Bath sponge aquaculture:

Aspects of culture and quality.

Thesis submitted by

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I acknowledge that Stefanie Inderbitzin, a fourth year mechanical engineering student, determined the initial sponge size and shape for the compression and tensile testing of *Spongia graminae* and also conducted these tests for this species.

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Table of Contents

Statement of Access ... i
Statement on Sources ... ii
Acknowledgements ... iii
Table of Contents ... iv
List of Figures ... vi
Abstract ... x

Chapter 1: General Introduction ... 1

1.1 Sponge Biology ... 1
1.2 Morphological Responses to Environmental Conditions ... 2
1.2.1 Water Flow ... 3
1.2.2 Food Availability ... 6
1.2.3 Sedimentation ... 7
1.2.4 Light Intensity ... 9
1.2.5 Predation ... 10
1.2.6 Other environmental and biological factors ... 10
1.3 Sponge Supply and Demand ... 11
1.4 Production Methods and Limitations ... 13
1.5 Project Aims ... 15

Chapter 2: Explant survival, growth and recovery for *Rhopaloeides odorabile* and *Coscinoderma* n. sp. ... 17

2.1 Introduction ... 17
2.2 Materials and Methods ... 21
2.2.1 Sponge Collection ... 21
2.2.2 Explant Survival ... 21
2.2.3 Explant Growth ... 23
2.2.4 Explant Recovery ... 23
2.2.4.1 Macroscopic Surface Examination ... 23
2.2.4.2 Microscopic Surface Examination ... 24
2.2.4.3 Cross-sectional Examination ... 24
2.2.5 Statistical Analysis ... 25
2.3 Results ... 26
2.3.1 Explant Survival ... 26
2.3.2 Explant Growth ... 29
2.3.3 Explant Recovery ... 34
2.3.3.1 Macroscopic Surface Examination ... 34
Rhopaloeides odorabile ... 34
Coscinoderma sp. ... 35
2.3.3.2 Microscopic Surface Examination ... 37
Rhopaloeides odorabile ... 37
Coscinoderma sp. ... 37
2.3.3.3 Cross-section Examination ... 39
Rhopaloeides odorabile ... 39
Coscinoderma sp. ... 44
2.4 Discussion ... 49
2.4.1 Explant Survival ... 49

2.4.2 Explant Growth 52
2.4.3 Explant Recovery 55
2.5 Conclusion 57

Chapter 3: Development of a New Protocol for Testing Bath Sponge Quality .. 59

3.1 Introduction 59
3.2 Materials and Methods 61
3.2.1 Sponge Collection and Preparation 61
3.2.2 Physical Properties 61
3.2.2.1 Density 62
3.2.2.2 Fibre Dimensions 62
3.2.3 Quality Tests 63
3.2.3.1 Compression Tests 63
3.2.3.2 Tensile Tests 64
3.2.3.3 Water Absorption Tests 67
3.2.10 Statistical Analysis 68
3.3 Results 70
3.3.1 Physical Properties 70
3.3.2 Quality Tests 73
3.3.2.1 Compression Tests 73
3.3.2.2 Tensile Tests 75
3.3.2.3 Water Absorption Tests 79
3.3.3 Inter-variable Relationships 80
3.3.3.1 Quality Variables versus Quality Variables 80
3.3.3.2 Independent Variables versus Quality Variables 83
3.4 Discussion 87
3.5 Conclusion 91

Chapter 4: General Discussion 92

References 97

List of Figures

Chapter 1

Fig. 1.1 Schematic representation of the aquiferous system within a sponge 2

Chapter 2

Fig. 2.1 The sponge species investigated in this study. Photos were taken *in situ* within the Palm Island Group. **A**. *R. odorabile* **B**. *Coscinoderma* n. sp. 20

Fig. 2.2 Percentage survival of *R. odorabile* and *Coscinoderma* sp. in *in vitro* (**A**) and *in situ* (**B**) cultures over time 27

Fig. 2.3 Survival of *R. odorabile* (**A**) and *Coscinoderma* sp. (**B**) in *in vitro* and *in situ* cultures grouped according to parentage. 28

Fig. 2.4 Total percentage growth of *R. odorabile* and *Coscinoderma* sp. cultured *in situ* ± standard error. 29

Fig. 2.5 Seasonal changes in daily growth rate of *R. odorabile* (**A**) and *Coscinoderma* sp. (**B**) cultured *in situ* ± standard error 30

Fig. 2.6 The effect of size dependent growth rates demonstrated through correlations between initial growth and initial explant size and the effect of this differential growth rate on final sponge size for *R. odorabile* (**A** and **B**) and *Coscinoderma* sp. (**C** and **D**) 31

Fig. 2.7 Total percentage growth for each individual explant of *R. odorabile* (**A**) and *Coscinoderma* sp. (**B**) 32

Fig. 2.8 Average daily growth rate (± standard error) grouped according to parentage for *R. odorabile* (**A**) and *Coscinoderma* sp. (**B**).. 33

Fig. 2.9 The newly exposed sponge surface immediately after cutting for *R. odorabile* (**A**) and *Coscinoderma* sp. (**B**)... 35

Fig. 2.10 Explants healing over a cut surface; **A** to **C**. *R. odorabile*; **A**. Day 1. All small canals have been covered by a protective layer, although a number of large exposed canals remain uncovered (*eg*. arrow); **B**. Day 2. Most large canals have been covered (*eg*. arrow); **C**. Day 7. Exposed spongin fibres have begun to fall away revealing the new protective layer beneath; **D** to **F**. *Coscinoderma* sp.; **D**. Day 1. Exposed canals have been covered; **E**. Day 7. The exposed fibres have begun to darken; **F**. Day 14. Most of the new surface remains obscured from view. 36

Fig. 2.11 *R. odorabile* surface; **A**. 0hrs after exposure. Spongin fibres and tissue are exposed; **B**. 12hrs after exposure. A new protective layer covers much of the underlying tissue; **C**. 24hrs after exposure. A protective layer completely covers the underlying tissue and most fibres; **D**. 23 days after exposure. Ostia are found with scattered and grouped distributions. A new fibre ending is seen protruding from the surface.. 38

Fig. 2.12 *Coscinoderma* sp. surface; **A**. 0hrs after exposure. Tissue and fibres are exposed; **B**. 5 days after exposure. A gap in the exposed fibres allows view of the new protective layer below. 38

Fig. 2.13 *R. odorabile*; **A** to **C**. Natural variation of the subsurface cellular make-up; **D**. Spicules incorporated into the surface crust; **E**. Subsurface skeletal support structure; **F**. Spongin distribution 41

Fig. 2.14 *R. odorabile*; **A**. Day 0. No surface layer; **B**. Day 1. Newly formed surface; **C**. Day 2. Cell transformation is in progress; **D**. Day 2. Collagen coat lifting off the new surface; **E**. Day 3. Ostia development is obvious; **F**. Day 10. Cellular composition is approaching that of an undamaged sponge............................... 42

Fig. 2.15 *R. odorabile*; **A**. Day 10. Cells are largely absent here leaving only layers of collagen behind; **B**. Day 41. Pigment cells are again common and the surface

resembles that of an undamaged surface; **C**. Day 41. Development of ostia is complete 43

Fig. 2.16 *Coscinoderma* sp.; **A** to **C**. Natural variation of the subsurface cellular make-up; **D**. Natural regression of the sponge surface; **E**. Spicules incorporated into the surface crust; **F**. Subsurface skeletal support structure 46

Fig. 2.17 *Coscinoderma* sp.; **A**. Distribution of spongin in an undamaged sponge; **B**. Day 0. No surface layer; **C**. Day 1. Cellular debris and spongin fibres above the newly formed surface; **D**. Day 1. Cells in a state of transformation and rearrangement below a clearly defined surface; **E**. Day 2. Lower cell density with a collagen layer being shed; **F**. Day 2. Collencytes are common and are used to form pinococytes at the surface 47

Fig. 2.18 *Coscinoderma* sp.; **A**. Day 3. Organised cell structure with choanoderm below; **B**. Magnified image of Fig. 3A. Hanging pinococytes are visible below the surface; **C**. Day 10. Subsurface region largely consisting of collagen layers; **D**. Day 41. Pigment cells are again numerous amongst the many collagen layers; **E**. Day 41. The collagen structure and cell density and organisation resembles that of an undamaged sponge 48

Chapter 3

Fig. 3.1 Photo of internal secondary fibres (*R. odorabile*) showing the method for measuring fibre width and fibre length. 63

Fig. 3.2 Stress-strain curve produced from a compression test (for a silk sponge) showing the calculation for firmness and the compression modulus (65% strain / 25% strain). 64

Fig. 3.3 Stress-strain curve produced from the tensile test (for a *Coscinoderma* sp. individual) showing the calculation for the tensile strength, elastic limit, elastic strain, modulus of elasticity, and modulus of resilience 67

Fig. 3.4 Sponge density, measured as the weight of the sponge over its entire volume including spaces between the fibres. Different letters indicate a significant difference ($p < 0.05$).. 71

Fig. 3.5 Fibre width, including only secondary fibres 71

Fig. 3.6 Fibre length, measured as the length between fibre nodes or branches 72

Fig. 3.7 Firmness, measuring the resistance to pressure, with lower values indicating a softer sponge 74

Fig. 3.8 Compression modulus, measuring the ability of a sponge to cushion a load with larger numbers indicating greater cushioning ability................................ 75

Fig. 3.9 Compressive strength, measuring the point at which a sponge is no longer easily compressed 75

Fig. 3.10 Tensile strength, measuring the maximum strength of the sponge 77

Fig. 3.11 Elastic limit, measuring the maximum stress prior to permanent damage occurring through tearing or breaking.. 77

Fig. 3.12 Elastic strain, demonstrating the amount of stretch within elastic limits ... 78

Fig. 3.13 Modulus of elasticity, measuring the resistance to stretching.................... 78

Fig. 3.14 Modulus of resilience, measuring elastic energy...................................... 79

Fig. 3.15 Absorbency.. 80

Fig. 3.16 Water retention efficiency 80

Fig. 3.17 **A.** Scatter plot of firmness and compressive strength; **B.** Scatter plot of the elastic limit and tensile strength; **C.** Scatter plot of the elastic limit and the modulus of elasticity; and **D.** Scatter plot of the elastic limit and the modulus of resilience 831

Fig. 3.18 **A.** Scatter plot of density and firmness; **B.** Scatter plot of density and compressive strength; and **C.** Scatter plot of density and absorbency............... 83

Abstract

With increased demand on sponges for cosmetic and biomedical purposes, natural sponge fishing grounds are no longer able to cope with the demand. With sufficient research and development, sponge aquaculture is likely to supply demand while allowing the preservation of natural sponge stocks. However, due to the array of different environmental conditions found amongst the habitats of different species, and differences amongst the species themselves, detailed research on the amenability to common culture methods, survival and growth rates, recovery processes, and the environmental requirements for any one species is required before commercial culture may be considered. Furthermore, due to the diverse morphological variation that occurs in sponges due to the environment, quantitative quality testing protocols must be established to experimentally determine the optimal environmental conditions required for production of quality bath sponges.

In this study, survival (*in situ* and *in vitro*), growth rates (*in situ*), and recovery processes (*in vitro*) were measured for two potential sponge aquaculture candidates, *Rhopaloeides odorabile* Thompson *et al* (1987) and *Coscinoderma* n. sp. [Phylum Porifera: Order Dictyoceratida Minchin (1900): Family Spongiidae Gray (1867)], cultured at the Palm Islands, central Great Barrier Reef. Sponge survival was dependent on species, culture method, and time with the highest mortality occurring soon after excision of sponge material from the parent stock. *R. odorabile* had the greatest survival of the two species *in vitro*, and the lowest survival *in situ*. Growth rates of 85.9 ± 23.7% and 115.2 ± 23.4% per annum, for *R. odorabile* and *Coscinoderma* n. sp. respectively, were not significantly different over the 21 month experimental period. Both species demonstrated initial size dependent growth rates with smaller explants growing fastest through the first season (78 days). Explant recovery rates were rapid for both species with a protective layer of collagen forming

over the surface within 24hrs. This layer was later replaced by pinacoderm as the subsurface tissue was reorganised to recreate a functional surface including redevelopment of the aquiferous system.

A quantitative testing protocol was developed to assess the quality of sponges using mechanical engineering techniques. Techniques quantified the directly measurable physical properties of sponges (density, fibre width, and fibre length) and the quality characteristics of firmness, compression modulus, compressive strength, tensile strength, elastic limit, elastic strain, modulus of elasticity, modulus of resilience, absorbency, and water retention efficiency. These were measured for *R. odorabile* and *Coscinoderma* n. sp. and three commercial species. There were significant differences between species for all quality parameters creating a unique profile for each species. *R. odorabile* was the firmest (37.8 ± 4.3kPa), strongest (157.4 ± 17.3kPa), and most rigid (838.7 ± 53.5kPa) species tested, while *Coscinoderma* n. sp. was one of the softest sponges (7.3 ± 1.1kPa) with the highest elastic energy (30.5 ± 3.5kJ/m^3) and water retention efficiency (40.1 ± 1.4%) of all species tested. These quality tests enable comparisons of quality between and within species with scientific rigour. Comparisons between species may be used as a marketing tool to promote aquaculture products for specific applications. Within species testing will allow quantification of differences in quality caused by genetic or environmental factors.

This research provides a foundation for the further research necessary to establish sponge aquaculture in Australia.

Chapter 1: General Introduction

1.1 Sponge Biology

Sponges are benthic, filter-feeding invertebrates that display a wide variety of morphological forms (described and illustrated in Boury-Esnalt and Rützler, 1997) and occupy a wide array of habitats including fresh and salt water, although the latter are predominant (reviewed by Bergquist, 1978). The sponge body is essentially a pump containing many tiny pumping units, or choanocyte chambers. Water is delivered to, and removed from, choanocytes *via* the aquiferous system, which forms an intricate network throughout the sponge body (Fig. 1.1). The water provides a source of food and oxygen, and transport for waste removal and gamete dispersion (reviewed by Simpson, 1984). Entrance to the aquiferous system is *via* the ostia, and exit *via* the oscules. Ostia are tiny and numerous (ranging in size from 1 to 50μm in most species), while oscules are generally large and few in number, varying greatly in size from one species to another (reviewed by Simpson, 1984). The body and aquiferous system of the sponge are covered by the exopinacoderm and endopinacoderm respectively. Supporting the sponge body is a skeleton consisting of organic collagen or spongin fibres and/or inorganic siliceous or calcareous spicules. Spicules are common and highly variable in shape and have become a key characteristic for species identification and classification in all but four orders of the phylum.

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Fig. 1.1 Schematic representation of the aquiferous system within a sponge (modified from Hentschel *et al*, 2003; Bergquist, 1978). Large arrows show the direction of water movement. (cc. choanocyte chamber, mes. mesohyl)

Sponges are divided into three classes. The largest and most abundant class, of which bath sponges are a member, is the Class Demospongiae. The Order Dictyoceratida, within this class, is characterised by having an internal skeleton comprised only of spongin, which makes them ideal for bath sponges. The other classes are Hexactinellida and Calcarea (summarised by Hooper and van Soest, 2002).

1.2 Morphological Responses to Environmental Conditions

Beyond the natural variation in morphology among species, the morphology of some sponges is highly plastic and can be influenced by environmental variables including water flow (*eg*. Palumbi, 1986), food availability (*eg*. Storr, 1976), sedimentation (*eg*. Bell *et al,* 2002b), light intensity (*eg*. Wilkinson and Vacelet, 1979), predation (*eg*. Hill and Hill, 2002), temperature (Mercurio *et al*, 2000), and exogenous and endogenous chemical concentrations (Krasko *et al*, 2000; Mercurio *et al*, 2000). Due to the close relationship between some environmental variables, many studies have

failed to establish which of these factors has been the cause for observed changes in morphology. For example, an increase in ambient water flow may decrease the sedimentation rate (Bell and Barnes, 2000) and ensure the provision of a continual supply of new water and food material (Barthel, 1991). Water flow, sedimentation, and food availability will be considered independently in this review.

1.2.1 Water Flow

Water flow produces a significant change in the morphology of many sessile marine organisms and sponges are no exception (Kaandorp, 1999). Increased water flow influences sponge morphology and growth in two main ways. Firstly, it provides a supply of new water and removes filtered water and waste products from the sponge, which may in turn stimulate the sponge to change shape or orientation in order to best utilise the local currents (Wilkinson and Vacelet, 1979; Vogel, 1974). Secondly, it increases the physical load upon the sponge thus increasing the need for structural stability or support (Palumbi, 1986; Bell and Barnes, 2000; Bell *et al*, 2002a).

Some sponge species “detect” the direction of the prevailing water currents and orientate themselves accordingly so as to best utilise water flow. For example, the normally vase-shaped sponge, *Spongia agaricina*, is able to assume an elephant-ear shape, positioning itself so that the inhalant surface faces the oncoming current when positioned on an exposed cliff face (Pronzato *et al*, 1998). A massive *Spongia* sp. (Darwin Harbour, Australia) also orients itself according to the prevailing water current so as to have its longest axis perpendicular to the current (McDonald *et al*, 2003). Interestingly, if reorientated, the sponge begins to grow laterally increasing its total volume.

Another morphological adaptation arises from the potential for sponges to utilise passive water flow induced by ambient water currents. Vogel (1974, 1977) demonstrated the effects of increased water flow on the passive flow of water through several sponge species using live and dead specimens. However, some authors regard passive flow, or the benefits derived from passive flow, as relatively minor (Barthel, 1991; Riisgård *et al*, 1993). There is one species however, that provides evidence in support of the theory that sponges demonstrate morphological adaptation to allow or enhance passive flow to increase food availability in nutrient deficient conditions. The shallow water sponge, *Ircinia felix*, changes from a massive form to become flattened with large chimney-like processes extending upwards from the base when transplanted to the cooler dark waters of 200m depth where the nutritional benefits from symbiotic cyanobacteria are lost (Maldonado and Young, 1998). This new growth form enhances the physical characteristics of the sponge that are required for passive flow.

The external morphology of sponges may also change in response to physical load (*ie*. the force of increased flow), with changes varying according to the environment. For example, in areas exposed to high water currents, the arborescent sponges, *Raspailia ramosa* and *Stelligera stuposa*, generally have a greater trunk diameter and basal attachment, and a more compact form, *ie*. many nodes and short branches. However, the authors suggested the compact form is more likely to be due to reduced sedimentation, as the simple erect forms found more frequently in areas of high sedimentation reduce the area on which sediment may settle (Bell *et al*, 2002a). Kaandorp and Kluijver (1992) also found *Haliclona oculata* to exhibit a more compact form in high flow environments, although they hypothesised that this was

due to an increase in food availability in higher velocity water currents, as new water is delivered to the sponge and the old water flushed away. The inseparable nature of water velocity, sedimentation and food availability remains a major issue in most *in situ* studies of the plasticity of sponge morphology and awaits controlled laboratory experiments to separate their effects.

Internal morphology such as skeletal structures and the aquiferous system can also vary depending on environmental conditions. For example, *Halichondria panicea* exhibits an increase in the number and size of spicules, while the oscules become smaller and more numerous due to the need for increased stiffness to withstand an increase in wave force (Palumbi, 1986). Similarly, the spicule content of *Cinachyrella australiensis* increases and oscule diameter decreases with an increase in water current (McDonald *et al*, 2002). Spicule content also increases for *H. panicea* when in an environment of increased water movement (Barthel, 1991), although in contrast to Palumbi's study, oscules were larger and fewer in high energy environments than for individuals in sheltered environments. In a similar study, the spicules of *Cliona celata* became less numerous, narrow and long, and the number of oscules decreased when subjected to increased water flow (Bell *et al*, 2002b).

Understanding the effects of the environment, and in particular water flow, has important ramifications for sponge aquaculture. Sponge skeleton quality is partially dependent on fibre density. If increased water flow or stress upon the sponge induces an increase in structural elements in some species, it is feasible that fibre density could increase in sponges lacking spicules, thus influencing sponge quality and potential application. Reduced oscule number and size would have a similar

impact. Furthermore, there is likely to be a species specific growth response according to the favoured conditions for any one species which would have further implications for sponge aquaculture site selection.

1.2.2 Food Availability

In addition to the morphological effects (on shape and structure) of food availability correlated with water movement, sponge nutrition also causes changes in sponge morphology, growth rates and colour. When cultured *in vitro, Halichondria melanadocia* developed “outgrowths” in low food treatments while normal and high food treatments promoted growth of rounded sponges. Furthermore, sponges cultured using more concentrated and varied diets demonstrated higher growth rates (Duckworth and Pomponi, 2005). In a similar study, an optimal food concentration was found for *Axinella corrugata* in the weeks following artificial propagation with low or high food concentrations significantly reducing growth (Duckworth et al, 2003). *In situ*, *Spheciospongia* sp. grows to almost one and a half metres high and one metre across in nutrient rich waters, whereas the largest individuals in nutrient deficient waters reach a diameter of up to one and a half metres, but are low and doughnut shaped (Storr, 1976). The effects on the shape and size of these sponges due to other environmental parameters were not discussed. Similarly, there was no investigation on the effects of size on internal morphology. In the only study relating nutrition to colour change, *Halisarca nahantensis*, which is normally whitish to brownish in colour, varies between green, orange, and grey when fed different microalgae diets in a closed system (Chen, 1976).

Surprisingly, there is little information regarding the effects on morphology caused by food availability or sponge nutrition *in situ* given their potential impact on growth. Numerous studies have been conducted that investigate the composition of sponge diets (*eg*. Reiswig, 1971; Pile *et al*, 1996; Ribes et al, 1999), however they do not relate dietary nutrition or food abundance to sponge growth or morphology.

The implications for sponge aquaculture regarding food availability are obvious, as sponges grown in a site where food availability is limited will exhibit reduced growth and potentially irregular shapes. Shape is important for bath sponge production, with rounded massive sponges being of the greatest value. As the sponge surface is lost during processing, surface colour is of no importance to quality.

1.2.3 Sedimentation

Exposure to sedimentation can directly affect sponge growth and morphology. Sedimentation reduces pumping rates (Gerrodette and Flechsig, 1979), increases energy expenditure to remove the unwanted particles from the sponge surface or canals (Gerrodette and Flechsig, 1979), and provides a substrate for fouling organisms (Teragawa, 1986a). Some sponges, *eg*. *Verongia aerophoba* and *Verongia cavernicola*, demonstrate significantly higher growth rates in the absence of sedimentation (Wilkinson and Vacelet, 1979). Furthermore, the arborescent sponges *Raspailia ramosa* and *Stelligera stuposa* have a reduced maximum size in areas of high sedimentation (Bell *et al*, 2002a).

In some species, sediment is used to the sponges' advantage as sand and spicules are selectively incorporated into the collagenous skeleton (Teragawa, 1986b; Bavestrello

et al, 1995; Bavestrello *et al*, 1996, In: Bavestrello *et al*, 1998; Pronzato *et al*, 1998). Incorporated inorganic material is beneficial as it strengthens or stiffens the skeletal support (Teragawa, 1985, In: Teragawa, 1986b; Bavestrello *et al*, 1995) and reduces the spongin requirement, thus enhancing skeletal growth (Teragawa, 1986a).

Sponges in low flow environments are generally exposed to a higher level of sedimentation as more particles fall out of suspension making it difficult to distinguish between the effects of flow and sedimentation (*eg*. Bell *et al,* 2002a,b). One sponge that shows a dramatic morphological response to increased sedimentation is *Cliona celata* (Bell *et al,* 2002b). In areas of low flow and high sedimentation these sponges generally have a reduced number of oscules, which lowers the probability of clogging from sediment (Bell *et at*, 2002b). Not only is the area in which sediment may settle reduced, but the velocity of exhalent water is increased, further inhibiting the settlement of particles in these openings. In areas of very high sedimentation this same species is abundant in a burrowing form. This remarkable form has its ostia below the substrate, which prevents clogging (Bell *et al*, 2002b).

Dramatic morphological changes due to sedimentation such as those seen in *Cliona celata* appear to be an exception, as most species exhibit little to no gross morphological change due to changes in sedimentation, with mortality or changes in growth rate or total size being the only visible effects, and inorganic inclusions the only internal change. These inclusions are an important consideration for the production of commercial sponges where such inclusions are undesired. Combined

with the higher growth rates achievable in the absence of sedimentation, low sedimentation sites are clearly superior from an aquaculture perspective.

1.2.4 Light Intensity

Light plays an important role in sponge growth and colour. Many sponges contain photosynthetic symbionts (usually cyanobacteria) that utilise light and provide the host sponge with up to 50% or more of its nutritional requirements (Wilkinson, 1983, 1987). The relationship between sponges and symbionts is for some species obligate and for some facultative, while other species have no photosynthetic symbionts (Maldonado and Young, 1998).

Wilkinson and Vacelet (1979) demonstrated the necessary role of light in the phototrophic sponge, *Verongia aerophoba,* with transplantation experiments using artificial shading. After almost one year, sponges under black shields had reduced chlorophyll content, surface colour, growth, and bud size. Most phototrophic sponges have a high surface area to volume ratio with encrusting, dish, and fan shaped morphologies (Wilkinson, 1983). However, there is no evidence of gross morphological change due to changes in light exposure as is commonly reported for corals (*eg*. Muko *et al*, 2000).

