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Ecology of eelgrass, *Zostera novazelandica* Setchell, in
Otago Harbour, Dunedin, New Zealand

By

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Abstract

The seagrass *Zostera novazelandica* Setchell forms extensive intertidal beds at Harwood in Otago Harbour, south-eastern New Zealand. However, despite being an important feature of the Harbour ecosystem, knowledge of spatial and temporal patterns, and of the ecology of this seagrass habitat is very limited. Major aims of this study were to map the distribution and areal extent of *Z. novazelandica*, and to understand how temporal changes in seagrass growth and production are controlled by environmental factors.

Thematic maps of the seagrass habitat area were developed using digitised aerial photographs and image processing techniques. Seagrass covered about 80 ha of the intertidal area, of which most was assigned to a 'sparse *Zostera*' category. Integration of these maps with a geographic information system enabled the detection and quantification of areal changes that occurred between April 1997 and April 1998.

Field studies carried out in permanent plots at Harwood during the period October 1996 to December 1998 documented defined seasonal changes in the rates of leaf growth and primary production, above-ground biomass, leaf length and leaf area index of *Zostera novazelandica*. These growth parameters were typically higher in summer and lower in winter. Biomass of above-ground parts showed a 2-fold increase from winter/spring values (mean of 40 g DW m⁻²) to summer values (mean of 97 g DW m⁻²). Mean leaf proportional growth rate, which was estimated using a leaf-marking method, ranged from 0.005 g DW g⁻¹ day⁻¹ in winter to 0.028 g DW g⁻¹ day⁻¹ in summer. The mean primary production rate varied from 0.2 g DW m⁻² d⁻¹ in winter to 2.0 g DW m⁻² d⁻¹ in summer. Results from a multiple regression analysis indicated that the variation in above-ground biomass was controlled mainly by air temperature. The seasonality in leaf proportional growth rate was, however, significantly influenced by air temperature and photon flux density (PFD). These findings were supported by the results of controlled laboratory culture experiments in which leaf growth rate was not influenced by the interactive effect of seawater nutrient enrichment with PFD and temperature. Furthermore, leaf growth rate did not respond to artificially elevated pore-water ammonium during a summer *in situ* enrichment study. *Z. novazelandica*, however, responded to the ammonium enrichment by increasing the canopy height, below-ground biomass, and chlorophyll *a* and *b* concentrations. These findings may indicate that growth of *Z. novazelandica* is nutrient limited during the summer.

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List of abbreviations

ANOVA	Analysis of variance
DMF	N, N-dimethylformamide
DW	Dry weight
$F_v:F_m$	Ratio of variable to maximal chlorophyll fluorescence
GCP	Ground control point
GIS	Geographic information system
GPS	Global positioning system
LAI	Leaf area index
LNS	Low nutrient seawater
PAM	Pulse amplitude modulated fluorometer
PFD	Photon flux density
PGR	Proportional leaf growth rate
SPOT	Systeme Pour l'Observation de la Terre
SST	Sea surface temperature

CHAPTER 1

GENERAL INTRODUCTION

1.1 SEAGRASSES AND THEIR STUDY IN NEW ZEALAND

Seagrasses are flowering plants (Angiosperms) living immersed in seawater, and have adapted to reproducing entirely underwater (Larkum *et al.* 1989). These marine plants often develop extensive meadows in shallow, sheltered, soft-bottom coastal areas and estuaries throughout the world (in McKenzie 1994 and literature cited therein). They are important primary producers, and form one of the most productive aquatic ecosystems (Hillman *et al.* 1989). Their relatively high rate of primary production contributes large amounts of detritus and dissolved organic matter (Moriarty *et al.* 1984, cited in Long *et al.* 1994) that are significant in supporting coastal marine communities and maintaining diverse biotas (Coles *et al.* 1995). Furthermore, the importance of seagrass habitats to commercial fisheries has been recognised in recent decades (in Long *et al.* 1993 and list of literature cited therein).

All seagrasses are monocotyledonous (Phillips and Menez 1988), and at present about 58 species are recognised world-wide and placed in one of three families: Hydrocharitaceae, Posidoniaceae and Zosteraceae (Kuo and McComb 1989). Eleven of these described species belong to the genus *Zostera* of the family Zosteraceae. Seagrasses of this genus are commonly known as eelgrasses and are generally confined to temperate regions of both hemispheres, except *Z. noltii*, which extends into tropical waters (Larkum and Hartog 1989).

Of the eleven species of *Zostera* known at present, two are found in New Zealand, *Z. novazelandica* Setchell and *Z. capricorni* Aschers., and both belong to the subgenus *Zosterella* (see Kuo and McComb 1989). Cheeseman (1925) first recognised the presence

of two forms of seagrass in New Zealand, which he designated as *Z. nana* Roth and *Z. tasmanica* Martens ex Aschers. Later, however, Setchell (1933) after a world-wide survey on the genus, concluded that *Z. nana* was confined to the northern hemisphere, and *Z. tasmanica* (now *Heterozostera tasmanica*) to Australia (in Armiger 1964). He thus erected a new species, *Z. novazelandica*, describing plants that are probably the same as Cheeseman's *Z. nana*, and this specific name is still recognised today. Furthermore, Setchell (1933) noted a resemblance of Cheeseman's *Z. tasmanica* with *Z. capricorni* Aschers. in Australia (Armiger 1964) and subsequently referred the former as *Z. capricorni*.

Despite a recent surge in research activity (c.f. Long *et al.* 1993), particularly on temperate seagrasses, relatively little research has been conducted on seagrass vegetation in New Zealand. Knowledge on such aspects as basic ecology and distribution, is very limited when compared, for example, to that from temperate Australian waters. Some of the earliest New Zealand studies concentrated on specific sites, generally near Auckland in North Island. These included the disappearance of *Zostera* from Stanley Bay as recorded by Hounsel in 1935 (in Dromgoole and Foster 1983) and a listing of faunal elements associated with *Zostera* beds (Oliver 1923; Wood 1962: cited in Dromgoole and Foster 1983). This was followed by Armiger (1964) who reported the occurrence of a persistent and systematic dying-of of *Zostera* due to a disease caused by *Labyrinthula* slime mould. Later, Byrom and Davidson (1992) recorded the activity of swans feeding on *Zostera* at Farewell Spit and in Whanganui Inlet, while Turner *et al.* (1996) documented spatial and temporal dynamics of seagrass patches in Manukau Harbour and Whangapoua Harbour. Recently, a few researchers have undertaken studies in seagrass habitats in the South Island. These include studies of the reproductive ecology and patch dynamics of *Zostera novazelandica* on the Kaikoura Peninsula (Ramage and Schiel 1998, Ramage and Schiel 1999). The importance of these seagrass beds as a habitat and as food for a burrowing

crab, *Macrophthalmus hirtipes*, has also been established (Woods and Schiel 1997). In Otago Harbour near Dunedin, seagrass beds at Harwood have been the focus for few recent studies. Israel and Fyfe (1996) initiated a programme to map the seagrass beds using SPOT XS satellite imagery. Later, Miller (1998) studied the effects of human disturbance on the seagrass and its associated benthic macrofauna. Most recently, Heiss *et al.* (2000) reported on the influence of the seagrass beds in reducing tidal current velocities and thereby the accumulation of finer sediment.

1.2 CHANGES IN SEAGRASS VEGETATION PATTERNS

Although seagrasses provide valuable resources, these habitats are subject to various threats and appear to be highly vulnerable to environmental change (Kamermans *et al.* 1999) and within recent years they have received a great deal of scientific and political attention (Pearce, 1991 in Kirkman 1996). They can change in several ways, both spatially and temporally, including changes in meadow areal extent, meadow shape, shoot density, species composition, plant productivity and depth distribution (see Coles *et al.* 1995).

Global changes in seagrass areal extent due to a decline and disappearance of meadows have widely been reported (see review by Short and Wyllie-Echeverria 1996). Apart from natural causes, such as wasting disease (*Labyrinthula* infections), the passage of high-energy storms and grazing by herbivores, decline in seagrass meadows has been attributed more commonly to human-induced deterioration of water clarity and quality (see Short and Wyllie-Echeverria 1996). These include the input of nutrient and sediment from runoff and sewage disposal, dredging and filling, pollution, upland development, and certain fishing practices (Short and Wyllie-Echeverria 1996).

Anthropogenic nutrient enrichment of coastal waters often stimulates the development of fast-growing phytoplankton and other algae, both epiphytic on seagrasses

and unattached macroalgae (Duarte 1995 in McMahon and Walker 1998, Kamermans *et al.* 1999). Proliferation of these primary producers causes shading of the seagrass plants. High sediment loads in the water column can also shade seagrass plants (Kirkman 1996). Light attenuation caused by shading can lower rates of seagrass photosynthesis (McMahon and Walker 1998) and growth (Shepherd *et al.* 1989 in Kirkman 1996). Under heavy shading conditions, seagrasses may eventually die, as they cannot photosynthesise and their carbon-storage reserves are used up (Gordon *et al.* 1993 in Kirkman 1996). An example of the detrimental effect of shading due to nutrient eutrophication was shown by Den Hartog (1994) in which thick green algal mats of *Enteromorpha radiata*, formed under the influence of sewage effluent discharge, smothered a *Zostera* bed in Langstone Harbour, Hampshire, UK in 1991. As a consequence, shading and long-term anaerobic conditions caused by the algal mats led to the suffocation and demise of about 10 ha of seagrass beds in the harbour (Den Hartog 1994). The effects of light reduction on the survival of seagrass beds have also been demonstrated in *in situ* light manipulation experiments (e.g. Alberte 1982, Bulthuis 1984, Czerny and Dunton 1995, Dennison and Gordon *et al.* 1994). Depending on the experimental light levels being used, prolonged shading resulted in the disappearance of seagrasses from some of the plots while in others caused changes to the plant's structure and productivity.

Seagrass decline has also been attributed to land reclamation and changes in land use. In Cockburn Sound, Western Australia, for example, depletion of seagrass habitat occurred as the area was developed as an industrial centre and port (Cambridge and McComb 1984). Construction of tide deflectors caused the formation of a tidal stream in Stanley Bay, Auckland Harbour, and is believed to be linked to the loss of *Zostera* in the harbour (Hounsell, 1935 cited in Droomgoole and Foster 1983). In Pensacola Bay, Florida, a complete demise of seagrasses during the period of 1949 – 1979 has been

attributed to urbanisation, industrial waste discharge, dredging and filling, and cultural eutrophication (in Short and Wyllie-Echeverria 1996 and literature cited therein).

Changes in other seagrass parameters, including abundance (e.g., shoot density, biomass), leaf growth rate and plant productivity, have frequently been observed on a regular seasonal basis, particularly in temperate waters. The period of maximum growth was generally found to be summer while the minimum was in winter. This has been shown for members of the family Zosteraceae in Australia (e.g. Bulthuis and Woelkerling 1983b, Larkum *et al.* 1984, Kerr and Strother 1990) and in the northern hemisphere (e.g. Jacobs 1979, Thom 1990, Auby and Labourg 1996, Iizumi 1996, Sfriso and Ghetti 1998) as well as for other seagrass species (e.g Kenyon *et al.* 1997, Rismondo *et al.* 1997, Zupo *et al.* 1997). Along with seasonality in growth, physiological changes have also been reported, which include variations in tissue nutrient content (e.g. Duarte 1990, Perez-Llorens and Niell 1993, Van Lent and Verschuur 1994, Kraemer and Mazzella 1999), pigment concentrations (Jimenez *et al.* 1987), and storage of carbohydrates (De Rosa *et al.* 1990).

1.3 SEAGRASS MAPPING AND ECOLOGICAL MONITORING

The sensitivity of seagrasses to changes in environmental variables which often leads to potential loss of seagrass beds, indicates a need to inventory and monitor changes of these habitats (Ward *et al.* 1997). Areas requiring conservation measures can therefore be identified before significant areas and species are lost (Coles and Kuo 1995). The choice of seagrass parameters for detecting changes will depend on the questions to be answered (Coles *et al.* 1995). Generally, seagrass changes can be documented through indirect observation using remote sensing techniques, and/or direct observation of

permanently established monitoring transects (Kirkman 1996, in Norris *et al.* 1997) or stations (Ackleson and Klemas, 1987, in Macleod and Congalton 1998).

Monitoring the spatial distribution of seagrass habitat is an important part of understanding the changes in the habitat (in Macleod and Congalton 1998). The distribution and areal extent of existing seagrass habitats can be mapped using airborne (e.g., scanned aerial photography, airborne scanners), or space-borne (satellite) remote sensing techniques. Depending on the scale, thematic maps obtained from the classified images can provide an inventory of the habitat as well as a spatial framework for baseline description and monitoring programmes (Kirkman 1996). Through periodic mapping and integration of the data with GIS, changes in the health of seagrass meadows (c.f. Kirkman 1996) can thereby be detected by comparing thematic maps of different dates.

Measures of change in other seagrass parameters (e.g., abundance, growth patterns, productivity, morphology or tissue nutrient content) caused either by natural conditions or human activities, can effectively be documented by monitoring seagrasses on permanent transects (e.g. Kirkman 1996). These data will be more useful if information on physico-chemical environmental variables (e.g., temperature, light, water column nutrient concentration) is also measured (Long *et al.* 1996). The information on the environmental variables helps to assess the causes and scale of seagrass loss and the mechanisms for its recovery (Coles *et al.* 1995).

1.4 THESIS OBJECTIVES AND LAYOUT

Harwood intertidal area is an important seagrass bed in Otago Harbour, South Island, New Zealand and was the focus of this study. The main objectives of this study were:

- To develop base maps for the distribution and areal extent of *Zostera novazelandica* at Harwood intertidal area and to detect its changes through time using image processing techniques from digitised aerial photographic data.
- To understand biological and ecological aspects of the seagrass at Harwood intertidal area so as to provide a baseline reference for future management of marine vegetation. Parameters measured include seasonal variation in seagrass growth rate, productivity and abundance, and the environmental parameters potentially affecting the seasonal patterns.

Chapter 2 focuses on the suitability of using scanned aerial photographs for mapping *Zostera novazelandica* beds at Harwood intertidal area, Otago Harbour. A supervised classification of the images is employed to generate thematic maps of the distribution and areal extent of the existing seagrass categories, namely dense *Zostera*, dense *Zostera* mixed with macroalgae, medium dense *Zostera*, and sparse *Zostera*. In addition, the magnitude of temporal change in areal extent for these seagrass categories is analysed using a post-classification change detection procedure applied to thematic maps obtained on three different dates.

Chapter 3 describes field experiments to determine the seasonal changes in abundance (biomass, shoot density), leaf proportional growth rate and production, and leaf morphometrics of *Zostera novazelandica* of Harwood intertidal seagrass beds. The growth parameters of the seagrasses are monitored in permanent plots and the findings compared

to the seasonality of other seagrass species of temperate regions. Along with the growth parameters, physico-chemical factors at the site are also measured. Using a best model approach of multiple-regression analysis, the seasonal variation in the growth parameters is compared to variations in environmental factors to determine environmental factors most likely to influence seasonality in the seagrass.

A further investigation of *Zostera novazelandica* growth in response to environmental parameters (temperature, PFD and seawater nutrient enrichment), is undertaken in Chapter 4. The plants were grown under constant laboratory conditions, and their response to the interactive effect of light, temperature and nutrient concentration is examined.

Growth and physiological responses of *Zostera novazelandica* to artificially enhanced sediment pore-water nitrogen (N) are examined in Chapter 5. To increase N loadings for the experiments, the sediment at Harwood intertidal seagrass beds was enriched using N-Osmocote®, a slow-release fertiliser, applied during the plant's active growth season. The responses obtained are used to infer the nutrient status of the *Zostera* at the study site.

The concluding chapter, Chapter 6, discusses the findings of this thesis and its implications to seagrass management in the Otago Harbour. Recommendation for further research in Otago Harbour is also made.

Chapter 2

Mapping seagrass habitat using digitised aerial photography

2.1 INTRODUCTION

Seagrasses are widely acknowledged as important components of coastal ecosystems along tropical, temperate and subarctic coasts. Seagrass beds are recognised for their significant role as breeding and nursery areas (e.g. Adams 1976 and Kikuchi 1974 in Hemminga and Nieuwenhuize 1991, Perkins-Visser *et al.* 1996) as well as providing shelter and food for a wide range of organisms including finfish and shellfish populations (e.g. Klumpp *et al.* 1989, Woods and Schiel 1997). The plants can also dampen wave action and stabilise sediment in which they grow (e.g. Orth 1977, Fonseca and Fisher 1986, Ginsburg and Lowenstam 1958 in Heiss *et al.* 2000).

Despite the importance of seagrass habitats, the plants are vulnerable to damage from natural events and human activities and are experiencing world-wide decline (see Short and Wyllie-Echeverria 1996). Therefore for effective, long-term management of these habitats, it is very important to gather accurate and complete information on their location, abundance, and spatial and temporal change. Periodic mapping and monitoring is needed in order to protect and preserve these critical marine resources (Short and Wyllie-Echeverria 1996).

Aerial photography provides for rapid collection of data for detailed synoptical survey, which is very important for studying the distribution, monitoring the dynamics or estimating the biomass of marine plant assemblages (Meulstee *et al.* 1986). Traditionally, investigators have employed a manual photographic interpretation technique to interpret aerial photographs. Computer image processing which is widely used in satellite teledetection has, however, recently been increasingly applied to the interpretation of such

photographs (e.g. Scarpace *et al.* 1981, Ferguson *et al.* 1993, Thamrongnawasawat and Catt 1994, Gao and O'Leary 1997). Although this technique has not been widely exploited in New Zealand, work on kelp beds off the Otago coast (Fyfe and Israel 1996) has confirmed its potential as a tool for future marine research.

Within Otago Harbour, a narrow-leaved seagrass, commonly called eelgrass, *Zostera novazelandica*, forms extensive beds. These marine plants contribute to the production of organic detritus making them an important component of the harbour ecosystem (ORC and DCC 1991). The primary direct grazer is the black swan (*Cygnus atratus*). At low tide, numerous shorebirds use these habitats to feed on the associated fauna that are supported by the eelgrass detritus. Despite their ecological importance to Otago Harbour, the aerial extent and bed dynamics of the eelgrasses are not well documented. This study aims to establish maps of the eelgrass beds' general distribution using digitised aerial photography and image processing techniques. This study is also attempt to quantify the temporal change in the seagrass aerial extent based on 4 categories of seagrass, i.e. dense, medium, sparse and *Zostera* mixed with macroalgae, in different seasons.

2.2 MATERIAL AND METHODS

2.2.1 Study site

The present study was conducted at Harwood intertidal area ($45^{\circ} 49' S$, $170^{\circ} 40' E$), located in the lower part of Otago Harbour, southern New Zealand (Figure 2-1). This intertidal area is inhabited by the seagrass *Zostera novazelandica*, which forms monospecific and extensive meadows of different densities and is a major feature of the site. Other habitats such as shell banks, beds of cockles and sand banks are also present. Furthermore, the area is easily accessible at low tide making it possible to view and compare these different substrata and vegetation types (Fyfe *et al.* 1999).

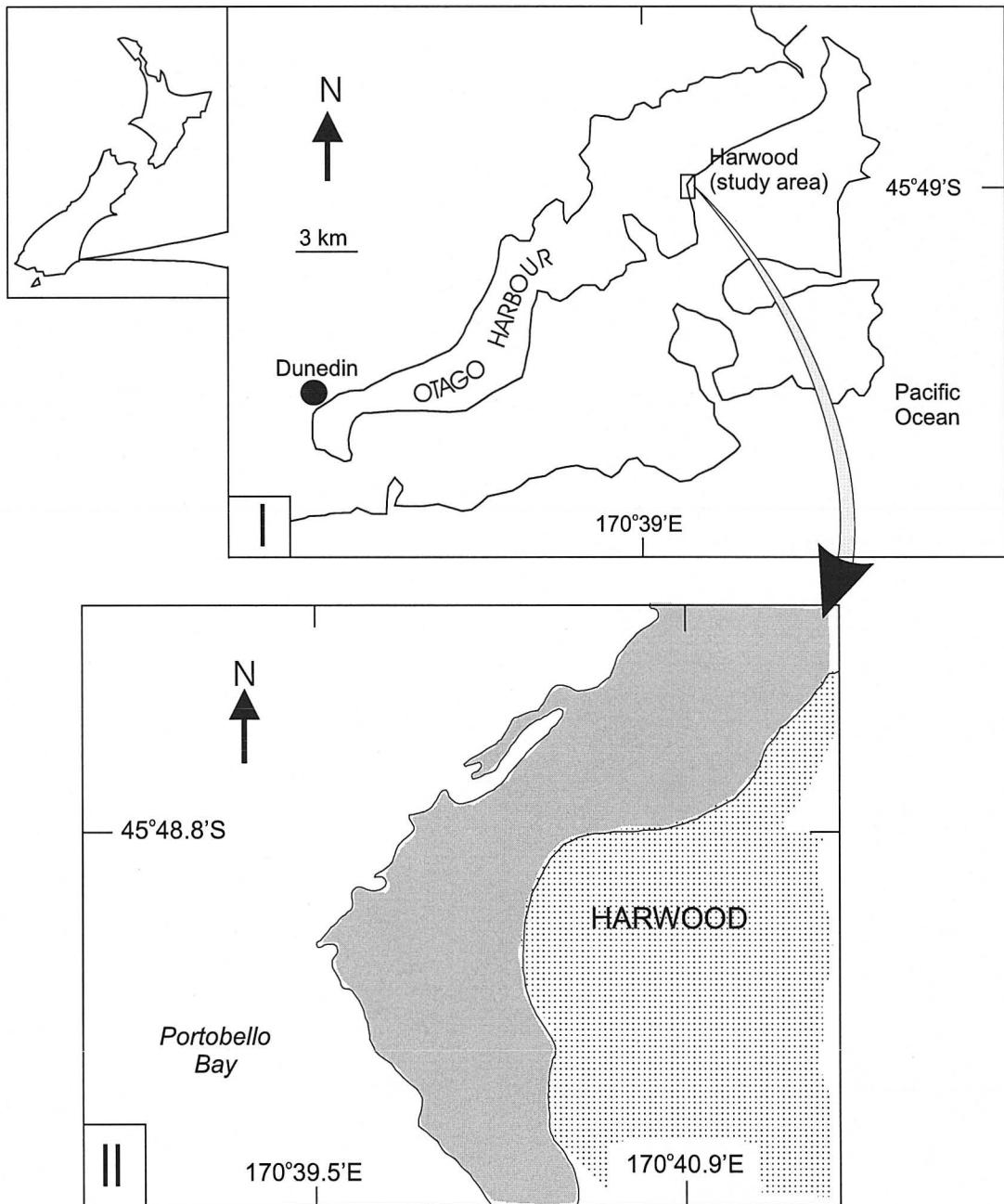


Figure 2-1: Map of Otago Harbour (I), New Zealand; showing location of the Harwood study area. Detail of Harwood (II): shaded area indicates the intertidal zone where seagrass are the dominant benthic vegetation; stippled areas indicates the terrestrial portion.

2.2.2 Aerial photography

Colour aerial photographs of the study site were acquired in April 1997 (early autumn), November 1997 (early summer) and April 1998 by the Otago University Surveying Department in conjunction with the Marine Science and Botany Departments.

Prior to the flights, white PVC plastic strips of 3 x 0.5 m for use as artificial targets were pegged to the substratum at different locations over the seagrass beds (Figure 2-2). Targets were also placed on land at the study site prior to the flight in April 1998 by painting white crosses on the black street surfaces. These targets were later geographically co-ordinated with a global positioning system (GPS).

Flights were at an approximate altitude of 1800 m and coordinated with morning low tide, low sun angle and favorable weather conditions (e.g. no clouds below the aircraft, minimal wind). Morning flights were preferred to avoid glare from the surface of tide pools distributed over the seagrass beds. Vertical photographs were obtained using a 60 X 90-mm format camera (Mamiya) with a focal length of 90 mm. Kodak 220 colour negative film and a UV filter were used (c.f. Fyfe *et al.* 1999). The colour aerial photographs were captured with a one-third overlap for each successive plate in a run (Kirkman 1996). In addition, about 500 to 1000 m of land was also included in each photograph (Fyfe *et al.* 1999).



Figure 2-2: One of the artificial targets (T) pegged on cockle and sand substratum.

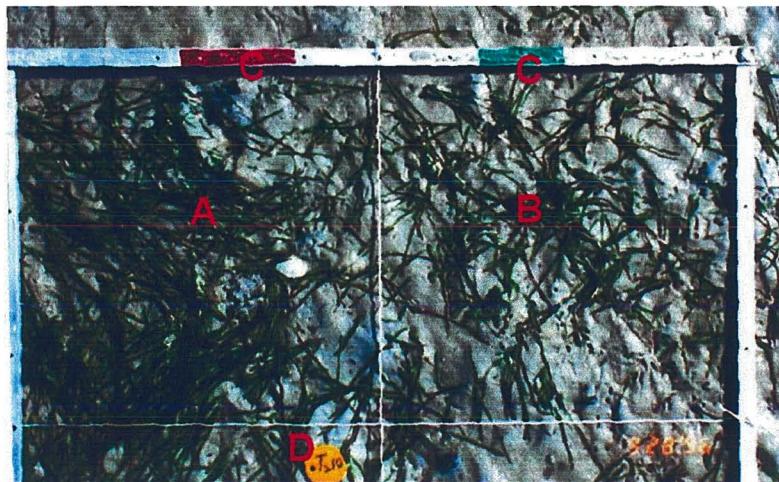


Figure 2-3: Photograph showing a pair of sub-quadrats (A, B), color codes (C) and quadrat's identification tag (D) used in the estimation of *Zostera novazelandica* ground cover.

2.2.3 Ground data collection

Several field visits were carried out during low tide within 2 weeks of the aerial photographs being acquired, to assign spectral classes and refine the apparent covertypes. The covertypes identified by (Israel and Fyfe 1996) were used as a reference, but some additions and modifications were necessary.

During the field surveys, 7 representative nearly homogeneous seagrass patches, comprising of various percentage of plant ground cover, were selected and their positions were recorded on the aerial photographs. They encompassed the range of *Zostera novazelandica* percent cover likely to be encountered throughout the Harwood intertidal seagrass beds. The seagrass percent cover was assessed using a photographed quadrat (Lanyon and Marsh 1995). At each of the seven selected seagrass patch, a 0.5 x 0.5 m quadrat was placed at intervals of 5 m along a 30 m transect. This quadrat was divided into four (0.25 x 0.25 m) colour-coded sub-quadrats. These sub-quadrats were photographed (Nikon 35 mm camera, 50 mm lens, Kodachrome ASA 100) in pairs i.e. 2 photos per quadrat (0.5 x 0.5 m) (Figure 2-3). A labelled plastic disc (transect no., quadrat no.) that was used as identification tag, was placed outside the area of the pair sub-quadrats and included in the photograph.

In the laboratory, the slide photograph of each sub-quadrat was projected and superimposed onto graph paper, on which a 0.25 x 0.25 m grid square was drawn (Foster *et al.* 1991). The grid in the square comprised 100 randomly distributed dots. The number of dots directly overlying or intercepting the seagrass material was counted to give the plant percent cover estimates. The average count obtained from the 4 sub-quadrats was the approximate percent cover of the *Zostera* present within an individual quadrat in the field. Percent ground cover was later used to categorise the seagrass covertypes. The major

categories considered were dense, medium dense, sparse, and a mixture of *Zostera* and macroalgae, in addition to bare sand and cockle/sand covertypes (Table 2-1).

After familiarisation with the range of percent cover of these reference patches and based on author's knowledge of the area, the surveys were later extended to other patches of *Zostera novazelandica* at the study site. At these locations, the percent cover was visually estimated and then categorised according to the above predetermined categories (Table 2-1). This sampling strategy was employed because of the limited access to the study site at low tide, photographic costs and limited time for photo analysis, and limited human resources.

2.2.4 Image processing

Each colour aerial photograph was first converted into digital images by scanning at 300 dpi resolution using a Hewlett Packard ScanJet IIC image scanner, to give three-band (red, green and blue) tagged file (tiff) format images. In this process, the relative intensity of light reflected from the photographs is measured by the scanner after passing through filters that allow the transmission of red, green, and blue wavelengths (c.f. Tomer *et al.* 1997).

The scanned photographs for April 1997 were imported into ERDASTM Imagine software version 8.2, running on an NT workstation, for further processing. They were later mosaicked to produce a single image view of Harwood intertidal area (Figure 2-4). The mosaics were constructed using the central portion of each photograph, whenever possible, to minimise vignetting (Lillesand and Kiefer 1994). A minimum of six common ground control points (GCPs) including those from the terrestrial parts (e.g. road intersections, buildings, artificial targets) were used for geometric rectification and to orient adjacent photographs.

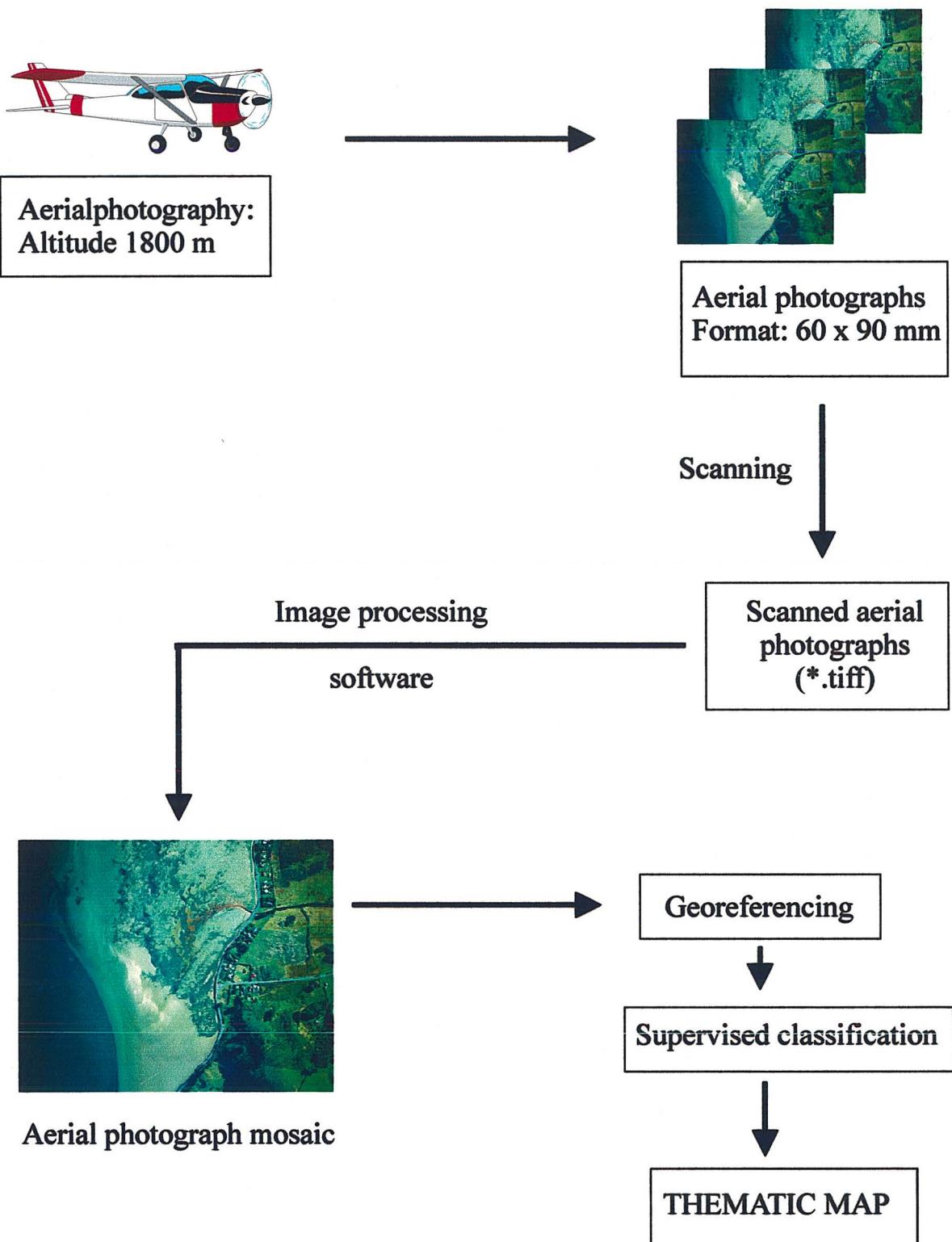


Figure 2-4: The schematic of methodology used for the mapping of Harwood intertidal seagrass habitat.

The accuracies of the geometric correction calculated as root-mean-square error (RMSE) during the mosaicking process were less than 1 pixel size (3 m). A linear contrast stretching was later applied to the spectral bands of the mosaicked image to enhance features previously obscured during the mosaicking processes.

Scanned photographs for November 1997 and April 1998 were imported into ILWIS (Integrated Land and Water Information System, The Netherlands), a Geographic Information System (GIS) with image processing software. Using this software, the photographs were separately mosaicked using with a procedure similar to that previously discussed for the April 1997 photographs.

2.2.4.1 Image registration and rectification

In this study, the April 1998 mosaicked image was first registered to the New Zealand Map Grid (NZMG) using the GPS geographically co-ordinated targets (see section 2.2.2) as ground control points (GCPs). The April 1997 and November 1997 images were later rectified to that of April 1998 image.

2.2.4.2 Land cover classification

Prior to the classification procedures, the terrestrial and water portions of the raw images were masked out to enhance the classification process (Macleod and Congalton 1998) of the covertypes existing along the exposed intertidal area. Known covertypes identified from field visits (Table 2-1) were located in the images and homogeneous examples were delineated to create 'signature' or 'reference' areas of the covertypes. For each of the covertypes, approximately six reference areas were collected. Four reference areas were used as training sites and the remaining 2 as test sites. Due to the limited extent and localised nature of some of the covertypes, region-extracted sampling was applied

(Campbell 1996). Each sample area contained approximately 70 pixels and these were evaluated for their separability from other classes using two-dimensional feature space plots. These larger areas ensured that sufficient variation was acquired to fully characterise the covertype population. Supervised classifications using a maximum likelihood classifier algorithm were performed separately on the April and November 1997 and the April 1998 images to produce biological maps of the study site. The resulting classified maps were filtered with a 3 x 3 majority filter to eliminate some of the speckling that occurred during the classification (c.f. Macleod and Congalton 1998). Results for map accuracy assessment were also generated using error matrices.

2.2.4.3 Land-cover change detection

In this study, a post-classification change-detection technique (Macleod and Congalton 1998, Mas 1999) was adopted to monitor the land-cover changes of the intertidal seagrass beds (Figure 2-5). This technique compares pixel-by-pixel the 2 independently classified maps obtained in section 2.2.4.1 to create a change image map. The classified maps of April 1997 and November 1997 were first overlaid using a ‘cross operation’ (ILWIS 1997) so that pixels at the same positions in both maps were compared and changes that occurred between classes were tracked. Subsequently, the classified map for November 1997 was overlaid with that of April 1998. The results of the post-classification change-detection procedure were a ‘cross table output’ (ILWIS 1997) showing detailed ‘from’ and ‘to’ changed class information of one map with respect to another.

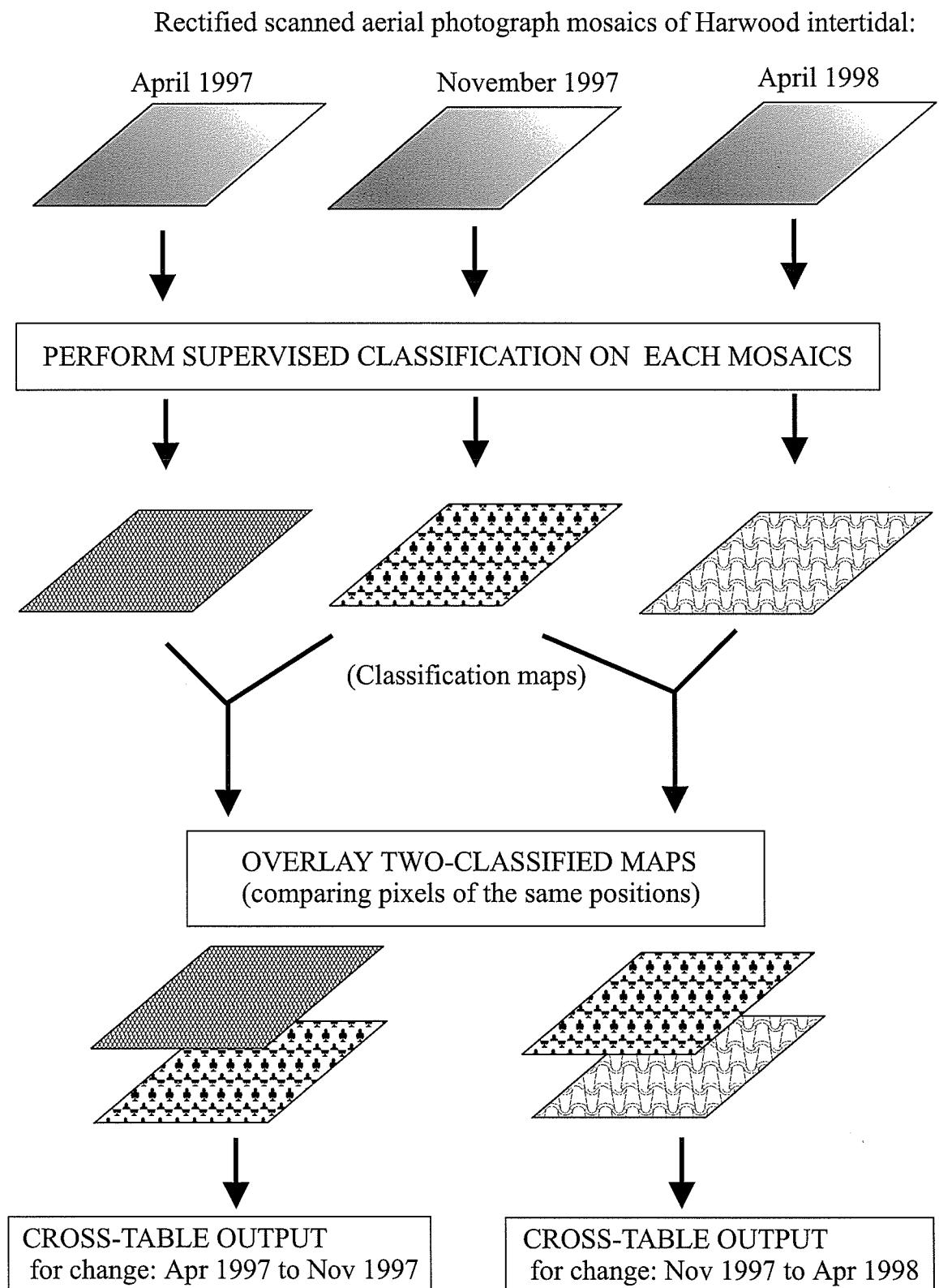


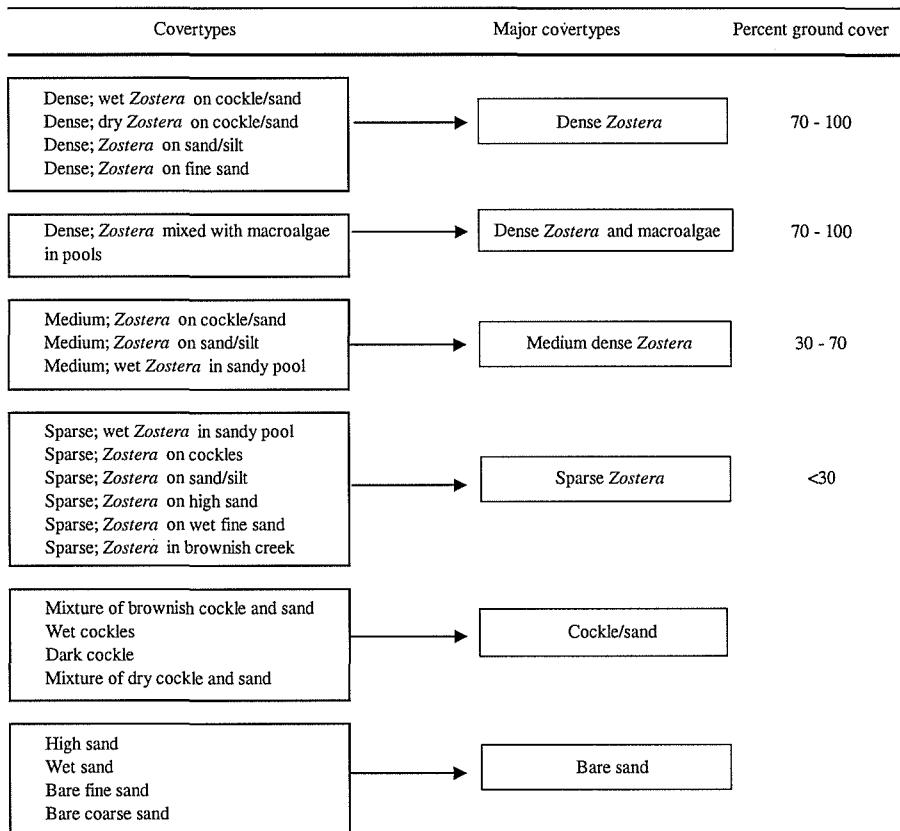
Figure 2-5: Schematic representation of post-classification change detection procedure used in this study.

