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Australian coastal lakes and lagoons

Peter Brendan Barnes
University of Wollongong

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**ENVIRONMENTAL IMPACTS AND THE ECOLOGY OF
SPONGES AND ASCIDIANS IN SOUTH-EASTERN
AUSTRALIAN COASTAL LAKES AND LAGOONS**

**A thesis submitted in fulfilment of the requirements for the award of
the degree**

DOCTOR OF PHILOSOPHY

from the

UNIVERSITY OF WOLLONGONG

by

Peter Brendan Barnes

Department of Biological Sciences

2009

Frontispiece: *Suberites* sp. in Wallis Lake



Declaration

I, Peter Brendan Barnes declare that this thesis submitted in fulfilment of the requirements for the award of Doctor of Philosophy in the Department of Biological Sciences, University of Wollongong is wholly my own work unless otherwise referenced or acknowledged. This document has not been submitted for qualifications at any other academic institution.

.....

Peter Brendan Barnes

15th July 2009

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Abstract

Estuaries worldwide are under threat from urbanisation and development and will need effective management for their successful conservation. Coastal lakes and lagoons have been identified as one of the estuary types most susceptible to human impacts largely because of their isolated nature and slow flushing times. Management of estuaries will be most effective when based on a sound scientific understanding of the patterns of distribution, biology and ecology over the full range of biodiversity of these systems, however, such an understanding is lacking for many systems and many taxa. Studies of sponges and ascidians in coastal lakes and lagoons are rare compared to other taxa, particularly in the southern hemisphere. This study represents the first detailed scientific investigation of the ecology of sponges and ascidian in coastal lakes and lagoons of southeastern Australia. Consequently, a large part of this thesis was devoted to quantifying basic patterns of distribution. I started with a pilot study to develop an effective sampling design, followed by large-scale comparisons among different types of lake, comparisons among habitats within lakes and an environmental impact study. I concluded with a manipulative experiment to examine processes responsible for small-scale patterns of distribution of sponges in seagrass meadows.

In the pilot study, distributions of sponges and ascidians were quantified at a hierarchy of three spatial scales in each of two coastal lakes. Nested analyses of variance were then used to identify spatial scales at which variation was significant. Most sponges and ascidians were very patchily distributed at a range of spatial scales from 10s of metres up to 100s of kilometres. Unlike other published examples of cost–benefit analyses, very few taxa were widespread over the larger spatial scales. Cost–benefit analyses done to determine the optimal sampling design revealed inclusion of

patchily distributed taxa in analyses improved the overall precision of sampling for comparisons of assemblages among lakes.

Large-scale comparisons of assemblages of sponges and ascidians were made among lakes of different size (big versus small), opening regime (mostly open to the ocean versus mostly closed) and level of environmental modification (extensively modified versus more pristine). Similar to other taxa studied in coastal lakes, in general there were more species in lakes mostly open to the ocean compared to the mostly closed lakes, and importantly, no sponges and only one species of ascidian was found in the small closed lakes. There also appeared to be an effect of the level of modification of a lake with relatively smaller abundances of ascidians in extensively modified lakes, and a complete absence of sponges from one of the extensively modified lakes.

Habitat-associated patterns were examined at smaller spatial scales by comparing the distributions of sponges and ascidians with the species composition and percentage cover of seagrass and macroalgae within two lakes; St Georges Basin and Wallis Lake. Several patterns of association were observed, but these varied among species of sponge and ascidian. In St Georges Basin, the most common sponge, *Aplysinella* cf. *rhax* and the native ascidian *Pyura stolonifera* were positively correlated with the seagrass, *Posidonia australis*. In contrast, the introduced ascidian, *Styela plicata* was more abundant in areas dominated by the seagrass, *Zostera capricorni*. In Wallis Lake, sponges were most diverse and some species most abundant in large beds of the macroalga, *Lamprothamnion* sp., while other sponges were found only on the holdfasts of brown macroalgae. In both lakes, sponges were generally less common in areas dominated by dense meadows of the seagrass, *Zostera capricorni*.

Among the many anthropogenic impacts threatening the ecology of coastal lakes, the discharge of cooling water from coal-fired power stations represents an

almost ideal case study from which to develop appropriate sampling regimes for detecting impacts on sponges and ascidians. Using reference locations both within and outside Lake Macquarie which has two cooling water outlets, I found assemblages of sponges and ascidians were often more diverse, more abundant and less temporally variable near to the outlets compared to reference locations.

Based on the observation that the sponge, *Suberites* sp. which contains photosynthetic symbionts was absent from meadows of dense *Zostera capricorni*, I used *in situ* manipulative experiments in Smiths Lake to investigate processes which may be affecting their distribution. Individual *Suberites* sp. were shaded, had water flow reduced and were transplanted into areas of dense *Z. capricorni*. There were no measurable short-term effects of shading or reduced water flow, but transplanted sponges were quickly eaten and I concluded predation by fish was likely to be a key process determining small-scale patterns of distribution of *Suberites* sp. in seagrass meadows. This result was in stark contrast to the majority of previous studies of the effects of seagrass habitat complexity on predation which have found predation to decrease with increasing density or complexity.

In conclusion, I have sought to provide sound scientific information to aid in the management of these systems. A simple, but nevertheless key finding was that sponges and ascidians are indeed present and widespread in coastal lakes and lagoons of southeastern Australia and should not be continually overlooked in the management and conservation of these systems. Conservation will be complex and requires an understanding of environmental impacts and the consequences of management on the full range of biodiversity. The distributions of sponges and ascidians at large 'lake-wide' scales appear to behave similarly to other taxa. Management strategies which change the characteristics of a lake at these large spatial scales such as artificial

openings of entrances could therefore be predicted to have similar effects across a range of taxa including sponges and ascidians. In contrast, at smaller spatial scales such as the complexity of seagrass meadows, some species of sponges and ascidians may behave very differently from other taxa. At present, our understanding of these naturally variable and complex systems is incomplete and will require ongoing scientific investigation to identify natural patterns of distribution, environmental impacts, important natural processes and the ecological consequences of management strategies.

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CHAPTER 1: GENERAL INTRODUCTION

1.1 ENVIRONMENTAL IMPACTS IN ESTUARIES AND THE NEED FOR EFFECTIVE MANAGEMENT

Estuaries worldwide are suffering ongoing environmental degradation from a myriad of human impacts (Kennish 2002; Lee *et al.* 2006), including fishing (Blaber *et al.* 2000), loss of habitat (Alongi 2002), nutrient enrichment (Bricker *et al.* 2008), industrial and urban contamination (Mathiessen & Law 2002; Thompson *et al.* 2007), changes to freshwater flows (Gillanders & Kingsford 2002), clearing of catchments (Ruiz-Fernandez *et al.* 2002) and introduced species (Ruiz *et al.* 1999). In addition to protecting their intrinsic values, the conservation of estuaries is of paramount importance because of the many resources and benefits they provide for coastal cities and communities including food, transport, recreation, aesthetic qualities and waste disposal (Beaumais & Laroutis 2007; Ronnbak *et al.* 2007). Because many estuaries have already suffered significant environmental impacts and large-scale modifications to their catchments, their long-term conservation will rely heavily on management. Such management will be most effective when based on a sound scientific understanding of the patterns of distribution, biology and ecology over the full range of biodiversity of these systems.

One of the key steps for the effective and long-term conservation of estuaries is the management of environmental impacts. The management of impacts, however, can be a challenging task for a number of reasons. First, because estuaries are extremely variable and complex in nature it cannot be assumed that impacts will be uniform in their effects among different types of estuaries or across different habitats within estuaries. Similarly, impacts are unlikely to have consistent biological effects for different organisms (Thompson *et al.* 2007). The detection of impacts can be complex

and difficult because of a background of often large natural variability that exists in natural systems (e.g. Morrissey *et al.* 1992; Archambault & Bourget 1999). For impacts to be unambiguously detected, changes in species abundances, distributions or other characteristics must be differentiated from natural background patterns of variability (Underwood 1991). The detection and identification of impacts is, however, extremely important because it allows management to be more effective by targeting prevention strategies at specific impacts and targeting restoration or conservation measures in particular habitats or for species which are under threat.

It is, of course, not only desirable to detect current impacts, but also to prevent or reduce the severity of impacts occurring in the future. A key requirement for prevention is to be able to predict the biological or ecological effects of planned (e.g. deforestation in catchments) or unplanned but inevitable changes (e.g. climate change) on the biota and ecology of natural systems. This will be best achieved by not only learning from previous mistakes, but also by understanding the processes important in structuring estuarine communities.

1.2 TYPES OF ESTUARIES: COASTAL LAKES AND LAGOONS

Coastal water bodies are extremely variable in their physical, geological, chemical, hydrological and biological characteristics (Wolfe & Kjerfve 1986) and as such, different types are expected to vary in their vulnerability to anthropogenic disturbances (Haines *et al.* 2006). Here, for clarity, I develop some working definitions of types of estuaries. A diverse range of classification schemes has been proposed for coastal water bodies (e.g. Day 1981; Kennish 1986; Kjerfve 1994; Roy *et al.* 2001). While Day (1980) suggested there was ‘an infinite variety of estuaries’ which were grouped only for convenience, groupings can nevertheless be extremely useful for

experimentation and in understanding the ecological processes operating within these systems. While there are many overlaps and similarities among classification schemes, there are also many differences and the terminology can be confusing.

First, while most people (scientists and the general public alike) have an intuitive understanding of what an estuary is, scientific definitions vary and have been refined over time. In this thesis, I use Day's (1980) definition of an estuary as 'a partially enclosed coastal body of water which is either permanently or periodically open to the sea and within which there is a measurable variation of salinity due to the mixture of sea water with fresh water derived from land drainage'. Estuaries can then be further categorised based on various criteria including geomorphology, hydrography, salinity, tidal characteristics and sedimentation (Kennish 1986). Day's (1980) definition encompasses most coastal water bodies ranging from large drowned river valleys to small coastal creeks. Most authors would also consider coastal lakes and lagoons as types of estuary (e.g. Day, 1981; Kennish 1986; Roy *et al.* 2001, but see Kjerfve 1994).

While definitions for coastal lakes and lagoons are broad, they generally share a number of common physical attributes. They are generally shallow, less than 5 metres and rarely deeper than 10 metres (Kennish 1986; Kjerfve 1994). Water exchange between the ocean is restricted with a relatively narrow inlet channel (McComb 1995) often as a result of sand deposits in the estuary mouth (Roy *et al.* 2001). They may become isolated from the sea for extended periods of time (Haines 2003). Tidal ranges are usually considerably less than in the ocean (approximately 5-10 %; Roy *et al.* 2001) and they often occur behind sand barriers (Roy *et al.* 2001).

The terminology used to describe water bodies with these characteristics varies from place to place. In south eastern Australia, they are commonly called 'coastal

lakes', in south western Australia they are called 'inlets', but internationally they are more commonly recognised as 'coastal lagoons'. To add to this complexity, in New South Wales, a relatively new term, 'Intermittently Closed and Open Lake or Lagoon' (ICOLL) has been used to describe coastal water bodies that are intermittently open and closed to the ocean (Haines *et al.* 2006). In this thesis, I use the term, 'lake and lagoon', in order to be both consistent with the local New South Wales nomenclature, and to acknowledge the more widely accepted international terminology of 'lagoon'.

Coastal lakes and lagoons are common throughout the world and occupy an estimated 13 % of coastal areas: 5.3 % for Europe, 13.8 % for Asia, 17.6 % for North America, 5.3 % for South America, 17.9 % for Africa and 11.4 % for Australia (Barnes 1980). They are considered the estuary type most sensitive to anthropogenic impacts largely due to their restricted or intermittent connection with the ocean and relatively slow flushing times (West 1990; Haines *et al.* 2006). Many suffer from severe eutrophication, which may cause algal blooms and fish kills (Lardicci *et al.* 2001). Coastal lakes and lagoons are common in New South Wales with ninety identified in a recent study (Healthy Rivers Commission of NSW 2002). These systems are under extreme pressure with a large and growing proportion of the Australian population living on or near the coast (Zann 1995). Indeed, just six of these ninety in New South Wales are considered pristine, while twelve are considered severely affected by development and a further seventeen moderately affected (Healthy Rivers Commission 2002). Because of the pressures of urbanisation and development, these systems are becoming increasingly managed. For example, up to 30 % of New South Wales coastal lakes and lagoons are artificially opened to the sea in an attempt to improve water quality and/or reduce the risk of flooding property (Haines 2004). It has been

suggested, however, that decision-making to date has been haphazard with little consideration for ecological outcomes (Thompson 2006).

While some types of estuaries are among the most intensively studied ecosystems in the world (e.g. Chesapeake Bay in North America, Kemp *et al.* 2005), the ecology and biology of many types of estuaries, particularly in some parts of the world remain poorly understood. While there have been many studies of coastal lakes and lagoons in Europe (e.g. Barnes 1988; Millet & Guelorget 1994; Benedetti-Cecchi *et al.* 2001; Lardicci *et al.* 2001; Zaldivar *et al.* 2003; Duport *et al.* 2007), particularly in the Mediterranean, and North America (e.g. Bricker *et al.* 2008), there has been relatively much less research in the southern hemisphere (but see Day 1981; Teske & Woolridge 2001, 2003; Nozais *et al.* 2005; Anandraj 2008 for South African examples). Research in temperate Australia has increased in recent years (e.g. Griffiths 1999; Cummins *et al.* 2004a & b; Dye & Barros 2005a & b; Dye 2006; Gladstone *et al.* 2006; Gray *et al.* 2009), but despite lakes and lagoons being centres of urbanisation, industry and agriculture, the ecology of these systems and the effects of management remain poorly understood.

1.3 HABITATS IN COASTAL LAKES AND LAGOONS: SOFT SEDIMENTS AND SEAGRASSES

The substrata of coastal lakes and lagoons in southeastern Australia, like many parts of the world, are dominated by soft sediments (Roy *et al.* 2001). In comparison, hard rocky substratum is relatively less common. Because of the prevalence of soft sediments and relatively shallow depths with ample light available for photosynthesis, seagrass meadows and macroalgal beds are common in coastal lakes and lagoons in New South Wales (West *et al.* 1985) and in shallow coastal waters throughout the world (Green & Short 2003).

Because of their prevalence, seagrass meadows, algal beds and soft sediments provide most of the available habitat for sessile invertebrates in these systems. In particular, seagrasses provide a large potential source of hard substrata on which sessile invertebrates may settle. Seagrasses are becoming increasingly recognised for their ecological and economic importance in supporting a diverse range of fauna and flora (Heck *et al.* 2003). Large-scale losses and fragmentation of seagrass meadows primarily as a result of human impacts and coastal development have been reported worldwide (Orth *et al.* 2006). Seagrasses in estuaries are particularly vulnerable to certain types of anthropogenic impacts, particularly contamination, more so than coastal seagrasses (West 1990; Ralph *et al.* 2006). With these impacts comes a growing need for conservation, which will require a sound ecological understanding of the effects of habitat loss and fragmentation on the full range of associated fauna and flora.

To date, most studies of fauna in seagrass habitats (see review by Bostrom *et al.* 2006) have focussed on fish (Connolly & Hindell 2006), crustaceans (Heck & Coen 1995), infauna (Bowden *et al.* 2001) or molluscs (Irlandi 1997). In contrast, for other faunal groups (e.g. sessile invertebrates such as sponges and ascidians), there have been very few studies (but see, Thorhaug & Roessler 1977; Fell & Lewadrowski 1981; Kuenen & Debrot 1995; Wulff 1995, 2008; for sponges, Petersen & Svane 1995 for ascidians and Lemmens *et al.* 1996 for ascidians and sponges). Distributions of fauna in seagrass habitats vary with a range of physical attributes including the species of seagrass or macroalgae (Rotherham & West 2002), shape of leaves (Schneider and Mann 1991), patchiness of seagrass beds (Reusch & Williams 1999), proximity to edges (Bologna & Heck 2000) and quality of surrounding habitats (Tanner 2006).

Associations of fauna with seagrasses are often complex and vary among taxa (Bostrom *et al.* 2006). It is likely that the associations of sessile filter-feeding organisms such as

sponges and ascidians may be very different from other taxa (e.g. mobile fish or crustacea). Effective conservation of the full range of biodiversity of these systems will require an ecological understanding of the various taxa, but to date, this understanding has been largely absent for sponges and ascidians.

1.4 SPONGES AND ASCIDIANS IN COASTAL LAKES AND LAGOONS

Sponges and ascidians are rarely mentioned in textbooks on estuaries or coastal lakes and lagoons (e.g. McLusky 1989; Morrissey 1995). The same is true for seagrass textbooks. In Short and Coles (2001) textbook on ‘Global Seagrass Research Methods’ individual chapters are devoted to methods for studying ‘macroalgal biomass’, ‘infauna and epifauna’ and ‘fish, crabs, shrimps and other large mobile epibenthos’, but sponges and ascidians are not mentioned. This would of course be for good reason if they were indeed absent, but studies from many parts of the world, in particular the Mediterranean, along with anecdotal and some scientific evidence from temperate Australia suggest otherwise (Table 1.1). For example, while sponges were not mentioned in a recent and excellent book describing the morphology and ecology of the many estuaries and lagoons of temperate Western Australia (Brearley 2005), the author has in fact seen them in many of those waterways (Anne Brearley, pers. comm.).

In New South Wales lakes and lagoons, the only conclusion that can be drawn for sponges and ascidians is that they are or have at least been present in these systems (see Table 1.1). The only published study directly relating to sponges in New South Wales coastal lakes and lagoons is a nineteenth century inventory which reported up to eight species from ‘The Illawarra’^a (von Lendenfeld 1888). In more recent times, although there appear to have been no studies specifically relating to sponges in New

^a It is assumed when von Lendenfeld used the term, ‘The Illawarra’ in 1888 he was referring to what is now known as Lake Illawarra.

South Wales lakes and lagoons, they have been reported occasionally in studies focussing on other taxonomic groups (Hutchings *et al.* 1978; Robinson *et al.* 1982; Day & Hutchings 1984). Anecdotal evidence from commercial fishermen working in New South Wales coastal lakes and lagoons who have found sponges (colloquially and affectionately referred to as ‘sponge cakes’ or ‘monkey shit’) in their set-nets or beach seines suggests they may actually be widespread and periodically abundant (Les Biles, pers. comm. and Lyle Bramble, pers. comm.).

Our scientific knowledge of sponges and ascidians in coastal lakes and lagoons comes largely from the Mediterranean, with some examples from the Caribbean, the anchialine lakes of Indonesia and Palau and a recent study from the karst lakes of Vietnam (See Table 1.1 for examples of studies). Research on sponges in Mediterranean lagoons is not new (e.g. Topsent 1925 and other references cited in Corriero 1987), however most examples have been descriptive studies of the patterns of distribution or the biology of 1 or 2 species within a small number of lakes and lagoons. One of the key observations from the Mediterranean relevant to New South Wales lakes and lagoons is the importance of seagrasses and in particular seagrass rhizomes as suitable substrata for the attachment of sponges (Mercurio *et al.* 2000, 2007). Some of the studies from the Caribbean (Kuenen & Debrot 1995) and Florida (Thorhaug & Roessler 1977) were broader studies examining whole seagrasses communities which happened to include sponges and ascidians and included limited descriptions of their patterns of distribution with respect to habitat and currents. The two studies from Indonesia and Vietnam appear to be first time surveys of sponges in these previously unsampled environments, which quite appropriately were largely qualitative descriptions of the diversity of these systems, with some preliminary ecological observations. For example, de Voogd *et al.* (2006) found diversity was positively

correlated with the size of Indonesian lakes and Azzini *et al.* (2007) suggested that growth for at least some of the species was seasonally variable and correlated with physical variables such as water temperature. Rutzler *et al.* (2000) suggested large diversity of sponges was dependent on availability of suitable hard substrata, low turbidity and proximity of 'sponge- rich coral reefs'. It must be noted, however, that the Indonesian, Vietnamese and Caribbean systems appear to be physically quite different compared to the lakes and lagoons of New South Wales, with more rocky substrata and mangroves, but less soft substrata and presumably no seagrasses.

The paucity of studies of sponges and ascidians in coastal lakes and lagoons worldwide may reflect their often difficult taxonomy (particularly for sponges, Hooper & Widenmeyer 1994), scarcity of researchers or perceived lack of economic importance in comparison to other taxa such as commercially valuable species of fish, prawns and lobsters (Heck & Orth 2006). Research on benthic invertebrates in coastal lakes and lagoons has largely focussed on molluscs (e.g. Gribbin & Wright 2007; Zettler & Daunys 2007), benthic macrofauna including polychaetes, nematodes, amphipods (e.g. Dye & Barros 2005a; Maggiore & Keppel 2007) and meiofauna (Dye & Barros 2005b). In conclusion, ascidians and in particular sponges represent relatively understudied taxa, in both seagrasses habitats (but see Wulff 2008) and coastal lakes and lagoons. It appears the sponges may indeed warrant their title as one of the 'neglected groups' as highlighted in a recent edition of the Canadian Journal of Zoology (2006; Volume 84) devoted to the 'neglected' phylum Porifera.

1.5 THE DETECTION OF ENVIRONMENTAL IMPACTS

The detection of anthropogenic impacts is essential for the effective management and conservation of natural environments (Osenberg & Schmitt 1996; Benedetti-Cecchi

2001). The identification of impacts, however, is often a complex and difficult task against the natural background variability that exists in nature (Underwood 1992), uncertainties about the spatial extent of disturbances (Raimondi & Reed 1996) and the choice of organisms to be included (Underwood & Peterson 1988; Jones and Kaly 1996).

An environmental impact study must be able to distinguish changes caused by a human impact from natural background variability (Underwood 1991). Experimental designs for detecting impacts have developed greatly over the last thirty years (Thomas *et al.* 1978; Green 1979; Hurlbert 1984; Stewart Oaten *et al.* 1986) culminating in the 'Beyond BACI' designs developed by Underwood (1991,1992,1993,1994). One of the key developments has been the need to include multiple reference (often called control) locations (Underwood 1992).

There have been numerous environmental impact studies examining sessile assemblages on hard substrata which have included sponges and ascidians (e.g. Underwood & Chapman 1996; Glasby 1997; Roberts *et al.* 1998). There have been relatively fewer that have focussed solely on sponges or ascidians (Muricy 1989; Carballo *et al.* 1996; Naranjo *et al.* 1996; Carballo & Naranjo 2002; Alcolado 2007). One of the observations from these studies and other studies examining natural patterns of distribution (Farnsworth & Ellison 1996; Roberts & Davis 1996; Hooper & Kennedy 2002; Newton *et al.* 2007) is that many sponges and ascidians are very patchily distributed with assemblages often including a high proportion of rare or uncommon species. Because of this patchiness, some studies investigating assemblages of sessile invertebrates (e.g. Glasby 1997; Roberts *et al.* 1998) have grouped species together and analysed them as Porifera or Ascidiacea or individual species have been reported as too spatially variable for formal analysis. When the taxa being investigated are extremely

rare, uncommon or patchily distributed, the design of sampling programmes becomes much more difficult (McDonald 2004). Environmental impact studies that have specifically targeted sponges or ascidians have generally included many sampling locations to acquire adequate estimates of natural variability (Carballo & Naranjo 2002). Given their patchy distributions, it is important that the design of any environmental impact study of sponges and ascidians is based on good estimates of natural patterns of distribution and variability. When this information is absent, which is the case for sponges and ascidians in coastal lakes, it is almost essential to include a pilot study to provide the necessary information to design appropriate sampling regimes (Green 1979; Andrew & Mapstone 1987).

1.6 THIS STUDY

The aims of this study were to broaden our scientific understanding of the estuary type considered most vulnerable to anthropogenic impacts (coastal lakes and lagoons) by examining the natural patterns of distribution, ecology and potential environmental impacts for two of the relatively understudied groups of organisms in these systems; sponges and ascidians. Comparisons and experiments were conceived with the intention of providing sound scientific information relevant for conservation and management.

More specifically, the aims of this thesis were to:

1. Develop a suitable sampling technique and sampling design for quantifying distributions of sponges and ascidians in coastal lakes (Chapter 2)
2. Examine natural patterns of distribution of sponges and ascidians within lakes and among different types of lake (Chapters 2 and 3)
3. Examine patterns of association of sponges and ascidians with habitat (seagrass and macroalgal assemblages) (Chapter 4)

4. Test for the effects of a specific environmental impact (hot water discharge from coal burning power stations) (Chapter 5)
5. Identify processes which may be affecting the distribution of sponges in meadows of the seagrass *Zostera capricorni* (Chapter 6)

The development of an appropriate sampling design for quantifying the distributions of sponges and ascidians was particularly important because to the best of my knowledge there had been no previous studies done on these animals in these habitats on which designs could be reliably based. An appropriate design was developed by estimating variances at a hierarchy of spatial scales in two lakes and then using cost-benefit analyses to optimise replication to improve precision within an allocated time-budget.

Similarly, given sponges and ascidians had not been studied in these habitats before, a broad understanding of natural patterns of distribution was a particularly important step in this research programme (Underwood *et al.* 2000). The identification of spatial scales at which variation is significant may point to scales at which important processes may be operating and help propose logical models to identify those processes (Underwood & Chapman 1996a). A series of comparisons were made to examine patterns of distribution. First, sampling was done at a hierarchy of four spatial scales (10s of metres, 100s of metres, kilometres) within lakes, and 100s of kilometres between two lakes. Second, to examine larger scale effects of lake-type, sponge and ascidian assemblages were compared among lakes of different sizes, opening regimes and general levels of human impact. Smaller-scale patterns of distribution of sponges and ascidians within lake were examined by investigating associations with assemblages of seagrasses and macroalgae in two lakes.

Based on the findings of the studies examining natural patterns, a sampling programme was designed to examine potential effects of a specific environmental impact on assemblages of sponges and ascidians (i.e. cooling water discharge from coal burning power stations). Similarly, based on patterns of distributions of sponges associated with complexity of seagrass meadows, some of the processes which may affect the distribution of sponges in seagrass meadows; shading, reduced water-movement and predation were investigated for a cyanobacteria-containing sponge, *Suberites* sp., using manipulative field experiments in Smiths Lake. Finally, in my general discussion, I compare my findings for sponges and ascidians in New South Wales coastal lakes, with other systems, with other taxa in coastal lakes and then consider the implications for sampling, detecting impacts, management and their conservation in coastal lakes and lagoons.

Table 1.1. Examples of studies reporting sponges or ascidians in coastal lakes and lagoons. Numbers in parentheses indicate number of lakes and lagoons sampled. Lakes and lagoons that were also studied in this thesis are identified by name (see Figure 3.1).

Location	Sponges	Ascidians	Comments	Source
South-eastern Australia (Lake Illawarra)	8 species		Inventory of sponges only	Von Lendenfeld 1888
South-eastern Australia (Wallis & Smiths Lakes)	Porifera reported, but no species names	<i>Styela etheridgei</i>	Both very patchily distributed. Study focussed on benthic macrofauna, not sponges or ascidians.	Hutchings <i>et al.</i> 1978
South-eastern Australia (Smiths Lake)	One unnamed species	<i>Styela plicata</i> Lesueur, 1803	Only presence reported. Study focussed on benthic macrofauna, not sponges or ascidians.	Robinson <i>et al.</i> 1982
South-eastern Australia (3)	Porifera reported, but no species names	Ascidacea reported	Both appear very uncommon. Study focussed on benthic macrofauna, not sponges or ascidians.	Day & Hutchings 1984
Caribbean (1)	9 species	<i>Styela partita</i> Stimpson	Sponges patchily distributed but abundant in some sites.	Kuenen & Debrot 1995
Caribbean (7)	182 species		Large differences in species composition among tropical mangrove ponds	Rutzler <i>et al.</i> 2000
Florida (1)	<i>Chondrilla nucula</i> and other unnamed species		<i>C. nucula</i> and other sponges dominant invertebrate in areas with patchy seagrass or strong tidal currents	Thorhaug & Roessler 1977
Florida (1)	19 species		Sponges may be important in controlling phytoplankton blooms	Peterson <i>et al.</i> 2006
Indonesia (4)	45 species		Positive correlation between number of species and size of lake	de Voogd <i>et al.</i> 2006
Italy (1)	42 species	Tunicates reported	Sponges found on rocks, seagrasses, algae and free-living on sediment	Corriero 1987
Italy (1)	<i>Tethya aurantium</i> <i>T. citrinia</i>		Both species common on <i>Posidonia oceanica</i> rhizomes. <i>T. aurantium</i> more abundant in areas with strong water currents and <i>T. citrinia</i> more abundant in still water	Corriero <i>et al.</i> 1989
Italy (1)	<i>Halichondria panicea</i> (Pallas)	<i>Botryllus schlosseri</i> (Pallas)	Found encrusting on wooden poles and on both natural hard and soft substrata	Marzano <i>et al.</i> 2003
Italy (2)	<i>Pellina semitubulosa</i> (Lieberkühn)		Both free living and sessile forms found. Sessile forms often attached to <i>Posidonia oceanica</i> rhizomes	Mercurio <i>et al.</i> 2000
Italy (1)	42 species		Species found on both rocky and soft substrata	Mercurio <i>et al.</i> 2001
Italy (2)	<i>Geodia cydonium</i> (Jameson 1811)		Sessile individuals found on rocky substrata or seagrass rhizomes. Non-sessile often found on soft substrata.	Mercurio <i>et al.</i> 2007
Italy (1)	<i>Geodia cydonium</i> (Jameson 1811)		Sexual reproduction correlated with water temperature	Mercurio <i>et al.</i> 2007
Italy (1)	<i>Halichondria panicea</i> (Pallas)		Temporally variable, but periodically abundant	Nonnis Marzano <i>et al.</i> 2003
Italy, France and Greece (11)	81 species		Diversity largest in locations with high seawater exchange. Few common species among lagoons.	Mercurio <i>et al.</i> 2004
Palau (not stated)	<i>Lamellodysidea chlorea</i> and <i>Xestospongia exigua</i>		Both species reported as 'common sponges in the shallow lagoons of Palau'.	Thacker 2005
Spain (1)		<i>Ecteinascidia turninata</i>	Grow on soft sediments, seagrass and algae	Carballo 2000
Venezuela (1)	18 species	9 species	Species were part of fouling assemblages on mangrove roots.	Orihuela & Diaz 1991
Vietnam (8)	46 species		Large variability in species composition among lakes. 50% of species not found in adjacent coastal sites.	Azzini <i>et al.</i> 2007

CHAPTER 2: SPATIAL SCALES OF VARIATION AND THE OPTIMISATION OF SAMPLING FOR SPONGES AND ASCIDIANS IN COASTAL LAKES AND LAGOONS

2.1 INTRODUCTION

Estuaries worldwide are under ever-increasing threat from urbanisation and development (Kennish 2002). Threats range in scale (Underwood & Chapman 1996a), from very large estuary-wide impacts (e.g. changes to the Nile Delta caused by the construction of the Aswan Dam; Stanley & Warne 1993) to much smaller impacts affecting smaller patches of an estuary (e.g. impacts of boat wash, Bishop 2004; effects of storm-water drains, Roberts *et al.* 2007). The variety and complexity of threats and range of scales over which impacts may occur provide serious challenges for the management of these systems.

In southeastern Australia, coastal saline lakes and lagoons are under extreme pressure with an estimated 85 % of the population living near the coast (Zann 1995). Conservation of these lakes will require effective management, which will, in turn, require anthropogenic impacts to be identified and their effects on the ecology of these systems to be understood. While research effort has increased in recent years (Dye 2006; Gladstone *et al.* 2006), the ecology of these systems and the exact nature of the impacts are poorly understood.

The identification of ecologically important impacts and processes is a complex and difficult task, particularly against a background of natural variability. A useful starting point in identifying and understanding processes is first to identify patterns and important scales of variability in the distribution of organisms (Underwood *et al.* 2000). Spatial scales at which significant variation exists often then reveal the scales at which processes are operating. Thus, once appropriate scales have been

identified, informed causative models can be proposed and examined to understand the relevant processes (Underwood & Chapman 1996a).

The reliable identification of patterns of distribution of organisms is therefore a key component of a research programme. The design of any study examining patterns of distribution of organisms should include adequate replication at spatial scales at which variation is significant (Morrisey *et al.* 1992). Inadequate replication at these spatial scales may confound results and reduce the power of statistical tests to detect differences (Underwood & Chapman 2003). Various strategies and techniques have been developed to help design experiments with appropriate replication to overcome this problem. Such strategies may involve up to three stages. First, most rely on having preliminary estimates of variances, which may be obtained from existing data, the literature, or pilot studies. Second, spatial scales at which variation is significant are identified. Third, replication at each spatial scale may then be optimised to obtain a statistical test with a desired level of power (Benedetti-Cecchi 2001) or to keep within an allocated budget.

The use of spatially nested designs followed by analyses of variance (ANOVA) has been identified as a powerful technique for identifying scales at which variation is significant (Green 1979, Andrew & Mapstone 1987, Morrisey *et al.* 1992, Underwood 1997). Procedures are relatively straightforward for studies examining a single taxon or variable. For studies examining assemblages (i.e. many taxa), optimising sample size is not as straightforward, because there are no procedures available for calculating the power of a multivariate statistical test. In studies comparing assemblages of organisms, it is common practice first to compare whole assemblages (i.e. multivariate sets of data) and then compare specific taxa of interest using univariate techniques (e.g. ANOVA; e.g. Bishop 2004). If preliminary estimates of variances exist, the design of such

experiments may be optimised to sample abundances of a particular taxon or another univariate measure using ANOVAs and cost–benefit analyses. An experimental design would then be optimised for certain taxa. It is, of course, unlikely that cost–benefit analyses done for several taxa would all produce the same optimised experimental design (e.g. Benedetti-Cecchi *et al.* 1996). Compromises will need to be made in the design of experiments in terms of which taxa to sample most precisely. Ultimately, an experiment should be designed to test the hypotheses of interest. It is, however, sometimes unclear which species of an assemblage are or will be of most interest before the start of a research programme. This is particularly the case for unsurveyed habitats, where taxa may be undescribed or very patchy in their distribution. For example, I recently searched the published literature and unpublished reports, but found no studies directly relating to sponges, no quantitative descriptions of their distribution, nor any reliable names of species in NSW coastal lakes. It is clear in this case that it is impossible to identify particular species of interest before the start of a research programme. Under these circumstances, the first objective of a research programme will be to identify the species present and describe their basic patterns of distribution.

Detailed case studies examining variation at a hierarchy of spatial scales exist for soft-sediment macrofauna (Morrissey *et al.* 1992) and intertidal rocky shore assemblages (Underwood & Chapman 1998) in temperate eastern Australia. Spatial variation in sponge assemblages has been quantified in shallow (<5 m; Underwood *et al.* 1991) and deeper (20 to 50 m; Roberts & Davis 1996) rocky reefs on the open coast of NSW and in semi-enclosed or isolated bodies of water elsewhere in the world (e.g. on mangrove roots in Caribbean lagoons; Farnsworth & Ellison 1996; tropical estuaries; Kuenen & Debrot 1995; freshwater lakes; Rader 1984, De Santo & Fell 1996), but it is unclear whether the extrapolation of such results to the considerably different habitats of

seagrasses and soft substrata of relatively shallow and sheltered temperate coastal lakes is likely to be useful.

This chapter presents a pilot study examining spatial variation in the distribution of sponges and ascidians in two NSW coastal lakes. The aims of this study were 2-fold. First, to identify spatial scales at which variation was significant and, hence, at which scales important to processes may be operating. Second, to assist in the design of further larger scale experiments to examine spatial and temporal changes among and within several NSW coastal lakes. Variation in the abundance^b of sponge and ascidian fauna was examined at a hierarchy of spatial scales using fully nested sampling designs. When a species was absent from some replicate levels of a spatial scale, variation was examined within the levels of the subsequently nested spatial scale(s) where the species was present. Cost-benefit analyses were used to determine the optimal numbers of locations, sites and replicate samples to be used in future work.

2.2 MATERIALS AND METHODS

2.2.1 Study sites and sampling methods

Individual sponges and solitary ascidians were counted in relatively shallow (0.5 to 2 m depth) seagrass meadows at a hierarchy of spatial scales in each of two saline coastal lakes in New South Wales, Australia, in January and February 2002. Wallis Lake and St Georges Basin (Figure 2.1) were chosen as representative of relatively large lakes, moderately affected by development, with entrances that usually remain open to the ocean (Roy *et al.* 2001). In each lake, six locations (kilometres apart), each with four sites (100s of metres apart), each with 20 replicate 10 × 2 m transects (10s of metres apart) were sampled using SCUBA or by snorkelling depending

^b In this thesis, I use the term abundance to mean number of individuals unless otherwise stated

on depth. Sites were approximately 80 m in diameter. This design allowed spatial variation to be examined at four spatial scales: (1) between lakes 100s of kilometres apart, (2) among locations kilometres apart, (3) among sites 100s of metres apart and (4) among transects within sites 10s of metres apart. Voucher specimens of sponges were lodged with the Queensland Museum, Brisbane, Australia.

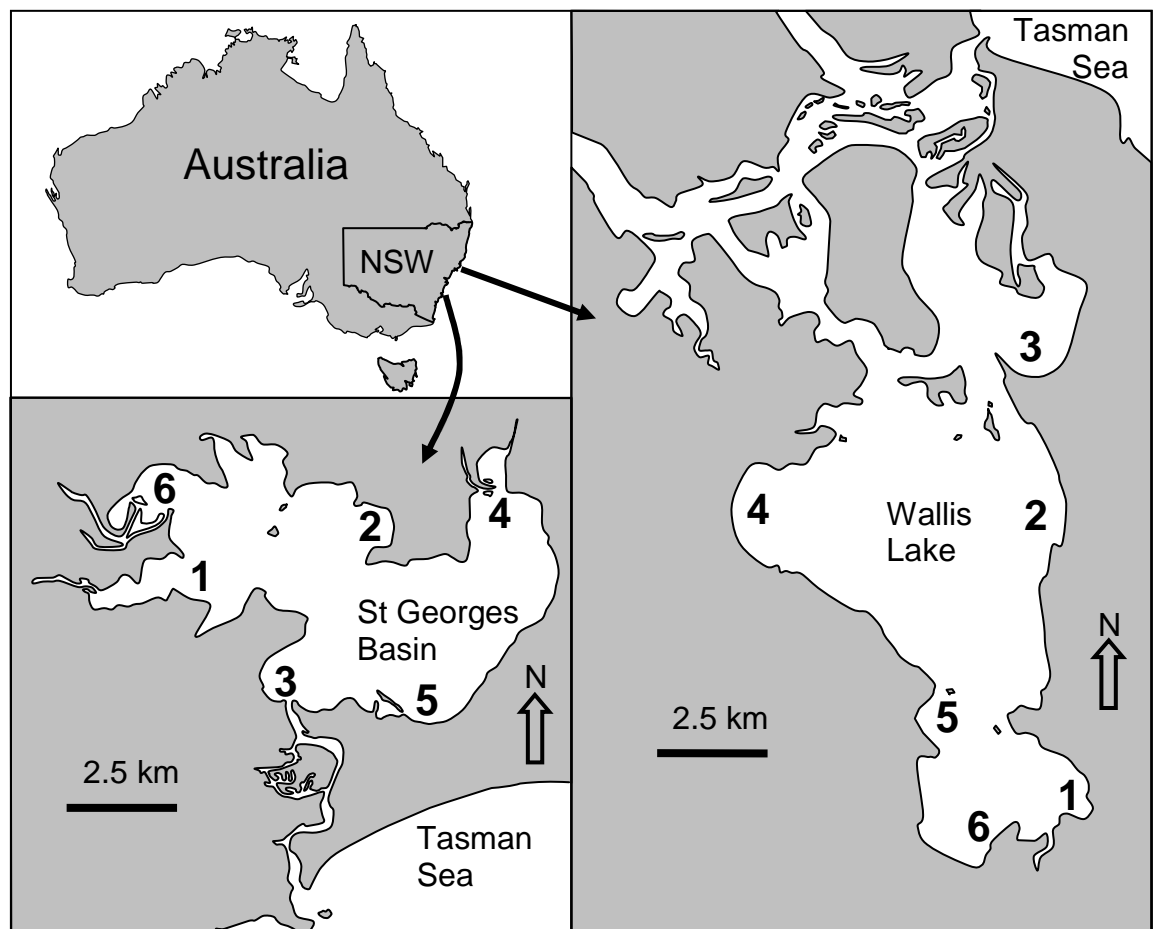


Figure 2.1. Locations sampled in St Georges Basin and Wallis Lake.

2.2.2 Statistical analyses: *Analyses of variance*

Three sets of fully nested ANOVAs were used to identify spatial scales at which significant variation occurred. First, three variables (the ascidian *Styela plicata* Lesueur, total numbers of taxa and total numbers of individual sponges) were identified

as widespread in both lakes, with non-zero values at most sites in most locations. Each was analysed with a 3-factor (lake, location and site) nested ANOVA, with all factors random.

The remaining taxa were patchily distributed and restricted to a single lake or some locations or sites within a lake. Preliminary examination of these data suggested that 3-factor ANOVAs (as used above) would be inappropriate because of the large numbers of zero values. These species, however, represented the majority of taxa and may presumably occur in other lakes or at subsequent times of sampling. The omission of such taxa from analyses may result in important processes operating at smaller scales and affecting patterns of distribution being overlooked. In the absence of widespread taxa, a broader understanding of spatial variation can be obtained by analysing these patchily distributed taxa in the places where they do occur. Therefore, a second set of ANOVAs was done to test for significant spatial variation among locations and sites within St Georges Basin only, for the solitary ascidian *Pyura stolonifera*^c Heller and the sponge *Aplysinella* cf. *rhax*. Each was analysed with a 2-factor (location and site) nested ANOVA.

Finally, using the same logic as above, a third set of ANOVAs was done for those species found in only one or a few locations in Wallis Lake. Abundances of *Halichondria* spp., *Mycale* sp. and *Suberites* sp. were analysed by a 1-factor (site) ANOVA to test for significant variation among sites in the locations where they occurred. Abundances of very uncommon taxa (<10 ind. lake⁻¹) were not analysed.

The assumption of homogeneity of variances was tested using Cochran's test (Winer *et al.* 1991). Data were transformed to $\ln(x + 1)$ when significant. When

^c Although *Pyura stolonifera* is now *P. praeputialis*, in Australia it is still more commonly referred to and known as *P. stolonifera*.

transformations did not remove heterogeneity, analyses proceeded, because ANOVA can be robust to deviations from heterogeneity of variances, particularly with fully balanced designs with many independent estimates of variance (Underwood 1981).

In addition, the relative contribution of each spatial scale to the total variation was examined. Variance estimates were calculated for each taxon or derived variable for each spatial scale using ANOVAs of untransformed data (see standard procedures in Underwood 1997).

2.2.3 Statistical analyses: *Cost–benefit analyses*

Cost–benefit analyses were done to determine the experimental design appropriate for sampling most taxa most effectively. Analyses were done using variance estimates calculated from ANOVAs of untransformed data (see standard procedures described in Winer *et al.* 1991, Underwood 1997). The limiting cost was time. Given the relatively large amount of travelling and preparation time needed to get to a lake, it was inefficient for lakes to be sampled in fractions of days. Thus, it was important that a lake could be sampled within a single day. Therefore, the number of replicate locations, sites and transects were optimised to keep within a budget of 1 day lake⁻¹ (i.e. 360 min on the water, excluding travelling to and from a lake). The average time to sample 1 transect was 2 min, time to manoeuvre the boat between sites was 10 min and between locations was 20 min. Cost–benefit analyses were done only within levels of spatial scales in which taxa were present.

The number of replicate transects per site (n) was determined using:

$$n = \sqrt{\frac{S_e^2 \times C_b}{S_{B(A)}^2 \times C_n}} \quad (1)$$

The number of replicate sites per location (b) was determined using:

$$b = \sqrt{\frac{S_{B(A)}^2 \times C_a}{S_A^2 \times C_b}} \quad (2)$$

The optimal number of locations per lake (a) was then determined as:

$$a = \frac{\text{Cost of lake}}{C_a + bC_b + bnC_n} \quad (3)$$

where S_e^2 is the estimated variance among transects, $S_{A(B)}^2$ is the estimated variance among sites, S_A^2 is the estimated variance among locations, C_a is the cost per location, C_b is the cost per site and C_n is the cost per transect.

2.3 RESULTS

2.3.1 List of taxa

In Wallis Lake, 9 species of sponges and 3 species of ascidians were found and, in St Georges Basin, 2 species of sponges and 2 species of ascidians (Table 2.1). Of the 9 species of sponges collected, only 2 could be identified to species level. The remainder were either undescribed, or we were presently unable to assign them to known taxa given the plethora of taxa in the older literature the identities of which still remain a mystery (Hooper & Wiedenmayer 1994). Because it was not possible to distinguish among species of *Halichondria* in the field, all species in this genus were simply recorded as *Halichondria* spp.

Table 2.1. Taxa found in each location (1 to 6) in St Georges Basin and/or Wallis Lake (+ species present; – species absent)

Taxa	St Georges Basin						Wallis Lake					
	1	2	3	4	5	6	1	2	3	4	5	6
Porifera												
<i>Aplysilla</i> cf. <i>sulphurea</i>	–	–	–	–	–	–	+	–	–	–	–	–
<i>Aplysinella</i> cf. <i>rhax</i>	+	+	–	+	+	+	–	–	–	–	–	–
<i>Halichondria</i> spp.	+	–	+	+	+	+	–	–	+	–	–	–
<i>Haliclona</i> sp. 1	–	–	–	–	–	–	–	–	+	–	–	–
<i>Haliclona</i> sp. 2	–	–	–	–	–	–	–	–	–	+	–	–
<i>Dysidea</i> sp.	–	–	–	–	–	–	+	–	–	–	–	–
<i>Myscale</i> sp.	–	–	–	–	–	–	+	–	–	–	+	–
<i>Raspailia</i> sp.	–	–	–	–	–	–	–	–	–	–	+	–
<i>Suberites</i> sp.	–	–	–	–	–	–	+	+	–	–	–	+
Ascidacea												
<i>Microcosmus</i> sp.	–	–	–	–	–	–	+	–	+	–	–	–
<i>Pyura stolonifera</i>	+	+	+	+	+	+	–	–	+	+	–	–
<i>Styela plicata</i>	+	+	–	+	+	+	+	+	+	+	+	+

The taxa identified differed markedly between the two lakes, with 2 of the 3 ascidians, *Styela plicata* and *Pyura stolonifera*, but only 1 sponge, *Halichondria* spp., found in both lakes (Table 2.1). In addition, patterns of presence/absence of individual taxa within lakes differed greatly between the two lakes. While *Aplysinella* cf. *rhax*, *Halichondria* spp., *P. stolonifera* and *S. plicata* were widespread throughout St Georges Basin, most taxa in Wallis Lake were found in 1 or a limited number of locations, with the exception of the introduced *S. plicata*.

