The ecology and demography of the introduced macroalga *Undaria pinnatifida* (Harvey) Suringar in Port Phillip Bay, Victoria, Australia.

Juanita Saulé Bité

A thesis submitted for the degree of Masters of Science

Department of Life Sciences and Technology

Victoria University

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Declaration

This thesis is submitted in accordance with the regulations of Victoria University of Technology in fulfillment of the requirements for the degree of Masters of Science. This thesis contains no material which has been accepted for the award of any other degree or diploma in ant university and no material published or written by another person except where duly acknowledged or referenced.

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Index to figures

Index to tables

List of Abbreviations

ANOVA	Analysis of variance
B	Biomass
DIN	Dissolved inorganic nitrogen
DIP	Dissolved inorganic phosphorus
DON	Dissolved organic nitrogen
EPA	Environment Protection Authority
n.a.	Not applicable
n.s.	Not significant
NRE	Natural Resources & Environment
PPB	Port Phillip Bay
PFD	Photon Flux Density
psu	Practical salinity unit
RuBP	ribulose-1,5-biphosphate carboxylase oxygenase (Rubisco)
SCUBA	Self-contained underwater breathing apparatus

All other symbols represent SI units

Glossary of algal authorities

* indicates no authorities provided by author

Chlorophyta

Caulerpa taxifolia (Vahl) C. Agardh

Codium fragile (Sur.) Hariot ssp. *tomentosoides* (van Goor) Silva

Dunaliella tertiolecta Dun. (Butcher)

Solieria chordalis J. Agardh

Ulva fasciata Delile

Ulva lactuca Linnaeus

Ulva pertusa *

Rhodophyta

Bangia fuscopurpurea (Dillw.) Lyngb.

Gracilaria sordida Nelson

Halymenia floresia (Clemente) C. Agardh

Polysiphonia breviarticulata (C. Agardh) Zanardini

Porphyra luecosticta *

Porphyra tenera Kjellman

Wrangelia penicillata C. Ag.

Phaeophyta

Chorda filum (L.) Stackh.

Ecklonia radiata (C. Agardh) J. Agardh

Fucus vesiculosus Linnaeus

Fucus virsoides (Don) J. Ag.

Hormosira banksii (Turner) Decaisne

Laminaria digitata (Huds.) Lamour

Laminaria hyperborea (Gunn.) Foslie

Laminaria saccharina (L.) Lamour

Lessonia corrugata Lucas

Macrocystis angustifolia Bory

Macrocystis angustifolia Bory

Macrocystis pyrifera (L.) C.

Nereocystis luetkeana (Mertens *f.*) Postels *et* Ruprecht

Phyllospora comosa C. Agardh

Sacchorhiza polyschides (Lightf.) Batt.

Sargassum muticum, (Yendo) Fensholt

Scytosiphon lomentaria (Lyngb.) J. Ag.

Sphaerotrichia divaricata (C. Agardh) Kylin

Undaria Pinnatifida (Harvey) Suringar

Diatom

Phaedodactylum tricornutum (TFX-1)

List Of Publications And Conference Presentations

Publications and Reports

Bité, J. S. 1998. The ecology and reproductive biology of the introduced Japanese macroalga *Undaria pinnatifida* (Harvey) Suringar in Port Phillip Bay. Prepared for Australian Quarantine Inspection Service, Melbourne.

Bité, J. S., S. J. Campbell, and T. R. Burridge. 1997. The ecology and demography of the introduced Japanese macroalga *Undaria pinnatifida* (Harvey) Suringar in Port Phillip Bay. Preared at Victoria University of Technology for Fisheries, Department of Natural Resources, Melbourne.

Campbell, S. J., J. S. Bité, and T. Burridge. 1999. Seasonal patterns in the photosynthetic capacity, tissue pigment and nutrient content of different developmental stages of *Undaria pinnatifida* (Phaeophyta: Laminariales) in Port Phillip Bay, South-Eastern Australia. *Botanica Marina* 42: 231-241.

Talman, S., J. S. Bité, S. J. Campbell, M. Holloway, M. Mc Arthur, D. J. Ross, and M. Storey. 1999. Impacts of introduced marine species in Port Phillip Bay.

Conference presentations

The ecology and demography of the introduced seaweed *Undaria pinnatifida* (Harvey) Suringar in Port Phillip Bay, Victoria. Paper presentation. *Australian*

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Abstract

In 1996 *Undaria pinnatifida* (Harvey) Suringar (Laminariales: Phaeophyta) was found growing in coastal waters of Port Phillip Bay, Victoria, Australia. *Undaria pinnatifida* is an opportunistic colonizer capable of high rates of reproduction and fast growth rates producing high density populations. It is the dominant macroalgal species at the site of invasion during winter and spring and has the potential for further spread from its current distribution in the northern part of Port Phillip Bay. This is the first study in Australia examining the effects of temperature, nitrogen concentration, photon flux density and photoperiod on germination of zoospores, gametophyte growth and reproduction of *U. pinnatifida* in culture. Information on its recruitment, growth and reproductive capacity in the field is also presented for the first time for a population in Australia.

Undaria pinnatifida zoospores, germlings and gametophytes showed substantive resilience to a range of physico-chemical conditions in the laboratory. Zoospores were able to germinate within the range of salinity concentrations (28-32 psu) and ammonium concentrations (0-30 μM NH_4-N) found in Port Phillip Bay. Germination was also found to be successful over the range of temperatures found in Port Phillip Bay (i.e. 10°C to 25°C) but is likely to be limited should temperatures fall outside this range. The initial growth of the germination tube (germling) was resilient to the range of salinities in Port Phillip Bay but elevated ammonium concentrations (>28 μM) encountered near sewage outfalls and riverine inputs may limit germling growth.

Responses of germling and gametophyte growth to photon flux and temperature suggests that the growth of microscpoic stages is favoured by low temperatures and low light conditions, consistent with the ability of *Undaria pinnatifida* to establish and grow during winter. Gametophyte growth and gametogenesis appear to follow seasonal patterns in temperature, ammonium nitrogen concentrations and photoperiod. The optimal growth and reproduction of gametophytes at low temperatures, low light and high inorganic nitrogen availability characterizes *U. pinnatifida* as a winter annual able to take advantage of high nutrient concentrations. The response of *Undaria pinnatifida* gametophyte growth in culture to photoperiod is possibly due to an increase in available light and therefore further studies are necessary to distinguish photoperiodic responses from responses to quantum dose of light.

In Port Phillip Bay the life cycle and growth of *Undaria pinnatifida* is typical for brown algae from a warm temperate climate, characterized by the appearance of sporophytes in late autumn, a distinct sporophyte growth period during winter and spring and the disappearance of sporophytes with a resting gametophyte stage over summer. Its reproductive capacity coincided with changes in daylength, temperature and inorganic nitrogen concentrations, indicative of a strong seasonal influence on its growth and reproduction. High temperatures appear to inhibit gametogenesis and sporophyte growth over summer, although genetic factors undoubtedly control the senescence of sporophytes that dictate a sporophyte longevity of less than one year. This is in contrast with the dynamics of *U. pinnatifida* populations from cool temperate waters where sporophyte generations are present year round.

Information from this thesis provides a critical understanding of the environmental factors that influence the growth and reproduction of different life stages of *Undaria pinnatifida* in Port Phillip Bay. Such information is important towards understanding the potential spread of this invasive species and may provide insight into methods that can be used to limit its expansion in southern Australian waters.

Chapter 1

1.1 General Introduction

1.1.1 Biological invasions

Biological invasions result from the transport, arrival, and establishment of species in a community where they did not previously exist. The extent of biological invasions is becoming apparent as exotic species continue to establish around the globe, often over long distances and across natural barriers, using human activity as the vector of dispersal (Carlton 1989). Invaders are more likely to become established in anthropogenically disturbed communities arising from increased turbidity due to agricultural clearing of land and logging; increased nutrients caused by sewage inputs and pollution due to industrial discharges (Orians 1986, Vitousek 1986). Many invaders are accidentally introduced and threaten commercially important industries such as fisheries and may cause damage to infrastructure in the marine environment (e.g. blocking of discharge pipes by mussels) (Dahlsten 1986).

Species invasions are serious threats to biodiversity (D' Antonio and Vitousek 1992) and ecosystems (Vitousek 1986) and it has been suggested that this loss of biodiversity will irreversibly damage the functioning of ecosystems world-wide (Low 1999). In many cases the biology and ecology of introduced species, as well as the impacts they are having on local ecosystems, are poorly understood. As a result of this poor understanding, control programs to eradicate or minimize the spread of exotic species have not been widely accepted (Dahlsten 1986).

1.1.2 Introduced marine species

Extensive literature is available on the introductions of terrestrial and freshwater species (Pieterse and Murphy 1990, di Castri et al. 1990, Drake et al. 1989, Mooney and Drake 1986, Diamond and Case 1986, Elton 1958). The impacts of marine species introductions are often difficult to document due to the absence of information on the distribution of native marine species prior to the invasion (Grosholz and Ruiz 1995, Carlton 1989, Posey 1988). In 1973, scientists warned that exotic fishes were being introduced into Australia (Friese 1973, Grainger 1973). In 1975, marine invertebrates were reported to have survived the voyage from Japan to Australia in the ballast water of a ship (Medcof 1975). Numerous introductions of exotic marine vertebrates, invertebrates and algae have subsequently been reported in Australia (Reichelt et al. 1994, Jones 1991, Pollard and Hutchings 1990a, b, Pollard and Hutchings 1990a, Hallegraef et al. 1988).

1.1.3 Introduced algae

The ecological effects of invasion and the subsequent spread of non-endemic marine macroalgae in nearshore environments is not well understood. Introduced marine macroalgae can have serious impacts on native marine communities and long term ecological effects (Rueness 1989). The establishment of foreign taxa in a particular locality depends on both the environmental conditions and the ability of a species to adapt to a particular habitat (Peters and Breeman 1992, Floc'h et al. 1991, Sanderson and Barrett 1989, Breeman 1988). Foreign species that become established usually have few predators, competitors and pathogens in the new habitat, which often allows

them to establish, persist and displace indigenous species and become economic and ecological pests (Trowbridge 1995).

Reports on the spread and new establishments of invasive marine algae in the Northern and Southern Hemispheres are becoming more frequent (Delgado et al. 1996, Sant et al. 1996, De Wreede 1996, Verlaque 1993, Rueness 1989, Fletcher 1980, Farnham 1980). Reports of invasions of foreign macroalgae include *Solieria chordalis* in the United Kingdom (Farnham 1980), *Sargassum muticum* in Canada (Scagel 1956) and southern England (Farnham 1980), *Caulerpa taxifolia* in the Mediterranean Sea (Sant et al. 1996, Verlaque 1993), *Codium fragile* ssp. *tomentosoides* in England (Silva 1955) and New Zealand (Trowbridge 1996, Rueness 1989), *Ulva fasciata* Delile in Japan, *Polysiphonia breviarticulata* in North America (Morand and Briand 1996) and *Undaria pinnatifida* in New Zealand (Hay 1988), the UK (Fletcher and Manfredi 1995), France (Floc'h et al. 1996), Argentina (Casas and Piriz 1996) and Italy (Curiel et al. 1996). Although many macroalgal species are recognized as possible introductions into Australian waters, only *Codium fragile* ssp. *tomentosoides* and *Undaria pinnatifida* have been reported in any detail (Campbell 1999, Campbell and Burridge 1998, Sanderson and Barrett 1989).

1.1.4 *Undaria pinnatifida*

Undaria pinnatifida is a native seaweed of Japan, Korea and parts of China, and has invaded environments where it is not endemic. It is an important cultivated sea vegetable known commonly as Wakame in Japan (Tseng 1983, Akiyama and Kurogi 1982). It has been successfully cultivated in France (Pérez et al. 1992b, Pérez et al.

1992a), with attempts to cultivate it in Tasmania, Australia (Craig Sanderson, personal communication, 1996). The interest in cultivation and the invasive nature of *U. pinnatifida* has led to the increased need to understand its biology and ecology.

Undaria pinnatifida has a capacity to spread from its initial site of colonisation and to establish in other areas (Floc'h et al. 1996, Casas and Piriz 1996, Fletcher and Manfredi 1995, Brown and Lamare 1994, Hay and Villouta 1993, Sanderson and Barrett 1989, Boudouresque et al. 1985). Many of the initial *U. pinnatifida* populations in a new country are situated near shipping ports (Casas and Piriz 1996, Fletcher and Manfredi 1995, AQIS 1994, Hay 1990, Sanderson 1990, Hay and Luckens 1987). The dispersal of *U. pinnatifida* is most likely through spores released from ballast water of ships or through mature reproductive plants attached to ships' hulls. The reported conditions and requirements for its growth suggest that it has the potential to spread and establish itself along the southern Australian coast from Cape Leeuwin in the south west of Western Australia to Woolongong in the south east of NSW (Sanderson 1990, Sanderson and Barrett 1989).

In the taxonomic system, *Undaria pinnatifida* is a member of the order Laminariales, which also includes the Australian native kelps *Ecklonia radiata* and *Macrocystis angustifolia* (Papenfuss 1951). As an annual macroalga, *U. pinnatifida* differs from most other Laminariales, which are perennials. It has a heteromorphic life cycle, alternating between the diploid macroscopic sporophyte and the haploid microscopic gametophyte (Floc'h et al. 1991). Sporophytes arise from the microscopic

gametophytes in winter, reaching 1-2 metres in length and become senescent in early summer. By late summer sporophytes are often absent (Brown and Lamare 1994).

The sporophyll is a specialised reproductive structure, developing at the base of the sporophyte thallus (Hay and Villouta 1993, Saito 1975). Asexual spores are produced by meiosis in the sporophyll of mature plants throughout the growing season (Hay and Villouta 1993, Sanderson and Barrett 1989). A mature sporophyll releases up to 10,000,000 zoospores (Saito 1975). The motile zoospores usually settle 1-6 hours after release (Hay 1991), but can remain motile in the water column for up to two days after release (Pérez et al. 1992b, Tamura 1966, Kanda 1936). Once attached to the substrate, spores germinate within hours of settling. In optimal conditions, a female or male gametophyte develops within 7 days of settlement (Kanda 1936). The egg in the female gametophyte is fertilized approximately 7 days later with motile spermatozoa produced by the male gametophytes. The zygote then develops into a sporophyte (Hu et al. 1981).

Numerous factors have been associated with the ecological success and spread of *U. pinnatifida* (AQIS 1994, Floc'h et al. 1991, Lee and Brinkhuis 1988, Saito 1975, Akiyama 1965). Factors that influence the growth of *U. pinnatifida* and other macroalgae include: temperature, light intensity, photoperiod, nutrient availability, salinity, depth, competitive ability and predation (Floc'h et al. 1991, Laing et al. 1989, Lee and Brinkhuis 1988, Fain and Murray 1982, Lüning and Neushul 1978, Lüning and Dring 1975, Saito 1975, Akiyama 1965, Kain 1964). These factors may interact

in complex ways to determine if a macroalga can colonize and establish in a particular environment.

For example, the geographical limits of macroalgae may be dictated by their ability to grow and reproduce at different temperatures (Peters and Breeman 1992, Breeman 1988). Light availability is also an important factor controlling growth rate, reproduction and recruitment (Breeman 1988, Ramus 1985, Novaczek 1984a, Lüning and Neushul 1978). The distribution of seaweeds along salinity gradients in estuaries suggests that salinity may also determine the distribution of macroalgae (Lobban et al., 1985b) and the availability of nutrients is also one of the primary factors regulating the growth, reproduction and physiology of algae (De Boer 1981). In order to contain the potential spread of *U. pinnatifida* it is important to understand the interactive effects of these factors on its distribution, growth and reproduction (Sanderson and Barrett 1989).

The effects of temperature on the growth and development of *U. pinnatifida* in its native habitats are well documented (Arasaki and Arasaki 1983, Saito 1975). Gametophytes are generally tolerant to a wide range of temperatures (5-30°C), but sporophytes are unable to tolerate temperatures higher than 20°C (Arasaki and Arasaki 1983). The interactive effects of temperature and other physico-chemical factors (e.g. photon flux density (PFD), daylength, nutrients) on the development of gametophytes and sporophytes of *U. pinnatifida* have not been examined. There is no information available on the responses of *U. pinnatifida* in Australia where the range of seawater temperatures and photon flux rates are different to those in its native

environment. In addition, the periodicity of recruitment, age and size structure and longevity of *U. pinnatifida* populations in Australia, including Port Phillip Bay, are not well documented (Campbell et al. 1999).

