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ECOPHYSIOLOGY OF *ECKLONIA RADIATA* (ALARIACEAE: LAMINARIALES) IN DOUBTFUL SOUND, FIORDLAND.

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ABSTRACT

This is the first study to quantify temporal and spatial variations in growth rates and productivity, inorganic N uptake and photosynthetic performance of the subtidal brown macroalga, *Ecklonia radiata*, in relation to an environmental gradient in Doubtful Sound, Fiordland. Doubtful Sound has a unique marine environment created by a low-salinity layer (LSL) that reduces light transmission through the water column. Three sites were chosen from across the environmental gradient; two sites near the entrance of the sound, Causet Cove and Thompson Sound, and a site further in towards the head of the fiord, Seymour Island.

Blade and stipe growth rates, erosion rates, morphological parameters and productivity of *Ecklonia radiata* were measured at the three sites between September 1998 and March 2000. *E. radiata* displayed a temporal variation in growth rates, with maximum growth rates in September and October 1998 and minimum rates between June and July 1999. Blade growth rates in September and October 1998 were double those for the same period the following year. Blade growth rates at Seymour Island were lower than rates at Causet Cove and Thompson Sound on five occasions. Stipe growth rates also displayed a temporal pattern with higher rates between October and February and lower rates between June and August. Erosion rates were generally similar between sites throughout the study. Eight morphological parameters were measured but only stipe length and blade thickness were significantly different between sites. Stipes of *E. radiata* at Seymour Island were longer and the primary blades thinner than those at Causet Cove and Thompson Sound. Daily individual sporophyte productivity (g DW d⁻¹ and g C d⁻¹) exhibited a similar temporal pattern to growth rates. *E. radiata* at Seymour Island exhibited the lowest daily population productivity rates (g DW m⁻² d⁻¹). The population density of *E. radiata* was low and reached a maximum of 11 individuals per m² at Causet Cove and Thompson Sound. The low population productivity was attributed to the low population density of *E. radiata* in Doubtful Sound.

C:N ratios and low tissue N indicated that *E. radiata* was N-limited year-round, although more strongly between February and April 1999. Inorganic N pools showed a temporal variation, with less inorganic N accumulated between November 1999 and March 2000. *Ecklonia radiata* showed a preference for NH₄⁺ over NO₃⁻; this was reflected in the larger
intracellular NH$_4^+$ pools. The low intracellular NO$_3^-$ pools reflected the low ambient seawater inorganic NO$_3^-$ concentrations.

Time-course experiments examined the ability of field collected *Ecklonia radiata* tissue to remove NO$_3^-$ and NH$_4^+$ at 5 and 30 μM from media over 300 minutes. *E. radiata* showed three patterns of depletion: linear, surge and no depletion. High rates of NO$_3^-$ depletion occurred at both 5 and 30 μM between March and May, while NH$_4^+$ depletion rates were higher at 5 μM than at 30 μM and showed no temporal pattern. Multiple-flask experiments were conducted between June 1999 and May 2000 to determine uptake kinetic parameters for *E. radiata*. Uptake rates were higher for NH$_4^+$ than for NO$_3^-$ uptake rates. There was no consistent temporal or spatial variation in K$_s$ and α indicating that *E. radiata* had no preference for either form of inorganic N investigated in this study. The ability of *E. radiata* to take up inorganic N at an ecologically (relevant) low concentration was evaluated using the uptake rate at 2 μM ($V_2$). $V_2$ values were low overall and this may reflect a low N requirement as a result of low growth rates, due in part to low ambient seawater inorganic N concentrations throughout the study period.

The temporal photosynthetic performance of *E. radiata* was investigated using photosynthesis versus photon flux density (PFD) curves (PE) and pigment content between September 1998 and March 2000. $P_{\text{max}}$ at all study sites was highest between June and August 1999 and ranged from 0.8 – 1.2 mgO$_2$ gDW$^{-1}$ h$^{-1}$. *E. radiata* at Causet Cove and Thompson Sound also exhibited higher $\alpha_p$ compared to kelp at Seymour Island during these months. Chl a and c contents displayed a temporal pattern with contents maximal in November and December 1999 while no defined temporal pattern for fucoxanthin content was exhibited. *E. radiata* at Seymour Island exhibited greater photoinhibition compared to *E. radiata* at Causet Cove or Thompson Sound, with inhibition occurring at PFD ≥ 100 μmol photons m$^{-2}$ s$^{-1}$.

The interactive effect of N availability and light on growth rates and productivity of *Ecklonia radiata* is summarized and the implications of the low productivity exhibited by *E. radiata* are discussed in relation to the nearshore marine ecosystem.
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This thesis is dedicated to my grandparents.
Doubtful Sound from Wilmot Pass, Fiordland, New Zealand (photo author).

Inside Doubtful Sound – looking back towards Deep Cove (photo author).
Tagged *Ecklonia radiata* at Thompson Sound (photo author).

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Chapter One

General Introduction

1.1 Introduction

Macroalgae form the basis of marine food webs in intertidal and subtidal benthic ecosystems (Correa and Potin 2000). In temperate regions of the northern and southern hemisphere, the subtidal zone is dominated primarily by laminarian kelps, which are typically characterized by large biomass and high primary productivity (e.g. Mann, 1973; Kain, 1979; Lüning, 1979; van den Hoek, 1982; Egan and Yarish, 1988). In New Zealand, *Ecklonia radiata* (Alariaceae, Laminariales) is a dominant, subtidal, canopy-forming kelp that occurs from the low intertidal to > 25 m on moderately sheltered and exposed coasts (Choat and Schiel, 1982; Adams, 1994; Schiel, 1990). *E. radiata* forests are important ecologically to inshore marine fauna, such as wrass and monocanthids which recruit among the fronds and feed extensively on invertebrates associated with *E. radiata* (Jones, 1984a, b). Economically, interest in alginate extraction from *E. radiata* has been expressed although there has only been minimal harvesting in New Zealand (Schiel and Nelson, 1990). In the northern regions of New Zealand, *E. radiata* may reach densities of up to 75 mature individuals m\(^{-2}\) with a biomass of 1 kg (dry wt) m\(^{-2}\) (Choat and Schiel, 1982; Novaczek, 1984).

Previous work on the genus *Ecklonia* has focused primarily on growth rates and productivity of species from Australia, Japan, South Africa and northern New Zealand (Mann *et al.*, 1979; Kirkman, 1984, 1989; Novaczek, 1984; Larkum, 1986; Hatcher *et al.*, 1987; Maegawa and Kida, 1987). Growth rates of *E. radiata* range from 0.76 to 1.96
cm d\(^{-1}\) (Larkum, 1986) while annual productivity ranges from 0.3 to 3.4 kg dw m\(^{-2}\) y\(^{-1}\) (Kirkman, 1984, 1989; Novaczeck, 1984; Larkum, 1986). Population density of \(E.\ radiata\) on the northeastern coast of New Zealand may reach up to 20 individuals per m\(^2\), with the greatest density occurring at depths of 3 - 6 m and from 10 - 17 m depth (Choat and Schiel, 1982). To date, there are no published studies of ecophysiological aspects of \(E.\ radiata\) from southern New Zealand.

Studies of \(Ecklonia\ radiata\) from northern New Zealand and Australia have found that the optimum temperature for growth and reproduction of \(E.\ radiata\) sporophytes was 12 – 20 °C (Noveaczek, 1984). Noveaczek (1984) also demonstrated that photon flux density (PFD) levels ≥ 1000 \(\mu\)mol photons \(m^{-2}\) \(s^{-1}\) were fatal to gametophytes and sporophytes less than 2 mm in length. Respiration rates of \(E.\ radiata\) increased with temperature up to 25°C and with pH up to 8.3 (Berquist, 1957). Percentage pigment composition of \(E.\ radiata\) did not change with depth although the total amount of pigment per unit area decreased with depth (Alcock, 1966), while the chemical constituents such as alginic acid, mannitol, laminarian and fucoidan, varied seasonally (Stewart \textit{et al.}, 1961). Studies on \(Ecklonia\ maxima\) from South Africa demonstrated that inorganic nitrogen (N) uptake was linearly related to ambient seawater concentrations and did not saturate at the highest concentration encountered \textit{in situ} (\(NO_3^-\) - 1.7 \(\mu\)M, \(NH_4^+\) - 0.2 \(\mu\)M; Probyn and McQuaid, 1985).

1.2 Taxonomy and Description of \textit{Ecklonia radiata}

\textit{Heterokontophyta}

\textit{Laminariales}

\textit{Alariaceae}

\textit{Ecklonia radiata} (C. Agardh) J. Agardh

\textit{Ecklonia radiata} is a perennial brown macroalga divided into holdfast, stipe and blade (Fig. 1.1). The holdfast is compact with (usually) dichotomous haptera from which a generally smooth, cylindrical, unbranched stipe, 5 to 60 cm in length, arises. The stipe then flattens and expands into a smooth or corrugated primary blade, the morphology (width, length, texture) of which varies primarily with water motion and depth, but also
Figure 1.1: Picture of *Ecklonia radiata* (Adams, 1994) and a close-up of a holdfast (photo: author).
with habitat and age (Lindauer et al., 1961). Smooth blades predominate in wave-sheltered areas while blades in wave-exposed regions are often corrugated (Lindauer et al. 1961). Mature sporophytes have lateral, foliar 'secondary' blades. *E. radiata* is golden brown in colour, occasionally variegated with lighter longitudinal stripes (Lindauer et al., 1961). The gametophyte is microscopic and similar in form to other laminarians.

### 1.2.1 Life History of *Ecklonia radiata*

*Ecklonia radiata* exhibits an alteration of generations life cycle (Fig. 1.2). Thalli are monoecious, producing unilocular sporangia in diffuse, mucilaginous sori on the surface of the primary blade (Adams, 1994). *E. radiata* is fertile from May to November in New Zealand but spores are only released over a short time period (approximately one-month), generally during October (Novaczek, 1984; Kennelly, 1987; Schiel, 1988; Jones and Andrews, 1990; Schiel and Nelson, 1990). The average dispersal of spores is only few meters from their origin (Schiel and Nelson, 1990).

### 1.3 Study Site

Doubtful Sound (45° 18' 00" S; 166° 58' 45" E) is one of fourteen fiords that indent the southwestern coastline of South Island, New Zealand (Fig. 1.3). The fiords were created during the retreat of the last glacial period, some 20 000 years ago and are characterized by steep mountainous walls, deep basins and shallow entrance sills (Grange and Singleton, 1988; Grange 1985; Ryan and Paulin, 1998). Doubtful Sound is the second largest of the fiords, penetrating approximately 40 km inland, and incorporating a number of branching arms. It has a maximum depth of 421 meters and a mean width of 1.2 km, which provides an area of 83.7 km² within the fiord (Stanton and Pickrill, 1981; Grange, 1990; Gibbs et al., 2000). Doubtful Sound connects with two other sounds, Thompson and Bradshaw, and is often termed the Doubtful Sound system or complex. However for simplicity, the term Doubtful Sound will be used throughout this dissertation to refer to the system as a whole.

While all of the fiords in the Fiordland region are subject to high orographic rainfall, Doubtful Sound has an additional freshwater input. Doubtful Sound receives an average rainfall of 465 mm mth⁻¹, an annual riverine input of 135 m³ s⁻¹ and an annual
Figure 1.2: Life history of *Ecklonia radiata*, which displays an alternation of generations life cycle (Kingsford and Battershill, 1998).
Figure 1.3: Map of Doubtful Sound, Fiordland, New Zealand, showing the locations of *Ecklonia radiata* populations studied in the first three experimental chapters (2-4). The main axis of Doubtful Sound is 40 km in length.
anthropogenic input from the Manapouri Hydroelectric Power Station of $\sim 450$ $\text{m}^3 \text{s}^{-1}$ (Bowman and Dietrich, 1995; Lamare, 1998; Gibbs et al., 2000). The large freshwater input results in the occurrence of a quasi-permanent, tannin-stained, low-salinity surface layer (LSL) that persists year-round (Gibbs et al., 2000). Salinity of the LSL ranges from 5 to 10 practical salinity units (psu) while the thickness varies both spatially and temporally from 0 to 12 m (Lamare, 1998). Sea surface water temperature in the LSL varies between 9 and 17°C annually with a relatively stable water temperature underneath (B. Stewart, pers. comm.). An estuarine circulation prevails with seaward movement of the LSL over incoming seawater, which is restricted due to the shallow sill at the entrance of the fiord and the arms (Schneider, 1999).

The transmission of incident light through the water column is significantly reduced due to the tannin-stained LSL, creating a globally unique marine environment (Grange et al., 1981). A low PFD regime prevails at depth, where even in summer light levels at 15 m are typically 1-2% of surface PFD (Grange, 1990). Due to the mountainous terrain, PFD is reduced in certain areas of the fiord, particularly the northern aspect, adding to the already low subtidal PFD levels. Underwater there is often a green tinge at the inner sites due to the attenuation of blue light through the LSL (Ryan and Paulin, 1998; author pers. obs.). The unusual combination of climate, topography, terrestrial vegetation, and physical and biological oceanographic processes play a major role in controlling the intertidal and subtidal community structure in Doubtful Sound (Grange and Singleton, 1988; Parker et al., 1997; Gibbs et al., 2000). This is most evident, and well studied, in the black coral (*Antipathes fiordensis*). *A. fiordensis* is normally a deep-dwelling organism (>100 m) but can be observed at depths as shallow as 10 m in Doubtful Sound (e.g. Grange, 1986; Grange and Singleton, 1988; Parker et al., 1997; Kai, 2000; Kregting, 2001).

### 1.4 Objectives of this study

The overall aim of this PhD was to investigate three basic eco-physiological aspects (growth, nitrate and ammonium uptake rates, and photosynthetic rates) of *Ecklonia radiata* from three sites in Doubtful Sound across an environmental gradient created by the LSL within a globally unique marine environment. Growth rates and subsequent productivity
estimates of *E. radiata* are investigated in chapter 2 and were measured monthly from September 1998 to December 1998 then bi-monthly thereafter until March 2000. Growth was measured using the hole punch technique (Parke, 1948) while production rates were determined using the area of constant biomass model (Mann *et al.*, 1979). In addition, sporophyte morphology (blade and stipe) and environmental parameters (ambient PFD levels and ambient seawater NO$_3^-$ and NH$_4^+$ concentrations) were measured. In chapter 3, inorganic N (NO$_3^-$ and NH$_4^+$) uptake rates of *E. radiata* were determined on a temporal and spatial basis using both time-course and multiple flask methods. From the multiple flask experiments the kinetic parameters, maximum uptake rate ($V_{\text{max}}$), half saturation constant ($K_s$) and alpha ($\alpha = V_{\text{max}}/K_s$) were determined. The ability of *E. radiata* to take up NO$_3^-$ and NH$_4^+$ at an ecologically relevant concentration, in this case 2 $\mu$M ($V_2$), was also determined. Percent tissue carbon (C) and N, C:N ratio and soluble tissue NO$_3^-$ and NH$_4^+$ of *E. radiata* were also determined on a temporal and spatial scale. Chapter 4 considers the photosynthetic rate of *E. radiata* measured using oxygen ($O_2$) evolution while an underwater pulse amplitude modulated (PAM) fluorometer was used to measure $F_v/F_m$ to provide an indication of the 'health' of *E. radiata*. The photosynthetic parameters, maximum photosynthetic rate ($P_{\text{max}}$), respiration ($R_d$), alpha ($\alpha_p$), saturation PFD ($E_k$) and compensation PFD ($E_c$), were determined from photosynthesis versus PFD (PE) curves and were used to investigate the ability of *E. radiata* to use varying levels of PFD on a temporal and spatial basis. Chlorophyll $a$, chl $c$ and fucoxanthin were determined to investigate photoacclimation on a temporal and spatial basis. In chapter 5, photoacclimation of *E. radiata* at five sites throughout the Doubtful Sound complex was investigated using PE curves, pigment concentrations, % tissue C and N, C:N ratios and morphological parameters. The summary relates inorganic N uptake rates and photosynthetic capacity of *E. radiata* to its growth rates, productivity and distribution, and highlights areas of future research.
Chapter Two

Growth rate and productivity of *Ecklonia radiata*.

2.1 INTRODUCTION

Laminarian kelps are the dominant subtidal macroalgae in many temperate regions and are major contributors to the productivity of near-shore marine ecosystems either directly through herbivory or indirectly via bacterial decomposition of detrital material (Mann, 1972, 1973; Chapman and Craigie, 1977; Chapman, 1978, 1987; Dunton and Schell, 1986; Duggins *et al*., 1990; Yoshikawa *et al*., 2001). Investigations have been conducted to determine the seasonal variation in kelp growth rates and productivity due to their importance to the marine ecosystem (e.g. Sargent and Lantrip, 1952; Buggeln, 1974; Chapman and Lindley, 1980; Larkum, 1986; Stuart, 1997). The results of these studies have shown that kelp growth and productivity are controlled by a number of abiotic and biotic factors, the main factors being light (specifically, the number of photons of photosynthetically active radiation [PAR] received at the macroalgal surface, hereafter referred to as photon flux density [PFD]) and spectral composition (Falkowski and Raven, 1997; Franklin and Forster, 1997); nutrient availability (Jackson, 1977; Chapman, 1978; Wheeler and North, 1980, 1981; Hanisak, 1983), temperature (Dean and Jacobson, 1984; van Tussenbroek, 1989; Davison, 1991) and water motion (Gerard and Mann, 1979; Wheeler, 1980a, 1988; Koehl and Alberte, 1988; Hurd *et al*., 1996; Hurd, 2000).

Growth rates of perennial laminarians typically follow a seasonal pattern with maximum rates in late winter/early spring and minimum rates in late summer/autumn (e.g. Gangé *et al*., 1982; Kain, 1989; Lüning, 1993). *Ecklonia radiata* from Australia, *Macrocystis*
*pyrifera* from the Falkland Islands and California, and *M. integrifolia* from Canada display this type of seasonal growth (Zimmerman and Kremer, 1986; Lobban, 1987; van Tussenbroek, 1989). However, growth rates of laminarians vary with species and locality and do not always follow the typical seasonal pattern (Kain, 1989). For example, the growth rate of *Laminaria longicuris* from Quebec peaks in summer and declines during winter (Gendron, 1985) while *Macrocystis pyrifera* from New Zealand exhibits higher growth during autumn (Brown *et al.*, 1997). These growth patterns may be related to seasonal variations in limiting environmental factors, particularly nutrient availability and PFD, as well as water motion (Kain, 1989).

### 2.1.1 Measuring kelp growth rate

Growth of *Ecklonia radiata* occurs from a meristem situated at the base of the blade. New tissue is continually added at the blade base while older tissue is eroded at the distal end (Parke, 1948; Mann, 1972; Dieckman, 1980; Gangé and Mann, 1987; Stuart, 1997). There are various methods for determining growth rates of macroalgae and these can be both non-destructive and/or destructive (Parke, 1948; Chapman, 1973; Brinkhuis, 1985). In kelps with a basal meristem such as *E. radiata* and *Laminaria* spp., the common method for measuring growth is to punch a hole distal to the meristematic region and measure the distance moved by the hole over time (Fig. 2.1). Mann (1972) estimated that *Laminaria longicuris, L. digitata* and *Agarum cribrosum* completely renew their blade tissue between one and five times a year. The resultant elongation of the blade provides a realistic measure of the amount of new tissue added at the meristem (Mann and Kirkman, 1981).

### 2.1.2 Modelling kelp production

The term (net) production is defined here as the quantity of new organic matter incorporated into biomass, and includes both the increase in plant biomass over a given period as well as any losses occurring as a result of erosion or grazing (Russell-Hunter,
Figure 2.1: Movement of punched holes in growing blades of *Ecklonia radiata* over time (not to scale). (Redrawn from Mann, 1973).
Chapter Two: Growth rate and productivity of *Ecklonia radiata*

1970; Issacs *et al.*, 1999). Production rates of kelps are frequently modelled using growth increments. There are three models commonly used to estimate kelp productivity (Table 2.1). The power (or exponential) and chordal models estimate productivity from an empirical relationship between blade biomass, blade length and blade growth (Mann, 1972). However, these two models do not take into account the effect of blade erosion or grazing which leads to inflated values of biomass production (Mann and Mann, 1981). The area of constant biomass model, developed by Mann *et al.* (1979), avoids the use of a generalised relationship between blade weight and biomass (Mann and Mann, 1981). The model is based on the assumption that the blade can be divided into three zones; (1) a basal zone where all growth results in increasing blade biomass, (2) a zone of maximum biomass, and (3) a zone of erosion (Fig. 2.2). Biomass per unit length, using the zone of maximum biomass, can be calculated and multiplied by the growth increment, as obtained using the hole punch technique, to estimate blade production. Comparisons of the three models using *Laminaria longicruris* by Gangé and Mann (1987) show that the power and chordal models overestimate production by as much as 65-100%. They consider the area of constant biomass model gives the best estimate of productivity therefore, this model has been used to estimate the productivity of *Ecklonia radiata* in the present study.
Table 2.1: The three commonly used models for estimating the productivity of kelps.

<table>
<thead>
<tr>
<th>Model</th>
<th>Equation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Power (exponential)</td>
<td>$P = a(L_1 + G)^b - aL_1^b$</td>
<td>Mann, 1972</td>
</tr>
<tr>
<td>Chordal</td>
<td>$P = G[a(L_1 + 1)^b - aL_1^b]$</td>
<td>Mann et al., 1979</td>
</tr>
<tr>
<td>Area of constant biomass</td>
<td>$P = W_{\text{max}}/l \times G$</td>
<td>Mann and Kirkman, 1981</td>
</tr>
</tbody>
</table>

where $P =$ productivity

a and b = co-efficients of weight to length relationship ($W = aL^b$)

$L_1 =$ initial blade length

$G =$ linear growth increment of blade (using the hole punch method)

$W_{\text{max}} =$ weight of section cut in area of maximum biomass (DW)

$l =$ length of cut section (5 cm)
Figure 2.2: Diagram illustrating the Area of Constant Biomass model. A) *Ecklonia radiata* at the beginning of the growth period. B) The distribution of weight per unit length (W/l) along the blade axis, defining an area of growth (a→b), maximum biomass (b→c) and erosion (c→). C) The original plant in A (unshaded) with new growth at the blade base (shaded). D) The new biomass distribution showing the area of growth (a′→b′), region of added biomass (b′→b), region of maximum biomass (b′→c) and erosion (c→). (Redrawn from Mann and Mann, 1981).
Chapter Two: Growth rate and productivity.

Beginning of growth period

End of growth period
2.1.3 Factors influencing macroalgal growth and productivity

Changes in growth and production rates of macroalgae are often related to changes in available PFD (Russell-Hunter, 1970; Falkowski and Raven, 1997). More specifically, it is the amount of PFD received at the blade surface and its spectral composition that affects growth and production rates. Some laminarians, such as Laminaria longicruris, L. pallida and Ecklonia radiata, at shallow depths grow more rapidly and have higher productivity than those at greater depths (Dieckman, 1980; Novaczek, 1980; Gendron, 1985). Kirkman (1989) found that artificial shading of E. radiata at 5 m reduced productivity to levels similar to kelps growing at 10 m. These differences in growth and productivity between depths were attributed to a reduction in PFD with depth. The LSL present in Doubtful Sound influences PFD penetration through the water column in much the same way as artificial shading does, therefore it is highly likely that growth and production rates of E. radiata in the present study will be lower than rates for other temperate laminarians due to the reduced PFD levels imposed by the LSL.

Light reaching the thallus of macroalgae is reduced by particulate matter in the water column that leads to absorption, scattering and reflection of light. Macroalgae that form canopies also reduce light levels through self-shading and shading of other macroalgae underneath. Wave or swell-induced movement of the macroalgal thallus disrupts self-shading producing a fluctuating light regime via light flecks (Dromgoole, 1987, 1988; Koehl and Alberte, 1988; Greene and Gerard, 1990; Wing and Patterson, 1993; Wing et al., 1993). A few macroalgae, including Ecklonia radiata, show increased growth and photosynthetic rates in fluctuating relative to steady PFD (Dromgoole, 1987, 1988; Greene and Gerard, 1990). In contrast, studies on the rhodophytes, Palmaria palmata and Lomentaria articulata found no enhancement of growth or photosynthetic rates in fluctuating relative to constant PFD (Kübler and Raven, 1996a and b). The ability of macroalgae to adjust their photosynthetic performance to the dominant light regime may contribute to significant gains in growth and primary productivity via carbon (C) gain (Dromgoole, 1987, 1988; Greene and Gerard, 1990; Wing and Patterson, 1993).
Water motion regulates the growth, production and distribution of macroalgae both directly and indirectly (Madsen et al., 2001). Fast flowing water (> 6 cm s\(^{-1}\)) can dislodge whole macroalgae and prevent spore settlement as well as decrease the thickness of the diffusion boundary layer (DBL), while slow flows (< 2 cm s\(^{-1}\)) increase the DBL thickness thereby reducing rates of photosynthesis and nutrient uptake, and ultimately growth and production rates (Wheeler, 1980a and b, 1988; Vadas et al., 1990; Koch, 1993; Gonen et al., 1995; Viejo et al., 1995; Blanchette et al., 1996; Hurd et al., 1996; Hurd, 2000). Studies have shown that macroalgae modify their hydrodynamic environment, alleviating drag and diffusion stresses while generating their own turbulence (Wheeler, 1980a, 1988; Anderson and Charters, 1982; Hurd et al., 1996, 1997). This has the potential to enhance growth and production rates through increases in the availability of nutrients and gases at the macroalgal blade surface due to a reduction in the DBL (Larkum, 1981) although this has yet to be demonstrated. The term water motion as used here only considers unidirectional and surge flow, and it is acknowledged that that there are several types of water motion such as laminar, turbulent, currents, surge and oscillatory motion, all of which may influence macroalgal growth and production rates. For a more detailed discussion on the influence of water motion on macroalgal physiology and production see Hurd (2000).

Macroalgal morphology typically varies with the hydrodynamic environment in which they grow (Hurd 2000 and references therein). Ecological and physiological functions of macroalgae are often closely related to their morphology. Kelps, such as *Ecklonia radiata* and *Macrocystis pyrifera*, often display wave-sheltered and wave-exposed morphologies. Wave-sheltered morphologies typically have a wider, thinner and undulate blade while wave-exposed blade morphologies are flat, thick and strap-like (e.g. Norton et al., 1981; Koehl and Alberte, 1988). Each morphological variation influences macroalgal growth and ultimately, productivity rates. For example, blades of *Nereocystis luetkeana* in fast flowing water (maximum 0.39 m s\(^{-1}\) in the middle of the bed) ‘clump’ together and experience lower drag forces but increased self-shading while blades in slow-moving water (maximum 0.10 m s\(^{-1}\)) spread out and ‘flap’ at amplitudes 2–12 times greater than those of wave-exposed blades (Koehl and Alberte, 1988). Additionally, C
uptake and photosynthetic rates were greater in *N. luetkeana* blades that flap in slow moving water compared to blades held stationary. Increased photosynthetic rates (on a dry weight basis) have also been demonstrated in macroalgae with thinner thalli compared to macroalgae with thick thalli (e.g. Littler, 1980; Johansson and Snoejis, 2002). Morphological variations, such as blade undulations, may reduce DBL thickness and increase the surface area available for light harvesting. Furthermore, an increase in surface area to volume (SA / V) may also reduce the demand for resources required for structural material and maximises light harvesting (e.g. Gerard and Mann, 1979).

2.1.4 Objectives of chapter two

The underwater light climate was examined at three sites in Doubtful Sound by measuring PFD over a depth of 9 m. PFD was expected to be higher from September through to February (spring/summer) and lower from June to August (winter). PFD at depth was predicted to be lower at Seymour Island due to reduced light penetration through the LSL. Spectral composition of PFD (PAR, 350-700 nm) was also measured at each site and greater attenuation within the blue region was expected at Seymour Island, again due to a thicker, more tannin-stained LSL. Water velocity adjacent to the primary blade of *Ecklonia radiata* was investigated by determining the dissolution rate of plaster of Paris cubes attached to the primary blade. Water velocity was expected to be higher at Causet Cove and Thompson Sound as these sites are more exposed to the open ocean.

Spatial and temporal variations in growth, erosion and production rates of three *Ecklonia radiata* populations in Doubtful Sound were examined using the hole-punch technique and the area of constant biomass model over a one and a half year period. Growth and production rates of *E. radiata* were expected to display a temporal pattern with maximum rates from June to August (winter) and minimum rates from November to February (late spring/summer). Spatially however, it was predicted that *E. radiata* at Seymour Island would have lower growth and production rates than *E. radiata* at Causet Cove and Thompson Sound. This would result from a greater reduction in PFD reaching the blade surface of *E. radiata* at Seymour Island and a change in the spectral composition as a result of greater attenuation of blue light due to a thicker LSL at this site thereby
influencing nutrient uptake and photosynthetic rates. Erosion rates were expected to be greater from September through to February (spring/summer) coinciding with higher PFD and nutrient availability, and greater blade growth. It was predicted that *E. radiata* at Causet Cove and Thompson Sound would have higher erosion rates as these two sites are closer to the open ocean and subjected to surge and swell opposed to the unidirectional current at Seymour Island. The substratum also differs between the sites; both Causet Cove and Thompson Sound are composed of large rocks while Seymour Island consists of shelly debris and sand, therefore greater abrasion of the blade was expected at the two outer sites.

The population density of mature *Ecklonia radiata* at each of the three study sites was also recorded. It was expected that the density of *E. radiata* at Causet Cove and Thompson Sound would be greater than at Seymour Island due to the unidirectional current and substratum at Seymour Island not being conducive for spore settlement. The LSL was also expected to influence the spectral composition at Seymour Island by attenuating wavelengths within the blue region. This may affect the reproductive ability of *E. radiata* at this site, resulting in a lower density although reproductive ability was not investigated in the present study.

Finally, morphological characteristics such as the primary blade thickness and stipe length and diameter of *Ecklonia radiata* were measured. *E. radiata* at Seymour Island was expected to have longer stipes and longer and wider but thinner blades than *E. radiata* at Causet Cove or Thompson Sound. The morphological differences could be interpreted as an adaptation to reduced PFD at depth imposed by the thicker LSL at this site. An increase in stipe length would lift the photosynthetic blade closer to the water surface and higher PFD, while an increase in the surface area and decrease in blade thickness would allow greater light-harvesting at reduced PFD. Alternatively, morphological differences may be the result of a variation in water velocities between the three sites; thinner blades and longer stipes would decrease the drag force and allow the blade to maintain a parallel orientation to the water surface.
2.2 METHODS
In April 1999, growth data recorded on a dive slate was lost over the side of the boat, resulting in missing values for growth rates of *Ecklonia radiata* from Seymour Island and Causet Cove. As a result, the values for *E. radiata* from Thompson Sound have been excluded from the analyses. Unforeseen circumstances delayed field trips in October 1999 and February 2000; therefore growth measurements and collection of *E. radiata* for nutrient uptake and photosynthetic experiments were carried out in the month following, i.e. in November 1999 and March 2000.

2.2.1 Site Descriptions

2.2.1.1 Causet Cove (45°16.92'S; 166°53.86'E)
Causet Cove is situated on the southern aspect of the fiord. It is a semi-sheltered cove, leveling out at 15-16 m depth before deepening to approximately 400 m. The site is often subjected to 1-2 m swell as well as surge from the open ocean. The LSL is approximately 1-3 m deep. Large mixed beds of *E. radiata* and *Carpophyllum flexuosum* persist from just beneath the LSL to 25 m. The substrate is composed of large rocks interspersed with sandy, shelly areas.

2.2.1.2 Outer Thompson Sound (45°07.45'S; 166°58.37'E)
Outer Thompson Sound (hereafter referred to as Thompson Sound) is also semi-sheltered by a rocky reef close to the open ocean and levels out at approximately 20 m. The site is situated on the northern side of the fiord and is subject to 1-3 m swells. *Ecklonia radiata* is the dominant macroalga at this site, with large beds persisting down to 20 m. The substrate was similar to that at Causet Cove.

2.2.1.3 Seymour Island (45°18.42'S; 167°00.26'E)
Seymour Island is a small island in the shallow reach between the three sounds. A unidirectional current is present, which at times is strong enough to sweep a diver at 15 m depth around the point of the island. The LSL extends to 6-8 m (*pers. obs.*). *Ecklonia radiata* was the only large macroalga found at this site, with the exception of algae in the LSL. The area was sparsely populated, and *E. radiata* was always heavily encrusted with
bryozoans and epiphytes. This was a sloping habitat with large areas of shell debris and sand.

2.2.2 Environmental parameters

Solar irradiance (irradiance [290 – 3000 nm] received directly at the earth’s surface, MJ m\(^{-2}\) d\(^{-1}\)) was collected at the Manapouri Aerodrome (approximately 30.6 km from Deep Cove is) and provided by the National Institute of Water and Atmospheric Research Limited (NIWA), Wellington from September 1998 to March 2000. Values were first converted from MJ m\(^{-2}\) d\(^{-1}\) to J m\(^{-2}\) d\(^{-1}\) (1 MJ = 10\(^{6}\) J), divided by the number of seconds in a day, then converted to μmol m\(^{-2}\) s\(^{-1}\) using a conversion factor 1 J m\(^{-2}\) s\(^{-1}\) = 4.2 μmol m\(^{-2}\) s\(^{-1}\) (Luning, 1981). At each of the three study sites in Doubtful Sound, PFD (μmol m\(^{-2}\) s\(^{-1}\)) was measured monthly from September 1998 until December 1998, then bi-monthly from February 1999 to March 2000 using a LiCOR spherical quantum sensor (model LI-193SB, USA) attached to a LiCOR Photometer (model LI-189, USA). Measurements were taken at the water surface and then at 1 m intervals to a depth of 9 m, which was the maximum extension of the cable attached to the underwater light sensor. A LiCOR underwater spectroradiometer (model LI-1800UW, Nebraska) was used to measure the spectral composition of PAR (350-700 nm) at the three study sites in February 1999. Wavelengths were measured every 10 nm beginning at the water surface and then at 1 m intervals to a depth of 10 m. This was only done on a single occasion due to unavailability of the spectroradiometer on subsequent field trips. PFD and spectroradiometer measurements could not be made at the same time every day due to the amount of work undertaken at each site, diving restrictions and the distance between sites. Consequently, the underwater PFD and spectroradiometer measurements represent a mere ‘snapshot’ in time at each site, and will have been influenced by factors such as the time of day that the measurements were taken, cloud cover, the aspect of the mountains as well as the depth of the freshwater layer.

Monthly attenuation coefficients were calculated from measurements of PFD over a depth profile taken at each of the three sites at the same time as collection of *Ecklonia radiata* occurred (Eq. 2.1). Attenuation coefficients were also calculated for selected
wavelengths over a depth profile in the blue (400-500 nm) and red (600-700 nm) region using the spectroradiometer data. These representative wavelengths were selected as chl $a$ of kelps absorbs strongly in these regions (Kirk, 1983). The PFD penetrating the water column to 10 and 20 m, corresponding to the depth from which $E. \text{radiata}$ at each site was collected, was calculated monthly using Eq. 2.2. Eq. 2.1 and 2.2 are taken from Dring (1982):

$$\text{Attenuation coefficient, } k \text{ (m}^{-1}) = \frac{\ln I_1 - \ln I_2}{d_2 - d_1} \tag{Eq. 2.1}$$

$$\text{PFD at given depth (LnI}_2) = \ln I_0 - (kz) \tag{Eq. 2.2} \text{ (μmol m}^{-2} \text{ s}^{-1})$$

where

- $I_1$ and $I_2$ = PFD at depths 1 and 2 (μmol m$^{-2}$ s$^{-1}$)
- $d_1$ and $d_2$ = depths of two measurements ($d_2 > d_1$) in meters
- $I_0$ = solar irradiance at water surface converted to PAR (μmol m$^{-2}$ s$^{-1}$)
- $z$ = depth (m)
- $k$ = attenuation coefficient (m$^{-1}$)

Daily measurements of rainfall were provided by NIWA, Wellington and were collected from a weather station on Secretary Island (Fig. 1.3). Data for ambient seawater temperature and salinity were measured monthly using a Sea-Bird SB-19 conductivity-temperature-depth profiler (CTD, Sea-Bird Electronics, Inc., USA) at 0.5 m intervals to a depth of 25 m between January 1998 and January 1999 at Malaspina Reach (refer to Fig. 1.3). Ambient seawater temperature and salinity data are courtesy of Nicole Goebel (Department of Marine Science, University of Otago, Dunedin).
2.2.3 *In situ* measurement of water velocity at the blade surface of *Ecklonia radiata*

Plaster of Paris cubes were used to estimate the relative water velocity at the blade surface of *Ecklonia radiata* bi-monthly from June 1999 until March 2000. Cubes were prepared according to the methods of Doty (1971). Plaster of Paris was mixed with water (500 g: 335 ml) and poured into plastic ice cube trays. The trays were tapped vigorously several times to dislodge air bubbles that may have formed whilst pouring the mixture. Cubes were left to air dry for three days before being removed from the trays. The cubes were cut in half and the base of each cube filed so that all cubes were a uniform weight (8.5 g ± 0.5 g). A groove was made in the base of each cube and a numbered paper clip (95 mm) was glued in place (Fig. 2.3). Cubes (with attached paper clip) were weighed two days prior to each field trip.

![Figure 2.3: Dimensions of plaster of Paris cubes used to measure *in situ* water velocity at the blade surface of *Ecklonia radiata.* Bottom surface of cubes were sanded to adjust weight to 8 ± 0.5 g. Cubes were made according to Doty (1971). Drawing not to scale.](image-url)
At each of the three study sites each month, divers attached two cubes to the primary blade of five *Ecklonia radiata* individuals; one at the blade/stipe junction and one at the distal tip. The cubes were left attached for approximately 24 h and then collected. Upon collection, cubes were rinsed in freshwater, dried in a drying room (65-70°C) overnight and their dry weight recorded.

### 2.2.3.1 Calibration of dissolution rates with water speed

Dissolution rates of the plaster of Paris cubes were calibrated by placing cubes of the same dimensions as those used *in situ* in a recirculating flume (University of Waikato; Fig. 2.4). Three cubes were threaded onto 0.3 mm fishing line and glued in place 11 cm apart. Each set of three cubes was suspended from a bar and attached to the bottom of the flume using velcro™ dots. In the flume, three sets of pre-weighed cubes were placed 12.5 cm apart across the tank at a distance of 12.5 cm from the inflow giving a total of nine cubes per velocity for 24 hr (Fig. 2.4 inset). Water velocity was measured using an acoustic Doppler velocimeter (ADV; SonTek, San Diego, USA) and ranged from 5.6 - 29.2 cm s⁻¹. Water velocity varied depending upon placement within the flume; the velocity quickened towards the water surface. Cubes were removed from the flume and left to dry for 24 - 48 h and a dry weight obtained. Seawater temperature in the flume was 12°C and salinity was 28.5 psu.

A linear regression equation was obtained for weight loss of the cubes against mean water velocity within the flume (Eq. 2.4). Weight loss values from *in situ* cubes were then entered into the equation to give an estimate of the cumulative water velocity at the blade surface of *Ecklonia radiata* from each of the three sites.

$$\text{Water velocity (cm s}^{-1}\text{)} = 7.623x - 1.729 \quad \text{Eq. 2.4}$$

where \(x\) = the mean weight loss of the plaster of Paris cubes per individual sporophyte.
Figure 2.4: Diagram and measurements of flume used to calibrate dissolution rates of plaster of Paris cubes. Cubes were glued to 0.3 mm fishing line 11 cm apart forming a line of cubes. Triplicate lines were placed in the flume 12.5 cm apart at each speed for 24 h. Lines of cubes were placed in the middle channel (shaded area). Inset: position of cubes within flume channel, front view (vertical lines indicate cubes). Scale is for flume diagram only. Arrows indicate the direction of water flow. (Diagram courtesy of Dr. C. Pilditch, University of Waikato).
Chapter Two: Growth rate and productivity.

Lines suspended from perpendicular rod on top of flume.

General Layout

scale 1 cm = 50 cm

polyethylene return section

acrylic flow channel (723 x 50 x 50 cm)

sediment box (90 x 50 x 15 cm)

support frame

return pipe (40 cm dia)

1 hp electric motor
Water velocity was similar at the tip and base of individual *Ecklonia radiata* primary blades \((t\text{-test}, P=0.362)\). Values were therefore averaged to give an estimate of cumulative water velocity over the entire blade.

