Fucus distichus L. and Related Species In the British Isles in Relation to Sea Temperature Change

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Abstract

Seawater temperature rise may have adverse effects on *Fucus* species in the British Isles with changes in distribution, abundance or loss of northern species. The use of the *F. distichus* complex as an indicator of sea temperature rise is investigated. Plants were cultured at 5, 10 and 15ºC to investigate the temperature tolerances in both juvenile and adult life stages of various *Fucus* species and taxa from different geographic locations. Head, rhizoid and total length of germlings were measured. Surface area, wet (fresh) weight, photosynthesis and respiration measurements were taken for apical tips of adult plants. Extra growth, regeneration and formation of receptacles was noted. Phenotypic variations between populations of the subspecies *anceps* from Scotland and Ireland were examined.

Germlings of Orkney species *F. distichus* subsp. *anceps*, *F. serratus*, *F. spiralis* and *F. vesiculosus* grew best at 15ºC; *F. vesiculosus* var. *linearis* initially best at 15ºC, changing thereafter to 10ºC; *F. spiralis* f. *nanus* grew best at 5ºC. *F. distichus* subsp. *anceps* from Ireland grew best at 10ºC. In Orkney adult plants optimum temperature for growth was 15ºC for *anceps* and *serratus*, 10ºC and 15ºC for *spiralis*, *vesiculosus*, *nanus* and *linearis*. In Ireland populations, adult plants of *anceps* and *linearis* showed a similar response to temperature with no optimum temperature preference for growth. *F. distichus* subsp. *edentatus* from Moray Firth grew best at 5ºC. No temperature preference was noted for *edentatus* from Shetland. In *serratus*, *spiralis* and *vesiculosus* from South Queensferry, Firth of Forth, only *spiralis* showed a temperature preference, at 10ºC. Formation of receptacles, new growth and regeneration was present on apical tips of Orkney *anceps* cultivated at 5ºC. Reproductive tips were noted at temperatures of 5, 10, 15ºC in Ireland *anceps*.

Key findings were:

- The effects of different temperatures on growth rates of head, rhizoid or both and total length varied between *Fucus* species and within species.
- Ecophysiological preferences may be different between the geographically different populations of *anceps* and *linearis* from Orkney and Ireland.
- Populations of *anceps* from Scotland and Ireland showed no evidence of two discrete phenotypic entities. However differences were seen with respect to size and form and aspects of reproduction with smaller oogonia in *anceps* from Ireland.

The use of the subsp. *anceps* from Ireland as an indicator of climate change and sea temperature rise in the British Isles seems appropriate. In Britain, increased sea temperatures may not directly determine the distribution of the subsp. *edentatus*, distribution possibly determined by nutrient enrichment in seawater and/or daylength. There is little evidence to suggest that sea temperature rise and climate change will have any immediate effect on the distribution of the other *Fucus* species investigated.

Dedication

This work is dedicated to family and friends for their undying support throughout my years of study. In particular I wish to thank the women in the Jefferson clan, my grandmother, mother and sisters, for their resilience, sense of fun and humour and love for life.

Acknowledgements

I would like to thank a number of people who have made this thesis possible. To the teaching and technical staff for their help and support and my colleagues in the department who have made my time at Heriot-Watt so enjoyable. I wish to thank Professor Martin Wilkinson for his support, advice, help and never ending enthusiasm but particularly his friendship over the years.

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ºC	degrees centigrade
(º)	degree of angle
%	percent
s	second
min	minute
hrs	hours
d	day
μm	micrometre ($m \times 10^{-6}$)
mm	millimetre ($m \times 10^{-3}$)
cm	centimetre ($m \times 10^{-2}$)
ml	millilitre (1×10^{-3})
mg	milligramme
l	litre
g	gramme
PAR	Photosynthetically Active Radiation
CT	Constant Temperature

Chapter 1: Introduction

Several marine species and taxa have been put forward as possible indicators of climate change with respect to sea temperature rise affecting their geographical distribution. One such species is the brown seaweed *Fucus distichus* L. emend. Powell and its subspecies *anceps* and *edentatus*. This sub-arctic species only occurs at a few localities in the British Isles and is described by Scottish Natural Heritage (SNH) as "species with a limited geographical range whose distribution may change due to global warming" (Hiscock *et al*., 2001). Both subspecies are designated as northern plants which may either decrease in abundance or disappear in or near Scotland. The Joint Nature Conservation Committee (JNCC) states warming is likely to see the disappearance of *Fucus distichus* L. from the British Isles and predicts the decline of *Fucus vesiculosus* in moderately wave exposed sites in the west and south with increasing temperatures (Burrows *et al*., 2009). *Fucus serratus* and *Fucus spiralis* are also flagged as possible candidates as climate indicator species. *Fucus distichus* L. also appears in the current UK List of Priority Species and Habitats (Biodiversity Reporting and Information Group, 2007) which highlights the need for research and monitoring for marine species and states it is likely to become extinct in the British Isles in the next 10 years.

The aim of this project is to investigate how seawater temperatures affect the establishment, growth, survival and distribution of various *Fucus* species and taxa found at different geographical locations in the British Isles. In this respect experiments will be performed on both juvenile and adult life stages as conditions for the development of sporelings of fucoids may not be the same as for mature plants (Knight and Parke, 1950) and evalution of the effects of temperature on one life phase alone will be of limited value in identifying any factors affecting distribution (Lüning, 1980; Chapman, 1995). Temperature preferences inferred from the laboratory experiments will be compared in relation to local sea temperatures for the species investigated and other factors that may be involved in the distribution of *Fucus* spp. will be discussed.

Fucus distichus is a distinctly northern species in Europe which reaches its southerly limits in the British Isles and therefore it is desirable to investigate the physiological tolerance of geographically different populations, since although morphological similar, they may be physiologically distinct. The culture of germlings and ecophysiological

experiments on adult plants, along with phenotypic variation studies will investigate any differences in populations of *Fucus distichus* from Ireland and Orkney. The problem of taxonomy and nomenclature of *Fucus* species in general and the taxonomic confusion surrounding *Fucus distichus* is also discussed. When all the experimental evidence is taken into account then the effects of seawater temperature on *Fucus* species and the question of how useful *Fucus distichus* is as an indicator of sea temperature rise and climate change in the British Isles will be discussed.

1.1 The Role of Climate Change on the Geographical Distribution of the World's Ecosystems

The important role that the climate plays in the geographical distribution of the world's ecosystems and of the wildlife they support has been well documented. Anthropogenically-induced climate change is one of the major factors likely to affect the Earth's ecosystems in the coming years and centuries (Stern, 2007). The Intergovernmental Panel on Climate Change (2007) has stated that "warming of the climate system is unequivocal, as is now evident from observations of increases in global average air and ocean temperatures". The earth's climate has warmed by approximately 0.6ºC over the past 100 years, greater than at any other time during the last 1,000 years (Walther *et al*., 2002; Parmesan and Yohe, 2003).

1.1.1 Climate change on a global scale

On a global scale marked changes have been seen in both terrestrial and aquatic ecosystems in response to rising surface temperatures. There is already compelling evidence of the ecological impact that recent climate change has had on a wide range of flora and fauna with diverse geographical distributions (Hughes, 2000). Plants and animals have been shown to respond to climate change in a number of ways i.e. phenology and physiology, range and distribution of a species, the composition of and interactions within communities and the structure and dynamics of ecosystems. Phenological trends have been revealed in Europe and North America that very likely reflect responses to climate change (Root *et al.* 2003). Some examples are: early leaf unfolding in numerous European plant species (Menzel and Estrella, 2001); the early appearance of butterfly species in the UK (Roy and Sparks, 2000); early breeding of amphibians in the UK (Beebee, 1995); earlier spring migration and breeding in

numerous bird species in Europe and North America (Bairlein and Winkel, 2001; Brown *et al*., 1999; Crick *et al*., 1997; Crick and Sparks, 1999; Dunn and Winkler, 1999; Inouye *et al*., 2000). In general spring activities have occurred progressively earlier in the year since the 1960s.

The distribution of species and range shifts are also predicted in response to climate change (Hoffman and Parsons, 1997). With general warming trends some species are expected to track the shifting climate (Woodward, 1987). Shifts in species ranges have occurred poleward in latitude and upward in elevation across a wide range of taxonomic groups and geographical locations during the twentieth century (McCarty, 2001). Some examples are: the treeline in Europe and New Zealand advancing towards higher altitudes (Kullman, 2001; Wardle and Coleman, 1992); Arctic shrub vegetation in Alaska expansion to previously shrub-free areas (Sturm *et al*., 2001); elevation shift in alpine plants in the European Alps (Grabherr *et al*., 1994); distribution changes in Antarctic plants and invertebrates (Kennedy, 1995); northward range shift of 39 butterfly species in North America and Europe (Parmesan,1999); 12 bird species in Britain with range movement northwards (Thomas and Lennon, 1999); increasing abundance of zooplankton, intertidal invertebrate and fish communities of the Californian coast of North Atlantic (Southward *et al*., 1995; Sagarin *et al*., 1999). There is also evidence (Epstein, 1998) that a steady rise in annual temperatures has caused the expansion of mosquito-borne diseases in the highlands of Asia, East Africa and Latin America.

Climate change may also cause non-native species from adjacent areas to cross frontiers and become new elements of the biota. While human activities are often the cause of species movement, particularly over long distances, any reproduction and spread at a new location would suggest altered site conditions due, for example, to climate change (Metzger *et al*., 2008). Shifts in species ranges are a predicted effect of global climate change (Hulme, 2003). A study by Sorte *et al*. (2010) used databases and citation searches to identify 129 marine species experiencing range shifts. The results showed that 75% of the range shifts found were in the poleward direction, consistent with climate change scenarios.

1.1.2 Climate change and the effect on the marine environment

The ocean climate can be defined by its temperature, salinity, ocean circulation and the exchange of heat, water and gases, including CO_2, with the atmosphere and climate change affects all of these (MCCIP, 2010). There is little doubt that the earth's oceans are warming and climate-linked changes are becoming evident in the marine environment and on species associated with it. Plankton constitutes the main primary and secondary biomass in marine ecosystems and plays a fundamental role in marine food-webs. Warmer water species are now increasing in the North Sea due to regional climate warming and the North Atlantic Oscillation (NAO) (Edwards *et al*., 2010). This change is considered detrimental as warmer-water species are not replacing colder-water species in similar abundances which has negative impact on other trophic levels. In the last 40 years there has been a northerly movement of warmer water plankton by 10° latitude in the north-east Atlantic and a similar retreat of colder water plankton to the north. In the North Sea plankton have been extensively studied using Continuous Plankton Recorder (CPR) data and results have shown that the previously dominant and important cold-water zooplankton species *Calanus finmarchicus* has declined in biomass by 70% since the 1960s.

Changes in marine commercial fish stocks have also been observed over the last few decades in the Atlantic and Pacific Oceans (Pinnegar and Heath, 2010). Northerly geographical range extensions or changes in the geographical distribution of fish populations have been recently documented for European Continental shelf seas and along the European Continental shelf edge and have been related to regional climate warming (Cheung *et al*., 2009). These include the movement of sardines and anchovies northward in the North Sea and red mullet and bass extending their ranges northward to western Norway (Stebbing *et al*., 2002). Around the UK warmer temperatures have been correlated with poor conditions for cod recruitment via changes at the base of the food web as cod, like many other fish species, are highly dependent on the availability of planktonic food during their pelagic larval stages (Beaugrand *et al*., 2003). Some fish distributions have moved northwards over the past 30 years by between 50 to 400km, with coldwater species such as monkfish and snake blenny moving the furthest (Perry *et al*., 2005). Abundances of warm-water fish species such as seabass, red mullet, John Dory, anchovy, squid and triggerfish have increased in UK waters during recent decades (Arvedlund, 2009). The stock biomass of adult seabass has quadrupled since 1985 from

500t, to over 2000t in 2004/2005. Diadromous species such as salmon and eel (which spend some of their life in both fresh and marine waters) have been shown to be particularly vulnerable to climate change with impacts on both the freshwater and marine phases (Jonsson and Jonsson, 2009, Lassalle and Rochard 2009).

Seabirds are an integral part of marine ecosystems. Population and breeding success in seabirds is complex and vary between species and geographical location and include factors such as climate, sea-surface temperature, plankton biomass and prey-fish stocks (Mavor *et al*., 2007). Major changes in plankton abundance in the North Sea have contributed to the reduction in quality and abundance of prey species such as lesser-sandeel (*Ammodytes marinus*) (Deurs *et al*., 2009) and snake pipefish (*Entelurus aequoreus*) (Harris *et al*., 2008) and have had a profound effect on some seabird colonies. In Britain and Ireland breeding success of seabirds such as skuas, black-legged kittiwake, terns and shag (Frederiksen *et al*., 2004a, 2004b) has declined since 2000 due to decreased food availability linked to climate change. More recently auks such as guillemots and Atlantic puffins have also declined (JNCC, 2009). Between 2000 and 2008, the total number of seabirds breeding in the UK decreased by approximately 9% with a decline in breeding success also noted (Mitchell and Daunt, 2010).

Sea surface temperature is a good predictor for marine mammal distributions and therefore marine mammals are sensitive indicators of changes in ocean environments (Robinson *et al*., 2005; Kaschner *et al*., 2006). The responses of some marine mammal species will depend on changes in prey abundance due to increased sea temperature. In the temperate zone, some species of toothed whales and dolphins are showing shifts in distribution which may be linked to increasing sea temperatures (Simmonds and Isaac, 2007). A recent large-scale survey of the European Atlantic continental shelf suggests there has also been a redistribution of harbour porpoises *Phocoena phocoena* in the North Sea over the last 10 years, with a notable increase in densities in the southern region (Learmonth *et al*. 2006). A study on cetacean in the north-west of Scotland (MacLeod *et al*., 2005) has suggested that warming of local waters has led to changes in the cetacean community with a decline in occurrence of the white-beaked dolphin *(Lagenorhynchus albirostris)* (cold water species) and an increase in the occurrence of the common dolphin *(Delphinus delphis)* (warm water species). This may be direct evidence that a pole-ward shift is happening in cetacean species however evidence of impacts from climate change may be difficult to distinguish from the impacts of human

activities such as prey depletion, incidental capture in fishing gear, pollution and disturbance.

1.2 Climate Change in the Marine Environment of the British Isles

There now appears to be scientific consensus that the planet is warming (Hughes, 2000; Walther *et al*., 2002; Parmesan and Yohe, 2003, Stern, 2007). Climate change is not a new phenomenon, however what is unusual is the rate and scale of change. The Marine Climate Change Impacts Report (MCCIP, 2010) states that several key components of the marine environment are likely to change as a result of climate change. Direct changes include sea surface temperature (SST), sea level rise and increasing UV-B penetration and indirect changes to wave climate, storminess, thermohaline circulation and alterations to nutrient supply. Despite considerable year-to-year and even decade-to-decade variability, SST around the UK coast has risen over the past three decades by about 0.7ºC **(Figure 1.1)** while seven of the warmest years in UK coastal waters since records began in 1870 have occurred in the last decade (Jenkins *et al*., 2008).

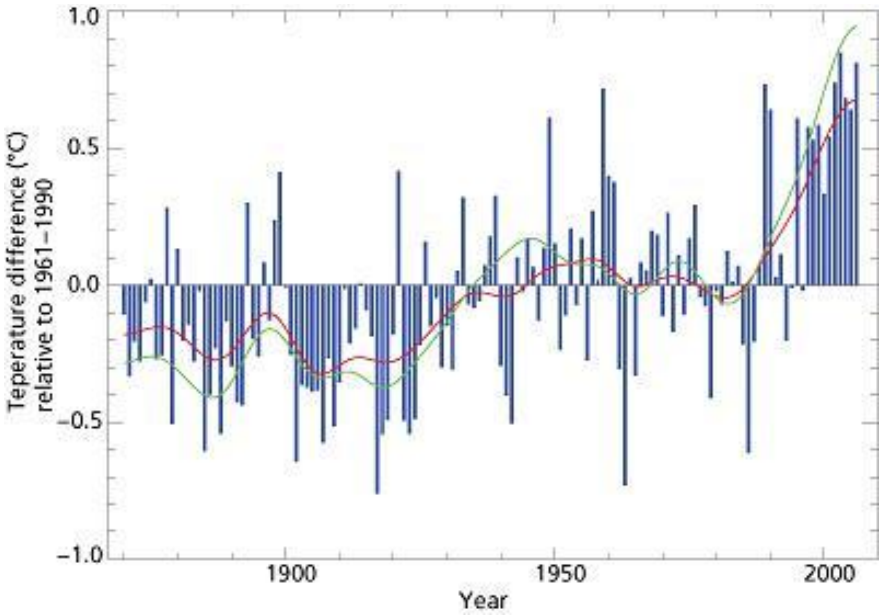


Figure 1.1: Annual-mean sea-surface temperature averaged around the UK coastline, for the period 1870-2006 (blue bars extending from the 1961-90 average of 11.3 ºC); the smoothed red line emphasises decadal variations. The green curve shows night marine air temperature over roughly the same area, with the same smoothing (taken from Met Office Hadley Centre HadISST1.1).

The coastal waters surrounding Britain and Ireland have warmed over the past 50 years, due partly to human-induced climate change and is part of a global rise in sea and air surface temperatures. Sea-surface temperatures (SST) around the UK coast have risen over the past three decades by about 0.7 ºC (Jenkins *et al*., 2008). Current indications (UK Climate Projections 2009) are that SST will continue to rise in all waters around the UK but with strong regional variations (Walther *et al*., 2002). **Figure 1.2** shows projected SST change however it should be noted that this in only one example and predictions vary. The largest increases have occurred in the English Channel and the southern North Sea than within Scottish continental shelf waters. The largest increase in air temperature has been over the southern North Sea at a rate of around 0.6°C per decade. It is predicted that by the 2050's, SST's may be as much as 2.5°C higher in summer and 2.3°C higher in winter than in 2000, average air temperatures relevant to rocky shores may be 2.1°C higher than at present (Austin *et al*., 2001) and sea-level may have risen by up to 80cm. Climate scenarios for Ireland waters suggest a future warming of approximately 0.28°C per decade (Sweeney and Fealy, 2002).

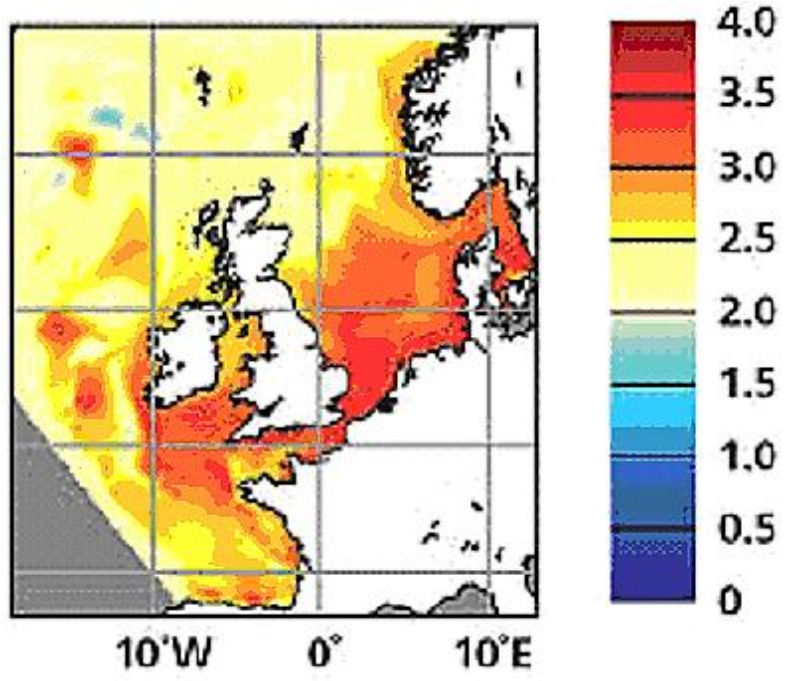


Figure 1.2: Projected autumn sea surface temperature change (ºC) by end of century (taken from UK Climate Projections, 2009).

1.2.1 Climate change and the effect on distribution of marine species in the British Isles

Increasing temperatures in the seas around Britain and Ireland appear to be affecting the abundance, distribution and health of some marine species and have been linked to the establishment, reproductive capabilities and spread of invasive non-native species. In the UK the honeycomb worm *Sabellaria alveolata* (Picton and Costello, 1998) has extended its northern range in the Irish Sea and North Channel. A rise in sea temperatures has been linked with disease outbreaks in the pink sea fan *Eunicella verrucosa* (Hall-Spencer *et al.*, 2007) in the Western English Channel, Celtic Sea and South-West Approaches. Rising water temperatures may also have contributed to the expansion in range of the bryozoan *Bugula neritina* (Hayward and Ryland, 1979; Arenas *et a*l., 2006) previously restricted to warm water areas such as power station outlets. The cold-water calcareous algae, maerl, may also be vulnerable to future climate impacts (Birchenough *et al*., 2010). Of the three major European maerl-forming species, two appear to be limited by temperature in UK waters. *Lithothamnion glaciale* is confined to Scotland and Northern Ireland and *Lithothamnion coralloides* reaches only as far north as the south-west of the British Isles (Birkett *et al.*, 1998). *L. coralloides* from the west coast of Ireland has been shown to respond positively to a 4°C increase in temperature (Blake and Maggs, 2003), suggesting the species may benefit from warming waters. Maerl is most commonly found on west coast of Scotland, the Western Isles, Orkney and Shetland (Hall-Spencer *et al.*, 2008) therefore temperature-related changes in these regions would affect a large proportion of the UK's maerl beds.

Introduced species that are not native and become established in the waters around the British Isles are termed 'marine non-natives'. A species can be considered invasive if it spreads rapidly and causes economic or environmental harm, or is harmful to human health (Reid *et al*., 2009). Marine non-natives may be introduced by human activities via aquaculture, ballast water or fouling on ships, natural range expansions or the availability of new niches as a consequence of climate change. Most non-indigenous marine species are spreading through natural processes independent of any contribution from climate change however some species that arrived through natural range expansions now appear to have increased the speed of expansion and there is now evidence to show that many non-native species are increasing in abundance and range extent due to rising temperatures (Mineu *et al*., 2008).

A number of key non-native marine species found in the UK are being closely monitored for the occurrence, expansion, means of dispersal and impacts on biodiversity by the Marine Aliens project being led by the Scottish Association for Marine Science (SAMS) in Scotland with MarLIN at the Marine Biological Association (MBA) in Plymouth. The species are: *Caprella mutica*, the Japanese skeleton shrimp, first found in a Scottish fish farm in 2000 (Willis *et al.*, 2004) and since found in over 120 locations throughout Europe; the invasive form of the green algae *Codium fragile* which replaces native species of the same genus, originating from Japan, first found in Devon in 1939 now widespread throughout Ireland, Scotland and on the south and west coasts of England (Trowbridge and Farnham, 2009); *Sargassum muticum* first recorded on the Isle of Wight in 1973 possibly imported with oysters from the Pacific and now found extensively on the western margins of Britain and Ireland (Harries *et al.* 2007a); *Undaria pinnatifida* (Ashton *et al.* 2006), brown seaweed first found in the Solent estuary in 1994 and now at sites along the south coast of England; *Styela clava*, the leathery sea squirt first found in Plymouth in 1953 and now widespread in Europe (Eno *et al.*, 1997; Davis and Davis 2004); *Perophora japonica* (Ashton *et al.* 2006) a colonial sea squirt first found in Plymouth in 1999 and since in the Channel Islands and further along the south coast.

The dispersal of more recent introductions is also being monitored by this project. These include the southern hemisphere tunicate *Corella eumyota* first found in Brighton, Gosport and Weymouth in 2004 (Arenas *et al.*, 2006) and now on the south coast of England and in south Wales and *Tricellaria inopinata*, a bryozoan native to the Pacific, first found in 1998 on the south coast of England (Dyrynda 2000) and recently found in marinas on the west coast of Scotland in 2007. Other non-native species that have been resident for some time, but are continuing to expand their range and numbers are *Crepidula fornicata*, the slipper limpet originating from the USA (Maggs *et al.*, 2010) and *Dreissena polymorpha*, the zebra mussel in both freshwater and estuarine environments.

The introduced Pacific oyster (*Crassostrea gigas*) spread from oyster farms in the early 1990s, becoming established in southern England. Similarly new self-sustaining populations are now established in Northern Ireland with recruitment occurring in favourable years (Nehls *et al.*, 2006). Current sea temperature projections are thought likely to result in *C. gigas* recruiting every year in Northern Ireland, Wales and south-

west England by 2040. Rising water temperatures may also have contributed to the expansion in range of the red seaweed *Caulacanthus ustulatus* which was introduced from Japan and spread rapidly to Devon in 2004, Cornwall in 2005 and Kent in 2009 (Zuccarello *et al*., 2002; Mineur *et al*., 2008).

1.2.2 The effect of climate change on rocky shores species

The above examples are evidence that climate change is affecting the distribution and range of many plants and animals in both the terrestrial and marine environment. However it should be noted that many marine non-natives are introduced by human activities via aquaculture, ballast water or fouling on ships. Many non-indigenous marine species are spreading through natural processes independent of any contribution from climate change however some species that arrived through natural range expansions now appear to have increased the speed of expansion and there is now evidence to show that many non-native species are increasing in abundance and range extent due to rising temperatures (Mineu *et al*., 2008).

Many studies of the biological impacts of climate change have focused on migratory species however these often exhibit large fluctuations from year to year making it hard to detect long-term range shifts (Easterlings, 2000). Range changes in more sedentary species, such as those found on rocky shores, follow slower processes which may make it easier to detect true geographic shifts in relation to increases in sea temperature. The effect of increased temperatures on the plants and animals inhabiting rocky shores has also been documented.

Changes in the distribution and abundance of northern and southern native species have been recorded with the range limits of some southern species moving further north as in the topshell *Osilinus lineata* (Mieszkowska *et al.* 2006). Population abundances of the topshell *Gibbula umbilicalis* (Kendall and Lewis, 1986) have increased throughout the UK and in warmer southern areas have switched to having two periods of gonad maturation per year. This was observed for the first time in 2008/2009 and is more characteristic of populations inhabiting warm waters and lower latitudes. Recent observations along the English Channel show that the warm water barnacle, *Balanus perforatus*, has extended its range eastward by upwards of 100 km in the past 25 years (Herbert *et al*., 2003) and is now found in areas in the eastern English Channel that were

formerly considered too cold for it in winter. Another warm water barnacle *Chthamalus montagui* has extended its range in eastern Scotland (Burrows *et al*., 1999). The warm-water barnacle *Solidobalanus fallax* has been recorded for the first time in the English Channel off Plymouth is also linked to sea temperature increase (Southward *et al*., 2004). The cold water barnacle *Semibalanus balanoides* is expected to retreat north and become very rare in the south-west of England as early as 2025 at the same time as the southern *Chthamalus* spp. increase and spread (Southward, 1967, 1991).

A study carried out on the intertidal species of Ireland (Simkanin *et al*., 2005) found that the abundance and range of some northern species had decreased over the entire coastline due to, as suggested by the authors, global climate warming. These species were the algae *Alaria esculenta*, *Laminaria saccharina* and *Laminaria hyperborea* and the barnacle *Balanus crenatus*. A decline in *Littorina littorea* was also noted but this was thought likely as a direct result of its commercial exploitation (Fisheries Science Services, 2003). The increase of the Australasian barnacle *Elminius modestus* around the Irish coastline has been well documented since it was introduced with shipping in 1955 (Crisp, 1958; O'Riordan, 1996). *E. modestus* is known to have a number of competitive advantages over native barnacles. Lawson *et al*. (2004) suggested that the significant increase of *E. modestus* may be a classic example of a successful invasion, reflecting a rapid colonisation of a new area by a non-native species entirely unrelated to climate change. However, climate change may indirectly affect the interactions between introduced and native species by causing increased stress in native populations and earlier recruitment in introduced species (Stachowicz *et al*., 2002).

Increased seawater temperatures have also been linked with changes in other algae distributions. Isolated individuals of the warm-water seaweed *Bifurcaria bifurcata* were found at Portland Bill in 2002, and have since become abundant at this location establishing a new range boundary (Mieszkowska *et al*, 2006). This southern species found on the Atlantic coast of France, Spain and Portugal and extending to the south and west coasts of England and the west coast of Ireland, had not been recorded east of Devon in the English Channel since the end of a warm period at the start of the 1900s. The warm water kelp *Saccorhiza polyschides* has shown a massive increase in abundance while the cold water species *Alaria esculenta* has decreased in abundance (Elliot *et al*., 2008). A scientific review for MCCIP 2007-2008 (Mieszkowska *et al*., 2006) on intertidal species for Scotland suggests that abundances of northern cold-water

species *Fucus vesiculosus*, *Halidrys siliquosa*, *Ascophyllum nodosum* and *Pelvetia canaliculata* have decreased between 2002 and 2006.

There are many examples of southern and northern species of plants and animals expected to shift and/or expand northwards under the predicted warming climate conditions (Hiscock *et al*., 2000) but very few examples of northern species moving south. However some examples have been noted as in the case of the pod weed, *Halidrys siliquosa* recorded for the first time in 2006 at Praia do Carreço on the Portuguese coast and extending southward (~80 km) from its previous southern distributional boundary at Ría de Pontevedra in Spain (Lima *et al.*, 2009). The authors stated that this shift was inconsistent with general predictions of species migrations under warming climate conditions given that in the last few decades an increase in temperature has been documented for the area.

A previously unrecorded population of *Fucus distichus* subsp. *edentatus* was discovered in 1980 in the Moray Firth area in north-east Scotland growing on harbour walls in Macduff. This was the first record for mainland Britain and marked a new southern limit and was described by Powell (1980) as 'a striking example of a northern species extending its range southwards in the North Sea'. In Scandinavia *edentatus* is common in north and west Norway and has extended its range southwards to Sweden (Wikström *et al*., 2002), Denmark (Lund, 1949) and the western Baltic Sea (Schueller and Peters, 1994). These shifts are inconsistent with general predictions of species migrations under warming climate conditions, which anticipate poleward shifts rather than southern expansions (Hughes, 2000; Walther *et al*., 2002) and therefore it can be assumed that other factors are contributing to the southern expansion of the subspecies *edentatus*. Wikström *et al*. (2002) suggested that low salinity will limit the distribution and colonisation of this subspecies in the Baltic Sea proper.

1.2.3 Climate change and its possible effect on the distribution of Fucus species

In order to consider the use of *Fucus* species and taxa in relation to monitoring of climate change it is first necessary to review the extent of climate change that is expected in British waters and to stress the considerable growing evidence that climate change may affect the distributions of marine organisms, both animal and plant, around

the British Isles. Following this, the utility of *Fucus* species in monitoring such change can then be discussed.

All species have limits of temperature tolerance and their distribution may change in response to climatic variation (Hughes, 2000; Walther *et al*., 2002; Reid, 2009). Climate change, as a warming of seawater temperatures, may have adverse effects on some marine species in the British Isles with the decline in abundance or loss of a number of northern species. With a changing climate, the 'climate space' for each species or habitat, that is the geographical and altitudinal zone to which it is well adapted, is likely to move, usually northwards (Southward, 1995; Southward *et al*., 1995, 2005). As previously discussed *F. distichus* subsp. *anceps* and *F. distichus* subsp. *edentatus* reach their southern geographical limits in the British Isles and are two species which may either decrease in abundance and extent or disappear as a result of sea temperature rise. The change in the distribution and abundance of *Fucus* species at existing locations in response to increases in sea temperature may be determined by a number of factors acting either on adult plants, germling stages or both. These include:

- The longevity of individuals in existing populations – if climate change has a negative effect on reproduction and local recruitment then existing populations will persist until the end of their natural life span (Li and Brawley, 2004).

- The lethal temperature limits of adult plants (Pollock, 1969; Pearson and Davidson, 1993).

- There is evidence that increased sea temperature can affect hold-fast and adhesion mechanism in *Fucus* species (McLachlan *et al*., 1971) and settling behaviour of algal spores (Charters *et al*., 1971; Coon *et al*., 1971).

- Indirect species interactions such as environmental control on predators and competitors (Sagarin *et al*., 1999).

It has been suggested that climate change may not only lead to a simple poleward shift in the distribution of intertidal organisms on rocky shores but cause localised extinctions of a species due to the inability of that species to spread to suitable habitats. This indeed may be the case for *F. distichus* subsp. *anceps* where it has found an

ecological niche in the upper part of sloping reefs on very exposed shores. As a geologically isolated ecotype at its most southern limit it may be very susceptible to environmental changes such as sea temperature rise.

The possible methods for study of environmental tolerances of *Fucus* species are now briefly discussed in this introduction.

1.3 Experimental Approaches in the Study of *Fucus* Species

Fucus species are equipped with a genotype that allows a wide range of phenotypic expression which often responds to differences in the environment (Russell, 1978). Perennial species such as *Fucus* are large and relatively long lived and can experience prolonged environmental effects and therefore responses to the environment are often very marked. Experimental studies may provide data on environmental factors affecting establishment, growth, survival and distribution of *Fucus* species. Members of the genus *Fucus* have been subjected to several experimental approaches, these include: cultivation under standard conditions; transplantation experiments; hybridisation studies; numerical taxonomy and molecular phylogenetics.

1.3.1 Cultivation under standard conditions

The gametes, embryos and adults plants of various *Fucus* species have provided material for experimental research to look at reproduction, recruitment, settlement and growth (Sideman and Mathieson, 1983a; Keser and Larson, 1984; Ang, 1991a, 1991b, 1992; Fletcher and Callow, 1992; Pearson and Brawley, 1996). Culturing of embryos and adults plants under standard conditions is important in experimental taxonomy of *Fucus* species and in analysis of environmental factors determining distribution and phenotypic variability. Different growth or reproductive patterns between isolates of a single species under controlled culture conditions can indicate a degree of genetic variation within the species.

1.3.2 Transplantation experiments

Culturing and transplant experiments carried out by McLachlan *et al.* (1971) on *Fucus distichus, Fucus edentatus, Fucus serratus and Fucus vesiculosus* resulted in each

species retaining its characteristic morphology under identical conditions of culture. The authors maintained these results strongly suggested that environmental or phenotypic plasticity was not the major factor for the considerable morphological variation amongst *Fucus* species, concluding that phenotypic plasticity contributes relatively little to intraspecific variation in *Fucus* species. Pollock (1969) carried out interzonal transplantation of embryos and mature plants of two distinct forms of *Fucus distichus* that co-exists in the intertidal region of the San Juan Archipelago, USA. Results indicated that there was no selection against embryos of either form by the environment and it was reasonable to conclude that the two forms were distinct in ways other than morphology.

1.3.3 Hybridisation studies in Fucus species

Hybridisation between different *Fucus* species is a possible source of variation and taxonomic confusion. Observations made by Burrows and Lodge (1951) on a strip of sea-shore cleared three years earlier, showed that the majority of *Fucus* plants re-colonising the strip could not be readily assigned to one species. The resultant hybrids developed morphologies intermediate in character between those of the parent species, *Fucus serratus*, *Fucus spiralis* and *Fucus vesiculosus*. Molecular phylogenetic studies carried out by Coyer *et al*. (2002) confirmed the occurrence of natural hybridisation in a field population between *Fucus serratus* and *Fucus evanescens* at the Kattegat Sea, Denmark. Authors such as Burrows and Lodge (1951, 1953) and Powell (1963) regarded *Fucus* species as being systematically distinct yet inter-fertile. Barriers to complete merging appear to be primarily ecological i.e. competition from parent plants, non-coincidence of fruiting periods, resistance to desiccation and changes in salinity etc. (Evans *et al*., 1982). While hybridisation between *Fucus* species has been reported in the field it may be that these intermediate forms represent ecotypes or phenotypic variants of parental type species.

1.3.4 Numerical taxonomy

Numerical taxonomy can be used to look at phenetic variation patterns within a *Fucus* species and have been used to describe morphology in fucoids (Anderson and Scott, 1998; Ruunskanen and Bäck, 1999; Scott *et al*. 2001). Mathematical procedures such

as multivariate analysis have been used to assess similarities and differences that make it possible to classify and identify taxa using measurements of observable characteristics and variation in large numbers of population samples. Rice and Chapman (1985) used this method to analysis the phenetic variation patterns within *Fucus distichus* L. emend. Powell collected from 51 sites in the North Atlantic to examine environmental and regional variation as well as taxonomic structure. Their analysis suggested that there were no significant differences between populations of species in Europe and North America and revealed two major sub-units within the taxon namely *Fucus distichus* L. and *Fucus evanescens* C. Ag., concluding that both species should not be divided into subspecies in the North Atlantic.

1.3.5 Molecular phylogenetics

Molecular phylogenetics uses the primary structures of macromolecules to construct a hypothetical course of macromolecular evolutionary history and plays an increasingly important role in algal taxonomic studies. ITS sequences have been used to survey fucoid genera at the family level (Rousseau *et al*., 1997; Lee *et al*., 1998) and explore relationships at the genus and species level (Serrao & Brawley, 1997; Leclerc *et al*., 1998; Serrao *et al*., 1999). Phylogenetic analysis by Coyer *et al*. (2006) on the genus *Fucus* and the resulting mtDNA tree revealed three monophyletic groups or species: Lineage 1A (*F. distichus*), Lineage 1B (*F. serratus*) and Lineage 2 (*F. vesiculosus, F. spiralis, F. cottonii, F. ceranoides* and *F. radicans*). Within Lineage 2 a clear separation between *F. spiralis, F. vesiculosus* and *F. ceranoides* as well as among *F. vesiculosus* and the newly described *F. radicans* was revealed. The molecular techniques used could not distinguish *F. distichus, F. evanescens* and *F. gardneri* and consequently the authors considered all species, subspecies and formae of Lineage 1A to be synonymous with *F. distichus*.

1.4 The Use of Different Life Stages in Experimental Studies of *Fucus* Species

Many algae have complex life histories involving two or more alternate phases. Fucales are monomorphic, that is they have a single morphological diploid phase (2n) producing haploid gametes (n). Laboratory culture studies can indicate that the life history and

growth of Fucales may be controlled by a variety of environmental conditions, one of the most influential being temperature (McLachlan, 1973; Bird and McLachlan, 1976; Strömgren, 1977; Terry and Moss, 1981; Li and Brawley, 2004).

1.4.1 Growth measurements of Fucus germlings

The reproductive early development stages of algae have been found to be extremely useful in algal growth studies. The development of germlings usually has a predictable growth pattern, are easy to handle in culture and require simple techniques and apparatus. Germlings can also be obtained in large numbers suitable for replication of genetically identical material (Fletcher and Callow, 1992). Many authors have used comparative growth rate of *Fucus* germlings over a fixed period of time to monitor the environment in relation to: bioassays (Scanlan and Wilkinson, 1987); desiccation (Thomas and Turpin, 1980); nitrogen uptake (McLachlan, 1977; Thomas *et al*., 1985); substratum (Hardy and Moss, 1979); irradiance (Terry and Moss, 1981; McLachlan and Bidwell, 1983); temperature (McLachlan, 1973, 1977; Terry and Moss, 1981; Li and Brawley, 2004).

1.4.2 Surface area measurements using apical tips of Fucus adult plants

For many algae the determination of growth can be measured by changes in surface area. The concentration of growth in *Fucus* to a specific area enables accurate measurement by using only small portions of plant excised from the tips of branches. This method of using apical segments has been developed by a number of workers in *Fucus* species (Burrows, 1971; Telfer, 1995; Grundy, 1996) to measure the change in area before and after treatment.

1.4.3 Wet (fresh) weight measurements of apical tips of Fucus adult plants

Weight measurements can be used for growth estimations when dealing with macroscopic portions of algae such as *Fucus*. This method was used by Schonbeck and Norton (1980) to measure increase in fresh weight of fucoid algae as part of their study on factors controlling lower limits of algae on the shore and by Fletcher and Fletcher (1975) to follow the growth of segments of *Sargassum muticum*. Measurement of the fresh weight of *Fucus* apical tips can be used in conjunction with surface area

experiments to see if growth increase is due to something other than length and breadth as an increase in bulk is equally important in consideration of growth (McLachlan *et al*., 1971).

1.4.4 Use of photosynthesis and respiration rates of adult Fucus plants

Various physiological criteria have been used as measures of the extent of stress on metabolism of algal species. Photosynthesis is a basis of primary production and its measurement is often used to indicate a plant's response to environmental variables owing to the ease with which it can be measured (Lobban *et al.*, 1985). The rates of metabolic process can be estimated by measuring rate of exchange of O_2 in water. Most studies of gas exchange in water employ some version of the light and dark bottle technique where portions of algae are enclosed in bottles filled with seawater, incubated under laboratory conditions and the dissolved oxygen measured after a fixed time (1 to 8 hours are common) using a dissolved oxygen meter. A number of authors have used this method in the study of *Fucus* and its response to environmental factors such as: freezing (Davidson *et al*., 1989; Pearson and Davidson, 1993); photoperiod (Strömgren, 1978); desiccation (Thomas and Turpin, 1980); emergence/submergence (Quadir *et al*., 1979); temperature (Newell and Pye, 1968, Healey, 1972).

1.5 Life History, Reproduction and Growth in *Fucus* Species

The genus *Fucus* has frequently been used to monitor and assess the marine environment in many parts of the world (Chapman, 1995). Temperature has quantitative effects on reproduction in a number of seaweeds (Lüning, 1980; Dring, 1992) and qualitative effects on the life history. Within one species there may be considerable genotypic variation in temperature tolerance and optimum temperature for growth (Lobban *et al*., 1985). Such variation may be great enough for geographically diverse populations to appear as distinct strains. As *Fucus* spp. and in particular, *F. distichus* L. and its associated subspecies *anceps* and *edentatus* have been proposed as possible indicators of climate change and sea temperature rise in the British Isles then knowledge of their life cycle with respect to the effects of environmental parameters such as temperature on both juvenile and adult life stages is a pre-requisite for this study.

1.5.1 Life cycle and reproduction of Fucus species

Fucus species are among some of the most morphologically and anatomically advanced groups of Phaeophyta possessing a diplontic life cycle with the haploid cells being gametes. Within the *Fucus* genus, *F. ceranoides*, *F. distichus* and *F. spiralis* **(Figure 1.3)** are monoecious/hermaphrodite producing gametes of both sexes in each conceptacle. *F. serratus* and *F. vesiculosus* are dioecious where conceptacles contain either male or female gametes on separate male and female plants. *Fucus* sexual reproduction is oogamous, that is, the fusion of a large, wall-less, nonmotile egg (~75 μm) and a small, motive, laterally biflagellate sperm (~5μm) (Quatrano, 1980). When plants reach reproductive maturity the tips of vegetative branches become receptacles with small openings on the surface. Each opening leads to a sunken flask-shaped conceptacle where antheridia and/or oogonia form along with sterile paraphyses (South and Whittick, 1987). Oogonia are borne on stalks and undergo meiosis followed by mitosis to produce eight egg cells. Antheridia are borne on branched paraphyses and produce numerous haploid spermatozooids. When incoming tides wash slightly desiccated receptacles this causes them to expand and extrude the gametes into the open sea where fertilisation occurs. Following fertilisation the spherical egg begins to divide and grow. The egg elongates into a three or four celled structure with the lowest, forming the rhizoid and the upper cell producing a quadrant of cells by successive division at right angles to one another. Growth of cells in the apical region is accompanied by cell division in other parts so that the young plant is roughly cylindrical.

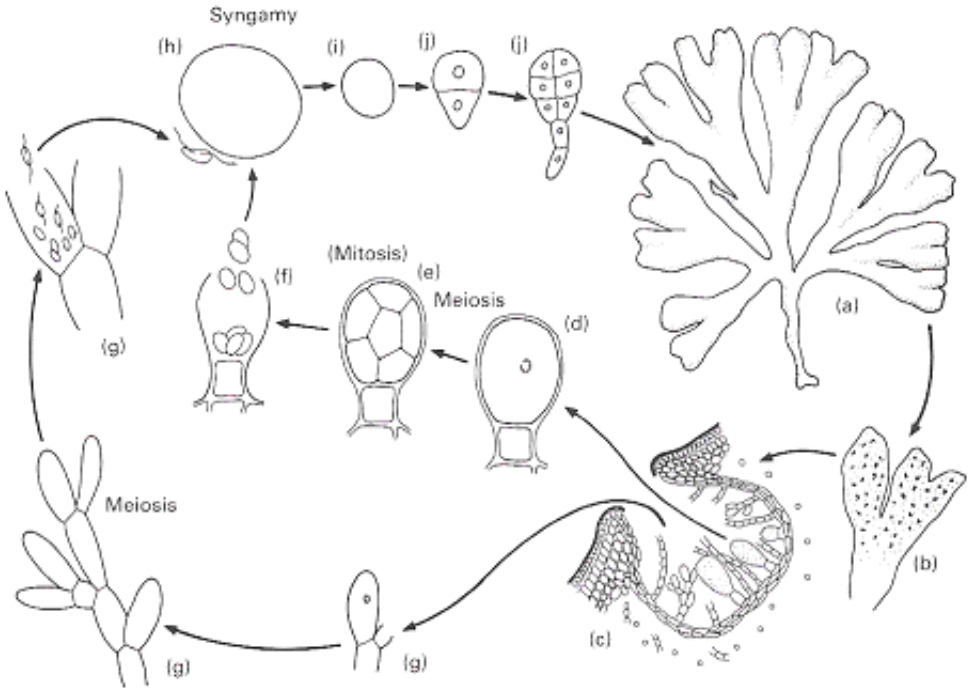


Figure 1.3: Life cycle of a monoecious species of *Fucus*. (a) Mature sporophyte. (b) Receptacles develop at tips of branches. (c) Conceptacles within receptacles contain oogonia, antheridia and sterile hair cells (paraphyses). (d) Oogonium. (e) Meiotic then mitotic division within the oogonium produces eggs. (f) Release of eggs. (g) Development of antheridial branch and antheridia, meiosis followed by mitosis in the antheridium produces sperm. (h) Syngamy. (i) Zygote. (j) Development of embryonic sporophyte. (taken from Scagel, 1966).

1.5.2 Settlement and attachment of Fucus species

The primary requirement for colonisation by large perennial algae such as *Fucus* is secure attachment of the reproductive cells to the substratum in a zone where the young plants will be able to develop. Fucoid eggs are initially covered by a mucilage layer consisting of alginates and fucoidan which attaches them to the oogonium wall. The eggs are expelled from the conceptacle still enclosed in this layer called the mesochiton which breaks down and attachment is by the zygote wall (Fletcher and Callow, 1992). A number of post-fertilisation changes take place including the secretion of other cell wall polymers, particularly sulphated fucans which are thought to be instrumental in anchoring the zygote to the substratum (Evans *et al*., 1982). Attachment is consolidated by hardening of the mucilage and then by the development of rhizoids. As rhizoids lengthen they closely adhere to the substrate and 3.5 hours after settlement embryos generally adhere quite strongly to the substrate (Peterson and Torrey, 1968).

1.5.3 Thallus growth in Fucus species

Fucoid algae undergo meristematic growth initiated by an apical cell located in an apical groove or pit and the meristematic cells remain the functional source of growth in length for the rest of the plant's life (Fritsch, 1945). Thallus growth comes from the primary meristem (usually the apical cell) and by activity of the surface layer (meristoderm). *Fucus* species exhibit some of the highest levels of differentiation and organisation in the algae containing several different tissue and cell types including photosynthetic, epidermis, cortex, medulla, sieve tubes and mucilage ducts (Lobban and Harrison, 1997). In general the cells of brown algae are uninucleate with relatively large nuclei and with cytoplasm containing numerous small vacuoles (Moss, 1966). The cells walls are composed of an inner layer of cellulose and outer layers of algin. Distinguishing features of *Fucus* species is the presence of conceptacles and their sterile counterparts the cryptostomata (Clayton, 1984) and in the case of *F. distichus*, caecostomata. These structures are described in greater detail in **Section 2.4.1**.

1.5.4 Wound healing and regeneration in Fucus species

Thallus damage and wounding in seaweeds may be caused by herbivores, sand abrasion and wave forces (McLachlan and Chen, 1972). In fucoids the thin perforated cross-walls of the medullary filaments are plugged after about 6 hours with newly synthesised sulphated polysaccharide, presumable fucoidan (Lobban *et al*., 1985). Later there is a general accumulation of polysaccharide at the wound surface and medullary cells adjacent to the damaged cells round off and become pigmented. After a week they give rise to lateral filaments which elongate and push through the wound surface where they branch repeatedly to form a protective layer. Cortical cells undergo longitudinal division parallel to the wound surface and outer cells assume the cytological and functional characteristics of epidermal cells e.g. they become pigmented. Regeneration is a well know phenomenon associated with wounding and has been studied thoroughly in *F. vesiculosus* (Moss, 1964a; Fulcher and McCully, 1969, 1971; McLachlan and Chen, 1972). This replacement of lost tissue by redifferentiation of medullary cells in *Fucus* from a wound surface has been referred to as adventive embryony.

1.5.5 Reproductive variation in Fucus species

The life-span of *Fucus* varies between species i.e. *F. distichus* 2 - 3 yrs., *F. spiralis* and *F. serratus* 3 – 4 yrs. and *F. vesiculosus* 4 – 5 yrs. Within the *Fucus* genus different species may reach reproductive maturity at different ages and reproduce at different times of the year. The following species all require two years to reach reproductive maturity: *F. distichus* subsp. *anceps* (Powell, 1957b); *F. distichus* subsp. *distichus* (Edelstein and McLachlan, 1975); *F. distichus* subsp. *edentatus*; *F. distichus* subsp. *evanescens*; *F. serratus* (Knight and Parke, 1950); *F. spiralis* and *F. vesiculosus* (Niemeck and Mathieson, 1976). Some of these species have been shown to produce receptacles on a small number of branches at the end of the first year's growth (Knight and Parke, 1950; Powell, 1957b; Edelstein and McLachlan, 1975). In some species reproductive plants may be found throughout the year although they have periods of maximum production in the Britain Isles i.e. *F. vesiculosus* (March – May), *F. spiralis* (Oct – June), *F. serratus* (Oct – Dec). Powell (1957b) recorded fertile *anceps* plants in all the months from April to August but suggested that receptacles may start to develop as early as January and that fruiting ceases during August at all sites in the British Isles. Powell also suggested that peak fruiting may occur some weeks earlier at the southern limits of distribution in Ireland than in Scottish localities.

Within species reproduction can differ in relation to geographic location. For example, *F. serratus* showed peak production in September in Wembury, Devon and November in Port Erin, Isle of Man (Knight and Parke, 1950). Ang (1991a) found *F. distichus* L. *emend.* Powell (no distinction made of subspecies) from Vancouver, British Columbia achieved peak reproduction in fall to winter while in Puget Sound it was found to peak in June (Thom, 1983). *F. vesiculosus* in the Baltic Sea has shown to exhibit two distinct strategies of reproduction, early summer (May – June) or late autumn (September – November) (Berger *et al*., 2001). One of the most important ways in which algae respond to their environment is in the timing of reproduction and temperature is an obvious seasonal cue in *Fucus* species.

Of the above methods those chosen for investigations in the time available in this thesis were:

- Growth measurements of *Fucus* germlings
- Surface area measurements using apical tips of *Fucus* adult plants
- Wet (fresh) weight measurements of apical tips of *Fucus* adult plants
- Photosynthesis and respiration rates of adult *Fucus* plants
- Phenotypic variation between geographically different populations of *Fucus distichus* subsp. *anceps*

To set the scene for this work it is also necessary to review the members of the genus *Fucus* that will be used in these experiments.

Chapter 2: Taxonomy, Nomenclature and Distribution of *Fucus distichus* L.