1.1.5 *Undaria pinnatifida* in Port Phillip Bay, Australia

Undaria pinnatifida (Harvey) Suringar was first reported in Australian waters off the coast of Tasmania in 1989 (Sanderson and Barrett 1989) and was subsequently found in Port Phillip Bay (PPB), Victoria in 1996. The initial site of introduction in Port Phillip Bay was located near Point Wilson (Figure 1.1) on a basalt reef at a depth of two to four metres (Campbell and Burridge 1998). Within three years *U. pinnatifida* has spread in an easterly direction and has become established at nearby Kirk Point and Long Reef. In 1999, *U. pinnatifida* was reported for the first time near Melbourne at St Kilda Pier, Princes Pier and Station Pier, approximately 60 km from its initial site of infestation (personal observations and communication with Stuart Campbell, EPA and Greg Parry, NRE).

Port Phillip Bay is a large, shallow marine embayment in southern Australia, that has a highly urbanized and agriculturally developed catchment. The maximum depth of the bay is 24m and half the volume is in waters shallower than 8m. Over an annual cycle, water temperatures in Port Phillip Bay range from 8°C to 24°C and salinity concentrations range from 28 to 32 psu. Nearshore waters in Port Phillip Bay have high concentrations of dissolved inorganic nitrogen (DIN) (0 - 28 μM) and dissolved inorganic

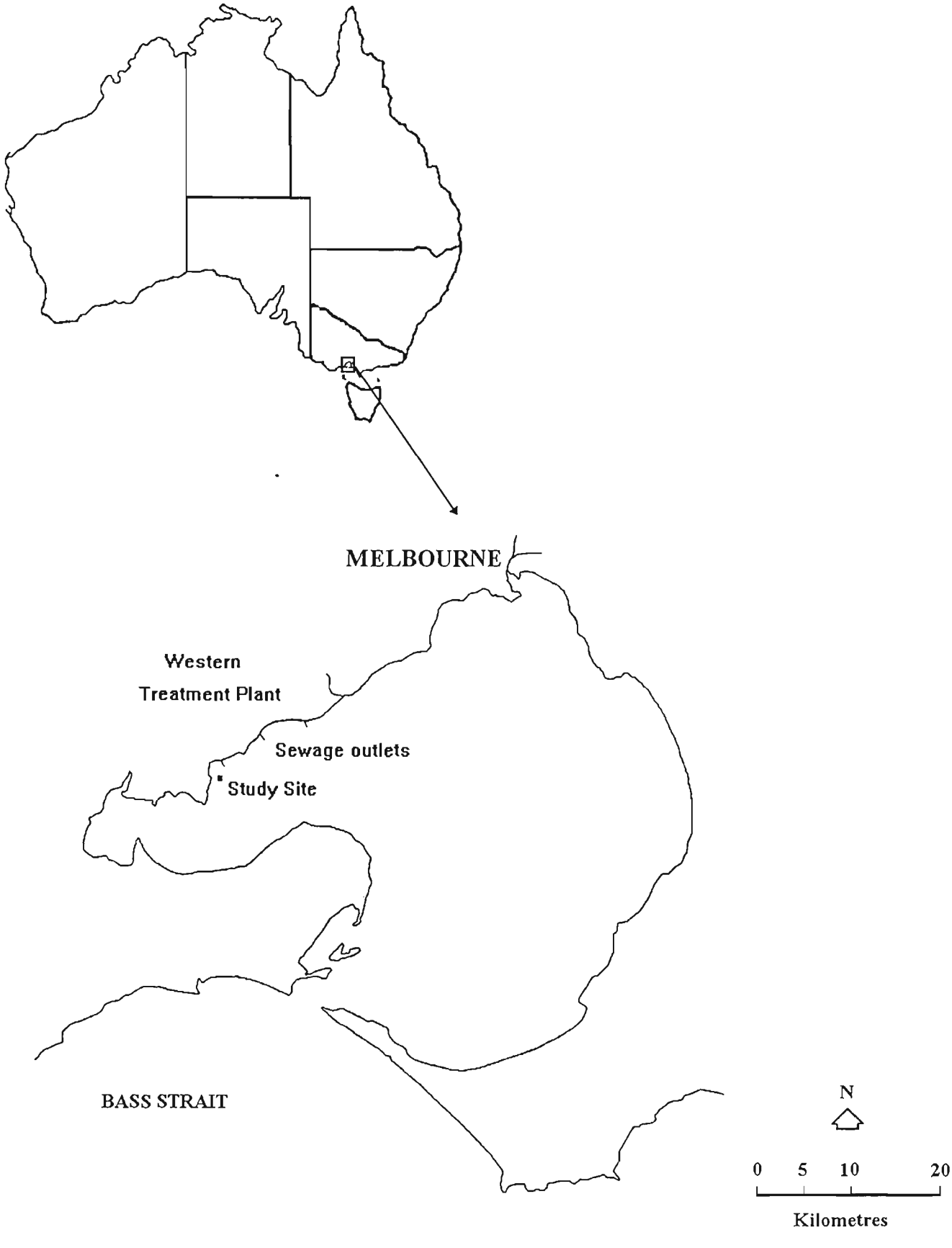

Fig. 1.1 Study site location in proximity to the Western Treatment Plant, Werribee, Port Phillip Bay and location of sewage outlets.

phosphorus (DIP) (10 - 15 μM). Concentrations of ammonium (1 to 30 μM NH_4^+-N) are often 5-10 times higher than nitrate concentrations (1-5 μM NH_4^+-N) and it is believed that ammonium is the primary source of DIN utilized by seaweeds in this region, with high photosynthetic and ammonium uptake rates reported for *U. pinnatifida* (Campbell 1999, Campbell et al. 1999). The population of *U. pinnatifida* examined here is situated in close proximity to fluctuating ammonium concentrations arising from
sewage inputs from the Western Treatment Plant, approximately 60 km west of Melbourne (Figure 1).

Information on the life cycle and biology of introduced seaweeds is necessary to determine the ecological effects of biological invasion and the strategies that may be used to control the spread of these species (Diamond and Case 1986, Mooney and Drake 1986). Ammonium (NH_4^+) was chosen as the nitrogen source, since high ammonium concentrations are found in waters where introduced *U. pinnatifida* populations have become established (Campbell and Burridge 1998, Curiel et al. 1994). This study aims to contribute to the knowledge of the ecology and reproductive biology of the kelp *Undaria pinnatifida* introduced into Port Phillip Bay, with the aim of a better understanding of its likely spread and ecological impact. Using the results of this study, the possibility of controlling the spread of *Undaria* in Port Phillip Bay and other parts of Australia, and potential eradication methods, may be determined.

The study first uses a series of laboratory experiments to determine the response of *Undaria pinnatifida* zoospore germination and germination tube development, the initial stage of its life cycle, to a range of different physico-chemical factors (Chapter 2). The next series of experiments examines the effect of physico-chemical factors on *U. pinnatifida* gametophytes. These experiments aim to determine if there is an interactive effect of ammonium, photon flux density (PFD) and photoperiod with temperature on the growth and development of gametophytes (Chapter 3). Chapter 4 presents the main conclusions of the study.

Chapter 2

The effect of environmental factors on the germination and germination tube growth of *Undaria pinnatifida* zoospores.

2.1 Introduction

The zoospore is the primary dispersal mechanism of laminarian kelps (Reed et al. 1988) and the spread and establishment of laminarians depends on the ability of its spores to germinate, develop germination tubes (germtubes) and grow (Bumidge et al. 1996, Reed 1990, Dean and Jacobson 1986, Vadas 1972, Kain 1964). Abiotic factors such as temperature, photon flux density and nutrients affect the settlement, germination and the initial growth of zoospores, and therefore these factors may directly influence recruitment, population size and community structure (Dean and Jacobson 1986). Studies on motile and germinating zoospores suggest laminarian zoospores show an affinity for nutrients by moving and growing in the direction of concentrated nutrients (Pillia et al. 1992, Amsler and Neushul 1990, Amsler and Neushul 1989, Henry and Cole 1982, Toth 1976). Kain (1964) examined the effect of photon fluxes on spore germination in *Laminaria hyperborea* (Kain 1964), and suggested that laminarian spores were well adapted to low light conditions and survival was possible for long periods in the dark. The effects of abiotic factors, such as light and temperature, on gametogenesis and subsequent sporophyte development has also been examined for a few laminarian species (Lee and Brinkhuis 1988, Deysher and Dean 1986, Deysher and Dean 1984).

Undaria pinnatifida zoospores are pear shaped, lack a cell wall and have two flagella (Pérez et al. 1992b, Henry and Cole 1982, Tamura 1966, Kanda 1936). During settlement, the zoospore becomes spherical, the flagella are absorbed into the cell, and in some cases fuse with the plasmalemma as a cell wall begins to develop (Henry and

Cole 1982). After deposition of 3 wall layers the spore produces a germination tube usually within 24 hours of settlement (Toth 1976). The germination tube increases in length and the spore's cytoplasm migrates towards the distal end. The tip of the germination tube swells as the cytoplasm and cell organelles move into the tube. Cell division takes place approximately 48 hours after settlement. Under favourable conditions, male and female gametophytes can be distinguished seven days after spore settlement (Pillia et al. 1992, Toth 1976, Kurogi and Akiyama 1957, Kanda 1936).

Investigations on the germination of spores of *Undaria pinnatifida* have been restricted to the effects of salinity (Saito 1962, Saito 1956a) and temperature (Saito 1975) on Japanese *U. pinnatifida*. These studies reported optimal adherence and germination of zoospores at temperatures below 20°C with decreasing germination rates at temperatures above 20°C and no germination at temperatures above 27°C (Saito 1975). The adherence of *U. pinnatifida* zoospores was reported to be inhibited at salinities less than 10 psu and optimal between 11 psu and 18 psu (Saito 1975). In Australia, *Undaria pinnatifida* is exposed to temperatures of a similar range to that reported for Japan, except that temperatures do not fall below 8°C. Although it is possible that the temperature and salinity responses of *Undaria pinnatifida* in Australia are similar to those reported for in Japan, there have been no studies undertaken to quantify the effects of salinity temperature, photon flux density and dissolved inorganic nitrogen on the germination and initial germination tube growth of populations in the southern hemisphere.

Studies on the biology of *Undaria pinnatifida* have primarily been undertaken for the purposes of commercial production of 'Wakame' in Japan. There is little information on the environmental factors affecting zoospore germination and germination tube growth of introduced populations of *U. pinnatifida*. This chapter aims to quantify the effects of salinity, temperature, nitrogen concentration and photon flux density on these microscopic stages in Port Phillip Bay, Australia. Using this information it is possible to determine the abiotic conditions suitable for the establishment of *U. pinnatifida* gametophyte populations.

2.2 Methods

2.2.1 Collection methods

Sporophytes were collected from 2 m depth off Point Wilson in Port Phillip Bay (38° 04.04′ S, 144° 31.35′ E) (Figure 1). Plants were kept chilled, transported to the laboratory and maintained overnight at 4°C in a cloth bag moistened with seawater, to enhance spore release.

2.2.2 Zoospore release

The method for release and settlement of spores is a modification of procedures employed for spore release in a number of other laminarian sporophytes (Pérez et al. 1992b, Reed 1990, Anderson and Hunt 1990, Novaczek 1984a, Toth 1976). Sporophylls of approximately 7 cm diameter were excised from mature plants and wiped with paper toweling to clean and remove potential contaminants. The sporophylls were washed in 0.2 μm membrane filtered seawater and 8 sporophylls were placed in 3 L of filtered seawater at 15°C, stirring intermittently for approximately 1 min., until zoospores were released. Zoospore release was confirmed microscopically by pipetting 1ml of zoospore solution onto a haemocytometer and examining at 400x magnification. If the zoospore count exceeded 10^4 cells ml^{-1} the suspension culture was diluted with seawater to achieve ~10^4 cells ml^{-1}, previously determined to produce optimal growth of laminarian gametophyte cultures. (Anderson and Hunt 1990).

2.2.3 Culture techniques

Germinated zoospores (germlings) were cultured in 25 ml glass beakers. Microscope coverslips were placed at the bottom of 25 ml beakers and acted as a substratum for spore settlement and germination tube growth. Each beaker was filled with 25 ml of the zoospore suspension, covered with polyethylene plastic wrap and maintained for one h under experimental conditions. The zoospore suspension was then gently decanted from the beakers and replaced with 25 ml of nutrient enriched seawater (Steele and Thursby 1988) (excluding N for ammonium enrichment experiments) (Appendix 1).

2.2.4 Experimental design

Five replicate cultures (beakers) were established for each treatment in the photon flux density and salinity experiments. Temperature and nitrogen availability (range finding) were conducted with experiments four replicates and three replicates were employed in the definitive range nitrogen availability experiment. In temperature evaluations a single-factor design was employed where cultures were grown in combination of different temperatures with treatments of 5°C, 10°C, 15°C, 20°C, 25°C, 28°C and 30°C. A two-factor design was employed for the light experiment where cultures were grown in combination of photon flux densities of 10 μmol m^{-2} s^{-1}, 30 μmol m^{-2} s^{-1}, 60 μmol m^{-2} s^{-1} and 80 μmol m^{-2} s^{-1} and temperatures of 10°C, 15°C and 20°C. Cultures were also grown under different salinities with treatments of 32 psu, 24 psu, 16 psu, 8 psu and 0 psu (freshwater). For the nitrogen availability experiments, an initial range finding concentration series of 0, 3.57, 7.14, 14.28, 28.57

and 57.14 μM NH_4^+-N was employed, followed by a two-factor experiment with a definitive range of 0, 0.45, 0.90 and 1.80 μM NH_4^+-N was incorporated temperatures of 10°C, 15°C and 20°C. Where physical parameters were not subject to experimental manipulation, all experiments were conducted at 15°C under a 12:12 light:dark cycle with a photon flux density of 80 μmol m^{-2} s^{-1} for 48 h.

2.2.5 Germination and growth rate analyses

Forty eight hours after zoospore release, cultures were examined for spore germination and rate of germling (germination tube) growth. Coverslips were removed from the beakers, placed on a microscope slide and examined at 400x magnification. The spores were identified as either germinated or non-germinated by observing the presence or absence of a germination tube growing from the settled spores. A minimum of 30 spores were examined, always completing the count for each field of view. For each replicate culture, the length of 10 germination tubes (if present) from germinated spores were measured using a graduated graticule. The percentage of germinated spores and mean growth rate (μm d^{-1}) of germinated spores was calculated for each replicate.

2.2.6 Statistical analyses

Data were tested for assumptions of normality and heterogeneity of variance and where necessary, data were either $\log_e$ (growth rates) or arcsin square root (% germinated) transformed. One and two-factor analysis of variance (ANOVA) were employed to test for significant effects of treatments. Tukeys post-hoc range test was

employed to determine significantly different groups (Zar 1996). The level of significance for hypothesis testing was $p < 0.05$, unless otherwise stated.

2.3 Results

The results indicate that germination and the subsequent growth of *Undaria pinnatifida* zoospores were maximal at temperatures between 10°C and 25°C, and at salinities greater than 16 psu. The effect of temperature on germination (Figure 2.1, Table 2.1) was highly significant (ANOVA: $F_{[5, 18]} = 9.48$, $p < 0.001$) with reduced germination at temperatures greater than 25°C and lower than 10°C; no germination occurred at 30°C. There was little difference in germination rates at temperatures from 10°C to 25°C, with a mean germination rate of 92.7 % over this range. Germination tube growth rate was also significantly affected (ANOVA: $F_{[5, 18]} = 68.11$, $p < 0.001$) by temperature with a maximum growth rate of 21.8μm d^{-1} at 20°C (Figure 2.2). The significantly lower rates of growth at 5°C and 28°C, are consistent with reduced germination at the same temperatures. Sensitivity to high temperatures was indicated by a significantly reduced rate of growth at 25°C (3.5 μm d^{-1}) when compared to 10°C, 15°C and 20°C (18.6, 16.8 and 21.8 μm d^{-1} respectively). Mean growth rate for the 10°C to 25°C temperature range was 17.3 μm d^{-1}.