### 2.2.4 Measurement of growth rates of *Ecklonia radiata*

#### 2.2.4.1 Identification of the meristematic region

Growth rates of *Ecklonia radiata* were measured using the hole punch technique (Parke, 1948). Growth was measured by punching holes near the distal end of the meristematic region in the blades and following the distance they moved from the blade/stipe junction over a certain time period. Holes must be punched distal to the blade meristem otherwise the measured increment of growth will not be representative of the total blade growth. A preliminary trial was undertaken in September 1998 to determine the location of the meristematic region in the primary blade of *E. radiata*. Ten sporophytes, each with a primary blade length \(\geq 30\) cm, were randomly selected at each of the three study sites and tagged *in situ* using a combination of white electrical insulation tape, string and electrical cable markers (Fig. 2.5). The length of the primary blade for each tagged individual was recorded and holes of 1 cm diameter were punched at 5 cm ± 0.5 cm increments along the entire primary blade beginning at the blade-stipe junction. The tagged kelps were harvested 21 days later and the distances between each hole and the blade/stipe junction, as well as the length of the primary blade, were re-measured. The distance each separate hole moved from the blade/stipe junction was calculated, giving an estimate of elongation, which was expressed as a percentage of total blade elongation. This determined that the maximum elongation occurred within the first 10 cm of the blade-stipe junction, i.e. within the meristematic region.

#### 2.2.4.2 Tagging and measurement of growth rates of *Ecklonia radiata*

Once the meristematic region had been identified, ten mature *Ecklonia radiata* sporophytes (primary blade \(\geq 30\) cm) at each of the three study sites were tagged and a hole punched 10 cm from the blade/stipe junction. Sporophytes were tagged monthly for the first three months of the study period, from October 1998 until December 1998, and
Figure 2.5: *Ecklonia radiata* stipes tagged using white insulation electrical tape before having an ID number attached. Arrow points to tip of blade showing eroded tissue. Note rugosity of secondary blades compared to smooth primary blades (photos author).
Chapter Two: Growth rate and productivity of *Ecklonia radiata*

bi-monthly thereafter until March 2000. Data collected during the preliminary trial in September were also used in subsequent spatial and temporal analyses. Stipe length and diameter, blade length, width and thickness, and distance to the hole from the blade/stipe junction were measured using a tape measure to the nearest 1 mm underwater and recorded (Fig. 2.6). The number of secondary (and tertiary, if any) blades were counted along one side of the primary blade and multiplied by two to give the total number of secondary blades for each individual *E. radiata*. At the end of each tagging period the sporophytes were collected, keeping the holdfast as intact as possible, and all parameters were re-measured in the laboratory. Primary blade thickness was measured at the blade/stipe junction, in the middle of the primary blade (half the blade length) and at the apical tip with calipers to 0.05 mm.

The linear increase in the primary blade of each sporophyte from each of the three study sites was calculated for each tagging period using the formula of Mann (1973; Eq. 2.5):

\[
\text{Blade growth rate (G, cm d}^{-1}\text{)} = \frac{H_2 - H_1}{t}
\]

Eq. 2.5

where

- \(H_1\) = distance of punched hole from blade/stipe junction at the start of the tagging period (cm)
- \(H_2\) = distance of punched hole from the blade/stipe junction at the end of the tagging period (cm)
- \(t\) = time (d)

The increase in stipe length was calculated by substituting the stipe length at the start of the tagging period \((S_{t1})\) and the stipe length at the end of the tagging period \((S_{t2})\) with \(H_1\) and \(H_2\) in Eq. 2.5, respectively (Fig. 2.6). Erosion of the primary blade was calculated as the difference between the total primary blade increase (total primary blade length at end of tagging period minus the primary blade length at the start of the tagging period) and the linear primary blade growth (measured as the distance the punched hole has moved over the given time period) (Yoshikawa *et al.*, 2001). If growth occurred in the primary blade above the initial punched hole, calculation of erosion resulted in a negative value of
Figure 2.6: Morphological measurements made on *Ecklonia radiata* during the study period, September 1998 to March 2000. \( S_T \) = stipe length, \( B_P \) = primary blade length, \( H_1 \) = distance the hole punched from blade/stipe junction (10 cm), \( H_2 \) = distance the punched hole moved over time and \( W_B \) = the width of the primary blade. Stipe diameters, number of secondary blades present and primary blade thickness at the apical tip, in the middle of the blade and at the blade/stipe junction were also measured.
erosion. Total sporophyte growth was calculated as the increase in blade and stipe length plus erosion.

### 2.2.4.3 Production rates of *Ecklonia radiata*

Production rates were estimated for *Ecklonia radiata* from each of the study sites using the area of constant biomass model (Mann *et al.*, 1979; Mann and Kirkman, 1981). Once all growth parameters had been re-measured, sporophytes were divided into holdfast, stipe and blade sections, and the blade further divided into 5 cm segments. All segments were weighed before being wrapped in pre-weighed tin foil and dried at 80 °C for 24 - 36 hours to obtain dry weights. Mean production per individual sporophyte was estimated using Eq. 2.6:

\[
\text{Daily individual sporophyte production (g DW d}^{-1}\text{)} = (W_{\text{max}}/l) \times G \quad \text{Eq. 2.6}
\]

where:
- \(W_{\text{max}}\) = segment in which maximum biomass occurred per sporophyte (g DW)
- \(l\) = length of segment of maximum biomass (5 cm)
- \(G\) = blade growth rate (cm d\(^{-1}\))

Daily individual sporophyte production on a C basis (g C d\(^{-1}\)) was calculated by multiplying daily sporophyte production on a DW basis by the monthly % C (Yokohama *et al.*, 1987). Daily population production (g DW or C m\(^{-2}\) d\(^{-1}\)) was calculated by multiplying daily individual sporophyte production (g DW or C d\(^{-1}\)) by the mean monthly density of mature *Ecklonia radiata* sporophytes per m\(^2\) (refer section 2.2.6). Annual production (kg DW or C m\(^{-2}\) y\(^{-1}\)) of *E. radiata* at the three sites was calculated by multiplying the average production rate for the given year by 365.
Chapter Two: Growth rate and productivity of *Ecklonia radiata*

2.2.5 Correlation between growth and erosion rates of *Ecklonia radiata* and environmental parameters.

Correlations between the average monthly blade growth and erosion rates of *Ecklonia radiata* from each of the three sites and the three main environmental factors likely to influence these rates in Doubtful Sound, specifically PFD, inorganic N (NO$_3^-$ and NH$_4^+$; data from chapter 3) and water velocity were examined using a Pearson Product Moment correlation (v2.03, SigmaStat™, SPSS Inc, USA, 1997). The level of significance was $P=0.05$. As environmental factors are frequently correlated with each other, they were first examined for any correlation.

2.2.6 *Ecklonia radiata* population density

The population density of *Ecklonia radiata* (primary blade ≥ 30 cm) at each study site was determined using a 1 m$^2$ quadrat from September 1998 to March 2000. The 1 m$^2$ quadrat was randomly placed within each site at the depth from which *E. radiata* were collected (~10-20 m) and the number of individual sporophytes recorded. The quadrat was then moved four fin kicks to the right, dropped and the number of *E. radiata* individual sporophytes present recorded again. This process was repeated a further three times, giving replication of five quadrats per site monthly or bi-monthly.

2.2.7 Data analysis

For all data analyses, the level of significance chosen was $P=0.05$. When the analysis of variance (ANOVA) indicated a significant effect, post-hoc multiple comparisons using Tukey tests were performed. All analyses were performed using SigmaStat computer software (v2.03, SPSS Inc, USA, 1997). Where data were missing, the best estimate of these values was calculated using a general linear model in SigmaStat computer software (v2.03, SPSS Inc, USA, 1997).

A two-way ANOVA was used to examine if water velocity adjacent to the blade surface of *Ecklonia radiata* varied either spatially or temporally. One-way ANOVA were used to examine if 1) the growth and erosion rates at each site varied on a temporal basis, and 2) whether growth and erosion rates varied between the three sites each month. One-way
ANOVA were used based on the recommendation of Dytham (1999) as data failed normality and equal variance tests for a two-way ANOVA, even after being transformed and ranked. Data met the assumptions of a one-way ANOVA. A two-way ANOVA was used to examine if individual sporophyte production and population production rates (on both a DW and C basis) varied temporally and spatially. Morphological parameters were also examined for temporal and spatial variation using two-way ANOVA.

One-way and two-way ANOVA were used to analyse the growth rates of *Ecklonia radiata* from the three sites as a different sporophyte was measured each month. However, as the monthly growth measurements are not entirely independent of each other (data was collected from the same population at each site) data could also have been analysed using split-plot or repeated measures ANOVA. This also applies to the nutrient uptake (Chapter 3) and photosynthetic parameters (Chapter 4).
2.3 RESULTS

2.3.1 Environmental parameters

Solar irradiance (µmol m⁻² s⁻¹), measured at the Manapouri Aerodrome, exhibited a temporal trend, increasing from November to February (late spring and summer) and decreasing from June to August (winter; Fig. 2.7). PFD measured over a depth profile of 9 m at each of the three study sites on the day that *Ecklonia radiata* was collected generally exhibited the same temporal pattern as solar radiation during the first half of the study period although there was a decline in PFD in December at all three sites (Fig. 2.8a-c). PFD levels remained low at Seymour Island from November 1999 to March 2000 (spring/summer) while levels increased from June to August 1999 (winter) at Causet Cove.

The spectral composition of PAR indicated maximum energy at 580 nm (green/yellow region) at Seymour Island, 470 nm (blue region) at Causet Cove and 490 nm (blue region) at Thompson Sound (Fig. 2.9a-c). Average monthly attenuation coefficients were similar between the three sites, ranging from 0.25 to 0.58 m⁻¹ at Seymour Island, 0.29 to 0.56 m⁻¹ at Causet Cove and 0.21 to 0.53 m⁻¹ at Thompson Sound and varied over time at all three sites (Fig. 2.10a). The attenuation coefficient of blue light was higher than that of red light at Seymour Island (t-test, P=0.02), with no significant difference detected between attenuation coefficients of blue and red light at Causet Cove or Thompson Sound (Fig. 2.10b). PFD reaching the three *Ecklonia radiata* populations at 10 and 20 m was typically < 50 µmol m⁻² s⁻¹ except for increases in February and November 1999 (Fig. 2.11a and b). PFD was typically lowest at Causet Cove throughout the study.

Rainfall exhibited no seasonal pattern and was maximal in January 1999 (Fig. 2.12). Ambient seawater temperature ranged from 8 °C in winter to 17 °C in summer (Fig. 2.13). Salinity ranged from 8 psu at 1 m increasing to 35 psu at 25 m, and showed no temporal pattern (Fig. 2.13).
Figure 2.7: Solar irradiance measured daily from September 1998 through to March 2000. Solar irradiance (MJ m\(^{-2}\) d\(^{-1}\)) was measured at the Manapouri Aerodrome (approximately 30.6 km from Deep Cove, Doubtful Sound) and converted to \(\mu\text{mol m}^{-2} \text{s}^{-1}\) using a conversion factor of 1 J m\(^{-2}\) s\(^{-1}\) = 4.2 \(\mu\text{mol m}^{-2} \text{s}^{-1}\) (Lüning, 1981). (Courtesy of the National Institute of Water and Atmospheric Research Ltd, Wellington).
Figure 2.8: Monthly PFD measurements at each of the three sites in Doubtful Sound on the day that *Ecklonia radiata* was collected. PFD was measured every meter beginning at the water surface (0 m) and continuing to 9 m using a LiCOR spherical quantum sensor attached to a LiCOR Photometer. Measurements were only made to 9 m which was a far as the cable attached to the quantum sensor extended. The legend shows the depth in meters.
Figure 2.9: Spectroradiometer data collected in February 1999 at each of the three sites in Doubtful Sound. The spectral composition of PAR (350 – 700 nm) was measured every 10 nm at 1 m intervals to a depth of 6 m.
Chapter Two: Growth rate and productivity of *Ecklonia radiata*

![Graph](image)

**Figure 2.10:** Mean monthly attenuation coefficients for *Ecklonia radiata* at each study site in Doubtful Sound. A) Using measurements of PFD collected at each site over a depth profile (0 - 9 m) between September 1998 and March 2000; and b) of blue (400-500 nm) and red (600-700 nm) wavelengths over a depth profile (0 - 6 m) using spectroradiometer data collected in February 1999. * indicates a significant difference (t-test, *P*=0.02). Points in the top graph are monthly means ± 1 SD (n=9) while bars in the bottom graph are the means over the selected wavelengths ± 1 SD (n=10).
Figure 2.11: Average monthly photon flux density (PFD) reaching *Ecklonia radiata* at a) 10 m and b) 20 m at the three study sites in Doubtful Sound between September 1998 and March 2000. PFD at 10 and 20 m depth was estimated using the average monthly attenuation coefficients for each site and solar radiation measured at the Manapouri Aerodrome (~30.6 km from Deep Cove, Doubtful Sound). Points represent monthly mean ± 1 SD (n=number of days in given month).
Figure 2.12: Daily rainfall measured on Secretary Island, Doubtful Sound, between September 1998 and March 2000. Data collected from Secretary Island and provided by NIWA, Wellington.
Figure 2.13: Salinity and temperature data collected from Malaspina Reach, Doubtful Sound, between February 1997 and December 1998. Salinity and temperature were measured to a depth of 25 m using a conductivity-temperature-depth (CTD) profiler. (Data courtesy of Nicole Goebel and presentation thanks to Louise Kregting).
2.3.2 *In situ* water velocity at the blade surface of *Ecklonia radiata*

Mean water velocity at the blade surface of *Ecklonia radiata* at Seymour Island ranged from 5 to 15.5 cm s\(^{-1}\), from 4 to 10 cm s\(^{-1}\) at Causet Cove, and from 4 to 12 cm s\(^{-1}\) at Thompson Sound (Fig. 2.14). Water velocity at the blade surface of *E. radiata* at all three sites was maximum in June 1999, at which time the velocity was highest at Seymour Island and lowest at Thompson Sound (*P*<0.001). Minimum water velocity at *E. radiata* blade surface at Seymour Island occurred in November 1999 while Causet Cove and Thompson Sound had minimum water velocity a month later in December 1999.

2.3.3 Blade elongation of *Ecklonia radiata*

The region of greatest blade elongation occurred within the first 5 cm from the blade/stipe junction of *Ecklonia radiata* at all three sites (Fig. 2.15), and more than 93% of blade elongation occurred within the first ten centimeters. No elongation occurred at distances > 25 cm from the blade/stipe junction.

2.3.4 Growth and erosion rates of *Ecklonia radiata*

Total sporophyte growth rates of *Ecklonia radiata* displayed a temporal pattern (*P*<0.001 for all three sites) with maximum total sporophyte growth rates observed between September and December 1998 at all three sites (Fig. 2.16a). Total sporophyte growth rates decreased April to June 1999. Although a slight increase was observed in total sporophyte growth rates at all three sites in August 1999, rates during the second growth period in 1999 were approximately half those in 1998. Total sporophyte growth rates of *E. radiata* were similar at the three sites in December 1998, June, August and December 1999 (*P*>0.05). Spatially, *E. radiata* from Causet Cove had significantly higher total sporophyte growth rates than those from either Seymour Island or Thompson Sound in September and November 1998 and again in November 1999. *E. radiata* from Thompson Sound had significantly higher total sporophyte growth rates in October 1999 while total sporophyte growth rate of *E. radiata* from Seymour Island was significantly lower than rates from the two outer sites.
Figure 2.14: A comparison of in situ relative water velocity adjacent to the blade surface of *Ecklonia radiata* from the three study sites in Doubtful Sound. Points represent monthly mean ± 1 SD (n=5). Dashed line represents suggested water velocity that may limit rates of nutrient uptake and photosynthesis based on Hurd et al. (1996).
Figure 2.15: Histogram showing primary blade elongation in *Ecklonia radiata* from three sites in Doubtful Sound. Bars represent monthly mean ± 1 SD (n=10).
Figure 2.16: Growth rates of *Ecklonia radiata* from three sites in Doubtful Sound measured monthly from September 1998 to December 1998 then bi-monthly from February 1999 to March 2000. A) total sporophyte growth rate is the summation of blade and stipe growth plus erosion, b) blade growth was measured using the hole punch method and represents the linear increase in the primary blade, c) stipe growth is the linear increase in stipe length over a given period and d) erosion rate is the amount of tissue lost during the tagging period from the distal tip. Negative erosion rates are the result of growth in the primary blade above the punched hole. Points represent the monthly mean (n=7-10). * indicates a significant difference between the three sites in the given month (P=0.05), refer to text for results of Tukey test that identifies which sites are significantly different each month. Note the different Y-axes.
Chapter Two: Growth and productivity of *Ecklonia radiata*

![Graph showing total sporophyte growth over time, with data points for Seymour Island, Causet Cove, and Thompson Sound.](image)

**a. Total sporophyte growth**

- Seymour Island
- Causet Cove
- Thompson Sound

The graph plots sporophyte growth in cm d⁻¹ over time, with peaks in sporophyte growth indicated by asterisks. The data is collected from 1998 to 2000, showing fluctuations in growth across different locations.
Chapter Two: Growth and productivity of *Ecklonia radiata*

b. Blade growth

- Seymour Island
- Cauvet Cove
- Thompson Sound

**cm d^-1**

<table>
<thead>
<tr>
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<th>Time (months)</th>
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</thead>
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<td></td>
</tr>
<tr>
<td>1999</td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td></td>
</tr>
</tbody>
</table>

* denotes significant difference.
Chapter Two: Growth and productivity of *Ecklonia radiata*

### c. Stipe growth

- **Seymour Island**
- **Causet Cove**
- **Thompson Sound**

<table>
<thead>
<tr>
<th>Month</th>
<th>1998</th>
<th>1999</th>
<th>2000</th>
</tr>
</thead>
<tbody>
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<td></td>
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* cm d

Note: * indicates significant growth variation.
Chapter Two: Growth and productivity of *Ecklonia radiata*

**d. Erosion**

- Seymour Island
- Cautset Cove
- Thompson Sound

![Graph showing erosion rates over time with markers for significant events.](image-url)
Ecklonia radiata blade growth rates also displayed a temporal pattern at all three sites ($P<0.001$ for all sites), with high rates from September to December, low rates from February through June and rates increasing from August to November (Fig. 2.16b). Blade growth rates were up to four and a half times higher from September to December 1998 at the three study sites compared to the same period in 1999. Maximum blade growth rates occurred at Seymour Island in December 1998 (0.27 cm d$^{-1}$), at Causet Cove in September 1998 (0.42 cm d$^{-1}$) and at Thompson Sound in October 1998 (0.45 cm d$^{-1}$). Blade growth rates of $E. \text{radiata}$ were lower at Seymour Island than at Causet Cove or Thompson Sound in September, October and November 1998 and February and November 1999 ($P<0.05$). Spatially, blade growth rates of $E. \text{radiata}$ from Causet Cove were significantly higher in September, November and December 1998 and again in November 1999 than those from either Thompson Sound or Seymour Island. In October 1998 $E. \text{radiata}$ from Thompson Sound had significantly higher blade growth rates.

$Ecklonia \text{radiata}$ displayed a temporal pattern in stipe growth rates at all three sites ($P<0.05$; Fig. 2.16c) that was similar to the temporal pattern observed for total sporophyte growth rates. Stipe growth rates ranged from 0.01 to 0.06 cm d$^{-1}$ at Seymour Island, 0.02 to 0.15 cm d$^{-1}$ at Causet Cove and 0.02 to 0.08 cm d$^{-1}$ at Thompson Sound. Stipe growth rates at the three sites were similar in December 1998, June, August and December 1999, and March 2000 ($P>0.05$). $E. \text{radiata}$ from Causet Cove had significantly higher stipe growth rates than those from Thompson Sound or Seymour Island from September to November 1998 and again in November 1999 while $E. \text{radiata}$ from Seymour Island had significantly lower stipe growth rates in December 1998 and February 1999.

$Ecklonia \text{radiata}$ at Seymour Island and Thompson Sound exhibited a temporal pattern in erosion rates ($P<0.001$) while $E. \text{radiata}$ at Causet Cove did not ($P=0.07$, Fig. 2.16d). Although negative values for erosion obscure the pattern, erosion of $E. \text{radiata}$ at Seymour Island was low in February, June and November 1999 while rates at Thompson Sound were low April and June 1999. Maximum erosion rates occurred at Seymour Island and Causet Cove in September 1998 (0.12 and 0.22 cm d$^{-1}$, respectively), and at
Thompson Sound in February 1999 (0.18 cm d\(^{-1}\)). Spatially, erosion rates at Thompson Sound were significantly higher than those at Seymour Island and Causet Cove in October 1998 but were significantly lower in November 1998. \textit{E. radiata} from Seymour Island had significantly lower erosion rates than those from the other two sites in February 1999 while sporophytes from Causet Cove had significantly higher erosion rates in November 1999.

\subsection*{2.3.5 Correlation between growth and erosion rates and environmental factors}
Total sporophyte growth rate of \textit{Ecklonia radiata} at Causet Cove was negatively correlated with water velocity (\(r=-0.901, P=0.03\)). Erosion rates of \textit{E. radiata} at Seymour Island were positively correlated with PFD (\(r=0.62, P=0.04\)) but negatively correlated with water velocity (\(r=-0.9, P=0.04\)). There was no correlation between any of the environmental factors and blade growth and erosion rates of \textit{E. radiata} at Thompson Sound. At all three sites the nitrate concentration in the water column (~1Om) was positively correlated with nitrate concentration in the surface water (\(r=0.969\) at Causet Cove and Seymour Island, and \(r=0.983\) at Thompson Sound, \(P<0.001\)). The same occurred for ammonium at Thompson Sound (\(r=0.783, P<0.001\)). There was no significant relationship between water motion and PFD or water motion and nutrient concentration at any of the study sites.

\subsection*{2.3.6 \textit{Ecklonia radiata} population density}
There was no temporal pattern in population density of \textit{Ecklonia radiata} evident at any of the three sites (\(P=0.114\)). However, the density of \textit{E. radiata} at Seymour Island was significantly lower than the density at Causet Cove and Thompson Sound over the entire study period (\(P<0.001\); Fig. 2.17).

\subsection*{2.3.7 Morphological characteristics of \textit{Ecklonia radiata}}
At all three sites, a temporal pattern in stipe length of \textit{Ecklonia radiata} was evident, with minimum stipe lengths between September and December 1998 and maximum stipe lengths from February through June 1999 (\(P<0.001\); Fig. 2.18a). The exception was stipe length at Thompson Sound, which was minimal in November 1999. During the second
Figure 2.17: Population density of *Ecklonia radiata* at three study sites in Doubtful Sound between September 1998 and March 2000. Points represent monthly mean ± 1 SD (n=5).
half of the study period, stipe lengths at Seymour Island were generally longer than those at either Causet Cove or Thompson Sound ($P=0.008$). Stipe diameter of *E. radiata* from the three sites exhibited no temporal pattern (Fig. 2.18b). *E. radiata* stipes from Seymour Island were wider in diameter than stipes of *E. radiata* at either Causet Cove or Thompson Sound in August 1999 and March 2000 ($P=0.02$ and 0.013 respectively).

Blade length of *Ecklonia radiata* from Causet Cove and Thompson Sound followed a similar temporal pattern to stipe length (Fig. 2.19a). Maximum blade length at Thompson Sound occurred in October 1998 and at Causet Cove in February 1999 (49.5 and 46.4 cm respectively). Minimum blade length occurred in August 1999 at both sites. In contrast, blade length of *E. radiata* at Seymour Island was maximal in June 1999 and minimal in December 1999 (57.9 and 23.3 cm respectively). Blade lengths of *E. radiata* were similar for all three sites ($P=0.06$).

Blade width of *Ecklonia radiata* was 21 – 31 cm at Seymour Island, 17 – 35 cm at Causet Cove and 19 – 35 cm at outer Thompson (Fig. 2.19b). Maximum blade width occurred in February 1999 at Causet Cove and Thompson Sound and in June 1999 at Seymour Island. Minimum blade width at Seymour Island and Thompson Sound occurred in December 1998 and in August 1999 at Causet Cove.

The total number of secondary blades remained relatively constant over the experimental period at all three sites ranging from 20 - 47 at Seymour Island, 18 – 33 at causet Cove and 21 – 34 at Thompson Sound (Fig. 2.19c). In June 1999 however, *Ecklonia radiata* at Seymour Island had approximately twice the number of secondary blades on *E. radiata* at Causet Cove or Thompson Sound.

The primary blade thickness of *Ecklonia radiata* decreased towards the blade tip (Fig. 2.20a-c). The primary blade of *E. radiata* at Seymour Island was significantly thinner than for *E. radiata* at Causet Cove and Thompson Sound over the entire experimental period ($P<0.001$). The apical tip was the thinnest region of the primary blade at all three sites ($P<0.001$).
Figure 2.18: A) Stipe length and b) stipe diameter of *Ecklonia radiata* from three sites in Doubtful Sound between September 1998 and March 2000. Points represent monthly mean ± 1 SD (n=10).
Chapter Two: Growth rate and productivity of *Ecklonia radiata*

Figure 2.19: A) Primary blade length, b) primary blade width and c) number of secondary blades of *Ecklonia radiata* from three sites in Doubtful Sound between September 1998 and March 2000. Points represent monthly mean ± 1 SD (n=10).
Figure 2.20: Primary blade thickness of *Ecklonia radiata* from three sites in Doubtful Sound between September 1998 and March 2000. a) thickness at base of blade, b) thickness in the middle of the blade and c) thickness at the distal tip of the primary blade. Points represent mean ± 1 SD (n=10).
Figure 2.21: Temporal changes in mean daily individual sporophyte production on a dry weight (DW) basis of *Ecklonia radiata* from three sites in Doubtful Sound from September 1998 to March 2000. Daily individual sporophyte production was determined using the area of constant biomass equation (Mann and Kirkman, 1981). Points represent mean ± 1 SD (n=7-10).
2.3.8 Production rates of *Ecklonia radiata*

2.3.8.1 Daily individual sporophyte production

Daily individual sporophyte production of *Ecklonia radiata* from all sites varied both spatially and temporally ($P<0.001$ and $P<0.001$, respectively; Fig. 2.21). However, a significant interaction between time and site ($P<0.001$) makes it difficult to interpret where these significant differences occurred, and only just significant $P$ values were treated with caution. The highest daily production rates occurred in October 1998 at Thompson Sound and Seymour Island (1.38 and 0.76 g DW d$^{-1}$ respectively) and in November 1998 at Causet Cove (0.98 g DW d$^{-1}$), while the lowest daily production at all sites occurred in March 2000.

On a carbon basis, daily individual sporophyte production also varied spatially and temporally ($P<0.001$ and $P<0.001$ respectively; Fig. 2.22) however, there was a significant interaction between time and site ($P<0.001$). Production rates on a C basis at Seymour Island ranged from 0.03 – 0.20 g C d$^{-1}$, at Causet Cove 0.05-0.28 g C d$^{-1}$ and at Thompson Sound 0.05-0.35 g C d$^{-1}$.

2.3.8.2 Daily population production rates of *Ecklonia radiata*

Daily population production rates (g DW m$^{-2}$ d$^{-1}$) of *Ecklonia radiata* from all three sites displayed both spatial ($P<0.001$) and temporal ($P<0.001$) variation however, there was also a significant interaction between time and site ($P<0.001$). The daily population production rate at Seymour Island was significantly lower than at either Causet Cove or Thompson Sound during the entire study period except in December 1998 when no significant differences were observed ($P<0.05$ for all months except December 1998; Fig. 2.23). Population production on a carbon basis by *E. radiata* from the three sites exhibited the same temporal pattern as population production on a dry weight basis (Fig. 2.24).
Figure 2.22: Temporal changes in mean daily individual sporophyte production on a C basis of *Ecklonia radiata* from three sites in Doubtful Sound from September 1998 to March 2000. Daily sporophyte production on a DW basis was multiplied by the mean monthly percent C to give daily productivity on a C basis. Note error bars go below 0 in graph b. Points represent mean ± 1 SD (n=7-10).
Chapter Two: Growth and productivity of *Ecklonia radiata*
Figure 2.23: Temporal changes in mean daily population production on a dry weight (DW) basis of *Ecklonia radiata* from three sites in Doubtful Sound from September 1998 to March 2000. Daily population production was calculated by multiplying the daily sporophyte production rate (g DW d$^{-1}$) with the population density (number of individuals m$^{-2}$) for that given month. Points represent mean ± 1 SD (n=7-10).
Chapter Two: Growth and productivity of *Ecklonia radiata*
Figure 2.24: Temporal changes in mean daily population production on a C basis of *Ecklonia radiata* from three sites in Doubtful Sound from September 1998 to March 2000. The daily population production on a C basis was calculated by multiplying the daily sporophyte production rate (g C d⁻¹) with the population density (number of individuals m⁻²) for that given month. Points represent mean ± 1 SD (n=7-10).
Chapter Two: Growth and productivity of *Ecklonia radiata*

![Graph showing production of *Ecklonia radiata* at different locations over time. The graph includes data from Seymour Island, Causet Cove, and Thompson Sound, with production measured in g C m$^{-2}$ d$^{-1}$. The data spans from 1998 to 2000, with peaks and troughs indicating variations in productivity across different months.]
2.4 DISCUSSION

2.4.1 Environmental factors in Doubtful Sound

Solar irradiance, measured at Manapouri Aerodrome, exhibited a seasonal pattern of high irradiance during late spring and summer and low irradiance during winter, which is typical of New Zealand (Novaczek, 1984; Brown et al., 1997; Stuart, 1997) and temperate regions globally (e.g. Gange et al., 1982; Kirkman, 1989; González-Fragoso et al., 1991). However, the amount of solar irradiance received directly by surface waters in Doubtful Sound is further influenced by meteorological conditions such as cloud cover and rainfall, and the topography (steep-sided, shadow casting mountains and a narrow channel) of the fiord. Greater emphasis could have been placed on the effect of underwater light quantity and spectral quality on the growth of *Ecklonia radiata* had data loggers been used to measure PFD at the water surface and at the depth from which *E. radiata* was collected at each site rather than rely on light measurements made only once during the day. Other studies on the attenuation of irradiance through the water column in Doubtful Sound indicate low levels of underwater irradiance (Grange et al., 1991; Rutherford et al., 2000; Gibbs, 2001). Sub-surface irradiance measurements collected from opposing sides of the fiord mid-way along Doubtful Sound demonstrated that during winter the northern side of the fiord blocked approximately 95 % of irradiance received by the fiord (Kregting, 2001). Furthermore, in an extensive study of the underwater environment of Doubtful Sound, Grange et al. (1991) found only 25 % of surface irradiance penetrates the LSL to 9 m and was sometimes as low as 1 %. During the present study, maximum transmission of irradiance through the water column occurred during winter, although transmission can decline markedly during heavy rainfall (Grange et al., 1991) as a result of a thickening LSL and increased particulate matter.

The predominance of green/yellow wavelengths (550 – 650 nm) at Seymour Island was expected due to greater attenuation of blue wavelengths by the tannin-stained LSL. However these results are based on just one unreplicated sampling period, February 1999, and should be treated with caution due to diurnal and temporal variations caused by tidal fluctuations and rainfall, which may alter terrestrial runoff. Blue light is a requirement for normal macroalgal development, and is a critical factor affecting the reproductive
ability of laminarians (Dring, 1986; Lobban and Harrison, 1997). Investigations have demonstrated that many laminarian species require a specific blue quantum dose to become fertile (Lüning and Neushul, 1978; Deysher and Dean, 1984; Lüning and Dring, 1985) and although this has yet to be tested experimentally for *Ecklonia radiata* it is probable that *E. radiata* shares this trait. It is known that gametophytes of *E. radiata* do not become fertile in red light (Novaczek, 1984a, b; Bolton and Levitt, 1985; Bolton and Anderson, 1987). The attenuation of blue light at Seymour Island in the present study may have an adverse effect on *Ecklonia radiata*, such as a reduction in fertility and reproductive capacity resulting in lower densities. Subsequent experimentation is needed to clarify the effects of blue light on reproductive ability, gametogenesis, growth rates, and ultimately survival of *E. radiata* in Doubtful Sound.

There was no seasonal trend in daily rainfall, sea-surface water temperature or salinity in Doubtful Sound between September 1998 and March 2000. A LSL was evident in Doubtful Sound during the study period and has been identified by numerous authors (Garner, 1964; Batham, 1965; Jillet and Mitchell, 1973; Stanton, 1978, 1984, 1986; Stanton and Pickard, 1981; Pickrill, 1987). Average attenuation coefficients at 9 m at the three study sites ranged from 0.21 to 0.58 m\(^{-1}\). This variation may be attributed to rain causing rapid fluctuations in the LSL, during which time light attenuation increases within the first few meters of the water column (Grange et al., 1991). This is similar to the freshwater layers produced during melt-water inputs in Arctic fiords at which time light attenuation is close to 1 m\(^{-1}\), indicative of a turbid, sediment-rich freshwater layer and decreasing light penetration through the water column (Hanelt et al., 2001). Grange *et al.* (1991) found that heavy rain caused rapid fluctuations in salinity below the surface layer in Doubtful Sound. Stanton (1986) recorded an annual sea-surface temperature ranging from 11.5 to 14.9°C in Doubtful Sound between 1979 and 1983 that is comparable to the range recorded in the present study (8 – 17°C). *E. radiata* is typically described as a warm temperate laminarian. The minimum winter water temperature at which it can survive is approximately 9°C (Bolton and Anderson, 1994). The effect of salinity and temperature on physiological aspects and growth rates of *Ecklonia radiata*
were not determined during this study, and subsequent investigations may help clarify which factor/s control these functions.

2.4.2 In situ water velocity at the blade surface of *Ecklonia radiata*

In the present study, dissolution rates were calibrated to steady-flow velocities determined in the laboratory. However, Porter *et al.* (2000) found that while the relation between dissolution rates and steady- and fluctuating-flow environments showed significant correlation, the relationship between dissolution rates and mixed-flow environments was weak. It was noted that two types of water motion occurred in Doubtful Sound: at Seymour Island a unidirectional current existed while at Causet Cove and Thompson Sound surge and swell dominated (*pers. obs.*). The effect of temperature and salinity has also been investigated on dissolution rates (Jokiel and Morrissey, 1993; Thompson and Glenn, 1994). Dissolution rates increased linearly between 18 and 32°C (Jokiel and Morrissey, 1993). A linear relationship was also observed between dissolution rates and salinity between 20 and 34 psu (Thompson and Glenn, 1994). Temperature and salinity remained constant in Doubtful Sound throughout the experimental period although temperature increased and salinity decreased slightly in the flume used for calibration.

Water velocities at the blade surface of *Ecklonia radiata* in Doubtful Sound were higher compared to similar studies. Gerard (1982) recorded *in situ* water velocities ranging from 4 to 6 cm s$^{-1}$ while velocities measured by Koehl and Alberte (1988) ranged between 0.58-0.6 cm s$^{-1}$. However, Gerard (1982) used *Macrocystis pyrifera* while Koehl and Alberte (1988) used *Nereocystis luetkeana* from a wave-sheltered area. Both *M. pyrifera* and *N. luetkeana* are more densely bladed than *E. radiata*, and form canopies at the water surface. This may result in lower relative water velocity at the blade surface as blades ‘go with the flow’ (Hurd, 2000). *In situ* dissolution rates may be increased due to fluctuations in temperature and salinity, abrasion of the plaster by other kelp blades or material in the water column, movement of the kelp blade itself or a combination of the aforementioned factors (Koehl and Alberte, 1988; Thompson and Glenn, 1994; Porter *et al.*, 2000). The effect of the high water velocities on growth rates and morphological
characteristics of *Ecklonia radiata* in Doubtful Sound is discussed separately under the relevant sections further on.

### 2.4.3 Growth rates of *Ecklonia radiata*

Total sporophyte growth rates of *Ecklonia radiata* from the present study will not be discussed, as these rates are the summation of blade and stipe growth rates and erosion rates which are discussed separately. Total sporophyte growth rates also exhibited similar temporal and spatial patterns to those observed for blade and stipe growth rates. Focusing on blade growth rates will allow comparisons with studies using the same technique, namely the hole punch method to measure the linear increase in blade size over a given period.

Blade growth rates of *Ecklonia radiata* in Doubtful Sound displayed a temporal pattern; rates were generally high September through December (spring/early summer) and low March through June (autumn/winter) with maximum blade growth rates occurring in September and October 1998. This temporal pattern is similar to that observed for *E. radiata* from Marmion Reef, Western Australia (Kirkman, 1984) and from New South Wales, Australia (Steinberg, 1995), although growth rates at the New South Wales site were only measured over a four week period in spring and again in autumn and may have missed the period of maximum growth which typically occurs in late winter/early spring for many perennial laminarians (Parke, 1948; Mann, 1972, Chapman and Craigie, 1977; Gerard and Mann, 1979; Kain, 1989; Luning, 1993). Growth rates of *E. radiata* in Doubtful Sound are similar to the temporal pattern of growth demonstrated for many laminarians. The peak in blade growth in winter of many laminarians is often attributed to an increase in ambient seawater inorganic N concentrations, however in Doubtful Sound seawater inorganic N concentrations remained low year-round in the present study (typically \( \leq 3 \) \( \mu \)M, chapter 3) and were not correlated to blade growth rates of *E. radiata* at any of the three study sites.

Blade growth rates during the first growth period in 1998/99 were at least double the rates during the second growth period a year later. Such a discrepancy between years has
not been reported in any published temporal studies of growth rates of laminarian species before. As already stated, the low ambient seawater inorganic N concentrations do not explain the difference in blade growth rates between the two growth periods. No significant correlations were found between ambient seawater inorganic N concentrations and blade growth rates of *E. radiata* from any of the three sites in the present study. Furthermore, seawater NO$_3^-$ concentrations at both the surface and at depth increased to 10 $\mu$M in November 1999 (data from chapter 3) therefore if N affected growth rates, rates would be expected to show an increase during the following tagging period, which they did not. On several occasions, PFD reaching *E. radiata* in Doubtful Sound at 20 m was less than 0.05 % of the surface radiation. The lower growth rates observed during the second half of the study could be attributed to these reduced light levels reaching the three *Ecklonia radiata* populations. However, further research on the underwater light regime in Doubtful Sounded is needed to quantify diurnal and seasonal changes. Furthermore, it is suggested that the temporal pattern in blade growth rates may be the result of reduced C accumulation imposed by the low PFD. It is likely that a combination of the low ambient inorganic N concentrations and the low levels of light reaching the thallus may have caused the lower blade growth rates during 1999/2000.

A comparison of blade growth rates, obtained using the hole-punch method, of various members of the Laminariales indicates that values differ greatly between kelp species (Table 2.2). *Undaria pinnatifida*, *Nereocystis luetkeana* and *Pelagophycus porra* had blade growth rates that were substantially higher than those of *Ecklonia radiata* from Doubtful Sound. The high growth rates recorded for *U. pinnatifida* (Castric-Fay *et al.*, 1999) and *U. pinnatifida* f. *distans* (Yoshikawa *et al.*, 2001) were attributed to the use of small line-cultured specimens whereas mature, wild specimens of *Ecklonia radiata* were used in the present study. The relatively high blade growth rates of *N. luetkeana* were possibly the result of a thinning of the population resulting in an increase in PFD availability, although this was not tested experimentally (Maxwell and Miller, 1996). Coyer and Zaugg-Haglund (1982) hypothesized that the high growth rates exhibited by *P. porra* were due to a combination of optimum PFD at 17 –21 meters and periodic
Table 2.2: A comparison of minimum and maximum blade growth rates (cm d\(^{-1}\)) of Laminarian kelps obtained using the hole-punch method of Parke (1948).

