Macroalgal assemblages as indicators of the broad-scale impacts of fish farms on temperate reef habitats

by

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Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma in any tertiary institution, and to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

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This thesis is an uncorrected text as submitted for examination.

Abstract

Intensive fish culture in open sea pens can deliver large amounts of nutrients to coastal ecosystems. Sheltered areas with high water quality are predominately chosen for this type of mariculture, and these systems may be adversely affected by the presence of the farms. Since macroalgal community composition has been shown to be a good indicator of environmental disturbance on reef, the present study investigated the effect of salmon farms on macroalgae in a semi-enclosed coastal waterway in southern Tasmania. Data on the macroalgal community were collected from two depths at 44 sites of varying distance from twelve active fish farm leases. This included reference sites at distances of 5 km or more. The sites were widely distributed throughout the study area, and varied in their exposure to wave action. The macroalgal community composition differed significantly between sites at 100 m from fish farms and sites at 5 km or more. Sites at 400 m varied in their response to farms, with some sites showing characteristics similar to 100 m sites. Impacts varied between swell exposed sites and sites only subjected to wind-generated waves. *Chaetomorpha* spp. and *Ulva* spp. were abundant near fish farms at exposed sites, whereas the abundance of filamentous green algae increased at sites near fish farms in sheltered sites. The percentage cover of indicator groups such as epiphytes and opportunistic algae in total provided the best indicators of fish farm impacts on a broad scale. The percent cover of canopy forming perennial algae did not decrease near fish farms indicating that their growth and recruitment has not been greatly affected by high levels of sedimentation from fish farms or prolonged fouling by opportunistic algal epiphytes to the present, however further study is needed to examine this in more detail.

The above analysis utilised photographic quadrats to quantify community composition. Most other broad-scale sampling methods used to measure macroalgal composition require expertise to identify species *in situ*. However, this reduces the capacity of monitoring programs to collect large amounts of data. Using data collected from a subset of 36 sites, the photographic method was compared with a manual quadrat sampling method. The two methods produced similar multivariate results but manual quadrats had a slightly greater capacity to detect the impacts of the fish farms. This indicates that photographic quadrats are likely to be conservative in quantifying effects of fish farms, but still deliver appropriate resolution to detect major changes in dominant macroalgal cover and composition. Some adjustments to the photographic methods used will allow better resolution for algae obstructed by canopy or epiphytic overgrowth.

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Chapter 1 Introduction

1.1 Background

1.1.1 Macroalgal environmental indicators

Coastal ecosystems, as the natural transition zones from land to sea, experience a high degree of pressure from anthropogenic activity. Humans have altered hydrological cycles and the flux of nutrients to coastal habitats (2002), causing system-wide impacts to estuaries, embayments, and large areas of semi-enclosed seas in many developed countries (Boesch 2002). In particular, excessive nutrients and increased rates of sedimentation have caused changes to habitat structure and diversity in temperate reef ecosystems (Worm et al. 1999; Airoldi 2003; Connell et al. 2008; Krause-Jensen et al. 2008). These changes impact on the delivery of ecosystem services to society (Costanza et al. 1997), as well as marine conservation objectives for reef areas, which are regarded as key habitats. Consequently there is a need to monitor and assess the ecological changes occurring as a result of altered water quality (Airoldi 2004).

Monitoring of nutrient levels is not solely effective in quantifying pollution pressure or impact in dynamic marine environments. The release of nutrients from a pollution source may vary diurnally, by quantity and by the nature of dispersal, creating a need for frequent sampling (Dalsgaard & Krause-Jensen 2006). Nutrient concentrations in the water column are also influenced by algal uptake (Goodsell et al. 2009). In addition, ecological impacts can rarely be predicted from nutrient concentrations, as there may be large differences among estuarine-coastal systems in their sensitivity to nutrient enrichment (Cloern 2001). Macroalgal communities on reef integrate the effects of long term exposure to altered local environmental conditions, as they are sessile and respond to pollution over time (Munda 1993; Pinedo et al. 2007). Consequently, macroalgae are now regarded as relevant and useful indicators of environmental impact (Morand & Briand 1996; Juanes et al. 2008).

A well documented consequence of excessive nutrients in coastal reef environments is the disproportionate growth of certain types of productive, fast growing macroalgae (Bokn et al. 2003; Krause-Jensen et al. 2008; Teichberg et al. 2008) at

the expense of habitat forming perennial species (Valiela et al. 1997; Worm & Sommer 2000; Gorgula & Connell 2004). These fast growing algae have been termed, 'opportunistic', 'bloom forming' or 'nuisance' macroalgae (Littler & Littler 1980; Valiela et al. 1997; McGlathery 2001; Krause-Jensen, 2007a).

In temperate waters, opportunistic green algae in the genera *Ulva,* (which now includes the genus *Enteromorpha*)*, Cladophora,* and *Chaetomorpha* (Lavery & McComb 1991) are the most common macroalgae reported to form blooms (Valiela et al. 1997). These algae are typically ephemeral, with a filamentous or sheet-like form, a relatively undifferentiated thallus, and a high thallus area to volume ratio (Littler & Littler 1980). Such attributes allow for fast growth and rapid reproduction when environmental conditions are ideal for growth (Littler & Littler 1980). These algae often have a high demand for nitrogen (Barr & Rees 2003), and their growth is favoured under a variety of pollution types (Guinda et al. 2008), such as sewage pollution (Soltan et al. 2001; Arevalo et al. 2007), sedimentation (Eriksson & Johansson 2005), and pollution from urbanisation (Gorgula & Connell 2004; Mangialajo et al. 2007). In eutrophic systems, dense blooms of opportunistic algae can form, and influence nutrient dynamics beyond their role as nutrient sinks (Lavery & McComb 1991), substantially altering marine community structure and function (Nelson et al. 2008).

Macroalgal blooms have been associated with the decline in coral cover in tropical waters (Fabricius et al. 2005; Littler & Littler 2007), as well as the loss of seagrass and changes in macroalgal community composition in temperate marine systems (Valiela et al. 1997; McGlathery 2001; Arevalo et al. 2007). Many opportunistic species grow as epiphytes on habitat forming algae, with epiphytic overgrowth increasing with nutrient enrichment over large spatial scales (Russell et al. 2005). Prolonged epiphytic fouling has the potential to impair the growth of canopy-forming species through light limitation, and to decrease the flux of dissolved substances to the host plant (Sand-Jensen 1977; Sand-Jensen et al. 1985; Worm et al. 1999). Light limitation has been shown to be the primary cause of seagrass decline in eutrophic waters (Hauxwell et al. 2001; Hauxwell et al. 2003). In numerous cases, increased over-growth by opportunistic algae accompanies a decrease in species richness and

total macroalgal cover (due to the loss of canopy forming perennials) (Wells et al. 2007).

On South Australian temperate reefs, algal turfs (filamentous assemblages of algae <5 mm in height) have replaced canopy forming algae along urbanised coastlines, with canopy algae declining up to 70% in cover on reefs (Connell et al. 2008). Experimental tests showed that algal turf could rapidly colonise and retain space at high rates of sedimentation and nutrient enrichment (Gorgula & Connell 2004). A potential mechanism for the loss of canopy-forming algae is the inability for recruitment to occur amongst algal turf (Gorgula & Connell 2004), as described by Kennelly (1987) and Airoldi (2003). Additionally, recruits of the opportunistic alga *Ulva* and *Cladophora* in the Baltic Sea have been experimentally shown to have a high tolerance for sedimentation, whilst the perennial brown alga *Fucus vesiculosus* and *Sphacelaria arctica* did not (Eriksson & Johansson 2005). Benthic communities in the Baltic Sea change along a gradient of eutrophication, with canopy forming algae replaced by bloom forming algae towards pollution sources (Worm et al. 1999; Worm & Lotze 2006).

Recent experimental studies highlight the importance of other environmental and ecological variables that interact with the growth and dominance of opportunistic macroalgae when nutrient enrichment occurs. Opportunistic algal growth tends to decrease with increasing grazing pressure (Bokn et al. 2003; Worm & Lotze 2006), increasing canopy cover (Bokn et al. 2003; Eriksson et al. 2007), physical disturbance (Worm et al. 2002), short water residence times (Valiela et al. 1997), or where recruitment is limited by the lack of a propagule bank (Worm et al. 1999; Worm et al. 2001). Light limitation also occurs seasonally or when over-shading by phytoplankton blooms occur, decreasing the depth penetration of algal growth (Krause-Jensen et al. 2007b; Krause-Jensen et al. 2008).

Given that different marine systems vary in their tolerance to altered water quality there is a need to determine the ecological responses to anthropogenic impacts on an ecosystem basis. Improved understanding of biogeochemical dynamics and nutrient budgets is important for science and conservation management. However, it is equally important to understand the response of key marine communities to altered

nutrient dynamics, as they are often the subjects of conservation and do not necessarily respond in a simple manner to biogeochemical processes. It is now widely recognised that macroalgal richness, opportunistic species and cover of macroalgae provide relevant and useful indicators when monitoring environmental disturbance on temperate reefs (Guinda et al. 2008; Juanes et al. 2008). The suitability of these indicators has been tested through the application of macroalgal indices for environmental monitoring purposes within the European Water Framework Directive (Wilkinson et al. 2007; Guinda et al. 2008), and observed in long-term studies of macroalgal assemblages (Shepherd et al. 2009).

1.1.2 Fish farming in coastal environments

Whilst much attention has been focussed on terrestrial derived pollution, eutrophication from marine fish farms is also a threat to coastal aquatic ecosystems. In 2006, aquaculture contributed 47% to the world's fish production, and continues to grow (FAO 2009). Finfish culture in open water cages accounts for a significant part of total aquaculture production and has rapidly expanded in many coastal systems since the 1980s and 90s (HEST 2000; Sowles & Churchill 2004; Pérez et al. 2008). An increasing need exists to monitor the impacts from fish farm derived nutrient and organic matter pollution as the industry continues to expand.

Farmed finfish (excluding mullet and rabbitfish) rely on nutrient-rich compound aquafeeds as an external food source (Tacon 2004). This type of intensive fish husbandry has the potential to alter sediment and water chemistry in and around the farm area (Woodward et al. 1992). Although improved feeding technology has provided a reduction in wasted feed input, Sanderson et al. (2008) suggested that about 70% of the nitrogen and 80% of the phosphorus input to a salmon farm is released to the environment as feed wastage, fish excretion, faeces production and respiration.

The level of impact from marine fish farms is highly dependent on the species farmed, the standards of animal husbandry and the dynamics of the receiving environment (Naylor et al. 2000). The impact of particulate waste on the sea bed

under and around cages has been widely documented. Negative impacts include: altered benthic infaunal species composition, increased sulphate reduction, the growth of bacterial mats of *Beggiatoa* spp. on the sediment surface, decreased oxygen and increased fluxes of ammonia, methane and hydrogen sulphide from sediments (Holmer & Kristensen 1992; Black 2001; Nickell et al. 2003; Navarro et al. 2008). The presence of these conditions can have deleterious effects on fish growth (Black, 1996); consequently the monitoring of the chemical environment is most often incorporated into fish farming practice, as is the periodic fallowing of farmed areas (Pereira et al. 2004).

Whilst the impacts of particulate waste from marine fish farms on sediments have been widely studied, impacts on the pelagic environment have not (Navarro et al. 2008). This is despite most of the nitrogen input to fish farms being lost to the environment in dissolved form, through fish excretion and remineralisation from sediments (HEST 2000). Additionally, the effect of particulate waste on sediments is often localised (< 30 m) (Ye 1991), whilst relatively broad scale and cumulative impacts may occur as a result of dissolved nutrients dispersing throughout the receiving ecosystem (Sowles & Churchill 2004).

In the pelagic environment, high ammonia and dissolved organic nitrogen levels were correlated with elevated abundances of heterotrophic microorganisms near fish farms, indicating that these microbes may be directly or indirectly affected by nutrients from fish farms (Navarro et al. 2008). Elevated abundances of phytoplankton have also been found surrounding fish farms (Buschmann et al 2001). However this is not always the case, as many studies failed to find clear associations between phytoplankton abundance and the presence of fish farms (HEST 2000; Alongi et al. 2003; Navarro et al. 2008; Pitta et al. 2009). This variation has been attributed to several factors. Pitta et al. (2009) recently demonstrated that phytoplankton growth rates increased near fish farms, but that microplankton grazers kept abundances at low levels, rapidly transporting the biological effects of nutrients from fish farms up the food chain. Also, low water residence times can disperse nutrients and phytoplankton accumulations before the biomass of a bloom increases significantly (HEST 2000; Navarro et al. 2008). In contrast, sessile communities may respond differently, because they accumulate the effects of locally elevated

nutrients over a longer time period (Munda 1993). Growth in macroalgae may also be easier to monitor than the nutrients themselves, as concentrations of dissolved nutrients vary significantly over the day, requiring intense sampling (Dalsgaard & Krause-Jensen 2006).

The link between macroalgal growth and fish farm effluent is well established, due to the development of integrated aquaculture schemes. In these cases, nitrogen-assimilating macroalgae reduce environmental pollution from fish farms whilst creating a profitable resource (Neori et al. 2004). The production of 92 tons of salmon can yield 385 tons (of *Ulva*) or 500 tons (of red algae) fresh weight of seaweed through assimilation of nutrient waste (Neori et al. 2004). Research into such polyculture systems have found that macroalgae such as *Ulva* (Hernández et al. 2008) effectively uptake fish farm derived nutrients, and that many macroalgal species have a preference for ammonia-nitrogen which is released from fish as metabolic waste (Sanderson et al. 2008).

From the perspective of conservation management, macroalgae of natural benthic communities near fish farms are also likely to respond to elevated nutrient levels, with potential implications for the diversity and composition of species (indicative of those already observed in eutrophic systems). Few studies have addressed this issue (Ruokolahti 1988; Ronnberg 1991; Ronnberg et al. 1992; Boyra et al. 2004; Vadas et al. 2004; Hemmi et al. 2005), although it is one of ecological and economical significance. Ronnberg (1992), and Hemmi et al. (2005) investigated the growth of epiphytes on *Fucus* in the Baltic Sea, and found an increased growth and biomass of epiphytes on *Fucus* near fish farms, with a shift from brown and red epiphytes to green epiphytes towards fish farms. Vadas et al. (2004) found increases in the foliose green alga *Ulva* near fish farms in Cobscook Bay, Maine. Boyra et al. (2004) also found significant differences between intertidal macrobenthic assemblages near fish farms and those at control locations. The presence of two pollution tolerant species and filter feeding anemones at impacted sites distinguished them from control sites. In the Mediterranean, significant losses of seagrass communities have been associated with fish farms (Dolenec et al. 2006; Holmer et al. 2008; Pérez et al. 2008).

Such effects could be expected to occur on a large scale, relative to most fish farm impacts on benthic communities which result from enriched particulate matter, and occur at the scale of tens of metres (Ye 1991). Due to advection, the effects of impaired water quality are likely to be detectable beyond fish farm lease boundaries (HEST 2000). Given this, the possibility of broad scale effects of open cage fish culture on benthic macrophytic communities should be considered as a part of ecosystem based aquaculture management. The issue is of particular relevance where numerous fish farms exist in close proximity, and where the fish farm industry is likely to expand.

This study investigates the scale and nature of fish farm impacts on temperate macroalgal communities in south-eastern Tasmania, where salmon farming has become a dominant form of aquaculture. The most concentrated area of salmon farming in Tasmanian is in the semi-enclosed water body of the D'Entrecasteaux channel and the adjoining Huon Estuary, where the industry rapidly expanded throughout the late 1980s and 90s. The waters of the area are considered relatively pristine, and fish farming may be considered a major source of anthropogenic nutrient input into the area (Macleod & Helidoniotis 2005).

Farmed salmon are carnivores, and are solely reliant on an external source of fish feed, which contains a high amount of protein. The Huon Estuary Study Team (2000) estimated that of the nitrogen contained in fish feed, 36% is retained as harvested fish, and the remaining 64% released into the estuary through metabolic waste or uneaten feed. Of this 13% is particulate and 87% is dissolved nitrogen (HEST 2000).

Biogeochemical models created for the Huon Estuary predicted that the impact of fish farm derived nutrients would vary seasonally. In winter, dissolved inorganic nitrogen (DIN) levels are already high (due to the presence of nutrient rich marine waters), flushing rates are high and biological uptake is low due to low light availability and low temperatures (HEST 2000). By contrast, in summer phytoplankton growth and biomass become nitrogen limited, and waters are more stratified. Biogeochemical models indicated that, during the 1997 summer, fish farm derived nitrogen loads contributed 25% to total DIN and chlorophyll levels (a proxy

for phytoplankton biomass). Simulations showed that doubling the 1997 fish farm loads would raise dissolved inorganic nitrogen (nitrate, nitrite and ammonia) levels by 50%, and carry risk of increased phytoplankton blooms (HEST 2000). Consequently, the industry voluntarily put a moratorium on the amount of feed used in the Huon Estuary (Crawford 2003), however significant growth of the industry has continued in the adjacent D'Entrecasteaux Channel. Between 1996/97 and 2006/07 Tasmanian salmon production levels have tripled from 7,647 to 23,637 tonnes (ABARE 2008), with most of the growth occurring in the D'Entrecasteaux Channel.

The Tasmanian salmon aquaculture industry recognises that economic sustainability requires environmental sustainability, which in turn requires an understanding of the impacts of fish farms (McLeod et al. 2004). Collaborative studies between industry and scientists have investigated; (i) the impacts of organic enrichment to the sediments near fish farms, (ii) appropriate monitoring techniques to detect these impacts, and (iii) the positive effects of fallowing practices (McLeod et al. 2004). A benthic monitoring program has incorporated this research to monitor for unacceptable impacts of organic enrichment to the seafloor extending to 35 m from aquaculture leases. The CSIRO have undertaken biogeochemical modelling of the whole D'Entrecasteaux Channel system, including fish farm inputs. These models are extrapolated to predict the occurrence of phytoplankton blooms and conditions leading to eutrophication of the system.

The effects of fish farms on water column nutrients and dissolved oxygen are likely to extend hundreds of metres from farm sites (HEST 2000), and may impact upon macroalgal community composition in reef habitats. Such effects would be considered relatively broad-scale in comparison to other benthic impacts of fish farms. Given the recent growth of fish farming in the D'Entrecasteaux region, knowledge is needed on the full extent and nature of impacts from fish farms so that cumulative and regional effects can be considered as a part of aquaculture management practices. This has significant implications, as current legislation states that fish farming activities should have no "unacceptable" impact beyond 35 m of the lease area.

Impacts to reef habitats from fish farms in the D'Entrecasteaux Channel have not yet been researched, neither has the potential use of macroalgae as a monitoring tool been investigated. Despite this, significant reef habitats are located along the coast of this region, with two areas designated as "no-take" marine reserves (Tinderbox and Ninepin Point). These reserves are managed for biodiversity conservation, recreation, and scientific research, three aims that could be compromised by eutrophication associated with excessive regional nutrient input. This thesis seeks to contribute to the understanding of broad scale biological responses to fish farming in the D'Entrecasteaux Channel. Such knowledge is relevant to ecosystem based management in the study area and other locations with intensive fish farming operations. Outcomes are also relevant to the long term conservation of reef biodiversity.

1.1.3 Monitoring macroalgae

Ecological data is inherently variable, and large sample sizes are generally needed to provide the statistical power needed to describe significant patterns. An obvious issue with intensive monitoring programs is the cost and expertise required for data collection. Upon designing a sampling regime, there is often a trade off between the resolution of the data and the sample size obtained. Ultimately the choice of a sampling method strongly depends on the specific question to be answered (Dumas et al. 2009).

Using manual sampling techniques, long term monitoring of macroalgae has occurred in four marine reserves and reference sites in east and south-eastern Tasmania, including Tinderbox and Ninepin Point reserves (Barrett et al. 2009). Alternately, the use of photographic sampling has been integrated into methodologies used by Reef Life Survey volunteers to collect information on the percentage cover of sessile invertebrates and macroalgae around Australia. Manual sampling of benthic community composition by SCUBA usually requires intensive field work performed by skilled staff (Alvaro et al. 2008). Visual census methods provide fine-scale data suitable for scientific research (Dumas et al. 2009), but may be limited where sampling is conducted under some degree of time constraint, due to the nature

of SCUBA safety requirements, the physical pressures of SCUBA diving, and the costs of field data collection.

Digital sampling methods can be advantageous through speeding up data collection *in situ* and eliminating the need for species identification *in situ*. They also provide permanent sample records which may be re-sampled at a later date, directly compared as part of a future time series (Teixidó et al. 2009), or used as a form of visual communication about the underwater characteristics of a sample/site (a picture speaks a thousand words). Additionally, the image analysis process is not under significant time pressure and additional information resources may be utilised. One drawback of this technique is that the image quality can be affected by environmental conditions such as water clarity, swell, and light intensity. Species may also be obstructed in a photo by shadows, canopy layer algae, or light reflection.

Nevertheless, photographic and video methods have been increasingly utilised as a consequence of improved technologies that have allowed the collection of high resolution digital images (Alvaro et al. 2008). Regardless, few previous studies have investigated the use of photographic techniques for sampling macroalgal communities. Some recent studies have demonstrated that photographic sampling can provide enough resolution to identify inter-tidal algal species (Ducrotoy & Simpson 2001), sub-tidal biotypes (Alvaro et al. 2008), and species groups of tropical benthic biota (Dumas et al. 2009), all of which are potentially useful indicators for environmental monitoring. These studies have advocated the possible application of digital sampling techniques for monitoring benthic communities where expertise can be used for the analysis of samples, but is not necessarily available for field data collection.

This study utilises the application of photographic monitoring techniques to assess the impacts of fish farms on subtidal temperate macroalgal community composition. A comparison of this method with manual sampling techniques is also conducted, in order to compare the application and data resolution of these two different approaches in sub-tidal temperate reef systems. This comparison will have particular relevance to the compatibility of monitoring techniques already used to sample algal communities in Tasmania.

1.2 Research aims

The rationale behind this project is to provide insight into broad scale ecological impacts of fish farming, whilst investigating the application of a digital monitoring technique that may prove useful in a general management context for monitoring macroalgal assemblages.

Primary aims of this project are:

* to reduce current knowledge gaps concerning the nature and extent of salmonid fish farm impacts on reef systems,
* to identify responses of macroalgal communities on Tasmanian temperate reefs to altered water quality, and
* to investigate the use of photographic sampling of macroalgae for monitoring purposes, as compared with manual sampling methods.

1.3 Thesis structure

Chapter 2 provides further details of the environmental context and seasonal dynamics of the study area. It also outlines the design of the study, the sampling protocol, the methods used, and the statistical analyses performed to answer the research questions previously outlined.

Chapter 3 compares photographic and manual sampling methodologies.

Chapter 4 reports the results of the research on the impact of fish farms on macroalgal community composition. Details are given for the nature, significance and distribution of the observations made throughout the study region.

Chapter 5 includes an overall discussion on the significance of the information obtained in Chapters 3 and 4. Relevance of the results for both scientific and management purposes are discussed, as well as potential constraints of the data collected. This chapter also provides a summary of the research outcomes, management recommendations and direction for future studies on this topic.

Chapter 2 Methods

2.1 Study region

This study is focussed within the D'Entrecasteaux channel and Port Esperance, in south-eastern Tasmania (Figure 2-1). Since the mid 1980's this region has been the main scene for the development of marine fish farming practices in Tasmania. Sites are also included on the western side of the Tasman Peninsula, near fish farms located in Wedge Bay. The D'Entrecasteaux Channel, Port Esperance, and Wedge Bay, are all part of the Bruny Bioregion, identified as part of the *Interim Marine and Coastal Regionalisation of Australia* (IMCRA) scheme (Edgar et al. 1997).