2.3 RESULTS

2.3.1 *Habitat maps*

Field surveys carried out at Harwood intertidal area initially identified a total of 22 covertypes or subclasses. After supervised classification these 22 covertypes were merged into six major covertypes (Table 2-1). Vegetation covertypes were composed mainly of monospecific stands of *Zostera novazelandica* at various densities. Based on ground truth data, these covertypes were categorised into: high percent ground cover, 70–100% (dense *Zostera*; dense *Zostera* /macroalgae); medium percent ground cover, 30 –

Table 2-1: Classification system for land-cover data sets for the Harwood intertidal area. Related classifications were grouped into six major covertypes. Percent ground cover for each seagrass covertype is also shown.



70% (medium dense *Zostera*); low percent ground cover, <30% (sparse *Zostera*). The denser seagrass beds were typically found in discrete patches and were better developed higher on the shore, and in poorly drained areas (Figure 2-6). Wide areas of sparse seagrass cover were also associated with sand (Figure 2-7). Macroalgae were common in shallow tidal pools and gullies that were about 20-30 cm deep, growing attached to the *Zostera* leaves or anchored to shell fragments, or occasionally forming clumps that completely covered the *Zostera* shoots (Figure 2-8). Covertypes lacking *Zostera* included bare sand and shelly sand (Table 2-1; Figure 2-9 and 2-10), which were found in wide areas of the northeast and southwest of Harwood respectively.

Distributions of most of these covertypes were clearly visible on the scanned aerial photograph mosaics (Figure 2-11). Darker coloration was obvious in tidal pools or drainage depressions where *Zostera* and macroalgae occurred together (Figure 2-11). There was considerable contrast between the dense *Zostera* areas and sandbars, but less contrast between sparse *Zostera* and sand and shell substrata. A supervised classification on different dates of the aerial photograph mosaics produced detailed thematic maps of the distributions of the covertypes present (Figure 2-12 to 14).

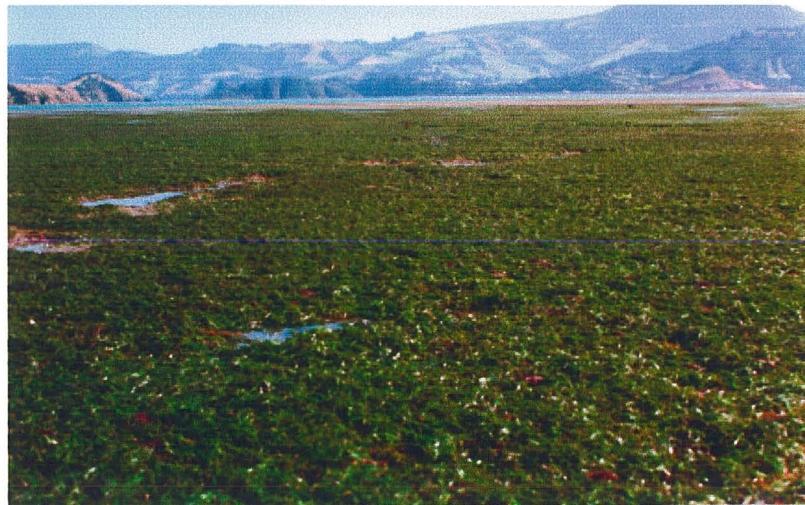


Figure 2-6: Dense *Zostera* covertyp.

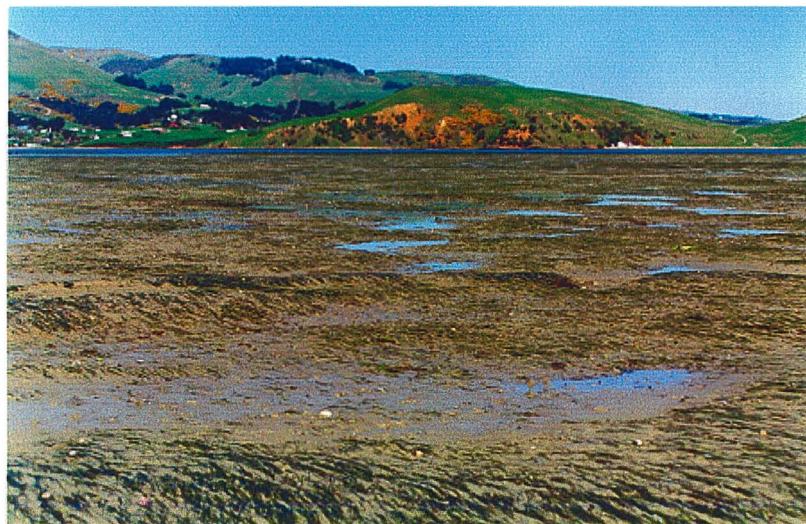


Figure 2-7: Sparse *Zostera* covertyp on wet sandy substratum.



Figure 2-8: *Zostera*/macroalgae covertyp found in tidal pool.



Figure 2-9: Sandbar located in the north-east of Harwood.

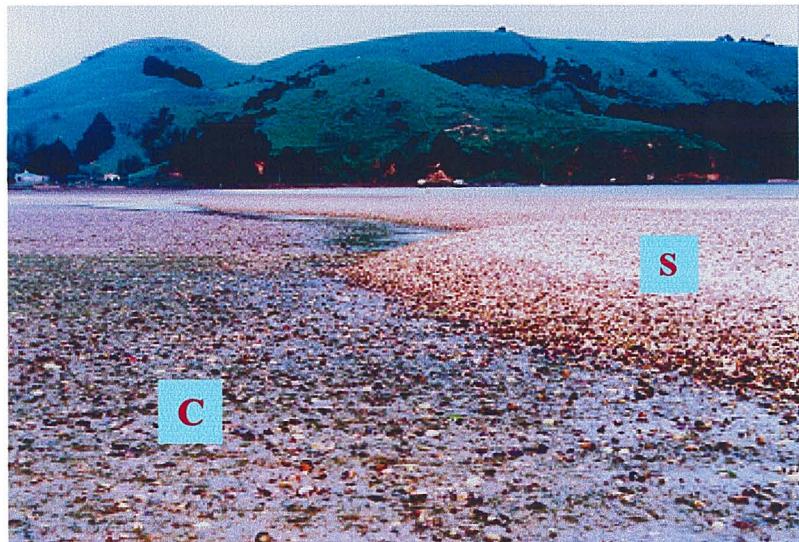


Figure 2-10: Sand/cockle covertype showing wet cockle (C) and shelly bar (S) substrata located in the south-west of Harwood.

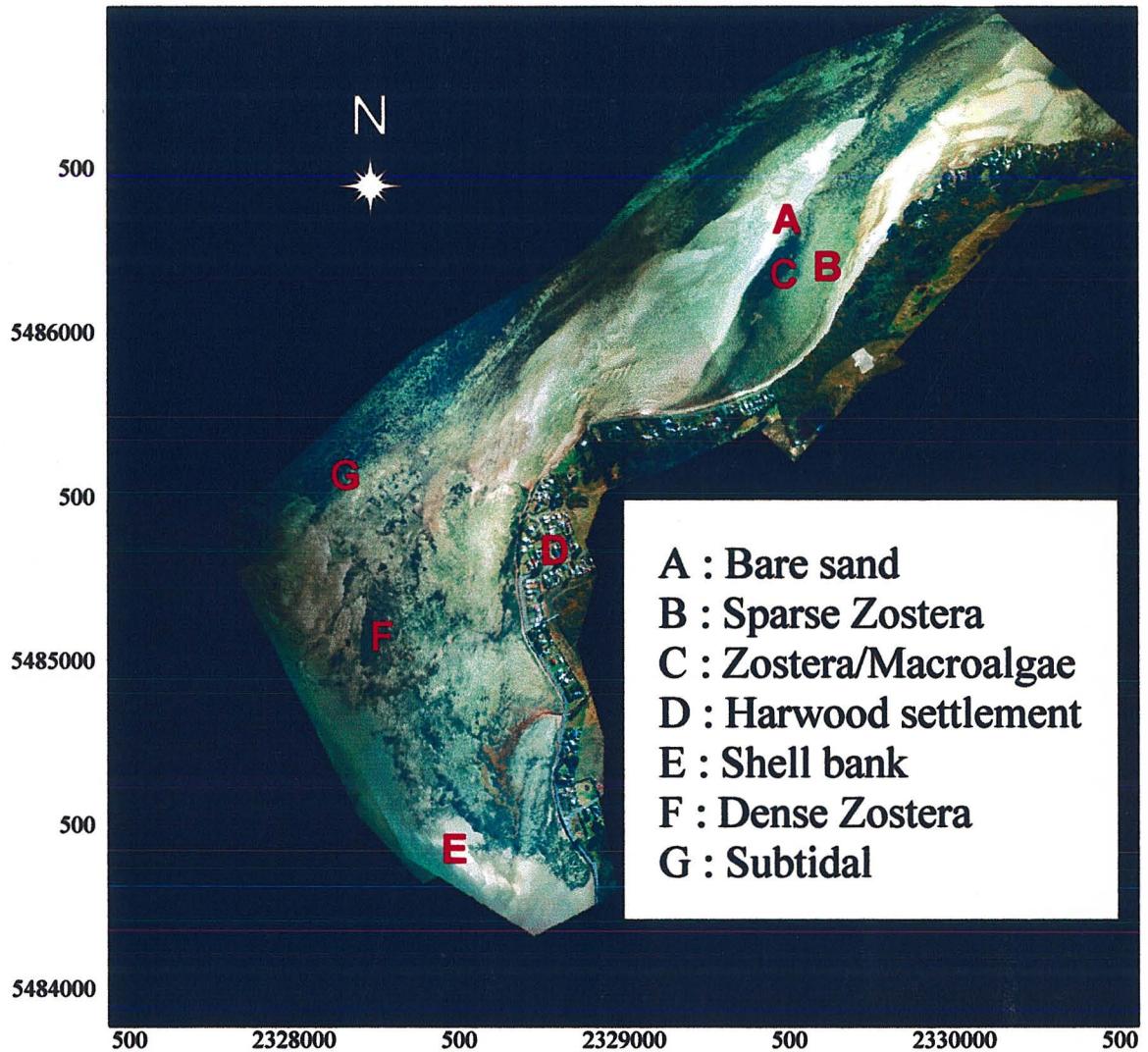


Figure 2-11: Example of an aerial photograph mosaic of Harwood intertidal area, showing the seagrass and substrata distribution in April 1997. The coordinate system is the New Zealand Map Grid: each grid represents 500 m on the ground.

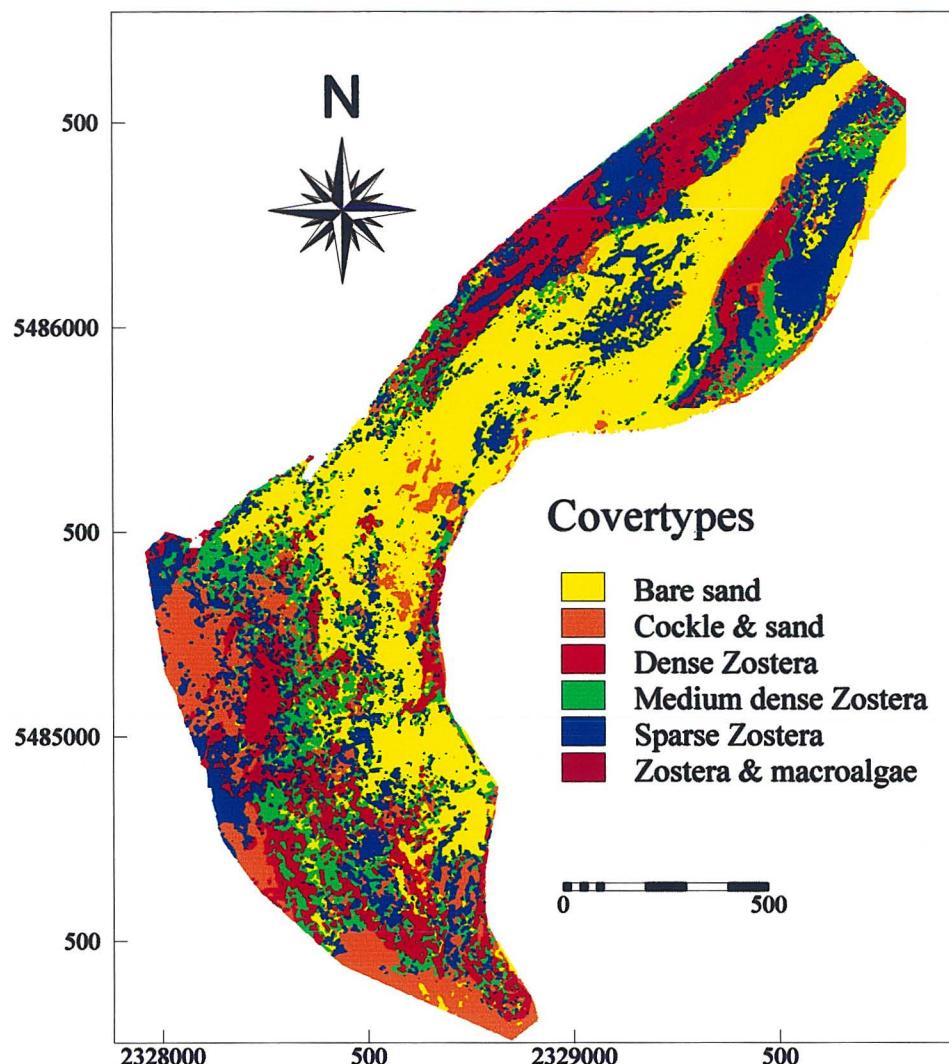


Figure 2-12: Distribution of major covertypes for the Harwood intertidal area in April 1997, produced using supervised classification.

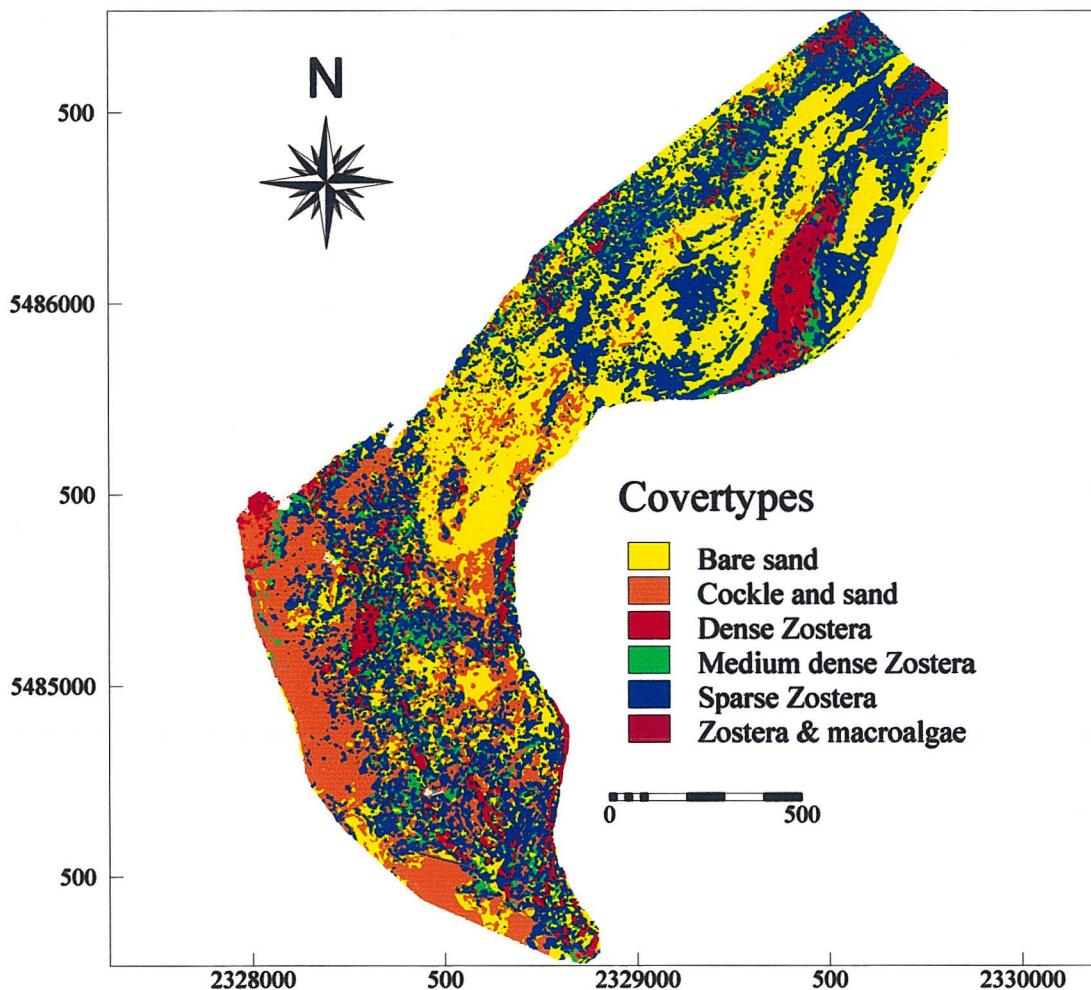


Figure 2-13: Distribution of major cover types for the Harwood intertidal area in November 1997, produced using supervised classification.

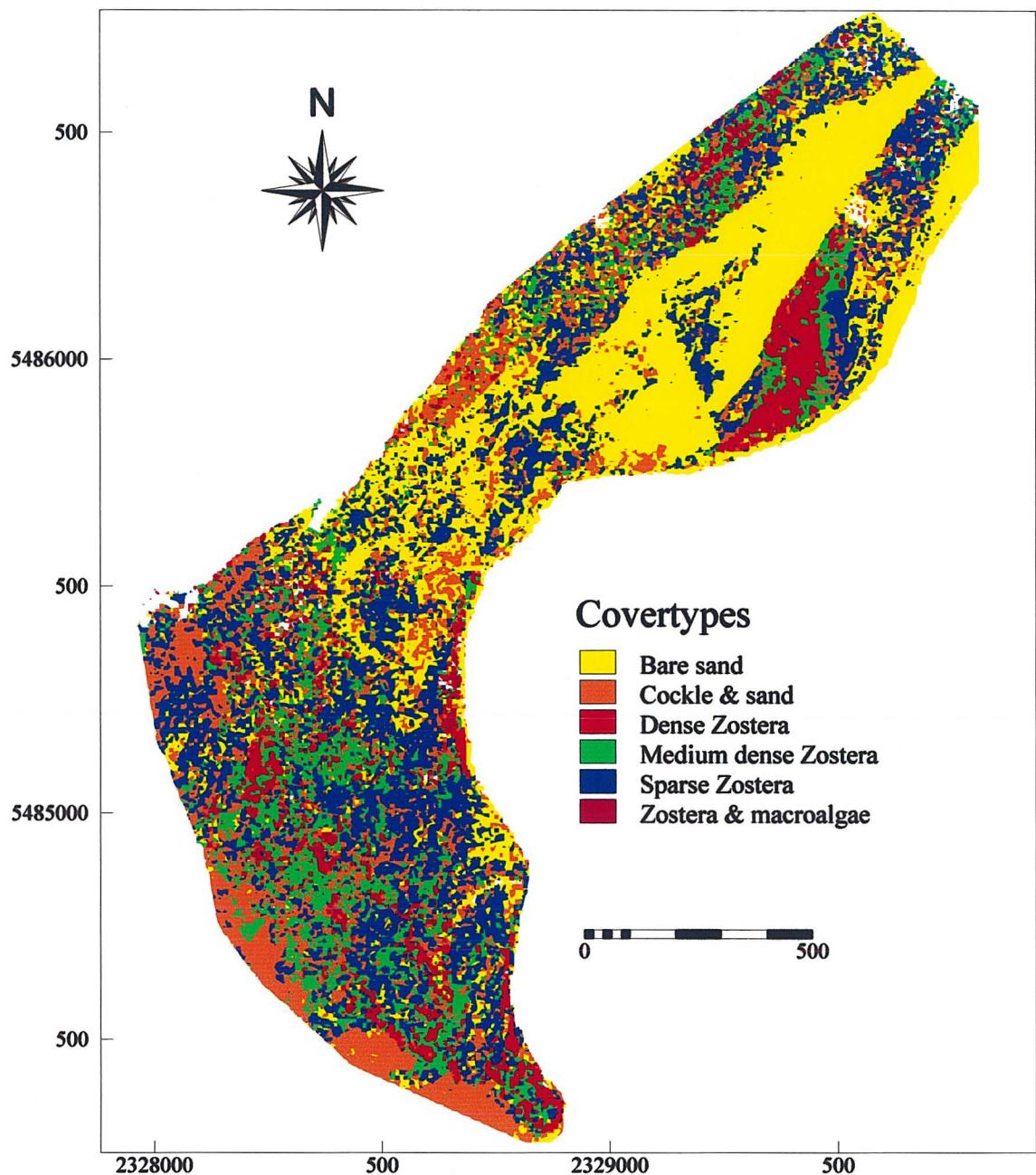


Figure 2-14: Distribution of major covertypes for the Harwood intertidal area in April 1998, produced using supervised classification.

2.3.2 Accuracy assessment of a single date classification

Discrimination was possible between various densities of seagrasses and between seagrass and non-seagrass vegetation, and these covertypes were readily mapped using image-processing techniques on the scanned aerial photograph images. Assessments on the reliability of the proposed maps were calculated from the error matrices produced (Table 2-2 to 4). In the error matrices, the number of pixels assigned to a particular covertype was arranged in columns and rows. Columns represented the reference data, while rows the classification generated from the images.

Table 2-2: Confusion matrix of April 1997 test data sets of Harwood intertidal area from supervised classifications.

Cover types	DZ	ZA	MZ	SZ	CS	BS	Total	User's Accuracy
DZ	365	0	24	1	10	0	400	91.3
ZA	6	142	0	0	0	0	148	95.9
MZ	10	0	329	53	0	0	392	83.9
SZ	1	0	9	552	20	19	601	91.8
CS	52	0	2	8	684	31	777	88.0
BS	0	0	0	79	18	464	561	82.7
						2536		
Total	434	142	364	693	732	514	2879	
Producer's Accuracy	84.1	100.0	90.4	79.7	93.4	90.3		88.1
						kappa = 0.852		

DZ = Dense *Zostera*
ZA = Dense *Zostera*/algae
MZ = Medium dense *Zostera*
SZ = Sparse *Zostera*
CS = Cockles/sand
BS = Bare sand

Of the three image classifications, the April 1997 classification produced the highest overall mapping accuracy (88%) (Table 2-2). It also had a kappa or Khat value of 0.85, which indicates this classification was 85% better than one resulting from chance. User's accuracy (error by commission) for each covertype was also high, and ranged from 83 – 96% (Table 2-2). Thus, when considering, for example, a dense *Zostera* covertype which had an accuracy of about 91%, a user has only 9% probability of observing anything other than dense *Zostera*. It should be noted that there was a minor confusion between

covertypes; for example, areas mapped as a dense *Zostera* may be mistaken for medium dense *Zostera*, cockle/sand and sparse *Zostera*.

The April 1997 classification also exhibited a high producer's accuracy (error by omission), ranging from 80 – 100% (Table 2-2). This index indicates the probability of a reference pixel being correctly classified for each covertype, which is of interest to the producer (the analyst) of the classification. For example, within the classification process, 100% of a 'dense *Zostera* and macroalgae' area has been correctly identified for this covertype.

Table 2-3: Confusion matrix of November 1997 test data sets of Harwood intertidal area from supervised classifications.

Cover types	DZ	ZA	MZ	SZ	CS	BS	User's Accuracy	
							Total	
DZ	25	0	30	0	6	31	92	27.2
ZA	1	80	45	0	1	0	127	63.0
MZ	20	15	155	10	64	3	267	58.1
SZ	0	0	2	51	21	0	74	68.9
CS	28	0	5	0	238	60	331	71.9
BS	0	0	0	0	0	72	72	100.0
						621		
Total	74	95	237	61	330	166	963	
Producer's Accuracy	33.8	84.2	65.4	83.6	72.1	43.4	64.5	
						Kappa = 0.542		

DZ = Dense *Zostera*
 ZA = Dense *Zostera*/algae
 MZ = Medium dense *Zostera*
 SZ = Sparse *Zostera*
 CS = Cockles/sand
 BS = Bare sand

The overall mapping accuracy for the November 1997 and April 1998 classification were 65% and 73% respectively, slightly lower than for April 1997 (Table 2-3 to 4). The kappa value for the November 1997 and April 1998 classifications was 0.54 and 0.66 respectively. Despite a low overall accuracy, there was a reasonably high user's accuracy for each covertypes in both classifications, with the exception of dense *Zostera* for the November 1997 data sets (Table 2-3). The results from the November 1997 classification showed a large numbers of errors of commission for the dense *Zostera* covertype, which was mistaken for medium dense and bare sand.

Table 2-4: Confusion matrix of April 1998 test data sets of Harwood intertidal area from supervised classifications.

Cover types							User's Accuracy	
	DZ	ZA	MZ	SZ	CS	BS	Total	
DZ	74	0	0	10	0	0	84	88.1
ZA	0	90	0	0	2	5	97	92.8
MZ	0	21	53	0	4	22	100	53.0
SZ	16	0	0	15	0	0	31	48.4
CS	0	0	2	0	77	41	120	64.2
BS	0	9	5	0	23	118	155	76.1
						427		
Total	90	120	60	25	106	186	587	
Producer's Accuracy	82.2	75.0	88.3	60.0	72.6	63.4		72.7
						Kappa = 0.661		

2.3.3 Temporal change in seagrass areal coverage

Post-classification change-detection using supervised classification was accomplished for the April 1997, November 1997 and April 1998 data. Overlay of the classified images, i.e. April 1997 and November 1997, and November 1997 and April 1998 resulted in 36 change detection categories (Table 2-5). The seagrass categories were subsequently merged into four major categories, while bare sand and cockle/sand were grouped into a single “non-vegetation” category, since changes in the seagrass categories are the main focus of the present detection studies (see Table 2-6).

Seagrass was found throughout Harwood intertidal area covering, for example, about 80 ha in April 1997 of the total area studied (about 158 ha). The area covered with the seagrasses was reduced to 72 ha in November 1997. A little change in total seagrass cover was, however, detected between November 1997 and April 1998 (Table 2-6). Based on the areal extent of each seagrass major category, about 43 – 75% of the intertidal seagrass habitat in Harwood was classified as sparse *Zostera*. The area covered by this

category increased from about 34.2 ha to 53.8 ha between April 1997 and November 1997, then slightly declined to 41.1 ha in April 1998.

Table 2-5: Thirty-six change-detection categories that occurred at Harwood intertidal seagrass habitat between April 1997 to April 1998.

	Apr 1997 to Nov 1997 and Nov 1997 to Apr 1998	
Dense <i>Zostera</i>	to	Dense <i>Zostera</i>
Dense <i>Zostera</i>	to	Medium dense <i>Zostera</i>
Dense <i>Zostera</i>	to	Sparse <i>Zostera</i>
Dense <i>Zostera</i>	to	Dense <i>Zostera/algae</i>
Dense <i>Zostera</i>	to	Bare sand
Dense <i>Zostera</i>	to	Cockle/sand
Medium dense <i>Zostera</i>	to	Dense <i>Zostera</i>
Medium dense <i>Zostera</i>	to	Medium dense <i>Zostera</i>
Medium dense <i>Zostera</i>	to	Sparse <i>Zostera</i>
Medium dense <i>Zostera</i>	to	Dense <i>Zostera/algae</i>
Medium dense <i>Zostera</i>	to	Bare sand
Medium dense <i>Zostera</i>	to	Cockle/sand
Sparse <i>Zostera</i>	to	Dense <i>Zostera</i>
Sparse <i>Zostera</i>	to	Medium dense <i>Zostera</i>
Sparse <i>Zostera</i>	to	Sparse <i>Zostera</i>
Sparse <i>Zostera</i>	to	Dense <i>Zostera/algae</i>
Sparse <i>Zostera</i>	to	Bare sand
Sparse <i>Zostera</i>	to	Cockle/sand
Dense <i>Zostera/algae</i>	to	Dense <i>Zostera</i>
Dense <i>Zostera/algae</i>	to	Medium dense <i>Zostera</i>
Dense <i>Zostera/algae</i>	to	Sparse <i>Zostera</i>
Dense <i>Zostera/algae</i>	to	Dense <i>Zostera/algae</i>
Dense <i>Zostera/algae</i>	to	Bare sand
Dense <i>Zostera/algae</i>	to	Cockle/sand
Bare sand	to	Dense <i>Zostera</i>
Bare sand	to	Medium dense <i>Zostera</i>
Bare sand	to	Sparse <i>Zostera</i>
Bare sand	to	Dense <i>Zostera/algae</i>
Bare sand	to	Bare sand
Bare sand	to	Cockle/sand
Cockle/sand	to	Dense <i>Zostera</i>
Cockle/sand	to	Medium dense <i>Zostera</i>
Cockle/sand	to	Sparse <i>Zostera</i>
Cockle/sand	to	Dense <i>Zostera/algae</i>
Cockle/sand	to	Bare sand
Cockle/sand	to	Cockle/sand

Table 2-6: Estimated changes (in ha) 'from – to' major categories of *Zostera novazelandica* at Harwood intertidal area, between April 1997 and November 1997, and November 1997 and April 1998 using post-classification change-detection techniques. Total area covered with seagrass on each date is also shown.

Period	to from ↓	Cover type (in ha)					Total area (ha.)	Area (ha) covered with seagrass
		Dense	Medium dense	Sparse	<i>Zostera</i> and macroalgae	Unvegetated		
Apr-97	Total	23.6	18.8	34.2	3.5	78.3	158.4	80.1
Apr-97	Dense	3.3	2.0	9.6	0.4	8.1	23.5	15.4
	Medium dense	1.5	1.9	7.7	0.1	7.6	18.8	11.1
	Sparse	2.0	1.6	13.8	0.2	16.7	34.2	17.5
	<i>Zostera</i> and macroalgae	0.6	0.3	1.0	0.4	1.3	3.5	2.2
	Unvegetated	2.0	2.2	21.8	0.1	51.6	77.7	26.2
Nov-97	Total	9.4	8.0	53.8	1.3	85.3	157.7	72.4
Nov-97	Dense	3.0	1.8	1.5	0.4	2.5	9.2	6.7
	Medium dense	0.8	1.8	2.0	0.2	3.0	7.8	4.8
	Sparse	3.0	7.9	15.8	1.0	25.5	53.2	27.6
	<i>Zostera</i> and macroalgae	0.4	0.3	0.1	0.2	0.2	1.2	1.0
	Unvegetated	2.6	8.3	21.6	0.9	51.6	85.0	33.5
Apr-98	Total	9.7	20.2	41.1	2.7	82.8	156.4	73.6

Furthermore, change detection analysis indicated considerable reductions in the dense *Zostera* and the medium dense *Zostera* categories between April 1997 and November 1997 (Table 2-6). Dense covertype declined from 23.6 ha to 9.4 ha between these dates and remained at low level in April 1998. Medium dense declined from 18.8 ha to 8.0 ha, but recovered to 20.2 ha during similar periods. Sparse *Zostera* increased between April 1997 and November 1997, then slightly declined to 41.1 in April 1998. The area covered by the mixture of *Zostera* and macroalgae, and unvegetated habitat were, however, generally similar throughout the study periods.

2.4 DISCUSSION

2.4.1 *Digitised aerial photography*

The present mapping studies of Harwood intertidal area represent the first comprehensive assessment of the seagrass distribution in Otago Harbour using a digitised aerial photography method. Although the seagrass habitat had initially been mapped by Israel and Fyfe (1996) using SPOT XS satellite imagery, there were considerable differences between the satellite observation and the field survey due to a lag of nearly a year between image acquisition and the ground reference data.

Results from this study indicate the potential value of computer image processing applied to aerial photographs in the type of ecological setting found at Harwood intertidal area. Using the technique, thematic maps of the vegetation and substratum distributions present in April 1997, November 1997 and April 1998 were achieved. Total areas covered by seagrass and other covertypes in the final maps were calculated automatically far more quickly (e.g. Thamrongnawasawat and Hopley 1995), without the need for extensive field efforts. This provided a significant advantage where field work in the present studies was limited by manpower and period of low tide exposure. Thus the use of this technique, as in the present seagrass habitat mapping, represents a significant advance over the analysis of aerial photographs by eye or direct mapping by intensive field survey (Sotheran *et al.* 1997).

Spatial resolution is one important factor for remote sensing research and small-scale habitat management. Aerial photographs have the advantage, compared to satellite imagery, of providing a resolution that can be adjusted according to the desired end results (c.f. Pasqualini *et al.* 1998). This is achieved by digitising the aerial photographs in addition to the possibility of selecting a suitable scale for the aerial photographs (Walker, 1989 in Pasqualini *et al.* 1998). High resolution and small-scale maps produced permit

detailed mapping of the seagrasses and substrata distributions. This is indicated by the more distinct classified covertypes obtained between sparse *Zostera* and bare sand than similar covertypes observed using SPOT imagery (Israel and Fyfe 1996).

In aerial photography, aeroplane flights may be scheduled to coincide with ideal conditions for data acquisition such as low tide, low sun angle, little wind and no cloud cover, where the windows of opportunity for these conditions are normally very limited in this region. This is a significant advantage over the use of satellite imagery, in which temporal resolution is specific (e.g. SPOT imagery observes a specific area of the earth every 4 to 5 days).

2.4.2 *Mapping accuracy*

Features at the study site presented a variety of signatures in the mosaicked images where their distribution has been successfully mapped and merged into six major covertype categories for clarity. The vegetation covertypes predominantly consisted of monospecific seagrass, *Zostera novazelandica*, and were categorised as dense seagrass, dense seagrass mixed with macroalgae, medium dense, and sparse seagrass. Bare sand and cockle/sand were noted as important non-vegetation covertypes.

Classifications of signatures into covertype categories involved the examination of the reflectance or spectral characteristics of pixels contained within the images. In the present studies, a number of useful *Zostera novazelandica* categories were distinguished with high user's accuracy for the April 1997 classification. Considerable confusion between some covertype categories due to spectral overlap occurred, however, in the classifications of the November 1997 and April 1998 images. Accordingly, user's accuracy for the categories had reduced in both maps. Misclassification frequently

occurred between different seagrass categories as well as between seagrass and substratum categories. These included:

- between dense seagrass and medium dense, sparse seagrass, bare sand.
- between *Zostera*/macroalgae and medium dense seagrass.
- between medium dense and bare sand, cockle/sand.

From *in situ* observations, the wide spread of macroalgal patches covering dense and medium dense seagrass areas were also recorded during November 1997. This may have increased the spectral overlap and hence confusion between the two seagrass categories for the November 1997 classification.

Confusion between the covertypes could also partly be attributed to the quality of the mosaicked images. For example, scene radiance along the flight path in the images changed markedly. This was indicated by the variability in the brightness values between consecutive aerial photographs which may have been related to minor changes in radiance associated with the proportion of different covertypes within a scene, as has previously been reported in Fyfe *et al.* (1999). Hence, it was difficult to distinguish between medium dense *Zostera* and some cockle areas in the image. Variations in reflectance values within similar categories of *Zostera* were also noticed. Difference in sediment wetness, or in *Zostera* leaf length, shoot density and the abundance of associated epiphytes may have contributed to this (Fyfe *et al.* 1999).

Thematic maps of marine ecosystems obtained through remote sensing studies are often presented with neither an error matrix calculated nor data on final field validation, despite their being fundamental to the map reliability (Chauvaud *et al.* 1998). In the present studies, overall mapping accuracy calculated from error matrices varied between classification dates, being 88% (April 1997), 65% (November 1997) and 74% (April 1998). These figures were, however, within a threshold accuracy requirement

(approximately 65 – 70%) for successful habitat mapping using remote sensing technique, as suggested by Mumby *et al.* 1998 .

The overall mapping accuracy in the present mapping was unlikely to improve by random or systematic sampling of single pixels, due to the localised nature of some covertypes (Fyfe *et al.* 1999). Furthermore, at 3-m resolution, there is uncertainty about the match between field and pixel location in the mosaicked images. Although this uncertainty may be minimised by the use of GPS in positioning the field samples, it is still difficult to correlate those samples with their coordinates on the images because of the geometric errors contained in the rectified and mosaicked images (Gao and O'Leary 1997).

2.4.3 Seagrass aerial extent and change detection

The total mapped area (vegetations and bare substrata) covered approximately 158 ha. The most *Zostera* habitats, with an estimated area of approximately 80 ha were detected in April 1997. Unfortunately published estimates of seagrass extent are lacking within this region for comparison. However, Israel *et al.* (1996) suggested that the seagrass beds range in size from several hectares for the beds adjacent to the harbour channel wall to 200 hectares at Harwood. Thus, results from present studies confirm that the Harwood site represents one of the largest seagrass stands in Otago Harbour.

Seagrass beds can change in several ways, among others, by changes in shoot density, biomass, meadow area, meadow shape, species composition, plant productivity and depth distribution (see Coles *et al.* 1995). The changes in these seagrass parameters may occur naturally and regularly as a seasonal phenomenon (Coles *et al.* 1995). Thus maps of benthic habitats such as of those obtained in these studies are important for the measurement of habitat dynamics and disturbance effects on the habitat coverage (in Mumby *et al.* 1998). Detecting changes in seagrass standing crop (Mellors 1991 in

Thamrongnawasawat and Hopley 1994) and seagrass areal coverage (Ferguson and Korfmacher 1997, Robbins 1997, Macleod and Congalton 1998) using remote sensing techniques have previously been reported elsewhere.

Application of digitised aerial photographs technique in short-term monitoring of *Zostera novazelandica* vegetation coverage at Harwood intertidal revealed that no large-scale changes in the total area of the habitats were detected between April 1997 and November 1997, and between November 1997 and April 1998. This suggested that the habitats had generally been stable over these periods. Their persistence throughout the study periods also indicates that the intertidal seagrass populations are perennial, similar to some *Zostera* populations in the Northern Hemisphere such as *Z. marina* from the intertidal area of Lake Grevelingen (Nienhuis 1983).

However, large reductions occurred based on seagrass categories, particularly in the area of dense and medium dense *Zostera* categories, the estimated changes being about 60% and 57% respectively between April 1997 and November 1997. Subsequently, in April 1998, the areal cover of dense *Zostera* was maintained, but the area of medium dense doubled from that in November 1997. Sparse *Zostera* expanded between these dates, and then declined in April 1998. Based on the present change detection analysis, it is suggested that no actual loss in area of seagrass vegetations was evident at Harwood during these short-term periods, although changes between categories were frequent.