Another effect of light on sponges is colour change. Dark coloured sponges are most commonly found in shallow waters, while the deep water is dominated by white or yellow sponges. Furthermore, single species common across a range of depths are commonly darker in shallower waters (Maldonado and Young, 1996). The reason for these changes is largely unknown, although it may be attributable to an increase

in symbionts and/or pigmentation to protect the sponge from damaging UV radiation (Sarà, 1971; Jokiel, 1980).

The only common morphological difference in sponges due to light appears to be surface colour, although as previously discussed this is not relevant for bath sponge quality. Therefore, the light levels chosen for a bath sponge farm would be selected according to factors such as sponge growth, where light may provide the benefit of increased energy contribution through photosynthetic symbionts, competition for space or food recourses by colonising organisms, or smothering from autotrophic fouling organisms.

1.2.5 Predation

Sponges can also change their internal morphology according to predation pressure. Hill and Hill (2002) examined the effect of damage, such as that caused by predation, on the morphology of *Anthosigmella varians* by removing small portions of tissue from healthy sponges. Spicule content increased in response to the level of damage sustained by the sponge. It is unlikely such a response could prove beneficial for an aquaculture situation even if the same effect could be observed on spongin production considering the drain on energy resources required for tissue repair.

1.2.6 Other environmental and biological factors

The temperature and silica concentration of the seawater and the reproductive state of sponges also alters the morphology of some species. For example, the total spicule mass of the Mediterranean sponge, *Pellina semitubulosa*, varies between sites according to silica concentration in the water, and within a single site changes are

positively correlated to temperature change (Mercurio *et al*, 2000). Gamete and larval release also stimulate the formation of new spicules (Mercurio *et al*, 2000). Furthermore, in the spicule producing sponge, *Suberites domuncula*, artificially increasing exogenous silicate concentration or a myotrophin-related polypeptide causes significant increases in the expression of a collagen-producing gene (Krasko *et al*, 2000). Myotrophin is thought to be part of the natural biochemical pathway responsible for the initiation of collagen production and may act independently or in unison with spicule production (Krasko *et al*, 2000). The application of the up-regulation of the genes controlling spongin production in sponges may provide a unique biotechnology application for bath sponge production to produce sponges of specific spongin densities.

1.3 Sponge Supply and Demand

Dictyoceratid sponges are used for a range of applications including bath, cosmetic, decorative, personal hygiene, art creation, painting and car detailing and they have been harvested by Greek fishermen for at least 3000 years (de Laubenfels and Storr, 1958). The Mediterranean Sea is still a major source of sponges with most sponges in the region being harvested from Tunisia, Greece and Turkey (Josupeit, 1990). Sponges are also sourced from further afield, in particular Cuba and Florida, where sponges have been fished since the 1850s (Josupeit, 1990; Witzell, 1998) and now supply the majority of the world market (Corriero *et al*, 2004). The number of sponges harvested has fallen heavily due to a combination of synthetic sponge production, over-fishing, and disease (Josupeit, 1990; Corriero *et al*, 2004). Despite the current demand for natural bath sponges, over fishing and disease continue to

limit supply (Corriero *et al*, 2004). Aquaculture is a promising solution to meet this demand whilst preserving natural populations.

Research into bath sponge aquaculture dates back to the late 1800s (reviewed by Witzell, 1998), however sponge aquaculture is still in its infancy. Pohnpei, Federated States of Micronesia, has the only commercial sponge aquaculture in the world with three farms currently in operation (R. Croft, pers. com.). Unfortunately, production problems have prevented entrance into the international market. Elsewhere, research continues, although numerous problems continue to prevent significant development within the industry.

Sponge aquaculture also has applications in the supply of biologically active compounds that exhibit anticancer, antibacterial, anti-inflammatory or antiviral properties (reviewed by Sennett, 2001). The number of sponges required to produce sufficient amounts of desired chemicals usually far exceeds that available in wild stocks as the target chemicals are usually found at only trace levels requiring a huge wet weight harvest to obtain sufficient material for clinical trials (Mendola, 2003). As metabolite production is beyond the scope of this report it will not be discussed any further except to say that the potential market for sponge aquaculture is large and varied and there are significant efforts to further the production of sponge secondary metabolites (Müller *et al*, 1999; de Caralt *et al*, 2003; Duckworth and Battershill, 2003a; Mendola, 2003; Nickel and Brümmer, 2003; van Treeck *et al*, 2003; Hadas *et al*, 2005).

1.4 Production Methods and Limitations

The most common method of sponge propagation is to cut a mature sponge into a number of pieces, or explants, each of which will recover to form a new genetically identical individual. Many methods of holding explants have been trialled with varying degrees of success, however, for commercial aquaculture development, species-specific research is required, as the response to culture methods differs markedly between species. For example, Duckworth and Battershill (2003a) have examined the survival, growth, and metabolite production of *Latrunculia* sp. nov, *Polymastia croceus*, and *Raspailia agminata* using four basic methods with minor variations for each. All three species had the greatest survival when farmed in mesh and lowest when farmed on rope, however the negative attributes of the mesh lanterns used, including biofouling, would limit commercial use of this method. Growth was negative, negligible or variable within each general method for all species and method groupings over the three month experiment. The bioactivity of the sponges was high for some specific variations of the general methods, although not necessarily common between species.

Other methods used to hold explants in previous research have included fixing them in position between two layers of mesh (van Treeck *et al*, 2003) or placing explants within plastic or nylon mesh bags or nets (Duckworth and Battershill, 2003a; Kelly *et al*, 2004; Hadas *et al*, 2005), threading them onto plastic coated wire (Verdenal and Vacelet, 1990) or a rope or thin line made of cotton (Duckworth and Battershill, 2003a), hemp (Duckworth and Battershill, 2003a), PVA (Duckworth and Battershill, 2003a), or nylon (Corriero *et al*, 2004), and tying them to various artificial media including glass (Barthel and Theede, 1986) PVC (Hadas *et al*, 2005), steel mesh

(Müller *et al*, 1999), and manila, sisal, nylon or polypropylene rope (Duckworth and Battershill, 2003a). The success of each culture was as variable as the number of methods used. Successful methods required a substance that did not degrade and promoted basal attachment by the sponge. Again, the success of any one method was heavily dependent on the target species. Species-specific research will provide the knowledge required to implement culture techniques that maximise sponge survival, growth and quality.

Explant survival and growth is also known to vary greatly beyond the effects of the culture method. Factors such as local environmental conditions during and post recovery, genetics (Kelly-Borges, 1996) and cellular composition (Kelly-Borges, 1994) are known or suspected to play an important role, although these and other unknown factors are not well understood. A crucial part of this understanding requires knowledge of the recovery process. To understand how explant growth and survival are affected by recovery, they must be examined during and proceeding this phase of development. Previous studies have noted changes in growth rates during or post-recovery (Osinga *et al*, 1999; Belarbi *et al*, 2003) or have examined recovery (Pronzato et al, 1999; Nickel and Brümmer, 2003), but few studies have performed detailed examination of the recovery process and related this to the longer term impacts on survival and growth. Breaking new ground, Hoffmann *et al* (2003) studied the growth and survival of *Geodia barretti* combined with a detailed study of the recovery process, discussing the likely impact of this process on the observed growth. In another study, de Caralt *et al* (2003) measured the survival and growth of *Corticium candelabrum* and detailed the recovery process, however few conclusions

were drawn regarding the relationship between the recovery process and its impact on survival and growth.

As well as finding optimal conditions for survival, growth and recovery, it is essential to understand the culture method and environment required to produce high quality sponges. The ideal characteristics of a commercial bath sponge are high water absorbency, strength, softness, and elasticity, absence of coarse foreign inclusions, and a suitable appearance and colour. The extent of variation in these characteristics due to the environment or culture method is likely to vary between and within species as previously discussed, but is largely unknown. At present there are no established protocols for testing sponge quality. Therefore, environmental conditions that may enhance or degrade sponge quality are unable to be assessed. Once developed, tests of quality would have three valuable applications in sponge aquaculture. Firstly, they would allow the assessment of environmentally induced morphological variation in sponges to aid in aquaculture site selection. Secondly, potential sponge aquaculture species could be tested against known commercial species to determine their market potential in the absence of experience. Thirdly, once established, a quality measure could be used as a marketing tool to promote cultured species.

1.5 Project Aims

The aim of this study was to provide a detailed examination of the recovery process combined with survival and growth measures for the tropical sponges *Rhopaloeides odorabile* Thompson *et al* (1987) and *Coscinoderma* n. sp. using two culture methods. Simultaneously, this study provides insight into the potential of two local

species for sponge aquaculture development. Furthermore, quantitative measures of sponge quality have been established for the first time, which will enable further research into the environmental impacts on sponge quality and provide key marketing tools. The specific aims were:

1. To compare the survival of *in vitro* and *in situ* culture of *R. odorabile* and *Coscinoderma* n. sp. explants; to monitor *in situ* explant growth of *R. odorabile* and *Coscinoderma* n. sp. during and following recovery; and to document the recovery processes for *R. odorabile* and *Coscinoderma* n. sp. as evidenced by cellular reorganisation and reconstruction.
2. To develop quantitative measures for sponge quality resulting in the measurement of the density, fibre width, fibre length, firmness, compression modulus, compressive strength, tensile strength, elastic limit, elastic strain, modulus of elasticity, modulus of resilience, absorbency and water retention efficiency of *R. odorabile* and *Coscinoderma* n. sp. and three commercial bath sponges, *Hippospongia lachne* (de Laubenfels, 1936) (wool sponge), *Spongia zimocca* (Schmidt, 1862) (silk sponge), and *Spongia graminae* (Hyatt, 1877) (grass sponge).

Chapter 2: Explant survival, growth and recovery for *Rhopaloeides odorabile* and *Coscinoderma* n. sp.

2.1 Introduction

The current demand for bath sponges outstrips supply due to overfishing and devastating outbreaks of disease, which together have severely depleted natural sponge populations, particularly in the Mediterranean (Corriero *et al*, 2004). Aquaculture of bath sponges remains in its infancy with only one species, *Coscinoderma mathewsi*, currently in commercial production in Pohnpei, Federated States of Micronesia (Kelly-Borges, 1994; R. Croft pers. com.). Cultivation experiments at the site resumed in 1984, after it was first initiated by the Japanese prior to the Second World War (Croft, 1995). Commercial production began in the early 1990s, however production problems have prevented entrance to the international market (R. Croft, pers. com.). Although there is a clear need for further research, there are limited studies examining potential candidates for bath sponge aquaculture (Verdenal and Vacelet, 1990; Pronzato *et al*, 1999; Corriero *et al*, 2004; Kelly *et al*, 2004).

Most sponge aquaculture research has been dedicated to the study of temperate species for the purpose of producing bioactive metabolites (*eg*. Duckworth *et al*, 1997; Muller *et al*, 1999; Duckworth and Battershill, 2003b; Hoffmann *et al*, 2003; van Treeck *et al*, 2003). Even the limited work on bath sponges has been primarily based in temperate climates (Verdenal and Vacelet, 1990; Pronzato *et al*, 1999; Kelly *et al*, 2004). Seasonal variation in growth rates of sponges in temperate regions has commonly been reported with periods of inhibited or even reversed sponge growth

(*eg*. Verdenal and Vacelet, 1990; Duckworth and Battershill, 2003b; Duckworth *et al*, 2004; Kelly *et al*, 2004). In the tropics, consistently high water temperatures provide the potential to see increased and sustained growth rates in sponges.

Unfortunately, most studies dealing with sponge growth have been confounded by the fact that sponges invariably demonstrate varying degrees of healing response following artificial propagation, where explant growth is potentially enhanced (Belarbi *et al*, 2003) or inhibited (Osinga *et al*, 1999), rather than growth response being solely due to seasonal or other environmental effects. To better understand the significance of this healing period in relation to the growth and survival of explants, studies examining growth and survival must be accompanied with a detailed examination of recovery (see de Caralt *et al*, 2003; Hoffmann *et al*, 2003).

The survival rate of sponge explants ranges dramatically according to species and culture method (*eg*. Duckworth and Battershill, 2003a). Reported survival rates vary from 0% (Duckworth and Battershill, 2003a; Hoffmann *et al*, 2003; van Treeck *et al*, 2003) to 100% (Verdenal and Vacelet, 1990) although many studies are conducted over a period of only a few months and thus give no more than an indication of the expected long term success for any one culture method (*eg*. Duckworth and Battershill, 2003a). Estimates of the minimal requirements for an economic sponge culture include at least 90% survival per annum (Verdenal and Vacelet, 1990).

In this study, two tropical sponges, *Rhopaloeides odorabile* and *Coscinoderma* n. sp. (Fig. 2.1), were investigated for their potential as aquaculture candidates. These species were chosen because their skeletal structure and local abundance make them

suitable candidates for aquaculture. *R. odorabile* has been well researched in regard to its natural products chemistry (Thompson *et al*, 1987), microbial symbiont populations (Webster and Hill, 2001; Webster *et al*, 2001) and abundance and distribution on the Great Barrier Reef (Wilkinson and Evans, 1989), and in particular within the Palm Island Group (Bannister *et al*, in prep). *Coscinoderma* n. sp. is an undescribed species that is abundant and widely distributed within the Palm Island Group (Bannister *et al*, in prep) and beyond. Throughout the years, numerous species of *Coscinoderma* have been described although only two of these species are considered valid (not including synonyms of the type species) (P. Bergquist, pers. com.). The valid species are *C. lanuginosum* Carter (1883) and *C. mathewsi* (Lendenfeld, 1886). The growth forms of these species are clearly distinct from the conulose spreading *Coscinoderma* of this study and they are further separated by their geographical distribution (P. Bergquist, pers. com.).

The specific aims of this chapter were to compare the survival of *in vitro* and *in situ* culture of *R. odorabile* and *Coscinoderma* n. sp. explants; to monitor *in situ* explant growth of *R. odorabile* and *Coscinoderma* n. sp. during and following recovery; and to document the recovery processes for *R. odorabile* and *Coscinoderma* n. sp. as evidenced by cellular reorganisation and reconstruction.

A

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B

Fig. 2.1 The sponge species investigated in this study. Photos were taken *in situ* within the Palm Island Group. **A**. *R. odorabile* **B**. *Coscinoderma* n. sp. (reproduced with permission from the Australian Institute of Marine Science)

2.2 Materials and Methods

2.2.1 Sponge Collection

R. odorabile and *Coscinoderma* n. sp. (hereafter referred to as *Coscinoderma* sp.) were collected from north-western Pelorus Island, North Queensland (18°32'S, 146°29'E). Specimens were cut from the hard substrate leaving a portion of the sponge attached for regeneration. Samples were maintained underwater at all times. In August 2002, sponges were collected for the quantification of explant survival and growth, and for quantification of the external closure of the exposed aquiferous system following excision as a measure of recovery. Specimens for the examination of surface recovery using scanning electron microscopy (SEM) were collected in October 2002, and sponges used for a histological examination of the subsurface cellular reorganization during recovery were collected in August 2004 and May 2005.

2.2.2 Explant Survival

For the measures of survival, growth and the closure of the exposed aquiferous system, five individuals of *R. odorabile* and *Coscinoderma* sp. were each cut into eight explants of approximately 5 × 5 × 5cm and measured exactly to determine individual sizes. Sponges were cut underwater with a sharp blade and threaded onto 4mm polypropylene rope using a marlinspike to skewer the explants, as per methodology previously employed by the Australian Institute of Marine Science for these species. Knots tied either side of each explant prevented them from sliding on the rope. Four explants from each of the five individuals of each species were randomly allocated to either the *in vitro* or *in situ* culture. Each rope was allocated an explant from each individual of a single species giving five explants per rope.

One rope for each species was then strung across each of four 80cm square PVC frames.

Frames containing explants for the *in vitro* culture were maintained at the Orpheus Island Research Station in separate raceways (40cm deep) with filtered shade and supplied with unfiltered seawater. Flow rate varied throughout the experiment from a minimum of 1.1×10^{-4}m/s to a maximum of 9.9×10^{-4}m/s. Vegetative fouling on the explants was removed throughout the study.

Sponges to be cultured *in situ* were taken by SCUBA to a depth of 11m, in close vicinity to the original collection site. The frames were placed in a line facing into the predominant current and attached to railway iron to prevent drifting. The flow rate was measured during mid-tide at approximately 0.5m/s. No fouling organisms colonised the *in situ* culture.

Survival of explants in the *in vitro* culture was monitored after 7, 14, 26, 36, 54 and 78 days and for the *in situ* culture, after 78, 166, 259, 386 and 621 days. Variation in the initial explant size was used to test for size dependent survival in the *in situ* culture after 78 days. Comparisons could only be made where replication was sufficient in both “living” and “dead” groupings. Survival data was also grouped according to the parental origin of the explants to determine if mortality within the experiment was related to parentage.

2.2.3 Explant Growth

The explants described in Section 2.2.2 Explant Survival were also used for measurement of growth. The growth of *in vitro* explants was not measured due to poor explant survival. The growth of explants in the *in situ* culture was measured as the percentage increase in sponge volume (multiple of the maximum depth, width, and height to within 0.5cm). Explants were measured at the beginning of the experiment and after 78, 166, 256, 386 and 621 days as for the monitoring of explant survival. Variation in the initial explant size was used to test for size dependent growth rates and the effect on final explant size. Initial explant size was plotted against the daily growth rate for each time period (*i.e*. between each time explant size was measured) and against the final explant size to determine correlations. To allow comparison between the initial and final effects on explant size, only explants surviving the full term of the experiment were included in the analysis. Growth was also compared between explants of common parentage to determine if total growth was influenced by parentage.

2.2.4 Explant Recovery

2.2.4.1 Macroscopic Surface Examination

The first measure of recovery was quantified as the time required for the closure of the exposed aquiferous system after cutting. This technique was non-invasive and allowed recovery to be monitored concurrently with the survival and growth measures of the *in vitro* culture. Photographs of the cut surface of *R. odorabile* and *Coscinoderma* sp. were taken after 1, 2, 7, 14, and 36 days using a Cannon 35mm camera. The coverage of exposed canals in a newly cut sponge were described for each species.

2.2.4.2 Microscopic Surface Examination

The second measure of recovery used SEM to allow the description of surface and ostia development of *R. odorabile* and *Coscinoderma* sp. after cutting. Sponges were held in raceways as previously described except that a sponge specimen of each species was placed on the floor of the raceway having been cut into two pieces to provide a large single cut surface for each species from which samples could be removed. The newly cut surface was maintained facing up at about a 60 degree angle to the raceway floor to avoid contact with the floor of the raceway and ensure the flow of ambient water past the cut surface was not restricted. A small cubic sample was taken from the individual immediately after cutting and every 12hrs thereafter over a five-day period. Samples were fixed and stored for up to seven days in 4% formaldehyde in seawater before being soaked in Marine Bouin's Solution for two hours. Over a five-hour period, samples were dehydrated through a graded series of ethanol baths beginning at 70% ethanol. Samples were air dried for at least two hours before being mounted and gold coated with a Jeol JUC-5000 Magnetron Sputtering Device. Samples were examined using a Jeol JSM-5410LV Scanning Electron Microscope. To test the sufficiency of air-drying, samples of both species (n = 3) were dried using a Pelco CPD2 Critical Point Dryer. Minor cracking was observed in samples treated using both methods, and air-drying used for further sample treatment. Surface recovery and distribution of newly formed ostia were described.

2.2.4.3 Cross-sectional Examination

The third measure of recovery used light microscopy to allow the description of subsurface cellular reorganization. Three individuals of each species were cut into

seven explants from which samples would later be taken for examination. These explants were also cultured on the floor of the raceway. A vertical cross-section of a healing surface was taken from three previously unsampled explants of each species at 0, 1, 2, 3, 4, 10 and 41 days after cutting and from eight uncut specimens per species. Samples were fixed in Formaldehyde-Acetic Acid-Calcium Chloride (FAACC), sub-sampled, dehydrated through a graded series of ethanol baths, cleared, infiltrated and embedded in paraffin, sectioned to 5µm and stained using Mayer's Haematoxylin and Young's Eosin Erythrosin (Woods and Ellis, 1994). This stain preferentially stains acidophilic tissue red (including most cellular material), and basophilic tissue blue (including DNA and RNA). Samples were examined using an Olympus BX40 light microscope in conjunction with an Olympus DP12 Digital Camera System. The subsurface cellular reorganization and the change in subsurface skeletal features and aquiferous system rearrangement are described. Terms and descriptions of sponge morphology and cell types were derived from Boury-Esnalt and Rützler (1997).

2.2.5 Statistical Analysis

Analysis of the relationship between species and culture method for the survival of *R. odorabile* and *Coscinoderma* sp. was performed using a chi-square test. Size dependent explant survival was tested for *R. odorabile* using a t-test, as was the difference in total explant growth between species. Differences in daily growth rate over time were analysed separately for each species using a repeated measures ANOVA and simple contrasts used to distinguish between seasons. Data were transformed to fit the assumptions of the ANOVA using $X' = \sinh^{-1}(100X)$. A variation of this transformation is described by Beall (1942). Homogeneity of

variance was tested using a plot of standardised residuals over predicted means (Quinn and Keough, 2002). Growth rate of explants relative to initial explant size were analysed using Pearson's correlation coefficient. Differences in the initial and total growth rates of explants relative to parentage were analysed for *Coscinoderma* sp. using a Kruskal-Wallis test. Statistical analyses were performed using SPSS 12.0 for Windows.

2.3 Results

2.3.1 Explant Survival

Survival of explants was significantly different between culture methods and species with *R. odorabile* demonstrating the highest survival in *in vitro* culture and the lowest in *in situ* culture (χ^2 = 4.898; df = 1, p = 0.027). Survival in the raceways (*in vitro*) dropped during the first two weeks to 90 and 50% for *R. odorabile* and *Coscinoderma* sp. respectively and stabilised after eight weeks at 75 and 30% respectively (Fig. 2.2A). Similarly, in the *in situ* culture, most mortalities occurred before the second examination of the culture (which coincided with the termination of the raceway culture at 11 weeks). Survival in the *in situ* culture at this time was 60 and 90% for *R. odorabile* and *Coscinoderma* sp. respectively and ended (after 21 months) with 50 and 80% survival respectively (Fig. 2.2B).

To determine if the initial survival was dependent on initial explant size the original size of dead and living explants of the *in situ* culture were compared (for the purpose of relating survival to size dependent growth rates). *R. odorabile* survival was higher for larger explants although this was not significant (t = 1.843, df = 18, p = 0.082). Due to the high survival rate of *Coscinoderma* sp. during this time (giving a

replication of only two dead explants), size comparisons for this species were not appropriate.

Survival was also compared between explants of common parentage. There was no obvious relationship for either *R. odorabile* or *Coscinoderma* sp. linking a single parent to poor survival in both *in situ* and *in vitro* cultures (Fig. 2.3). Insufficient replication prevented the use of statistical analysis.

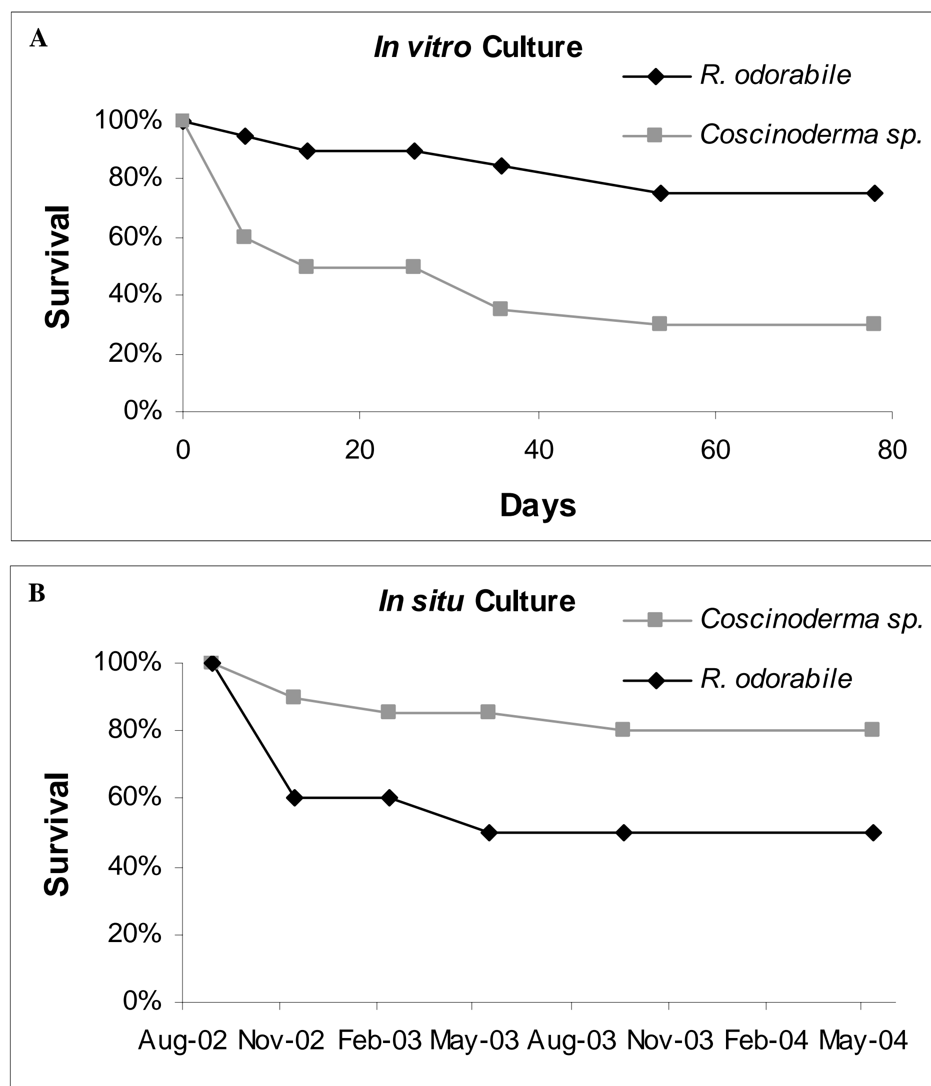


Fig. 2.2 Percentage survival of *R. odorabile* and *Coscinoderma* sp. in *in vitro* (**A**) and *in situ* (**B**) cultures over time.

