Community Structure of Coral-Associated Fauna of Reefs in the Chagos Archipelago

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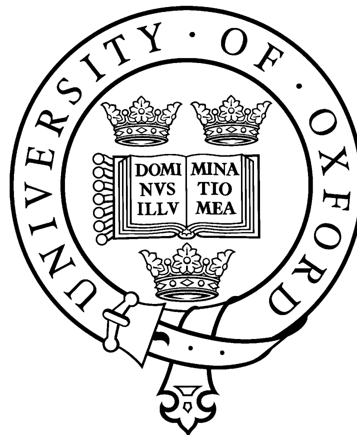

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My thesis is dedicated to my beloved family

"The more clearly we can focus our attention on the wonders and realities of the universe about us, the less taste we shall have for destruction" - Rachel Carson

"Never doubt that a small group of thoughtful, committed citizens can change the world. Indeed, it is the only thing that ever has" - Margaret Mead

"The greatness of a nation and its moral progress can be judged by the way its animals are treated" - Mahatma Gandhi

"I would rather be amongst forest (or marine!) animals and the sounds of nature, than amongst city traffic and the noise of man!" - Anthony Douglas Williams

"The cure for everything is salt water – sweat, tears, or the sea" - Isak Dinesen

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Declaration

I declare that the work presented here is my own, under the supervision of Professor Alex Rogers, as well as Dr Heather Koldewey and Professor Morgan Pratchett. Chapter two has been published as a scientific report in the Journal of Coral Reefs, with contributions from my aforementioned supervisors as well as Professor Michael Bonsall and Professor Martin Speight. In the instances where others have contributed, such as in the appendices, the contributions have been specifically acknowledged. I have clearly indicated and referenced where I have quoted and discussed others' work throughout the document. With regards to data access; all sequence data has been archived in GenBank, rare specimens (where not used for molecular purposes) have been catalogued in the University of Oxford Natural History Museum or Raffles Museum in Singapore where appropriate, and species inventories are listed as appendices I and II.

I can confirm this thesis has not been submitted for any other qualification at any institution. I hereby confirm this thesis is submitted in fulfilment of the requirements for the degree of Doctor of Philosophy.

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October 2015

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Abstract

The aim of this study is to assess the biodiversity of the reef cryptofauna (with a focus on the decapods, Crustacea) on dead coral microhabitats, and to begin to understand the processes underpinning their community structure in the Chagos Archipelago. The majority of reef biodiversity is comprised of the cryptofauna, defined as small, often cryptic, mainly invertebrates, which inhabit the reef structure. Despite this the cryptofauna are severely understudied relative to the fish and coral faunal components. An estimated 168,000 species of reef invertebrates have been described on coral reefs, and approximately 20% of reef invertebrates are crustaceans, making them one of the most speciose taxa on coral reefs.

The Chagos Archipelago represents one of the most resilient reefs globally, partly because of its remote location, away from the majority of human pressures. Consequently, it serves as an unaffected reference site for biodiversity and ecosystem function studies. The decapod species richness estimate for Chagos (at least 217 species) exceeds that of any other location globally. A high proportion (32%) were observed to be rare species (singletons), this may be an artefact of incomplete sampling, however if this observation represents true rarity it would suggest this component of biodiversity could be more vulnerable to biodiversity loss than previously thought. Furthermore, any biodiversity loss could also have implications for ecosystem function if rare species contribute disproportionately more to vulnerable ecosystem processes than dominant species. Data presented on determining the most accurate species delimitation method for estimating decapod species richness utilising DNA barcoding. Performance of species delimitation methods was taxon-specific within the decapods, and delimitation of singletons was challenging for all methods. However, the Poisson tree processes (PTP) approach was generally the most accurate at delimiting decapod putative species.

Whilst assessing decapod diversity a high prevalence of obligate coral-dwellers on dead coral microhabitats were discovered. Obligate coral-dwellers are almost universally found on live coral, inferring they have a strong reliance on live coral for food, habitat and/or recruitment. The prevalence of obligate coral-dwellers on dead coral suggests that these decapods are not simply persisting on coral hosts that have died but may be explicitly recruiting to or moving to dead coral hosts at certain stages in their life cycle.

Finally, the processes influencing community assembly and maintenance of a family of decapods, the Palaemonidae, on dead coral colonies was investigated. There was spatial hierarchy in trait and phylogenetic diversity, with environmental filtering acting only at the local level (within atolls and between coral colonies). Whilst phylogenetic signal at the metacommunity level (the archipelago) was inconclusive, trait convergence and lability of trait evolution were key processes determining species distribution at the local level.

This thesis represents the first biodiversity estimation of the cryptofauna in Chagos on any microhabitat and subsequently provides a baseline against which to compare this component of biodiversity in other areas experiencing higher levels of anthropogenic stressors, at least in the Indian Ocean. I also produce a rare empirical evaluation of species delimitation methods, which will provide guidance for future decapod molecular studies. The prevalence of obligate coral-dwellers on dead corals demonstrates the complexity of these organisms' habitat associations and highlights the need for further investigation to establish their vulnerability to habitat degradation on coral reefs. Furthermore the identification of some of the deterministic processes driving community structure of the Palaemonidae contributes to understanding of ecosystem function.

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1. CHAPTER ONE:

Introduction

CHAPTER ONE

1.1. Introduction

Biological diversity literally translates as the 'variety of life', it incorporates three biological levels; genes, species, and ecosystems across different spatial and temporal scales. Biodiversity is intrinsically linked to ecosystem function, because species and their interactions play a major role in regulating environmental properties and processes (Loreau et al. 2001; Hooper et al. 2005; Loreau 2008). Today the global extinction rate is 1000 times higher than the background rate (Millennium Ecosystem Assessment 2005), and this acceleration is attributed to the activities of modern humans. As we lose species and biodiversity patterns are altered through anthropogenic impacts, it follows that ecosystem function will also be altered, often to the detriment of the ecosystem and the humans that rely on it for goods and services. Nowhere is this paradigm more acute than in coral reef ecosystems, which is one of the most biodiverse ecosystems (Reaka-Kudla 1997), and one of the most threatened (Wilkinson 2008; Burke et al. 2011). Here I will review our understanding of the biodiversity-ecosystem functioning relationship and the consequences of biodiversity changes, caused by anthropogenic activities, on coral reef ecosystem functioning. Furthermore, the review will focus on the most biodiverse and yet understudied component of coral reefs, the so-called cryptofauna (Reaka-Kudla 1997; Knowlton and Jackson 2008), communities of animals that live hidden within the coral framework.

1.2. Coral Reefs

Coral reefs are the most biodiverse marine ecosystem (Reaka-Kudla 1997; Sala and Knowlton 2006), and they are also amongst the most valuable providing humankind with an estimated \$172-\$375 billion US per annum in goods and services (Costanza et al 1997; TEEB 2010). They represent 0.2% of the ocean floor and yet they are estimated to harbour a quarter to a third of all marine life, most of which are found nowhere else (Reaka-Kudla 1997; Sala and Knowlton 2006). However coral reefs are also one of the most threatened ecosystems (WWF.

2015). 19% of coral reefs have been lost (Wilkinson 2008) and 75% of the world's reefs are threatened by local and thermal stressors (Burke et al. 2011).

To date approximately 250,000 species have been described from coral reefs, but published estimates for actual reef biodiversity range from 1 to 10 million species (Reaka-Kudla 1997; Small et al., 1998). There are approximately 5000 species of fish (Lieske and Myers 1994; Depczynski and Bellwood 2003; Bellwood et al. 2012), 700 species of coral (Veron 2000; Hughes et al. 2002) but an overwhelming richness of approximately 168,000 described species of reef invertebrates (excluding corals) belonging to at least 31 phyla (Reaka-Kudla 1997; Ruppert et al. 2004; Stella et al. 2011a). Coral reefs likely support the greatest number of metazoan phyla of any ecosystem on the planet (Reaka-Kudla 1997). The cryptofauna, e.g. crabs, brittlestars, and shrimp, often compared to rainforest insects, makes up the majority of the reef metazoan diversity, a large proportion of the biomass, and are important trophic links, crucial to ecosystem function (Ginsburg 1983; Reaka-Kudla 1997; Enochs and Manzello 2012b; Kramer et al. 2014).

1.3. Biodiversity & Ecosystem Function

Ecosystem functioning encompasses a variety of ecosystem properties related to 'pools" and "fluxes" of matter and energy and the ecosystem provision of goods and services (Loreau 2001). There are numerous properties and processes which contribute to ecosystem functioning, and there are often strong interactions between them. Giller et al. (2004) listed over 20 ecosystem processes potentially affected by diversity loss. For instance the biomass of groups of species is an ecosystem property, and reef-building is an ecosystem process that can be measured by a change in reef perimeter or calcite precipitation rate. However, there is no single response that can capture the entire scope of ecosystem properties and processes to characterize the overall functioning of an ecosystem (Giller et al. 2004; Tilman et al. 2014).

Biodiversity, i.e. genotypes, species, and functional groups, can be considered explanatory variables of ecosystem function (Gamfeldt and Hillebrand, 2008). Species richness is an important and readily quantified component of biodiversity (Done et al. 1996; Giller et al. 2004; Magurran and McGill 2011). However there are other facets of biodiversity such as functional diversity, phylogenetic diversity, habitat or beta diversity, and species evenness, which are also important measurements of biodiversity. In order to adequately link biodiversity to ecosystem function we must look at richness and evenness (the Species diversity concept) as well as some measure of abundance per unit area (Done et al. 1996). For example, reefs may have identical species richness and relative abundances but the one with the greatest total biomass will contribute more to limestone and/or protein accumulation (Done et al. 1996). Also it has been demonstrated in plant communities that anthropogenic disturbances can bring about significant changes in evenness without associated changes in richness (Connolly et al. 2001; Giller et al. 2004) and it is important to look at these over different taxonomic levels. The mechanisms by which biodiversity may influence ecosystem function are often more related to some functional traits of species, e.g. body size, rather than species richness and composition itself (Tilman et al. 2014; Giller et al. 2004; Hooper et al. 2005; Magurran and McGill 2011). Therefore studying diversity at the functional group level is often most valuable but care is required when assigning species to functional groups (Giller et al. 2004; Hooper et al. 2005; Magurran and McGill 2011). Phylogenetic diversity (a measure of phenotypic and genetic diversity) is often used in a comparative way with trait diversity to determine how historical and/or environmental factors affect the formation of species assemblages across different spatial and/or temporal scales (Webb et al. 2002; Pavoine and Bonsall 2011). Both phylogenetic and trait diversity can be used to identify the relative important of ecological deterministic processes such as environmental filtering and limiting similarity from alternative processes such as random speciation and extinction, random dispersal and ecological drift (Webb et al. 2002; Kraft and Ackerly 2010; Pavoine et al. 2014).

Another facet of biodiversity is species interactions. Human impact on biodiversity is usually measured by reduction in species abundance or richness but just as important, though more difficult to discern, is the anthropogenic elimination of ecological interactions (Bascompte 2009). At a remote Pacific atoll McCauley et al. (2012) found evidence of an interaction chain at least five linkages long between trees and manta rays. Seabirds roosting on native trees fertilize the soils, increasing coastal nutrients and the abundance of plankton, thus attracting manta rays to native forest coastlines. At regions of this atoll where native trees have been replaced by human propagated palms this complex interaction chain breaks down. This example demonstrates how anthropogenic disturbance may cause reductions in ecological interaction chain length, consequently isolating and simplifying ecosystems and reducing ecosystem function (McCauley et al. 2012).

1.4. Natural & Human Disturbance

Disturbance is important for the maintenance of biodiversity and has a long history for coral reefs (Connell 1978; Nyström et al. 2000). Coral reef disturbances range from frequent minor pulses such as grazing and predation to larger more infrequent events such hurricanes and sea level changes. This dynamic set of disturbances is referred to as the natural disturbance regime of coral reefs. The “intermediate disturbance hypothesis” (Connell 1978) holds that a low rate of disturbance allows competitively dominant species to monopolise areas, a high rate allows only the most rapid colonizers to dominate, and an intermediate rate favours coexistence of many species.

In recent decades human activities are altering the natural disturbance regimes of coral reefs. Overfishing and destructive fishing methods are the most immediate and prevalent local human activity acting on coral reefs, with 55% of reefs threatened globally by this activity

alone (Burke et al. 2011). Other major local threats include marine-based pollution and damage (e.g. toxins from oil and gas installations and shipping), watershed pollution (e.g. fertilizer run-off from agriculture), and coastal development (e.g. coastal construction leading to sediment run-off). Global threats are largely a result of rising carbon dioxide (CO_2) levels in the atmosphere from burning fossil fuels (Veron et al. 2009). The oceans are a Co_2 sink, and increasing absorption of heat-trapping Co_2 particles results in higher oceanic CO_2 levels, and consequently increases in sea temperature. This causes disturbance to the symbiotic coral-zooxanthellae relationship resulting in coral bleaching, which put simply is when corals eject their symbiotic algae, zooaxanthellae, responsible for much of the coral's energy production (Veron et al. 2009; Hoegh-Guldberg et al. 2007; Hoegh-Guldberg. 1999). Mass coral bleaching can kill corals outright but even if mortality does not occur corals are weakened, reducing their ability to reproduce, reducing growth rate, and increasing their vulnerability to disease (Veron et al. 2009; Pandolfi et al. 2011). This is compounded by local human impacts such as over-fishing and nutrient run-off. Current projections predict that half of all reefs will experience severe bleaching annually by 2030s assuming CO_2 levels continue along current trajectories (Veron et al. 2009; Burke et al. 2011). In addition to this CO_2 levels are altering ocean chemistry and increasing ocean acidity, reducing the saturation level of aragonite available for coral growth (Pandolfi et al. 2011). At current CO_2 trajectories by 2050 only 15% of reefs will be in areas adequate for reef growth (Burke et al. 2011; Pandolfi et al. 2011), and yet the potential impacts of ocean acidification are still not being incorporated into many coral reef refugia models (Keppel and Kavousi 2015).

1.5. Reef Resilience

Reef resilience can be described as the ability to absorb recurrent disturbances or shocks, resist phase shifts, and regenerate after natural and human-induced disturbances (Nyström et al. 2000). Increasingly reefs are unable to recover from recurrent disturbances as they have

done through their evolutionary history because their resilience has been reduced often by a synergy of human impacts (Hughes et al. 2010). On a regional scale the Caribbean shows significantly lower resilience than Indo-pacific reefs, with 83% of Caribbean reefs showing either no evidence of recovery (47%) or no decline (36%) following disturbance, compared with 39% of Indo-Pacific reefs exhibiting either no recovery (24%) or no decline (15%) (Connell 1997; Roff and Mumby 2012). There are obviously many key biogeographical differences between the Caribbean and Indo-Pacific regions including faunal composition, trophic structure, grazing pressure, life-history traits, habitat connectivity, coral reproductive traits, and species richness. These characteristics will influence reef resilience. Roff and Mumby (2012) have put forward specific key factors that might explain the Caribbean's general trend of lower resilience. These include faster rates of macroalgal growth, higher rates of algal recruitment, basin-wide iron-enrichment of algal growth, Aeolian dust, a lack of acroporid corals, lower herbivore biomass, and missing groups of herbivores. Roff and Mumby (2012) concluded that Indo-Pacific systems would need to be pushed considerably harder to exhibit instability or even experience a coral-macroalgal phase shift due to their higher resilience.

Once a reef has been pushed from its equilibrium state, e.g. coral-dominated, by an episodic disturbance or shock to an alternative state, e.g. a macro-algae dominated state, this state will be maintained if it is reinforced by a set of positive feedbacks (Nystrom et al. 2000; Hughes et al. 2010; Nystrom et al. 2012; Roff and Mumby 2012). For instance a macro-algae dominant state will be maintained if herbivore populations are depleted, preventing the return of corals, as recruits are shaded and/or overgrown, and microbial communities are destabilised which this promotes coral disease (Smith et al. 2006; Birrell et al. 2008; Hughes et al. 2010; Ainsworth et al. 2010). There is much evidence of phase-shifts from corals to alternative assemblages, and their underlying processes and mechanisms (Nystrom et al. 2000; Hughes et al. 2010; Nystrom et al. 2012). However it seems phase shift reversals are rare. Roff and Mumby (2012) analysed long-term reef health data of 38 Caribbean reefs following phase

shifts and found only a single case documenting recovery and reversal of macroalgal phase shifts, which was on a Jamaican reef.

The capacity of reefs to cope and recover from disturbance is determined by characteristics such as genetic variability within populations, diversity within and among functional groups, variability and connectedness of habitats, and distribution, abundance and dynamic interactions of species (Done et al. 1996; Nystrom et al. 2000; Pratchett et al. 2014) - all facets of 'biodiversity'.

1.6. Biodiversity loss & Ecosystem function

Over the last decade much research has attempted to understand how ecosystems change as their diversity and community structure are compromised. However, our understanding of how ecosystems respond to losses in biodiversity remains rudimentary. The central question is whether ecosystems with increasing biodiversity loss can maintain functional properties and processes to the same level and rate as unaffected ecosystems (Loreau et al. 2001; Kinzig et al. 2002; Giller et al. 2004; Tilman et al. 2014). The majority of this research to date has been at small spatial, temporal, and taxonomic scales where species diversity and composition can be manipulated. Many of these studies have demonstrated that biodiversity loss is often detrimental to ecosystem properties (Loreau et al. 2001; Hooper et al. 2005; Srivastava and Vellend 2005; Cardinale et al. 2006). However, several counterexamples have questioned the generality of these biodiversity effects (Finke et al. 2004; Fridley 2002; Petchey et al. 1999). As a result it has also been argued that the consequences of biodiversity loss are likely to be idiosyncratic, differing between trophic levels and ecosystems (Emmerson et al. 2001; Covich et al. 2004). Nevertheless, Cardinale et al. (2006) presented a meta-analysis of 111 studies that experimentally manipulated species diversity to examine how it affects the functioning of numerous trophic groups across different ecosystems. They focused on experiments that

varied the richness of three or more species in a given trophic group and measured either of two response variables: the aggregate abundance or biomass of species, and/or the total amount of resources depleted. The analysis demonstrated that the average species loss does negatively affect functioning of a wide variety of organisms and ecosystems (Cardinale et al. 2006).

The scaling up of these findings to actual human scenarios is contentious due to the difficulty of simulating the full complexity of natural systems (Duffy 2009; Hillebrand and Matthiessen 2009). In a rare global analysis of coral reef fish diversity Mora et al. (2011) showed that coral reef ecosystem functioning (measured as standing biomass) accelerates with the addition of new species, implying that each species makes a unique contribution to the functioning of the ecosystem. This non-saturating relationship is in contrast to many experiential studies that describe saturating relationships between biodiversity and ecosystem functioning (Hooper et al. 2005) and it indicates that the implications of biodiversity loss on coral reefs could be more worrying than previously thought. However, this study also demonstrates a negative effect of human population density on reef fish functioning, finding that for the same number of people the loss of standing biomass is significantly larger in more diverse systems (Mora et al. 2011). Human population density was highly significantly related to three human activities: fishing, coastal development, and land use. The authors suggest that selective extinction of large species, which is well known in coral reef systems (Bellwood et al. 2004; DeMartini et al. 2008), is potentially responsible for this pattern. Danovaro et al (2008) also found an exponential relationship between biodiversity and ecosystem function in deep-sea ecosystems, as well as an exponential relationship between functional biodiversity and ecosystem efficiency. It is suggested that mutually positive functional interactions (ecological facilitation) can be common in deep-sea ecosystems and drives this relationship (Danovaro et al. 2008; Loreau 2008).

These two large-scale empirical studies (Danovaro et al. 2008; Mora et al. 2011) suggest that species loss can have a substantially larger effect on functioning of natural ecosystems than predicted from manipulative experiments. Mora et al (2014) provide three hypotheses that, individually or combined, could explain the contrast between manipulated experiments and large-scale observational studies: i) use of functional richness instead of species richness, ii) more ecological interactions lead to an increased production efficiency of species in producing biomass, iii) communities are likely assembled in an ordered succession of species from low to high ecological efficiency.

1.7. Mechanisms of effects of diversity on ecosystem function

Ecosystem processes and properties can respond to changes in species or functional diversity in several ways. The patterns depend on the degree of dominance of the species lost or gained, the strength of their interactions with other species, the order in which species are lost, the species remaining, and the relative abundance of biotic and abiotic control over process rates (Lawton and Brown 1993; Naeem et al. 1995; Naeem 1998; Hooper et al. 2005; Mouillot et al. 2013). More than 50 potential response patterns have been proposed (Loreau et al. 1998; Naeem 2002). Increases in ecosystem functioning with increasing diversity can arise from two primary mechanisms: sampling effect or selection probability, and positive interactions between species (complementarity and facilitation), described below.

Ecosystem functioning can increase in higher-diversity assemblages solely because the probability is increased that the functionally dominant species are present; this is known as a sampling effect (Loreau et al. 2001; Giller et al. 2004; Hooper et al. 2005). In some experiments this effect may be due to an experimental artefact (Huston 1994), or it might be a valid ecological mechanism by which diversity translates into enhanced ecosystem functioning (Loreau et al. 2001). This assumes that the dominant species are those that

contribute most to the measured process or property (Giller et al. 2004). This is important to the management of coral reefs because if the critical ecosystem functions are mostly maintained by dominant species and only rare species went extinct then ecosystem function would be largely unaltered by species losses. However contrary to this, more recent studies have demonstrated that rare species can have a disproportionately large affect on maintaining ecosystem processes (Lyons et al. 2005; Bracken and Low 2012; Mouillot et al. 2013). Related to the sampling effect is the effect of species identity. Some species are more efficient than others at driving particular processes (Hooper and Vitousek 1997; Giller et al. 2004) e.g. there are strong species identity effects in the nutrient recycling of fish (Vanni 2002). Therefore if a species is lost the effect on the system will depend on that species' specific effect on influencing ecosystem processes. In addition the contribution of individual species to ecosystem processes can vary with environmental conditions (Cardinale et al. 2000).

Species or functional richness could increase ecosystem properties through positive interactions among species (Loreau et al. 1998; Danovaro et al. 2008). Complementarity results from reduced interspecific competition through niche partitioning (Hooper et al. 2005). If species use different resources, or the same resources but at different times or different points in space, more of the total available resources are expected to be used by the community (Hooper et al. 2005). If those resources limit growth, then increasing functional richness should lead to greater total productivity and decreased loss of resources from the ecosystem. Facilitative interactions among species could also lead to an increase in ecosystem functioning through increased species richness, for instance if certain species alleviate certain conditions which benefit others or provide a critical resource for other species (Bruno et al. 2003; Hooper et al. 2005; Danovaro et al. 2008). Grazing herbivores, for example, facilitate coral growth by preventing algal overgrowth of newly settled recruits and juvenile corals (Done et al. 1996).

Of course complementarity, facilitation, selection or sampling effects are not likely to be mutually exclusive in complex ecosystems (Loreau et al. 2001; Hooper et al. 2005) such as coral reefs. More complex ecosystems with multi-trophic levels will likely have more complex responses because a higher number of factors will come into play such as the degree of top-down vs. bottom-up control, food web connectivity, trophic level and functional characteristics of the species gained or lost, and immigrations vs. emigration (Loreau et al. 2001; Hooper et al. 2005).

1.7.1. Biodiversity as insurance

Organismal functional traits are one of the key controls of ecosystem properties (Loreau et al. 2001; Giller et al. 2004; Hooper et al. 2005). The number of traits, represented either by species or functional groups, forms the basis of the insurance hypothesis of biodiversity (Giller et al. 2004; Hooper et al. 2005). The insurance hypothesis states that biodiversity maintains ecosystem functioning in the face of disturbance and environmental fluctuations, by increasing the likelihood that there are some well-adapted or resistant species that maintain ecosystem functioning (Yachi and Loreau 1999). In other words even when high diversity is not critical for maintaining ecosystem processes under constant or benign environmental conditions, it might be important for maintaining them under changing conditions. Therefore a species may be functionally redundant for an ecosystem process at a given time but is not redundant through time (Loreau et al. 2001; Tilman et al. 2014). This should lead to greater stability of ecosystem properties (Hooper et al. 2005; Tilman et al. 2014).

Using experimental ocean warming conditions in seagrass ecosystems Eklof et al. (2012) have shown that climate change can weaken the insurance effect of biodiversity by reducing the relative efficiency of resistant species. A meta-analysis showed that herbivore consumption rate trades off with predation resistance in three common seagrass herbivores The experiment then showed that the herbivores together controlled macroalgae cover and

facilitated seagrass dominance regardless of climate change (Eklof et al. 2012). When the predation-vulnerable herbivore was excluded in normal conditions the two resistant herbivores maintained top-down control, but under warming conditions increased algal growth outstripped control by herbivores and the system became algal-dominated (Eklof et al. 2012). This short-term experiment is an inevitable simplification of the natural world but it demonstrates a mechanism by which rising sea temperatures may act on an ecosystem closely connected to reefs, though arguably less complex, to reduce ecosystem function.

In reef ecosystems functional diversity, the existence of species that fill similar ecological roles, is particularly important for resilience as it can buffer against changing conditions and species loss (Nystrom et al. 2000; Bellwood et al. 2004; Nystrom et al. 2012). High functional diversity will provide alternative ways to maintain key processes of ecosystem function, e.g. herbivory, following environmental change. Put another way, high diversity provides the potential for functional redundancy i.e. where the loss of one species is potentially compensated for by the actions of another (Nystrom et al. 2000; Bellwood et al. 2004; Obura 2005). So, areas with limited redundancy, e.g. Caribbean reefs, often exhibit lower resilience to impact (Roff and Mumby 2012). However, even in high-diversity ecosystems redundancy in critical functional groups can be limited (Bellwood et al. 2004; Mouillot et al. 2013), and the activities of lost species can only be compensated for to some extent by an increase in population size of the remaining species (McGrady-Steed and Morin 2000).

Human activity can result in a loss of biodiversity within and among functional groups, leading to simplification of the habitat, reduced functional plasticity, and a decreased ability to buffer the effect of future disturbance (Nystrom et al. 2000). Reefs with decreased diversity within functional groups might still maintain ecological function but when faced with an additional disturbance they might reach a critical threshold and shift to another less-desirable stable state (Nystrom et al. 2000; Bellwood et al. 2004). For instance, in many areas of the Caribbean following depletion of fish stocks sea urchins became the primary herbivore and

prevented a rapid phase shift to a macro-algae dominant state, until disease targeting *Diadema* populations lead to a die-off (Lessios 1988; Knowlton 1992; Bellwood et al. 2004). Another cost of this reduction in functional diversity is that although echinoids and fish can substitute for each other in a herbivore role, echinoids are far more destructive bioeroders; they burrow into and erode the reef structure (Bellwood et al. 2004).

Reef resilience also strongly relies on the range of responses by species within a functional group to environmental change so that not all species in the group are equally affected by all disturbances (Elmqvist et al. 2003; Bellwood et al. 2004). For example, the response diversity of fish to chronic overfishing is extremely low resulting in few fish species remaining on the reef. In this instance the insurance value of redundancy and of high species richness is negligible highlighting that the right combination of functional traits, not just functional diversity, plays a major role (Bellwood et al. 2004).

One of the major challenges of understanding effects of biodiversity on ecosystem functioning is whether patterns observed at small scales also occur at larger scales. Natural systems can be viewed as heterogeneous landscapes composed of numerous patches that differ in both their biotic and abiotic attributes (Giller et al. 2004). These patches taken together represent a fundamentally different type of biodiversity than the diversity of species, other taxonomic units or functional groups (Giller et al. 2004). Reef ecosystem function is also highly dependent on habitat diversity and connectivity through community interactions, particularly dispersal and immigration, with other reefs and ecosystems, e.g. mangroves, in the seascape (Roberts 1997; Nystrom et al. 2000). For instance many reefs rely to some extent on larval sources from outside their own reef boundaries. This may provide the sink reef with an element of spatial reef resilience because if the sink reef suffers substantial mortality, for instance following a bleaching event, then reef recovery may still occur due to larval recruitment from source reefs which perhaps survived the bleaching relatively unscathed due to local resilience factors such as shading (Roberts 1997). However, over-reliance on larval

recruitment from other reefs may be problematic because dispersal routes from source reefs may be cut off or altered by human activities, e.g. through channel construction or reef mining leading to changes in currents, or by creating local pollution barriers reducing larval survival rates.

1.8. Shifting Baselines

We have very little understanding of how reefs functioned in the absence of any human-impacts (Knowlton and Jackson 2008). Many studies seek to explain current conditions, such as patterns of biodiversity, by recent events and activities only, mainly because long-term datasets do not exist, i.e. pre-human disturbances; a paradigm known as the problem of shifting baselines (Pauly 1995; Jackson et al. 2001; Knowlton and Jackson 2008; Hughes et al. 2010). Even studies of the Caribbean that began in the 1950s were based on reefs that were already severely overfished (Pandolfi et al. 2003; Knowlton and Jackson 2008). Remote uninhabited atolls, such as the Northern Line Islands in the Pacific (Sandin et al. 2008) and the Chagos Archipelago in the Indian Ocean (Sheppard et al. 2012), represent probably the closest existing true baseline. These remote reefs could be key to investigating biodiversity effects on ecological processes and are vital for monitoring of global climate change impacts under optimal conditions of minimal local anthropogenic impacts (Knowlton and Jackson 2008; Hughes et al. 2010).

1.9. Cryptofauna Biodiversity

The cryptofauna are an important food source for fish (Stella et al. 2011) 70% of fish are invertivores, and 60% of invertivores prey predominantly on benthic Crustacea (Williams and Hatcher 1983; Randall et al. 1997; Froese and Pauly 2014). They have high productivity, that is the ability of a group of organisms to transfer energy to higher trophic levels, with productivity estimates for Crustacea from all microhabitats only 3 times less than productivity

estimates for reef fishes (Kramer et al. 2014). Cryptofauna living in live coral have also been shown to protect the coral from predators, e.g. crown-of-thrown starfish *Acanthaster planci*, and the harmful effects of bleaching (Glynn 1980; Glynn 1983; Pratchett 2001). Despite the importance of cryptofauna in coral reef ecosystems very little is known about the biodiversity of this suite of organisms and the biological and environmental interactions that affect their distribution and life histories. However, several large-scale initiatives to inventory coral reef biodiversity have recently been undertaken. These include the investigation of mollusc fauna in New Caledonia (Bouchet et al. 2002), the marine biodiversity survey of Guam and the Marianas (Paulay 2003), the Santo 2006 Expedition in Vanuatu (http://www.santo2006.org), the Moorea Biocode Project (http://bscit.berkeley.edu/biocode), and the Census of Marine Life – Census of Coral Reefs (http://www.creefs.org) survey of French Frigate Shoals, northwestern Hawaiian Islands in 2006, and in Australia in 2008.

The lack of knowledge to date about this component of coral reef biodiversity is partly because sampling of the cryptofauna is often difficult due to the concealed nature of this biota and their close association with ecologically sensitive coral structures. In addition traditional taxonomic methods of species identification are extremely slow and taxonomy requires specialised skills for which few people are trained (Plaisance et al., 2009) meaning assessments of cryptofauna biodiversity are rare.

1.9.1. Species Diversity Estimates using DNA Barcoding

The revolution in molecular genetics now offers alternative methods to make progress in estimating cryptofauna biodiversity. DNA barcoding can be used to identify species (Hebert et al. 2003), but perhaps more importantly it provides a faster way to understand trends and patterns of biodiversity by identifying operational taxonomic units (OTUs), which can be used to estimate species richness. It is particularly useful for identifying cryptic species that are hard to identify using traditional taxonomic methods (Knowlton 1993; Knowlton 2000) as

specimens are, for example, juvenile and therefore not catalogued in many keys and species descriptions.

The core idea of DNA barcoding is that short pieces of DNA can be found that vary only to a very minor degree within species, such that this variation is much less than between species (Savolainen et al. 2005). The gene of choice for many DNA-based animal identifications is cytochrome *c* oxidase I gene (COI), one of 13 protein-coding genes in the animal mitochondrial genome, which are preferable for barcoding because indels are rare (Hebert et al. 2003). COI has two important advantages over other protein-coding genes; the universal primers for this gene are robust (Folmer et al. 1994), and it appears to possess a greater range of phylogenetic signal than any other mitochondrial gene (Hebert et al. 2003). The ability of the 650-base pair sequence of the COI gene to identify organisms to species level has been demonstrated for large taxonomic assemblages of animals such as insects and fish (Hebert et al. 2003). It has also been demonstrated for the subphylum Crustacea (Costa et al. 2007), species of which are numerous within the cryptofauna (Plaisance et al. 2009). Studies on assemblages of decapods has shown that levels of nucleotide sequence divergences were from 19 to 48 times greater between congeneric species than between individuals of a species, indicating that the COI gene should be very effective for discriminating species of Crustacea (Costa et al. 2007). Using DNA barcodes to identify unknown samples is easier if a well-studied, well-sampled sequence database is available (Meyer and Paulay 2005), and databases such as GenBank (http://www.ncbi.nlm.nih.gov/genbank) and Biocode (http://biocode.berkeley.edu) now contain a number of species-level identified sequences for identification of organisms found within the cryptofauna community, though of course these databases are far from complete.

On reefs in the Pacific of Panama, Enochs and Mazello (2012) utilized barcoding techniques to estimate motile cryptofauna species richness and reported a total of 289 OTUs from six phyla in live and dead coral across a gradient of substrate erosion. Species richness was

higher in dead frameworks (261-370 OTUs) than live coral (112-219 OTUs). This is likely due to the defences of live corals making them an inhospitable place to live (Stella et al. 2009), and the greater niche diversity relative to live coral (Idjadi and Edmunds 2006). Plaisance et al. (2009) investigated the crustacean cryptofauna inhabiting dead *Pocillopora* coral heads of similar size, from 10m depth, and one type of reef exposure, from atolls in five central Pacific locations. Crustaceans accounted for 30-40% of the macrofaunal diversity encountered in this habitat. 403 individuals were sequenced from 22 dead coral heads yielding 125 distinct taxa using a species level criterion of 5% similarity (Plaisance et al. 2009). Species richness statistics suggested total number estimates in Moorea of at least 90 crustacean species and 150 in the Northern Line Islands (Plaisance et al. 2009). Generally these high estimates of cryptofauna species richness found on coral reefs can be attributed to the intricate topographic structure built by scerlactinian corals, and the diversity of habitats which creates multiple niches ideal for speciation (Gratwicke and Speight 2005; Wilson et al. 2007). Even so these estimates of crustacean species richness are surprisingly high for one component of the cryptofauna, using a limited sampling effort, in one habitat type, and also in a region of moderate species diversity well to the east of the center of diversity (Hoeksema 2007). Interestingly 44% of OTUs were represented by only a single individual, suggesting a high proportion of rare individuals, and an additional 33% were represented by several specimens at only one locality, suggesting a high level of endemism (Plaisance et al. 2009).

1.9.2. Cryptofauna abundance inhabiting live and dead coral colonies

Dead and live coral habitats have differing cryptofauna community structures, with live coral having many specialised associates (Stella et al. 2011). For instance, at least 51 species of invertebrates feed on coral tissue or the mucus it produces (Rotjan and Lewis 2008). It is estimated that 10-25% of small invertebrates have close associations with live coral, particularly branching coral heads, but it is not known to what extent most of these organisms are dependent on the live coral and have specialised (Bruce 1972; Abele and Patton 1976;

Cole 1980; Tsuchiya et al 1992; Stella et al. 2011). Organisms that live within live corals can be divided into live coral obligates and facultative (or opportunistic) coral dwellers (Pratchett et al. 2009; Stella et al 2011). Facultative coral dwellers may also inhabit dead coral habitats, sediment, rubble, or intact but dead coral structures, whereas live coral obligates are dependent on their live coral hosts to some extent.

High numbers of invertebrates have been found living on both dead and living branching coral heads (Enochs 2012; Kramer et al. 2014). The abundance of invertebrates has been shown to be correlated with the structure of the framework, with branching coral colonies hosting higher abundances than massive coral colonies (Edwards and Emberton 1980; Coles 1980; Stella et al. 2011). Branching coral colonies are more complex structures than massive corals and therefore afford a higher level of protection from predators (Edwards and Emberton 1980; Coles 1980). Studies have also shown a positive correlation between branching structure density and the abundance of motile cryptofauna (Edwards and Emberton 1980; Vytopil and Willis 2001).

Estimates of cryptic reef crustacean and mollusc density are as high as 5,200 and 570 individuals per m^{-2} respectively (Klumpp et al. 1988). Total cryptofauna abundance recorded from a single live coral colony of *Pocillopora damicornis* in Australian waters stands at 2,000 individuals (Grassel 1973). A more recent study by Enochs (2012) found a mean of 2,147 individuals m^{-2} on live *Pocillopora* colonies in Panama, whilst Kramer et al. (2014) found dead coral colonies to be the most important microhabitat in terms of crustacean abundance (7838 ± 628 individuals per $100cm^{-2}$, mean ± SE). Differences in abundance estimates are likely a result of a variety of variables such as biogeography, coral colony size and structure, and reef health (Coles 1980; Enochs et al. 2011; Bellwood et al. 2012).

1.9.3. Cryptofauna biomass and trophic structure

Ginsburg (1983) proposed that the biomass of cryptic taxa actually exceeds that of the surface biota. To investigate this hypothesis Enoch (2012) compared the cryptofauna biomass from the most eroded structures sampled in his study to fish biomass densities published for other reef regions and found that the cryptofauna biomass was only slightly less than that of the epibenthic fish biomass estimated by Ackerman and Bellwood (2000) for Orpheus Island, Great Barrier Reef, and by Opitz (1996) for all fishes on a theoretical Caribbean coral reef. Although the comparison is limited because the regions differ in morphology and complexity, it should also be noted that Enoch's study only encompassed the motile metazoans, just one component of the cryptofauna. Ginsburg's proposal has yet to be proved, but it does serve to highlight the high biomass of this component of biodiversity. High biomass levels are partly a result of efficient nutrient capture and recycling within coral reef ecosystems, for instance sessile suspension feeding cryptic species have been shown to account for 22% of gross reef metabolism (Richter 2001).

The cryptofauna consists of a high biomass of organisms from all trophic levels (Enochs 2012; Enochs and Manzello 2012a). Of great importance are the deposit feeders, detritivores, and omnivorous trophic groups for the scavenging and recycling of organic matter e.g. coral mucus and fish faeces back into the food web (Rothans and Miller 1991; Depczynski and Bellwood 2003; Glynn 2011). The dominant herbivores known for playing a significant role in controlling algae competition on coral reefs are fish, e.g. scarids, and to some extent larger invertebrates such as *Diadema* (Harborne et al. 2009; Mumby 2009), but herbivorous cryptofauna also play an important part at the local scale. Many herbivorous crustaceans, molluscs, and echinoderms identified in the cryptofauna are micrograzers, translating primary productivity into the wider food web (Klumpp et al. 1988; Enochs 2012). For instance, Klumpp et al. (1988) demonstrated that cryptic micrograzers have high grazing rates at the local scale, of 2.16 g/C/m$^{-2/}$day^{-1} inside damselfish territories. High concentrations of carnivores have also been recorded (Glynn 2006; Enochs and Manzello 2012) including cryptic predatory fish, gastropods, polychaetes, isopods, and stomatopods (Sheppard 1984;

Reaka 1987; Grutter et al. 2008) suggesting that intricate food webs exist within the coral colonies microhabitats themselves. In addition, cryptofauna are also a principle food source for epibenthic and nektonic reef fish (Peyrot-Clausade 1980), with crustaceans being the predominant prey of invertivore fish (Kramer et al. 2015). Some fish species have been observed to actively break apart small reef structures to forage for prey (Randal, 1967). These multiple trophic levels demonstrate that this component of reef biodiversity is an important element of the larger coral reef ecosystem.

1.9.4. Cryptofauna and reef health

Declines in coral and fish populations are well documented on coral reefs and the effects of these declines on ecosystem functioning are at least partially understood (Bellwood et al. 2004; Hughes et al. 2010; Mora et al 2012; Nystrom et al. 2012; Mouillot et al 2013). However, we have almost no understanding of the impact of human activities on the cryptofauna, the largest component of reef biodiversity. The relatively few studies of reef cryptofauna biodiversity have focused on patterns and estimates of biodiversity, reviewed above, and have largely not addressed the impacts of human activities.

However, in an experiment, using artificial reef framework (ARF) units containing unconsolidated dead rubble placed *in-situ* on reef structures, Enochs et al. (2011) found that the presence of live coral cover, often used as an indicator of reef degradation, did not have a significant effect on the species richness or abundance of motile cryptofauna occupying the dead coral framework. They suggest that this indicates the dead-framework dwelling fauna have some degree of resilience to coral mortality. However, this experiment was limited and does not necessarily reflect natural reef scenarios as presence and abundance of live coral cover was represented by placing colonies of live or dead *Pocillipora* corals respectively on top of the ARFs. The effect of two other environmental parameters, water flow and porosity (e.g. large rubble fragments had greater amounts of void space and therefore higher porosity

than small fragments), were also tested by Enochs et al. (2011). Cryptofauna abundance and biomass were higher in low-porosity ARTs and biomass was greater in slow-flow environments, highlighting the importance of sheltered low-porosity habitats, such as back-reef rubble plains. In addition, Idjadi and Edmunds (2006) documented a significant positive relationship between reef structural complexity, and the diversity of 15 reef-associated invertebrates. Although these abiotic factors are not indicators of reef health they do go some way to explaining the patterns and trends of cryptofauna biodiversity.

In another study Enochs and Manzello (2012) sampled cryptofauna from four successive levels of degraded reef framework, as proxies of reef health, on an eastern Pacific pocilloporid reef. The density of cryptofauna per volume substrate was highest on dead coral frameworks of intermediate degradation, where complex eroded substrates provide abundant shelter, perhaps indicating that the effects of coral reef degradation will not be reflected by a reduction in cryptofauna populations until potentially years later when the dead coral framework has fully eroded, assuming no coral reef recovery takes place (Enochs and Manzello 2012).

1.10. Study location: The Chagos Archipelago, central Indian Ocean

The Indian Ocean harbours 13% of the world's coral reefs and has more than 65 million people living within 30km of the coastline (Burke et al. 2011). Located in the middle of the Indian Ocean (Fig. 1.1 and 1.2) the Chagos Archipelago represents some of the most un-impacted and resilient reefs in the world (Burke et al. 2011; Sheppard et al. 2012) and one of the closest existing baselines for natural healthy coral reef ecosystems (Knowlton and Jackson 2008). These reefs are biographically important as 'stepping stones' between the Western Indian Ocean and Indonesian region which are connected through the east-west flow of the South Equatorial Current (SEC) which reverses for a few months a year (Obura 2012) (Fig.

1.1). Chagos is currently the world's largest no-take marine protected area (MPA) at approximately 640,000km^2 and is estimated to contain at least 25% of the world's reefs under 'low threat' (Burke et al. 2011) and contains probably the largest continuous tracts of reefs under 'low threat' globally (Sheppard et al. 2012).

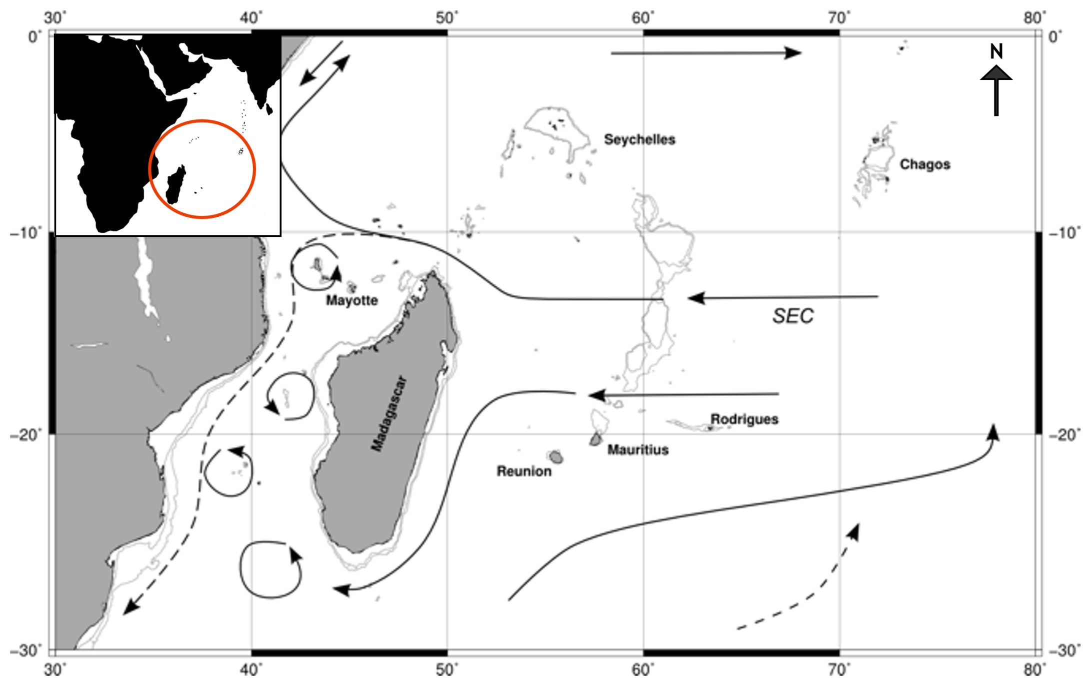

Figure 1.1. Map of the western Indian Ocean showing the direction of the major currents (from Obura et al. 2012). 200m and 1000m depth contours shown. SEC= South Equatorial Current. The in-laid map illustrates the geographical location in the wider Indian Ocean and the red circle highlights the area of focus in the main map.

However, Chagos reefs have suffered from global impacts. Chagos reefs were decimated in 1998 by bleaching-related mortality (Sheppard 1999b; Sheppard et al. 2002; Sheppard et al. 2012), which heavily impacted reefs throughout the Indian Ocean, many of which have not recovered (Ateweberhan et al. 2011). In Chagos there was variability across the archipelago with the southern atolls suffering mortality to deeper depths, for example to 40m in Diego Garcia, while in northern atolls mortality occurred only to approximately 10-15m (Sheppard

et al. 2002). Lagoon areas also fared much better than outer reef slopes at retaining high coral cover and species richness levels (Sheppard et al. 2002). Three years after the 1998 bleaching event Chagos outer reef slopes showed high levels of coral recruitment with juvenile densities of up to 28 m^{-2}, the highest reported globally at the time (Sheppard et al. 2002; Harris and Sheppard 2008). Now over a decade after, Chagos reefs have shown widespread recovery with coral cover returning to pre-1998 levels of between 40-50% at 6 -15m depth (Sheppard 1980; 1999a; 1999b; Sheppard et al 2008; 2012) with colonies forming canopies indicating an increase in structural complexity (O'Farrell 2007).

Bleaching events of sufficient magnitude to cause coral mortality are expected to increase globally (Hoegh-Guldberg et al. 2007; Veron et al, 2009). However, models based on recruit availability scaled to the present coral cover, suggest that Chagos reefs will long be able to withstand recurring strong mortality events, even each decade, and still maintain high coral cover, such is Chagos' high resilience (Riegl et al. 2012). Significant declines in cover are expected only if both larval supply decreases and coral mortality events increase in intensity and frequency (Riegl et al. 2012).

Taken as a whole Chagos healthy reefs fit with a global pattern of healthier coral reefs with increased distance from human populations (Riegl et al. 2012; Sheppard et al. 2012) as has been shown in other locations, such as NW Hawaiian Islands (Kittinger et al. 2011), Kiribati (Sandin et al. 2008; Dinsdale et al. 2008) and New Caledonia (Fichez et al. 2011). The archipelago has been uninhabited for the last 40 years with the exception of a military base on Diego Garcia, the southern-most atoll (Fig.1.2). This facility does not depend on local food resources but is provisioned and supported entirely from outside the archipelago, and has for the last 15 years at least had strong environmental management (Sheppard et al. 2012). The lack of fishing pressure means the fish populations have remained remarkably high and stable (Graham and McClanahan 2013; Graham et al. 2013). Fish biomass levels are orders of magnitude higher than anywhere else in the Indian Ocean (Graham et al. 2013), matched only

in remote, un-fished atolls in the Pacific (Sandin 2008; Williams 2011). Conversely, as a result of reef degradation, declines in fish species richness, and in the abundance of corallivores, planktivores and small-bodied fish (<20 cm maximum attainable size) have been seen across the Indian Ocean (Graham, et al. 2008; MacNeil and Graham 2010; Graham et al. 2013). However, poaching by illegal vessels is an issue in Chagos and reef shark populations have declined by over 90% in the 30-year period from 1975 to 2006 (Anderson et al. 1998; Graham et al. 2010).

Chagos waters are also some of the cleanest in the world (Sheppard et al. 2012). Extensive pollution monitoring takes place in Diego Garcia and of over 100 metals and organic substances analysed according to US operating procedures almost all analyses report levels below detectable or reporting limits (Sheppard et al. 2012). In addition independent studies of chemicals of particular interest have shown negligible evidence of contaminants e.g. Hydrocarbons (Readman et al. 1999), PCBs and organic pesticides (Wolschke et al. 2011), antifouling booster biocides and triazine herbicides (Guitart et al. 2007), and metals except copper (Everaarts et al. 1999).

In addition to few human activities there are other contributing factors that likely aid the resilience of Chagos reefs. Modeling studies indicate that intact deep reef areas could be at least partially responsible for relatively rapid re-colonization of shallow reefs (Riegl and Piller 2003), and good connectivity within the Archipelago itself (Riegl et al. 2012). Also there are regular incursions of deep, cool water that rise to cover reefs, including during the annual periods of greatest warming (Sheppard 2009). And, strong light adapted 'Clade A' forms of symbiotic zooxanthellae have been identified in shallow corals in Chagos, occurring in approximately half of the shallow water *Acropora* colonies that were heavily affected by warming but which are now recovering strongly (Yang et al. 2012).