<table>
<thead>
<tr>
<th>Species</th>
<th>Growth Rate (cm d(^{-1}))</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Alaria esculenta</em></td>
<td>0.1-0.5</td>
<td>Buggeln, 1974</td>
</tr>
<tr>
<td><em>Costaria costata</em></td>
<td>1.8</td>
<td>Maxell and Miller, 1996</td>
</tr>
<tr>
<td><em>Ecklonia radiata</em></td>
<td>0.76-1.96</td>
<td>Larkum, 1986</td>
</tr>
<tr>
<td><em>E. radiata</em> – Seymour Island</td>
<td>0.06-0.27</td>
<td>Present study</td>
</tr>
<tr>
<td>– Cauet Cove</td>
<td>0.09-0.42</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>– Thompson Sound</td>
<td>0.07-0.45</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td><em>Laminaria digitata</em></td>
<td>0.02-0.62</td>
<td>Conolly and Drew, 1985</td>
</tr>
<tr>
<td><em>L. longicruris</em> – Black Rock</td>
<td>0.15-1.1</td>
<td>Gerard and Mann, 1979</td>
</tr>
<tr>
<td>– Callahan Island</td>
<td>0.2-1</td>
<td>Gerard and Mann, 1979</td>
</tr>
<tr>
<td>– Boutilier Point</td>
<td>0.4-1.0</td>
<td>Gagné et al., 1982(^a)</td>
</tr>
<tr>
<td>– Pubnico</td>
<td>0.7-1.5</td>
<td>Gagné et al., 1982(^a)</td>
</tr>
<tr>
<td>– Centreville</td>
<td>0.4-1.8</td>
<td>Gagné et al., 1982(^a)</td>
</tr>
<tr>
<td><em>L. longicruris</em></td>
<td>0.92-1.6</td>
<td>Chapman and Craigie, 1977</td>
</tr>
<tr>
<td><em>L. saccharina</em></td>
<td>0.5-2.1</td>
<td>Parke, 1948</td>
</tr>
<tr>
<td></td>
<td>0.04-1.26</td>
<td>Conolly and Drew, 1985</td>
</tr>
<tr>
<td></td>
<td>0.1-1.2</td>
<td>Sjøtun, 1993</td>
</tr>
<tr>
<td></td>
<td>0.1-0.8</td>
<td>Sjøtun and Gunnarsson, 1995</td>
</tr>
<tr>
<td><em>Nereocystis luetkeana</em></td>
<td>3.2-6.1</td>
<td>Maxell and Miller, 1996</td>
</tr>
<tr>
<td><em>Pelagophycus porra</em></td>
<td>2.2-6.8</td>
<td>Coyer and Zaugg-Haglund, 1982</td>
</tr>
<tr>
<td><em>Phyllariopsis purpurascens</em></td>
<td>0.3-0.38</td>
<td>Flores-Moya et al., 1993</td>
</tr>
<tr>
<td><em>Saccorhiza polyschides</em></td>
<td>0.2-2.1</td>
<td>Norton and Burrows, 1969</td>
</tr>
<tr>
<td><em>Undaria pinnatifida</em></td>
<td>1.8-2.1</td>
<td>Castric-Fey et al., 1999</td>
</tr>
<tr>
<td><em>U. pinnatifida</em></td>
<td>0.02-1.16</td>
<td>Stuart, 1997</td>
</tr>
<tr>
<td><em>U. pinnatifida f. distans</em></td>
<td>1-1.8</td>
<td>Yoshikawa et al., 2001</td>
</tr>
</tbody>
</table>

\(^a\) Blade growth rates of *Laminaria longicruris* were examined from three sites situated around Nova Scotia, Canada. The sites were chosen for their contrasting pattern of inorganic N availability. At Boutilier Point N was intermittent, at Pubnico N was abundant from November through February and at Centreville N was always greater than 3 \(\mu\)g at.\(^{-1}\).
intrusions of nutrient-rich water. Rates of blade growth for *E. radiata* in Doubtful Sound were similar to rates for *Laminaria digitata* from Fife, Scotland (Connelly and Drew, 1985) and *Phyllariopsis purpurascens* from the Straits of Gibraltar (Flores-Moya *et al.*, 1993). The low rates of blade growth recorded for *P. purpurascens* resulted from growth in low PFD (0.1-1.5% of incident PFD; Flores-Moya, 1997) while *L. digitata* was more than likely N-limited year-round (Connelly and Drew, 1985). *E. radiata* was both N-limited year-round (data from chapter 3) and subject to extremely low PFD (0-1.7% of surface PFD at 20 m), particularly during winter months, which may account for the low growth rates observed in the present study. *U. pinnatifida*, *N. luetkeana* and *P. porra* are also annuals (or at least *P. porra* located off Santa Catalina Island, California is thought to be; Coyer and Zaugg-Haglund, 1982) whereas many of the laminarians with lower growth rates, including *E. radiata*, are perennial. However, there is no clear differentiation between blade growth rates of annual and perennial species, or between species with different morphologies (i.e. simple vs. digitate thalli). Nor was there a distinct correlation of blade growth rates with any of the environmental factors investigated which suggests that *Ecklonia radiata* in Doubtful Sound has physiologically adapted to the low ambient inorganic N supply and low PFD levels reaching the populations. This is investigated further in chapter 5 where photosynthetic rates and pigment content of five populations of *E. radiata* were examined along the main fiord axis.

*Ecklonia radiata* from Seymour Island generally had lower blade growth rates than those from Causet Cove or Thompson Sound, as was expected due to the LSL, although statistical analysis revealed that rates were similar between sites on four occasions. A reduction in growth rate of macroalgae is often attributed to a reduction in PFD reaching the thallus. Artificial shading of *Ecklonia radiata* sporophytes in Australia at 5 m reduced growth rates to rates similar to those recorded at 10 m (Kirkman, 1989), although artificial shading was only investigated in winter and autumn when solar radiation was minimal. Studies have demonstrated that the LSL present in Doubtful Sound reduces PFD penetration through the water column by up to 75% (Grange *et al.*, 1991; Rutherford *et al.*, 2000; Gibbs, 2001). In addition, attenuation coefficients calculated for
each site in the present study indicate that the water is typically turbid which would further reduce transmission. Estimated PFD reaching *E. radiata* populations at 10 m ranged from 0.5-10% of surface irradiance and 0-1.7% of surface irradiance at 20 m in the present study. It is therefore plausible that the low blade growth rates of *E. radiata* in Doubtful Sound are a result of lower photosynthetic rates due to greatly reduced PFD at depth. As already stated however, no correlation was found between PFD and blade growth rates of *E. radiata* at any of the study sites in the present study.

The observed differences in blade growth rates may be the result of differences in the depth from which *E. radiata* was collected. Noveaczek (1984) found that growth rates of *E. radiata* from Goat Island, New Zealand at 15 m were lower and lagged a month behind rates of sporophytes at 7 m. Lower blade growth rates were also recorded for *E. radiata* at 10 m compared to sporophytes at 5 m at Perth, Australia (Kirkman, 1989). Similar results have also been demonstrated for *Laminaria pallida* in South Africa (Dieckman, 1980) and *L. longicururs* in the Bay of Chaleurs (Gendron, 1985). In the present study *E. radiata* sporophytes were collected from depths ranging from 10 to 20 m. This was due to population patchiness and low densities at each site, and may account for some of the variability in growth rates. Future studies should ensure that the macroalgae under investigation is collected from the same depth to minimize variability.

2.4.3.1 Stipe growth rates of *Ecklonia radiata*

Stipes of *Ecklonia radiata* may vary in length depending on locality and environment. Larkum (1986) stated that Australian *E. radiata* sporophytes often have a short stipe (≤ 20 cm) whereas sporophytes in New Zealand generally have long stipes (≤ 150 cm). The lengths of *Ecklonia radiata* stipes in Doubtful Sound ranged from 6 to 53 cm (with an average length of 20 cm) at all three sites which are more similar to Australian populations. Maximum stipe growth rates of *E. radiata* from the three study sites in Doubtful Sound were lower than maximum rates reported for other laminarians with the exception of *Saccorhiza polyschides* (Table 2.3). The high maximum stipe growth rates recorded for *Nereocystis luetkeana* and *Macrocystis pyrifera* may be attributed to either
life history (\textit{N. luetkeana} is an annual alga), pattern of growth (\textit{M. pyrifera} grows from an apical scimitar) or the fact that both form surface canopies whereas most other kelps form sub-surface canopies. Although the maximum stipe growth rate of \textit{E. radiata} at Causet Cove was approximately double the rates observed at Seymour Island and Thompson Sound, there was no significant difference between the sites due to the large inter-plant variation, which possibly resulted from the difference in depth from which sporophytes were collected.

Table 2.3: Maximum stipe growth rates (cm d\(^{-1}\)) for various Laminarian kelps.

<table>
<thead>
<tr>
<th>Species</th>
<th>Growth rate (cm d(^{-1}))</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{Ecklonia radiata}</td>
<td>0.06</td>
<td>Present study</td>
</tr>
<tr>
<td>- Seymour Island</td>
<td>0.15</td>
<td>“</td>
</tr>
<tr>
<td>- Causet Cove</td>
<td>0.15</td>
<td>“</td>
</tr>
<tr>
<td>- Thompson Sound</td>
<td>0.08</td>
<td>“</td>
</tr>
<tr>
<td>\textit{Laminaria longicruris}</td>
<td>0.78</td>
<td>Gendron, 1989</td>
</tr>
<tr>
<td>\textit{Macrocystis pyrifera}</td>
<td>4.30</td>
<td>Lobban, 1978</td>
</tr>
<tr>
<td></td>
<td>3.40</td>
<td>Cribb, 1954</td>
</tr>
<tr>
<td>\textit{Nereocystis lutekeana}</td>
<td>0.90</td>
<td>Kain, 1987</td>
</tr>
<tr>
<td></td>
<td>3.60</td>
<td>Nicholson, 1970</td>
</tr>
<tr>
<td></td>
<td>5.10</td>
<td>Maxell and Millar, 1996</td>
</tr>
<tr>
<td></td>
<td>7.80</td>
<td>Duncan, 1973</td>
</tr>
<tr>
<td>\textit{Pelagophycus porra}</td>
<td>0.70</td>
<td>Coyer and Zaugg-Hagland, 1982</td>
</tr>
<tr>
<td>\textit{Saccorhiza polyschides}</td>
<td>0.17</td>
<td>Norton and Burrows, 1969</td>
</tr>
<tr>
<td>\textit{Undaria pinnatifida}</td>
<td>0.30</td>
<td>Stuart, 1997</td>
</tr>
</tbody>
</table>

Furthermore, it has been demonstrated that the rate of stipe growth varies with depth and wave exposure; in regions of greater wave action stipes are typically stunted while growth of stipes is slower at depths > 14 m compared to shallower depths (Duncan, 1973;
Novaczek, 1980). Water velocity adjacent to *E. radiata* blades was similar between sites in the present study although velocity was higher compared to other studies. The high water velocity experienced by *E. radiata* in Doubtful Sound may have resulted in internal reserves of inorganic N and C being allocated to structural components in order to increase the flexibility, or rigidity, of the stipe rather than into growth. Future studies could investigate the different structural components and biomechanical properties of the stipe in relation to water motion.

### 2.4.3.2 Erosion rates of *Ecklonia radiata*

Erosion rates of *Ecklonia radiata* from Seymour Island and Thompson Sound in Doubtful Sound exhibited a temporal pattern with rates at both sites declining between March and July (autumn/winter), while *E. radiata* at Causet Cove did not display any temporal pattern. This is opposite to the pattern of erosion found for *E. radiata* from southern Australia, which began declining in July and generally remained low through to November (Kirkman, 1984). Erosion rates at Seymour Island were positively correlated with PFD; greatest erosion occurred as PFD increased. This may be the result of the blade remaining parallel to the water surface due to the unidirectional current, instead of being swept back and forth as would occur in a swell and surge dominated habitat. As the blade could remain parallel to the surface for some time it may be subjected to periods of high irradiance that may have a detrimental effect on the blade tissue due to photoinhibition and subsequent damage to the photosystems. Negative erosion rates were occasionally observed at Seymour Island and Thompson Sound, and were the result of elongation of the blade above the punched hole during some of the tagging periods. Negative erosion rates were also observed for line-cultured *Undaria pinnatifida* in Japan for the same reason (Yoshikawa *et al*., 2001). Although a preliminary trial at the beginning of the present study found 93% blade elongation occurred within the first 10 cm from the blade/stipe junction, it is recommended based on these results that the hole be punched further away (e.g. 15 cm from the blade/stipe junction) or that additional holes be punched at regular intervals along the length of the blade.
It has been demonstrated that kelp-derived carbon (eroded tissue) contributes to all aspects of the marine food web, either directly via grazing, indirectly via detrital pathways or through the release of dissolved organic matter (DOM) (e.g. Mann, 1973; Stuart et al., 1982; Branch and Griffiths, 1988; Duggins et al., 1989; Lobban and Harrison, 1997; Yoshikawa et al., 2001). Although there was evidence that _Ecklonia radiata_ had been grazed upon directly, at no time during the present study was grazing observed (pers. obs.). There are a number of faunal species that may graze directly upon _E. radiata_ in Doubtful Sound, such as the sea urchin or kina, _Evechinus chloroticus_, however, there is no published data identifying which faunal species do graze _E. radiata_ in Doubtful Sound. Future research could identify and quantify animals that graze directly upon _E. radiata_ in Doubtful Sound, and perhaps continue with feeding trials to see whether _E. radiata_ is a preferred food source.

It has been estimated that less than 10% of kelp-derived carbon is eaten directly; the remainder is released as particulate organic matter (POM) (Mann, 1973). Mann further suggests that erosion at the blade tip of _Laminaria longicruris_ is a continuous process and is the result of senescence. Necrotic tissue was observed at the distal end of the primary blade of _Ecklonia radiata_ year-round in the present study and may be sloughed off due to abrasion as a result of direct grazing or wave action. Duggins et al., (1989) demonstrated that kelp-derived C was found throughout the near-shore food chain using stable carbon isotope (δ¹³C) analyses of kelp-dominated (_Laminaria groenlandica_, _L. longipes_ and _Alaria fistulosa_) near-shore communities from the Aleutian Islands. Furthermore, growth rates of the filter feeders _Mytilus edulis_ (mussel) and _Balanus glandula_ (an intertidal barnacle) transplanted to kelp-dominated areas were two to four times greater than rates from sea-urchin dominated areas. Branch and Griffiths (1988) and Duggins et al. (1989) present studies and illustrations pertaining to the fate of kelp productivity in the near-shore ecosystem, which have been summarized in Lobban and Harrison (1997; Fig. 4.3a and b). A similar study should be undertaken in Doubtful Sound to assess the importance of _E. radiata_ and other macroalgal-derived carbon to the growth and production of consumers, such as sea urchins, within the Sounds. Knowledge of this process could have important implications for the survival of higher trophic
species (e.g. fish and sea urchins) as well as for the management of the pristine waters of the Fiordland World Heritage Park.

2.4.4 Morphological characteristics of *Ecklonia radiata*

Against predictions, only two morphological parameters differed on a spatial and temporal basis. *Ecklonia radiata* at Seymour Island had thinner primary blades throughout the study while stipes were longer between June 1999 and March 2000 compared to *E. radiata* at the two outer sites. A reduction in blade thickness is often indicative of a deeper (Larkum, 1972; Drew, 1974) and lower light environment. The LSL in Doubtful Sound could be as deep as 6-7 m at Seymour Island compared with 3-4 m at Causet Cove and Thompson Sound (*pers. obs.*), however, attenuation coefficients indicate that the turbidity of the water was similar between sites. The greatest difference observed between sites was in the spectral composition of PAR; green/yellow wavelengths dominated at Seymour Island while blue wavelengths dominated at Causet Cove and Thompson Sound. The lack of blue light at Seymour Island may have reduced photosynthetic rates thereby reducing C and inorganic N uptake resulting in lower internal C and N reserves available for structural material. Also, kelp blades may be several cell layers thick, resulting in shading of the innermost cells (Lobban and Harrison, 1997). A thinner blade would therefore increase transmission of PFD through the blade due to fewer cell layers and perhaps increase the light-harvesting ability of the macroalga as more light would reach more chloroplasts. Morphological changes may also affect the surface area:volume (SA:V) ratio. A higher SA:V ratio results in a reduction in resources required for structural material as well as maximizing the area available for light harvesting (Gerard and Mann, 1979; Hurd, 2000).

Stipe lengths of *Ecklonia radiata* from the study sites in Doubtful Sound averaged 35 cm or less and had maximum growth rates of 0.5 cm d⁻¹ at all three sites. *E. radiata* in New Zealand typically has stipes up to 150 cm in length with short-stiped plants more noticeable in wave-exposed regions compared to wave-sheltered areas (Lindauer *et al.*, 1961; Novaczek, 1980; Larkum, 1989). Morton and Miller (1968) noted three forms of *E. radiata* in New Zealand with a short-stipe, frilled form, such as found in Doubtful
Sound, commonly found where light penetration was reduced in turbid waters. The longer stiped sporophytes observed at Seymour Island during the present study may reflect an adaptation to the low PFD imposed by the LSL whereby stipes lengthen with decreasing PFD in order to place the blade as close as possible to the water surface.

Many studies have demonstrated that the morphology of macroalgae, particularly the blade, varies with exposure to water motion (e.g. Lindauer et al., 1961; Norton and Burrows, 1969; Larkum, 1972; Russell, 1978; Gerard and Mann, 1979; Druehl and Kemp, 1982; Koehl, 1986; Hurd et al., 1996; Kawamata, 2001). Blade morphology of *Ecklonia radiata* from Causet Cove and Thompson Sound displayed a wave-exposed morphology whereby blades were thicker and more rigid than those at Seymour Island, which displayed a wave-sheltered morphology regardless of the fact that water velocity was similar between the three sites. Wave-exposed morphologies may necessitate an increase in structural tissue (Norton et al., 1981) via changes in alginic acid composition (Kraemer and Chapman, 1991), decreasing the amount of photosynthetic tissue thereby reducing linear growth rates. Alternatively, the thin, wide bladed, long stiped sporophytes present at Seymour Island were very flexible, had a higher SA:V ratio as a result of less necessity for structural tissue and hence, had growth rates similar to those of *E. radiata* at Causet Cove and Thompson Sound. It seems likely that it is the type of water motion (swell and surge versus a uni-directional current), and not the velocity adjacent to the blade surface, which may account for the morphological differences of *E. radiata* in the present study. Recent research by Stevens et al. (2003) supports this contention. Reciprocal transplants could be carried out to investigate whether the morphological differences observed in the present study are a result of differences in water motion, or whether some other factor (i.e. reduce PFD, geneotypic differences between populations) is influencing morphology.

### 2.4.5 *Ecklonia radiata* population density

The population density of *Ecklonia radiata* at Seymour Island (~2.5 m²) was four to five times lower than the density at Causet Cove and Thompson Sound (averaging 7.5 and 8 sporophytes m², respectively). However, compared to the densities of *E. radiata*
recorded in other regions of New Zealand and Australia, those in Doubtful Sound were extremely low overall. Novaczek (1984) recorded densities of *E. radiata* up to 50 plants m$^{-2}$ at Goat Island, New Zealand, Schiel and Nelson (1990) suggest that *E. radiata* in New Zealand may reach densities of up to 75 (mature) plants m$^{-2}$ at depths of 4-15 m, while Larkum (1986) estimated the mean density of mature *E. radiata* at Port Jackson, Australia, at 22.5 plants m$^{-2}$. Sporophyte density is determined by spore settlement and gametophyte survival, which is affected by substratum, PFD and spectral composition, water motion, temperature and salinity, or a combination of these factors. The substratum at Seymour Island was composed of shelly debris and sand with most *E. radiata* found attached to dead horse-mussels (*Atrina zealandica*; pers. obs.). The water motion at this site was unidirectional with the velocity reaching 15.5 cm s$^{-1}$. The LSL was also thicker at this site than at Causet Cove and Thompson Sound, and attenuation coefficients indicated that the water was turbid more often than not. There was also a reduction in the blue region of the action spectrum. The low population density at Seymour Island in comparison with densities at Causet Cove and Thompson Sound can therefore be explained by the combination of a lack of suitable substrata, a fast, unidirectional water flow, turbid water and lack of blue light that are not conducive for either spore settlement or gametophyte survival. Sporophytes themselves are influenced by storm events and turbulent water motion that may dislodge and rip whole sporophytes from the substratum, as well as break stipes and tear blades removing the meristematic region, resulting in a decline in density. However, these events are typically episodic and may actually help the maintenance of the macroalgal population, as is the case for *E. radiata* in Australia (Kirkman, 1981; Kennelly, 1987).

2.4.6 Application of the area of constant biomass model to *Ecklonia radiata*

The area of constant biomass model has been assessed as the more reliable model for estimating biomass productivity in laminarians compared with the exponential or chordal models. However this method, while independent of the generalizations influencing the other two models, necessitates that the shape and size of the plants under investigation remain relatively constant over the growth period (Gendron, 1985). The primary blade of
Ecklonia radiata maintained a uniform shape during the experimental period, thereby meeting the criteria of the model.

The location of the meristematic region varies according to species and as linear growth increases with blade size, it is therefore necessary to determine the location of the meristematic region at the onset of the experiment (Kain, 1987). A preliminary trial on Ecklonia radiata from Doubtful Sound found up to 93% of blade elongation occurred within 10 cm of the blade/stipe junction. The area of constant biomass model is only reliable provided all meristematic tissue is included below the punched hole. Blade growth, and subsequent productivity estimates, in laminarian kelps have often been based on growth increments measured at a distance of 10 cm from the blade/stipe junction (Chapman and Craigie, 1977; Luning, 1979; Mann and Kirkman, 1981; Gangé et al., 1982; Conolly and Drew, 1985; Larkum, 1986; Kirkman, 1989; Flores-Moya et al., 1993; Sjøtun and Gunnarsson, 1995). However, several studies have shown meristematic activity in laminarian kelps distal of 10 cm (Buggeln, 1974; Kain, 1976; Sjøtun, 1983; Gangé and Mann, 1987; Ishikawa, 1993; Stuart, 1997). Furthermore, it is difficult to assess errors due to temporal factors. It is recommended that a preliminary trial is essential to follow blade elongation in laminarians such as E. radiata, however if the meristematic region increases with blade size or over time this may not be observed in a one off preliminary trial such as undertaken in the present study. It is recommended that instead of increasing the distance of the hole from the blade/stipe junction (i.e. from 10 to 20 cm distance), that holes be punched at regular intervals along the entire blade.

To determine productivity using the area of constant biomass model requires destructive sampling. Destructive sampling often leads to a change in community structure, particularly when the density of the macroalga being investigated is not high to begin with. Removal of Ecklonia radiata in the present study was restricted to ten-tagged sporophytes used to measure growth and five additional sporophytes for experimental work (determination of nutrient uptake and photosynthetic parameters) from each site each field trip to limit the effects on the community. Ideally a larger sample size would have reduced variation at each site and provided a better estimate of growth and
productivity rates. For example, Gendron (1985) had a sample size of 50-100 plants, and Sjøtun et al. (1998) tagged up to 25 Laminaria hyperborea individuals. The sample size must also be large enough to accommodate the loss of tagged plants. In the present study, 70 % of tagged plants survived with losses reduced over shorter time periods (i.e. one month compared to two months).

2.4.6.1 Productivity of Ecklonia radiata

The daily productivity of individual Ecklonia radiata sporophytes on a dry weight basis was generally greater during 1998 than the rest of the study period. Productivity of E. radiata at Thompson Sound was approximately two-fold higher than sporophytes from Causet Cove and Seymour Island in October 1998. This can be attributed to higher blade growth rates at Thompson Sound at this time. Daily individual productivity values (g DW d\(^{-1}\)) for Ecklonia radiata from two locations in Australia were within the range recorded for E. radiata in the present study while rates for E. radiata from northern New Zealand were slightly lower (Table 2.4). The lower individual productivity values for E. radiata from Goat Island may be the result of smaller blades at this site (10 –30 cm at 15 m) compared to blade size in Doubtful Sound (23 – 58 cm). Furthermore, productivity of E. radiata at Goat Island was estimated using length-weight regressions compared to the use of the area of constant biomass in the present study and this may account for the slight discrepancy.

Values of daily population productivity (g DW m\(^{-2}\) d\(^{-1}\)) of Ecklonia radiata at Causet Cove and Thompson Sound in the present study were similar to values documented for other laminarians (Table 2.5), with the lower population density at Seymour Island resulting in relatively low productivity rates that were comparable to those at 7 m in northern New Zealand (Novaczek, 1984). The extremely low value recorded for Phyllariopsis purpurascens may by attributed to it being a summer annual as well as being collected from a depth of 30 m (Flores-Moya et al., 1993). Population productivity of E. radiata from Causet Cove and Thompson Sound on a C basis was similar to E. cava from Japan, E. radiata from Sydney, Australia and Laminaria longicruris from Canada (Table 2.5). For E. radiata from Seymour Island population productivity was similar to
Chapter Two: Growth rate and productivity of *Ecklonia radiata*

Table 2.4: Daily individual sporophyte productivity (g DW d$^{-1}$) of *Ecklonia radiata* from various locations and depths. *E. radiata* at the three study sites in Doubtful Sound were collected between 10 and 20 m.

<table>
<thead>
<tr>
<th>Locality and depth</th>
<th>Productivity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goat Island, NZ* - 7 m</td>
<td>0.14</td>
<td>Novaczek, 1984</td>
</tr>
<tr>
<td>- 15 m</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>Perth, Australia - 5 m</td>
<td>0.49</td>
<td>Kirkman, 1989</td>
</tr>
<tr>
<td>- 10 m</td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td>Sydney, Australia - 1.5 m</td>
<td>0.27 – 0.5</td>
<td>Larkum, 1986</td>
</tr>
<tr>
<td>Doubtful Sound - Seymour Island</td>
<td>0.14-0.76</td>
<td>Present study</td>
</tr>
<tr>
<td>- Causet Cove</td>
<td>0.19-0.98</td>
<td>“ “</td>
</tr>
<tr>
<td>- Thompson Sound</td>
<td>0.16-1.38</td>
<td>“ “</td>
</tr>
</tbody>
</table>

*NZ = New Zealand

that of *Undaria pinnatifida* from Dunedin, New Zealand, and may be attributed to the low structural C component as a result of thin blades at these two sites.

Values of annual population productivity (kg DW m$^{-2}$ y$^{-1}$) for *Ecklonia radiata* from the three sites in the present study were similar to those estimated for *E. radiata* from northern New Zealand at 15 m, and Perth, Australia, at 10 m (Table 2.6). The slightly higher productivity estimates for *E. radiata* from Port Jackson and Perth, Australia and *E. cava* from Japan can be attributed to the plants being collected from a shallower depth. This also reflects the trend of higher growth rates at shallower depths (e.g Novaczek, 1984; Kirkman, 1989). Although the density of *E. radiata* from northern New Zealand and Australia was greater than that in Doubtful Sound, annual productivity was similar as *E. radiata* in Doubtful Sound could attain a greater sporophyte size as there was less self-shading and greater water movement that may reduce sedimentation as well as maintain the supply of nutrients to the blade. The rates of annual population productivity for *E.
Table 2.5: Comparison of daily population productivity estimates on a dry weight (DW) and carbon (C) basis for various laminarians.

<table>
<thead>
<tr>
<th>Species</th>
<th>Productivity (g DW m(^{-2}) d(^{-1}))</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ecklonia cava</em></td>
<td>7.7</td>
<td>Hayashida, 1977</td>
</tr>
<tr>
<td><em>E. radiata</em> – Seymour Island</td>
<td>0.43-2.16</td>
<td>Present study</td>
</tr>
<tr>
<td>– Causet Cove</td>
<td>1.6-8.1</td>
<td>“”</td>
</tr>
<tr>
<td>– Thompson Sound</td>
<td>1.6-10.7</td>
<td>“”</td>
</tr>
<tr>
<td>– 5 m</td>
<td>4.9</td>
<td>Kirkman, 1989</td>
</tr>
<tr>
<td>– 10 m</td>
<td>12.1</td>
<td>“”</td>
</tr>
<tr>
<td>– 7 m</td>
<td>0.8-1.4</td>
<td>Novaczek, 1984</td>
</tr>
<tr>
<td>– 15 m</td>
<td>8.2</td>
<td>“”</td>
</tr>
<tr>
<td><em>Laminaria longicruris</em></td>
<td>4.1-19.2</td>
<td>Egan and Yarish, 1990</td>
</tr>
<tr>
<td><em>Macrocystis pyrifera</em></td>
<td>10.9-19.2</td>
<td>Gerard and North, 1984</td>
</tr>
<tr>
<td><em>Phyllariopsis purpurascens</em></td>
<td>0.03</td>
<td>Flores-Moya <em>et al.</em>, 1993</td>
</tr>
<tr>
<td><em>Undaria pinnatifida</em></td>
<td>0.15-4.4</td>
<td>Stuart, 1997</td>
</tr>
</tbody>
</table>

(g C m\(^{-2}\) d\(^{-1}\))

<table>
<thead>
<tr>
<th>Species</th>
<th>Productivity (g C m(^{-2}) d(^{-1}))</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Durvillaea antarctica</em></td>
<td>7.1</td>
<td>Haxen and Grindley, 1985</td>
</tr>
<tr>
<td><em>Ecklonia cava</em></td>
<td>2.6</td>
<td>Yokohama <em>et al.</em>, 1987</td>
</tr>
<tr>
<td><em>E. maxima</em></td>
<td>5.56</td>
<td>Mann <em>et al.</em>, 1979</td>
</tr>
<tr>
<td><em>E. radiata</em></td>
<td>2.2</td>
<td>Larkum, 1986</td>
</tr>
<tr>
<td><em>E. radiata</em> – Seymour Island</td>
<td>0.08-0.48</td>
<td>Present study</td>
</tr>
<tr>
<td>– Causet Cove</td>
<td>0.41-2.29</td>
<td>“”</td>
</tr>
<tr>
<td>– Thompson Sound</td>
<td>0.38-2.74</td>
<td>“”</td>
</tr>
<tr>
<td><em>Laminaria longicruris</em></td>
<td>0.4-1.2</td>
<td>Hatcher <em>et al.</em>, 1977</td>
</tr>
<tr>
<td><em>L. solidungula</em></td>
<td>3.6</td>
<td>Chapman and Lindley, 1980</td>
</tr>
<tr>
<td><em>Macrocystis integrifolia</em></td>
<td>3.56</td>
<td>Wheeler and Druehl, 1986</td>
</tr>
<tr>
<td><em>M. laevis</em></td>
<td>7.7-11.5</td>
<td>Attwood <em>et al.</em>, 1991*</td>
</tr>
<tr>
<td><em>M. pyrifera</em></td>
<td>9.5</td>
<td>Jackson, 1977</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Jackson, 1987</td>
</tr>
<tr>
<td><em>Undaria pinnatifida</em></td>
<td>0.04-1.19</td>
<td>Stuart, 1997</td>
</tr>
</tbody>
</table>

* Based on growth measurements in April/May 1988 and August 1989.
Table 2.6: A comparison of the annual population productivity of *Ecklonia* species from New Zealand, Australia and Japan.

<table>
<thead>
<tr>
<th>Location</th>
<th>Species</th>
<th>kg DW m$^{-2}$ y$^{-1}$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doubtful Sound – Seymour Island</td>
<td><em>E. radiata</em></td>
<td>0.17-0.61</td>
<td>Present study</td>
</tr>
<tr>
<td>– Causet Cove</td>
<td></td>
<td>0.78-2.11</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>– Thompson Sound</td>
<td></td>
<td>0.68-2.48</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>North Island, NZ – 7 m</td>
<td><em>E. radiata</em></td>
<td>3</td>
<td>Novaczek, 1984</td>
</tr>
<tr>
<td>– 15 m</td>
<td></td>
<td>0.3-0.5</td>
<td></td>
</tr>
<tr>
<td>Port Jackson, Australia</td>
<td><em>E. radiata</em></td>
<td>2.9</td>
<td>Larkum, 1986</td>
</tr>
<tr>
<td>Perth, Western Australia – 5 m</td>
<td><em>E. radiata</em></td>
<td>3.4</td>
<td>Kirkman, 1989</td>
</tr>
<tr>
<td>– 10 m</td>
<td></td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>Japan</td>
<td><em>E. cava</em></td>
<td>2.8</td>
<td>Hayashida, 1977</td>
</tr>
</tbody>
</table>

*E. radiata* from Doubtful Sound are possibly underestimated as 1) estimates are based on bimonthly blade growth rates and 2) productivity of the stipe and hapteron were not estimated in this study although they are unlikely to contribute much more tissue.

2.4.7 Summary

Solar irradiance was higher from November through February and lower from June to August. PFD was greatest at the water surface, declining with depth and was lower during the second half of the study period, particularly at Seymour Island. At Seymour Island green/yellow wavelengths dominated while blue wavelengths were prevalent at Causet Cove and Thompson Sound. Attenuation coefficients indicated turbid water at all three sites while there was greater attenuation in the blue region of the action spectra at Seymour Island. Water velocity adjacent to *Ecklonia radiata* blades ranged from 4 -15.5 cm s$^{-1}$, which is considerably higher compared to similar studies. Elongation of the primary blade was greatest in the first 10 cm with no elongation occurring after 25 cm. Growth rates of *Ecklonia radiata* in Doubtful Sound were high between September and October and lower between June and July with growth rates in September and October
1998 up to four and a half times greater than the same period in 1999. Spatially, *E. radiata* from Seymour Island generally had lower blade growth rates than those from Causet Cove (which were typically the highest) or Thompson Sound, as was expected due light reduction as a result of the LSL. *E. radiata* at Seymour Island and Thompson Sound showed a temporal pattern in erosion rates while sporophytes at Causet Cove did not. Erosion rates were similar between sites. The population density at Seymour Island was up to four fold lower than densities at Causet Cove and Thompson Sound although the overall density in Doubtful Sound was lower than for other locations in northern New Zealand and Australia. Only two of the six morphological parameters measured, blade thickness and stipe length, differed between the three sites with *E. radiata* at Seymour Island having thinner blades and longer stipes compared to *E. radiata* at Causet Cove and Thompson Sound. These differences in morphology are thought to be an adaptation to the reduced PFD imposed by the LSL. Daily individual sporophyte productivity on a dry weight and C basis were similar between the threes sites and were similar to previous studies of *E. radiata* in northern New Zealand and Australia. Population productivity per unit dry weight and C of *E. radiata* from Seymour Island was lower than rates at Causet Cove and Thompson Sound due to the lower population density. Annual population productivity was comparable to rates for *E. radiata* from 10 and 15 m in Australia and northern New Zealand. Two of the factors commonly thought to affect growth rates and productivity of macroalgae, nutrient uptake and photosynthetic rates, are examined in further detail in the following chapters.
Chapter Three

Inorganic nitrogen uptake by *Ecklonia radiata*

3.1 INTRODUCTION

3.1.1 Nitrogen availability and sources in temperate marine regions

Nitrogen (N) is a limiting factor of macroalgal growth as well as being an important component of cellular proteins, chlorophyll, nucleic acids, amino acids and other cellular compounds (Chapman and Craigie, 1977; Chapman, 19782; Hanisak, 1979; Wheeler and North, 1981; Rosenberg and Ramus, 1982; Lapointe and Duke, 1984; Codispoti, 1997; Pavia and Toth, 2000). Macroalgae can utilize three inorganic N forms: ammonium (NH$_4^+$), nitrate (NO$_3^-$) and nitrite (NO$_2^-$) (DeBoer, 1981). In temperate coastal waters ambient seawater NO$_3^-$ concentrations range from 0 – 10 μM and are typically maximal in winter decreasing during the summer months while NH$_4^+$ concentrations are relatively low but constant year-round (< 4 μM; e.g. Ryther and Dunstan, 1971; Dugdale, 1976; Nixon and Pilson, 1983; Sharp, 1983; Lobban and Harrison, 1997).

Sources of inorganic N available to macroalgae are sometimes categorised as ‘new’ N or regenerated N (O’Neil *et al.*, 1996). ‘New’ N occurs via physical advection, or upwelling events, but also includes atmospheric input via rain or N$_2$ fixation by microbes, anthropogenic inputs such as terrestrial run-off, sewage and agricultural fertilizer and submarine discharge of ground water in coastal areas, especially NO$_3^-$ (Nixon *et al.*, 1976; Johannes, 1980; Capone and Bautista, 1985; Paerl, 1985; Paerl *et al.*, 1990). Regenerated N, mostly NH$_4^+$, may come from the sediment via NH$_4^+$ regenerated through bacterial reduction of NO$_3^-$ and NO$_2^-$, excretion from zooplankton and deamination of
organic nitrogenous compounds (Gilbert et al., 1982; Goldman et al., 1985; Verity, 1985; Flynn and Butler, 1986; Hanson et al., 1990).

3.1.1.1 Nitrogen availability and sources in Doubtful Sound

In Doubtful Sound the concentration of macronutrients, particularly N and phosphorus (P) is low in the LSL with little temporal variation although concentrations increase with depth (Walls, 1995; Goebel, 2001; Peake et al., 2001). The low levels of macronutrients in the LSL are attributed to the low anthropogenic influence (Peake et al., 2001) as Doubtful Sound is situated within the Fiordland National Park, a world heritage site. Anthropogenic inputs have been shown to contribute greatly to nutrient cycles in other fiordic systems (e.g. Jones et al., 1995). As the LSL flows towards the open ocean, it drives a counter current layer of oceanic water into the fiord. The largest source of inorganic N to benthic species may therefore be from this density-driven estuarine circulation that is responsible for the entrainment (or mixing) of the oceanic waters with the underlying marine waters in Doubtful Sound (Bowman et al., 1999; Gibbs et al., 2000; Gibbs 2001).

Each of the three sites in the present study may therefore be subject to different nutrient availability as a result of environmental influences such as the mixing of oceanic waters with the LSL and underlying salt water. This influence is likely to be greater at Causet Cove and Thompson as these sites are closest to the coastal entrance of the fiord. As a consequence, Ecklonia radiata may encounter different N sources and availability, and employ different uptake strategies as Chapman and Craigie (1977) demonstrated with Laminaria longicuruis from Nova Scotia, Canada. Causet Cove and Thompson Sound also experience surge-type water motion and have a thinner LSL than at Seymour Island therefore E. radiata is less likely to be affected by the nutrient concentration gradient surrounding the thallus (termed the diffusion boundary layer [DBL]) and light limitation. In contrast, a unidirectional current dominates at Seymour Island and the influence of the LSL maybe greater (i.e. greater reduction of PFD at depth). E. radiata at Seymour Island may therefore be more dependent on episodic storm events and wind-driven entrainment of the LSL into underlying marine waters to replenish nutrients.
3.1.2 Mechanisms of N uptake in macroalgae

Uptake of nutrients by macroalgae occurs through one or a combination of the following mechanisms. Ions can be transported across the cell membrane via passive diffusion, facilitated diffusion, or active transport (detailed in Lobban and Harrison, 1997). Briefly, passive diffusion is the transport of ions down an electrochemical gradient and as such it is not directly energy dependent, lacks ion selectivity and does not saturate. Facilitated diffusion is similar to passive diffusion but is mediated by carrier proteins, accelerating the rate of ion transport across the cell membrane. This process does not require energy but does exhibit ion selectivity and can become saturated. Active transport is energy-dependent, is mediated by carrier proteins and ion selective. It is thought that active transport most commonly occurs for NO$_3^-$ uptake; however, the mechanism often depends on pH, temperature and enzymatic activity (DeBoer, 1981; Reed, 1990). Ammonium is most commonly taken up via passive diffusion of NO$_3^-$ (DeBoer, 1981; Lobban and Harrison, 1997).