General descriptions of hydrology, ecology and biogeochemical dynamics in these areas have been presented in several research publications and management reports, including those compiled for aquaculture management. The Huon Estuary Study (HEST 2000) was designed to improve understanding of chemical, physical, and biological dynamics of the estuary with particular emphasis on the potential impact of salmon farming. Jordan *et al.* (2002) provided an overview of hydrodynamics, nutrients and habitats in North West Bay, whilst Clementson *et al.* (1989) investigated the temporal dynamics of chemical and biological parameters in Storm Bay. An overall description and mapping of inshore habitats throughout the south-east region of Tasmania was presented by Barrett *et al.* (2001), for the purpose of Marine Protected Area (MPA) planning. A description of subtidal benthic macroalgae in the D'Entrecasteaux Channel was also provided by Sanderson (Sanderson 1984), and a description of seasonal variations in south-east Tasmanian phytal animal communities was provided in Edgar (1983a). The State of the D'Entrecasteaux Channel report (Phillips 1999) also provided an overview of the waterway and its catchments, their uses, values and threats.

The study area is part of Tasmania's southern Natural Resource Management (NRM) region, which is managed by both local and state government. NRM planning involves the monitoring, management and reporting of water quality and coastal values. One aspect of this has been the evaluation and mapping of foreshore values, condition and pressures throughout the southern NRM region, as presented by Migus

(2008). The index of 'foreshore pollution pressure' provides information about the distribution of stormwater, sewage, heavy industry, intensive agriculture, marina, and rural or aquaculture runoff along the coastline (Migus 2008).

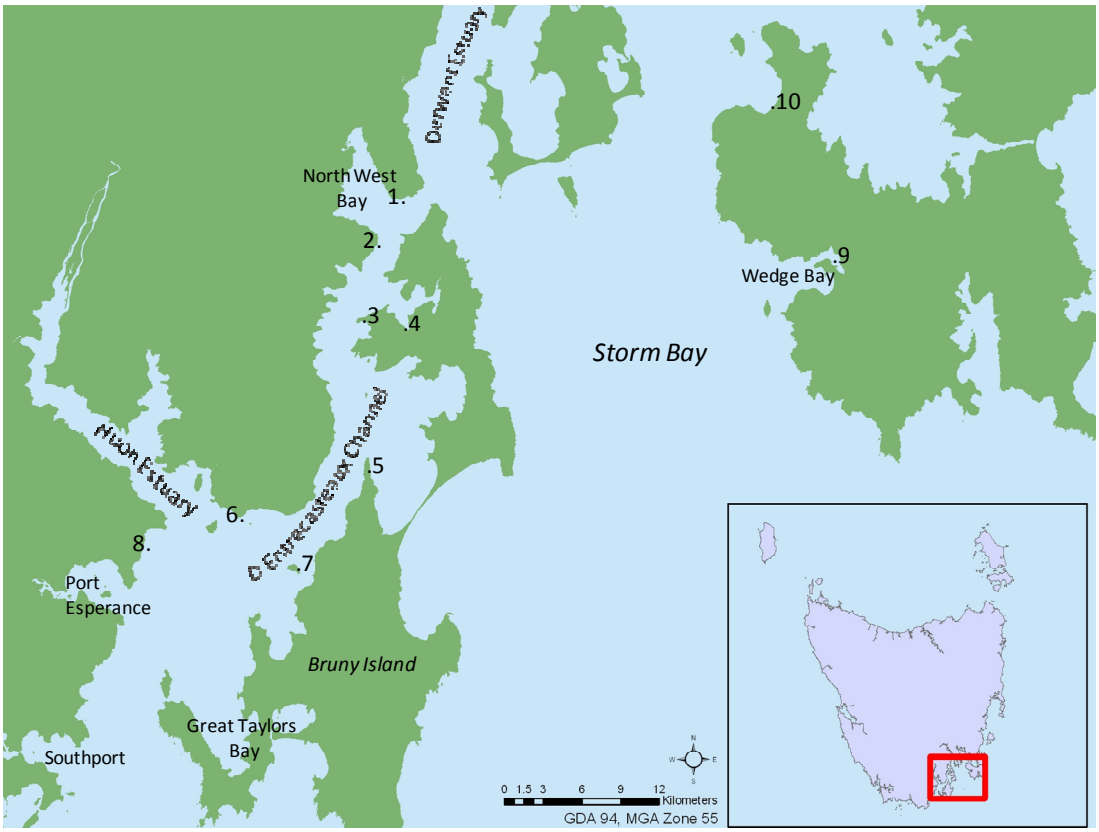

Figure 2-1 Study region. Locality of other areas mentioned in this document, 1. Tinderbox, 2. The Sheppards, 3. Roberts Point, 4. Sykes Cove, 5. Simpsons Point, 6. Ninepin Point, 7. Satellite Island, 8. Roaring Bay, 9. Parsons Bay, 10. Sloping Main

2.1.1 D'Entrecasteaux region

The D'Entrecasteaux Channel is the narrow body of water between Bruny Island and the mainland of south eastern Tasmania, extending around 50 km from the north, to the south, (Figure 2-1). It is a multi-use area popular for boating, sailing, and recreational fishing, and has areas of significant historical and cultural value (Phillips 1999). Surrounding land use is primarily for agriculture, pasture, or rural residential use, and significant areas of native vegetation remain intact (Phillips 1999).

The channel receives water from Storm Bay in the north, the Southern Ocean in the south, and freshwater from the Huon and North West Bay rivers. Flushing rates are variable throughout the channel, and the maximum tidal range is 1 metre (DPIWE, 2002). The depth generally exceeds 10 m and reaches 53 m in some places (Phillips 1999). The northern end of the channel adjoins the Derwent Estuary between Pierson Point and Dennes Point, and the coast near Tinderbox is subject to some swell action, receiving water from the adjacent Storm Bay. The middle region of the channel is largely sheltered, protected from oceanic swell by Bruny Island. Here, winds may be a major influence on the movement of surface waters. In addition, current speeds increase where water flows though narrow parts of the channel with shallow depths. The southern third of the channel is influenced by swell from the adjoining Southern Ocean, and is also influenced by the inflow of tannin stained freshwater from the Huon River. Distinctive macroalgal communities that exist at the mouth of the Huon Estuary, are adapted to low light conditions created by the overlying tannin stained waters.

Rocky shores along the coastline are primarily of sedimentary or dolerite origin. In the middle region of the channel, reefs associated with the shoreline, are low profile, rarely extending beyond 5 m depth (Barrett et al. 2001). Deeper reefs occur in areas with higher levels of exposure or current flow. Macroalgal communities found on the reef consist of temperate species. The degree of water movement over the reef influences the dominant algal species present, and the depth they extend to, with a general transition from *Phyllospora comosa, Lessonia corrugata* and *Ecklonia radiata*, to Fucoid and *Sargassum* species with decreasing wave exposure (Barrett et al. 2001).

2.1.2 Wedge Bay

Wedge Bay is located on the western coast of the Tasman Peninsula and opens into Storm Bay. The coastline is generally exposed to oceanic swell (with the exception of the very sheltered inlet of Parsons Bay), although blocked from the full extent of south-westerly swell by Bruny Island. The area is less subject to freshwater inflow and runoff than areas in the D'Entrecasteaux channel. Rocky shores of this area also

consist of sedimentary rocks or dolerite, and share most species of macroalgae with reefs in the D'Entrecasteaux Channel.

2.1.3 Seasonal dynamics

The Bruny bioregion experiences a cold temperate marine climate, and strong seasonal influences owing to its position within the Subtropical Convergence. Water temperatures peak at 19^{o}C in February and drop to approximately 9^{o}C in July (Jordan et al. 2002). In winter and spring nutrient-rich subantarctic waters have a particularly large influence across southern Tasmania (Harris et al. 1987). The influence of these waters decreases over the summer period, as the oligotrophic waters of the East Australian Current (EAC) extend southwards. The strength of the EAC is subject to substantial inter-annual variability, but summer conditions within the study region are nevertheless comparatively warmer, stratified and nutrient poor than winter conditions. Nitrate levels in North West Bay and the upper D'Entrecasteaux Channel (Jordan et al. 2002), Storm Bay (Clementson et al. 1989), and at the mouth of the Huon Estuary (HEST 2000) have been observed to show patterns indicative of these oceanographic processes, where nitrogen concentrations are high in winter and decrease throughout spring and summer.

Algal growth does not respond directly to these seasonal fluctuations in nutrient levels. Flushing rates, at a local scale, will govern the residence time of nutrients and hence the opportunity for these to be assimilated. The Huon Estuary Study Team (2000) reported that in winter the waters are largely biogeochemically inactive with large fluxes of dissolved inorganic nitrogen (DIN) of marine origin remaining unused as a consequence of high flushing rates, low light conditions and low temperatures. Throughout spring, as light and temperatures increase, algal growth increases, and becomes increasingly nitrogen limited. High inputs of nitrogen in spring and summer can result in algal blooms (Jordan et al. 2002). The biomass of algal epiphytes in south-eastern Tasmania is at its highest in February and March (Edgar 1983a).

2.2 Experimental design

2.2.1 Site selection

Potential sites were identified in ArcGIS 9.3 (ESRI) by overlaying the following factors on a digital coastline map for the Bruny Bioregion:

* marine farming leases currently licensed for finfish farming (supplied by DPIW)
* benthic marine habitats of the region displaying coastal subtidal reef (supplied by the habitat mapping section of Tasmanian Aquaculture and Fisheries Institute)
* foreshore pollution pressure (supplied by NRM South)
* an index of exposure calculated for possible samples sites

Data were analysed using the Mercator projection, Geocentric Datum of Australia 1994, and Map Grid of Australia zone 55.

In order to investigate the impacts of fish farming, sample sites were selected at different distances from fish farming lease areas. In ArcGIS, buffer zones were created around active fish farm lease areas at distances of; 100 m, 400 m, 2 km and 5 km (Figure 2-2). These distances were chosen because the effects of fish farms are likely to decrease along an exponential gradient with distance from fish farms. Possible sample sites for the 100 m, 400 m, and 2 km distance categories were identified throughout the channel where these distances intersected with subtidal reef habitat, and where the area did not have significant levels of foreshore pollution pressure from sources other than fish farms. Reference sites were positioned on coastal reef areas, without significant foreshore pollution pressure, at distances from fish farms of 5 kilometres or more.

An index of wave exposure was then calculated for possible sample sites, based on fetch, wind energy, and the influence of open-ocean swell at each site. Fetch distances (F) in 16 compass directions were calculated, from a grid based data layer of the coastline of 25 m grain size using a model developed by Burrows et al. (2008). Wind data was supplied by the Bureau of Meteorology. Wind data was sourced from the automated weather stations at Cape Bruny (for sites in the D'Entrecasteaux

channel) and Hobart Airport (for sites on the Tasman Peninsula). It consisted of averages of wind speed and direction over the period January 1995 to December 2007. Wind energy (*WE*) was calculated as the square of the average wind speed (in knots) for each sector multiplied by the proportion of time the wind blew in that sector (Burrows et al. 2008). The exposure index value for numerous possible sites was then calculated as the average of *WE* multiplied by *F* at each site, plus the addition of a constant for sites directly or indirectly exposed to open ocean swell, as in Barrett et al. (2001). For sites indirectly exposed to swell, this constant was set at the maximum exposure value for coasts affected only by local wind waves. For sites directly exposed to swell this constant was set at 1.5 times the maximum exposure value for coasts affected only by local wind waves. The distinction between swell exposed sites and sheltered (non swell exposed) sites was identified from exposure maps generated by Barrett et al. (2001), and provided a 2 level categorical version of the exposure index to be used for statistical tests.

In order to determine the effect of fish farming at different levels of exposure, sites ranging in wave exposure were included in each of the four distance categories (Figure 2-3). Sites were also spread throughout the study region as much as possible, in order to encompass the effect of fish farming over a regional scale (Figure 2-3). Selection of sample sites occurred through a process of elimination, with the aim to maximise these two variables (exposure range and spatial coverage) within each distance class. The selection of 100 m sites was limited by the availability of fish farms leases near a shoreline with reef. Choice of 5000 m reference sites was limited because of the density of fish farms within the D'Entrecasteaux area. Two reference sites were chosen at Southport, having similar aspect to those near fish farms in Port Esperance. Similarly, a reference site north of Sloping Main had similar exposure conditions to those in Wedge Bay. Ten priority sample sites were identified for each distance class, with alternative back-up sites also identified in case these were determined unsuitable when in the field.

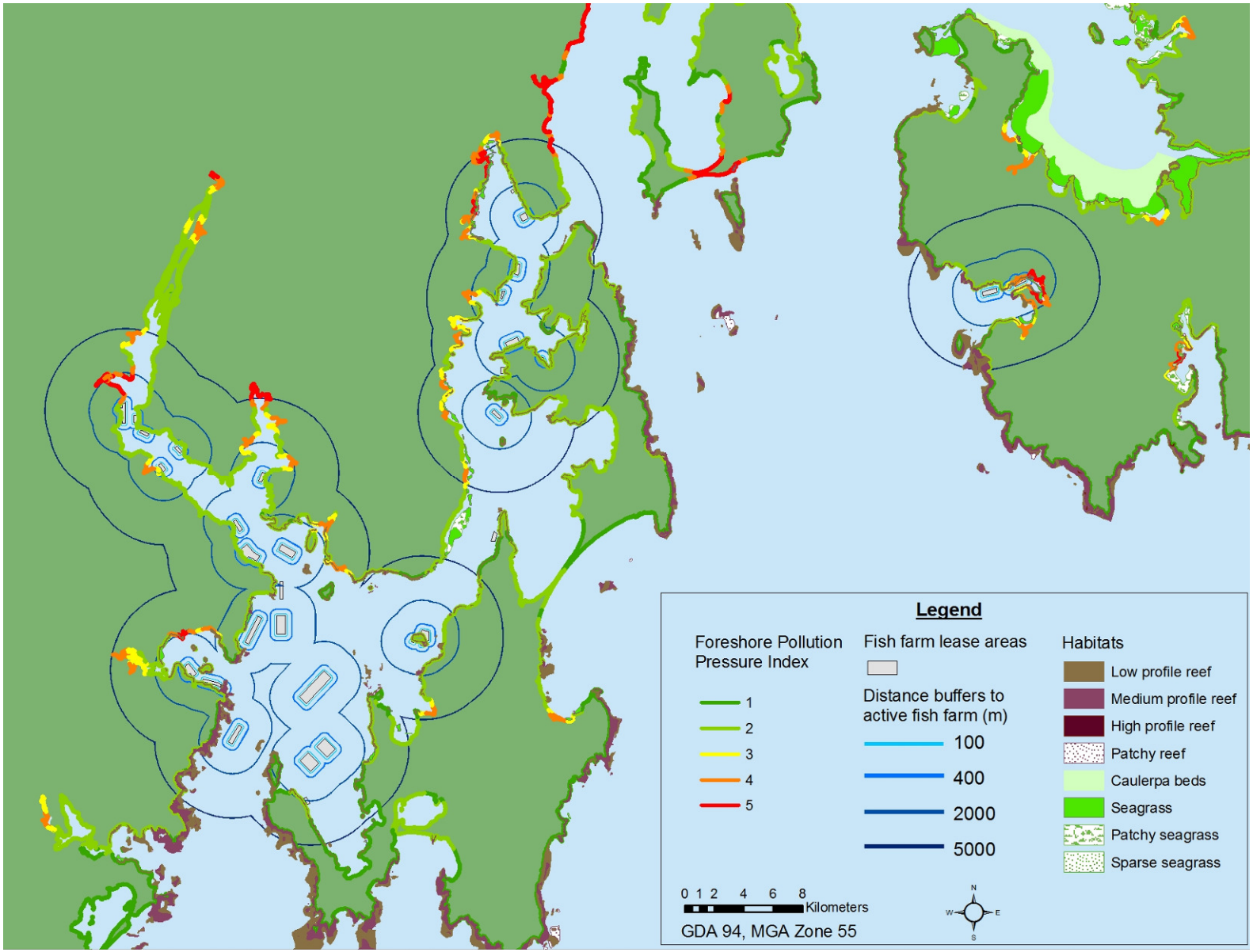

Figure 2-2 Distribution of subtidal habitats, foreshore pollution, and fish farm leases throughout the study region. Buffers around fish farm leases represent radius distances of 100 m, 400 m, 2000 m , and 5000 m. Foreshore pollution index as defined by Migus (2008): 1=no pressure, 2=slight pressure, 3=moderate pressure, 4=heavy pressure, and 5=extreme pressure from pollution. **NB.** "Likely aquaculture run-off" is responsible for high values in Parsons Inlet (Wedge Bay).

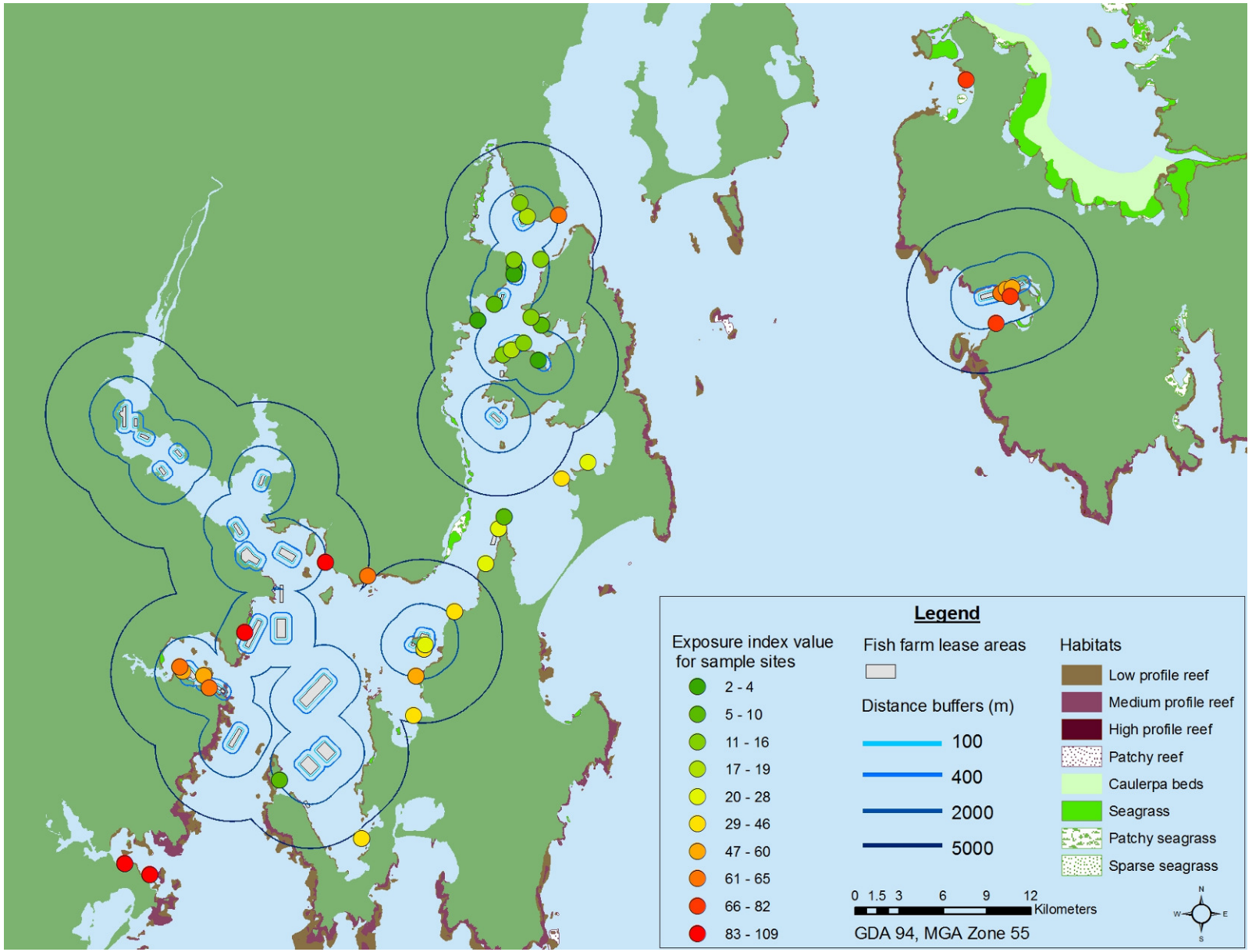

Figure 2-3 Relationship between sites sampled, exposure, fish farm leases and the distribution of subtidal reef habitats in the study region.

2.3 Data Collection

2.3.1 Species Identification

Macroalgae were identified in the water to the highest possible taxonomic resolution. In cases where macroalgae could not be identified to species *in situ*, they were grouped at generic level or into a functional group. For example filamentous algae were grouped into red, green and brown groups. Despite this coarse level of identification, the majority of filamentous green algae are likely to be *Cladophora* spp., the majority of filamentous browns are likely to be *Hinksia* spp., and the majority of reds are likely to be *Polysiphonia* spp. The coverage of benthic sessile invertebrates such as sponges, bryozoans, cnidarians and ascidians were also recorded.

2.3.2 Photographic quadrats

A total of 73 photo quadrat transect samples, from 44 sites, were collected throughout the D'Entrecasteaux channel and around Nubeena. GPS coordinates extracted from ArcGIS were used to locate predetermined field sites. Each site was first scoped with a depth sounder to determine the reef depth and extent. If the site was determined unsuitable due to a lack of reef, an alternative *a priori* identified site was sampled instead. Sampling was conducted between 17 November and 17 December, 2008.

As the effect of fish farms on macroalgal composition may vary with depth, sites were sampled at two depths (2 m and 5 m) where the reef extent was large enough. A transect tape of 50 m length was laid out along the reef following a contour line of 2 m depth. Photographs encompassing 50 cm of transect tape (approximately 0.25 cm^2 quadrat size) were taken at every 5 m interval (n = 10 photos per transect). This process was repeated along the 5 m contour at sites where the reef extended to this depth. Photographs were taken with an 8 mega-pixel digital Olympus camera, with a 28mm wide angle lens and strobe.

2.3.3 Manual quadrats

To compare the use of the 'manual-quadrat' sampling technique with the use of 'photo-quadrats', manual quadrat samples were also conducted at a subset of field sites. A total of 36 manual quadrat transects were conducted from 35 sites. Manual quadrat samples were collected along one (or both) of the transect lines used for the photo quadrat samples at 5 m intervals (n=10 per transect), using a 0.25 cm^2 quadrat with a grid of 7 wires crossing perpendicularly. Within each quadrat, the species occurring under 50 grid positions (one corner of the quadrat plus 49 intersection points) were recorded (Figure 2-4). Macroalgal species from all layers, encrusting to understorey to canopy, were recorded under each position. Time constraints on the dive meant that abundant species were estimated to some degree in most quadrats, i.e. if a species covered about half the quadrat, 25 points were recorded.

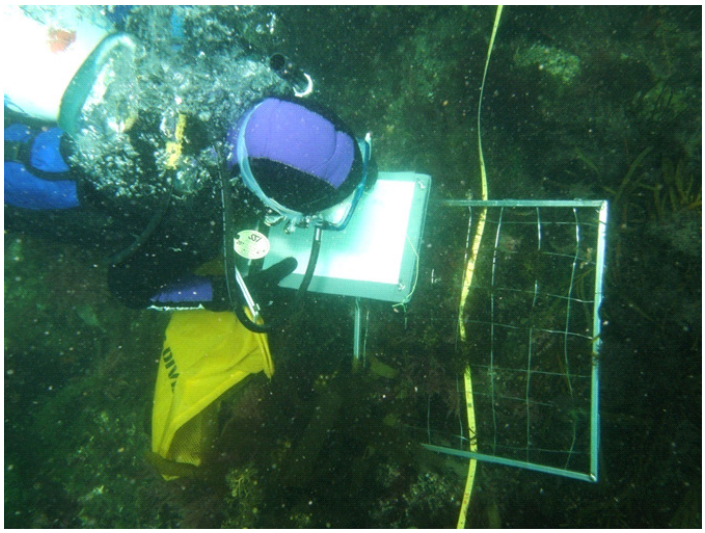

Figure 2-4 Manual quadrat sampling technique

2.4 Data Analyses

2.4.1 Photographic quadrats

Photos were cropped and adjusted for brightness and contrast before being imported into CPCe (Coral Point Count with excel extension) for analysis. The process was a point count based method where points are overlayed over an image and the species beneath each point visually identified and recorded to a database (Kohler & Gill 2006). For each image a grid of 56 points, was overlayed with an image border of

100 pixels. Points where the underlying algae could not be identified to species were lumped into a higher category, such as '*Sargassum* sp.', or 'foliose red algae'. For the instances where the area was in shadow, too blurry or covered by transect line, 'shadow' was recorded.

