Aspects of structure, growth and morphogenesis in a new filamentous red alga (Ceramiaceae, Rhodophyta).

by

Wendy Ann Stirk

Submitted in partial fulfilment of the
requirements for the degree of
Doctor of Philosophy

in

the Department of Botany,
University of Natal,
Pietermaritzburg.

1993

Declaration

The experimental work described in this thesis was carried out in the Department of Botany, University of Natal, Pietermaritzburg, under the supervision of Doctor M.E. Aken and Professor J. van Staden.

These studies represent original work by the author and have not been submitted in any form to another University. Where use was made of the work of others it is duly acknowledged in the text.

Wendy Ann Stirk
December 1993

Acknowledgements

I wish to extend my sincerest thanks to:

- Doctor M.E. Aken for his enthusiastic supervision of the work detailed in this thesis and for his encouragement shown at all times during the preparation of this dissertation.

- Professor J. van Staden for all his interest in my work and for taking on the task of being my supervisor. I would also like to thank him for the generous use of his equipment.

- Doctor R.P. Beckett for participating in my research meetings and for the many words of encouragement he gave me over the years.

- The Natal Sharks Board, especially Mr. Sheldon Dudley, for keeping me informed of any seaweed drifts occurring along the Natal south coast and for the seawater temperature data.

- Staff of the Electron Microscope Unit, University of Natal, Pietermaritzburg for showing me the "tricks of the trade".

- Mr. M.J.C. Hampton and other technical staff of the Department of Botany, University of Natal, for capable support.

- Members of the Science Workshop and the Electrical Workshop, University of Natal, Pietermaritzburg, for making some of the experiments possible.

- Ian Crouch and Sally Upfold for helping me with the Mung Bean Bioassay and HPLC separation of polyamines, respectively.

- Gail Smerdon for correcting my grammatical errors.

- My friends for showing an interest in my research and for all their encouragement.

- The FRD for their generous financial support during my studies.

Abstract

Pteroceramium, a descriptive name given to an undescribed winged species closely related to *Ceramium*, has uniaxial filamentous thallus construction with pseudodichotomous branching. Alternate branches become dominant. This pattern of growth is referred to as cellulosympodial growth. All growth is from an apical cell which cuts off subapical cells. The subapical cells develop into axial cells. Each axial cell cuts off six pericentral cells in a ring around its apical pole. The pericentral cells divide further to form the cortical band. Pc1 always forms on the outer face of the thallus as determined by the preceding pseudodichotomy and gives rise to the larger outer wing which is a lateral expansion of the cortical band. The smaller inner wing forms from Pc6 on the inner face. The other pericentral cells give rise apically to uniseriate spines. The pericentral cells also give rise to rhizoids and adventitious lateral branches.

Each axial cell has a large central vacuole with a few peripheral chloroplasts, mitochondria and floridean starch granules. The smaller wing cells have a much denser cytoplasm with fewer small vacuoles, many chloroplasts which are more closely packed together and more floridean starch granules than axial cells. Chloroplasts have a typical Rhodophyta ultrastructure with single, evenly spaced thylakoids with phycobilisomes. Pit connections have a plug core but no plug cap.

Pteroceramium has a typical *Polysiphonia*-type triphasic life history. The carposporophyte is naked and tetraspores are produced in a characteristic decussate cruciate arrangement.

The effect of a number of physical and chemical factors on growth and morphogenesis was investigated. *Pteroceramium* grew best at irradiance levels between 79 μmol m^{-2} s^{-1} and 129 μmol m^{-2} s^{-1} with growth being limited at 30 μmol m^{-2} s^{-1}. The largest axial cells and wings were obtained from the material grown at 79 μmol m^{-2} s^{-1} and the smallest measurements for material grown at 129 μmol m^{-2} s^{-1}. Monochromatic light fields of red, green and blue caused reduced growth rates compared to the control replicates grown in a white light from both incandescent and fluorescent lights. Light

quality had no effect on morphogenesis. The critical daylength for maximum rates of cell elongation was 10 hours or longer, although 16 hours light caused a decrease in final axial cell volume. Optimum temperatures for growth of *Pteroceramium* were between 20°C and 25°C with growth decreasing at 15°C and 30°C. Axial cell volume was reduced and wing size was stunted at these two extreme temperatures tested. Scouring by sand caused axial cells to decrease in volume although the wings were unaffected. Smothering by sand did not prevent growth although axial cells and wings were greatly decreased in size, with the wings consisting of only one or two other cells. Tumbling to disrupt gravity did not affect the angle of each pseudodichotomy. Decreased levels of nitrogen and phosphorus limited growth but had little effect on axial cell volume and wing development. *Pteroceramium* was stenohaline with maximum growth at 34‰ and reduced growth at 30‰ and 40‰. *Pteroceramium* grew best at pH 7.5 and pH 8.5 with decreased growth at pH 6.5 and pH 5.5. The various pHs tested had little effect on morphogenesis.

The best photosynthetic responses were obtained from material preconditioned at 80 μmol m^{-2} s^{-1} compared with that at 30 μmol m^{-2} s^{-1} and 150 μmol m^{-2} s^{-1}. There was a decrease in pigment content with increasing irradiance at which the alga was grown. Phycoerythrin was the dominant pigment. Exposure to a high irradiance (3000 μmol m^{-2} s^{-1}) for 30 minutes or longer inhibited photosynthesis. Plants did not fully recover even 24 hours later, indicating that this damage was permanent. *Pteroceramium* was able to acclimatize slowly over a week to temperature changes within the range of 15°C to 25°C. Rapid increases of 5°C within this temperature range increased photosynthetic performance and a rapid drop of 5°C decreased photosynthetic performance. However, a 10°C increase or drop reduced *Pteroceramium*'s photosynthetic performance. Photosynthetic rates were decreased in alkaline conditions and increased in acidic conditions.

Pteroceramium has well defined developmental patterns with basal band growth of axial cells and tip growth in the rhizoids. The pericentral cells are formed in a set sequence similar to *Ceramium* species with Pc1 forming on the outer face, Pc2 and Pc3 forming on the lower and upper surface nearest to Pc1 respectively, Pc4 and Pc5 forming on

the lower and upper surface respectively farthest from Pc1, and Pc6 forming on the inner face. This sequence is unaffected by the direction of illumination or gravity.

Exogenous application of plant hormones (IAA, GA_3 and kinetin) in the concentration range of 10^{-9} M to 10^{-5} M had no effect on growth and morphogenesis in *Pteroceramium*. Application of polyamines and their precursors caused a decrease in growth and a reduction in cell size at concentrations higher than 10^{-4} M. Polyamine inhibitors caused a reduction in growth and cell size at concentrations higher than 10^{-5} M. Arginine increased growth at concentrations 10^{-5} M and 10^{-6} M. High power liquid chromatography (HPLC) separation of *Pteroceramium* extracts indicated that spermidine was present in *Pteroceramium* at approximately 38 μg spermidine g^{-1} fresh weight. The apical tip exerts an apical dominance effect on the subordinate branches, suppressing their elongation. Removal of the dominant apical tip increased adventitious branch formation. This effect was not reversed by application of exogenous IAA at concentrations of 10^{-9} M to 10^{-4} M.

Table of contents

List of figures

Chapter 1

Chapter 3

Chapter 4

Chapter 5

Chapter 6

List of tables

Chapter 1

Chapter 4

Chapter 5

Chapter 6

List of plates

Chapter 2

Chapter 3

Chapter 6

Chapter 1

Introduction

1.1 The experimental organism - a note regarding nomenclature

The experimental organism under investigation in this work has been referred to by the descriptive name *Pteroceramium*, meaning "winged *Ceramium*". This name has been used for convenience in preference to *gen. nov.* throughout the text. The alga belongs to a new monotypic genus that is being described elsewhere. The alga has previously been referred to as Ceramieae indet. (STEGENGA and BOLTON, 1992; STIRK and AKEN, 1993). A voucher specimen of the clonal material used in this study has been deposited in the University of Natal Herbarium (NU 10678). The formal description done elsewhere (AKEN and NORRIS, in prep.) will cite this voucher specimen and the published name will have priority.

1.2 The Rhodophyta

Rhodophyta are a monophyletic taxon of eukaryotes (GABRIELSON, GARBARY and SCAGEL, 1985) showing greater diversity in form, life history and reproductive strategies than any other algal division. There are approximately 4100 species in 675 genera, found mainly in temperate and tropical marine waters (LEE, 1980; KRAFT, 1981).

Rhodophyta are characterised by:

1) phycobiliproteins as accessory pigments organized in phycobilisomes attached to thylakoids;

2) chloroplasts with unstacked, single thylakoids;

3) absence of flagella and vestigial basal bodies (centrioles) at any stage in their life history;

4) chlorophyll *a* with chlorophyll *d* as a minor component; and

5) floridean starch (α(1,4)-linked glucans) as a storage product found in the cytoplasm (GABRIELSON, GARBARY and SCAGEL, 1985).