Measuring reef health and resilience focuses heavily on two components of biodiversity; corals and fish biomass, as these contain highly important functional groups e.g. the reef builders and the grazers (Bellwood et al. 2004; Hughs et al. 2010). Monitoring in Chagos is no exception and consequently the cryptofauna biodiversity on Chagos reefs has never been assessed. However this group of organisms cannot be ignored for it makes up the largest component of biodiversity (Reaka-Kudla 1997), spans all trophic levels, and contains many important functional groups e.g. filter feeders and detritivores. It is even possible that the cryptofauna contains a higher biomass than the surface fauna (coral and fish) (Ginsbury 1983).

1.11. Project Aims

The true scale of species diversity associated with coral reefs and how it is affected by human impacts is not fully understood (Knowlton and Jackson 2008). This is especially the case for the reef cryptofauna (Plaisance et al. 2009; Stella et al. 2011; Enochs 2012), as is evident from the above review. Given the high level of threats to coral reefs and the volume of goods and services humans receive from them (TEEB 2010; Burke et al. 2011; WWF 2015) it is critical that we understand the consequences of biodiversity changes on coral reef ecosystem function and resilience. The relatively 'pristine' nature of the Chagos environment and therefore relatively natural functioning of its coral reefs makes the archipelago an ideal location to establish a baseline cryptofauna biodiversity estimate for the Indian Ocean (Sheppard et al. 2008). Studying coral reefs without most anthropogenic stressors (acknowledging that global climate change pressures are unavoidable) means we can attribute findings to natural environmental rather than anthropogenic-induced effects.

This thesis will assess the diversity of one of the predominant groups of reef cryptofauna, the decapods, on branching dead coral colonies microhabitats in the Chagos Archipelago, and

investigate whether and how their diversity differs across five atolls within the Archipelago (Fig. 1.2). While assessing the decapod biodiversity we discovered decapod obligate live coral-dwellers to be prevalent on dead coral colonies in Chagos, and because this has not been reported anywhere else we will examine this unusual finding in depth in Chaper 1. In Chapter 2 an integrative approach will be taken, using both morphological and molecular techniques, to assess the complete decapod species richness from dead coral microhabitats, and the techniques used will also be evaluated. Biodiversity indices will then be employed in Chapter 3 to measure the diversity of the decapods and compare these estimates to cryptofauna studies from other locations, and discuss the possible factors that affect decapod diversity. Finally in Chapter 4, the processes driving the community structure of one family of decapods, the Palaemonidae, will be investigated using trait and phylogenetic diversity. This work will form the first step towards understanding the role of cryptofauna biodiversity in reef ecosystem function and resilience on Chagos reef systems, and more widely.

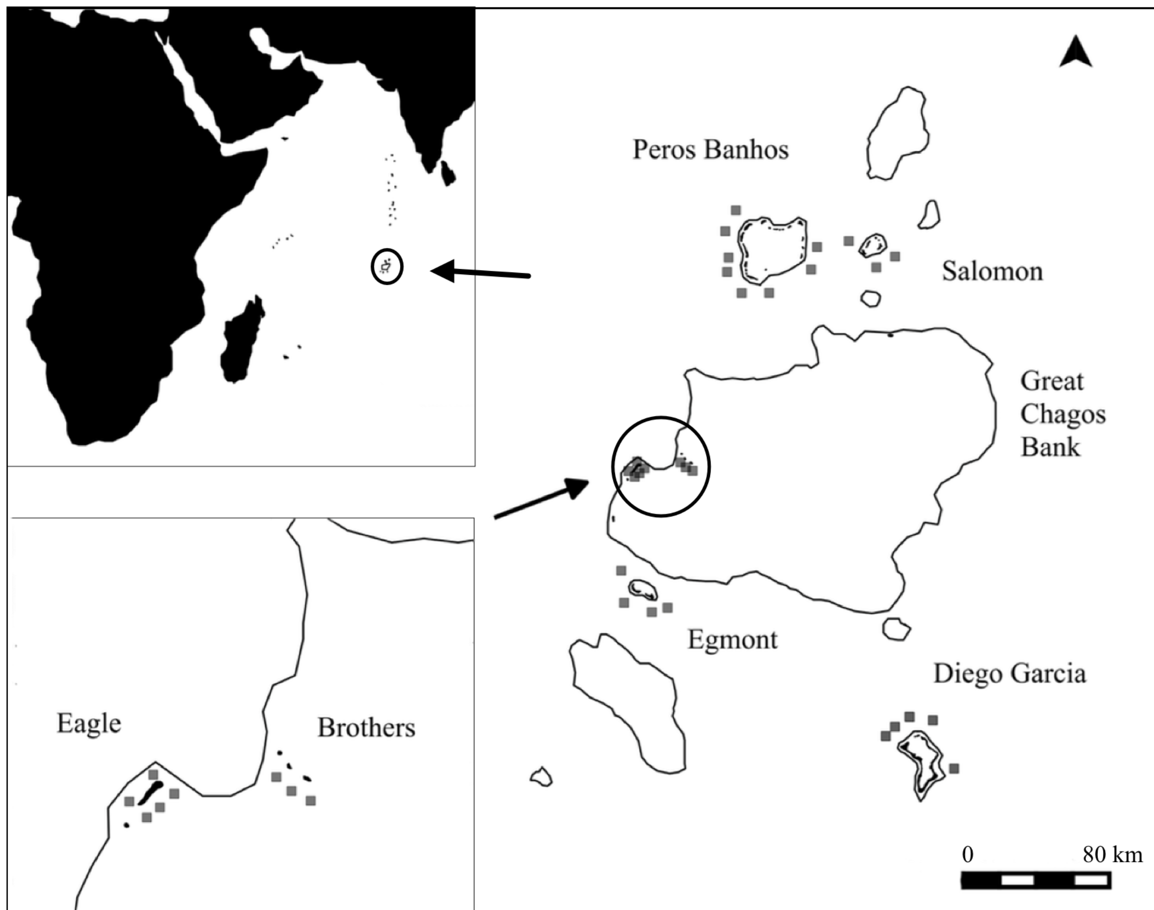

Figure 1.2. The Chagos Archipelago; grey squares represent the 28 site where dead coral colonies were collected on the 2012 and 2013 expeditions. A close up of Eagle and Brothers Islands (part of the Great Chagos Bank) in the bottom left corner shows the distribution of the 8 sites around these two islands.

2. CHAPTER TWO:

High prevalence of obligate coral-dwelling decapods on dead corals in the Chagos Archipelago, central Indian Ocean

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2.1. Abstract

Small and cryptic organisms that live within the interstices of reef habitats contribute greatly to coral reef biodiversity, but are poorly studied. Many species of cryptofauna have seemingly obligate associations with live coral and are therefore considered to be very vulnerable to coral mortality. Here we report the unanticipated prevalence of obligate coral-dwelling decapod crustaceans on dead colonies of branching corals in the Chagos Archipelago (British Indian Ocean Territory) in the central Indian Ocean. A total of 205 obligate coral-dwelling decapods, including *Trapezia* crabs, were recorded from 43 (out of 54) dead coral colonies of *Acropora* and *Pocillopora* collected across five different atolls. *Trapezia* individuals found on dead corals were mainly juveniles and the few adults were almost exclusively male. Among the shrimps (Palaemonidae), however, it was predominantly adult females found on dead corals. Obligate coral-dwelling species that typically occur only on live *Pocillopora* hosts (e.g. *Trapezia* spp.) were recorded on dead *Acropora*. These findings suggests that these obligate coral-dwelling decapods are not simply persisting on coral hosts that have died, but may be explicitly recruiting to or moving to dead coral hosts at certain stages in their life cycle. Variation in the abundance of live coral among sites had no affect on the presence or abundance of obligate coral-dwelling decapods on dead corals. This study shows that habitat-associations of obligate coral-dwelling organisms, and their reliance on different habitat-types, are complex and further work is required to establish their vulnerability to widespread habitat degradation on coral reefs.

2.2. Introduction

Coral reefs exhibit extremely high levels of biodiversity with estimated species richness ranging from 172,000 to over nine million species worldwide (Reaka-Kudla 1997; Small et al.

1998; Ruppert et al. 2004). The contributions of conspicuous and well-studied taxa, such as scleractinian corals (Veron 2000) and reef fishes (Lieske and Myers 1994), to coral reef biodiversity are minimal. Rather, the vast majority of species living on coral reefs are small, cryptic invertebrate species that live within the interstices of coral reef habitats (Reaka-Kudla 1997). Many of these invertebrates have evolved to form close mutualistic associations with corals, particularly branching Scleractinia, which provide food (mostly, in the form of coral mucus and associated detritus), as well as a physical refuge from predators (Castro 1988; Stella et al. 2011a). In return, corals often benefit from the presence of coral-dwelling invertebrates. For instance, obligate coral-dwelling crabs of the family Trapeziidae actively defend their coral hosts against corallivores, e.g., *Acanthaster planci* (Glynn 1982; Pratchett et al. 2000; Pratchett 2001; McKeon and Moore 2014), as well as removing excess sediment in turbid conditions which may otherwise smother the coral (Stewart et al. 2006, 2013).

Coral-dwelling invertebrates are generally divided into those that have obligate versus facultative associations with live coral hosts (Castro 1976; Stella et al. 2011a). Obligate coral-dwelling invertebrates are defined as those that are almost universally found on live coral colonies (Stella et al. 2011a), inferring that they have strong reliance on live corals for food, habitat, and/or recruitment. Most obligate coral-dwelling invertebrates also exhibit very specific host coral preferences, and are generally associated with either *Acropora* or *Pocillopora* corals (e.g. Bruce 1998; Pratchett 2001; Stella et al. 2011a). Meanwhile, facultative coral-dwelling invertebrates opportunistically inhabit live coral colonies as one of the predominant structural microhabitats available within reef systems, but are not considered to be fundamentally dependent upon abundant live coral for their local persistence (Castro 1976; Stella et al. 2011a). Stella et al (2011a) reported that 56% of coral-dwelling invertebrates appeared to have an obligate reliance on live corals, largely because these species had not been recorded outside of live coral habitats. This suggests that these species, at least 487 species (collated by Stella et al. 2011a), would be extremely vulnerable to increasing coral loss (e.g. Burke et al. 2011), though few studies have explicitly tested their

responses to localised coral loss, or explicitly tested occupation rates on recently dead (but intact) coral heads (but see Coles 1980; Tsuchiya 1999; Leray et al. 2012). Perhaps the best indication we have of the impact of reef condition on the live coral cryptofaunal communities is a study by Idjadi and Edmunds (2006) who showed positive significant relationships for reef topography with the diversity of live coral associates but this was not the case for coral diversity and live coral cover.

Dead coral microhabitats have a different community structure of purely facultative species that tend to be smaller in body size (Coles 1980; Leray et al. 2012), though the occasional observations of obligate species on dead corals have been reported (Preston and Doherty 1990; Stella et al. 2011b). Motile cryptofaunal species diversity estimates have been shown to be much higher on dead coral than on live coral microhabitats across a range of geographical regions (Patton 1994; Plaisance et al. 2009; Enochs and Manzello 2012). Dead corals are also thought to be the most important microhabitats for crustacean abundance, biomass, and productivity (Kramer et al. 2014). Pronounced differences in crustacean community structure patterns have been shown on similar microhabitats between different parts of the reef, e.g., lagoonal, reef slope, and reef flat (Peyrot-Clausade 1989; Plaisance et al. 2011), as well as across the reef shelf (Klumpp et al. 1988; Preston and Doherty 1990). Coral colony size, complexity, and surface area of dead and live coral microhabitats have consistently been positively correlated with species richness and abundance of their decapod communities (Abele and Patton 1976; Coles 1980; Leray et al. 2012).

In this study, we report on the unanticipated abundance of obligate coral-dwelling decapod species on dead colonies of *Acropora* and *Pocillopora* in the Chagos Archipelago. Dead coral colonies (e.g. Fig. 2.1) were collected from five different atolls in the Chagos Archipelago in order to explore patterns of biodiversity within this habitat-type (e.g., Plaisance et al. 2009). We did not however, expect to find large numbers of decapod crustaceans that have been widely reported to occur only on live coral hosts (Castro 1976; Stella et al. 2011a). We refer

to the live coral obligates reported here as ‘obligate coral-dwelling’ decapods as we found these individuals on dead coral and hence it is unclear whether these organisms are completely dependent on live coral throughout their life cycle. This study investigates the abundance, habitat-associations, size, life-history stage, and gender of *Trapezia* crabs and shrimps from the family Palaemonidae, the only live obligate taxa found on the dead corals. One possibility is that these obligate coral-dwelling species are simply persisting for a period of time on established coral hosts following host coral mortality, in which case, we would expect to find relatively large, mature individuals continuing to live on preferred coral hosts. Alternatively, dead corals may represent marginal habitats that are only used in the absence of suitable live coral hosts, in which case the occupation of dead corals would be highest in areas with low abundance of suitable live coral hosts. Testing these hypotheses is potentially very important to understand the biological reasons for this spill-over to dead coral microhabitats and whether these microhabitats may have a poorly known functional role in the life cycles of these species.

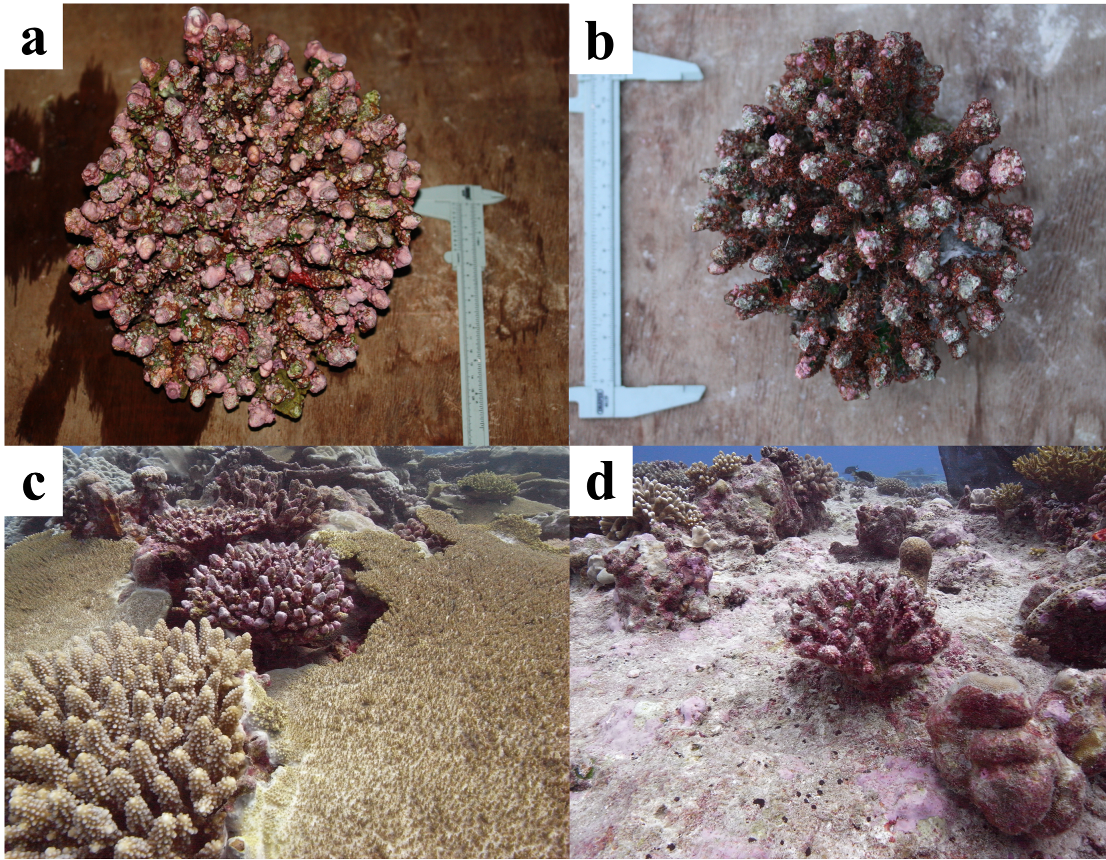


Figure 2.1 Photographs of two dead coral colonies collected: **(a)** and **(c)** show a dead coral colony *ex situ* and *in situ* at Peros Banhos surrounded by live coral respectively, **(b)** and **(d)** show a dead coral colony *ex situ* and *in situ* at Brothers Island on bare bedrock with live coral in the background.

2.3. Methods

2.3.1. Study Site

Data were collected from the Chagos Archipelago during the 2012 Chagos Scientific Expedition (Fig. 1.2). The Chagos Archipelago (British Indian Ocean Territory) is isolated from major anthropogenic disturbances (e.g. sedimentation and pollution) affecting most reefs throughout the world (Burke et al. 2011; Sheppard et al. 2012) and are a biographically important 'stepping stone' connecting reefs in the Western Indian Ocean to the highly diverse coral triangle (Sheppard et al. 2012; Fig. 1.1).

2.3.2. Sampling design

Dead branching coral colonies of approximately 20 cm in diameter were collected from 25 sites across the Chagos Archipelago at Diego Garcia Atoll, Peros Banhos Atoll, Salomon Atoll, Eagle and Brothers Islands of the Great Chagos Bank, and Egmont Atoll (Fig. 1.2). Sites were separated by at least 250 m and located on the outer reef. At each site, 2-4 individual dead *Acropora* or *Pocillopora* coral colonies were collected from between 8-12 m depths. Coral colonies were defined as being dead if they had no observable polyps, evidence of turf and crustose coralline algae, and sometimes erosion (e.g. Fig. 2.1). Benthic transects were undertaken in a cross formation, 10 m x 10 m, with the dead coral colony located at the centre, to enable percentage coral cover estimates within a 5 m radius of the dead coral colony. Photographs (using a Olympus Xz-1 digital camera) were taken every 0.5 m along the transect using a fixed camera frame to keep the camera a standard 1 m above the reef.

2.3.3. Sample collection

Prior to collection, coral colonies were enclosed within a water-tight (250 Micron gauge) polythene bag to contain all motile fauna. Colonies were then chiselled and/ or prised from the reef substrate ensuring minimal disruption to their physical integrity; colonies that did break apart or crumble during the collection process were abandoned. Care was taken to ensure that there were no living corals or other large sessile fauna (e.g., sponges) attached to the coral head. Once detached from the reef, colonies were completely enclosed in the bag for transport. Colonies were enclosed in individual bags for up to two hours prior to processing.

All cryptofauna were removed and sorted first by immersing the coral colony in a bucket of freshwater for approximately one minute, following Stella et al. (2010), and then passing the water through a 1-mm sieve. The seawater in which the coral colonies were stored and transported was also sieved. All specimens were recorded and preserved in 90% ethanol for

transport. Selected fauna for which colour patterns are known to be useful in identification, such as the Alpheidae, were photographed before preservation in ethanol. The coral colony was also placed in a full bucket of seawater and the displaced seawater measured to calculate volume as a proxy for coral colony size. Finally the coral colony was inspected and carefully broken up, using a hammer and chisel to collect any remaining hidden fauna.

Of the cryptofauna collected we chose to focus on the crustaceans, as these were the most abundant taxa, alongside the molluscs, inhabiting the dead coral microhabitats. These taxa were further sorted into morphotypes and then identified to the lowest taxonomic level possible using standard morphological characteristics. Within the crustacean taxa all live coral obligates observed were within the Trapeziidae family and Palaemonidae family (Bruce 1969, 1998, 2004; Castro et al. 2004). Special care was then taken to identify these live coral obligates to the lowest taxonomic level possible (Castro et al. 2004; S. de Grave pers comm). In most cases it was possible to identify these obligates to species but for a few individuals only genus level identification was possible (see results). For each of these individuals, body size, life-stage, and gender were also recorded. Carapace width for *Trapezia* crabs and carapace length for the Palaemonidae were used as standardised proxies for body size (Anger and Moreira 1998). Palaemonidae carapace length was measured under a graduated microscope lens as the linear length of the carapace from the posterior of the orbital cavity to the most posterior tip of the carapace (Anger and Moreira 1998), and for *Trapezia* crabs the linear maximum carapace width was measured. The life-history stage of an individual was classified as adult or juvenile by determining whether or not the individual had fully developed sex organs. If the individual had fully developed sex organs, its gender was recorded.

2.3.4. Data Analysis

Rank abundance models were fitted to explore patterns of abundance for obligate coral-dwelling species within the Archipelago. Comparisons of rank abundance models were made using chi-squared goodness of fit tests (X^2). Rarefied species richness was calculated to compare species richness with uneven sample sizes between atolls. Rarefaction corrects for bias in species number resulting from unequal sample sizes by standardisation to the number of species expected in a sample if it had the same total size as the smallest sample (Magurran and McGill 2011). Shannon-Weaver Diversity Index, Simpson Diversity Index, and Pielou's Evenness Coefficient were all calculated for the Archipelago and individually for all six atolls and islands. Species diversity indices were analysed in Species Diversity & Richness IV (Seaby and Henderson 2006). Benthic photo-transects were analysed using Coral Point Count (CPC) software (Kohler and Gill 2006). Ten points were randomly assigned to each photo and the benthic cover beneath that point was recorded. From this dataset, live branching *Acropora* and *Pocillopora* species counts were combined and percentage cover calculated for use in this analysis. All other analyses were undertaken in R (R Development Core Team 2008). A binomial test was used to test the hypothesis that the probability of coral obligates present on dead coral colonies was significantly greater than the absence. Sign tests were used to investigate the probability of each decapod obligate species being present on dead *Acropora* versus dead *Pocillopora* colonies. Chi-squared goodness of fit tests (X^2) were used to determine whether a certain life-history stage and/or gender predominated in the obligate coral-dwelling assemblages.

Generalised linear models (GLMs) were used to describe presence and absence, abundance, body size, life-history stage, and gender of the obligate coral-dwellers, and to relate these indices and traits to at least two of the following environmental predictor variables: percentage local live branching coral cover (data were first arcsine transformed), genera of the dead coral colony, biogeography (atoll location), and niche structure of the coral colony habitat using volume of the coral colony as a proxy (coral colony size). It should be noted that coral colonies of approximately 20 cm diameter were collected, but inevitably there was still

some variation in coral colony size and therefore this was included as an environmental variable in the statistical analysis. Because of the binomial nature of the dependent variables of presence/absence, life-history stage, and gender of obligate coral-dwellers, the logit-link function and binomial family function were used in these models. For the dependent variable of obligate coral-dweller abundance, the log-link function and Poisson family function were used. Finally, for the mean body size model, the Gaussian family function and identity-link function were used. A series of GLMs were fitted for each dependent variable to the appropriate predictor variables and their interactions and then simplified. Model significance was assessed using F-values or X^2 values depending on the nature of the dependent variable. All models that demonstrated over or under-dispersion (residual deviance higher/lower than degrees of freedom) were fitted with the quasi-family function to introduce a dispersion parameter and obtain a quasi-likelihood estimate (Crawley 2005).

2.4. Results

2.4.1. Prevalence & diversity of obligate coral-dwellers

Obligate coral-dwelling decapods were present on 43 of the 54 dead coral colonies collected from across the Archipelago. There was a significant probability (0.80) of an obligate coral-dweller occurring on a dead coral colony (*95% CI* =0.66-0.89, p <0.01). The Palaemonidae shrimp and *Trapezia* crabs were found on 59% (32 of the 54) and 62% (34 of the 54) dead coral colonies, respectively.

Two-hundred and five obligate coral-dwelling decapod individuals, from seven identified species were recorded across 43 of the 54 dead coral colonies sampled during this study, including two species of *Trapezia* and five species of shrimps from the family Palaemonidae (Fig. 2.2). In addition, there was a high abundance of *Trapezia* juveniles (*Trapezia spp*.) that

could not be identified to species and some Palaemonidae shrimp from the *Harpiliopsis* and *Harpilius* genera (*Harpiliopsis* spp. and *Harpilius* spp.) that could not be identified to species, as the individuals were juveniles or because of damage to the samples. Therefore, for all following analyses, the obligate coral-dwellers observed were categorised into ten taxa (seven identified to species level, plus three taxa identified to genus: *Trapezia spp., Harpiliopsis spp.* and *Harpilius spp*.). *Trapezia* spp. (juveniles) and a shrimp species, *Jocaste lucina*, were the dominant taxa accounting for 77 and 60 individuals, respectively (Fig. 2.2). Many of the taxa occurred rarely in the samples, with *Coralliocoris graminea* and *Trapezia bidentata* each found only once (Fig. 2.2). These eight obligate coral-dwelling decapod taxa (seven species and *Harpilius spp*., as no *Harpilius species* were otherwise identified) comprised 6.5% of the decapod species identified on the 54 dead coral colonies, and they comprised approximately 11% of all decapod individuals present.

All models fitted to the rank abundance data (Fig. 2.2) were statistically different from the data (broken stick model χ^2=31.27 with 9*df*, p <0.001; geometric model χ^2=173.95 with 9*df*, p <0.001; exponential model χ^2=131.79 with 9*df*, p <0.001), which demonstrates the high heterogeneity within the coral-dwelling obligate assemblage on dead corals.

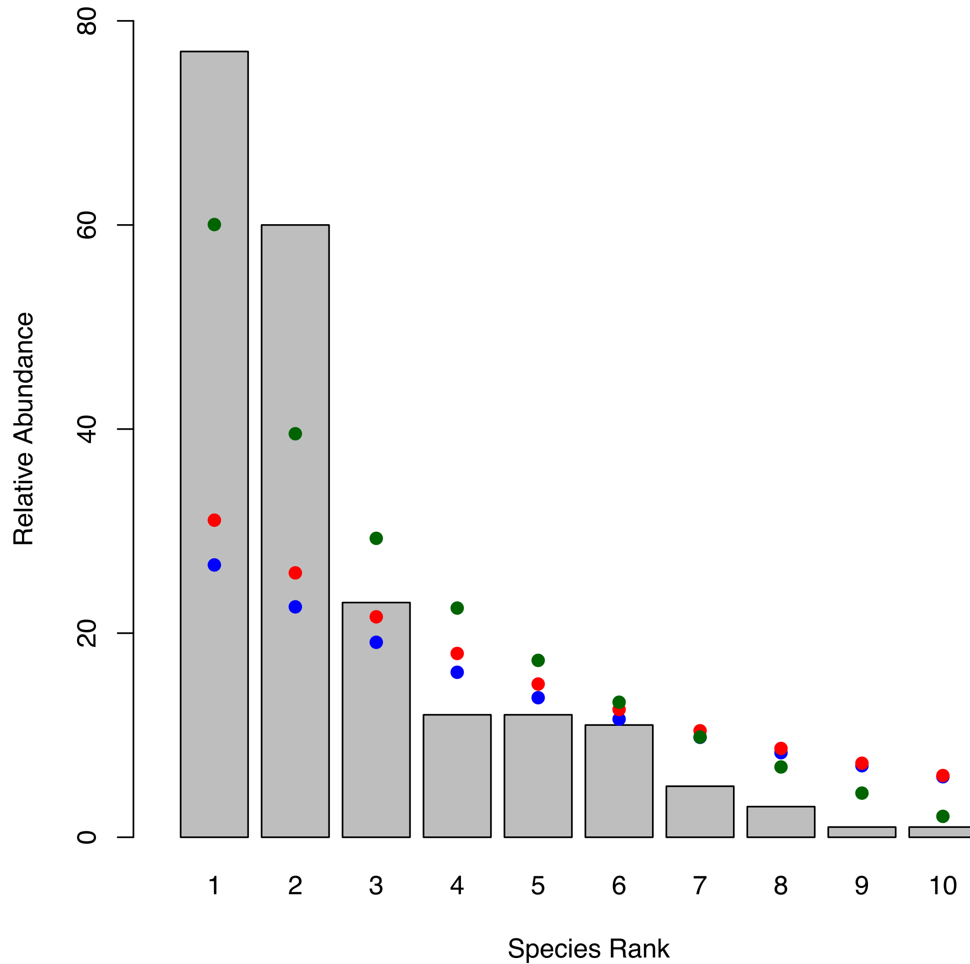


Figure 2.2 Fits of broken stick (green points), geometric (blue points), and exponential (red points) to the relative abundance of ten species groups across the Chagos Archipelago. Species ranks are: (1) *Trapezia spp*. (juveniles) (2) *Jocaste lucina* (3) *Harpiliopsis spinigera* (4 & 5) *Harpiliopsis* spp. and *Jocaste japonica* (6) *Trapezia tigrina* (7) *Harpilius spp.* (8) *Harpiliopsis depressa* (9 & 10) *Coralliocaris graminea* and *Trapezia bidentata.*

2.4.2. Habitat associations & specialisation

The prevalence and abundance of obligate coral-dwellers on dead *Acropora* versus dead *Pocillopora* colonies differed for each taxa: There was a significantly higher probability (0.86) of finding *Trapezia* spp. (juveniles), which are considered live *Pocillopora* obligates

(Castro 1976; Castro et al. 2004), on dead *Acropora* rather than dead *Pocillopora* colonies (*95% CI* =0.76-0.93, *p* <0.01). Other live *Pocillopora* specialists found on dead *Acropora* were *Trapezia tigrina*, *Harpilius* spp., *Harpiliopsis spinigera*, and *Harpiliopsis* spp., whilst *Harpiliopsis depressa* and *T. bidentata* were found exclusively on dead *Pocillopora* colonies. Of the three live *Acropora* obligates, two species, *Jocaste japonica* and *C. graminea*, were found exclusively on dead *Acropora* colonies, whilst there was a significantly higher probability (0.80) of finding *J. lucina* on dead *Acropora* than dead *Pocillopora (95% CI* =0.68-0.89, *p* <0.01).

2.4.3. Local live branching coral cover

The obligate coral-dweller prevalence model showed no significant effect of live branching coral cover (from within a 5-m radius of the dead coral colony) on obligate presence across any of the atolls. The total obligate coral-dweller assemblage showed over-dispersion (*dispersion parameters* =1.14) suggesting that other ecological processes are driving the obligate coral-dwellers presence on dead coral colonies. Similarly, local live branching coral abundance had no effect and showed over-dispersion (*dispersion parameters* =4.59).

2.4.4. Dead coral colony size

There was a significant positive effect of coral colony size on total obligate coral-dweller abundance (*f* = 4.85 with 50*df*, *p* = 0.03). However, coral colony size only explained 9.3% of the variation and large over-dispersion in the model (*dispersion parameter* =4.16) also suggested variation in obligate coral-dweller abundance that was not explained by coral colony size or biogeography. Brothers had the highest abundance, at six obligates coral-dwellers per coral colony, and Eagle had the lowest, at 2.2 per coral colony (Table 2.1). However, the total obligate coral-dweller abundance model showed no significant difference in abundance between any of the atolls or islands. Brothers and Eagle are geographically the

closest but still at least 20 km apart (Fig. 1.2). Both islands are part of a large atoll called the Great Chagos Bank (Fig. 1.3), so Eagle and Brothers were first considered separately and then combined in a second model to reflect the Great Chagos Bank. This showed no change in the non-significance of atoll effect on obligate abundance.

Palaemonidae shrimp obligate abundance also had a significant positive relationship with coral colony size (f =4.09 with 47*df*, p =0.048), which explained 9.68% of the variation in abundance. *Trapezia* obligate abundance increased with coral colony size but this relationship was not significant, suggesting that the Palaemonidae obligate assemblage drives the overall affect of coral colony size on total obligate abundance. Large over-dispersion in the *Trapezia* (*dispersion parameter* = 2.44) and Palaemonidae (*dispersion parameter* = 3.50) obligate abundance models suggest there was variation in both assemblages not explained by either environmental parameters tested.

Table 2.1 Biodiversity indices for the Chagos Archipelago overall and for the 6 surveyed atolls and islands. Indices include: total abundance of obligates *(Total N),* mean abundance of obligates with standard error *(Mean N ± se),* rarefied species richness with standard error *(Rarefied d ± se),* Shannon-Weaver diversity index *(H'),* Simpson's diversity index *(l - λ)* and Pielou's species evenness coefficient *(J'*). All indices are measures per dead coral colony, except *Total N* that measures the species abundance per atoll.

Location	*Total N*	*Mean N ± se*	*Rarefied d ± se*	*H'*	*l - λ*	*J'*
Brothers	36	6.00 ± 9.17	5.56 ± 0.88	0.62	0.49	0.45
Diego Garcia	51	4.64 ± 4.64	4.85 ± 1.04	0.54	0.41	0.49
Eagle	13	2.17 ± 2.32	4.83 ± 0.39	0.42	0.59	0.45
Egmont	15	3.75 ± 4.11	5.72 ± 0.49	0.71	0.62	0.48
Peros Banhos	57	3.56 ± 2.99	3.85 ± 0.90	0.46	0.54	0.51
Salomon	33	3.00 ± 3.00	4.55 ± 0.89	0.43	0.36	0.43
Chagos Archipelago	205	3.80 ± 4.80	4.97 ± 1.15	0.50	0.50	0.72

2.4.5. Body size

The majority of obligate coral-dwellers had a carapace length of between 1 mm and 4 mm (Fig. 2.3.a and b). Carapace length was used as a standardised measure of body size (see methods). The mean obligate coral-dwellers body size was highest at Brothers and smallest at Eagle (Fig. 2.3.a). However there was no significant relationship between obligates' mean body size and reef location, or coral colony size. The data were over-dispersed (*dispersion parameter* = 2.66) indicating variation in body size is controlled by other environmental factors (e.g., food source availability).

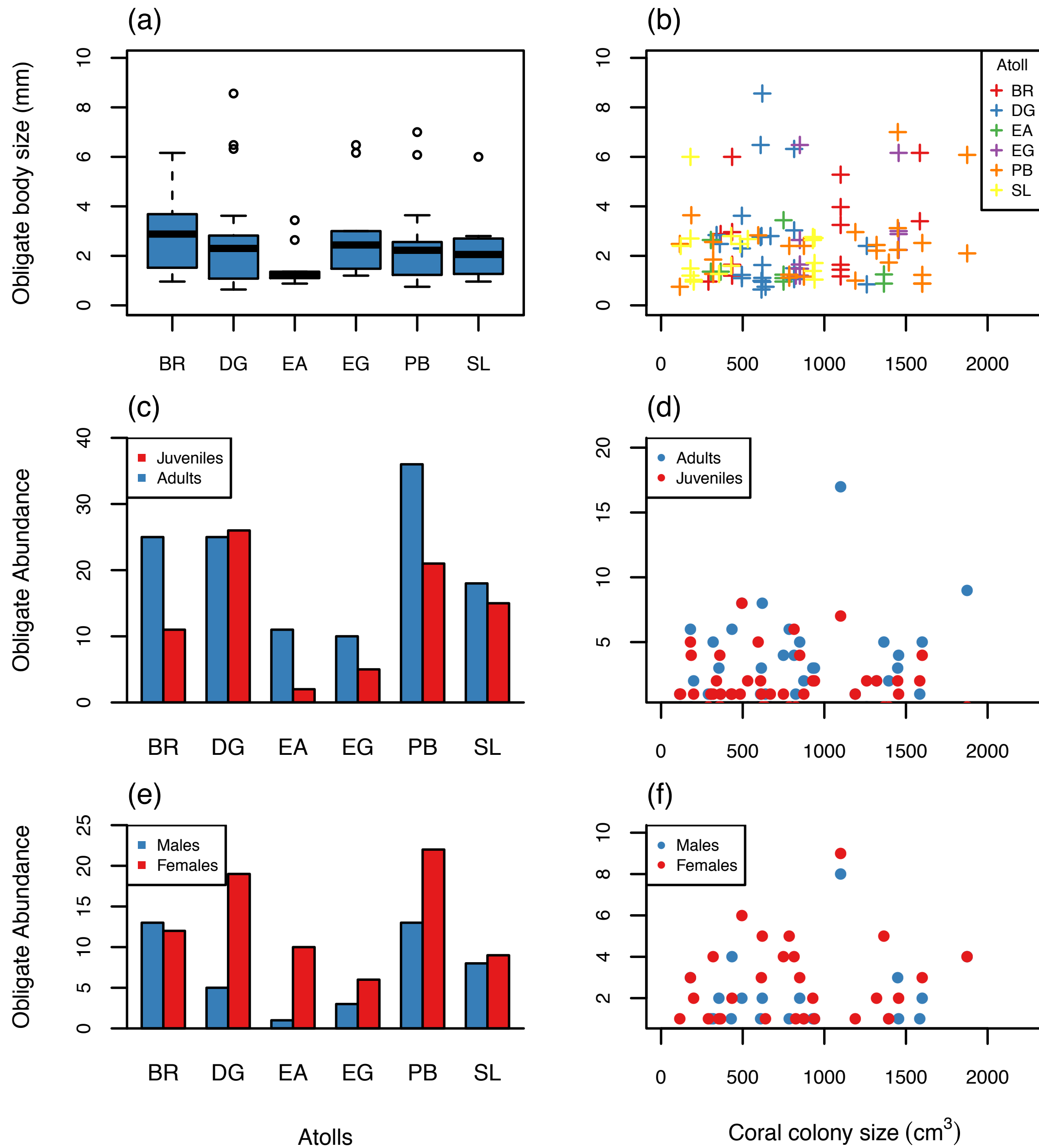


Figure 2.3 Mean body sizes (mm) of the total coral obligates assemblage, **(a)** at each atoll or island regardless of coral colony size, **(b)** on coral colonies of varying sizes (cm^3) separated by atoll or island location. Abundance of juveniles versus adults in the total coral obligate assemblage, **(c)** at each atoll or island location, **(d)** with coral colony size (cm^3). Abundance of males verses females in the total coral obligate assemblage, **(e)** at each atoll or island location, **(f)** and with coral colony size (cm^3). Atoll legend: BR= Brothers, DG= Diego Garcia, EA= Eagle, EG= Egmont, PB= Peros Banhos, SL= Salomon.

2.4.6.Life-history stage & gender

Chi-squared goodness of fit demonstrated there were significantly more adults than juveniles in the obligate coral-dwellers assemblage (Table 2.2). This trend was clearly driven by the larger Palaemonidae shrimp obligate assemblage, which had significantly more adults than juveniles (Table 2.2). In contrast, the *Trapezia* obligate assemblage had significantly more juveniles than adults (Table 2.2). The largest difference in the proportion of total obligate adults and juveniles occurred at Eagle with the lowest difference at Diego Garcia (Fig. 2.3.c). Coral colony size had no significant effect on the proportions of the two life-stages (Fig. 2.3.d).

Table 2.2 Chi-squared goodness of fit values (X^2) and abundance of **(a)** adults and juveniles in; the total coral obligate assemblage, the *Trapezia* obligate assemblage and the Palaemonidae shrimp obligate assemblage, **(b)** the abundance of males and females (adults only) in; the total coral obligate assemblage, the *Trapezia* obligate assemblage and the Palaemonidae shrimp obligate assemblage (gender could not be identified for 4 shrimp species).

	Abundance		X^2	*P value*
(a) Life-Stage	*Adults*	*Juveniles*		
Coral obligates	125	80	9.88	0.001
Trapezia obligates	12	77	47.47	0.001
Shrimp obligates	113	3	9.88	$5.58e^{-12}$
(b) Gender	*Males*	*Females*		
Coral obligates	43	78	10.12	0.001
Trapezia obligates	10	2	5.33	0.02
Shrimp obligates	33	76	16.96	$3.81e^{-05}$

It is only possible to accurately sex adult decapods, therefore, only the adults were included in the gender analysis. Chi-squared goodness of fit test showed there were significantly more female than male decapods; this was driven by the larger assemblage of Palaemonidae shrimp obligates which also had a significantly higher female to male ratio (Table 2.2). In contrast, the *Trapezia* assemblage had a significantly higher male to female ratio (Table 2.2), but were dominated by juveniles so the adult assemblage for which gender could be determined was very small and comprised of only two species, *T. tigrina* and *T. bidentata* (Fig. 2.2).

Across Chagos, neither coral colony size nor biogeography was a driver of the proportion of females to males in the total obligate assemblage (Fig. 2.3.e and f). However, at the local assemblage level, Eagle did have a significantly higher proportion of females to males compared to other locations (t =2.05 with 114*df*, p =0.04). Diego Garcia and Peros Banhos also had a much higher proportion of females to males, but this was not significant (Fig. 2.3.e).

2.5. Discussion

This study revealed an unanticipated prevalence of obligate coral-dwelling decapods on dead coral colonies within the Chagos Archipelago. All ten decapod taxa considered in this study are widely regarded as ‘live coral-dwelling obligate species’ with strong host specificity for either *Pocillopora* or *Acropora* corals; *T. bidentata* (Castro et al. 2004), *T. trigrina* (Castro et al. 2004), *Trapezia spp.* (juveniles) (Castro et al. 2004), *H. depressa* (Bruce 1998), *Harpilius spp*. (Bruce 2004), *C. graminea* (Bruce 1998; Vytopil and Willis 2001), *J. lucina* (Bruce 1969; Patton 1994), *J. japonica* (Bruce 1969; Patton 1994), *Harpiliopsis spp*. (Bruce 1998), and *H. spinigera* (Abele and Patton 1976; Bruce 1998). *C. graminea*, *J. lucina*, and *J. japonica* are all considered to be live *Acropora* obligates (Bruce 1969, 1998; Vytopil and Willis 2001). The other five species are regarded as live *Pocillopora* obligates (Bruce 2004; Castro et al. 2004), though both *H. spinigera* and *H. depressa* have also been observed on live *Stylophora* (Edwards and Emberton 1980; Stella et al. 2011a). To the best of our knowledge there have been only two other recorded observations of obligate coral-dwelling decapods on dead coral hosts, specifically *Trapezia cymodoce* (Stella et al. 2011b) and *H. depressa* (Preston and Doherty 1990), both on *Pocillopora* on the Great Barrier Reef (GBR). The observations of *H. depressa* were relatively rare comprising less than 1% of the agile shrimp population (Preston and Doherty 1990). In the case of *T. cymodoce*, the observation was made in the weeks immediately following a major coral mortality event, and of 20 bleached colonies, 14 retained both individuals of their *T. cymodoce* pairs (Stella et al. 2011b). Chagos has been subject to major bleaching events leading to substantial coral mortality in the past, most notably in 1998 (Sheppard 1999; Sheppard et al. 2002), but no mass coral mortality events have been recorded since (Sheppard et al. 2012).

This study observed a mean species richness of 4.97 obligate coral-dweller species (se ± 1.15) per dead coral colony (with a mean volume of 764 cm^3, and a mean abundance of 3.8 individuals but with large variation between coral colonies (se ± 4.80; Table. 2.1). Patton (1994) reported a mean of 4.5 obligate coral-dweller species and abundances of 9.1 on live *Acropora* colonies on the GBR, with a larger mean volume of 4900 cm^3 in comparison to this study. Whilst Abele and Patton (1976) reported nine species (cumulative species number) on live *Pocilliopora* colonies of approximately 1500 cm^3 size in the Gulf of Panama. However, it is not possible to make direct comparisons of obligate coral-dweller diversity found here with the above records from live coral colonies due to differences in geographical location, coral colony sizes, host colony genera and species, and coral complexity, which are all known to affect the associated species inhabiting live coral and their diversity (e.g. Abele and Patton 1976; Vytopil and Willis 2001).

The high prevalence of obligate coral-dwelling decapods on dead, but intact corals in Chagos is hard to explain, especially given limited knowledge about the life history and ecology of these organisms. It seems plausible that at least some of these individuals and species have persisted since these coral colonies actually died. However, based on extensive growth of both turf and crustose coralline algae (CCA) on all colonies (Fig. 2.1), it appears likely that the corals surveyed in this study had been dead for at least several months (if not years) and there have been no reports of coral infauna persisting this long following host coral mortality. Stella et al (2011b) found that some *Trapezia* remained with original host colonies for at least five weeks following bleaching, although their fitness was adversely affected as fecundity levels were reduced. Our results also show that the host specificity of these coral-dwelling obligates for *Acropora* or *Pocillopora* does not always hold on the dead coral colonies and depends on the species. Most notably, *Trapezia* spp., which are normally found exclusively on *Pocillopora* colonies (Castro 1976; Castro et al. 2004) were significantly more prevalent on dead *Acropora* versus dead *Pocillopora* colonies. This may be because of the small size of the *Trapezia* juveniles making them more vulnerable to predation so the generally more

complex branching structure of dead *Acropora* colonies likely affords them higher protection (Vytopil and Willis 2001). This was also the case for *Harpilius* spp., *Harpiliopsis* spp., and *H. spinigera*. These observations suggest that these organisms are not simply persisting on host corals with which they established strong affinities before they died. Rather, it appears that many of the coral-dwelling species recorded in this study had actively recruited to dead coral hosts. Many of the dead coral colonies in our study were in very close proximity to live branching coral (Fig. 2.1.c), hence it is possible that the adult obligate coral-dwellers were moving between live versus dead coral hosts. *Trapezia* spp., for example, are fiercely territorial (Stewart et al. 2013), but have been known to move among coral colonies at night possibly to secure a larger, healthier colony or secure a mate (Castro 1978; Stella et al. 2011b). Where live coral colonies are fully saturated with these cryptofaunal species, there may be temporary or recurrent spill over to nearby dead corals. However, further research will need to be conducted to test for movement between live and dead coral microhabitats, as well as testing for differences in individual fitness of organisms occupying these different habitats.

The occurrence of significant sex bias in the Palaemonidae shrimps and in the *Trapezia* suggests that subordinate individuals may be forced to occupy sub-optimal habitats over the course of their life cycle. If so, this would emphasise the importance of the mosaic of habitats on healthy reef ecosystems. Juveniles were significantly more abundant than adults in the *Trapezia* assemblage, suggesting that dead coral colonies maybe acting as an essential or preferred habitat for *Trapezia* juveniles, potentially as a refuge from the highly territorial adults. In live coral colonies *Trapezia* juveniles have predominantly been found inhabiting the dead base of the colony (Abele and Patton 1976; Castro 1976) possibly for better protection from predators, or as the result of competition by the larger adult pairs that prefer the live tissue.

Stomach content analysis of *Trapezia* collected from live coral hosts has revealed that they feed predominantly on coral mucus and some coral tissue, as well as various detrital particles (Knudsen 1967; Preston 1973; Patton 1974; Castro 1976; Rotjan and Lewis 2008) presumed to be ingested within coral mucous (Gerlach 1960; Castro 1976). Strong dependence on corals for food has been the central basis of the tenet that *Trapezia* will be extremely vulnerable to coral loss (e.g. Stella et al. 2011a). However, it is known that *Trapezia*, as well as *Coralliocaris* and *Jocaste* species at least, can utilise additional food sources as they have been observed consuming zooplankton (Patton 1994), and all of these species have appendages well developed for suspension feeding (Bruce 1976). Nonetheless, the lack of preferred food resources on dead coral microhabitats may cause these obligates to become nutritionally starved in the longer-term, thereby affecting their individual fitness (e.g. Stella et al. 2011b).

The abundance of live *Pocillopora* colonies has been reported to be the major limiting factor for populations of *Trapezia* at most reef locations (Castro 1978; Stella et al. 2011b) with high levels of occupation across suitable coral hosts and strong competition among individuals and species for preferred corals. For example, Stewart et al. (2006) observed 95% of live *Pocillopora* colonies to be occupied by *Trapezia.* Competition for space is likely to increase further as coral cover declines (Glynn 1976), potentially forcing obligate coral-dwelling organisms to utilise dead or marginal coral microhabitats, though there may be increasing competition from cryptofaunal species that specifically utilise recently dead coral habitats (Coles 1980; Plaisance et al. 2009; Enochs 2012). However, we do not know how inhabiting sub-optimal dead coral microhabitats affects the fitness of obligate coral-dwelling organisms, and hence how long they can actually persist on dead coral hosts.

Coral assemblage dynamics differ dramatically on a local scale and the small size of these obligates means that the local abundance of live coral hosts could be a driver of their presence on dead coral colonies. The abundance of these obligate coral-dwellers was highest at

Brothers and lowest at Eagle, two of the geographically closest locations, which were the two locations surveyed that form part of the Great Chagos Bank. These two islands also had the highest and lowest mean local live branching coral cover at 14% and 5% respectively. At Eagle, low cover of branching corals was directly attributable to localised outbreaks of crown-of-thorns starfish (Appendix III/ Roche et al. 2015). However, we found no correlation between the presence or abundance of obligate coral-dwellers on dead corals and the local abundance of live branching corals between or within the atolls. Similarly Enochs et al. (2011) found that live coral cover had no significant affect on the motile cryptofauna occupying dead corals but rather dead coral porosity (gaps in rubble structure) and water flow had a greater affect, with low-porosity, slow-flow environments supporting a higher abundance and biomass of motile cryptofauna. Idjadi and Edmunds (2006) also observed no significant correlation with live coral cover or diversity and cryptofauna diversity, but there was a significant positive correlation between reef structural complexity and cryptofauna diversity.

The abundance of Palaemonidae shrimp obligates was found to be driven at least partly by the dead coral colony size, as shown previously for live coral colonies (Abele 1976; Abele and Patton 1976; Coles 1980; Enochs 2012). However, colony size only explained approximately 10% of the variation in abundance and so many other factors, including inter- and intra-species interactions, proximity to live coral colonies, dispersal characteristics, predation pressure, and habitat heterogeneity are likely to be important (Abele and Patton 1976). We attempted to control for coral colony size by collecting dead corals of approximately 20 cm in diameter. As even small variations in coral colony size showed a significant correlation with Palaemonidae abundance it is likely that coral colony size is an important driver of Palaemonidae obligate abundance. The complexity of the coral colonies has also been shown to affect the abundance and species richness of coral associates, as well as the host coral genera preferred by different coral associates, with more complex habitats thought to provide better refuge from predation and probably better niche separation (Castro 1988; Patton 1994;

Vytopil and Willis 2001; Stella et al. 2010). It is also likely that the abundance of individual species is strongly affected by the abundance, diversity, and composition of cryptofaunal assemblages within each coral colony.

