DEMOGRAPHY AND IMPACTS OF HABITAT DEGRADATION ON THE GIANT BARREL SPONGE *Xestospongia* spp. IN THE INDO-PACIFIC

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A thesis submitted to the Victoria University of Wellington

in fulfilment of the requirements for the degree of

Doctor of Philosophy

2018
Abstract

Coral cover worldwide is in decline largely due to anthropogenic influence. In some areas reefs are transitioning into alternative states dominated by sponges, which remain largely understudied despite their abundance and functional importance. Coral reefs in the Wakatobi National Marine Park (WNMP), Indonesia are among the most diverse in the world but remain vulnerable to a multitude of stressors, including coastal development and the resultant sedimentation. Some degraded reefs are characterized by high levels of sedimentation and low coral cover in this area, but support large populations of the ecologically important giant barrel sponge *Xestospongia* spp. Giant barrel sponges in the genus *Xestospongia* may be among the largest benthic invertebrates providing habitat and fulfilling ecosystem services on reefs where coral is declining. The large size of these sponges is of particular importance as body size is mechanistically linked to pumping and nutrient cycling. This thesis examines the demographic structure and connectivity of *Xestospongia* spp. in four core sites within the WNMP, and attempts to elucidate the mechanisms allowing them to tolerate sedimentation.

In my first data chapter I examined the influence of environmental variability on *Xestospongia* spp. growth and longevity over the course of two years at four sites. Specific growth rate, density, mean volume, and environmental variables were examined and compared. Four candidate growth models were fitted to the volume data each site and compared using Akaike’s Information Criterion. Best fit models were determined using a multi-model inference (MMI) approach. Models were averaged to extrapolate size-at-age, which were validated by sponge growth on an artificial structure of known age. There was no difference in model-averaged growth rates between depths or sites of varying habitat quality despite differences in density and mean volume, perhaps suggesting that *Xestospongia* spp. may be less reliant on their photosynthetic symbionts and feeding heterotrophically, or are able to switch between these trophic modes to maintain growth. Size-at-age estimates placed the largest measured sponges between 24 and 30 years, in contrast with published estimates of Caribbean *Xestospongia muta* of over 240 years for sponges of comparable size. My results highlight the accelerated growth of these massive sponges compared to estimates from the Caribbean; these differences have important implications for how these ecologically important species should be managed.
In the second data chapter I used empirical data to construct an integral projection model (IPM) to explore population dynamics at two sites. My aim was to quantify the extent that the relationship between sponge size and growth, survival, and fecundity (vital rate parameters) influences population-level outcomes (growth or decline). Indicators of asymptotic population dynamics (stable size distributions and long term growth rate) and elasticities of vital rate parameters were calculated. The importance of recruitment to population growth was examined by simulating demographically open and closed systems. To assess the importance of size-dependent survival on population growth at each site, recruit and adult mortality was simulated for each system. IPM analyses suggest that in the absence of large sponges population growth would show a substantial decline but both populations are resilient to instances of poor recruitment, and that maintaining *Xestospongia* spp. size should be considered a principal element in management and conservation. Finally, this chapter emphasizes the importance of recruit source to population dynamics on a small scale. The results of this chapter highlight that population biomass at both sites is increasing and *Xestospongia* spp. are likely to remain the dominant component of these reef system in the WNMP. However, one large-scale mortality event affecting large sponges could severely impact populations with a subsequent slow recovery.

My third data chapter examined the physiological effects of short-term exposure of *Xestospongia* spp. to suspended sediment in an effort to quantify mechanisms of local adaptation. In the Wakatobi National Marine Park, Indonesia, some degraded reefs are characterized by high levels of sedimentation and low coral cover, but support large populations of *Xestospongia* spp. Respiration rates increased compared to controls when sponges were exposed to environmentally relevant suspended sedimentation concentrations of 75 and 150 mg l$^{-1}$. For the first time sponge mucus production was observed as a mechanism to remove settled sediment, and sediment clearance was filmed *in situ* over the course of 24 h. Sponges produced mucus in response to sediment addition, with a mean clearance rate of 10.82 ± 2.04% h$^{-1}$ (sediment size fractions 63–250 µm). Mucus production is an effective, but slow mechanism supporting barrel sponge survival in habitats experiencing high levels of sedimentation. My results suggest that there are likely to be energetic consequences for sponges living in sedimented environments, which may influence the energy available for other demographic processes and therefore have implications for barrel sponge population sustainability.
My final chapter explored genetic connectivity and structuring of *Xestospongia* spp. at four sites using nine multilocus microsatellite markers. Genetic analyses demonstrated strong genetic structuring supporting a cryptic species complex of five genetic clusters, likely representing separate species or subspecies. Fine-scale relatedness was measured to identify potential sources of larval recruits using assignment tests, sibship analyses, and maximum-likelihood estimation of relatedness among and between study sites. Mean Maximum-likelihood estimation of relatedness estimates revealed that for three sites self-recruitment does not appear to dominate, but one site was characterized high levels of self-recruitment.

In summary, *Xestospongia* spp. populations in the sites sampled during my study are composed of a species complex, the structure of which appears to be driven by self-recruitment and is heavily reliant upon large sponges. The study site is characterized by the highest levels of settled and suspended sediment features large and stable *Xestospongia* spp. populations that appear to be tolerant to varying levels of sedimentation, possibly due to the production of mucus as a sediment clearance mechanism. This tolerance comes at a cost, however, and results in elevated respiration rate which may require energy otherwise utilized for demographic processes (growth, reproduction, etc.). Growth curves suggested that sponges at sites of increased sedimentation took longer to grow to an equivalent size, although there was no difference in growth between sites; this may suggest that these sponges can shift to heterotrophic feeding in conditions less favourable to photosynthesis by its symbionts. *Xestospongia* spp. population dynamics appear to be heavily reliant on large individuals, and the results of my study suggest that management efforts should be targeted accordingly.
This thesis is dedicated to my father, Brien M Grath
Acknowledgements

The biggest and heartiest thanks must first go to my primary academic supervisor, Assoc. Prof. James Bell. James has supported me through the myriads of challenges and failures that accompany remote field work, as well as the provision of endless feedback on manuscripts and drafts. He is an inspiration who drives me to be a better scientist and for that I am extremely grateful. I must also thank the Hoga Island contingent of the “Sponge Club”, without whom I surely would have lost my mind along with much of my data. Huge thanks go to Megan Shaffer for being the world’s best RA (as well as an amazing friend). The entirety of the Sponge Club kept me sane throughout this process, even if we’re terrible at pub trivia.

I would like to thank James Seager for providing patient and continual assistance with EventMeasure software and advice on stereo photogrammetry techniques. Shirley Pledger and Lisa Woods were instrumental in statistical assistance, and together with Boyd Anderson helped me finally crack R (sort of). Daniel McNaughton (Snout) is sincerely thanked for all of his help with dive support and training. Without John van der Sman I would not have the clever respiration chambers that survived multiple trips to Indonesia, nor would I have the illustrations to demonstrate their use without Jen Matthews and David Young. Estate Khmaladze is thanked for his input on estimating parentage for Xestospongia, as is Joe Marlow and Tracey Bates for the majority of the photos that begin each chapter in this thesis. I am grateful to the Geology Department, Jane Chewings in particular, who shared resources and knowledge in processing sediment samples.

I am grateful for the Victoria University of Wellington Doctoral, Submission, and Hardship Scholarships that have provided stipend support and covered enrolment fees. The PADI foundation is thanked for supporting a portion of the research carried out on Hoga. I would like to thank Operation Wallacea for their funding associated with my field work and an emphatic thanks go to the dive operations managers, science staff, dive staff, research assistants, dissertation students and local Indonesian staff that were instrumental to the completion of this thesis.

Finally, my friends and family are owed a tremendous debt of gratitude; without them this process would have been impossible. My sisters are thanked for providing support, as is my father for constant encouragement. I must also dedicate a particularly huge thank you to Dan for keeping me going, keeping me fed, and keeping me relentless. Thank you for your patience, support, and love.
Chapter contributions and publications

The experimental design, statistical analyses, and writing of all chapters was executed by Emily McGrath with guidance from Assoc. Prof. James Bell. Additional guidance on growth model analyses and R coding was provided by Dr. Lisa Woods in Chapter 2, and assistance with integral projection modeling by Dr. Shirley Pledger in Chapter 3. Assistance on respiration chamber construction was provided by John van der Sman and Dan Crossett, and respiration chamber figure design was provided by Jennifer Matthews and David Young in Chapter 4. All fieldwork, data processing, and laboratory work was performed by Emily McGrath with the exception of DNA sequencing which was performed by Macrogen Inc. (South Korea) for Chapter 5. Data chapters have been written into the following manuscripts:

Chapter 2:

Chapter 3:

Chapter 4:

Chapter 5:
Table of Contents

List of Figures .................................................................................................................. 15
List of Tables .................................................................................................................... 19
Chapter 1: General Introduction ......................................................................................... 23
  1.1 Global decline of coral reefs ...................................................................................... 25
  1.2 Shifts from coral dominated states .......................................................................... 25
  1.3 The functional roles of marine sponges ................................................................. 25
  1.4 Sponges on coral reefs ............................................................................................ 27
  1.5 Sedimentation and the benthic community ........................................................... 27
     1.5.1 Sedimentation impacts on sponges ................................................................. 28
  1.6 Xestospongia spp. ................................................................................................... 29
  1.7 Demography ............................................................................................................ 31
     1.7.1 Growth and size ............................................................................................... 31
     1.7.2 Population dynamics ....................................................................................... 31
     1.7.3 Population connectivity ................................................................................... 32
  1.8 The Wakatobi National Marine Park: a system under threat ..................................... 33
  1.9 Study sites ............................................................................................................... 34
  1.10 Xestospongia spp. demography .............................................................................. 35
  1.11 Goals and Aims .................................................................................................... 36
Chapter 2: Growth and longevity in giant barrel sponges: Redwoods of the reef or Pines in the Indo-Pacific? .................................................................................................................. 39
  2.1 Abstract .................................................................................................................... 41
  2.2 Introduction ............................................................................................................. 41
  2.3 Methods .................................................................................................................. 44
     2.3.1 Study sites ........................................................................................................ 44
     2.3.2 Environmental parameters ............................................................................. 45
     2.3.3 Sponge volume measurement ......................................................................... 45
     2.3.4 Barrel sponge demography ............................................................................ 46
     2.3.5 Candidate models .......................................................................................... 47
     2.3.6 Data Analysis .................................................................................................. 47
  2.4 Results .................................................................................................................... 50
     2.4.1 Environmental data ......................................................................................... 50
     2.4.2 Xestospongia spp. demography ....................................................................... 53
     2.4.3 Growth models ............................................................................................... 58
Chapter 3: Size matters in population dynamics of giant barrel sponges on Indonesian coral reefs

4.4 Results
4.3 Methods
4.2 Introduction
4.1 Abstract

2.5 Discussion
2.5.4 Implications of barrel sponge life-history traits for management and conservation
2.5.3 Model choice and parameter uncertainties
2.5.2 Redwoods of the reef or Pines of the Indo-Pacific?
2.5.1 Growth of giant barrel sponges

2.4.5 Sponge age on the USAT Liberty Wreck
2.4.4 Size-at-age estimates
2.4.3 Recruitments and mortality
2.4.2 Integral projection model (IPM)

3.5 Discussion
3.5.4 Evidence for the support of size-based management
3.5.3 Model assumptions
3.5.2 The effects of larval source on population growth
3.5.1 The importance of size in Xestospongia populations

3.4 Results
3.4.2 Integral projection model (IPM)
3.4.1 Recruitment and mortality

3.3 Methods
3.3.2 Demographic model
3.3.1 Data collection

3.2 Introduction
3.2.1 Integral projection models

3.1 Abstract

2.4 Methods
2.3 Introduction
2.2 Redwoods of the reef or Pines of the Indo-Pacific?
2.1 Growth of giant barrel sponges

2.1 Abstract
2.0 Introduction

1.0 Introduction
6.5 Management implications .................................................. 161
6.6 Research limitations .......................................................... 162
   6.6.1 Integral Projection Models .............................................. 163
6.7 Future directions ............................................................... 163
   6.7.1 *Xestospongia* spp. symbiont reliance ................................ 163
   6.7.2 Spatial competition ...................................................... 164
   6.7.3 Far-reaching effects of sedimentation ................................ 165
6.8 Concluding remarks .......................................................... 166

References .............................................................................. 167
Appendix .................................................................................. 191
Model diagnostics ................................................................. 191
Numerical parameterization .................................................... 192

List of Figures

Figure 1.1. *Xestospongia* spp. in the Wakatobi National Marine Park, southeast Sulawesi, Indonesia. 31

Figure 1.2. Map of the study area in relation to Indonesia and the Wakatobi, as well as the study sites (black dots) with the proximity to Kaledupa Island, Hoga Island and Sampela village (adapted from Powell et al. 2014). 34

Figure 2.1. Mean *Xestospongia* spp. density (sponges m$^{-2}$ ± SE) across the wider Wakatobi (2014). Site abbreviations are as follows: Sampela 1 (S1), Buoy 1 (B1), Karang Gurita (KG), Ridge 1 (R1), Kaledupa Double Spur (KDS), Wanci (W), Tomea (T). 54

Figure 2.2. Mean *Xestospongia* spp. volume (cm$^3$) at each site (± SE); A) mean volume gained from 2014-2015 and B) mean volume across years at each site. Asterisks (*) and letters denote significant differences between sites. 55

Figure 2.3. Sponge growth from 2014-2016 at Kaledupa Double Spur (sponge 1, specific growth rate (SGR = 1.51), Buoy 1 (sponge 2, SGR = 2.46), and Sampela 1 (sponges 3, SGR = 2.15, and 4, SGR = 0.99). Scale bars = 15.0 cm. 57

Figure 2.4. Sponge mortality over time; likely causes burial by sediment and rubble (1a-c), tissue necrosis (2a-c), and possible anchor shearing (3a,b). Scale bars = 10.4 cm. 58

Figure 2.5. Size in 2016 predicted by 2014 data with model averaged estimates at each site. 59

Figure 2.6. Estimated size-at-age for the models of best fit per site (Buoy 1 [B1]: specialized von Bertalanffy; Sampela 1 [S1]: specialized von Bertalanffy; Kaledupa Double Spur [KDS]: Gompertz; Ridge 1 [R1]: Gompertz), including model averages. The dashed line represents the largest sponge volume recorded in this study. 61

Figure 3.1. Survival and growth kernel (A, B) and fecundity kernel (C, D) for Sampela 1 and Buoy 1. The dashed line in the survival and growth kernel indicates stasis where sponges along the line neither shrink nor grow; presence above the line indicates growth, below indicates shrinkage. Lighter colours indicate a higher probability of a transition occurring. 81

Figure 3.2. The transition surface the integral projection model for each site: A) Buoy 1, B) Sampela 1. 82
Figure 3.3. Stable size distribution of *Xestospongia* spp. populations in equilibrium and the observed size distribution for Buoy 1 (A, B) and Sampela 1 (C, D).

Figure 3.4. Proportional elasticities of vital rate parameters (survival and growth, fecundity) for the population growth rate for Buoy 1 (A, C), and Sampela 1 (B, D). Lighter colours indicate a higher sensitivity of the population growth rate to change.

Figure 3.5. Population growth rates at Buoy 1 and Sampela 1 as a result of simulating size-dependent survival in both open and closed populations. A: recruit and B: largest sponges (0 and 100% mortality). The dashed line represents self-sustainability, or the population neither growing nor decreasing.

Figure 4.1. Respiration chamber design with model *Xestospongia* spp. sponge, asterisks (*) denote cable clamps throughout the chamber, arrows denote direction of water flow. Note: the chamber was blacked out for actual respiration experiments.

Figure 4.2. Mean sediment fraction percent composition (± SE) collected in sediment traps in depths ranging from 5-10 m at Sampela 1, Wakatobi National Marine Park (n = 30).

Figure 4.3. Mean grain size distribution of suspended sediment (± SE) collected at 10 m from Sampela 1, Wakatobi National Marine Park (n = 3).

Figure 4.4. Time series of mucus production as a sediment clearance mechanism in two *Xestospongia* spp. sponges following 125-250 µm sediment addition; a) immediately following sediment addition; b) one hour post sediment addition; c) two hours post sediment addition; d) four hours post sediment addition. Arrows indicate the presence of mucus aggregates and buoyant threads.

Figure 4.5. Mean increase in respiration rate (± SE) over the course of the experiment for control (no sediment; n = 7) and sediment treatments: 75 mg l$^{-1}$ (n = 6) and 150 mg l$^{-1}$ (n = 7) standardized by seawater blank (T3-T0, 45 minutes total) corrected for changes in partial pressure of O$_2$. Superscripts denote significant differences (Tukey’s HSD post-hoc, P < 0.01).

Figure 4.6. Mean respiration rates (± SE) of *Xestospongia* spp. sponges during exposure to suspended sediment treatments over the course of 45 minutes and corrected for changes in partial pressure of O$_2$. Control treatment represents individual sponges not exposed to a suspended sediment. Time 0 represents the baseline respiration rate per treatment immediately before the addition of sediment treatments, while 15-45 represent the time (min) post sediment addition.
Figure 5.1. Results of Principle Coordinate Analysis (PCA) using genetic distances between individuals and a genetic distance matrix in GENALEX 6.5. Circles were added manually based on the genetic groups identified by STRUCTURE; the five groups were further analysed for within group population differentiation (AMOVA).

Figure 5.2. STRUCTURE-derived large-scale population genetic differentiation inferred via Bayesian clustering for all samples. Individual membership coefficients are divided into five sections representing the proportion of membership for different genetic clusters (Groups A-E). Prior population information was given and the best inferred clustering scheme was K = 3.

Figure 5.3. STRUCTURE-derived large-scale population genetic differentiation inferred via Bayesian clustering for all groups.

Figure 5.4. Plots generated in STRUCTURE Harvester demonstrating the mean log likelihood of the data \([L(K)]\) and Evanno’s delta K statistic for Groups A through E. Group C was omitted from analysis due to small sample sizes.

Figure 5.5. Mean maximum-likelihood estimation of relatedness (MLE) values between individual sponges within and between (overlapping portion) sites for each genetic group (A-D). Group C was omitted from analyses due to low sample size. Values in bold indicate a significant influence of site pairing on MLE (Bonferroni post-hoc tests, \(P < 0.05\)).

Figure 5.6. A comparison of the maximum-likelihood estimation of relatedness estimates (MLE) ± SE across genetic groups for each site pairing: A) Buoy 1 to Buoy 1 (B1-B1), Sampela 1 to Sampela 1 (S1-S1), and Buoy 1 to Sampela 1 (B1-S1), and B) Kaledupa Double Spur to Kaledupa Double Spur (KDS-KDS), Ridge 1 to Ridge 1 (R1-R1), and Kaledupa Double Spur to Ridge 1 (KDS-R1). Letters and asterisks (*) represent differences in mean values (\(P < 0.05\)).
List of Tables

Table 2.1. Difference equations for candidate growth models. 47

Table 2.2. Environmental parameters for Hoga Island sites. a indicates data collected solely herein, b indicates data from Rowley (2014), c indicates data from Powell et al. (2014), d indicates the inclusion of data from Powell et al. (2014). Remaining data sources were obtained and denoted as follows: e Hennige et al. (2008), f Hennige et al. (2010), g Crabbe and Smith (2002), h Salinas de Leon (2010), i Biggerstaff (2016). NTU refers to nephelometric turbidity units, STU refers to standard turbidity units. All values are expressed as mean (± SE). 51

Table 2.3. Among site variation in environmental parameters (one-way ANOVA; Buoy 1, Sampela 1, Kaledupa Double Spur, Ridge 1). Asterisks denote a significant effect ($P < 0.05$). 52

Table 2.4. Spatial variation in mean density (sponges 100 m$^{-2}$) across sites (one-way ANOVA; Buoy 1 [B1], Sampela 1 [S1], Kaledupa Double Spur [KDS], Ridge 1 [R1], Karang Gurita [KG]). Asterisks (*) denote a significant effect ($P < 0.05$). 53

Table 2.5. Spatial variation in mean volume (cm$^3$) across each site (two-way ANOVA; Buoy 1, Sampela 1, Kaledupa Double Spur, Ridge 1) and over time (2014, 2015, 2016). Asterisks (*) denote a significant effect ($P < 0.05$). 56

Table 2.6. Parameter estimates for candidate models at each site. 59

Table 2.7. AIC$_c$ criteria across sites ranked by fit following model elimination based on unreliable parameter estimates: parameter number in the model (+1) to account for variance ($\sigma^2$), residual sum of squares (RSS), bias-corrected Akaike information criterion (AIC$_c$), Akaike differences ($\Delta_i$), Akaike weights ($w_i$). 60

Table 4.1. Repeated measures ANOVA model examining Xestospongia sp. mean respiration rate exposed to suspended sediment concentrations and no sediment control sponges. Significant values are derived from Greenhouse-Geisser corrections. 116

Table 5.1. Hierarchal analysis of molecular variance (AMOVA) used to estimate levels of genetic differentiation between five genetic groups derived from PCA ($P < 0.001$). 133
Table 5.2. Standard genetic diversity indices for five genetic groups at nine loci for Buoy 1 (B1), Sampela 1 (S1), Kaledupa Double Spur (KDS), and Ridge 1 (R1). \( N_A \) = number of alleles per locus, \( H_o \) = observed heterozygosity, \( H_e \) = expected heterogeneity, \( F_{IS} \) = inbreeding coefficient. \( F_{IS} \) values that were significant after sequential Bonferroni correction are denoted as bold, sample sizes for each site are in parentheses.

Table 5.3. Pairwise loci comparisons for Hardy Weinberg Equilibrium (HWE) between at each site for genetic Groups A-E. Values in bold indicate significant deviance from HWE (\( P < 0.05 \)).

Table 5.4. Hierarchal analysis of molecular variance (AMOVA) used to estimate levels of genetic differentiation between each group individually (Group A, \( P = 0.001 \); Group B, \( P < 0.001 \); Group D, \( P < 0.001 \); Group E, \( P = 0.08 \)).

Table 5.5. Pairwise fixation index values (\( F_{ST} \)) for Groups B (below diagonal), D (above diagonal), and A (below diagonal), E (above diagonal). Bold values indicate significance based on 10,000 permutations (\( P < 0.001 \)).

Table 5.6. Assignment tests conducted in GeneClass v. 2.0 for Xestospongia recruits for genetic Groups B and D.

Table 5.7. One-way ANOVA results examining pairwise maximum-likelihood estimation of relatedness estimates (MLE) within each site and between sites for each genetic group. Bonferroni post-hoc tests were used to examine the significant main effects between site pairings. Asterisks (*) denote significant effects (\( P < 0.05 \)).

Table 5.8. One-way ANOVA results examining pairwise maximum-likelihood estimation of relatedness estimates (MLE) between genetic groups within and between sites. Bonferroni post-hoc tests were used to examine the significant main effects between groups for Buoy 1 and Sampela 1 pairings only. Asterisks (*) denote significant effects (\( P < 0.05 \)).

Table 5.9. Putative full sibship dyad reconstruction analysis in COLONY showed the occurrence of full sibling pairs among Xestospongia recruits for Group A (\( P > 0.75 \)); some of the pairings are for one individual with multiple siblings. Distance (m) represents the distance between the matched pairs.
Chapter 1: General Introduction

*Xestospongia* spp. at Kaledupa Double Spur in the Wakatobi National Marine Park
(photo credit: Joe Marlow)
1.1 Global decline of coral reefs

It is widely recognized that coral reefs are among the most highly productive and biologically diverse ecosystems on Earth, providing ecosystem goods and services vital to tropical and subtropical nations (Moberg and Folke 1999). Despite their value over half of all coral reefs worldwide are considered under threat (Burke et al. 2011), and the natural and anthropogenic pressures associated with global coral reef decline have been well documented (Hoegh-Guldberg et al. 2007; Wild et al. 2011; Perry et al. 2013). Major anthropogenic risk factors contributing to this deterioration include water pollution from terrestrial runoff and dredging, destructive fishing practices, overfishing, coastal development (Burke et al. 2011), and this decline is only expected to continue with the effects of climate change (Hoegh-Guldberg et al. 2007). These factors, in conjunction with natural pressures, may lead to coral mortality, long-term regime shifts in benthic community structure, and decreased net carbonate accretion, thereby threatening economically important reef systems (Wild et al. 2011; Kennedy et al. 2013).

1.2 Shifts from coral dominated states

There is a growing body of evidence to suggest that declines in coral cover are often closely followed by a transition into a community structure dominated by alternative benthic organisms, which is often referred to as a ‘phase’ or ‘regime’ shift. The majority of regime shift case studies are from coral to algal-dominated states following the widespread coral declines in the Caribbean associated with habitat degradation and decreases in herbivore abundance (Hughes 1994). These shifts often include feedback loops that prevent coral recruitment and therefore re-establishment of a coral dominated state (Norström et al. 2009). Reports of regime shifts to communities dominated by organisms other than algae are few, although there have been increasing reports of coral-dominated states to those dominated by other benthic organisms, including sponges (Norström et al. 2009; Bell et al. 2013a).

1.3 The functional roles of marine sponges

Sponges are one of the most ancient and simple metazoans belonging to the phylum Porifera and have evolved into an abundant, diverse and ecologically important group in both marine and
freshwater habitats (Hooper and Van Soest 2002; Becerro 2008). First appearing in the Precambrian era over 700 million years ago, sponges were the major contributors to reef structure during the Devonian, Palaeozoic and Mesozoic eras (Li et al. 1998; Love et al. 2009). There are an estimated 12,000 known sponge species worldwide, with many more still likely to be identified (Van Soest et al. 2012).

The sponge body plan is designed for suspension feeding; water is drawn through surface pores (ostia) and flows through inhalant canals to choanocyte chambers. Each chamber possesses flagella-bearing choanocytes that pump water through collar-filters that filter small suspended particles and out through exhalant canals that merge into oscula on the sponge surface (Riisgard and Larsen 1995). This simple system is very efficient; some species can filter up to 72,000 times their body volume per day and retain particles with > 90% efficiency (Reiswig 1971; Pile et al. 1997; Koopmans et al. 2010). Highly efficient particle retention, coupled with the ability to pump large quantities of water relative to their size, means that sponges have the potential to strongly modify water column characteristics by removing a large portion of available food (e.g. picoplankton; Perea-Blazquez et al. 2012) and dissolved organic carbon (de Goeij et al. 2008). Pile et al. (1997) reported that feeding efficiency in the freshwater sponges *Baikalospongia intermedia* and *B. bacillifera* was sufficient to cause picoplankton-depleted layers over the benthos.

Sponges provide an important function in linking water column productivity and the benthic community (Pile et al. 1997; Lesser 2006), and are considered a critical component in structuring benthic communities. They have been shown to facilitate carbon transport (Jiménez and Ribes 2007; de Goeij et al. 2008), as well as influence nitrogen (Jiménez and Ribes 2007) and silica cycling (de Goeij et al. 2013). Sponges can therefore play a large role in ecosystem functioning, and any changes in sponge populations have the potential to impact pelagic ecosystems (Bell 2008).
1.4 Sponges on coral reefs

In coral reef ecosystems sponges perform a number of functional roles that greatly influence ecosystem health (Wulff 2006). Many sponges are phototrophic, where over 50% of their energy requirements are derived from photosynthetically fixed carbon produced by photosymbiotic microbes and zooxanthellae (Webster and Taylor 2012). In addition to hosting microbial communities, many sponges form complex benthic structures utilized by other species for habitat, both internally and externally (Henkel and Pawlik 2005; Kersken et al. 2014). Furthermore, sponges can physically alter reef structure via substrate consolidation and bioerosion (Diaz and Rutzler 2001; Wulff 2001), and produce secondary metabolites of both ecological and pharmaceutical interest (Paul and Ritson-Williams 2008). Reduction in sponge abundance, biomass, and species richness can result in cascading impacts on marine ecosystems (Peterson et al. 2006; Bell 2008), yet sponges generally are globally understudied due in large part to difficulties in their identification and quantification (Diaz and Rutzler 2001; de Voogd et al. 2006).

1.5 Sedimentation and the benthic community

The impacts of sedimentation on sponges is receiving increasing global attention (Bell et al. 2015a; Schönberg 2016). Sedimentation generating events, such as storms, are naturally occurring for most benthic organisms. Increasing evidence suggests, however, that there is a global increase of land-based sediments making their way into coastal ecosystems (Lohrer et al. 2006; Stender et al. 2014; Capuzzo et al. 2015). Other sediment sources include agriculture-derived runoff and unsustainable land-use practices, such as coastal construction and dredging, deforestation, and mangrove and seagrass removal (McKergow et al. 2005; Syvitski et al. 2005; Pineda et al. 2016). Sediment deposition and resuspension is one of the most common causes of coastal degradation and can impact the benthos in a variety of ways, both directly and indirectly, likely at all life stages (Airoldi 2003; Carballo 2006).

The effects of sedimentation on marine invertebrates remains largely unstudied in spite of the ubiquitous nature of this threat and the variety of potential impacts. Increased sediment loads can alter coastal ecology through reduction in light availability (Capuzzo et al. 2015), introduction of
nutrients (Smith et al. 2001), and direct organismal contact (e.g. smothering; Pineda et al. 2016). High suspended sediment loads have been shown to reduce physiological condition and clearance rates in mussels (Ellis et al. 2002), and alter feeding behaviour in several species of bivalve (Bayne et al. 1993; Jorgensen 1996). Sediment exposure may result in the consumption of sub-optimal levels of nutrients, as well as overall decreases in dietary absorption (Newell and Jordan 1983; Ward and MacDonald 1996).

1.5.1 Sedimentation impacts on sponges

Several studies have focused on the effect of sedimentation on the population dynamics of sponge assemblages and have revealed species-dependent responses (Airoldi 2003; Tjensvoll et al. 2013; Pineda et al. 2016). Many species are commonly found in highly sedimented areas (Bell and Barnes 2000a; Bell and Smith 2004; Knapp et al. 2013), and some species demonstrate a variety of tolerances and adaptations to sediment exposure and burial (Ilan and Abelson 1995; Cerrano et al. 2007; de Voogd 2012; Schönberg 2016). On coral reefs, increased sedimentation and reduced light availability may result in environments becoming uninhabitable by hard coral but beneficial to some sponge species. In the Palmyra Atoll, habitat degradation and the introduction of non-native sponge species is thought to have resulted in a sponge-dominated lagoon system (15-33% sponge cover; Knapp et al. 2013). Furthermore, sponge densities in Indonesia have increased at some sites over a 6-7 year period (Bell and Smith 2004; Powell et al. 2010), while coral cover and habitat quality have decreased (Powell et al. 2010).

While some sponge species are able to tolerate and even thrive in highly sedimented habitats, there is evidence that sedimentation may be deleterious to sponges at the individual and population level (Bell et al. 2015a). Settled sediment can directly affect sponges via burial/smothering (Wulff, 1997) and cause tissue scour and abrasion (Rogers 1990; Ilan and Abelson 1995), resulting in partial mortality and reduced survival (Wulff 1997; Maldonado et al. 2008). At the physiological level, settled and suspended sediment can exert a major influence on sponge functioning. Sponges are obligate suspension feeders and exposure to suspended sediment may clog the inhalant canals and filtering system responsible for pumping. The experimental addition of fine suspended sediments have been shown to reduce or arrest pumping rates in several sponge species (Gerrodette
and Flechsig 1979; Leys et al. 1999; Tompkins-MacDonald and Leys 2008; Bannister et al. 2012). As pumping is required to feed, clogging by fine sediment may reduce feeding efficiency and particle retention (Lohrer et al. 2006), as well as respiration (Gerrodette and Flechsig 1979).

Altered pumping have the potential to effect the pelagic community (Lohrer et al. 2006). Furthermore, turbidity, or re-suspension of fine sediments, may result in shading which has been shown to reduce reproductive efficiency (Roberts et al. 2006; Whalan et al. 2007a), as well as photosynthetic efficiency of symbionts (Biggerstaff et al. 2015), which could result in fewer yearly recruits and therefore decreases in sponge abundance over time. Sedimentation has previously been shown to reduce larval settlement rates in coral (Babcock and Davies 1991; Gilmour 1999), and may therefore be expected to affect sponge larval settlement rates but has not yet been examined.

1.6 Xestospongia spp.

Some of the most conspicuous sponges on coral reefs fall into the genus Xestospongia, which include the giant barrel sponges. The Caribbean X. muta is one of the most intensely studied sponges in the world (e.g. López-Legentil and Pawlik 2008; López-Legentil et al. 2008; McMurray et al. 2008, 2010, 2014, 2017; Pawlik et al. 2013), yet Indo-Pacific species have received far less attention despite their ubiquitous nature (but see de Voogd et al. 2003; Powell 2013; Swierts et al. 2013; Bell et al. 2014). Among the largest known sponges, Xestospongia species can grow up to several meters in diameter and live to be hundreds or possibly thousands of years old (McMurray et al. 2008). In the Caribbean, X. muta populations may cover > 9% of surface area and have a greater biomass and filtering capacity than any other benthic invertebrate (Zea et al. 1994; Handley et al. 2003). X. muta consume dissolved organic carbon (DOC; 70%) and detritus (20%) as the majority of their diet (McMurray et al. 2016), and are capable of pumping vast quantities of water per day (McMurray et al. 2014). Barrel sponge size scales directly to ecosystem impact as large sponges are expected to pump larger quantities of water, as well as increase habitat availability for infauna (Westinga and Hoetjes 1981; Tanaka and Aoki 1999; Henkel and Pawlik 2005). X. muta populations have also been reported to harbour a variety of macro-fauna (Lewis and Finelli 2015; Hammerman and García-Hernández 2017), as well as a dense microbial community that
contributes to primary production (Montalvo and Hill 2011). On reef systems where coral cover is declining in the Indo-Pacific, *Xestospongia* spp. are likely the largest remaining benthic invertebrates.

Indo-Pacific barrel sponges were believed to solely include *X. testudinaria* and *X. berguista*, but recent work in this region has revealed complications to this classification; as such *Xestospongia* spp. will be used throughout this thesis. Swierts et al. (2013) and Bell et al. (2014) reported the presence of a *Xestospongia* species complex in the Sulawesi using nuclear (ATP synthase β intron) and mitochondrial (CO1 and ATP6 genes) markers, as well as microsatellites, respectively. Both authors reported four genetically differentiated *Xestospongia* morphotypes that corresponded with morphological differences. Furthermore, the two species examined by Bell et al. (2014) demonstrated differing patterns of genetic structuring; the authors suggest this may be attributed to differing processes influencing gene flow between populations. While *X. muta* population structure is believed to be driven by oceanic currents in the Caribbean (López-Legentil and Pawlik 2008; Richards et al. 2016), the forces structuring Indo-Pacific barrel sponge genetic structure remain unknown. For Chapters 2 through 4 sponges were sampled based on morphology consistent with *X. testudinaria*, following the work of Bell et al. (2014) in the Wakatobi National Marine Park. In Chapter 5, however, molecular analyses revealed a possible *Xestospongia* species complex, indicating that morphology was not a reliable indicator of species. Due to the demographic nature of the contents of Chapters 2–5, the discovery of a subspecies is not expected to compromise the reported results, as multiple species were treated at the level of genus. These results, in conjunction with previous work in Indonesia, demonstrates the need for further taxonomic clarification for sponges in this genus.

Reduced coral competition and fewer storm events have resulted in an increase in successful recruitment of *X. muta* in the Florida Keys where they remain as the second most common sponge in terms of percent cover (Loh and Pawlik 2014). *X. muta* abundance has increased by 45% from 2000–2006 in this area, and are now considered the most important habitat-forming reef organism (McMurray et al. 2010). Although previous work in the Indo-Pacific suggests that *Xestospongia*
spp. are present across a spectrum of habitat quality (Bell et al. 2014), there remains a paucity of information on the population dynamics in this area.

![Figure 1.1 Xestospongia spp. in the Wakatobi National Marine Park, Indonesia.](image)

1.7 Demography

1.7.1 Growth and size

An individual’s size will inherently affect the magnitude of its influence on the surrounding environment (Ayling 1983; Werner and Gilliam 1984; Pansini and Pronzato 1990). Large benthic organisms may dominate specific ecosystem functions, particularly in the case of species that mediate nutrient flux (Norkko et al. 2013), such as *Xestospongia* spp. Sponges are dominant benthic competitors (Engel and Pawlik 2005), and *Xestospongia* spp. are large and thought to be long-lived, characteristics that render species susceptible to extinction (Thrush and Dayton 2010).