2.1 Brief History of Taxonomy and Nomenclature in the Genus *Fucus*

The naming of species and the construction of classification schemes are governed by The International Code of Botanical Nomenclature (ICBN) (McNeill, 2006) which sets out rules and recommendations dealing with the formal botanical names that are given to plants. Its intent is that each taxonomic group of plants has only one correct name that is accepted worldwide with the binomial system of classification having the species as its baseline unit. Two concepts have arisen for what constitutes a species, the morphological concept, widely used by Victorian naturalists, and the more objective biological species concept which requires that members of the same species be freely inter-fertile with each other, producing viable offspring that are capable of successfully backcrossing with the parents. This is a reflection of the fact that members of the same species should have virtually identical karyotypes, thus allowing successful meiosis and fusion of gamete nuclei. By contrast members of different species should be sterile with each other (South and Whittick, 1987). There is a problem with the biological species concept with regard to *Fucus* species as they are seen as being systematically distinct yet inter-fertile (Burrows and Lodge, 1951; Powell, 1963) and therefore by definition are not true species but that ecological factors maintain them as separate entities at different positions on the ecological gradient up the intertidal seashore.

The very large number of sub-generic taxa previously recognised was reduced by Powell in 1963 when he grouped over a hundred species, varieties and forms of *Fucus* into just a few species. **Table 2.1** shows the characters Powell used, based on eco-morphological criteria, in the separation of the most common *Fucus* species found in the British Isles.

Table 2.1: Characters used in the separation of *Fucus* species in the British Isles (taken from Boney, 1978).

Character	*F. ceranoides*	*F. distichus*	*F. serratus*	*F. spiralis*	*F. vesiculosus*
Shape of frond	flat	flat	flat	spirally twisted	flat
Vesicles	none	none	none	none	present*
Margin of frond	even	even	serrated	even	even
Midrib and wings	midrib well marked	midrib not so apparent	both prominent	both prominent	both prominent
Shape of fruiting receptacles	bifid or spindle-shaped - may be sharply pointed	narrowly cylindrical to fusiform	extended growth	rounded	ellipsoidal-elongate, pointed
Sterile rim of receptacles	no definite rim	no definite rim	no definite rim	present	no definite rim
Inflation of receptacle	inflated	inflated	flattened	inflated	inflated
Sex of plant	hermaphrodite‡	hermaphrodite†	dioecious	hermaphrodite	dioecious
Caecostomata§	none	present	none	none	none
Habitat	mainly in estuaries and harbours; under influence of brackish water	upper part of rocky shores	lower part of rocky shore	upper part of rocky shores	upper and middle parts of rocky shores

Notes: * on shores exposed to severe wave action a form lacking vesicles is found; *F. vesiculosus* var. *linearis*
‡ whilst frequently hermaphrodite, numerous records of unisexual plants are known
† may appear to be unisexual when antheridia are all discharged prior to release of oogonia
§ caecostomata are small internal cavities lying within the cortical region, lined with flattened cells and containing a few slightly branched paraphyses (Caecostomata are described in more detail in Section 2.4.1)

The genus *Fucus* is widely distributed throughout the cool temperate waters of the North Atlantic and North Pacific oceans (Lüning, 1990; Wynne and Magne, 1991) and species are found in habitats ranging from the rocky intertidal to brackish salt marshes. The genus is represented worldwide by a number of highly polymorphic species and a great many forms of imprecise systematic status (Evans *et al.*, 1982). This confusion is primarily due to the large degree of phenotypic variability exhibited by the genus. At present 20 species have been flagged as currently accepted taxonomically by Algaebase, a database composed by the National University of Galway, Rep. of Ireland. These are: *F. ceranoides* L.; *F. cottonii* M.J. Wynne & Magne; *F. distichus* L.; *F. evanescens* C. Agardh.; *F. gardneri* P.C. Silva; *F. lagasca* Clemente; *F. parksii* Gardner; *F. radicans*

L. Bergström & L.Kautsky, F. *serratus* L.; *F. setaceus* Poiret; *F. spataeformis* Esper; *F. spiralis* var. *platycarpus* Batters; *F. spiralis* L.; *F. vesiculosus* L.; *F. vesiculosus* f. *mytili* (Nienburg) Nienhuis; *F. vesiculosus* var. *volubilis* Goodenough & Woodward; *F. vesiculosus* var. linearis (Hudson) Kutzing; *F. vesiculosus* var. *vardorum* (Areschoug); *F. vesiculosus* var. *compressus* Kjellman; *F. virsoides* J. Ag.

2.2 Taxonomic History of *Fucus distichus* L.

The species was first described by Linnaeus in *Systema Natura* (1767) as '*Fucus fronde plana dichotoma integerrima lineari fructificationibus tuberculatis mucronatis*' translated as '*Fucus* fronds plane [flat], dichotomously branched, entire [margins], linear [i.e. very narrow], with fructifications [receptacles] having round humps [i.e. conceptacles probably] and sharply pointed'. However Powell (1957a) stated that this short diagnosis could not by itself delimit any particular species of *Fucus*. The many different morphological forms of this species led to conflicting ideas regarding their nomenclature and as a consequence many different authors have used different names. The European authors Rosenvinge (1893), Börgesen (1902) and Jónsson (1903) were of the opinion that the different forms found in the North Atlantic were all one species but that each form was the result of the ecology of its surroundings. The authors showed to an extent that between the more extreme, distinctive forms found, there existed many intermediate forms. The name *Fucus inflatus* L. was generally used by European phycologists for this complex of forms and several characteristic forms were widely recognised. However, the American authors Taylor (1937), Gardner (1922) and Setchell and Gardner (1925) rejected the name as they believed Linneaus's short description of *Fucus inflatus* L. and accompanying specimens in the Linnaean Herbarium could not be associated with any one species and instead gave each form a separate species name.

After full consideration of all that had been published on the autecology and world distribution of the above forms, Powell (1957a) in agreement with Börgesen (1902), Jónsson, (1903) decided that the numerous forms described were not sufficiently distinctive to warrant separate specific status and that the use of the disputed specific epithet '*inflatus* L.' was not justifiable. Rice and Chapman (1985) later concurred with Powell in the rejection of the name *F. inflatus*.

2.3 Case for Using the Name *Fucus distichus* L. emend. Powell

Powell (1957a, 1957b) showed that the name *Fucus distichus* L. applied to a distinctive fucoid. Powell decided to use sub-specific status rather than specific designation and divided the genus *Fucus distichus* L. into four subspecies: *anceps*, *distichus*, *edentatus* and *evanescens* **(Figure 2.1)**. Powell maintained that the numerous forms he studied were best interpreted as forms of a single, extremely plastic, highly successful and widely distributed species. Powell based his statement on the fact that, at least near the centres of distribution of the species (e.g. Faeröe Islands and Iceland, and probably in northern Europe and on the Pacific coast of North America), whole series of forms intermediate in character between the extreme forms were very common and that near the centres of distribution of the species it was possible to interpret the extreme forms as products of their ecological environment, with the intermediate forms developing under intermediate environmental conditions. Powell also stated that towards the southern and northern limits of distribution the species is often represented by populations of only one or two of the more distinctive and best adapted forms, confined to restricted habitats and often geographically isolated and that these isolated populations could well be regarded as genetically adapted ecotypes. Further emphasis on the close affinity of the various forms is demonstrated by the occurrence of caecostomata **(Section 2.4.1)**.

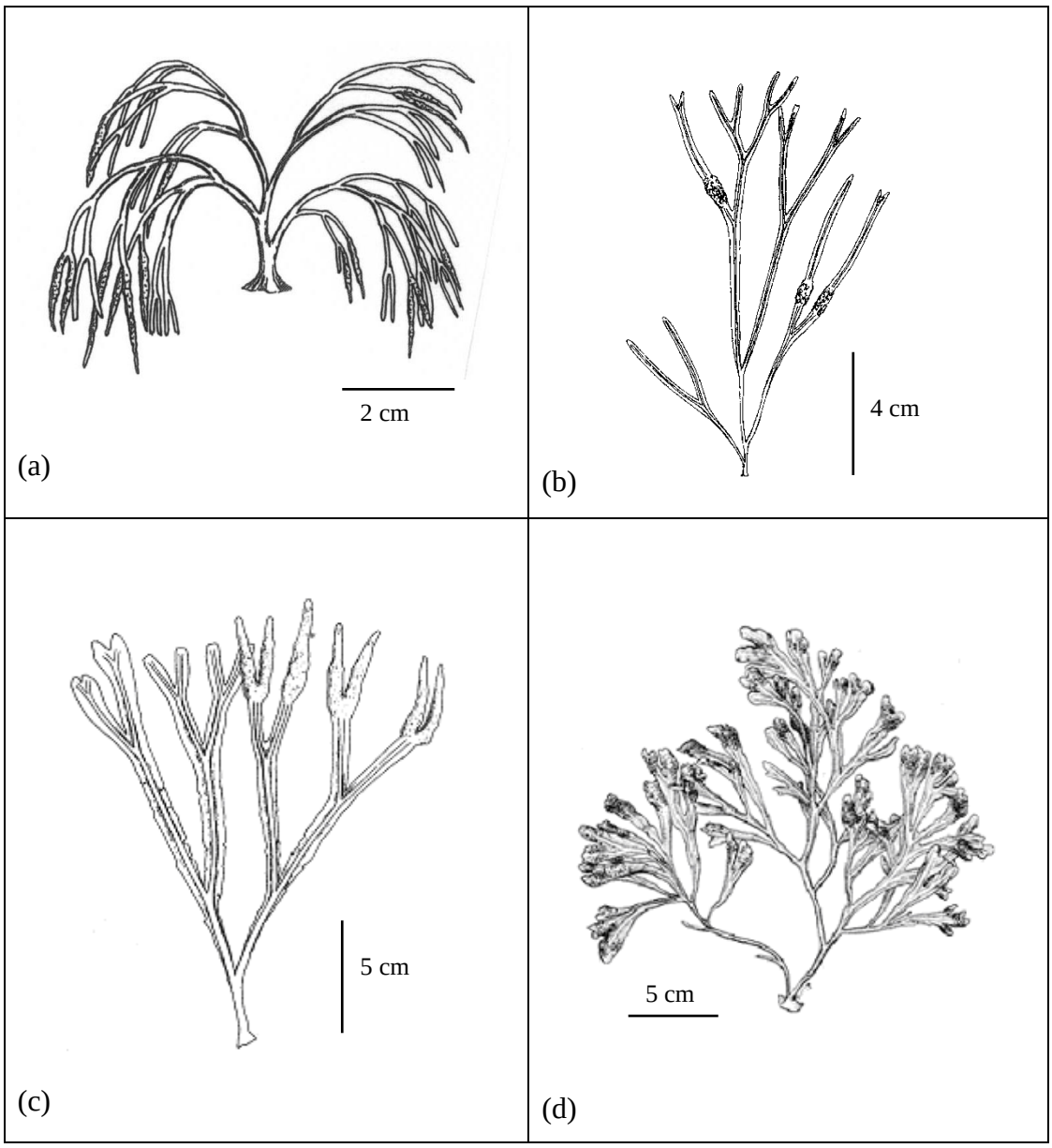


Figure 2.1: Four subspecies of *Fucus distichus* L. described by Powell (1957a). (a) *anceps* (b) *distichus* (c) *edentatus* (d) *evanescens* (taken from Hiscock 1979, Silva 1979).

2.4 General Characteristics and Ecology of *Fucus distichus* L. emend. Powell

Powell (1957a, 1957b) described the principle characteristics of the species as a whole as: (1) the conceptacles of all forms are invariably hermaphrodite (gametes of both sexes in each conceptacle) and (2) closed cavities called caecostomata are found in variable numbers in the fronds of most forms of the species but not in any other species of the *Fucus* genus. **Table 2.2** gives descriptions and the ecology of the subspecies described by Powell.

Table 2.2: Description and ecology of *Fucus distichus* L. subspecies described by Powell (1957b).

Subspecies	**Description**	**Ecology**
F. distichus* subsp. *anceps	Plants relatively small but very sturdy usually (4-) 6-10 (-15) cm in length, yellowish brown to dark brown in colour with a well developed holdfast up to 2 cm in diameter giving very firm attachment. The stipe is short, relatively thick, almost terete and stands erect while the more lax distal branches arch over characteristically. Branching is distichous and usually dichotomous but may be unilateral in part and the angle between older branches is often wide and between youngest branches acute so that the young terminal branches are closely crowded and run almost parallel.	Found only on extreme exposed coasts which are subject to considerable swell and wave-action, in the upper part of the littoral zone.
F. distichus* subsp. *distichus	Plants are relatively small and delicate, 5-15 (-20) cm in length, light brown in colour with a relatively small holdfast. The stipe is very thin, lax, round or oval in section with branching dichotomous and usually distichous and axils rather acute. Branches have entire margins, evesiculate, narrow, linear, 1.5-3 (-4) mm wide with principle branches showing a definite but not very prominent midrib and narrow lateral alae. Although cryptostomata and caecostomata are both usually present they are few, small and obscure. Receptacles are apical, narrowly cylindrical to fusiform, generally inflated, broader than the distal parts of the fronds which bear them, 0.5-3cm long and 1-4mm broad, unbranched or once forked. Conceptacles are hermaphrodite.	Largely occurs in rock pools (but may occur also on open rocks in some northern parts of its range) in the upper part of the littoral zone, at both exposed and sheltered sites.

Table 2.2: (continued)

F. distichus* subsp. *edentatus	Plants typically large and sturdy, 20-45(-60) cm in length, dark brown in colour with branching regularly dichotomous, usually distichous and axils generally acute. Branches with entire margins, evesiculate, leathery and more or less flaccid, alate above but rather narrow for a fucoid of this size, (5-) 9-15 (-20) mm broad but a little broader just below the dichotomies. Relatively thick midrib very distinct below but may become very indistinct above, especially immediately below the receptacles. Towards the base the midrib has no alae and forms a firm stipe. Holdfast a conical disk and cryptostomata are typically few but can be quite numerous, usually rather small and inconspicuous in older branches. Caecostomata are usually present, from few to very abundant (up to 500 per cm^2). Receptacles are apical, typically elongated and swollen, cylindrical or somewhat flattened, 2-10 (-22) cm long, 5-15 (-25) mm broad below, often tapering to acute (sterile) tips, usually divided 1-3 times into antler-like subdivisions and often not very sharply demarcated from sterile tissue below. Vegetative growth beyond the receptacle may occur and conceptacles are invariably hermaphrodite.	Plant is typically larger than subsp. *distichus* or subsp. *anceps* and grows best at sheltered or semi-exposed sites in the sublittoral fringe and lower mid-littoral zones. It can be found however, at any level from the upper mid-littoral zone down into the sublittoral zone with plants growing at high levels or in more exposed situations generally shorter and narrower. Suggested that this plant grows best under conditions of sewage and other organic pollution (Powell, 1963). Often found in harbours.
F. distichus* subsp. *evanescens	Most variable and therefore the most difficult to define of the four subspecies. Principal features are plant large with broad fronds, flattened receptacles, not markedly elongated and relatively broad. Midrib indistinct in apical parts of plant. As in subsp. *edentatus* characters such as the midrib becoming indistinct at top of frond, and the numbers of cryptostomata and caecostomata, are very variable and if Arctic forms are included then the relative width of the frond also varies widely. The only characteristic feature of subsp. *evanescens* found throughout its geographical range relates to the shape of the receptacles which are relatively short and broad, rather flattened and distinct from the rest of the frond.	Plants usually found in lower part of littoral zone (and in sheltered situations) in some of the more southerly parts of the over-all range of the subspecies and also applied to a number of relatively small and often rather narrow plants found on Arctic and other Northern shores. Thought to be only subsp. which is circumpolar in distribution and not found as far south as subsp. *edentatus*. Convenient to recognise subsp. *evanescens* as subspecies best adapted to Arctic conditions.

2.4.1 Caecostomata

The closed cavities known as caecostomata are found only in *Fucus distichus* L. and are of taxonomic importance and are therefore discussed. Caecostomata originate in the same way as conceptacles and cryptostomata in *Fucus* spp. Cryptostomata can be regarded as sterile conceptacles, having paraphyses but no sexual organs. These three structures originate just behind the growing point from a single initial cell which becomes lodged at the base of a deep and narrow cavity owing to rapid growth and division of the surrounding cells (Round, 1981). The cavity then enlarges and becomes lined with several layers of flattened cells which delimit these structures from the compact cortical tissue and loose medullary tissue. From this point onwards the development of caecostomata differs from cryptostomata and conceptacles as the pores communicating with the exterior become blocked by cell division of the meristoderm and the cavities soon become completely roofed over.

Caecostomata **(Figure 2.2)** appear as small, lighter coloured (less dense), rounded spots in the frond and when very numerous gives a stippled appearance. Powell (1957a) found caecostomata in all of the North Atlantic forms of *Fucus distichus* he studied. In British material the presence of caecostomata in *F. distichus* subsp. *edentatus* can easily be detected both in fresh and herbarium material by holding the frond up to light and can be extremely abundant with up to a maximum density of 500 per cm^2 with cryptostomata few or absent. Both *F. distichus* subsp. *distichus* and *F. distichus* subsp. *anceps* have very reduced fronds and caecostomata and cryptostomata occur in small numbers and caecostomata may only be detected by sectioning the frond. Laboratory examination of *F. distichus* subsp. *edentatus* taken from the island of Mousa, Shetland Islands, showed that fully formed caecostomata are first visible in sporelings as small as 5 mm in length (Powell, 1963a).

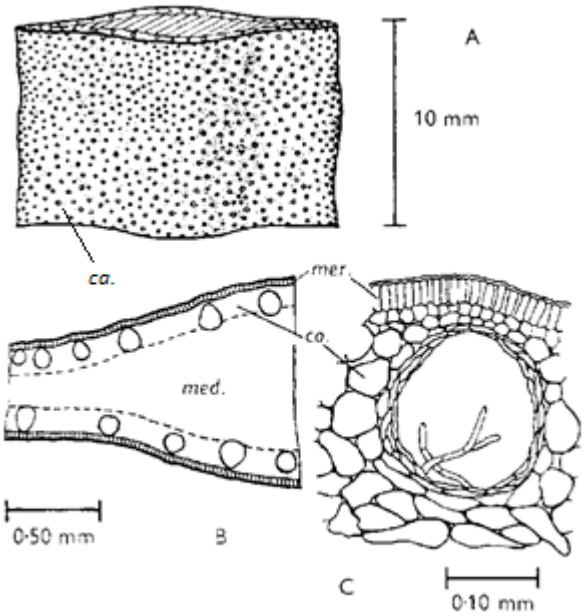


Figure 2.2: Caecostomata in *Fucus distichus* subsp. *edentatus (*plant from Lerwick harbour, Shetland Islands). (A) Surface view of part of thallus; *ca.* (internal) caecostomata. (B) Transverse section of part of thallus showing caecostomata situated mostly in the cortex. (C) Enlarged diagram of a single caecostomata, containing two colourless paraphyses (taken from Powell, 1957a).

2.4.2 Powell's distinction between Fucus vesiculosus var. linearis (L.) Huds., Fucus spiralis f. nanus (Stackh.) Batt. and Fucus distichus subsp. anceps

In Britain three species of *Fucus* can be found on exposed coasts and with increasing exposure all three species show reduction along similar lines, all becoming both shorter and narrower. Of the three forms *F. vesiculosus* var. *linearis* **(Figure 2.3a)** is the most variable in form and in extreme exposure may be reduced to bladderless erect tufts a few inches in length with narrow fronds. In certain localities and circumstances *F. vesiculosus* var. *linearis* can closely resemble either *F. distichus* subsp. *anceps* **(Figure 2.1a)** or to a lesser extent, *F. spiralis* f. *nanus* **(Figure 2.3b)** and Powell stated that all three forms can and have been confused by investigators not familiar with all three species. The following is a description, using nomenclature of the time, given by Powell by which the three forms may be distinguished from each other.

"F. vesiculosus f. *linearis* is unisexual and dioecious while *anceps* is hermaphrodite (old plants may have released antheridia and may only show oogonia). The midrib in *F. vesiculosus* f. *linearis* in younger plants is very distinct with lateral alae clearly

differentiated. In *anceps* the midrib and lateral wings are not sharply differentiated with distal branches oval in section. In addition *anceps* usually has a small number of small caecostomata. *F. spiralis* f. *nana* varies in length with narrow strap-like thalli either unbranched or with a few dichotomies and has a very distinct midrib and lateral wings. Receptacles are small, terminal, globular with a sterile rim with conceptacles hermaphrodite. The prominent midrib and shape of the receptacles distinguish this form from *anceps* and the hermaphrodite conceptacles and sterile rim on receptacles distinguish it from the reduced form of *linearis*".

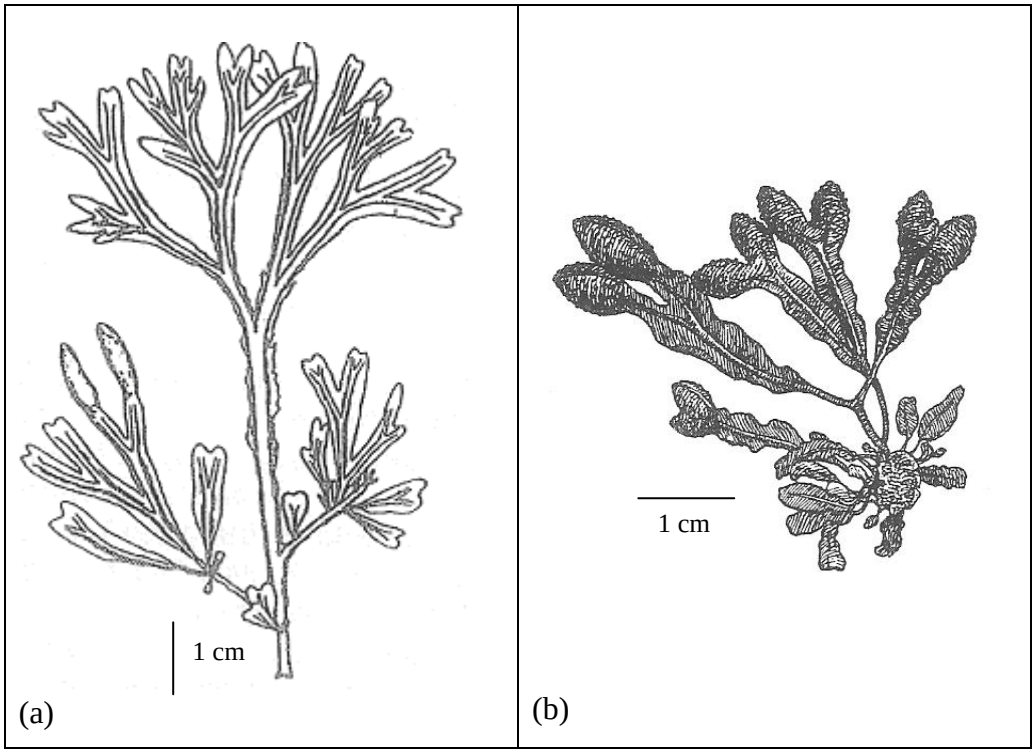


Figure 2.3: (a) *Fucus vesiculosus* var. *linearis* (b) *Fucus spiralis* f. *nanus* (taken from Hiscock, 1979).

A common feature of both *anceps* and *nanus* is for plants to have all the apices fertile at the same time, this is seen to a lesser extent in *linearis*. Powell (1957b) stated that this feature in the least favourable environmental circumstances is doubtless of value for the survival of the species.

2.4.3 Current taxonomic status of Fucus distichus since the major review by Powell

Powell's work was later questioned by Rice & Chapman (1985) who stated that their numerical taxonomic study on the same genus from fifty one sites on the North Atlantic actually showed two distinct species, *F. distichus* L. and *F. evanescens* C. Ag. They argued that Powell had only limited plant material outside Europe and that of the time of Powell's study only the most rudimentary methods of multivariate analysis were readily available. However authors Sideman & Mathieson (1983) on their studies in the Gulf of Maine, North-East America agreed with Powell's subspecies status. Further confusion is seen in the common *Fucus* found in the North-East Pacific where Japanese workers (Wakana & Abe, 1992) have used the name *Fucus distichus* subsp. *evanescens* for some time while others workers (Silva, 1979; Scagel *et al*., 1989; Blanchette,1997; Haring, 2002) favour retention of the name *Fucus gardneri*. Coyer *et al*. (2006) on their molecular phylogeny work on genus *Fucus* stated that the distinction among *F. distichus*, *F. evanescens* and *F. gardneri* in the northeastern Pacific remained unclear. There does appear to be uncertainty about the taxonomy and nomenclature of this common *Fucus* found along the coast of Pacific North America and indeed *Fucus distichus* worldwide and to illustrate this **Appendix A** lists various synonyms used, past and present, by different workers for this very complex circumpolar taxon.

2.4.4 Taxonomy and nomenclature of Fucus distichus in the British Isles

In Britain and Ireland the more recent publications and check-lists have warranted the use of separate specific status for *Fucus distichus* (incorporating subsp. *anceps*) and *Fucus evanescens* C. Ag. (incorporating subsp. *edentatus*). **Table 2.3** shows checklists and names used by various organisations along with descriptions and notes where available.

Table 2.3: Checklists and names used for *Fucus distichus* L. in the British Isles.

Checklist	**Nomenclature**	**Description/notes where available**
Parke & Dixon, 1964 Parke & Dixon, second revision, 1968 Parke & Dixon, third revision, 1976	*Fucus distichus* L. subsp. *distichus* subsp. *anceps* (Harv. et Ward ex Carr.) Powell subsp. *edentatus* (Pyl.) Powell	
South & Tittley, 1986	*Fucus distichus* L.	Incorporates *Fucus distichus* ssp. *anceps* (Harv. Et Ward ex Carr) Powell.
	Fucus evanescens C. Agardh	Incorporates *Fucus distichus* ssp. *edentatus* (Bach. Pyl.) Powell
M. D. Guiry, 1997	*Fucus distichus* L.	
	Fucus evanescens C. Agardh	
Scottish Natural Heritage (Hiscock *et al*., 2000)	*Fucus distichus distichus*	Northern species recorded from Scotland to the Arctic. Not recorded in Northern Ireland. Incorporates *anceps*.
	Fucus evanescens	Present from Shetland and the Danish Baltic to the Arctic. The only British records are from Shetland. Incorporates *edentatus*.
Hardy, G. M. D. Guiry, 2003	*Fucus distichus*	A northern wrack, superficially resembling *Pelvetia canaliculata*, found in the upper intertidal on very exposed and wave-battered shores. Incorporates *anceps*.
	Fucus evanescens	A quite large wrack found on sheltered northern shores. Incorporates *edentatus*.

Although there is an unwritten rule amongst British marine phycologists that wherever possible the nomenclature of the latest checklist is used, the author is in agreement with Powell's 1957a description of the subspecies. In this study, populations of *Fucus distichus* from the most southerly limits of distribution of the species in Britain and Ireland were investigated, and therefore from populations that exhibit the greatest difference in form in response to varying environmental conditions. The sub-specific status will be used throughout in this investigation as it readily distinguishes the markedly different forms. This work concentrates primarily on *Fucus distichus* subsp. *anceps* which is the subspecies most widely distributed in the British Isles and most likely therefore to be limited by sea temperature rise.

2.5 Temperature as a Primary Factor of Algal Geographical Distribution

It has long been accepted that temperature is a primary environmental factor, past and present, in controlling geographical distribution of marine plants (Van den Hoek, 1975; Druehl, 1981). Setchell (1920) applied the concepts of stenothermy (narrow temperature tolerance) and eurythermy (wide temperature tolerance) and proposed a series of coastal zones separated by 5ºC and 10ºC isotheres (isothere, mean temperature for the warmest six weeks) and isocrymes (isocryme, mean temperature for coldest six weeks). Isocrymes **(Figure 2.4)** are generally thought to limit poleward distributions and isotheres **(Figure 2.5)** equatorward distributions. Hutchins (1947) suggested that there are four critical temperatures 1) the minimum for survival, which may determine the winter poleward boundary for a species 2) the minimum for reproduction, controlling the summer poleward boundary 3) the maximum for reproduction, controlling the winter equatorward boundary 4) and the maximum temperature for survival, determining the summer equatorward boundary. Van den Hoek (1982b) added two more temperatures, upper and lower limits for growth. Hutchins proposed that either isocrymes or isotheres could limit both poleward and equatorward distributions, therefore a species may be restricted by survival temperatures north and south, by reproduction temperatures north and south, or by one survival limit and one reproduction limit.

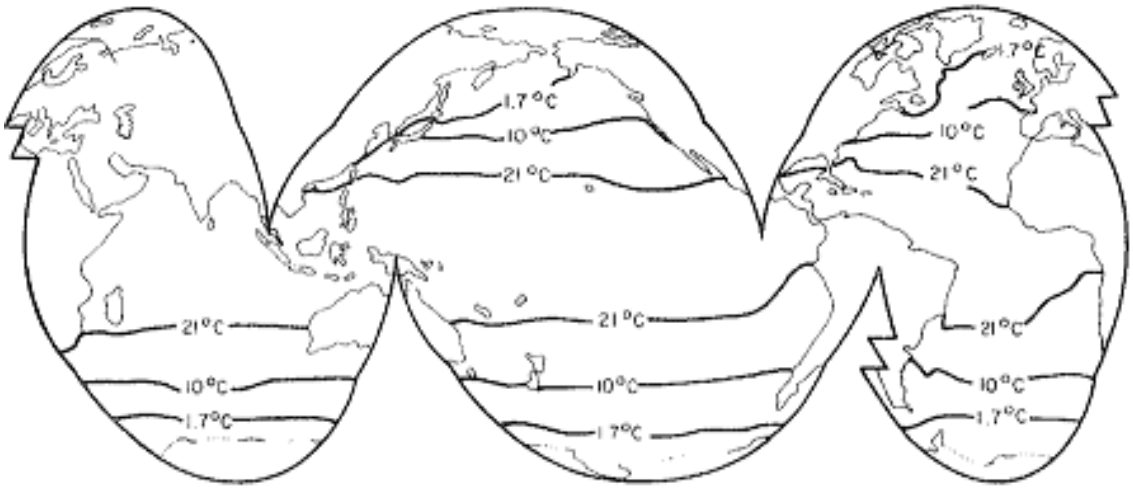


Figure 2.4: Isocrymes, mean temperature for coldest six weeks (Setchell, 1920).

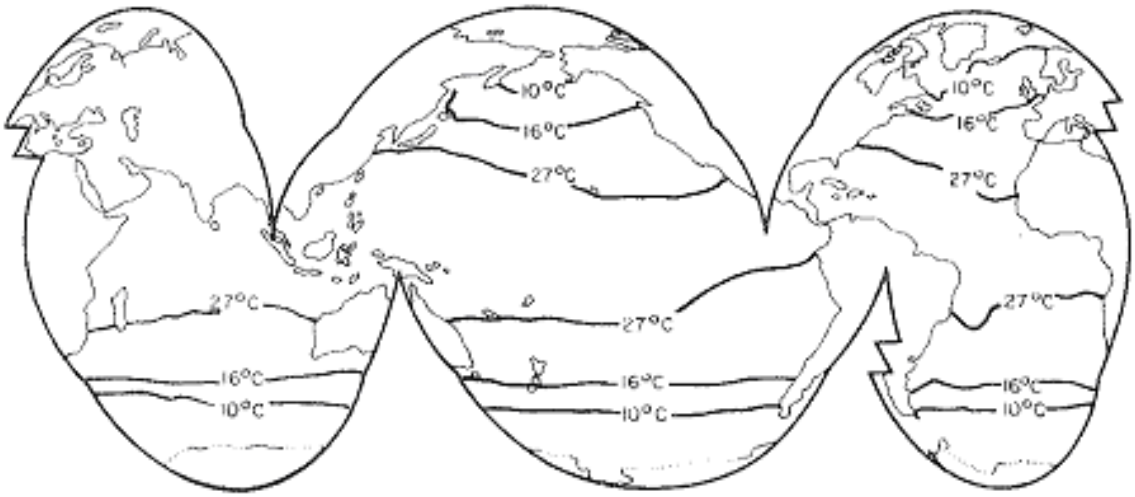


Figure 2.5: Isotheres, mean temperature for the warmest six weeks (Setchell, 1920).

2.5.1 Marine biographical regions

Seven groups of biogeographical regions of the world's seas are recognised (Van den Hoek, 1975; Lüning, 1990).

1) Arctic group (one region).
2) Cold temperate group, Northern Hemisphere (three regions).
3) Warm temperate group, Northern Hemisphere (four regions).
4) Tropical group (four regions).
5) Warm temperate group, Southern Hemisphere (five regions).
6) Cold temperate group, Southern Hemisphere (five regions).
7) Antarctic group (one region).

The boundaries of each group of biogeographical regions are primarily recognised by drastic changes in the composition of the coastal flora and fauna and, with some exceptions, the boundaries follow certain surface seawater isotherms (Luning, 1990). Temperature is a very pronounced variable along many coasts with average water temperatures varying from around 0ºC to about 27ºC and such temperatures are bound to affect algal distribution (Round, 1981). The cold temperate waters are among the highest productive of the oceans and are characterised by strong seasonality. Winter temperatures lie between zero and +10ºC with annual temperature variations in coastal surface waters around 10-20ºC (Michanek, 1979). Phycologically the cold temperate

zone has the highest developed rockweed and kelp vegetation, particularly *Fucus* and *Laminaria* in the Atlantic although these genera do stretch into neighbouring regions.

2.5.2 Phytogeographical zones

Temperature regimes are used in delineating phytogeographic regions and provinces and seaweed distributions on a global scale. Phytogeography involves the determination of the extent of the spread of individual species and also the classification of regions into distinct biological zones based on species complements (Round, 1981). Van den Hoek (1975) distinguished five phytogeographic regions along the coasts of the northern Atlantic Ocean which can be attributed to surface water temperatures **(Figures 2.6 and 2.7)**. The much larger extension of the cold and warm temperate regions along the eastern Atlantic coast compared with that along the western Atlantic coast is caused by a system of currents which bring relatively warm water to northern Norway.

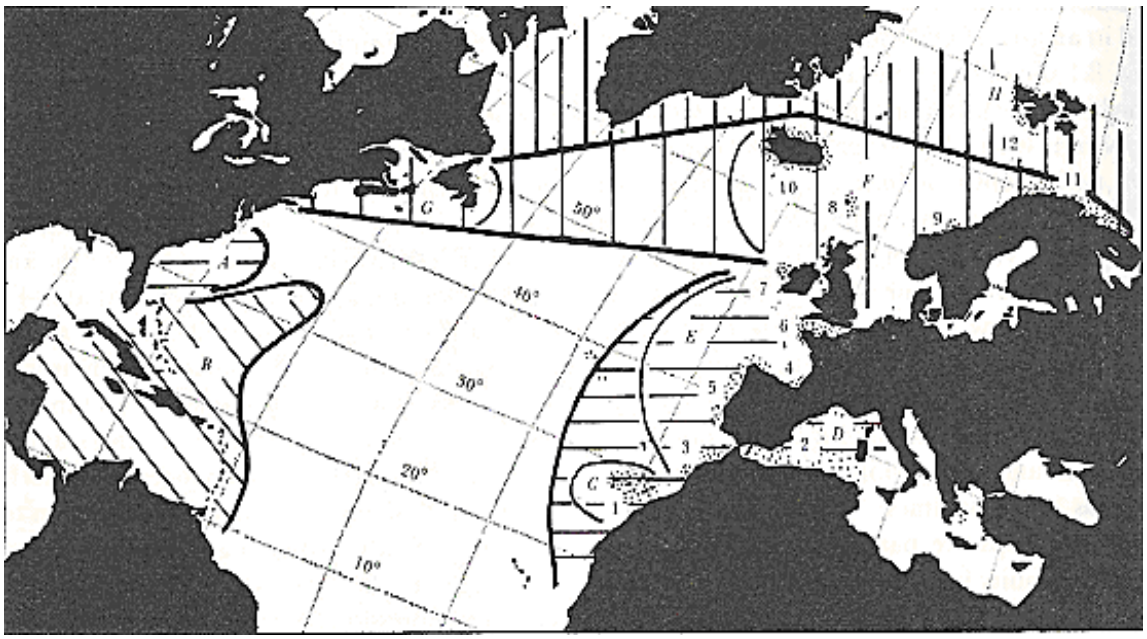


Figure 2.6: The phytogeographic regions and provinces along the northern Atlantic coasts. A, warm temperate Carolina region; B, tropical western Atlantic region; C, Canaries province; D, Mediterranean province; E, Lusitanian province of warm temperate Mediterranean-Atlantic region; F, Eastern province; G, Western province of cold temperate Atlantic-Boreal region; H, Arctic region (taken from van den Hoek, 1975).

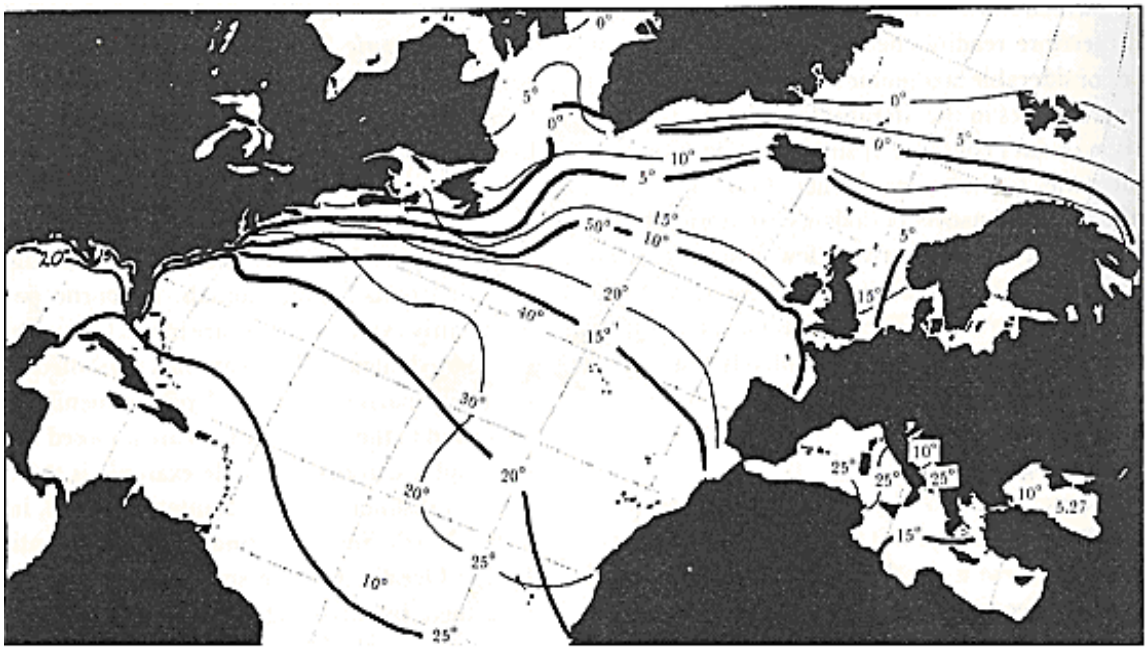


Figure 2.7: Surface temperatures in February (thin lines) and August (thick lines) (taken from van den Hoek, 1975).

The phytogeography of macroalgae such as *Fucus* along the coasts is complicated by their association with certain types of substrata, degree of wave action and vertical zonation on the shore however comparable habitats can usually be found over wide geographic ranges. One of the difficulties with coastal phytogeography is that isolated records can occur outside the main zone of growth as in the case of *F. distichus* subsp. *anceps* and *F. distichus* subsp. *edentatus*. Algal phytogeography is dependent upon precise knowledge of species such as *Fucus* where taxonomic problems are considerable and therefore data must be examined critically (Dring, 1992).

2.5.3 Floristic discontinuities

Floristic discontinuity can be seen in the analysis of eleven seaweed floras from the N.E. Atlantic ranging from Morocco to Spitzbergen **(Figure 2.8)** (van den Hoek, 1975). There is a gradual change in the flora between Morocco and N.W. Brittany with some northern species replacing southern ones. However between N.W. Brittany and the Faeroes, a sharp change can clearly be seen. The Clare Island flora on the west coast of Ireland is clearly transitional with a large decrease in the number of species. The coasts around the British Isles constitutes a border region where the southern limits of many northern species are situated and a marked difference can be seen between the oceanic influenced west coasts and the neritic regions of the North Sea and the eastern English

Channel (Lewis, 1964; Dring, 1992). The distributions of many species in the British Isles follow summer or winter isotherms **(Figure 2.9)**. It is predicted that the main effect of climate change and warming will be a shift in species' boundaries (Hiscock *et al.*, 2004).

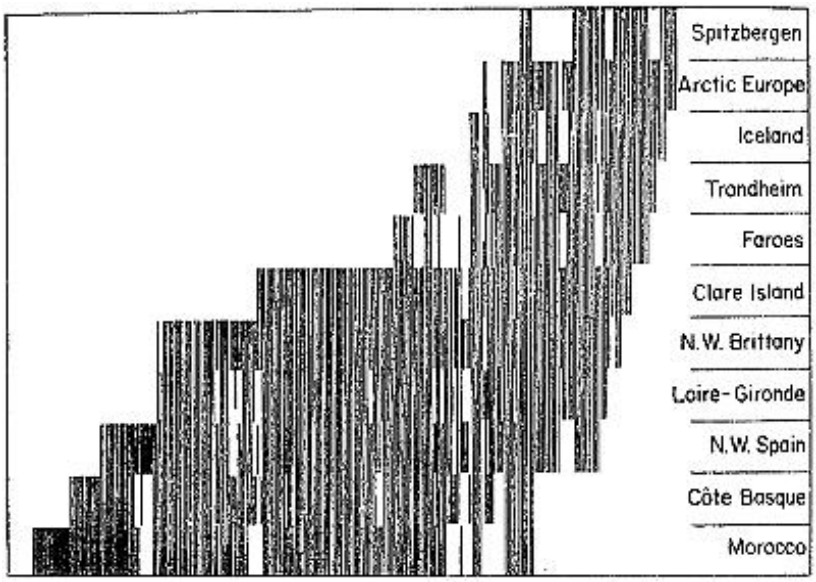
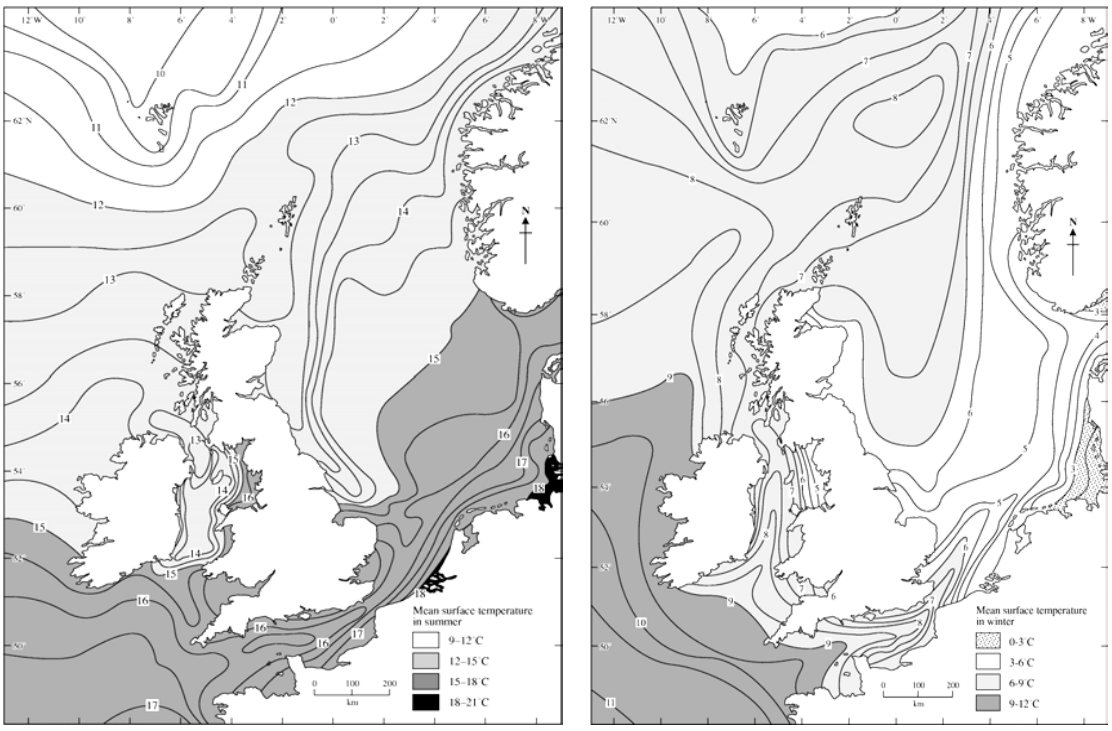


Figure 2.8: Latitudinal distribution of seaweed species on 11 coasts in the N.E. Atlantic. Each line represents the distribution span of one species (taken from van den Hoek, 1975).



(a) (b)

Figure 2.9: Summer (a) and winter (b) isotherms for surface waters around the British Isles (taken from Hiscock *et al.*, 2004).

2.6 Local Factors Affecting Distribution of *Fucus* Species on Intertidal Rocky Shores

The worldwide distribution patterns of seaweeds are mainly determined by global temperature gradients but these patterns are then locally modified by both biotic (competition and grazing) and abiotic (physical and chemical) factors. Many environmental factors affect the distribution, growth and morphology of *Fucus* species on intertidal rocky shores e.g. desiccation (Thomas and Turpin, 1980; Haring *et al*., 2002), light (Terry and Moss, 1981; McLachlan and Bidwell, 1983), nutrient availability (Schonbeck and Norton, 1979; Thomas *et al*., 1985), wave exposure (Denny *et al*., 1989; Kiirikki, 1996; Blanchette *et al*., 1999) and temperature (Strömgren, 1977; Pearson and Davidson, 1993, 1994; Li and Brawley, 2004). Observations have indicated that temperature is important not only on a geographical scale but locally as well (Norton *et. al*., 1981).

2.6.1 Vertical and horizontal distribution of Fucus species

The zonation of dominant rocky shore organisms is a well-known feature of seashore ecology where the vertical zonation patterns that are often seen are the vertical distribution limits of many species, each species having an upper and lower limit. A vertical strip of the shore may logically be divided into zones determined by the organisms present e.g. a *Fucus* zone, a barnacle zone etc. rather than in terms of tide levels because of the modifying effect of wave action (Dayton, 1971). As well as zonation patterns of organisms there is a decline in species number with increasing height, this being a reflection of the increasing harshness of physical conditions going up the shore. The upper and lower limits of many intertidal algae may be controlled by competitive exclusion although propagule dispersal, settlement and predation (Charters *et al*., 1971; Fletcher and Callow, 1992) are possible explanations to vertical distribution on intertidal rocky shores. The horizontal distribution of species along a shore is due to the modification of zonation by local topographic features such as slope, aspect, rock pools, crevices and wave exposure. Wave exposure is one of the best known modifying factors and the results can be changes to species present, heights at which they occur on the shore, the physical form of an organism within a single species such as *Fucus* and the vertical extent on shore.

2.6.2 Sea temperature and its effect on intertidal Fucus species

Several authors have investigation the effect of sea temperature on the growth of *Fucus* species using laboratory studies on juvenile and adult life stages. Strömgren (1977) showed that growth rate of apical sections of five species of intertidal Fucales, *P. canaliculata, F. spiralis, F. vesiculosus, F. serratus* and *Ascophyllum nodosum* from Trondheims-fjorden, Norway, showed an optimum temperature below 17.5ºC and that increased temperature may greatly enhance the growth of brown seaweeds in the intertidal zone but that high temperatures of 30-35ºC were lethal to all species with a survival time corresponding to their vertical zonation in the natural habitat. McLachlan *et al*. (1971) on studies of *Fucus* species from the Nova Scotia, Canada cultured *F. distichus* subsp. *distichus, F. distichus* subsp. *edentatus, F. serratus* and *F. vesiculosus* from zygotes at different temperatures for a two year period. The results showed that initially all species grew more rapidly at 15ºC, with *F. serratus* maintaining increased rate compared to other temperatures. *F. d.* subsp. *distichus* and *F. d.* subsp. *edentatus* showed thallus deterioration after 2-3 months at 15ºC however thalli remained healthy at 5-13ºC. All species grew slowly at 5ºC. However, further studies by McLachlan (1973) of *edentatus* and *distichus* showed that there was no indication that temperature, at least up to 15ºC, had any morphogenetic effects on growth and development of embryos. At 5ºC growth was very slow but it was impossible to prevent germination either in darkness or light and when transferred to higher temperatures growth and development proceeded rapidly. The authors also showed that in *distichus* receptacles can mature at low temperatures, confirming their ecological observations.

The early development stages, in particular the germination of the zygote, represents a critical stage in the life history of algae. Temperature experiments on the germination of four species of Fucales *P. canaliculata, F. spiralis, F. vesiculosus* and *F. serratus* (Terry and Moss, 1981) showed that successful germination in laboratory experiments related to the species zonation on the shore and to their seasonal release of gametes. The three species of *Fucus* were able to germinate in the dark unlike *P. canaliculata* which was strongly inhibited under dark conditions. The authors suggested that distribution of this species in nature is largely controlled by factors acting on embryos and that these responses may be different from those of mature plants and that optimum temperature range may vary with stage of development. The response of embryos to environmental parameters is significant in considering the distribution of *Fucus* species

and temperature is a major controlling factor both on a geographical and local scale. Investigation of the life-history stages is necessary to gain a complete understanding of factors controlling macroalgal distribution (Thomas, *et al*., 1985).

2.6.3 Wave exposure and its effect on intertidal Fucus species

Lewis (1964) showed that biological zones are determined by the actual exposure time during which desiccation develops so that there is a broadening and raising of the zones as the degree of coastal exposure to waves is increased **(Figure 2.10)**. For exposure forms of *Fucus* such as *F. distichus* subsp. *anceps*, *F. spiralis* f. *nanus* and *F. vesiculosus* var. *linearis* this 'extending' of the intertidal area due to wave exposure may allow these species to find an ecological niche at the top of the shore where they are out-competed by other species further down the shore. **Figure 2.11** represents some patterns of zonation found on exposed shores. Wave exposure can affect the biology of algae from a change in size, morphology and form with a general trend of decreasing thallus size with increasing wave exposure (Burrows and Lodge, 1951). Studies of *F. gardneri* (= *F. d.* subsp. *evanescens*) showed that wave exposure set a limit to plant size (Blanchette *et al*., 1999).