For *Trapezia* spp., colony size did not influence abundance, but this is perhaps not unexpected. In live coral hosts, *Trapezia* spp. are highly territorial, and each coral colony supports just one breeding pair, regardless of colony size (Preston 1973; Patton 1974), and often territory size is thought to exceed their nutritional requirements (Huber and Coles 1986). However, of the 13 *Trapezia* adults found on the dead coral colonies only two adults occurred on the same coral colony, and both of these were male. The lack of adult mating pairs on the dead coral colonies suggests that these species could not persist in the absence of live coral habitats. Furthermore, *Trapezia* body size was not significantly affected by coral colony size. This is in contrast to live coral colony size that has been shown to correlate with body size of *Trapezia ferruginea* (Abele and Patton 1976).

This study has revealed high prevalence of at least seven obligate coral-dwelling species on dead coral colonies, predominated by *Trapezia* juveniles and female Palaemonidae shrimp, indicating that dead coral colonies may play an important role in the life cycle of some coral-dependent organisms. Dead coral colonies may also act as sub-optimal habitat for these obligate coral-dwelling taxa, allowing them to survive a period of time when there is insufficient live coral habitat, or when they are searching for new territories. However, dead branching corals are temporary habitats that erode over time (Sheppard et al. 2002; Perry et al. 2012), therefore even availability of this sub-optimal habitat for these taxa will be dependent on long-term coral loss. Results from this study add further complexity to our limited knowledge of the relationships between corals and the high diversity of motile invertebrates that inhabit live and dead colonies. These relationships are, however, fundamental to understanding the consequences of coral loss on species persistence and biodiversity.

3. CHAPTER THREE:

A comparative species delimitation approach to estimating Decapoda species richness from dead coral colonies in the Chagos Archipelago

3.1. Abstract

The Decapods are a speciose group of Crustacea and comprise one of the largest components of coral reef cryptofauna. To assess the species richness of Decapods from dead coral colonies in the Chagos Archipelago we use an integrative approach to molecular species delimitation. DNA barcodes, short DNA sequences, can be used to assign organisms into putative species and a variety of species delimitation methods have been designed to do this. Among the most popular methods are phylogenetic approaches, which use coalescence theory to delimit species, and a 'classic' barcoding gap approach, which uses a threshold distance between intra- and inter-specific genetic variations. Here, we provide an empirical evaluation of decapod species estimates using two coalescence-based methods and a relatively new barcoding gap approach: General Mixed Yule Coalescent (GMYC) model, the newer Poisson tree processes (PTP) method, and the Automatic Barcoding Gap Discovery (ABGD) method. When an integrative approach is taken, using morphological identifications, all methods underestimated decapod species richness. This is thought to be largely a result of the high number of singletons in the dataset, which no method delimited accurately. The GMYC method produced very mixed results, probably partly because of the number of singletons and also in the case of the Palaemonoidea and Brachyura because of the occurrence of numerous short branch lengths. The PTP and ABGD methods provided relatively congruent results, but in our opinion the PTP method preformed slightly better as it produced higher species estimates that aligned more closely with expert morphological identifications. In addition the ABGD requires prior information to make informed choices regarding a molecular threshold. This study provides a rare evaluation of three species delimitation methods and supports the use of an integrative approach to species delimitation where resources allow.

3.2. Introduction

The Decapoda (subphylum: Crustacea) are a very ecologically diverse order inhabiting most marine and freshwater environments, and some groups have also adapted to terrestrial environments. The group are functionally important, spanning all trophic levels including herbivores, omnivores, predators and scavengers, and are also important prey for many fish species. The decapods comprise of approximately 233 families, containing 2,725 genera and an estimated 17,635 extant and fossil species (De Grave et al. 2009). The largest groups include the Brachyura (true crabs), Anomura (including squat lobsters, hermit crabs, and porcelain crabs) and Caridae (shrimp). Anatomically the group is characterised by having ten legs, five pairs of thoracic appendages on five thoracic segments, with the front three pairs functioning as mouthparts (maxillipeds).

Cryptofauna are defined as small, often cryptic, invertebrates and vertebrates that live within coral reef framework (Reaka-Kudla 1997; Plaisance et al. 2011a). Decapods are one of the most species rich groups of organisms and they make-up one of the largest components of reef cryptofauna (Reaka-Kudla 1997; Enochs and Manzello 2012). Here we focused sampling on the cryptofauna inhabiting dead coral colonies, the most productive reef microhabitat (Kramer et al. 2014) and yet understudied (Plaisance et al. 2009; Enochs and Manzello 2012). The cryptofauna inhabiting dead but intact branching coral colonies, rather than live coral, are considered facultative, that is they opportunistically inhabit reef microhabitats (but see Head et al. 2015/Chapter 2).

By their small and cryptic nature the decapods inhabiting coral colonies are hard to identify and study, leading to a bottleneck in identifying the biodiversity of this functionally important component of reef fauna (Plaisance et al. 2011b). However, over the last decade a revolution in molecular sequencing techniques now means DNA sequences can be used as taxon ‘barcodes’ (Hebert et al. 2003). By analysing the variability in at least one standard molecular

marker it is often possible to discriminate biological entities. Accordingly DNA barcodes can be used as an identification tool, overcoming the bottleneck created by a shortage of taxonomic expertise available to identify complex groups, such as the decapods (Casiraghi et al. 2010). When a reference data set is available from previously sequenced and taxonomically identified species, then the barcode sequence can be used to identify the species. However, reference data sets are insufficient for many taxon groups and so it is not yet possible to identify the majority of even common marine species through DNA barcoding. Instead the molecular sequences can be clustered into entities that provide a set of species hypothesises, known as molecular operational taxonomic units (MOTUs), and used as an exploratory tool in biodiversity assessments (Hebert et al. 2003; Puillandre et al. 2012). A great advantage of molecular tools is the ability to identify species or MOTUs from only fragments of specimens, and from all life-history stages. For instance, it is often not possible to identify juveniles or larvae taxonomically to species, as is commonly the case with the decapods. DNA barcoding is also a tool for discovering new species, such as cryptic species (De Salle 2006).

Hebert et al. (2003) first proposed barcoding used the mitochondrial cytochrome *c* oxidase subunit-I (COI) gene as the universal gene for animals. For a gene to be a suitable genetic marker the genetic variation within the gene must be greater between species than the variation between individuals of the same species - this is known as the 'DNA barcoding gap' (Hebert et al. 2003). In addition, its priming sites must be sufficiently conserved to allow for reliable binding for amplification, and the amplification and sequencing process must be robust to variable lab conditions and protocols (Vences et al. 2005; Lefébure et al. 2006). The gene must also convey sufficient phylogenetic information to allow assignment of species to higher order taxa using simple phenetic approaches (Vences et al. 2005; Lefébure et al. 2006). Despite the presence of multiple insertions and deletions in the large ribosomal subunit (16S) rRNA gene (Hebert et al. 2003), there is evidence in some taxa that the 16S rRNA gene also fulfils these requirements, e.g. amphibians (Vences et al. 2005), crustacean (Lefébure et al.

2006; Tang et al. 2010), fish and land snails (Steinke et al. 2005). In Crustacea there is a correlation between molecular divergence (both COI and 16S rRNA) and the morphological taxonomy (Lefébure et al. 2006). However generally 16S evolves more slowly than COI, and so COI is seen as a more effective marker, but 16S is easier to amplify than COI (Lefébure et al. 2006). Where resources allow and particularly where taxonomic information is not available it is advocated that COI and 16S should both be used (Vences et al. 2005).

Analysing the variation in the marker and assigning a DNA sequence to a species or MOTU relies on the existence of a barcoding gap. The classical genetic similarity approach uses a relative threshold of genetic distance to assign DNA sequences to species, such as the 10x rule of inter- versus intra-specific divergence (Hebert et al. 2003). However assigning the same cut-off across clades can be problematic (Will and Rubinoff 2004) and the distributions of intra- versus inter-specific divergence typically overlap, particularly in diverse clades of rapidly speciating organisms (Meier et al. 2006). Newer methods of species delimitation have sought to overcome these issues; Methods include (but are not limited to): coalescent theory based approaches (Pons et al. 2006; Monaghan et al. 2009; Zhang et al. 2013); Bayesian assignment (Munch et al. 2008a); fuzzy sets (Zhang et al. 2012); and automatic barcode gap discovery (ABGD) (Puillandre et al. 2012). All methods have their shortcomings but coalescent theory-based approaches and ABGD are perhaps those that have been shown to perform best and/or have been most widely used (Paz and Crawford 2012; Fujisawa and Barraclough 2013; Zhang et al. 2013).

The ABGD method like the ‘classical’ DNA barcode gap analysis uses a threshold distance to consider whether two sequences are from two different groups, but it statistically infers the barcoding gap from the data and partitions the dataset accordingly (Puillandre et al. 2012). It has three main advantages over the traditional analysis; firstly the threshold is obtained from the data itself rather than *a priori*, secondly through a repetitive process different ‘clades’ within the dataset may be assigned different thresholds, and thirdly, the algorithm assigns

potential clustering patterns based on a wide range of potential thresholds (Paz and Crawford 2012; Puillandre et al. 2012).

One of the most widely used evolutionary-based species delimitation methods for single-loci datasets, which is based on neutral coalescent theory, is the General Mixed Yule-Coalescent approach (GMYC) (Fujisawa and Barraclough 2013). It uses a maximum likelihood method to identify transition points between the inter- / intra-specific branch rates on a time-calibrated ultrametric tree (Pons et al. 2006; Fujisawa and Barraclough 2013). The Poisson tree processes approach (PTP), a relatively new evolutionary-based species delimitation method, is similar to the GMYC approach in that it identifies significant changes in the pace of branching events on the tree (Zhang et al. 2013). In contrast to GMYC, which uses time to identify branching rates transition points, the PTP directly uses the number of substitutions (Zhang et al. 2013) and therefore it uses a non-ultrametric phylogeny. The fundamental assumption of the PTP is that the number of substitutions between species is significantly higher than the number of substitutions within species (Zhang et al. 2013).

The aim of this study is to determine the best species delimitation methodology to quantify the species richness of decapods inhabiting dead scleractinian coral colonies across the Chagos Archipelago. Using a single-locus, 16S rRNA, we compare the performance of three species delimitation methods: ABGD, GMYC and PTP. We choose to use the 16S rRNA gene as, similar to other studies (Lefébure et al. 2006), we found it difficult to amplify the COI gene across a sufficient number of samples. There is evidence that 16S rRNA is also an appropriate marker in crustaceans for DNA barcoding delineation (Lefébure et al. 2006; Tang et al. 2010). For some families of decapods, where taxonomic expertise was available, it was also possible to undertake an integrative approach (Vences et al. 2005; Vogler and Monaghan 2007; Padial et al. 2010; Ross et al. 2010) by combining information on their morphological taxonomy with DNA barcodes. ABGD and PTP species delimitation methods where chosen for their novelty and promise as improved species delimitation methods (Paz and Crawford

2012; Zhang et al. 2013), and GMYC because it is has been frequently used in empirical studies (e.g. Pons et al. 2006; Monaghan et al. 2009; Vuataz et al. 2011; Paz and Crawford 2012). By undertaking a comparative analysis of these three species delimitation analyses and their concordance with taxonomic identifications we also hope to inform future biodiversity assessments on species rich groups.

3.3. Methods

3.3.1. Sampling design

Collections of dead branching corals were taken from the Chagos Archipelago in 2012 to quantify the diversity of the cryptofauna inhabiting this one sub-habitat (Head et al. 2015/Chapter 1). Fifty-four dead branching coral colonies of approximately 20 cm in diameter were collected from 28 sites across the Chagos Archipelago at Diego Garcia Atoll, Peros Banhos Atoll, Salomon Atoll, Eagle and Brothers Islands of the Great Chagos Bank, and Egmont Atoll (Fig. 1.2). Sites were separated by at least 250 m and located on the outer reef. Coral colonies were defined as being dead if they had no observable polyps, evidence of turf and crustose coralline algae, and often erosion. Between 2 to 4 dead *Acropora* or *Pocillopora* coral colonies of approximately 20cm in diameter were identified at each site at approximately 8-10m depth. A water-tight polythene bag (250 Micro gauge) was secured around the coral to ensnare all inhabiting organisms, before the coral was chiselled off the reef. The coral was then completely enclosed in the bag for transport and securely cable tied and bought to the surface where it was soaked in a bucket of freshwater for no longer than 2 minutes to remove inhabiting motile fauna (Following the protocol of Stella et al. 2010). Both the seawater from the plastic bag and the freshwater were then filtered through a 1mm sieve and all fauna caught was sorted, photographed, catalogued and preserved in 95% ethanol. Finally the coral colony was inspected and carefully broken up, using a hammer and chisel to collect any remaining hidden fauna.

3.3.2. Sorting and identification of specimens

Brachyura, Galatheidae, Hippolytidae and Palaemonoidea specimens were identified to species by taxonomic experts (Brachyura identified by Prof. P. Ng Kin Lee, Galatheidae identified by Dr. E. Macpherson, Hippolytidae and Palaemonoidea identified by Dr. S. De Grave respectively). Rare species were catalogued into the Raffles Museum, Singapore (Brachyura) and Oxford University Natural History Museum (Palaemonoidea, Hippolytidae and Galatheidae) collections. Identification to species level in these groups enabled sequencing of fewer individuals, typically 1-3 individuals per species. The Alpheidae and Porcellanidae were sorted into morphotypes where possible, but uncertainties in identification resulted in the majority of specimens being sequenced. Paguroidea were considered too difficult to morphotype and so all organisms from this family were sequenced. All larvae and juvenile forms for which it was not possible to obtain identifications were sequenced.

3.3.3. DNA Extraction, PCR amplification, and sequencing

Pleopods, eggs or abdominal tissue were excised for DNA extraction using Qiagen's DNeasy Blood and Tissue kits resulting in 100µl elutions. In some cases, when organisms were particularly small, whole organisms were ground up for extraction. Polymerase chain reaction (PCR) amplifications were carried out in 18µl volume reactions using; 12µl Master mix HotStarTaq (Qiagen), 3µl DNA template, 0.6µl RNase-free water (Qiagen), and 2.4µl (4µM) primer mix (2µl forward, 2µl reverse, and 96µl RNase-free water). Partial fragments of the 16S ribosomal RNA (rRNA) gene (~520bp) were amplified using the forward and reverse primers 16S-l2/1472 (Schubart et al. 2002) for the Caridae, 16S AR/BR for the Brachyura (Palumbi et al. 1991), 16S AR/BRDR for the Porcellanidae and Paguroidae (Palumbi et al. 1991), and 16S AR/BR or 16SLRN/J for the Galatheoidae (Morrison et al. 2002). Amplification required testing of different primer set combinations for many specimens. PCR thermal cycle for 16S differed slightly for all primer sets. The thermal cycle using the

L2/1472 primers was an initial 15 min at 95°C; with 40 cycles of 60s at 94°C, 110s at annealing temperature of 46°C, and 110s at 72°C; followed by 10 min at 72°C. For AR/BR it was an initial 15 min at 95°C; with 35-40 cycles of 60s at 94°C, 60s at annealing temperature of 50-55°C, and 120s at 72°C; followed by 10 min at 72°C. Whilst for AR/BRDR it was initial 15 min at 95°C; with 40 cycles of 30s at 94°C, 60s at annealing temperature of 50°C, and 120s at 72°C; followed by 5 min at 72°C. Finally for LRN/J it was initial 15 min at 95°C; with 30 cycles of 30s at 94°C, 30s at annealing temperature of 50°C, and 90s at 72°C; followed by 7 min at 72°C. PCR reactions were checked using gel electrophoresis, and successful amplifications were purified using ExoSap-IT (Affymetrix) following the manufacturer's instructions. Automated sequencing was performed on purified products in both directions with the aforementioned primers using an Applied Biosystems 3730xl DNA Analyzer.

3.3.4. Sequence alignment

Sequences were paired and edited using chromatograph visualisations in Geneious 6.1.5 (Biomatters Ltd., Auckland, New Zealand). Seven multiple alignments were compiled, one for each of the following Decapoda groups; Brachyura, Palaemonoidae, Hippolytidae, Alpheoidae, Porcellanidae, Papaguroidae and Galatheoidae. Each multiple alignment was compiled using the Geneious alignment function under default settings (Biomatters Ltd.), and then checked and edited by eye. Some regions of the 16S gene were highly variable and difficult to align with confidence. In order to eliminate the most highly divergent regions using a reproducible set of conditions 16S alignments were run through the GBlocks server using the less stringent option which allows for less strict flacking positions and for gaps (Castresana 2000). Table 3.1 shows the alignment lengths pre- and post- GBlocks. The GMYC species delimitation method requires the multiple alignment to contain only unique sequences to avoid polytomies on the ultrametric tree (Fujisawa and Barraclough 2013). To keep the multiple alignments used for all species delimitation methods consistent we therefore

used those containing only unique sequences. MOTHUR was used to identify any identical sequences (Schloss et al. 2009). Table 3.1 details the total number of sequences versus the number of unique sequences for each multiple alignment. All unique sequences were catalogued in GenBank.

Table 3.1 Sequencing information for all decapod families/superfamilies. Models of evolution: HKY (Hasegawa, Kishino and Yano 1985) distinguishes between the rate of transitions and transversions (but allows only one rate for each) and allows unequal base frequencies, and TIM3 (transition model) allows variable transition rates, two transversion rates and variable base frequencies. Gamma distributed rate variation among sites (G) and a proportion of Invariable sites are allowed (I).

Taxa	% of specimens successfully sequenced	No. of sequences	No. of unique sequences	Length of sequence pre-GBlocks	Length of sequences post-GBlocks	Model of evolution
Brachyura	57%	87	84	469	406	TIM3+I+G
Palaemonoidea	95%	20	20	521	444	HKY+I+G
Hippolytidae	59%	16	11	556	556	HKY+I+G
Alpheidae	80%	252	154	623	501	TIM3+I+G
Porcellanidae	85%	98	51	543	522	HYG+I
Paguroidea	86%	163	134	552	511	HYK+I+G
Galatheidae	60%	9	8	637	542	HKY+G

3.3.5.Species delimitation methods

Automatic Barcode Gap Discovery (ABGD)

The ABGD method was implemented using the Web interface: http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html. The multiple alignment Fasta file was inputted under default parameters, using Kimura 2-parameter (K2P) distances that correct for transition rate bias in the substitution process (Kimura 1980). The minimum relative gap width was set to a value between 0.5 – 1.5. This relative gap width should be informed by prior knowledge where possible (Puillandre et al. 2012). In this case information on a molecular threshold for the 16S gene in Crustaceans is available (Lefébure et al. 2006). However the partitions are stable over a wide range of prior values (Puillandre et al. 2012). This produces recursive partitions, which may result in a different number of groupings being reported. The best partition should be chosen using independent data (e.g. previously defined species, other barcoding studies) and the aid of the pairwise distribution histogram (Puillandre et al. 2012). For this reason this method is viewed as a primary species hypothesis testing method, on which further analysis should be undertaken (Puillandre et al. 2012).

Poisson Tree Processes Model (PTP)

The PTP method requires a non-ultrametric tree, so a tree was constructed for each multiple alignment using Bayesian Inference analysis in MrBayes v.3.2 (Ronquist et al. 2012). First jModelTest v.2 software was used to select an appropriate model of molecular evolution on which accurate phylogenetic inference largely depends. The models of evolution that obtained the highest support under Bayesian Information Criterion (BIC) are detailed in Table 3.1. In

MrBayes, Metropolis-coupled Monte Carlo Markov Chains (MCMC) were run for 100 million generations (x8 chains, temp =0.05), with trees sampled every 10,000 generations, resulting in 10 million trees. The parameters of nucleotide frequencies, substitution rates, gamma shape, and invariant-sites proportion were unlinked across partitions. Tracer v.1.6 was used to ascertain if convergence had been obtained. If standard deviation of partition frequencies was <0.01, potential scale reduction factor (PSRF) was ~1.00, effective sample sizes (ESS) were >200, and the shape of the stationary posterior-distribution trace was a 'straight hairy capillary' (viewed in Tracer v 1.6; Rambaut and Drummond 2007) the data were considered to have converged (Ronquist et al. 2012). Once convergence was reached a summary tree was constructed by discarding the first 10% of trees as burn-in, and then using a 50% majority rule consensus tree to construct the summary tree and estimate posterior probabilities (PP) in MrBayes. The summary tree was visualised in FigTree.

The PTP analysis was undertaken using the PTP Web interface at http://species.h-its.org/ptp/. The tree file was uploaded and default parameters selected. The default parameters included: selecting an un-rooted tree, using 100,000 MCMC generations, thinning of 100 and burn-in of 10%. For the multiple alignments with over 50 sequences the number of MCMC generations was increased to the maximum 500,000.

General Mixed Yule-Coalescent approach (GMYC)

GMYC uses genealogical information rather than simple genetic distances and therefore requires an ultrametric gene genealogy as input (Monaghan et al. 2009). BEAST v.2.1 was used to obtain the ultrametric tree (Drummond and Rambaut 2007) using the models of evolution attained from jModelTest (Table 3.1). A Yule prior, and a log-normal relaxed molecular clock for the variation of substitution rates, were selected and run for 100 million MCMC generations, with trees sampled every 10,000 generations. Tracer v.1.6. (Rambaut and Drummond 2007) was used to investigate if convergence had been met using the same

parameters as stated previously. The phylogenetic time-trees produced by BEAST were summarised using the TreeAnnotator programme, with a 10% burn-in, to find the best-supported tree with a posterior clade probability for each node (Drummond and Rambaut 2007). The GMYC model is implemented using the SPLITS package (Fujisawa and Barraclough 2013) for R (R-Development-Core-Team 2008). The ape package is also needed to import the tree file (Paradis et al. 2004). Both the single and multiple threshold GMYC models were applied. The single threshold model estimates a single point of transition between intra- and inter-specific rates of coalescence, whereas the multiple threshold model allows the point of transition to vary across the genealogy (Pons et al. 2006; Monaghan et al. 2009).

3.4. Results

3.4.1. *Brachyura*

Morphological identifications of adult specimens revealed at least 45 species. However less than half of these specimens were successfully sequenced, therefore of the 84 unique sequences, it is known from morphological identifications that there are at least 17 species (Table 3.2). However, 58 of the 84 sequences are larva megalopa specimens for which it is not possible to identify species using adult morphology, and therefore it is possible there are more than 17 Brachyura species represented by the 84 sequences. The single threshold and multiple threshold GMYC detected a very different number of putative species, at 3 and 36 respectively (Table 3.3). The single threshold GMYC underestimated the number of putative species. It is also likely that this is the case for the multiple threshold GMYC as it has grouped different morphological species into the same MOTUs in at least 5 occasions. For instance it groups *Lophozozymus anaglyptus* and *Pilodius* sp. together as well as grouping *Domecia glabra*, *Quadrella* sp. and *Tylocarcinus styx* together. The last 3 species are all from different families. The PTP method estimated 56 putative species with a confidence interval

of between 49 and 63 species. ABGD detected between 41 and 54 species across the recursive partitions, depending on the prior intraspecific divergence chosen, with 45 MOTUs emerging as the most appropriate number of groupings from this method (Fig. 3.1.a. and Table 3.3). The PTP method does not group any known species into the same MOTU (Fig. 3.2), with the exception of *Cryptodomia* sp. and *Lahaina* sp., which are morphologically classified in different families. The grouping of sequences from specimens of different families and genera suggests that either the families need revision or that the species delimitation methods may be underestimating the number of species. The PTP method estimates the highest putative species number, and is therefore probably the most accurate species estimation of all the methods tested, assuming that the Brachyura taxonomic classification system is correct.

3.4.2. *Palaemonoidea*

Morphologically there are 20 species of Palaemonoidea (Table 3.2). As with the Brachyura, the GMYC method underestimated the number of putative species, but here the multiple threshold approach makes little difference, only increasing the estimated number of species from 2 (single threshold) to 3 (Table 3.3). The PTP method and ABGD provide higher species estimations, which more closely correspond to the morphological estimation. The ABGD method consistently estimated 15 MOTUs across all prior intraspecific divergence distances (Fig.3.1b). This was re-run with different gap widths to try and identify the prior intraspecific divergence at which the MOTU estimates would reduce but a gap width larger than 1 (used here in Fig. 3.1.b) failed to distinguish between MOTUs. The PTP estimates 17 putative species with a confidence interval of between 4 and 20 putative species (Table 3.3). The PTP method groups together *Cuapetes elegans* with *Cuapetes ensifrons*, *Palaemonella spinulata cf.* with *Palaemonella tenuipes cf.*, and *Jocaste japonica* with *Periclimenaeus pettihouarsi*, making the species estimates conservative in comparison to the morphological identifications (Fig. 3.3).

3.4.3. *Hippolytidae*

At least 8 species of Hippolytidae were morphologically identified (Table 3.2), but like the Brachyura there were some juveniles that could not be identified to species level. Of the 8 known species, 5 were successfully sequenced along with the representative juvenile morphotypes. It is unknown whether these juvenile morphotypes belong to the identified adult species or different species. The species delimitation methods gave relatively congruent results. The ABGD method estimated 5 putative species across all prior intraspecific divergence distances and a clear barcoding gap demonstrated by the pairwise distance distribution histogram (Fig. 3.1.c.). However, this barcoding gap should be treated with caution and not seen as definitive for the Hippolytidae as the multiple-alignment in this study only contains 11 sequences (Table 3.1). Both of the GMYC methods estimated 6 species, and the PTP method estimated 7 species using Bayesian Inference with a confidence interval between 5 and 8 species (Table 3.3). However there was some disparity in the groupings of the sequences between methods. The PTP and GMYC single threshold both grouped 4 *Saron* species together as one putative species (Fig. 3.4). From the morphological identifications it is known there should be at least two *Saron* species; *S. marmoratus* and *S. neglectus*, and the other two specimens represent juvenile morphotypes, which could possibly be *S. marmoratus* or *S. neglectus* juveniles. The PTP differed by separating the *Thorella* sp. and one of the *Thorina maldivensis* (Fig. 3.4), whereas the GMYC single threshold grouped these two morphologically identified species together as one putative species. In contrast, the GMYC multiple threshold seems to be less accurate, grouping different genera (*Thorina maldivensis*, *Thorella* sp., *Thor ambiomensis* and *Thor* sp.) into two putative species and separating all *Saron* species into different putative species. Therefore the PTP method again appears to have calculated the most accurate species estimate.

3.4.4. *Alpheidae*

This family of Caridae were identified to genus level only, as taxonomic expertise for this family is scarce and so it was not possible for us to take morphological identifications to species level. At least 6 genera were identified from the 252 individuals successfully sequenced (Table 3.1). These included; *Alpheus*, *Synalpheus*, *Athanas*, *Metalpheus*, *Racilius*, and *Rugathanas* species. As with the Brachyura the single threshold GMYC appeared to vastly underestimate the number of species (Table 3.3). In contrast the multiple threshold GMYC estimated 51 putative species, the highest species estimate from all the methods, and the likelihood ratio (LR), a measure of the certainty of the estimation, was significant (LR=7.68, p=0.022). The ABGD and PTP methods both produced putative species estimates of 42 species, with a confidence interval of 38 to 62 species for the PTP method. The ABGD method estimated different species estimates for different partitions but the presence of a barcoding gap in the pairwise distance distribution histogram, though not well defined, helped identified an appropriate partition at the prior intraspecific divergence of 0.0028 (Fig. 3.1.d). However, it is evident from the Bayesian inference tree produced by the PTP method (Fig. 3.5) and the species estimate groupings from the multiple threshold GMYC that different genera have been grouped into the same putative species on multiple occasions by both methods. Therefore the multiple threshold GMYC may have produced the closest species estimate at 51 putative species, as it is the highest of the three estimates, but even this is very likely an underestimate because this method also groups species from different genera together as putative species.

3.4.5. *Porcellanidae*

Morphological identifications are not available for this family and therefore an integrative approach cannot be undertaken for the species groupings produced by these methods. Again the putative species estimates from the PTP and ABGD method are in agreement at 7 putative species (Table 3.3) from the 51 unique sequences (Table 3.1). ABGD found a clear barcoding gap (Fig. 3.1.e), and the PTP method had a confidence interval of between 7 and 12 species.

Both the single and multiple threshold GMYC methods estimated higher species estimations at 15 and 13 putative species respectively, but only the multiple threshold method likelihood ratio was significant (*LR*=6.33, *p*=0.042). However, the 7 putative species maybe an underestimate as it is evident from one of the branches on the Bayesian Inference tree (Fig. 3.6) that the PTP method has grouped 31 sequences into one MOTU, which may constitute two different MOTUs.

3.4.6. *Paguroidea*

Morphological identifications were not available for this family and therefore an integrative approach to the species groupings could not be taken. As was the case for the Porcellanidae, both GMYC estimates were higher than that of the PTP and ABGD, with the single threshold method estimating 16 species and the multiple threshold method estimating 18 species (Table 3.3), and both methods had significant likelihood ratios (*LR*=18.17, *p*=0.0001 & *LR*=27.93, *p*=8.60e^{-07}respectively). The PTP estimated 13 putative species with a confidence interval between 10 and 21 species. The lack of a clear barcoding gap in the pairwise distance distribution histogram by the ABGD method makes objective selection of the best species estimate from this method difficult (Fig. 3.1.f). However, the species estimation across all prior intraspecific divergence rates only varied between 12 and 9 putative species (Fig. 3.1.f), which is similar to the PTP estimate. Groupings for the two GMYC and the PTP methods broadly agreed, with the exception that the GMYC methods split some of the larger species groupings further into different species, thereby obtaining the higher species estimate (Fig. 3.7 and Table 3.3).

3.4.7. *Galatheidae*

Of the 8 species of Galatheidae identified morphologically, only 3 *(Galathea pilosa*, *Galathea tanegashimae*, and *Galathea aegyptiaca*) were successfully sequenced along with larval forms, for which identification was not possible (Table 3.2). All species delimitation

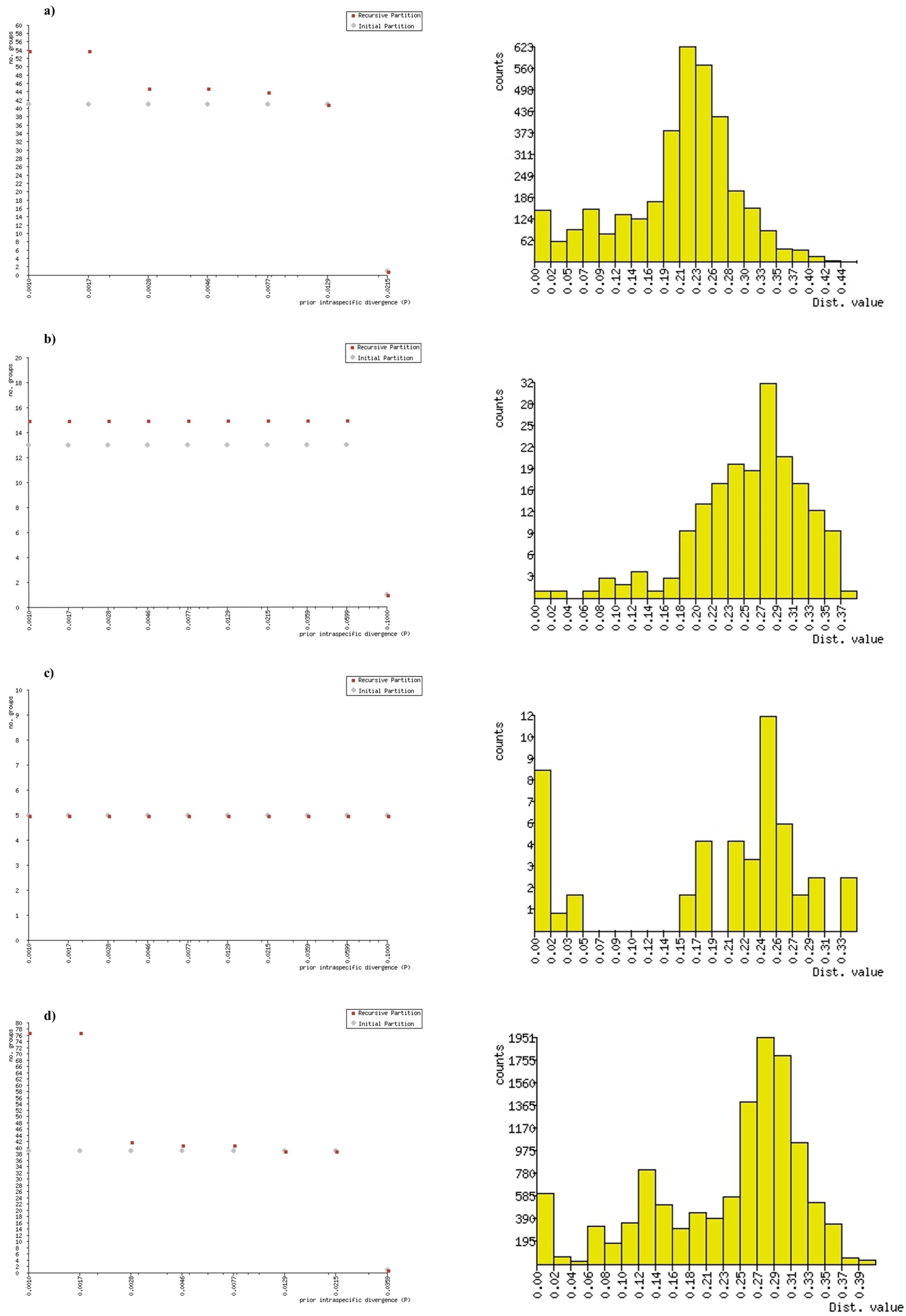
methods, with the exception of the multiple threshold GMYC method, were in agreement in estimating 6 putative species (Table 3.3). This suggests that 3 of the larval Galatheidae forms are separate species (Fig. 3.8). The single threshold GMYC had a significant likelihood ratio (LR=5.99, p=0.05), whereas the multiple threshold method did not (LR=5.37, p=0.07), making the multiple threshold estimate potentially less reliable. The PTP method had a confidence interval of between 3 and 7 putative species, and the ABGD method consistently estimated the same number of species groupings across all partitions (Fig. 3.1.g).

Table 3.2 Taxonomic estimates for each group of decapods, based on morphological identifications, and the number of these taxonomic species that were successfully sequenced. Where no information is provided, taxonomic expertise was not available. It should be noted that the number of species successfully sequenced cannot be directly compared to the number of MOTUs in Figure 3 because larvae forms, for which taxonomic identification are not possible, were also included in molecular sequence delimitation

Taxa	Species richness from morphological identifications	Number of morphological species successfully sequenced
Brachyura	≥45	≥18
Palaemonoidea	21	20
Hippolytidae	≥8	≥5
Alpheidae	--------	------
Porcellanidae	--------	------
Paguroidea	--------	------
Galatheidae	≥ 9	≥3

Table 3.3 Number of MOTUs estimated by 3 different species delimitation methods. * indicates that the results likelihood ratios were significant (GMYC tests only). It should be noted that an appropriate partition could not be chosen with confidence for the Paguroidea species estimate using the ABGD method and therefore a range of estimates from all partitions are listed.

	Number of MOTUs			
Taxa	GMYC (Single threshold)	GMYC (Multi threshold)	PTP	ABGD
Brachyura	3	36	56	45
Palaemonoidea	2	3	17	15
Hippolytidae	6	6	7	5
Alpheidae	3	51*	42	42
Porcellanidae	15	13*	7	7
Paguroidea	16*	18*	13	12 - 9
Galatheidae	6*	4	6	6


a)
Recursive Partition
Initial Partition
no. groups
prior intraspecific divergence (P)
counts
623
560
498
436
373
311
249
186
124
62
Dist. value
b)
Recursive Partition
Initial Partition
no. groups
prior intraspecific divergence (P)
counts
32
28
25
22
19
16
12
9
6
3
Dist. value
c)
Recursive Partition
Initial Partition
no. groups
prior intraspecific divergence (P)
counts
Dist. value
d)
Recursive Partition
Initial Partition
no. groups
prior intraspecific divergence (P)
counts
1951
1755
1560
1365
1170
975
780
585
390
195
Dist. value

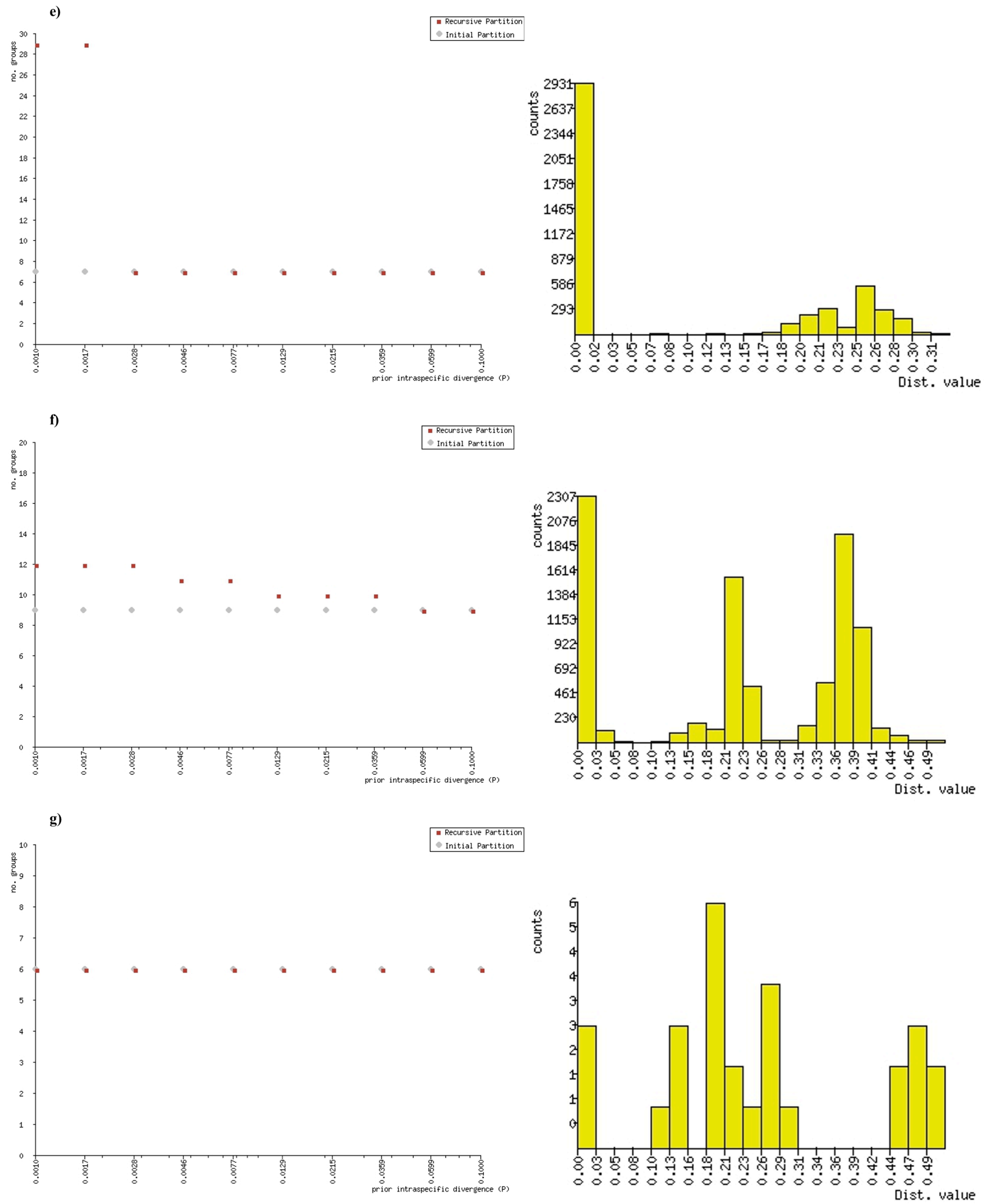


Figure 3.1 ABGD method outputs for **(a)** Brachyura, **(b)** Palaemonoidea, **(c)** Hippolytidae, **(d)** Alpheidae, **(e)** Porcellanidae, **(f)** Paguroidea, **(g)** Galatheidae. The plots (left hand-side) illustrate the number of MOTU groupings at prior interspecific divergence distances, and the bargraphs (right hand-side) illustrate the 'barcoding gap' for each taxon.

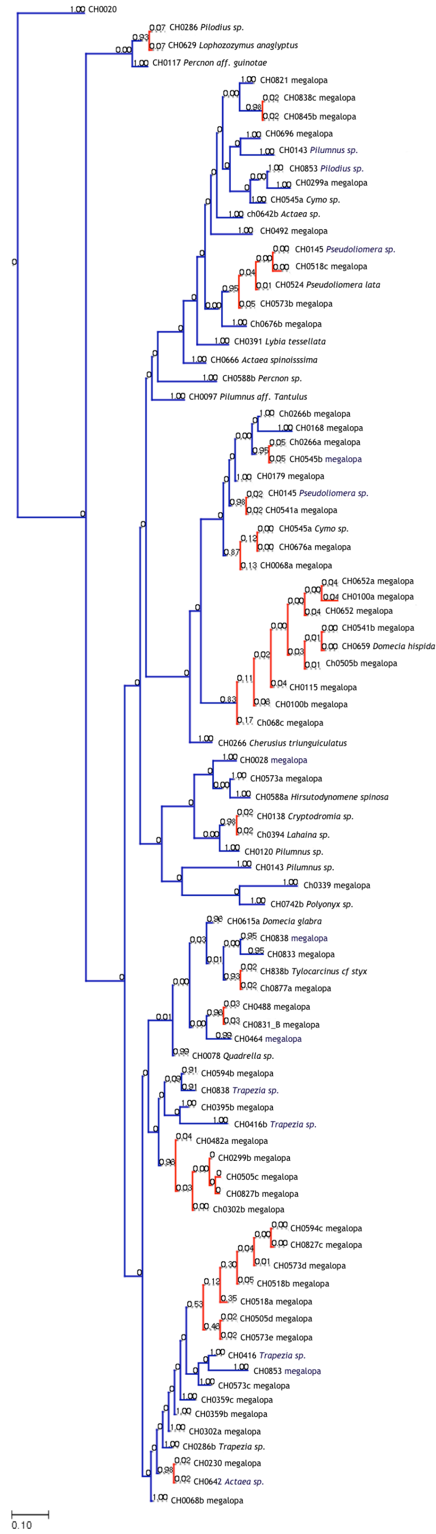


Figure 3.2 PTP Bayesian Inference Tree for the Brachyura illustrating MOTU clusters with posterior probabilities displayed on the branches. Blue lines represent unique MOTUs. Red lines represent individuals clustered into an MOTU.

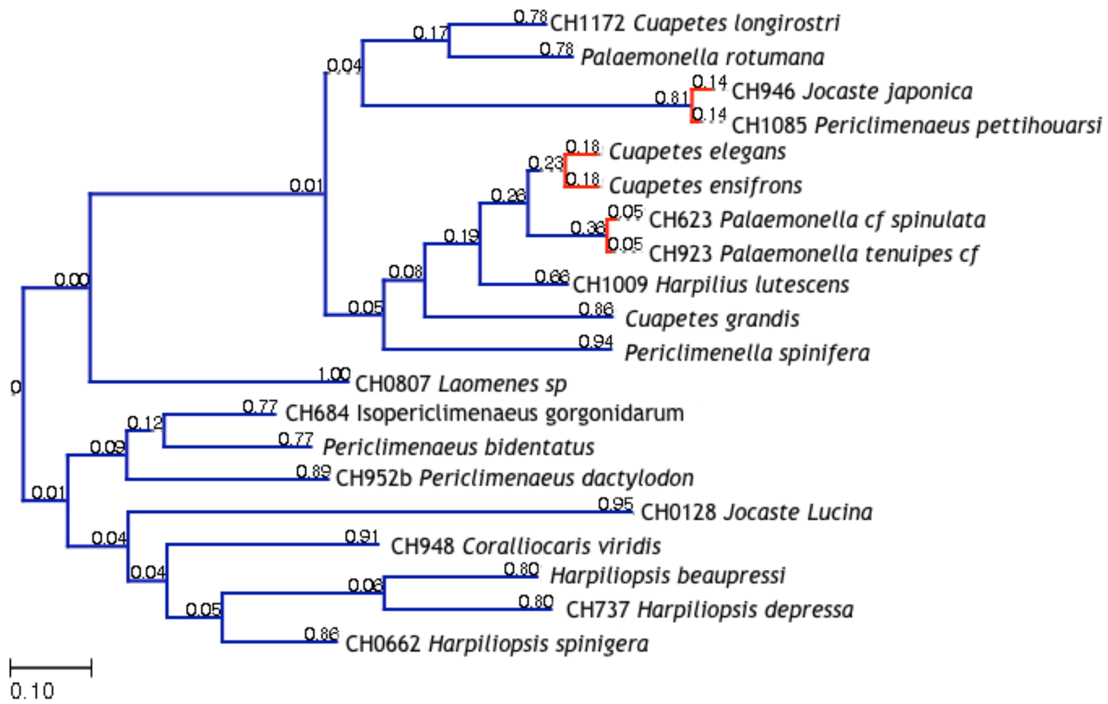


Figure 3.3 PTP Bayesian Inference Tree for the Palaemoniodea illustrating MOTU clusters with posterior probabilities displayed on the branches. Blue lines represent unique MOTUs. Red lines represent individuals clustered into an MOTU.

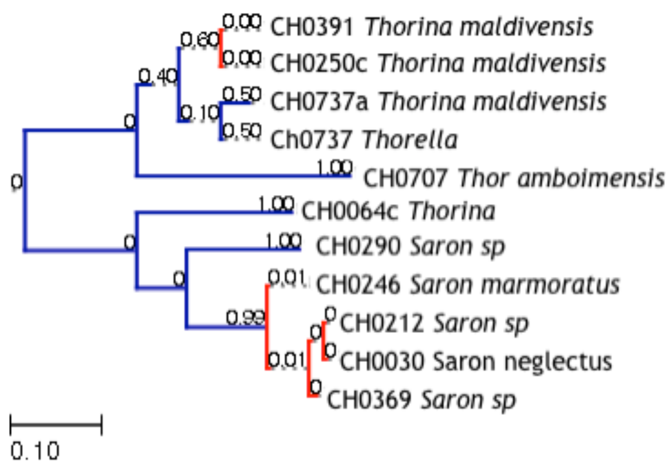


Figure 3.4 PTP Bayesian Inference Tree for the Hippolytae illustrating MOTU clusters with posterior probabilities displayed on the branches. Blue lines represent unique MOTUs. Red lines represent individuals clustered into an MOTU

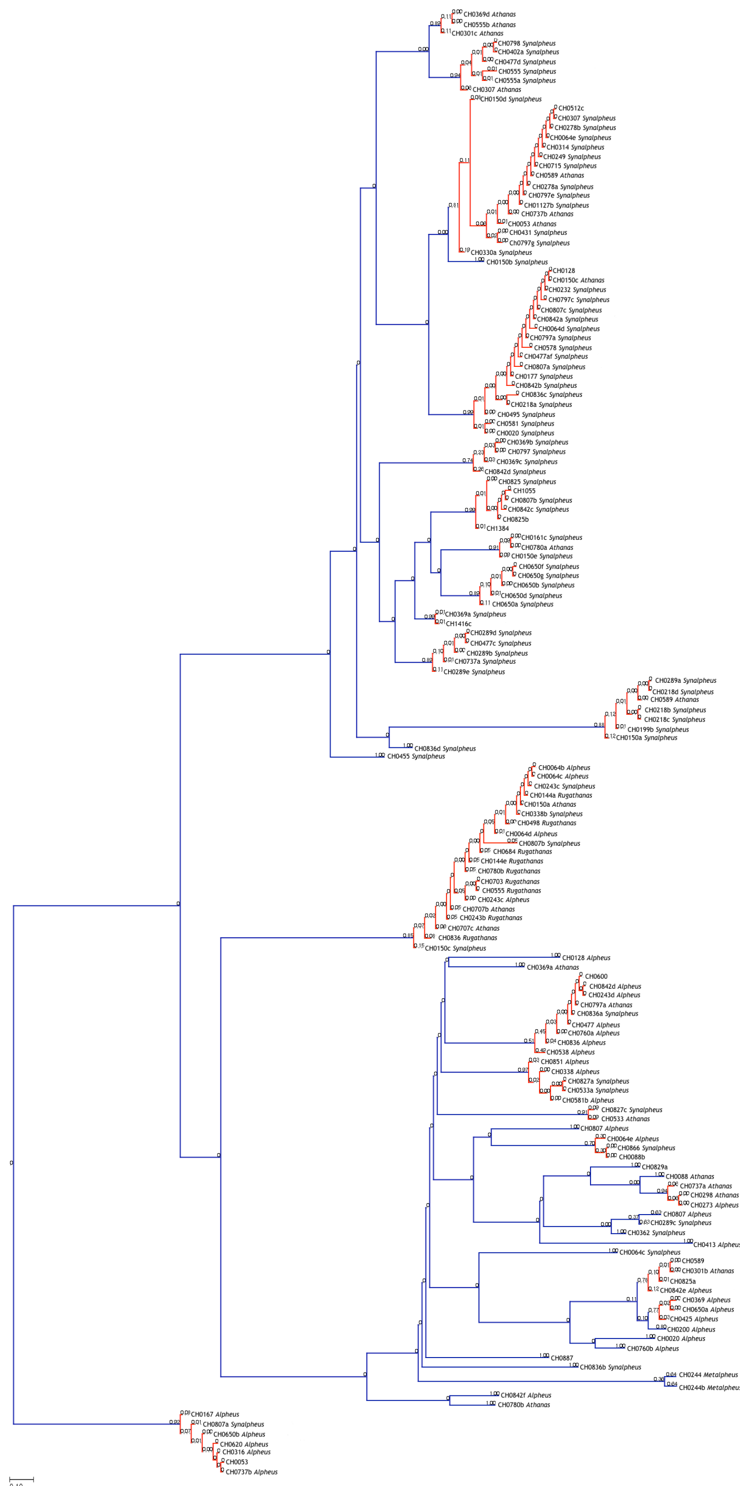


Figure 3.5 PTP Bayesian Inference Tree for the Alpheidae illustrating MOTU clusters with posterior probabilities displayed on the branches. Blue lines represent unique MOTUs. Red lines represent individuals clustered into an MOTU.