Changes in the areal cover of the medium dense and sparse categories may have been related to seasonal variation. Seasonal variations in shoot density and growth rate are typical for this temperate species (as described later in Chapter 3). Furthermore, the expansion of unvegetated sand bottom areas at the expense of the sparse seagrass category was also detected. The frequent close association of the sparse category with the sandy substratum may explain this. During stormy weather, movement of sandbars, particularly on the northeast section of the study area, appeared to smother living seagrass plants, as

evidenced during site investigations. Localised impact from this sedimentation prevented a good *Zostera* colonisation and hence reduced the area covered by the plants covertypes. Therefore, it is more likely that the sedimentation during periods of stormy sea conditions mainly controlled this extensive and important class of sparse *Zostera*.

Verification of the considerable reduction in dense *Zostera* coverage between April 1997 and November 1997 is difficult to ascertain as the user's accuracy for this seagrass category was low in the latter classification. In addition to the confusion between dense *Zostera* with several other covertypes as previously described, the spatial misregistration of the multiday images may also have contributed to the large areal reduction of this covertype as this problem can cause high rates of error around edges of scene (Townshend *et al.* 1992 cited in Cohen *et al.* 1996). Spatial misregistration (sometimes called "positional error") in the context of change detection is a misalignment between multitemporal dataset layers. However, the possible implication of the spatial misregistration to the present seagrass change detection error is unknown, as no standard accuracy assessment technique for change detection has been developed to date (Congalton and Green, 1999). The topic of image misregistration for change detection is also still a topic of current research.

This research has demonstrated how integration of remote sensing data with GIS registration of data layers and overlay operations can document trends in seagrass cover over time at Harwood intertidal area. The present thematic maps may be used as baseline maps for future long-term monitoring of changes to the intertidal seagrass habitats, such as from impacts of associated macroalgae and black swans' grazing activities on the seagrass. Furthermore, as these maps are geo-referenced and presented on a standard GIS map format, this allows other information to be superimposed and making data queries possible for the management plan of the Harwood intertidal area.

Chapter3

Seasonal changes in biomass, leaf morphometrics and leaf production of *Zostera novazelandica*

3.1 INTRODUCTION

Seagrass meadows occur intertidally and subtidally in estuaries and shallow coastal waters of tropical, temperate and subarctic regions (Den Hartog 1970). Their seasonal growth patterns have been extensively studied and documented in temperate populations along the coasts of Europe, North America and Australia (e.g. Harrison and Mann 1975, Bulthuis and Woelkerling 1983b, Kerr and Strother 1989, Thom 1990, Alcoverro *et al.* 1995, Vermaat and Verhagen 1996, Rismondo *et al.* 1997). Seasonal growth cycles may also be evident in tropical seagrass (e.g. Erfiemeijer and Herman 1994, McKenzie 1994, Kenyon *et al.* 1997, Stapel *et al.* 1997).

For temperate seagrass species, seasonality of growth has been well studied in *Zostera marina*, a northern hemisphere eelgrass (e.g. Sand-Jensen 1975, Jacobs 1979, Harrison 1982b, Orth and Moore 1986, lists of the extensive literature in Duarte 1989, Olesen and Sand-Jensen 1994b, Iizumi 1996, Rigollet *et al.* 1998, Sfriso and Ghetti 1998). Other important species include *Z. japonica* (Thom 1990), and intertidal communities of *Z. noltii* Hornem (Vermaat *et al.* 1987, Perez-Llorens and Xavier Niell 1993, Auby and Labourg 1996, Vermaat and Verhagen 1996, Sfriso and Ghetti 1998) and *Z. americana* (Harrison 1982b). In the southern hemisphere, however, information on seasonal variation is mostly limited to species in Australia including *Amphibolis antartica* and *A. griffithii* (Walker, in Hillman *et al.* 1989), *Heterozostera tasmanica* (Bulthuis and Woelkerling 1983b), *Posidonia australis* (Kirkman and Reid 1979, West and Larkum 1979, Cambridge and Hocking 1997), *P. sinuosa* (Cambridge and Hocking

1997), *Z. muelleri* (Kerr and Strother 1989, Kerr and Strother 1990) and *Z. capricorni* (Kirkman and Cook 1982, Larkum *et al.* 1984).

3.1.1 Biomass and production

Biomass, leaf growth rates and productivity are the major growth parameters in temperate seagrasses that have frequently been observed to vary seasonally. These parameters have generally been observed to reach their maximum and minimum values in summer and winter respectively (e.g. Jacobs 1979, Bulthuis and Woelkerling 1983b, Larkum *et al.* 1984). The magnitude of the fluctuation differs, however, according to species and geographical location (see Hillman *et al.* 1989). Along with seasonal growth, physiological changes have also been observed in temperate seagrasses, including variations in tissue nutrients contents (Duarte 1990, Perez-Llorens and Xavier Niell 1993, Van Lent and Verschuur 1994, Sfriso and Marcomini 1997, Rigollet *et al.* 1998, Kraemer and Mazzella 1999), pigment concentrations (Jiménez *et al.* 1987), and storage of carbohydrates (De Rosa *et al.* 1990).

"Biomass" is often applied to the entire weight of living seagrass material, including roots and rhizomes (above- and below-ground material), and "standing crop" to above-ground material only (Zieman and Wetzel 1980). Unfortunately, as pointed out by Hillman *et al.* 1989, most studies report only the above-ground biomass, because of the difficulty of recovering the below-ground material. Nevertheless, data available from a few complete measurements of both above- and below-ground parts showed that biomass of *Zostera marina* in Danish waters increased about 3-fold in summer as compared to values in autumn (Sand-Jensen 1975). A more than 50-fold variation in biomass had also been reported for an intertidal seagrass *Z. noltii* in Netherland (Vermaat and Verhagen 1996), while similar species in southern Spain varied by only 1.5- to 4-fold (Perez-Llorens and Xavier Niell 1993). Partitioning of biomass

into above- and below-ground portions indicates greater variability in the above-ground biomass, while the below-ground portion usually shows little variation, possibly because it is protected from mechanical abrasion (e.g. Sand-Jensen 1975, Jacobs 1979, Thom 1990, Perez-Llorens and Xavier Niell 1993, Rismondo *et al.* 1997, Rigollet *et al.* 1998, Sfriso and Ghetti 1998). This is illustrated by the changes in the ratio of above to below-ground biomass from winter to summer e.g. 1:2 to 1:1 for *Z. marina* and *Halophila ovalis* (Sand-Jensen 1975, Hillman *et al.* 1995), 1:4 to 4:1 for *Cymodocea nodosa* (Sfriso and Ghetti 1998).

3.1.2 Influence of environmental factors on seasonal growth rates

Temperate seagrass populations frequently display marked growth seasonality compared to tropical and subtropical populations (Duarte 1989). The results of non-manipulative studies suggest that such variation is often associated with periodical fluctuations in solar radiation (Sand-Jensen 1975, Dennison 1987, Iizumi 1996) and temperature (Kirkman and Cook 1982, Bulthuis 1987, Perez and Romero 1992, Zupo *et al.* 1997), although the difficulty of separating these parameters is acknowledged (cf. Kerr and Strother 1990). Others attributed this seasonal variation of growth to day-length (Harrison and Mann 1975), water salinity (Pinnerup 1980, Hillman *et al.* 1995, Rigollet *et al.* 1998), nutrient availability (Short 1987, Rigollet *et al.* 1998) and grazing by herbivorous migratory waterfowl (Vermaat and Verhagen 1996).

The effects of light and temperature on the seasonality of some seagrass growth parameters has been confirmed through field manipulative experiments (Bulthuis 1983a) and laboratory studies (Harrison 1982a), and also on the short-term responses of photosynthetic rates (Bulthuis 1983b, Kerr and Strother 1985, Perez and Romero 1992, Masini and Manning 1997). Computer simulation models have also been

developed for some seagrasses such as *Posidonia oceanica* (Zupo *et al.* 1997) and *Zostera marina* (Wetzel and Neckles 1986). These models also suggested that temperature and light, or a combination of both, are important factors governing the seasonal variation in seagrass growth rates and production.

Some seagrasses, however, may deviate from the above seasonality pattern, with growth maxima not in summer. An early spring biomass peak was observed in *Zostera marina* from North Carolina with lower values maintained during the remainder of the year (Penhale 1977). Bulthuis and Woelkerling (1983) reported an anomalous bimodal seasonal pattern of above-ground biomass for *Heterozostera tasmanica* with peaks in early spring and autumn, and minima in winter and midsummer. Both studies attributed these unusual seasonal patterns to high temperature and stress associated with exposure to air.

Another seasonal deviation was shown by *Posidonia oceanica* which had a high growth rate during winter (e.g. Bay 1984), suggesting the importance of factors other than light and temperature in regulating the seasonal variation. Recent studies by Alcoverro *et al.* (1995) indicated that seasonal variability in growth of this species resulted partly from changes in light and temperature in addition to the local variation in environmental variables (e.g. nutrients, dissolved inorganic carbon, redox potential). However, Marba *et al.* (1996) demonstrated that species living in the same locality and subjected to similar seasonal changes in irradiance and temperature did not exhibit parallel seasonal growth pattern, indicating that seagrass seasonality has both extrinsic (e.g. light and temperature), and intrinsic components (e.g. resource allocation, reproduction) which may buffer, or amplify, the external seasonal forcing. Regulation of intrinsic components includes the storing of more photoassimilates that could be transported over a longer distance, as suggested to occur in seagrass with thick and

long-living rhizomes (e.g. *P. oceanica*). Hence in the larger species, growth can be relatively independent of environmental conditions compared to smaller seagrass species with thinner and shorter living rhizomes (e.g. *Cymodocea nodosa*) (Marba *et al.* 1996).

3.1.3 Objectives

Despite extensive work on the seasonal growth patterns of seagrasses elsewhere, there have been no similar studies published for seagrasses in New Zealand, namely *Zostera novazelandica* and *Z. capricorni*. This chapter presents results of field studies on seasonal variation in the growth parameters of a monospecific stand of *Z. novazelandica* in established meadows at Harwood intertidal area, Otago Harbour, in southern New Zealand. Biological data (biomass, shoot density, leaf morphometrics, proportional leaf growth rate and production) of the seagrass along with environmental parameters of the study site (seawater ammonium, nitrate and phosphate, air and seawater temperature, and PFD) were regularly collected from October 1996 to December 1998. The relationship between seasonal patterns in above-ground biomass and leaf growth rates of *Z. novazelandica* and changes in the physico-chemical parameters were explored using multiple regression analysis.

3.2 MATERIALS AND METHODS

3.2.1 Study site

The study was carried out in the Harwood intertidal area (Figure 3-1), previously described in Chapter 2. This site was chosen because of its easy access during low tide. It was also the most extensive seagrass habitat in the harbour, colonising from the lower to upper intertidal zone. The intertidal area was also topographically gentle with pools and shallow gullies about 20-30 cm deep being a common landscape during the low tide.

Three permanent sampling plots (H1, H2 and H3) were established in a dense (about 70% seagrass cover) *Zostera novazelandica* bed in the southern tidal flats of Harwood (Figure 3-1).

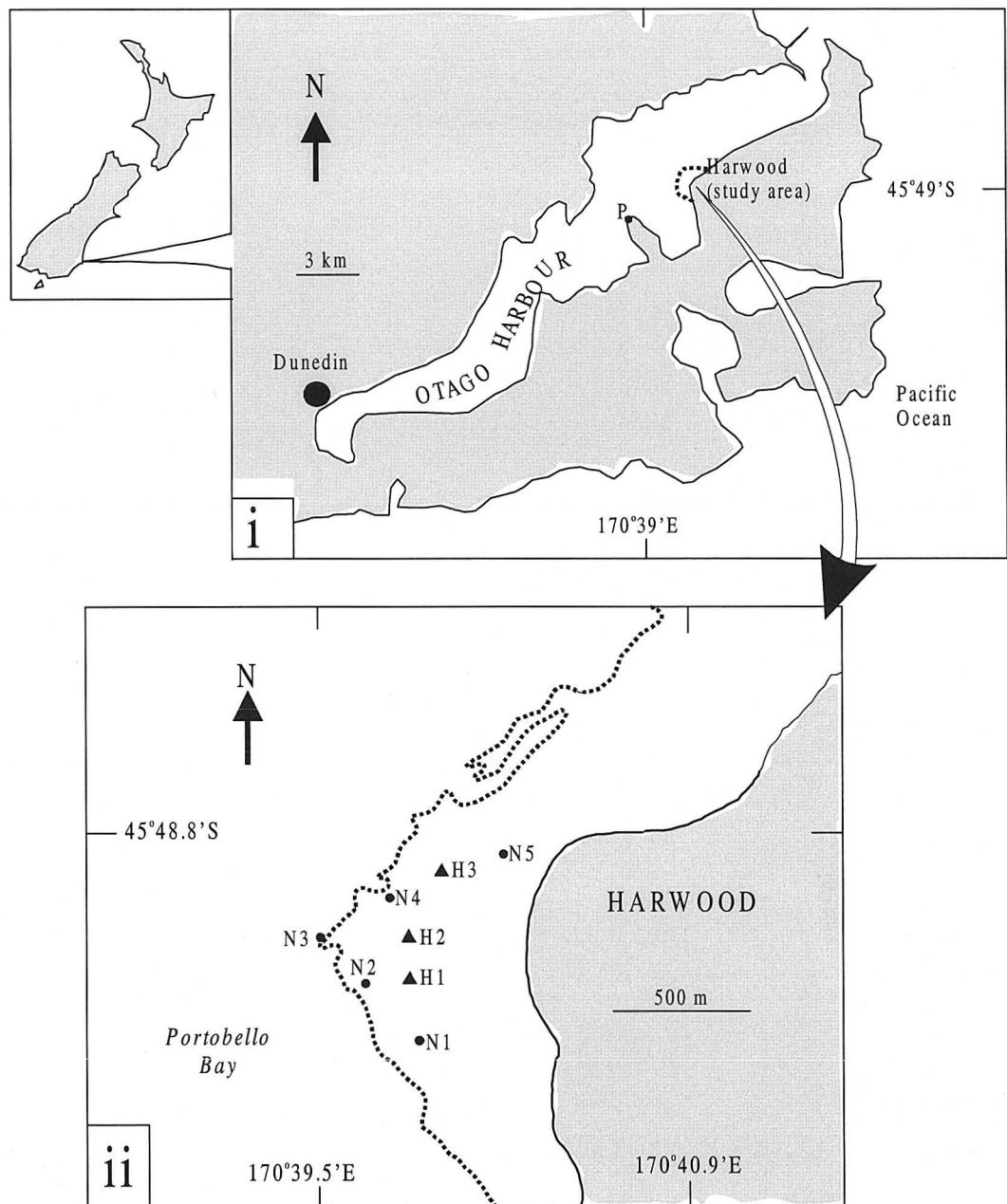


Figure 3-1: Map of Otago Harbour (i), New Zealand; showing the location of the Harwood intertidal area and Portobello Marine Laboratory (P). Detail of Harwood (ii), including permanent plots (\blacktriangle) and seawater nutrient sampling stations (\bullet). Dotted line indicates the edge of the intertidal area.

The distance between H1 and H2 was about 200 m; and 400 m between H2 and H3. The plots were located on the lower zone of the mid-tide level and were each covered with seawater for about the same length of time, 4-6 hours depending on the tidal cycle (Heiss 1998).

The plots had similar substrata, consisting mainly of a mixture of fine sand and cockle shell (*Austrovenus stutchburyi*). Plots H1 and H2 were sampled from October 1996 until December 1998. Due to logistical problems, sampling at plot H3 was begun in July 1997. All sampling was carried out during daytime low tides.

3.2.2 Seagrass sampling

The three 20 x 20 m permanent plots (H1, H2 and H3) were marked with a bright coloured steel peg at each corner. On the first day of each (approximately bimonthly) sampling period, seagrass samples from these plots were taken for subsequent measurements of the following parameters (Figure 3-2):

- biomass
 - *above-ground biomass*
 - *below-ground biomass*
- shoot density
- leaf morphometrics
 - *number of leaves per shoot*
 - *leaf length*
 - *leaf area index*

On the second day of each sampling period, *Zostera novazelandica* plants in each plot were selected randomly (using random number tables) ($n \geq 24$), and their leaves marked for growth and production rate measurements.

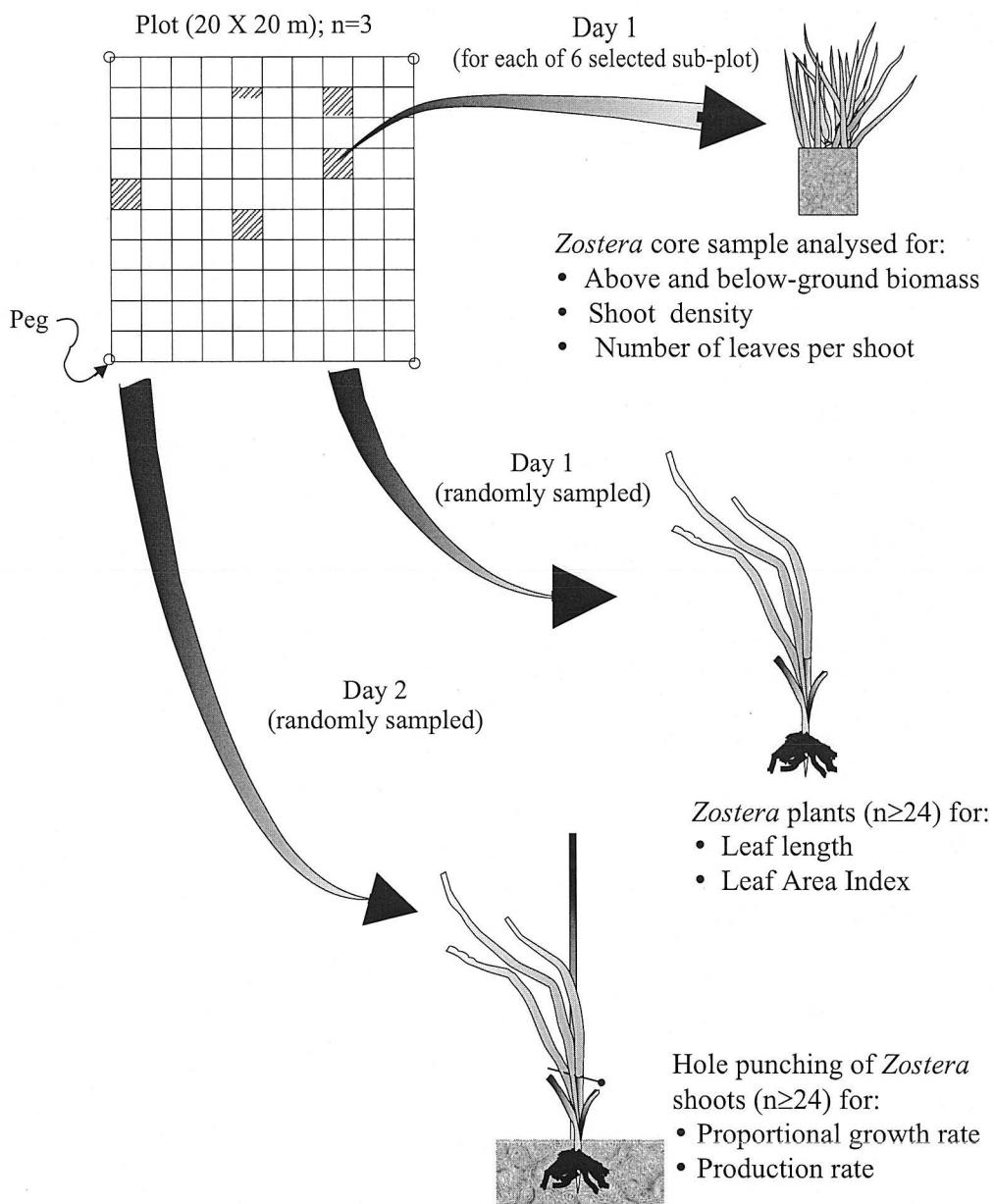


Figure 3-2: Division of *Zostera novazelandica* experimental permanent plots (H1, H2, H3) into 2 x 2 m sub-plots, established at Harwood intertidal area. Shaded boxes represent six-subplots that were selected randomly at each approximately bi-monthly sampling.

3.2.2.1 Shoot density, number of leaves and living biomass

Each plot (H1, H2 and H3) was divided into 100 sub-plots (2 X 2 m) (Figure 3-2). For each plot on each sampling occasion, six sub-plots, disregarding the bare areas (Kirkman 1996), were selected with a random number generator. A seagrass core sample was then taken from each of the six sub-plots ($n=6$ per plot) using a stainless steel corer of 10 cm diameter and 25 cm length. The core samples were sieved underwater through a 2 mm mesh sieve, to remove sediment, and transported to the laboratory in polyethylene bags in a cool box. The materials were then kept in a freezer at -20°C until further analyses.

Each frozen core sample was washed in tap water to remove salts and remaining sediment and then rinsed with deionised water. If present, epiphytes were carefully removed with a razor blade. The number of shoots per core sample was counted. In this study, 'shoot' refers to the vertical part of the seagrass that bears leaves, consisting of a colourless sheath and a green photosynthetic blade. The mean number of shoots from each plot was calculated from the average counts of the six core samples. Data were expressed as number of shoots m^{-2} . Five seagrass shoots from each core were then randomly selected and the number of leaves per shoot counted and averaged giving the mean number of leaves per shoot for each plot.

Following the above measurements, the seagrass material from each core sample was fractionated into below and above-ground material. The above-ground fraction was the leaf blade (photosynthetic) while below-ground material mainly comprised leaf sheath, rhizome and roots, according to Lanyon and Marsh (1995). Dead biomass, consisting of dark brown leaves and dark/black rhizomes and roots, was discarded. The living biomass fractions were dried in an oven at 60-80°C to a constant

weight (Van Lent *et al.* 1991). The average above and below ground biomass was obtained from the six core samples from each plot and the data were expressed as g dry weight m⁻² (gDW m⁻²). Below-ground biomass sampling ended in March 1998 as the data from plots H1, H2 and H3 showed no clear seasonal variation.

3.2.2.2 Leaf morphometrics

Individual *Zostera novazelandica* plants ($n \geq 24$) with intact leaf tips were randomly collected using a trowel from each permanent plot on day 1 (Figure 3-2). These samples were used to obtain the leaf length and Leaf Area Index (LAI) (Rismondo *et al.* 1997). Prior to analyses, the samples were treated in a similar manner to that described in section 3.2.2.1. The individual leaves of each plant were cut at the level of their ligule to separate them from the leaf sheath. The length of the longest leaf and total leaf area of each shoot were then measured with a Skye leaf area image analyser (Leaf Measurement System software v1.1; Skye Instruments LTD, 1995). Leaf area index (m².m⁻²) was determined by multiplying the mean surface area of shoots (one-sided) by shoot density per m⁻² of each plot (Rismondo *et al.* 1997).

3.2.2.3 Leaf growth and production rates

On each sampling occasion, a minimum of 24 seagrass plants inside each plot were randomly selected and marked using the hole-punching method of Kirkman and Reid (1979). A fine hole was made with a small needle through the leaf bundles, typically just above the level of the ligule of leaf number 3 (Figure 3-3). Since the older leaves grow very little, this needle hole was used as a datum mark. Each marked seagrass shoot was tied with a soft wire to a small coloured bamboo skewer (as a marker) pushed into the sediment. After approximately 6 - 10 days the individual

seagrass plants were dug out with a trowel, wrapped in wet tissue paper and brought back to the laboratory. Each shoot was first cut at the datum mark (Figure 3-4). The individual leaves were then cut through the centre of their needle marks and separated into two fractions, the previous growth tissue and new growth increments. The material was washed with deionised water and dried in an oven at 80°C for 48 hours and the dry weight determined.

The proportional leaf growth rate (PGR) of *Zostera novazelandica* in each plot at every sampling time was calculated according to Larkum *et al.* (1984), summarised as follows:

$$\text{PGR (gDW g}^{-1} \text{ day}^{-1}\text{)} = \frac{W_1}{W_1 + W_2} \quad \frac{1}{T_2 - T_1} \quad \text{Equation 3-1}$$

Where W_1 = dry weight of new growth increment (g)

W_2 = dry weight of the previous growth tissue (g)

T_1 = time (day) at the beginning of the experiment

T_2 = time (day) at the end of the experiment

The above ground rate of primary production ($\text{gDW m}^{-2} \text{ day}^{-1}$) in each plot at every sampling time was then estimated according to Larkum *et al.* (1984),

$$\text{Primary production} = \text{PGR} \times \text{AGB} \quad \text{Equation 3-2}$$

Where PGR = mean proportional leaf growth rate ($\text{gDW g}^{-1} \text{ day}^{-1}$)

AGB = mean above ground biomass (gDW m^{-2})

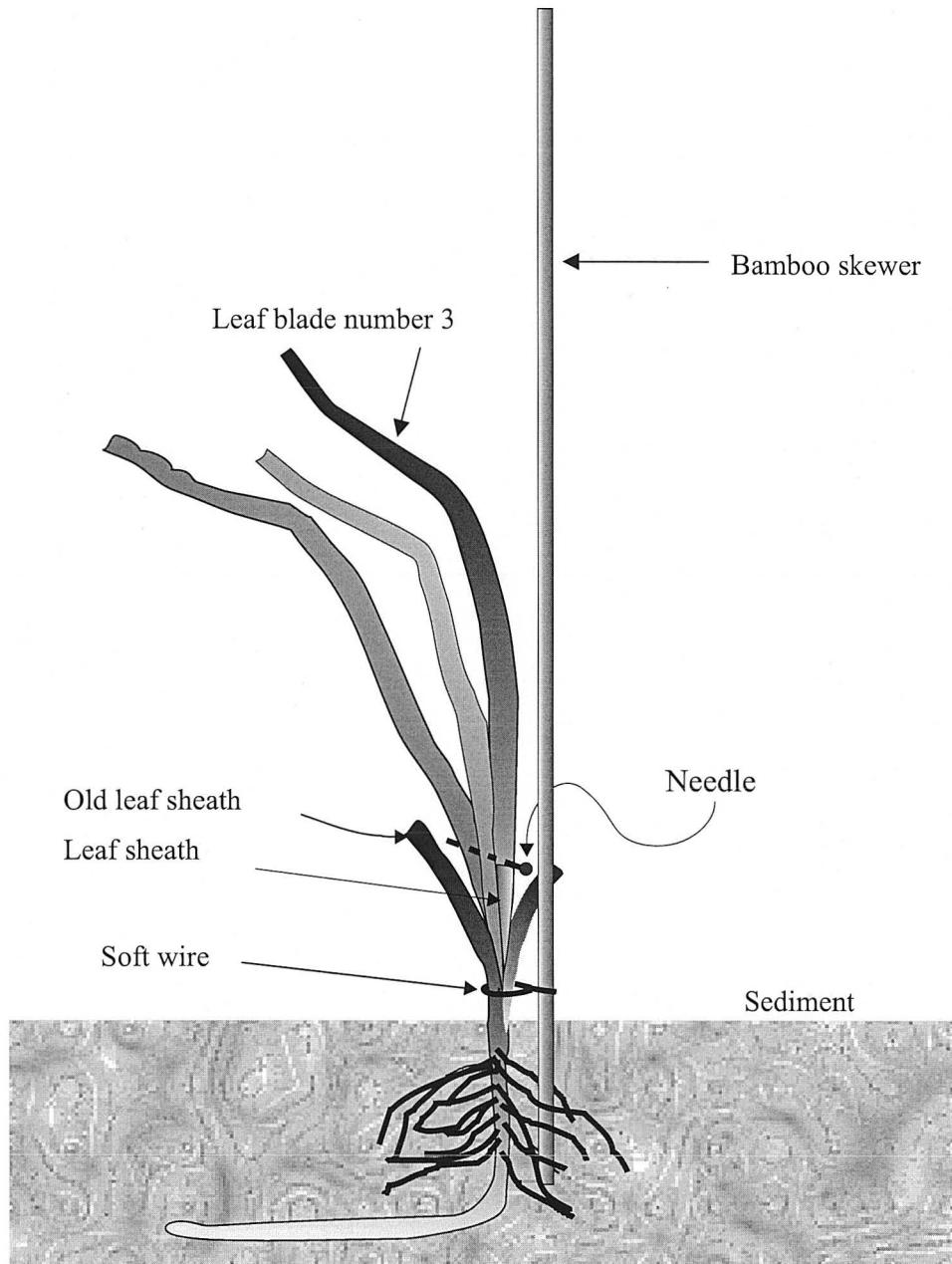


Figure 3-3: Hole punch method employed to measure growth and production rates of *Zostera novazelandica*.

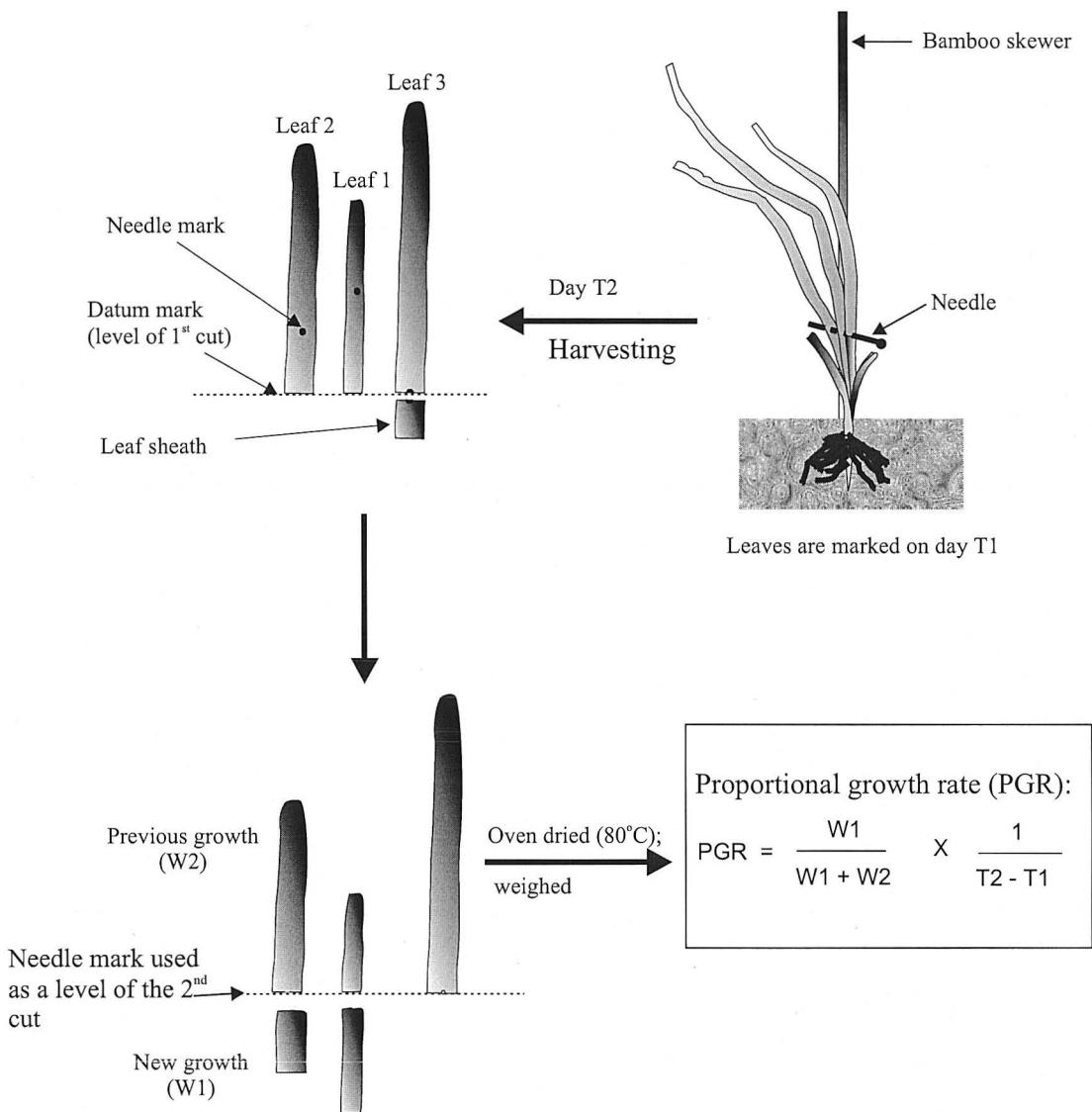


Figure 3-4: Schematic diagram for the measurement of leaf growth rate on each shoot of *Z. novazelandica* in the study.

3.2.3 *Environmental parameters*

3.2.3.1 Seawater ammonium, nitrate and phosphate

Seawater samples for the analysis of ammonium, nitrate and phosphate were taken at high tide from five stations at Harwood (Figure 3-1). These stations were relocated on each sampling occasion using a global positioning system (GPS). Each month, triplicate samples of seawater above the seagrass beds were taken using a 60 mL syringe at a depth of about 0.5 m. The sampling started in October 1996 and continued until December 1998. Due to logistical problems, seawater samples in October and December 1996, and March 1997, were taken from only 2 or 3 stations. All samples were brought back to the laboratory in a cool box, filtered through Whatman™ GF/C millipore filter into acid-washed 30 mL screw-cap Nalgene™ tubes. The samples were frozen at -20°C until further analyses. Duplicate seawater samples from each station were then analysed for ammonium, nitrate and phosphate with an automated ion analyser (QuickChem® 8000, Lachat Instruments) and a mean for each station taken. The third sample was kept frozen as a spare in case a sample of that particular station was lost during processing.

3.2.3.2 Sea surface temperature and light levels

Monthly mean sea surface temperature (SST) and air temperature were obtained from a database of records taken at Portobello Marine Laboratory, located about 3 km from the study site. Mean daily solar radiation was supplied by Dunedin Airport weather station located about 40 km to the south-west (National Institute of Water and Atmospheric Research Ltd (NIWA)). The solar radiation (mJ m^{-2}) was converted to

Photon Flux density (PFD, mol quanta m⁻² d⁻¹) by using a conversion equation, PFD = 4.5 X (solar radiation) (M. D. Stuart. *pers.com.*)

3.2.4 Data analyses

The three plots (H1, H2, H3) were treated as replicates as they were located at nearly the same level of the mid-tidal zone and had a similar type of substratum. Therefore the biological parameters from within each of the three plots were pooled and the mean taken to give one value each month per plot. The mean value for the three plots was then obtained (n=3). As the plot H3 was sampled only from July 1997, a mean of each of the biological parameters prior to this sampling month was taken from plot H1 and H2 (n=2).

Differences between monthly mean values of these parameters were first tested with a one-way ANOVA using the software package SPSS for Windows Release.2.0 (SPSS Inc.,Chicago). Where necessary, data were log-transformed prior to analysis to satisfy the ANOVA assumptions of normality and variance homogeneity. If the results of ANOVA showed significant differences (at $p<0.05$), pairwise multiple comparisons using a Tukey Test were employed to distinguish between means of each sampling month.

The means of nitrate, ammonium and phosphate concentrations for each sampling month, at each station were obtained from the duplicate samples. The monthly means of these nutrients were then calculated from the means of all stations (2 to 5 stations).

The relationship between above-ground biomass with shoot variables (shoot density, leaf length, number of leaves per shoot) was determined by multiple-regression analysis. The best combination of independent variables (best model) that

contributed to the explained above-ground biomass was first selected based on Mallow's C_p and adjusted R^2 (Helsel and Hirsch 1992, McKenzie 1994), using SPSS best subset regression approach (SPSS Inc.,Chicago). The relationship between growth parameters (above-ground biomass and leaf proportional growth rate) and environmental variables (air temperature, SST, PFD, nitrate, ammonium, phosphate) were determined using a similar approach. Air temperature, surface seawater temperature and PFD had no immediate effect on seagrass survival and growth (McKenzie 1994) and so values for the previous month were used as the independent variables in the selection of the best model for above-ground biomass. Subsequently, multiple-regression analysis followed by ANOVA were performed on the best models selected, and the level of significance determined.

3.3 RESULTS

3.3.1 Seagrass biomass

Flowering shoots of *Zostera novazelandica* were observed in samples taken in December and March 1997 and 1998. However, as the flowering shoot biomass was less than 1% of the leaf standing crop, these data were pooled with the above-ground biomass.

There were highly significant differences in the mean above-ground biomass over time (one-way ANOVA, $p<0.001$) (Figure 3-5 and Table 3-1). Above-ground biomass showed a clear seasonal trend that was consistent between years. Biomass was maximal during March (autumn), and the magnitude of this peak biomass was similar for March 1997 and 1998, about 97 and 93 gDW m⁻² respectively (Tukey Test; $p<0.05$). Minimum biomass was recorded during winter and spring, with values of 50

gDW m^{-2} (October 1996), 45 gDW m^{-2} (July 1997) and 40 gDW m^{-2} (October 1998) being 2-fold lower than the March values (Tukey Test, $p<0.05$). In a multiple regression analysis (best model approach) of above-ground biomass to shoot parameters, leaf length and shoot density explained most (84%) of the above-ground biomass variance (Table 3-1). Leaf length appeared to be the most important factor ($\beta=0.79$) followed by shoot density ($\beta=0.67$).

Table 3-1: Results of a multiple regression analysis describing the relationship (best model presented) between above-ground biomass and shoot length and density of *Zostera novazelandica* at Harwood.

Biological Variable	Coefficient	P	Standardised Coefficient (β)
Leaf length	13.67	0.003	0.79
Shoot density	0.01	0.007	0.67
$N = 9$		Overall $r^2 = 0.84$	

The mean below-ground biomass of *Z. novazelandica* did not vary over the course of the study (one-way ANOVA; $P>0.05$) (Figure 3-5; Table 3-2). The below-ground biomass contributed, however, most of the total biomass throughout the study, ranging from 180 - 235 gDW m^{-2} .

3.3.2 Shoot density

Shoot density of *Zostera novazelandica* did not vary with season (one-way ANOVA, $p>0.05$) (Figure 3-6 and Table 3-2). Shoot density ranged from 4800 to 8700 shoots m^{-2} .

Table 3-2: A summary of the one-way ANOVA (at $p<0.05$) results to test for significant differences between months of the growth parameters of *Zostera novazelandica* at the Harwood intertidal site, Otago Harbour. ** Denotes significant difference; NS = not significant. ^a indicates data were log-transformed to satisfy assumption of normality and variance homogeneity.

Parameter	Source of variation	df	SS	F-ratio	p-value
Above-ground biomass	Month	10	9852.02	8.73	<0.001**
Below-ground biomass	Month	7	4278.83	0.21	0.976 NS
Shoot density	Month	10	29639776.13	0.79	0.639 NS
LAI	Month	9	21.10	2.68	0.038**
No. of leaves per shoot	Month	9	1.56	2.34	0.063 NS
Leaf length	Month	9	28.78	5.13	0.002**
^a PGR	Month	10	1.39	58.62	<0.001**
Production rate	Month	10	9.89	33.24	<0.001**

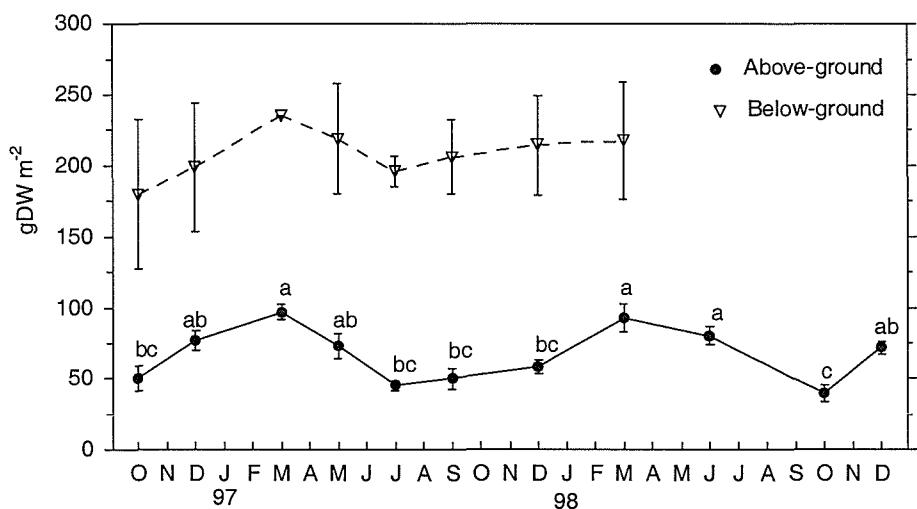


Figure 3-5: Seasonal variations in above and below-ground biomass of *Zostera novazelandica* at Harwood intertidal area (means \pm 1 S.E.). The means from October 1996 to May 1997 were obtained from site H1 and H2 ($n=2$), and those from July 1997 to December 1998 from site H1, H2 and H3 ($n=3$). Like letters (a, b and c) above points indicate that means are not significantly different (Tukey Test at $p<0.05$).