2.3.2 Spatial scales of variation

At the lake scale (100s of kilometres apart), there was no significant variation in mean numbers of taxa, total numbers of individual sponges, nor abundances of *Styela plicata* per transect (Table 2.2; Figure 2.2) In contrast, there was significant variation of these variables at the smaller scales of location (kilometres apart) and site (100s of metres apart) within lakes (Table 2.2; Figure 2.2). Similarly, in St Georges Basin, there was significant variation in the abundances of *Aplysinella* cf. *rhax* and *Pyura stolonifera* at the scales of location and site (Table 2.3; Figure 2.2)

Table 2.2. ANOVAs to examine variation for selected variables between and within St Georges Basin and Wallis Lake (n.s.- not significant, *** $p < 0.001$)

Source of variation	df	Total no. of taxa			Total no. of individual sponges			No. of <i>Styela plicata</i>		
		Mean square	<i>F</i>	<i>p</i>	Mean square	<i>F</i>	<i>p</i>	Mean square	<i>F</i>	<i>p</i>
Lake	1	12.02	1.03	n.s.	32.53	1.20	n.s.	104.46	1.06	n.s.
Location (La)	10	11.67	9.40	***	27.00	5.04	***	98.66	10.78	***
Site (Lo(La))	36	1.24	10.98	***	5.36	11.76	***	9.16	29.86	***
Residual	912	0.11			0.46			0.31		
Transformation		Ln($x + 1$)			Ln($x + 1$)			Ln($x + 1$)		

Table 2.3. ANOVAs to examine variation among locations and sites for abundances of *Aplysinella* cf. *rhax* and *Pyura stolonifera* in St Georges Basin (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$)

Source of variation	df	<i>A. cf. rhax</i>			<i>P. stolonifera</i>		
		Mean square	<i>F</i>	<i>p</i>	Mean square	<i>F</i>	<i>p</i>
Location	5	30.63	3.39	*	41.92	4.35	**
Site (Lo)	18	9.04	13.68	***	9.64	11.87	***
Residual	456	0.66			0.81		
Transformation		Ln($x + 1$)			Ln($x + 1$)		

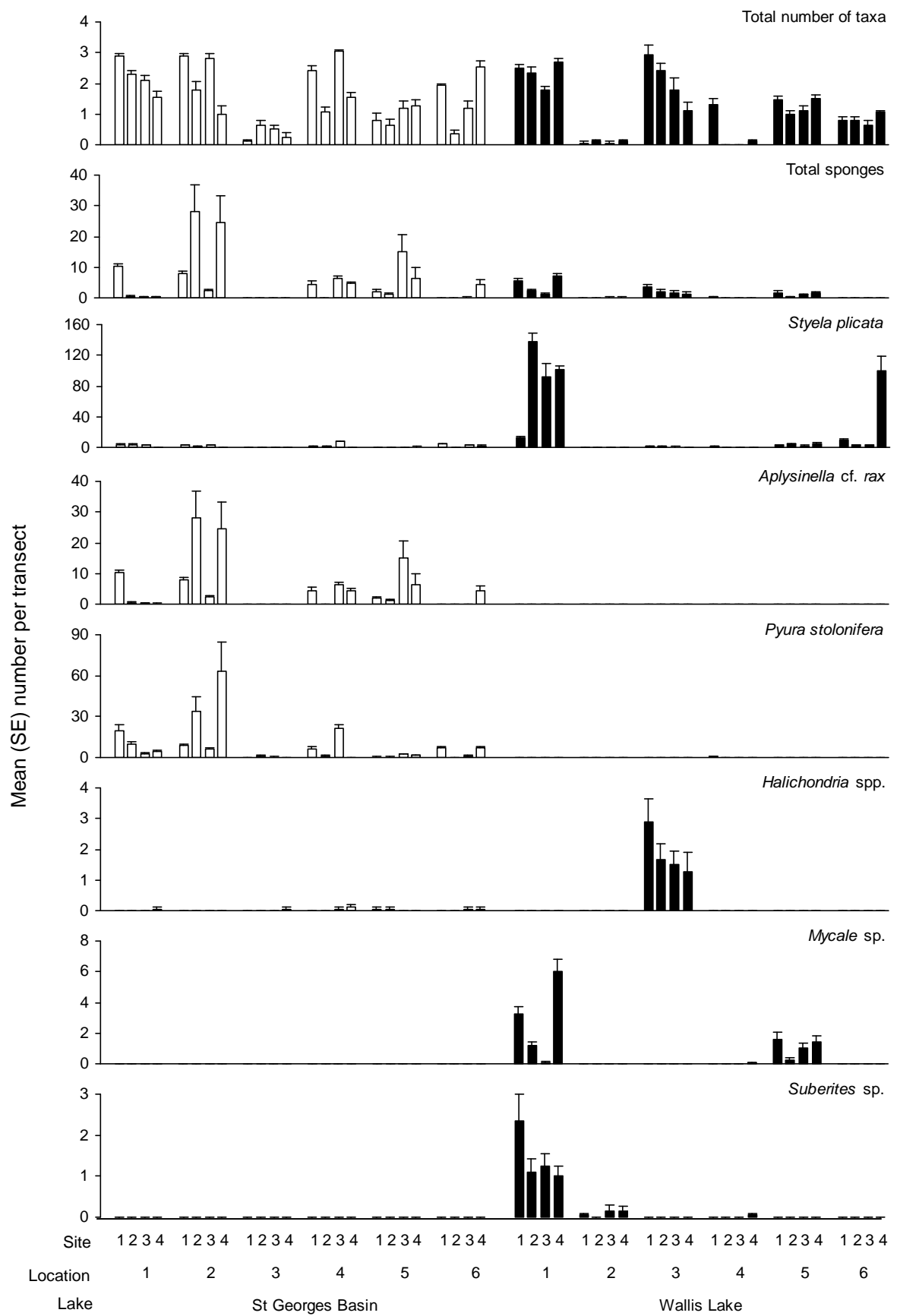


Figure 2.2. Mean (SE) numbers of taxa per transect ($n = 20$) at each site.

In Wallis Lake, preliminary examination of raw data for each taxon showed obvious patchiness at the scale of location (kilometres apart) (Table 2.2, Figure 2.2). ANOVAs were therefore done only within locations where specific taxa occurred and where abundances were large enough for ANOVA to be appropriate. Variation among sites was not significant for *Halichondria* spp. (Location 3), *Mycale* sp. (Location 5), or *Suberites* sp. (Location 1). In contrast, there was significant variation among sites for *Mycale* sp. at Location 1 (Table 2.4, Figure 2.2).

Table 2.4. ANOVAs to examine variation among sites within specified locations in Wallis Lake for each of *Halichondria* spp., *Mycale* sp. and *Suberites* sp. Locations are indicated in parentheses (n.s. - not significant, *** $p < 0.001$)

Source of variation	df	<i>Halichondria</i> spp. (L3)			<i>Mycale</i> sp. (L1)			<i>Mycale</i> sp. (L5)			<i>Suberites</i> sp. (L1)		
		Mean square	<i>F</i>	<i>p</i>	Mean square	<i>F</i>	<i>p</i>	Mean square	<i>F</i>	<i>p</i>	Mean square	<i>F</i>	<i>p</i>
Site	3	10.82	1.42	n.s.	11.54	38.19	***	6.77	2.48	n.s.	0.44	1.04	n.s.
Residual	76	7.59			0.30			2.73			0.42		
Transformation		None			Ln($x + 1$)			None			Ln($x + 1$)		

Estimates of variance components for each variable are shown in Table 2.5.

Caution should be used in the interpretation of variance components across taxa because the size of the residual variance will affect the contribution of each spatial scale to the total variance (Morrissey *et al.* 1992, Underwood & Petraitis 1993). When the proportion of the residual variance differs among taxa, proportions of other spatial scales should not be compared across taxa. The size of residual variance, however, will not affect the ratio among other variance components within a taxon (Underwood & Petraitis 1993). Therefore, ratios of variance estimates can be compared. In the present study, there was considerable residual variance (i.e. among transects within sites) for all taxa examined. The proportion of residual variance ranged from 27 to 73% for variables examined in both lakes, 74 to 76% for *Aplysinella* cf. *rhax* and *Pyura stolonifera* in St Georges Basin

and 43 to 98% for *Suberites* sp., *Mycale* sp. and *Halichondria* spp. in Wallis Lake.

These relatively large contributions suggest that there was also considerable patchiness at small spatial scales of 10s of metres within sites.

In addition, variance components confirm that there was little variation between lakes for number of taxa, total number of sponges and *Styela plicata*, but most variation was at the smaller spatial scales of locations, sites and transects.

Table 2.5. Variance estimates derived from ANOVA for selected variables calculated from untransformed data. Locations are indicated in parentheses (–: variances were not calculated at that spatial scale)

Source of variation	Total no. of taxa	Total no. of sponges	<i>Styela plicata</i>	<i>Aplysinella</i> cf. <i>rhax</i>	<i>Pyura stolonifera</i>	<i>Suberites</i> sp. (L1)	<i>Mycale</i> sp. (L1)	<i>Mycale</i> sp. (L5)	<i>Halichondria</i> spp. (L3)
Lake	0.03	4	68	–	–	–	–	–	–
Location (La)	0.53	12	478	22	67	–	–	–	–
Site (Lo(La))	0.28	16	405	31	119	0.23	6.53	0.20	0.16
Residual	0.50	86	344	169	537	3.24	5.00	2.73	7.60

2.3.3 Optimising replication: Cost–benefit analyses

Appropriate replication was determined in 3 stages. First, cost–benefit analyses were done to determine the optimal replication for comparisons among lakes. Analyses were done for numbers of taxa, total numbers of sponges and for *Styela plicata* using data from both lakes and for *Aplysinella* cf. *rhax* and *Pyura stolonifera* using data from St Georges Basin. Because fractions of replicates cannot be sampled, numbers of replicates were rounded to whole numbers keeping near to the budget of 360 min lake^{–1}. These analyses produced 3 different designs: (1) 10 locations, 1 site and 3 transects for numbers of taxa; (2) 6 locations, 2 sites and 5 transects for total numbers of sponges, *A. rhax* and *P. stolonifera*; and (3) 11 locations, 1 site and 2 transects for *S. plicata* Table 2.6. Because *S. plicata* is an introduced species and the primary aim of the research programme was to examine native species, Option 3 was not considered further.

Next, to determine which design would be the best compromise for sampling most taxa, cost–benefit analyses were done for *Suberites* sp., *Mycale* sp. and *Halichondria* spp. within locations in Wallis Lake (Table 2.6). The limiting cost in these analyses was the time available to sample 1 location (including 20 min of travelling time between locations). Analyses were done using: (1) 10 locations lake⁻¹ (i.e. 36 min location⁻¹) and (2) 6 locations lake⁻¹ (i.e. 60 min location⁻¹). For Option 1 (10 locations lake⁻¹), although there were originally differences among species in the numbers of sites (0.4 to 1.2) and transects (2.0 to 15.4), when the numbers were rounded to stay within the time budget, the design became the same for all species (1 site and 3 transects location⁻¹; Table 2.6). For Option 2 (6 locations lake⁻¹), the design varied from 1 to 3 sites location⁻¹ and 15 to 2 transects site⁻¹ (Table 2.6).

Table 2.6. Replication at each spatial scale derived from cost–benefit analyses for sampling sponges and ascidians. Values in parentheses have not been rounded. Numbers in bold have been rounded to whole units of sampling. In cases where there was a choice between rounding up or down, the replication that produced the more precise estimate of the mean is given.

	Both lakes		St Georges Basin			Wallis Lake			
	No. of taxa	Sponges	<i>Styela plicata</i>	<i>Aplysinella</i> cf. <i>rhax</i>	<i>Pyura stolonifera</i>	<i>Suberites</i> sp. (L1)	<i>Mycale</i> sp. (L1)	<i>Mycale</i> sp. (L5)	<i>Halichondria</i> spp. (L3)
Locations	10 (10.0)	6 (6.0)	11 (10.6)	6 (6.7)	6 (6.0)	Option 1: 10 locations lake⁻¹			
Sites (Lo)	1 (1.0)	2 (1.6)	1 (1.3)	2 (1.7)	2 (1.9)	1 (0.6)	1 (1.2)	1 (0.6)	1 (0.4)
Transects (S(Lo))	3 (3.0)	5 (5.2)	2 (2.1)	5 (5.3)	5 (4.8)	3 (8.4)	3 (2.0)	3 (8.2)	3 (15.4)
Locations						Option 2: 6 locations lake⁻¹			
Sites (Lo)						2 (1.5)	3 (2.9)	2 (1.5)	1 (1.0)
Transects (S(Lo))						5 (8.4)	2 (2.0)	5 (8.2)	15 (15.4)

Finally, to determine the best compromise in replication, the precision of estimating the means of each design was compared among the different variables. Precision was calculated as the estimated standard error of the mean (SEM, number per sample) and expressed as a percentage. The estimated SEM was calculated as the square root of the estimated variance of the means, V , where:

$$V = \frac{(S_e^2 + n \times S_{B(A)}^2 + n \times b \times S_A^2)}{n \times b \times a} \quad (4)$$

for calculating SEM per lake and:

$$V = \frac{(S_e^2 + n \times S_{B(A)}^2)}{n \times b} \quad (5)$$

for calculating SEM per location.

At the location scale, the precision of sampling patchily distributed taxa (*Suberites* sp., *Mycale* sp. and *Halichondria* spp.) was greatly improved by using 2 sites and 5 transects compared to 1 site and 3 transects Table 2.7. In comparison, there was only a relatively small loss in precision of the mean at the scale of lake when sampling number of taxa and *Styela plicata* with 6 locations, 2 sites and 5 transects compared to 10 locations and 3 transects. Therefore, it was concluded that the best allocation of resources would be to use 6 locations, 2 sites and 5 transects lake⁻¹.

Table 2.7. Precision of estimating means measured as the standard error of the mean for selected variables at the scales of lake and location, using different numbers of locations, sites and transects.

Numbers of locations, sites, transects	Precision at the scale of lake	Precision at the scale of location				
	No. of taxa	<i>Styela</i> <i>plicata</i>	<i>Suberites</i> sp. (L1)	<i>Mycale</i> sp. (L1)	<i>Mycale</i> sp. (L5)	<i>Halichondria</i> spp. (L3)
10, 1, 3	24.6%	92.9%	80.3%	108.0%	100.4%	89.9%
6, 2, 5	27.2 %	101.5%	46.5%	73.2%	58.2%	50.2%

2.4 DISCUSSION

Two key patterns of distribution of sponges and ascidians in coastal lakes are highlighted by this study. First, most of the sponges and ascidians were clearly very patchily distributed at a range of spatial scales from 10s of metres up to 100s of kilometres, which appears common for many species of sponges and ascidians in other habitats (Roberts & Davis 1996, Ferdeghini *et al.* 2000, Hooper & Kennedy 2002, Hooper *et al.* 2002). Second, few taxa were widespread over the larger spatial scales. While similar patterns have been found in other enclosed bodies of water (e.g. Kuenen & Debrot 1995), the organisation of assemblages in these lakes appears fundamentally different to those on the open coast, where assemblages of sponges and ascidians usually consist of several patchy but widespread species and numerous very uncommon taxa (e.g. Wilkinson & Evans 1989, Farnsworth & Ellison 1996, Roberts & Davis 1996,).

Such variability suggests there may be many different processes operating and influencing these patterns at a range of scales from a few metres to an entire lake (Underwood & Chapman 1996b). In addition, patterns were complex and not consistent between lakes, suggesting different processes may be operating in different lakes. Numerous mechanisms, including predation (Wulff 2000), availability of substrata to settle on (Keough 1984), water quality (Burns & Bingham 2002), hydrodynamics (Guichard & Bourget 1998), competition, dispersal and recruitment (Farnsworth & Ellison 1996) have been proposed and examined to explain these distributions.

At the largest spatial scale of lakes (100s of kilometres apart), while the mean number of taxa and mean abundance of sponges per transect did not differ, the composition of assemblages differed greatly between the two lakes. Only 1 of 9 genera of sponges was found in both lakes, and these (*Halichondria* spp.) may be different

species. Differences between lakes point to processes operating at large spatial scales of the entire lake and/or region. Although little is known of many of the taxa found in this study, it is logical to suggest different species may have different tolerances to the physiological stresses imposed by these environments and hence different distributions. For example, water quality can vary greatly among NSW lakes (Pollard 1994a, West & Jones 2000). Large-scale floods can dramatically change physical variables such as salinity, temperature, turbidity and pH and affect entire estuaries and assemblages of animals (Moverley *et al.* 1986). Further, the magnitude and duration of changes after input of freshwater may vary greatly among different NSW coastal lakes (e.g. Pollard 1994a). Similarly, the regime of opening and closing of entrances of NSW lakes is known to affect water quality, which can be correlated with the distribution of some organisms (Dye & Barros 2005a & b). Differences in species composition may also be due to limited dispersal between lakes. Dispersal may be limited because: (1) coastal lakes in NSW are separated by 10s to 100s of kilometres of open coast, (2) it appears that the distributions of many of these sponges are not continuous along the coast and may be restricted to lakes or estuaries (P. Barnes unpubl. data) and (3) many sponges have short dispersal distances (Zea 1993, Farnsworth & Ellison 1996, Maldonado & Young 1996, but see Davis *et al.* 1996).

Patchiness was also clear at smaller spatial scales from 10s and 100s of metres to kilometres apart within lakes. This was most evident in Wallis Lake where only 2 of the 8 species of sponge were found in >1 location. Nevertheless, some species were relatively abundant at some places. Similarly, the abundances of those more widespread taxa were significantly variable within the spatial scales in which they occurred. Again, numerous processes have been proposed to explain smaller-scale patterns. For example, abiotic factors which may affect sponges such as sedimentation rates (Burns &

Bingham 2002) and turbidity (Bell & Barnes 2000a) are known to differ among areas within lakes kilometres apart (Roberts 2001, Sloss *et al.* 2004). Larval recruitment and small dispersal distances have been proposed as important in explaining aggregated patterns of distribution of epibionts (including sponges and ascidians) over relatively small scales of metres to 100s of metres (Farnsworth & Ellison 1996). Asexual propagation via fragmentation or budding is common in sponges and may be important in determining distributions or maintaining populations in some areas (Wulff 1991; Bingham & Young 1995). Predation by fish has been found to play a key role in structuring assemblages of sponges in some habitats (Pawlik 1998, Wulff 2000, 2005), and abundances of fishes are often patchy and differ among habitats within and among NSW lakes (Pollard 1994b).

The shallow areas sampled in these lakes are often a mosaic of patches of different species of seagrasses and macroalgae and patches of bare sediment (West *et al.* 1985, Cummins *et al.* 2004b), which vary over scales of metres to kilometres. Such patchiness in habitat may have a number of consequences for the distribution of sponges and ascidians. For example, sponges and ascidians were found attached to a variety of surfaces, including seagrasses, macroalgae and fragments of shells, and unattached on top of patches of sediment. Small-scale patchiness in the distribution of sponges and ascidians may therefore be related to the availability of suitable substratum on which to settle (Keough 1984). In addition, assemblages of potential predators may differ among types of vegetation and, therefore, affect distributions of sponges and ascidians. Overall, it is likely that many processes are interacting to influence patterns of distribution.

Greater understanding of the ecology of sponges and ascidians in coastal lakes and, hence, long-term conservation will best be achieved by experimental examination of the processes causing small- and large-scale patterns of variation. Also, because

patterns of distribution varied greatly among species, further experiments should include examination of specific species (Cummins *et al.* 2004a). However, because assemblages of sponges and ascidians in these habitats are virtually unknown, it would be beneficial first to test the generality or otherwise of these patterns through time and among different lakes. The findings of this study have several important implications for the design of such research programmes.

At the lake scale, although there were no significant differences in the mean number of taxa, individual sponges, or *Styela plicata* per transect, there were obvious differences in the composition of assemblages between Wallis Lake and St Georges Basin. Two-thirds of the taxa were exclusively found in one or the other lake. Wallis Lake had more taxa (11) compared to St Georges Basin (4) and taxa were widespread throughout St Georges Basin, but in Wallis Lake most were restricted to 1 or a few locations. Such obvious differences emphasise the need to include adequate replication at the lake scale for studies examining differences in composition of assemblages among large spatial scales (e.g. regions of coast) or types of lake (e.g. urbanised versus relatively pristine, open versus intermittently open or closed to the sea). For such comparisons, inclusion of sampling at a hierarchy of spatial scales will further improve the power of tests for differences (Morrissey *et al.* 1992). Further, adequate replication at the smaller scales of 10s and 100s metres and kilometres will be needed to ensure differences between lakes are not masked by significant small-scale variation. Very patchy distributions at the scale of locations kilometres apart (as in Wallis Lake) also have important consequences for finding sponges in a lake. The number of locations sampled will determine the probability of a particular species being found. For example, *Mycale* sp. was widespread in only 2 of the 6 locations in Wallis Lake. Logically, the probability of sampling at least 1 location with *Mycale* sp. will increase with the number

of locations sampled (for theory on sampling rare species see Kovalak *et al.* 1986, Green & Young 1993).

In the present study, unlike other published examples of spatial variation and cost–benefit analyses (Kennelly & Underwood 1985, Morrissey *et al.* 1992, Bennedetti-Cecchi *et al.* 1996, Bartsch *et al.* 1998), there were very few taxa widespread over all spatial scales. Such studies quite appropriately chose to analyse taxa that were ‘consistently present’ (Morrissey *et al.* 1992), because they presumably represented a large proportion of and were therefore representative of the assemblage. In contrast, this study found that it was often the patchily distributed taxa that represented the largest proportion of the assemblage. In this case, it was important to optimise sampling designs for those taxa. It should not be assumed that derived variables such as total number of taxa are appropriate surrogates for designing experiments to sample individual species. Rather, if the aim of the sampling programme is to sample many taxa as precisely as possible, designs can be improved by including patchily distributed taxa in cost–benefit analyses. In this study, it was found that the selection of a sampling design that led to relatively large increases in precision of sampling patchily distributed taxa, resulted in only relatively small compromises in the precision of sampling widespread variables.

CHAPTER 3: HUMAN IMPACTS AND PATTERNS OF DISTRIBUTION OF SPONGES AND ASCIDIANS AMONG NEW SOUTH WALES COASTAL LAKES AND LAGOONS

3.1 INTRODUCTION

Estuaries worldwide are common focal points for cities, towns, industry and agriculture (Kennish 2002). Increasing threats and impacts from development have resulted in the need for increased intervention and management of these environments to ensure their long-term ecological functioning and conservation (Lee *et al.* 2006; Lotze *et al.* 2006). While estuaries are among the most intensively studied ecosystems in the world, they constitute a diversity of habitats and forms, with the ecology and biodiversity of many types of estuaries remaining poorly understood. The more intensively studied estuaries include many of the larger and river dominated examples such as Chesapeake Bay (Kemp *et al.* 2005), San Francisco Bay (Thompson *et al.* 2007) and Sydney Harbour (Bulleri *et al.* 2005). Coastal lagoons, however, are morphologically very different types of estuaries. They are common in many parts of the world including Africa, South America, the Mediterranean and Australia (Whitfield 1992; Suzuki *et al.* 1998; Roy *et al.* 2001). While considerable work has been done on lagoons in Europe and North America and in recent times attention has increased elsewhere in the world, in temperate Australia they remain relatively understudied.

There are also large imbalances in the amount of research done on different taxonomic or functional groups in these systems. Groups such as fish and seagrasses have been studied relatively intensively (Bostrom *et al.* 2006; Orth *et al.* 2006a) compared to other groups such as sponges and ascidians. Sponges have been rarely studied in coastal lagoons with the exception of Italian lagoons (see Table 1.1). Further,

physical and biological processes differ among estuary types and it cannot be assumed all taxonomic groups will respond in the same way (Roy *et al.* 2001). Without a broad scientific understanding of how different groups respond in different estuaries and under different estuarine conditions, management may be left to guesswork and conservation cannot be assured.

In south-eastern Australia, a high proportion of the human population live near the coast and this is steadily increasing (Zann 1995). In this region, saline coastal lakes and lagoons are a common type of estuary (Roy *et al.* 2001) and encompass a diversity of morphologies, hydrology, levels of human impact, uses and management. They range in size from small coastal creeks of less than a few hectares in water surface area to large lakes tens of km² in area (Geoscience Australia 2001; Roy *et al.* 2001; Haines 2003). Regimes of opening with the sea vary in duration and frequency from lakes and lagoons that are permanently open, to those that open and close numerous times a year, to those which are closed for periods of years (Haines 2003). Furthermore, the majority of New South Wales' ninety lakes and lagoons have some degree of development (agriculture, industry or urbanisation) in their catchments with only six classified as near pristine (Healthy Rivers Commission of NSW 2002). Many sustain commercial and recreational fisheries, oyster farms and are popular tourist destinations (Turner *et al.* 2004). Finally, because of the increased pressures of urbanisation and development, these systems are becoming increasingly managed. For example, up to 30% of lakes and lagoons in NSW are artificially opened to the sea usually with the justification of improving the water quality or reducing the risk of flooding (Haines 2004).

Despite their proximity to development and varied uses, scientific knowledge of the biology and ecology of these systems is limited to some groups of organisms in a subset of lakes and lagoons. For example, seagrasses (e.g. West *et al.* 1985; King

1986), phytoplankton (Cummins *et al.* 2004a), fish (Pollard 1994a; West & Jones 2000; Griffiths & West 1999) and meiobenthic (Dye 2005; Dye & Barros 2005a) and macrobenthic assemblages (Dye & Barros 2005b) have been studied in a number of NSW lakes and lagoons. Virtually nothing, however, is known of the distribution, biology nor ecology of epibenthic fauna, namely sponges and ascidians in these systems. With the exception of von Lendenfeld's (1888) inventory for Lake Illawarra, a recent search of the scientific literature found no studies directly relating to sponges, no quantitative descriptions of their distribution, nor any reliable names of species in NSW coastal lakes.

The conservation of biodiversity in these systems will rely heavily on effective management, which will in turn rely on a sound scientific understanding of natural processes, unplanned human impacts (Underwood & Chapman 2003) and the effects (positive or deleterious) of managerial actions. The logical first step in this process, however, is to understand the basic patterns of distribution of species (Underwood *et al.* 2000). Sponges and ascidians in NSW coastal lakes represent an unusual group of organisms to study compared to other groups because of the paucity of information regarding even broad scale patterns of distribution. For example, for groups such as fishes, seagrasses and macrofauna it is at least known in which lakes they occur, but for sponges and ascidians even their presence or absence in particular lakes has yet to be determined. In systems where little is known about the distribution of organisms, it is sensible first to examine relatively broad scale patterns of distribution and then refine theories to examine the processes that underpin these patterns.

When choosing the spatial or temporal factors to include in such initial studies much can be gained, in terms of the ecological relevance of the results, by examining known patterns of distribution of other organisms in similar habitats. One of the key

attributes that has been examined for coastal lakes is the regime of opening and closing to the open ocean (Pollard 1994a; Teske & Wooldridge 2003; Dye 2006). A pattern common to many groups is higher species diversity, but smaller abundances in permanently open compared to intermittently closed systems (e.g. fish – Pollard 1994a; macrobenthos - Teske & Wooldridge 2001; Dye & Barros 2005a; Meiofauna – Dye & Barros 2005b). It has been suggested high diversity may arise from processes such as recruitment from the ocean or more stable environmental conditions in permanently open systems. Although little is known of the recruitment processes and environmental requirements of sponges and ascidians in these systems, it is likely they may differ from other taxa. The size of an estuary has also been identified as important to the structure of macrobenthic assemblages (Teske & Wooldridge 2001).

Based on these observations, in this study, I tested the hypothesis that assemblages of sponges and ascidians would differ among lakes of different size and opening regime. Further, in other coastal environments, distribution of sponges and ascidians may be affected by a variety of human impacts (Carballo *et al.* 1996; Carballo & Naranjo 2002) including sewage and silt deposition (Roberts *et al.* 1998; Roberts *et al.* 2006). I therefore tested a second hypothesis that assemblages in lakes extensively impacted by human development would be different from relatively less impacted lakes.

3.2 METHODS

3.2.1 Study-sites and sampling methods

To test for differences among lakes of different sizes and different opening regimes, lakes were paired according to two opening regimes and two arbitrarily chosen size classes (Table 3.1, Figure 3.1). Categories of opening regime follow those of Haines (2003). Haines (2003) reported that the opening regimes of the majority of

NSW coastal lakes could be divided into two distinct groups; Lakes with entrances closed to the sea more than 60% of the time (classified as ‘mostly closed’) and lakes with entrances closed less than 20% of the time (classified as ‘mostly open’). In this study, lakes were classified as mostly closed or mostly open based on opening records in the three years (1999-2002) previous to this study (Geoscience Australia 2001, Roberts & Dickinson 2005, Lake Illawarra Authority, pers. comm.). Lakes with a surface area between three and five km² were arbitrarily classified as small and larger than six km² as large. Ideally, all large lakes would have been of a similar size. However, given the restricted number and types of lakes to choose from, it must be noted that the two large closed lakes (Smiths and Coila, Figure 3.1) were relatively smaller than the two large open lakes (Wallis Lake and St Georges Basin, Figure 3.1) and caution should be used when interpreting comparisons between the lake types.

To test for differences in assemblages between lakes with different levels of human impact, two extensively impacted lakes (Tuggerah and Illawarra) were compared to two relatively less impacted lakes (Wallis and St Georges Basin). We used the classifications of estuaries and their catchments found in the Ozestuaries database (Geoscience Australia 2001). The Ozestuaries’ classifications are based on a number of indicators of environmental condition and increase in severity of human impact from ‘pristine’, ‘near pristine’, ‘largely unmodified’, ‘modified’ to ‘extensively modified’ as the highest level of impact (Walker & Veitch 2001). Lake Illawarra and Tuggerah Lake were classified as ‘extensively modified’, and Wallis Lake and St Georges Basin as ‘modified’. All four lakes were relatively large and mostly open to the sea (Figure 3.1). Tuggerah Lake had been open to the sea since 1993 (Roberts & Dickinson 2005) and Lake Illawarra was open from 1998 (Lake Illawarra Authority, pers. comm.).

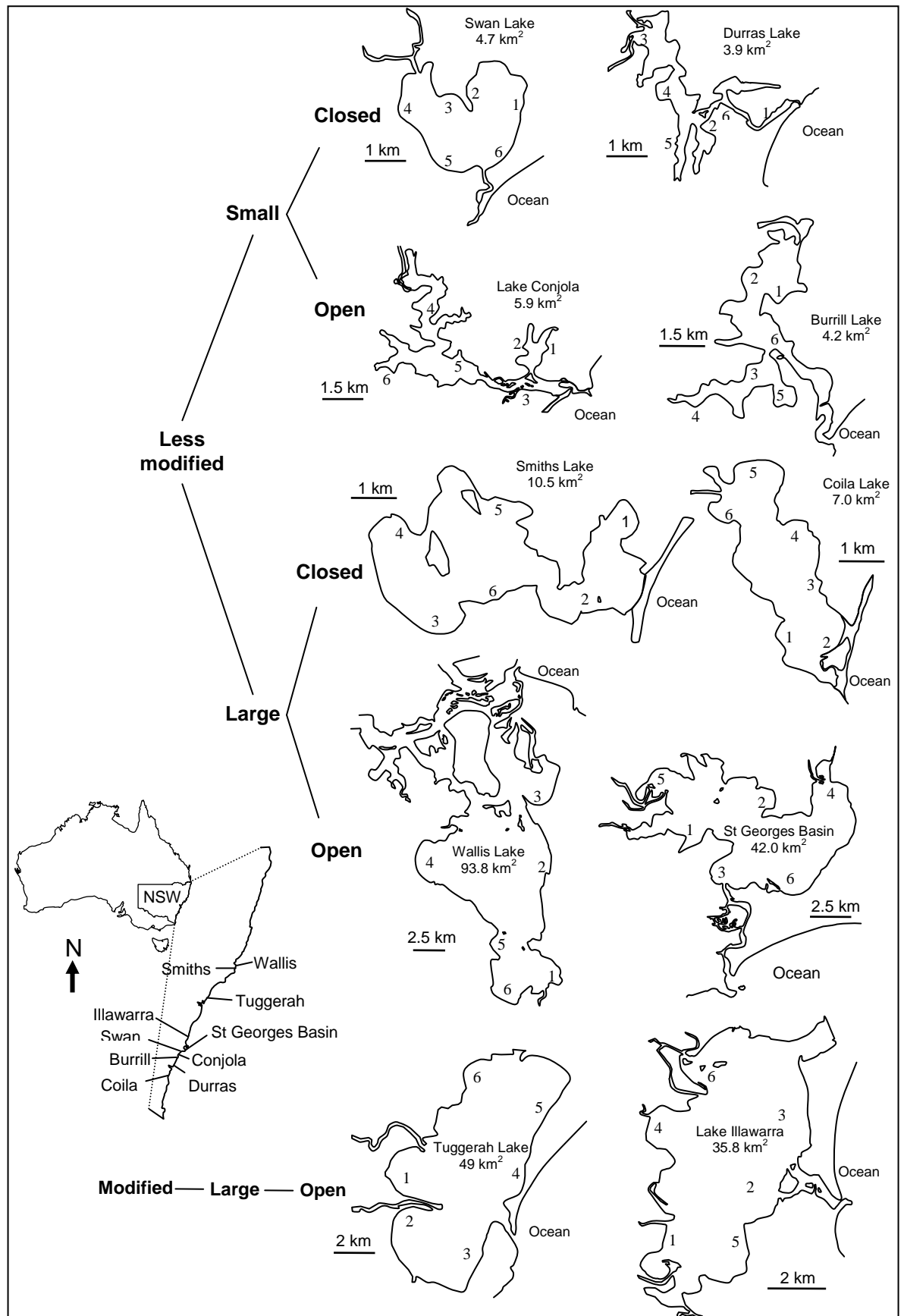


Figure 3.1. Locations sampled in 10 New South Wales lakes and lagoons.

Pilot surveys suggested that sponges and ascidians in NSW coastal lakes were most diverse and abundant in the relatively shallow (0 to 2.5 metres depth) seagrass and macroalgal meadows of coastal lakes (Barnes *et al.* 2006). In the current study, the distributions of sponges and ascidians were quantified in ten coastal lakes in New South Wales Australia between January and May 2002, using two methods. First, transects were used to quantify the relatively abundant species of sponges and ascidians. Individual sponges, solitary and colonial ascidians were counted by a snorkeller in replicate 10 x 2 m transects. Second, because many species of sponge and ascidian are very uncommon and sparsely distributed in NSW coastal lakes (Barnes *et al.* 2006), and are therefore unlikely to be found in relatively small transects, timed searches were used to determine the presence or absence of species. Replicate timed searches in which a snorkeller swam haphazardly for five minutes within a site allowed more area to be searched than within transects and hence greatly increased the probability of finding the less common species. Each method was used at a hierarchy of spatial scales in each lake. In each lake, six locations (kilometres apart), each with four sites (100s of metres apart), each with six replicate 10 x 2 metre transects (10s of metres apart) and four replicate five minute timed searches were done (See Barnes *et al.* 2006 for optimisation of sampling design). Sites were approximately 80 metres in diameter.

Voucher specimens of sponges were identified by and lodged with the Queensland Museum, Brisbane, Australia. Voucher specimens of ascidians are held at the University of Wollongong.

3.2.2 Statistical analyses

Non metric MDS ordinations (PRIMER, Clarke 1993) were used to illustrate patterns of difference among lakes for sponge and ascidian assemblages separately.

Data were pooled to provide a centroid for each lake and square-root transformed to reduce the influence of very abundant taxa.

Two sets of analysis of variance were used to test for differences in abundances of some of the relatively more widespread and abundant taxa (total sponges, total ascidians, *Styela plicata* Lesueur and *Pyura stolonifera* Heller). First, five factor analyses of variance were used to test for differences among lakes with different opening regimes and different sizes. The factors were; Opening Regime (fixed and orthogonal with two levels; mostly open and mostly closed), Size (fixed and orthogonal with two levels; small and large), Lake (random and nested in the interaction of Opening Regime and Size with two levels), Location (random and nested in Lake with six levels) and Site (random and nested in Location with two levels) with $n = 6$ replicate transects per site. Second, four factor analyses of variance were used to test for differences between Lakes with different levels of human impact. The factors were; level of Impact (fixed and orthogonal with two levels; extensively modified and less modified), Lake (random and nested in level of Impact with two levels), Location (random and nested in Lake with six levels) and Site (random and nested in Location with two levels) with $n = 6$ replicate transects per Site.

The assumption of homogeneity of variances was tested using Cochran's Test (Winer *et al.* 1991). Data were transformed to $\text{Ln}(x + 1)$ when significant. When transformations did not remove heterogeneity, analyses proceeded because analysis of variance can be robust to deviations from heterogeneity of variances, particularly with fully balanced designs with many independent estimates of variance (Underwood 1981). Post-hoc pooling was used to construct tests for higher order effects when $p > 0.25$.

3.3 RESULTS

3.3.1 List of taxa

Eighteen species of sponge and six species of ascidian were found in the ten Lakes (Table 3.1). Of the eighteen species of sponge collected, only two could be tentatively identified to species. The remainder are likely to be undescribed or presently unable to be assigned to a known taxon given the plethora of taxa in the older literature whose identities still remain a mystery (Hooper & Wiedenmayer 1994).

3.3.2 Sponges: Open versus closed and big versus small lakes

In the relatively less impacted lakes, the presence of sponges was related to the combination of opening regime and size, with sponges found in all, but the two small closed lakes (Table 3.1). In the four open and two large closed lakes where sponges were found, there were no widespread species and accordingly there were striking differences in the composition of assemblages among lakes with few clear patterns of similarity between lakes of similar types (Table 3.1, Figure 3.2a). The two small open lakes, Conjola and Burrill, were the most similar in terms of species present, but still had only three out of a total of seven species in common (Table 3.1). Most species of sponge were found only in a single lake (10 of 18 species) or two lakes (5 of 18 species) (Table 3.1). Only three species were found in more than two lakes. *Mycale* sp. and *Aplysilla* cf. *sulphurea* were found in the same three lakes (Wallis, Conjola and Burrill), all mostly open to the ocean (Table 3.1), but were relatively uncommon within these lakes with only two specimens of *Mycale* sp. found in each of Burrill and Conjola. *Suberites* sp.1 was found in the most northern lakes (Wallis and Smiths) and the open, but relatively impacted Tuggerah Lake.

Table 3.1. Locations in which species were found in each of ten NSW coastal lakes. Includes data from timed searches and transects. Refer to Figure 3.1 for positions of Locations in each lake.

Condition	Less modified								Extensively modified	
Opening-regime	Mostly closed				Mostly open				Mostly open	
Size	Small		Large		Small		Large		Large	
Lake	Swan	Durras	Smiths	Coila	Conjola	Burrill	Wallis	St Georges	Tuggerah	Illawarra
Porifera										
<i>Aplysilla</i> cf. <i>sulphurea</i>					2	6	1			
<i>Aplysinella</i> cf. <i>rhax</i>								1,2,4,5,6		
<i>Dysidea</i> sp.							1			
<i>Halichondria</i> sp. 1					1		3			
<i>Halichondria</i> sp. 2					1					
<i>Halichondria</i> sp. 3							3			
<i>Halichondria</i> sp. 4								5		
<i>Halichondria</i> sp. 5									4,6	
<i>Haliclona</i> sp. 1							3		4	
<i>Haliclona</i> sp. 2							3,4			
<i>Hymeniacidon</i> sp. 1						3				
<i>Hymeniacidon</i> sp. 2				3	4					
<i>Mycale</i> sp.					6	6	1,4,5			
<i>Niphates</i> sp.						6				
<i>Raspailia</i> sp.							5			
<i>Suberites</i> sp. 1			1,3,4,5,6				1,2,4		4,6	
<i>Suberites</i> sp. 2					4	6				
<i>Tetilla</i> sp.			5				4			
Total number of sponge species per lake	0	0	2	1	6	5	10	2	3	0
Asidiacea										
<i>Botrylloides leachi</i>						6				
<i>Eudistoma laysani</i>		2,6			3	1,2,3,4,5,6				
<i>Herdmania grandis</i>									4	
<i>Microcosmus squamiger</i>							1,3			
<i>Pyura stolonifera</i>				1,2,3,4,5	1,2,3		3,4	1,2,3,4,5,6	5	
<i>Styela plicata</i>			3,4,6	2,3	4	1,2,3,4,5,6	1,3,4,5,6	1,2,3,4,5,6	1	1,4
Total number of ascidian species per lake	0	1	1	2	3	3	3	2	3	1

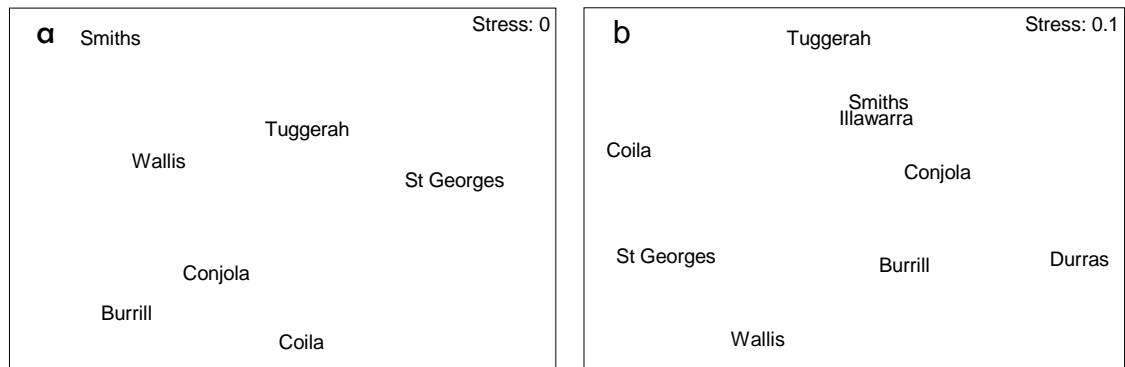


Figure 3.2. MDS plots comparing assemblages of (a) sponges and (b) ascidians among lakes. Each point represents the centroid for that lake. Note: Durras, Swan and Illawarra lakes are absent from (a) because no sponges were found and similarly Swan Lake is absent from (b) because no ascidians were found.

The abundance of all sponges (all species pooled together) did not vary significantly among types of lake (Table 3.2, Figure 3.3a). However, on average, the large and open St Georges Basin had the most sponges, consisting mostly of *Aplysinella* cf. *rhax*. Abundances of individual species of sponge were not compared statistically among types of lake because few were widespread and many were only found in timed searches.

Within each lake, most sponges were very patchily distributed with most found in only one location (Table 3.1). Only *Aplysinella* cf. *rhax* which was found in five of the six locations in St Georges Basin and *Suberites* sp. 1, which was found in five and three of the Locations in Smiths and Wallis Lakes respectively, could be considered widespread within a lake (Table 3.1).

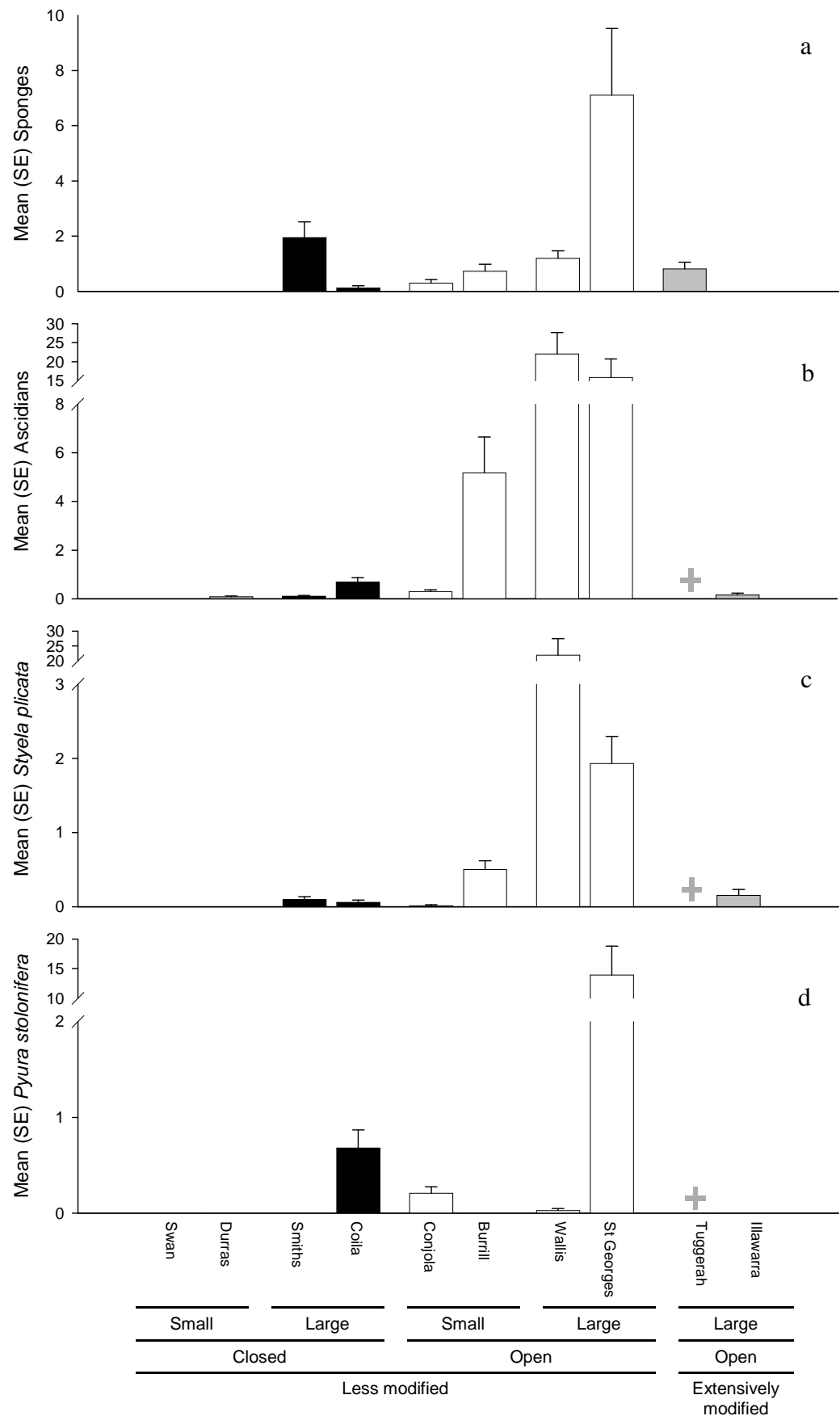


Figure 3.3. Mean (SE) numbers per transect in each lake. Note: axes are not to scale in histograms which are split. + indicates taxa were found in timed searches only.

3.3.3 Ascidians: Open versus closed and big versus small lakes

In general, assemblages of ascidians showed similar patterns of distribution among and within lakes as the sponge assemblages (Table 3.1, Figure 3.2b). Ascidiens were not found in the small and closed Swan Lake and only one species (*Eudistoma laysani*) was found in the other small and closed lake (Durras). Three of the six species were each found in single but different lakes (Table 3.1). Significantly, the introduced ascidian *Styela plicata* was the most widespread and abundant species among the lakes and was found in all but the two small closed lakes (Table 3.1, Figure 3.3c). The native *Pyura stolonifera* was the next most widespread.

The abundance of *Styela plicata* varied with the combination of opening regime and size with significantly more per transect in the large and open lakes compared to all other types of lake (Table 3.2, Figure 3.3c). The abundance of *Pyura stolonifera* did not vary significantly among types of lake, but did vary among the smaller scales between and within lakes (Table 3.2, Figure 3.3d). The abundances of the remaining ascidians were not compared statistically because they were not widespread and had very small abundances.

Table 3.2. Analyses of variance to test for differences among Lakes with different opening regimes and sizes for selected variables. ns - not significant, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. Tests for main effects were constructed after non-significant sources of variation were pooled ($p > 0.25$).

Source of variation	df	Total individual sponges			Total ascidians		
		MS	<i>F</i>	<i>p</i>	MS	<i>F</i>	<i>p</i>
Opening Regime	1	14.51	3.25	ns	111.65	16.62	***
Size	1	21.90	4.90	ns	44.00	6.55	*
OR x S	1	1.92	0.43	ns	22.30	3.32	ns
Lake (OR x S)	4	4.47	2.41	ns	5.80	0.85	>0.25
Location (La(OR x S))	40	1.85	2.47	***	6.81	6.50	***
Site (Lo(La(OR x S)))	48	0.75	2.67	***	1.05	3.12	***
Residual	480	0.28			0.34		
Transform		Ln($x + 1$)			Ln($x + 1$)		

Source of variation	df	<i>Styela plicata</i>			<i>Pyura stolonifera</i>		
		MS	<i>F</i>	<i>p</i>	MS	<i>F</i>	<i>p</i>
Opening Regime	1	40.04	7.67		13.61	0.75	ns
Size	1	27.71	5.31		23.10	1.28	ns
OR x S	1	21.65	4.15	*	8.71	0.48	ns
Lake (OR x S)	4	3.52	0.68	> 0.25	18.07	14.77	***
Location (La(OR x S))	40	5.22	8.16	***	1.22	1.89	*
Site (Lo(La(OR x S)))	48	0.64	6.16	***	0.65	3.59	***
Residual	480	0.10			0.18		
SNK tests for OR x S		Large Open >> Large Closed Large Open >> Small Open					
Transform		Ln($x + 1$)			Ln($x + 1$)		

3.3.4 Sponges: Extensively modified versus less modified lakes

Three species of sponge were found in the extensively modified Tuggerah Lake, but none was found in the extensively modified Lake Illawarra (Table 3.1). In comparison, in the two less modified lakes of similar size and opening regime (Wallis and St Georges Basin), ten and two species of sponge were found respectively. The abundance of all sponges (all species pooled together) was not statistically different between the two types of lake because of large variation at small spatial scales (Table 3.3, Figure 3.2a). The average abundances of individual species, however, did differ between the lakes. For example, in Tuggerah Lake, *Suberites* sp. 1 were not found in

transects and only two individuals were found in Timed Searches compared to a total of 8 and 137 found in Transects in Wallis and Smiths Lakes respectively. Similarly, 512 *Aplysinella* cf. *rhax* were found in St Georges Basin. Conversely, 44 *Haliclona* sp. 1 were found in Transects in Tuggerah Lake compared to four in Wallis and none in St Georges Basin.

