Ecological Role and Potential Value of Sponges to Torres Strait

Final Report on Project Activities, June 2010

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Australian Institute of Marine Science
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Acronyms and abbreviations used in this report

AIMS ..................Australian Institute of Marine Science
ANOVA ...............analysis of variance
ARP .......................Annual Research Plan
BOM ....................Bureau of Meteorology
cm ..........................centimetre
CRC .......................Cooperative Research Centre
GBR .....................Great Barrier Reef
KEL .....................Kaileg Enterprises Limited
m .........................metres
MANOVA ..................Multivariate analysis of variance
min ........................minutes
MTSRF .................Marine and Tropical Sciences Research Facility
NPARC .................Northern Peninsula Area Regional Council
pers. ob ..................personal observation
RRRC ..................Reef and Rainforest Research Centre Limited
sec ............................seconds
TS ............................Torres Strait
TSC ......................Torres Shire Council
TSI .......................Torres Strait Islanders
TSRA ..................Torres Strait Regional Authority
TSRC .....................Torres Strait Regional Council
YICC .................Yorke Island Community Council
Acknowledgements

This project received funding from the Australian Government’s Marine and Tropical Sciences Research Facility (MTSRF), Torres Strait Regional Authority (TSRA) and the Australian Institute of Marine Science (AIMS). We also acknowledge significant in-kind and logistical support from Kailag Enterprises Limited. The project team is grateful to research managers at RRRC, especially Joelle Prange and Melissa Jess.

The editors and authors of this final report wish to gratefully acknowledge many individuals who have helped and supported this project during the past four years. On Masig Island, the project has benefited throughout by the local knowledge and professional maritime and diving expertise of Mr John Morris and Mr Samson Lowatta and the gentle mentoring of Uncle Dan Mosby, and more recently, the expert coxswain skills of Mr Gavin Mosby. Other Masig islanders to assist the project include Mr Solomon Elia, Mr Joey Billy, Ms Ella Lowatta and Ms Hilda Mosby. Additional field assistance has been provided by Ms Michelle Jonker, Mr Stanley Lui, Mr Eric Matson and Mr Tim Hyndes. We also thank Mr Andrew Dennison from Tagai College for arranging a surveyed boat for one field trip. We are grateful to Mr Vic McGrath for providing liason and advice, and also Mr Damian Miley from TSRA. We also thank the former chair, councilors and staff of Yorke Island Community Council and later the Torres Strait Regional Council office at Masig, for logistical support and advice. We wish to especially acknowledge the late chairman Mr Donald Mosby, for his vision and innovation in the original initiation of this project.

We also particularly thank and acknowledge the help and advice from Mr John Mosby – chair of KEL; and TSRA member for Masig. We also thank others from KEL, especially directors Mr John Morris, Mr Douglas Billy, and Mr Daniel Mosby, chief executive officer Ms Phillipa Bauer, the sponge farm project manager Mr Chris Robertson, and the entire sponge farm team. Our link to your advice, local knowledge, insights into sponge and reef ecology, and vision as Australia’s pioneering sponge farmers has made an enormous contribution to this project. We also thank the traditional owners at Masig, including the PBC chair Mr Frances Nai, who welcomed our team to Masig on many occasions. Finally, we say a big Esso to the community of Masig, who always made the team feel welcome, and ensured we all ate many Wongai to ensure our continued return.
Summary

- Sponges are an important component of benthic communities throughout the coral reefs of Torres Strait. They play important functional roles in coral reef ecosystems and several species show commercial potential for use as biomaterials. One suitable commercial bath sponge species, *Coscinodera matthewsi*, occurs throughout Torres Strait with very high abundance around Masig in Central Torres Strait.

- A previous project, funded through the CRC Torres Strait, and undertaken by AIMS and the (then) Yorke Island Community Council, established farming protocols and best-environmental-practice guidelines to grow *C. matthewsi* at Masig Island. That project has since underpinned the commencement of Australia’s first commercial sponge farm at Masig, by Kailag Enterprises Limited.

- The current project, conducted over four years within the MTSRF with funding and in-kind support from MTSRF, AIMS, TSRA and KEL, was initiated from two perspectives. Firstly, as sponges are a major determining component of Torres Strait benthic communities, there was a need to understand the ecology and demography of sponges, especially *C. matthewsi*, as a possible surrogate for ecosystem connectivity and health of benthic communities in Torres Strait. Secondly, there was a need to establish a firmer knowledge base to underpin the sustainable environmental management of Torres Strait’s newest marine industry – sponge farming.

- Benthic surveys at a range of scales (between and within island groups) have confirmed that *C. matthewsi* occurs throughout eastern and central Torres Strait, with extremely high abundance around Masig and Kodal Islands in central Torres Strait. Preferred habitat is reef slope between 2-14m, and consolidated or mobile hard substrate (e.g. coral rubble), with smaller sponges found more often on the latter. *C. matthewsi* at Masig is typically lobate in morphology.

- Benthic surveys at Masig have been carried out throughout this project, and combined with survey data from the previous CRC project to allow a long term assessment of distribution and abundance of different sponge size classes over a fine spatial scale. Sponge abundance was highly variable and dominated by sponges <20cm. Much of the observed variability may be explained by a potential reliance of this species on fragmentation as a means of reproduction within the population. Supporting evidence for an important role for fragmentation includes: Prevalence of unstable (rubble) substrate and the lobate morphology of sponges both facilitating fragmentation; higher numbers of small sponges at sites exposed to strong south-easterly weather (and therefore more prone to fragmentation caused by disturbance); documented cases of sponges attached to rubble moving short distances; and the patchy distribution of sponge abundance (with patches possibly focused around original parent sponges). Larval recruitment and mortality events also undoubtedly have roles in determining sponge demographics at Masig. Future fine-scale genotyping studies could confirm the relative importance of larval vs. fragment recruitment.

- Despite intensive benthic surveys between 2004-2010 around Masig and elsewhere in Torres Strait, sponge disease was mostly absent, and only noted in a very small proportion of the sponge community, at the very end of the current project. Future research into the microbiology of this species and the epidemiology of potential pathogens is recommended, if the health of this species can be used as a measure of reef health in the future.
Distribution and abundance patterns reported at Masig during this project combined with conservative (underestimated) habitat area measurements, suggest that seed stock harvest guidelines previously recommended (target sponges >15cm; ensure at least 30% of each donor sponge remains attached to the substrate; harvest up to 10% of the population) would support the sustainable harvest of at least 700 donor sponges per year. Thus, the current operation can be sustained by the existing population at masig.

Recruitment dynamics of sponges and other benthic sessile organisms to the Masig Island group were assessed using experimental settlement surfaces. Recruitment activity for six taxonomic groups was observed. Polychaetes dominated recruitment abundance while algae showed the highest percentage cover.

An assessment of the larval ecology and behaviour of *C. matthewsi* was undertaken using the facilities of Orpheus Island Research Station (GBR) during the summer spawning season in December 2009. Field observations at Masig confirmed that the Masig population of this species reproduced during the same period. This species was shown to be viviparous, releasing tufted parenchymella larvae during daylight hours over an extended period. Larval swimming at the surface was observed for 18-24 hours, and settlement to substrate was enhanced on established biofilms, with 80% of larvae settled after 42 hours. These results indicate that, coupled with strong currents in Torres Strait, this species is certainly capable of broad scale distribution through larval dispersal. Results also suggest that, depending on post-settlement mortality and growth rates, larval production and selective settlement onto farming structures has potential for future development of more efficient sponge farm seeding methodology.

Partial 28S rRNA sequences of sponges, and DGGE of 16S rDNA sequences from sponges’ microbial in-fauna, coupled with larval biology and hydrodynamics data, support a high degree of connectivity in *C. matthewsi* populations between central and eastern Torres Strait reefs, and consequential low risk in translocation at that scale.

A series of experiments were undertaken to explore a range of collection and handling strategies to see if they could enhance sponge farm growth and survival. While undertaken within and administered by the MTSRF program, these experiments were funded entirely by a special appropriation from TSRA. The results show that: sponges can withstand limited exposure to air without any detrimental affect on survival and growth; the squeezing of sponges required to place them into mesh panels has a negative impact on survival and growth; and survival and growth can be improved if sponge explants are given a nursery stage in a mesh tray to recover from being cut into explants and prior to deployment to farm panels. The results also showed that explant growth and survival is not dependent on the size of the donor sponge, nor the area of the donor sponge from which the explant is taken. These results can help optimize operational strategies on the sponge farm.

Observations and anecdotal evidence from sponge farm operations indicate that the mesh panel farming methods recommended by previous research have proven to be unsuitable for the farm site, due to unexpected excessive levels of fouling away from the reef edge and without the controlling benefit of grazing fishes. Farm operations have moved from panels to individual sponge attachment using cable ties, but this is also proving problematic. Future research is needed to trial alternative methods of sponge attachment to low-fouling farming structures.

Torres Strait Islanders have been actively engaged throughout this project at a range of levels. Masig Island locals John Morris, Samson Lowatta and Gavin Mosby were employed on the project during the study, and were instrumental to its success.
Objectives and outcomes

<table>
<thead>
<tr>
<th>Objective</th>
<th>Outcome</th>
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</table>
| 1. Undertake an assessment of the distribution and abundance of wild commercial sponge species in Torres Strait, identifying elements of environmental risk (evidence of disease). | • Distribution and abundance at a range of scales in eastern-central TS and at Masig.  
• Demonstrated low incidence of disease in wild populations |
| 2. Determine connections between sponge populations and risks in translocation; | • Evidence for broad-scale connectivity across eastern and central TS, and low translocation risk at that scale. |
| 3. Determine patterns of sponge recruitment/mortality and the environmental risk of seed stock harvest leading to development of a sustainable seed collection strategy. | • Five years of temporal trends in sponge demographics at fine spatial scale around Masig.  
• Evidence that existing protocols for seed stock harvest are sustainable.  
• Evidence for importance of fragmentation as a reproductive strategy for this species.  
• Evidence for competent larvae also contributing to reproduction, with potential for future sponge farm seeding techniques. |
| 4. Develop optimal handling guidelines to improve sponge explant growth and survival | • Experimental support for improvements in explant handling and attachment. |

Outputs (see Appendix for links)

- Six direct and related peer-reviewed publications
- Additional four publications in preparation
- Two public meetings in Torres Strait
- Eight conference presentations
- Metadata and e-Atlas links
Introduction

Background

Sponges occupy all aquatic biotopes from the tropics to the poles with an estimated diversity of over 15000 species (Hooper and Levi 1994). They are implicated in the functioning of reefal ecosystems, playing important ecological roles including reef bio-erosion and consolidation, infaunal habitat refuges and benthic–pelagic coupling (review Bell 2008). In addition, there are several species that are commercially important as biomaterials, including their use as bath sponges (Prozonto 1999).

Commercial bath sponges (Order: Dictyoceratida) are characterised by a skeletal composition with high quality spongin which is targeted for both household and industrial applications. The supply of bath sponges has relied heavily on wild-harvest effort predominantly in the Mediterranean and Caribbean (Prozonto 1999). However, unregulated harvest effort, coupled with disease outbreaks, has placed enormous pressure on wild sponge populations in these regions with subsequent impacts on the supply of bath sponges (Prozonto 1999). The short fall in supply, originating from wild- harvest effort, provides an opportunity for the development of a bath sponge aquaculture industry, with the potential to meet future demand for bath sponges in a sustainable manner. Since 1999, the commercial potential of two Australian coral reef sponges, *Rhopaloides odorabile* and *Coscinoderma matthewsi*, has been explored (Evans-Illidge et al. 2006, Duckworth et al. 2007, Duckworth 2009), and a small scale community based aquaculture venture with *C. matthewsi* has been established in Micronesia (ref for Phonpei).

*Coscinoderma matthewsi* was first identified in Torres Strait during surveys in 2004. Reef surveys have shown that Torres Strait has a rich and often highly abundant sponge community, and that Masig is the site of the largest population of *C. matthewsi* found to date.

A market analysis of *C. matthewsi*, sourced from the Great Barrier Reef, revealed this sponge to comprise commercial grade spongin (Figure 1 a-b). *Coscinoderma matthewsi* occurs throughout several islands of the eastern and central Torres Strait, but shows highest abundances in the vicinity of Masig Island (Duckworth et al. 2007b). The key priority of CRC Torres Strait task 1.6 was to optimise the culture of *C. matthewsi*. The outcome of this project established that clonal propagules (explants) could be collected and grown in pearl panels (Figure 2), achieving both high survival and growth, thereby suggesting bath sponge aquaculture in the Torres Strait would be a viable industry. That project has since underpinned the commencement of Australia’s first commercial sponge farm at Masig, by Kailag Enterprises Limited.

MTSRF Project 1.3.2 has built on the output of previous sponge research to both further develop the sustainability of a future sponge industry in Torres Strait, and also to provide a knowledge base for sponges as an important and often dominant component of Torres Strait benthic communities. This project has benefited from several research leaders over the four operational years, who have contributed expertise to sometimes cross-disciplinary subject matter in the following chapters. Thus, individual authorship has been assigned based on these chapters.
Figure 1. (a) *C. matthewsi* live sponge and (b) skeleton displayed as a bath sponge.

Figure 2. Sponge explants deployed in pearl panels on long-lines for grow-out.
Field Work Program

Involvement of Torres Strait Islanders

The involvement of Torres Strait Islander has been integral to this project (Table 1). Field work has been undertaken with close assistance and guidance from Masig Islanders, with John Morris and Samson Lowatta employed for diving and boating assistance throughout the project, and more recently Gavin Mosby engaged as a regular and expert coxswain. The marine expertise and detailed local knowledge of these gentlemen has been instrumental during this project. The project also benefited more recently with close links to the sponge farm at Masig, and further interactions with local sponge knowledge from the sponge farm team at Kailag Enterprises Limited.

Table 1. Summary of field work undertaken for this project.

<table>
<thead>
<tr>
<th>Dates</th>
<th>Work undertaken</th>
<th>TS Islanders Involved</th>
</tr>
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<tbody>
<tr>
<td>11-18 September 2006</td>
<td>Public meeting, council meeting, Masig surveys</td>
<td>John Morris, Samson Lowatta, YICC</td>
</tr>
<tr>
<td>10-25 November 2006</td>
<td>Eastern and Central TS surveys</td>
<td>John Morris, Samson Lowatta</td>
</tr>
<tr>
<td>26 February to 12 March 2007</td>
<td>Masig surveys, monitor experiments</td>
<td>John Morris, Samson Lowatta</td>
</tr>
<tr>
<td>28 May to 4 June 2007</td>
<td>Masig surveys, monitor experiments</td>
<td>John Morris, Samson Lowatta</td>
</tr>
<tr>
<td>3-10 September 2007</td>
<td>Masig surveys, monitor experiments</td>
<td>John Morris, Samson Lowatta</td>
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<tr>
<td>6-13 November 2007</td>
<td>Masig surveys, monitor experiments</td>
<td>John Morris, Samson Lowatta</td>
</tr>
<tr>
<td>26 May to 6 June 2008</td>
<td>Masig surveys, monitor experiments</td>
<td>John Morris, Samson Lowatta</td>
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<tr>
<td>5-14 November 2008</td>
<td>Masig surveys</td>
<td>John Morris, Samson Lowatta</td>
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<tr>
<td>13-25 February 2009</td>
<td>Masig surveys</td>
<td>John Morris, Samson Lowatta, Gavin Mosby</td>
</tr>
<tr>
<td>1-15 June 2009</td>
<td>Masig surveys</td>
<td>John Morris, Samson Lowatta, Gavin Mosby, Solomon Elia and Joey Billy</td>
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<tr>
<td>2-9 November 2009</td>
<td>Masig surveys</td>
<td>John Morris, Samson Lowatta, Gavin Mosby</td>
</tr>
<tr>
<td>5-20 December 2009</td>
<td>OIRS larval behavior studies</td>
<td>John Morris, Samson Lowatta, Gavin Mosby</td>
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Evans-Illidge and Battershill

Distribution and abundance of C. matthewsi at two spatial scales: (1) across Central and Eastern Torres Strait; and (2) within the Yorke Islands.

Alan Duckworth, Carsten Wolff and Heidi Luter

Introduction

Distribution and size frequency patterns of sessile organisms such as sponges may vary among and within neighbouring islands and regions. Heterogeneity over both small and large spatial scales results from the interaction of physical, biological and stochastic factors influencing the distribution and abundance of individual species (Wilkinson and Cheshire 1989, Zea 2001). Sponges are an important component in many benthic communities and can dominate the benthos in some regions in terms of biomass and diversity (Schmahl 1990, Wilkinson and Cheshire 1990). Being efficient filter feeders of small particulate matter, sponges also represent an important energy coupling between the benthic and pelagic communities (Reiswig 1971, Pile et al. 1996, Duckworth et al. 2006).

The abundance and distribution patterns of sponges can be influenced by water flow and depth (Wilkinson and Evans 1989, Roberts and Davis 1996), larval dispersal and recruitment patterns (Maldonado and Young 1996), asexual fragment and propagule development and dispersal (Wulf 1985, Kelly-Borges and Bergquist 1988, Battershill and Bergquist 1998), predation (Dunlap and Pawlik 1996), light intensity (Wilkinson and Trott 1985) and substrate and habitat type (Reiswig 1973, Adjeroud 1997). Environmental and biological factors such as disease can also generate randomness in sponge distribution (Zea 2001). The influence or impact of each factor varies between sponge species, often restricting species to a specific area or depth (Wilkinson and Evans 1989) and exacerbating heterogeneity in community structure between and within reefs or islands.

Structuring factors that promote patchy distributions may also influence size frequency patterns of sponges over short spatial scales. The effect of a physical or biological factor on size frequency patterns is often complex (Turon et al. 1998, Bell et al. 2002) and may have a positive or negative impact depending on its level of intensity. For example, sponge growth rates will generally increase as water flow increases because of the greater availability of suspended food (Wilkinson and Vacelet 1979, Duckworth and Battershill 2003), however, high water flow can also damage sponges, remove tissue, and decrease their size (Trautman et al. 2000)

Two survey experiments were done in Torres Strait examining the abundance and size frequency distributions of C. matthewsi. The first experiment surveyed around islands in the central and eastern Torres Strait, to determine where C. matthewsi is most abundant. This survey also collected information on the distribution and abundance of several commercially and ecologically important species such as coral trout, crown-of-thorn-starfish, sea cucumbers and snappers, and these results have been reported separately and submitted to ReefCheck. The second survey was based in the area where C. matthewsi is most common, to further determine what factors influence its size and abundance.
Ecological Role and Potential Value of Sponges in Torres Strait: Final Report 2010

Methods

Central and Eastern Torres Strait survey

Study area and sampling strategy

The study area in Torres Strait is situated between Papua New Guinea and northern Queensland, Australia, and is bordered by the Warrior Reefs to the west and the edge of the continental shelf to the east (Figure 3); this represents the known distribution of *C. matthewsi* in Torres Strait (Duckworth et al. 2007). Surveys for *C. matthewsi* were done at five island-groups: Ugar (Stephen Is) and Erub (Darnley Is) in eastern Torres Strait and Masig (Yorke Is), Poruma (Coconut Is) and Warraber (Sue Is) in central Torres Strait (Figure 3). All five islands are inhabited, with populations in the low hundreds. The island-groups were, on average, 66 km (±SE = 11) km apart from each other. Ugar and Erub are both volcanic islands, rising above 30 m, while the three central Torres Strait island-groups consist entirely of sand cays and are generally low-lying (<10 m in height). All surveyed islands are small in size (<10 km²) and are enclosed by a fringing coral reef. The reef slope generally starts at a depth of 5 m (mean low water) and stops at 15 m, descending at an angle ranging from 20 to 60°. Maximum depth between neighbouring islands and reefs is approximately 30 m, with the substrate consisting of muddy sand (Harris 1988). South-easterly trade winds are common from April to December, while monsoonal weather patterns with more northerly winds dominate in summer.

![Figure 3. Map of Torres Strait showing the five island-groups surveyed for *C. matthewsi*.](image)
Sponge surveys were done at 7 or 8 randomly selected locations in each island-group, with each location ≥2 km apart, averaging 8 km. The locations were situated around each main island (e.g. Ugar, Masig) and neighbouring islands and reefs in each island-group. Each location was divided into two sites, approximately 200 m apart. At each site, three 30 x 1 m strip transects were quantitatively surveyed for *C. matthewsi* sponges. Transects were separated by at least 20 m apart to retain independence and all were done between 7-11 m, a depth where *C. matthewsi* is common in Torres Strait (Duckworth and Wolff 2007). Transect depth did not vary significantly between island-groups (Nested ANOVA: $F_{DF=4,32}=1.48; P=0.231$) nor between locations nested within island-groups (Nested ANOVA: $F_{DF=32,37}=1.53; P=0.105$).

To examine size frequency distributions patterns, the greatest dimension of every *C. matthewsi* was measured by a ruler attached to the dive slate and recorded. For graphical interpretation, sponges were grouped into 2 or 5 cm size classes. For any diseased sponge, the percent of infected or necrotic pinacoderm was noted. All surveys were done in November 2006, thus limiting possible temporal variation in sponge size and abundance influencing the results.