This range of diversity suggests an ancient lineage (PUESCHEL, 1990) although there is a poor fossil record. The most extensive fossil records are found from the Cretaceous period (160 million years ago) onwards. Most of these fossils are calcareous, referable to the Corallinaceae with the most significant deposits being found in tropical seas as coral reefs (DIXON, 1973).

The nature of the ancestral red alga is unknown. Rhodophyta are taxonomically distant from other algal divisions owing to the presence of phycobiliprotein pigments and lack of flagella and basal bodies. This may indicate early divergence from a common eukaryotic ancestor (BRAWLEY and WETHERBEE, 1981). Another possible theory is that the Rhodophyta are derived from blue-green algae as both have similar floridean starch, phycobiliprotein pigments and absence of flagella. The strongest argument against this theory is the difference in eukaryotic and prokaryotic states (DIXON, 1973). Another proposal is that Rhodophyta are a sister group to higher fungi and share a common ancestor independent of all other eukaryotes. This relationship is based on Rhodophyta and fungi both having similar life histories, plugged pits and absence of flagella. However, this proposal is not supported by ultrastructural studies (GABRIELSON, GARBARY and SCAGEL, 1985).

1.3 Classification of the Rhodophyta

Historically, the Rhodophyta have been subdivided into two subclasses with the less advanced Bangiophycidae lacking pit connections, apical growth and sexual reproduction, and the more advanced Florideophycidae characterized by pit connections, apical growth and sexual reproduction. However, some Bangiophycidae have been found to have apical growth; e.g. *Conchocelis* stage of *Porphyra* in the Bangiales, sexual reproduction, and pit connections; e.g. Rhodochaetales and Bangiales. Likewise, some Florideophycidae have intercalary growth; e.g. Delesseriaceae and Corallinaceae, and lack a triphasic life history.

It has recently been proposed to drop the subclasses and place all the orders in a single class, the Rhodophyceae (LEE, 1980). This proposal is supported by a cladistics study involving 35 different characters (GABRIELSON and GARBARY, 1987). Characters chosen were mainly reproductive, although morphological, ultrastructural and biochemical characters were also considered. The subclass Bangiophycidae was shown to be paraphyletic (a group that includes a common ancestor and some but not all of its descendants). Also, no synapomorphies (shared derived characters) were found to unite the Bangiophycidae into a monophyletic group. The Florideophycidae were shown to be monophyletic with synapomorphic characters being the formation of tetrasporangia and gonimoblast filaments. This classification with a single class, the Rhodophyceae, will be followed in this study (Table 1.1).

There is disagreement over the most primitive Rhodophyta order. The most common suggestion is an Acrochaetiaceae species with early divergence of the former Bangiophycidae orders, implying a reduction from, rather than ancestor to, the more complex orders (KRAFT, 1981). The order Acrochaetiales is characterized by extreme simplicity of the thallus with the absence of a differentiated carpogonial branch. Carpogonia are either sessile on the side of a vegetative branch, intercalary or terminal (PAPENFUSS, 1966).

It is generally agreed that the Ceramiales "represent a sort of culmination or end point of specialization" (KRAFT, 1981) and are the most clearly defined order. The Ceramiales are considered a monophyletic order, exhibiting unique bipolar spore germination with highly derived characters. The Ceramiaceae is the most primitive family in the order with the other three families being derived from a ceramiacean ancestor (GARBARY and GABRIELSON, 1990).

Table 1.1 Classification of the Rhodophyta followed in this study highlighting the position of *Pteroceramium*. Former Bangiophycidae orders are shown by *.

Division	Class	Order	Family
Rhodophyta	**Rhodophyceae**	Porphyridiales*	
		Erythropeltidales*	
		Rhodochaetales*	
		Compsopogonales*	
		Bangiales*	
		Acrochaetiales	
		Nemaliales	
		Palmariales	
		Corallinales	
		Batrachospermales	
		Hildenbrandiales	
		Gelidiales	
		Gracilariales	
		Bonnemaisoniales	
		Gigartinales	
		Rhodymeniales	
		Ceramiales	**Ceramiaceae**
			Delesseriaceae
			Rhodomelaceae
			Dasyaceae

1.4 Morphology of *Ceramium* Roth

Most *Ceramium* species are small delicate plants with their diagnostic feature being the pattern of cortical cell development (WOMERSLEY, 1978). The erect or prostrate thallus has uniaxial construction and is pseudodichotomously or irregularly branched (SIMONS, 1966). The thallus is differentiated into axial and cortical cells. Axial cells are large (up to 1.5 mm in length), ovoid to cylindrical in shape and are hyaline or faintly pigmented (DIXON, 1960a). The cortex is an aggregation of filaments of limited growth which may partially or totally cover the axial cells (DIXON, 1960a; SIMONS, 1966).

Filaments of unlimited growth arise from transverse divisions of a principal apical cell. The subsequent subapical cell divides further, giving rise to 3-10 pericentral cells, depending on the species (WOMERSLEY, 1978). The remaining mother cell enlarges to form an axial cell (DIXON, 1960a). This enlargement is an important factor contributing to longitudinal growth and is correlated with development of large vacuoles. When mature, an axial cell has a peripheral layer of cytoplasm with a few chromophores. There may also be a central strand of cytoplasmic material running between pit connections. This strand contains a nucleus but eventually both the strand and nucleus disintegrate (DIXON, 1960a).

Pericentral cells form in a definite sequence with a fixed orientation. The first formed pericentral cell (Pc1) is cut off on the outer face of each axial cell as determined by the previous pseudodichotomy. The remaining pericentral cells are cut off successively in pairs in an alternating sequence to form a ring at the apical pole of each axial cell. The orientation of other structures; e.g. spines and hairs, depend on the orientation of the pericentral cells (DIXON, 1960a).

Lateral branches of limited growth are formed when each pericentral cell divides to produce four apical cells - two directed acropetally and two basipetally. These cells continue to divide, producing successively smaller cells. Usually acropetal development is more extensive than basipetal development (WOMERSLEY, 1978). These lateral branches of limited growth form an encircling band appressed to the axial cell (DIXON, 1960a).

There are two types of lateral branches of unlimited growth:

1) in the apical region, lateral branches of unlimited growth form in a pseudodichotomous manner. The apical cell divides along the two inclined walls, cutting off two new apical cells. The first is formed on the inner face and the second on the outer face. These two new apical cells divide transversely a number of times before the next pseudodichotomy. Growth is usually unequal between the dominant and subordinate branches (DIXON, 1960a) with the dominant branches forming the main axis. There are usually 6-10 axial cells for each branch between pseudodichotomies (DIXON, 1960b). This branching pattern can be distorted by profuse adventitious branching (WOMERSLEY, 1978); and

2) in mature parts of the thallus, lateral branches of unlimited growth form by the transformation of an apical cell of a lateral branch of limited growth and are called adventitious branches (DIXON, 1960a).

1.5 Ultrastructure of the Rhodophyta

There is a very distinctive absence of flagella and centrioles.

The cell wall is composed of cellulose microfibrils in an electron transparent matrix. The inner layer consists of randomly orientated microfibrils and the outer layer has a parallel arrangement of microfibrils. The cell wall is surrounded by a thick, protein-rich mucilage layer which contributes to thallus integrity (PUESCHEL, 1990).

The presence of pit connections is correlated with apical growth and a fundamental filamentous construction (PUESCHEL and COLE, 1982). Pit connections are formed by centripetal ingrowth of a septum being halted short of completion, resulting in a plasmalemma-lined aperture. Pit connections between daughter cells are termed primary pit connections while non-kindred cells have ultrastructurally similar connections termed secondary pit connections. A common constituent in all pit connections is a plug core which is a homogenously granular mass of protein occluding the aperture. There have been no successful attempts of enzymatic digestion of the plug core, suggesting that these proteins are so highly cross-linked that they are resistant to digestion (TRICK and PUESCHEL, 1990). Some orders have one or two electron opaque layers (plug caps) covering the plug core. The outer cap layer is composed of glycoproteins, taking up carbohydrate specific stains and protein specific stains and being digested by proteolytic enzymes (RASCIO, MARIAIN, DALLA VECCHIA and TREVISAN, 1991a; TRICK and PUESCHEL, 1991). The outer cap layer varies from dome-shaped in the Corallinales and Batrachospermales to plate-like in the Nemaliales and Palmariales (TRICK and PUESCHEL, 1991). There is also a cap membrane on each side of the plug core. The structure and organization of pit connections is considered taxonomically significant, especially at familial and ordinal levels (Table 1.2; PUESCHEL and COLE, 1982). There is no difference in the ultrastructure of pit connections between heteromorphic life history stages, increasing the taxonomic importance of this feature (PUESCHEL, 1989). Ceramiales have pit plugs covered by a cap membrane and a consistent absence of plug caps (PUESCHEL and COLE, 1982).