3.3.5 Ascidiaceae: Extensively modified versus less modified lakes

The number of species of ascidian found in the extensively modified Tuggerah Lake (3), was similar to the two less modified lakes, Wallis (3) and St Georges Basin (2) (Table 3.1). Only one species, *Styela plicata* was found in Lake Illawarra. Furthermore, the distribution of ascidians was restricted to a single location for each species in Tuggerah Lake compared to multiple locations in Wallis Lake and St Georges Basin (Table 3.1). There were significantly more *Styela plicata* per transect in the less modified compared to the extensively modified lakes (Table 3.3, Figure 3.3c).

Table 3.3. Analyses of variance to test for differences between Lakes with different levels of human impact. ns - not significant, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. Tests for main effects were constructed after non-significant sources of variation were pooled ($p > 0.25$).

Source of variation	df	Total individual sponges			Total ascidians			<i>Styela plicata</i>		
		MS	F	p	MS	F	p	MS	F	p
Impact	1	22.86	3.68	ns	146.18	72.24	*	62.10	6.21	*
Lake(I)	2	6.21	2.48	ns	2.02	0.17	ns	5.91	0.57	> 0.25
Location(La(I))	20	2.50	1.17	ns	11.68	7.80	***	10.40	8.59	***
Site(Lo(La(I)))	24	2.14	6.18	***	1.50	4.12	***	1.21	7.82	***
Residual	240	0.35			0.36			1.00		
Transform		Ln(x + 1)			Ln(x + 1)			Ln(x + 1)		

3.4 DISCUSSION

This chapter highlights a number of key patterns of distribution of sponges and ascidians among NSW coastal lakes. First, there was a clear relationship between the presence and abundance of sponges and ascidians and the combination of opening regime and size of lake. In general, there were more species in lakes mostly open to the ocean regardless of size compared to the mostly closed lakes, and importantly, no sponges and only one species of ascidian were found in the small closed lakes. Second, there appeared to be an effect of the level of modification of a lake with relatively smaller abundances of ascidians in the extensively modified Tuggerah and Illawarra, and no sponges in Lake Illawarra.

The general pattern of absence of sponges from small and mostly closed lakes was further supported by qualitative searches after this study of five other small and mostly closed NSW lakes and lagoons (Wamberal 0.41 km², Tabourie 1.57 km², Mummaga 1.40 km², Fairy Lagoon 0.03 km² and Bellambi Lagoon 0.03 km²) where similarly no sponges and only one species of ascidian (*Eudistoma laysani* in Lake Mummaga) were found (Barnes unpublished data; Appendix 2). The most obvious explanations for absences of sponges and ascidians from small and mostly closed lakes relate to differences in water quality. Large changes in water quality, especially the input of freshwater, have long been known to affect the survival of sponges in estuaries (von Lendenfeld 1885; Fell *et al.* 1989; Roberts *et al.* 2006). Historical records of water quality for NSW lakes are relatively scarce and incomplete; however, sufficient data exist for salinity to propose some preliminary theories. It must be noted that reductions in salinity occur with the input of freshwater into a lake from rainfall and terrestrial runoff, and other measures of water quality (e.g. temperature, turbidity, pH, dissolved oxygen, etc.) often also vary with changes in salinity (e.g. Suzuki *et al.* 1998; see

Roberts & Barnes 2004 for examples from Tuggerah Lake). Therefore, any one or combination of these variables may affect the distribution of sponges and ascidians, and in this discussion, historical records of salinity are considered as measures of freshwater input. The patterns of distribution of animals in this study correlate well with historical ranges of salinity (Appendix 1). In New South Wales, smaller mostly closed lakes often have larger ranges, different frequencies and more rapid changes than mostly open and/or larger lakes. In particular, salinities can become very low in small closed lakes (Appendix 1). For example, in lakes where sponges were not found (e.g. Swan and Durras) or were very rare (e.g. Coila), historically salinities have fallen below 10 ‰ compared to lakes where sponges were more common and salinities did not fall below 13 ‰ (Appendix 1). In addition, when the small lakes are closed, periods of low salinity often last longer as freshwater builds up until the lake opens to the sea (Pollard 1994b; West & Jones 2000). Furthermore, the rate of change of salinity can also affect the survival of sponges. For example, Fell *et al.* (1989) found that the sponge, *Microciona prolifera* could tolerate gradual reductions of salinity from 30 ‰ to 10 ‰, but direct reductions were lethal. It should be noted that Smiths Lake, which has the second most abundant population of sponges, experiences relatively stable salinities (Appendix 1) because it has a relatively small catchment for its size compared to other NSW lakes (Haines 2003).

Another major finding of this study was a general increase in species diversity in mostly open compared to mostly closed lakes. It is difficult to assess the generality of this finding for sponges and ascidians because of the absence of comparable studies, but similar patterns have been documented for other groups of organisms such as fish in NSW coastal lakes (Pollard 1994b) and macrobenthos in NSW (Dye & Barros 2005b) and South African (Teske & Wooldridge 2001) coastal lakes and estuaries. The two

most likely explanations for this pattern are 1) increased opportunity for recruitment from the sea via the open entrances either from marine populations or populations in other nearby estuaries, or 2) favourable environmental conditions within a lake as mentioned above. It is, however, very difficult to determine the geographical distribution of these sponges from the literature and whether they may occur in the ocean, because most in this study are likely to be undescribed (Hooper & Wiedenmayer 1994). Despite this, preliminary surveys of marine waters near the lakes for some of the larger and upright sponges that were easily recognisable in the field (e.g. *Dysidea* sp., *Mycale* sp., *Raspailia* sp., *Suberites* spp. and *Tetilla* sp.) failed to find these species, which suggests they may indeed be restricted to lakes or other estuarine systems. The remaining sponges were mostly smaller, encrusting and cryptic. They were generally difficult to identify in the field and subsequently, it was extremely difficult to estimate their range of distribution.

The patterns of distribution within lakes also support the theory that some species may be restricted to lakes (Table 3.1, Figure 3.1). If recruitment of larvae from the ocean was important, it could be hypothesised that sponges will be more abundant and or more diverse in locations nearest to the ocean. Presumably, larvae would have less distance to travel and environmental conditions would be more similar to the ocean nearer to the entrance. However, for most species in most lakes, this was not the case (Table 3.1, Figure 3.1). For example, in Wallis Lake, sponges were most abundant and equally most diverse in the furthest location from the ocean (Location 1) and similarly in Smiths Lake, *Suberites* sp. 1 were most abundant in the two locations furthest from the ocean. Only in Burrill Lake, was diversity of sponges largest in the location nearest to the ocean. Although very little is known about the dispersal capabilities of marine sponges, it is probably limited for many species (Nichols & Barnes 2005). In this study,

most species of sponge were very patchily distributed within a lake and only found in one or two locations. Aggregated patterns of distribution such as these further suggest these species may have short dispersal distances with larvae settling near to the parents (Uriz *et al.* 1998). This also suggests exchange of larvae among lakes may be limited.

Restricted distributions of some species of sponges to lakes or estuaries also appear to occur elsewhere in the world. Interestingly, two species of *Suberites* morphologically very similar to the *Suberites* spp. in this study appear to be restricted to estuaries along the Pacific coast of Mexico (Carballo *et al.* 2004) and saline mangrove lakes of Indonesia (N. de Voogd, pers. comm.). The two sponges that could be tentatively named to species, *Aplysilla* cf. *sulpurea* and *Aplysinella* cf. *rhax*, however, have cosmopolitan distributions occurring over a wide geographical range (Hooper & Wiedenmayer 1994). Therefore, overall it appears likely there is a combination of marine species of sponges that are at their limits of distribution in lakes and some species that may be restricted to these environments.

In contrast to the sponges, all the ascidians found in this study are much more widely distributed and are not restricted to coastal lakes. For example, *Pyura stolonifera* are common on rocky shores along the rocky coastlines of southern Australia and South Africa (Kott 1985). *Herdmania grandis* (Heller) have an Indo-West Pacific distribution (Kott 2002). *Styela plicata* have a worldwide distribution (da Rocha & Kremer 2005) and are considered an introduced species in Australian waters. Similarly, *Botrylloides leachi* (Savigny), *Eudistoma laysani* (Sluiter) and *Microcosmus squamiger* Michaelson, are also cosmopolitan species.

Finally, the results indicate the level of human impact may affect the diversity of sponges in some cases and abundances in others. The most obvious support was that no sponges were found in the extensively modified Lake Illawarra. Interestingly,

historical information suggests there may have been up to eight species of sponge in Lake Illawarra in the late 1800s before large-scale development in the area (von Lendenfeld 1888). In Tuggerah Lake, although the number of species was similar to St Georges Basin and overall abundances were similar to Wallis Lake, the majority of sponges were of a different growth form. In Tuggerah, most were small encrusting forms on the holdfasts the brown algae, *Cystophyllum onustum* (Mertens) J. Agardh by May, or *Sargassum* spp., compared to larger and more upright forms dominating in Wallis Lake and St Georges Basin. Changes in sponge and ascidian assemblages have been correlated with proximity to development in other coastal environments (Carballo *et al.* 1996; Carballo & Naranjo 2002). Numerous possible mechanisms may explain the absence of sponges in Lake Illawarra and smaller forms in Tuggerah Lake. For example, increases in turbidity and sedimentation which have been reported in these lakes (Sloss *et al.* 2004), can affect the survival and growth of sponges (Gerrodette & Flechsig 1979; Roberts *et al.* 2006). Similarly, high levels of heavy metals (Cebrian *et al.* 2003) as have been reported from Lake Illawarra (Chenhall *et al.* 1994) and other forms of pollution such as sewage (Roberts *et al.* 1998) have also been correlated with decreases in sponge survival.

Together these findings have important implications for the management and conservation of biodiversity in these systems. First, it appears high levels of human modification of these systems may adversely affect the distribution of sponges and ascidians. Given that they are susceptible to human impacts and the coastal fringe of eastern Australia is becoming increasingly developed and urbanised, conservation of biodiversity in these systems will heavily rely on a number of lakes being given an adequate level of protection. It appears an important requirement for sponges and ascidians in these systems is the maintenance of adequate water quality, which will

largely be achieved by appropriate management of catchments. Presumably, along an increasingly urbanised coastline, not all lakes and catchments can remain unmodified. Importantly, if indeed many of these sponges are restricted in distribution to coastal lakes, and given their very patchy distributions many may be restricted to a small subset of lakes, it is paramount that lakes designated for protection are chosen carefully, taking into account existing scientific information on the distribution of all types of organisms. Further, sponges appear to be more patchily distributed among NSW lakes than other organisms such as seagrasses (West *et al.* 1985) and fish (Pollard 1994b; West & Jones 2000). It cannot, therefore, be assumed that protection of a small number of lakes based on the presence of other types of organisms will also conserve sponges on a regional basis.

A final key consideration in conserving the biodiversity of coastal lakes is the complex issue of management of opening regimes with the ocean. Lakes that are mostly open generally have more species of sponges and ascidians than mostly closed lakes. Lakes that were once intermittently closed and open are now being deliberately kept open for management reasons – usually with the general aims of protecting property from flooding or improving water quality. To add to the complexity of current opening regimes, there is also concern in other parts of the world such as South Africa (Teske & Wooldridge 2001), that some estuaries are now closing more frequently due to alteration to catchments, which may restrict freshwater reaching estuaries (Gillanders & Kingsford 2002) and increase sediment loads, which may then prematurely close entrances. By artificially keeping entrances open, we may be inadvertently increasing the diversity of these systems above what would be expected under a natural regime of opening and closing. Such an impact of a managerial strategy can be difficult to interpret as good or bad, because increases in diversity are usually considered positive,

but these impacts are presumably moving away from a natural condition, which is usually considered negative (Bulleri *et al.* 2007). Regardless of whether increases in diversity are considered positive or negative, it must be acknowledged that such managerial actions will very likely change the biodiversity of these systems. It is therefore important that management of entrances be done with clearly set goals and knowledge of the potential consequences to the ecology of these systems. Management of opening regimes may indeed provide us with a powerful tool for conservation.

CHAPTER 4: ASSOCIATIONS OF SPONGES AND ASCDIANS WITH AQUATIC VEGETATION

4.1 INTRODUCTION

A fundamental goal in ecology is to understand how habitat structure may influence patterns of distribution of associated fauna and flora. Seagrass landscapes, which are found in shallow coastal waters and estuaries over most temperate and tropical regions, are becoming increasingly recognised for their ecological and economic importance in supporting a diverse range of fauna and flora (Jackson *et al.* 2001; Heck *et al.* 2003). With global declines, increasing fragmentation and changes to the structure of seagrass habitats caused by anthropogenic impacts (Duarte 2002), it is imperative to understand the range of ecological processes operating in these systems. A better understanding of processes will enable further insight into the effects of anthropogenic impacts and allow informed management for long-term conservation.

The logical first step in understanding processes is to understand the basic patterns of distribution of fauna within seagrass habitats (Underwood *et al.* 2000). Seagrass habitats are often described as heterogeneous landscapes or mosaics consisting of patches of different species of seagrasses and macroalgae, interspersed with patches of bare substrata (usually sand or mud) (Bostrom *et al.* 2006). At smaller scales, the physical attributes of those patches also vary in size, shape, perimeter, density and height of plants, etc. (Larkum *et al.* 2006).

Numerous associations of sponges with macroalgae have been identified at relatively small spatial scales in which the sponge and algae exist interspersed with each other (e.g. where the macroalgae may form the primary skeleton for the sponge, Rutzler 1990; see review by Wulff 2006). Such associations have been observed on tropical

coral reefs (Gaino & Sara 1994; Trautman *et al.* 2000) and temperate rocky reefs (Huisman 2000), but to the best of my knowledge have not been investigated in seagrass meadows.

Associations of fauna with seagrasses (see review by Bostrom *et al.* 2006) have been studied for fish (Connolly & Hindell 2006), crustaceans (Murphey & Fonesca 1995; Hovel 2003), mobile epifauna (Turner *et al.* 1999), infauna (Bowden *et al.* 2001) and molluscs (Irlandi 1997; Bologna & Heck 2000a). Distributions of fauna can vary with a range of attributes including species of seagrass (Rotherham & West 2002), shape of leaves (Schneider and Mann, 1991), patchiness of seagrass beds (Reusch & Williams 1999), proximity to edges (Bologna & Heck 2000b) and quality of surrounding habitats (Tanner 2006). In contrast, we know much less about associations of sponges or ascidians with seagrass habitats. We do know they occur in seagrass meadows (Wulff 1995, 2008) and seagrass leaves and rhizomes provide hard substrata on which they grow (Fell & Lewandrowski 1981; Mercurio *et al.* 2000, 2007), but only a few studies have examined associations in detail (Thorhaug & Roessler 1977; Kuenen & Debrot 1995; Petersen & Svane 1995). Without a good understanding of the full range of faunal associations with seagrasses, it may be difficult to conserve the overall biodiversity of these systems.

Most of our knowledge of associations of sponges with seagrasses comes from the Mediterranean lagoons and in particular Italy (Corriero *et al.* 1989; Mercurio *et al.* 2000, 2007). One of the common patterns found in these studies was the common occurrence of sponges on the rhizomes of seagrasses (Mercurio *et al.* 2000, 2007). Of the handful of other studies of sponges and ascidians that have been done in seagrass habitats, many are limited by the lack of reported detail in patterns of distribution and few have examined patterns of association with habitat. For example, some have been

multi-phyla studies and appear not to have targeted sponges or ascidians. In many cases, it appears the techniques and replication used have been appropriate to sample other taxa, but it is unclear whether they are also adequate to quantify distributions of sponges and ascidians. For example, Robinson *et al.* (1982) appropriately used 20 cm diameter corers to sample soft sediment macrofauna in Smiths Lake, Australia. It is, however, extremely unlikely that small corers were also adequate to quantify distributions of the unnamed sponge they reported. In Smiths Lake, *Suberites* sp. is the most common sponge, but rarely exceeds densities of > 1 individual per 10 m^2 (Chapter 3) and is therefore unlikely to be collected in a relatively small core. Similarly, it is unclear whether the 20×20 cm box-corers used by Marzano *et al.* (2003) to sample soft sediment fauna in a Mediterranean lagoon were also adequate to quantify the distribution of the sponge they reported. Similar examples can be found in Hutchings and Recher (1974) and Day and Hutchings (1984). While such studies provide invaluable information on the presence of sponges and ascidians in these habitats, they do not provide the detailed quantitative information on smaller scale patterns of distribution within seagrass habitats needed to develop models to examine ecological processes.

Others studies have examined patterns of distribution at larger spatial scales, comparing seagrass habitats to other habitats such as coral reefs, mangroves, soft substrata, boulders and manmade structures (Pawlik 1998; Barnes 1999; Lehnert & Fisher 1999, Marzano *et al.* 2003). Still others have examined life histories (Fell & Lewandrowski 1981) or morphology (Mercurio *et al.* 2006). Overall, it appears sponges and ascidians may be a common component of seagrass fauna throughout the world, but despite their apparent widespread occurrence, there has been a lack of studies explicitly examining their distributions within seagrass landscapes. Of the two studies

that have made comparisons at smaller spatial scales, both reported sponge assemblages to differ widely with the composition and structure of seagrass and macroalgal habitats (Thorhaug & Roessler 1977; Kuenen & Debrot 1995). These results suggest smaller scale patchiness of seagrasses and macroalage are important in determining the distributions of sponges and ascidians. Overall, with the exception of Wulff (1995, 2008) and Pawlik (1998) who highlighted the importance of predation in structuring sponge assemblages in Caribbean seagrass meadows, there is a general lack of knowledge of the processes structuring these assemblages. Without a good ecological understanding of sponges and ascidians in seagrasses it will be difficult to ensure their conservation in these increasingly impacted landscapes.

Seagrasses and macroalgal beds dominate the relatively shallow areas of New South Wales coastal lakes (West *et al.* 1985). They have been identified as important habitats for fish and crustaceans and considerable effort is made to protect them and their associated faunal biodiversity (Creese & Breen 2003). Sponges and ascidians often make up a conspicuous component of this biodiversity (Chapters 2 & 3), yet to the best of my knowledge, no studies have examined associations of sponges and ascidians with seagrasses in New South Wales coastal lakes or any coastal water bodies elsewhere in Australia. While several elegant studies have examined the effects of more complex attributes of seagrass landscapes on the associated fauna (e.g. patch size - Irlandi 1997; proximity to edges - Tanner 2005), because virtually nothing is known of associations of sponges and ascidians, a logical starting point is to examine relatively simple patterns of association.

In this study, I examined associations of individual sponges and ascidians with species and percentage cover of seagrasses and macroalgae. This was done in two steps, by first describing spatial patterns of aquatic vegetation (seagrasses and

macroalgae), which were then overlain by spatial patterns of distribution of sponges and ascidians. I predicted that if associations did exist, then abundances of individual species of sponges or ascidians would show similar patterns of spatial distribution as assemblages of aquatic vegetation. Further, the importance of understanding the spatial scales at which associations occur and processes operate is being increasingly highlighted (Turner *et al.* 1999; Jackson *et al.* 2006). For example, the spatial arrangement of seagrass habitats varies from small scales of centimetres to metres (e.g. shoots, rhizomes), to metres to 10s of metres (small patches of species and/or bare space), to 100s to 1000s of metres (combinations of smaller patches). It is important that the scales at which associations occur are identified. For example, a sponge or ascidian may be associated with a particular species of seagrass at a relatively small spatial scale by only growing on the leaves or rhizomes of individual plants. Alternatively, the same sponge or ascidian may be associated with the same species of seagrass at a larger scale by being evenly distributed in a meadow which contains patches of seagrass and bare sediment. Understanding the spatial scales at which associations occur allow more informed models to be proposed to examine processes, and provides better information for management to determine areas of habitat to conserve. In this study, this was done at two spatial scales; 1) relatively small patches of habitat (20 m² transects) and relatively larger patches (sites approximately 5000 m² in area).

4.2 MATERIALS AND METHODS

4.2.1 Study-sites

The study sites are the same as those used in Chapter 2. Note, however, that the numbering of the Sites differs from Chapter 2 because of the analyses used (Figure 2.1, Figure 4.1).

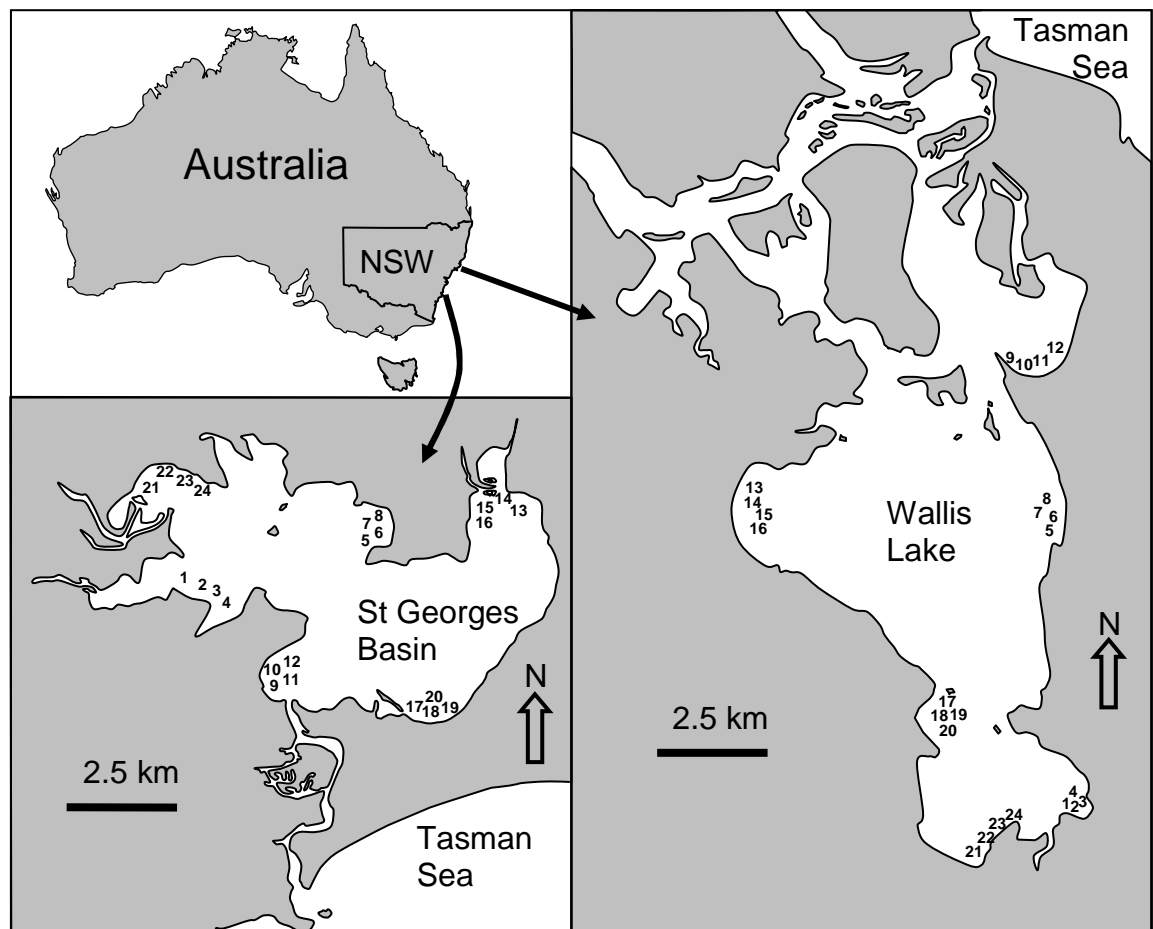


Figure 4.1. Sites sampled in St Georges Basin and Wallis Lake.

4.2.2 Sampling methods

Sponges and ascidians

Sponges and ascidians were counted according to the methods described in Chapter 2.

Rapid assessment of aquatic vegetation

In this study, because the spatial distributions of sponges and ascidians were very patchily distributed and some species were very uncommon, it was necessary to sample as many replicates as possible to get a good estimate of their distributions. Subsequently, it was necessary to sample replicate transects as quickly as possible. Quantifying assemblages of aquatic vegetation, particularly seagrasses, however, can be very time consuming and costly. Various techniques have been used to reduce the time needed for sampling (Short & Coles 2001).

In this study, to reduce the time of sampling aquatic vegetation, a modification of the Braun-Blanquet technique (Mueller-Dombois & Ellenberg 1974) was used to categorise percentage cover of vegetation. Similar methods have been successfully used to quantify the abundances of seagrasses in other NSW estuaries (King and Hodgson 1995). Total percentage cover for each species or type of aquatic vegetation was estimated as one of five categories: < 1%; 1- 33 $\frac{1}{3}$ %; 33 $\frac{1}{3}$ - 66 $\frac{2}{3}$ %; 66 $\frac{2}{3}$ - 100%. Categories were chosen for ease of estimation by an observer underwater. It must be noted, however, that reductions in time taken for sampling, can also result in a trade off with precision and accuracy, and caution should therefore be used in interpreting the results. For example, in transects where several taxa could be classed in the same category of cover (e.g. 4 taxa as 1-33 $\frac{1}{3}$ %), total percentage covers could be larger than

100%. Nevertheless, this study represents a sensible preliminary investigation of associations of sponges and ascidians with aquatic vegetation.

Finally, individual plants of the brown macro-alga, *Cystoseiras trinodus* were counted because preliminary observations suggested sponges were sometimes common on its holdfasts or stems.

4.2.3 Statistical analyses

Associations with assemblages of aquatic vegetation were examined for each species of sponge or ascidian separately, in each lake at each of two spatial scales: i) patches of habitat approximately 1000s m² in area (i.e. Sites) and ii) patches of habitat 20 m² (i.e. transects). For all multivariate analyses, the mid-point of each category of percentage cover was used. For example, 0.5 was used as the midpoint of the < 1 % category and 50 was used as the midpoint of the 33 ⅓ - 66 ⅔ % category. All analyses were done on untransformed data.

First, Cluster analyses based on group averages and using Bray-Curtis measures of dissimilarity (Clarke & Warwick 1994) were used to identify groups of Sites with relatively similar assemblages of aquatic vegetation. Next, nMDS ordinations were used to illustrate differences in assemblages of aquatic vegetation among Sites. Average percentage covers of each taxa for each site ($n = 20$ replicate transects) were used in both analyses. To illustrate associations of sponges and ascidians with assemblages of aquatic vegetation, ‘bubble plots’ were used to superimpose the relative abundances of each species on each Site in the nMDS ordinations (see Clarke and Warwick 1994). In this technique, the diameter of a circle is proportional to the abundance of a variable of choice. In the present study, it was predicted that if sponges or ascidians were associated with particular assemblages of aquatic vegetation, then

relatively large circles (i.e. relatively large abundances of sponges or ascidians) would show similar patterns of clustering as assemblages of vegetation.

Associations of sponges and ascidians with aquatic vegetation were also examined at the smaller scale of transect within each Site. Tests of associations at this smaller spatial scale were reliant on adequate variability in vegetation among transects. For example, in sites consisting largely of a monoculture of a single species (e.g. dense beds of *Lamprothamnion* at Site 1 in Wallis Lake), there was inadequate variation among transects to test for patterns of association at this smaller spatial scale. Using the same logic as above, Cluster analyses based on group averages and using Bray-Curtis measures of dissimilarity (Clarke & Warwick 1994) were used to identify groups of transects with similar assemblages of aquatic vegetation within each Site. MDS ordinations were then used to illustrate differences in assemblages of aquatic vegetation among transects within each Site and 'bubble plots' were used to superimpose the relative abundances of each species on each transect in the nMDS ordinations. In addition, analyses were not done in Sites where abundances of sponges and ascidians were too small (< 5 individuals per species) to detect patterns of association.

To test for a relationship between *Halichondria* spp. and the presence of the large brown algae, *Cystoseira trinodus*, the number of plants with *Halichondria* spp. was correlated with the total number of *C. trinodus* plants. This was done only in sites 9-12 in Wallis Lake where abundances of *C. trinodus* were large enough for valid tests to be done.

4.3 RESULTS

4.3.1 St Georges Basin

Four species of seagrass, *Posidonia australis*, *Zostera capricorni*, *Halophila ovalis* and *Ruppia megacarpa*, and six macroalgal taxa, *Chaetomorpha* spp., *Cystoseira trinodus*, *Dictyota* sp., *Hormosira banksii*, *Microdictyon umbilicatum* and Rhodophyta were recorded in St Georges Basin. Rhodophyta were grouped together because they were difficult and time consuming to identify to species in the field. It is likely however, that most of the Rhodophyta were either *Chondria* spp. or *Gracilaria* spp. *P. australis*, *Z. capricorni* and *H. ovalis* were all abundant in patches, but in comparison *R. megacarpa* was less abundant (Figure 4.2). *Chaetomorpha* sp., *Hormosira banksii* and *Microdictyon umbilicatum* were relatively uncommon and, therefore, are not included in figures.

At the scale of Site in St Georges Basin, 7 groups of aquatic vegetation were identified using Cluster analysis (Figure 4.4a). In contrast to patterns in Wallis Lake, differences in assemblages did not correspond to location within the Lake. Sites in Group 1 were characterised by a combination of *Posidonia australis* and bare sediment, with small amounts of *Zostera capricorni*, *Halophila* spp. and the brown alga *Dictyota* sp. Group 2 had similar amounts of *P. australis* to group 1, but with relatively more *Z. capricorni* and *Halophila* spp. and less bare sediment. Group 3 had little to no *P. australis*, relatively large covers of *Z. capricorni* and *Halophila* spp. and small patches of bare sediment. Group 4 was characterised by a large cover of *Halophila* spp. with small amounts of *Z. capricorni*. The remaining three groups consisted of single Sites. Group 5 (Site 4) had similar amounts of *Z. capricorni* and *H. ovalis* as group 4, but was the only Site with large amounts of the brown alga *Hormosira banksii*. Group 6 (Site

14) had a large amount of bare sediment. Group 7 (Site 16) was dominated by *H. ovalis*, with small patches of *Dictyota* sp. and bare sediment.

Two species of sponge and two species of ascidian were found (Table 2.1). The sponge, *Aplysinella* cf. *rhax* was found in a range of Sites, but was on average most abundant in sites in Group 1 with relatively large covers of *Posidonia australis* (Figure 4.3 & Figure 4.5b). *Styela plicata* was found throughout the lake, however, abundances were consistently larger in Sites in Groups 3 or 4, characterised by large covers of *Z. capricorni* and/or *Halophila ovalis* (Figure 4.2), and illustrated by the cluster of large circles in Figure 4.5d. In contrast, *S. plicata* was found in much smaller abundances in Sites in Groups 1 and 2 characterised by large covers of *P. australis* and bare sediment. *Pyura stolonifera* was found throughout the lake with no clear patterns of association with vegetation at the scale of site (Figure 4.5c).

At the smaller spatial scale of 10s of metres among transects within sites, the degree of patchiness of aquatic vegetation varied from site to site. In some Sites, aquatic vegetation was very similar among transects. For example, in Site 2 all transects were very similar with > 66% cover of *Halophila ovalis* and < 33% cover of *Zostera capricorni*, and in Site 16 most transects had > 66 % cover of *Z. capricorni* and < 33 % bare sediment. Such homogeneous Sites are characterised by relatively small measures of dissimilarity produced by Cluster analyses (e.g. Appendix 3.1 - Sites 2 & 5). Because of the homogeneity of vegetation, it was not possible to examine associations of sponges and ascidians at the scale of 10s metres within those sites.

Aquatic vegetation in other sites was more patchy among transects. In several sites there were two or three distinct groups of transects identified using Cluster analyses (Appendix 3.1). For example, in Site 8, half of the transects had > 66 % cover of *Posidonia australis*, while the other half had predominantly bare sediment. In Sites

with differences in aquatic vegetation among transects, several associations of sponges and ascidians were identified. Associations were most obvious in Sites in Groups 1 and 2, with patches of *P. australis*, *Zostera capricorni* and/or *Halophila ovalis*. *Aplysinella* cf. *rhax* and *Pyura stolonifera* were on average more abundant in transects with > 66 % cover of *P. australis* than in transects with little *P. australis* and larger covers of *Zostera capricorni*, *Halophila ovalis* and/or bare sediment (Figure 4.2, Figure 4.3; Appendix 3.2 – Sites 6, 8 & 19). For example, in Site 6, the mean abundance of *Aplysinella* cf. *rhax* in transects with > 33 % cover of *P. australis* was 45.9 ± 12.1 ($n = 12$) compared to 1.0 ± 0.6 ($n = 8$) in transects with < 33 % cover of *P. australis* and relatively more *Z. capricorni*. In Site 8, the mean abundance of *Aplysinella* cf. *rhax* in transects with > 33 % cover of *P. australis* was 65.9 ± 12.5 ($n = 7$) compared to 0.01 ± 0.6 ($n = 12$) in transects with < 33 % cover of *P. australis* and relatively more *H. ovalis* and bare sediment. Similarly, in Site 19, the mean abundance of *A* cf. *rhax* in transects with > 33 % cover of *P. australis* was 20.3 ± 7.2 ($n = 14$) compared to zero ($n = 6$) in transects with < 33 % cover of *P. australis* and relatively more *H. ovalis* and bare sediment.

In contrast, *Styela plicata* showed no clear patterns of association among transects within Sites in Groups 3-7, which had large covers of *Zostera capricorni* and/or *Halophila ovalis* and no *Posidonia australis* (Appendix 3.1). Rather, *S. plicata* were more evenly distributed within these Sites as illustrated by the lack of clustering of circles in the nMDS plots in Appendix 3.3.

4.3.2 Wallis Lake

Four species of seagrass, *Posidonia australis*, *Zostera capricorni*, *Halophila ovalis* and *Ruppia megacarpa*, and five types of macroalgae, *Lamprothamnion* sp., *Chaetomorpha* sp., *Cystoseira trinodus*, *Dictyota* sp. and Rhodophyta were recorded in Wallis Lake. Rhodophyta were grouped together because they were difficult and time

consuming to identify to species in the field. It is likely however, that most of the Rhodophyta were either *Chondria succulenta* (J. Agardh) Falkenberg, or *Gracilaria* spp. *Z. capricorni* was the most abundant seagrass (Figure 4.3). In comparison, *P. australis*, *H. ovalis* and *R. megacarpa* were less abundant and very patchily distributed. *Chaetomorpha* sp. was relatively very uncommon and is therefore not included in figures. *C. trinodus* were found only in Sites 9-12 in Location 3 (Figure 4.1). Unlike St Georges Basin, a large area of Wallis Lake was dominated by a dense bed of *Lamprothamnion* sp. (Figure 4.1, Figure 4.2; Sites 1-4).

At the scale of Site in Wallis Lake, three distinct groups of assemblages of aquatic vegetation were identified using Cluster analysis (Figure 4.4a). It must be noted that differences in assemblages largely corresponded to position within the lake. Sites in Group 1 had a dense cover of the charophyte, *Lamprothamnion*, with relatively small patches of *Zostera capricorni*, *Halophila ovalis*, *Ruppia megacarpa* and/or bare space (Figure 4.2). Group 1 consisted of the 4 sites in Location 1 in the southern portion of the Lake. The remaining two Groups differed in the relative contribution of *Zostera capricorni* and bare sediment. Sites in Group 2 were dominated by *Z. capricorni* with little bare sediment, while group 3 had relatively more bare sediment and less *Z. capricorni* (Figure 4.2).

Nine species of sponges and 3 species of ascidians were found with most very patchily distributed and restricted to a subset of sites (Table 2.1). There were three clear patterns of association of sponges and ascidians with the assemblages of vegetation at the scale of Site. The sponge, *Suberites* sp. was on average most abundant in the Group 1 sites dominated by *Lamprothamnion*, but was relatively uncommon in other sites. Note the relatively large abundances of *Suberites* sp. in Sites 1-4 illustrated by relatively large circles in Figure 4.5g. *Mycale* sp. was also relatively abundant growing on

Lamprothamnion sp. in sites in Group 1 (Figure 4.5f). *Mycale* sp. was also found on the blades of *Z. capricorni* in Sites 17-20 in Group 2 which had a dense cover of *Z. capricorni* and relatively little bare sediment. *Halichondria* spp. and *Haliclona* sp. 1 were found only in Sites 9-12 and only on the brown alga *Cystoseiras trinodus*. *C. trinodus* was not found elsewhere in the Lake. The ascidian, *Styela plicata*, was the most widespread sessile invertebrate found in this study, but was on average most abundant in Group 1 sites with a dense cover of *Lamprothamnion* sp. (Figure 4.5h).

The magnitude of variability in vegetation among transects differed among the sites. In Sites 1-4, there was relatively small variability among transects with most dominated by *Lamprothamnion*. In the remainder of the Sites, there was more variability among transects, generally caused by differences in cover of *Zostera capricorni* and bare sediment among transects and to a lesser extent, patches of *Halophila ovalis* and bare macroalgae (Appendix 3.2). Despite the patchiness among transects, in contrast to St Georges Basin, there was only one discernable association with aquatic vegetation at the smaller scale of transect within sites. In Sites 9-12, *Halichondria* spp. were found only on and positively correlated with *Cystoseiras trinodus* (Figure 4.6). *Haliclona* sp. 1 was also only found on *C. trinodus* in Sites 9-12, however, abundances were too small to test for correlations. In the remainder of Sites there were no discernable associations at the scale of transect as evidenced by the absence of clustering in the nMDS ordinations in Appendix 3.4.

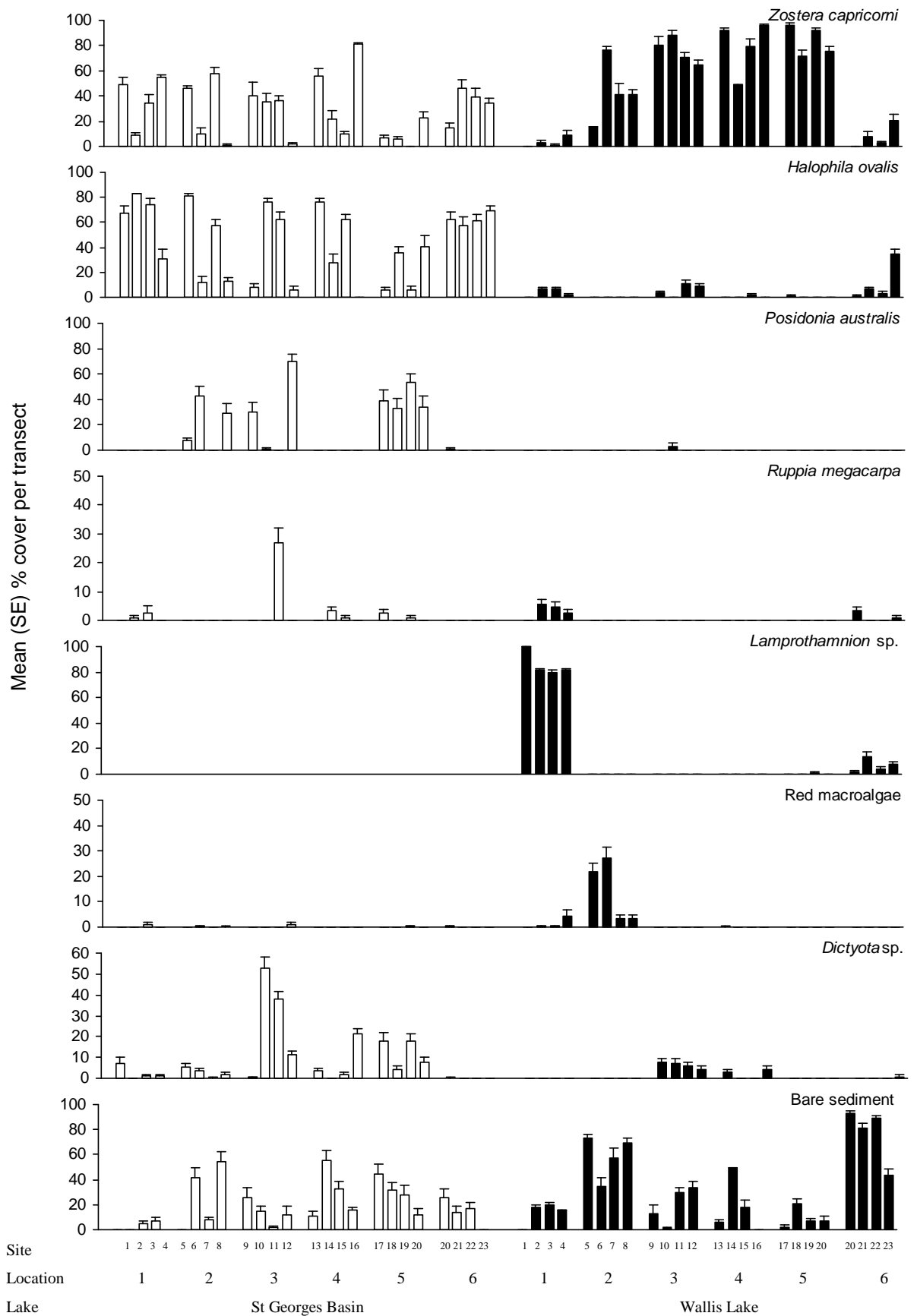


Figure 4.2. Mean (SE) percentage covers of seagrasses and macroalgae in St Georges Basin and Wallis Lake (n=20).

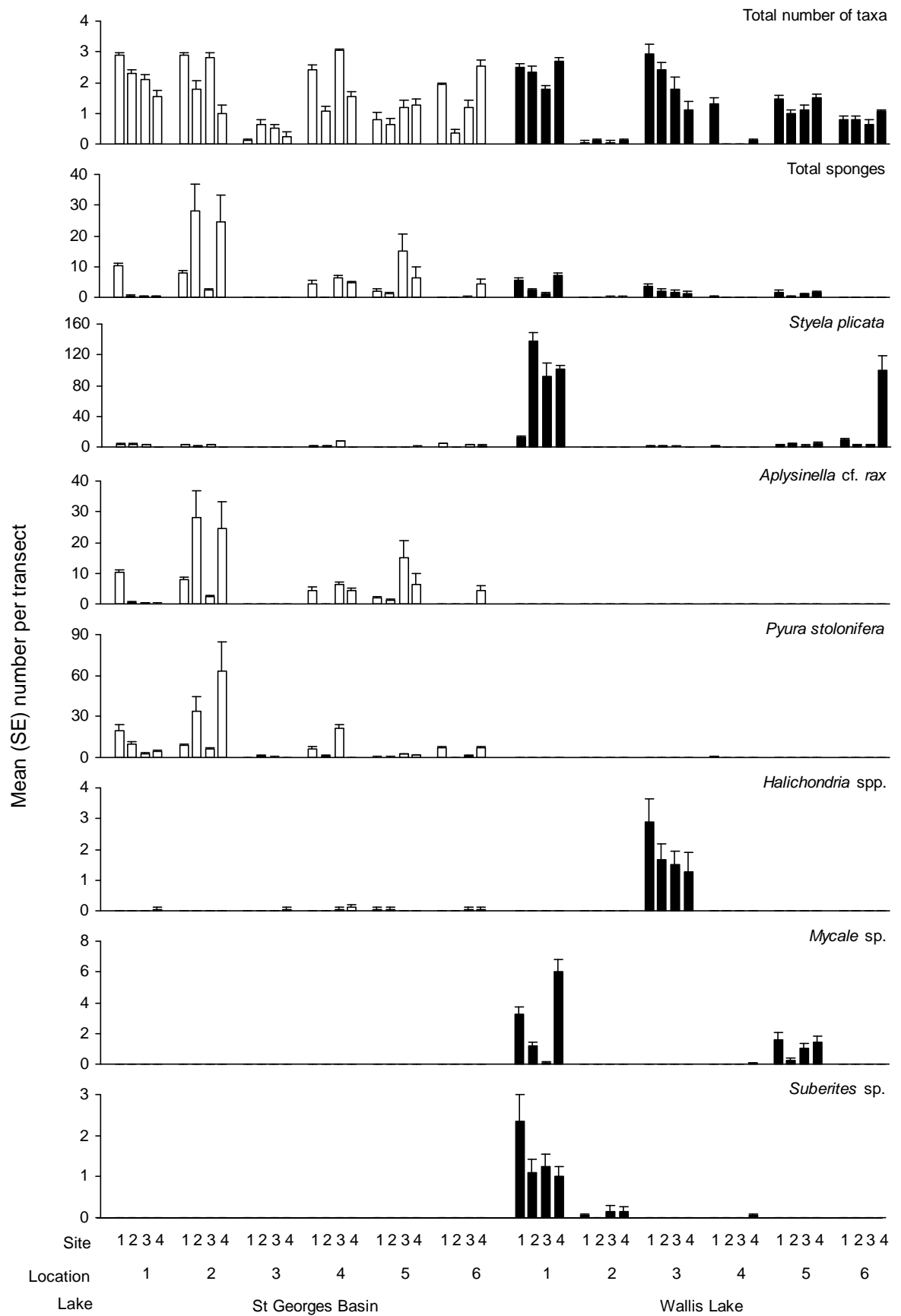


Figure 4.3. Mean (SE) numbers of taxa per transect ($n = 20$) at each site

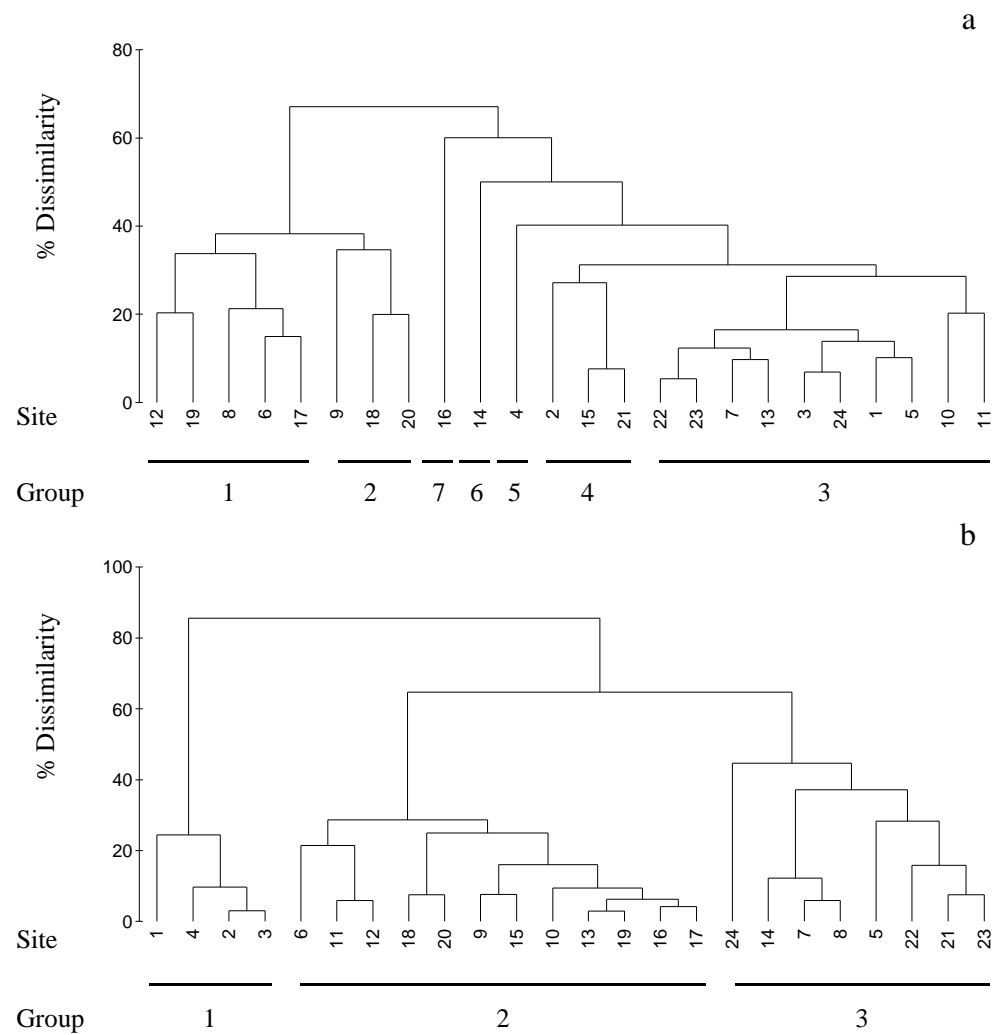


Figure 4.4. Dendrograms from Cluster analyses illustrating groups of Sites with relatively similar assemblages of aquatic vegetation in St Georges Basin (a) and Wallis Lake (b).