Once taken up, inorganic N is assimilated within the cells before being incorporated into various cellular components (Fujita et al., 1988; Lobban and Harrison, 1997). Assimilation of NO$_3^-$ is a two-step reduction process requiring energy in the form of electrons (eight in total) derived from light reactions during photosynthesis (Falkowski and Raven, 1997; Lobban and Harrison, 1997). In contrast, NH$_4^+$ is already reduced and can readily be incorporated into amino acids (Nasr et al., 1968, Lobban and Harrison 1997). Ammonium utilization may therefore be energetically cheaper than NO$_3^-$ (Syrett, 1962). Ammonium uptake is often more rapid than NO$_3^-$ uptake in many macroalgae when both NO$_3^-$ and NH$_4^+$ are available due to its reduced form, and the fact that NH$_4^+$ uptake is usually light independent (e.g. D’Elia and DeBoer, 1978; Haines and Wheeler, 1978; Wallentinus, 1984; Thomas and Harrison, 1985, 1987; O’Brien and Wheeler, 1987; Ahn et al., 1998; Harrison and Hurd, 2001). High NH$_4^+$ uptake rates may also be the result of diffusion of ammonia (NH$_3$) into cells following conversion from NH$_4^+$ at the cell surface due to high pH (Hurd, 2000).
3.1.3 Methods of measuring N uptake in macroalgae

Uptake rates of nutrients by macroalgae are most commonly measured by following the depletion of a nutrient from the seawater medium (Lobban and Harrison, 1997). There are two experimental methods employed: time-course (also called batch or perturbation) and multiple-flask. Briefly, the time-course method is where a known concentration of the nutrient is added to media that is then sampled at varying time intervals over a period of hours until the nutrient is exhausted (Caperon and Meyer, 1972; Topinka, 1978; Fujita, 1985; Harrison et al., 1989; Henley and Dunton, 1995, 1997; Hurd and Dring, 1990; Pedersen, 1994). Time-course experiments are typically used to reveal variations in N uptake rates over time to determine the incubation period for the multiple-flask method (Harrison et al., 1989). In the multiple flask method a range of nutrient concentrations are each added to separate flasks, an initial water sample is taken and after a short incubation period (i.e. 5 - 60 min) a final water sample is taken (McCarthy and Goldman, 1979; Wallentinus, 1984; Harrison et al., 1989; Hurd and Dring, 1990; Pedersen, 1994; Taylor and Rees, 1999). There are advantages and disadvantages to each method, which will be elaborated on in the discussion. Generally however, both methods are used as each determines different aspects of nitrogen uptake kinetics of a macroalga.

The kinetics of nutrient uptake will depend on which uptake mechanism transports inorganic N across the cell membrane (Lobban and Harrison, 1997). If the rate of N uptake is proportional to the external substrate concentration, passive diffusion is implied (Lobban and Harrison, 1997; Harrison and Hurd, 2001). However, if the uptake rate increases rapidly at low external substrate concentrations but a plateau is reached at high concentrations, uptake is saturated signifying that all transport mechanisms are working at maximal rates (Harrison and Druehl, 1982). Facilitated diffusion or active transport is then implied if a plot of N uptake rate vs. external substrate concentration yields a rectangular hyperbola (Harrison and Hurd, 2001). The rectangular hyperbola is analogous to the Michaelis-Menten equation for enzyme kinetics (Eq. 3.1 and Fig. 3.1):

\[ V = \frac{V_{\text{max}} \times K_s}{K_s + S} \]  

Eq. 3.1
where \( V \) is the initial uptake rate (\( \mu\text{mol g DW}^{-1} \text{ s}^{-1} \)), \( V_{\text{max}} \) is the maximum uptake rate at the saturating substrate concentration, \( S \) is the substrate concentration (\( \mu\text{M} \)) and \( K_s \) is the half-saturation constant (\( \mu\text{M} \)), the nutrient concentration where \( V = \frac{V_{\text{max}}}{2} \) (Harrison and Druehl, 1982; Harrison et al., 1989; Harrison and Hurd, 2001).

**Figure 3.1: Diagram of the Michaelis-Menten hyperbola and the associated uptake kinetic parameters.** \( V \) is the initial uptake rate (\( \mu\text{mol g DW}^{-1} \text{ s}^{-1} \)), \( V_{\text{max}} \) is the maximum uptake rate at the saturating substrate concentration, \( S \) is the substrate concentration (\( \mu\text{M} \)), \( K_s \) is the half-saturation constant (\( \mu\text{M} \)), the nutrient concentration where \( V = \frac{V_{\text{max}}}{2} \), and alpha (\( \alpha \)) is the ratio \( V_{\text{max}} / K_s \).
The maximum uptake rate ($V_{\text{max}}$) is used to compare the ability of macroalgae to take up nutrients at high concentrations while $K_s$ is used to compare the ability of macroalgae to take up nutrients at low concentrations (Harrison and Hurd, 2001). Macroalgae with a high $V_{\text{max}}$ are better able to take up nutrients at high concentrations than macroalgae with a low $V_{\text{max}}$. In comparison, a low $K_s$ indicates a greater affinity for nutrients at low concentrations (DeBoer, 1981). The ratio of $V_{\text{max}} / K_s$ gives an estimate of the steepness of the linear portion of the rectangular hyperbola, termed alpha ($\alpha$; Lobban and Harrison, 1997). However, $\alpha$ invariably incorporates the associated errors of both $V_{\text{max}}$ and $K_s$, as determination of the $K_s$ value is dependent upon $V_{\text{max}}$. An alternative method is to compare uptake of inorganic N at a low (relevant) concentration (i.e. 2 µM), with the uptake rate ($V$) at this concentration denoted by a subscript (e.g. $V_2$; Healey, 1980; Hurd and Dring, 1990).

Most nutrient uptake experiments are carried out in the laboratory as in the present study, and while it is preferable to use whole macroalgae, allowances must be made to accommodate large macroalgae, such as *Ecklonia radiata*. Cut portions of the blade may be used but cutting may alter uptake rates due to wound healing, often with increased accumulation of polysaccharides at the location, and increased respiration (Hatcher, 1977). Therefore, many researchers place pieces of macroalgal tissue into constant conditions for several hours to allow wound healing to take place as was done in the present study.

### 3.1.4 Factors influencing inorganic N uptake by macroalgae

There are several abiotic and biotic factors that influence nutrient uptake rates in macroalgae. Consideration of the experimental conditions under which N uptake is measured is important when making inferences about the uptake mechanism involved. Uptake rates frequently vary over time therefore the maximum uptake rate will depend on the duration of the experiment (Harrison *et al.*, 1989). Uptake rates are also influenced by the past nutritional history, internal N storage capacity, age and type of tissue of the macroalgae being investigated. Many macroalgae have the capacity to store inorganic N,
particularly NO$_3^-$, in the vacuole and cytoplasm for future use when external substrate concentrations are low (Harrison and Hurd, 2001). It has been demonstrated in perennial macroalgal populations that rates of NO$_3^-$ and NH$_4^+$ uptake, as well as internal N content, decrease with increasing age (Harrison et al., 1986; Sjøtun et al., 1996). It is also common for younger tissue to have higher uptake rates than mature tissue (Topinka, 1978; Wheeler and Srivastava, 1984; Thomas et al., 1985; Harrison et al., 1986).

In Doubtful Sound the concentration of inorganic N in the seawater, amount of PFD available and water motion will influence uptake rates by *Ecklonia radiata*. Light drives the photosynthetic process that provides the reductant and energy (in the form of ATP) for the active transport and reduction of NO$_3^-$ as well as providing organic C skeletons for the assimilation of N into amino acids (DeBoer, 1981; Turpin et al., 1988; Turpin, 1991; Kopczak, 1994; Lobban and Harrison, 1997). Therefore, PFD will influence inorganic N uptake indirectly, although the effect may be greater on NO$_3^-$ uptake than NH$_4^+$ as NO$_3^-$ has to be reduced prior to incorporation into amino acids. A reduction in PFD as imposed by the LSL may influence the photosynthetic ability of *E. radiata* thereby affecting NO$_3^-$ reduction and C fixation. Higher PFD levels and/or light flecks at the outer sites may also stimulate macroalgal growth thereby increasing demand for inorganic N and protein synthesis (Koehl and Albert, 1988; DeBoer, 1981; Wing and Patterson, 1993; Lobban and Harrison, 1997). Water motion supplies nutrients to the surface of the macroalga and also determines the thickness of the DBL, which may limit the availability of nutrients at the surface of the macroalga, and hence influence uptake rates (Lobban and Harrison, 1997; Hurd, 2000).

### 3.1.5 Objectives of chapter three

There were three objectives of this chapter. The first objective was to determine the temporal variation in nutrient availability in Doubtful Sound by measuring the ambient seawater concentration of NO$_3^-$ and NH$_4^+$ in the surface LSL, and at the depths at which *Ecklonia radiata* was collected (10 – 20 m). It was expected that ambient seawater inorganic N would display the typical pattern observed in temperate regions (refer 3.1.1). The second objective was to investigate the inorganic N status (% tissue C and N, C:N ratio and soluble tissue NO$_3^-$ and NH$_4^+$ content) of *E. radiata* growing in Doubtful Sound
on both a temporal and spatial scale to examine whether N may limit growth and productivity, and whether *E. radiata* employs nutrient storage as a strategy to optimise growth. The third objective was to determine the inorganic N uptake kinetics ($V_{\text{max}}$, $K_s$, $\alpha$ and $V_2$) of *E. radiata* in Doubtful Sound using time-course and multiple-flask experiments to compare NO$_3^-$ and NH$_4^+$ uptake kinetics between the three study sites over time.
3.2 METHODS

3.2.1 General laboratory preparation
All glassware was washed using De-con® 90, acid washed (10% HCl) and rinsed (18-Ω high purity water) before each experiments.

3.2.2 Preparation of seawater for experiments
Natural filtered (0.90 µm Whatman GF/C) seawater (< 5 µM NO₃⁻ + NH₄⁺) was used for all experiments and was sourced from Portobello Marine Laboratory located 10 km northwest of Dunedin. Filtered seawater was stored in 20 L opaque plastic carboys in darkness at 4°C.

3.2.3 Inorganic N concentration of ambient seawater
Triplicate 30 mL seawater samples were collected from the water surface and the same depths from which Ecklonia radiata were collected from each site (10-20 m). Samples were collected monthly from September 1998 until December 1998, then bi-monthly from February 1999 to March 2000. Samples were stored in 30 ml polyethylene vials (TechnoPlas, Code P-9025-UU) and placed in a fridge (4°C) for 36-48 h until transported to the laboratory in Dunedin. Samples were then filtered through Whatman™ GF/C glass microfibre filters (0.45 µm) and frozen until analysis. Seawater samples were analysed colormetrically for inorganic NO₃⁻ and NH₄⁺ using a QuikChem® 8000 Automated Ion Analyzer (Lachat Instruments Inc., Wisconsin, USA, hereafter referred to as an auto-analyser). Omninon Flow Injection Analysis software (v 1.3, Lachat Instruments Inc., Wisconsin, USA) was used to correct for salinity effects at low N concentrations. The auto-analyser could detect a minimum level of 0.03 and 0.07 µM of NO₃⁻ and NH₄⁺, respectively (Lachat Instruments Inc., Wisconsin, USA).

3.2.4 Collection methods for Ecklonia radiata
Destructive sampling of Ecklonia radiata sporophytes was a trade-off between having enough material for adequate replication during experiments, whilst minimizing the impact on the E. radiata populations at each site. Five mature (> 60 cm) sporophytes were collected and placed in 20 L opaque covered buckets underwater at depth (10-20 m).
from each of the three sites no more than 12 h before returning to Dunedin. The sporophytes were aerated overnight using Silent-Flo® 5000 aquarium air pumps, before being transported back to the laboratory in Dunedin (Fig. 1.3; ~ 5 h away by car). Sporophytes could not be collected on the day of return as no diving is permitted on the day of leaving Doubtful Sound due to the height of Wilmot Pass (~ 600 m above sea level). The five *E. radiata* sporophytes from each site were numbered and used for the nutrient uptake experiments, as well as obtaining tissue to determine % tissue C and N, C:N ratios and soluble tissue NO$_3^-$ and NH$_4^+$ content. The sporophyte material was also used to obtain photosynthetic parameters and pigment content (Chapter 4). *E. radiata* sporophytes were maintained for the duration of the experiments in outdoor tanks with flowing seawater at the Portobello Marine Laboratory.

3.2.5 Inorganic N status of *Ecklonia radiata*

3.2.5.1 Percent tissue C and N, and C:N ratios

Percent tissue C and N were determined for five *Ecklonia radiata* sporophytes from each site monthly during 1998, beginning in September and then bi-monthly from February 1999 to May 2000. The primary blade of each of the five *E. radiata* collected from each site was divided into five sections: base of the blade, 2/5 from the blade base, middle of the blade, 4/5 from the blade base and distal tip of the blade. From each of the five sections, a piece of macroalgal tissue was cut and dried at 80°C for 24-36 h. The tissue from each sporophyte was pooled (n=5 per site), finely ground using a mortar and pestle, placed in ziplock plastic bags and stored on silica gel in a sealed plastic container. Ground tissue was analysed using a Carlo Erbo CHNS-O Elemental Analyser (EA1108) for % C and % N. The % tissue C and % tissue N were standardised to algal dry weight (g) and carbon to nitrogen (C:N) ratios were calculated on an atomic weight basis.

3.2.5.2 Soluble tissue NO$_3^-$ and NH$_4^+$

Soluble tissue NO$_3^-$ and NH$_4^+$ were determined for *Ecklonia radiata* bi-monthly from April 1999 until May 2000 using a boiling-water extraction method (Hurd et. al., 1996). The primary blades of each of the five *E. radiata* sporophytes collected from each site were visually divided into five sections (as above). From each of the five sections, pairs of tissue discs (SA double sided = 8.3 cm$^2$, n=5 per site) adjacent to each other were cut
using a cork borer. Each pair of discs were cleaned of sediment and epiphytic material, rinsed under running seawater and blotted dry. One disc of each pair was placed into a 50 mL boiling tube containing 40 mL of 18-Ω high purity water. The tubes were placed in a boiling water bath for 20 min, cooled, the supernatant decanted and filtered using 0.45 μm Whatman™ GF/C glass microfibre filters into 10 mL polyethylene vials and then frozen for subsequent NO₃⁻ and NH₄⁺ analysis using an auto-analyser. This process was repeated twice more as preliminary experiments indicated that boiling the same macroalgal tissue disc three times extracted all soluble tissue NO₃⁻ and NH₄⁺. The second disc from each pair cut was blotted dry and dried at 80°C for 24 – 36 hours to obtain a dry weight (g). The variation of soluble tissue inorganic N was minimal between discs taken from the five sections of each sporophyte blade therefore, the amounts of soluble tissue inorganic N for each sporophyte were pooled to give five replicates per site.

The amount of soluble tissue NO₃⁻ or NH₄⁺ (μmol g DW⁻¹) was determined as follows:

\[
\text{Soluble tissue NO}_3^- \text{ or NH}_4^+ = \frac{(B_1 + B_2 + B_3) \times 0.04}{DW} \quad (\text{Eq. 3.2})
\]

where

- \(B_1 \ldots B_3\) = NO₃⁻ or NH₄⁺ concentration (μM) in the supernatant after the first, second and third extractions, respectively
- 0.04 = extraction volume (L)
- DW = dry weight of macroalgal tissue disc (g)

3.2.6 Methods of measuring inorganic N uptake

Nitrate and NH₄⁺ uptake rates of *Ecklonia radiata* from three study sites were determined using time-course and multiple-flask experiments. Time-course experiments were conducted monthly from September 1998 to December 1998 then bi-monthly from February to April 1999 and were used to examine if the disappearance of the nutrient in question from the seawater media was linear or non-linear. Multiple-flask experiments
were conducted bi-monthly from June 1999 until May 2000 and were used to determine the inorganic N uptake kinetic parameters ($V_{\text{max}}$, $K_s$ and $\alpha$) for the three *E. radiata* populations being investigated.

### 3.2.6.1 Time-course of inorganic N uptake by *Ecklonia radiata*

#### 3.2.6.1.1 Pre-treatment of macroalgal material and experimental procedure

Sediment and epiphytes were removed from the sporophytes by cleaning with an absorbent paper towel under running seawater. For each of the five sporophytes collected per site four tissue discs were randomly cut (SA double sided =38.48 cm$^2$) from the primary blade and each set of discs per sporophyte placed in separate 1.25 L glass containers containing approximately 500 mL of filtered seawater. Containers were covered with parafilm to prevent spillage and placed overnight on orbital shakers (model SS70, Chiltem Scientific) set at 110-130 rpm to maintain water motion and reduce the formation of a boundary layer. Orbital shakers were situated in a controlled environment growth cabinet (Conviron model E15, Controlled Environments Ltd, Canada) set at 12°C. Cool white fluorescent tubes (160 W, Sylvania F72T12CW-VHO) and 40 W incandescent light bulbs (Osram) provided a PFD of 350 – 380 μmol photons m$^{-2}$ s$^{-1}$.

Treatments consisted of 350 mL filtered seawater added to 1.25 L glass containers containing either 5 μM NO$_3^-$, 5 μM NH$_4^+$, 30 μM NO$_3^-$ or 30 μM NH$_4^+$ (as KNO$_3$ or NH$_4$Cl respectively). The two concentrations were chosen based on data that indicated that nutrient concentrations in the water column in Doubtful Sound ranged from < 1 μM to ≤ 30 μM at the surface (N. Goebel, *pers. comm.*, 2001). The containers were randomly placed on orbital shakers in the growth cabinet under the same conditions maintained during pre-treatment approximately ten minutes before the commencement of the experiment to allow thorough mixing of the respective nutrient with the seawater. Randomisation also minimized confounding effects caused from unequal PFD within the growth cabinet or from the raised edges of the orbital shakers. An initial 8 mL water sample ($t=0$) was taken from each container to determine the initial concentration before one macroalgal disc was added per treatment. There were a total of five replicates per
treatment for each of the three sites. Controls consisted of filtered seawater only for each treatment and were used to monitor any changes in the concentration of inorganic NO$_3^-$ or NH$_4^+$ due to microbial activity (Harrison et al., 1986). Further water samples were taken at 30, 60, 90, 120, 180, 240, 300 and 360 minutes. Water samples were collected using silicon tubing attached to acid-washed (10% HCl) 25 mL syringes. Syringes were rinsed twice between samples using 18-Ω high purity water to avoid contamination. Samples were stored in 10 mL polyethylene vials (Techno-Plas, P-9716-UU) and frozen at –20 °C for later analysis. At the completion of each experiment, the macroalgal discs were removed and dried at 80 °C for 24-36 hrs to obtain a dry weight (g).

Water samples were analysed using an auto-analyser. The change in either NO$_3^-$ or NH$_4^+$ concentration over time was averaged between the five replicates per site for each monthly sampling period and graphed. The change in concentration was not standardized to dry weight as the same surface area was used for each replicate.

3.2.6.2 Determination of inorganic N kinetic parameters for *Ecklonia radiata* using the multiple-flask method

3.2.6.2.1 Pre-treatment of macroalgal material and experimental procedure

Fourteen tissue discs (SA double sided =29.04 cm$^2$) were randomly cut from the primary blade of each of the five sporophytes collected per site. Pre-treatment for tissue discs used in the multiple-flask experiments followed the same method used for time-course experiments (refer section 3.2.6.1.1).

An initial volume of 160 mL of natural filtered (0.45 μm, Whatman™ GF/C) seawater was added to 250 mL conical flasks and a known amount of either NO$_3^-$ or NH$_4^+$ (as KNO$_3$ or NH$_4$Cl respectively) was added from stock solutions. Treatments consisted of seven initial concentrations for each inorganic N form used (2, 4, 8, 16, 32, 48 and 64 μM) with five replicates per treatment for each site. Controls consisted of each nutrient at each concentration without macroalgal tissue. Flasks were randomly placed on an orbital shaker in the growth cabinet (refer section 3.2.6.1.1). An initial 9 mL water sample was removed from each flask using a 25 mL syringe with silicon tubing attached
before one tissue disc was added per flask. A final 9 mL water sample was collected from each flask one hour later. Samples were stored in 10 mL polyethylene vials (TechnoPlas, P-9716-UU) and frozen for later analysis. Each tissue disc was removed, blotted dry and dried at 80 °C for 24-36 hrs to obtain a dry weight (g). Nitrate and NH$_4^+$ uptake rates were expressed on a dry weight basis. Water samples were analysed using an auto-analyser.

3.2.6.2.2 Calculation of inorganic N uptake rates

Uptake rates at each nutrient concentration from the multiple-flask experiments were determined using the equation:

$$
V = \frac{(I - F) \times \text{vol}}{\text{DW} \times t}
$$

(Eq. 3.3)

where

- $V$ = uptake rate ($\mu$mol g DW$^{-1}$ s$^{-1}$)
- $I$ = initial substrate concentration ($\mu$M)
- $F$ = final substrate concentration ($\mu$M)
- vol = volume (L)
- $t$ = time (s)
- DW = dry weight (g)

3.2.6.2.3 Inorganic N uptake rates vs. substrate concentration, and kinetic parameters

Replicate data sets were pooled due to the pattern of uptake rate vs. concentration of individual replicates (Hurd and Dring, 1990), which displayed high variability within months (some replicates displayed saturated uptake while others displayed linear uptake, refer section 3.3.5.1). The five replicate data sets for each nutrient examined from each site each month were graphed together and the relationship between NO$_3^-$ uptake rate and NO$_3^-$ concentration, and NH$_4^+$ uptake rate and NH$_4^+$ concentration as described using the Michaelis-Menten equation (Eq. 3.1). Data were fitted to the Michaelis-Menten equation (Eq. 3.1) by means of an iterative non-linear least squares regression using SigmaPlot.
software (v 4.01, SPSS Inc, USA). The resultant curve for the average uptake rate from each site vs. substrate concentration each month was graphed to compare the monthly uptake patterns and to illustrate whether or not NO$_3^-$ and NH$_4^+$ uptake displayed saturation kinetics.

Kinetic parameters ($V_{\text{max}}$ and $K_s$) were determined each month for NO$_3^-$ or NH$_4^+$ using SigmaPlot software to calculate averages and standard error for each site. Alpha was calculated using the ratio $V_{\text{max}} / K_s$. In some instances saturation kinetics was not displayed and kinetic parameters could not be determined.

Comparisons could not be made between the various NO$_3^-$ and NH$_4^+$ kinetic parameters between sites or over time when uptake was linear as kinetic parameters could not be determined. Therefore, uptake rates at an ecologically relevant (low) concentration were used to compare the ability of each *Ecklonia radiata* replicate from the three sites to take up either NO$_3^-$ or NH$_4^+$. A concentration of 2 \( \mu \text{M} \) was selected based on the ambient seawater NO$_3^-$ and NH$_4^+$ concentrations monitored in Doubtful Sound in the present study. For data displaying saturation kinetics, uptake rates at 2 \( \mu \text{M} \) (denoted $V_2$) for *E. radiata* were calculated using the Michaelis-Menten equation (Eq. 3.1) while $V_2$ for data displaying linear uptake were estimated using linear regressions. Estimated uptake rates at 2 \( \mu \text{M} \) for each set of replicates per site (n=5) were averaged and the standard deviation calculated.

### 3.2.7 Data analysis

One-way ANOVA were used to examine monthly variation in % C, % N and C:N ratios of *Ecklonia radiata* between the three sites as well as temporal variation at each site. Spatial and temporal variations in soluble tissue NO$_3^-$ and NH$_4^+$ were also analysed using one-way ANOVAs. As a result of pooling replicate data and determining the average values for each kinetic parameter ($V_{\text{max}}$, $K_s$ and $\alpha$), statistical analysis using ANOVA could not proceed, as there was only one value per site per month. Therefore, trends displayed by *E. radiata* for inorganic N kinetic parameters have been described. Nitrate and NH$_4^+$ uptake rates at 2 \( \mu \text{M} \) were analysed using two-way ANOVA, with the factors being time and site.
The level of significance for ANOVA was $P<0.05$. Where ANOVA indicated significant differences between factors, post-hoc multiple comparisons were performed using Tukey tests. All analyses were performed using SigmaStat computer software (v2.03, SPSS Inc, USA).
3.3 RESULTS

3.3.1 Ambient seawater inorganic NO$_3^-$ and NH$_4^+$ concentrations
In general, both ambient seawater NO$_3^-$ and NH$_4^+$ concentrations in the surface and subsurface waters were low (generally < 3 μM) throughout the study with no temporal or spatial variation evident (Fig. 3.2). However, a sharp increase in NO$_3^-$ concentration occurred in surface and subsurface (10-20 m) samples during November 1999 (10 μM, Fig. 3.2) at all three sites.

3.3.2 Percent tissue C and N, and C:N ratios
Percent tissue C ranged from 24-34 % at Seymour Island, 25-33 % at Causet Cove and 25-31 % at Thompson Sound (Fig. 3.3a). Values were generally similar between sites during the study period although % tissue C at Causet Cove during November 1998 was significantly higher than values at either Seymour Island or Thompson Sound ($P=0.001$, respectively) while % tissue C at Seymour Island during December 1999 was significantly lower than at either Causet Cove ($P=0.009$) or Thompson Sound ($P=0.011$). Percent tissue C at all three sites were similar in February ($P=0.761$) and April 1999 ($P=0.220$) but these two months were significantly higher than any other month ($P<0.005$).

Values of % tissue N ranged from 0.8 – 1.6 % at Seymour Island, 0.9-1.5 at Causet Cove and 1.1-1.7 % at Thompson Sound (Fig. 3.3b). Percent tissue N showed greater variation than % tissue C between sites with values decreasing in February and April 1999. Percent tissue N at Causet Cove was significantly lower than at Seymour Island and Thompson Sound during November ($P=0.001$ and 0.009, respectively) and December 1998 ($P<0.001$, respectively). *Ecklonia radiata* at Thompson Sound had significantly higher % N than *E. radiata* at Seymour Island in April 1999 ($P<0.05$), March ($P<0.01$) and May 2000 ($P=0.22$).

C:N ratios of *Ecklonia radiata* ranged from 19-53 at Seymour Island, 21-39 at Causet Cove and 22-34 at Thompson Sound, and exhibited a significant temporal variation at all three sites ($P<0.001$, respectively). C:N ratios increased during February and April 1999
Figure 3.2: Ambient seawater inorganic N concentrations from the three study sites in Doubtful Sound. Water samples were collected monthly from September 1998 to December 1998, then bi-monthly from February 1999 to May 2000. a) NO₃⁻ at water surface, b) NO₃⁻ at the depth from which *Ecklonia radiata* was collected, c) NH₄⁺ at water surface and d) NH₄⁺ at the depth from which *E. radiata* was collected. Points represent mean ± 1 SD (n=3).
Figure 3.3: A) percent tissue C, b) percent tissue N and c) C:N ratio (atomic weight basis) for *Ecklonia radiata* from three sites in Doubtful Sound. Dashed line in bottom graph (c) is the critical C:N limit for kelps proposed by Hurd *et al.* (1996); higher values indicate N-limitation. Points represent mean ± 1 SD (n=5). * indicates significant differences between sites at *P*<0.05.
with the highest values occurring during April 1999 at all three sites (Fig. 3.3c). During these two months, the C:N ratio of *E. radiata* at Seymour Island was significantly higher than ratios at either Causet Cove or Thompson Sound (*P*<0.005, respectively).

### 3.3.3 Soluble tissue NO$_3^-$ and NH$_4^+$
Soluble tissue NO$_3^-$ content of *Ecklonia radiata* displayed temporal (*P*<0.001) and spatial (*P*=0.027) variations (Fig. 3.4a). However, significant interactions over time and between sites (*P*=0.001, respectively) suggest that differences in soluble tissue NO$_3^-$ content were neither constant over time nor independent. Soluble tissue NO$_3^-$ content of *E. radiata* ranged from 0.3 – 3.8 μmol g DW$^{-1}$ at all three sites and was minimal in December 1999. Maximum soluble tissue NO$_3^-$ of *E. radiata* varied between the three sites.

Soluble tissue NH$_4^+$ of *Ecklonia radiata* also displayed a temporal (*P*<0.001) and spatial (*P*=0.002) difference similar to soluble tissue NO$_3^-$ content (Fig. 3.4b). *E. radiata* from Causet Cove typically had a lower soluble tissue NH$_4^+$ content than at either Seymour Island or Thompson Sound (*P* =0.012 and 0.003, respectively). Soluble tissue NH$_4^+$ also ranged from 0.3-3.8 μmol g DW$^{-1}$ and was maximal at Causet Cove in August 1999 and at Seymour Island and Thompson Sound in May 2000.

### 3.3.4 Time-course of NO$_3^-$ and NH$_4^+$ depletion by *Ecklonia radiata*
The depletion of NO$_3^-$ and NH$_4^+$ at low and high initial concentrations by *Ecklonia radiata* from all three sites was typically non-linear over time. The amount of NO$_3^-$ or NH$_4^+$ removed from the media by *E. radiata* tissue discs are expressed as percentages within the text for comparative purposes.

#### 3.3.4.1 Nitrate depletion at a low initial concentration (5 μM)
*Ecklonia radiata* removed < 50% of the initial NO$_3^-$ concentration from the media in September and October 1998 (Fig. 3.5a-b). On three occasions, *E. radiata* removed > 90% of the initial concentration over 300 minutes and this was independent of site (Fig. 3.5a-f). The least amount of NO$_3^-$ removed from the media was 6% in October 1998 by
Figure 3.4: Monthly average soluble tissue a) NO$_3^-$ and b) NH$_4^+$ content of Ecklonia radiata from three sites in Doubtful Sound. Samples were collected bi-monthly from April 1999 to May 2000 using a boiling water extraction method (Hurd et al., 1996). Points represent mean ± 1 SD (n=5).
Figure 3.5: Time-course of NO$_3^-$ depletion from seawater media at a low (5 µM) initial NO$_3^-$ concentration by *Ecklonia radiata* from three sites in Doubtful Sound. Time-course experiments were conducted monthly between September and December 1998, and bimonthly from February to April 1999. Points represent mean ± 1 SD (n=5). Note different Y-axis for graph e. Surface area was 38.48 cm$^2$ (double sided) per flask, and dry weights ranged from 0.13 to 1.15 g.
Chapter Three: Inorganic N uptake

a. September

b. October

c. November
d. December

e. February

f. April
E. radiata from Seymour Island. In comparison, 97% of the initial NO$_3^-$ concentration was removed in April 1999 by E. radiata from the same site.

### 3.3.4.2 Nitrate depletion at a high concentration (30 µM)
During the first three months of the experiment, Ecklonia radiata from the three sites removed < 50% of the initially high NO$_3^-$ concentration (Fig. 3.6a-c). In November 1998, E. radiata from Seymour Island had removed only 2% of the initial concentration after 300 minutes (Fig. 3.6c). The greatest amount of NO$_3^-$ removed from the media was 98% by E. radiata from Thompson Sound in December 1998 and April 1999 and 96% by E. radiata from Causet Cove in April 1999 (Fig. 3.6d, f).

### 3.3.4.3 Ammonium depletion at a low concentration (5 µM)
In all but five instances, Ecklonia radiata removed > 50% of the initial NH$_4^+$, and on six occasions > 90% NH$_4^+$ was removed after 300 minutes (Fig. 3.7a-f). E. radiata from Thompson Sound and Causet Cove removed 38 and 30% of the initial NH$_4^+$ concentration in November 1998, respectively. In contrast, E. radiata from all three sites removed > 80% of initial NH$_4^+$ concentration from the medium in December 1998 and February 1999.

### 3.3.4.4 Ammonium depletion at a high concentration (30 µM)
On two occasions > 90% of the initial NH$_4^+$ concentration was removed from the medium by Ecklonia radiata from Thompson Sound (Fig. 3.8a-f). Typically, E. radiata from the three sites removed < 50% of NH$_4^+$ from the medium over 300 minutes. In November 1998, ≤ 31% of the initial NH$_4^+$ concentration was removed by E. radiata from the three sites over 300 minutes.
Figure 3.6: Time-course of NO$_3^-$ depletion from seawater media at a high (30 µM) initial NO$_3^-$ concentration by *Ecklonia radiata* from three sites in Doubtful Sound. Time-course experiments were conducted monthly between September and December 1998, and bimonthly from February to April 1999. Points represent mean ± 1 SD (n=5). Note different Y-axis for graph e. Surface area was 38.48 cm$^2$ (double sided) per flask, and dry weights ranged from 0.13 to 1.18 g.
Chapter Three: Inorganic N uptake

a. September

b. October

c. November
d. December

e. February

f. April
Figure 3.7: Time-course of NH$_4^+$ depletion from seawater media at a low (5 µM) initial NH$_4^+$ concentration by *Ecklonia radiata* from three sites in Doubtful Sound. Time-course experiments were conducted monthly between September and December 1998, and bimonthly from February to April 1999. Points represent mean ± 1 SD (n=5). Note different Y-axis for graph e. Surface area was 38.48 cm$^2$ (double sided) per flask, and dry weights ranged from 0.12 to 1.14 g.
Chapter Three: Inorganic N uptake

a. September

b. October

c. November

d. December

e. February

f. April

Time (minutes)
Figure 3.8: Time-course of $\text{NH}_4^+$ depletion from seawater media at a high (30 $\mu$M) initial $\text{NH}_4^+$ concentration by *Ecklonia radiata* from three sites in Doubtful Sound. Time-course experiments were conducted monthly between September and December 1998, and bimonthly from February to April 1999. Points represent mean $\pm$ 1 SD (n=5). Note different Y-axis for graph e. Surface area was 38.48 cm$^2$ (double sided) per flask, and dry weights ranged from 0.12 to 1.06 g.
3.3.5 Patterns of inorganic N uptake for _Ecklonia radiata_ determined using the multiple-flask method

Controls showed < 1% change in \( \text{NO}_3^- \) or \( \text{NH}_4^+ \) concentration over the incubation period, therefore changes in substrate concentration of the treatments were attributed to uptake by _Ecklonia radiata_. Uptake rates from pooled replicates were graphed against either \( \text{NO}_3^- \) or \( \text{NH}_4^+ \) concentration to compare monthly uptake kinetics by _E. radiata_ from the three study sites.

_Ecklonia radiata_ did not always display saturation kinetics that could be described by the Michaelis-Menten model over the range of concentrations examined (Fig. 3.9, Table 3.1, appendix A). Nitrate uptake by _E. radiata_ at Seymour Island showed unsaturated uptake rates in March and May 2000 while \( \text{NO}_3^- \) uptake by _E. radiata_ at Causet Cove did not saturate in August and November 1999 or March 2000. In contrast, \( \text{NO}_3^- \) uptake by _E. radiata_ at Thompson Sound saturated at all experimental concentrations examined for all months. _E. radiata_ from Seymour Island had the highest \( \text{NO}_3^- \) uptake rate every month with the exception of uptake in December 1999, which was similar to \( \text{NO}_3^- \) uptake rates by _E. radiata_ at Causet Cove and Thompson Sound.

Ammonium uptake rates were also variable, displaying both saturated and unsaturated uptake with increasing substrate concentration. _Ecklonia radiata_ at Seymour Island showed saturating \( \text{NH}_4^+ \) uptake rates every month except in May 2000 when uptake was linear. _E. radiata_ from Causet Cove showed the opposite trend with saturated uptake displayed in December 1999 only. During 1999, \( \text{NH}_4^+ \) uptake by _E. radiata_ from Thompson Sound displayed saturation while \( \text{NH}_4^+ \) uptake in March and May 2000 was linear.

### 3.3.5.1 Comparison of kinetic parameters (\( V_{max}, K_m, \alpha \) and \( V_2 \))

Replicate data sets were pooled due to the pattern of uptake rate vs. concentration of individual replicates, which displayed high variability within months (some replicates displayed saturated uptake while others displayed linear uptake). For example, there was a five-fold difference in \( \text{NO}_3^- \) uptake rate between _Ecklonia radiata_ replicates 3 and 4 at 43 \( \mu \text{M} \) from Seymour Island in November 1999 while \( \text{NH}_4^+ \) uptake rates at 80-85 \( \mu \text{M} \) for
Figure 3.9: Representative graphs of the variation in replicate NO$_3^-$ and NH$_4^+$ uptake each month depicting saturated and non-saturated (linear) uptake for _E. radiata_ from Doubtful Sound. A) Saturated NO$_3^-$ uptake, Seymour Island, November 1999; b) non-saturated NO$_3^-$ uptake, Causet Cove, August 1999; c) saturated NH$_4^+$ uptake, Thompson Sound, November 1999; and d) non-saturated NH$_4^+$ uptake, Causet Cove, August 1999. Nitrate and NH$_4^+$ uptake were measured at the beginning and end of 1 hr experiments (refer to section 3.2.6.2 for conditions) using the multiple flask method bi-monthly from June 1999 to May 2000. Curves were fitted to pooled replicates (n=5) using the Michaelis-Menten hyperbolic equation. Results are summarised in Table 3.1 while all graphs can be referred to in appendix A.
Table 3.1: Synthesis of NO$_3^-$ and NH$_4^+$ uptake kinetics for *Ecklonia radiata* in Doubtful Sound, June 1999 to May 2000. Parameters were determined using the multiple-flask technique and fitting a Michaelis-Menten hyperbola to pooled replicates for each site each month. The column marked ‘pattern’ indicates whether the pooled data from each site each month displayed saturated uptake (S) or linear (non-saturated) uptake (L). $V_{\text{max}} =$ maximum uptake rate (μmol g DW$^{-1}$ s$^{-1}$), $K_s =$ half saturation constant (μM) and $\alpha = V_{\text{max}}/K_s$ (L gDW$^{-1}$ s$^{-1}$). Numbers are means of pooled replicates with standard errors calculated by SigmaPlot in parenthesis. -- indicates kinetic parameter could not be obtained using Michaelis-Menten equation due to non-saturated uptake.
<table>
<thead>
<tr>
<th>Site</th>
<th>Date</th>
<th>Pattern</th>
<th>( V_{\text{max}} )</th>
<th>( K_s )</th>
<th>( \alpha )</th>
<th>Pattern</th>
<th>( V_{\text{max}} )</th>
<th>( K_s )</th>
<th>( \alpha )</th>
</tr>
</thead>
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<tr>
<td>Seymour Island</td>
<td>June</td>
<td>S</td>
<td>2.14 (0.63)</td>
<td>56.14 (30.98)</td>
<td>0.03</td>
<td>S</td>
<td>16.28 (12.85)</td>
<td>215.32 (217.89)</td>
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<td></td>
<td>August</td>
<td>S</td>
<td>3.54 (2.50)</td>
<td>68.56 (83.83)</td>
<td>0.03</td>
<td>S</td>
<td>1.44 (0.28)</td>
<td>23.86 (11.02)</td>
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<tr>
<td></td>
<td>November</td>
<td>S</td>
<td>3.68 (4.51)</td>
<td>14.26 (23.92)</td>
<td>0.26</td>
<td>S</td>
<td>12.40 (23.45)</td>
<td>62.09 (47.28)</td>
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<tr>
<td></td>
<td>December</td>
<td>S</td>
<td>0.93 (0.20)</td>
<td>2.78 (2.89)</td>
<td>0.36</td>
<td>S</td>
<td>0.48 (0.12)</td>
<td>4.44 (5.77)</td>
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<tr>
<td>March</td>
<td>L</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>0.11</td>
<td>L</td>
<td>--</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>May</td>
<td>L</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>0.11</td>
<td>L</td>
<td>--</td>
<td>--</td>
<td></td>
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<tr>
<td>Causet Cove</td>
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<td>S</td>
<td>1.24 (0.62)</td>
<td>26.51 (28.77)</td>
<td>0.05</td>
<td>L</td>
<td>--</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td></td>
<td>August</td>
<td>L</td>
<td>--</td>
<td>--</td>
<td>0.11</td>
<td>L</td>
<td>--</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td></td>
<td>November</td>
<td>L</td>
<td>--</td>
<td>--</td>
<td>0.11</td>
<td>L</td>
<td>--</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>Thompson Sound</td>
<td>June</td>
<td>S</td>
<td>1.35 (1.33)</td>
<td>53.92 (95.06)</td>
<td>0.02</td>
<td>S</td>
<td>10.22 (2.85)</td>
<td>72.37 (36.31)</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>August</td>
<td>S</td>
<td>1.09 (0.19)</td>
<td>5.70 (2.22)</td>
<td>0.19</td>
<td>S</td>
<td>1.61 (0.23)</td>
<td>11.54 (5.92)</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>November</td>
<td>S</td>
<td>1.15 (0.15)</td>
<td>10.86 (4.90)</td>
<td>0.11</td>
<td>S</td>
<td>2.34 (0.34)</td>
<td>9.59 (5.23)</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>December</td>
<td>S</td>
<td>1.07 (0.19)</td>
<td>2.55 (2.72)</td>
<td>0.42</td>
<td>S</td>
<td>2.46 (0.53)</td>
<td>8.32 (4.78)</td>
<td>0.30</td>
</tr>
<tr>
<td>March</td>
<td>S</td>
<td>1.92 (5.03)</td>
<td>120.96 (430.08)</td>
<td>0.02</td>
<td>L</td>
<td>--</td>
<td>--</td>
<td></td>
<td></td>
</tr>
<tr>
<td>May</td>
<td>S</td>
<td>1.91 (1.59)</td>
<td>42.59 (68.02)</td>
<td>0.04</td>
<td>L</td>
<td>--</td>
<td>--</td>
<td></td>
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</tr>
</tbody>
</table>
replicates 2 and 4 from Thompson Sound displayed a 19-fold difference. This frequently limited the number of replicates that could be used to obtain kinetic parameters and in some instances the resulting kinetic parameters gave nonsense values. For example, the mean $V_{\text{max}}$ and $K_s$ values for *E. radiata* from Causet Cove in November 1999 were 15154.89 µmol g DW$^{-1}$ s$^{-1}$ and 157505.49 µM, respectively. Extremely high $V_{\text{max}}$ and $K_s$ values may indicate linear uptake and were clearly identifiable (refer to appendix A), therefore have not been included in Table 3.1 that summarises uptake kinetic parameters for saturating data.