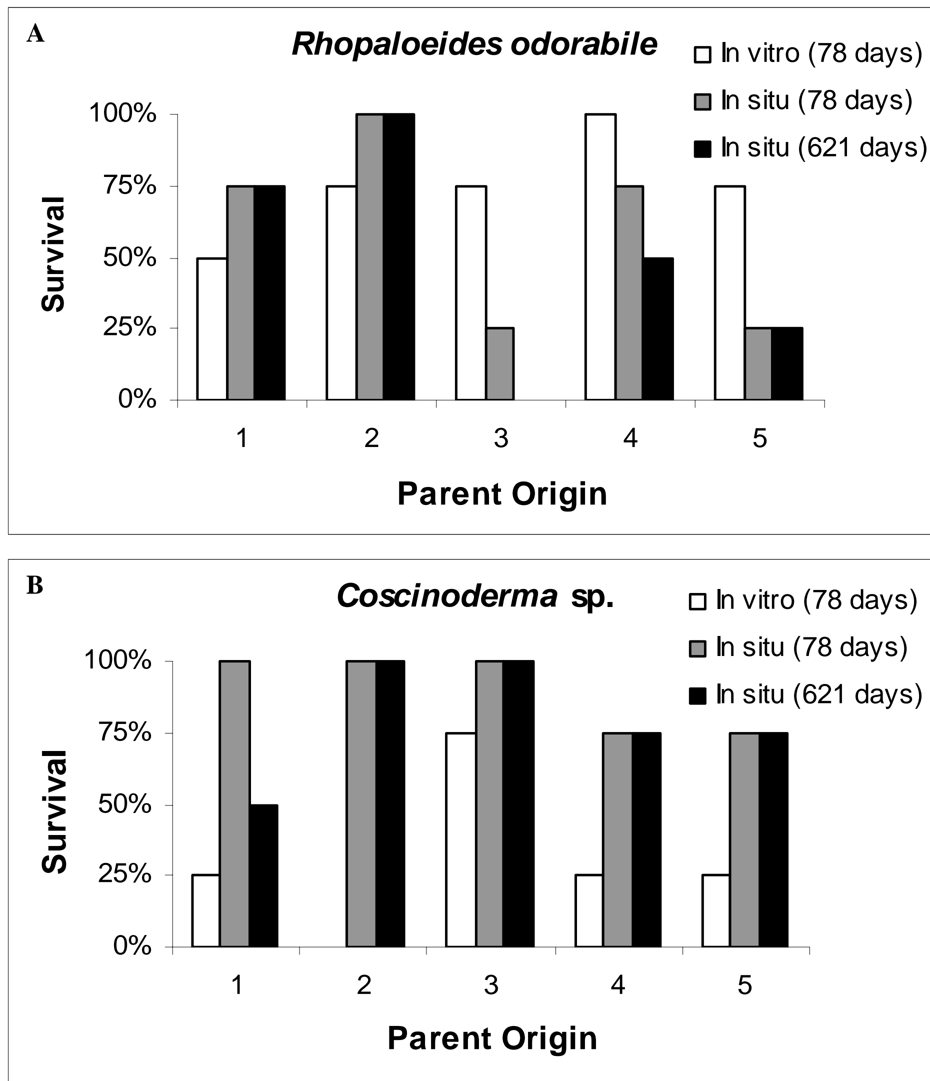


Fig. 2.3 Survival of *R. odorabile* (**A**) and *Coscinoderma* sp. (**B**) in *in vitro* and *in situ* cultures grouped according to parentage. *In situ* survival is given for 78 days to coincide with the termination of the *in vitro* culture, and at 621 days.

2.3.2 Explant Growth

Over the course of the 21 month *in situ* culture, there was no significant difference in growth between the species (t-test: t = 1.51, df = 119, p = 0.134). *R. odorabile* grew 146.0 ± 40.3%, with an average annual growth rate of 85.9 ± 23.7% (Fig. 2.4). *Coscinoderma* sp. grew 195.9 ± 39.8% (mean ± S.E.) with an average annual growth rate of 115.2 ± 23.4%. There was no seasonal effect on the growth rate of *R. odorabile* (Fig. 2.5A; Repeated measures ANOVA: $F_{(4,36)}$ = 0.646, p = 0.634). However, *Coscinoderma* sp. grew significantly faster during the summer of 2002/03 than during all other periods except the winter of 2003 (Fig. 2.5B; Repeated measures ANOVA: $F_{(4,60)}$ = 3.7, p = 0.009).

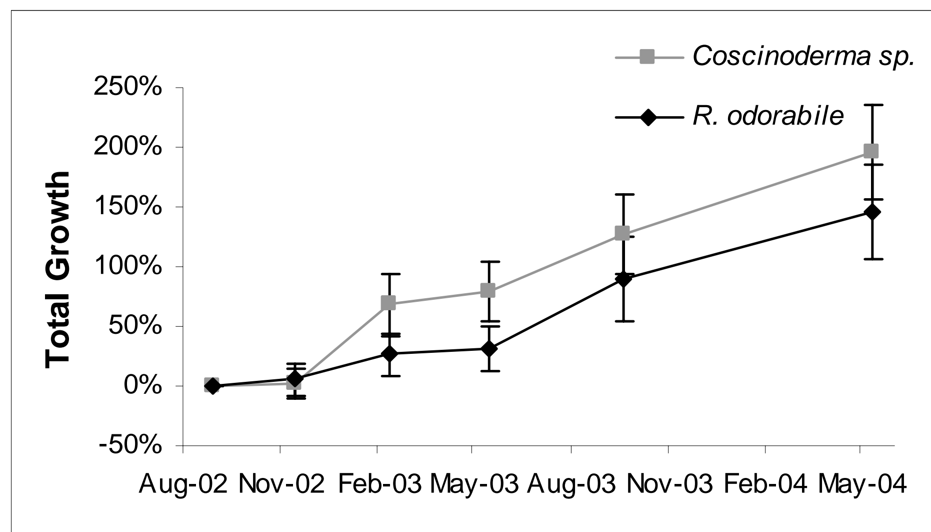


Fig. 2.4 Total percentage growth of *R. odorabile* and *Coscinoderma* sp. cultured *in situ* ± standard error.

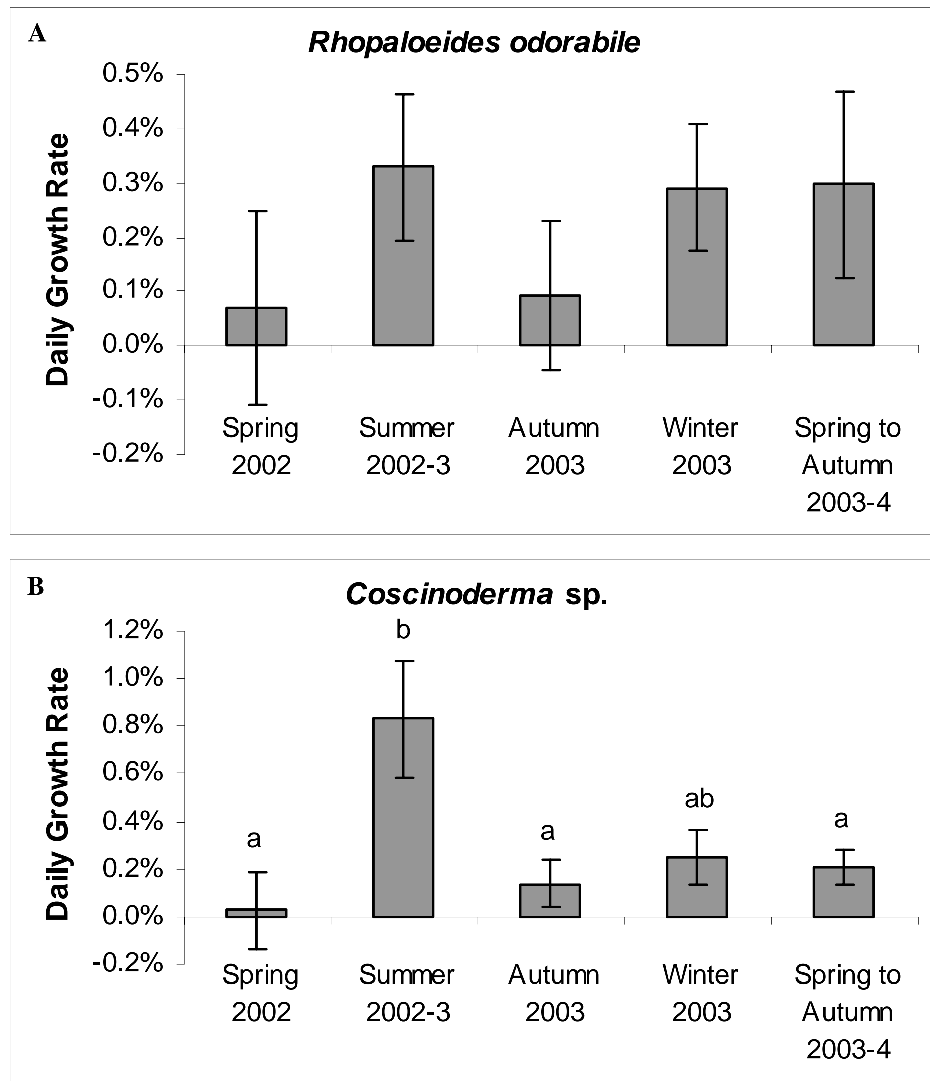


Fig. 2.5 Seasonal changes in daily growth rate of *R. odorabile* (**A**) and *Coscinoderma* sp. (**B**) cultured *in situ* ± standard error. Different letters represent significant differences ($p < 0.05$).

Variation in initial explant size was used to test for size dependent growth rates during each season throughout the study. Smaller initial explant size significantly increased the initial growth rate (first 11 weeks of culture) of explants for both *R. odorabile* (Fig. 2.6A; Pearson's correlation coefficient: $r = -0.760$, n = 10, $p = 0.011$) and *Coscinoderma* sp. (Fig. 2.6C; Pearson's correlation coefficient: $r = -0.735$, n = 16, $p = 0.001$). Initial size had no influence on the growth rate beyond the first season. Due to the size dependent growth rate during the first season, where smaller

explants caught up to larger explants, initial explant size showed no relationship to the final explant size (Fig. 2.6B and D).

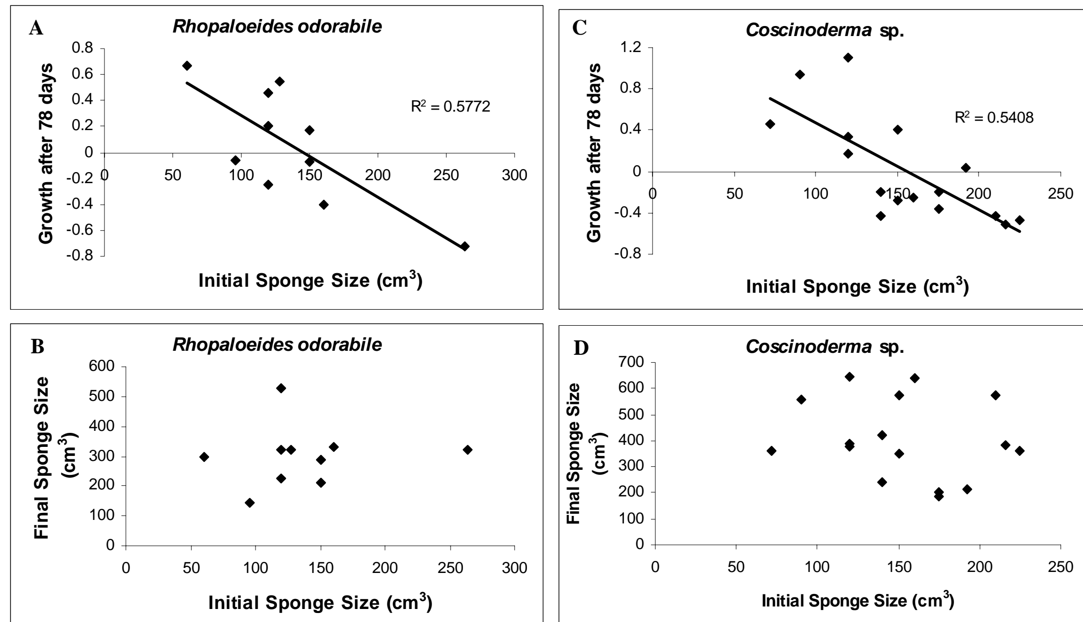


Fig. 2.6 The effect of size dependent growth rates demonstrated through correlations between initial growth and initial explant size and the effect of this differential growth rate on final sponge size for *R. odorabile* (**A** and **B**) and *Coscinoderma* sp. (**C** and **D**). The line of best fit and r^2 values are shown where appropriate.

Differences in the growth rate of individual explants over the course of the study were large. The annual growth rate of individuals ranged from 13.4 to 235.3% for *R. odorabile* (Fig. 2.7A) and 4.2 to 307.2% for *Coscinoderma* sp (Fig. 2.7B). The effect of parentage on explant growth was examined. Variation in explant growth was high between individuals with common parentage (Fig. 2.7). Average daily growth rates varied greatly between explants of common parentage as well as between parental origins for *R. odorabile* and *Coscinoderma* sp. (Fig. 2.8). Differences in the mean growth rates of Coscinoderma sp. from different parents were not significant for the initial 78 days (Kruskal-Wallis test: $\chi^2_{(4)} = 6.075$, $p = 0.194$) or over the entire growth period (Kruskal-Wallis test: $\chi^2_{(4)} = 6.667$, $p = 0.155$).

The high mortality of R. odorabile prevented the statistical analysis of growth differences due to parentage.

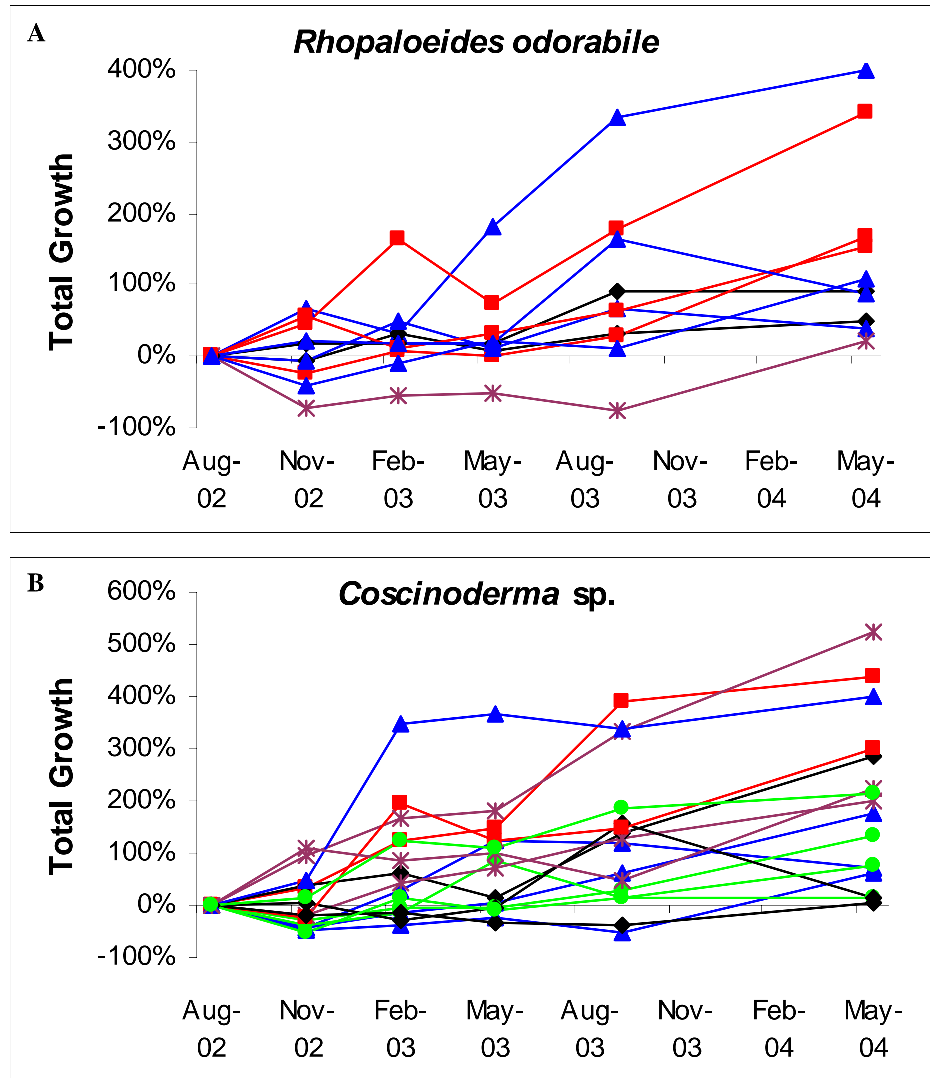


Fig. 2.7 Total percentage growth for each individual explant of *R. odorabile* (**A**) and *Coscinoderma* sp. (**B**). Individuals within each species graphed with the same colour have common parentage.

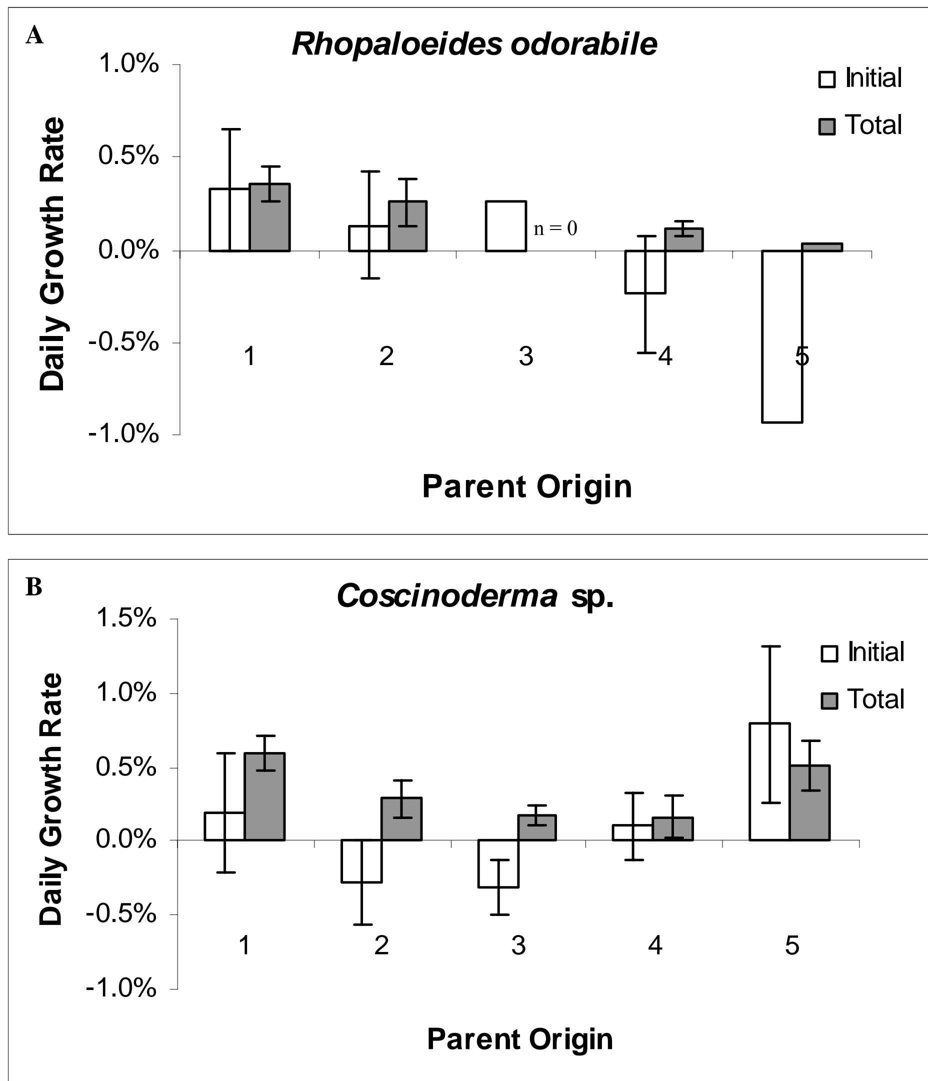


Fig. 2.8 Average daily growth rate (± standard error) grouped according to parentage for *R. odorabile* (**A**) and *Coscinoderma* sp. (**B**). Initial growth (first 78 days) and total growth (621 days) are shown separately. Means lacking SE bars have a replication of n = 1.

2.3.3 Explant Recovery

In each experiment documenting the recovery process, the newly exposed sponge surface was covered over by a layer of protective collagen or pinacoderm. Without high powered microscopy the nature of the new surface could often not be determined. For this reason, any newly developed surface covering will henceforth be referred to as a "protective layer" unless its composition is known.

2.3.3.1 Macroscopic Surface Examination

Rhopaloeides odorabile

When first cut and exposed, the new surface of the sponge was covered with hundreds of small openings ranging in size up to 3mm in diameter caused by the exposure of the many canals of the aquiferous system (Fig. 2.9A). Within 24hrs, a protective layer had covered all but the largest of canals (greater than 1.5 to 2mm), and most of these were greatly reduced in size. After 48hrs, over half of the remaining canals had been covered (Fig. 2.10A-B). Within seven days, 10 of the 12 explants containing exposed large canals after 24hrs had complete surface coverage. Remaining canals were greatly reduced in size and were completely covered after 14 days. A thin layer of exposed spongin fibres covered the cut surface during the first seven days of the recovery process, after which it fell away fully exposing the new protective layer beneath (Fig. 2.10C). Within 14 days, the original rumpled texture and light brown colour of the surface of most explants had returned to normal.

***Coscinoderma* sp.**

Similar to *R. odorabile*, the newly exposed surface of *Coscinoderma* sp. had many exposed canal openings ranging in size up to 3mm (Fig. 2.9B). Although half the explants died within two weeks, even these individuals healed over most canals before dying. Of the remaining 10 sponges, all sponges had complete coverage of small canals (less than 1.5 to 2mm) within 24hrs and 7 of these also had complete coverage of larger canals. Within 48hrs, 9 of the 10 sponges had complete coverage of all canals, and the one remaining sponge had complete coverage after one week. Two explants also contained a canal in excess of 10mm. Instead of healing over the canals, the sponge shape formed around them, transforming the canal walls into an external surface. The remainder of the surface was covered by a dense layer of exposed spongin fibres obscuring the view of the development of the new surface beneath (Fig. 2.10D-F). On several of the surviving individuals the cream coloured exposed canal surfaces darkened over a period of 36 days to match the purple-grey colour of the undamaged surfaces.

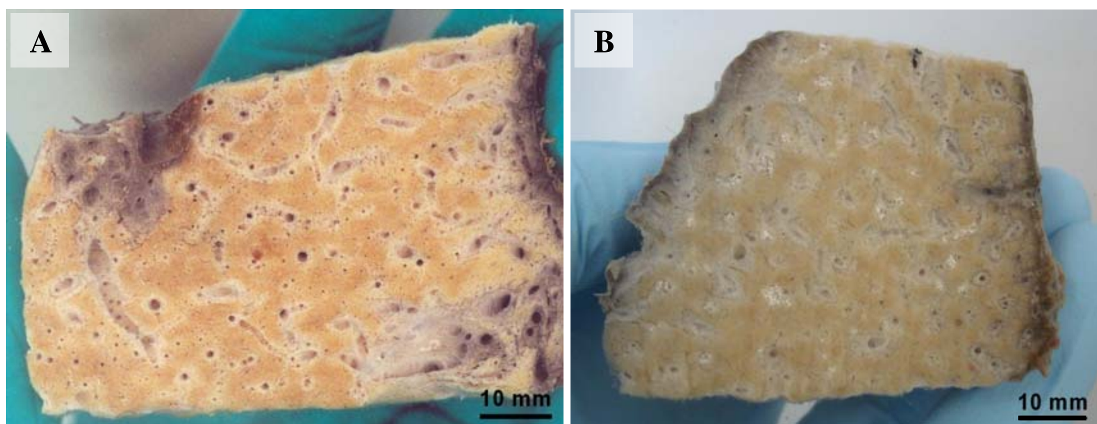


Fig. 2.9 The newly exposed sponge surface immediately after cutting for *R. odorabile* (**A**) and *Coscinoderma* sp. (**B**; representative photo taken outside of experimental period).