**Environmental factors**

For each transect reef slope was also recorded and the percentage of consolidated limestone rock (hereafter rock), dead coral rubble (rubble) and sand. These environmental variables are quick to measure, necessary for the area covered and were previously found to partially influence the distribution patterns of dictyoceratid sponges such as *C. matthewsi* in Torres Strait (Duckworth *et al.* 2007).

**Data analysis**

Abundance of *C. matthewsi* across spatial scales was analysed using a nested ANOVA, with island-group as a fixed factor, and location and site as nested factors. To meet assumptions of ANOVA, data was log (x+1) transformed and island-groups with less than 25 sponges were not included in the analysis. The Tukey-Kramer Multiple Comparison (TKMC) Test was used to determine which island-group(s) differed from each other in sponge abundance (and size).

Sponge size was compared among island-groups using one-way ANOVA. Only island-groups with greater than 25 recorded sponges were analysed to reduce the probability of a Type I error occurring due to different sample sizes (Zar 1999). Data was log transformed. For each island-group, mean sponge size, standard-deviation, 95th percentile and coefficient of variation (CV) were calculated on raw, untransformed data. Because the maximum size of sessile invertebrates can be influenced by chance events, the 95th percentile is used instead to compare the upper size limit across space (Soong 1993, Meesters *et al.* 2001). The CV is a measure of the amount of variation in a population irrespective of mean size. Sponge size for each island-group was also analysed using single Kolmogorov-Smirnov tests with Lilliefors adjustment (P>0.05) to determine if the size data has a normal distribution. To ensure that each test had a similar statistical power, 50 *C. matthewsi* individuals were randomly selected if sample number from an island group exceeded 60 recorded sponges. Skewness ($g_1$) values were calculated as well, with a positive value indicating a greater proportion of small individuals and a negative value indicating that the population is dominated by large individuals. For each island-group, Kolmogorov-Smirnov and skewness tests were done on both raw and log-transformed data, to determine if a log-transformation can normalise the size data for a sponge as shown for many hard coral species (Bak and Meesters 1998, Vermeij and Bak 2003).

To investigate variation in size distribution over small spatial scales, sponge size was analysed in the island-group where *C. matthewsi* was most abundant using a nested ANOVA.
with location as a fixed factor and site nested; data was log-transformed. Only locations where both sites had >10 individuals were analysed to reduce the likelihood of a Type I error. For each analysed site, mean sponge size, standard-deviation, 95th percentile and coefficient of variation (CV) were calculated.

Separate nested ANOVAs were done on each environmental factor to test for differences among island-groups, locations and sites. All environmental factors were arcsine transformed.

**Masig island-group survey**

**Study area and sampling strategy**

This study was done around Keats, Kodall and Masig Islands, located in central Torres Strait and where *C. matthewsi* is most abundant. Keats, Kodall and Masig are sand cays, low-lying (<10 m in height) and small in size (<5 km²). Fringing coral reef surrounds all islands, with broken reef connecting Kodall and Masig. The coral reef slope generally starts at a depth of 6 m (MLW) and stops on sand at 15 m, descending at an angle ranging from 20 to 60°. South-easterly trade winds (15-20 knots) are common from April to December, while monsoonal weather patterns with more northerly winds.

![Figure 4. Map of Torres Strait showing the surveyed sites (1-11) around Keats, Kodall and Masig.](image.png)
Abundance between depths and microhabitats

In March 2007, C. matthewsi was surveyed at 11 sites on coral reef at Keats, Kodall and Masig, with locations at least 1 km apart. At each site, C. matthewsi was surveyed at both shallow (4-6 m) and deep (10-12 m) depths, with the former generally on the reef flat. Three 20 x 1 m transects were examined at each depth, with transects separated by at least 20 m to retain independence. For each transect, divers recorded every C. matthewsi sponge found within 1 m of one side of the transect line. Basic environmental factors were also noted and for each transect we estimated the degree of reef slope and the percentage of dead coral rubble (rubble), sand and consolidated limestone rock (hereafter rock) free of living organisms.

For each sponge, the substrate type that it was attached to was recorded. Three types were identified: rock; rubble; and sand, with grain size ranging from 0.5-2 mm. These three substrate types vary in their level of stability for sponge attachment and growth, with rock providing a secure immobile base for sponges, while sand being easily moved by water currents is a relatively insecure and fluid substrate for attachment. Rubble provides an intermediate level of stability; a separate study at Masig has found that rubble pieces (with a sponge attached) can move several meters between monitoring events (3 months), at times causing the sponge to become partially buried by rubble or sand. For each sponge we also recorded whether it was living in an exposed microhabitat, such as on top of rock fully exposed to the ambient water flow, or in a sheltered microhabitat, such as under an overhang or protected between surrounding rocks.

To examine size frequency distribution patterns, the greatest length, width and height of every C. matthewsi was measured by a ruler attached to the dive slate and recorded. For graphical interpretation, sponges were grouped into 2 cm size classes. Some individuals of C. matthewsi in Torres Strait have a palmate morphology, where large lobes project upwards from the main sponge base. For each measured sponge, the number of lobes was also counted and recorded.

Data analysis

A MANOVA was done to examine the influence of site (random factor), depth (fixed factor) and their interaction on the four environmental factors (slope, %rock, %rubble and %sand), with factors arcsine transformed (Zar 1999). For all environmental factors (and size comparisons; see below), within-cell correlation analyses detected no multicollinearity problems (r-squared <0.99) while Bartlett-Box homogeneity tests determined that the covariance matrices are equal (P>0.05). Wilks’ lambda statistic was used to compute F-ratios and data was then analysed further by ANOVA.

The abundance of C. matthewsi between sites and depth was analysed using a two-way ANOVA, with location and depth as a random and fixed factor, respectively. Data was log (x+1) transformed to meet the assumptions of ANOVA. Chi-Square analysis was used to compare observed and expected frequencies of sponges living on rock and rubble at 6 and 12 m. If a significant result was obtained, the Chi-Square analysis (Zar 1999) was subdivided to determine which substrate-depth treatment(s) primarily contributed to the nonconformity of the data.

Differences in sponge length, width and height and lobe number between depths and substrate types were analysed using MANOVA. To ensure similar sample sizes between treatments to reduce the probability of a Type I error (Zar 1999), 50 individuals were randomly selected if sample number from a treatment exceeded 50 sponges; data was log (x+1) transformed. Size frequency distributions for C. matthewsi do not vary greatly over distances of km’s (previous study), thus data in this study was pooled across sites. Wilks’
lambda statistic was used to compute F-ratios and data was then analysed further by ANOVA.

For significant factors (e.g. depth, depth*substrate), mean size, standard deviation, 95th percentile and coefficient of variation (CV) were calculated on raw, untransformed data for sponge length, width and height. Because the maximum size of sessile invertebrates can be influenced by chance events, the 95th percentile is used instead to compare the upper size limit across space (Soong 1993, Meesters et al. 2001). The CV is a measure of the amount of variation in a population irrespective of mean size. Each depth- substrate class was also analysed using single Kolmogorov-Smirnov tests with Lilliefors adjustment (P>0.05) to determine if sponge length, width and height have a normal distribution. Sample numbers need to be similar between classes to ensure that each test has a similar statistical power. If need be, a minimum of 50 C. matthewsi individuals were randomly selected to ensure similar sample numbers. Skewness (g1) values were calculated as well, with a positive value indicating a greater proportion of small individuals and a negative value indicating that the population is dominated by large individuals. For each depth- substrate class, Kolmogorov-Smirnov and skewness tests were done on both raw and log-transformed data. Exposure (sheltered vs. exposed) was not included in this analysis because sample number in some depth- substrate- exposure classes would be too small.

Results

Central and Eastern Torres Strait survey

Spatial variability of environmental factors

The degree of reef slope and percentage of rock and sand varied significantly among the five island groups (Table 2). Reef slope was steepest at Masig where it averaged 46°; at the four other island-groups it averaged 23-30°. Reef slope also varied significantly between locations nested within island-groups (Table 2), ranging 10-56° at Erub for example. The percentage of rock was greatest at Ugar, Erub and Masig where consolidated limestone constituted about half of the reef substrate, while at Poruma and Warraber it averaged less than 35%. Approximately one-third of the rock substrate at each island-group was occupied by living organisms, predominantly Scleractinian and sponge species (unpublished data), leaving two-thirds as ‘free space’. The percentage of sand was highest at Warraber (>30%) and lowest at Ugar (<20%). At all island-groups, approximately 33% of the substrate consisted of dead coral rubble. All four environmental factors differed significantly among sites (Table 2), indicating variation over short spatial scales (~200 m).

Table 2. Summary of nested ANOVA’s examining variation of the four environmental factors among island-groups, locations and sites. All factors were arcsine transformed. F-ratios shown. Probability: * = <0.05; **= <0.001; *** = <0.001.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Reef slope</th>
<th>% Rock</th>
<th>% Rubble</th>
<th>% Sand</th>
</tr>
</thead>
<tbody>
<tr>
<td>Island-group</td>
<td>4</td>
<td>3.52*</td>
<td>4.25**</td>
<td>2.10</td>
<td>3.88*</td>
</tr>
<tr>
<td>Location (Island)</td>
<td>32</td>
<td>4.78***</td>
<td>0.91</td>
<td>1.47</td>
<td>0.72</td>
</tr>
<tr>
<td>Site (Location(Island))</td>
<td>37</td>
<td>2.10***</td>
<td>5.08***</td>
<td>5.58***</td>
<td>4.54***</td>
</tr>
<tr>
<td>Error</td>
<td>148</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Spatial variability in abundance

In total, 436 *C. matthewsi* individuals were counted and measured during the survey, with the sponge species found at all five island-groups (Figure 5). However, *C. matthewsi* was uncommon at Warraber with only 19 sponges recorded and therefore Warraber was not included in the statistical analysis for abundance comparisons. The abundance of *C. matthewsi* varied significantly among island-groups in Torres Strait (Nested ANOVA: $F_{DF=3,26}=9.94$; $P<0.001$), being most abundant at Masig with 5.5 sponges per 30 m$^2$ on average (Figure 5). On coral reefs at Masig, *C. matthewsi* is a dominate sponge species in both abundance and biomass; in this survey, 233 *C. matthewsi* sponges were recorded from Masig. In contrast, mean abundance (and total number) was similar according to the TKMC Test among Ugar, Erub and Poruma being 1.7 (81), 1.1 (53) and 1.2 (50) sponges per 30 m$^2$ respectively. At Warraber, mean abundance was 0.5 *Coscinoderma* per transect.

Sponge abundance varied significantly among locations within each island group (Nested ANOVA: $F_{DF=26,30}=1.93$; $P=0.042$). At Erub and Poruma, for example, *C. matthewsi* was relatively common at some locations but not recorded at neighbouring locations a few kilometres away (Figure 5). Among the seven locations at Masig, mean abundance ranged from 0.8 to 8.8 sponges per 30 m$^2$.

The abundance of *C. matthewsi* also varied significantly among sites (Nested ANOVA: $F_{DF=30,120}=2.28$; $P<0.001$). In some locations at Ugar, Erub and Poruma, *C. matthewsi* was found at one site but not recorded 200 m away (Figure 5). In locations where *C. matthewsi* was recorded at both sites, density could vary by a factor of 10. Of the 24 locations within *C. matthewsi*, almost half (11/24) had >75% of its individuals recorded from one site. Variation in *C. matthewsi* abundance between neighbouring sites was therefore a common phenomenon in Torres Strait.

Of the 436 *C. matthewsi* individuals recorded from Torres Strait, only one was diseased with approximately 20% of its pinacoderm necrotic. The diseased sponge was found at Erub, living within several meters of healthy, non-diseased *C. matthewsi* individuals.

Spatial variability in size among island-groups

The size frequency distribution of *C. matthewsi* varied significantly among island groups in Torres Strait (One-Way ANOVA: $F_{DF=3,414}=16.93$; $P<0.0001$). The TKMC Test determined that sponge size was similar between Masig and Poruma, and between Ugar and Erub (Table 3; Figure 6). At Warraber, which was not included in the statistical analysis because of low sponge abundance, mean length was similar to Ugar and Erub (Table 3). Size results for the 95th percentile showed a similar pattern, being highest at Masig and Poruma and smallest at the three remaining island-groups. At Masig and Poruma, *C. matthewsi* could be expected to grow to over 20 cm in length. The coefficient of variation varied little among the island-groups (Table 3), indicating that variation around the mean was similar throughout Torres Strait.

The Kolmogorov-Smirnov Normality test on raw size data was significant for Ugar, Erub and Masig (Table 3), indicating that the untransformed size structure of *C. matthewsi* at three of the five island-groups did not have a normal distribution. After a log-transformation, the size structure at Ugar, Erub and Masig became normally distributed. The untransformed size distributions at all island-groups were positively skewed, indicating that small individuals dominant the *C. matthewsi* population at each island-group. The proportion of small individuals was greatest at Ugar and Masig (Table 3), where *C. matthewsi* was most abundant. Log-transforming the size data reduced the level of skewness ($g_1$ value closer to zero) at four of the five island-groups. The one exception was at Poruma, where the raw, untransformed size data had a normal distribution and was only slightly positively skewed (Table 3).
Figure 5. Mean abundance of *C. matthewsi* at each island-group, with nested sites and locations.
Table 3. Size distribution parameters for *C. matthewsi* in each island-group giving sample number (N), mean size, standard deviation (SD), coefficient of variation (CV) and the 95th percentile (95th); all measurements in centimetres. Also shown are results from the single Kolmogorov-Smirnov Normality Test (KS) and skewness (g1) tests for untransformed and log (+1) transformed data. Probability for KS either non-significant (n.s.) or significant (<0.05); *test done on 50 randomly chosen sponges.

<table>
<thead>
<tr>
<th>Island-group</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>CV</th>
<th>95th</th>
<th>Untransformed data</th>
<th>Log-transformed data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>KS</td>
<td>g1</td>
</tr>
<tr>
<td>Ugar</td>
<td>81</td>
<td>6.9</td>
<td>4.1</td>
<td>0.6</td>
<td>15.0</td>
<td>&lt;0.05*</td>
<td>1.24*</td>
</tr>
<tr>
<td>Erub</td>
<td>53</td>
<td>7.6</td>
<td>5.2</td>
<td>0.7</td>
<td>18.4</td>
<td>&lt;0.05</td>
<td>1.08</td>
</tr>
<tr>
<td>Masig</td>
<td>233</td>
<td>10.3</td>
<td>6.1</td>
<td>0.6</td>
<td>22.0</td>
<td>&lt;0.05*</td>
<td>1.22*</td>
</tr>
<tr>
<td>Poruma</td>
<td>50</td>
<td>12.7</td>
<td>6.4</td>
<td>0.6</td>
<td>24.1</td>
<td>n.s.</td>
<td>0.34</td>
</tr>
<tr>
<td>Warraber</td>
<td>19</td>
<td>7.5</td>
<td>6.1</td>
<td>0.8</td>
<td>20.1</td>
<td>n.s.</td>
<td>0.96</td>
</tr>
</tbody>
</table>

Spatial variability in size among locations and sites

At Masig, the size frequency distribution of *C. matthewsi* was similar between locations (Nested ANOVA: F_{DF=4,5}=0.93; P=0.515); two Masig locations were not included in the analysis because <10 sponges were recorded from at least one site. *C. matthewsi* size patterns varied significantly, however, between sites nested within locations (Nested ANOVA: F_{DF=4,191}=4.27; P=0.001), indicating that size frequency distributions can vary greatly over short spatial scales (~200 m). At location 2, for example, 60% of *C. matthewsi* sponges at site 1 were larger than 10 cm while >90% of individuals at site 2 were smaller than 10 cm (Figure 7). A greater proportion of large sponges at site 1 resulted in mean size and 95th percentile values double that recorded from site 2 (Table 4). Although the results shown in Table 4 have to be treated cautiously because of low sample number at some sites, some patterns emerge. The coefficients of variation were generally similar between neighbouring sites, which suggests the amount of variation around a mean was similar across short spatial scales. The mean size and 95th percentile value can vary greatly between sites at some but not all Masig locations.

Table 4. Size distribution parameters for *C. matthewsi* between sites at Masig locations showing sample number (N), mean size, standard deviation (SD), coefficient of variation (CV) and the 95th percentile (95th); all measurements in centimetres. Only locations where both sites had ≥10 individuals were compared.

<table>
<thead>
<tr>
<th>Location</th>
<th>Site</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>CV</th>
<th>95th</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>1</td>
<td>20</td>
<td>13.9</td>
<td>5.8</td>
<td>0.4</td>
<td>21.2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>14</td>
<td>6.4</td>
<td>3.3</td>
<td>0.5</td>
<td>10.7</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>33</td>
<td>8.5</td>
<td>4.9</td>
<td>0.6</td>
<td>18.0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>12</td>
<td>9.3</td>
<td>8.7</td>
<td>0.9</td>
<td>23.7</td>
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<tr>
<td>4</td>
<td>1</td>
<td>21</td>
<td>9.0</td>
<td>4.8</td>
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<td>15.0</td>
</tr>
<tr>
<td></td>
<td>2</td>
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<td>12.3</td>
<td>7.8</td>
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<td>25.0</td>
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<tr>
<td>5</td>
<td>1</td>
<td>20</td>
<td>9.9</td>
<td>5.6</td>
<td>0.6</td>
<td>18.3</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>20</td>
<td>7.4</td>
<td>3.3</td>
<td>0.5</td>
<td>12.1</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>12</td>
<td>13.8</td>
<td>8.4</td>
<td>0.6</td>
<td>27.3</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>17</td>
<td>12.5</td>
<td>4.6</td>
<td>0.4</td>
<td>18.8</td>
</tr>
</tbody>
</table>
Figure 6. Size frequency distributions of *C. matthewsi* between island-groups.
Figure 7. Size frequency distributions of *C. matthewsi* between sites and locations at Masig.
**Masig island-group survey**

**Environmental factors**

A MANOVA of the four environmental factors found a significant interaction between site and depth (Wilks’ Lambda: $F_{df=10,157}=2.92; P<0.001$). Of the ANOVAs examining the four factors, the interaction term was significant for reef slope ($F_{df=10,44}=4.68; P<0.001$) and %rubble ($F_{df=10,44}=2.48; P=0.018$). Although the difference in reef slope between 6 and 12 m varied greatly across space (sites), deep reef at all sites was steeper (mean=37°) than shallow reef (14°) (Figure 8). Both shallow and deep reef had similar percentages of rubble (~30%), but rubble cover at 6 and 12 m varied greatly between neighbouring sites (Figure 8). The percentage of rock was similar between depths, sites and there was no interaction effect (all with $P>0.05$). On average, 18% of the substrate in the study area consisted of rock free of living organisms (Figure 8). Percent sand did not have a significant interaction term but varied significantly between depths ($F_{df=1,10}=25.21; P<0.001$). On average, shallow reef had half the amount of sand (16%) than deep reef (33%) (Figure 8), probably because sand is more easily swept away in the shallows. The percentage of sand also varied significantly between sites ($F_{df=10,44}=5.41; P<0.001$), ranging from 12% to 43% among neighbouring sites. In addition to rock, rubble and sand, living organisms occupied the remaining substrate cover on each transect. Living organisms, dominated by scleractinian corals, occupied more space on shallow reef (39%) than deep reef (18%).

![Figure 8. Mean values of reef slope, %rock, %rubble and %sand between depths at each surveyed site. Error bars represent variation (1 standard error) among transects.](image-url)
Abundance between sites and depths

In total, 269 *C. matthewsi* sponges were counted and measured during the survey. The abundance of *C. matthewsi* varied significantly between sites (ANOVA: $F_{df=10,44}=3.09$; $P=0.005$), ranging from 1.2 to 7.0 individuals on average per 20 m$^2$ (Figure 9). Reef depth also had a significant effect on abundance (ANOVA: $F_{df=1,10}=60.25$; $P<0.001$), with sponge density almost three times greater at 12 m (mean=5.9 sponges per 20 m$^2$) than at 6 m (2.2) (Figure 9). There was no significant interaction term (ANOVA: $F_{df=10,44}=0.71$; $P=0.713$). No diseased sponge was found.

![Figure 9](image)

**Figure 9.** Mean abundance of *C. matthewsi* between depths at each surveyed site. Error bars represent variation (1 standard error) among transects.

Abundance between depth, substrate types and exposures

All individuals of *C. matthewsi* except two were found attached to and living on either rock or rubble. The exceptions included one sponge was found growing on sand at a depth of 12 m in an exposed microhabitat. This sponge appeared healthy, was of average size, and had its base buried ~5 cm into sand; moving the sand away from the sponge’s base determined that it was only attached to sand. The second sponge was found growing directly on coral (*Porites* sp.). This sponge was relatively small in size and appeared healthy. Because only one sponge each was found living in sand or on coral, the Chi Square test analysed frequencies of sponges recorded from rock and rubble substrate only.