In general, NH$_4^+$ $V_{\text{max}}$ values for *Ecklonia radiata* were higher than those for NO$_3^-$ $V_{\text{max}}$ at all three sites for the respective month, particularly at Seymour Island in June and November 1999 (Table 3.1). Comparison of NO$_3^-$ and NH$_4^+$ $V_{\text{max}}$ values at Causet Cove was difficult due to linear uptake in four out of the six months.

Maximum NO$_3^-$ uptake rates for *Ecklonia radiata* were similar over time at each respective site, however, NO$_3^-$ $V_{\text{max}}$ for *Ecklonia radiata* from Seymour Island were up to three and a half times higher than rates at either Causet Cove or Thompson Sound (Table 3.1). The exception was in December 1999 when NO$_3^-$ $V_{\text{max}}$ were similar between the sites. Nitrate $K_s$ values were highly variable over time and ranged from 2.78 to 56.14 µM at Seymour Island, 2.59 – 62.63 µM at Causet Cove and 2.55-120.96 µM at Thompson Sound (Table 3.1). Similar NO$_3^-$ $K_s$ values were observed in December 1999 at all three sites and in June 1999 at Seymour Island and Thompson Sound. The highest NO$_3^-$ $\alpha$ values were recorded in December at all three sites but displayed high variation between months (Table 3.1). For example, at Seymour Island there was a 12-fold difference in NO$_3^-$ $\alpha$ between June and December while at Thompson Sound there was a 21-fold difference for the same months.

Ammonium $V_{\text{max}}$ values for *Ecklonia radiata* displayed high variability over time and between sites. The highest NH$_4^+$ $V_{\text{max}}$ values were recorded in June 1999 at all three sites (Table 3.1), although NH$_4^+$ $V_{\text{max}}$ was approximately two times higher at Seymour Island than at Causet Cove. *E. radiata* at Seymour Island also had a high NH$_4^+$ $V_{\text{max}}$ in
November 1999 compared to Thompson Sound with linear uptake displayed at Causet Cove at this time. Ammonium $K_s$ values were also highly variable over time and ranged from 4.44 to 215.32 μM at Seymour Island, 2.61-225.01 μM at Causet Cove and 8.32-72.37 μM at Thompson Sound (Table 3.1). The highest $NH_4^+$ $K_s$ values for $E. radiata$ were observed in June 1999 while the lowest values occurred in December 1999 at all three sites. Values for $NH_4^+$ $\alpha$ were highest in December at all three sites although $NH_4^+$ $\alpha$ at Seymour Island was approximately three times lower than at either Causet Cove or Thompson Sound. Ammonium $\alpha$ values at Thompson Sound were typically higher than values at either Seymour Island or Causet Cove.

Uptake of both $NO_3^-$ and $NH_4^+$ at 2 μM by $Ecklonia radiata$ from the three study sites displayed a temporal difference ($P<0.05$). A significant interaction between time and site for both $NO_3^-$ and $NH_4^+$ uptake ($P=0.037$ and $P<0.01$, respectively) however, implies that $NO_3^-$ and $NH_4^+$ $V_2$ were not constant over time or between sites. In November, both $NO_3^-$ and $NH_4^+$ $V_2$ for $E. radiata$ at Seymour Island were significantly higher than at either Causet Cove or Thompson Sound (Fig. 3.10a, b). At this time, $NH_4^+$ $V_2$ at Thompson Sound was also significantly higher than at Causet Cove ($P<0.01$).
Figure 3.10: Rates of a) NO$_3^-$ and b) NH$_4^+$ uptake at 2 $\mu$M ($V_2 = \mu$mol gDW$^{-1}$ s$^{-1} * 10^3$) by Ecklonia radiata from three sites in Doubtful Sound between June 1999 and May 2000. $V_2$ for data showing saturation kinetics were determined for each replicate data set using the Michaelis-Menten equation while data that did not saturate at the highest concentration examined (64 $\mu$M) were estimated from a linear regression fitted to the replicate data sets. Bars represent mean $\pm$ 1 SD (n=5). * on graphs indicates a significant difference between sites ($P<0.05$).
Chapter Three: Inorganic N uptake

3.4 DISCUSSION
This study is one of only a few that has investigated inorganic N uptake rates of macroalgae on a time scale of months. Wheeler and Srivastava (1984) investigated NO$_3^-$ uptake rates of *Macrocystis integrifolia* every 4 – 8 weeks over a one and a half-year period. Dean (1998) determined NO$_3^-$ and NH$_4^+$ uptake rates for *Undaria pinnatifida* monthly over a seven-month period. Other studies have either investigated uptake rates at one point in time or looked at differences between summer and winter rates (e.g. D’Elia and DeBoer, 1978; Haines and Wheeler, 1978; Rosenberg *et al.*, 1984; Wallentinus, 1984; Hurd and Dring, 1990; Phillips, 2001). The discussion focuses primarily on members of the Laminariales for comparative purposes, however, other macroalgal examples are occasionally considered when appropriate.

3.4.1 Ambient seawater inorganic N concentrations
The pattern of high seawater NO$_3^-$ concentration in winter and low concentration in summer typically recorded for temperate coastal waters (e.g. Chapman and Craigie, 1977; Wheeler and North, 1980; Wheeler *et al.*, 1984) was not observed in the present study.

The small variation in the NO$_3^-$ concentration of seawater from Doubtful Sound was lower than other reported findings from Doubtful Sound but similar to the summer minimum reported for Otago Harbour (Chin, 1989; Walls, 1995; Brown *et al.*, 1997; Stuart, 1997; Dean, 1998), Katiki Point (80 km north of Dunedin; Gillanders and Brown, 1994a and b) and Mokohinau Islands, northeastern New Zealand (Taylor *et al.*, 1998).

The observed peak in NO$_3^-$ concentration in November 1999 (summer) is similar to the winter maximum observed in Otago Harbour (Stuart, 1997) and at Katiki Point (Gillanders and Brown, 1994a and b). This peak is possibly a result of a storm event and subsequent mixing between surface and nutrient-rich deeper water. Seawater NH$_4^+$ concentration from the present study was consistently low (2 – 4 \( \mu \)M) throughout the year and showed no temporal pattern. This is comparable to reports of seawater NH$_4^+$ concentrations for coastal waters of New Zealand (Brown *et al.*, 1990; Gillanders and Brown, 1994a and b; Taylor *et al.*, 1998, 1999) and temperate regions elsewhere (e.g. Chapman and Craigie, 1977; Asare and Harlin, 1983; Probyn and Chapman, 1983) but contrasts with NH$_4^+$ levels reported for Otago Harbour (~ 0.8 \( \mu \)M; Chin, 1989, Brown *et al.*, 1997). Further, the typical temporal pattern commonly observed for ambient
seawater N concentrations may have been missed in the present study due to the timing of the sampling regime (generally once every two months). Greater resolution of sampling would resolve this discrepancy.

3.4.2 N status of Ecklonia radiata

Percent tissue N content has been recommended as an indicator of N-limitation in macroalgae due in part to the ability of macroalgae to store N in several forms such as amino acids, proteins and chlorophyll (Gangé et al., 1982; Hanisak, 1983; Ekman et al., 1989; Fujita et al., 1988; Wheeler and Björnsäter, 1992; Naldi and Wheeler, 1999). Furthermore, a high tissue N content may reflect a high demand for N or critical N content required to sustain growth (Fujita et al., 1988; Pedersen and Borum, 1996). The proposed critical N concentration for macroalgae is ca. 2 % of dry weight, below which growth may be N-limited (Hanisak, 1979, 1983; DeBoer, 1981; O’Brien and Wheeler, 1987). The tissue N content of Ecklonia radiata in Doubtful Sound was always lower than 2 %, values ranged from 0.77 – 1.67 % of dry weight, suggesting either N-limitation or a low N requirement for growth and/or photosynthetic process. The range observed in the present study was slightly lower than the range reported for E. maxima from South Africa (Probyn and McQuaid, 1985) but was similar to values for Macrocystis integrifolia from Vancouver Island, British Columbia (Rosell and Srivastava, 1985; Hurd et al., 1994; Table 3.2). Occasionally, E. radiata from Causet Cove and Thompson Sound had higher % tissue N than E. radiata at Seymour Island. This may be due to greater N availability at the two outer sites through oceanic exchange and water motion. It has been suggested however, that since the N requirement for growth and photosynthesis is modified by light, macroalgae cannot be assigned one critical N concentration (Lapointe and Duke, 1984; Shivji, 1985) therefore, C:N ratios are frequently used in combination with internal tissue N content to assess N-limitation of macroalgae.

C:N ratios of temperate laminarians typically exhibit a temporal pattern; ratios increase during summer and decrease during winter (Hanisak, 1979; Rosell and Srivastava, 1985; Henley and Dunton, 1995; Sjøtun et al., 1996; Neill, 2001; Phillips, 2001). Ecklonia radiata from Doubtful Sound displayed a similar temporal pattern during the 1998/1999
Table 3.2: Comparison of % tissue C, % tissue N and C:N ratios (seasonal range where possible) for several subtidal species of Laminariales.

<table>
<thead>
<tr>
<th>Macroalgae</th>
<th>% C</th>
<th>% N</th>
<th>C:N</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ecklonia maxima</em></td>
<td>29.61-31.45</td>
<td>1.79-2.12</td>
<td>14.99-17.75</td>
<td>Probyn and McQuaid 1985</td>
</tr>
<tr>
<td><em>E. radiata</em></td>
<td>21.83-34.24</td>
<td>0.77-1.67</td>
<td>18.89-52.67</td>
<td>cited Atkinson and Smith 1983</td>
</tr>
<tr>
<td><em>E. radiata</em></td>
<td>36</td>
<td></td>
<td>26</td>
<td>Mann 1972</td>
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<tr>
<td><em>L. hyperborea</em></td>
<td>24-33</td>
<td>2.4-3</td>
<td>6-14</td>
<td>Sjøtun <em>et al.</em> 1996</td>
</tr>
<tr>
<td>(first-year plants)</td>
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<tr>
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<td></td>
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<td>Sjøtun <em>et al.</em> 1996</td>
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<td>(canopy plants)</td>
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<td>Mann 1972</td>
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<td><em>L. saccharina</em></td>
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<td>11.50-28</td>
<td>Henley and Dunton 1995</td>
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<td><em>L. solidungula</em></td>
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<td>11.21</td>
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<td>0.83-2.96</td>
<td>10-37</td>
<td>Rosell and Srivastava 1985</td>
</tr>
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<td><em>M. integrifolia</em> (exposed site)</td>
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<td>1.08</td>
<td>28.79</td>
<td>Hurd <em>et al.</em> 1994</td>
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<td><em>M. integrifolia</em> (sheltered site)</td>
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<td>Hurd <em>et al.</em> 1996</td>
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<td></td>
<td>Atkinson and Smith 1983</td>
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<td></td>
<td></td>
<td>Stuart 1997</td>
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<td><em>U. pinnatifida</em></td>
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<td>1.47-2.89</td>
<td>12.50-23.60</td>
<td>Dean 1998</td>
</tr>
</tbody>
</table>

* % C, % N and C:N for present study is the range observed between the three sites
  * Values estimated from graph as no numbers available
  * C:N ratio calculated on atomic weight
  * cultivated in the laboratory before being transplanted to the sea
study period although the highest C:N ratios at all three sites occurred in April 1999 (autumn). The increase in C:N ratios during February and April 1999 for *E. radiata* was a result of high % tissue C and low % tissue N. High % tissue C indicates that *E. radiata* was storing C, possibly to maintain growth during periods of low external N concentration and reduced light. C:N ratios then decreased to approximately 25 and remained fairly stable for the rest of the study period. The range of C:N ratios exhibited by *E. radiata* in Doubtful Sound were typically higher than other laminarian species (Table 3.2) although ratios were similar to those of *E. maxima* from South Africa (Probyn and McQuaid, 1985) and of canopy plants of *Laminaria hyperborea* from Norway (Sjøtun *et al.*, 1996).

The proposed critical limit for C:N ratios of macroalgae is 10; values greater than this indicate N-limitation while lower values indicate N-storage (D’Elia and DeBoer, 1978; Harrison and Druehl, 1982; Hanisak, 1983). However, Heterokontophytes typically have higher C:N ratios compared to Chlorophytes and Rhodophytes (Niell, 1976; Lapointe, 1989). Laminarians, such as *Ecklonia radiata*, typically have a tough pliable thallus, which require large quantities of structural C. Hurd *et al.* (1996) proposed a C:N ratio of 20 as indicative of mild N limitation for *Macrocystis integrifolia* from Canada. C:N ratios of *E. radiata* from all sites in Doubtful Sound exceeded 20 on all but three occasions indicating *E. radiata* was N-limited year-round in Doubtful Sound. The range of C:N ratios exhibited by *E. radiata* in Doubtful Sound were typically higher than C:N ratios for other laminarian species (Table 3.2). The increase in C:N ratios for *E. radiata* in the present study was a result of an increase in % tissue C and decrease in % tissue N. Even though C:N ratios are a function of both C and N metabolism (Hanisak, 1979; Hanisak and Harlin, 1978) as demonstrated in the present study, they are still useful in describing the N status of macroalgae.

The N status of macroalgae can further be described by investigating intracellular N pools. Large intracellular N pools allow macroalgae to sustain growth during periods of low, or sporadic, external N availability (e.g. Chapman and Craigie, 1977; Asare and Harlin, 1983; Probyn and Chapman, 1983; McGlathery *et al.*, 1996; Harrison and Hurd, 2001). *Ecklonia radiata* in Doubtful Sound had relatively low but similar sized
in intracellular NO$_3^-$ and NH$_4^+$ pools. Ammonium is, however, highly soluble and may be toxic to macroalgae at high concentrations (Waite and Mitchell, 1972; McGlathery et al., 1996) with efflux and feedback inhibition preventing the accumulation of large internal NH$_4^+$ pools (McGlathery et al., 1996). Furthermore, large NH$_4^+$ pools may be difficult for macroalgae to maintain in a low NH$_4^+$ environment (Fujita et al., 1988) such as occurs in Doubtful Sound. In contrast, intracellular NO$_3^-$ pools of macroalgae typically vary on a temporal basis in response to ambient seawater NO$_3^-$ availability (Chapman and Craigie, 1977; Rosenberg and Ramus, 1982; Asare and Harlin, 1983; Wheeler and Srivastava, 1984; McGlathery et al., 1996). As the ambient seawater NO$_3^-$ concentration in Doubtful Sound was low year-round this may account for the low NO$_3^-$ pools observed for *E. radiata*. Low internal soluble N pools provide further evidence of N-limitation of *E. radiata* in Doubtful Sound. Furthermore, intracellular N pools often contain a mix of inorganic and organic N compounds such as amino acids, chlorophyll and accessory pigments, enzymes and other proteins (Bird, 1982; Gagné et al., 1982; Rosenberg and Ramus, 1982; Duke et al., 1987; Lignell and Pedersen, 1987; Fredriksen and Rueness, 1989; Pedersen, 1994; Naldi and Wheeler, 1999). However, the proportion of different forms of internal N compounds varies among species (Germann et al., 1987) and it is not know the extent to which they contribute to N metabolism. As *E. radiata* inhabits a dynamic light environment, a greater amount of inorganic N may be apportioned to maintaining high levels of chlorophyll and accessory pigments thereby accounting for the low soluble tissue NO$_3^-$ pools.

Low soluble NO$_3^-$ pools may also be the result of constant high enzyme activity, particularly of enzymes involved in N uptake processes such as nitrate and nitrite reductase (NR and NiR respectively) and glutamine synthetase (GS). Accumulation of NO$_3^-$ in intracellular pools has been observed in *Porphyra perforata* and *Gracilaria tikvahiae* when NR is inactive (Thomas and Harrison, 1985; Hwang et al., 1987). *Ecklonia radiata* may take advantage of high NR/NiR/GS activity to sustain growth rather than storing N.
3.4.3 Choice of technique for determining and estimating uptake kinetics

Many researchers have used a combination of time-course and multiple-flask methods to determine nutrient uptake kinetics of macroalgae (e.g. Probyn and Chapman, 1982; Fujita, 1985; Harrison et al., 1989; Hurd and Dring, 1990; Pedersen, 1994; Pedersen and Borum, 1996; Campbell et al., 1999; Lotze and Schramm, 2000). Time-course experiments are often used to determine whether uptake is constant over prolonged periods (i.e. 3-6 hrs) and whether there is evidence of lag or surge phases in uptake rates (Harrison et al., 1986; Lobban and Harrison, 1997; Phillips, 2001). This technique is not recommended for estimating uptake kinetic parameters as the cellular nutritional history of the macroalga tissue changes over the incubation period (Harrison et al., 1989; Lobban and Harrison, 1997). In comparison, the multiple-flask method allows a more accurate determination of uptake kinetic parameters due to a shorter incubation period (10-60 minutes) and the use of a different specimen of the species being studied (in the present study, tissue discs randomly cut from the primary blade of each *Ecklonia radiata* sporophyte) for each nutrient concentration (Lobban and Harrison, 1997). This incorporates the natural variability of the species under investigation (discussed further in section 3.4.5.3) and provides a better indication of population variability (Topinka, 1978; Harrison and Druehl, 1982; Pedersen, 1994). Furthermore, the regulation of uptake rates by the macroalga's nutritional history is diminished (Harrison et al., 1989; Lobban and Harrison, 1997). A time-course of N uptake should always be performed first, however, to determine if uptake rates vary over time and the period over which uptake rates are maximal (Harrison et al., 1989).

Nutrient uptake kinetics of phytoplankton and macroalgae have typically been described using the Michaelis-Menten rectangular hyperbola (e.g. Dugdale, 1976; Caperon and Meyer, 1972; Conway and Harrison, 1976; Topinka, 1978; Goldman and Gilbert, 1982; Probyn and Chapman, 1982; Harrison et al., 1986, 1989; Hurd and Dring, 1990; Hein et al., 1995; Campbell, 1999), even though the equation has traditionally been used to describe enzyme kinetics (e.g. Cornish-Bowden, 1979). Although there are inherent problems in using the Michaelis-Menten equation to describe uptake kinetics, most notably the assumption that uptake rate is constant over time (Goldman and Gilbert, 1982; Wheeler, 1982), it has been demonstrated that it is the best option for comparing
uptake abilities between species or sites as well as for estimating uptake kinetic parameters (Hurd and Dring, 1990). There are several transformations that can be used to linearize nutrient uptake data (e.g. Hanes-Woolf, \( S/V \) vs. \( S \), Lineweaver-Burk double reciprocal, \( 1/V \) vs. \( 1/S \); Eadie-Hofstee, \( V \) vs. \( V/S \)) from which kinetic parameters (\( V_{\text{max}} \) and \( K_s \)) can be calculated and the fit of the data to the equation tested (Dowd and Riggs, 1965; DeBoer, 1981; Berges et al., 1994; Lobban and Harrison, 1997). However, all transformations have inherent problems as outlined by Dowd and Riggs (1965) and Berges et al. (1994), and all make certain assumptions about the data (Wilkinson, 1961; Dowd and Riggs, 1965). Greater accuracy in the estimation of kinetic parameters can now be obtained using computer programs, such as SigmaPlot, that fit uptake data directly to the Michaelis-Menten equation using an iterative process, making data transformation unnecessary (Berges et al., 1994).

### 3.4.4 Time-course of inorganic N uptake by *Ecklonia radiata*

*Ecklonia radiata* displayed two patterns of depletion of inorganic N from media during the time-course experiments undertaken in the present study: non-linear, where inorganic N depletion from the media was not constant over time, and linear, where depletion was constant for the duration of the experiment.

Non-linear uptake in macroalgae may result from an initial lag in N uptake rate, particularly NO$_3^-$, and is often associated with N-limited macroalgae (Thomas and Harrison, 1985, 1987; Harrison et al., 1986; McGlathery et al., 1996). A lag phase is often indicative of a partially inactive transport mechanism that has to be reactivated by exposure to high NO$_3^-$ concentrations (\( \geq 30 \mu\text{M} \)), or inhibition of NO$_3^-$ uptake in the presence of intracellular NO$_3^-$ pools (Thomas and Harrison, 1985; McGlathery et al., 1996). A lag phase in NO$_3^-$ uptake has been reported for the laminarians, *Macrocystis integrifolia* and *Laminaria groenlandica*, lasting up to one hour (Wheeler and Srivastava, 1984; Harrison et al., 1986). Typically, no lag phase in NO$_3^-$ uptake was evident for *Ecklonia radiata* from Doubtful Sound. The one exception was NO$_3^-$ uptake by *E. radiata* from Causet Cove in February 1999 when the initial NO$_3^-$ concentration was approximately 160 \( \mu\text{M} \). It is possible, however, that a lag phase in NO$_3^-$ uptake did occur...
Alternatively, as a subtidal macroalga, *Ecklonia radiata* is constantly exposed to NO$_3^-$, albeit in varying concentrations, therefore may have no need to deactivate its NO$_3^-$ uptake system as some intertidal species do in order to conserve metabolic energy (Thomas and Harrison, 1985). In this instance, no lag phase in uptake would be observed. If the NO$_3^-$ uptake system is still active the implication is that NR remains active, preventing the inhibition of uptake from intracellular NO$_3^-$ pools due to continued assimilation of NO$_3^-$.

Although NR activity was not investigated in the present study, low internal NO$_3^-$ pools support the contention that NR in *E. radiata* remains active. Furthermore, the lack of a lag phase in *E. radiata* may represent an adaptation to an environment where inorganic N is low and/or episodic.

In contrast, N uptake in macroalgae may also be non-linear as a result of an initial phase of surge, or enhanced, uptake. Surge uptake is common in N-deficient macroalgae and is attributed to the rapid uptake of the nutrient under investigation into apparent free space such as cell walls and intercellular spaces or the filling of internal N storage pools (Harrison and Druhel, 1982; Fujita *et al.*, 1988; Brinkhuis *et al.*, 1989; Harrison *et al.*, 1989; McGlathery *et al.*, 1996). An initial phase of surge uptake of NH$_4^+$ is common and has been observed in several macroalgae (e.g. Haines and Wheeler, 1978; Rosenberg *et al.*, 1984; Harrison *et al.*, 1986; Pedersen, 1994; Campbell, 1999), however, surge uptake of NO$_3^-$ in laminarians has only been alluded to (e.g. Chapman *et al.*, 1978). *Ecklonia radiata* from Doubtful Sound displayed an initial phase of surge uptake for both NH$_4^+$ and NO$_3^-$ at high (30 μM) and low (5 μM) concentrations, although it was of variable duration. The observed surge uptake of NH$_4^+$ and NO$_3^-$ in *E. radiata* may be the result of the sporophytes being N-limited year-round as indicated by high C:N ratios, therefore *E. radiata* has a greater demand for the nutrient and takes it up rapidly when available.

Surge uptake is advantageous in systems with episodic nutrient supply (Pedersen, 1994; Pedersen and Borum, 1996) such as occurs in Doubtful Sound where episodic pulses of inorganic N are introduced to the water column by rainfall events and entrainment of the
LSL and underlying marine water by wind stress and wave action (Gibbs et al., 2000; Gibbs, 2001).

*Ecklonia radiata* also displayed linear patterns in the depletion of both NO$_3^-$ and NH$_4^+$ from media in the present study. This indicates that either NO$_3^-$ and NH$_4^+$ uptake rates were consistent over the incubation period or that uptake was saturated at all experimental concentrations used. This may be a result of depleted inorganic N storage pools that have not filled over the course of the experiment. Linear depletion rates for macroalgae most commonly occur for NH$_4^+$ uptake (Thomas and Harrison, 1985; Thomas et al., 1985; Harrison et al., 1986; Taylor et al., 1998; Campbell, 1999) although linear patterns of NO$_3^-$ have been illustrated (Probyn and McQuaid, 1985; Harrison et al., 1986). For example, *Ecklonia maxima*, from South Africa, displayed linear depletion for both NO$_3^-$ and NH$_4^+$, although the experiment was carried out *in situ* at ambient inorganic N seawater concentrations (Probyn and McQuaid, 1985). Linear uptake of NO$_3^-$ and NH$_4^+$ may be the result of a high demand by *E. radiata* for N.

In October and November 1998, *Ecklonia radiata* from Seymour Island, although displaying a linear pattern of depletion, removed only 6 and 2 % NO$_3^-$ from the initial NO$_3^-$ concentration, respectively. The low NO$_3^-$ depletion rates at this site may indicate that *E. radiata* was not utilizing inorganic N. This contention is supported by C:N ratios for *E. radiata* from this site (~ 20), suggesting that *E. radiata* was not strongly N-limited at this time. Alternatively, NR and/or NiR may have been inactive thereby reducing NO$_3^-$ assimilation, which could be examined in future work.

3.4.5 Multiple flask method

3.4.5.1 Patterns of inorganic N uptake in *Ecklonia radiata* at a range of N concentrations

In the present study, *Ecklonia radiata* displayed both linear and saturated NO$_3^-$ and NH$_4^+$ kinetics; however, neither were generally consistent on either a spatial or temporal scale (Table 3.4). The exception was NO$_3^-$ and NH$_4^+$ uptake in December 1999 that saturated at all three sites. Saturation kinetics described by the Michaelis-Menten equation typically indicates active transport, particularly for NO$_3^-$ (Harlin and Craigie, 1978;
DeBoer, 1981). However, while saturated $\text{NH}_4^+$ uptake also implies that uptake is carrier-mediated it is often difficult to distinguish between active transport and facilitated diffusion, which display similar characteristics, although active transport is generally assumed (DeBoer, 1981). Saturated $\text{NO}_3^-$ and $\text{NH}_4^+$ uptake has been reported for the subtidal laminarians, *Laminaria abyssalis* (Braga and Yoneshigue-Valentin, 1996), *L. longicruris* (Espinoza and Chapman, 1983), *Macrocystis pyrifera* (Wheeler, 1977; Haines and Wheeler, 1978; Gerard, 1982; Kopczak, 1994) and *Undaria pinnatifida* (Campbell, 1999).

Saturation of $\text{NO}_3^-$ and $\text{NH}_4^+$ was not always achieved by *Ecklonia radiata* at the highest substrate concentration examined (64 $\mu$M) in the present study. Linear increases in $\text{NO}_3^-$ and $\text{NH}_4^+$ uptake rates with increasing substrate concentration have been exhibited by many macroalgal species including the laminarians, *Ecklonia maxima* (Probyn and McQuaid, 1985), *Laminaria groenlandica* (Harrison *et al.*, 1986; Druehl *et al.*, 1989) and *Macrocystis pyrifera* (Haines and Wheeler, 1978). However, saturation may not have been observed in some studies due to lack of data points at high substrate concentrations (e.g. $< 30$ $\mu$M for $\text{NH}_4^+$ uptake in *Macrocystis pyrifera*, Haines and Wheeler, 1978). Linear increases in inorganic N may indicate a diffusion component (Lobban *et al.*, 1985), acting in combination with an active transport mechanism (Harrison *et al.*, 1986). Furthermore, non-saturating uptake of $\text{NH}_4^+$ by macroalgae indicates either a large internal storage capacity or a high assimilatory rate with linear uptake of $\text{NH}_4^+$ often attributed to passive diffusion after conversion of $\text{NH}_4^+$ to $\text{NH}_3$ (Raven, 1984; Rees, 1995; Hurd, 2000). The rate of assimilation of inorganic N may control N uptake (Rees *et al.*, 1998; Taylor *et al.*, 1998), however, the extent of that control, if any, requires further investigation.

**3.4.5.2 Relevance of kinetic parameters determined using the multiple-flask method**

Experimental conditions, such as temperature and PFD, for nutrient uptake experiments vary greatly between studies, as does the type of macroalgal tissue used (e.g. apical vs. basal). Therefore, comparison of uptake kinetics and inference from laboratory to *in situ* uptake rates of macroalgae should be made with caution. Intertidal and subtidal
macroalgae are considered to have different strategies for nutrient uptake (Wallentinus, 1984), because intertidal macroalgae are subject to periods of emersion. Consequently, comparison of uptake kinetic parameters has been restricted to subtidal macroalgae, particularly laminarians.

Kinetic parameters such as $V_{max}$ and $K_s$, as calculated using a rectangular hyperbola, give an indication of uptake ability of the macroalgae being studied at high and low nutrient concentrations, respectively (Haines and Wheeler, 1978). However, as demonstrated in the present study, uptake often deviates from saturation kinetics (discussed in section 3.4.5.3), and while kinetic parameters for near-linear plots can be derived using a computer program the relevance of these values is questionable. For example, $K_s$ values greater than the highest substrate concentration (in the present study this was 64 μM) used have little biological or ecological validity; such high concentrations seldom occur in the natural environment with the possible exception of areas near sewage outlets (Sharp, 1983). Furthermore, $NH_4^+$ at high concentrations (> 30 - 50 μM) may be toxic to macroalgae (Waite and Mitchell, 1972; McGlathery et al., 1996), although Ecklonia radiata continued to remove $NH_4^+$ at concentrations greater than 30 μM during the time-course and multiple-flask experiments. This could also be the result of depleted internal inorganic N pools, which have been shown to influence uptake rates of some laminarians (e.g. Laminaria groenlandica, Druehl et al., 1989; Macrocystis pyrifera, Kopczak, 1994).

3.4.5.3 Variation in inorganic N uptake rates by *Ecklonia radiata*

High inter-plant variability was observed for *Ecklonia radiata* in the present study for both $NO_3^-$ and $NH_4^+$ uptake rates, which resulted in variable kinetic parameters. Inter-plant variability in nutrient uptake rates by macroalgae has been demonstrated previously (Harrison et al., 1986; Hurd and Dring, 1990; Campbell, 1999), although uptake rates for intertidal species such as those investigated by Hurd and Dring (1990) will be influenced by factors such as desiccation, relative humidity and position on the shore that will not influence subtidal macroalgae. Slight desiccation of tissue discs of *Ecklonia radiata* may have occurred during the preparation period however effects of desiccation on uptake rates would have been eliminated as discs were preconditioned in natural filtered seawater overnight before experiments proceeded. In the present study, *Ecklonia radiata*
was collected from different depths within each site due to low population densities. Individual *E. radiata* sporophytes were also collected from different microhabitats within each site. For example, a single sporophyte may have been collected from the sheltered side of a rock at 10 m while another may have been collected from an exposed sandy area at 20 m within the same site. As individual sporophytes were exposed to different microclimates this may account for some of the inter-plant variation in N uptake rates.

Inter-plant variability in N uptake may result from the past-nutritional history of the macroalga under investigation, which has been shown to influence internal N-status and subsequent N uptake rates (Harrison *et al.*, 1986; Hurd and Dring, 1990). Nitrogen uptake rates are often inversely correlated with internal N-status with lower uptake rates observed in N-replete macroalgae compared to N-deficient macroalgae (e.g. D'Elia and DeBoer, 1978; Hanisak, 1983; Fujita, 1985; Thomas and Harrison, 1985; O'Brien & Wheeler 1987; McGalthery *et al.*, 1996). Ambient seawater inorganic N concentrations and C:N ratios of *Ecklonia radiata* in the present study indicate that sporophytes were N-limited prior to uptake experiments, however, the extent of N-limitation differed between sporophytes and between sites, possibly accounting for some of the inter-plant variation.

Differences in age and type of tissue may have influenced N uptake rates observed for *Ecklonia radiata* in the present study. Many macroalgae, such as *E. radiata*, are perennial and populations consist of a range of age classes (Harrison *et al.*, 1986; Sjøtun *et al.*, 1996; Lobban and Harrison, 1997). Age is often determined using stipe growth rings (Novaczek, 1981) which are analogous to tree rings. The age of *E. radiata* in the present study was investigated but no stipe rings were evident (Prof. Isolde Ullmann *pers. comm.*), therefore the age of *E. radiata* sporophytes was not determined. It has been demonstrated using *Laminaria groenlandica* that uptake of NO$_3$$^-$ and NH$_4$$^+$ decreases with increasing age (Harrison *et al.*, 1986). Furthermore, Sjøtun *et al.* (1996) found a higher N content in first-year *L. hyperbora* individuals compared to older individuals of the same population and suggested that these first-year individuals may have a higher potential for the uptake of N. Considerable variations in uptake rates have also been reported for different types of blade tissue (e.g Topinka, 1978; Davison and Stewart, 1983, 1984).
In the present study whole sporophytes could not be used due to the large size of the thallus, hence tissue discs were randomly cut from the primary blade with reproductive tissue excluded. Discs used for each replicate set of treatments (refer section 3.2.6.2) were therefore a mixture of basal (young), apical (mature) and intercalary tissue. It is common for younger tissue to have higher uptake rates than mature tissue (Topinka, 1978; Wheeler and Srivastava, 1984; Thomas et al., 1985; Harrison et al., 1986). Topinka (1978) suggests this difference reflects a division in metabolic activities with higher uptake rates for younger plants suggestive of higher growth demands for N compared to slower growing, older plant material. Gerard (1982) measured in situ uptake rates for varying parts of *Macrocystis pyriforma* (mature vs. apical) by enveloping whole thalli in plastic bags. This method could be applied to *E. radiata* to determine uptake rates by whole sporophytes in situ.

Additional factors that may have influenced N uptake rates by *Ecklonia radiata* in Doubtful Sound includes stress of *E. radiata* sporophytes after removal from the natural environment and that sporophytes may have encountered higher PFD levels than usual. This was mitigated, however, by placing sporophytes in opaque buckets filled with surrounding seawater at the depth collected and sealing the buckets before bringing the sporophytes to the surface. Sporophytes may have endured higher temperatures when kept un-aerated in the buckets due to the length of time taken to return to shore. Furthermore, experiments did not begin until approximately 24 hrs after collection due mainly to the location of the study site in proximity to the laboratory. However, all of these additional factors would have been eliminated, or at least minimized, by preconditioning procedures. Inter-plant variation was also observed in growth and productivity rates (chapter 2), and photosynthetic rates (chapter 4) of *E radiata* in Doubtful Sound. The inter-plant variation demonstrated by *Ecklonia radiata* sporophytes in growth and productivity, photosynthesis and N-uptake in the present study probably reflects the naturally occurring variation of a field population (Harrison et al., 1986). It may also be a strategy used by *E. radiata* populations to cope with a rapidly fluctuating environment (i.e. PFD and N availability).
3.4.5.4 Comparison of NO$_3^-$ and NH$_4^+$ $V_{\text{max}}$ with other studies

High $V_{\text{max}}$ values indicate the ability of macroalgae to take up N at high concentrations. As discussed earlier, experimental conditions vary greatly making comparison of uptake kinetic parameters between studies difficult, however there is a trend for NO$_3^-$ $V_{\text{max}}$ to be lower than NH$_4^+$ $V_{\text{max}}$ for many macroalgae (e.g. D’Elia and DeBoer, 1978; Wallentinus, 1984; Thomas and Harrison, 1985; O’Brien and Wheeler, 1987; Pedersen and Borum, 1997). This is because NO$_3^-$ uptake is assumed to be an active process that requires energy (DeBoer, 1981; Lobban and Harrison, 1997). In general, this trend was supported by the findings in the present study, particularly at Thompson Sound where NH$_4^+$ $V_{\text{max}}$ rates for *Ecklonia radiata* were one and half to seven and a half times greater than NO$_3^-$ rates (Table 3.3). NH$_4^+$ $V_{\text{max}}$ for *Laminaria abyssalis*, *L. groenlandica*, and *Macrocystis pyrifera* fell within the ranges observed for *E. radiata* from Doubtful Sound, while the range of NH$_4^+$ $V_{\text{max}}$ for *Undaria pinnatifida* was considerably higher than for the other laminarians (Table 3.3). However, *U. pinnatifida* is considered a fast growing, opportunistic annual (Campbell, 1999) which may account for the high NH$_4^+$ uptake rate, and also confers a competitive advantage over slower-growing, perennial macroalgae such as *E. radiata*.

Although the linear uptake of NH$_4^+$ is thought to be largely due to passive diffusion of NH$_3$ at the cell surface (Rees, 1998; Taylor and Rees, 1998; Hurd, 2000), several studies suggest high NH$_4^+$ uptake rates by N-deficient macroalgae are due to the possession of an NH$_4^+$ transport system (D’Elia and DeBoer, 1978; Rosenberg *et al.*, 1984; Fujita, 1985; Pedersen, 1994; Rees, 1995; McGlathery *et al.*, 1996). The high values of NH$_4^+$ $V_{\text{max}}$ displayed by *E. radiata* at Seymour Island and Thompson Sound appear to support this hypothesis, however, the mechanisms of NH$_4^+$ uptake remain unclear as does the degree to which NH$_4^+$ assimilation controls uptake (Rees *et al.*, 1998; Taylor *et al.*, 1998), therefore further investigations are needed.

The range of $V_{\text{max}}$ values for NO$_3^-$ for *Ecklonia radiata* from Doubtful Sound, particularly Causet Cove and Thompson Sound, were comparable to values recorded for *Laminaria abyssalis* and *L. longicurris*, while the upper limit of the range at Seymour Island was similar to rates recorded for *Laminaria longicurris* at 9 and 18°C (Table 3.3).
Table 3.3: A comparison of kinetic parameters, $V_{\text{max}}$ (μmol gdw$^{-1}$ s$^{-1} \times 10^3$), $K_s$ (μM) and $\alpha$ ($V_{\text{max}}/K_s$), for NO$_3^-$ and NH$_4^+$ uptake in subtidal Laminarians.

<table>
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<td>$K_s$</td>
<td>$\alpha$</td>
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</tr>
<tr>
<td><strong>Ecklonia radiata</strong></td>
<td>12</td>
<td>0.93 - 3.68</td>
<td>2.78 - 66.56</td>
<td>0.03 - 0.36</td>
</tr>
<tr>
<td>- Causet</td>
<td>12</td>
<td>1.06 - 1.76</td>
<td>2.59 - 62.63</td>
<td>0.03 - 0.41</td>
</tr>
<tr>
<td>- Thompson</td>
<td>12</td>
<td>1.07 - 1.92</td>
<td>2.55 - 120.96</td>
<td>0.02 - 0.42</td>
</tr>
<tr>
<td><strong>Laminaria abyssalis</strong></td>
<td>18</td>
<td>1.39</td>
<td>14</td>
<td>0.36</td>
</tr>
<tr>
<td><strong>L. groenlandica</strong></td>
<td>13</td>
<td>5.56</td>
<td>5.56</td>
<td>5.56</td>
</tr>
<tr>
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<td>13</td>
<td>1.67</td>
<td>1.67</td>
<td>1.67</td>
</tr>
<tr>
<td><strong>L. longicruris</strong></td>
<td>10</td>
<td>1.94</td>
<td>5.9</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>2.67</td>
<td>4.1</td>
<td>0.65</td>
</tr>
<tr>
<td><strong>Macrocystis pyrifera</strong></td>
<td>16</td>
<td>8.47</td>
<td>13.1</td>
<td>0.65</td>
</tr>
<tr>
<td><strong>Undaria pinnatifida</strong></td>
<td>12</td>
<td>6.9-41.7</td>
<td>4-23.5</td>
<td>11.1-48.6</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>25.92</td>
<td>4</td>
<td>6.73</td>
</tr>
</tbody>
</table>

*estimated using Hanes-Woolf linear transformation  
*b first-year sporophytes  
*c second-and third-year sporophytes  
*d summer tissue  
*e winter tissue
Temperature has been shown to influence the nutrient status and the rate of diffusion and carrier-mediated uptake (DeBoer, 1981), and this may account in part for differences in uptake rates between the various laminarians. The low NO$_3^-$ $V_{\text{max}}$ for *E. radiata* may indicate that sporophytes were not utilizing NO$_3^-$ or that *E. radiata* was unable to respond to, and exploit, episodic pulses of high ambient NO$_3^-$ concentrations. Furthermore, the typically low N supply in Doubtful Sound implies that there was a limited amount of NO$_3^-$ for *E. radiata* to take up. Alternatively, Doubtful Sound is a low light environment (e.g. Grange et al., 1981; Ryan and Paulin, 1998; Rutherford, 2000; Gibbs, 2001; Goebel, 2001), therefore *E. radiata* may not be able to procure enough light to provide energy for the active transport of NO$_3^-$. Nitrate uptake of *Macrocystis pyrifera* has been shown to decrease with a reduction of light (Wheeler, 1982), although a more recent study found no influence of light on NO$_3^-$ uptake by *M. pyrifera* (Kopczak, 1994). Spatial and temporal variation in photosynthesis of *E. radiata* is investigated in chapter 4.