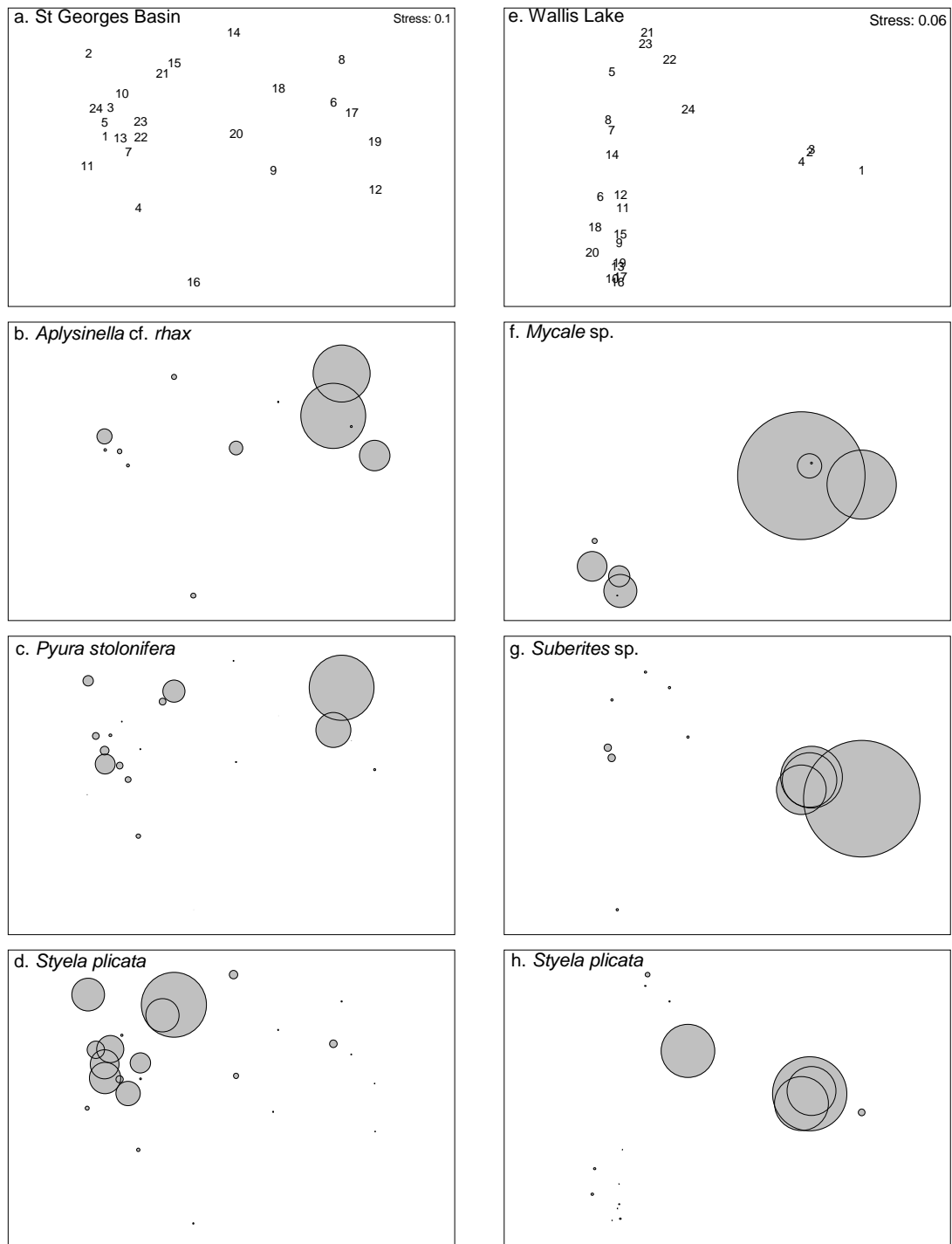


Figure 4.5. nMDS ordinations illustrating associations of sponges and ascidians with assemblages of aquatic vegetation at the scale of Site. Figs a and e illustrate differences in assemblages of aquatic vegetation among Sites in St Georges Basin and Wallis Lake respectively. In Figures b-e, abundances of *Aplysinella cf. rhax*, *Pyura stolonifera* and *Styela plicata*, respectively, have been superimposed on Fig. a. In Figures f-h, abundances of *Mycale* sp., *Suberites* sp. and *S. plicata*, respectively, have been superimposed on Fig. e. The diameter of each circle is proportional to the abundance of each species at that Site. Abundances are relative to each figure and cannot be compared among figures.

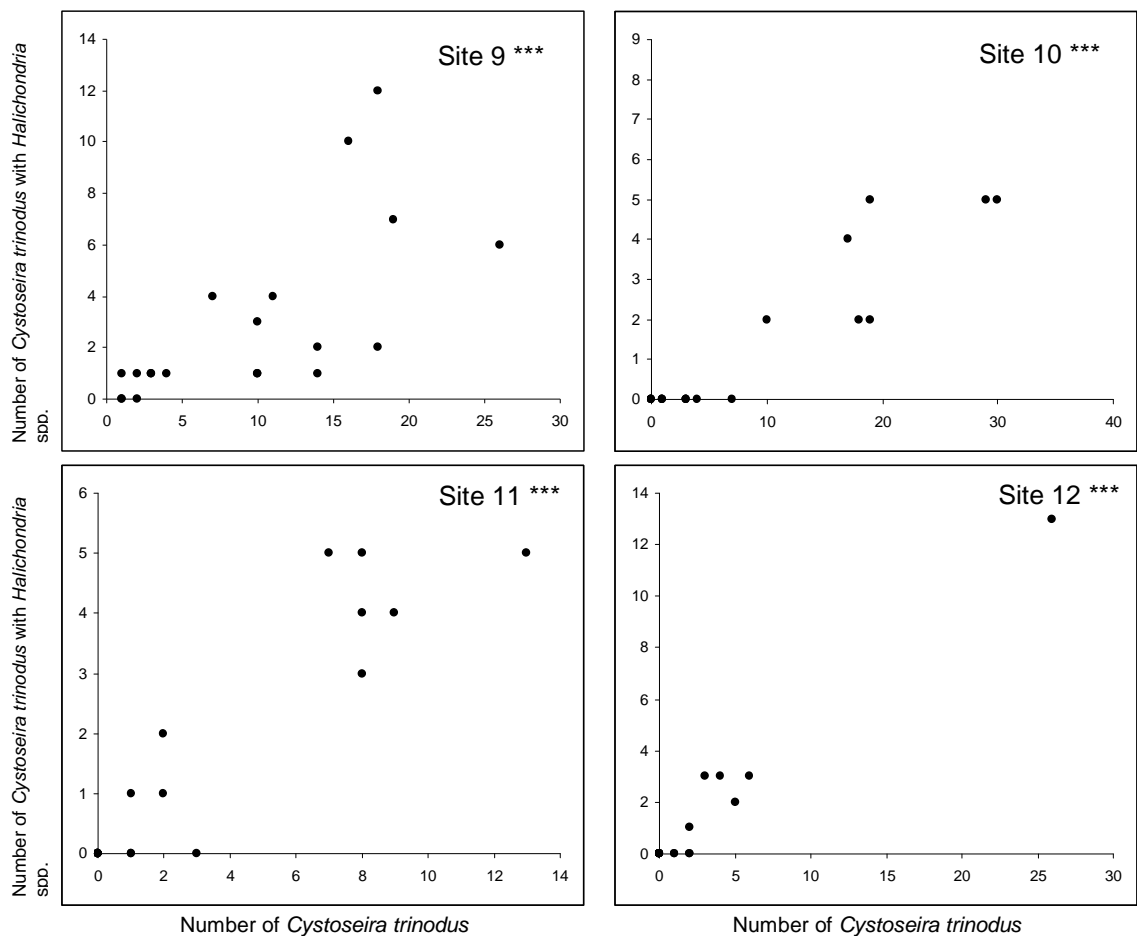


Figure 4.6. Correlations between number of *Cystoseira trinodus* with *Halichondria* spp. and total number of *C. trinodus* per transect at each of 4 sites in Location 3. $n = 20$ transects per site. *** correlations were significant at $p < 0.001$.

4.4 DISCUSSION

There were several clear patterns of association of sponges and ascidians with assemblages of aquatic vegetation in both St Georges Basin and Wallis Lake. Patterns, however, in general differed between the two lakes. This may have partly been due to differences in the composition of assemblages of vegetation and different species of sponges between the two lakes. In St Georges Basin, the sponge, *Aplysinella* cf. *rhax* was clearly associated with *Posidonia australis* at both the scales of Site and Transect. In Sites with patches of *P. australis* and *Zostera capricorni*, *A. cf. rhax* was consistently more abundant in transects dominated by *P. australis*. The ascidian, *Pyura stolonifera*

was similarly associated with patches of *P. australis* within Sites. In contrast, the introduced ascidian, *Styela plicata*, was relatively less abundant in *P. australis* and was more common in transects dominated by *Z. capricorni* or *H. ovalis*.

In Wallis Lake, the clearest patterns of association were the large abundances of *Suberites* sp., *Mycale* sp. and *Styela plicata* in the Sites dominated by the macroalga, *Lamprothamnion* sp. Kuenen and Debrot (1995) found a similar pattern in a Venezuelan lagoon, where sponges including *Mycale angulosa* and *M. microsigmatosa* were typical in habitats dominated by macroalga (*Cladocephalus* and *Caulerpa verticillata* or *Halimeda opuntia*), but absent from habitats dominated by the seagrass, *Thalassia testudinum*. In contrast Thorhaug & Roessler (1977), reported a relatively dense *Thalassia* community dominated by sponges in estuarine lagoons of South Florida. A second clear pattern in Wallis Lake was the occurrence of *Halichondria* spp. and *Haliclona* sp. 1 only on the thalli of the brown alga *Cystoseira trinodus*. These associations, however, must be interpreted with caution because of the distributions of *Lamprothamnion* sp. and *Cystoseira trinodus* within Wallis Lake. Because both species of algae were restricted to particular areas of Wallis Lake, it is not possible to determine whether the presence of sponges and ascidians was due to the presence of the algae or the position within the lake. For example, distributions of sponges have been correlated with other physical factors in sheltered or enclosed water bodies such as aspect (leeward versus windward; Farnsworth and Ellison, 1996) and tidal flow (Thorhaug and Roessler, 1977). However, in Wallis Lake, it is unlikely that patterns of winds and currents would differ greatly among the sites sampled. The only pattern common to both lakes was that abundances of sponges were in general smaller in Sites or Transects dominated by *Zostera capricorni* compared to Sites or Transects dominated by other species of seagrass or macroalgae or large areas of bare sediment.

Several models have been proposed to explain patterns of distribution of fauna in seagrasses including differences in water flow, physical disturbance, sediment characteristics, life history traits, movement of adults, availability of food and predation (see reviews by Bostrom *et al.* 2006; Connolly & Hindell 2006). While most models have been developed to explain distributions of fauna such as fish, crustaceans, molluscs and macrofauna, most are also applicable to sponges and ascidians. An additional model which is appropriate to consider for sessile invertebrates such as sponges and ascidians, but is rarely examined for other fauna, is the availability of suitable substrata on which to settle (Russell *et al.* 2003).

The first group of models relates to the physical structure of the seagrasses and macroalgae. The simplest of these is the availability of suitable substrata on which to grow. Availability of substrata appears important for *Halichondria* spp. and *Haliclona* sp 1 in Wallis Lake, where they were found only on the thalli of *Cystoseira trinodus*. Interestingly, two species of the sponge, *Mycale* are also common on *Cystoseira* spp. thalli in Mediterranean bays (Corriero *et al.* 1998). In comparison, other species of sponges and ascidians appeared not to be as substrata-specific. *Suberites* sp. were, in general, not attached to seagrasses nor macroalgae, but were found on top of *Lamprothamnion* sp. or on bare sediment amongst patches of *Zostera capricorni*. *Mycale* sp. were most abundant attached to *Lamprothamnion* sp., but were also found on the leaves of *Z. capricorni* in other Sites. Similarly, in St Georges Basin, *Aplysinella* cf. *rhax* were most abundant attached to and amongst *Posidonia australis*, but were also found growing on *Halophila ovalis*, *Z. capricorni* and *C. trinodus*. Overall, it appears suitable substrata on which to attach may be a limiting factor for some species, but not for the majority of sponges and ascidians in the seagrasses of coastal lakes.

The physical structure of seagrass and macroalgae may also have indirect effects on the distribution of sponges and ascidians. In Wallis Lake, the clearest differences in physical structure exist between the Sites dominated by *Lamprothamnion* sp. and those dominated by *Zostera capricorni*. *Lamprothamnion* sp. in Wallis Lake form relatively dense homogeneous beds, but rarely exceed more than 20 cm above the substrata. Sponges and ascidians were in general found on top of the *Lamprothamnion*. In contrast, *Z. capricorni* in general forms taller beds with a canopy 50 cm to a metre above the substrata. These differences in physical structure may have a number of consequences for sponges and ascidians. For example, patches of seagrass may decrease horizontal water movement, which may in turn decrease the availability of food for filter feeders, as demonstrated for bivalves in seagrass beds (Bologna & Heck 1999; Reusch & Williams 1999). Reusch and Williams (1999) correlated reduced growth of the mussel, *Musculista senhousia*, inside *Zostera marina* (which is morphologically similar to *Z. capricorni*) with reduced water flow and suggested mussels receive less food.

Canopies formed by *Zostera capricorni* may also restrict the amount of light reaching the substrata below. Many sponges contain photosynthetic symbionts and require adequate light to survive and grow (Wilkinson & Vacelet 1979; Cheshire *et al.* 1997). The *Suberites* sp. in this study contains photosynthetic cyanobacteria. Although, it is not known whether this *Suberites* sp. can survive without its cyanobacteria, its absence in areas of dense *Z. capricorni* may potentially be explained by low light levels. In contrast, *Suberites* sp. was most abundant on top of *Lamprothamnion* sp., where light levels were not affected by a canopy.

The movement of adults is not an obvious model which comes to mind when explaining patterns of distribution of sponges. However, several species of sponge are

known to be passively moved by water currents. For example, spherical specimens of *Geodia cydonium* can be rolled across soft substratum in Mediterranean bays by slow circular currents (Mercurio *et al.* 2006). Similarly, the same species of *Suberites* as found in Wallis Lake can be moved distances of metres by wind-driven currents in nearby Smiths Lake (Barnes, unpublished data). Although reproductive strategies and life histories are not clear for this species of *Suberites*, if asexual reproduction and fragmentation is important, individual sponges may be prevented from colonising areas with dense *Zostera capricorni* by the edges of the patches forming a physical barrier to movement of the adults.

Differential predation is the most frequently proposed model to explain patterns of distribution of fauna within seagrass landscapes (Connolly & Hindell 2006). One of the few ecological studies of sponges in seagrasses, found that predation by the starfish, *Oreaster reticulatus*, prevented reef sponges from colonising Caribbean seagrass meadows (Wulff 1995). Predation is also frequently proposed to explain distributions of sponges among other types of habitats (Dayton 1975; Farnsworth & Ellison 1996 or see review by Wulff 2006). The predation model is based on the premise that the distribution of predators and/or their feeding behaviour differs among habitats. Although, little is known of potential predators of sponges in New South Wales Coastal lakes, in the absence of abundant echinoderms and nudibranchs, fish are the most likely predators. Indeed, predation by fish plays an important role in structuring sponge assemblages in many habitats (Dunlap & Pawlik 1996; Pawlik 1998; Wulff 2000). Numerous studies have identified differences in fish assemblages with physical characteristics of seagrass landscapes (see review by Connolly & Hindell 2006). More specifically however, in New South Wales coastal lakes, *Zostera capricorni* and *Posidonia australis* meadows can support different assemblages,

abundances and sizes of fish (Rotherham & West 2002). Such differences in abundances of potential predators provide a plausible explanation for differences in abundances of *Aplysinella* cf. *rhax* and *Pyura stolonifera* between *P.australis* and *Z.capricorni* in St Georges Basin.

This study provides the logical starting points for investigating explanatory models as proposed above to examine the ecological processes operating within the seagrass meadows of New South Wales Coastal lakes. Further correlative investigations should consider including a combination of quantitative sampling and qualitative observations of small scale associations between sponges and the structure of the plants on which they're growing. Greater understanding of the processes, however, will most likely be achieved through carefully designed manipulative experiments.

CHAPTER 5: ANTHROPOGENIC IMPACTS IN ESTUARIES: COOLING WATER DISCHARGE FROM POWER STATIONS AFFECTS ASSEMBLAGES OF SPONGES AND ASCIDIANS IN LAKE MACQUARIE

5.1 INTRODUCTION

The detection of anthropogenic impacts is essential for the effective management and conservation of natural environments (Schmitt & Rosenberg 1996). The identification of impacts, however, is often a complex and difficult task against the natural background variability that exists in nature (Underwood 1992), uncertainties about the spatial extent of disturbances (Raimondi & Reed 1996), variability in biological responses (Warwick & Clarke 1993) and the choice of organisms to be included (Underwood & Peterson 1988; Jones and Kaly 1996). It can be particularly complex for some groups of organisms which are rare, patchily distributed or for which there is a paucity of information regarding even broad scale patterns of distribution.

An environmental impact study must be able to distinguish changes caused by a human impact from natural background variability. Protocols for detecting impacts have developed greatly over the last thirty years (Green 1979; Stewart-Oaten *et al.* 1986; Underwood 1992, 1993, 1994). One of the key developments has been the inclusion of multiple control or control sites (Underwood 1992). Many environmental impact studies include a relatively small number (2, 3 or 4) of control sites (e.g. Morrissey *et al.* 2003; Klaoudatos *et al.* 2006; Roberts *et al.* 2007). While a small number of control sites maybe adequate for estimating the natural variability of organisms that are relatively common and/or widespread (e.g. sessile assemblages on hard substrata, Glasby 1999; soft sediment macrofauna Gray *et al.* 1990; assemblages of fish, Rogers *et al.* 1999), this may not be the case for rare or patchily distributed taxa.

For example, many species of sponges and ascidians in coastal lakes occur only in a small subset of sites sampled (often less than 20% of sites; Chapters 2 and 3). For these species, it is clear that the inclusion of only a small number of control sites will not provide a good estimate of their natural spatial variability.

A second consideration in designing impact studies is determining the spatial extent of any impact (i.e. over how large an area any impact has occurred). This has important implications for the choice of control sites, which is usually based on the logic that they should resemble the putatively impacted site in as many ways as possible (e.g. habitat type, aspect, geomorphology, position in estuary, etc.), but not be affected by the purported impact (Glasby & Underwood 1998). In many studies, however, the spatial extent of an impact maybe unclear at the outset, which then causes uncertainty in choosing appropriate control sites. In these cases, it maybe desirable to test for impacts at a range of spatial scales by including control sites at increasing distances from the source of the purported impact (Underwood *et al.* 2003).

Environmental impacts may also cause a range of biological responses from changes in growth (Cebrian *et al.* 2003), diversity or species composition of assemblages (Zvereva *et al.* 2008), changes in average abundances of particular species (Roberts *et al.* 1998) to changes in spatial or temporal patterns of variation (Warwick & Clarke 1993). Different biological responses may have different consequences for the long-term conservation and management of natural systems and it is often desirable to examine a range of responses.

Finally, there is much debate over the choice of organisms to be included in environmental impact studies (Jones & Kaly 1996). Choices are often made for good practical reasons (e.g., organisms that are easily identified, easily sampled and are

common and in large enough abundances for statistical tests to be valid). Inclusion of taxa with these characteristics can allow impacts to be detected quickly and cost-efficiently. There are, however, also good reasons for investigating taxa that are less common. Jones & Kaly (1996) suggest there is a clear case for examining 'rare' species, which may become extinct as a result of human impacts. Further, while environmental impacts are often associated with declines in abundances, diversity or distributions they may also cause increases. For example, some types of human impacts (e.g., human-built structures in estuaries) have been identified as potential footholds from which introduced species may colonise new habitats (Glasby *et al.* 2007). Introduced species, may persist in relatively small abundances or patchy populations before becoming more widespread and problematic (e.g. Meinesz 1999). The management and potential eradication or control of potentially harmful introduced species will benefit from the early identification of invasion sites.

Cooling water discharge from coal powered and nuclear power stations is a common source of environmental impact on estuarine, coastal and freshwater systems throughout the world (Bamber 1995). Impacts vary and range from no measurable effect, to increases in growth and abundance, to high mortality depending on the taxa studied and severity of such factors as temperature of the discharge water (Suresh *et al.* 1993; Ambrose *et al.* 1996; Keser *et al.* 2005). Lake Macquarie, the largest saline lake on the New South Wales coast with almost 200 km of shoreline, 114 km² of surface area and large seagrass meadows, has two coal fired power stations, Eraring and Vales Point. In addition, like many other NSW estuaries, it is under increasing pressure from urbanisation, industry and alterations to the catchment (Lee *et al.* 2006). For example, a recent Healthy Rivers Commission of NSW (2002) inquiry rated Lake Macquarie's catchment condition as 'modified' and lake condition as 'severely affected' - the most

severe level of environmental stress. One of the many activities often purported as a potential impact on the natural environments of Lake Macquarie is the extraction and discharge of cooling water for the power stations (Nicholls 1999; Eyre 2005). While potential impacts of power stations in Lake Macquarie have been studied for seagrasses (King 1986), macroalgae (Nicholls 1999), molluscs (Wallis 1976; Jolley *et al.* 2004) and fish (Hannan & Williams 1998; Kirby *et al.* 2001), there have been no studies investigating the potential impacts on assemblages of sponges or ascidians.

Observations from a preliminary survey near the outlet of the Eraring power station suggested the diversity and abundance of sponges might be relatively large in that area compared to the rest of the lake (P. Barnes, pers. obs).

Sponges and ascidians in NSW coastal lakes represent an unusual group of organisms to study compared to other groups because of the paucity of information regarding even basic patterns of distribution. Because knowledge is still limited for the design of impact studies that focus on sponges and ascidians, in this study I took a broad approach for detecting impacts over a range of spatial and temporal scales, which included descriptive comparisons of diversity among sites within Lake Macquarie and six other control estuaries, and quantitative comparisons of spatial and temporal distributions within lake Macquarie and three other control lakes:

1. To examine potential localised impacts, the diversity of sponges and ascidians near the two cooling-water outlets was compared to multiple control sites within Lake Macquarie.
2. To examine larger-scale impacts, the diversity of sponges and ascidians in Lake Macquarie was compared to six other lakes and estuaries without power stations.
3. To test for potential localised impacts on spatial and temporal patterns of distributions, abundances of some of the more common species, the sponges,

Mycale sp. and *Suberites* sp. and the ascidians, *Polyclinum nudum* and

Botrylloides leachi were compared to populations at control sites within Lake Macquarie over three times of sampling.

4. Similarly, as a further test for potential impacts on spatial and temporal patterns of distributions, abundances of the sponges, *Mycale* sp. and *Suberites* sp. near the cooling water outlets were compared to populations in other lagoons without power stations over three times of sampling.

5.2 MATERIALS AND METHODS

5.2.1 Site description and sampling methods

Two coal-burning power stations (Vales Point and Eraring; Figure 5.1) extract and discharge water that is heated up to 6-10° above ambient temperatures into Lake Macquarie (Nicholls 1999; Kirby *et al.* 2001). The outlets from each of these power stations flow into relatively shallow waters (<2 metres) over mostly soft substratum which support meadows of seagrass and patches of macroalgae (King 1986; Nicholls 1999). They discharge large volumes of water (up to 146 m³/s when operating at full capacity) creating current velocities which are generally larger than elsewhere in the lake (Nicholls 1999; NSW Department of Natural Resources 2008).

Populations of sponges and ascidians are often very patchily distributed within the seagrass meadows of NSW coastal lakes and estuaries with some species restricted to only a few small areas of a lake (Chapters 2 & 3). It is, therefore, very important to maximise the area searched in order to increase the probability of finding sponges and ascidians in a lake. To maximise the area searched in this study, two intensities of sampling were used; 1) rapid searches and 2) transects. Because rapid searches were

relatively quick to do, they allowed a relatively large number of sites to be sampled and hence a relatively large area of Lake Macquarie to be searched. Rapid searches were used to determine the presence of sponges and ascidians in a site and involved either an observer using a viewing tube from a boat or a snorkeller searching in the water depending on the depth and visibility of the water. For each technique, approximately 400 m² of the lake was searched, the presence of sponges and ascidians was recorded and the cover of seagrasses and macroalgae was estimated (see below). A preliminary study to compare the two methods found there were no differences in the species found when each method was used to search the same area of lake (Barnes, unpublished data).

In impact studies, it is often desirable to choose control sites that are as similar as possible to the purportedly impacted site, but remain unaffected by that impact (Glasby & Underwood 1998). On initial inspection, the seagrass meadows close to the Vales Point and Eraring outlets, however, were observed to be very different from elsewhere in the lake. There were dense meadows of *Halophila ovalis* of which similar could not be found in other sites in Lake Macquarie or in any of the other estuaries sampled at that time. It is likely, that these patterns of growth of *Halophila ovalis* may have been associated with the impact of the cooling water discharge and may therefore be part of a secondary impact affecting the distributions of sponges and ascidians. Therefore, in order to place any impacts detected in context with possible differences in habitats, associations of sponges and ascidians with type of habitat including the cover of seagrasses, macroalgae and unvegetated sediment were estimated at each site. A rapid assessment technique was used to estimate habitat using three categories of cover; dense – greater than 66% cover, patchy – 33-66% cover, and sparse – 1-33% cover (see Chapter 4). Because in other NSW lakes, the presence of some species of sponges appears to be correlated with the height of seagrasses (Chapter 4), in this study, the

seagrass *Zostera capricorni* (eelgrass) was further classified as either short (less than 10cm) or tall (greater than 10cm). In addition, the presence of mussel beds, the large bivalve *Pinna bicolor* and the brown alga *Cystoseira trinodus* were recorded because sponges and ascidians have been found growing on these organisms in other NSW coastal lakes (Chapter 4) and elsewhere in Australia (Pitcher & Butler 1987).

On the first time of sampling in March 2004, in sites where populations of sponges and ascidians were found using rapid searches, an adaptive-type sampling approach was then used to quantify abundances at that site (Smith *et al.* 2004). In each site, individual sponges, solitary and colonial ascidians were counted by a snorkeller in six replicate 10 x 2 m transects (see Chapter 3 for optimisation of sampling design). Sites were approximately 80 m in diameter. These sites were then re-sampled in August 2004 and March 2005 to examine temporal changes in abundances.

5.2.2 Localised impacts on diversity within Lake Macquarie

To examine potential localised impacts of cooling water discharge on the diversity of sponges and ascidians within Lake Macquarie, 29 sites were sampled in the lake in March 2004 (Figure 5.1). Two potentially impacted sites were chosen in close proximity to the outlets at each of the Vales Point and Eraring power stations and an additional 25 Control sites were chosen haphazardly in seagrass meadows around the lake. Because at Time 1 relatively few Control sites contained sponges and ascidians, 18 new Control sites were searched at Time 2 and a further 21 new Control sites were searched at Time 3 (Figure 5.2; Figure 5.3). The inclusion of new control sites at each time of sampling improved the ability of the study to test for impacts by providing three separate comparisons and increased the probability of finding new populations of sponges and ascidians which appeared extremely patchily distributed within the lake. In

August 2004, four of the sites sampled were near the cooling water inlet for Eraring Power Station.

5.2.3 Larger scale impacts on diversity: comparing Lake Macquarie to other estuaries

To examine larger-scale impacts, the diversity of sponges and ascidians in Lake Macquarie was compared to six other NSW coastal lakes and estuaries (Wallis Lake, Smiths Lake, Lake Conjola, Brisbane Water, Pittwater and Port Hacking; Appendix 2). Because Lake Macquarie is the largest coastal lake in New South Wales, it was not possible to include multiple control lakes of similar size. Therefore, a range of estuaries were chosen based on their relative proximity to Lake Macquarie (within 200 km), relatively large sizes, presence of large areas of seagrass or macroalgae (West *et al.* 1985), and importantly, no power stations. All six of these estuaries were sampled once in January to March 2004, using the same methods of rapid assessment and similar numbers of sites as in Lake Macquarie (**Table 5.4**).

5.2.4 Localised impacts within Lake Macquarie: Spatial and temporal patterns of abundance of *Mycale* sp., *Suberites* sp. and *Polyclinum nudum*

Potential localised impacts on spatial and temporal patterns of distribution were investigated for those species that were found to be relatively common within Lake Macquarie at Time 1 (*Mycale* sp., *Suberites* sp., *Polyclinum nudum* and *Botrylloides leachi*). Abundances of each species were compared over three times of sampling between the four impact sites near the cooling water outlets and the five control sites identified as having relatively large populations of sponges at Time 1 (Control Sites 5, 6, 7, 8 and 9; Figure 5.1). Formal statistical analyses were not done because in the majority of comparisons there were clear patterns of presence versus absence.

5.2.5 Larger scale impacts: Spatial and temporal patterns of abundance of *Mycale* sp. and *Suberites* sp. in Lake Macquarie and coastal lakes

To test for impacts on spatial and temporal patterns of distribution, abundances of the sponges, *Mycale* sp. and *Suberites* sp. in the sites near the cooling water outlets were compared to sites in other lakes where these species were known to occur (Chapters 2 & 3). Abundances of *Mycale* sp. were compared to two sites in each of Wallis Lake and Lake Conjola. Abundances of *Suberites* sp. were compared to two sites in each of Wallis Lake and Smiths Lake (Figure 3.1). Sponges were counted in six replicate 10 x 2 m transects in each site and sampling was done within one month of each time of sampling in Lake Macquarie. Three factor asymmetrical analyses of variance were used to test for differences in temporal patterns of distribution between Lake Macquarie and each of the two control lakes. The logic and construction of asymmetrical analyses of variance are described by Underwood (1991, 1994) and illustrated with an example by Glasby (1997). The assumption of homogeneity of variance was tested using Cochran's test (Winer *et al.* 1991). Data were transformed to $\ln(x + 1)$ when significant. When transformations did not remove heterogeneity, analyses proceeded because ANOVA can be robust to deviations from homogeneity of variances, particularly with designs with balanced sample sizes and many independent estimates of variance (Underwood 1981). Vales Point and Eraring were compared to the Control Lakes separately.

5.3 RESULTS

Five species of sponge and five species of ascidian were found in Lake Macquarie (Table 5.1, Table 5.2, Table 5.3). Of the five species of sponge collected, only one (*Apysilla* cf. *sulphurea*) could be tentatively identified to species. The remainder (*Haliclona* sp., *Mycale* sp., *Phorospongia* sp. and *Suberites* sp.), are likely to

be undescribed or are presently unable to be assigned to a known taxon given the plethora of taxa in the older literature whose identities remain a mystery (Hooper & Wiedenmayer 1994). The three colonial ascidians, *Botrylloides leachi* (Savigny), *Polyclinum nudum* Kott and *Symplegma oecania* Tokiola, and the solitary ascidian *Pyura stolonifera* (Heller) are native to Australian waters, while the solitary ascidian *Styela plicata* Leseur is considered an introduced species in Australia (NIMPIS 2006).

5.3.1 Localised impacts on diversity within Lake Macquarie

There were a number of patterns found consistent with localised impacts on the diversity of sponges and ascidians near the cooling water outlets (Table 5.1, Table 5.2, Table 5.3; Figure 5.1, Figure 5.2, Figure 5.3). First, although overall there were relatively few species of sponges found in the Lake, diversity was generally largest near the outlets, particularly Vales Point. Sponges were generally extremely uncommon elsewhere in the lake with two exceptions; *Mycale* sp. was periodically present and abundant on *Posidonia australis* leaves near Belmont (sites 6 and 7, Figure 5.4), and the majority of the other sites where sponges were found were in relative close proximity to the outlets (e.g. Myuna Bay, Vales Point) or inlet canal for Eraring Power Station (i.e. Bonnells Bay in August 2004). The only other sponges found in the lake were a single *Mycale* sp. and a single *Suberites* sp. in Crangan Bay.

Second, the ascidian *Polyclinum nudum* was found at Vales Point on all three times and at Eraring at Times 2 and 3, but in no other seagrass habitats in the lake on any occasion. *P. nudum* was most abundant near Vales Point outlet in March 2004 and March 2005 with on average approximately 15 and 27 colonies per transect respectively (Figure 5.4). Abundances were smaller in August 2004 with on average one colony per transect. Colonies of *P. nudum* were also serendipitously found on the wooden pilings and netting of the swimming baths at Vales Point in August 2004. Subsequent to this

observation, to determine if *P. nudum* occurred on other similar structures in Lake Macquarie, searches were made on jetties, netting and rocky reefs in Bonnells Bay, Wangi Wangi, Crangan Bay and Belmont, however, no other colonies were found. It must also be noted that *P. nudum* is normally considered a sessile organism that grows attached to hard substrata. Near the Vales Point outlet, however, most colonies were not attached to hard substrata, but on top of soft sediment or *Halophila ovalis* and appeared healthy with no signs of necrosis.

Of the other ascidians, *Botrylloides leachi* was found only near the outlets or in sites near to the Swansea channel connecting the lake to the ocean, *Symplegma oecania* and *Pyura stolonifera* were found only near the Swansea channel, and *Styela plicata* showed no trend in its pattern of distribution being the only species that could generally be considered widespread (Table 5.1, Table 5.2, Table 5.3). *S. plicata* was not found near the Vales Point outlet. *B. leachi* and *S. oecania* were relatively abundant on the blades of the seagrass *Zostera capricorni* at Sites 8 and 9 near the Swansea Channel in March 2004 and March 2005, but were not found at these Sites in August 2004 (Figure 5.4). *B. leachi* was also found in relatively small abundances (i.e. only in rapid searches) in March 2004 at the Eraring outlet, and in August 2004 and March 2005 at the Vales Point outlet.

Table 5.1. Sponges, ascidians and habitat found in each Site at Time 1 (March 2004).

Site name and number		Sponges	Ascidians	Habitat
Sites sampled at all 3 Times				
Vales Point outlet	1	<i>Mycale</i> sp. <i>Suberites</i> sp. <i>Haliclona</i> spp.	<i>Polyclinum nudum</i>	Dense <i>Halophila ovalis</i> , sparse bare sediment
Vales Point outlet	2	<i>Mycale</i> sp. <i>Suberites</i> sp.	<i>Polyclinum nudum</i>	Dense <i>H. ovalis</i> , patchy bare sediment
Eraring outlet	3		<i>Botrylloides leachi</i>	Dense <i>Halophila</i>
Eraring outlet	4	<i>Mycale</i> sp. <i>Suberites</i> sp.	<i>Styela plicata</i>	Patchy <i>Zostera capricorni</i> , dense <i>H. ovalis</i>
Myuna Bay	5	<i>Mycale</i> sp. <i>Phorospongia</i> sp.		Short dense <i>Z. capricorni</i> , dense <i>H. ovalis</i>
Belmont	6	<i>Mycale</i> sp.		Dense <i>Posidonia australis</i>
Belmont	7	<i>Mycale</i> sp.		Dense <i>P. australis</i>
Elizabeth Island	8		<i>Botrylloides leachi</i> <i>Styela plicata</i> , <i>Symplegma oceania</i> ,	Patchy <i>Z. capricorni</i> , sparse <i>H. ovalis</i> , patchy bare sediment
Elizabeth Island	9		<i>Styela plicata</i> , <i>Symplegma oceania</i> , <i>Botrylloides leachi</i>	Dense <i>Z. capricorni</i> , sparse <i>H. ovalis</i> , patchy bare sediment
Sites sampled at Time 1 only				
Cams Wharf	10			Dense <i>Z. capricorni</i>
East Crangan Bay	11			Dense <i>Z. capricorni</i>
Southern Crangan Bay	12			Short dense <i>Z. capricorni</i> , sparse <i>H. ovalis</i>
Gwandalan	13			Dense <i>Z. capricorni</i> , sparse <i>H. ovalis</i>
West Crangan Bay	14	One individual <i>Mycale</i> sp. on <i>Pinna bicolor</i>		Dense <i>Z. capricorni</i> , sparse <i>H. ovalis</i> , abundant <i>P. bicolor</i>
Point Wolstoncroft	15			Dense <i>Zostera</i> , sparse <i>H. ovalis</i> , abundant <i>Pinna bicolor</i>
Chain Valley Bay	16			Sparse <i>Z. capricorni</i> , dense <i>Microdictyon umbilicatum</i>
Bird Cage Point	17			Dense <i>Z. capricorni</i>
Bird Cage Point	18			Dense <i>Z. capricorni</i> , patchy <i>Halophila</i> , patchy <i>Ruppia megacarpa</i>
Bird Cage Point	19			Dense <i>Z. capricorni</i>
Myuna Bay	20	One individual <i>Suberites</i> sp.		Short dense <i>Z. capricorni</i> , dense <i>H. ovalis</i>
South of Wangi Wangi Point	21			Patchy <i>Z. capricorni</i> , patchy <i>H. ovalis</i> , patchy bare sediment
South of Wangi Wangi Point	22			Dense <i>Z. capricorni</i> , dense <i>H. ovalis</i> , mussel beds
Wangi Wangi Point	23		<i>Styela plicata</i>	Dense <i>Z. capricorni</i> , dense <i>H. ovalis</i> , mussel beds
Koorooro Bay	24			Dense <i>Z. capricorni</i> , patchy red algae
Koorooro Bay	25			Dense <i>Z. capricorni</i>
Awaba Bay	26			Dense <i>Z. capricorni</i>
Awaba Bay	27		<i>Styela plicata</i>	Dense <i>Z. capricorni</i> , patchy bare sediment
Warners Bay	28			Dense <i>Z. capricorni</i> , patchy bare sediment
Warners Bay	29			Dense <i>Z. capricorni</i> , patchy red algae

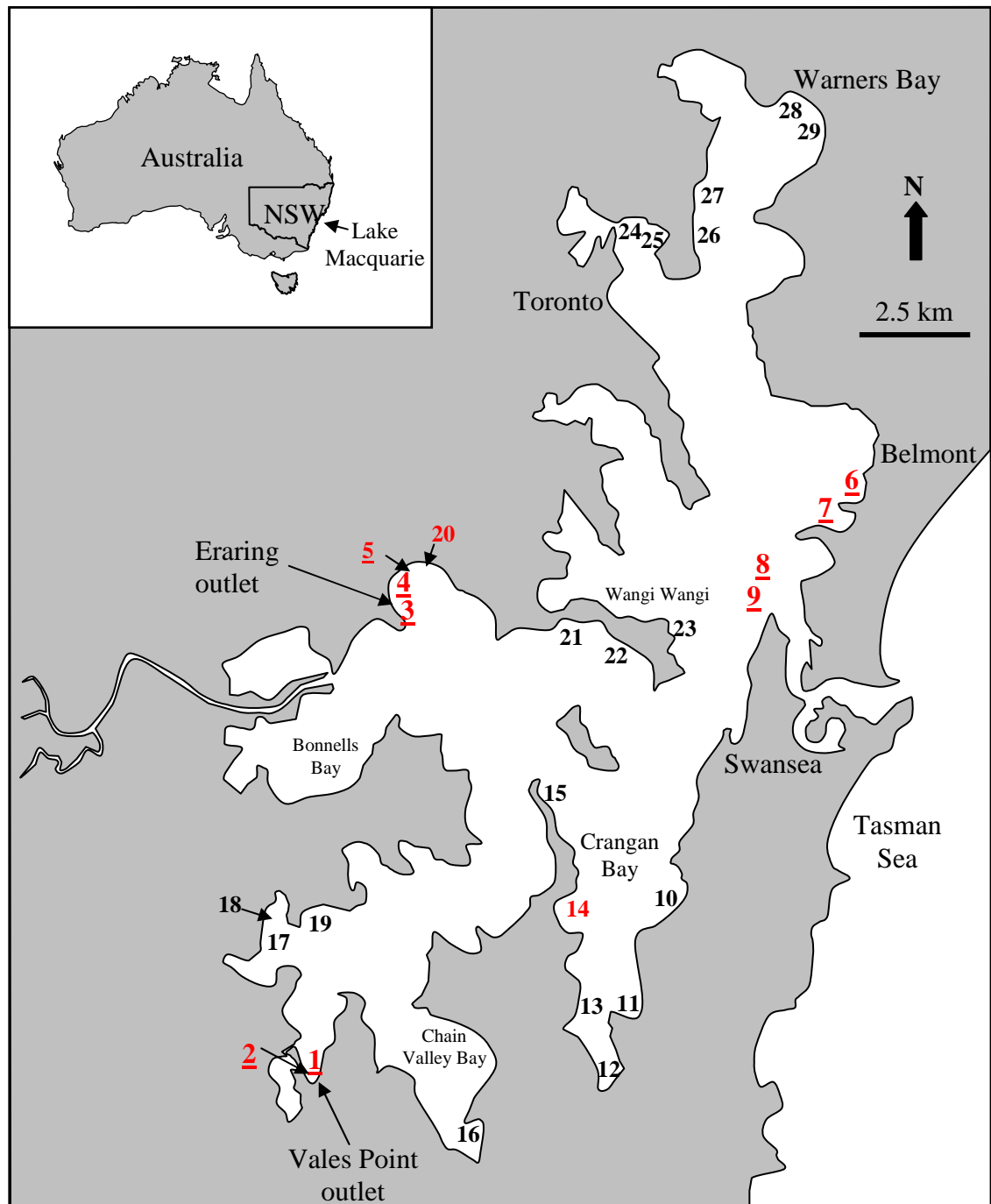


Figure 5.1. Sites sampled at Time 1 (March 2004). Underlined sites were also sampled at Times 2 & 3. Sites in red indicate presence of sponges and/or ascidians. See Table 5.1 for details.

Table 5.2. Sponges, ascidians and habitat found in each Site at Time 2 (August 2004)

Site name and number		Sponges	Ascidians	Habitat
Sites sampled at all 3 Times				
Vales Point	1	<i>Mycale</i> sp. <i>Suberites</i> sp.	<i>Polyclinum nudum</i> <i>Botrylloides leachi</i>	Dense <i>Halophila ovalis</i> , sparse bare sediment
Vales Point	2	<i>Mycale</i> sp.		Dense <i>H. ovalis</i> , patchy bare sediment
Eraring	3		<i>Polyclinum nudum</i>	Dense <i>H. ovalis</i>
Eraring	4	<i>Suberites</i> sp.	<i>Polyclinum nudum</i> <i>Styela plicata</i>	Patchy <i>Zostera capricorni</i> , patchy <i>H. ovalis</i> , patchy filamentous green algae, <i>Cystoseira trinodus</i> , patchy bare sediment
Myuna Bay	5			Short dense <i>Zostera</i> , patchy <i>H. ovalis</i>
Belmont	6			Dense <i>Posidonia australis</i>
Belmont	7	<i>Mycale</i> sp.		Dense <i>P. australis</i>
Elizabeth Island	8			Patchy <i>Z. capricorni</i> , sparse <i>H. ovalis</i> , patchy bare sediment
Elizabeth Island	9			Patchy <i>Z. capricorni</i> , sparse <i>H. ovalis</i> , patchy bare sediment
Sites sampled at Time 2 only				
Swan Bay	30		<i>Botrylloides leachi</i> <i>Styela plicata</i>	Dense <i>Z. capricorni</i> , sparse <i>H. ovalis</i>
Swan Bay	31		<i>Styela plicata</i>	Dense <i>Z. capricorni</i> , sparse <i>H. ovalis</i>
Galgabba Point	32			Dense <i>P. australis</i>
Galgabba Point	33			Dense <i>P. australis</i>
Vales Point	34	<i>Suberites</i> sp.		Patchy <i>Z. capricorni</i> , patchy <i>H. ovalis</i> , <i>Cystoseira trinodus</i> , mussels
Vales Point	35			Patchy <i>Zostera</i> , patchy <i>H. ovalis</i> , <i>C. trinodus</i> , mussels
Bonnells Bay	36	<i>Suberites</i> sp.	<i>Styela plicata</i>	Short dense <i>Z. capricorni</i> , sparse <i>H. ovalis</i>
Bonnells Bay	37		<i>Styela plicata</i>	Patchy <i>Z. capricorni</i> , sparse <i>H. ovalis</i> , patchy bare sediment
Bonnells Bay	38	<i>Aplysilla</i> cf. <i>sulpurea</i> <i>Haliclona</i> sp. <i>Mycale</i> sp. <i>Suberites</i> sp.	<i>Styela plicata</i>	Patchy <i>Z. capricorni</i> , dense <i>H. ovalis</i> . mussel beds
Bonnells Bay	39	<i>Haliclona</i> sp. <i>Suberites</i> sp.		Patchy <i>Z. capricorni</i> , dense <i>H. ovalis</i> . mussel beds
Bonnells Bay	40		<i>Styela plicata</i>	Patchy <i>Z. capricorni</i> , patchy <i>H. ovalis</i> ,
Myuna Bay	41			Short dense <i>Z. capricorni</i> , patchy <i>H. ovalis</i>
Wangi Wangi Point	42			Dense <i>Z. capricorni</i> , mussels beds
Wangi Wangi Point	43		<i>Styela plicata</i>	Dense <i>Z. capricorni</i> , sparse <i>H. ovalis</i> , mussels beds
Arcadia Vale	44		<i>Styela plicata</i>	Dense <i>Z. capricorni</i> , patchy red algae
Arcadia Vale	45		<i>Styela plicata</i>	Dense <i>Z. capricorni</i> , patchy red algae
Coal Point	46			Dense <i>Z. capricorni</i>
Cold Tea Canal	47			Patchy <i>Z. capricorni</i> , patchy <i>H. ovalis</i>

Figure 5.2. Sites sampled at Time 2 (August 2004). Underlined sites were also sampled at Times 1 & 3. Sites in **red** indicate presence of sponges and/or ascidians. See Table 5.2 for details.