1.7.2 Population dynamics

While growth provides information on individual-level processes, it is also of interest to quantify the dynamics of a population. The success of a population depends on its overall health (Caswell 2001), including growth as well as survival and reproduction (Clark et al. 2011), characteristics
integral to persistence in a changing environment. On coral reefs, the use of traditional mechanistic models that utilize information derived from the present state (i.e. presence/absence, distribution, percentage cover) are common. Data such as percentage cover are a result of many processes (dispersal, ecological interactions, demography, behaviour, etc.). Their use to assess the changes in community structure or population dynamics may be misleading as these measures do not incorporate the mechanisms behind any measured changes (Edmunds and Elahi 2007; Darling et al. 2013). For example, high levels of percentage cover may be masking long-term population decline (Hughes and Tanner 2000).

Population modelling allows the incorporation of the current state of a population into a modelling framework that describes changes in a population over time in order to predict future behaviour (Caswell 2001). Using size-structured models it is possible to link the contribution of individual demographic characteristics, such as growth, fecundity, and mortality, to population performance (Elahi et al. 2016). Integral projection models (IPMs) are a relatively new and powerful method used in many ecological applications to describe the dynamics of a population over time (Easterling et al. 2000). Transitions between size classes are integrated to inclusively quantify how the state of an individual changes over time (Edmunds et al. 2014). IPMs describe how sponges enter the population through recruitment and leave through death, incorporating immigration and emigration where appropriate (Edmunds et al. 2014).

1.7.3 Population connectivity

An organism’s dispersal among geographic locations determines genetic population structure through the exchange of alleles. Successful dispersal and settlement significantly affects the dynamics of a population, and can be used to quantify gene flow between populations via connectivity estimates (Cowen et al. 2000; Shanks et al. 2003). While genetic connectivity estimates provide information on the degree that population growth and demographic processes are affected by genetic dispersal and recruitment, demographic connectivity refers to the relative contribution of net immigration to total recruitment (Lowe and Allendorf 2010), and can be estimated using relatedness analyses.
Larval behaviour and small-scale oceanographic forces may also be responsible for limiting the spatial extent of dispersal by enhancing self-recruitment. Self-recruitment is defined as the proportion of sampled larvae identified as the offspring of adults from the same location (Berumen et al. 2012), the scale of which depends on the organism in question. Self-recruitment is common among a variety of taxa and can be used to assess the degree of openness of a population (Bode et al. 2006; Jones et al. 2009). Previously, Bell et al. (2014) showed that barrel sponges in the Wakatobi National Marine Park are likely self-recruiting, with low levels of gene flow between populations at scales of 5-100 km and evidence of inbreeding across all populations. A reliance on self-recruitment in conjunction with this species’ longevity, slow growth, and small population size may make them vulnerable to environmental disturbances (Bell et al. 2014).

1.8 The Wakatobi National Marine Park: a system under threat

The Indonesian Archipelago has been described as having the richest areas of marine biodiversity worldwide (Unsworth et al. 2010). The Wakatobi National Marine Park (WNMP; 05°29.6S, 123°45.26E; Figure 1.1), located off the southeast coast of Sulawesi, Indonesia), is a World Biosphere Reserve (Clifton 2013) as well as the third largest and most populated marine national park in Indonesia. The WNMP contains some of the highest marine diversity in the world (Unsworth et al. 2010), yet is impacted by the local population of 100,000 people that are heavily reliant on this resource (Cullen 2010). Unfortunately, the WNMP has historically lacked efficient enforcement, sufficient funding, community participation in management, and appropriate zonation (Unsworth et al. 2010).

The reefs in the WNMP are subject to a range of local-scale impacts including coral mining and unsustainable fishing practices (e.g. blast and cyanide fishing; Clifton 2013). These practices, in conjunction with the potential impacts from climate change and ocean acidification (Elliott et al. 2001; Pandolfi et al. 2003; Hoegh-Guldberg et al. 2007), are expected to directly (via physical damage) and indirectly (via the introduction of sediment) affect coral health. Coral reefs in this area have already declined substantially in the last decade; hard coral cover surveys conducted from 2000 to 2007 revealed that coral decreased an average of 45% across six sites over that time (McMellor and Smith 2010).
1.9 Study sites

The four core sites used in this study were surveyed around Hoga Island (Figure 1) between 2014 and 2016. These included: Sampela 1, Ridge 1, Buoy 1, and Kaledupa Double Spur. Buoy 1 and Ridge 1 are located on the western side of Hoga Island, while Sampela 1 and Kaledupa Double Spur are located on the northeast side of Kaledupa Island (Figure 1). Sampela 1 is on the fringing reef surrounding Kaledupa Island adjacent to the local Bajo village of Sampela. The reef at Sampela 1 is an important and exploited resource that is considered degraded due to high level of sedimentation (Crabbe and Smith 2002a) and decreasing hard coral abundance (35 to 5% from 2000-2011; Curtis-Quick 2013). The sediment at Sampela 1 is made up of fine particles settling from suspension that forms a layer of sediment (< 5 mm thick) on the benthos. The remaining
study sites experience varying rates of sedimentation; Buoy 1 is classified as moderate site quality based on levels of coral cover, and proximity to human populations (1.5 km). Ridge 1 and Kaledupa Double Spur experience low levels of sedimentation.

Three further sites were surveyed in 2014 as part of this thesis to estimate population densities in the wider WNMP: Karang Gurita, Wanci, and Tomea. Bell et al. (2014) described Wanci as a low quality site with very low coral cover (5-10%) and fish biomass. These sites are also located < 1 km from a large population and experience heavy sedimentation and high turbidity. Tomea is considered to have moderate site quality with moderate coral cover (25-35%), good water clarity, low sedimentation, and is located a distance of 3-5 km from large populations. Karang Gurita is considered a high quality site with the high coral cover (over 35%), high water clarity, and is the greatest distance from a large human population (Bell et al. 2014).

1.10 Xestospongia spp. demography

There are several factors contributing to the lack of basic information on sponge demography. Measuring sponge growth can be difficult due to complex external morphology and internal physiology, as well as a lack of measurable features, such as otoliths in fish (e.g. Mercier et al. 2011). Previous work on sponges has described highly variable growth among individuals, possibly due to a difference in individual fitness in the face of environmental variability (de Caralt et al. 2008). This could be explained by the energetic requirements for different strategies of handling suboptimal conditions that could come at a cost to life history processes (Reiswig 1971; Roberts et al. 2006; Whalan et al. 2007a; Bannister et al. 2012). Examining the life histories of functionally significant species allows an understanding of the processes influencing their dynamics. Since Xestospongia spp. have the potential to significantly affect reef functioning, understanding size and growth reflects their impact on coral reefs. Furthermore, previous work in the Caribbean suggests that barrel sponges may live in excess of hundreds if not thousands of years (McMurray et al. 2008). The same is true in the Indo-Pacific; a long life, coupled with large size, may result in vulnerability to extinction in changing habitats (Thrush and Dayton 2010).
1.11 Goals and Aims

Given the decline of coral cover in the WNMP, *Xestospongia* spp. are now one of the largest remaining benthic invertebrates on many reefs. The lack of basic demographic information, as well as the paucity of information regarding the effect of sediment exposure on *Xestospongia* spp. health, results in a large gap in our understanding of how reefs in the WNMP function. The primary goals of this thesis are to establish a baseline of *Xestospongia* spp. demography on an individual and population level, and explore mechanisms of acclimation of these sponges to sedimentation. With this information it will be possible to understand how the *Xestospongia* spp. community in the WNMP will be affected by habitat degradation and enable focused management efforts in the future.

The aims of my thesis were to:

1. Quantify *Xestospongia* spp. growth and longevity across four sites to explore the influence of environmental variability on individual level demography. I measured and compared demographic parameters including specific growth, mean volume, and density, as well as environmental parameters across sites. I fitted and compared four growth models across sites using multi-model inference. I also extrapolated size-at-age across sites and validated the accuracy of my estimates using sponges from a structure of known age.

2. Explore the mechanisms driving population growth dynamics of *Xestospongia* spp. across two sites using integral projection models (IPMs). I quantified the extent that the relationship between sponge size and growth, survival, and fecundity (vital rate parameters) influences population-level outcomes (growth or decline). I assessed the importance of recruitment source and size-dependent survival on population growth at each site.

3. Assess the adaptive mechanisms and physiological effects of suspended and settled sediment on barrel sponges. I quantified the ambient suspended and settled sediment at Sampela 1, and measured the physiological response of barrel sponges with *in situ* respirometry to two environmentally relevant suspended sedimentation treatments (as well as a control treatment). I also quantified the efficacy of mucus production as a sediment
removal mechanism and assessed the prevalence of mucus bound sediment both in the spongocoel and outer surface of sponges at Sampela 1.

4. Utilize nine multi-locus microsatellite markers to investigate the genetic connectivity and structure of *Xestospongia* spp. populations at four sites. I assessed the prevalence of cryptic speciation across sites and using assignment tests, kinship analysis, and mean-likelihood of relatedness estimates, quantified the degree of relatedness and prevalence of self-recruitment between and within sites.
Chapter 2: Growth and longevity in giant barrel sponges: Redwoods of the reef or Pines in the Indo-Pacific?

*Xestospongia* spp. on the wreck of the USAT Liberty
2.1 Abstract

Describing how environmental variability influences life history dynamics of functionally important species is critical for successful conservation and management. The massive barrel sponges *Xestospongia* spp. have important functional roles on coral reefs, particularly as a result of their water column interactions. Earlier Caribbean studies suggested that barrel sponges are ‘Redwoods of the Reef’ that may be 1000s of years old. However, nothing is known of how such growth rates are influenced by environmental variability, or for barrel sponges from outside the Caribbean. We assessed the influence of environmental variability on Indo-Pacific barrel sponge demography. Growth rates were measured for 133 *Xestospongia* spp. (2 years across 4 sites) experiencing different environmental conditions in the Wakatobi National Marine Park, Indonesia. I compared specific growth rate, density, and mean volume, while four candidate growth models were fitted to the volume data at each site and compared using Akaike’s Information Criterion. Models of best fit models were determined using a multi-model inference (MMI) approach. Estimates were validated based on the size of barrel sponges on the USAT Liberty wreck which sank in 1962. Sponge volume was variable, yet strongly influenced by site (19.99 to 552,937.89 cm$^3$). Different best fit growth models were found at the different sites; in conjunction with differences in density, volume gained, and mean volume, this suggests that sponges living in different environmental conditions have differing growth trajectories. Size-at-age estimates placed the largest measured sponges between 24 and 30 years across sites, contrasting with published estimates of Caribbean *Xestospongia muta* of over 240 years for similar sized sponges. These ages were corroborated with the maximum age of sponges on the USAT Liberty wreck placed between 24 and 30 years old. My results highlight the accelerated growth to a large size of these massive sponges, compared to estimates from the Caribbean, and suggest that growth rates in the Indo-Pacific might be more comparable to Pines rather than Redwoods. These differences have important implications for how these ecologically important species should be managed.

2.2 Introduction

Understanding the life history traits of an organism, such as growth, recruitment, and mortality, are central to quantifying its contribution to ecosystem functioning (Pardo et al. 2013), and in managing species in response to environmental perturbations (Beardsley and Britton 2012). The
size of an organism, and the population within which it resides, will likely affect the magnitude of its influence on other organisms (Werner and Gilliam 1984). Size is typically related to life-history processes such as mortality, growth and reproduction (Meesters et al. 2001), as well as its spatial competitiveness (Chadwick and Morrow 2011) and ability to consume resources. However, these processes are not independent of the environment, and are likely to be influenced by a range of abiotic and biotic factors (Ehrlén and Morris 2015).

An organism’s lifespan, along with its population and individual growth rate, can potentially be used to predict its resilience to environmental disturbance or exploitation (Hamidan and Britton 2015). For example, long-lived organisms with small population sizes that have sporadic or infrequent recruitment, low fecundity and slow growth rates (K-strategies) are likely to be more sensitive to disturbance compared to fast growing, short-lived, highly fecund species with large population sizes (r-strategies). Therefore, accurate measures of growth, recruitment, mortality, and age-structure are needed to support appropriate conservation and management strategies (Mumby et al. 2015).

Sponges are one of the most ancient and simple metazoans that have evolved into an abundant, diverse and ecologically important group in both marine and freshwater habitats (Hooper and Van Soest 2002; Becerro 2008). Highly efficient particle retention, coupled with the ability to pump large quantities of water relative to their size, results in the potential to strongly modify water column characteristics by removing a large portion of available particulate food (e.g. Perea-Blazquez et al. 2012) and dissolved organic carbon (de Goeij et al. 2013). Sponges link water column productivity and the benthic community (benthic-pelagic coupling; Pile et al. 1997; Lesser 2006), facilitating carbon transport (Jiménez and Ribes 2007; de Goeij et al. 2008), and nitrogen (Jiménez and Ribes 2007) and silicon cycling (Maldonado et al. 2005). Sponges therefore have a number of important functional roles on reefs, and any changes in sponge populations has the potential to impact ecosystem function (Bell 2008).

While sponges show a range of life-history strategies, some species are thought to be very long lived, with estimated life spans ranging from decades to thousands of years old (Dayton 1979; Leys and Lauzon 1998; McMurray et al. 2008; Webster et al. 2008; Teixidó et al. 2009). Trophic
relationships, physiology, and ecological impacts are all affected by size (Bluweiss et al. 1978; Peters 1986; Sebens 1987; Caswell 1988; Teixidó et al. 2009) and the presence of large sponges in a population typically suggests longevity (Ayling 1983; Pansini and Pronzato 1990). Among the largest known sponges, sponges in the genus *Xestospongia* can grow up to several meters in diameter (McMurray et al. 2008) and pump large quantities of water. McMurray et al. (2014) reported that *X. muta* process the equivalent of a 30 m water column in 2.3-18 days. These sponges rarely stop pumping (McMurray et al., 2014), and are highly efficient in retaining picoplankton (62-97%), while also consuming dissolved organic carbon (DOC; McMurray et al. 2016). In fact, they have recently been shown to have a higher carbon flux rate than other reef species on Conch Reef in Florida, and this rate has increased in recent years (McMurray et al. 2017). Despite this highly efficient feeding, McMurray et al. (2008) suggested that Caribbean *X. muta* individuals are slow growing and could live to be hundreds or possibly even thousands of years old. These features of *X. muta* would be expected to make this species susceptible to environmental perturbations. Whether these features are applicable to *Xestospongia* spp. in the Indo-Pacific or how the environment influences such parameters is unknown.

Growth models are widely used to describe increases in size or volume over time, particularly in fisheries biology (Lugert et al. 2014), where otolith measurements or known fish size are used to validate model projections and produce size-at-age models. Choosing the appropriate models is critical as poor model selection may lead to errors in parameter estimation and subsequent inferences about growth dynamics (Symonds and Moussalli 2011). In the event of inappropriate candidate model choice, high model parsimony may be achieved while producing biologically meaningless parameter estimates, resulting in incorrect growth trajectories and age/size estimations (Burnham and Anderson 2003; Karkach 2006; Pardo et al. 2013). Rather than choosing arbitrary models *a priori* and identifying the “best” candidate model or models, multi-model inference (MMI) using model averaging can be used to estimate parameters from multiple or an entire set of candidate models in order to reduce model selection uncertainty (Burnham and Anderson 2003). This method entails examining the fit of a range of candidate models to the data based on parsimony according to Akaike Information Criteria (AIC; Akaike 1974; Grueber et al. 2011), allowing for robust comparisons between models which could not otherwise be compared (Symonds and Moussalli 2011). MMI should be considered when Akaike weights ($w_i$) support
more than one model, creating uncertainty in model selection (Burnham and Anderson 2003; Katsanevakis 2006; Katsanevakis and Maravelias 2008). MMI is able to provide an averaged model incorporating information from multiple well-fitting models, rather than choosing one model that risks a poor fit to the dataset. As sponges lack features comparable to otoliths and absolute size-at-age is difficult to quantify in slow growing species, MMI should be particularly useful for reducing model selection uncertainty when estimating sponge growth. Furthermore, MMI approaches allocate Akaike weights to a set of models at each site. The weights can differ from site-to-site, meaning that there is no need to identify one consistent model across all sites, which is important where sponges may be experiencing different growth trajectories at different sites.

While there has been considerable study of *Xestospongia muta* in the Caribbean, the demographics of Indo-Pacific *Xestospongia* spp. has been poorly studied, despite being widespread across the region and likely to fulfil similar functional roles as in the Caribbean. Understanding barrel sponge demography, and how it is influenced by environmental variability is particularly important given current trends of habitat degradation in the Indo-Pacific and elsewhere. Here I examined the influence of varying habitat quality on the demography of *Xestospongia* spp. by quantifying individual growth, recruitment and mortality rates, and population sizes. We used a multi-model inference (MMI) approach with Akaike weights to model average four candidate growth models. Akaike differences were examined to select models of best fit; these models were used to estimate size-at-age across sites, providing important insight into growth dynamics and potential resilience to environmental perturbations.

### 2.3 Methods

#### 2.3.1 Study sites

Four core sites were surveyed around Hoga Island between 2014 and 2016: Sampela 1, Ridge 1, Buoy 1, and Kaledupa Double Spur. Buoy 1 and Ridge 1 are located on the western side of Hoga Island, while Sampela 1 and Kaledupa Double Spur are located on the northeast side of Kaledupa Island (Figure 1.1). The three further sites were surveyed to estimate population densities in the wider Wakatobi in 2014: Karang Gurita, Wanci Harbour, and Tomea. No environmental data were
collected for the sites sampled in the wider Wakatobi, although previous studies have provided environmental descriptions.

2.3.2 Environmental parameters

Throughout March-August 2014, May-June 2015, and June-August 2016 a XR-420 CTD data logger (RBR, Ottawa) was deployed at the four Hoga Island sites for a minimum of three independent dates per site. Deployment occurred for a minimum of 24 hours on haphazardly selected dates to measure turbidity and chlorophyll-\(a\) (as a proxy for food availability). The CTD was set to record every minute with no averaging. Averages were based on 24 hour deployments with minutes as subsamples within each period. Mean daily reef PAR was quantified using three separate 24 hour deployments of an Odyssey PAR logger (Dataflow Systems, Christchurch NZ) at 10 m. The logger was set to record every minute and data was averaged over daylight hours. In order to gain a longer time series of the environmental conditions at these sites, we also considered the data collected as part of a number of previous studies. Full details of the environmental data at the four core sites (including data collected in the present study) can be found in Table 2.2.

2.3.3 Sponge volume measurement

Data were collected from June to August in 2014, 2015 and 2016. Sponge images were collected yearly from Sampela 1, Buoy 1, Ridge 1, and Kaledupa Double Spur, while wider WNMP sites (Wanci, Tomea, and Karang Gurita) were visited in 2014. Each Hoga Island study site was mapped at the beginning of the study. Preliminary surveys were conducted to assess the extent of the populations; this was determined when no additional sponges were found over a 50 m distance from either side of the reef. All sponges within this area were then located and recorded using x and y coordinates from 1 to 30 m. All sponges were marked with a unique tag to facilitate subsequent identification. While target sponges were identified based on morphology described in Bell et al. (2014) for \textit{X. testudinaria}, the discovery of a potential species complex (Chapter 5) revealed that this method is inaccurate. Sponges were therefore treated at the level of genus for the duration of analyses.
Digital images for stereo photogrammetric analysis were taken with a Fujifilm FinePix Real 3D W3 Digital Camera with corresponding underwater camera housing. Volumetric measurements were calculated using stereo calibration and measurement software (CAL and PhotoMeasure) created by J. Seager (http://www.seagis.com.au). The use of stereo photogrammetry allows for accurate repeated 3D measurements in order to best calculate true external and spongocoel volume, though the internal canal system remains difficult to quantify (Abdo et al. 2006). Furthermore, stereo photogrammetry allows for multiple measurements to be made for a variety of sponge parameters. Volume was calculated by approximating geometric shapes for each sponge shape and corrected for spongocoel volume after McMurray et al. (2008). Due to the highly diverse morphologies of Indo-Pacific *Xestospongia* spp., sponges were categorized as either cylinder, barrel, sphere, inverted truncated elliptical cone, or frustrum of a cone. Spongocoels were categorized as either cylinders or inverted truncated elliptical cones (depending on the sponge) and volume was calculated accordingly. In the event that a sponge had multiple barrels, the volume of each was measured as appropriate and the volumes were combined. Finally, if sponge morphology changed over time formulae were adapted as to reflect changes in shape.

2.3.4 Barrel sponge demography

Specific growth rate (SGR) was calculated as the difference in sponge volume (cm$^3$) divided by the number of years between sampling events (after McMurray et al. 2008).

$$SGR = \frac{(V_t - V_i) * V_i^{-1}}{t}$$

Negative SGRs were confirmed from photographs and severely damaged sponges or those with severe necrosis were removed from further analysis. Sponge density was calculated by dividing the total area sampled by the sponge number at each event: Buoy 1 (4,500 m$^2$), Sampela 1 (5,450 m$^2$), Kaledupa Double Spur (5,952 m$^2$), Ridge 1 (5,238 m$^2$), Wanci (3,500 m$^2$), Tomea (11,880 m$^2$), Karang Gurita (22,780 m$^2$).
2.3.5 Candidate models

Five growth models were used to investigate the growth of *Xestospongia* spp.: specialized von Bertalanffy, generalized von Bertalanffy, Gompertz, Richards, Tanaka; Table 2.1. The Richards equation produced values identical to those in the generalized von Bertalanffy model and as such was removed across sites to avoid model redundancy (Katsanevakis 2006). Difference equations were obtained from Brey (2001) and Johnson (2012) and modified for size-increment data (Table 1). The biological meaning for each parameter associated with each function is as follows (Brey, 2001): \( S_{\infty} \) is the size (volume) reached after an infinite growth period, \( K \) equates to the growth rate, \( t_0 \) is the theoretical size at time 0, \( t^* \) is the age of growth inflection, and \( D \) determines the shape of the curve (in most cases approximately sigmoid). The Tanaka growth model for indeterminate growth includes the following parameters (Brey 2001): \( a \) is related to maximum growth rate \((\sim 1/\sigma^{0.5})\), \( c \) is the age at which growth is maximum, \( d \) shifts body size at which growth is maximum, and \( f \) is the measure of rate of change of the growth rate.

Table 2.1. Difference equations for candidate growth models.

<table>
<thead>
<tr>
<th>Function name</th>
<th>Function</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specialized von Bertalanffy</td>
<td>( S_2 = S_1 + (S_{\infty} - S_1) \times (1 - e^{-K \times dt}) )</td>
<td>( S_{\infty}, K, t_0 )</td>
</tr>
<tr>
<td>Generalized von Bertalanffy</td>
<td>( S_2 = (S_{\infty}^{-1/D} \times (1 - e^{-K \times dt}) + S_1^{1/D} \times (e^{-K \times dt})) \times D )</td>
<td>( S_{\infty}, K, t_0, D )</td>
</tr>
<tr>
<td>Gompertz</td>
<td>( \ln(S_2) = \ln(S_{\infty}) \times (1 - e^{-K \times dt}) + \ln(S_1) \times (e^{-K \times dt}) )</td>
<td>( S_{\infty}, K, t^* )</td>
</tr>
<tr>
<td>Richards</td>
<td>( S_2 = (S_{\infty}^{-1/D} \times (1 - e^{-K \times dt}) + S_1^{-1/D} \times (e^{-K \times dt})) \times D )</td>
<td>( S_{\infty}, K, t^*, D )</td>
</tr>
<tr>
<td>Tanaka</td>
<td>( S_2 = 1/(f^{0.5}) \times \ln(2 \times G + 2 \times (G^2 + f \times a^{0.5}) + d) )</td>
<td>( a, c, d, f )</td>
</tr>
</tbody>
</table>

\( G = E/4 - f \times a/E + f \)
\( E = \exp\left(\left(f^{0.5} \times (S_1 - d)\right)\right) \)

2.3.6 Data Analysis

All statistical analyses were performed by SPSS v. 22 and in R (version 3.3.3) and plotted with SigmaPlot v. 11.0 and R. Data collected for this thesis were tested for normality and homogeneity of variance; volume, density, and SGR were log_{10}-transformed. Mean temperature, turbidity, and chlorophyll-a data were compared across sites using one-way ANOVAs.
Sponges were grouped into depth ranges of 1-9.9, 10-19.9, and 20-30 m at each site (McMurray et al. 2008). The influence of depth on sponge volume was tested using a one-way ANCOVA with depth (10, 20, 30 m) as a fixed factor for each site. One-way ANOVAs were used to examine the effect of site on specific growth rate, yearly gains in volume, and sponge density averaged across depths. A two-way ANOVA was used to examine the effect of site on mean sponge volume across sites and years samples. Specific growth rates (SGR) from 2014-2016 were used as it was the longest available time interval. Mean volume gain was not used for the 2014-2015 sampling event due to the small sample size of sponges at Ridge 1. Significant effects were investigated further with Tukey post-hoc tests.

2.3.6.1 Model selection

Size increment volume data ranging from 2014 and 2016 (n = 113) was fitted to the candidate growth models by nonlinear least-squares regression (nlsLM, R) using the Levenberg-Marquardt algorithm. Sponge volume data, corrected for spongocoel volume, was cube root transformed for model input, and the difference equation for each function was applied to the transformed data (McMurray et al. 2008, Katsanevakis and Maravelias 2008). Models were discarded from further analysis if they failed in predicting reliable asymptotic lengths (Diouf et al. 2009).

Model fit and biological accuracy of parameter estimates for *Xestospongia* spp. growth were examined with an information theory approach. This method entails consideration of all candidate models, their parsimony with Akaike’s information criteria (AIC), and the accuracy of parameter estimation (Katsanevakis and Maravelias 2008). This approach is reliant on the strength of evidence in the data and thereby more relevant than classically used tools such as $R^2$ (Diouf et al. 2009; Katsanevakis 2006). The bias-corrected Akaike Information Criterion ($\text{AIC}_c$) was utilized to compare growth as the ratio of sample size to parameter number ($n/k$) was less than 40 (Shono, 2000; Symonds and Moussalli, 2011).

$$\text{AIC}_c = \text{AIC} + \frac{2k(k + 1)}{n - k - 1}$$
Akaike weights ($w_i$) represent the relative likelihood to the candidate model of best fit (Burnham and Anderson 2002). In the event that data have a $w_i$ above >0.9, the model in question is deemed the only appropriate model for the dataset. Should the Akaike weight ($w_i$) support more than one model, uncertainty in model selection cannot be ignored (Burnham and Anderson, 2003; Katsanevakis, 2006) and multi-model inference (MMI) using model averaging should be considered (Katsanevakis and Maravelias 2008). Model averaging weighs potential uncertainty in each candidate model in order to make more confident inferences and estimates the predicted response variable from multiple candidate models (Katsanevakis and Maravelias 2008).

After the growth rate at each site was considered independently with unique Akaike weights calculated, MMI with model averaging was utilized for each site and size-at-age was extrapolated. Then, in order to compare differences in growth rates between sites, a second model analysis approach was take. AICc values were calculated across models for each site and pooled. The model with highest support was then used to compare growth across sites using an analysis of the residual sum of squares (ARSS).

2.3.8 Size-at-age predictions

In order to retrospectively extrapolate age at size for each site when the actual age was unknown, the predicted size (volume) at time $t$ was estimated for each growth function using the parameter estimates from the size increment data for each site. Integrated versions of the relevant growth functions were solved for $t_0$ using $t = 0$ and the smallest sponge measured at each site as size at $t_0$ (McMurray et al. 2008). Given $t_0$, size-at-age $t$ is predicted for each growth model, and then weighted by $w_i$, to obtain the model averaged estimate of size-at-age $t$. Values were then cubed to obtain size (volume [cm$^3$])-at-age plots for each site.

2.3.9 Model validation

In order to validate my size-at-age estimates I used an opportunity where the barrel sponge *Xestospongia* spp. has settled on a shipwreck in northern Bali. The USAT Liberty was torpedood in 1942 during WWII and ran onto shore in northern Bali (8°16'28.48"S; 115°35'35.02"E), where it rested until it sank in 1963 after the tremors associated with the eruption of Mount Agung caused
the ship to slip into the sea. This provides an earliest possible date (1963) when *Xestospongia* spp. could have recruited to the wreck. I obtained multiple measurements of the gross morphology of 30 haphazardly distributed sponges to estimate the total volume of each sponge (based on McMurray et al., 2008) and determine the volume of the largest sponges (Bell unpublished data). Size-at-age models were used from each of the sites to estimate the approximate age of the sponges on the wreck and compared to the known maximum possible ages of the sponges in the WNMP.

### 2.4 Results

#### 2.4.1 Environmental data

Environmental parameters for each site are summarized in Table 2. There was no significant difference in temperature across sites ($F_{3,28} = 0.219, P = 0.882$). Sampela 1 had the highest chlorophyll-\(a\) and turbidity values ($F_{3,40} = 5.233, P = 0.004$; 1.68 ± 0.11 µg l\(^{-1}\) and 4.62 ± 0.773 STU, respectively), while Kaledupa Double Spur was characterized by the lowest (0.31 ± 0.083 µg l\(^{-1}\) and 1.85 ± 0.012 STU). Turbidity varied slightly between sites ($F_{3,46} = 2.799, P = 0.05$, Table 3). The biotic composition was quite variable across sites; Rowley (2014) reported hard coral cover at 11.11 ± 7% at Sampela 1 compared to 35.7 ± 13.62% at Kaledupa Double Spur. These data support the relative classifications of habitat quality proposed by Bell et al. (2014).
Table 2.2. Environmental parameters for Hoga Island sites. \(^a\) indicates data collected solely herein, \(^b\) indicates data from Rowley (2014), \(^c\) indicates data from Powell et al. (2014), \(^d\) indicates the inclusion of data from Powell et al. (2014) with data collected herein. Remaining data sources were obtained and denoted as follows: \(^e\) Hennige et al. (2008), \(^f\) Hennige et al. (2010), \(^g\) Crabbe and Smith (2002), \(^h\) Salinas de Leon (2010), \(^i\) Biggerstaff (2016). NTU refers to nephelometric turbidity units, STU refers to standard turbidity units. All values are expressed as mean (± SE).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Units</th>
<th>Site</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sampela 1</td>
</tr>
<tr>
<td><strong>abiotic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(^a) Temperature</td>
<td>ºC</td>
<td>28.82 ± 0.34</td>
</tr>
<tr>
<td>(^b) Salinity</td>
<td>PSU</td>
<td>32.5 ± 0.45</td>
</tr>
<tr>
<td>(^c) Flow</td>
<td>m s(^{-1})</td>
<td>0.05 ± 0.022</td>
</tr>
<tr>
<td>(^d) Substrate angle</td>
<td>º</td>
<td>46.67 ± 31.09</td>
</tr>
<tr>
<td>(^g) Rugosity index</td>
<td></td>
<td>13.94 ± 0.95</td>
</tr>
<tr>
<td>(^b) Light</td>
<td>K(_{d} PAR)min-max</td>
<td>0.31-3.14</td>
</tr>
<tr>
<td>(^e) Chlorophyll-(a)</td>
<td>µg l(^{-1})</td>
<td>2.42 ± 0.49</td>
</tr>
<tr>
<td>(^c) Turbidity</td>
<td>NTU</td>
<td>4.62 ± 0.77</td>
</tr>
<tr>
<td>(^b) STU</td>
<td></td>
<td>6.596 ± 1.099 (5m)</td>
</tr>
<tr>
<td>(^c) Sediment</td>
<td>g dry weight day(^{-1})</td>
<td>0.26 ± 0.04</td>
</tr>
<tr>
<td>(^b)</td>
<td></td>
<td>0.2 ± .06</td>
</tr>
<tr>
<td>(^c)</td>
<td></td>
<td>0.33 ± 0.03</td>
</tr>
<tr>
<td>(^d)</td>
<td></td>
<td>0.357 ± 0.168</td>
</tr>
<tr>
<td>(^e)</td>
<td></td>
<td>0.23 ± 0.04</td>
</tr>
<tr>
<td>(^i)</td>
<td></td>
<td>0.221 ± 0.03</td>
</tr>
</tbody>
</table>
### Table 2.3. Among site variation in environmental parameters (one-way ANOVA; Buoy 1, Sampela 1, Kaledupa Double Spur, Ridge 1). Asterisks denote a significant effect ($P < 0.05$).

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>(a) chlorophyll a</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Site</td>
<td>3</td>
<td>11.867</td>
<td>5.233</td>
<td>0.004</td>
<td>3</td>
<td>20.712</td>
<td>2.799</td>
<td>0.05</td>
</tr>
<tr>
<td>Error</td>
<td>40</td>
<td>2.268</td>
<td></td>
<td></td>
<td>46</td>
<td>7.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Tukey post-hoc tests</strong></td>
<td></td>
<td></td>
<td></td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>B1-S1</td>
<td>-1.999</td>
<td>0.002*</td>
<td></td>
<td></td>
<td>B1-KDS</td>
<td>0.1102</td>
<td>0.878</td>
<td>0.2</td>
</tr>
<tr>
<td>B1-KDS</td>
<td>0.1102</td>
<td>0.878</td>
<td></td>
<td></td>
<td>B1-R1</td>
<td>-0.8767</td>
<td>0.2</td>
<td>0.8707</td>
</tr>
<tr>
<td>S1-KDS</td>
<td>-2.1094</td>
<td>0.002*</td>
<td></td>
<td></td>
<td>S1-R1</td>
<td>-1.1226</td>
<td>0.072</td>
<td>2.5</td>
</tr>
<tr>
<td>KDS-R1</td>
<td>-0.9868</td>
<td>0.175</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-2.5497</td>
<td>0.030*</td>
</tr>
</tbody>
</table>
2.4.2 Xestospongia spp. demography

Xestospongia spp. density varied across sites across Hoga Island and the wider Wakatobi (one-way ANOVA, $F_{6,65} = 15.057$, $P < 0.01$; Table 2.4; Figure 2.1). Sites with the highest sponge densities were Buoy 1, Sampela 1, and Karang Gurita (Figure 2.1).

Table 2.4. Spatial variation in mean sponge density (sponges 100 m$^2$) across sites (one-way ANOVA; Buoy 1 [B1], Sampela 1 [S1], Kaledupa Double Spur [KDS], Ridge 1 [R1], Karang Gurita [KG]). Asterisks (*) denote a significant effect ($P < 0.05$).

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>MS</th>
<th>$F$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between sites</td>
<td>6</td>
<td>1.042</td>
<td>15.232</td>
<td>$&lt; 0.001^*$</td>
</tr>
<tr>
<td>Within sites</td>
<td>49</td>
<td>0.068</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Tukey post-hoc tests

<table>
<thead>
<tr>
<th>Difference</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1-S1</td>
<td>0.196</td>
</tr>
<tr>
<td>B1-KDS</td>
<td>0.673</td>
</tr>
<tr>
<td>B1-R1</td>
<td>0.497</td>
</tr>
<tr>
<td>B1-W</td>
<td>0.814</td>
</tr>
<tr>
<td>B1-T</td>
<td>0.974</td>
</tr>
<tr>
<td>B1-KG</td>
<td>0.339</td>
</tr>
<tr>
<td>S1-KDS</td>
<td>0.478</td>
</tr>
<tr>
<td>S1-R1</td>
<td>0.301</td>
</tr>
<tr>
<td>S1-W</td>
<td>0.618</td>
</tr>
<tr>
<td>S1-T</td>
<td>0.778</td>
</tr>
<tr>
<td>S1-KG</td>
<td>0.143</td>
</tr>
<tr>
<td>KDS-R1</td>
<td>-0.176</td>
</tr>
<tr>
<td>KDS-W</td>
<td>0.140</td>
</tr>
<tr>
<td>KDS-T</td>
<td>0.301</td>
</tr>
<tr>
<td>KDS-KG</td>
<td>-0.334</td>
</tr>
<tr>
<td>R1-W</td>
<td>0.316</td>
</tr>
<tr>
<td>R1-T</td>
<td>0.477</td>
</tr>
<tr>
<td>R1-KG</td>
<td>-0.159</td>
</tr>
<tr>
<td>W-T</td>
<td>0.161</td>
</tr>
<tr>
<td>W-KG</td>
<td>-0.474</td>
</tr>
<tr>
<td>T-KG</td>
<td>-0.636</td>
</tr>
</tbody>
</table>
Figure 2.1. Mean *Xestospongia* spp. density (sponges 100 m$^{-2}$ ± SE) across the wider Wakatobi (2014). Site abbreviations are as follows: Sampela 1 (S1), Buoy 1 (B1), Karang Gurita (KG), Ridge 1 (R1), Kaledupa Double Spur (KDS), Wanci (W), Tomea (T).