There is increasing evidence that N-limitation may be the result of low iron (Fe) availability, particularly in phytoplankton (Milligan and Harrison, 2000; Soria-Dengg et al., 2001; Wang and Dei, 2001) but also in some macroalgae (Berges et al., 1995; Liu et al., 2000). In macroalgae Fe may influence N assimilation, as it is a major component of the enzymes (i.e. NR, NiR and GS) involved in the reduction of NO$_3^-$ to NH$_3$, as well being required for the synthesis of chlorophyll (Lobban and Harrison, 1997). While there have been no investigations of Fe concentration in Doubtful Sound, Fe concentration in nearby Lake Manapouri, from which freshwater is discharged into Doubtful Sound via the hydroelectric power station, is extremely low by global standards (< 70 nmol L$^{-1}$; Reid et al., 1999). Low Fe supply in Doubtful Sound may therefore influence the ability of *Ecklonia radiata* to sequester inorganic N, particularly NO$_3^-$. However, further research is needed on the concentration and availability of Fe in Doubtful Sound, and how these two factors may influence inorganic N uptake and assimilation in *E. radiata*.

A high $V_{\text{max}}$ for inorganic N at unnaturally high concentrations is generally considered of limited ecological significance, particularly as NH$_4^+$ concentrations in seawater are typically low in temperate regions (Sharp, 1983). However, high $V_{\text{max}}$ values may imply the potential for macroalgae to exploit high N pulses. Although the ambient seawater
inorganic N concentration in Doubtful Sound was low year-round, the timing of sampling may have missed periodic increases in N supply due to terrestrial run-off, upwelling and storm events, and animal excretion. Furthermore, as *Ecklonia radiata* appeared N-limited year-round the range of NH$_4^+$ and NO$_3^-$ $V_{\text{max}}$ rates may indicate that *E. radiata* was utilizing inorganic N as soon as it became available.

3.4.5.5 Uptake rate at low inorganic N concentrations

Nutrient uptake rates of macroalgae at low concentrations are frequently compared using the half saturation constant (K$_s$), which reflects the affinity of the carrier site for a particular ion (Braga and Yoneshigue-Valentin, 1996). Low K$_s$ values are commonly used to indicate high-affinity for a nutrient by a macroalga (Hurd and Dring, 1990), and infers a competitive advantage at low substrate nutrient concentrations (Healey, 1980). However, K$_s$ is somewhat dependent upon $V_{\text{max}}$, therefore the value of K$_s$ may change when $V_{\text{max}}$ increases or decreases (Braga and Yoneshigue-Valentin, 1996; Lobban and Harrison, 1997). The ratio $V_{\text{max}} / K_s$, also termed alpha (\(\alpha\)), has been suggested as a better index of the preference of a species for certain ions (Braga and Yoneshigue-Valentin, 1996) or as an indicator of competitive ability in taking up nutrients (O’Brien and Wheeler, 1987; Hurd and Dring, 1990). These two terms, however, often give contradictory results. For example, at low nutrient concentrations, K$_s$ values for *Laminaria abyssalis* from Brazil indicated that phosphate (PO$_4^{3-}$) was taken up more efficiently than NO$_3^-$ and NH$_4^+$ while \(\alpha\) values indicated NH$_4^+$ was taken up faster. In the present study a third method, the rate of uptake at an ecologically relevant concentration ($V_2$), was used to compare the ability of *Ecklonia radiata* to take up inorganic N at low concentrations as there were several occasions when kinetic parameters could not be determined due to non-saturated uptake. Despite the deficiencies outlined above, K$_s$ remains the most widely employed term in the literature to compare the nutrient uptake ability of macroalgae at low concentrations. The ability of *Ecklonia radiata* to take up inorganic N at low ambient concentrations is therefore discussed in terms of K$_s$ values, with occasional reference to \(\alpha\).
Generally, there was no consistent spatial or temporal pattern in $K_s$ values for *Ecklonia radiata* in Doubtful Sound indicating that *E. radiata* had no preference for either form of inorganic N investigated in this study. The one exception occurred in December 1999 (early summer) when lower $K_s$ values for both NO$_3^-$ and NH$_4^+$ were observed compared with other sampling times, indicating a greater affinity for inorganic N ions at this time. Higher $\alpha$ values support this contention, although NO$_3^-$ $\alpha$ values were slightly higher than NH$_4^+$ values indicating that NO$_3^-$ was taken up more rapidly than NH$_4^+$. This suggests that *E. radiata* utilizes whichever inorganic N form is available in the greatest concentration, which in Doubtful Sound is NO$_3^-$. 

Values of NO$_3^-$ and NH$_4^+$ $K_s$ for other subtidal laminarians vary between species but generally fall within the ranges reported for *Ecklonia radiata* in the present study (Table 3.3). The upper range of the $K_s$ values suggest that *E. radiata* from Doubtful Sound were not as efficient at taking up NO$_3^-$ and NH$_4^+$ at low concentrations compared to other laminarians (Table 3.3). Variation in $K_s$ values for *E. radiata* may imply an adaptive response to the periodic nutrient supply as a result of terrestrial runoff, mixing events and oceanic exchange in Doubtful Sound. The temporal variation and range in $K_s$ values suggests that *E. radiata* may adjust its inorganic N uptake rates to coincide with other physiological mechanisms such as growth and/or photosynthesis, although further research is needed.

**3.4.5.5.1 Inorganic N uptake at 2 $\mu$M ($V_z$)**

Although $\alpha$ better reflects uptake rates at low concentrations than do $K_s$ values alone, it does not provide a direct measure of uptake (Harrison et al., 1989; Hurd and Dring, 1990). Healey (1980) suggested comparing the ability of macroalgae to take up nutrients at an ecological relevant low concentration. High inter-sporophyte variation in $V_{max}$ and $K_s$ values, as well as linear uptake for some replicates of *Ecklonia radiata* in the present study, necessitated the use of this method. As far as the author is aware, only three studies have adopted Healey’s suggestion. Wallentinus (1984) re-calculated N and P uptake abilities of a range of macroalgae at three concentrations (5, 25 and 100 $\mu$M), Hurd and Dring (1990) compared P uptake by five intertidal fucoids at a concentration of...
1 μM ($V_1$), and Phillips (2001) calculated NO$_3^-$ and NH$_4^+$ uptake rates for two red and two brown intertidal species. Wallentinus (1984) found uptake rates to be affected by ambient seawater nutrient concentrations, water temperature and thallus morphology with low uptake rates characteristic of late successional, long-lived, coarse-species with low surface to volume ratios while high uptake rates were characteristic of short-lived, opportunistic species. Furthermore, low uptake rates at ecologically relevant concentrations may reflect a slow growth rate and therefore a low requirement for the nutrient under investigation as demonstrated by low $V_1$ values for $P$ uptake in five intertidal fucoids (Hurd and Dring, 1990). Generally, the rates of NO$_3^-$ and NH$_4^+$ uptake at 2 μM for *E. radiata* in Doubtful Sound were similar between the three study sites. Although direct comparison cannot be made with either of the earlier studies using this method, the low $V_2$ values observed for *E. radiata* from Doubtful Sound fit Wallentinus' late successional, long-lived group. Furthermore, the low NO$_3^-$ and NH$_4^+$ $V_2$ values for *E. radiata* may reflect a low N requirement as a result of low growth rates, due in part to low ambient seawater inorganic N concentrations throughout the study period.

### 3.4.6 Summary

Ambient seawater inorganic N concentrations at the water surface and in subsurface samples were low-year round, with the exception of a peak in NO$_3^-$ concentrations in November 1999.

C:N ratios, % tissue N and soluble tissue N content indicated that *Ecklonia radiata* was N-limited throughout the study.

*Ecklonia radiata* displayed both non-linear and linear patterns of NO$_3^-$ and NH$_4^+$ uptake. There was no evidence of a lag phase in uptake but there was some evidence of surge uptake, which is common for N-deficient macroalgae and may be advantageous for *E. radiata* in an environment where N supply is episodic. Linear depletion may have occurred due to depleted internal N pools filling over the course of the experiment or because *E. radiata* had a high demand for N.
Ecklonia radiata also exhibited both saturated and non-saturated uptake for both NO$_3^-$ and NH$_4^+$. $V_{\text{max}}$ for NO$_3^-$ uptake rate were low indicating that *E. radiata* was not utilizing, or was unable to respond to, episodic pulses of NO$_3^-$. $V_{\text{max}}$ for NH$_4^+$ uptake rates were greater than for NO$_3^-$ rates although high NH$_4^+$ $V_{\text{max}}$ values are of limited ecological significance given that ambient NH$_4^+$ concentration is typically low naturally. At low inorganic N concentrations, uptake kinetic parameters indicated that *E. radiata* had no preference for either NO$_3^-$ or NH$_4^+$, suggesting that *E. radiata* utilizes whichever inorganic N form is available.

Estimates of the uptake ability of *Ecklonia radiata* to take up NO$_3^-$ and NH$_4^+$ at the ecologically relevant low concentration of 2 µM ($V_2$) were similar, supporting the contention that *E. radiata* exhibited no preference for either inorganic N form. Furthermore, the low $V_2$ values may reflect a low N requirement as a result of low growth rates.
Chapter Four

Photosynthetic performance of *Ecklonia radiata*

4.1 INTRODUCTION

Growth and production of macroalgae rely on the photosynthetic processes that provide chemical energy, such as ATP, to supply C for the synthesis of biochemical compounds such as lipids, amino acids and pigments, as well as for N uptake and assimilation (Kirk, 1983; Lapointe *et al.*, 1984; Turpin, 1991; Raven *et al.*, 1992). In many laminarians the end products of photosynthesis, such as laminaran and mannitol, may be stored and used as a C source to support growth during periods of unfavourable conditions such as low PFD and nutrient availability (Lüning, 1971; Mann, 1973; Hatcher *et al.*, 1977; Chapman and Craigie, 1978; Dunton and Schell, 1986).

4.1.1 A description of the light climate in Doubtful Sound

In Doubtful Sound several factors influence the quality and quantity of light available to *Ecklonia radiata* for photosynthesis. These factors include fiord topography, altitude of the sun (see Drew, 1983a for discussion on solar angles and consequences), meteorological conditions (e.g. cloud cover and rain) and concentrations of phytoplankton, gilvin and particulate matter within the water column, as well as the LSL. As a result of the LSL, ca. 90 % PFD is attenuated in the top 4 m and a further 5-10 % over the next 15 m, giving a photic zone of ~20 m depth (Rutherford *et al.*, 2000; Gibbs, 2001). The total PFD reaching the water surface varies on a seasonal basis with PFD penetrating to greater depths during winter (Grange *et al.*, 1991, chapter 2). Furthermore, a light gradient exists along the Sound with higher light levels at the water surface and increased transmission through the water column at sites near the coastal entrance. This
is a result of a general decrease in the average height of the mountainous walls, increase in the width of the Sound and a thinning of the LSL. It is likely that *E. radiata* at Causet Cove and Thompson Sound will receive a higher level of PFD than sporophytes at Seymour Island, which may result in higher photosynthetic rates. There is also a shift in spectral composition (chapter 2) in the underlying marine waters from the head of the Sound to its coastal entrance. Greater attenuation of blue wavelengths occurs at Seymour Island where the LSL is generally thicker than at either Causet Cove or Thompson Sound (chapter 2). The low light conditions and change in spectral composition from blue to green wavelengths may be partially responsible for the lack of macroalgae at the head of the Sound (Grange et al., 1991; Boyle et al., 2001).

4.1.2 Determination of the photosynthetic parameters of macroalgae

Photosynthesis provides fixed C and energy for growth and production, and can be used to give an indication of primary productivity (Lobban and Harrison, 1997). Photosynthetic rates of macroalgae are typically measured using O$_2$ release or $^{14}$C uptake by the macroalgal tissue at varying irradiances. A photosynthetic versus PFD curve (PE curve) is generated and any one of a number of mathematical models, such as a tangential hyperbola, fitted (refer Henley, 1993). Note that in this thesis the more recent terminology of P vs E, as opposed to P vs I, curves has been adopted (for an explanation see Falkowski and Raven, 1997). The PE curve can typically be split into three regions; a region of light-limitation where photosynthesis increases with increasing irradiance, a light-saturated region where the photosynthetic rate ceases to increase with increasing irradiance, and a region where photosynthesis decreases with increasing irradiance (Fig. 4.1). Several physiological parameters can then be obtained from the PE curve and used to assess the ability of the macroalgae to harvest available light.

There are three primary parameters derived from the PE curve. The maximum rate of photosynthesis ($P_{\text{max}}$) is considered the photosynthetic limit of the light-independent reactions and is primarily limited by C procurement (Sukenik et al., 1987; Levavasseur et al., 1991; Raven and Osmond, 1992). The initial slope of the tangential hyperbola, termed alpha (denoted $\alpha_p$ to distinguish the photosynthetic parameter from $\alpha$ used for N uptake [chapter 3]), determines the efficiency of light utilization at low PFD, and is a
Figure 4.1: Generalized diagram of gross photosynthesis versus photon flux density (PFD) with a tangential hyperbola (Jassby and Platt, 1976) fitted showing how the photosynthetic parameters relate to it. $P_{\text{max}}$ is the maximum rate of photosynthesis, alpha (or $\alpha_p$) is the initial slope, $R_d$ is respiration, $E_k$ is the light saturation parameter and $E_c$ is the compensation PFD. The more recent terminology of $P$ vs. $E$, as opposed to $P$ vs. $I$, has been adopted (for an explanation see Falkowski and Raven, 1997). Consequently $E_k$ and $E_c$ are equivalent to $I_k$ and $I_c$. 
function of both light-harvesting efficiency and photosynthetic energy conversion efficiency (Geider and Osborne, 1992; Henley, 1993). Higher $\alpha_p$ values indicate a greater efficiency of light utilization at low PFD (Cheshire et al., 1996). Respiration ($R_d$) consumes $O_2$ and releases $CO_2$ (Lobban and Harrison, 1997), limiting the amount of carbon converted to new biomass (Round and Chapman, 1994).

Secondary photosynthetic parameters can then be derived using the primary parameters. The light saturation parameter, $E_k$, is considered by Henley (1993) to be the most used (and misused) parameter. $E_k = P_{\text{max}}/\alpha_p$, and is therefore dependent on both $P_{\text{max}}$ and $\alpha_p$, incurring errors associated with both of these parameters (Talling, 1957; Gerard, 1986). $E_k$ is indicative of the physiological adaptation of a macroalga to its light environment (Chalker, 1981; Henley, 1993; Cheshire et al., 1996). The compensation PFD, $E_c$, is the PFD at which gross photosynthesis and respiration balance each other or alternatively, the physiological minimum PFD required for survival (Schaffelke et al., 1996). $E_c$ sets the physiological constraints for the depth limit of attached macroalgae (Geider and Osborne, 1992; Markager and Sand-Jensen, 1992). $E_c$ is derived from $R_d/\alpha_p$ and is not fixed but varies with $R_d$ and $\alpha_p$, both of which depend on the environmental conditions (PFD, nutrient availability and temperature) under which the macroalga is growing (Geider and Osborne, 1992). A third, and less commonly used, parameter defines photoinhibition ($\beta$), or the steepness of decline in photosynthesis at high PFD (Platt et al., 1980).

4.1.3 Photosynthetic pigments in macroalgae

In macroalgae, photosynthesis occurs within the thylakoid membranes associated with chloroplasts. Each thylakoid membrane contains light-harvesting complexes made up of a series of pigments designed to capture and transfer photons to a reaction center of chlorophyll-protein complexes. There are three main light-harvesting pigments in the Heterokontophyta. Chlorophyll $a$ (chl $a$) is the main photosynthetic pigment and is ubiquitous to all photosynthesizing terrestrial and aquatic plants. There are two types of accessory pigments in Heterokontophyta: chl $c$ and fucoxanthin. Light energy is transferred (as excited electrons) via the accessory pigments to chl $a$, which is situated at
the reaction center of the two photosystems (PSI and PSII). These pigments have different absorption bands, allowing greater utilization of photosynthetically active radiation (PAR) thereby allowing macroalgae to maximize photosynthetic rates (Fig. 4.2, Lobban and Harrison, 1997).

Figure 4.2: *In vitro* absorption spectra of the three main pigments (chl *a*, chl *c* and fucoxanthin) found in members of the Heterokontophyta extracted in DMSO followed by acetone. Redrawn from Lobban and Harrison (1997) and Campbell *et al.* (1999a).
Furthermore, pigment contents of macroalgae tend to increase with a decrease in PFD (e.g. Rosenberg and Ramus, 1982; Machalek et al., 1996; Campbell et al., 1999b) although there are exceptions to this, particularly amongst laminarian species (Dunton and Jowalis, 1988; Markager, 1993; Rodrigues et al., 1993). Increasing the pigment content allows for greater amounts of PFD to be captured and used within the photosynthetic process while a decrease may reflect greater growth and high PFD (Lewey and Gorham, 1984; Fillet, 1995). A decrease in the pigment ratios, chl c/chl a and fucoxanthin/chl a, may be indicative of acclimation to low light whereby chl a production is maintained or increased at the expense of chl c (Campbell et al., 1999b). Fucoxanthin content in the brown algae, Ascophyllum nodsum and Fucus vesiculosus, has been shown to decrease relative to chl a as irradiance decreases (Ramus et al. 1977). However, it is also reported that absorption of light by fucoxanthin in vivo extends from 500 to 590 nm (Ramus et al. 1977).

Additionally, light absorption and photosynthesis in macroalgae has been suggested to be a function of chlorophyll concentration and thickness of the photosynthetic tissues (Agusti et al, 1994; Enriquez et al, 1996). Photosynthesis is directly related to light absorption, the hypothesis being that chlorophyll concentration increases with decreasing tissue thickness (Björkman and Demmig, 1987; Henley and Dunton, 1995). Levitt (1993) suggested that an advantage of being thin was to increase the surface area:volume ratio (SA:V) thereby allowing rapid uptake of nutrients as well as presenting a larger photosynthetic area to incident light. It may also help to alleviate internal self-shading by non-photosynthetic cell wall and cytoplasmic components (Levitt, 1993).

4.1.4 The use of chlorophyll fluorescence for assessing 'algal health'
The photosynthetic efficiency of photosystem II (PSII) can be assessed by measuring chlorophyll-fluorescence using a pulse-amplitude-modulated (PAM) fluorometer (Diving-PAM, Walz, Effeltrich, Germany; e.g. Schreiber et al., 1995; Durako and Kunzelman, 2002; Schreiber et al., 2002). The ratio of variable to maximal chlorophyll fluorescence (Fv/Fm) in dark-adapted macroalgal tissue can be used as an indicator of the physiological status ('algal health'), or photosynthetic stress (such as photoinhibition), of that macroalga (Krause and Weis, 1991; Bruhn and Gerard, 1996; Dring et al., 1996;
PAM fluorometry measures the initial fluorescence \((F_0)\) using a low PFD, when PSII reaction centres are open (oxidized), and the maximal fluorescence \((F_m)\) that occurs during a saturating pulse of PFD, when the reaction centres are closed (reduced; e.g. \(\text{Flores-Moya et al., 1998; Aguilera et al., 2000; Beer et al., 2000; Kromkamp and Forster, 2003}\)). Variable fluorescence \((F_v)\) is the difference between \(F_m\) and \(F_0\) (\(\text{Bruhn and Gerard, 1996}\)). The ratio \(F_v/F_m\) of unstressed plants is high, approximately 0.8 for most terrestrial angiosperms and Chlorophytes (\(\text{Björkman and Demming, 1987; Franklin et al., 1992}\)). Reported \(F_v/F_m\) values for Heterokontophytes range from 0.7 to 0.8 and for Rhodophytes from 0.6 to 0.7 (\(\text{Bruce et al., 1983; Popovic et al., 1983; Bose et al., 1988; Huppertz et al., 1990; Hanelt et al., 1992}\)).

4.1.5 Factors influencing macroalgal photosynthesis

Subtidal macroalgae in Doubtful Sound are subject to a constantly changing underwater environment. Photosynthesis in macroalgae is influenced by a number of factors including temperature, salinity, nutrients, \(O_2\), and bicarbonate \([\text{HCO}_3^-]\) availability. The most important abiotic factor influencing photosynthesis of subtidal macroalgae is however, light availability. Light and its spectral composition change with depth and also vary according to attenuation, absorption, scattering and reflection by particulate matter and phytoplankton within the water column (\(\text{Kirk, 1983}\)). It has been shown that light penetration in Doubtful Sound at 9 m depth is approximately 25 % of surface light (chapter 2) but may be reduced to less than 1 % during days of heavy rain (\(\text{Grange et al., 1991}\)). The LSL may also cause a change in the underwater spectral composition between the study sites in Doubtful Sound.

4.1.5.1 The influence of PFD on macroalgal photosynthesis

Every macroalgal species has a PFD level at which photosynthesis becomes light-saturated, commonly termed \(E_k\). Typically, \(E_k\) values for intertidal macroalgae are 400 – 600 \(\mu\)mol photons \(m^{-2} s^{-1}\), upper and mid sublittoral species require 150 – 200 \(\mu\)mol photons \(m^{-2} s^{-1}\) while deep sublittoral species require less than 100 \(\mu\)mol photons \(m^{-2} s^{-1}\) (\(\text{Lüning, 1981; Lobban and Harrison, 1997}\)). \(E_k\) will depend on the habitat and the depth
that the macroalga is found in the water column as well as pigment concentration. For example, blades from the top of the canopy forming subtidal macroalga, *Macrocystis pyrifera*, had higher photosynthetic rates than blades near the holdfast (Gerard, 1986). The higher photosynthetic rates were attributed to higher light levels at the surface water than at depth.

Many macroalgae exhibit a reduction in photosynthesis at extremely high light levels (e.g. Drew, 1974; Herbert and Waaland, 1988; Franklin *et al.*, 1992; Hanelt, 1992, 1996, 1998; Hanelt *et al.*, 1997). The reduction of photosynthesis at high light levels may be caused by damage to components in the photosystems (particularly PSII) due to excessive energy, known as photoinhibition (Powles, 1983; Henley, 1993; Long *et al.*, 1994; Lobban and Harrison, 1997). Osmond (1994) further defined photoinhibition as either dynamic or chronic, based primarily on recovery from damaging effects. Dynamic photoinhibition involves the dissipation of excess energy as heat without a decline in $P_{\text{max}}$ and is rapidly reversible (on a scale of minutes) (Franklin and Foster, 1997). Chronic photoinhibition involves a decrease in quantum yield and $P_{\text{max}}$ and is reversible on a scale of hours to days (Franklin and Foster, 1997). Alternatively, the downturn in photosynthetic rates may be a form of protecting the photosynthetic apparatus from photo-oxidative damage caused by high light levels (Falkowski and Raven, 1997; Franklin and Foster, 1997).

The spectral composition of underwater light also affects the photosynthetic activity in many macroalgae, particularly the availability of blue light (Kirk, 1976; Dring, 1989; Schmid *et al.*, 1992). For example, blue light stimulated photosynthesis in the brown macroalga, *Ectocarpus siliculosus*, via activation of inorganic carbon acquisition (Schmid, 1998; Hillrichs and Schmid, 2001). Similarly, Lüning and Dring (1985) and Dring (1989) found photosynthetic rates of brown macroalgae in blue light were often double the rates in red light. Blue light may stimulate photosynthesis in brown macroalgae either via Calvin-cycle enzymes, a bicarbonate-uptake mechanism, or activation of the release of $CO_2$ from an internal store (Falkowski and LaRoche, 1991; Schmid and Dring, 1996).
4.1.5.2 The influence of water motion on macroalgal photosynthesis
The effects of light on photosynthesis may be further influenced by other environmental factors such as water motion. Water motion affects metabolic processes that occur at the macroalgal surface, such as \( O_2 \) evolution and respiration, which depend on chemical exchange with the environment (Patterson, 1992). Water motion stirs up particulate matter within the water column thereby decreasing light penetration resulting in reduced photosynthetic rates. Alternatively, water motion also increases movement of the macroalgal blade (photosynthetic surface). Subtidal macroalgae often form a canopy that reduces the amount of light reaching understory seaweed. Water movement through these canopies often produces momentary flashes of high light called light- or sun-flecks. Wing and Patterson (1993) measured the effect of light-flecks on photosynthetic rates in the intertidal algae, *Postelsia palmaeformis* and *Hedophyllum sessile*. They concluded that wave-induced light-flecks increased photosynthetic rates, contributing to significant gains in the primary productivity of macroalgae.

4.1.6 Objectives of chapter four
The LSL in Doubtful Sound decreases towards the open coast. Consequently, light penetration would be expected to increase along this gradient of decreasing LSL thickness and an associated decrease in detrital material. As a result of differences in the levels of light reaching the photosynthetic surface of *Ecklonia radiata* in Doubtful Sound, a temporal change in pigment content and photosynthetic parameters (\( P_{\text{max}}, \alpha_p, E_k, \) and \( \beta \)) between the three study sites was predicted.

*Ecklonia radiata* at Seymour Island was expected to have higher photosynthetic pigment concentrations, particularly of the accessory pigments (chl \( c \) and fucoxanthin) due to reduced PFD as a result of decreasing light penetration through the LSL. The increase in accessory pigments would be utilized to optimize available light. It was also expected that the three pigments studied would be higher between September and February (spring/summer) and lower between June and August (winter) due to the amount of incident light received at each site. The amount of light received over the study period would be influenced by the temporal shift in the declination of the sun, and the effect of the fiord walls casting shadow over certain regions of the Sound.
Ecklonia radiata at Seymour Island would also require a lower light level to saturate photosynthesis than E. radiata at either Causet Cove or Thompson Sound. It was expected that E. radiata at Seymour Island would have lower $P_{\text{max}}$ and $E_k$ values but higher $\alpha_P$ values compared to E. radiata at Causet Cove or Thompson Sound, because E. radiata at the inner site would display greater acclimation to low light. It was also predicted that E. radiata at Seymour Island would show greater photoinhibition ($\beta$) at higher light levels than E. radiata at either Causet Cove or Thompson Sound. Photoinhibition was expected to vary over time and between sites with greater $\beta$ predicted between September and February due to a decrease in the amount of detrital material in the LSL and subsequent increase in light penetration compared to winter months.

Furthermore, overall $F_v/F_m$ (a proxy for algal health) for Ecklonia radiata from Causet Cove and Thompson Sound would be higher than the $F_v/F_m$ ratio for E. radiata from Seymour Island. This would be a result of a thinner LSL and consequently higher light levels at depth, as well as a greater availability of nutrients, at the two outer sites. However, it was predicted that an $F_v/F_m$ gradient would occur along the macroalgal blade with lower $F_v/F_m$ at the apex of the primary blade compared to the blade base, regardless of site. This would occur as the apex is furthest away from the meristematic region, closer to the water surface and therefore higher light levels, and is the region of tissue erosion due to necrosis and grazing.
4.2 METHODS

*Ecklonia radiata* sporophyte material used for the nutrient uptake experiments in (section 3.2.3, chapter 3) was also used for the photosynthetic and pigment experiments. There were five replicate *E. radiata* sporophytes per site per sampling period.

**4.2.1 Determination of photosynthetic pigment contents**

Chl *a*, *c* and fucoxanthin were extracted following the method of Seely *et al.* (1972).

**4.2.1.1 Determination of DW/FW ratio**

For each *Ecklonia radiata* sporophyte collected, five pairs of discs were cut using a cork borer (SA = 9.82 cm$^2$) from the primary blade at equal distances from the base of the blade to the tip. All discs were cleaned of epiphytic material as previously described (section 3.2.9). For each pair of discs, one disc was rinsed, blotted dry and the wet weight recorded, then dried at 80 °C for 24-36 h until a constant dry weight was obtained.

**4.2.1.2 Extraction method for pigments**

Pigments were extracted from the second disc by immersion in 4 mL of dimethyl sulphide oxide (DMSO) and agitating for 3 minutes in darkness. The disc was then placed in 4 mL of 90 % acetone and left for 30 minutes in darkness, swirling occasionally. The liquid was decanted into a clean test tube. The absorbance of extracts was measured using a spectrophotometer (Hitachi U-1100). The absorbance of DMSO extracts was measured at the following wavelengths; 665, 631, 582 and 480 nm. Acetone extracts were measured at 661, 628, 580 and 470 nm.

Pigment concentrations were calculated from the equations of Seely *et al.* (1972; refer to appendix B for equations) and expressed on a dry weight (gDW) basis. Data from each blade were averaged to give an overall estimate of pigment content per sporophyte.
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4.2.2 Photosynthetic and respiration rates of *Ecklonia radiata*

4.2.2.1 Experimental setup and measurement of O$_2$ evolution

Discs (SA = 29.04 cm$^2$) were randomly cut from the primary blade of each of the five sporophytes collected per site each sampling period and were pre-treated as for nutrient uptake experiments (section 3.2.9).

Photosynthetic and respiration rates of *Ecklonia radiata* were determined by measuring changes in oxygen (O$_2$) concentration at a range of PFD levels using a slightly modified method from Thomas (1988). Biological oxygen demand (BOD) bottles (266 ml) were wrapped in varying layers of neutral density screening that provided a range of PFD levels. Two bottles were left uncovered (providing an 'initial' and a light bottle) and a further bottle was wrapped in tin foil (dark bottle) to provide an estimate of dark respiration. The initial oxygen concentration (mg L$^{-1}$) of 20 L filtered seawater was measured using an oxygen meter (Orion Dissolved Oxygen Meter, model 860). N$_2$ gas was bubbled through the seawater until the oxygen concentration was approximately half the initial concentration. Each oxygen bottle was filled with the de-oxygenated seawater, a disc placed in each bottle, except the 'initial' bottle, and all the bottles stoppered. Initially, from September 1998 to December 1998, six PFD levels were used ranging from 0 to 200 μmol photons m$^{-2}$ s$^{-1}$. In February 1999, PFD levels were increased to twelve and ranged from 0 to 350 μmol photons m$^{-2}$ s$^{-1}$. There were five replicate blades per site with six or twelve discs cut from each blade (one disc for each PFD level used per replicate blade). Due to the number of replicates, the experiment was run three times during the day starting at approximately 9am, 12pm and 3pm, and the PE curve for each blade per site obtained at the same time.

Each blade was randomly assigned to one of three runs as well as shaker position. Bottles were positioned on an orbital shaker in the growth cabinet (see section 3.2.4 for further details), and the O$_2$ concentration of the ‘initial’ bottle immediately measured using an oxygen meter. After one hour the disc was removed and the oxygen concentration within each bottle measured and recorded. PFD levels for each bottle were measured by placing the light sensor of a LiCor light meter (model LI-189) inside a 250 mL beaker covered in the appropriate layers of neutral density screening while all other
oxygen bottles remained in their respective positions on the shaker. Discs were blotted dry to determine wet weight, then dried at 80 °C for 24-36 h until a constant dry weight was obtained. All photosynthetic parameters ($P_{\text{max}}$, $\alpha_p$, $R_d$, $E_k$, $E_c$ and $\beta$) were expressed per dry weight.

**4.2.2.2 Determination of PE parameters**

The gross photosynthetic rate for each replicate was calculated as follows:

\[
\text{Gross photosynthesis} = \frac{(\text{LB} - \text{IB}) \times 0.266}{t \times \text{DW}} + \frac{(\text{DB} - \text{IB}) \times 0.266}{t \times \text{DW}} \quad \text{(Eq. 4.1)}
\]

where
- $\text{LB} =$ light bottle $O_2$ concentration (mg L$^{-1}$)
- $\text{IB} =$ initial bottle $O_2$ concentration (mg L$^{-1}$)
- $\text{DB} =$ dark bottle $O_2$ concentration (mg L$^{-1}$)
- 0.266 = $O_2$ bottle volume (L)
- $\text{DW} =$ dry weight (g) of tissue disc
- $t =$ incubation time (h)

Respiration, $R_d$, was calculated for the dark bottle as follows:

\[
\text{Respiration} = \frac{(\text{DB} - \text{IB}) \times 0.266}{t \times \text{DW}} \quad \text{(Eq. 4.2)}
\]

The gross photosynthetic rate was plotted against PFD for each replicate. Data were fitted to a tangential hyperbola (Jassby and Platt, 1976) by means of an iterative non-linear least squares regression using SigmaPlot software (v 4.01, SPSS Inc, USA) and the photosynthetic parameters ($P_{\text{max}}$, $\alpha_p$, and $R_d$) obtained.
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\[
P = P_{\text{max}} \times \tanh(\alpha_P \text{PFD}/P_{\text{max}}) + R_d \quad \text{Eq. 4.3}
\]

where
- \(P\) = gross photosynthetic rate (mg O\(_2\) g DW\(^{-1}\) h\(^{-1}\))
- \(P_{\text{max}}\) = the maximum photosynthetic rate (mg O\(_2\) g DW\(^{-1}\) h\(^{-1}\))
- \(\alpha_P\) = alpha (mg O\(_2\) g DW\(^{-1}\) h\(^{-1}\) (\(\mu\)mol photons m\(^{-2}\) s\(^{-1}\))\(^{-1}\))
- \(R_d\) = respiration (mg O\(_2\) g DW\(^{-1}\) h\(^{-1}\))

As inhibition occurred for all replicates, data was also fitted to the photoinhibition equation of Platt *et al.* (1980) by means of an iterative non-linear least squares regression using SigmaPlot software (v 4.01, SPSS Inc, USA) and used to determine the inhibition (\(\beta\)) parameter only. Although the photo-inhibition equation also determines \(P_{\text{max}}\) and \(E_k\), they are frequently overestimated and were therefore not used in the present study.

\[
P = P_{\text{max}}(1-\exp(-1/E_k))\exp(-\beta\star E/P_{\text{max}}) \quad \text{Eq. 4.6}
\]

where
- \(P\) = gross photosynthetic rate (mg O\(_2\) g DW\(^{-1}\) h\(^{-1}\))
- \(P_{\text{max}}\) = the maximum photosynthetic rate (mg O\(_2\) g DW\(^{-1}\) h\(^{-1}\))
- \(E\) = PFD (\(\mu\)mol photons m\(^{-2}\) s\(^{-1}\))
- \(E_k\) = saturating PFD (\(\mu\)mol photons m\(^{-2}\) s\(^{-1}\))
- \(\beta\) = inhibition parameter (mg O\(_2\) g DW\(^{-1}\) h\(^{-1}\))

Although measurements of \(R_d\) were made, consistent negative \(R_d\) values during the later half of the study could not be explained fully although various factors such as contamination of the seawater, epiphytic material on algal tissue, air bubbles in the de-oxygenated seawater or light entering the dark bottle were explored at the time but dismissed. Therefore, \(R_d\) values for *Ecklonia radiata* from Doubtful Sound have not been reported here. Consequently, compensation PFD (\(E_c\)) values have not been reported either as this value relies on the use of \(R_d\).
4.2.3 Chlorophyll fluorescence of *Ecklonia radiata*

The Fv / Fm ratio of five randomly chosen *Ecklonia radiata* individuals at each of the three study sites was measured *in situ* from October 1998 to March 2000. Fv / Fm measurements could not be made at the same time every day due to the amount of work undertaken at each site, diving restrictions and the distance between sites. It is acknowledged that Fv / Fm values typically decrease during the morning and increase in the late afternoon and early evening *in situ* (i.e. Hanelt *et al.*, 1993). However, Fv / Fm was used only as a proxy for 'algal health' and not as a measurement of photosynthetic efficiency (Hanelt, 1992; Hanelt *et al.*, 1995). In October 1998, each primary blade was divided into five regions: blade/stipe junction, 2/5 from the blade base, middle, 4/5 from the blade base and apex (Fig. 4.3). A dark leaf clip (hereafter referred to as a clip) was attached at each region and the tissue underneath dark-adapted for 10-15 minutes after which Fv/Fm was measured using an underwater pulse-amplitude-modulated fluorometer (Diving-PAM, Walz, Effeltrich, Germany). No significant variation was observed between the five regions of each blade during October 1998, therefore *in situ* measurements from November 1998 onwards were taken at the blade/stipe junction and apex of the primary blade only. The number of sporophyte replicates used per site was increased to six to utilize the total number of clips available.

4.2.4 Data analysis

Monthly variations between the three sites in chl a, c, and fucoxanthin contents on a dry weight, and the photosynthetic parameters (P_max, α_P, E_k, and β) were analyzed using a one-way ANOVA. Temporal variation in chl a, c, and fucoxanthin contents on a dry weight and the photosynthetic parameters (P_max, α_P, E_k, and β) were analyzed using two-way ANOVA (time x site). Fv/Fm ratios at the blade base and at the apex were tested separately using a two-way ANOVA. Factors were site and position along blade. ANOVA are relatively robust to violations of assumptions therefore even if data failed tests for equal variance or normality, ANOVA were continued (Underwood 1981, 1997, Zar 1996). For all data analyses, the level of significance chosen was P=0.05. When the ANOVA indicated a significant effect, post-hoc multiple comparisons using Tukey tests
were performed. All analyses were performed using SigmaStat computer software (v2.03, SPSS Inc, USA, 1997).
Figure 4.3: Diagram showing placement of Diving-PAM fluorometry clips used to measure $F_v/F_m$ along the primary blade of *Ecklonia radiata* in October 1998, and in the subsequent months.
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4.3 RESULTS

4.3.1 Pigment contents of *Ecklonia radiata*

The chl \(a\) content of *Ecklonia radiata* ranged from 0.7 to 3.6 mg gDW\(^{-1}\) at Seymour Island, from 0.5 to 3.2 mg gDW\(^{-1}\) at Causet Cove, and from 0.5 to 2.6 mg gDW\(^{-1}\) at Thompson Sound (Fig. 4.4a). A significant temporal pattern was exhibited \((P<0.001)\) with the lowest chl \(a\) content at all three sites in November and December 1998 and maximum chl \(a\) content one year later. As a percentage of total pigment content *E. radiata* from Causet Cove had the lowest amounts of chl \(a\) (41 - 52 %), followed by *E. radiata* from Thompson Sound (48 -57 %) and Seymour Island (50 - 59 %).

Chl \(c\) content of *Ecklonia radiata* ranged from 0.5 to 2.8 mg gDW\(^{-1}\) at Seymour Island, from 0.4 to 3.1 mg gDW\(^{-1}\) at Causet Cove, and from 0.4 to 2.0 mg gDW\(^{-1}\) at Thompson Sound (Fig. 4.4b). *E. radiata* exhibited significant temporal \((P<0.001)\) and spatial \((P=0.003)\) variations in chl \(c\). Generally, lower chl \(c\) content was recorded in 1998 than in 1999/2000, although the minimum chl \(c\) content of *E. radiata* at Seymour Island occurred in June 1999. Maximum chl \(c\) content occurred in November 1999 at Causet Cove and Thompson Sound, and in December 1999 at Seymour Island. The value of chl \(c\) content for *E. radiata* at Causet Cove was typically higher than values for sporophytes at either Seymour Island \((P=0.01)\) or Thompson Sound \((P=0.005)\), particularly during 1999 and 2000. When expressed as a percentage of total pigment content, chl \(c\) of *E. radiata* ranged from 19 - 41 % from Seymour Island, 34 - 47 % from Causet Cove, and 21 - 43 % from Thompson Sound over the experimental period.