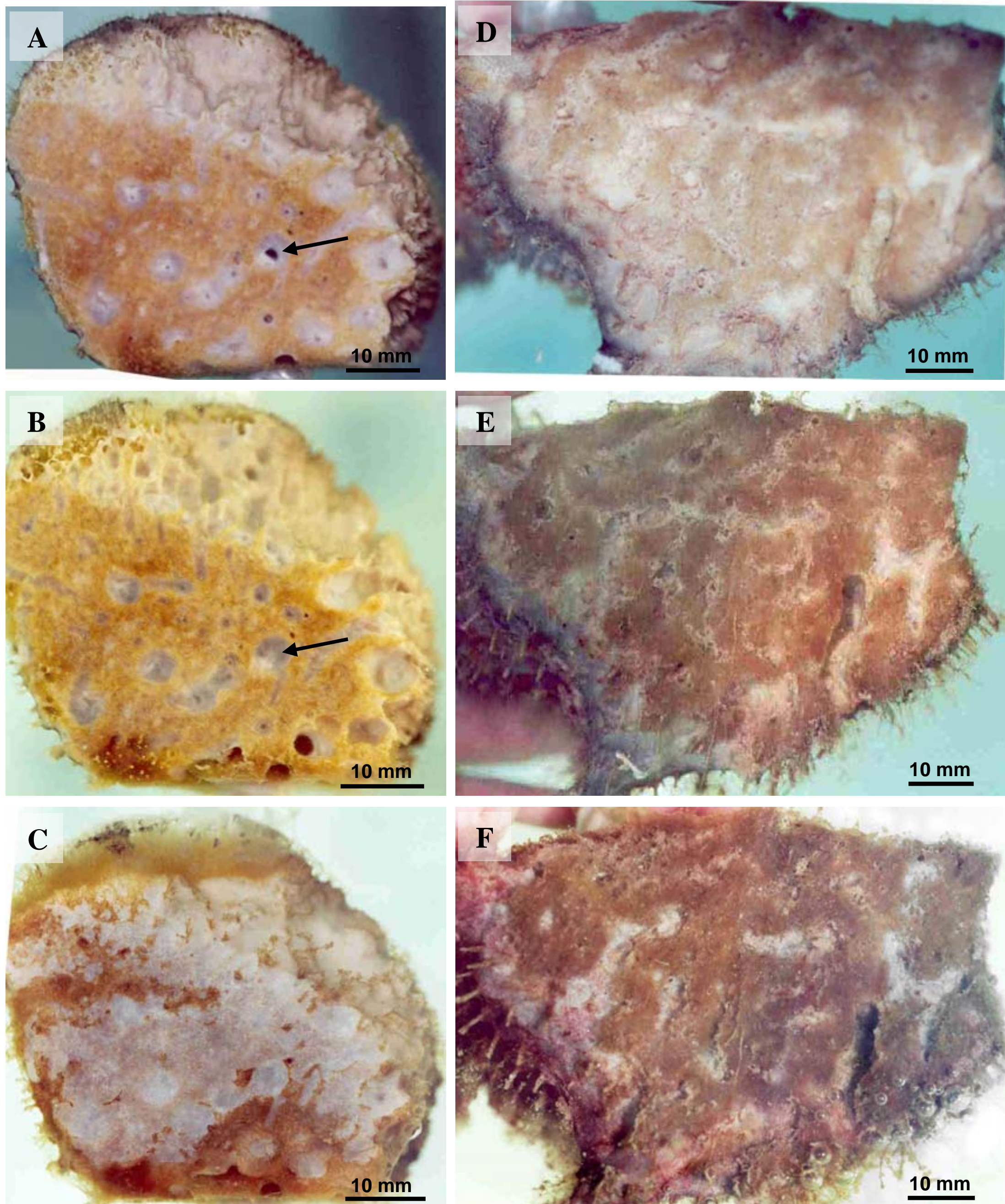


Fig. 2.10 Explants healing over a cut surface; **A** to **C**. *R. odorabile*; **A**. Day 1. All small canals have been covered by a protective layer, although a number of large exposed canals remain uncovered (*eg*. arrow); **B**. Day 2. Most large canals have been covered (*eg*. arrow); **C**. Day 7. Exposed spongin fibres have begun to fall away revealing the new protective layer beneath; **D** to **F**. *Coscinoderma* sp.; **D**. Day 1. Exposed canals have been covered; **E**. Day 7. The exposed fibres have begun to darken; **F**. Day 14. Most of the new surface remains obscured from view.

2.3.3.2 Microscopic Surface Examination

To further determine the external recovery process, samples taken over a period of five days were examined using SEM.

Rhopaloeides odorabile

The newly cut sponge surface revealed coarsely cut spongin fibres and underlying tissue (Fig. 2.11A). Twelve hours after being cut, a protective layer began to cover the exposed tissue (Fig. 2.11B). After only 24hrs, development of the protective layer was complete (Fig. 2.11C). In some areas this new layer developed around protruding fibres. The last time at which a cut fibre was observed protruding from the surface was 48hrs after exposure. New fibre endings seen protruding from the surface four days after exposure also protruded from a 23 day old surface (Fig. 2.11D). Ostia first became apparent after 24hrs. Samples taken at various times had areas of absent, scattered, and grouped ostia. A sample taken 23 days after exposure also had ostia with scattered and clustered distributions (Fig. 2.11D).

***Coscinoderma* sp.**

The *Coscinoderma* sp. surface also displayed the coarsely cut spongin fibres and underlying tissue immediately after cutting (Fig. 2.12A). By the first day, tissue had retracted onto the dense mesh of fibres and the surface remained in this condition for the remaining four days of observation. Surface recovery was first observed on a sample taken 36hrs after cutting, where a gap in the fibre covering allowed a view of the new surface below (Fig. 2.12B). No ostia were observed on samples taken over the five-day experiment duration.

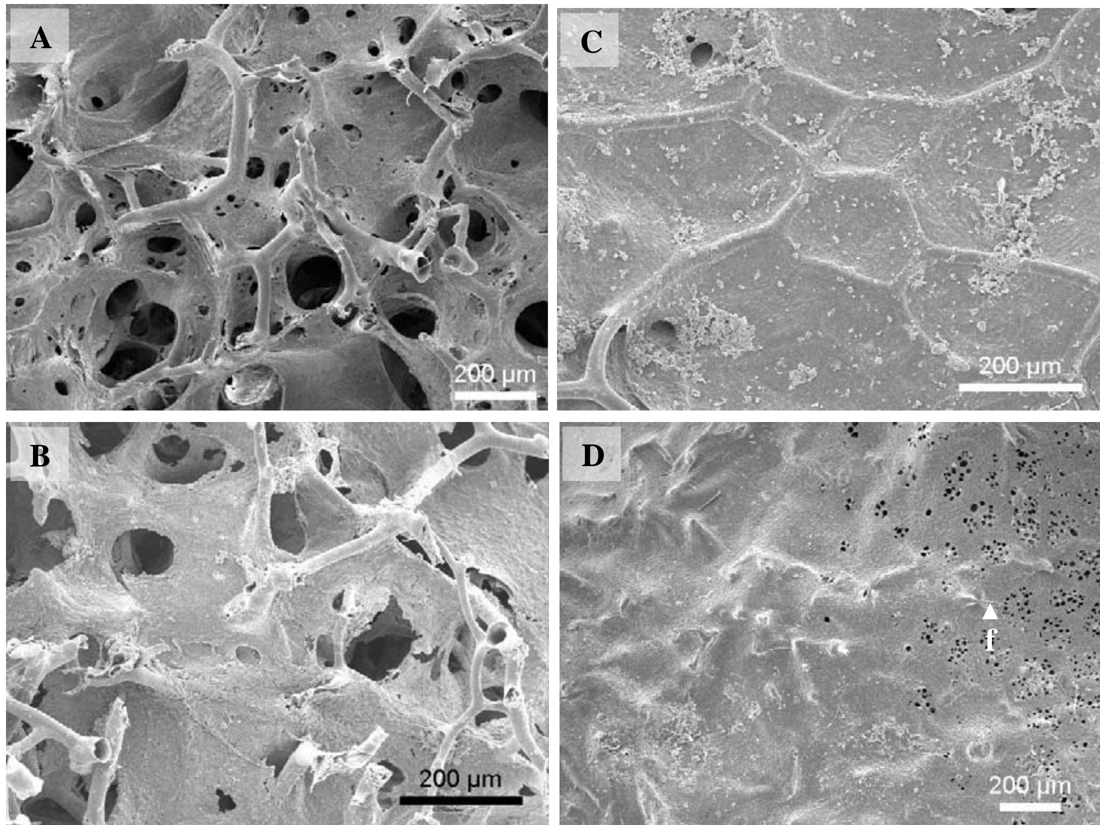


Fig. 2.11 *R. odorabile* surface; **A**. 0hrs after exposure. Spongin fibres and tissue are exposed; **B**. 12hrs after exposure. A new protective layer covers much of the underlying tissue; **C**. 24hrs after exposure. A protective layer completely covers the underlying tissue and most fibres; **D**. 23 days after exposure. Ostia are found with scattered and grouped distributions. A new fibre ending is seen protruding from the surface. (f. fibre ending)

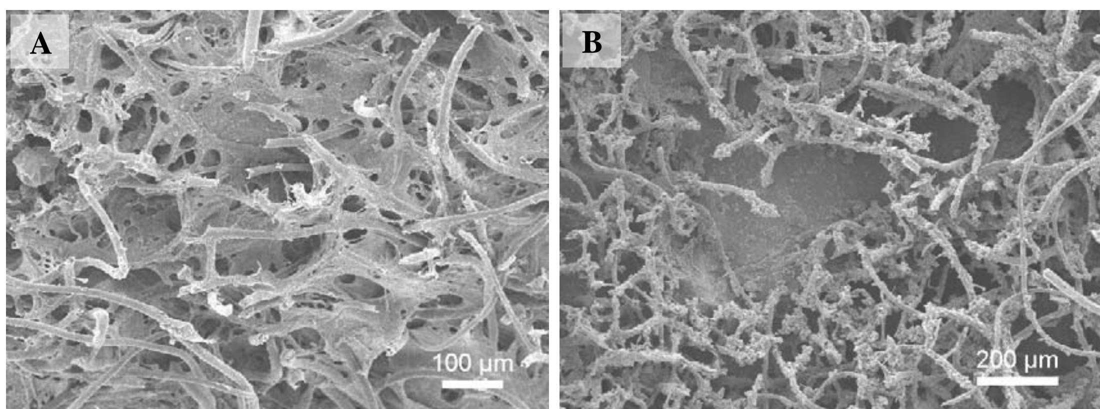


Fig. 2.12 *Coscinoderma* sp. surface; **A**. 0hrs after exposure. Tissue and fibres are exposed; **B**. 5 days after exposure. A gap in the exposed fibres allows view of the new protective layer below.

2.3.3.3 Cross-section Examination

To document the subsurface cellular reorganisation, cross-sections of healing surfaces were taken over a six-week period and compared to samples of undamaged surfaces using light microscopy.

Rhopaloeides odorabile

The undamaged surface and underlying tissue of *R. odorabile* was highly variable in its density and cellular composition (Fig. 2.13A-C). The most common cells present were pigment cells (evidenced by their melanic inclusions, seen in Fig. 2.13A as intracellular black spots). Although not always obvious, the surface was covered with a thin pinacoderm layer (Fig. 2.13B-C). Ostia were commonly found, although their distribution was variable (Fig. 2.13E). Some ostia were open, while others had a thin cellular covering. Foreign spicules were found randomly incorporated into the surface and underlying tissue, sometimes well below the surface regions (Fig. 2.13D). The skeletal support structure for the sponge surface consisted of clumps of fibres including primary and secondary fibres and foreign spicules (Fig. 2.13E). The secondary spongin network was almost completely restricted to the choanoderm (Fig. 2.13F).

Immediately after cutting, the surface was in disarray as a result of cutting (Fig. 2.14A). However, within the first 24hrs, sponges had developed a new external surface layer (Fig. 2.14B). This layer was formed below a layer of cut spongin fibres, which remained with the sponge for several days. The new subsurface region was highly variable in its cell composition and density, potentially attributable to the new function of the local area, *eg*. to develop mesohyl or the aquiferous system.

This variation remained evident throughout the study. Despite the variation, most areas consisted of high numbers of collencytes and archeocytes, while pigment and spherulous cells were absent (Fig. 2.14C). A protective collagen layer quickly formed on the surface and was subsequently shed (Fig. 2.14D). This process of collagen production and shedding continued to occur throughout the healing process (not shown). Development of new ostia became evident after three days (Fig. 2.14E).

By day 10, most areas of the sponge were well advanced in the cellular transition with most sections densely cellular (Fig. 2.14F) and development of ostia near complete (not shown). Many regions had collagen laced throughout the tissue (Fig. 2.14F) and the first pigment cells since repair began had become evident (not shown). Only small patches of the new surface remained in an early state of transition (Fig. 2.15A). After 41 days, most surface regions contained high numbers of pigment cells (Fig. 2.15B), much like most regions of an undamaged surface, and ostia development was complete (Fig. 2.15C). By this time, the old spongin matrix above the newly developed surface had been lost. Despite the high level of development of most surface regions, other sections remained in poor state of repair with a disorganized cell structure and the surface formed by a collagen layer (not shown).

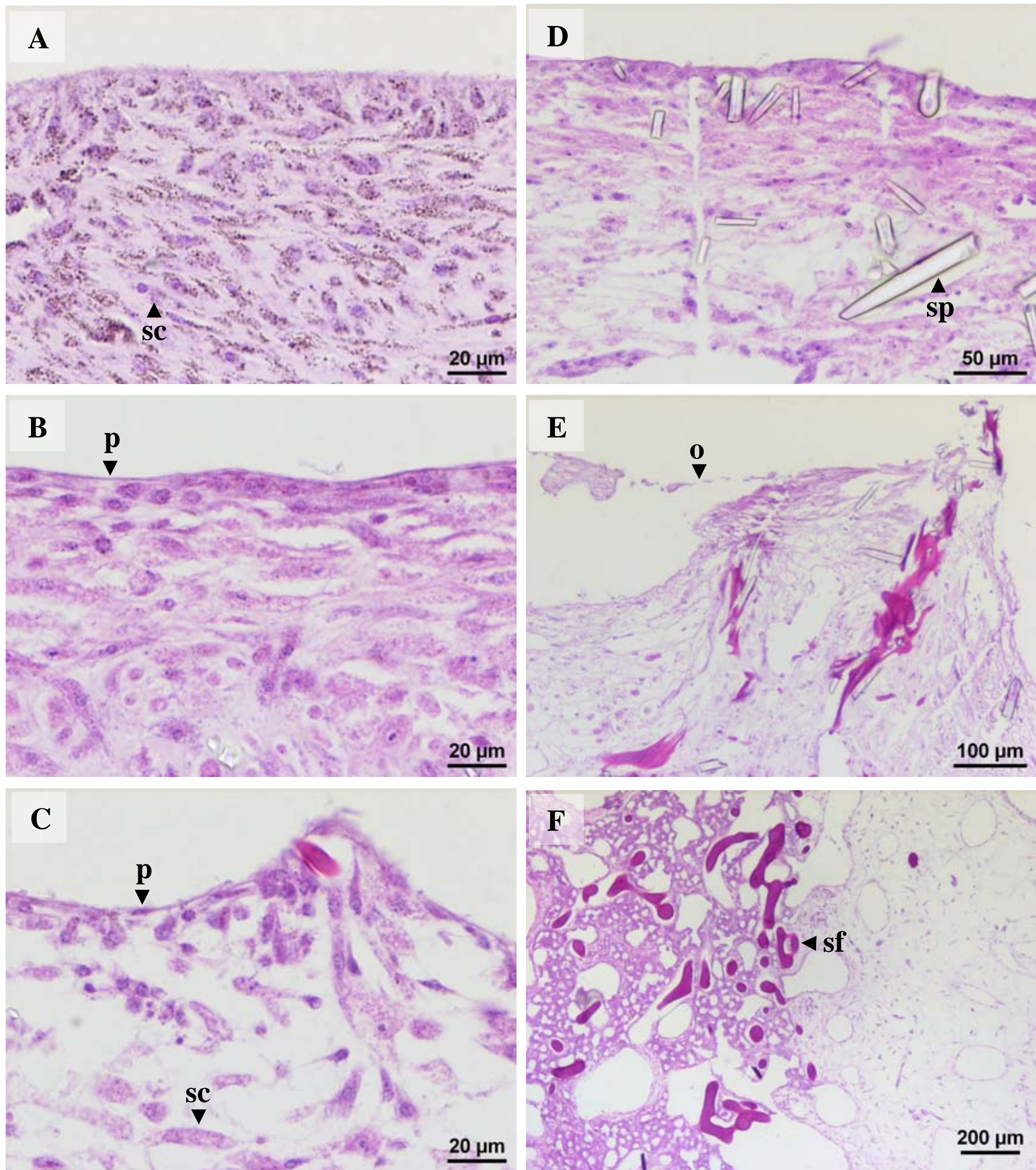


Fig. 2.13 *R. odorabile*; **A** to **C**. Natural variation of the subsurface cellular make-up; **D**. Spicules incorporated into the surface crust; **E**. Subsurface skeletal support structure; **F**. Spongin distribution. (sc. spherulous cell, p. pinacocyte, sp. spicule, o. ostia, sf. secondary fibre)

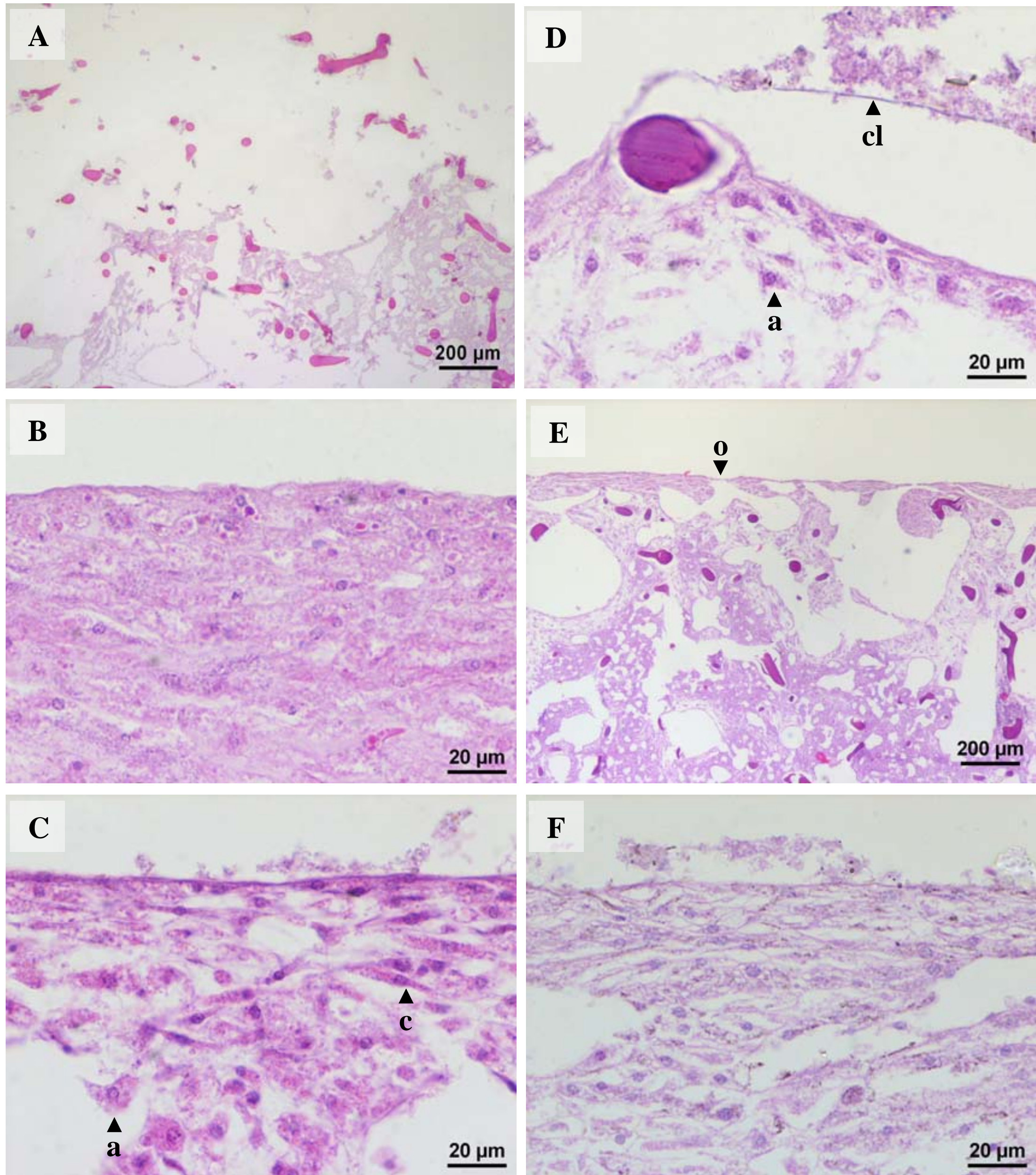


Fig. 2.14 *R. odorabile*; **A**. Day 0. No surface layer; **B**. Day 1. Newly formed surface; **C**. Day 2. Cell transformation is in progress; **D**. Day 2. Collagen coat lifting off the new surface; **E**. Day 3. Ostia development is obvious; **F**. Day 10. Cellular composition is approaching that of an undamaged sponge. (c. collencyte, a. archeocyte, cl. collagen layer, o. ostia)

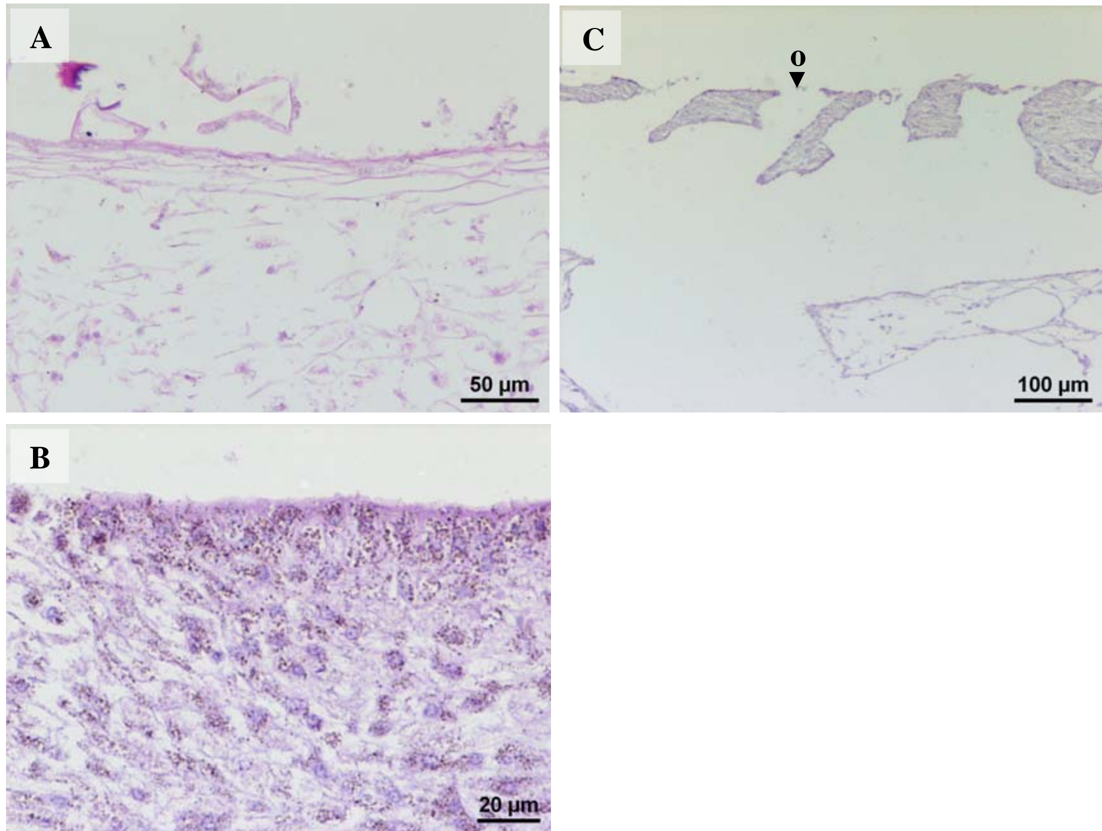


Fig. 2.15 *R. odorabile*; **A**. Day 10. Cells are largely absent here leaving only layers of collagen behind; **B**. Day 41. Pigment cells are again common and the surface resembles that of an undamaged surface; **C**. Day 41. Development of ostia is complete. (o. ostia)

***Coscinoderma* sp.**

In an undamaged sponge, the density and thickness of the subsurface mesohyl of *Coscinoderma* sp. was highly variable although it typically consisted of numerous pigment cells with archeocytes and collencytes sometimes found in the tissue below (Fig. 2.16A-C). The surface was lined with a thin layer of pinacocytes (Fig. 2.16B). Despite the organised state of most areas, the surface of some specimens was in a state of natural regression and rearrangement, evidenced by the presence of spongin fibres above the sponge surface (Fig. 2.16D). Ostia were commonly found although their distribution was highly variable (not shown). Spicules and other foreign matter were sometimes incorporated into the surface layers to form a more ridged protective surface (Fig. 2.16E). The principle skeletal support structure for the sponge surface consisted of clumps of primary and secondary fibres, spicules and sand (Fig. 2.16F). The choanoderm, found below the subsurface region, was normally heavily supported by secondary fibres, which were sparse or completely absent throughout the superficial regions of the tissue (Fig. 2.17A).

Immediately after cutting, the surface was in disarray as a result of cutting (Fig. 2.17B). Within the first 24hrs a defined surface layer had been developed. This layer was hidden below spongin fibres and cell debris (Fig. 2.17C), which would remain with the sponge for an unknown period of time (greater than 41 days) or until overgrown by new tissue growth. The subsurface region was densely cellular for the first day (Fig. 2.17D). Collencytes quickly constructed a surface collagen layer that was subsequently shed. Formation and shedding of this layer continued throughout the healing process (Fig. 2.17E).