The volume gained for each sponge from yearly measurements (2014-2016) was compared across sites. Mean yearly volume gained varied across sites (one-way ANOVA: F$_{3,220} = 3.168$, $P = 0.025$) and was greatest at Kaledupa Double Spur (41,277 ± 12,479 cm$^3$; Figure 2.2A). There was no influence of depth (one-way ANCOVA, F$_{2,99} = 2.232$, $P = 0.089$) or year (two-way ANOVA, F$_{2,489} = 1.124$, $P = 0.326$) on mean *Xestospongia* spp. volume but it did vary spatially (two-way ANOVA, F$_{1,489} = 1282$, $P < 0.001$; Figure 2.2B; Table 2.5). Individual sponge volume was highly variable and ranged from 19.99 to 552,937.89 cm$^3$ across sites and years. Buoy 1 had smallest mean sponge volume (23,221 ± 5,082 cm$^3$), while the largest was at Karang Gurita (116,721 ± 29,275 cm$^3$).
Figure 2.2. Mean *Xestospongia* spp. volume (cm³) at each site (± SE); A) mean volume gained from 2014-2015 and B) mean volume across years at each site. Asterisks (*) and letters denote significant differences between sites.
Table 2.5. Spatial variation in mean volume (cm$^3$) across each site (two-way ANOVA; Buoy 1, Sampela 1, Kaledupa Double Spur, Ridge 1) and over time (2014, 2015, 2016). Asterisks (*) denote a significant effect ($P < 0.05$).

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>5</td>
<td>2064.407</td>
<td>8.602</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Volume x Site</td>
<td>1</td>
<td>397820.2</td>
<td>1282.562</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Volume x Year</td>
<td>2</td>
<td>269.872</td>
<td>1.124</td>
<td>0.326</td>
</tr>
</tbody>
</table>

Tests of Between-Subjects Effects

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>MS</th>
<th>$F$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td>3</td>
<td>3339.418</td>
<td>13.914</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Error</td>
<td>489</td>
<td>240.004</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Tukey post-hoc tests

<table>
<thead>
<tr>
<th>Difference</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1 - KDS</td>
<td>-13.6</td>
</tr>
<tr>
<td>B1 - R1</td>
<td>-7.03</td>
</tr>
<tr>
<td>B1 - S1</td>
<td>-7.75</td>
</tr>
<tr>
<td>KDS - R1</td>
<td>6.57</td>
</tr>
<tr>
<td>KDS - S1</td>
<td>5.85</td>
</tr>
<tr>
<td>R1 - S1</td>
<td>-0.71</td>
</tr>
</tbody>
</table>

Specific growth was highly variable but was not influenced by location (one-way ANOVA: $F_{3,109} = 1.103$, $P = 0.390$). Only 8.9% of sponges showed negative SGRs, the remaining were positive (Figure 2.3). The mean SGR for across sites was 0.45 ± 0.06, with the negative values removed, and specific SGRs were as fast as 3.14 yr$^{-1}$ (Sampela 1) and as slow (non-negative) as 0.01 yr$^{-1}$ (Kaledupa Double Spur).
Figure 2.3. Sponge growth from 2014-2016 at Kaledupa Double Spur (sponge 1, specific growth rate (SGR = 1.51), Buoy 1 (sponge 2, SGR = 2.46), and Sampela 1 (sponges 3, SGR = 2.15, and 4, SGR = 0.99). Scale bars = 15.0 cm.

There were instances of tissue loss and partial mortality evident in photographs, typically present in the form of shearing where up to half of the sponge was missing (Figure 2.4). Although there was no direct cause visible in the photographs, the nature of the injuries were suggestive of anchor damage. Also common was tissue loss due to smothering by coral rubble and sedimentation.
Figure 2.4. Sponge mortality over time; likely causes include burial by sediment and rubble (1a-c), tissue necrosis (2a-c), and possible anchor shearing (3a,b). Scale bars = 10.4 cm.

2.4.3 Growth models

The cube root estimates of sponge volume data from 2014 and 2016 revealed a nearly linear relationship that demonstrated a general increase in size over time (Figure 2.5). Several different growth models were identified across sites as supported by the Akaike Information Criterion with a correction for sample size ($AIC_c$). There was not one clear model of best fit ($w_i > 0.9$) for the remaining candidate models at any site. The generalized von Bertalanffy equations failed to predict reliable parameter estimates (Table 2.5) and were therefore removed from any further analyses of Buoy 1 and Kaledupa Double Spur data. Furthermore, the specialized von Bertalanffy equation was also removed for the analyses of Kaledupa Double Spur and Ridge 1. The removal of these models was further supported by the general lack of support based on $AIC_c$ criteria. Following the removal of these models Akaike weights were recalculated for each site, and the comparison of
the residual sums of squares and AICc weights for the remaining models at each site suggested that different growth models were best fitted at the different sites (Table 2.6).

Figure 2.5. *Xestospongia* spp. size (volume [cm$^3$]) in 2016 predicted by 2014 data with model averaged estimates at each site.

Table 2.6. Parameter estimates for candidate models at each site.

<table>
<thead>
<tr>
<th></th>
<th>Gompertz</th>
<th>Specialized von Bertalanffy</th>
<th>Generalized von Bertalanffy</th>
<th>Tanaka</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$S_\infty$</td>
<td>$K$</td>
<td>$S_\infty$</td>
<td>$K$</td>
</tr>
<tr>
<td>B1</td>
<td>139.1</td>
<td>0.053</td>
<td>184.0</td>
<td>0.015</td>
</tr>
<tr>
<td>KDS</td>
<td>370.6</td>
<td>0.035</td>
<td>5.94E+06</td>
<td>5.06E-07</td>
</tr>
<tr>
<td>R1</td>
<td>108.3</td>
<td>0.079</td>
<td>3.05E+06</td>
<td>8.84E-07</td>
</tr>
<tr>
<td>S1</td>
<td>97.2</td>
<td>0.07</td>
<td>251.3</td>
<td>0.011</td>
</tr>
</tbody>
</table>
Table 2.7. AICc criteria across sites ranked by fit following model elimination based on unreliable parameter estimates: parameter number in the model (+1) to account for variance (\(\sigma^2\)), residual sum of squares (RSS), bias-corrected Akaike information criterion (AICc), Akaike differences (\(\Delta_i\)), Akaike weights (\(w_i\)).

<table>
<thead>
<tr>
<th>Site</th>
<th>Function</th>
<th>K</th>
<th>RSS</th>
<th>AICc</th>
<th>(\Delta_i)</th>
<th>(w_i)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>SvB</td>
<td>3</td>
<td>668.6</td>
<td>100.0</td>
<td>0.000</td>
<td>0.7263</td>
</tr>
<tr>
<td></td>
<td>Tanaka</td>
<td>4</td>
<td>658.3</td>
<td>102.3</td>
<td>2.2142</td>
<td>0.240</td>
</tr>
<tr>
<td></td>
<td>Gompertz</td>
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<td>820.5</td>
<td>106.2</td>
<td>6.1419</td>
<td>0.0340</td>
</tr>
<tr>
<td>S1</td>
<td>SvB</td>
<td>3</td>
<td>582.3</td>
<td>133.5</td>
<td>0.000</td>
<td>0.5528</td>
</tr>
<tr>
<td></td>
<td>GvB</td>
<td>4</td>
<td>581.8</td>
<td>135.9</td>
<td>2.3419</td>
<td>0.1714</td>
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<tr>
<td></td>
<td>Tanaka</td>
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<td>582.3</td>
<td>135.8</td>
<td>2.2958</td>
<td>0.1754</td>
</tr>
<tr>
<td></td>
<td>Gompertz</td>
<td>3</td>
<td>621.0</td>
<td>136.9</td>
<td>3.4123</td>
<td>0.1004</td>
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<td>KDS</td>
<td>Gompertz</td>
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<td>103.9</td>
<td>35.7</td>
<td>0.0000</td>
<td>0.7889</td>
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<tr>
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<td>Tanaka</td>
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<td>91.2</td>
<td>38.3</td>
<td>2.6371</td>
<td>0.2110</td>
</tr>
<tr>
<td>R1</td>
<td>Gompertz</td>
<td>3</td>
<td>178.5</td>
<td>46.6</td>
<td>0.0000</td>
<td>0.7161</td>
</tr>
<tr>
<td></td>
<td>GvB</td>
<td>4</td>
<td>173.5</td>
<td>49.7</td>
<td>3.1830</td>
<td>0.1458</td>
</tr>
<tr>
<td></td>
<td>Tanaka</td>
<td>4</td>
<td>174.7</td>
<td>49.9</td>
<td>3.2930</td>
<td>0.1380</td>
</tr>
</tbody>
</table>

In the second model analysis examining the influence of site on *Xestospongia* spp. growth, only the Gompertz and Tanaka growth curves were common to all sites. AICc weights (\(w_i\)) were combined across sites and ranked as follows: Gompertz (\(w_i = 0.609\)) > Tanaka (\(w_i = 0.391\)). As there was no clear model of best fit the Gompertz and Tanaka models were averaged and used to examine the effect of site on *Xestospongia* spp. growth. Residual sum of squares analyses (ARSS) revealed no influence of site on growth (\(P = 0.541\)).

2.4.4 Size-at-age estimates

The relationship between volume (cm\(^3\)) at age (years) was determined at each site by predicting size-at-age \(t\), and then weighting by \(w_i\) to obtain the model averaged estimate of size-at-age \(t\). The smallest sponge size at each site were as follows: Buoy 1 = 19.99 cm\(^3\), Sampela 1 = 20.60 cm\(^3\), Kaledupa Double Spur = 411.0 cm\(^3\), Ridge 1 = 287.0 cm\(^3\). The resulting curves appear to vary by site with Kaledupa Double Spur producing a more rapid growth to larger sizes (Figure 2.6). Extrapolating the growth curves to 50 years it is evident that sponges at Buoy 1 and Sampela 1
have periods of slow initial growth with a gradual increase over time (Figure 2.6). Sponges at Kaledupa Double Spur have a shorter initial period of slow growth followed by rapid growth increase (Figure 2.6).

Figure 2.6. Estimated size-at-age for the models of best fit per site (Buoy 1 [B1]: specialized von Bertalanffy; Sampela 1 [S1]: specialized von Bertalanffy; Kaledupa Double Spur [KDS]: Gompertz; Ridge 1 [R1]: Gompertz), including model averages. The dashed line represents the largest sponge volume recorded in this study.

2.4.5 Sponge age on the USAT Liberty Wreck

The USAT Liberty Wreck sank in 1962, providing a maximum sponge age of 56 years. Sponges ranged in volume from approximately 80,000 cm$^3$ (n = 4) to 310,000 cm$^3$ (n = 6). Depending on the site-specific growth model used, the age of the largest sponge is estimated to be between 24 (Ridge 1 model) and 30 years old (Buoy 1 model).
2.5 Discussion

Despite the ecological importance of barrel sponges there has only been one previous study quantifying their growth rate, which did not consider effects of habitat variability or sponges in the Indo-Pacific. Here we estimated important life history characteristics for *Xestospongia* spp. populations and found an effect of spatial influence on growth trajectories (but not modelled growth) at different sites over a relatively small spatial scale (< 5 km). Interestingly, the lowest quality site (Sampela 1) supported some of the highest sponge densities and mean volumes, while the largest increases in size over the study period were found at higher quality sites. The estimates of size-at-age using a multi-model inference approach obtained from this study provided considerably lower age estimates for comparable sized barrel sponges in the Caribbean *X. muta* (McMurray et al. 2008), and rather than being the Redwoods of the reefs, their faster growth rates means *Xestospongia* spp. growth rates in the Indo-Pacific make them more comparable to Pines. These results highlight the importance of environmental variation on barrel sponge growth dynamics, and particularly how these dynamics might be influenced by environmental degradation.

2.5.1 Growth of giant barrel sponges

The variability of sponge growth is well reported in the literature; some growth is seasonal (Leys and Lauzon 1998; Turon et al. 1998; Garrabou and Zabala 2001), while in some species growth measurements are confounded by shrinkage (Barthel and Tendal 1993; Turon et al. 1998; Garrabou and Zabala 2001). Previous studies have shown that sponge growth trends vary with environmental quality, though most of this research has emphasized the seasonal effects of temperature (Leys and Lauzon 1998; Turon et al. 1998; Garrabou and Zabala 2001; McMurray et al. 2008). Teixidó et al. (2009) reported long term net growth of zero for larger, healthy *Crambe crambe* individuals. Slow seasonal growth rates may be due to resource limitation or necessary regeneration, which may come at the expense of other processes, such as growth (Henry and Hart 2005). It is possible that the timeframe of the present study did not include any periods of rapid adaptive growth during less stressful conditions, however, the differing models of best fit at each site suggest that *Xestospongia* spp. exhibited differing growth strategies per site (although not overall growth rate). This may be
reflective of abiotic characteristics at the sites, although a more specific experimental approach (e.g. through transplantation) will be needed to identify causality.

Surprisingly, *Xestospongia* spp. density was high at sites characterized by comparatively higher levels of turbidity and decreased light availability. When combined with the lack of influence of depth on mean sponge volume, we propose that barrel sponges at these sites may be less reliant on their photosynthetic symbionts and heterotrophic feeding. There is evidence that Caribbean *X. muta* share a commensal relationship with their photosynthetic cyanobacteria (Thacker 2005; López-Legentil et al. 2008a), but this is unknown for Indo-Pacific species. Cleary and de Voogd (2007) reported that *Xestospongia* spp. were constrained to deeper depths in other areas of Sulawesi, supporting a limited reliance on symbionts. Furthermore, recent observations from deeper water areas in the Wakatobi (50-85 m) have found large populations of *Xestospongia* that all appear completely ‘bleached’ but otherwise healthy, suggesting that barrel sponges can survive in almost complete absence of the photosymbionts (Bell unpublished data). An alternative hypothesis is that *Xestospongia* spp. can shift to heterotrophic feeding in conditions less favourable to photosynthesis by its symbionts. The Sampela 1 site is also characterized by elevated chlorophyll-a concentrations, previously used as a proxy for potential sponge food sources (Powell et al. 2014). It is important to note, however, that the picoplankton ingested by sponges (McMurray et al. 2016) are likely to have a variety of chlorophyll types. Further research on the feeding biology of *Xestospongia* spp. is necessary to confirm their dependence on photosynthetic symbionts and ability to switch to heterotrophy when light availability is low, and how this translates in different growth rates.

The wide range of habitat characteristics present in this study may contribute to differences in mean volume between sites. Both abiotic and biotic factors are likely to affect the population and individual sponge dynamics at each site; these include factors not measured in this study such as food supply and consumption, hydrodynamics, and spatial competition. In particular, the importance of spatial competition in shaping the ecology of sponges has received considerable attention (Jackson and Buss 1975; Wulff 2000, 2006; Bell and Barnes 2003a; López-Victoria et al. 2006; González-Rivero et al. 2011), and may have a large influence on *Xestospongia* spp. dynamics. At Sampela 1, for instance, competition with other benthic taxa is far reduced than at
the other sites due to low coral cover, which would be expected to influence density, mean volume, and potentially growth rates.

McMurray et al. (2008) reported seasonality in *X. muta* growth, which was not measured in this study; the volume gained per year in the summer months was in line with growth measured herein from 2014 to 2015 (4,195.53 ± 4,080 cm³ McMurray et al., 2008; 4,572.60 ± 1,394.66 cm³, Buoy 1). From 2015-2016, however, the volume gained at Kaledupa Double Spur was over eight-fold larger than the volumes reported by McMurray et al. (2008; 40,676.99 ± 12,479.05 cm³). The high variation in volume gained among years highlights the importance of interannual variation in environmental conditions as a possible driver of growth variability.

2.5.2 Redwoods of the reef or Pines of the Indo-Pacific?

Variation in age extrapolated from growth model projections is not uncommon due to differences in model selection, sampling methods, reproductive life history or morphological differentiation (Hesp et al. 2004). McMurray et al. (2008) examined *Xestospongia muta* growth at one location, determining the model of best fit by comparing AICc scores. There was evidence of substantial model support throughout multiple candidate models based on AICc scores, however, such that estimates are strongly supported (Burnham and Anderson 2003). In the same study, a sponge with a volume of 632,912.80 cm³ was predicted to be nearly 242 years old. Although the largest sponge measured in the present study was slightly smaller at 552,937.89 cm³, we estimated it to be a maximum of only 36 years old based on the model-averaged growth curve from that site (Sampela 1, Figure 2.6). While there are likely regional differences in growth rates (for example due to differences in average sea surface temperature), based on the growth models herein, we estimated the sponges on the USS Liberty wreck to be between 24 and 30 years old. As the ship sank in 1963, the maximum age the sponges could be at the time of measurements (2009) is 46 years old. It seems reasonable that sponges would not have recruited immediately to the wreck until suitable biofilms had formed, and therefore the age estimates from the wreck provide strong independent support for size-at-age estimates in the Wakatobi National Marine Park. In contrast, using the age at size relationships from McMurray et al. (2008) from the Caribbean, would age the biggest sponge on the wreck at approximately 100 years old, much older than the wreck. There are further
examples where large sponge size does not correlate with long life in sponges. For example, Rohde and Schupp (2012) found that individuals of *Ianthella basta* nearly 2 m high were only 10 years old.

2.5.3 Model choice and parameter uncertainties

The size-at-age extrapolation used in this study followed multi-model inference (MMI) with a model averaging method commonly employed in other fields (see Mercier et al. 2011) to ensure a robust estimation of size at any given age. A distinction of note between this practice and that data presented herein, however, is the ability to “ground truth” using actual size-at-age data, typically otolith-derived age and fish length (Diouf et al. 2009; Mercier et al. 2011). As this was not possible in this study, *Xestospongia* size at *t*₀ was by proxy represented by the smallest sponge in the data based on McMurray et al. (2008). It is therefore likely that my size-at-age estimates are due to the potential for overestimation of volume at the time of larval settlement.

My results reinforce the importance of carefully examining candidate models as part of the model selection process in order to reduce selection uncertainty. The multi-model selection process resulted in the removal of several candidate models at different sites due to unreliable parameter estimates based on AIC<sub>c</sub> selection criteria (Diouf et al. 2009). This resulted in an inability to compare the models of best fit for each site using residual sum of squares analyses (ARSS). The models that were averaged and used for this comparison, Gompertz and Tanaka, were among the least supported models at each site, potentially limiting the robustness of the site comparison. Furthermore, there was strong support for different models at each site, suggesting a spatial influence on growth trajectories. The differing shapes of the model-averaged size-at-age curves, coupled with the differing mean volumes and mean volume gained support differing growth trajectories employed by sponges at different sites.

Models with extremely large standard errors associated with parameter estimates were disregarded from multi-model averaging as they were deemed unreliable. Some parameter estimates in the remaining models had moderately large standard errors, likely due to the large parameter number in candidate models (given the apparent linear trend between 2014 and 2016 sizes) or due to the
relatively small sample size (Benzekry et al. 2014). Although the specific values of the parameters estimated by the model fit may therefore be of moderate confidence, their descriptive power remains unaffected (Benzekry et al. 2014). Employing multi-model averaging is widely considered superior in lieu of a priori model choice, but it is possible that the data support another model that I did not examine.

2.5.4 Implications of barrel sponge life-history traits for management and conservation

Previous research on barrel sponges suggests that they should be susceptible to environmental disturbance. They have a large body size, low population connectivity (see Bell et al., 2014), slow growth, are long-lived (McMurray et al. 2008), and have high larval mortality common to broadcast spawning (Maldonado 2006). However, despite these features, we found barrel sponges were abundant and large at the site with the lowest quality, which is characterized by low coral cover, and high levels of sedimentation and turbidity. The much faster growth rates described in this study compared to those reported in the Caribbean may partly explain the high abundance at this low quality site. The reduction in coral cover at Sampela 1 is largely thought to have occurred in the last 10-20 years, with coral cover declining from 30% to < 8% cover (McMellor and Smith 2010), which will likely have released barrel sponges from spatial competition, and potentially allowed a larger population size. However, we do not currently have long-term population data on barrel sponges in the Wakatobi, which would be needed to test this hypothesis.

This study is the first to identify the role that environmental variation plays in determining the growth rates in Xestospongia spp. Interestingly, my results demonstrate that Indo-Pacific barrel sponges achieve a comparable size to that of their Caribbean cohorts much faster, and therefore large barrel sponges on Indo-Pacific reefs are more comparable to Pine trees rather than the Redwoods proposed by McMurray et al. (2008). This chapter highlights how changes in environmental conditions, such as through degradation, may influence these functionally important species. However, barrel sponges also have the potential to dominate in environments where there is low coral abundance. This is particularly the case for sedimented habitats, although there may be energetic costs associated with living in these suboptimal conditions that negatively impact growth rates.
Chapter 3: Size matters in population dynamics of giant barrel sponges on Indonesian coral reefs

A recruit in the genus Xestospongia in Karang Gurita, Indonesia. (photo credit: Joe Marlow)
3.1 Abstract

The Indo-Pacific contains up to 75% of the world’s coral reefs and is considered the epicentre of marine diversity, yet is estimated to be losing coral cover at an increasing rate. On some reef systems where coral cover is declining sponges in the genus *Xestospongia* are now among of the largest benthic invertebrates. The large size of these sponges is of particular importance as body size is mechanistically linked to the ability to process water and subsequent nutrient cycling, which is a key functional role of sponges. Despite their abundance and ecological influence, sponge population dynamics remain largely unstudied. An integral projection model (IPM) was parameterized for Indo-Pacific barrel sponges using empirical data from two sites in the Wakatobi Marine National Park (WMNP), with the aim of examining the population level consequences of changes in the demographic processes underlying two populations of varying habitat quality. This was accomplished by examining how the relationship between sponge size and growth, survival, and fecundity (vital rate parameters) influences long term population growth. The importance of recruit source to population growth was examined by creating IPMs in demographically open and closed systems for each site. Both *Xestospongia* spp. recruitment and mortality were variable, ranging both temporally and spatially. IPMs revealed differing population growth rates (\( \lambda \)) at each site; the Buoy 1 populations is projected to increase by 30% per annum (\( \lambda = 1.30 \)), while the Sampela 1 population is nearly self-sustaining (\( \lambda = 1.02 \)). Mean fecundity, or size-dependent reproductive output, was minimal for both sites (Buoy 1: 0.2 ± 0.044, Sampela 1: 0.05 ± 0.0094). Simulations of these IPMs were used to gain insight into the implications of disturbance to size-specific demographic rates. Simulations indicated that populations at both Sampela 1 and Buoy 1 are resilient to low recruitment rates, but the loss of large individuals resulted in a substantial population decline (closed populations: \( \lambda = 1.306 \) to 0.663 at Buoy 1; \( \lambda = 0.997 \) to 0.81 at Sampela 1; open populations: \( \lambda = 0.999 \) to 0.61 and 0.972 to 0.79 at Buoy 1 and Sampela 1, respectively). Therefore, ensuring that large *Xestospongia* spp. remain in the populations should be considered a principal element in sponge management and conservation. The results of this chapter highlight that the number of individuals are increasing at both sites, and are likely to remain dominant components of the reef system in the WMNP. However, one large-scale mortality event that disproportionately affects large sponges could severely impact populations that would be expected to recover very slowly.
3.2 Introduction

Population dynamics refers to the structural composition of a population and the biological or environmental processes driving them. A widely used extension of population dynamics analysis is population viability, which is defined as the predicted likely future status of a population (Morris and Doak 2002). Both population dynamics and viability can actively inform management and conservation, and allow managers to assess the extinction risk of a population. For example, it is possible to estimate if a population is growing, static, or declining, and using this information determine key management targets can be developed (i.e. life stages or demographic processes). Populations of the same taxon in different locations can also be compared to determine if extinction risk varies based on physical location; in the event of limited resources a comparison of this nature allows managers to evaluate relative risk and prioritize conservation appropriately (Morris and Doak 2002).

Understanding the life history traits of an organism is central to accurately quantifying its biology (Pardo et al. 2013) and can inform how it interacts with other components of the ecosystem in which it is found. On coral reefs, sponges have a variety of functional and ecological roles that greatly influence ecosystem health. Sponges act as benthic competitors, consumers, and prey (Diaz and Rutzler 2001), and can contain dense microbial communities that contributes to reef primary production (Montalvo and Hill 2011). By converting planktonic carbon into sponge biomass, sponges provide an important function in linking water column productivity and the benthic community (Pile et al. 1997; Lesser 2006), and are considered a critical component in structuring the benthic community. Sponges have been shown to facilitate carbon transport (Jiménez and Ribes 2007; de Goeij et al. 2008), as well as influence nitrogen (Jiménez and Ribes 2007) and silicon cycling (de Goeij et al. 2013), and may explain how oligotrophic coral reefs can support a diverse array of fauna (de Goeij et al. 2013). Sponges can therefore play a large role in ecosystem functioning, and any changes in sponge populations have the potential to impact pelagic ecosystems (Bell 2008).

Sponges in the genus *Xestospongia* are among the largest known coral reef sponges with life-spans that may exceed half a century (see Chapter 2). Barrel sponges can also mediate water column
characteristics. For example, McMurray et al. (2016) reported that *X. muta* consumed DOC (70%) and detritus (20%) as the majority of their diet. Furthermore, they are capable of pumping up to a 12.9 m column of water per day (McMurray et al. 2014). The large size, expected longevity, and filtering capability of *Xestospongia* spp. means that they are major contributors to coral reef functioning. In the Florida Keys *X. muta* are the second most common sponge in terms of percentage cover as a result of increases in successful recruitment following coral decline (Loh and Pawlik 2014), and its abundance has increased by 45% from 2000-2006 in this area (McMurray et al. 2010). In the WNMP *Xestospongia* spp. are commonly found in dense population in sites of high sedimentation and low coral cover (see Chapter 2; Bell et al. 2014). *Xestospongia* spp. often remain one of the largest remaining benthic invertebrates on reef systems where coral cover is declining, yet the population dynamics of these ecologically important species has not been examined in the Indo-Pacific.

### 3.2.1 Integral projection models

Integral projection models (IPMs) are a relatively new and powerful method used to describe population dynamics, specifically how individuals enter a population through recruitment and leave through death (Edmunds et al. 2014). The flexibility of the model structure supports nearly any type of growth measurement or fecundity process, from spawning to recruitment (Edmunds et al. 2014). IPMs relate continuous state variables (e.g. size, age, etc.) to explanatory variables in order to reveal how changes in individual performance (summarized by their vital rates: survival, growth, and fecundity) influence the long term dynamics of a population (Coulson 2012; Merow et al. 2014). A variety of informative population statistics can then be derived from IPM analyses that provide insight into the contribution of the state variable to population growth and reproduction (Edmunds et al. 2014). In this way IPMs are able to link an individual’s performance to population outcomes on a demographic scale (Easterling et al. 2000).

IPMs were originally developed to improve upon assumptions made in the widely used population projection matrix models (PPMs). PPMs rely on dividing populations into discrete size classes in order to describe population dynamics over time (Caswell 2001). In the event an organism lacks biologically relevant justification for such a grouping, delineation becomes arbitrary as similar size
may not necessarily indicate a comparable response to stressors (Easterling et al. 2000). In the case of sponges, inappropriate size assignment could lead to false conclusions on the importance of size contribution to the dynamics of a population. Instead, IPMs assign size-specific values to population-level dynamics that take individual variation in performance into account (Easterling et al. 2000). This is particularly important in populations that are comprised of differing sizes that have varying contributions to population growth (Morris and Doak 2002). Individual barrel sponges examined in this chapter have a large range of possible sizes, from small recruits (19.99 cm$^3$) to very large adults (approximately 600,000 cm$^3$), and the capacity of IPMs makes them well suited to capture the possible range of transitions. Finally, IPMs can deal with missing or finite data, which is important as complete sampling of an entire population is rarely feasible (Ramula et al. 2009).

To date IPMs have been widely applied to terrestrial species, with only few investigations of the marine species (but see scleractinian coral: Burgess 2011; Madin et al. 2012; Edmunds et al. 2014, and octocoral species: Bruno et al. 2011). The flexibility of IPMs allows for modelling of vital rates on a variety of life history types. Previous work has included covariates such as genotype (Coulson et al. 2011), competition (Adler et al. 2010), successional stage (Metcalf et al. 2009a, 2009b), density dependence (Rebarber et al. 2012), species interactions (Bruno et al. 2011), and abiotic parameters (Edmunds et al. 2014; Vindenes et al. 2014; Elahi et al. 2016). IPMs have also been used to explore the mechanisms underpinning demographic response to and long-term consequences of climate change (Ozgul et al. 2010; Madin et al. 2012; Radchuk et al. 2013; Vindenes et al. 2014). IPMs can also be built for both demographically open and closed systems depending on the level of population connectivity. As marine populations are neither strictly open or closed, modelling populations in both circumstances informs management priorities based on self-recruitment at a small scale (Yau et al. 2014).

Previous evidence suggests that coral cover in the WMNP has declined and Xestospongia spp. populations have been found to be dense in sites considered degraded (Chapter 2). In some areas Xestospongia spp. are now the largest benthic invertebrates. Furthermore, as individual sponge size is directly related to functional importance on coral reefs due to pumping capability, any
increase or decrease in barrel sponge populations would be expected to have influence on overall reef functioning. IPMs were used to investigate \textit{Xestospongia} spp. population dynamics at two sites of varying habitat quality in the WMNP. First, I explored patterns of recruitment and mortality, and using individual demographic data, parameterized an IPM for each site in order to further examine the components of population dynamics and quantify their importance. I then explored properties of the IPM to compare population statistics for each site, including stable size distributions and long-term population growth rates. Elasticity analyses were then used to identify the relative importance of changes in vital rate parameters to the population growth rate. Finally, I used the IPMs in simulation studies to examine the role of recruitment on population growth at each site by modelling demographically open and closed systems. Using the IPMs for each system and site, I simulated recruit and adult mortality for each system in order to assess the implications of disturbances to size-specific demographic rates at each site.

3.3 Methods

3.3.1 Data collection

Vital rate parameters (growth, reproduction, survival) are influenced by abiotic and biotic factors that, in some instances, may vary on small spatial scales (Ruttenberg et al. 2005). Slight changes in these parameters across local scales can therefore result in significant population-wide differences, emphasizing the importance of spatial scale in demographical assessments and subsequent management decisions (Wilson et al. 2012). In Chapter 2 I explored \textit{Xestospongia} spp. demography at an individual level and presented site-specific characteristics at four core sites in the WMNP. Although Buoy 1 and Sampela 1 shared high sponge densities, they differed in mean volume (Chapter 2). Sponges at Buoy 1 were smaller and less numerous than at Sampela 1, an important distinction as size is the principle component of the IPMs used in this study. The sites themselves varied in chlorophyll-\textit{a} concentration, and although not explicitly measured in this study, previous work suggests that they differ in a multitude of abiotic and biotic factors, which might be expected to affect vital rate parameters (Prince 2010; Chapter 2). The site characteristics are also quite different; Sampela 1 is a shallow reef system (< 13 m) located less than one kilometre from the large populations of Sampela Village and Kaledupa Island, whose inhabitants are reliant on the reef as a resource. Buoy 1 is characterized by a vertical wall and moderate coral cover (20-
25%, see Chapter 2, Table 2.2). Therefore, in an effort to accurately quantify both the life history differences and variation in environmental quality driving *Xestospongia* spp. population dynamics, each site was analysed separately.

All sponges were mapped and tagged at Sampela 1 and Buoy 1 (as detailed in Chapter 2). While target sponges were identified based on morphology described in Bell et al. (2014) for *X. testudinaria*, the discovery of a potential species complex (Chapter 5) revealed that this method is inaccurate. As such sponges were treated on the level of genus for the duration of the study. Photos of all sponges from each location were taken yearly (2014-2016) and volume was calculated using stereo photogrammetry. Digital images for photogrammetric analysis were taken with a Fujifilm FinePix Real 3D W3 Digital Camera and corresponding underwater camera housing. Volumetric measurements were calculated using stereo calibration and measurement software (CAL and PhotoMeasure) created by J. Seager (http://www.seagis.com.au). Sponge and spongocoel volumes were calculated based on similarity to 3-D geometric solids. Final volumes were obtained by subtracting the spongocoel volume from the whole sponge volume.

Mortality was recorded for each individual yearly. Indications of overall health (i.e. presence of necrosis or disease) was noted. Mortality was determined when sponges were missing but the tag was successfully located. *Xestospongia* recruits were also counted yearly. Sponges were considered recruits if they were not present in mapped areas on the previous year’s survey. Small sponges located in 2014 were confirmed as recruits by comparing their size to the yearly mean growth rates of known recruits.

### 3.3.2 Demographic model

Analyses were conducted in R version 3.3.3 using IPMpack (Metcalf et al. 2013). The basic structure of an IPM is as follows:

\[
n(y, t + 1) = \int k(y, x, \theta(t))n(x, t)dx,
\]
where the number of individuals at time \( t + 1 \) is represented as \( n(y, t + 1) \). The given state variable, which in this case is defined by size, \( y \) for \( t + 1 \) is a product of the number of individuals at time \( t \), or \( n(x, t) \), with state variable \( x \). Between these time points sponges can grow, remain the same size (stasis), shrink, die, and reproduce; these functions are represented by kernels (Easterling et al. 2000). Kernel \( k(y, x, \theta(t)) \) is analogous to the population projection matrix and describes all possible transitions from \( x \) (at time \( t \)) to \( y \) (at time \( t + 1 \)) under the environment \( \theta(t) \) and integrated over all states \( x \), providing a population level response to change (Rees et al. 2014). The kernel is composed of the vital rates of the individual, further broken down into three main components: the growth kernel: \( g(x, y, \theta(t)) \), the survival kernel: \( s(x, \theta(t)) \), and the fecundity kernel: \( f(x, y, \theta(t)) \). The kernel relationships are defined as:

\[
k(y, x, \theta(t)) = s(x, \theta(t))g(x, y, \theta(t)) + f(x, y, \theta(t)),
\]

The transition matrix \( k(y, x, \theta(t)) \Delta x \) is developed for each time step by discretizing all possible states into bins (\( \Delta x \)), and then applying kernel analysis across the matrix of \( x \) and \( y \) combinations. From this matrix the population growth factor (\( \lambda \)) can be determined (Easterling et al. 2000). Prior to fitting vital rate functions and IPM parameterization it is necessary to determine the combination of functions that best fits the data using model diagnostics.

### 3.3.2.1 Growth

The relationship between size at time \( t \) (2014) and \( t+1 \) (2016) was determined using the growth kernel for each site. Growth kernel development was based on volume data (cm\(^3\)) collected for all tagged sponges at each respective site (\( n = 77 \) for Buoy 1 and \( n = 95 \) for Sampela 1). Size data was log transformed in order to approximate linear growth models with error variance independent of size, increasing accuracy (Rees et al. 2014). Growth was modelled as a linear regression of size in 2014 as a function of size in 2016 (volume [cm\(^3\)]); corresponding AIC values were used to guide model choice (Table 1). In order to reduce the likelihood for eviction, or the exclusion of sizes on the lower and upper extremes of the data set (Williams et al. 2012), upper and lower size boundaries (\( L \) and \( U \) respectively) for the model were determined. This was accomplished by
multiply the smallest \((l)\) and largest \((u)\) value by constants: \([L, U] = [0.9l, 1.1u]\); Rees and Rose 2002. For both sites model diagnostics available through IPMpack were completed to ensure bin choice and model size range was appropriate. A lack of density dependence in \textit{Xestospongia} spp. was also assumed based on \textit{X. muta} population dynamics as reported in the Caribbean (McMurray et al. 2015).

3.3.2.2 Survival

The probability that an individual survived from 2014 to 2016 was determined by the survival kernel. Survival data was binary \((0 = \text{mortality}, 1 = \text{survival})\) and measured from yearly surveys. The probability of individual \textit{Xestospongia} spp. survival was estimated by a logistic regression with survival as a function of size.

3.3.2.3 Fecundity kernel

The fecundity kernel describes per capita contribution to new individuals in the next time step (Merow et al. 2014). The lack of basic information on barrel sponge reproduction requires several assumptions in building the fecundity portion of the IPM. Fecundity was defined as the presumed reproductive output proportional to body size. This was calculated by dividing individual sponge volume (non-recruits only) by the total non-recruit sponge volume and then multiplying by the number of successful recruits, or those that settled and grew over time, at each site. Although size-dependent fecundity has not been explicitly studied in \textit{Xestospongia} spp. it has been reported in other sponge species (Uriz et al. 1995; Whalan et al. 2007b), and utilized as a proxy for fecundity in Caribbean \textit{Xestospongia muta} matrix modelling (McMurray et al. 2017). The assumption was also made that sponges in the recruit phase were too small to be capable of reproduction (Whalan et al. 2007b; McMurray et al. 2017) and that every individual was reproductively active.