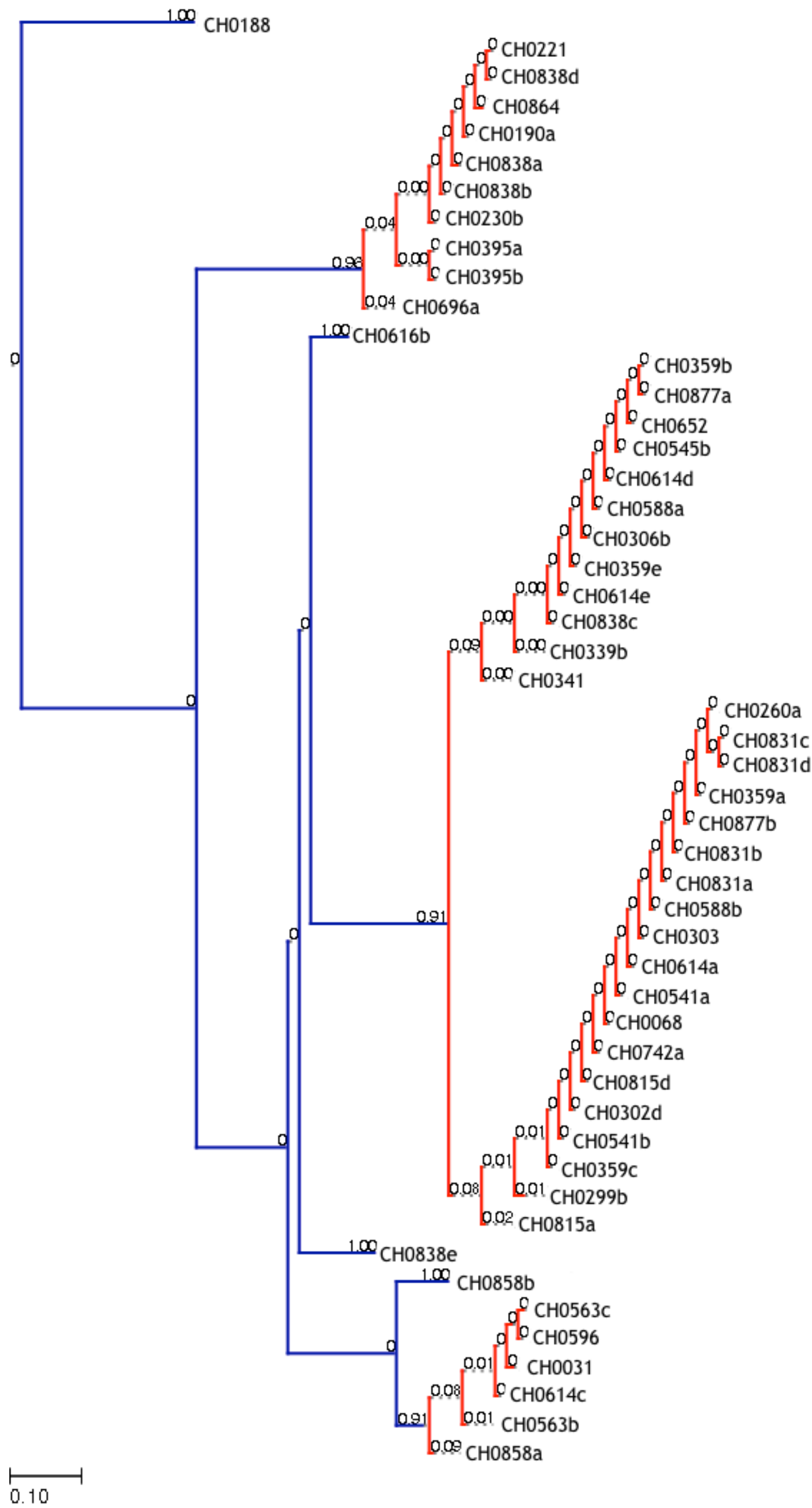


Figure 3.6 PTP Bayesian Inference Tree for the Porcellanidae illustrating MOTU clusters with posterior probabilities displayed on the branches. Blue lines represent unique MOTUs. Red lines represent individuals clustered into an MOTU.

Figure 3.7 PTP Bayesian Inference Tree for the Paguroidea illustrating MOTU clusters with posterior probabilities displayed on the branches. Blue lines represent unique MOTUs. Red lines represent individuals clustered into an MOTU.

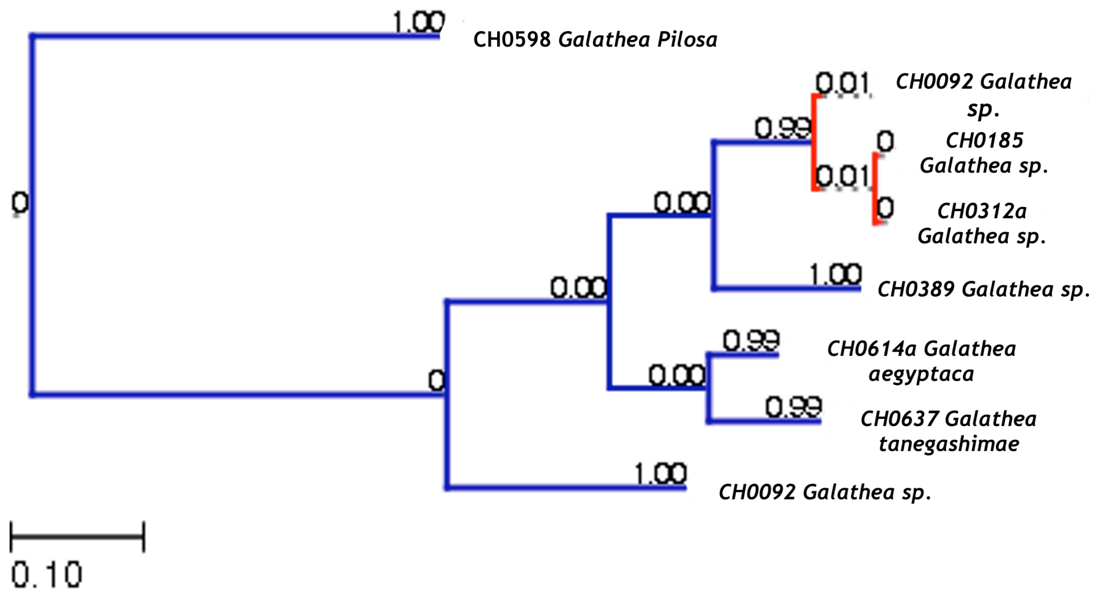


Figure 3.8 PTP Bayesian Inference Tree for the Galaetheidae illustrating MOTU clusters with posterior probabilities displayed on the branches. Blue lines represent unique MOTUs. Red lines represent individuals clustered into an MOTU.

3.5. Discussion

When an integrative approach is taken in comparing the molecular decapod species estimates to morphological identifications all three species delimitation methods produce conservative estimates of putative species numbers. The PTP and ABGD methods generally calculated similar species estimates to each other, giving some extra validation to these estimates. However, the PTP species delimitation method generally performed marginally better as its species estimates were slightly higher for most families, and therefore more aligned with morphological estimates based on expert taxonomic identifications. In contrast, the GMYC method produced mixed results, which were often different to the other two species delimitation methods, and morphological identifications.

There are many singletons within the decapod biodiversity samples from Chagos and singletons can be problematic for all species delimitation approaches (Lim et al. 2012), which may account for the conservative species estimates. It has been argued that because coalescent-based approaches mainly use points of coalescence to delimit species, which by definition is lacking for singletons (i.e. coalescence is the merging of two or more branches into one), they are not expected to deal well with singletons (Lim et al. 2012). However, reports of how successful different methods are at delimiting singletons are mixed (Paz and Crawford 2012; Modica et al. 2014; Kekkonen et al. 2015; Lang et al. 2015), suggesting this is not just dependent on the species delimitation method but also taxon dependent e.g. how many singletons there are and how evolutionary distinct they are from their nearest neighbour.

It is important that species delimitation methods can identify singletons as unique species and not assign them incorrectly to the nearest species (Lim et al. 2012) because rarity is common

in biodiversity samples (Magurran and McGill 2011). Some reviews suggest 30% of terrestrial tropical arthropods are represented by only one known specimen (Coddington et al. 2009). Rare species are also important from the point of view of conservation, ecology and evolutionary history. The definition of a rare species can vary (Coddington et al. 2009; Magurran and McGill 2011; Mouillot et al. 2013) but in this study we consider singletons to be rare species. For the five decapod groups for which taxonomic expertise were available many singletons were identified. Comparing the morphological identified singletons with the results of the species delimitation methods it is suggested that the PTP approach was the most successful at independently identifying singletons. With the exception of the Galatheidae, every method for each family clustered at least one singleton per group together incorrectly with another species; across the board this probably accounts for the underestimation of species. The Palaemonoidea represented the most extreme case, with the multi-alignment being comprised of exclusively singletons, which could explain the poor estimation from GMYC for this group. Although the PTP and ABGD performed far better (in comparison to species numbers derived from morphological identifications) they still grouped at least six singletons together into 3 putative species (*Cuapetes elegans* with *Cuapetes ensifrons*, *Palaemonella spinulata cf.* with *Palaemonella tenuipes cf.*, and *Jocaste japonica* with *Periclimenaeus pettihouarsi)*.

Gene choice may also partially explain the underestimation of species diversity because the 16S gene is known to be a slower evolving gene than the commonly used COI gene (Hebert et al. 2003). However 16S is accepted as a suitable gene for barcoding in crustaceans (Lefébure et al. 2006; Tang et al. 2010), and it was chosen for this study as, similar to reports elsewhere for Crustacea and some other taxa (Vences et al. 2005; Lefébure et al. 2006), we experienced difficulties in amplifying COI. Future studies, where resources allow, would ideally take a multi-loci approach, sequencing both 16S and COI genes.

Of the three methods tested here the GMYC approach is the most widely used species delimitation method in empirical studies (E.g. Pons et al. 2006; Monaghan et al. 2009; Vuataz et al. 2011; Lang et al. 2015). However, it performed the least well of the three methods tested, providing estimates that often were not congruent with the PTP and ABGD methods (Table 3.3) and/or morphological identifications (Table 3.2). Recent studies comparing GMYC with the newer PTP approach, which are both based in a sound phylogenetic framework, also show PTP to be more accurate (Zhang et al. 2013; Toussaint et al. 2015). In this study the Galatheidae and Hippolytidae species estimates were exceptions, where the GMYC estimates were closely aligned with those of the PTP and ABGD delimitation methods. Interestingly, these two families had the lowest number of sequences in the multi-alignments of all the groups, with 11 and 51 sequences respectively. However, the GMYC method has not been reported to have difficulties with larger multi-alignments. Instead its difficulties in estimating accurate species estimates for many of the decapod families may result from the use of an ultrametric tree, whose reconstruction can be an error-prone process, and, in addition, is more computationally intensive than the other methods (Paz and Crawford 2012; Zhang et al. 2013). The presence of many short branches (close to zero) in the tree is also known to yield potentially inaccurate results (Zhang et al. 2013). This may have been the case for the Palaemonoidea and Brachyura trees, which contained many short branch lengths, as the GMYC method produced particularly low estimates. Likelihood ratios for the Palaemonoidea, Brachyura and Hippolytidae groups were also not significant, indicating the uncertainty in delimitation results.

The GMYC method offers two approaches: the single and multiple threshold versions. The single threshold approach assumes that speciation events are older than all coalescent events in the gene tree, whereas the multiple threshold relaxes this assumption searching for alternative models, iteratively splitting and fusing existing species clusters until it finds the best solution (Monaghan et al. 2009). The multiple threshold method has been found to yield relatively similar results to the single threshold method but with a tenancy to over–split the

species clusters, leading to suggestions it should be used with caution (Fujisawa and Barraclough 2013). However, the multiple threshold approach actually produced more accurate species estimates for three of the seven families (Brachyura, Alpheidae and Porcellanidae), suggesting that speciation in some lineages on the tree may be older than coalescent in other lineages in the different group trees. This would seem likely for large, higher order groups, such as the Brachyura, and more speciose groups such as the Alpheidae.

Despite generally performing well, a substantial disadvantage of the ABGD for our purposes, i.e. to obtain a decapod species estimate with a maximum level of certainty and carry out subsequent biodiversity statistics, is choosing among the different partitions, as it is intended to be a primary species partition hypothesis on which further work can be carried out (Puillandre et al. 2012). We therefore used it as a validation method to compare to other methods. ABGD also requires prior information about divergence levels for the study taxa. In this case some information on a molecular threshold for 16S rRNA is available for crustaceans (Lefébure et al. 2006; Tang et al. 2010). It is argued that methods using a molecular pairwise distance threshold, such as the ABGD, suffer from a lack of connection to evolutionary history and that an evolutionary model is needed to assign certainty to the resulting species delimitations (Fujisawa and Barraclough 2013). It is therefore interesting to note that the species delimitation results using the ABGD method in this study align closer to the PTP method results, which uses an evolutionary model, than the PTP results and GMYC results align (both using evolutionary models). A big advantage of the ABGD method is that it is fast with a user-friendly web interface. The ABGD approach has also been advocated for its rapid and accurate delimitation in other studies (e.g. Paz and Crawford 2012; Modica et al. 2014).

Biodiversity inventories, such as ours (Appendix I: species list), focus on specific geographical localities (sympatric data) rather than on taxonomic clades, unlike most barcoding applications (Crawford et al. 2010; Paz and Crawford 2012). Therefore the

potentially sparse phylogenetic sampling may mean that sympatric datasets are less likely to contain sister species leading to greater between species genetic distances, presuming rapid local divergence has not occurred (Paz and Crawford 2012). In addition sympatric datasets normally sample a single or a few populations of conspecific species rather than the whole species range and therefore intraspecific genetic diversity should be lower (Meyer and Paulay 2005; Crawford et al. 2010). Thus in theory high inter-specific variation, coupled with lower intra-specific variation should lead to an ideal scenario for barcoding, particularly those based on genetic distances (Hebert et al. 2003; Meyer and Paulay 2005). Paz and Crawford (2012) found that clustering methods based on genetic distance (ABGD) preformed well on a sympatric dataset of amphibians but the coalescent-based method (GMYC) preformed poorly. However, this theory for sympatric datasets may not hold true for our biodiversity inventory on small invertebrates sampled across a large area (six atolls comprising an area of over 60 000 km^2 (Sheppard et al. 2012) as it is highly likely that many populations of decapod species were sampled, potentially leading to a higher level of intraspecific variation. Moreover, only Hippolytidae and Porcellanidae had well-defined barcoding gaps (Fig. 1c, 1e). That said, our results on ABGD and GMYC are congruent with the Paz and Crawford (2012) study, in that in our opinion the ABGD method preformed better than the GMYC approach. However, the PTP, also a coalescent-based approach performed well in our study but was not tested by Paz and Crawford (2012).

The species delimitation of the decapods from one microhabitat in the Chagos Archipelago has provided rare data for empirical evaluation of three species delimitation methods (but also see Paz and Crawford 2012 comparative study), and demonstrates taxon dependent method performance within the decapods (Kekkonen et al. 2015). The delimitation of singletons was challenging for all methods, but particularly the GMYC approach, resulting in under-estimation of the species richness garnered from morphological identifications. The PTP approach consistently provided the best species estimates across the groups in our opinion, when compared with morphological identifications (when available). The ABGD species

estimates, a primary species hypothesis method, were also relatively closely aligned with those of the PTP. However, the popular GMYC method generally performed poorly possibly because of inaccurate ultrametric tree reconstructions, which can be error-prone, and, in some cases, short branch lengths (Zhang et al. 2013). Overall we would therefore recommend the PTP species delimitation method for species richness assessments of the decapods using a molecular approach, though caution is needed when the sample contains a high proportion of rare species. These results also lend support to the integrative approach, which advocates combining single-loci species delimitation with morphological knowledge or additional genetic information for improved species estimates (Padial et al. 2010; Lang et al. 2015; Toussaint et al. 2015). However, limited resources mean this is not always possible and we believe the use of single-loci molecular species delimitation are still valuable, depending on the aim of the study and as long as the method's limitations are recognised. We encourage the collection of species from understudied groups, such as the decapods, into reference libraries, e.g. GenBank and BOLD, enabling species names to be assigned to MOTUs with confidence, but again limited resources mean a complete reference library is likely unrealistic for ecologically cryptic and species rich groups such as these. Nevertheless even for taxon where reference libraries are insufficient, molecular approaches are still a useful tool for establishing species richness estimates.

4. CHAPTER FOUR:

The biodiversity of the decapods, a component of the cryptofauna, inhabiting dead coral colonies in the Chagos Archipelago

4.1. Abstract

Coral reefs are the most biodiverse marine ecosystem, and whilst the biodiversity of the fish and coral fauna has been quantified, that of the cryptofauna component is still largely unknown. The cryptofauna are described as the small, often cryptic species, mainly invertebrates that live within the reef framework itself. Here, we quantify the diversity of the decapods, a dominant component of reef cryptofauna, inhabiting dead coral colonies across six atolls and islands in the Chagos Archipelago, central Indian Ocean. Dead coral colonies are thought to be one of the most productive marine microhabitats but are regularly over-looked. We find 164 observed species and an abundance of 1,868 decapods, from 54 dead coral colonies, but total species estimates calculate at least 217 decapod species across the Archipelago. Galatheids were the most dominant decapod taxa, and alpheids and hippolytids were also very abundant, with the four most dominant species belonging to the galatheids and hippolytids. This is the highest decapod species richness estimate for this microhabitat from any location globally to date although we only sampled 75% of estimated species diversity. A striking result of the study was the high proportion (32%) of seemingly rare species, and the high proportion (38%) of species that were found only at a particular atoll, however this could also be an artefact of incomplete sampling. Nevertheless if a high proportion of the decapod diversity are indeed rare, then any biodiversity loss could have very negative implications for ecosystem function as recent studies report rare species can contribute disproportionately more to vulnerable ecosystem processes than dominant species. The species richness per dead coral colony was significantly lower at Eagle Island than at any other atoll/island, possibly as a result of a crown-of-thorns (*Acanthaster planci*) outbreak around the island at the time of surveying which may have had indirect affects on the island's reef ecosystem. Peros Banhos atoll and Diego Garcia had the highest decapod species richness estimates of all the atolls/islands. Diego Garcia also had the highest number of

species (25 species) occurring at just one atoll. High species richness at Peros Banhos could be related to the high reef structural complexity at this atoll, an environmental factor that has previously been linked to higher cryptofauna diversity. The Chagos Archipelago is geographically remote, removed from most anthropogenic pressures, with some of the most resilient reefs globally, hence it acts as a reference site for biodiversity and ecosystem process studies in the Indian Ocean. This study is the first to assess any component of reef cryptofauna diversity in Chagos and can now be used as a baseline against which other cryptofauna in the Indian Ocean can be compared.

4.2. Introduction

There have been many predictions of the number of marine species on Earth, which span many orders of magnitude (Grassel and Maciolek 1992; May 1994). One of the most recent estimates predicts ~2.2 million eukaryote marine species, which would mean that over 91% of species in the ocean still await description (Mora et al. 2011a). Coral reefs are the most diverse ocean ecosystem on a per area basis (and perhaps absolutely too), despite covering less than 0.2% of the ocean floor, with the deep sea being the other major repository (Sala and Knowlton 2006). Reef biodiversity can be divided into three main components; fish, reef-building organisms and cryptofauna (Reaka-Kudla 1997). The cryptofauna is defined as the small, often cryptic mainly invertebrates that live within the reef framework itself (Reaka-Kudla 1997; Plaisance et al. 2011). An estimated 168,000 species of reef invertebrates have been described on coral reefs (Ruppert et al. 2004; Stella et al. 2011a), far surpassing the number of fish species (~5000 species; Bellwood et al. 2012) and the approximately 700 species of reef-building corals (Veron 2000). The majority of these reef invertebrates can be described as cryptofauna, but the cryptofauna also contains many poorly-known groups and are hard to sample as a result of their small and cryptic nature (Plaisance et al. 2009). Hence this component of biodiversity is understudied and we currently do not know even to the nearest order of magnitude how many species inhabit coral reef ecosystems (Reaka-Kudla 1997; Small et al. 1998; Plaisance et al. 2009). However, in recent years there have been several large-scale initiatives undertaken, such as the Census of Marine Life (http://www.creefs.org) and the Moorea Biocode Project (http://bscit.berkeley.edu/biocode), which have emphasised the importance of documentation of small and understudied organisms such as invertebrate and microbial species.

Approximately 20% of reef invertebrates are crustaceans, making them one of the most speciose groups on coral reefs (Kramer et al. 2014). Crustacea play a major role in the trophic dynamics of detrital-based food webs on coral reefs and are an extremely important link between primary production and higher consumers, as well as between microbial- and detrital-based food webs (Enochs and Manzello 2012a; Kramer et al. 2014). Their productivity, that is the ability of a group of organisms to transfer energy to higher trophic levels, is estimated at approximately 0.066g wet weight $m^{-2}d^{-1}$ (Kramer et al. 2014), only 3 times less than the estimated productivity of fishes (Depczynski et al. 2007). Crustacea are considered one of the most important dietary components of reef fish assemblages because 70% of reef fish are invertivores, and 60% of invertivores prey predominantly on benthic Crustacea (Williams and Hatcher 1983; Randall et al. 1997; Froese and Pauly 2014). In addition, decapods also have important functional roles in maintaining coral reef health with species cleaning fish of parasites, e.g. cleaner shrimp (Becker and Grutter 2004), and some species defending coral colonies from predators and clearing excess sediment preventing smothering of coral polyps, e.g. *Trapezia* crabs (Pratchett 2001; McKeon and Moore 2014).

Crustaceans inhabit all reef microhabitats and are major components of invertebrate communities on live corals, dead corals, coral rubble, the epilithic algal matrix (Kramer et al. 2013), and sand (Kramer et al. 2014). However, most cryptofauna studies have focused on live corals habitats (Stella et al. 2011a). The limited number of comparative studies on cryptofauna biodiversity across microhabitats have identified dead coral habitats to be the most biodiverse microhabitat (Enochs 2012; Kramer et al. 2014). This is probably because of the structural relief of the coral still remaining intact to provide habitat and shelter from predators, in comparison to the other microhabitats, such as sand and coral rubble where the structural complexity is lower. The heterogeneity of the benthic substrata increases on dead branching coral compared to live coral as sessile organisms, such as Porifera and Ascidiacea, colonise recently dead corals providing a variety of niches for motile cryptofauna (Enochs and Manzello 2012b), resulting in higher biodiversity through complementarity and

facilitative interactions (Hooper et al. 2005). In addition, the productivity of the complete faunal assemblage of dead coral colonies is estimated to be up to 149g Ash-free Dry Weight (AFDW) m^{-2} yr^{-1} (Kramer et al. 2014), suggesting this microhabitat is one of the most productive in the world, surpassed only by Californian macrophyte detritus and mussel beds in the Wadden Sea (Asmus 1987; Taylor 1998).

The recent Living Blue Planet Report documents a 49% decline in the size of marine vertebrate populations between 1970 and 2012 (WWF. 2015), suggesting it is now probably impossible to find a true baseline against which to measure biodiversity levels and assess how ecosystems functioned before human disturbance (Knowlton and Jackson 2008). This is especially acute for tropical coral reefs, which are estimated have lost more than half their reef-building coral cover globally over the last 30 years (WWF. 2015). Our best strategy perhaps is to use the few remaining reefs approaching 'pristine' conditions, as a result of their remote locations away from direct human impacts, as baselines for measuring biodiversity and ecosystem processes (Knowlton and Jackson 2008; Sandin et al. 2008). The Chagos Archipelago, or British Indian Ocean Territory (BIOT), in the middle of the Indian Ocean represents such a reference site for the Indian Ocean (Sheppard et al. 2012) (Fig. 1.2).

This study investigates the biodiversity of decapods (Crustacea), inhabiting the most productive microhabitat, dead coral colonies, on coral reefs across the Chagos Archipelago. Dead coral colonies of similar size and structure were sampled from sites across the archipelago, including: Diego Garcia Atoll, Peros Banhos Atoll, Salomon Atoll, Egmont Atoll, and Brothers and Eagle Islands (the latter two both part of the Great Chagos Bank) (Fig. 1.2). Extrapolation techniques were used to standardise richness data to account for uneven sampling between atolls and islands enabling biodiversity comparisons between the locations within the Archipelago. Coral colonies were sampled from the same depth and reef type and similarities in decapod community structure between the atolls and islands were evaluated. This is the first inventory of reef decapod cryptofauna on any microhabitat from

the Chagos Archipelago and therefore represents a baseline biodiversity estimate for decapods away from direct human impacts in the Indian Ocean.

4.3. Methods

4.3.1. Sampling design summary

Fifty-four dead coral colonies from 25 sites across five atolls in the Chagos Archipelago were sampled. All dead coral colonies were of approximately 20 cm in diameter and were either dead *Pocillopora* or *Acropora* coral species. Between 2-4 colonies were collected from each site on the outer reef of the atoll/island, at a depth of approximately 9 m. Sites were separated by at least 250 m. Please refer to Chapter 3 for further details of the sampling methodology.

4.3.2. Species richness estimates

Different species delimitation methods were used for each superfamily/family depending on the performance of the method, which were often taxon specific (evaluated in Chapter 3). Brachyura, Galatheidae, Hippolytidae and Palaemonoidea specimens were identified to species by taxonomic experts (Brachyura identified by Prof. P. Ng Kin Lee, Galatheidae identified by Dr. E. Macpherson, Hippolytidae and Palaemonoidea identified by Dr. S. De Grave). Rare species were catalogued into the Raffles Museum, Singapore (Brachyura) and Oxford University Natural History Museum (Palaemonoidea, Hippolytidae and Galatheidae) collections. For the groups where morphological identifications were available they were used in preference to molecular delimitation methods (Table 4.1) as they also allowed species names to be given and were generally thought to be more reliable than the molecular species delimitations where many had problems with singletons, of which there are many in this dataset (see Chapter 3). It was not possible to identify Brachyura and Galatheidae larval and megalopa forms morphologically to species level, so only molecular techniques could be

used. However, difficulties in amplifying the 16S rRNA gene for many of the adult specimens meant morphological identifications could not be matched with molecular sequences in many cases. Therefore to ensure no duplication in species counts and resulting over-estimation of species numbers, we chose to disregard the Brachyura and Galatheoidae larval and megalopa species counts, but their abundances were counted (Table 4.1). This will likely result in conservative estimates of species richness.

4.3.3. Data Analysis

Species rarefaction curves, which plot the species richness as a function of the number of individuals sampled, were used to establish whether the sampling design reflected the 'true' species richness (Magurran and McGill 2011). Non-parametric species estimators Chao1 and Abundance-based Coverage Estimator (ACE) were calculated to estimate the total species richness of the community from those observed from a sample, enabling estimates to be compared across samples. They use a mark-release-recapture like ratio to estimate richness by adding a correction factor to the observed number of species (Magurran and McGill 2011). The Chao1 estimator is particularly useful for data sets skewed towards the low-abundance classes, as is likely to be the case for diverse communities such as decapods (Magurran and McGill 2011). The ACE incorporates data from all species with fewer than 10 individuals, rather than just singletons or doubletons (Magurran and McGill 2011). Mean species richness and abundance was calculated per coral colony because the number of coral colonies collected per atoll/island were uneven as a result of the limited expedition time. Generalised linear models (GLMs) were used to test for significance in the affect of atoll on the response variables mean species richness and mean abundance per coral colony. GLMs were appropriate as the dataset is count data and therefore a Poisson model needs to be fitted. All data were under or over-dispersed so Quasi-Poisson models were fitted to introduce a dispersion parameter and obtain a quasi-likelihood estimate (Crawley 2005).

A Venn diagram was used to visualise overlap in species occurrence between atolls. Non-metric multidimensional scaling (nMDS) based on Bray-Curtis similarity (Bray and Curtis 1957) was used to visualise the ordination of the decapod community structure. A similarity profile (SIMPROF) was used to test whether the similarities observed in the data are smaller and/or larger than those expected by chance in combination with a cluster analysis (Clarke and Somerfield 2008). Permutational MANOVA (PERMANOVA), a multivariate analysis of variance, was used to test for significance differences between decapod communities in response to atoll location. It uses permutations to make the data distribution free, allowing it to handle non-normally distributed data and more complex unbalanced sampling designs. All analysis was undertaken in R (R-Development-Core-Team 2008) using the vegan package (Oksanen et al. 2015), or in PRIMER v.6 (Clarke 1993).

4.4. Results

4.4.1. Total species richness and abundance

In total 1,868 decapods individuals were recorded inhabiting 54 coral heads from 25 sites across five atolls in the Chagos Archipelago (Table 4.1). Total species richness of the decapods was 164 species, and 32% of these species were rare (singletons) in the community. The Caridea were the most species rich and abundant component of the decapods and had the highest proportion of rare species (Table 4.1). At the family/superfamily level the Alpheidae were the most species rich at 51 species, and the Galatheidae were the most abundant at 343 individuals (Table 4.1). However, these species richness values are conservative as it was not possible to morphologically identify some of the Brachyura and Galatheidae larvae and megalopa forms, and some morphotypes failed to amplify when using molecular techniques (See Chapter 3). *Galathea aff. spinosorostris* and *Saron neglectus*, a galatheid and hippolytid species respectively, were the two most abundant species, at 115 and 103 individuals respectively across Chagos (Table 4.2). Only 38 species were represented by 10 or more individuals across the Archipelago (Appendix I & II).

The rarefaction curves were yet to plateau (Fig. 4.1) suggesting that the true species richness of the three infraorders and total decapod species richness is higher, and further sampling would be needed to capture the actual total species richness of this group. Only at Salomon and Egmont atolls do the rarefaction curves indicate that the Anomura species richness may be beginning to plateau (Fig. 4.1.b). The Chao1 and ACE estimators, which estimate total species richness whilst accounting for uneven sampling across the atolls, calculate the total decapod species richness for the Archipelago at 217±19.53 (Chao1) / 218.22 ±7.58 (ACE) species (Table 4.3). This suggests that our sampling effort has captured approximately 75% of the decapod species richness inhabiting dead branching corals in the Chagos Archipelago. Diego Garcia and Peros Banhos atolls are estimated to have the highest decapod species richness at 119.75 ±13.26 (Chao1)/ 130.92 ±6.15 (ACE) and 123.05 ±12.68 (Chao1)/ 130.69 ±5.90 (ACE) species respectively, and Eagle Island the lowest at 30.8 ±2.86 (Chao1)/33.47 ±2.29 (ACE) species (Fig. 4.2). Generally the two species estimator results were closely aligned, though Chao1 tended to produce larger variation around the mean, suggesting less certainty in the Chao1 estimations (Fig. 4.2 and table 4.3). Interestingly the species richness estimators suggest that there is little Anomura species richness still to be captured, with ACE estimating there are only approximately 8 more Anomura species to be discovered on this microhabitat (Table 4.3), despite the rarefaction curve not yet approaching a plateau. Instead most of the remaining species richness to be captured are Caridae species (Table 4.3).

Table 4.1 Biodiversity metrics for each decapod family/superfamily, with totals given for each infra-order and for decapods as a whole.

Infraorder	Superfamily/ Family	Species delimitation method	Species richness	Abundance	Singletons	% Singletons
Caridea	Alpheidae	GMYC	51	222	20	
Caridea	Palaemonoidea	Morphology	20	170	7	
Caridea	Hippolytidae	Morphology	8	262	2	
Caridea	Total		79	654	29	37%
Anomura	Galatheidae	Morphology	8	343	3	
Anomura	Galatheidae (larvae)	-	-	34	-	-
Anomura	Paguroidea	PTP	13	163	1	
Anomura	Porcellanidae	PTP	7	98	4	
Anomura	Total		28	638	8	29%

Brachyura	Dromioidea	Morphology	3	11	0	
Brachyura	Eriphioidea	Morphology	2	9	1	
Brachyura	Grapsoidea	Morphology	2	4	1	
Brachyura	Majoidea	Morphology	7	98	2	
Brachyura	Pilumnoidea	Morphology	7	63	1	
Brachyura	Trapezioidea	Morphology	8	144	2	
Brachyura	Xanthoidea	Morphology	28	151	8	
Brachyura	Megalopa	-	-	96	-	-
Brachyura	Total		57	576	15	26%
Decapoda	Total		164	1,868	52	32%

Table 4.1 Abundances of the ten most common decapod species. It should be noted that Porcellanidae sp.4 and Paguroidea sp.4 are putative species defined by molecular methods (see Chapter 3).

Species	Abundance
Galathea aff *spinosorostris*	115
Saron neglectus	103
Galathea platycheles	92
Thorina maldivensis	92
Trapezia juveniles	82
Porcellanidea sp.4	76
Galathea eulimene	65
Jocaste luncina	63
Paguroidae sp.4	52
Tylocarcinus styx	50

Table 4.2 Total species richness estimators Chao1 and ACE compared to the observed species richness from 54 sampled dead coral colonies, for each infra-order and for the decapods as a whole.

	Observed species richness	Chao1	Standard Error	ACE	Standard error
Anomura	28	31.5	±3.44	36.41	±3.21
Brachyura	57	72	±10.33	68.71	±3.91
Caridea	79	112.83	±16.87	112.61	±5.72
Decapods	164	217	±19.53	218.22	±7.58

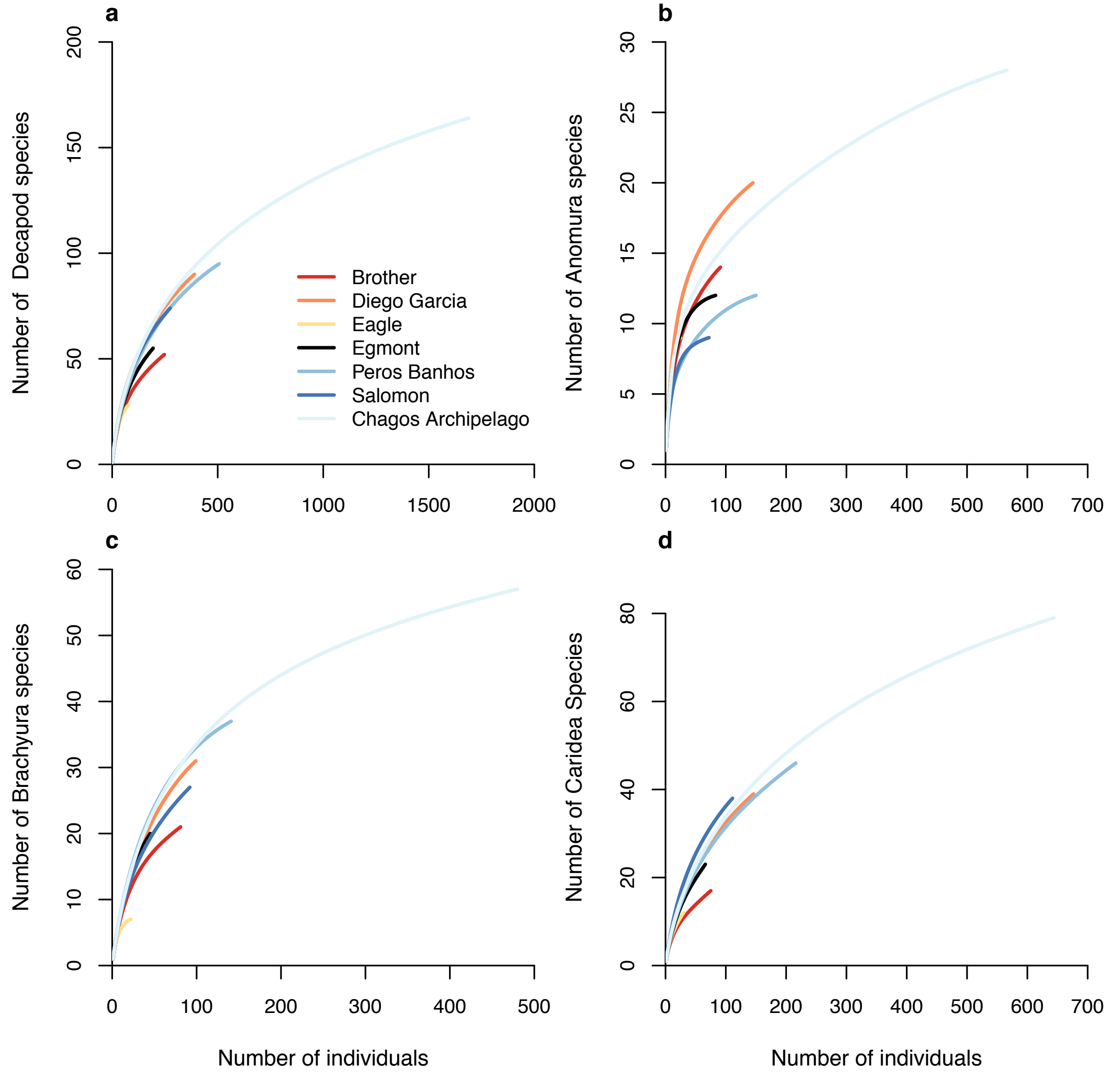


Figure 4.1 Rarefraction curves for **(a)** the decapods; and divided into the decapod's three infra-orders **(b)** Anomura, **(c)** Brachyura, **(d)** Caridea.

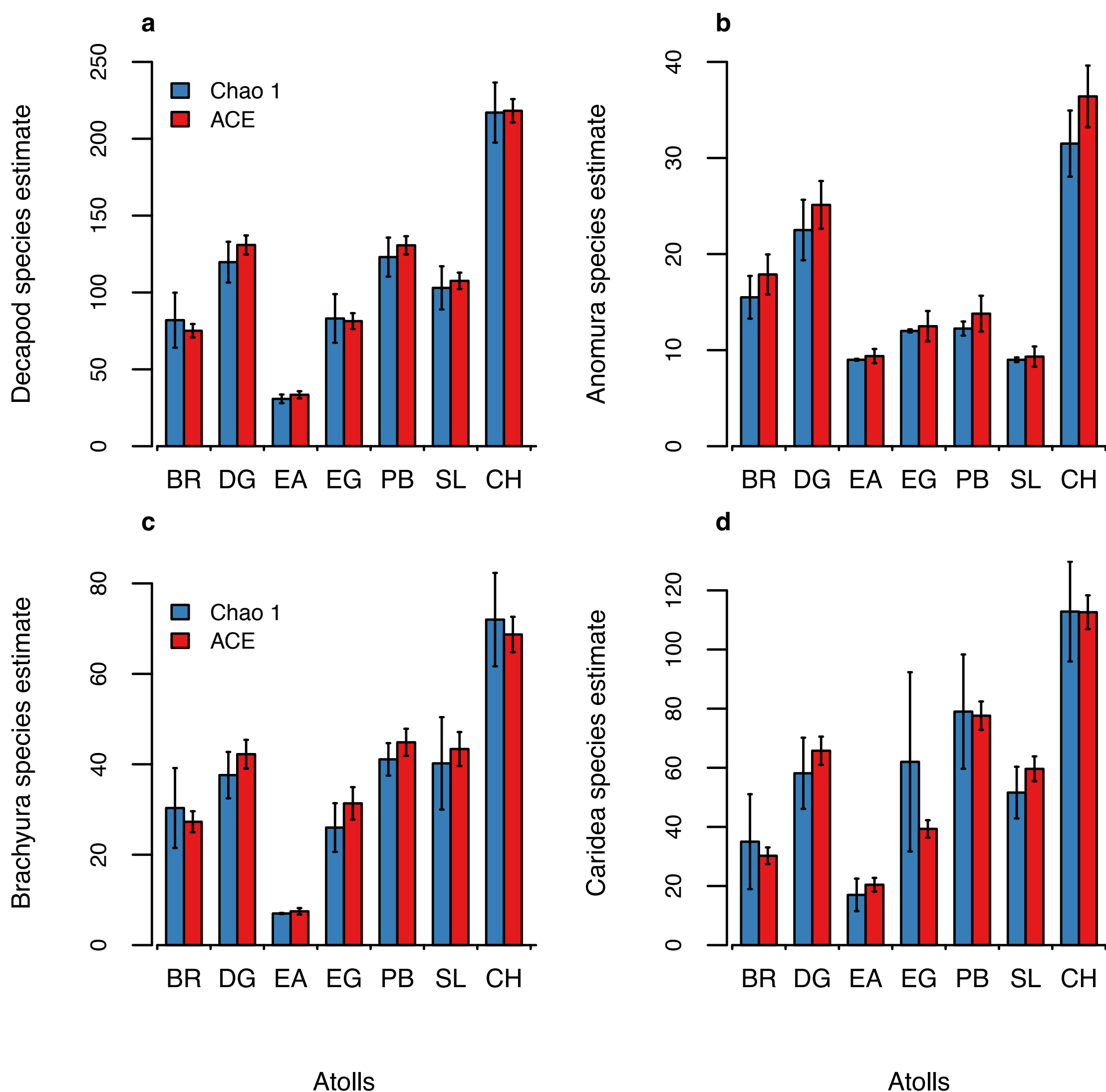


Figure 5.2 Bargraphs illustrating species richness estimates and associated standard error calculated from two estimators: Chao1 and ACE across each atoll/island and for the archipelago in total, for the **(a)** decapods, and divided into the decapod's three infra-orders; **(b)** Anomura, **(c)** Brachyura, and **(d)** Caridea. Atoll/island abbreviations: BR=Brothers, DG=Diego Garcia, EA=Eagle Islands, EG=Egmont, PB=Peros Banhos, SL=Salomon, and CH=Chagos Archipelago.

4.4.2. Species richness per coral colony

The mean decapod species richness per coral head was 15 ±1.14 species across the Chagos Archipelago. Egmont, Peros Banhos, Diego Garcia and Brothers had a higher than average mean decapod species richness per coral colony than the Archipelago, with Egmont having the highest mean species richness at 22.5 ±5.39 (Fig. 4.3.a). The effect of the atoll on species richness was significant ($f=2.53, p=0.04$) and this significance lies between Eagle Island ($t=-2.17, p=0.04$) and the other atolls and islands, which had a significantly lower species richness at 7.17 ±2.44 species (Fig. 4.3.a). All data were over-dispersed (Table 4.4) suggesting there is variation in species richness controlled by other factors, such as food availability and species interactions.

When the decapod species richness is divided into the three major infra-orders, the Anomuras comprise the lowest fraction of 3.7 ±0.31 mean species across the Archipelago, whilst Brachyura mean species richness was 4.86 ±0.53, and the Caridea mean species richness 6.38 ±0.64 (Fig.4.3). The effect of atoll on the Anomura mean species richness was significant (Table 4.4) and the pattern across atolls slightly different to that of the Brachyura and Caridea, with Eagle Island ($t=-2.44, p=0.37$) and Salomon atoll ($t=2.05, p=0.05$) having significantly lower species richness than the other atolls. Diego Garcia had the highest mean species richness alongside Egmont at 6 ±0.65 and 6 ±1.08 mean species respectively. There was no significant effect of atoll on the mean Brachyura or Caridea species richness, although the same general trends occurred of the lowest species richness at Eagle Island and the highest at Egmont atoll as with the decapods (Fig. 4.3.c and d).

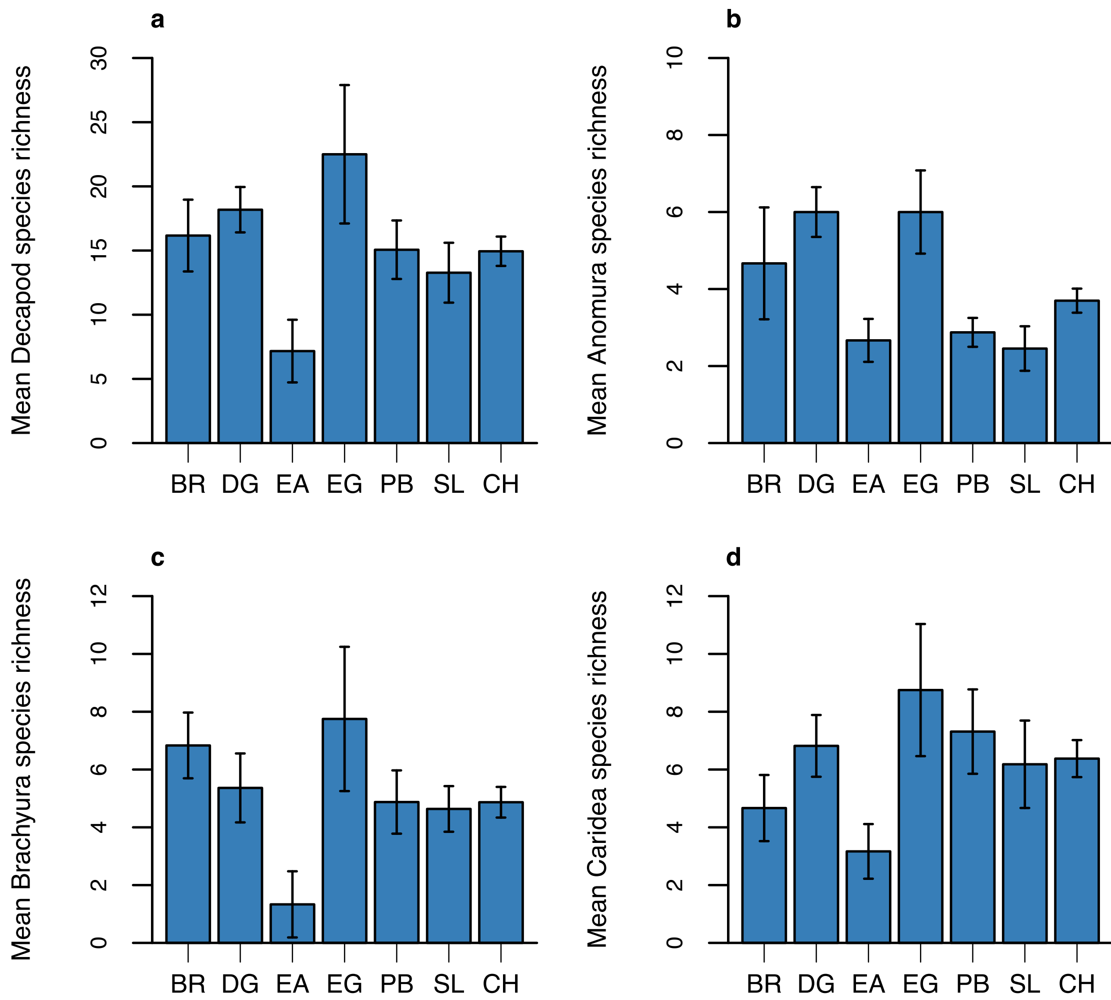


Figure 4.3 Bargraphs illustrating the mean species richness and standard error per coral colony for each atoll/island and for the archipelago as a whole for the **(a)** decapods, and divided into the decapod's three infra-orders; **(b)** Anomura, **(c)** Brachyura, and **(d)** Caridea. Atoll/island abbreviations: BR=Brothers, DG=Diego Garcia, EA=Eagle Islands, EG=Egmont, PB=Peros Banhos, SL=Salomon, and CH=Chagos Archipelago.

Table 4.4 The GLM results demonstrating the effect of atoll/island location on the mean species richness and abundance per coral colony. *df*=degrees of freedom, * indicates significant *p values.*

	Mean species richness per coral colony				Mean abundance per coral colony			
	p value	*f statistic*	*df*	*Dispersion parameter*	*p value*	*f statistic*	*df*	*Dispersion parameter*
Anomura	0.01*	3.32	48	1.12	0.01*	3.32	48	7.12
Brachyura	0.08	2.9	48	3.08	0.39	1.06	48	7.33
Caridea	0.28	1.29	48	3.19	0.44	0.99	48	9.62
Decapods	0.04*	2.53	48	4.2	0.085	2.07	48	15.95

4.4.3. Abundance per coral colony

The trends across the atolls in mean decapod species abundance per coral colony were similar to the trends in mean species richness, but unlike the species richness they were not significant (Table 4.4). The mean decapod abundance per coral colony across the Chagos Archipelago was 31.98 ±3.26. Egmont atoll had the highest mean at 48.5 ±13.67 species, and Eagle had the lowest mean at 12.8 ±5.95 species (Fig. 4.4.a). The data were very over-dispersed (Table 4.3), suggesting high variability in species abundances influenced by other factors. A lack of significance in the trends across the atolls may be partly a result of relatively large variation around the mean abundances, particularly at Egmont atoll and Brothers Islands (see error bars on fig. 4.4.a).

There was a correlation in the abundance of decapods and all three infra-orders across the atolls and islands (Fig. 4.4) with the mean species richness values (Fig. 4.3), with the exception of the mean decapod abundance at Brothers Island which exceeded that of Diego Garcia, whereas the reverse was true of its mean species richness. The effect of atoll on the

Anomura abundance was also significant (Table 4.4), but this was not the case for the Brachyura and Caridae. The Anomura mean abundance was significantly lower at both Eagle Island (*t=-2.14, p=0.04*) and Salomon atoll (*t=-2.05, p=0.05*) than at all other atolls.