Results for each transect were then exported to Microsoft Excel 2007. Percentage coverage (per transect) was calculated for each cover type. Adjustments were made so that obstruction by ephemeral epiphytic algae did not overly bias the coverage estimate for the more permanent underlying algal community. Percentage data for the underlying community was calculated as:

> Percentage (*i*) = points covered by type *i* * 100 / (total number of points - the points attributed to ephemeral coverage – the points in shadow)

Percentage data for ephemeral species was calculated as:

> Percentage (*i*) = points covered by type *i* * 100 / (total number of points – the points in shadow)

2.4.2 Statistical Analysis

2.4.2.1 Comparing manual quadrats with photo quadrats

Data from the 36 manual quadrat (MQ) transect samples were paired with the appropriate subset of data from the photographic quadrat (PQ) transect samples. This dataset was analysed on a compositional, multivariate basis to compare the community information gathered by different methods. Bray-Curtis similarity matrices of the square root data for MQ and PQ methods were generated. The correlation between these matrices was tested in Primer 6+ using the 2STAGE function, selecting the Spearman rank correlation option. Non-metric MDS plots were also generated for MQ and PQ data sets in Primer 6+ to visualise how each method described the relationship between sites.

Bray-Curtis similarities were calculated for the MQ and PQ data at each site. These values were treated as the response variable in a general linear model based on the categorical factors of exposure (2 levels: swell exposed, non swell exposed), depth (2

levels: 2 m and 5 m) and distance (4 levels: 100 m, 400 m, 2000 m, and 5000 m), in order to see whether the similarity between PQ and MQ methods varied over these factors. Results should be interpreted with caution as interaction terms could not be included, hence assumed non-significant, due to the sample size being too small to provide appropriate replication for a full model.

Algal species were then organised into groups according to their height dominance on the reef, so that the methods could be compared for each algal layer. Six layer categories were generated:

- Upper canopy: canopy species that grow tall/erect, e.g. *Macrocystis pyrifera,* and *Sargassum fallax* (seasonally forming a tall canopy in sheltered sites) These may be captured differently in photo quadrats than canopy species which spread out over other algal layers
- Lower canopy: other canopy layers, e.g. *E. radiata, P. comosa, D. potatorum*
- Middle storey: includes most foliose red algae, and species such *Carpoglossum confluens,*
- Under storey: prostrate growing plants such as *Sonderapelta coriacea*, *Homeostrichus olsenii*, and *Caulerpa* beds
- Encrusting: encrusting algae, sponges, and cnidarians including: crustose coralline algae, encrusting *Peyssonnelia* spp., and the octocoral *Erythropodium hicksoni*

To investigate the agreement between the sampling methods at each algal layer, the percentage cover estimate of each algal layer, was calculated for MQ and PQ data for each sample. Shannon diversity (H') and Margalef species richness (d) variables were also calculated using the diverse function in Primer 6+. The differences between these estimates (MQ – PQ) were tested using pair-wise t-tests in Minitab 15. It was also of interest to see whether these differences changed over different levels of exposure, distance and depth, i.e. whether the trends detected over exposure, distance and depth were similar using both methods. This was tested using general linear modelling for normally distributed variables and Kruskall Wallis tests for

those without a normal distribution. Again, results should be interpreted with caution as interaction terms could not be included, due to the sample size being too small to provide appropriate replication for a full model.

Finally, to test the use of photo-quadrats in detecting the impact of fish farming on macroalgal assemblages, the estimates of components of variation were compared from PERMANOVA analyses (see section 2.4.2.2) conducted on each multivariate data set. The components of variation estimate the importance of each term in explaining the overall variation in dataset (Anderson et al. 2008), and thus can be used to compare the relative success of photo-quadrats in detecting the effect of each factor. The components of variation are analogous to the *sums of squared fixed effects* (divided by the appropriate degrees of freedom) in a univariate AVOVA, for fixed terms (Anderson et al. 2008). Factors included in the model were exposure, depth and distance (all fixed).

2.4.2.2 Community composition

Using the full dataset collected by photo-quadrats (N = 72), the percentage abundance data of macroalgae and sessile invertebrates on each transect sample was square root transformed for multivariate procedures so that analysis was not overly biased towards the dominant species but still relied on variations in abundance. All multivariate tests based on resemblance matrices of the species variables used Bray-Curtis as the distance measure. Exposure was included in categorical tests by separating swell-exposed sites from non-swell exposed sites, creating a two-level factor.

Multivariate ordination procedures were conducted in Primer 6+ (Primer-E 2008) to visualise the distance between sites according to their benthic community composition. Both non-metric multidimensional scaling (MDS), and principal coordinates analysis (PCO) ordinations were examined for 2D and 3D solutions. Like principal components analysis (PCA), PCO ordinations project sample points onto axes that minimises residual variation, however unlike PCA, PCO can be based on a resemblance matrix using any type of distance measure. This produces an

alternative ordination procedure to non-metric MDS, which sets an *a priori* number of axes, and preserves the rank order of inter-point dissimilarities, rather than the dissimilarity values themselves. Ordination plots were explored for patterns and clusters, with the use of data labels and vector overlays. Vectors were produced in Primer using Pearson correlation of graph axes and the independent variables of distance, exposure and depth (these variables were first normalised).

To test the null hypothesis that community composition was not significantly different between samples with different attributes, a PERMANOVA test was conducted using the PERMANOVA+ extension in Primer 6 (Primer-E 2008). PERMANOVA is a multivariate analogue of analysis of variance, which can be based on any distance matrix, and uses permutation methods to calculate significance values (Anderson 2001). In this case the model included the fixed categorical factors of depth, distance and the exposure index (swell-exposed versus non swell-exposed), and all interaction terms. Calculation of the Pseudo-F ratio and P value (α=0.05) was based on 999 permutations of the residuals under a reduced model. The components of variation attributed to each factor were displayed, which explain the relative importance of different terms in the model towards explaining the overall variation (Anderson et al. 2008, p. 54). The calculation of components of variation in PERMANOVA can give negative values to insignificant terms in the model (Anderson et al., 2008). Terms with negative estimates of components of variation were consecutively pooled with the residuals starting with the one with the smallest MS, as suggested in Anderson et al. (2008). A restricted set of appropriate *a posteriori* pair-wise tests was also conducted if the term distance or its interaction with another term was found to be significant. First, 100 m sites were tested against 5000 m reference sites, to test for a significant difference. If this test was significant then 400 m sites were tested against 5000 m sites, and so on. This was done separately at the two levels of depth and/or exposure if the interaction terms were significant. This method acknowledged the use of 5000 m sites as reference sites and the order of the distance scale used (instead of assuming distance values were unrelated categories), and reduced the chance of a 'Type I error' occurring, as pair-wise comparisons for interaction effects otherwise involved 12 tests unadjusted for Type I error (in contrast to using other univariate pair-wise tests such as Tukey's test).

In order to identify taxa that were correlated with the effect of distance, a constrained ordination procedure was conducted using the CAP function in PERMANOVA+ extension (Primer-E 2008). The CAP procedure involved a canonical discriminant analysis (between distance groups) on the PCO axes. The CAP axes are fitted through the multivariate data cloud to best discriminate between predefined groups. Diagnostics are conducted by permutation, using two test statistics (trace and largest root), and cross-validation of groups by the "leave one out" procedure. Species variables were then correlated with the CAP axis using Pearsons Correlation Coefficient.

2.4.2.3 Nutrient pollution indicators

Following earlier authors (Steneck & Dethier 1994; Krause-Jensen et al. 2007b; Juanes et al. 2008), species were separated into categories representing their functional growth habits. 'Opportunistic greens' were green algal species that respond to elevated nutrients with rapid growth. This class included *Chaetomorpha* spp. (Juanes et al. 2008), *Cladophora* spp. (including filamentous green algae) (Juanes et al. 2008), *Enteromorpha* spp. (Munda 1993), and *Ulva* spp (Munda 1993). 'Opportunistic total' were the opportunistic greens, brown filamentous algae, red filamentous algae (including *Ceramium* spp), and algal turf (Gorgula & Connell 2004; Juanes et al. 2008). 'Epiphytic species' included *Chaetomorpha billardierii,* filamentous algae, *Colpomenia* spp. and *Asparagopsis armata*. 'Canopy brown' species were identified as those perennial brown algae which form a canopy over the mid-storey, under-storey and encrusting species. Individual species which may respond negatively to pollution have not been identified *a priori* from other studies as these are likely to be perennial 'competitive' algae (Littler & Littler 1980; Krause-Jensen et al. 2007b) which vary by region.

The affect of pollution on the diversity and richness of macroalgae communities is also well cited (Munda 1993; Ducrotoy 1999; Wells et al. 2007; Guinda et al. 2008; Juanes et al. 2008). Shannon diversity (H') and Margalef species richness (d) variables were calculated using the diverse function in Primer 6+.

Indicator variables and species categories that were identified *a priori* from the literature as likely to respond to nutrification or pollution were grouped and analysed

using univariate tests. Indicators were only tested individually where they had a high rate of occurrence amongst the samples (occurring >19 samples). General linear models were performed in Minitab15, using distance, depth, and exposure categories, and all interaction factors. Variables were transformed and tested for normality and heteroskedacity using the Ryan-Joiner test and model diagnostics. Tukeys pairwise tests were used to determine which classes were significantly different from each other.

The spatial distribution patterns of significant indicator variables throughout the study area were also investigated. This was done by labelling each sample site with the percentage cover of the indicator variables, using ArcGIS 9.3. This was done separately for 2 m and 5 m depths.

Chapter 3 Results – Comparison of sampling techniques

3.1 Relationship between sites

A comparison of data gained from a subset of 34 sites indicated that both manual quadrat and photographic quadrat data distinguished sites from one another in a similar pattern. There was a high correlation between MQ and PQ resemblance matrices for macroalgal community data (Spearman correlation = 0.86377). MDS ordinations of MQ data (Figure 3-1) and PQ data (Figure 3-2) show a similar relationship between sample sites for a 2D solution. The placement of samples along MDS axis 1 on the MQ plot was highly correlated with that on the PQ plot (Pearson correlation = – 0.955, $P < 0.001$). Similarly, sample coefficients on MDS axis 2 were highly correlated between the two methods (Pearson correlation = 0.701, $P < 0.001$).

The resemblance between PQ and MQ compositional data at each site did not differ significantly with the factors of distance ($F = 1.24$, $P = 0.321$) and depth ($F = 0.01$ $P = 0.919$), but did differ significantly with exposure ($F = 10.77$, $P = 0.004$). Pair-wise tests showed that PQ and MQ data was more similar at sheltered sites than swell-exposed sites (difference in means (exposure 2 – exposure 1) = –10.93, $T = –3.281$, $P = 0.0036$). Two-way interaction factors were all non-significant (distance*exposure: $F = 0.38$, $P = 0.766$, depth*exposure: $F = 3.95$, $P = 0.060$, distance*depth: $F = 2.14$, $P = 0.125$).

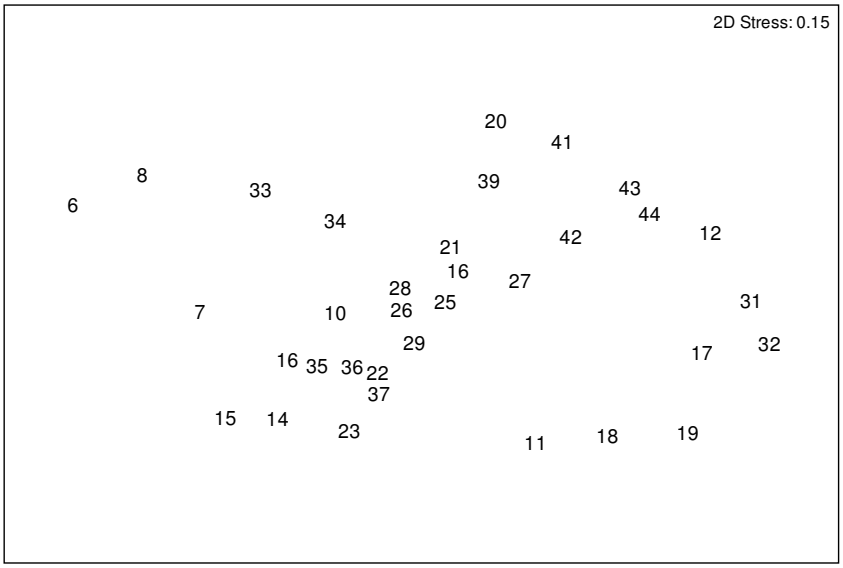

Figure 3-1 The 2D MDS solution for the manual quadrat data. Square-root transformed data and Bray Curtis distance measure were used. Data labels represent site numbers for each sample.

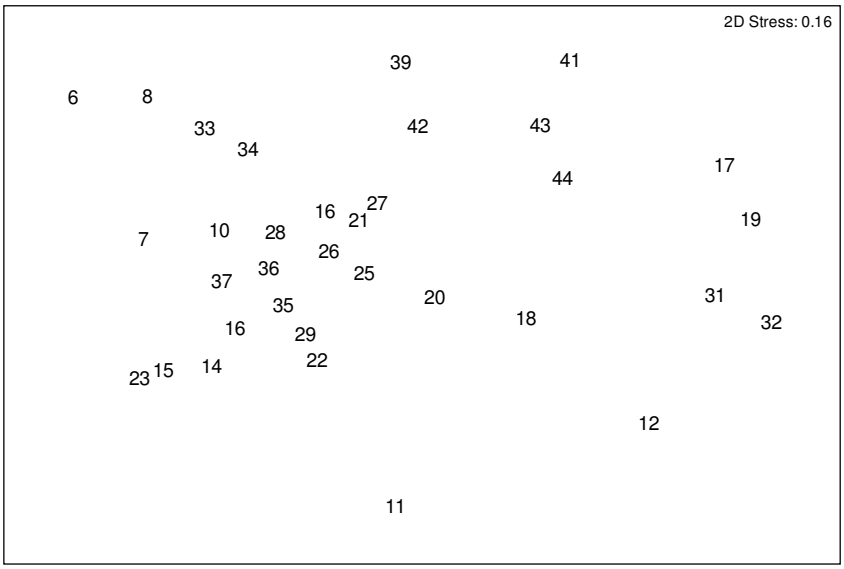

Figure 3-2 The 2D MDS solution for the photographic quadrat data. Square-root transformed data and Bray Curtis distance measure were used. Data labels represent the site number for each sample.

3.2 Algal community structure

The average cover of epiphytes, lower canopy and middle storey algae was very similar using MQ and PQ methods (Table 3-1). Photo-quadrats estimated a significantly higher cover for upper canopy than manual quadrats. The difference between methods was most pronounced for understorey and encrusting layers, where photographic quadrats detected significantly less coverage than manual quadrats (Table 3-1). MQ sampling detected significantly more total algal cover, and slightly more algal richness, and diversity. Standard error values for each variable were similar using MQ and PQ methods.

Table 3-1 Average percent cover of algal layers, total algal cover, richness and diversity measured using photographic quadrats and manual quadrats

Variable	Method	Average % cover	Standard Error	Standard Deviation	T-value	P-value
epiphyte	MQ	39.1	5.24	30.6	-1.17	0.252
	PQ	41.4	4.44	25.9		
	difference	*-2.3*				
upper canopy	MQ	29.0	3.90	22.7	**-3.02**	**0.005**
	PQ	35.6	4.06	23.7		
	difference	*-6.6*				
lower canopy	MQ	7.77	1.96	11.4	-0.40	0.693
	PQ	8.02	1.98	11.6		
	difference	*-0.25*				
mid-storey	MQ	16.7	2.80	16.3	1.73	0.094
	PQ	14.1	2.43	14.2		
	difference	*2.7*				
under-storey	MQ	52.8	4.40	25.7	**2.99**	**0.005**
	PQ	37.9	4.86	28.3		
	difference	*14.9*				
encrusting	MQ	21.3	4.15	24.2	**4.62**	**0.000**
	PQ	3.65	1.52	8.88		
	difference	*17.6*				
total algal cover	MQ	166.8	5.86	34.2	**3.37**	**0.002**
	PQ	140.7	4.38	25.6		
	difference	*26.1*				
richness	MQ	5.22	0.24	1.39	**2.46**	**0.017**
	PQ	4.75	0.32	1.86		
	difference	*0.47*				
diversity	MQ	2.72	0.07	0.395	**4.81**	**0.000**
	PQ	2.52	0.07	0.411		
	difference	*0.20*				

Variation in algal structure, richness and diversity over distance were similar for both methods (Figure 3-3). The difference between methods over distance did not vary significantly for any of the variables, according to general linear models and Kruskal-Wallis tests (Appendix 1). However there appeared to be a tendency for photographic sampling to disproportionately overestimate upper-canopy cover when epiphytic cover was high (100 m sites). Additionally, manual quadrats detected a slight increase in diversity and richness at reference sites (where lower-canopy cover was high), but this was not apparent using photo-quadrats. For encrusting algae, the difference between photo-quadrat data and manual quadrat data was larger at 100 m sites and reference sites. Manual sampling also detected a pattern of increased cover in under-storey algae at distances more than 100 m from fish farms, whilst photo-quadrats did not. Trends were ambiguous for middle-storey algae, which varied around 15 % cover across distance categories. Patterns detected over distance were relatively similar using both methods for epiphytes and lower canopy.

Trends in algal structure, diversity and richness over the exposure and depth categories were also similar using both methods (Figure 3-4 and Figure 3-5). However, for under-storey and encrusting algae, the differences between methods were significantly greater at exposed sites than sheltered sites, for which PQs estimated very small percentage covers. Agreement between the methods also seemed to be slightly more compromised at shallow depths where PQs recorded comparatively higher upper-canopy and epiphytic algae, and underestimated understorey algae more than at 5 m depths. However general linear modelling did not indicate these differences to be statistically significant. Encrusting algae was heavily underestimated using photo-quadrat methods at both 2 m and 5 m depths.

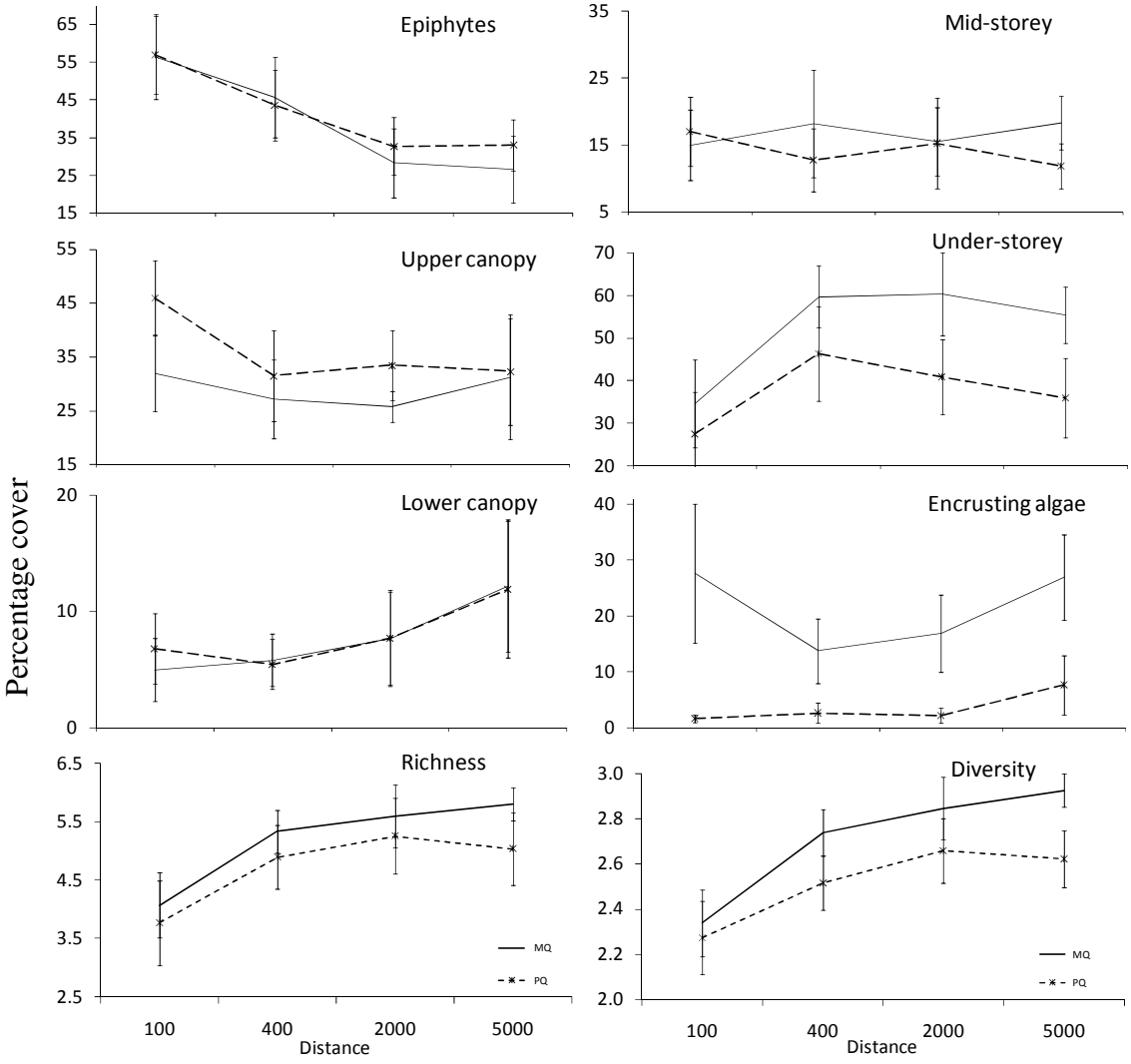

Figure 3-3 Variation in percentage cover of algal layers, richness and diversity with distance using photo-quadrat methods and manual quadrat methods. Differences between methods in trends over exposure were tested in a general linear model including exposure, depth and distance or using Kruskal-Wallis tests for variables without a normal distribution.

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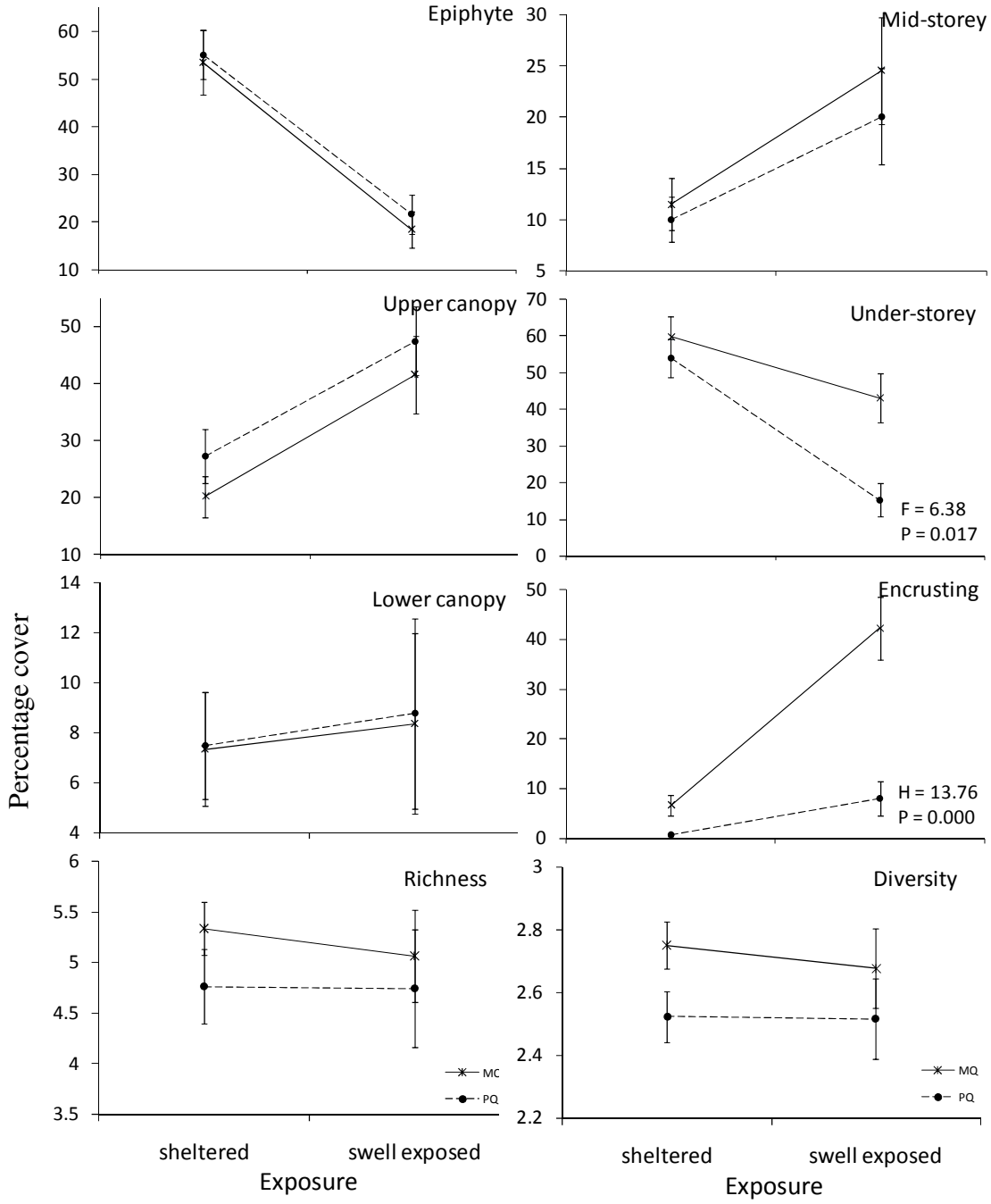

Figure 3-4 Variation in percentage cover of algal layers, richness and diversity with exposure using photo-quadrat methods and manual quadrat methods. Differences between methods in trends over exposure were tested in a general linear model including exposure, depth and distance or using Kruskal-Wallis tests for variables without a normal distribution.