Photon flux density did not affect zoospore germination, while germination tube growth (Figure 2.3, Table 2.2) was significantly affected (ANOVA: $F_{[3, 48]} = 3.35$, $p < 0.05$) . There was a significant (ANOVA: $F_{[6, 48]} = 11.65$, $p < 0.001$) interaction between photon flux density and temperature on germination tube growth rate, but not on germination. Inspection of the data showed that growth

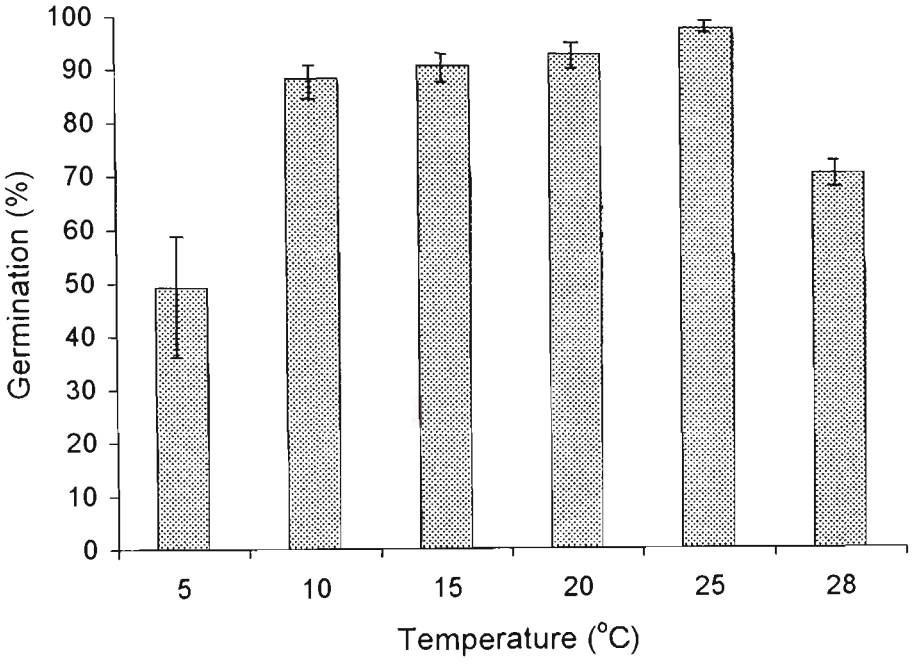

Figure 2.1. Germination rates (%) of *Undaria pinnatifida* zoospores at different temperatures. Values are means ± s.e. (n = 4).

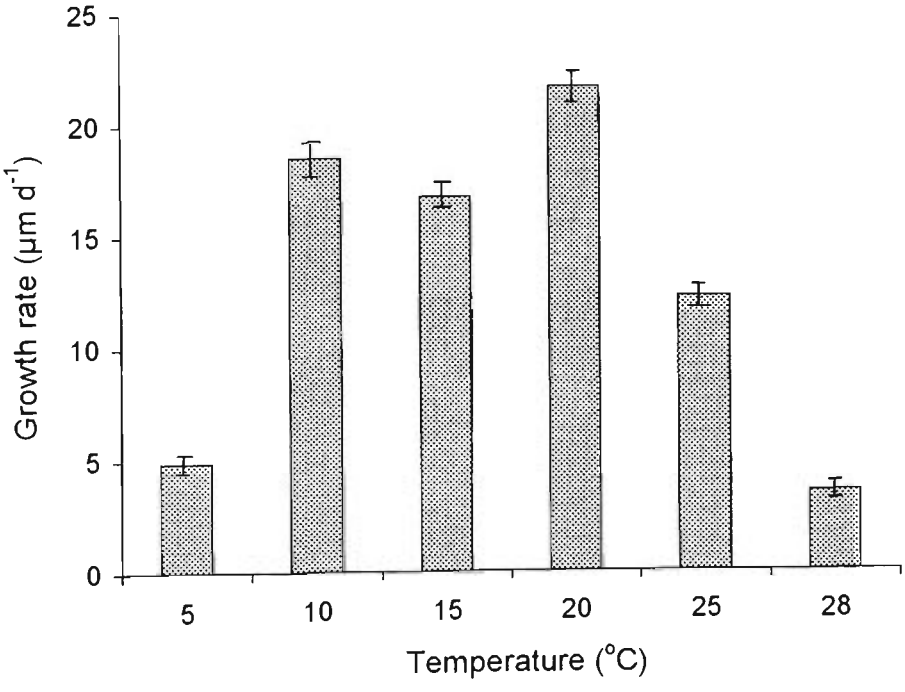

Figure 2.2. *Undaria pinnatifida* germination tube growth rates (µm d^{-1}) at different temperatures. Values are means ± s.e. (n = 4).

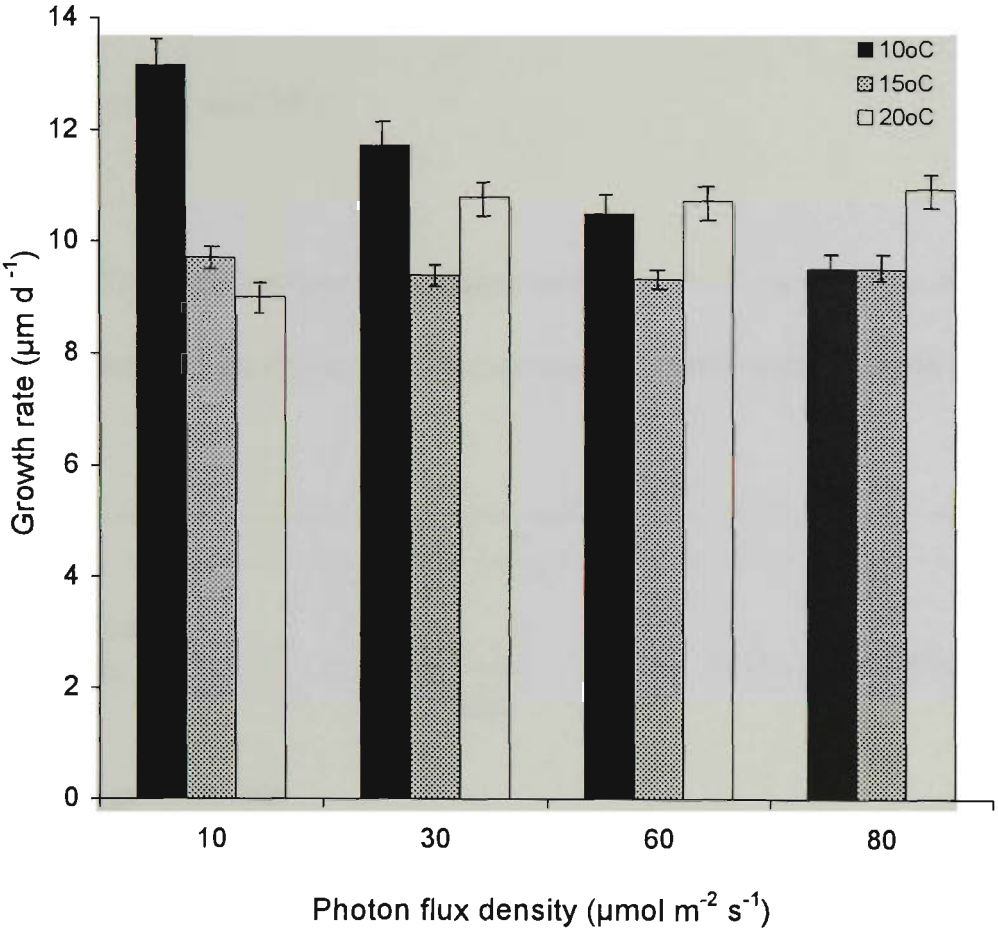

Figure 2.3. *Undaria pinnatifida* germination tube growth rates (µm d^{-1}) at various temperatures and photon flux densities. Values are means ± s.e. (n = 5).

rates decreased with increasing photon flux density at 10°C, but that photon flux density had little or no effect on growth rates at 15°C and 20°C. Germination tube growth was highest (13.2 μm d^{-1}) at 10 μmol m^{-2} s^{-1} and 10°C and lowest (6.8 μm d^{-1}) at 10 μmol m^{-2} s^{-1} and 20°C.

Table 2.1. One-way analyses of variance on the effect of temperature, nitrogen concentration and salinity on germination and germination tube growth rates.

Factor	SS	df	F	p
Germination				
Temperature	26.556	5	9.48	0.001
Error	10.088	18		
Germtube growth rate				
Temperature	11.537	5	68.11	0.001
Error	0.610	18		
Germination				
Ammonium (0-57.14 μM NH_4^+-N)	0.034	5	0.32	0.893
Error	0.384	18		
Germtube growth rate				
Ammonium (0-57.14 μM NH_4^+-N)	12.141	5	236.02	0.001
Error	0.185	18		
Germination				
Salinity	3.693	4	25.72	0.001
Error	0.538	15		
Germtube growth rate				
Salinity	28.277	4	3841.77	0.001
Error	0.028	15		

Reduced salinity had a highly significant effect on germination and growth (Table 2.1) with decreased rates of both germination and growth below 24 psu and no significant difference (for each endpoint) between 32 psu and 24 psu. Mortality of all spores occurred, as expected, at 0 psu salinity. Dose response curves for reduced salinity (Figures 2.4 and 2.5) indicate a similar dose response relationship for each endpoint with median effect concentrations (EC_{50}) for germination and growth of 11.2 psu and 10.4 psu respectively.

Ammonium concentrations from 0 to 57 μM NH_4^+-N had no significant effect on germination, with a mean germination rate over all treatments (including control) of 93.5 ± 1.5%. Ammonium did, however, have a significant effect (Table 2.1) on the growth rates of developing germlings (Figure 2.6), with growth rates almost 75% less at 57 μM NH_4^+-N than at 0 μM NH_4^+-N. The median effect dose (EC_{50}) for growth inhibition was 25.6 μM NH_4^+-N.

There was a significant interaction between ammonium and temperature (Table 2.2) at ammonium concentrations from 0 to 1.8 μM NH_4^+-N (Figure 2.7) on germination, but not on germination tube growth rates. This interaction was explained by significantly higher germination rates at 20°C compared to rates at 10°C and 15°C (at 1.8 μM NH_4^+-N) and no affect of temperature at ammonium concentrations below 1.8 μM NH_4^+-N.

Table 2.2. Two-way analysis of variance on the effect of temperature, nitrogen concentration and photon flux density on germination and germination-tube (germtube) growth rates.

Factor	SS	df	F	p
Germination				
Temperature	0.113	2	2.64	0.092
Ammonium (0-1.8 μM NH_4^+-N)	0.097	3	1.50	0.240
Temperature x Ammonium	0.337	6	2.62	0.042
Error	0.515	24		
Germtube growth rate				
Temperature	8.774	2	5.27	0.013
Ammonium (0-1.8 μM NH_4^+-N)	1.097	3	0.44	0.727
Temperature x Ammonium	8.715	6	1.74	0.154
Error	57.125	24		
Germination				
Temperature	0.612	2	21.06	0.001
Photon flux density (PFD)	0.120	3	2.74	0.053
Temperature x PFD	0.083	6	0.956	0.465
Error	0.698	48		
Germtube growth rate				
Temperature	118.816	2	107.70	0.001
Photon flux density (PFD)	5.550	3	3.35	0.026
Temperature x PFD	38.561	6	11.65	0.001
Error	26.476	48		

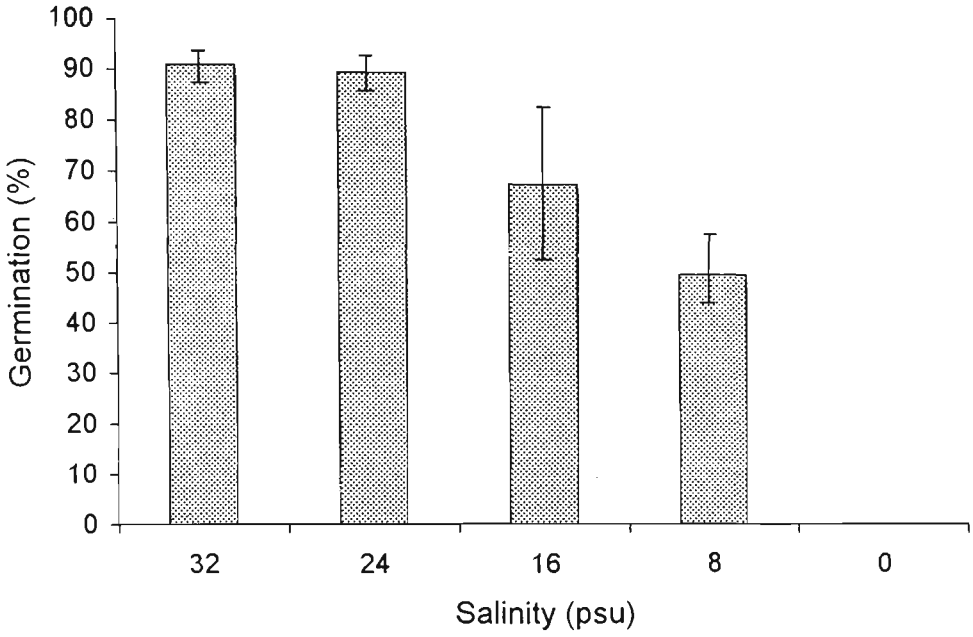

Figure 2.4. Germination rates (%) of *Undaria pinnatifida* zoospores at 15°C and various salinity concentrations. Values are means ± s.e. (n = 4).

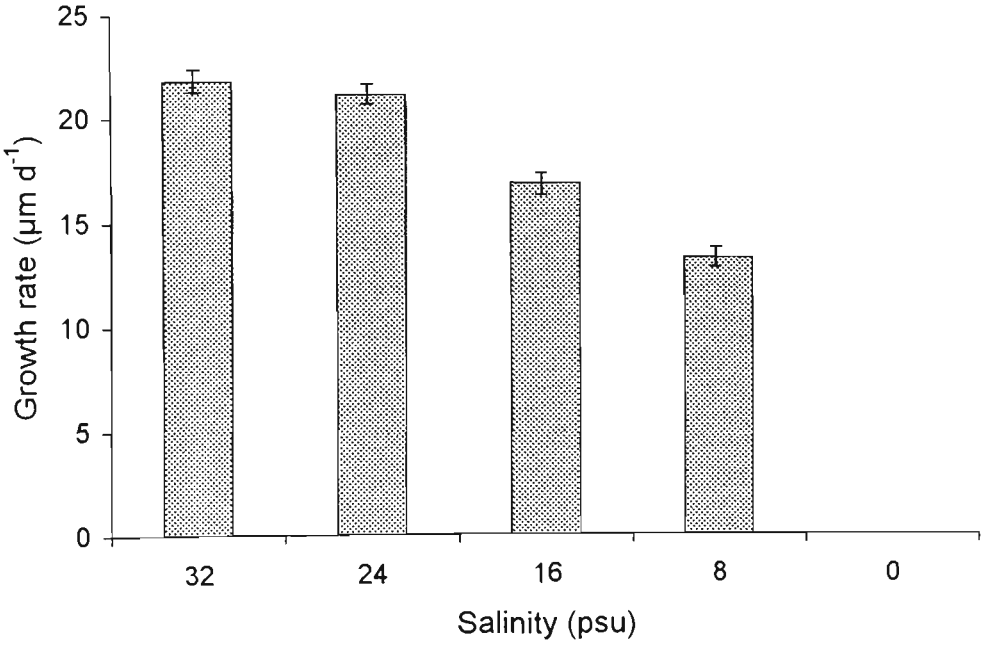

Figure 2.5. *Undaria pinnatifida* germination tube growth rates (µm d^{-1}) at 15oC and various salinity concentrations. Values are means ± s.e. (n = 4).