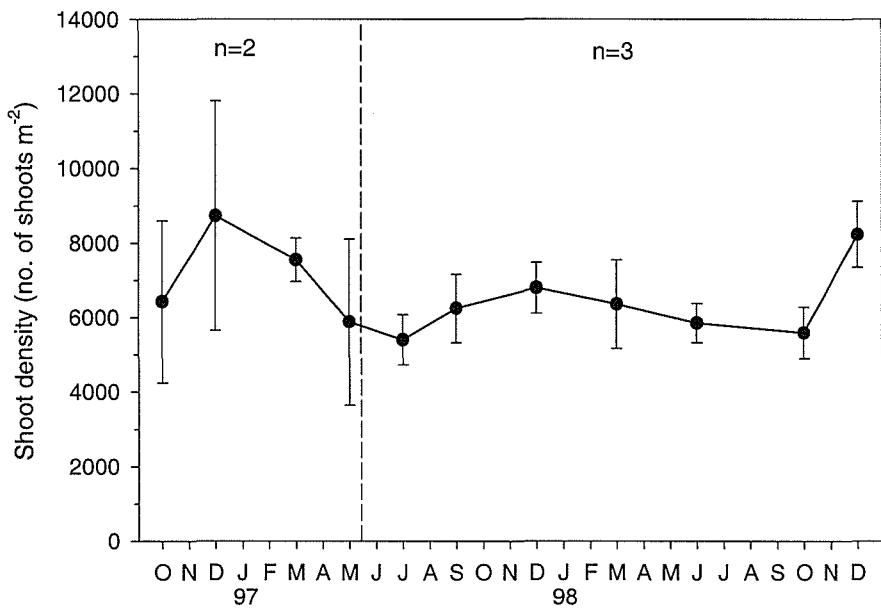


Figure 3-6: Seasonal variation in shoot density of *Zostera novazelandica* at Harwood intertidal, Otago Harbour. Vertical bars indicate ± 1 S.E. of the mean. The means from October 1996 to May 1997 were obtained from site H1 and H2 ($n=2$) and those from July 1997 to December 1998 from site H1, H2 and H3 ($n=3$).

3.3.3 Number of leaves, leaf blade length and leaf area index (LAI)

The mean number of leaves per shoot of *Zostera novazelandica* ranged from 2.8 to 3.5 (Figure 3-7A). The number of leaves per shoot did not vary between sampling months (Tukey Test; $p>0.05$; Table 3-2).

There was a clear seasonal variation in the *Zostera novazelandica* leaf length (Figure 3-7B). The longest seagrass leaves were observed in March 1997 (11.3 cm) and March 1998 (10.7 cm) (Tukey Test, $p<0.05$; Table 3-2). The leaf blades were shortest in December 1996, December 1997 and October 1998, ranging from 7.9 to 9.2 cm.

The seasonal variation of *Zostera novazelandica* leaf area index is shown in Figure 3-7C. Peaks of LAI occurred in March 1997 and March 1998 with values of 4.6 and $3.2 \text{ m}^2 \cdot \text{m}^{-2}$ respectively. The peak in March 1997 was significantly higher than

that in March 1998 (Tukey Test; $p<0.05$; Table 3-2). The LAI was lower, at between 2.1 to $2.9 \text{ m}^2 \cdot \text{m}^{-2}$, from May-December 1997. In 1998, the lowest mean value occurred in October ($1.3 \text{ m}^2 \cdot \text{m}^{-2}$) which was significantly different from those in 1997 (Tukey Test; $p<0.05$).

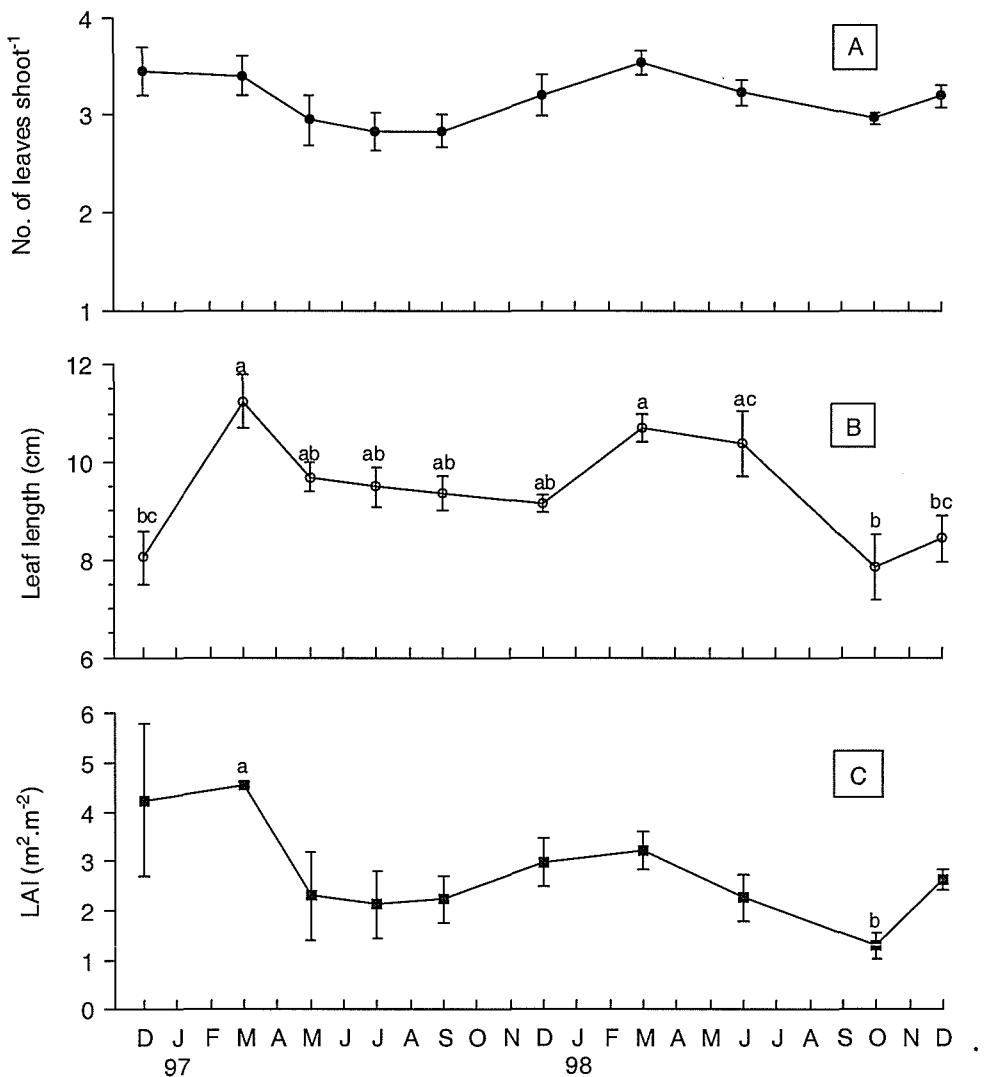


Figure 3-7: Seasonal variation in number of leaves per shoot (A), leaf length (B) and leaf area index (C) of *Zostera novazelandica* at Harwood intertidal, Otago Harbour. Vertical bars indicate ± 1 S.E. of the mean. The means from December 1996 to May 1997 were obtained from site H1 and H2 (n=2), and those from July 1997 to December 1998 from site H1, H2 and H3 (n=3). Like letters (a, b and c) above points indicate that means are not significantly different (Tukey Test at $p<0.05$).

3.3.4 Leaf growth rate and production

The proportional growth rate of *Z. novazelandica* leaves was significantly different between sampling months (Tukey Test; $p<0.001$) as shown in Figure 3-8 and Table 3-2. In 1997 and 1998, growth rates were minimal during winter (June-July) at $< 0.01 \text{ gDW g}^{-1} \text{ day}^{-1}$. The growth rates gradually increased as the season progressed towards spring and summer. The periods of maximum growth differed slightly between years, with peaks in October - December 1996 (spring/summer); December 1997 - March 1998 (summer/autumn) and December 1998, attaining rates of 0.023, 0.021 and $0.028 \text{ gDW g}^{-1} \text{ day}^{-1}$ respectively.

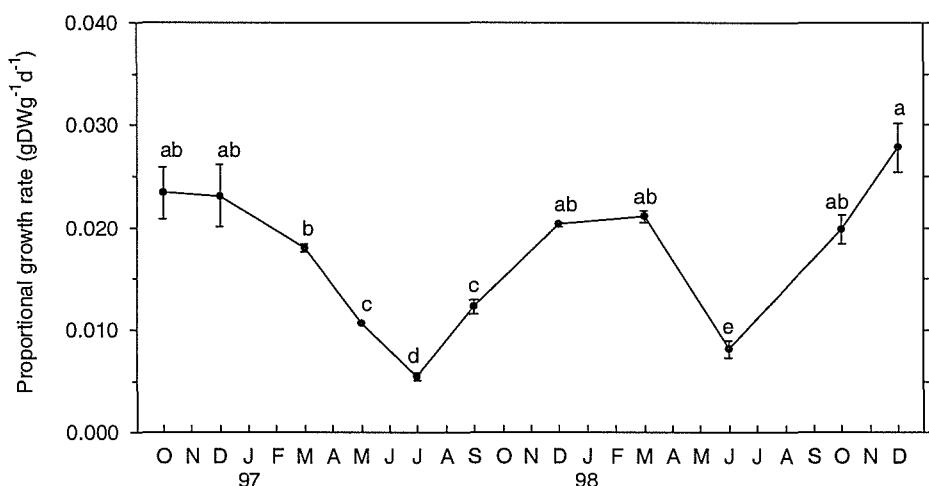


Figure 3-8: Seasonal variation in proportional growth rate of *Zostera novazelandica* leaves in Harwood intertidal, Otago Harbour. Vertical bars indicate ± 1 S.E. of the mean. The means from October 1996 to May 1997 were obtained from site H1 and H2 ($n=2$), and those from July 1997 to December 1998 from site H1, H2 and H3 ($n=3$). Like letters (a, b, c, d and e) above points indicate that means are not significantly different (Tukey Test at $p<0.05$).

The estimate of leaf production was a product of the mean proportional growth rate and the mean above-ground biomass of *Zostera novazelandica* (Equation 3-2). In 1997 and 1998, the summer and autumn production rates were 7 and 3 times greater

than those of winter respectively (Tukey Test; $p<0.001$) (Figure 3-9 and Table 3-2). The reduction of production rate during winter coincided with the reduced above-ground biomass and growth rates in those periods. The peak production rate was between 1.8 and 2.0 gDW m⁻² d⁻¹, while minimum rate was 0.2 and 0.7 gDW m⁻² d⁻¹.

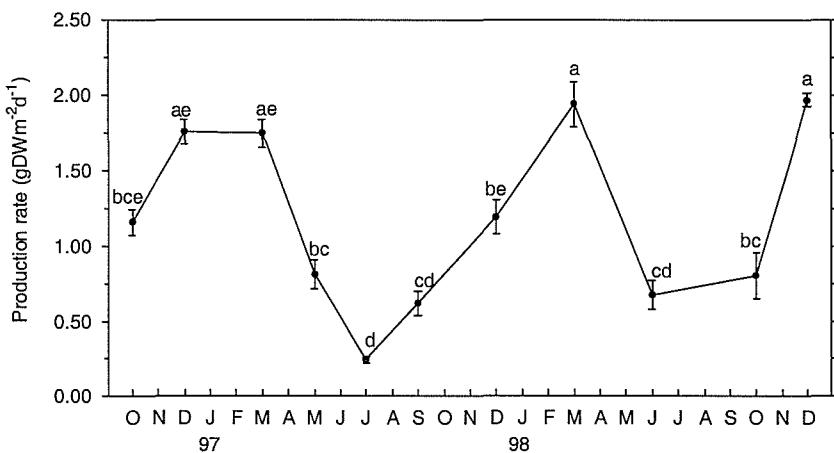


Figure 3-9: Seasonal variation in production rate of *Zostera novazelandica* leaves in Harwood intertidal, Otago Harbour. Vertical bars indicate ± 1 S.E. of the mean. The means from October 1996 to May 1997 were obtained from site H1 and H2 ($n=2$), and those from July 1997 to December 1998 from site H1, H2 and H3 ($n=3$). Like letters (a, b, c, d and e) above points indicate that means are not significantly different (Tukey Test at $p<0.05$).

3.3.5 Environmental parameters

The environmental parameters at the study site during the sampling period are illustrated in Figure 3-10. Air temperature (Figure 3-10A) showed the expected annual variation, peaking during the summer, at 15.5 and 15.7°C in February 1997 and February 1998 respectively. Minimal values were observed in July 1997 (7.6°C) and August 1998 (8.7°C).

The sea surface water temperature (Figure 3-10B) was very close to that of air, with maxima in February 1997 (16.1°C) and February 1998 (15.7°C). The temperature

gradually dropped to minimum values during the winter months, at 7.4°C in July 1997 and 8.0°C in July -August 1998. Its annual variation was about 8-9°C. Incident PFD (Figure 3-10C) fluctuated between winter minima of $18.9 \text{ mol m}^{-2} \text{ d}^{-1}$ (June 1997) and $19.8 \text{ mol m}^{-2} \text{ d}^{-1}$ (June 1998), and summer maxima of $95 \text{ mol m}^{-2} \text{ d}^{-1}$ (December 1996) and $91.4 \text{ mol m}^{-2} \text{ d}^{-1}$ (January 1998).

The concentrations of nitrate, ammonium and phosphate in the surface waters of Harwood seagrass beds are presented in Figure 3-11. Nitrate (Figure 3-11A) had an overall mean concentration during the study period of about $0.71 \mu\text{M}$. Peak values were detected in June 1997 and May-June 1998 (winter), with the concentrations of $3.4 \mu\text{M}$ and $2.9 \mu\text{M}$ respectively. Concentrations below the limits of detection occurred occasionally during spring/summer.

Ammonium concentrations (Figure 3-11B) were more variable than those for nitrate, with the overall mean concentration during the study period of about $2 \mu\text{M}$. Ammonium peaked at $2.5 \mu\text{M}$ in June and December 1997 and remained low ($<1 \mu\text{M}$) in other sampling months of that year. A higher concentration of $> 7 \mu\text{M}$ was measured during September - November 1998, coinciding with the period when nitrate and phosphate were depleted from the water column.

The mean concentration of phosphate was $0.18 \mu\text{M}$ during the study period and no clear seasonal variation was exhibited (Figure 3-11C). Peak values occurred in December 1996 ($0.64 \mu\text{M}$) and March 1997 ($0.47 \mu\text{M}$), and fluctuated between $0 - 0.34 \mu\text{M}$ in the rest of the sampling months. Phosphate was not detected in the seawater from September to November 1998.

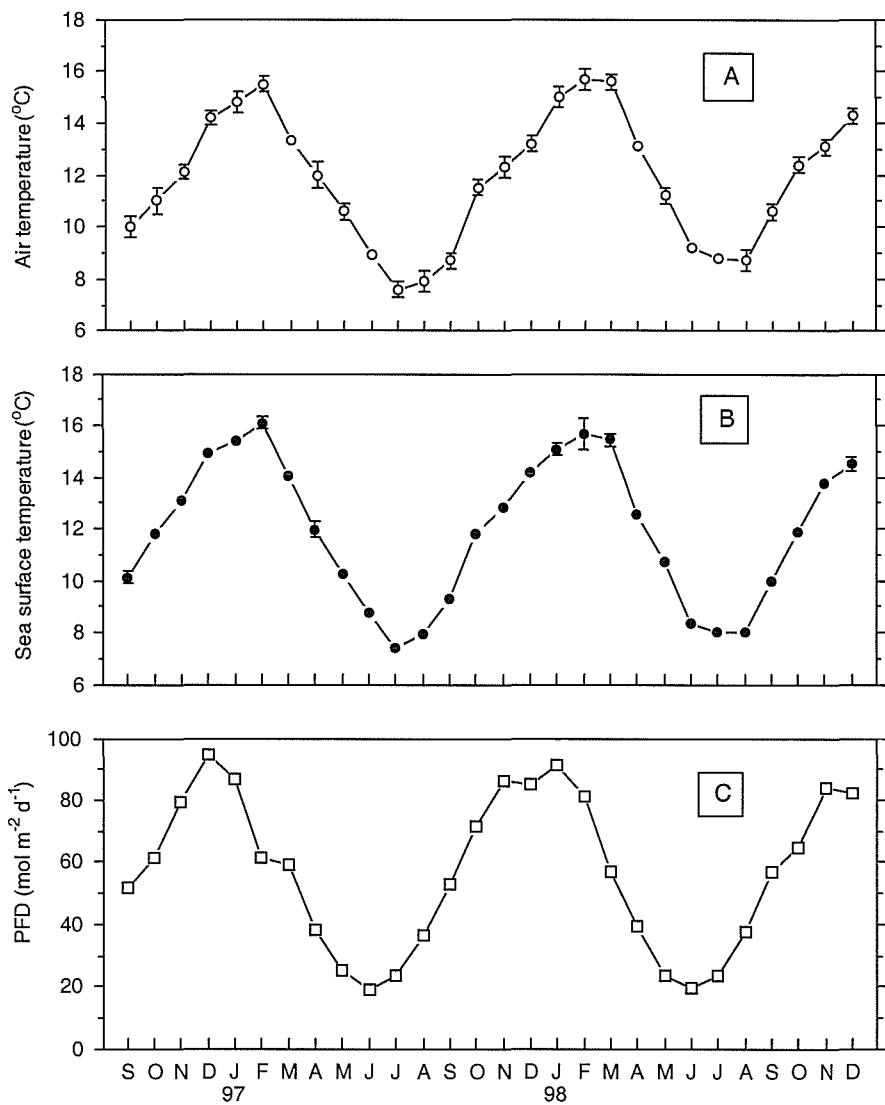


Figure 3-10: Monthly mean of air temperature (A) and sea surface temperature (B) recorded at Portobello Marine Laboratory; and mean daily PFD (C) of Dunedin area for the period of September 1996-December 1998. Points indicate the mean ± 1 S.E.

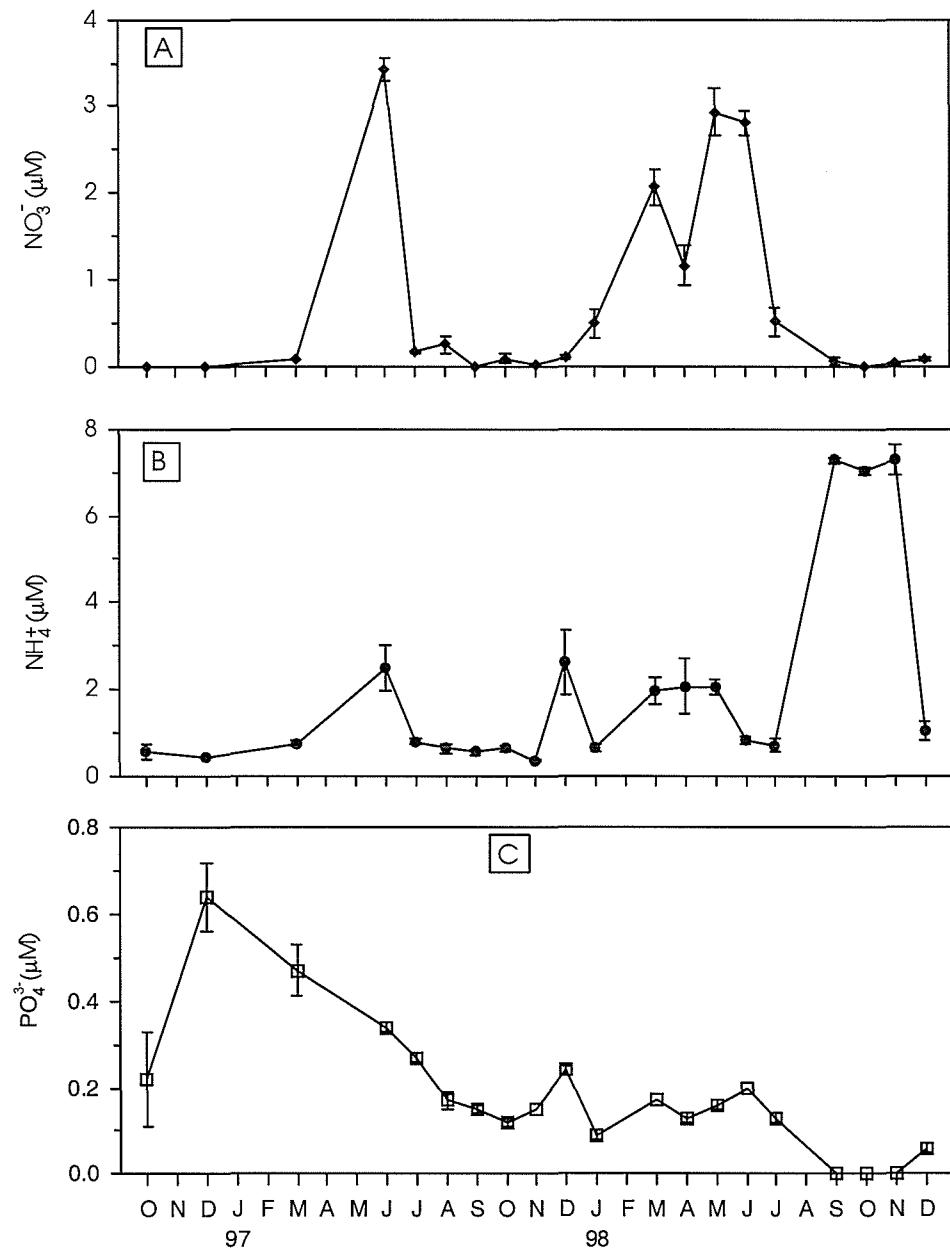


Figure 3-11: Nutrient concentrations in the water column above the *Zostera novazelandica* beds at Harwood, based on frequent sampling during high tide from October 96 - December 1998: (A) nitrate (NO_3^-); (B) ammonium (NH_4^+); (C) phosphate (PO_4^{3-}).

3.3.6 Relationship between environmental factors and the growth of *Zostera novazelandica*

A multiple regression of above-ground biomass of *Zostera novazelandica* against the environmental parameters indicated that 95% of the variance in above-

ground biomass may be explained by air temperature from the previous month, seawater nitrate and ammonium concentrations ($p<0.001$) (Table 3-3). Biomass was positively correlated with air temperature from the previous month and seawater ammonium concentration and negatively correlated with nitrate concentration. Air temperature was the most important predictor for the biomass in the model, as shown by its high standardised coefficient ($\beta=0.84$), followed by ammonium and nitrate (Table 3-3).

The best model to explain the variance in leaf proportional growth rate of *Zostera novazelandica* (89%) included air temperature, PFD and seawater ammonium concentration ($p<0.05$) (Table 3-4). Ammonium concentration did not, however, account for a significant proportion of the overall variance in leaf proportional growth rate. Air temperature and PFD were the most important growth rate predictors in the model, having nearly similar values of standardised coefficient (β), 0.59 and 0.55 respectively. Both these environmental parameters were positively related to leaf proportional growth rate.

Table 3-3: The relationship between the above-ground biomass of *Zostera novazelandica* and air temperature from the previous month, nitrate and ammonium concentrations in the surface seawater.

Variable	Coefficient	P	Standardised Coefficient (β)
Intercept	-10.038	0.437	
Air temperature from previous month	6.638	<0.001	0.84
Seawater nitrate	-2.681	0.011	-0.41
Seawater ammonium	25.148	0.004	0.51
N=10		Overall $r^2 = 0.95$	

Table 3-4: The relationship between the leaf proportional growth rate of *Zostera novazelandica* and air temperature, PFD and seawater ammonium concentrations.

Variable	Coefficient	P	Standardised Coefficient (β)
Intercept	-0.011	0.096	
Air temperature	0.0016	0.038	0.59
PFD	0.0002	0.036	0.55
Seawater ammonium	0.0002	0.119	0.29
N = 10		Overall $r^2 = 0.89$	

3.4 DISCUSSION

3.4.1 Seasonal growth parameters of *Zostera novazelandica*

3.4.1.1 Biomass

Zostera novazelandica is endemic to southern New Zealand where it commonly forms prominent patches in the intertidal zone (Ramage and Schiel 1998). The present results demonstrate, for the first time, growth seasonality in this species. Above-ground biomass ranged from 40 to 97 gDW m⁻² and below-ground biomass from 180 to 235 gDW m⁻². These are comparable to values for *Z. capricorni* in Port Hacking, Australia (Kirkman and Cook 1982), of 40 - 97 gDW m⁻² for above- and 160 - 200 gDW m⁻² for below-ground biomass respectively. As expected for a temperate species, *Z. novazelandica* exhibited a distinct unimodal seasonal pattern in biomass of the above-ground parts, being maximal in late summer and minimal during winter/early

spring. A wide seasonal variation in above-ground biomass published for *Zostera* spp. from different geographical locations and for the closely related *Heterozostera tasmanica* is shown in Table 3-5. The amplitude in above-ground biomass for *Z. novazelandica* from summer maximum to winter minimum was about 2-fold and fell into the lower range of the listed values. The fluctuations were roughly comparable to those of *Z. marina* in Roscoff, France and some Australian seagrasses including *Z. capricorni* and *Heterozostera tasmanica* which showed a 2.5 to 4-fold increase in summer to winter above-ground biomass (Jacobs 1979, Kirkman and Cook 1982, Larkum *et al.* 1984).

3.4.1.2 Flowering

There appeared to be a low incidence of flowering of *Zostera novazelandica* shoots at the study site observed during December and March 1997 and 1998, contributing to <1% of the leaf standing crop. In contrast, a population at the Kaikoura Peninsula, about 450 km north-east of Otago Harbour, began flowering in October and persisted until June, with peak flower production from January to March (Ramage and Schiel 1998). Moreover, reproductive shoots for this population corresponded to >15% of its leaf standing crop during the peak season, suggesting a substantially different allocation of resources than for population at Harwood. In the present study, however, flowering of seagrass was not followed in detail and the data obtained were limited only to plants in the experimental plots. Patchiness of flowering shoots could have resulted in the brief period of flowering, and the low biomass of reproductive shoots recorded at Harwood, as compared to the population at Kaikoura Peninsula.

3.4.1.3 Root-rhizome

At Harwood, the root-rhizome system of *Zostera novazelandica*, which commonly occurred in a mixture of cockleshells (live and dead material) and sand substratum, was rarely found deeper than 20 cm in the sediment, but contributed a major portion of the plant biomass. Biomass of the below-ground parts constantly exceeded those of the above-ground parts, as illustrated by the small variation in the ratio of above to below-ground biomass between winter (0.25) to summer (0.5). In contrast, a perennial population of *Z. marina* in the northern hemisphere had a low winter ratio of above:below-ground biomass (0.59) compared to the ratio in summer (1.31), as a consequence of a greater reduction (e.g. Sand-Jensen 1975) or even complete disappearance of the above-ground materials during the winter (Van Lent and Verschuur 1994). Below-ground parts of *Z. novazelandica* at Harwood did not vary with sampling time, appearing to remain constant throughout the year. Similarly, in the temperate waters of Australia, Kirkman and Cook (1982) reported very little variation in the below-ground biomass of *Z. capricorni*. Also, no seasonal variation was observed for below-ground biomass of *Posidonia australis* (Kirkman and Reid 1979) and estuarine populations of *Halophila ovalis* (Hillman *et al.* 1989). Thus results of the present study further support the statement by Larkum *et al.* (1984) that variation in biomass for southern hemisphere seagrasses is generally less pronounced than for northern hemisphere species. Comparisons made with several studies indicate that seasonal biomass variation in *Z. novazelandica* at Harwood closely resembled that of *Z. capricorni* in Port Hacking, Australia (Kirkman and Cook 1982). Both species show a similar above-ground biomass peak in March (late summer), with a comparable range of minimum and maximum biomass values and with limited variation in the below-ground biomass.

Table 3-5: Comparison of seasonal variation in above-ground biomass for species of *Zostera* and *Heterozostera*.

Species	Location	Ratio of summer to winter above- ground biomass	Reference
<i>Z. novazelandica</i> (intertidal)	Harwood, Otago Harbour, New Zealand	2	This study
<i>Z. capricorni</i> (intertidal)	New South Wales, Australia	2.5	Kirkman <i>et al.</i> (1982)
<i>Z. capricorni</i> (intertidal)	New South Wales, Australia	4	Larkum <i>et al.</i> (1984)
<i>Z. muelleri</i> (intertidal)	Victoria, Australia	7-40	Kerr and Strother (1990)
<i>H. tasmanica</i> (Intertidal/subtidal)	Victoria, Australia	2-4	Bulthuis and Woelkerling (1983b)
<i>Z. marina</i> (intertidal)	Roscoff, France	2.8	Jacobs (1979)
<i>Z. marina</i> (subtidal)	Venice, Italy	6.6	Rigollet (1998)
<i>Z. noltii</i> (intertidal)	Spain	4 - 7	Perez-Llorens and Niell (1993)

3.4.1.4 Shoot density

Populations of *Zostera novazelandica* at Harwood had overwintering shoots, and it can thus be considered perennial, similar to the population reported on the Kaikoura Peninsula (Ramage and Schiel 1998). Shoot density at Harwood ranged from 5,400 to 8,700 shoots m⁻² which far exceeded the highest counts (550 shoots m⁻²) in April measurements for *Z. novazelandica* populations at other localities in the North Island of New Zealand (Turner *et al.* 1996). Shoot density was comparable to that of an intertidal *Z. noltii* population in Venice lagoon, which ranged from 2,000 to 14,000 shoots m⁻² (Sfriso and Ghetti 1998), but lower than a summer maximum of 24,000 shoots m⁻² for the same species in the Netherlands (Vermaat and Verhagen 1996). In

contrast to *Z. noltii*, variation in shoot densities of *Z. novazelandica* did not show a clear seasonal pattern.

Since flowers were found in a very small numbers, it was probable that little recruitment through sexual reproduction occurred during this study. Furthermore, overwintered seeds that germinate in spring as reported for the Kaikoura Peninsula ((Ramage and Schiel 1998), was not observed during this investigation, suggesting that seed germination is not an important propagation mechanism at Harwood. Therefore, reproduction of the plants was probably mainly vegetative, through production of shoots by branching of rhizomes. Most of the plants sampled comprised individual shoots with dead rhizomes, which may indicate that the shoots were derived from old branching rhizomes with disintegration of their connection to lateral shoots, as reported in *Z. marina* (Olesen and Sand-Jensen 1994a).

Maximum development of *Z. novazelandica* vegetative shoots, as observed during summer, might in part be controlled by local substratum characteristics. As previously mentioned, *Z. novazelandica* grew on cockle beds, and a high density of living cockles may limit space availability, although bivalve densities were not determined. Nutrient enrichment of the sediment did not significantly increase the shoot density in summer (Chapter 5), implying that these seagrasses were not nutrient limited during this study. Recently Reusch (1996) revealed negative correlations between infaunal mussel biomass (*Musculista senhousia*) and *Z. marina* shoot densities in a survey conducted in southern California bays. Results of manipulative experiments suggested that the negative interaction was due to mechanical interference of rhizome elongation and branching by the bivalve's byssal mats. Data were not available, however, to enable a seasonal comparison of shoot densities of *Z. novazelandica* inhabiting different types of substratum such as silt, sand or cockle beds

around Otago Harbour or at other localities in New Zealand. Such future studies would be useful in interpreting the shoot density pattern of *Z. novazelandica* communities in New Zealand.

Self-shading at low tide is another factor which may be important for the observed small seasonal differences in shoot density of *Z. novazelandica* at Harwood. With experiments in shaded and unshaded areas, Backman and Barilotti (1976) provided evidence that shoot density in *Zostera marina* was a function of the irradiance the plants receive (in Jacobs, 1979). Similarly, reduced shoot density has been recorded in *Heterozostera tasmanica* under *in situ* screen-reduced irradiance (Bulthuis 1983a). For submerged macrophytes, an indicator that has been used to show self-shading is the biomass density, i.e. the ratio of plant biomass (B) to plant height (V) (in gDW m⁻³) (Duarte and Kalff 1987). The B/V ratio is closely related to the increase in the extinction coefficient of light due to the presence of the macrophytes, as shown by Owens *et al.* (1968) (cf. Duarte and Kalff 1987). Duarte and Kalff (1987) calculated a biomass density of 1000 gDW m⁻³ as a critical limit for the occurrence of self-shading in submerged angiosperms, with values above 1000 gDW m⁻³ indicating self-shading. Similarly, Vermaat *et al.* (1996) calculated a low-tide biomass per water volume ratio (also designated as B/V) for an intertidal *Zostera noltii* population at Zandkreek, Netherlands, since most of the daily production achieved was during low tide. Using maximum above-ground biomass (53 and 64 gDW m⁻²) and low-tide water depth, which was rarely more than 1 cm, the estimated biomass density (B/V) values were 5300 and 6400 gDW m⁻³ respectively. Applying a similar approach for *Z. novazelandica*, with a maximum above-ground biomass of 97 and 93 gDW m⁻² in March 1997 and 1998 respectively, and a low-tide water depth of 1 cm, gives a biomass density of 9700 and 9300 gDW m⁻³. The results of Vermaat *et al.*

(1996) and for *Z. novazelandica* in the present study exceeded the critical limit of 1000 gDW m⁻³ as previously calculated by Duarte and Kalff (1987). Thus, maximum shoot densities observed in *Z. novazelandica* in this study were also likely to be influenced by self shading at low tide under crowded conditions similar to those reported for *Z. noltii* (Vermaat *et al.* (1996)).

3.4.1.5 Leaf numbers

Zostera novazelandica shoots at Harwood generally consisted of 3-4 leaf blades with occasionally 5 blades per shoot. The mean number of leave blades on a single shoot ranged from 2.8 to 3.5, but the number did not differ statistically between summer and winter. This may indicate that the new leaves were continuously produced as old ones were shed. Information on leaf dynamics in seagrasses is, however, very scarce (Ibarra-Obando *et al.* 1997), and few workers have examined seasonal changes in leaf biometry of temperate populations (Hillman *et al.* 1995). This is also the case for *Z. novazelandica* where available data on leaf number are limited to the non-continuous measurements of Turner *et al.* (1996). Turner *et al.* (1996) recorded 2.95 and 3.16 mean leave blades per shoot respectively in April 1995 for seagrass populations at Wairoa Island and Clark Beach in North Island, New Zealand,

3.4.1.6 Leaf morphometrics

The *Zostera novazelandica* leaf blades were longer during March and shorter during October to December although the variation in leaf length was generally less pronounced in 1997 than in 1998. The occurrence of a seasonal trend in leaf length shown in the present study was also reported for *Posidonia sinuosa* and *Z. marina* (Hillman *et al.* 1995 and literature cited therein). In contrast, no seasonal variation was found either for leaf length or width of *Heterozostera tasmanica* in Australia (Bulthuis and Woelkerling 1983) and *Z. marina* in Baja California (Ibarra-Obando *et*

al. 1997). The minimum length of *Z. novazelandica* leaves (7 to 9 cm) at Harwood was usually recorded during spring, probably due to the formation of some new shoots, and shedding of dead leaves from previous winter. This idea is supported by the observation that seagrass shoots bearing brown and darker dead leaves were obvious from July to September 1997, and in October 1998. Isolated patches of seagrass shoots with brownish "burned" looking leaves were also observed in the lower and mid-tidal zone, including some plants in the experimental plots during sampling in December 1997 and 1998. The cause for this localised mid-summer die-back suffered by the seagrass population has yet to be determined. However, the occurrence seemed to coincide with the highest recorded irradiance. Subsequent recovery in late summer/early autumn resulted in very green leaves and high vigour shoots, as seen in March.

Seasonal changes in shoot density (i.e. shoot demography) (Vermaat *et al.* 1987, Olesen and Sand-Jensen 1994a, Vermaat and Verhagen 1996), shoot size and leaf dimension, or both (Rismondo *et al.* 1997), have been correlated to seasonal variation in seagrass biomass. Shoot density and biomass were positively correlated for *Zostera noltii*, *Cymodocea nodosa* and some *Z. marina* populations (e.g. Sfriso and Ghetti 1998, Meling-Lopez and Ibarra-Obando 1999). The increase in biomass may result both from continued development of the old shoots and the addition of young plants during the spring recruitment, or principally by an increase in shoots that survived the winter. As shoot density and number of leaves per shoot of *Z. novazelandica* in the present study was rather constant throughout the year, one would predict that most of the observed variation in above-ground biomass was probably due to changes in leaf dimensions. Consistent with this prediction, leaf length was the

most important factor influencing above-ground biomass, although shoot density was also important.

Analysis of published data compiled from various localities has revealed that *Z. marina* predominantly allocate their biomass increase to increasing shoot size and maintained a stable shoot density (Olesen and Sand-Jensen 1994a). This pattern is normally observed in eelgrass populations growing in stable environments and is also expected to occur in other species that develop dense stands (Olesen and Sand-Jensen 1994a). These characteristics of stable environments and dense stands were observed in the habitat at the present study site. Thus, as for other *Zostera* species, seasonal biomass changes for *Z. novazelandica* at Harwood appear to be influenced most strongly by changes in leaf length rather than shoot density.

The significant changes in leaf dimension found in this study may also have partly contributed to changes in leaf area index (LAI), which fluctuated seasonally from 1.3 to 4.6 m² m⁻². The highest value recorded was 4.6 m² m⁻² in March 1997, which compared favourably with a summer value of 4.0 m² m⁻² in a seagrass population on the Kaikoura Peninsula (Ramage and Schiel 1998). The present leaf area index was also similar to the annual range reported for *Heterozostera tasmanica* (1.0 to 5.9 m² m⁻²) in Australia (Bulthuis and Woelkerling 1983b) and *Z. marina* in Baja California (0.7 to 4.1 m² m⁻²) (Ibarra-Obando *et al.* 1997). However, it was lower than the index range of 4 to 9.5 m² m⁻² for *Z. marina* in Roscoff, France (Jacobs, 1979) and maximum values of 21 m² m⁻² for the same species in Alaska (McRoy, 1970). Leaf area index (LAI) is a useful indicator of potential productivity of a stand of plant vegetation (Hillman *et al.* 1989) as it also indicates the entire photosynthetic surface area of the leaves (Van Lent *et al.* 1991). Since LAI combines leaf size and density, the seasonal pattern of LAI for *Z. novazelandica* was more similar to the temporal

above-ground biomass pattern than to the seasonal pattern of production, as also found by Jacobs (1979), Bulthuis and Woelkerling (1983b) and Hillman *et al.* (1989).

3.4.1.7 Leaf growth rate and above-ground production

The leaf-marking technique, although widely used to measure leaf growth rate, has been criticised in that marking, either by staples or hole punching, may injure the plants (Walker 1985). However, the hole punching method employed in this study had no apparent detrimental effect on the marked *Zostera novazelandica* leaves. A problem was, however, encountered in that the leaf sheaths used for the datum mark were frequently smothered by sediment. Thus, before marking, the sediment had to be mechanically removed to expose the leaf sheath. This problem along with smaller leaf dimensions (average width about 2 mm), rendered the marking process for this species very time consuming. Despite this, results obtained clearly indicated that leaf proportional growth rate for *Z. novazelandica* varied through time, being normally higher from late spring through late summer and lower in winter. Regardless of the monthly variation, the younger leaves of each shoot consistently grew faster than the older leaves.