Two discrete stages were defined for the initial time step \(t\), recruit and adult, while the second time step \(t + 1\), had the same stages with the addition of ‘dead’ for those sponges that died over the course of the survey period. Mean and standard deviation of recruits was calculated at each site and included in the respective fecundity object. Recruits and dead sponges were classified as being unable to produce offspring. A transition matrix was included using probabilities calculated from
existing data to describe the movement between recruits to alternate stages if appropriate, i.e. the probability of a recruit remaining a recruit, dying, or moving to an adult stage from one time step to the next. Adults were able to shrink in size but not revert to the recruit stage. The function was then incorporated into the IPM for the remainder of the analyses.

Populations were assumed to be “closed” for the majority of IPM analyses based on previous evidence that *Xestospongia* spp. populations in the Wakatobi are characterized by high levels self-recruitment (Bell et al. 2014; Chapter 5). In the case of IPMs, a closed system indicates that all recruitment is dependent on the size of the local population, or in this case study site (Edmunds et al. 2014). Further investigation, however, suggests that although self-recruitment is common at both sites examined here, the population at each site is not fully closed but instead shares some recruits between sites (Chapter 5). By omitting the fecundity kernel and thereby excluding recruitment, a demographically open population may be simulated where none of the recruits present are a product of local adults (Yau et al. 2014). The benefits of this analysis are two-fold: examining open population dynamics gives an insight into both recruitment extremes, and allows for exploration of population dynamics in the absence of recruitment (Edmunds et al. 2014). The population growth rate then becomes population decline (Yau et al. 2014).

3.3.2.4 Model analysis

To examine further the long-term changes in population dynamics for *Xestospongia* spp. I constructed and analysed separate IPMs for Buoy 1 and Sampela 1. The long term, or asymptotic, dynamics of a population can be observed when a population remains unperturbed for a duration of time in a constant environment. Indicators of asymptotic dynamics include the stable size distribution and the population growth rate. The population growth rate is described by the dominant eigenvalue \( \lambda \), and expresses the growth or decline of a population over time. If \( \lambda \) is > 1 then the population is increasing and < 1 if it is in decay (Moore et al. 2016). The stable size distribution provides a snapshot of the size structure of the population once it reaches the stable state assumed by the population growth rate. The relative importance of changes in each parameter on population growth is described by the elasticity (Metcalf et al. 2013). This method highlights which vital rates are the most changeable and what \( \lambda \) is the most sensitive to. Using the
“sensParams” function in IPMpack each parameter was perturbed by $10^{-4}$ and the resultant $\lambda$ was calculated, providing an estimation of the ratio of change in the population growth rate in respect to the altered parameter (see Merow et al. 2014). To examine the population-level consequences of changes in demographic rates at each site, each IPM was analysed to obtain $\lambda$, stable size distributions, and elasticities. Confidence limits (95%) were constructed for $\lambda$ to facilitate direct comparisons between sites using bootstrapping. Sponges were randomly selected from the original dataset with replacement and IPMs were constructed and analysed with 300 iterations.

Finally, I examined the importance of recruitment to population growth by simulating demographically open and closed systems in IPMs for each site. Using each IPM I performed two separate simulations: closed scenarios included site specific fecundity information, while open populations do not. In a closed system when projected through time the population is expected to converge to the stable size distribution, at which point $\lambda$ reflects growth. The IPM for the open population infers a population in decline over time. Next, I examined the importance of size-dependent survival rates to population growth (closed populations) and decline (open populations). This was accomplished by fitting the survival model using the current dataset and varying the magnitude of the intercept and slope for the survival term at each site while leaving the other model parameters fixed. Recruit and large adult sponge mortality was simulated for each system and each site, relative to the default, unaltered IPM (Merow et al. 2014), and the resultant $\lambda$ was examined.

3.4 Results

3.4.1 Recruitment and mortality

*Xestospongia* spp. mortality was variable; the highest mortality recorded was 11 individuals in one year (Sampela 1; 2014) and the lowest 3 (Buoy 1, 2015; Figure 1.1). No evidence of disease was observed; partial necrosis was uncommon (Buoy 1: n = 2.5%; Sampela 1: n = 6.25%). Recruitment ranged across sites and years from seven (Sampela 1; 2014) to 20 recruits (Buoy 1; 2016).
3.4.2 Integral projection model (IPM)

Model diagnostics were completed using the diagnostics function in IPMPack (see Appendix for diagnostic information and numerical parameterization). The size-dependent fecundity function with recruit transition matrix comprised the fecundity kernel, and together with the size and growth kernel, constituted a high dimensional IPM with 100 size classes (Figure 3.1). For Buoy 1 sponges in the largest size classes exhibited growth, stasis, and shrinkage over the sample period, while Sampela 1 sponges demonstrated minimal shrinkage and were instead dominated by growth and stasis in larger size classes (Figure 3.1, A, B; dashed line indicates stasis). In contrast to Sampela 1, Buoy 1 size classes were not continuous. Instead, a large recruit component was present coupled with a group of larger sponges (Figure 3.1 A, B). The fecundity kernels were similarly structured in both sites due to the size-dependent nature of *Xestospongia* spp. fecundity utilized in this study (Figure 3.1 C, D).

![Figure 3.1](image-url)

Figure 3.1. Survival and growth kernel (A, B) and fecundity kernel (C, D) for Sampela 1 and Buoy 1. The dashed line in the survival and growth kernel indicates stasis where individuals along the line neither shrink nor grow; individuals grow above the line and shrink below it. Lighter colours indicate a higher probability of a transition occurring.
The matrix of the IPM is represented as a 3D histogram (Figure 3.2); the x axis (denoted IPM) represents individual sponge size at time $t$, the y axis represents sponge size at $t + 1$, and the z axis illustrates the transition rates between sizes. The ridge running diagonally from left to right indicates the growth and survival for individuals of increasing size and represents survival and transition rates on a scale between 0 and 1. The triangular shape on the top of the matrix represents the fecundity kernel, or size-dependent reproductive output, and therefore has a larger scale than the growth and survival ridge.

While the fertility kernel is similarly shaped for each site, the growth and survival ridge is more prominent at Sampela 1, indicating higher probabilities of a transition occurring between sizes from 2014 to 2016 (Figure 3.2). In both sites the size transition ridge peaks on the right extremity, representing the probability that large sponges remain in the largest size class (stasis; Figure 3.2). This is particularly prominent at Sampela 1, whereas at Buoy 1 large sponges may shrink or grow.

![Figure 3.2](image)

Figure 3.2. The transition surface the integral projection model for each site: A) Buoy 1, B) Sampela 1.

The population growth rate ($\lambda$) generated by the Buoy 1 IPM suggests that populations are projected to increase by 30% per annum ($\lambda = 1.30$, bootstrap 95% confidence intervals [1.25,
The Sampela 1 IPM, however, suggests a population that is nearly self-sustaining, with a 2% population increase per annum ($\lambda = 1.02$, bootstrap 95% confidence intervals [0.961, 1.017]). Mean fecundity, or size-dependent reproductive output, was $0.2 \pm 0.044$ at Buoy 1 and $0.05 \pm 0.0094$ at Sampela 1. The projected population growth is supported by empirical observations of low mortality in relation to the high number of yearly recruits at both sites.

3.4.2.1 Model output vs. empirical evidence

Assuming a constant environment and a stabilized population growth rate, the proportion of *Xestospongia* spp. individuals produced by the IPM in each size class varied per site. The stable size distribution reflects the structure of the population once stability is reached and the population growth rate is estimated. For Buoy 1, the distribution of sponge size at equilibrium is skewed to the very largest sponge sizes (Figure 3.3A). This contradicts the actual current size distribution which features a more even spread of intermediated sizes (Figure 3.3B). The stable size distribution at equilibrium at Sampela 1, however, featured a peak in the intermediate size class (Figure 3.3C), which was nearly reflected in the current observed sponge size distribution (Figure 3.3D).

![Figure 3.3](image)

**Figure 3.3.** Stable size distribution of *Xestospongia* spp. populations in equilibrium and the observed size distribution for Buoy 1 (A, B) and Sampela 1 (C, D)
3.4.2.2 Vital rate elasticities

Elasticities, or the relative contribution of changes in parameter to the population growth rate, were measured by analyses via perturbation of the base IPM were used to identify vital rate parameters which, when adjusted slightly, had the greatest change in population growth rate (λ) for each site. Overall survival and growth dominated the elasticities of λ; for each site changes in λ were due to survival and growth (84.8% and 97.9% at Buoy 1 and Sampela 1, respectively). The elasticities attributed to fecundity, however, were comparably less (15.1 and 2.0%, at Buoy 1 and Sampela 1, respectively).

The importance of sponge size in influencing λ was evident in both sites; elasticities demonstrated in both cases that future population abundance is most sensitive to changes in growth and survival of the largest size class (Figure 3.4 A, B). The population growth rate at Buoy 1 appears to be more sensitive to changes in the largest three size classes, whereas the growth rate at Sampela 1 is most sensitive to changes in the largest size class (Figure 3.4 A, B). At Buoy 1, population growth is most influenced by transitions into the largest size class where fecundity is at its highest (Figure 3.4 C). No sensitivity to fecundity was detectable for Sampela, however (Figure 3.4 D).
3.4.2.3 Closed vs. open populations

*Buoy 1*

The effect of size-dependent survival rates on population growth was examined for both demographically open and closed populations. This was first explored in a closed population, including site-specific fecundity data. Simulating recruitment failure (100% recruit mortality), Buoy 1 populations will continue to grow each year, albeit at a reduced rate compared to current population projections (a growth rate of 21.7% as compared to the current growth rate of 30% per annum; Figure 3.5A). In a scenario where the complete mortality of the largest sponges occurs, however, the population reverts to a decline of 33.7% per year (Figure 3.5A).
By removing the fecundity kernel from the IPM, an open population reveals reliance on recruitment and reflects population decline. The open population at Buoy 1 appears to be mostly self-sustaining ($\lambda = 0.999$, or yearly decrease in size of 0.1%), but begins to decline at a rate of 0.5% per year if recruitment fails (Figure 3.5A). If an event results in mortality of the largest sponges, however, this rate increases to a decline of 39% per year.

**Sampela 1**

A complete loss of recruits (simulating a failed recruitment year) did not result in a major change in either the population rate of increase (closed) or decrease (open) for Sampela 1 ($\lambda = 0.989$ and 0.972, respectively; Figure 3.5). In the event of complete mortality of the largest sponges, the closed population will decline 18% per year, while the open population 21% (Table 3.5). In both instances of simulated mortality, the closed and open population projections remained of similar scale at Sampela 1. At Buoy 1, however, recruitment scenario had a large influence on projections of population growth rate (Figure 3.5).

Figure 3.5. Population growth rates at Buoy 1 and Sampela 1 as a result of simulating size-dependent survival in both open and closed populations. A: recruit and B: largest sponge individuals (0 and 100% mortality). The dashed line represents self-sustainability, or the population neither growing nor decreasing.
3.5 Discussion

Coral cover decline is well documented on many Indo-Pacific coral reefs despite their recognized importance in global marine diversity. Sponges in the genus *Xestospongia* are now some of the largest benthic invertebrates on some reefs where coral is declining, yet little is known about their population dynamics. Integral projection models (IPMs) are flexible and powerful methods of exploring population dynamics using empirical data for individuals, allowing an examination of how individual performance influences population health (Edmunds et al. 2014). In this chapter the primary goal was to quantify how the relationship between sponge size and growth, survival, and fecundity influences population-level outcomes (growth or decline) at two different sites of varying habitat quality. My results suggest that the current population at Buoy 1 is increasing in size by 30% per year and has not reached a stable size distribution. Sampela 1, however, has a stable population with a growth of just 2% per annum and sponges near to their stable size despite low levels of recruitment. The populations at both sites are minimally affected by recruitment failure and can persist for long periods of time with minimum larval input. The apparent difference in population stability at each site is further reflected in elasticity analyses where the future population abundance at Buoy 1 is more sensitive to changes in *Xestospongia* spp. growth and survival than those at Sampela 1. Comparisons of the 95% confidence intervals support that projected population growth differs between sites.

3.5.1 The importance of size in Xestospongia populations

The dominance of large sponges in contributing to population growth is likely due to both sites being characterized by large sponges that comprise a higher proportion of the stable size distribution, which reflects size structure at equilibrium (and therefore population increase). IPMs for both sites were characterized by a level of plasticity, particularly important in larger sponges that were capable of growing, shrinking, or remaining in stasis. Smaller sponges always grew in size, while transitions of small colonies or recruits had a negligible effect on population performance. The dominant contribution of large sizes to population growth is also reflected by the vital rate elasticities, which showed that the retention of large sponges (via the combined effect of survival and growth) has the largest effect. This influence is reinforced by the fact that
Xestospongia spp. fecundity is size-dependent, so even reproductive contributions are dependent on size.

An individual’s size will inherently affect the magnitude of its influence on the surrounding environment (Ayling 1983; Werner and Gilliam 1984; Pansini and Pronzato 1990). Large benthic organisms may dominate specific ecosystem functions, particularly in the case of species that mediate nutrient flux (Norkko et al. 2013). Sponges are recognized as having mechanistic links to reef functioning through altering nutrient and organic matter content in the water column. Large sponges are expected to pump larger quantities of water, and sponge size scales with habitat available for infauna (Westinga and Hoetjes 1981; Tanaka and Aoki 1999; Henkel and Pawlik 2005). Sponges are dominant benthic competitors (Engel and Pawlik 2005) and the persistence of a population comprised of an abundance of large sponges could continue the preservation of an alternative community structure. In the event of significant or recurrent disturbances reducing the abundance of large Xestospongia spp. individuals, however, substantial implications might be expected for the surrounding reef due to the potential for altered stability and rate of nutrient cycling. Although recruitment may assist recovery, the establishment of adults through recruit growth is likely to take decades (see Chapter 2).

3.5.2 The effects of larval source on population growth

Although the persistence of populations at both sites is most influenced by adult fecundity (via large size), the directional transport of larvae was demonstrated to affect long-term population growth rates. Increased gene flow (e.g. connectivity) between study sites would be expected to be beneficial for overall population growth (Jones et al. 2009). However, increased larval exchange between populations simulated via open population scenarios always resulted in a lower population growth rate compared to closed populations that simulated self-recruitment. Upon simulation of recruitment failure, the rate of population growth decreased for closed populations but still remained stable (Buoy 1 continued to grow yearly while Sampela remained in stasis).

The results of this chapter suggest that due to the longevity of Xestospongia spp., several years of limited recruitment in a closed system may result in a slight decrease in population growth but
overall persistence of population structure, perhaps suggesting resilience for short-term disturbances. Adult mortality, however, resulted in a precipitous population decline for both sites in closed populations. By omitting the fecundity kernel and thereby excluding recruitment, a demographically open population is simulated where none of the recruits present are a product of “local” adults (e.g. at each site; Yau et al. 2014), revealing reliance on recruitment from other sites and reflecting population decline (Yau et al. 2014). Upon simulating recruit mortality (representing recruitment failure), the same slight decline in population growth occurred, reinforcing the idea that the population dynamics are mainly reliant on the persistence and performance of large sponges. As in the closed systems, mortality of large sponges resulted in population decline for both sites.

The magnitude of the difference between demographically closed and open populations was large in Buoy 1 scenarios, potentially suggesting that the source of larval recruits exerts a more substantial influence on Xestospongia spp. population dynamics at this site (Figure 3.5). An investigation of Xestospongia spp. connectivity at Buoy 1 and Sampela 1 (see Chapter 5) suggests that although these sites appear to be genetically undifferentiated and are therefore connected via larval exchange to some degree, there is also evidence of substantial within-site relatedness likely due to self-recruitment. This was particularly true for Sampela 1, suggesting that the closed scenario is more accurate for the Sampela 1 population. For Buoy 1, mean relatedness was typically comparable with within sites and between sites, indicating no bias towards strict self-recruitment. If Buoy 1 populations are more open, or reliant on the production of recruits from another site (such as Sampela 1), the projected population growth rates are much closer to stasis and far more reactive to increased adult mortality.

As they are reliant on larval influx from outside sources, open populations are sensitive to larval dispersal ability, while a dependency on local recruitment demonstrated by closed populations is inherently associated with internal factors like demographic rates (Figueira 2009). Considering demography in association with larval dispersal potential is therefore integral when accurately assessing population dynamics (Figueira and Crowder 2006). For instance, if larval supply significantly decreases due to mortality of larger sponges, or “breed stock”, then slower
colonization would be expected even if free space is plentiful. This may be tempered, however, in populations of long-lived individuals that exhibit the “storage effect”. In this case strong recruitment events are “stored” when the recruits survival into numerous adults, which allows for population persistence in poor recruitment years (Warner and Chesson 1985). A similar trend has been demonstrated in fisheries where long-lived, slow growing fish are severely affected by exploitation, taking far longer to recover than short-lived species. Through the storage effect, however, they are less sensitive to recruitment fluctuations (Jennings and Kaiser 1998).

3.5.3 Model assumptions

Previous work on the Great Barrier Reef suggests that *Xestospongia* spp. (*X. testudinaria*, *X. exigua*, and *X. bergquistia*) are oviparous, gonochoric, and highly fertile (Fromont and Bergquist 1994). It is possible that the assumptions included in the development of the fecundity kernel herein are not fully representative; i.e. all individuals may not be reproductively active, nor reproductive year to year. Further investigation on the basic reproductive biology for Indo-Pacific *Xestospongia* spp. is required. Caution in elasticity interpretation is also required, as elasticities have been shown to incorrectly identify vital rates as important based on the mean size of the rate itself (Morris and Doak 2002).

When populations are at or close to their steady state, asymptotic model parameters are useful in describing short-term dynamics as demonstrated in this study. It is highly unlikely that sites included in this study are at their stable state, however, and unaccounted for environmental variability could alter individual performance. Sudden changes in habitat conditions are likely to cause deviations from a steady state, in which case transient dynamics are more informative (Koons et al. 2005). Transient dynamics provide information on population size and structure changes prior to reaching asymptotic growth (Maron et al. 2010). A further exploration into such dynamics, in conjunction with the inclusion of environmental variability and age structure could provide a more robust theoretical insight to the factors driving *Xestospongia* spp. dynamics. Similarly, benthic competition plays a central role in population dynamics and an in depth exploration of benthic composition at each site may inform on the importance of site on barrel sponge population.
On average the sponges at Buoy 1 are smaller, indicating that the population is likely younger than that at Sampela 1 (see Chapter 2). The projected population growth rate of 30% per year would be required to allow a steady state to be reached. This projected rate may be overestimated, however, as the increase projected would require conditions to remain exactly as they were when the data was collected. The estimated increase in population size would also require that the conditions of high recruitment and low mortality persist in order for yearly growth to occur. As populations do not exist in equilibrium and coral reefs do not exist in a completely unchanging environment it is likely to vary over time. Furthermore, as Buoy 1 populations are characterized by smaller sponges than those dominating the steady state required to reach equilibrium, some instability in projected growth rates is expected. For instance, Hughes and Tanner (2000) detailed the long-term population decline of the massive coral *Monastrea annularis* using matrix models; the authors revealed that because the initial population structure was characterized by smaller colonies than the stable population distribution, the population took 26 years to reach the predicted population growth rate and fluctuated until it was reached.

### 3.5.4 Evidence for the support of size-based management

Successful management may require targeting a subset of an organism’s life cycle or even vital rates in the event that population growth is affected by one stage over another (Wallace et al. 2013). Large size and longevity are characteristics that render species susceptible to extinction (Thrush and Dayton 2010), and the loss of larger, older individuals is a sign of substantial population decline (Hughes and Tanner 2000) that would be expected to take decades to recover from. A disturbance event removing a majority of the large sponges at these sites has the potential to decimate the population even if recruitment remains constant, particularly at Buoy 1. Comparing population viability, such as population growth in this case, allows for triaging populations that require the most protection. I would advocate that as *Xestospongia* spp. populations at Buoy 1 are less stable and more sensitive to the loss of large sponges, any management efforts should be focused at this site.
Due to the substantial decline of coral cover in the Wakatobi Marine National Park, sponges in the genus *Xestospongia* are now some of the largest benthic invertebrates providing habitat and fulfilling ecosystem services in some areas. Although the IPMs presented in this chapter are relatively simple, they can be used to gain theoretical insight into the implications of disturbances to size-specific demographic rates. In Chapter 2 I concluded that Buoy 1 and Sampela 1 do not have differing growth trajectories on an individual level. A more detailed investigation into population-level dynamics, however, reveals that the populations at each site are increasing at different rates due to variation in life histories. The results from this Chapter indicate that *Xestospongia* spp. populations are increasing in size and will continue to be a dominant component of coral reefs at both sites. The implications of this increase on an ecosystem-wide scale are currently unknown but expected to be substantial via water column interactions (e.g. via nutrient cycling and heterotrophic feeding). My results suggest that in the absence of large individuals population growth shifts to substantial decline, and that size should be considered a principal element in management and conservation. Finally, this study emphasizes the importance of recruit source to population dynamics on a small spatial scale. These results, in conjunction with the known importance of large barrel sponge individuals in ecosystem functioning on coral reefs, suggests that for Indo-Pacific *Xestospongia* spp. size does in fact matter.
Chapter 4: Adaptive mechanisms and physiological effects of suspended and settled sediment on barrel sponges

Fluorescein dye is pumped through a *Xestospongia* spp. at Sampela 1
(photo credit: Tracey Bates)
4.1 Abstract

Coral reefs across the Indo-Pacific are among the most diverse in the world but like reefs globally, they remain vulnerable to a multitude of stressors including coastal development and the resultant sedimentation. In the Wakatobi National Marine Park, Indonesia, some degraded reefs are characterised by high levels of sedimentation and low coral cover, but support large populations of the ecologically important giant barrel sponge Xestospongia spp. Barrel sponges can have a strong influence on water characteristics, yet tolerance and responses to sedimentation are unknown. This study examined the physiological effects of short-term exposure of barrel sponges to suspended sediment. Respiration rates increased compared to controls when sponges were exposed to environmentally relevant suspended sedimentation concentrations of 75 and 150 mg l\(^{-1}\). Sponge mucus production was observed as a mechanism to remove settled sediment for the first time and sediment clearance was filmed in situ over the course of 24 hours. Sponges produced mucus in response to sediment addition, with a mean clearance rate of 10.82 ± 2.04% h\(^{-1}\) (sediment size fractions 63-250 µm). Mucus production is an effective, but slow mechanism supporting barrel sponge survival in habitats experiencing high levels of sedimentation. Our results show that there are likely to be energetic consequences for sponges living in sedimented environments, which may influence the energy available for other demographic processes, and therefore have implications for barrel sponge population sustainability.

4.2 Introduction

It is widely recognized that coral reefs are among the most highly productive and biologically diverse ecosystems on Earth, providing ecosystem goods and services vital to tropical and subtropical nations (Moberg and Folke 1999). Despite their value, over half of coral reefs worldwide are considered under threat (Burke et al. 2011), and the multitude of natural and anthropogenic pressures associated with global coral reef decline have been well documented (Hoegh-Guldberg et al. 2007; Wild et al. 2011; Perry et al. 2013). Unsustainable land conversion practices such as urbanization, deforestation, and increased agricultural pressures result in runoff and erosion (Airoldi 2003; McLaughlin et al. 2003; Syvitski et al. 2005), increasing terrigenous sediment loads that may reach near shore waters (Thrush et al. 2004; Bannister et al. 2012; Stender et al. 2014). The impacts of sedimentation are diverse and have been shown to be deleterious to
scleractinian corals (see Fabricius 2005 for a review), but its impact on other reef organisms is less clear.

Sponges are important components of corals reefs, yet we have a much poorer understanding of how they are impacted by sedimentation compared to corals (see Bell et al. 2015 for review). Although some sponge species are able to tolerate and even thrive in highly sedimneted habitats, there is strong evidence that sedimentation is usually deleterious to sponges at the individual and population level (see Bell et al. 2015 for a review). Settled sediment can directly affect sponges via burial or smothering (Wulff 1997) and through transport cause tissue scour/abrasion (Rogers, 1990; Ilan and Abelson 1995), resulting in partial mortality and reduced survival (Wulff 1997; Maldonado et al. 2008). At the physiological level, settled and suspended sediment can exert a major influence on sponge functioning. Sponges are obligate filter feeders and exposure to suspended sediment may clog the inhalant canals and filtering system responsible for pumping. The experimental addition of fine suspended sediments has been shown to reduce or arrest pumping rates in several sponge species (Gerrodette and Flechsig 1979; Leys et al. 1999; Tompkins-MacDonald and Leys 2008; Bannister et al. 2012). As pumping is required to feed, clogging by fine sediment may reduce feeding efficiency and particle retention (Lohrer et al. 2006), as well as respiration (Gerrodette and Flechsig 1979).

Despite sedimentation generally being considered to have negative impacts on sponges, high sponge diversity (e.g. Bell and Smith 2004; Knapp et al. 2013) and abundance (Powell et al. 2014) have been reported from some sedimneted sites. For example, in Indonesia sponge densities have increased over the last decade at some highly sedimneted sites (Bell and Smith 2004; Powell et al. 2010; 2014), while habitat quality and coral cover have simultaneously decreased (Powell et al. 2010). Some sponge species appear to tolerate sedimentation and turbid conditions, and demonstrate specific adaptations that allow them to persist in conditions classically considered sub-optimal for suspension feeders. Active and passive responses are employed by sponges to rid the sponge surface of settled sediment or prevent it from settling (Bell 2004). Active responses to sedimentation include an alteration or cessation of pumping rates (Gerrodette and Flechsig 1979; Tompkins-MacDonald and Leys 2008), physically moving away from sedimneted areas in the case of larvae (Maldonado and Uriz 1999), and the production of mucus (Turon et al. 1999; Bannister
et al. 2012). Passive responses include morphological and structural modifications (Barthel and Tendal 1993; Bell et al. 2002; McDonald et al. 2002; Bell 2004), and positioning of the inhalant ostia and osculum to prevent sediment from settling (Bell 2004).

Changes in respiration rates in response to sediment addition have been examined in a number of sponge species with contrasting results. Following exposure to sediment, sponge respiration rates have been shown to both increase (Bannister et al. 2012) and decrease (Lohrer et al. 2006; Tjensvoll et al. 2013). Increased respiration rates may reflect the energetic requirements of sediment clearance mechanisms, such as mucus production following short term exposure, whereas respiration rates may decrease due to a reduction in pumping rate to prevent sediment ingestion (Bell et al. 2015). Sediment size, mineralogy (Bannister et al. 2012), and concentration (Tjensvoll et al. 2013) may influence these responses. The energetic costs of producing a sediment response is expected to incur additional metabolic costs to the sponge, presumably at the expense of other demographic processes such as growth and reproduction (Reiswig 1971; Roberts et al. 2006; Whalan et al. 2007; Bannister et al. 2012). Other benthic organisms, such as corals, have been reported to produce energetically costly mucus as a sediment removal mechanism (Riegl and Branch 1995). The production of mucus as a sediment clearing mechanism has also been observed in several sponge species (Gerrodette and Flechsig 1979; Turon et al. 1999; Kowalke 2000; Bannister et al. 2012), though the energetic costs have yet to be determined.

Some of the most conspicuous sponges on coral reefs fall into the genus *Xestospongia*, which include the giant barrel sponges. *Xestospongia* species can grow up to several meters in diameter and live to be hundreds or possibly even thousands of years old (McMurray et al. 2008; McClain et al. 2015). The Caribbean species *X. muta* has been thoroughly studied and found to be ecologically important on coral reefs largely due to their ability to modify water quality characteristics (e.g. López-Legentil and Pawlik 2008; López-Legentil et al. 2008; McMurray et al. 2008; 2010; 2015; Southwell 2008; McClain et al. 2015). Indo-Pacific *Xestospongia* species, however, have received far less attention despite their abundance and likely similar function in reef ecosystems (but see Fromont and Bergquist 1994; Swierts et al. 2013; Bell et al. 2014). Sponges, including *Xestospongia* spp., play a variety of functional roles that mediate water column processes (Bell 2008; Maldonado et al. 2012), including highly efficient removal of picoplankton
and bacteria (Pile et al. 1997; Perea-Blazquez et al. 2012) and nutrient cycling (Southwell 2008; de Goeij et al. 2013; Fiore et al. 2013). In addition, Xestospongia spp. are phototrophic and contain a dense microbial community (Montalvo and Hill 2011), so also contribute to primary production on reefs. Due to their size, ability to pump vast quantities of water (McMurray et al. 2014), and influence on biogeochemical processes, changes in Xestospongia productivity and abundance could have significant impacts on reef function, particularly in systems impacted by reduced water quality.

Previous research on Xestospongia spp. populations in the Indo-Pacific has demonstrated that this species has high levels of self-recruitment, small population sizes, and low larval dispersal rates (Bell et al. 2014). These characteristics, in addition to the slow growth rates reported for their Caribbean congener X. muta, suggests that these populations should be susceptible to environmental disturbance (Bell et al. 2014). However, Bell et al. (2014) reported that Xestospongia spp. were very abundant at sites experiencing high levels of sedimentation and habitat degradation. These factors, in conjunction with the presence of large and likely old sponges in these habitats, support the hypothesis that barrel sponges likely possess physiological traits enabling them to tolerate high-sediment environments (Bell et al. 2014).

Given the current trends in coastal development and resultant sedimentation expected to reach coral reefs, it is important to understand the effects that sedimentation may have on Xestospongia spp. In this study we aimed to quantify settled and suspended sediment on a degraded reef dominated by Xestospongia spp., enabling environmentally relevant sediment addition experiments. Further, I observed the occurrence, location, and rate of sediment accumulation on Xestospongia spp., as well as quantified mucus production as a settled sediment removal mechanism. Finally, I examined the effects of sedimentation on in situ sponge respiration rates for different suspended sediment treatments.
4.3 Methods

4.3.1 Study site

This study was conducted in the Wakatobi National Marine Park (WNMP), located in southeast Sulawesi, Indonesia. Hoga Reef is a shallow sloping reef on the southwest corner of Hoga Island in the centre of the WNMP, located adjacent to Buoy 1 (Figure 1.1), and was the location where barrel sponges were collected from for our experiments. This site has moderate coral cover (30-40% cover), and low turbidity and sedimentation rates. Sponges were moved from the Hoga Reef to Sampela 1 (approx. 100 m away). Sampela 1 has low environmental quality due to close proximity (< 1 km) to the large populations of Sampela Village and Kaledupa Island. The site has high sedimentation rates, low (and decreasing) coral cover and low fish abundance compared to other sites in the vicinity (Crabbe and Smith 2002; Curtis-Quick 2013; Bell et al. 2014). Due to the limited availability of suitably small sponges for use in our in situ respiration chamber at Sampela 1, sponges used in the respiration experiment were harvested from Hoga Reef and moved the short distance to a single location at Sampela 1. While the sedimentation and turbidity rates differ between the sites, the flow rates are low at both sites (approximately 5 cms$^{-1}$; see Powell et al. 2014). The sponges were left to acclimate to the new conditions for two weeks and inspected prior to experimentation for any signs of new tissue necrosis. In situ respiration experiments were performed from June to August 2015, mucus clearance experiments were conducted from June to August 2014 and 2015, and sediment data was collected in May-July 2015 and 2016. All statistical analyses were performed by SPSS v. 22 and plotted with SigmaPlot v. 11.0. Data were tested for normality and homogeneity of variance and met the assumptions of the statistical analyses that we used. All results are presented with a mean ± standard error unless noted otherwise.

4.3.2 Sedimentation data

4.3.2.1 Sediment deposition rates

Sediment deposition rates were estimated using plastic cylindrical sediment traps equipped with an inverted funnel to minimize resuspension using locally available materials. Sediment traps were approximately 30 cm in height, had a trapping area of 50.26 cm$^2$, and a height to width aspect ratio of 3:1 for the trap mouth, and 10:1 to the funnel opening. Traps were deployed at Sampela 1 at 5
m and 10 m below the reef crest (n = 15 per depth) in a vertical orientation at least 20 m apart for three independent, but consecutive two week periods from June to August 2015. A Ziploc bag was placed over the trap openings upon collection to reduce sediment loss during removal and transportation. Samples were wet sieved into major sediment size factions (> 250, 125-250, 63-125, 38-63, and < 38 µm) and dried to a constant weight at 200°C. Sedimentation rate (g m⁻² d⁻¹) was adjusted to the area of the trap opening and standardised per day; given the constraints of the aspect ratio, the traps were used to estimate the abundance of targeted sediment fractions pertinent to experiments herein. Settled sediment percentage composition data obtained from sediment traps were normally distributed and subsequently analysed using an independent t-test for each sediment size fraction to assess differences between sampling depths (5 and 10 m).

4.3.2.2 Suspended sediment

Ambient seawater samples (50 ml, n = 3) were collected for suspended sediment analyses over the same time period as the sediment traps. Samples were collected at 10 m, filtered through glass fibre filter paper (0.7 µm, Membrane Solutions), and dried to a constant weight at 80°C. The filtered sediment was re-suspended to 120 ml in a 4% NaCl solution and stirred for 20 minutes using an overhead stirrer at 1000 rpms (Chiltern Scientific PS41) to break up any sediment aggregates. Sediment grain size distributions were analysed with a Beckman Coulter Multisizer 3 using a 4-120 µm aperture. Ambient suspended sediment concentration (mg l⁻¹) was calculated by standardising the weight of the sediment retained on the filter by the total volume of water sampled.

4.3.2.3 Sediment accumulation

Qualitative visual surveys were completed at Sampela 1 to identify barrel sponges with sediment present within the osculum (n = 93), as well as those with mucus-bound sediment present on the external surface of the sponge (n = 215). For the latter, sediment was manually disturbed and deemed mucus-bound if the sediment was difficult to remove and if mucus was directly observed.
In order to observe the sediment accumulation time on the external surface of sponges, a high-definition GoPro Hero® fitted with a CamDo time lapse intervalometer was deployed for 24 hours following sediment removal (n = 9). The intervalometer was programmed to take a picture every 10 minutes. Individual sponges were manually cleared of sediment and the camera was positioned to capture one side of each sponge. Sediment accumulation time was defined as the time (min) required before 100% of the cleaned external sponge surface was recovered by sediment. The flow of fluorescein dye was used to ensure that the sponge was pumping before sediment clearance and at the end of the recording time.

4.3.3 Sediment clearance experiment

To examine the production of mucus as a settled sediment response mechanism, previously tagged sponges in a known population at Sampela 1 were chosen haphazardly and filmed using a GoPro Hero® fitted with a CamDo time lapse intervalometer and mounted on a tripod, following the application of sediment treatments. Sediment application was intended to replicate the natural accumulation of settled sediment on the sponges and the amount of sediment to be added was determined by calculating the mean sedimentation rate experienced at Sampela 1 (expressed in g m⁻² d⁻¹) and was standardised for sponge surface area.

Measuring sponge volume can be difficult due to complex external morphology. The use of stereo photogrammetry allows for accurate repeated 3D measurements in order to accurately calculate the total and spongocoel volume (Abdo et al. 2006), though the internal canal system remains difficult to quantify. Sponge surface area (cm²) and oscula volume (cm³) were calculated from digital images taken with a Fujifilm FinePix Real 3D W3 Digital Camera and corresponding underwater camera housing, and measured using stereo calibration and measurement software (CAL and EventMeasure) created by J. Seager (http://www.seagis.com.au). EventMeasure allows for multiple, precise, three-dimensional measurements to be collected from photos taken from several angles and from above, looking into the osculum. This method was used to estimate sponge surface area, and also for measuring sponge volume for the respiration experiment (see below).
Sediment treatments included the most prevalent size fractions as determined from sediment traps (25.43 ± 1.26%: 63-125, and 23.02 ± 0.83%: 125-250 µm, respectively). Size fractions > 250 µm consisted mainly of large pieces of shell, rock, and algae and therefore are unlikely to be effectively removed from the sponge osculum via mucus clearance (personal obs.). Preliminary experiments revealed that the addition of sediment smaller than 63 µm was immediately pumped out of the osculum and prevented from settling, therefore < 38 and 38-63 µm fractions were not used in the study. Surface sediment was collected from the reef, wet filtered to a fraction of 38-63 µm, and dried to a constant weight at 180°C (for a minimum of 12 hours) to ensure the removal of any microbial communities or trace organic material.

Sediment was carefully added to the base of the sponge osculum (n = 15), and the GoPro was positioned directly on the rim of the osculum, taking care not to damage the tissue. The camera was positioned over the sponge in order to have an unobscured view of the base of the osculum; in some cases this necessitated placement directly over the osculum. Due to the small size of the camera compared to the much larger size of the osculum, there was no perceived effect of the camera presence on sponge pumping.