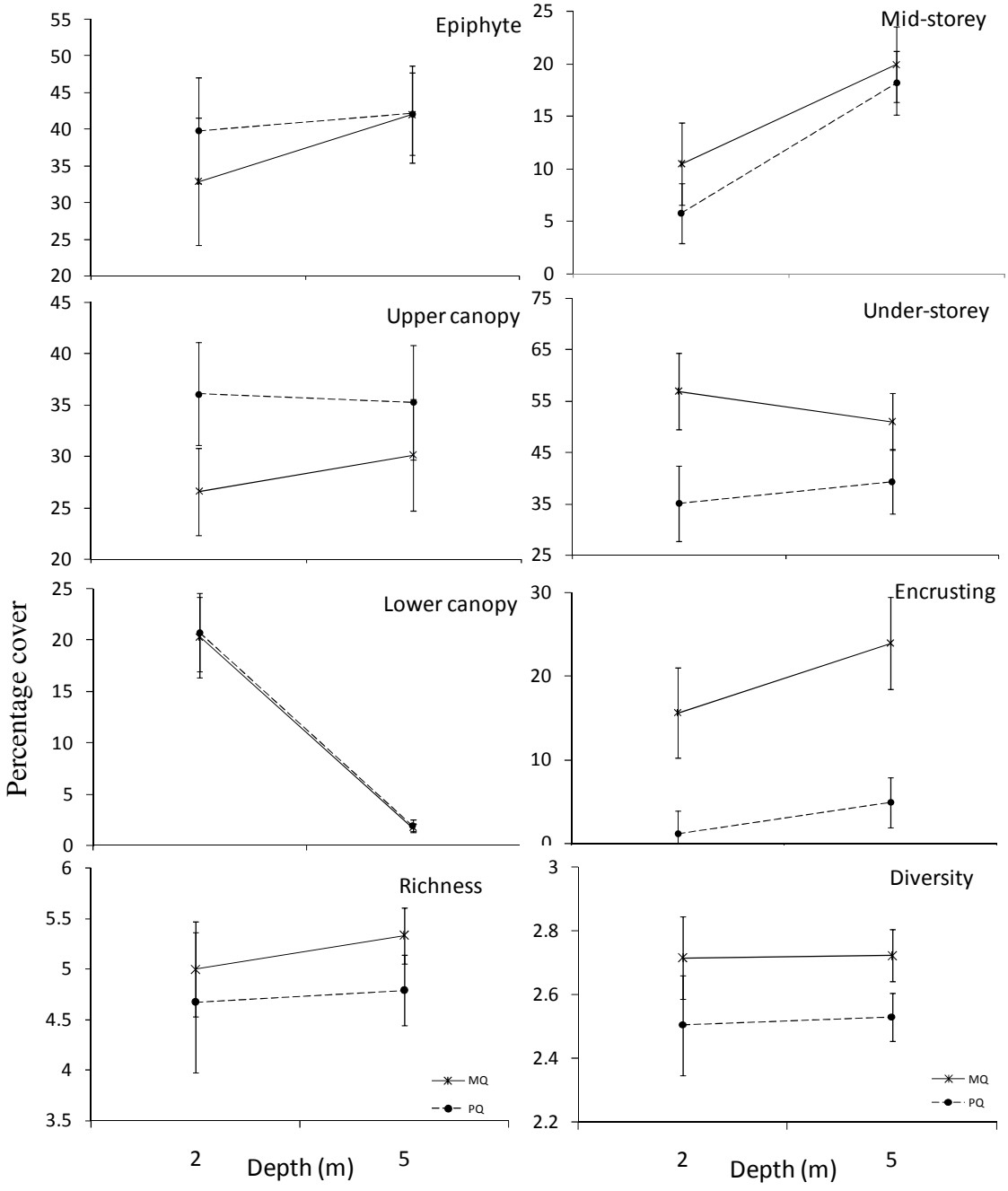

Figure 3-5 Variation in percentage cover of algal layers, richness and diversity with depth using photo-quadrat methods and manual quadrat methods. Differences between methods tested were tested in a general linear model including exposure, depth and distance.

3.3 Detecting distance effects

The components of variation in PERMANOVA indicated that the two methods attributed a similar amount of variation in the data to each of the model factors and their interaction terms (Table 3-2). The difference between the components of variation was greatest for the factor *distance*. Components of variation derived from the PERMANOVA on the manual quadrat data attributed 14.9 % of the variation in the data to the factor of distance. Comparatively, the photographic method picked up a weaker signal for distance (10.7 %). The difference between methods for the interaction of exposure and distance was comparatively small. Photographic methods also attributed a slightly smaller amount of variation to the exposure and depth interaction. Slightly more of the data was explained by the PERMANOVA model when manual quadrat data was used, as the component of variation for the residual term is slightly smaller.

Table 3-2 Comparison of the estimates of components of variation given by PERMANOVA using manual quadrat data and photographic quadrat data.

Estimates of components of variation for MQ data			*Estimates of components of variation for PQ data*	
Source	Estimated component of variation	**Square root**	Estimated component of variation	**Square root**
S(exposure)	799.0	**28.27**	907.9	**30.13**
S(depth)	321.4	**17.93**	349.5	**18.70**
S(distance)	221.5	**14.88**	114.4	**10.70**
S(exposure x depth)	284.0	**16.85**	194.1	**13.93**
S(exposure x distance)	172.8	**13.15**	196.9	**14.03**
S(depth x distance)	195.5	**13.98**	181.1	**13.46**
*S(exp x depth x dist)***	222.0	**14.90**	388.2	**19.70**
V(Residuals)	1349	**36.73**	1481	**38.49**
Total				
** Term has one or more empty cells				

Chapter 4 Results - The effect of fish farms

4.1 Community composition

A total of 120 taxa were identified from 73 samples in the photo-quadrat analysis. Community composition was patterned over an exposure gradient, with a clear distinction between swell-affected sites and non-swell-affected sites (Figure 4-1).

The effect of distance was independent from that of exposure (Figure 4-1 and 4-2). Both the continuous and categorical variables for exposure achieved a high correlation with PCO axis one, which explained 31.6 % of the variance (Table 4-1). Distance had a higher correlation with PCO axis 2 which explained 11.6% of the variance in the data. The distribution of samples from each distance class along PCO axis 2 appears different at different levels of exposure, indicating an interaction effect. An interaction term between the two variables achieved a high correlation with the first two axes.

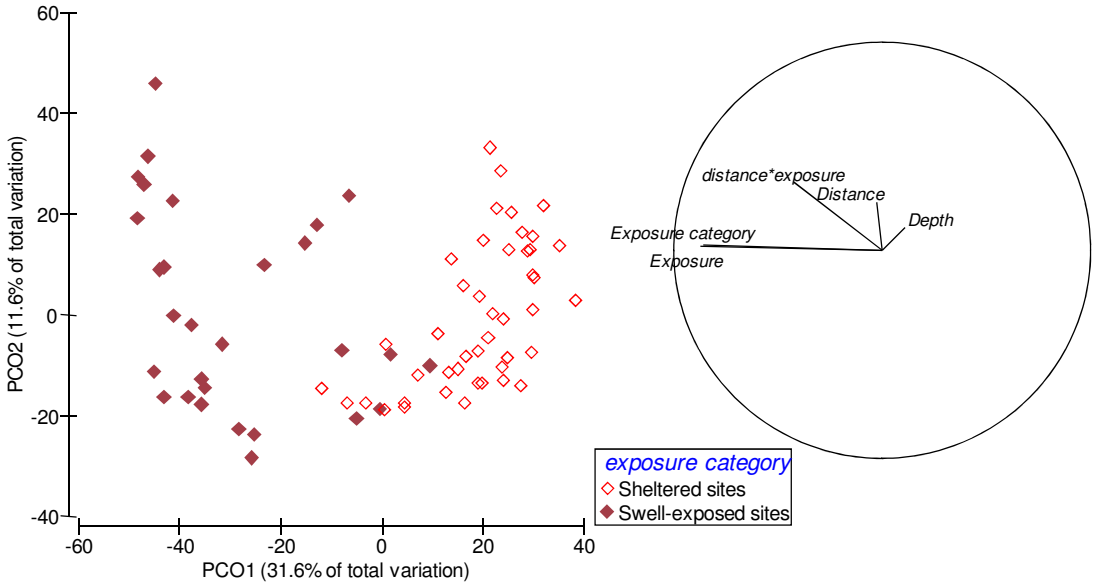

Figure 4-1 PCO ordination showing exposure categories. Ordination is based on Bray Curtis similarity matrix of square root data. Fitted environmental vectors based on Pearson correlation. The circle represents perfect correlation of 1

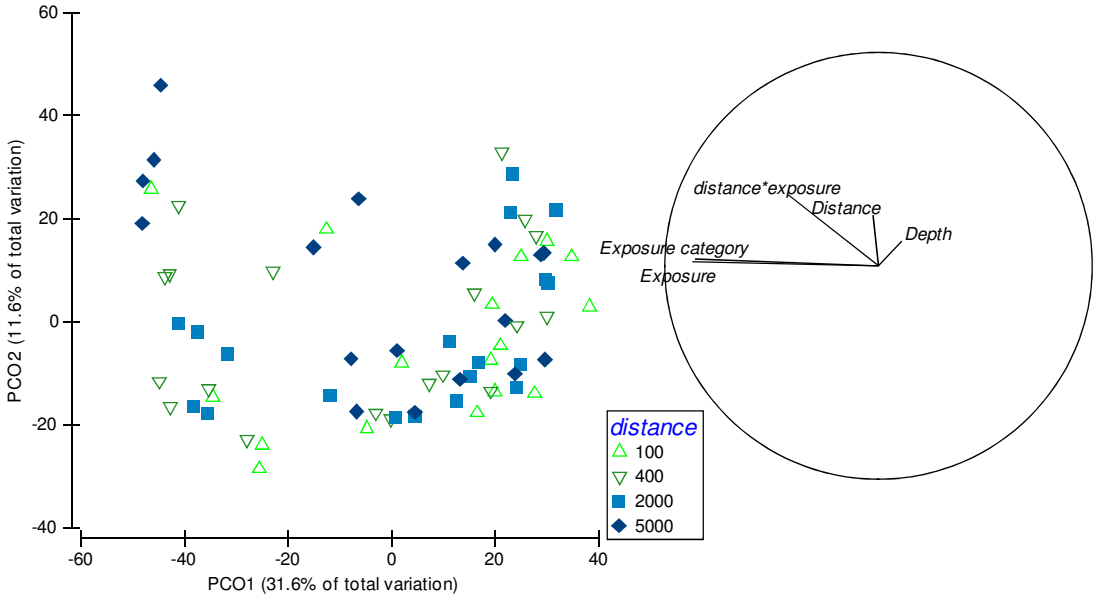

Figure 4-2 PCO ordination showing distance categories. Ordination is based on Bray Curtis similarity matrix of square root data. Fitted environmental vectors based on Pearson correlation. The circle represents perfect correlation of 1.

	Distance	Depth	Exposure	Exposure category	Distance* Exposure
PCO AXIS1 (31.6% of total variation)	-0.0282	0.1079	-0.8708	-0.8622	-0.4262
PCO AXIS2 (11.6% of total variation)	0.2301	0.1106	0.0218	0.0301	0.3284
PCO AXIS3 (9% of total variation)	0.1322	0.1854	-0.0109	-0.1174	0.1290

Table 4-1 Pearson correlation values for environmental variables with the first three PCO axis

The PERMANOVA analysis (Table 4-2) revealed significant effects for the factors: exposure (*Pseudo-F*=25.70, *P*=0.001), distance (*Pseudo-F*=2.41, *P*=0.001), depth (*Pseudo-F*=2.70, *P*=0.001), and the interaction factors: exposure by distance (*Pseudo-F*=2.41, *P*=0.001), and exposure by depth (*Pseudo-F*=2.46, *P*=0.001). The components of variation attributed to each factor revealed that exposure explained the most variation within the data (34.8%), followed by the interaction factor exposure by distance (16.4%). Distance alone explained a further 11.6%.

Pair-wise comparisons for the interaction factor of distance and exposure showed that macroalgal composition at 100 m sites was clearly different from 5000 m reference sites in both exposed and sheltered sites (Table 4-3). Sites 400 m metres away from fish farms were not significantly different from sites 5000 m away from fish farms.

Table 4-2 Table of results, and estimates of components of variation for PERMANOVA on square-root species abundance data and Bray-Curtis distance matrix. *SS and degrees of freedom for terms *depth x distance* and *exposure x depth x distance* were pooled with the residuals as they had negative estimates of components of variation.

PERMANOVA table of results							***Estimates of components of variation***	
Source	**Degrees of freedom**	**Sum of squares**	**Mean Square**	**Pseudo-F ratio**	**P value (by permutation)**	**Unique permutations**	**Estimate**	**Square root**
exposure	1	40993	40993	25.699	0.001	998	1210.2	34.79
depth	1	4307.1	4307.1	2.7002	0.001	999	80.009	8.945
distance	3	11544	3848.1	2.4124	0.001	997	134.75	11.61
exposure x depth	1	3930.7	3930.7	2.4642	0.004	998	137.81	11.74
exposure x distance	3	11517	3838.9	2.4067	0.001	997	268.4	16.38
*Pooled residuals**	63	100000	1595.1				1595.1	39.94
Total	72	172000						

Table 4-3 Pariwise comparisons for distance groups within sheltered sites and swell exposed sites. Tested using PERMANOVA with 9999 permutions. All results obtained with >9921 unique permutations. NB these are not adjusted for Type 1 error.

Exposure	Distance groups compared	t-value	P-value
Sheltered	100, 5000	1.704	0.002
Swell exposed	100, 5000	1.596	0.028
Sheltered	400, 5000	1.213	0.165
Swell exposed	400, 5000	1.291	0.113

A CAP analysis revealed significant differences between distance groups by permutation tests (*Trace statistic* = 0.74883, P = 0.0004, and *First squared canonical correlation* = 0.46613, *P* = 0.0005) under 9999 permutations. The best separation among groups along the distance continuum was achieved along CAP axis 1 (Figure 4-3). Correlation of the species variables with CAP axis1 indicated that *Chaetomorpha billardierii, Ulva* spp. and *Chaetomorpha coliformis* decreased with increasing distance from fish farms (Table 4-4). Species that increased in abundance with increasing distance were all red algae, mainly consisting of understorey and mid-storey species.

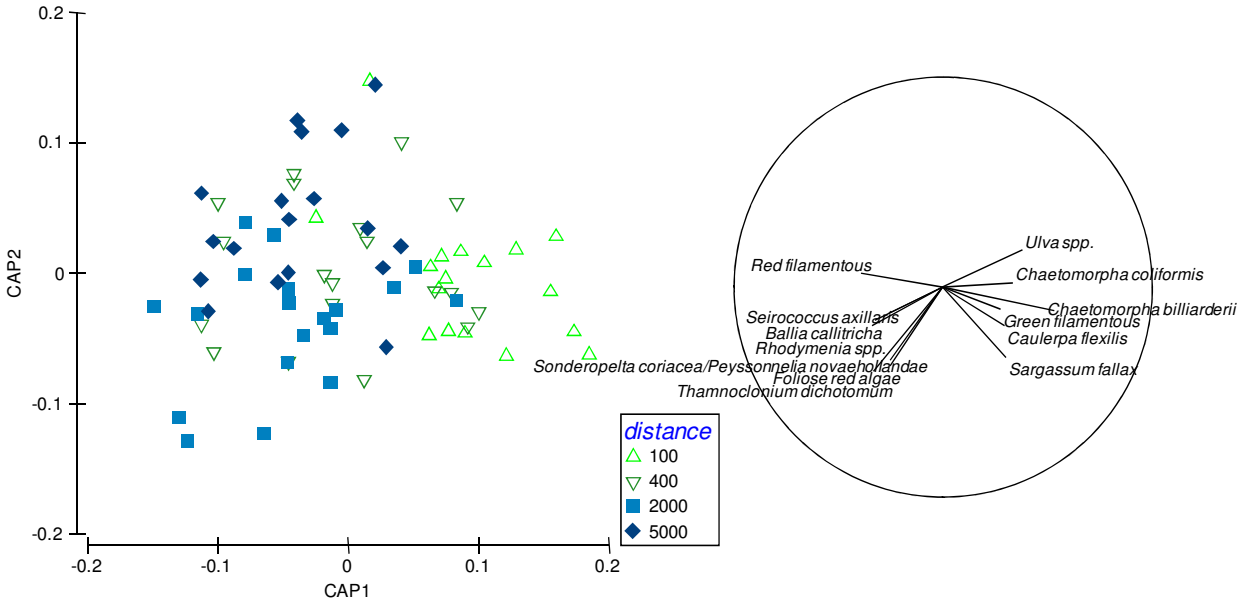

Figure 4-3 a) CAP ordination from a discriminant analysis by distance, using a Bray-Curtis matrix of square root species abundance data. b) Fitted vectors of species variables correlating with CAP axis 1 (Pearsons correlation coefficient > 0.25)

Table 4-4 Species variable correlations with CAP axis 1. Only correlations over 0.25 using Pearson correlation are shown.

Species Variable	Pearson correlation with CAP axis 1
Chaetomorpha billardierii	0.53
Ulva spp.	0.38
Chaetomorpha coliformis	0.33
Sargassum fallax	0.30
Caulerpa flexilis	0.29
Green filamentous	0.27
Foliose red algae	-0.26
Sonderopelta coriacea/Peyssonnelia novaehollandae	-0.26
Seirococcus axillaris	-0.26
Rhodymenia spp.	-0.33
Thamnoclonium dichotomum	-0.33
Ballia callitricha	-0.34
Red filamentous	-0.39

The 'leave one out' allocation procedure in the CAP analysis showed that sites 100 m from fish farms shared the most consistent macroalgal community composition, achieving correct classification for 76.5% of the samples in that group (Table 4-5). Sites from other distances, particularly 400 m sites, were much more variable. Misclassification of 400 m sites into 100 m categories occurred in 5 cases. Misclassification of 400 m sites into 2000 m sites and 5000 m sites occurred 5 and 6 times respectively. Sites 2000 m and 5000 m from fish farms had low misclassification rates for the 100 m group. This indicates, as in the PERMANOVA pairwise tests, they were particularly distinct from the 100 m sites.

Table 4-5 Leave-one-out allocation of observations to groups, for the choice of m=9

	Classified					
Original group	100	400	2000	5000	Total	%correct
100	13	1	1	2	17	76.5
400	5	4	4	6	19	21.1
2000	2	4	9	4	19	47.4
5000	0	6	4	8	18	44.4

4.2 Nutrient Pollution Indicators

All of the groups identified *a priori* as potential nutrient indicators had high frequencies of occurrence amongst the transect samples (Table 4-6). Canopy brown alga dominated most sites, and covered an average of 46.06% of each transect. Filamentous brown algae and algal turf were also widespread and abundant on many transects. The most abundant species of the genus *Chaetomorpha* was *C. billardierii* an epiphyte, but *C. coliformis* was also present. The high cover of filamentous brown algae meant that this taxon was the dominant component of the opportunistic algae indicator group.

Table 4-6 Average abundance and occurrence of indicator species groups over the 73 transects

Taxon	Average percentage abundance	Occurrence (number of transects present)
Canopy Brown algae	46.06	69
Filamentous green algae	6.70	48
Filamentous brown algae	24.08	60
Filamentous red algae	3.11	55
Chaetomorpha spp.	2.73	25
Ulva spp.	0.79	24
Algal turf	11.58	69

General linear models indicated that all groups responded significantly to exposure, or returned a significant interaction between exposure and distance (Table 4-7). Canopy brown alga, *Chaetomorpha* spp. and *Ulva* spp. increased with increasing exposure. Filamentous species, opportunistic species in total, and algal turf, decreased with increasing exposure (Appendix 4).

Box plots of indicator species and groups indicated several trends in abundance over the four distance categories (Figure 4-4). General linear models revealed that the abundance of total opportunistic algae, epiphytic algae, *Ulva* spp., diversity and richness were significantly different between distance categories (Table 4-7). Filamentous brown algae accounted for a large proportion of the total opportunistic algae category (Table 4-8). Tukeys pair-wise tests showed that the abundance of

opportunistic algae and epiphytic algae was significantly higher for 100 m sites than for 400 m, 2000 m, and 5000 m sites, and the analysis of *Ulva* spp. separately showed that its abundance was higher at 100 m sites than 5000 m sites (Table 4-8). The response of diversity and richness to distance was more variable with a significant increase occurring between the 100 m to 2000 m distance categories (Table 4-8).

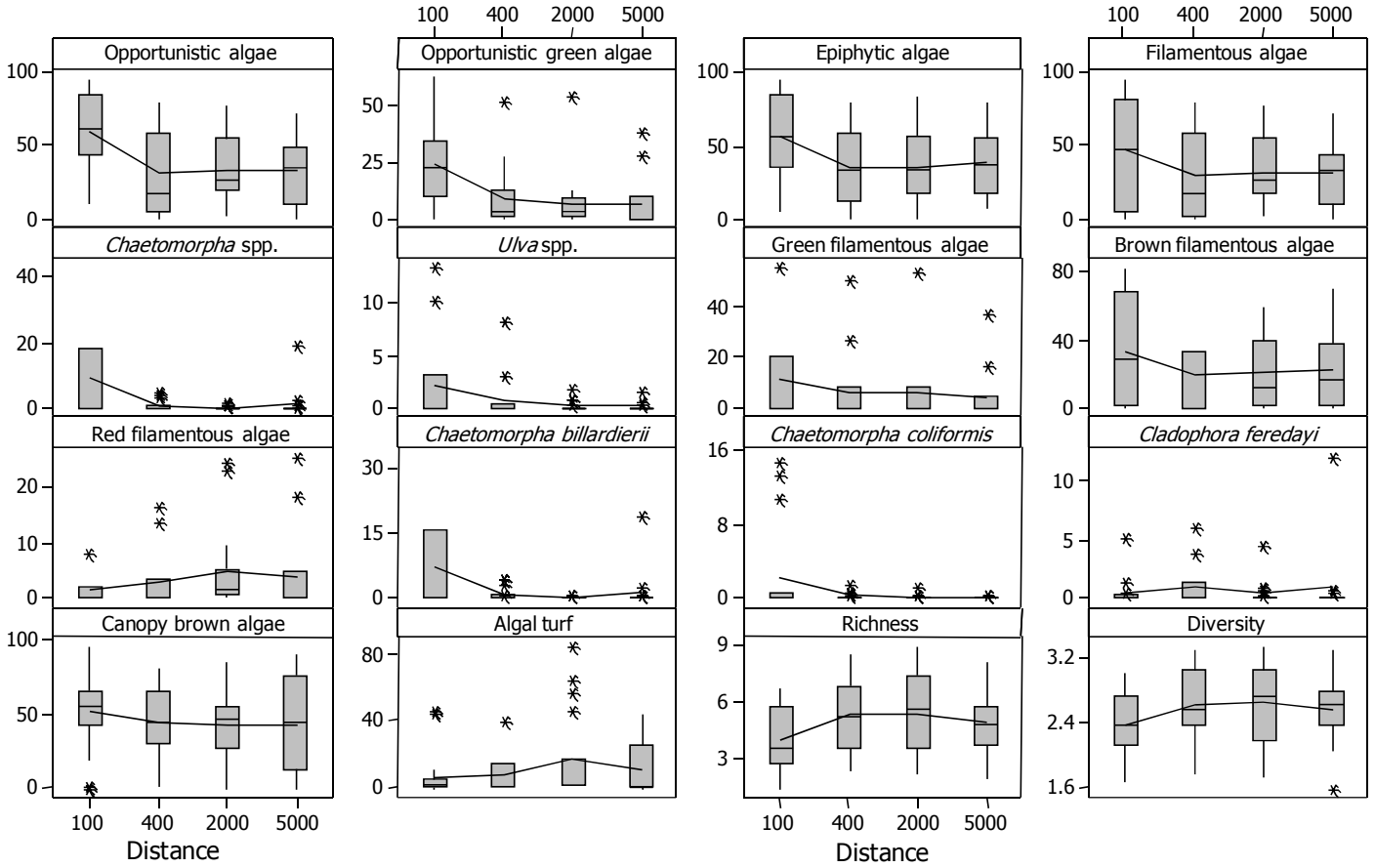

Figure 4-4 Boxplots of percentage cover of indicator variables (raw data) against distance categories. Connect line for mean values shown. Outliers are represented as an asterix.