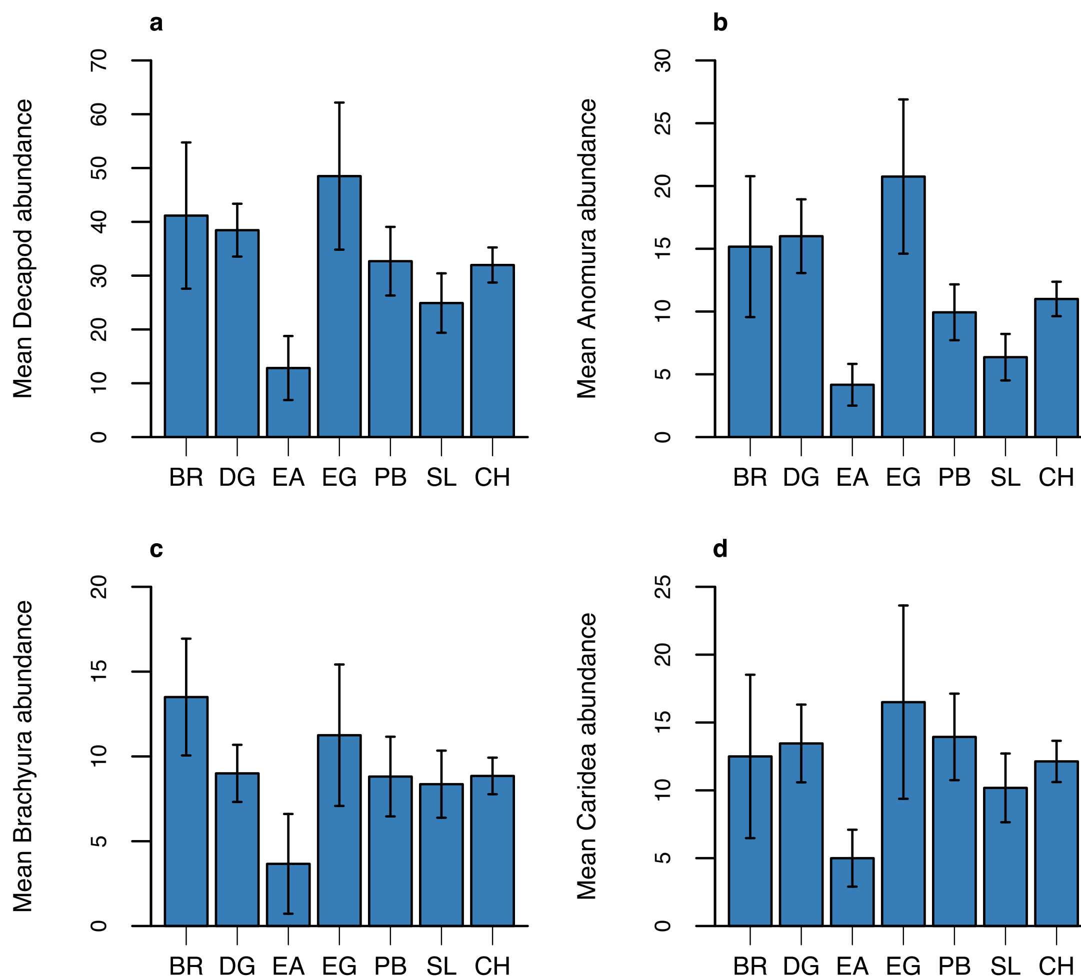


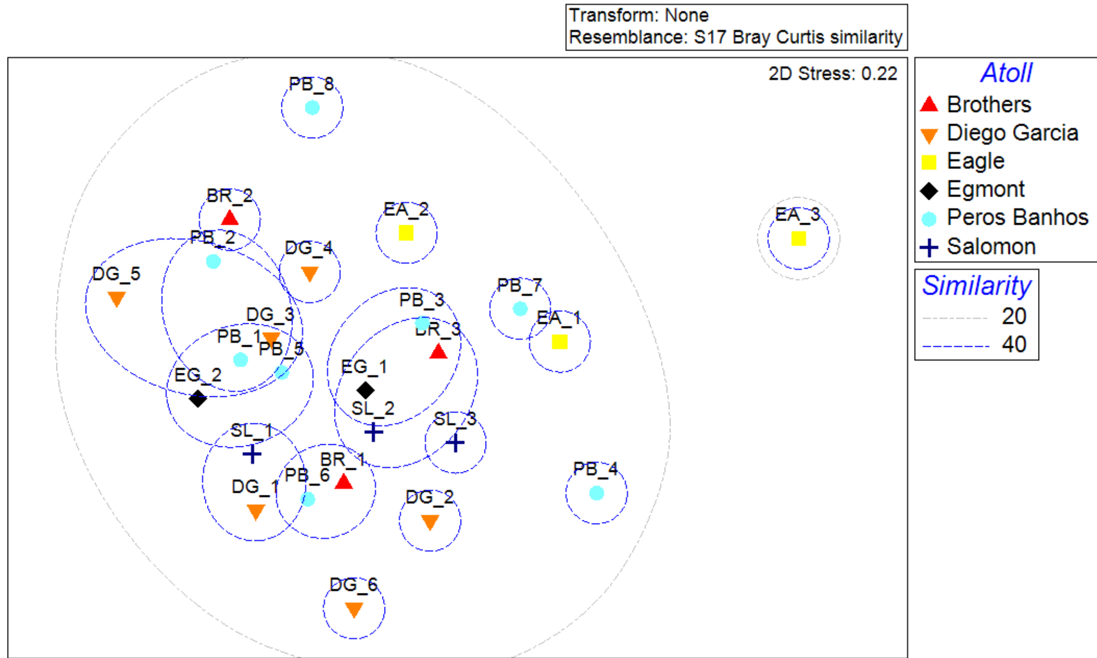
Figure 4.4 Bargraphs illustrating the mean species abundance and standard error per coral colony for each atoll/island and for the archipelago as a whole, for the **(a)** decapods, and divided into the decapod's three infra-orders; **(b)** Anomura, **(c)** Brachyura, and **(d)** Caridea. Atoll/island abbreviations: BR=Brothers, DG=Diego Garcia, EA=Eagle Islands, EG=Egmont, PB=Peros Banhos, SL=Salomon, and CH=Chagos Archipelago.

4.4.4. Community structure

PERMANOVA test showed no significant effect of atoll on decapod community structure, nor on any of the three infraorders that comprise the decapods (Table 4.5). Eagle Island and Brothers Island are geographically at least 20 km apart but are also both part of a large atoll, called the Great Chagos Bank (GCB), so the PERMANOVA test was run twice to consider these islands together as the GCB and separately as islands. Separately the effect of atoll accounted for 23% of the variation in decapod community structure, and when pooled together the affect of atoll explained 17%, giving validation to the separation of these islands in the analysis (See R^2 in Table 4.5). The nMDS plot (Fig. 4.5.a) illustrates this lack of significant structure in the community between atolls, and demonstrates that only one site on the southern tip of the Eagle island was significantly dissimilar in its community structure (*sample stat=0.007, p=0.05*) at a 20% similarity level. The nMDS plot also shows some clustering of sites at a 40% similarity level, however, only the 20% similarity level is supported by the SIM prof test, as demonstrated by the cluster plot (Fig. 4.5.b). Despite the lack of community structure between atolls, the Venn diagram (Fig. 4.6) shows that only 24 of 164 species were shared between all atolls, and each atoll had many unique species, for instance Diego Garcia had the highest number of unique species at 25 species. The ranking of unique species per atoll in the Venn diagram reflects the ranking of species richness per atoll (Fig. 4.2), suggesting that rare species are perhaps driving these differences in species richness between atolls. So the nMDS was repeated with transformed data ($^2\surd$ transformed) to account for the low abundance of some species but the lack of community structure remained the same. The Bray-Curtis dissimilarity indices (Table 4.6) demonstrate that Peros Banhos and Eagle Island are the least similar in community structure and Peros Banhos and Diego Garcia are the most similar.

Table 4.5 PERMANOVA statistics evaluating the significant difference in community structure with atoll/island location. R^2 shows the proportion of variance explained by atoll/island. GCB is an abbreviation for the Great Chagos Bank.

	p value	*Pseudo-f statistic*	R^2
Anomura	0.09	1.32	0.26
Brachyura	0.08	1.26	0.25
Caridea	0.38	1.03	0.22
Decapods	0.11	1.15	0.23
Decapods (Brothers & Eagle Islands combined as GCB)	0.37	1.04	0.17



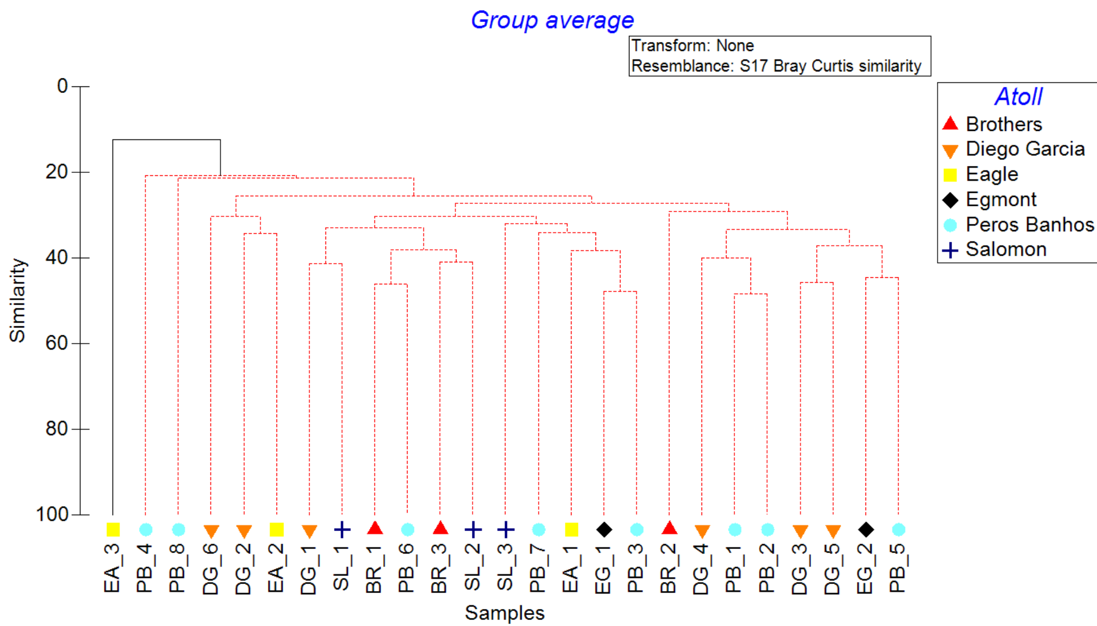


Figure 4.5 (a) nMDS plot using Bray Curtis similarity illustrates the lack of community structure between the atolls/islands at a 20% similarity level. **(b)** The cluster diagram illustrates that only one site at Eagle Island was significantly dissimilar at a 20% similarity level supported by a SIM prof test.

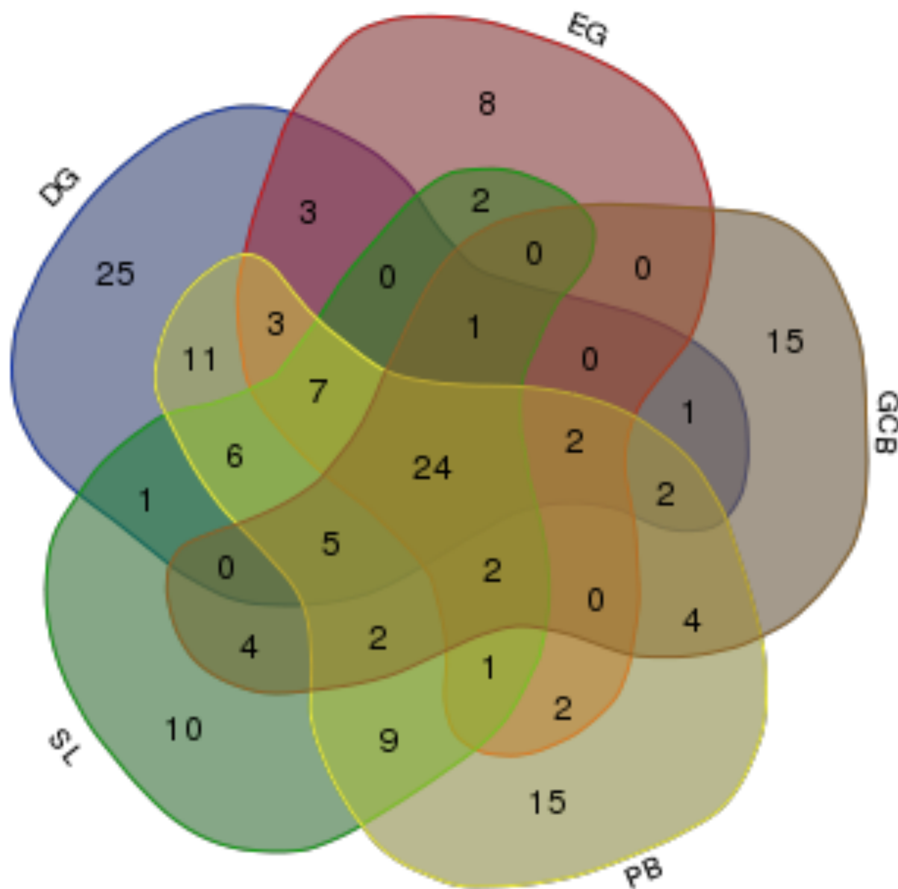


Figure 4.6 Venn diagram illustrating the species overlap between atolls/islands in Chagos. Atoll abbreviations: DG=Diego Garcia, EG=Egmont, PB=Peros Banhos, SL=Salomon, and GCB=Great Chagos Bank (Eagle and Brothers Islands combined into the wider GCB).

Table 4.6 Bray-Curtis Dissimilarity indices for each atoll/island across the Chagos Archipelago

	Brothers Islands	Diego Garcia	Eagle Island	Egmont	Peros Banhos
Diego Garcia	0.60				
Eagle Island	0.65	0.73			
Egmont	0.61	0.46	0.6		
Peros Banhos	0.54	0.41	0.77	0.59	0.44
Salomon	0.52	0.49	0.69	0.56	0.53

4.5. Discussion

The observed decapod species richness for the Chagos Archipelago was 164 species, with an abundance of 1,868 individuals from 54 dead coral colonies collected from six atolls and islands across the Archipelago. However, rarefaction curves did not plateau suggesting that this sample size is still too low, and species estimators calculated the total decapod species richness to be at least 217 species (Chao1). A high proportion, 32% of observed species richness (52 species), were singletons, and only 23% of species (40 species) were represented by ten or more individuals across the Archipelago. Whilst only 15% of species (24 species) were found across all atolls, with 38% of species (73 species) appearing to be unique to one atoll, Diego Garcia having the highest number of species, at 25 species, found at no other atoll, perhaps suggesting high levels of endemism. Although this could also be a consequence, at least in part, of incomplete sampling. High levels of seemingly rare species are a common pattern in reef cryptofaunal samples, such as molluscs (Bouchet et al. 2002) and isopods (Kensley 1998), implying that much of the reef cryptofauna could be comprised of low-abundance species. For instance, a study of crustacean communities on dead coral colonies in Moorea and the Northern Line Islands, in the Pacific Ocean, found 44% to be singletons and a further 33% represented by several specimens found only at one locality, but rarefaction curves also showed sampling to be insufficient (Plaisance et al. 2009). For Chagos the mean decapod species richness per coral colony was approximately 15 species and mean decapod species abundance per coral colony was approximately 32 species. The only significant difference across the atolls and islands was the low species richness at Eagle Island at approximately 7 decapod species per coral colony, this island also had the lowest mean abundance per coral colony and the lowest total species estimate.

4.5.1. Rare Species

If the observed high proportion of rare species in this decapod community are truly rare, rather than purely an artefact of incomplete sampling, then this raises the question of the role of rare species in ecosystem function. Rare species are generally more vulnerable to being lost, but the ecological consequences are frequently overlooked (Lyons et al. 2005; Mouillot et al. 2013). Until recently it was often assumed that dominant species likely share common sets of traits with rare species and this thus insures against the loss of ecosystem function supported by rare species (Loreau et al. 2001; Hooper et al. 2005). However contrary to this, Mouillot et al. (2013) recently demonstrated that in three diverse ecosystems rare species (of reef fishes, alpine plants, and tropical trees) supported the most distinct combination of traits, and moreover species that have low functional redundancy and are likely to support the most vulnerable functions are rarer than expected by chance. This emphasises the importance of rare species to ecosystem function (Zavaleta and Hulvey 2004; Bracken and Low 2012). Recent studies have also shown non-saturating patterns between biodiversity and functioning in marine ecosystems, suggesting that loss of species may have a substantially larger effect on the functioning of ecosystems than anticipated (Danovaro et al. 2008; Loreau 2008; Mora et al. 2011a; Mora et al. 2014), and if a high proportion of these species are rare there is a greater risk of biodiversity loss. This positive relationship between biodiversity and functioning is likely a result of interspecific facilitation and complementarity (Cardinale et al. 2002; Hooper et al. 2005; Danovaro et al. 2008)

4.5.2. Comparisons with other crustacean studies

Very few studies have investigated the cryptofauna biodiversity of dead coral microhabitats (but see Coles 1980; Preston and Doherty 1990; Plaisance et al. 2009; Enochs 2011), but of those that have this is the highest decapod species richness estimate to date for any location globally. Plaisance et al. (2009) found total Crustacea species richness estimates of 90 Operational Taxonomic Units (OTUs) for Moorea and 150 OTUs for the Northern Line Islands, both remote atolls in the Pacific. Off the coast of Panama, total estimated cryptofauna

species richness was 261-370 OTUs (Enochs and Manzello 2012a), however, the arthropods only accounted for approximately 27% of the observed richness, putting a maximum arthropod species richness estimate at approximately 100 OTUs. On Hawaiian reefs, Coles (1980) reported 115 observed decapod species on 18 dead corals, however sizes of the corals varied and no total species estimates are available so direct comparisons cannot be made. Preston and Doherty (1990) sampled 1080 corals from the Great Barrier Reef (GBR) and yielded 28 species of agile shrimp (families: Hippolytidae, Pandalidae, Palaemonoidae and Processidae) from 25,324 individuals. In Chagos we had a much smaller sample size but also found 28 species from just the Hippolytidae (20 species) and Palaemonoidae (8 species) (none from Pandalidae and Processidae), and there was an overlap of at least 4 species with the GBR study. This is surprising as we would except a higher species richness on the GBR in comparison to the Chagos Archipelago, because the GBR is much closer to the Coral Triangle, the epicentre of coral reef biodiversity (Bellwood et al. 2012).

Decapod community structure also varies between these studies. In Chagos, galatheids were the most dominant, and alpheids and hippolytids were also very abundant (Table 4.1), with the four most dominant species belonging to the galatheids and hippolytids (Table 4.2). The high abundance of palaemonoids and *Trapezia* crabs was unexpected as most of these species are considered obligate live coral dwellers and populations have not been reported on dead coral colonies elsewhere as far as we are aware (Discussed in Chapter 2/Head et al. 2015). In comparison decapod communities on dead corals in Hawaii were dominated by xanthids, pagurids and alpheids (Coles 1980). Whilst in Moorea and Northern Line Islands Brachyura dominated the communities (Plaisance et al. 2009), the Chagos community was comparatively more even across the infra-orders but the Caridea were the most abundant over all.

4.5.3. Factors affecting cryptofauna diversity

Eagle Island had significantly lower mean species richness per coral colony then the other atolls and islands, and the island's mean abundance per coral colony and total estimated richness was also the lowest across the Archipelago. In this study, one site on the southern tip of Eagle Island also stands out in its community structure, because of the particularly low decapod richness and abundance on coral colonies at this site compared to all others. At the time of surveying the reefs around Eagle Island were suffering from a crown-of-thorns (COTs), *Acanthaster planci,* outbreak (Appendix III/ Roche et al. 2015). Whilst only coral colonies that had been dead for months, if not years, were sampled (see sampling design) and therefore their mortality would not have been as a result of the current COTs outbreak, it is possible that such outbreaks have indirect affects on the local ecosystem potentially resulting in this low decapod diversity. There are reports of reduced diversity of live coral associates following COTs outbreaks (Leray et al. 2012), but the affect of COTs outbreaks on other cryptofauna communities is unknown.

Egmont consistently had the highest species richness and abundance per coral colony across the infra-orders, except in Brachyura abundance, though none were significant. Whilst Peros Banhos and Diego Garcia had the highest total decapod species richness estimates of at least 119.75 ±13.26 and 123.05 ±12.68 (Chao1) species respectively. Peros Banhos and Diego Garcia are the largest atolls (not including the Great Chagos Bank) and therefore there may be a higher habitat availability and diversity of niches across these atolls promoting diversity. Diego Garcia is also geographically the most isolated atoll within the archipelago (Fig. 1.2), which could result in higher levels of endemism. This theory is supported by Diego Garcia also having the highest number of species (25 species) unique to a particular atoll (Fig. 4.6). On live coral microhabitats, neither surrounding live coral cover, nor coral diversity, has been shown to correlate with cryptofauna diversity but reef structural complexity was significantly positively correlated (Idjadi and Edmunds 2006). Reef structural complexity in Peros Banhos is the highest of all Chagos atolls, and it is significantly greater than on Diego Garcia reefs, whilst structural complexity in Diego Garcia is lower than all other atolls (Graham et al.

2013). So this could account for Peros Banhos' high species richness but not Diego Garcia's. Structural complexity potentially needs to be investigated at a more local level surrounding the coral colonies sampled.

Our knowledge of the factors affecting cryptofauna diversity on any microhabitat are very limited, but some studies have been undertaken (e.g. Idjadi and Edmunds 2006). Enochs et al (2011) found that low-porosity (gaps in rubble structure) and slow-flow environments supported a higher abundance and biomass of motile cryptofauna on dead coral and coral rubble microhabitats. The size of the coral colonies, their structural complexity and surface area have also been demonstrated to be positively correlated with the abundance and species richness of decapod communities on both live and dead coral colonies, with more complex corals thought to provide better refuge from predators and better niche separation (Abele and Patton 1976; Coles 1980; Vytopil and Willis 2001; Leray et al. 2012). In this study the size of the coral colony was controlled to a certain extent by selecting colonies of approximately 20 cm in diameter, however, even small variations in coral colony size can affect cryptofauna abundances (Head et al. 2015/Chapter 2), so this may have accounted for some variation in decapod abundances. It is also likely that decapod diversity is affected by the abundance and composition of the wider cryptofauna community on the dead coral colonies, e.g. molluscs, through predation, competition and other interspecific interactions. With crustaceans found in the diet of more than 50% of reef fish, predation by invertivore fish species will also likely impact cryptofauna abundance. Quantitative dietary information, though essential to understanding reef trophic dynamics, is only just emerging for invertivore fish. Most notably a recent study by Kramer et al (2015) found that wrasse (Labridae), a speciose and abundant reef fish family, over >90mm in length had a predominantly 'macro-crustacean' (i.e. Brachyura, Anomura, Caridea, Stomatopoda) diet consuming mostly Brachyura (40%), with *Gomphosus* and *Novaculichthys* being the greatest consumers of macro-Crustacea.

4.5.4. Comparisons with other reef fauna

Molluscs made up a large proportion of the remaining cryptofauna inhabiting the dead coral colonies in Chagos. The molluscs numbered 976 individuals, most of which were gastropods (820 individuals) with a species richness of 72 observed species (Marjot 2013) compared to the 164 observed species of decapods and 1,868 individuals. Therefore decapods comprised more than double the species richness and abundance across the Archipelago than the gastropods. Panama's reefs demonstrated an opposing trend, with molluscs having a higher species richness than arthropods, at 132 to 77 OTUs respectively (Enochs and Manzello 2012a).

The estimated fish species richness in Chagos is at least 784 species (Graham et al. 2013). This compares to at least 217 estimated Decapod species from just one microhabitat, but the total decapod species richness across all microhabitats is likely much higher (Kramer et al. 2014). If decapod mean abundance per coral colony (20 cm diameter) of 32 individuals is scaled up to an estimate per m^2, and mean fish abundance per $500m^2$ (774 individuals per $500m^2$; Graham et al. 2013) is scaled down to per m^2, then a comparison can be made between mean decapod abundance and mean fish abundance (160 and 1.5 individuals per m^2, respectively) in Chagos, demonstrating that the abundance of decapods on dead coral colonies is approximately 2 orders of magnitude greater than that of fishes. This difference in abundance is less than that estimated at Lizard Island, GBR, where Crustacea, pooled from all microhabitats, were found to be 4 orders of magnitude greater than that of fishes (Kramer et al. 2014). However, this measured all crustaceans and perhaps more importantly it included smaller size-classes of organisms than our study (we included organisms >1mm), and was therefore dominated by small crustacean taxa such as harpacticoid copepods, substantially increasing the abundance estimates, which likely explains the greater difference in crustacean and fish abundance estimates compared to Chagos. In addition, Kramer et al (2014) found the biomass of fishes to exceed the biomass of cryptofaunal Crustacea by only one order of

magnitude, likely a result of the high abundance of these small organisms, and the study did not include very large decapods such as lobsters, which would further decrease the biomass difference between fishes and Crustacea. Decapod biomass was not measured in our study but it would be interesting to see if the difference in biomass might be larger in Chagos because of the high fish biomass recorded here, which exceeds all other locations in the Indian Ocean by an order of magnitude and is comparable only to remote islands in the Pacific Ocean (Sandin et al. 2008; Graham et al. 2013).

Coral reefs worldwide are under immense anthropogenic pressures, altering reef biodiversity and structure, and often creating more depauperate ecosystems (Hughes et al. 2010; Burke et al. 2011). The effects of anthropogenic stressors on the reef fish and coral fauna components are relatively well known (e.g. Mora et al. 2011b; McClanahan et al. 2014), especially in comparison to the effects on the cryptofaunal component. The Chagos reef ecosystem is one of the most resilient reefs globally, and one of the most removed from direct human impacts, representing a reference site for biodiversity (Burke et al. 2011; Sheppard et al. 2012). Here we have shown higher levels of decapod diversity, on one microhabitat in Chagos, than reported anywhere else to date. This biodiversity assessment can now be used as a baseline against which to compare this component of biodiversity in other areas experiencing higher levels of anthropogenic stressors at least in the Indian Ocean. This study also highlights the prominence of dead coral colonies as microhabitats for decapod diversity and the importance of corals in supporting diverse invertebrate fauna even after their death.

5. CHAPTER FIVE:

Examining functional trait and phylogenetic diversity to understand the processes driving the community structure of a family (Palaemonidae) of the cryptofauna in the Chagos Archipelago

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5.1. Abstract

The processes influencing community assembly have long fascinated ecologists and are essential to understanding how ecosystems function. Two important deterministic mechanisms in shaping community structure are environmental filtering and limiting similarity. Here, we use trait and phylogenetic diversity to examine which of these mechanisms are the key processes driving the assembly and maintenance of a marine cryptofauna community, the palaemonids, inhabiting dead coral colonies on coral reefs across the Chagos Archipelago. We also investigate the role of the phylogeny in structuring the community. Palaemonidae is a family of Caridae shrimp, a speciose group of decapods, which often have specific host-associations with other marine organisms, such as corals and echinoderms. Like the majority of the cryptofauna, defined as the small and cryptic organisms living within the reef framework, the palaemonids are relatively understudied. To investigate the key processes affecting species composition and distribution within this family, we measured the fundamental life-history traits of body size and fecundity, alongside host association. We found spatial hierarchy in trait and phylogenetic diversity, with environmental filtering and the phylogeny acting at the local level, within site and between coral colonies. At the metacommunity level, across the whole archipelago, the presence of phylogenetic signal was inconclusive and requires further investigation. At the local level (between coral colonies, and within atolls) the predominant environment filter was the size of the habitable space within the branches of the coral colony acting on the body size of the palaemonids. The weak phylogenetic signal suggests trait convergence and lability of trait evolution were also key processes determining species distribution.

5.2. Introduction

Many processes are involved in determining which species co-exist and assemble into communities. The niche-based model of community assembly recognises environmental filtering and limiting similarity as two important deterministic mechanisms responsible for shaping and maintaining communities (Webb et al. 2002). Environmental filtering is the process by which abiotic conditions favour species with certain adaptive traits necessary for survival in that environment (Webb et al. 2002). Limiting similarity refers to biotic interactions such as competition, mutualism, and facilitation, which tend to limit niche overlap and similar species coexisting leading to competitive exclusion (MacArthur and Levins 1967). These processes act through density-dependent mechanisms (limiting similarity) and density-independent mechanisms (filtering) (Chesson 2000; Clark 2009; Chase and Leibold 2003). According to 'the neutral model' communities are primarily shaped by stochastic processes including; population dynamics unrelated to fitness differences between species, and random speciation and extinction (Hubbell 2001). However, the neutral model is suggested to be 'a special case', that only occurs when density-dependent mechanisms are absent and species are equally fit (Adler et al. 2007; Clark 2009). This is supported by several studies of trait and phylogenetic diversity that indicate that communities are structured by ecological processes such as competition and environmental filtering. Many of these studies are based on plant assemblages (e.g. Mayfield et al. 2005; Cavender-Bares et al. 2006; Kraft and Ackerly 2010) but also extend across a variety of taxa and ecosystems including fish (Ingram and Shurin 2009; Pavoine et al. 2009), amphipods (Best et al. 2013), birds (Lovette and Hochachka 2006), bats (Stevens et al. 2003) and butterflies (Pavoine et al. 2014). In one example of taxa and ecosystems, Helmus et al (2007) studied 11 sunfish species (Centrarchidae) across 890 lakes and found that both environmental filtering, through water quality and latitude, and competition were likely to occur simultaneously.

Patterns in trait and phylogenetic diversity can be used to identify which ecological and evolutionary processes are at work in shaping communities (Webb et al. 2002; Pavoine and Bonsall 2010). For instance, clustering of both trait diversity and phylogenetic diversity suggest that environmental filtering is the driving mechanism behind community assembly, and that the trait has phylogenetic signal (Pavoine et al. 2010) (Table 5.1). Incorporating phylogenetic information can demonstrate how evolutionary history has shaped ecological processes and helps untangle the mechanisms behind community assembly (Webb et al. 2002; Pavoine and Bonsall 2010). However it is necessary to understand how traits evolve and change in order to interpret phylogenetic over-dispersion versus clustering patterns as these patterns can occur through different mechanisms (Pavoine and Bonsall 2010). Most notably phylogenetic over-dispersion within a community can be a result of competition associated with traits that are conserved through evolutionary time, or environmental filtering processes associated with traits that have converged through evolution (Cavender-Bares et al. 2004; Losos 2008; Kraft and Ackerly 2010). However taking phylogenetic and trait diversity together can distinguish between these mechanisms, for instance communities that display high phylogenetic diversity, and high trait diversity suggests that competition on phylogenetically conserved traits is the main driver, whereas high phylogenetic diversity but low trait diversity is evidence for environmental filtering on convergent traits (Mayfield and Levine 2010; Pavoine and Bonsall 2010).

The influence of environmental filtering versus limiting similarity depends on the habitat, spatial (Swenson and Enquist 2009; Kraft and Ackerly 2010), temporal (Pavoine et al. 2011) and taxonomic or phylogenetic scales (Cavender-Bares et al. 2006), therefore interpretation of trait and phylogenetic analyses must consider scale. Spatially at the largest continental scale phylogenetic and/or trait clustering reflects biogeographic rather than ecological processes (Webb et al. 2002). A regional scale, or metacommunity (set of local communities linked by dispersal, γ) can be divided into a local (α) diversity component and a component associated

with the difference between local communities (β diversity) (Veech et al. 2002; Swenson and Enquist 2009; Pavoine and Bonsall 2010). Processes at a regional or metacommunity level can drive local communities and the size of the regional species pool will also affect the composition of the local community (Ricklefs 1987). Any process that causes local speciation, extinction or character displacement may influence the regional species pool (Ricklefs 1987).

In this study we investigate the processes that drive the palaemonid shrimp (Caridea: Palaemonidae) metacommunity structure, on dead branching coral hosts, across the Chagos Archipelago, a coral reef system of approximately 200 x 300 km (Sheppard et al. 2012) in the central Indian Ocean. Palaemonidae is a family of Caridae shrimp, a speciose group of decapods, with 981 recognised species across 143 genera (De Grave and Fransen 2011; De Grave et al. 2015). They inhabit all oceans accept the Arctic and Antarctic regions, and their central of biodiversity is on coral reefs in the Indo-Pacific (De Grave 2001). The majority of species inhabit shallow water habitats, e.g. coral reefs, sea grass, kelp forests, but a few species are known to inhabit the deep sea, e.g. *Periclimenes boucheti*. An interesting characteristic of many members of the family is the symbiotic associations they form with a range of hosts including molluscs, echinoderms, hard corals (Scleractinia), tunicates (ascidians), anemones (Actiniaria) and sponges (Porifera) (Bruce 1977). These associations are normally described as commensal, but it has recently been suggested that some maybe better described as parasites (Duris et al. 2011). It is estimated about 60 -70% form symbiotic associations with a host, though this is likely an underestimate as many are not well known, and those that do not form associations are considered free-living (De Grave 2001) e.g. *Palaemonella rotumana*. Free-living palaemonids have the general palaemonid body structure including well-developed dentate rostrum and long slender chelae and pereiopods (Bauer 2004). Palaemonid species with symbiotic associations with a host have evolved morphological adaptations in body shape, rostrum, mouthparts, eye-design and ambulatory legs (Bauer 2004; Kou et al. 2013; Dobson et al. 2014; Kou et al. 2014). For instance,

Coralliocaris and *Jocaste* spp., which are considered live obligate coral-dwellers (but see Head et al. 2015), have modified walking legs with a special appendage, called a dactyl, to improve their grip on their coral hosts (Bruce 1977; Patton 1994).

Here we explore the role of environmental filtering and limiting similarity on the palaemonid metacommunity on dead coral colonies in the Chagos Archipelago, within the context of community assembly theory, by using select functional traits and phylogenetic information combined with select environmental variables and spatial co-ordinates. The samples comprised of 207 individuals from 20 palaemonid species inhabiting 65 dead coral colonies across 28 sites surveyed across six atolls and islands within the archipelago. Our main hypothesis is that either environmental filtering or limiting similarity is driving community structure. If environmental filtering is acting then we ask; what factors are filtering which combinations of trait states, and which lineages are affected by these filters? Clustering of trait values within the population would indicate environmental filtering, conversely if the traits show an over-dispersed pattern then limiting similarity is at work (Table 5.1). The phylogenetic pattern may also be clustered, over-dispersed, or randomly distributed, depending on the evolutionary conserved or convergent nature of the traits (Table 5.1). This relates to our second hypothesis; that traits have phylogenetic signal i.e. closely related species have more similar trait states than distantly related species. We then investigate how this compares across distinct spatial scales from the site level, atoll scale, to the whole archipelago. We also consider how species abundance affects these phylogenetic and trait diversity patterns. To test the primary hypothesis that limiting similarity or environmental filtering structure the palaemonid communities we made use of a novel extended version of traditional RLQ analysis which allows us to link species occurrences with geographical space, environmental variables, species traits, and phylogeny to investigate complex assembly rules (Doledec et al. 1996; Pavoine et al. 2011). To investigate the secondary hypothesis we use phylogenetic signal statistics (Ives et al. 2007), and for the two remaining hypotheses we utilise the quadratic entropy index (QE) to understand phylogenetic and trait diversity

(Pavoine et al. 2010). Together these analyses allow us to combine several indices of biodiversity: species presences/absences, species abundance, trait diversity, phylogenetic diversity and correlation between traits and phylogeny to begin to understand the processes underpinning Palaemonidae community structure.

Table 5.1 Possible explanatory mechanisms for each phylogenetic and trait pattern combination (adapted from Pavoine et al. 2010). EF=Environmental filtering, LS=Limiting similarity.

Phylogenetic pattern	Trait pattern		
	Clustering	Overdispersion	Randomness
Clustering	EF; phylogenetic signal; low α trait diversity	LS; convergence; high α trait diversity	Critical conserved traits involved in EF have been omitted; α random diversity
Overdispersion	EF; convergence; low α trait diversity	LS; phylogenetic signal; high α trait diversity	Critical conserved traits involved in LS have been omitted; random α trait diversity
Randomness	EF; labile traits; low α trait diversity	LS; labile traits; high α trait diversity	Neutral processes; balance between EF and LS

5.3. Methods

5.3.1. Sampling design

Collections of dead branching corals were undertaken from the Chagos Archipelago in 2012 and 2013 to quantify the diversity of the cryptofauna, that is the small often hidden organisms that live within the reef structure itself including palaemonid species, inhabiting this one sub-habitat (Head et al. 2015/Chapter 2). 65 dead branching coral colonies of approximately 20 cm in diameter were collected from 28 sites across the Chagos Archipelago at Diego Garcia Atoll, Peros Banhos Atoll, Salomon Atoll, Eagle and Brothers Islands of the Great Chagos Bank, and Egmont Atoll (Fig. 1.2). Sites were separated by at least 250 m and located on the outer reef. Coral colonies were defined as being dead if they had no observable polyps, evidence of turf and crustose coralline algae, and sometimes erosion. Between two to four dead *Acropora* or *Pocillopora* coral colonies of approximately 20cm in diameter were identified at each site at approximately 8 -10m depth. A water-tight polythene bag (250 Micro gauge) was secured around the coral to contain all inhabiting organisms, before the coral was chiselled off the reef. The coral was then completely enclosed in the bag for transport and securely cable tied and bought to the surface, where it was soaked in a bucket of freshwater for no longer than 2 minutes to remove inhabiting motile fauna (Following the protocol of Stella et al. 2010). Both the seawater from the plastic bag and the freshwater were then filtered through a 1mm sieve and all fauna caught in the sieve were sorted, photographed, catalogued and preserved in 95% ethanol. Finally the coral colony was inspected and carefully broken up, using a hammer and chisel to collect any remaining hidden fauna. All Caridea specimens were identified to genera, and palaemonid shrimp were identified to species, and rare species were catalogued in the Oxford University Museum of Natural History collections.

5.3.2.Phylogeny

The pleopods, eggs or abdominal tissue were used for DNA extraction using Qiagen's DNeasy Blood and Tissue kits resulting in 100μl elutions. Four genes were targeted in accordance with a previous Palaemonidae phylogenetic study (Kou et al 2013); partial

fragments of the 16S ribosomal RNA (rRNA) gene (~368bp), and partial fragments of three nuclear genes enolase (~405bp), PEPCK (~521bp), and NaK (~620bp). Polymerase chain reaction (PCR) amplifications were carried out in 18ml volume reactions using; 12µl Master Mix HotStarTaq (Qiagen), 3µl DNA template, 0.6µl RNase-free water (Qiagen), and 2.4µl (4µM) primer mix (2µl forward, 2µl reverse, and 96µl RNase-free water). For the 16S gene and enolase genes the forward and reverse primers 16S-l2/1472 (Schubart et al. 2002) and EA2/ES2 (Tsang et al. 2011) amplified the respective gene consistently. However amplification of PEPCK and NaK genes required testing of different primer set combinations for each specimen. The successful forward and reverse primer combinations were either For2/Rev or For/Rev3 for PEPCK and For-b/Rev or For-b/Rev2 for NaK (Tsang et al. 2008; Kou et al. 2013). PCR thermal cycle conditions for the enolase, NaK and PEPCK genes were; initial denaturation of 15min at 94°C, followed by 40 cycles of denaturisation at 94°C for 30s, annealing at 55°C/56.5^{o}C/56°C accordingly for 40s, extension at 72°C for 1 min, and a final extension at 72°C for 10mins. Thermal cycle parameters for the 16S was slightly different after the initial 15 min at 95°C; with 40 cycles of 60s at 94°C, 110s at annealing temperature of 46°C, and 110s at 72°C; followed by 10 min at 72°C. PCR reactions were checked using gel electrophoresis, and successful amplifications were purified using ExoSap-IT (Affymetrix) following the manufacturer's instructions. Automated sequencing was performed on purified products in both directions with the aforementioned primers using an Applied Biosystems 3730xl DNA Analyzer.

Sequences were paired and edited using chromatograph visualisations in Geneious 6.1.5 (Biomatters Ltd., Auckland, New Zealand). Each gene multi-alignment was compiled using the Geneious alignment function using default settings (Biomatters Ltd.), and then checked and edited. Some regions of the 16S gene appeared highly variable and were difficult to align with confidence. So the 16S alignment was run through the GBlocks server using the less stringent option which allows for less strict flanking positions (Castresana 2000), in order to eliminate the most highly divergent regions using a reproducible set of conditions.

Consequently 133 nucleotides (nt) were eliminated reducing the partial 16S rRNA fragment to 368nt. We retained 55 species in the phylogenetic analysis for which we had reliable sequences for at least two genes (Table 5.2), as it has been demonstrated that utilising even incomplete data in phylogenies can be beneficial (Wiens 2003; Wiens and Tui 2012). These included 19 species from the Chagos metacommunity (Table 5.2). Sequences were catalogued on GenBank.

Phylogenetic trees were constructed for the consensus alignment (all four genes combined where available; Table 5.2), 16S and enolase combined, and for each gene separately. To root the tree, each tree included an outgroup, *Macrobrachium nipponense,* a species belonging to the superfamily Palaemonoidea within which the family Palaemonidae resides (Kou et al. 2013). To select appropriate models of molecular evolution on which accurate phylogenetic inference largely depends (Simon et al. 2006), partitioning was undertaken in PartitionFinder v.1.1 software (Lanfear et al. 2012). PartitionFinder groups together sites in the alignment that are assumed to have been shaped by similar evolutionary processes, and then estimates independent substitution models for each group of sites (Lanfear et al. 2012). A 10-partition scheme obtained the highest support under Bayesian Information Criterion (BIC), detailed in Table 2. Using these models of evolution (Table 5.3), phylogenies were constructed under Bayesian Inference (BI) analysis in MrBayes v.3.2 (Ronquist et al. 2012), on the on-line CIPRES Science Gateway (Miller and Schwartz 2010). Metropolis-coupled Monte Carlo Markov Chains (MCMC) were run for 30 million generations (x8 chains, temp =0.05), with trees sampled every 3000 generations, resulting in 10 million trees. The parameters of nucleotide frequencies, substitution rates, gamma shape, and invariant-sites proportion were unlinked across partitions. A flat Dirichlet prior distribution allowed rates to vary, to account for rate variation among partitions (Marshall et al. 2006). Tracer v.1.6 was used to ascertain if convergence had been obtained (Rambaut and Drummond 2007). If standard deviation of partition frequencies was <0.01, potential scale reduction factor (PSRF) was ~1.00, effective sample sizes (ESS) were >200, and the shape of the stationary posterior-

distribution trace was a ‘straight hairy capillary’ the data were considered to have converged (Drummond and Rambaut 2007). Once convergence was reached a summary tree was constructed by discarding the first 10% of trees as burn-in, and then using a 50% majority rule consensus tree to construct the summary tree and estimate posterior probabilities (PP) in MrBayes.

5.3.3. Traits

Trait selection was constrained by the amount of information available on the palaemonids, as is often the case (e.g. Gayraud et al. 2003). Consequently we chose to measure the most important and fundamental functional traits of body size and fecundity as measures of fitness. In addition we gathered information from the literature on the species- host association, e.g. hard coral, an important characteristic of the sub-family. As this community of palaemonids were collected from dead branching corals we split the host-associations into four appropriate categories; hard coral (Scleractinia) associates, free-living, free-living and coral associates (palaemonid species which are generalists and have been recorded both free-living and inhabiting corals), and sessile invertebrate associates. The last category refers to sessile invertebrates, such as sponges and tunicates, which are often found encrusting dead coral colonies. The palaemonid species we refer to here as ‘coral associates’ are widely considered to be obligate live coral-dwelling associates (Bruce 1969; Bruce 1972; Bruce 1998) but we found large numbers of these species on dead coral colonies (Head et al. 2015/Chapter 2) and consequently refer to them more generally as coral associates.

Carapace length was used as a proxy for body size and measured under a graduated microscope lens as the linear length of the carapace from the posterior of the orbital cavity to the most posterior tip of the carapace (Anger and Moreira 1998). To measure fecundity both mean egg size and total egg number were recorded. A dissecting microscope was used to carefully remove the egg mass from the pleopods of ovigerous females. The longest and

shortest diameter of ten randomly selected eggs from each female were measured to the nearest 4µm, under a compound microscope using a graduated microscope lens and connected monitor screen, and a mean value was calculated. Total egg number per female was also counted under the compound microscope, and where egg mass was large (>1000 eggs approximately) graph paper was inserted into the field of view and eggs were distributed as evenly as possible, then a sub-sample of ten squares worth of eggs were counted, a mean taken, and multiplied by the number of squares occupied by eggs. Egg number was then adjusted to take account of egg size as a measure of fecundity (see results and discussion).

5.3.4. Environmental variables & spatial data

Live coral cover, fish biomass, invertivore fish biomass (predators), and coral colony habitable space were included in the analysis as environmental variables. Benthic transects were undertaken in a cross formation, 10 m x 10 m, with the dead coral colony located at the centre, to enable percentage coral cover estimates within a 5 m radius of the dead coral colony. Photographs (using a Olympus Xz-1 digital camera) were taken every 0.5 m along the transect using a fixed camera frame to keep the camera a standard 1 m above the reef. Benthic photo-transects were analysed using Coral Point Count (CPC) software (Kohler and Gill 2006). Ten points were randomly assigned to each photo and the benthic cover beneath that point was recorded. Percentage coral cover was then calculated and arc-sine transformed. The habitable space between the branches of each dead coral colony was estimated by placing the coral colony in a full bucket of seawater and the displaced seawater measured to calculate volume as a proxy for habitable space within the coral colony structure (Head et al. 2015/Chapter 2). From previous work fish biomass was recorded per site (Graham and McClanahan 2013; Graham et al. 2013), and divided into functional groups so potential fish predators of palaemonids, i.e. fish invertivore biomass, could be tested as a potential environmental filter. Spatial data was acquired from GPS co-ordinates collected at each site.

5.3.5.Statistical methods

High intra-specific variation means all subsequent analyses were undertaken at the individual palaemonid level rather than at a species level. To establish the best measure of fecundity linear regression and ANOVA were used to evaluate the relationship between egg number with egg size and with female body size. The relationships were plotted and a generalised additive model (gam) used to obtain a line of best-fit. To test for phylogenetic signal in the traits we used the K-statistic (Blomberg et al 2003) incorporating sampling error following Ives et al. (2007), as our data have within-species variation which is not accounted for in most methods. To investigate phylogenetic patterns in trait diversity across distinct spatial scales we used phylogenetic quadratic entropy (PQE) and trait quadratic entropy (TQE) developed by Pavoine et al (2010) and based on Rao's measure of diversity (Rao 1982). The index uses the phylogenetic tree, distributions of relative abundances of species in a community, and a matrix of trait distances among species, obtained by an Euclidean metric, to assess whether there is any phylogenetic and trait clustering in the Archipelago (Pavoine et al. 2010). We measured PQE and TQE at three 3 distinct spatial scales (1) between atolls, (2) between sites within atolls, and (3) between coral heads within sites, to determine if there was spatial structure to the community. The index also allows us to evaluate the influence of species abundances on the phylogenetic pattern of trait diversity.

We used an extended version of the RLQ analysis to test our main hypothesis that environmental filtering rather than limiting similarity is driving the community structure. The RLQ analysis, a three-way ordination method, connects environmental variables at each site (R matrix: sites as row and environmental variables as columns), traits of species inhabiting these sites (Q matrix: species as rows and traits as columns), linked by species occurrences at sites (L matrix: sites as rows and species abundance or occurrences as columns). Pavoine et al (2011) developed an extended version, which we refer to as ESLTP analysis. This combines analysis of environmental variables (E), geographical space (S), species compositions in

sampling units (L), biological traits (T) and phylogeny (P) in the same way as the RLQ but spatial variables along with environmental variables are introduced as columns of the R matrix, and phylogenetic variables together with trait variable are introduced as columns of the matrix Q. The environmental matrix E was analysed by principal coordinate analysis (PCoA). Matrix S used eigenvectors of a Gabriel neighbour matrix (Pavoine et al. 2011) and was analysed using principal component analysis (PCA). Matrix T used pairwise distance between species because traits were of different statistical types, i.e. continuous and categorical, and was analysed by PCoA. Matrix P used pairwise phylogenetic distances among species, calculated as the square root of the sum of branch lengths along the shortest path that connects species. This provides Euclidean distances (Ollier et al. 2006) that were analysed using the PCoA. For further details see Pavoine et al. (2011). To test the significance of the connections between matrices we applied a significance test developed to matrices E and T (hypothesis: species traits are associated with the environment), E and P (hypothesis: species phylogenies are associated with the environment), S and T (hypothesis: species traits are structured spatially), and S and P (hypothesis: species phylogenies are structured spatially). Finally a Moran's test was used to test the significance of spatial structure in environmental variables. All analyses were undertaken in R (R-Development-Core-Team 2008) using the packages gam, ade4 (Dray and Dufour 2007), phytools (Revell 2012) and APE (Paradis et al. 2004).