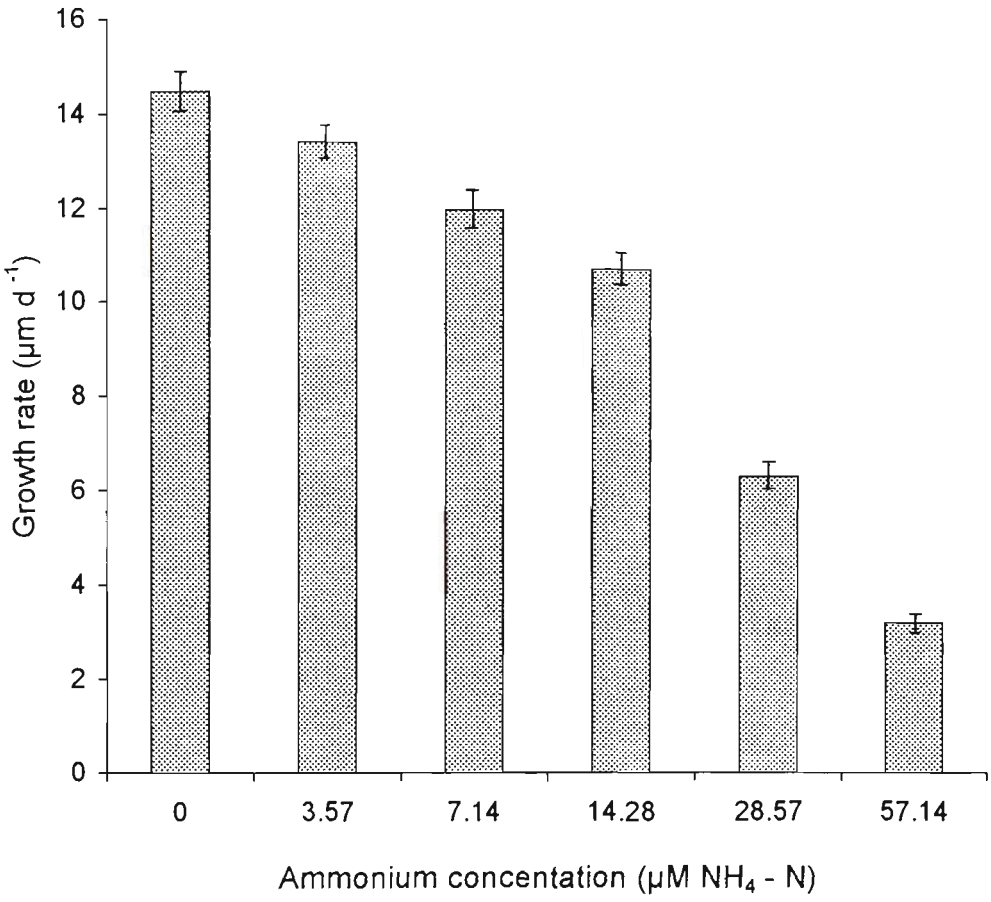

Figure 2.6. *Undaria pinnatifida* germination tube growth rates (µm d^{-1}) at 15°C and various ammonium concentrations. Values represent means ± s.e. (n = 4).

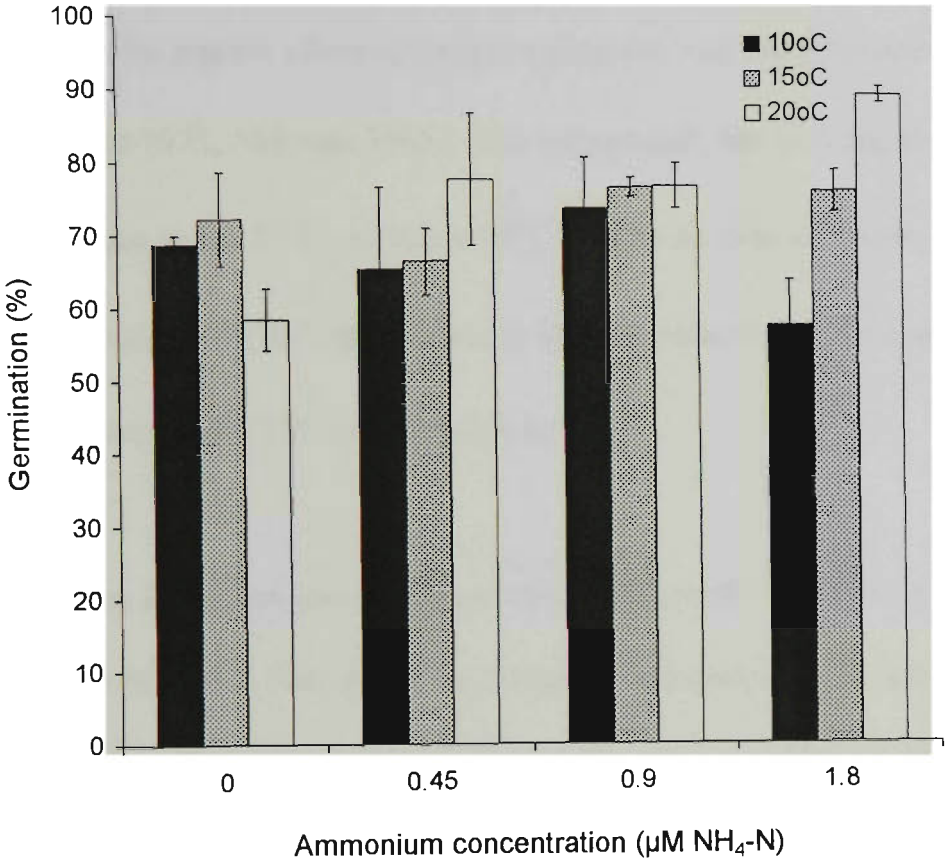

Figure 2.7. *Undaria pinnatifida* zoospore germination rates (%) at various temperatures and ammonium concentrations. Values represent means ± s.e. (n = 4).

2.4 Discussion

The results of this study suggest that high germination rates of *Undaria pinnatifida* zoospores occur over a range of temperatures from 10°C to 25°C, a finding that differs from previous reports where optimal germination occurred only at temperatures below 20°C (Saito 1975, Akiyama 1965). The substantially reduced germination rates at temperatures above 25°C and below 10°C and the absence of germination at temperatures above 28°C are consistent with previous reports for zoospore germination (Saito 1975, Akiyama 1965).

Differences in optimal germination rates between strains of *Undaria pinnatifida* zoospores from Port Phillip Bay and Japan could be attributed to genotypic variation or possibly to the acclimation of zoospores to water temperatures at the time of collection. In the present study the collection of sporophylls for germination temperature experiments was made when waters were 18°C. The temperature of waters from which Japanese sporophylls were collected was not recorded (Saito 1975), but if they were lower than 18°C this may explain the lower germination success at 25°C relative to the PPB population. It has been suggested that algae adapt to changing temperatures by altering the concentrations of certain enzymes or by the introduction of isoenzymes with different temperature dependencies (Lüning and Neushul 1978, Küppers and Weidner, 1980). The latter may account for any variation in growth rates between experiments under similar conditions.

The optimal rate of germination tube growth between 10°C and 20°C is consistent with previous reports for optimal growth of *U. pinnatifida* gametophytes (Akiyama 1965) and also characteristic of warm temperate algae (Lüning 1990b). Growth of *U. pinnatifida* germination tubes at 15°C was rapid, with a mean growth rate (16.8 μm d^{-1}) almost twice that reported for other laminarians, such as *Ecklonia radiata* (8.7 μm d^{-1}) grown under similar conditions (Burridge et al. 1999a). The effect of temperature on growth of the germination tube may be due to changes in photosynthesis regulated by temperature-dependent enzymes under conditions of saturating light (Lüning 1990b). A low growth rate at 5°C could be caused by a decrease in the activity of enzymes involved in photosynthesis and other metabolic processes, which generally decrease at low temperatures in macroalgae (Lobban et al. 1985a). The decrease in growth with increasing temperatures above 20°C may be explained by irreversible heat damage to thermoliable proteins as temperature rises (Lüning 1990b, Lobban et al. 1985a), leading to zoospore death at 30°C .

The high growth rate of germination tubes at 10°C under low photon fluxes suggests a capacity for optimal growth during winter when zoospores were collected. At low photon flux densities, a change in temperature may have little or no effect on the rate of photosynthesis in macroalgae (Lüning 1990b), a response that is due to the acclimation of the principal enzymes involved in photosynthesis (e.g. ribulose biphosphate carboxylase) to ambient temperature (Davison et al. 1991). Therefore photosynthesis and subsequent growth would remain efficient at low temperatures during low light availability, as found in this study. As *U. pinnatifida* is a winter

annual, its spores must be able to germinate and grow at low temperatures and utilize minimal light resulting from shorter daylengths and shading from the sporophyte canopy. This is consistent with previous studies where optimal responses of brown algal zoospores in the laboratory have occurred under conditions which closely reflect those from which plants were collected (Lee and Brinkhuis 1988, Novaczek 1984b, Yarish et al. 1979, Sheader and Moss 1975).

Light availability does not appear to play a significant role in the initial stages of zoospore germination in *Undaria pinnatifida*; similar findings have also been reported for zoospore germination in other members of the Laminariales (Lee and Brinkhuis 1988, Lüning 1980b, Kain 1964). Kain (1964) postulated that zoospores of *Laminaria hyperborea* have a high carbohydrate storage and low photosynthetic capacity relative to respiration rate, suggesting that light has little influence on the carbohydrate content of zoospores. This could also explain why laminarian zoospores can survive extended periods of darkness (tom Dieck 1993, Lüning 1980b, Kain 1964). Following germination, growth would lead to increased photosynthetic demand and therefore respond to changes in light availability (Kain 1964), as demonstrated in this study.

The decline in zoospore germination and growth in response to decreasing salinity is also consistent with previous reports for *Undaria pinnatifida* and other algal macrophytes (Burridge et al. 1999b, Shir and Burridge 1998, Saito 1975). Saito (1975) reported germination of Japanese *U. pinnatifida* zoospores at salinities between 7 and 21 psu, with optimal germination at salinities above 15 psu and

retarded development below 13 psu. *U. pinnatifida* zoospores appear to be relatively tolerant to low salinity in comparison to other laminarian kelps. The salinity median effect concentration (EC_{50}) for both germination (11.2 psu) and growth (10.4 psu) were lower than comparable values reported for the two laminarian algae *Macrocystis angustifolia* Bory (18 psu and 18 psu respectively) (Shir and Burridge 1998) and *Ecklonia radiata* (C. Ag.) J. Agardh (16.4 psu and 12.58 psu respectively) (Burridge et al. 1999b); and also for zygote germination in the fucoid macrophytes *Phyllospora comosa* C. Agardh (19 psu) and *Hormosira banksii* (Turner) Decaisne (17 psu) (Shir and Burridge 1998).

The mechanism that enables *Undaria pinnatifida* zoospores and germlings tolerate relatively low salinity is uncertain. It has been suggested that the tolerance of algal cells to low salinity may be determined by cell wall strength and the ability of the algal cells to adjust their internal osmotic potential to become less negative (Lobban et al. 1985b). In response to changing salinities algal cells can alter their internal osmotic pressure by pumping ions into or out of the cell or by the interconversion of monomeric and polymeric metabolites (Hellebust 1976). Therefore the ionic composition of the surrounding water is an important factor influencing the ability of algal cells to tolerate low salinity (Gessner and Schramm 1971, Guillard 1962, Provasoli 1958). For example, calcium ions (Ca^{2+}) have been associated with salinity tolerance of algae (Robinson and Jaffe 1975), as have increased cell membrane width (Yarish et al. 1980) and decreased membrane permeability (Poovaiah and Leopold 1976). It has been postulated that the germination of brown algal embryos involves Ca^{2+}, where movement of cellular vesicles into the basal pole of the cell and site of

rhizoid outgrowth is thought to be initiated by the generation of a Ca^{2+} driven transcellular electrical field (Robinson and Jaffe 1975). This process may also be limited by changes in membrane permeability in response to unfavourable salinities that affect both germination and growth of the germination tube. It is possible that *U. pinnatifida* zoospores are highly efficient at maintaining internal ionic composition to achieve stable osmoregulation, thereby withstanding relatively low salinities.

The results of this study suggest that germination of *Undaria pinnatifida* zoospores occurs independent of ammonium availability, while higher concentrations of ammonium decreased germination tube growth. Germination is likely to be independent of ammonium availability with internal storage of nutrients in the zoospore. At high ammonium concentrations physiological activity in macroalgae has been shown to be impaired and germination tube growth inhibited (Azov and Goldman 1982, Prince 1974, Waite and Mitchell 1972). The response of germination tube growth to ammonium availability may be due to the time taken for ammonium uptake and assimilation to occur and influence physiological processes. Such responses may be caused by a decrease in cellular ribulose biphosphate (RuBP) which in turn suppresses photosynthetic carbon fixation (Elrifi et al. 1988). The decrease in RuBP is possibly due to competition for metabolites between the Calvin cycle and nitrogen assimilation pathways (Elrifi and Turpin 1986). It has also been suggested that increased ammonium concentrations inhibits stimulation of productivity by other nutrients (Waite and Mitchell 1972), which may further contribute to decreasing growth rates at high ammonium concentrations.

Enhanced germination rates of *Undaria pinnatifida* spores at the optimal ammonium concentration of 1.8 μM NH_4^+-N with an increase in temperature is likely to be due to the effect of temperature on nutrient uptake. Temperature can alter the activity of enzymes and influence the rate of nutrient uptake (Riccardi and Solidoro 1996, Harrison 1985, Lobban et al. 1985a, Wheeler and Weidner 1983, De Boer 1981, Küppers and Weidner 1980). At sub-optimal ammonium concentrations (< 1.5 μM NH_4^+-N), temperature does not influence germination since nitrogen (NH_4^+) becomes the limiting factor.

Germination tube development of *U. pinnatifida* zoospores appears to be more responsive to changes in environmental conditions than germination alone. This supports the contention by Anderson (1988) that the development of the germination tube is not as ecologically important as germination. Without germination further growth cannot occur, whereas the growth of the germination tube does not necessarily reflect the 'fitness' of the spore. Indeed 'healthy' germinated spores may exhibit delayed growth responses and develop into gametophytes once favourable environmental conditions become established (Anderson and Hunt 1988).

Undaria pinnatifida zoospores show substantive resilience to a range of environmental factors when compared to the limited data on other macroalgal zoospores. Temperature is perhaps the principal factor governing germination and germination tube growth rates in *U. pinnatifida* from Port Phillip Bay, and it has also been found to have a major influence on other life stages (Hay and Villouta 1993, Saito 1956a). Maximum germination tube growth at low light intensities and low

temperatures also reflects the conditions found during winter and spring in Port Phillip Bay and other temperate climates. This study has also shown that *U. pinnatifida* has a greater tolerance to reduced salinity compared to native laminarian kelps. This may explain the capacity for growth of *U. pinnatifida* in coastal waters subject to freshwater inputs. High germination rates at high ammonium concentrations demonstrates a tolerance to waters subject to high nutrient loads resulting from sewage inputs and in part may explain the occurrence of *U. pinnatifida* in polluted waters in Port Phillip Bay. Although laminarian spores appear phenotypically similar there appear to be some variations between species (Amsler and Neushul 1989, Henry and Cole 1982) and these may contribute to the physiological differences found between some species of the Laminariales and *U. pinnatifida.*

Zoospore germination and the initial growth of the germination tube may not be a good indicator for later success of *Undaria pinnatifida.* Germination is largely self-sustained by internal supply of nitrogen and carbon in the zoospore, possibly contributing to the robust nature of the initial growth stage. Hence, gametophyte development, gametogenesis and the early sporophyte development may play a more important role in the success of *U. pinnatifida.*

Chapter 3

The effect of environmental factors on the growth and development on *Undaria pinnatifida* gametophytes.

3.1 Introduction

The development of *Undaria pinnatifida* gametophytes following zoospore germination is a crucial stage in the successful growth and survival of these macroalgae. The influence of environmental factors (e.g. temperature, light and nutrients) on gametophyte growth is intrinsic to our understanding of *U. pinnatifida*'s potential for growth and spread in Australian waters. The development of laminarian gametophytes has been widely investigated in the Northern Hemisphere (Deysher and Dean 1984, Lüning and Neushul 1978, Lüning and Dring 1975, Hsiao and Druehl 1973c, Hsiao and Druehl 1973b, Hsiao and Druehl 1973a, Vadas 1972, Lüning and Dring 1972, Hsiao and Druehl 1971, Anderson and North 1969, Kain 1969, Cole 1968, Kain 1964, Yabu 1964), only a few studies have investigated the effects of environmental factors on gametophyte development of the Laminariales in the Southern Hemisphere (Bolton and Levitt 1985, Novaczek 1984b, Novaczek 1984a, Branch 1974).

Only a single study has examined the environmental factors influencing gametophyte growth and reproduction of the Laminariales in Australia. tom Dieck(1993) examined the tolerance of gametophytes to temperature and darkness, including three native Australian species; *Ecklonia radiata*, *Lessonia corrugata* and *Macrocystis*

angustifolia. Studies on *U. pinnatifida* gametophyte growth have been carried out in Japan were it is native (Akiyama 1965, Saito 1956b, Saito 1956a), but no studies have been published on the response to environmental factors of *U. pinnatifida* gametophytes from introduced populations. Hence, there are no data available on the effects of light, temperature and nutrients on gametophyte development of *U. pinnatifida* in Australia.