Of the 267 individuals included in this analysis, the number of *C. matthewsi* individuals found growing on rock and rubble at each depth is shown in Table 5. This table also shows the expected frequencies, calculated using the availability of rock and rubble at each depth. The Chi-Square analysis determined a difference between the observed and expected frequencies ($\chi^2_{df(3)}=33.0$, $P<0.0001$), indicating that *C. matthewsi* individuals were found at one or more substrate-depth combinations at frequencies too different to be attributed to stochastic processes. Subdividing the Chi-Square analysis (Zar 1999) determined that the observed and expected frequencies were similar for sponges found in the treatments rock-shallow, rock-deep and rubble-deep ($\chi^2_{df(2)}=5.54$, $P=0.063$). When the frequency of sponges found in the rubble-shallow treatment is tested against the combined frequencies for the other three treatments, the Chi-Square analysis is highly significant ($\chi^2_{df(1)}=28.3$, $P<0.0001$). This result indicates that nonconformity of sponge abundance data between substrates and
depths results largely from significantly less *C. matthewsi* found growing on rubble at shallow depths than expected considering the availability of rubble at 6 m (Table 5).

<table>
<thead>
<tr>
<th>Substrate, depth</th>
<th>Observed</th>
<th>Expected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rock, shallow</td>
<td>40</td>
<td>46</td>
</tr>
<tr>
<td>Rock, deep</td>
<td>91</td>
<td>55</td>
</tr>
<tr>
<td>Rubble, shallow</td>
<td>33</td>
<td>83</td>
</tr>
<tr>
<td>Rubble, deep</td>
<td>103</td>
<td>83</td>
</tr>
</tbody>
</table>

Depth interacting with exposure also influences the abundance of *C. matthewsi* (Figure 10). *C. matthewsi* is more common in sheltered microhabitats at shallow depths, but more abundant in exposed microhabitats on deeper reef. Similar findings between rock and rubble at each depth, suggests that substrate type does not influence this generalisation.

**Figure 10.** Percent of sponges found in sheltered and exposed microhabitats per depth-substrate treatment. The number on each scale represents the number of individuals per treatment.

**Size frequency distributions between depths and substrates**

A MANOVA of sponge length, width, height and lobe number found a significant effect of depth (Wilks’ lambda $F_{df(4,166)}=9.13; P<0.0001$) and substrate (Wilks’ lambda $F_{df(4,166)}=2.77; P=0.028$) and no significant depth*substrate interaction (Wilks’ lambda $F_{df(4,166)}=0.88; P=0.479$). Subsequent ANOVA’s determined that survey depth had a significant effect on all four sponge parameters: length ($F_{df=1,169}=6.90; P=0.009$); width ($F_{df=1,169}=5.46; P=0.021$); height ($F_{df=1,169}=7.47; P=0.007$); and lobe number ($F_{df=1,169}=32.3; P<0.0001$). ANOVA’s also
determined that substrate type influenced both sponge length \( (F_{df=1,169}=8.97; \ P=0.003) \) and width \( (F_{df=1,169}=4.40; \ P=0.037) \) but not height or lobe number \( (P>0.05) \).

For sponge length, individuals generally reached largest size when attached to rock and occurring on deeper reefs (Table 6; Figure 11). The result of the TKMC test, showing most similarities between substrate types, indicates that depth had the greatest influence on sponge length. This is supported by the 95\(^{th}\) percentile results, with sponges growing on rubble at 12 m expected to grow to twice the size of sponges on rubble at 6 m (Table 6). Further comparing maximum size between depths, only 1 individual was≥30 cm in length at 6 m while 11 sponges were≥30 cm in size on deep reef; in addition, 3 individuals were recorded from 12 m that were >40 cm in length (Figure 11). The coefficient of variations were largest for sponges growing on deep reef (Table 6), indicating that sponges found at 12 m vary more in length regardless of substrate type. Although substrate type is comparatively less important to size structure compared with depth, it can influence sponge length. In shallow water, for example, mean length of individuals growing on rock was more than 1½ times the length of sponges living on rubble. For all depth*substrate classes, the length frequency distributions using raw, non-transformed data were positively skewed (Table 6), indicating a greater proportion of small sponges (Figure 11). The skewness for each class was generally not improved after a log-transformation. The raw length data in the shallow*rubble, deep*rock and deep*rubble treatments produced significant Kolmogorov-Smirnoff tests (Table 6), indicating that the length structure of sponges in these three treatments did not have a normal distribution. A log-transformation “corrected” these distribution patterns.

Table 6. Length distribution statistics for C. matthewsi growing on rock and rubble substrate at shallow and deep depths. This table gives sample number (N), mean size, standard deviation (SD), coefficient of variation (CV) and the 95\(^{th}\) percentile (95\(^{th}\)); all measurements in centimetres. For mean size, depth*substrate treatments with the same letter have similar sized sponges according to the TKMC test. Also shown are results from the single Kolmogorov-Smirnov Normality Test (KS) and skewness \( (g_1) \) tests for untransformed and log (+1) transformed data. Probability for KS either non-significant (n.s.) or significant (<0.05); *test done on 50 randomly chosen sponges.

<table>
<thead>
<tr>
<th>Depth* substrate</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>CV</th>
<th>95(^{th}) Untransformed data</th>
<th>Log-transformed data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>KA</td>
<td>g_1</td>
</tr>
<tr>
<td>shallow, rock</td>
<td>40</td>
<td>12.0B</td>
<td>7.2</td>
<td>0.6</td>
<td>26.2</td>
<td>n.s</td>
</tr>
<tr>
<td>Shallow, rubble</td>
<td>33</td>
<td>7.2A</td>
<td>4.2</td>
<td>0.6</td>
<td>14.0</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Deep, rock</td>
<td>91</td>
<td>13.4B</td>
<td>9.8</td>
<td>0.7</td>
<td>30.0</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Deep, rubble</td>
<td>103</td>
<td>11.6AB</td>
<td>8.5</td>
<td>0.7</td>
<td>28.5</td>
<td>&lt;0.05*</td>
</tr>
</tbody>
</table>

Considering sponge width, individuals were generally widest when growing on rock and at deeper depths (Table 7; Figure 12). The TKMC test found that sponges growing on rubble at 6 m were significantly different in size to sponges attached to rock at 12m, with mean width of rubble*shallow sponges only two-thirds that found for rock*deep sponges. The 95\(^{th}\) percentile results indicate that sponges attached to rock at 12 m are expected to grow to double the width of sponges growing on rubble at 6 m (Table 7). Sponges attached to rock at 12 m also have the highest coefficient of variation (Table 7), indicating that these sponges show the most variation in width among the C. matthewsi population in the study area. The Kolmogorov-Smirnov tests on non-transformed width data for all depth*substrate treatments were significant, indicating that raw width data does not have a normal distribution regardless of depth or substrate type. Each Kolmogorov-Smirnov test was non-significant after a log-
transformation (Table 7), indicating that the log-width frequency patterns are normally distributed. The raw width data for each depth*substrate treatment was positively skewed (Table 7), indicating more small than large sponges across depths and substrate types. Similar to sponge length, a log-transformation on width data did not generally improve the skewness values (Table 7).

Table 7. Width distribution statistics for *C. matthewsi* growing on rock and rubble substrate at shallow and deep depths. See Table 6 for explanation of codes.

<table>
<thead>
<tr>
<th>Depth*substrate</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>CV</th>
<th>95th</th>
<th>KS</th>
<th>g₁</th>
<th>KS</th>
<th>g₁</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Untransformed data</td>
<td>Log-transformed data</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>KS</td>
<td>g₁</td>
<td>KS</td>
<td>g₁</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shallow, rock</td>
<td>40</td>
<td>6.4&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>3.7</td>
<td>0.6</td>
<td>13.1</td>
<td>&lt;0.05</td>
<td>1.01</td>
<td>n.s.</td>
<td>-0.67</td>
</tr>
<tr>
<td>Shallow, rubble</td>
<td>33</td>
<td>4.9&lt;sup&gt;A&lt;/sup&gt;</td>
<td>2.6</td>
<td>0.5</td>
<td>10.0</td>
<td>&lt;0.05</td>
<td>0.63</td>
<td>n.s.</td>
<td>-0.68</td>
</tr>
<tr>
<td>Deep, rock</td>
<td>91</td>
<td>7.7&lt;sup&gt;B&lt;/sup&gt;</td>
<td>5.3</td>
<td>0.7</td>
<td>20.0</td>
<td>&lt;0.05*</td>
<td>1.04*</td>
<td>n.s.*</td>
<td>-0.15*</td>
</tr>
<tr>
<td>Deep, rubble</td>
<td>103</td>
<td>6.7&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>4.2</td>
<td>0.6</td>
<td>14.5</td>
<td>&lt;0.05*</td>
<td>0.54*</td>
<td>n.s.*</td>
<td>-0.72*</td>
</tr>
</tbody>
</table>

Figure 11. Length frequency distribution of *C. matthewsi* between depths and substrate types.
Height of *C. matthewsi* varied between depths only, with mean height of individuals at 6 m approximately three-quarters the height of sponges at 12 m (Table 8). The 95th percentile results were more similar between depths (Table 8) however, showing that *C. matthewsi* in shallow water has only a slightly smaller maximum height than sponges living on deeper reef (Figure 13). These differences in mean size and 95th percentile results explain the high coefficient of variation and the high positive skewness value for sponges found at 6 m (Table 8). A log-transformation did help normalise the skewness of shallow-water sponges. In contrast, a log-transformation did not fully normalise the height data according to the Kolmogorov-Smirnov test, although it did improve the overall size structure (Table 8). Positive skewness values for sponges at 6 and 12 m indicates that *C. matthewsi* in the study area have a greater proportion of short than tall individuals (Figure 13).

Table 8. Height distribution statistics for *C. matthewsi* growing at shallow and deep depths. See Table 3 for explanation of codes. *test done on 73 randomly chosen sponges.

<table>
<thead>
<tr>
<th>Depth</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>CV</th>
<th>95th</th>
<th>Untransformed data</th>
<th>Log-transformed data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>KS</td>
<td>g&lt;sub&gt;1&lt;/sub&gt;</td>
</tr>
<tr>
<td>Shallow</td>
<td>73</td>
<td>6.5</td>
<td>5.1</td>
<td>0.8</td>
<td>17.2</td>
<td>&lt;0.05</td>
<td>2.16</td>
</tr>
<tr>
<td>Deep</td>
<td>194</td>
<td>8.6</td>
<td>5.7</td>
<td>0.7</td>
<td>19.4</td>
<td>&lt;0.05*</td>
<td>0.95*</td>
</tr>
</tbody>
</table>

**Figure 12.** Width frequency distribution of *C. matthewsi* between depths and substrate types.
Individuals of \emph{C. matthewsi} living at 6 and 12 m also varied greatly in lobe number. Almost 75\% of shallow-water sponges had no lobes, while most (62\%) deep-water individuals had one or more lobes (Figure 14). In addition, sponges with 5 of more lobes were rare at 6 m (1\%) but reasonably common at 12 m (17\%).
Discussion

The central and eastern Torres Strait survey determined that *C. matthewsi* is most abundant around Masig, supporting the findings from a Torres Strait wide survey in 2004 (Duckworth *et al.* 2007). *C. matthewsi* has not been found in western Torres Strait. High abundance of the bath sponge at Masig is due to the interaction of physical, biological and stochastic processes (Duckworth and Wolff 2007). One physical factor that can have a significant effect on distribution patterns of sponges is reef slope (Bell and Barnes 2000), with some species excluded from flat habitats due to high sedimentation levels clogging their inhalant canals preventing them filtering efficiently. In the present study, reef slope was greatest at Masig. Perhaps *C. matthewsi* in Torres Strait prefers steep habitats to reduce the smothering effects of fine sediments and detritus, which are common throughout the region. Masig also has relatively high levels of rock substrate, which the second survey determined to be an important requirement for both high abundance and large size of *C. matthewsi*. Rock substrate provides a secure substrate for attachment and subsequent high growth.

The *C. matthewsi* populations at Ugar, Erub, Masig, Poruma and Warraber were dominated by small individuals, with the size frequency distributions positively skewed at each island-group. The proportion of small sponges varied between island-groups, however, with a higher proportion of small individuals at Ugar and Masig than at Poruma. Several factors could cause these size differences between island-groups, with one major factor likely to be that successful sponge recruitment varies across space in Torres Strait. One reason for the relatively low recruitment of *C. matthewsi* to Poruma could be due to the lower levels of secure substrate (rock) for settlement. Objective 3, investigating the recruitment of bath sponges between island, depth and seasons, will help explain what factors and processes influence the recruitment of *C. matthewsi* to Torres Strait coral reefs.

Water movement greatly affects sponge recruitment and distribution patterns, through influencing larval and sponge fragment transport. Dictyoceratid sponges such as *C. matthewsi* typically produce well developed larvae with poor swimming abilities that settle within a few hours or days (Bergquist and Sinclair 1968, Maldonado and Young 1996). In Torres Strait, eddies may form around islands (Wolanski *et al.* 1984), which could potentially trap larvae and promote high settlement and abundance of dictyoceratids in one localised area. This probably explains why sponge abundance can vary significantly between neighbouring locations or sites within a location. Currents between islands in central and eastern Torres Strait can exceed 50 cm s\(^{-1}\) (Wolanski and Ruddick 1981), however, which suggests that some sponge larvae could be quickly transported among neighbouring island-groups. The transport of larvae of *C. matthewsi* between Ugar, Erub, Masig, Poruma and Warraber probably explains the low levels of variation in genetic profiles and sponge-associated microbial communities between the five island-groups (see Objective 2).

Differences in water flow across depth help explains the variation in abundance and size frequency patterns of *C. matthewsi* between 6 and 12 m on reefs at Masig. Ambient water flow generally decreases with depth which can greatly influence the abundance and diversity of coral reef organisms (Wilkinson and Evans 1989, Vermeij and Bak 2003, Duckworth and Wolff 2007, Penin *et al.* 2007). A separate study at Masig using an Acoustic Doppler Current Profiler has determined that average current speeds at 6 and 12 m are 0.25 and 0.11 ms\(^{-1}\) (unpublished data). Sessile organisms such as sponges at 6 m would therefore experience ambient water flows twice as high as organisms living on reef at 12 m. Differences in water flow between depths would be greater during storms, when most of the wave energy is concentrated in the shallows.
Population Demographics of *C. matthewsi* at Masig: Abundance, Size and Disease

Elizabeth Evans-Illidge, Craig Symns, Chris Battershill, Stephen Whalan, Alan Duckworth and Heidi Luter

Introduction

An understanding of distribution and abundance patterns for sessile invertebrates must take into account biotic and abiotic processes including reproductive/recruitment strategies that contribute to distribution patterns across a wide range of spatial and temporal scales. Sponges exhibit remarkable diversity in reproductive processes with many species, including *C. matthewsi*, able to reproduce both sexually and asexually. Research effort must therefore examine the mobile larval phase (e.g. to examine availability of suitable settlement habitat - Whalan *et al.* 2008) and post-settlement processes following recruitment as juveniles and adults (e.g. to examine predation and disturbance events – Maldonado and Uriz 1998). It must also consider the role of fragmentation and propagule formation and fate (Battershill and Bergquist 1990 and 2010, Wulff 1985, Kelly-Borges and Bergquist 1988, Maldonado and Uriz 1999). The interaction of these processes for both larval, propagule and adults stages is a contributing influence to the patchy distributions often seen in sponge populations (e.g. Barnes *et al.* 2006).

Other population demographic characters such as size frequencies can also show considerable patchiness in how sponges respond to their micro-environment, particularly over localized spatial scales (Duckworth and Wolff 2007b, Duckworth *et al.* 2008 and 2009). Both physical and biological processes influence size structures of sponge populations and can have either detrimental or beneficial impacts on growth and therefore size structures. For example, strong water currents can deliver more food have a positive effect on growth for some species (Duckworth and Battershill 2003), but for other more fragile species strong currents can damage sponges and reduce growth (Trautman *et al.* 2000).

Bath sponges have been sourced for centuries for a range of commercial and cosmetic uses (Pronzato 1999, Catharios 1998). Traditionally, the bath sponge market was supplied through the collection of wild sponges mostly from the Mediterranean and Caribbean. More recently, there has been an increasing interest in developing sustainable culture techniques to supply markets, thereby alleviating pressure on wild sponge populations. The ability of sponges to grow from fragments (explants) excised from donor wild sponges, and then seeded on a range of standard aquaculture infrastructure, makes them ideal candidates for aquaculture (Evans-Illidge *et al.* 2006, Duckworth 2009, Duckworth and Wolff 2007a). Harvesting from donor wild stock and subsequent culture of explants is a feasible method to obtain commercial quantities of sponge (Evans-Illidge *et al.* 2006, Duckworth 2009, Duckworth and Wolff 2007a). A research program explored the potential for sponge farming in Torres Strait at Masig (Duckworth *et al.* 2007b), and on the basis of this research, Australia’s first sponge farm was established at Masig, by the indigenous company Kailag Enterprises Limited, in 2009.

To support the regulation of seed stock harvests for this new industry, a brand new commercial fishery for Torres Strait has recently been recommended for formal declaration and management. Sustainable management of such a seed-stock harvest fishery must be informed by a robust understanding of population demographics, including data on abundance and distribution of the target species. Proceeding with a commercial venture without this information...
poses potential risks of localised extinction due to over-harvest at inappropriate spatial scales, which is what has happened elsewhere due to over-fishing of wild stocks (Pronzanto 1999, Catharios 1998).

Determining key population demographic patterns for *C. matthewsi* has been a central focus of MTSRF project 1.3.2 and, as described in the previous chapter of this report, has confirmed that throughout Torres Strait this species is most abundant in the Masig Island group. The previous chapter considers patterns at a large and medium spatial scale. This chapter focuses on a smaller scale, and addresses temporal patterns at the within-reef scale at Torres Strait’s demonstrated sponge hot-spot (Masig) and site of Australia’s first commercial sponge industry.

**Methods**

The study area is in central Torres Strait at Masig and Kodal Islands (Figure 15). These islands are sand cays, low-lying (<10 m in height) and small in size (<5 km²), and are joined by a contiguous reef system (Figure 16) comprising both sheltered and exposed aspects with respect to the south-easterly trade winds which dominate the area from April to December. Monsoonal weather patterns with sometimes strong north-westerly winds affect the area for the rest of the year (BOM 2010). This reef system has been shown to contain the largest density of *C. matthewsi* in Torres Strait in habitat characterized by reef slope with consolidated and unconsolidated reef substrate, including mobile rubble of various sizes. The coral reef slope generally starts at a depth of 6 m (MLW) and stops on sand at 15 m, descending at an angle ranging from 20 to 60° (see previous chapter and Duckworth *et al.* 2007, Duckworth *et al.* 2008, Duckworth *et al.* 2009, and Whalan 2009). Most *C. matthewsi* in this region are of a lobate growth morphology, rather than clumped and massive morphology typical in other areas (Figure 14 previous chapter, and Figure 17).

![Figure 15. Map of Torres Strait showing position of Masig and Kodal Islands.](image-url)
Figure 16. Aerial photo showing continuous reef habitat around Masig and Kodal Islands.

Figure 17. Examples of *C. matthewsi* spreading its lobes over substrate of variable mobility (and therefore fragmentation potential).

**Survey methods**

Strip transects were used to quantify the size and distribution of sponges around Masig. As part of this project, the following two survey methods have been used at the times indicated in table 19, with a switch from the first to the second method due to concerns that randomly placed transects over a species distribution known to be patchy may be producing unrealistically variable patterns in abundance (Whalan 2009). Sponges were measured using a cm scale, and the dimension of the longest axis was used in this analysis as a measure of sponge size. For each sponge counted and measured, disease state was also noted.

**Method 1:**

At each site, *C. matthewsi* was surveyed at both shallow (4-6 m) and deep (10-12 m) depths, with the former generally on the reef flat. However, as patterns in abundance were shown to be consistent between depths from analysis of this data (Whalan 2009), only deep data was used for the analysis reported here. At each survey event, between 6 and 12 sites were
surveyed around Masig. At each site, three strip transects were examined (either 20 x 1 m or 30 x 1m), with transects separated by at least 20 m to retain independence. At each transect, divers recorded and measured every *C. matthewsi* sponge found on the transect, and noted its disease state. Basic environmental factors were also noted and are reported in Duckworth *et al.* 2008 and Whalan 2009.

**Method 2:**
At each of six permanent sites around Masig, three 2 x 25m transects were surveyed at a single depth (10-12m) only. Surveys were not undertaken at a second shallower depth, as previous surveys using method 1 had shown a consistent pattern in abundance between survey times at both depths. Each transect was marked at its beginning and end with a permanent star picket. At each survey event, every sponge was measured and noted for disease status. Further environmental data was also recorded but is not analysed here.