From the second day, collencytes were numerous (Fig. 2.17F) and highly active constructing many layers of collagen through and over the new surface cell matrix. The subsurface mesohyl, typically became depleted of cells as they were evacuated or transformed (Fig. 2.17E-F). Archeocytes were common (Fig. 2.17F). Little changed from days 2 to 4 as the sponge constructed the new surface and performed the cellular rearrangement necessary throughout the rest of the sponge to reorganise canals and mesohyl. In some regions the surface remained depleted of cells for between 10 and 41 days, while other sections transformed quickly (within 3 days) into functional tissue (Fig. 2.18A-B). By day 10, the first pigment cells had developed (not shown), although in many areas there was little more than layers of collagen present (Fig. 2.18C).

After 41 days, the subsurface cells had become far more numerous and pigment cells were abundant (Fig. 2.18D-E), much like most regions of an undamaged surface. Most of the cell debris originally held within the old spongin fibres above the newly developed surface had been lost although the fibres now held layers of collagen shed by the developing surface over the previous weeks. At no time throughout the study were new ostia observed.

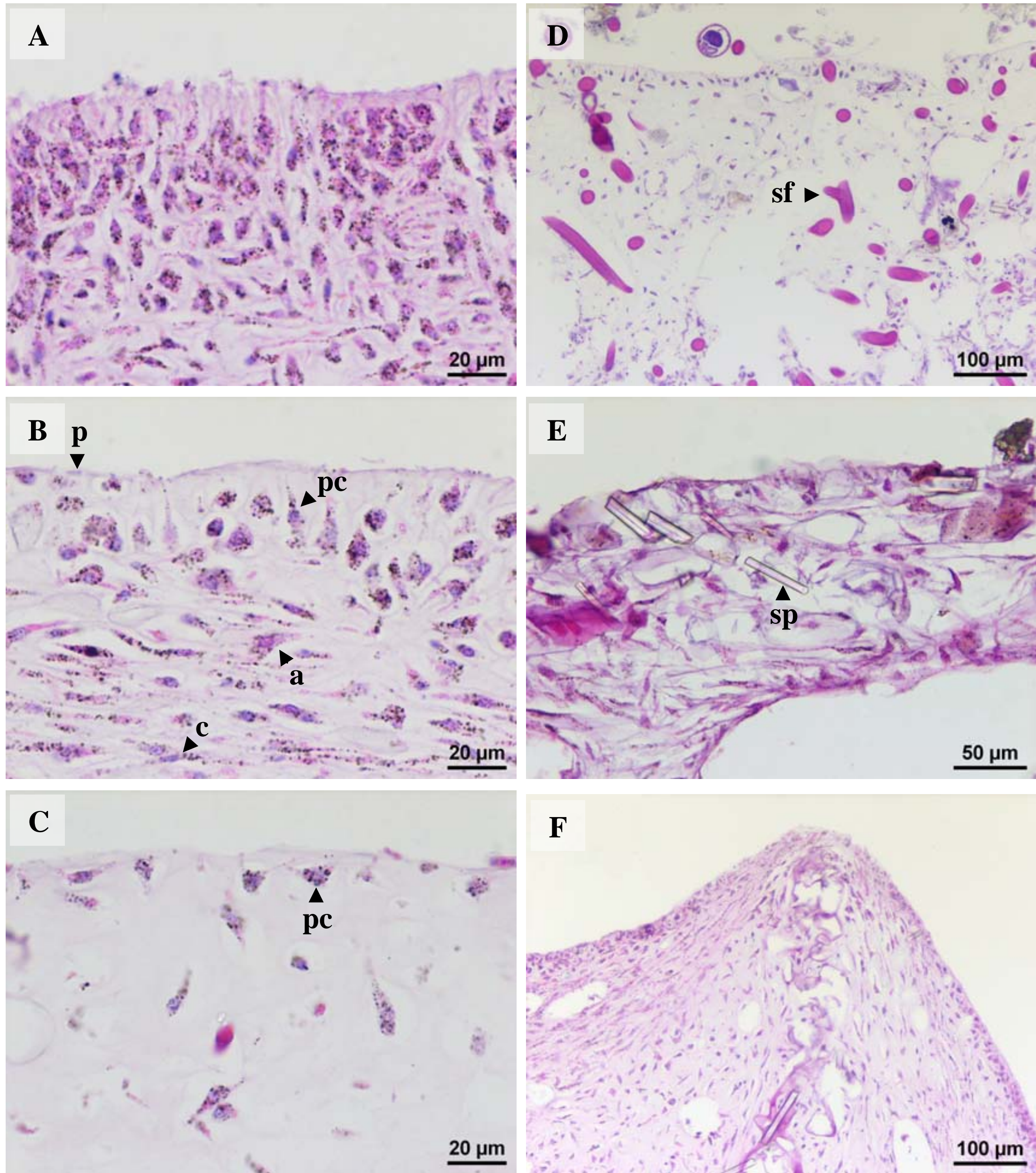


Fig. 2.16 *Coscinoderma* sp.; **A** to **C**. Natural variation of the subsurface cellular make-up; **D**. Natural regression of the sponge surface; **E**. Spicules incorporated into the surface crust; **F**. Subsurface skeletal support structure. (p. pinacocyte, pc. pigment cell, a. archeocyte, c. collencyte, sf. secondary fibre, sp. spicule)

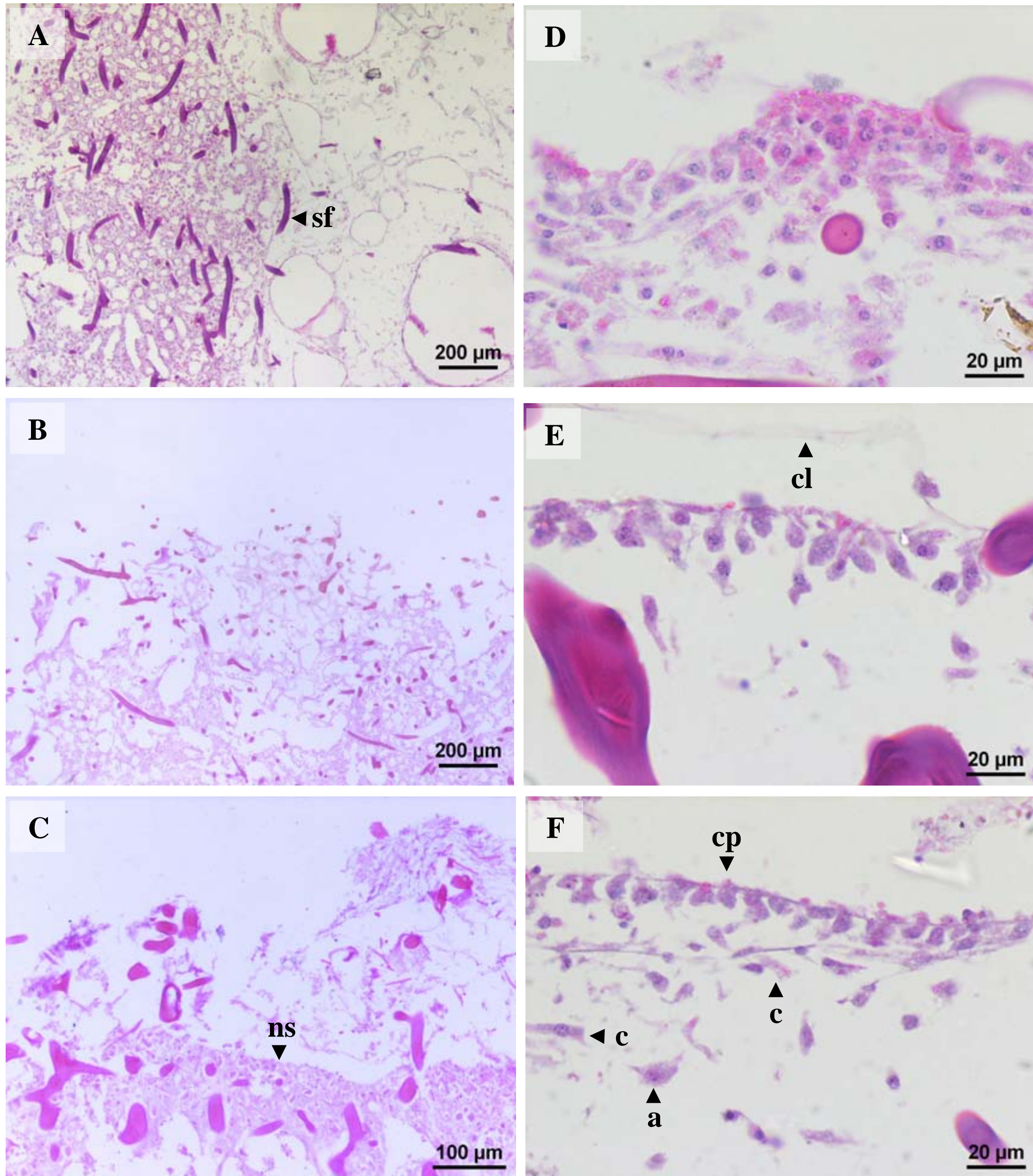


Fig. 2.17 *Coscinoderma* sp.; **A**. Distribution of spongin in an undamaged sponge; **B**. Day 0. No surface layer; **C**. Day 1. Cellular debris and spongin fibres above the newly formed surface; **D**. Day 1. Cells in a state of transformation and rearrangement below a clearly defined surface; **E**. Day 2. Lower cell density with a collagen layer being shed; **F**. Day 2. Collencytes are common and are used to form pinococytes at the surface. (sf. secondary fibre, ns. new surface, cl. collagen layer, cp. collencyte transforming into a pinacocyte, c. collencyte, a. archeocyte)

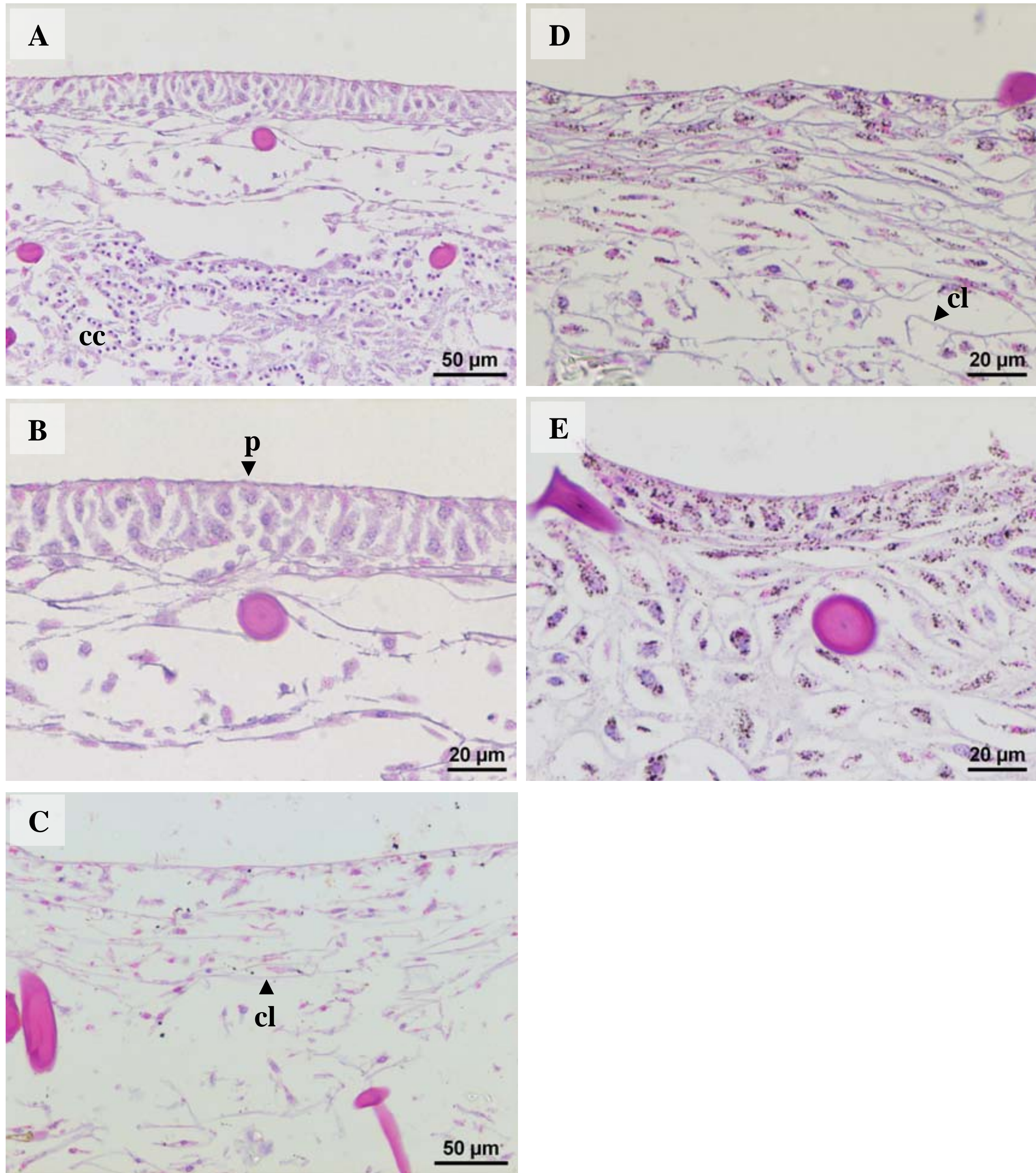


Fig. 2.18 *Coscinoderma* sp.; **A**. Day 3. Organised cell structure with choanoderm below; **B**. Magnified image of Fig. 2.18A. Hanging pinococytes are visible below the surface; **C**. Day 10. Subsurface region largely consisting of collagen layers; **D**. Day 41. Pigment cells are again numerous amongst the many collagen layers; **E**. Day 41. The collagen structure and cell density and organisation resembles that of an undamaged sponge. (cc. choanocyte chamber, p. pinacocyte, cl. collagen layer)

2.4 Discussion

Explant survival was heavily dependent on species, culture method and time, with the highest mortality occurring soon after cutting. Growth rates were not significantly different between the species with average growth rates of approximately 100% per annum. Both species demonstrated size dependent growth rates for the first season of growth with smaller explants growing fastest. Recovery rates were rapid for both species with a protective laminar plate of collagen forming over the surface within 24hrs. This layer was later replaced by pinacoderm as the subsurface tissue was reorganised to recreate a functional surface including redevelopment of the aquiferous system.

2.4.1 Explant Survival

Explant survival was related to the time after cutting, the species and whether cultured *in vitro* or *in situ*. The mortality rate decreased over time for both species. Survival of *R. odorabile* was highest in the raceways at 75% compared to 50% *in situ*, and *Coscinoderma* sp. highest in the *in situ* culture at 80% compared to only 30% *in vitro*. The greatest difference between the two culture environments was that the water flow of the *in situ* culture was about 1000 times greater than the flow in the *in vitro* culture. Secondly, light was qualitatively assessed to be greater in the *in vitro* culture. Sedimentation and temperature differences were not assessed.

The difference in water flow may have been a major factor in the difference in survival between the species. Although passive water flow through a new explant is unlikely since the structural characteristics required for such flow (Vogel, 1974) have been destroyed, some degree of water flow is necessary to supply oxygen and

remove waste products such as ammonia. The difference in survival was greatest for *Coscinoderma* sp. suggesting it was the most sensitive to changes in water flow. The greater survival of *R. odorabile* in the low flow environment seemingly contradicts its patterns of natural distribution as it is generally confined to exposed locations on the reef (Bannister *et al*, in prep). This may be explained by larval settlement or post-settlement survival (recruitment) rather than limitations of the adult sponge (see also Maldonado and Young, 1998).

Light may have several effects on sponge explants. Vegetative fouling on the sponge surface may be considered an indirect effect of increased light. The presence of fouling can restrict regeneration processes (Hoppe, 1988) and cause tissue death (Kaandorp and Kluijver, 1992). Fouling by epiphytic red and green algae occurred on both species in the *in vitro* culture, but not in the *in situ* culture. Unlike the fouling on *R. odorabile*, fouling on *Coscinoderma* sp. could not be easily removed by hand which may have negatively impacted this species. Secondly, the process of producing explants leaves much of the sponge unprotected by pinacoderm or underlying pigments and bacteria which can shade underlying tissue (Sarà, 1971). Therefore, light in the form of UV radiation may be damaging to explants, as it is known that cryptic sponges without UV protection are highly susceptible to UV damage (Jokiel, 1980).

Sedimentation was not measured in this study although this too may also have influenced explant survival between the cultures. Sedimentation reduces pumping rates and increases energy expenditure to remove the unwanted particles from the sponge surface or canals (Gerrodette and Flechsig, 1979). High levels of

sedimentation are often correlated with a decrease in water flow (*eg*. Bell *et al*, 2002a) suggesting *Coscinoderma* sp. may be well suited to dealing with high sediment loads considering its broad natural distribution (Bannister *et al*, in prep). Furthermore, it is often found with its surface partially covered by fine sediment (pers. obs.). Despite this, the optimal conditions required for explant recovery have received minimal attention and may be different to the environmental conditions of the sponge's natural habitat.

The effect of season on the survival of *R. odorabile* and *Coscinoderma* sp. was not determined in this study. However, it may be important in improving survival rates as seasonal effects on explant survival occur for some species. Explants of *Psammocinia hawere* (Duckworth *et al*, 1997) and *Latrunculia wellingtonensis* (Duckworth *et al*, 2004) demonstrate higher survival if prepared during winter rather than in summer. Similarly, *Polymastia croceus* has higher survival if transplanted in spring rather than summer (Duckworth *et al*, 2004). Further research is required to determine the seasonal survival of *R. odorabile* and *Coscinoderma* sp. The difference in temperature between the *in situ* and *in vitro* cultures is likely to have been negligible, as the residence time of water in the raceways was normally little more than an hour.

The minimum survival considered necessary for an economically viable sponge culture is 90% per annum (Verdenal and Vacelet, 1990). Although the survival of both species in this study (*in situ* culture) fell short of this value, most mortalities occurred in the first 9 months, giving survival rates of 100 and 94% (one mortality) over the last 12 months for *R. odorabile* and *Coscinoderma* sp. respectively. Given

that the method used in the present study was unlikely to be optimal due to the high level of stress imposed upon the sponge explants during setup and a lack of knowledge regarding the optimal environmental conditions for the survival of these species, both species show commercial potential in relation to explant survival. *R. odorabile* in particular requires further research to improve initial survival rates. Given the higher survival of this species in the raceways, nursery culture of *R. odorabile* also warrants further investigation.

2.4.2 Explant Growth

R. odorabile and *Coscinoderma* sp. both demonstrated an initial period (11 weeks) of size dependent growth. In *R. odorabile*, this was followed by a continued period of relatively stable growth (0.25%/day), while in *Coscinoderma* sp., the growth spiked before settling into a steady growth rate of 0.18%/day. *Coscinoderma* sp. displayed the greatest total growth, although it was not significantly different to that of *R. odorabile*. Growth of sponges has been shown to vary in response to explant size (van Treeck *et al*, 2003), extent of damage (Schmahl, 1999; Duckworth, 2003), season (Turon *et al*, 1998) and species (Duckworth, 2003). Growth can also vary within a species exposed to the same environmental conditions (Hoppe, 1988; Turon *et al*, 1998). It is possible all of these factors influenced sponge growth observed in the present study, however it was beyond the aim of the study to differentiate between these. Determining optimal environmental parameters for explant growth is clearly the next stage of development to follow from this study.

Size dependent growth rates were only found in the initial recovery stage for each species although the effect was evident over the entirety of the study. This is not the

first time smaller sponges have been found to exhibit faster growth rates (Dayton, 1979; Hoppe, 1988) although this has not been a consistent finding (Turon *et al*, 1998; Hadas *et al*, 2005). An optimum initial size for *Ircinia variabilis* explants of 9.95 to 16.32g was suggested by van Treeck *et al* (2003) after finding a tendency for high growth rates for explants of this size range. Few studies appear to have tested for this phenomenon, and of these, most relate to the growth of undamaged sponges (Dayton, 1979; Hoppe, 1988; Turon *et al*, 1998).

In a study investigating the growth rate of massive sponges after damage, Duckworth (2003) found *Latrunculia wellingtonensis* exhibited a greater growth rate directly following 50, 75, and 90% tissue removal than it did in undamaged specimens. When tissue was removed from *Polymastia croceus* it demonstrated a similar growth rate to that of control specimens. In contrast, Schmahl (1999) found growth rate to be inversely related to the degree of damage inflicted upon the giant barrel sponge, *Xestospongia muta*. The removal of large portions of tissue requires sponges to undergo significant internal reorganisation. By their very nature, explants also have a high level of internal reorganisation that must occur before the sponge may function effectively. Studies describing explant culture demonstrate that a lack of growth and even regression in new explants is not uncommon (*eg*. Duckworth *et al*, 1997, 2004), however few studies have shown the long term impacts of recovery on growth. This study demonstrated that seasonal growth of *R. odorabile* and *Coscinoderma* sp. is relatively steady. Furthermore, size dependent growth was only observed in explants in the period immediately following propagation. Therefore, the size dependent growth was most likely related to the recovery process. Further

research is required to determine how recovery rates and processes differ according to explant size which may in turn help explain this size dependent growth.

After growth rates stabilised, season appears to have little effect on the growth of either species, suggesting the consistently warm water of the tropics may not fluctuate sufficiently to cause seasonal changes in growth rate. However, seasonal variation in temperature has a significant impact upon the growth rates of *Crambe crambe* (Turon *et al*, 1998), *Haliclona permollis* (Elvin, 1976), and *Xestospongia muta* (Schmahl, 1999) with higher growth occurring during late spring, summer, and autumn when the water is warmest. Some sponges even exhibit no growth or regression during the cooler parts of the year (*eg*. Elvin, 1976; Turon *et al*, 1998; Duckworth and Battershill, 2003b). In the Palm Islands, ultraplankton abundance is significantly greater during the summer (Bannister, 2003), although further research is required to establish if sponges obtain nutritional benefit during this time or whether food availability may even exceed sponge requirements throughout the year.

In this study, high variability in growth was observed between the two species and between individuals of the same species, including explants from the same parent. Variation within a single species has also been reported by a number of authors for a variety of sponge species (*eg*. Verdenal and Vacelet, 1990; Turon *et al*, 1998; Osinga *et al*, 1999). Reasons for the phenomenon are poorly understood with several factors likely to be responsible. The cell composition of an explant is at least partially dependent on the region of the parent sponge from which the explant was taken and has been suggested to be a significant factor in the healing and growth of explants (Kelly-Borges, 1994). Genetics are also likely to play an important role in variation

observed between individuals with uncommon parentage (Kelly-Borges, 1996), although no research has been published that investigates these possibilities. The present study demonstrated that even if genetics are a significant factor in explant growth, they were not the cause of the greatest variation observed in the current experiment.

Overall, *Coscinoderma* sp. demonstrated its potential as a commercial species with an annual growth rate of 115.2%. The minimum growth rate of a sponge with commercial potential is considered to be 100% per annum (Verdenal and Vacelet, 1990). *R. odorabile* had an annual growth rate of 85.9% although further research is likely to improve this given a better understanding of its specific culture requirements and improved culture techniques.

2.4.3 Explant Recovery

The surface of *R. odorabile* and *Coscinoderma* sp. healed rapidly over a period of 24hrs. For *R. odorabile*, a protective layer covered large exposed canals of most explants within seven days, while most large canals in *Coscinoderma* sp. explants were covered in only two days. Surface colour of *R. odorabile* and *Coscinoderma* sp. darkened over a period of two and five weeks respectively to match that of an undamaged surface.

The recovery rates of *R. odorabile* and *Coscinoderma* sp. in this study are comparable to those found by others. Within 24hrs of cutting, a new surface layer becomes evident on the surface of *Hippospongia communis* and *Spongia officinalis* (Pronzato *et al*, 1999). Colour was restored to the new surface within a week of

exposure and over a period of a month reorganization of the aquiferous system had occurred. In *Ircinia strobilina*, the generation of a new protective layer took less than 24hrs, with colour returning to normal after three days (Storr, 1976). Hoppe (1988) also found regeneration of a surface layer later followed by a restoration of the original colour for the three species, *I. strobilina*, *Neofibularia nolitangere*, and *Agelas clathrodes*. In contrast, Hoppe (1988) reported much longer average recovery periods (1, 2 and 3-4 weeks respectively) with recovery varying according to species and wound size and location. The return of normal colour took 4, 3 and 9-10 weeks respectively.

More recent studies have made use of microscopic (SEM, TEM, or light microscopy) examination to describe the finer cellular details of the recovery process (*eg*. Pronzato *et al*, 1999; de Caralt *et al*, 2003; Hoffmann *et al*, 2003). In this study, SEM and histological preparations confirmed the development of a defined surface within the first 24hrs. Previous studies on sponge surface recovery have reported the development of a "skin" (Storr, 1976), "thin cell layer" (Hoffmann *et al*, 2003), thin "tissue" (Ayling, 1983) or "scar tissue" (Hoppe, 1988) as the exposed tissue and aquiferous system was covered over by a protective layer. According to Pronzato *et al* (1999), the recovery process of bath sponges (Order Dictyoceratida) involves the development of a fibrous collagen layer on the surface as opposed to the other sponge orders that redevelop the exopinacoderm. This study confirmed the development of a protective collagen coat, although not fibrous, and this was later replaced by pinacoderm. The time taken to replace the collagen layer with pinacoderm was highly variable, being dictated by the speed of recovery in the local area which took days or weeks for some explants.