Table 5.3. Sponges, ascidians and habitat found in each Site at Time 3 (March 2005)

Site name and number		Sponges	Ascidians	Habitat
Sites sampled at all 3 Times				
Vales Point	1	<i>Haliclona</i> sp. <i>Mycale</i> sp. <i>Suberites</i> sp.	<i>Polyclinum nudum</i>	Dense <i>Halophila ovalis</i> , patchy filamentous green algae, sparse bare sediment
Vales Point	2	<i>Mycale</i> sp. <i>Suberites</i> sp.	<i>Botrylloides leachi</i>	Dense <i>H. ovalis</i> , patchy bare sediment
Eraring	3	<i>Mycale</i> sp.		Dense <i>H. ovalis</i>
Eraring	4		<i>P. nudum</i> <i>Styela plicata</i>	Patchy <i>H. ovalis</i> , patchy bare sediment
Myuna Bay	5			Patchy <i>Zostera capricorni</i> , patchy bare sediment
Belmont	6	<i>Mycale</i> sp.		Dense <i>Posidonia australis</i>
Belmont	7			Dense <i>P. australis</i>
Elizabeth Island	8		<i>B. leachi</i> <i>Pyura Stolonifera</i> <i>S. plicata</i>	Patchy <i>Z. capricorni</i> , sparse <i>H. ovalis</i> , patchy bare sediment
Elizabeth Island	9		<i>Botrylloides leachi</i> <i>Styela plicata</i>	Patchy <i>Z. capricorni</i> , patchy bare sediment
Sites sample at Time 3 only				
Swansea	48	<i>Mycale</i> sp.		Patchy <i>Z. capricorni</i> , dense <i>Posidonia</i>
Point Morisset	49		<i>Styela plicata</i>	Patchy <i>Z. capricorni</i> , mussel reefs
Point Morisset	50	<i>Suberites</i> sp	<i>Styela plicata</i>	Dense <i>Z. capricorni</i>
West Crangan	51			Dense short <i>Z. capricorni</i>
Point Wolstoncroft	52			Dense <i>Z. capricorni</i>
Summerland Point	53			Dense <i>Z. capricorni</i>
Frying Pan Bay	54		<i>Styela plicata</i>	Dense <i>Z. capricorni</i> , <i>Cystoseira trinodus</i>
Frying Pan Point	55		<i>Styela plicata</i>	Dense <i>Z. capricorni</i>
Bluff Point	56			Dense <i>Z. capricorni</i>
Bardens Bay	57			Mussel reefs
Bonnells Bay	58			Dense <i>Z. capricorni</i> , mussel reefs
Rocky Point	59		<i>Styela plicata</i>	Patchy <i>Z. capricorni</i> , sparse <i>H. ovalis</i> , patchy bare sediment
Rocky Point	60		<i>Styela plicata</i>	Patchy <i>Z. capricorni</i> , sparse <i>H. ovalis</i> , patchy bare sediment
Myuna Bay	61			Dense <i>Z. capricorni</i>
Pulbah Island	62			Short dense <i>Z. capricorni</i> , abundant <i>Pinna bicolour</i>
Pulbah Island	63			Short dense <i>Z. capricorni</i> , abundant <i>P. bicolour</i>
Wangi Wangi	64			Dense <i>Z. capricorni</i>
Eraring Bay	65		<i>Styela plicata</i>	Dense <i>Z. capricorni</i>
Catalina Park	66			Dense <i>Z. capricorni</i>
Coal Point	67			Dense <i>Z. capricorni</i>
Belmont	68			Dense <i>Z. capricorni</i>

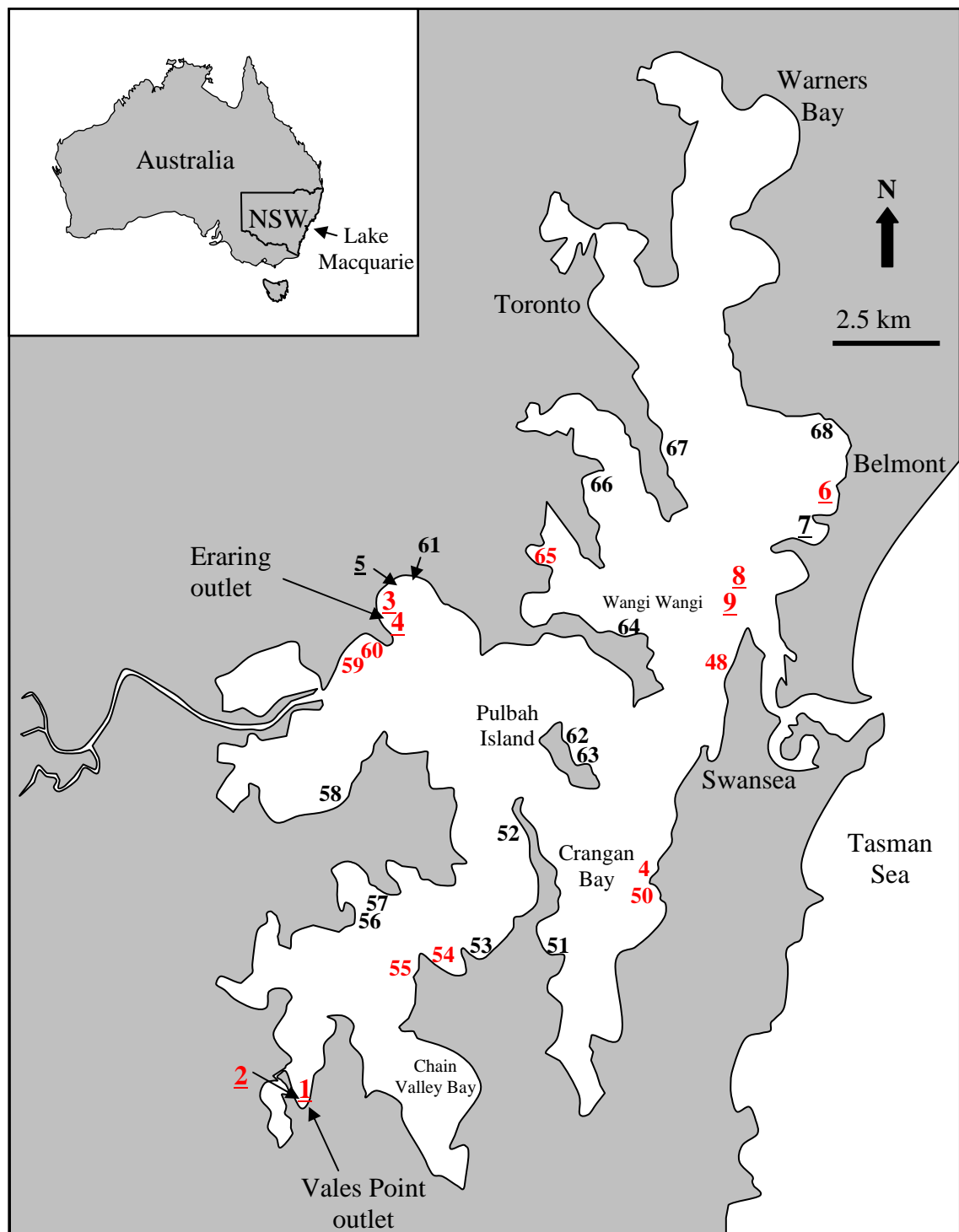


Figure 5.3. Sites sampled at Time 3 (March 2005). Underlined sites were also sampled at Times 1 & 2. Sites in red indicate presence of sponges and/or ascidians (See Table 5.3 for details).

5.3.2 Larger-scale impacts on diversity: Comparing Lake Macquarie with other estuaries

Overall, there were no patterns to suggest there were large lake-scale impacts of cooling water outlets on the diversity of sponges in Lake Macquarie compared to other estuaries (Figure 5.4). Regardless if sites near to the outlets were included in comparisons, the species and numbers of species of sponges found were similar to those in the seagrass meadows of other similar New South Wales estuaries. For example, three of the four species found in Lake Macquarie in March 2004 were also found in Wallis Lake. In contrast, with the exception of *Styela plicata*, the diversity of ascidians in Lake Macquarie was very different from all the other estuaries sampled. *Botrylloides leachi*, *Symplegma ocellata* and *Polyclinum nudum* were found only in Lake Macquarie, with *P. nudum* found only near the outlets.

Table 5.4. Distributions of sponges and ascidians found in the seagrass meadows of Lake Macquarie compared to Wallis Lake, Smiths Lake, Brisbane Water, Pittwater, Port Hacking and Lake Conjola in January-April 2004. Condition as classified by Healthy Rivers Commission (2002). Numbers in () indicate total number of sites where those species were found.

Estuary	Lake Macquarie	Wallis Lake	Smiths Lake	Brisbane Water	Pittwater	Port Hacking	Lake Conjola
Condition	Severely affected	Moderately affected	Slightly affected	na	na	na	Slightly affected
Sites sampled	29	16	16	20	17	17	24
Sponges found in Lake Macquarie							
<i>Haliclona</i> spp.	Uncommon (1)	Uncommon (2)		On mussel reefs, but uncommon (2)			
<i>Mycale</i> sp.	Abundant in patches near outlets and on <i>Posidonia australis</i> near Swansea (7)	Very patchy, growing on alga, <i>Lamprothamnion</i> sp. (4)		Abundant in patches, mainly on <i>P. australis</i> (5)		Very uncommon, one individual found on <i>P. australis</i> (1)	Abundant in patches, growing on the alga <i>Caulerpa taxifolia</i> (6)
<i>Phorospongia</i> sp.	Very uncommon, two individuals found (1)						
<i>Suberites</i> sp.	Found near outlets, but otherwise uncommon (4)	Patchily distributed amongst the alga, <i>Lamprothamnion</i> sp. and patchy <i>Zostera capricorni</i> (6)	Widespread, abundant in patches amongst patchy <i>Z. capricorni</i> (11)				
Total number of species of sponges	4	6	2	5	2	1	2
Ascidians found in Lake Macquarie							
<i>Botrylloides leachi</i>	Abundant on <i>Z. capricorni</i> near Swansea Channel (3)						
<i>Polyclinum nudum</i>	Abundant near Vales Point outlet (2)						
<i>Styela plicata</i>	Uncommon (5)	Widespread, abundant in patches (8)	Widespread, abundant in patches (6)	Uncommon (4)			Widespread (11)
<i>Symplegma ocellata</i>	Abundant on <i>Z. capricorni</i> near Swansea Channel (2)						
Total number of species of ascidians	4	3	1	2	0	1	2

5.3.3 Localised impacts on spatial and temporal patterns of abundance of *Mycale* sp, *Suberites* sp., *Polyclinum nudum* and *Botrylloides leachi* within Lake Macquarie

Statistical analyses of abundances were considered unnecessary within Lake Macquarie because sponges and ascidians were absent from many of the sites and times making patterns relatively straight forward (Figure 5.4). *Mycale* sp. was the most abundant sponge and was recorded at sites 1 and 2 near the Vales Point outlet on all three times of sampling. In contrast, elsewhere in the lake there were large temporal changes in patterns of presence and absence. For example, in March 2004, *Mycale* sp. was found at Site 4 near the Eraring outlet, site 5 in Myuna Bay, sites 6 and 7 near Belmont and a single specimen was found on the bivalve *Pinna bicolor* at Site 14 in Crangan Bay. In August 2004 and March 2005, *Mycale* sp. was very uncommon at sites distant from the outlets.

Suberites sp. was on average most abundant at sites 1 and 2 near the Vales Point outlet and in March 2004 near the Eraring outlet (Figure 5.4). *Suberites* sp. was found in only in relatively small numbers (i.e. 1 or 2 individuals in rapid searches) in a small subset of the sites distant from the outlets (Table 5.1, Table 5.2, Table 5.3; Figure 5.1, Figure 5.2, Figure 5.3). *Haliclona* spp. were found at site 1 near the Vales Point outlet in March 2004 and March 2005 and in Bonnells Bay in August 2004 (Table 5.1, Table 5.2, Table 5.3; Figure 5.1, Figure 5.2, Figure 5.3). *Aplysilla* cf. *sulphurea* was very uncommon with only three individuals found on mussel reefs at site 38 in Bonnells Bay in August 2004. Similarly, *Phorospongia* sp. was also very uncommon with two individuals found at site 5 in Myuna Bay in March 2005.

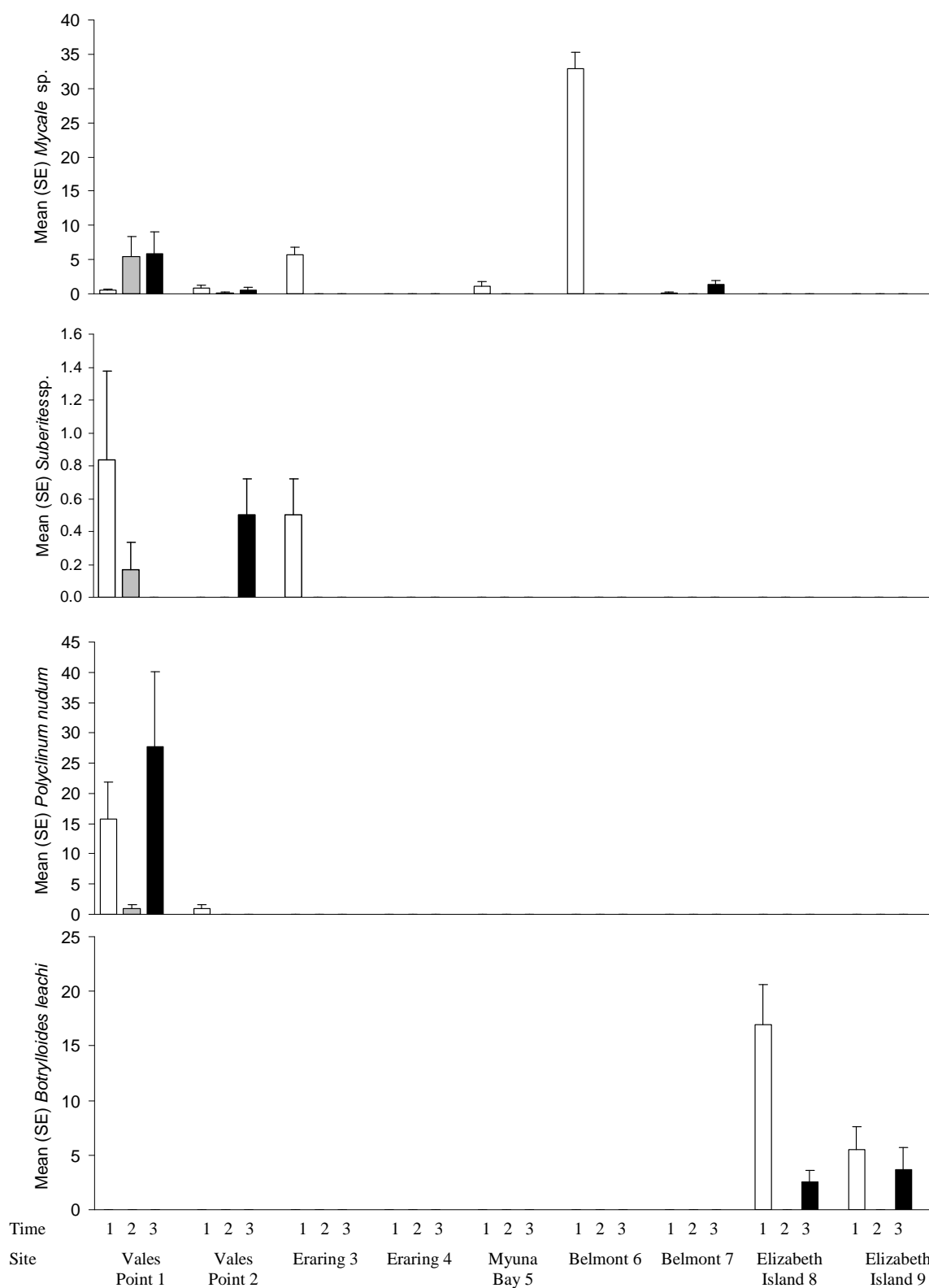


Figure 5.4. Means (SE) of abundances of invertebrates per transect in each of nine locations in March 2004 (Time 1), August 2004 (Time 2) and March 2005 (Time 3). $n = 6$.

5.3.4 Impacts on spatial and temporal patterns of abundance of *Mycale* sp., and *Suberites* sp: Comparisons of Lake Macquarie with other coastal lakes

Patterns of abundance of *Mycale* sp. were complex, varying through time, between sites and also between the two control lakes (Table 5.5, Figure 5.5). Although there were no statistically significant differences in abundances between Vales Point and the two Control lakes, there was a clear difference among lakes in the pattern of presence or absence among times. *Mycale* sp. was present at both sites at Vales Point on all three sampling times, but was found at only one time in each of the sites in Wallis Lake and Lake Conjola. In contrast, the temporal patterns of presence versus absence at the Eraring outlet were very similar to the two control lakes with *Mycale* sp. found only at Time 1, when abundances were also largest in Wallis Lake and Lake Conjola.

Abundances of *Suberites* sp. were significantly larger in the control lakes compared to sites at Vales Point or Eraring (Table 5.6, Figure 5.6). In contrast to *Mycale* sp., *Suberites* sp. were present on all occasions in each of the sites in Wallis and Smiths lakes, while occurring on fewer occasions in the sites at Vales Point and Eraring outlets.

Table 5.5. Asymmetrical analyses of variance to test for differences in abundances of *Mycale* sp. in Lake Macquarie compared to Wallis Lake and Lake Conjola. ^a Tests were constructed after lower order sources of variation were eliminated at $p > 0.25$. ^b Transformations did not remove heterogeneity of variances.

Source of variation	df	Vales Point v Controls			Eraring v Controls			F versus
		MS	F	p	MS	F	p	
Time	2							
Lake	2							
Impact v Controls	1	0.474	0.36	ns	0.921	0.62	ns	T x S(L) ^a
Between Controls	1	9.568	0.86	> 0.25	9.568	0.86	> 0.25	T x Between Cs ^a
Time × Lake	4	11.053	2.78	ns	5.920	4.01	ns	T x S(L)
Time × Impact v Controls	2	10.941	0.98	> 0.25	0.676	0.06	> 0.25	T x Between Cs
Time × Between Controls	2	11.164	8.41	**	11.164	7.56	**	T x S(L)
Sites(Lake)	3	0.310	0.23	> 0.25	1.343	0.91	> 0.25	T x S(L)
Time × Sites(Lake)	6	1.328	3.98	**	1.476	10.02	***	Residual
Residual	90	0.334			0.147			
Transform		Ln(x + 1) ^b			Ln(x + 1) ^b			

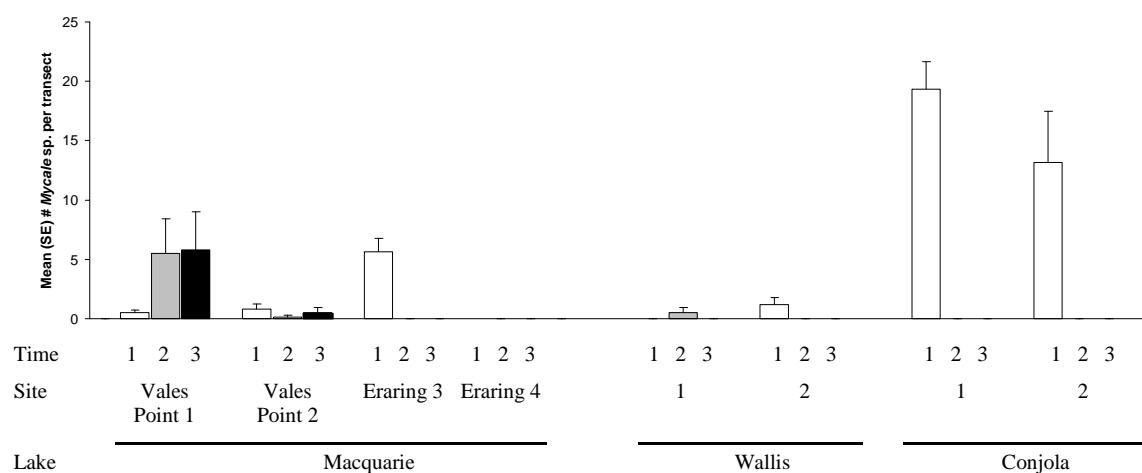


Figure 5.5. Means (SE) of abundances of *Mycale* sp. per transect in Sites near the cooling water outlets compared to Sites in Wallis Lake and Lake Conjola in March 2004 (Time 1), August 2004 (Time 2) and March 2005 (Time 3). n = 6.

Table 5.6. Asymmetrical analyses of variance to test for differences in abundances of *Suberites* sp. in Lake Macquarie compared to Wallis Lake and Smiths Lake. ^a Tests were constructed after lower order sources of variation were eliminated at $p > 0.25$.

Source of variation	df	Vales Point v Controls			Eraring v Controls			F versus
		MS	F	p	MS	F	p	
Time	2							
Lake	2							
Impact v Controls	1	10.205	31.96	**	13.156	61.93	***	T x S(L) ^a
Between Controls	1	3.965	1.01	> 0.25	3.965	1.00	> 0.25	T x Between Cs ^a
Time x Lake	4	0.090	0.28	ns	0.065	0.31	ns	T x S(L)
Time x Impact v Controls	2	0.170	15.80	ns	0.119	11.06	ns	T x Between Cs
Time x Between Controls	2	0.011	0.03	> 0.25	0.011	0.05	> 0.25	T x S(L)
Sites(Lake)	3	3.935	12.33	**	3.964	18.66	**	T x S(L)
Time x Sites(Lake)	6	0.319	1.05	> 0.25	0.212	0.77	> 0.25	Residual
Residual	90	0.305			0.277			
Transform		Ln(x + 1)			Ln(x + 1)			

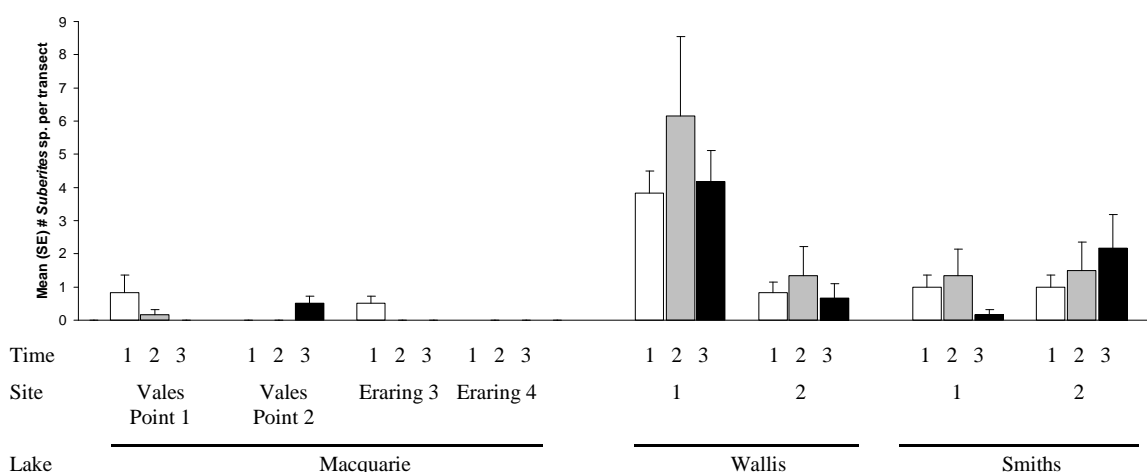


Figure 5.6. Means (SE) of abundances of *Suberites* sp. per transect in Sites near the cooling water outlets compared to Sites in Wallis Lake and Smiths Lake in March 2004 (Time 1), August 2004 (Time 2) and March 2005 (Time 3). $n = 6$.

5.3.5 Associations of sponges and ascidians with habitat

In order to interpret the potential impacts of the power stations on assemblages of sponges and ascidians it is important to consider differences in habitat. In general, the habitats in sites near to the power station outlets were very different from sites

elsewhere in Lake Macquarie (Table 5.1, Table 5.2, Table 5.3) or in any of the other estuaries sampled. These sites were characterised by beds of dense *Halophila ovalis* with occasional patches of bare sand or gravelly substrata. The only similar habitats found in Lake Macquarie or in any of the other estuaries were near the inlet canal for Eraring Power Station (Table 5.1, Table 5.2, Table 5.3). Although not quantitatively measured, relatively strong currents flowing in the direction of the inlet canal were observed at sites 37, 38 and 39 near the inlet canal (pers. obs.). In general, in Lake Macquarie, sponges were most diverse and abundant in habitats dominated by *H. ovalis*, *Posidonia australis* or where *Zostera capricorni* was patchy and/or short. With the exception of *Styela plicata*, which was found in almost all habitats, sponges and ascidians were not found where *Z. capricorni* was very dense and tall (Table 5.1, Table 5.2, Table 5.3). *Mycale* sp. was found in a number of different habitats. The largest abundances of *Mycale* sp. recorded in Lake Macquarie were on the blades of *P. australis* in March 2004 at site 6. *Mycale* sp. was also found on *H. ovalis*, bare sediment and gravel in sites near to the Vales Point and Eraring outlets.

5.4 DISCUSSION

This study identified several patterns of distribution of sponges and ascidians that were consistent with localised impacts of cooling water outlets of power stations including increased diversity, increased abundances and reduced temporal variability near to the outlets. Patterns, however, were complex, varied in effect among species, between the two outlets and their interpretation was dependant on the spatial or temporal scales examined.

Localised impacts: When diversity was examined within Lake Macquarie, two key patterns emerged to suggest there were localised increases in sponge and

ascidian diversity and abundance near the outlets. Although sponges were very patchily distributed, they were most diverse and/or abundant in sites near the cooling water outlets of the two power stations (particularly at Vales Point) and the inlet canal to Eraring power station. Elsewhere in the lake, with the exception of sites near Belmont, they were extremely uncommon. Ascidians, however, showed more variable patterns among species. There was strong evidence to suggest *Polyclinum nudum* occurred only in close proximity to the outlets. *P. nudum* was found nowhere else in Lake Macquarie or in any other of the estuaries studied in this thesis. *Botrylloides leachi* was most abundant near to the Swansea Channel and was not found in any other sites distant from the Swansea Channel with the exception of sites adjacent to the outlets. *Symplegma oceania* and *Pyura stolonifera* were found only near to the Swansea Channel and it is likely their distribution was correlated with larval supply and proximity to source populations on the open coast (Ayre *et al.* 1997). Together, these patterns suggest sponges and the ascidians, *P. nudum* and *B. leachii* would likely to have been absent or at least uncommon in these areas of Lake Macquarie if there were no power stations. These patterns are in contrast to Nicholls (1999) study of macroalgae which showed a decrease in diversity in close proximity to the outlets.

Impacts that increase diversity or abundances often require different considerations and logic for management than those impacts that cause decreases. One important consideration is to determine whether species introduced to areas pose ecological threats to other native organisms. Because the majority of sponges and ascidians in this study were likely to have been native to Lake Macquarie and abundances were increased only in relatively small areas near to the outlets, it is unlikely any serious threats would be posed to the ecology of the lake. In contrast, threats can become more serious when new species are introduced. Several authors

have suggested that some types of human impacts may provide footholds from which introduced species may then move on to colonise surrounding habitats. For example, Glasby *et al.* (2007) suggested artificial structures in estuaries such as pontoons and jetties may provide footholds for invasive sessile invertebrates. Piola & Johnston (2008) found heavy metal pollution to favour non-indigenous sessile invertebrates (including ascidians and sponges) over indigenous species. In the case of the power stations in Lake Macquarie, it appears the discharge of cooling water may be creating just such a foothold for new species to colonise new habitats. More specifically, it is likely that *Polyclinum nudum* was beyond its natural range of distribution in Lake Macquarie and only able to survive because of the presence of the power stations. Records for the range of *P. nudum* are rare, but its previously southern most collection was approximately 400km north at Coffs Harbour (Kott 1992). Introduced species, which are able to gain a foothold because of anthropogenic impacts, may then persist in relatively small abundances or patchy populations before becoming more widespread and problematic (e.g. Meinesz 1999). The management and potential eradication or control of potentially harmful introduced species will benefit from the early identification of invasion sites. In addition, the effectiveness of management will also be improved by an understanding of the physical and biological processes causing these impacts.

Models to explain increases in diversity and abundance: The patterns identified in this study provide the logical starting points to propose possible ecological models to explain the relatively high diversity and abundances of sponges and ascidians near the cooling water outlets including; higher water temperatures, increased food supply, increased water movement and alterations to physical habitat. Each of these possible models is discussed below.

High water temperatures: Historically water temperatures in Lake Macquarie have ranged between winter minima of 13-15 °C and summer maxima of 26-28 °C (Roberts & Barnes 2004; Eyre 2005; Nicholls 1999). Water temperatures near the cooling water outlets, however, can be up to 10 °C and on average 6 °C warmer (Nicholls 1999) than elsewhere in the Lake. Increased water temperatures can have varying effects on marine and estuarine fauna and flora depending on the magnitude of change (see Bamber 1995). For example, in Long Island Sound in the US, increased temperatures caused by the cooling water discharge from a power station increased growth of the large brown alga *Ascophyllum nodosum* (L.) Le Jolis, until temperatures exceeded 27-28 °C and the alga died (Keser *et al.* 2005). In contrast, in the Mediterranean, thermal increases of approximately 2 °C caused by a power station had no detectable effects on meiobenthic and macrobenthic invertebrate assemblages in soft sediments (Lardicci *et al.* 1999). In India, fouling communities (including ascidians) died when thermal discharges from a nuclear power plant caused water temperatures to rise above 37 °C (Suresh *et al.* 1993).

In this study, there were a number of correlative patterns to support the explanation that higher water temperatures were at least partially responsible for sustaining populations of at least two of the species: *Mycale* sp. and *Polyclinum nudum*. *Mycale* sp. was most widespread and abundant in March 2004 and March 2005 when ambient water temperatures were relatively warm throughout the lake (approximately 24 °C), compared to winter (August 2004) when water temperatures were relatively cooler (approximately 13 °C) and *Mycale* sp. was only relatively abundant near the outlet to Vales Point power station where temperatures were 23-24 °C. Further, *Mycale* sp. was only found in Wallis Lake and Lake Conjola in March 2004 when water temperatures were relatively warm (20-22 °C), and was very uncommon or absent in

August 2004 when water temperatures were much cooler (12-15 °C). *P. nudum* shows an even stronger correlation with water temperature; found only near the outlets where water temperatures remained above approximately 23-24 °C. Higher water temperature in a Mediterranean lagoon relative to the adjacent sea has been suggested as the key environmental factor sustaining populations of the ascidian, *Ecteinascidia turbinata* to survive through colder winter periods when populations outside of the lagoon do not survive (Carballo 2006). To add further support to the temperature model, *P. nudum* was not found in other areas of Lake Macquarie that could be considered to have similarly strong water movement (e.g. near the entrance to the Swansea Channel or the inlet canal to Eraring Power Station) or similar habitats characterised by dense beds of *Halophila ovalis* (i.e. near the inlet to the Eraring Power Station). *P. nudum* appears to be a more tropical species typically found in warmer waters north of Lake Macquarie (Kott 1985).

Increased supply of food: A second explanation for patterns of high diversity and abundance at the outlets relates to the possibility of increased availability of food for filter feeders. For example, Ambrose *et al.* (1996) suggested that increases in the abundances of other filter feeding invertebrates (sessile polychaetes and bivalves) near the cooling water outlets from Californian power stations maybe due to increased organic material, which originated from plankton killed in the cooling system and discharged in the plume. Mortality of plankton due to the stresses of entrainment within cooling-water systems of power station appears to be common (Bamber & Seaby 2004).

Increased movement of water: The combined discharge from the outlets is up to 146 m³/s when operating at full capacity, which creates current velocities that are generally much larger than elsewhere in the lake (Nicholls 1999; NSW Department of Natural Resources 2008). It has been proposed that changes in water movement can

increase abundances of filter feeders including ascidians and sponges (Glasby 2001). Large abundances of ascidians have been correlated with areas of strong currents or tidal flow (Lambert & Lambert 2003) and experimental work has shown increased growth and survival of some sponge species with increased water velocity (Wilkinson & Vacelet 1979). In Lake Macquarie, this model is supported by the observation that there was a large diversity and abundance of sponges near the inlet canal to Eraring power station where water movement was large, but water temperature was not above ambient.

Changes in seagrass habitat: Finally, the composition of the seagrass meadows near the outlets was very different from those found elsewhere in the lake. Sites near the outlets were characterised by very dense *Halophila ovalis* with occasional patches of bare substrata and sparse *Zostera capricorni*. This contrasted to most other sites within the lake, which were dominated by patchy to dense *Z. capricorni* and in some places *Posidonia australis*. Dense meadows of *H. ovalis* are structurally very different, with relatively short leaves and canopy (less than 5 cm), compared to *Z. capricorni* and *P. australis* meadows which are in general much taller (up to 1 metre high). The structure and patchiness of seagrass habitats affects assemblages of many types of animals including fish (Connolly & Hindell 2006; Jackson *et al.* 2006), molluscs (Irlandi 1997; Bologna & Heck 2000) and crustaceans (Tanner, 2006). Although, little to no work has been done to examine the effects of seagrass structure on sponges or ascidians, it is likely they are also affected. Differences in seagrass complexity may also have direct or indirect effects on the associated fauna (Bostrom *et al.* 2006). For example, dense *Halophila ovalis* may have a direct positive effect by providing a suitable habitat for sponges and ascidians to attach. Conversely, dense *Z. capricorni* may have a direct negative effect by out-competing sponges for space on the

substrata (Cebrian & Uriz 2006). It has been suggested, however, that indirect effects of seagrass complexity are more likely to influence distributions of fauna (Bostrom *et al.* 2006). For example, the patchiness of seagrasses can affect predation rates on fish (Hindell *et al.* 2000) and bivalves (Bologna & Heck 1999).

Overall, it is likely that processes may be interacting synergistically and probably affect different organisms in different ways. For example, from the observations made in this study, it could be hypothesised that *P. nudum* will be influenced by high water temperatures, while sponges may respond more to stronger currents and habitat structure. The disentanglement and identification of these specific processes and mechanisms is likely only to be achieved with manipulative experiments.

Larger scale impacts: While differences in diversity and abundances within Lake Macquarie were relatively easy to interpret as localised to the outlets, larger scale comparisons with other estuaries were more complicated (Table 5.4). With the outlet sites included, assemblages of sponges in Lake Macquarie could be considered to have a similar diversity, but with smaller abundances and more patchy distributions than other large estuaries (e.g. Wallis Lake and Brisbane Water). In contrast, if the sites near outlets are omitted, diversity and abundances in Lake Macquarie would be considered small. This suggests additional impacts may have or are affecting the distributions of sponges. Lake Macquarie is classified as severely affected by catchment modification (Healthy Rivers Commission of NSW 2002) with various impacts including eutrophication and macroalgal blooms caused by nutrient inputs from terrestrial run-off (Nicholls 1999) and heavy metal contamination in molluscs caused by industry (Batley 1987).

In conclusion, the patterns observed in this study suggest that discharge of cooling water from the power stations in Lake Macquarie have a localised impact that increases the diversity and abundances of sponge and ascidian assemblages.

CHAPTER 6: EFFECTS OF SHADING, WATER FLOW AND PREDATION ON THE SPONGE, *SUBERITES* SP. IN THE SEAGRASS MEADOWS OF A TEMPERATE AUSTRALIAN COASTAL LAKE

6.1 INTRODUCTION

The structure and complexity of seagrass landscapes can dramatically influence the distributions of a diverse range of associated fauna and flora (Bostrom *et al.* 2006; Connolly & Hindell 2006). Seagrass landscapes are naturally heterogeneous environments often described as a mosaic of patches of different species of seagrass, macroalgae and unvegetated substratum (Turner *et al.* 1999; Bell *et al.* 2006b). Patches vary at large scales in terms of their size, shape, perimeter, proximity to other habitats, etc., and at smaller scales in terms of the attributes of the individual plants (e.g. shoot height, shoot density, biomass) that make up those patches (West 1990; Cunha & Duarte 2007). In recent times, the natural structure of seagrass landscapes has been dramatically changed on a worldwide scale by coastal development and associated anthropogenic impacts (Orth *et al.* 2006a; Ralph *et al.* 2006). Dredging, eutrophication, sedimentation, contamination, boating, introduced species and shoreline modification have lead to extensive losses of seagrass meadows, increased fragmentation, reductions in patch size and number, changes in species composition and changes to the attributes of the plants themselves (Cambridge & McComb 1984; Cunha *et al.* 2005). Seagrass landscapes are also increasingly recognised as ecologically and economically important habitats as nursery grounds for many species (Jackson *et al.* 2001; Heck *et al.* 2003), for nutrient cycling, stabilising sediments and preventing shoreline erosion (Costanza *et al.* 1997; Orth *et al.* 2006a). With increasing impacts comes a need for better management of these important habitats which must include an understanding of the effects of changes to seagrass habitats on the associated fauna.

Associations with seagrasses have been extensively studied for several faunal groups, including crustaceans (Murphey & Fonesca 1995; Hovel 2003), molluscs (Irlandi *et al.* 1995; Bologna & Heck 2000a; Peterson & Heck 2001), mobile macrofauna (Turner *et al.* 1999) and in particular, much work has focussed on fish (Connolly & Hindell 2006; Jackson *et al.* 2006). In contrast, there have been exceedingly few studies examining patterns of distribution of sponges with seagrass landscapes (but see Thorhaug & Roessler 1977; Kuenen & Debrot 1995). Bostrom *et al.* (2006) and Connolly & Hindell (2006) proposed several possible underlying processes to explain distributions of nekton within seagrass habitats including environmental disturbance, encounter rates with seagrass patches, food availability, predation, larval supply, migration or movement of adults and reproductive success. While these models were developed largely from studies of mobile fauna, they also provide logical and plausible processes which may explain the distributions of sessile fauna such as sponges.

In particular, the availability of food has been frequently proposed to explain higher and lower densities of filter feeding invertebrates inside patches or under seagrass canopies (Irlandi *et al.* 1999; Reusch & Williams 1999). Decreases in the availability of food for filter feeding bivalves have been directly related to decreases in water flow and particles of food in patches of dense seagrass (Reusch & Williams 1999). The effects of water flow can also be more complex than the simple delivery of food, and may influence feeding capability and respiration for many sessile filter feeders (Knott *et al.* 2004). Water flow and current velocities can be dramatically affected by the structure of seagrass meadows (Koch *et al.* 2006). For example, Heiss *et al.* 2000 found that in beds of *Zostera novazelandica* (Setchell), which were 12 cm high, velocities ranged from 1.2 - 4.6 cm s⁻¹ outside and 1.9 - 7.1 cm s⁻¹ above the seagrass

patch compared to $0.1 - 1.8 \text{ cm s}^{-1}$ inside, indicating slower and less variable water flow. Given that numerous studies have demonstrated the importance of adequate water flow for the survival and growth of sponges in non-seagrass habitats (e.g. Wilkinson & Vacelet 1979; Duckworth *et al.* 2004; Bell & Barnes 2000a & b), it also provides a plausible mechanism to explain the distribution of sponges within seagrass habitats.

One attribute of many shallow water sponges, which is unusual in the animal kingdom, is the presence of photosynthetic symbionts within the sponge tissue (Rutzler 1990). In this respect, sponges which contain photosynthetic symbionts may be affected by processes more widely studied for plants. Therefore, a mechanism additional to those proposed by Bostrom *et al.* 2006 and Connolly & Hindell 2006 for nekton can be proposed for sponges: i.e. the availability of light in seagrass habitats. Significant effects of canopy shading on understoreys have been extensively studied and are well known in the terrestrial realm (Keddy 2005) and to a lesser extent in kelp forests of the marine realm (Dayton 1975; Connell 2003). In seagrass habitats, effects of self-shading by seagrass canopies are prevalent and well understood (Zimmerman 2008). The effects of shading by seagrasses on other species and algae are less well understood, but there is some evidence to suggest shading can have strong influences on understorey species. For example, Ceccherelli & Cinelli (1999) found shading by meadows of very dense *Posidonia oceanica* in the Mediterranean restricted the growth of the alga *Caulerpa taxifolia*. In non-seagrass environments experimental shading of sponges which contain photosynthetic symbionts has lead to significant and sometimes rapid reductions in size and growth leading to eventual mortality (Roberts *et al.* 2006). Using manipulative experiments, Thacker (2005) found the cyanobacteria containing sponge, *Lamellodysidea chlorea*, lost substantial biomass and surface area (up to 40%)

in relatively short periods of time (2 weeks). Wilkinson and Vacelet (1979) found the growth of *Verongia aerophoba* which contains cyanobacteria to be 'enhanced in light'.

The cyanobacteria containing sponge, *Suberites* sp. can be a common and conspicuous inhabitant of the seagrass meadows and algal beds of several New South Wales coastal lakes (Chapters 2, 3, 4 and 5). The distribution of *Suberites* sp. within these lakes is characterised by its absence from areas where the seagrass, *Zostera capricorni* has relatively high shoot density and long leaves, and its presence in areas where *Z. capricorni* is absent, sparse, patchy or has relatively short leaves (Chapters 4 and 5). Based on these observations, I examined three possible models to explain the absence of *Suberites* sp. from dense and tall *Z. capricorni* meadows using *in situ* manipulative experiments: i) *Suberites* sp. are absent from areas with dense and tall *Z. capricorni* because there is inadequate water flow for them to survive, ii) *Suberites* sp. are absent from areas with dense and tall *Z. capricorni* because there is inadequate light for them to survive, iii) *Suberites* sp. are absent because of other processes associated with areas of dense and tall *Z. capricorni*.

6.2 MATERIALS AND METHODS

6.2.1 Study-sites and sampling methods

Experiments were done in the seagrass meadows of Smiths Lake on the New South Wales mid-north coast (Figure 6.1). Smiths Lake is intermittently closed and open to the ocean with salinities ranging from 15-36 ‰. Four sites were chosen in a continuous meadow of *Zostera capricorni* on the north-western shore of Big Island: Sites 1 and 2 were in areas characterised by short (< 10 cm leaf height) *Zostera capricorni*, with small patches (less than 1 m²) of taller *Z. capricorni* (approximately 50-100 cm leaf height), bare sediment and sparse *Halophila ovalis* where *Suberites* sp.

were relatively abundant (approximately 5-10 individuals per 10 m²); Sites 3 and 4 were in areas characterised by dense (approximately 90-100 % cover) and tall *Z. capricorni* (approximate height of canopy of 90-110 cm) where *Suberites* sp. were absent.

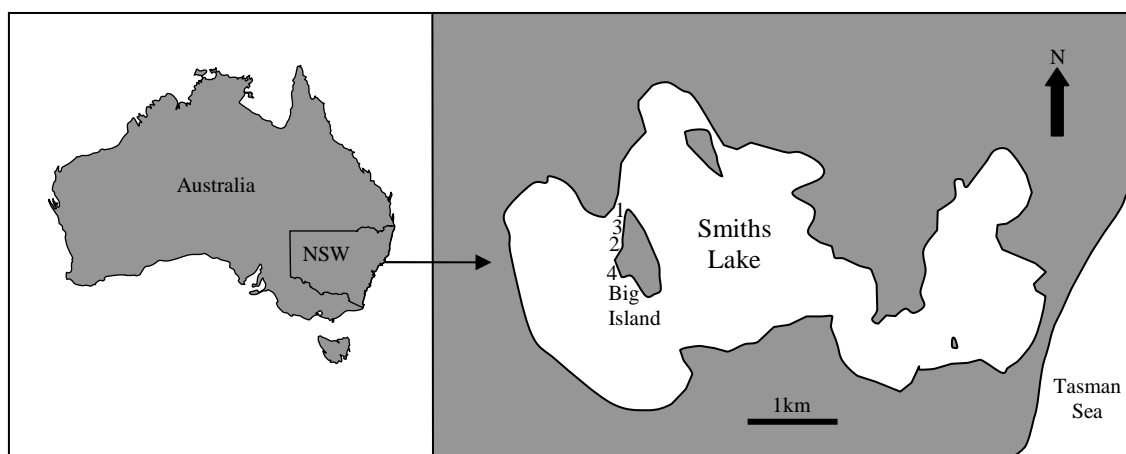


Figure 6.1. Study Sites in Smiths Lake

Experiment 1: Shading: In order to simulate the shading produced by dense *Zostera capricorni*, the intensity of light under dense canopies where *Suberites* sp. were absent (Sites 3 and 4) and in nearby unshaded areas where *Suberites* sp. were present (Sites 1 and 2) were measured using a Licor® PAR sensor. To test for the effects of this level of shading on the survival and growth of *Suberites* sp., four individual sponges were then separately shaded with a sheet of rigid opaque black plastic (50 cm × 70 cm) that was secured approximately 40 cm above the substratum by four plastic pipes (Figure 6.2). Fifty 5 mm □ holes were drilled into each sheet of plastic to simulate the reduced light intensity experienced under a dense *Z. capricorni* canopy. The flow of water under the shade-structure was measured by tracking the movement of coloured dye and was found to be similar to adjacent areas. To test for artefacts of placing a physical structure over the sponges, procedural controls with same dimensions and number of holes as the shade-structure, but made from ‘Acrylite® OP-4 Ultraviolet-

Transmitting Acrylic Sheet' which transmits Photosynthetically Active Radiation and Ultraviolet Radiation, and therefore maintained a close to natural light regime, were placed over four individual sponges (Figure 6.2). The procedural controls and shade-structures were checked every 2-3 days for epiphytes and cleaned as necessary.

Experiment 2: Reduced water flow: Water movement in Smiths Lake is temporally variable and greatest when the entrance is open to the sea, after heavy rain or when there are moderate to strong winds (Everett 2007; Barnes pers. obs). Currents are generally negligible at other times (Barnes pers. obs). Relative differences in the movement of water within and outside meadows of dense *Zostera capricorni* were estimated using a simplified dye tracking technique (Koch & Verduin 2001). A small blob of red dye was released approximately 15 cm above the substratum (i.e. at a similar height as the top of the sponges) and the distance it moved in a set period of time was estimated by a snorkeller by reference to wooden stakes placed at 20 cm intervals from the point of release. This process was repeated four times within beds of dense *Z. capricorni* (Sites 3 and 4) and four times outside beds of dense *Z. capricorni* (Sites 1 and 2). When there were relatively strong winds (15 – 25 knots) from the northeast, the velocity of water was estimated to range from 0.5 to 2.0 cm⁻¹ outside beds of dense *Z. capricorni*, compared to inside meadows of dense *Zostera capricorni* where velocities were negligible and estimated to be close to zero. When there were relatively weak winds (< 5 knots), water velocity was negligible in each of the habitats and again estimated to be close to zero. While it is acknowledged this technique may not provide accurate estimates when velocities are very small, it did demonstrate a clear relative difference from inside to outside beds of dense *Z. capricorni*.

To test for the effects of reduced water flow on the survival and growth of *Suberites* sp., rectangular plastic barriers (45 cm × 70 cm × 42 cm tall) were placed

around four individual sponges (Figure 6.2) to simulate conditions within a meadow of dense *Z. capricorni*. Approximately one hundred holes, 5 cm in diameter were drilled into the sides of each barrier to allow a reduced movement of water. The plastic barriers remained open at the top and there were no measurable differences in light intensity compared to adjacent areas. To test for potential artefacts of placing a physical barrier around the sponges, procedural controls consisting of plastic barriers with the same dimensions, but with large holes cut into all four sides to allow normal movement of water was placed around four individual sponges (Figure 6.2).

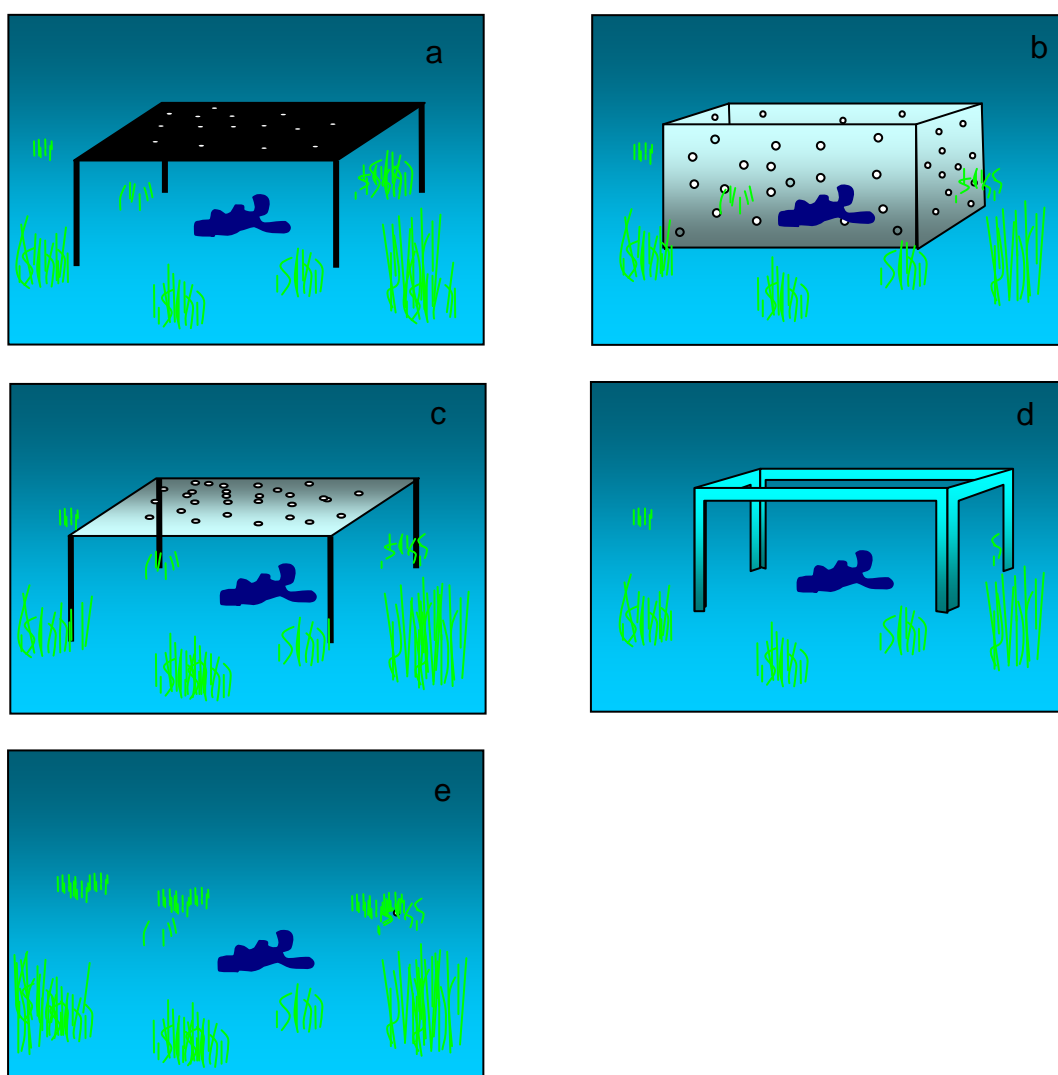


Figure 6.2. *In situ* experiments: a) reduced light, b) reduced water flow, c) procedural control for reduced light, d) procedural control for reduced current and e) untouched control. Experiments were repeated at each of two sites with $n = 4$ for each treatment at each site.