**Table 9.** Field schedule survey method type for demographic surveys.

<table>
<thead>
<tr>
<th>Survey date</th>
<th>Survey method</th>
</tr>
</thead>
<tbody>
<tr>
<td>November 2006</td>
<td>1</td>
</tr>
<tr>
<td>March 2007</td>
<td>1</td>
</tr>
<tr>
<td>November 2007</td>
<td>1</td>
</tr>
<tr>
<td>May 2008</td>
<td>1</td>
</tr>
<tr>
<td>November 2008</td>
<td>1</td>
</tr>
<tr>
<td>June 2009</td>
<td>2</td>
</tr>
<tr>
<td>November 2009</td>
<td>2</td>
</tr>
<tr>
<td>May 2010</td>
<td>2</td>
</tr>
</tbody>
</table>

This data collected as outlined above was combined with historical survey data from December 2005 collected during a previous project, with methods outlined in Duckworth *et al.* 2007 and Duckworth *et al.* 2008. Appropriate statistical adjustments were made when combining data from different sampling designs such that results err on the side of conservativeness (described below).

The position of each site surveyed was recorded with GPS, and assigned an aspect category of either sheltered or exposed, with respect to exposure to predominant south-easterly trade winds.

**Analysis methods**

Temporal and seasonal changes in sponge size structure and their covariation with habitat characteristics are central to understanding the demographics and maintenance of the population. Working with size data collected with different levels of temporal resolution, transect sizes and sampling intensity at the site level, and different seasons presented some analytical challenges. Additionally, not all combinations of factors such as year and season occurred in the data set, so this structural imbalancing required a series of targeted tests to resolve particular questions.

**Data treatment**

Where possible, size categories were treated at the finest possible resolution. Size classes in 2005 were grouped at a resolution of 0-5cm, 5-10cm, 10-20cm, 20-50cm and >50cm. After
2005, sizes were recorded to the nearest cm and provided the best resolution of pattern. To make the most of the temporal series of available data from Masig, we conducted analysis abundance of size classes the coarse 5 category scale to include 2005, and closer examination of size differences at the centimeter scale of later sampling programs.

Combining different sample programs that have used different transect sizes and sampling intensity at a site into a single analysis presented the primary analytical hurdle to be dealt with. Transect size differences in the various sample programs were dealt with by re-expressing all numbers of sponges per size category as a value per site, where the site was standardized to a size of 500m². This enabled size-frequency analyses to be independent of both transect size and sample effort at a given site. Consequently, analyses were conducted at a Site, rather than Transect level of replication; due to a combination of differences in transect sizes, their allocation to sites and exposure aspect, and number of site sampled between years, and low numbers within individual size categories at lower levels of aggregation.

**Analytical framework**

As it was clear that there were temporal patterns that differed between exposure aspects, we focused the analysis on measuring patterns in size frequency distributions over time and exposure regime. Size-frequency data require careful attention to analytical assumptions and appropriate models. Size frequencies are often skewed in their distribution, and errors are usually not normally distributed. In addition, multimodality can cause problems, but was not observed in these data. A multinomial Generalized Linear Model approach was used for all size-frequency analyses, with a cumulative logit link-function. This approach had the advantage that the ordered structure of sizes (e.g. 2cm is smaller than 10cm) was taken into account in the analysis, and correct statistical hypothesis tests based on appropriate errors could be carried out. In addition to classical p-value interpretation of model effects, tests based on likelihood ratios and Information Criteria (Akaike’s & Schwarz-Bayes) were used to examine whether the model could be simplified by removing, in particular, higher-order interactions.

As it was clear from initial examination that there was considerable temporal variability in abundance, we conducted two sets of formal tests to examine patterns in *C. matthewsi* size structure, and the potential drivers of variability. First, did size-frequency distribution of sponges differ between years, and how did this covary with exposure regime (Aspect) and Season. Not all Seasons were sampled in all Years, but all Aspects were sampled in a given Year and Season combination. Consequently, we analyzed each Season separately under the model: Size=Year+Aspect+Year*Aspect. Secondly the interaction between Season and Aspect across two sample years (2008,2009) in *C. matthewsi* size frequency distributions was examined. These were the only two years in which both Summer and Winter were sampled. This was tested using the model:

Size=Year+Aspect+Season+Year*Aspect+Year*Season+Aspect*Season+ Year*Aspect*Season

As the harvestable sponge size (for seed stock collection) is >15cm, abundance of this size class was plotted through time with standard error bars. To facilitate discussion regarding harvest management, observed abundance of harvestable sponges was extrapolated to a conservative under-estimate of available sponge habitat area in the Masig/Kodal reef complex, using the polygon measurement tool in Google Earth™.

Disease occurrence was so low as to preclude formal analysis. Our interpretation was restricted to graphical presentation of the per capita occurrence of disease, i.e. the proportion of sponges within the five size classes that had some disease; and a plot of the relationship
between degree of disease and sponge size. Data from both years in which disease was measured were combined due to low numbers.

**Results**

Temporal abundance of different size classes were clearly different between sheltered and exposed aspects (Figure 18). Smaller size classes were, unsurprisingly, more abundant than larger classes with 0-5cm, 5-10cm, 10-20cm almost equally abundant, followed by the 20-50cm size class, and sponges >50cm were relatively uncommon regardless of exposure. However the temporal variability of different classes differed widely with exposure regime. The abundances of all size classes were more temporally stable at Sheltered sites than Exposed. This was particularly obvious for size classes <20cm, the abundance of which varied widely through time at Exposed sites. In contrast the temporal abundance of sponges >20cm appeared to be far more stable at both Sheltered and Exposed sites.

![Figure 18](image-url) Densities of different size classes of *C. matthewsi* at Sheltered and Exposed sites on Masig Island. ◇ 0-5cm; ▽ 5-10cm; ◊ 10-20cm; ■ 20-50cm; ▲ >50cm.
There was a significant interaction between Year and Aspect for both summer and winter samples (Table 10), largely driven by temporal rather than aspect effects. In Summer, this interaction appeared to be driven by the 2008 and 2009 samples (Figure 19). The size distributions of *C. matthewsi* in the sheltered sites in 2006, 2007 and 2008 were remarkably similar, and very similar to exposed sites in 2006 and 2007 but in 2008 the size frequencies at the exposed site appeared to undergo some disturbance. In 2009 both sheltered and exposed sites underwent a shift to smaller size classes.

This interaction effect was far more pronounced in Winter (Figure 20). In particular, there was a very large difference between sheltered and exposed sites in 2008, with exposed sites containing only a few size classes ranging from 7 to 18 cm. This difference between aspects was reduced in 2009 and 2010. The temporal patterns indicated that on exposed sites there is some ability to recover reasonably rapidly from disturbance events.

**Table 10.** Generalized Linear Mixed Model testing whether the size distributions of 2006-2010 data differed between Year and Aspect during Summer and Winter. Counts were first standardized to a common area of 1000m$^2$ to enable the comparison of different methods. The link function was the cumulative logit, the error distribution was multinomial.
Figure 19. Size-frequency distributions of *C. matthewsi* during Summer in Sheltered and Exposed Aspects, from 2006-2009. Sheltered aspects are in the left column, Exposed aspects in the right.
Figure 20. Size-frequency distributions of *C. matthewsi* during Winter in Sheltered and Exposed Aspects, from 2008 to 2010. Sheltered aspects are in the left column, Exposed aspects in the right.
Formal comparison of winter and summer samples was restricted to 2008 and 2009, in which both seasons were sampled. There was a strong Year*Season*Impact interaction (Table 11). In Sheltered sites this was driven by an abrupt shift to smaller size classes in Summer 2009, followed by a general increase in size with lower numbers the following winter (Figure 21). In the Exposed sites, the fragmented size structure in Summer 2008 became more restricted to 7-18 cm the following winter. These appeared to fragment in the 2009 Summer resulting in a large number of fragments less than 8cm. As with the sheltered sites, this was followed by a general increase in size and decrease in abundance in Winter 2009.

Table 11. Generalized Linear Mixed Model testing whether the size distributions of winter and summer samples for 2008 and 2009 data differed across Years, Aspect, and their interaction. The link function was the cumulative logit, the error distribution was multinomial.
Figure 21. Seasonal and Aspect effects on *C. matthewsi* size-frequencies for 2008 and 2009. There are strong seasonal differences in abundance, with few sponges across the size range in Winter. The degree of difference is dependent on both the Aspect, and the Year.
In summary, there is considerable temporal variability in *C. matthewsi* abundance in different size classes over time. Size structure was constant over 2006-2008 in the sheltered sites, but appeared to undergo some disruption in the exposed sites before the November 2007 sampling program. In addition, it appears the sheltered sites underwent a similar event by the November 2009 sampling program. There is a clear seasonal signal, with winter size classes often larger and in lower abundance, in the absence of disturbance. It was particularly evident on exposed sites that there is considerable resilience, which results in greater temporal and seasonal variability.

![Figure 22. Temporal variation in numbers of harvestable (≥15cm) C. matthewsi per 1000m² across all sites and exposure types.](image)

When considering sponges >15cm only (which is the harvestable sponge size for seek-stock collection for the sponge farm), the numbers were highly variable up until 2008, and ranged from a minimum of three sponges per 1000m² in November 2006 to a maximum of 27 sponges per 1000m² in March 2007 (Figure 22). The polygon measurement tool in Google Earth™ was used to calculate a conservative underestimate of available sponge habitat around Masig and Kodal reefs, at 2.4km². Using this total area estimate to extrapolate sponge abundance figures, the total stock of harvestable sponges at Masig and Kodal reefs is conservatively estimated at between 7200 and 64800 sponges.

Disease occurrence was very low. Of 817 sponges counted during the Summer 2009 and Winter 2010 sampling, only 40 contained some level of disease. Within size classes, the per capita rates for the highest occurrence levels did not extend beyond 0.05 (5%), with the exception of the single >50cm sponge observed, which did have some disease (Figure 23). There was a decreasing relationship with percent of disease cover and sponge size (Figure 24). Disease on smaller sponges occupied a greater percentage of sponge area, and larger sponges typically had <20% diseased area, although this is undoubtedly and artefact of disease detection and spurious relationship with sponge area.
Figure 23. Per capita occurrence of disease across different size classes (left) and number of sponges within each size class (right) for Summer 2009 and Winter 2010 samples combined. The numbers are the observed numbers of diseased and total sponges in each size class. The single >50cm sponge observed was diseased, so its per capita disease rate was 1.

Figure 24. Percent of diseased area vs. sponge size.
Discussion

The long-term fluctuations in abundance of *C. matthewsi* over time shown in Figures 18 and 19 for the period 2006 – 2010, are consistent with trends described at deep and shallow sites in previous annual reports of this project (Whalan 2009), and the findings of broader surveys undertaken for this species in Torres Strait (see previous chapter of this report, plus Duckworth and Wolff 2007, Duckworth et al. 2008, Duckworth et al. 2009). The current report is the first to include data from surveys after the method was changed from randomly placed transects at two depths, to permanently placed transects at one depth (10-12m) from 2009. This indicates that the high variability in sponge abundance observed earlier is probably a real phenomenon, and not simply an artefact of randomly placed transects over a patchily distributed species, as suggested previously (Whalan 2009). In previous surveys which included two depths, *C. matthewsi* was more abundant in the deep (10-12m) compared to the shallow sites (5-6m), but the trend in abundance between survey times was consistent between the sites (Whalan 2009). However, this chapter presents an analysis that includes data from the deeper (10-12m) sites only.

While the size frequency distributions (Figures 18-21) are consistent with previous findings with the longest axis of the majority of sponges being less than 20cm (Duckworth et al. 2009, Whalan 2009), the current analysis shows several interesting trends in the abundance of different size classes between exposure aspect, seasons and through time. There is considerable temporal variability in *C. matthewsi* abundance in different size classes over time, with fluctuations in small size classes driving much of the variability (Figure 18).

Processes influencing abundance patterns of sessile organisms, such as sponges, can be complex and include biotic and abiotic factors acting on all life stages of a species (Ayling 1980, Morgan 2001, Duckworth et al. 2008, Duckworth et al. 2009). Biotic and abiotic factors with potential to influence sponge demographics include mortality due to disease (Ayling 1981, Pronzanto 1999, Webster 2007), predation (Randall and Hartman 1968, Ayling 1978), reef slope (Bell and Barnes 2000, Bell et al. 2002, Duckworth et al. 2009), and substrate composition (Trautman et al. 2003, Duckworth et al. 2009). However, none of these factors are suspected to drive sponge survival and mortality to explain the observed trends through time. While disease was observed at this site for the first time during this study, observations show extremely low levels of partial infection which are not expected to impact overall mortality, although a potential role for partial disease in facilitating fragmentation is discussed below. Few animals are known to eat sponges, and there are no anecdotal reports of any animal feeding on *C. matthewsi* at Masig. While hawksbill turtles are known to eat sponges, a recent survey of the gut contents of captured hawksbill turtles at Masig failed to demonstrate that *C. matthewsi* makes up any part of this turtle species’ diet there (Fuentes 2009). Thus, predation pressures on *C. matthewsi* at Masig are expected to be low and consistent throughout the study period. While substrate type is known to affect sponge abundance at Masig (Duckworth et al. 2007, Duckworth and Wolff 2008, Duckworth et al. 2009), the data for sites examined in this study indicate that overall substrate type has been consistent between periods (Whalan 2009) and therefore unable to explain the observed trends.

Previous reports of *C. matthewsi* in Torres Strait have considered the possibility that pulses in larval recruitment and mortality events may help explain the trend in size frequencies (Whalan 2009, Duckworth et al. 2009). Although sponges are sessile organisms as adults, they have a mobile larval phase with most species’ producing larvae that disperse over limited spatial scales (Whalan et al. 2005, 2008) before locating a suitable site to metamorphose into a sponge. Preliminary larval studies for *C. matthewsi* have been undertaken and are described in detail in the relevant chapter of this report. This species is viviparous, and releases larvae during daylight hours over summer. Larvae can stay at the surface for up to 24 hours (see larval behavior chapter). This initial data is consistent with
what has previously been reported for closely related dictyoceratid sponges on the Great Barrier Reef (Ettinger-Epstein et al. 2008, Whalan et al. 2008). Despite typically strong currents in Torres Strait, eddies often form around island and reef groupings in Torres Strait (Wolanski et al. 1984). While the presence of eddies has not been examined around Masig, it is possible that if eddies were to exist, a proportion of larvae from this species around Masig may well be captured within the Masig area, with limited further dispersal to other island groups. However, besides endogenous recruitment, complex hydrodynamics coupled with daily spawning of larvae over several weeks for some sponge species (including C. matthewsi), can result in long distance larval dispersal that retains population connectivity among more distant sites (Whalan et al. 2008b). While inconclusive on their own, due to limitations on methods employed, the data discussed in the connectivity chapter also supports connectivity of C. matthewsi on a very large scale across central and eastern Torres Strait.

Once metamorphosis occurs, the sponge is unable to escape any adverse habitat/environmental influences sometimes resulting in mortality. Therefore, limited larval dispersal capabilities, stochastic larval recruitment, coupled with high vulnerability to post-settlement mortality due to unfavourable habitats (e.g. increased competition from coral or algae, excessive wave exposure, incidental grazing from herbivores), may all contribute to observed abundance patterns (Wilkinson and Evans 1989, Maldonado and Young 1996, Bell 2008). The substrate available for recruitment may also be critical for larval recruitment (e.g. Pawlik 1992, and see also the larval biology and behaviour chapter in this report). The presence of open space represented by rock or rubble around Masig may be influential and support high larval settlement of this species around Masig. The settlement study reported in the relevant chapter of this report does not record high settlement of this species at Masig, although this should not be taken as evidence that high levels of larval settlement does not occur within the wild population as discussed in that chapter.

The potential for C. matthewsi to rely on asexual methods of reproduction should also be considered. Asexual reproduction has been shown to play a critical role in determining population demographics for many sponges (e.g. Corriero et al. 1998, Zilberberg et al. 2006, Wulff 1985, Kelly-Borges and Bergquist 1988, Ereskovsky and Tokina 2007, Ayling 1980, Hammel et al. 2009, Meroz-Fine et al. 2005), and in some species has been demonstrated as the most dominant reproduction method, with the larger size of asexual propagules providing a survival advantage over new larval recruits (Battershill and Bergquist 1990, Battershill and Bergquist 2010). Asexual propagules are a sub-clone of the parent sponge that becomes a new, functional sponge unit, and can be broadly split into two categories. The first category involves the formation of specific sponge buds on the parent. True buds become complete functional new sponges during development on the parent sponge through a cellular differentiation process that in some species has been likened to larval morphogenesis processes (Ereskovsky and Tokina 2007). The second category involves simple fragmentation of a piece of the parent sponge, without special cellular differentiation prior to the fragment's release, and often during a disturbance event (Ayling 1980, Bell and Barnes 2003). Some sponge morphologies are more prone to this method of asexual reproduction (Wulff 1985).

Differentiated buds have not been observed on C. matthewsi during the period of this study. However, there are many characteristics of C. matthewsi and its habitat at Masig that provide a high potential for fragmentation to play an important role in this species. This species at Masig does not conform to the hemispherical massive morphology reported elsewhere (Bergquist 1995), but rather is characterized by long fleshy lobe-like projections that extend out to cover multiple adjacent substrates of varying stability and therefore movement potential (see Figure 17 and Figure 14 and discussion in previous chapter). The substrate type in C. matthewsi habitat at Masig is characterized by mobile rubble of varying sizes. The potential for this complex of ephemeral sponge substrate to move apart during periods of

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high-energy disturbance is high, and since the ramose, lobate \textit{C. matthewsi} is attached to the substrate at various intervals, the potential for these events to cause fragmentation of sponge fragments already attached to substrate (albeit mobile rubble) is also high. Resettlement of fragments at the appropriate orientation (rubble side down) after the disturbance event would also be optimized due to gravity. Should two sponge fragments then come into contact with each other, re-coalescence is possible (Zilberberg \textit{et al.} 2006, Battershill and Bergquist 2010).

Fragmentation as described above, and the potential for high-energy disturbance events could explain the size frequency patterns reported in this study. For most of the year, Torres Strait including Masig is subjected to frequent periods of consistently strong south-easterly trade winds which often exceed 40km/hr (BOM 2010). The highly mobile substrate coupled with the sponges growth morphology and the constant potential for fragmentation during strong south-easterly winds, may explain why the <20cm size classes are always most abundant at all sites, and to a greater extent at exposed locations (Figure 18). This is consistent with the high correlation found between smaller sponges and unconsolidated rubble substrate (Figure 11 and previous chapter). The disruption at exposed sites before the November 2007 sampling program, causing a proliferation of smaller sponges (Figure 18) was likely caused by a strong south-easterly wind period. Similarly, the apparent but smaller disruption at sheltered sites before the 2009 sampling program (Figure 18), could have been caused by a North-Westerly monsoonal storms which can occur at that time of year and to which the ‘sheltered’ locations would actually be exposed. High temporal and seasonal variability, especially at exposed sites and probably in relation to disturbance, provides an indication of resilience, partly due to this sponges apparent adaptation to fragmentation.

Fragmentation studies with other species have shown that fragments rarely move more than a few metres from their parent (Wulff 1985). In observations from an unpublished study at Masig, rubble pieces with sponge attached had moved several meters between monitoring events (3 months) (Duckworth \textit{et al.} unpublished data). The possibility that \textit{C. matthewsi} relies on fragmentation with limited fragment dispersal is consistent with the patchy distribution described for this species at Masig (Duckworth \textit{et al.} 2008 and Duckworth \textit{et al.} 2009). The extraordinarily high abundance of this species at Masig compared to other places in Torres Strait, despite extensive surveys elsewhere (Duckworth \textit{et al.} 2008) could be explained by the compounding effect of continuous fragmentation of individuals after growth.

The likely reliance on fragmentation in this species for wild population demographics further positions this species as an ideal sponge farm candidate, as sponge farm methods also utilize fragmentation for farm production. It also reinforces the established seed stock harvest protocols, with only partial removal of each donor sponge ensuring zero impact on wild mortality due to harvest activities. Previous research has found that \textit{C. matthewsi} will survive and regrow after two-thirds of an individual’s biomass is removed or harvested (Duckworth \textit{et al.} 2007a,b). However, a full understanding of the relative importance of larval recruitment compared to fragmentation would require further research, ideally including a fine-scale genotype study of the population around Masig.

While sponge disease has been an important feature of sponge populations elsewhere including commercial species (Webster 2007, Pronzanto 1999, Catharios 1998), disease was not observed in \textit{C. matthewsi} for most of the current study, with very low disease incidence reported in November 2009 and May 2010 only. However, the possibility of un-detected small focal areas of disease should be considered, such as under accumulated sediment in creases of the sponge, such as at the base of lobes. If small areas of disease and collagen weakening were to occur in these areas, this would contribute to the susceptibility to fragmentation processes described earlier. Thus, while low levels of disease formally observed to date in this species at Masig are encouraging, future surveys to observe
sediment covered areas, and research into the microbiology of this species and the epidemiology of potential pathogens, is recommended.