The proposed function of the pit connections is symplastic communication controlled by a membranous barrier (PUESCHEL, 1990). Secondary pit connections are common in red algae; e.g. *Hildenbrandia* crustose species may have up to eight secondary pit connections per cell. The suggested function for these is improved coherence between vertical filaments, increasing thallus toughness (PUESCHEL, 1988).

Chloroplasts have a double unit membrane (BRAWLEY and WETHERBEE, 1981). They vary in shape from stellate to discoid, ramifying to highly lobed. Unstacked photosynthetic membranes (thylakoids) are evenly spaced throughout the stroma. Water soluble phycobiliprotein pigments form electron dense granules termed phycobilisomes which are attached to the outer thylakoid surface. Phycobilisomes have a diameter of approximately 35 nm (DUCKETT and PEEL, 1978). The chloroplast is usually encircled by a single peripheral thylakoid which may be closed or open. Variations on this are several peripheral thylakoids or a total absence as in the lower Rhodophyceae orders. This is thought to be a derived character (SCOTT and BROADWATER, 1989). A genophore is an electron

Table 1.2 Ordinal distribution of pit plug features in the Rhodophyceae (PUESCHEL, 1990)

Order	No. of cap layers	Cap membrane
Rhodochaetales	0	-
Compsopogonales	0	-
Bangiales	1	-
Acrochaetiales	2	+
Nemaliales	2	+
Palmariales	2	+
Corallinales	2	-
Batrachospermales	2	?
Hildenbrandiales	1	+
Gelidiales	1	+
Gracilariales	0	+
Bonnemaisoniales	0	+
Gigartinales	0	+
Rhodymeniales	0	+
Ceramiales	0	+

transparent region within the stroma composed of DNA (PUESCHEL, 1990). There may also be electron dense "globules" in the stroma which could possibly be involved in lipid synthesis and storage (BRAWLEY and WETHERBEE, 1981). Floridean starch is deposited free in the cytoplasm compared to higher plants and green algae where amylopectin is deposited in the chloroplast. When viewed with a scanning electron microscope, the floridean starch granules have concentric layering and a radiating fibrillar substructure (PUESCHEL, 1990).

Mitochondria are similar to those found in higher plants, consisting of a double unit membrane with an infolding of the inner membrane to form cristae (PUESCHEL, 1990). The electron transparent region has DNA fibrils (BRAWLEY and WETHERBEE, 1981).

Vacuoles are generally large with a single membrane and are most prominent in mature cells. Their main role is in osmotic regulation (PUESCHEL, 1990).

Golgi apparatus are most common in vegetative cells, especially during sporogenesis. There are 4-15 stacked cisternae. The forming face (CIS), as in most eukaryotes, is associated with endoplasmic reticulum. However, in the Rhodophyta, the golgi apparatus is often associated with mitochondria, possibly facilitating energy transfer. Endoplasmic reticulum may either be rough or smooth. It is usually found in the cell periphery and around the nucleus, often increasing during mitosis and meiosis (OATES and COLE, 1989; PUESCHEL, 1990).

The nucleus is typically eukaryotic consisting of a nuclear envelope with nuclear pores and a nucleolus. During interphase the nucleus is generally small (3-5 μm). Multinuclearity is common, arising from cell fusion or uncoupling of karyokinesis and cytokinesis (PUESCHEL, 1990).

Microbodies have a granular content and a single unit membrane. In other plant groups, microbodies contain a number of enzymes. Their activity in the red algae is uncertain (PUESCHEL, 1990).

1.6 Reproduction in the Rhodophyta

The basis for the ordinal classification in the Rhodophyceae is usually the pre- and post-fertilization development in the female reproductive structures (GUIRY, 1978). Post-fertilization varies in different genera but is constant for each species (DIXON, 1973; BOLD and WYNNE, 1978).

There is great diversity in Rhodophyta reproductive strategies with both sexual and asexual stages being found. There are three basic algal life history patterns:

1) monophasic, usually with only asexual stages; e.g. *Porphyridium* species;

2) biphasic, with alternation of phases between gametophyte and sporophyte generations. This is common in the primitive Rhodophyceae orders; and

3) triphasic, found in the more advanced Rhodophyceae orders, including the Ceramiales (ANG and DE WREEDE, 1990).

Sexual organ differentiation patterns are highly conserved with all spore types showing similar ultrastructural characteristics to male gametes (HOMMERSAND and FREDERICQ, 1990). In the Rhodophyta all gametes and spores are non-motile, a condition found elsewhere only in the Prokaryotes, Cyanophyta and Prochlorophyta (GUIRY, 1990). Three patterns of sexual organ development can be distinguished in the Rhodophyta, each being diagnostic for a distinct evolutionary line:

1) intercalary reproductive cells with spermatia and spores being cut off from a continuously dividing mother cell. This is characteristic of the orders Rhodochaetales, Compsopogonales and Erythropeltidales;

2) spermatia and carpospores formed in packets by successive perpendicular divisions of a mother cell. Conchospores are generated in fertile rows and monospores are formed terminally in the filamentous *Conchocelis* stage. This is diagnostic of the other primitive Rhodophyceae orders; and

3) spermatia and all other spores produced through transformation of apical initials. This is characteristic of the more advanced orders (HOMMERSAND and FREDERICQ, 1990).

Facultative asexual reproduction is important as an alternative reproductive strategy (HAWKES, 1990). Examples of mitospores involved in asexual reproduction include:

1) parasporangia that produce more than four spores but are not homologous with tetrasporangia as meiosis does not occur (BOLD and WYNNE, 1978) and they do not replace the tetrasporangia (DIXON, 1973). They form by cellular proliferation and separation and can be initiated by a single terminal cell. In *Plumaria elegans* (Bonnemaison) Schmitz there are 6-7 paraspores per parasporangium while in some *Ceramium* species, several hundred paraspores have been noted (DIXON, 1973); and

2) monosporangia produce single uninucleate spores by mitosis. They occur either on haploid or diploid plants (DIXON, 1973; GUIRY, 1978) and repeat the parental phase. They can be borne individually, in groups or in chains (seirosporangia). This is a common method of reproduction in the more primitive orders that lack a triphasic life history (MAGGS, 1988).

Parthenogenesis may also occur. This happens when gametes do not undergo sexual union but develop into gametophyte plants (BOLD and WYNNE, 1978).

1.6.1 Generalized triphasic life history

Uninucleate spermatangial mother cells, derived from specialized cortical cells, each produce 2-5 spermatangia which are cut off subterminally from the mother cell. Each spermatangium produces a single non-motile spermatium (HOMMERSAND and FREDERICQ, 1990). Most spermatia are small, spherical to ovoid in shape, lack plastids but have 1-2 nuclei, a few mitochondria, dictyosomes, ribosomes and endoplasmic reticulum (BROADWATER, SCOTT and WEST, 1991). They lack a cell wall but are surrounded by a thick mucilaginous coat (BOLD and WYNNE, 1978; LEE, 1980).

Spermatia have pronounced polar orientation with the nucleus in the apical portion and vacuoles, filled with striated fibrous material, in the basal portion (DIXON, 1973; LEE, 1980). The gelatinisation of the spermatangial wall near the apex and the concurrent release of fibrous material from the vacuole cause a swelling that pushes out the spermatium. Spermatia are carried passively through the water column (DIXON, 1973).

Carpogonia (haploid female reproductive structures) develop from an apical cell of a lateral or terminal filament which ceases to grow (DIXON, 1973, HOMMERSAND and FREDERICQ, 1990). Carpogonia may be sessile or borne on a short 3-4 celled carpogonial branch which can arise laterally, as a secondary filament produced by an adventitious initial or terminally, by transformation of an apical cell (HOMMERSAND and FREDERICQ, 1990). In the more advanced orders, carpogonial branch cells are often different in size and shape from the surrounding vegetative cells and lack chloroplasts (DIXON, 1973).

Carpogonia consist of an inflated basal portion and a narrow gelatinous elongated tip, the trichogyne (DIXON, 1973; LEE, 1980). Trichogynes vary in size; e.g. in Gigartinales the trichogyne is a short 5 μm cylinder while in Ceramiales it is up to 100 μm in length. However, the relative volumes of the trichogyne and basal portions are usually similar. Trichogynes are the receptive sites for spermatia, with contact usually occurring near the distal rather than the proximal end of the trichogyne (DIXON, 1973). Attachment appears to require a binding substance secreted by exocytosis from the tip of the trichogyne (HOMMERSAND and FREDERICQ, 1990). In *Aglaothamnion neglectum* Feldmann-Mazoyer the spermatia are not sticky as they do not bind to each other or to the male gametophyte. They only adhere to a trichogyne or hairs found on the female gametophyte. Water movement causes the hairs and trichogyne to come into contact, transferring spermatia to the trichogyne. This increases the probability of successful fertilization. Binding onto the trichogyne is not species specific but can occur between closely related species although there is no fertilization (MAGRUDER, 1984).