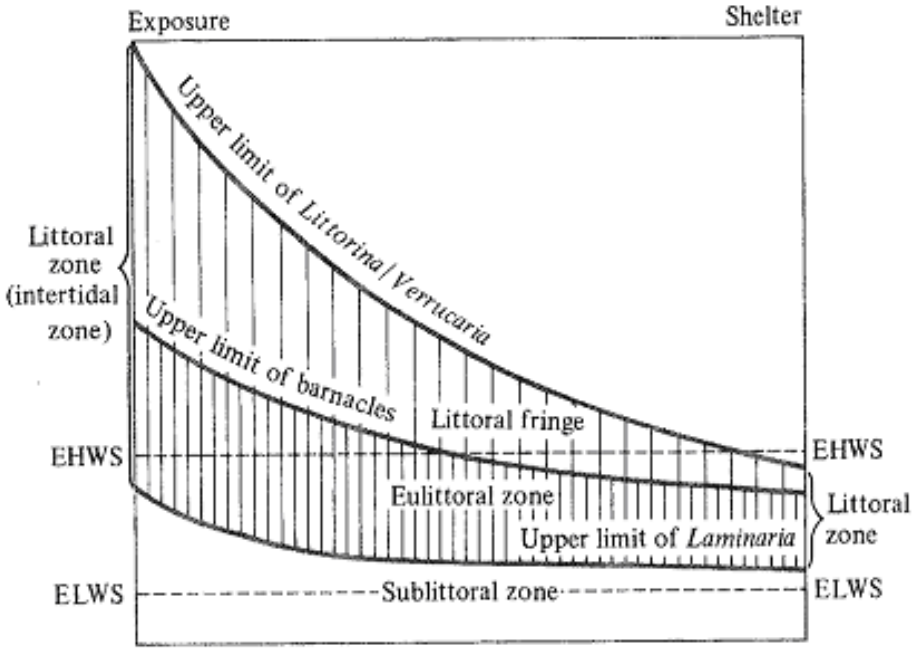


Figure 2.10: Lewis's scheme for intertidal zonation illustrating the effects of wave exposure in broadening and raising the zones. EHWS, extreme high water of spring tides. ELWS, extreme low water of spring tides (taken from Lewis, 1964).

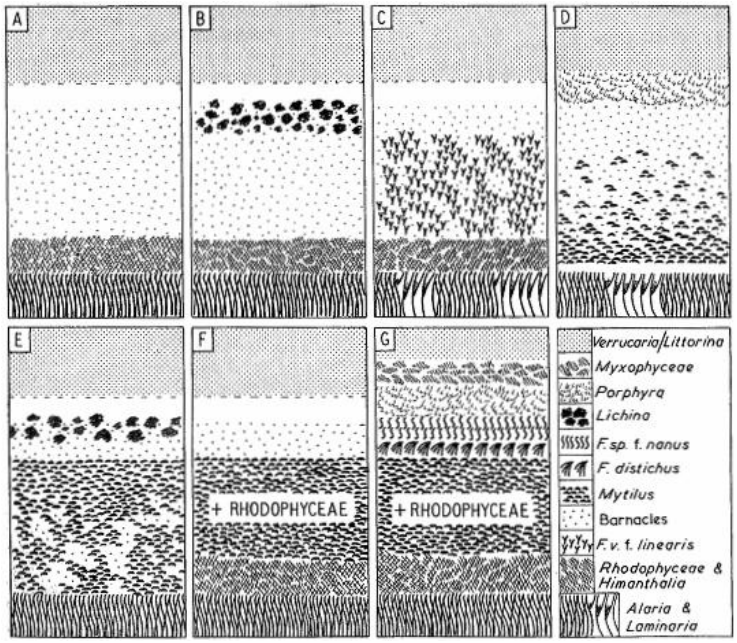


Figure 2.11: Representation of some patterns of zonation of exposed shores (taken from Lewis, 1964).

2.7 Worldwide Distribution of *Fucus distichus* L. emend. Powell

Fucus distichus is a species with a wide Arctic to temperate latitudinal range. Worldwide distribution and geographical limits of the species are given in **Figure 2.12** and **Table 2.4** respectively (no differentiation is made between subspecies). **Table 2.5** denotes worldwide distribution specific to the four subspecies of *Fucus distichus* described by Powell (1957a).

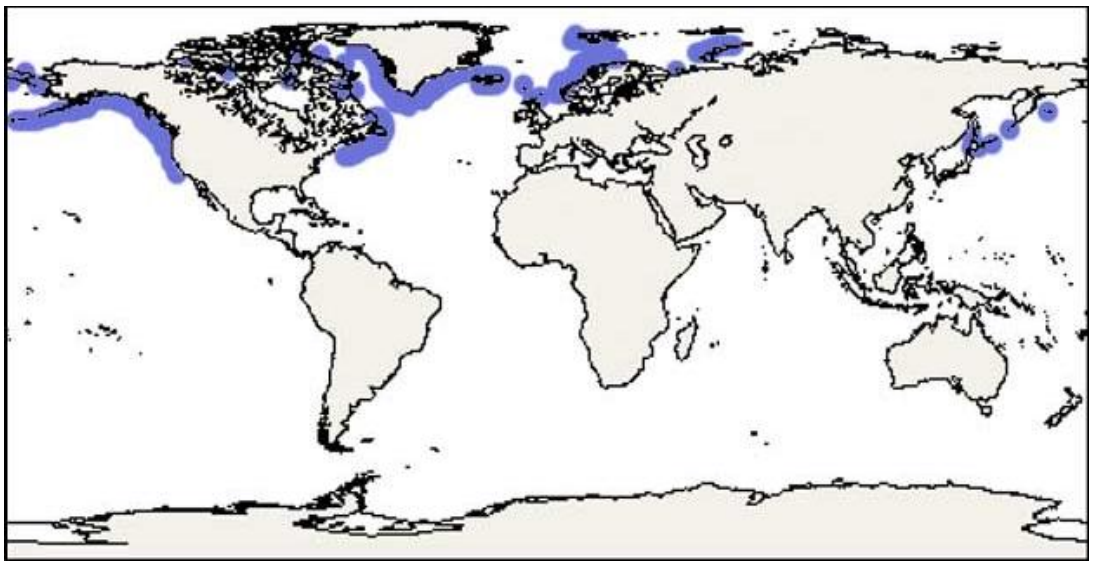


Figure 2.12: Worldwide distribution of *Fucus distichus* (taken from The Marine Life Information Network (MarLIN, 2008)).

Table 2.4: Geographical limits of *Fucus distichus* L.

Northern limits	Southern limits			
Middle-Arctic	Atlantic		Pacific	
	Europe/Africa	North America	North America	North Asia
	Rep. of Ireland	Virginia	Middle-California	North Japan

Table 2.5: Worldwide distribution of the four subspecies proposed by Powell (1957a).

Subspecies	**Worldwide Distribution**
F. distichus subsp. *anceps*	North-west Russia (Barents Sea and White Sea); North, West and South Norway; Iceland; Greenland; Faeroe Islands; British Isles (North and West Scotland and Ireland); Canada (Quebec, Newfoundland, Nova Scotia); Atlantic U.S. (Maine, New Hampshire, Massachusetts).
F. distichus subsp. *edentatus*	North-west Russia (Barents Sea and White Sea); North and West Norway; West Sweden, Denmark; Iceland; Faeroe Islands; Shetland Isles, Fair Isle, Outer Hebrides, Macduff Harbour N.E. Scotland; Greenland; Atlantic coast of Canada and U.S.A.; Pacific coast of Canada and U.S.A.; Sea of Okhotsk, North Japan.
F. distichus subsp. *distichus*	Kara Sea; Barents Sea; White Sea; North and West Norway; Iceland; Faeroe Islands; Greenland; Atlantic coast of Canada and U.S.A.; Sea of Okhotsk, North Japan; British Isles on Shetland Mainland.
F. distichus subsp. *evanescens*	Arctic and sub-arctic, circumpolar. Siberian Polar Sea; North-west Russia; Arctic Ocean, Novaya Zemlya, Svalbard, Bear Island; Greenland; Iceland; American Polar Sea; Atlantic Canada; Atlantic U.S.A.; Pacific Canada; Pacific U.S.A.; Bering Sea; Aleutian Islands; North Japan, Sea of Okhotsk, Kurile Islands. *F. distichus* subsp. *evanescens* has not been found in Britain and the nearest locality is probably Iceland.

2.7.1 Distribution of Fucus distichus L. emend. Powell in the British Isles

Of the four subspecies re-established and emended by Powell (1957a) only three of the subspecies, *anceps*, *edentatus* and *distichus*, have been found in the British Isles. **Figure 2.13** and **Figure 2.15** show the distribution of *anceps* and *edentatus* respectively, taken from The National Biodiversity Network (NBN) (Hardy and Guiry, 2003; Hiscock and Kimmance 2003). At present subspecies *distichus* has only been recorded on the Shetland mainland. **Table 2.6** gives the localities of each subspecies. NBN distribution maps are based on species records from numerous national and regional surveys and recording schemes submitted to the NBN by a large number of organisations including The British Phycological Society (BPS), Joint Nature Conservation Committee (JNCC) and Scottish Natural Heritage (SNH). The majority of these records come from published scientific papers by renowned phycologists however there are some instances where the recorder is unknown and/or it is unclear whether these records have been verified and plants correctly identified.

A case in point can be seen in **Figure 2.13** where three recordings of *anceps* are suspected of being incorrect. The two records denoted by circles from Bressay, Shetland and Macduff Harbour are most certainly records for *edentatus* as *anceps* has not been recorded from either of these sites. These may be administrative and/or map compilation errors rather than incorrect identification. However the record denoted by the square in **Figure 2.13** for Loch Alsh, The Highlands is a particularly dubious record. The ecology of this subspecies and other records for *anceps* show that it is only found on extreme wave exposure coasts which are subject to considerable swell and wave-action. It can be seen from **Figures 2.13 - 2.14** that this is not the case at Loch Alsh, being a sea loch and therefore sheltered from the full force of the Atlantic Ocean by land mass. It is very doubtful whether this recording is indeed *anceps*. This subspecies has in the past been mistaken for *Pelvetia canaliculata* due to its small form, location in the upper part of the littoral zone and characteristic arching of the distal branches.

F. distichus subsp. *anceps* is rare in the British Isles and proper identification and accurate recordings are vital if this species is to be used as a possible indicator of sea temperature rise and climate change.

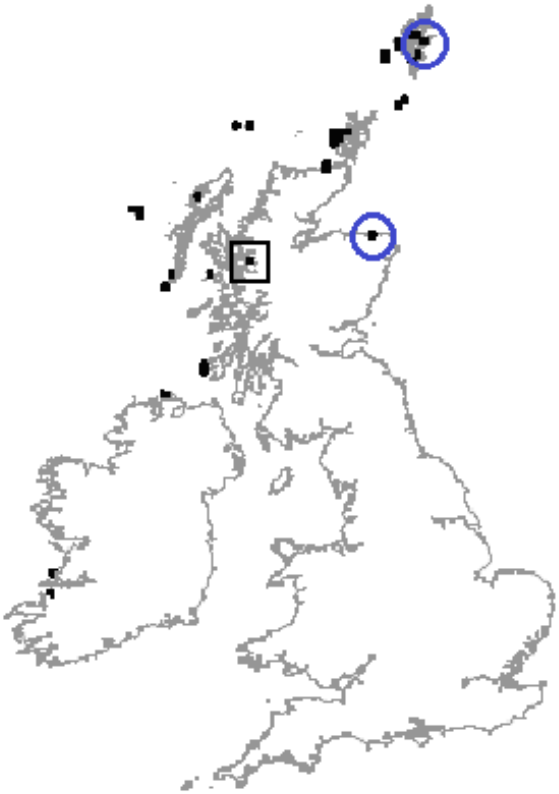


Figure 2.13: Map showing distribution of *Fucus distichus* subsp. *anceps* in Britain and Ireland (Crown Copyright reserved, 2004). Suspected incorrect recordings are denoted by a square for Loch Alsh, Highlands and circles for Bressay, Shetland and Macduff Harbour, Moray Firth.

Figure 2.14: Map showing location of suspected incorrect recording of *F. distichus* subsp. *anceps* at Loch Alsh, Highlands (from Ordnance Survey, Crown Copyright reserved, 2004).

Figure 2.15: Map showing distribution of *F. distichus* subsp. *edentatus* in Britain (Crown Copyright reserved, 2004).

Table 2.6: Distribution of *F. distichus* L. *emend.* Powell in Britain and Ireland.

Subspecies	Distribution
F. distichus subsp. *anceps*	**Scotland:** Orkney Islands (Powell, 1957b, 1963a). Old Man of Hoy (Hiscock, 1995). Shetland mainland (British Phycological Society, 1962; Powell, 1963a; Irvine, 1972; Russell, 1974). Fair Isle (Powell, 1957b; Burrows, 1963; Hiscock, 1987). North mainland Britain, Caithness (Powell, 1957b). Outer Hebrides: Island of Lewis-Harris (Powell, 1957b); North Rona (Powell, 1958; Gilbert *et al*. 1973; Norton & Powell, 1979); St. Kilda (Powell, 1957b; Russell, 1974; Norton & Powell, 1979); Sula Sgeir*; Mingulay, Barra (Northen, 1996)*. Inner Hebrides: Canna*. Islay (Hiscock, 1982). Loch Alsh, Western Isles** **Ireland:** Kilkee, Co. Clare. Malin Head, Co. Donegal. Kerry Head, Co. Kerry (Powell, 1957b)
F. distichus subsp. *edentatus*	Shetland Islands (Powell, 1957b, 1963a; British Phycological Society, 1962; Burrows, 1963; Russell, 1974). Fair Isle (Powell, 1957b). Outer Hebrides: North Rona (Powell, 1957b, 1963a; Gilbert *et al*. 1973; Norton & Powell, 1979). Macduff harbour, north-east Scotland (Powell, 1980)
F. distichus subsp. *distichus*	In Britain subspecies *distichus* has been found only on the Shetland Mainland (British Phycological Society, 1962; Powell, 1963a; Russell, 1974)

*Records where identification unverified. **Probable incorrect identification due to sheltered site location.

Chapter 3: General Methods

This chapter sets out the general techniques used to investigate the effects of temperature on the growth of *Fucus* spp. in both juvenile and adult life stages. The methods used to obtain gamete release and fertilisation, measurement of growth of germling and adult plants, the metabolic effects of temperature on adult plants and the method used to look at phenetic variation patterns within *Fucus* species are discussed. Any variations to these for individual experiments are specified in the relevant sections for those experiments. *Fucus* species investigated and population sampling sites are given in **Table 3.1** and **Figures 3.1 – 3.5**.

The method of germling measurement used by Scanlan (1985) on toxicity testing of *Fucus* germlings was adopted in this instance. The technique used for growth measurement of excised tips of adult *Fucus* plants for change in surface area was adapted from previous workers Burrows (1964) and Telfer (1995). Variation in rates of photosynthesis and respiration using the light and dark bottle technique (Strickland and Parsons, 1972) and a method used by Thomas (1988) for aquatic macro-flora was used to assess the effects of temperature on the performance and metabolism of adult *Fucus* plants.

The principles of numerical taxonomy (Sokal and Rohlf, 1969; Sneath & Sokal, 1973) and multivariate analysis (Ho, 2006) were used to assess similarities and differences between geographically different *Fucus distichus* population samples using measurements of observable characteristics and variation. The characters used were based on previous work by Rice and Chapman (1985).

3.1 Algae Used in this Study

Table 3.1: *Fucus* populations studied in this investigation with collection sites, national grid references and present author's codes. (Fda = *F. distichus* subsp. *anceps*; Fspna = *F. spiralis* f. *nanus*; Fveslin = *F. vesiculosus* var. *linearis*; Fspir = *F. spiralis*; Fserr = *F. serratus*; Fves = *F. vesiculosus*; Fde = *F. distichus* subsp. *edentatus*: O = Orkney; I = Ireland; M = Macduff; Shet = Shetland; SQ = South Queensferry).

Species	Location	Nat. grid	Code
Fucus distichus subsp. *anceps*	Bay of Skaill (South), Orkney Islands	HY226190	OFda
Fucus distichus subsp. *anceps*	George's Head, Kilkee, Co. Clare, Rep. of Ireland	W088162	IFda
Fucus distichus subsp. *edentatus*	Bressay, Shetland Islands	HU475434	ShetFde
Fucus distichus subsp. *edentatus*	Macduff Harbour, Moray Firth, Scotland	NJ702645	MacFde
Fucus serratus	Bay of Skaill (North), Orkney Islands	HY232196	OFserr
Fucus serratus	South Queensferry, West Lothian, Scotland	NT140784	SQFserr
Fucus spiralis	Bay of Skaill (North), Orkney Islands	HY232196	OFspir
Fucus spiralis	South Queensferry, West Lothian, Scotland	NT140784	SQFspir
Fucus spiralis f. *nanus*	Bay of Skaill (South), Orkney Islands	HY226190	OFspna
Fucus vesiculosus	Bay of Skaill (North), Orkney Islands	HY232196	OFves
Fucus vesiculosus	South Queensferry, West Lothian, Scotland	NT140784	SQFves
Fucus vesiculosus var. *linearis*	Bay of Skaill (South), Orkney Islands	HY226190	OFveslin
Fucus vesiculosus var. *linearis*	George's Head, Kilkee, Co. Clare, Rep. of Ireland	W088162	IFveslin

3.2 The Study Areas and Collection Sites

3.2.1 Bay of Skaill, Orkney Islands

Facing the Atlantic Ocean, this very exposed site consists of a central sandy bay changing progressively to a rocky substratum on both the north and south ends of the bay. The north part of the shore is composed of gradually sloping rock ridges and platforms with large boulders on the upper shores and displays typical patterns of algae zonation. At the southern end of the bay there is a large extremely exposed rocky outcrop where the large splash zone results in algal cover to a substantial height above high water.

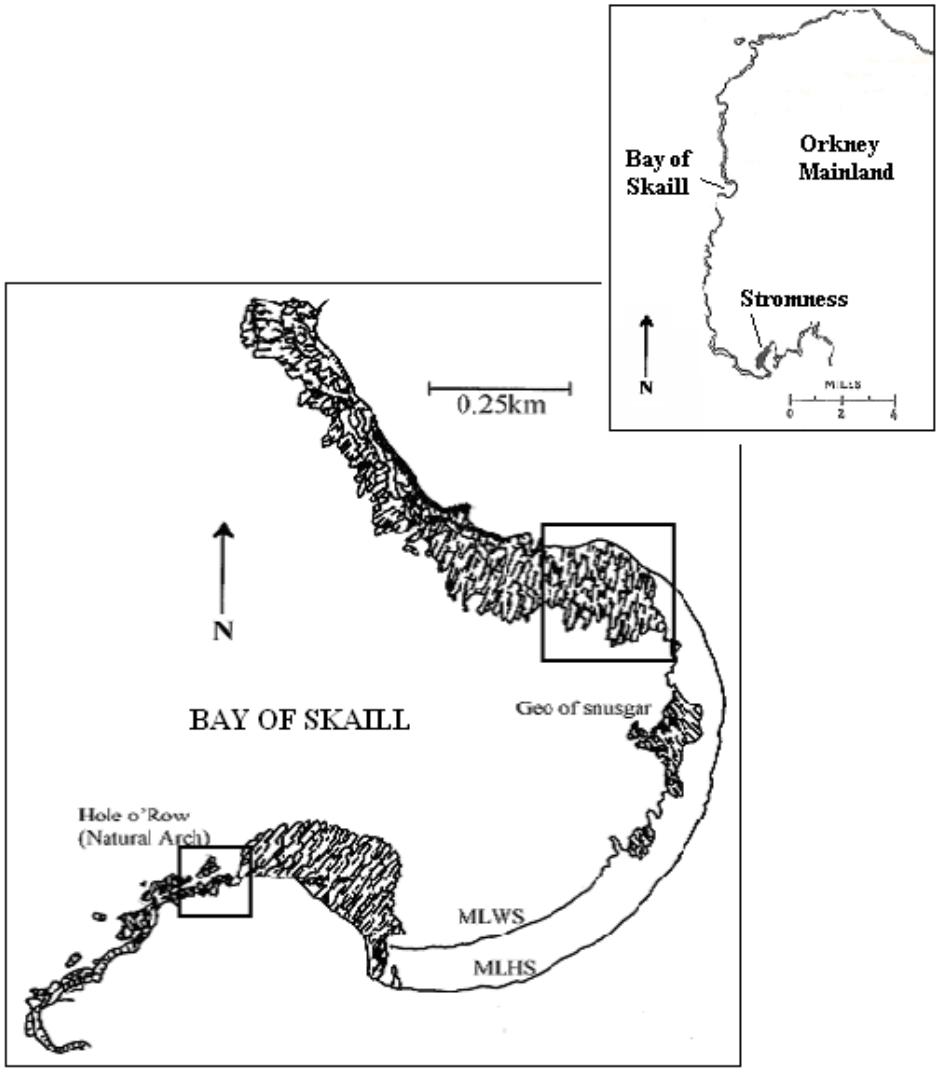


Figure 3.1: Map of Bay of Skaill, Orkney with collection sites for OFspir, OFserr, OFves, (North) and OFda, OFveslin (South), indicated by boxes. MLWS = mean low water of spring tides. MLHS = mean high water of spring tides (adapted from JNCC, 1997).

3.2.2 George's Head, Kilkee, Co. Clare, Rep. of Ireland

Facing N.N.W. and the Atlantic Ocean this exposed reef slopes smoothly down into deep water with an angle of slope estimated to be 10-15º (Powell, 1957b) and is much affected by swell and waves.

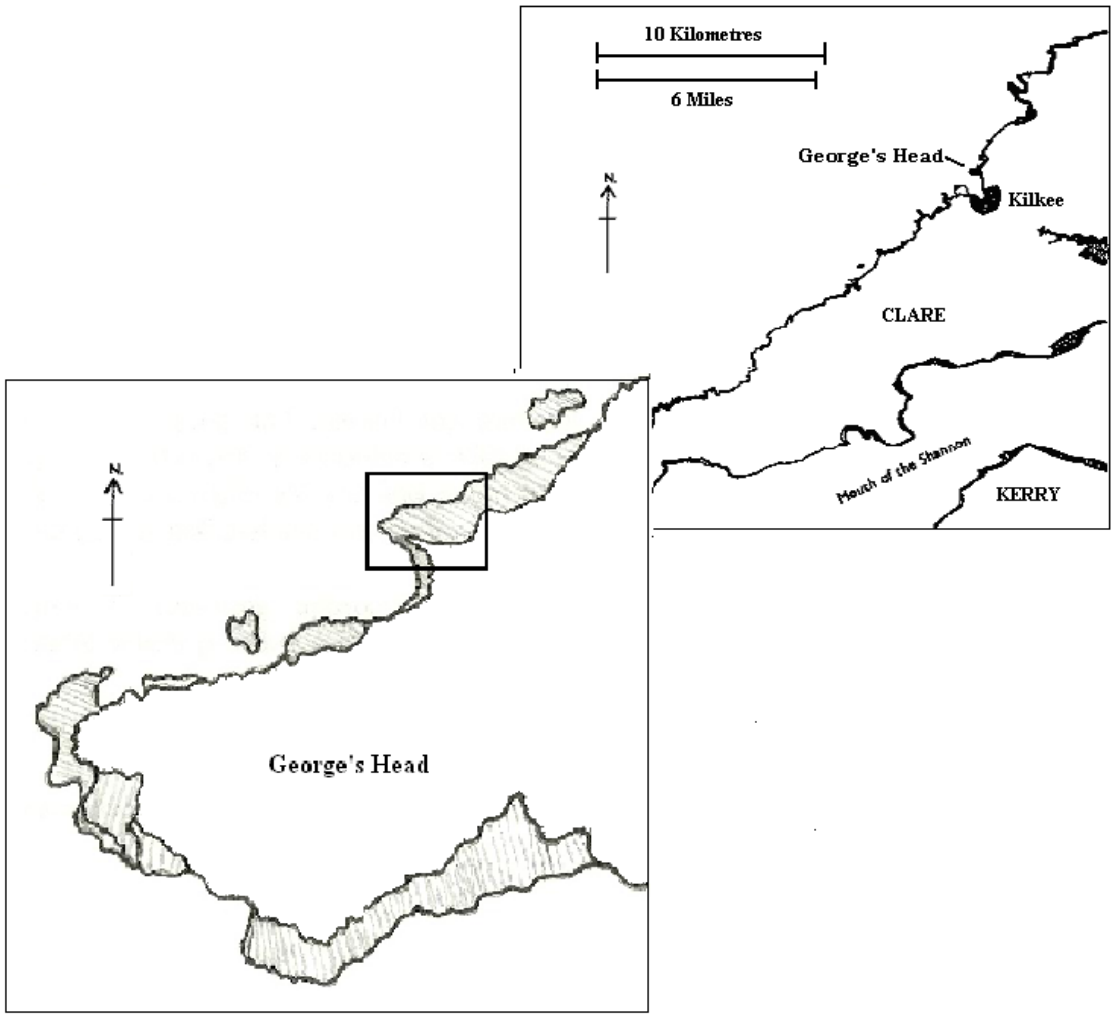


Figure 3.2: Map to show location of George's Head, Kilkee, Co. Clare, Ireland with collection site for IFda and IFveslin indicated by box (adapted from Powell, 1957b).

3.2.3 Macduff Harbour, Moray Firth, Scotland

Growing on harbour walls and marks the southern limit and the only known occurrence of subspecies *edentatus* on the British mainland.

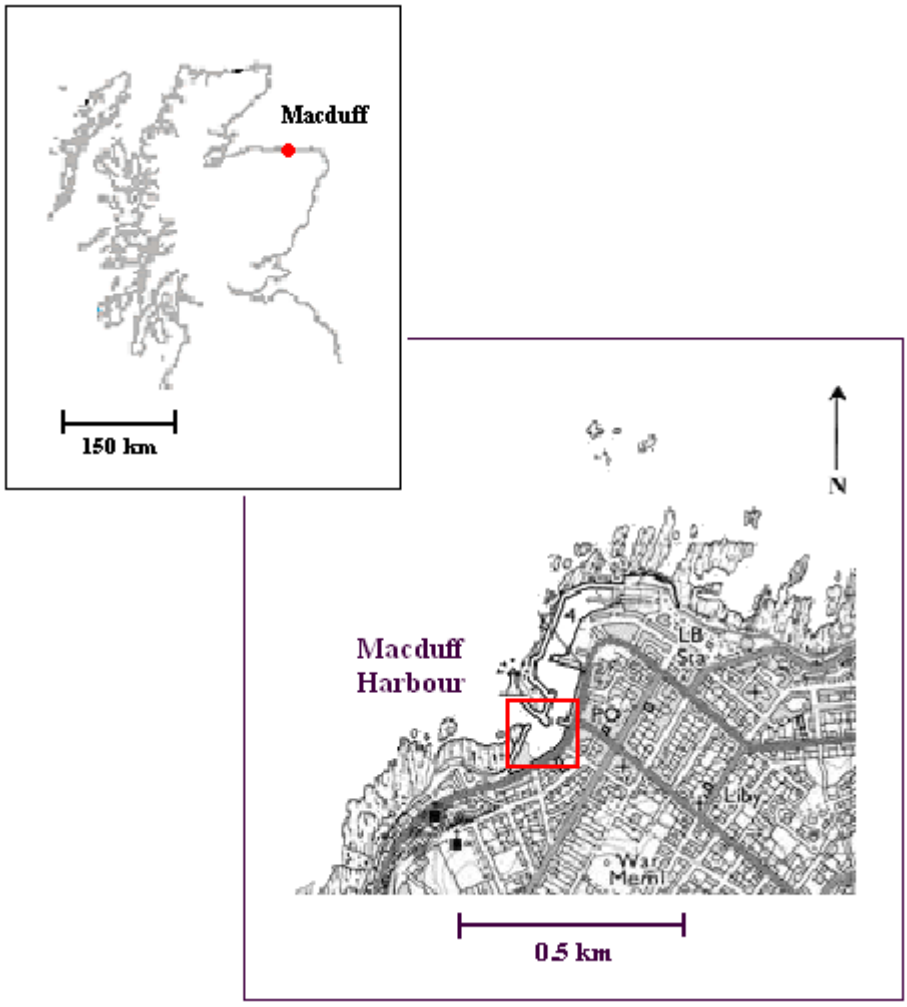


Figure 3.3: Map to show location of Macduff Harbour, Banffshire with collection site for MacFde indicated by box (from Ordnance Survey, Crown Copyright reserved, 2009).

3.2.4 Bressay, Shetland Islands

The site is situated on an area of sheltered shore near a breakwater.

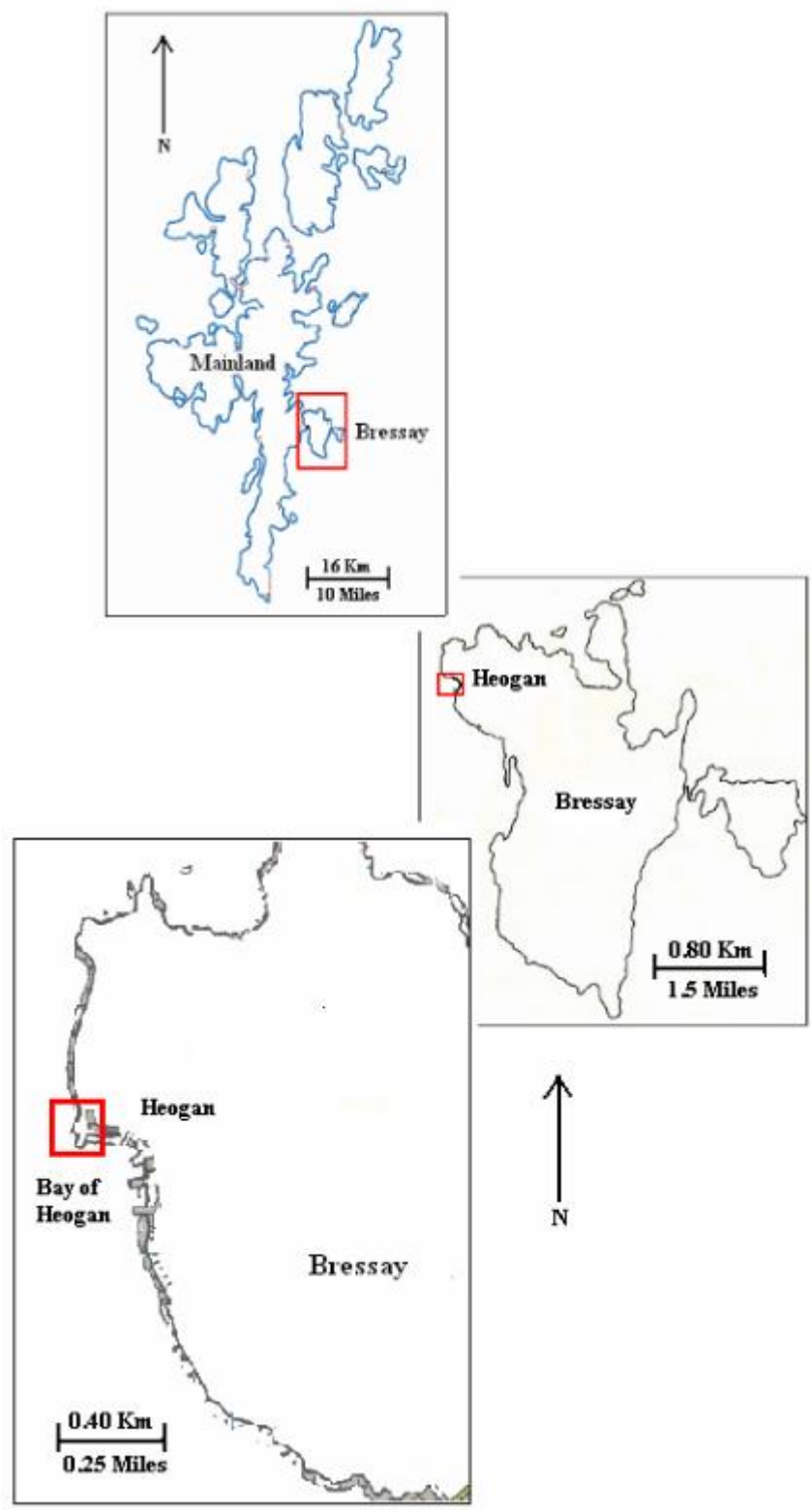


Figure 3.4: Map to show location of Bressay, Shetland Islands with collection site for ShetFde indicated by boxes (Crown Copyright reserved, 2009).

3.2.5 South Queensferry, Firth of Forth, West Lothian, Scotland

The site is situated on a gently sloping sheltered shore on the Firth of Forth.

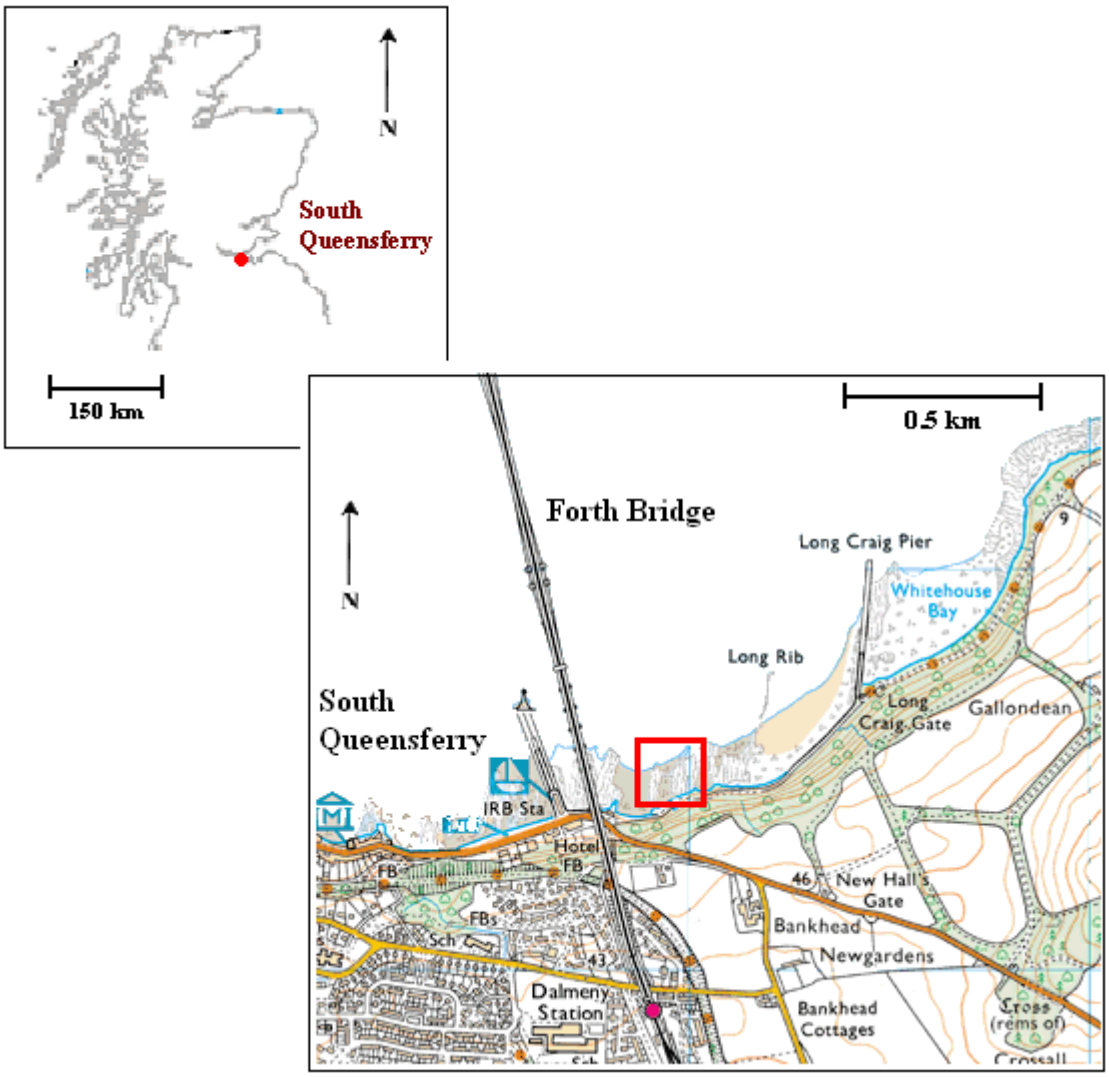


Figure 3.5: Map to show location of South Queensferry, West Lothian with collection site for SQFserr, SQFspir and SQFves indicated by box (from Ordnance Survey, Crown Copyright reserved, 2009).

3.3 Collection, Storage and Preparation of Plants and Equipment

Collection of plants for both germling and adult growth experiments concentrated on healthy specimens as free from epiphytes as possible. Plants were gently shaken to remove any animals attached and placed in double polythene bags for transportation. On return to the laboratory plants were placed immediately in large plastic tanks containing seawater and aerated with air pumps and left to acclimatise in constant temperature rooms (CT) at 5°C, 10°C and 15°C for 24 hours. If, as in the case of Ireland, Orkney and Shetland collections, this acclimatisation procedure was not immediately possible then plants were kept refrigerated until such time as laboratory facilities were available and transported in cool bags containing ice packs before acclimatisation in CT rooms.

For successful gamete release plants with mature, well developed receptacles with a slight mucus discharge were selected. In dioecious *Fucus* species, female plants have an olive green colouration to the receptacles whereas male plants exude a yellow/orange antheridia discharge. Plant collection was taken from all areas of the intertidal zone for that particular seaweed where reasonably possible to ensure a true representation of the population. Wherever possible, seawater for the adult and germling growth experiments was collected from the site area. To minimise contamination from diatoms, unicellular and filamentous green algae all seawater used in experiments was filtered through Whatman GF/C glass fibre paper, approximate pore size ~1μm.

3.3.1 Preparation of glass and plastic ware

All glass, plastic ware and filter meshes used in experimental work were washed in detergent (Decon 90) using a soft brush and rinsed 3 times under cold tap water and where appropriate rinsed in distilled water or test medium.

3.3.2 Culture conditions

Throughout the investigations all growth experiments and cultures including fertilisation, gamete release and germling production were carried out in CT rooms at 5°C, 10°C and 15°C (±1°C) using GF/C filtered seawater. Photosynthetically active

radiation (PAR) of 80μE $m^{-2}s^{-1}$ at water surface was provided by Philips White-light fluorescent tubes and photoperiod set at 12:12, light:dark (07.00 – 19.00 hrs).

3.4 Gamete Release and Germling Production

After some preliminary work and using techniques adapted by other workers (Peterson and Torrey, 1968; Pollock, 1970; McLachlan *et al*., 1971; Quatrano, 1980), a technique was developed to obtain large numbers of fertilised eggs using an easily repeatable method.

3.4.1 Preparation of receptacles

For dioecious species such as *F. serratus*, *F. spiralis*, *F. vesiculosus* and *F. vesiculosus* var. *linearis*, plants were initially separated by sex by sight. Male reproductive plants exude an orange discharge whereas female plants produce a yellow discharge. Once in the laboratory the sex of plants was confirmed by sectioning receptacles and examining under a compound microscope for either antheridia (male) or oogonia (female). Although this procedure is not needed for monoecious/hermaphrodite species as gametes of both sexes are formed in the same conceptacle, it was very useful in identification between dioecious *Fucus vesiculosus* var. *linearis* and monoecious *Fucus distichus* subsp. *anceps* and *F. spiralis* f. *nanus* which are often confused **(Section 2.4.2)**. Only receptacles from plants which could be assigned unequivocally to a specific taxon were used as a source of gametes for culture. Once acclimatised in CT rooms at 5°C, 10°C and 15°C excised receptacles were prepared by lightly brushing with a soft nail brush to remove epiphytes and then rinsed in cold tap water for 1 minute to remove gross contaminants. This procedure was repeated two more times with blotting of receptacles between washes. Receptacles not immediately used were blotted dry, placed in plastic bags and kept in the dark under refrigeration at 4°C with sexes stored separately until required. Receptacles can be stored on ice for up to two weeks as noted by Peterson & Torrey (1968) but McLachlan (1971) found fresh receptacles to have a more consistent development than those stored on ice. In this investigation fresh receptacles were used throughout and wherever possible within a few days of collection.

3.4.2 Gamete release

In dioecious species the sexes were kept and treated separately throughout. Receptacles were rinsed in cold tap water for 1-2 minutes to remove any previously released gametes. This ensured that gametes required for experimentation were released at the same time. After rinsing, female receptacles were placed in CT rooms on blotting paper for 1 hour to dry. Drying of receptacles caused eggs to release from receptacles when later placed in seawater. Receptacles were placed in clear plastic dishes (220 × 115 × 70 mm) containing GF/C filtered seawater and left overnight with a minimum 8 hours illumination (PAR) of 80μE $m^{-2}s^{-1}$ in CT room at temperature relevant to that particular experiment. Male receptacles were placed on moist blotting paper in plastic bags and kept refrigerated overnight at 4°C in the dark. When male receptacles were placed in fresh GF/C filtered seawater in the light, copious amounts of antheridia were released. Monoecious species receptacles were rinsed as described above and gamete release achieved using the process as for female receptacles.

3.4.3 Fertilisation of gametes

Fertilisation of gametes was achieved by a series of filtration and rinses to obtain clean suspension for settlement **(Figure 3.6)**. This process varied for dioecious and monoecious species. In dioecious species the initial filtration and rinses for male and female plants were done separately. Initial filtration of the solution containing sperm or egg suspension was rinsed firstly through 120 μm and then 45 μm nylon mesh to remove debris, clumped antheridia or possible fertilised eggs. This process used large volumes of GF/C filtered seawater to prevent lysing of eggs. A small volume of sperm suspension was added to the egg suspension. This was then gently swirled and placed in a CT room under a light source (PAR) of 80μE $m^{-2}s^{-1}$ for 30 minutes. To encourage fertilisation at this stage a black sheet was placed under the plastic container to reduce the light intensity at the bottom of the dish. Because sperm are negatively phototactic they migrate to the bottom of the dish where the eggs have settled and fertilisation can occur. This combined egg/sperm solution was then filtered through 45 μm nylon mesh to collect the fertilised eggs with repeated rinsing using filtered seawater to remove excess sperm and unfertilised eggs. Fertilised eggs have a diameter of approximately 70-80 μm and are sufficiently rigid to be retained on the nylon mesh whereas unfertilised eggs lack a cell wall and are squeezed through the mesh.

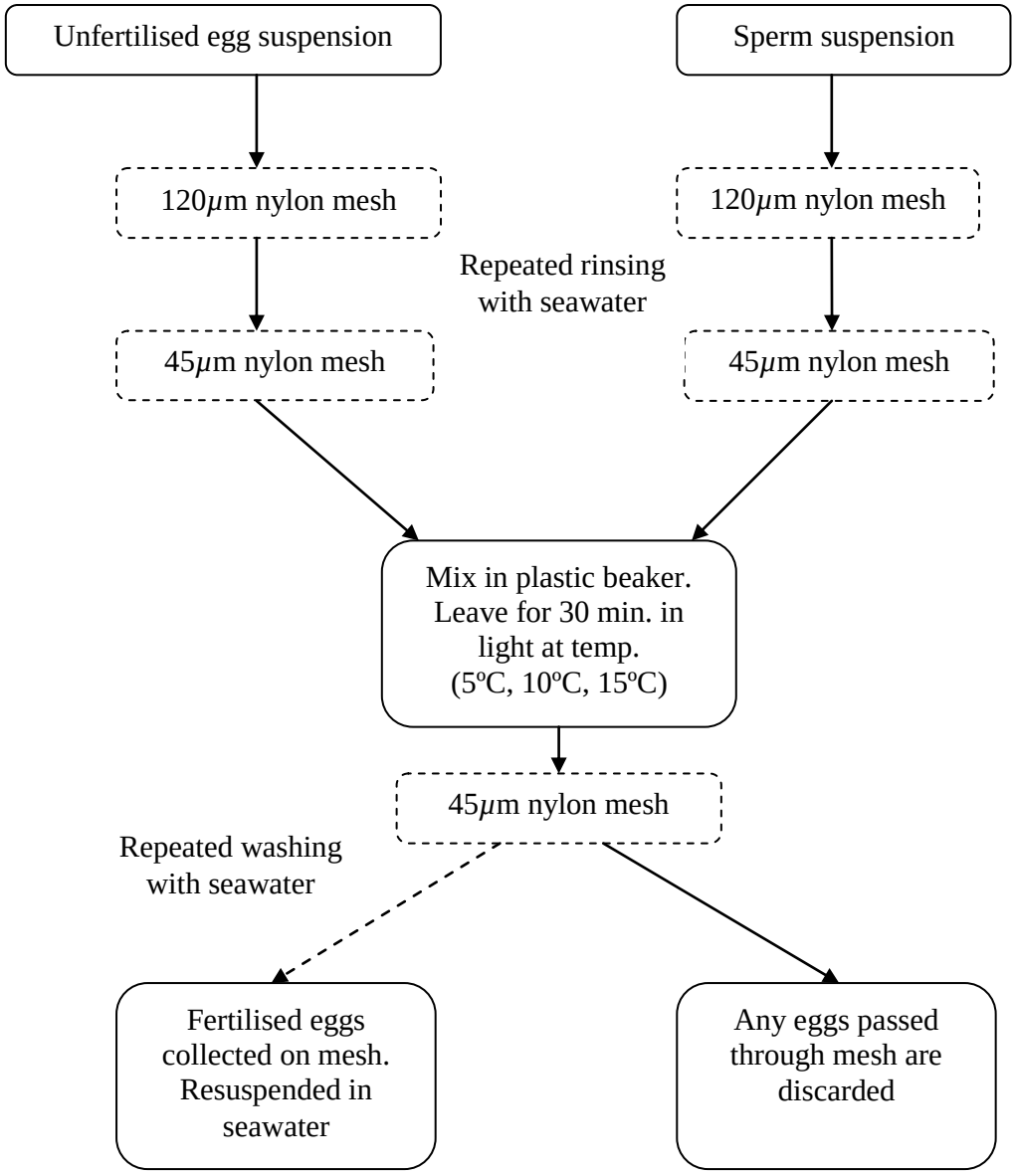


Figure 3.6: Flow diagram illustrating filtration process used to obtain fertilised eggs in dioecious *Fucus* species. Broken lines represent the filtrate and solid lines the supernatant (adapted from Grundy, 1996).

In monoecious species the filtration process was simplified. Receptacles of both sexes were placed in clear plastic dish (220 × 120 × 85 mm) containing GF/C filtered seawater and left overnight with a minimum 8 hours illumination in CT room. The mixed egg and sperm suspension was filtered firstly through 120 μm nylon mesh to remove debris, clumped antheridia and possible fertilised eggs and then through 45 μm nylon mesh to collect the fertilised eggs. In both dioecious and monoecious species fertilised eggs were re-suspended in seawater and this suspension was pipetted directly onto plastic slides in clear plastic containers and left overnight to settle in the CT room

of the appropriate temperature for that experiment. The following day the slides were removed to clean containers with 400ml of medium added which was thereafter changed every 3 to 4 days.

3.5 Growth Measurement of *Fucus* Germlings using the Light Microscope Method

A compound microscope incorporating a graticule was used to measure germlings (Scanlan, 1985). Authors such as Grundy (1996) felt this method difficult due to the curved nature of germlings, particularly those transplanted to the field but preliminary investigations found this the most efficient method at this time of measuring laboratory cultivated germlings.

3.5.1 Measurements of change in head and rhizoid length and total length of Fucus germlings

To allow for comparison increase in growth measurement of different parts of the embryo three measurements were taken for each germling. The component length of head and rhizoid and the total length were measured **(Figure 3.7)**. Scanlan (1985) in her toxicity tests on *Fucus* germlings considered the total length of germlings to be the more accurate due to the unclear division between the head and rhizoid but in this case it was felt that a boundary could clearly be delineated. The length of the longest rhizoid was taken as this was deemed to be the first to grow. One possible problem in taking head measurements was that as the germlings grew and became upright then a foreshortened view of the head was presented. This was resolved by using fine needle forceps to gently tip the germling over on its side thereby allowing viewing of the full head length for accurate measuring. In these instances the rhizoid was always measured first as this procedure could cause the germling to be dislodged from the slide. These "casualties" of the measuring process were removed by rinsing with the treatment medium and using a Pasteur pipette before returning to culture conditions. This loss of germlings was not a problem due to high germling production and settlement rate at the initial stages of cultivation.

1) Egg

2) Early development of rhizoid

3) Development of germling into distinct differentiated areas.

Figure 3.7: Early development of *Fucus* embryo and head and rhizoid components used in germling measurements (taken from Scanlan, 1985).

3.5.2 Method

Each slide contained between 100 – 200 fertilised eggs, each slide being a replicate. 5 replicates and 20 germlings measured per replicate slide giving a total of 100 samples measured for each *Fucus* species at temperatures of 5°C, 10°C and 15°C. Measurements were taken using a graticule (μm) in a compound microscope at ×10 objective lens (magnification × 100). Medium was changed every 3 to 4 days. To ensure accurate and consistent measurements the setting up of germling growth experiments were staggered. Slides were carefully removed from the culture dishes and gently rinsed with the treatment medium using a Pasteur pipette to remove any loose germlings and diatom growth. Germlings were measured using a compound light microscope with a thin layer of water added to prevent desiccation. Stratified random sampling (Dytham, 2003) was used where the total area of the slide was divided into 5 equal plots and 4 measurements taken at random from each plot given a total of 20 measurements per slide. Random sampling of plots was done by moving over the area without looking down the eyepiece and when the movement stopped the germling closest to the centre of field of view was measured. Measurements were taken as quickly as possible and slides returned to the culture conditions to prevent desiccation.

3.6 Growth Measurement of Apical Tips of Adult *Fucus* Plants Using Change in Surface Area

Measurement of percentage change in surface area was used to examine the growth rate of excised tips of *Fucus* plants before and after treatment. The method used here was developed from work carried out by Burrows (1964), Telfer (1995) and Grundy (1996). The technique used by Burrows consisted of calculating the surface area of individual excised tips using graph paper to count the squares or using photographic contact prints to record images and measure the image area with a planimeter. Telfer (1995) determined the change in surface area using a photocopier to record the images of excised tips and analysed these using a magnetic tablet to draw round them. In this present study images were electronically scanned to a computer and the image analysis program ImageJ (Abramoff *et al*., 2004) used to determine the surface area.

3.6.1 Method

Non-reproductive adult plants were used for this laboratory investigation. Excised tips were placed in CT rooms of appropriate temperatures for experiments and left to acclimatise for 24 hours. Apical tips were chosen randomly from the different plants but from the same population. Twenty excised tips of 3 cm in length were used for each replicate, three replicates per species. Adult sections were placed in clear plastic containers with 400 ml of GF/C filtered seawater. Each replicate was placed in a separate culture vessel with lid to prevent contamination and evaporation and incubated at 5°C, 10°C and 15°C. Care was taken to ensure no tips overlapped to prevent shading. The medium was changed every 3 to 4 days.

Prior to and after treatment the tips of each replicate were quickly blotted dry, placed between two A4 sheets of acetate and scanned at 100% original size. Care was taken to prevent overlapping of tips which would give an inaccurate measurement. The before and after images were scanned electronically and measured using an image analysis program ImageJ. Measurements were transferred to spread sheets (Microsoft Excel) and the before and after differences in surface area calculated. Growth was calculated as percentage change in the surface area of the tips.

3.7 Growth Measurement of Apical Tips of Adult *Fucus* Plants Using Wet (Fresh) Weight

Weight measurements can be used for growth estimations when dealing with macroscopic portions of algae such as *Fucus*. This method was used by Schonbeck and Norton (1980) to measure increase in fresh weight of fucoid algae as part of their study on factors controlling lower limits of algae on the shore and by Fletcher and Fletcher (1975) to follow the growth of segments of *Sargassum muticum*. Fresh weight was used to measure growth of the same *Fucus* apical tips used in the surface area experiments to see if there was any growth increase due to something other than length and breadth. Increase in bulk is equally important in consideration of growth (McLachlan *et al*., 1971).

3.7.1 Method

The apical tips prepared for the surface area experiments were weighed before and after the culture period of 100 days. The apical tips were dried between paper towels (consistently for 1 minute) to remove excess water and weighed (g) per replicate on digital scales. 3 replicates per *Fucus* species, each replicate containing 20 apical tips. This was carried out as quickly as possible to prevent shrinkage before returning to relevant CT rooms. General observations on the health of apical sections were noted with regards to texture and discolouration along with any additional growth in the form of reproductivity and regeneration.