A minor difference in the healing process of *R. odorabile* noted between the macroscopic and cross-sectional examination, as opposed to SEM, was that in the SEM study the outermost fibres were covered during healing, while in the other studies, they remained exposed after healing to later to fall away. This apparent discrepancy in the healing process may also be found by comparing work by Storr (1976) and Hoppe (1988) who both studied the healing surface of *I. strobilina*. Storr (1976) found that the fibres were covered, while Hoppe (1988) found that the exposed fibres and surrounding tissue were shed leaving behind the new surface layer. It is likely this difference was due to the quality of the cut through the tissue, with a damaging cut resulting in minor tissue loss.

Ostia were first observed after only 24hrs in *R. odorabile* but not at all in *Coscinoderma* sp. over the six-week sampling period. The early appearance of ostia in *R. odorabile* indicates that the sponge is able to utilise the ambient water from an early stage in recovery. Flow measurements are required to ascertain the degree to which explants are able to pump water for food, respiration or waste removal. Much of the energy required for recovery is suspected to come from energy reserves in the sponge (Simpson, 1984).

2.5 Conclusion

Understanding the dynamics of explant recovery, survival, and growth according to species, culture methodology, and environmental conditions is a necessary step in developing an effective and commercially viable sponge culture. This study provided a detailed examination and documentation of the healing process for *R. odorabile* and *Coscinoderma* sp. at a macroscopic and microscopic level. Initial

survival rates were poor, however, survival throughout the latter half of the experiment were well above the requirements for commercial culture. Both species exhibited size dependent growth rates following propagation and demonstrated consistent average growth rates throughout the year following the fluctuations due to the recovery period. Consequently, *R. odorabile* and *Coscinoderma* sp. are suitable candidates for commercial aquaculture. Further research should focus on the environmental requirements for optimal survival and growth, optimum explant size to maximise initial growth and explant yield, reasons for variation in growth rates between explants of common and uncommon parentage (*ie*. non-genetic and genetic factors), and time efficient culture techniques specific to these species.

Chapter 3: Development of a New Protocol for Testing Bath Sponge Quality

3.1 Introduction

Bath sponges have been used for more than 3000 years (de Laubenfels and Storr, 1958) for bathing, cleaning equipment, painting, medical uses, padding for battle armour, and as a vessel for drinking water (Cresswell, 1922). Today they still have many applications despite the introduction of synthetic products. In countries such as Australia, where sponge aquaculture is under research and development, new dictyoceratid sponge species are under the spotlight as potential commercial species. At present, industry and scientists alike rely on the experienced hand of sponge traders to determine the quality and value of bath sponges. This completely subjective method prevents the comparative assessment of species or individuals using objective, quantitative indices. To date, information regarding what constitutes a high quality sponge or the various quality characteristics needed for specific applications is scarce forcing reliance upon these traditional means of assessment. Traditional quality assessments can be performed only by experienced sponge traders who squeeze the sponge by hand to feel the elasticity and softness of the sponge and then decide how this compares with other commercial species.

Development of quantitative quality testing protocols would enable direct comparisons to be made between new aquaculture candidates and known commercial sponges. Not only does sponge quality vary between species, but it may also vary within a single species according to its original habitat. Quality determining characteristics that change according to environmental conditions include shape (Maldonado and Young, 1998) and density (Palumbi, 1986). An increase in density

means an increase in load bearing material. Therefore, changes in the mechanical properties of the processed sponge may also be expected. Although there is an abundance of literature describing the morphological plasticity of sponges according to their environment (reviewed in Chapter 1), these have not been quantified in regard to the effects of these changes on sponge quality. Understanding the environment necessary for producing sponges of optimal quality will aid in aquaculture site selection and the success of the industry.

In addition to natural variation, post-harvest treatment protocols applied to the sponge also influence quality. After the harvested sponge has been flushed of all cellular material leaving behind the collagenous skeleton, sponges are either sold as they are, or chemically treated to soften and bleach the sponge. Unfortunately, chemical treatment also reduces the sponge's durability (Dobson, 2003). With the development of sponge quality testing protocols, chemical treatments may be optimised for each species and tailored for specific applications.

Rhopaloeides odorabile and *Coscinoderma* n. sp. (hereafter referred to as *Coscinoderma* sp.) are two bath sponges with promising potential for commercial production in Australia (see Chapter 2). The specific aim of this chapter was to develop quantitative measures for sponge quality resulting in the measurement of the density, fibre width, fibre length, firmness, compression modulus, compressive strength, tensile strength, elastic limit, elastic strain, modulus of elasticity, modulus of resilience, absorbency and water retention efficiency of *R. odorabile* and *Coscinoderma* sp. and three commercial bath sponges, *Hippospongia lachne* (wool sponge), *Spongia graminae* (grass sponge), and *Spongia zimocca* (silk sponge).

3.2 Materials and Methods

3.2.1 Sponge Collection and Preparation

R. odorabile was collected from several reefs throughout the central Great Barrier Reef in May 2004 and October 2005. *Coscinoderma* sp. was collected from western Orpheus Island, North Queensland (18°37'S, 146°29'E), in May 2004 and October 2005. The commercial sponges (wool, grass, and silk sponges) were sourced from Mediterranean Natural Sponges, Sydney, Australia (grass sponge) and Aegean Sponge Co., Cleveland, USA (silk and wool sponges; species names were provided by the supplier).

Purchased sponges had been chemically treated prior to shipment and arrived in a state ready for testing. The only modification made to the sponges was to their shape, and this is described specifically for each test below. *R. odorabile* and *Coscinoderma* sp. were collected and allowed to decay in seawater for approximately one week to clear non-skeletal tissue. Specimens were then thoroughly rinsed in fresh water, left to dry and cut to size as described for each test. *R. odorabile* and *Coscinoderma* sp. were not chemically treated, although this could be considered for specific future applications.

3.2.2 Physical Properties

The direct physical properties of density, fibre width, and fibre length, were measured to determine their use as predictors of quality, including characteristics such as firmness, strength, elasticity, and absorbency (see Section 3.2.3 Quality Tests).

3.2.2.1 Density

Density was measured for all five species. Sponges of each species were cut into cubes and the density determined as the dry weight of each cube divided by the volume of the entire cube, including open and closed air spaces or pores within the cube. This measure of density is referred to as bulk density according to the Australian standard for physical test methods for refractories and refractory materials (AS1774.5 2001). Alternative measures are apparent solid density and true density. In these measures, the calculation of volume includes only closed pores for apparent solid density and no pores for true density. Measuring bulk density is standard practise in determining the density of foam when measuring properties that affect polyurethane foam performance (In Touch, 1994a). Cubes ranged in size between 30 and 50mm according to species. Cube sizes were approximately 30, 40 and 50mm for the silk, grass, and wool sponges respectively and 40mm for *R. odorabile* and *Coscinoderma* sp. Each species was replicated 18 times except the grass sponge with 16 replicates.

3.2.2.2 Fibre Dimensions

Fibre dimensions were measured for each sponge by cutting to expose a random area below the sponge surface. For each sponge, a photo of this surface was taken using a Leica DMLB light microscope in conjunction with Leica IM50 software. The area captured by each photo was large enough to show a representative sample of fibre dimensions for each individual. As for density, each species was replicated 18 times except the grass sponge with 16 replicates. The **fibre width** (µm) and **fibre length** (µm) of each sample was measured by taking the average of ten to fifteen randomly selected secondary fibres within each photo (Fig. 3.1). Fibre length was defined as

the internodal distance, *ie*. the length of a fibre between branches. Fibre length could not be determined for *Coscinoderma* sp. and the wool sponge because some or all the fibres of these species were too long to examine using this method (greater than 1mm). Long fibres could not be measured using lower magnification due to the three-dimensional arrangement of long fibres preventing full view of any one fibre.

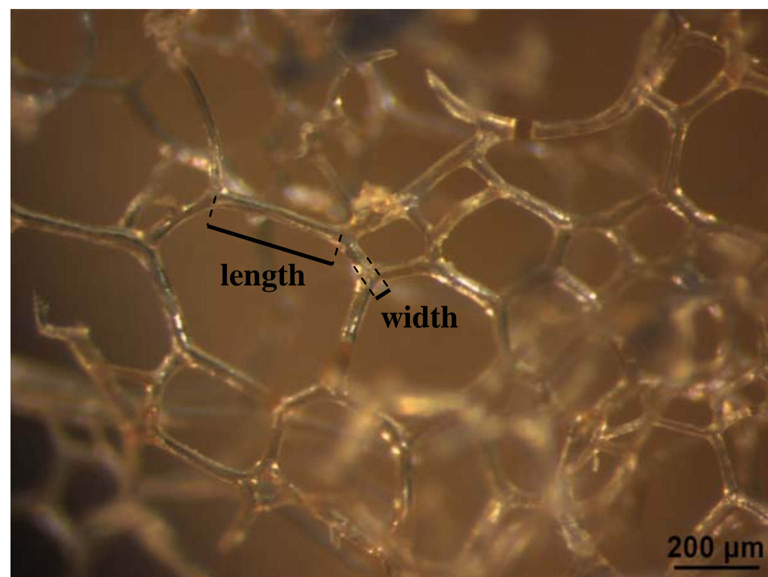


Fig. 3.1 Photo of internal secondary fibres (*R. odorabile*) showing the method for measuring fibre width and fibre length.

3.2.3 Quality Tests

3.2.3.1 Compression Tests

The same cubes used to measure density were compressed using an Instron 4302 testing machine at a rate of 3mm/min. A stress-strain curve was plotted for each sample (Fig. 3.2). Stress, measured in Pascals (Pa), equals force divided by cross-sectional area. Strain (mm/mm) is a measure of the sample displacement divided by the sample length (de Garmo *et al*, 2003). The difference in cube sizes between species was methodologically sound because the compression properties of any material remain the same regardless of size or shape (Hibbeler, 2003). As for

density, each species was replicated 18 times except the grass sponge with 16 replicates. **Firmness** was recorded as the stress required to compress the sponge by 25% (In Touch, 1994b; Fig. 3.2). The **compression modulus** is a measure of the sponge's ability to cushion or support a weight and was quantified as the stress required to compress the sponge by 65% divided by its firmness (In Touch, 1993; Fig. 3.2). **Compressive strength** is the measure of the point at which the sponge is no longer easily compressed and was calculated as the stress required to cause an increase in strain of only 0.35% per kPa.

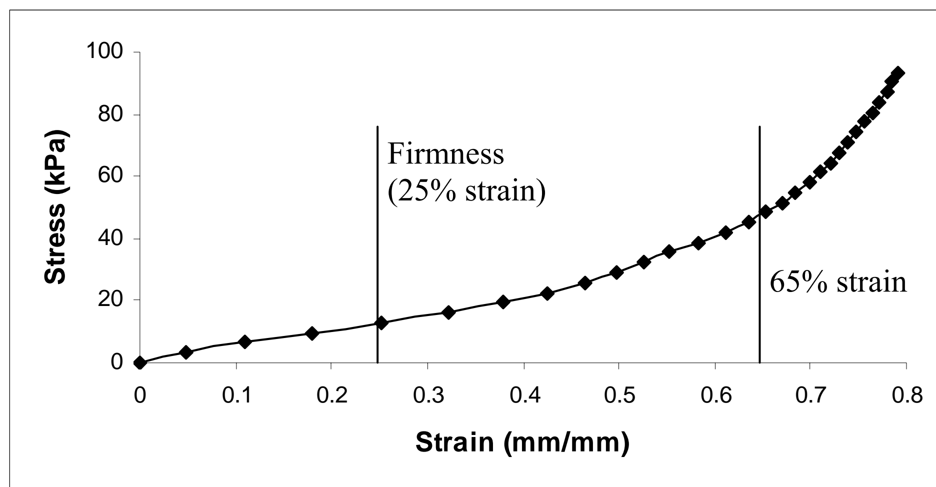


Fig. 3.2 Stress-strain curve produced from a compression test (for a silk sponge) showing the calculation for firmness and the compression modulus (65% strain / 25% strain).

3.2.3.2 Tensile Tests

To measure tensile properties, sponges were cut into square-edged dog-bone shaped pieces with a uniform cross-sectional area for a given length (de Garmo *et al*, 2003). Most test pieces had a cross-sectional area of approximately 20mm^2 for a length of 70mm, however, test pieces varied in size according to the available material (sponge size) and sponge density, with low density sponges requiring a larger sample to ensure the larger air spaces did not interfere with obtaining a representative sample.

As for the compression tests, the difference in test piece sizes between species was methodologically sound because the tensile properties of any material remain the same regardless of size or shape (Hibbeler, 2003). The number of replicates for each species were *R. odorabile* (n = 18), *Coscinoderma* sp. (n = 18), the wool sponge (n = 18), the grass sponge (n = 13), and the silk sponge (n = 16). The thicker ends of each test piece were embedded in epoxy resin to allow a solid grip for testing. Testing was performed using an Instron 1342 testing machine and Instron Fast Track Console 3.3 and Fast Track 2 Wavemaker Editor software. Samples were stretched to breaking point at a rate of 10mm/min. Data were used to create a stress-strain curve for each sample from which strength and elasticity were measured (Fig. 3.3).

Tensile strength was determined as the highest level of stress applied to the sample before it broke (Askeland and Phulé, 2006; Fig. 3.3). The **elastic limit** (σ) measures the maximum stress able to be applied before permanent damage occurs and was calculated as the stress at which the stress-strain curve ceased to follow a linear relationship (Hibbeler, 2003; Fig. 3.3). **Elastic strain** (ε) is the maximum strain (or stretch) able to be applied to the sponge before permanent damage occurs and was calculated as the strain corresponding with the elastic limit (Hibbeler, 2003; Fig. 3.3). For example, a specimen with an elastic strain of 0.4 (or 40%) would be able to stretch to 40% more than its original length before permanent damage occurred.

Elasticity is the quality of any material that enables it to return to its original form following deformation. This encompasses the degree to which the material resists deformation when a force is applied (rigidity), and the amount the material is able to stretch without causing permanent damage and thus the potential energy gained by

the material when stretched to this point. Therefore, two measures must be provided to quantify the elasticity of a material. Firstly, the **modulus of elasticity** (E) is a measure of the rigidity or stiffness of a material and was calculated as the slope of the linear section of each plot (Askeland and Phulé, 2006; Fig. 3.3). Secondly, the **modulus of resilience** (E_r) is a measure of the energy absorbed by a material when stretched to its elastic limit (and thus the energy released by the material when returning from its elastic limit to its original form) (Askeland and Phulé, 2006; Fig. 3.3). The modulus of resilience is calculated as the area under the elastic region of the stress-strain curve and is thus calculated as:

$$E_r = \frac{\sigma \times \varepsilon}{2} = \frac{\sigma^2}{2E} \qquad \text{(Hibbeler, 2003)}$$

If the initial part of the stress-strain curve is flattened (*ie*. non-linear), the first equation for the modulus of resilience will overestimate the true area, while the second will underestimate it, although only marginally. Since this flattening of the curve was true for most of the sponges tested (*eg*. Fig 3.3), the second formula was used.

Some sponges used for the tensile tests were not sufficiently large to obtain cubes for measuring density (as previously described in Section 3.2.2.1 Density) from the same individuals. For these small individuals, density, fibre width, and fibre length were determined using tensile test specimens prior to cutting the samples to shape and setting them in resin. The measures of density, fibre width, and fibre length using these tensile specimens were used only for correlating the different quality variables with physical properties as is described in Section 3.2.10 Statistical Analysis.

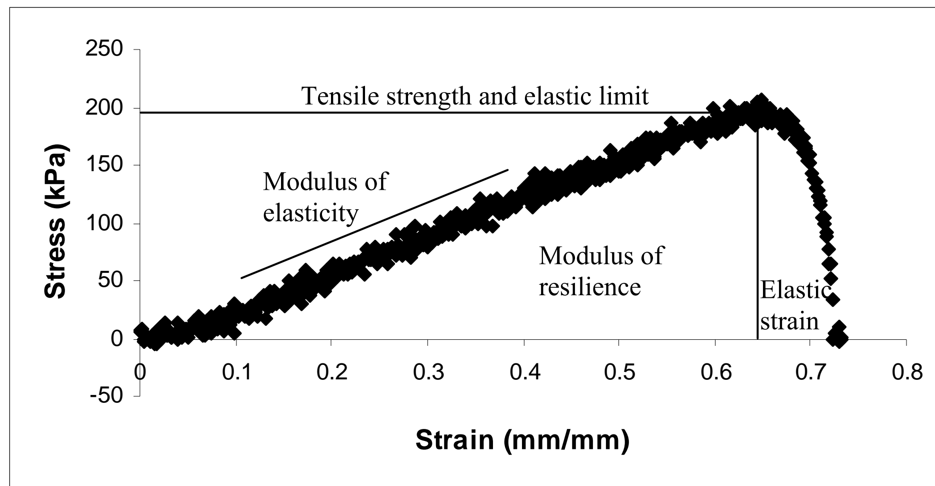


Fig. 3.3 Stress-strain curve produced from the tensile test (for a *Coscinoderma* sp. individual) showing the calculation for the tensile strength, elastic limit, elastic strain, modulus of elasticity, and modulus of resilience. In this case, tensile strength equals the elastic limit.

3.2.3.3 Water Absorption Tests

To measure water absorption, the same cubes used to measure density and compressive properties were used as these tests were non-destructive. As for density, each species was replicated 18 times except the grass sponge with 16 replicates. A dry cube from each individual was weighed (Wt_D) before being submersed in water and manipulated to remove trapped air bubbles. Once saturated, the sponge was lifted from the water and drained for 30 seconds in a horizontal position and reweighed (Wt_S). This is the Australian standard method for measuring the water absorption of surgical materials (AS2836.6 1998). The water laden sponge was then centrifuged using an Eppendorf Centrifuge (5810) at 10rcf (relative centrifugal force) for 1min and reweighed (Wt_C). **Absorbency** and **water retention efficiency** (WRE) were determined as follows:

$$\text{Absorbency} = \frac{Wt_S - Wt_D}{Wt_D}$$

$$\text{WRE} = \frac{Wt_C - Wt_D}{Wt_S - Wt_D}$$

Correlations made between test piece size and absorbency showed no relationship within the tested size range for *Coscinoderma* sp. which was the only species to have large variation in test piece size (Pearson's correlation coefficient: $r = -0.091$, $n = 17$, $p = 0.729$). Therefore differences in average test piece size for different species were considered to be within the limits of this test.

3.2.10 Statistical Analysis

Analysis of variance

Analyses were performed for each variable using one-way analysis of variance (ANOVA) and Tukey's post-hoc tests. Homogeneity of variance was tested using a plot of residuals against standardised means (Quinn and Keough, 2002). Data were transformed to meet the assumptions of an ANOVA according to the formulas presented in Table 3.1. Statistical analyses were performed using SPSS 12.0 for Windows.

Correlations

To determine relationships between various measures of sponge quality each species was analysed individually. Bivariate correlations were conducted using Spearman's rank correlation coefficient between all quality variables (where possible) to determine the existence of common relationships that would allow simplification of techniques. Only relationships between variables that were common among four or

five species are presented. Relationships common to less than four species are not as likely to be representative of general relationships commonly held by other species. Variables obtained using the tensile tests could not be correlated with variables from the compression and water absorption tests due to the small size of some sponges limiting the number of tests that could be carried out using any one individual. Statistical analyses were performed using SPSS 12.0 for Windows. The r^2-values were determined using Microsoft Office Excel 2003.

Multiple linear regressions were performed between the directly measurable properties (*ie*. density, fibre width, and fibre length) and all quality variables (*ie*. firmness, compression modulus, compressive strength, tensile strength, elastic limit, elastic strain, modulus of elasticity, modulus of resilience, absorbency, and water retention efficiency). The standard approach was used, meaning the three variables independent of quality (density, fibre width, and fibre length) were analysed simultaneously, as opposed to the stepwise and hierarchical regressions (Francis, 2001). Because the analysis considers the independent variables simultaneously, an r^2-value is produced for an equation that uses all three independent variables. An ANOVA determines the significance of this relationship. The analysis then determines which of the independent variables contributes significantly to the equation, producing a t-value, significance value (p), and a partial r-value for each independent variable. The partial r-value is statistically controlled for the other independent variables. Only independent variables that had a significant relationship ($p < 0.05$) are presented in the results. Homogeneity of variance was checked using a plot of residuals versus predicted values. Outliers were checked for using the Mahalanobis distance (Francis, 2001). Variables specified in Table 3.3 were log

transformed to meet statistical assumptions. Statistical analyses were performed using SPSS 12.0 for Windows.

3.3 Results

3.3.1 Physical Properties

The physical properties of density, fibre width, and fibre length, were measured directly from the sponge sample to determine if quality characteristics were predictable using quick, simple methods. There were significant differences between species for all parameters. Four of the five species exhibited a similar density ranging from 30.4 ± 1.6 to 35.8 ± 2.1kg/m^3. The exception was the grass sponge with a significantly lower density of 16.5 ± 0.7kg/m^3 (Fig. 3.4; Table 3.1). All three commercial species had similar fibre widths ranging from 21.9 ± 0.4 to 22.8 ± 0.5µm. The fibre width of *R. odorabile* was significantly greater at 30.9 ± 1.0µm, while *Coscinoderma* sp. was significantly lower than all other species at 17.5 ± 0.3µm (Fig. 3.5; Table 3.1). *R. odorabile* had the shortest fibres of all species at 166.7 ± 6.1µm, significantly less than the grass sponge at 257.4 ± 11.0µm and the silk sponge at 300.3 ± 13.7µm, which were also significantly different from each other (Fig. 3.6; Table 3.1). Fibre length could not be determined for *Coscinoderma* sp. and the wool sponge due to their long fibres in excess of 1mm.

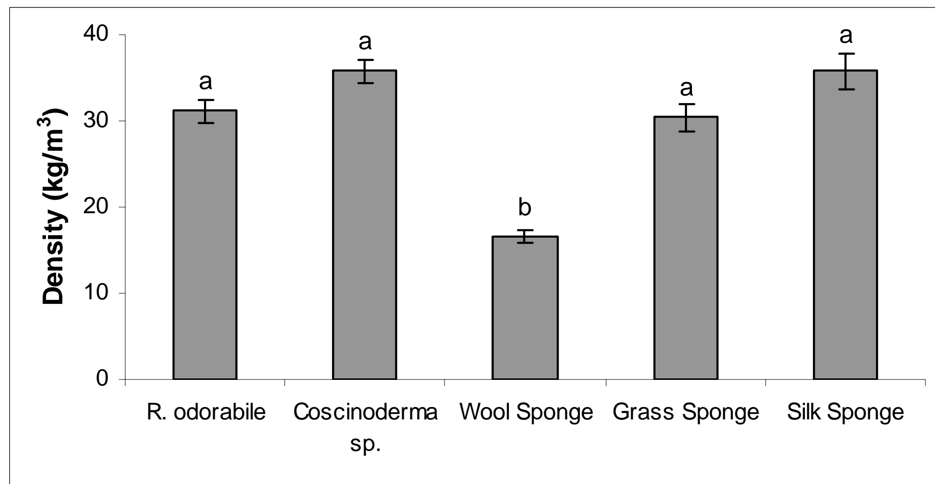


Fig. 3.4 Sponge density, measured as the weight of the sponge over its entire volume including spaces between the fibres. Different letters indicate a significant difference ($p < 0.05$).

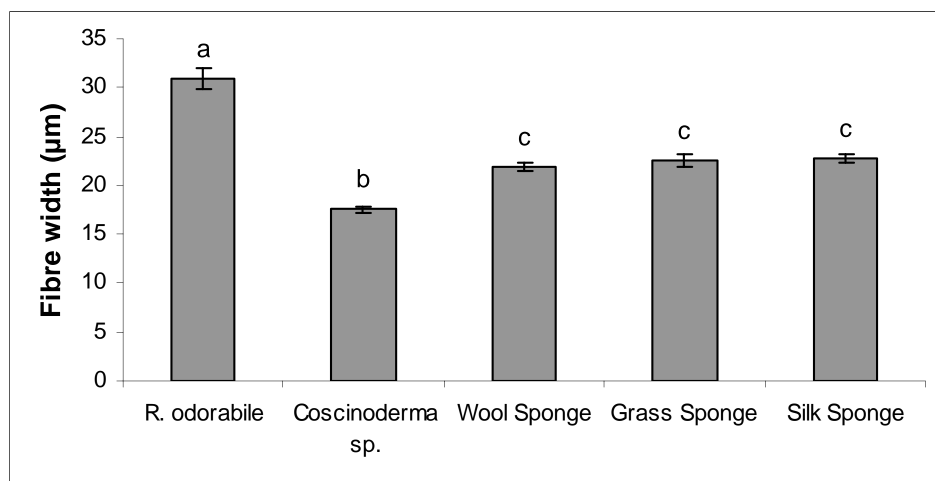


Fig. 3.5 Fibre width, including only secondary fibres. Different letters represent significant differences ($p < 0.05$).