The carpogonium has two nuclei. One, located in the trichogyne, degenerates soon after the carpogonium has matured. The other nucleus is located in the basal portion and functions as the female gamete or egg nucleus (DIXON, 1973; LEE, 1980). On contact, the walls of both the spermatium and trichogyne dissolve by enzymatic digestion (HOMMERSAND and FREDERICQ, 1990), allowing the male nucleus to migrate into the carpogonium and to fuse with the egg nucleus (LEE, 1980). The trichogyne has a narrow base which admits only a single nucleus. The carpogonial vacuole contracts immediately after a male nucleus has entered the basal portion of the carpogonium, drawing it into contact with the egg nucleus and pinching off the trichogyne cytoplasm. This separates the trichogyne from the basal portion, effectively preventing supernumary fertilization (HOMMERSAND and FREDERICQ, 1990).

The zygote remains attached and dependent on the carpogonium, undergoing complex post-fertilization development to form the carposporophyte. The carposporophyte has a number of gonimoblast filaments, each with a terminal carposporangium producing a single carpospore. The carpogonium may either directly (more primitive orders) or indirectly (more advanced orders) produce the carposporophyte. For direct development, the carpogonium divides to produce a mass of cells making up the carposporophyte. For indirect development, the carposporophyte is derived from one or more auxillary cells. In the Ceramiales and Rhodymeniales, the carpogonium produces connecting cells which fuse with the auxillary cells. Connecting cells are minute and uninucleate with minimal amounts of cytoplasm. Either the gonimoblast filaments may be produced from the connecting cells in proximity with an auxillary cell or the zygote nucleus is transferred to an auxillary cell which then gives rise to the gonimoblast filaments (HOMMERSAND and FREDERICQ, 1990).

Three types of connecting cells have been identified:

1) those not involved in carposporophyte development but which are rather a vestigial system of rapidly degenerating cells; e.g. *Faucheocolax attenuata* Setchell;

2) those which form when the auxillary cell which initiated carposporophyte development, degenerates; e.g. *Plocamiocolax pulvinata* Setchell and *Ceramium strictum* Greville *et* Harvey; and

3) those that continue to be active for longer periods of time within the developing carposporophyte; e.g. *Gracilaria verrucosa* (Hudson) Papenfuss and *Nemalion helminthoides* (Velley) Batters (DELIVOPOULOS, 1990).

If the carposporophyte is surrounded by haploid tissue, it is termed a cystocarp (DIXON, 1973). The cystocarp is divided into three tissue types:

1) an outer gametophytic photosynthetic tissue layer consisting of unmodified vegetative cells and secondary photosynthetic cells. There may be a cuticle;

2) an inner non-photosynthetic gametophytic tissue layer in close contact with the developing carposporophyte. These cells have a high protein content and enlarged nuclei; and

3) the developing carposporophyte with connecting filaments or cells, auxillary cells and gonimoblast filaments (HOMMERSAND and FREDERICQ, 1990).

Non-motile carpospores are released by rupturing of the carposporangial wall and germinate into a free-living diploid phase, the tetrasporophyte. The tetrasporophyte produces tetrasporangia, usually on filaments of limited growth which may be partially or totally embedded within an aggregation of filaments. Each tetrasporangium gives rise to four haploid, non-motile tetraspores by meiosis. At first, the tetraspore primordia are relatively small and uninucleate but increase rapidly in size while the first meiotic division is in progress (DIXON, 1973). Each tetraspore has a prominent golgi apparatus with associated mitochondria (LEE, 1980).

Tetraspore arrangement is a useful taxonomic criterion (GUIRY, 1978). There are three basic spore arrangements:

1) cruciate tetrasporangia usually have a median division along their short axis. Orientation of the other two cleavages along the long axis is more irregular, being either in a single plane or in different planes with the degree of

divergence varying through 180°. Most commonly, the two longitudinal cleavages are at 90° to each other (decussate cruciate). There are many variations to the cruciate pattern; e.g. oblique division of cleavage along the longitudinal axis. Successive cleavage is more common than simultaneous cleavage (GUIRY, 1990). In successive cleavage, the first transverse cytoplasmic cleavage occurs before the second meiotic nuclear division (Fig. 1.1A and B). Cruciate tetrasporangia have been reported in all orders having a triphasic life history but appear to be the most primitive sporangial type (GUIRY, 1978);

2) zonate tetrasporangia most commonly have successive cleavage with initial division being median followed by two other roughly parallel divisions. The nuclei divide once before transverse cleavage and again after it has occurred (Fig. 1.1C). Simultaneous cleavage found only in the Corallinales (GUIRY, 1978 and 1990) establishes a quadrinucleate condition prior to the first cytoplasmic cleavage (Fig. 1.1D); and

3) tetrahedral tetrasporangia are the most common form of tetraspores and are thought to be derived from cruciate tetrasporangia (GUIRY, 1990). There is mainly simultaneous cleavage with the second nuclear division occurring prior to the correlated development of the invagination furrows although successive cleavage does also occurs (Fig. 1.1E and F).

After cleavage, a new cell wall is laid down within the existing cell wall. A third cell wall is then laid down around each spore. Fibrous vesicles are present within the spores although not in as high a concentration as in the spermatangia (DIXON, 1973).

The non-motile tetraspores are released, either by splitting or breakdown of the wall, and develop into the gametophyte generation. The tetrasporangium mother cell may develop into a new tetrasporangium (DIXON, 1973). Usually there is a single period of spore discharge over a 24 hour period which may correspond to spring tide. This would aid spore dispersal by water movement (NGAN and PRICE,

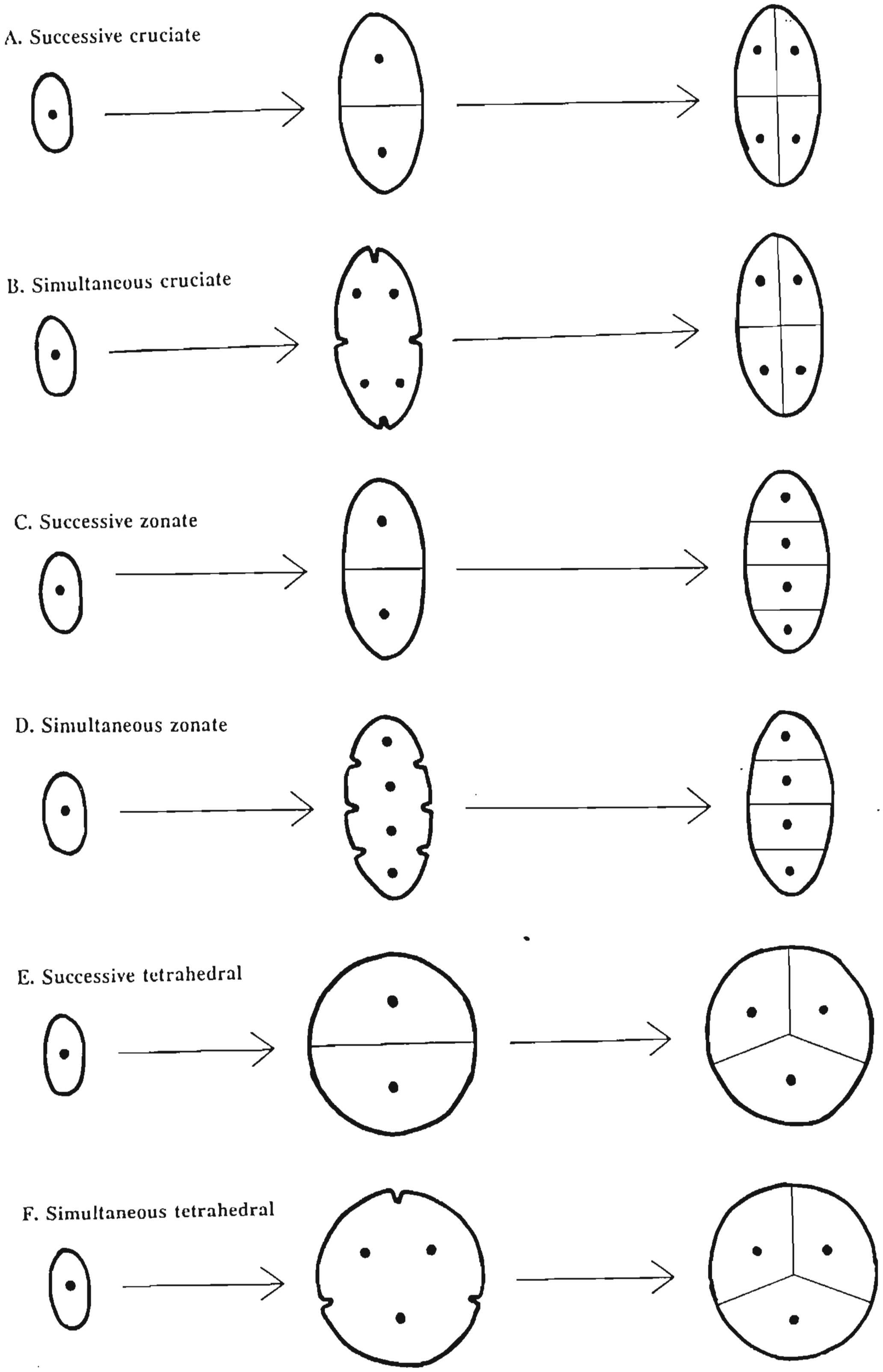


Fig. 1.1 Different cleavage types found in the tetrasporangia of the Rhodophyta. Nuclei are shown as dark spheres. A) Successive cleavage of a cruciate tetrasporangium. B) Simultaneous cleavage of a cruciate tetrasporangium. C) Successive cleavage of a zonate tetrasporangium. D) Simultaneous cleavage of a zonate tetrasporangium. E) Successive cleavage of a tetrahedral tetrasporangium. F) Simultaneous cleavage of a tetrahedral tetrasporangium (modified from GUIRY, 1978).