3.8 Light and Dark Bottle Method for Measuring Rate of Photosynthesis and Respiration in Adult *Fucus* Plants

Photosynthesis is an important, easily measured metabolic process routinely used as a gauge of environmental effects on seaweeds. This procedure was used to investigate the effect of temperature between and within *Fucus* species from different geographic locations. Non-reproductive apical tips taken from the same populations of *Fucus* species as in the surface area experiment were used in this part of the investigation. This method had been used by previous workers for different algae species: exposure to various biocides in *Fucus*, *Ulva* and *Enteromorpha* (Scanlan and Wilkinson, 1987);

effects of sulphide-rich seepage on macrobenthos (Cruz, 1988); the effects of coastal pollution on *Fucus* germlings (Grundy, 1996). Evolution and consumption of oxygen was used to measure the net photosynthetic and respiration rates using the light and dark bottle method with a dissolved oxygen meter and a small amount of plant material. The method used here is taken from Strickland and Parsons (1972) and based on one used by Thomas (1988) for aquatic macro-flora.

3.8.1 Use of light and dark bottles

Dark bottles containing plants consume oxygen due to respiration so that in sealed bottles containing seawater and plants kept in the dark the dissolved oxygen concentration decreases. In light bottles any change in dissolved oxygen is the net result of photosynthesis and respiration which may increase or decrease depending on which of the two processes has the greater rate at the time. Light bottles have an oxygen change due to net photosynthesis where:

Net photosynthesis (NP) = gross or true photosynthesis (GP) – respiration (R).

Therefore:

- Respiration is the oxygen change in dark bottles which must decrease.
- Net photosynthesis in the oxygen change in light bottles which may increase or decrease.
- Gross photosynthesis is the sum of oxygen changes in light and dark bottles.

3.8.2 Method

To calculate the rates of photosynthesis (P) and respiration (R) for *Fucus* species known weights of plant were incubated in sealed light and dark bottles containing seawater for a known length of time in front of an artificial light source at 5ºC, 10ºC and 15ºC in CT rooms. Measurements of dissolved oxygen (DO) in bottles were taken using an YSI model 58 Dissolved Oxygen Meter at the end of incubation. Control light and dark bottles containing only seawater and no plants were used to allow for any changes in dissolved oxygen that may have occurred due to causes other than P or R. The difference in DO between control and treatment bottles is due to P or R. 10 replicates

with 3 controls were set up for both light and dark bottles and allowed to incubate for a minimum period of 1.5 hours. After this time dissolved oxygen measurements were taken and plants sections placed in 80ºC oven and dried at a constant temperature overnight. The full method is given in **Appendix B**.

To calculation rate of P and R results obtained were put into following equation:

NP or R = 60xy/mt

Where x is dissolved oxygen change in mg/l, y is bottle volume in ml, m is dry weight of plant used in mg, t is incubation time in minutes. To calculate gross photosynthesis (GP) magnitudes of light and dark bottle changes were either added or subtracted according to whether or not there was a decrease in light bottles as shown below:

- If dissolved oxygen went up in light bottles then GP was represented by increase in dissolved oxygen in light bottles plus decrease in dissolved oxygen in dark bottles.

- If dissolved oxygen went down in light bottles then GP was represented by decrease in dissolved oxygen in light bottles minus decrease is dissolved oxygen in dark bottles.

3.9 Phenotypic Variation in *Fucus distichus* subsp. *anceps* Populations

Multivariate analysis was used to examine the similarities and differences between two geographically different populations of *F. distichus* subsp. *anceps* from Orkney and Ireland. This part of the investigation examined phenetic variation patterns within *anceps* populations using the principles of numerical taxonomy (Sneath and Sokal, 1973; Rice and Chapman, 1985; Rice *et al*., 1985; Scott *et al*. 2001). Many factors can affect the amount of intraspecific variability, that is, the variation between members of a species. Important factors to intraspecific variability are breeding systems and selection pressures. Inbreeding, which is possible with monoecious species such as *Fucus distichus* can lead to a reduction of within-population variation but also allows wide differences between populations. If a species has widely differing latitudes and longitudes, its physiology and ecological parameters may be quite different (Lobban

and Harrison, 1997). At the southern limit of its range, *Fucus distichus* is represented by populations of the best adapted forms, particularly subsp. *anceps* in Ireland, which are confined to restricted habitats and geographically isolated. If, as suggested by Powell (1957b), such restriction and isolation resulted in the gradual evolution of genetically distinct ecotype or even species then how appropriate is the use of *Fucus distichus* as an indicator of sea temperature rise and climate change in the British Isles?

3.9.1 Collection of plant material

Locations and populations were selected taking into account the author's prior knowledge of the distribution of the populations of *anceps* under investigation and from previous records (Powell, 1957b). At each site an arbitrary point was selected in the stand of *anceps* and one hundred mature plants (i.e. plants with ripe receptacles) were removed from the vicinity without selection. The use of exclusively mature plants reduced the effects of age on morphology in the data sets (Powell, 1957a; Edelstein and McLachlan, 1975; Sideman and Mathieson, 1983a). Plants were placed in plastic bags and kept in refrigeration until laboratory facilities were available. Upon return to laboratory plants were acclimatised in seawater in CT room at 10ºC.

3.9.2 Method

Principal components analysis was used to assess similarities and differences using measurements of observable characteristics and variation. The type of character i.e. morphometric (continuous) and meristic (discrete) were analysed separately because of the fundamental differences in their distributional characteristics (Sokal and Rohlf, 1969, Rice and Chapman, 1985). To obtain normal distribution and to meet the assumptions of parametric statistics the data of morphometric characters were log (x + 1) transformed. The analyses were performed using Statistical Package for the Social Sciences (SPSS), Version 12.0 for Windows. Each plant was analysed on the basis of fifteen characters, here defined as all tissue arising from a single holdfast **(Table 3.2)**. These character definitions were based on previous work by Rice and Chapman (1985) and are given in precise detail in **Appendix C**. Macroscopic characters were measured to an accuracy of 1 mm. Microscopic characters were measured to the nearest μm using a graticule in a compound microscope at ×10 objective lens (magnification × 100). Angles were measured using a protractor to the nearest 5º.

The main aim of factor analysis is the orderly simplification of a large number of intercorrelated measures to a few representative constructs or factors which can then be used for subsequent analysis and is based on the assumption that all variables are correlated to some degree. Those variables that share similar underlying dimensions should be highly correlated and those variables that measure dissimilar dimensions should yield low correlations. High/low correlation coefficients are apparent in the correlation matrix because they form clusters indicating which variables "hang" together (Ho, 2006). Factor analysis was carried out in four steps: computation of the correlation matrix; extraction of initial factors; rotation of the extracted factors to make them more interpretable; scores for each factor were computed for each case and these scores were used for further analysis.

To determine the number of initial unrotated factors to be extracted, eigenvalues criterion was used. Only factors with eigenvalues of 1 or greater were considered to be significant (Ho, 2006) and all factors with eigenvalues less than 1 were disregarded since an eigenvalue greater than 1 indicates that more common variance than unique variance is explained by that factor. The rotation phase sharpened the factors by identifying those variables that loaded on one factor and not on another. The ultimate affect was to achieve a simpler, theoretically more meaningful factor pattern. Even after rotation there may be significant cross-loadings across several factors. At this stage it must be decided which factors are substantively meaningful (either theoretically or intuitively) and retain only these for further rotation. This will result in "clean" factors making interpretation of the factors easier. The Rotated Component Matrix presents factors after orthogonal varimax rotation. To identify what these factors represent it is necessary to consider what items are loaded on each of the factors. The clustering of the items in each factor give some idea of the meaning of that factor. When interpreting factors, the size of the factor loadings help with interpretation with large loadings indicating that they are representative of the factor whereas small loadings are not. Factor loadings greater than ±0.33 are considered to meet the minimal level of practical significance (Ho, 2006). The grouping of variables with high factor loadings should suggest what the underlying dimension is for that factor.

Table 3.2: List of characters measured on each plant.

Number	Character	Type of character
1	Plant length (mm)	Morphometric
2	Plant width (mm)	Morphometric
3	Receptacle length (mm)	Morphometric
4	Receptacle width (mm)	Morphometric
5	Conceptacle length (μm)	Morphometric
6	Conceptacle width (μm)	Morphometric
7	Distance to oldest dichotomy (mm)	Morphometric
8	Diameter of oogonium	Morphometric
9	Midrib width (mm)	Morphometric
10	Stipe width (mm)	Morphometric
11	Diameter of holdfast at widest point (mm)	Morphometric
12	Angle of oldest dichotomy (º)	Meristic
13	Angle of youngest dichotomy (º)	Meristic
14	Number of lower dichotomies	Meristic
15	Number of upper dichotomies	Meristic

Scatter plots of the factors (principal components) for individual plants on selected pairs of components were prepared. The positions of individuals on the axes were used to see if the populations of *anceps* varied and a one-way analysis of variance (ANOVA) performed on the principal components to look at any significant difference between populations. Independent samples *t*-test was used to determine significant differences in the mean values measured between populations.

Chapter 4: Investigation into the Effect of Temperature on the Establishment and Growth of *Fucus* Germlings

4.1 Laboratory Investigation into Germling Growth of Various *Fucus* Species and Taxa Collected from Different Locations and Cultivated at Different Temperatures

The following experiments were used to investigate the effects on germling growth of *Fucus* species and taxa collected from geographically different locations **(Table 4.1)** and cultured at different temperatures in a controlled environment. The establishment of germlings and comparisons between populations of *Fucus distichus* subsp. *anceps* from Orkney and Ireland and other fucoid species were investigated. Unfortunately comparison of germling growth between different populations of *F. vesiculosus* var. *linearis* from Orkney and Ireland was not possible due to unsuccessful settlement of the Ireland population.

4.1.1 Method

The method used in this experiment for germling cultures is set out in detail in **Chapter 3**, **Sections 3.3 - 3.4**. Reproductive plants collected from Bay of Skaill, Orkney and Co. Clare, Ireland were kept refrigerated until such time as laboratory facilities were available (usually no more than 3 days). In dioecious species the sexes were kept and treated separately throughout. On return to the laboratory, plants were left to acclimatise for 24 hours at temperatures of 5°C, 10°C and 15°C. Receptacles were excised from adult plants and prepared firstly by lightly brushing with a soft nail brush to remove epiphytes and then by rinsing in cold tap water for 1 minute to remove gross contaminants. This procedure was repeated two more times with blotting of receptacles between washes.

Twenty germlings were measured for each replicate, five replicates per species giving a total of 100 germlings for each species at temperatures of 5°C, 10°C and 15°C. Each slide represented a replicate. Slides were placed in clear plastic containers with 400ml of filtered seawater, the medium was replaced every 3 to 4 days. Photosynthetically active radiation (PAR) of 80μE $m^{-2}s^{-1}$ at water surface was provided by Philips White-light fluorescent tubes and photoperiod set at 12:12, light:dark (07.00 – 19.00 hrs).

Measurements of germlings were carried out as described in **Section 3.5.1**., after fertilisation and over a 100 day period. To investigate the effects different temperatures had on the establishment and growth of germlings, measurements were taken to examine the comparative growth of different parts of embryos of different *Fucus* species. These measurements included the length of 'head' and 'rhizoid' component parts, total length and rhizoid as a percentage of the total length. The last measurement may give some indication as to where the greatest amount of growth effort was concentrated, particularly over the first few days, and at what temperature. As rhizoids are essential in the establishment of a *Fucus* population then the distinction of the different component parts and measurements thereof were deemed important in this investigation. Recording of head and rhizoid measurements started when the component parts of the germling became apparent.

To investigate any statistically significant differences of germling growth between treatments all datasets were tested for normality (Kolmogorov-Smirnov test) and homogeneity of variance (Levene's Test) to see if a one-way Analysis of Variance (ANOVA) could be used. If one-way ANOVA appropriate then *post hoc* (Tukey's HSD) test performed for pairwise comparison between mean values to identify which treatment groups were significantly different from other groups. Where datasets failed the above tests then a non-parametric (Kruskal-Wallis test) equivalent was performed. Where significant results for Kruskal-Wallis test were found then *post hoc* pairwise Mann-Whitney *U* test performed to identify significant difference between median values for treatment groups.

4.1.2 Fucus species and taxa used to investigate the effect of different temperatures on germling growth.

Table 4.1: The *Fucus* species and taxa used in this part of the present investigation along with National Grid (NG) references, author's codes, fruiting periods, life spans and collection dates. (Codes: Fda = *Fucus distichus* subsp. *anceps*; Fspna = *Fucus spiralis* f. *nanus*; Fveslin = *Fucus vesiculosus* var. *linearis*; Fspir = *Fucus spiralis*; Fserr = *Fucus serratus*; Fves = *Fucus vesiculosus*; O = Orkney; I = Ireland).

Species	Location	NG Ref.	Code	Fruiting Periods	Life Span	Collection Date
Fucus distichus subsp. *anceps*	Bay of Skaill (South), Orkney	HY226190	OFda	April - August*	2 - 3 years ‡	Mid April '06
Fucus distichus subsp. *anceps*	George's Head, Kilkee, Co. Clare, Ireland	W088162	IFda	March - August*	2 - 3 years	Early March '06
Fucus serratus	Bay of Skaill (North), Orkney	HY232196	OFserr	Peak fruiting period Oct – Dec but reproductive plants found throughout the year.	3 to 4 years	Mid April '06
Fucus spiralis	Bay of Skaill (North), Orkney	HY232196	OFspir	Peak fruiting period Oct – June but reproductive plants found throughout the year.	3 to 4 years	Mid April '06
Fucus spiralis f. *nanus*	Bay of Skaill (South), Orkney	HY226190	OFspna	Fruiting period vague.	3 to 4 years	Mid April '06
Fucus vesiculosus	Bay of Skaill (North), Orkney	HY232196	OFves	Peak fruiting period March – May but reproductive plants found throughout the year.	4 to 5 years	Mid April '06
Fucus vesiculosus var. *linearis*	Bay of Skaill (South), Orkney	HY226190	OFveslin	Fruiting period vague.	3 to 4 years	Mid April '06

* Receptacles may start to develop as early as January. Fruiting ceases during August at all sites in the British Isles (Powell, 1957b).
‡ More usual for plants to survive for a least 3 years at Scottish sites (Powell, 1957b).
Note: Dates given for reproductivity in this investigation only apply to *Fucus* species of the British Isles.

4.2 Laboratory Investigation into Germling Growth of *Fucus distichus* subsp. *anceps* Collected from Bay of Skaill (South), Orkney and Cultivated at Different Temperatures

4.2.1 Results

The mean 'head' and rhizoid lengths of germlings cultured at different temperatures are given in **Tables 4.2** and **4.3** respectively. **Table 4.4** gives the rhizoid lengths as a percentage of the total germling length. The mean total lengths of the germlings cultured at different temperatures are given in **Table 4.5**. These results are graphed in **Figures 4.1 – 4.4**. Statistically significant differences among treatment groups using non-parametric Kruskal-Wallis test and *post hoc* pairwise Mann-Whitney *U* test were performed. Results are given in **Table 4.6**.

Table 4.2: Mean head lengths (μm) of 5 replicates of Orkney *F. distichus* subsp. *anceps* germlings cultivated at different temperatures over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Standard deviations of the replicate means are given in italics.

	Number of days following fertilisation							
Temp.	**7**	**16**	**36**	**50**	**62**	**74**	**86**	**100**
5ºC	**79.1**	**149.5**	**216.7**	**247.0**	**308.0**	**361.4**	**407.2**	**457.8**
	11.73	*19.04*	*27.89*	*29.63*	*37.71*	*38.74*	*67.63*	*75.42*
10ºC	**100.6**	**189.9**	**239.8**	**265.4**	**279.7**	**349.8**	**401.3**	**428.9**
	13.99	*24.06*	*29.88*	*34.33*	*46.83*	*61.56*	*77.25*	*81.62*
15ºC	**130.0**	**262.3**	**412.9**	**492.9**	**574.2**	**582.7**	**675.3**	**764.9**
	23.44	*43.46*	*62.07*	*69.84*	*109.8*	*102.43*	*125.77*	*152.67*

Table 4.3: Mean rhizoid lengths (μm) of 5 replicates of Orkney *F. distichus* subsp. *anceps* germlings cultivated at different temperatures over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Standard deviations of the replicate means are given in italics.

	Number of days following fertilisation							
Temp.	**7**	**16**	**36**	**50**	**62**	**74**	**86**	**100**
5ºC	**108.5**	**205.9**	**371.4**	**432.5**	**541.3**	**573.3**	**595.8**	**601.3**
	21.71	*40.08*	*119.55*	*114.16*	*159.04*	*201.58*	*198.4*	*213.18*
10ºC	**128.0**	**243.3**	**478.0**	**638.5**	**670.3**	**677.7**	**635.5**	**628.7**
	23.95	*101.24*	*188.17*	*165.75*	*199.2*	*159.66*	*206.48*	*211.47*
15ºC	**127.1**	**237.6**	**770.0**	**1009.9**	**1244.3**	**1519.5**	**1648.5**	**1683.0**
	52.75	*84.36*	*248.72*	*295.41*	*423.28*	*432.57*	*387.07*	*300.61*

Table 4.4: Mean rhizoid lengths as a percentage of the total lengths(μm) of 5 replicates of Orkney *F. distichus* subsp. *anceps* germlings cultivated at different temperatures over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Standard deviations of the replicate means are given in italics.

	Number of days following fertilisation							
Temp.	**7**	**16**	**36**	**50**	**62**	**74**	**86**	**100**
5ºC	**57.45**	**57.69**	**62.55**	**62.86**	**62.64**	**59.72**	**57.52**	**55.32**
	5.88	*6.60*	*10.06*	*7.37*	*7.84*	*8.66*	*9.99*	*10.33*
10ºC	**54.62**	**54.14**	**60.53**	**69.45**	**69.3**	**65.7**	**61.13**	**58.04**
	6.77	*10.1*	*16.53*	*7.34*	*7.65*	*6.97*	*9.30*	*10.18*
15ºC	**47.91**	**46.52**	**63.64**	**66.1**	**66.76**	**71.28**	**72.44**	**68.55**
	9.64	*9.12*	*9.07*	*6.94*	*9.26*	*6.75*	*5.72*	*6.11*

Table 4.5: Mean total lengths (μm) of 5 replicates of Orkney *F. distichus* subsp. *anceps* germlings cultivated at different temperatures over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Standard deviations of the replicate means are given in italics.

	Number of days following fertilisation									
Temperature	**1**	**3**	**7**	**16**	**36**	**50**	**62**	**74**	**86**	**100**
5ºC	**63.8**	**99.81**	**187.64**	**354.61**	**581.1**	**679.62**	**848.93**	**935.71**	**1002.2**	**1059.11**
	4.88	*10.63*	*25.43*	*45.18*	*121.03*	*116.7*	*160.92*	*203.16*	*204.94*	*206.99*
10ºC	**62.92**	**118.8**	**234.61**	**433.2**	**777.11**	**908.15**	**950.71**	**1023.63**	**1033.9**	**1057.5**
	5.74	*13.35*	*32.02*	*108.23*	*158.61*	*171.92*	*211.12*	*177.83*	*225.72*	*232.41*
15ºC	**64.71**	**118.31**	**257.13**	**499.9**	**1181.82**	**1503.51**	**1818.8**	**2102.71**	**2227.48**	**2446.9**
	6.43	*16.4*	*64.37*	*96.95*	*253.26*	*313.96*	*450.53*	*439.7*	*429.68*	*337.47*

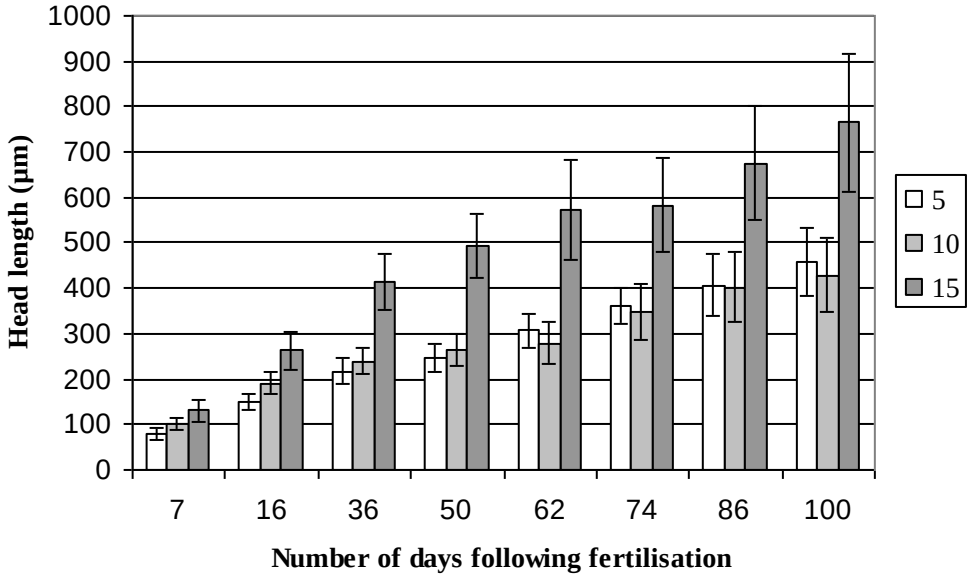


Figure 4.1: Mean head lengths (μm) of 5 replicates of Orkney *F. distichus* subsp. *anceps* germlings cultivated at temperatures 5, 10, 15ºC over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Error bars signify standard deviation.

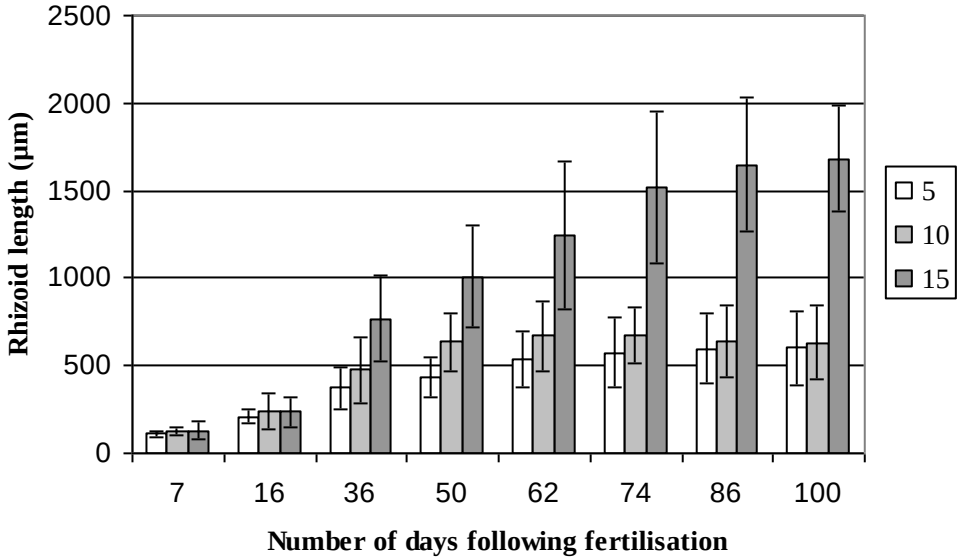


Figure 4.2: Mean rhizoid lengths (μm) of 5 replicates of Orkney *F. distichus* subsp. *anceps* germlings cultivated at temperatures 5, 10, 15ºC over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Error bars signify standard deviation.

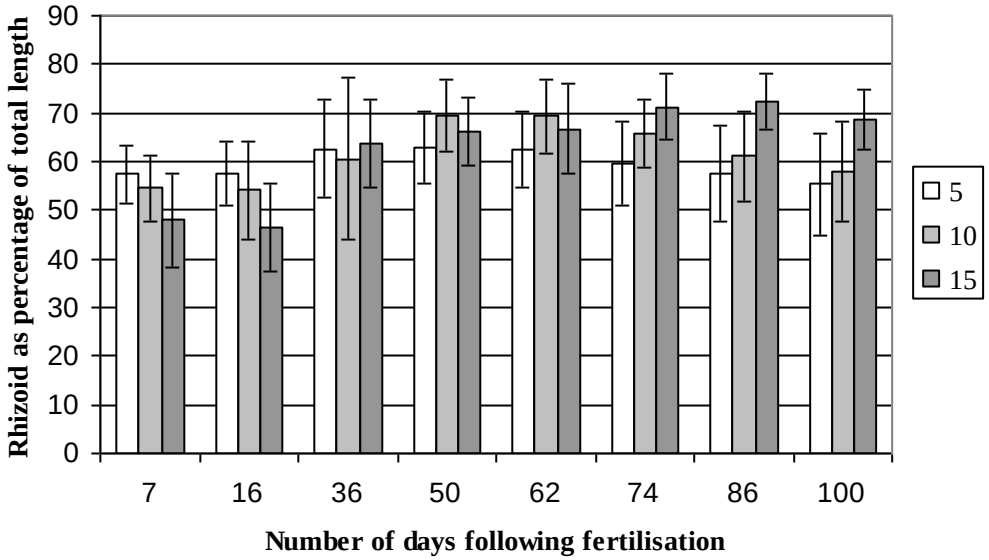


Figure 4.3: Mean rhizoid lengths as a percentage of the total length of 5 replicates of Orkney *F. distichus* subsp. *anceps* germlings cultivated at temperatures 5, 10, 15ºC over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Error bars signify standard deviation.

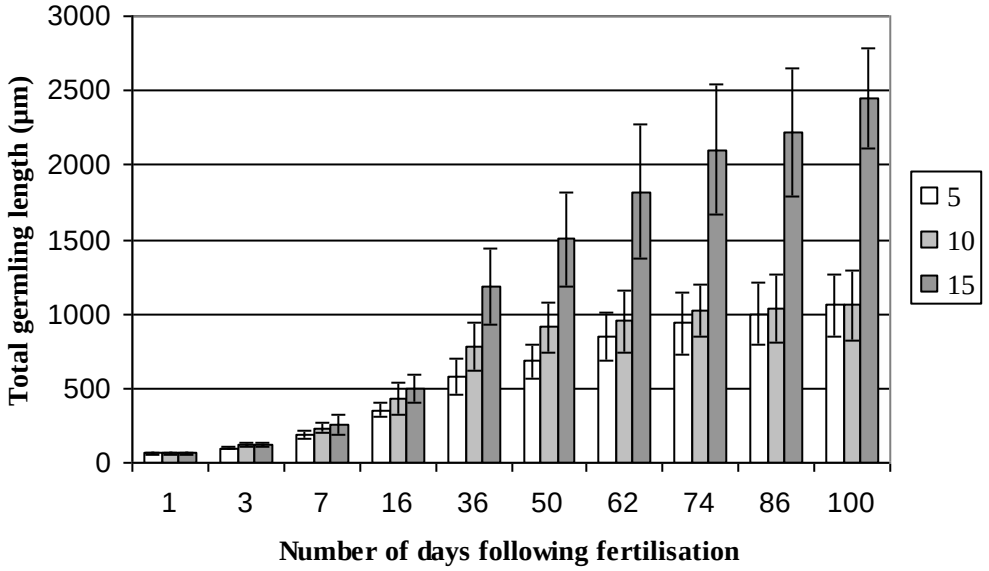


Figure 4.4: Mean total lengths (μm) of 5 replicates of Orkney *F. distichus* subsp. *anceps* germlings cultivated at temperatures 5, 10, 15ºC over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Error bars signify standard deviation.

Table 4.6: Matrices of results for Kruskal-Wallis test and *post hoc* Mann-Whitney *U* pairwise test of median values for germling growth of Orkney *F. distichus* subsp. *anceps* cultivated at different temperatures after a 100 day period. PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. 5 replicates, each replicate represents the mean of 20 germlings (* significant at p = <0.05, *** highly significant at p = 0.001, NS = not significant).

Measurement (μm)		**Temperature ºC**	
		5	10
Head	10	*	
	15	***	***
		5	10
Rhizoid	10	NS	
	15	***	***
		5	10
Rhizoid as % of total length	10	*	
	15	***	***
		5	10
Total length	10	NS	
	15	***	***

Mean head length **(Figure 4.1)** was consistently greater at 15ºC over the 100 days and followed the pattern as for total length of germlings. Head length increased throughout at all temperatures however by day 36, growth at 15ºC was significantly greater than at 5ºC and 10ºC. 5ºC was the least favourable temperature up to 50 days. At 62 days and after the lowest growth rate was at 10ºC

Mean rhizoid length **(Figure 4.2)** growth was approximately equal at all temperatures up to 16 days. At 36 days and after growth at 15ºC was highest and at 100 days was more than twice that at 5ºC and 10ºC. 5ºC was the least favourable temperature for rhizoid growth throughout. Growth at 5ºC and 10ºC increased gradually to 50 days where after growth slowed and plateaued with no significant difference between these temperatures at 100 days.

Mean rhizoid length as a percentage of the total length **(Figure 4.3)** showed some initial preference for 5ºC up to day 16, for 10ºC at 50 and 62 days and for 15ºC at 74 to 100 days. These results are dependent on how temperature affects head and/or rhizoid growth. At 5ºC and 10ºC this was the result of lower head growth rates compared to rhizoid growth while at 15ºC was mainly due to an increase in rhizoid length.

Initial total length growth **(Figure 4.4)** was approximately equal up to 3 days. At 7 days growth was best at 15ºC and throughout and at 100 days was significantly greater than at 5ºC and 10ºC having more than twice the growth (p = 0.001). 5ºC was the least favourable temperature throughout but at 100 days showed no significant difference to growth at 10ºC. At 15ºC germling growth increased throughout the experimental period whereas at 5ºC and 10ºC growth slowed at 62 days and started levelling off.

4.2.2 Conclusions

Results show that germlings grew at all temperatures but that 15ºC was the optimum temperature for total length growth while that at 5ºC and 10ºC was significantly less. Both the associated head and rhizoid components increased in length at 15ºC throughout the experimental period while at 5ºC and 10ºC growth slowed at 50 days and levelled off. Results suggest that different temperatures affected the growth rate of the component parts of the germlings, differently. For example, at temperatures of 5ºC and 10ºC low germling growth rates can be attributed to low growth rate of rhizoid length and to a lesser extent head length, affecting the total length. The higher mean rhizoid length as a percentage of the total length at the lowest temperature of 5ºC over the first 16 days is undoubtedly the result of the lower rate of head growth at that temperature. However, it may also indicate that at this temperature where germling growth was inhibited, growth effort was concentrated on rhizoid development at the start of the culture period.

4.3 Laboratory Investigation into Germling Growth of *Fucus distichus* subsp. *anceps* collected from Kilkee, Co. Clare, Ireland and Cultivated at Different Temperatures

4.3.1 Results

The mean 'head' and rhizoid lengths of germlings cultured at different temperatures are given in **Tables 4.7** and **4.8** respectively. **Table 4.9** gives the rhizoid lengths as a percentage of the total germling length. The mean total lengths of the germlings cultured at different temperatures are given in **Table 4.10**. These results are graphed in **Figures 4.5 – 4.8**. Statistically significant differences among treatment groups using non-parametric Kruskal-Wallis test and post hoc pairwise Mann-Whitney U test were performed. Results are given in **Table 4.11**.

Table 4.7: Mean head lengths (μm) of 5 replicates of Ireland *F. distichus* subsp. *anceps* germlings cultivated at different temperatures over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Standard deviations of the replicate means are given in italics.

	Number of days following fertilisation								
Temp.	**3**	**7**	**16**	**36**	**50**	**62**	**74**	**86**	**100**
5ºC	**79.5**	**89.5**	**113.1**	**299.4**	**289.2**	**287.6**	**319.5**	**370.3**	**393.3**
	12.74	*12.75*	*14.26*	*54.12*	*34.13*	*41.54*	*58.25*	*49.86*	*72.21*
10ºC	**84.0**	**164.8**	**196.5**	**361.1**	**426.1**	**429.6**	**450.1**	**463.9**	**462.3**
	11.89	*20.77*	*22.49*	*35.33*	*60.4*	*51.89*	*67.89*	*64.46*	*65.81*
15ºC	**87.4**	**142.3**	**171.4**	**350.3**	**401.6**	**428.6**	**440.9**	**448.6**	**461.0**
	9.81	*19.48*	*14.77*	*42.34*	*56.26*	*51.56*	*58.88*	*64.89*	*63.68*

Table 4.8: Mean rhizoid lengths (μm) of 5 replicates of Ireland *F. distichus* subsp. *anceps* germlings cultivated at different temperatures over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Standard deviations of the replicate means are given in italics.

	Number of days following fertilisation								
Temp.	**3**	**7**	**16**	**36**	**50**	**62**	**74**	**86**	**100**
5ºC	**67.2**	**131.3**	**180.4**	**426.0**	**576.5**	**687.6**	**816.3**	**931.9**	**1029.2**
	12.07	*15.35*	*19.84*	*88.17*	*112.8*	*162.38*	*224.57*	*204.0*	*162.19*
10ºC	**141.8**	**246.7**	**332.7**	**576.5**	**579.0**	**1251.0**	**1388.4**	**1558.5**	**1678.1**
	24.18	*25.03*	*23.56*	*63.38*	*86.36*	*129.93*	*231.45*	*252.77*	*244.89*
15ºC	**127.2**	**224.8**	**287.4**	**468.8**	**572.4**	**934.4**	**868.5**	**816.0**	**753.1**
	22.83	*26.3*	*23.34*	*87.61*	*131.33*	*195.96*	*202.59*	*208.39*	*154.31*

Table 4.9: Mean rhizoid lengths as a percentage of the total length of 5 replicates of Ireland *F. distichus* subsp. *anceps* germlings cultivated at different temperatures over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Standard deviations of the replicate means are given in italics.

	Number of days following fertilisation								
Temp.	**3**	**7**	**16**	**36**	**50**	**62**	**74**	**86**	**100**
5ºC	**45.7**	**59.44**	**61.41**	**58.42**	**66.14**	**69.78**	**71.19**	**70.98**	**72.24**
	6.12	*4.16*	*3.72*	*4.89*	*5.22*	*5.94*	*4.99*	*5.58*	*4.6*
10ºC	**62.55**	**59.86**	**62.78**	**61.27**	**57.51**	**74.32**	**75.24**	**76.77**	**78.08**
	5.7	*4.45*	*3.08*	*3.41*	*5.02*	*3.42*	*3.98*	*4.01*	*4.01*
15ºC	**59.16**	**61.25**	**62.6**	**56.94**	**58.27**	**68.14**	**65.76**	**63.94**	**61.68**
	5.36	*4.04*	*3.23*	*5.11*	*5.42*	*5.07*	*5.83*	*5.72*	*4.59*

Table 4.10: Mean total lengths (μm) of 5 replicates of Ireland *F. distichus* subsp. *anceps* germlings cultivated at different temperatures over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Standard deviations of the replicate means are given in italics.

	Number of days following fertilisation									
Temperature	**1**	**3**	**7**	**16**	**36**	**50**	**62**	**74**	**86**	**100**
5ºC	**99.7**	**146.9**	**221.1**	**293.6**	**724.1**	**865.7**	**976.2**	**1135.8**	**1303.3**	**1424.7**
	15.34	*16.74*	*20.44*	*25.64*	*123.16*	*121.36*	*167.54*	*257.83*	*209.02*	*190.8*
10ºC	**152.5**	**225.7**	**411.7**	**530.1**	**939.5**	**1005.3**	**1680.6**	**1838.9**	**2022.0**	**2142.8**
	19.92	*26.22*	*27.96*	*33.59*	*74.31*	*104.59*	*130.24*	*246.06*	*257.67*	*249.84*
15ºC	**134.5**	**214.0**	**366.9**	**458.3**	**819.1**	**974.5**	**1361.3**	**1309.2**	**1264.6**	**1214.1**
	19.66	*24.86*	*34.6*	*28.82*	*98.16*	*159.25*	*207.83*	*214.08*	*233.78*	*183.13*

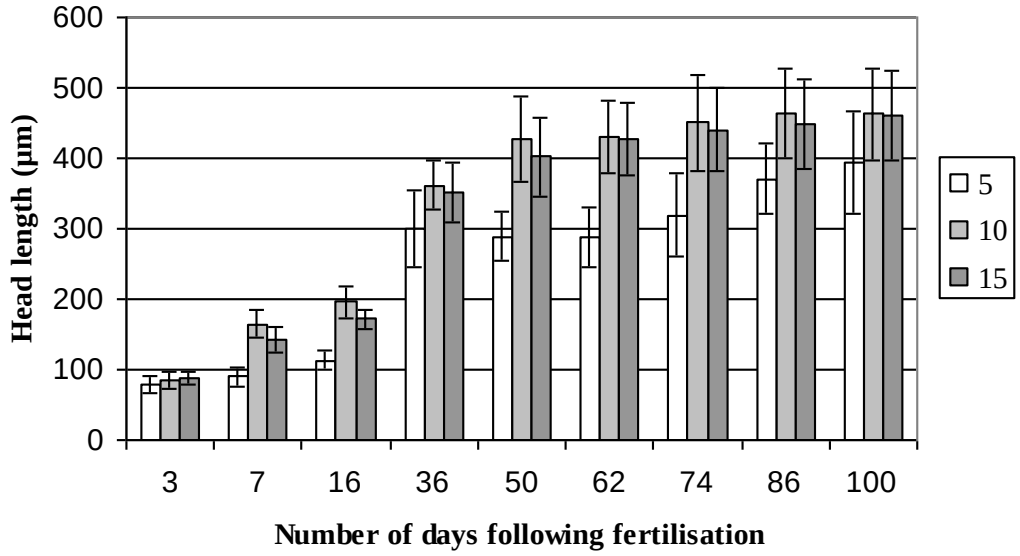


Figure 4.5: Mean head lengths (μm) of 5 replicates of Ireland *F. distichus* subsp. *anceps* germlings cultivated at temperatures 5, 10, 15ºC over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Error bars signify standard deviation.

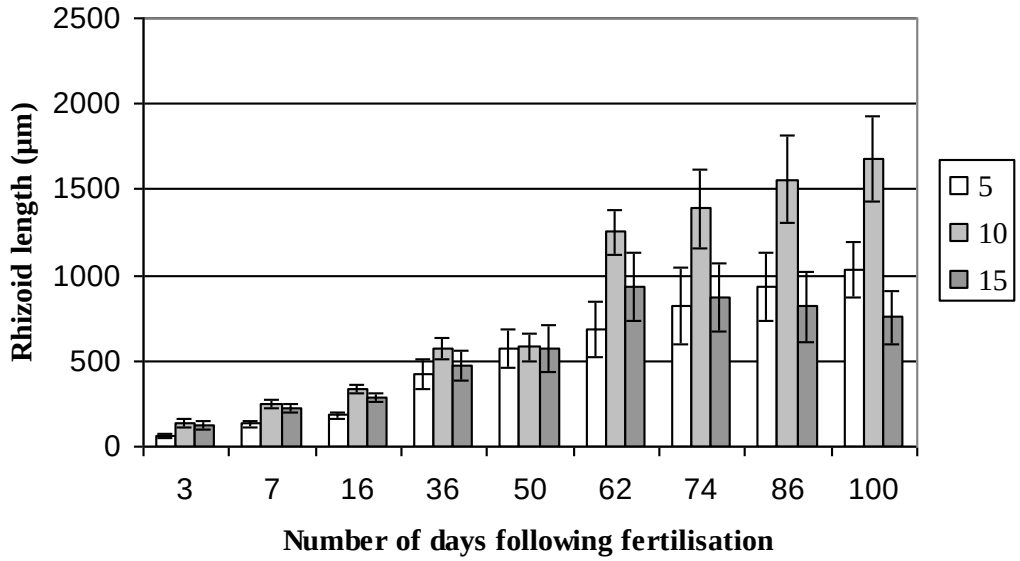


Figure 4.6: Mean rhizoid lengths (μm) of 5 replicates of Ireland *F. distichus* subsp. *anceps* germlings cultivated at temperatures 5, 10, 15ºC over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Error bars signify standard deviation.

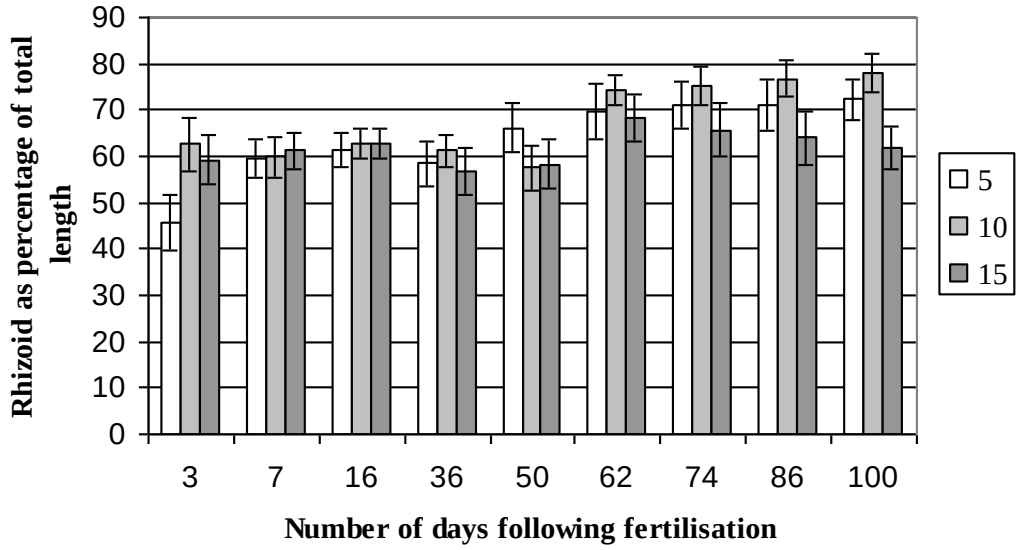


Figure 4.7: Mean rhizoid lengths as a percentage of total length of 5 replicates of Ireland *F. distichus* subsp. *anceps* germlings cultivated at temperatures 5, 10, 15ºC over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Error bars signify standard deviation.

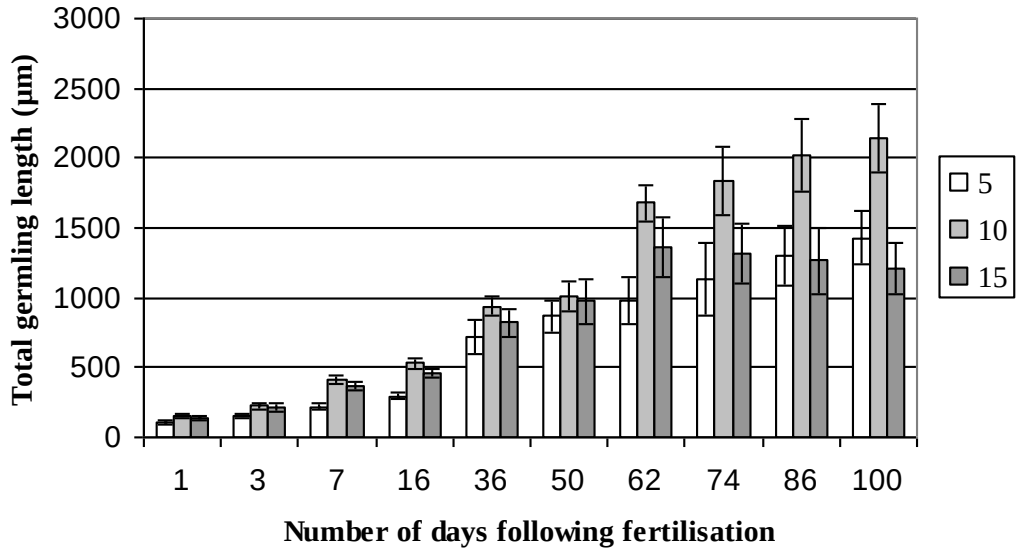


Figure 4.8: Mean total lengths (μm) of 5 replicates of Ireland *F. distichus* subsp. *anceps* germlings cultivated at temperatures 5, 10, 15ºC over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Error bars signify standard deviation.

Table 4.11: Matrices of results for Kruskal-Wallis test and *post hoc* Mann-Whitney *U* pairwise test of median values for germling growth of Ireland *F. distichus* subsp. *anceps* cultivated at different temperatures after a 100 day period. PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. 5 replicates, each replicate represents the mean of 20 germlings (*** highly significant at $p = 0.001$, NS = not significant).

Measurement (μm)	**Temperature ºC**		
		5	10
Head	10	***	
	15	***	NS
		5	10
Rhizoid	10	***	
	15	***	***
		5	10
Rhizoid as % of total length	10	NS	
	15	***	***
		5	10
Total length	10	***	
	15	***	***

Mean head length **(Figure 4.5)** was best at 10ºC and marginally better than 15ºC throughout but at 100 days showed no significant difference to growth at 15ºC. Growth slowed at 50 days at both 10ºC and 15ºC before starting to level off. 5ºC was the least favourable temperature although growth did increase throughout but at 100 days was significantly less than both 10ºC and 15ºC ($p = 0.001$).

Mean rhizoid length **(Figure 4.6)** growth was best at 10ºC, increasing throughout and at 100 days was significantly greater than both 5ºC and 15ºC ($p = 0.001$). 5ºC was the least favourable temperature although growth increased throughout and at 86 and 100 days was significantly greater than 15ºC ($p = 0.001$). At 15ºC growth peaked at 62 days, dipping thereafter and at 100 days had the lowest growth rate.

Mean rhizoid length as a percentage of total length **(Figure 4.7)** showed that initially the warmer temperatures of 10ºC and 15ºC had the best growth rates at 3 days, the result of lower rhizoid growth at 5ºC. At days 7 and 16 results are similar at all temperatures indicating that the head to rhizoid ratio are roughly equal. At 62 days onwards the greatest percentage growth of rhizoids is at 10ºC due to a lower growth rate of both head and rhizoid at 5ºC and a noticeable reduction in rhizoid growth at 15ºC.

Total length growth **(Figure 4.8)** was best at 10ºC, increasing throughout and at 100 days was significantly greater than at 5ºC and 15ºC (p = 0.001). 5ºC was the least favourable but growth did increase gradually and at 86 days and after was greater than 15ºC. At 15ºC growth peaked at 62 days, dipping thereafter.

4.3.2 Conclusions

Results show that germlings grew at all temperatures but that 10ºC was the optimum temperature with growth increasing throughout the experimental period. Lower growth rates were recorded at 5ºC and 15ºC where temperature inhibited growth of one or both of the component parts. For example, the lowest growth rate at 5ºC is a result of reduced growth of both head and rhizoid length. However the low growth at 15ºC is mainly attributed to a reduction in rhizoid length effecting the total length growth.

One interesting point noted was the reduction of rhizoid growth at 15ºC from 62 days onwards while growth continued to increase at 5ºC and 10ºC. This suggests that either rhizoid growth was inhibited at 15ºC after this time or that growth effort was concentrated on development of the head component. One further point noted was the clear differentiation and early development of rhizoids in the Ireland population of *anceps* and subsequently measurements of the different components parts began at 3 days as opposed to 7 days for all other species including Orkney *anceps*.

4.4 Laboratory Investigation into Germling Growth of *Fucus serratus* Collected from Bay of Skaill (North), Orkney and Cultivated at Different Temperatures

4.4.1 Results

The mean 'head' and rhizoid lengths of germlings cultured at different temperatures are given in **Tables 4.12** and **4.13** respectively. **Table 4.14** gives the rhizoid lengths as a percentage of the total germling length. The mean total lengths of the germlings cultured at different temperatures are given in **Table 4.15**. These results are graphed in **Figures 4.9 – 4.12**. Statistically significant differences among treatment groups using non-parametric Kruskal-Wallis test and *post hoc* pairwise Mann-Whitney *U* test were performed. Results are given in **Table 4.16**.

Table 4.12: Mean head lengths (μm) of 5 replicates of Orkney *F. serratus* germlings cultivated at different temperatures over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Standard deviations of the replicate means are given in italics.

	Number of days following fertilisation							
Temp.	**7**	**16**	**36**	**50**	**62**	**74**	**86**	**100**
5ºC	**68.4**	**94.0**	**378.0**	**431.3**	**464.3**	**485.1**	**517.3**	**531.5**
	9.82	*24.12*	*54.74*	*74.95*	*90.27*	*91.14*	*91.9*	*107.76*
10ºC	**84.9**	**292.6**	**356.4**	**401.5**	**392.8**	**384.5**	**372.3**	**382.4**
	11.59	*57.22*	*74.34*	*91.03*	*89.95*	*99.8*	*90.5*	*87.11*
15ºC	**70.4**	**395.4**	**613.7**	**934.4**	**1003.0**	**1091.1**	**1121.3**	**1203.5**
	7.64	*60.58*	*121.49*	*227.51*	*261.97*	*314.17*	*301.65*	*273.24*

Table 4.13: Mean rhizoid lengths (μm) of 5 replicates of Orkney *F. serratus* germlings cultivated at different temperatures over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Standard deviations of the replicate means are given in italics.

	Number of days following fertilisation							
Temp.	**7**	**16**	**36**	**50**	**62**	**74**	**86**	**100**
5ºC	**20.0**	**100.9**	**275.7**	**331.0**	**375.3**	**413.7**	**471.2**	**508.9**
	25.86	*69.63*	*86.33*	*106.71*	*134.18*	*222.27*	*205.19*	*251.03*
10ºC	**107.9**	**329.3**	**674.5**	**749.6**	**704.8**	**669.4**	**641.6**	**623.3**
	27.42	*110.53*	*258.26*	*276.65*	*272.47*	*270.92*	*205.67*	*163.67*
15ºC	**100.4**	**354.7**	**846.4**	**987.0**	**1044.6**	**1074.1**	**1154.6**	**1213.5**
	26.32	*156.87*	*295.8*	*347.69*	*355.67*	*370.59*	*389.09*	*335.21*

Table 4.14: Mean rhizoid length as a percentage of the total length of 5 replicates of Orkney *F. serratus* germlings cultivated at different temperatures over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Standard deviations of the replicate means are given in italics.

	Number of days following fertilisation							
Temp.	**7**	**16**	**36**	**50**	**62**	**74**	**86**	**100**
5ºC	**41.01**	**57.6**	**41.61**	**42.99**	**44.04**	**43.84**	**46.61**	**49.67**
	9.71	*5.09*	*8.73*	*9.32*	*9.4*	*12.89*	*11.71*	*14.15*
10ºC	**55.39**	**52.0**	**63.11**	**63.26**	**62.4**	**61.59**	**62.26**	**61.37**
	6.33	*9.48*	*13.3*	*10.27*	*10.78*	*12.61*	*10.19*	*8.81*
15ºC	**57.63**	**45.43**	**56.66**	**50.64**	**50.55**	**49.19**	**50.47**	**49.91**
	7.39	*11.33*	*11.49*	*10.89*	*10.66*	*10.91*	*11.71*	*9.22*

Table 4.15: Mean total lengths (μm) of 5 replicates of Orkney *F. serratus* germlings cultivated at different temperatures over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Standard deviations of the replicate means are given in italics.