The gametophyte stages of many laminarian species, including *Undaria pinnatifida*, generally show a similar course of development. Zoospores germinate to form an equal number of male and female dioecious, filamentous gametophytes (Kain 1964, Papenfuss 1951). In the early stages of development the dumb-bell shaped male and female gametophytes are identical. Under optimal conditions, male and female gametophytes are identifiable approximately seven days after spore settlement. Females form large-celled gametophytes, usually possessing one or very few cells with limited branching, while males form small-celled gametophytes with multiple branching (Hu et al. 1981, Kanda 1936, Yendo 1911). The male gametophytes form antheridia, each of which produces a single spermatozoid, and the antheridia usually die after the sperm release. The often single cell of a typical female gametophyte develops into an oogonium with a single ovum. The ovum usually remains attached to the gametophyte during fertilization and early development of the sporophyte (Kain 1979, Jennings 1967, Papenfuss 1951, Kanda 1936). The egg can also develop parthenogenically, but the resulting sporophyte is often malformed compared with the diploid form (Zhongxi et al. 1982, Kain 1964, Yabu 1964).

The size, form and fertility of laminarian gametophytes varies with temperature, light and nutrient availability (Kain 1964, Fritsch 1945). Vegetative growth and gametogenesis have different physiological requirements (Kain 1979, Lüning and Neushul 1978). Temperature is the primary factor that regulates reproduction, development and growth in macroalgae due to its effect on cellular metabolism (Lee and Brinkhuis 1988, Lobban et al. 1985a, Novaczek 1984b, Saito 1975). Temperature may also interact with other environmental factors (e.g. nitrogen concentration, light availability) to influence metabolic processes such as nitrogen uptake (Hanisak 1983), photosynthesis, growth and reproduction (Lobban et al. 1985a).

Nitrogen is an important nutrient necessary for the growth, development and reproduction of macroalgal gametophytes (Hsiao and Druehl 1973b, Hsiao and Druehl 1973a, Hsiao and Druehl 1971). Nitrogen is necessary for algal growth as it is incorporated into important compounds essential for life, such as amino acids, purines, pyrimidines, porphyrins, amino sugars, amines and photosynthetic pigments (Harrison 1985, Lüning 1981a). The most important forms of nitrogen utilized by macroalgae are ammonium (NH_4^+) and nitrate (NO_3^-) (Harrison 1985, Hanisak 1983). Ammonium may be directly incorporated into compounds and is usually taken up at a higher rate, sometimes inhibiting the uptake of NO_3^- , but this depends on the NH_4^+ concentration and the algal species (Harrison 1985, Hanisak 1983). Uptake of NH_4^+ can be achieved by facilitated diffusion or active transport which utilize transport mechanisms (e.g. enzyme activated systems) requiring energy or passive diffusion with no energy requirement (Harrison 1985, De Boer 1981, Hanisak and Harlin 1978).

Light is absorbed by algal pigments and used as energy in the process of photosynthesis. The amount of light available for photosynthesis therefore influences the amount of photosynthates produced for growth and other metabolic functions (Ramus 1981). The effect of light on gametophyte growth of Laminariales (Lee and Brinkhuis 1988, Bolton and Levitt 1985, Novaczek 1984a, Lüning 1980b, Lüning and Neushul 1978, Anderson and North 1969), including *Undaria pinnatifida* (Saito 1975, Akiyama 1965, Saito 1956a) is well documented. The low light requirements for photosynthesis (Fain and Murray 1982, Kain 1964) and growth (Lüning and Neushul 1978, Vadas 1972) of laminarian gametophytes has characterized them as 'shade plants' (Han and Kain 1996, Lee and Brinkhuis 1988, Lüning and Neushul 1978).

Gametophyte development in response to photoperiod has received little attention. The daily light period (daylength) is measured in algae by sensor pigments not involved in photosynthesis; daylength is used as an environmental signal to trigger a change in the pattern of metabolism (photoperiodism) (Lüning 1981a). Photoperiodic responses in macroalgae have been reported for *Porphyra tenera* (Dring 1967), *Bangia fuscopurpurea* (Richardson 1970) and *Scytosiphon lomentaria* (Dring and Lüning 1975). The responses of laminarian gametophytes to photoperiod are not distinct and has not been well studied (Lüning 1980b). Deysher (1984) found that the effect of photoperiod on *Macrocystis pyrifera* gametophytes at 15°C was negligible and could be accounted for by quantum dosage effects (i.e. the sum of irradiance and length of light exposure). In contrast, Akiyama (1965) reported maximum growth rates and induction of gametogenesis for *U. pinnatifida* gametophytes with increased photoperiod, whereas Saito (1975) reported that *U. pinnatifida* gametophytes

exhibited higher growth and gametogenesis with decreased photoperiod. In France, *U. pinnatifida* gametogenesis has been reported to correspond with short day length *in situ* (Floc'h et al. 1991).

This chapter investigates the effects and interactive effects of environmental factors such as temperature, ammonium concentration, light and photoperiod on gametophyte growth and development of *U. pinnatifida* gametophytes.

3.2 Methods

3.2.1 Collection and zoospore release

Sporophytes were collected as for germination experiments in Chapter 2 (2.2 Methods, 2.2.1 Collection). The method for release and settlement of spores was repeated as in Chapter 2 (2.2 Methods, 2.2.2 Zoospore release). Sporophylls from eight parent sporophytes were used to reduce genetic influences in spore cultures (Novaczek 1984a).

3.2.2 Culture techniques

The culture techniques employed for the gametophyte cultures were as for zoospore germination and germination tube growth experiments in Chapter 2 (2.2.3 Culture techniques). The cultures were placed in incubators under experimental conditions for up to 28 days. The culture medium was replaced with fresh seawater three times a week. For the photon flux density and photoperiod experimental procedures five replicate cultures (beakers) were established for each treatment, whilst for the temperature and salinity experiments four replicates were utilized. Three replicates were employed in the nitrogen availability experiments.

3.2.3 Experimental design

In the first two experiments a single-factor design was employed where cultures were grown for 21 days under different salinities of 32 psu, 24 psu, 16 psu, 8 psu and 0 psu (freshwater) and temperatures of 5°C, 10°C, 15°C, 20°C, 25°C and 28°C. For the

nitrogen availability experiments a two-factor design incorporating temperatures of 10°C, 15°C and 20°C was employed, with ammonium concentrations of 0, 3.57, 7.14, 14.28, 28.57 and 57.14 μM NH_4-N to find the response range, and a subsequent experiment using concentrations of 0, 0.45, 0.9, 1.5, 3.57 and 7.14 μM NH_4-N. A two-factor design was employed for the photon flux density experiment where cultures were grown under light regimes of 10 μmol m^{-2} s^{-1}, 30 μmol m^{-2} s^{-1}, 60 μmol m^{-2} s^{-1} and 80 μmol m^{-2} s^{-1} and at 10°C, 15°C and 20°C. The photoperiod experiment employed a two-factor design with three photoperiods of 8 h light (16 h dark), 12 h light (12 h dark) and 16 h light (8 h dark) each at 10°C, 15°C and 20°C. Where physical parameters were not subject to experimental manipulation, all experiments were conducted at 15°C under a 12:12 light:dark cycle with a light intensity of 80 μmol m^{-2} s^{-1}.

3.2.4 Growth rate and gametogenesis analyses

Cultures were examined 7, 14, 21 and 28 d after zoospore release for gametophyte growth and gametogenesis. Coverslips were removed from the beakers, placed on a microscope slide and examined at 400x magnification. For each replicate culture, the longest axis of 10 random gametophytes were measured using a graduated graticule and the growth rate (μm d^{-1}) of gametophytes calculated, by dividing the length by the number of days after spore germination. Gametogenesis was recorded as the presence or absence of sporophytes. Gametophytes were considered dead when they lost their colour and appeared to have no cell contents.

3.2.5 Statistical analyses

The growth rate data were tested for assumptions of normality and heterogeneity of variance and where appropriate data was either log or square root transformed. Single and two-factor analysis of variance (ANOVA) were employed to test for significant effects of treatments and Tukeys post-hoc range was employed to determine significant groups (Zar 1996). The level of significance for hypothesis testing was $p < 0.05$, unless otherwise stated. All statistical analysis were carried out using SYSTAT, version 5.0.

3.3 Results

Undaria pinnatifida zoospores germinated within 24 h, developing into immature gametophytes within 7 d after settlement. Male and female gametophytes could be distinguished within 14 d for most cultures, depending on experimental conditions. Gametophytes reached sexual maturity, giving rise to sporophytes within 14 to 30 d, depending on experimental conditions. The length of gametophytes did not necessarily correlate with sporophyte production.

3.3.1 Effects of temperature

Gametophyte growth was significantly affected by temperature (ANOVA: $F_{[5, 234]} = 229.8$, $p < 0.001$) (Table 3.1, Figure 3.1). After 7 d, gametophyte growth rates were highest at 10°C (8.06 μm d^{-1}) and 15°C (7.79 μm d^{-1}), and were significantly higher than growth rates at 5°C (3.35 μm d^{-1}), 20°C (4.43 μm d^{-1}), 25°C (3.36 μm d^{-1}) and 28°C (1.44 μm d^{-1}).

Table 3.1. One-way ANOVA on the effect of temperature on gametophyte growth after 7d, n = 4. Data were $\log_e(x)$ transformed.

Factor	SS	df	F	p-value
7 d Gametophytes				
Temperature	78.525	5	229.84	0.001
Error	15.989	234		

Table 3.2 summarises the survival of *Undaria pinnatifida* gametophytes and onset of sporophyte production at a range of temperatures over the 28 d experimental period. Gametophytes grown at 10°C and 15°C matured and gave rise to sporophytes within 14 d (Table 3.2). At 20°C and 25°C many of the gametophytes were dead after 14 d, but at 21 d, a few gametophytes had become large and multi-branched, resembling parthenogenic gametophytes (Zhongxi et al. 1982, Yabu 1964). A few sporophytes were found amongst the masses of gametophyte branches at 20°C and 25°C after 21 d. Sporophyte production was not achieved within 21 d (the course of the experiment) at 5°C, most likely due to retarded gametophyte maturation and at 28°C due to gametophyte death.

Table 3.2 Gametophyte and sporophyte survival in culture at different temperatures over 21 days following zoospore release.

	5°C	10°C	15°C	20°C	25°C	28°C
7 days	G	G	G	G	G	G
14 days	G	S	S	G (D)	G (D)	G (D)
21 days	G	S	S	P (S)	P (S)	D

G = living gametophytes, S = living sporophytes, G (D) = gametophyte appears dead, D = dead gametophytes, P (S) = parthenogenic gametophytes (a few sporophytes present).

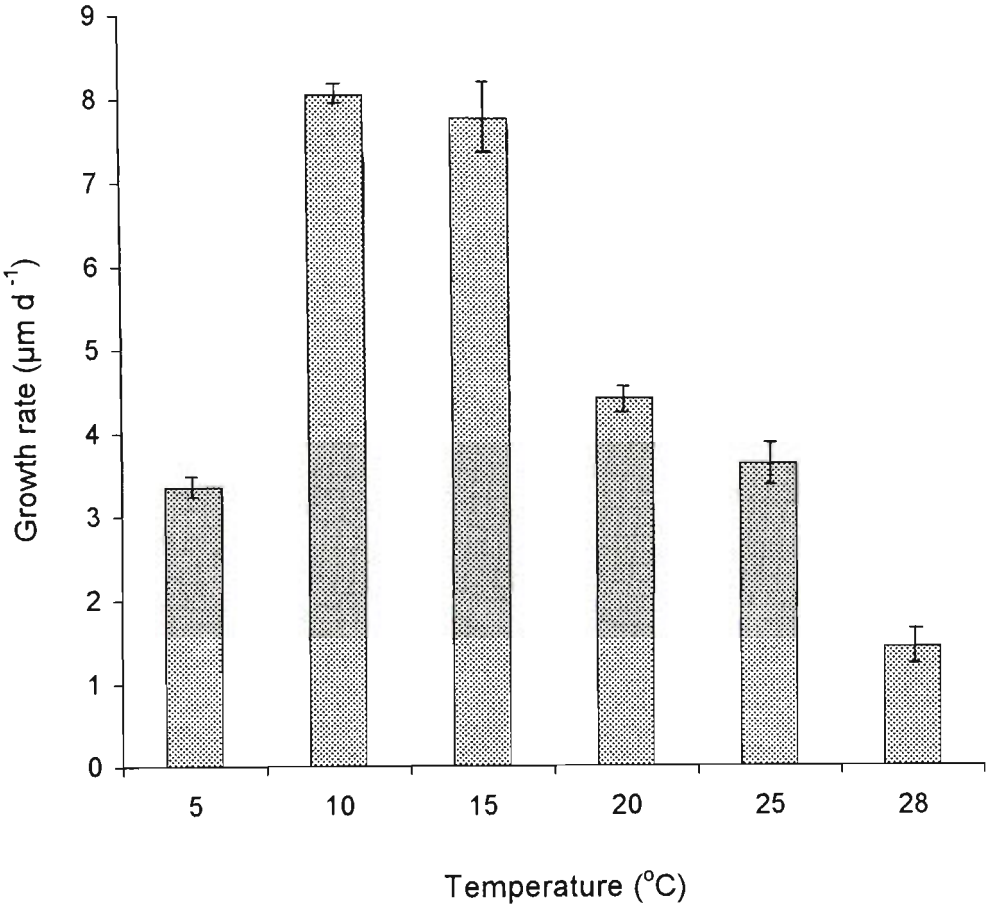

Figure 3.1. *Undaria pinnatifida* gametophyte growth rates (µm d^{-1}) after 7d over a range of temperatures. Values are means ± s.e. (n = 4).

3.3.2 Salinity

Undaria pinnatifida gametophyte growth was significantly (ANOVA: $F_{[4, 195]}$ = 2530.9, $p < 0.001$) affected by salinity after 7 d, with growth rates at salinities of 8 psu and 16 g psu, significantly lower than at 24 psu and 32 g psu (Figure 3.2, Table 3.3). No growth occurred at 0 psu salinity. After 21 days, the mean growth rate at 32 psu was significantly (ANOVA: $F_{[4, 45]} = 148.383$, $p < 0.001$) higher than at 24 g psu (Figure 3.3).

Table 3.3. One-way ANOVA on the effect of salinity on gametophyte growth rates after 14 d, n=4. Data were $\log_e(x)$ transformed.

Factor	SS	df	F	p-value
7 d Gametophytes				
Salinity	366.872	4	2530.944	0.001
Error	7.067	195		
21 d Gametophytes				
Salinity	6.013	4	148.383	0.001
Error	0.456	45		

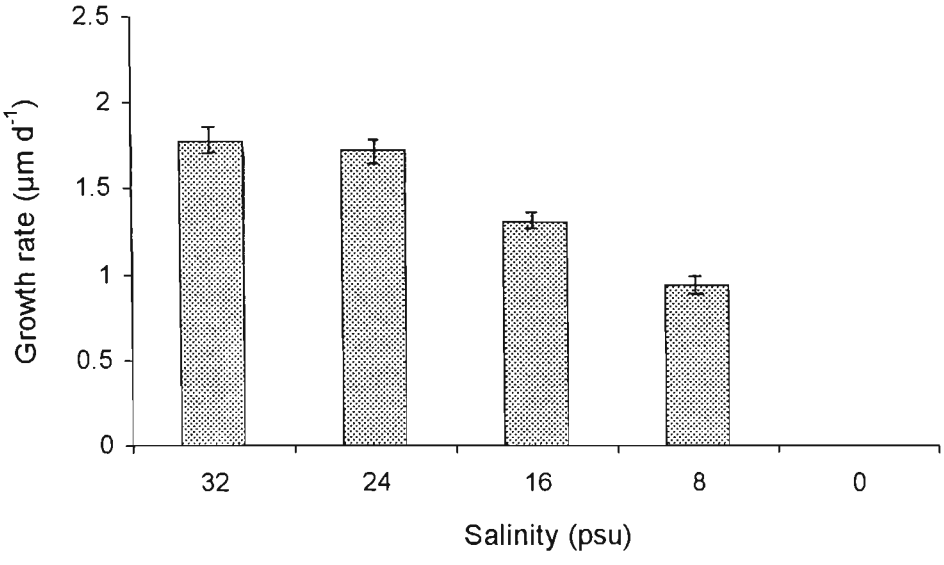

Figure 3.2. *Undaria pinnatifida* gametophyte growth rates (µm d^{-1}) after 7d at 15°C over a range of salinities. Values are means ± s.e. (n = 4).