Table 5.1 Genes sequenced for the 55 species comprising the partial Pontoniinae phylogeny, including 19 species from the Chagos metacommunity

Genus	Species	Species in Chagos metacommunity	GenBank accession number/ Sequence IDs			
			16S	Enolase	NaK	PEPCK
Family: Palaemonidae						
Anchistus	*Anchistus demani*	No	CH0996	CH0996	CH0996	CH0995
Ancylomenes	*Ancylomenes holthuis*	No	JX025220	JX537915	JX467438	JX435436
	Ancylomenes luteomaculatus	No	JX025222	JX537916	JX467439	JX435437
Apopontonia	*Apopontonia falcirustris*	No	MA0150b	MA150b	MA150b	MA150b
Brucecaris	*Brucecaris tenuis*	No	JX025218	JX537917	JX467440	JX435438
Conchodtytes	*Conchodtytes mezeagrinae*	No	CH0902	CH0902	CH0902	CH0902
Coralliocaris	*Coralliocaris graminea*	No	KF38361	KF738298	KF738343	------------
	Coralliocaris sandyi	No	KF38362	KF738299	KF738344	------------
	Coralliocaris viridis	Yes	CH0948	CH0948	CH0948	CH0948
Crinotonia	*Crinotonia attenuatu*	No	-----------	JX537918	JX467441	JX435439
Cuapetes	*Cuapetes amymone*	No	JX025216	JX537919	JX467442	JX435440
	Cuapetes anacanthus	No	JX025215	JX537920	JX467443	JX435441

	Cuapetes andamanensis	No	JX025214	JX537921	JX467444	JX435442
	Cuapetes elegans	Yes	JX025213	JX537922	JX467445	JX435443
	Cuapetes ensifrons	Yes	JX025212	JX537923	JX467446	JX435444
	Cuapetes grandis	Yes	JX025211	JX537924	JX467447	JX435445
	Cuapetes longirostris	Yes	CH1172	CH1172	CH1172	CH1172
	Cuapetes tenuipes	No	JX025209	JX537925	JX467448	JX435446
Harpiliopsis	*Harpiliopsis beaupressi*	Yes	MA312b	MA312b	MA312b	JX435447
	Harpiliopsis depressa	Yes	CH0737	CH0836	CH0836	CH0836
	*Harpiliopsis spinigera_*	Yes	CH0662	CH0662	CH0662	CH0662
Harpilius	*Harpilius consobrinus*	No	CH1019	CH1019	CH1019	CH1019
	Harpilius lutescens	Yes	CH1009	CH1009	JX467450	CH1009
Isopericlimenaeus	*Isopericlimenaeus gorgonidarum*	Yes	CH0684	-----------	CH0684	CH0684
Jocaste	*Jocaste japonica*	Yes	CH0946	CH0333	CH0333	CH0333
	Jocaste lucina	Yes	CH0128	CH0737	CH0737	CH0128
Laomenes	*Laomenes ceratophthalmus*	No	JX025203	JX537928	JX467451	JX435449
	Laomenes pardus	No	JX025202	JX537929	JX467452	JX435450

	Laomenes sp	Yes	CH0807	CH0807	CH0807	CH0807
Leptomenaeus	*Leptomenaeus dolichosternum*	No	JX025201	JX537930	JX467453	JX435451
Palaemonella	*Palaemonella pottsi*	No	JX025198	JX537932	JX467455	JX435453
	Palaemonella rotumana	Yes	MA540	MA540	MA540	MA056
	Palaemonella spinulata cf	Yes	CH0623	KF738304	KF738352	-----------
	Palaemonella tenuipes cf	Yes	CH0923	CH0923	CH0923	CH0923
Periclimenaeus	*Periclimenaeus bidentatus*	Yes	KF738368	CH0402	KF738353	-----------
	Periclimenaeus diplosomatis	Yes	CH0952b	CH0952	CH1200	CH0952
	Periclimenaeus pettihouarsi	Yes	CH1085	CH1085	------------	-----------
Periclimenella	*Periclimenella spinifera*	No	JX025194	JX537933	JX467456	JX435454
Periclimenes	*Periclimenes boucheti*	No	JX025192	JX537934	JX467457	JX435455
	Periclimenes brevicarpalis	No	JX025191	JX537935	JX467458	JX435456
	Periclimenes commensalis	No	JX025190	JX537936	JX467459	JX435457
	Periclimenes dentidactylus	No	JX025189	JX537937	JX467460	JX435458
	Periclimenes digitalis	No	JX025188	JX537938	JX467461	JX435459
	Periclimenes hertwigi	No	JX025186	JX537939	JX467462	JX435460

	Periclimenes imperator	No	JX025185	JX537940	JX467463	JX435461
	Periclimenes laccadivensis	No	JX025184	JX537941	JX467464	JX435462
	Periclimenes soror	No	JX025178	JX537942	JX467465	JX435463
Philarius	*Philarius gerlachei*	No	JX025177	JX537943	JX467466	JX435464
Phycomenes	*Phycomenes cobourgi*	No	JX025174	JX537944	JX467467	JX435465
Unguicaris	*Unguicaris panglaonis*	No	JX025172	JX537945	JX467468	JX435466
	Unguicaris pilipes	No	JX025171	JX537946	JX467469	JX435467
Vir	*Vir colemani*	No	CH0989	CH0989	CH0989	CH0949
	Vir philippinensis	No	JX025170	JX537947	JX467470	JX435468
Zenopontonia	*Zenopontonia noverca*	No	CH1141	CH1141	-----------	CH1141
Superfamily: Palaemonoidea						
Macrobrachium	*Macrobrachium nipponense*	No	JX435435	JX537948	JX467471	JX435469

Table 5.3 Partition scheme and best-fit models of evolution used in Bayesian Inference (BI) analysis.

Gene and codon position number	Partition delineation	Best-fit model of evolution
Enolase 1st	1-405\3	K80+I+G
Enolase 2nd	2-405\3	K80+I+G
Enolase 3rd	3-405\3	GTR+G
NaK 1st	406-1026\3	GTR+I+G
NaK 2nd	407-1026\3	GTR+I+G
NaK 3rd	408-1026\3	GTR+G
PEPCK 1st	1027-1548\3	GTR+I+G
PEPCK 2nd	1028-1548\3	GTR+I+G
PEPCK 3rd	1029-1548\3	GTR+I+G
16S	1549-1992	K80+I+G

5.4. Results

5.4.1. Phylogeny

Nineteen of the twenty species from the metacommunity were represented by at least two genes in the consensus phylogeny (Fig. 5.1), from which a composite metacommunity phylogeny was produced (Fig. 5.2). Only *Exoclimenella maldevensis* was not included in the consensus phylogeny as we were only able to amplify the 16S gene for this species. As this species was rare in the community, occurring only once, it was excluded from further analysis. An additional 26 species were included in the phylogeny to provide more information on the evolutionary relationships between species in the metacommunity. The

consensus phylogeny consists of two major clades, clade I is well supported (Bayesian posterior probability (PP)=1) and consists of only five species from the genera *Periclimenes* and *Laomenes*, species from both these genera are also placed in clade II. Clade II is moderately well supported (PP=0.7) but the basal relationships with its nested clades are not well resolved. All of the Chagos metacommunity species are dispersed throughout clade II but with clusters of seven species and eight species in two of the largest nested clades, clade II.i (PP=0.71) and II.ii (PP=0.81) respectively (Fig. 5.1). The seven species from the metacommunity all clustered in clade II.i are all scleractinian associates, with the exception of the free-living species *Periclimenella pettihouarsi*. The eight species dispersed throughout clade II.ii are all free living and sometimes associated with scleractinian corals, with the exception of *Harpilius lutescens,* which is purely a Scleractinia associate. The four remaining species from the metacommunity not located in these two larger nested clades are all associates of sessile organisms encrusting on the branches of the dead corals; *Laomenes* spp. (crinoid associate), *Isopericlimenaeus gorgonidarum* (encrusting sponge associate), *Periclimenaeus bidentatus* (encrusting sponge associate) and *Periclimenaeus dactylodon* (encrusting ascidian associate). *I.gorgonidarum, P.bidentatus* and *P.dactylodon* are all clustered together in a small clade. Whilst *Laomenes spp.* appears to be a sister species of three other crinoid associates; *Perclimenes commensalis, Unguicaris panglaonis, Unguicaris pilipes*.

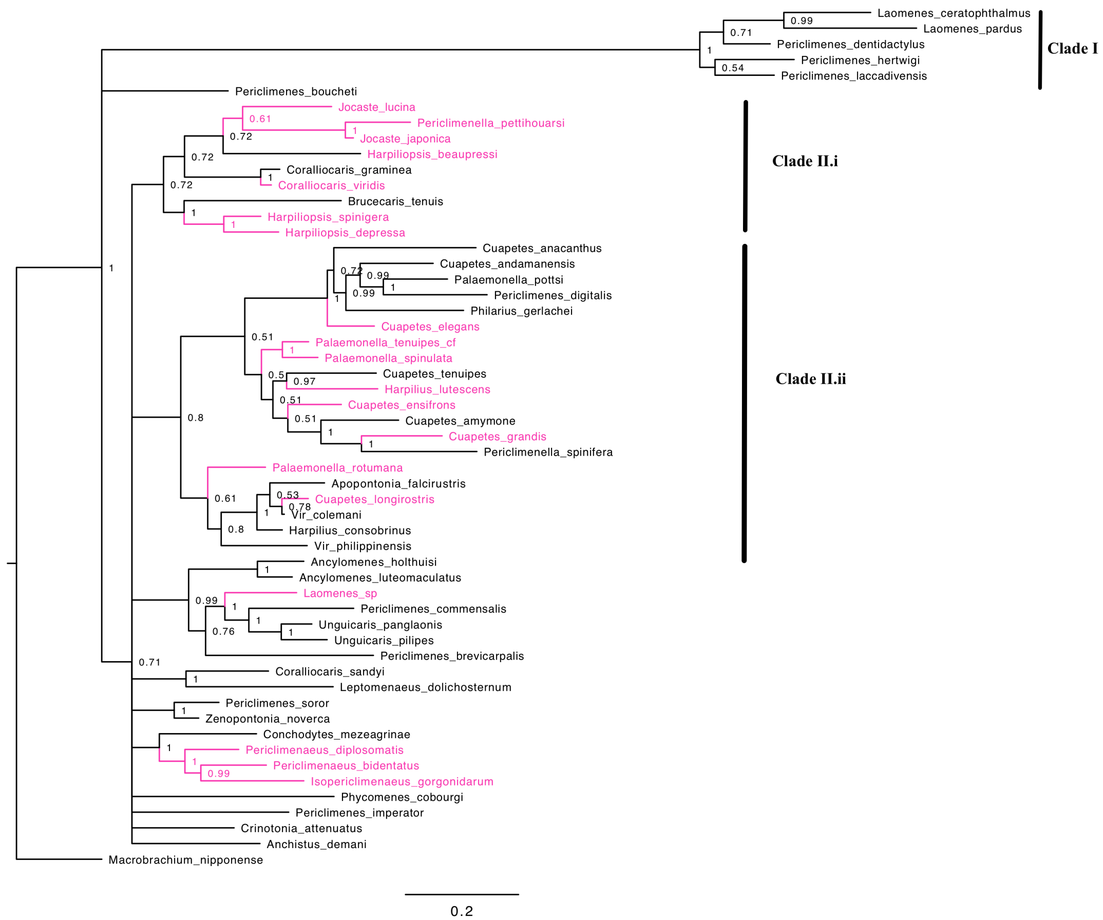

Figure 5.1 Bayesian inference phylogeny of 55 species from the family Palaemonidae, using a consensus sequence of four genes; 16S, Enolase, NaK, and PEPCK. Node support values represent Bayesian posterior probabilities. The species in magenta are those present in the Chagos metacommunity.

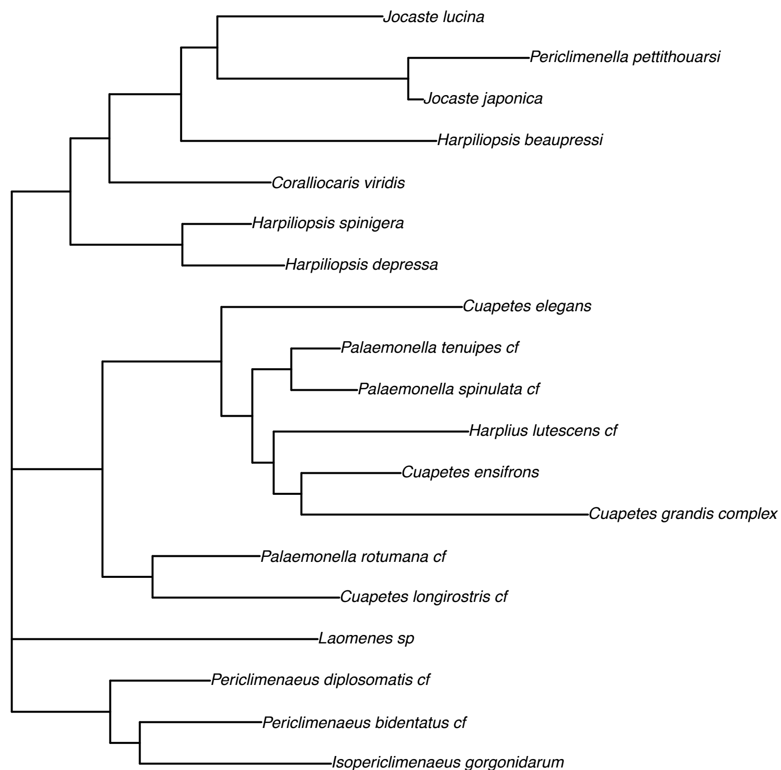

Figure 5.2 A composite Palaemonidae phylogeny of the Chagos metacommunity (corresponds species highlighted in magenta in figure 1).

5.4.2. Traits

24 gravid females were recorded from eight of the 20 species in the metacommunity (Fig. 5.3.a). However four gravid females were excluded from the analysis as a result of damage to the egg sac and/or suspected shedding of eggs during collection.

Linear regression demonstrated that egg size was significantly negatively correlated with egg number (t = -2.65, p = 0.02), but neither egg number (t = 0.15, p = 0.88) nor egg number adjusted for egg size (t = 0.37, p = 0.72) had a significant linear relationship with female body size (Fig. 5.3). Therefore egg number was adjusted to account for egg size only (egg number was multiplied by egg size), and used as a measure of fecundity.

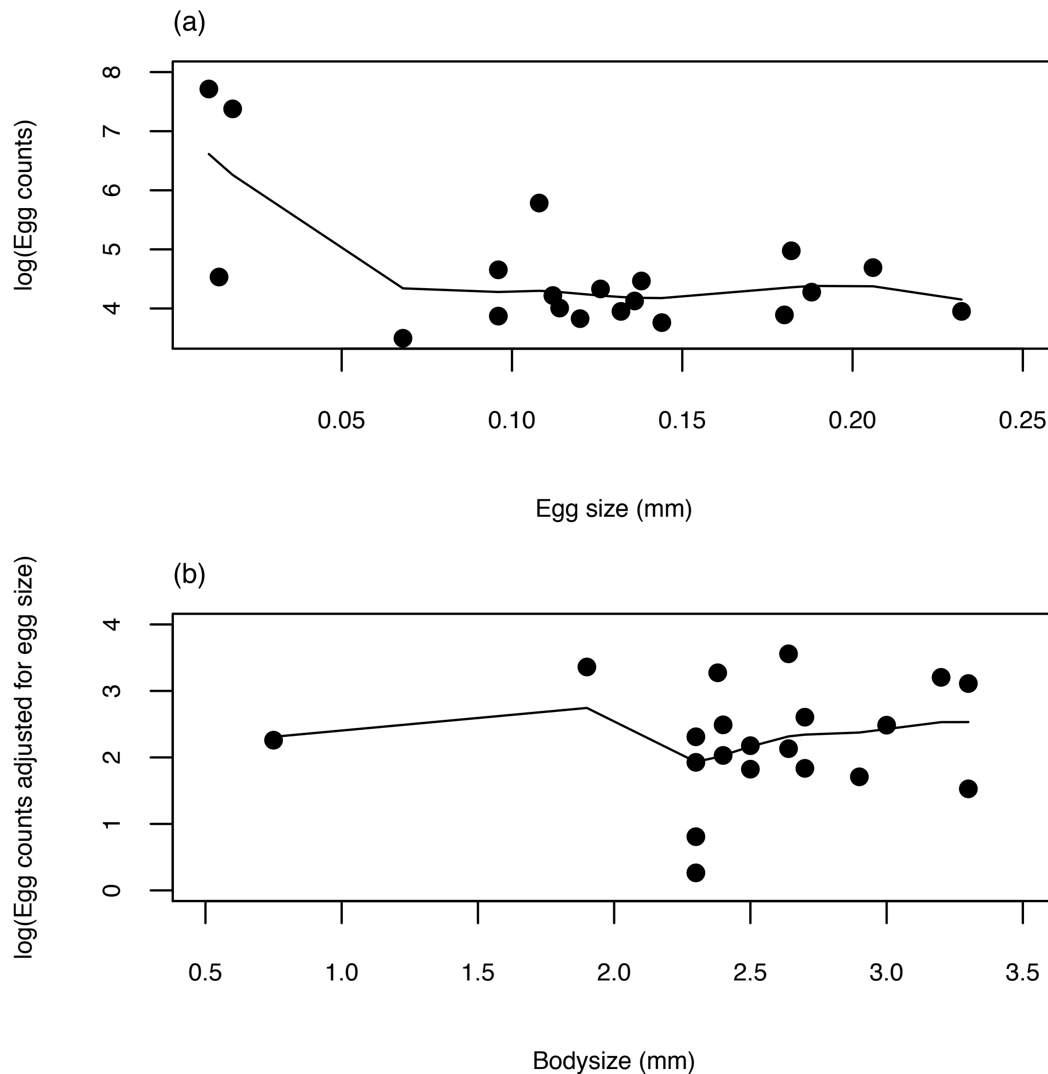

Figure 5.3 Scatterplots, with lines of fit best, demonstrating **(a)** the significant relationship between the number of eggs and average egg size, and **(b)** the non-significant relationship between number of eggs adjusted for egg size and female body size. Hence, egg number adjusted for egg size is used as a measure of fecundity.

5.4.3. Test for phylogenetic signal

At the metacommunity level, body size has a weaker phylogenetic signal (i.e. closely related species are less similar in body size) than would be expected under a Brownian motion model of evolution, when accounting for within-species variation (K = 0.47, σ = 3.58). Whilst, fecundity also had a weak phylogenetic signal (K = 0.91), the among species variation, once within-species variation had been controlled for, was high (σ = 62.23). Host association could not be tested as the K statistic can only evaluate the phylogenetic signal of continuous traits. Phylogenetic signal for body size at the local scale (sites within atolls) was also tested and the same trends occurred with body size at each atoll having a weaker phylogenetic signal than would be expected under a Brownian motion model of evolution, when accounting for within-species variation (see Table 5.4 for K and σ statistics). At a local scale, body size at Diego Garcia and Salomon had the strongest phylogenetic signals. Phylogenetic signal could not be tested for fecundity at the local scale because the number of gravid females per atoll was too small to produce meaningful results.

Table 5.4 Phylogenetic signal in body size per atoll using the Blomberg's K statistic. If K is less than 1 there is less phylogenetic signal than would be expected by chance under a Brownian model of evolution. σ shows the variation around the K statistic after controlling for intra-specific variation.

Atoll	Body size	
	K statistic	σ
Brothers	0.69	2.70
Diego Garcia	0.92	0.80
Eagle	0.82	0.56
Egmont	0.85	0.44
Peros Banhos	0.47	4.02
Salomon	0.98	0.60

5.4.4. Test for TQE and PQE

There was no significant trait or phylogenetic clustering among atolls within the archipelago (TQE $p = 0.42$, PQE $p = 0.31$). However, there was significant trait and phylogenetic clustering among sites within atolls (TQE $p = 0.01$, PQE $p = 0.04$), and among coral colonies within sites (TQE $p = 0.002$, PQE $p = 0.03$). When the analysis was weighted by abundance of species at each spatial scale the results remained the same.

5.4.5. Extended RLQ Analysis

The correlation between both traits and environmental variables (matrix Q) and both environmental and spatial variables (matrix R) was significant ($p = 0.01$). This significant association was between traits and the environment ($p = 0.001$), phylogeny and the

environment (p = 0.01), but not between traits and space (p = 0.32), or between phylogeny and space (p = 0.15). Extended RLQ analysis was also carried out on both body size and fecundity separately and the same significant correlations were obtained. A Moran's test showed that environmental variables had a significant (p = 0.03) but weak (Obs = 0.12) spatial structure.

The proportion of variation explained by the first and second axes of the ESLTP analysis was 87% (56% and 30% respectively). Spearman correlations with the axes of the ESLTP analyse indicate that body size (Spearman correlation with 1st axis 1 -0.75, and 2nd axis -0.93) was most closely correlated with the habitable space of the host coral (Spearman correlation with 1st axis -0.65, and 2nd axis -0.59) in the RLQ analysis. When phylogenetic distance between species and spatial co-ordinates were also considered (ESLTP analysis) then correlation patterns remained the same with body size (Spearman correlation with 1st axis -0.48, and 2nd axis -0.71) and habitable space being most strongly correlated (Spearman correlation with 1st axis -0.63, and 2nd axis -0.45) (Fig. 5.4.a and b). Species are broadly clustered in body size by their host association, with coral associates generally having the smallest body size (Fig. 5.5.b). The exception to this was *Laomenes spp*., an associate of an encrusting sessile organism, but this species was rare in the community with only one individual sampled. Free living and associates of encrusting sessile organisms had the largest body sizes, and species considered free living and coral associates were intermediate in body size (Fig. 5.5.b). In Peros Banhos, Salmon and Brothers Islands smaller specialist coral associate species dominate the community (Fig. 5.5 and Fig. 5.4.c taken together). In contrast at Egmont and Eagle Islands the larger free-living species were generally more common, as well as those less specialist species considered free-living and coral associates (Fig. 5.5 and Fig. 5.4.c taken together). From a phylogenetic perspective, the species that dominated the community in Peros Banhos, Salomon and Brothers all belonged to clade II.i (Fig. 5.1). The free-living species, and free-living and coral associate species, which were more common at Eagle and Egmont, were dispersed throughout clade II.ii (Fig. 5.1).

When average trait and phylogenetic Spearman correlation values were plotted against the composite community phylogeny (Fig. 5.6) we can identify that certain sister species, e.g. *Harpiliopsis spinigera* and *Harpiliopsis depressa,* and certain clades share similar life history traits (body size and fecundity together), e.g. clade II.ii, suggesting some conserved evolution, but there was also much variation in correlation values between sister species and within clades suggesting convergent evolution. For instance, *Harpiliopsis beaupressi* and *P. pettithouarsi* appear convergent in trait and phylogenetic values from their sister species. These results should to be taken alongside the phylogenetic signal K-statistics (above) that accounts for intraspecific variation.

Fecundity was most closely associated (Spearman correlation with 1st axis 0.23, and 2nd axis -0.44) with live coral cover within a 5m radius of the dead coral host (Spearman correlation with 1st axis 0.53, and 2nd axis -0.71), and also fish biomass (Spearman correlation with 1st axis 0.12, and 2nd axis -0.61). The ESLTP analysis showed a similar pattern but also a correlation between fecundity (Spearman correlation with 1st axis 0.47, and 2nd axis -0.50) with coral cover (Spearman correlation with 1st axis 0.56, and 2nd axis -0.65) and fish biomass (Spearman correlation with 1st axis 0.19, and 2nd axis -0.64) (Fig. 5.4.a and 5.4.b), indicating that there was also some phylogenetic pattern in trait diversity (Also see TQE and PQE). Peros Banhos, Salomon and Brothers Islands had the highest fish biomass and coral cover (Fig. 5.4.b and 5.4.c). Eagle Islands and Egmont appear to be similar in environmental conditions, whilst Diego Garcia was different from all other atolls and islands (Fig. 5.7). Species fecundity was broadly clustered by their host association, and the most fecund species were the coral associates *Jocaste japonica* and *Jocaste lucina*. Free-living species and those considered free-living and coral associates generally had lower fecundity (Fig. 5.5.a).

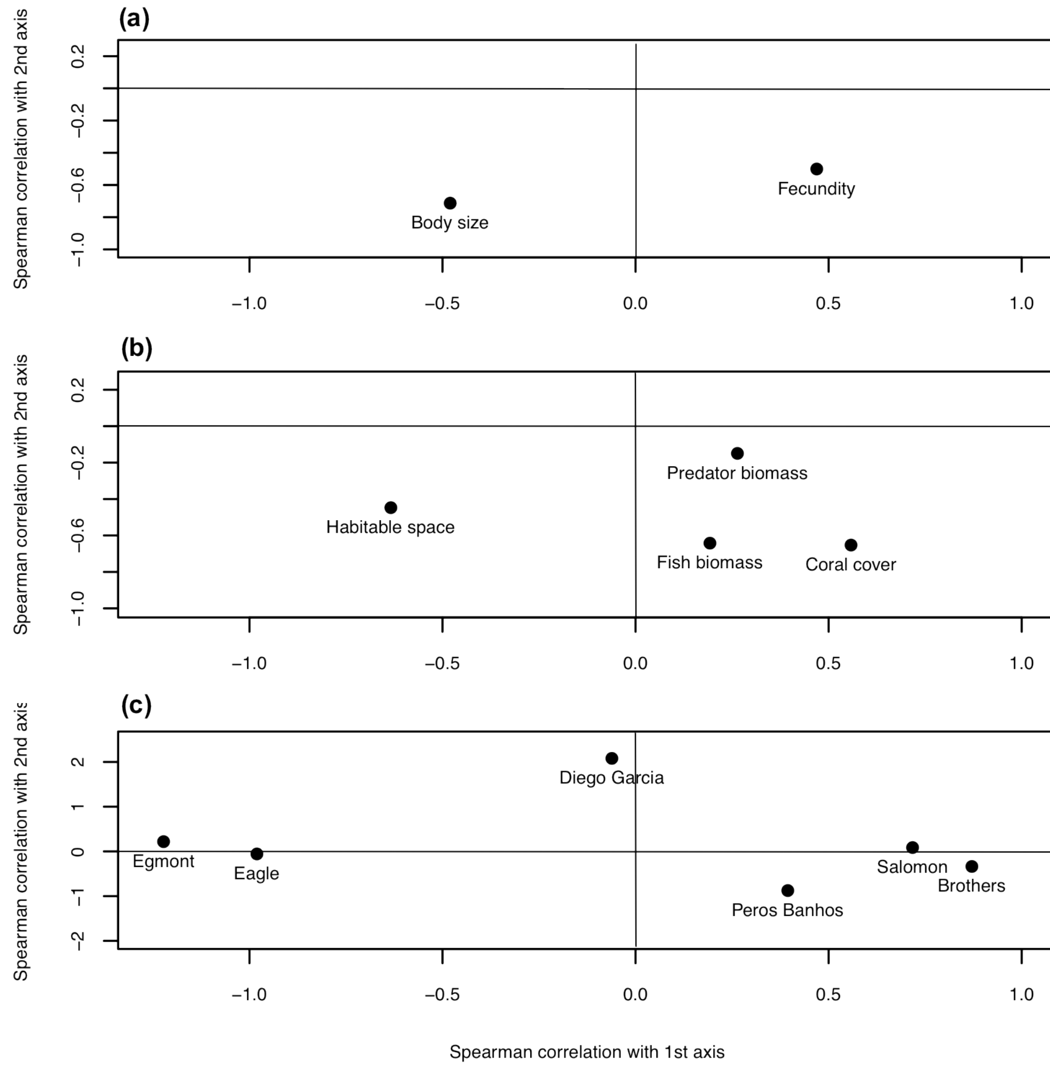

Figure 5.4 Scatterplot of the average correlation values for the whole community from the extended RLQ for **(a)** trait and phylogenetic values, **(b)** environmental variables, and **(c)** atolls clustered by environmental variables.

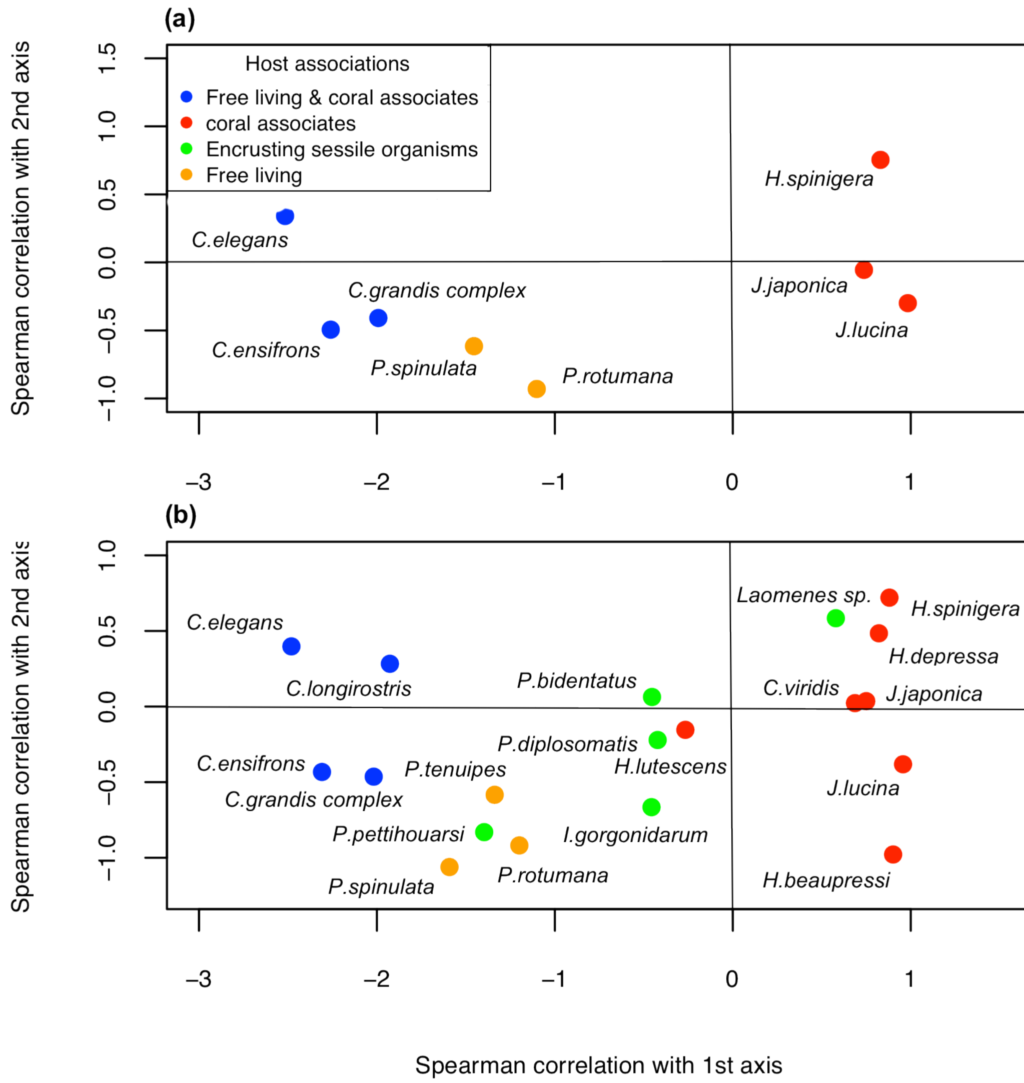

Figure 5.5 Scatterplot of the average trait and phylogenetic correlation values for each species from the extended RLQ for **(a)** fecundity, **(b)** body size. The legend indicates each species' host association.

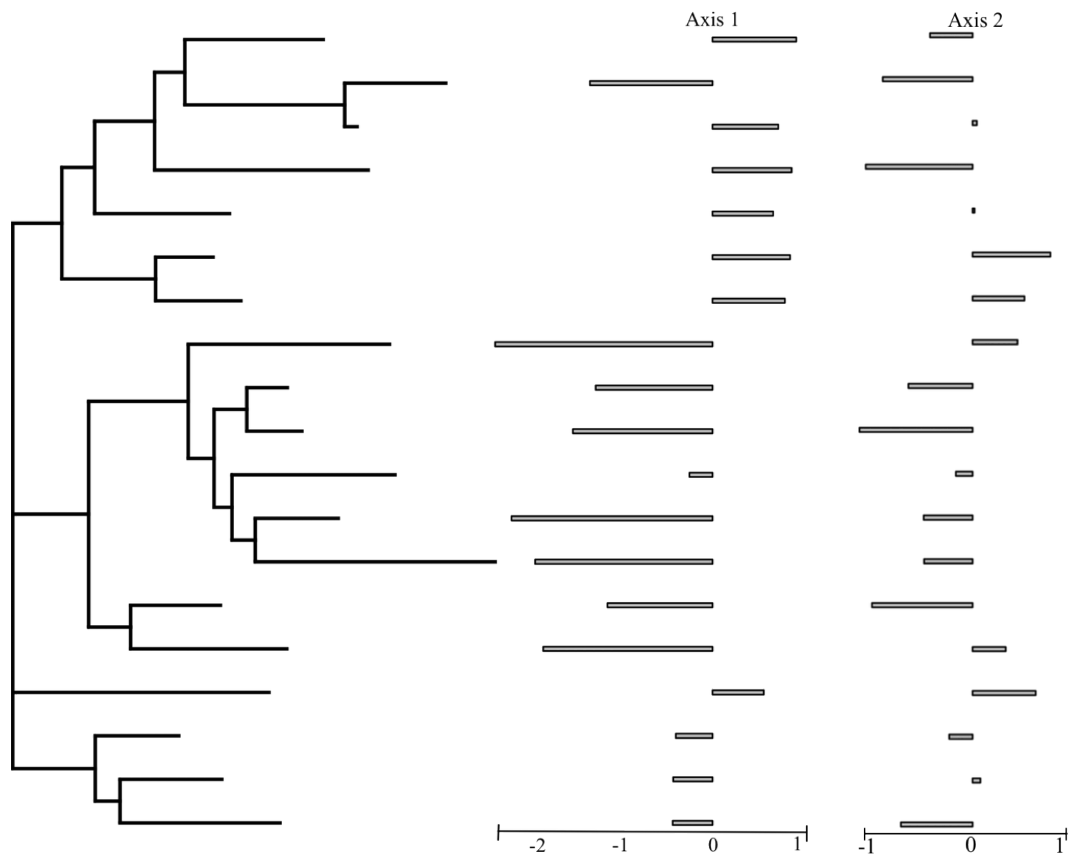

Figure 5.6 The Palaemonidae phylogenetic tree for the community (see figure 2 for species names) and coordinates of palaemonid species on the 1st and 2nd axis of the extended RLQ analysis based on a combination of the traits (body size and fecundity) and phylogenetic distances. Both axes account for a certain proportion of the variation (56% and 30% respectively) and therefore should be taken together.

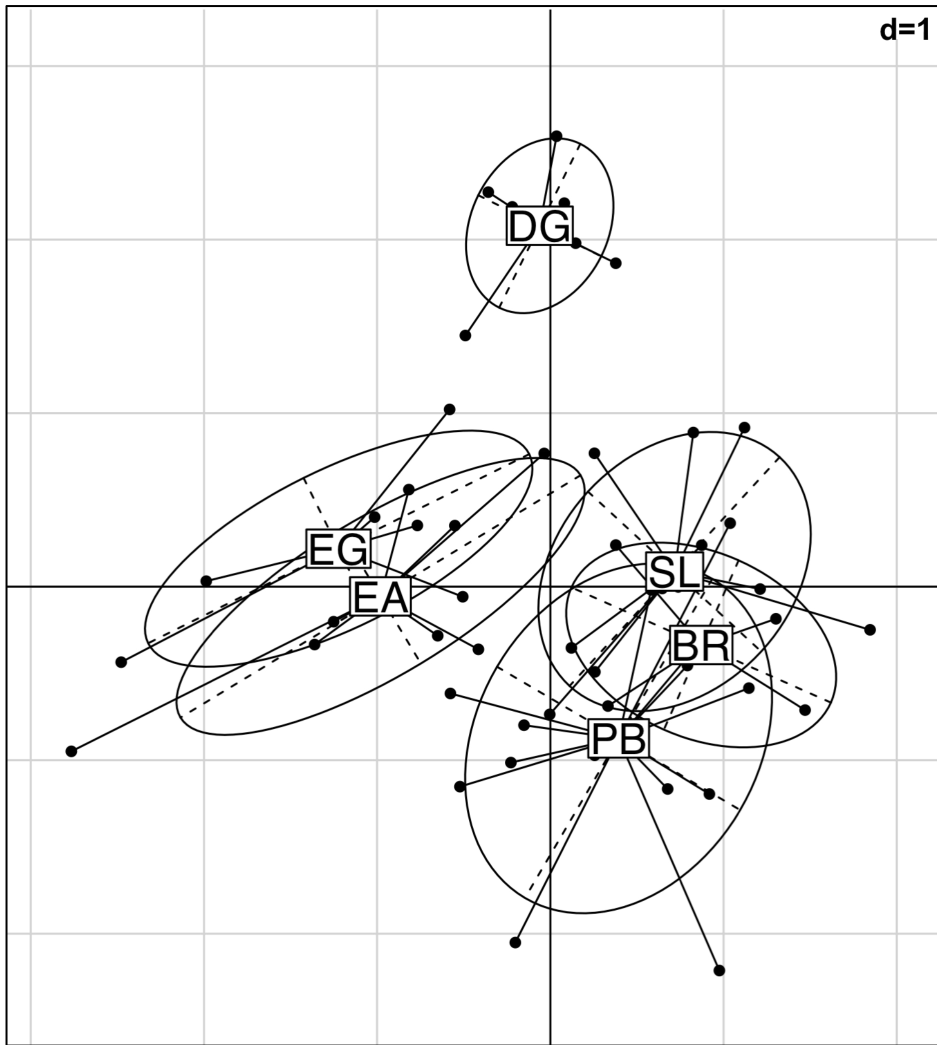

Figure 5.7 Average environmental and spatial variable correlation values per site, and clustered by atoll, from the extended RLQ. Legend: BR = Brothers Islands, DG= Diego Garcia, EA= Eagle Islands, EG= Egmont, PB= Peros Banhos, SL= Salomon.

5.5. Discussion

The mechanisms driving palaemonid community assembly and maintenance in Chagos show distinct spatial patterns. Different processes are known to act at different spatial scales and this has been demonstrated particularly clearly in forest ecosystems (Ricklefs 1987; Kraft and Ackerly 2010). On Chagos reefs the QE tests demonstrate spatial hierarchy with significant trait clustering found within atolls and within sites but not between atolls. This suggests that environmental filtering is acting at the local community level but not at the metacommunity Archipelago scale (Table 5.1) (Webb et al. 2002; Pavoine and Bonsall 2010). The RLQ significance test supports this, as it shows no significant spatial structure in traits between atolls. There is also weak phylogenetic signal at both the metacommunity level and within local communities indicating traits are regulated by the phylogeny through both trait conservatism and convergence at all spatial levels. The QE also demonstrated phylogenetic clustering at the local levels but, contrary to the phylogenetic signal analysis, there was no clustering at the metacommunity level and nor did the RLQ significance test show significant spatial structure in phylogenetic diversity between atolls. Abundance did not affect the clustering patterns of traits or phylogenetic diversity at each spatial scale, suggesting that abundance is not correlated with the phylogenetic positions of species and/or with their trait states, and that individual abundances per coral colony are fairly evenly distributed.

Phylogenetic signal at all spatial scales

Analysis on body size and fecundity revealed that these traits were only weakly phylogenetically conserved in communities within Chagos and across the metacommunity. So while traits were regulated by phylogeny and environment (the latter only at the α level), trait convergence within the phylogeny was considerable, consequently eroding much of the

phylogenetic structure associated with habitat use. This weak or partial phylogenetic conservatism could be a result of evolutionary lability in traits, where some lineages experience higher rates of trait evolution than others (Blomberg et al. 2003; Ackerly 2009; Pavoine et al. 2014).

The erosion of phylogenetic structure and potentially labile traits could explain the lack of phylogenetic clustering at the metacommunity level in the QE test and the RLQ significance test. The *K* phylogenetic signal test also controls for intraspecific variation (Ives et al. 2007), which is high in this community, possibly allowing for detection of signal where the other tests do not. Alternatively if there is no phylogenetic structure, as the QE and RLQ tests suggest, this would indicate that stochastic processes, such as ecological drift, are predominating at this scale (Clark 2009).

A common hypothesis in community assembly studies in the past has been that phylogenetic relatedness can act as a surrogate for key life history traits without having to measure the traits themselves (Losos 2008; Wiens 2010). However this depends on how traits associated with environmental filtering or limiting similarity have evolved, i.e. the degree of phylogenetic conservatism (Webb et al. 2002). Congruent functional and phylogenetic patterns shaped by phylogenetic conservatism have been found in communities (Willis et al. 2009), and random or weak phylogenetic patterns have been found in other communities (e.g. Purschke et al. 2013; Pavoine et al. 2014). In this study because both trait conservatism and trait convergence appear to have shaped trait values of palaemonids inhabiting dead coral colonies at the local community and metacommunity level (Fig. 5.6), phylogenetic distances alone would not be sufficient to describe the distribution of species.

Environmental filtering at the local community scale

Environmental filtering is an important mechanism underpinning palaemonid species distribution within local communities in the Chagos. Filtering results in the evolutionary selection of species with a similar set of traits adapted to the specific environmental conditions (Webb et al. 2002). In the Chagos archipelago the habitable space of the dead coral host is a dominant filter that acts on the body size of palaemonids at the local level (Fig. 5.4). It is well reported that coral associate abundance, species richness and biomass increases with coral colony size, and complexity in live coral colonies (Abele 1976; Abele and Patton 1976; Coles 1980; Vytopil and Willis 2001) and to a lesser extent dead coral colonies (Enochs et al. 2011; Enochs and Manzello 2012). However, the relationship of coral associates' body size with coral colony size has been neglected, possibly because the small size of these organisms makes them hard to measure. The exception is a study on live coral associates in dead coral colonies which found that live coral associates body size (palaemonids only) was significantly correlated with coral colony size at Egmont atoll (Head et al. 2015/ Chapter 2). The abundance of palaemonids on the coral colonies had no effect on body size patterns. We might have expected the higher abundance to be correlated with smaller body size as competition for space intensifies. However the relationship of species abundance with body size is well studied in ecology (Nee et al. 1991; Brown et al. 2004; White et al. 2007), and generally the frequency distribution of individual body sizes in a community is highly variable and complex (White et al. 2007).

Fecundity is correlated with live coral cover within a 5m radius of the coral colonies, and fish biomass (Fig. 5.4). It is unlikely that these abiotic variables act directly as filters on palaemonid fecundity rather that underlying mechanisms drive this relationship. It is probable that conditions that promote a healthy reef ecosystem with high coral cover and fish biomass (Sandin et al. 2008; Hughes et al. 2010; Graham et al. 2013) also provide good resources for other reef associates such as palaemonids, allowing them to invest more energy in reproduction resulting in higher fecundity rates. We also isolated the functional group of invertivore fish from the fish biomass estimates and included this as an environmental

variable, expecting that if invertivore fish were important predators of palaemonid shrimp we would find a negative correlation with body size and fecundity. However they were not correlated. It is likely that we would need to isolate explicitly the invertivore fish groups predating palaemonids such as the coral associated coral crouchers (*Caracanthus*) and certain species of gobies (Gobiidae) to investigate predator-prey relationships in this niche. Also, although cryptic invertebrates are known to be important prey for many epibenthic and necktonic fish species (Depczynski and Bellwood 2003; Glynn 2006; Kramer et al. 2015), and have been found to make up a substantial component of gut contents of at least 212 fish species (Randall 1967), it is likely that palaemonids, because of their small size, might only be an important food source for juvenile fish or species of very small body size (Kramer et al. 2015). In addition, species of molluscs (Kohn 1983), annelids (Ott and Lewis 1972), and arthropods (Reaka 1987) are also cryptic carnivores and the prevalence of palaemonids in these food webs is unknown but may affect their distribution at least on a local scale.

The approach taken here to measure fecundity should also be considered when interpreting our findings. In this study we used egg numbers adjusted for egg size as a measure of fecundity, which is a standard measure of realized fecundity in Caridea. However, the stage of embryonic development will affect the number of eggs mainly through brood loss as egg size increases through development (Kuris 1991), hence egg size was measured as a proxy to account for developmental stage (Anger and Moreira 1998). In addition, in many organisms there is a well-known trade-off between egg number and size under different conditions, e.g. food availability, to increase reproductive success (Guisande et al. 1996; Bertini and Baeza 2014). Body size of the female is also shown to affect the energy investment in fecundity (Anger and Moreira 1998; Bertini and Baeza 2014). However, we found that female body size was not correlated with egg number or egg size in this community (Fig. 5.3), possibly because of the small range in gravid female body sizes, and as a result was not accounted for in the fecundity measure.

Lack of environmental filtering at the metacommunity level

In contrast to the local scale, environmental filtering was not found to be a driver at the metacommunity level (between atolls). The lack of environmental filtering could occur as these environmental variables had only a weak spatial structure at the atoll level, although spatial structure was significant. Nevertheless it is useful to note these environmental spatial patterns at the atoll level and their possible explanations. Most interesting perhaps is that the environmental conditions at Diego Garcia were not closely associated with any other atoll (Fig. 5.7), which may be a result of human disturbance as it is the only atoll in the archipelago with human inhabitants, and is known for instance to have recreational fishing pressure (Sheppard et al. 2012; Graham et al. 2013). Diego Garcia is also the most geographically distinct, located furthest south and at the greatest distance from another atoll (Fig 1.2). The highest coral cover and fish biomass were found at Peros Banhos, Salomon and Brother Island (Fig. 5.4.b and c). Egmont Atoll and Eagle Island had lower coral cover and fish biomass and were similar in these environmental conditions (Fig. 5.7). This may in part be the result of crown-of-thorns, *Acanthaster planci*, outbreaks, observed at both Egmont and Eagle in recent years, resulting in reduced coral cover (Roche et al. 2015/Appendix III).

A noteworthy spatial pattern in trait diversity was that the most fecund palamonid species were predominantly found in Peros Banhos, Salomon and Brothers (but not solely), and these were the atolls with the highest fish biomass and coral cover (Fig. 5.4). Despite high intraspecific variation between species and their traits, we can link certain species lineages with trait patterns. For instance, the most fecund species were *Jocaste lucina* and *Jocaste japonica,* both coral associate species, whilst *Cuapetes elegans*, a free living and coral associate species, had the lowest fecundity in the community (Fig. 5.4.a and 5.5.a taken together). In the case of body size, *Harpiliopsis spinigera* and *Harpiliopsis depressa*, both coral associates, and *Laomenes spp.* (an associate of a crinoid found inhabiting a dead coral colony) have the smallest body size, and *Isoperclimenlla gorgonidarum* has the largest (Fig. 5.4.a and 5.5.b taken together). However *Laomenes spp.* and *I.gorgonidarum* were both rare

in the community with only one species each. The coral associates *Harpiliopsis*, *Jocaste*, and *Coralliocaris* are all generally smaller in body size, though there was much intraspecific variation, and their dorsoventrally compressed body is an adaption to living within the branches of coral hosts (Bruce 1969; Kou et al. 2014). *Cuapetes* and *Harpilius* (free living & coral associates, and coral associates respectively) have a sub-cylindrical to slightly laterally compressed body (Bruce 1977; Kou et al. 2014), a different adaptation to living in coral hosts, reflected here by their larger dorsoventral body size compared to *Harpiliopsis*, *Jocaste*, and *Coralliocaris*. These different adaptations to coral host living may partly explain their divergent evolutionary pathways (Fig. 5.1) (Kou et al. 2014). All three free-living species were correlated with large body sizes, which also relates to their life strategy i.e. they have no size-constraint because there is no strict continuous association with the host organism (Dobson et al. 2014).

In summary both environment and the phylogeny explain trait diversity and patterns of coexistence to some degree within the local α community. The strongest filter within local communities is habitable space of the coral colonies acting on the size of the Palaemonidae shrimps. There are also likely to be other environmental filters not identified here. Furthermore, phylogenetic signal was weak, both within local communities and at the metacommunity level, though conflicting evidence in phylogenetic structure at the metacommunity level means further investigation is required to identify the processes acting at this scale. The weak phylogenetic signal, at least at the local level, suggests trait convergence and lability of trait evolution could be key processes determining species distribution. Evidence of trait convergence means evolutionary history must be used in conjunction with life-history traits to understand the patterns and processes underpinning community composition, as has recently been advocated by others (Kraft and Ackerly 2010; Pavoine et al. 2014). As relatively little is known about the life-history strategies of palaemonid species (Dobson et al. 2014; Kou et al. 2014) we choose to focus on the fundamental traits of body size and fecundity. However, as our knowledge increases it will

be important to identify other key functional traits associated with the sub-family, to improve our understanding of community assembly. Overall in this study we have isolated some key ecological (environmental filters – acting on α community only) and evolutionary mechanisms (trait convergence, labile rates of trait diversification) driving compositional patterns in palaemonid local communities in the Chagos Archipelago and possibly across the archipelago metacommunity.

6. CHAPTER SIX:

Concluding Remarks

6.1. Key Findings & Implications

To conserve and manage biodiversity we must first know what is being lost, and where and why. To effectively monitor biodiversity we must know what is there to begin with (Kowlton and Jackson 2008; Reaka-Kudla 1997). Measuring biodiversity has been the focus of many ecologists' research for decades (e.g. Loreau et al. 2001; Mora et al. 2011). What aspect of biodiversity is measured, e.g. species richness, phylogenetic diversity, functional diversity, trait diversity, largely depends on the aim of the study and the target audience (Magurran and McGill 2011). For instance, a conservation practitioner might largely be interested in species richness and abundance and perhaps functional diversity, whilst an ecologist interested in the mechanisms driving community assembly and maintenance might investigate trait and phylogenetic diversity. However, it is strongly advocated to measure more than one aspect of biodiversity for a more complete understanding of the intricacies of the ecosystem (Pavoine and Bonsall 2011). In this thesis we have increased our understanding of the biodiversity of a dominant component of the reef cryptofauna, the decapods, using a variety of biodiversity measures including; species richness, evenness, diversity, abundance, phylogenetic diversity, and trait diversity. We carried out this study in a particularly interesting site, the Chagos Archipelago, a large marine reserve in the Indian Ocean which is considered 'near pristine' (Sheppard et al., 2012). The overarching aim of this study is to assess the diversity and community structure of the decapods on one microhabitat, dead coral colonies, in the Chagos Archipelago and begin to understand the processes driving their community structure.