Fucoxanthin content of *Ecklonia radiata* also displayed a significant temporal pattern \((P<0.001)\), with the lowest fucoxanthin content typically recorded during 1998 (Fig. 4.4c). In general, *E. radiata* at Seymour Island had a significantly higher fucoxanthin content than *E. radiata* at Thompson Sound, however it was only-just-significant \((P=0.043)\) and should be treated with caution. The amount of fucoxanthin present in *Ecklonia radiata* from the three study sites contributed < 25 % of the total pigment content.
Figure 4.4: Temporal and spatial variation of photosynthetic pigments on a dry weight basis in the primary blade of *Ecklonia radiata* from three sites in Doubtful Sound. A) Chl a, b) chl c, and c) fucoxanthin measured from September 1998 to March 2000. * indicates significant differences between sites in a given month. Points represent mean ± 1 SD (n=5).
Pigment content differed not only over time but also between sites. *E. radiata* at Seymour Island had significantly higher chl $a$ and chl $c$ contents (on a dry weight basis) than those at either Causet Cove or Thompson Sound from September to December 1998 and again in December 1999 (Fig. 4.4). Chl $a$ and $c$ contents of *E. radiata* were generally similar between Causet Cove and Thompson Sound during the study period with the exception of chl $a$ at Causet Cove in June 1999 and chl $c$ at Causet Cove in June and November 1999 which were significantly higher than at the other two sites. *E. radiata* from Seymour Island also had significantly lower chl $a$ and $c$ than the other two sites in June 1999. *E. radiata* had significantly higher fucoxanthin at Seymour Island from September to December 1998, in December 1999 and in March 2000. Fucoxanthin contents were similar in the three sites during the middle of the study period.

**4.3.1.1 Pigment ratios of Ecklonia radiata**

There was a significant temporal pattern in chl $c$/chl $a$ ratios of *Ecklonia radiata* from Doubtful Sound ($P<0.001$; Fig. 4.5a) with a decrease in ratios at all three sites in February 1999. Significant spatial variation ($P<0.001$) in chl $c$/chl $a$ ratios was also observed with ratios at Causet Cove higher than those at Seymour Island or Thompson Sound in November and December 1998 and February 1999. However, significant temporal and spatial interactions ($P<0.001$) imply that variations in chl $c$/chl $a$ ratios between sites were neither constant over time nor independent.

The fucoxanthin/chl $a$ (hereafter referred to as fucox/chl $a$ for brevity) ratio for *Ecklonia radiata* from Seymour Island ranged from 0.15-0.45, from Causet Cove 0.07-0.32 and from outer Thompson 0.15-0.4 (Fig. 4.5b). Fucox/chl $a$ ratios of *E. radiata* from the three sites exhibited a significant temporal variation ($P=0.015$) with an increase in values observed in February 1999. The ratio of fucox/chl $a$ at Seymour Island was generally significantly higher than those at Causet Cove or Thompson Sound ($P=0.018$), particularly from June 1999 onwards.
Figure 4.5: Ratios of a) chl c/chl a and b) fucoxanthin/chl a for *Ecklonia radiata* from three sites in Doubtful Sound. Data were collected between September 1998 and March 2000. Points represent mean ± 1 SD (n=5).
between October and December 1998 and then generally decreased to a minimum in November and December 1999. In March 2000, ratios increased and were similar to those at the beginning of the study period. Ratios at Seymour Island were typically lower than those at either Causet Cove or Thompson Sound ($P=0.023$).

### 4.3.2 Photosynthetic performance of *Ecklonia radiata*

*Ecklonia radiata* also exhibited a significant temporal pattern ($P<0.001$) in $P_{\text{max}}$ on a dry weight basis (Fig. 4.6a). $P_{\text{max}}$ values were low between September and December 1998 then increased to a maximum at Thompson Sound and Seymour Island in June 1999 and at Causet Cove two months later. Values for $P_{\text{max}}$ then decreased to values similar to those in September to December 1998. Significant variation in monthly $P_{\text{max}}$ values between sites occurred twice during the study period. In November 1998 $P_{\text{max}}$ values at Seymour Island were significantly higher than at Thompson Sound (Tukey test $P=0.032$) but similar to those at Causet Cove, while in August 1999 values at Thompson Sound were significantly lower than those at Seymour Island (Tukey test $P=0.022$) and Causet Cove (Tukey test $P=0.004$).

There was significant variation in the initial slope of the PE curve, $\alpha_p$, over time ($P=0.010$) for *Ecklonia radiata* (Fig. 4.6b). However, a Tukey test indicated that only $\alpha_p$ values in August 1999 were significantly higher than values in September and October 1998. This was due in part to the maximum $\alpha_p$ value at Causet Cove in August 1999 being up to 12 times greater than values at the three sites in September 1998 and up to 39 times greater than in October 1998. Maximum $\alpha_p$ values at Seymour Island and Thompson Sound occurred in November 1998. Spatial variation in $\alpha_p$ values was observed in November and December 1998. In November 1998, $\alpha_p$ values at Seymour Island were significantly higher than at Causet Cove (Tukey test, $P=0.027$), while a month later in December $\alpha_p$ values at Seymour Island were significantly higher than at either Causet Cove (Tukey test, $P=0.016$) or Thompson Sound (Tukey test, $P=0.031$).

There were no temporal variations in $E_k$ for *Ecklonia radiata* on a dry weight basis ($P=0.098$ and 0.057, respectively; Fig. 4.6c). In November 1999, $E_k$ values at Causet
Cove were significantly higher than at Thompson Sound (Tukey test, \(P=0.015\)) but similar to values at Seymour Island (Turkey test, \(P=0.063\)). \(E_k\) values ranged from 2.2 - 12.7 \(\mu\text{mol photons m}^{-2} \text{s}^{-1}\) at Seymour Island, 3.6 - 19.6 \(\mu\text{mol photons m}^{-2} \text{s}^{-1}\) at Causet Cove and 1.6 - 9.4 \(\mu\text{mol photons m}^{-2} \text{s}^{-1}\) at Thompson Sound. Minimum and maximum \(E_k\) varied according to site.

Inhibition (\(\beta\)) of photosynthetic rates of *Ecklonia radiata* showed a significant temporal and spatial variation (\(P<0.001\) and 0.013, respectively; Fig. 4.6d). Inhibition was generally similar between the three sites from September 1998 and February 1999, with the exception of inhibition at Causet Cove in October 1998. At this time inhibition at Causet Cove was maximal and was significantly higher than at either Thompson Sound (Tukey test, \(P=0.026\)) or Seymour Island (Tukey test, \(P=0.021\)). Inhibition increased to a maximum at Thompson Sound and Seymour Island in April and June 1999, before declining. In June 1999, \(\beta\) at Causet Cove was significantly lower than values at Thompson Sound (Turkey test, \(P=0.013\)) or Seymour Island (Tukey test, \(P=0.011\)).

Spatially, \(P_{\text{max}}\) was generally similar between the three sites each month (Fig 4.6a-d). Differences were observed between sites in November 1998 when \(P_{\text{max}}\) at Seymour Island was significantly higher than \(P_{\text{max}}\) at Thompson Sound (\(P=0.001\)) and in August 1999 when \(P_{\text{max}}\) of *Ecklonia radiata* from Thompson Sound was significantly lower than \(P_{\text{max}}\) at Causet Cove (\(P=0.023\)) and Seymour Island (\(P=0.014\)). Alpha and \(E_k\) were similar between the three sites each month. Photoinhibition was also generally similar between the three sites each month except in October 1998 when *E. radiata* at Causet Cove had significantly higher inhibition than at either Seymour Island or Thompson Sound (\(P=0.015\) and 0.013). In June 1999 the opposite occurred with inhibition at Causet Cove being significantly lower than at either Seymour Island or Thompson Sound (\(P=0.031\) and 0.025).
Figure 4.6: Temporal and spatial variation in photosynthetic parameters on a dry weight basis (DW) basis for *Ecklonia radiata* in Doubtful Sound measured between September 1998 and March 2000. A) The maximum rate of photosynthesis ($P_{\text{max}}$), b) the initial slope ($\alpha_p$), c) light saturation parameter ($E_k$), and d) the photoinhibition parameter ($\beta$). $P_{\text{max}}$ and $\alpha_p$ were determined using the equation of Jassby and Platt (1976), $E_k$ was determined from the ratio $P_{\text{max}}/\alpha_p$ while $\beta$ was determined using the photoinhibition equation of Platt *et al.* (1980). * indicates a significant difference ($P=0.05$) between the three sites for the given month. Points represent mean ± 1 SD (n=5).
Chapter Four: Photosynthetic performance of Ecklonia radiata

(a) $P_{\text{max}}$ (mg O$_2$ gDW$^{-1}$ h$^{-1}$)

(b) Alpha

(c) $E_s$ (umol photons m$^{-2}$ s$^{-1}$)

(d) Inhibition

Time (months)

1998 1999 2000
4.3.3 $F_v/F_m$ of *Ecklonia radiata*

The ratio of variable to maximal chlorophyll fluorescence ($F_v/F_m$) of *Ecklonia radiata* from all three sites, measured *in situ* in October 1998, showed a significant decrease at the apex of the primary blade compared with values from the other four regions ($P<0.001$, Fig. 4.7). Generally, $F_v/F_m$ values for *E. radiata* from Seymour Island were significantly lower than values from the other two sites during the entire study period. The exception was in October and December 1998 when the $F_v/F_m$ values were similar between the three sites ($P=0.884$ and 0.730). $F_v/F_m$ values measured at the apical tip showed a similar pattern to basal values with $F_v/F_m$ at Seymour Island being significantly lower in all months except June 1999. At this time *E. radiata* at Seymour Island had significantly higher $F_v/F_m$ values than *E. radiata* from Causet Cove (Tukey test, $P=0.009$) or Thompson Sound (Tukey test, $P=0.011$). Apical $F_v/F_m$ values from the three sites were consistently lower than values measured at the blade/stipe junction throughout the experimental period (Fig. 4.8).
Figure 4.7: Variable to maximum chlorophyll fluorescence, $F_v/F_m$, along the blade of *Ecklonia radiata* from three sites in Doubtful Sound in October 1998. The initial blade length was measured and divided into five regions. From each region, $F_v/F_m$ was measured *in situ* using a pulse-amplitude-modulated fluorometer (Diving-PAM, Walz, Effeltrich, Germany). Bars represent mean ± 1 SD (n=5). Note y-axis begins at 0.5.
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Figure 4.8: *In situ* measurements of $F_v/F_m$ at a) the base of the primary blade and b) the apical tip of the primary blade for *Ecklonia radiata* from three sites in Doubtful Sound. Measurements were made using a Diving–PAM fluorometer from October 1998 until March 2000. * indicates significant differences between the three sites in the given month. Points represent mean ± 1 SD (n=6). Note y-axis begins at 0.5.
4.4 DISCUSSION

Subtidal macroalgae in Doubtful Sound are subject to rapidly changing PFD, ranging from 1-2 \( \mu \text{mol m}^{-2} \text{ s}^{-1} \) to 350 \( \mu \text{mol m}^{-2} \text{ s}^{-1} \) at approximately 10 m depth. This chapter examined the temporal photosynthetic performance of *Ecklonia radiata* from three sites within the sound, along a light gradient. The ability of *E. radiata* to capture and utilize low levels of PFD was examined using photosynthetic parameters \( (\text{P}_{\text{max}}, \alpha_p \text{ and } E_k) \) obtained from PE curves. The content of the three main photosynthetic pigments was determined to examine how *E. radiata* responds to the fluctuating PFD environment created by the LSL. Chlorophyll fluorescence was used as a proxy to investigate the ‘health’ (Kromkamp and Forster, 2003) of *E. radiata in situ*. The photosynthetic capacity of *E. radiata*, as determined from \( \text{P}_{\text{max}}, \alpha_p \text{ and } E_k \), is compared to other literature focusing on laminarian species, however, occasionally other macroalgal examples are considered.

Pigment content and photosynthetic rates can be normalised using various denominators, such as dry weight (DW), wet or fresh weight (WW, FW), surface area (SA), surface area to volume (SA/V) and chl \( \alpha \), with varying results (Ramus, 1981; Lobban and Harrison, 1997). Normalization parameters also vary with environmental parameters and between species (Ramus, 1981). In the present study, pigment contents and photosynthetic parameters of *Ecklonia radiata* were normalized to DW. Data was normalized to DW primarily as the main objective of the study was to investigate the growth and productivity of *E. radiata*. The DW of brown macroalgal tissue may include C storage products, such as laminaran and mannitol, which contribute to growth by providing energy or C for biosynthesis of structural components (Lobban and Harrison, 1997). However, in relation to photosynthesis and pigment content expressing data on a SA basis may be more appropriate considering that light, as well as nutrients, are absorbed across the entire surface of the thallus. However, the thickness of the thallus, which may change over time, was not taken into account. Thallus thickness has been shown to influence pigment content in particular (Lüning, 1981). In the present study, only the
temporal pattern of pigment contents changed according to what basis they were expressed.

4.4.1 Pigment contents of *Ecklonia radiata*

Variation in pigment content in macroalgae is often attributed to changes in light over time, be it on a scale of minutes, hours, days or months. High pigment content generally reflects low light availability while a decline in pigment content reflects saturating light and often increased growth (Brinkhuis, 1977; Ramus, 1981; Rosenberg and Ramus, 1982; Lewey and Gorham, 1984; Henley and Ramus, 1989a and b; Gao, 1990; Fillit, 1995). This was not fully supported by temporal pigment data expressed on a DW basis, particularly chl a and c contents, for *Ecklonia radiata* from the present study. Although chl a and c contents were low between September and December 1998, both increased between August and December 1999, corresponding with increasing light and growth rates. The lower pigment content during 1998 coincided with low NO$_3^-$ uptake rates, low C:N ratios and high growth rates, while the higher pigment content for the same period in 1999 coincided with a peak in ambient seawater NO$_3^-$ concentration and an increase in PFD and growth rates. This temporal pattern is similar to the annual *Undaria pinnatifida* from south-eastern Australia, but contrasts with *Macrocystis integrifolia* and *Nereocystis luetkeana* which exhibit the opposite trend (Smith *et al.*, 1983; Wheeler *et al.*, 1984; Campbell *et al.*, 1999b).

Spatially, *Ecklonia radiata* from Seymour Island had higher pigment contents than sporophytes from Causet Cove and Thompson Sound, particularly during spring and summer. This may be the result of a combination of environmental factors; a thicker LSL, a unidirectional current, a greater amount of terrestrial material in the water column absorbing light and shadowing by both the mountainous terrain and the overhanging terrestrial growth (*pers. obs*) at the inner site which reduces light at depth. By increasing its pigment content, *E. radiata* at Seymour Island could capture a greater number of photons and maintain a similar photosynthetic rate to those sporophytes at the two outer sites. Further, the lower pigment contents of sporophytes from the two outer sites may reflect diversion of N into growth rather than pigment synthesis as these sporophytes had
significantly thicker thalli than those at Seymour Island. Spatial differences in pigment content, and photosynthetic rates, are investigated further in Chapter 5.

The range of chl \( a \) values for *Ecklonia radiata* from Doubtful Sound were higher than values reported for *Phyllariopsis purpurascens* but lower than ranges for *Undaria pinnatifida* and the fucoid, *Sargassum muticum* (Lewey and Gorham, 1984; Flores-Moya et al., 1995; Campbell et al., 1999b). The high ranges for *U. pinnatifida* and *S. muticum* can be attributed to the fact that both are fast growing macroalgae with a thin or branching thallus as opposed to *E. radiata* which is relatively slow growing and typically has a thick thallus. The relatively low chlorophyll content for *E. radiata* from the three sites may be attributed to the low ambient seawater N concentration observed in Doubtful Sound during this study, as there was strong evidence to suggest that *E. radiata* in Doubtful Sound was N-limited year-round. N-deficiency in macroalgae often results in a decline in nitrogenous photosynthetic pigments, such as chlorophylls, as well as a change in the ratio of chl \( a \) to accessory pigments (Turpin, 1991 and references therein). An increase in ambient N concentration has been shown to increase chl \( a \) and \( c \) content in brown macroalgae (e.g. Chapman et al., 1978; Campbell et al., 1999b). It is possible that available N was immediately utilized by *E. radiata* in Doubtful Sound to sustain growth rather than being stored or used to synthesize new pigment compounds.

Additionally, lower pigment contents may reflect the dilution of pigments via increased biomass (Lewey and Gorham, 1984; Rodrigues et al., 2000). This contention is partially supported by data from the present study. *Ecklonia radiata* had higher blade growth rates between September and December 1998 at all three sites (chapter 2) that coincided with low pigment contents. However, the opposite pattern emerged during the same period the following year; growth rates were lower and pigment contents increased. Increasing pigment contents during periods of low growth would allow *E. radiata* to capture and utilize greater amounts of PFD thereby fixing more C for synthesis into biomass.

### 4.4.2 A comparison of photosynthetic parameters

In this section of the discussion, each photosynthetic parameter as determined from a PE curve will be addressed separately in relation to the literature before the overall
photosynthetic performance of *Ecklonia radiata* is discussed. However, comparison of photosynthetic parameters between studies is often difficult as many researchers give measurements of photosynthetic parameters normalized to DW only and do not provide DW/SA ratios for conversion.

**4.4.2.1 \( P_{\text{max}} \)**

The range of \( P_{\text{max}} \) values for *Ecklonia radiata* from each site in Doubtful Sound were similar to values reported for *Laminaria digitata* and *L. saccharina*, while the upper limits of \( P_{\text{max}} \) for *E. radiata* fell within the range reported for perennial macroalgae (Table 4.1). \( P_{\text{max}} \) for *Undaria pinnatifida*, an annual, was up to 84 times higher than \( P_{\text{max}} \) for *E. radiata* and may be attributed to its thin thallus and high growth rates (Campbell *et al.*, 1999b). Further, King and Schramm (1976a) demonstrated that the average photosynthetic rate of perennial macroalgae (4.4 mgO₂ gDW⁻¹ h⁻¹) was significantly lower than for annuals (25.3 mgO₂ gDW⁻¹ h⁻¹). \( P_{\text{max}} \) of *E. radiata* displayed a temporal pattern being maximal between June and August and minimal between December and February. This temporal pattern was similar to that reported for *Macrocystis integrifolia* but contrasted with patterns reported for *Laminaria setchelli*, *M. pyrifera* and *Nereocystis luetkeana* (Smith *et al.*, 1983; Wheeler *et al.*, 1984; Cabello-Pasini and Alberte, 1997).

Temporal variations in \( P_{\text{max}} \) depend on environmental parameters, such as transmission of light through the water column and ambient seawater N concentration therefore, macroalgae from different locations may exhibit different temporal patterns in \( P_{\text{max}} \).

In several species of macroalgae, \( P_{\text{max}} \) increases during the growing period with a complementary increase in pigment content (e.g. Drew, 1983a and b; Smith *et al.*, 1983; Wheeler *et al.*, 1984; Gutkowski and Maleszewski, 1989). In contrast, the increase in \( P_{\text{max}} \) exhibited by *Ecklonia radiata* coincided with a period of low growth (chapter 2) and low pigment contents. This is similar to the pattern observed in the fucalean macroalga, *Sargassum muticum* (Lewey and Gorham, 1984). During periods of high \( P_{\text{max}} \) and low growth rates, *E. radiata* may be partitioning energy from the photosynthetic process to other metabolic process such as nutrient uptake and assimilation.
Table 4.1: Comparison of PE parameters for members of the Heterokontophyta. 

\[ P_{\text{max}} = \text{mgO}_2 \text{ gDW}^{-1} \text{ h}^{-1} \], \[ \alpha_P = \text{mgO}_2 \text{ gDW}^{-1} \text{ h}^{-1} (\mu\text{mol photons m}^{-2} \text{ s}^{-1})^{-1} \], \[ E_k = (\mu\text{mol photons m}^{-2} \text{ s}^{-1}) \]. Site abbreviations as follows: SI = Seymour Island, CC = Causet Cove, TS = outer Thompson Sound.

<table>
<thead>
<tr>
<th>Species</th>
<th>( P_{\text{max}} )</th>
<th>( \alpha_P )</th>
<th>( E_k )</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascoseira mirabilis</td>
<td>20 - 60</td>
<td></td>
<td></td>
<td>Gómez et al. 1995</td>
</tr>
<tr>
<td>Ecklonia cava</td>
<td>185</td>
<td></td>
<td></td>
<td>Maegawa et al. 1987(^a)</td>
</tr>
<tr>
<td><strong>Ecklonia radiata</strong> – SI</td>
<td>0.7 – 4.2</td>
<td>0.09 – 0.7</td>
<td>6.54 – 26.60</td>
<td>This study</td>
</tr>
<tr>
<td>– CC</td>
<td>0.5 – 3.9</td>
<td>0.03 – 1.1</td>
<td>8.03 – 46.69</td>
<td>“ “</td>
</tr>
<tr>
<td>– TS</td>
<td>0.7 – 4.2</td>
<td>0.1 – 0.6</td>
<td>7.26 – 49.23</td>
<td>“ “</td>
</tr>
<tr>
<td>Eisenia bicylis</td>
<td>185</td>
<td></td>
<td></td>
<td>Maegawa et al. 1987(^a)</td>
</tr>
<tr>
<td>Laminaria abyssalis</td>
<td>70</td>
<td></td>
<td></td>
<td>Rodrigues et al. 2000</td>
</tr>
<tr>
<td>L. digitata</td>
<td>1.4 – 4</td>
<td></td>
<td></td>
<td>King and Schramm, 1976(b)</td>
</tr>
<tr>
<td>L. saccharina</td>
<td>2</td>
<td></td>
<td></td>
<td>King and Schramm, 1976(a)</td>
</tr>
<tr>
<td>L. solidungula(^b)^(^c)</td>
<td>0.31</td>
<td></td>
<td></td>
<td>Henley and Dunton 1997</td>
</tr>
<tr>
<td><strong>Macrocytis pyrifera</strong></td>
<td>22 - 64</td>
<td></td>
<td></td>
<td>Chin 1989</td>
</tr>
<tr>
<td>Nereocystis luetkeana</td>
<td>4.1</td>
<td></td>
<td></td>
<td>Wheeler et al. 1984</td>
</tr>
<tr>
<td>Pelvetia canaliculata</td>
<td>2</td>
<td></td>
<td></td>
<td>Pfetzing et al. 2000</td>
</tr>
<tr>
<td>Phycopods rubens</td>
<td>1.4 – 4.3</td>
<td></td>
<td></td>
<td>King and Schramm, 1976(a)</td>
</tr>
<tr>
<td>Phyllariopsis purpurascens</td>
<td>12 - 53.7</td>
<td></td>
<td></td>
<td>Flores-Moya et al. 1995</td>
</tr>
<tr>
<td>Phyllophora brodiaei</td>
<td>1.1 – 2</td>
<td></td>
<td></td>
<td>King and Schramm 1976(a)</td>
</tr>
<tr>
<td><em>Polysiphonia nigrescens</em></td>
<td>2.5 – 5.1</td>
<td></td>
<td></td>
<td>Campbell et al. 1999(b)</td>
</tr>
<tr>
<td>Sargassum muticum(^d)</td>
<td>15 - 42</td>
<td>0.15 – 0.3</td>
<td></td>
<td>Campbell et al. 1999(b)</td>
</tr>
<tr>
<td>Undaria pinnatifida</td>
<td>0 - 42</td>
<td></td>
<td></td>
<td>Oh and Koh 1996</td>
</tr>
<tr>
<td></td>
<td>19 – 21.3</td>
<td></td>
<td></td>
<td>Matsuyama 1983</td>
</tr>
<tr>
<td></td>
<td>12.5 – 20.5</td>
<td></td>
<td></td>
<td>Wu et al. 1984</td>
</tr>
<tr>
<td>Annuals(^e)</td>
<td>14 – 47.7</td>
<td></td>
<td></td>
<td>King and Schramm 1976(a)</td>
</tr>
<tr>
<td>Perennials(^f)</td>
<td>2 – 8.8</td>
<td></td>
<td></td>
<td>Buesa 1990</td>
</tr>
<tr>
<td>Cuban browns</td>
<td></td>
<td></td>
<td></td>
<td>Cheshire et al. 1996</td>
</tr>
<tr>
<td>South Australian macroalgal community</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Measurements for PE curves carried out at 20 °C; \(^b\) values estimated from graphs; \(^c\) estimates using old blades in treatment with 25 \(\mu\)mol photons m\(^{-2}\) s\(^{-1}\) and no N; \(^d\) experiments carried out at 10 °C and 300 \(\mu\)mol photons m\(^{-2}\) s\(^{-1}\); \(^e\) annuals included reds; \(^f\) brown algae excluding *Fucus* species.
Interestingly, the greatest $P_{\text{max}}$ for *Ecklonia radiata* from Causet Cove occurred two months after the other two study sites. This may reflect habitat differences, particularly aspect, which influences incident PFD. Causet Cove was situated on the south side of the fiord, the site at Thompson Sound was situated on the northern side while the site at Seymour Island was situated on the west side of the island. The south side of Doubtful Sound receives up to 95% more incident PFD on sunny days at the water surface than the north side, although the difference was reduced to 10% on rainy and/or cloudy days (Kregting, 2001). A low $P_{\text{max}}$ combined with fiord aspect, the LSL and variation in PFD levels in Doubtful Sound suggests that *E. radiata* acclimates to the fluctuating PFD at the three sites via changes in the number of photosynthetic units (PSU) (e.g. Ramus, 1981; Kirst and Wiencke, 1995). While incident PFD was measured at the sites at the time of collection, further research, both on a temporal and spatial scale, is needed to elucidate the influence of the fiord aspect and LSL on PAR at depth.

It was expected that *Ecklonia radiata* from Seymour Island would have a lower $P_{\text{max}}$ than *E. radiata* at the other two sites. However, in general $P_{\text{max}}$ was similar between the three study sites, regardless of changes in light and absorption of different wavelengths at each site (refer Chapter 2). This pattern was also observed for the other photosynthetic parameters measured ($\alpha_p$, $E_k$ and $\beta$). *E. radiata* may have maintained similar $P_{\text{max}}$ values at the three sites through morphological changes (Chapter 2). *E. radiata* at Seymour Island had thinner and wider primary blades than those at Causet Cove or Thompson Sound allowing a greater surface area for light capture. Furthermore, had the experiments been conducted immediately following collection of the sporophytes, greater variation in photosynthetic parameters from the three sites may have been evident.

### 4.4.2.2 Alpha ($\alpha_p$)

It was expected that *Ecklonia radiata* from the inner site, Seymour Island, would have higher $\alpha_p$ values than *E. radiata* at the two outer sites due to the presence of a deeper LSL, and therefore lower PFD levels. Contrary to this expectation, it was *E. radiata* from the two outer sites that exhibited higher $\alpha_p$ values between June and August 1999 although only $\alpha_p$ values for *E. radiata* from Causet Cove were significantly higher. This
suggests that *E. radiata* at the two outer sites were more efficient at utilizing low PFD than *E. radiata* at the inner site. *E. radiata* at the inner site had a thinner blade than *E. radiata* at the outer sites (chapter 2), which may result in greater transmission of PFD through the thallus to underlying pigments. *E. radiata* at Seymour Island may be better acclimated to low PFD than *E. radiata* at the outer sites as a result of longer periods at low PFD over time due to the thicker LSL. Acclimation to low PFD is considered in chapter 5, which investigates photoacclimation of five populations of *E. radiata* from Doubtful Sound.

Higher $\alpha_p$ values indicate greater photosynthetic efficiency at low PFD (Cheshire *et al.*, 1996). The range of $\alpha_p$ values for *Ecklonia radiata* were higher than reported ranges for other heterokontophytes (Table 4.1). Regardless whether expressed on a DW or SA basis, the highest $\alpha_p$ values for *E. radiata* occurred between June and August 1999, during seasonally low PFD (chapter 2). High $\alpha_p$ of *E. radiata* during this period demonstrates an acclimation to a low light environment similar to many species of polar algae (Chapman and Lindley, 1980; Dunton, 1985; Dunton and Jodwalis, 1988; Wiencke, 1990; Thomas and Wiencke, 1991; Drew and Hastings, 1992; Wiencke *et al.*, 1993).

### 4.4.2.3 $E_k$

The light saturation parameter, $E_k$, for *Ecklonia radiata* from Seymour Island was half that of *E. radiata* from Causet Cove or Thompson Sound, indicating that *E. radiata* at Seymour Island exhibits greater acclimation to low PFD than *E. radiata* at Causet Cove and Thompson Sound. While the range of $E_k$ for *E. radiata* in Doubtful Sound was similar to those reported for other laminarians, such as *Laminaria saccharina* and *Macrocystis integrifolia*, values were towards the lower end of the range (Table 4.1, Smith *et al.*, 1983; Chin, 1989; Clendennen *et al.*, 1996; Machalek *et al.*, 1996; Dean, 1998). As for $\alpha_p$, $E_k$ of *E. radiata* in Doubtful Sound was, however, similar to brown macroalgae of the Antarctic and Arctic. For example, Arctic *Laminaria* had $E_k$ values ranging from 18.6 to 52.6 $\mu$mol photons m$^{-2}$ s$^{-1}$ while $E_k$ of *L. solidungula* ranged from 19.7 to 42.3 $\mu$mol photons m$^{-2}$ s$^{-1}$ (Dunton and Jodwalis, 1988; Wiencke *et al.*, 1993). These low $E_k$ values are a reflection of the environment where sea ice up to 1 m thick,
with additional snow cover, persist for variable periods during winter (Drew, 1977). The low $E_k$ values for *E. radiata* in Doubtful Sound are consistent with macroalgae living in a low-light environment.

*Ecklonia radiata* from all three sites exhibited no temporal variation in $E_k$ values. This contrasts with other studies where $E_k$ values were generally lower during winter months (e.g. King and Schramm, 1976; Smith *et al.*, 1983; Wheeler *et al.*, 1984; Cheshire *et al.*, 1996), correlating with low incident irradiance. Lower $E_k$ values indicate a degree of photoacclimation to lower light environments (Cheshire *et al.*, 1996) giving macroalgae a greater ability to utilize low PFD resulting from shorter daylengths during winter months (Campbell *et al.*, 1999b). The low temporal $E_k$ values for *E. radiata* are possibly a result of the year-round LSL and provide further evidence that *E. radiata* in Doubtful Sound has characteristics of a shade-adapted macroalga.

### 4.4.2.4 Photoinhibition ($\beta$)

Many studies which investigate the photosynthetic properties of marine algae (including phytoplankton) often restrict the range of PFD levels used to intensities below the threshold of photoinhibition, concentrating on the photosynthetic rate at low PFD (e.g. Jassby and Platt, 1976; Henley, 1993). However, photoinhibition at higher PFD levels has been shown to occur for a number of macroalgae independent of species or habitat. *Ecklonia radiata* at all three sites in Doubtful Sound exhibited photoinhibition throughout the entire experimental period, typically at PFD levels $> 100$ μmol photons m$^{-2}$ s$^{-1}$. Measurements of PFD at depth at each of the three study sites showed that *E. radiata* was subjected to irradiances $> 100$ μmol photons m$^{-2}$ s$^{-1}$ on a number of occasions throughout the experimental period, therefore photosynthesis is likely to become naturally inhibited on a regular basis.

Photoinhibition of *Ecklonia radiata* from Doubtful Sound during the laboratory experiments was expected as the range of PFD used in the experiments often exceeded ambient PFD levels at depth. Macroalgae that inhabit deeper waters have been shown to be more susceptible to photoinhibition at a lower PFD than intertidal macroalgae (e.g. Rodrigues *et al.*, 2000). For example, *Laminaria abyssalis* showed photoinhibition at a
PFD of 50 μmol photons m⁻² s⁻¹ whereas the intertidal alga, *L. digitata*, showed no inhibition at 1000 μmol photons m⁻² s⁻¹. *L. abyssalis* also had lower pigment concentrations than *L. digitata* that may have accounted for photoinhibition. Similarly, *E. radiata* also had low pigment concentrations so that when PFD levels increased above saturation, excess energy in the form of excited electrons may not have been properly dissipated. The excess energy can lead to photodamage of PS II explaining the observed photoinhibition (Anderson and Osmond, 1987). Photoinhibition may therefore be used as a protective mechanism by macroalgae to protect cells from damage by high levels of PFD (Bruhn and Gerard, 1996). Further research is required however, to interpret photoinhibition in *E. radiata*.

4.4.3 Variable to maximal chlorophyll fluorescence of *Ecklonia radiata*

The $F_v / F_m$ ratio of mature thalli of brown algae range between 0.7 and 0.8 (Büchel and Wilhelm, 1993). *Ecklonia radiata* $F_v / F_m$ values ranged between 0.64 – 0.76 at the blade base and 0.53-0.68 at the apical tip. These values were similar to yields reported for other brown algae (Bruce *et al.*, 1983; Popovic *et al.*, 1983; Huppertz *et al.*, 1990). For example, mature sporophytes of *Laminaria* species yielded values of 0.75 while young sporophytes and gametophytes of the same species had lower values, 0.50-0.65, which were similar to values recorded at the apical tip of *E. radiata* in this study (Dring *et al.*, 1996). Lower $F_v / F_m$ ratios at the apical tip compared to values at the blade base of *E. radiata* were most likely due to tissue senescence. Cheshire *et al.* (1996) noted that *E. radiata* at West Island, South Australia, had a constant loss of tissue from necrotic tips. Many *E. radiata* in Doubtful Sound were bleached and/or eroded at the apical tip, which is also older tissue, and may account for the lower $F_v / F_m$ values. Additionally, as $F_v / F_m$ measurements were made at different times of the day at each site diurnal variation in irradiance is likely to influence the values obtained although tissue sections used for the measurements were dark adapted first and were used only as a ‘proxy’ for algal health.

As expected, *E. radiata* at the two outer sites typically had higher $F_v / F_m$ than sporophytes at Seymour Island at both the apical tip and blade base. When measured in the dark (as done in this study) $F_v / F_m$ is a measure of the potential electron transfer quantum yield of PSII (Beer *et al.* 2000). Therefore, the lower $F_v / F_m$ observed for
sporophytes from Seymour Island may indicate a lower photosynthetic efficiency in PSII system in comparison to *E. radiata* from Causet Cove and Thompson Sound. However, similar \( \alpha_p \) values between sporophytes from the three sites do not support this contention. Differences in \( F_v / F_m \) are most likely a result of morphological variations (i.e. thinner thalli and longer stipes at Seymour Island) between *E. radiata* at the inner and two outer sites. While *E. radiata* at Seymour Island had higher pigment contents, and could maintain a similar \( P_{\text{max}} \) to *E. radiata* at the two outer sites, the increased capacity to capture light combined with a lower sporophyte density (and therefore less self-shading) may have caused greater photoinhibition resulting in a lower \( F_v / F_m \). Overall however, the \( F_v / F_m \) ratios for *E. radiata* from Seymour Island, whilst lower than for sporophytes from Causet Cove or Thompson Sound, were similar to ratios reported for various other brown algae (Bruce *et al.*, 1983; Popovic *et al.*, 1983; Huppertz *et al.*, 1990).

### 4.4.4 Overall photosynthetic performance of *Ecklonia radiata*

Against expectation, \( P_{\text{max}} \), \( \alpha_p \), \( E_k \) and \( \beta \) were generally similar between the three sites throughout the study period. Comparison of spatial differences in photosynthetic parameters with other *Ecklonia* or laminarian species, from other places is difficult as most literature only investigates temporal differences hence only use species from one site. Similar photosynthetic parameters between the three sites could be the result of differences in thallus morphology. For example, *E. radiata* from Seymour Island typically had a thinner thallus (Chapter 2), which would lead to less self-shading and a greater chance to capture light via all photosynthetic cells. Changes in pigment content, such as higher chl \( a \) and \( c \) content in *E. radiata* at Seymour Island during spring and summer months, may also have helped maintain similar photosynthetic rates between *E. radiata* at the three sites. *E. radiata* at Seymour Island also had a higher fucoxanthin content during certain months of the study. The absorption spectra of fucoxanthin is extended when bound into pigment protein complexes so although light composition at Seymour Island may differ from the two outer sites, *E. radiata* is effectively able to use additional wavelengths to maintain its photosynthetic rate (Anderson and Barrett 1979). Additionally, sporophyte density will also partially determine the amount of light reaching the thallus and hence influence photosynthetic rates. The density of *E. radiata*
at Seymour Island was significantly lower than at the two outer sites year-round (Chapter 2) therefore, although light levels may generally be lower at Seymour Island more photosynthetic area is available to capture light. At Causet Cove and Thompson Sound, the higher density combined with surging water motion may produce self-shading of sporophytes below the canopy plants. Furthermore, as sporophytes were kept in an outdoor tank under ambient light conditions (that probably differed from those in Doubtful Sound) after collection for up to three days before experiments were performed, *E. radiata* from the three sites may have acclimated accounting for the similar photosynthetic parameters. Shade cloth was used to cut ambient light levels to those measured in Doubtful Sound. Future research into photosynthetic rates of *E. radiata* would benefit from the use of PAM fluorometer to measure electron transport rates (ETR) in situ, as well as running PI experiments as soon as possible after collection of sporophytes from the field.

The photosynthetic performance of *Ecklonia radiata* from Doubtful Sound exhibits many of the photosynthetic characteristics of a shade-adapted alga, such as low $P_{\text{max}}$ and $E_k$ values and high $\alpha$ values. All of these characteristics combined enhance the capacity of *E. radiata* to efficiently utilize the low PFD levels that prevail at depth in Doubtful Sound. The one exception to the list of shade-adapted features was that *E. radiata* had relatively low photosynthetic pigment concentrations during periods of low ambient PFD (i.e. winter). However, this is not uncommon and has been recorded for other macroalgae (e.g. Dunton and Jodwalis, 1988; Hsiao, 1990; Kirst and Wiencke, 1995; Machalek et al., 1996).

Many kelps, such as *Ecklonia radiata*, are constructed of thick, complex tissue which reduces PFD absorption as well as the quantity of photosynthetic units (PSU) per unit biomass compared to macroalgae with thin thalli (Osborne and Raven, 1986; Lüning, 1990; Ramus, 1990; Krause-Jensen and Sand-Jensen, 1998). $P_{\text{max}}$ and $E_k$ values of *E. radiata* suggest saturation at PFD levels < 50 \(\mu\text{mol} \text{ photons m}^{-2} \text{ s}^{-1}\), similar to polar macroalgae. This is consistent with an inverse relationship found between photosynthetic activity and thallus complexity and is further investigated in chapter 5 (Khailov et al.,...
Chapter Four: Photosynthetic performance of *Ecklonia radiata*  


Macroalgae often respond to changes in PFD through morphological modifications such as an increase in surface area to maximize photosynthetic potential, either through enhanced light-capturing abilities, gas exchange or nutrient absorption (Riechert and Dawes, 1986; Reiskind *et al.*, 1989). While there were no significant differences in the overall size of the primary blades of *Ecklonia radiata* between the study sites, *E. radiata* at Seymour Island had significantly thinner blades than *E. radiata* at the two outer sites. A thinner blade would therefore allow greater PFD transmission through the blade (photosynthetic surface) increasing the amount of energy received by the photo-systems. This may explain why $P_{\text{max}}$ values of *E. radiata* between the three study sites were similar.

### 4.4.5 Limitations of the PE experiments and fitting equations to data

The advantages and disadvantages of mathematical formulae used to determine photosynthetic parameters in macroalgae have been reviewed elsewhere (e.g. Jassby and Platt, 1976; McBride, 1992; Henley, 1993). Most researchers recommend the exponential equation of Jassby and Platt (1976) for determining $P_{\text{max}}$, $\alpha_P$ and the subsequent $E_K$ parameters. However, this equation does not include a term for photo-inhibition ($\beta$), which occurred for *Ecklonia radiata* throughout this study. Although the photo-inhibition equation can be used to determine $P_{\text{max}}$, $\alpha_P$ and $E_K$, it typically overestimates $P_{\text{max}}$ and $E_K$ values, therefore $P_{\text{max}}$, $\alpha_P$ and $E_K$ were determined using a tangential hyperbola (Jassby and Platt, 1976) in the present study.

Estimates of the maximum photosynthetic rate ($P_{\text{max}}$) and the initial slope of the PE curve ($\alpha_P$) may vary between seaweed species due to discrepancies in the methods and models used to obtain the PE parameters. For example, $P_{\text{max}}$ has been calculated using the highest two or three photosynthetic points (e.g. Wheeler, 1980b) or using one of the reported equations such as the tangential hyperbola or a non-linear curve fit with Marquardt-Levenberg algorithm (e.g. Henley and Dunton, 1997). Similarly, $\alpha_P$ may be calculated by fitting a regression through points under a certain PFD level or by using
one of the reported mathematical formulae. Comparison with much of the literature is therefore difficult and has been limited to studies using a form of the tangential hyperbola.