1983). Both carpospores and tetraspores have a greater density than seawater and therefore sink fairly rapidly to the substratum. Initial attachment is by mucilage (CHAMBERLAIN and EVANS, 1973).

1.6.2 Variations to the triphasic life history

There are five basic variations to the triphasic life history:

1) the *Polysiphonia*-type life history (Fig. 1.2A) was first described by YAMANOUCHI (1906) for *Polysiphonia flexicaulis* (as *Polysiphonia violacea* Greville). This is the most common form of triphasic life history, consisting of a sequence of gametangial, carposporangial and tetrasporangial phases. Gametangial and tetrasporangial phases are isomorphic with the carposporophyte developing on the female gametophyte (DIXON, 1973 and 1982);

2) the *Bonnemaisonia hamifera* Hariot-type life history (Fig. 1.2B) is similar to the *Polysiphonia*-type life history except that the gametangial and tetrasporangial phases are heteromorphic (DIXON, 1973 and 1982). This type of life history is most common in the Nemaliales (MARTIN, 1969);

3) the *Lemanea*-type life history (Fig. 1.2C) consists of a sequence of heteromorphic gametangial and carposporangial phases. The carpogonial phase develops on the gametangial phase with meiosis occurring somatically during the development of the carpogonium. This type of life history is only established for *Lemanea* and *Batrachospermum* (DIXON, 1973 and 1982);

4) the *Liagora tetrasporifera* (Børgesen)-type life history (Fig. 1.2D) consists of a sequence of heteromorphic gametangial and tetrasporangial phases with the tetrasporangial phase developing on the gametangial phase and meiosis occurring in the tetrasporangia (DIXON, 1973 and 1982). Examples of this type of life history are found in other *Liagora* species and *Helminthocladia*

hudsonii J.Agardh (as *Helminthocladia agardhiana* Dixon). All these examples have cruciate tetrasporangia (MARTIN, 1969); and

5) the *Palmaria*-type life history (Fig. 1.2E) consists of two gametangial phases with one phase being small (carpogonium) and the other large (spermatangia). The tetrasporangial phase is isomorphic with the larger gametangial phase. Examples of this type of life history are found in *Palmaria palmata* (Linnaeus) Stackhouse, *Halosaccion ramentaceum* (Linnaeus) J.Agardh and *Rhodophysema elegans* (Crouan *et* Crouan) Batters; DIXON, 1982).

There are many other intraspecific variations to the five life history types. Development of contrasting reproductive structures on the same individual which typically occur on separate plants is called "mixed phases". This is a common phenomenon in the Ceramiales. For example, *Polysiphonia harlandii* Harvey had tetrasporangia and cystocarps on the same thallus but the viability and genetic status of these spores is unknown (CHEUNG, LEE and HODGKISS, 1984). *Polysiphonia urceolata* (Dillwyn) Greville had perfectly formed carposporophytes and tetrasporangia on a single thallus (EDELSTEIN and McLACHLAN, 1967; LAWSON and RUSSELL, 1967). *Callithamnion tetragonum* (Withering) S.F.Gray had tetrasporophytes bearing gametangia in addition to normal tetrasporangia (RUENESS and RUENESS, 1985). *Callithamnion baileyi* Harvey produced spermatangia or procarps on tetrasporophytes (WHITTICK and WEST, 1979). *Aglaothamnion diaphanum* L'Hardy-Halos *et* Maggs produced bisporangia and spermatangia on the same thallus with the bisporangia developing into bisporophyte plants (L'HARDY-HALOS and MAGGS, 1991). *Campylaephora crassa* (Okamura) Nakamura showed a *Polysiphonia*-type life history but parasporangial plants with 17-20 spores (mitotic diploids) are also found. These parasporangial plants later developed into normal tetrasporophyte plants (BOO, FREDRIKSEN, RUENESS and LEE, 1991).

Although tetraspores are the most common type of meiospore, there are other spore types:

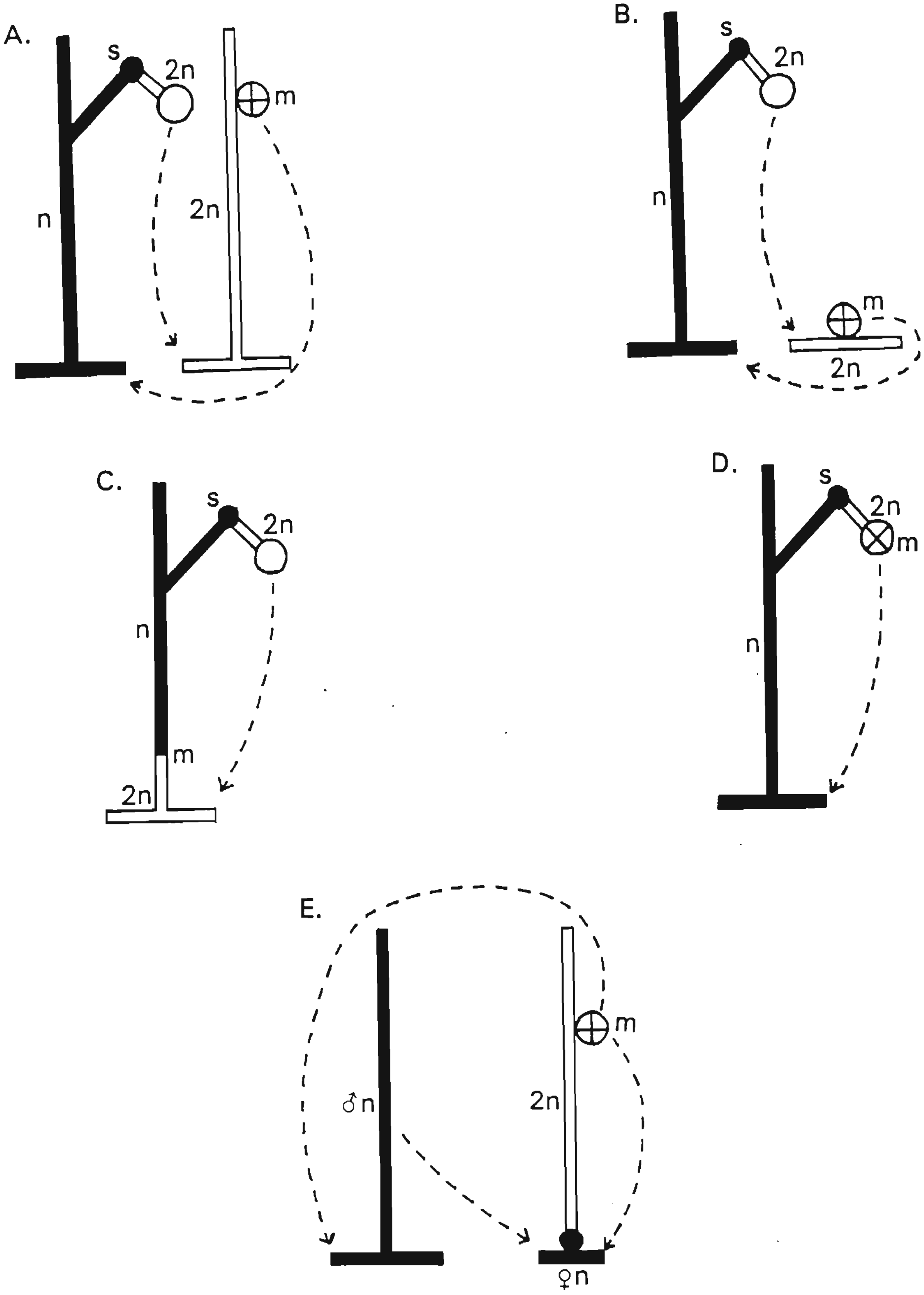


Fig. 1.2 Five variations of the triphasic life history pattern currently established for the Rhodophyceae. A) *Polysiphonia*-type life history. B) *Bonnemaisonia hamifera*-type life history. C) *Lemanea*-type life history. D) *Liagora tetrasporifera*-type life history. E) *Palmaria*-type life history. m = position of meiosis, s = position of syngamy, n = haploid, 2n = diploid, ♂ = male and ♀ = female (DIXON, 1982).