The leaf growth rate showed a 4-fold difference between maximum and minimum values. This is comparable to *Z. marina* in Europe for which growth rates are 2-3 fold faster during summer than winter (Sand-Jensen 1975, Jacobs 1979). Leaf growth rates in some Australian seagrasses also exhibit a 2 to 3-fold difference between summer and winter as recorded for *Posidonia australis* and *P. sinuosa* (Kirkman and Reid 1979, Cambridge and Hocking 1997), and between 1.4 - 2-fold for *Z. capricorni* (Kirkman and Cook 1982, Larkum *et al.* 1984). Faster summer leaf growth rates were also observed in *Heterozostera tasmanica* (Bulthuis and

Woelkerling 1983b) and *Z. muelleri* (Kerr and Strother 1989), up to 5-fold and 2 to 4-fold greater than the winter rate respectively.

Above-ground production of *Zostera novazelandica* also showed a seasonal trend with a progressive increase during spring, reaching a maximum in summer/early autumn, corresponding to the maximum biomass and leaf proportional growth rate. Seasonal changes in production rate for this species were of greater magnitude than changes in biomass, as has generally been reported for other seagrasses (Hillman *et al.* 1989). Table 3-6 compares the leaf primary production rate in *Z. novazelandica* with other species of temperate seagrasses from different geographical locations, in which the leaf-marking technique was used.

Table 3-6: Published values (minimum - maximum) of leaf production in some temperate seagrasses using leaf-marking method.

Species	Locality	Leaf production (gDWm ⁻² d ⁻¹)	Reference
<i>Z. novazelandica</i>	Harwood, Otago Harbour, N. Zealand	0.2 - 2.0	This study
<i>Z. marina</i>	Otsuchi Bay, Japan	1.0 - 22.0	Iizumi, (1996)
<i>Z. marina</i>	Baja California, Mexico	0.21 - 1.9	Ibarra-Obando <i>et al.</i> (1997)
<i>Z. marina</i>	Roscoff, France	1.0 - 3.0	Jacobs (1979)
<i>Heterozostera tasmanica</i>	Western Ports & Port Phillip Bay, Australia	0.34 - 4.2	Bulthuis and Woelkerling (1983b)
<i>Z. capricorni</i>	Port Hacking, Australia	0.3 - 2.5	Kirkman and Cook (1982)
<i>Z. capricorni</i>	Botany Bay, Australia	1.37 - 8.92	Larkum <i>et al.</i> (1984)
<i>Posidonia australis</i>	N.S.W. Australia	0.7 - 5.5	West and Larkum (1979)
<i>Cymodocea nodosa</i>	Northern Adriatic Sea	0 - 4.0	Pedduzi and Vukovic (1990)

It appears that the range of seasonal fluctuation of leaf primary production of *Zostera novazelandica* is very much narrower than that reported for a *Z. marina* community from Otsuchi Bay, Japan (Iizumi 1996). However, the summer maximal production of $2.0 \text{ gDW m}^{-2} \text{ d}^{-1}$ measured in the present study compared well with *Z. marina* populations in France (Jacobs 1979) and Baja California (Ibarra-Obando *et al.* 1997), and with *Z. capricorni* in Port Hacking Australia (Kirkman and Cook 1982). These data were also within the range of leaf production values reported for other southern hemisphere species such as *Heterozostera tasmanica* (Bulthuis and Woelkerling 1983b) and *Posidonia australis* (West and Larkum 1979). In contrast to *Cymodocea nodosa* (Pedduzi and Vukovic 1990), leaf primary production of *Z. novazelandica* did not stop during winter, when a rate of $0.2 - 0.7 \text{ g DW m}^{-2} \text{ d}^{-1}$ was measured.

3.4.2 Relationship with environmental parameters

Zostera novazelandica at Harwood is subject to a wide range of temperatures including seasonal changes in air and sea temperatures, and daily fluctuation due to tidal rise and fall. The monthly mean sea surface water temperature was very close to that of air, ranging from a minimum of about 7°C in July - August to a maximum of 16°C in February. In shallow tidepools, seagrass plants experienced slightly higher water temperature, for example, about 23°C during *in situ* measurements taken at noon in December 1996 during a clear sky (data not shown). The maximum temperature recorded was below the optimum temperature of $25 - 35^\circ\text{C}$ for photosynthesis at light saturation recorded for most seagrasses (Bulthuis 1987). Mean daily PFD ranged from $18.9 \text{ mol m}^{-2} \text{ d}^{-1}$ (June 1997) to $95 \text{ mol m}^{-2} \text{ d}^{-1}$ (December 1996). Pattern of seasonality for these three physical environmental parameters (i.e. seawater and air temperatures, PFD) clearly indicated a lag in seawater and air temperatures.

Mean concentrations of dissolved nutrients in seawater during the study period, for nitrate, ammonium and phosphate were 0.71, 2.0 and 0.18 μM respectively. Concentrations of ammonium and phosphate obtained were similar to the typically low mean values reported for the water column of seagrass meadows world-wide, being approximately 3.1 and 1.0 μM respectively (Hemminga 1998). The nitrate concentration was usually high during winter and low during summer, probably due to an annual cycle of vertical mixing of the surface and deep water in Otago Harbour. Winter peak values of nitrate at Harwood (2.0 - 3.4 μM) were, however, slightly lower than those reported (7-11.5 μM) in the water column of kelp beds, *Undaria pinnatifida*, at Carey's Bay (Stuart 1997, Dean 1998), located about 5 km to the north-west of the present study site. Ammonium concentrations in seawater above the seagrass meadow were more variable, while phosphate did not show any specific trend through time.

An interactive combination of climatic factors is believed to be important in determining the seasonal growth cycle in seagrasses. There is general agreement that light and temperature are the first two environmental variables that need to be considered when growth and production are evaluated (c.f. Ibarra-Obando *et al.* 1997 and list of literature cited therein). In the case of biomass, some workers have inferred the importance of light and temperature by comparing the seasonal curves of the abiotic parameters with those of the biomass pattern (e.g. Sand-Jensen 1975, Kirkman and Reid 1979, Aioi 1980, Kirkman and Cook 1982, Bulthuis and Woelkerling 1983b, Larkum *et al.* 1984, Orth and Moore 1986). Others have relied on correlations of the measured environmental variables with variation in biomass (e.g. Kerr and Strother 1990, McKenzie 1994, Rigollet *et al.* 1998). In the present work, however, results of multiple regression analyses indicated that air temperature of the previous month and

water column ammonium and nitrate concentrations were significant factors contributing to above-ground biomass variation. Results of the analyses also suggested that air temperature alone had sufficient ability to predict the above-ground biomass ($\beta=0.84$), and was hence the most important controlling abiotic factor. Whether this interpretation is correct is difficult to elucidate, because the monthly means of this variable were similar to those for seawater, and the latter is largely determined by the amount of incoming solar radiation (Hillman *et al.* 1989). Yet, the above-ground biomass declined when temperature and light were minimal during the short day lengths of winter.

The present result is similar to that of (Vermaat and Verhagen 1996), in which air temperature (max. of $<20^{\circ}\text{C}$) was positively correlated with biomass, suggesting that it accounted for the seasonal variation in intertidal *Z. noltii* in The Netherlands. In contrast, a dominant but negative influence of air temperature on above-ground biomass had been reported for tropical intertidal meadows of *Z. capricorni* in northern Queensland, Australia (McKenzie 1994). In this case, reduction in biomass was suggested to be due to longer duration of daytime exposure when air temperature (25.3°C) was higher than that of seawater (19.8°C). Similarly, during summer, the temperate seagrass *Heterozostera tasmanica* in Victoria (Australia) showed above-ground biomass reduction and a decline in photosynthesis due to exposure and desiccation stress at spring low tides when air and seawater temperatures differed, 40°C and 35°C respectively during summer (Bulthuis and Woelkerling 1983b).

Temperature can affect seagrasses by regulating processes such as nutrient uptake and availability (Bulthuis 1987). In this study, N availability was responsible for (at least part of) the observed seasonal changes in above-ground biomass, which was positively correlated with ammonium concentration, and inversely related to

nitrate concentration in sea water. The greater importance of seawater ammonium compared with nitrate as a source of nitrogen, has also been reported by Hemminga *et al.* (1994) for an intertidal *Z. marina* population at Zandkreek (The Netherlands). As seasonality in porewater nutrients at the study site were not determined in this study, effects of rhizosphere nutrients on seasonal production is not known. Hemminga *et al.* (1994) also speculated that leaves, which were lying on a moist sediment surface at low tide, might be capable of direct uptake of nutrients from the sediment porewater. Although this has yet to be confirmed, the direct uptake may help *Zostera novazelandica* to incorporate more nutrient particularly during high summer temperature. The assimilated nutrient may be used by the plant in the production of new tissues thus stimulating the increase in leaf extension that mostly contributed to the above-ground biomass increase. This idea is supported by the results of an *in situ* enrichment experiment described in Chapter 5, in which a significant increase in shoot height was observed in fertilised compared to unfertilised plots.

Seasonal variation in leaf primary production measured in this study is a function of leaf growth rate and above-ground biomass. Any factor that influenced the seasonality of these two parameters would have directly affected the leaf production. The rate of leaf growth of *Zostera novazelandica* was highest in late spring - late summer, when PFD and air and surface seawater temperatures were high, and lowest in winter when these variables were low. Results of statistical analyses suggested that variation in the leaf growth rate of *Z. novazelandica* was positively correlated with a combination of air temperature, PFD and seawater ammonium, although the latter did not account for a significant contribution. Interaction of light and temperature in field situations makes the individual effects of these environmental variables difficult to study, and has led to some disagreement and confusion as to whether light or

temperature, is predominantly responsible for seasonality in growth rates of seagrasses (c.f. West and Larkum 1983). In some studies, irradiance was highly correlated with leaf growth rates, (e.g. *Z. marina* (Sand-Jensen 1975, Jacobs 1979, Aioi *et al.* 1981)) while others reported temperature as a more significant factor (e.g., *Posidonia australis* (Kirkman and Reid 1979) and *Z. capricorni* (Kirkman and Cook 1982, Larkum *et al.* 1984)). High correlation of leaf growth rate with irradiance and daylength was also reported in *Zostera muelleri* (Kerr and Strother 1989). An indication of the importance of irradiance and temperature provided by the statistical analyses of field data was further supported by the results of controlled laboratory experiments described in Chapter 4. Here, the interaction between irradiance and temperature had a significant influence on the leaf growth rate of *Z. novazelandica*.

Moreover, lack of significant correlation of ammonium with the observed leaf growth rate was in agreement with results of leaf growth rate responses to summer field N-enrichment (chapter 5). It seems, therefore, less likely that the temporal variation of leaf growth rate for *Zostera novazelandica* at the study site was controlled by nutrient availability.

3.4.2.1 Turnover rate

The mean turnover rate is a measure of the relative efficiency of plants in producing new leaf material (Cambridge and Hocking 1997) and can be estimated from the ratio of production to above-ground biomass (P/B) (Brouns 1985). For *Zostera novazelandica*, the mean turnover rate of the leaves (percentage change in leaf biomass day⁻¹), was 1.7% day⁻¹ with a higher rate of 2% day⁻¹ recorded during October - March. This turnover rate is comparable to the rates of 1.3 - 1.7 in *Heterozostera tasmanica* in Australia (Bulthuis and Woelkerling 1983b), and a maximum value of 1.8% day⁻¹ for *Posidonia australis* (West and Larkum 1979). Furthermore, the estimated annual leaf

production during this study was about 434 gDW m⁻², similar to *Heterozostera tasmanica* (414 gDW m⁻²) and *Zostera marina* (407 gDW m⁻²) (Nienhuis and de Bree 1980, Bulthuis and Woelkerling 1983b).

3.4.2.2 Epiphytes

Another factor that could have contributed to differences between summer and winter growth rates was changes in epiphytic algal load, probably through effects of shading, as has been observed in *Heterozostera tasmanica* (Bulthuis and Woelkerling 1983a) and *Posidonia australis* (Silberstein *et al.* 1986). Although the present study did not attempt to investigate epiphytes quantitatively, they were very obvious during June-August and epiphytic algae persisted within the study plots until December. The epiphytes (including *Enteromorpha* sp., *Polysiphonia decipiens*, *Cerramium flaccidum*) grew on surfaces of older leaves, and other seaweeds (*Tenocladia novae-zelandiae*, *Adenocystis utricularis*, *Colpomenia peregrina*, *Codium fragile*) were anchored to the cockle shells on the sediment. Epiphytes, however, were seldom apparent on the surfaces of marked *Z. novazelandica* leaves, but the seagrass plants were occasionally covered by a drift macroalga, *Ulva* sp., at low-tide which would have blocked out the light supply.

3.4.2.3 Importance of seagrass production to local ecology

Otago Harbour, particularly the Harwood intertidal area, provides a favourable environment with respect to light and temperature so that growth and production of *Zostera novazelandica* occurs all year round. The majority of biomass production probably stays in the ecosystem after shedding or fragmentation, as evidenced by the substantial amount of particulate organic matter, from seagrass that was normally observed in the core samples. Seagrass production in Otago Harbour, which until recently has not been given any attention, may be significant in supporting local food

webs, considering the high production rate, and the stability and wide area of coverage (Chapter 2) of the seagrass population there. This is also reflected by the fact that the area attracts various wading birds as a feeding ground and is also habitat for benthic invertebrates. Black swans, which directly consumed the plants, frequently leave patches of bare sediment when they dig up live rhizome material. The mud crab *Macrophthalmus hirtipes*, a species endemic to New Zealand, has been reported living as deposit feeder and as a grazer of seagrass on the Kaikoura Peninsula (Woods and Schiel 1997). The activity of waterfowl and crabs may have affected the abundance of seagrass at Harwood during this study. However, the extent of their grazing pressure is unknown since no quantitative estimates are available.

Chapter 4

Laboratory examination of the influence of temperature, light and nutrients on the growth rate and physiology of *Zostera novazelandica*

4.1 INTRODUCTION

It has been widely demonstrated from various field studies that seagrass habitats in temperate regions fluctuate seasonally in their biomass and productivity, being normally higher in summer and lower during the winter (e.g. Vermaat and Verhagen 1996, Rigollet *et al.* 1998, Sfriso and Ghetti 1998). It is generally accepted that these seasonal variations are attributable to periodic fluctuations of environmental factors, particularly irradiance and temperature (e.g. Sand-Jensen 1975, Perez and Romero 1992). Local factors (e.g. nutrient availability, dissolved inorganic carbon, redox potential) have also been suggested, but are considered secondary controlling factors that influence local variability of the seasonal pattern (Alcoverro *et al.* 1995).

Most ecological studies of seagrass have been descriptive, in which factors controlling growth and development have been inferred from correlations observed in the field (Backman and Barilotti 1976). Due, however, to interactions between these controlling factors in nature, it is difficult to evaluate the relative importance of individual factors on the growth of aquatic plant using field data alone (Hillman *et al.* 1995). For example, it is difficult to separate the effects of light and temperature, since in most aquatic systems seasonal variations in insolation cause corresponding variations in water temperature (in Hillman *et al.* 1995). Similarly, in *Zostera marina*, lack of photosynthesis at night hinders the seagrass roots from assimilating nitrogen available in the sediments. As nutrient uptake is significantly affected by light, this would confound the effects of

nutrient availability (Zimmerman *et al.* 1987). Therefore, controlled laboratory experiments are desirable to investigate the combined influences of those inferred controlling factors on the growth of the seagrasses.

A few laboratory experiments have been conducted to study the response of seagrass to environmental parameters. These include studies on the single and interactive effects of seawater nutrient concentration and photosynthetic active radiation (PAR) on the growth response of *Zostera marina* and its epiphytes (Moore and Wetzel 2000). Results from the 4–6 week microcosm experiments performed in a greenhouse supplied with flow-through seawater suggested that light availability was the principal factor governing seagrass growth (Moore and Wetzel 2000). Short and Burdick (1995) examined the mechanisms responsible for the eutrophication-related decline in *Z. marina* using an outdoor mesocosm. The response of the seagrass to excess nutrient loading and reduced light, which simulated coastal eutrophication, was noted. Reduced light and elevated nutrient levels in the water column each significantly affected the seagrass growth rate, morphology, density and biomass (Short and Burdick 1995). Outdoor aquaria have also been used by Tomasko and Lapointe (1991) in their studies on the productivity and biomass of *Thalassia testudinum* in relation to water column nutrient availability and epiphyte levels. The results of these aquarium studies were shown to approximate those from the field studies, where elevated nutrient levels caused the increase in epiphyte levels and decreased blade turnover rates (Tomasko and Lapointe 1991). Finally, the individual effects of salinity, temperature and light on growth rate have been studied in *Halophila ovalis* cultured in the laboratory (Hillman *et al.* 1995). It was found that their growth rates were high at salinities of 10-40‰, and under low light intensity, but severely restricted at temperature below 15°C (Hillman *et al.* 1995).

Growth rates of intertidal populations of *Zostera novazelandica* in Otago Harbour, southern New Zealand are highest in summer and lowest during winter (Chapter 3). Field

studies suggested that seasonal variation in growth rate, production, biomass and morphometry are linked in part to changing environmental conditions (Chapter 3). Some of the seagrass growth parameters such as growth rate were positively correlated with temperature and light. Since the field data provide only correlative information on the controlling factors associated with the seagrass seasonal variation, it cannot be certain that these factors were the causative agents. Although laboratory experiments on this species have previously been carried out (Ramage and Schiel 1998), the studies were limited only to factors that may control its reproduction. Therefore, the present study was designed to investigate on the possible interactive effects of temperature, photon flux density (PFD) and nutrient enrichment (N and P) on the growth of *Zostera novazelandica*. In this study, the growth responses of seagrass plants (proportional leaf growth rate, chlorophyll *a* and *b* pigment contents, ratio of variable to maximal chlorophyll fluorescence ($F_v:F_m$) and tissue nutrient contents) to changes in these physico-chemical parameters were monitored in indoor aquarium experiments.

4.2 MATERIAL AND METHODS

Experiments to test the interactive effects of temperature, PFD and nutrient addition on the growth rate and physiological parameters of *Zostera novazelandica* were based on a split-plot design, with 2 levels of temperature (high: 18°C; low: 8°C), PFD (high: approx. 150 µmol quanta m⁻² s⁻¹; low: approx. 45 µmol quanta m⁻² s⁻¹), and nutrients (unenriched; enriched). These studies required eight, 40 x 25 x 25 cm glass aquaria, each of which was randomly assigned to the treatments as shown in Figure 4-1 and Table 4-1.

These laboratory studies were conducted in two replicate 21-day experiments. Space limitations and the time required to process samples meant that replicate experiments could not be conducted simultaneously and the whole experiment was

therefore conducted twice, during October (Experiment 1) and November 1998 (Experiment 2).

4.2.1 Plant collection

Samples of *Zostera novazelandica* were collected on the day the experiments began, from Harwood intertidal area, between plots H2 and H3, details of which are described in previous chapter (Chapter3, Figure 3-1). Between 70 and 80 seagrass cores were randomly taken at low tide, using a stainless steel corer of 10 cm diameter and 25 cm length. The core samples were sieved underwater through a 2 mm mesh sieve, to remove sediment, and placed in cool boxes filled with seawater collected from the site. They were transported to Portobello Marine Laboratory, University of Otago, 10 minutes away, and processed on the same day.

Seagrass materials were cultured under laboratory conditions using a method modified from Ramage and Schiel (1998). All core samples were combined, and then ten individual *Zostera novazelandica* plants, each of similar appearance, were selected from the samples and grouped as a planting unit (Figure 4-1). A total of 64 of these planting units with similar wet weights (12 - 15 g) were used for each of 2 replicate experiments. Each planting unit was placed immediately in a 250 mL polystyrene cup, which was filled with fine sand to about 2 cm from the top to cover the below-ground parts of the seagrass plants. The sand was collected from the sandflat adjacent to the seagrass sampling area.

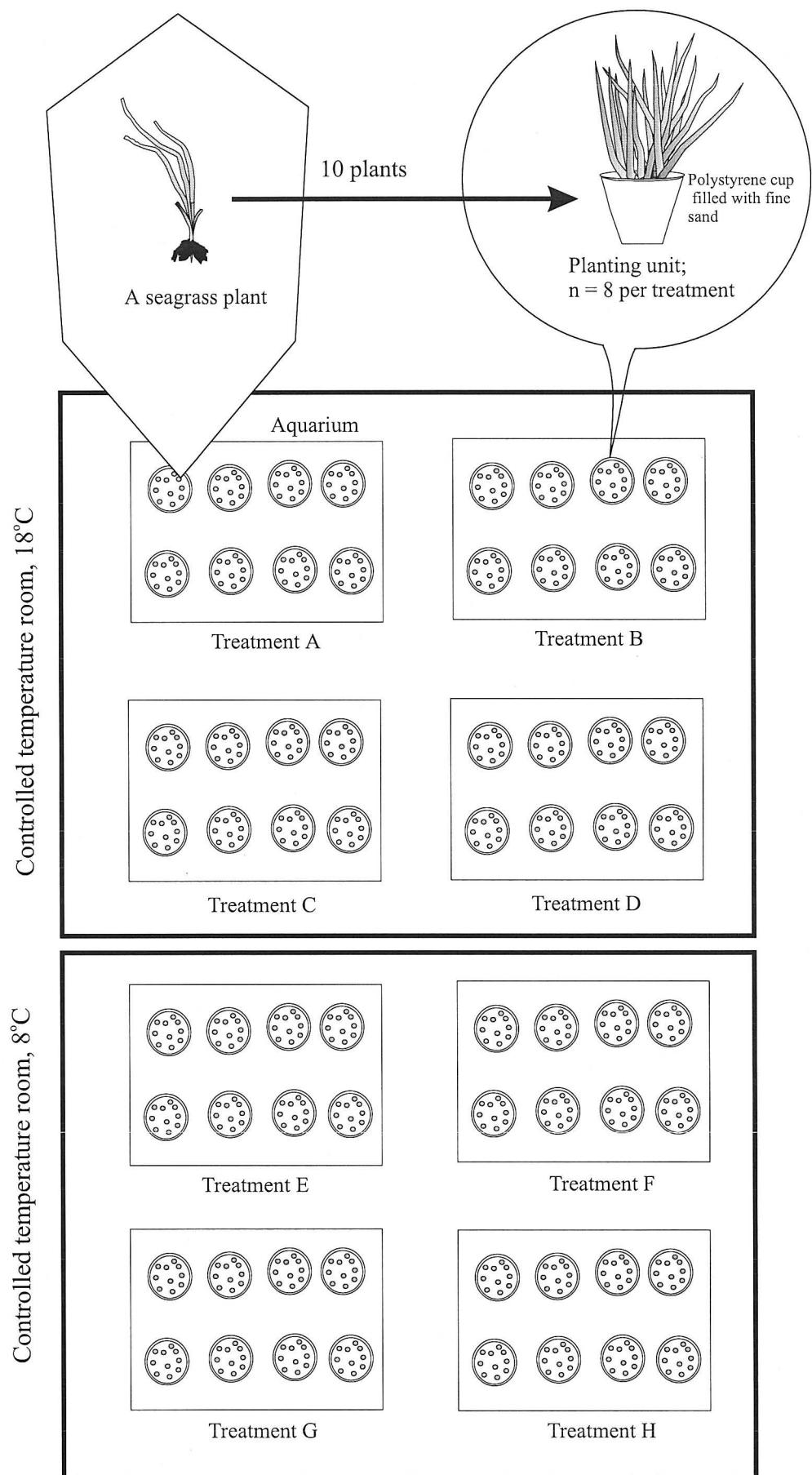


Figure 4-1: Schematic of the split-plot experimental design used to test the interactive effects of temperature, PFD and nutrients on *Zostera novazelandica* growth rate and physiology. Details of treatments A to H are explained in the text and in Table 4-1.

Table 4-1: The combination of environmental variables used for each treatment.

Treatment	Environmental factors		
	Temperature °C	PFD μmol quanta m ⁻² s ⁻¹	Nutrient
A	18	150	Unenriched
B	18	150	Enriched
C	18	45	Unenriched
D	18	45	Enriched
E	8	150	Unenriched
F	8	150	Enriched
G	8	45	Unenriched
H	8	45	Enriched

4.2.2 Experimental design

Eight replicate *Zostera novazelandica* planting units (n=8) were randomly placed in each of the 8 aquaria (Figure 4-1 and Figure 4-2). The aquaria assigned for treatments A, C, E and G were filled with 16 L of low nutrient seawater (LNS) (described below). The other four aquaria were used for treatments B, D, F and H and received 16 L of LNS enriched with 1.6 g L⁻¹ fertiliser (N : P : K = 25 : 2 : 4; Yates™ Gro-plus Professional). Aeration was provided by an air pump with a manifold to which were connected airline tubing and 6 evenly positioned air-stones in each aquarium.

Experiments were performed in two temperature-controlled rooms (8° and 18°C), and PFD for each aquarium was supplied by 2 fluorescent tubes, a cool-white fluorescent (36Watt; Osram Sylvania®) and a full spectrum light tube (36 Watt; Aquarella®), hanging

overhead, on a 12/12 Light : Dark cycle. PFD was manipulated to give 150 and 45 μmol quanta $\text{m}^{-2} \text{s}^{-1}$ using different thicknesses of shade cloth, suspended about 10 cm above the top of the aquaria. PFD in each aquarium was measured at the surface of the seawater, at 5-6 different points using a Li-Cor quantum sensor and the average taken. The PFD variance within a single aquarium, between aquaria of low light treatments and between aquaria of high light treatments was <15%, 7% and 21% respectively.

All aquaria were scrubbed clean of periphyton (about once a week) using a scouring pad. Seawater in each aquarium was replaced every 3 days using stock (filtered) that had previously been pre-conditioned to the respective temperatures. To reduce the effect of variation in PFD that occurred within each aquarium, planting units were repositioned every 3 days.

4.2.3 Low nutrient seawater (LNS)

Stocks of low nutrient seawater (LNS) were prepared by immersing over night three blades of the kelp *Macrocystis pyrifera* in buckets containing 20 L of 1- μm filtered seawater. The seaweed blades were of nearly equal size, collected from the pier off the Portobello Marine Laboratory and wiped clean with tissue paper before use. The LNS was prepared in the 18°C controlled-temperature room. The seawater in the buckets was aerated, with a PFD provided during the day from the ceiling lighting system of the controlled-temperature room (the light level was not determined). The seawater was re-filtered (1- μm) and then acclimatised in the respective controlled-temperature rooms prior to the experiment. Stocks of nutrient-enriched seawater were prepared using fertiliser granules (1.6 g L^{-1}) dissolved in the LNS.



Figure 4-2: Experimental set up within the 18°C temperature-controlled room of the *Zostera novazelandica* laboratory studies under (i) high and (ii) low PFD. Photographs show the *Z. novazelandica* planting units (A), light source (B) and shade cloth (C) to reduce the PFD. Seawater was not enriched in aquaria 1 and 3, but was enriched in aquaria 2 and 4.

Duplicate 30 mL seawater samples were taken from the LNS bucket and the nutrient-enriched seawater bucket for nutrient analysis using the automated ion analyser (QuickChem® 8000, Lachat Instruments).

4.2.4 Physiological responses measured

4.2.4.1 Proportional leaf growth rate

The proportional growth rate of *Zostera novazelandica* leaves was measured using a hole punch marking technique of Kirkman and Reid (1979). At the beginning of the experiment, a single seagrass shoot from each planting unit within each aquarium was randomly selected ($n=8$ per aquarium) and a pinhole was made through its leaves (as previously described in Chapter 3). Each of the selected seagrass plants was tagged for identification using a coloured, thin, plastic-coated wire tied around its base near the sediment surface.

The shoots of the individually marked seagrass plants in each planting unit of each aquarium were harvested on day 7, at which time another eight shoots were marked. This procedure was repeated on days 14 and 21. All samples for growth rate measurements were processed as previously described (Section 3.2.2.3 of Chapter 3). The proportional leaf growth rate (PGR) of *Zostera novazelandica* in each aquarium was calculated according to Larkum *et al.* (1984) as previously described.

4.2.4.2 Pigment content

The second youngest leaf of a single *Zostera novazelandica* in 3 or 4 separate planting units of each aquarium ($n=3$ or 4) was cut and used to determine levels of chlorophyll *a* and *b*. Sampling was carried out at the start of the experiment and then weekly on days 7, 14 and 21. The samples were placed in polyethylene bags, sealed and

immediately frozen at -20°C before being analysed, which was normally carried out within a week.

The leaf materials (approximately 15 mg wet weight) were chopped into small pieces and then immersed in 5 mL of N, N-dimethylformamide (DMF) in screw-cap glass tubes for chlorophyll extraction (Dunton and Tomasko 1994). The tubes were wrapped in aluminium foil and stored for 24 hours at room temperature (12° - 15°C). The absorbance of the chlorophyll extracts was then measured in a UV spectrophotometer (Pharmacia) at 664, 647, and 750 nm, and concentrations of chlorophyll *a* and *b* were determined (Porra *et al.* 1989).

4.2.4.3 Tissue carbon and nitrogen

Four of the 8 dried seagrass samples taken on day 14 of the growth rate measurements were ground to a fine powder with a mortar and pestle (n=4 per aquarium). Due to funding restrictions, it was not possible to analyse C and N of all samples, and because seagrasses did not survive beyond the end of 21 days in some treatments, plants sampled on day 14 were, therefore, used for comparisons. Samples were analysed for elemental C and N using a Dumas elemental analyser (Carlo Erba Instruments-CHNS-0 EA1108).

4.2.5 $F_v:F_m$ ratio

Photosynthetic activity of the *Zostera novazelandica* was assessed by a chlorophyll *a* fluorescence technique, a non-destructive probe of the photosystem II (PSII) photochemical processes, using a recently developed pulse-amplitude modulated (PAM) fluorometer (Waltz, Germany) (Ralph *et al.* 1998). Eight shoots of *Z. novazelandica* (one from each planting unit) were randomly selected from each aquarium (n=8) at noon on

days 7, 14, 21, and its photochemical efficiency which is the ratio of variable fluorescence to maximal fluorescence ($F_v:F_m$ ratio) was measured. On each sampling occasion, two leaves of each selected seagrass shoot were first dark adapted for 10 min using a dark-adaptation clip (Ralph 1998). The clip was placed at a standard position, approximately in the middle of the leaf blades and the fluorescence signal was later determined.

4.3 DATA ANALYSIS

Experiment 1 and experiment 2, which were conducted over two different time periods, were treated as replicates. Data for growth rate, chlorophyll *a* and *b* content and $F_v:F_m$ ratio were averaged for each of the 8 treatment aquaria. The mean values from the 2 replicate experiments were then calculated ($n=2$) and the results plotted ($\pm 1S.E.$; $n=2$). The means of C, N and C:N for the period 7 - 14 day were calculated in the same way ($n=2$).

Data were analysed using a split-plot ANOVA, using the software package Statistix (Novell, Statistix. Ink). Temperature was assigned to the main plots, while PFD and nutrients were subplots. Results of each seagrass growth parameter for each treatment from the two replicates were pooled to increase the number of degrees of freedom for analysis of variance (Sokal and Rohlf 1995, Ramage and Schiel 1998).

For growth rate, chlorophyll *a* and *b* content, and $F_v:F_m$ ratio, the data were separately analysed for each week. The significant effects of the interaction between temperature, PFD and nutrient additions were tested against the *Zostera* growth parameters with the following model statement:

Model statement:

- Temperature X PFD
- Temperature X PFD X Replication (Error)
- Temperature X Nutrient
- Temperature X Nutrient X Replication (Error)
- Temperature X Nutrient X PFD
- Temperature X Nutrient X PFD X Replication

Because of the experimental design, the analysis has no statistical power to test for the individual effects of temperature, PFD and nutrient, thus only the interactive effects of these variables were tested.

4.4 RESULTS

4.4.1 Seawater nutrient concentration

The concentrations of ammonium, nitrate and phosphate in the LNS seawater samples measured during the experiments ranged between 1-5, 2-6 and 0.2-2 µM respectively. The concentrations of these nutrients in enriched seawater were 1000x higher, approximately 3.5, 5.0 and 3 mM, for ammonium, nitrate and phosphate respectively.

4.4.2 Proportional growth rate

Changes in the proportional growth rates of *Zostera novazelandica* in response to different treatments in the laboratory experiments are shown in Figure 4-3. Between days 0 - 7, the growth rates in treatments A, B and C were more than 0.02 g DW g⁻¹ day⁻¹ but low in other treatments with rates generally < 0.015 g DW g⁻¹ day⁻¹. No growth rate data were obtained between days 15 - 21 in treatments B and D as a greater proportion of the shoots in these treatments were dead. The maximum growth rate of about 0.031 g DW g⁻¹ day⁻¹ was measured in seagrass shoots under treatment A, and the minimum of about 0.012 g DW g⁻¹ day⁻¹ in treatment G (Figure 4-3).

Results of the split-plot ANOVA (Table 4-2) suggested that the growth rates of the *Zostera novazelandica* leaves were highly significantly controlled by the interaction

between temperature and PFD, which was clearly shown between days 0 - 7 and 8 - 14 ($p<0.05$, 15 df).

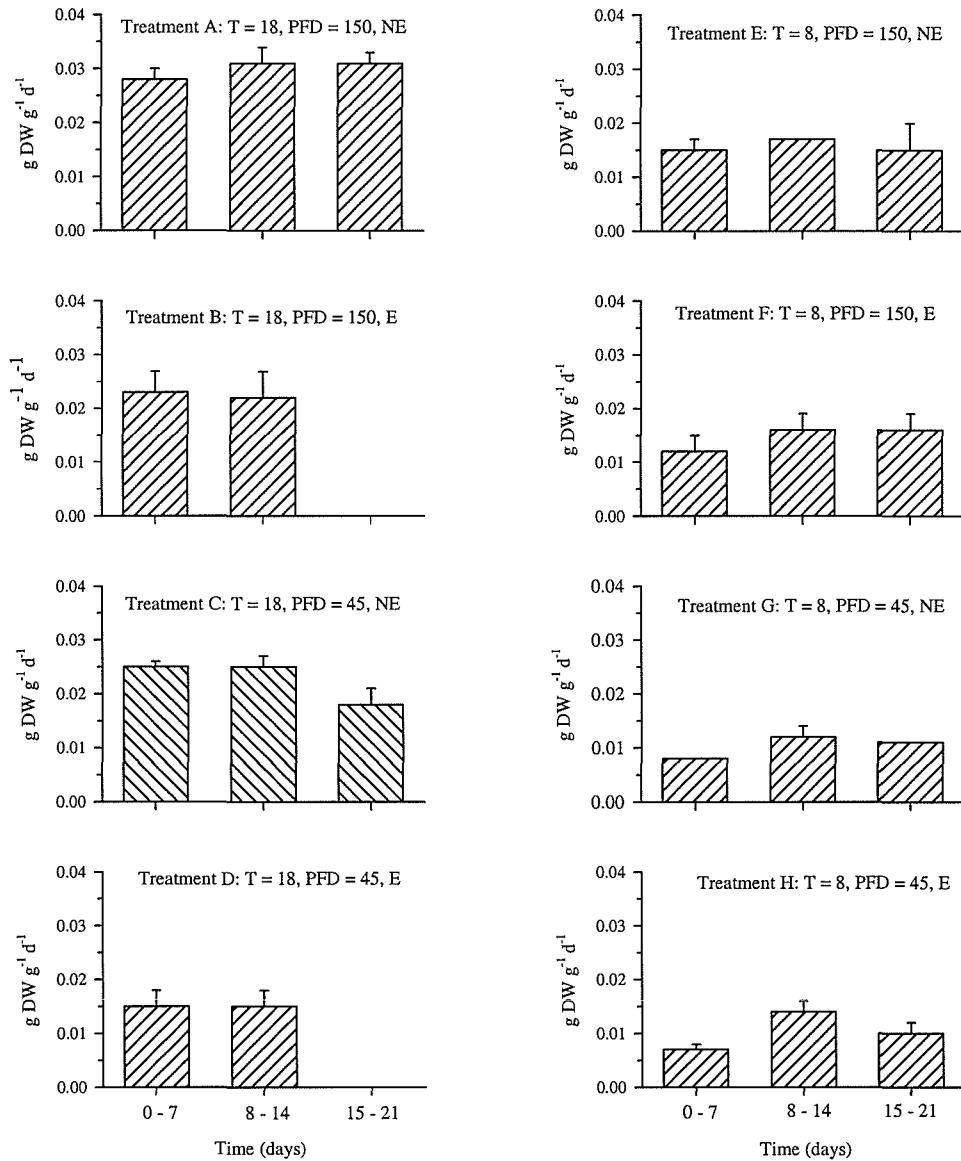


Figure 4-3: Rates of leaf growth for *Zostera novazelandica* in treatments A - H, under laboratory experiments during October and November 1998. Vertical bars indicate $\pm 1\text{S.E.}$ of the mean ($n=2$). T = experimental temperature ($^{\circ}\text{C}$), PFD = photon flux density ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$), E = enriched and NE = not enriched.

Table 4-2: Results of the split-plot ANOVA showing the interactive effects of temperature, PFD and nutrient levels on the growth rates of *Zostera novazelandica* in days 0 - 7 and 8 - 14. Data for days 15 - 21 were not analysed due to a reduced number of samples, hence reduced degrees of freedom.

Source of variation	DF	MS		<i>F</i> value		<i>P</i> value	
		Days 0 - 7	Days 8 - 14	Days 0 - 7	Days 8 - 14	Days 0 - 7	Days 8 - 14
Temperature (A)							
PFD (B)							
A*B	3	2.468 X 10 ⁴	1.295 X 10 ⁴	91.40	21.01	<0.001	0.007
Replication (C)							
A*B*C	4	2.700 X 10 ⁶	6.164 X 10 ⁶	1.66		0.376	
Nutrient (D)							
A*D	2	5.825 X 10 ⁵	8.664 X 10 ⁵		1.94		0.340
A*C*D	2	3.503 X 10 ⁵	4.464 X 10 ⁵				
A*B*D	2	7.052 X 10 ⁶	3.259 X 10 ⁷	1.20	0.09	0.454	0.919
A*B*C*D	2	5.863 X 10 ⁶	3.701 X 10 ⁶				
Total	15						

4.4.3 Chlorophyll a and b

Chlorophyll *a* concentrations in the leaves of *Zostera novazelandica* at the start of the experiments were about 1.1 mg g⁻¹ tissue wet weight (Figure 4-4). Throughout the culture experiments, the plants in treatment C developed more chlorophyll *a* amounting to a 2.4-fold increase by day 21 (2.75 mg g⁻¹ tissue wet weight). Pigment content of treatments A and G also increased (1.91 and 1.81 mg g⁻¹ tissue wet weight respectively), but this increase was considerably lower than that of treatment C. The chlorophyll *a* in other treatments, however, either showed a slight increase, or declined (Figure 4-4). The minimum chlorophyll *a* content was measured in plants of treatment B (1.19 mg g⁻¹ tissue wet weight) on day 14 and treatment F (1.3 mg g⁻¹ tissue wet weight) on day 21. Split-plot ANOVA showed that the chlorophyll *a* concentration was significantly affected by the interaction between temperature and nutrient levels as observed in day 7 ($p<0.05$, 15 df.; Table 4-3). This analysis and the results in Figure 4-4 indicate that unenriched plants growing in high temperature produced more chlorophyll *a* than all other treatments. Plants maintained at low temperature had a low chlorophyll *a* content in both enriched and unenriched conditions. By day 14, however, the interaction of these variables had little effect on the chlorophyll *a* content ($p = 0.056$, 15 df.).