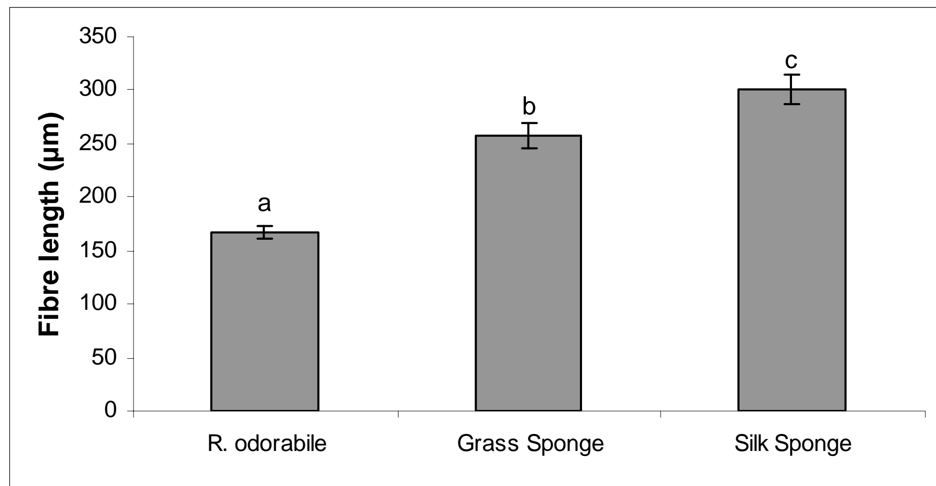


Fig. 3.6 Fibre length, measured as the length between fibre nodes or branches. Different letters represent significant differences ($p < 0.05$).

Table 3.1 One-way ANOVAs for physical properties and quality characteristics of species including the transformations used to meet the statistical assumptions of ANOVA.

Variable	**Transformation**	***df***	**MS**	***F***	***p***
Density	$X' = \sqrt[4]{X}$	4	0.559	41.328	< 0.001
Fibre width	$X' = \sqrt{\log(X)}$	4	0.025	68.677	< 0.001
Fibre length	$X' = \sqrt[5]{X}$	2	0.569	49.632	< 0.001
Firmness	$X' = \log(X)$	4	2.938	60.123	< 0.001
Compression modulus	$X' = \sqrt{\log(X)}$	4	0.120	41.455	< 0.001
Compressive strength	$X' = \log(X)$	4	0.477	63.181	< 0.001
Tensile strength	$X' = \log(X)$	4	1.164	27.958	< 0.001
Elastic limit	$X' = \sinh^{-1}(X)$	4	6.429	27.881	< 0.001
Elastic strain	$X' = \log(10X)$	4	0.619	38.698	< 0.001
Modulus of elasticity	$X' = \log(X)$	4	1.762	38.533	< 0.001
Modulus of resilience	$X' = \sqrt[4]{X}$	4	1.851	24.276	< 0.001
Absorbency	$X' = X$	4	325.954	13.505	< 0.001
WRE	$X' = \sin^{-1}(\log(\sqrt{X}))$	4	0.149	53.906	< 0.001

3.3.2 Quality Tests

3.3.2.1 Compression Tests

Compression tests were used to construct stress-strain curves for each individual of each species to measure sponge firmness, compression modulus, and compressive strength. As for the directly measurable physical properties, there were significant differences between species for all parameters measured. For the measure of firmness (25% compression), the wool sponge was the softest at 3.2 ± 0.3kPa.

R. odorabile was the firmest sponge at 37.8 ± 4.3kPa, an order of magnitude firmer than the wool sponge (Fig. 3.7). All species were significantly different to each other with the exception of the grass and wool sponges (Table 3.1). Compression modulus (with higher numbers representing a better ability to cushion a load) was similar between commercial species, ranging from 3.0 ± 0.2 to 3.5 ± 0.1 (Fig 3.8). *R. odorabile* was significantly lower at 2.1 ± 0.1, while *Coscinoderma* sp. was significantly greater at 4.3 ± 0.2 (Table 3.1). Compressive strength mirrored the results of the firmness tests with the wool sponge being the weakest at 33.7 ± 1.0kPa and *R. odorabile* the strongest, almost three times higher at 92.4 ± 5.3kPa (Fig. 3.9). All species were significantly different to each other with the exception of the grass and wool sponges (Table 3.1).

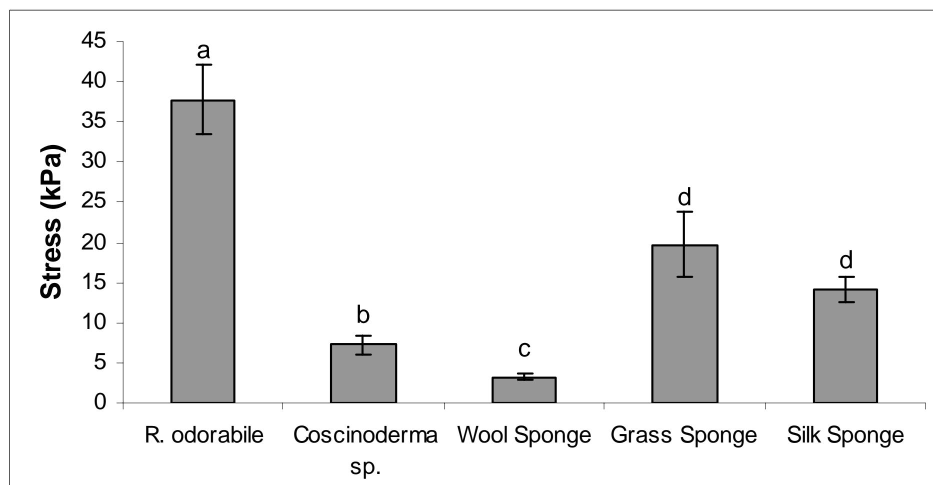


Fig. 3.7 Firmness, measuring the resistance to pressure, with lower values indicating a softer sponge. Different letters represent significant differences ($p < 0.05$).

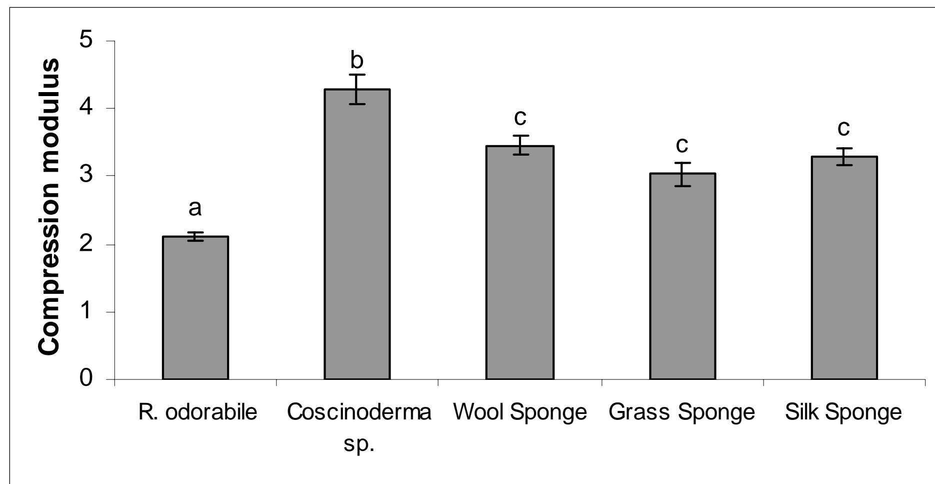


Fig. 3.8 Compression modulus, measuring the ability of a sponge to cushion a load with larger numbers indicating greater cushioning ability. Different letters represent significant differences ($p < 0.05$).

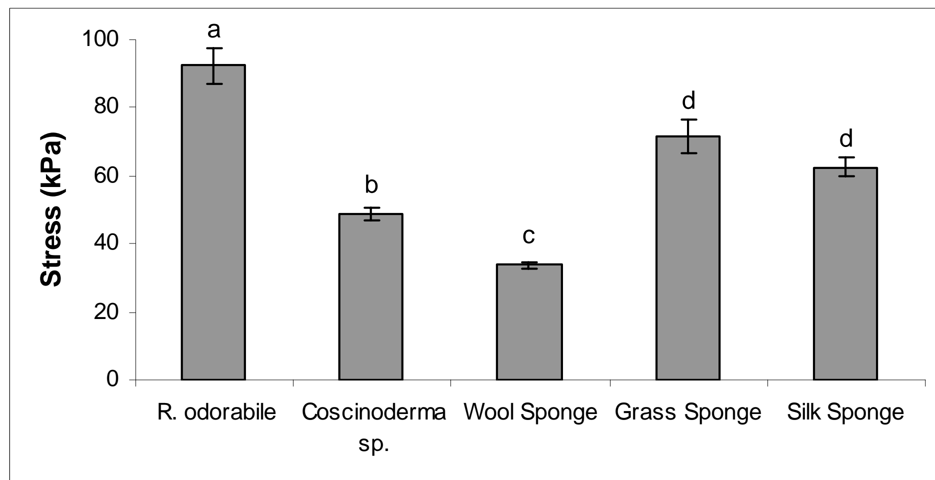


Fig. 3.9 Compressive strength, measuring the point at which a sponge is no longer easily compressed. Different letters represent significant differences ($p < 0.05$).

3.3.2.2 Tensile Tests

Tensile tests were used to construct stress-strain curves for each individual of each species to measure tensile strength, elastic limit, elastic strain, modulus of elasticity, and modulus of resilience. Again, there were significant differences between species for all properties measured. *R. odorabile*, *Coscinoderma* sp. and the silk sponge were significantly stronger than the wool and grass sponges that had tensile strengths of 38.5 ± 3.1 and 62.3 ± 12.4kPa respectively (Fig. 3.10, Table 3.1). *R. odorabile*

was the strongest species at 161.8 ± 17.9kPa, four times stronger than the wool sponge. Similarly, *R. odorabile*, *Coscinoderma* sp. and the silk sponge had a significantly higher elastic limit than the wool and grass sponges that had limits of 36.3 ± 3.1 and 60.9 ± 12.6kPa respectively. *R. odorabile* had the highest elastic limit at 157.4 ± 17.3kPa, over four times greater than the wool sponge (Fig 3.11; Table 3.1). The elastic strain of *Coscinoderma* sp. was significantly greater than the commercial species at 0.58 ± 0.05 (*ie*. it could stretch to 58% more than its original length before permanent damage occurred), while *R. odorabile* was significantly less than all other species at 0.19 ± 0.01, three times lower than *Coscinoderma* sp. (Fig. 3.12; Table 3.1).

The wool sponge was clearly the least elastic, giving the lowest results for both the modulus of elasticity at 121.8 ± 11.7kPa (Fig. 3.13) and the modulus of resilience at 5.5 ± 0.5kJ/m^3 (Fig. 3.14). *R. odorabile* and *Coscinoderma* sp. demonstrated very different elastic properties to each other. *R. odorabile* had significantly greater resistance to stretching (modulus of elasticity) at 838.7 ± 53.5kPa compared to *Coscinoderma* sp. at only 221.4 ± 28.3kPa (Fig. 3.13), however it had significantly less elastic energy (modulus of resilience) at only 15.7 ± 2.7kJ/m^3 compared to *Coscinoderma* sp. at 28.5 ± 3.2kJ/m^3 (Fig. 3.14; Table 3.1).

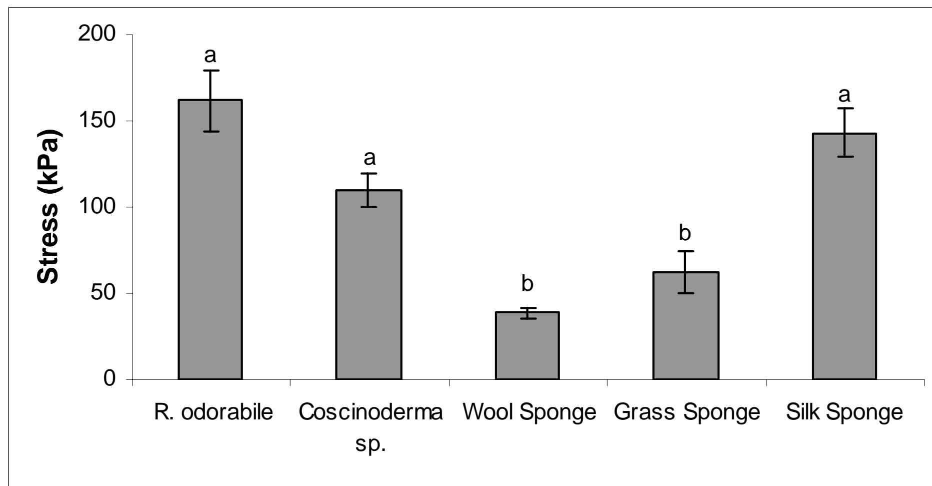


Fig. 3.10 Tensile strength, measuring the maximum strength of the sponge. Different letters represent significant differences ($p < 0.05$).

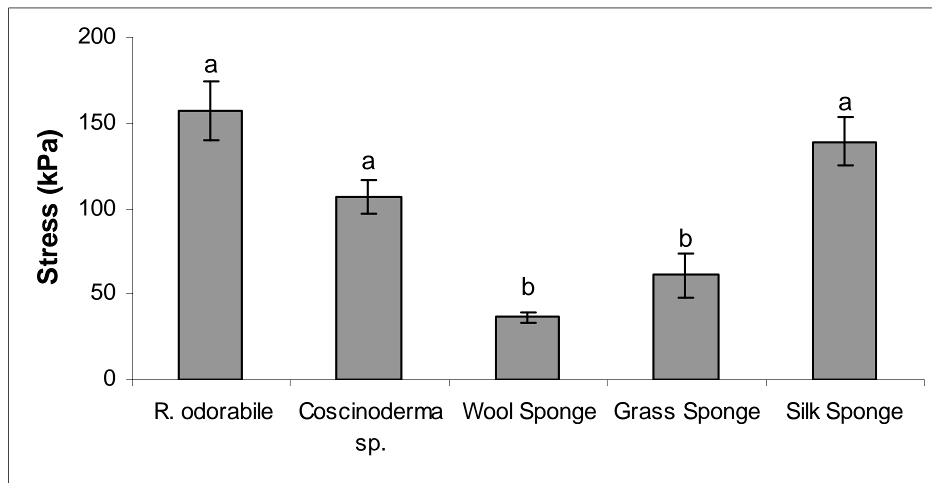


Fig. 3.11 Elastic limit, measuring the maximum stress prior to permanent damage occurring through tearing or breaking. Different letters represent significant differences ($p < 0.05$).

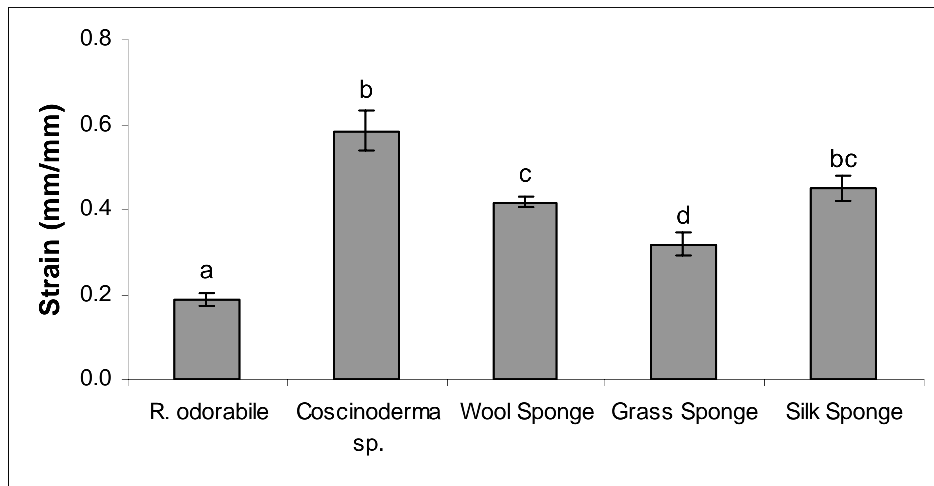


Fig. 3.12 Elastic strain, demonstrating the amount of stretch within elastic limits. Different letters represent significant differences ($p < 0.05$).

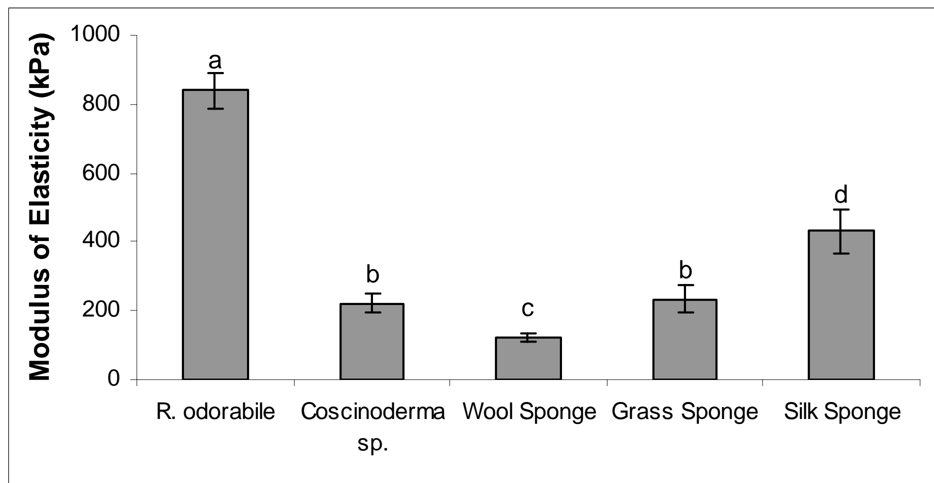


Fig. 3.13 Modulus of elasticity, measuring the resistance to stretching. Different letters represent significant differences ($p < 0.05$).

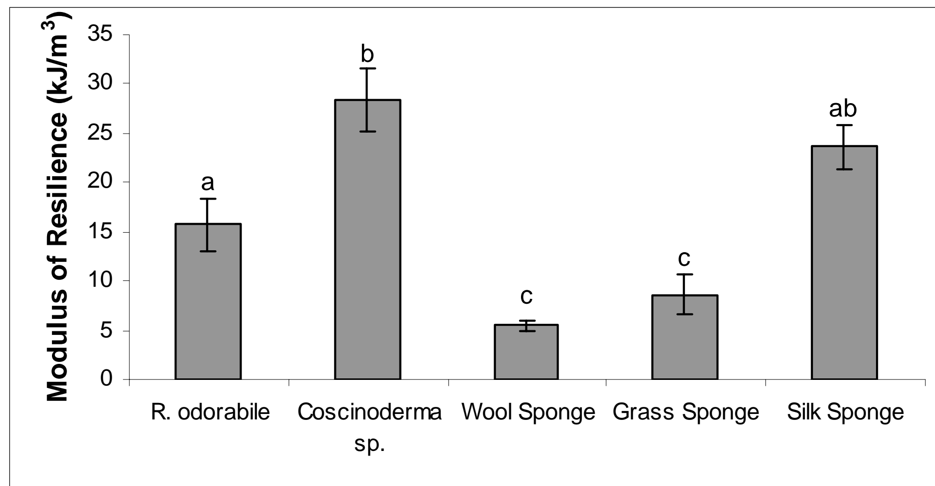


Fig. 3.14 Modulus of resilience, measuring elastic energy. Different letters represent significant differences ($p < 0.05$).

3.3.2.3 Water Absorption Tests

Absorbency was measured as the total weight of water able to be absorbed per dry weight sponge, while water retention efficiency measured the percentage of water retained after centrifugation. Overall, there was little difference among species, with absorbency ranging from 19.7 ± 0.8 (*Coscinoderma* sp.) to 31.0 ± 1.1 (wool sponge) (Fig. 3.15). The wool sponge had significantly greater absorbency than all other species (Table 3.1) and the silk sponge had significantly greater absorbency than *Coscinoderma* sp. Conversely, *Coscinoderma* sp. had the greatest water retention efficiency at 40.1 ± 1.4%, significantly greater than all species except the silk sponge (Fig. 3.16; Table 3.1). *R. odorabile* had significantly less water retention than all other species at 20.6 ± 0.6%.

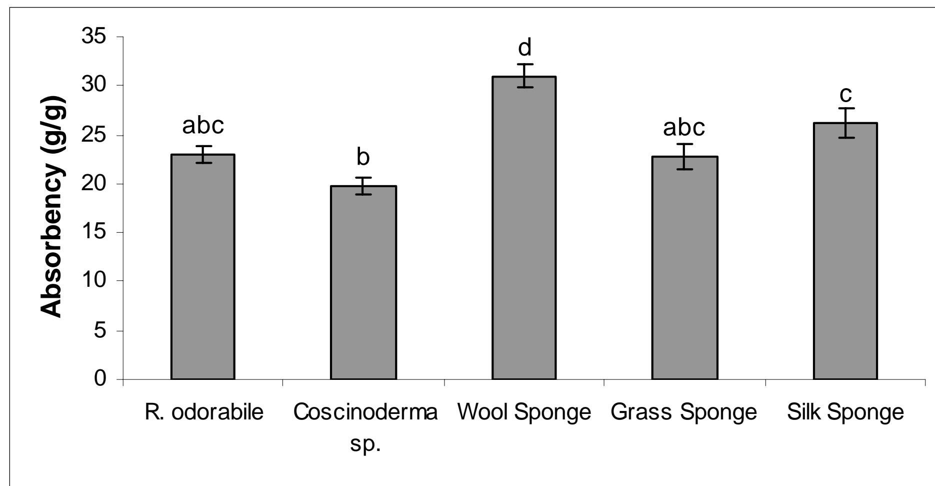


Fig. 3.15 Absorbency. Different letters represent significant differences ($p < 0.05$).

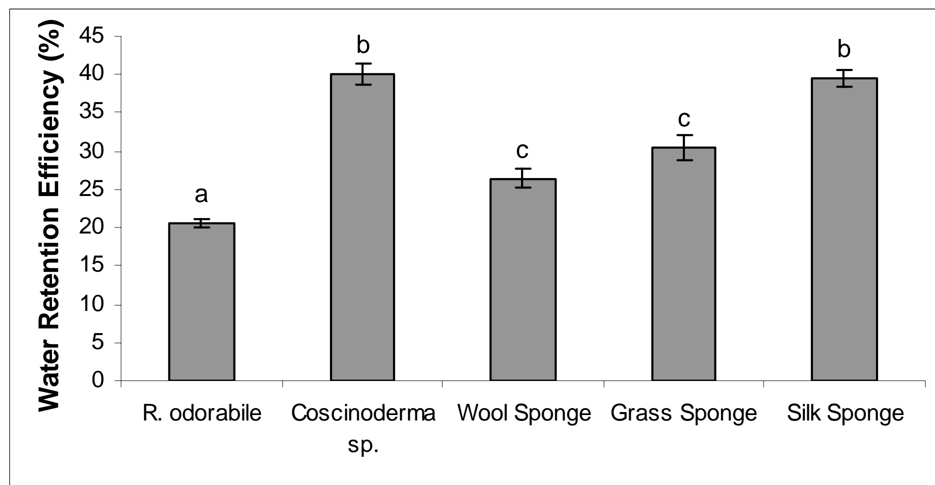


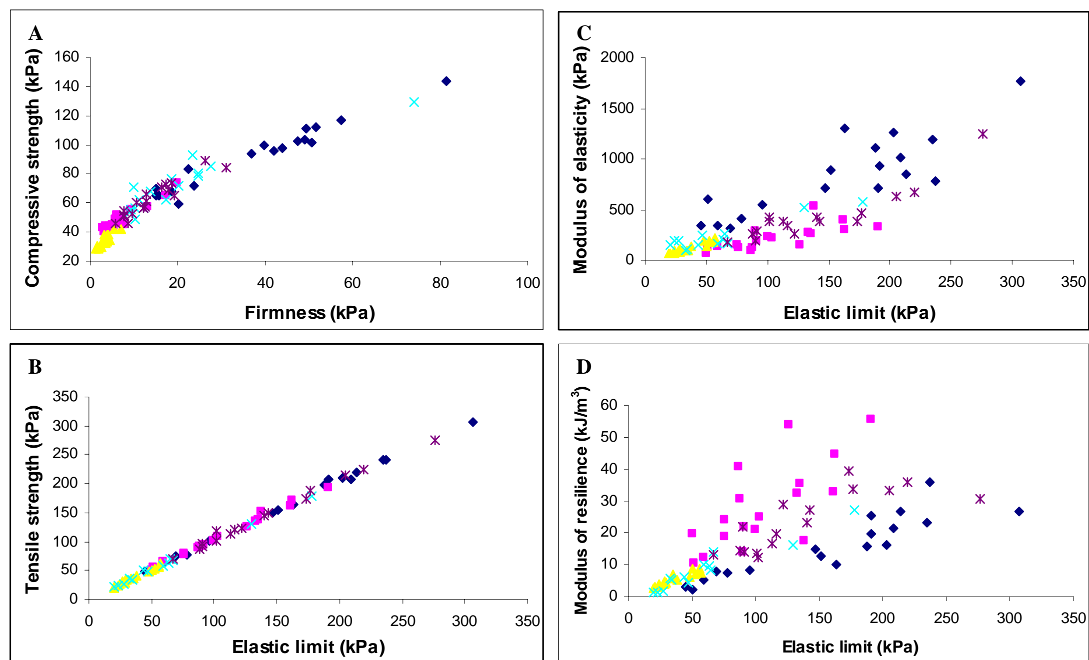
Fig. 3.16 Water retention efficiency. Different letters represent significant differences ($p < 0.05$).