Table 4-7 Significant environmental variables affecting the abundance of predicted macroalgal indicators. Adjusted R-squared values, F ratios and P values are shown, calculated from a fully factorial general linear model of the factors distance, depth and exposure against transformed univariate response variables. Non-significant results omitted, but variables of interest approaching significance shown in grey text.

Model Response	**distance**		**depth**		**exposure**		**dist*exp**		**depth*exp**		**R-sq (adjusted) %**
	F ratio	*P value*	*F ratio*	*P value*	*F ratio*	*P value*	*F ratio*	*P value*	*F ratio*	*P value*	
Canopy Brown algae			**25.81**	**0.000**	**23.04**	**0.000**					40.75
Opportunistic Green algae	**7.87**	**0.000**					**3.42**	**0.023**			23.33
Opportunistic algae total	**5.56**	**0.002**	3.28	0.075	**64.07**	**0.000**	2.32	0.085			51.26
Filamentous algae	**4.77**	**0.005**	**6.04**	**0.017**	**171.79**	**0.000**	**3.96**	**0.012**	**5.35**	**0.024**	73.87
Epiphytic algae	**8.10**	**0.00**	**5.50**	**0.023**	**103.53**	**0.000**			**5.20**	**0.026**	63.65
Filamentous green algae					**25.46**	**0.000**					22.75
Filamentous brown algae	2.26	0.091	**5.13**	**0.027**	**148.37**	**0.000**					68.56
Filamentous red algae											0.00
Chaetomorpha spp.	**9.10**	**0.000**			**13.99**	**0.000**	**8.04**	**0.000**			39.5
Ulva spp.	**4.89**	**0.004**	**11.86**	**0.001**	**8.710**	**0.005**			**7.44**	**0.008**	36.37
Algal turf											0.00
Shannon diversity (H')	**2.96**	**0.040**			**24.68**	**0.000**					30.16
Richness (d)	**4.71**	**0.005**			**45.26**	**0.000**					42.25

Table 4-8 Indicator group average abundances over distance categories. Significant relationships presented in bold. Pair-wise test groupings presented for significant terms where there was no significant interaction term between distance and exposure or depth.

Distance (m)	100			400			2000			5000		
	mean	*sd*	Grp	mean	*sd*	Grp	mean	*sd*	Grp	mean	*sd*	Grp
Canopy browns	51.7	*25.7*		45.8	*23.8*		43.5	*23.2*		43.7	*32.3*	
Opportunistic greens	**23.1**	***19.1***		**8.62**	***12.6***		**6.45**	***11.9***		**6.27**	***11.1***	
Opportunistic species total	**58.31**	***25.3***	**A**	**31.0**	***27.9***	**B**	**32.3**	***22.3***	**B**	**32.3**	***23.0***	**B**
Filamentous species total	**46.2**	***34.8***		**28.6**	***29.4***		**31.7**	***22.7***		**30.2**	***23.0***	
Epiphytic species total	**55.4**	***28.2***	**A**	**34.3**	***25.6***	**B**	**34.1**	***23.8***	**B**	**37.6**	***22.7***	**B**
Filamentous green	10.9	*15.2*		6.16	*12.8*		5.84	*12.2*		4.21	*9.24*	
Filamentous brown	33.9	*32.1*		19.8	*25.6*		21.5	*20.6*		22.1	*22.0*	
Filamentous red	1.39	*2.15*		2.60	*4.69*		4.43	*7.26*		3.87	*7.01*	
***Chaetomorpha* spp.**	**9.49**	***13.8***		**0.75**	***1.41***		**0.12**	***0.27***		**1.21**	***4.49***	
***Ulva* spp.**	**2.22**	***3.94***	**A**	**0.77**	***1.90***	**AB**	**0.18**	***0.44***	**AB**	**0.11**	***0.33***	**B**
Diversity	**2.39**	***0.41***	**A**	**2.63**	***0.47***	**AB**	**2.67**	***0.49***	**B**	**2.58**	***0.39***	**AB**
Richness	**4.06**	***1.69***	**A**	**5.39**	***2.02***	**AB**	**5.43**	***2.16***	**B**	**4.99**	***1.55***	**AB**
Samples	17			19			19			18		

The interaction term between distance and exposure was significant for *Chaetomorpha* spp., filamentous algae and opportunistic green algae. *Chaetomorpha* spp. were most abundant at swell exposed sites 100 m from fish farms, covering an average of 21.4% of each transect. Tukeys pair-wise test showed that this group of sites was significantly different from all other sites (Table 4-9). Filamentous algae were much more abundant at sheltered sites than exposed sites. At sheltered sites, the abundance of these algae was significantly higher at 100 m sites than 2000 m and 5000 m reference sites, however, no significant distance effect was seen at exposed sites. Filamentous green algae showed a pattern of decreasing abundance with increasing distance from fish farms, in total (Table 4-8) and at sheltered sites (Table 4-9), however the factor of distance or the interaction of distance and exposure were not significant (Table 4-7). Opportunistic greens were dominated by filamentous green alga at sheltered sites, and *Chaetomorpha* spp. at swell exposed sites (Table 4-9). At sheltered sites, the abundance of opportunistic

greens decreased with increasing distance from fish farms, however, Tukeys pair-wise tests indicated that this pattern was not statistically significant.

Table 4-9 Indicator group average abundance and pair-wise groupings for distance and exposure relationship. Terms with a significant *distance x exposure* relationship are shown in bold. Sub-components of opportunistic green algae and filamentous algae are also shown.

Sheltered sites (exposure category 1)												
Distance (m)	100 m			400 m			2000 m			5000 m		
	Mean	*Sd*	Grp	Mean	*Sd*	Grp	Mean	*Sd*	Grp	Mean	*Sd*	Grp
Opportunistic green algae	**17.2**	***18.6***	**A**	**13.6**	***16.8***	**A**	**8.36**	***13.5***	**A**	**8.16**	***13.1***	**A**
Filamentous algae	**71.5**	***17.4***	**C**	**56.6**	***14.8***	**BC**	**41.0**	***18.2***	**B**	**42.0**	***18.9***	**B**
***Chaetomorpha* spp.**	**1.13**	***2.30***	**A**	**0.00**	***0.00***	**A**	**0.06**	***0.16***	**A**	**1.96**	***5.72***	**A**
Ulva spp.	0.28	*0.52*		0.52	*0.95*		0.05	*0.19*		0.15	*0.41*	
Green filamentous algae	15.7	*16.9*		12.6	*16.6*		7.93	*13.7*		5.95	*11.5*	
Brown filamentous algae	54.1	*25.4*		39.6	*24.8*		29.0	*19.0*		33.9	*20.4*	
Red filamentous algae	1.62	*2.59*		4.47	*6.24*		4.11	*6.47*		2.13	*2.40*	
Algal turf	3.73	*3.35*		10.8	*9.76*		23.4	*27.7*		11.9	*15.7*	
Samples	*10*			*9*			*14*			*11*		
Swell exposed sites (exposure category 2)												
Distance (m)	100 m			400 m			2000 m			5000 m		
	Mean	*Sd*	Grp	Mean	*Sd*	Grp	Mean	*Sd*	Grp	Mean	*Sd*	Grp
Opportunistic green alga	**31.5**	***17.7***	**B**	**4.17**	***4.53***	**AB**	**1.13**	***1.00***	**A**	**3.29**	***6.77***	**A**
Filamentous algae	**9.98**	***13.2***	**A**	**3.27**	***5.34***	**A**	**5.79**	***10.5***	**A**	**11.7**	***15.6***	**A**
***Chaetomorpha* spp.**	**21.4**	***14.7***	**B**	**1.43**	***1.70***	**AB**	**0.28**	***0.44***	**A**	**0.03**	***0.07***	**A**
Ulva spp.	5.00	*5.07*		0.99	*2.51*		0.54	*0.73*		0.05	*0.13*	
Green filamentous algae	4.00	*9.22*		0.34	*0.49*		0.00	*0.00*		1.46	*2.50*	
Brown filamentous algae	4.90	*10.8*		2.30	*4.83*		0.46	*0.52*		3.62	*5.44*	
Red filamentous algae	1.07	*1.45*		0.97	*1.65*		5.32	*9.99*		6.60	*10.7*	
Algal turf	13.7	*21.7*		7.40	*12.2*		1.82	*1.32*		10.4	*16.9*	
Samples	*7*			*10*			*5*			*7*		

The distribution of opportunistic green algae, opportunistic algae in total, and epiphytic algae at 2 m depth throughout the sample sites is shown in Figure 4-5, Figure 4-6, and Figure 4-7 respectively. Similar distribution patterns occurred for 5 m transects (see Appendix 6, Appendix 7 and Appendix 8). The pattern of increasing cover of opportunistic green algae near fish farms can be seen throughout the channel with highest percentage covers recorded at sites near Satellite Island, Wedge Bay, and The Sheppards (see Figure 2-1 for site names).

Notable exceptions include 2000 m and 5000 m sites within the shallow waters of Great Taylors Bay in the south of the channel, which had a high proportion of opportunistic green algae and large fish farms at the entrance to the bay. Additionally, sites at Roberts Point in the north had low cover of opportunistic green algae, but a large abundance of opportunistic algae in total, due to the prevalence of filamentous brown algae at these sites. Although the site near Roaring Bay is 400 m from a fish farm, it is directly exposed to southerly ocean swells, and had a low abundance of opportunistic green algae.

The distribution of opportunistic algae throughout the channel indicates that the sheltered northern part of the D'Entrecasteaux Channel has a higher abundance of opportunistic algae than the more exposed parts to the south. A pattern of increasing opportunistic algae towards fish farms can also be seen in most areas throughout the channel, with the most affected areas being north of Roberts Point, The Sheppards Syke's Cove and Satellite Island. Some sites within Port Esperance that were close to fish farms had high abundances of opportunistic green algae but relatively low abundances of total opportunistic algae. It was evident that there was a high density of sea urchins at these sites (Appendix 10).

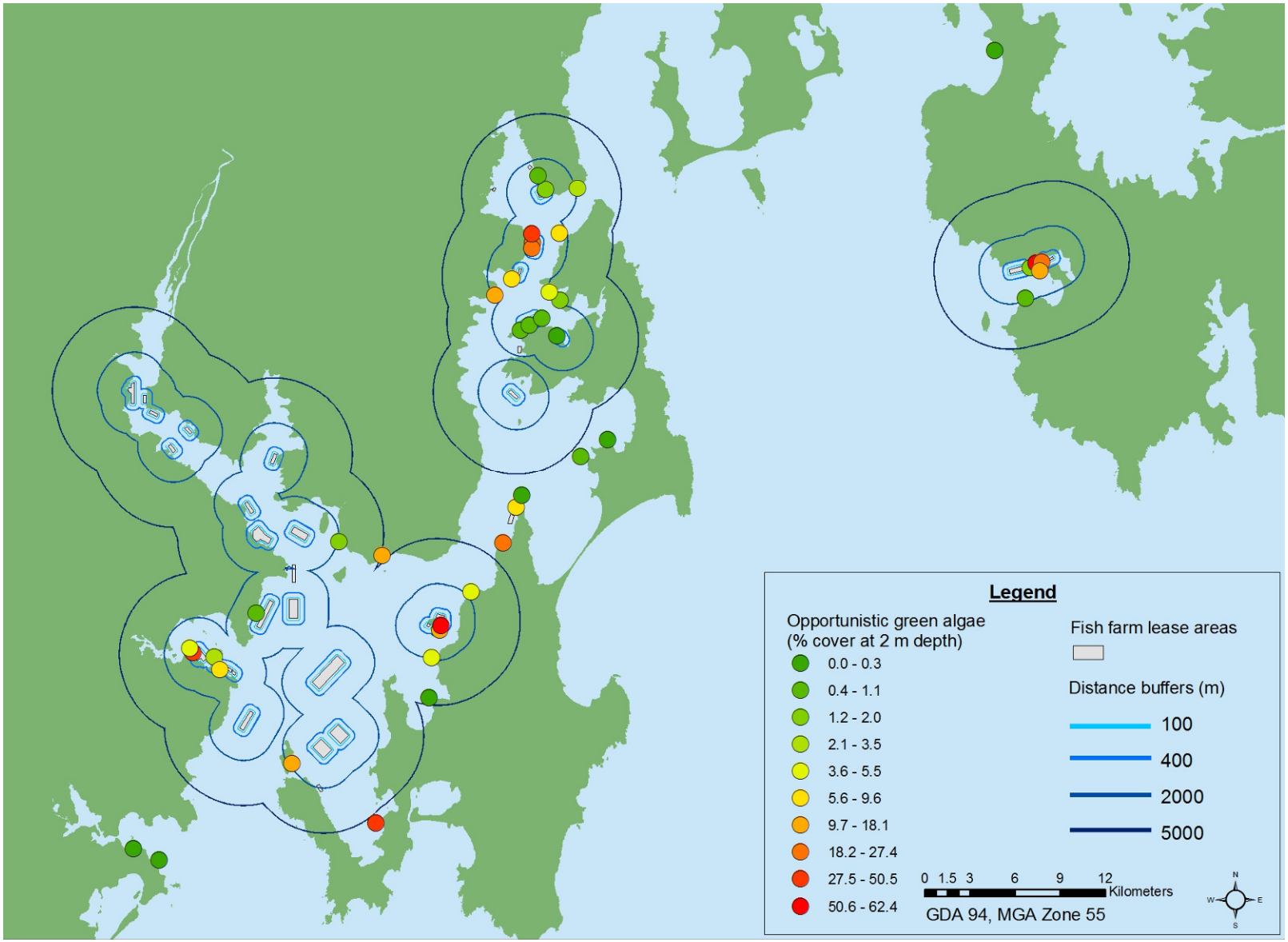

Figure 4-5 Percentage cover of opportunistic green algae throughout the sample sites on 2m depth transects. Data are divided into 10 categories using ArcGIS 9.1 natural breaks (Jenks) function.

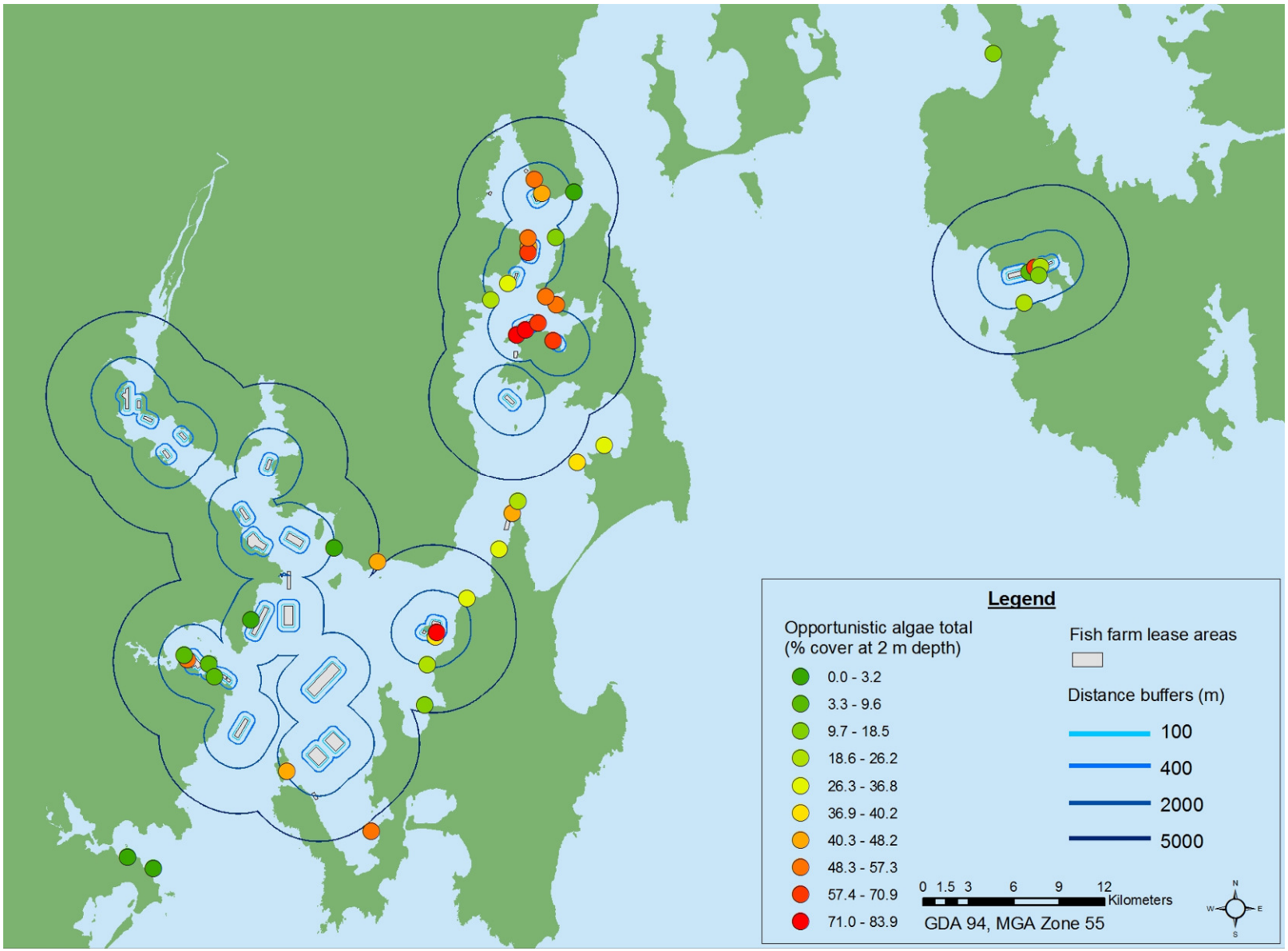

Figure 4-6 Percentage cover of opportunistic algae in total throughout the sample sites on 2m depth transects. Data are divided into 10 categories using ArcGIS 9.1 natural breaks (Jenks) function.

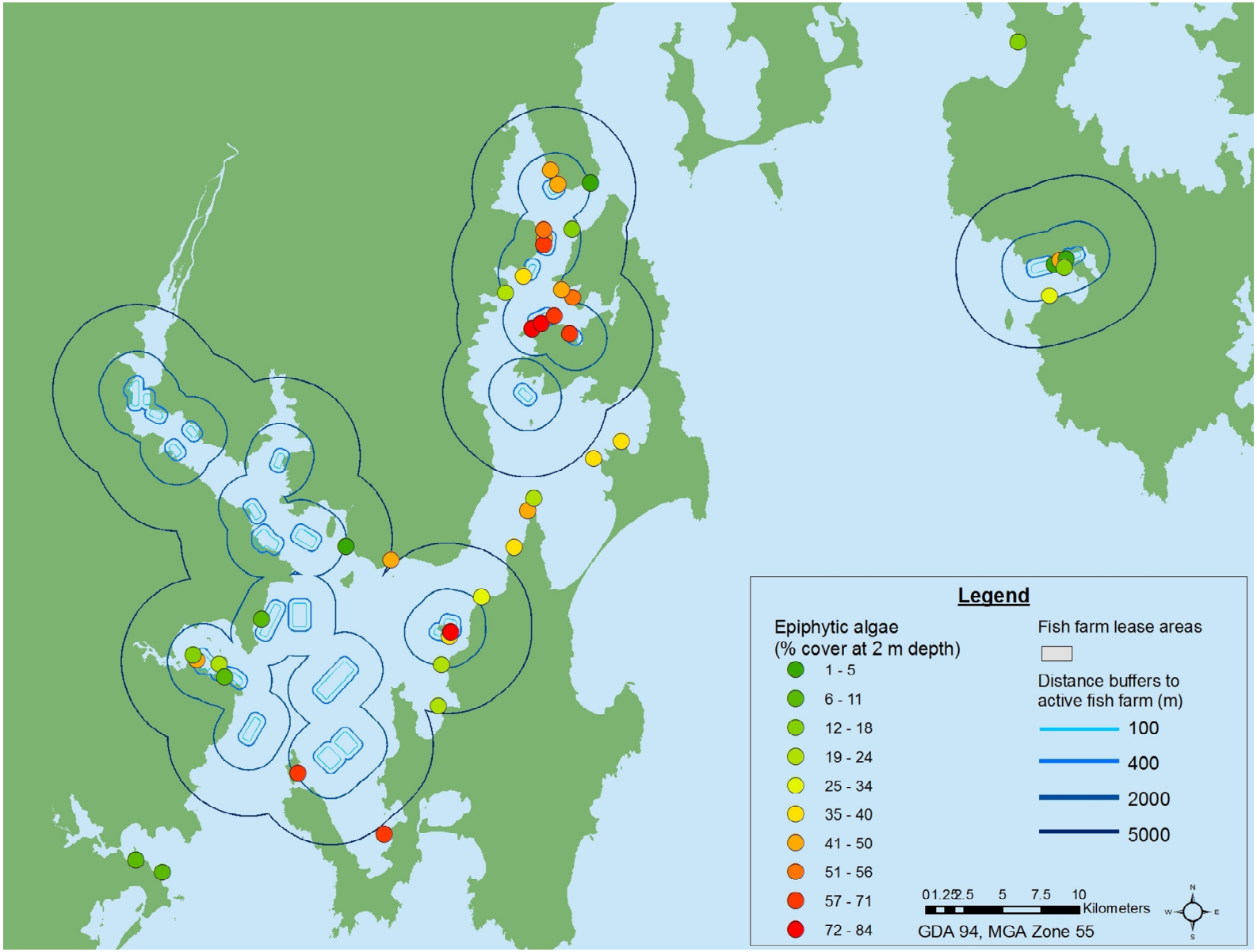

Figure 4-7 Percentage cover of epiphytic algae in total throughout the sample sites on 2m depth transects. Data are divided into 10 categories using ArcGIS 9.1 natural breaks (Jenks) function.

Chapter 5 Discussion and conclusion

5.1 Comparison of methods

The photographic sampling methods described in this study were able to detect the major differences in macroalgal composition between sites at a similar resolution to *in situ* manual methods. This was indicated by the high correlation between the multivariate resemblance matrices and MDS solutions for 34 sample sites. A pilot analysis on this dataset indicated that both methods allocated a similar importance to the independent factors of exposure, depth and distance in explaining the overall variation in benthic composition. A slightly smaller effect size was attributed to distance using photographic data. This indicated that photographs were a conservative method for detecting fish farm effects, compared to the use of manual sampling techniques.