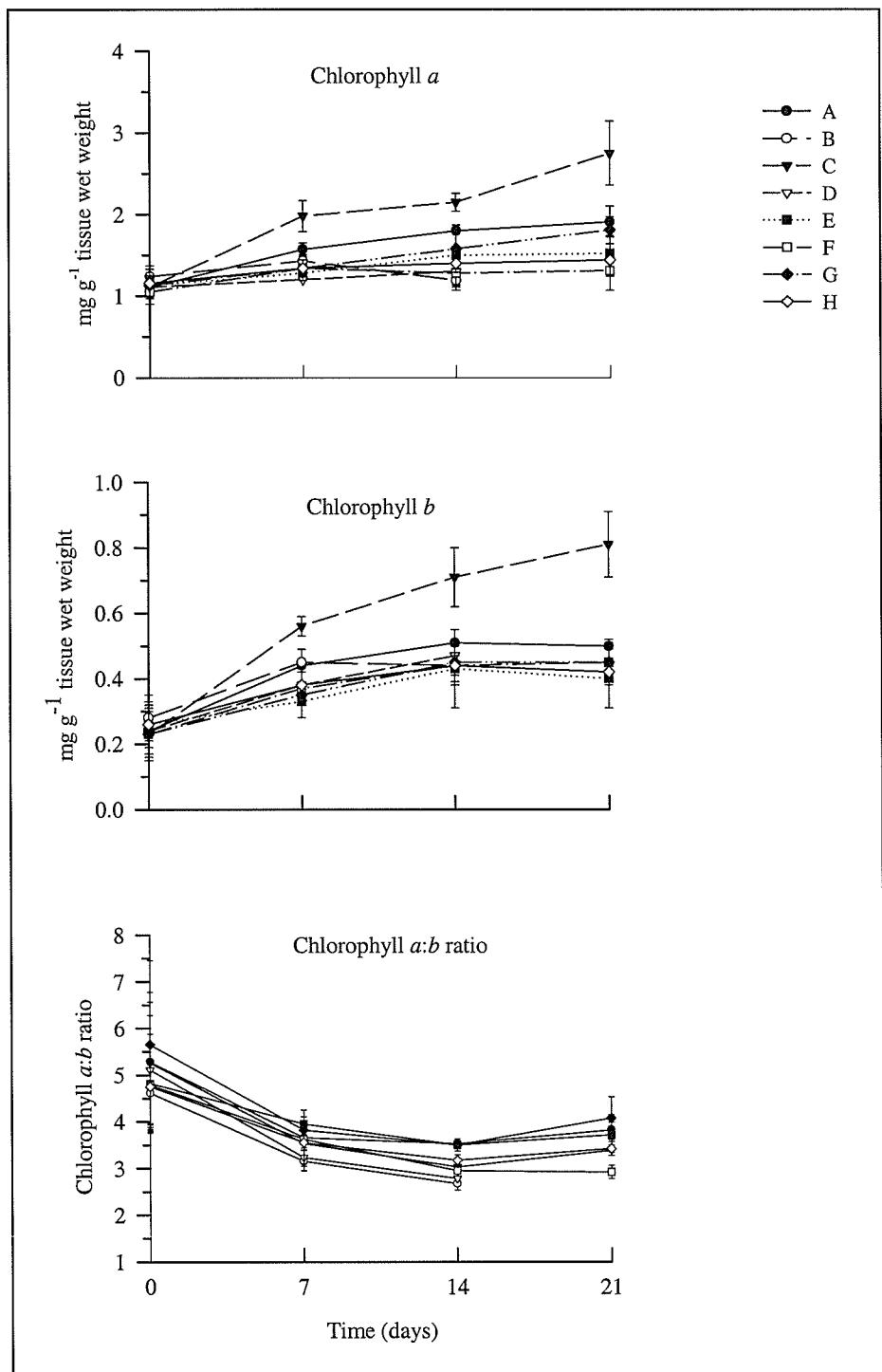


Figure 4-4: Weekly changes in the leaf chlorophyll *a* and *b* and chlorophyll *a:b* ratio of *Zostera novazelandica* in treatments A - H, under laboratory experiments during October and November 1998. Vertical bars indicate $\pm 1\text{S.E.}$ of the mean ($n=2$).

The initial concentration of chlorophyll *b* in all treatments was about 0.24 mg g⁻¹ tissue wet weight (Figure 4-4). All treatments showed a steady increase in chlorophyll *b* content during the first week of the experiment. As for chlorophyll *a*, the chlorophyll *b* content of plants in treatment C increased further between days 14 and 21, resulting in a final chlorophyll *b* content of 0.81 mg g⁻¹ tissue wet weight. This value was 3.5x greater than at the start of the experiment. Also similar to chlorophyll *a*, treatment A had the second greatest increase in chlorophyll *b*, 2-fold, while the increase in other treatments were about 1.5-fold. Results of the split-plot ANOVA (Table 4-4) indicate that interactions of both temperature and PFD ($p<0.05$, 15 df.), and temperature and nutrient levels ($p<0.001$, 15 df) significantly affected the chlorophyll *b* concentration on day 7. On day 14, the interaction between temperature, PFD and nutrient levels was a significant factor controlling the concentration of chlorophyll *b* in the *Zostera novazelandica* leaves ($p<0.05$, 15 df).

Chlorophyll *a:b* ratios, in contrast, showed a steady decline in all treatments from day 0 to day 14. The biggest reduction of the chlorophyll *a:b* ratio during this period was recorded in treatment D (about 1.8x), followed by treatments B and C (about 1.7x) (Figure 4-4).

Table 4-3: Results of the split-plot ANOVA showing the interactive effects of temperature, PFD and nutrient levels on the chlorophyll *a* content of *Zostera novazelandica* at the start (day 0), day 7 and day 14 of the culture experiments. Data for day 21 were not analysed due to a reduced number of samples, hence the reduced degrees of freedom.

Source of variation	DF	MS			<i>F</i> value			<i>P</i> value		
		day 0	day 7	day 14	day 0	day 7	day 14	day 0	day 7	day 14
Temperature (A)										
PFD (B)										
A*B	3	0.0053	0.0710	0.0789	0.11	5.12	0.75	0.952	0.074	0.579
Replication (C)										
A*B*C	4	0.0502	0.0139	0.1059						
Nutrient (D)										
A*D	2	0.0053	0.2127	0.5686	0.47	138.11	16.83	0.679	0.007	0.056
A*C*D	2	0.0111	0.0015	0.0338						
A*B*D	2	0.0069	0.1016	0.0142	1.6	2.69	4.63	0.385	0.271	0.178
A*B*C*D	2	0.0043	0.0378	0.0031						
Total	15									

Table 4-4: Results of the split-plot ANOVA showing the interactive effects of temperature, PFD and nutrient levels on the chlorophyll *b* content of *Zostera novazelandica* at the start (day 0), day 7 and day 14 of the culture experiments. Data for day 21 were not analysed due to a reduced number of samples, hence the reduced degrees of freedom.

Source of variation	DF	MS			F value			P value		
		day 0	day 7	day 14	day 0	day 7	day 14	day 0	day 7	day 14
Temperature (A)										
PFD (B)										
A*B	3	4.78×10^4	0.0134	0.0195	0.02	8.06	1.27	0.996	0.036	0.397
Replication (C)										
A*B*C	4	0.0226	0.0017	0.0153						
Nutrient (D)										
A*D	2	5.38×10^4	0.0081	0.0243	3.74	1750.88	6.56	0.211	<0.001	0.132
A*C*D	2	1.44×10^4	4.65×10^6	0.0037						
A*B*D	2	0.0012	0.0104	0.0080	26.37	1.90	169.86	0.037	0.345	0.006
A*B*C*D	2	4.62×10^5	0.0055	4.71×10^5						
Total	15									

4.4.4 $F_v:F_m$ ratio

The response of the $F_v:F_m$ ratio of *Zostera novazelandica* to experimental treatments is illustrated in Figure 4-5. Measurements made at day 0 and 7 showed the plants in all treatments had a high $F_v:F_m$ ratio (approximately 0.8 to 0.9). In treatments A, C, E, G and H, the high values were maintained until day 14. For the remaining treatments $F_v:F_m$ ratios declined and a minimum value of 0.64 was observed in treatment D. Results of the split-plot ANOVA indicated no significant difference in the $F_v:F_m$ ratio of *Z. novazelandica* due to interactions of temperature, PFD and nutrient addition ($p>0.05$; 15 df.) (Table 4-5).

4.4.5 Leaf tissue carbon and nitrogen content, and C:N ratio

The proportion of carbon, nitrogen and C:N ratio in the leaf tissues of *Zostera novazelandica* from each treatment measured on day 14 is shown in Figure 4-6. The carbon content of leaves was very similar for all treatments, ranging from 35.47% (treatment H) to 37.44% of the tissue dry weight (treatment D). The nitrogen content ranged between 1.75% (treatment A) and 2.29% (treatment B) of the tissue dry weight. The C:N ratio was between 18.46 (treatment H) and 24.45 (treatment E). ANOVA suggested that there were no interactive effects of temperature, PFD and nutrient levels on the leaf nutrient content of *Z. novazelandica* ($p>0.05$, 15 df.; Table 4-6).

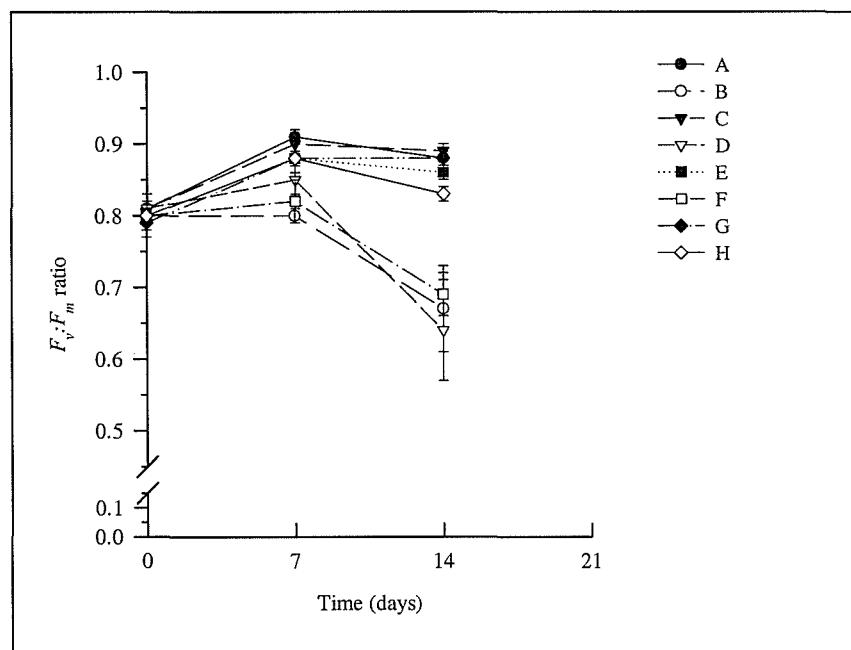


Figure 4-5: Weekly changes in the PS II photochemical efficiency ($F_v:F_m$ ratio) of *Zostera novazelandica* in treatments A - H, under laboratory experiments during October and November 1998. Vertical bars indicate ± 1 S.E. of the mean, obtained from 2 replicate experiments ($n=2$). No measurements were made on day 21.

Table 4-5: Results of the split-plot ANOVA showing the interactive effects of temperature, PFD and nutrient levels on the $F_v:F_m$ ratio of *Zostera novazelandica* at the start (day 0), day 7 and day 14 of the culture experiments.

Source of variation	DF	MS			F value			P value		
		day 0	day 7	day 14	day 0	day 7	day 14	day 0	day 7	day 14
Temperature (A)										
PFD (B)										
A*B	3	3.14 X 10 ⁴	0.0027	0.0072	0.02	0.65	0.83	0.998	0.621	0.544
Replication (C)										
A*B*C	4	0.0203	0.0041	0.0087						
Nutrient (D)										
A*D	2	4.75 X 10 ⁵	0.0127	0.0634	0.37	6.47	6.59	0.729	0.134	0.132
A*C*D	2	1.28 X 10 ⁴	0.0020	0.0096						
A*B*D	2	1.54 X 10 ⁴	0.0052	0.0036	0.78	3.48	2.11	0.560	0.223	0.321
A*B*C*D	2	1.96 X 10 ⁴	0.0015	0.0017						
Total	15									

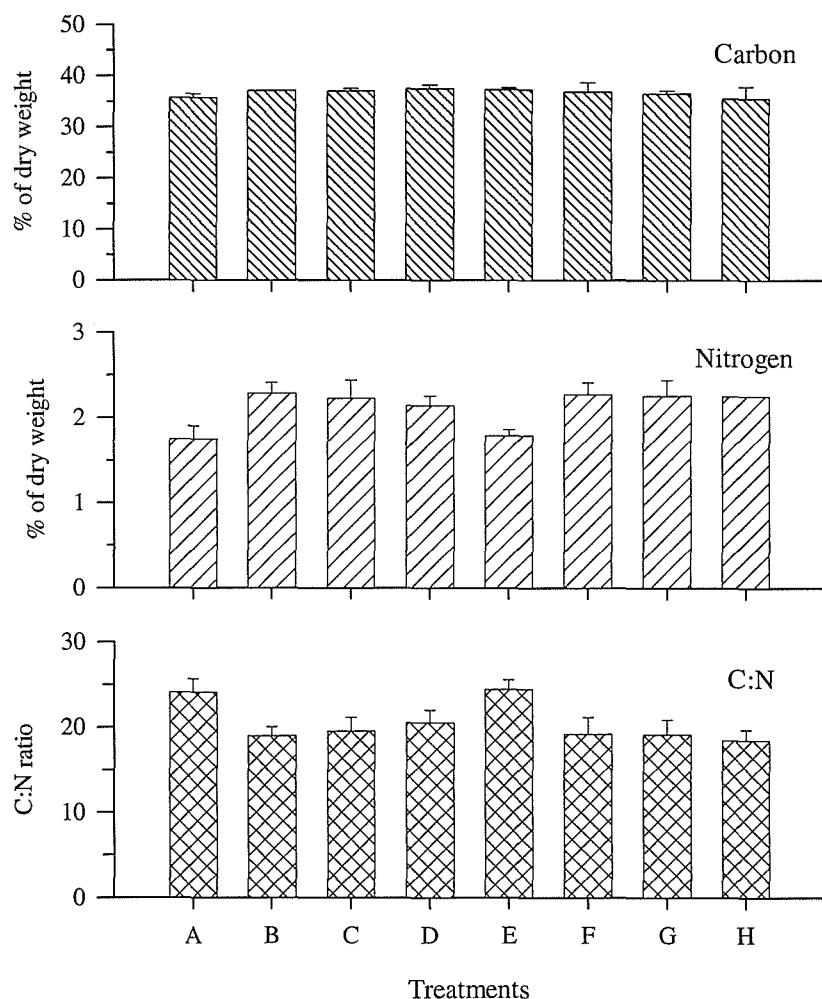


Figure 4-6: Carbon and nitrogen content as % dry weight, and C:N ratio, in the leaf tissues of *Zostera novazelandica* in treatments A - H, on day 14 under laboratory experiments during October and November 1998. Vertical bars indicate $\pm 1\text{S.E.}$ of the mean, obtained from 2 replicate experiments ($n=2$).

Table 4-6: Results of the split-plot ANOVA showing the interactive effects of temperature, PFD and nutrient levels on carbon, nitrogen and C:N ratio content of *Zostera novazelandica* on day 14 of the culture experiments.

Source of variation	DF	MS			F value			P value		
		Carbon	Nitrogen	C:N	Carbon	Nitrogen	C:N	Carbon	Nitrogen	C:N
Temperature (A)										
PFD (B)										
A*B	3	1.326	0.053	7.902	0.40	1.14	1.12	0.764	0.434	0.44
Replication (C)										
A*B*C	4	3.355	0.047	7.050						
Nutrient (D)										
A*D	2	1.354	0.107	13.091	0.36	2.67	7.13	0.737	0.273	0.12
A*C*D	2	3.797	0.040	1.837						
A*B*D	2	0.272	0.158	14.520	4.35	6.77	7.50	0.187	0.129	0.12
A*B*C*D	2	0.063	0.023	1.935						
Total	15									

4.5 DISCUSSION

Temperature, irradiance and nutrient availability are the main factors regulating primary production of marine vascular plants (c.f. Zupo *et al.* 1997 and literature cited therein). In the present study, interactive effects of temperature, PFD availability and water column nutrient concentration on the growth of *Zostera novazelandica* were investigated. The levels of seawater temperature used in this aquarium study reflect the summer (18°C) and winter (6°C) conditions under which the seagrass grow in Otago Harbour. The average PFD of 45 and 150 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ are within the range (30 - 300 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$) employed in the experiments on this species from Kaikoura Peninsula, New Zealand (Ramage and Schiel 1998). It was not possible to increase the maximum level of PFD in the present culture set up as it caused a corresponding increase in the water temperature in the aquaria. Thus, it is recognised that the PFD levels in the present experiment do not correspond to PFD levels of about 70-90 mol $\text{m}^{-2} \text{d}^{-1}$ reaching seagrass leaves in field conditions (see Figure 3-10 in Chapter 3). Higher level of PFD (about 1,600 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$) at the natural seagrass habitat was also recorded by the underwater PAM light meter during a field N-enrichment study in December 1998 (see Chapter 5, section 5.4.3.3). No attempt was made to determine whether or not the growth of *Z. novazelandica* in the aquaria experiments was light saturated and, moreover, no published values on light saturation parameters are available for this species. The growth of the related species *Z. marina* is light saturated between 80 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ (Dennison and Alberte 1986) and 280 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ (Zimmerman *et al.* 1995). The molar concentrations of ammonium, nitrate and phosphate sources in the enriched treatments were, however, respectively 500, 1400 and 4600 times higher than the typical range of their seasonal fluctuations measured in the water column around the *Z. novazelandica* natural habitat in Otago Harbour (see Chapter 3).

4.5.1 Plant mortality

In this study, *Zostera novazelandica* grown under different combinations of temperature, PFD and nutrient levels displayed a variety of growth responses. The most obvious response was a higher mortality rate for nutrient enriched plants, in both low and high PFD levels at 18°C, which was not observed in the enriched plants at low temperature. Dark green leaf blades with weakened tissue structure were the first symptoms, and subsequently defoliation of the leaves occurred. Moreover, low $F_v:F_m$ values of the shoots for treatments B and D, exhibited on day 14 also indicated that the plants were under physiological stress. This suggests that an interactive effect of high temperature and nutrient enrichment caused the seagrass mortality in the enriched aquaria.

Toxicity of seawater ammonium at a concentration as low as 25 μM caused necrosis in *Zostera marina*, with the toxicity more pronounced in plants grown on sand than mud, and at higher temperatures (20°C) (van Katwijk *et al.* 1997). The toxic effects of ammonium in *Z. marina* were also found to be correlated with a leaf nitrogen content of 3.5% of dry weight (van Katwijk *et al.* 1997). In the present study, however, necrosis was not observed for *Z. novazelandica* and the leaf tissue nitrogen content in the enriched treatment was about 2.2% of dry weight. Therefore it is unlikely that the negative effects of seawater nutrient enrichment were correlated with ammonium toxicity.

Burkholder *et al.* (1992) reported evidence of direct nitrate toxicity on *Zostera marina* in their microcosm experiments with similar symptoms to the present study, in which plants enriched with NO_3^- at an approximate concentration of 200 to 300 μM and at high temperature, showed a bright green colour and weakened leaf tissue structure, especially in the region of the meristem. In another study, an axenic seagrass culture of *Halophila ovalis* turned brown and died within a few weeks when grown on a medium supplemented with nitrate at concentration of 1.7 mM (Bird *et al.* 1998). Whether

ammonium or nitrate toxicity caused the mortality of *Z. novazelandica* shoots in the present study is difficult to determine, and would be a subject of detailed future study.

4.5.2 Proportional growth rate responses

In these experiments, the proportional leaf growth rate of unenriched *Zostera novazelandica* was similar to that recorded in the field monitoring programme. For example, the maximum growth rate achieved for the seagrass at high temperature and high PFD (treatment A) was $0.031 \text{ g DW } \text{g}^{-1} \text{ d}^{-1}$ comparable to $0.028 \text{ g DW } \text{g}^{-1} \text{ d}^{-1}$ of maximum *in situ* growth rate recorded in December 1998 (Chapter 3). Prolonged shading caused the growth rate to decline with rapid reduction occurring under low PFD and at low temperature (e.g., treatment G). Maximum growth rate measured from this treatment on days 8-14 (mean about $0.012 \text{ g DW } \text{g}^{-1} \text{ d}^{-1}$) approximates to *in situ* winter growth rate of the seagrass recorded in June 1998 (mean $0.009 \text{ g DW } \text{g}^{-1} \text{ d}^{-1}$) (Chapter 3). This may indicate a limited use of storage carbohydrate to support normal growth rate under this condition (e.g. Longstaff *et al.* 1999). The reduction of growth rate under reduced PFD was also recorded in *Z. capricorni* (Abal *et al.* 1994), *Posidonia sinuosa* (Gordon *et al.* 1994) and *Halophila ovalis* (Longstaff *et al.* 1999).

Seasonality in the proportional growth rate for *Zostera novazelandica* at Harwood was discussed in Chapter 3 where it was suggested that temperature and PFD were the principal predictors explaining the variance of leaf growth rate in the *Z. novazelandica* population. This suggestion is supported by the present experiment in which the interaction between temperature and PFD significantly affected the leaf proportional growth rate. At lower temperature and lower PFD (treatment G), leaf growth rates were reduced to about half those of high temperature and high PFD (treatment A). Thus, these

aquaria experiments support results of field studies and regression analyses described in Chapter 3.

Seawater nitrogen enrichment in this study did not, however, enhance the rates of proportional leaf growth of the *Zostera novazelandica* at the tested levels of temperature and PFD. This also indicates that interactive effect of seawater nutrient enrichment with PFD and temperature did not influence the leaf growth rate of the seagrass, which also supported the regression analysis of the field data (Chapter 3).

4.5.3 Pigment responses

Chlorophyll *a* is a primary photosynthetic pigment in all plants including marine macrophytes (Lobban *et al.* 1985), and in seagrasses, chlorophyll *b* is also present in addition to the chlorophyll *a* (e.g., Dennison 1990). Also, the chlorophyll concentrations in aquatic macrophytes are known to fluctuate in response to variation in temperature and to changing light environment (in Czerny and Dunton 1995 and literature cited therein). Under reduced availability of PFD, increased chlorophyll *a* and *b* contents, and changes in the ratio of chlorophyll *a:b* have been shown to occur in some seagrasses in response to this condition (e.g., Abal *et al.* 1994, Czerny and Dunton 1995, Longstaff *et al.* 1999).

It is unlikely that the *in situ* growth of the intertidal *Zostera novazelandica* samples used during this investigation (i.e. late spring) was light limited as the plants there were exposed to high availability of PFD during daytime low tide thus their photosynthetic apparatus is expected to be well adapted to high irradiance conditions. However, the high levels of PFD provided in the present culture were lower than in the field, thus the experiment may have been conducted at PFD levels lower than light-saturation. This was indicated during the first week of the start of the experiment, in which *Z. novazelandica* showed some evidence of acclimation to some of the treatments given. Plants in

treatments A (high PFD, high temperature, ambient nutrient level) and C (low PFD, high temperature, ambient nutrient level) showed a significant increase in their chlorophyll *a* and *b* compared to plants in other treatments. Statistical analyses suggested that this increase was attributable to a combined effect of high temperature and unenriched seawater condition.

At the end of the 21-day experiment, plants in treatment C had the highest concentration of both chlorophyll *a* and *b*. This may indicate that the pigments are probably related to PFD and temperature effects, although this was not statistically tested due to the high mortality rates of the samples in treatments B and D and hence the reduced degrees of freedom. By increasing the pigment concentrations under low PFD, the plants may have improved their photosynthetic efficiency (Dennison and Alberte, 1986 in Goodman *et al.* 1995), as increasing seawater temperature increased the metabolic demands imposed by growth and tissue maintenance (Czerny and Dunton 1995). Whether their growth was light limited under the present low-light treatment was not, however, examined. The photo-adaptive response (Longstaff *et al.* 1999) shown by the *Z. novazelandica* in treatment C is similar to that of its close relative *Z. capricorni*, in which plants grown under low PFD ($\leq 20\%$ of incident light) had higher pigment (chlorophyll *a* + *b*) content than those grown under high PFD (50 - 100% of incident light) when measured at the end of a 2-month outdoor aquaria shading experiment, using 23°C flowing seawater (Abal *et al.* 1994). Higher concentration of chlorophyll *a* + *b* in response to low PFD was also reported in *Halophila ovalis* (Longstaff *et al.* 1999), and *Z. marina* and *Z. noltii* (Jiménez *et al.* 1987).

A decrease in the ratios of chlorophyll *a:b* pigments was also observed in *Zostera novazelandica*, with greater reduction generally occurring in all treatments during the first week of the culture experiments. The adaptive response shown may have helped increase the light absorption efficiency of the seagrass (Abal 1996 and Lee and Dunton 1997, cited

in Longstaff and Dennison 1999). However, for the plants in unenriched treatments (under low and high PFD levels), the ratios of the chlorophyll *a:b* pigments were similar, with values ranging from 3.4 to 4.1 at the end of the experimental period. This may suggest that the ratios of chlorophyll *a:b* pigments of this species remain constant under different light levels. Therefore, the behaviour exhibited by *Z. novazelandica* in the present study agrees with the Oltmann's theory (1892), which postulates that under low light intensity, all pigment concentrations increase and, in consequence, the ratio chlorophyll *a*:auxiliary pigments remains constant when irradiance changes (c.f. Jiménez *et al.* 1987). This behaviour is also similar to that of *Z. marina* specimens used in the experiments conducted by Jiménez *et al.* (1987).

Inorganic nitrogen (NH_4^+ , NO_3^-) taken from the seawater is generally assimilated into simple organic compounds (e.g., amino acids and amino compounds) in an intracellular residual organic N pool, which is later incorporated into proteins and other macromolecules such as chlorophyll pigments as known to occur in macroalgae (see McGlathery *et al.* 1996). This may suggest that more chlorophyll pigments are synthesised by N-limiting plants in the presence of an elevated external N supply. This was previously demonstrated in some seagrasses in The Philippines where increased chlorophyll *a* and *b* along with tissue N-content was recorded in response to *in situ* N-enrichment of their sediment pore-water (Agawin *et al.* 1996). Similar results were also reported in *Zostera marina* and macroalgae, *Fucus vesiculosus*, in seawater enrichment experiments (Pedersen 1995). However, N-enrichment of the seawater in the present aquaria study apparently did not enhance the synthesis of chlorophyll pigments neither at high PFD nor at low PFD treatments. Their tissue N concentrations were also not significantly different from unenriched treatments (see the following section). These results thus indicate that the *Z. novazelandica* used in this study was not under N-limiting conditions. It may also support the results of field sediment N-enrichment for this seagrass conducted during summer

1998, in which only a marginal increase of chlorophylls *a* and *b* pigments was observed (see Chapter 5). With regard to treatment C (low PFD and low nutrient), an increase in chlorophylls *a* and *b* may possibly indicate that the levels of N required to synthesise these pigments as a photo-adaptive response to low PFD, are sufficient in the unenriched seawater hence also reflecting that N is not limiting. In contrast, it remains to be explained why there is a lack of photo-adaptation in N-enriched plants under low PFD such as in treatment D. It is speculated that the physiological stresses imposed by the effects of N addition and high temperature may have necessitated diversion of the plants' N from synthesis of chlorophyll pigments to other protein molecules. Increased protein synthesis in N-enriched plants following physiological stresses imposed by enrichment has been reported in eelgrass and other angiosperms, while decreasing the production of their antimicrobial phenolics. However, these have occurred in light replete condition (in Burkholder *et al.* 1992 and literature cited therein).

4.5.4 Effect on $F_v:F_m$ ratio

Pulse amplitude modulation (PAM) chlorophyll fluorescence parameters have been used to monitor light stress effects on photosynthesis in marine macrophytes including macroalgae (see review by Franklin and Forster 1997) and seagrasses (Dawson and Dennison 1996, Ralph 1996). One of the fluorescence parameters widely used is the ratio of 'dark-adapted' variable to maximum fluorescence, $F_v:F_m = (F_m - F_o)/F_m$. The initial fluorescence (F_o) is a measure of fluorescence when a large majority of the PSII reaction centres are open (the primary acceptor or Q_A molecules are oxidised), and maximum fluorescence (F_m) represents the fluorescence when PSII reaction centres are closed (the primary acceptor or Q_A molecules are fully reduced) (Bolhar-Nordenkampf *et al.* 1989 cited in Longstaff *et al.* 1999). The ratio of $F_v:F_m$ is also used as an indicator for the

photochemical efficiency of photosystem II (PSII) primary reaction centres (Kraus and Wies 1988 in Longstaff *et al.* 1999).

$F_v:F_m$ is normally measured by the application of a saturating flash on a dark adapted leaf. In healthy leaves of green plants, a typical $F_v:F_m$ value of 0.82 has been reported (Franklin and Forster 1997). For plants under stress conditions, a lower value is normally recorded indicating a proportion of their PSII reaction centres are damaged, a phenomenon called photoinhibition (Frachebound 2001). In the present study, however, the maximum $F_v:F_m$ value of 0.9 was recorded in treatments A and C, a week after the initiation of the experiments and this coincided with the period when the plants were actively synthesising their chlorophyll *a* and *b* in response to these treatments, as previously described. The value obtained was higher than the published maximum $F_v:F_m$ ratios measured for *Posidonia australis*, *Amphibolis antarctica*, *Halophila ovalis* and *Halodule wrightii* in the field (Ralph *et al.* 1998, Beer and Bjork 2000); and *H. ovalis* plants cultured in the laboratory (Ralph 1998), in which the ratios of about 0.8 were usually recorded. This was also higher than the ratio reported in a common reed *Phragmites australis* (about 0.83) (Adams and Bate 1999). It is possible that the high value obtained in this study may have been due to an error associated with the PAM instrument itself or the *Zostera novazelandica* plants in these treatments may have photosynthesised optimally during this period, as $F_v:F_m$ ratio is also an estimate of photosynthetic capacity (Adams and Bate 1999). Unfortunately, there have been no published data on $F_v:F_m$ ratios of *Z. novazelandica* neither in the field nor in culture conditions to make a comparison.

All plants in unenriched treatments in the present study consistently maintained high ratio of $F_v:F_m$ on days 7 and 14, suggesting that the photochemical efficiency of their PSII was not impaired (c.f. Longstaff *et al.* 1999) by the combination effects of the physico-chemical parameters provided in the culture set up for the time-period of the exposure. Except for enriched plants in treatment H, however, the $F_v:F_m$ ratios of plants in other

enrichment aquaria (B, D and F) declined to <0.7 when measured on day 14. A decrease in these $F_v:F_m$ values indicates a reduced photosystem II activity (Beer *et al.* 2000) hence the occurrence of photoinhibition, when a saturating flash was applied to dark adapted leaves of the *Z. novazelandica*. This also shows that the plants were physiologically stressed (e.g., Adams and Bate 1999) under these conditions, although the rates of their proportional leaf growth remained stable. Therefore, their photosynthetic characteristics seem to be a more sensitive indicator of stress to the treatments than leaf growth rates.

The plants in treatments B and D with $F_v:F_m$ ratios of 0.67 and 0.64 respectively, however, seem to be more stressed than those in treatment F ($F_v:F_m$ ratios = 0.69). This was indicated by weakened tissue structures and subsequent defoliation of their leaves, followed by shoot mortality during week 3 (as previously described). This did not occur in treatment F. Furthermore, no sign of stress was observed for the enriched plants in treatment H (under low PFD, low temperature), paralleled by their high $F_v:F_m$ ratio (about 0.83 on day 14). These results may indicate that the stress symptoms exhibited by the *Z. novazelandica* are probably not an effect of the nutrient enrichment per se. Results from the statistical analysis were also unable to indicate a significant interaction effect between temperature, PFD and nutrient addition on the response shown by the ratios of $F_v:F_m$. Thus, the present results do not indicate a clear cause for the reduction in the $F_v:F_m$ ratios in treatments B, D and F. It is, therefore, suggested that further studies in this area are needed for *Z. novazelandica* by measuring the effect of a single factor (PFD, temperature, nutrient enrichment) as well as combined effects of these factors on seagrass growth.

4.5.5 Tissue nutrient response

Nutrient concentrations in various seagrass tissues have often been used to infer the nutrient limitation for seagrass growth (c.f. Lee and Dunton 1999a and literatures cited

therein). The nutrient concentrations are usually positively correlated with the level of nutrient in the environment (c.f., Stapel and Hemminga 1997). Duarte (1990) compiled data on nutrient concentrations of 27 seagrass species from wide ranges of geographical locations and found that seagrasses with N contents of <1.8% on dry weight basis or C:N ratio of >20 are N-limited. Additions of nitrogen to the N-limited seagrass results in increased nitrogen concentration in their tissues (Duarte 1990 and literatures cited therein).

In the present study, statistical analyses indicated that there was no significant interactions effect of seawater nitrogen enrichment, temperature and PFD on the concentration of nitrogen in the leaf tissue of the *Zostera novazelandica*. For the enriched treatments, mean nitrogen concentrations in the leaf tissues measured on day 14 were about 2.2% of dry weight, which were higher than the critical value of the suggested N limitation. This implies that the growth of the seagrass in these treatments was not under nitrogen limited conditions, as was expected by the high concentration of nitrogen added in the seawater. The measured leaf nitrogen concentration was quite similar to that of the *in situ* nitrogen enrichment experiment on sediment pore-water conducted for this species during summer 1998 (Chapter 5), which showed mean values of about 2.8% of dry weight.

Nitrogen concentration in the leaf tissue of unenriched *Zostera novazelandica* cultured in high PFD treatment tended, however, to decrease (although not statistically significantly) both at high and low temperatures (treatments A and E). The values of N concentration recorded in these treatments were about 1.7% of dry weight, which according to the relationship above would be indicative of slight N-limitation. This idea is supported by the high atomic C:N ratio of about 24%, which is above the suggested critical value for N-limitation (Duarte 1990). According to Abal *et al.*, (1994) plants under higher PFDs have higher rates of photosynthesis and the nutrient absorbed would be rapidly utilised for growth. Thus, high growth rate plants require high nutrient supply rates to maintain cell synthesis resulting in an increased nutrient demand and a higher probability

of nutrient deficiency (c.f. Pedersen 1995). This idea is supported by the present study whereby the growth rate of *Z. novazelandica* was highest in the treatment A although their photosynthesis was not measured. In order to meet the N demands required for growth, the plants in treatment A had probably used up their stored-N, as nitrogen supply from the seawater was not sufficient and therefore led to a reduced intracellular nitrogen pool. This gradual ‘dilution’ (Stocker, 1980 cited in Perez-Llorens and Xavier Niell 1993) of the stored nitrogen resources during growth may explain the lower concentration of N in the leaf tissue of the *Z. novazelandica* in this treatment compared to those in the low light condition. *Zostera* spp. have been shown to be capable of taking up nutrients both from water column and sediment pore-water (see Perez-Llorens and Xavier Niell 1993 and literatures cited therein). The seagrass would pump nutrient from the sediment through its root-rhizome system when nutrient availability in the seawater become critical (Sfriso and Marcomini 1999). However, in the present culture study, the *Z. novazelandica* must have relied heavily on the water column for inorganic N as the sediment (fine sand) for the seagrass substratum was washed prior to the experiment and so lacked a significant sediment pore-water N pool. Furthermore, the leaf uptake of inorganic N may have also reduced the N uptake by roots, as reported to occur in *Z. marina* (Thursby and Harlin 1982).

However, this does not explain the suggested nutrient limitation in plants of treatment E that had lower growth rates than those in treatment A. Results of *in situ* short-term experiments by Short and McRoy (1984) show a concentration-dependent uptake of nitrogen (both ammonium and nitrate) in the leaves of *Zostera marina* at ambient nitrogen concentrations in the water column. Thus, it is possible that the low concentrations of seawater nitrogen in the aquarium E in the present study may have reduced the plants’ nutrient uptake, which was then followed by a reduction in intracellular nitrogen pools, as has been shown in other marine plants (Bird et al., 1982, in Pedersen 1995). Furthermore,

lower seawater temperature in this treatment may have also affected the enzymatic and physiological rates of the seagrasses as temperature lower by 10°C (-Q₁₀) would reduce all the chemical reaction rates by half (Lobban and Harrison 1994) including nutrient uptake and dark reaction of the photosynthesis. This would result in a lower growth rate and lower demand for nutrients in these plants. Also, there was a possibility that nitrogen in the seawater that was adsorbed and absorbed (Short and McRoy 1984) by the leaf in this treatment was translocated to other part of the plants (see Thursby and Harlin 1982) such as rhizomes and roots systems, and leaf sheaths, although this was not examined in the present study.

According to Duarte (1990) the concentration of carbon in seagrass leaves worldwide was less variable than that of nitrogen or phosphorous. The low variability in the carbon concentration suggests that carbon-limited seagrass growth is rare in nature because of high carbon availability in seawater (Duarte 1990). However, low availability of dissolved inorganic carbon (DIC) normally used by many seagrass species i.e. CO₂ and bicarbonate (HCO₃⁻), may occur under natural condition that renders the seagrass photosynthesis (and hence, growth) to frequently become carbon-limited (Beer and Rehnberg 1997, Short and Neckles 1999). This occurred in plants inhabiting the environment with low current velocities in which a thick diffusion boundary layer developed surrounding their leaf surfaces, and also in plants with a relatively inefficient uptake system (see review by Short and Neckles 1999). Low availability of DIC in seawater could also occur during periods of high water pH values due to, for example, a massive growth of phytoplankton (Riebesell et al., 1993 cited in Invers *et al.* 1997) and macrophytes (e.g., *Ulva*) (Sfriso and Marcomini 1999). In the present study, the mean carbon concentration in the leaf of *Zostera novazelandica* measured on day 14 ranged from 35.5% to 37.4% of dry weight and also the C content was not significantly affected by the interaction between treatments. Generally, the carbon content in the leaf of *Z.*

novazelandica in this experiment is comparable to its field values of 37%, such as those measured in the control plots described in Chapter 5. The carbon values obtained were also in the range of carbon content in the leaf of laboratory cultured *Z. capricorni* (34.9% to 39.3%) from Australia (Abal *et al.* 1994).

According to Burkholder *et al.* (1992), nitrate enrichment may cause disappearance of *Zostera marina* by a direct physiological effect such as carbon limitation, phosphorus limitation, or other internal nutrient imbalance. However, with regard to carbon, no changes were observed in tissue carbon content between treatments. The carbon concentration was also above the critical value of 33.6% (Duarte 1990), indicating that the growth of *Z. novazelandica* in the present culture experiments was not under carbon limitation. This situation is expected as the seawater in the aquaria was continuously aerated, and completely renewed at every 3 days, thus the development of a boundary layer could have been minimised.

4.6 CONCLUSIONS

Results from the present laboratory aquaria study demonstrated a strong response of some growth parameters of *Zostera novazelandica* (proportional growth rate, pigments, ratio of variable : maximum fluorescence ($F_v:F_m$) and tissue nutrient content) to interactive effects of altered temperature, PFD and inorganic nitrogen (N_i) nutrient concentrations in the seawater column. *Z. novazelandica* in the culture can grow at least as quickly as the field population under high levels of PFD and temperature. The experimental evidence also suggested that the ambient N concentration available in the seawater is probably enough to allow the high growth rates, although a smaller reduction in tissue nitrogen content was observed. This study supports results of field studies and regression analyses

described in Chapter 3 in which seasonal growth rate is controlled by the interaction of PFD and temperature.