The results of this project support the finding of previous research, that the reef surrounding Masig and Kodal Islands is a ‘hot spot’ of *C. matthewsi* abundance, and that sponges from this island-group could be sustainably harvested for seed-stock supply to a sponge farm. Previous research (Duckworth *et al.* 2007b) has recommended at least 30% of each donor sponge remains attached to the substrate to facilitate zero mortality and regrowth, that only large sponges over 15 cm in length should be harvested to maximise the number of sponge pieces or explants obtained from the harvested sponges and minimize the number of wild donor sponges damaged to less than 10% of the total population. The current project has extrapolated abundance figures to cautiously calculate a conservative under-estimate of the total harvestable (>15cm) sponge stock at Masig and Kodal at a minimum 7200 sponges. While this figure could be tightened in future with more accurate mapping of actual sponge habitat area, it provides an indication that at least 700 sponges could be harvested while keeping the impact on harvestable size classes below 10%. This figure accommodates the sponge farm’s seed stock needs, indicating that the current operation can be sustained by the existing population at Masig.
Recruitment Dynamics of Sessile Invertebrates

Steve Whalan, Alan Duckworth and Carsten Wolff

Introduction

Population demographics for sessile marine invertebrates are shaped by a myriad of interacting processes that include biotic and abiotic influences (Morgan 2001). Moreover, for sessile invertebrates, which display a bi-partite life cycle, the mobile larval phase also has significant implications for population demographics. The complexity of these processes can contribute to remarkable variability in population abundances and distributions within species (e.g. Barnes et al. 2006), both on temporal and spatial scales and with particular regard to larval recruitment. Moreover, intra-specific variation in larval recruitment can influence the composition and diversity of benthic communities (Smith and Witman 1999), and can help explain community dynamics both temporally and spatially.

While traditional survey techniques (e.g. transects) that rely on quantifying patterns of sessile invertebrate distributions have often highlighted their patchy nature (e.g. sponges – Barnes et al. 2006), population genetic data also corroborate the highly variable nature of larval dispersal and recruitment. For example, several coral species show high levels of endogenous recruitment but also wide-scale dispersal that spans the Great Barrier Reef (Ayre and Hughes 2000). Similar patterns are also demonstrated for dictyoceratid sponges (e.g. Whalan et al. 2008a). Despite the difficulty in quantifying patterns of recruitment in sessile marine invertebrates, the importance of developing our understanding of the role these processes play in structuring populations cannot be underestimated. This is particularly relevant for commercially targeted marine resources where our understanding of processes that maintain populations are pivotal to how we manage harvest activities, both temporally and spatially, and ultimately how the resource can be conserved (yields optimized) into the future. With specific reference to this study, the sustainable management of commercial species in Torres Strait, such as *C. Matthewsi*, will rely on robust recruitment data. The objective of this study is to quantify the recruitment of bath sponges and other sessile organisms to reefs in the Masig Island group.

Methods

Study site and plate deployment

The study was conducted at Masig and Marsden islands, (central Torres Strait). Both Islands comprise sand cays with fringing coral reefs, the reef profile typically comprising a reef slope descending at an angle ranging from 20-60° from 6m terminating at a sand bottom at 15m. Approximately 5 km of open water reaching 30 m in depth and with a bottom composition of mud and sand, separates the two islands (Harris 1988).

To quantify recruitment patterns of sessile marine invertebrates, settlement plates were deployed at three locations on the northern side of each island, each location separated by 200m. Each location was divided into three sites separated by 20 m, with each site having two depth categories: shallow (6m) and deep (12m). Five settlement plates were deployed at each depth using the direct attachment method of Mundy (2000). Briefly, 11x11cm terracotta tiles were anchored 1cm above the reef to provide settlement surfaces on both sides of each plate. Tiles were placed 1m apart.
In accordance with data collections from previous years, to allow temporal (i.e. seasonal) patterns to be assessed, seasonal sets of plates were deployed at the start of summer (i.e. November) and the start of winter (i.e. May). Plates were left for six month periods to allow comparisons over summer and winter. In summary, this study ran for approximately three years, from November 2006 to June 2009. Each season, 180 plates were deployed at Marsden and Masig equating to 30 plates per location. Each plate was used once. At the end of each season, the top and underside of each plate were photographed in situ and a new plate was then deployed.

**Photographic analysis**

Photos of each tile were undertaken following the exact protocol as in previous years. An underwater close-up frame was constructed to photograph settlement tiles at a fixed distance and to record site and tile information on its frame. As the aspect ratio of the digital images allowed for the recording of extra information on each image, due to the tiles being square, a 4-digit code wheel was built into one side of the frame. The framer was adapted to accommodate either an Olympus C-7070 or Canon IXUS 850IS camera in underwater housings. Both these cameras have identical lenses and sensor-resolution, hence images produced are comparable in quality and view. The recruitment of sessile organisms to central Torres Strait was determined for both abundance and percent cover. To determine the abundance of each taxon, an overhead transparency marked with a square was overlaid on a PC-screen. All images of tiles were displayed by Microsoft Windows XP “Picture and Fax Viewer™” and enlarged by clicking the zoom-in button sufficient times to identify each organism. To measure surface area occupied by each taxon a 40 point grid was overlaid on the PC-screen image. For both abundance and percent cover, the square or grid was reduced by a 1cm margin to eliminate any potential edge effects” (Duckworth and Wolff 2008).

**Data analysis**

Tile recruitment data for both abundance and percent cover for each season was analysed separately for each taxonomic group using ANOVA. Following Duckworth’s experimental design for previous surveys in this project (e.g. Duckworth and Wolff 2008): season, island and depth were fixed factors, while location (island) and site (location(island) were nested factors. Data for all species in each taxonomic group was pooled, and if necessary log or arcsine transformed to meet assumptions of ANOVA.

**Translocation of recruitment tiles**

A separate experiment was undertaken in 2008 to assess the effect of habitat on sponge recruit performance. Tiles with recruits were collected in May 2008, photographed and then re-deployed as follows: One treatment included deploying tiles from natal sites at Masig and translocating them to Marsden and one treatment included deploying tiles from natal sites at Marsden and translocating them to Masig Island. Controls for each were included at each island. Tiles were then photographed in November 2008. The number of sponge recruits from all tiles was recorded from the photos and analysed using repeated measures ANOVA.
**Results**

**Recruitment – all groups**

Seven broad taxonomic groups recruited to experimental tiles placed around Masig Island group over two seasons and at two depths during 2008-09 (Figure 25). Tiles were placed at fixed depths and for ease of discussion these are referred to as shallow (6 m) and deep (12 m). Notably, both sessile invertebrate and algal recruits recruited to the bottom surface of tiles with no evidence of successful recruitment to the top surface of tiles due to their undeveloped state. Identification to species or genus level was not established for many of the recruits.

With the exception of sponge recruitment, numbers of recruits for all taxonomic groups showed similarities between the two sampling periods (corresponding to summer and winter). Polychaetes dominated tiles with the mean number of this group being four-fold higher than any other taxa (Figure 25). In contrast however, polychaetes occupy the least surface area of tile (2.74±0.29 %) in comparison to other groups which have several species with encrusting, prostrate morphologies such as algae (24.65±1.94%) and sponges (20.74±1.5%) (Figure 26). Very low numbers of cnidarians were recorded for summer recruitment (0.5±0.1) and none were observed for winter surveys and as such have not been included in the formal statistical analyses.

Overall, when taxonomic groups are analysed separately there were significant interactive effects of location, season and depth for all taxa. These are discussed more comprehensively for each group.

**Sponges**

**Abundance:** At least 11 species of sponges recruited to the underside of tiles, including several with encrusting morphologies (*Clathria* sp. and three other unidentified encrusting species), *Iotrochota* sp., *Callyspongia* spp., *Dysidea* spp., *Hyrtios erecta*, *Nara nematifera* and *Leucetta* sp. Overall, sponge recruitment per 100cm² tile surface at Masig Island sites was 7.61(±1.78) at deep sites and 5.24(±1.45) at shallow sites during the winter compared to 6.64(±2.19)(deep) and 2.93(±0.47) (shallow) at Marsden during the same time period (Figure 27). Sponge recruitment in summer at Masig Island was 16.23(±3.33) at deep sites and 20.82(±7.35) at shallow sites compared to 29.47(±6.13) (deep) and 17.58(±4.7) (shallow) at Marsden Island. There was a significant interactive effect of location and depth in addition to a significant main effect of season on sponge recruitment with more sponges recruiting during summer (ANOVA: F 4, 359 = 6.99, p<0.05).

**Percent cover:** Encrusting species dominated tiles in terms of coverage with *Clathria* sp. occupying up to 35% of the tile surface (mean –winter recruitment) and with a combined coverage for other encrusting species being 28% of the total tile surface (mean –winter recruitment). There was a significant main effect of island (Figure 27; ANOVA: F 1, 359 = 6.48, p<0.05) on the extent of percent cover for sponges.
Figure 25. Mean number of recruits (±1 SE) to settlement tiles at Masig and Marsden Islands over two seasons in 2008-2009 for sessile invertebrates and algae.

Figure 26. Mean percentage cover of recruits (±1 SE) occupying settlement tiles at Masig and Marsden Islands over two seasons in 2008-2009 for sessile invertebrates and algae.

Figure 27. Mean number of sponge recruits (±1 SE) left to settlement tiles at Masig and Marsden Islands over two seasons in 2008-2009 and mean percentage cover sponge recruits (±1 SE) to settlement tiles (right). (W) is winter, (S) is summer.
**Ascidians**

*Abundance*: At least seven species of ascidians recruited to the underside of tiles, including *Didemnum* spp. *Botryllus* spp. and *Clavelina* spp. and a further species identified from the Styelidae family. Other species were unidentified at this time. Overall, ascidian recruitment 100cm\(^{-2}\) at Masig Island sites was 3.91(±1.37) at deep sites and 5.56(±1.99) at shallow sites during the winter compared to 2.58(±1.04) (deep) and 4.18(±1.23) (shallow) at Marsden during the same time period (Figure 28). Ascidian recruitment in summer at Masig Island was 2.07(±0.33) at deep sites and 4.84(±2.02) at shallow sites compared to 2.51(±0.64) (deep) and 3.44(±0.74) (shallow) at Marsden Island. There were significant interactive effects of season and site (Figure 28; ANOVA: F\(_{12, ~359}\) = 1.85 p<0.05), in addition to significant interactions of depth and location (ANOVA: F\(_{4, ~359}\) = 3.88 p<0.05) on ascidian recruit abundance.

*Percent cover*: Didemnid species dominated percent cover for ascidians with a mean coverage of 52% in winter and 25% in summer per 100cm\(^2\) tile. Styelid and Botrylid species showed less dominance with mean percent covers being 13% and 6% (winter) and 5% and 4.5% (summer) respectively. There was a significant interaction effect of depth and location on ascidian percentage cover (Figure 28; ANOVA: F\(_{4, ~359}\) = 3.02 p<0.05). In addition, there was also a significant main effect of season with higher coverage observed in winter (ANOVA: F\(_{1, ~359}\) = 7.71, p<0.05).

![Figure 28. Mean number of ascidian recruits to settlement tiles at Masig and Marsden Islands over two seasons in 2008-2009 (±1 SE) (left), and mean percentage cover ascidian recruits (±1 SE) to settlement tiles (right). (W) is winter, (S) is summer.](image-url)
Bryozoans

**Abundance:** At least six species of bryozoans recruited to the underside of tiles. Species identification was completed on colony morphology and colour and still requires formal taxonomic confirmation. Bryozoan recruitment was dominated in both seasons by an encrusting white species (total numbers equivalent to 84.4% in winter and 79.3% in summer) and a grey erect branching species (11.5% in winter and 8.4% in summer). Overall, bryozoan recruitment 100cm$^{-2}$ at Masig Island sites was 3.1 (±0.51) at deep sites and 3.62 (±0.74) at shallow sites during the winter compared to 4.28(±0.91) (deep) and 3.25(±0.24) (shallow) at Marsden during the same period (Figure 29). Bryozoan recruitment in summer at Masig Island was 3.31 (±0.56) at deep sites and 2.5 (±0.45) at shallow sites compared to 2.68 (±0.52) (deep) and 3.31 (±0.54) (shallow) at Marsden Island. There was a significant interactive effect of season and depth explaining differences across seasons and locations (ANOVA: $F_{12, 359} = 1.96$ $p = 0.03$).

**Percentage cover:** Bryozoans showed variability in space occupied on the settlement tiles with a significant interaction of depth, site and season (Figure 29; ANOVA: $F_{12, 359} = 1.82$ $p<0.05$).

![Figure 29. Mean number of bryozoan recruits to settlement tiles at Masig and Marsden Islands over two seasons in 2008-2009 (±1 SE) (left), and mean percentage cover bryozoan recruits (±1 SE) to settlement tiles (right). (W) is winter, (S) is summer.](image-url)
Polychaetes

Abundance: Two genera of polychaetes recruited to the underside of tiles (*Metalaeospira* spp. and *Galeotaria* spp.), both of which form calcareous tubes. Overall, polychaete recruitment 100cm⁻² at Masig Island sites was 46.29 (±5.91) at deep sites and 40.93 (±7.94) at shallow sites during the winter compared to 39.47(±2.82) (deep) and 43.49(±8.9) (shallow) at Marsden during the same period (Figure 30). Polychaete recruitment in summer at Masig Island was 29.4 (±5.4) at deep sites and 36.6 (±4.45) at shallow sites compared to 30.67 (±5.1) (deep) and 40.2 (±5.14) (shallow) at Marsden Island (Figure 30). There was a significant interactive effect of site and depth explaining the spatial variation among islands (Figure 30; ANOVA $F_{12, 359} = 2.052; p = 0.02$). A significant interaction effect of season and island also accounting for spatial and temporal recruitment differences (ANOVA: $F_{12, 359} = 2.052; p = 0.02$).

Percent cover: Although polychaetes recruited in higher numbers to tiles, the percent cover for this group was the lowest in comparison to all other taxa (with the exception of cnidarians). For example, the highest mean coverage of tiles by polychaetes was 3.11(±0.91%) at Masig Island (deep) during winter and the lowest was 0.56(±0.21%) during summer also at Masig Island deep sites. Percent cover was consistent across all factors with the exception of a significant main effect of season, percent cover being higher in winter.

![Figure 30](image-url)

**Figure 30.** Mean number of polychaete recruits (±1 SE) left, to settlement tiles at Masig and Marsden Islands over two seasons in 2008-2009 and mean percentage cover polychaete recruits (±1 SE) to settlement tiles (right). (W) is winter, (S) is summer.
**Bivalves**

*Abundance:* At least two species, from two genera, of sessile bivalves recruited to the underside of tiles. Identification to species level was not completed due to the small size of bivalves and formal identification still needs to be confirmed, however tentative identification indicates species to be from the genera *Pindacta* and *Pteria*. Overall, bivalve recruitment 100cm$^{-2}$ at Masig Island sites was 5.35(±1.28) at deep sites and 1.51(±0.51) at shallow sites during the winter compared to 3.48(±0.79) (deep) and 1.91(0.4) (shallow) at Marsden during the same time period (Figure 31). Bivalve recruitment in summer at Masig Island was 2.43(±0.49) at deep sites and 1.96(±0.45) at shallow sites compared to 4.33(±0.7) (deep) and 2.79(±0.45) (shallow) at Marsden Island. There was a significant interactive effect of location and depth (ANOVA: $F_{4,359} = 3.73$, $p<0.05$) in addition to a significant main effect of season (ANOVA: $F_{1,359} = 5.07$, $p<0.05$) explaining recruitment variability at spatial scales of locations within islands and between summer and winter recruitment abundance.

*Percent cover:* There were significant interactive effects of season and depth (Figure 31; ANOVA: $F_{1,359} = 8.71$, $p<0.05$) in addition to interactions of depth and location (ANOVA: $F_{4,359} = 3.33$, $p<0.05$) on bivalve percent cover.

![Figure 31](image-url). Mean number of bivalve recruits (±1 SE) left, to settlement tiles at Masig and Marsden Islands over two seasons in 2008-2009 and mean percentage cover bivalve recruits (±1 SE) to settlement tiles (right). (W) is winter, (S) is summer.
Algae

At least five different groups of algae recruited to the underside of tiles. Identification was completed on algal morphology (encrusting or filamentous) and basic taxonomic grouping (red, green, brown), but still requires formal taxonomic confirmation. As such, filamentous and encrusting green and red algae were found in addition to brown algae. Overall, algal recruitment 100cm$^{-2}$ at Masig island sites was 9.19(±1.11) at deep sites and 8.48(±1.19) at shallow sites during the winter compared to 10.44(±1.09) (deep) and 11.64 (±1.28) (shallow) at Marsden during the same time (Figure 32). Algal recruitment in summer at Masig Island was 2.84 (±0.77) at deep sites and 4.56(±0.75) at shallow sites compared to 4.91 (±0.14) (deep) and 8.86(±1.65) (shallow) at Marsden Island. There was a significant interactive effect of site and depth (ANOVA: F$._{12, 359} = 1.99$, p<0.05) in addition to a significant main effect of season (ANOVA: F$_{1, 359} = 110.49$, p<0.05) explaining recruitment differences between summer and winter and at spatial scales among locations within the island groups.

Percent cover: Space occupied by algae on the settlement tiles varied significantly in accordance with interactions of site and depth (Figure 32; ANOVA: F$._{12, 359} = 2.21$, p<0.05) and also season and island. For example, algae occupied more space on tiles at Marsden during the winter in comparison to Masig in summer (ANOVA: F$_{1, 359} = 8.11$; p <0.05).

Translocation of sponge recruits

There was no significant effect of translocation on numbers of sponge recruits translocated between the two islands in comparison to control treatments that were not translocated, over the period of this experiment (May – November 2008).
Discussion

Recruitment patterns for many marine organisms exhibit considerable variation over spatial (Ayre and Hughes 2000) and temporal scales (Johnson and Black 1984), corroborated by the patchy distributions observed for numerous sessile invertebrates (e.g. Barnes et al. 2006 Bannister et al. 2007). A complex suite of processes including variability in levels of adult reproductive output (e.g. Whalan et al. 2007), and pre and post larval settlement selection processes (Morgan 2001, Whalan et al. 2008) contribute to fluctuating recruitment trends. The overall findings in this study are consistent with other studies that have identified spatial variability in recruitment patterns for sessile marine invertebrates (e.g. Barnes et al. 2006, Bannister et al. 2007). Notably, that recruitment of sessile invertebrates at Masig and Marsden Island showed complex patterns as discerned by significant interactive effects across a range of spatial (e.g. depth, site, location and island) and temporal scales (i.e. season).

Although the groups of taxa recruiting to Masig and Marsden Island reefs are consistent among the three years of this MTSRF funded project the variability exhibited at both temporal and spatial scales among each year of survey make generalised conclusions difficult. For example, ascidian recruitment showed consistent levels of recruitment regardless of season and location during the 2007- 2008 surveys (Duckworth and Wolff 2008) while the present study showed that interactive effects of location, season and depth, are responsible for different levels of recruitment. Whilst the last three years of surveys have collected valuable baseline data on recruitment patterns, further information is required before more conclusive interpretations can be drawn regarding temporal and spatial patterns of sessile invertebrate recruitment at Masig and Marsden Island. For example, recruitment for a number of groups varied between seasons. For some sessile invertebrates reproduction occurs according to discrete seasons and reproductive outputs (usually summer- e.g. Whalan et al. 2007) so this finding is not unexpected. The findings of year round recruitment however, suggest a continuous reproductive effort, although the collection of winter recruitment tiles (i.e. tiles deployed in May/June and collected in November) may also contain results of recruitment as late as November. An understanding of reproductive ecology including timing of seasonality and reproductive output are central in developing our understanding of population maintenance and this information would be valuable in partly explaining recruitment patterns of sessile invertebrates in Torres Strait.

One overwhelming and uniform finding among the three years of surveys is that recruitment has not been successful to the light exposed (topside) surface of tiles suggesting that larval settlement to top surfaces are either not occurring or are being disturbed by post-settlement processes, including incidental grazing (Maldonado and Uriz 1998). Settlement to the underside of tiles may therefore be un-representative of total settlement; it was expected that coral recruits would play a role in the recruitment dynamics, so this proposal is clearly supported with the low numbers of corals recruiting to tiles, many of which rely on access to light. Sponge larvae can also have specific cues for settlement to light (Whalan et al. 2008a). The presence of adult C. matthewsi in light exposed, coral rubble habitats (see chapter 2) suggest that this sponge would be more likely to recruit to light exposed surfaces rather than the underneath of tiles. The low numbers of C. matthewsi recruits may therefore be a reflection of larvae settling to the topside surfaces but failing to recruit to populations, succumbing to mortality through processes such as incidental grazing. Given the diversity and density of the settlement community and the observation that settlers may seldom become recruits or established adults, particularly on upper surfaces, suggests that the process of successful recruitment is a rare event. If this is the case then there is a likelihood that the reef biodiversity is vulnerable to disturbance events and the observed community may well have been established over a long period.
While there are no data detailing the larval ecology of *C. matthewsi* (e.g. larval behaviour, competency, settlement cues), for some sponges successful settlement is mediated by a range of behaviours linked to both specific chemical and physical cues (Whalan et al. 2008). The discrete habitat associated with the experimental design (tiles) may be insufficient for larval settlement of *C. matthewsi*. Although terracotta tiles have proved successful for coral and other invertebrate settlement studies, the specificity of larval settlement cues for other invertebrates such as *C. matthewsi* may have prevented realistic estimates of recruitment in this study. *Coscinoderma matthewsi* has a strong association with light exposed, dead coral rubble habitats (pers. ob). Settlement assays coupled with permanent quadrats to quantify *C. matthewsi* recruitment planned in 2009-2010 will provide further information on recruitment dynamics for *C. matthewsi*.