	Number of days following fertilisation									
Temperature	**1**	**3**	**7**	**16**	**36**	**50**	**62**	**74**	**86**	**100**
5ºC	**66.5**	**82.0**	**88.4**	**194.9**	**651.7**	**759.3**	**837.7**	**897.6**	**988.3**	**1039.4**
	8.33	*16.39*	*27.33*	*85.27*	*112.77*	*118.25*	*156.82*	*213.58*	*194.28*	*209.23*
10ºC	**75.6**	**157.9**	**192.5**	**621.8**	**1031.1**	**1153.6**	**1099.6**	**1053.9**	**1013.9**	**1008.2**
	9.46	*32.95*	*33.47*	*126.07*	*265.55*	*288.85*	*291.47*	*278.31*	*224.45*	*192.35*
15ºC	**83.2**	**132.5**	**171.4**	**751.9**	**1457.6**	**1921.6**	**2021.7**	**2160.7**	**2244.0**	**2411.0**
	8.27	*26.83*	*28.28*	*164.15*	*300.41*	*371.31*	*470.63*	*480.84*	*517.35*	*427.91*

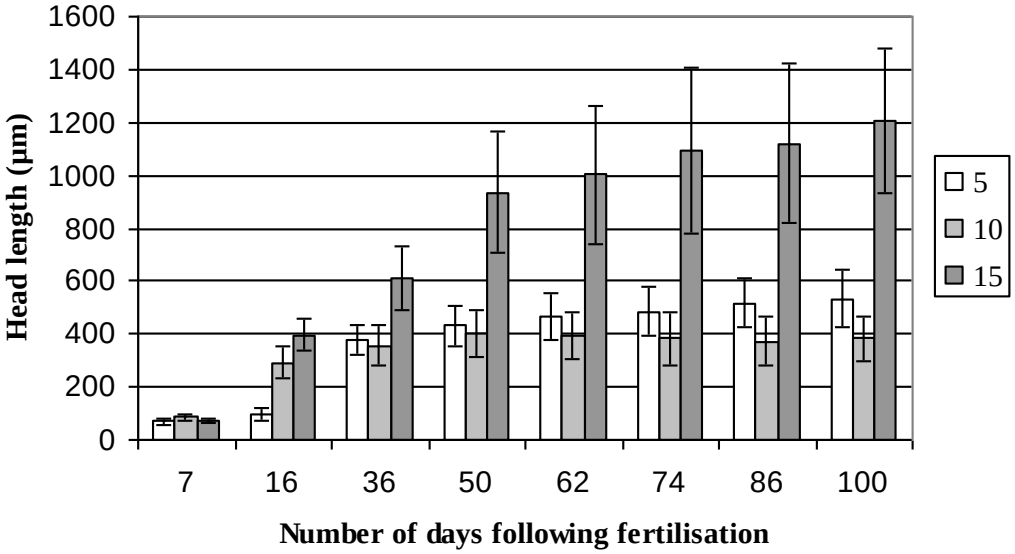


Figure 4.9: Mean head lengths (μm) of 5 replicates of Orkney *F. serratus* germlings cultivated at temperatures 5, 10, 15ºC over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Error bars signify standard deviation.

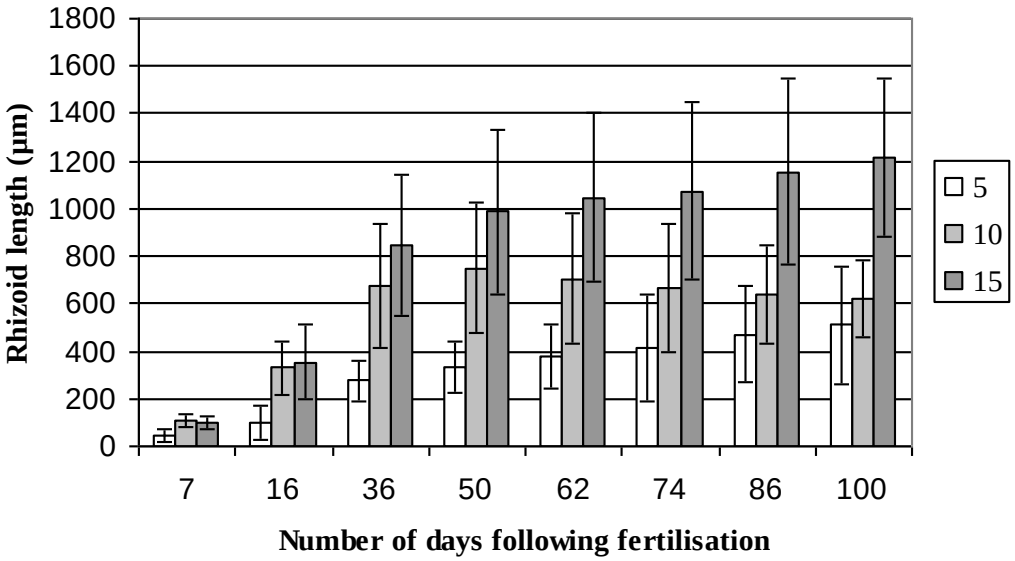


Figure 4.10: Mean rhizoid lengths (μm) of 5 replicates of Orkney *F. serratus* germlings cultivated at temperatures 5, 10, 15ºC over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Error bars signify standard deviation.

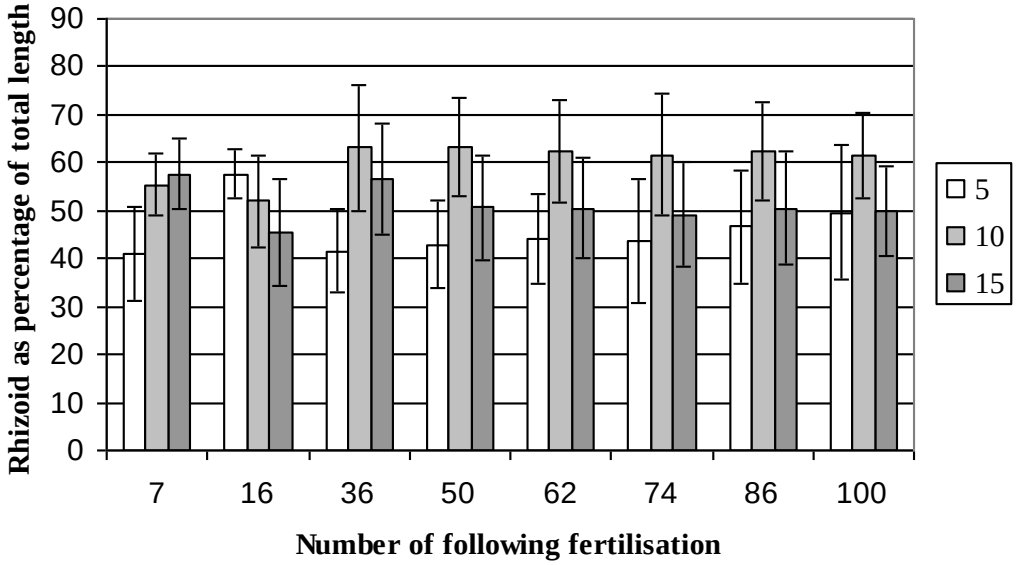


Figure 4.11: Mean rhizoid lengths as a percentage of the total length of 5 replicates of Orkney *F. serratus* germlings cultivated at temperatures 5, 10, 15ºC over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Error bars signify standard deviation.

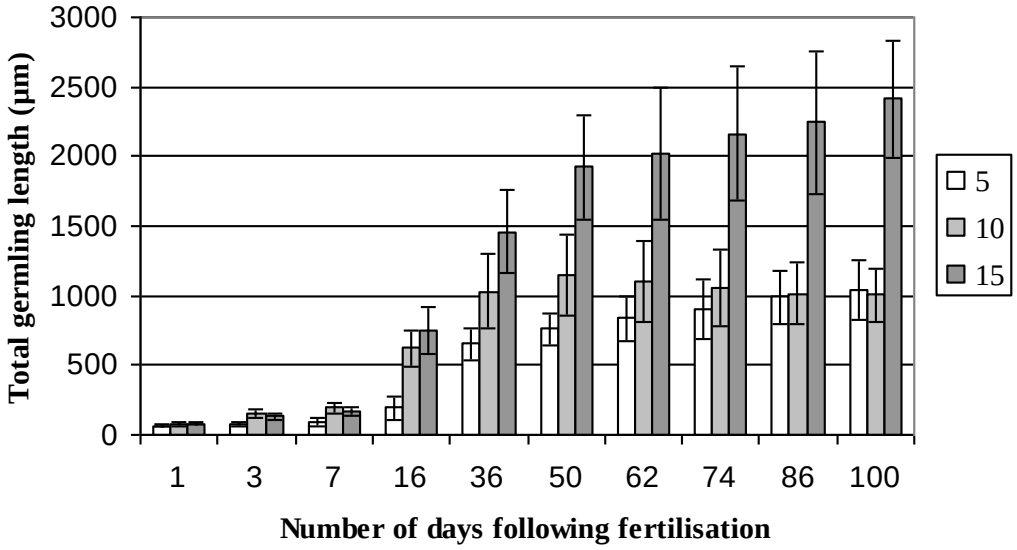


Figure 4.12: Mean total lengths (μm) of 5 replicates of Orkney *F. serratus* germlings cultivated at temperatures 5, 10, 15ºC over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Error bars signify standard deviation.

Table 4.16: Matrices of results for Kruskal-Wallis test and *post hoc* Mann-Whitney *U* pairwise test of median values for germling growth of Orkney *F. serratus* cultivated at different temperatures after a 100 day period. PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. 5 replicates, each replicate represents the mean of 20 germlings (*** highly significant at $p = 0.001$, NS = not significant).

Measurement (μm)	**Temperature ºC**		
		5	10
Head	10	***	
	15	***	***
		5	10
Rhizoid	10	***	
	15	***	***
		5	10
Rhizoid as % of total length	10	***	
	15	NS	***
		5	10
Total length	10	NS	
	15	***	***

Mean head length **(Figure 4.9)** growth was greatest at 15ºC and by 36 days and after was significantly greater than at both 5ºC and 10ºC. Growth increased at 15ºC throughout the experimental period while growth slowed and plateaued around 50 days at 5ºC and 10ºC. At 100 days there was significantly difference in growth between all temperatures ($p = 0.001$), but 15ºC had more than twice the growth rate of 5ºC and 10ºC.

Mean rhizoid length **(Figure 4.10)** was again greatest at 15ºC with growth at 5ºC showing the lowest rate. While 5ºC and 15ºC showed a similar growth profile, increasing throughout, growth at 10ºC peaked at 50 days and dipped thereafter. At 100 days there was significant difference in growth between all temperatures ($p = 0.001$).

Mean rhizoid length as a percentage of the total length **(Figure 4.11)** was initially best at 7 days at 10ºC and 15ºC due to lower rhizoid growth at 5ºC. The high percentage of rhizoid growth at 10ºC from 36 days onwards was the result of a reduction in head growth at this temperature.

The optimum temperature for total length **(Figure 4.12)** growth was 15ºC, increasing throughout and at 100 days was significantly greater than both 5ºC and 10ºC (p = 0.001), at more than twice the length. The least favourable temperature was 5ºC although growth did increase over the 100 days. At 10ºC growth peaked at 50 days, dipping thereafter and at 100 days was similar to 5ºC with no significant difference between these temperatures.

4.4.2 Conclusions

The optimum temperature for total length growth was 15ºC while at 5ºC and 10ºC was much less. Growth profiles showed an increase at 15ºC throughout whereas at 5ºC and 10ºC growth slowed at 36 days and levelled off, the result of differences in growth rates of the component parts of germlings. Temperatures of 5ºC and 10ºC appeared to inhibit the growth of both head and rhizoids which subsequently affected the total length.

The results suggest that a high temperature may be required for both the establishment and continued growth and development of Orkney populations of *F. serratus*.

4.5 Laboratory Investigation into Germling Growth of *Fucus spiralis* Collected from Bay of Skaill, (North) Orkney and Cultivated at Different Temperatures

4.5.1 Results

The mean 'head' and rhizoid lengths of germlings cultured at different temperatures are given in **Tables 4.17** and **4.18** respectively. **Table 4.19** gives the rhizoid lengths as a percentage of the total germling length. The mean total lengths of the germlings cultured at different temperatures are given in **Table 4.20**. These results are graphed in **Figures 4.13 – 4.16**. Statistically significant differences among treatment groups using non-parametric Kruskal-Wallis test and *post hoc* pairwise Mann-Whitney *U* test were performed. Results are given in **Table 4.21**.

Table 4.17: Mean head lengths (μm) of 5 replicates of Orkney *F. spiralis* germlings cultivated at different temperatures over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Standard deviations of the replicate means are given in italics.

	Number of days following fertilisation							
Temp.	**7**	**16**	**36**	**50**	**62**	**74**	**86**	**100**
5ºC	**87.1**	**156.4**	**200.6**	**234.5**	**240.0**	**250.9**	**262.1**	**279.4**
	9.57	*21.3*	*24.9*	*26.3*	*29.2*	*30.69*	*46.48*	*52.68*
10ºC	**116.3**	**174.2**	**195.4**	**227.3**	**236.5**	**253.0**	**271.1**	**278.7**
	12.36	*24.79*	*28.16*	*25.34*	*29.45*	*33.38*	*39.05*	*37.16*
15ºC	**175.5**	**222.3**	**245.0**	**307.6**	**312.1**	**319.0**	**342.3**	**356.2**
	20.9	*32.03*	*30.27*	*42.81*	*43.47*	*42.96*	*55.41*	*54.73*

Table 4.18: Mean rhizoid lengths (μm) of 5 replicates of Orkney *F. spiralis* germlings cultivated at different temperatures over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Standard deviations of the replicate means are given in italics.

	Number of days following fertilisation							
Temp.	**7**	**16**	**36**	**50**	**62**	**74**	**86**	**100**
5ºC	**90.5**	**257.9**	**390.0**	**495.2**	**508.8**	**540.4**	**551.8**	**555.5**
	19.25	*49.22*	*103.62*	*120.66*	*112.78*	*137.22*	*141.22*	*153.76*
10ºC	**171.2**	**462.9**	**621.7**	**686.8**	**629.8**	**593.0**	**616.2**	**641.1**
	31.15	*74.5*	*121.81*	*153.41*	*141.57*	*161.48*	*130.4*	*162.37*
15ºC	**338.8**	**720.1**	**775.2**	**928.4**	**945.9**	**988.2**	**1171.5**	**1290.5**
	69.58	*145.07*	*125.25*	*158.73*	*206.99*	*277.17*	*336.22*	*341.1*

Table 4.19: Mean rhizoid lengths as a percentage of the total lengths (μm) of 5 replicates of Orkney *F. spiralis* germlings cultivated at different temperatures over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Standard deviations of the replicate means are given in italics.

	Number of days following fertilisation							
Temp.	**7**	**16**	**36**	**50**	**62**	**74**	**86**	**100**
5ºC	**50.6**	**61.84**	**64.87**	**67.28**	**67.34**	**67.42**	**66.8**	**65.53**
	7.29	*5.75*	*7.45*	*6.06*	*5.2*	*6.4*	*7.92*	*8.29*
10ºC	**59.15**	**72.29**	**75.57**	**74.57**	**72.19**	**69.15**	**69.09**	**68.88**
	5.26	*5.49*	*4.28*	*4.93*	*5.98*	*6.83*	*5.95*	*6.36*
15ºC	**65.38**	**76.07**	**75.59**	**74.81**	**74.63**	**74.3**	**76.51**	**77.48**
	7.04	*4.2*	*4.08*	*3.87*	*4.15*	*5.99*	*5.39*	*5.53*

Table 4.20: Mean total lengths (μm) of 5 replicates of Orkney *F. spiralis* germlings cultivated at different temperatures over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Standard deviations of the replicate means are given in italics.

	Number of days following fertilisation									
Temperature	**1**	**3**	**7**	**16**	**36**	**50**	**62**	**74**	**86**	**100**
5ºC	**77.6**	**102.3**	**178.0**	**414.3**	**593.1**	**728.0**	**749.1**	**791.4**	**816.0**	**836.6**
	8.3	*9.41*	*21.51*	*52.99*	*106.07*	*116.67*	*118.25*	*137.38*	*133.2*	*146.05*
10ºC	**101.5**	**133.7**	**287.5**	**637.9**	**818.3**	**913.2**	**863.9**	**845.1**	**885.4**	**920.8**
	15.66	*16.86*	*34.77*	*80.16*	*131.18*	*154.8*	*139*	*160.43*	*135.12*	*165.01*
15ºC	**112.9**	**285.0**	**513.0**	**941.6**	**1021.6**	**1236.3**	**1260.0**	**1313.3**	**1512.9**	**1646.7**
	23.63	*68.26*	*71.22*	*155.04*	*130.51*	*169.4*	*223.83*	*271.87*	*357.98*	*353.16*

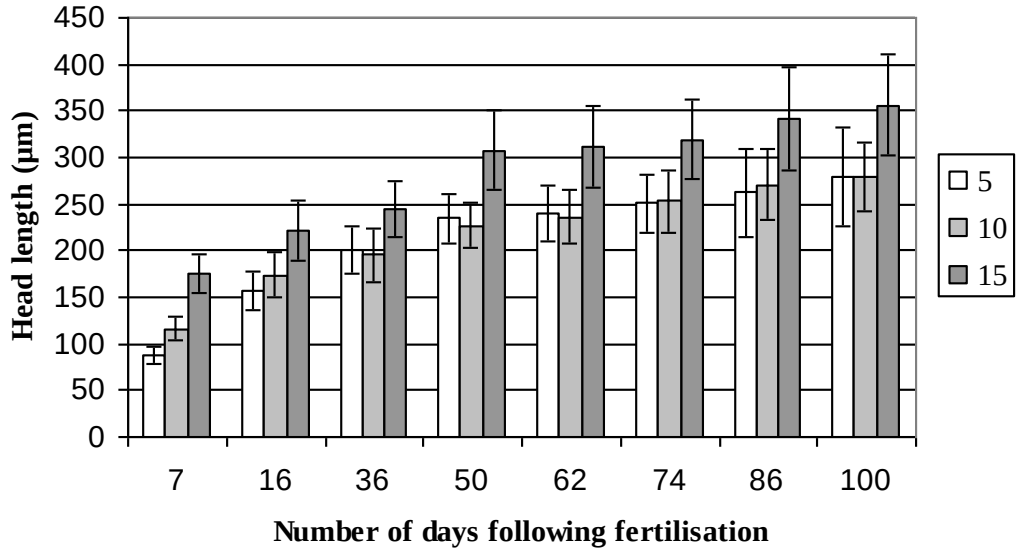


Figure 4.13: Mean head lengths (μm) of 5 replicates of Orkney *F. spiralis* germlings cultivated at temperatures 5, 10, 15ºC over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Error bars signify standard deviation.

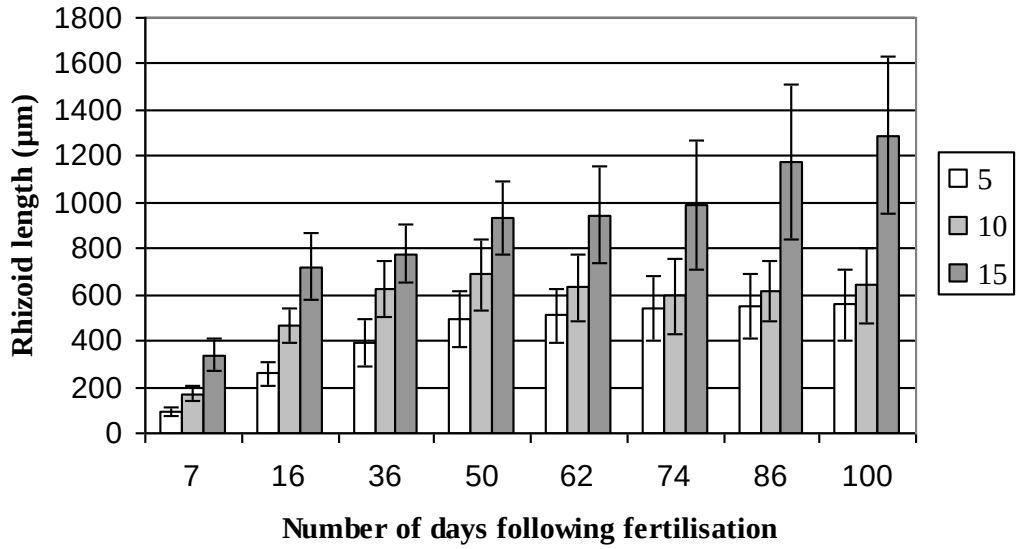


Figure 4.14: Mean rhizoid lengths (μm) of 5 replicates of Orkney *F. spiralis* germlings cultivated at temperatures 5, 10, 15ºC over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Error bars signify standard deviation.

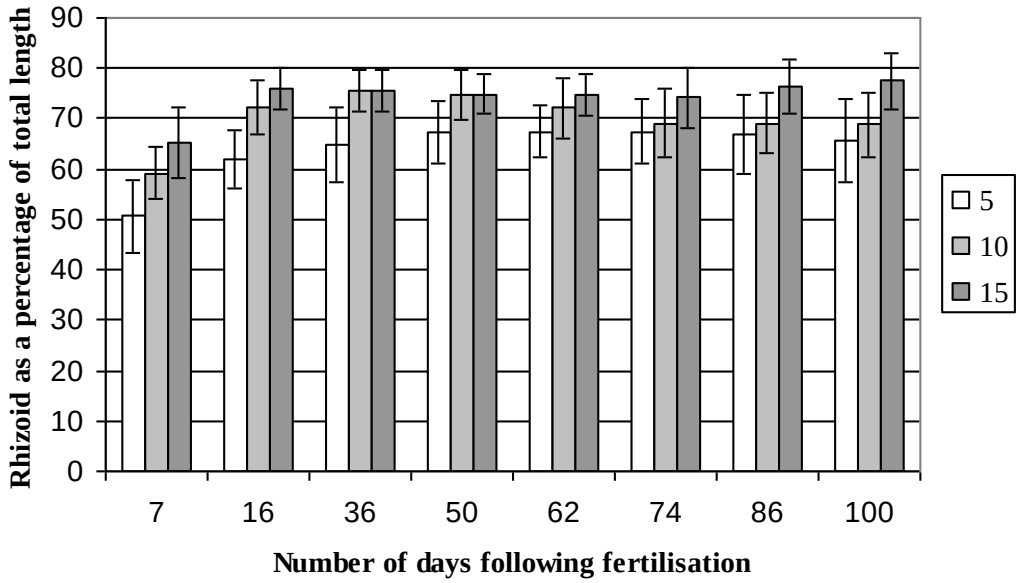


Figure 4.15: Mean rhizoid lengths as a percentage of the total lengths of 5 replicates of Orkney *F. spiralis* germlings cultivated at temperatures 5, 10, 15ºC over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Error bars signify standard deviation.

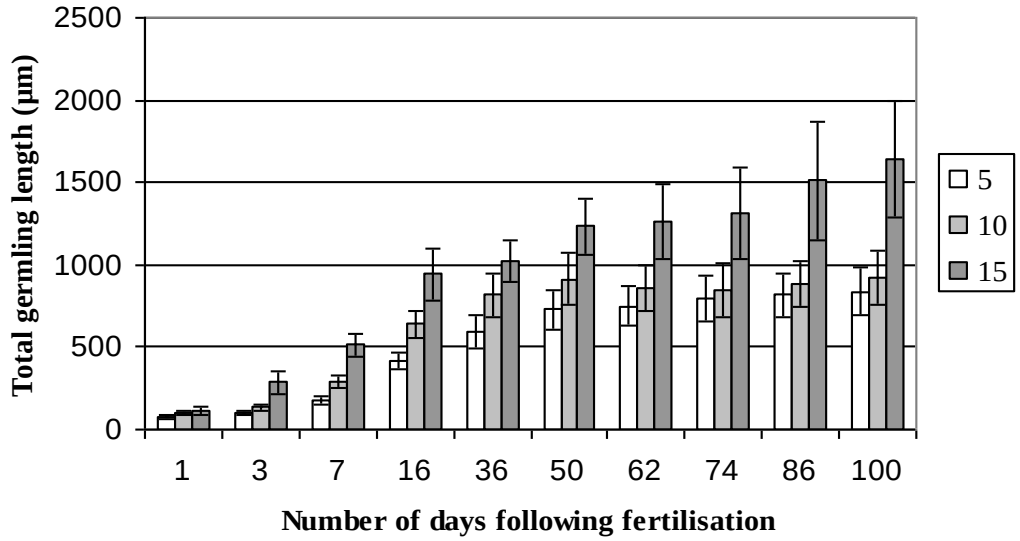


Figure 4.16: Mean total lengths (μm) of 5 replicates of Orkney *F. spiralis* germlings cultivated at temperatures 5, 10, 15ºC over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Error bars signify standard deviation.

Table 4.21: Matrices of results for Kruskal-Wallis test and *post hoc* Mann-Whitney *U* pairwise test of median values for germling growth of Orkney *F. spiralis* cultivated at different temperatures after a 100 day period. PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. 5 replicates, each replicate represents the mean of 20 germlings (*** highly significant at $p = 0.001$, NS = not significant).

Measurement (μm)	**Temperature ºC**		
		5	10
Head	10	NS	
	15	***	***
		5	10
Rhizoid	10	***	
	15	***	***
		5	10
Rhizoid as % of total length	10	NS	
	15	***	***
		5	10
Total length	10	***	
	15	***	***

Mean head length results **(Figure 4.13)** showed very similar growth profiles at all temperatures, increasing throughout. The optimum temperature for growth was at 15ºC throughout and at 100 days was significantly greater than both 5ºC and 10ºC ($p = 0.001$) with no significant difference between 5ºC and 10º.

Mean rhizoid length **(Figure 4.14)** was also greatest at 15ºC throughout and at 100 days was significantly greater than both 5ºC and 10ºC ($p = 0.001$). Growth at 5ºC and 10ºC slowed at 50 days and levelled off thereafter with 5ºC having the lowest growth rate at the end of the culture period of 100 days.

Mean rhizoid length as a percentage of the total length **(Figure 4.15)** was greatest at 15ºC due to lower rhizoid growth at 5ºC and 10ºC.

Mean total length **(Figure 4.16)** growth showed an optimum temperature for growth at 15ºC throughout and at 100 days was significantly greater than both 5ºC and 10ºC (p = 0.001). At all temperatures growth slowed at 50 days and started to levelled off. At 5ºC and 10ºC growth levels plateaued thereafter whereas at 15ºC growth rate increased at 86 days and after.

4.5.2 Conclusions

The optimum temperature for growth was at 15ºC while at the lower temperatures of 5ºC and 10ºC growth was much reduced. Results showed that different temperatures affected the growth rate of the component parts of the germlings, differently. For example, at 15ºC both head and rhizoid length increased throughout while at 5ºC and 10ºC rhizoid growth was reduced at these temperatures but had less effect on head length. It may indeed be that germling growth was inhibited after the period of 50 days at 5ºC and 10ºC however it may be the case that growth effort was concentrated on head development as this increased at this time.

The results suggest that a high temperature may be required not only for the initial establishment of Orkney populations of *F. spiralis* but also for growth and development.

4.6 Laboratory Investigation into Germling Growth of *Fucus spiralis* f. *nanus* Collected from Bay of Skaill (South), Orkney and Cultivated at Different Temperatures

4.6.1 Results

The mean 'head' and rhizoid lengths of germlings cultured at different temperatures are given in **Tables 4.22** and **4.23** respectively. **Table 4.24** gives the rhizoid lengths as a percentage of the total germling length. The mean total lengths of the germlings cultured at different temperatures are given in **Table 4.25**. These results are graphed in **Figures 4.17 – 4.20**. Statistically significant differences among treatment groups were sought using either one-way ANOVA and *post hoc* (Tukey's HSD) test for pairwise comparison or non-parametric Kruskal-Wallis test and *post hoc* pairwise Mann-Whitney *U* test. Results are given in **Table 4.26**.

Table 4.22: Mean head lengths (μm) of 5 replicates of Orkney *F. spiralis* f. *nanus* germlings cultivated at different temperatures over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Standard deviations of the replicate means are given in italics.

	Number of days following fertilisation							
Temp.	**7**	**16**	**36**	**50**	**62**	**74**	**86**	**100**
5ºC	**63.9**	**93.9**	**104.9**	**126.9**	**162.8**	**171.4**	**172.1**	**181.3**
	7.09	*20.74*	*23.76*	*34.19*	*36.82*	*38.46*	*33.4*	*51.18*
10ºC	**62.7**	**77.4**	**109.4**	**122.8**	**138.6**	**140.3**	**150.7**	**162.2**
	8.27	*15.08*	*30.08*	*34.53*	*32.91*	*30.37*	*36.58*	*39.76*
15ºC	**75.4**	**91.4**	**111.3**	**125.8**	**137.8**	**135.2**	**144.0**	**139.5**
	11.41	*14.91*	*17.21*	*31.88*	*30.24*	*16.85*	*30.25*	*24.51*

Table 4.23: Mean rhizoid lengths (μm) of 5 replicates of Orkney *F. spiralis* f. *nanus* germlings cultivated at different temperatures over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Standard deviations of the replicate means are given in italics.

	Number of days following fertilisation							
Temp.	**7**	**16**	**36**	**50**	**62**	**74**	**86**	**100**
5ºC	**71.8**	**202.3**	**477.5**	**588.4**	**802.1**	**831.2**	**833.5**	**816.1**
	24.43	*34.98*	*90.74*	*131.8*	*145.4*	*193.98*	*191.46*	*180.01*
10ºC	**64.9**	**244.5**	**464.8**	**489.1**	**529.1**	**466.0**	**475.3**	**519.2**
	14.32	*45.99*	*90.99*	*108.42*	*130.36*	*167.14*	*177.41*	*147.35*
15ºC	**111.5**	**383.1**	**519.6**	**555.4**	**562.8**	**405.7**	**366.8**	**377.6**
	30.33	*101.98*	*174.66*	*186.17*	*186.92*	*152.55*	*90.18*	*98.65*

Table 4.24: Mean rhizoid lengths as a percentage of the total lengths(μm) of 5 replicates of Orkney *F. spiralis* f. *nanus* germlings cultivated at different temperatures over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Standard deviations of the replicate means are given in italics.

	Number of days following fertilisation							
Temp.	**7**	**16**	**36**	**50**	**62**	**74**	**86**	**100**
5ºC	**51.1**	**67.92**	**81.62**	**82.12**	**82.48**	**76.96**	**79.86**	**79.42**
	9.93	*6.23*	*4.84*	*4.93*	*4.79*	*10.88*	*8.97*	*7.79*
10ºC	**48.15**	**75.63**	**80.76**	**86.54**	**78.82**	**75.44**	**74.7**	**75.43**
	7.93	*4.41*	*4.55*	*5.41*	*4.7*	*6.46*	*7.64*	*4.89*
15ºC	**58.81**	**79.93**	**81.25**	**80.67**	**79.12**	**74.02**	**72.08**	**72.71**
	7.05	*4.87*	*4.93*	*5.53*	*5.63*	*4.61*	*7.36*	*7.06*

Table 4.25: Mean total lengths (μm) of 5 replicates of Orkney *F. spiralis* f. *nanus* germlings cultivated at different temperatures over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Standard deviations of the replicate means are given in italics.

	Number of days following fertilisation									
Temperature	**1**	**3**	**7**	**16**	**36**	**50**	**62**	**74**	**86**	**100**
5ºC	**62.2**	**99.7**	**135.7**	**298.0**	**583.3**	**716.5**	**970.7**	**1068.9**	**1035.3**	**1022.1**
	9.38	*8.58*	*27.31*	*44.54*	*97.56*	*155.59*	*155.79*	*138.95*	*168.44*	*171.75*
10ºC	**63.2**	**120.8**	**135.4**	**322.0**	**575.1**	**612.6**	**668.4**	**607.0**	**623.9**	**682.5**
	11.27	*15.35*	*24.35*	*53.12*	*106.35*	*125.38*	*147.26*	*181.19*	*192.65*	*176.3*
15ºC	**62.8**	**127.5**	**186.9**	**475.2**	**630.8**	**680.7**	**700.6**	**541.3**	**505.1**	**513.2**
	7.8	*14.45*	*34.63*	*105.64*	*181.53*	*194.31*	*200.8*	*155.18*	*98.77*	*99.27*

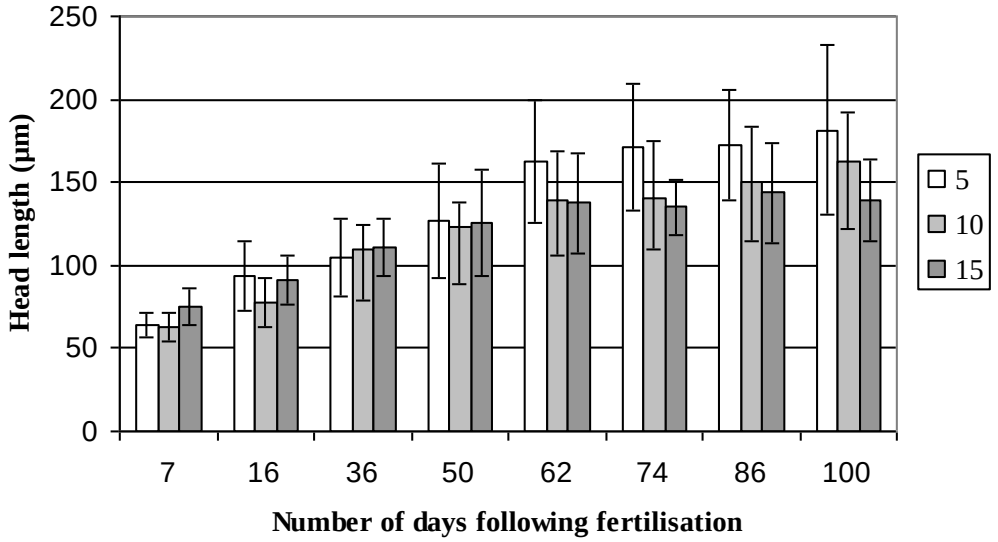


Figure 4.17: Mean head lengths (μm) of 5 replicates of Orkney *F. spiralis* f. *nanus* germlings cultivated at temperatures 5, 10, 15ºC over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Error bars signify standard deviation.

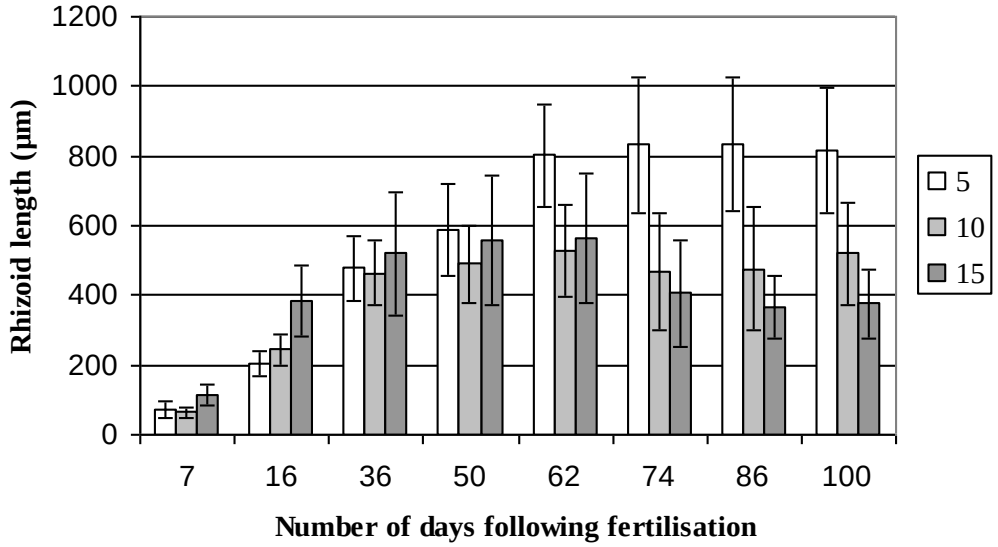


Figure 4.18: Mean rhizoid lengths (μm) of 5 replicates of Orkney *F. spiralis* f. *nanus* germlings cultivated at temperatures 5, 10, 15ºC over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Error bars signify standard deviation.

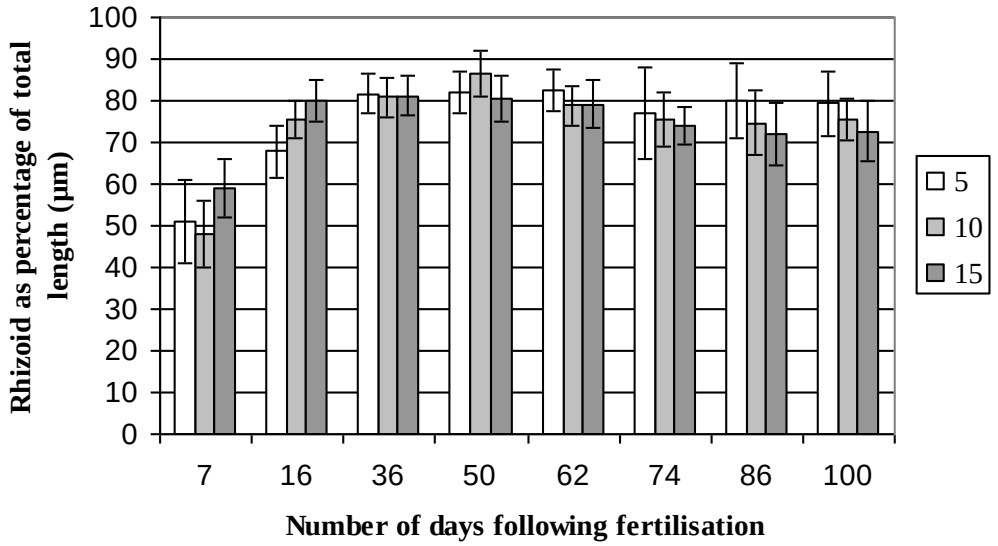


Figure 4.19: Mean rhizoid lengths as a percentage of the total length of 5 replicates of Orkney *F. spiralis* f. *nanus* germlings cultivated at temperatures 5, 10, 15ºC over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Error bars signify standard deviation.

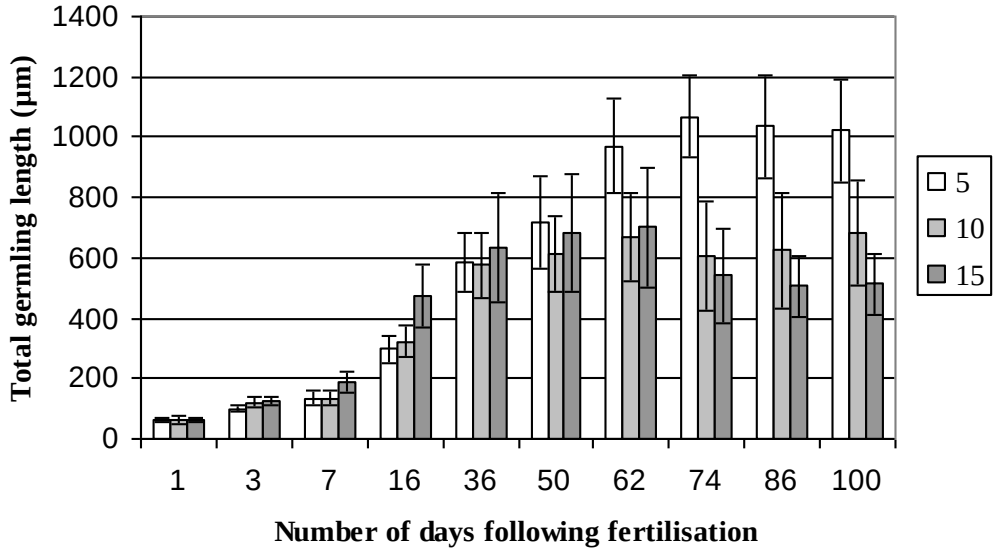


Figure 4.20: Mean total lengths (μm) of 5 replicates of Orkney *F. spiralis* f. *nanus* germlings cultivated at temperatures 5, 10, 15ºC over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Error bars signify standard deviation.

Table 4.26: Matrices of results for Kruskal-Wallis test with *post hoc* Mann-Whitney *U* pairwise test for head, rhizoid and total length of median values and one-way ANOVA and *post hoc* Tukey's HSD pairwise test for significant difference in mean values for rhizoid as % of total length measurement for germling growth for Orkney *F. spiralis* f. *nanus* cultivated at different temperatures after a 100 day period. PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. 5 replicates, each replicate represents the mean of 20 germlings (*** highly significant at $p = 0.001$, NS = not significant).

Measurement (μm)	**Temperature ºC**		
		5	10
Head	10	***	
	15	***	***
		5	10
Rhizoid	10	***	
	15	***	***
		5	10
Rhizoid as % of total length	10	NS	
	15	***	NS
		5	10
Total length	10	***	
	15	***	***

Mean head length **(Figure 4.17)** was marginally best at 15ºC at 7 days after which no real temperature preference was noted until 62 days when 5ºC was the optimum temperature for growth thereafter. At 5ºC and 10ºC growth increased gradually throughout while at 15ºC growth peaked at 62 days and plateaued and at the end of the culture period had the lowest growth rate. At 100 days there was significant difference in growth at all temperatures ($p = 0.001$).

Mean rhizoid length **(Figure 4.18)** was initially better at 15ºC up to 16 days. By day 62 and after 5ºC was the best temperature for growth however growth slowed at this time

and levelled off. Growth at 10ºC and 15ºC slowed at 36 days levelling out to 62 days after which growth dipped particularly at 15ºC which had the lowest growth rate at 100 days. At 100 days there was significant difference in growth at all temperatures (p = 0.001) with rhizoid length at 5ºC more than twice that at 15ºC.

Mean rhizoid length **(Figure 4.19)** as a percentage of the total length showed a similar pattern to rhizoid length with growth initially best at 15ºC, the result of reduced rhizoid growth at 5ºC and 10ºC up to 16 days.

Mean total length **(Figure 4.20)** initially showed best growth at 15ºC at 7 to 16 days. This changed and by 62 days onwards the optimum temperature was 5ºC however growth slowed at this temperature at 74 days levelling off thereafter. Growth slowed at 62 days at both 10ºC and 15ºC dipping thereafter. At 100 days growth was significantly greater at 5ºC than at 10ºC and 15ºC (p = 0.001) with 15ºC having the lowest growth rate.

4.6.2 Conclusions

The results suggest that different temperatures affected the growth rate of the component parts of the germlings, differently and at different stages of development. The total length of germlings increased at all temperatures up to day 36 with some indication of an initial preference for 15ºC. After this time however the optimum temperature for total length growth changed to 5ºC. This was the result of lower growth rates of rhizoids at 10ºC and both head and rhizoids at 15ºC. At the optimum temperature of 5ºC growth was also affected where at 74 days rhizoid growth slowed and in turn influenced total length however head length continued to increase. Results suggest that a higher temperature may be favourable for the initial establishment of Orkney populations of *F. spiralis* f. *nanus* evidenced by the increased rhizoid growth in the first two week period but that this requirement may change to a lower temperature as plants develop and grow.

4.7 Laboratory Investigation into Germling Growth of *Fucus vesiculosus* Collected from Bay of Skaill (North), Orkney and Cultivated at Different Temperatures

4.7.1 Results

The mean 'head' and rhizoid lengths of germlings cultured at different temperatures are given in **Tables 4.27** and **4.28** respectively. **Table 4.29** gives the rhizoid lengths as a percentage of the total germling length. The mean total lengths of the germlings cultured at different temperatures are given in **Table 4.30**. These results are graphed in **Figures 4.21 – 4.24**. Statistically significant differences among treatment groups using non-parametric Kruskal-Wallis test and *post hoc* pairwise Mann-Whitney *U* test were performed. Results are given in **Table 4.31**.

Table 4.27: Mean head lengths (μm) of 5 replicates of Orkney *F. vesiculosus* germlings cultivated at different temperatures over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Standard deviations of the replicate means are given in italics.

	Number of days following fertilisation							
Temp.	**7**	**16**	**36**	**50**	**62**	**74**	**86**	**100**
5ºC	**77.1**	**153.5**	**166.9**	**242.1**	**244.8**	**252.6**	**271.9**	**298.2**
	11.75	*25.32*	*29.57*	*29.99*	*51.53*	*49.58*	*64.03*	*63.41*
10ºC	**130.0**	**194.5**	**202.4**	**258.9**	**278.3**	**274.0**	**300.0**	**316.5**
	20.58	*29.66*	*28.5*	*42.21*	*44.79*	*51.13*	*55.05*	*57.92*
15ºC	**209.2**	**315.6**	**358.5**	**423.5**	**451.7**	**477.9**	**532.2**	**583.1**
	28.7	*47.98*	*57.76*	*65.48*	*92.56*	*107.67*	*135.44*	*140.1*

Table 4.28: Mean rhizoid lengths (μm) of 5 replicates of Orkney *F. vesiculosus* germlings cultivated at different temperatures over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Standard deviations of the replicate means are given in italics.

	Number of days following fertilisation							
Temp.	**7**	**16**	**36**	**50**	**62**	**74**	**86**	**100**
5ºC	**83.4**	**231.8**	**368.7**	**522.4**	**559.8**	**604.1**	**615.6**	**615.9**
	18.6	*74.49*	*131.7*	*159.21*	*163.99*	*165.65*	*166.3*	*172.44*
10ºC	**187.8**	**554.4**	**713.8**	**831.6**	**795.0**	**839.5**	**861.6**	**933.4**
	43.01	*105.37*	*170.99*	*192.8*	*172.96*	*148.41*	*167.98*	*242.3*
15ºC	**268.4**	**512.3**	**811.4**	**1069.3**	**1063.0**	**1078.5**	**1076.3**	**1056.8**
	112.96	*152.08*	*219.59*	*209.85*	*281.29*	*305.53*	*302.77*	*283.1*

Table 4.29: Mean rhizoid lengths as a percentage of the total lengths of 5 replicates of Orkney *F. vesiculosus* germlings cultivated at different temperatures over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Standard deviations of the replicate means are given in italics.

	Number of days following fertilisation							
Temp.	**7**	**16**	**36**	**50**	**62**	**74**	**86**	**100**
5ºC	**51.53**	**58.29**	**66.99**	**66.88**	**68.6**	**69.71**	**68.85**	**66.53**
	7.32	*10.71*	*9.56*	*7.58*	*8.5*	*7.34*	*8.25*	*8.27*
10ºC	**58.27**	**73.7**	**77.19**	**75.25**	**73.5**	**74.78**	**73.85**	**73.75**
	8.47	*4.63*	*4.99*	*5.78*	*5.96*	*5.56*	*5.36*	*6.2*
15ºC	**53.72**	**60.74**	**68.47**	**71.09**	**69.3**	**68.34**	**66.04**	**63.8**
	12.82	*7.93*	*6.81*	*5.01*	*8.07*	*8.77*	*9.73*	*9.5*

Table 4.30: Mean total lengths (μm) of 5 replicates of Orkney *F. vesiculosus* germlings cultivated at different temperatures over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Standard deviations of the replicate means are given in italics.

	Number of days following fertilisation									
Temperature	**1**	**3**	**7**	**16**	**36**	**50**	**62**	**74**	**86**	**100**
5ºC	**71.1**	**102.6**	**160.7**	**385.2**	**536.4**	**765.0**	**803.6**	**855.7**	**885.8**	**913.4**
	7.64	*9.91*	*21.57*	*75.34*	*125.26*	*159.95*	*166.59*	*182.04*	*178.93*	*181.93*
10ºC	**76.7**	**147.6**	**319.6**	**747.9**	**916.2**	**1095.8**	**1072.7**	**1116.5**	**1160.7**	**1253.0**
	10.74	*14.78*	*43.09*	*115.15*	*181.29*	*193.58*	*166.71*	*144.9*	*178.37*	*252.52*
15ºC	**89.1**	**291.3**	**477.6**	**828.0**	**1169.4**	**1495.5**	**1515.4**	**1557.4**	**1609.5**	**1639.9**
	20.89	*67.8*	*112.61*	*160.71*	*228.19*	*226.24*	*278.52*	*306.32*	*293.09*	*269.73*

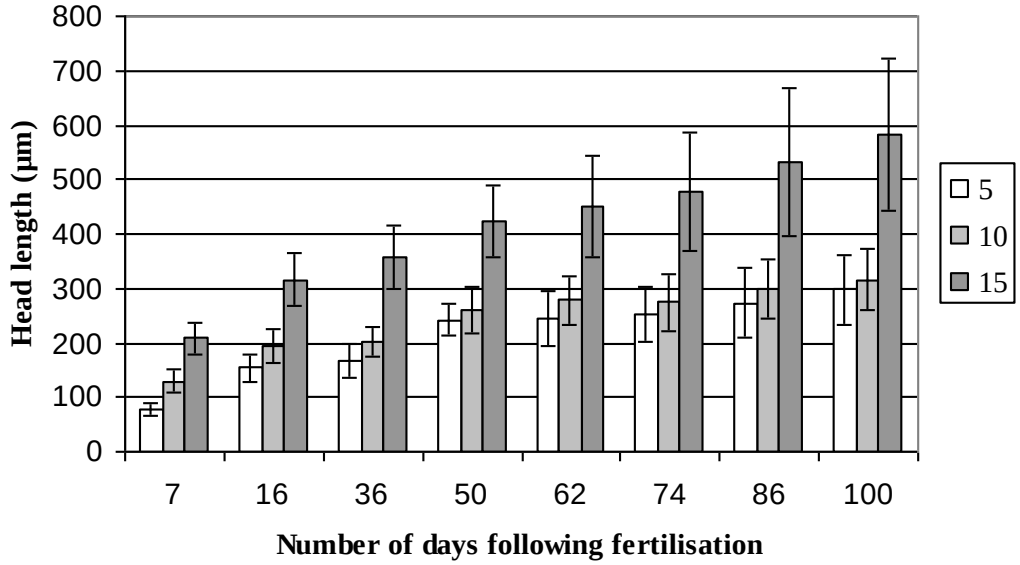


Figure 4.21: Mean head lengths (μm) of 5 replicates of Orkney *F. vesiculosus* germlings cultivated at temperatures 5, 10, 15ºC over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Error bars signify standard deviation.

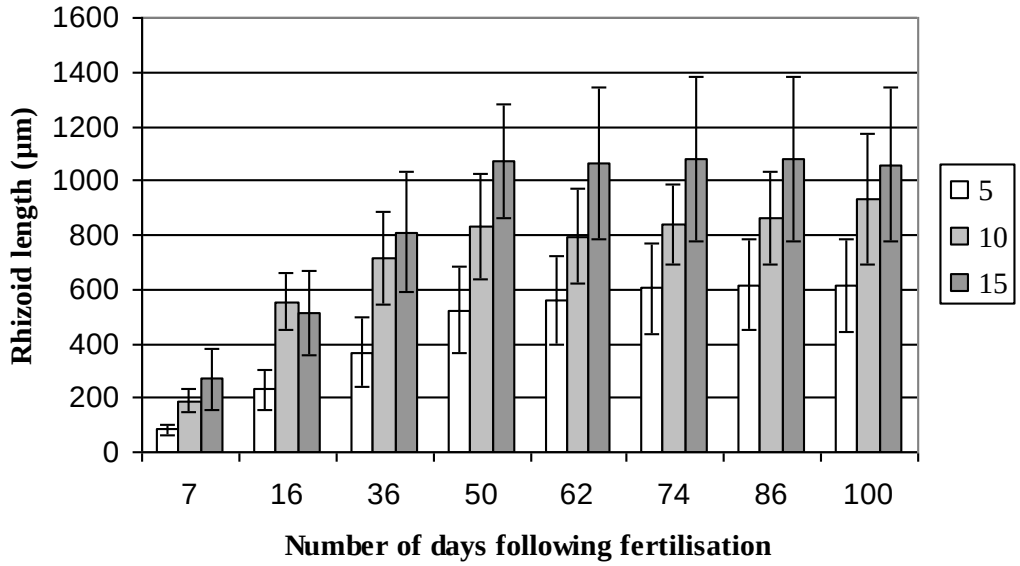


Figure 4.22: Mean rhizoid lengths (μm) of 5 replicates of Orkney *F. vesiculosus* germlings cultivated at temperatures 5, 10, 15ºC over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Error bars signify standard deviation.