The impact of moderate light reductions indicate that *Zostera novazelandica* was able to survive in an environment of high temperature in ambient nitrogen concentration during the 21-day experimental period while maintaining comparatively high growth rates. Under this ‘shade’ condition, the seagrass exhibited a photo-adaptive response or acclimation by increasing chlorophyll *a* and *b* concentrations and reducing its chlorophylls *a:b* ratios. More chlorophyll being manufactured means more light harvesting pigments to capture reduced availability of photons under the shade condition in the aquarium. *Z. novazelandica* also acclimatised to quality of light (colour) or spectral distribution by synthesising more accessory pigments (i.e. chlorophyll *b*) indicated by the reduction of chlorophylls *a:b* ratios. Thus, light that was not significantly absorbed by chlorophyll *a*, would instead be captured by the chlorophyll *b*. This acclimation might have helped in extending the period of their survival under below the minimum light requirements (see Longstaff and Dennison 1999 and literature cited therein). The minimum light requirements (i.e. the quantity of light under which the seagrass can grow) is generally high in seagrasses as compared to other marine flora, with an average of about 11% of surface light (see Longstaff and Dennison 1999 and literature cited therein). With regard to this study, the efficiency of the photo acclimations observed, however, could not be ascertained since in some low light treatments, growth was reduced and mortality higher, which suggest that photosynthetic rate was also reduced and respiration may be elevated. Results of the present study may also suggest that field *Z. novazelandica* populations may increase their chlorophyll concentration as a method of adaptation to reduced light levels and seasonal increases in temperature. This response may also be a factor that enables the plants to successfully exploit the intertidal and subtidal areas of Otago Harbour.

Addition of excessive nitrogen concentration in the seawater under the levels of temperature and PFD provided in the culture did not stimulate the rate of leaf proportional growth nor increase the synthesis of chlorophyll pigments or leaf tissue nutrient contents of the seagrass. These results are paralleled with the responses recorded in *in situ* sediment N-enrichment studies described in Chapter 5, which indicate that nitrogen is not a limiting nutrient to the growth of *Z. novazelandica*. However, elevating the concentration of nitrogen in the seawater at high temperature had imposed physiological stresses to the seagrass, as indicated by lower values of leaf $F_v:F_m$, weakened tissue structure and subsequent defoliation of the leaves, and consequently the plants died before the end of the experimental period. By contrast, plant mortalities were not observed in seawater N-enrichment at low temperature although their leaf $F_v:F_m$ values were low. This may suggest that the adverse effects of the excessive N-enrichment in seawater are less severe in cooler seasons.

Chapter 5

The effects of sediment nitrogen enrichment on the growth, morphology and physiology of *Zostera novazelandica*

5.1 INTRODUCTION

Seagrasses are evolutionarily descended from terrestrial vascular plants (Den Hartog 1970) and able to absorb nutrients (nitrogen and phosphorus) both through their leaves and root-rhizome systems (e.g., Iizumi and Hattori 1982, Thursby and Harlin 1982, Short and McRoy 1984, Short 1987, Hemminga *et al.* 1994, Stapel *et al.* 1996). Ammonium and nitrate are the major sources of N for leaf uptake from the water column for seagrass meadows, while ammonium is the most abundant form in the sediment pore water for roots (Lee and Dunton 1999b). Phosphorus occurs as phosphate both in the water column and sediment pore water (Hemminga 1998). Ammonium and phosphate concentrations are typically low in the water column but much higher concentrations are present in the porewater (Hemminga 1998). Nitrate (nitrate and nitrite) concentrations are similar in the water and sediment. Furthermore, nitrate uptake in eelgrass is inhibited by high ammonium concentration in the rhizosphere (Short and McRoy 1984).

5.1.1 Nutrient limited growth

Despite high nutrient concentrations in the sediment porewater, evidence of nutrient limited growth and productivity has been reported for some seagrasses in their natural habitats. For example, N-limited seagrasses have been documented in coastal waters of Europe (Pedersen and Borum 1993, Van Lent *et al.* 1995), Australia (Bulthuis and Woelkerling 1981, Bulthuis *et al.* 1992, Udy and Dennison 1997, Udy *et al.* 1999) and

Atlantic coast of the USA (Harlin and Thorne-Miller 1981, Kenworthy and Fonseca 1992, Lee and Dunton 1999a). Phosphorus limitation has been reported for subtropical and tropical seagrass species in the Mediterranean (Perez *et al.* 1991), the Caribbean (Short *et al.* 1990, Jensen *et al.* 1998) and Florida Bay (Powell *et al.* 1989, Fourqurean and Zieman 1992, Fourqurean *et al.* 1992). Evidence of simultaneous N and P limitation has also been found in a few seagrass beds (Murray *et al.* 1992, Agawin *et al.* 1996, Alcoverro *et al.* 1997). Moreover, iron limitation has recently been indicated in seagrasses in the Mexican Caribbean (Duarte *et al.* 1995) and Bermuda (Jensen *et al.* 1998).

Nutrient availability is considered an important regulating factor for the macrophyte-dominated marine ecosystems where light and temperature is sufficient (c.f. Ceccherelli and Cinelli 1997). For seagrass, nutrient concentrations in the sediment are considered more important in determining whether seagrass growth is nutrient limited (see Bulthuis *et al.* 1992 and literatures cited therein). In temperate seagrass ecosystems, higher nutrient uptake during the seagrass growing season could deplete the nutrient availability (Alcoverro *et al.* 1995, Sfriso and Marcomini 1999) resulting in seasonal (mid-spring and late-summer) nutrient limited growth (e.g., Perez *et al.* 1991, Van Lent *et al.* 1995, Alcoverro *et al.* 1997). In tropical and subtropical coastal waters, adsorption of dissolved inorganic phosphate onto calcium carbonate particles often limits the primary production of seagrass growing in carbonate sediments (Short *et al.* 1990, Fourqurean *et al.* 1992, Jensen *et al.* 1998). Furthermore, nutrient uptake by below-ground parts of seagrasses may be insufficient to meet total plant demands even in the presence of high nutrient concentrations in the pore water (Stapel *et al.* 1996). This was due to the formation of a microgradient of nutrient depletion around the seagrass roots resulted in a slow nutrient diffusion rates from pore water to the surface of the roots hence limit the nutrient availability (in Bulthuis and Woelkerling 1981, Stapel *et al.* 1996).

5.1.2 Tools for nutrient limitation studies

'Nutrient limitation' in aquatic ecology refers to the limitation of net production of key plant components or of total system net production (in Taylor *et al.* 1995, literatures cited therein). In the case of seagrasses, this has been evaluated using several approaches. Some investigators have measured the nutrient content (carbon, nitrogen and phosphorus) in the seagrass tissue and subsequently inferred nutrient limitation when the respective nutrient concentrations fell below suggested critical values (e.g., Duarte 1990, Fourqurean and Zieman 1992, Perez-Llorens and Xavier Niell 1993). Potential nutrient limitation has also been determined through the analysis of the nutrient budget of seagrasses (e.g., Patriquin 1972, Pedersen and Borum 1993, Lee and Dunton 1999b), nutrient uptake kinetics (Stapel *et al.* 1996) and development of a numerical simulation model (Zimmerman *et al.* 1987). Others have directly monitored responses of plants following *in situ* fertilisation (normally N, P or N+P) of the water column (e.g., Harlin and Thorne-Miller 1981, Williams and Ruckelshaus 1993) or sediments (e.g., Perez *et al.* 1991, Bulthuis *et al.* 1992, Murray *et al.* 1992, Van Lent *et al.* 1995, Agawin *et al.* 1996, Udy *et al.* 1999).

Responses obtained from *in situ* nutrient enrichment experiments have been considered the most conclusive evidence for nutrient limitation (Alcoverro *et al.* 1997), with the greatest effects being in response to sediment fertilisation (c.f. Dennison *et al.* 1987). Response of seagrasses to enrichment varied greatly, however, among habitats, hence demonstrating different types of growth limitation. In general, the exposure to high nutrient concentrations of a nutrient-limited seagrass population will influence their growth rates, biomass or morphology (i.e. leaf length, canopy height, shoot density, density of leaves clusters) or combination of these variables (e.g., Bulthuis *et al.* 1992, Kenworthy and Fonseca 1992, Murray *et al.* 1992, Van Lent *et al.* 1995). Physiological responses

have been observed in a few seagrasses, which include increasing tissue C, N and P content (e.g., Bulthuis and Woelkerling 1981, Perez *et al.* 1991, Lee and Dunton 1999a), and the concentration of N-rich amino acids, particularly glutamine and asparagine (e.g., Udy and Dennison 1997, Udy *et al.* 1999). Increases in blade chlorophyll content (Lee and Dunton 1999a), and maximum rates of photosynthesis (Agawin *et al.* 1996) have also been reported.

Despite the positive responses obtained in a few direct tests, nutrient limitation is not considered a general phenomenon in seagrass (in Van Lent *et al.* 1995) and still a subject of controversy (c.f. Agawin *et al.* 1996, literatures cited therein). Based on the simulation model of seagrass growth, Zimmerman *et al.* (1987) disputed the suggestion that productivity of seagrasses in their natural habitats is nitrogen limited. This contention was further supported by the results of some *in situ* enrichment experiments, which did not demonstrate nutrient limitation, as evidenced by the growth of *Zostera marina* in Great Harbour, USA (Dennison *et al.* 1987) and SE Asian seagrasses that inhabit both terrigenous and carbonate sediments (Erfemeijer *et al.* 1994). Also no significant response was reported in the growth rate and P_{max} of *Z. marina* following N-enrichment in Danish Estuary which led to a suggestion that resources other than N were limiting (Pedersen 1995). Recently, Ceccherelli and Cinelli (1997) suggested that the growth of *Cymodocea nodosa* in the Mediterranean may not be under nutrient limitation following results of a short-term sediment enrichment experiment. The authors had also suggested, however, that the lack of response for the shoot density and leaf length of the enriched plants may be attributable to the small plot sizes used. It appears, therefore, that the occurrence of nutrient-limited growth for seagrasses is site-specific.

To date there are no published data on the effects of *in situ* nutrient enrichment on the growth of seagrass in New Zealand. The present study focused on the responses of *Zostera novazelandica* to sediment N enrichment in seagrass meadows at Harwood, Otago

Harbour, southern New Zealand. This *in situ* manipulation experiment was designed to test if *Zostera* growth was nutrient limited during summer growing period when seawater nutrients are low. The response of *Z. novazelandica* to sediment ammonium enrichment was evaluated through changes in the plant growth rate, biomass, shoot density, canopy height (actually shoot height was measured as a proxy), tissue nutrient content, leaf chlorophyll content, and PSII photochemical efficiency ($F_v:F_m$ ratio). In addition, ammonium concentrations in the sediment porewater of the seagrass habitat were measured.

5.2 MATERIAL AND METHODS

5.2.1 Study site

The study was located at the Harwood intertidal seagrass bed (Figure 5-1), details of which are described in previous chapters. The enrichment experimental plot was established on the lower intertidal zone, between plots H2 and H3 used in the seasonal variation studies of *Zostera novazelandica* discussed in Chapter 3. The substratum at this site was a mixture of cockleshells and sand.

5.2.2 Experimental design

The enrichment experiment was based on that Bulthuis *et al.*, (1992), using a randomised block design. Three replicate blocks, each consisting of a fertilised treatment paired with an unfertilised control plot, were established in a relatively dense seagrass bed (Figure 5-2). Each plot (0.5 X 0.5 m) was marked at its corners with coloured bamboo stakes of about 7 cm height.

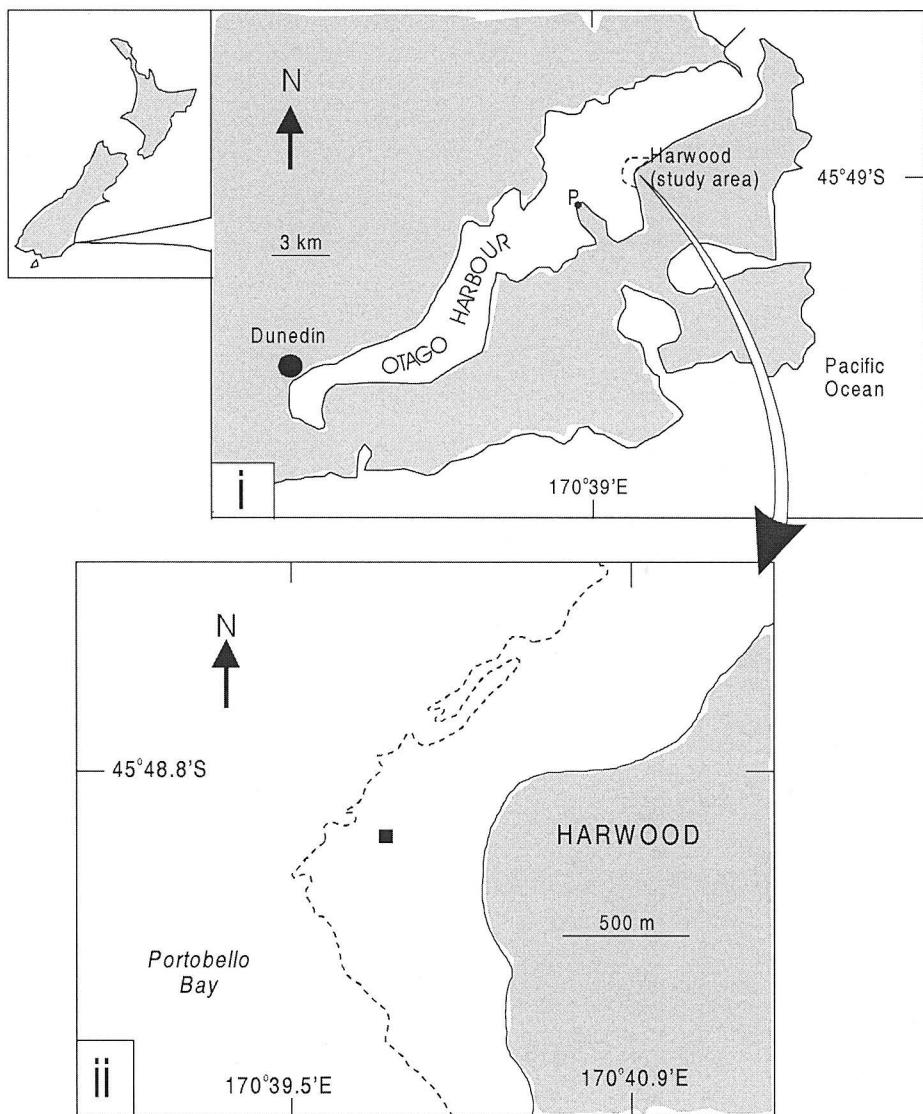


Figure 5-1: Map of Otago Harbour (i), New Zealand; showing the location of the Harwood intertidal area, and Portobello Marine Laboratory (P). Detail of Harwood (ii), including enrichment experimental plot (■). Dotted line indicates the approximate edge of the intertidal area.

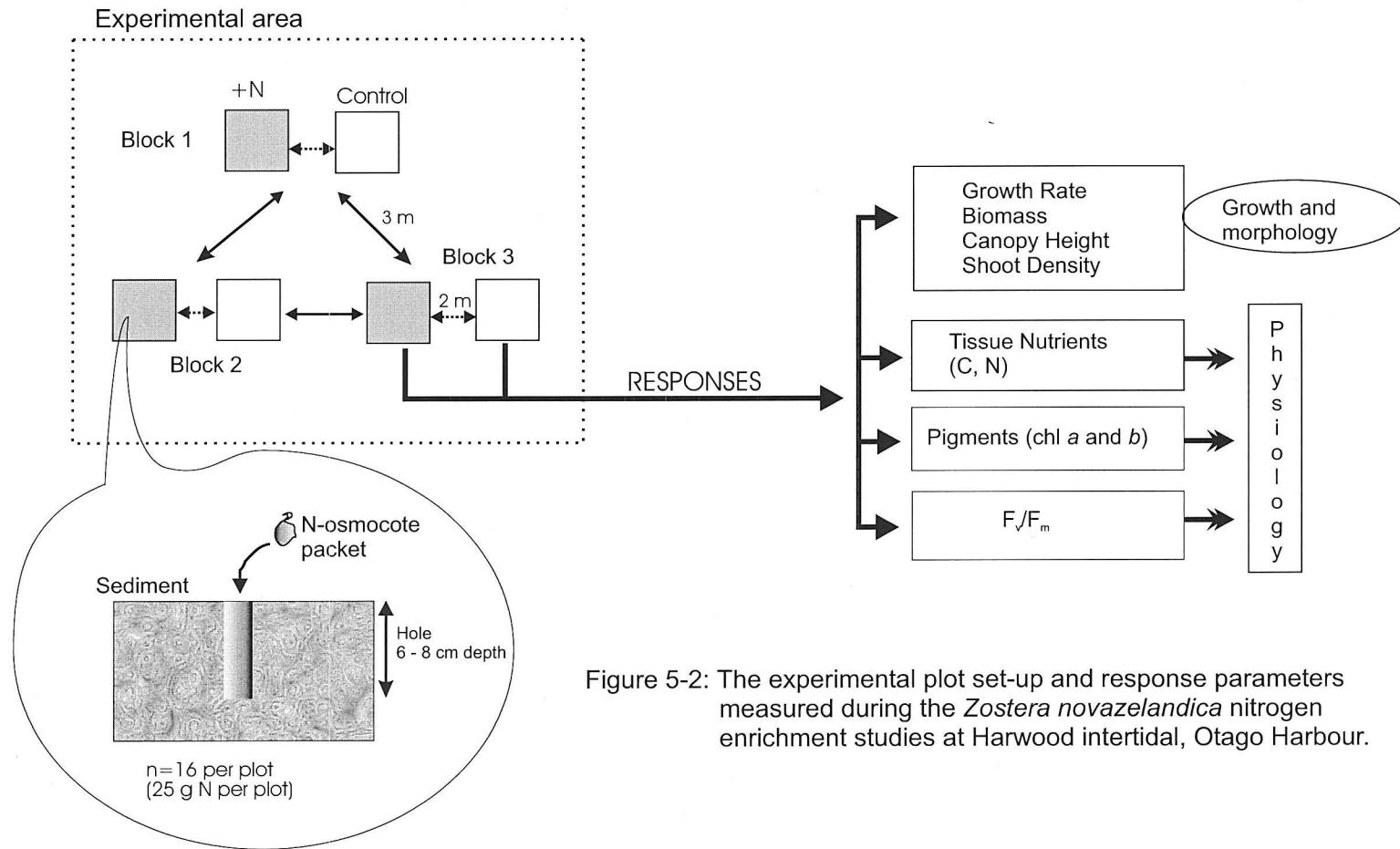


Figure 5-2: The experimental plot set-up and response parameters measured during the *Zostera novazelandica* nitrogen enrichment studies at Harwood intertidal, Otago Harbour.

The replicate plots were about 3 m from each other and there was about 2 m between the treatment and control plots. These distances were expected to be sufficient to avoid the exchange of nutrients between plots through the rhizome system, since the rhizomes of *Zostera novazelandica* at the site did not extend longer than a metre (pers. observation). Manipulation of the level of sediment porewater nitrogen was achieved by addition of slow-release nitrogen-OsmocoteTM fertiliser, containing 11.5% nitrate and 11.5% ammonium (release time at 16°C: 6 - 7 months; OsmocoteTM, Scotts Australia). Osmocote slow-release fertiliser has been widely used in seagrass enrichment studies (e.g., Bulthuis *et al.* 1992, Erftemeijer *et al.* 1994, Udy and Dennison 1997).

5.2.3 Fertilisation method

The experiment was initiated in early summer (27 November 1998) and ended in autumn (30 March 1999) spanning the period when *Zostera novazelandica* growth was expected to be at its annual maximum (see Chapter 3, Figure 3-8). Prior to the addition of the fertiliser, each treatment plot was divided into 25 sub-plots of 5 X 5 cm. Sixteen of these sub-plots were randomly selected and allocated for the fertiliser additions. A small sediment core (2 cm diameter) was taken from the centre of the sub-plots to a depth of 6 - 8 cm where the densest root development zone of *Zostera* occurred (Figure 5-2). Fertiliser packets each containing 6.8 g of Osmocote wrapped in gauze cloth were then buried in the holes created by the removal of the sediment cores. The holes were re-filled with their original sediment cores. The amount of fertiliser added corresponded to 108.7 g Osmocote (25 g N) per plot, similar to the amount used by Bulthuis *et al.*, (1992) in the enrichment of *Heterozostera tasmanica* in Port Phillip Bay, Australia.

5.2.3.1 Porewater ammonium concentration

In this study, only ammonium concentration in the sediment porewater was determined, as it is preferred over nitrate by seagrasses, for example by *Zostera marina* (Short and McRoy 1984, Hemminga *et al.* 1994). Concentrations of ammonium from each plot were determined prior to the treatment and then every month following fertilisation to assess the effectiveness of the enrichment protocol. Porewater samples were taken using a sediment sipper (Udy and Dennison 1997, Kendrick *et al.* 1998, McMahon and Walker 1998). The sipper used in this study was 9 cm long and pushed into the sediment to draw water at a depth of between 5 - 6 cm.

Sampling was carried out during low tide. At each plot, the sipper was initially flushed with porewater by withdrawing 20 mL samples using a 60 mL syringe and the sample then discarded. The porewater sample was then collected in a separate syringe which was immediately emptied into a screw cap plastic vial containing 1 mL mercuric II chloride ($11.0 \text{ g Hg}^{2+}\text{L}^{-1}$) to remove sulphides (Airey *et al.* 1984). Duplicate porewater samples were randomly taken from each plot at each sampling month ($n=2$ per plot). All samples were kept on ice and transported back to the laboratory. Filter holders, screw-cap plastic tubes, syringes and sipper were acid washed using 10% (v/v) hydrochloric acid before use.

Samples were filtered (WhatmanTM, GF/F, 0.7 μm) into acid washed screw-cap tubes to remove yellow precipitates of sulphide and frozen at -20°C until further analysis. Ammonium concentration was analysed spectrophotometrically at 640nm according to methods of Parsons *et al.*, (1984) .

5.2.3.2 Plant responses monitored

The parameters used to assess the response of *Zostera novazelandica* to nitrogen enrichment were growth and morphology (growth rate, biomass, shoot density and shoot height as a proxy for a canopy height) and physiology (tissue C and N content, pigment content, photosystem II photochemical efficiency or $F_v:F_m$) (Figure 5-2). These parameters, with the exception of $F_v:F_m$, have been commonly used in previous nutrient enrichment studies (Bulthuis and Woelkerling 1981, Bulthuis *et al.* 1992, Murray *et al.* 1992, Erftemeijer *et al.* 1994, Van Lent *et al.* 1995, Udy and Dennison 1997). A summary of the sampling plan for the response parameters is presented in Table 5-1.

Table 5-1: Parameters investigated during the nitrogen enrichment study.

<i>Response parameters</i>	<i>Sampling time</i>	<i>No. of samples/plot</i>
Pore water ammonium	Nov 98 - March 99	2
Shoot height (used as a proxy for canopy height)	March 1999	20
Shoot density	March 1999	3
Biomass	March 1999	3
Leaf growth rate	March 1999	8-12
Tissue nutrient (C,N)		
a. leaves	March 1999	3
b. below-ground		3
Chlorophyll <i>a</i> and <i>b</i>	Nov 1998 - Mar 1999	3
$F_v:F_m$ ratio	Dec 98, Feb., Apr 1999	8

5.2.3.3 Growth and morphological responses

a. Proportional growth rate

A minimum of 8 seagrass plants inside each experimental plot were randomly selected and marked in March 1999 (after 4 months of enrichment), using the hole-punch method of Kirkman and Reid (1979). Each marked seagrass shoot was tied with a soft wire to a small, coloured bamboo skewer (Chapter 3: Figure 3-3). The individual seagrass plants were collected on day 7 after hole punching and processed as described in Section 3.2.2.3 of Chapter 3. The proportional leaf growth rate (PGR) of *Zostera novazelandica* in each plot was calculated according to Larkum *et al.* (1984) as previously described.

b. Above and below ground biomass

All plant materials in 3 randomly allocated subplots were harvested from each experimental plot at the conclusion of the experiment (March 1999). Samples were dug using a trowel for the estimation of both above and below-ground biomass. These samples were taken after the completion of the leaf growth rate experiment. All samples collected for biomass were handled and processed according to the procedure described in Section 3.2.2.1 of Chapter 3.

c. Shoot density and canopy height

Shoot densities of *Zostera novazelandica* were determined *in situ* from 3 randomly allocated subplots in each experimental plot ($n=3$ per plot). The mean number of shoots in each plot was calculated from these subplot samples. Seagrass canopy height was obtained by measuring from the sediment surface to the top of the tallest leaf (Bulthuis *et al.* 1992) for 20 randomly selected shoots in each plot.

5.2.3.4 Physiological response

a. *Tissue carbon and nitrogen*

Dried seagrass samples used for above and below-ground biomass measurements (section 5.2.3.3b) were ground to a fine powder with a mortar and pestle (n=3 per plot). Samples were analysed for elemental carbon and nitrogen using a Dumas elemental analyser (Carlo Erba Instruments-CHNS-0 EA1108).

b. *Chlorophyll content*

The chlorophyll concentrations were analysed using the second youngest leaf from randomly selected shoots to standardise for leaf age (Abal *et al.* 1994). Sampling was carried out before additions of fertiliser and then monthly during the enrichment studies. Between 3 - 5 shoots were sampled in each of 3 randomly selected sub-plots from the treatment and control plots (n=3 per plot). The samples were brought back to the laboratory in polyethylene bags in a cool box, immediately frozen and analysed within 4 days.

The leaf chlorophyll *a* and *b* was extracted according to Dunton and Tomasko (1994). Preweighed cleaned leaf material (approximately 50 mg wet weight) were chopped into small pieces and then immersed in 5 mL of N, N-dimethylformamide (DMF) in screw-cap glass tubes. The tubes were wrapped in aluminium foil at room temperature (12 - 15°C) for 24 hours. The absorbance of the chlorophyll extracts was then measured in a UV spectrophotometer (Pharmacia) at 664 and 647 nm, and all readings were corrected for turbidity scattering by subtracting the 750 nm absorbance. Chlorophyll *a* and *b* content was determined using the equations of Porra *et al.* (1989) .

c. $F_v:F_m$ ratio

The ratio of variable to maximal chlorophyll fluorescence for *Zostera novazelandica* was determined using an underwater pulse amplitude modulated chlorophyll fluorescence meter (PAM) (Waltz, Germany). Eight shoots were randomly selected from each plot ($n=8$) on each sampling occasion. Before the measurement was made, two leaves from a seagrass shoot were dark adapted for 10 min using a dark-adaptation clip (Ralph 1998). The clip was positioned approximately in the middle portion of the leaf blade to ensure that the fluorescence signal determined was at a standard position on the leaves (Ralph 1998). The dark adaptation time of 10 min used for *Z. novazelandica* in this study was selected based on dark acclimation period of 5–15 min, commonly used for measurements of chlorophyll fluorescence signals of marine plants such as seagrasses (Ralph 1998, Ralph *et al.* 1998, Beer and Bjork 2000) and macroalgae (Collen and Davison 1999). Furthermore, no significant difference was observed between the signals values measured using 5 min dark adaptation than that of 15 min (Ralph 1996).

Measurements were made at almost the same time i.e. late morning low tide, on each sampling month. Solar irradiance and air temperature were also measured by the PAM chlorophyll fluorescence meter. This study was carried out monthly during the nitrogen enrichment period, except in March 1999 when the instrument was not available. The final measurement was taken in April 1999.

5.3 DATA ANALYSIS

The mean values of canopy height, shoot density, biomass, growth rate, and tissue C and N content within each enriched and control plot ($n=3$) were obtained. Differences between the means were then analysed using a two-way ANOVA with factors being A = blocks and B = treatments (control and enriched).

For chlorophyll *a* and *b* and the $F_v:F_m$ ratio, the mean values for treatment and control plots for replicate blocks were obtained. The monthly means for enriched and control plots were then calculated from the 3 replicate plots ($n=3$). The test of significance on the monthly means between control and enriched plots was then performed using a 3-way ANOVA with factors being A = blocks, B = treatments and C = time. These data were also separately analysed each month using a two-way ANOVA to detect significant responses of these parameters to N additions (Bulthuis and Woelkerling 1981). Statistical analyses were performed using SPSS software package for Windows Release.2.0 (SPSS Inc.,Chicago). Where necessary data were transformed to satisfy the ANOVA requirements of normality and variance homogeneity.

5.4 RESULTS

5.4.1 Porewater ammonium concentration

The concentration of ammonium in the control sediment porewater declined throughout the course of the experiment, from 74 μM (November 1998) to about 9 μM (March 1999) (Figure 5-3). In contrast, the porewater ammonium concentration increased dramatically in the enriched plots, from the initiation to the conclusion of the experiment. Mean values increased 4-fold during the first month of fertiliser additions (December 1998). The ammonium peaked at more than 5200 μM in March 1999, which was 500-times higher than that in control plots.

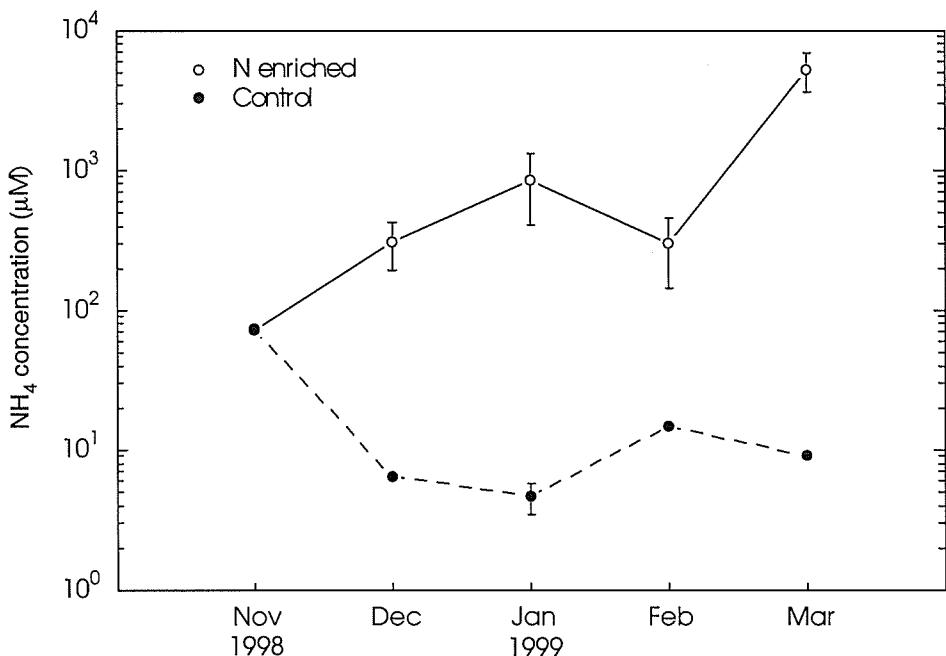


Figure 5-3: Porewater ammonium concentration (mean of triplicate samples \pm 1S.E.) in control and enriched plots during the enrichment experiment at Harwood intertidal area.

5.4.2 Morphology and growth responses

Responses of plant canopy height, shoot density, biomass and leaf proportional growth rate of *Zostera novazelandica* to nitrogen enrichment are shown in Figure 5-4 and Table 5-2. These parameters were assessed in March 1999, 4 months after fertiliser was added to the sediments.

The canopy of *Zostera novazelandica* growing in the nitrogen enriched treatment was taller (11.4 cm) than those in control plot (9.4 cm) ($p<0.05$) (Figure 5-4). Shoot density of the enriched treatment was 34% higher than that of the control but this increase was not significant ($p=0.07$).

There was no significant difference in above-ground biomass between control and enriched plots in March 1999. A marginally significant increase in below ground biomass (13%) was observed in the enriched plots compared to the controls ($p=0.05$) (Table 5-2).

There was no significant effect of nitrogen additions to the leaf proportional growth rate of *Zostera novazelandica*, with treatment and control plots having a similar growth rate ($0.024 \text{ gg}^{-1} \text{ day}^{-1}$).

Table 5-2: Summary of two-way ANOVA on morphology, biomass and growth rate of *Zostera novazelandica* in enriched and control plots at the end of the enrichment experiment.

Growth variable	Source of variation	d.f.	SS	F-value	P-value
Canopy height	Blocks	2	3.98	33.395	0.03
	Treatments	1	4.46	74.850	0.01
Shoot density	Blocks	2	1967841.00	1.17	0.46
	Treatments	1	10225981.50	12.19	0.07
Above-ground biomass	Blocks	2	2909.06	28.81	0.03
	Treatments	1	4.10	0.08	>0.50
Below-ground biomass	Blocks	2	9825.27	110.74	0.01
	Treatments	1	815.97	18.39	0.05
Growth rate	Blocks	2	9.10×10^{-5}	7.38	0.12
	Treatments	1	1.07×10^{-5}	1.73	0.32

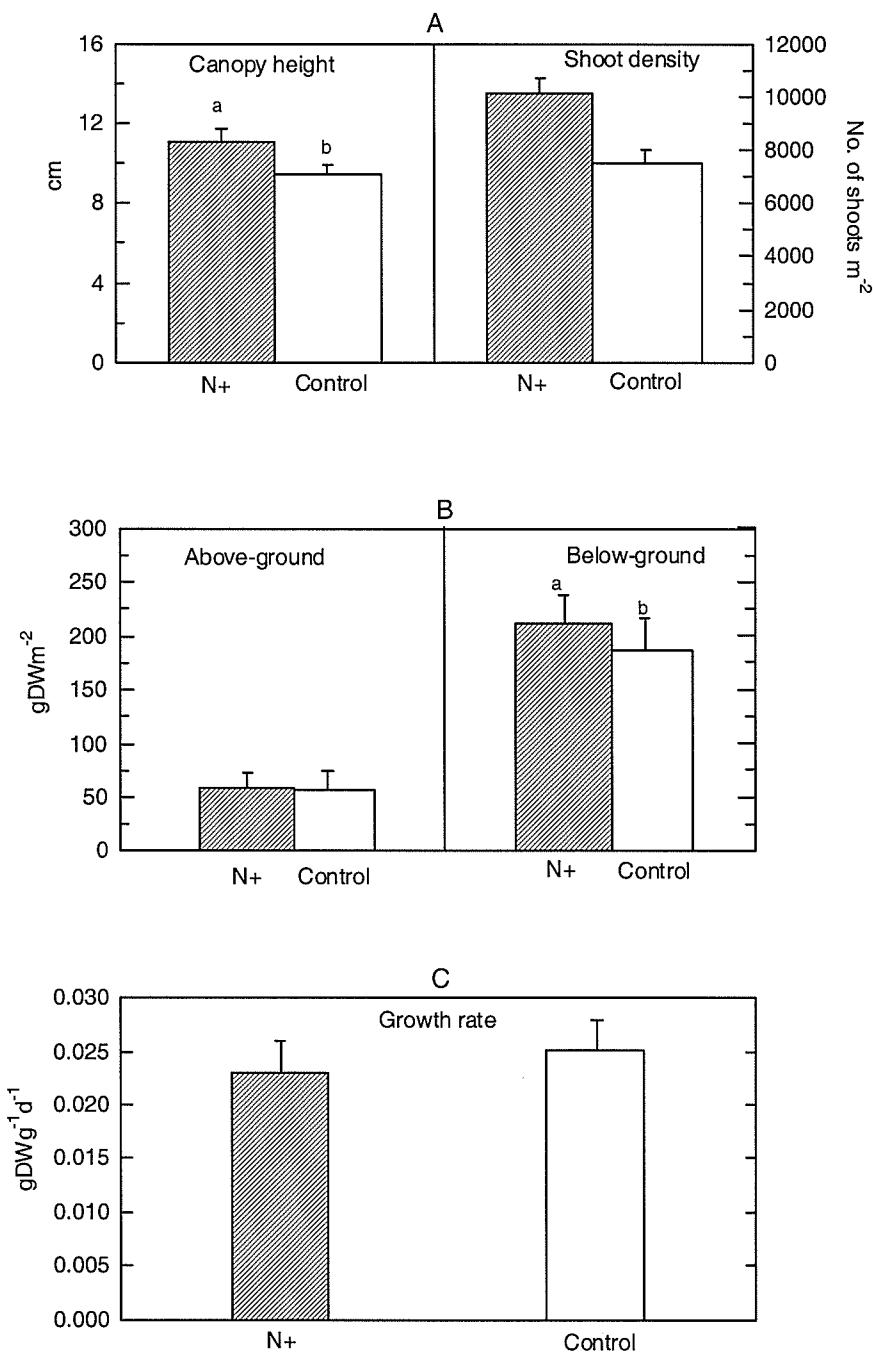


Figure 5-4: Growth and morphological responses of *Zostera novazelandica* in March 1999 after 4 months of enrichment with nitrogen fertiliser compared to untreated controls. Bars represent mean of triplicate samples \pm 1S.E. A, canopy height and shoot density; B, above and below ground biomass; C, leaf proportional growth rate. Different letters (a, b) indicate significant differences between means ($p<0.05$).

5.4.3 Physiological responses

5.4.3.1 Plant tissue C and N

Nitrogen enrichment did not affect the carbon or nitrogen content of the tissues of *Zostera novazelandica* at the study site (Figure 5-5; Table 5-3). The leaves had a higher C and N content than the below-ground tissue both in the enriched and control plots (two-way ANOVA, $p>0.05$). Nitrogen enrichment did not significantly affect the C:N atomic ratio of leaves and below ground tissues of *Z. novazelandica* (two-way ANOVA, $p>0.05$).

5.4.3.2 Chlorophyll *a* and *b* concentration

A three-way ANOVA on the chlorophyll *a* concentration in the leaves of *Z. novazelandica* showed a significant variation through time and between treatments ($p<0.05$; Table 5-4). The initial concentration (before nutrient additions) in November 1999 was about 1.4 mg g⁻¹ tissue wet weight. Both control and enriched plots showed, however, a significant reduction in February 1999 and then increased in March 1999 (Figure 5-6). A significant effect of nitrogen enrichment on chlorophyll *a* was observed only in March 1999 (Table 5-5, $p<0.05$).

The concentration of chlorophyll *b* did not vary during the course of the enrichment experiment ($p>0.05$) and varied only marginally significantly between treatments (three-way ANOVA; $p = 0.047$) (Table 5-6; Figure 5-6). Separate two-way ANOVA performed on each month's data failed, however, to detect significant response between treatments ($p>0.05$, Table 5-7).

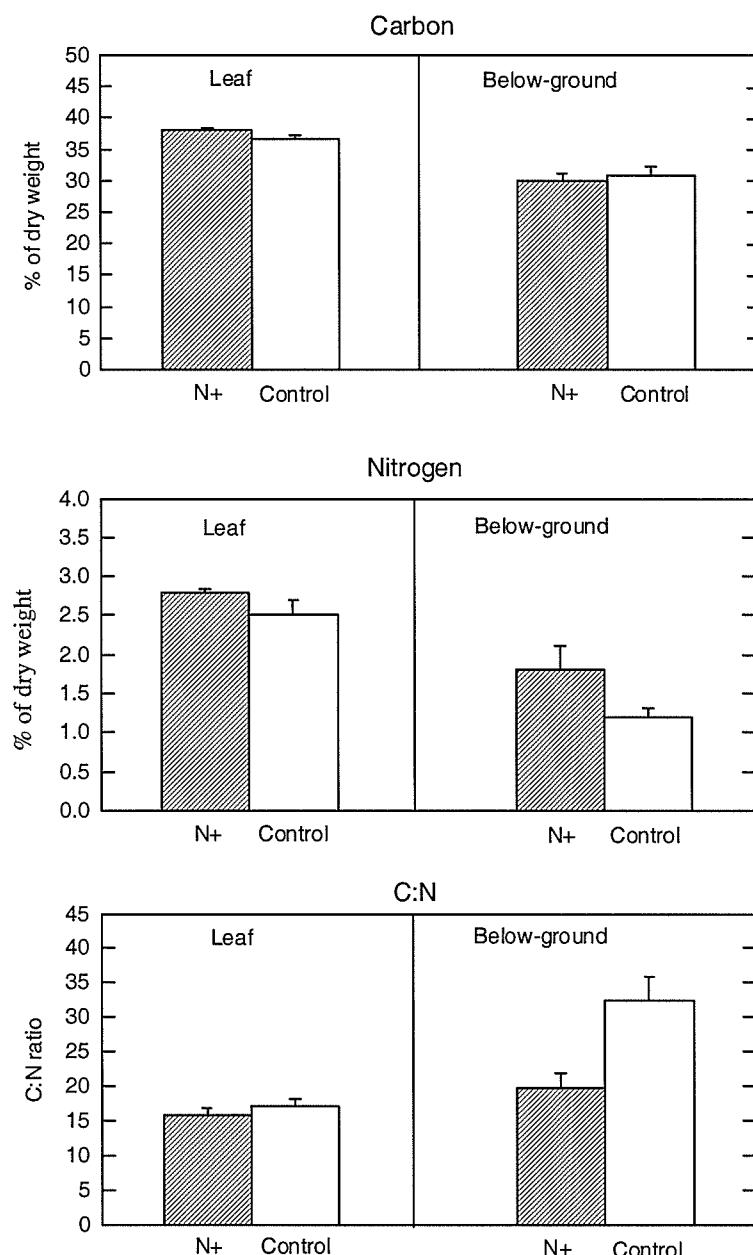


Figure 5-5: Carbon and nitrogen content as % per g dry weight, and C:N ratio in the leaf and below ground tissues of *Zostera novazelandica* plants in March 1999 after 4 months of enrichment with nitrogen fertiliser compared with untreated controls. Mean of triplicate samples ± 1S.E.