Finally, the translocation of tiles with sponge recruits between Marsden and Masig Islands showed no effect on post-settlement survival. Given the presence of species that recruit to the tiles are common to both habitats this is not entirely surprising. The abundance of *C. matthewsi* recruits to tiles has generally been not detectable in comparison to dominating forms (e.g. encrusting species) so drawing conclusions on the effects of island habitat for this species are difficult with the data associated with the present study. There are clearly differences in abundances of *C. matthewsi* adults between these two islands, and it would be valuable to include translocations of adults (e.g. explants) as well as recruits of *C. matthewsi*, with adequate replication, to assess this question further.
Larval Biology and Behaviour

Steve Whalan and Chris Battershill

Introduction

Larval dispersal, settlement and recruitment of marine organisms are key processes in defining adult distributions, particularly for sessile invertebrates which exhibit a mobile larvae phase. Larval dispersal can be influenced by both large scale oceanic processes (Sponaugle et al. 2002) and intrinsic biological traits, including larval competencies (Miller and Mundy 2003, Nozawa and Harrison 2005), swimming ability (Metaxas 2001), and vertical migration behaviours (Raimondi and Morse 2000, Queiroga et al. 2002). Fine scale habitat influences also mediate settlement with successful recruitment, in part, relying on larvae identifying favourable habitats to settle. Therefore, for many invertebrate larvae, settlement is often in response to environmental cues signalling favourable habitats.

Light, salinity, temperature, pressure and gravity all contribute to the settlement process (Young 1995, Underwood and Keough 2000, Maldonado 2006). In addition, cues associated with physical surfaces and chemicals affect larval settlement. Surface micro-topography can provide settlement adhesion points (Verran and Boyd 2001) and micro refuges (Maldonado and Uriz 1998, Peterson et al. 2005). Chemical cues have been widely investigated for marine invertebrate larvae and include cues associated with biofilms of micro-organisms (Pawlik 1992, Hadfield and Paul 2001, Negri et al. 2001, Huang and Hadfield 2003), conspecifics (Raimondi 1991, Head et al. 2004), and host organisms (Swanson et al. 2004).

Larval settlement in many sessile marine invertebrates is well documented, however holistic approaches quantifying the entire pre-settlement phase from larval release to recruitment are rare (Harrison and Wallace 1990, Raimondi and Morse 2000). There are fewer studies demonstrating these processes in other conspicuous benthic taxa such as sponges (but see Maldonado and Young 1996, Maldonado and Uriz 1998, Maldonado 2006, Whalan et al. 2008a). Studies undertaken to date indicate depth regulation (Uriz et al. 1998), phototaxis (Leys and Degnan 2001, Maldonado et al. 2003), and settlement surface micro-refuge characters (Maldonado and Uriz 1998), to be important for successful sponge larval settlement. In addition some species respond to cues associated with micro-organism biofilms before successfully settling (Keough and Raimondi 1995).

The objective of this study was to develop our understanding of the fundamental larval ecology of Coscinoderma matthewsi, the target sponge for the applied elements of this MTSRF program, and a useful and highly relevant model for examining recruitment process of important Torres Strait reef organisms in general. Information of this nature enhances our understanding of population distributions, including the processes that influence and maintain them, and is fundamental to the conservation and management of marine benthic environments. In addition, an overall understanding of larval settlement behaviour would provide the first step necessary to underpin future exploration of hatchery production of seed sponges, thereby increasing the sustainable management of this resource. Using manipulative laboratory experiments, this study examined the behavioural characteristics of C. matthewsi larvae from release to settlement and metamorphosis. Specifically, the pre-competency periods and swimming abilities of larvae are determined in addition to quantification of settlement responses to cues associated with biofilms.
Methods

Spawning synchronicity between Orpheus Island and Masig Island sponges

This research was undertaken at Orpheus Island Research Station rather than Masig Island because of the necessity to use laboratory based aquarium facilities allowing experimental manipulations of larval assays under controlled conditions (i.e. filtered (25 um) flow-through sea water with regulation of photoperiod and temperature). The small size of larvae (0.5 mm in length) also requires access to labs with microscopes to establish and monitor larval experiments. The facilities at Masig Island are currently unsuitable as a base for conducting this research. Given *C. matthewsi* occurs both at Orpheus Island and Masig Island, information on larval behaviours should be relevant regardless of where the experiments take place. However, to establish if spawning cycles are consistent between Orpheus and Masig sponge populations, Torres Strait Islander colleagues assessed the spawning cycles of *C. matthewsi* at Masig during the same period the larval work was being undertaken at Orpheus. Both TS and Orpheus crews established the same experimental protocols on Masig, based on established methodologies (e.g. Whalan *et al.* 2008a), prior to the summer project. To establish spawning cycles of *Coscinoderma matthewsi* at Masig Island, larval traps based on a Whalan modified design from Lindquist *et al.* (1997) were placed on ten large adult sponges in the late afternoon during the same period that spawning was observed in sponges from Orpheus Island (December 2009). Traps were checked and removed the following day (late morning).

Study site and sample collection – larval settlement OIRS

Larvae were collected from adult female sponges located on the fringing reefs off Orpheus Island on the central GBR. Experimental manipulations were conducted at Orpheus Island Research Station (OIRS) during the spawning season of *C. matthewsi* in December 2009. *C. matthewsi* is a brooding species releasing larvae in the day time by dribble spawning (Whalan unpub data). Larvae were collected using the modified larval traps placed over sexually mature sponges at dawn. Larvae were collected mid to late morning providing larvae that were up 6 hours old. Following collections, larvae were transported to OIRS to examine pre-settlement larval mobility and settlement behaviour. The collection of larvae from several sponges maintained in flow-through aquaria at OIRS, were also used.

Settlement assays

Larval settlement assays were based on the methods of Whalan *et al.* (2008a) which undertook investigations of larval settlement for a closely related sponge *Rhopaloeides odorabile*. Briefly, settlement assays were conducted in 70 ml plastic jars (n=5) filled with 10ml of 25µm filtered seawater (FSW). Biofilm treatments included jars that had been deployed in Pioneer Bay at 10m depth for 18 days prior to starting assays. *Coscinoderma matthewsi* is commonly found in Pioneer Bay at 10m. Prior to commencing assays, biofilmed jars were carefully shaken underwater to remove incidental and loose sediment. Each of the biofilmed and control treatment jars (i.e jars without a biofilm), were then filled with 10 ml of FSW and 10 larvae added. Numbers of larvae that had settled or undergone metamorphosis was then recorded, initially at 6 hours then every 12 hours thereafter until all larvae had either settled or died. Qualitative records of larval behaviour were also noted using the control treatments to ascertain larval pre-settlement competencies. Larval settlement was analysed using a repeated measures analysis of variance (RM ANOVA), with time of settlement as the within factor and settlement as the between factor.
Results

**Spawning behaviour of *C. matthewsi* –Orpheus Island**

At Orpheus Island, twenty sponges were investigated for spawning activity, each having a larval trap placed over the sponge. The release of fully developed larvae confirmed viviparity in this species. During the experimental period (Dec 2009) all sponges exhibited spawning activity, with sponges dribble spawning up to 600 larvae per day during daylight hours (Whab and Whalan unpub data). Larvae were released just after dawn and terminating just before dusk (0600 and 1700 hours). No spawning was observed during the night. Larvae are tufted parenchymellae, typical for dictyoceratid sponges, prolate spheroid in shape with a mean size of 572 µm in length (Figure 33 Wahab and Whalan unpublished data). At release, larvae exhibit sustained swimming for up to 24 hours, at which time swimming is replaced by intermittent periods of substrate exploration and temporary attachment, followed by settlement by the anterior pole, completing metamorphosis by larval invagination to form a disk like morphology (Figure 34). Growth to a juvenile sponge continues with the formation of a central accumulation of body mass and the extension of a single oscular tube, visible from 6-8 weeks (Figure 35).

Figure 33. *C. matthewsi* larva showing anterior (a) and posterior ends (p), posterior end showing ciliated pigment ring supporting very fine cilia (c) used to maintain and direct movement.

Figure 34. *C. matthewsi* following metamorphosis, showing a clear transition from larva to a flattened disk like morphology with the pigmented cilia ring (c) evident in the centre of the disk.

Figure 35. *C. matthewsi* juvenile sponge at 60 days post metamorphosis, showing the development of an oscular tube (o). Sponge is approximately 2mm long including oscular tube. Image produced with permission – Muhammad Abdul Wahab
**Larval release cycle at Masig island**

Six out of the ten sponges which had larval traps placed over at Masig Island were observed to each contain several hundred larvae. Larval release occurred in concert with spawning at Orpheus Island confirming spawning synchronicity of this species at these two locations. Consistent with larval release at Orpheus Island, sponges at Masig Island also released larvae during the day, with no evidence of larvae being released at night.

**Larval pre-competencies**

Larval pre-settlement activity, without the presence of ‘chemical’ (biofilm) cues extended up to 48 hours (Figure 36). During this time all larvae displayed characteristic behaviours at release whereby they swam to the surface of the experimental jar, and maintained this position from 18-24 hours. Following this time period larvae migrated to the bottom of experimental jars retaining a demersal habit until settlement.

**Larval settlement**

Mean larval settlement to treatments with biofilms was 76% with a corresponding mortality of 24 %. In filtered sea water, (i.e. no established biofilm) the mean larval settlement was 70 % and 30 % mortality (Figure 36).

There was a significant interactive effect of biofilm and time influencing larval settlement for *C. matthewsi* (RM ANOVA: $F_{1,45.1} = 5.86, p=0.03$). Larval settlement occurred from 18 hours, regardless of the presence of a biofilm, and terminated at 30 hours for biofilm treatments and 42 hours for treatments without any biofilm cue. The significant interaction of biofilm and time is clearly seen with numbers of larvae settling at 18 to 30 hours being markedly higher for biofilm treatments. For example, at 18 hours mean larval settlement was 46% in comparison to 10% for treatments without a biofilm. This pattern continues at 24 hours and 30 hours, until 42 hours where settlement for biofilmed treatments was 76 % in comparison to 70 % for non biofilmed treatments.

![Figure 36](image-url)  
**Figure 36.** Mean *C. matthewsi* larval settlement (± 1 SE) over time in response to treatments with an established 10 day old biofilm and control treatments with no biofilm.
Discussion

*Coscinoderma matthewsi* is viviparous releasing tufted parenchymella larvae, during daylight hours for extended periods over the Austral summer. While comprehensive reproductive data for this sponge is still to be undertaken this initial data are consistent with what is known for closely related dictyoceratid sponges. Both *Rhopaloeides odorabile* and *Luffarella variabilis* are viviparous and spawn tufted parenchymella larvae during daylight over 4-6 weeks during December and January (Ettinger-Epstein et al. 2008, Whalan et al. 2008a).

Comprehensive detail on the larval ecology in sponges is largely missing from the literature. The few accounts that are available suggest that sponge larvae have poor mobility and exhibit short pre-competency periods, processes which may contribute to subdivided population genetic structures (Whalan et al. 2005, 2008b). For example, the sponge *Rhopaloeides odorabile* has pre-competency periods similar to *C. matthewsi* in this study and exhibits population genetic differentiation over kilometers (Whalan et al. 2008b). While there are still knowledge gaps surrounding the larval ecology of *C. matthewsi* the consistencies between pre-settlement larval behaviour would suggest that similar patterns of larval dispersal and population genetic structure may exist.

Larval settlement to an established biofilm was not unexpected; biofilms in nature occur everywhere. Moreover, this result has been found for other sessile invertebrates (Pawlik 1992) as well as sponges (Ettinger-Epstein et al. 2008, Whalan et al. 2008a). This result raises two important considerations. Firstly, for the motile larval stage, finding a suitable habitat is critical because once metamorphosis is completed the decision is irreversible. Biofilms are indicative of open space, and the decision to settle to this cue should therefore facilitate growth without impeding competition from other sessile taxa. Secondly, larval settlement to biofilmed surfaces occurred for *C. matthewsi* more rapidly in comparison to non-biofilmed surfaces. This finding raises important questions central to larval and juvenile fitness. Sponge larvae are lecithotrophic (Maldonado 2006) and the ability to find a suitable settlement habitat earlier in the swimming phase will likely mean that more energy (lipid reserves) can be directed to development and growth of the juvenile rather than exhausted through additional swimming and exploration of a suitable settlement habitat.

While biofilms are implicated in more rapid settlement of *C. matthewsi*, larval settlement is often more complex, involving a hierarchical range of physical and chemical cues to promote settlement (Ettinger-Epstein et al. 2008, Whalan et al. 2008a). This can include cues associated with coral rubble (Whalan et al. 2008a) and conspecifics (Ettinger-Epstein et al. 2008). Moreover, it can include physical cues including light (Whalan et al. 2008a, Ettinger-Epstein et al. 2008) and surface micro-topography (Maldonado and Uriz 1998), all of which contribute to survival (recruitment) to the mature population. There is clearly more work required before the larval ecology of *C. matthewsi* is comprehensively known, however, this study outlines information that forms a foundation in this task. In addition, it provides ancillary information critical to closing the life cycle of this sponge which is currently being developed as an aquacultured bath sponge. Fundamental data on the larval cycle will remove the reliance of sourcing seed stock from wild harvest and facilitate the recruitment of sponges onto artificial surfaces for grow-out, improving the overall sustainability of this industry.
Connectivity across East and Central Torres Strait

Alan Duckworth, Rose Cobb, Nicole Webster and Carsten Wolff

Introduction

Genetic markers are a powerful tool for population structure analyses in sponges and other taxa, and different categories of molecular markers have already been applied to these types of investigations for sponges (Duran et al. 2004a). In the past, the most commonly used markers for sponge population studies were allozymes. However, these markers can mask genetic divergence, especially within populations (Klautau et al. 1999). Increasingly, population structures have been examined with DNA sequence analyses, which are considered more powerful as cryptic changes in genetic loci can be identified (Shearer et al. 2002, Duran et al. 2004a, Duran et al. 2004b).

For fine-scale population structure studies, the ribosomal internal transcribed spacer region (ITS) is the predominantly used marker (Nichols and Barnes 2005). Sequences from the ribosomal subunits (5.8S rDNA, 18S rDNA and 28S rDNA) have also been useful in determining the genetic structure of sponge populations (Worheide et al. 2002, Watkins and Beckenbach 1999). Microsatellites are very useful markers for describing population structures (Duran et al. 2004a), as subtle divergences have been detected when other markers failed to show genetic heterogeneity (Hughes and Queller 1993, Jarne et al. 1994). Duran et al. (2004b) examined mitochondrial DNA (mtDNA) sequence data of Crambe crambe but found very low levels of intraspecific variation, and therefore mtDNA markers may not be useful for population-level studies of sponges.

Many sponge species are host to a huge density and diversity of microorganisms with bacterial cells comprising 40-60% of the tissue volume in some species (Hentschel et al. 2006). A variety of sponge-microbe associations have been described in tropical, temperate and Antarctic regions and these involve a diverse range of heterotrophic bacteria (including facultative anaerobes), cyanobacteria, unicellular algae, fungi and archaea (Wilkinson 1980, Preston et al. 1996, Holler et al. 2000, Webster and Hill 2001, Webster et al. 2001, Hentschel et al. 2002, Taylor et al. 2004, Webster et al. 2004).

There are numerous descriptions of specific sponge-microbe relationships in which the host may benefit from: the provision of nutrition (Wilkinson and Garrone 1980); transportation of waste products or active metabolites (Wilkinson 1978a, Wilkinson 1983, Borowitzka et al. 1988); chemical defence (Unson et al. 1994); or contribution to mechanical structure (Wilkinson 1978b). Presumably the microorganisms benefit from the provision of a protective supporting medium and nutrient flow, although little evidence has been published to support this. Sponge-associated microbial communities have a phylogenetic signature distinctly different from that of microorganisms residing within the marine plankton and sediments (Webster and Hill 2001, Hentschel et al. 2002, Taylor et al. 2004, Webster et al. 2004, Taylor et al. 2005) with some evidence for uniformity in sponge microbial communities between oceans and species (Hentschel et al. 2002). Sponges however, can filter large quantities of seawater (Bell et al. 1998), hence a proportion of microbes residing within the tissue may be transient components of the ambient seawater. Even with the assistance of molecular techniques it can be extremely difficult to distinguish between true ‘sponge-associated bacteria’ and seawater-derived contaminants.

The bath sponge Coscinoderma matthewsi is being farmed in Torres Strait, with the first pilot venture located at Masig. If this farm proves commercially viable it is likely that additional island communities will want to establish sponge farms in their local waters. This may require
C. matthewsi individuals to be moved from regions or islands where they are abundant (e.g. Masig) to regions where they are not. A potential problem of sponge translocations between populations, such as in Torres Strait, is a decrease in the genetic diversity of wild populations (Cognetti et al. 2006). Translocation may also introduce new sponge-associated microbe types into a region.

As sponge communities are prevalent in Torres Strait, information about connectivity between populations will also provide insights into the connectivity of Torres Strait benthic communities in general. To inform the dual perspectives of aquaculture translocation and ecological assessment, we examine and compare the genetic structure and microbial populations of C. matthewsi populations from central and eastern Torres Strait. For microbial analysis, a DNA fingerprinting technique (denaturing gradient gel electrophoresis – DGGE) was used to determine the stability of bacterial associations within C. matthewsi across wide spatial scales.

Methods

Genetic analysis

Specimen collection and preservation
During the central and eastern Torres Strait survey in November 2006, tissue samples from 10 individuals of C. matthewsi were collected from each island-group (Table 12). In addition, 10 sponges were collected from each of two sites at Masig (Table 12). All sponge samples were placed in separate cryo-tubes and preserved in liquid nitrogen until they could be stored at -80°C.

<table>
<thead>
<tr>
<th>Island-group</th>
<th>Site</th>
<th>Sample number codes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Masig</td>
<td>1, Kodall Is</td>
<td>1-10</td>
</tr>
<tr>
<td>Masig</td>
<td>2, Keats Is</td>
<td>11-20</td>
</tr>
<tr>
<td>Erub</td>
<td>1</td>
<td>21-30</td>
</tr>
<tr>
<td>Ugar</td>
<td>1</td>
<td>31-40</td>
</tr>
<tr>
<td>Poruma</td>
<td>1</td>
<td>41-50</td>
</tr>
<tr>
<td>Warraber</td>
<td>1</td>
<td>51-60</td>
</tr>
</tbody>
</table>

DNA extraction, amplification and sequencing
Approximately 2 g of tissue were homogenised in liquid nitrogen and 750 μl of lysis buffer [100 mM Tris pH 9, 100 mM EDTA, 1% SDS, 100 mM NaCl, 0.5 mg/ml Proteinase K], and subsequently it was incubated at 65°C for 1 hour with gentle agitation. KoAc was added to a final concentration of 1 M, followed by incubation on ice for 30 minutes. The samples were centrifuged at 8000 rpm for 15 minutes, and the supernatant was reserved for DNA precipitation with isopropanol using the standard protocol.

A fragment of nuclear DNA containing part of the 28S rRNA gene was amplified for all individuals using RD3A (5’-GACCGTCTTGAAACAGA) and RD5B2 (5’-ACACACTCTTACGGA) primers. Recombinant Pfu Polymerase (Fermentas) was used for the PCR. A total of 50 μl of reaction mixture were prepared for each sample according to the protocol. PCR was performed under the following conditions: initial denaturation at 95°C
for 3 minutes; 35 cycles of 95°C for 30 seconds, 50°C for 20 seconds, 72°C for 1 minute; a
final extension step of 72°C for 10 minutes. Products were purified with QIAquick (Qiagen)
columns according to protocol. Sequencing was performed at Macrogen Inc. with a 3730xl
DNA analyser using both forward and reverse primers.

Data analysis
Sequences were edited and formed into contigs using Vector NTI (Invitrogen), then aligned
in Sequencia and trimmed using MacClade. A BLAST search of GenBank was used to
confirm the taxonomic origin of the sequences. The final length of the sequences was 690
bp. Two of the sequences (one each from Poruma and Warraber) were removed from the
analysis due to poor sequence data. The data was analysed with MEGA3.1. To determine
nucleotide differences, the following calculations were done:

Mean Diversity within Subpopulations
In a subpopulation, the mean diversity is defined as
\[
\alpha_i = \frac{1}{q-1} \sum_{i=1}^{q} \sum_{j=1}^{q} \chi_{ij}d_{ij}
\]
where \(\chi_{ij}\) is the frequency of i-th sequence in the sample from subpopulation i, and q is the
number of different sequences in this subpopulation.

Mean Diversity for Entire Population
For the entire population, the mean diversity is defined as
\[
\alpha_T = \frac{1}{q-1} \sum_{i=1}^{q} \sum_{j=1}^{q} \chi_{ij}d_{ij}
\]
where \(\chi_{i}\) is the estimate of average frequency of the i-th allele in the entire population, and q
is the number of different sequences in the entire sample.