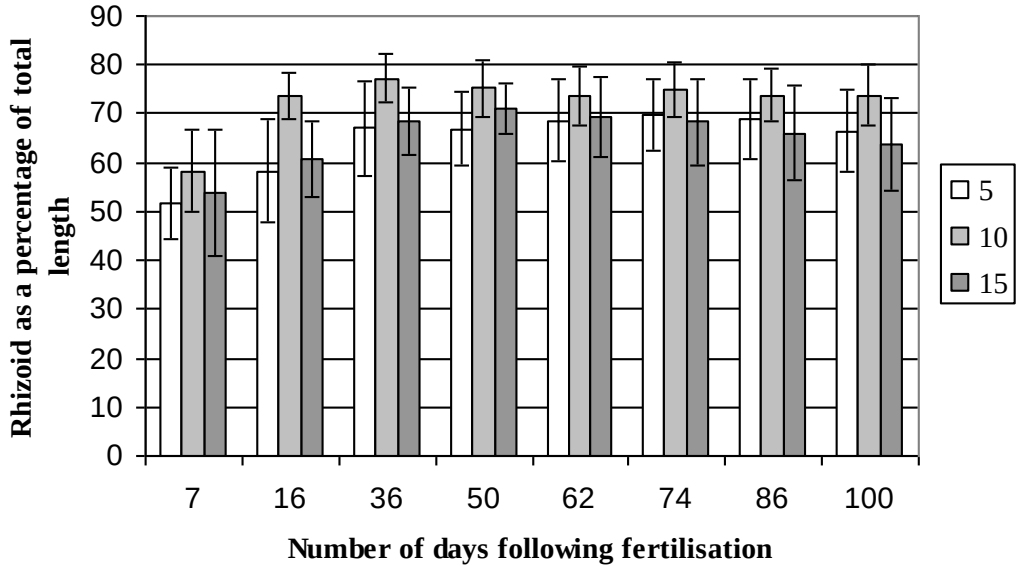


Figure 4.23: Mean rhizoid lengths as a percentage of the total length of 5 replicates of Orkney *F. vesiculosus* germlings cultivated at temperatures 5, 10, 15ºC over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Error bars signify standard deviation.

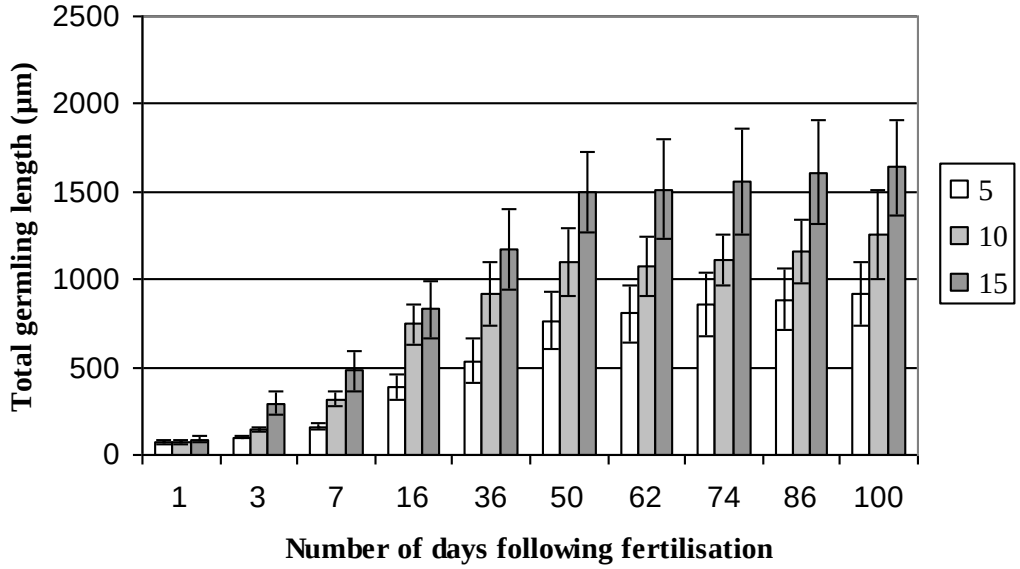


Figure 4.24: Mean total lengths (μm) of 5 replicates of Orkney *F. vesiculosus* germlings cultivated at temperatures 5, 10, 15ºC over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Error bars signify standard deviation.

Table 4.31: Matrices of results for Kruskal-Wallis test and *post hoc* Mann-Whitney *U* pairwise test of median values for germling growth of Orkney *F. vesiculosus* cultivated at different temperatures over a 100 day period. PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. 5 replicates, each replicate represents the mean of 20 germlings (* significant at p = <0.05, *** highly significant at p = 0.001, NS – not significant).

Measurement (μm)	**Temperature ºC**		
		5	10
Head	10	*	
	15	***	***
		5	10
Rhizoid	10	***	
	15	***	***
		5	10
Rhizoid as % of total length	10	***	
	15	NS	***
		5	10
Total length	10	***	
	15	***	***

Mean head **(Figure 4.21)**, rhizoid **(Figure 4.22)** and total length **(Figure 4.23)** were greatest at 15ºC while 5ºC was the least favourable for growth. Head length increased throughout at 15ºC while at both 5ºC and 10ºC growth slowed at 50 days and levelled off. Growth profiles for rhizoid growth were similar at all temperatures where after initial increase up to 50 days at 10ºC and 15ºC and 74 days at 5ºC growth slowed and levelled off. At 100 days significant differences in growth were noted at all temperatures for both head and rhizoid component parts and subsequently the total length.

Mean rhizoid length as a percentage of the total length **(Figure 4.23)** showed high levels at 5ºC and 10ºC however this is the result of greatly reduced head lengths compared to rhizoid length at these temperatures.

4.7.2 Conclusions

Results show that although germlings grew at all temperatures the optimum temperature for growth was 15ºC with 5ºC the least favourable temperature. Results also show that different temperatures appear to affect the growth rate of the component parts of the germlings, differently. For example, at temperatures of 5ºC and 10ºC germling growth was greatly reduced and this can be attributed to slow growth rate of head length and to a lesser extent rhizoid length affecting the total length.

The results suggest a preference for higher temperatures for the establishment and growth of populations of *F. vesiculosus* from Orkney.

4.8 Laboratory Investigation into Germling Growth of *Fucus vesiculosus* var. *linearis* Collected from Bay of Skaill (South), Orkney and Cultivated at Different Temperatures

4.8.1 Results

The mean 'head' and rhizoid lengths of germlings cultured at different temperatures are given in **Tables 4.32** and **4.33** respectively. **Table 4.34** gives the rhizoid lengths as a percentage of the total germling length. The mean total lengths of the germlings cultured at different temperatures are given in **Table 4.35**. These results are graphed in **Figures 4.25 – 4.28**. Statistically significant differences among treatment groups using non-parametric Kruskal-Wallis test and *post hoc* pairwise Mann-Whitney *U* test were performed. Results are given in **Table 4.36**.

Table 4.32: Mean head lengths (μm) of 5 replicates of Orkney *F. vesiculosus* var. *linearis* germlings cultivated at different temperatures over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Standard deviations of the replicate means are given in italics.

	Number of days following fertilisation							
Temp.	**7**	**16**	**36**	**50**	**62**	**74**	**86**	**100**
5ºC	**71.7**	**80.0**	**114.0**	**116.0**	**130.4**	**136.8**	**139.2**	**137.2**
	10.55	*13.26*	*16.45*	*19.23*	*28.1*	*29.64*	*33.66*	*30.85*
10ºC	**72.6**	**80.6**	**135.5**	**149.6**	**159.6**	**189.6**	**192.2**	**187.3**
	12.68	*15.49*	*18.66*	*23.09*	*28.32*	*29.4*	*20.23*	*21.78*
15ºC	**115.8**	**132.0**	**163.9**	**189.8**	**235.5**	**207.5**	**286.9**	**270.7**
	25.63	*25.54*	*32.25*	*24.62*	*50.42*	*50.88*	*69.89*	*80.05*

Table 4.33: Mean rhizoid lengths (μm) of 5 replicates of Orkney *F. vesiculosus* var. *linearis* germlings cultivated at different temperatures over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Standard deviations of the replicate means are given in italics.

	Number of days following fertilisation							
Temp.	**7**	**16**	**36**	**50**	**62**	**74**	**86**	**100**
5ºC	**84.1**	**121.4**	**328.2**	**517.1**	**564.5**	**631.9**	**613.8**	**593.1**
	26.52	*40.92*	*83.38*	*90.76*	*165.05*	*185.02*	*174.15*	*180.99*
10ºC	**82.6**	**117.7**	**414.6**	**556.4**	**644.4**	**747.5**	**732.1**	**724.0**
	21.49	*33.48*	*127.86*	*136.44*	*142.3*	*149.48*	*119.38*	*155.11*
15ºC	**123.0**	**351.2**	**636.3**	**767.5**	**824.3**	**552.2**	**475.4**	**439.5**
	36.11	*94.49*	*146.35*	*152.32*	*230.61*	*212.87*	*184.33*	*161.8*

Table 4.34: Mean rhizoid lengths as a percentage of the total lengths (μm) of 5 replicates of Orkney *F. vesiculosus* var. *linearis* germlings cultivated at different temperatures over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Standard deviations of the replicate means are given in italics.

	Number of days following fertilisation							
Temp.	**7**	**16**	**36**	**50**	**62**	**74**	**86**	**100**
5ºC	**52.39**	**59.63**	**73.74**	**81.58**	**80.29**	**81.13**	**80.4**	**79.97**
	9.14	*7.51*	*5.51*	*4.07*	*5.62*	*6.46*	*6.7*	*6.56*
10ºC	**50.95**	**58.56**	**74.29**	**77.83**	**79.26**	**79.04**	**78.76**	**78.97**
	7.05	*6.12*	*5.84*	*6.26*	*5.12*	*4.39*	*3.05*	*3.77*
15ºC	**50.93**	**71.74**	**78.71**	**79.7**	**76.97**	**71.17**	**60.54**	**60.66**
	9.86	*6.63*	*5.4*	*3.21*	*6.25*	*7.6*	*13.34*	*13.34*

Table 4.35: Mean total lengths (μm) of 5 replicates of Orkney *F. vesiculosus* var. *linearis* germlings cultivated at different temperatures over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Standard deviations of the replicate means are given in italics.

	Number of days following fertilisation									
Temperature	**1**	**3**	**7**	**16**	**36**	**50**	**62**	**74**	**86**	**100**
5ºC	**59.1**	**61.6**	**155.8**	**199.8**	**441.0**	**631.8**	**695.3**	**768.7**	**754.8**	**732.1**
	8.77	*13.31*	*32.35*	*45.48*	*89.14*	*96.36*	*172.26*	*181.56*	*171.64*	*182.82*
10ºC	**59.2**	**61.2**	**160.9**	**198.3**	**549.5**	**706.3**	**807.0**	**942.1**	**925.3**	**912.3**
	7.34	*13.8*	*30.69*	*43.21*	*139.16*	*139.11*	*142.6*	*160.01*	*127.16*	*164.77*
15ºC	**56.8**	**78.6**	**238.8**	**483.2**	**802.2**	**960.4**	**1059.8**	**759.7**	**761.6**	**710.0**
	7.23	*38.3*	*43.63*	*108.04*	*149.97*	*162.26*	*235.87*	*233.52*	*160.93*	*147.02*

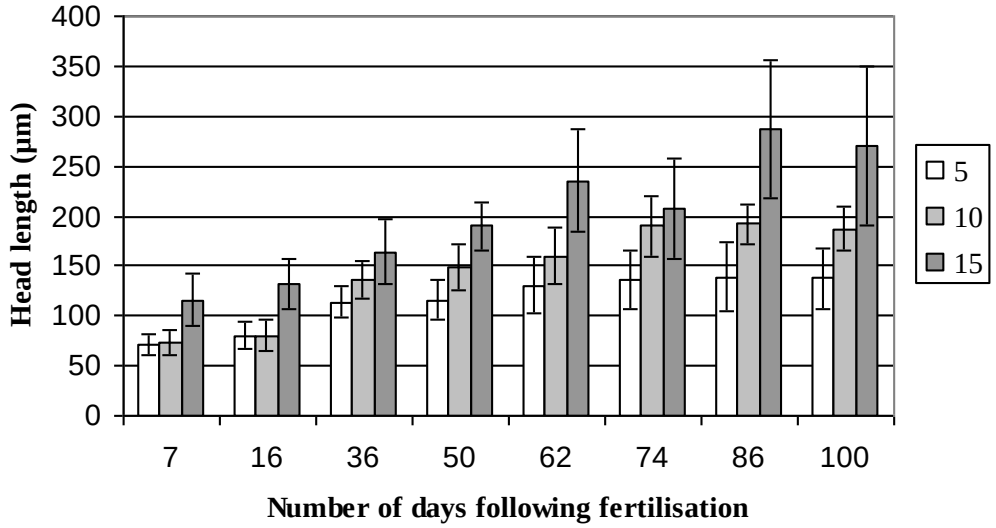


Figure 4.25: Mean head lengths (μm) of 5 replicates of Orkney *F. vesiculosus* var. *linearis* germlings cultivated at temperatures 5, 10, 15ºC over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Error bars signify standard deviation.

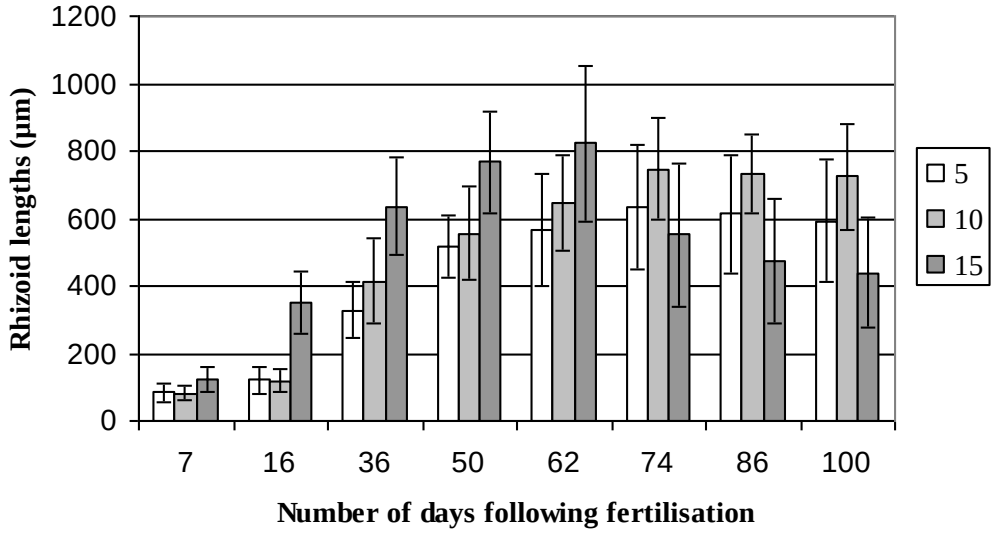


Figure 4.26: Mean rhizoid lengths (μm) of 5 replicates of Orkney *F. vesiculosus* var. *linearis* germlings cultivated at temperatures 5, 10, 15ºC over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Error bars signify standard deviation.

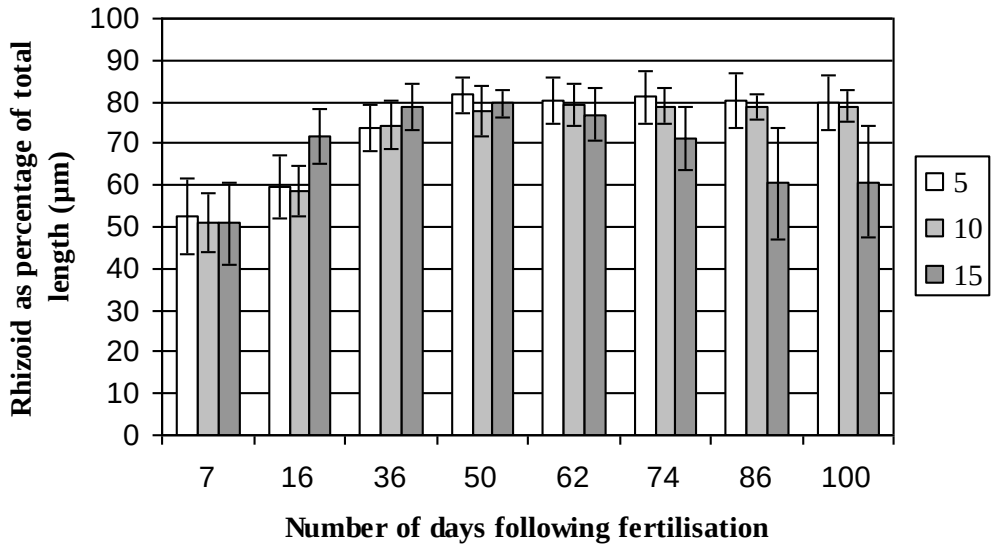


Figure 4.27: Mean rhizoid lengths as a percentage of the total length of 5 replicates of Orkney *F. vesiculosus* var. *linearis* germlings cultivated at temperatures 5, 10, 15ºC over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Error bars signify standard deviation.

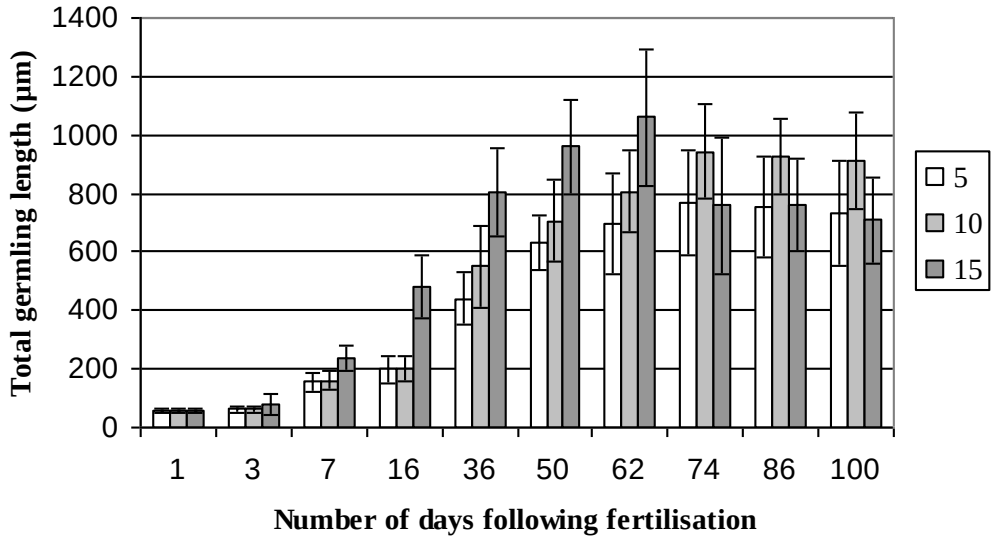


Figure 4.28: Mean total lengths (μm) of 5 replicates of Orkney *F. vesiculosus* var. *linearis* germlings cultivated at temperatures 5, 10, 15ºC over a 100 day period, measured at times after fertilisation (days). PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. Each replicate represents the mean of 20 germlings. Error bars signify standard deviation.

Table 4.36: Matrices of results for Kruskal-Wallis test and *post hoc* Mann-Whitney *U* pairwise test of median values for germling growth of Orkney *F. vesiculosus* var. *linearis* cultivated at different temperatures after a 100 day period. PAR 80μE $m^{-2}s^{-1}$, photoperiod light:dark 12:12. 5 replicates, each replicate represents the mean of 20 germlings (*** highly significant at $p = 0.001$, NS = not significant).

Measurement (μm)	**Temperature ºC**		
		5	10
Head	10	***	
	15	***	***
		5	10
Rhizoid	10	***	
	15	***	***
		5	10
Rhizoid as % of total length	10	NS	
	15	***	***
		5	10
Total length	10	***	
	15	NS	***

Mean head length **(Figure 4.25)** was consistently greatest at 15ºC throughout with 5ºC the least favourable temperature for growth. Growth generally increased at 15ºC up to 86 days with a dip at 74 days and 100 days. Growth increased at 5ºC and 10ºC up to around 62 days and 74 days respectively were growth slowed and levelled off. At 100 days there was significant difference in growth between all temperatures ($p = 0.001$).

Mean rhizoid length **(Figure 4.26)** was greatest at 15ºC up to 62 days after which growth dipped, becoming the least favourable temperature thereafter and 10ºC was then the best for growth. 5ºC was the least favourable temperature up to 62 days. Growth profiles for 5ºC and 10ºC were similar, peaking at 74 days and levelling out thereafter. At 100 days there was significant difference in growth between all temperatures ($p = 0.001$).

Mean rhizoid growth as a percentage of total length **(Figure 4.27)** showed no initially preference for temperature at 7 days suggesting that growth effort was spread over both components parts at all temperatures in the early stages of development. At days 16 and 36 the slight preference at 15ºC was due to greater increase in rhizoid growth at this temperature. At days 50 and 62 again growth effort is over both head and rhizoid. The lower rate noted at 15ºC at 74 days onwards is the result of a dip in rhizoid growth.

The results for mean total length **(Figure 4.28)** reflect the growth patterns for head and rhizoid growth with 15ºC the optimum temperature for grown up to 74 days after which growth dipped and 10ºC was the optimum temperature. 5ºC was the least favourable temperature for growth up to 62 days and thereafter equalled 15ºC as the least favourable temperatures. Total length levelled out at 74 days at all temperatures. At 100 days growth was significantly greater at 10ºC (p = 0.001) with no significant difference in growth at 5ºC and 15ºC.

4.8.2 Conclusions

Results show that 15ºC was initially the optimum temperature for growth however the sharp dip in rhizoid growth at 62 days meant that 10ºC was the optimum temperature after this time. The least favourable temperature for growth was at 5ºC, particularly for head growth.

The sharp dip in rhizoid growth noted at 15ºC may have been due to some condition of culture however head length at this temperature was not affected although a dip was noted at 74 days before recovery. Such a severe response was not recorded for other species however a dip in rhizoid growth was noted at 15ºC in *nanus* from Orkney at 74 days and *anceps* from Ireland at 62 days, two other 'dwarf' forms. If the response seen in *linearis* was due solely to the highest temperature of 15ºC then either rhizoid growth was inhibited as this temperature or growth effort was concentrated on head development at that time.

4.9 Conclusions from germling growth experiments

The results from the germling experiments show a number of key points.

- Germlings of all the species investigated grew at all three temperatures but the majority of species and taxa grew best at the highest temperature of 15ºC. These were, in order of greatest total length at 100 days, *F. distichus* subsp. *anceps*, *F. serratus*, *F. spiralis* and *F. vesiculosus* all from Orkney. *F. distichus* subsp. *anceps* from Ireland showed a preference for 10ºC while the only species which favoured the lowest temperature of 5ºC was *F. spiralis* f. *nanus* from Orkney. *F. vesiculosus* var. *linearis* from Orkney was the only species were a change in optimum temperature preference was noted over the culture period, going from 15ºC to 10ºC.

- The optimum temperature for germling growth for a species was mainly the result of continuous growth of both head and rhizoid components at that temperature over the experimental period.

- The effects of different temperatures on growth rates of head, rhizoid or both varied not only within a species but between species. At non-optimum temperatures for growth the reduction in head and/or rhizoid growth varied as follows: reduction in rhizoid growth in *F. distichus* subsp. *anceps* from Ireland and Orkney and *F. spiralis* and *F. spiralis* f. *nanus*; reduction in head growth in *F. vesiculosus*; low rates of both rhizoid and head growth in *F. serratus* and *F. vesiculosus* var. *linearis*.

- Because the reduction in growth of component parts of germlings did not occur at all temperatures and that both head and rhizoid length increased at the optimum temperatures then it can be assumed that temperature was the limiting factor for growth of *Fucus* germlings at non-optimal temperatures. This assumption is within the context of this experiment as only one factor that of temperature was tested.

A brief summary of the results for all species are given in **Table 4.37**.

Table 4.37: Summary table showing: optimum temperature for *Fucus* germling growth; mean head, rhizoid and total length; growth increase or decrease of head and rhizoid components at different temperatures at end of 100 days.

Species	**Optimum temperature for germling growth at end of 100 days**	**Mean head, rhizoid and total length growth at end of 100 days (μm)**			**Growth increase or decrease of germling head and/or rhizoid components at different temperatures**	
		Head Length	**Rhizoid Length**	**Total Length**	**Head**	**Rhizoid**
OFda	15ºC	764.9	1683.0	2446.90	Growth increased at 5ºC, 10ºC and 15ºC.	Increased at 15ºC. At 5ºC and10ºC growth levelled out at 62 days.
IFda	10ºC	462.3	1678.1	2142.8	Growth increased at 5ºC, 10ºC and15ºC.	Increased gradually at all temperatures but dipped at 15ºC after 62 days
OFserr	15ºC	1203.5	1213.5	2411.0	Increased at 15ºC. At 5ºC and10ºC growth levelled out at 62 days.	Increased at 5ºC and 15ºC. Dipped at 10ºC after 50 days.
OFspir	15ºC	356.2	1290.5	1646.7	Increased at 5ºC, 10ºC and 15ºC.	Increased at 15ºC. Levelled out at 74 days at 5ºC, dipped after 50 days at 10ºC.
OFspna	5ºC	181.3	816.1	1022.1	Growth increased at 5ºC and10ºC. Levelled out at 62 days at 15ºC.	Growth levelled out at 5ºC at 74 days At 62 days levelled out at10ºC and dipped 15ºC.
OFves	15ºC	583.1	1056.8	1639.9	Growth increased at 15ºC. At 5ºC and10ºC growth levelled out at 62 days.	Growth levelled out at 50 days at 10ºC and 15ºC, and 74 days at 5ºC.
OFveslin	15/10ºC*	270.7	724.0	912.3	Increased at 15ºC. Levelled out at 74 days at 10ºC and 62 days at 5ºC.	Increased at 15ºC to 62 days. Levelled out at 74 days at 10ºC. Dipped at 74 days at 5ºC.

* Only species where optimum temperature preference changed over the culture period, initially at 15ºC up to 62 days, thereafter at 10ºC to end of the culture period at 100 days.

4.10 Discussion

The response of embryos to environmental parameters is significant in considering distribution of *Fucus* species with temperature a major controlling factor (McLachlan, 1973). The attachment to the substratum is of prime importance to *Fucus* species (Hardy and Moss, 1979) and rhizoids are essential in the establishment of a population therefore the present work investigated, not only the effect temperature had on the total length of germlings but also on the associated head and rhizoid component parts.

Rhizoid length growth was greatest for *F. distichus* subsp. *anceps* from Orkney (1683.0 μm) and Ireland (1678.1 μm). This may not be surprising for a species found on extreme wave exposed shores where a strong attachment is essential. A similar pattern may have be expected therefore in *F. spiralis* f. *nanus* (816.1 μm) and *F. vesiculosus* var. *linearis* (824.3 μm), two other wave exposure species, but this was not the case as both species had the lowest rhizoid growth rates. This may however reflect the overall small size of the plants found in nature as both these species also exhibited the lowest head lengths.

At the end of the experimental period growth rates appeared slow for all plants with the greatest length only about 2.5 mm in just over three months. It was impossible to replicate under laboratory conditions all the environmental factors that are found on intertidal rocky shores and consequently there may have been some limiting factors to germling growth. Apart from controlled temperature variables, two consistent factors were incorporated into the experimental setup that were deemed important, light and photoperiod.

Although germination and initial growth of *Fucus* zygote over the first few days is independent of light (McLachlan, 1973; Terry and Moss, 1981) further development and growth of embryos require light. In the present study light was provided by Philips White-light fluorescent tubes with photosynthetically active radiation (PAR) of 80μE $m^{-2}s^{-1}$ at water surface. Similar irradiance was used by Grundy (1996) on her studies on *F. serratus* germlings in relation to coastal pollution. Although Grundy's experimental period only ran for up to 12 days, her results showed that germling growth for *F.*

serratus were comparable to the current study over the same time periods. Work by Scanlan and Wilkinson (1987) on biocide toxicity testing using *F. serratus* showed germlings with average length of only 50 μm at 10ºC after 20 days. This was much less than the 621.8 μm recorded at 16 days in the present study at the same temperature. A lower irradiance of (PAR) of 40-50μE $m^{-2}s^{-1}$ and a photoperiod of light:dark 16:8h may explain the difference in growth.

Work by McLachlan (1973) on *F. edentatus* embryos, a species not unlike *F. serratus* in size and morphology, showed similar growth rates to the present study at a period of 14 days. Long term studies on the culture of *Fucus* species by McLachlan (1971) showed thalli growth for *F. vesiculosus* up to 2 cm after four months and *F. serratus* exceeding 13 cm in a year. *F. distichus* subsp. *distichus* and *edentatus* exceeded 4 cm over 2 years. McLachlan stated that for all species rate of growth was less than observed in nature. It may be that any negative effects in the present investigation due to limiting factors occur in the later stages of plant growth. Incubation of embryos under continuous light can result in abnormal development, including suppression of the rhizoid system therefore photoperiod was set at light:dark 12:12h as this ratio had been shown to be adequate for normal development (McLachlan, 1973).

Natural unsupplemented seawater has been used successfully by a number of workers (Terry and Moss, 1981; Scanlan and Wilkinson, 1987). In this study membrane-filtered (pore size ~1μm) natural seawater was used in laboratory cultures and collected from the same site as the *Fucus* species investigated wherever possible. However McLachlan (1977) suggested that unfortified seawater repressed the growth and development of embryos. In the present study the medium was changed every 3/4 days and this was felt adequate at the time, however nutritional requirements may change with maturation and a depletion of nutrients may account for overall small plant size at the end of the experimental period.

Studies on *F. distichus* by Thomas *et al*. (1985) indicated that periodic exposure to air was necessary for optimum germling development and that secondary rhizoid development was greatly reduced if germlings were not exposed. However (McLachlan

et al, 1971) found no requirement for a tidal cycle for *F. serratus* and *F. vesiculosus* which grew well under submerged conditions. Any regime of daily tidal cycle was beyond the scope of this investigation but was not thought essential. It is interesting to note that *F. serratus* had some of the best growth rates in this study and is a species which occurs in the lowest zone on the intertidal shore and subsequently is submerged for long periods of time. Do the results reflect the natural position of this species on the shore and was this noted for other *Fucus* species? *F. distichus* subsp. *anceps* occupies the top of the shore and if the same theory is applied then it would be expected that continuous submersion would have a detrimental effect on growth. That was not the case as both populations, from Orkney and Ireland, had high growth rates with *anceps* from Orkney having the highest. However two other species occupying the top of the shore, *F. spiralis* f. *nanus* and *F. vesiculosus* var. *linearis*, did show very low growth rates, in fact the lowest of all species investigated, but this may reflect the small size of both these species in nature rather than any negative effect from continuous submersion.

Is there any evidence to suggest that growth rates of *Fucus* germlings in culture were related to collection dates and seasonality? *F. distichus* subsp. *anceps* from Orkney and Ireland were collected in April and March respectively. Fruiting periods are from April to August which may suggest laboratory cultivation was in the early part of their reproductive season. However receptacles may start to develop as early as January and both populations showed high growth rates and may in fact have been at their peak fruiting period. *F. serratus* which has a peak fruiting period from October to December, although reproductive plants can be found throughout the year, was collected and cultivated in April yet this species had the second highest growth rate. The results do not appear to show any seasonal preference however the work involved in this study provide only limited observations. Culture experiments carried out by McLachlan (1973) over a period of several years and at all seasons showed no indication of seasonal variation.

Laboratory experiments have shown that substratum type can affect the morphology of rhizoids of *Fucus* species (Hardy and Moss, 1979). The smooth surfaces used in this experiment are an unnatural environment for species which normally settle on rocky

seashores. On rough surfaces rhizoids can penetrate into crevices and any weak areas. Preliminary investigations on settlement using stone tiles proved impractical for various reasons. Firstly it was not possible to take accurate measurements using a compound microscope as tiles were too thick for the high magnification needed and secondly the tiles did not allow any light through. A dissecting microscope was tested but again no accurate measurements could be taken. The smooth surfaces used here produced germlings with long, straight, narrow rhizoids and although may not have reflected natural morphology did in fact make it easy to get accurate measurements of the primary rhizoids. As the material used was consistent throughout then it was felt that the effects of temperature alone could be measured using this method.

Despite the possibility of some limiting factors to growth, experimental conditions were constant throughout for each species with temperature the only variable and results show that temperature did affect germling growth of *Fucus* species. Growth rates of head, rhizoid or both and the subsequent effect on the total length varied between species and within species, at different temperatures and often over different time periods.

For a population of *Fucus* species to become established then germlings of that species must develop and grow, reach maturity and become reproductive. It has been suggested that the conditions for the development of sporelings of fucoids may not be the same for mature plants (Knight and Parke, 1950). If this is true then evalution of the effects of the environmental conditions on germlings alone will be of limited value in identification of factors limiting distribution (Lüning, 1980; Chapman, 1995). The following chapter therefore will investigate the effect temperature had on adult *Fucus* species with regards to growth, survival and performance.

Chapter 5: Investigations into the Effect of Temperature on Growth of Adult *Fucus* Plants

The following experiments were used to investigate the effect of temperature on growth, survival and performance of various *Fucus* species and taxa using the measurements of change in surface area, wet (fresh) weight and photosynthetic and respiration rates.

5.1 Laboratory Investigations into the Effect of Temperature on Adult Growth of Various *Fucus* Species and Taxa using Change in Surface Area Measurements

Mean growth of replicates was used to investigate the change in surface area of various *Fucus* species and taxa collected from different geographical locations and cultivated at different temperatures in a controlled environment. *Fucus* species investigated along with collection sites, date of collection and sea surface temperatures for month of collection are given in **Table 5.1**. To avoid any issue of aged plants affecting results care was taken to include a broad representation of the population when collecting the *Fucus* species investigated.

5.1.1 Method

The method used in this experiment is set out in **Section 3.3**. Vegetative plants collected from Orkney, Shetland and Ireland were kept refrigerated until such time as laboratory facilities were available (usually no more than 3 days). On return to the laboratory, plants were placed immediately in large plastic tanks containing seawater and aerated with air pumps and left to acclimatise for 24 hours at temperatures of 5°C, 10°C and 15°C. Vegetative apical tips of 3cm in length were excised and placed in seawater in the CT rooms at the appropriate temperature for that experiment and allowed to fully hydrate before initial measurements were taken. Laboratory cultures were under the same conditions as for the germling experiments and apart from controlled temperature variables, two consistent factors were incorporated into the experimental setup, light and photoperiod. Any limiting factors to growth of adult apical tips were the same as for germling growth and covered in **Section 4.10**.

Twenty adult sections were used for each replicate, three replicates per species at temperatures of 5°C, 10°C and 15°C cultivated over a period of 100 days. The adult sections were placed in clear plastic containers with 400ml of GF/C filtered seawater which was changed every 3 to 4 days. Photosynthetically active radiation (PAR) of 80μE $m^{-2}s^{-1}$ at water surface was provided by Philips White-light fluorescent tubes and photoperiod set at 12:12, light:dark (07.00 – 19.00 hrs). Measurement of the tips was carried out as described in **Section 3.7.1.**, before and after the treatment period using scanned images of the sections and analysed with the image analysis program ImageJ (Abramoff *et al*., 2004). Unfortunately some species were subject to a smaller temperature range of 10°C and 15°C and reduced culture period of 50 days. This was due to serious malfunction and failure of CT rooms leaving them inoperable for many months. These species are denoted with asterisks in **Table 5.1**.

To investigate any statistically significant differences of percentage change of surface area growth of plants between treatments all datasets were tested for normality (Kolmogorov-Smirnov test) and homogeneity of variance (Levene's Test) to see if a one-way Analysis of Variance (ANOVA) could be used. If one-way ANOVA appropriate then *post hoc* (Tukey's HSD) test performed for pairwise comparison to identify which groups were significantly different from other groups. Where datasets failed the above tests then a non-parametric (Kruskal-Wallis test) equivalent was used to look at differences between median values. Where significant results for Kruskal-Wallis test were found then *post hoc* pairwise Mann-Whitney *U* test performed.

Table 5.1: *Fucus* species and taxa used in this part of the investigation along with present author's codes, locations, National Grid references, sea surface temperatures for the month of collection and collection dates. (Codes: Fda = *Fucus distichus* subsp. *anceps*; Fspna = *Fucus spiralis* f. *nanus*; Fveslin = *Fucus vesiculosus* var. *linearis*; Fserr = *Fucus serratus*; Fspir = *Fucus spiralis*; Fves = *Fucus vesiculosus*; Fde = *Fucus distichus* subsp. *edentatus*. O = Orkney; I = Ireland; Mac = Macduff Harbour; Shet = Shetland Islands; SQ = South Queensferry).

Species	Codes	Location	NG Ref.	Sea Surface Temps.†	Collection Dates
Fucus distichus subsp. *anceps*	OFda	Bay of Skaill (South), Orkney	HY226190	7 - 8ºC	Mid April '06
Fucus distichus subsp. *anceps*	IFda	George's Head, Kilkee, Co. Clare, Ireland	W088162	8 - 10ºC	Early March '06
Fucus spiralis f. *nanus*	OFspna	Bay of Skaill (South), Orkney	HY226190	7 - 8ºC	Mid April '06
Fucus vesiculosus var. *linearis*	OFveslin	Bay of Skaill (South), Orkney	HY226190	7 - 8ºC	Mid April '06
Fucus vesiculosus var. *linearis*	IFveslin	George's Head, Kilkee, Co. Clare, Ireland	W088162	8 - 10ºC	Early March '06
Fucus serratus	OFserr	Bay of Skaill (North), Orkney	HY232196	7 - 8ºC	Mid April '06
Fucus spiralis	OFspir	Bay of Skaill (North), Orkney	HY232196	7 - 8ºC	Mid April '06
Fucus vesiculosus	OFves	Bay of Skaill (North), Orkney	HY232196	7 - 8ºC	Mid April '06
Fucus distichus subsp. *edentatus*	MacFde	Macduff Harbour, Moray Firth, Scotland	NJ702645	10 - 11ºC	Mid Oct '06
Fucus distichus subsp. *edentatus* *****	ShetFde	Bressay, Shetland Islands	HU475434	10 - 11ºC	Mid July '05
Fucus serratus *****	SQFserr	South Queensferry, Midlothian, Scotland	NT140784	12 - 13 ºC	Mid July '05
Fucus spiralis *****	SQFspir	South Queensferry, Midlothian, Scotland	NT140784	12 - 13 ºC	Mid July '05
Fucus vesiculosus *****	SQFves	South Queensferry, Midlothian, Scotland	NT140784	12 - 13 ºC	Mid July '05

† Average sea surface temperature for the month of collection.
***** Reduced culture period of 50 days at 10ºC and 15ºC only due to serious malfunction of CT rooms and eventual failure.

5.1.2 Results

The results of the changes in surface area are expressed as both the total change in surface area in centimetres as well as percentage change of surface area. The results are given in **Table 5.2** and the changes expressed as a percentage change in surface area are graphed in **Figures 5.1 – 5.2.**

Table 5.2: Mean growth of adult *Fucus* tips cultured at different temperatures after a period of 100 days. PAR 80μEm^{-2} s^{-1}. Photoperiod light:dark 12:12. Growth is expressed as change in surface area in cm^2 and percentage change. 3 replicates, each replicate represents 20 vegetative tips. Standard deviations of replicate means given in italics.

	Change in Surface Area					
	Temperature					
	5ºC		10ºC		15ºC	
Species	cm²	percent	cm²	percent	cm²	percent
OFda	12.55	54.15	18.4	29.47	86.58	134.82
	1.88	*0.51*	*0.89*	*3.33*	*6.48*	*13.04*
IFda	13.38	67.1	8.86	52.18	11.52	81.44
	0.86	*3.14*	*0.72*	*3.32*	*2.35*	*28.86*
OFspna	8.29	15.5	114.37	190.62	95.73	183.61
	1.85	*3.86*	*8.42*	*18.74*	*5.96*	*6.02*
OFveslin	19.41	45.09	114.23	181.58	102.16	178.99
	14.01	*34.18*	*10.97*	*17.03*	*11.92*	*4.96*
IFveslin	8.62	28.49	4.53	16.43	2.49	18.99
	0.24	*2.18*	*1.71*	*7.82*	*2.13*	*9.42*
OFserr	27.81	29.34	186.29	196.35	202.39	230.24
	3.73	*5.01*	*1.94*	*7.27*	*23.4*	*5.72*
OFspir	12.11	21.44	84.81	129.18	62.27	121.50
	3.2	*4.28*	*38.47*	*3.62*	*24.23*	*1.73*

Table 5.2 (continued)

OFves	13.97	27.39	98.51	129.82	77.93	123.12
	10.23	*26.8*	*44.90*	*2.77*	*32.96*	*4.76*
MacFde	122.5	188.61	111.91	169.61	104.73	162.48
	2.38	*1.64*	*3.57*	*4.38*	*5.47*	*6.44*
ShetFde	N/A	N/A	61.99	86.3	51.32	90.37
	N/A	N/A	*0.74*	*2.82*	*12.55*	*16.67*
SQFserr	N/A	N/A	85.89	140.61	94.36	141.44
	N/A	N/A	*4.16*	*1.55*	*26.32*	*44.83*
SQFspir	N/A	N/A	84.35	163.45	71.18	130.34
	N/A	N/A	*3.29*	*10.7*	*6.19*	*6.48*
SQFves	N/A	N/A	79.96	169.43	90.97	164.39
	N/A	N/A	*9.66*	*0.68*	*11.37*	*40.76*

N/A not applicable due to CT room failure.

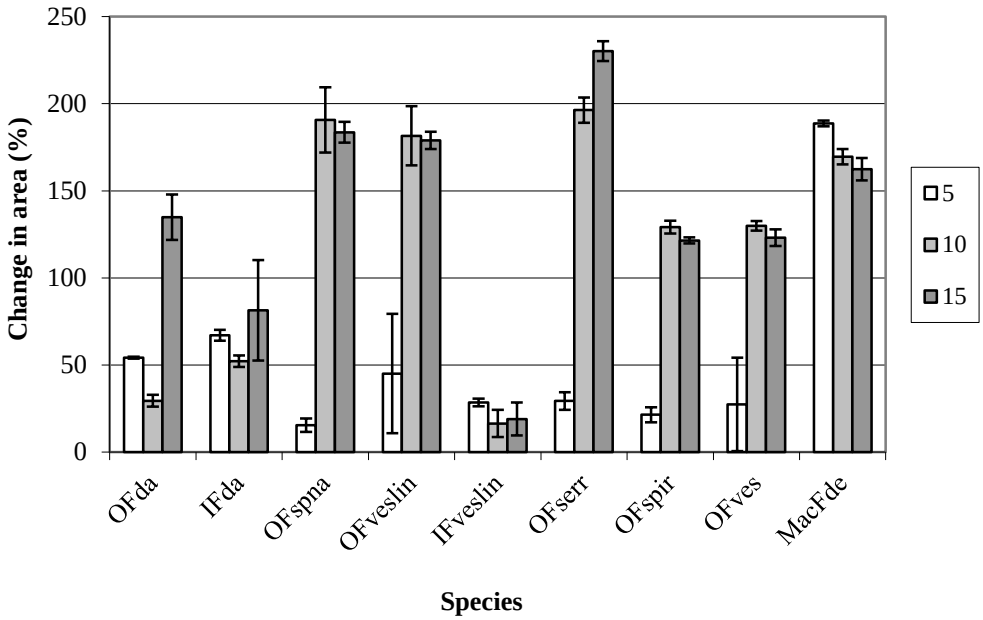


Figure 5.1: Mean percentage change in surface area (cm^2) of adult *Fucus* tips cultured at temperatures 5, 10, 15ºC after a period of 100 days. PAR 80μE $m^{-2}s^{-1}$. Photoperiod light:dark 12:12. 3 replicates, each replicate represents 20 tips. Error bars signify standard deviation for the replicate means.

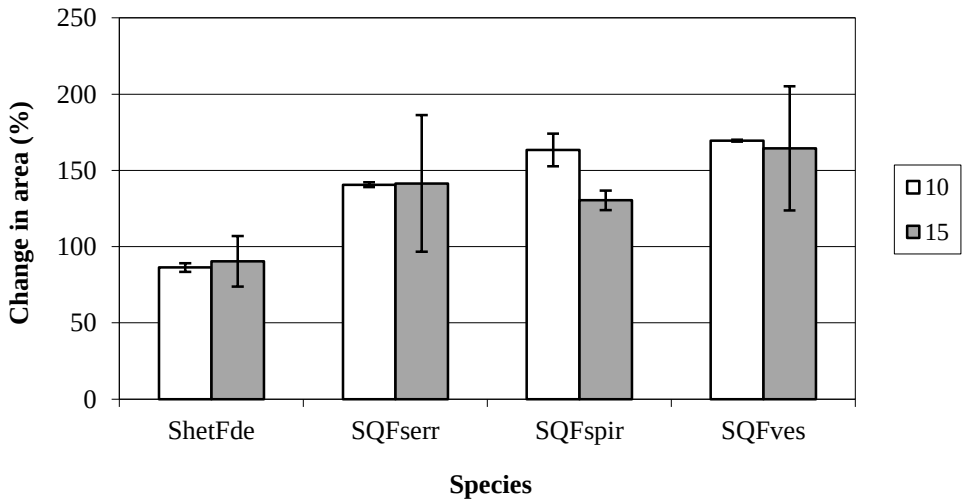


Figure 5.2: Mean percentage change in surface area (cm^2) of adult *Fucus* tips cultured at temperatures of 10ºC and 15ºC after a period of 50 days. PAR 80μE $m^{-2}s^{-1}$. Photoperiod light:dark 12:12.3 replicates, each replicate represents 20 tips. Error bars signify standard deviation for the replicate means.

All the species investigated showed an increase in surface area at the end of the experimental period. Statistical tests were performed on the percentage change in surface area of the apical tips to investigate any significant differences in growth at different temperatures. The results are given in **Table 5.3**.

Table 5.3: Matrices of results of one-way ANOVA and *post hoc* Tukey's HSD pairwise test for significant difference in mean values and non-parametric Kruskal-Wallis test and *post hoc* Mann-Whitney *U* pairwise test for significant difference in median values. Tests performed on percentage change in surface area of adult *Fucus* tips cultured at temperatures 5, 10, 15ºC after a period of 100 days. PAR 80μE m^{-2} s^{-1}. Photoperiod light:dark 12:12. 3 replicates, each replicate represents mean of 20 tips (***significant at p = < 0.001; *significant at p = 0.05; NS= not significant).

Species		**Temperature**	
OFda		5	10
	10	***	
	15	***	***
IFda		5	10
	10	NS	
	15	NS	NS
OFspna		5	10
	10	***	
	15	***	NS
OFveslin		5	10
	10	***	
	15	***	NS
IFveslin		5	10
	10	NS	
	15	NS	NS
OFserr		5	10
	10	***	
	15	***	***
OFspir		5	10
	10	***	
	15	***	NS
OFves		5	10
	10	***	
	15	***	NS
MacFde		5	10
	10	***	
	15	***	NS

Results show that the majority of species had greatest increase in growth at the higher temperatures of 10°C and 15°C. These species were, in order of greatest surface area increase, *F. serratus*, *F. spiralis* f. *nanus*, *F. vesiculosus* var. *linearis*, *F. distichus* subsp. *anceps*, *F. vesiculosus* and *F. spiralis* from Orkney. Of these *F. serratus* and *F. distichus* subsp. *anceps* had a clear preference for 15°C. In all the above species there was significant difference in surface area increase at the higher temperatures compared to that at 5°C with *nanus* having the lowest growth rate overall. *F. distichus* subsp. *edentatus* from Macduff grew best at the lowest temperature of 5°C with no significant difference in growth at 10°C and 15°C, however surface area did increase at all temperatures. The Ireland populations of *linearis* and *anceps* showed the lowest growth rates overall with no significant difference in surface area between temperatures at the end of the experimental period.

Unfortunately results for *F. serratus*, *F. spiralis* and *F. vesiculosus* from South Queensferry and *F. distichus* subsp. *edentatus* from Shetland were very limited due to CT room failure at 5ºC and are therefore treated separately. Results show that *vesiculosus* had the greatest growth increase in surface area at 10ºC and 15ºC although only marginally greater at 10ºC than *spiralis*. For *serratus* there was little difference between 10ºC and 15ºC with growth at 10ºC less than that for *spiralis* and *vesiculosus* and at 15ºC greater than *spiralis* but less than *vesiculosus*. *F. distichus* subsp. *edentatus* showed the lowest growth rate overall at both temperatures. Due to large standard deviations between the means only one statistically valid difference between temperatures was noted in *spiralis* ($p = < 0.05$) with a preference for growth at 10ºC.

5.1.3 Conclusions

In the Orkney species, with the exception of *anceps*, there was a clear preference for growth at the warmer temperatures of 10ºC and 15ºC with very reduced growth at the lowest temperature of 5ºC. In the case of *F. distichus* subsp. *anceps* the greatest increase in growth was at 15ºC. The southern population of *anceps* from Ireland showed low growth at all temperatures, a trend noted in the other southern species from Ireland, *F. vesiculosus* var. *linearis* and both species showed no significant difference in

growth between temperatures. Both *anceps* and *linearis* are species associated with wave exposed shores and are small in nature and this may have accounted for the small increase in growth in the Ireland populations. However results show that the same two species from Orkney including the other wave exposure form *F. spiralis* f. *nanus*, all had much greater growth increase, at least at higher temperatures.

F. distichus subsp. *edentatus* from Macduff grew well at all temperatures but had a growth temperature preference at 5°C. Unfortunately this preference for cooler temperatures found in the Macduff population could not be compared to *edentatus* from Shetland due to CT room failure at 5ºC and reduced culture period. The limited results for the Shetland population show that surface area did increase at both temperatures however there was no significance in growth at 10ºC and 15ºC.

Similarly it is difficult to compare results of the same *Fucus* species from geographically different populations of Orkney and South Queensferry due to the reduced temperature and cultivation period. However both *spiralis* and *vesiculosus* showed greater surface area growth in the populations from South Queensferry despite being cultivated for only 50 days, half the time period of the Orkney populations. It may be that South Queensferry species are larger in nature or that they grow faster than Orkney species although this was not recorded for *serratus* from South Queensferry. Another possibility may be that some limiting factor to growth was present later in the experimental period which affected the Orkney populations; however *serratus* from Orkney showed high rates of surface area increase under the same experimental conditions.

The results show that there was an increase in surface area growth for all *Fucus* species over the experimental period but that this often varied between species and temperatures. Strömgren (1977, 1985) suggested that it is possible that meristematic cells respond to temperature in different ways. The author also stated that the observed growth response of *Fucus* apices was representative of intact plants and that estimates of average growth rates from the length of intact apices confirmed that the observed growth rates in cut apices were realistic.

5.2 Laboratory Investigations into the Effect of Temperature on Adult Growth of Various *Fucus* Species and Taxa using the Wet (Fresh) Weight Method

Weight measurements can be used for growth estimations when dealing with macroscopic portions of algae such as *Fucus*. This method was used by Schonbeck and Norton (1979, 1980) to measure increase in fresh weight of fucoid algae as part of their study on factors controlling lower limits of algae on the shore and by Fletcher and Fletcher (1975) to follow the growth of segments of *Sargassum muticum*. In this part of the investigation it was used to measure the fresh weight of the *Fucus* apical tips used in the surface area experiments in **Section 5.1**. Unfortunately *F. serratus*, *F. spiralis* and *F. vesiculosus* from South Queensferry and *F. distichus* subsp. *edentatus* from Shetland, the species cultivated at a reduced time period and temperature, were not included in this part of the investigation due to lost data. Whereas measurement of surface area showed increase due to any changes in length and breadth, fresh weight may indicate changes due to bulk. McLachlan *et al*. (1971) stated that increase in bulk is equally important in consideration of growth.