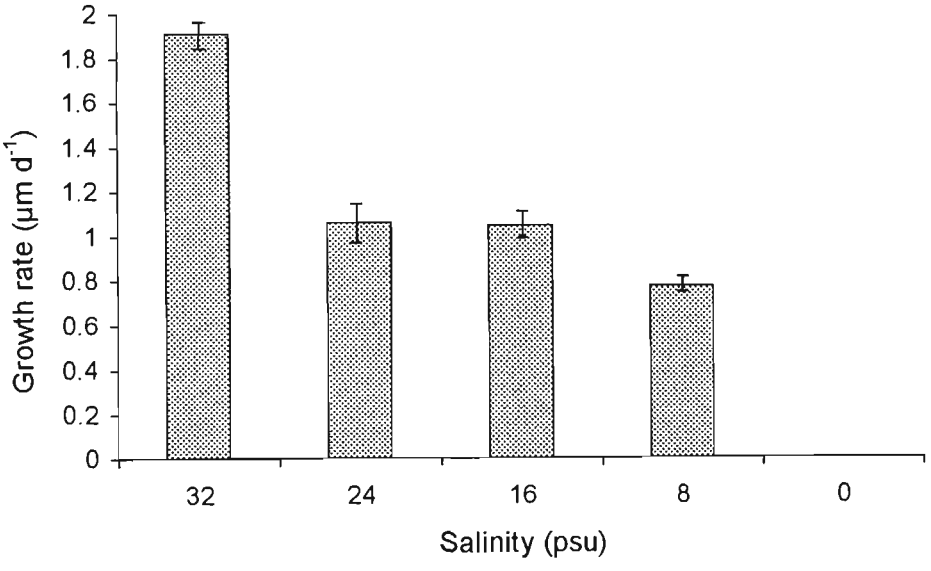

Figure 3.3. *Undaria pinnatifida* gametophyte growth rates (µm d^{-1}) after 21d at 15°C over a range of salinities. Values are means ± s.e. (n = 4).

3.3.3 Ammonium nitrogen and temperature

Over a period of 7 d, gametophyte growth was significantly (ANOVA: $F_{[5,36]}$ = 1169.49, $p < 0.001$; $F_{[5,36]}$=11.07) affected by ammonium concentration in both the low and high concentration ranges employed (Figure 3.4 & 3.5; Table 3.4). There was no interaction (Appendix 3) between ammonium concentration and temperature in the high concentration range (0 to 57.14 μM NH_4-N), due to a consistent decrease of growth rates at all temperatures as ammonium concentrations increased. In contrast, there was a significant interaction (ANOVA: $F_{[10, 36]} = 5.00$, $p < 0.001$) (Appendix 2) between ammonium concentration and temperature on gametophyte growth rate in the low concentration range of 0.45 to 7.14 μM NH_4-N (Figure 3.5; Table 3.4). This was due to the significant decline in growth rates from 0.45 to 7.14 μM NH_4-N at 20°C, while growth rates changed little across ammonium-N concentrations at 10°C or at 15°C. At 15°C, gametophyte growth rate was significantly higher at 0.45 μM NH_4-N (5.57 μm d^{-1}) compared with 7.14 μM NH_4-N (4.60 μm d^{-1}), while at 10°C there was no difference in gametophyte growth rates over 0.45 to 7.14 μM NH_4-N.

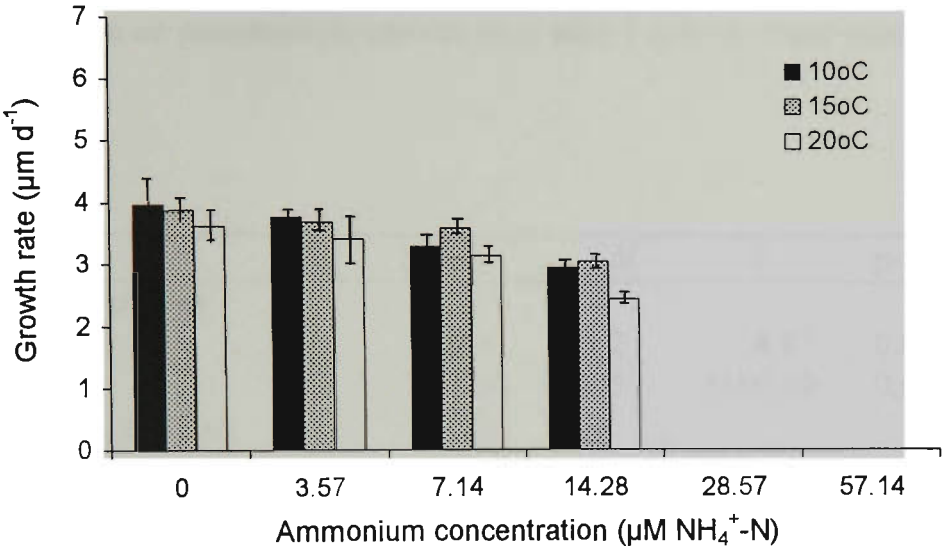

Figure 3.4. *Undaria pinnatifida* gametophyte growth rates (µm d^{-1}) after7 d over a range of temperatures and ammonium concentrations. Values are means ± s.e. (n = 3).

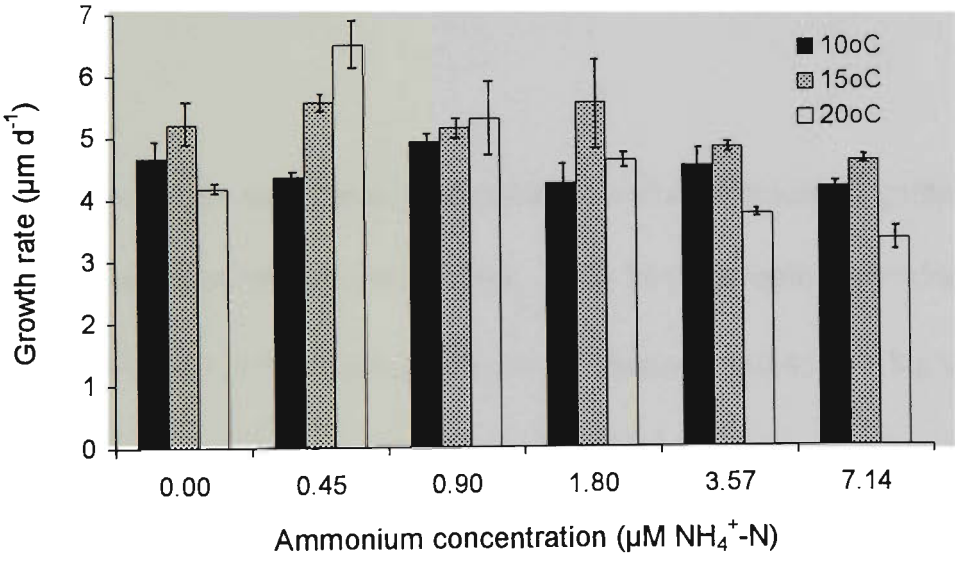

Figure 3.5. *Undaria pinnatifida* gametophyte growth rates (µm d^{-1}) after 7 d over a range of temperatures and ammonium concentrations. Values are means ± s.e. (n = 3).

Table 3.4. Two-way ANOVA on the effect of temperature and ammonium nitrogen concentration on gametophyte growth rates after 7 d, n=4. Data were $\log_e(x)$ transformed.

Factor	SS	df	F	p-value
7 d Gametophytes				
Temperature	0.044	2	4.85	0.014
Ammonium (0-57.14 μM NH_4-N)	26.423	5	1169.49	0.001
Temperature x Ammonium	0.040	10	0.885	0.555
Error	0.163	36		
7 d Gametophytes				
Temperature	0.145	2	11.31	0.001
Ammonium (0-7.14 μM NH_4-N)	0.354	5	11.07	0.001
Temperature x Ammonium	0.320	10	5.00	0.001
Error	0.230	36		

Table 3.5 summarizes the effects of temperature and ammonium on gametophyte development and maturation over 28 days. After 14 d sporophyte development was initiated at 15°C and 20°C in ammonium concentrations of 0.45 to 1.8 μM NH_4-N. In contrast, sporophyte development at 10°C was evident only after 21 d in the above range of ammonium concentrations. In the higher ammonium concentration range (3.57-57.14 μM NH_4-N) no sporophyte development occurred, gametophytes surviving up to 14 d at 10 and 15°C but not at 20°C.

Table 3.5 Gametophyte and sporophyte survival at different ammonium concentrations and temperatures over 28 days following zoospore release.

		Ammonium-N concentration (μM)								
Temp	Days	0	0.45	0.90	1.80	3.57	7.14	14.28	28.57	57.14
10°C	7	G	G	G	G	G	G	G	D	D
	14	GD	G	G	G	G	G	D		
	21	D	S	G	G	G	D			
	28		S	S	S	G				
15°C	7	G	G	G	G	G	G	G	D	D
	14	G	S	S	S	G	G	G		
	21	D	S	S	S	G	D	D		
	28		S	S	S	GD				
20°C	7	G	G	G	G	G	G	G	D	D
	14	D	S	S	S	D	D	D		
	21		S	S	S					
	28		S	S	S					

G = extant gametophytes, S = extant sporophytes, G D = gametophyte appears dying or dead, D = dead gametophytes, Blank area = experiment discontinued due to gametophyte death.

3.3.4 Photon flux density (PFD) and temperature

The two factor experiment revealed significant (ANOVA: $F_{[2,48]} = 161.08$, $p < 0.001$; $F_{[3,48]} = 17.14$, $p < 0.001$) effects of both temperature and PFD and a significant ($F_{[6,48]} = 25.25$, $p < 0.001$) (Appendix 4) interaction between temperature and photon flux density (PFD) on *Undaria pinnatifida* gametophyte growth rate after 7 d (Table 3.6; Figure 3.6) and 14 d (Table 3.6; Figure 3.7). At 7 d this interaction was explained by significantly higher gametophyte growth rates at low PFD's (10 - 30 μmol m^{-2} s^{-1}) compared to high PFD's (60 - 80 μmol m^{-2} s^{-1}) at 10°C. The highest growth rates occurred at 10°C and 30μmol m^{-2} s^{-1} (mean = 10.42 μm d^{-1}) (Figure 3.6).

After 14 d the significant ($F_{[6,48]} = 25.25$, $p < 0.001$) (Appendix 4) interaction between temperature and PFD (Table 3.6) was due to significantly higher growth rates of gametophytes grown at 10°C and 10 μmol m^{-2} s^{-1} (mean = 8.39 μm d^{-1}) compared to gametophytes grown at all other temperatures and PFD's, with the exception of gametophytes grown at 20°C and 30 μmol m^{-2} s^{-1} (mean = 7.22 μm d^{-1}). There was no effect of PFD on growth of gametophytes grown at 15°C, at 7 d or 14 d.

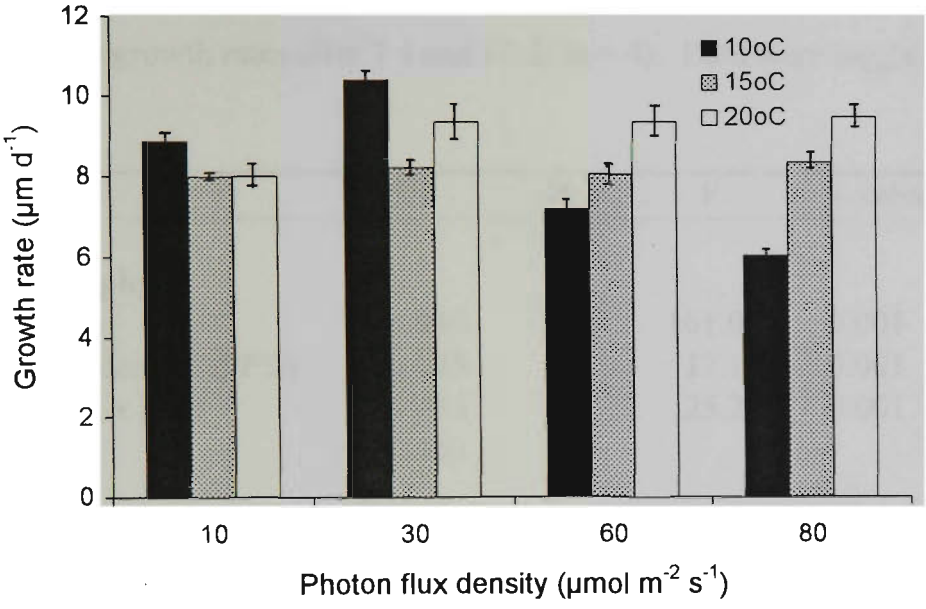

Figure 3.6. *Undaria pinnatifida* gametophyte growth rates (µm d^{-1}) at 7 d over a range of temperatures and photon flux density. Values are means ± s.e. (n = 5).

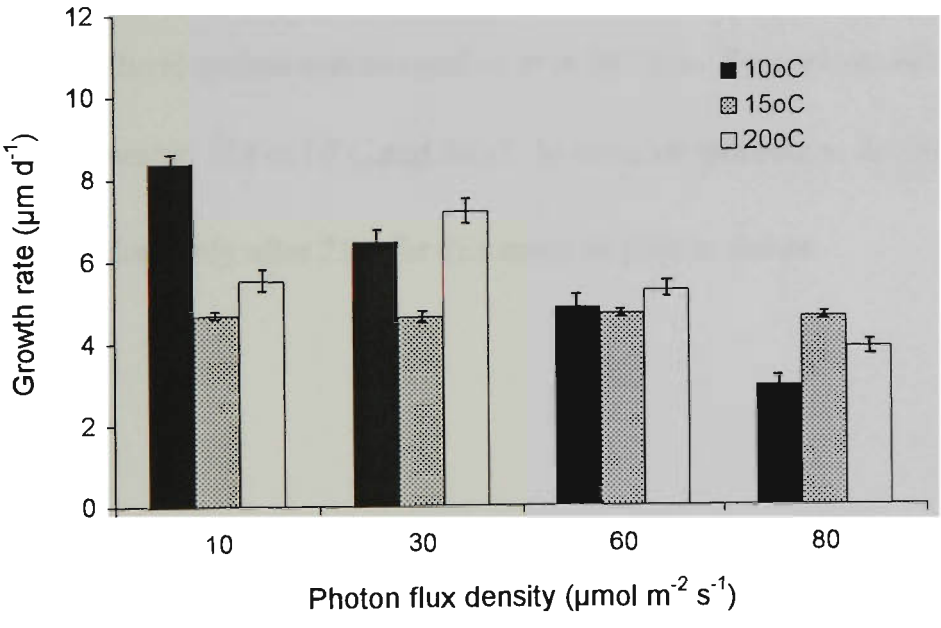

Figure 3.7. *Undaria pinnatifida* gametophyte growth rates (µm d^{-1}) at 14 d over a range of temperatures and photon flux density. Values are means ± s.e. (n = 5).

Table 3.6 Two-way ANOVA on the effect of temperature and photon flux density on gametophyte growth rates after 7 d and 14 d, (n = 4). Data were $\log_e(x)$ transformed.

Factor	SS	df	F	p-value
7 d Gametophytes				
Temperature	3.040	2	161.08	0.001
Photon flux density (PFD)	0.485	3	17.14	0.001
Temperature x PFD	1.430	6	25.25	0.001
Error	0.453	48		
14 d Gametophytes				
Temperature	2.013	2	47.06	0.001
Photon flux density (PFD)	2.703	3	42.12	0.001
Temperature x PFD	2.362	6	18.40	0.001
Error	1.027	48		

Table 3.7 summarises the effects of temperature and photon flux density (PFD) on gametophyte development and maturation over 28 days. Sporophyte development was initiated within 21d at 10°C and 20°C. In contrast sporophyte development at 15°C was evident only after 21 d for this range of photon fluxes.

Table 3.7 Gametophyte and sporophyte development at different PFD's and temperatures over 28 days following zoospore release.

		Photon Flux Density (μmol m^{-2} s^{-1})			
Temp	Days	10	30	60	80
10°C	7	G	G	G	G
	14	G	G	G	G
	21	S	S	S	S
	28	S	S	S	S
15°C	7	G	G	G	G
	14	G	G	G	G
	21	G	G	G	G
	28	S	S	S	S
20°C	7	G	G	G	G
	14	G	G	G	G
	21	S	S	S	S
	28	S	S	S	S

G = extant gametophytes

S = extant sporophytes

3.3.5 Photoperiod and temperature

There was a significant (ANOVA; $F_{[4, 36]} = 6.37$, $p < 0.001$) (Appendix 5) interactive effect between temperature and photoperiod on *Undaria pinnatifida* gametophyte growth rates after 7 d (Table 3.8; Figure 3.8) and 14 d (Table 3.8; Figure 3.9). The 7 d data showed that at 15°C gametophyte growth rates were higher at a 12 h photoperiod, while at 20°C growth rates increased with increasing photoperiod, but at 10°C the growth rates were very similar for the 3 photoperiods. The significant (ANOVA; $F_{[4, 36]} = 40.38$, $p < 0.001$) (Appendix 5) interaction between photoperiod and temperature after 14 d at 20°C continued to show increased gametophyte growth with increasing photoperiod, but no difference in growth rates between photoperiods at both 10°C and 15°C (Figure 3.9).