Mean Interpopulational Diversity
The estimate of interpopulational diversity is given by
\[
\delta_{ST} = \chi_T - \chi_S
\]

Coefficient of Differentiation
The estimate of the proportion of interpopulational diversity is given by
\[
N_{ST} = \delta_{ST}/\chi_T
\]

Microbial analysis

DNA Extraction
Four replicate sponges were analysed from sites 1 and 2 at Masig and three replicate
sponges were analysed from Erub, Ugar, Poruma and Warraber. DNA was extracted from
individual sponges by homogenising approx 1g of tissue from each individual in 0.5 ml of
grinding buffer (2 ml 1 M Tris, 4 ml 0.5M EDTA, 2 ml 10% SDS, 400 µl 5 M NaCl and 11.6 ml
distilled water). Tubes were immersed in liquid nitrogen and ground with plastic pestles.
Samples were incubated at 65°C for 60 min prior to addition of 187 µl 5 M potassium acetate.
Samples were incubated on ice for 30 min and centrifuged at 8000 x g for 15 min. The
supernatants were transferred to fresh tubes and DNA was precipitated with 0.8 vol of
isopropanol.
**DGGE**

The 16S rDNA from each sample was amplified by PCR with universal bacterial primers 1055f: 5′-ATG GCT GTC GTC AGC T-3′ and 1406r: 5′-ACG GGC GGT GTG TAC-3′, (Ferris et al. 1996). The reverse primer was modified to incorporate a 40 bp GC clamp (Muyzer et al. 1993). Primers 1055f and 1406r match over 56,000 and 62,800 sequences respectively in the Ribosomal Database Project. PCR reactions were performed as described by (Ferris et al. 1996). Products from triplicate PCR reactions were combined and 15 μl applied to duplicate 40% wt/vol polacrylamide (37:5:1) gels containing a 50-70% denaturing gradient of formamide and urea. Gels were electrophoresed at 60°C for 17 h in 1 x TAE buffer at 50V using the Ingeny D-Code system. Gels were stained with 1 x Sybr Gold for 30 min, visualised under UV illumination and photographed.

**Results**

**Genetic analysis**

The 690 bp sequence was analysed for all 58 individuals. The mean interpopulation diversity was 0.000 with a standard error of 0.000 when analysed with the Kimura 2-parameter model. However, there was variation observed in the sequences, and Table 13 shows that this was mostly within populations. Furthermore, after constructing a maximum parsimony tree with 500 bootstrap replicates, there were 293 equally probably trees indicating that there was no group structure, and this was supported by low bootstrap values (Figure 37).

**Table 13. Number of nucleotide differences (distance).**

<table>
<thead>
<tr>
<th>Distance</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within Kodall (Masig)</td>
<td>1.578</td>
</tr>
<tr>
<td>Within Keats (Masig)</td>
<td>1.556</td>
</tr>
<tr>
<td>Within Erub</td>
<td>0.956</td>
</tr>
<tr>
<td>Within Ugar</td>
<td>0.528</td>
</tr>
<tr>
<td>Within Poruma</td>
<td>0.400</td>
</tr>
<tr>
<td>Within Warraber</td>
<td>2.833</td>
</tr>
<tr>
<td>Mean diversity within subpopulations</td>
<td>1.308</td>
</tr>
<tr>
<td>Mean diversity for the entire population</td>
<td>1.390</td>
</tr>
<tr>
<td>Mean interpopulational diversity</td>
<td>0.082</td>
</tr>
<tr>
<td>Coefficient of Differentiation</td>
<td>0.059</td>
</tr>
</tbody>
</table>

**Microbial analysis**

DGGE analysis for replicate C. matthewsi samples at each site revealed 5 predominant bands in all replicate sponges (Figure 38). This indicates that a major component of the microbial community is conserved in all individuals across a wide spatial scale. Some intersponge variability in banding patterns was observed. For example, sponge 15 from Masig site 2 and sponge 41 from Poruma both contained a larger number of bands indicating a more diverse microbial community than other samples.
Figure 37. Maximum parsimony tree of the 6 sponge populations with 500 bootstrap replicates. The five island-groups are: Ugar = Stephens, Erub = Darnley, Masig = Yorke, Poruma = Coconut, and Warraber = Sue.
Discussion

The variability observed in the partial 28S sequences across all individuals from the six sample areas showed little to no group structure correlating with island-groups but variation occurred within island groups. This may be due to gene flow across the spatial scale examined, and the variation that was observed within island groups may have been a result of intragenomic variation rather than variation between individuals as it has been determined that sponges have multiple copies of rRNA genes within a single genome (Wörheide et al. 2004). While sponge populations that are further apart than those reported here have also shown low levels of spatial structure, measurement of genetic population structure in sponges can be problematic and is highly method dependant. For example, Duran et al. (2004b) described one single polymorphic nucleotide in mtDNA sequences of Crambe crambe populations over 3000km in the western Mediterranean and Atlantic range, but in later studies based on ribosomal ITS sequences (Duran et al. 2004c) and on microsatellite sequence data (Duran et al. 2004a) it was found that these populations were genetically distinct. Identical mtDNA sequences were also found across four species and two genera (Lubomirskia and Baikalospongia) of Lake Baikal freshwater sponges (Schroder et al. 2003, cited in Erpenbeck et al. 2005). In contrast, Wörheide et al. (2002) identified four different clades of Leucetta ‘chagosensis’ over the western Pacific region with rDNA sequence data, although the geographical distances between the genotypes were relatively large. Two distinct clades of Clavelina lepadiformis (Ascidiaeae) were found over short distances (<10 km) in different environments from mtDNA sequences, a result attributed to the species’ short larval life-span (Tarjuelo et al. 2001).

While the results of the current study have failed to identify island-based groupings, this result should be treated with caution. Sample sizes from each island-group or site may have been insufficient to accurately assess population structure of these sponges. Other DNA markers such as microsatellite (Duran et al. 2004a) or other ribosomal or ITS markers (Duran et al. 2004c) may be more variable, and could be used to confirm the lack of population
structure and associated risks involved with sponge translocation within Torres Strait. Unfortunately, such analysis was outside the scope of the current study.

The pattern in microbial bands also failed to correlate to any particular island-group or site, and instead indicated that no site specific variability exists in microbial communities associated with *C. matthewsi* sampled from central and eastern Torres Strait. These results are in accordance with previous studies of tropical (Webster and Hill 2001) and Antarctic (Webster *et al.* 2004) sponge-associated microbial communities which demonstrated highly conserved microbial assemblages across wide spatial scales.

While the lack of genetic population structure correlating with island-groups coupled with consistent sponge-associated microbial communities observed here is consistent with a finding of connectivity across the spatial scale sampled, such a conclusion cannot be made on these results alone due to the limitations of the methods used. However, when combined with data on larval behaviour and biology plus hydrodynamics of the region, the results reported here can make an important contribution to our understanding of connectivity in Torres Strait.

Larval biology studies for *C. matthewsi* have demonstrated daily spawning of larvae over several weeks, with swimming ability observed for up to 24 hours (see previous chapter in this report). Coupled with typically complex hydrodynamics (Wolanski *et al.* 1984, 1988), and strong currents in Torres Strait (Lemckert *et al.* 2009), *C. matthewsi* larvae would be expected to disperse far and frequently enough to maintain population connectivity beyond the scale assessed in this study. It has been argued that sponge larvae may travel even greater distances than expected from their swimming capabilities by currents (Mariani *et al.* 2006).

Thus, gene flow facilitated by larval dispersal in strong Torres Strait currents, coupled with the corroborating lack of island-group based population structure and microbial communities found in this study, all point to connectivity in this species between areas sampled: Ugar, Erub, Masig, Poruma and Warraber. While this indicates there is little risk in translocation of *C. matthewsi* between central and eastern Torres Strait, further genotyping studies using larger sample sizes is recommended to confirm this finding.
Optimising Sponge Farm Practice

Steve Whalan and Elizabeth Evans-Illidge

Introduction

Sponge aquaculture currently relies on growing out clonal fragments, or explants, which have been cut from wild donor sponges. The production of explants for grow-out involves cutting and exposing the surface of sponges, which in turn demands a recovery stage before the new sponge explants can invest energy in growth.

To the explant, this recovery process involves some combination of two stages: firstly, the production of a new epithelial layer over the cut surface to seal the new explant’s aquiferous system (analogous to the first-aid application of a band-aid); and secondly, the re-organisation and alignment of internal cells and structures to create a brand new functional sponge (Kelly-Borges and Bergquist 1988, Wulff 1985, Baldacconi 2010, Hammel et al. 2009). Successful recovery may depend on the health of the donor sponge (Duckworth and Wolff 2007a, Baldacconi et al. 2010, Kelly-Borges and Bergquist 1988, Henry and Hart 2005), the types of parent tissues included with the explant (Kelly-Borges and Bergquist 1988), and the overall size of the explant (Kelly-Borges and Bergquist 1988, Battershill and Bergquist 2010). Recovery may require significant cellular re-organisation and energy investment to achieve (Korotkova 1970, Henry and Hart 2005, Louden et al. 2007).

Nevertheless, the recovery of some sponges following damage from cutting can be rapid. The sponge that is the focus of this project, Coscinoderma matthewsi, produced a protective collagen layer over the cut surface within 24 hours and achieved full cellular integrity of the pinacoderm (i.e. outer skin of the sponge) and aquiferous systems within six weeks after cutting, in field experiments conducted on the Great Barrier Reef (Louden et al. 2007).

Australia’s first sponge farm was established in 2008 by Kailag Enterprises Limited, to commercially grow C. matthewsi at Masig, following recommendations from Duckworth et al. (2007b,c). There is a need to improve farm production with higher growth and survival of farmed sponges. This study, which was carried out in the context of the MTSRF program but separately funded by the Torres Strait Regional Authority, aimed to identify ways to improve explant collection and handling techniques to maximize speed and degree of recovery and therefore minimize energy investment required for recovery.

These key questions were addressed in this research, which was separately funded by the Torres Strait Regional Authority. The outcomes will be highly transferrable to other potential sponge farm ventures elsewhere, to optimise the collection and handling of sponges prior to grow-out. The following key questions were considered:

1. How much does the exposure of explants to air influence survival and growth?

Collection of explants can result in some exposure to air, during the transfer of explants from in-sea collection to holding tanks for transport around work areas, to the grow-out panel framework. While the time of exposure can be restricted to relatively short time frames (i.e. seconds – minutes) we know little of the effect on survival or growth from this exposure, in order to advise best-practice handling guidelines. Although some species that inhabit intertidal regions frequently experience long periods of exposures to air (e.g. Phyllospongia lamellose), C. matthewsi is a subtidal species and the effects of subjecting these sponges to ‘out of water conditions’ (air, sunlight and temperature) in a damaged (fresh explant) state
are unknown. This study will assess a range of air exposure treatments to identify the optimum balance between handling convenience and growth/survival performance.

2. **Would explant growth and survival benefit from a transitional nursery stage for recovery, following collection from donor sponges but prior to inclusion on-farm?**

The process of explant cutting and deployment to the farm includes two distinct phases that cause damage. The initial cutting results in direct damage and exposure of internal tissue, and an exposed aquiferous system. The second step, of squeezing the explant into a mesh panel at the farm, often causes cell-loss through the exposed surface. A nursery phase to allow recovery from cutting prior to further potential trauma, may enhance overall recovery from the two. This study will assess benefits of an intermediary nursery phase in improving growth and survival.

3. **Does the size of donor sponge affect explant growth and survival?**

Energy investment into growth is mediated by investments into other key life history traits, including reproduction, and adaptive internal reorganization and regeneration (Meroz-Fine *et al.* 2005, Wulff 2006, Henry and Hart 2005). Also, genotypic controls on somatic growth have been suggested (Kelly-Borges and Bergquist 1988). It is possible that smaller, sexually immature sponges, that are not yet contributing to the bio-energetic investment of reproduction, or are still in an early rapid growth phase, may exhibit faster growth rates compared to large sponges, and if so, that this trait may transfer to clonal explants. This study will assess growth and survival performance of explants sourced from different size classes of donor sponges.

4. **Does the body region of donor sponges from which explants are taken, affect explant growth and survival?**

The recovery rate of a damaged sponge is influenced by its efficiency in regeneration of compromised cellular and skeletal architecture. New explants have to take their start-tissue package, which is a piece of a donor sponge, and turn it into a new functional individual. Kelly-Borges and Bergquist (1988) have proposed that the inclusion of donor sponge oscules in the explant will promote the more rapid development of a new aquiferous system and regeneration of the new sponge. Oscules and associated canal architecture are typically located along the upper surface of *C. matthewsi* to facilitate water (i.e. food and oxygen) exchange. This study will assess whether or not explant survival and growth can be improved if the new explant comes from the dorsal, oscule-rich, region of donor sponges (compared to central or basal regions).

5. **Does explant squeezing (i.e. the loss of sponge cells) effect growth and survival?**

Damage caused during the handling of explants during their collection and production, is often unavoidably exacerbated through squeezing to fit the explants into sponge farm equipment (mesh panels). This is because a tight fit in the panel has been shown to be important to prevent excess movement causing further surface damage. Squeezing usually results in cell loss which can be easily visualized as cloudy water. This study will assess the extent to which different levels of squeezing has an impact on explant growth and survival.

Besides reporting the results of these investigations into explant collection and handling methods, this chapter also documents anecdotal observations of sponge-farm grow-out experience in the uptake of recommendations from previous sponge farming research into commercial practice. While the sponge farm initially followed recommendations in Duckworth *et al.* (2007b,c) in the use of sponge panels as the most efficient method of sponge grow-out, the farm workers report unexpectedly excessive levels of fouling, which smothered and adversely affected the sponges. To alleviate this problem, farm procedures were revised,
and workers removed the mesh from the panels and attached sponges using cable ties, in order to minimize fouling surfaces.

**Materials and methods**

**General collection and culture methods**

Explants were sourced from several donor sponges surrounding Masig Island in 2008. Approximately one third of donor sponges were collected for production of explants (the remainder of the donor remains attached to the reef and heals and regrows (Duckworth and Wolff, 2007a)).

After application of relevant treatments (see below), explants were cultured following methods modified from Duckworth and Wolff (2007a). Briefly, explants were measured and randomly placed into 8-pocket PVC mesh panels (Australian netmakers – see Figure 39), commonly used to farm pearl oysters. Each panel pocket had four explants, forming subsamples with each experimental treatment, which was replicated four times. Each panel was attached to a riser (rope) line, which was fixed to the bottom and held upright with a sub-surface buoy, so that each panel was established between 9-12 metres.

For the ‘nursery’ and ‘squeezing’ experiments, explants were also loosely placed into plastic mesh oyster baskets (TTP plastics-aquatray) which were fixed horizontally to the sea-floor. Oyster baskets are purpose made plastic mesh (25mm mesh) rectangular crates (93cm x 91cm x 10cm) with 9 internal partitions and enclosed with a mesh lid (Figure 39). To quantify growth during the experimental period, explants were measured along the greatest axis of length, width and depth, at the experiment deployment (February 2009), monthly for the duration of the experiments, and then at the end of the experiment in June 2009. Explant survival was also recorded. For simplicity, analyses of results relied on incremental growth from the beginning and final measurements. Data was analysed using one-way Analysis of Variance (ANOVA) except for the nursery experiments, which was analysed using t-tests. Data for the survival of explants in the handling experiments did not meet the assumption of homoscedasticity and was analysed using the non-parametric test of Kruskal-Wallis.

![Figure 39. Photo showing (a) an oyster basket used in the squeezing (handling/ nursery experiments); and (b) standard mesh panel on riser.](image-url)
Specific methods for individual experimental treatments

Exposure of explants to air

To quantify the effects of exposing explants to air, as may be expected during the seeding process, we exposed explants to three different exposures: 10 sec, 30 sec and 2 min. Following collection, explants were suspended in a mesh catch bag at approximately 2 metres under the dive boat. Explants were removed and placed into a large plastic tub filled with seawater ensuring that no explants were exposed to air. Explants were then individually exposed to ambient atmospheric conditions (air, sunlight and temperature) for each of the experimental exposures within the experiment and subsequently returned to the catch bag pending seeding into the culture panels. Exposure of explants took place during the early afternoon, on a clear, sunny day. A control treatment included explants undergoing all the handling described excluding any exposure to air. Explants were then placed into mesh panels following the general methods outlined above.

Collecting from different size classes of donor sponge

At Masig Island Coscinodera matthewsi exhibits a size range of 1-60cm (Duckworth and Wolff 2008). Accordingly, we targeted three different size class to determine if the size of donor sponges influenced subsequent explant growth, under the premise that small individuals were immature and medium to larger sponges were mature individuals and more likely to invest energy into reproduction at the expense of growth. The size classes of donor sponges were determined by the length of the greatest axial dimension and were arbitrarily defined as: small <10cm, medium 11-15cm and large >20. Explants were made in situ and placed into the plastic mesh panels following the general methods outlined above.

Collecting from the top or base of donor sponges

To test the effect on growth and survival of explants originating from different regions of donor sponge we collected explants from the top half of donor sponges and others from the basal portion of donor sponges. As a control we also collected explants that were not divided, but comprised both top and bottom portions. Explants were made in situ and placed into the plastic mesh panels following the general methods outlined above.

The effect of squeezing caused by placing explants into panel

To assess the effect of squeezing normally imposed during seeding into mesh panels, two treatments were compared. First, explants were placed into panels and then removed to mimic the squeezing pressure normally experienced during seeding into panels. These explants were then placed into oyster baskets and gently held in place with a loose covering of plastic to prevent them rolling around and incurring incidental tissue damage. In the second treatment, explants were not squeezed at all and placed directly into the oyster baskets as described above.

The effects of a transitional nursery stage

To determine if growth and survival are enhanced if explants are placed into a transitional nursery for recovery from cutting and prior to placement in panels, explants were placed into a nursery for one month. The nursery comprised an oyster basket (Figure 39) as outlined in the general methods. After one month of recovery in the nursery, which is the period suggested by Louden et al. (2007) as the time required for complete pinacoderm and aquiferous system recovery in this species, explants were removed and seeded onto mesh panels for the remainder of the experiment. Growth and survival over the experimental period was compared to explants seeded directly onto mesh panels without a nursery stage.
Anecdotal observations of sponges on the sponge farm

In May 2010, divers inspected the commercial sponge farm operation at Masig with the sponge farm’s head diver. In one dive, eight individual risers were observed and photographed along two longlines.

Results

Effects of exposure of explants to air on growth and survival

There was no significant effect of exposure to air on explant growth regardless of exposure period (i.e. 10s, 30s or 2 min) when compared to explant controls not exposed to air (Figure 40 a-c; ANOVA, $F_{3, 10} = 1.69 p > 0.05$). The mean size of explants increased for the 30s and 2 min treatments, including controls, but for the 10 sec treatment the mean size of explants decreased from 98.7 (± 36.2cm³) in Feb to 55.4 (±11.6 cm³) in June. Mean survival of explants ranged from 43 (±24%) to 75 (±25%) for controls, but overall there was also no significant effect of air exposure on the survival of explants (Figure 41; ANOVA $F_{3, 1} = 0.87$, $p > 0.05$).

Effects of donor sponge size classes on growth and survival

Explants collected from different sized donor sponges did not exhibit different growth profiles with explants collected from small donor sponges (<10cm) showing consistent growth rates to those collected from either medium (11-15cm) or larger (>20cm) donor sponges (Figure 42, ANOVA $F_{2, 9} = 2.46$, $p > 0.05$). The mean size of explants sourced from all size classes of donor sponges decreased over the experimental period with small, medium and large samples showing a mean decrease of 31.4%, 41.8% and 54.9% respectively. Survival of sponges was also consistent regardless of the size of donor sponge the explant was sourced from (Figure 43, ANOVA $F_{2, 1} = 0.91$, $p>0.05$).
Figure 40. Mean sponge explant size (volume) (±1 SE) following three different exposure times to air. Controls include explants not exposed to air.
Figure 41. Mean survival (±1 SE) of sponge explants following different exposure times to air.

Figure 42. Mean size (±1SE) of explants prepared from different size classes of donor sponges. Size classes are based on explants taken from donor sponges - small <10cm, medium >10 <15cm and large >20cm.

Figure 43. Mean survival (±1SE) of explants prepared from different size classes of donor sponges. Size classes are based on explants taken from donor sponges - small <10cm, medium >10 <15cm and large >20cm.
Effects of collecting from the top or base of donor sponges on growth and survival

Both explants sourced from top and basal donor sponge regions exhibited a mean reduction in size, explants from top regions decreasing by 13.5% while basal sourced explants shrank by 16.8%. In contrast, controls, comprising both top and basal regions, showed a mean increase of 4.6%. Overall, there was no significant effect on growth in relation to where the explant was collected with growth profiles being consistent for either top, basal or controls (top/basal) sourced explants (Figure 44; ANOVA: F_{2,8} = 0.58, P > 0.05). Mean survival of explants was consistent among all treatments and ranged from 58 (±8.3%) in controls (top and basal) to 75 (±14.4%) in top and 75(±8.8%) in bottom sourced explants (Figure 45; ANOVA: F = 1.72 p>0.05).