1) polysporangia which produce more than four spores, although usually in multiples of four (GUIRY, 1978). Polysporangia are defined as "sporangia which are initially multinucleate, undergo meiosis to multiply the number of nuclei by four and then cleave to form a cluster of uninucleate spores" (MAGGS, 1988). Polyspores are considered homologous with tetraspores as meiosis occurs during spore formation (DIXON, 1973). As polysporangia occur only in families which have simultaneously dividing tetrahedral tetrasporangia, it is thought that polysporangia are derived from these (GUIRY, 1978 and 1990). In most cases, polysporangia occur in place of tetrasporangia although examples where both occur on the same plant are known; e.g. *Chylocladia reflexa* (Meneghini *ex* Zanardini) Kützing and *Gastroclonium coulteri* (Harvey) Kylin. Polysporangia occur predominantly in the more advanced orders, being recorded in 13 genera of the Ceramiaceae (GUIRY, 1990);

2) bisporangia which produce two bispores are common in the Corallinaceae (MAGGS, 1988) and Ceramiaceae (DIXON, 1973). Bisporangia may occur alone or mixed with cruciate or zonate tetrasporangia. Bispores are thought to be homologous with the tetraspores as they are not found on gametangial or carpogonial plants (Dixon, 1973; GUIRY, 1990). These spores may either be binucleate (mitotically derived) or quadrinucleate (meiotically derived; MAGGS, 1988); and

3) carpotetrasporangia are formed on gonimoblast filaments by the cleavage of gonimoblast cells; e.g. some species of *Liagora* and *Helminthocladia* have cruciately divided carpotetrasporangia (GUIRY, 1978).

FELDMANN (1952 in GUIRY, 1987) proposed that the *Polysiphonia*-type life history is the ancestral reproductive pattern for all orders with a triphasic life history as this is the most common life history type. All other life histories were secondarily derived by reduction (HAWKES, 1990), forming the fundamental linkage between all former Florideophycideae orders (GABRIELSON and GARBARY, 1987; HOMMERSAND and FREDERICQ, 1990). However, it is interesting to note that the Rhodymeniales and Ceramiales, which are widely regarded as the most advanced and highly specialized orders, are known to have only a *Polysiphonia*-type life history. Acrochaetiales and Nemaliales, which are regarded as the more primitive orders, show many examples of the *Bonnemaisonia hamifera*-type life history. GUIRY (1987) proposed that both the *Polysiphonia* and *Bonnemaisonia*-type life histories arose independently from an acrochaetoid ancestor which was similar to extant Acrochaetiales.

Carposporophyte evolution has been one of increasing dependency on the gametophyte, the ancestral carposporophytes being essentially autotrophic. This condition is the closest to being realised today in the order Acrochaetiales (HOMMERSAND and FREDERICQ, 1990). There are two main schools of thought on the origin of the carposporophyte:

1) FELDMANN (1952 in GUIRY, 1987) proposed that the original life history contained three morphologically similar free-living generations. This was succeeded by one where the zygote was not immediately released but divided within the carpogonium, giving rise to the carposporophyte which lived parasitically upon the gametophyte. Evidence for this is in a cultured strain of *Acrochaetium pectinatum* (Kylin) Hamel where the zygote may be released as a "sporozygote" to give rise to a free-living tetrasporophyte or develops *in situ* to produce an attached carposporophyte; or

2) the carposporophyte evolved *in situ* as a means of zygote amplification to increase the number of progeny from a single fertilization (GUIRY, 1987). Zygote amplification came about in two ways: either by formation of a mitosporangial generation and a meiosporangial generation (triphasic) or by the formation of a meiosporangial generation directly from the zygote (biphasic; HOMMERSAND and FREDERICQ, 1990).

1.7 Environmental variation in marine habitats affecting algal distribution

Environmental factors affect both spatial (horizontal and vertical) and temporal (seasonal) distribution of benthic macroalgae. This may be from a small local scale to a large geographical scale (EDWARDS, 1979). The boundaries of species are determined by a variety of physicochemical and biotic factors. The lower limits of algae are generally determined by competition for light and space and grazing and predation pressure while the upper limits are determined by abiotic factors. Usually the more stress-tolerant species are confined to the adverse, stressful habitat in the eulittoral and supralittoral zones by the more competitive, less stress-tolerant species (LÜNING, 1990).

The euphotic zone is the lighted layer inhabited by autotrophic plants and extends as deep as 200 m-270 m. Below this is the aphotic zone inhabited by heterotrophic organisms and extends as deep as 11 022 m. The euphotic zone has a number of divisions:

1) the supralittoral zone which is only reached by spray water;

2) the eulittoral zone (intertidal) which is either periodically or aperiodically submersed and emersed; and

3) the sublittoral zone is submersed with the upper part at extreme low water levels occasionally being emersed. The lowest limit of this zone is set by the deepest occurring algae with the main limiting factor being light (LÜNING, 1990).

A season is viewed as a continuum of change from one extreme to another. The driving force in a seasonal environment is the change in incoming radiation as determined by daylength and the altitude of the sun. Incoming radiation is further altered by the variable atmosphere such as cloud cover (KAIN, 1989).

Up to 50% of the incoming irradiation is composed of visible and ultraviolet light while the other 50% is in the infrared range. Between 2%-6% of this incoming radiation may be reflected at the water surface, depending on wave action, solar angle, cloud cover, water turbidity and tides (LOBBAN, HARRISON and DUNCAN, 1985; COUTINHO and ZINGMARK, 1987; REISKIND, BEER and BOWES, 1989; LÜNING, 1990). There is exponential attenuation along the depth gradient owing to scattering and absorption by water molecules, phytoplankton and dissolved and suspended particles called "gelbstoff" which are dissolved organic and humic substances (KIRK, 1979; LÜNING, 1990). Scattering increases the optical pathway, thereby increasing absorption. Generally, less than 20% of the incident irradiation reaches below 10 meters although this depends on water clarity with scattering being more pronounced in turbid waters (REISKIND, BEER and BOWES, 1989).

This reduction in irradiance is accompanied by a change in the spectral composition owing to selective attenuation along the depth gradient. With increasing depth, the spectral distribution becomes increasingly narrower (LÓPEZ-FIGUEROA and NIELL, 1990). The longer wavelengths (red) are almost completely absorbed within the upper five meters of the water column (LEVRING, 1969). In clear oceanic waters, there is maximum transmittance of the short wavelengths (blue) while in coastal waters containing suspended and dissolved particles, short wavelengths are absorbed (LOBBAN, HARRISON and DUNCAN, 1985; LÜNING, 1990; RÜDIGER and LÓPEZ-FIGUEROA, 1992).

Irradiance is "radiant energy which is incident upon an unit area in unit time" and intensity is "radiant flux emitted by a source". Irradiance is the more important measure in biological work (LÜNING, 1981a; LOBBAN, HARRISON and DUNCAN, 1985). In the work described in this thesis, irradiance was measured as μmol m^{-2} s^{-1} although these units are interchangeable with μE m^{-2} s^{-1} (LÜNING, 1981a). Only photosynthetically active radiation in the range 350 nm-700 nm was measured. This is an approximate measure of the irradiance available for photosynthesis (KIRK, 1979).

There is a general increase in seawater temperature from the poles (0°C) to the tropics (28°C) owing to variable incoming radiation. This pattern is modified by ocean currents, upwelling and seasonal changes, with greatest change occurring in the mid-latitudes compared to the tropics and poles. Thermal stratification also results in sharp boundaries called thermoclines with surface water being warmer. Vertical mixing is slow unless there is wave action. Once wave action begins, there is a rapid change in temperature (LOBBAN, HARRISON and DUNCAN, 1985).

Nutrient concentrations are generally higher in coastal than oceanic waters (LOBBAN, HARRISON and DUNCAN, 1985). There are higher concentrations of dissolved nitrogen and phosphorus in spring and winter and lower levels in summer owing to phytoplankton assimilation (KAIN, 1989; CHOPIN, HOURMANT, FLOC'H and PENOT, 1990; LYNGBY, 1990). Nutrient levels in coastal waters may be affected by domestic and industrial effluent and surface drainoff from agricultural lands (BROWN, DUCKER and ROWAN, 1977; DeBOER, GUIGLI, ISRAEL and D'ELIA, 1978).

Salinity gradients are more pronounced in coastal waters while oceanic waters have a relatively stable salinity. On a global scale, there is a decrease in salinity from the tropics to the poles of 37°/∞ to 33°/∞ (LOBBAN, HARRISON and DUNCAN, 1985; BIRD and McLACHLAN, 1986; KIRST, 1990). Salinity is increased by evaporation which is greatest in the tropics and decreased by rainfall and snow melt; e.g. the Mediterranean, which, with high evaporation and low rainfall has a salinity of 38.4°/∞-39°/∞. Salinity variation is local rather than global. It is altered by freshwater plumes from major rivers, local weather conditions and meltwater (LOBBAN, HARRISON and DUNCAN, 1985; BIRD and McLACHLAN, 1986). There are also large diurnal fluctuations in salinity which are more pronounced in the eulittoral region owing to tidal cycles (BIRD and McLACHLAN, 1986).