Sediment applications and GoPro deployment occurred between 7 and 11 am to allow for the maximum amount of daylight, and each sponge was filmed for 24 hours following each sediment application. The GoPro was programmed to take a photo once every hour. Fluorescein dye was used to ensure that the sponge was pumping before sediment additions and following camera retrieval; though *Xestospongia* pumping rates may vary over the course of 24 hours, a complete pumping cessation is uncommon (McMurray et al. 2014).

Photos (n = 24 per sponge) were analysed with ImageJ 1.43 software (developed at the National Institutes of Health, Washington, DC, USA) to measure the area of settled sediment present in the sponge osculum every hour for the duration of the video. The ImageJ *Freehand Sketcher* was used to manually trace the boundary of the sediment in the digital images and the area of the shape was calculated automatically. Strings of mucus were not included in the area estimation. Sediment clearance rates were calculated as the hourly percentage decrease in sediment area in the osculum.
and on the surface of the sponge. Independent t-tests were used to examine the effect of osculum volume (as a proxy for sponge volume reported by McMurray et al. [2014]), and sediment fraction on sediment clearance rates.

4.3.4 In situ respiration experiment

The large size of barrel sponges creates difficulties in measuring respiration in a laboratory setting, necessitating in situ data collection using a submersible respiration chamber. Sponges of suitable small size were harvested from Hoga Reef within predefined selection criteria (no mortality or necrosis, and the size being limited by the size of the respiration chamber) from 10 m and were transported a short distance (approx. 100 m) to a single location at Sampela 1 where the experiment was conducted. Entire sponges, still attached to coral rock, were removed from the substrate and placed in a stable location at the same depth at Sampela 1. Sponges were cleaned of any epibionts (mainly algae and synaptid sea cucumbers) before harvest and once again before the start of each experiment. Sponges were allowed to acclimate for two weeks at Sampela 1 prior to experimentation, consistent with previous laboratory experiments (Fang et al. 2013; Wisshak et al. 2014; Lesser et al. 2016; Bennett et al. 2017). As such respiration results are unlikely to be the result of the sponge be transported to a central location for experiments.

4.3.4.1 Respiration chamber design

A single 73 litre Perspex respiration chamber was developed for in situ measurements; once sealed, the chamber was completely water-tight. The chamber contained a fully closed, manual circulation system based on a series of hand pumps to provide standardised water flow for the time that the sponge was in the chamber (Figure 4.1). The hand pumps were compressed every two seconds and delivered a mean volumetric flow of 90 l h⁻¹. Sponge pumping activity was also expected to increase flow within the chamber and maintain water circulation. If Indo-Pacific Xestospongia spp. pumping rates are comparable to those measured by McMurray et al. (2014) for X. muta of similar morphotypes (0.06 ± 0.04 s⁻¹ l⁻¹ sponge tissue), then the sponges themselves contribute 7.8 l h⁻¹ on top of the hand pump flow rate of 90 l h⁻¹. The mean cross flow rate in the chamber was 0.066 ± 0.013 m s⁻¹, which is comparable to the ambient flow rate at Sampela 1 (0.063 ± 0.044 m s⁻¹);
Powell et al., 2014). The incurrent pump was positioned 10 cm lower than the out-current pump to reduce sediment falling out of suspension.

Water samples and sediment additions were drawn and added, respectively, through two ports affixed with reinforced T-valves that were water tight when closed. Water samples for oxygen analysis were drawn from the port with a 35 ml syringe through a fitted rubber stopper; concentrated sediment was added using the same method. As *Xestospongia* spp. contain a dense photosynthetic microbial community (Montalvo and Hill 2011), whole sponge oxygen consumption would represent both the metabolic activity of the sponge and the microbial community which may be difficult to distinguish (Cheshire et al. 1997). For this reason the chamber was blacked out (after Bennett et al. 2016) so the symbionts would not be photosynthesising and therefore producing oxygen. Therefore the respiration rates that we measured are a combination of the sponge and its associated microbial community. Furthermore, there is no evidence in the literature to suggest different sponge respiration rates occur in the light compared to the dark.
Figure 4.1 Respiration chamber design with model *Xestospongia* spp. sponge, asterisks (*) denote cable clamps throughout the chamber, arrows denote direction of water flow. Note: the chamber was blacked out for actual respiration experiments.

### 4.3.4.2 Experimental design

Individual sponges of the same gross morphology were chosen haphazardly and placed in the chamber at 10 m to dark adapt for 10 minutes, while still being exposed to ambient water circulating within the chamber. The chamber was then sealed and baseline water samples ($T_0$) were immediately drawn, followed by the addition of the sediment treatment (mg dry weight l$^{-1}$). Water samples were taken every 15 minutes thereafter for 45 minutes while pumping continued to ensure adequate circulation.
Suspended sediment concentration measurements collected from Hoga Reef at 10 m revealed the mean ambient suspended sediment load to be $134.67 \pm 2.26 \text{ mg} \text{l}^{-1} \pm \text{SE}$. In order to quantify potential threshold levels of sponge physiological responses to suspended sediment, sponges were exposed to initial concentrations of $75 \text{ mg} \text{l}^{-1} \text{(n} = 6\text{)},$ $150 \text{ mg} \text{l}^{-1} \text{(n} = 7\text{)},$ and a control treatment (no sediment, $n = 7$). Sediment for each treatment was collected and processed in the same manner as the sediment clearance experiment. There is the potential that respiration rates may be influenced as a result of bacterial activity on the sediment or in response to organic residue remaining on the particles. However, any potential influence of sediment-derived microbial populations or trace nutrient modification on sponge respiration rates are likely to be negligible due to the temperature and duration of the heating processes, which would have removed an organic residues.

The suspended sediment composition of water samples at 10 m ($n = 3$) revealed that sediment $< 38 \mu \text{m}$ comprised the largest fraction of suspended sediment ($73.64 \pm 1.02\%$), but was difficult to isolate reliably using wet sieving techniques. Therefore, the second most abundance sediment fraction ($38-63 \mu \text{m}; 21.28 \pm 0.03\%$) was chosen for the respiration experiments as this will still be experienced by sponges filtering water from the water column.

Respiration rates were calculated based on the chamber volume (l), volume of the sponge ($\text{cm}^3$), and the amount of concentrated suspension injected into the chamber ($\text{g}$ dry weight of sediment $\text{l}^{-1}$ seawater). Separate, sealed Perspex chambers were used to collect baseline respiration data for seawater samples in the absence of sponges; water samples for respiration measurements were collected at the beginning of every sponge respiration experiment ($T_0$), and after 45 minutes ($T_3$, $n = 20$). The uptake of fluorescein dye by the target sponge was used to ensure that the sponge was pumping at the start of the experiment and at the conclusion of the experiment. The presence of mucus was also noted.

Water samples were kept at constant ambient water temperature and oxygen concentration was immediately analysed at the surface using a FIBOX 3, 505 nm oxygen probe (combined with FIBOX 5.20 Software for data logging; Precision Sensing GmbH, Regensburg, Germany). The
oxygen electrode was calibrated prior to each oxygen measurement to 0% oxygen saturation (water containing 1 g of sodium sulphite [Na$_2$SO$_3$]) and 100% oxygen saturation (water bubbled for 10 minutes) in 100 ml of seawater. Chamber samples were kept in the dark and sealed in an airtight container fitted with a rubber stopper to prevent oxygen exchange. As samples were collected at 10 m and analysed at sea level final oxygen concentration values for sponges and seawater blanks were corrected for changes in partial pressure of O$_2$.

Sponge volume was calculated from three-dimensional photographs following the methods utilized in the in situ sediment clearance experiment, but expanded to include the entire sponge. Volume was calculated by approximating geometric shapes for each sponge shape and corrected for spongocoel volume, and ranged from 71.95 to 583.43 cm$^3$. Due to the highly diverse morphologies of Indo-Pacific Xestospongia sp., sponges were categorized as either cylinder, barrel, sphere, inverted truncated elliptical cone, or frustrum of a cone. Spongocoels were categorized as either cylinders or inverted truncated elliptical cones (depending on the sponge) and volume was calculated accordingly. A dry weight/volume ratio was calculated by measuring the smaller sponge fragments by hand, and then drying and weighing them (n = 179). Whole sponge volume was extrapolated from this data using linear regression (F$_{1,178}$ = 216.70, R$^2$ = 0.574, p < 0.001) in lieu of sacrificing whole sponges.

Suspended sediment concentration was expected to fluctuate due to settlement in the chamber and movement through the pump system. The actual concentration of sediment within the chamber was calculated by taking water samples of known volume (n=3) immediately after the addition of sediment and at the end of the experiment. Samples were filtered through glass fibre filter paper (0.7 µm, Membrane Solutions), and dried to a constant weight at 80°C.

Sponge respiration rate was calculated based on chamber volume (corrected for individual sponge volume), time (h), seawater sample (blanks), and calculated as follows:

\[
\text{Respiration rate} = \frac{V_{\text{chamber}(t)}}{DW_{\text{sponge}}} \times \left[ \text{sponge} \left( mgO_2 \left[ T_0 \right] - mg \ O_2 \left[ T_3 \right] \right) - \text{blank} \left( mg \ O_2 \left[ T_0 \right] - mg \ O_2 \left[ T_3 \right] \right) \right]
\]
where $T_0$ is the beginning and $T_3$ is the end of the sampling period, $V_{\text{chamber}}$ is the volume of the chamber, and $DW_{\text{sponge}}$ is the estimated dry weight of the sponge. The change in respiration rate over time was calculated for each sponge as above for each time point ($T_0$ and $T_3$). Logistical constraints of *in situ* sample collection rendered continuous oxygen consumption curves impossible. However, Mills et al. (2014) demonstrated the linear relationship of *Halichondria panacea* oxygen consumption in a closed system over time, supporting the use of point measurements.

A One-way ANOVA was employed to test the differences in respiration rate in response to suspended sediment exposure (75 and 150 mg l$^{-1}$) and control treatments (no sediment), followed by Tukey’s HSD *post-hoc* test to examine the significant main effects between treatments. The effect of short term suspended sediment exposure on sponge respiration rate was analysed with a One Way repeated measure ANOVA. Sampling time ($T_0$-$T_3$) measured at 15 minute intervals comprised the within factor treatment, and the suspended sediment treatments (75 and 150 mg l$^{-1}$) and the no sediment control compromised the between factor treatment ($n = 20$). Greenhouse-Geisser adjusted probability was used to estimate statistical significance to avoid violating sphericity. A Bonferroni *post-hoc* test was used to examine the relationship between respiration rates and sediment treatment.

### 4.4 Results

#### 4.4.1 Sedimentation data

At the conclusion of the two week sampling period three 5 m and two 10 m traps were unable to be collected (final $n = 25$). There were no significant differences between depths in any size fraction and the data was pooled (> 250 µm: $t(23) = -0.084$, $P = 0.934$; 125-250 µm: $t(23) = 0.123$, $P = 0.903$; 63-125 µm: $t(23) = -0.213$, $P = 0.834$; 38-63 µm: $t(23) = 0.917$, $P = 0.369$; < 38 µm: $t(23) = -0.012$, $P = 0.990$). The mean sediment deposition rate obtained from the sediment traps at Sampela 1 was 44.40 ± 3.0 g dry weight m$^{-2}$ day$^{-1}$; the mean sediment size fraction composition was primarily 63-125 µm sediment (25.41 ± 1.26%), followed by 125-250 µm (23.15 ± 0.61%), 38-63 µm (20.55 ± 1.98%) and < 38 µm (11.41 ± 1.84%; Figure 4.2).
Mean settled sediment fraction percent composition (± SE) collected in sediment traps in depths ranging from 5-10 m at Sampela 1, Wakatobi National Marine Park (n = 30).

Suspended sediment grain sizes derived from water samples collected from Hoga Reef at 10 m ranged from 6 to 106 µm (Figure 4.3). The dominant grain size was < 38 µm with 73.64 ± 1.02% composition, followed by 38-63 µm (21.28 ± 0.03%), and 63-106 µm (5.58 ± 0.09%).
Visual sponge surveys revealed that 60% of sponges had sediment observed within their osculum at Sampela 1 (n = 93), while 99% of sponges had mucus-bound sediment present on their external surface (n = 215). Footage from sediment accumulation videos revealed that sponges cleared of sediment were 100% re-covered in sediment in a mean of 135.11 ± 10.93 minutes (n = 9).

**4.4.2 Settled sediment clearance**

Mucus production as a settled sediment clearance mechanism was observed in every sponge following sediment addition. Mucus was evident after one hour post sediment application. Sediment fraction size did not influence the mean rate of sediment clearance ($t(13) = 0.817, P = 0.429$); sponges rid themselves of 125-250 μm sediment at a rate of 12.62 ± 3.83% hr$^{-1}$, and 63-125 μm sediment at a rate of 9.24 ± 2.47% hr$^{-1}$. There was no influence of sponge osculum volume (used as a proxy for sponge size) on the efficiency of sediment clearance ($t(11) = -0.175, P = 0.864$).
The typical progression of sediment clearance by mucus production proceeded as follows: 1) mucus production began in the base of the osculum; 2) sediment was aggregated within a concentrated mass of mucus; and 3) strings of buoyant, mucus aggregate were propelled upward and out of the sponge by the force of sponge pumping (Figure 4.4). In some instances reef fish were observed within the sponge osculum following the production of mucus, though scavenging was not directly observed.

Figure 4.4. Time series of mucus production as a sediment clearance mechanism in two *Xestospongia* spp. sponges following 125-250 µm sediment addition; a) immediately following sediment addition; b) one hour post sediment addition; c) two hours post sediment addition; d) four hours post sediment addition. Arrows indicate the presence of mucus aggregates and buoyant threads.

4.4.3 In situ respiration experiment

Throughout the course of the experiment sponges were exposed to less suspended sediment than initial treatment concentrations due to sediment settlement within the pumping system and in the
The actual range of suspended sediment that sponges were exposed to for the 75 mg l\(^{-1}\) treatment was between 52.46 ± 3.14 and 64.33 ± 5.63 mg l\(^{-1}\), and for the 150 mg l\(^{-1}\) initial application between 123.33 ± 8.03 and 138.57 ± 8.43 mg l\(^{-1}\).

There was a clear effect of suspended sediment exposure on *Xestospongia* spp. respiration rate over the course of the experiment (one-way ANOVA, \(F_{2,19} = 7.277, P < 0.01\); Figure 4.5). Control sponges that were not exposed to sediment had an 18.8 ± 8.02% mean increase in respiration rate, as compared to 95.05 ± 22.11% and 71.56 ± 11.33% (in 75 and 150 mg l\(^{-1}\) treatments, respectively). Exposure to both the 75 and 150 mg l\(^{-1}\) suspended sediment treatments resulted in a greater increase in sponge respiration rate than the control (75 mg l\(^{-1}\): Tukey HSD *post-hoc*, \(P < 0.01\); 150 mg l\(^{-1}\): Tukey HSD *post-hoc*, \(P < 0.05\)). There was no significant difference in respiration rates at any given time point between the low and high suspended sediment treatment (75 and 150 mg l\(^{-1}\); Tukey HSD *post-hoc*, \(P = 0.553\); Figure 4.5).

![Figure 4.5. Mean increase in respiration rate (± SE) over the course of the experiment for control (no sediment; \(n = 7\)) and sediment treatments: 75 mg l\(^{-1}\) (\(n = 6\)) and 150 mg l\(^{-1}\) (\(n = 7\)) standardized by seawater blank (T3-T0, 45 minutes total) corrected for changes in partial pressure of O\(_2\). Superscripts denote significant differences (Tukey’s HSD *post-hoc*, \(P < 0.01\)).](image)
Mean respiration rates increased steadily over the course of 45 minutes for all treatments beginning with variable baseline respiration rates of 0.03 ± 0.04, 0.09 ± 0.06, and 0.08 ± 0.02 mg O₂ g⁻¹ DW h⁻¹, in control, 75 and 150 mg l⁻¹ treatments, respectively (Figure 4.6). There was a highly significant interaction between sampling time and treatment in the repeated measures ANOVA (Table 4.1). Between sponge effect tests revealed a significant effect of exposure to suspended sediment on sponge respiration rate within the sediment treatments. Bonferroni multiple comparison post-hoc tests revealed that sponges exposed to sediment treatments had a greater increase in respiration rates than that of the control (Table 4.1).

![Figure 4.6](image.png)

Figure 4.6. Mean respiration rates (± SE) of *Xestospongia* spp. during exposure to suspended sediment treatments over the course of 45 minutes and corrected for changes in partial pressure of O₂. Control treatment represents individual sponges not exposed to a suspended sediment. Time 0 represents the baseline respiration rate per treatment immediately before the addition of sediment treatments, while 15-45 represent the time (min) post sediment addition.
Table 4.1. Repeated measures ANOVA model examining Xestospongia spp. mean respiration rate exposed to suspended sediment concentrations and no sediment control sponges. Significant values are derived from Greenhouse-Geisser corrections.

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4.5 Discussion

Elevated suspended sediment loads reaching coastal habitats can have major consequences for the ecology of the organisms inhabiting these environments, particularly benthic suspension feeders (Smith and Kukert 1996; Henley et al. 2000). Increased sedimentation is deleterious to a variety of species (Ellis et al. 2002; Fabricius 2005) and mechanisms for tolerance are often at the expense of demographic processes, resulting in decreased growth and reproduction. Here we measured the physiological response of Xestospongia spp. to suspended sediment in the Indo-Pacific and demonstrated that mucus production is likely an important sediment clearance mechanism. We also found increased respiration rates in response to suspended sediment treatments. Given there is likely to be an ongoing energetic cost to continually remove sediment in sedimented environments, we propose that some demographic processes (such as growth and reproduction) of sponges in sedimented environments may be lower than in non-sedimented sites. If energy is devoted away from demographic processes this may have wider implications for population sustainability and maintenance. For example, Xestospongia recruitment is known to be low and sporadic (McMurray 2008; Bell et al. 2014), and any reduction in reproductive output may tip populations into decline with wider ecosystem impacts, given the strong influence these species exert over the water column.
4.5.1 The effect of suspended sediment on Xestospongia spp. respiration

Sponge exposure to 38-63 µm suspended sediment rapidly increased respiration rates in both treatments more than eight fold in only 45 minutes (Figure 4.6). The rapid respiratory response demonstrated in such a short time emphasizes the impact of sediment on Xestospongia spp. respiration rate. As the sponges did not stop pumping (indicated by the uptake of fluorescein dye at both the start and end of the experiment), exposure to suspended sediment must affect barrel sponge physiology. We propose that these sponges have a broad tolerance to sediment exposure, as the highest sediment treatment (150 mg l\(^{-1}\)) did not significantly increase respiration more than the lower sediment treatment (75 mg l\(^{-1}\); Table 4.1; Figure 4.6). Due to logistical constraints it was not possible to observe recovery rates following suspended sediment exposure though this would have provide relevant information on Xestospongia spp. recovery mechanisms and should be a focus of future research.

Our study demonstrates the initial response of Xestospongia spp. following short-term exposure to high levels of suspended sediment. The rapid increase in respiration rate after this short period of time suggests that these environmental concentrations are sub-optimal for Xestospongia spp., yet density is among the highest in the WNMP (see Chapter 2). Assuming a gain in biomass over time and using growth models developed for the Caribbean congener X. muta (McMurray et al. 2008), barrel sponges at Sampela 1 are on average likely 29-38 years old. This means they are nearly as old as the settlement of the nearby Bajo village built in the 1950’s (Bell et al. 2014) that has resulted in heavy exploitation of the Sampela reef in the subsequent years, and likely contributes to the current high levels of sedimentation. The maturity of this sponge population suggests that they are better able to cope with environmental perturbations, whether chronic or acute, compared to other components of the reef, such as corals.

Preliminary trial respiration experiments revealed that smaller sediment size factions (38-63 µm) are prevented from settling into the sponge osculum due to the velocity of water pumped through the sponge, suggesting that in moderation, sponges may be able to rid themselves of small particles as part of normal pumping activities. In the Caribbean, Xestospongia muta have been shown to pump large quantities of water for long periods of time (McMurray et al. 2014), rendering this a
potentially effective method of prevention against fine sediment settlement. It is important, however, to understand how barrel sponges rid themselves of sediment with grain sizes smaller than 38 µm as they may have alternative physiological impacts than those examined in this study. Both the suspended and settled sediment grain sizes measured in this study are likely to affect the physiology of pumping and feeding of barrel sponges. According to Bannister et al. (2012), sediments with grain sizes between 10 and 100 µm may be filtered into the aquiferous system of many sponges, and those smaller than 10 µm may be filtered into the choanocyte chambers themselves.

Unlike other fields of study there is no one standard unit for sponge respiration, which makes comparisons difficult. Respiration rates vary widely between and even within species, with units reported including nmol O$_2$ min$^{-1}$g$^{-1}$ (Cheshire et al. 1997), mg O$_2$ l$^{-1}$ (Coma 2002), ml O$_2$ h$^{-1}$ g$^{-1}$ AFDW (Kowalke et al. 2000), cm$^3$O$_2$h$^{-1}$ per cm$^3$ sponge (Reiswig 1974) and µmol h$^{-1}$g DW$^{-1}$ (Tjensvoll et al. 2013). We reported low (T$_0$) respiration rates for Xestospongia spp. (between 0.014 ± 0.0207 to 0.0677 ± 0.0317 mg O$_2$ g$^{-1}$DW h$^{-1}$) at the start of the experiment. We confirmed pumping prior to each experiment starting, and therefore Xestospongia spp. likely has low basal respiration rates compared to other sponge species. Furthermore, while Osinga et al. (1999) reviewed respiration studies and reported a range between 0.2 and 25 µmol O$_2$ h$^{-1}$ (Osinga et al. 1999), our apparent low respiration rates are still comparable with those reported in earlier studies (e.g. Kowalke 2000).

Previous studies have found that elevated suspended sediment can result in chronic stress in corals, and that the energetic cost of sediment removal may increase with continual low-level sedimentation (McLaughlin et al. 2003). Sponges experiencing high rates of sedimentation may have high metabolic costs associated with active sediment removal mechanisms along with a reduction of symbiont productivity, though this remains poorly understood (Tompkins-MacDonald and Leys 2008; Bell et al. 2015). The production of mucus would therefore be expected to increase respiration rates (Bell et al. 2015), and this is supported by the results of the in situ respiration experiment. Following the conclusion of the experiment, mucus production
originating from both the external surface of the sponge and interior of the osculum was evident in sponges exposed to both sediment treatments, but not the control.

4.5.2 Mucus as a sediment clearance mechanism

Although not previously examined in sponges, mucus production has been well studied in corals. Mucus is often produced when corals are exposed to air or sedimentation, with mucus moving nutrients and carbon to off-reef locations (Wild et al. 2004; Huettel et al. 2006). Previous work has suggested that continuous mucus production in corals allows for the high productivity of coral ecosystems in an oligotrophic system (Huettel et al. 2006), and was recently demonstrated to play an important role in the “sponge loop” that facilitates nutrient cycling (Rix et al. 2016). Coral mucus can be a significant source of carbon (Riegl and Branch 1995), phosphate, silicate, ammonium, and nitrate/nitrite (Wild et al. 2005). As such, a chronically stressed sponge population continually producing mucus could not only affect the demographic processes of the sponges themselves, but could have a larger impact on the recycling of matter and release of nutrients on coral reefs. The presence of mucus-bound sediment within the oscula suggests that 60% (n = 93) of sponges surveyed may utilize mucus as a clearance mechanism. Mucus production at this scale would therefore be expected to have a contribution to local nutrient cycling at Sampela 1, though this has yet to be quantified.

4.5.3 Experimental limitations

This study is the first to measure respiration rates of a barrel sponge species, and the first to examine the effects of suspended sediment exposure on Xestospongia in the Indo-Pacific. Measuring respiration rates in situ eliminates the stress of transporting large barrel sponges into a lab and maintaining them in aquaria, resulting in more natural measurements. It is possible that the brief transplantation between sites described here may have influenced barrel sponge respiration. In order to mitigate this effect a two-week acclimation time was observed which is longer than many previous experimental studies that have taken sponges to the laboratory (Fang et al. 2013; Stubler et al. 2014; Lesser et al. 2016).
There are drawbacks to our *in situ* method, including a limited measurement time due to local dive restrictions, which prevented observations being made on the recovery from sediment exposure. In addition, the large size of the study species resulted in the small sample size of sponges of appropriate size to fit into the chamber. Although the method utilized for our study was a simple and inexpensive one, it is important to note that simple recirculating, closed-system chambers of this nature are incapable of recreating the complex flow found on the benthic boundary layer of a coral reef. Furthermore, as the sponge was observed to be pumping both before and after the experiment, it is likely that pumping throughout the course of the experiment increased water flow and therefore exchange rate in the chamber (Patterson et al. 1991). Finally, the inclusion of a disturbance control (Crowe and Underwood 1999) would have allowed for quantification of sponge handling and transportation.

Suspended sediment concentration is likely to be variable at a range of temporal and spatial scales. The WNMP is subject to wet and dry seasons with varying levels of rainfall (Crabbe and Smith 2005; Salinas-de-Leon et al. 2013), which affect turbidity levels. Although the small sample size and spot sampling method utilized in this study is likely not representative of the full range of suspended sediment concentrations that *Xestospongia* spp. may experience throughout the year, the presence of a mature, well developed population at Sampela 1 indicates that these sponges have mechanisms allowing them to survive varying degrees of sedimentation.

Despite the recognized importance of sponge conservation (Bell et al. 2015), there remains a general lack of understanding of the effects of both suspended sediment concentrations and settled sedimentation on sponges, as well as the potential adaptations allowing some sponges to persist in these habitats. This chapter experimentally demonstrates that Indo-Pacific *Xestospongia* spp. respond quickly to exposure to sedimentation with a likely energetic cost, which may have influence sponges demographic processes and subsequent reef ecosystem functioning.
Chapter 5: Limited larval dispersal structures *Xestospongia* spp. populations in the Wakatobi National Marine Park

The collection of *Xestospongia* spp. samples for molecular analysis, Karang Gurita (photo credit: Joe Marlow)
5.1 Abstract

An organism’s dispersal and successful recruitment determines population genetic structure and influences population dynamics. Relatedness analyses can provide information on recruitment events and provide insight into spatial patterns of larval dispersal, which are critical for understanding population connectivity. The sponges in the genus *Xestospongia* are widely recognized as important contributors to coral reef functioning and have been shown to exhibit high within-species genetic differentiation at local spatial scales in the Indo-Pacific. Understanding fundamental population characteristics, such as genetic connectivity and dispersal potential, are vital for informing management and conservation strategies, particularly for ecologically important species. In this chapter I used nine multilocus microsatellites to examine *Xestospongia* diversity and structure at four sites in the Wakatobi National Marine Park (WNMP). I also explored *Xestospongia* spp. dispersal ability and level of self-recruitment using assignment tests, kinship analyses, and maximum-likelihood estimation of relatedness. Genetic analyses demonstrated strong genetic structuring and evidence for five genetic groups over a small spatial scale (2 km$^2$), coupled with high levels of inbreeding within each ‘species’ group ($F_{is}$ ranged from 0.028 to 0.467 at Ridge 1 and Sampela 1, respectively). Relatedness analyses suggested that the species complex might be a product of limited connectivity due at least in part to low larval dispersal and high levels of self-recruitment. The limited demographic connectivity among *Xestospongia* spp. populations, in conjunction with large size and longevity, are characteristics generally believed to make populations susceptible to local extinction in changing environments. However, reduced genetic exchange between populations via limited larval dispersal may enable local adaptation in a population, potentially allowing the persistence of dense *Xestospongia* spp. populations in poor quality habitats in the WNMP.

5.2 Introduction

5.2.1 Marine larval dispersal

Throughout the 20th century it was widely believed that marine populations were demographically open and demonstrated panmixia (e.g. Palumbi 1994; Caley et al. 1996), due to the lack of perceived physical barriers coupled with the ability of some larvae to remain in the water column
for extended periods of time. In “open” populations without restrictions to gene flow, individuals immigrate and emigrate among populations, reducing the accumulation of genetic differentiation among populations (Wright 1931). In contrast, “closed” populations experiencing low gene flow have a limited exchange of individuals (Cowen and Sponaugle 2009), and genetic drift and natural selection may result in population differentiation. Recent research suggests that rarely is a system either open or closed (Christie et al. 2010), and in fact many populations are more in line with closed models due to barriers to larval dispersal relative to the scale of dispersal potential (Hellberg et al. 2002; Cowen and Sponaugle 2009). An understanding of both the levels of larval dispersal from the natal habitat and the connectivity between other populations is required to adequately manage an ecosystem (Planes et al. 2009). Effective management must ensure that a loss of individuals from a populations is replenished by larvae from outside populations (Shanks et al. 2003).

5.2.2 Connectivity and relatedness

The use of population genetics is the most common approach for estimating dispersal and connectivity in marine populations (Hellberg 2007), and entails comparing allelic or genotypic frequencies over a range of spatial scales (Hedgecock 2010). Although widely used, there are limitations to these methods when applied to larval dispersal; estimates are averaged over thousands of generations and in some cases may reflect past rather than current dispersal patterns (Hedgecock et al. 2007; Jones et al. 2009). In contrast, assignment-based estimates focus specifically on recruit assignment to a population of origin based on their multilocus genotype (Manel et al. 2005; Saenz-Agudelo et al. 2009). These estimates provide information comparable to the more logistically complex methods, such as directly observing larval dispersal (Leis et al. 2007), or larval tagging (Almany et al. 2007). Measurements of population structure using traditional genetic approaches (e.g. F-statistics) do not incorporate measures of relatedness which omit a valuable insight into larval dynamics. For example, kinship structure may be identical between populations, yet have differing genetic differentiation (Iacchei et al. 2013).

Relatedness analyses of marine invertebrate cohorts have been shown to influence the success and spatial arrangement of larval settlement, which indirectly mediates genetic diversity, phenotype,
and abundance (Aguirre et al. 2013), and therefore resilience to disturbance (Saavedra-Sotelo et al. 2011). Such analyses can provide information on the directionality of larval dispersal, including where larvae come from and where the progeny of a population go (Jones et al. 2009). In that way measures of relatedness can reveal ongoing genetic exchange and inform the potential range of larval dispersal (Kanno et al. 2011; Schunter et al. 2014). In addition to providing insight into spatial patterns of larval dispersal, relatedness analyses can provide mechanistic and temporal information on recruitment events themselves (Selkoe et al. 2006; Veliz et al. 2006). The most fundamental aspect underlying relatedness is the concept of identity by descent (IBD), or the probability that sets of genes are inherited from one ancestral gene (Weir et al. 2006). Estimates of maximum-likelihood of relatedness (MLE) measures IBD between pairs of individuals by estimating the probability of observing a given pairwise allelic pattern (Huang et al. 2015). Unlike continuous maximum-likelihood estimation, kinship analyses determine the level of relatedness from a set of discrete possibilities (i.e. half- or full-siblings). This information can then be used to elucidate the potential range of larval dispersal and reproductive mode (Kanno et al. 2011; Schunter et al. 2014). Assignment testing provides direct measure of demographic connectivity by assigning individuals to their population of origin based on their multilocus genotype (Berry et al. 2004; Manel et al. 2005; Underwood et al. 2007; Saenz-Agudelo et al. 2009). Together, these estimates provide an insight into demographic connectivity between sites.

5.2.3 Indo-Pacific Xestospongia spp.

Some of the most conspicuous sponges on coral reefs fall into the genus Xestospongia, which include the giant barrel sponges. Among the largest known sponges, Xestospongia species can grow up to several meters in diameter and live to be in excess of 30 years of age in the Wakatobi Marine National Park (see Chapter 2). In addition to their large size and longevity, Xestospongia species are phototrophic and contain a dense microbial community, contributing to reef primary production (Montalvo and Hill 2011). Bell et al. (2014) identified a species complex in Indo-Pacific Xestospongia spp., with four groups of genetically differentiated clusters from what was perceived to be one species. The authors suggested that the differences between these species may be affected by the processes influencing gene flow between populations. Furthermore, limited
larval dispersal distances, coupled with reliance on self-recruitment, are conditions that could support local adaptation.

Understanding fundamental characteristics such as genetic connectivity and dispersal potential are vital to informing management and conservation for ecologically important species. The aim of this chapter was to assess the genetic diversity and structure of four sites surrounding Hoga Island in the Wakatobi National Marine Park. I also explored the both the directionality of *Xestospongia* spp. dispersal, as well as dispersal potential. Recruit source of origin (e.g. natal habitat) was determined by using assignment tests. Dispersal potential was examined in two ways; first, sibship analyses were used to estimate recruit kinship to adult individuals at each site, informing the degree of self-recruitment. Next, I compared maximum-likelihood estimation of relatedness in adults among and between study sites in order to assess the degree of non-random mating or limited dispersal.

5.3 Methods

Samples were collected between May and August 2014 to 2016. Four study sites were sampled in the Wakatobi National Marine Park. These included Sampela 1, Buoy 1, Kaledupa Double Spur, and Ridge 1. Sampela 1 is characterized by high level of sedimentation and declining hard coral abundance (Crabbe and Smith 2002b; Curtis-Quick 2013; Chapter 2). Buoy 1 is a steep wall near the southwest corner of Hoga Island and is characterized by moderate levels of coral cover (20-25%; Powell et al. 2014), yet has substantial exposure to sedimentation (Chapter 2). Kaledupa Double Spur is located on the northeast side of Kaledupa Island and features moderate coral cover (25-35%). Ridge 1 is located on the western side of Hoga Island and is also characterized by moderate coral cover. Sponges from Sampela 1 and Buoy 1 were extensively mapped and tagged with individual identifiers from depths of 1 to 30 m (see Chapter 2). Care was taken to include all sponges across the reef; the extent of the population was noted when no further sponges were located within 50 m of the last tagged sponge in every direction.
Tissues samples for DNA extraction were collected from all sponges at Buoy 1 and Sampela 1 and preserved in 90% ethanol (n = 117 and 99, respectively). In order to expand the analyses, microsatellite information for Kaledupa Double Spur and Ridge 1 was obtained from Bell et al. (2014; n =26 and 10, respectively). Approximately 25 mm² tissue samples were used for DNA extraction using a Qiagen DNeasy Blood and Tissue extraction kit following the manufacturer’s instructions. DNA concentration was quantified using an Implen Nanophotometer. Following the protocol used by Bell et al. (2014), nine microsatellite loci were amplified in multiplex polymerase chain reaction (PCR). The reaction volumes (12 µl) contained the following: approximately 50 ng DNA template, 1X Bioline MyTaq Red Mix (.11 units/µl) Taq DNA polymerase, 82.5 mM Tris–HCl pH 8.5, 22 mM (NH4)2SO4, 1.65 mM MgCl2, 0.22 mM dNTPs), ddH2O, and forward and reverse primers in equal amounts (detailed in Bell et al. 2013). The 5’ ends of the forward primers were tagged with the fluorescent labels FAM, VIC, NED or PET and loci were arranged into the multiplex PCR panels described by Bell et al. (2014). Samples were amplified on a GeneAmp 2700 (Life Technologies) thermocycler with the following cycling conditions: 94ºC for 5 minutes; followed by 40 cycles of 94ºC for 30 seconds, 60ºC for 45 seconds, 72ºC for 60 seconds; followed by a final extension at 72ºC for 10 minutes. PCR products were visualized on an agarose gel using RedSafe Nucleic Acid Staining Solution and sent to Macrogen Inc. (Geumcheon-gu, Seoul, Republic of Korea) for allele sizing. Alleles were binned manually as well as using the Excel Add-in Autobin (http://www4.bordeaux-aquitaine.inra.fr/biogeco/Media/Ressources/Logiciels/Autobin).

5.3.1 Preliminary analyses

As Bell et al. (2014) found evidence for a Xestospongia species complex around Hoga Island, an exploratory Principle Coordinate Analysis (PCA) was conducted in GENALEX v. 6.3 (Peakall and Smouse 2006), using pairwise matrices of Nei’s genetic distance (D; Nei 1972) to identify the patterns of differentiation in all sponges across all sampled populations. Based on the identified clusters in the PCA, coupled with support from Bell et al. (2014), five genetically distinct groups were separated for further separate analysis. GENALEX v. 6.3 was used to perform a hierarchal analysis of molecular variance (AMOVA, n = 10,000 permutations) to support this separation by
identifying the proportion of genetic variation between the five groups. Populations were grouped into five clusters for the subsequent analyses and classified as A-E.