Experiment 3: Transplantation to dense *Zostera capricorni*: To examine the model that *Suberites* sp. were absent from dense and tall *Zostera capricorni* meadows because they could not survive there, four individual sponges from each of two Sites (Sites 1 and 2) were transplanted into meadows of dense and tall *Z. capricorni* (Sites 2 and 4) as per Figure 6.3. Sponges were placed in plastic containers and kept submerged in water while being transported between Sites. To test for potential artefacts of transplanting sponges to a new location, four individual sponges were translocated into similar habitats from which they had been removed (i.e. four sponges were translocated from Site 1 to Site 2 and another four from Site 2 to Site 1). See Chapman (1986) for logic of controls necessary for experiments involving transplantation of organisms.

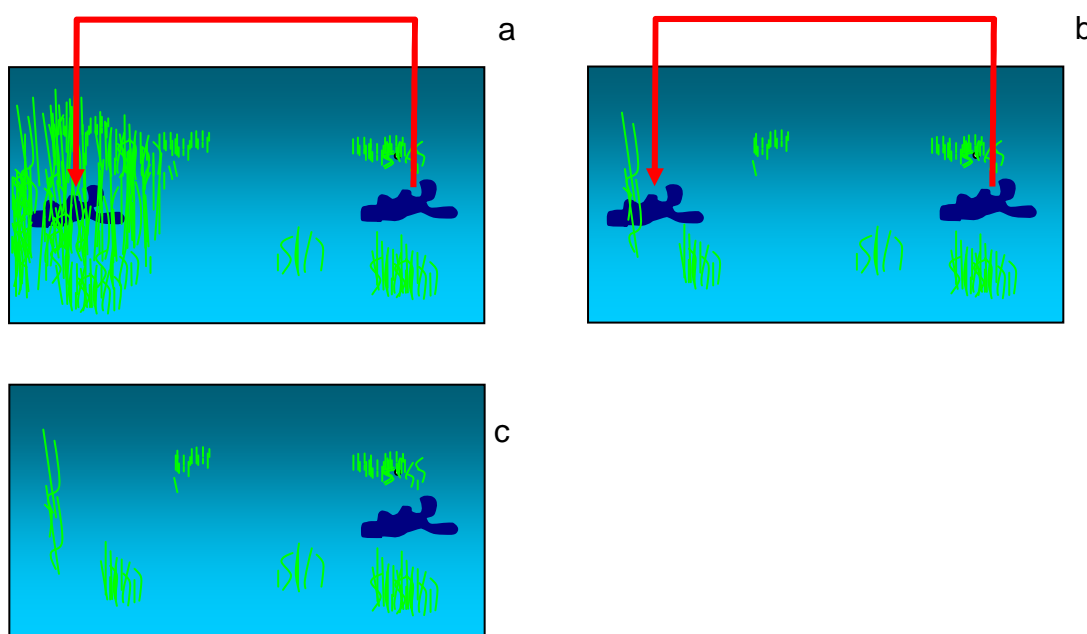


Figure 6.3. *In situ* experiment to test for effects of transplanting *Suberites* sp. to beds of dense and tall *Zostera capricorni*: a) transplantation into dense seagrass, b) translocation procedural control and c) untouched control. Experiments were repeated in each of two sites with $n = 4$ for each treatment at each site.

All sponges were individually tagged with small plastic shellfish labels that were secured with dental floss sewn through the tissue of the sponge. The results of a pilot study suggested this method of tagging had no detectable effects on the sponges (Barnes, unpublished data).

The survival and change in size of each individual sponge were recorded and measured from before to five weeks after the start of the experiments. Each sponge was photographed from above with a digital camera to estimate a 2-dimensional area. In addition, the number and diameter of individual oscula were counted and measured. For some species of sponges, the number and size of oscula are known to change when exposed to environmental stress (e.g. shading of the cyanobacteria containing *Verospongia aeropoba*, or exposure to light Wilkinson & Vacelet 1979; increased salinity, Leamon & Fell 1990) including some species of *Suberites* (e.g. physical disturbance of *S. domuncula*, Hamer *et al.* 2007). Similar, the *Suberites* sp. in this study in Smiths Lake, was observed to reduce both the size and numbers of oscula within 2 days of being placed under stress in a manipulative experiment to examine the effects of freshwater flood events (P. Barnes, unpublished data).

6.3 RESULTS

There were no measurable effects of any procedural artefacts of shading, reduced water flow or transplantation on the survival and growth of sponges or number or size of oscula (Figure 6.4; Appendix 4). Similarly, there were no measurable effects of shading or reduced water flow (Figure 6.4; Appendix 4).

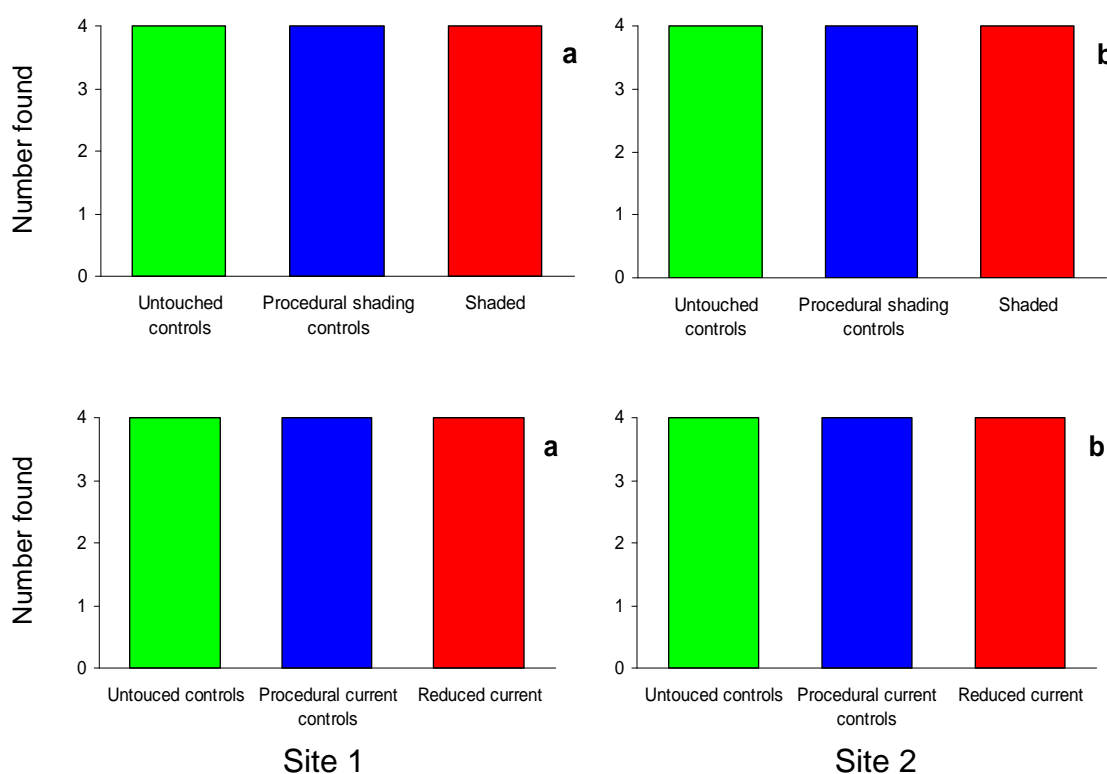


Figure 6.4. Number of sponges found one month after water flow was reduced compared to controls in Sites 1 and 2.

In contrast, however, there were large and clear effects on the survival of *Suberites* sp. transplanted into dense *Zostera capricorni*. Only three of the eight individual sponges transplanted into dense *Z. capricorni* were found (Figure 6.5). Further, the three that were found were severely reduced in size, had a reduced number of oscula and had very clear signs of predation including obvious bite marks typical of Monocanthid fishes (Figure 6.6). No bite marks were observed on any of the sponges in the remainder of the treatments. Following these observations, a further four sponges from each of Sites 1 and 2 were transplanted into dense *Z. capricorni* (Sites 3 and 4 respectively) and then inspected at regular intervals. After 1 hour of transplanting, there were obvious bite marks on six of the eight sponges. After 1 day, all sponges had numerous bite marks and were reduced in size and after 2 days, six of the sponges had been completely consumed. During this time, repeated attempts were made to observe

and identify fish or other animals feeding on the sponges, however, none was observed directly feeding on the sponges. Fortuitously, information on potential predators was provided by a commercial fisher netting in Smiths Lake in dense *Z. capricorni* meadows near to the study sites and during the same time as these experiments who reported catching large numbers of juvenile Monocanthids of two species (*Meuschenia trachylepis* and *Scobinichthys granulatus*).

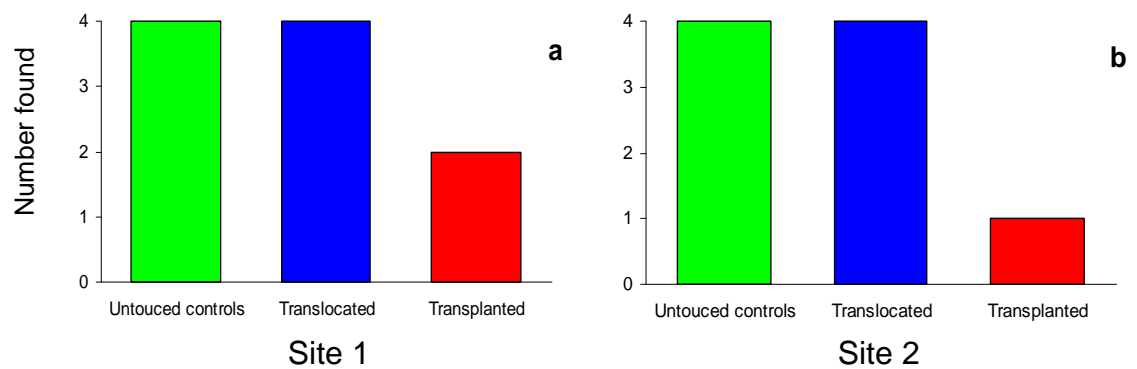


Figure 6.5. Number of sponges found one month after being transplanted to dense seagrass compared to controls in Sites 1 and 2.



a) Typical healthy *Suberites* sp. before being transplanted into dense *Zostera capricorni*



b) *Suberites* sp. one hour after being transplanted into dense *Zostera capricorni*



c) *Suberites* sp. one day after being transplanted into dense *Zostera capricorni*



d) Juvenile *Meuschenia trachylepis* are likely predators of *Suberites* sp.

Figure 6.6. Examples of effects of predation on *Suberites* sp. transplanted into dense *Zostera capricorni* meadows and a likely predator, *Meuschenia trachylepis*. Note two obvious bite marks in b) and numerous bite marks in c).

6.4 DISCUSSION

The results of this study strongly suggest that predation by fish is likely to be a key process determining small-scale patterns of distribution of *Suberites* sp. in seagrass meadows. Although, predation cannot be unequivocally attributed to fish, the absence of any other obvious sponge-feeders in Smiths Lake, the shape of the bite marks, the rapidity with which sponges were consumed and the catches by commercial fishers in nearby seagrass meadows in the lake strongly suggest that juvenile Monocanthids, most likely *Meuschenia trachylepis* and/or *Scobinichthys granulatus* were consuming the *Suberites* sp. soon after they were transplanted into dense and tall *Zostera capricorni*

meadows. This model is further supported by the observation that *M. trachylepis* and also *M. freycineti* are known to be significant consumers of epiphyte-covered leaves of the seagrass, *Posidonia australis* in other New South Wales estuaries, to the extent that these two fish species may directly affect seagrass biomass and potentially alter the trophodynamics of *P. australis* beds (Wressnig & Booth 2007, 2008).

Predation on sponges in seagrass meadows has also been observed elsewhere in the world. For example, in tropical seagrass meadows of the Caribbean, the species composition of sponge assemblages is maintained at least in part by predation by the large starfish, *Oreaster reticulatus* (Wulff 1995, 2008).

Increased predation of sponges with increased complexity of seagrass habitat was somewhat unexpected and is in stark contrast to the findings of a large volume of literature on the effects of seagrass structure on the survival of fauna. Heck & Orth (2006) in a review of predation in seagrass beds came to the conclusion that ‘On balance, the existing literature shows that, although there are differences in details, seagrass presence is almost always negatively related to predation effectiveness, and that increasing seagrass abundance is usually associated with decreasing predator effectiveness’. There appear to be few exceptions to this conclusion. Although, in a recent laboratory study, Matilla *et al.* (2008) concluded predation on grass shrimp by pinfish was not affected by increasing densities of artificial seagrass leaves, there appear to be no examples of increased predation on fauna correlated with increased complexity of seagrass habitats.

While the results of this study are in direct contrast to studies of predation on fauna in seagrass habitats, there are similarities with patterns of predation on some types of flora. Orth *et al.* (2006b) found predation rates by Portunid crabs on *Posidonia sinuosa* seeds were generally higher in complex vegetated habitats than in sand. The

authors attributed this pattern to the greater structural complexity of the seagrass canopies providing greater refuge for the crabs which were rapidly preyed upon by fish in sandy or less complex habitats. This observation suggests predation of *Suberites* sp. maybe caused by confinement of the sponge predators to a particular habitat (i.e. relatively dense and tall *Zostera capricorni*). Wulff (1995, 2008) observed similar differential predation on sponges between seagrass meadows and reefs in the Caribbean and confinement of the sponge predator, the starfish *Oreaster reticulatus*) to seagrass meadows. Alternatively, in Smiths Lake, the predator consuming *Suberites* sp. might not be confined to dense and tall seagrass, but might only feed in that type of habitat. It is also likely that the predator may be usually herbivorous or omnivorous and is an opportunistic feeder on sponges which are only available on occasional or unusual circumstances (Wulff 2006). For example, in Smiths Lake, it is possible that periodic broad-scale recruitment of *Suberites* sp. or movement of individuals into seagrass beds during strong winds and storms (P. Barnes, pers. obs.) may occasionally increase abundances within dense *Z. capricorni*. The restricted distribution of *Suberites* sp. may result from being eaten whenever they become available in dense and tall seagrass (Wulff 1995).

Two additional aspects of the patterns of predation on sponges observed in this study appear unusual. First, predation on sponges in temperate waters is generally considered to be dominated by invertebrates (Wulff 2006), although there is some evidence to suggest fish may play a role in some southern hemisphere systems (Ayling 1981). Second, the complete removal of sponges by predators in natural environments appears rare (Wulff 2006). It is more common, for bites to be taken with the bulk of the sponge left and able to survive. The complete removal of *Suberites* sp. may be related to an artefact of being transplanted to a new habitat. In other experiments, sponges

transplanted to new habitats have been completely consumed very quickly (Dunlap & Pawlik 1996).

There were no measurable effects of shading or reduced water flow on the survival, growth or oscula of *Suberites* sp. over the one-month period of this study. Negative effects cannot be completely ruled out in the longer-term, but these may be unlikely because sub-lethal responses to stress can occur over relatively short time periods. For example, *Suberites* sp. from Smiths Lake have responded quickly to artificial inputs of freshwater with reductions in the size and number of oscula within 2 days (Barnes, unpublished data). Hamer *et al.* (2007) suggested contractions and loss of oscula as a response to stress might be common for this genus. Responses to shading for other species can also be rapid with *Lamellodysidea chlorea* losing half its percentage cover after 2 weeks of artificial shading (Thacker 2005).

Changes to the structure of seagrass landscapes have important implications for the management and conservation of biodiversity in seagrass meadows. Anthropogenic impacts can change the natural structure of seagrass meadows in different ways. It could be hypothesised that seemingly deleterious impacts to seagrass meadows may actually have beneficial effects for some sponges. For example, reductions in the numbers or sizes of patches of seagrass (Cunha *et al.* 2005) or density of plants may provide suitable habitat for some species. In contrast, it could also be hypothesised that increases in seagrass densities or canopy height as a result of environmental impacts (e.g. nutrient enrichment, Lee & Dunton 2000) may create habitats which are unsuitable for some species of sponge. Clearly, extremes of unnatural removal or growth of seagrasses are not desirable. It is, however, imperative that the natural patchiness of seagrass meadows and its importance in structuring the associated assemblages of animals are recognised, understood and protected.

In conclusion, it appears *Suberites* sp. in areas in which seagrass is absent or sparse, is protected from predators that are confined to dense seagrass. This pattern was somewhat unexpected, is contrary to patterns observed for other faunal groups examined in seagrasses and highlights the complexity of ecological interactions among species and trophic levels in seagrass habitats.

CHAPTER 7: GENERAL DISCUSSION

7.1 OVERVIEW

To the best of my knowledge this study represents the first comprehensive and quantitative investigation of sponges and ascidians in both coastal lagoons and seagrass environments in temperate Australia. Previous studies of sponges and ascidians in these systems were virtually absent or extremely limited in detail. The first logical step in any investigation of organisms or habitats which have not been previously studied or surveyed is to investigate their basic patterns of distribution (Underwood *et al.* 2000). A large component of this thesis, therefore, was devoted to quantifying and understanding patterns of distribution. Patterns were investigated and identified at a range of spatial scales from associations of sponges and ascidians with patches of seagrass and species of macroalgae (Chapter 4) to much larger lake-wide patterns correlated with size of lake and opening regime with the ocean (Chapter 3). In addition, specific localised impacts of cooling water discharge from power stations (Chapter 5) and broader impacts correlated with the general effects of urbanisation and development were also identified (Chapter 3). The identification of patterns then provided the necessary observations to propose and investigate logical and informed models about processes and mechanisms operating in these systems. For example, based on the observation that some species of sponges were absent from dense seagrass meadows, I used manipulative experiments to identify predation by fish as an important mechanism in structuring assemblages of sponges in coastal lakes and lagoons (Chapter 6). In this chapter, I discuss these findings in relation to sponges and ascidians in other systems, other organisms in coastal lagoons, considerations for sampling and finally, the implications for management and conservation of these environments.

7.2 PATTERNS OF DISTRIBUTION

One of the most basic patterns of distribution that can be examined in ecology is the simple presence or absence of a species or taxonomic group. Before this study, even this basic information was virtually absent for sponges and ascidians in coastal lagoons and also seagrass habitats in New South Wales. A simple, but nevertheless key finding of this study, therefore, was their patchy but widespread occurrence in these systems. Sponges and ascidians were found in twelve of the nineteen lakes and lagoons that were surveyed over 800 km of the New South Wales coastline during the course of this thesis (Appendix 2). In general, with the exception of the large, but heavily modified Lake Illawarra, it was only in the small or very small lakes and lagoons where sponges or ascidians were not found (Chapter 3).

Diversity in the lakes and lagoons could generally be considered small when compared to other systems where sponges and ascidians have been studied. The largest number of species of sponges found in a single lake or lagoon was ten (in Wallis Lake), which was similar to a Caribbean lagoon (Kuenen & Debrot 1995), but generally less compared to Mediterranean lagoons where it appears not uncommon to find over 40 species (Corriero 1987; Mercurio *et al.* 2001, 2004). Most studies of diversity in Mediterranean lagoons, however, sampled rocky substratum in addition to seagrasses. Similar to the Mediterranean studies, relatively large numbers of species have been found in Vietnamese (up to 29 species in a single lake, Azzini *et al.* 2007) and Indonesian lakes (up to 45 species, de Voogd *et al.* 2006), but again sampling appeared to be largely on rocky substrata or mangrove roots rather than soft sediments and seagrasses. In contrast, rocky substratum was rarely encountered in the lakes and lagoons sampled in this thesis (Roy *et al.* 2001).

The number of species of sponges and ascidians found in New South Wales lagoons also appears small when compared to similar sized sampling areas in the marine waters of New South Wales. Roberts *et al.* (1998) identified over 100 species of sponges and 37 ascidians using photo-quadrats on subtidal rocky reefs over a few kilometres of New South Wales coastline and Roberts and Davis (1996) identified over 50 species of sponges over 20 km of New South Wales coastline compared to the 18 sponges and 7 ascidians identified in lagoons spanning over 800 km of coastline in this thesis.

There are several possible explanations for these relatively small diversities and patterns of distribution in lakes and lagoons compared to other systems. These include; limited availability of suitable substrata, limitations to recruitment from the open ocean and physical stresses caused by large fluctuations in abiotic variables associated with the unstable nature of these isolated water bodies (Barnes 1988; Woolridge 1999). Numerous studies have highlighted the importance of suitable hard substrata for the settlement and survival of sessile invertebrates including sponges and ascidians (Connell & Keough 1985; Davis *et al.* 1997; Rutzler *et al.* 2000). Diversity of ascidians is often larger in other types of estuaries in New South Wales which have more rocky substratum. For example, Newton *et al.* (2007) found 15 ascidians on rocky reefs in Port Stephens which is a large and permanently open water body with substantial areas of rocky reef and geographically close to Wallis and Smiths lakes. Rutzler *et al.* (2000) suggested the abundance of solid substrata in the form of mangrove roots was a principal factor in promoting the diversity of sponges in mangrove ponds in Belize. Overall, it is likely that the diversity of sponges in New South Wales lakes and lagoons may at least in part be limited by the lack of suitable hard substrata.

Recruitment or migration of organisms from the sea is also often invoked as important in structuring estuarine assemblages (Woolridge 1999; Bilton *et al.* 2002). Recruitment to lakes and lagoons which are periodically open and closed maybe erratic and depend on the timing and duration of the connection to the sea. Periodic openings of estuary mouths and subsequent recruitment and immigration have been identified as important in structuring assemblages of mobile fauna such as fish (Young & Potter 2002; James *et al.* 2008). Recruitment or lack thereof, is also likely to be important for structuring assemblages of sponges and ascidians. Most sponge larvae are considered to have relatively short planktonic periods of a few minutes to a few days and generally less than two weeks (Maldonado 2006). Ascidian larvae are similarly philopatric, particularly colonial species (Davis & Butler 1989), although the larvae of some solitary species have shown evidence of further dispersal (Ayre *et al.* 1997). Short dispersal distances (Nichols & Barnes 2005) combined with limited opportunity for access into these lakes and lagoons because of narrow inlet channels, restricted tidal exchange and in some cases, short opening periods with the sea (Roy *et al.* 2001; Haines *et al.* 2006), likely restrict recruitment of sponge and ascidian larvae into these isolated systems. The consequences of such limited opportunities for recruitment probably include the restriction of the number of typically marine species which are incapable of forming self sustaining populations within lakes and lagoons. The probability of larvae or other propagules (e.g. fragments) of those marine species entering lakes would presumably be relatively small. This theory is supported by the results of Chapter 3, where more species were found in mostly open lakes and lagoons than mostly closed.

There is also evidence to suggest populations of some species may be self sustaining and may not occur or are rare on the open coast (see Discussion in Chapter 3). For example, *Suberites* sp. were found on each of ten occasions over four years of

sampling in Wallis and Smiths lakes (Barnes, unpublished data). For such 'lake-bound' populations restricted exchange with the sea may actually be an advantage in that it may reduce the loss of propagules from suitable habitats. In contrast, exchange of propagules between lakes would likely be extremely restricted and depend on a combination of corresponding opening periods and favourable currents and tides.

One of the most often proposed mechanisms for structuring assemblages in coastal lakes and lagoons is the physical stress placed on biota caused by large fluctuations in abiotic variables associated with the unstable nature of these isolated systems (Barnes 1988, 1994; Kjerfve 1994; Millet & Guelorget 1994; Woolridge 1999). For example, rainfall may reduce salinity, change other components of water quality such as dissolved oxygen, pH, turbidity and temperature in relatively short periods of time (Wilson *et al.* 2002), and lead to the opening of estuary mouths, and physical scouring of channels to the sea (Gillanders & Kingsford 2002; Anandraj *et al.* 2008). Opening of estuary mouths may then lead to influxes of seawater and further rapid changes. Such rapid changes in water quality and flow in estuaries often result in mortality of organisms such as fish (Wilson *et al.* 2002), macrofauna (Moverley *et al.* 1986) and algae (Anandraj *et al.* 2008). Similarly, rapid or large changes in water quality in estuaries have been long known to cause mortality of sponges (von Lendenfeld 1885; Pawlik *et al.* 2007). It is obvious that very large changes will result in the mortality or dislodgement of sponges and ascidians in coastal lakes and lagoons and will be important in structuring these assemblages. What is more ecologically interesting and more important for management, however, is the magnitude or speed of changes which may affect sponges and ascidians. The correlations of sponge and ascidian diversity with the opening regime of coastal lakes identified in Chapter 3, suggest at least some of the species may be very tolerant to large changes in water

quality. For example, if *Suberites* sp. form self sustaining populations in Smiths Lake, they must be able to tolerate ranges in salinity from close to seawater (36 ‰) to less than 20 ‰ (Chapter 3). Tolerances to changes in water quality including large reductions in salinity have been observed for other estuarine sponges (Fell *et al.* 1988) and are likely to be important for sustaining populations in New South Wales lakes and lagoons.

Overall, it is likely there is a combination of mechanisms including the availability of suitable habitat, limitations to recruitment and physical stress which is structuring assemblages. These mechanisms are, however, likely to be altered by human intervention in the form of artificial opening of entrances for example, which have implications for the conservation and management of these systems.

7.2.1 Comparisons with other taxa in coastal lakes and lagoons

Management decisions may be complicated by the fact that environmental impacts or natural characteristics of an estuary may not affect all species in the same way. For example, contaminants in estuaries are often not consistently related to effects on the biota (e.g. Stark 1998a & b; Thompson *et al.* 2007). When asking the question whether patterns of distribution of sponges and ascidians are similar to other biota in coastal lakes and lagoons, the answer depends on what spatial scale and variables are examined. Of the many large-scale physical characteristics of coastal lakes and lagoons that have been examined around the world, opening regime appears to be a common factor in determining the composition and abundance of fauna (Table 7.1). In the majority of studies I examined, when effects of opening regime were detected, there were generally larger diversities found in open lakes and lagoons compared to closed, or diversity increased after previously closed lakes were opened (Table 7.1). In contrast,

larger abundances were often found in closed lakes, although there were occasional exceptions and the pattern was not as consistent across taxa as was diversity (Table 7.1). At this scale of considering a whole-lake, diversity of sponges and ascidians behaved similarly to other taxa with more species in open lakes and lagoons. In contrast to many other taxa, however, abundances of sponges and ascidians were generally larger in the open compared to closed lakes and lagoons.

At smaller spatial scales within lakes, I found sponges may respond very differently from what would be expected for other taxa. This was particularly evident for the sponge *Suberites* sp. which was largely absent from areas of dense seagrass, most likely due to high rates of predation by fish. This result is in contrast to the prevailing paradigm that increased seagrass complexity usually reduces the rate of predation on both mobile and sessile fauna (Heck & Orth 2006).

7.2.2 Considerations for sampling

There are a number of issues in relation to sampling that should be considered for any future work on sponges and ascidians in lakes and lagoons. First, there is no doubt that the identification of the sponges to the level of species would have greatly aided in the interpretation of the results, particularly in gaining a better understanding of species biogeography. Given that the majority of the species found may be undescribed (J. Hooper pers. comm.) and given the time and expertise needed, more detailed identifications were beyond the scope of this project. It was also somewhat fortuitous that because of the relatively small number of species found, most identifications could be made in the field after initial identifications were made by the Queensland Museum. Nevertheless, a more comprehensive understanding of the importance of lakes and

lagoons as habitats for these sponges could be gained from future investment in expert taxonomic identifications incorporating molecular techniques (Worheide *et al.* 2005).

In addition to counting individuals, measuring morphology (e.g. volume, shape and surface area) or other characteristics (e.g. algal content, reproductive status) of individual sponges can provide useful information to quantify and compare populations of sponges (Wulff 2001; Bell *et al.* 2006a). In particular Wulff (2001) has stressed how the conclusions drawn among assemblages of sponges may differ greatly depending on the variable measured. In the experiments investigating patterns of distribution in this thesis (Chapters 2-5), individuals were counted, but other characteristics were not measured. This was a conscious decision made as a trade-off between the need to maximise the area of a lake searched to increase the chances of finding very patchily distributed species and the time needed to measure other characteristics of individuals. In the initial stages of this research programme in which I was examining previously unstudied and unknown taxa, I decided to maximise the time invested in determining the species present and their geographical distributions at the expense of collecting autecological data. Once patterns or populations have been identified, it may then be desirable to collect more detailed morphological information depending on the biological or ecological models being examined. For example, size of individuals, number of oscula and size of oscula were measured for *Suberites* sp. in the manipulative experiments described in Chapter 6. With improvements in technology, methods for accurately measuring sponge morphology are becoming less costly and more accessible (e.g. Abdo *et al.* 2006).

When making comparisons among lakes or among areas within lakes, consideration should be given to the possible effects of differences in habitat (e.g. seagrasses and algae). Ideally, sampling could be stratified across particular habitats.

Stratified sampling may be practical in lakes and lagoons where habitats are well mapped, but in less well known systems it would likely add time and cost to identify suitably similar habitats prior to sampling sponges and ascidians. Further, habitats may change over repeated sampling times and potentially confound comparisons. Alternatively, habitat could be measured as a co-variable to examine its contribution to differences among places or times. Ultimately, the strategy chosen should be appropriate for the hypotheses being tested.

Another key consideration for sampling is the need for adequate spatial replication and replication at appropriate spatial scales as discussed in Chapter 2. Many taxa had very patchy distributions (Chapters 2, 3 and 5) and would require relative large numbers of locations and replicates to obtain reliable estimates of their distribution within a lake. Again, the replication and sampling methods chosen should be appropriate for the hypotheses being tested. For example, transects (10×2 m) may be adequate for comparing abundances of some of the more abundant species among lakes or locations within lakes, but would be too small to compare diversity because many taxa were very uncommon and unlikely to be encountered in transects (Chapter 3). In studies comparing diversity of sponge or ascidian assemblages, it would be desirable to include much larger sampling units such as the timed searches used in Chapter 3.

Finally, as with all ecological studies, the generality of the findings will be improved by sampling on multiple occasions. The generality of the findings of Chapters 2, 3 and 4 were somewhat limited by including only a single time of sampling. Whereas the results of Chapter 5 examining the impacts of power stations could be interpreted with more confidence because sampling was repeated three times.

7.3 IMPLICATIONS FOR CONSERVATION AND MANAGEMENT

The coastal lakes and lagoons of temperate Australia are becoming increasingly impacted by development which will in turn necessitate increased management. Management, however, often proceeds without a good understanding of the ecological consequences of its actions (Thompson 2006). For example, Jones and West (2005) suggested the effects on fish of artificially opening lake entrances in New South Wales were largely unpredictable and should be done with great caution. Several goals have been set for the management of these systems including protection of property from flooding, maintenance of water quality and conservation of biodiversity (Creese & Breen 2003; Haines 2004). To add to the complexities of management, in recent times hard substrata in the form of artificial reefs are being deliberately added to several New South Wales lakes with the aim of improving recreational fishing (NSW DPI, 2008). This study identifies a number of important considerations and knowledge gaps, which are relevant in particular to conservation and management of biodiversity.

7.3.1 Ecological significance

One of the key knowledge gaps is the ecological significance of sponges and ascidians in these systems. The results of Chapter 5 suggest at least some sponges are eaten by some species of fish (family: Monacanthidae), but it is unclear whether they are important components of the diet of these fish or are consumed by other animals within the lakes. Filter feeders including sponges and ascidians are becoming increasingly recognised for their effects on water quality (Lemmens *et al.* 1996; Peterson *et al.* 2006). Peterson *et al.* (2006) recently suggested the filtering activities of sponges in Florida Bay in the state of Florida had a key role in the prevention of detrimental phytoplankton blooms. Sponges may also form important habitats for a variety of other invertebrates (Henkel & Pawlik 2005).

7.3.2 Geographical range.

Another of the key knowledge gaps is uncertainty about the broader geographical ranges of the species found. Determining whether species are restricted to these systems or are normally marine species at the limits of their range has important implications for their status and priority for protection. The majority of sponges were not able to be identified to species and therefore, it was very difficult to estimate their geographical ranges. Nevertheless, as discussed in Chapter 3, the available information suggests there is likely to be a combination of marine sponges at their limits of distribution in lakes and some species that may be restricted to these environments. Endemism or restricted geographical distributions appear not uncommon for sponges, particularly among habitats which are isolated by large geographic distances or long periods of time such as freshwater lakes (Meixner *et al.* 2007) or oceanic seamounts (Xavier & van Soest 2007). There is also some evidence to suggest endemism may occur over smaller scales such as among coastal lakes and lagoons. In a study of Vietnamese lakes (Azzini *et al.* 2007), twenty two of a total of forty seven species found inside lakes were not found in surrounding coastal sites. Similar to this thesis, however, it is difficult to determine whether such restricted distributions are real or are rather an artefact of inadequate sampling of marine habitats for species which maybe naturally sparsely distributed. In Mediterranean lagoons, which have been more thoroughly studied and where the sponge fauna are far better described it appears the majority of species are probably marine. For example, in a study of 11 Mediterranean lagoons (Mercurio *et al.* 2004), although 58 of 81 species of sponge were considered to occur occasionally and only 4 species were widespread, the majority of species were considered marine.

In contrast and as previously discussed in Chapter 3, the ascidians found in this study were relatively easily identified to the level of species and at least some information was available on their geographic distributions (Kott 1985, 1990, 1992; Newton *et al.* 2007). Many were cosmopolitan and the remaining could be considered common or widespread in coastal or other estuarine habitats and possibly at the limits of distribution or only occasionally present within coastal lakes and lagoons.

In addition to the detection of impacts, another important tool for the conservation and management of estuaries is the establishment of marine parks and marine protected areas. The goals of establishing these areas are usually to protect representative habitats, fauna, flora (particularly rare or endangered species or habitats) and/or ecological processes (Creese & Breen 2003). The choice of which areas to protect requires careful consideration of many factors including the geographical range of species. Given the large differences in species compositions among lakes and possible restricted distributions of sponges, it is likely many lakes would need to be protected for comprehensive conservation of a wide range of species.

7.3.3 Utility as bio-indicators.

It seems increasingly popular in the management of the natural environment to search for organisms which can be used as bio-indicators (e.g. Bongers & Ferris 1999; Davis *et al.* 2001). In any discussion of management of lakes and lagoons, it therefore seems appropriate to comment on the value of sponges and ascidians as bio-indicators. In managerial terminology, a good bio-indicator will supposedly indicate whether a system is ecologically healthy or unhealthy. In more scientific terms, a change or difference in the abundance or other attribute of a particular taxon may indicate whether a system is in a condition which is definable as desirable or undesirable. Numerous

groups of organisms have been proposed as indicators of environmental change or impact in marine environments (Jones & Kaly 1996), including sponges and ascidians (Muricy 1989; Carballo *et al.* 1996; Naranjo *et al.* 1996; Roberts *et al.* 1998; Alcolado 2007). Sessile invertebrates have been proposed at least in part because of their constant exposure and inability to remove themselves physically from the source of an impact. Relatively fewer taxa have been proposed for estuaries, possibly because of large variability in the physical qualities and biological communities within these systems. Whitfield and Elliot (2002) advocated using fish as indicators of environmental change and impact in estuaries, but also highlighted the importance of understanding the large variability in fish assemblages when interpreting differences. Similarly, the presence, absence or diversity of sponges and ascidians in a lagoon may indeed be a very good indication of whether that particular system is relatively pristine or impacted, but large natural variability among lakes may make it very difficult or impractical to identify species which are good indicators across a range of lakes and lagoons. For example, no sponges were found in Durras Lake, but it is considered one of the most pristine in New South Wales (Healthy Rivers Commission of NSW 2002). Durras Lake is also relatively small, mostly closed to the sea and experiences relatively large changes in water quality. In this case, the absence of sponges is probably a normal condition indicative of a 'healthy' system.

Large variability in species composition among lakes, together with the difficult taxonomy of sponges and large sampling effort required to get reliable estimates of distributions of sponges and ascidians probably makes them impractical to use widely as bio-indicators over the full range of lakes and lagoons in New South Wales. Rather, studying sponges and ascidians may be more valuable for management when used to test hypotheses about specific impacts (e.g. Chapter 5; Impacts of power stations),

ecologically processes (e.g. Chapter 6; Predation by fish in seagrass beds) or examine the effectiveness of management strategies (e.g. artificial opening of lake entrances) in particular lakes or subsets of lakes.

7.3.4 Effects of opening regime

Opening regime is important to consider because it is one of the physical characteristics of coastal lagoons often deliberately altered to achieve management outcomes in New South Wales (Haines *et al.* 2006). In most cases, closed lagoons are artificially opened with aims of alleviating flooding to property or supposedly increasing recruitment of recreationally or commercially important species of fish or crustaceans (Griffiths & West 1999). It appears, however, that to date little consideration has been given to the broader effects on the biodiversity and ecology of systems which are naturally predominantly closed and isolated from the sea (Jones & West 2005; Dye 2006). The existing evidence suggests artificially opening lakes will in turn artificially increase the diversity and change abundances of most taxa including sponges and ascidians. While it may be tempting to interpret an increase in diversity as somehow being a positive impact, it still represents a change from the ‘natural’ condition (Bulleri *et al.* 2007). Careful consideration must then be given to the potential effects on the broader ecology of the whole system. For example, Jones and West (2005) suggested artificially opening entrances may have long-term detrimental impacts of losses in fish diversity and reductions in abundances of some species. At present, it seems inevitable that lakes and lagoons will continue to be opened artificially, regardless of the broader ecological consequences. This practice does however provide opportunities for experimental examination of its potential impacts, which can be used to refine the practice if necessary and allow more scientifically informed decisions to be made in the future.

7.4 CONCLUSION

The fact that sponges and ascidians have previously gone unstudied and mostly unnoticed in New Wales Coastal lakes and lagoons seems somewhat surprising given the results of this study. Although many species had very patchy distributions, not all species were small, cryptic or hidden away in inaccessible areas of a lake. Rather, a number of species were relatively large, abundant in patches, brightly coloured, found in relatively shallow and clear water, and therefore conspicuous and easily seen from a boat or by swimmers. Further, many were in areas regularly frequented by anglers, water-skiers, swimmers and other users of the lake. For example, several individual *Mycale* sp. in Lake Conjola each covered several m² of lake floor, were bright red or orange in colour and in shallow and clear areas of the lake regularly used by water skiers, swimmers and anglers (P. Barnes, pers. obs.). Despite such observations, sponges are invariably omitted from estuary process studies and inventories of biodiversity in coastal lakes in New South Wales (but see, Roberts 2001). The lack of scientific studies is likely due to a lack of sponge researchers (particularly in this region of the world) and an understandable focus on other organisms such as fish which are perceived as more economically important (Heck *et al.* 2003). Nevertheless, sponges and ascidians comprise an often conspicuous component of the biodiversity of lakes and lagoons and should be included in biodiversity assessments and considered in management decisions. Management of these varied and complex systems will in itself be complex. As this study has highlighted, it cannot be assumed all organisms will respond or behave in the same way to impacts or changes in environment. Conservation will only be successful through management when based on a sound scientific understanding of the ecology of these systems. The fundamental and logical steps in

gaining this understanding are to identify natural patterns of distribution, impacts and processes including the effects of management.

Table 7.1. Selected examples of studies investigating the effects of the physical characteristics of coastal lakes and lagoons on biota. Generalised conclusions on effects of opening regime on diversity and abundance are included. Numbers in brackets represent the numbers of lakes and lagoons sampled in the respective studies.

Location	Factors investigated	Taxa studied	Effects of opening regime on:		Other findings	Source
			Diversity	Abundance		
South-eastern Australia (8)	Opening regime and distance from sea	Macrofauna	Not reported	Closed > Open (In inner reaches) Open > Closed (near entrances)	Abundances larger near mouths.	Dye 2006
South-eastern Australia (8)	Opening regime and distance from sea	Meiofauna	Open \neq Closed	Closed \neq Open	Diversity and abundance decreased with distance from sea – lowest where salinity was most variable.	Dye & Barros 2005a
South-eastern Australia (8)	Opening regime and distance from sea	Macrofauna	Open > Closed	Closed > Open (In inner reaches)	Diversity and abundance larger in inner reaches.	Dye & Barros 2005b
South-eastern Australia (4)	Opening regime	Macrofauna near entrance	No detectable effects	No detectable effects	Opening of an estuary did not affect macrofauna near entrance	Gladstone <i>et al.</i> 2006
South-eastern Australia (3)	Opening regime	Fish	Open > Closed	Open > Closed (for some species)	Abundances of some species increased after lakes were opened	Griffiths 1999
South-eastern Australia (6)	Opening regime, temporal and spatial variability	Fish	Open > Closed	Closed > Open	Changes in assemblage structure including increased diversity after lakes opened to the sea	Jones & West 2005
South-eastern Australia (3)	Opening regime	Fish	Open > Closed	Closed > Open	Diversity larger in permanently open, but closed supported larger catches	Pollard 1994
South-eastern Australia (1)	Open and closed phases, and water quality	Plankton	Not reported	Not reported	Increased productivity when the lake was open	Everett <i>et al.</i> 2007
Southern Africa (13)	Opening regime and size	Macrofauna	Open > Closed	Closed > Open	Diversity largest in permanently open lakes	Teske & Wooldridge 2001
Southern Africa (13)	Opening regime, salinity, sediment size	Macrofauna		Closed > Open	More marine species nearer to entrance. Sediment characteristics more important than salinity.	Teske & Wooldridge 2003
Southern Africa (1)	Open and closed phases	Meiofauna	Not reported	Closed > Open	Abundances largest after prolonged closure of estuary mouth	Nozais <i>et al.</i> 2005
France (1)	Hydrodynamic features	Macrofauna			Seasonal variations of macrofauna correlated with tidal extent and sediment size	Millet & Guelorget 1994
Greece (1)	Distance from sea, spatial and seasonal variability	Meiofauna			Assemblages varied with distance from sea	McArthur <i>et al.</i> 2000

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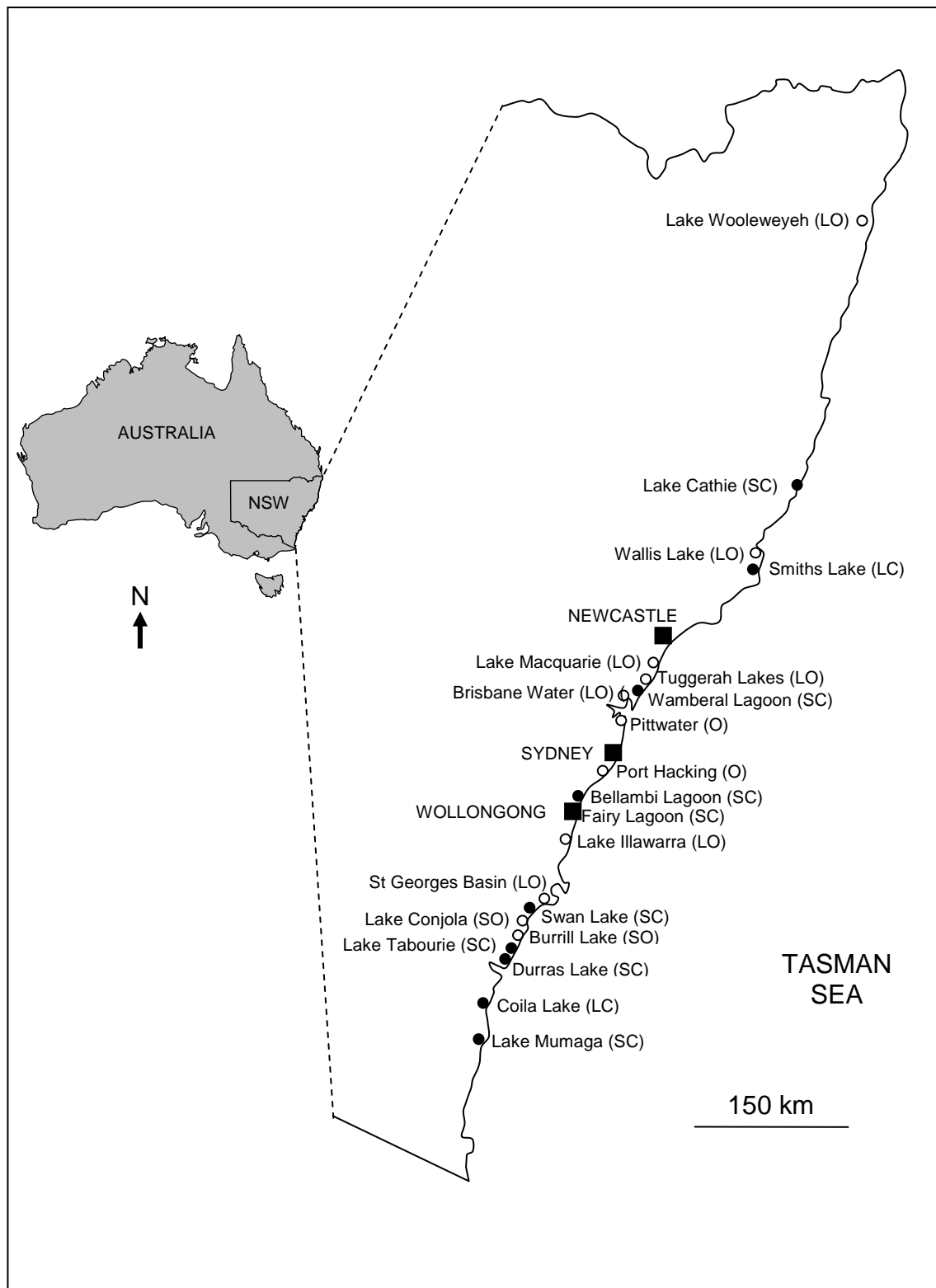
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APPENDICES

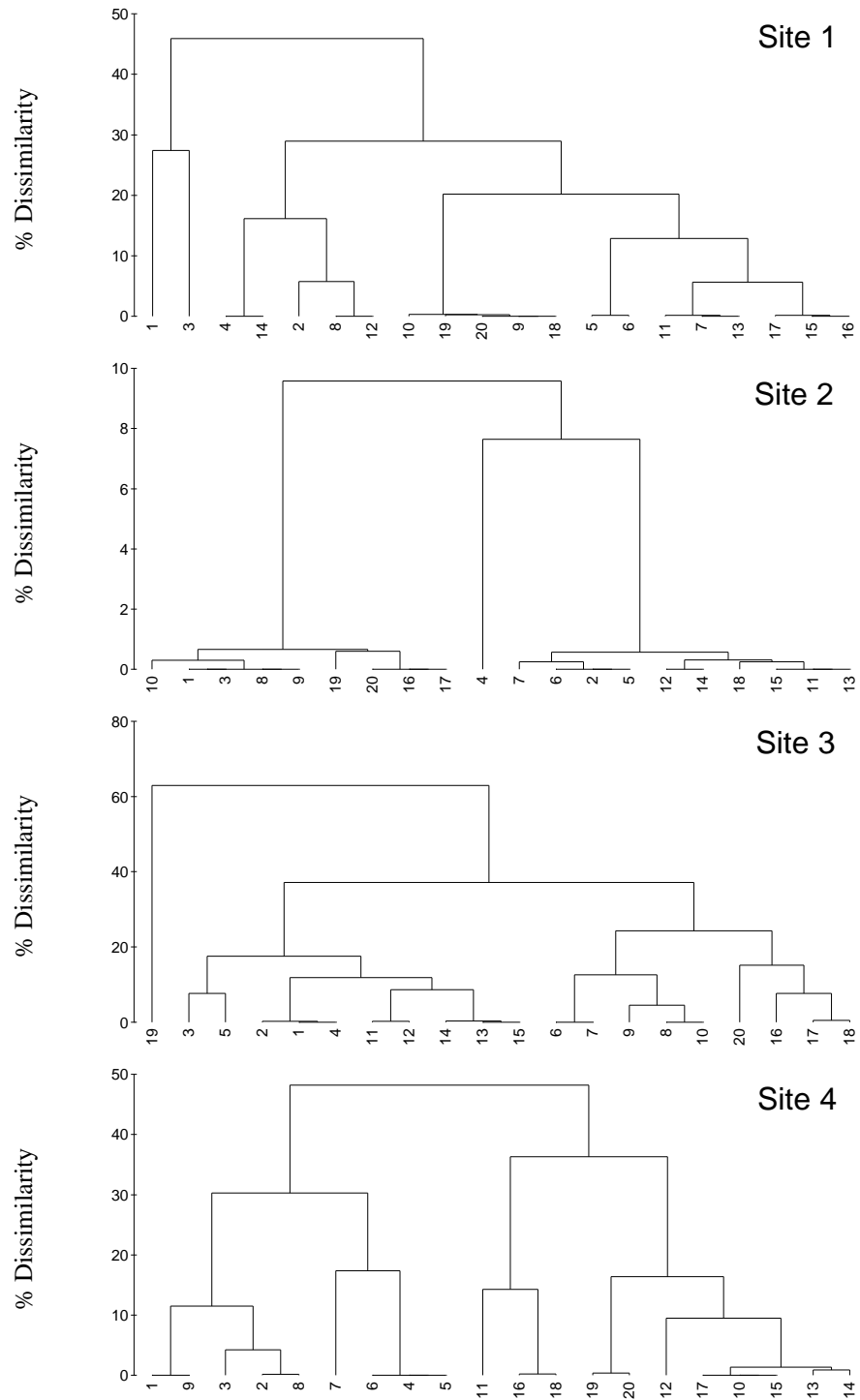
Appendix 1. Salinity ranges for lakes sourced from the literature. Data for Wallis Lake refer to measurements taken in the main body of the Lake south of the entrance.