5.2.1 Method

The apical tips prepared for the surface area experiments were weighed before and after the culture period of 100 days. The apical tips were dried between paper towels to remove excess water and weighed per replicate on digital scales (g). 3 replicates per *Fucus* species, each replicate containing 20 apical tips. This was carried out as quickly as possible to prevent shrinkage before returning to the relevant CT rooms. General observations on the health of apical sections were noted with regards to texture and discolouration along with any changes in the form of reproductivity, regeneration and new growth.

5.2.2 Results

The results of growth increase at different temperatures expressed as percentage change of the wet (fresh) weight after a period of 100 days are given in **Table 5.4** along with observations on condition and growth. Examples of regeneration and new growth

beyond apical tips are shown in **Figure 5.3 (a – f)**. Changes expressed as a percentage change in wet (fresh) weight over the experimental period are graphed in **Figure 5.4**.

Table 5.4: Mean growth of adult *Fucus* tips cultured at different temperatures after a period of 100 days along with observations. PAR 80μE m^{-2} s^{-1}. Photoperiod light:dark 12:12. Growth is expressed as percentage change of wet (fresh) weight. 3 replicates, each replicate represents 20 vegetative tips. Standard deviations of the replicate means given in italics.

Species	Temp. ºC	Wet weight %	Observations after 100 days in culture
	5	88.76 (*20.44)*	Texture firm. Discolouration, tips pale, some dark areas. Some new growth at tips. Some plants reproductive with new growth beyond reproductive area. Regeneration from cut end (wound surface) of plant.
OFda	10	37.24 (*1.76)*	Texture firm with no disintegration. Discolouration: some plants very pale, some plants have dark patches. Small amount of new growth present on some tips.
	15	13.37 (*2.44)*	Apical tips show some deterioration with texture soft and slimy in some plants. Some discolouration with light and black areas present. Small amount of new growth at some tips.
	5	146.12 (*3.75)*	Texture firm, no disintegration. Pale in colour with occasional dark patches. Plants becoming reproductive.
IFda	10	82.12 (*3.69)*	Texture fairly firm, little disintegration. Discolouration, plant pale colour with black patches. Some tips reproductive with new growth beyond reproductive area. Regeneration growth at cut end of plants.
	15	115.73 (*16.46)*	Texture firm, no disintegration. Discolouration, some tips very pale, dark patches. Some tips reproductive.
	5	44.94 (*8.74)*	Texture firm with no disintegration. Discolouration, pale areas and black patches. Some plants becoming reproductive.
OFspna	10	44.80 (*7.63)*	Texture firm. Some discolouration with light and black areas. Some new growth on tips.
	15	47.46 (*2.01)*	Texture firm. Some discolouration with black areas, especially on midrib. New growth on tips.
	5	79.73 (*22.19)*	Texture firm, very little disintegration. Discolouration, light and black areas. Some new growth at tips. Some plants reproductive. Very small amount of regeneration at cut end.
OFveslin	10	54.68 *(8.46)*	Texture firm. Discolouration with light and black areas, particularly on tips. Some new growth on tips.
	15	47.18 (*2.87)*	Texture firm. Some discolouration with light and black areas. Lot of new growth on tips.

Table 5.4 (continued)

	5	89.31 (*6.28)*	Texture firm, no disintegration. Pale in colour with some plants having dark patches. Plants reproductive.
IFveslin	10	53.23 (*8.66)*	Texture fairly firm, some disintegration. Tips very pale in colour, black areas present. Few plants becoming reproductive.
	15	27.89 (*3.56)*	Texture fairly firm but some disintegration. Tips pale in colour, black areas present. Small amount of regeneration at cut end of plants.
	5	45.46 (*12.85)*	Texture firm, no disintegration. Some discolouration with light areas and black patches. Small amount of new growth starting on tips.
OFserr	10	44.12 (*2.56)*	Texture firm. Some discolouration. Some plants reproductive.
	15	67.63 (*5.50)*	Texture firm. Some discolouration with light and dark areas. Some new growth on tips. Some plants reproductive.
	5	39.91 (*1.61)*	Texture firm, no disintegration. Some discolouration on tips, occasional black areas.
OFspir	10	22.92 (*1.84)*	Texture firm, no disintegration. Some discolouration on tips, light and dark areas.
	15	27.23 (*2.78)*	Texture firm, no disintegration. Some discolouration, areas of black on some tips and edges.
	5	35.34 (*6.36)*	Texture firm, no disintegration. Some discolouration on tips, light and dark areas.
OFves	10	18.93 (*3.13)*	Texture firm, no disintegration. Some discolouration on tips, some tips black.
	15	13.76 (*3.74)*	Texture firm, no disintegration. Some discolouration, black areas on all apical tips.
	5	65.49 (*1.28)*	Texture firm. Some discolouration with light and black areas. Some plants reproductive.
MacFde	10	42.00 (*7.57)*	Most apical tips texture firm but some flaccid. Some discolouration especially at tips where black. Some tips becoming reproductive with new growth beyond reproductive area.
	15	48.23(*9.50)*	Most apical tips texture firm but some flaccid. Some discolouration with light and dark areas. New growth on some tips.

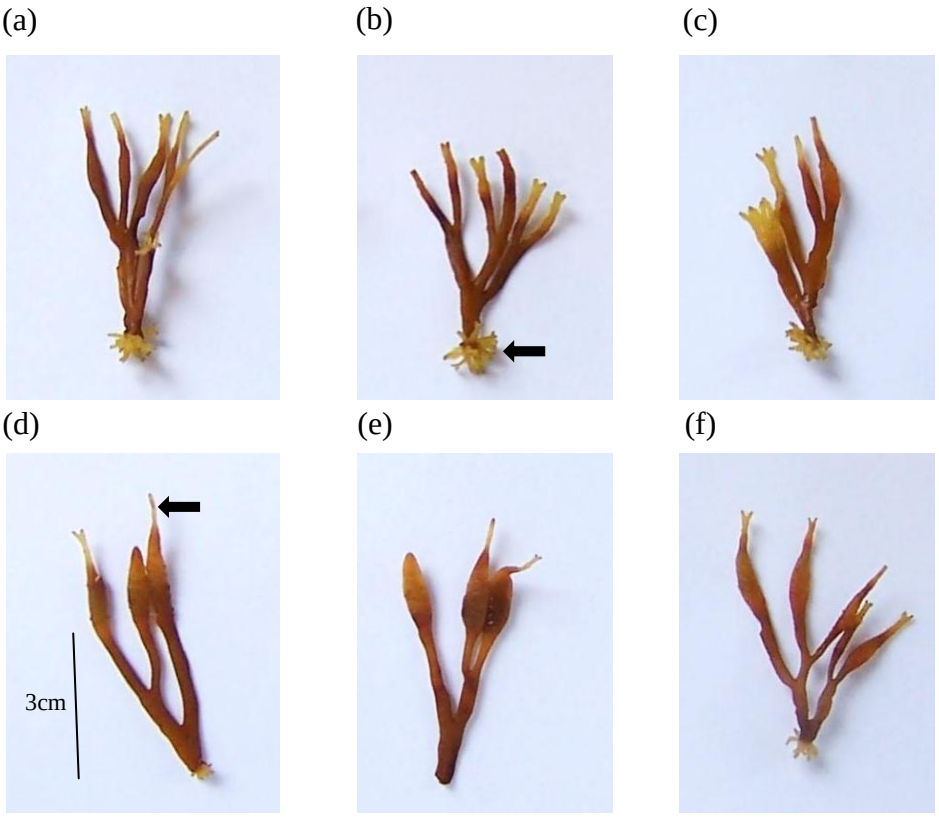


Figure 5.3 Examples of regeneration at wound end (a – c) and new growth beyond reproductive tips (d – f) in *F. distichus* subsp. *anceps* from Orkney.

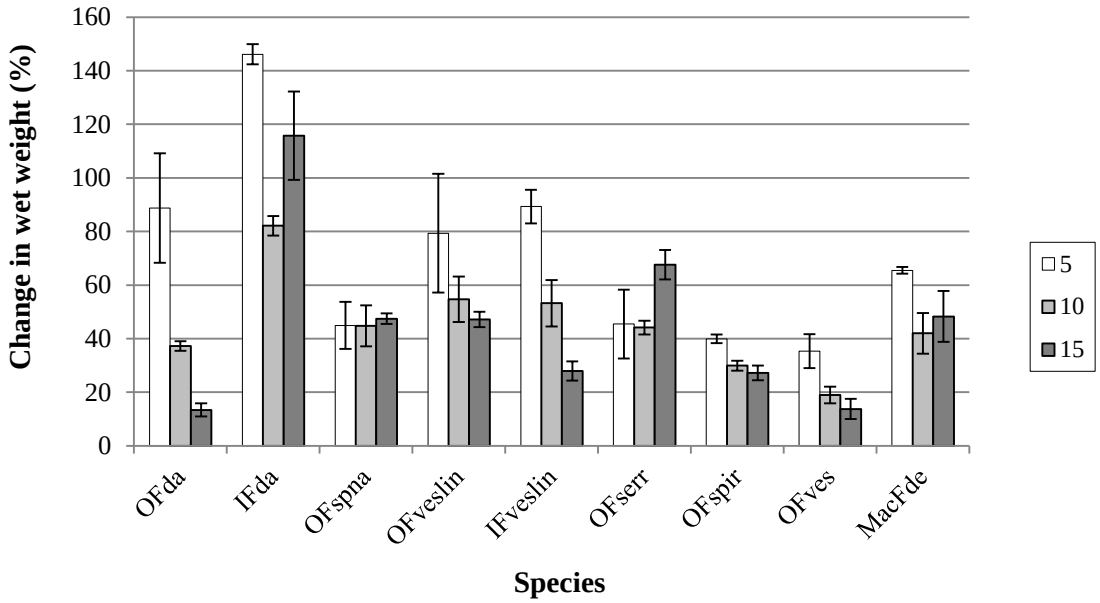


Figure 5.4: Mean percentage change in wet (fresh) weight (g) of adult *Fucus* tips cultured at temperatures 5, 10, 15ºC after a period of 100 days. PAR 80μE m^{-2} s^{-1}. Photoperiod light:dark 12:12. 3 replicates, each replicate contained 20 tips. Error bars signify standard deviation for the replicate means.

The results show that the majority of species had the greatest increase in wet weight at the lower temperature of 5ºC. The exceptions were for Orkney species *F. serratus* at 15ºC, *F. vesiculosus* var. *linearis* at 5ºC and 10ºC and *F. spiralis* f. *nanus* which showed no significant increase in fresh weight between temperatures. *F. distichus* subsp. *anceps* from Ireland had the greatest increase in fresh weight of all the species at all temperatures.

5.2.3 Conclusions

The use of fresh weight may seem a fairly crude method to investigate growth increase however when using portions of macroscopic algae it can show differences in growth in relation to different temperatures. In this part of the investigation the same apical tips as used for surface area measurements were used and whereas measurement of surface area showed increase due to changes in length and breadth, fresh weight may indicate changes due to bulk and/or other forms of growth.

The first point to note is that wet weight increased for all the *Fucus* species and taxa investigated at all temperatures. However, this change in wet weight varied at different temperatures for a species and between species. In many species an increase in wet weight at a certain temperature differed from that of surface area at that same temperature. For example, the greatest increase in surface area for *anceps* from Orkney was at 15ºC however the greatest increase in wet weight was at 5ºC. Comparison of percentage changes in wet weight and surface area results for all species are given in **Table 5.7** and discussed in detail in **Section 5.4**. Differences between the two measurements were also noted in *anceps* and *linearis* from Ireland and *linearis*, *spiralis* and *vesiculosus* from Orkney. The only species showing the same temperature preference from both measurements were *edentatus* from Macduff at 5ºC and *serratus* from Orkney at 15ºC. What was it then, other than an increase in length and/or breadth as indicated by surface area measurements that contributed to the increase in fresh weight?

Observations in **Table 5.4** show that for many of the species the increase in fresh weight may be attributed to some pieces of plants becoming reproductive. This was certainly the case for *anceps* and *nanus* at 5ºC and *linearis* at 5ºC and 10ºC from Orkney and from Ireland, *anceps* at all temperatures and *linearis* at 5ºC and 10ºC.

Mature receptacles have been obtained from apical segments in culture by other authors (Burrows, 1964; Moss, 1966; McLachlan *et al*., 1972) suggesting that reproduction in *Fucus* is an intrinsic characteristic.

Another contributory factor for increase in fresh weight was growth regeneration from the cut end of the apical tips in some species. This was very prevalent in *anceps* from Orkney grown at 5ºC **(Figure 5.3 a - c)** but much less so in *anceps* from Ireland at 10ºC. This growth was also noted, although very limited, in *linearis* from Orkney at 5ºC and in the Ireland population at 15ºC. Regeneration is a well know phenomenon associated with wounding (Moss, 1964; Fulcher and McCully, 1969, 1971). This replacement of lost tissue by redifferentiation of medullary cells in *Fucus* from a wound surface has been referred to as adventive embryony (Fulcher and McCully, 1969, 1971, McLachlan and Chen, 1972) and is initiated by increased meristematic activity at loci on the new epidermal layer.

The increase in fresh weight at 5ºC for both *spiralis* and *vesiculosus* cannot be attributed to either reproductivity or regeneration and must therefore be caused by increase in bulk. Moss (1964b, 1966) stated that isolated thallus apices of mature *Fucus* plants will undergo some growth and differentiation when cultured in seawater.

One interesting observation noted for the subspecies *anceps* and *edentatus* was the occurrence of continued vegetative growth beyond the receptacle. This was previously described by Powell (1957a) and in this investigation was present in *edentatus* from Macduff at 10ºC and 15ºC and both *anceps* populations, at 5ºC for Orkney and 10ºC for Ireland **(Figure 5.3 d - f)**.

Observations from **Table 5.4** show that in all species and at all temperatures varying degrees of discolouration were noted with some species having areas of blackened tissue. This has been noted in studies of populations of *Macrocystis pyrifera* in California where warm water promotes black-rot disease (Andrews, 1976) and in cultivated *Porphyra* in Japan (Tseng, 1981). Schonbeck and Norton (1978, 1980a) described temperature damage in *F. spiralis* as consisting of reddish spots of decaying tissue. This discolouration may be an indication of possible deterioration in health of the apical tips. However the fact that some tips had become reproductive and the presence of regeneration and new growth would seem to indicate some degree of good

health. The following section therefore may indicate the state of health of the *Fucus* plants by investigating the effect of temperature on the metabolic process of photosynthesis.

5.3 Laboratory Investigations into the Effect of Temperature on Adult Growth of Various *Fucus* Species and Taxa using Photosynthetic and Respiration Rates

Photosynthesis is a major, easily measured metabolic process routinely used as a gauge of environmental effects on seaweeds (Lobban and Harrison, 1997). Various physiological criteria have been used as measures of the extent of stress on metabolism with photosynthesis and respiration often used owing to the ease with which they can be measured (Newell and Pye, 1968; Healey, 1972; Davison *et al*., 1989). The complex processes of photosynthesis and respiration are affected by many variables, one of which is temperature. The following method investigated the effect of temperature on the performance of adult *Fucus* plants using light and dark bottles to measure rates of photosynthesis and respiration. This method yields reproducible, representative results with the advantage of giving all three components, gross and net production and respiration (Quadir *et al*., 1979). It is assumed that oxygen exchange in light represents net photosynthesis, that is, the difference between gross photosynthesis and respiration and therefore the sum of oxygen exchanged in light and uptake in the dark gives the gross (true) rate of photosynthesis.

5.3.1 Method

Collection and pre-experimental preparation of adult plants is set out in **Section 3.3**. The same species and populations as above were investigated **(Table 5.1)**. The method used here is taken from Strickland and Parsons (1972) and based on one used by Thomas (1988) for aquatic macro-flora and set out in detail in **Section 3.8**. The procedure was carried out before and after a culture period of 100 days at temperatures of 5°C, 10°C and 15°C using vegetative tips of 3 cm in length. It is known that wounding and consequent loss of internal fluids affects photosynthesis and therefore excised tips were placed in full seawater and left to acclimatise overnight in CT rooms at the appropriate temperature for that experiment. As previously mentioned CT room failure meant that some species were subject to a smaller temperature range of 10°C and 15°C with reduced culture period of 50 days.

5.3.2 Results

Results of mean oxygen consumption before and after the experimental period are expressed as net photosynthesis (NP), respiration (R) and gross photosynthesis (GP) and are given in **Table 5.5**. Percentage change of NP, R and GP at end of the culture period are also given and graphed in **Figures 5.5 – 5.7** respectively.

To investigate any statistically significant differences in plant growth between treatments all datasets were tested for normality (Kolmogorov-Smirnov test) and homogeneity of variance (Levene's Test) to see if a one-way Analysis of Variance (ANOVA) could be used. If one-way ANOVA appropriate then *post hoc* (Tukey's HSD) test performed for pairwise comparison to identify which groups were significantly different from other groups. Where datasets failed the above tests then a non-parametric (Kruskal-Wallis test) equivalent was used to look at differences between median values. Where significant results for Kruskal-Wallis test were found then *post hoc* pairwise Mann-Whitney *U* test performed. Results are given in **Table 5.6**. In the case of the species with reduced culture period and temperatures datasets were similarly tested for normality (Kolmogorov-Smirnov test) and homogeneity of variance (Levene's Test). If datasets passed the above tests then one-way ANOVA used to compare the treatment means. Where datasets failed the above tests then the non-parametric equivalent, Mann-Whitney *U* test was used to look at differences between median values.

Table 5.5: Mean photosynthesis and respiration rates of apical tips of various *Fucus* species and taxa recorded before and after culture at different temperatures over a period of 100 days. PAR 80μE m^{-2} s^{-1}. Photoperiod light:dark 12:12. 10 replicates per species. Change is expressed as net photosynthesis (NP), respiration (R) and gross photosynthesis (GP) as well as percentage change of NP, R and GP at start and end. Standard deviations of replicate means given in italics. *Species cultivated at reduced time period and temperature due to CT room failure.

		Change in Photosynthesis and Respiration								
		mg O_2 g (dry wt)$^{-1}$ h^{-1}								
		Before			After			% Change		
Species	Temp. ºC	NP	R (-)	GP	NP	R (-)	GP	NP	R (-)	GP
OFda	5	1.01	0.23	1.24	0.36	0.22	0.58	-64.36	-4.35	-53.23
		0.18	*0.10*		*0.13*	*0.14*				
	10	1.24	0.34	1.58	0.44	0.13	0.57	-64.52	-61.76	-63.92
		0.28	*0.11*		*0.13*	*0.05*				
	15	2.17	0.26	2.43	0.44	0.16	0.60	-79.72	-38.46	-75.31
		0.35	*0.06*		*0.19*	*0.12*				
IFda	5	0.68	0.40	1.08	0.31	0.10	0.41	-54.41	-75.00	-62.04
		0.11	*0.09*		*0.11*	*0.06*				
	10	0.58	0.37	0.95	0.43	0.16	0.59	-25.86	-56.76	-37.23
		0.12	*0.15*		*0.09*	*0.06*				
	15	0.64	0.30	0.94	0.32	0.21	0.52	-50.00	-30.00	-44.68
		0.09	*0.08*		*0.06*	*0.08*				

Table 5.5 (continued)

Species	Temp. ºC	NP	R (-)	GP	NP	R (-)	GP	NP	R (-)	GP
	5	1.07	0.40	1.47	0.43	0.20	0.63	-59.81	-50.00	-57.17
		0.17	*0.18*		*0.21*	*0.14*				
OFspna	10	1.05	0.44	1.49	0.31	0.11	0.42	-70.48	-75.00	-71.81
		0.12	*0.14*		*0.10*	*0.04*				
	15	1.30	0.26	1.57	0.28	0.15	0.43	-78.46	-42.31	-72.61
		0.13	*0.16*		*0.18*	*0.06*				
	5	0.93	0.18	1.11	0.51	0.06	0.60	-45.16	-66.67	-45.94
		0.15	*0.13*		*0.10*	*0.03*				
OFveslin	10	0.99	0.30	1.29	0.40	0.13	0.53	-59.60	-56.67	-58.91
		0.12	*0.13*		*0.08*	*0.05*				
	15	0.60	0.24	0.84	0.35	0.18	0.52	-41.67	-25.00	-38.10
		0.13	0.08		*0.19*	*0.11*				
	5	0.91	0.32	1.23	0.29	0.18	0.47	-68.13	-43.75	-61.79
		0.30	*0.11*		*0.07*	*0.04*				
IFveslin	10	0.92	0.30	1.22	0.39	0.11	0.50	-57.61	-63.33	-59.02
		0.11	*0.09*		*0.10*	*0.03*				
	15	0.98	0.37	1.35	0.41	0.16	0.57	-58.16	-56.76	-57.77
		0.14	*0.10*		*0.10*	*0.04*				

Table 5.5 (continued)

Species	Temp. ºC	NP	R (-)	GP	NP	R (-)	GP	NP	R (-)	GP
	5	1.06	0.40	1.46	0.52	0.31	0.84	-50.94	-22.50	-42.47
		0.13	*0.23*		*0.18*	*0.17*				
OFserr	10	1.02	0.23	1.25	0.71	0.14	0.85	-30.39	-39.13	-32.00
		0.10	*0.11*		*0.09*	*0.08*				
	15	1.24	0.21	1.45	0.96	0.14	1.09	-22.58	-33.33	-24.83
		0.23	*0.06*		*0.20*	*0.05*				
	5	0.94	0.10	1.04	0.62	0.07	0.69	-34.04	-30.00	-33.65
		0.13	0.03		*0.12*	*0.05*				
OFspir	10	1.14	0.22	1.36	0.44	0.11	0.55	-61.40	-50.00	-59.56
		0.06	0.04		*0.08*	*0.04*				
	15	0.70	0.26	0.96	0.42	0.09	0.51	-40.00	-61.54	-46.88
		0.11	*0.10*		*0.07*	*0.02*				
	5	1.03	0.28	1.31	0.36	0.21	0.57	-65.05	-25.00	-56.49
		0.14	*0.05*		*0.07*	*0.06*				
OFves	10	1.14	0.20	1.34	0.34	0.15	0.49	-70.18	-25.00	-63.43
		0.14	*0.05*		*0.10*	*0.11*				
	15	0.91	0.19	1.10	0.41	0.08	0.50	-54.95	-57.89	-54.55
		0.05	*0.02*		*0.04*	*0.02*				

Table 5.5 (continued)

Species	Temp. ºC	NP	R (-)	GP	NP	R (-)	GP	NP	R (-)	GP
MacFde	5	1.40	0.25	1.65	0.67	0.20	0.87	-52.14	-20.00	-47.27
		0.31	*0.11*		*0.22*	*0.18*				
	10	1.15	0.17	1.32	0.34	0.10	0.41	-70.43	-41.18	-68.94
		0.07	*0.14*		*0.10*	*0.04*				
	15	1.16	0.24	1.40	0.19	0.13	0.32	-83.62	-45.83	-77.14
		0.12	*0.09*		*0.06*	*0.05*				
ShetFde*	10	0.96	0.36	1.32	0.42	0.29	0.71	-56.25	-19.44	-46.21
		0.08	*0.15*		*0.15*	*0.10*				
	15	1.01	0.22	1.23	0.61	0.19	0.80	-39.60	-13.64	-34.96
		0.12	*0.04*		*0.03*	*0.02*				
SQFserr*	10	0.87	0.23	1.10	0.55	0.07	0.62	-36.78	-69.57	-43.64
		0.06	*0.04*		*0.04*	*0.02*				
	15	0.84	0.21	1.05	0.33	0.08	0.41	-60.71	-61.90	-60.95
		0.06	*0.03*		*0.04*	*0.02*				
SQFspir*	10	1.02	0.19	1.21	0.58	0.12	0.70	-43.14	-36.84	-42.15
		0.13	*0.09*		*0.04*	*0.02*				
	15	1.06	0.25	1.31	0.38	0.18	0.56	-64.15	-28.00	-57.25
		0.10	*0.06*		*0.11*	*0.06*				
SQFves*	10	1.06	0.22	1.28	0.66	0.13	0.79	-37.74	-40.91	-38.28
		0.11	*0.07*		*0.04*	*0.04*				
	15	0.90	0.22	1.12	0.82	0.09	0.91	-8.89	-59.09	-18.75
		0.05	*0.08*		*0.04*	*0.04*				

Table 5.6: Matrices of results of one-way ANOVA and *post hoc* Tukey's HSD pairwise test for significant difference in mean values and non-parametric Kruskal-Wallis test and *post hoc* Mann-Whitney *U* pairwise test for significant difference in median values. Tests performed on net photosynthesis (NP) and respiration (R) of adult *Fucus* tips cultured at temperatures 5, 10, 15ºC after a period of 100 days. PAR 80μE m^{-2} s^{-1}. Photoperiod light:dark 12:12. 10 replicates per species. (NS = Not significant; *significant at p = < 0.05, ** significant at p < 0.01; ***significant at p = 0.001).

Change in Photosynthesis and Respiration												
	Before						After					
Species	NP			R			NP			R		
		5	10		5	10		5	10		5	10
OFda	10	NS		10	*		10	NS		10	NS	
	15	***	***	15	NS	NS	15	NS	NS	15	NS	NS
		5	10		5	10		5	10		5	10
IFda	10	NS		10	NS		10	*		10	NS	
	15	NS	NS	15	NS	NS	15	NS	*	15	***	NS
		5	10		5	10		5	10		5	10
OFspna	10	NS		10	NS		10	NS		10	NS	
	15	***	***	15	NS	NS	15	NS	NS	15	NS	NS
		5	10		5	10		5	10		5	10
OFveslin	10	NS		10	NS		10	NS		10	NS	
	15	***	***	15	*	NS	15	*	NS	15	***	NS

Table 5.6 (continued)

	NP			R			NP			R		
		5	10		5	10		5	10		5	10
IFveslin	10	NS		10	NS		10	*		10	***	
	15	NS	NS	15	NS	NS	15	*	NS	15	NS	*
		5	10		5	10		5	10		5	10
OFserr	10	NS		10	NS		10	*		10	***	
	15	*	*	15	*	NS	15	***	**	15	***	NS
		5	10		5	10		5	10		5	10
OFspir	10	***		10	***		10	***		10	NS	
	15	***	***	15	***	NS	15	***	NS	15	NS	NS
		5	10		5	10		5	10		5	10
OFves	10	**		10	*		10	NS		10	NS	
	15	**	***	15	***	***	15	NS	NS	15	***	NS
		5	10		5	10		5	10		5	10
MacFde	10	*		10	NS		10	***		10	NS	
	15	*	NS	15	NS	NS	15	***	NS	15	NS	NS

Table 5.6 (continued)

		NP			R			NP			R	
		5	10		5	10		5	10		5	10
ShetFde	10	N/A		10	N/A		10	N/A		10	N/A	
	15	N/A	NS	15	N/A	**	15	N/A	**	15	N/A	***
		5	10		5	10		5	10		5	10
SQFserr	10	N/A		10	N/A		10	N/A		10	N/A	
	15	N/A	NS	15	N/A	NS	15	N/A	***	15	N/A	NS
		5	10		5	10		5	10		5	10
SQFspir	10	N/A		10	N/A		10	N/A		10	N/A	
	15	N/A	NS	15	N/A	NS	15	N/A	***	15	N/A	***
		5	10		5	10		5	10		5	10
SQFves	10	N/A		10	N/A		10	N/A		10	N/A	
	15	N/A	***	15	N/A	NS	15	N/A	***	15	N/A	*

N/A not applicable due to CT room failure

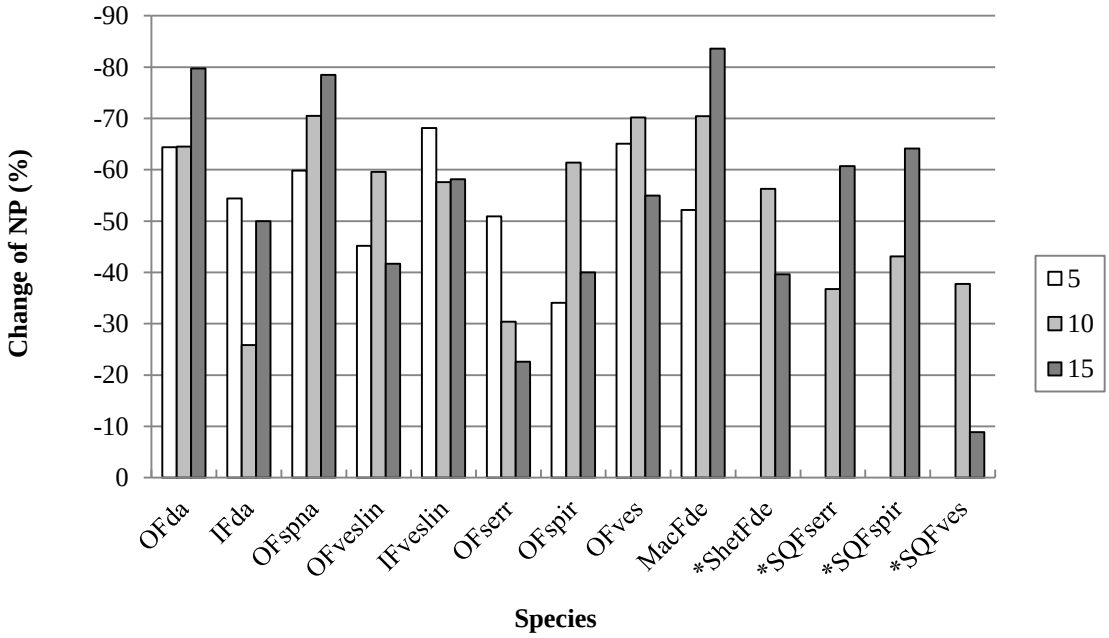


Figure 5.5: Mean net photosynthesis rates of 10 replicates of adult *Fucus* species cultured at temperatures 5, 10, 15ºC after a period of 100 days. PAR 80μE m^{-2} s^{-1}. Photoperiod light:dark 12:12. Asterisks indicate *Fucus* species cultured at temperatures of only 10ºC and 15 ºC at a reduced period of 50 days due to CT room failure.

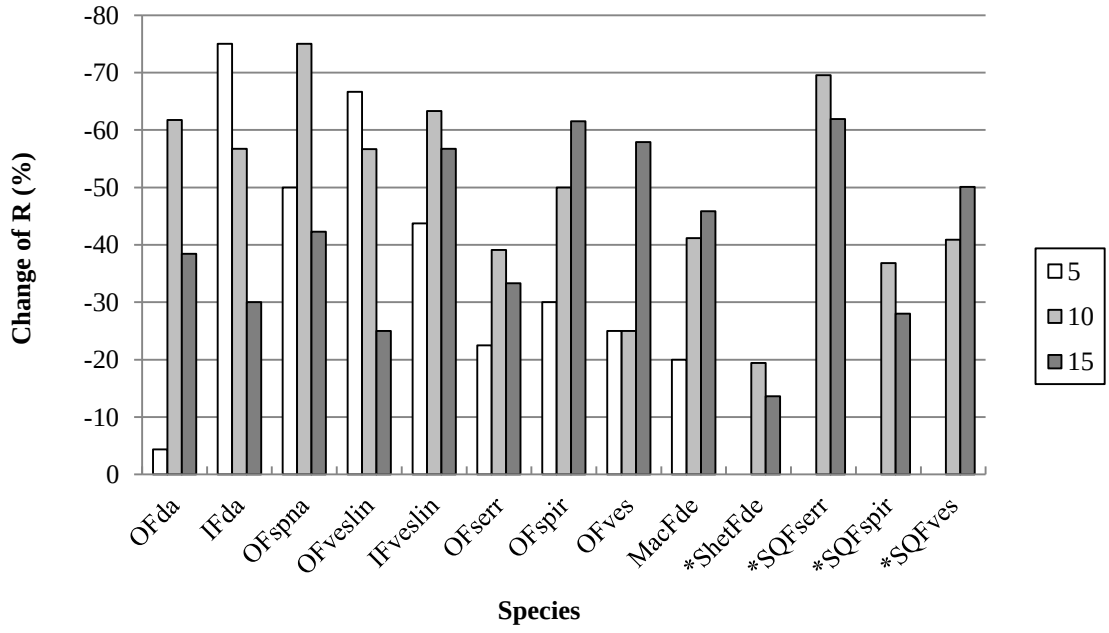


Figure 5.6: Mean respiration rates of 10 replicates of adult *Fucus* species cultured at temperatures 5, 10, 15ºC after a period of 100 days. PAR 80μE m^{-2} s^{-1}. Photoperiod light:dark 12:12. Asterisks indicate *Fucus* species cultured at temperatures of only 10ºC and 15 ºC at a reduced period of 50 days due to CT room failure.

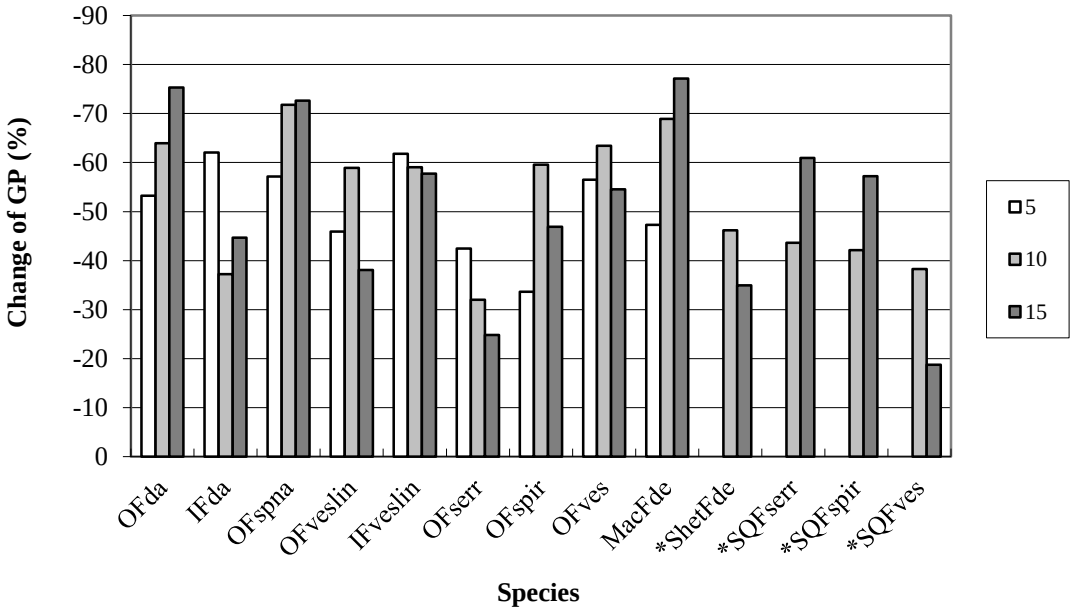


Figure 5.7: Mean gross photosynthesis rates of 10 replicates of adult *Fucus* species cultured at temperatures 5, 10, 15ºC after a period of 100 days. PAR 80μE m^{-2} s^{-1}. Photoperiod light:dark 12:12. Asterisks indicate *Fucus* species cultured at temperatures of only 10ºC and 15 ºC at a reduced period of 50 days due to CT room failure.

Examination of the initial results taken at the start of the culture period show that net photosynthesis in the Orkney population of *F. distichus* subsp. *anceps* was much greater at the higher temperature of 15ºC with a gross photosynthesis rate of almost twice that recorded at 5ºC. *F. vesiculosus* var. *linearis* from Orkney showed gross photosynthesis rates greater at 5ºC and 10ºC and significantly less at 15ºC while *F. spiralis* f. *nanus* from Orkney showed an initial preference for 15ºC. The results for the Ireland populations of *anceps* and *linearis* show no initial temperature preference. Initial results for the other Orkney species show *F. serratus* favoured 15ºC, *F. spiralis* 10ºC and *F. vesiculosus* 5ºC and 10ºC. Initial results for *F. distichus* subsp. *edentatus* from Macduff showed a preference for 5ºC. Unfortunately it is difficult to compare this response with the *edentatus* population from Shetland due to reduced temperatures and culture periods, however the limited results show some preference for 10ºC. The South Queensferry species also show only limited results with *F. serratus* and *F. spiralis* having no initial temperature preference and *F. vesiculosus* with a temperature preference at 10ºC at the start of the culture period.

At the end of the culture period the repeated experimental results show a reduction in net photosynthesis, respiration and gross photosynthesis rates in all the species investigated and that this reduction varied with temperature. The Orkney *Fucus* species *anceps* and *nanus* showed no preference for temperature while *linearis* showed some preference for 5ºC. Ireland *anceps* and *linearis* showed some preference for 10ºC and 15ºC. Results for the other Orkney species show that *F. serratus* retained its temperatures preference for 15ºC while *F. spiralis* and *F. vesiculosus* temperature preference had changed to the lower temperature of 5ºC. *F. d.* subsp. *edentatus* from Macduff retained its preference for 5ºC. As with the initial results is difficult to compare this response with *edentatus* from Shetland due to reduced temperatures and culture periods however the limited results show a preference now for 15ºC. The South Queensferry species also show only limited results with *F. serratus* showing no temperature preference and *F. spiralis* a preference at 10ºC and *F. vesiculosus* at 15ºC.

5.3.3 Conclusions

The first comment on this experiment is that rates of net photosynthesis, respiration and gross photosynthesis varied not only for a species at different temperatures but also between species and populations **(Tables 5.5 & 5.6)**. The second point is that rates of change and temperature preferences often varied at start and end of the cultural period.

Initial results taken at the start of the culture period show that gross photosynthesis for the Orkney population of *anceps* was not only greater at all temperatures when compared to the Ireland population but much greater at the higher temperature of 15ºC while Ireland *anceps* showed no initial temperature preference. Similarly the two populations of *linearis* from Orkney and Ireland responded differently to temperature with the Orkney population showing an initial preference for 10ºC and 15ºC while the Ireland population had no preference for temperature.

At the end of the experimental period all species and populations showed a reduction in rates of net photosynthesis and respiration with respect to different temperatures. It was also noted that temperature preference had changed for some species. This was the case for both populations of *anceps* from Orkney and Ireland, *nanus* from Orkney and *linearis* from Ireland. The interesting point to note here is that species of *anceps* and *linearis* from Ireland went from having no temperature preference to having a

preference for warmer temperatures. This trend was reversed for Orkney species *anceps* and *nanus* from having a preference for 15ºC to no preference. In *linearis* from Orkney an initial preference for 5ºC and 10ºC went to 5ºC. The only two species to retain a clear temperature preference at the start and end of the cultivation period was *serratus* from Orkney at 15ºC and *edentatus* from Macduff at 5ºC.

The *edentatus* population from Shetland showed some initial preference for 10ºC at the start and 15ºC at the end, however missing values for the lower temperature of 5ºC mean the results are limited. Similarly it is hard to compare results from the geographical different populations of *serratus*, *spiralis* and *vesiculosus* from South Queensferry with those from Orkney due to reduced culture period and temperature range, however some differences between the species can be seen. *F. serratus* showed no preference for temperature at start and end, *spiralis* went from no preference to 10ºC and *vesiculosus* from 10ºC to 15ºC. Even these limited results appear to show some differences to the species from Orkney.

The percentage changes calculated from the before and after results also show that the *Fucus* species and populations investigated responded differently to temperature and may indicate which temperatures inhibited the metabolic processes involved in photosynthesis over a period of time. These results are particularly useful for any species which showed the same temperature preferences before and after the culture period as in the case of *serratus* from Orkney and South Queensferry and *edentatus* from Macduff. For example in *serratus* from Orkney where 15ºC was the optimum temperature the results show that 5ºC was the inhibitory temperature.

Observations from **Table 5.4** show that in all species and at all temperatures varying degrees of discolouration were noted. It may be that these areas of discolouration would have some effect on the light harvesting capabilities and subsequently the photosynthetic rates in some species. It has been stated that when measurements are made for complex reactions such as photosynthesis and respiration, the overall rate is a composite of all the individual reaction rates and any rate limiting reaction will not necessarily be the same one at all temperatures (Davison, 1991). In this investigation this did appear to be the case as results show that both net photosynthesis and respiration rates varied for *Fucus* species at different temperatures.

5.4 Discussion

Results from the surface area, wet (fresh) weight and photosynthetic and respiration experiments on *Fucus* apical tips were obtained under extremely artificial conditions and this may have contributed to the effects of different temperatures during cultivation of the adult plants. However assuming the responses seen are due solely to temperature then the following will examine the results for the three experiments and how these relate to the sea surface temperatures found in the natural habitat for that species. The comparative temperature preferences from surface area and wet weight for each species at the end of the experimental period are given in **Table 5.7**. Temperature preferences for oxygen consumption before and after the culture period are also given along with temperature at which the greatest percentage changes were noted.

Table 5.7: Comparisons of temperature preferences of *Fucus* apical tips taken from surface area and wet (fresh) weight results after the culture period. Gross photosynthesis before and after the culture period are also given along with temperature (ºC) at which greatest percentage changes occurred (NS = no significant differences between temperatures; NA = not applicable).

Species	Surface Area	Wet (Fresh) Weight	Gross Photosynthesis		
			Before	After	Greatest % change
OFda	15	5	15	NS	15
IFda	NS	5	NS	10 & 15	5
OFspna	10 & 15	NS	15	NS	10 & 15
OFveslin	10 & 15	5	5 & 10	5	10
IFveslin	NS	5 & 10	NS	10 & 15	5 & 10
OFserr	15	15	15	15	5
OFspir	10 & 15	5	10	5	10
OFves	10 & 15	5	5 & 10	5	10
MacFde	5	5	5	5	15
ShetFde*	NS	NA	10	15	10
SQFserr*	NS	NA	NS	NS	15
SQFspir*	10	NA	NS	10	15
SQFves*	NS	NA	10	15	10

*Species cultivated at reduced time period and temperature due to CT room failure.

It may be expected that the northern Orkney species would have a preference for cooler seawater temperatures. All Orkney species were collected in April where local sea surface temperatures are between 7 - 8°C (Fisheries Research Services, 2004). Orkney sea surface temperatures peak at 13°C in August however records for 2003 show temperatures up to 15.1°C. Lowest temperatures are in February at an average of around 6°C. The experiments ran from mid-April to late July covering a good period of the range of temperatures found locally in nature.

Comparison of the results for the Orkney species shows some conflicting temperature requirements over the three experimental procedures and culture period. The only species which showed a clear temperature preference for growth and performance was *serratus* at the highest temperature of 15°C. Surface area results show the greatest increase was at the higher temperatures of 10°C and 15°C for all Orkney species. Wet weight results however show a preference for the lowest temperature of 5°C for most species with the exception of *linearis* at 5°C and 10°C and *nanus* which showed no temperature preference. For species *anceps* and *linearis* this increase in wet weight at 5°C was most certainly due to reproductivity, new growth and regeneration **(Table 5.4)**. As these growth forms were not recorded for *spiralis* and *vesiculosus* then it can be assumed that weight gain was due to increase in bulk. All apical tips used in photosynthesis and respiration experiments were non-reproductive with no new growth or regeneration so as not to influence oxygen consumption rates. With the exception of *serratus* temperature preferences changed for all species before and after the culture period with a general trend going from warmer temperatures to cooler or no temperature preference.

The waters around Ireland's coasts are remarkably warm, 7° to 8°C higher than the average global sea temperature at these latitudes due to the warm North Atlantic Drift. Sea temperatures off the west coast range from an average of 8 - 10°C during February or March and from 14 - 16°C in August (Met Eireann, 2010). In March, the date of collection of the Ireland populations of *anceps* and *linearis*, the average monthly sea surface temperatures are 8 – 10ºC. The experiments ran from early March to mid-June again covering a good period of the range of temperatures found locally. It may be assumed therefore that in contrast to northern Orkney species, southern species from Ireland would prefer warmer sea temperatures. The first thing to note is that both *anceps* and *linearis*, with the exception of wet weight, showed different temperature

preferences when compared to the Orkney populations of the same species. The second point is that Ireland *anceps* and *linearis* showed exactly the same temperature preferences throughout. Results show no temperature preference for surface area or for oxygen consumption at the start of the culture period however a preference for warmer temperatures of 10°C and 15°C was recorded at the end. Reproductivity, new growth and regeneration were noted in some form at all temperatures in both species **(Table 5.4)** however the greatest increase in wet weight was at 5°C.

Results for *edentatus* from Macduff show a clear temperature preference for 5°C across all three experimental procedures. Local sea surface temperatures at date of collection in October are 10 – 11ºC while temperatures peak in August at 12ºC with the lowest in February at around 5.6°C (Fisheries Research Services, 2004). Unfortunately it is difficult to compare the responses in the Macduff population with those of *edentatus* from Shetland due to CT room failure at 5ºC and reduced culture periods of 50 days at 10ºC and 15ºC. Wet weight measurements are also not available due to lost data. Results for Shetland *edentatus* show no temperature preference for surface area; however despite the limited results gross photosynthesis rates do show some preference towards warmer temperatures. Local sea temperatures at date of collection in July are 10 – 11ºC.

Species *serratus*, *spiralis* and *vesiculosus* from South Queensferry were also cultivated at reduced time periods and temperatures. Local sea temperatures in the North Sea at date of collection are 12 - 13ºC in July with temperatures varying from 5 - 6ºC in February to 13 – 14ºC in August (Fisheries Research Services, 2004) however sea temperatures may be slightly higher in the Firth of Forth. Without the use of the lower temperature of 5ºC results are limited; however some comparisons can be made with the geographically different populations from Orkney. Unlike *serratus* from Orkney which showed optimum growth and performance at 15ºC the South Queensferry population had no real temperature preference although there was some indication that 15ºC inhibited oxygen consumption. Results from the two other South Queensferry species suggest that *spiralis* may have had some preference towards 10ºC, however as 5ºC was unavailable this could have been the favoured temperature. The only temperature preference noted for *vesiculosus* was for gross photosynthesis at 15ºC.

Studies investigating the effects of temperature on photosynthesis and respiration and growth have found that often maximum rates correlated with the temperature regime in an algae's habitat (Dring, 1992). In Orkney species where local sea temperatures at date of collection in July were around 12-13°C this response was not recorded for gross photosynthesis as the general trend was an initial preference for warmer temperatures changing to cooler or no preference. Similarly wet weight measurements show no relation to local sea temperatures as 5°C was the optimum temperature for increase. Surface area optimum temperatures were the only results that showed some correlation to local sea temperatures with greatest increase at 10°C and 15°C. *F. serratus* was the only Orkney species to show a clear preference for 15°C. Results for the Ireland populations of *anceps* and *linearis* show that temperature preferences of 10°C and 15°C for gross photosynthesis at the end of the experimental period are the only results that relate to the local sea surface temperatures of 10 – 11ºC.

Results for *edentatus* from Macduff showed a clear temperature preference for 5°C for growth and performance and show no correlation to local sea temperatures of 10 – 11ºC for the October collection date. Results for the Shetland population of *edentatus* show a slight trend towards warmer temperatures for gross photosynthesis and therefore some correlation to local sea surface temperatures of 10 – 11ºC. It has been suggested by authors Powell (1957b) and Russell (1974) that *edentatus* is very tolerant of polluted waters and grows best under conditions of sewage and other organic pollution giving it an advantage over less tolerant local *Fucus* species, reducing competition. Powell also suggested that physical environments such as sea and air temperatures may not directly determine the distribution of this subspecies in Britain and these results suggest that this may be the case for the Macduff population of *edentatus*. The limited results for the South Queensferry species of *serratus*, *spiralis* and *vesiculosus* where local sea temperatures in the North Sea in July are 12 - 13ºC, suggest some preference for warmer sea temperatures. The exception was *serratus* which showed no temperature preference.

Instances in which the optimum temperature has not been near that for natural conditions have also been noted by Fries (1966). The author stated that there may be a physiological optimum for algae alone and an ecological optimum in nature and that the temperature optimum for algae under laboratory conditions where temperature is constant may appear narrower than in nature.

A number of key points can concluded from the above investigations:

- Results show that temperature did effect the growth of the *Fucus* adult plants investigated but that the form of growth varied between species and taxa, and at different temperatures.

- Rates of net photosynthesis, respiration and gross photosynthesis associated with metabolism and performance varied with temperature both between and within the *Fucus* species investigated.

- Percentage changes of gross photosynthesis calculated before and after the culture period show that *Fucus* species did not respond equally with temperature over a period of time.

- The effects of temperature on growth and performance did not always correlate with the sea surface temperatures found locally for that species.

- The response to temperature varied for geographically different populations of *Fucus* species and taxa.

- The results demonstrate the need for varied and complimentary experimental procedures along with basic observations to understand the effects of different sea temperatures on *Fucus* species.

It has been speculated that there may be ecotypic differentiation in physiological characteristics among populations from different geographic locations (Russell, 1978). Powell (1957b) suggested that in the case of the population of *F. distichus* subsp. *anceps* in Ireland at its southern limit of distribution being confined to a restricted habitat and geographically isolated may result in the gradual evolution of a genetically distinct ecotype or even species. To investigate this further and because of the differences noted in the above experiments, a numerical study of variation will now look at any similarities and differences between the two geographically different populations of *anceps* from Orkney and Ireland.

Chapter 6: Phenotypic Variation in *Fucus distichus* subsp. *anceps* Populations

Fucus species are equipped with a genotype that allows a wide range of phenotypic expression. As a result this phenotype often responds with considerable sensitivity to differences in the environment (Russell, 1978). If a species occurs at widely different latitudes and longitudes, its physiology and ecological parameters may be quite different (Lobban and Harrison, 1997). Inbreeding, which is possible with monoecious species such as *Fucus distichus*, can lead to a reduction of within-population variation but also allows wide differences between populations. In this part of the investigation the principles of numerical taxonomy (Sokal and Rohlf, 1969) and multivariate analysis (Ho, 2006) were used to examine the similarities and differences between two geographically different populations of *F. distichus* subsp. *anceps* in the British Isles, from Orkney **(Section 3.2.1, Figure 3.1)** and Ireland **(Section 3.2.2, Figure 3.2)**, using measurements of observable characteristics and variation.

6.1 Method

Collection of plant material is covered in detail in **Section 3.9.1**. At both sites one hundred mature plants (i.e. plants with ripe receptacles) were removed. Each plant was analysed on the basis of fifteen characters as **(Table 6.1)**. These character definitions were based on previous work by Rice and Chapman (1985) and are given in precise detail in **Appendix C**. The type of character i.e. morphometric (continuous) and meristic (discontinuous) were analysed separately because of the fundamental differences in their distributional characteristics (Sokal and Rohlf, 1969; Rice and Chapman, 1985). Morphometric characters were log (x + 1) transformed to meet the assumptions of parametric statistics. All statistical analyses were carried out using Statistical Package for the Social Sciences (SPSS), Version 12.0 for Windows.

Principal components analysis was performed on the correlation matrix of transformed morphometric variables **(Table 6.2)** and meristic variables **(Table 6.3)**. The regression factors scores for the principal components were used to generate graphs of the outputs **(Figure 6.1 a - d)**. The positions of individuals on the axes can be used to see if the species vary and therefore a one-way analysis of variance (ANOVA) was performed on

the principal components to look at any significant difference between populations **(Tables 6.4 and 6.5)**. The mean values and standard deviations for the measured variables for the populations of *anceps* from Orkney and Ireland are given in **Table 6.6**. All datasets were tested for normality (Kolmogorov-Smirnov test) and homogeneity of variance (Levene's Test). Independent samples *t*-test was used to determine significant differences in the mean values. Where datasets failed the above tests then the non-parametric Mann-Whitney test was used.