STRUCTURE v. 2.3.2 (Pritchard et al. 2000) was utilized to perform Bayesian clustering analysis to further support the number of distinct species in the dataset. First, a Bayesian evaluation of genetic partitioning was performed ($K = 1-7$; Bell et al. 2014), followed by calculation of the $\Delta K$ statistic based on the probability of the log probability ($L(K)$). The runs were performed using a burn-in period of $10^4$ and $10^5$ Markov chain Monte Carlo (MCMC) iterations. The $\Delta K$ statistic was estimated using STRUCTURE HARVESTER (Earl and VonHoldt 2012), and clustering outcomes were visualized in DISTRUCT v. 1.1 (Rosenberg 2004).

5.3.2 Population genetic analyses

Genetic analyses were conducted for genetic groups A-E. The number of alleles per locus ($N_A$) and Weir and Cockerham’s (1984) inbreeding coefficients ($F_{IS}$) were calculated using FSTAT v. 2.9.3 (Goudet 1995). Deviation from Hardy-Weinberg equilibrium (HWE) across loci was evaluated in GENEPOP 4.1 (Rousset 2008). Linkage disequilibrium between all loci and population combinations was tested using Arlequin v. 3.5.1.2 (Excoffier et al. 2005) and adjusted using Bonferroni corrections (Rice 1989). Markov-chain parameters included 10,000 dememorization steps, 1,000 batches and 10,000 iterations per batch. Arlequin v. 3.5.1.2 was also used to estimate observed ($H_o$) and expected ($H_e$) heterogeneity.

5.3.3 Within group population differentiation

The proportion of genetic variation that could be attributed to among site differences within each genetic group (A-E) was examined using an AMOVA ($n = 10,000$ permutations) in GENALEX v. 6.3. Population differentiation ($F_{ST}$), assuming the Infinite Allele Model (IAM), was quantified across all loci in Arlequin v. 3.5.1.2. Sample sites were used as population units and significance values were based on 10,000 permutations. Due to the debate regarding the use of Stepwise Mutation Models (SMM) and $R_{st}$ indices for studies using microsatellite loci when the repeating region is compound or interrupted (van Herwerden et al. 2003; Meirmans and Hedrick 2011), $R_{st}$
was not used in this study. Among site differentiation for each group was supported by Bayesian clustering analysis in STRUCTURE v. 2.3.2.

5.3.4 Relatedness

Assignment tests for the recruits from each genetic group (A-E) was conducted in GENECLASS2 using a Bayesian approach (Berry et al. 2004; Piry et al. 2004). Adult sponges from each genetic group were used as reference populations. Recruit assignment was conducted using an exclusion threshold approach where individuals were excluded from their corresponding population when the probability of assignment to the reference population was less than 0.05 (Type I error; 50). Recruits were reassigned to a source population when the probability of assignment was greater than 10%. Individuals assigned to more than one population (P > 0.10) were excluded. Individuals that were not assigned to any population were considered to have originated from a non-sampled population. Group E was omitted from this analysis as it only contained recruits from one genetic population (Sampela 1/Buoy 1). No recruits were located at either Kaledupa Double Spur or Ridge 1 and were therefore not included in assignment testing.

Larval dispersal potential was examined using two different approaches: relatedness analysis and kinship analyses. The pairwise maximum-likelihood estimation of relatedness (MLE) for all sampled sponges were calculated using ML-Relate (Kalinowski et al. 2006). ML-Relate calculates the pairwise likelihood of each relationship and produces a matrix of relationships on a continuous scale with the highest likelihood per pair of sponges in the population. One-way ANOVAs were then conducted on the estimates of relatedness between and within sites as well as between genetic groups for each site pairing. Greater relatedness within sites than among sites would suggest the occurrence of non-random mating or limited dispersal (Giles et al. 2015).

Kinship analyses were completed for Sampela 1 and Buoy 1 only, as there was insufficient recruit information for Kaledupa Double Spur and Ridge 1. Unlike continuous maximum-likelihood estimation, kinship analyses determine the level of relatedness from a set of discrete possibilities (i.e. half- or full-siblings). COLONY v. 2.0.6.3 (Jones and Wang 2010) was then used to assign
known recruits within each group to half or full sibling groups using the remaining adult population as a reference group. COLONY uses a maximum-likelihood group-wise method to find the most likely configuration of full-sibs (individuals that share both parents) and half-sibs (individuals that only share one parent) over the entire population sampled, rather than for pairs of individuals. This group-wise method splits the data into three groups: a candidate father, mother, and the offspring. Individuals in the subsamples are then assigned to family clusters; those between clusters are considered unrelated. This algorithm generates many clusters which are evaluated and compared to new pedigrees until the best cluster is identified by likelihood scores (Wang and Santure 2009; Jones and Wang 2010; Wang 2013). Furthermore, COLONY also utilizes locus-specific error rates to infer relationships with higher confidence (Wang 2004). Geographical distances between individuals of each sibling pair were also calculated based on study site maps. Putative parental genotypes were provided as the full set of genotypes from the respective genetic cluster with the recruit genotypes removed. Predefined parameters were used with the exception of the mating system, which was set to polygamy as well as possible inbreeding. Kinship results were then cross-validated with ML-Relate following the scale for the index of relatedness (full-siblings: $r \geq 0.5$, half-sibs $\geq 0.25$).

5.4 Results

5.4.1 Preliminary genetic analyses

The Principle Coordinate Analysis (PCA) between all samples from across all four sites defined a priori as populations revealed a clustering of five groups generally inconsistent with population location (Figure 5.1). Kaledupa Double Spur and Ridge 1 were generally clustered along the same axis. AMOVA analyses demonstrated that 20% of variation could be attributed to among group differences (Table 5.1, $P < 0.001$).
Figure 5.1. Results of Principle Coordinate Analysis (PCA) using genetic distances between individuals and a genetic distance matrix in GENALEX 6.5. Circles were added manually based on the genetic groups identified by STRUCTURE; the five groups were further analysed for within group population differentiation (AMOVA).

Table 5.1. Hierarchal analysis of molecular variance (AMOVA) used to estimate levels of genetic differentiation between five genetic groups derived from PCA ($P < 0.001$).

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>Est. Var.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among Pops</td>
<td>4</td>
<td>339.420</td>
<td>84.855</td>
<td>0.758</td>
<td>20%</td>
</tr>
<tr>
<td>Among Indiv</td>
<td>270</td>
<td>1133.164</td>
<td>4.197</td>
<td>1.248</td>
<td>34%</td>
</tr>
<tr>
<td>Within Indiv</td>
<td>275</td>
<td>467.500</td>
<td>1.700</td>
<td>1.700</td>
<td>46%</td>
</tr>
<tr>
<td>Total</td>
<td>549</td>
<td>1940.084</td>
<td>3.707</td>
<td></td>
<td>100%</td>
</tr>
</tbody>
</table>

STRUCTURE analysis also revealed genetic differentiation among samples; maximum loglikelihood scores and Delta $K$ values indicated $K = 3$ to be the most likely number of populations (Figure 5.2). However, upon further inspection of the STRUCTURE data, five delineated groups were found, which consistently matched the genetic clusters identified in the PCA plot. The groups
were referred to as: A (corresponding with STRUCTURE group 1), B (corresponding to Group 2), C (corresponding to Group 3), D (corresponding to Group 4) and E (corresponding to Group 5; Figure 5.2). Group C was removed from the duration of analyses due to small sample size. *Xestospongia* individuals Groups A and E were present at Buoy 1 and Sampela 1 only, whereas Groups B and D were present at all four sites.

Figure 5.2. STRUCTURE-derived large-scale population genetic differentiation inferred via Bayesian clustering for all samples. Individual membership coefficients are divided into five sections representing the proportion of membership for different genetic clusters (Groups A-E). Prior population information was given and the best inferred clustering scheme was $K = 3$.

5.4.2 Population genetic analyses

The mean number of alleles per locus ranged from 3.22 (Group E, Sampela 1) to 7.56 (Group A, Buoy 1; Table 5.2). The inbreeding coefficient $F_{IS}$ was positive for all sites and all groups and ranged between 0.028 (Groups B and D, Ridge 1) and 0.467 (for both Groups B and D, Sampela 1; Table 5.2).
Table 5.2. Standard genetic diversity indices for five genetic groups at nine loci for Buoy 1 (B1), Sampela 1 (S1), Kaledupa Double Spur (KDS), and Ridge 1 (R1). \( N_A \) = number of alleles per locus, \( H_o \) = observed heterozygosity, \( H_e \) = expected heterogeneity, \( F_{IS} \) = inbreeding coefficient. \( F_{IS} \) values that were significant after sequential Bonferroni correction are denoted as bold, sample sizes for each site are in parentheses.

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
<th>Group D</th>
<th>Group E</th>
</tr>
</thead>
<tbody>
<tr>
<td>( N_A )</td>
<td>7.56 5.89</td>
<td>3.67 5.33 5.33 5.22</td>
<td>5.67 6.33 4.11 3.78</td>
<td>5.22 3.22</td>
</tr>
<tr>
<td>( H_o )</td>
<td>0.398 0.291</td>
<td>0.418 0.478 0.553 0.298</td>
<td>0.418 0.438 0.398 0.517</td>
<td>0.372 0.373</td>
</tr>
<tr>
<td>( H_e )</td>
<td>0.635 0.482</td>
<td>0.592 0.585 0.569 0.553</td>
<td>0.624 0.708 0.656 0.73</td>
<td>0.514 0.449</td>
</tr>
<tr>
<td>( F_{IS} )</td>
<td><strong>0.378</strong> <strong>0.400</strong></td>
<td>0.316 <strong>0.186</strong> 0.028 <strong>0.467</strong></td>
<td>0.316 <strong>0.116</strong> 0.028 <strong>0.357</strong></td>
<td><strong>0.279</strong> 0.178</td>
</tr>
</tbody>
</table>

Pairwise loci comparisons for Hardy Weinberg Equilibrium were made between loci at each site for each group (Table 5.3). Occurrences of significant deviations from HWE were present at each site for each group with the exception of Ridge 1 (Group D) and Sampela 1 (Group E; Table 5.3). Loci 2 and 3 were monomorphic at Buoy 1 (Table 5.3).
Table 5.3. Pairwise loci comparisons for Hardy Weinberg Equilibrium (HWE) between at each site for genetic Groups A-E. Values in bold indicate significant deviance from HWE ($P < 0.05$).

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
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<th>Group B</th>
<th></th>
<th>Group D</th>
<th></th>
<th>Group E</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Locus 1</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001 0.005 &lt; 0.001 &lt; 0.001 &lt; 0.001</td>
<td>&lt; 0.001 0.241 0.125 &lt; 0.001</td>
<td>0.001 0.987</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Locus 2</td>
<td>n/a 0.915</td>
<td>0.046 0.851 0.602 0.705</td>
<td>&lt; 0.001 0.174 0.261 0.172</td>
<td>&lt; 0.001 0.029</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Locus 3</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001 n/a 0.045 0.007 &lt; 0.001</td>
<td>&lt; 0.001 0.055 0.375 0.006</td>
<td>&lt; 0.001 0.240</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Locus 4</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001 &lt; 0.001 0.470 0.004 0.002</td>
<td>0.019 0.113 0.396 0.307</td>
<td>&lt; 0.001 0.511</td>
<td></td>
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</tr>
<tr>
<td>Locus 5</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001 0.072 0.482 0.020 &lt; 0.001</td>
<td>0.004 0.019 0.484 &lt; 0.001</td>
<td>0.998 0.875</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Locus 7</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001 0.860 &lt; 0.001 0.013 &lt; 0.001</td>
<td>&lt; 0.001 0.000 0.217 &lt; 0.001</td>
<td>&lt; 0.001 0.164</td>
<td></td>
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</tr>
<tr>
<td>Locus 8</td>
<td>&lt; 0.001</td>
<td>0.894 &lt; 0.001 &lt; 0.001 &lt; 0.001 0.073</td>
<td>&lt; 0.001 0.006 0.440 0.001</td>
<td>0.653 0.875</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Locus 11</td>
<td>&lt; 0.001</td>
<td>0.035 0.613 &lt; 0.001 0.006 &lt; 0.001</td>
<td>0.002 0.005 0.180 0.211</td>
<td>&lt; 0.001 0.880</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Locus 12</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001 0.029 &lt; 0.001 &lt; 0.001 &lt; 0.001</td>
<td>0.028 0.003 0.339 &lt; 0.001</td>
<td>&lt; 0.001 0.004</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Genotypic disequilibrium was examined for each locus pair for the nine microsatellite loci for each population and across loci for each genetic group. Significant values ($P < 0.05$) indicating disequilibrium were detected for every population in every group; however, upon Bonferroni correction linkage was no longer significant in any group.

5.4.3 Within group population differentiation

AMOVA for each group suggested that only a small proportion of genetic variability can be explained by differences in populations for Groups A and E (2%), whereas Groups B and D had a higher proportion of genetic variation explained by between site differences (11 and 10%, respectively; Table 5.4).

Table 5.4. Hierarchical analysis of molecular variance (AMOVA) used to estimate levels of genetic differentiation between each group individually (Group A, $P = 0.001$; Group B, $P = 0.001$; Group D, $P = 0.001$; Group E, $P = 0.08$).

<table>
<thead>
<tr>
<th>Group</th>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>Est. Var.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Among Pops</td>
<td>1</td>
<td>7.926</td>
<td>7.926</td>
<td>0.064</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Among Indiv</td>
<td>64</td>
<td>236.149</td>
<td>3.690</td>
<td>1.087</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>Within Indiv</td>
<td>66</td>
<td>100.000</td>
<td>1.515</td>
<td>1.515</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>131</td>
<td>344.076</td>
<td>2.667</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Among Pops</td>
<td>3</td>
<td>48.869</td>
<td>16.290</td>
<td>0.334</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Among Indiv</td>
<td>78</td>
<td>268.899</td>
<td>3.447</td>
<td>0.794</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>Within Indiv</td>
<td>82</td>
<td>152.500</td>
<td>1.860</td>
<td>1.860</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>163</td>
<td>470.268</td>
<td>2.987</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Among Pops</td>
<td>3</td>
<td>41.290</td>
<td>13.763</td>
<td>0.354</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Among Indiv</td>
<td>58</td>
<td>243.250</td>
<td>4.194</td>
<td>1.149</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>Within Indiv</td>
<td>62</td>
<td>117.500</td>
<td>1.895</td>
<td>1.895</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>123</td>
<td>402.040</td>
<td>3.399</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>Among Pops</td>
<td>1</td>
<td>4.350</td>
<td>4.350</td>
<td>0.038</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Among Indiv</td>
<td>40</td>
<td>124.817</td>
<td>3.120</td>
<td>0.763</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Within Indiv</td>
<td>42</td>
<td>67.000</td>
<td>1.595</td>
<td>1.595</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>83</td>
<td>196.167</td>
<td>2.396</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Pairwise $F_{ST}$ values supported the AMOVA results; significant values were detected between Kaledupa Double Spur/Ridge 1 and Buoy 1/Sampela 1 populations for Groups B and D, but not between these site pairs (Table 5.5). No significant $F_{ST}$ values were found for Groups A or E (Table 5.5).
Table 5.5. Pairwise fixation index values (F_{ST}) for Groups B (below diagonal), D (above diagonal), and A (below diagonal), E (above diagonal). Bold values indicate significance based on 10,000 permutations (P = 0.001).

<table>
<thead>
<tr>
<th>Group</th>
<th>Site</th>
<th>B1</th>
<th>KDS</th>
<th>R1</th>
<th>S1</th>
</tr>
</thead>
<tbody>
<tr>
<td>B, D</td>
<td>B1</td>
<td>0.193</td>
<td>0.138</td>
<td>0.030</td>
<td></td>
</tr>
<tr>
<td></td>
<td>KDS</td>
<td>0.120</td>
<td>0.006</td>
<td>0.228</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R1</td>
<td>0.108</td>
<td>0.017</td>
<td>0.185</td>
<td></td>
</tr>
<tr>
<td></td>
<td>S1</td>
<td>0.024</td>
<td>0.164</td>
<td>0.164</td>
<td></td>
</tr>
<tr>
<td>A, E</td>
<td>B1</td>
<td>0.000</td>
<td>0.024</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S1</td>
<td></td>
<td>0.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B1</td>
<td>0.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S1</td>
<td>0.023</td>
<td>0.000</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

STRUCTURE plots for each group further supported differences between populations; the best inferred clustering scheme for Group A was K = 3 measured using Evanno’s delta K determined from the mean estimated Ln(K). Similarly, the best clustering scheme for Groups B and D was K = 2 (Figures 5.3, 5.4). The STRUCTURE plot for Group E, however, suggested high gene flow between sites (Figure 5.3, 5.4). These results support the separation of clusters into two populations for Groups B and D: Sampela 1 and Buoy 1, and Kaledupa Double Spur and Ridge 1. Groups A and E were treated as one population including both Sampela 1 and Buoy 1.

Figure 5.3. STRUCTURE-derived large-scale population genetic differentiation inferred via Bayesian clustering for all groups.
Figure 5.4. Plots generated in STRUCTURE Harvester demonstrating the mean log likelihood of the data \([L(K)]\) and Evanno’s delta K statistic for groups A through E. Group C was omitted due to small sample sizes.
5.4.4 Fine-scale relatedness

The number of new recruits found varied between years and sites (see Chapter 3), although no recruits were ever found at Kaledupa Double Spur or Ridge 1. Assignment tests of *Xestospongia* recruits revealed high levels of self-recruitment in Groups B and D (Table 5.6), with no recruits assigned to the Kaledupa Double Spur/Ridge 1 genetic group. Unassigned recruits, or those attributed to non-sampled populations, accounted for 14.3% of Group B and 5.8% of Group D. Group A was omitted from the analysis as it only contained one genetic population, and Group C was omitted due to small sample size.

Table 5.6. Assignment tests conducted in GeneClass v. 2.0 for *Xestospongia* recruits for genetic Groups B and D.

<table>
<thead>
<tr>
<th>Group</th>
<th>Site</th>
<th>n</th>
<th>% of Population</th>
<th>Assigned Population</th>
<th>Not Assigned</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>B1/S1</td>
<td>KDS/R1</td>
</tr>
<tr>
<td>B</td>
<td>B1/S1</td>
<td>7</td>
<td>86%</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>KDS/R1</td>
<td>0</td>
<td>0%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D</td>
<td>B1/S1</td>
<td>17</td>
<td>94%</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>KDS/R1</td>
<td>0</td>
<td>0%</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

It was expected that the maximum-likelihood estimation of relatedness (MLE) would be higher within sites than among sites based on previous findings that found high levels of self-recruitment in *Xestospongia* spp. (see Bell et al. 2014). In the event of self-recruitment, relatedness is expected to be higher within sites than between sites due to limited larval dispersal which prevents gene flow between populations. A comparison of mean MLE for the site pairings for each group (within Buoy 1, within Sampela 1, and between Buoy 1 and Sampela 1; within Kaledupa Double Spur, within Ridge 1, and between Kaledupa Double Spur and Ridge 1) revealed that relatedness was group and site dependent (Figure 5.5; Table 5.7).

For Groups A, B, and E, the mean MLE within Sampela 1 was higher than at Buoy 1 or between sites (Buoy 1 and Sampela 1), indicating that the individuals at Sampela 1 were more related to the individuals nearby than those farther away (Figure 5.5A, Table 5.7). Buoy 1 relatedness, however, was equivalent to that between sites, suggesting no influence of distance on degree of
relatedness (Figure 5.5). For the Kaledupa Double Spur and Ridge 1 complex, genetic group influenced the mean MLE. Group B did not demonstrate any differences between Kaledupa Double Spur, Ridge 1, or a combination of the two, suggesting that relatedness is the same whether nearby or far (one-way ANOVA, $F_{2,1553} = 1.054, P = 0.349$). In Group D, however, the mean MLE at both Kaledupa Double Spur and Ridge 1 was higher than between sites (Figure 5.5C; Table 5.7).

![Venn diagrams showing relatedness between sites for different genetic groups](image)

Figure 5.5. Mean maximum-likelihood estimation of relatedness (MLE) values between individual sponges within and between (overlapping portion) sites for each genetic group (A-D). Group C was omitted from analyses due to low sample size. Values in bold indicate a significant influence of site pairing on MLE (Bonferroni post-hoc tests, $P < 0.05$).
Table 5.7. One-way ANOVA results examining pairwise maximum-likelihood estimation of relatedness values within each site and between sites for each genetic group. Bonferroni *post-hoc* tests were used to examine the significant main effects between site pairings. Asterisks (*) denote significant effects ($P < 0.05$).

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P</th>
<th>Source</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tests of Within-Subjects Effects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>2</td>
<td>1.835</td>
<td>78.742</td>
<td>&lt; 0.001*</td>
<td>Time</td>
<td>2</td>
<td>0.299</td>
<td>3.97</td>
<td>0.020*</td>
</tr>
<tr>
<td>Error</td>
<td>4288</td>
<td></td>
<td></td>
<td></td>
<td>Error</td>
<td>195</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bonferroni <em>post-hoc</em> tests</td>
<td>Difference</td>
<td>$P$</td>
<td></td>
<td></td>
<td>Bonferroni <em>post-hoc</em> tests</td>
<td>Difference</td>
<td>$P$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B1-B1 - S1-S1</td>
<td>-0.0717</td>
<td>&lt; 0.001*</td>
<td></td>
<td></td>
<td>KDS-KDS - R1-R1</td>
<td>-0.0432</td>
<td>0.492</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B1-B1 - S1-B1</td>
<td>-0.0099</td>
<td>0.288</td>
<td></td>
<td></td>
<td>KDS-KDS - R1-KDS</td>
<td>0.0973</td>
<td>0.022*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S1-S1 - S1-B1</td>
<td>-0.0617</td>
<td>&lt; 0.001*</td>
<td></td>
<td></td>
<td>R1-R1 - R1-KDS</td>
<td>-0.1405</td>
<td>0.025*</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Tests of Within-Subjects Effects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Time</td>
<td>2</td>
<td>0.04</td>
<td>1.054</td>
<td>0.349</td>
<td>Time</td>
<td>2</td>
<td>0.101</td>
<td>3.943</td>
<td>0.020*</td>
</tr>
<tr>
<td>Error</td>
<td>1553</td>
<td></td>
<td></td>
<td></td>
<td>Error</td>
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<tr>
<td>Bonferroni <em>post-hoc</em> tests</td>
<td>Difference</td>
<td>$P$</td>
<td></td>
<td></td>
<td>Bonferroni <em>post-hoc</em> tests</td>
<td>Difference</td>
<td>$P$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KDS-KDS - R1-R1</td>
<td>-0.017</td>
<td>0.244</td>
<td></td>
<td></td>
<td>B1-B1 - S1-S1</td>
<td>-0.0089</td>
<td>0.403</td>
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</tr>
<tr>
<td>KDS-KDS - R1-KDS</td>
<td>0.0026</td>
<td>0.817</td>
<td></td>
<td></td>
<td>B1-B1 - S1-B1</td>
<td>0.0115</td>
<td>0.267</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R1-R1 - R1-KDS</td>
<td>-0.0196</td>
<td>0.152</td>
<td></td>
<td></td>
<td>S1-S1 - S1-B1</td>
<td>-0.0204</td>
<td>0.105</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Tests of Within-Subjects Effects</strong></td>
<td></td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>Time</td>
<td>2</td>
<td>0.687</td>
<td>16.621</td>
<td>&lt; 0.001*</td>
<td>Time</td>
<td>2</td>
<td>0.791</td>
<td>17.921</td>
<td>&lt; 0.001*</td>
</tr>
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<td>Error</td>
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<td>Difference</td>
<td>$P$</td>
<td></td>
<td></td>
<td>Bonferroni <em>post-hoc</em> tests</td>
<td>Difference</td>
<td>$P$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B1-B1 - S1-S1</td>
<td>0.0285</td>
<td>0.223</td>
<td></td>
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<td>B1-B1 - S1-S1</td>
<td>-0.093</td>
<td>&lt; 0.001*</td>
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<td>B1-B1 - S1-B1</td>
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<td>0</td>
<td></td>
<td></td>
<td>B1-B1 - S1-B1</td>
<td>0.029</td>
<td>0.006</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S1-S1 - S1-B1</td>
<td>-0.0557</td>
<td>&lt; 0.001*</td>
<td></td>
<td></td>
<td>S1-S1 - S1-B1</td>
<td>-0.1223</td>
<td>&lt; 0.001*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
A comparison of the mean maximum-likelihood estimation of relatedness (MLE) values across genetic groups for each site pairing (within Buoy 1, within Sampela 1, and between Buoy 1 and Sampela 1; within Kaledupa Double Spur, within Ridge 1, and between Kaledupa Double Spur and Ridge 1) revealed significant differences between genetic groups among site pairings (Figure 5.6, Table 5.8). Within Buoy 1, Group A relatedness was only similar to Group D (one-way ANOVA $F_{3,2238} = 7.41, P < 0.001$; Bonferroni post-hoc, $P = 0.872$), while within Sampela 1 Group E had significantly higher relatedness than the remaining groups (Figure 5.6). Between Sampela 1 and Buoy 1, however, there was no influence of genetic group on mean relatedness (Figure 5.6, Table 5.8). Conversely, the mean MLE within and between sites for Kaledupa Double Spur and Ridge 1 differed significantly (one-way ANOVA $F_{1,277} = 34.061, P < 0.001$, one-way ANOVA $F_{1,291} = 14.886, P < 0.001$, one-way ANOVA $F_{1,865} = 35.789, P < 0.001$, respectively, Table 5.8), although no post-hoc tests were possible due to only two groups existing at these sites.

![Figure 5.6](image)

Figure 5.6. A comparison of the maximum-likelihood estimation of relatedness (MLE) values ± SE across genetic groups for each site pairing: A) Buoy 1 to Buoy 1 (B1-B1), Sampela 1 to Sampela 1 (S1-S1), and Buoy 1 to Sampela 1 (B1-S1), and B) Kaledupa Double Spur to Kaledupa Double Spur (KDS-KDS), Ridge 1 to Ridge 1 (R1-R1), and Kaledupa Double Spur to Ridge 1 (KDS-R1). Letters and asterisks represent differences in mean values ($P < 0.05$).
Table 5.8. One-way ANOVA results examining pairwise maximum-likelihood estimation of relatedness (MLE) values between genetic groups within and between sites. Bonferroni *post-hoc* tests were used to examine the significant main effects between groups for Buoy 1 and Sampela 1 pairings only. Asterisks (*) denote significant effects ($P < 0.05$).

<table>
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<th>Source</th>
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<th>F</th>
<th>P</th>
<th>Source</th>
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<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
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<td></td>
<td></td>
<td></td>
<td><strong>Tests of Within-Subjects Effects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>B1-B1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><strong>S1-B1</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Time</td>
<td>3</td>
<td>0.179</td>
<td>7.41</td>
<td>&lt; 0.001*</td>
<td>Time</td>
<td>3</td>
<td>0.719</td>
<td>27.564</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Error</td>
<td>2238</td>
<td></td>
<td></td>
<td></td>
<td>Error</td>
<td>3543</td>
<td></td>
<td></td>
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<tr>
<td>Bonferroni <em>post-hoc</em> tests</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Bonferroni <em>post-hoc</em> tests</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group A - Group B</td>
<td>0.003</td>
<td>0.005*</td>
<td></td>
<td></td>
<td>Group A - Group B</td>
<td>0.056</td>
<td>0.122</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group A - Group D</td>
<td>-0.029</td>
<td>0.872</td>
<td></td>
<td></td>
<td>Group A - Group D</td>
<td>0.054</td>
<td>0.101</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group A - Group E</td>
<td>-0.031</td>
<td>&lt; 0.001*</td>
<td></td>
<td></td>
<td>Group A - Group E</td>
<td>0.043</td>
<td>0.053</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group B - Group D</td>
<td>-0.032</td>
<td>0.039*</td>
<td></td>
<td></td>
<td>Group B - Group D</td>
<td>-0.0041</td>
<td>0.860</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group B - Group E</td>
<td>-0.035</td>
<td>0.116</td>
<td></td>
<td></td>
<td>Group B - Group E</td>
<td>-0.0127</td>
<td>0.162</td>
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<td></td>
</tr>
<tr>
<td>Group D - Group E</td>
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<td>0.806</td>
<td></td>
<td></td>
<td>Group D - Group E</td>
<td>-0.011</td>
<td>0.156</td>
<td></td>
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</tr>
<tr>
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<td></td>
<td></td>
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<td><strong>KDS-KDS</strong></td>
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<tr>
<td>Group A - Group B</td>
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<td>Group A - Group D</td>
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<td>Group A - Group E</td>
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<tr>
<td>Group B - Group D</td>
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<td>Group B - Group D</td>
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<tr>
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<td>Group D - Group E</td>
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<tr>
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<td>35.789</td>
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<tr>
<td>Error</td>
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<td></td>
<td>Error</td>
<td>865</td>
<td></td>
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</tbody>
</table>
Kinship analyses in COLONY revealed full sibship occurrence for juvenile pairs of sufficient confidence for Group A only (16% of individual relationships identified; Table 5.9). The remaining groups lacked sufficient confidence of sibship accuracy and were not included ($P > 0.75$). Of these full sibling pairs, dispersal distance ranged from 60 to 131.1 m (Table 5.9). All of the individual pairs classified by COLONY as full-siblings belonged to the same kingroup as identified by ML-Relate.

Table 5.9. Putative full sibship dyad reconstruction analysis in COLONY showing the occurrence of full sibling pairs among *Xestospongia* recruits for Group A ($P > 0.75$); some of the pairings are for one individual with multiple siblings. Distance (m) represents the distance between the matched pairs.

<table>
<thead>
<tr>
<th>Group A (n = 25)</th>
<th>Sibling 1</th>
<th>P</th>
<th>Distance (m)</th>
<th>Sibling 2</th>
<th>Site</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Site</td>
<td></td>
<td></td>
<td>Site</td>
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</tr>
<tr>
<td>B1</td>
<td>0.999</td>
<td>131.1</td>
<td>B1</td>
<td>0.919</td>
<td>69.5</td>
</tr>
<tr>
<td>B1</td>
<td>0.828</td>
<td>86.3</td>
<td>B1</td>
<td>0.77</td>
<td>60.0</td>
</tr>
</tbody>
</table>

5.5 Discussion

In order to provide adequate protection to an ecosystem, managers need to understand both the levels of self-recruitment to the natal habitat and connectivity with other populations (Planes et al. 2009). Indo-Pacific *Xestospongia* spp. are recognized as being ecologically important contributors to coral reef functioning, yet information on larval dispersal patterns is limited. Within the Wakatobi National Marine Park (WNMP) there appears to be a species complex of reproductively isolated *Xestospongia* species. Fisher’s exact test ($F_{ST}$), STRUCTURE Bayesian clustering, and AMOVA results all supported the presence of multiple genetic clusters, suggesting that *Xestospongia* spp. individuals could be partitioned into as many as five distinct species in an area approximately 2 km$^2$. These results also suggest *Xestospongia* spp. have limited larval dispersal potential and are generally dependent on self-recruitment. Assignment tests, kinship analyses, and estimates of relatedness all support recruits staying within their population and, in many cases, the preferential retention of larvae at their natal site. Dispersal distances varied between species and in some cases may be restricted by site characteristics. This study also highlights the importance of examining small scale relatedness
in self-recruiting organisms, as the occurrence of larval retention to natal sites may be overlooked with connectivity estimates alone.

5.5.1 Cryptic speciation in Xestospongia spp.

The presence of a species complex in a small area (2 km$^2$) supports the findings of Bell et al. (2014) who examined population structure on a larger scale in the WNMP. In the Indo-Pacific, barrel sponges were previously believed to include X. testudinaria, X. exigua, and X. berguista, but recent work has revealed many more potential species. Swierts et al. (2013) reported the presence of a Xestospongia species complex in the Sulawesi, Indonesia using nuclear (ATP synthase β intron) and mitochondrial (CO1 and ATP6 genes) markers. More recently, Setiawan et al. (2016) reported a species complex in the Indo-Pacific comparable to Sweirts et al. (2013) on a much wider scale (6,000 km). Cryptic speciation is common among cosmopolitan sponge species, where sponges that look identical in the field may be genetically distinct (Klautau et al. 1999; Diaz and Rutzler 2001; Miller et al. 2001; Blanquer and Uriz 2007; Blanquer et al. 2008). Increasingly, advanced molecular tools have identified numerous examples of unexpected species complexes in what was previously thought to be one species (e.g. Wörheide et al. 2002; Zilberberg et al. 2006; Xavier et al. 2010; Swierts et al. 2013). An explanation for this could be the strong population structuring and restricted gene flow that appears to be common in sponge populations (Wörheide et al. 2002; Duran et al. 2004; Chaves-Fonnegra et al. 2007; Bell et al. 2014; Pérez-Portela et al. 2015), possibly as a result of short larval dispersal distances (Ilan and Loya 1990; Meroz-Fine et al. 2005; Maldonado 2006).

Swierts et al. (2013) further reported that the four genetically differentiated groups had unique morphotypes that may be related to habitat quality. The potential for morphological plasticity in sponges is considerable owing to the lack of basal laminae that prevents organization beyond the cellular level (Degnan et al. 2005). Macro-morphological adaptation in response to environmental conditions is common in sponges (e.g. Bell et al. 2002; McDonald et al. 2002; Meroz-Fine et al. 2005) due to considerable plasticity potential. Nearly twice as many morphological groups were identified in this study than by Sweirts et al. (2013; personal obs.), who examined Xestospongia populations in the nearby Sulawesi. It is possible that environmental drivers may explain the increased number of morphological groups observed herein.
I was unable to identify the species present in this study, but there is a possibility that two of the genetic groups identified in this study may be hybrids (which would be supported by the Bayesian approach; STRUCTURE: K = 3, genetic Group C and E). Moving forward, speciation of the five putative species identified as part of a complex on the reefs surrounding Hoga Island would continue to add to the body of work on Xestospongia taxonomy. It is likely that there are undescribed species as part of this complex (Bell et al. 2014), and the expansion of the nuclear and mitochondrial markers used by Swierts et al. (2013) would allow for further clarification. Expanding the number of sampling sites to the wider WNMP and further afield into other locations in Indonesia may also shed light onto the composition of species complexes in this region.

5.5.2 Xestospongia spp. relatedness

A combination of assignment tests, kinship analyses, and comparisons of mean maximum-likelihood estimation of relatedness (MLE) all support that self-recruitment is any important feature of Xestospongia spp. populations around Hoga Island, but only in some of the species groups. Sponges within sites were expected to be more related than those between sites based on the findings of Bell et al. (2014), who provided evidence for high levels of self-recruitment and inbreeding in the WNMP. Comparisons of mean MLE suggests that mean relatedness, and therefore larval dispersal, may differ at fine spatial scales.

Mean MLE was compared within Buoy 1, within Sampela 1, and between Buoy 1 and Sampela 1. For species A and E, relatedness of recruits within Sampela 1 was higher than between sites. This was also the case at Buoy 1 for species B. Higher mean relatedness within sites suggests a deviation from random mating, likely due to the occurrence of self-recruitment, as larvae are more likely to stay at the natal site than disperse to other available locations. The successful settlement of recruits at their natal habitat would likely result in an assemblage comprised of related sponges. This then increases the potential for inbreeding which was identified in some cases in this study. Why larvae appear to be retained at Sampela 1 but not at Buoy 1 in these species may be due to the physical features of the sites themselves. Sampela 1 is a shallow reef located in a lagoon, which may act as a physical barrier in preventing dispersal and retaining recruits, while Buoy 1 is characterized by a wall and is adjacent to a channel that may assist larval transport. Currents and physical barriers can be causative agents of self-recruitment.
(Guardiola et al. 2012; Giles et al. 2015), and the patchy nature of some coral reef environments can influence limit the dispersal capability of pelagic larvae (Pinsky et al. 2012).

Buoy 1 and Sampela 1 were not identified as genetically differentiated despite the high levels of self-recruitment for some species. It is possible that populations at each site are maintained by local recruitment with occasional exchanges of recruits between sites. Only a few migrants per generation are required to maintain genetic homogeneity among populations (Slarkin 1985), which is small compared to the possible number of recruits produced by a population in recruitment events (Swearer et al. 2002). Furthermore, in a population dominated by local recruits, the influence of few externally sourced recruits has a minimal influence on long term population growth or size (Strathmann et al. 2002).