6.1.1. Prevalence of obligate coral-dwelling species

Whilst investigating the decapod diversity on dead coral microhabitats we discovered a high prevalence of obligate coral-dwellers on the dead coral colonies, species that are thought to have a mutualistic relationship with live corals and are strongly dependent on them for their

survival (Stella et al. 2011a). Of the seven obligate coral-dwelling species, *Trapezia* juveniles and female palaemonid shrimps predominated suggesting perhaps that the dead coral microhabitats might be important in certain stages of these species' life cycles. Alternatively, dead coral colonies could be marginal habitats for these obligate coral-dwelling species, allowing them to survive for at least a period of time when live coral habitats are scarce or saturated or when they are searching for new territories. The implications of these findings are not yet clear: these organisms could be more resilient to coral loss than originally thought, at least in the short-term, or they may be reliant on a mosaic of microhabitats making them even more sensitive to disturbance. For instance, Enochs and Manzello (2012) and Kramer et al. (2014) have recently highlighted the importance of all coral microhabitats for ecosystem function (measured in standing biomass). We can conclude that the habitat associations of obligate coral-dwellers and their reliance on different habitat types is complex and further work is needed to establish their vulnerability to habitat degradation on coral reefs.

6.1.2. Comparative species delimitation

A robust approach to estimating decapod species richness was undertaken by comparing putative species estimates from a variety of species delimitation methods (Table 3.3). Overall the PTP approach was considered most accurate across decapod families/super-families, though often the performance of the methods was taxon-specific. Morphological identifications for many of the taxa made these comparisons in species delimitation results possible and highlighted the irreplaceability of taxonomic expertise (Kim and Byrne 2006). Until a comprehensive reference database matching barcodes to species names is available for all taxa this will likely remain the case, while molecular studies must continue in order to make this a reality. Many scientists now advocate for an integrative approach to species identification, combining both morphological and molecular approaches (Vogler and Monaghan 2007; Padial et al. 2010; Ross et al. 2010), which although certainly more robust, seems in some ways to defeat the point of molecular sequencing providing an alternative tool

for assessing biodiversity. If an integrative approach cannot be taken, then using two genes is increasingly 'the norm' in an attempt to increase the genetic information available to delimit species (Vences et al. 2005). However, where resources are scarce, as is often the case in biodiversity assessments, this is often not possible and cruder analyses using only one gene still have value, depending on the aims of the study (Vences et al. 2005). The taxon-specific nature of the species delimitation methods, as found here, does not provide a definite guideline for future studies to follow but rather suggests a comparative approach should be followed for a robust approach. Although more time-consuming this does not incur any additional financial costs. Alternatively, where available, past information on a species delimitation method's performance at estimating putative species for that specific taxon should be used. In the case of the decapods our study can be used as guidance by future decapod species delimitation studies. For example, we found the PTP method was most accurate for taxon Palaemonoidea and Hippolytidae and can therefore recommend this method for future studies for these taxa.

6.1.3. Decapod biodiversity on dead coral microhabitats

Decapod species richness on dead coral colonies in Chagos was found to be the highest for this microhabitat of any location studied globally to date. This provides further evidence of the 'pristine' nature of Chagos reef system, and its importance as a refuge in the Indian Ocean (Burke et al. 2011; Sheppard et al. 2012). The high decapod biodiversity estimates also highlight the importance of this microhabitat in supporting a diverse array of cyrptofauna. The role of dead coral skeletons in the ecosystem should not be underestimated because crustaceans play key trophic roles in coral reefs food webs (Enochs and Manzello 2012; Kramer et al. 2014). The high abundance of rare species was also a key finding suggesting, in accordance with other studies (e.g. Plaisance et al. 2009), that a high proportion of the cryptofauna are of low abundance. However, observed rare species could also be a consequence, at least in part, of incomplete sampling indicated by higher species estimates

and a lack of plateauing of the species rarefaction curves. If a high proportion of the decapods are truly rare this could potentially have important implications in relation to biodiversity loss of the cryptofauna and subsequently ecosystem function (See Broader Implications below). Eagle Island had a significantly lower species richness and abundance per coral colony than any other location, and the island's reefs were experiencing a crown-of-thorns (COTS) (*Acanthaster planci*) outbreak at the time of surveying (Roche et al. 2015/Appendix III). It seems likely that this could be at least partially the cause of the lower decapod diversity, implying that COTS outbreaks may have a wider impact on the ecosystem beyond the often extensive coral morality, which has previously been reported (e.g. Leray et al. 2012).

6.1.4. Processes underpinning Palaemonidae diversity

A focus on the processes underpinning the community structure of the Palaemonidae, a family of the decapods, revealed some interesting results. The community had spatial structure, indicating that different processes were shaping and maintaining communities at different spatial scales from the coral colony level to the whole Archipelago metacommunity. Environmental filtering was driving the community structure at the local scale (between coral colonies, and within atolls), but not limiting similarity, both key processes in community assembly and maintenance (Webb et al. 2002). The predominant environmental filter was the size of the coral colony acting on the shrimp body size. Phylogeny, i.e. the relatedness of species, also explained some trait diversity and patterns of coexistence at the local level, within atolls and between coral colonies. However, conflicting evidence at the metacommunity scale means processes acting at this scale require further investigation. Weak phylogenetic signal, suggests that both trait convergence and lability of trait evolution were key evolutionary processes shaping species distributions, at least at the local scale. Evidence of trait convergence highlights the importance of incorporating trait diversity alongside evolutionary history into biodiversity studies to gain a deeper understanding of the evolutionary and ecological processes underpinning community composition (Purschke et al.

2013; Pavoine et al. 2014). In summary, environmental filtering at the local scale, and trait convergence and labile rates of trait diversification are key processes in assembly and maintenance of this community. Understanding how these cryptofauna communities are assembled and maintained will help determine their responses to human disturbance.

6.2. Broader Implications

We have very little understanding of how direct or indirect human impacts affect cryptofauna biodiversity. However habitat degradation is likely to be largest threat (Stella et al. 2011a; Kramer et al. 2014). The availability of dead coral microhabitats is completely reliant on the turnover of live coral colonies. Therefore, increasing levels of coral loss globally, as a result of a synergy of anthropogenic pressures (Burke et al. 2011; WWF. 2015), will eventually also lead to the loss of dead coral microhabitats, though there could be an increase in habitat availability in the short-term. Ocean acidification, one of the anthropogenic induced processes driving these synergistic affects, is causing changes in ocean chemistry including reductions in aragonite saturation and lower pH of the water column (Veron et al. 2009). This is predicted to result in lower coral growth rates, more brittle coral skeletons, and more rapid erosion of dead and live coral structures, reducing the habitat availability for cryptofauna biodiversity (Veron et al. 2009; Stella et al. 2011a). Coralline algae, which are essential for cementing coral rubble, are even more vulnerable to acidification because of the type of carbonate, magnesium calcite, they secrete (Kuffner et al. 2007), further exacerbating dead coral and coral rubble microhabitat degradation. CO_2-induced pH reduction has also been shown to negatively affect crustaceans' exoskeleton mineralization, and biophotonics in shrimp, potentially leaving them more vulnerable to physical damage and predation (Taylor et al. 2015).

Coral loss threatens the maintenance of cryptofauna biodiversity, mainly through habitat degradation, and can result in biodiversity loss, as has been shown in coral-dwelling fish and corallivorous fish (Munday 2004; Pratchett et al. 2006). Biodiversity is thought to provide insurance such that should one species be lost there will be another species with a similar combination of traits available to maintain the functional processes it delivered, i.e. it provides redundancy to the system (Nystrom et al. 2000; Bellwood et al. 2004; Nystrom et al. 2012). This suggests that Chagos with its high cryptofauna biodiversity should have a higher insurance against loss of ecosystem function. However, biodiversity has recently been shown to have an exponential relationship with ecosystem function, for instance in global reef fish populations and in deep-sea sediments (Danovaro et al. 2008; Loreau 2008; Mora et al. 2011). If this is also true of the cryptofauna component of biodiversity, then this could imply that even a small loss in biodiversity could reduce ecosystem function. Furthermore, if the high proportion of rare decapod species observed inhabiting dead coral microhabitats in Chagos is true, rather than an artefact of incomplete sampling, this could make Chagos reefs vulnerable to losses in ecosystem functioning. This is because, despite previous assumptions that rare species do not play a particularly strong role in maintaining ecosystem function (e.g. Grime 1998), recent studies have demonstrated that rare species tend to support the most vulnerable functions in an ecosystem, and therefore the loss of rare species would more strongly influence ecosystem processes and properties (Zavaleta and Hulvey 2004; Lyons et al. 2005; Bracken and Low 2012; Mouillot et al. 2013). Further investigation into the functional redundancy within cryptofauna biodiversity is now needed before any conclusions can be drawn.

6.3. Limitations

Limited resources are almost always a constraint in biological research and this study was no exception. All data were collected from a 2.5 week and 3 week expedition to the Chagos

Archipelago in 2012 and 2013 respectively, made financially possible by the Darwin Initiative and Selfridges' Project Ocean funding. One of the limitations of the study was therefore limited fieldwork time. The remote location of the archipelago makes fieldwork expensive, as well as logistically difficult, and a lack of long-term funding makes planning expeditions challenging. Expeditions are multidisciplinary, meaning research activities have to be co-ordinated across scientists and are rarely optimal for a single study. The sampling design was therefore uneven across the atolls because fieldwork had to be delivered as part of shared research expeditions. In addition to financial limitations, restricted access to Chagos by the UK Foreign and Commonwealth Office was also a factor, though it is becoming far more open in recent years, and greatly supported by access to the Pacific Marlin as a research vessel. Limited fieldwork time meant sample size was too small to capture all cryptofauna biodiversity (Fig. 4.1), capturing an estimated 75% of decapod biodiversity, though every effort was made to maximise sample size following similar results of insufficient sample size from previous cryptofauna studies at different locations (Plaisance et al. 2009; Enochs and Manzello 2012). The main consequence of a limited sample size is the potential for bias in the representation of rare species, i.e. rare species in a community will be found to be less represented in a smaller sample both with regard to their occurrence and their relative abundance, while common species will often be adequately represented (Beck et al 2013; Magurran and McGill 2011). Undersampling is commonplace in species-rich tropical assemblages and possible repercussions are well-studied (e.g. Chao et al. 2006; Beck et al. 2013; Meyer et al 2015). Many species estimators of alpha diversity have been developed to correct for undersampling, e.g. Chao and ACE, but undersampling can also bias the measurement of beta diversity (here we used the Bray-Curtis Dissimilarity metric to measure beta diversity), and this bias is harder to account for (Chao et al 2006; Beck et al 2013). A recent study on the consequences of information loss due to species undersampling in tropical bat surveys found that sampling capturing 85% of bat biodiversity was reliable at revealing patterns of species richness, rarity and temporal or spatial turnover in most assemblages

(Meyer et al. 2015). However estimation of the sampling required for adequate species detection will differ for different faunal communities because of variation in biotic factors.

The lack of taxonomic expertise was another major limitation. Even if collecting more coral colonies evenly across the archipelago had been possible, it might not have been possible to identify all resulting cryptofauna specimens because of a lack of taxonomic expertise and limited finances for molecular work. Indeed this is also why some of the samples collected on the 2013 expedition were not included in Chapter 3 and 4. Whilst it should also be noted that the sequencing success rate for the Brachyura and Hippolytidae was relatively low, at 57% and 59% respectively, limited finances meant PCRs could not be repeated to obtain a higher success rate. Ideally we would also have had morphological identifications for the Paguridae and Porcellanidae (Chapter 3) to provide an integrative approach to species identification, but finding a taxonomist willing to identify these groups within the timeframe proved too challenging, and taxonomical guides for these groups are not available.

In a comparative study of the analytical methods of species delimitation, Chapter 3, we highlighted some of the limitations of these methods, such as delimiting rare species, which given the high proportion of rare species in reef cryptofauna samples (Chapter 4), could result in substantial under-estimation of species richness in cryptofauna taxa. The main restriction in using molecular barcoding methods for reef cryptofauna is the limited ability to put species names to molecular barcodes because of a deficiency in cryptofauna species barcodes in molecular reference databases, such as GenBank and BOLD. Presumably this is a result of the lack of cryptofauna studies, high species richness, and potentially a high number of rare species within this component of biodiversity. However, molecular methods can still be very useful in quantifying biodiversity, if not identifying species per se. We recognise the major advances that have been made in molecular techniques over the past two decades (Hebert et al. 2003; Thomsen and Willerslev 2015), and hope that comparative studies such as this will aid further methodological improvements.

With regards to Chapter 5, the functional traits of most cryptofauna taxa are poorly understood, limiting our investigation of the trait diversity of the Palaemonidae to the fundamental traits of body size and fecundity, alongside habitat association. As our knowledge of important functional traits for each cryptofauna group increases, more can be learned about the evolutionary and ecological mechanisms shaping community assembly. In addition, the construction of a phylogenetic tree of the Palaemonidae is still in its infancy, though many advances have been made in recent studies (Kou et al. 2013; Kou et al. 2014; De Grave et al. 2015). To overcome this limitation we sequenced four genes for each species from the community to produce a composite community phylogeny, enabling phylogenetic diversity within the community to be used as a measure of biodiversity.

6.4. Future Directions

6.4.1. Advances in tools

Reef health and resilience monitoring programmes, such as the IUCN's Reef Resilience protocol (Obura and Grimsditch 2009) and the popular citizen science Reef Check Programme (Freiwald et al. 2013), focus on fish and coral composition, and sometimes also larger invertebrates. However, we have demonstrated here that the cryptofauna is species rich, and actually the most biodiverse component of coral reefs (Reaka-Kudla 1997), spanning many important trophic groups, with high productivity and biomass levels (Enochs 2012; Kramer et al. 2014). Therefore by failing to incorporate this aspect of biodiversity into reef health indicators we risk missing vital clues to losses in ecosystem functioning. At present it still remains impractical to quickly measure this component of biodiversity, alongside fish and coral, partly because of the skills required and the time involved, but new sampling and molecular tools are fast evolving, offering great potential for the future.

6.4.2. Autonomous Reef Monitoring Structures (ARMS)

To improve quantitative sampling of the cryptofauna 'non-natural reef structures' have been developed (Zimmerman and Martin 2004), which provide a standardised collection tool and remove the destructive nature of sampling. The census of coral reef ecosystem project (CReefs), which began in 2005, developed prototype Autonomous Reef Monitoring Structures (ARMS) consisting of stacked PVC layers of different sizes and shaped openings allowing organisms to settle or shelter with the structure. ARMS are designed to mimic to some degree the complexity of a coral reef, be practical, and cause minimal disturbance to the reef in deployment and collection (www.creefs.org/research_arms.html). The project has deployed devices globally in shallow fore-reef habitats in the Pacific, Atlantic and Indian Oceans, though very few devices were deployed in the Indian Ocean (Plaisance et al. 2011). Plaisance et al. (2011), the first to publish research utilising ARMS, examined the crustacean spatial diversity patterns for three habitat types (fore-reef, back-reef, and lagoon) across four Pacific Ocean locations. Informed and implemented by this study, ARMS have now been deployed in Chagos, which will offer useful comparative insights into the future.

6.4.3. Environmental DNA and metabarcoding

Environmental DNA (eDNA) is defined as genetic material obtained directly from environmental samples (water, sediment etc.) without any obvious signs of biological source material (Thomsen and Willerslev 2015). It is an efficient, non-invasive and easy-to-standardise sampling approach, which when coupled with cost-efficient, fast-evolving DNA sequencing techniques is emerging as a promising and effective tool to meet the challenges of biodiversity assessments (Kelly et al. 2014; Thomsen and Willerslev 2015). Metabarcoding is a rapid method of biodiversity assessment, which combines DNA based identification with high-throughput DNA sequencing (Ji et al. 2013). This next generation sequencing (NGS)

uses universal PCR primers to mass-amplify DNA barcodes from the eDNA sample, then the PCR product is sent through a next generation sequencer producing hundreds of thousands of sequences that need to be filtered (Ji et al. 2013). The translation of DNA sequence diversity obtained from metabarcoding to actual 'species' richness and diversity is not straightforward and probably presents the biggest challenge of the technique (Coissac et al. 2012). However, a range of pipelines have been developed to aid processing of NGS data and progress continues in standardising and improving clustering, e.g. EPA-PTP (Coissac et al. 2012; Puillandre et al. 2012; Zhang et al. 2013). To date most studies utilising eDNA and metabarcoding have focused on verifying the presence of species identified from eDNA against traditional fieldwork techniques (Ji et al. 2013; Thomsen and Willerslev 2015). In the marine environment initial studies, for instance on marine fish fauna (Thomsen et al. 2012), show promise for future assessments and monitoring of marine biodiversity and resources (Kelly et al. 2014; Thomsen and Willerslev 2015).

6.4.4. Advancing Biological Understanding

This study advances our knowledge of cryptofauna biodiversity, and whilst undertaking this research many areas of potential further study in this field became evident. Perhaps partly because reef cryptofauna biodiversity and its role in ecosystem function is relatively understudied, especially in comparison to the fish and coral faunal components on coral reefs. Below I have highlighted what we believe are the most important and interesting directions for future investigation in this field as a result of this study:

- The high prevalence of coral-dwelling obligates on dead coral microhabitats in Chagos presents many questions, and understanding the biological reasons for the spill-over of obligate coral-dwellers to dead coral microhabitats could be important to understanding how coral associates might respond to predicted coral loss. A starting point would be to investigate the abundance of obligate coral-dwellers on live corals

in Chagos and whether these microhabitats are saturated with decapods as a possible reason for their spill-over to dead coral microhabitats.

- Research into the functional redundancy within the decapods would give insight into the potential impact of biodiversity loss on ecosystem function, especially considering the high-proportion of rare species identified here.

- To date we have quantified the decapod and mollusc components of the cryptofauna, but other large components of the cryptofauna remain to be quantified such as polychaetes and echinoderms. In addition, quantification of cryptofauna diversity on other microhabitats would provide a more complete baseline assessment of cryptofauna biodiversity on coral reefs.

- This study has provided a baseline to which we can compare other reefs under higher levels of anthropogenic pressure to understand how this component of biodiversity is responding to multiple stressors. However, this study also demonstrated that even a small difference in coral colony size can affect cryptofauna diversity, therefore to standardise for this effect ARMS could be used.

- Here we began to understand the processes underpinning Palaemonoidea community structure. One of the mechanisms likely to regulate decapod diversity is predation because decapods are an extremely important prey source for reef fish species (Williams and Hatcher 1983; Randall et al. 1997), and yet hardly any quantitative dietary informative is available (But see Kramer et al. 2015), relative to for instance the grazers e.g. parrotfish (Scaridae). Given that decapods support such a high proportion of higher consumers further research to quantify and understand the predator-prey relationship is necessary to improve our knowledge of trophic

dynamics on coral reefs ecosystems and the mechanisms shaping decapod communities.

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8. APPENDIX

Appendix I: Decapod species list and abundances

Infra-order	Species	Abundance
Anomura	*Galathea eulimene*	65
Anomura	*Galathea pilosa*	2
Anomura	*Galathea tanegashimae*	28
Anomura	*Galathea aegyptiaca*	1
Anomura	*Galathea aff spinosorostris*	115
Anomura	*Galathea platycheles*	92
Anomura	*Paguroidea Species 1*	1
Anomura	*Paguroidea Species 2*	3
Anomura	*Paguroidea Species 3*	2
Anomura	*Paguroidea Species 4*	52
Anomura	*Paguroidea Species 5*	2
Anomura	*Paguroidea Species 6*	2
Anomura	*Paguroidea Species 7*	13
Anomura	*Paguroidea Species 8*	2
Anomura	*Paguroidea Species 9*	19
Anomura	*Paguroidea Species 10*	2
Anomura	*Paguroidea Species 11*	46
Anomura	*Paguroidea Species 12*	17
Anomura	*Paguroidea Species 13*	2
Anomura	*Phyladiorhynchus sp*	1
Anomura	*Porcellanidae Species 1*	1
Anomura	*Porcellanidae Species 2*	12
Anomura	*Porcellanidae Species 3*	1
Anomura	*Porcellanidae Species 4*	76
Anomura	*Porcellanidae Species 5*	1
Anomura	*Porcellanidae Species 6*	1
Anomura	*Porcellanidae Species 7*	6
Anomura	*Sadayoshia edwardsii*	1
Brachyura	*Actaea sp*	2
Brachyura	*Actaea spinoisssima*	1
Brachyura	*Actumnus sp*	1
Brachyura	*Cherusius triunguiculatus*	3
Brachyura	*Chlorodiella laevissima*	3
Brachyura	*Chlorodiella sp*	9
Brachyura	*Chlorodopsis melanospinis*	19
Brachyura	*Cryptodromia sp*	2
Brachyura	*Cyclodius granulosus*	7
Brachyura	*Cyclodius sp*	2
Brachyura	*Cymo sp*	6
Brachyura	*Domecia glabra*	39

Brachyura	*Domecia hispida*	5
Brachyura	*Dynomene sp*	6
Brachyura	*Eupilumnus sp*	8
Brachyura	*Euxanthus sp*	1
Brachyura	*Globopilumnus sp*	1
Brachyura	*Hirsutodynomene spinosa*	3
Brachyura	*Hyastenus cf uncifer*	8
Brachyura	*Lahaina sp*	1
Brachyura	*Liocarpilodes sp*	1
Brachyura	*Liomera cf rubra*	3
Brachyura	*Liomera cf rugata*	1
Brachyura	*Liomera cf tristis*	3
Brachyura	*Liomera cinctimanus*	1
Brachyura	*Liomera sp*	1
Brachyura	*Lophozozymus anglyptus*	15
Brachyura	*Lybia tessellata*	1
Brachyura	*Pilumnus sp*	5
Brachyura	*Paraxanthodes sp*	5
Brachyura	*Percnon aff guinotae*	1
Brachyura	*Percnon sp*	3
Brachyura	*Perinia cf uncifer*	1
Brachyura	*Perinia tumida*	13
Brachyura	*Pilodius aff spinipes*	2
Brachyura	*Pilodius melanospinis*	7
Brachyura	*Pilodius sp*	12
Brachyura	*Pilumnidae sp*	12
Brachyura	*Pilumnus aff tantulus*	7
Brachyura	*Pilumnus cf longicornis*	3
Brachyura	*Pilumnus sp*	16
Brachyura	*Platypodia anglyplus*	4
Brachyura	*Pseudoliomera lata*	3
Brachyura	*Pseudoliomera sp*	5
Brachyura	*Quadrella sp*	1
Brachyura	*Schizophrys sp*	9
Brachyura	*Tetralia sp*	2
Brachyura	*Tiarinia sp*	16
Brachyura	*Trapezia bidentata*	1
Brachyura	*Trapezia sp*	82
Brachyura	*Trapezia tigrina*	11
Brachyura	*Tweedieia odhneri*	29
Brachyura	*Tylocarcinus styx*	50
Brachyura	*Viaderiana typica*	19
Brachyura	*Xanthias cherbonnieri*	5
Brachyura	*Xanthias sp 1*	2
Brachyura	*Xanthias sp 2*	1

Caridea	*Coralliocaris viridis*	2
Caridea	*Cuapetes grandis complex*	10
Caridea	*Harpiliopsis spinigera*	43
Caridea	*Harplius lutescens*	5
Caridea	*Jocaste lucina*	63
Caridea	*Palaemonella spinulata*	1
Caridea	*Periclimenaeus diplosomatis*	2
Caridea	*Cuapetes elegans*	11
Caridea	*Harpiliopsis depressa*	3
Caridea	*Jocaste japonica*	12
Caridea	*Laomenes sp*	1
Caridea	*Isopericlimenaeus gorgonidarum*	1
Caridea	*Periclimenaeus bidentatus*	3
Caridea	*Palaemonella tenuipes*	1
Caridea	*Cuapetes ensifrons*	1
Caridea	*Cuapetes longirostris*	2
Caridea	*Exoclimenella maldivensis*	1
Caridea	*Harpiliopsis beaupressi*	3
Caridea	*Palaemonella rotumana*	4
Caridea	*Periclimenes pettithouarsi*	1
Caridea	*Hippolyte ventricosa*	1
Caridea	*Saron marmoratus*	1
Caridea	*Saron neglectus*	103
Caridea	*Saron sp*	20
Caridea	*Thor amboimensis*	3
Caridea	*Thor sp*	37
Caridea	*Thorella cobourgi*	5
Caridea	*Thorina maldivensis*	92
Caridea	*Alpheid species 1*	2
Caridea	*Alpheid species 2*	17
Caridea	*Alpheid species 3*	3
Caridea	*Alpheid species 4*	3
Caridea	*Alpheid species 5*	5
Caridea	*Alpheid species 6*	2
Caridea	*Alpheid species 7*	9
Caridea	*Alpheid species 8*	8
Caridea	*Alpheid species 9*	6
Caridea	*Alpheid species 10*	3
Caridea	*Alpheid species 11*	9
Caridea	*Alpheid species 12*	3
Caridea	*Alpheid species 13*	2
Caridea	*Alpheid species 14*	1
Caridea	*Alpheid species 15*	3
Caridea	*Alpheid species 16*	7
Caridea	*Alpheid species 17*	22

Caridea	*Alpheid species 18*	5
Caridea	*Alpheid species 19*	2
Caridea	*Alpheid species 20*	11
Caridea	*Alpheid species 21*	7
Caridea	*Alpheid species 22*	3
Caridea	*Alpheid species 23*	22
Caridea	*Alpheid species 24*	3
Caridea	*Alpheid species 25*	2
Caridea	*Alpheid species 26*	3
Caridea	*Alpheid species 27*	2
Caridea	*Alpheid species 28*	5
Caridea	*Alpheid species 29*	14
Caridea	*Alpheid species 30*	5
Caridea	*Alpheid species 31*	1
Caridea	*Alpheid species 32*	1
Caridea	*Alpheid species 33*	1
Caridea	*Alpheid species 34*	1
Caridea	*Alpheid species 35*	1
Caridea	*Alpheid species 36*	1
Caridea	*Alpheid species 37*	1
Caridea	*Alpheid species 38*	2
Caridea	*Alpheid species 39*	1
Caridea	*Alpheid species 40*	1
Caridea	*Alpheid species 41*	1
Caridea	*Alpheid species 42*	1
Caridea	*Alpheid species 43*	1
Caridea	*Alpheid species 44*	1
Caridea	*Alpheid species 45*	1
Caridea	*Alpheid species 46*	1
Caridea	*Alpheid species 47*	1
Caridea	*Alpheid species 48*	1
Caridea	*Alpheid species 49*	1
Caridea	*Alpheid species 50*	1
Caridea	*Alpheid species 51*	2

Appendix II: Decapod species richness and abundance per site matrix.

Appendix III: Co-authored research note published in the course of the D.Phil.

Site	***Alpheid species 1***	***Alpheid species 2***	***Alpheid species 3***	***Alpheid species 4***	***Alpheid species 5***	***Alpheid species 6***	***Alpheid species 7***	***Alpheid species 8***	***Alpheid species 9***
Brothers 1	0	0	0	0	0	0	0	0	0
Brothers 2	1	0	0	0	0	0	0	0	6
Brothers 3	0	0	0	0	0	0	0	0	0
Diego Garcia 1	0	1	0	0	1	0	2	0	0
Diego Garcia 2	0	1	0	0	0	0	0	0	0
Diego Garcia 3	0	0	0	0	0	0	0	0	0
Diego Garcia 4	0	0	0	0	0	0	0	0	0
Diego Garcia 5	0	0	0	0	0	0	0	0	0
Diego Garcia 6	0	0	0	0	0	0	0	0	0
Eagle 1	0	0	0	0	0	0	0	0	0
Eagle 2	0	3	1	0	0	0	0	0	0
Eagle 3	0	0	0	0	0	0	0	0	0
Egmont 1	0	1	0	0	0	0	0	0	0
Egmont 2	0	2	0	0	0	0	1	1	0
Peros Banhos 1	0	0	0	1	0	1	0	1	0
Peros Banhos 2	0	1	0	1	0	0	0	0	0
Peros Banhos 3	0	1	0	0	1	0	0	0	0
Peros Banhos 4	0	0	0	0	0	0	0	0	0
Peros Banhos 5	0	0	0	0	1	0	1	0	0
Peros Banhos 6	0	0	0	0	0	0	0	0	0
Peros Banhos 7	0	2	1	1	2	0	0	1	0
Peros Banhos 8	0	0	0	0	0	0	1	0	0
Salomon 1	0	6	1	0	0	0	2	0	0
Salomon 2	0	0	0	0	0	0	1	0	0
Salomon 3	1	0	0	0	0	1	1	5	0

Site	***Alpheid species 10***	***Alpheid species 11***	***Alpheid species 12***	***Alpheid species 13***	***Alpheid species 14***	***Alpheid species 15***	***Alpheid species 16***	***Alpheid species 17***	***Alpheid species 18***
Brothers 1	0	0	0	0	0	0	0	0	0
Brothers 2	0	0	0	0	0	0	0	1	0
Brothers 3	0	1	0	0	0	0	0	0	0
Diego Garcia 1	0	0	0	1	0	1	0	0	0
Diego Garcia 2	0	0	0	0	0	0	0	2	0
Diego Garcia 3	0	0	0	0	0	0	0	0	0
Diego Garcia 4	0	0	1	0	1	1	0	0	0
Diego Garcia 5	0	0	0	0	0	1	3	0	0
Diego Garcia 6	0	0	0	0	0	0	0	0	0
Eagle 1	0	0	0	0	0	0	0	0	0
Eagle 2	0	0	0	0	0	0	0	1	0
Eagle 3	0	0	0	0	0	0	0	0	0
Egmont 1	0	1	0	0	0	0	0	1	0
Egmont 2	0	1	0	0	0	0	1	3	0
Peros Banhos 1	0	4	0	1	0	0	0	5	1
Peros Banhos 2	1	0	1	0	0	0	0	1	0
Peros Banhos 3	0	0	0	0	0	0	0	0	0
Peros Banhos 4	0	0	0	0	0	0	0	1	0
Peros Banhos 5	0	1	0	0	0	0	0	7	0
Peros Banhos 6	0	0	1	0	0	0	0	0	0
Peros Banhos 7	0	0	0	0	0	0	1	1	0
Peros Banhos 8	0	0	0	0	0	0	0	1	1
Salomon 1	1	0	0	0	0	0	1	1	0
Salomon 2	0	1	0	0	0	0	1	2	1
Salomon 3	1	0	0	0	0	0	0	2	2

Site	*Alpheid species 19*	*Alpheid species 20*	*Alpheid species 21*	*Alpheid species 22*	*Alpheid species 23*	*Alpheid species 24*	*Alpheid species 25*	*Alpheid species 26*	*Alpheid species 27*
Brothers 1	0	0	0	0	1	0	0	0	0
Brothers 2	0	1	0	0	0	0	0	0	0
Brothers 3	0	0	0	0	0	0	0	0	0
Diego Garcia 1	0	0	0	0	0	0	0	0	0
Diego Garcia 2	0	0	0	0	1	1	0	1	0
Diego Garcia 3	0	0	1	0	1	0	0	0	0
Diego Garcia 4	0	2	1	0	2	0	0	0	0
Diego Garcia 5	0	1	0	1	6	0	0	0	0
Diego Garcia 6	0	0	0	0	0	0	0	0	0
Eagle 1	0	0	0	0	0	0	0	0	0
Eagle 2	0	0	0	0	0	0	0	0	0
Eagle 3	0	0	0	0	0	0	0	0	0
Egmont 1	0	1	1	0	2	0	0	1	0
Egmont 2	0	0	0	1	2	0	1	0	0
Peros Banhos 1	0	2	1	0	1	0	0	1	0
Peros Banhos 2	0	1	1	1	3	0	0	0	0
Peros Banhos 3	0	1	1	0	0	0	0	0	0
Peros Banhos 4	0	0	0	0	0	0	0	0	0
Peros Banhos 5	0	0	0	0	1	1	0	0	0
Peros Banhos 6	0	0	0	0	1	0	0	0	0
Peros Banhos 7	0	0	0	0	1	0	0	0	0
Peros Banhos 8	0	1	0	0	0	0	0	0	0
Salomon 1	0	0	1	0	2	0	1	0	2
Salomon 2	2	0	0	0	0	0	0	0	0
Salomon 3	0	1	0	0	0	1	0	0	0

Site	*Alpheid species 28*	*Alpheid species 29*	*Alpheid species 30*	*Alpheid species 31*	*Alpheid species 32*	*Alpheid species 33*	*Alpheid species 34*	*Alpheid species 35*	*Alpheid species 36*
Brothers 1	0	0	0	0	1	0	0	0	0
Brothers 2	0	0	0	0	0	0	0	0	0
Brothers 3	0	0	0	0	0	0	0	0	0
Diego Garcia 1	0	1	1	1	0	0	0	0	0
Diego Garcia 2	1	3	0	0	0	0	0	0	0
Diego Garcia 3	3	0	0	0	0	0	0	0	0
Diego Garcia 4	0	0	1	0	0	0	0	0	0
Diego Garcia 5	0	1	0	0	0	0	1	0	0
Diego Garcia 6	0	0	0	0	0	0	0	0	0
Eagle 1	0	0	0	0	0	0	0	0	0
Eagle 2	0	1	0	0	0	0	0	0	0
Eagle 3	0	0	0	0	0	0	0	0	0
Egmont 1	0	0	0	0	0	0	0	0	0
Egmont 2	0	0	0	0	0	0	0	0	0
Peros Banhos 1	0	2	1	0	0	0	0	0	1
Peros Banhos 2	0	0	0	0	0	0	0	0	0
Peros Banhos 3	0	0	0	0	0	0	0	0	0
Peros Banhos 4	0	0	0	0	0	0	0	1	0
Peros Banhos 5	1	1	0	0	0	0	0	0	0
Peros Banhos 6	0	0	1	0	0	0	0	0	0
Peros Banhos 7	0	0	0	0	0	0	0	0	0
Peros Banhos 8	0	0	1	0	0	0	0	0	0
Salomon 1	0	3	0	0	0	1	0	0	0
Salomon 2	0	0	0	0	0	0	0	0	0
Salomon 3	0	2	0	0	0	0	0	0	0

Site	*Alpheid species 37*	*Alpheid species 38*	*Alpheid species 39*	*Alpheid species 40*	*Alpheid species 41*	*Alpheid species 42*	*Alpheid species 43*	*Alpheid species 44*	*Alpheid species 45*
Brothers 1	0	0	0	0	0	0	0	0	0
Brothers 2	0	0	0	0	0	0	0	0	0
Brothers 3	0	0	0	0	0	0	0	0	0
Diego Garcia 1	0	1	0	0	0	1	0	1	0
Diego Garcia 2	0	0	0	0	0	0	0	0	0
Diego Garcia 3	0	0	0	0	1	0	0	0	0
Diego Garcia 4	0	0	0	0	0	0	0	0	0
Diego Garcia 5	0	0	0	0	0	0	0	0	0
Diego Garcia 6	0	0	0	0	0	0	0	0	0
Eagle 1	0	0	0	0	0	0	0	0	0
Eagle 2	0	0	0	0	0	0	0	0	0
Eagle 3	0	0	0	0	0	0	0	0	0
Egmont 1	0	0	0	0	0	0	0	0	1
Egmont 2	0	0	0	0	0	0	0	0	0
Peros Banhos 1	0	0	0	0	0	0	0	0	0
Peros Banhos 2	0	0	0	1	0	0	1	0	0
Peros Banhos 3	0	0	0	0	0	0	0	0	0
Peros Banhos 4	0	0	0	0	0	0	0	0	0
Peros Banhos 5	0	0	0	0	0	0	0	0	0
Peros Banhos 6	0	1	0	0	0	0	0	0	0
Peros Banhos 7	0	0	0	0	0	0	0	0	0
Peros Banhos 8	0	0	0	0	0	0	0	0	0
Salomon 1	0	0	0	0	0	0	0	0	0
Salomon 2	0	0	1	0	0	0	0	0	0
Salomon 3	1	0	0	0	0	0	0	0	0

Site	*Alpheid species 46*	*Alpheid species 47*	*Alpheid species 48*	*Alpheid species 49*	*Alpheid species 50*	*Alpheid species 51*	*Coralliocaris viridis*	*Cuapetes elegans*	*Cuapetes ensifrons*
Brothers 1	0	0	0	0	0	0	0	0	0
Brothers 2	0	0	0	0	0	0	1	0	0
Brothers 3	0	0	0	0	0	0	0	0	0
Diego Garcia 1	0	0	0	0	0	0	0	0	0
Diego Garcia 2	0	1	0	0	0	0	0	3	0
Diego Garcia 3	0	0	0	0	0	0	0	1	0
Diego Garcia 4	0	0	0	0	0	0	0	0	0
Diego Garcia 5	1	0	0	0	1	0	0	0	0
Diego Garcia 6	0	0	0	0	0	0	0	0	0
Eagle 1	0	0	0	0	0	0	0	0	0
Eagle 2	0	0	0	0	0	0	0	0	0
Eagle 3	0	0	0	0	0	0	0	0	0
Egmont 1	0	0	0	0	0	0	0	0	0
Egmont 2	0	0	0	0	0	1	0	0	0
Peros Banhos 1	0	0	0	0	0	0	0	4	0
Peros Banhos 2	0	0	0	1	0	0	0	1	1
Peros Banhos 3	0	0	0	0	0	0	0	0	0
Peros Banhos 4	0	0	0	0	0	0	0	0	0
Peros Banhos 5	0	0	0	0	0	0	0	0	0
Peros Banhos 6	0	0	0	0	0	0	0	0	0
Peros Banhos 7	0	0	0	0	0	0	0	0	0
Peros Banhos 8	0	0	0	0	0	0	0	0	0
Salomon 1	0	0	0	0	0	1	1	0	0
Salomon 2	0	0	1	0	0	0	0	0	0
Salomon 3	0	0	0	0	0	0	0	2	0

Site	*Cuapetes grandis complex*	*Cuapetes longirostris cf*	*Exoclimenella maldivensis*	*Harpiliopsis beaupressi*	*Harpiliopsis depressa*	*Harpiliopsis spinigera*	*Harplius lutescens cf*	*Hippolyte ventricosa*	*Isopericlimenaeus gorgonidarum*
Brothers 1	1	0	0	0	0	0	0	0	0
Brothers 2	0	0	0	0	0	7	5	0	0
Brothers 3	0	0	0	0	0	2	0	0	0
Diego Garcia 1	0	0	0	0	0	0	0	0	0
Diego Garcia 2	0	0	0	0	0	0	0	0	0
Diego Garcia 3	0	0	0	0	0	4	0	0	0
Diego Garcia 4	4	0	0	0	0	0	0	0	0
Diego Garcia 5	0	0	0	0	2	8	0	0	0
Diego Garcia 6	0	0	0	0	0	0	0	0	0
Eagle 1	0	0	0	0	0	1	0	0	1
Eagle 2	0	0	0	0	0	2	0	0	0
Eagle 3	0	0	0	0	0	0	0	0	0
Egmont 1	1	0	0	0	1	2	0	0	0
Egmont 2	2	0	0	0	0	1	0	0	0
Peros Banhos 1	0	0	0	1	0	5	0	0	0
Peros Banhos 2	0	0	1	0	0	1	0	0	0
Peros Banhos 3	0	1	0	0	0	0	0	1	0
Peros Banhos 4	0	0	0	0	0	0	0	0	0
Peros Banhos 5	1	0	0	0	0	2	0	0	0
Peros Banhos 6	0	0	0	0	0	0	0	0	0
Peros Banhos 7	0	0	0	0	0	0	0	0	0
Peros Banhos 8	0	0	0	0	0	0	0	0	0
Salomon 1	1	1	0	0	0	2	0	0	0
Salomon 2	0	0	0	2	0	5	0	0	0
Salomon 3	0	0	0	0	0	1	0	0	0

Site	*Jocaste japonica*	*Jocaste lucina*	*Laomenes sp*	*Palaemonella rotumana cf*	*Palaemonella spinulata cf*	*Palaemonella tenuipes cf*	*Periclimenaeus bidentatus cf*	*Periclimenaeus diplosomatis cf*	*Periclimenes pettithouarsi*
Brothers 1	0	0	0	0	1	0	0	0	0
Brothers 2	0	4	0	0	0	0	0	2	0
Brothers 3	0	3	0	0	0	0	0	0	0
Diego Garcia 1	0	0	1	0	0	0	0	0	0
Diego Garcia 2	1	2	0	0	0	0	0	0	0
Diego Garcia 3	0	4	0	0	0	0	0	0	0
Diego Garcia 4	0	0	0	0	0	0	0	0	0
Diego Garcia 5	1	3	0	0	0	0	0	0	0
Diego Garcia 6	0	0	0	0	0	0	0	0	0
Eagle 1	2	4	0	0	0	0	1	0	0
Eagle 2	0	2	0	0	0	0	0	0	0
Eagle 3	0	1	0	0	0	0	0	0	0
Egmont 1	1	2	0	0	0	1	0	0	0
Egmont 2	0	2	0	0	0	0	0	0	0
Peros Banhos 1	0	2	0	0	0	0	0	0	0
Peros Banhos 2	3	2	0	1	0	0	0	0	0
Peros Banhos 3	2	4	0	0	0	0	1	0	0
Peros Banhos 4	0	0	0	0	0	0	0	0	0
Peros Banhos 5	0	6	0	1	0	0	0	0	1
Peros Banhos 6	0	0	0	0	0	0	1	0	0
Peros Banhos 7	0	10	0	0	0	0	0	0	0
Peros Banhos 8	0	1	0	0	0	0	0	0	0
Salomon 1	2	1	0	2	0	0	0	0	0
Salomon 2	0	0	0	0	0	0	0	0	0
Salomon 3	0	7	0	0	0	0	0	0	0

Site	*Saron marmoratus*	*Saron neglectus*	*Saron sp*	*Thor amboimensis*	*Thor sp*	*Thorella cobourgi*	*Thorina maldivensis*	*Actaea sp*	*Actaea spinoisssima*
Brothers 1	0	7	0	0	4	0	2	0	0
Brothers 2	0	19	0	0	1	0	0	1	0
Brothers 3	0	2	0	0	1	0	1	0	1
Diego Garcia 1	0	1	0	0	3	0	6	0	0
Diego Garcia 2	0	1	0	0	9	0	1	0	0
Diego Garcia 3	0	6	4	1	0	0	9	0	0
Diego Garcia 4	0	1	0	0	0	0	0	0	0
Diego Garcia 5	0	0	0	0	0	0	14	0	0
Diego Garcia 6	0	0	0	0	1	0	3	0	0
Eagle 1	0	5	0	0	0	0	0	0	0
Eagle 2	0	0	0	2	1	0	0	0	0
Eagle 3	0	2	0	0	0	0	0	0	0
Egmont 1	0	5	0	0	0	5	0	0	0
Egmont 2	0	0	0	0	0	0	20	0	0
Peros Banhos 1	0	7	7	0	4	0	7	0	0
Peros Banhos 2	0	20	3	0	0	0	5	0	0
Peros Banhos 3	0	3	2	0	0	0	0	0	0
Peros Banhos 4	0	1	0	0	0	0	0	0	0
Peros Banhos 5	0	2	0	0	2	0	10	0	0
Peros Banhos 6	0	4	0	0	1	0	8	0	0
Peros Banhos 7	0	6	0	0	1	0	0	0	0
Peros Banhos 8	0	0	0	0	0	0	0	0	0
Salomon 1	1	4	1	0	2	0	5	0	0
Salomon 2	0	7	3	0	6	0	1	0	0
Salomon 3	0	0	0	0	1	0	0	1	0

Site	***Actumnus sp***	***Cherusius triunguiculatus***	***Chlorodiella laevissima***	***Chlorodiella sp***	***Chlorodopsis melanospinis***	***Cryptodromia sp***	***Cyclodius granulosus***	***Cyclodius sp***	***Cymo sp***
Brothers 1	0	0	0	0	2	0	1	0	0
Brothers 2	0	0	0	0	4	0	0	0	0
Brothers 3	0	0	0	0	0	0	0	0	0
Diego Garcia 1	0	0	0	0	1	0	0	0	0
Diego Garcia 2	0	1	0	4	1	0	0	0	0
Diego Garcia 3	0	0	0	0	0	0	0	0	0
Diego Garcia 4	0	0	0	0	0	0	0	0	0
Diego Garcia 5	0	0	0	0	0	0	0	0	0
Diego Garcia 6	0	0	0	1	0	0	0	0	0
Eagle 1	0	0	0	0	0	0	0	0	0
Eagle 2	0	0	0	0	0	1	4	0	0
Eagle 3	0	0	0	0	0	0	0	0	0
Egmont 1	0	0	0	2	0	0	0	0	0
Egmont 2	0	0	0	1	2	0	0	0	0
Peros Banhos 1	0	0	0	0	3	0	0	0	0
Peros Banhos 2	0	0	0	0	0	0	0	0	0
Peros Banhos 3	0	0	0	1	0	0	0	1	0
Peros Banhos 4	0	0	2	0	0	0	0	0	0
Peros Banhos 5	0	0	0	0	0	0	0	0	0
Peros Banhos 6	0	0	0	0	1	0	2	0	6
Peros Banhos 7	0	0	0	0	0	0	0	0	0
Peros Banhos 8	0	0	0	0	1	0	0	0	0
Salomon 1	0	2	0	0	3	1	0	0	0
Salomon 2	0	0	1	0	1	0	0	0	0
Salomon 3	1	0	0	0	0	0	0	0	0

Site	***Domecia glabra***	***Domecia hispida***	***Dynomene sp***	***Eupilumnus sp***	***Euxanthus sp***	***Globopilumnus sp***	***Hirsutodynomene spinosa***	***Hyastenus cf uncifer***	***Lahaina sp***
Brothers 1	3	0	0	0	0	0	0	4	0
Brothers 2	3	2	0	0	0	0	0	0	0
Brothers 3	13	1	0	0	0	0	0	0	0
Diego Garcia 1	1	0	0	0	0	0	0	1	0
Diego Garcia 2	0	0	0	0	0	0	0	0	0
Diego Garcia 3	0	0	0	0	0	0	0	0	0
Diego Garcia 4	0	0	0	2	0	1	0	0	0
Diego Garcia 5	0	0	0	1	0	0	0	0	0
Diego Garcia 6	0	0	1	2	0	0	0	0	0
Eagle 1	0	0	0	0	0	0	0	0	0
Eagle 2	0	0	0	0	0	0	0	0	0
Eagle 3	0	0	0	0	0	0	0	0	0
Egmont 1	0	0	0	0	0	0	0	0	0
Egmont 2	0	0	0	0	0	0	0	1	0
Peros Banhos 1	0	1	0	0	0	0	0	0	0
Peros Banhos 2	0	0	1	0	0	0	0	0	0
Peros Banhos 3	2	0	0	0	0	0	0	0	1
Peros Banhos 4	0	0	0	2	0	0	0	0	0
Peros Banhos 5	1	1	0	0	0	0	0	0	0
Peros Banhos 6	4	0	1	0	0	0	0	0	0
Peros Banhos 7	4	0	0	1	0	0	0	0	0
Peros Banhos 8	1	0	0	0	0	0	3	0	0
Salomon 1	0	0	3	0	0	0	0	0	0
Salomon 2	0	0	0	0	0	0	0	0	0
Salomon 3	7	0	0	0	1	0	0	2	0

Site	*Liocarpilodes sp*	*Liomera cf rubra*	*Liomera cf rugata*	*Liomera cf tristis*	*Liomera cinctimanus*	*Liomera sp*	*Lophozozymus anaglyptus*	*Lybia tessellata*	*Nanopilumnus sp*
Brothers 1	0	0	1	0	0	0	1	0	0
Brothers 2	0	0	0	0	0	0	0	0	0
Brothers 3	0	0	0	0	0	0	1	0	0
Diego Garcia 1	0	0	0	0	0	0	0	0	0
Diego Garcia 2	0	0	0	0	0	0	0	0	0
Diego Garcia 3	0	0	0	0	0	0	0	0	0
Diego Garcia 4	0	0	0	0	0	0	0	0	0
Diego Garcia 5	0	0	0	0	0	0	0	0	5
Diego Garcia 6	0	1	0	0	0	0	0	0	0
Eagle 1	0	0	0	0	0	0	0	0	0
Eagle 2	0	0	0	0	0	0	0	0	0
Eagle 3	0	0	0	0	0	0	0	0	0
Egmont 1	0	0	0	0	1	1	2	0	0
Egmont 2	1	0	0	2	0	0	1	0	0
Peros Banhos 1	0	0	0	0	0	0	1	1	0
Peros Banhos 2	0	1	0	0	0	0	0	0	0
Peros Banhos 3	0	0	0	0	0	0	0	0	0
Peros Banhos 4	0	0	0	0	0	0	0	0	0
Peros Banhos 5	0	0	0	0	0	0	2	0	0
Peros Banhos 6	0	1	0	1	0	0	1	0	0
Peros Banhos 7	0	0	0	0	0	0	1	0	0
Peros Banhos 8	0	0	0	0	0	0	1	0	0
Salomon 1	0	0	0	0	0	0	4	0	0
Salomon 2	0	0	0	0	0	0	0	0	0
Salomon 3	0	0	0	0	0	0	0	0	0