3.3.3 Inter-variable Relationships

3.3.3.1 Quality Variables versus Quality Variables

Correlations between all quality variables (*ie*. firmness, compression modulus, compressive strength, tensile strength, elastic limit, elastic strain, modulus of elasticity, modulus of resilience, absorbency, and water retention efficiency) revealed several significant relationships. Only relationships common among four or five species have been presented. Firmness and compressive strength were significantly

correlated for all species (Fig. 3.17A; Table 3.2), while the elastic limit was significantly correlated with tensile strength for all species (Fig. 3.17B; Table 3.2). The elastic limit was also significantly correlated with the modulus of elasticity (Fig. 3.17C; Table 3.2) and the modulus of resilience (Fig. 3.17D; Table 3.2) for all species, although this latter relationship is a direct result of the method by which the modulus of resilience is calculated (see Section 3.2.3.2 Tensile Tests). Tensile strength was also significantly correlated with the modulus of elasticity and the modulus of resilience for all species due to the close relationship already observed between tensile strength and the elastic limit in Fig. 3.17B (Table 3.2).



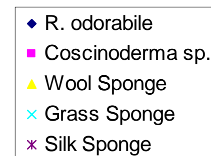


Fig. 3.17 A. Scatter plot of firmness and compressive strength; **B.** Scatter plot of the elastic limit and tensile strength; **C.** Scatter plot of the elastic limit and the modulus of elasticity; and **D.** Scatter plot of the elastic limit and the modulus of resilience. Different species are shown in different colours according to the key. The r^2-values for each species are given in Table 3.2.

Table 3.2 Spearman's rank correlation analysis for related variables significant across at least four species. The r^2-values are also given.

Correlations	Species	*rho*	n	*p*	r^2
Firmness by compressive strength	*R. odorabile*	0.953	18	<0.001	0.955
	Coscinoderma sp.	0.930	18	<0.001	0.934
	Wool sponge	0.876	18	<0.001	0.776
	Grass sponge	0.876	16	<0.001	0.859
	Silk sponge	0.932	18	<0.001	0.882
Elastic limit by tensile strength	*R. odorabile*	0.994	18	<0.001	0.997
	Coscinoderma sp.	1.000	18	<0.001	0.993
	Wool sponge	0.992	18	<0.001	0.982
	Grass sponge	0.984	13	<0.001	0.998
	Silk sponge	0.988	16	<0.001	0.993
Elastic limit by modulus of elasticity	*R. odorabile*	0.759	18	<0.001	0.722
	Coscinoderma sp.	0.858	18	<0.001	0.542
	Wool sponge	0.936	18	<0.001	0.899
	Grass sponge	0.654	13	0.015	0.853
	Silk sponge	0.815	16	<0.001	0.813
Elastic limit by modulus of resilience	*R. odorabile*	0.953	18	<0.001	0.805
	Coscinoderma sp.	0.655	18	0.003	0.521
	Wool sponge	0.930	18	<0.001	0.865
	Grass sponge	0.940	13	<0.001	0.928
	Silk sponge	0.844	16	<0.001	0.632
Tensile strength by modulus of elasticity	*R. odorabile*	0.792	18	<0.001	0.710
	Coscinoderma sp.	0.858	18	<0.001	0.593
	Wool sponge	0.946	18	<0.001	0.915
	Grass sponge	0.648	13	0.017	0.847
	Silk sponge	0.809	16	<0.001	0.797
Tensile strength by modulus of resilience	*R. odorabile*	0.930	18	<0.001	0.807
	Coscinoderma sp.	0.655	18	0.003	0.477
	Wool sponge	0.922	18	<0.001	0.814
	Grass sponge	0.945	13	<0.001	0.925
	Silk sponge	0.853	16	<0.001	0.633

3.3.3.2 Independent Variables versus Quality Variables

All quality characteristics were also correlated against the three physical properties independent of quality (density, fibre width, and fibre length) using multivariate linear regression analysis. Most variables showed at least some relationship to one or more of the directly measurable physical properties, although the relationships were often not common among species (Table 3.3). Firmness was positively correlated with density for all species except *Coscinoderma* sp. (Fig. 3.18A; Table 3.3). Compressive strength was also positively correlated with density for all species except *Coscinoderma* sp. (Fig. 3.18B; Table 3.3). Finally, absorbency was negatively correlated with density for all species (Fig. 3.18C; Table 3.3). Water retention efficiency was the only quality characteristic to show a significant relationship to all three physical properties with some species positively correlated with density and / or negatively correlated with fibre width or length, however no relationships were common between more than two species (Table 3.3).

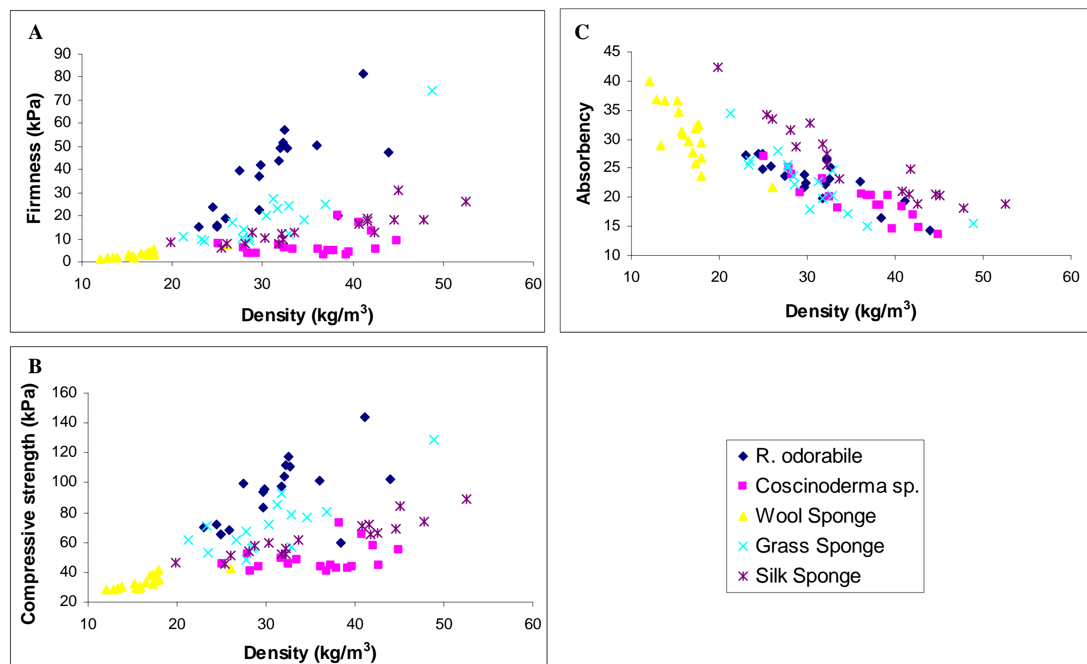


Fig. 3.18 A. Scatter plot of density and firmness; **B.** Scatter plot of density and compressive strength; and **C.** Scatter plot of density and absorbency. Different species are shown in different colours according to the key. The r^2-values for each species are given in Table 3.3.

Table 3.3 Standard multivariate linear regression between the physical properties of density, fibre width, and fibre length, and each quality characteristic. The r^2-values indicate the goodness of fit for the regression line created to fit all three independent variables. The partial r-value is statistically controlled for the other independent variables. Species marked with an asterisk indicate that the dependent variable was log transformed to meet the assumptions of the analysis. Only significant factors have been presented.

			ANOVA				Significant Factor(s)			
	Species	r^2	***df***	**MS**	***F***	***p***	**Factor**	***t***	***p***	**Partial r**
Firmness	*R. odorabile*	0.640	3,14	1217.669	8.306	0.002	Density	3.570	0.003	0.690
	Coscinoderma sp.	0.098	2,15	19.032	0.812	0.462				
	Wool sponge	0.843	2,15	15.605	40.139	<0.001	Density	7.540	<0.001	0.890
	Grass sponge	0.789	3,12	988.251	14.952	<0.001	Density	6.265	<0.001	0.875
	Silk sponge	0.871	3,14	225.743	31.417	<0.001	Density	8.207	<0.001	0.910
							Fibre length	-4.236	0.001	-0.750
Compression modulus	*R. odorabile*	0.254	3,14	0.099	1.593	0.236				
	Coscinoderma sp.	0.012	2,15	0.089	0.089	0.915				
	Wool sponge	0.173	2,15	0.454	1.572	0.240				
	Grass sponge	0.433	3,12	0.906	3.050	0.070				
	Silk sponge	0.547	3,14	0.712	5.627	0.010	Fibre length	4.043	0.001	0.734
Compressive strength	*R. odorabile*	0.581	3,14	1650.422	6.471	0.006	Density	2.715	0.017	0.587
	Coscinoderma sp.	0.106	2,15	71.575	0.885	0.433				
	Wool sponge	0.685	2,15	114.675	16.299	<0.001	Density	4.728	<0.001	0.774
	Grass sponge	0.675	3,12	1319.524	8.325	0.003	Density	4.743	<0.001	0.808
	Silk sponge	0.906	3,14	775.285	45.016	<0.001	Density	10.504	<0.001	0.942

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			ANOVA				Significant Factor(s)			
	Species	r^2	*df*	MS	*F*	*p*	Factor	*t*	*p*	Partial r
Tensile strength	*R. odorabile*	0.196	3,14	6524.663	1.138	0.368				
	Coscinoderma sp.	0.034	2,15	495.809	0.266	0.770				
	*Wool sponge	0.277	2,15	0.054	2.878	0.087	Density	2.271	0.038	0.506
	*Grass sponge	0.342	3,9	0.099	1.558	0.266				
	*Silk sponge	0.075	3,12	0.010	0.327	0.806				
Elastic limit	*R. odorabile*	0.204	3,14	6616.414	1.195	0.347				
	Coscinoderma sp.	0.028	2,15	391.238	0.217	0.808				
	Wool sponge	0.263	2,15	397.667	2.673	0.102	Density	2.250	0.040	0.502
	*Grass sponge	0.347	3,9	0.106	1.592	0.259				
	Silk sponge	0.090	3,12	1468.054	0.394	0.759				
Elastic strain	*R. odorabile*	0.268	3,14	0.004	1.712	0.210				
	Coscinoderma sp.	0.062	2,15	0.022	0.496	0.619				
	Wool sponge	0.204	2,15	0.004	1.917	0.181				
	*Grass sponge	0.192	3,9	0.023	0.715	0.567				
	Silk sponge	0.281	3,12	0.020	1.566	0.249				
Modulus of elasticity	*R. odorabile*	0.406	3,14	354370.899	3.191	0.057				
	**Coscinoderma* sp.	0.057	2,15	0.027	0.455	0.643				
	*Wool sponge	0.274	2,15	0.070	2.838	0.090	Density	2.346	0.033	0.518
	*Grass sponge	0.315	3,9	0.064	1.380	0.310				
	*Silk sponge	0.153	3,12	0.034	0.722	0.558				

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	Species	r^2	*df*	MS	*F*	*p*	Factor	*t*	*p*	Partial r
			ANOVA				Significant Factor(s)			
Modulus of resilience	*R. odorabile*	0.155	3,14	79.133	0.858	0.486				
	**Coscinoderma* sp.	<0.001	2,15	<0.001	0.001	0.999				
	Wool sponge	0.237	2,15	8.483	2.328	0.132				
	*Grass sponge	0.277	3,9	0.179	1.148	0.381				
	*Silk sponge	0.065	3,12	0.010	0.280	0.839				
Absorbency	*R. odorabile*	0.835	3,14	64.197	23.689	<0.001	Density	-7.191	<0.001	-0.887
							Fibre width	-3.181	0.007	-0.648
	Coscinoderma sp.	0.790	2,15	85.891	28.294	<0.001	Density	-6.944	<0.001	-0.873
	*Wool sponge	0.622	2,15	0.026	12.348	0.001	Density	-4.777	<0.001	-0.777
	*Grass sponge	0.723	3,12	0.034	10.465	0.001	Density	-4.951	<0.001	-0.819
	Silk sponge	0.888	3,14	228.258	36.984	<0.001	Density	-10.003	<0.001	-0.937
Water retention efficiency	*R. odorabile*	0.733	3,14	26.494	12.782	<0.001	Density	4.397	0.001	0.762
							Fibre length	-2.741	0.016	-0.591
	Coscinoderma sp.	0.540	2,15	164.815	8.815	0.003	Density	4.017	0.001	0.720
							Fibre width	-2.606	0.020	-0.558
	Wool sponge	0.053	2,15	13.002	0.422	0.663				
	Grass sponge	0.275	3,12	55.877	1.514	0.261				
	Silk sponge	0.323	3,14	35.631	2.229	0.130	Fibre width	-2.243	0.042	-0.514

3.4 Discussion

These experiments demonstrate that direct quantitative comparisons can be made between species with regard to firmness, strength, elasticity, and absorbency. *R. odorabile* and *Coscinoderma* sp. compared well with the commercial species, *S. zimocca* (the silk sponge, a high quality Mediterranean species) and *H. lachne* and *S. graminae* (the wool and grass sponges, lower grade Caribbean sponges) (de Laubenfels and Storr, 1958; species names were provided by the supplier). The silk sponge consistently outperformed the two Caribbean sponges, regarding measures of strength and elasticity. For every test except absorbency, the test score for the silk sponge fell in between that of *R. odorabile* and *Coscinoderma* sp. However, unlike the silk sponge, the test scores for *R. odorabile* and *Coscinoderma* sp. ranged dramatically between different quality characteristics. This variability means each species may be suited to a unique application as is later described.

Quality tests revealed some tests are more useful than others. Firmness, compression modulus, the elastic limit, modulus of elasticity, modulus of resilience, absorbency, and water retention efficiency all demonstrate valuable aspects of quality that may be used to describe individual species. The elastic strain may also be a useful measure, although if necessary, it may be closely approximated using the modulus of resilience and the modulus of elasticity. More importantly, correlations between quality characteristics suggest that the measures of compressive strength and tensile strength may be less useful. Compressive strength was strongly correlated to firmness. Firmness has direct implications for the feel of the sponge and thus is an important quality characteristic to be measured. However, compressive strength is

more useful for materials that rupture or brake under high pressure (unlike sponges) such as plastic or wood so is not likely to be regarded as an important measure of quality for most applications. Without the necessity to measure compressive strength, the method for measuring the other compression test variables is also simplified. Instead of creating a stress-strain curve, it will be possible to measure only two points of stress, one at 25% strain (firmness) and the other at 65% strain (to calculate compression modulus). To produce results comparable to this study, it will be necessary to maintain a compression speed of only 3mm/min, as compression speed, or strain rate, impacts the way in which a material responds to these forces (Askeland and Phulé, 2006). However, for future work it may prove more efficient to set a new standard of 10mm/min to equal that of the tensile test. This will not only increase commonality between methods, but reduce compression testing time threefold.

Secondly, tensile strength may be less useful as tensile strength was closely correlated to the elastic limit. The elastic limit is a necessary measure for the calculation of the modulus of resilience, and is also valuable in its own right. Although in many sponges tensile strength equals the elastic limit, the defining feature of the elastic limit is that this is the point at which damage first occurs. Therefore, the elastic limit may be a more valuable and appropriate measure of sponge strength. As a note of caution, changes in terminology must be made with care to avoid confusion with definitions already set in place regarding the physical and mechanical properties of materials.

Significant correlations between other quality variables included between the elastic limit and the modulus of elasticity and between the elastic limit and the modulus of resilience. The relationship between the elastic limit and the modulus of elasticity may be expected as high rigidity (modulus of elasticity) might also be expected to give the sponge greater strength. Despite this, there is no guarantee of this relationship holding true for other species. For example a brittle sponge would have a high modulus of elasticity, but a low elastic limit. As previously mentioned, the relationship between the elastic limit and the modulus of resilience is a result of the means by which the modulus of resilience is calculated.

Each of the quality characteristics tested revealed significant differences between species, creating a unique profile for each species. This unique profile can be used to determine which species is best suited to any one application. For example, a sponge used for industrial cleaning may require a minimum elastic limit (strength) of 100kPa, minimum modulus of elasticity (rigidity) of 700kPa, minimum firmness of 30kPa, and a minimum water retention efficiency of 20%. Of the five species tested, only *R. odorabile* fits this set of requirements. Alternatively, a sponge used for medical purposes may have no minimum requirement for its elastic limit, require a maximum modulus of elasticity of 400kPa, maximum firmness of 15kPa, and a minimum water retention efficiency of 35%, to which only *Coscinoderma* sp. meets all specifications. With chemical treatments available to soften sponges, firm sponges such as *R. odorabile* potentially have multiple applications, using various levels of treatment.

Prediction of quality characteristics based on directly measurable physical properties proved unviable. However, correlations between these physical properties and other quality characteristics revealed significant relationships between density and firmness, density and compressive strength, and density and absorbency. Therefore, density is a key parameter to indicate quality differences within a species when comparing individuals from different environments. For example, if high flow environments cause a particular sponge species to become more dense, when cultured in such an environment the processed bath sponge may be expected to be firmer and less absorbent. Sponge fishing in the Bahamas, has already revealed differences in sponge quality (subjectively assessed) for the sponge *Hippospongia gossypina* (*c.f. Equina gossipina* van Soest, 2005), between different locations, with one location producing smoother, tougher sponges with more closely woven fibres (Cresswell, 1922). It was not determined if differences were due to environmental or genetic factors. The effects of the environment are highly variable between species (see Chapter 1), and significant relationships between physical properties and quality characteristics in this study were often not common between species. Further research is required to quantify the effect the environment may have on the quality of any one species. Given significant differences in quality between habitats, each species in consideration for aquaculture production could be quality tested using samples taken from various habitats prior to site selection in order to optimise product value.

Finally, to continue the development of these methods and further demonstrate their usefulness to scientists and entrepreneurs alike, further quality testing is needed to broaden the range of commercial species that can be used as guides for finding

culturable species that have marketable properties. To set a high standard for the industry, commercial species should include the more valuable Mediterranean species such as the honeycomb sponge (*Hippospongia communis*) or the elephant ear sponge (*Spongia agaricina*). Secondly, another valuable addition to the protocol could include testing the sponges when wet, as variables such as firmness are greatly influenced by the moisture content of the sponge. Further research is required to establish whether the differences in wet and dry measured quality characteristics are directly proportional.

3.5 Conclusion

For the first time, quantitative methods for determining sponge quality have been developed allowing the comparison of new species against high quality commercial species. The tests presented here demonstrated the potential of *R. odorabile* and *Coscinoderma* sp. as at least one of the two species consistently outperformed the commercial species in each of the quality tests. *R. odorabile* was the firmest, strongest (elastic limit), and most rigid (modulus of elasticity) sponge of the five species. On the other hand, *Coscinoderma* sp. was one of the softest, highest supporting (compression modulus), and with the highest elastic energy (modulus of resilience) and water retention efficiency of all the species. As more commercial species are quality tested, an acceptable range of quality values will become more apparent. Furthermore, quality tests may now be used to quantify the effects of the environment or chemical treatments on sponge quality allowing optimisation of sponge quality for the aquaculture industry and providing a key opportunity to capitalise on niche markets that require sponges with specific quality characteristics.

Chapter 4: General Discussion

This study provides an initial assessment of the aquaculture potential of the two bath sponge species, *Rhopaloeides odorabile* and *Coscinoderma* n. sp (hereafter referred to as *Coscinoderma* sp.). It also provides a detailed description of the recovery process of sponge explants for these species, and links recovery of sponges to the survival and growth patterns of explants cultured *in situ*. Furthermore, a quantitative quality testing protocol for sponges has been developed allowing the direct comparison of quality to be made between and within species with scientific rigor.

R. odorabile and *Coscinoderma* sp. appear to be strong candidates for aquaculture, however further research is required prior to the establishment of commercial aquaculture of these species. This is because both species suffered heavy mortality in the initial stages of the experiment causing them to fall short of the 90% survival required for commercial sponge aquaculture (Verdenal and Vacelet, 1990). However, in the later stages of the culture sequence, survival was near 100% for both species. Most mortalities occurred due to the initial damage inflicted on the sponge, as has been found by some previous studies of explant culture (*eg*. Pronzato *et al*, 1999; de Caralt *et al*, 2003). However, the main cause of mortality in some studies has been due to the culture method or environment resulting in sustained mortality over the course of the experiment (*eg*. Verdenal and Vacelet, 1990; Duckworth and Battershill, 2003b; van Treek *et al*, 2003). Improvements in aquaculture practices are needed to reduce initial mortality rates, including minimising the damage inflicted upon explants during excision and threading of culture lines, and optimising the environmental conditions during the first month of culture to provide optimal conditions for explant recovery.

Sponge growth was heavily impacted by recovery. Both species exhibited size dependent growth rates immediately following propagation with smaller explants growing the fastest. This is only the second time that size dependent growth rates have been reported in explants. The other, a study by van Treeck *et al* (2003), found an optimum size for *Ircinia variabilis* explants with faster growth for explants within a given size range. Duckworth *et al* (1997) found a minimum size threshold below which survivorship and growth were significantly reduced, however the other sizes classes (27 to 125 cm^3) had no influence on growth rate. *Coscinoderma* sp. also exhibited higher than average growth rates the season following this size dependent growth despite the relatively steady average growth rates throughout the remainder of the study. Research is required to determine how the recovery processes influences the growth of these species to capitalise on these growth effects. The growth rates of approximately 100% per annum in this study bordered upon the minimum requirements for commercial aquaculture (Verdenal and Vacelet, 1990).

Further research is also required to understand the driving factor behind growth rates. Unlike many studies of sponge growth (Verdenal and Vacelet, 1990; Duckworth and Battershill, 2003b; Duckworth *et al*, 2004; Kelly *et al*, 2004), this study was done in the tropics where the year-round warm water temperatures may have been responsible for the relatively consistent average growth rates. However, like many other studies, growth rates were highly variable between individuals including those of common parentage (Verdenal and Vacelet, 1990; Turon *et al*, 1998; Osinga *et al*, 1999). If all sponge explants could be cultivated with the growth rates measured of the fastest growing individuals (up to 235 and 307% per annum for *R. odorabile* and *Coscinoderma* sp. respectively), the potential of sponge aquaculture would be

dramatically improved. To achieve this, research must investigate the driving forces behind rapid growth rates, potentially including different cell or symbiont ratios within explants, or genetic heterogeneity within individual parent sponges caused by mosaicism or chimerism (described by Pineda-Krch and Lehtilä, 2004).

The process by which sponge recovery occurred was remarkably similar between individuals and species, however the speed of recovery was highly variable from one individual to another (days to weeks). In common with other studies, the exposed surface was rapidly covered with a new protective layer (Storr, 1976; Hoppe, 1988; Pronzato *et al*, 1999; Hoffmann *et al*, 2003). However, this is the first time the recovery process has detailed the transition from a protective surface layer of collagen to pinacoderm and established that ostia and incurrent canals redevelop within days (in *R. odorabile*). Finally, the subsurface cellular composition returned to normal, becoming densely populated with pigment cells, normally within 41 days. The optimal environmental requirements during the recovery phase need to be determined to maximise survival and growth of the species during the early stages of culture.

As an alternative to current aquaculture techniques, the sexual reproduction of sponges has the potential to produce high numbers of stock with minimum labour and minimal use of broodstock, but this has yet to receive any attention from the research community. Current methods use up to 25% or more of the year's produce as seed stock (explants) to replace the harvested sponges (MacMillan, 1999). This is done despite sponges being highly fecund invertebrates (Corriero *et al*, 1996; Uriz *et al*, 1998). In a single reproductive season, sponges may release in excess of 10000

larvae per individual (P. Ettinger-Epstein, unpublished data). Sexual propagation of sponges would also bypass the excision and recovery process which caused high mortality in the current experiment. Furthermore, it would allow the use of selective breeding to increase average growth rates. Larval recruitment and maturation time are key issues to examine to determine the viability of this method.

Finally, the development of a quantitative quality measuring protocol now allows direct comparisons to be made between and within species allowing the assessment of new aquaculture species and providing a tool by which intra-species variation in quality can be determined. The high quality of *R. odorabile* and *Coscinoderma* sp. was well established as they meet or outperformed the tested commercial sponges in many of the quality tests. For example, *R. odorabile* was the firmest, strongest, and most rigid species tested, while *Coscinoderma* sp. was one of the softest sponges with the highest elastic energy and water retention efficiency of all the species.

Comparison of bath sponge species has two main benefits. Firstly, researchers can use the protocols to identify the suitability of new species compared to existing commercial species in an objective manner, without the need for cooperation from experienced sponge traders. Secondly, the quality testing protocol provides a key marketing tool for new species. Using the unique profile created by the quality testing procedure, any sponge can be chosen for a given application based on quantitative values of softness, strength, elasticity, or absorbency. For example, *R. odorabile* and *Coscinoderma* sp. may be used for applications requiring high elasticity or strength that may be beyond the limits of some commercial sponges.

Comparisons of sponge quality between individuals of a single species will also have several benefits as field experiments, combined with a quality testing protocol, will allow for the determination of factors responsible for variation in quality and identify how these factors may be managed to produce a sponge of optimal quality. Factors that may influence sponge quality include genetic variation, environmental variation (for morphologically plastic species), and post-harvest chemical treatments.

Research into sponge biology has provided the essential foundation on which the Australian sponge aquaculture industry will be founded. Despite this, there remains much work to be done before sponge aquaculture is able to reach its full potential. With continued research, sponge aquaculture may in time provide an environmentally sound, renewable resource and a source of income for remote and regional communities.

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