There are many microclimates in the eulittoral zone created by shore topography, overhangs, crevices and clumps of algae. Rockpools, estuaries and other coastal sites are subjected to sudden and massive changes in several environmental factors. Especially rockpools are subjected to heating and drying effects of sun and wind, diluting effects of rain, as well as biological activities changing salinity, temperature, pH and O_2 and CO_2 tensions during ebb tide (BLACKWELL and GILMOUR, 1991). Sheltered rockpools have a slower change than the more exposed pools higher up on the eulittoral zone. This change depends on length of exposure, surface:volume ratio of the pool, the amount of mixing and stratification and climatic factors including precipitation, air temperature, wind velocity, humidity and fresh water runoff (LOBBAN, HARRISON and DUNCAN, 1985; REED, 1990).

1.8 Photosynthesis

Photosynthesis consists of two major groups of reactions:

1) light reactions convert electromagnetic energy from the sun to chemical potential energy (ATP and NADPH). After energy absorption by antenna pigments, most of the energy is transferred via chlorophyll *a* to the reaction centres. The photosynthetic enzyme complexes become progressively more oxidised with each photochemical reaction until there are four electrons which split water to release O_2. With H^+ buildup, ADP is phosphorylated and NADP reduced to NADPH. For every one ADP to be converted to ATP, three H^+ are needed (LOBBAN, HARRISON and DUNCAN, 1985; BUCHANAN, 1992). All the apparatus for the light reactions are localised in the chloroplast membranes and are called photosynthetic units. Photosynthetic units vary between species regarding their component macromolecules, spatial distribution and kinetic properties (RAMUS, 1981); and

2) dark reactions are the sequence of reactions using chemical potential energy to fix inorganic carbon. These reactions are called the Reductive Phosphate Pathway, Calvin cycle or C_3 cycle. This is an enzymatic process and so is strongly affected by temperature, pH and salinity. Irradiance is also important as many enzymes are light activated and so operate only in the light with a direct supply of ATP and NADPH. The key enzyme in the Calvin cycle is the bifunctional ribulose-1.5-bisphosphate carboxylase/oxygenase enzyme (RUBISCO) which needs Mg^{2+} and CO_2 for

activation. The net effect of the Calvin cycle is 6 CO_2 + 18 ATP + 12 NADPH $\longrightarrow$ hexose + 18 ADP + 18 PO_4^{3-} + 12 NADP. There are supplementary pathways to the Calvin cycle; e.g. C_4 and Crassulacean acid metabolism (CAM) pathways. Following fixation, carbon accumulates in various low molecular weight compounds. In the Ceramiales, digeneaside is formed and in other Rhodophyceae orders, floridoside is the principal product (LOBBAN, HARRISON and DUNCAN, 1985).

Another reaction of photosynthesis is photorespiration. Photorespiration arises because RUBISCO can act as an oxygenase binding O_2 rather than a carboxylase binding CO_2. The carboxylase activity is inhibited by O_2 so that this effect is more pronounced in high O_2 or low CO_2 concentrations. This can be overcome by CO_2 concentrating mechanisms in the chloroplasts (LOBBAN, HARRISON and DUNCAN, 1985).

Chlorophyll *a* is the primary photosynthetic pigment and is found in all algae (RAMUS, 1981; LOBBAN, HARRISON and DUNCAN, 1985). Accessory pigments extend the light absorbency capacity (LÜNING, 1990), filling in where chlorophyll absorption is low (Table 1.3). Rhodophyta are able to utilise most of the visible spectrum (400-700 nm). All O_2 liberating organisms have two photosystems which are connected via an electron transport chain. Each system has its own reaction centre and antenna pigments which capture photosynthetically active radiation and pass the light energy to reaction centres within the thylakoid membrane, sequentially through the accessory pigments to chlorophyll *a*. Photosystem I's reaction centre is P_{700} (absorption maximum peaking at 700 nm) with chlorophyll *a* as the main antenna pigment. Photosystem II's reaction centre is P_{680} (absorption maximum peaking at 680 nm) and in the Rhodophyta, the antenna pigments are water-soluble phycobiliproteins located in phycobilisomes (LOBBAN, HARRISON and DUNCAN, 1985).

Six types of biliproteins have been identified in the Cyanophyta and Rhodophyta, based on their absorption spectra. Allophycocyanin is considered the ancestral form and is structurally the simplest (HOLZWARTH, 1991). There are two types of phycocyanin with the C-type exclusive to the Cyanophyta and three types of

Table 1.3 Action spectrum of the various pigments showing absorption maxima (TALARICO and KOSOVEL, 1978; ALGARRA, THOMAS and MOUSSEAU, 1990; LÜNING, 1990; HANELT, HUPPERTZ and NULTSCH, 1992).

Pigment	Absorption maxima
chlorophyll *a*	435 (Soret band); 679 nm
R-phycoerythrin	495; 542; 563 nm
R-phycocyanin	615 nm
allophycocyanin	650 nm

phycoerythrin with the R-type found mainly in the former Florideophycidae orders and the B-type found mainly in the former Bangiophycidae orders. The distribution of these pigments may have some phylogenetic significance (Table 1.4; HONSELL, KOSOVEL and TALARICO, 1984).

Table 1.4 Distribution of biliproteins in the Ceramiales. Similar icons indicate pigments only found together in a species. R-PE = R-type phycoerythrin, B-PE = B-type phycoerythrin, R-PC = R-type phycocyanin, C-PC = C-type phycocyanin and APC = allophycocyanin (HONSELL, KOSOVEL and TALARICO, 1984).

Family	No. species tested	R-PE	B-PE	R-PC	C-PC	APC
Ceramiaceae	43	43	0	16†	8†	0
Delesseriaceae	9	9	0	2◇	0	1◇
Dasyaceae	10	10	0	0	0	0
Rhodomelaceae	14	14	6⊙	3■	3⊙	2■

Phycobilisomes are supramolecular extra-thylakoidal complexes attached to the stromal surface of the thylakoid membrane and consist of phycobiliproteins bound together by linker polypeptides that ensure proper orientation of the phycobiliprotein chromophores (KOSOVEL and TALARICO, 1982; KURSAR and ALBERTE, 1983; HOLZWARTH, 1991). On the periphery of the phycobilisomes are rods attached to a central core located nearest to the thylakoid (GANTT, 1990; HOLZWARTH, 1991). The protein structure is highly conserved in red algae according to immunological reactivity tests (GANTT, 1990). The size and shape of the phycobilisomes vary with species. Most commonly, they are hemidiscoidal while other variations are hemiellipsoidal or hemispherical. Hemispherical phycobilisomes are generally larger (ALGARRA, THOMAS and MOUSSEAU, 1990; GANTT, 1990; HOLZWARTH, 1991).

The phycobiliproteins are arranged in an energetically favourable pattern with transfer in the direction of decreasing energy levels (Fig. 1.3). There is a close connection between pigments to allow efficient energy transfer (TALARICO and KOSOVEL, 1978; KOSOVEL and TALARICO, 1979; KURSAR and ALBERTE, 1983) with the primary acceptor within 10 nm of the secondary acceptor. Transfer times are in the nanosecond range. There is a close association between the phycobilisomes and photosystem II, often within one supramolecular complex. Morphological evidence of this close association is obtained by freeze-fracture techniques which show the photosystem II core particles associated with the exoplasmic face of the thylakoid membranes being directly aligned with rows of phycobilisomes on the outer membrane surface (McKAY and GIBBS, 1990). There are 3-4 photosystem II reaction centres functionally linked to each phycobilisome (LEVY and GANTT, 1988).