Site	***Paraxanthodes sp***	***Percnon aff guinotae***	***Percnon sp***	***Perinia cf uncifer***	***Perinia tumida***	***Pilodius aff Spinipes***	***Pilodius sp***	***Pilodus melanospinis***	***Pilumnidae sp***
Brothers 1	0	0	0	0	0	0	0	0	1
Brothers 2	0	0	0	0	0	2	0	1	0
Brothers 3	0	0	0	0	0	0	0	0	0
Diego Garcia 1	0	0	0	0	1	0	0	1	0
Diego Garcia 2	0	0	0	0	1	0	0	0	2
Diego Garcia 3	0	1	0	0	0	0	0	0	0
Diego Garcia 4	1	0	0	0	2	0	0	0	0
Diego Garcia 5	0	0	2	0	0	0	0	0	0
Diego Garcia 6	0	0	0	0	1	0	2	1	0
Eagle 1	0	0	0	0	0	0	0	0	0
Eagle 2	0	0	0	0	0	0	0	0	0
Eagle 3	0	0	0	0	0	0	0	0	0
Egmont 1	0	0	0	1	2	0	0	0	0
Egmont 2	1	0	0	0	2	0	0	0	3
Peros Banhos 1	0	0	0	0	2	0	5	2	2
Peros Banhos 2	1	0	0	0	0	0	0	1	0
Peros Banhos 3	0	0	0	0	0	0	0	0	0
Peros Banhos 4	0	0	0	0	0	0	4	0	1
Peros Banhos 5	0	0	0	0	1	0	0	0	0
Peros Banhos 6	0	0	0	0	0	0	0	0	0
Peros Banhos 7	0	0	0	0	0	0	0	1	0
Peros Banhos 8	0	0	1	0	0	0	0	0	0
Salomon 1	0	0	0	0	1	0	0	0	1
Salomon 2	1	0	0	0	0	0	0	0	2
Salomon 3	1	0	0	0	0	0	1	0	0

Site	*Pilumnus aff tantulus*	*Pilumnus cf longicornis*	*Pilumnus sp*	*Platypodia anaglyplus*	*Pseudoliomera lata*	*Pseudoliomera sp*	*Phyladiorhynchus sp*	*Quadrella sp*	*Schizophrys sp*
Brothers 1	0	0	1	0	0	0	1	0	2
Brothers 2	0	0	0	0	0	0	0	0	1
Brothers 3	0	0	0	0	0	0	0	0	0
Diego Garcia 1	0	0	0	0	0	1	0	0	0
Diego Garcia 2	0	0	0	1	0	0	0	1	0
Diego Garcia 3	0	0	1	0	0	0	0	0	0
Diego Garcia 4	2	3	1	0	0	0	0	0	0
Diego Garcia 5	0	0	2	0	0	0	0	0	0
Diego Garcia 6	0	0	0	0	0	1	0	0	0
Eagle 1	0	0	0	0	0	0	0	0	0
Eagle 2	0	0	0	0	0	0	0	0	4
Eagle 3	0	0	0	0	0	0	0	0	0
Egmont 1	0	0	0	1	0	0	0	0	0
Egmont 2	0	0	0	0	1	0	0	0	0
Peros Banhos 1	1	0	0	1	0	0	0	0	0
Peros Banhos 2	0	0	0	0	0	0	0	0	0
Peros Banhos 3	0	0	0	0	0	0	0	0	0
Peros Banhos 4	1	0	0	0	0	0	0	0	0
Peros Banhos 5	0	0	0	0	0	1	0	0	0
Peros Banhos 6	0	0	6	0	1	0	0	0	2
Peros Banhos 7	0	0	0	0	0	0	0	0	0
Peros Banhos 8	0	0	0	0	1	0	0	0	0
Salomon 1	3	0	4	1	0	2	0	0	0
Salomon 2	0	0	0	0	0	0	0	0	0
Salomon 3	0	0	1	0	0	0	0	0	0

Site	***Tetralia sp***	***Tiarinia sp***	***Trapezia bidentata***	***Trapezia sp***	***Trapezia tigrina***	***Tweedieia odhneri***	***Tylocarcinus styx***	***Viaderiana typica***	***Xanthias cherbonnieri***
Brothers 1	0	4	0	2	1	2	0	3	1
Brothers 2	0	1	0	7	1	1	4	0	0
Brothers 3	0	0	0	2	1	1	1	3	0
Diego Garcia 1	2	0	0	4	0	1	0	0	0
Diego Garcia 2	0	0	0	1	0	2	2	1	0
Diego Garcia 3	0	0	0	10	0	0	0	0	0
Diego Garcia 4	0	0	0	3	1	0	1	1	0
Diego Garcia 5	0	0	1	6	1	0	2	0	0
Diego Garcia 6	0	1	0	1	0	3	3	1	0
Eagle 1	0	0	0	0	0	0	4	0	0
Eagle 2	0	0	0	2	0	2	3	2	0
Eagle 3	0	0	0	0	0	0	0	0	0
Egmont 1	0	1	0	3	1	1	3	1	0
Egmont 2	0	0	0	1	1	1	6	1	0
Peros Banhos 1	0	1	0	8	0	1	5	2	3
Peros Banhos 2	0	0	0	5	0	0	0	0	0
Peros Banhos 3	0	0	0	4	0	1	0	0	0
Peros Banhos 4	0	0	0	5	0	0	2	0	0
Peros Banhos 5	0	0	0	1	0	0	0	0	0
Peros Banhos 6	0	1	0	0	0	1	3	3	0
Peros Banhos 7	0	0	0	0	1	2	0	0	0
Peros Banhos 8	0	0	0	2	2	0	0	0	0
Salomon 1	0	3	0	8	1	1	5	0	0
Salomon 2	0	4	0	3	0	9	1	1	0
Salomon 3	0	1	0	4	0	0	5	0	1

Site	*Xanthias sp*	*Xanthias sp 2*	*Galathea eulimene*	*Galathea pilosa*	*Galathea tanegashimae*	*Galathea aegyptiaca*	*Galathea aff platycheles*	*Galathea aff spinosorostris*	*Galathea platycheles*
Brothers 1	0	0	0	2	0	1	0	5	9
Brothers 2	0	0	9	0	6	0	0	0	23
Brothers 3	0	0	0	0	6	0	0	3	1
Diego Garcia 1	0	0	0	0	0	0	0	11	4
Diego Garcia 2	0	0	2	0	1	0	1	1	1
Diego Garcia 3	0	0	3	0	0	0	0	0	1
Diego Garcia 4	0	0	14	0	2	0	0	5	1
Diego Garcia 5	0	0	23	0	0	0	0	0	0
Diego Garcia 6	0	0	0	0	0	0	0	0	7
Eagle 1	0	0	0	0	0	0	0	3	0
Eagle 2	0	0	6	0	2	0	0	0	2
Eagle 3	0	0	0	0	0	0	0	1	0
Egmont 1	0	0	0	0	0	0	0	6	4
Egmont 2	0	0	8	0	2	0	0	4	10
Peros Banhos 1	0	0	17	0	2	0	0	18	0
Peros Banhos 2	0	1	15	0	0	0	0	2	0
Peros Banhos 3	0	0	3	0	1	0	0	6	1
Peros Banhos 4	0	0	0	0	0	0	0	3	1
Peros Banhos 5	0	0	10	0	0	0	0	4	6
Peros Banhos 6	1	0	0	0	0	0	0	5	1
Peros Banhos 7	0	0	0	0	0	0	0	2	0
Peros Banhos 8	0	0	7	0	0	0	0	0	0
Salomon 1	0	0	0	0	0	0	0	20	6
Salomon 2	0	0	0	0	6	0	0	5	1
Salomon 3	1	0	0	0	0	0	0	7	2

Site	*Sadayoshia edwardsii*	*Paguroidea species 1*	*Paguroidea species 2*	*Paguroidea species 3*	*Paguroidea species 4*	*Paguroidea species 5*	*Paguroidea species 6*	*Paguroidea species 7*	*Paguroidea species 8*
Brothers 1	0	0	0	0	3	0	2	0	0
Brothers 2	0	0	0	0	0	0	0	0	0
Brothers 3	0	1	0	0	2	0	0	0	0
Diego Garcia 1	1	0	0	0	0	0	0	0	0
Diego Garcia 2	0	0	0	0	4	0	0	0	0
Diego Garcia 3	0	0	0	1	5	0	0	3	0
Diego Garcia 4	0	0	0	0	1	0	0	0	0
Diego Garcia 5	0	0	3	1	10	0	0	5	2
Diego Garcia 6	0	0	0	0	1	0	0	0	0
Eagle 1	0	0	0	0	1	0	0	0	0
Eagle 2	0	0	0	0	1	0	0	0	0
Eagle 3	0	0	0	0	1	0	0	0	0
Egmont 1	0	0	0	0	3	0	0	0	0
Egmont 2	0	0	0	0	10	2	0	5	0
Peros Banhos 1	0	0	0	0	0	0	0	0	0
Peros Banhos 2	0	0	0	0	0	0	0	0	0
Peros Banhos 3	0	0	0	0	3	0	0	0	0
Peros Banhos 4	0	0	0	0	0	0	0	0	0
Peros Banhos 5	0	0	0	0	1	0	0	0	0
Peros Banhos 6	0	0	0	0	1	0	0	0	0
Peros Banhos 7	0	0	0	0	0	0	0	0	0
Peros Banhos 8	0	0	0	0	0	0	0	0	0
Salomon 1	0	0	0	0	4	0	0	0	0
Salomon 2	0	0	0	0	1	0	0	0	0
Salomon 3	0	0	0	0	0	0	0	0	0

Site	*Paguroidea species 9*	*Paguroidea species 10*	*Paguroidea species 11*	*Paguroidea species 12*	*Paguroidea species 13*	*Porcellanidae species 1*	*Porcellanidae species 2*	*Porcellanidae species 3*	*Porcellanidae species 4*
Brothers 1	0	0	3	0	0	0	0	1	4
Brothers 2	0	0	0	0	0	0	0	0	0
Brothers 3	0	0	1	0	0	0	0	0	6
Diego Garcia 1	0	0	1	1	0	0	0	0	7
Diego Garcia 2	2	0	0	0	0	0	0	0	1
Diego Garcia 3	5	1	3	1	0	0	2	0	3
Diego Garcia 4	1	0	2	0	0	0	0	0	5
Diego Garcia 5	1	0	4	3	0	0	3	0	2
Diego Garcia 6	4	0	1	2	1	0	0	0	2
Eagle 1	1	0	0	0	0	0	0	0	2
Eagle 2	1	0	2	0	0	0	1	0	0
Eagle 3	0	0	1	0	0	0	0	0	0
Egmont 1	0	0	4	2	0	0	0	0	3
Egmont 2	3	1	13	2	0	0	0	0	1
Peros Banhos 1	0	0	2	0	0	0	0	0	12
Peros Banhos 2	0	0	0	0	0	0	0	0	11
Peros Banhos 3	0	0	2	2	0	0	2	0	0
Peros Banhos 4	0	0	2	0	0	0	0	0	0
Peros Banhos 5	0	0	0	0	0	0	0	0	1
Peros Banhos 6	0	0	0	0	0	0	0	0	4
Peros Banhos 7	1	0	2	0	1	0	0	0	1
Peros Banhos 8	0	0	1	0	0	0	0	0	4
Salomon 1	0	0	2	1	0	0	0	0	1
Salomon 2	0	0	0	1	0	0	3	0	4
Salomon 3	0	0	0	2	0	1	1	0	2

Site	*Porcellanidae species 5*	*Porcellanidae species 6*	*Porcellanidae species 7*	*Porcellanidae species 8*
Brothers 1	0	0	1	1
Brothers 2	0	0	0	0
Brothers 3	0	0	0	0
Diego Garcia 1	0	0	0	0
Diego Garcia 2	0	0	0	1
Diego Garcia 3	0	0	0	0
Diego Garcia 4	0	0	0	0
Diego Garcia 5	1	0	0	0
Diego Garcia 6	0	1	0	1
Eagle 1	0	0	0	0
Eagle 2	0	0	0	0
Eagle 3	0	0	0	0
Egmont 1	0	0	0	0
Egmont 2	0	0	0	0
Peros Banhos 1	0	0	0	0
Peros Banhos 2	0	0	0	0
Peros Banhos 3	0	0	0	0
Peros Banhos 4	0	0	0	0
Peros Banhos 5	0	0	0	0
Peros Banhos 6	0	0	0	0
Peros Banhos 7	0	0	0	2
Peros Banhos 8	0	0	0	0
Salomon 1	0	0	0	0
Salomon 2	0	0	0	0
Salomon 3	0	0	0	0

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CrossMark

SHORT NOTE

Localized outbreaks of *Acanthaster planci* at an isolated and unpopulated reef atoll in the Chagos Archipelago

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Abstract Outbreaks of the crown-of-thorns starfish (COTS), *Acanthaster planci*, have occurred at many locations throughout the Indo-Pacific and are a major contributor to widespread coral loss and reef degradation. The causes of outbreaks remain controversial, but are commonly attributed to anthropogenically elevated nutrients and/or over-fishing. If so, it seems unlikely that outbreaks would occur in reef systems that are largely isolated from anthropogenic disturbances. However, high densities of COTS were recently observed on reefs in the Chagos Archipelago, a remote group of atolls and banks within the central Indian Ocean, which experience very limited anthropogenic influence. Aggregations of COTS were first noticed at Eagle Island in 2012, which, although unquantified, appeared to be at outbreak levels, and very high densities (1624 km^{-2}) were subsequently recorded at Danger Island in 2013. While these islands are uninhabited by humans, it is possible that nutrient inputs result from upwelling zones around the Archipelago, or high densities of breeding seabirds. Among islands within the Great Chagos Bank, densities of the red-footed booby *Sula sula* ranged from 8 to 7888 individuals km^{-2}, with associated guano input ranging from 96 to 25,381 kg $island^{-1}$ $year^{-1}$. However, Danger and Eagle Islands where high COTS densities were recorded, had both high and low levels of guano production, respectively, which suggests that outbreaks may not be directly linked to guano nutrient enrichment. Other factors which might be responsible for intermittent COTS outbreaks should be considered in isolated reef systems such as the Chagos Archipelago.

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Introduction

The corallivorous crown-of-thorns starfish (COTS), *Acanthaster planci*, is a native inhabitant of coral reef ecosystems throughout the Indo-Pacific (Birkeland and Lucas 1990; Pratchett et al. 2014). While normally found at low densities (Moran and De'ath 1992), sporadic population outbreaks of COTS can cause significant localized coral loss. These outbreaks, first noted in widely separated sites in the Indo-Pacific in the 1960s (Endean and Chesher 1973), have been highlighted as an important contributor to ongoing reef degradation in the Indo-Pacific (Bruno and Selig 2007; Pratchett 2010). Surveys of 214 reefs on Australia's Great Barrier Reef (GBR) have revealed a 50.7 % decline in coral cover, from 28.0 % in 1985 to 13.8 % in 2012 (De'ath et al. 2012), and 42 % of the estimated coral loss is attributed to COTS outbreaks, however, as only three threats were considered this may be an overestimate. De'ath et al. (2012) suggest that if there had been

no outbreaks during the study period, coral cover would have actually increased despite other episodic disturbances. There is therefore increasing focus on ways to minimize COTS outbreaks, either by direct control (e.g., Rivera-Posada et al. 2012), or by addressing the root cause of outbreaks (e.g., Fabricius et al. 2010), especially if they are attributable to anthropogenic activities (Dulvy et al. 2004; Timmers et al. 2012).

The principal hypotheses linking COTS outbreaks to increasing anthropogenic pressure on Indo-Pacific reefs are: (1) a predatory release for post-settlement COTS life stages resulting from overfishing and associated trophic cascades (Endean 1976; Dulvy et al. 2004; Sweatman 2008), and (2) increased larval survival due to elevated nutrient levels and associated eutrophication of near shore waters (Birkeland 1982; Brodie et al. 2005). However, outbreaks of COTS are known to occur on isolated reef systems, often with no local human inhabitation (Wilson and Stoddart 1988; Timmers et al. 2012; Pratchett et al. 2014), requiring an altogether different explanation for the initiation of outbreaks. It may be that major fluctuations in COTS abundance are simply linked to their life-history characteristics (e.g., high fecundity, short generation times, and highly variable mortality during their early life history; Birkeland 1989). Indeed, COTS underpinned the proposal by Uthicke et al. (2009) that echinoderms are a 'boom or bust' phylum. Understanding the incidence of COTS outbreaks on small, isolated, or unpopulated reefs is very important for establishing putative causes of outbreaks (Wilson and Stoddart 1988; Pratchett et al. 2014).

The purpose of this study was to document and explore potential drivers of COTS outbreaks in the Chagos Archipelago located in the central Indian Ocean. Large COTS aggregations, which appeared to experienced observers to be at outbreak densities, were first noted in Chagos during a research expedition carried out during 2012. With the exception of Diego Garcia, which houses a US military support facility, atolls of the Chagos Archipelago have been uninhabited since the early 1970s. Moreover, the entire area of the northern atolls and surrounding 640,000 km^2 of water were declared a no-take marine reserve in 2010 and contain some of the most pristine ecosystems on the globe (Sheppard et al. 2012). Given limited fishing, pollution, agriculture, or coastal development in the Chagos Archipelago, it would be very difficult to attribute localized outbreaks of COTS to anthropogenic activities. Still, there may be natural sources of nutrients that could contribute to the initiation of outbreaks (e.g., Houk and Raubani 2010).

Two potential sources of nutrients within the Chagos archipelago are: (1) nutrient input by seabirds, particularly after heavy rainfall events preceded by a drought, which will wash accumulated guano into nearshore waters; and (2) oceanographic upwelling. Nutrient input by seabirds has been shown to be a significant source of nutrients within nearshore waters surrounding coral reef atolls (Allaway and Ashford 1984). In the Chagos Archipelago, the red-footed booby, *Sula sula,* is the major contributor to the biomass of breeding seabirds, with significant variation in the densities present on different islands (Carr 2013). Upwelling of cooler water likely enriched in nutrients is known to occur in areas surrounding the Chagos Archipelago (Hermes and Reason 2008), and there is additional evidence of localized intrusions of cool water (Sheppard 2009). We examined chlorophyll-a concentrations derived from satellite data as a proxy for upwelling and biological productivity within the Chagos Archipelago.

Continuous direct monitoring of COTS populations in Chagos is unfeasible; therefore, we quantified coral cover and morphological composition around islands with high and low levels of guano input to examine for potential impacts associated with COTS outbreaks.

Methods

Surveys of shallow reef environments were conducted in February and March during 2013 at a total of six sites around four islands around the Great Chagos Bank: Nelson Island, Eagle Island, Middle Brother Island, and Danger Island (Fig. 1; Table 1). Underwater video surveys of coral reef benthos were conducted as part of long-term monitoring of the Chagos Archipelago; however, where COTS were encountered, a targeted survey of COTS abundance was carried out.

The relative abundance of live versus dead corals (defined as completely white skeleton, or white with some algal growth) was quantified at survey locations from 2013, divided into four depth zones: 25–20 m, 20–15 m, 15–10 m, and 10–5 m, and according to exposure as either seaward or lagoon sites. Underwater video transects were recorded using a Sony HDRCX550 camera in a Light and Motion Bluefin housing with a Fathom 90 wide angle port and cyan filter, onto which red lasers with a spacing of 10 cm were mounted. Surveys were conducted for 10 min within each depth range, aiming for a constant speed (~0.1 m s^{-1}), approximately 1 m above the substrate (Turner and Klaus 2005). Percentage cover of live versus dead coral, as well as composition of corals were assessed by randomly selecting 20 video frames from each depth range, and recording what lay beneath 15 randomly selected points per frame, assigned using Coral Point Count software (Kohler and Gill 2006). The composition of coral assemblages was based on the following morphological classifications: branching *Acropora* sp., other branching coral, *Pocillopora* sp., foliaceous, laminar, encrusting, and massive.

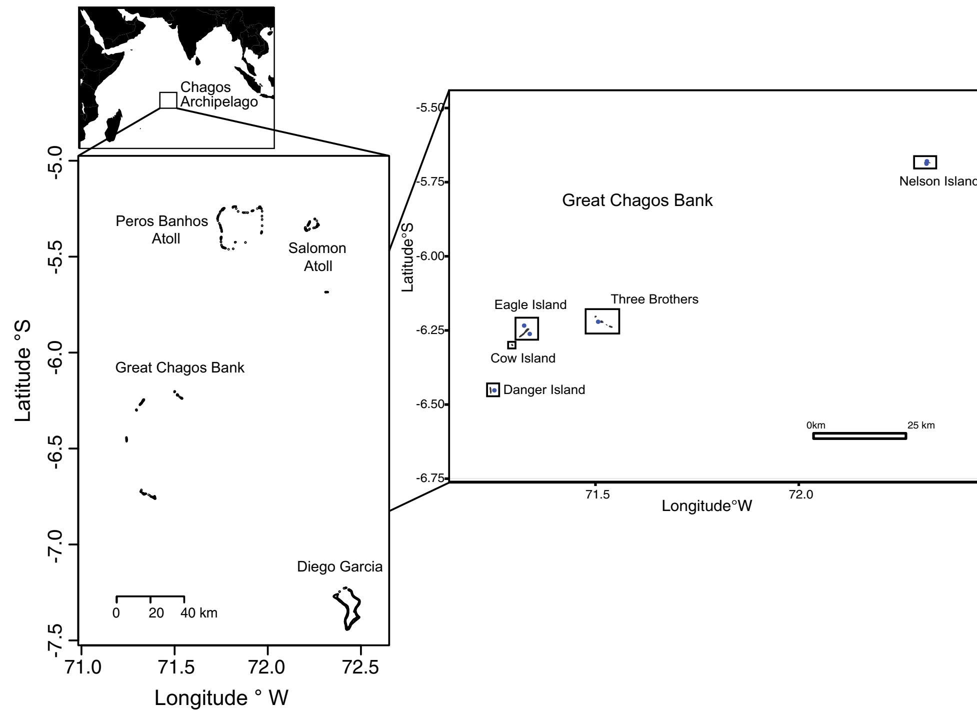

Fig. 1 Study sites around the Great Chagos Bank, Chagos Archipelago. Circles indicate coral survey sites, and squares mark islands on which bird surveys were conducted

Table 1 Locations and timings of coral reef surveys within the Chagos Archipelago

Island	Site	Location	Years surveyed	COTS outbreak
Nelson Island	Seaward	5 40.769°S, 72 18.906°E	2013, 2014	
	Lagoon	5 41.279°S, 72 18.847°E	2013	
Eagle Island	Lagoon	6 11.015°S, 71 20.630°E	2012, 2013, 2014	2012 (unquantified)
	Seaward	6 11.950°S, 71 18.942°E	2013, 2014	
Middle Brother	Seaward	6 08.950°S, 71 31.015°E	2012, 2013, 2014	
Danger Island	Lagoon	6 23.647°S, 71 14.630°E	2013, 2014	2013

Densities of COTS were quantified using underwater video along a 150 m × 2 m swath with a zig-zag pattern through areas occupied by COTS, aiming for a 0.1 m s^{-1} speed, ~2 m above the substrate. A total of 50 still images were randomly selected from the video sequence, avoiding duplicate images. COTS densities were estimated based on the number of starfish observed within each frame, using the measured diameter of starfish (18–30 cm) and the reef area covered within each frame.

To examine for anomalous periods of rainfall, data were obtained for 2008–2013 from the weather station situated on Diego Garcia, operated by the US Navy. Chlorophyll data were obtained from the MODIS-Aqua 4 km monthly chl-a data product, from a box centered on −7.5°S (5°–10°) and 70°E (65°–75°). Data were obtained from 2008 to 2013 for summer months JFM (January, February, March) as these generally correspond to peak chl-a values, and COTS spawning and larval release are likely to occur at this time in the Indian Ocean (Vogler et al. 2012).

Seabird abundances were recorded by counting *S. sula* apparently occupied nests (Bibby et al. 1992). The mean number of *S. sula* contributing guano per day was estimated by multiplying the number of nests by four (two parents, one chick, one fledged chick still on island). To estimate the guano production rate per island, *S. sula* defecation rates from Young et al. (2010a) were employed (0.66 g/36 min). Total guano production rates were estimated by multiplying the island production rate over a period of 12 h per day by the mean number of birds contributing and the number of 12 h periods in one year.

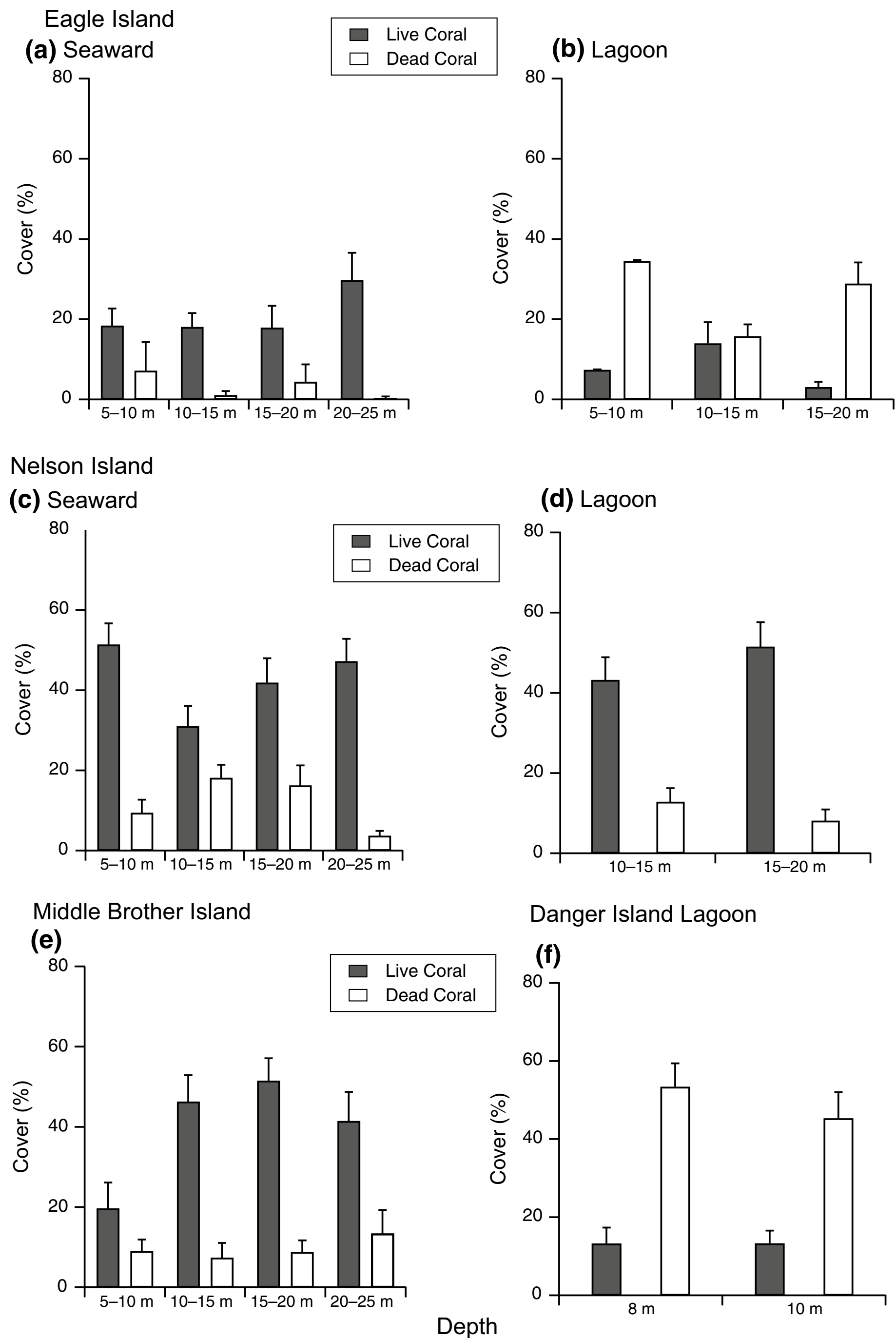

Fig. 2 Variation in live coral cover and dead coral cover (± SE) at **a** Eagle Island seaward site, **b** Eagle Island lagoon site, **c** Nelson Island seaward site, **d** Nelson Island lagoon site, **e** Middle Brother Island site and **f** Danger Island lagoon site

Results and discussion

COTS were recorded at two out of six sites surveyed on the Great Chagos Bank in 2013; only a single individual was recorded at Nelson Island, while an aggregation (estimated extent 2.5–3.5 km^2) was observed in the lagoon at Danger Island at 6–8 m depth. The mean COTS density recorded within each of the 50 frames from video transects was 1.6 m^{-2} (±0.3SE), ranging from 0 to 10.6 m^{-2}. Based on the known extent of the aggregation and mean densities of starfish, it was estimated that there were approximately 5684 individuals at Danger Island (1624 starfish km^{-2}),

The density of starfish recorded at Danger Island was above threshold densities considered to be indicative of an outbreak (>1000 km^{-2}, Keesing and Lucas 1992; >1500 km^{-2}, Moran and De'ath 1992). Despite the relatively moderate extent of the outbreak (~2.5–3.5 km^2), it was apparent that high COTS densities were causing

coral mortality, leading to areas of dead arborescent *Acropora* at Danger Island.

Live coral cover was highest at Nelson Island (44.8 % ± 2.3SE) and Middle Brother (39.6 % ± 3.5SE), and lower at Eagle Island (15.3 % ± 1.8SE) and Danger Island (13.0 % ± 2.6SE; Fig. 2). Danger Island had higher levels of dead coral cover (48.0 % ± 4.5SE) than the other sites surveyed (Fig. 2). Dead branching *Acropora* coral cover was highest at Danger Island (42.4 % ± 4.5SE), followed by Nelson (9.2 % ± 1.4SE), Eagle Island (4.1 % ± 1.0SE), and Middle Brother (3.0 % ± 1.2SE; Fig. 3). However, islands with higher guano input levels had lower levels of other dead branching corals (2.5 % ± 1.9SE), than those with low guano input (8.0 % ± 0.8SE).

While some of the dead *Acropora* cover found at Danger Island may be attributable to generally high levels of background mortality among *Acropora* corals (Pratchett et al. 2013), these high levels of coral mortality were restricted to the one site where high densities of COTS were also recorded actively feeding on branching *Acropora* (Fig. 4c). Corals in the genus *Acropora* are the preferred coral food source of COTS (Pratchett et al. 2014), and outbreaks recorded throughout the Indo-Pacific invariably have disproportionate impacts on *Acropora* corals (Baird et al. 2013).

While outbreaks of COTS may be an entirely natural phenomenon (Potts 1981), whereby subtle changes in the distribution or behavior of adult individuals translate into highly variable reproductive success and settlement (Babcock and Mundy 1992), there are several hypotheses linking COTS outbreaks to increasing anthropogenic pressures on coral reefs (Birkeland 1982; Brodie et al. 2005; Houk et al. 2007; Houk and Raubani 2010; Fabricius et al. 2010). Fabricius et al. (2010) indicated that outbreaks are correlated with anthropogenically elevated nutrients, at least on Australia's GBR. Most notably, this modeling study (Fabricius et al. 2010), showed that increased nutrient concentrations are associated with higher larval survival rates of COTS in laboratory experiments, building on earlier work by Lucas (1982). However, the longstanding larval resilience hypothesis points to the ability of COTS larvae to develop in the absence of a planktonic food source, suggesting that COTS recruitment success is not linked to food availability (Olson et al. 1987; Olson and Olson 1989). Recent experimental work by Wolfe et al. (2015), designed to test these competing hypotheses, found that optimal larval performance occurred within a moderate nutrient range, whereas both low and high nutrient levels impaired development. The concept that nutrient enrichment is universally implicated in COTS outbreaks is increasingly being questioned (Lane 2011; Miller et al. 2015), particularly given records of outbreaks in isolated locations (as in this study), with limited changes in nutrient concentrations.

While it is clear that outbreaks can occur on reefs located great distances from major anthropogenic pressures (e.g., overfishing and coastal development), this does not necessarily mean that fluctuations in nutrient concentrations are unimportant in initiating localized outbreaks (e.g., Houk et al. 2007). In Chagos, the major contributor to variable concentrations of nutrients within the immediate vicinity of islands is likely to be high densities of nesting seabirds. A recent study at Palymra Atoll in the Northern Pacific found waters surrounding islands with native vegetation had 26.5 times higher nitrogen loads than those surrounding palm forests, due to higher seabird numbers (McCauley et al. 2012), with estimates of red-footed boobies ranging from approximately 4000–10,000 individuals (Young et al. 2010b). Similarly, in Chagos, the total number of nesting seabirds, and therefore localized nutrient inputs, is directly related to variation in major vegetation types with higher bird numbers were recorded on islands with *Pisonia* and *Scaevola* as the dominant vegetation cover. Total numbers of nesting pairs of red-footed boobies (*S. sula*) varied greatly among islands, ranging from 5268 individuals on Nelson Island, to 20 individuals at Eagle Island (Table 2). Estimates of annual guano production by *S. sula* from islands of the Great Chagos Bank ranged from 96 to 25,381 kg year^{-1} (Table 2).

The ability to fully compare between islands with high and low guano within the Chagos Archipelago is limited by the difficulty of sampling sufficient numbers of islands in this remote location. At Danger Island, where outbreak densities of COTS were recorded, the estimated production of guano was 23,801 kg year^{-1}, which was the second highest of the surveyed islands, consistent with the hypothesis of a relationship between COTS outbreaks and seabird abundance. However, the island where COTS aggregations were first observed on the lagoon side in 2012 (Eagle Island), has very low numbers of roosting seabirds and guano inputs (96 kg year^{-1}), which means this hypothesis is difficult to accept within our present knowledge of the Chagos Archipelago. However, accurate data on localized current patterns are not presently available for areas such as the Great Chagos Bank—outbreaks appear to be highly geographically limited within Chagos, suggesting that site-specific processes (larval delivery, juvenile predation or food supply) influenced by local oceanography may be involved.

If localized outbreaks of COTS in Chagos are linked to nutrient inputs from land-based sources (i.e., nesting seabirds) outbreaks would be expected to arise approximately 3 years after heavy rainfall events preceded by a period of drought (sensu Birkeland 1982). This modeled 3-year lag accounts for the time required for elevated densities of

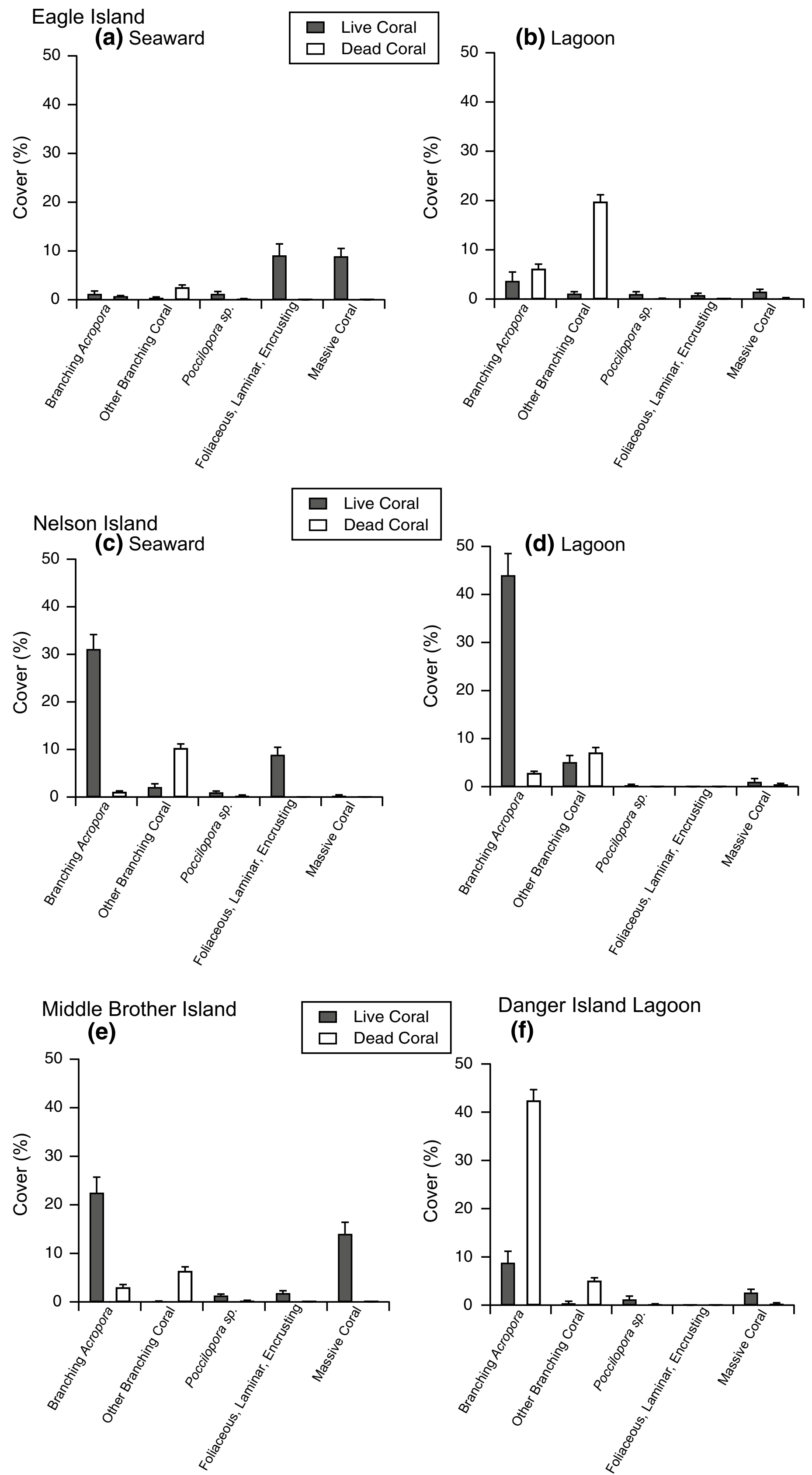

Fig. 3 Proportion of live and dead coral cover assigned to morphological groupings (±SE) at **a** Eagle Island seaward site, **b** Eagle Island lagoon site, **c** Nelson Island seaward site, **d** Nelson Island lagoon site, **e** Middle Brother Island site and **f** Danger Island lagoon site

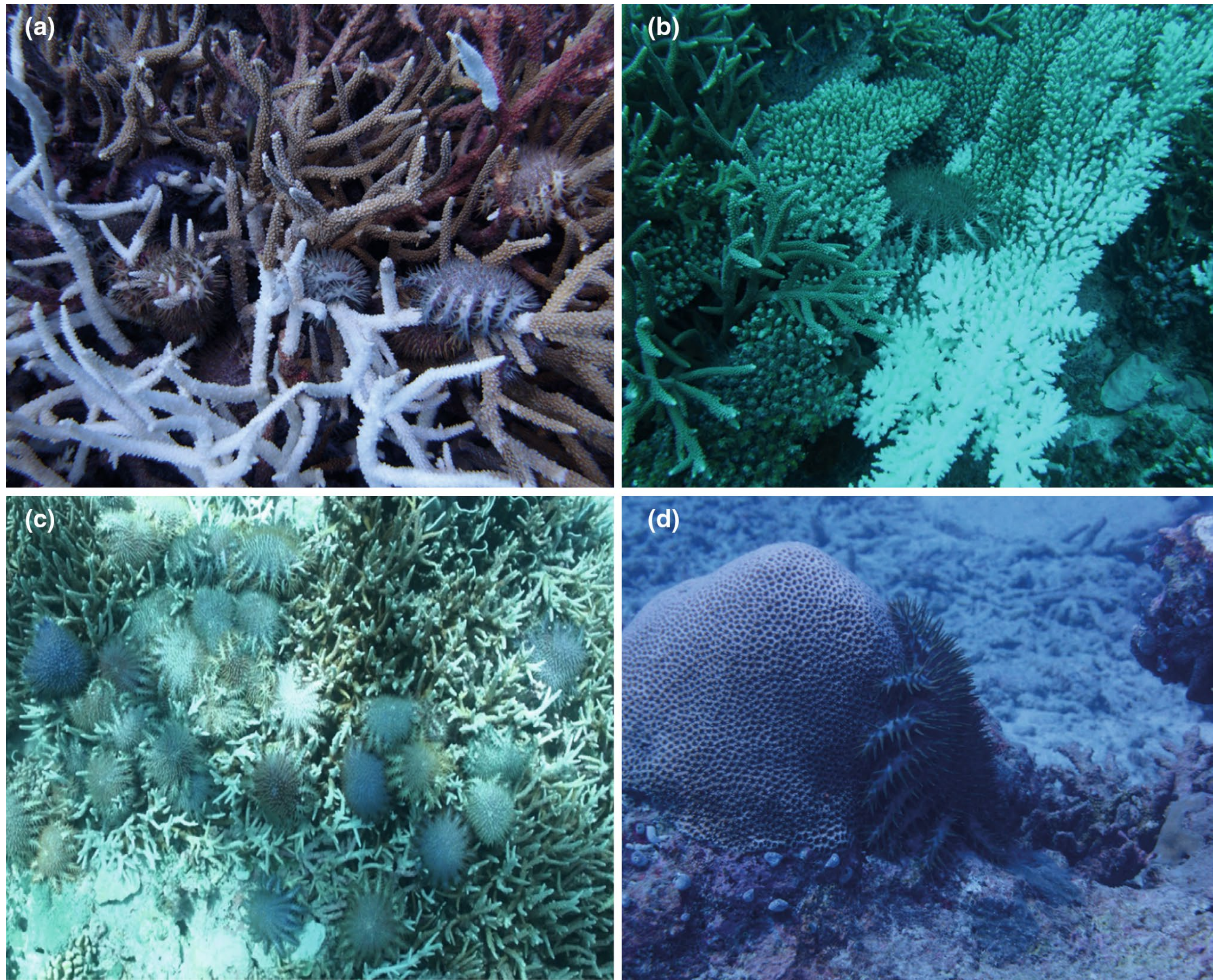

Fig. 4 COTS activity within the Chagos Archipelago: **a** high densities of COTS feeding on *Acropora* on Eagle Island in March 2012; **b** isolated COTS individual feeding on *Acropora* on Nelson Island in March 2013; **c** COTS outbreak on Danger Island in March 2013; **d** COTS at Danger Island in March 2013 (note that feeding on a non-preferred food source such as this massive coral may indicate a depletion of branching corals)

Table 2 Guano input for islands of the Chagos Archipelago based on surveys of the Red-footed booby *S. sula*

Island	Area (km^2)	Dominant vegetation	Mean no. birds contributing ($year^{-1}$)	Bird density individuals (km^{-2})	Guano produced (kg $island^{-1}$ $year^{-1}$)
North Brother	0.06	*Pisonia*	468	7800	2255
Middle Brother	0.08	Coconut	68	850	328
South Brother	0.23	Coconut	200	870	964
Eagle Island	2.44	Coconut	20	8	96
Cow Island	0.18	*Pisonia*/*Scaevola*	1420	7889	6842
Danger Island	0.66	*Pisonia*/*Scaevola*	4940	7485	23,801
Nelson Island	0.81	*Scaevola*/*Argusia*	5268	6504	25,381

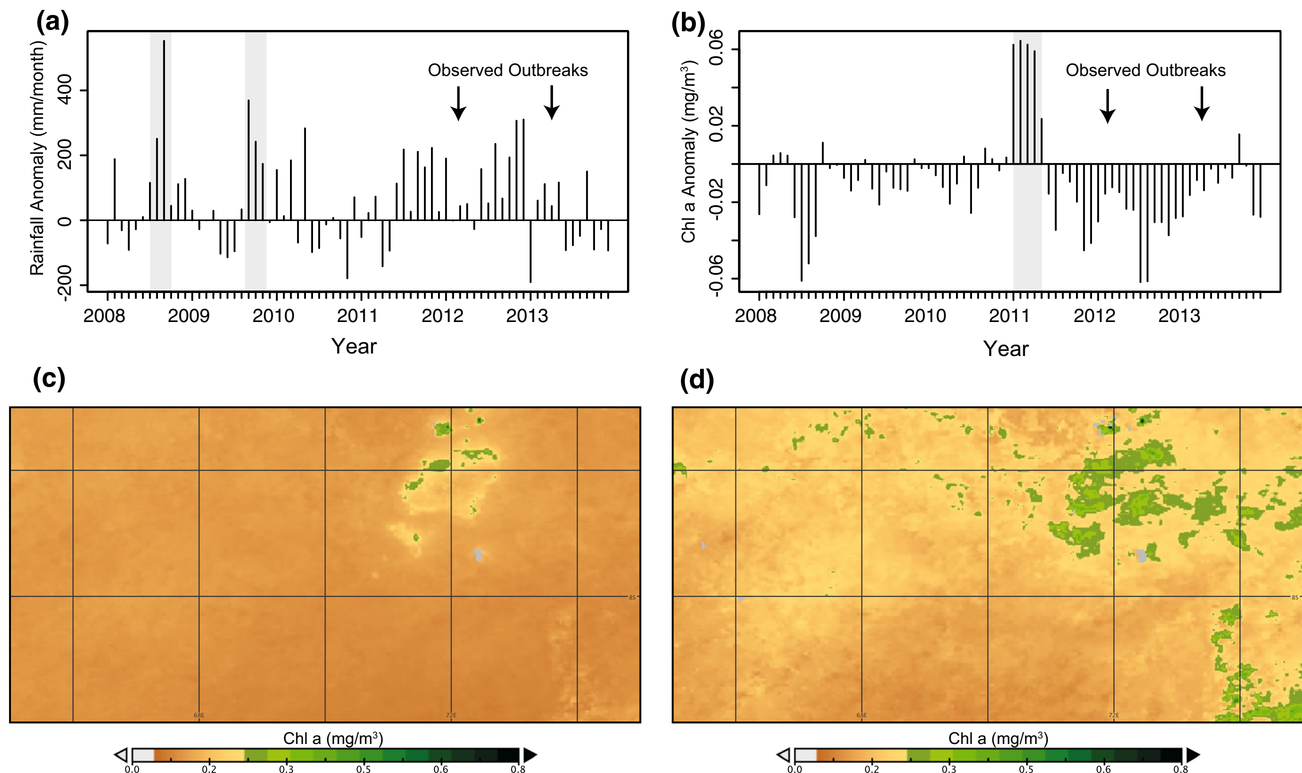

Fig. 5 **a** Monthly precipitation anomaly from rainfall recorded at Diego Garcia from 2008 to 2013. Shaded areas indicate time periods of high precipitation events. *Arrows* indicate the timing of the observed COTS outbreaks in 2012 and 2013. **b** Monthly JFM chlorophyll-a anomaly from 2008 to 2013 derived from MODIS-Aqua satellite product. *Shaded area* indicates anomalous chl-a during 2011. **c** Mean of JFM chl-a centered on −7.5°S (5°–10°) and 70°E (65°–75°) for 2008–2013. **d** Mean chl-a during JFM 2011 for the same region

larval starfish to settle on the reef, metamorphose, begin feeding, and attain sufficient size (200–300 mm) to emerge from the reef matrix (Birkeland 1982). Above-average rainfall was recorded in the Chagos Archipelago in late 2009, after very low rainfall throughout much of the year (Fig. 5). This would suggest that the annual accumulation of guano throughout 2009 would have been washed into the ocean in early 2010, potentially causing a plankton bloom and thereby increasing food availability for larval COTS. This event could explain the high densities of starfish recorded in early 2013, but not those observed in 2012, unless these consisted of subadult COTS. The remoteness of the Chagos Archipelago additionally means that it is not possible to accurately determine the start and end times of these COTS outbreaks.

Upwelling of nutrient-rich waters has been implicated in COTS outbreaks on coral reefs at several regions globally (Houk and Raubani 2010; Miller et al. 2015). In the southwest Indian Ocean, the Seychelles–Chagos thermocline ridge (SCTR) is an upwelling region between 55°–90°E, and 5°–12°S, which has been found to have high variability in surface chlorophyll-a concentration (Dilmahamod 2014). This variability represents an alternative possible driver (to guano-derived nutrients) of enhanced larval COTS survival leading to episodic outbreaks within the Chagos Archipelago. Satellite imagery shows widespread high chlorophyll-a concentration relative to mean monthly levels occurred in early 2011 around the Chagos Archipelago (Fig. 5b, d); however, no COTS outbreaks were observed during surveys in 2014. Recent population genetic work within the Pacific found high levels of genetic differentiation between COTS populations, even within archipelagos and atolls, pointing to COTS outbreaks being primarily influenced by localized conditions (Timmers et al. 2012). To more thoroughly investigate potential connections between nutrient sources and COTS populations within the Chagos Archipelago, data are required to assess how nutrient concentrations around the islands are influenced by both bird populations and localized oceanography, and to link these spatially and temporally to possible future outbreaks.

COTS are widely distributed throughout the Indian Ocean, and while outbreaks have been reported at a variety of locations (e.g., Colin 1977; Mendonça et al. 2010), the geographic extent and magnitude of outbreaks are very minor compared to more extreme outbreaks recorded in the western Pacific (Pratchett et al. 2014). This may be because COTS from the Indian Ocean are an altogether different species to that responsible for devastating outbreaks in the

Pacific (Vogler et al. 2008), and could, therefore, have fundamentally different life-history characteristics (Pratchett et al. 2014). Alternatively, environmental conditions (e.g., the geographic extent of well-connected reef systems) in the west Pacific may enable establishment of more extensive outbreaks. Nonetheless, high densities of COTS in Chagos are clearly capable of causing localized coral mortality. While coral assemblages in Chagos have exhibited considerable resilience in the recent past (Sheppard et al. 2002), this could be jeopardized by impending changes in climate, or changes in exposure to anthropogenic pressure. Continued monitoring within the Chagos Archipelago is therefore important, not only to test for changes in the frequency or extent of COTS outbreaks, but also to assess whether there is reduced capacity to recover from such disturbances.

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