Analysis of algal structure gave further insight into the discrepancies between the two methods. Differences were greatest for the benthic assemblage growing under the canopy, whereas the two methods detected similar trends in percentage cover for epiphyte, upper canopy and lower canopy layers, over the factors of distance, depth and exposure. PQ methods did estimate more upper canopy algae relative to manual quadrats, presumably because the fronds of these tall growing algae sit closer to the camera, thus covering disproportionately more of the photo than other algae. The differences between photographic and manual sample estimates of canopy algae were generally unaffected by lower level variation in epiphytic loading. This is because the percentage cover of non-epiphytic algae was calculated independently of epiphyte cover for the photo-quadrat analysis (see methods). However, a notable exception to this is that overestimates of canopy algae appeared slightly larger at 100 m sites, which had particularly high epiphytic coverage. This discrepancy may have been caused by epiphytic overgrowth reducing the area of the photo sampled for non-ephemeral algae. A bias towards upper canopy algae occurred, as algal layers underneath this were often in shadow. Better estimates could be achieved in future studies by collecting more photos per transect when epiphytic coverage is particularly high.

The comparison of methods for middle-storey, under-storey, and encrusting algae revealed that PQs detected significantly less cover than MQs. This is predictable since these algae are obstructed by canopy algae in photo-quadrat samples, but *in situ* manual sampling was able to detect these layers by brushing aside the canopy after recording its coverage. This bias is more relevant to the monitoring of macroalgal communities than coral communities (as in Dumas et al. 2009) which have fewer vertical layers.

In the present study, the 'canopy effect' reduced the ability of photo-quadrats to detect under-storey and encrusting layers at exposed sites (which had more canopy cover), and a slightly reduced detection of middle storey algae in shallow 2 m depths where lower-canopy algae were more abundant. The 'canopy effect' was also indicated by the multivariate resemblance data, where agreement between the methods was significantly greater at sheltered sites than exposed sites which have more canopy algae (refer to Figure 3-4). This reduces the capacity of photographic methods to describe trends relating to exposure and depth, where middle-storey, understorey or encrusting algae are particularly important, without further adjustment to the methods used in this study. Notably, this trend is opposite to that reported in Alvaro et al. (2008) where manual quadrats recorded less encrusting algae because sampling did not involve brushing the canopy from under the quadrat grid after recording its coverage.

Manual quadrats also detected significantly higher levels of benthic richness and diversity, but these differences were not significantly affected by the levels of exposure, depth or distance. This suggests that PQs could still detect changes in richness and diversity in response to major environmental factors. However, further study may be necessary to confirm this conclusion. Since middle storey algae, such as red algae, are particularly diverse in some reef habitats, there are likely to be instances where low growing benthos are highly diverse but canopy cover disproportionately reduces the capacity of photographs to detect this. This may be the case at 5000 m reference sites where manual quadrats detected an increase in diversity from 2000 m but photo-quadrats did not.

These results provide further insight into the application of photo-quadrats for temperate reef monitoring. For the methods used in the present study, the analysis provided good evidence that photo-quadrats were useful in detecting ecological change. They were particularly useful for detecting change in the cover of epiphytes, which are abundant and are ecologically important modifiers of habitat structure and diversity (Russell et al. 2005), and most opportunistic algae are epiphytic. Furthermore, the PQ data for community composition detected comparable variation in response to exposure, distance to fish farms and depth (between 2 and 5 m), and a similar relationship between sites. This indicates that discrepancies between the methods for measuring differences in entire communities were generally small. Nevertheless, there may be room to improve the accuracy of photographic methods in measuring overall diversity and richness, and algal layers that are obscured by canopy or epiphytes. This improvement may be achieved through some simple modifications to the methods described in this study, such as the collection of replicate photographic samples where canopy and epiphytes are brushed aside, or the collection of more samples where epiphytic or canopy cover is high.

5.2 The effect of fish farms

5.2.1 The nature of salmon farm impacts

Benthic community composition varied with distance to fish farms, and the nature of these changes varied between swell exposed areas and sheltered areas. A large effect was detected, with “distance” and its interaction with exposure collectively explaining 28.0 % of community variation between sites. The observed differences in macroalgal community with distance to fish farms are likely to be primarily related to nutrient enrichment of the waters surrounding farms. Suspended particulate matter from fish farms may also affect water quality and the resulting effects on macroalgal communities cannot be clearly separated from the effects of dissolved nutrients. Nevertheless, fish farms can be identified as the main contributors to the ecological effects of “distance” and “distance x exposure” in the present study, as sites with terrestrial sources of pollution were avoided in the sampling design, and sites within each distance class were spread throughout the region to encompass a diversity of local environmental conditions.

Analogous to the results of studies on macroalgal composition and nutrient enrichment from other anthropogenic sources (Lavery et al. 1991; Russell et al. 2005; Arevalo et al. 2007; Juanes et al. 2008), the effect of fish farms was characterised by increases in the cover of epiphytes and total opportunistic algae (which included opportunistic green algae, filamentous algae and algal turf). In particular, opportunistic green alga of the genera *Chaetomorpha, Ulva* and *Cladophora* (the main constituent of filamentous green algae in this region) were collectively responsive to the proximity of fish farms. The abundance of these algae was also identified by the discriminant analysis in CAP as correlating highly with distance to fish farms. Algal turf did not exhibit a notable increase towards fish farms. However, the ability to detect turf may have been compromised by the use of photo-quadrats, as it grows close to the rock substrate. Similarly, there was no obvious trend in red filamentous algae which often grows under other filamentous or foliose algae in reduced light conditions, and may be obstructed in photos. Neither of these variables was explained by general linear models containing the variable exposure, distance and depth.

Opportunistic algae are broadly regarded as indicators of nutrient enrichment due to their growth habits and life history (Mann 1973; Littler & Littler 1980), their affinity for nutrients, and their robustness to pollution and sedimentation (Eriksson & Johansson 2005; Liu et al. 2007). The high cover of opportunistic species near fish farms suggests that, for the seasonal period sampled, algal growth was nutrient-limited and that nutrients from fish farms disproportionately enhanced the growth of fast growing pollution-tolerant algae. It may also reflect, on a regional basis, the overall inability for the ecosystem to counteract the increased growth of opportunistic algae through grazing or physical disturbance over the short term.

The secondary impacts occurring from this epiphytic and opportunistic overgrowth were unclear. There was no apparent decline of canopy algae close to fish farms, as has been reported for other cases of eutrophication, and although average diversity and species richness were lowest at sites 100 m from fish farms, they were not significantly different from reference sites. This may indicate that the macroalgal communities in the region display some resilience to the full affects of nutrient over-enrichment during the period since fish farming has commenced. This is discussed

further in section 5.2.5. Although it is unlikely that any major decline in canopy cover or algal richness has occurred, it is likely that improved methods for the use of photo-quadrats may increase the ability of future monitoring to detect any smaller scale variation in canopy cover, richness and diversity between sites.

5.2.2 The influence of wave exposure on salmon farm effects

The importance of wave exposure in determining macroalgal composition was detected in this study, and is consistent with previous work (Edgar 1983b, 1984; Sanderson 1984; Dayton 1985; Barrett et al. 2001; Burrows et al. 2008). All of the algal variables tested in this study responded to exposure, or an interaction between exposure and another factor. A transition to more robust, wave-resistant algae in swell exposed areas was apparent. Algal indicator groups such as epiphytic algae, filamentous algae, and opportunistic algae were collectively more dominant in sheltered sites than exposed sites, whilst the opposite trend was apparent in canopy brown algae and encrusting algae. Delicate algae are generally limited in swell-exposed areas by waves and whip-lash from canopy algae which cause their detachment and export from the system (Kiirikki 1996; Pihl et al. 1999).

Multivariate and univariate analyses indicated that the impacts of fish farms were evident in both sheltered and swell-exposed areas. The collective groups of opportunistic and epiphytic algae increased in cover towards fish farms at both exposure levels, with the greatest cover of these algae being located at sites near fish farms in sheltered habitats. There was no significant interaction effect between distance from a fish farms and exposure for these groups. This aligns with the findings of Krauvfelin (2007) who concluded, from mesocosm experiments, that nutrient enrichment could enhance opportunistic algal growth and export in both sheltered and moderately wave exposed conditions. There are several possible reasons for this. Firstly, in studies by Kraufvelin (2007) and in the present study it was apparent that the levels of wave action tested were not energetic enough to limit the dominant growth of opportunistic algae altogether. In the present study, fish farms were generally situated in areas only subjected to low to moderate levels of wave exposure, presumably for logistical and safety reasons. Furthermore, the physical presence of fish cages in the area may actually absorb some of the wave

energy which would otherwise reach the adjacent reef. Nevertheless, since opportunistic algae are highly responsive to exposure, growth may be reduced in more exposed situations than those sampled widely in the present study. Whilst no active fish farms were located within 100 m of reef highly exposed to swell, a more exposed site with steep reef was located near Roaring Bay, 400 m from a fish farm, and was sampled. This site was directly exposed to southern ocean swell entering the southern D'Entrecasteaux. Although it was only 400 m from a large fish farm, the cover of opportunistic algae was particularly low.

It could also be expected that nutrients will not accumulate in exposed waters which have higher flushing rates. However assemblages existing within them may also be more susceptible to nutrient enrichment than those naturally adapted to withstand nutrient inputs for longer periods (e.g. those in the northern D'Entrecasteaux Channel). This was suggested by Russel et al. (2007), who observed that nutrient enrichment of communities in oligotrophic waters yielded a comparable percentage cover of epiphytes to those in nutrient rich waters, even though levels were only raised to <5% of the nutrients found in the eutrophic waters.

The composition of opportunistic algae and epiphytes characterised differences in the effect of fish farms between sheltered and exposed areas. Changes in composition were evident over a regional gradient in exposure, from the sheltered waters of the northern D'Entrecasteaux Channel to the more exposed parts of the southern channel and Wedge Bay. Opportunistic green algae from the genus *Chaetomorpha* were abundant close to fish farms in swell-exposed sites and showed a significant response to fish farms at 100 m sites, but were uniformly rare in sheltered waters. This taxon was dominated by the epiphyte *Chaetomorpha billardierii*. *Ulva* was also more abundant in swell-exposed areas. On the other hand, epiphytic filamentous green algae were better indicators of fish farm impacts in sheltered waters, where they showed a stronger pattern of decreased cover away from farms. Collectively, opportunistic green algae responded significantly to distance in exposed waters but it was possible that a lack of statistical power and high variability meant it did not respond significantly to distance in sheltered waters where its cover, on average, still showed a pattern of decline away from fish farms. Brown, green and red filamentous algae in total provided a better indicator for fish farm impacts in sheltered waters, as

they showed a significant decrease in abundance with distance from farms. However their cover was uniformly low in exposed areas.

The observed changes in the composition of opportunistic algae in relation to exposure are likely to reflect slight differences in their form and function, which determines, amongst other things, their ability to withstand wave action (Littler & Littler 1980). Uniseriate filamentous algae are susceptible to disturbance of their delicate nature, whilst single layer foliose algae such as *Ulva* are more robust (Steneck & Dethier 1994) and more strongly attached to the substratum. Although *Chaetomorpha billardierii* is not foliose like *Ulva* it has a more robust thallus than fine filamentous algae, and *Chaetomorpha coliformis* is well attached to its substratum.

Further variation in the impact of fish farms within each distance class may have been explained by variables other than exposure which mediated water movement, and thus the dispersal of nutrients. The magnitude of wind energy influencing a site seemed to add little information to the patterns described by exposure to swell, as both the continuous and categorical index of exposure had a similar correlation with the PCO axes. However, much of the water movement in the northern D'Entrecasteaux Channel is provided by currents which are accentuated by constrictions in the bathymetry and geomorphology of the water body (Barrett et al. 2001). Sites along Simpsons point were particularly affected by these currents which seemed to bring a large flux of suspended particles. These currents may also have increased the delivery of nutrients to communities at Simpson point, as nutrient flux is increased by water movement (Dayton 1985). These communities had a slightly larger cover of opportunistic algae than most other reference sites.

Current direction has been an important source of variation in the dispersal of nutrients from fish farms (Dalsgaard & Krause-Jensen 2006; Sanderson et al. 2008), as well as particulate wastes (MacLeod et al. 2004). Nutrient enhancement may extend to a much larger distance in the direction of the prevailing currents, but may not be detectable in the opposite direction (Sanderson et al. 2008). However, sites in the present study were identified purely on the basis of distance to a fish farm, reef location, a wide spatial distribution of sites, exposure and the absence of other

pollution types. This relatively simple design aimed to provide an overview of farm impacts on a regional scale. The prevailing current directions at each fish farm would have added random variation to the effects seen within each distance category. In addition, further variation in the data may be explained by spatial differences in the movement of water throughout the channel. As an example, a 2 km site and a reference site located in Great Taylors Bay had very high levels of opportunistic and opportunistic green algae. The bay is relatively shallow and sheltered, with very large fish farms located at its mouth. Consequently, it is possible that swells entering the southern D'Entrecasteaux and prevailing westerly winds, push water back into the bay from the direction of the fish farms providing a supply of fish farm derived nutrients to the majority of the bay.

5.2.3 The influence of depth

Although depth had a significant influence in the composition of macroalgal communities, no significant interaction between depth and distance was found. This indicates that nutrients disperse throughout the depth range sampled on the majority of reefs affected by fish farms, and have a similar effect at each depth. It is also likely that the maximum effect of fish farms was variable between these depths. Studies have found that eutrophication enhances light limitation at depth, due to suspended sediments, epiphytic shading or phytoplankton blooms (Krause-Jensen et al. 2007a; Krause-Jensen et al. 2007b). Nevertheless, due to natural differences in water clarity throughout the region, the depth at which nutrient-influenced light limitation becomes important may have been variable.

5.2.4 The scale of impacts

Previous studies on fish farm impacts in Tasmania have largely focussed on benthic soft sediment habitats (Crawford et al. 2002; Macleod et al. 2002; MacLeod et al. 2004; Edgar et al. 2005a), and found only minor effects at distances of 35 m from a lease boundary (Edgar et al. 2005a). The present study indicated that fish farms may have a significant effect on benthic reef communities at greater distances than this, and that the effects were observed over a regional scale.

The effect of fish farms on reef benthic communities extended to at least 100 m from fish farms at both sheltered and exposed sites, where the macroalgal community was significantly different from reference sites. Although 400 m sites were collectively not significantly different to reference sites, it is likely that effects extended to at least 400 m in some areas but not others. This was indicated by the leave-one-out procedure in the CAP analysis, which revealed that 5 of the 400 m sites showed characteristics akin to 100 m sites, compared to none of the 5000 m reference sites and only 2 of the 2000 m sites. This suggests that variations in the detectable effects of fish farms can be anticipated at scales of hundreds of metres, but these rarely reach distances of several kilometres away from farming areas.

Previous studies on the dispersal of nutrients from fish farms have concluded that nutrient enrichment occurred within a similar range. Algal growth in bioassays was significantly elevated to 150 m from fish farms in the Mediterranean Sea (Dalsgaard & Krause-Jensen 2006) and in Scottish waters ammonium levels at 4 m depth were elevated for extended periods of the day at distances exceeding 200 m (Sanderson et al. 2008). It is thus likely that distance from a fish farm, at the scale of hundreds of metres, is a fairly rudimentary representation of the "effect" of a fish farm due to complexities which affect the output and dispersal of nutrients from a farm, and factors which affect the susceptibility of the marine environment to these nutrients. It is likely that stocking levels, the distribution of occupied fish pens, feeding regimes, and hydrodynamics will vary between fish farms, thus changing the amount and distance of nutrients dispersing beyond a lease boundary.

Local scale variation in differences in the extent of fish farm impacts could not be tested in this study but is likely to be important in the management of possible cumulative effects of fish farms. Whilst 100 m sites, and some 400 m sites, clearly stand out as highly impacted from fish farms, a lack of baseline data collected before fish farming commenced in the region creates uncertainty in labelling other sites as totally 'unimpacted'. Such a concern is strongest in the northern region of the D'Entrecasteaux Channel where there is a high density of fish farms. As no reference sites could be identified in this region, and there is a lack of baseline macroalgal community data within the wider region, it is unsure whether the high dominance of opportunistic algae in the northern D'Entrecasteaux Channel is a result

of natural circumstances or more subtle broad scale effects of elevated nutrients throughout the system. In addition, the macroalgal composition prior to fish farming at reference sites in the middle and lower areas of the D'Entrecasteaux Channel is unknown, therefore it is possible that these have responded in some way to more subtle, ecosystem wide changes to nutrient regimes over the past two decades. Yet it is still apparent that these changes, if any, are not as major as those changes affecting sites hundreds of metres from fish farms, where elevated nutrient levels are commonly detectable, and that the effects do not differ between 2 km and 5 km from fish farms.

5.2.5 Further research directions

With continual input of fish farm derived nutrients into the D'Entrecasteaux Channel and Wedge Bay areas, it is likely that rapid summer growth in opportunistic algae will persist. Yet the long term consequences of opportunistic overgrowth in fish farm affected sites are relatively unclear. Seasonal fluctuations in temperature, light, nutrients and water movement have a dominant influence in the region, and play an important role in the variation in algal composition (Edgar 1983a). On reef in south-eastern Tasmania, the late summer and early autumn period coincides with the maximum biomass of epiphytic algae (Edgar 1983a). It also coincides with the maximum standing crop of some abundant perennial algae such as *Sargassum fallax* and *Caulocystis cephalornithos*, whose upper reproductive fronds senesce in winter (Edgar 1983a). Other kelps, such as *Ecklonia* and *Phyllosphora* possess relatively constant biomass year-round. Further investigations are needed to determine the relative abundance of opportunistic algae near fish farms throughout a longer time period, particularly in winter when flushing rates are higher and in late summer when fish farms are likely to have their maximum effect.

Over the long-term, the dominance and effect of opportunistic and epiphytic algal growth in benthic reef communities is also mediated by their recruitment success, the dominance of canopy algae, and trends in grazing pressure. In South Australia nutrient and sediment enrichment enhances the growth of algal turf, and rapidly inhibits recruitment in canopy algae. However, reef in South Australia lacks the

presence of a strong grazer community (Connell 2007), unlike Tasmania where grazers can have a major influence over algal growth (Ling 2008). Whilst opportunistic growth was dominant at many sites at the time of sampling, it remains unclear if grazing is an important mechanism controlling growth at other times of the year. In other studies, grazers have played an important role in counteracting nutrient impacts by their preferential consumption of opportunistic algae over thick perennial algae (Bokn et al. 2003). In addition, the presence of an established canopy can buffer a system against nutrient enrichment by up to 90 %, by modifying resource and consumer control of opportunistic algae (Eriksson et al. 2007). However, in the present study, a large percentage of opportunistic algae grew as epiphytes, so it is unlikely that canopy algae would limit their growth through resource competition.

Regardless, the relative dominance of canopy algae, grazers and opportunistic growth will be in important in understanding maintenance of habitat characteristics over the long term. In some systems continual nutrient enrichment may still override grazer control when annual opportunistic algae are at high densities and maintain reproductive propagule banks (Worm et al. 1999; Worm et al. 2001; Worm & Lotze 2006). Rapid 'regime' shifts have been previously recorded, where diversity and canopy algae decline once uncontrolled opportunistic growth begins to inhibit perennial recruitment and acquire space on the reef (Kraufvelin et al. 2006; Connell 2007). Shifts in stable states have occurred from a single nutrient pulse (Worm & Sommer 2000), whilst another study reported 5 years of persistent canopy cover under nutrient enrichment, followed by a rapid modification to an opportunistic algae-dominated state (Kraufvelin et al. 2006). Whilst the present study did not indicate a major decline in canopy cover near farms, it is possible that continual nutrient enrichment from fish farms remains a threat to macroalgal community structure and diversity over the long-term. The barren-state of a reef 100 m from a fish farm at Port Esperance which had low canopy cover, a high density of grazers, and a high opportunistic green algal cover stood out from the rest of the sites studied (Figure 5-1). The situation could be indicative of a complex relationship between grazers, nutrients, sedimentation and canopy loss. However, there is a lack of long-term ecological data and sufficient spatial coverage to support this supposition.

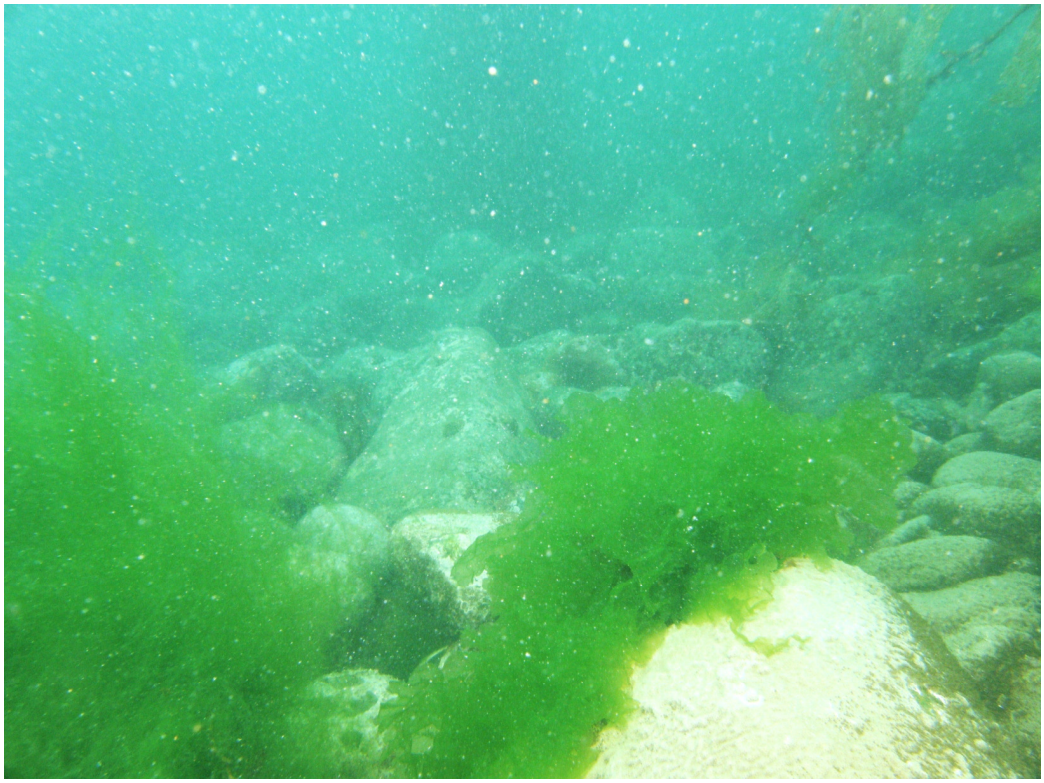

Figure 5-1 A reef site 100 m from a fish farm at Port Esperence

A comprehensive understanding of the long term resilience of reef communities affected by fish farm nutrients would involve knowledge of the relationships between grazers, canopy cover, and competition between perennial and opportunistic algal growth over seasonal periods. Studies in other regions have generally used experimental manipulations of grazers, nutrients, propagule banks, and/or disturbance, in order to properly identify their effects and interactions at reef sites over time (Worm et al. 1999; Worm et al. 2002; Bokn et al. 2003; Kraufvelin 2007). Such information would be of use in determining possible follow-on effects to invertebrate and fish communities that are a part of reef systems, and would be of great importance to managing the sustainability of marine resources and assets in areas affected by multiple anthropogenic threats.

5.3 Synthesis and implications for management

Previously few studies have made the link between the pelagic dispersal of nutrients from fish farms and the composition of nutrient-assimilating macroalgal communities on reef (Ruokolahti 1988; Ronnberg et al. 1992; Vadas et al. 2004). This is because research on the benthic impacts of fish farms has largely been focussed on organic enrichment impacts which are relatively localised. In addition, impacts of fish farm nutrients in the pelagic environment have been relatively hard to identify because nutrients may be assimilated by phytoplankton, then rapidly transported up the food web or flushed out of the system (Navarro et al. 2008). In recent years, sessile macroalgae have become more widely used as bioindicators of environmental disturbance. This study has identified macroalgal communities as relevant indicators for detecting the impacts of fish farm derived nutrients on the marine environment. It has provided observational evidence that nutrients from fish farms can alter the macroalgal composition on temperate reefs on a relatively broad scale compared to other benthic impacts of fish farms. Macroalgal compositional changes included the increased cover of opportunistic algae, particularly filamentous species in sheltered regions and other opportunistic green algae in exposed sites. On average the effects extended at least 100 m from fish farms, on a regional basis. In terms of fish farm impacts these are considered 'far-field' (Crawford 2003).