Condition	Opening regime	Size	Lake	Salinity range(%)	Time period (no. times of sampling)	Source
Less modified	Mostly closed	Small	Swan	6.4-19.1	10/1984-5/1988 (30)	Pollard (1994a)
			Durras	9.9-34	2/2001 (continuous)	NSW Department of Natural Resources (unpublished data)
		Large	Coila	6.2-35.6	10/1997-7/2000 (12)	West & Jones (2000)
			Smiths	19-29	4/1997-4/1998 (5)	Webb, McKeown & Associates (1998)
	Mostly open	Small	Conjola	13.4-35.9	8/1984-9/1986 (22)	Pollard (1994a)
			Conjola	15.9-36.3	10/1997-7/2000 (11)	West & Jones (2000)
			Burrill	25-37.5	10/1997-7/2000 (12)	West & Jones (2000)
		Large	St Georges Basin	22-35.1	10/1997-7/2000 (12)	West & Jones (2000)
			Wallis	29.6-36	7/1997-5/1998 (5)	Webb, McKeown & Associates (1999)
			Tuggerah	17.2-34.6	5/2000-3/2002 (13)	Roberts & Barnes (2004)
Extensively modified	Mostly open	Large	Illawarra	24-38.7	10/1997-7/2000 (11)	West & Jones (2000)

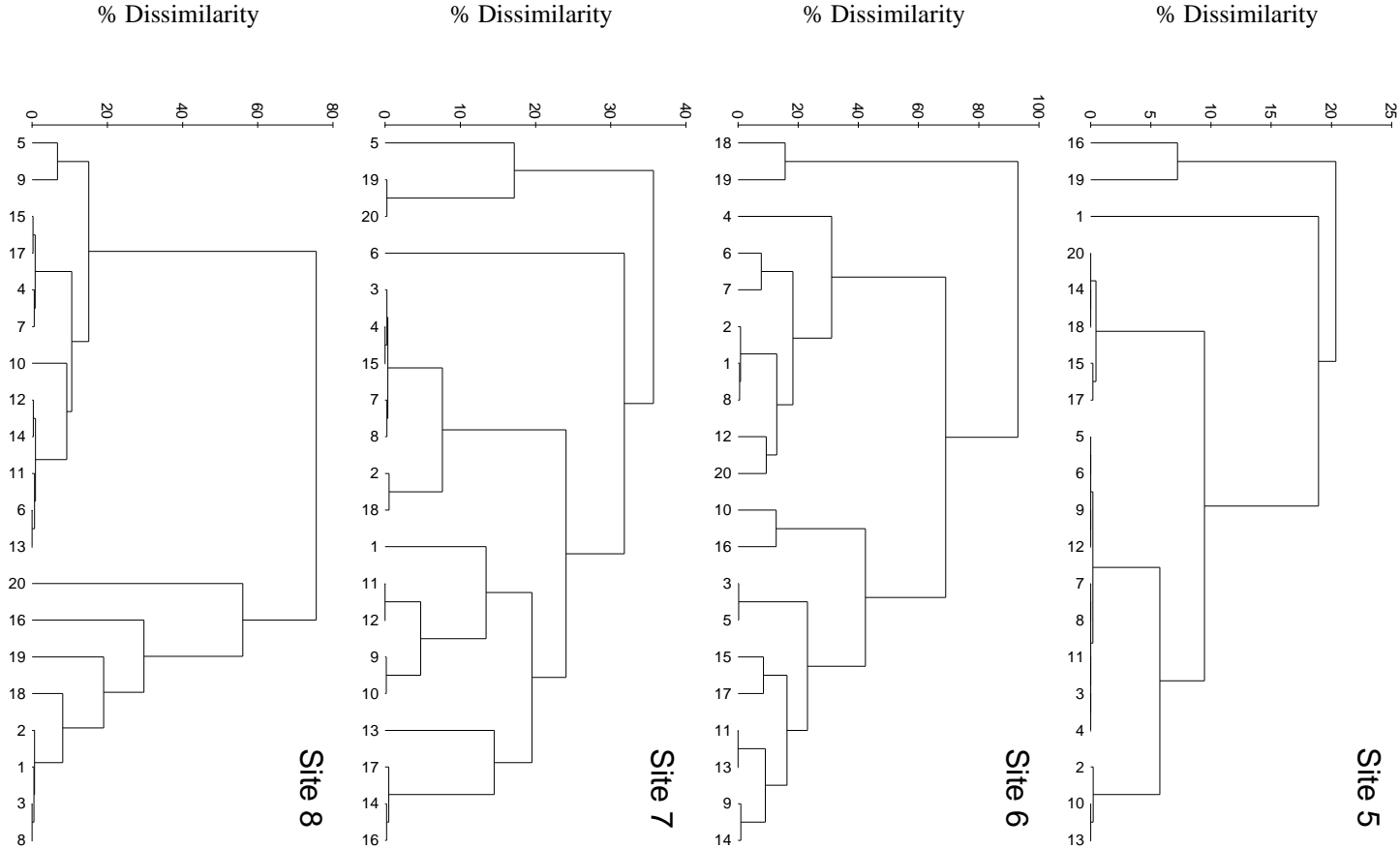
Appendix 2. Map of the New South Wales coast showing all estuaries sampled as part of this thesis. LO (Large Open), LC (Large Closed), SO (Small Open) and SC (Small Closed) refer to types of lakes and lagoons as defined in Chapter 3. Pittwater and Port Hacking are drowned river valley estuaries examined in chapter 5.



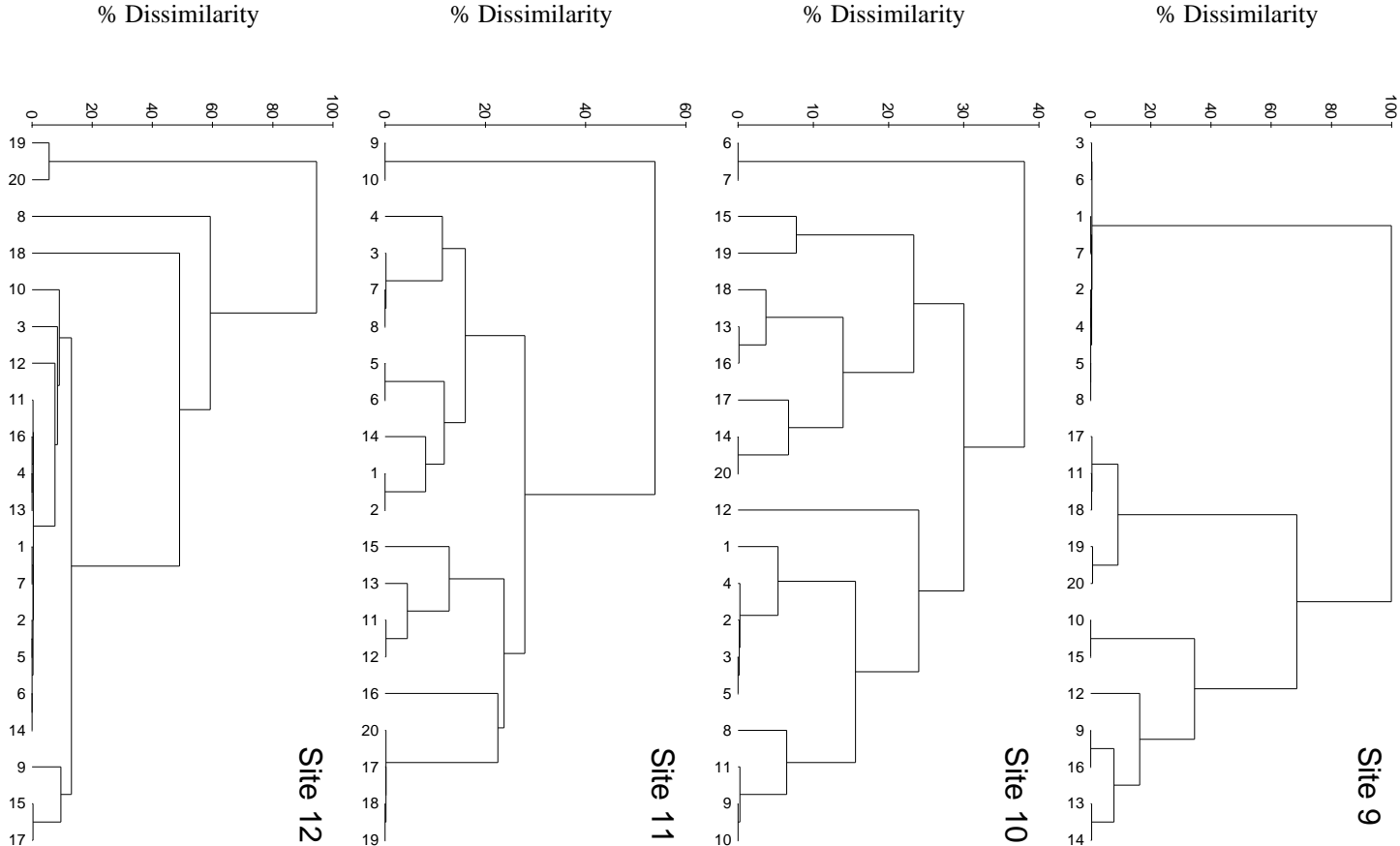
Appendix 3.1. Dendrograms from Cluster analyses illustrating groups of transects with relatively similar assemblages of aquatic vegetation in each Site in St Georges Basin (n = 20).



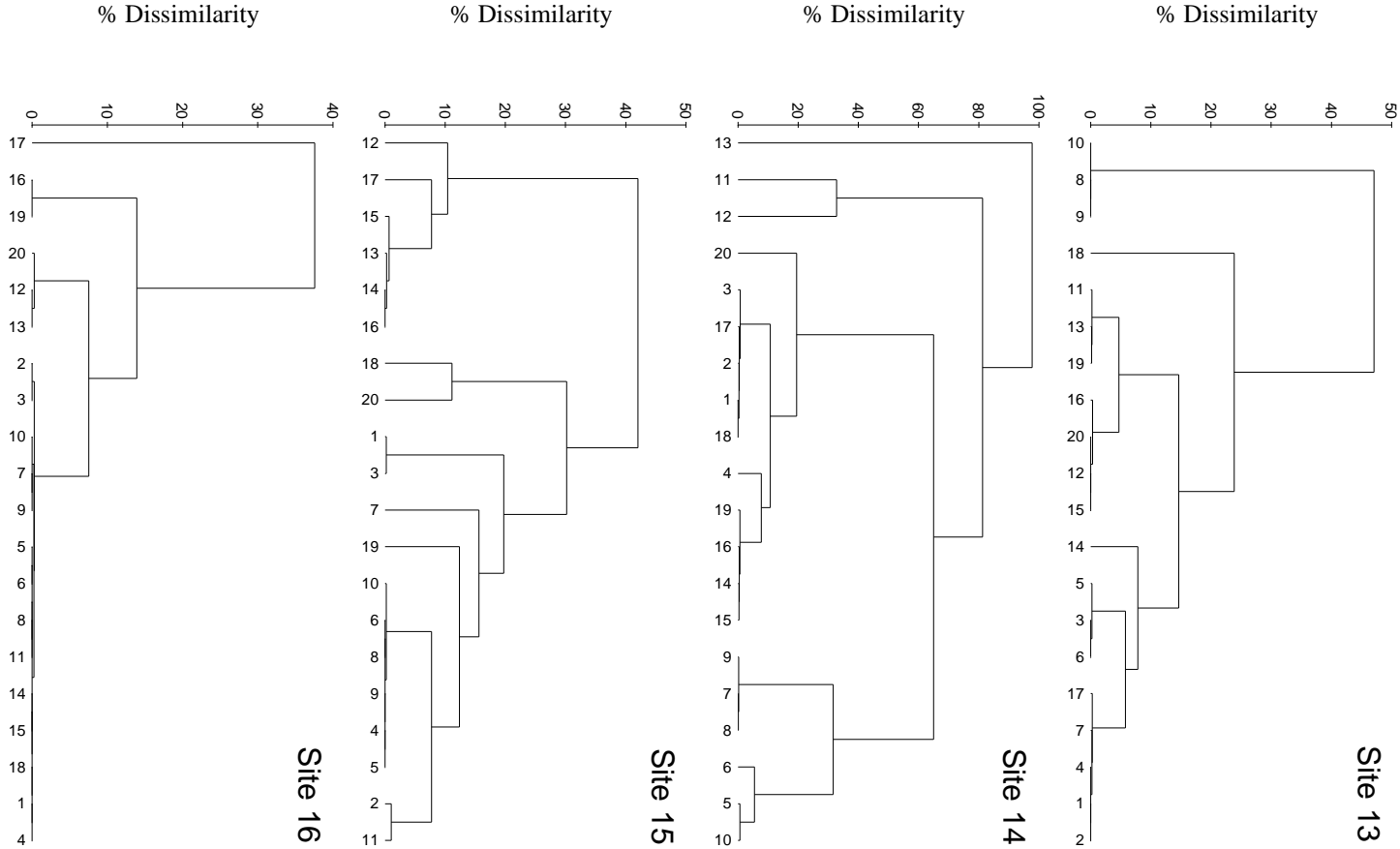
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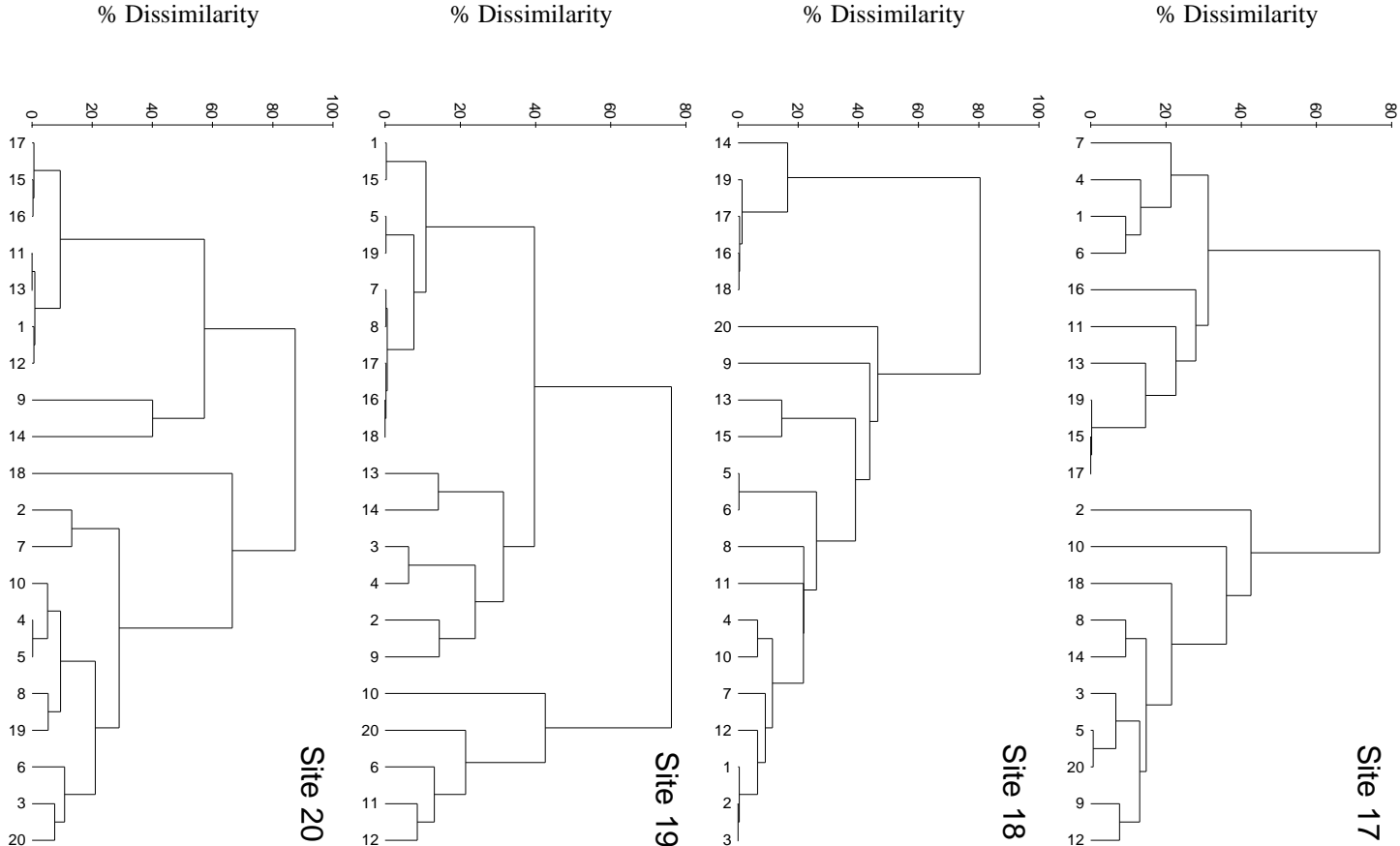
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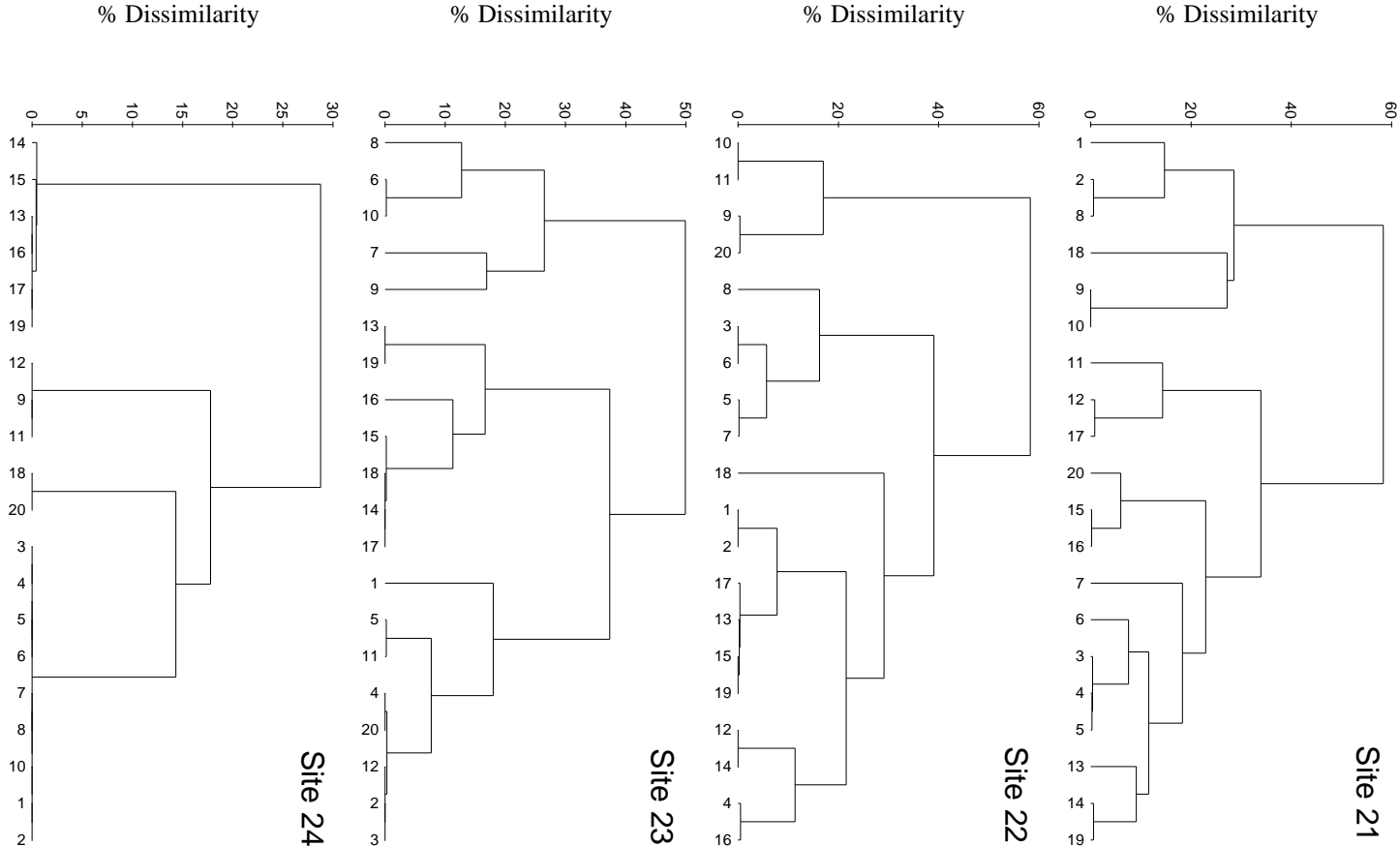
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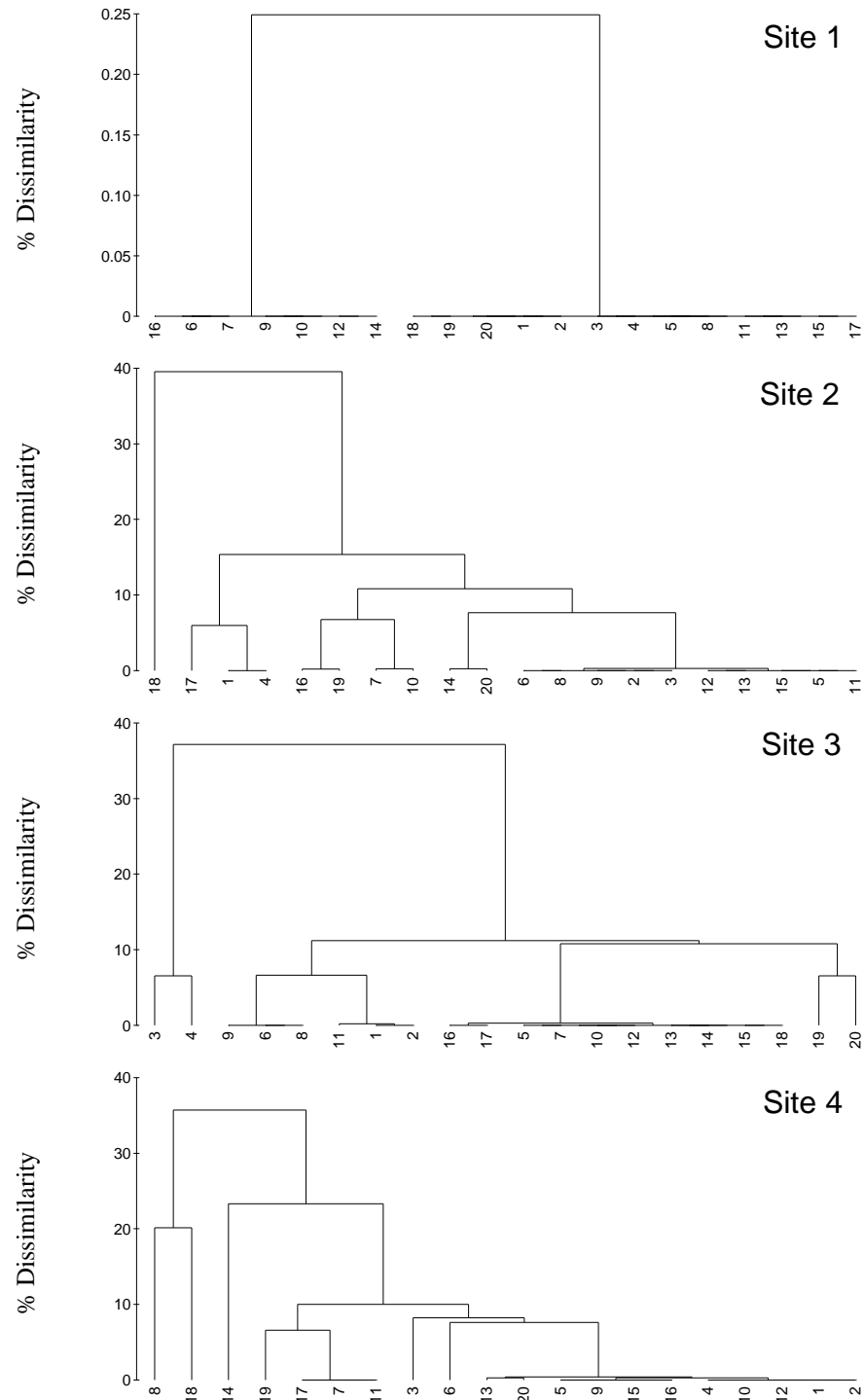
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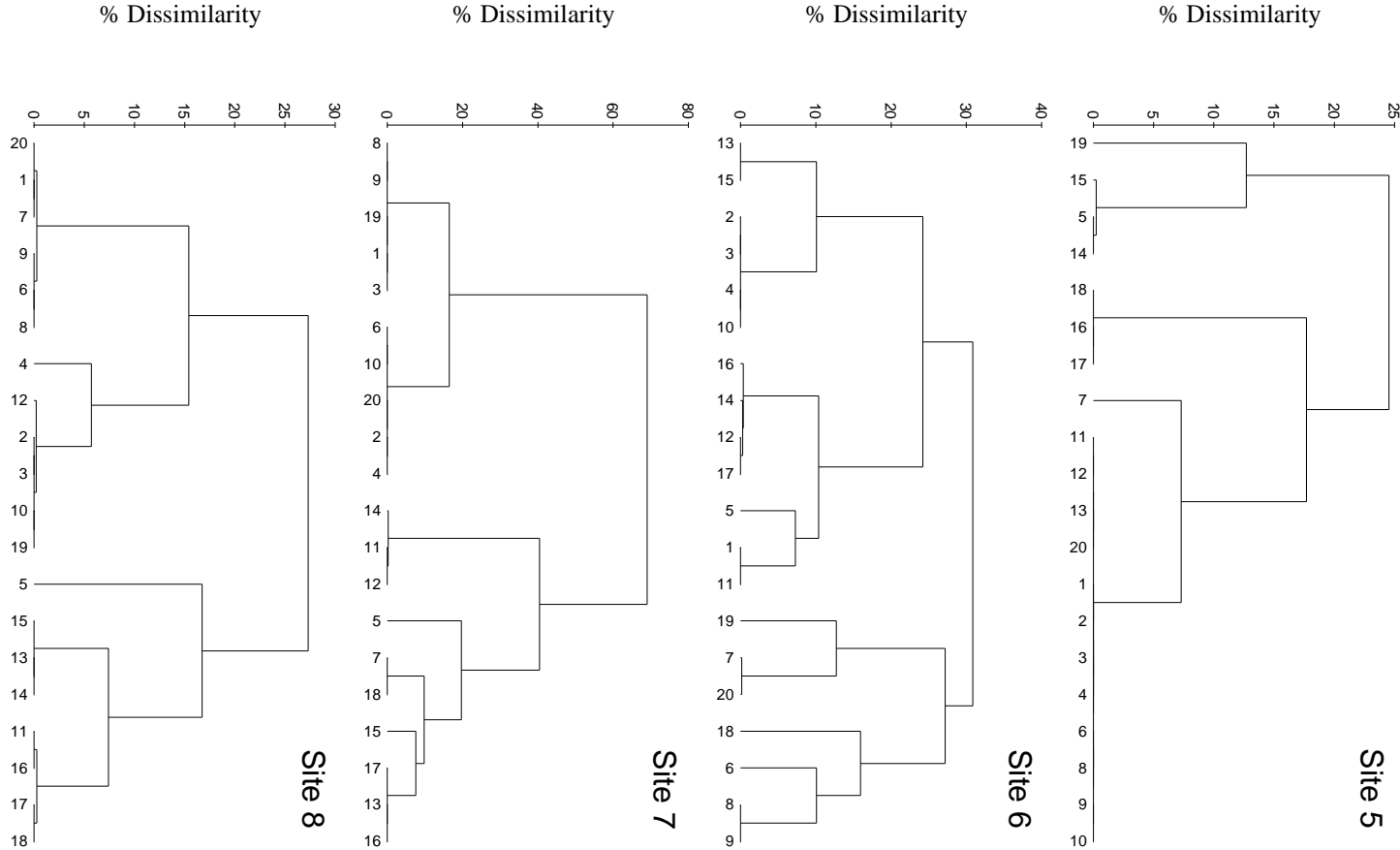
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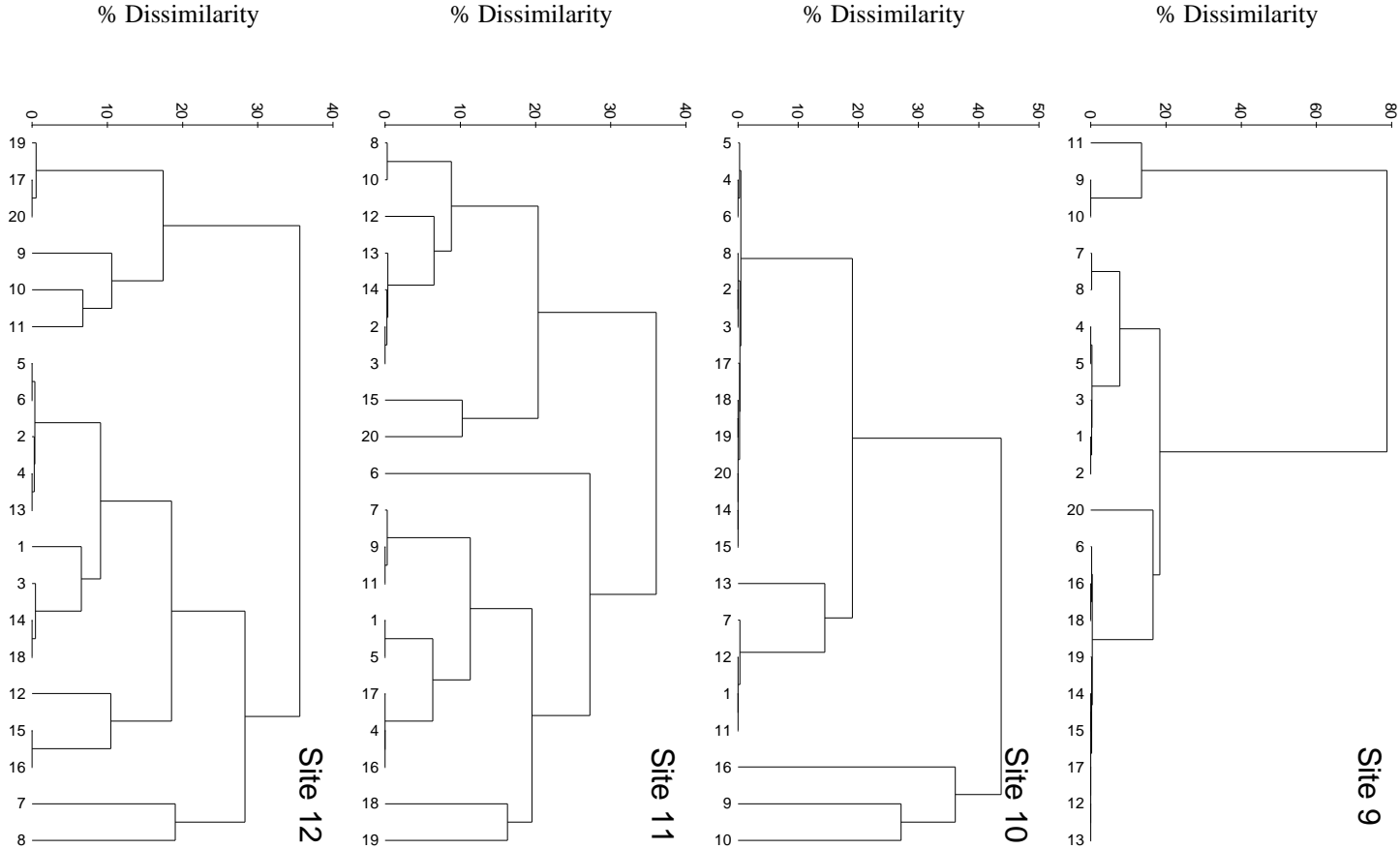
Appendix 3.2. Dendrograms from Cluster analyses illustrating groups of transects with relatively similar assemblages of aquatic vegetation in each Site in Wallis Lake (n = 20).



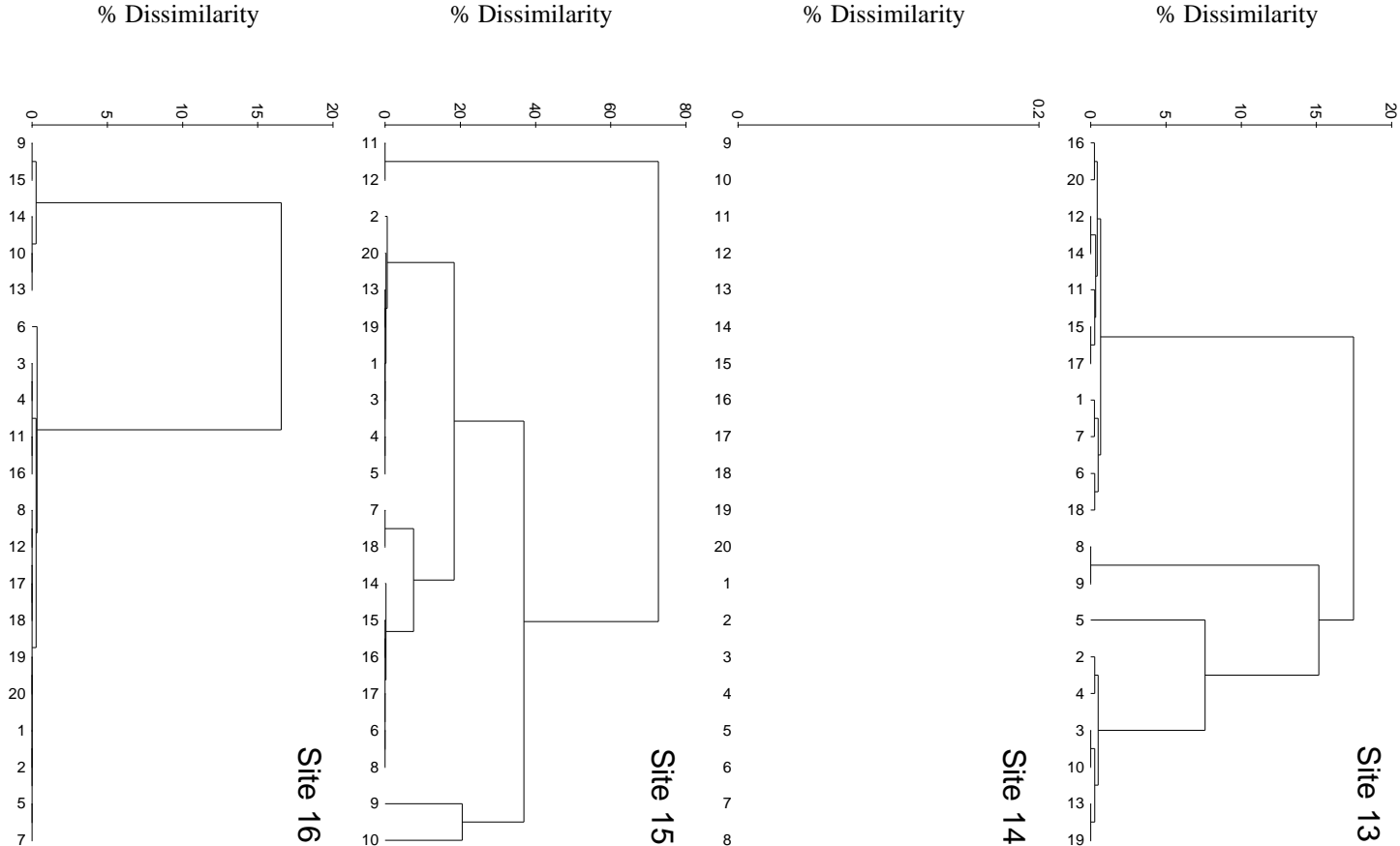
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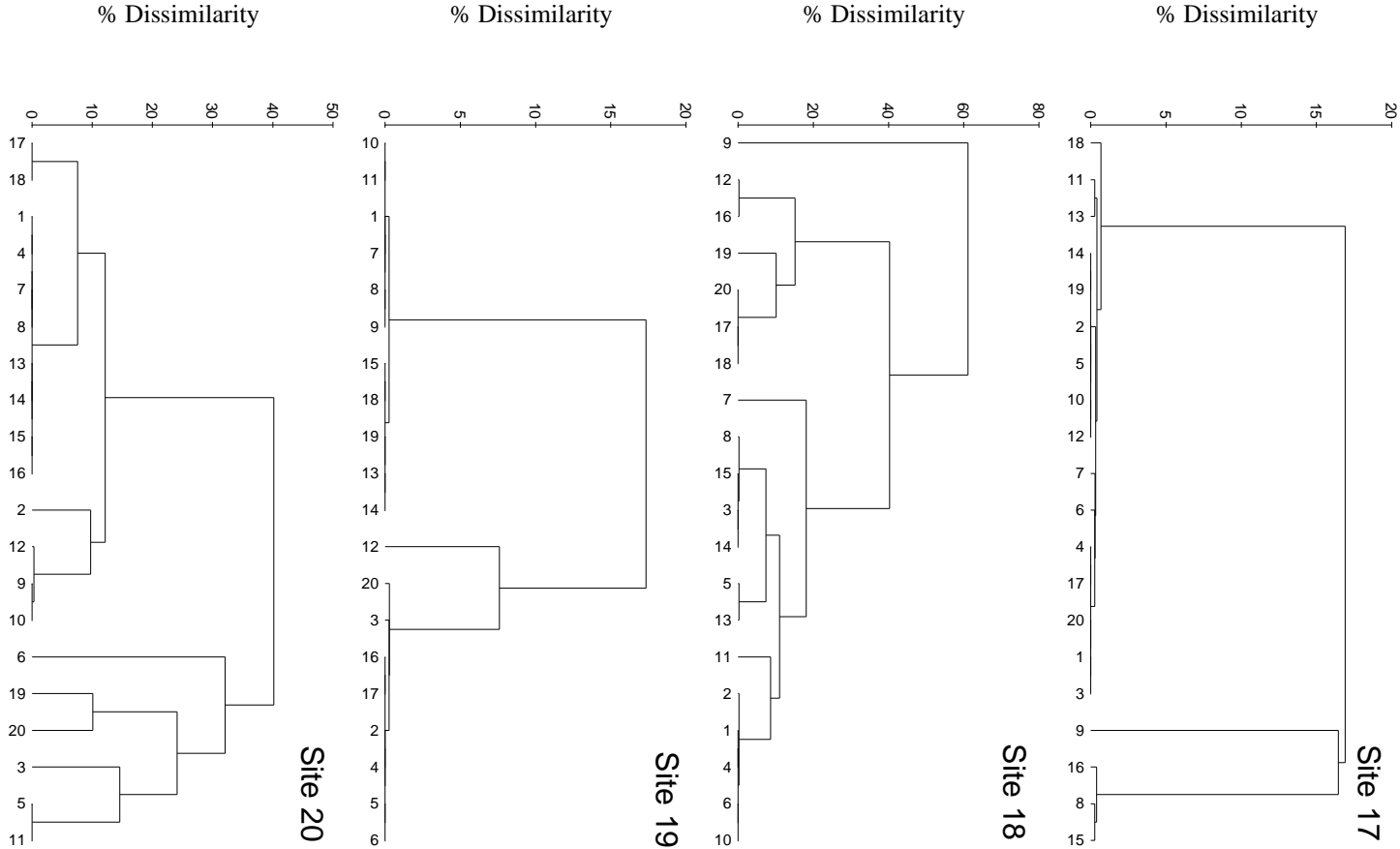
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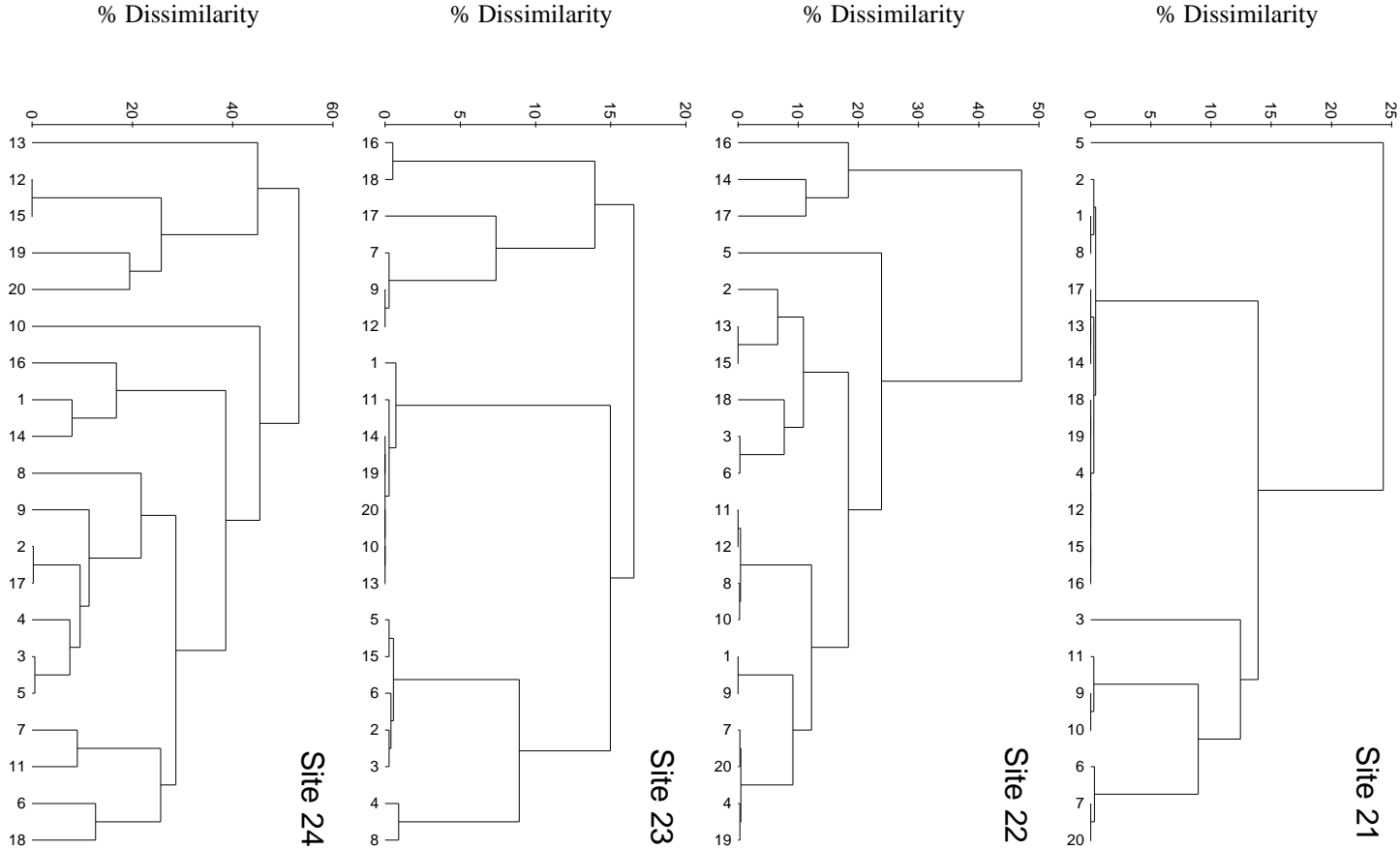
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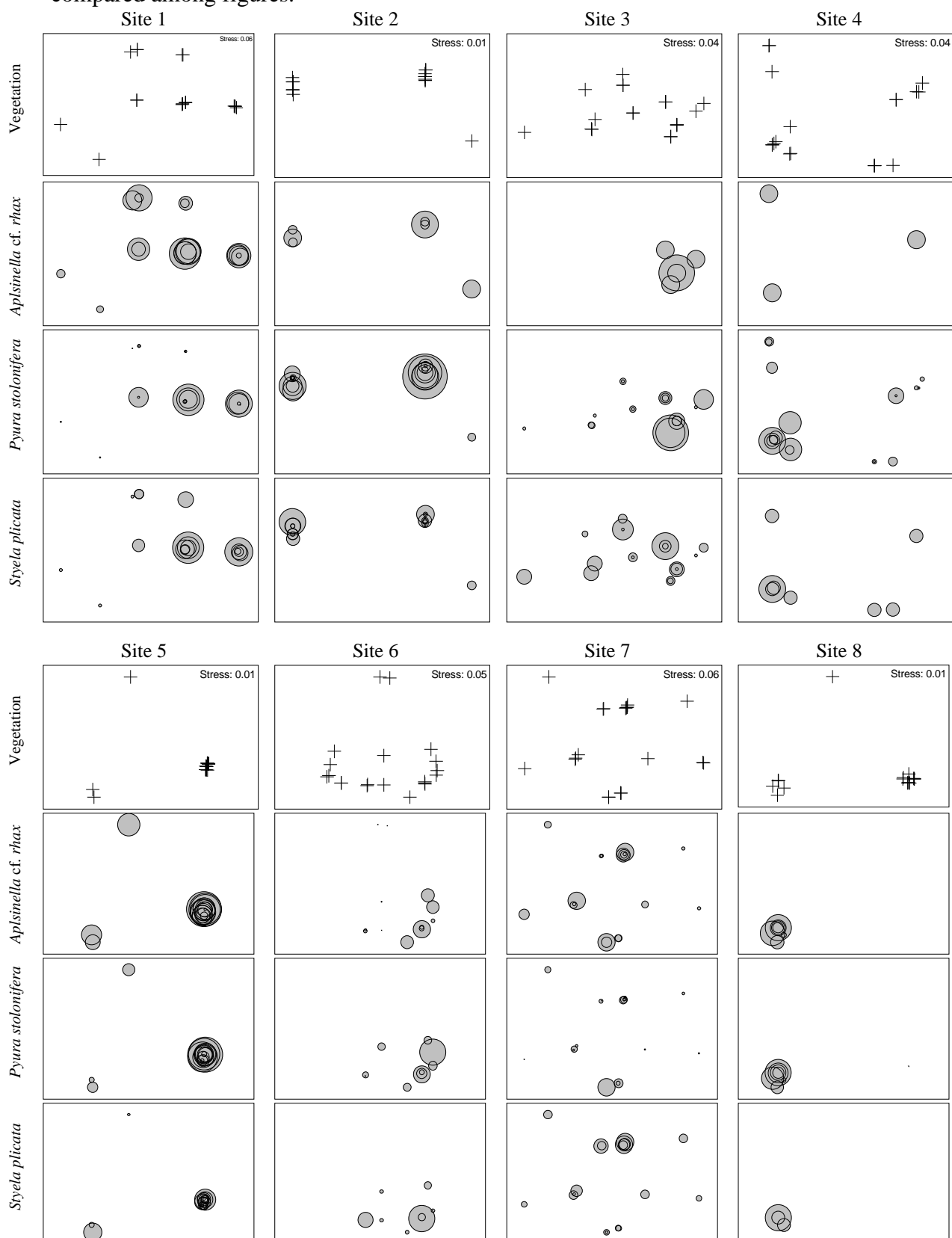
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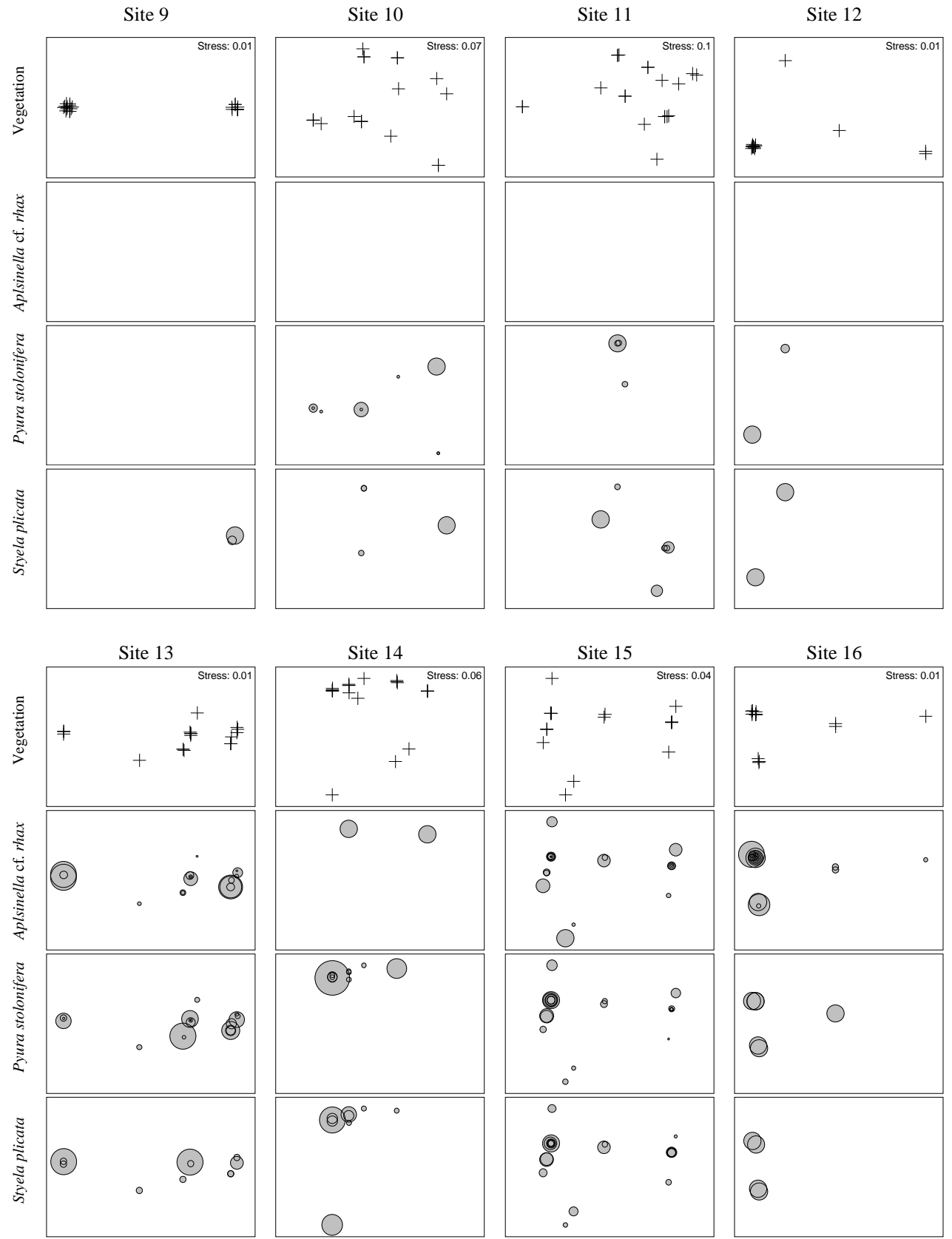
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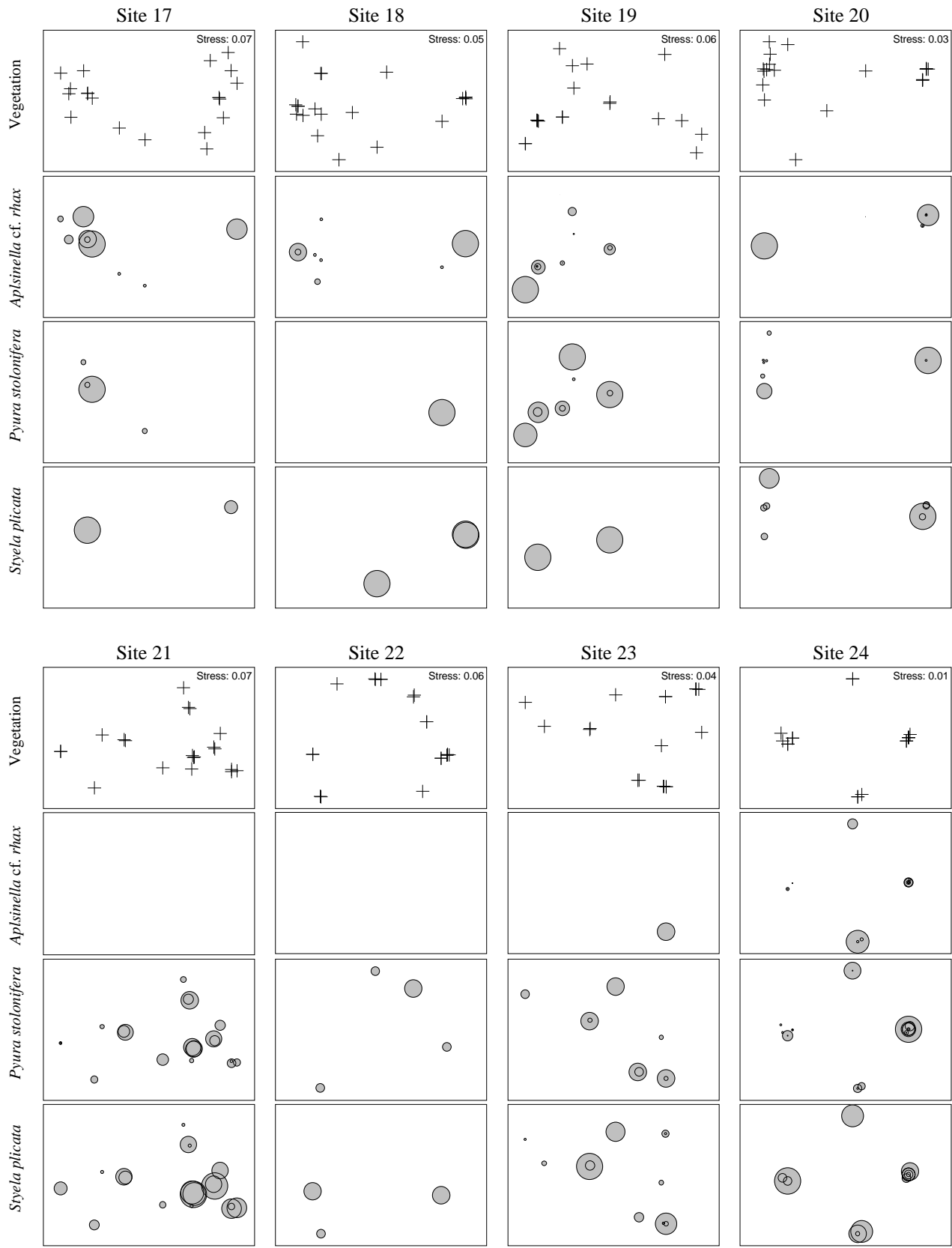
Appendix 3.3. nMDS ordinations illustrating associations of sponges and ascidians with assemblages of aquatic vegetation at the scale of Transect in each Site in St Georges Basin. The diameter of each circle is proportional to the abundance of each species at that Transect. Abundances are relative to each figure and cannot be compared among figures.