There is a paucity of information about kinship in sponges, but the few studies examining the important of relatedness in structuring populations reiterate the importance of scale. For example, Calderon et al. (2007) examined *Crambe crambe* relatedness from 0 to 7 m, and Blanquer et al. (2009) explored *Scopalina lophyropoda* kinship among and within three wall habitats within 25 m of each other. Both authors reported limited larval dispersal, which might be expected as both are encrusting species that rely on fusion and fission. *C. crambe*, however, was found to be the most related to other sponges within 100 cm (in the absence of asexual reproduction), despite their capability to disperse several meters from their population of origin (Calderon et al. 2007). Giles et al. (2015) examined the reef sponges *Stylissa carteri* at 36 sites throughout the Red Sea, as well as on finer scales ranging from 50-200 m. The authors reported that there was no evidence of non-random mating at the 50 m scale, yet at 100-200 m there was a deviation from random mating. My study supports these earlier studies, while larval dispersal potential is at least partially dependent on species, the scale at which relatedness is examined is important. On a broad scale the influence of environmental heterogeneity (Giles et al. 2015) or oceanographic processes (Richards et al. 2016) may structure populations, while on a small scale individual dynamics are more important.

5.5.3 Larval dispersal ability

Differences in mean MLE between species, as well as among sites, suggest that site characteristics alone may not explain barriers to relatedness. For the Buoy 1/Sampela 1
complex, species D had equivalent relatedness both within and between sites; for Kaledupa Double Spur and Ridge 1 this was the case with species B alone. Conversely, the site characteristics of Ridge 1 would be expected to encourage larval dispersal, yet mean MLE was equivalent across all site pairings. The seemingly contradictory relationship between mean relatedness for some species at some sites as compared to others might suggest a combination of dispersal ability and site characteristics, or realized dispersal. Realized dispersal is a combination of biological ability to disperse (which may be species dependent) and the ability to cross barriers to isolated habitats (Jones et al. 2007). Biological factors such as the capacity to settle, or competency time, may explain variation in larval settlement dynamics among cohorts (Richmond 1988), and failure to account for such factors may result in underestimating self-recruitment rates (Connolly and Baird 2010). It is also possible that the recruitment rate between sites is higher for some species but larval mortality between sampling events prevented the inclusion of those data. For example, assignment testing and sibship analyses were impossible owing to the lack of identified recruits for Ridge 1 or Kaledupa Double Spur. It is therefore only possible to infer self-recruitment based on mean MLE. Alternatively, the absence of recruits may indicate high mortality, perhaps due to increased spatial competition with other benthic organisms at these sites (see Chapter 2 for biotic site characteristics).

The two main Xestospongia groups examined Bell et al. (2014), Xestospongia spp. and X. testudinaria, showed different patterns of genetic structuring, which the authors attributed to differing dispersal potential; due to the overlap of some sites with this study it is likely that these species were included in this study. Bell et al. (2014) also suggest that these differences may be attributed to different processes affecting gene flow between populations. This is supported further by sibship analyses; Group A was the only genetic group where full-sibship was detected. The lack of half- or full-sibling relationships of high confidence for any other genetic group at any site likely reflects an influx of larval recruits from external sources, which may maintain gene flow and prevent further differentiation between locations. Extending sampling to a wider range of sites in the Wakatobi region and comparing relatedness, as well as mean MLE comparisons within and between sites, would allow for a more thorough understanding of these processes. Further, although mean MLE values exhibit lower standard errors than other metrics, it is important to note that this method may include bias if sample sizes are small (although in many cases bias still remains minimal; Milligan 2003).
Inbreeding coefficients were highly positive across the majority of species and sites, a trait also common in sponges (Duran et al. 2004; Chaves-Fonnegra et al. 2007; Blanquer et al. 2009; Bell et al. 2014). Previous work has attributed highly positive, multilocus $F_{IS}$ values to the Wahlund effect caused by the existence of multiple breeding units as well as the presence of null alleles. However, Bell et al. (2013) tested for and discarded loci that demonstrated null alleles and selection, rendering it unlikely that null alleles are responsible for my results. High levels of inbreeding are also in line with limited dispersal distances and self-recruitment demonstrated in this study. The two lowest $F_{IS}$ values were both for Ridge 1 (species B and D); this may be attributed to physical site characteristics; although fine scale oceanographic information is lacking for this region, Ridge 1 is features high currents (Powell et al. 2014), which in conjunction with a lack of physical barriers surrounding the site could increase dispersal potential as flow rates could assist larval dispersal away from Ridge 1. Reduced self-recruitment and influx of larvae from external sites may be expected to reducing the occurrence of inbreeding.

The high level of speciation in a small area, coupled with limited demographic connectivity and potential reliance on self-recruitment renders them vulnerable to disturbance. Dependence on their own recruitment at a small scale may have consequences for vulnerability and viability of local populations. To date this is the first study to examine sponge relatedness on Indo-Pacific coral reefs. The recognized importance of *Xestospongia* on coral reef functioning, coupled with the high levels of self-recruitment reported here, reinforce the necessity for barrel sponge conservation in the WNMP. Although there is evidence that Hoga Island *Xestospongia* populations have already displayed local adaptation resulting in a species complex, further habitat degradation may exceed their adaptive ability.
Xestospongia spp. at Ridge 1
(photo credit: Joe Marlow)
6.1 Summary of key findings

This PhD thesis examines the demographic structure and connectivity of Xestospongia spp. in four core sites of varying habitat quality within the Wakatobi National Marine Park using a combination of observational, modelling, experimental, and molecular methods. The primary aims of this thesis were to gain insight on individual and population level dynamics, as well as the connectivity patterns for these ecologically important sponges. In addition, I sought to quantify the physiological response of Xestospongia spp. to exposure to suspended and settled sediment that allows them to persist in a degraded habitat. In summary the main findings were: I) In the sites examined for this thesis Xestospongia spp. reach comparable size to Caribbean barrel sponges nearly five times faster, and may be less reliant on their photosynthetic symbionts or are capable of switching between these trophic modes to maintain growth; II) barrel sponge populations at two sites are increasing in size and are resilient to poor recruitment, but remain vulnerable to the mortality of large sponges; III) respiration rates increased when sponges were exposed to environmentally relevant suspended sedimentation concentrations, and the production of mucus is utilized as a slow but effective sediment clearance mechanism; and IV) the presence of a species complex is likely a product of limited connectivity due at least in part to reduced larval dispersal and high levels of self-recruitment.

Here I discuss my findings with respect to the uncertain future of coral reefs and implications for conservation and management.

6.2 Potential mechanisms of persistence of Xestospongia spp. in degraded habitats

An organism’s vulnerability to a changing environment is multi-faceted. Williams et al. (2008) proposed that several components dictate this vulnerability: the exposure of a species to environmental change and its sensitivity to it, its resilience, and its ability to adapt to said change. The inability of benthic invertebrates to escape changes in environmental quality results in the necessity to adapt or acclimate (Hoegh-Guldberg 1999). Xestospongia spp. in the WNMP appear to be fairly ubiquitous across a spectrum of habitat quality and have the highest density at Sampela 1 (of the four core sites). The reduction in coral cover at Sampela 1 is largely thought to have occurred in the last 10-20 years, with coral cover declining from 30% to < 8% cover from 2000 to 2007 (McMellor and Smith 2010). The oldest sponges at Sampela 1 are in excess of 35 years of age, indicating that they would have been present during substantial changes to environmental quality and biotic composition. Reduced genetic exchange between
populations via limited larval dispersal may enable localized adaptation in a population, potentially allowing the persistence of dense *Xestospongia* spp. populations in poor quality habitats in the WNMP.

Acclimation is an active organismal response whereby physiological processes are altered in order to better perform to new conditions (Hoegh-Guldberg et al. 2007). The production of mucus as a sediment clearing mechanism is one such an example of a potential acclamatory response. Adaptation, however, refers to elevated fitness levels of individuals in their native environment compared to genotypes from external sources (Kawecki and Ebert 2004). Through the maintenance of genetic variation among populations within a species, local adaption enables morphological or physiological speciation via ecological specialization (Sobel et al. 2010). In Chapter 5 I describe a potential species complex of highly differentiated species, a characteristic that may increase the potential for local adaptation (Leimu and Fischer 2008; Hereford 2009; Sanford and Kelly 2011).

A growing body of literature addressing adaption capacity in response to climate change has revealed that, not surprisingly, the capacity for adaptation is species dependent (Harley et al. 2006; Gibson et al. 2011), and influenced by generational turnover (Berteaux et al. 2004). As such, species that have long generation times (i.e. longer lived) are expected to have a limited ability to adapt. Furthermore, the capacity of adaptation is not infinite; the rate and extent of habitat degradation may exceed the mechanisms allowing a species to persist in the face of degrading habitats (Chevin et al. 2010). Indo-Pacific *Xestospongia* spp. may live to be upwards of 50 years of age, while other coral reef sponges, such as the already abundant *Lamellodysidea herbacea*, may have much faster generation times and therefore an accelerated adaptation potential. In these instances it is possible that *Xestospongia* spp. may lose the ‘adaptation race’ in changing environments.

6.2.1 Mucus as a sediment tolerance mechanism

In Chapter 4, I found that sponges at Sampela 1 produced mucus to aid in the removal of sediment from the sponge surface. These earlier experimental results, combined with relatively fast growth rate estimates (as compared to the Caribbean *X. muta*), suggest barrel sponges maybe more resilient to some environmental impacts, particularly increased sedimentation and turbidity, than might be expected. However, increased respiration rates when exposed to
suspended sediment treatments indicate that a metabolic trade-off is occurring as more energy is needed to rid the sponges of the sediment (Chapter 4). The encrusting Lamellodysidea herbacea has also been demonstrated to produce mucus as a sediment clearing mechanism with a subsequent respiration rate response (Biggerstaff et al. 2017), indicating that it may be a commonly used mechanism for sponges.

6.2.2 Size and shape

Possessing a larger body size in a habitat with high levels of sedimentation and turbidity may have several benefits. For instance, a large sponge would be expected to withstand partial smothering by sediment or rubble, and may be able to tolerate some clogging of the internal aquiferous structure. An increased surface area creates space in which to house phototrophic symbionts and therefore maximize productivity in a turbid environment. Although not captured in the data collection for this study, intermittent growth rates may allow for increased growth in times of favourable conditions. This has been reported for other long lived sponges (Teixidó et al. 2006, 2009), as well as for coral (Garrabou and Zabala 2001) and octocoral (Linares et al. 2007).

Sponges possess considerable potential for morphological plasticity due to the lack of basal laminae that prevents organization beyond the cellular level (Degnan et al. 2005). Macro-morphological adaptation in response to environmental conditions is common in sponges (e.g. Bell et al. 2002; McDonald et al. 2002; Meroz-Fine et al. 2005) due this plasticity potential. In this thesis I identified nearly twice as many morphological groups than reported by Sweerts et al. (2013; personal obs.), who examined Xestospongia populations in the nearby Sulawesi. It is possible that environmental influence can be attributed to the increased number of morphological groups reported in this study, and barrel sponge micro-morphology needs to be examined further in order to clarify these differences.

6.3 Redwoods versus Pines: Caribbean and Indo-Pacific Xestospongia demography

6.3.1 Growth and age

The predicted size-at-age values that I calculated vary substantially from those reported by McMurray et al. (2008), where the authors suggest that a sponge of comparable size to the largest in my study could be 127 years old, and larger sponges in the Caribbean upwards of
2,000 years of age. Sponges in the WNMP reach comparable size to those described by McMurray et al. (personal obs.), but my estimates suggest that these sponges would only be several hundred years old. Variation in size/age extrapolated from growth model projections of the same taxa is not uncommon. Hesp et al. (2004) compared their prediction of the Australian tarwhine *Rhabdosargus sarba* against others from the Arabian Gulf, India, and South Africa, and found stark differences in resultant maximum fish length in some instances. The authors attributed this to differences in differing sampling methods and model selection in other studies, reproductive life history or morphological distinction (Hesp et al. 2004). Furthermore, in sponges large size does not necessarily equate to a long life; Rhodes and Schupp (2012) found that individuals of *Ianthella basta* nearly 2 m in height were only 10 years old.

One possibility for the disparity in age estimations between this thesis and McMurray et al. (2008) is the use of differing volume measurement methods that may introduce error. Sponge volume is notoriously difficult to measure and barrel sponges are no exception. The authors used a hand measuring method, which, when used with sponges of complex morphologies, is subject to large amounts of error due to the abundance of irregularly shaped projections and ridges, necessitating the use of 3D stereo photogrammetry. This method is both more accurate and precise in measuring complex shapes (as compared to hand measuring; Abdo et al. 2006). The use of 3D photos allows for the quantification of error for each measurement to ensure maximum accuracy before storing values for future use, as well as for unlimited replication. As measurements are done *ex situ*, they would therefore be expected to be more accurate than *in situ* measurements limited by dive restrictions and the general inaccuracy of a measuring tape.

While dissimilar model outputs for similar taxa is not uncommon, I believe that the disparity in age estimates is due to the model choice by McMurray et al. (2008). The authors examined *Xestospongia muta* growth at one location, determining the model of best fit by comparing AICc scores. There was evidence of substantial model support throughout multiple candidate models based on AICc scores, however, suggesting that multiple models were strongly supported (Burnham and Anderson 2003). In this case multi-model inference and model averaging would have resulted in the most appropriate model of best fit to be chosen. The validation of my age estimates also strengthens them; large sponges are common on the USAT
Liberty and demonstrate that it is impossible for *Xestospongia* spp. on the wreck to be hundreds of years old.

Another possibility is that the differences in growth and age are due to differences in study locations. There is evidence to suggest that higher overall ocean productivity supports greater sponge biomass in the Caribbean as compared to Pacific sponges (the Great Barrier Reef; Wilkinson 1987; Wilkinson and Cheshire 1990). The authors also noted that the majority of sponges in the Caribbean study sites were heterotrophic and consumed nearly 15 times more organic carbon than their Pacific cohorts, which were dominantly small phototrophs. A biomass increase in heterotrophic sponges is therefore suggested as a warning signal for organic pollution (Wilkinson and Cheshire 1990). Sampela is heavily impacted by the nearby Bajau community, where human waste is directly introduced to the sea. Although the influence of such waste pollution has not been examined on water quality on Sampela, it might offer some explanation as to why barrel sponge growth at this site is accelerated compared to those in the Florida Keys. Yet, this does not explain the comparatively rapid growth of the other three study sites or on the USAT Liberty wreck.

6.3.2 Matrix models vs. IPMs

McMurray et al. (2015, 2017) used size-structured matrix models to examine *Xestospongia muta* population dynamics in the Florida Keys. The authors described the presence of a “base” size class with remnant like morphology and more than two oscules, which was not observed in this study. There is substantial evidence that suggests that the discretization required for such models can be restrictive and arbitrary in some systems (Easterling et al. 2000). In the presents study there was no biological justification to choose size categories. In contrast, discretization is particularly problematic in populations comprised of differing sizes which may have varying contributions to population growth (Morris and Doak 2002). Although McMurray et al. (2015) also determined that large barrel sponge sizes are important contributors to population growth, it is my opinion that caution should be exercised in interpretations of size class performance.
6.4 Implications of sponge dominance on coral reefs

Several studies have examined the sponge assemblages in the WNMP, primarily on reefs in the vicinity of Hoga Island. Bell and Smith (2004) discovered that that sedimentation may reduce sponge richness but does not impact abundance; sponges were actually more abundant in highly sedimented sites (Bell and Smith 2004). Recent shifts from coral- to sponge-dominance on some coral reefs has been ascribed to environmental degradation, the conditions of which are detrimental to coral but tolerable by sponges (Colvard and Edmunds 2011; Schils 2012; Kelmo et al. 2014; Bell et al. 2015b). Examples of shifts to dominance by a sediment tolerant sponge already exists at Sampela 1; the encrusting species Lamellodysidea herbacea is known to be the dominant component of the sponge assemblage (42% total sponge abundance by number; Powell et al. 2014). This sponge is capable of photoacclimation to turbid conditions within days, and its abundance is positively correlated to sedimentation (Biggerstaff et al. 2015), suggesting that this photoacclimatory ability may allow them to persist in turbid environments.

The slow growth of Xestospongia spp. to a large size renders them unlikely to settle as recruits and rise to dominance on a reef. A more realistic scenario would be the degradation of a reef system where barrel sponge populations currently exist (e.g. Kaledupa Double Spur), creating a habitat detrimental for other organisms (e.g. coral) but tolerable to sponges. Coral mortality would release barrel sponges from spatial competition, and potentially allow growth to a larger population size. A reef dominated by Xestospongia spp. could result in a loss of functional diversity in the sponge assemblage, as is likely to have occurred at Sampela 1. Pumping rates scale to sponge size (McMurray et al. 2014), so an increase in barrel sponge biomass would be reflected in the amount of water column interaction, and therefore the potential depletion of nutrients and microorganisms. Xestospongia spp. are highly efficient filter feeders that prefer cyanobacteria and heterotrophic bacteria, but mainly consume dissolved organic carbon and detritus (McMurray et al. 2016). Through pumping large quantities of water, barrel sponges may also possess the potential to deplete the water column of nutrients such as nitrogen, oxygen, and silica, as is noted for other species (Diaz and Rutzler 2001; Maldonado et al. 2005).

Sponges may contribute to coral reef productivity via sponge-facilitated carbon flow (de Goeij et al. 2013), and microbial symbionts contribute to primary productivity. This could have cascading effects on the plankton communities and nutrients available for other coral reef organisms. At the same time, increased sponge biomass would increase the availability of
carbon, sponge mediated nutrients, detritus, and sponge biomass for predation. If this population was structured similarly to the sponges at Sampela 1, this theoretical barrel sponge population would remain highly dependent on large sponges and, as is the case herein, be at risk of population decline should they experience an event causing wide-spread mortality. In such an event, the barrel sponges at Sampela 1 are likely to be very slow to recover, if at all, in which case the reef would remain widely barren until an opportunistic and faster growing species such as *Lamellodysidea herbacea* colonized the available space.

6.4.1 Mucus production: implications on a reef-wide level

In Chapter 4, I described the production of mucus as mechanism of sediment removal, which could have substantial ecological consequences if produced on a large scale. Although not previously examined in sponges, mucus production has been well studied in corals. Coral mucus is highly nutritious and energy rich; up to 80% dissolves in the water column and provides a food source for planktonic bacteria and sponges (Wild et al. 2004; Rix et al. 2016). Rix et al. (2016) traced coral mucus uptake into two sponge species and reported that 21-40% of the mucus-derived carbon and 32-39% of the mucus-derived nitrogen were released as detritus. Furthermore, coral-derived mucus provides an efficient method of trapping and transporting particles, carrying nutrients and energy to off-reef locations (Wild et al. 2004), and can require 2.5 times the amount of energy used for other physiological functions to produce (Riegl and Branch 1995). Wild et al. (2004) demonstrated that coral mucus stimulates microbial growth, and is an important energy source for the sedimentary community. Although not quantified, it was common to observe fish grazing on sponge surfaces with no evidence of physical damage. Instead, it appeared as though fish were feeding on mucus-bound sediment. Dozens to hundreds of such marks in the mucus-bound sediment surface were evident on nearly all sponges at Sampela 1 and appeared to scale with sponge size (and therefore surface area, personal obs.). It is likely that smaller organisms were feeding on this available nutrient source but were not observed. Finally, I was unable to observe nocturnal mucus production or possible mucus grazing at night. A further investigation into timing of production, as well as the differences in diurnal versus nocturnal grazing, would offer a complete insight into the daily cycles of mucus production and feeding response in the surrounding community.
6.5 Management implications

Coral reefs in the Indo-Pacific face significant risks from local threats; of particular concern is fishing pressure (the use of destructive fishing methods and overfishing) and pollution (e.g. sedimentation, eutrophication, etc.; Burke et al. 2011). The ubiquitous nature of plastic pollution, both macro and micro, should also be highlighted as a concern to suspension feeding sponges. The Wakatobi National Marine Park (WNMP) is designated as a World Biosphere Reserve (Clifton 2013) and has some of the richest areas of marine biodiversity worldwide (Unsworth et al. 2010). Unfortunately, the WNMP has historically lacked efficient enforcement, sufficient funding, community participation in management, and appropriate zonation (Unsworth et al. 2010). Destructive fishing methods, such as the use of dynamite, is still actively used on the reefs near Hoga Island, as close as Kaledupa Double Spur (personal obs.). Rubble fields and destroyed coral heads are common in this area despite the practice being illegal (Erdmann 2000). Further contributing to sedimentation increases are the common practices of mangrove removal for firewood and coral reef gleaning whereby seagrass beds are tread on and damaged. If current management and enforcement efforts remain as they are at present they are unlikely to reduce or reverse the impact of local stressors and resultant degradation of coral reefs in the WNMP.

In Chapter 2 I observed that typical causes of negative growth values confirmed by photographs were necrosis over time or sudden and dramatic loss of tissue. In the Caribbean, *Xestospongia muta* damage is often caused by vessel anchoring, marine construction, line fishing, and natural storm events (Schmahl 1999; Chiappone et al. 2002; Gilliam et al. 2008). This “shearing” may be caused by anchor damage (Gilliam et al. 2008) or slow burial by rubble over time (personal obs.). Sponge mortality over time was rarely documented in this thesis as sponges typically died between years. However, at Sampela 1 one large individual was found dislodged at the base and tipped over in the sand. Upon righting, the sponge regained colour within six weeks (personal obs.) but was missing and presumably dead by the next survey. As *Xestospongia* species require stabilization for successful reattachment (Gilliam et al. 2008), and unsustainable fishing practices are common around the WNMP (Curtis-Quick 2013), active management of anchoring and fishing debris would be required to protect these sponges. While the current presence of an abundant and large-bodied barrel sponge population at Sampela 1 suggests that significant anthropogenic damage has not been of significant magnitude to result
in population decline, the sensitivity of population growth rate to large sponge loss indicates that at the very least these threats should be restricted to their current level.

Cyclical and fatal bleaching had been documented in *X. muta* populations in the Florida Keys (López-Legentil et al. 2008b), while fatal bleaching is also caused by sponge orange band disease (SOB; Cowart et al. 2006). Seasonal bleaching may not be fatal but results in increased predation likely due to the reduction in symbiont-derived chemical defences (Dunlap and Pawlik 1998). To date there are no published incidences of either widespread bleaching events or disease in Indo-Pacific barrel sponge species. Furthermore, the dedication of resources to the continual clearance of settled sediment via mucus production could potentially affect the ability of sponges to recover from these stressors.

6.6 Research limitations

Once yearly field seasons that occurred at the in the same time of year prevented a thorough assessment of environmental and biotic factors that may be influencing *Xestospongia* spp. populations. The life expectancy of barrel sponges may exceed 50 years, in which case a study period of two years may be insufficient to detect changes at the population level. In addition, very little is known about *Xestospongia* spp. reproductive biology in the Indo-Pacific, and fecundity in this thesis were mainly based Great Barrier Reef-derived values (Fromont and Bergquist 1994). Fecundity at Sampela 1 may actually be quite low; there are fewer yearly recruits observed than at Buoy 1, although it is possible that they settled but died in the interim (e.g. smothered by sedimentation). Recruits were likely to settle, grow, and die within the course of a year, indicating that I do not have a full picture of settlement success and growth in small barrel sponges. The lack of observed recruits at Ridge 1 and Kaledupa Double Spur are also of concern; it is unclear if recruits were simply harder to find due to the higher complexity of the sites due to high coral cover, or if recruitment is very low. Similarly, I was unable to observe the direct causes of *Xestospongia* spp. mortality.

Perhaps the most restrictive, however, was the inability to fully quantify environmental data which may vary seasonally (Salinas-de-Leon et al. 2013). There are a multitude of factors that influence sponge diversity, abundance, and distribution. Past literature suggests that environmental variables of importance include depth (Wilkinson and Cheshire 1989; Bell and Barnes 2000b; de Voogd et al. 2006), wave action (Schubauer et al. 1990; Bannister et al.
2007), tidal amplitude (Barnes 1999), substrate type (Ginn et al. 2000; Carballo and Nava 2007), light (Wilkinson and Cheshire 1990), water flow (Bell and Barnes 2000a, 2003b; Ginn et al. 2000) and sedimentation (Bell and Barnes 2000a; Bell and Smith 2004; Cleary and De Voogd 2007), although it is likely a combination of abiotic and biotic factors in most habitats (Bell and Smith 2004). All of the data in this thesis was collected during the dry summer season when the prevailing wind comes from the east. The Wakatobi also has a rainy season when the prevailing wind is from the west; the differences in seasonality would be expected to influence environmental and biological conditions across sites. Specifically, seasonal changes in wind and rain are likely to affect abiotic variables of interest such as turbidity and chlorophyll α concentrations. The inability to collect data during the rainy season therefore results in a large gap in our understanding of inter-annual performance of Xestospongia spp. dynamics.

6.6.1 Integral Projection Models

In the case of Buoy 1, it does not appear that asymptotic growth has been reached as the current size structure does not closely match the stable size distribution, and the population growth rate is projected to increase significantly each year. Such a dramatic increase (30% per annum) is contingent upon current conditions remaining consistent, an unlikely circumstance on coral reefs affected by a variety of local and wider anthropogenic impacts. Stochastic events may influence a population that has yet to reach asymptotic growth at a stable stage distribution; fluctuations that describe how populations vary in such an event are referred to as transient dynamics (Tremblay et al. 2015) and could be beneficial to examine in the case of Buoy 1 sponges. Several more years of growth data would be beneficial for both growth and population models to ensure a better approximately of their multi-decadal lifespans. Perhaps of greatest interest is the inclusion of environmental variables in the IPMs for each site. The four core sites included in this study vary substantially in both abiotic and biotic composition, and having sufficient annual data could offer valuable insight into the ecosystem wide influences driving Xestospongia spp. populations.

6.7 Future directions

6.7.1 Xestospongia spp. symbiont reliance

In Chapter 3 I determined that Xestospongia spp. density was high at sites characterized by comparatively higher levels of turbidity and decreased light availability, which may indicate
that barrel sponges at these sites may be less reliant on their photosynthetic symbionts and are instead capable of shifting to heterotrophic feeding. There is evidence that Caribbean *X. muta* share a commensal relationship with their photosynthetic cyanobacteria (Thacker, 2005; López-Legentil et al., 2008), but this is unknown for Indo-Pacific species. McMurray et al. (2015) reported an inverse association of sponge depth and population decline and suggested this was due to greater picoplankton abundance at depth. Similarly, Cleary and de Voogd (2007) reported that *X. testudinaria*, were constrained to deeper depths in other areas of Sulawesi, supporting a limited reliance on symbionts. Furthermore, recent observations from deeper water areas in the Wakatobi (50-85 m) have found large populations of *Xestospongia* that all appear completely ‘bleached’ but appear otherwise healthy, suggesting that barrel sponges can survive in almost complete absence of photosymbionts (Bell unpublished data).

The ability to shift feeding strategies in conditions less favourable to symbionts could provide a great adaptive advantage in a changing environment. Both the ability of sponges to alternate between feeding strategies and the ecological implications are deserving of further investigation.

6.7.2 Spatial competition

Predation, spatial competition, and environmental factors may be important drivers of sponge assemblages in the WNMP based on correlative studies (Bell and Smith 2004; Bell et al. 2010; Powell et al. 2010). Of particular interest to the present study would be the importance of spatial competition on *Xestospongia* spp. populations, which has been demonstrated to influence other sponge distributions (de Voogd et al. 2003; Engel and Pawlik 2005; González-Rivero et al. 2011). Little is known about the competitive ability of *Xestospongia* spp. in the Indo-Pacific; the Caribbean *X. muta* has both physical and chemical defences used to deter predation (Chanas and Pawlik 1997; Jones et al. 2005), which may be beneficial in a competitive setting (e.g. chemical defence). I would expect that the numerous abiotic and biotic differences between sites would have a strong role in constraining barrel sponge population growth and recruit success. For instance, the much faster growth rates described in this study compared to those reported in the Caribbean may partly explain the high sponge abundance at this low quality site. The reduction in coral cover at Sampela 1 is likely to have only occurred in the last several decades, which will likely have released barrel sponges from spatial competition and potentially allowed a larger population size. The high barrel sponge density at sites such as Karang Gurita which are characterized by high coral cover, however, present a conflicting
scenario. Long term observations of interactions with a range of taxa across a spectrum of habitat quality are therefore necessary in order to quantify the occurrence, outcome, and mechanisms behind such competition.

6.7.3 Far-reaching effects of sedimentation

The impacts of sedimentation on sponges is recognized as an area of potential concern for management and conservation (Bell et al. 2015a). Terrigenous sediment exposure has been demonstrated to decrease reproduction in female *Rhopaloeides odorabile* (Whalan et al. 2007a), but there is currently very information available on *Xestospongia* spp. reproduction or the influence of sediment. Future work should examine the impacts of both settled and suspended sedimentation on all life stages, particularly pelagic larvae and recently settled recruits. It would be expected that even minimal amounts of settled sediment would be adequate to smother fragile new recruits, and it is currently unclear how suspended sediment levels affect the ability of pelagic larvae to disperse. This avenue of exploration is of paramount importance as it is inconsequential that adult sponges exhibit tolerance to sedimentation if the recruits are prevented from successful settlement and growth. The compounded effects of sediment tolerance and climate change is also of interest as sponges have varying degrees of tolerance to increased temperature and pH (Bennett et al. 2017), which may affect the resources available for acclimation.

Perhaps the most important direction for future work as it relates to this thesis is the exploration of both the metabolic consequences and ecological impacts of mucus production. Due to the once yearly field seasons for data collection herein, it is unclear if seasonality to turbidity and sedimentation is such that mucus production is not necessary during some parts of the year. If that were the case it is possible that the “extra” resources not otherwise dedicated to clearing sediment could be instead allocated to periods of compensatory growth and/or reproduction. The introduction of mucus and associated nutrients therein are certain to have cascading, ecosystem-wide effects, particularly if sponge mucus is of comparable composition to coral mucus. Future work should focus on determining the composition of sponge mucus, as well as quantifying total mucus production in an effort to understand the metabolic cost of its production.
6.8 Concluding remarks

To conclude, while significant headway has been made in the recognition for the need for sponge conservation (Bell et al. 2015b), the current knowledge base for even fundamental demographic information remains limited. *Xestospongia* spp. may be the largest benthic invertebrates providing habitat and fulfilling ecosystem services on some reefs in the WNMP, an area of noted biodiversity. This work adds to the body of research demonstrating that understanding sponge demography plays a critical role on coral reefs in changing environments. Furthermore, my thesis highlights several characteristics of *Xestospongia* spp. that render them vulnerable to anthropogenic impact, despite some evidence of mechanisms of sediment tolerance. My findings emphasize the importance of mitigating anthropogenic impacts that both directly and indirectly affect barrel sponges, particularly sedimentation, as changes in their abundance may have far-reaching consequences on coral reefs.
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Appendix

Model diagnostics

Model diagnostics were completed using the diagnostics function in IPMPack. Size bins for log-transformed size data ranged from 0 to 5.91 at Buoy 1 and 0 to 6.12 at Sampela 1. These included a size class extension to encompass individuals above and below current integration limits and reduce the likelihood of eviction (Figure A.1). The bin range chosen encompassed all of the size data included (Figure A.1A, D), and there was no evidence of under- or over-estimation of density, as evidenced by the linear relationship between predictions for the current and extended data range, and increased bin number plotted against the fitted survival model predictions (Metcalf et al. 2013; Figure A.1B, E). The theoretical density function was a close match to the discretized histograms for the current IPM for the 0.25, 0.5, and 0.75 quartile (Figure A.1C, F, top row), as well as for the same quartiles for an increased number of bins (Figure A.1C, F, bottom row), confirming appropriate bin choice.

Figure A.1. Model diagnostics for Buoy 1 and Sampela 1: A, B) bin range, C, D) plotted survival model predictions against the sum of the columns of the discretized matrix. The black line represents current bin choice, the red line represents the extended range, and the blue line represents an increased number of bins (A-D). Panels E, F represent discretized histograms for
the current IPM for the 0.25, 0.5, and 0.75 quartile (top row), as well as for the same quartiles for an increased number of bins (bottom row); red lines represent fit of the theoretical density function.

**Numerical parameterization**

The integral projection model (IPM) was parametrized with empirical data collected from 2014-2016. The growth model that included size in 2014, size in 2016, and size squared with size provided the best fit (Figure A.2A, C). Growth data was linear for both sites with the exception of sponge recruits which had an initial volume of zero as they appeared between survey years (Figure A.2A, C). As such, the model of best fit included the quadratic term which encompassed recruit growth. The probability of survival was estimated using a logistic regression using binary data (0 = mortality, 1 = survival). The survival model of best fit was also quadratic, including size, survival, and the interaction of size squared with size (Figure A.2B, D).

![Figure A.2](image)

Figure A.2. Functions used to parameterize the integral projection model for *Xestospongia* spp.: (A, B) growth and (C, D) survival. Red lines denote vital rate regression models of best fit, R denotes recruit growth.
Adaptive mechanisms and physiological effects of suspended and settled sediment on barrel sponges

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\section*{A R T I C L E   I N F O}

\textbf{Keywords:}
Sedimentation
Porifera
Acclimation
Mucous production
Coral reef
Degradation

\section*{A B S T R A C T}

Coral reefs across the Indo-Pacific are among the most diverse in the world but like reefs globally, they remain vulnerable to a multitude of stressors, including coastal development and the resultant sedimentation. In the Wakatobi Marine National Park, Indonesia, some degraded reefs are characterised by high levels of sedimentation and low coral cover, but support large populations of the ecologically important giant barrel sponge \textit{Xestospongia testudinaria} (Lamarck 1815). Barrel sponges can have a strong influence on water characteristics, yet tolerance and responses to sedimentation are unknown. This study examined the physiological effects of short-term exposure of \textit{X. testudinaria} to suspended sediment. Respiration rates increased compared to controls when sponges were exposed to environmentally relevant suspended sediment concentrations of 75 and 150 mg l\textsuperscript{-1}. Sponge mucus production was observed as a mechanism to remove settled sediment for the first time and sediment clearance was filmed \textit{in situ} over the course of 24 h. Sponges produced mucus in response to sediment addition, with a mean clearance rate of 10.82 \pm 2.04\% h\textsuperscript{-1} (sediment size fractions 63-250 \textmu m). Mucus production is an effective, but slow mechanism supporting barrel sponge survival in habitats experiencing high levels of sedimentation. Our results show that there are likely to be energetic consequences for sponges living in sedimented environments, which may influence the energy available for other demographic processes, and therefore have implications for barrel sponge population sustainability.

\section*{1. Introduction}

It is widely recognized that coral reefs are among the most highly productive and biologically diverse ecosystems on Earth, providing ecosystem goods and services vital to tropical and subtropical nations (Moberg and Folke, 1999). Despite their value, over half of coral reefs worldwide are considered under threat (Burke et al., 2011), and the multitude of natural and anthropogenic pressures associated with global coral reef decline have been well documented (Wilkinson, 1999; Hoegh-Guldberg et al., 2007; Wild et al., 2017; Perry et al., 2013). Un可持续的 land conversion practices such as urbanization, deforestation, and increased agricultural pressures result in runoff and erosion (Airoldi, 2003; McLaughlin et al., 2003; Sylwia et al., 2005), increasing tuffignous sediment loads that may reach near shore waters (Thrush et al., 2004; Bannister et al., 2012; Stender et al., 2014). The impacts of sedimentation are diverse and have been shown to be deleterious to scleractinian corals (see Fabricius, 2005 for a review), but its impact on other reef organisms is less clear.

Despite sponges being important components of corals reefs, we have a much poorer understanding of how they are impacted by sedimentation compared to corals (see Bell et al., 2015 for review). Although some sponge species are able to tolerate and even thrive in highly sedimented habitats, there is strong evidence that sedimentation is often deleterious to sponges at the individual and population level (see Bell et al., 2015 for a review). Settled sediment can directly affect sponges via burial or smothering (Wulff, 1997), while sediment can cause tissue scour/abrasion when carried in suspension (Rogers, 1990; Ilan and Abelson, 1995), resulting in partial mortality and reduced survival (Wulff, 1997; Maldonado et al., 2008). Sponges are obligate filter feeders and the experimental addition of fine suspended sediments has been shown to reduce or arrest pumping rates in several sponge species (Gerrodette and Flechsig, 1979; Leys et al., 1999; Tompkins-MacDonald and Leys, 2008; Bannister et al., 2012). As pumping is required to feed, clogging by fine sediment may reduce feeding efficiency and particle retention (Lohrer et al., 2006), as well as respiration (Gerrodette and Flechsig, 1979).