In Tasmania, both government and industry have become increasingly concerned over the far-field impacts caused by salmon farming (Crawford 2003). The issue is of particular relevance to the D'Entrecasteaux Channel as it is a multi-use area (Phillips 2000), where there has been some conflict over resource usage and increasing concern about the expansion of fish farming (Crawford 2003). Aquafin CRC, CSIRO Marine and Atmospheric Research (CMAR), and the Tasmanian Aquaculture and Fisheries Institute (TAFI) have played a major role in developing knowledge about pelagic impacts of fish farming in the region. These investigations have worked towards developing comprehensive 3D biogeochemical models of the D'Entrecasteaux and Huon regions, which are integral to understanding nutrient dispersal from fish farms and the carrying capacity of the farming region (MARLIN 2008). This study provides additional information that relates directly to the ecological response of benthic communities in the Bruny Bioregion. The

information could be incorporated into system-wide management considerations such as: 'what are the biodiversity and resource assets at risk from far field fish farm impacts?'. In order to fully understand the impacts of fish farms on reef organisms, further research is necessary to determine the broader scope of effects, including possible changes to invertebrates and fish assemblages. Such changes may have implications through the legislation regarding fish farm management (Living Marine Resources Act 1995 and the Marine Farming Development Planning Act 1995), which state "there shall be no unacceptable visual, chemical or biological impacts detectable on the benthos 35 m beyond the boundaries of the lease area". To date, there has been no clarification of "unacceptable" with regards to reef habitats.

In particular changes that may impact on the values of the two reef habitat marine reserves at Tinderbox and Ninepin point are relevant, as these areas are representative units of a wider marine protected area network. Both areas are recognised for the diversity of seaweeds (Phillips 1999). Tinderbox marine reserve was declared to provide a safe, sheltered marine study area for education, research and recreation (Phillips 1999), and lies roughly 800 m from a fish farm lease. In addition, any changes to habitats which may: (i) favour the proliferation of introduced species in the region, (ii) compromise recreational diving and fishing, or threaten reef-associated protected species in the area such as the Live-bearing seastar, *Pateriella vivipara* (endangered), and the rare seastar, *Smilasterias tasmaniae*, are also of particular relevance.

As well as further research, a broad scale macroalgal monitoring program would be useful in detecting any ongoing impacts to reef habitats throughout the region. Macroalgae are identified as an indicator of habitat quality for State of the Environment reporting (Ward 2000), yet there is a lack of guidance for their use in monitoring programs. It is likely that the photo-quadrat methods described in this study, possibly with a few alterations, would provide a pragmatic approach to sampling macroalgal communities at a large number of sites. Digital techniques have already been identified by the salmon farming industry as cost-effective ways to measure benthic impacts, with underwater video monitoring of sediment impacts already now a part of compliance monitoring (Crawford et al. 2001).

The monitoring of anthropogenic impacts on natural habitat assemblages is a central component of good environmental management. An over-reliance on the use of physiochemical data and a lack of systematic broad scale sampling has contributed to "shifting baselines" and over-looked losses of marine diversity (Edgar et al. 2005b). Monitoring of macroalgae already occurs in many marine protected areas in Tasmania. Combined with fish and invertebrate data this gives important insights into the impacts of fishing in unprotected areas (Barrett et al. 2009). This study has shown that macroalgae can also be utilised as an indicator of nutrient related environmental disturbance of reefs in Tasmania, and applied in an ecosystem wide approach. Since the macroalgal indicators identified in this study are broadly regarded as indicators of pollution disturbance, their use may be applicable to other sources of pollution such as sewage wastewater, and also applicable to reef in other sheltered areas, and including those experiencing low levels of wave exposure within Tasmania. In addition, a similar monitoring strategy could also be tested and applied for sea-grass systems. Such monitoring approaches will be important in the management of coastal systems that are impacted by a variety of anthropogenic activities. Information gained will be relevant to protecting a range of biodiversity assets, resource assets and ecosystem services within marine communities through the long term.

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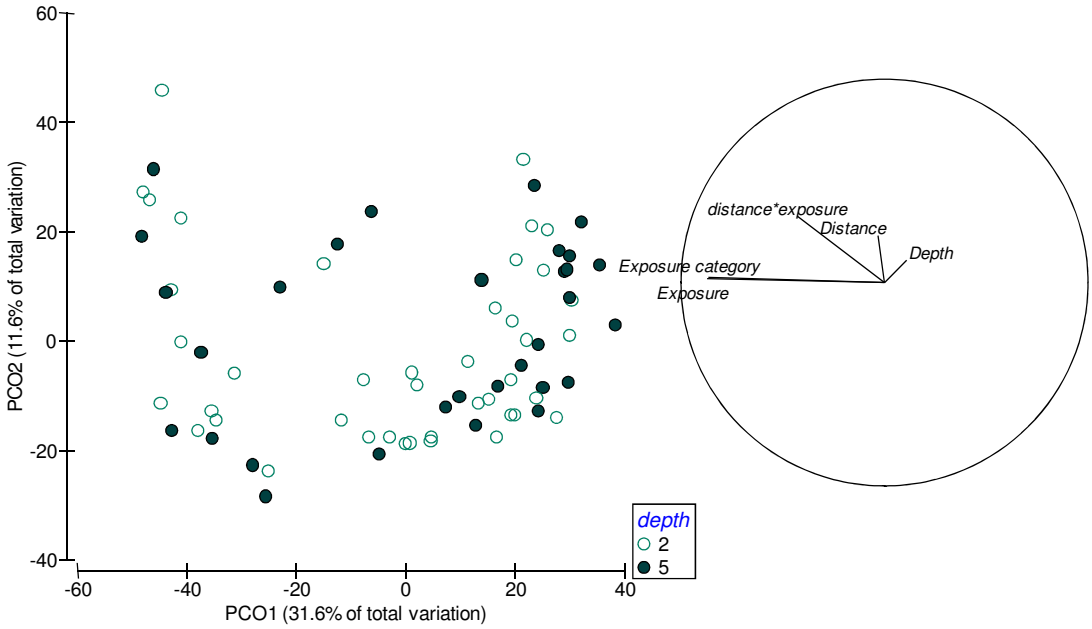
Appendix

Appendix 1 Significance tests for the difference between methods over the factors distance, depth, and exposure. Difference between the percentage estimates (MQ – PQ) was tested for the 6 algal layers, richness and diversity in each sample (N = 36). These were conducted using general linear modelling if the data were normal. Where the data did not have normal distribution (labelled with *) the Kruskal Wallis test was conducted on each of the three factors separately. The Kruskal Wallis test is adjusted for ties. Significant results shown in bold.

	Distance		**Exposure**		**Depth**	
	F / H Statistic	P Value	F / H Statistic	P Value	F / H Statistic	P Value
Epiphyte	1.31	0.291	0.08	0.780	0.87	0.360
Upper-canopy	1.58	0.217	0.07	0.795	0.63	0.435
Lower-canopy*	1.88	0.598	0.00	0.972	0.25	0.619
Mid-storey	4.87	0.181	0.59	0.441	0.15	0.699
Under-storey	0.66	0.587	**6.38**	**0.017**	1.37	0.252
Encrusting*	1.14	0.769	**13.83**	**0.000**	0.11	0.740
Richness	1.70	0.190	2.99	0.095	1.09	0.304
Diversity	0.98	0.416	0.21	0.648	0.83	0.369



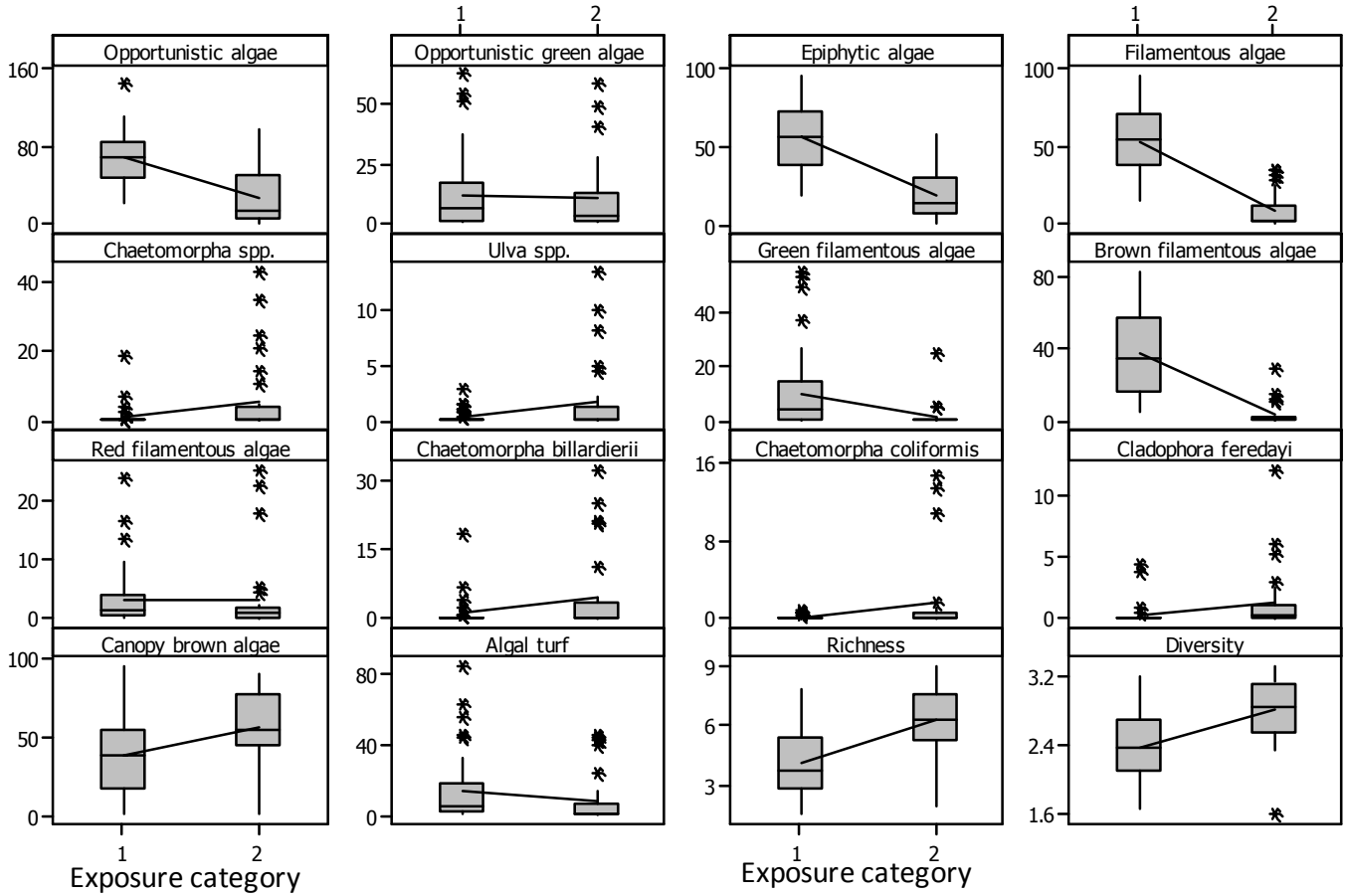
Appendix 2 PCO ordination showing depth categories. Ordination is based on Bray Curtis similarity matrix of square root data. Fitted environmental vectors based on Pearson correlation. The circle represents perfect correlation of 1

Appendix 3 Average percentage cover of taxa from all samples (N = 73) against distance categories at each level of exposure. Algal layer codes are: en = encrusting, ep = epiphyte, lc = lower canopy, m = middle-storey, u = under-storey, uc = upper canopy.

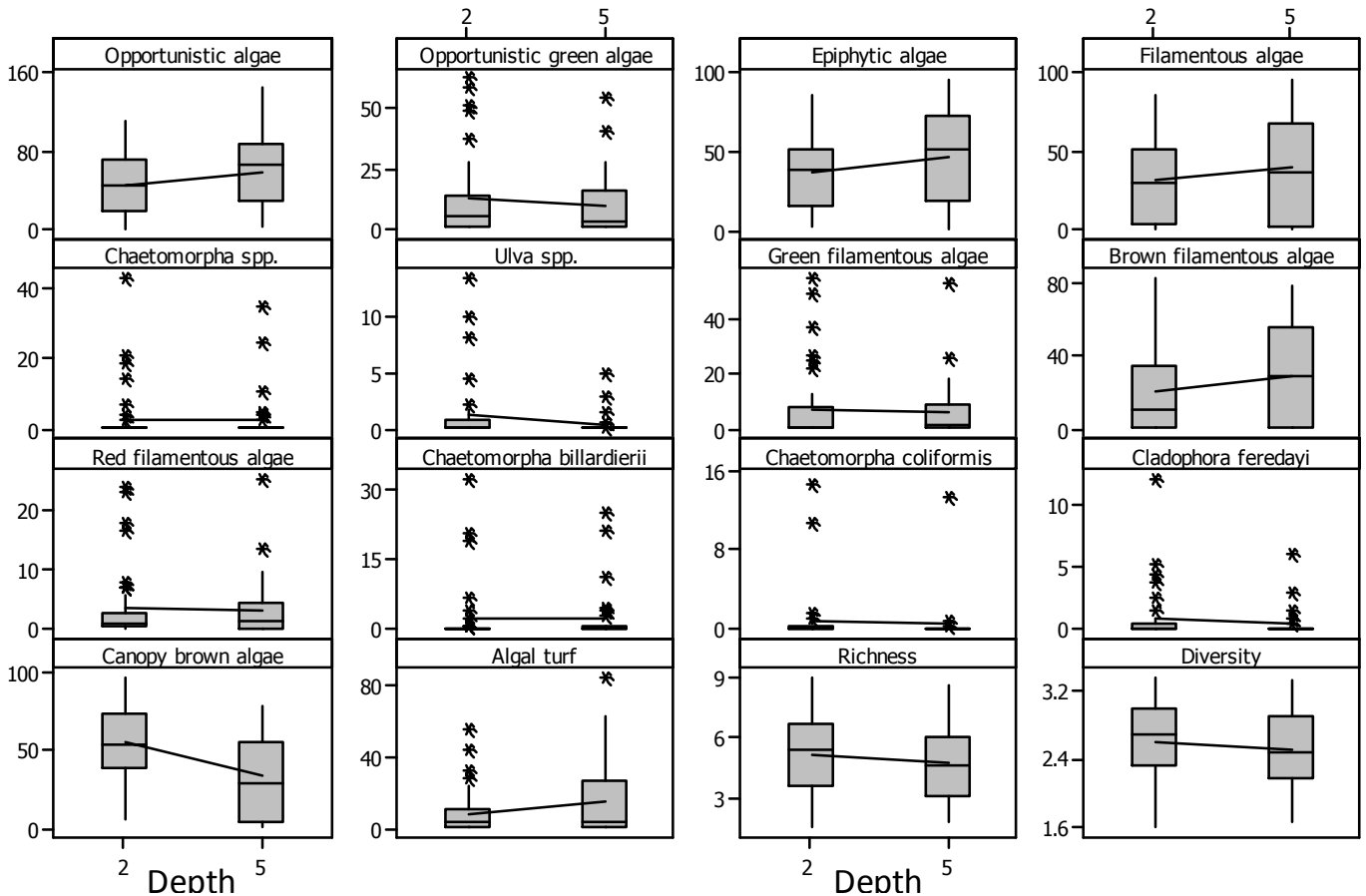
Average percentage cover								**Taxa**	**Layer**
Distance for sheltered sites				Distance for swell-exposed sites					
100	400	2000	5000	100	400	2000	5000		
14.53	7.27	7.75	17.04	0.77	1.04	1.27	6.82	Crustose coralline algae	en
1.53	0.00	0.00	0.00	0.00	0.00	0.00	0.00	Dead CCO	en
0.53	1.96	0.00	0.11	0.00	1.27	0.00	0.13	*Erythropodium hicksoni*	en
9.98	4.00	8.60	7.78	0.77	0.22	0.83	0.65	*Peyssonnelia* sp. *(encrusting)/Hildenbrandia* sp. *(encrusting)*	en
1.10	0.53	0.55	1.98	0.13	0.12	0.14	0.06	Sponge (encrusting)	en
29.35	27.29	16.08	16.16	37.27	31.41	18.13	23.60	Brown filamentous algae	ep
0.03	0.00	0.15	0.09	0.00	0.00	0.00	0.00	*Ceramium* spp.	ep
14.68	1.40	0.00	0.00	8.87	0.78	0.05	0.02	*Chaetomorpha billardierii*	ep
0.18	0.09	0.00	0.00	0.00	0.11	0.00	0.00	*Colpomenia* spp.	ep
5.38	8.67	6.85	3.40	8.42	5.56	9.29	2.39	Green filamentous algae	ep
0.00	0.00	0.08	0.07	0.00	0.00	0.15	0.03	*Hydroidea* spp.	ep
6.43	5.36	5.15	4.33	1.09	2.79	4.89	3.95	Red filamentous algae	ep
0.48	3.00	0.00	2.56	1.35	3.04	0.22	3.06	Red foliose epiphyte	ep
2.03	1.53	1.05	3.49	3.12	0.83	0.95	2.35	*Caulocystis cephalornithos*	lc
0.00	0.00	0.00	0.00	0.24	0.00	0.00	0.13	*Cystophora brownii*	lc
0.53	0.36	2.38	2.36	0.13	0.00	0.06	0.12	*Cystophora monilifera*	lc
2.03	0.00	0.00	0.00	2.50	0.27	1.92	3.24	*Cystophora moniliformis*	lc
0.00	0.00	0.00	0.29	0.00	0.00	0.00	0.10	*Cystophora platylobium*	lc
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	*Cystophora retorta*	lc
0.25	2.76	2.30	3.22	0.29	3.64	2.73	3.43	*Cystophora retroflexa*	lc
0.00	0.56	0.85	0.00	0.00	0.33	1.18	0.00	*Lessonia corrugata*	lc
0.00	0.00	0.65	0.58	0.00	0.00	0.45	1.13	*Sargassum decipiens*	lc
0.00	0.29	0.03	0.11	0.00	0.08	0.00	0.00	*Sargassum sonderi*	lc
0.00	0.00	0.00	0.00	0.29	0.00	0.10	0.00	*Sargassum* spp.	lc
0.18	0.36	0.48	2.16	0.27	0.34	0.33	1.34	*Sargassum verruculosum*	lc
2.78	1.69	1.33	0.44	0.06	1.43	1.76	0.00	*Acrocarpia paniculata*	m
1.90	2.62	2.10	3.47	0.46	1.98	2.65	3.50	*Asparagopsis armata*	m
0.08	0.29	0.20	0.96	0.03	0.47	0.23	0.18	*Callophyllis rangerifica*	m
2.58	2.18	3.85	0.89	1.50	1.57	4.27	0.68	*Carpoglossum confluens*	m
0.00	1.11	0.13	1.64	0.00	1.30	0.00	0.06	*Caulerpa brownii*	m
2.00	1.78	0.50	0.44	3.50	0.91	0.35	0.00	*Caulerpa flexilis*	m
0.25	1.11	0.25	0.00	1.72	0.09	0.00	0.00	*Chaetomorpha coliformis*	m
0.78	1.56	0.30	0.00	0.65	0.98	0.10	0.00	*Cladophora feredayi*	m
0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	*Cutleria multifida*	m
0.00	0.00	0.00	0.07	0.00	0.00	0.00	0.00	*Encyothalia cliftoni*	m
0.93	0.93	1.98	1.38	1.02	1.50	3.19	1.61	Foliose red algae	m

0.05	0.04	0.00	0.20	0.53	0.00	0.05	0.11	*Gracilaria* spp.	m
0.18	0.00	0.50	0.13	0.00	0.00	0.16	0.72	*Griffithsia* spp.	m
0.00	0.00	0.00	0.09	0.00	0.00	0.00	0.02	*Halopteris paniculata*	m
1.00	0.69	0.18	0.56	0.92	0.33	0.00	0.25	*Hemineura frondosa*	m
0.00	0.31	0.85	2.33	1.06	0.21	0.87	1.90	*Hypnea ramentacea*	m
0.00	0.00	0.48	0.00	0.00	0.13	0.10	0.00	*Jeannerettia lobata*	m
0.03	0.07	0.08	0.00	0.00	0.11	0.03	0.02	*Laurencia* spp.	m
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	*Perithalia caudata*	m
0.30	0.89	0.00	0.00	0.00	0.13	0.00	0.00	*Phacelocarpus* spp.	m
0.13	0.24	0.18	1.07	0.00	0.11	0.15	0.26	*Plocamium angustum*	m
0.00	0.00	0.00	0.51	0.00	0.00	0.00	0.13	*Plocamium dilatatum*	m
0.00	0.31	0.08	0.13	0.00	0.05	0.17	0.21	*Plocamium mertensii*	m
0.00	0.04	0.10	0.04	0.00	0.00	0.00	0.03	*Plocamium* spp.	m
0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	*Pyura gibbosa*	m
0.00	0.40	0.00	0.00	0.00	0.29	0.15	0.00	*Seirococcus axillaris*	m
0.68	1.11	0.75	2.96	3.53	0.60	0.09	1.12	Sponge (erect)	m
0.00	0.00	0.00	0.09	0.00	0.00	0.00	0.00	*Sporochnus* spp.	m
0.05	0.13	1.28	0.42	0.00	0.00	0.28	0.19	*Thamnoclonium dichotomum*	m
1.10	0.13	0.25	0.00	1.62	0.39	0.10	0.00	*Ulva* spp.	m
0.03	0.58	0.23	0.47	0.44	0.19	0.60	0.85	*Xiphophora gladiata*	m
0.23	0.00	0.00	0.00	0.00	0.00	0.00	0.02	*Xiphophora* spp.	m
0.85	0.27	0.00	0.00	0.02	0.09	0.00	0.00	Drift	n/a
0.00	0.00	0.00	0.00	0.29	0.24	0.09	0.20	Foliose brown algae	n/a
0.00	0.00	0.00	0.00	0.03	0.00	0.13	0.00	Foliose green algae	n/a
0.00	0.18	0.00	0.00	0.00	0.88	0.00	0.00	Oysters	n/a
0.00	0.00	0.00	0.00	0.00	0.45	0.00	0.00	Sediment	n/a
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	*Arthrocardia wardii*	u
0.90	0.53	0.35	0.84	0.00	0.00	0.00	0.03	Ascidians	u
0.00	0.40	0.00	0.38	0.00	0.00	0.05	0.05	*Ballia callitricha*	u
0.00	0.07	0.00	0.00	0.00	0.00	0.00	0.00	*Botryocladia sonderi*	u
20.70	28.58	16.15	26.44	8.84	13.75	13.66	14.14	Brown turf sediment matrix	u
0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	*Bryopsis gemellipara*	u
0.00	0.00	0.08	0.00	0.00	0.00	0.00	0.00	*Bugula dentata*	u
0.25	0.73	5.38	5.31	0.04	0.21	2.22	2.66	*Caulerpa geminata*	u
1.45	1.18	0.60	1.76	2.37	1.86	0.24	2.66	*Caulerpa longifolia*	u
0.10	1.56	0.28	0.16	0.00	0.48	0.12	0.44	*Caulerpa remotifolia*	u
0.00	0.00	0.00	0.07	0.00	0.00	0.00	0.04	*Caulerpa scalpelliformis*	u
0.93	0.00	2.80	0.93	0.73	0.00	1.14	0.27	*Caulerpa simplisciuscula*	u
0.00	0.00	0.00	0.00	0.27	0.05	0.00	0.11	*Caulerpa* spp.	u
3.53	8.87	21.65	10.73	9.97	12.56	19.37	14.63	*Caulerpa trifaria*	u
0.00	0.00	0.10	0.04	0.00	0.00	0.02	0.00	*Champia* spp.	u
0.00	0.00	0.03	0.02	0.00	0.00	0.00	0.00	*Codium pomoides*	u
0.08	0.04	0.08	0.00	0.00	0.05	0.00	0.00	*Codium* spp.	u
0.00	0.02	0.00	0.27	0.00	0.00	0.00	0.05	*Corallina officinalis*	u
0.00	0.00	0.20	0.00	0.00	0.00	0.00	0.00	Coralline turf and	u