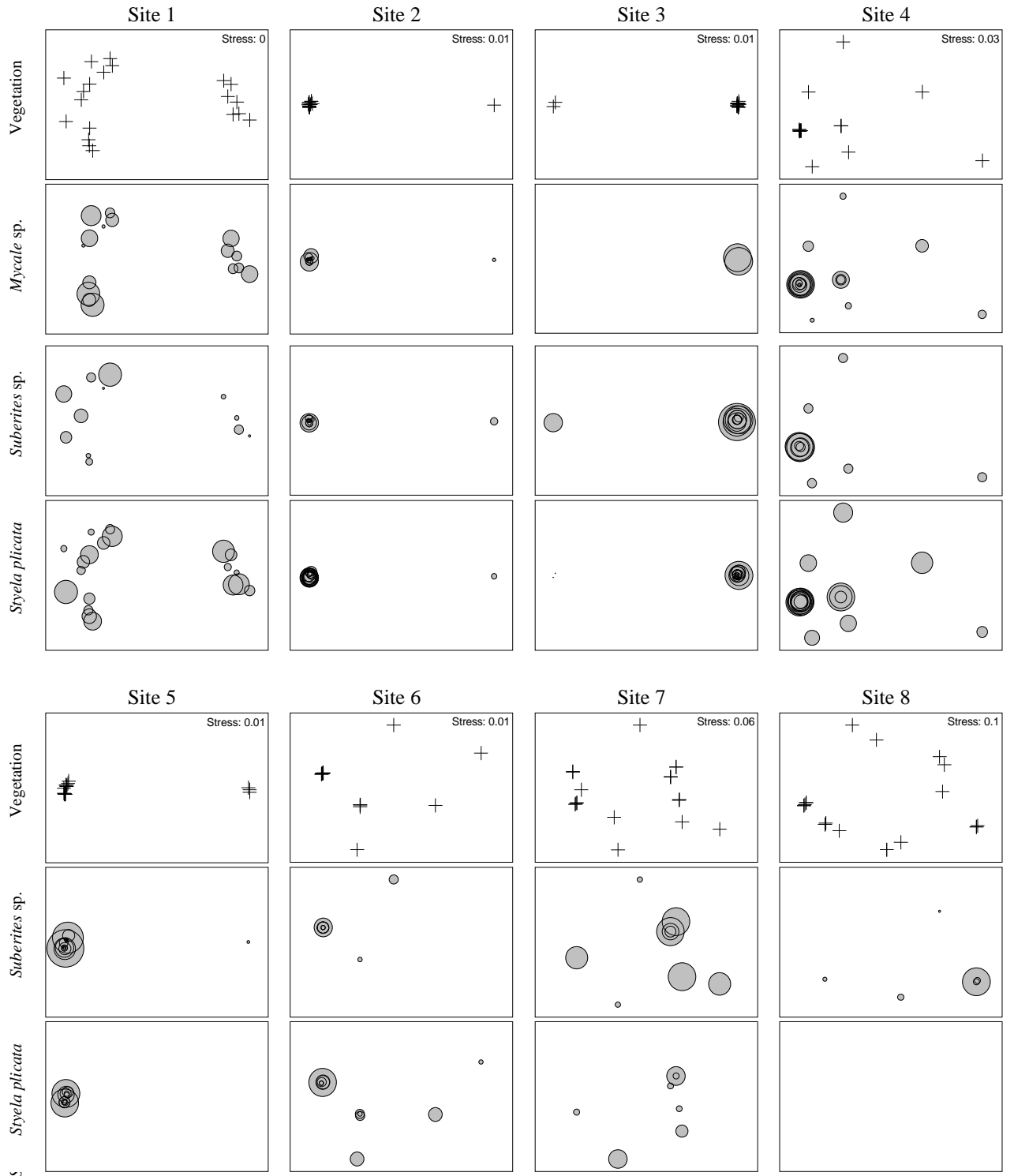
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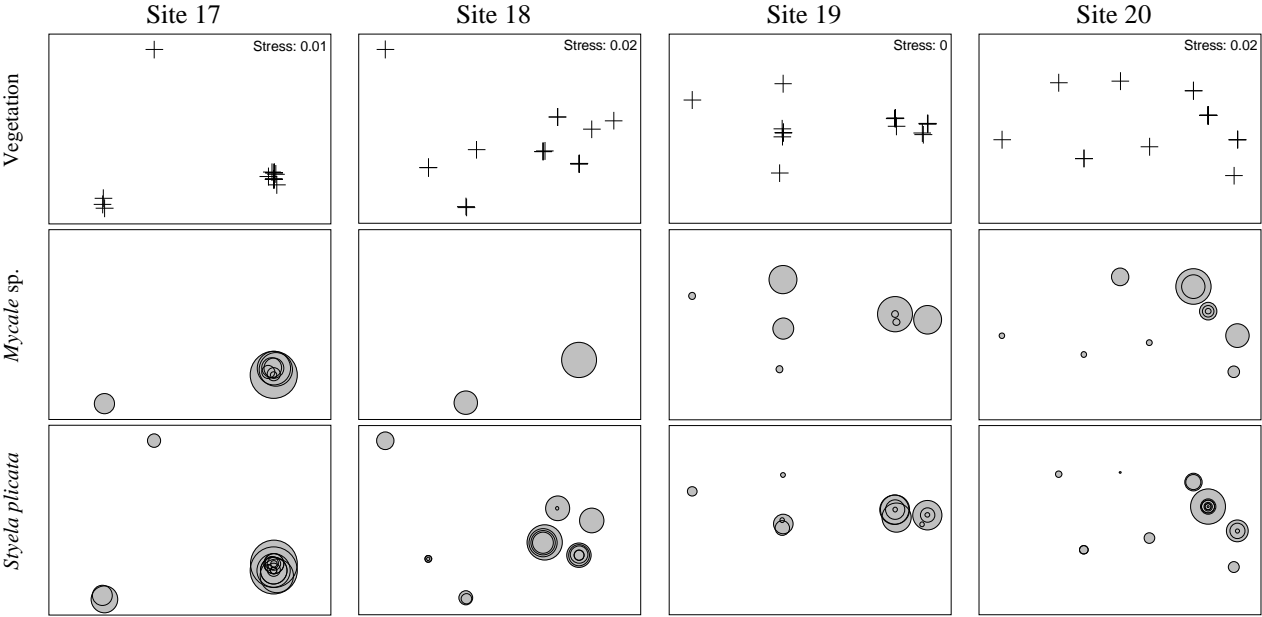
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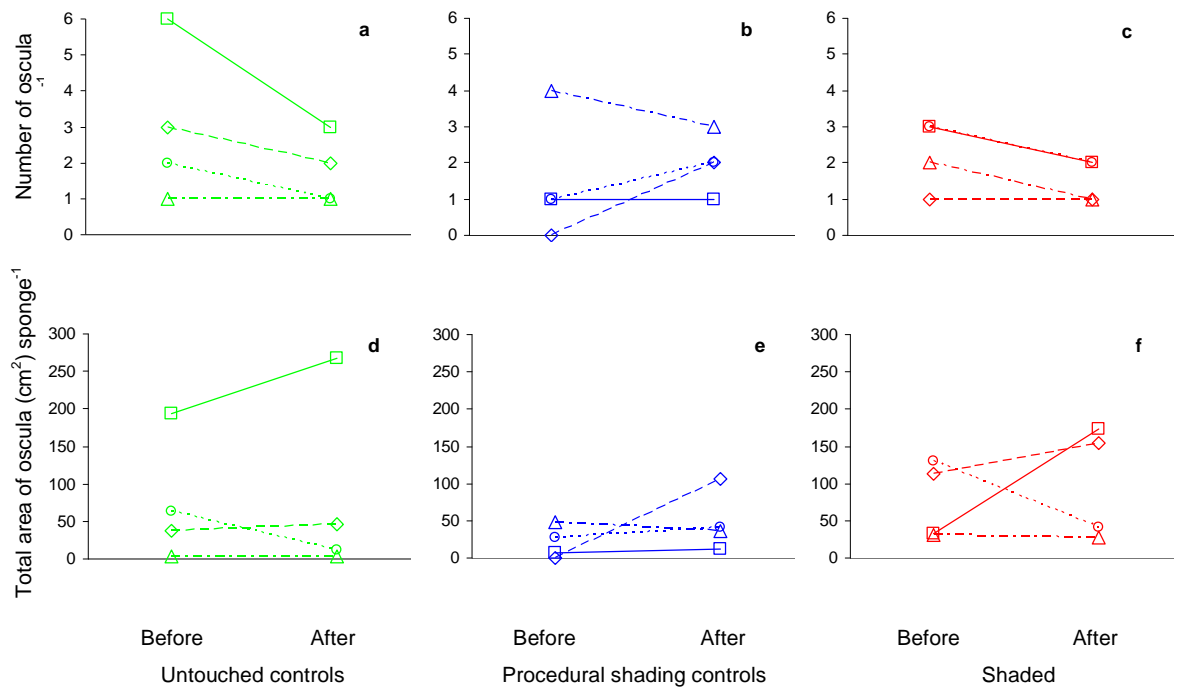
Appendix 3.4. nMDS ordinations illustrating associations of sponges and ascidians with assemblages of aquatic vegetation at the scale of Transect in each Site in Wallis Lake. The diameter of each circle is proportional to the abundance of each species at that Transect. Abundances are relative to each figure and cannot be compared among figures.



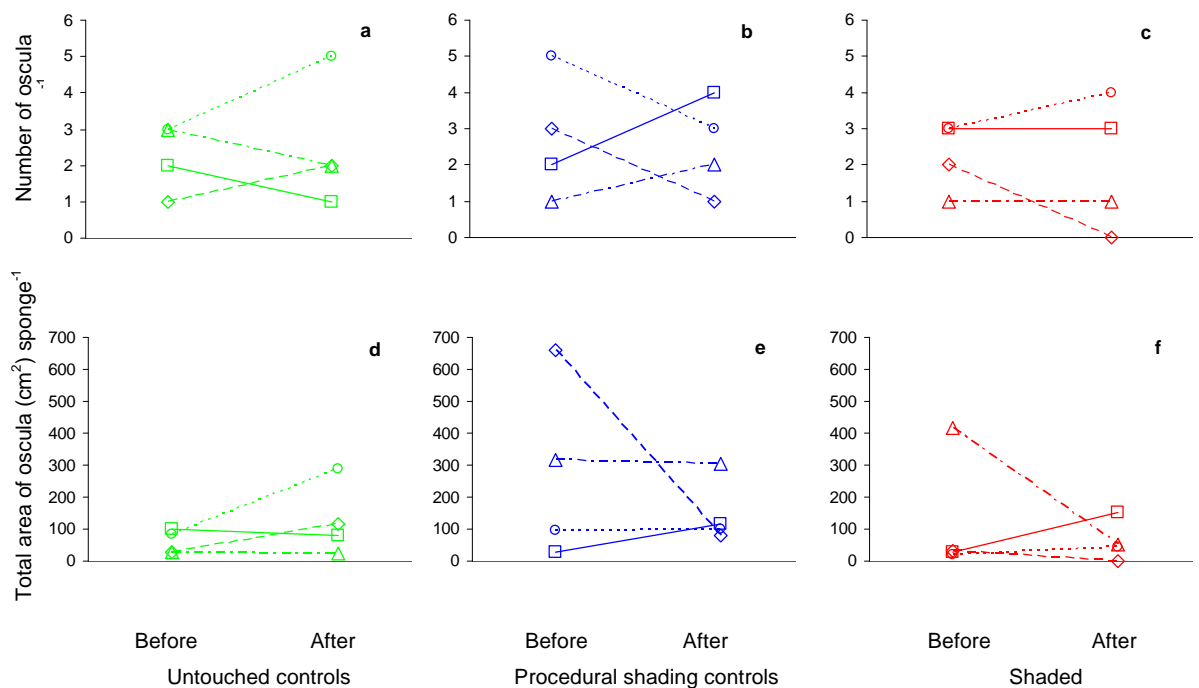
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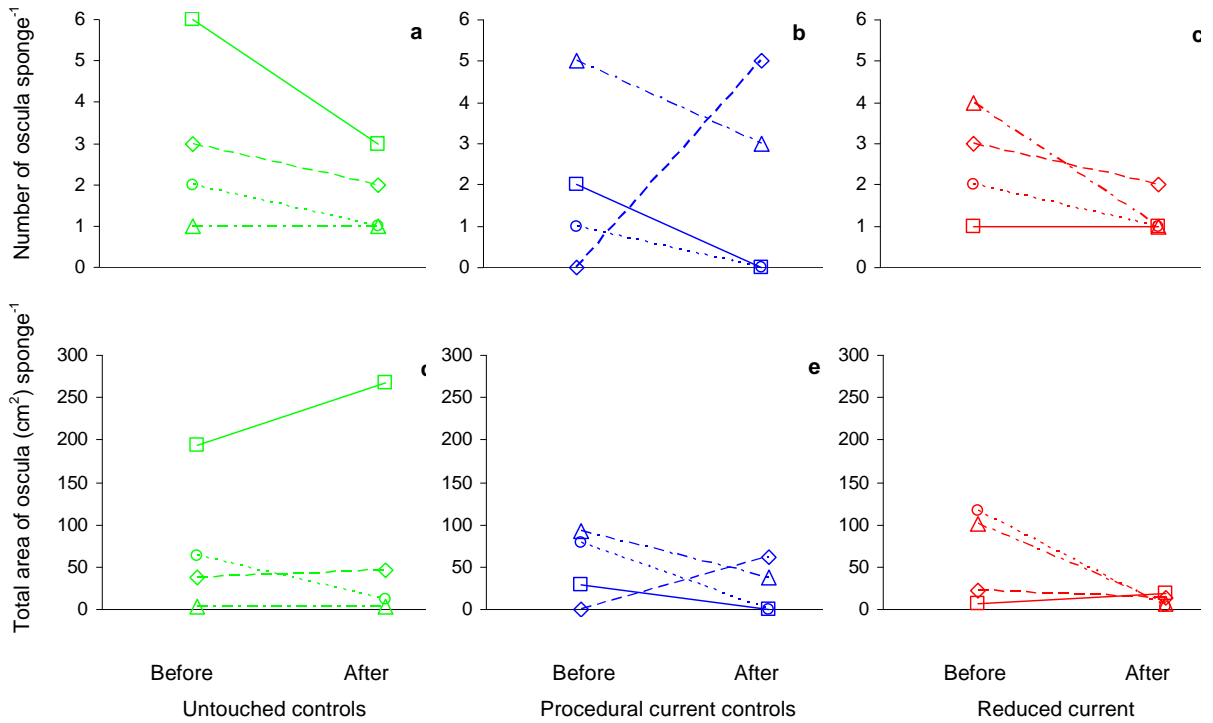
Appendix 4.1. Changes in numbers of oscula and total area of oscula openings (cm^2) from before to after shading in Site 1. Symbols represent individual sponges in each treatment.



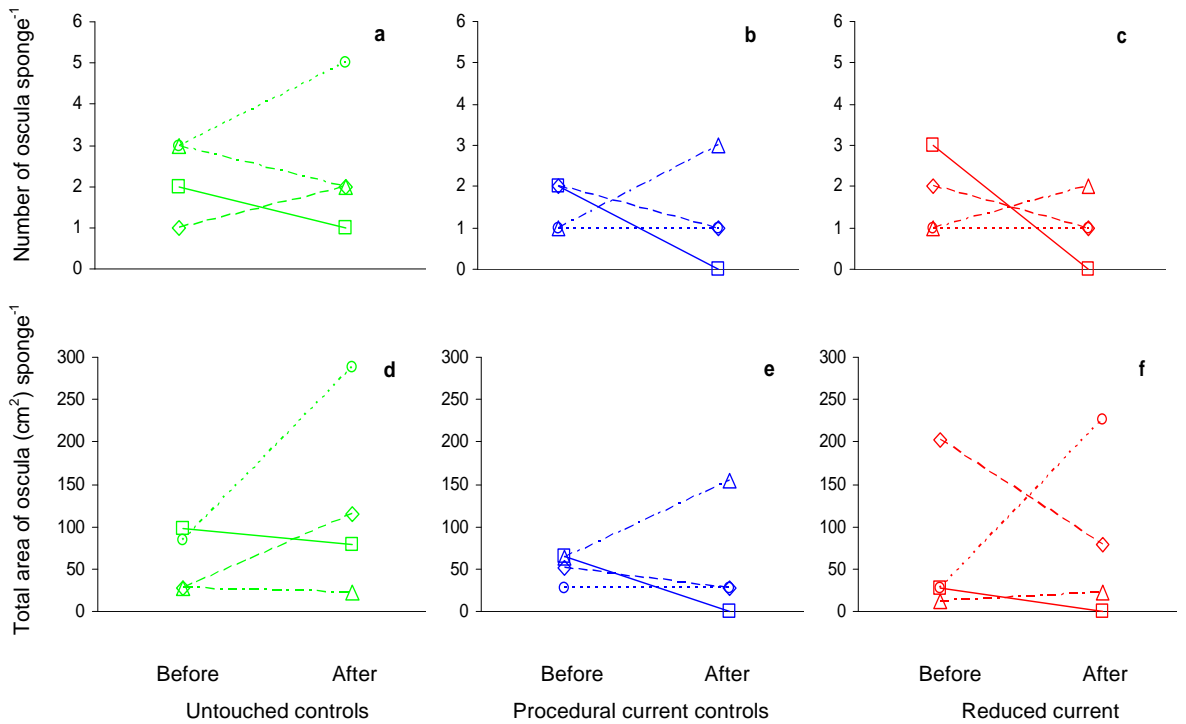
Appendix 4.2. Changes in numbers of oscula and total area of oscula openings (cm^2) from before to after shading in Site 2. Symbols represent individual sponges in each treatment.



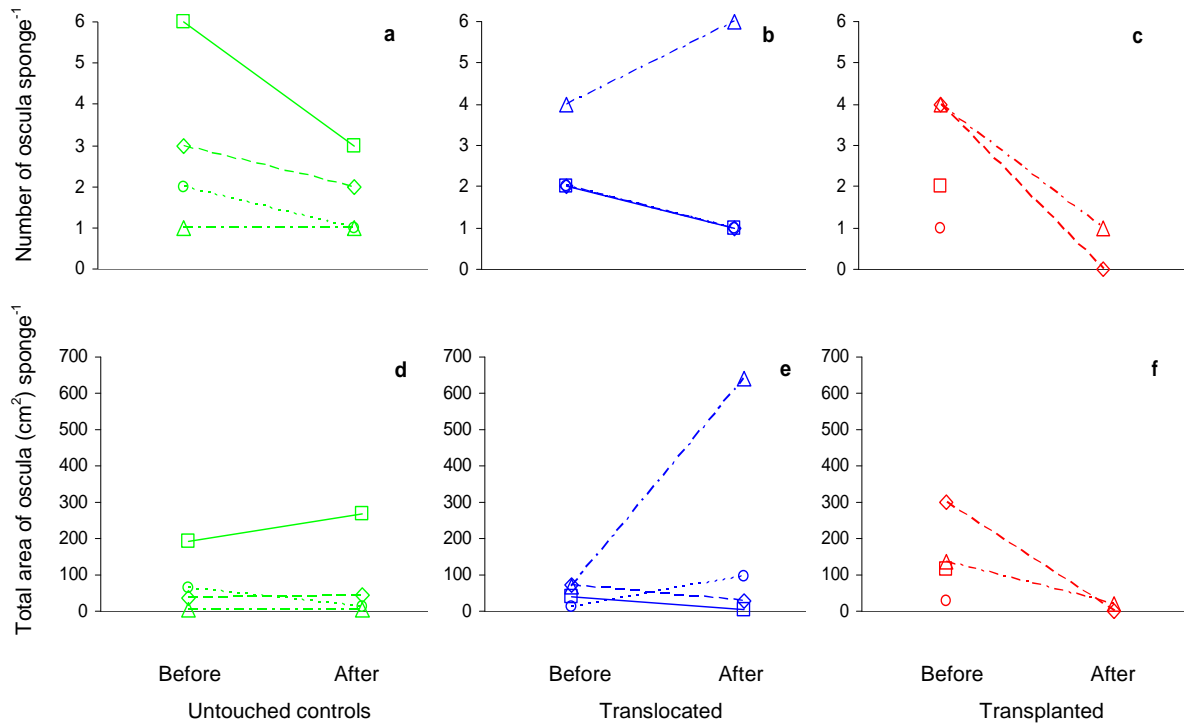
Appendix 4.3. Changes in numbers of oscula and total area of oscula openings (cm^2) from before to after current was reduced to sponges in Site 1. Symbols represent individual sponges in each treatment.



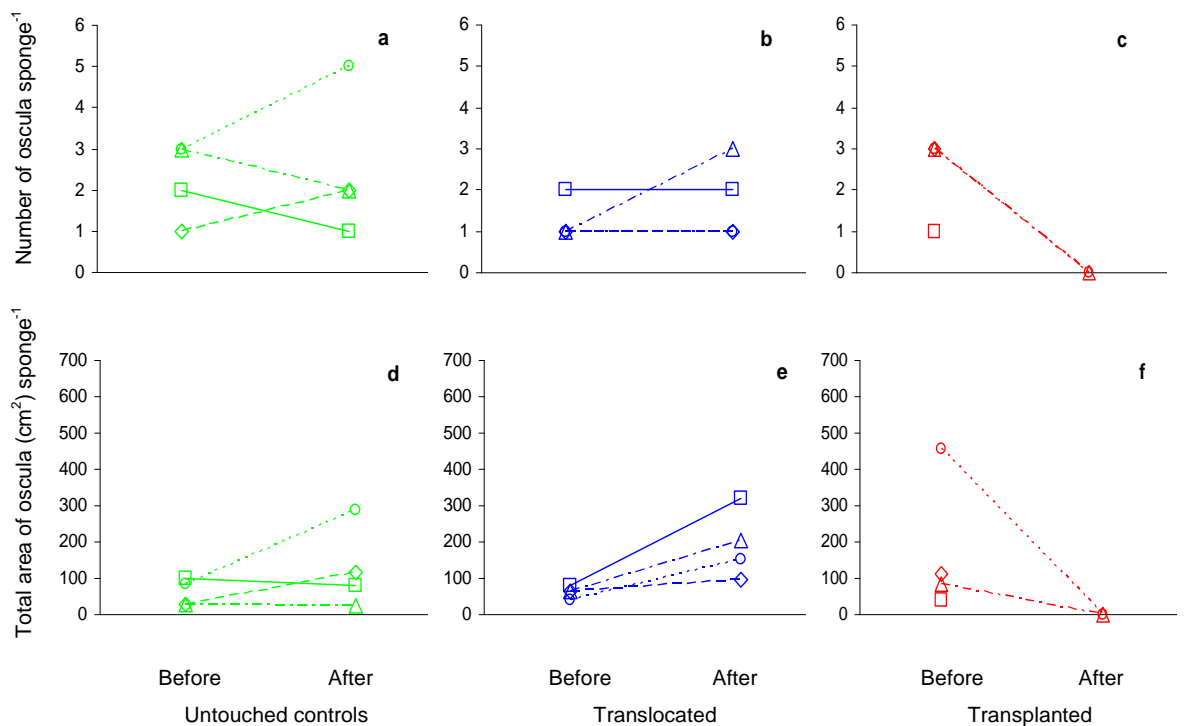
Appendix 4.4. Changes in numbers of oscula and total area of oscula openings (cm^2) from before to after current was reduced to sponges in Site 2. Symbols represent individual sponges in each treatment.



Appendix 4.5. Changes in numbers of oscula and total area of oscula openings (cm^2) from before to after sponges were transplanted from Site 1 into dense seagrass in Site 3. Symbols represent individual sponges in each treatment.



Appendix 4.6. Changes in numbers of oscula and total area of oscula openings (cm^2) from before to after sponges were transplanted from Site 2 into dense seagrass in Site 4. Symbols represent individual sponges in each treatment.



Appendix 5. Descriptive notes on distribution, colour, form and growth of sponge species found during the course of this thesis.

Aplysilla cf. sulphurea. (Wallis Lake, Lake Conjola and Burrill Lake). Bright yellow, irregular or encrusting on macroalgae (*Lamprothamnion* sp. or *Cystoseira trinodus* holdfasts). Surface ornamentation. Usually small.

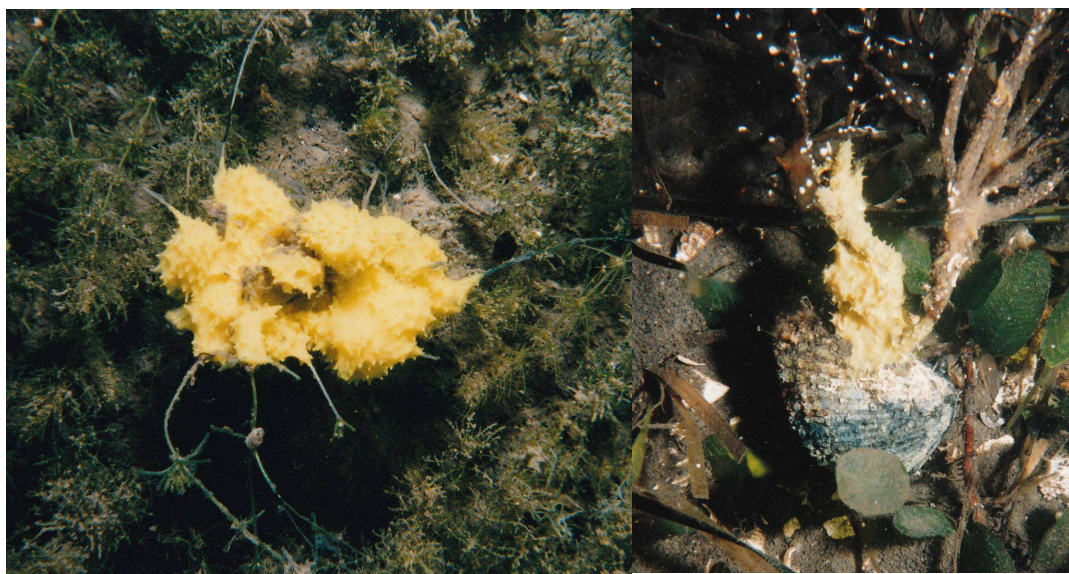


Figure A5.1. *Aplysilla cf. sulphurea*: typical specimens growing on *Lamprothamnion* sp. in Wallis Lake (left) and on a bivalve and *Cystoseira trinodus* in Lake Conjola (right)

***Aplysinella* cf. *rhax*.** Purple colour, thick encrusting to globular in shape. Soft and compressible. Largest specimens approximately size of a softball, but usually smaller. Found attached to leaf sheaths or rhizomes of seagrass (typically *Posidonia australis*), holdfasts of brown alga (predominantly *Cystoseira trinodus*) and bivalve molluscs (typically *Anadara* sp.).



Figure A5.2. *Aplysinella* cf. *rhax* growing in *Posidonia sinuosa* seagrass in St Georges Basin.

***Dysidea* sp. 1.** (Wallis Lake). Mauve to purple in colour, globular. Most specimens golf ball to cricket ball size. Growing on macroalgae (predominantly *Lamprothamnion* sp.).



Figure A5.3. *Dysidea* sp. (Wallis Lake). Small specimen in *Lamprothamnion* sp. meadow.

***Halichondria* spp.** Five species of *Halichondria* were found during the course of this thesis. All were small, irregular or thinly encrusting on macroalgae (typically *Cystoseira trinodus*), bivalves (*Anadara* sp. or *Mytilus* sp.) or rocks. In general, it was difficult to distinguish among species in the field. Colours ranged from white to yellow to orange to pink.

***Haliclona* sp. 1.** (Wallis Lake, Tuggerah Lake). Very thinly encrusting on algae (*Cystoseira trinodus*) creamy white to pinkish in colour.

***Haliclona* sp. 2.** Small pink individuals, irregular, bottle shaped with fistules, rarely larger than size of a golf ball. Very soft and compressible. Found growing in sand or base of seagrass (*Zostera capricorni*), bivalves (*Anadara* sp.) or encrusting on macroalgae (*Cystoseira trinodus*) .

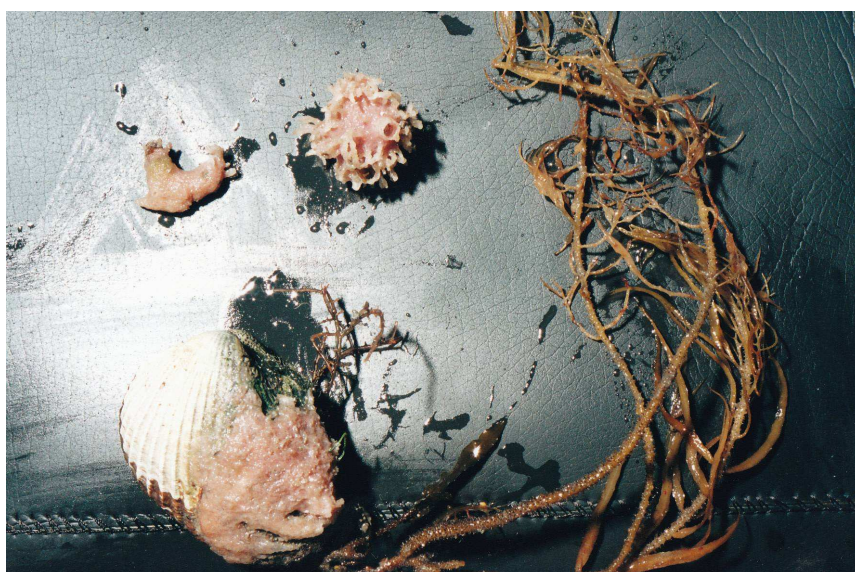


Figure A5.4. *Haliclona* sp. 2. Three small individuals removed from water in Wallis Lake. Note bottom specimen encrusting on the bivalve *Anadara* and holdfast of the alga, *Cystoseira trinodus*.

***Hymeniacidon* sp. 1.** Small yellow/orange irregular shaped, found partly buried in sand or encrusting on bivalves (*Anadara* sp.). Enlarged basal portion with a lot of thin fistules.



Figure A5.5. *Hymeniacidon* sp. 1 in soft sediment in Burrill Lake.

***Hymeniacidon* sp. 2.** Small yellow irregular shaped, found partly buried in soft sediment sand. Enlarged basal portion with a lot of thin fistules.

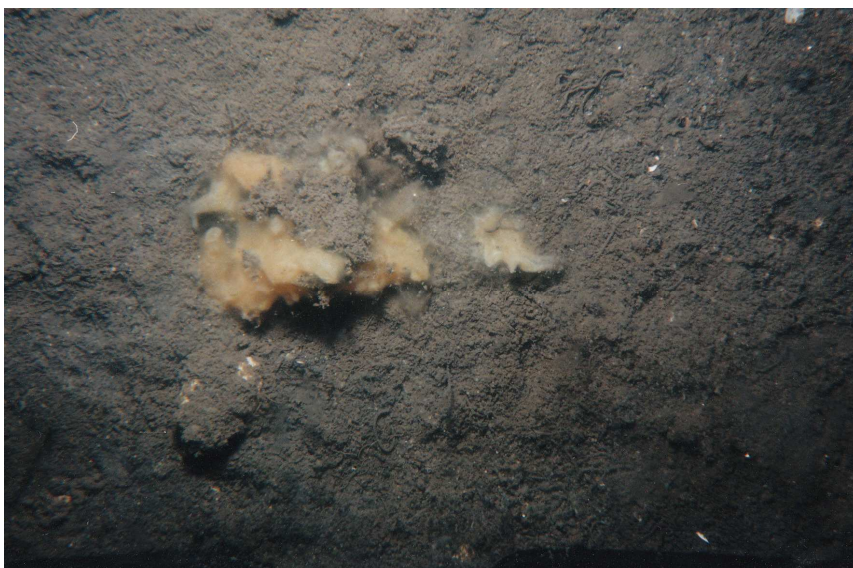


Figure A5.6. *Hymeniacidon* sp. 2 in soft sediment in Coila Lake.

***Mycale* sp.** Light blue to blue/grey with orange spots or speckles (typical of Wallis Lake) to bright orange with bright red spots or speckles (typical of Lake Conjola). Found mostly on macroalgae (*Caulerpa taxifolia* or *Lamprothamnion* sp.) and occasionally on seagrass leaves (*Zostera capricorni*). Generally encrusting and less than 2cm thick. Largest specimens observed covered several m² of seafloor (in Lake Conjola), but this was uncommon with most specimens less than 100cm².



Figure A5.7. *Mycale* sp. growing on the green alga, *Caulerpa taxifolia* in Lake Conjola (top) and in mixed meadow of seagrass and macroalgae in Wallis Lake (bottom)

***Niphates* sp.** Very small thinly encrusting yellow specimens found on brown alga (*Cystoseira trinodus*).

***Raspaillia* sp.** Small orange aborescent, branching, tree-like. Found on leaves of the seagrass, *Zostera capricorni* and bivalve, *Anadara* sp.

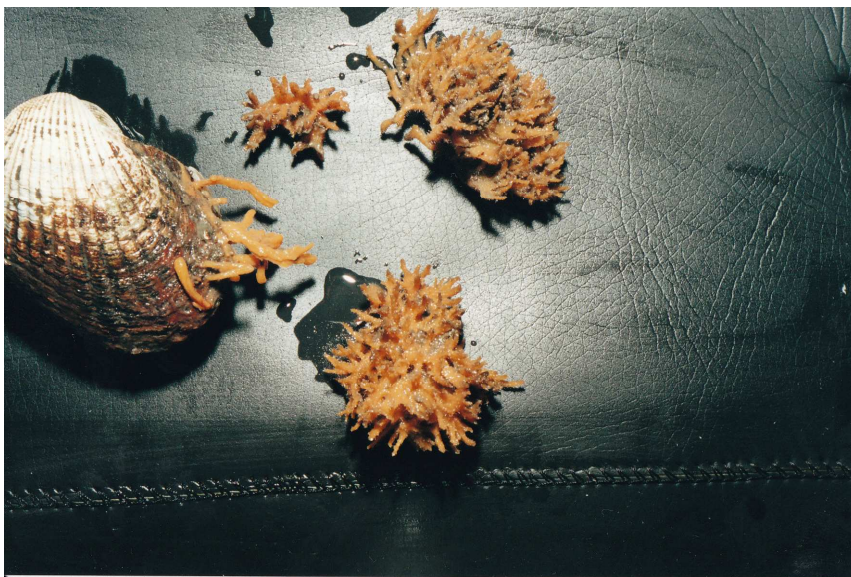


Figure A5.8. On-deck photograph of *Raspaillia* sp. from Wallis Lake

***Suberites* sp. 1.** (Wallis Lake, Smiths Lake, Lake Macquarie, Tuggerah Lakes). Green to blue in colour with some small cryptic specimens found under small stones being orange in colour. Irregular globular, lobate or digitate. Soft. Majority of specimens smaller than hand-size, but not uncommon to find specimens several times larger. Large oscula often visible. Found growing on unattached on soft sediment or attached to macroalgae (predominantly *Lamprothamnion* sp.), seagrass (*Zostera capricorni*), small bivalves (*Anadara* sp.). Occasionally observed on wooden pilings or jetties.

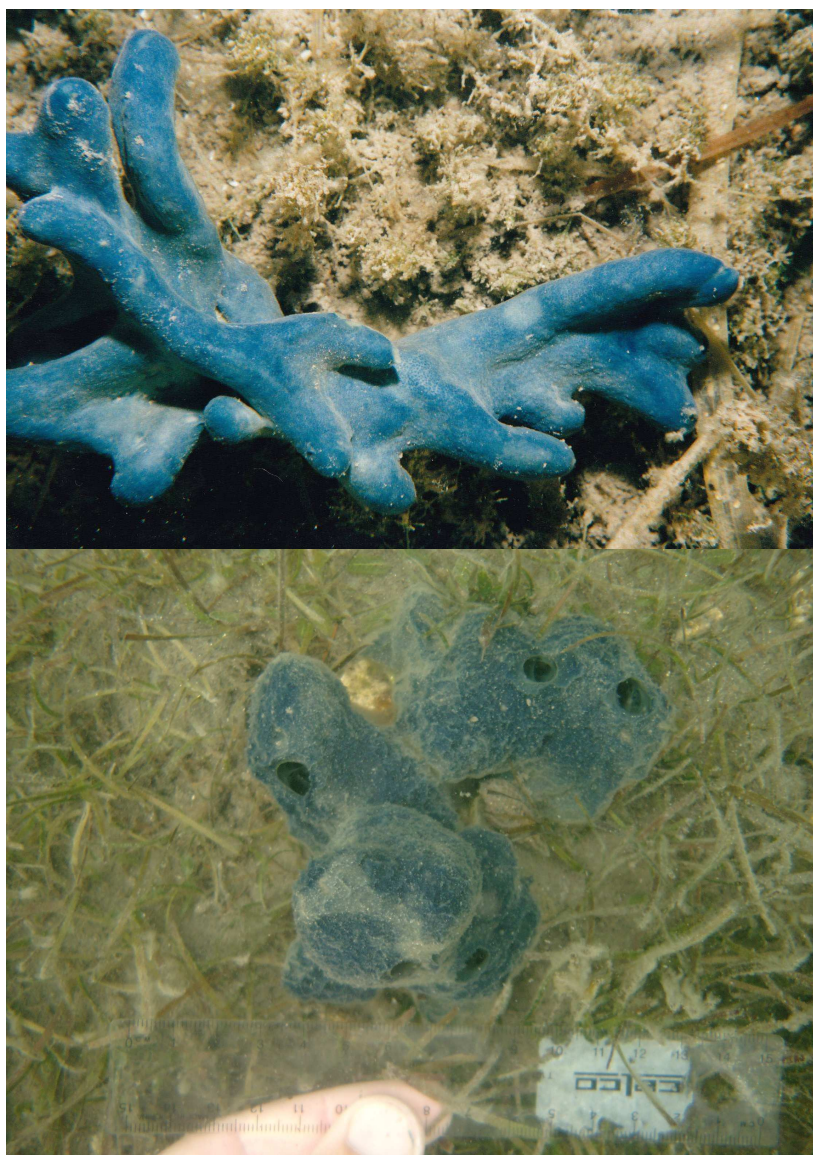


Figure A5.7. *Suberites* sp. 1 in Wallis Lake (top) and Smiths Lake (bottom).

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***Suberites* sp. 2.** Very thin small and encrusting yellow/orange specimens found on green alga (*Codium fragile*).

***Tetilla* sp.** Pink to apricot in colour. Massive, spherical-oval, stalked. Small- thumb sized. Found only in soft sediment. Usually in deeper water than other species (2-4m).

***Calcarea* sp.** Very small individuals rarely larger than size of a golf ball. White irregular and complex form. Mostly found on macroalgae (*Lamprothamnion* sp.).



Figure A5.?. Small *Calcarea* sp. growing on *Lamprothamnion* sp. in Wallis Lake.