Although sedimentation is generally considered to have negative impacts on sponges, high sponge diversity (e.g. Bell and Smith, 2004;
Knapp et al., 2013) and abundance (Powell et al., 2014) have been reported from some sedimented sites. For example, in Indonesia, sponge densities have increased over the last decade at some highly sedimented sites (Bell and Smith, 2004; Powell et al., 2010, 2014), while habitat quality and coral cover have simultaneously decreased (Powell et al., 2010). Some sponge species appear to tolerate sedimentation and turbid conditions, and demonstrate specific adaptations that allow them to persist in conditions classically considered sub-optimal for suspension feeders. Active and passive responses are employed by sponges to rid the sponge surface of settled sediment or prevent it from settling (Bell, 2004). Active responses to sedimentation include an alteration or cessation of pumping rates (Gerrodette and Flechsig, 1979; Tompkins-MacDonald and Leys, 2008), physically moving away from sedimented areas in the case of larvae (Maldonado and Uroz, 1999), and the production of mucus (Turon et al., 1999; Bannister et al., 2012). Passive responses include macro-morphological and skeletal-level structural modifications (Barthel and Tendal, 1993; Bell et al., 2002; McDonald et al., 2002; Bell, 2004; de Voogd and Cleary, 2007; Schönberg, 2014), and positioning of the inhalant ostia and osculum to prevent sediment from settling (Bell, 2004).

Changes in respiration rates in response to sediment addition have been examined in a number of sponge species with contrasting results. Following exposure to sediment, sponge respiration rates have been shown to both increase (Bannister et al., 2012) and decrease (Lohrer et al., 2006; Tjensvoll et al., 2013). Increased respiration rates may reflect the energetic requirements of sediment clearance mechanisms, such as mucus production following short term exposure, whereas respiration rates may decrease due to a reduction in pumping rate to prevent sediment ingestion (Bell et al., 2015). Sediment size, minerology (Bannister et al., 2012), and concentration (Tjensvoll et al., 2013) may influence these responses. The energetic costs of producing a sediment response is expected to incur additional metabolic costs to the sponge, presumably at the expense of other demographic processes such as growth and reproduction (Reiswig, 1971; Roberts et al., 2006; Whalan et al., 2007; Bannister et al., 2012). Other benthic organisms, such as corals, have been reported to produce energetically costly mucus as a sediment removal mechanism (Riegel and Branch, 1995). The production of mucus as a sediment clearing mechanism has also been observed in several sponge species (Gerrodette and Flechsig, 1979; Turon et al., 1999; Kowalke, 2000; Bannister et al., 2012), though the energetic costs have yet to be determined.

Some of the most conspicuous sponges on coral reefs fall into the genus Xestospongia, which include the giant barrel sponges. Xestospongia species can grow up to several meters in diameter and live to hundreds or possibly even thousands of years old (McMurray et al., 2008; McClain et al., 2015). The Caribbean species X. muta has been thoroughly studied and found to be ecologically important on coral reefs, largely due to its ability to modify water quality characteristics (e.g. López-Legentil and Pawlik, 2008; López-Legentil et al., 2008; McMurray et al., 2008, 2010, 2015; Southwell, 2008; McClain et al., 2015). Indo-Pacific Xestospongia species, however, have received far less attention, despite their abundance and likely similar function in reef ecosystems (but see Fromont and Bergquist, 1994; Swiers et al., 2013; Bell et al., 2014). Sponges, including Xestospongia spp., play a variety of functional roles that mediate water column processes (Bell, 2008; Maldonado et al., 2012), including highly efficient removal of picoplancton and bacteria (Pile et al., 1997; Perea-Blazquez et al., 2012) and nutrient cycling (Southwell, 2008; de Goede et al., 2013; Fiore et al., 2013). In addition, Xestospongia spp. are phototrophic and contain a dense microbial community (Montalvo and Hill, 2011), so also contribute to primary production on reefs. Due to their size, ability to pump vast quantities of water (McMurray et al., 2014), and influence biogeochemical processes, changes in Xestospongia productivity and abundance could have significant impacts on reef function, particularly in systems impacted by reduced water quality.

Previous research on Xestospongia spp. populations in the Indo-Pacific has demonstrated that this species has high levels of self-recruitment, small population sizes, and low larval dispersal rates (Bell et al., 2014). These characteristics, in addition to the slow growth rates reported for their Caribbean congener X. muta, suggest that these populations should be susceptible to environmental disturbance (Bell et al., 2014). However, Bell et al. (2014) reported that Xestospongia spp. were very abundant at sites experiencing high levels of sedimentation and habitat degradation. These factors, in conjunction with the presence of large and likely old individuals in these habitats, support the hypothesis that barrel sponges likely possess physiological traits enabling them to tolerate high-sediment environments (Bell et al., 2014).

Given the current trends in coastal development and resultant sedimentation expected to reach coral reefs, it is important to understand the effects that sedimentation may have on Xestospongia spp. In this study we aimed to: 1) quantify settled and suspended sediment on a degraded reef dominated by Xestospongia testudinaria, enabling environmentally relevant sediment addition experiments; 2) observe the occurrence, location, and rate of sediment accumulation on X. testudinaria individuals; 3) observe and measure X. testudinaria mucus production as a settled sediment removal mechanism; 4) examine the effects of sedimentation on in situ sponge respiration rates for different suspended sediment treatments.

2. Methods

2.1. Study site

This study was conducted in the Wakatobi Marine National Park (WMNP; 05°29.6S, 123°45.26E; Fig. 1). The WMNP, located in southeast Sulawesi, Indonesia, is the most populated marine national park in Indonesia (Clifton and Unsworth, 2010). Located in the coral triangle, the WMNP contains some of the highest marine diversity in the world, yet is heavily impacted by a local population of over 100,000 people that are reliant on the reef as a resource (Cullen, 2010). Hard coral cover in the WMNP has declined substantially over the last decade; surveys conducted from 2000 to 2007 revealed that coral cover decreased an average of 45% across six sites over that time (McMellor and Smith, 2010). Using microsatellite analyses and external morphological examination, Bell et al. (2014) revealed that barrel sponges in the WMNP are comprised of a species complex, likely including Xestospongia testudinaria, X. bergquistia, and one undescribed species. As such, only individuals corresponding to the X. testudinaria morphology, as previously determined by Bell et al. (2014), were chosen for this study.

Hoga Reef is a shallow sloping reef on the southwest corner of Hoga Island in the centre of the WMNP (Fig. 1), and was the collection site of barrel sponges for our experiments. This site has moderate coral cover (30–40% cover), and low turbidity and sedimentation rates. Sampela 1 is a small community in the WMNP that contains some of the highest marine diversity in the world, yet is heavily impacted by a local population of over 100,000 people that are reliant on the reef as a resource (Cullen, 2010). High coral cover in the WMNP has declined substantially over the last decade; surveys conducted from 2000 to 2007 revealed that coral cover decreased an average of 45% across six sites over that time (McMellor and Smith, 2010). Using microsatellite analyses and external morphological examination, Bell et al. (2014) revealed that barrel sponges in the WMNP are comprised of a species complex, likely including Xestospongia testudinaria, X. bergquistia, and one undescribed species. As such, only individuals corresponding to the X. testudinaria morphology, as previously determined by Bell et al. (2014), were chosen for this study.

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used. All results are presented with a mean ± standard error unless noted otherwise.

2.2. Sedimentation data

2.2.1. Sediment deposition rates

Sediment deposition rates were estimated using plastic cylindrical sediment traps equipped with an inverted funnel to minimize resuspension using locally available materials. Sediment traps were approximately 30 cm in height, had a trapping area of 50.26 cm², and a height to width aspect ratio of 3:1 for the trap mouth, and 10:1 to the funnel opening. Traps were deployed at Sampela 1 at 5 m and 10 m below the reef crest in a vertical orientation at least 20 m apart for three independent, but consecutive two week periods from June to August 2015 (n = 15 per depth across all three time periods). A Ziploc bag was placed over the trap openings upon collection to reduce sediment loss during removal and transportation. Samples were wet sieved into major sediment size factions (> 250, 125–250, 63–125, 38–63, and < 38 μm) and dried to a constant weight at 200 °C. Sedimentation rate (g m⁻² d⁻¹) was adjusted to the area of the trap opening and standardised per day; given the constraints of the aspect ratio, the traps were used to estimate the abundance of targeted sediment fractions pertinent to experiments herein. The percentage of different size factions in the settled sediment composition data obtained from sediment traps were normally distributed and subsequently analysed using a Three Way ANOVA (fraction size, depth and time as fixed factors).

2.2.2. Suspended sediment

Ambient seawater samples (50 ml, n = 3) were collected for suspended sediment analyses over the same time period as the sediment traps. Samples were collected at 10 m, filtered through glass fibre filter paper (0.7 μm, Membrane Solutions), and dried to a constant weight at 80 °C. The filtered sediment was re-suspended to 120 ml in a 4% NaCl solution and stirred for 20 min using an overhead stirrer at 1000 rpms (Chiltern Scientific PS41) to break up any sediment aggregates. Sediment grain size distributions were analysed with a Beckman Coulter Multisizer 3 using a 4–120 μm aperture. Ambient suspended sediment concentration (mg l⁻¹) was calculated by standardising the weight of the sediment retained on the filter by the total volume of water sampled.

2.2.3. Sediment accumulation

Qualitative visual surveys were completed at Sampela 1 to identify barrel sponge individuals with sediment present within the osculum (n = 93), as well as those with mucus-bound sediment present on the external surface of the sponge (n = 215). For the latter, sediment was manually disturbed and deemed mucus-bound if the sediment was difficult to remove and if mucus was directly observed.

In order to observe the sediment accumulation time on the external surface of sponges, a high-definition GoPro Hero® fitted with a CamDo time lapse intervalometer was deployed for 24 h following sediment removal (n = 9). The intervalometer was programmed to take a picture every 10 min. Individual sponges were manually cleared of sediment and the camera was positioned to capture one side of each sponge. Sediment accumulation time was defined as the time (min) required before 100% of the cleaned external sponge surface was recovered by sediment. The flow of fluorescein dye was used to ensure that the sponge was pumping before sediment clearance and at the end of the
2.3. Sediment clearance experiment

To examine the production of mucus as a settled sediment response mechanism, previously tagged sponges in a known population at Sampela 1 were chosen haphazardly and filmed using a GoPro Hero® fitted with a CamDo time lapse intervalometer and mounted on a tripod, following the application of sediment treatments. Sediment application was intended to replicate the natural accumulation of settled sediment on the sponges and the amount of sediment to be added was determined by calculating the mean sedimentation rate experienced at Sampela 1 (expressed in g m⁻² d⁻¹) and was standardised for sponge surface area.

Measuring sponge volume can be difficult due to complex external morphology. The use of stereo photogrammetry allows for accurate repeated 3D measurements in order to calculate the total and spongo-coel volume (Abdo et al., 2006), though the internal canal system remains difficult to quantify. Sponge surface area (cm²) and oscula volume (cm³) were calculated from digital images taken with a Fujifilm FinePix Real 3D W3 Digital Camera and corresponding underwater camera housing, and measured using stereo calibration and measurement software (CAL and EventMeasure) created by J. Seager (http://www.seagis.com.au). EventMeasure allows for multiple, precise, three-dimensional measurements to be collected from photos taken from several angles and from above, looking into the osculum. This method was used to estimate sponge surface area, and also for measuring sponge volume for the respiration experiment (see below).

Sediment treatments included the most prevalent size fractions as determined from sediment traps (25.43 ± 1.26%: 63–125, and 23.02 ± 0.83%: 125–250 μm, respectively). Size fractions > 250 μm consisted mainly of large pieces of shell, rock, and algae and therefore are unlikely to be effectively removed from the sponge osculum via mucus clearance (personal observation). Preliminary experiments revealed that the addition of sediment smaller than 63 μm was immediately pumped out of the osculum and prevented from settling, therefore < 38 and 38–63 μm fractions were not used in the study. Surface sediment was collected from the reef, wet filtered to a fraction of 38–63 μm, and dried to a constant weight at 180 °C (for a minimum of 12 h) to ensure the removal of any microbial communities or trace organic material.

Sediment was carefully added to the base of the sponge osculum (n = 15), and the GoPro was positioned directly on the rim of the osculum, taking care not to damage the tissue. The camera was positioned over the sponge in order to have an unobscured view of the base of the osculum; in some cases this necessitated placement directly over the osculum. Due to the small size of the camera compared to the much larger size of the osculum, there was no perceived effect of the camera presence on sponge pumping.

Sediment applications and GoPro deployment occurred between 7 and 11 am to allow for the maximum amount of daylight, and each sponge was filmed for 24 h following each sediment application. The GoPro was programmed to take a photo once every hour. Fluorescein dye was used to ensure that the sponge was pumping before sediment additions and following camera retrieval; though Xestospongia pumping rates may vary over the course of 24 h, a complete pumping cessation is uncommon (McMurray et al., 2014).

Photos (n = 24 per sponge) were analysed with ImageJ 1.43 software (developed at the National Institutes of Health, Washington, DC, USA) to measure the area of settled sediment present in the sponge osculum every hour for the duration of the video. The ImageJ Freehand Sketcher was used to manually trace the boundary of the sediment in the digital images and the area of the shape was calculated automatically. Strings of mucus were not included in the area estimation. Sediment clearance rates were calculated as the hourly percentage decrease in sediment area in the osculum and on the surface of the sponge.

Independent t-tests were used to examine the effect of osculum volume (as a proxy for sponge volume reported by McMurray et al., 2014), and sediment fraction on sediment clearance rates.

2.4. In situ respiration experiment

The large size of barrel sponges creates difficulties in measuring respiration in a laboratory setting, necessitating in situ data collection using a submersible respiration chamber. Sponges of suitable small size (sponges sizes between 20 and 39 cm high, and 19 and 30 cm wide) were harvested from Hoga Reef within predefined selection criteria (no mortality or necrosis, and the size being limited by the size of the respiration chamber) from 10 m and were transported a short distance (approx. 100 m) to a single location at Sampela 1 where the experiment was conducted. Entire sponges, still attached to coral rock, were removed from the substrate and placed in a stable location at the same depth at Sampela 1. Sponges were cleaned of any epibionts (mainly algae and synaptid sea cucumbers) before harvest and once again before the start of each experiment. Sponges were allowed to acclimate for two weeks at Sampela 1 prior to experimentation, consistent with previous laboratory experiments (Wisshak et al., 2012; Fang et al., 2013; Stabler et al., 2014; Bennett et al., 2016; Lesser et al., 2016; Vicente et al., 2016). As such respiration results are unlikely to be the result of the sponge being transported to a central location for experiments.

2.4.1. Respiration chamber design

A single 73 litre Perspex respiration chamber was developed for in situ measurements; once sealed, the chamber was completely water-tight. The chamber contained a fully closed, manual circulation system based on a series of hand pumps to provide standardised water flow for the time that the sponge was in the chamber (Fig. 2). The hand pumps were compressed every two seconds and delivered a mean volumetric flow of 90 l h⁻¹. Sponge pumping activity was also expected to increase flow within the chamber and maintain water circulation. If Indo-Pacific Xestospongia spp. pumping rates are comparable to those measured by McMurray et al. (2014) for X. muta of similar morphotypes (0.06 ± 0.04 s⁻¹−¹ sponge tissue), then the sponges themselves contribute 7.8 l h⁻¹ on top of the hand pump flow rate of 90 l h⁻¹. The mean cross flow rate in the chamber was 0.066 ± 0.013 m s⁻¹, which is comparable to the ambient flow rate at Sampela 1 (0.063 ± 0.044 m s⁻¹; Powell et al., 2014). The incumbent pump was positioned 10 cm lower than the out-current pump to reduce sediment falling out of suspension.

Water samples and sediment additions were drawn and added, respectively, through two ports affixed with reinforced T-valves that were water tight when closed. Water samples for oxygen analysis were drawn from the port with a 35 ml syringe through a fitted rubber stopper; concentrated sediment was added using the same method. As Xestospongia testudinaria contain a dense photosynthetic microbial community (Montalvo and Hill, 2011), whole sponge oxygen consumption would represent both the metabolic activity of the sponge and the microbial community which may be difficult to distinguish (Cheshire et al., 1997). For this reason the chamber was blacked out (after Bennett et al., 2016) so the symbionts would not be photosynthesising and therefore producing oxygen. Therefore the respiration rates that we measured are a combination of the sponge and its associated microbial community. Furthermore, there is no evidence in the literature to suggest different sponge respiration rates occur in the light compared to the dark.

2.4.2. Experimental design

Individual sponges of the same gross morphology were chosen haphazardly and placed in the chamber at 10 m to dark adapt for 10 min, while still being exposed to ambient water circulating within the chamber. The chamber was then sealed and baseline water samples (Tₒ) were immediately drawn, followed by the addition of the sediment
treatment (mg dry weight l\(^{-1}\)). Water samples were taken every 15 min thereafter for 45 min while pumping continued to ensure adequate circulation.

Suspended sediment concentration measurements collected from Hoga Reef at 10 m revealed the mean ambient suspended sediment load to be 134.67 ± 2.26 mg l\(^{-1}\) (± SE). In order to quantify potential threshold levels of sponge physiological responses to suspended sediment, sponges were exposed to initial concentrations of 75 mg l\(^{-1}\) (n = 6), 150 mg l\(^{-1}\) (n = 7), and a control treatment (no sediment, n = 7). Sediment for each treatment was collected and processed in the same manner as the sediment clearance experiment. There is the potential that respiration rates may be influenced as a result of bacterial activity on the sediment or in response to organic residue remaining on the particles. However, any potential influence of sediment-derived microbial populations or trace nutrient modification on sponge respiration rates is likely to be negligible due to the temperature and duration of the heating processes, which would have removed an organic residues.

The suspended sediment composition of water samples at 10 m (n = 3) revealed that sediment < 38 μm comprised the largest fraction of suspended sediment (73.64 ± 1.02%), but was difficult to isolate reliably using wet sieving techniques. Therefore, the second most abundance sediment fraction (38–63 μm; 21.28 ± 0.03%) was chosen for the respiration experiments as this would still be experienced by sponges filtering water from the water column.

Respiration rates were calculated based on the chamber volume (l), volume of the sponge (cm\(^3\)), and the amount of concentrated suspension injected into the chamber (g dry weight of sediment l\(^{-1}\) seawater). Separate, sealed Perspex chambers were used to collect baseline respiration data for seawater samples in the absence of sponges; water samples for respiration measurements were collected at the beginning of every sponge respiration experiment (T\(_0\)), and after 45 min (T\(_3\), n = 20). The uptake of fluorescein dye by the target sponge was used to ensure that the sponge was pumping at the start of the experiment and at the conclusion of the experiment. The presence of mucus was also noted.

Water samples were kept at constant ambient water temperature and oxygen concentration was immediately analysed at the surface using a FIBOX 3, 505 nm oxygen probe (combined with FIBOX 5.20 Software for data logging; Precision Sensing GmbH, Regensburg, Germany). The oxygen electrode was calibrated prior to each oxygen measurement to 0% oxygen saturation (water containing 1 g of sodium sulphite [Na\(_2\)SO\(_3\)] and 100% oxygen saturation (water bubbled for 10 min) in 100 ml of seawater. Chamber samples were kept in the dark and sealed in an airtight container fitted with a rubber stopper to prevent oxygen exchange. As samples were collected at 10 m and analysed at sea level final oxygen concentration values for sponges and seawater blanks were corrected for changes in partial pressure of O\(_2\).

Sponge volume was calculated from three-dimensional photographs following the methods utilized in the in situ sediment clearance experiment, but expanded to include the entire sponge. Volume was calculated by approximating geometric shapes for each sponge shape, corrected for spongocoel volume after McMurray et al. (2008), and ranged from 71.95 to 583.43 cm\(^3\). Due to the highly diverse morphologies of Indo-Pacific Xestospongia sp., sponges were categorized as either cylinder, barrel, sphere, inverted truncated elliptical cone, or frustum of a cone. Spongocoels were categorized as either cylinders or inverted truncated elliptical cones (depending on the sponge) and volume was calculated accordingly. A dry weight/volume ratio was calculated by measuring the smaller sponge fragments by hand, and then drying and weighing them (n = 179). Whole sponge volume was extrapolated from this data using linear regression (F\(_{1,178} = 216.70, p < 0.001\) in lieu of sacrificing whole individuals.

Suspended sediment concentration was expected to fluctuate due to settlement in the chamber and movement through the pump system. The actual concentration of sediment within the chamber was calculated by taking water samples of known volume (n = 3) immediately after the addition of sediment and at the end of the experiment. Samples were filtered through glass fibre filter paper (0.7 μm, Membrane Solutions), and dried to a constant weight at 80 °C.

![Fig. 2. Respiration chamber design with model Xestospongia spp. sponge, asterisks (*) denote cable clamps throughout the chamber, arrows denote direction of water flow. Note: the chamber was blacked out for actual respiration experiments.](image-url)
Sponge respiration rate was calculated based on chamber volume (corrected for individual sponge volume), time (h), seawater sample (blanks), and calculated as follows:

\[
\text{Respiration rate} = \frac{[\text{sponge} (\text{mgO}_2[T_0]) - mg \text{O}_2[T_3]) - \text{blank} (mg \text{O}_2[T_0] - mg \text{O}_2[T_3])]}{V_{\text{chamber}}(T)} 
\times \frac{1}{DW_{\text{sponge}}}
\]

where \(T_0\) is the beginning and \(T_3\) is the end of the sampling period, \(V_{\text{chamber}}\) is the volume of the chamber, and \(DW_{\text{sponge}}\) is the estimated dry weight of the sponge. The change in respiration rate over time was calculated for each sponge as above for each time point \(T_0\) and \(T_3\).

Logistical constraints of in situ sample collection rendered continuous oxygen consumption curves impossible. However, Mills et al. (2014) demonstrated the linear relationship of Halichondria panacea oxygen consumption in a closed system over time, supporting the use of point measurements.

A One-way ANOVA was employed to test the differences in respiration rate in response to suspended sediment exposure (75 and 150 mg l\(^{-1}\)) and control treatments (no sediment), followed by Tukey’s HSD post-hoc test to examine the significant main effects between treatments. The effect of short term suspended sediment exposure on sponge respiration rate was analysed with a One Way repeated measure ANOVA. Sampling time \((T_0 - T_3)\) measured at 15 min intervals comprised the within factor treatment, and the suspended sediment treatments \((75\) and \(150\) mg l\(^{-1}\)) and the no sediment control compromised the between factor treatment \((n = 20)\). Greenhouse-Geisser adjusted probability was used to estimate statistical significance to avoid violating sphericity. A Bonferroni post-hoc test was used to examine the relationship between respiration rates and sediment treatment.

3. Results

3.1. Sedimentation data

At the conclusion of the two week sampling periods, three 5 m and two 10 m traps were unable to be collected (final \(n = 25\)). There were no significant differences in depths in any size fraction and the data were pooled (Three Way ANOVA; \(F < 1\) in all cases, \(p > 0.05\)). The mean sediment deposition rate obtained from the sediment traps at Sampela 1 was 44.40 ± 3.0 g dry weight m\(^{-2}\) day\(^{-1}\); the mean sediment size fraction composition was primarily 63–125 μm sediment (25.41 ± 1.26%), followed by 125–250 μm (23.15 ± 0.61%), 38–63 μm (20.55 ± 1.98%) and < 38 μm (11.41 ± 1.84%; Fig. 3).

Suspended sediment grain sizes derived from water samples collected from Hoga Reef at 10 m ranged from 6 to 106 μm (Fig. 4). The dominant grain size was < 38 μm with 73.64 ± 1.02% composition, followed by 38–63 μm (20.55 ± 1.98%) and > 125 μm (20.55 ± 1.98%). Visual sponge surveys revealed that 60% of sponges had sediment observed within their osculum at Sampela 1 (\(n = 93\)), while 99% of sponges had mucus-bound sediment present on their external surface (\(n = 215\)). Footage from sediment accumulation videos revealed that sponges cleared of sediment were 100% re-covered in sediment in a mean of 135.11 ± 10.93 min (\(n = 9\)).

3.2. Settled sediment clearance

Mucus production as a settled sediment clearance mechanism was observed in every sponge following sediment addition. Mucus was evident after one hour post sediment application. Sediment fraction size did not influence the mean rate of sediment clearance (\((t(13) = 0.817, p = 0.429)\); sponges rid themselves of 125–250 μm sediment at a rate of 12.62 ± 3.83% hr\(^{-1}\), and 63–125 μm sediment at a rate of 9.24 ± 2.47% hr\(^{-1}\). There was no influence of sponge osculum volume (used as a proxy for sponge size) on the efficiency of sediment clearance (\((t(11) = -0.175, p = 0.864)\).

The typical progression of sediment clearance by mucus production proceeded as follows: 1) mucus production began in the base of the osculum; 2) sediment was aggregated within a concentrated mass of mucus; and 3) strings of buoyant, mucus aggregate were propelled upward and out of the sponge by the force of sponge pumping (Fig. 5; Supplementary Materials). In some instances reef fish were observed within the sponge osculum following the production of mucus, though scavenging was not directly observed.

3.3. In situ respiration experiment

Throughout the course of the experiment sponges were exposed to less suspended sediment than initial treatment concentrations due to sediment settlement within the pumping system and in the interior of
the chamber itself. The actual range of suspended sediment that sponges were exposed to for the 75 mg l\(^{-1}\) treatment was between 52.46 ± 3.14 and 64.33 ± 5.63 mg l\(^{-1}\), and for the 150 mg l\(^{-1}\) initial application between 123.33 ± 8.03 and 138.57 ± 8.43 mg l\(^{-1}\).

There was a clear effect of suspended sediment exposure on *Xestospongia testudinaria* respiration rate over the course of the experiment (one-way ANOVA, \(F_{2,19} = 7.277, p < 0.01\); Fig. 6). Control sponges that were not exposed to sediment had an 18.8 ± 8.02% mean increase in respiration rate, as compared to 95.05 ± 22.11% and 71.56 ± 11.33% (in 75 and 150 mg l\(^{-1}\) treatments, respectively). Exposure to both the 75 and 150 mg l\(^{-1}\) suspended sediment treatments resulted in a greater increase in sponge respiration rate than the control (75 mg l\(^{-1}\): Tukey HSD post-hoc, \(p < 0.01\); 150 mg l\(^{-1}\): Tukey HSD post-hoc, \(p < 0.05\)). There was no significant difference in respiration rates at any given time point between the low and high suspended sediment treatment (75 and 150 mg l\(^{-1}\); Tukey HSD post-hoc, \(p = 0.553\); Fig. 6).

For all treatments, mean respiration rates increased steadily over the course of 45 min beginning with highly variable baseline respiration rates of 0.03 ± 0.04, 0.09 ± 0.06, and 0.08 ± 0.02 mg O\(_2\) g\(^{-1}\) DW h\(^{-1}\), in control, 75 and 150 mg l\(^{-1}\) treatments, respectively (Fig. 7). There was a highly significant
interaction between sampling time and treatment in the repeated measures ANOVA (Table 1). Between sponge effect tests revealed a significant effect of exposure to suspended sediment on sponge respiration rate within the sediment treatments. Bonferroni multiple comparison post-hoc tests revealed that sponges exposed to sediment treatments had a greater increase in respiration rates than that of the control (Table 1).

4. Discussion

Elevated suspended sediment loads reaching coastal habitats can have major consequences for the ecology of the organisms inhabiting these environments, particularly benthic suspension feeders (Smith and Kukert, 1996; Henley et al., 2000). Increased sedimentation is deleterious to a variety of species (Ellis et al., 2002; Fabricius, 2005) and mechanisms for tolerance are often at the expense of demographic processes, resulting in decreased growth and reproduction. Here we measured the physiological response of Xestospongia testudinaria to suspended sediment in the Indo-Pacific, and demonstrated that mucus production is likely an important sediment clearance mechanism. We also found increased respiration rates in response to suspended sediment treatments. Given that there is likely to be an ongoing energetic cost to continually removing sediment in sedimented environments, we propose that some demographic processes (such as growth and reproduction) of sponges in sedimented environments may be lower than in non-sedimented sites. If energy is devoted away from demographic processes, this may have wider implications for population sustainability and maintenance. For example, Xestospongia recruitment is known to be low and sporadic (McMurray et al., 2008; Bell et al., 2014), and any reduction in reproductive output may tip populations into decline. Given the strong influence that these species exert over the water column, their loss could have broader ecosystem impacts.

4.1. The effect of suspended sediment on Xestospongia testudinaria respiration

Sponge exposure to 38–63 μm suspended sediment rapidly increased respiration rates in both treatments more than eight fold in only 45 min (Fig. 6). The rapid respiratory response demonstrated in such a short time emphasizes the impact of sediment on respiration rate of X. testudinaria. As the sponges did not stop pumping (indicated by the uptake of fluorescein dye at both the start and end of the experiment), exposure to suspended sediment must affect barrel sponge physiology through changes to respiration. We propose that these sponges have a broad tolerance to sediment exposure, as the highest sediment treatment (150 mg l$^{-1}$) did not significantly increase respiration more than the lower sediment treatment (75 mg l$^{-1}$; Table 1; Fig. 6). Due to logistical constraints it was not possible to observe recovery rates following suspended sediment exposure though this would have provide relevant information on X. testudinaria recovery mechanisms and should be a focus of future research.

Our study demonstrates the initial response of a sponge species following short-term exposure to high levels of suspended sediment. The rapid increase in respiration rate after this short period of time suggests that these environmental concentrations are sub-optimal for Xestospongia testudinaria, yet Bell et al. (2014) observed the highest X. testudinaria abundance in the Wakatobi at our study site, Sampela 1. Assuming a net gain in biomass over time, and using growth models developed for the Caribbean congener Xestospongia muta (McMurray et al., 2008), barrel sponges at Sampela 1, could be, on average 50–60 years old. Thus they may be at least as old as the settlement of the nearby Bajo village built in the 1950s (Bell et al., 2014) that has resulted in heavy exploitation of the Sampela reef in the subsequent years, and likely contributes to the current high levels of sedimentation. The maturity of this sponge population suggests that they are better able to cope with environmental perturbations, whether chronic or acute, compared to other components of the reef, such as corals, which cover < 5% and are comprised of mostly small colonies.

Preliminary respiration experiments revealed that smaller sediment size factions (38–63 μm) are prevented from settling into the sponge osculum due to the velocity of water pumped through the sponge, suggesting that in moderation, sponges may be able to rid themselves of small particles as part of normal pumping activities. In the Caribbean, Xestospongia muta individuals have been shown to pump large quantities of water for long periods of time (McMurray et al., 2014), rendering this a potentially effective method of prevention against fine sediment settlement. It is important, however, to understand how barrel sponges rid themselves of sediment with grain sizes smaller than 38 μm as they may have alternative physiological impacts than those examined in this study. Both the suspended and settled sediment grain sizes measured in this study are likely to affect the physiology of pumping and feeding of barrel sponges. According to Bannister et al. (2012), sediments with grain sizes between 10 and 100 μm may be filtered into the aquiferous system of many sponges, and those smaller than 10 μm may be filtered into the choanocyte chambers themselves.

Unlike other fields of study, there is no one standard unit for sponge respiration, which makes comparisons difficult. Respiration rates vary widely between and even within species, with units reported including mmol O$_2$ min$^{-1}$ g$^{-1}$ (Cheshire et al., 1997), mg O$_2$ l$^{-1}$ (Coma et al., 2002), ml O$_2$ h$^{-1}$ g$^{-1}$ AFDW (Kowalke, 2000), cm$^3$ O$_2$ h$^{-1}$ per cm$^3$ sponge (Reiswig, 1974) and μmol h$^{-1}$ g DW$^{-1}$ (Tjensvoll et al., 2013). We reported low (T$_o$) respiration rates for X. testudinaria (between 0.014 ± 0.0207 to 0.0677 ± 0.0317 mg O$_2$ g$^{-1}$ DW$^{-1}$) at the start of the experiment. We confirmed pumping prior to each experiment starting, and therefore X. testudinaria likely has low basal respiration rates compared to other sponge species. Furthermore, while Osinga et al. (1999) reviewed respiration studies and reported a range between 0.2 and 25 μmol O$_2$ h$^{-1}$, our apparent low respiration rates are still comparable with those reported in several earlier studies (e.g. Kowalke, 2000; McMurray et al. 2008).

Previous studies have found that elevated suspended sediment can result in chronic stress in corals, and that the energetic cost of sediment removal may increase with continual low-level sedimentation (McLaughlin et al., 2003). Sponges experiencing high rates of sedimentation may have high metabolic costs associated with active sediment removal mechanisms along with a reduction of symbiotic productivity, though this remains poorly understood (Tomkins-MacDonald and Leys, 2008; Bell et al., 2015). The production of mucus would therefore be expected to increase respiration rates (Bell et al., 2015), and this is supported by the results of the in situ respiration experiment. Following the conclusion of the experiment, mucus production originating from both the external surface of the sponge and interior of the osculum was evident in sponges exposed to both sediment treatments, but not the control.

4.2. Mucus as a sediment clearance mechanism

Although not previously examined in sponges, mucus production has been well studied in corals. Mucus is often produced when corals are exposed to air or sedimentation, with mucus moving nutrients and carbon to off-reef locations (Wild et al., 2004; Huettel et al., 2006). Previous work has suggested that continuous mucus production in corals allows for the high productivity of coral ecosystems in an oligotrophic system (Huettel et al., 2006), and was recently demonstrated to play an important role in the “sponge loop” that facilitates nutrient cycling (Rix et al., 2016). Coral mucus can be a significant source of carbon (Riegl and Branch, 1995), phosphate, silicate, ammonium, and nitrate/nitrite (Wild et al., 2005). As such, a chronically stressed sponge population continually producing mucus could not only affect the demographic processes of the sponges themselves, but could have a larger impact on the recycling of matter and release of a nutrient on coral reefs. The presence of mucus-bound sediment within the oscula
suggests that 60% (n = 93) of sponges surveyed may utilize mucus as a clearance mechanism. Mucus production at this scale would therefore be expected to have a contribution to local nutrient cycling at Sampela 1, though this has yet to be quantified.

4.3. Experimental limitations

This study is the first to measure respiration rates of a barrel sponge species, and the first to examine the effects of suspended sediment exposure on *Xestospongia* in the Indo-Pacific. Measuring respiration rates in situ eliminates the stress of transporting large barrel sponges into a lab and maintaining them in aquaria, resulting in more natural measurements. It is possible that the brief transplantation between sites described here may have influenced barrel sponge respiration. In order to mitigate this effect, a two-week acclimation time was observed, which is longer than many previous experimental studies that have taken sponges to the laboratory (Fang et al., 2013; Stubbler et al., 2014; Lesser et al., 2016; Vicente et al., 2016).

There are drawbacks to our *in situ* method, including a limited measurement time due to local dive restrictions, which prevented observations being made on the recovery from sediment exposure. In addition, the large size of the study species resulted in the small sample size of sponges of appropriate size to fit into the chamber. Although the method utilized for our study was a simple and inexpensive one, it is important to note that simple recirculating, closed-system chambers of this nature are incapable of recreating the complex flow found on the benthic boundary layer of a coral reef. Furthermore, as the sponge was observed to be pumping both before and after the experiment, it is likely that pumping throughout the course of the experiment increased water flow and therefore exchange rate in the chamber (Patterson et al., 1991). Finally, the inclusion of a disturbance control (Crowe and Underwood, 1999) would have allowed for quantification of sponge handling and transportation.

Suspended sediment concentration is likely to be variable at a range of temporal and spatial scales. The Wakatobi is subject to wet and dry seasons with varying levels of rainfall (Crabbe and Smith, 2005), which affect turbidity levels. Although the small sample size and spot sampling method utilized in this study is likely not representative of the full range of suspended sediment concentrations that *Xestospongia testudinaria* may experience throughout the year, the presence of a mature, well-developed population at Sampela 1 indicates that these sponges have mechanisms allowing them to survive varying degrees of sedimentation.

4.4. Conclusions

Despite the recognized functional importance of sponges and the need to apply conservation and management measures to mitigate environmental impacts (Bell et al., 2015), there remains a general lack of understanding of the effects of both suspended sediment concentrations and settled sedimentation on sponges, or the potential adaptations allowing some sponges to persist in coral reef habitats. This paper experimentally demonstrates that Indo-Pacific *Xestospongia testudinaria* respond quickly to exposure to sedimentation and there is an energetic cost to these responses, which may have influence sponges demographic processes and subsequent reef ecosystem functioning.

Acknowledgements

This research was funded by a Victoria University of Wellington Doctoral Scholarship awarded to Emily McGrath. Operation Wallacea provided funding for travel and accommodation facilitate data collection. A research permit for this research was issued to Mr. Joseph Marlow 182/SIP/FRP/ESS/Dit.kl/VI/2016) in collaboration with Prof Jamal Jompa by the Indonesian Ministry of Research and Technology (RISTEK). We would like to Jennifer Matthews and David Young for assistance with the figure illustration and John Van der Sman for assistance with the design of the respiration chamber. We are also grateful to the staff and volunteers of Hoga Island Marine Research Station.

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