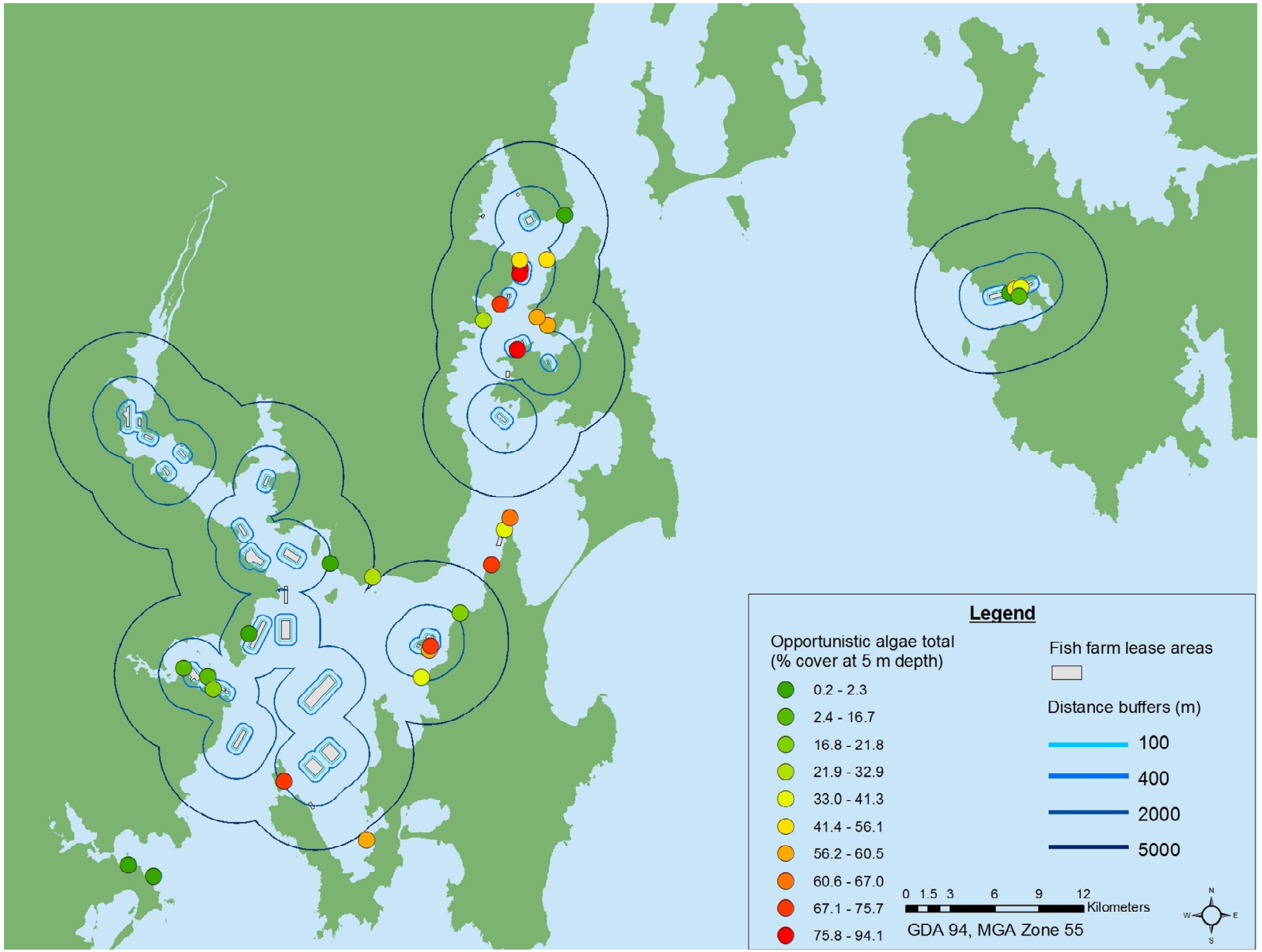
								sediment matrix	
0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.07	Coralline algae (branched)	u
0.00	0.07	0.03	0.13	0.00	0.02	0.00	0.00	*Culicia* spp.	u
0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.02	*Dictymenia* spp.	u
0.10	0.13	0.38	0.00	0.06	0.06	0.36	0.08	*Dictyopteris muelleri*	u
0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.00	*Dictyota* spp. (wide)	u
0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	*Distromium flabellatum*	u
0.00	0.04	0.08	0.00	0.00	0.00	0.05	0.00	*Erythremenia minuta*	u
0.00	0.00	0.03	0.00	0.00	0.00	0.10	0.00	*Gelidium australe*	u
0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	*Gelidium* spp.	u
0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.03	*Gloiosaccion brownii*	u
0.03	0.18	0.00	0.00	0.03	0.06	0.00	0.00	Green turf sedimentmatrix	u
1.05	1.18	0.10	0.07	0.28	0.08	0.07	0.09	*Haliptilon roseum*	u
0.00	1.40	0.00	0.00	0.00	0.33	0.00	0.00	*Halophila australis*	u
0.00	0.00	0.03	0.02	0.00	0.00	0.00	0.00	*Herdmania momus*	u
3.75	7.84	0.85	0.00	4.74	15.33	0.86	0.00	*Heterozostera* spp.	u
0.80	1.36	1.00	0.24	0.10	0.27	0.03	0.08	*Homoeostrichus olsenii*	u
0.03	0.00	0.00	0.29	0.00	0.00	0.06	0.00	*Jania* spp.	u
0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	*Kallymenia* spp.	u
0.00	0.04	0.58	0.09	0.00	0.03	0.35	0.00	*Lenormandia marginata*	u
0.00	0.11	0.00	0.00	0.03	0.00	0.00	0.00	*Lobophora variegata*	u
0.00	0.00	0.00	0.07	0.00	0.00	0.00	0.00	*Mastophoropsis canaliculata*	u
0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.00	*Metagoniolithon radiatum*	u
0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	*Metamastophora flabellata*	u
0.18	0.00	0.00	0.27	0.00	0.00	0.00	0.00	Red turf sediment matrix	u
0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	*Rhodophyllis multipartita*	u
0.20	2.31	2.38	2.67	0.00	0.05	0.08	0.12	*Rhodymenia* spp.	u
0.03	0.47	0.03	0.27	0.00	0.06	0.09	0.00	Soft erect bryozoans	u
0.00	0.24	3.58	1.71	0.00	0.13	1.55	0.15	*Sonderopelta coriacea/Peyssonnelia novaehollandae*	u
0.00	0.00	0.00	0.00	0.00	0.00	0.09	0.00	*Stenogramme interrupta*	u
0.00	0.76	2.33	0.64	0.00	0.74	0.28	0.04	*Strepsichordaia caliciformis (sponge)*	u
0.00	0.00	0.03	0.20	0.00	0.07	0.00	0.00	*Tethya* spp. (sponge)	u
0.08	0.07	0.18	0.49	0.00	0.00	0.03	0.03	*Triphyllozoon* spp. (sponge)	u
0.43	1.53	0.10	0.87	0.11	0.18	0.00	0.21	*Zonaria spp.*	u
13.18	7.40	6.03	9.13	13.96	14.43	11.28	8.71	*Ecklonia radiata*	uc
0.00	0.07	0.95	1.67	0.00	0.17	0.00	2.14	*Macrocystis pyrifera*	uc
4.43	8.96	0.00	14.96	6.89	8.53	0.00	13.75	*Phyllospora comosa*	uc
14.30	10.38	18.38	4.47	23.82	8.18	21.40	6.47	*Sargassum fallax*	uc
0.00	0.00	0.00	0.00	1.21	0.00	0.00	0.00	*Sargassum lacerifolium*	uc
0.00	0.31	0.33	1.00	0.08	0.14	0.74	1.21	*Sargassum vestium*	uc



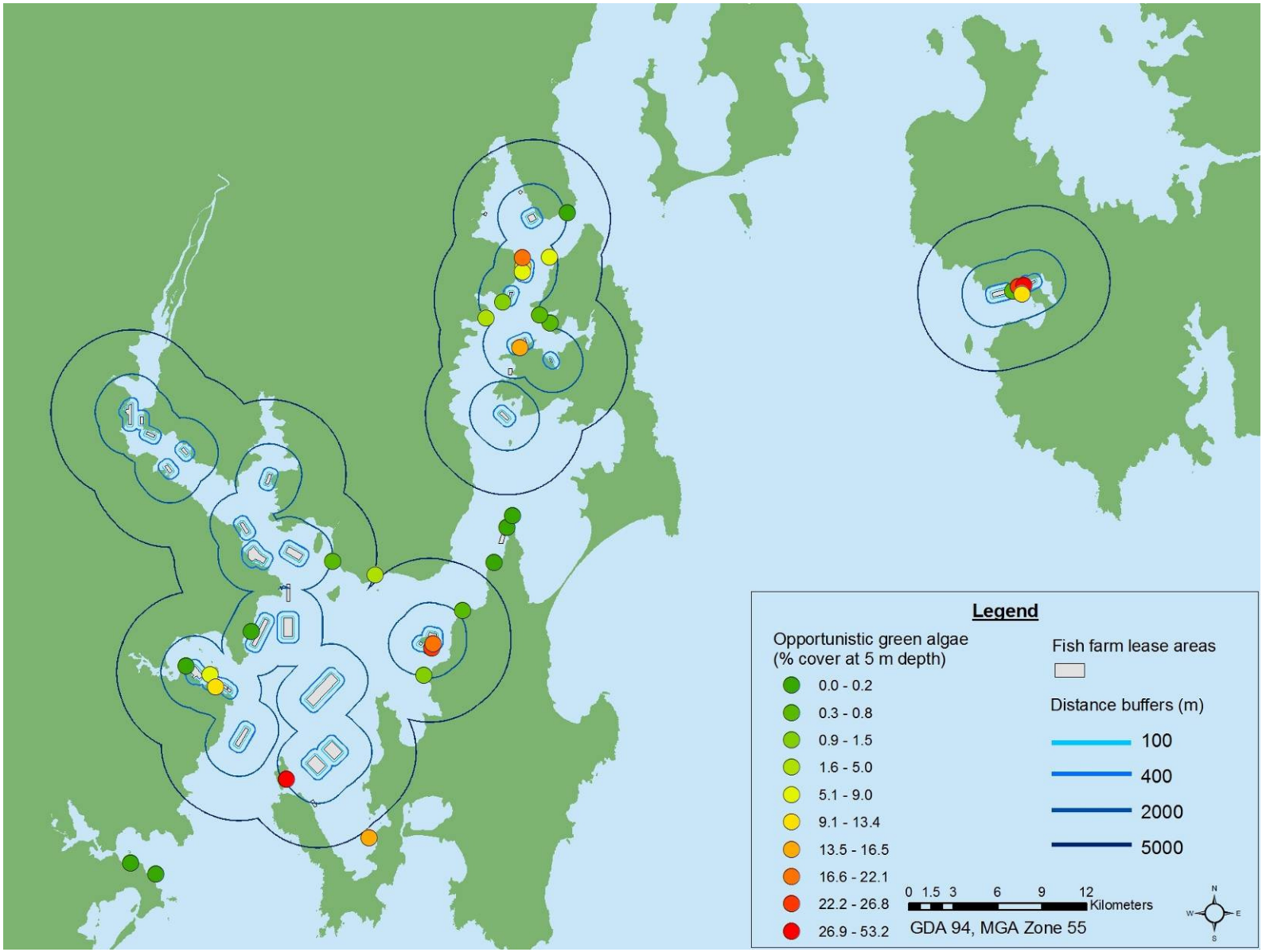
Appendix 4 Box plots of percentage cover of indicator variables (raw data) against exposure categories. Connect line for mean values shown. Outliers are represented as an asterix.



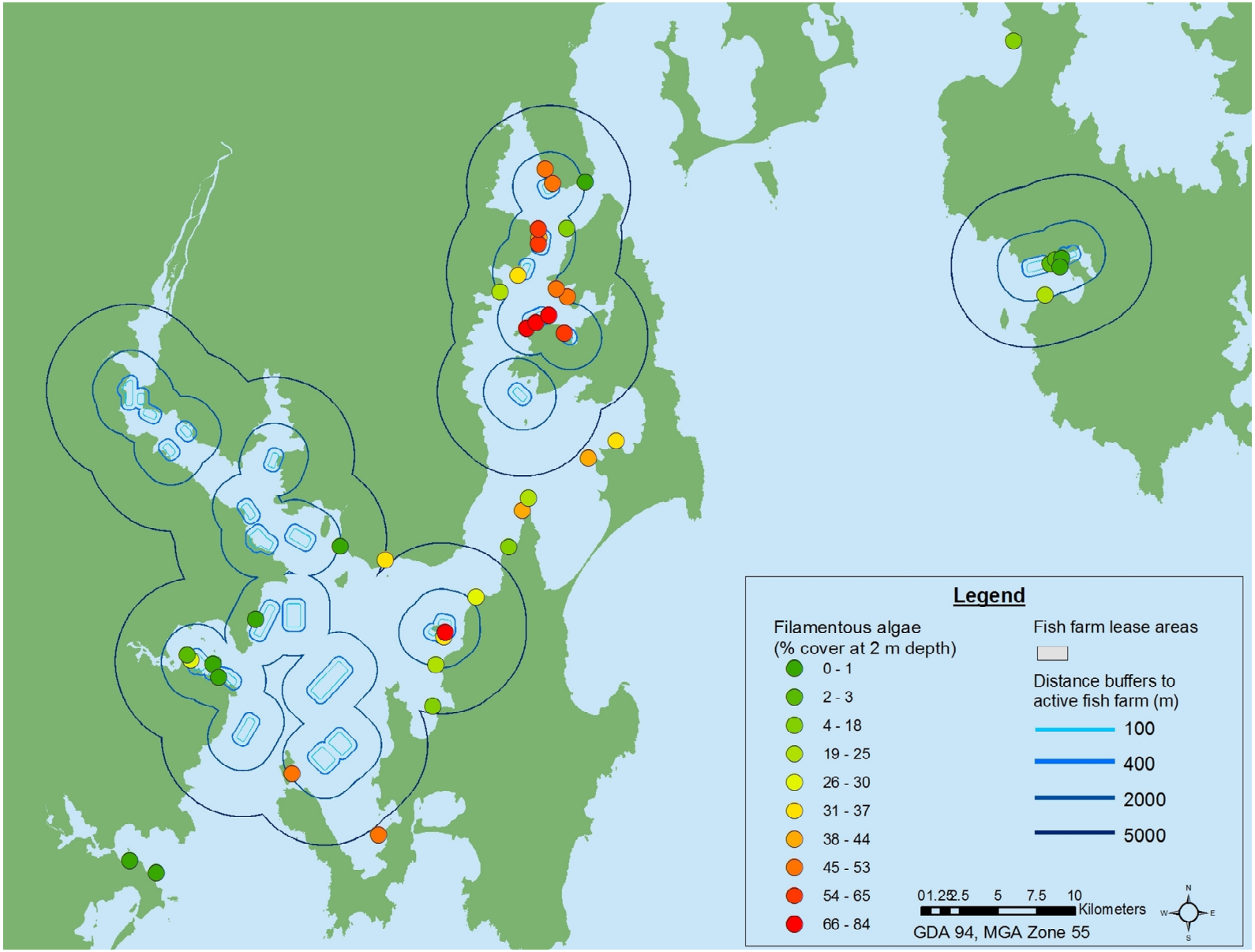
Appendix 5 Box plots of percentage cover of indicator variables (raw data) against depth categories. Connect line for mean values shown. Outliers are represented as an asterix.



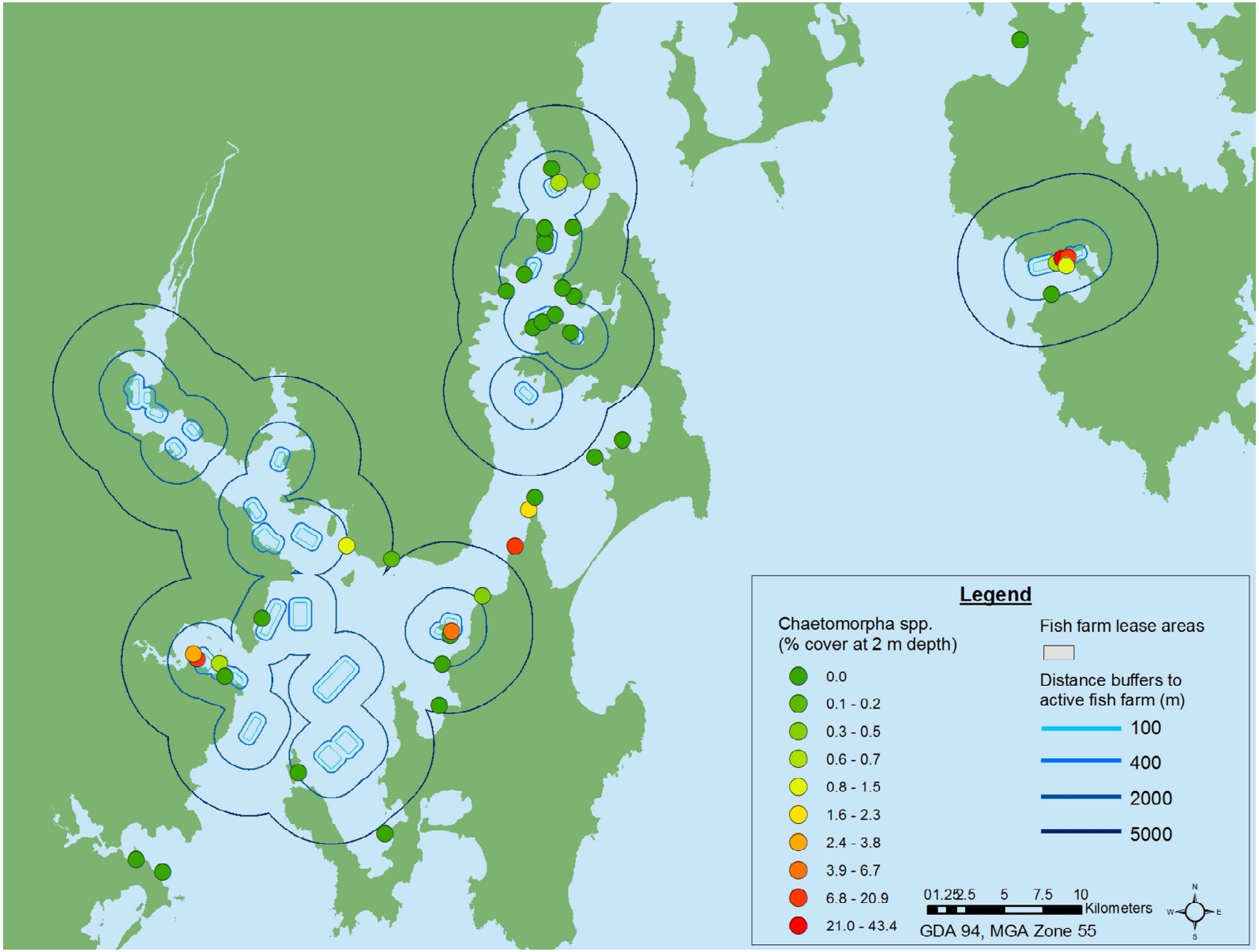
Appendix 6
Percentage cover of opportunistic algae in total throughout the sample sites on 5 m depth transects.
Data is divided into 10 categories using ArcGIS 9.1 natural breaks (Jenks) function.



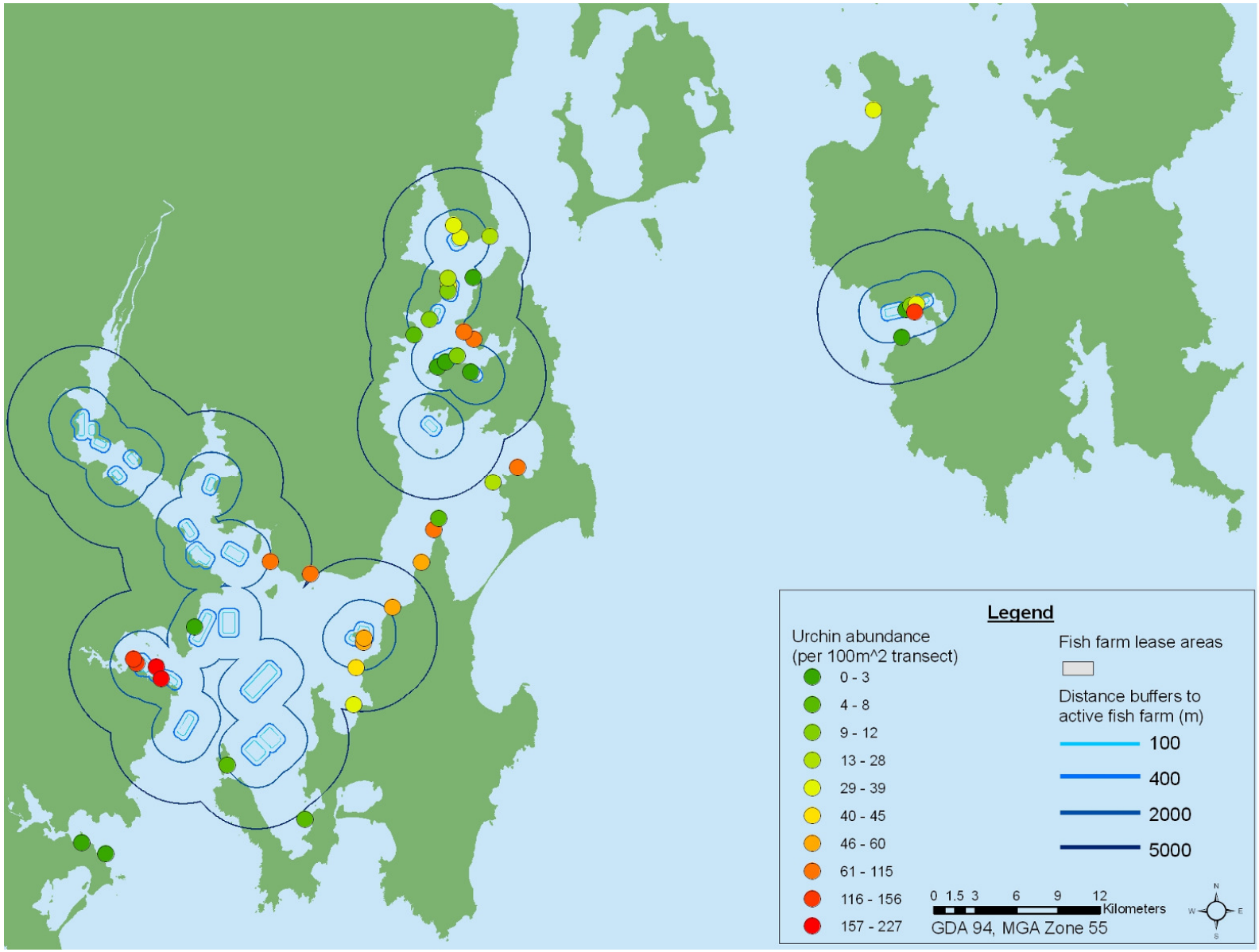
Appendix 7 Percentage cover of opportunistic green algae in total throughout the sample sites on 5 m depth transects. Data is divided into 10 categories using ArcGIS 9.1 natural breaks (Jenks) function.



Appendix 8 Percentage cover of filamentous algae in total throughout the sample sites on 2 m depth transects. Data is divided into 10 categories using ArcGIS 9.1 natural breaks (Jenks) function.



Appendix 9 Percentage cover of *Chaetomorpha* spp. algae in total throughout the sample sites on 2 m depth transects. Data is divided into 10 categories using ArcGIS 9.1 natural breaks (Jenks) function.



Appendix 10

Common sea urchin (*Heliocidaris erythrogramma*) abundance at each sample site. Counts were conducted along a 2m or 5m depth transect which was 2 m wide and 50 m long (data collected by Reef Life Survey, Tasmanian Aquaculture and Fisheries Institute (TAFI)).