Table 3.8 Two-way ANOVA testing the effect of temperature and photoperiod on gametophyte growth rates after 7 d and 14 d, (n = 4). 7 d and 14 d data were $\log_e(x)$ transformed.

Factor	SS	df	F	p
7 d Gametophytes				
Temperature	2.061	2	60.40	0.001
Photoperiod	0.312	2	9.15	0.001
Temperature x Photoperiod	0.434	4	6.37	0.001
Error	0.614	36		
14 d Gametophytes				
Temperature	0.114	2	8.88	0.001
Photoperiod	0.706	2	54.89	0.001
Temperature x Photoperiod	1.039	4	40.38	0.001
Error	0.232	36		

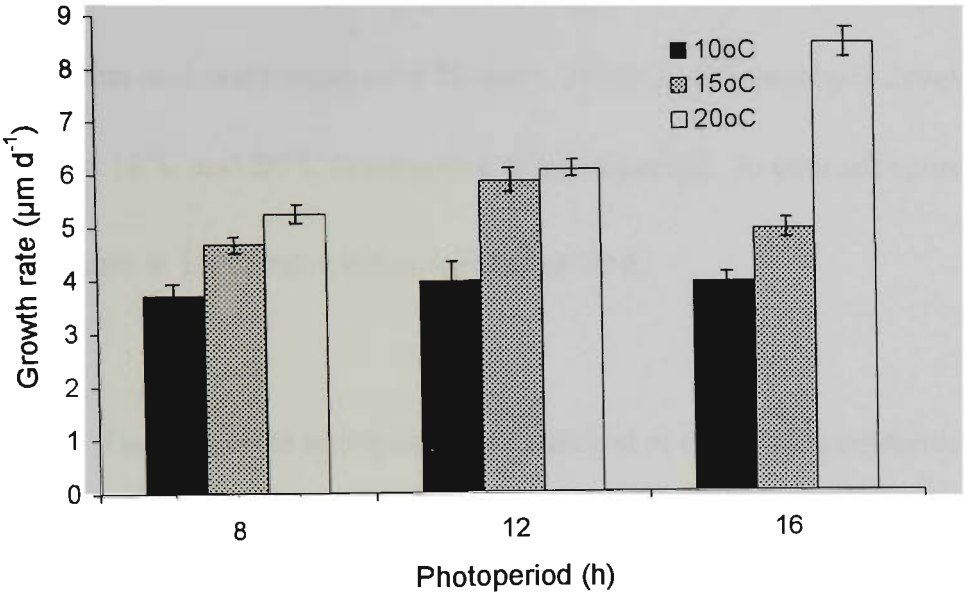

Figure 3.8. *Undaria pinnatifida* gametophyte growth rates (µm d^{-1}) at 7 d at different temperatures and photoperiods. Values are means ± s.e. (n = 5).

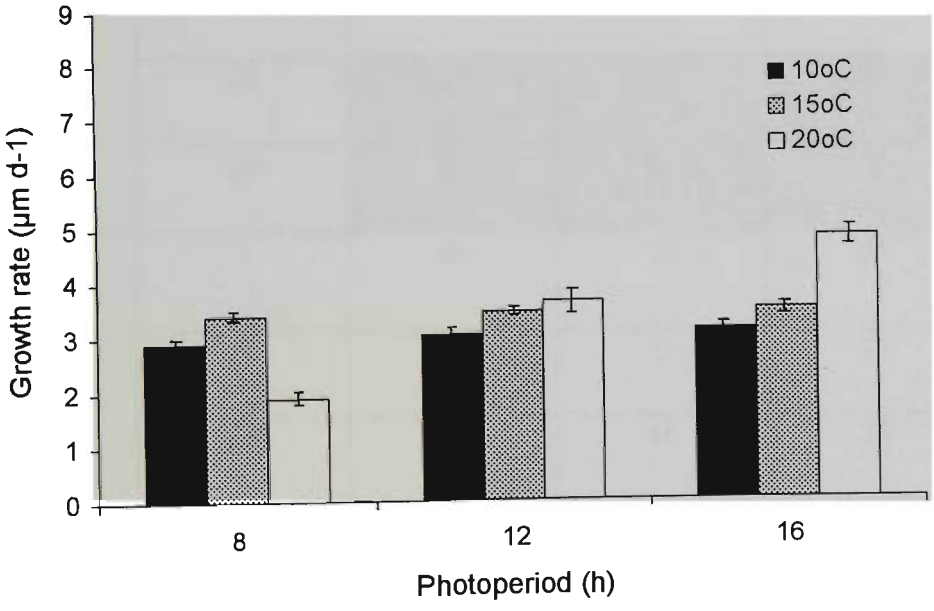

Figure 3.9. *Undaria pinnatifida* gametophyte growth rates (µm d^{-1}) at 14 d at different temperatures and photoperiods. Values are means ± s.e. (n = 5).

Table 3.9 summarises the effects of temperature and photoperiod on gametophyte development and maturation over 28 days. After 21 d sporophyte development was initiated at 10°C and 20°C irrespective of photoperiod. In contrast sporophyte development at 15°C was evident only after 28 d.

Table 3.9 Gametophyte and sporophyte survival at different temperatures and photoperiods over 28 d following zoospore release.

Temperature	No. days old	Photoperiod		
		8 hours	12 hours	16 hours
10°C	7	G	G	G
	14	G	G	G
	21	S	S	S
	28	S	S	S
15°C	7	G	G	G
	14	G	G	G
	21	G	G	G
	28	S	S	S
20°C	7	G	G	G
	14	G	G	G
	21	S	S	S
	28	S	S	S

G = extant gametophyte, S = developing sporophyte

3.4 Discussion

A major finding of this study on the introduced macroalga, *Undaria pinnatifida*, in Australia, was that growth and development of gametophytes were observed over a wide range of temperatures (5°C to 28°C), a finding consistent with previous reports on temperature tolerance of Japanese *U. pinnatifida* gametophytes from -1°C to 30°C (tom Dieck 1993, Saito 1975, Akiyama 1965, Saito 1956a). The optimal growth of *U. pinnatifida* gametophytes at 10°C and 15°C is also consistent with the range of temperatures for optimal growth of warm temperate algae (Lüning 1990b), but these temperatures are lower than those reported for optimal growth of *U. pinnatifida* gametophytes from Japan (i.e. 15°C to 24°C) (Saito 1975, Akiyama 1965, Saito 1956a).

The difference in optimal gametophyte growth between strains of *Undaria pinnatifida* in Port Phillip Bay and Japan could be attributed to genotypic variation or acclimation of algal spores to water temperature at the time of collection. It has been suggested that algae adapt to changing temperatures by altering the concentrations of certain enzymes or by the introduction of isoenzymes with different temperature dependencies (Küppers, 1980, Lüning and Neushul 1978). This may also account for any differences in growth rates and in the onset of gametogenesis between experiments under similar conditions. Within-species variation in optimal temperatures for gametophyte growth, development and reproduction have been reported for other laminarian species, such as *Laminaria saccharina* (Lee and

Brinkhuis 1988), *Ecklonia radiata* (Novaczek 1984b) and *Sphaerotrichia divaricata* (Peters and Breeman 1992).

The effect of temperature on photosynthesis may explain the differences in gametophyte growth rates over the range of temperatures employed. At low temperatures, such as 5°C, photosynthesis and other metabolic activities decrease (Lobban et al. 1985a), while high temperatures denature enzymes, inhibiting enzyme activity and photosynthetic capacity (Lüning 1990b, Lobban et al. 1985a). Conversely it has been suggested that the activity of photosynthetic enzymes, such as ribulose-1,5-biphosphate carboxylase oxygenase (Rubisco) and other Calvin Cycle enzymes, are not influenced by changes in temperature at low temperatures (Davison et al. 1991). This would enable growth of temperate macroalgae such as *U. pinnatifida* to be maintained at relatively low temperatures which they are commonly exposed to.

Retarded development of *Undaria pinnatifida* gametophytes at salinities below 24‰ is consistent with previous reports for the Laminariales (Yabu 1964) and other macroalga such as *Chorda filum* (Norton and South 1969). In contrast, a previous study on *U. pinnatifida* gametophytes reported retarded growth at salinities lower than 15‰, particularly at temperatures greater than 22°C (Saito 1975). Saito, 1975 does not provide information on which temperatures gametophytes were cultured and therefore it is possible the deviation in salinity effects between this study and the present study is due to differences in temperature at which cultures were maintained, with higher temperatures possibly allowing a greater tolerance to salinity reduction.

Reduced salinity suppresses the growth of macroalgae by altering water potential and ion movement, cell turgor and osmotic controls (Norton and South 1969). The decline in gametophyte growth rates with decreasing salinity may be associated with these effects which utilize carbon supplies (CO_2 and HCO_3^-) to produce osmolyte's, such as mannitol, otherwise used for growth (Lobban et al. 1985b, Gessner and Schramm 1971). Low salinities have also been implicated in the suppression of photosynthesis in a number of macroalgae such as *Halymenia floresia* (Gessner 1971), *Fucus virsoides*, *Ulva lactuca*, *Porphyra luecosticta* and *Wrangelia penicillata.* (Zavodnik 1975). It has been suggested that mannitol and the ionic composition of algal cells may be involved in the photosynthetic performance of some macroalgae at low salinities (Zavodnik 1975, Gessner and Schramm 1971). The relatively low salinity-related EC_{50} of *U. pinnatifida* gametophyte growth may therefore be attributed to the ionic composition of the culture medium, supplying ions which assist osmoregulation.

Undaria pinnatifida gametophytes did not survive beyond 21d in the absence of nitrogen (as ammonium) suggesting that nitrogen is essential to gametophyte growth (De Boer 1981). Nitrogen availability is likely to have a substantial impact on the metabolism of macroalgae, as it is an essential plant nutrient (Hanisak 1983), influencing the photosynthetic capacity (Pedersen 1995, McGlathery 1992, Lapointe 1987, Küppers and Weidner 1980) and growth (Pedersen 1995, Kuwabara and North 1980, Hanisak 1979, Topinka and Robbins 1976) of macroalgal species. It is possible that low nitrogen availability in Port Phillip Bay during summer could limit *U.*

pinnatifida gametophyte growth and contribute to the absence of sporophytes during this period.

The decline in *Undaria pinnatifida* gametophyte growth rates with increasing ammonium concentration and the mortality of gametophytes exposed to high ammonium concentrations (≥400 μg L^{-1} N) indicates that NH_4 was toxic to *U. pinnatifida* in high concentration, a toxic response to ammonium. Toxic effects of ammonium have been reported for many macroalgae, such as *Ulva lactuca* (Waite and Mitchell 1972), *Gracilaria sordida* (Laing et al. 1989), *Fucus vesiculosus* (Prince 1974) and microalgae, such as *Phaedodactylum tricornutum* and *Dunaliella tertiolecta* (Azov and Goldman 1982). Ammonium uptake may also reduce photosynthesis by diverting ATP (i.e. energy) away from the production of metabolites necessary for photosynthetic CO_2 fixation (Calvin Cycle) towards nitrogen assimilation and production of amino acids (Turpin 1983, Azov and Goldman 1982, Waite and Mitchell 1972, Elrifi and Turpin 1986). It has also been suggested that increased ammonium concentrations inhibit uptake of other nutrients (e.g. NO_3, PO_4) (Waite and Mitchell 1972) further contributing to a decrease in growth rates in macroalgae.

At 10°C to 15°C growth rates remain relatively unaffected by increased ammonium concentrations (up to 7.14 μM), possibly because the nitrogen taken up by the alga meets its requirements for growth. At 20°C however, nitrogen uptake would be higher and exceed growth requirements, and saturated cellular ammonium concentrations may become toxic to metabolic activities. Although nitrogen is an important element

for amino acid and enzyme production (Wheeler and Weidner 1983) at high temperatures the rate of nitrogen uptake increases (Riccardi and Solidoro 1996, Harrison 1985, Lobban et al. 1985a, Wheeler and Weidner 1983, De Boer 1981, Küppers and Weidner 1980) and excessive ammonium accumulation may inhibit the activity of enzymes and reduce growth. At relatively low ammonium concentrations (i.e. 0.45μM) an opposite trend was observed as an increase in temperature and nitrogen uptake is likely to satisfy the growth demands of the alga. Similar observations on the interaction of temperature and nitrogen and their influence on sporophyte growth in *Laminaria saccharina* have been attributed to the effect of temperature on nutrient uptake (Wheeler and Weidner 1983).

Light availability also affected the growth of gametophyte cultures with growth rates over the 14 d period remaining high at low PFD. The elevated growth of *Undaria pinnatifida* gametophytes observed at low PFD may contribute to their survival and development during periods of low light during winter and from canopy shading of adult sporophytes. These photo-adaptive responses are consistent with the characterization of laminarian gametophytes as 'extreme shade plants' (Lee and Brinkhuis 1988, Lüning and Neushul 1978) and with reports of optimal gametophyte growth and photosynthesis at low light (Lee and Brinkhuis 1988, Novaczek 1984a, Deysher and Dean 1984, Lüning and Neushul 1978, Vadas 1972, Anderson and North 1969, Kain 1964).

Conversely, *Undaria pinnatifida* gametophyte maturation and sporophyte production has been reported to improve with increasing PFD (Saito 1975, Akiyama 1965, Saito

1956b). Akiyama (1965) found that *U. pinnatifida* gametophyte growth and maturation increased with increasing irradiance from 570 to 4,000 lux (approximately 11 to 80 $\mu E\ m^{-2}\ s^{-1}$), but no information on other conditions of temperature or photoperiod were provided. Saito (1975) also reported that high PFD's promoted the maturation of *U. pinnatifida* gametophytes but that optimal growth varied with temperature (Saito 1956a).

The interactive effect of light and temperature on gametophyte growth may be explained in terms of their effect on photosynthesis. At low PFD's the principal enzymes involved in photosynthesis (e.g. ribisco) acclimatize to changing temperatures and remain efficient at low temperatures. Hence, light becomes the limiting factor controlling photosynthesis and is sustained independent of temperature (Davison et al. 1991). Such mechanisms may account for high *Undaria pinnatifida* gametophyte growth at low PFD, as spores and gametophytes acclimatize to low temperatures found in Port Phillip Bay. In addition, low PFD's are reported to enhance pigment production in a number of algal species, with consequent increases in rates of photosynthesis and growth (Rosenburg et al. 1995, Healey 1985, Rosenburg and Ramus 1982, Ramus 1981, Ramus et al. 1976a, Ramus et al. 1976b). These attributes would allow *U. pinnatifida* gametophytes to grow and reproduce at low temperatures and utilize low light arising from shorter daylengths and canopy shading from adult sporophytes.

The maturation of *Undaria pinnatifida* gametophytes irrespective of daylength is consistent with many reports for the Laminariales, where photoperiod has no effect on

gametogenesis (Novaczek 1984a, Deysher and Dean 1984, Lüning 1980a). In agreement, Akiyama(1965) found that gametogenesis in *U. pinnatifida* could be initiated under long or short daylengths. Differences in gametophyte growth rates found in this study at different photoperiods can be accounted for by the effect of quantum dose of light on photosynthesis, rather than photoperiod alone. Novaczek (1984a) also showed gametophyte growth rates for *Ecklonia radiata* increased at low photon flux density when subjected to long daylength, responding to the quantity of light received rather than photoperiod.

The effect of photoperiod on gametophyte growth rates is also influenced by temperature. At 10°C the absence of any photoperiod effect on growth may be due to acclimation of photosynthetic enzymes to low temperature, as explained previously (Davison et al. 1991). At 20°C, however, enhanced gametophyte growth at the 16 h photoperiod is likely to be due to increased enzyme activity, enhancing growth of gametophytes and the utilization of the higher light quantity (long day length). Elevated growth rates of gametophytes at 20°C and 16 h of light, however, did not induce early gametogenesis. Akiyama (1965) also found that growth of *Undaria pinnatifida* gametophytes was optimal under a long photoperiod, while gametogenesis occurred independent of daylength. These findings support suggestions that the environmental factors influencing gametophyte growth and gametogenesis in the Laminariales are controlled by separate developmental pathways, each with different physiological requirements (Kain 1979, Lüning and Neushul 1978).

Further studies investigating the responses of *Undaria pinnatifida* gametophytes to photoperiod and PFD are necessary to distinguish photoperiodic responses from responses to quantum dose of light. Such studies should include night-break regimes since many photoperiodic responses are actually responding to the period of darkness (Lüning 1980a). Further studies on blue light requirements for gametogenesis may also contribute further to our understanding of this subject (Lüning 1980b, Lüning and Dring 1975).