Table 5-3: Summary of two-way ANOVA on C and N content in leaf and below-ground tissues of *Zostera novazelandica* in enriched and control plots at the end of the enrichment experiment.

Variable	Source of variation	d.f.	F-value	P-value
% tissue carbon:				
i. leaf	Blocks	2	0.027	0.974
	Treatments	1	1.545	0.340
ii. below-ground	Blocks	2	13.797	0.068
	Treatments	1	2.494	0.255
% tissue nitrogen				
i. leaf	Blocks	2	2.111	0.321
	Treatments	1	4.000	0.184
ii. below-ground	Blocks	2	0.388	0.721
	treatments	1	5.224	0.150
C:N ratio				
i. leaf	Blocks	1	2.405	0.294
	Treatments	2	3.797	0.191
ii. below-ground	Blocks	2	0.320	0.758
	Treatments	1	6.418	0.127

Table 5-4: Results of three-way ANOVA on chlorophyll *a* in the leaf of *Zostera novazelandica* during nitrogen enrichment, from November 1998 through March 1999.

Source of variation	d.f.	SS	F value	P value
Blocks	2	0.0457	2.324	0.160
Treatments	1	0.0538	5.466	0.048
Months	4	0.4060	10.309	0.003
Residual	8	0.0787		

Table 5-5: Probabilities of the F ratio from two-way ANOVA performed on chlorophyll a concentration of *Zostera novazelandica*. Data were separately analysed each month from November 1998 to March 1999.

Source of variation	Nov. 98	Dec. 98	Jan. 99	Feb. 99	Mar. 99
Blocks	>0.5	0.335	0.019	>0.5	>0.5
Treatments	>0.5	0.338	0.133	0.273	0.029

Table 5-6: Results of three-way ANOVA on chlorophyll b in the leaf of *Zostera novazelandica* during nitrogen enrichment, from November 1998 through March 1999.

Source of variation	d.f.	SS	F value	P value
Blocks	2	0.000757	0.252	0.783
Treatments	1	0.00825	5.485	0.047
Months	4	0.0132	2.194	0.160
Residual	8	0.0120		

Table 5-7: Probabilities of the F ratio from two-way ANOVA performed on chlorophyll b concentration of *Zostera novazelandica*. Data were separately analysed each month from November 1998 through 1999.

Source of variation	Nov. 98	Dec. 98	Jan. 99	Feb. 99	Mar. 99
Blocks	0.424	0.304	>0.5	>0.5	0.250
Treatments	>0.5	>0.5	0.270	0.347	0.063

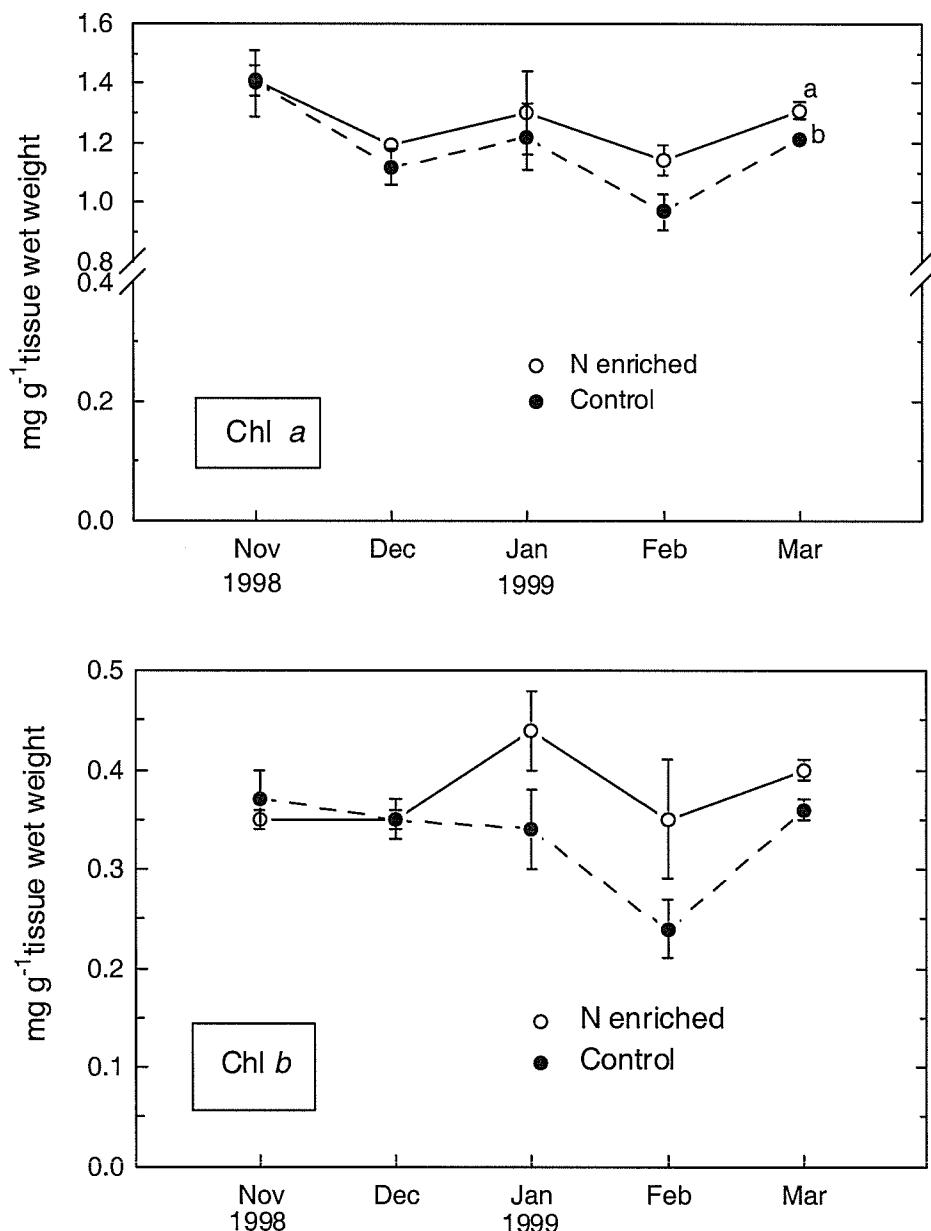


Figure 5-6: Monthly concentrations of chlorophyll *a* and *b* of *Zostera novazelandica* leaves in control and nitrogen enriched plots at Harwood intertidal area, Otago Harbour, from November 1998 through March 1999: mean \pm 1S.E. ($n=3$). Different letters (a and b) indicate significantly different means between treatments ($p<0.05$) in the respective month as explained in Table 5-5 and Table 5-7.

5.4.3.3 $F_v:F_m$ ratio

Figure 5-7 illustrates the $F_v:F_m$ ratio of *Zostera novazelandica* growing in nitrogen-enriched sediments and those of unenriched control plots when exposed during day low tide. The ratio showed a highly significant variation throughout the enrichment experiment ($p<0.001$) both in treatment and control plots, from below 0.6 (December 1998) to about 0.8 (April 1999) as shown in Table 5-8. There was also a significant nitrogen effect in the mean $F_v:F_m$ ratio ($P=0.001$).

$F_v:F_m$ was low (less than 0.6) when measurements were performed in summer (December 1998). The ratio measured was significantly higher in the enriched than that in control plots ($p<0.05$) (Table 5-9). Air temperature and light intensities recorded *in situ* using the PAM fluorometer were high, 19°C and $1600 \mu\text{mol m}^{-2}\text{s}^{-1}$ respectively, and it was a windy day. A significant nitrogen effect on $F_v:F_m$ ratio was also observed in February 1999 ($p<0.05$). The treatments were, however, marginally insignificant in January 1999, with an F ratio probability of 0.07 (Table 5-9).

The mean $F_v:F_m$ ratios were higher (around 0.8) in autumn (April 1999) with no significant differences between enriched and control plots. Although it was windy when $F_v:F_m$ measurements were carried out, light levels and air temperature recorded by PAM were lower than in the summer, $200-500 \mu\text{mol m}^{-2}\text{s}^{-1}$ and 13°C respectively.

Table 5-8: Results of three-way ANOVA on PS II photochemical efficiency ($F_v:F_m$ ratio) of *Zostera novazelandica* leaf tissue during nitrogen enrichment from December 1998 to April 1999.

Source of variation	d.f.	SS	F value	P value
Blocks	2	0.0197	8.15	0.019
Treatments	1	0.0427	35.24	0.001
Months	3	0.3470	95.48	<0.001
Residual	6	0.00727		

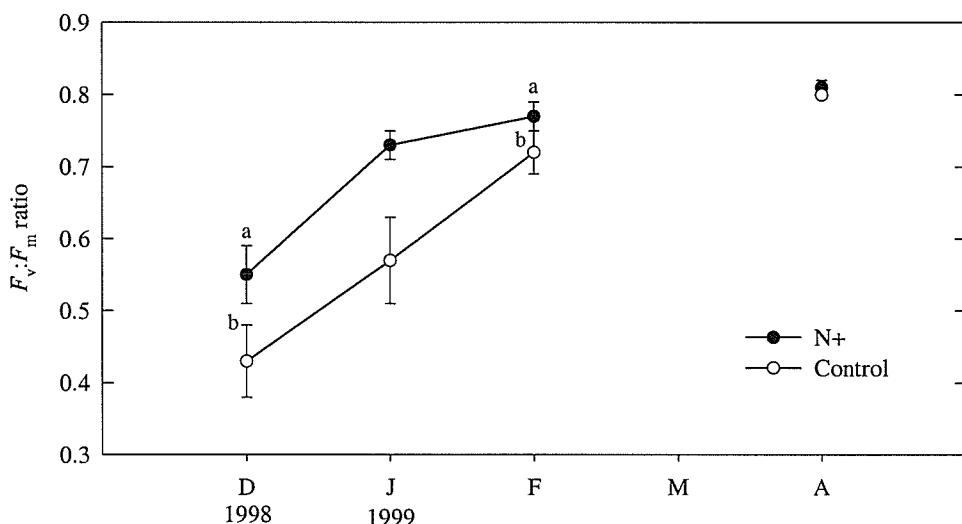


Figure 5-7: Monthly values of PS II photochemical efficiency ($F_v:F_m$) of *Zostera novazelandica* leaves in control and nitrogen enriched plots at Harwood intertidal area, Otago Harbour, from December 1998 - February and April 1999: mean \pm 1S.E. (n=3). Different letters (a and b) indicate significantly different means between treatments ($p<0.05$) in the respective month as explained in Table 5-9.

Table 5-9: Probabilities of the F ratio from two-way ANOVA performed on PS II photochemical efficiency ($F_v:F_m$ ratio) of *Zostera novazelandica*. Data were separately analysed each month. In March 1999 PAM was not available for measurements.

Source of variation	Dec. 98	Jan. 99	Feb. 99	Mar. 99	Apr 99
Blocks	0.034	0.0314	0.030	-	>0.5
Treatments	0.022	0.072	0.040	-	0.441

5.5 DISCUSSION

5.5.1 Porewater ammonium

At the onset of the present study, porewater ammonium concentrations of, for example 74 µM in November 1998 (control plots) were high when compared to <8 µM in the seawater (results in Chapter 3). However, the porewater ammonium gradually declined to about 9 µM in March 1999. This decline may have been related to the production of the *Zostera novazelandica* biomass as its growth is at annual peak during the summer period. Unfortunately, information is not presently available to determine whether or not *Z. novazelandica* primarily absorbed ammonium from the water column through its leaves or from the porewater via its roots, and nor is there information on the interaction of nutrient uptake between these two structures. Therefore, the reduction of porewater ammonium shown in the control plots cannot easily be explained.

The concentrations of porewater ammonium measured in the control plots throughout the study period are comparable to published values for seagrass beds elsewhere (Table 5-10). The range is, however, very much lower than that reported for *Heterozostera tasmanica* beds in Victoria (Australia) (Bulthuis and Woelkerling 1981). It

also appeared that throughout the summer period, porewater ammonium concentrations at Harwood were below the mean value of 86 µM for seagrass meadows worldwide which has been calculated from a literature survey by Hemminga (1998). The ammonium concentration is also below the threshold of 100 µM and 500 µM, values which were suggested to be limiting by Dennison *et al.* (1987) and Williams and Ruckelshaus (1993) respectively. This may imply that the growth of *Zostera novazelandica* at the study site could be under N limitation.

Table 5-10: Comparisons of porewater ammonium concentrations within sediments supporting seagrasses at various geographical locations. * Indicates data obtained from several sites; ** indicates data were collected on different dates; ⊕ DIN (ammonium, nitrate and nitrite).

Locality	Porewater NH ₄ ⁺ concentration (µM)	Reference
Harwood, Otago Harbour	9 - 74*	This study
Philippines	1.9 - 21.7*	Agawin <i>et al.</i> 1996
South Sulawesi, Indonesia	60	Erfemeijer <i>et al.</i> 1994
Moreton Bay, Australia	7.4	Udy and Dennison, 1997
Victoria, Australia	200 - 1700*	Bulthuis & Woelkerling, 1981
Port Phillip Bay, Australia	10.6 - 20.1**⊕	Bulthuis <i>et al.</i> 1992
Florida Bay, USA	98 - 160**	Fourqurean <i>et al.</i> 1992
The Netherlands	19.5 - 50.5**	Van Lent <i>et al.</i> 1995
Galenzana Bay, Italy	<1.5	Ceccherelli & Cinelli, 1999

Enriching the sediments with slow-released N-Osmocote fertiliser dramatically increased the levels of ammonium in the treatment plots. For example, at the end of the experimental incubation period, the porewater ammonium in these plots increased to about

5 mM, which is higher than the values obtained (about 1 mM) in the enrichment studies of Australian seagrasses by Udy and Dennison (1997). The high concentrations of ammonium measured indicate that the method of fertilisation employed was very effective in enhancing the N availability in the sediments. The slow-released N-Osmocote fertiliser used in this study is formulated for optimum release at a temperature of 16°C, and expected to be suitable for local summer temperature conditions. Moreover, fertilisers packaged in gauze cloth appeared to stay within the sediment as no washed-out material was observed during period of the study. A considerable amount of fertiliser granules still remained in the packets when they were retrieved at the end of the experiments, although their weights were not determined.

5.5.2 Seagrass responses

5.5.2.1 Shoot height, shoot density, growth rate and biomass

Generally, the growth responses of seagrasses to nutrient enrichment seemed to vary between species and/or levels of nutrient contained in their sediment porewater. For example, *Heterozostera tasmanica* growing under low N sediment porewater concentrations in Port Phillip Bay, Australia (see Table 5-10), had a significant increase in canopy height when enriched with N fertiliser (Bulthuis *et al.* 1992). Along with canopy height, above-ground biomass and density of leaf clusters of this seagrass also increased, thus indicating growth was N-limited (Bulthuis *et al.* 1992). In contrast, for those dominating higher concentrations of ammonium porewater in Victoria, only leaf growth rate were stimulated by fertiliser additions, and with no significant response in above-ground biomass or shoot density (Bulthuis and Woelkerling 1981). In another enrichment study, Van Lent *et al.* (1995) reported an enhancement of leaf growth rate, flowering and above-ground biomass in *Zostera marina* under low porewater ammonium (19.5 µM), but

a lack of response in plants at a site with relatively high ammonium concentration (50.5 µM). Conversely, Erftemeijer *et al.* (1994) reported no significant effects on tropical seagrass biomass, shoot density and leaf production to *in situ* nutrient enrichment. In the present study, enriched *Z. novazelandica* showed a limited growth response by having a slightly higher shoot canopy (22%) and a marginally significant increase (13%) of the below-ground biomass. This may indicate that during the growing season, *Z. novazelandica* was nutrient limited. The increase in shoot canopy height is similar to the increase in leaf-length recorded for *Z. marina* following nutrient enrichment, a morphological characteristic that for this species reflects the level of nutrient in its bed (in Harlin and Thorne-Miller 1981). The increase in below-ground biomass in the present results is contrary, however, to the reported response of *Thalassia testudinum* where, under low-sediment N availability the plant allocated more biomass to increase its root surface area for nutrient uptake (Lee and Dunton 1999b). However, neither the above-ground biomass nor other growth variables (shoot density and leaf growth rate) of *Z. novazelandica* were significantly effected by the enrichment manipulations. This may suggest that factors other than nutrients are a major regulator in the habitat as high leaf growth rates were maintained both in control and treatment plots. This is in agreement with the results of Chapter 3 in which temperature and PFD were suggested of being the principal predictors explaining the variance of leaf growth rate in the *Z. novazelandica* populations at Harwood. This suggestion is also supported by the results of laboratory-controlled aquaria experiment (Chapter 4) whereby the interaction between temperature and PFD had significantly affected the seagrass leaf proportional growth rate.

Udy *et al.* (1997) found that *Zostera capricorni* growing under low porewater ammonium (see Table 5-10) in Moreton Bay (Australia) did not respond to an enrichment with nitrogen alone fertiliser. When both N and P fertilisers were added together, *Z. capricorni* showed a significant increase in its canopy height, shoot density, above-ground

biomass and leaf growth rates (Udy and Dennison 1997). The authors suggested that seagrasses from very low nutrient environments would require multiple nutrients in order to stimulate their balanced nutrient limitation growth (i.e. neither N or P additions by themselves will stimulate growth) (Udy and Dennison 1997). Ideally, both N-Osmocote and P-Osmocote fertilisers should have been used in the present enrichment studies at Harwood. However, this was not possible, as the production of P-Osmocote fertiliser had been discontinued by the time the experiments were initiated and an alternative P-source could not be found. Therefore, it is possible that the small response observed in the growth of *Z. novazelandica* to N enrichment during this study was due to an un-balanced nutrient limitation. Moreover, the seagrass may have responded within the first month of the enrichment. Due to the time schedule of the present experiment, these enrichment effects may have been diluted (c.f. Erftemeijer *et al.* 1994) and thus undetected. However, the duration of the enrichment used is similar to that of Bulthuis *et al.* (1992) who reported a significant growth response to N-fertilisers in *Heterozostera tasmanica* after 5 months of enrichment.

5.5.2.2 Tissue nutrient response

Results of the present study show that the leaves of *Zostera novazelandica* had a relatively high carbon and nitrogen content with values of about 37% and 2.5% dry wt respectively, for the control plants. These values exceeded the mean carbon and nitrogen content of 33.6% and 1.92% dry wt respectively, calculated for the leaves of 27 seagrass species, in a literature comparison reported by Duarte (1990). The nutrient contents in the leaf of *Z. novazelandica* were also comparable to summer values reported for *Z. noltii* (32% C; 3% N) (Perez-Llorens and Xavier Niell 1993) and *Z. marina* (38% C and 2% N) (Pellikaan and Nienhuis 1988). Furthermore, the atomic ratio of C:N calculated for *Z.*

novazelandica in this study is about 15 which is quite similar to those for *Z. noltii* and *Z. marina*, 10 and 13 respectively (Pellikaan and Nienhuis 1988, Perez-Llorens and Xavier Niell 1993).

According to Duarte (1990), seagrasses with leaf carbon content below 33.6% and nitrogen level below 1.8% are probably nutrient limited. He also suggested that an atomic C:N ratio above 20 is evidence of nitrogen limitation. Nitrogen in the tissue also increases when nutrient limited plants are fertilised (Duarte 1990). The C and N content of *Zostera novazelandica* in the present study was higher than the suggested threshold values, and in conjunction with the C:N ratio of 15 indicate that the plant was not N-limited. Moreover, the tissue nitrogen concentration did not significantly increase in the enriched plants. This may suggest that the seagrass could have acquired sufficient nitrogen, and that nitrogen is not a limiting nutrient for summer growth. This inference seems consistent with the lack of a significant response in growth rate when *Z. novazelandica* was fertilised. However, it did not accord with the positive responses in its canopy height and below-ground biomass to N enrichment. Agawin *et al.* (1996) reported the occurrence of nutrient limitation in seagrasses from Philippines, despite the presence of high N content in its leaf tissues. Conversely, *Z. marina*, which contained lower tissue N in its leaves, did not show a growth rate increase when enriched with N (Pedersen 1995). Thus, Hemminga (1998) stated that the threshold values of seagrass leaf nutrient content suggested by Duarte (1990) have not been consistently corroborated by later studies. His statement also seems to be supported by the present results where positive responses were observed in the *Zostera* canopy height, below-ground biomass and chlorophyll *a* and *b* (described below) to an N enrichment despite a high nitrogen concentration in its leaf tissues. Recently, Lee and Dunton (1999a) found that tissue nutrient content may not always be representative of the ambient nutrient conditions of seagrass growth. Udy *et al.* (1997) proposed a species-specific variation of the tissue N content in seagrass that lead to growth limitation.

In this study, high % tissue nitrogen was measured in the leaves of *Zostera novazelandica* even though the concentration of nitrogen in the sediment porewater was below the critical values of nutrient limitation. This finding is similar to those reported for *Halodule uninervis* (2.4%) and *Cymodocea serrulata* (2.0%), growing on low concentration (7.4 μM) of sediment porewater NH_4^+ (Udy and Dennison 1997). It is possible that *Z. novazelandica* has a transient nutrient absorption ability whereby pulses of N available in the water column are quickly taken up by the leaves (e.g., Pedersen 1995) and subsequently stored in their tissues. Some seagrasses have significant N storage capacity in their rhizomes and leaves (Kraemer and Mazzella 1999). Such ammonium pulses could have occurred particularly at the beginning of flood-tide when a high concentration of ammonium is flushed from the intertidal sediment (e.g., Rocha 1998). Moreover, biodeposits (faeces and pseudofaeces) of some suspension feeding bivalves have been shown to contain a high concentration of N and P (Peterson and Heck Jr. 1999 and literature cited therein). Recently, it was demonstrated that sediment pore water nutrients (N and P) increase significantly in the presence of mussel (*Modiolus americanus*) and that the ratios of C:N and C:P in the leaf tissue of seagrass *Thalassia testudinum* subsequently declined with an increased of the mussel densities (Peterson and Heck Jr. 1999). As *Z. novazelandica* population in the present study is associated with live cockles (*Austrovenus stutchburyi*), it is possible that a high concentration of N from the deposition of the cockles' faeces and pseudofaeces is biologically available in the seagrass rhizosphere for uptake and thus, increased the nitrogen content within their leaf tissue. Also, a number of seagrass species have been shown capable of reclaiming part of nutrients (N, P) in their ageing leaves before these are lost (e.g., Hemminga *et al.* 1991, Pedersen and Borum 1993). Whether this resorption process (Hemminga *et al.* 1991) had contributed to the high tissue N-content in the *Z. novazelandica* leaf in the present study is yet to be determined.

Carbon and nitrogen content of *Zostera novazelandica* was found to be lower in the roots and rhizomes than in the leaves. This has also been observed in other seagrasses, even in the absence of nutrient limitation (e.g., Perez *et al.* 1994). However, it is not possible to draw inferences from the nutrient levels of the below-ground parts because comparative analysis of the critical nutrient levels in root and rhizome materials is not yet available (Duarte, pers. com.). The low nutrient content in the rhizomes and roots of *Zostera novazelandica* may indicate the mobilisation of the nutrient (Sfriso and Marcomini 1999, list of literatures cited therein) to other actively growing parts of the plants, especially the younger leaves.

5.5.2.3 Pigments concentrations and $F_v:F_m$ ratio

An increase in chlorophyll *a* and *b* along with tissue N-content in response to fertilisation has been observed in some seagrasses in the Philippines (Agawin *et al.* 1996). Similarly, Pedersen (1995) reported that an increase in tissue N in marine macrophytes (i.e. *Zostera marina* and *Fucus vesiculosus*) was followed by an increase in chlorophyll *a* and *b* because large fractions of the total tissue-N are associated with the photosynthetic apparatus. Conversely, in *Heterozostera tasmanica*, increased N tissue content was not, however, followed by higher chlorophyll concentration (Bulthuis *et al.* 1992). In the present study, however, marginal but significant increased in chlorophyll *a* and *b* pigments were observed in the enriched *Z. novazelandica* compared to the controls despite the presence of high N content in its leaf tissues.

During day time low tides, *Zostera novazelandica* at Harwood is exposed to high levels of summer solar irradiance and high air temperatures which together may stressful to the seagrasses. Higher levels of UV radiation typical of summer in New Zealand may also cause damage to PSII. Results indicate that the $F_v:F_m$ ratio was lowest in December 1998

(mid-summer) than in April 1999, both in control and enriched plants. The lower $F_v:F_m$ ratio obtained in mid-summer may suggest that *Zostera novazelandica* is displaying some degree of sensitivity to environmental desiccation stress during tidal emersion. Enrichment with nitrogen seems to reduce the sensitivity of *Z. novazelandica* around mid-day tidal emersion during midsummer. This is shown in the significantly higher $F_v:F_m$ ratio in the N-enriched plants compared to the controls. At the end of summer and early autumn, the stress effect is less severe in both treatments as ambient light and air temperature levels are declining. Moreover, overlapping of dense leaves that occurs in these periods may have reduced desiccation through evaporation at low-tide and hence a higher $F_v:F_m$ ratio recorded in both control and treatment plots. A higher ratio of $F_v:F_m$ also indicates that the growth of the plant is not inhibited at the high porewater ammonium level of the enriched seagrass.

Only the results of Bjork *et al.* (1999) appears to provide evidence of the desiccation-physiological response of intertidal seagrasses using a pulse amplitude modulated (PAM) fluorometer. However, these authors measured photosynthetic efficiencies as an electron quantum yield of PSII (Y), which showed a significant reduction when the seagrasses were exposed to air in their laboratory experiments.

CHAPTER 6

SUMMARY AND GENERAL CONCLUSION

6.1 REMOTE SENSING

Extensive beds of the seagrass *Zostera novazelandica* cover large areas of the intertidal zone at Harwood, Otago Harbour. The situation at Harwood, of a monospecific bed of seagrass, exposed and accessible at low tide, was ideal for mapping for the purpose of inventory and documenting temporal and spatial changes of this habitat using digitised aerial photographs and image processing techniques. Mapping using aerial photography enabled flights to be scheduled to coincide with appropriate weather conditions, and tide and sun angle for acquiring high quality images (Fyfe and Israel 1996).

Digitised aerial photographs from April 1997, November 1997 and April 1998 were interpreted to determine the extent of seagrass and unvegetated substrata, using a supervised classification technique. High-resolution thematic maps with an acceptable range of accuracy provided a detailed inventory of the spatial distribution of *Zostera* and range of covertypes that occurred with the seagrass vegetation. Six major covertypes were proposed in these maps, consisting of dense *Zostera*, dense *Zostera* mixed with macroalgae, medium dense *Zostera*, sparse *Zostera*, cockle/sand, and bare sand. The seagrass vegetation was categorised based on its percent ground cover. The sparse *Zostera* and bare sand areas appeared more distinct in these maps than those observed in the previous mapping of a similar habitat using SPOT imagery (Israel and Fyfe 1996). This indicates that digitised aerial photography may provide the best remote sensing option to investigate seasonal changes based on the distribution and areal cover of *Zostera* vegetation at the study site.

Integration of these thematic maps with GIS enabled the detection and quantification of changes that occurred in the habitat throughout the study period. Intertidal seagrass cover was estimated to total about 80 ha at Harwood, with sparse *Zostera* category dominating most of the habitat. While the total area of seagrass cover has remained relatively constant, it appears that changes between covertype categories were common in the habitat. For example, the areal extent of bare sand area increased at the expense of the sparse *Zostera* category. *In situ* ground surveys indicated that localised sedimentation, which smothered live seagrass plants, had caused these changes as both covertypes were normally found in closed association.

Changes within *Zostera* categories were, however, the most frequently detected and were matched by a corresponding seasonal variation in growth features of the plants (e.g., shoot density and leaf length), some of which were also detected in the permanent ecological monitoring plots established within the dense seagrass beds.

Since the maps provided in this study are in standard GIS format, they allow for future update and integration of other information, such as environmental parameters (e.g., current pattern, nutrient input, seawater temperature), or local development activities. Thus, the present maps provide a framework for impact assessment studies and identification of potential problem areas requiring management intervention at the study site.

Mapping of the *Zostera* beds is very important for their management and conservation. This is in line with Agenda 21 of the United Nations Conference on Environment and Development (UNCED) in 1992, which encourages the identification and conservation of 'marine ecosystems exhibiting high levels of biodiversity and productivity'. Therefore it is recommended that mapping be extended to *Zostera* beds in the whole Otago Harbour system. This is necessary due to the close proximity of the seagrass vegetation to human populations, and where harbour development activities,

dredging and nutrient loading may threaten the health of these plants. Maps of *Zostera* that are regularly acquired are needed to monitor future changes and to provide for predictive studies in documenting impacts of these threats. Whilst aerial photography is a useful tool when integrated with GIS, it has some processing limitations, such as variability in the brightness values between consecutive photographs. Therefore, satellite imagery such as SPOT should be considered as it provides good regional coverage and more uniform spectral information throughout the image (Fyfe *et al.* 1999). In areas where the plants indicate some localised impacts from environmental degradation, aerial photographs may be used for detail monitoring.

6.2 GROWTH PARAMETERS

Defined seasonal changes in the growth of *Zostera novazelandica* and its physico-chemical environmental factors were documented during a 27-month monitoring of the plants at ground level. The information generated has improved the current understanding of the relationship between seagrass growth and environmental factors in Otago Harbour.

Zostera plants in Otago Harbour flower during summer but are low in reproductive shoot biomass. Other growth parameters (rates of leaf growth and primary production, above-ground biomass, leaf length and leave area index) of the plants had a summer maximum and a winter minimum. Rates of leaf proportional growth were estimated using a leaf-marking technique and the values doubled from winter to summer. The variation in above-ground biomass was, however, less pronounced (2-fold) and fell into the lower range for some genera of *Zostera* and *Heterozostera* that have been studied elsewhere. It appears that the above-ground biomass was influenced mostly by variation in leaf length, and a substantial amount of standing crop remained during winter. The product of leaf growth rate and above-ground biomass gave an estimate of leaf production of the seagrass, which varied about 10 times between summer and winter. Growth

occurred, and above-ground biomass remained during winter, indicating that the production of the *Z. novazelandica* also continued year round in contrast to some *Zostera* species in the Northern Hemisphere. No seasonal variation was, however, observed in the below-ground biomass, shoot density, or number of leave blades per shoot.

This study failed, however, to estimate the productivity of *Zostera novazelandica* for the entire area of the seagrass habitat at Harwood intertidal area. Apart from being outside the objective of the current study, the seagrass habitat did not contain uniform stands but comprised different seagrass categories, as recognised in the mapping studies. As each of these categories may have a different biomass due to different shoot densities or due to variability in leaf length and width (West and Larkum 1983) or different leaf growth rate, it was not possible to estimate productivity simply by multiplying the present production data obtained from the permanent plots (i.e. of dense area) by the total seagrass area obtained from the maps. This is because production is a function of biomass and leaf growth rate. However, such an estimate could be obtained in the future by recording and combining the production data for each of the seagrass categories.

It is also necessary to understand the fate of the production of the *Zostera novazelandica* to assess the relative importance of the species to the ecology of the Harbour. This could be investigated by using, for example, a ^{15}N enrichment technique (see Winning *et al.* 1999) in a food web study. Given that a major proportion of the plant's detrital material stayed below the sediment surface and the abundance of benthic macrofauna (e.g., cockles, crabs), it is reasonable to assume that *Z. novazelandica* is a major contributor to the secondary production at Harwood intertidal area.

Seagrass growth parameters such as biomass, density and productivity have been described as indicators for the 'health' of seagrass meadows (Kirkman 1996). Therefore, repeated, long-term measurement of these parameters for *Zostera novazelandica* at Harwood is required to provide information on whether these parameters return to their

initial values. This would provide an indication of the occurrence of interannual changes, which may be related to year-to-year variation in environmental condition, through either natural processes or human activities. Subsequently, disturbance of the seagrass population could be detected at an early stage, and distinguished from natural variation (see Kirkman 1996).

6.3 EFFECTS OF PHYSICO-CHEMICAL PARAMETERS

Strong seasonality shown in the above-ground biomass and leaf growth rates of this intertidal *Zostera novazelandica* was demonstrated to be coupled to seasonal forcing through a multiple regression analysis between these growth parameters and the seasonal variations in its environmental variables. For example, variation in the above-ground biomass was suggested as being controlled mainly by air temperature, similar to the characteristic of an intertidal *Zostera noltii* from the Netherland (Vermaat and Verhagen 1996). Air temperature and PFD, however, accounted for significant seasonality in the leaf growth rate. It would be interesting, nevertheless, to determine whether growth characteristics of subtidal *Z. novazelandica* in the Harbour differ from the present intertidal population, where reduced submarine irradiance reaching subtidal plants may play a major controlling factor.

The similarity between monthly mean air temperature and mean seawater temperature at the study site, and the fact that sea temperature is largely determined by incoming solar radiation (Hillman *et al.* 1989) has, however, confounded the interpretation from the regression analysis. Thus, the *Zostera novazelandica* shoots were grown in seawater culture under controlled experiments to investigate the interactive effects of temperature (only seawater temperature tested), PFD and nutrient concentration (combination of ammonium, nitrate and phosphate) on the growth of these plants. Due to the experimental design, individual effects of these environmental variables could not be

tested. Despite this constraint, results of these experiments indicated that it was the interaction between temperature and PFD that significantly governed the plant's leaf growth rates, which supports the regression analysis from the field studies. The rate was highest at high temperature and high PFD, and the value obtained was closest to those measured in the field.

Plants grown at high temperature under low PFD initially had high growth rates but these declined after 14 days in the culture. This finding implies that the summer growth rate of *Zostera novazelandica* in Otago Harbour, particularly those in the subtidal area, would be severely affected under conditions of reduced water column light intensity or shading by epiphytes on the surface of their leaves. Reduced light intensity in the water column may indirectly be attributable, for example, to blooms of phytoplankton populations due to eutrophication, or directly from sediment loading originating from human activities and developments of the surrounding Harbour basin.

Data from these culture experiments also indicated that interactive effects of seawater nutrient enrichment with PFD and temperature did not influence the leaf growth rate of *Zostera novazelandica*, which also supported the regression analysis of the field data. However, prolonged exposure to high nutrient concentration at high temperature, both under high and low PFD, caused a detrimental effect on the morphology of the plants, as indicated by a bright green colour, weakened tissue structure and subsequent abscission of the leaves. Furthermore, a physiological stress effect was exhibited prior to shoot mortality by low $F_v:F_m$ values of the shoots exhibited on day 14. This finding illustrates the effectiveness of the use of $F_v:F_m$ as a tool for measuring seagrass vigour.

Mortality of *Zostera novazelandica* in the present controlled experiments occurred at high nutrient concentration and high temperature, and was not observed in the enriched plants at low temperature. This indicates an interactive effects of high temperature and nutrient enrichment. Furthermore, toxicity of ammonium in seawater at high temperature

(van Katwijk *et al.* 1997) and direct nitrate toxicity (Burkholder *et al.* 1992) has also been reported in *Z. marina*. Whether ammonium or nitrate toxicity caused the mortality is difficult to determine in the present study, and would be a subject of detailed future study. This would also include studies of lethal concentration of these nutrients for *Z. novazelandica* at the range of temperatures and irradiances found in the natural habitat. Although in a laboratory experiment reality can only be partly simulated (c.f. van Katwijk *et al.* 1997), this finding suggests that increased nutrient loading to Otago Harbour through, for example, agricultural run-off or urban waste water, would cause mortality of *Z. novazelandica* beds, particularly in the late summer when irradiance decreases and temperature is still high, a condition that would lower their carbon fixation rate (van Katwijk *et al.* 1999). In the case of eutrophication by ammonium, carbon is needed to assimilate this nutrient (van Katwijk *et al.* 1999). The adverse effects of increased nutrient loads are expected to be strongest in *Zostera* beds of the upper Otago Harbour basin where the residence time of the seawater is longer, between 5 to 30 days as compared to 12-24 hours in water of the lower basin (ORC and DCC 1991), thus helping to prolong the exposure to eutrophic conditions.

6.4 NUTRIENT STATUS

The present study also found that the concentration of ammonium at the study site was higher in the sediment pore-water ($74 \mu\text{M}$) than in the water column ($< 8 \mu\text{M}$). However, the level of pore-water ammonium available was below the mean value reported for seagrass meadows world-wide, and also below the threshold value for seagrass nutrient-limited conditions. This implied that the growth of *Zostera novazelandica* at Harwood could be under nutrient limitation. This hypothesis was tested through an *in situ* sediment N-enrichment experiment where growth, morphological and physiological

responses of *Z. novazelandica* to slow-released N-fertiliser were assessed. This experiment was conducted during summer when growth of the seagrass was at its peak.

Zostera novazelandica showed limited responses to the elevated pore-water ammonium concentration. These include an increase in their canopy height, biomass of below-ground parts, and chlorophyll *a* and *b* concentrations. These positive responses may indicate that growth of the plants is nutrient limited during the summer. However, this suggestion is confounded by a lack of response in their tissue C and N content, above-ground biomass and growth rate. Results from this study seem to support findings by others where growth responses of seagrasses to nutrient enrichment are generally varied between species and/or levels of nutrient in the sediment pore-water. However, it would be interesting to know whether the lack of some of these growth responses in the *Zostera novazelandica* is due to unbalanced nutrient limitation of growth (i.e., the plants need N+P, not just N). This could be demonstrated in future work by adding both N and P fertilisers together in the sediment in order to stimulate the growth response.

The lack of response in the leaf growth rate to the enrichment manipulation in this study may suggest that factors other than nutrients are a dominant regulator. Interestingly, this agreed well with the results of a regression analysis for data of the permanent plot studies and results from the culture studies, in which temperature and PFD were suggested as the most significant factors that affected the leaf growth rate of *Zostera novazelandica*, as previously discussed.

In contrast to the culture studies, the elevated concentration of ammonium in the sediment pore-water did not show signs of toxicity to the *Zostera novazelandica* plants. The enrichment may even have helped reduce the sensitivity of the plants to desiccation around mid-day tidal emersion, as shown by $F_v:F_m$ values of their shoots. This result, together with an increase in below-ground biomass and shoot height, indicates that the plants must have absorbed the ammonium from the sediment as source of nutrient for

growth. This suggestion is also supported by a reduction in the concentration of pore-water ammonium at the end of the plants' growth season. The question is, what proportion of ammonium is taken from sediment pore-water through the plants' roots as opposed to from seawater via leaves. These questions need to be addressed in future studies, as it will provide new information on the interaction between roots and leaves in the uptake of nutrients by the plants, especially during summer when nutrient concentration in the water is limiting. Results from such studies will also provide some indication of the importance of sediment pore-water N to the seagrass bed, which may be useful for the management of seagrass vegetation in the Harbour.

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