Table 6.1: List of 15 characters measured on each plant to examine similarities and differences between different populations of *F. distichus* subsp. *anceps* in the British Isles.

Number	Character	Type of character
1	Plant length (mm)	Morphometric
2	Plant width (mm)	Morphometric
3	Receptacle length (mm)	Morphometric
4	Receptacle width (mm)	Morphometric
5	Conceptacle length (μm)	Morphometric
6	Conceptacle width (μm)	Morphometric
7	Distance to oldest dichotomy (mm)	Morphometric
8	Diameter of oogonium	Morphometric
9	Midrib width (mm)	Morphometric
10	Stipe width (mm)	Morphometric
11	Diameter of holdfast at widest point (mm)	Morphometric
12	Angle of oldest dichotomy (º)	Meristic
13	Angle of youngest dichotomy (º)	Meristic
14	Number of lower dichotomies	Meristic
15	Number of upper dichotomies	Meristic

6.2 Results

A correlation matrix of transformed morphometric variables of *anceps* from Orkney and Ireland found correlations between some of the variables. Bartlett's Test of Sphericity yielded a value of 506.91 and an associated level of significance <0.001 therefore significant correlations were seen among at least some of the variables. The extraction was deemed robust by virtue of a high (0.600) Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy associated with in, indicating that factor analysis was appropriate.

Using the criterion of retaining factors with eigenvalues of 1 or greater, three factors were retained for rotation which revealed cross-loadings on some factors. To obtain meaningful factors these were subjected to orthogonal varimax rotation with a minimum level of significance of (±) 0.33 (Ho, 2006). These rotated factors accounted for 19.04%, 17.74% and 17.06% for a total of 53.84% variance in the data. **Table 6.2** shows the weighting of the eleven variables on the three components.

Table 6.2: Post extraction and rotation factor loadings of the eleven variables of transformed morphometric data subjected to principal components analysis.

Variable	First component	Second component	Third Component
Conceptacle length	0.870		
Conceptacle width	0.829		
Diameter of oogonium	-0.517		0.427
Distance to oldest dichotomy	0.511		
Plant width		0.780	
Midrib width		0.693	
Plant length		0.635	0.548
Receptacle width		0.575	
Diameter of holdfast			0.729
Stipe width			0.689
Receptacle length			0.507

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.

The meristic data were analysed in a similar way to the morphometric data. The principal components analysis was however based on a correlation matrix of raw data using Spearman's *r* as a non-parametric measure of correlation. Using the criterion of

retaining factors with eigenvalues of 1 or greater, two factors were retained for orthogonal varimax rotation accounting for 34.53% and 27.75% for a total of 62.28%. The weighting of the four variables on the two components are given in **Table 6.3**.

Table 6.3: Post extraction and rotation factor loadings of the four variables of meristic data subjected to principal components analysis.

Variable	First component	Second component
Number of upper dichotomies	0.816	
Number of lower dichotomies	0.623	
Angle of youngest dichotomy	0.563	
Angle of oldest dichotomy		0.928

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.

The position of the individuals on the axes is indicated by (±) of the weightings and is used to generate a graphs of the outputs **(Figure 6.1 a - d)**. Using the saved components is a preferred method as it is representative of all the original variables.

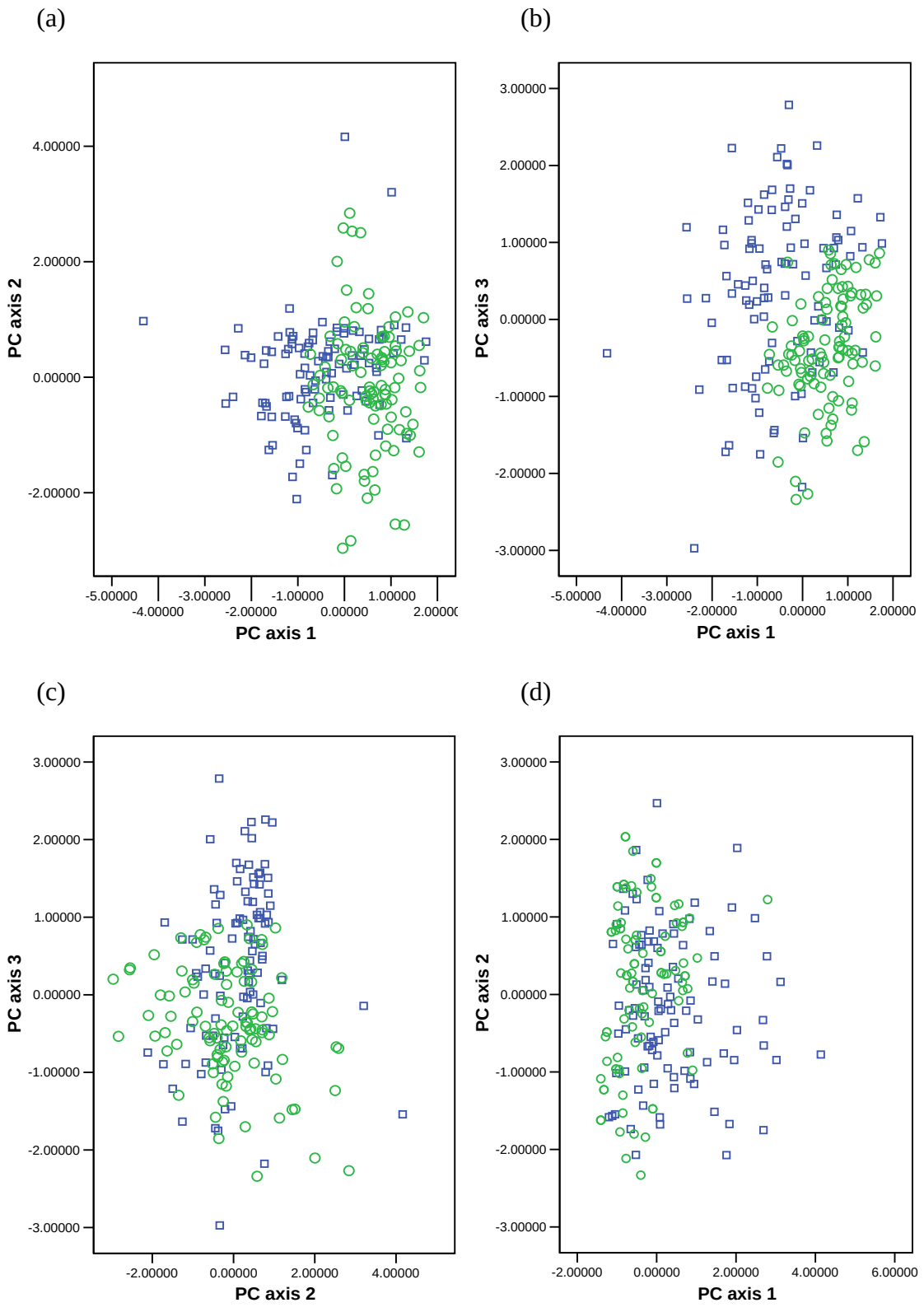


Figure 6.1: Plots for individual plants according to their principal components: a – c = morphometric; d = meristic. Species coded by shapes □OFda, ○IFda.

The positions of individuals on the axes can be used to see if the species vary and therefore a one-way analysis of variance (ANOVA) was performed on the principal components to look at any significant differences between populations.

Table 6.4: Results of one-way ANOVA performed on the principal components of morphometric characters to look at any significant differences between populations ***highly significant; NS = not significant).

Source	d.f.	SS	MS	*F*	*P*
REGR factor score 1 for analysis 1	1	57.88	57.88	81.21	< 0.001***
Residual	198	141.12	0.71		
Total	199	199.00	1.00		
REGR factor score 2 for analysis 1	1	3.18	3.18	3.22	NS (0.074)
Residual	198	195.82	0.99		
Total	199	199.00	1.00		
REGR factor score 3 for analysis 1	1	27.54	27.54	31.81	< 0.001***
Residual	198	171.46	0.87		
Total	199	199.00	1.00		

Table 6.5: Results of one-way ANOVA performed on the principal components of meristic characters to look at any significant differences between populations (*significant; ***highly significant).

Source	d.f.	SS	MS	*F*	*P*
REGR factor score 1 for analysis 1	1	26.31	26.31	30.17	< 0.001***
Residual	198	172.69	0.87		
Total	199	199.00	1.00		
REGR factor score 2 for analysis 1	1	4.82	4.82	4.91	0.028*
Residual	198	194.18	0.98		
Total	199	199.00	1.00		

Table 6.6: Results of *t*-test and Mann-Whitney test used to determine significant differences in the mean or rank values measured between populations of *anceps* from Orkney and Ireland (*significant; **very significant; ***highly significant; NS = not significant).

Variable	Population	Mean ± SD	*P*
Plant length (mm)	Orkney	133.24 ± 54.53	< 0.001***
	Ireland	103.86 ± 38.50	
Plant width (mm)	Orkney	2.58 ± 0.83	NS
	Ireland	2.43 ± 0.96	
Receptacle length (mm)	Orkney	37.58 ± 13.22	0.032*
	Ireland	33.33 ± 12.34	
Receptacle width (mm)	Orkney	3.92 ± 0.81	NS
	Ireland	3.89 ± 0.93	
Conceptacle length (μm)	Orkney	601.95 ± 147.01	< 0.001***
	Ireland	704.10 ± 136.99	
Conceptacle width (μm)	Orkney	527.75 ± 148.91	< 0.001***
	Ireland	633.50 ± 124.84	
Diameter of oogonium (μm)	Orkney	119.66 ± 12.70	< 0.001***
	Ireland	69.80 ± 9.43	
Distance to oldest dichotomy (mm)	Orkney	13.52 ± 10.03	0.008**
	Ireland	19.12 ± 12.74	
Stipe width (mm)	Orkney	3.76 ± 1.37	0.032*
	Ireland	3.30 ± 0.94	
Diameter of holdfast (mm)	Orkney	7.76 ± 3.35	< 0.001***
	Ireland	5.68 ± 2.39	
Midrib width (mm)	Orkney	1.02 ± 0.14	< 0.001***
	Ireland	0.98 ± 0.15	
No. of lower dichotomies	Orkney	13.96 ± 9.51	< 0.001***
	Ireland	7.79 ± 6.46	
No. of upper dichotomies	Orkney	41.12 ± 31.89	0.002***
	Ireland	28.49 ± 25.33	
Angle of oldest dichotomy (º)	Orkney	30.56 ± 13.27	NS
	Ireland	32.67 ± 13.89	
Angle of youngest dichotomy (º)	Orkney	20.77 ± 11.89	0.001***
	Ireland	15.47 ± 9.48	

6.3 Conclusions

Principal axis factors extraction has uncovered three latent factors that describe relationships between the morphometric variables **(Table 6.2)**. Inspection of weightings of the three components, indicate which variables contribute most to differences between individuals. The first component explains the most variation, second the next most variation and so on. Examination of the factor loadings for the morphometric variables show that eleven variables loaded highly on the three components. Components two and three had positive loadings while component one was bipolar. The first principal component is associated with conceptacle length and width, diameter of oogonium and distance to oldest dichotomy and mainly reflects aspects of differences in reproductive structures. Component two is associated with plant length and width, midrib width and receptacle width and mainly reflects differences in plant size. Component three is associated with diameter of holdfast, stipe width and receptacle length may reflect some aspects of the growth attachment mechanism. Cross-loadings were present for diameter of oogonium on components one and three and for plant length on two and three. The author must decide which components are meaningful (either theoretically or intuitively) and by examining the wording of the cross-loading variables, and based on their face-validity, assign them to the components of which they are most conceptually/logically representative (Ho, 2006). Size and shape are spread over the three components however the first component is closely associated with the size of reproductive structures.

Examination of the factor loadings for meristic variables **(Table 6.3)** show that four variables loaded highly on two components with all components having positive loadings. The first principal component is associated with the number of upper and lower dichotomies and the angle of youngest dichotomy. High correlations between the numbers of upper and lower dichotomies are inevitable relationships. The second component has a heavy loading for the angle of oldest dichotomy and both factors reflect differences in aspects of branching and shape.

Results of one-way ANOVA performed on the principal components of morphometric characters **(Table 6.4)** indicate significant differences on the first ($F_{1,198} = 81.21$, $P < 0.001$) and third ($F_{1,198} = 31.81$, $P < 0.001$) principal components showing differences in reproductivity structures and growth attachment mechanism respectively. Although the

results for the second principal component are higher than the critical *P*-value of 0.05 ($F_{1,198} = 3.22$, $P = 0.074$) it does indicate at least a 92% chance that there is a difference between the populations with respect to aspects of size. The results of one-way ANOVA performed on the principal components of meristic characters **(Table 6.5)** show highly significant difference between the populations on the first principal component ($F_{1,198} = 30.17$, $P = 0.001$) and significant difference on the second principal component ($F_{1,198} = 4.91$, $P = 0.028$). This indicates that there are differences between the populations with regards to branching and shape.

The plots of principal components **(Figure 6.1 a - d)** show overlapping for both morphometric and meristic variables with no clear separation between the two populations of *anceps*. However, morphometric plot scores on the first and third components show rather less overlap with the arrangements of plants elongated along these components. These are associated with conceptacle and oogonium size and aspects of plant attachment and size respectively. Although elongation is seen on the second component on the meristic plot there is no clear separation between the populations, however the first component shows some separation in the Orkney population associated with growth and shape.

The *t*-test and Mann-Whitney test used to determine differences in the mean and rank values measured between populations of *anceps* show some significant results **(Table 6.6)**. Plant length, conceptacle length and width, diameter of oogonium, diameter of holdfast, midrib width, number lower dichotomies and angle of the youngest dichotomy all show highly significant differences between the populations ($p < 0.001$) as does the number of upper dichotomies ($p < 0.002$). Distance to oldest dichotomy is very significantly different ($p = 0.008$) and stipe width ($p = 0.032$) is significant different between populations. Comparison of the mean values of the variables shows that Orkney plants were not only longer than Ireland plants but had a greater amount of dichotomous branching. Midrib and stipe width and diameter of holdfast were also greater, these variables are also associated with larger plants. In Ireland plants mean values for distance to oldest dichotomy, that is the length of the stipe, and conceptacle length and width were greater than for Orkney plants, diameter of the oogonium however were much smaller in Ireland plants.

6.4 Discussion

The results of this investigation show an overlap between the populations and therefore no evidence of two discrete entities. However they show differences in growth with respect to size and form and some aspects of reproductive structures, between the two populations of *anceps* from Orkney and Ireland. The Orkney plants were not only longer on average than Ireland plants but more highly branched having a greater amount of both lower and upper dichotomous branching. It should be noted that plant length recorded here for both populations were within the size parameters noted by other authors for this subspecies (Powell, 1957b; Russell, 1974). Orkney plants also showed greater growth in stipe width and diameter of the holdfast, both variables associated with larger plants and the strong attachment mechanism needed on wave exposed shores.

The differences in plant length and branching patterns noted in this investigation may be the result of ecological interactions such as herbivory and competition. *Fucus* species have a pronounced wound response which results in the formation of a new vegetative axis at the cut or grazed surface and heavily grazed plants may show increased numbers of dichotomies (Moss, 1966; Fulcher and McCully, 1971). An increase in plant length was found in relation to northward distribution in a study of intraspecific geographic-morphological variation patterns in *F. distichus* (=*anceps*) by Rice *et al*. (1985) and a similar pattern may be seen here in the longer plant length noted in the northern Orkney population. Ang (1992) stated that the cost of reproduction in *F. distichus* (no distinction made of subspecies) may manifest itself in the form of reduced growth. This may be the case for the Ireland population being at the southern limit of its range and, as suggested by Powell (1957b), less tolerant of adverse conditions and therefore greater reproductive effort required.

Strong wave exposure can cause tattering of the fronds in larger *Fucus* plants and therefore there is strong selection for optimally sized plants, smaller than the tattering threshold at a given location (Blanchette, 1997). Both the Orkney and Ireland collection sites are exposed to severe wave action and there may be other contributory factors present that account for the smaller and less branched growth form seen in the Ireland population. Powell (1957b) also stated that *anceps* may occur at lower levels at Irish sites than at Scottish sites and that this could be the result of severe desiccation at the

Irish site. In this study Ireland plants were recorded as having a smaller angle in youngest dichotomous branching. This was also noted by Powell where he stated that in Irish plants the angle between the youngest branches were very acute so that young terminal branches were closely crowded and ran almost parallel. This crowding of the terminal branches along with the characteristic arching of the frond as described in **Section 2.4** may trap moist air and reduce desiccation during periods of emersion. A similar growth form is seen in *Pelvetia canaliculata*, another species found in the high intertidal and subject to long periods of emersion and hence desiccation.

F. distichus subsp. *anceps* is hermaphrodite/monoecious producing gametes of both sexes in each receptacle. Culture experiments (McLachlan *et al*., 1971) have suggested that self-fertilising is high with the ova being fertilised before release. Pollock (1970) stated that post-discharge fertilisation involved antherozoids which had penetrated the oogonium's mesochite before leaving the conceptacle and therefore derived from the same conceptacle. It would appear then that sexual reproduction in this species involves a high degree of self-fertilising. Not much is known about the extent of dispersal of macroalgae spores however once a zygote is formed it can probably only be dispersed over limited distances. Estimates range from 1.5 – 3m in *Postelsia palmaeformis* (Dayton, 1973), 5m in *Macrocystis pyrifera* (Anderson and North, 1966), 10 – 30 m in *Alaria esculenta* (Sundene, 1962) and 60m for *Fucus* eggs (Burrows and Lodge, 1951). As previously discussed in **Section 5.2.3** adventive embryony occurs in *F. distichus* and may produce many clones. These processes of self-fertilisation, limited dispersal of zygotes and cloning may well result in very limited gene flow.

Results show that although conceptacles were on average larger in Ireland plants the oogonia within were significantly smaller ($P < 0.001$) than in Orkney plants. This may be attributed to immature oogonia as suggested by Ang (1991a) in his study on reproduction and recruitment in *F. distichus* (no distinction made of subspecies). Reproduction in *anceps* in the British Isles is recorded as being between April and August with fertile plants found in all these months although receptacles may start to develop as early as January (Powell, 1957b). In this investigation collections were made at the end of April and May for Orkney and Ireland plants respectively. It may indeed be the case that the oogonia were immature and would continue to develop and grow however if April is taken as the start of the reproductive period and as both populations were collected around this time then it may be assumed that immature

oogonia were present in both populations and yet oogonium were much smaller in Ireland plants.

Powell also suggested that plants of *anceps* frequently had all apices fertile at the same time and therefore to complement the results recorded for the other variables the numbers of reproductive and vegetative tips were noted for each plant. Counts for Orkney plants showed that 73% of tips were reproductive and 27% were vegetative while in Ireland plants 51% were reproductive and 49% were vegetative. This may suggest that plants had not reached peak fruiting, particularly in the Ireland population, however Powell also stated that peak fruiting is reached a few weeks earlier near the southern limits of distribution as in the Ireland plants, than in the Scottish localities. Possible explanations for the smaller oogonium found in Ireland *anceps* are: plants do not have all tips reproductive at the same time as suggested; peak fruiting period occurs later than previously thought in the Ireland population; oogonium are indeed smaller in the Ireland population. Is there any ecological advantage for the Ireland population in having smaller oogonium?

Fucus spp. release their eggs still held together in the oogonium and this mass of eight eggs will influence frictional drag and modify the settlement rate, sinking faster than would a single egg (Coon *et al*., 1971). Small eggs cost less energy to produce than large ones (Clayton, 1984) and a smaller size tends to confer a lower rate of sinking. This may give *anceps* in Ireland a greater potential for dispersal and colonisation of new areas. Amsler and Searles (1980) found supporting evidence for differential settlement rates of algal spores and their influence on dispersal capability in coastal waters and suggested these indicated differences in evolutionary tactics.

The high selfing rates in hermaphrodite/monoecious species such as in *anceps* may reduce intra-population genetic variation, effective population sizes and gamete dispersal however it may be advantageous in respect to reproductive assurance and colonising capacity (Perrin *et al*., 2007). Powell (1957b) stated that at its southern limit *F. distichus* is represented by populations of the best adapted forms which are confined to restricted habitats and geographically isolated as in the case of *anceps*. This restriction and isolation along with limited gene flow and various selection pressures may result in the gradual evolution of a genetically distinct ecotype. Powell also suggested that *anceps* is less tolerant of adverse conditions near the southern limit of its

range and a species or subspecies that are represented by a single ecotype confined to a limited area frequently exhibit exceptionally small 'ecological aptitude'. Russell (1974) stated that the precarious foothold of this subspecies may make it particularly sensitive to any change in habitat conditions. Any reduction in within-population variation can allow wide differences between populations where physiology and ecological parameters may be quite different (Lobban and Harrison, 1991).

The main points from this part of the investigation are:

- Although there is no evidence of two discrete entities, results do show differences between the two populations of *anceps* from Orkney and Ireland with respect to size and form and some aspects of reproduction.

- The smaller plants recorded in the Ireland population may be evidence that at the southern limit of its range *anceps* is less tolerant of adverse conditions and therefore any changes in sea temperatures.

- The smaller oogonium noted in *anceps* from Ireland may influence the dispersal capabilities of algal spores and indicate possible differences in evolutionary tactics between the geographically different populations.

Many factors including light, daylength, wave exposure and temperature can act on both the juvenile and adult life stages of *Fucus* species and affect their distribution both on a local and global scale. These factors and the question of how appropriate the use of *anceps* is as a possible indicator of sea temperature rise and climate change in the British Isles are discussed in the following chapter.

Chapter 7: Discussion

The main aims of this investigation were to look at how seawater temperatures affected the establishment, growth, survival and distribution of *Fucus* species. This was approached in a number of ways: to demonstrate the need for studies on both the juvenile and adult life stages to investigate any particular temperature preferences; whether any temperature preference differed between the life stages for the *Fucus* species investigated; to examine whether temperature preferences inferred from laboratory experiments showed any correlation to sea temperatures found locally for those species; what other factors may be involved in the distribution of *Fucus* species. To compare the temperature preferences for growth of juvenile and adult life stages **Table 7.1** gives a summary of the experimental results along with sea temperatures found locally for those species and dates of collection.

Table 7.1: Temperature preferences for growth and performance of juvenile and adult life stages of *Fucus* species inferred from the present investigation along with dates of collection and range of sea temperatures found locally for that species (- No data available; NS = not significant).

Germling Temperature Preferences (ºC)		**Adult Temperature Preferences (ºC)**					Average sea surface temperatures for month of collection	Range of sea surface temperatures found locally
Species	Optimum temperature after 100 days	Surface area change after 100 days	Wet (fresh) weight change after 100 days	Gross photosynthesis before and after the experimental period of 100 days				
				Before	After	Greatest % change		
Fucus distichus subsp. *anceps* Bay of Skaill (South), Orkney	15	15	5	15	NS	15	7 - 8ºC (April)	Low Feb 6ºC – High Aug 13ºC
Fucus distichus subsp. *anceps* Kilkee, Co. Clare, Ireland	10	NS	5	NS	10 & 15	5	10 - 11ºC (March)	Low Feb 10ºC – High Aug 16ºC
Fucus spiralis f. *nanus* Bay of Skaill (South), Orkney	5	10 & 15	NS	15	NS	10 & 15	7 - 8ºC (April)	Low Feb 6ºC – High Aug 13ºC
Fucus vesiculosus var. *linearis* Bay of Skaill (South), Orkney	15 then10*	10 & 15	5	5 & 10	5	10	7 - 8ºC (April)	Low Feb 6ºC – High Aug 13ºC
Fucus vesiculosus var. *linearis* Kilkee, Co. Clare, Ireland	-	NS	5/10	NS	10 & 15	5 & 10	10 - 11ºC (March)	Low Feb 10ºC – High Aug 16ºC
Fucus serratus Bay of Skaill (North), Orkney	15	15	15	15	15	5	7 - 8ºC (April)	Low Feb 6ºC – High Aug 13ºC

Table 6.7 (continued)

Fucus spiralis Bay of Skaill (North), Orkney	15	10 & 15	5	10	5	10	7 - 8ºC (April)	Low Feb 6ºC – High Aug 13ºC
Fucus vesiculosus Bay of Skaill (North), Orkney	15	10 & 15	5	5 & 10	5	10	7 - 8ºC (April)	Low Feb 6ºC – High Aug 13ºC
Fucus distichus subsp. *edentatus* Macduff, Moray Firth, Scotland	-	5	5	5	5	15	10 - 11ºC (Oct)	Low Feb 5.6ºC – High Aug 12ºC
Fucus distichus subsp. *edentatus*** Bressay, Shetland Islands	-	NS	-	10	15	10	10 - 11ºC (July)	Low Feb 5ºC – High Aug 12ºC
*Fucus serratus*** South Queensferry, West Lothian, Scotland	-	NS	-	NS	NS	15	12 - 13 ºC (July)	Low Feb 5-6ºC – High Aug 13-14ºC
*Fucus spiralis*** South Queensferry, West Lothian, Scotland	-	10	-	NS	10	15	12 - 13 ºC (July)	Low Feb 5-6ºC – High Aug 13-14ºC
*Fucus vesiculosus*** South Queensferry, West Lothian, Scotland	-	NS	-	10	15	10	12 - 13 ºC (July)	Low Feb 5-6ºC – High Aug 13-14ºC

* Only species where optimum temperature preference changed over the culture period, initially at 15ºC up to 62 days, thereafter at 10ºC to end of the culture period at 100 days.
**Reduced culture period of 50 days at 10ºC and 15ºC only due to serious malfunction of CT rooms and eventual failure.

7.1 Comparison of the temperature preferences for growth and performance of juvenile and adult life stages of *Fucus* species

Studies on the juvenile stages of *Fucus* species are important in understanding the effect sea temperature may have on the establishment and maintenance of populations. In this investigation it was shown that temperature affected the rate of germling growth of *Fucus* species. More importantly however was that different temperatures affected the growth rate of the component head and rhizoid parts differently. Subsequently the effect of temperature on the total length varied not only within a species but also between species. At the end of the experimental period the majority of species showed an optimum for growth at the highest temperature of 15ºC. These were *F. distichus* subsp. *anceps*, *F. serratus*, *F. spiralis* and *F. vesiculosus* from Orkney. *F. vesiculosus* var. *linearis* from Orkney was the only species where a change in temperature preference was noted for total length, initially growing best at 15ºC up to 62 days, thereafter at 10ºC to end of the culture period at 100 days. The only Orkney species which showed a preference for growth at the lowest temperature of 5ºC in the juvenile stage was *F. spiralis* f. *nanus*. In the Ireland population of *anceps* growth of the juvenile stage was greatest at 10ºC.

The study of the germling life stage is only part of the story. For a population of *Fucus* species to become established germlings must develop and grow, reach maturity and become reproductive. Knight and Parke (1950) suggested that conditions for the development of sporelings of fucoids may not be the same as for mature plants. If this is true then evalution of the effects of environmental conditions such as temperature on germlings alone will have limited value (Lüning, 1980; Chapman, 1995). How then did temperature preferences found in germlings compare to those for adult plants and do these growth responses to temperature reflect local sea temperatures for the species investigated?

The optimum temperature of 15ºC noted in the juvenile stage of *anceps* from Orkney was reflected in the adult life stage in the form of maximum surface area increase at 15ºC. The cooler temperature of 5°C resulted in new growth, regeneration and development of receptacles of the adult apical tips. Results for *nanus* from Orkney showed some changes in temperature preferences between juvenile and adult life stages. An optimum temperature for germling growth was found at 5ºC. This was not reflected

in adult plants where surface area increase was greatest at 10ºC and 15ºC. New growth also occurred at 10ºC and 15ºC. As noted in *anceps*, development of receptacles was present on apical tips at 5ºC. *F. vesiculosus var. linearis* from Orkney showed a preference for higher temperatures for growth in both juvenile and adult life stages. Initially 15ºC was the optimum temperature for germling growth up to 62 days after which growth at 10ºC was greatest. Adult growth reflected this preference for higher temperature with surface area increase greatest at 10ºC and 15ºC. Once again new growth, regeneration and development of receptacles were greatest at the lowest temperature of 5ºC.

Sea surface temperatures in Orkney range from a low in February at 6ºC, peaking in August at 13ºC however records for 2003 show temperatures up to 15.1°C (Fisheries Research Services, 2004). The experiments ran from mid-April to late July covering a large period of the range of temperatures found locally in nature. Results from **Table 7.1** show that optimum temperatures for germling growth in *anceps* (15ºC) and *linearis* (15ºC then 10ºC) and surface area increase in *anceps* (15ºC), *linearis* (10ºC and 15ºC) and *nanus* (10ºC and 15ºC) at the end of the culture period in late July, showed some correlation to local sea surface temperatures recorded for July and August in Orkney. The fruiting period for *anceps* runs from April to August (Powell, 1957a) therefore the reproductive apical tips noted at 5ºC only corresponded to local sea surface temperatures at the start of the fruiting period. If seasonality is responsible for reproduction of the apical tips then development of receptacles may have been expected at the higher temperatures later in the culture period to correspond with local sea surface temperatures for July, however this was not the case. In the case of *linearis* and *nanus* from Orkney fruiting records are vague. Plants were reproductive at date of collection in April where local sea surface temperatures are between 7 - 8ºC and therefore the reproductive tips noted at 5ºC show some relationship to local sea surface temperatures. It should be noted here that fruiting records given here only apply to the British Isles.

The other Orkney species *F. serratus*, *F. spiralis* and *F. vesiculosus* all showed preferences for the higher temperatures of 10ºC and 15ºC in both juvenile and adult life stages. In adult plants of both *F. spiralis* and *F. vesiculosus* no new growth, regeneration or formation of receptacles was noted however an increase in bulk at the lower temperature of 5ºC was recorded. *F. serratus* was the only species which showed a real affinity for 15ºC across all experimental procedures in both juvenile and adult life

stages and at this temperature new growth and the formation of receptacles were also noted.

The population of *anceps* from Ireland showed an optimum temperature for juvenile growth at 10ºC however in adult plants surface area increase was the same at all three temperatures. The formation of receptacles in adult apical tips was noted to some degree at all three temperatures but only at 10ºC was there additional new growth and regeneration. These results suggest a preference for 10ºC at the juvenile stage and at the adult stage in the form of new growth and reproductivity in *anceps* from Ireland. Unfortunately any comparison of the juvenile stage of *linearis* from Ireland could not be made due to unsuccessful settlement. In adult plants surface area increase was the same at all three temperatures, as noted for *anceps* from Ireland. Sea temperatures off the west coast range from an average of 8 - 10°C during February or March and from 14 - 16°C in August (Met Eireann, 2010). Experiments ran from early March to mid-June covering an early to mid-range period of the sea surface temperatures found locally in nature and show some correlation in the juvenile life stage to temperatures at collection date in March.

It is interesting to note that the two species from Ireland associated with wave exposure, *anceps* and *linearis*, showed the same temperature preferences as each other and that these differed from the corresponding populations from Orkney. In the Orkney populations of *anceps*, *linearis* and *nanus* differences were noted in temperature preferences for some aspect of growth and performance in either juvenile or adult life stages. What was particularly noticeable in *nanus* was that unlike *anceps* and *linearis*, the temperature preference in the juvenile stage was at 5ºC and only in adult plants was the preference for the highest temperature of 15ºC, as seen in *anceps* and *linearis*, reflected. However one aspect of growth that was observed in all three of these species from Orkney was the formation of receptacles on apical tips at 5ºC. This would suggest that the growth effort concentrated on reproduction at 5ºC at the expense of the increased surface area noted at 10ºC and 15ºC. This has been noted in *F. vesiculosus* where growth of branches arrested with the appearance of receptacles (Edelstein and McLachlan, 1975).

It may be that northerly Orkney populations of *anceps* have a preference for colder sea temperatures for reproduction. This preference for reproduction at the lowest

temperature of 5ºC in *anceps* was not observed in the Ireland population where the formation of receptacles was noted to some degree at all three temperatures and may suggest different reproductive strategies or even genetic differences between the geographically different populations of *anceps*. Previous work on *edentatus* from New England (Sideman and Mathieson, 1983) and central Puget Sound (Thom, 1983) found substantial differences in the characteristics between populations. This was attributed to either the adaptive abilities of the subspecies or the existence of separate species. Similarly, a study on *F. vesiculosus* in Baltic Sea (Berger *et al*., 2001) suggested having two strategies of reproduction was an expression of two distinct genotypes. Studies on *Pylaiella littoralis* (= *Pilayella*) found that the morphological differences between populations were accompanied by physiological and genetic differences (Russell, 1963).

F. distichus subsp. *edentatus* from Macduff were non-reproductive at the date of collection. Despite this adult plants showed a clear preference for 5ºC in all aspects of growth including the development of receptacles on apical tips at 5ºC and less so at 10ºC. Local sea surface temperatures range from a low in February at 5.6ºC to a high in August at 12ºC (Fisheries Research Services, 2004). Experiments ran from October, where sea temperatures are around 10 - 11ºC, to December and results show a temperature preference for growth lower than might be expected. It has been suggested by Powell (1957b) and Russell (1974) that *edentatus* is very tolerant of nutrient enriched waters and grows best under conditions of sewage and other organic pollution giving it an advantage over less tolerant local *Fucus* species, reducing competition. Powell also suggested that sea and air temperatures may not directly determine the distribution of this subspecies in Britain. It has been suggested that distribution of *edentatus* in Britain is most likely determined by daylength (Hiscock *et al*., 2001). In North America (McLachlan, 1973) and Norway (Strömgren, 1985) light in addition to temperature is considered a controlling factor in the distribution of *edentatus*.

Unfortunately growth results for *F. serratus*, *F. spiralis* and *F. vesiculosus* from South Queensferry and *edentatus* from Shetland are very limited. This was due to a series of malfunctions of CT rooms with eventual total failure leaving them inoperable for many months. This resulted in no data for juvenile growth and for adult plants culture at 10ºC and 15ºC only at a reduced culture period of 50 days. Sea surface temperatures for South Queensferry range from a low in February of 5 - 6ºC and high of 13 - 14ºC in August and for Shetland a low of 5ºC in February and high of 12ºC in August (Fisheries

Research Services, 2004). Experiments ran from mid-July to early September. The only results that can be taken from these reduced experiments is that adult growth occurred at 10ºC and 15ºC but without the use of the lower temperature of 5ºC and no results for juvenile growth, any relation to local sea surface temperatures would be purely speculative.

In some of the species investigated the optimum temperature for gross photosynthesis at the start of the culture period was the same as for surface area increase. This was noted for Orkney populations of *anceps*, *nanus* and *serratus* at 15ºC, for *spiralis* at 10ºC and for *edentatus* from Macduff at 5ºC. Both *linearis* and *vesiculosus* from Orkney showed different temperature optimums between the experimental procedures at 10ºC and 15ºC for surface area increase and 5ºC and 10ºC for gross photosynthesis. Once again growth results for *F. serratus*, *F. spiralis* and *F. vesiculosus* from South Queensferry and *edentatus* from Shetland are limited and only *serratus* showed similar results with no temperature preference for growth or performance. Similarly the results for *anceps* and *linearis* populations from Ireland showed the same response in surface area growth and gross photosynthesis in having no temperature preferences. At the end of the culture period the optimum temperature for gross photosynthesis had changed for most species. This may have been due to the artificial conditions of culture as observations showed some discolouration of apical tips. However there were exceptions in the case of *edentatus* from Macduff and *serratus* from Orkney and South Queensferry which retained their temperature preferences before and after the culture period.

As mentioned, laboratory conditions for culture are extremely artificial and consequently there may have been some limiting factors to growth and performance. In this investigation two consistent factors of light and photoperiod were incorporated in the culture conditions along with the controlled temperature variables. It was not possible to replicate all the environmental factors that are found on intertidal rocky shores. Wave action is a major controlling factor not only in the distribution of algae but also on the morphology and form of a species (Burrows and Lodge, 1951; Powell, 1963; Blanchette, 1997; Anderson and Scott, 1998; Blanchette *et al*., 1999).

Fucoids are only able to survive on wave exposed shores when they develop into narrow dwarf forms such as *F. distichus* subsp. *anceps*, *F. vesiculosus* var. *linearis* and *F. spiralis* f. *nanus* (Powell, 1957a; Lewis, 1964; Russell, 1978). A combination of

sloping shore, ocean swell and severe wave action results in the upward extension of the littoral zone and allows these species to find an ecological niche high on the shore where few other species can survive. Most of the time these high shore species are not affected by wave action but are frequently wetted by spray from breaking waves and ocean swell, indeed species such as *Alaria esculenta* which occupies the sublittoral fringe is subject to far greater swell and wave action. In relatively calm weather *anceps*, *nanus* and *linearis* are subject to severe desiccation, particularly in the summer months and particularly at the Irish sites. On wave exposed shores *anceps* forms a belt between *nanus* above and *linearis* below, often mingling with these species. Powell (1957b) suggested that these high level fucoids may occur at relatively lower levels at some Irish sites than at Scottish sites, the result of more severe desiccation at the Irish sites. It may be, and as suggested by Powell (1957b), that the eventual southern limit of *anceps* in Ireland will be determined by increasing desiccation as retreat further down the shore is limited by competition from other plant and animal species and severe wave action. As warm sea temperatures bring warm air temperatures then any increase in sea temperatures may indirectly affect the distribution of this species through increased desiccation.

In the British Isles *anceps* is only found under conditions of very considerable exposure to wave action and oceanic swell. Would a reduction in wave action affect the distribution, form or even survival of *anceps*? This may seem a hypothetical question as it is very unlikely to happen in nature. However work is currently underway at the European Marine Energy Centre (EMEC) in the development and deployment of wave and tidal energy convertors in Orkney and elsewhere. Seabed leases have been issued for marine renewable developments in the Pentland Firth and Orkney waters and the next few years will see the establishment and operation of wave and tidal energy conversion on a large scale. The scale of the environmental effects from a range of tidal and wave energy convertors remains uncertain (Side, 2010). A study by the International Centre for Island Technology (ICIT) and the Institute of Petroleum Engineering at Heriot-Watt University is currently underway to examine the effect any reduction in wave energy may have on the ecology of the wave exposed coast of the West Mainland of Orkney. Energy extraction by wave energy converting devises (WEC's) may be expected to change exposure characteristics shoreward of their location (Want *et al*., 2010). Four littoral species have been proposed as possible sentinel species; these are *Chthamalus stellatus*, *Gibbula umbilicalis*, *Patella*

ulyssiponensis and *Fucus distichus* subsp. *anceps*. The challenge will be to discriminate between environmental disturbances arising from climate change and rising sea temperatures and those occurring in response to the removal of marine energy.

The removal of wave and tidal energy from wave exposed shores could have serious implications for high shore forms such as *anceps, nanus* and *linearis*. The removal of this energy would surely lessen or even remove the upward extension of the littoral zone and reduce the amount of spray these species receive. If retreat down the shore is limited by competition from other plant and animal species then they will be subject to even greater desiccation than at present. One interesting question here is with the removal of wave action would *anceps*, *nanus* and *linearis* be able to out-compete the lower shore species, which presumably would also be subject to some change, allowing them to colonise the lower shore and would they then develop into their larger more sheltered forms i.e. *anceps* to *edentatus*, *nanus* to *spiralis* and *linearis* to *vesiculosus* or are these forms genetically 'fixed'? *Fucus* species possess a genotype that permits a wide range of phenotypic expression, and a phenotype that responds to differences in the environment (Russell, 1978). Field transplant experiments on *F. distichus* by Pollock (1969) suggest that the amount of phenotypic plasticity in this species is low. Culture and out-planting experiments to examine the growth, reproductive phenology and longevity of in situ populations of the *F. distichus* complex carried out by Sideman and Mathieson (1983) suggested genetic differences between the taxa. Inbreeding, which is possible with monoecious species such *F. spiralis* and *F. anceps* can lead to a reduction of within-population variation. This combined with geographic and reproductive isolation would suggest that any removal of wave energy would have little effect on the morphology of these species.

Near the centres of distribution of *F. distichus* extreme forms can be interpreted as products of their ecological environment with intermediate forms developing under intermediate conditions (Powell, 1957a; Russell, 1974). However at the southern limits of distribution the species is represented by populations of only one or two of the best adapted, more distinctive forms as seen in *anceps* from Orkney and Ireland and *edentatus* from Macduff. These populations are confined to restricted habitats, often geographically isolated, have a very limited range of form and are reproductively isolated and as such could be regarded as adapted ecotypes. An ecotype is a form possessing distinctive inherited characteristics that are assumed to enable it to succeed

in a particular habitat and may be regarded as the phenotypic expression determined to some extent by genetic differences (Russell, 1978).

Taxonomy of the *F. distichus* complex has been treated differently by different authors. Taylor (1962) treated the taxa as species while Rice and Chapman (1985) considered them to be two distinct species i.e. *anceps* as part of *distichus* and *evanescens* to include both *evanescens* and *edentatus*. Molecular work by Serrão *et al.* (1999) and Coyer *et al.* (2006) could not justify the separation of any of the taxa in the *F. distichus* complex at the species level, despite ecological and morphological differences. In the present investigation, and in agreement with Powell's (1957a) description, the sub-specific status was used for the *F. distichus* complex as the different morphologies found in the British Isles are adaptations to the particular environmental habitat that they occupy, irrespective of any genetic difference.

7.2. Are *Fucus* species suitable indicators of climate change?

In the British Isles there is little evidence at present to suggest that intertidal *Fucus* species such as *F. serratus*, *F. spiralis* and *F. vesiculosus* are affected by sea temperature rise directly. However increased temperatures may affect *Fucus* species indirectly through any detrimental effects to grazers and competition species. Studies have shown that grazing prevents the establishment of fucoids and other algae throughout much of Europe (Jenkins *et al*. 2005, Coleman *et al*. 2006). Any changes in species interactions could alter assemblage composition and are likely to have consequences for the balance and dynamics of fucoids on European rocky shores (Thompson *et al*. 1996). It has been suggested (Hiscock *et al*., 2004) that in a warmer climate, fucoid cover would be expected to lessen on shores in Britain and Ireland with implications for primary production and associated epiphytic species. *F. serratus, F. spiralis and F. vesiculosus* are included in a report 'Recommendations for Intertidal Biodiversity Surveillance' by JNCC (Burrows *et al*., 2009) as possible candidate indicator species for monitoring the effects of changes in marine biodiversity. In the British Isles where sheltered shores are dominated by fucoids it seems unlikely that *Fucus* species will to be adversely affected by increased temperatures in the immediate future. In the case of high shore wave exposure species such as *anceps*, *linearis* and *nanus* increased air temperatures as a result of warmer sea temperatures could lead to severe desiccation stress and be the limiting factor in the distribution of these species.

The short term effects of temperature on *Fucus* species attained from laboratory investigations are not easily related to the long term responses of plants in the field. Despite this results show that temperature did affect the *Fucus* species investigated be it to rhizoid and/or head growth at the juvenile stage or forms of growth, reproduction and performance in adult plants. It is interesting to note that temperature preferences for the geographically different populations of *anceps* appeared to contradict local sea temperatures with the Orkney population preferring a warmer temperature to the Ireland population. These results could be some condition of cultivation however if they are representative of temperature preferences then the fact that *linearis* populations mirrored the temperature preferences of the *anceps* populations from Ireland and Scotland suggests that the physiology and ecological parameters may be quite different between the geographically different populations of *anceps* and *linearis*. This difference between populations is further demonstrated in *anceps* where the Orkney population showed a temperature preference for 15°C in both juvenile and adult plants whereas the Ireland population showed a temperature preference for 10°C in juveniles but no species temperature preference in adult plants.

The relationship between sea temperature and distribution of seaweeds should be treated with some caution as temperature response ecotypes have evolved in some seaweed species (Breeman, 1988). For example, the broadly distributed brown algae *Ectocarpus siliculosus* has shown genotypic variation between geographically diverse isolates with respect to temperature optima (Russell and Bolton, 1975) likewise, *Laminaria saccharina* shows genetic adaption of temperature optima towards the edge if its range (Gerard and Du Bois, 1988). A similar picture may be seen in the geographically different populations of *anceps*. Although the phenotypic variation investigation carried out in **Chapter 6** showed no evidence of two discrete entities, the results did show differences between the two populations from Orkney and Ireland with respect to size and form and some aspects of reproductivity.

A report for Scottish Natural Heritage by the Marine Biological Association of the UK (Hiscock *et al*., 2001) describes *F. distichus* (=*anceps*) and *F. evanescens* (=*edentatus*) as northern species which may either decrease in abundance and extent or disappear from Scotland. This report states that *anceps* does not occur south of the summer 13°C isotherm in Britain and that extrapolation from the present distributional range would suggest that following a 1°C and 2°C rise in summer sea temperature the 13°C isotherm

would have moved north of the British Isles and *anceps* would therefore become extinct in Britain. The report also stated that a 2°C rise in sea temperature may make no difference to the distribution of populations in northern Scotland and that distribution is most likely determined by daylength rather than temperature. This is based on laboratory and field studies showing that both embryos and mature plants can tolerate higher temperatures of 15°C or more (Bird and McLachlan, 1976). The results from this investigation concur with this statement as both juvenile and adult plants of *anceps* grew best at 15°C. In the case of *edentatus*, and as discussed above, the southern expansion of this subspecies would suggest that increasing sea temperature does not affect distribution. It would appear that these 'obvious' candidates as indicators of climate change are not appropriate, at least for Scotland.

Fucus distichus (=*anceps*) is also included in the JNCC report (Burrows *et al*., 2009) as a climate indicator species and as a nationally scarce species likely to become extinct in the next 10 years if no action taken. The report also states that as *anceps* reaches the southern limit of its distribution in the British Isles, it will probably be very intolerant of increases in temperature. As this report refers to changes in the British Isles it is therefore relevant to *anceps* populations from Ireland. The *anceps* populations in Ireland are believed to be relics from a time when sea temperatures were much colder (Powell, 1957a) and therefore it can be assumed that they evolved over a long time period to survive at warmer temperatures. The Ireland population showed a temperature preference for 10°C in juveniles but no preference in adult plants and it may be that an increase in sea temperatures will have a detrimental effect on the early life stage and establishment of this population of *anceps*. The early stages of *Fucus* development and the processes influencing these early stages may not only affect the ecology of a species but also its evolution and role in communities (Vadas *et al*., 1992). It has been suggested (Breeman, 1990) that seaweeds are unable to form temperature ecotypes rapidly and adjust to the rapid environmental changes. Inbreeding, which is possible with monoecious species such as *anceps,* can cause a reduction in genetic variability and may result in low adaptability. The increasing rate of global warming and low adaptability of *anceps* combined with its geographical isolation at its southern limit in Ireland and limited suitable substrate for colonisation may mean that *anceps* in Ireland will become locally extinct.

In theory then it would appear that *anceps*, in Ireland at least, may indeed be a suitable candidate as an indicator of climate change. However in practice this may not be so easy to implement. Rocky shore species have long been known to provide excellent sentinels for detecting changes in biodiversity (Southward *et al.*, 1995). Rocky shores also provide for continued assessment and resurveys to explore changes in species communities and distributions. When choosing indicators, there will always be a level of judgement required and the choice of indicators reflect the knowledge of the experts present and driven by the current understanding of climate change impacts (Southward *et al.*, 2005). The role of indicators is to quantify and simplify phenomena and provide insight into complex realities. An indicator should be easy and/or cheap to measure. Ideally long-term time series of data should be used as these are essential to distinguish the signal of climate change from the noise of natural variability. In respect to population of *anceps* from Orkney, the site of investigation at Bay of Skaill has a good recorded history with extensive records from Traill in the 1880s and herbarium specimens at the Natural History Museum from the 1830s. Further results of field surveys undertaken in 1973 and 1998/9 (Wells *et al*., 2003) indicate no loss of species richness has occurred at this and other Orkney sites, suggesting stability in marine algal assemblages over the past two centuries despite increased inshore sea temperatures.

A suitable indicator species should be subject to a single pressure such as climate change rather than to a suite of other pressures i.e. exploitation or invasive species. The *anceps* population in Orkney is currently part of an investigation to determine any effects reduction in wave energy may have on the ecology of this and other wave exposure species (Want *et al*., 2010). The challenge here will be to discriminate between environmental disturbances arising from climate change and rising sea temperatures to those occurring in response to the removal of marine energy and may give an interesting insight into the dynamics on exposed shores. This is part of the large scale plan for wave and tidal energy in this area and may be another reason why this population of *anceps* is not appropriate as an indicator of climate change.

Evidence of observed changes can be biased towards areas that have had some form of biological monitoring in place over a consistently long period. In this investigation records for *anceps* in Orkney have been obtained either through personal observations, specific collection trips or as part of a larger survey. Indeed in this investigation collections from Orkney usually coincided with other research work and field trips. In

the case of the Ireland population in Co. Clare records are very limited. With the exception of Powell's work in 1957 and this present investigation no other recording has been done for this subspecies in Ireland and therefore the true extent of its abundance and distribution is unclear. To understand the effects rising sea temperatures may have on this population then a study over many years would be required. Unless a dedicated search is undertaken in the future it is difficult to see how this population will be monitored in relation to climate change.

The use of *anceps* as an indicator species is further hampered by the very nature of the shores on which they are found and the fact that correct identification of *anceps* from *linearis* and *nanus* **(Section 2.4.2)** relies on collection of samples. These shores are subject to extreme wave exposure and ocean swell and sites may at times be inaccessible. At the site in Orkney the platform on which *anceps* is established is given some protection, but only at low tide, from an offshore reef, however great care must still be taken. Location of populations from previous records is often difficult and time consuming. Indeed in this investigation location of the population at George's Head, Kilkee in Ireland, despite a good shore description from Powell (1957a), took three consecutive days to find due to bad weather, ocean swell and the size of the shore. At this time an attempt was made to locate populations recorded further south at Loop Head, however the huge sloping reefs combined with extreme ocean swell made this impossible.

Does change matter? Change is likely to matter most visibly if commercial species are adversely affected, harmful species such as toxic algae increase or, as in the case of *anceps*, a species of marine natural heritage importance that is nationally rare or scarce and declining in abundance (Hiscock *et al*., 2004; Burrows *et al*., 2009). The House of Lords Science and Technology Committee through its continuing commitment to the Convention on Biological Diversity (CBD) stated that halting the decline in biodiversity is a key international obligation (Systematics and Taxonomy: Follow-up, August 2008). Furthermore in a response to climate change the Government is committed to ensure environmental sustainability, which includes a significant reduction in the rate of biodiversity loss.

In conclusion, there does appear to be mounting evidence to support climate-induced impacts on species populations and distribution (Parmesan, 1996; Hughes, 2000;

McCarty, 2001). There are many factors which can affect the distribution of a species on both a global, regional and local scale, and these can be physical, anthropogenic and ecological processes. It is not simply the magnitude of change of global average temperature over the last century but the complex processes involved in systems that make responses to climate change difficult to predict (Elliot *et al*., 2008). For *Fucus* species such as *F. serratus, F. spiralis* and *F. vesiculosus* it seems unlikely that climate change will have any immediate effect on the distribution of these species. In theory the use of *F. distichus* subsp. *anceps* as an indicator of climate change and sea temperature rise in the British Isles seems appropriate for the population from Ireland at least, however from a practical point of view, added to the taxonomic confusion surrounding this species, this may be harder to implement.

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