Although a small wet 'laboratory' was available on site in Doubtful Sound, there were no facilities to undertake experiments immediately following collection of *Ecklonia radiata*. There was often a delay in beginning photosynthetic experiments of up to 40 to 50 hours. This may have caused degradation of cells and/or cellular leakage that may have resulted in a decline in the photosynthetic activity. This may also account for the low pigment contents recorded. PAM fluorometry (Walz, 2000) was used to measure the 'health' of the seaweed once transported back to the laboratory in Dunedin and an Fv/Fm of 0.65 and 0.75 indicated that the macroalgae were healthy following transportation. However, PAM fluorometry (Walz, 2000) was not used to measure the 'health' after discs had been cut from the thallus and pre-treated, and a wounding affect may have influenced respiration rates (Hatcher, 1977; Kain, 1979) contributing to an overall lower photosynthetic activity.

Photosynthetic parameters determined in the laboratory under constant conditions may not accurately describe photosynthesis under natural conditions of changing irradiance and nutrient levels (Gerard, 1984a and b; Dawes, 1995). There are limitations with investigating photosynthetic rates of large macroalgae in the laboratory as there is always the question of how closely a 'static' bottle experiment represents natural populations (Smith *et al*., 1983). One of the consequences of enclosing a piece of seaweed in a small volume of water is the downturn in photosynthetic rate and subsequent build-up of CO₂. Koch (1993) demonstrated the importance of stirring the medium to ensure optimal diffusion of CO₂ across the thallus and to prevent photosynthesis being limited by CO₂ availability. Levavasseur *et al*. (1991) provided evidence for the occurrence of photosynthetic limitation for phytoplankton when inorganic C concentration in the medium was low. In the present study, the shaker tables used for experiments were set at 135 rpm, which was the highest speed that could be used without tipping the BOD bottles over. However, this speed may not have been fast enough to adequately reduce the DBL
around the entire seaweed disc which often sank to the bottom of the bottle and appeared to stick, resulting in lower photosynthetic rates than would occur in situ.

Many aspects of the photosynthetic process, such as enzymes used for catalysing reactions, are affected by temperature (Davison, 1991). Temperature is often 'controlled' in laboratory experiments at an average level that reflects in situ measurements. While the air temperature inside the incubator used in these experiments was maintained at 12 °C, occasionally the water temperature in the BOD bottles had risen by up to 2 °C during the incubation period. However, according to Lüning and Dring (1985) the rate of light-saturated photosynthesis is independent of temperature, therefore the variation in temperature should have little effect on $P_{\text{max}}$. Ecophysiological experiments typically only measure changes related to one factor, such as PFD, while all other factors (e.g. temperature) are kept constant. In situ, however, temperature influences photosynthetic and nutrient uptake rates (e.g. Madson and Maberly, 1990).

4.4.6 Summary
This is the first study to investigate the photosynthetic performance of *Ecklonia radiata* in Doubtful Sound. On a dry weight basis, chl $a$ and $c$ contents of *E. radiata* displayed a temporal pattern with maximum contents in November and December 1999. Fucoxanthin content contributed ≤ 25 % of the total pigment content. $P_{\text{max}}$ was maximal during the winter months of June and August 1999 and coincided with a period of low growth and low pigment contents. Against expectation, *E. radiata* from Causet Cove and Thompson Sound exhibited higher $\alpha_p$ values between June and August 1999 than *E. radiata* from Seymour Island. $E_k$ values for *Ecklonia radiata* from Seymour Island indicate greater acclimation to low PFD at either Causet Cove or Thompson Sound. Photoinhibition occurred in *E. radiata* from all three study sites at PFD levels > 100 $\mu$mol photons m$^{-2}$ s$^{-1}$. $F_v/F_m$ values indicated that *E. radiata* was healthy except for the apical tips, which displayed signs of senescence, bleaching and grazing.

These findings present strong evidence to suggest that *Ecklonia radiata* in Doubtful Sound is a shade-adapted macroalga. Findings in this study indicate that temporal
changes in photosynthetic parameters for *E. radiata* reflected not only periods of increased growth but also changes in ambient PFD and seawater N concentration. The interpretation of PE curves, however, should take into account possible preceding events such as changes in PFD and nutrient levels (Dawes, 1995). Additional studies, on the photosynthetic enzymes and electron transfer interactions in PSUs as well as a comprehensive study at the underwater light regime present in Doubtful Sound, should give a better understanding of how these parameters influence the photosynthetic performance of *E. radiata*. 
Chapter Five
Photo-acclimation of *Ecklonia radiata* in Doubtful Sound

5.1 INTRODUCTION

At the onset of this study, *Ecklonia radiata* in the Doubtful Sound complex (hereafter referred to as Doubtful Sound) had not been recorded past Seymour Island. This was considered to be due to the LSL and subsequent reduction in PFD penetration. However, during a survey of the subtidal macroalgae community in Doubtful Sound conducted in October 2000, two new populations of *E. radiata* were discovered: one in Bradshaw Sound and the other situated at Elizabeth Island (pers. obs). The density of both populations of *E. radiata* was sparse, similar to the density at Seymour Island. The LSL reached a depth of 3 - 5 m in Bradshaw Sound and ≥ 6 m at Elizabeth Island, raising the question of how these two *E. radiata* populations were capable of survival at (the assumed) continual low PFD.

When a photosynthetic organism is placed for a long period in a given PFD regime it acclimates to that regime within the limits of its genetic potential and environmental constraints (Falkowski and Raven, 1997). Photo-acclimation occurs on a number of levels, including optical, biochemical, ultrastructural and physiological, all of which help optimize light harvesting and utilization (Dubinsky *et al.*, 1995; Falkowski and LaRoche, 1991). Physiological acclimation to PFD in macroalgae may involve changes in pigment content, photosynthetic efficiency and the size and number of the photosynthetic unit (PSU; Kübler and Davison, 1995; Fisher *et al.*, 1998). For example, macroalgal species typically increase photosynthetic pigment contents as PFD decreases (e.g. Ramus *et al*,...

Synthesis of photosynthetic pigments involved in the photosynthetic process, particularly chlorophyll, requires N (Chopin et al., 1995). N uptake rates, and therefore subsequent pigment synthesis, are a function of both N availability in seawater and the surface area of the macroalga (Nielsen and Sand-Jensen, 1990; Hein et al., 1995; Krause-Jensen and Sand-Jensen, 1998). For example, both chl a content and photosynthetic capacity of Laminaria saccharina increases with increasing external NO₃⁻ content (Chapman et al., 1978). Similarly, photosynthetic pigment levels of Nereocystis luetkeana showed a direct correlation with ambient NO₃⁻ content (Wheeler et al., 1984). A reduction in N availability and pigment synthesis may therefore result in a decrease in photosynthesis, ultimately reducing growth rates.

5.1.1 Objective of chapter 5

It has been shown that Ecklonia radiata in the Doubtful Sound complex was probably N-limited year-round (chapter 3) and subject to low PFD due to the LSL (chapter 2). One of the significant differences between E. radiata from the three study sites was a thinner blade at the inner site, Seymour Island. The observation that E. radiata was present at two sites further into the fiord, where the LSL was thicker (up to 15 m), suggested that inner populations had acclimated to reduced PFD levels. It was hypothesized that E. radiata would show a different photo-acclimation response towards the inner sound compared to sites at the outer sound. Photosynthetic pigment contents of E. radiata were expected to increase towards the inner sound thereby increasing the capture and transfer of PFD at low levels. It was expected that morphological characteristics, such as blade length and width, of E. radiata would also increase towards the inner site resulting in an increased photosynthetic area to capture greater amounts of PFD at low levels (Fig. 5.1). The primary blade thickness was expected to decrease to minimize internal self-shading. The content of the photosynthetic pigments (on a dry weight basis), particularly fucoxanthin, were expected to increase towards the inner site to maximize PFD capture.
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and transfer. *E. radiata* at the innermost site was expected to have a higher $P_{\text{max}}$ and $\alpha_P$ but lower $E_k$ and $E_c$ than *E. radiata* at the outer sites.
Figure 5.1: Representative *Ecklonia radiata* sporophytes from a) Causet Cove, an outer site, and b) Elizabeth Island, the innermost site that *E. radiata* was found. The ruler in a) is 1 m in length and in b) 40 cm.
5.2 MATERIALS AND METHODS

5.2.1 Collection of *Ecklonia radiata*

Five *Ecklonia radiata* sporophytes were collected from 10 m depth from each of five sites: Causet Cove, Thompson Sound, Seymour Island, Bradshaw Sound and Elizabeth Island (Fig. 5.2). Collection was carried out mid to late afternoon on the 28th November 2000. Collection and preparation methods were the same as used previously (refer to sections 3.2.1 and 3.2.4).

5.2.2 Environmental parameters: PFD and ambient seawater inorganic N

PFD was determined every meter to a depth of nine meters using an underwater light sensor (refer section 2.2.2 for further details). Attenuation coefficients were calculated using Eq. 2.1 for each site over a depth profile of 9 m (refer section 2.2.2). Five water samples were collected from the surface seawater and at 10 m depth, stored in 30 ml vials and frozen for later analysis of ambient seawater NO$_3^-$ and NH$_4^+$ contents (refer section 3.2.3 for further details).

5.2.3 Determination of photosynthetic pigment contents of *Ecklonia radiata*

Chlorophyll (chl) a, chl c and fucoxanthin contents of *E. radiata* were determined following the dimethyl sulfoxide (DMSO) and acetone extraction method of Seely *et al.* (1972; refer to section 4.2.1 for further details). Discs were randomly taken from the primary blade only.

5.2.4 Morphological characteristics of *Ecklonia radiata*

Each *Ecklonia radiata* sporophyte was tagged and numbered at the laboratory in Dunedin. Nine morphological parameters were measured (Table 5.1). Data from the five sporophytes per site were averaged (n=5) to give an overall estimate.
Chapter Five: Photo-acclimation of *Ecklonia radiata*

Figure 5.2: Map of the Doubtful Sound complex showing the five sites *Ecklonia radiata* was collected from on 28th November 2000. The main axis of Doubtful Sound is 40 km in length. For more detail refer to Fig. 1.3.
Table 5.1: Morphological characteristics, and units, measured for each *Ecklonia radiata* sporophyte from each of the five sites.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stipe – length</td>
<td>mm</td>
</tr>
<tr>
<td>– diameter</td>
<td>mm</td>
</tr>
<tr>
<td>Primary blade – length</td>
<td>mm</td>
</tr>
<tr>
<td>– width</td>
<td>mm</td>
</tr>
<tr>
<td>– thickness</td>
<td>mm</td>
</tr>
<tr>
<td>Secondary blade – number/sporophyte</td>
<td>mm</td>
</tr>
<tr>
<td>– length of longest one</td>
<td>mm</td>
</tr>
<tr>
<td>– width of longest one</td>
<td>mm</td>
</tr>
<tr>
<td>– thickness of longest one</td>
<td>mm</td>
</tr>
</tbody>
</table>

5.2.5 Determination of photosynthetic parameters of *Ecklonia radiata*

Photosynthetic parameters, $P_{max}$, $\alpha_p$, $R_d$, $E_k$, $E_c$ and $\beta$, were determined as previously described (refer section 4.2.2). Discs were randomly taken from the primary blade only however, reproductive tissue was excluded.

5.2.6 Data analysis

Variations between sites in each morphological characteristic, photosynthetic pigment content and photosynthetic parameters were analysed using one-way ANOVA. Significance was set at $P=0.05$, with post-hoc multiple comparisons using Tukey tests performed to isolate significant differences. All analyses were performed using SigmaStat computer software (v2.03, SPSS Inc, USA, 1997).
5.3 RESULTS

5.3.1 Environmental parameters

5.3.1.1 PFD levels

PFD levels at the surface of the water were lowest at the innermost site, and increased towards the two outer sites (Fig. 5.3). At 9 m depth, PFD levels were between 1 and 5 % of surface PFD levels for all sites. Mean attenuation coefficients were similar between the five sites ($\bar{\tau}=0.146$; Fig. 5.4).

5.3.1.2 Ambient seawater inorganic N concentration

Surface ambient seawater $\text{NO}_3^-$ concentration was approximately 3 $\mu$M at all sites compared to 1 $\mu$M $\text{NH}_4^+$ (Fig. 5.5). At 10 m depth, the $\text{NO}_3^-$ concentration was six times higher than $\text{NH}_4^+$ content at all five sites. In both cases, there were no discernible differences in ambient seawater N concentration between the inner and outer sites.

5.3.2 %tissue C and N, and C: N ratios of *Ecklonia radiata*

Percent tissue C of *Ecklonia radiata* ranged between 25 and 26 %, and levels were similar at all five sites (Fig. 5.6a). Percent tissue N levels of *E. radiata* ranged between 1.5 and 1.7 %, and also showed little variation between sites (Fig. 5.6b). C:N ratios of *E. radiata* ranged from 18.3 to 26.5, with no obvious pattern detected between sites (Fig. 5.6c).

5.3.3 Pigment composition of *Ecklonia radiata*

5.3.3.1 Photosynthetic pigment contents

There was significant variation in chl a content between the five sites ($P<0.001$), with *Ecklonia radiata* at Elizabeth Island having significantly higher chl a content than at Causet Cove and Thompson Sound (Fig. 5.7a; Tukey test, $P=0.001$ and $P=0.006$ respectively). No significant variation in chl c content between the five sites was observed (Fig. 5.7a; $P=0.084$). Fucoxanthin content of *E. radiata* varied significantly
Figure 5.3: PFD levels to a depth of 9 m at each of the five sites in Doubtful Sound, November 2000. *Ecklonia radiata* individuals were collected from 10 m depth but the cable attaching the underwater spherical bulb to the quantum sensor only extended to 9 m.
Figure 5.4: Attenuation coefficients ($k$) for *Ecklonia radiata* at each of the five sites in November 2000. Attenuation coefficients were calculated using PFD measured at each site over a depth profile (9m). Bars represent mean $\pm$ 1 SD (n=5). Site abbreviations are as follows, and go from the coastal entrance to the head of the fiord (left to right): CC = Causet Cove, TS = Thompson Sound, SI = Seymour Island, BS = Bradshaw Sound, and EI = Elizabeth Island.
Figure 5.5: Ambient seawater NO$_3^-$ and NH$_4^+$ concentration collected at a) the water surface and b) at 10 m depth from each of the five sites in Doubtful Sound in November 2000. Bars represent mean ± 1 SD (n=5). Refer to Fig. 5.4 for site abbreviations.
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Figure 5.6: Percent tissue C and N, and C:N ratio of *Ecklonia radiata* from the five study sites the Doubtful Sound, November 2000. Bars represent mean ± 1 SD (n=5). Refer to Fig. 5.4 for site abbreviations.
Figure 5.7: A) Photosynthetic pigment concentration and b) pigment ratios for *Ecklonia radiata* from the five sites in Doubtful Sound, November 2000. Pigments were extracted in DMSO and acetone. Bars represent mean ± 1 SD (n=5). Refer to Fig. 5.4 for site abbreviations.
between sites (Fig. 5.7a; \( P<0.001 \)). *E. radiata* at Elizabeth Island had a higher fucoxanthin content than *E. radiata* at either Causet Cove or Thompson Sound (Tukey test \( P<0.001 \) and \( P=0.003 \) respectively), while *E. radiata* at Causet Cove had a significantly lower fucoxanthin content than *E. radiata* at either Seymour Island or Bradshaw Sound (Tukey test \( P=0.002 \) for both).

5.3.3.2 Photosynthetic pigment ratios

There was a significant difference in the chl \( a/\text{chl c} \) ratio between the five sites (\( P=0.027 \), Fig. 5.7b). *Ecklonia radiata* at Elizabeth Island had a significantly higher chl \( a/\text{chl c} \) ratio than *E. radiata* at Thompson Sound (Tukey test, \( P=0.039 \)). There was also a significant variation between sites in the chl \( a/\text{fucoxanthin} \) ratio (\( P=0.018 \), Fig. 5.7b). *E. radiata* at Elizabeth Island had a significantly lower chl \( a/\text{fucoxanthin} \) ratio than *E. radiata* at Causet Cove (Tukey test, \( P=0.014 \)), Thompson Sound (Tukey test, \( P=0.009 \)), and Seymour Island (Tukey test, \( P=0.028 \)).

5.3.4 Morphological characteristics of *Ecklonia radiata*

*Ecklonia radiata* sporophytes from Seymour Island had significantly longer stipes than *E. radiata* from the other four sites (Fig. 5.8a; \( P=0.021 \); Tukey test, \( P=0.020 \), \( P=0.026 \), \( P=0.015 \), \( P=0.018 \) respectively). However, *E. radiata* from Causet Cove and Thompson Sound had a greater stipe diameter compared to *E. radiata* at the inner sites (Fig. 5.8b; \( P=0.03 \)). Primary blade length and width of *E. radiata* increased from the outer to the inner sites (Fig. 5.8c, d). In contrast, the primary blade thickness of *E. radiata* decreased from the outer to the innermost site (Fig. 5.8e).

The other five morphological parameters showed significant spatial variations. *Ecklonia radiata* at Causet Cove and Thompson Sound had the greatest number of secondary blades per sporophyte, with the number decreasing toward the innermost site (Fig. 5.9a). The length of the longest secondary blade per *E. radiata* sporophyte also decreased from the outer to the inner site (Fig. 5.9b). In contrast, the width of the longest secondary
Figure 5.8: Morphological characteristics for *Ecklonia radiata* from five sites in Doubtful Sound, November 2000. A) stipe length, b) stipe diameter, c) primary blade length, d) primary blade width, and e) primary blade thickness. Bars represent mean ± 1 SD (n=5). Refer to Fig. 5.4 for site abbreviations.
Figure 5.9: Morphological characteristics continued: a) Number of secondary blades per individual *Ecklonia radiata* sporophyte, b) length of longest secondary blade, c) width of the longest secondary blade and d) thickness of the longest secondary blade. Bars represent mean ± 1 SD (n=5). Refer to Fig. 5.4 for site abbreviations. Letters indicate significant differences between the sites at $P=0.05$. 
blade of *E. radiata* from each of the five sites was similar (Fig. 5.9c). The thickness of the longest secondary blade also decreased from the outer to the inner site (Fig. 5.8d).

5.3.5 Photosynthetic parameters of *Ecklonia radiata*

$P_{\text{max}}$ values of *Ecklonia radiata* were similar between the five sites (Fig. 5.10a; $P=0.113$). There was significant spatial variation in $\alpha_p$, $R_d$, $E_{k}$, $E_{c}$ and $\beta$ for *E. radiata* (Fig. 5.10b-e, $P=0.03$, 0.04, <0.001, <0.001 and 0.01, respectively). *E. radiata* at Causet Cove and Thompson Sound exhibited significantly lower $\alpha_p$ values than at the other three sites. The lowest $R_d$ values occurred at Elizabeth Island and generally increased towards Causet Cove and Thompson Sound. $E_{k}$ values were highest at Thompson Sound and decreased towards the innermost site, Elizabeth Island. $E_{c}$ values at Causet Cove and Thompson were double those recorded at the inner sites (Fig. 5.10e). *E. radiata* at Causet Cove and Thompson Sound had a greater value of $\beta$ than *E. radiata* at the inner sites (Fig. 5.10f).
Figure 5.10: Photosynthetic parameters, $P_{\text{max}}$, $\alpha_p$, $R_d$, $E_k$, $E_c$ and $\beta$, for Ecklonia radiata from Doubtful Sound, November 2000. Bars represent mean ± 1 SD (n=5). Refer to Fig. 5.4 for site abbreviations. Letters indicate significant differences between sites at $P=0.05$. 
5.4 DISCUSSION

There was evidence of photo-acclimation to low PFD by *Ecklonia radiata* in the Doubtful Sound complex, particularly at the two inner sites: Bradshaw Sound and Elizabeth Island. This was characterised by an increase in photosynthetic pigment contents, particularly chl *a*, and several morphological changes such as an increase in primary blade width and length and a decrease in blade thickness. Higher α, lower β and lower E<sub>k</sub> and E<sub>c</sub> values of *E. radiata* at the two inner sites also indicate acclimation to low PFD. Photo-acclimation by macroalgae not only maximizes the photosynthetic response at an optimum light level but also maintains an optimal photosynthetic capacity regardless of ambient light levels (Ramus et al., 1976a; Henley and Ramus, 1989a, b, c).

C:N ratios of *E. radiata* from all five sites were similar concuring with previous data (chapter 3), which indicated that *E. radiata* throughout the Doubtful Sound complex was N-limited year-round. Photo-acclimation is sensitive to N supply and synthesis of pigments may be inhibited by N-deficiency (Ferrar and Osmond, 1986; Henley et al., 1991). However, effective acclimation may necessitate diversion of N from synthesis of light-harvesting pigment-proteins to Rubisco (Henley et al., 1991 and references herein).

Photosynthetic pigments typically increase in content with depth and decreasing ambient PFD (Riechert and Dawes, 1986). All three photosynthetic pigments measured in *Ecklonia radiata* increased with decreasing ambient PFD levels, although the increase was greatest in chl *a* content compared to the accessory pigments. This is similar to other macroalgae regardless of division (e.g. Riechert and Dawes, 1988). The increase in chl *a* content in *Laminaria saccharina* grown at low PFD was associated with an increase in PSII reaction centres (Machalek et al., 1996). The higher content of chl *a* compared to chl *c* and fucoxanthin in *E. radiata* at the inner sites may be due to the spectral composition of PAR at depth in Doubtful Sound. Chl *a*/chl *c* ratios of *E. radiata* increased towards the innermost site while the inverse occurred with chl *a*/fucoxanthin ratios. These results are comparable to previous investigations, which report that photo-acclimation of algal species resulted in variation in pigment ratios (Ramus et al., 1977;
Chapter Five: Photo-acclimation of *Ecklonia radiata*


*Ecklonia radiata* at the innermost site, Elizabeth Island, had longer, wider but thinner primary blades compared to *E. radiata* at Causet Cove and Thompson Sound. This results in an increase in the photosynthetic area and minimization of internal self-shading (Levitt, 1993; Kübler and Davison, 1995). The numbers of lateral secondary blades per individual *E. radiata* decreased towards Elizabeth Island and were thinner. This finding is consistent with other studies (Riechert and Dawes, 1986; Russell *et al.*, 1998). Riechert and Dawes (1986) found that the surface area of *Caulerpa racemosa* var. *uvifera* increased with both depth and decreasing light. Furthermore, they suggested that the increase in surface area was to accommodate the increase in pigment density. When investigating photo-acclimation of macroalgae, the measurement of morphological changes such as blade width and thickness are warranted, as morphological changes are not restricted to changes in cell volume, the number and density of thylakoid membranes or the size of pyrenoids (Sukenik *et al.*, 1987; Berner *et al.*, 1989).

Differences in stipe length and diameter of *Ecklonia radiata* between the five sites were also found. Stipes of *E. radiata* were significantly longer at Seymour Island compared to the other four sites. However, stipe diameter decreased significantly towards Elizabeth Island. This morphological adaptation may be a result of differences in water motion rather than reduced PFD between the five sites, with a strong uni-directional current present at Seymour Island compared to surge at Causet Cove and Thompson Sound and little water motion at Bradshaw Sound and Elizabeth Island. This is supported by attenuation coefficients that were similar between the five sites. Druehl (1967) found that *Laminaria groenlandica* has two stipe morphologies; a long form found only in areas subject to heavy surf (> 50 cm) and a short form (< 5 cm) that dominates in quieter waters. He suggested that water motion may determine the morphological features of *L. groenlandica* and may thus determine its relative distribution. Both stipe and blade morphologies of *E. radiata* display phenotypic plasticity, with variation often attributed to differences in exposure to water motion (Morton and Miller, 1968; Novaczek, 1980,
1984). Alternatively, a longer stipe enables the macroalgae to raise its photosynthetic surface closer to the water surface and therefore, a higher light level. Transplanting *Ecklonia radiata* from areas of low water motion to high water motion, and vice versa, could elucidate if observed morphology is an adaptation to water motion rather than to low light levels.

Against expectation, the maximum photosynthetic rate ($P_{\text{max}}$) for *Ecklonia radiata* was similar between the five sites. $P_{\text{max}}$ values of *E. radiata* in November 2000 are slightly higher than values recorded previously (chapter 4). This may be due to a shorter time period between collection and beginning the experiment. They are also comparable with King and Schramm’s (1976b) photosynthetic studies on brown macroalgae. The similarity of $P_{\text{max}}$ from the five sites suggests that *E. radiata* acclimates to the low light levels through changes in morphology and pigment contents thereby increasing the capacity to effectively utilize available photons, regardless of ambient light levels.

The higher $\alpha_p$ values for *Ecklonia radiata* at the inner sites demonstrate a greater efficiency of capturing and transferring energy at low PFD levels compared to *E. radiata* at the outer sites. Alpha values for *E. radiata* at the inner sites are comparable to polar algae, such as *Laminaria solidungula*, which also contend with low PFD levels. The low PFD experienced by polar algae are a result of thick ice cover during winter as well as turbid meltwater in summer (Chapman and Lindley, 1980), analogous to the LSL and terrestrial runoff in Doubtful Sound. The efficient photon capture by *E. radiata* at the inner sites may be due to an increase in the size, not number, of the photosynthetic units (PSU). Ramus (1981) used various models to demonstrate that an increase in PSU size, rather than an increase in number, results in greater $\alpha$ values but equal $P_{\text{max}}$ values. As a consequence, a lower PFD ($E_k$) is required to saturate photosynthesis (Lobban and Harrison, 1997).

$E_k$ and $E_c$ values for *Ecklonia radiata* decreased towards the innermost site providing further evidence for acclimation to low PFD levels whilst optimizing the photosynthetic rate. $E_k$ values for *E. radiata* in Doubtful Sound are comparable to $E_k$ values reported for
Arctic and Antarctic brown macroalgae (Dunton and Jodwalis, 1988; Wiencke et al., 1993). The decrease in $E_k$ supports the suggestion that PSU size increased in *E. radiata* at the inner sites.

The ecological advantage in having the ability to acclimate to low, or high, light levels is to increase both productivity and distribution (Falkowski and LaRoche, 1991). As already stated, it was assumed that the distribution of *Ecklonia radiata* was restricted to near the outer coast primarily due to the thickening LSL, and therefore a decrease in light penetration at depth, further into the sound. The ability to adapt to fluctuating light levels means that *E. radiata* can extend its distribution further into the sound although the population density decreases (< 3 plants per m$^2$).

5.4.1 Summary

In summary, there is strong evidence for photo-acclimation to low PFD by *Ecklonia radiata* in Doubtful Sound. The greatest differences between *E. radiata* from the five sites were the changes in morphological characteristics, the photosynthetic parameters, $\alpha_P$, $E_k$ and $E_c$, and the photosynthetic pigment contents. Further investigation is warranted to elucidate what factor, or combination of factors, is causing these morphological changes in *E. radiata*. It has been hypothesized that *E. radiata* increases the size of its PSUs to enhance its photosynthetic capacity. However, this aspect needs to be further quantified. Similarly, the effect of N supply on photosynthesis and in particular pigment content of *E. radiata* also needs further investigation, in view of the fact that *E. radiata* may be N-limited year-round. It is suggested that photo-acclimation may therefore be a process by which *E. radiata* overcomes the reduced light at depth imposed by the LSL and extends its distribution throughout the Doubtful Sound complex.
Chapter Six
Summary

6.1 INTRODUCTION
At the onset of this study there was no quantitative ecophysiological data for subtidal macroalgae within the Fiordland region of New Zealand, and very few studies of subtidal macroalgae within New Zealand. The low-salinity layer (LSL) present in this region, which is thicker in Doubtful Sound compared to the other sounds, influences the floral and faunal community structure and distribution, and influences their depth limits. This study has provided new information on the growth and production rates of the laminarian, Ecklonia radiata, and elucidated how environmental factors (light, inorganic N availability and water motion) might influence growth and production of E. radiata in this globally unique marine environment. In this chapter, the major findings will be synthesised and the interactive effect of inorganic N and light on the ecophysiology of E. radiata considered. The contribution of E. radiata productivity to the nearshore marine ecosystem, particularly in Doubtful Sound, will be discussed before future research is highlighted. In the final section, concluding remarks are made.

6.1.1 Main findings of this study
Growth rates of Ecklonia radiata exhibited temporal variation similar to those reported for other laminarians, minimum rates were recorded between February and June (autumn/winter) while maximum rates occurred between September and December (spring/summer). In general, E. radiata from Seymour Island had the lowest growth rates. Individual daily productivity of E. radiata was similar between the three study sites, and
comparable to previous studies within New Zealand. However, a low population density at Seymour Island contributed to low population productivity at this site compared to Causet Cove and Thompson Sound. Annual productivity was comparable to rates for *E. radiata* at 10 and 15 m depth in Australia and other localities within New Zealand.

High C:N ratios and low internal soluble tissue NO$_3^-$ and NH$_4^+$ pools indicated that *Ecklonia radiata* was N-limited year-round. There was evidence of the continual utilization of NO$_3^-$ and NH$_4^+$ by *E. radiata* instead of storage. Furthermore, elevated C:N ratios and % tissue C in *E. radiata* at all three sites during winter months suggested that either C was being stored for use for growth or the concentration of inorganic N was too low to be taken up. The second contention is supported by the low external inorganic N concentrations. Soluble tissue NH$_4^+$ pools were larger than soluble tissue NO$_3^-$ pools. The low NO$_3^-$ pools may be the result of the low ambient seawater NO$_3^-$ concentrations or constant NR and/or NiR activity.

In the time-course experiments, *Ecklonia radiata* displayed two patterns of NO$_3^-$ and NH$_4^+$ depletion from the medium: surge and linear depletion. No lag phase was observed for either inorganic N form. This suggests both passive diffusion and active transport as mechanisms by which *E. radiata* takes up inorganic N from the external media. In multiple-flask experiments inorganic N, particularly NH$_4^+$, uptake kinetics of *Ecklonia radiata* did not always display saturation kinetics. In general, NH$_4^+$ exceeded uptake rates of NO$_3^-$. There was no evidence of a temporal trend in inorganic N uptake.

*Ecklonia radiata* in the Doubtful Sound complex is considered to be a shade-adapted macroalga, evidenced by high pigment content, low Ek and Ec values and phenotypic plasticity in morphology. This enabled *E. radiata* to maintain similar photosynthetic capacities at the three study sites regardless of distribution and fluctuating PFD levels encountered spatially throughout the fiord. Differences in blade thickness and pigment content (chl a increased towards the inner sites) of *E. radiata* from the three study sites suggested *E. radiata* had the ability to acclimate to a range of PFD. However, an increase
in pigment content and differences in morphology, such as decreasing blade thickness, suggest greater photo-acclimation at the inner sites.

6.1.2 Interactive effect of inorganic N and light on growth of *Ecklonia radiata*

The growth rate of *Ecklonia radiata* in Doubtful Sound showed both spatial and temporal variability, which may have been influenced by environmental factors, such as nutrient availability or reduced transmission of light through the water column, throughout the fiord. *E. radiata* had similar growth rates to those reported for other temperate laminarians, indicating that neither the low inorganic N uptake or photosynthetic rates observed for *E. radiata* in Doubtful Sound exerted as much influence over the kelp's growth as expected. $K_s$ and $V_2$ values suggest that *E. radiata* has the ability to exploit the continuous, low ambient seawater inorganic N concentration recorded in Doubtful Sound during the study. This potential may confer a competitive advantage for *E. radiata* over other subtidal macroalgae, allowing it to maintain growth rates and increase its distribution throughout the fiord. *E. radiata* also showed several characteristics common to shade-adapted macroalgae that included an increase in surface area and pigment content towards the head of the fiord, reflecting the reduction in PFD transmission as a consequence of the LSL. The maintenance of growth by *E. radiata*, regardless of low N uptake and photosynthetic rates, indicates a wide ecological tolerance that allows distribution across a wide range of environmental factors in Doubtful Sound.

However, although growth rates were similar to other temperate laminarians, productivity estimates for *Ecklonia radiata* were considerably lower due to the low population density. One explanation is the restriction of large *E. radiata* populations to near the coastal entrance of the fiord in response to environmental gradients. Nienhuis (1987) found macroalgal density greatest at the entrance to estuaries with density decreasing up stream as wave action decreased and turbidity and light extinction increased. Productivity may also be indirectly influenced by water motion. Water velocity was typically high (> 6 cm s⁻¹) throughout the fiord, which may have affected the ability of *E. radiata* spores to settle, particularly at the inner sites. Macroalgal spores are unlikely to live long unless
they settle (Santelices, 1990). Reduced spore settlement may have contributed to the lower population densities further into the sounds. Overall however, lower population density of *E. radiata* in Doubtful Sound contributed to the lower productivity, particularly at the inner site.

Temperate laminarians are commonly N-limited during spring and summer when seawater N concentration is low (Kain, 1989). This is the first study to report year-round N-limitation in a laminarian. However, N-limitation of *Ecklonia radiata* did not appear to have an effect on either nutrient uptake rates or photosynthesis compared to other studies. N-limitation may result from a complex interaction between physiological and abiotic factors, however, for phytoplankton and some macroalgae there is increasing evidence that N-limitation and utilisation may be a result of low iron (Fe) availability (Berges and Harrison, 1995; Boyd *et al.*, 1998; Liu *et al.*, 2000; Milligan and Harrison, 2000; Soria-Dengg *et al.*, 2001; Wang and Dei, 2001). Fe is required in the enzyme NR that reduces nitrate to nitrite. Further investigations are required to elucidate the effect of Fe-limitation on N-metabolism in seaweeds.

### 6.2 Contribution of *Ecklonia radiata* to the marine ecosystem

In temperate regions, where kelps attain high productivity, it has been implied that kelp-derived organic material could play an important role in nearshore temperate secondary production (Mann, 1972, 1973; Duggins, 1980). However, the importance and fate of kelp material to the nearshore ecosystem remains largely unknown (Duggins *et al.*, 1989). Duggins *et al.* (1989) demonstrated that the growth rates of benthic suspension feeders, *Mytilus edulis* and *Bilanus glandula*, increased in the presence of organic detritus derived from kelps. Wing *et al.* (unpublished data) found the sea urchin, *Evechinus chloroticus*, achieved larger sizes, had a higher calorific content and smaller Aristotle’s lantern index where there were dense stands of laminarian kelps. It was further suggested that sea urchin populations in New Zealand fiords are spatially structured by productivity gradients (Wing *et al.*, unpublished data). The low productivity of *Ecklonia radiata* in Doubtful Sound, as estimated in this study, are not enough to sustain marine secondary production.
However, *E. radiata* does not form monospecific beds at the outer sites, and the LSL has been observed to have a large diversity of macroalgae present (pers. ob). The productivity of other macroalgae, particularly those in the LSL, have not been investigated to date and may contribute significantly to the overall primary production in Doubtful Sound.

Much of the organic material derived from kelps is indirectly transported through the marine food chain as detritus and dissolved organic material. To assess the importance of kelp derived organic material to the nearshore marine ecosystem, stable isotopes, such as $\delta^{13}C$ and $\delta^{15}N$, have been used to follow material through the marine food chain (e.g. Duggins et al., 1989). Up to 65 % of kelp-derived carbon in the Alaskan marine food chain is found in the tissues of predator consumers such as *Hexagrammos lagocephalus* (rock greenling) and *Phalacrocorax pelagicus* (cormorant). The low productivity of *Ecklonia radiata* in Doubtful Sound has important implications for the population dynamics of marine fauna, such as *Evechinus chloroticus*, and the benthic community structure of the fiords (Wing et al., unpublished data).

### 6.3 Future research

Constant NR/NiR activity was suggested as an explanation for low internal NO$_3^-$ pools in *Ecklonia radiata* observed during this study. Although activity of enzymes involved in N uptake and assimilation have been investigated (Davison et al., 1984; Fujita et al., 1988; Berges et al., 1995; McGlathery et al., 1996; Rees et al., 1998), further research is required to gain a thorough understanding of enzyme activity in relation to N metabolism. *E. radiata* demonstrated an ability to adapt to low light, both physiologically and morphologically. Transplant experiments could be used to investigate the ability of *E. radiata* from a low light environment to adjust to higher light levels. This type of experiment could also be used to assess the morphological development of *E. radiata* in relation to both light and water motion. *E. radiata*, at its shallowest depth (~ 1 m), may become immersed within the LSL during wind-driven events for unknown periods. Short-term responses to an increase or decrease in salinity have been discussed in Russell (1987) in relation to osmotic potential and ionic composition however, the influence of salinity on growth, nutrient uptake and photosynthetic rates of *E. radiata* warrants further research.
6.4 Concluding remarks

This study is the first to investigate the subtidal macroalgae, *Ecklonia radiata*, from within the Doubtful Sound complex, and has contributed to the knowledge of subtidal macroalgal ecophysiology in New Zealand. It is also one of only a few studies that have investigated subtidal macroalgal growth at a temporal resolution greater than seasons. However, as factors, such as nutrient availability and incident PFD fluctuate on a temporal scale as well as spatially, further temporal (monthly) and spatial (meters) resolution of growth and productivity of *E. radiata* within the Doubtful Sound complex is therefore needed.
References


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Figure 1: Variation in monthly NO$_3^-$ uptake rate with initial NO$_3^-$ concentration for discs (29.04 cm$^2$) of Ecklonia radiata from three sites in Doubtful Sound: a) Seymour Island, b) Causet Cove and c) Thompson Sound. Nitrate uptake was measured over 1 hr using the multiple-flask method bi-monthly from June 1999 to May 2000, and curves were fitted to pooled replicates (n=5) using the Michaelis-Menten hyperbolic equation. Note the different Y-axes. Symbols represent replicate incubations.
Appendix A

a. Seymour Island

June

August

November

December

March

May

Concentration (μM)

Concentration (μM)
Appendix A

b. Causet Cove

June

August

November

December

March

May

Concentration (μM)

Concentration (μM)

V (μmol g DW⁻¹ s⁻¹ × 10³)
Appendix A

c. Thompson Sound

June

August

November

December

March

May

Concentration (µM)

Concentration (µM)
Figure 2: Variation in monthly ammonium uptake rate with initial ammonium concentration for discs (29.04 cm²) of *Ecklonia radiata* from three sites in Doubtful Sound: a) Seymour Island, b) Cau set Cove and c) Thompson Sound. Ammonium uptake was measured over 1 hr using the multiple-flask method bi-monthly from June 1999 to May 2000, and curves were fitted to pooled replicates (n=5) using the Michaelis-Menten hyperbolic equation. Note the different Y-axes. Symbols represent replicate incubations.
Appendix A

c. Thompson Sound

### June

![Graph showing data for June]

### August

![Graph showing data for August]

### November

![Graph showing data for November]

### December

![Graph showing data for December]

### March

![Graph showing data for March]

### May

![Graph showing data for May]

Concentration (μM) vs. \( V (\mu\text{mol g DW}^{-1} \text{s}^{-1} \cdot 10^7) \)
Appendix B

Equations taken from Seely *et al.* (1972) used to calculate pigment content after extraction in DMSO and 90% acetone using a Hitachi U-1100 spectrophotometer.

For DMSO extract:

\[
\text{chl } a \ (\text{g L}^{-1}) = \frac{A_{665}}{72.812} \quad \text{Eq. 1}
\]

\[
\text{chl } c \ (\text{g L}^{-1}) = A_{631} + A_{582} - \frac{0.297 A_{665}}{61.8} \quad \text{Eq. 2}
\]

 Fucoxanthin \ (\text{g L}^{-1}) = A_{480} - 0.722\left(A_{631} + A_{582}\right) - \frac{0.297 A_{665}}{130} - 0.049 A_{665}/130 \quad \text{Eq. 3}

For 90% acetone extract:

\[
\text{chl } a \ (\text{g L}^{-1}) = \frac{A_{664}}{73.6} \quad \text{Eq. 4}
\]

\[
\text{chl } c \ (\text{g L}^{-1}) = A_{628} + A_{580} - \frac{0.239 A_{661}}{62.0} \quad \text{Eq. 5}
\]

 Fucoxanthin \ (\text{g L}^{-1}) = A_{470} - \left[0.774\left(A_{6628} + A_{580} - 0.239 A_{661}\right) - 0.031 A_{661}\right]/142 \quad \text{Eq. 6}

Where, \(A_{***}\) = the absorbance in a 1 cm cuvette at a wavelength of *** nm.