3.4.1 Conclusion

The present study has shown that gametophyte growth occurs at 20°C, given sufficient light availability, however high growth rates are possible at low temperatures and low light availability, provided nitrogen is in adequate supply. These characteristics allow *Undaria pinnatifida* to exhibit rapid growth during winter and invade nutrient enriched sites in Port Phillip Bay. A major factor regulating gametophyte growth in *U. pinnatifida* is temperature, which is consistent with other reports on *U. pinnatifida* (Saito 1975) and other laminarian species (Lee and Brinkhuis 1988, Lüning 1980b). Temperature tolerance of *U. pinnatifida* gametophytes is amongst the widest (< 0°C to 30°C) reported for the Laminariales (tom Dieck 1993), permitting this macroalga to invade a wide variety of temperate environments and survive warm temperate summers.

Chapter 5

Synthesis

1.1 Summary of Findings

The three aims of this study were :-

1. To examine the physico-chemical parameters that control zoospore germination in *Undaria pinnatifida*;
2. To examine the physico-chemical parameters that control gametophyte growth and reproduction in *Undaria pinnatifida*;

Undaria pinnatifida zoospores showed substantive resilience to a range of physico-chemical conditions in the laboratory. Germination was possible at the range of salinity concentrations found in Port Phillip Bay (28-32 PPS) and was not affected by ammonium concentrations encountered in Port Phillip Bay waters (0-30 μM NH_4-N). Germination was also found to be successful over the range of temperatures found in Port Phillip Bay (i.e. 10°C to 25°C) and is likely to be limited if temperatures exceed this range. Because of the suitability for germination of the environmental conditions found in Port Phillip Bay and therefore *U. pinnatifida* has the potential for further spread from its current distribution in the northern part of Port Phillip Bay. Viable spore release and germination are crucial for the establishment, growth and reproduction of gametophyte and sporophyte stages and are more likely to be of ecological significance than the subsequent development of the germination tube,

which may be retarded but nevertheless result in the formation of a healthy sporophyte.

The initial growth of the germination tube was resilient to the range of salinities in Port Phillip Bay but elevated ammonium concentrations (>28 μM) encountered near sewage outfalls and riverine inputs may limit germination tube growth. An interaction between photon flux and temperature suggests that germination tube growth at low temperatures is favoured by low light conditions, consistent with the ability of *U. pinnatifida* to establish during winter. The ability of germination tubes to grow at low light suggests that the initial growth of the germination tube occurs independent of photosynthesis, and that germination tube growth is reliant on storage products from the zoospore (Kain 1964). Finally, reduced growth of the germination tube at high temperatures (> 20°C) suggests that any future increase in water temperature in Port Phillip Bay may limit the geographical distribution of *U. pinnatifida.*

Conditions that permit *Undaria pinnatifida* gametophyte growth and gametogenesis appear to follow seasonal patterns of temperature, nitrogen ammonium concentrations and photoperiod in PPB. The optimal growth and reproduction of gametophytes at low temperatures, low light and high nutrient availability characterizes *U. pinnatifida* as a winter annual able to take advantage of high nutrient concentrations. The response of *U. pinnatifida* gametophyte growth in culture to photoperiod is possibly due to an increase in available light and therefore further studies are necessary to distinguish photoperiodic responses from responses to quantum dose of light.

In Port Phillip Bay the life cycle and growth of *Undaria pinnatifida* is typical of a warm temperate climate with a distinct sporophyte growth period during winter and spring, and a resting gametophyte stage over summer. Its reproductive capacity coincided with changes in daylength and temperature. Germination of spores occurred when temperatures were low and daylength short. High temperatures appear to inhibit gametophyte development and hence gametogenesis and sporophyte growth over summer, although genetic factors undoubtedly control the senescence of sporophytes that dictate a sporophyte longevity of less than one year (Tsutsui and Ohno 1993, Koh and Shin 1990). This is in contrast with the dynamics of *U. pinnatifida* populations from cool temperate waters where sporophyte generations are present year round.

1.2 Concluding remarks

This study showed that *Undaria pinnatifida* exhibits a distinct seasonal life cycle in Port Phillip Bay, characterized by the appearance of sporophytes in late autumn and their disappearance during summer. It is an opportunistic colonizer capable of high rates of reproduction and fast growth rates producing high density populations. During winter and spring it is the dominant macroalgal species at the site of invasion in Port Phillip Bay. Its impact on the ecology of temperate reef communities is yet unknown but it appears to displace other native macrophytes which are a food source for local marine fauna such as urchins, abalone (Fleming 1995). A series of experimental investigations showed that spores, germlings and gametophytes of *Undaria pinnatifida* tolerated a wide range of temperatures, photon fluxes and

ammonium concentrations, undoubtedly contributing to its ability to persist in Port Phillip Bay. If precautionary measures are not taken this seaweed is likely to spread throughout the bay.

This thesis provides the first detailed study in Australia of the environmental factors that control the growth and reproduction of the different life stages of *Undaria pinnatifida*. Considerable recent interest on the introduction of exotic marine species in Port Phillip Bay (Campbell and Hewitt 1999) has clearly demonstrated that there is little known about the impacts of exotic seaweeds on marine communities. In order to control the potential impacts and further spread of *U. pinnatifida* in Australia it is necessary to understand the factors controlling the growth and reproduction of this invasive species. This thesis is a preliminary step towards this goal.

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APPENDIX 1

Nutrient Enriched Seawater

To make up 1 litre:- 10 ml Nutrient Enrichment Stock Solution
1 ml Germanium Dioxide Stock Solution
0.45 ml 1M Nitrogen Stock Solution

Add the above to a 1 litre volumetric flask
Make up to 1 litre with 0.2μm filtered seawater
Mix

NB. GeO_2 is added to give a final concentration of 0.175mg l^{-1} which has been found to control diatom growth without an observable effect on gametophyte growth (Markham & Hagmeier 1982)

Nutrient Enrichment Stock Solution

To make 1 litre:-

1.28g Sodium phosphate ($NaH_2PO_4.2H_2O$)
(Sodium dihydrogen orthophosphate)

0.266g Sodium EDTA ($Na_2EDTA.2H_2O$)
(Ethylene diamine tetra-acetic acid)

0.118g Sodium citrate ($Na_3C_6H_5O_7.2H_2O$)
(Trisodium citrate)

0.097g Ferrous sulfate ($FeSO_4.7H_2O$)
(Iron II sulfate)

Dissolve the above in approximately 800ml 0.2μm filtered seawater
Add 10ml Vitamin Stock Solution (thawed)
Make up to 1 litre in a volumetric flask
Mix thoroughly

Vitamin Stock Solution

To make up 500 ml:- 9.75g Thiamine HCl

0.005g Biotin

0.005g B_{12}

Dissolve the above in approximately 400ml of 0.2µm filtered seawater

Make up to 500ml in a volumetric flask

Dispense into 10ml aliquots and store at -18°C

Nitrogen Stock Solution (1M N)

To make 100ml:- 1.4g Ammonium chloride (NH_4Cl)

Dissolved in 100ml of filtered seawater

Germanium Dioxide Stock Solution (175mg l^{-1} GeO_2)

To make 100ml:-

0.0175g Germanium dioxide (GeO_2)

(Germanium (IV) oxide)

Dissolved in 100ml 0.2µm filtered seawater

APPENDIX 2

Table 2a. Treatments for ammonium (0 - 57.14 μM NH_4^+-N) and temperature (°C) experiments on *Undaria pinnatifida* gametophyte for 7 days

Treatment	Temperature	Ammonium (μM NH_4^+-N)
1	10°C	0
2		3.57
3		7.14
4		14.28
5		28.57
6		57.14
7	15°C	0
8		3.57
9		7.14
10		14.28
11		28.57
12		57.14
13	20°C	0
14		3.57
15		7.14
16		14.28
17		28.57
18		57.14

Table 2b. Tukeys post-hoc test results for ammonium (0 - 57.14 μM NH_4^+-N) and temperature (°C) effects on *Undaria pinnatifida* gametophyte growth rates at 7 days. (Matrix of pairwise comparison probabilities, p values)

Treatment	1	2	3	4	5
1	1.000				
2	1.000	1.000			
3	0.474	0.869	1.000		
4	0.023	0.109	0.989	1.000	
5	0.000	0.000	0.000	0.000	1.000
6	0.000	0.000	0.000	0.000	1.000
7	1.000	1.000	0.656	0.046	0.000
8	1.000	1.000	0.966	0.210	0.000
9	0.996	1.000	0.997	0.387	0.000
10	0.076	0.286	1.000	1.000	0.000
11	0.000	0.000	0.000	0.000	1.000
12	0.000	0.000	0.000	0.000	1.000
13	0.997	1.000	0.996	0.363	0.000
14	0.737	0.979	1.000	0.910	0.000
15	0.173	0.513	1.000	1.000	0.000
16	0.000	0.000	0.029	0.539	0.000
17	0.000	0.000	0.000	0.000	1.000
18	0.000	0.000	0.000	0.000	1.000

Treatment	6	7	8	9	10
6	1.000				
7	0.000	1.000			
8	0.000	1.000	1.000		
9	0.000	1.000	1.000	1.000	
10	0.000	0.139	0.471	0.703	1.000
11	1.000	0.000	0.000	0.000	0.000
12	1.000	0.000	0.000	0.000	0.000
13	0.000	1.000	1.000	1.000	0.677
14	0.000	0.880	0.998	1.000	0.994
15	0.000	0.290	0.723	0.902	1.000
16	0.000	0.000	0.000	0.001	0.255
17	1.000	0.000	0.000	0.000	0.000
18	1.000	0.000	0.000	0.000	0.000

Treatment	11	12	13	14	15	16	17
11	1.000						
12	1.000	1.000					
13	0.000	0.000	1.000				
14	0.000	0.000	1.000	1.000			
15	0.000	0.000	0.886	1.000	1.000		
16	0.000	0.000	0.001	0.010	0.119	1.000	
17	1.000	1.000	0.000	0.000	0.000	0.000	1.000
18	1.000	1.000	0.000	0.000	0.000	0.000	1.000

APPENDIX 3

Table 3a. Treatments for ammonium (0 - 7.14 μM NH_4^+-N) and temperature (°C) experiments on *Undaria pinnatifida* gametophyte for 7 days.

Treatment	Temperature	Ammonium (μM NH_4^+-N)
1	10°C	0
2		0.45
3		0.90
4		1.80
5		3.57
6		7.14
7	15°C	0
8		0.45
9		0.90
10		1.80
11		3.57
12		7.14
13	20°C	0
14		0.45
15		0.90
16		1.80
17		3.57
18		7.14

Table 3b. Tukeys post-hoc test results for ammonium (0 - 7.14 μM NH_4^+-N) and temperature (°C) effects on *Undaria pinnatifida* gametophyte growth rates for 7 days. (Matrix of pairwise comparison probabilities, p values)

Treatment	1	2	3	4	5
1	1.000				
2	0.922	1.000			
3	1.000	0.987	1.000		
4	1.000	0.513	0.997	1.000	
5	0.924	0.078	0.760	0.999	1.000
6	0.314	0.998	0.551	0.057	0.002
7	0.904	1.000	0.982	0.474	0.067
8	0.970	1.000	0.997	0.655	0.132
9	1.000	0.939	1.000	1.000	0.905
10	0.000	0.003	0.000	0.000	0.000
11	0.021	0.714	0.063	0.001	0.000
12	0.537	1.000	0.775	0.139	0.009

Treatment	6	7	8	9	10	11	12
6	1.000						
7	0.999	1.000					
8	0.991	1.000	1.000				
9	0.350	0.923	0.978	1.000			
10	0.090	0.004	0.001	0.000	1.000		
11	0.998	0.749	0.576	0.026	0.640	1.000	
12	1.000	1.000	1.000	0.578	0.034	0.977	1.000

APPENDIX 4

Table 4a. Treatments for PFD (μmol m^{-2} s^{-1}) and temperature (°C) experiments on *Undaria pinnatifida* gametophyte for 7 and 14 days.

Treatment	Temperature	PFD (μmol m^{-2} s^{-1})
1	10°C	10
2		30
3		60
4		80
5	15°C	10
6		30
7		60
8		80
9	20°C	10
10		30
11		60
12		80

Table 4b. Tukeys post-hoc test results for PFD (μmol m^{-2} s^{-1}) and temperature (°C) effects on *Undaria pinnatifida* gametophyte growth rates at 7 days. (Matrix of pairwise comparison probabilities, p values)

Treatment	1	2	3	4	5
1	1.000				
2	0.000	1.000			
3	0.000	0.000	1.000		
4	0.000	0.000	0.000	1.000	
5	0.000	0.000	0.000	1.000	1.000
6	0.000	0.000	0.002	1.000	0.979
7	0.000	0.000	0.000	1.000	1.000
8	0.000	0.000	0.027	0.984	0.675
9	0.214	0.000	0.163	0.000	0.000
10	0.935	0.104	0.000	0.000	0.000
11	0.972	0.065	0.000	0.000	0.000
12	0.613	0.390	0.000	0.000	0.000

Treatment	6	7	8	9	10	11	12
6	1.000						
7	1.000	1.000					
8	1.000	0.949	1.000				
9	0.000	0.000	0.000	1.000			
10	0.000	0.000	0.000	0.001	1.000		
11	0.000	0.000	0.000	0.003	1.000	1.000	
12	0.000	0.000	0.000	0.000	1.000	1.000	1.000

Table 4c. Tukeys post-hoc test results for PFD (μmol m^{-2} s^{-1}) and temperature effects on *Undaria pinnatifida* gametophyte growth rates at 14 days. (Matrix of pairwise comparison probabilities, p values)

Treatment	1	2	3	4	5
1	1.000				
2	0.000	1.000			
3	0.000	0.000	1.000		
4	0.000	0.000	0.000	1.000	
5	0.000	0.000	0.002	0.000	1.000
6	0.000	0.000	0.000	0.000	1.000
7	0.000	0.000	0.004	0.000	1.000
8	0.000	0.000	0.001	0.000	1.000
9	0.000	0.046	0.278	0.000	0.000
10	0.160	0.676	0.000	0.000	0.000
11	0.000	0.005	0.697	0.000	0.000
12	0.000	0.000	0.005	0.000	1.000

Treatment	6	7	8	9	10	11	12
6	1.000						
7	1.000	1.000					
8	1.000	1.000	1.000				
9	0.000	0.000	0.000	1.000			
10	0.000	0.000	0.000	0.000	1.000		
11	0.000	0.000	0.000	1.000	0.000	1.000	
12	1.000	1.000	1.000	0.000	0.000	0.000	1.000

APPENDIX 5

Table 5a. Treatments for photoperiod (h) and temperature (°C) experiments on *Undaria pinnatifida* gametophyte for 7 and 14 days.

Treatment	Temperature	Photoperiod (h)
1	10°C	8
2		12
3		16
4	15°C	8
5		12
6		16
7	20°C	8
8		12
9		16

Table 5b. Tukeys post-hoc test results for photoperiod (h) and temperature (°C) effects on *Undaria pinnatifida* gametophyte growth rates at 7 days. (Matrix of pairwise comparison probabilities, p values)

Treatment	1	2	3	4	5
1	1.000				
2	0.982	1.000			
3	0.857	1.000	1.000		
4	0.000	0.001	0.003	1.000	
5	0.000	0.000	0.000	0.000	1.000
6	0.000	0.000	0.000	0.961	0.008
7	0.000	0.000	0.000	0.205	0.356
8	0.000	0.000	0.000	0.000	0.992
9	0.000	0.000	0.000	0.000	0.000

Treatment	6	7	8	9
6	1.000			
7	0.907	1.000		
8	0.000	0.037	1.000	
9	0.000	0.000	0.000	1.000

Table 5c. Tukeys post-hoc test results for photoperiod (h) and temperature (°C) effects on *Undaria pinnatifida* gametophyte growth rates at 14 days. (Matrix of pairwise comparison probabilities, p values)

Treatment	1	2	3	4	5
1	1.000				
2	0.987	1.000			
3	0.826	1.000	1.000		
4	0.019	0.276	0.656	1.000	
5	0.005	0.114	0.387	1.000	1.000
6	0.002	0.061	0.253	1.000	1.000
7	0.000	0.000	0.000	0.000	0.000
8	0.001	0.037	0.176	0.997	1.000
9	0.000	0.000	0.000	0.000	0.000

Treatment	6	7	8	9
6	1.000			
7	0.000	1.000		
8	1.000	0.000	1.000	
9	0.000	0.000	0.000	1.000