![Figure 44](image1.png)

**Figure 44.** Mean sizes (±1 SE) of explants seeded from different regions of donor sponges. Controls are a cross section of donor sponge displaying both top and basal sections.

![Figure 45](image2.png)

**Figure 45.** Mean survival of explants seeded from different regions of donor sponge. Note trend line for top region obscured as a result of having the same values as bottom regions.
**Effects of squeezing from placing explants into panels, on growth and survival**

Both the mean size of handled and non-handled explants increased, (39.3% and 20.7% respectively.) The handling of sponges, via the action of placing newly cut explants directly into panels however, did not significantly influence growth (Figure 46; ANOVA: $F_{2,9} = 0.99$, $p>0.05$) or survival (Figure 47; ANOVA: $F_{2,9} = 0.81$, $p>0.05$).

![Figure 46](Image)

**Figure 46.** Mean sizes (±1 SE) of explants subjected to squeezing from placement into mesh panels, compared to explants not squeezed.

![Figure 47](Image)

**Figure 47.** Mean survival (±1 SE) of explants to squeezing from placement into mesh panels, compared to explants not squeezed.
Effects of a transitional nursery stage on growth and survival

Consistent with other experiments in this study explants decreased in size for both handled (54.5%) and non handled (10.5%) sponges, but growth rates between treatments was significant with nursery treatments performing better than non-nursery treatments (Figure 48; t-test, df = 6, p > 0.05). There was no effect of a transitional nursery stage on survival (Figure 49; t-test, df = 1, p= 0.059), although the significance level is very close to the arbitrary rejection alpha level of 0.05. The mean survival of nursery explants was 87.5 (±7.2%) compared to 50 (±14.4%) for explants not subjected to a nursery period.

Figure 48. Mean sizes (±1 SE) of explants subjected to a transitional nursery period enabling recovery of explanting period before seeding onto mesh panels compared to explants without a nursery stage.

Figure 49. Mean survival (±1 SE) of explants subjected to a transitional nursery period enabling recovery of explanting period before seeding onto mesh panels compared to explants without a nursery stage.
Figure 50. Examples of explants on the sponge farm at Masig: (a) recent explant attached by cable tie; (b) healthy larger explant attached by cable tie; (c) large explant with advanced cable tie ‘donut’; (d) large explant with cable tye donut forming; (e) large explant that has fallen off the riser due to cable tye donut; (f) fouling on riser and panel frames; (g) large explants surviving amongst fouling; (h) large explant struggling with overgrowth.
**Anecdotal observations of sponges on the sponge farm**

Sponges were observed on eight risers of two longlines at the Kailag Enterprises Limited sponge farm at Kailag. Sponges were attached by cable-ties to the frames of panels which had had the mesh removed. Some sponges were relatively recent explants (Figure 50a), and some were much larger (Figure 50b). Many sponges had worked loose on their cable-ties, and were developing a clear donut area around the cable tie (Figure 50c,d). Several sponges were found loose on the seafloor, with evidence that they had fallen off the riser due to cable-tie wear (Figure 50e). There was significant fouling covering many of the risers (Figure 50f), and while some sponges looked healthy amongst the fouling (Figure 50g), others were being overgrown (Figure 50h).

**Discussion**

Five separate experiments were conducted over a four-month period to establish the impacts of handling and collection procedures on explant growth and survival. Sponge farming has traditionally relied on collecting sections from donor sponges and then seeding them onto various farming structures, ranging from threading explants onto rope lines or placing them into other structures such as baskets or plastic mesh panels (e.g. Duckworth and Wolff 2007a). Regardless of the culture method employed to grow sponges, the creation of clonal fragments results in a proportion of the explants surface being cut and exposed as it is excised from the donor sponge. The ability of sponges to recover from this process, with full cellular integrity occurring within four to six weeks, demonstrates that this process is not detrimental to survival and is highly suitable as a farming collection procedure (Louden *et al.* 2007). This is further evidenced with the high survivorship and growth observed from many experimental trials for sponge aquaculture (e.g. Duckworth and Wolff 2007a, Louden *et al.* 2007, Baldacconi *et al.* 2010) and studies of the fate of fragments in nature (Wulff 1985, Kelly-Borges and Bergquist 1988, Mercurio *et al.* 2006, Battershill and Bergquist 2010). Nevertheless, recovery clearly requires significant cellular re-organisation and energy investment to achieve (Korotkova 1970, Henry and Hart 2005, Louden *et al.* 2007). Thus explant recovery along with overall health and growth potential should be maximized by minimizing the stress caused by explant collection and production. Thus, results from this study can inform future farm operational procedures to improve sponge farm production.

Exposing sponges to air provided some unexpected results in that exposures of up to two minutes did not lead to a significant reduction in growth or survival in comparison to explants not exposed. Given the subtidal habit of *C. matthewsi* (Duckworth and Wolff 2008) the exposure of explants to air for up to two minutes was expected to result in high mortalities due to challenges of exposure to sunlight (UV), and increased temperatures, and the potential for air-pocket formation in sponge canals. Environmental stress, particularly temperature, has been demonstrated to negatively affect sponge health and survival (e.g. Webster *et al.* 2008). However, the considerable variation surrounding the mean values for growth and survival for both treatments and controls warrants interpretive caution in the results. Thus, the lack of a statistically significant reduction in growth and survival due to air exposure in the current study should not be taken to clearly demonstrate that air exposure of two minutes causes no ill effects. While inconclusive, the results do not suggest a need to discontinue the current practice of allowing short exposures of explants to air, such as during the transfer of sponges between collection holding tanks and panels.

The finding that a nursery stage results in higher survival and growth rates is consistent with the findings of Duckworth *et al.* (2007), and identifies an easily implemented farm protocol that can significantly improve survival and growth of explants. This result shows that it is an advantage to implement a nursery phase to allow recovery from explant cutting, prior to the
further trauma of deployment into panels for growout. Sponge survival after damage depends on the extent and repeatedness of damage (Wulff 2006, Henry and Hart 2005). After damage, pinacoderm recovery plays a particular role in sealing off the aquiferous system of a new sponge fragment or explant (Kelly-Borges and Bergquist 1988, Wulff 1985, Baldacconi et al. 2010). *Coscinoderma matthewsi* can establish a protective collagen layer over the cut surface and a new pinacoderm in as little as three days (Louden et al. 2007). Therefore, a nursery stage after explant cutting to allow recovery prior to deployment to the farm is highly recommended for incorporation into sponge farming procedures.

In terms of collecting efficiencies, neither the size of the donor sponge nor the region from which the explant was collected appears to influence growth or survival of explants over the period of this study. It is important to note that decreased growth of explants observed in this study, compared to rates reported for this species by others over longer studies (e.g. Louden et al. 2007, Duckworth and Wolff 2007a, Duckworth et al. 2007c) may be a reflection of the short term over which the study was conducted. The results may reflect the focus of sponges on recovery at the expense of growth, during the short four month term of the study.

Observations of the sponge farm confirm that farm operations have moved away from use of mesh panels as recommended in Duckworth et al. (2007b), to direct attachment with cable ties to the panel frames after mesh removal. This action was taken because of unexpected and unacceptably high levels of fouling experienced from the use of mesh panels at the farm site, which had completely smothered the sponges and caused them to shrink rather than grow, and caused problems with the farm hardware, with the weight on the risers overpowering the buoyancy of sub-surface floats (Robertson, per comm. 2010). While the potentially damaging affect of fouling has been well documented previously (Duckworth and Battershill 2003), it was unanticipated at this site by Duckworth and colleagues (Duckworth et al. 2007b, Duckworth and Wolff 2007a), who had conducted several experiments in the Masig area using mesh panels without a significant fouling issue. However, Duckworth and colleagues’ experiments were always conducted immediately adjacent to the reef, while the sponge farm site is away from the reef and over a bare sandy bottom. Local marine experts believe that the fouling is worse away from the reef due to an absence of grazing fish over sand, coupled with a prevalence of predatory pelagic fish, should grazers stray from the reefal area (Morris pers comm. 2010, Lowatta pers comm. 2010). This problem may be alleviated in future, as the sponge farm becomes more populated with sponges and hence creates a more contiguous fish attracting device to provide a corridor from the reef edge to the sponges, along which grazing fish can safely travel and access the sponge risers for grazing purposes.

While the removal of mesh from farming structures and attachment of sponges by cable-tie clearly limits the fouling problem by reducing the available surface for fouling settlement, it unfortunately appears to have introduced another problem to sponge farming. The ‘donut effect’ observed on many sponges has been clearly documented by others (see Duckworth 2009, Duckworth et al. 2007c, Duckworth and Wolff 2007a), as a problem with this species when the attachment method involves piercing the sponge. To avoid loss of sponges when this problem becomes severe, further trials are needed to both addresses the need to limit fouling settlement surface and avoid piercing the sponge. It is recommended that options that promote direct sponge attachment be explored, to avoid the need for mesh enclosures.
Sponge Assets and Ecosystem Health in Torres Strait

Chris Battershill

Sponges are the lowest metazoans, and have been in existence for over 800 million years. They are arguably the most prevalent organism in the world’s oceans, characterizing by far the largest spatial extent of the sea floor of any taxonomic group. They are present in all ecosystems and can dominate some benthic habitats, occupying coral reefs, rocky reefs, reef extensions and sediment flats from shallow waters to the abyssal planes, tropics to the poles. Their morphology and mode of feeding is basic (with ingestion of dissolved organic material and particles including ultraplankton to 50µm diameter), and growth can be flexible with sponges attaining a size that is optimal for the micro-environmental conditions. They can be very important components of reef systems accounting for up to four tonnes of Carbon processing from the water column per square kilometre of seafloor for an average sponge community, thereby constituting an important and little studied component of Carbon flux and benthic pelagic coupling in marine systems (Battershill et al. in prep, Bell et al. 1998, Bannister et al. 2010).

Sponges have a highly sophisticated metabolic chemistry and can benefit from microbial symbionts. As such they represent an extremely useful taxonomic and ecological group by which to measure the status of ecosystem functioning and health. Examining their population demographics can inform understanding of how the local environment is performing, as sponges will reflect longer term prevailing biological and physical conditions in their population size frequency (Battershill and Bergquist 1990). Given their filter feeding mode of sustenance, they are vulnerable to pathogenic attack, again constituting a useful indicator organism of relevance. Sponges are important reef organisms in the sense that they play roles in both reef consolidation and destruction. Many species bind rubble areas providing stability, or alter sedimentary regimes a precursor to establishment of extensive sponge gardens which become important nursery grounds for fishes and other organisms. Other species erode reefs both coral and rocky by boring into the reef matrix (Schonberg 2001, Schonberg and Wilkinson 2001).

Sponges are also now known to be important sources for biologically active compounds, an obvious consequence of their need to maintain effective and flexible chemical defenses against pathogenic attack, feeding pressure and against competitive overgrowth by neighbors. These compounds have found their way into the clinic for Cancer and other indications with over three compounds now available as drugs (Newman et al. 2009). Importantly, sponges are the basis of an international industry based on their collagen skeletons. Such an industry is attractive to remote communities as the product is easily generated from the raw material, it is light weight thus transported easily and doesn't need freezing. As such they are useful targets to explore new industry potential, particularly invoking aquaculture as a sustainable production method.

A focus on sponge communities in the Torres Strait was therefore a high priority for a number of reasons:

- Very little ecological work has been carried out in the Torres Straits in general apart from broad scale biodiversity studies. (Of 138 papers published on the Torres Strait from 1977, only 7 concern coral or sponge communities with the 6 on sponges being authored by the MTSRF TS team, see Appendix 1).

- It was shown very early on that sponge communities in the Torres Strait were highly prevalent in their spatial extent, characterizing some benthic communities.
Sponge characterised reefs are known to be important nursery grounds for fishes and other important marine organisms such as rock lobsters.

Sponge reefs and gardens can be highly sensitive to disturbance and decreasing ecosystem health.

Sponges are highly relevant marine organisms to monitor due to this sensitivity and their importance as habitat constructors.

Sponges can be a good source of bioproducts constituting targets for future marine based industry.

The Torres Strait community is highly interested in reef health and ecosystem connectivity (TSRA et al. 2009).

The Torres Strait community is strongly interested in appropriate, sustainable new marine industry from which to expand educational and employment opportunity (TSRA et al. 2009).

There is a growing need, in the face of possible changes in prevailing ocean current systems, to examine biodiversity connectivity between the Torres Strait marine ecosystem south to the northern Great Barrier Reef, and west to the Gulf of Carpentaria. A start needs to be made in the Torres Strait.

Building on work carried out under the auspices of the Torres Strait CRC, the MTSRF Project 1.3.2 was designed to examine the ecology of sponge communities at the same time as advancing the sustainable use of sponges as an aquaculture target for commercial bath sponge production. The project represents a unique study where the same package of research tasks provides information relevant to ecological assessments of commercial bath sponge production. The project represents a unique study where the same package of research tasks provides information relevant to ecological assessments of Torres Strait marine benthic communities and their health, while advancing the aquaculture systems attendant to a new ‘bath sponge’ production industry. This is achieved by the fact that both outcome areas require fundamental information on sponge biology and ecology. The focal species *Cosncinoderma matthewsi* is the most common species in the Torres Strait and a highly relevant ‘model’ organism by which to track reef dynamics.

Specifically the Project examined:

1. **Undertake an assessment of the distribution and abundance of wild commercial sponge species in the Torres Strait, identifying elements of environmental risk (evidence of disease)**

   Masig Island is confirmed as the location of the most dense *C. matthewsi* populations and these resilient. Populations are characterized by large numbers of small individuals that do have significant variability in numbers over seasons and years, with sponges on the exposed side of the Island being more variable. This suggests a resilient population with rapid replacement of sponges (as evidenced by the consistently high numbers of small size classes, especially on the exposed side of the Island). The information is now available for sponge farming industry to accurately develop a sponge seed harvest program and stock size models. Storms and sedimentation phenomena are key factors in sponge population dynamics. Disease has been observed in later years of the project and signals a potential threat to sponge populations generally throughout the Torres Strait.
2. Determine connections between sponge populations and risks in translocation

The sponge populations examined to date in this project appear to be well mixed across central and eastern Torres Strait. This is unusual for sponge populations in general, as reproductive processes are limited in terms of how far larvae or asexually produced propagules can travel and hence highly patchy heterogeneous genetic groupings are the norm (see Whalan et al. 2005). This finding is novel scientifically, although few of these types of studies have been carried out internationally. It suggests that in Torres Strait there is a highly dynamic process of propagule spread and recruitment. As sponges are the least well dispersed taxa in terms of larvae swimming/travel capability, then it is fair to add that most sessile organisms such as corals and soft coral will also likely be very well mixed in terms of their population genetics. Therefore translocation of sponge clones from one Island to another is not viewed as being an issue. To be prudent however, it is always advisable that translocation distances be kept to an absolute minimum as this will ensure the best survival results (sponges are usually well attuned to local micro-environmental conditions). Minimum translocation also minimizes the spread of any disease organisms.

3. Determine patterns of sponge recruitment/mortality and the environmental risk of seed stock harvest leading to development of a sustainable seed collection strategy

Sponges of the Torres Strait as evidenced by *C. matthewsi*, have seasonal and highly synchronous spawning cycles. Indeed they spawn at the same time as the same species on Orpheus Island in the Palm Islands Group. The reproductive process and settlement behavior of the sponge is well discussed in the report. Also significant is the high degree of asexual reproduction that takes place with the sponge morphology well suited to fragmentation with pre-attachment to coral rubble. There is evidence both from sightings of asexual propagules *in situ* and analysis of sponge population demography that suggests that this form of reproduction is highly important for the species and reef systems in the Torres Strait. Such a finding is consistent with other works examining sponge population ecology world-wide (Battershill and Bergquist 1990, Battershill and Bergquist 2010, Corriero et al. 1998, Ereskovsky and Tokina 2007, Wulff 1985).

The factors that will influence larval recruitment are sediment regimes and the amount of ‘settleable’ space available. Settlement plate experiments showed that sponges do recruit to artificial tiles (undersides) along with many other fouling species. The fact that ‘discrete’ sponges (as opposed to thin encrusting species) were found at all on these tiles is significant as they generally do not either settle on such surfaces or are quickly outcompeted by other organisms. The implication is that a great deal of sponge recruitment in general is occurring in Torres Strait. This is important as attests to the dynamic nature of the ecosystem and the likely resilience that this infers. It also suggests a useful mechanism for enhancing generation of farmable seed stock.

4. Develop optimal handling guidelines to improve sponge explants growth and survival

Sponge aquaculture currently relies on growing out clonally produced fragments from wild donor sponges. The production of explants for grow out involves cutting and exposing surface of sponges which in turn involves a recovery stage before the sponge explants can grow. The growth and survival of sponge clonal explants within a culture environment is therefore largely determined by the health of those explants at the time of seeding. In a series of experiments it was shown that *C. matthewsi* is tolerant of most handling procedures apart from excessive compression, and that survival and growth of
explants can benefit from a nursery period. Explants can also be exposed to air for short periods of time. The implications are that a method for deployment needs to be developed where the clones are not unnecessarily compressed. They can however be handled above water for short periods which will aid future development of a mechanized system for periodic long line maintenance and re-emersion. The work further suggests that a system based on pre-settlement of larvae or clones (rather than threading onto a support structure) would be highly desirable and could improve initial growth rates substantially.

Flow on Developments and Outcomes from this MTSRF included:

- Kailag Enterprises launched Australia's first sponge farm in 2009.

- A sponge farming protocol has been developed and is available to Kailag Enterprises Limited. In addition a video of sponge farming operations has been created by Kailag Enterprises. The work associated with sponge farming, both in the Torres Strait and closely related work from the Palm Islands has been published (refer references). Workshops with Kailag have been held and issues that have arisen in the sponge farm (fouling, need for better support structure in the water column, faster seed stock generation) have been discussed and alternate mechanisms trialed. An FRDC pre-proposal has been submitted to focus on developing a seed stock generation and deployment technology based on larvae. With respect to larval generation of seed stock specifically, extra experiments were carried out over the 2009-2010 summer period to examine the efficacy of settling larvae onto artificial surfaces. The Kailag sponge farm now represents the most advanced sponge farm internationally.

- The Kailag Sponge farm has benefitted from experience made during the Palm Island sponge farming project (also research in Western Australia). There have been a number of sponge farm workshops, and although desirable, it has been deemed unadvisable to hold a more nationally scaled workshop until the Kailag farm has advanced further and the underpinning technologies in current development assessed for IP value and possible protection. The project has been presented at all Annual Conferences sponge farm steering group meetings. A sponge farming steering committee has been established (instigated by TSRA and DEEDI) and meets monthly.

- The project has been a launch pad for training and employment of 10-12 Torres Strait Islander staff, now employed by Kailag Enterprises.
References


Battershill CN, Bergquist PR (2010). The predominance of asexual modes of reproduction in storm disturbed temperate, subtidal sponge communities. Submitted manuscript.


Evans-Illidge and Battershill


**Vermeij** MJA, Bak RPM (2003) Species-specific population structure of closely related coral morphospecies along a depth gradient (5-60 m) over a Caribbean reefs slope. Bulletin of Marine Science 73: 725-744


**Whalan** S (2009) Ecological role and potential value of sponges to Torres Strait. Project annual report 2009, MTSRF project 1.3.2.


Appendix 1

Publications

Peer reviewed publications

The following publications either arose directly from this MTSRF project, or are closely related to it:


Publications in preparation

The following additional publications are currently in preparation, based on the results of this MTSRF project:

Duckworth A, Wolff C, Whalan S
► *Larval recruitment dynamics of sessile coral reef invertebrates.*

Evans-Illidge EA, Syms C, Battershill C, Whalan S, Duckworth A, Luter H
► *Disturbance induced fragmentation drives the population demographics of a coral reef sponge.*

Whalan S, Evans-Illidge EA
► *Optimising sponge farm handling practices to promote explant growth and survival.*

Whalan S, Wahab MA
► *The application of sponge larval behaviour and biology to commercial sponge farm seeding methods and coral reef connectivity.*
Metadata pages

ABUNDANCE OF COSCINODERMA SponGES IN CENTRAL TOrRES STRAIT, AUSTRALIA (MTSRF PROJECT 1.3.2)

RECRUITMENT OF COSCINODERMA SponGES IN CENTRAL TOrRES STRAIT, AUSTRALIA (MTSRF PROJECT 1.3.2)

SMALL SCALE VARIATION IN DICTYOCERID SponGES IN CENTRAL TOrRES STRAIT, AUSTRALIA (MTSRF PROJECT 1.3.2)

LARVAL SETTLEMENT BEHAVIOUR OF COSCINODERMA SponGES FROM THE TOrRES STRAIT, AUSTRALIA (MTSRF PROJECT 1.3.2)

EFFECTS OF HANDLING ON SponGES OF AQUACULTURE POTENTIAL FROM THE TOrRES STRAIT, AUSTRALIA (MTSRF PROJECT 1.3.2)