Energy flows from phycoerythrin to phycocyanin to allophycocyanin to the anchor polypeptide (terminal pigment) to the chlorophyll *a* molecule associated with photosystem II in the thylakoid membrane (KOSOVEL and TALARICO, 1979 and 1982; McKAY and GIBBS, 1990; HOLZWATH, 1991). The anchor polypeptide is the terminal pigment in the primary pathway and the main conduit between the phycobilisomes and photosystem II. From the terminal pigment, energy is transferred to the chlorophyll *a* antenna of photosystem II. From here, it can go in

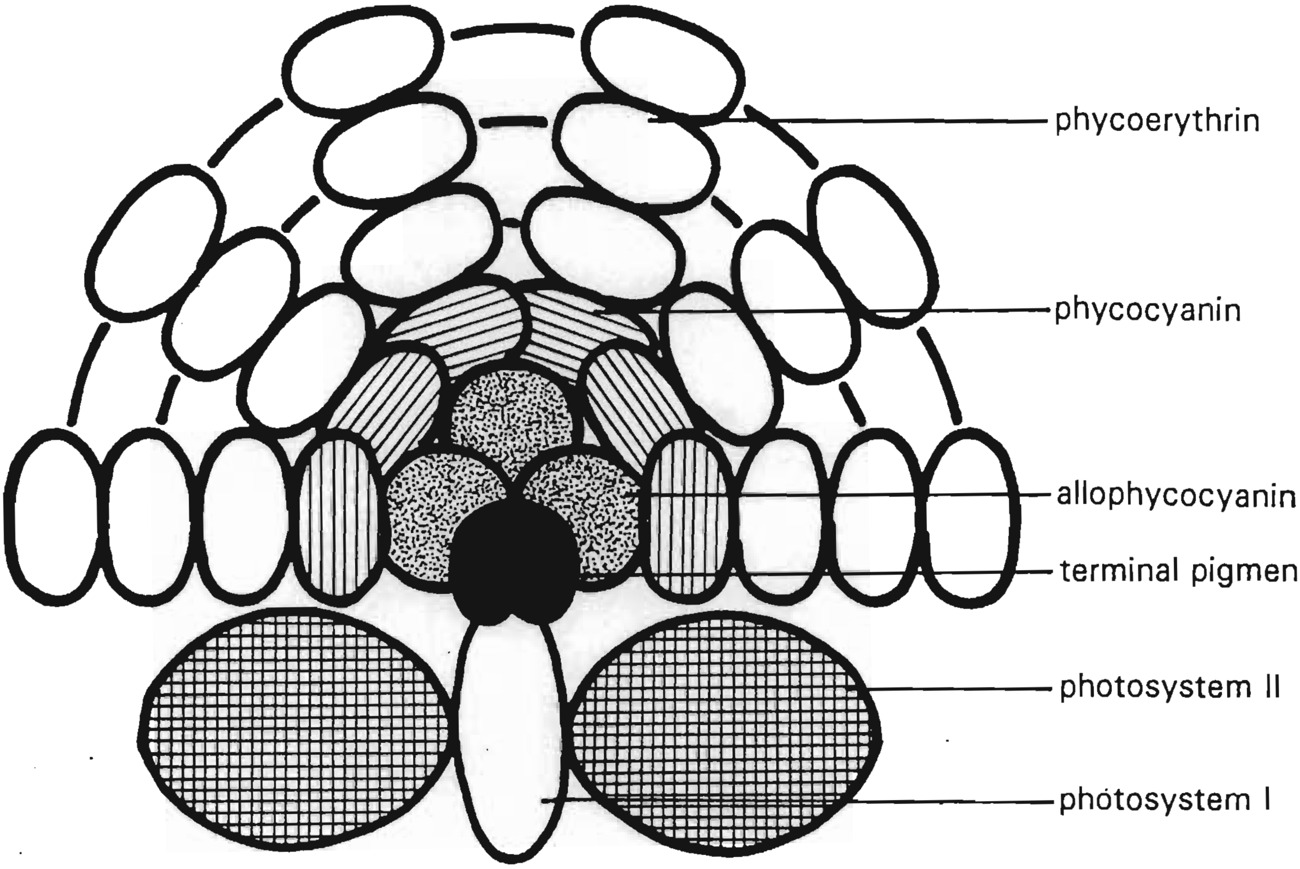


Fig. 1.3 Schematic arrangement of phycobiliproteins in a phycobilisome with the presumed arrangement of the photosystems (GANTT, 1990).

one of two ways depending on the light quality (REHM, GÜLZOW, MARQUARDT and RIED, 1990):

1) to the reaction centre of photosystem II. Once in the reaction centre, the energy is passed along an electron transport chain to the proton acceptor of photosystem I. This pathway is favoured in orange and green light; or

2) to the antennae of photosystem I. This is thought to be the "spillover" pathway (KURSAR and ALBERTE, 1983) favoured in blue and red light (GANTT, 1990).

1.9 Chemical regulation of growth and morphogenesis in plants

Growth and development in plants is a coordinated process and this implies the existence of some form of controlling mechanism (either external or internal) and a transport system (DIXON, 1973). It is generally agreed that higher plant growth and development is regulated by endogenously produced growth substances. A plant growth regulator is a "naturally occurring chemical that controls plant growth and development" (BRADLEY, 1991).

Algae are less advanced than higher plants but still have potential for chemical regulation of development. However, it is not known if this involves growth regulators (EVANS and TREWAVAS, 1991). "Growth regulating substances....are almost certainly present (in algae), although most evidence is circumstantial." (LOBBAN, HARRISON and DUNCAN, 1985). Such circumstantial evidence for plant hormones, similar to those found in higher plants, occurring in algae is due to apical dominance, photoperiodism and orderly differentiation of complex specialised structures (CONRAD and SALTMAN, 1962; PROVASOLI and CARLUCCI, 1974). Knowledge of algal response to plant growth regulators will help to improve algal tissue culture techniques (BRADLEY and CHENEY, 1990).

Plant hormones are found in almost all living cells and control a broad spectrum of processes (ALTMAN, 1989). Plant hormones work through some secondary messenger system, binding to a receptor molecule (BRADLEY, 1991) which is most probably a protein (VENIS and NAPIER, 1991). Plants change in their sensitivity to hormones as they develop. There is an integrated response to changing environmental stimuli and developmental stage (BRADLEY and CHENEY, 1990; TREWAVAS, 1991).

Auxins act at both cellular, organ and whole plant levels. Varied responses of higher plants to IAA include stimulation of cell division and shoot growth, inhibition of root growth, control of vascular system and tissue differentiation and apical dominance, delay of senescence and promotion of flowering, fruit set and ripening. It is assumed that IAA has one receptor and that the multiplicity of effects can be explained by second messengers (BANDURSKI and NONHEBEL, 1984).

Cytokinins are substituted purine compounds and promote cell division, cell elongation, retard senescence, cause redifferentiation in certain plant tissue cultures and induce nutrient mobilization in higher plants (MOONEY and VAN STADEN, 1986; HORGAN, 1984).

Gibberellins are diterpene acids with a 19- or 20-carbon atom skeleton. There are over 80 individually known gibberellins which have been isolated from both fungi and higher plants. The precursor is mevalonic acid. They are known to promote shoot elongation, flowering and seed germination, increase stem diameter and affect synthesis of RNA and protein, especially in fruits and seeds in higher plants (JONES and MacMILLAN, 1984).

Polyamines are ubiquitous in plant tissues, being found in all organisms including bacteria and animals although little is known about amines in algae (KNEIFEL, 1979; ALTMAN, 1989; EVANS and MALMBERG, 1989). They have been rigorously conserved through evolution, indicating some important cellular function (GALSTON, 1983; ADIGA and PRASAD, 1985; SMITH, 1985). The precise role of polyamines in cellular processes is not fully understood but they are assumed to have a regulatory role associated with cell proliferation and tissue regeneration; e.g. synthesis of nucleic acids and proteins, and to play a role in stress responses (COHEN, ARAD, HEIMER and MIZRAHI, 1982a; HAMANA and MATSUZAKI, 1982; PEGG and McCANN, 1982; GALSTON, 1983; ALTMAN, 1989).

Polyamines have a similar role to plant hormones but are rather classed as general bioregulators. It is postulated that polyamines are a type of plant growth regulator or act as hormonal secondary messengers mediating the effect of recognised hormones or may have other less direct roles (GALSTON, 1983; EVANS and MALMBERG, 1989). There are many interactions between plant hormones and polyamines affecting both their content and biosynthesis (ALTMAN, 1989). There appears to be little transport of polyamines in higher plants and what evidence there is, is inconclusive. As a result, polyamines are not classed as hormones (GALSTON, 1983).

Polyamines are required in higher concentrations than hormones (EVANS and MALMBERG, 1989). Exogenous application of plant hormones is between 10^{-5} M to 10^{-7} M, whereas polyamines are applied at least one order of magnitude higher, between 10^{-3} M to 10^{-5} M. The necessity for this higher concentration may be due to difficulties in penetration, difficulties in transport to the site of action, rapid metabolism and already sufficient endogenous concentrations (ALTMAN, 1989). Exogenous uptake is an energy dependent mechanism (PISTOCCHI, BAGNI and CREUS, 1987).

There are polyamine gradients in various higher plants, there generally being highest concentrations where cell division activity is highest (GALSTON, 1983). Polyamines are specific to the pattern of growth rather than the growth rate (FIENBERG, CHOI, LUBICH and SUNG, 1984). Polyamines are involved in various developmental processes and are physiologically important:

1) there is increased polyamine concentrations prior to cell division especially in dormant tissues; eg. the precursor ornithine increases in tobacco suspension cultures with the inhibitor α-difluoromethyl ornithine (DFMO) stopping cell division (MINOCHA, 1988; EVANS and MALMBERG, 1989). Polyamines are associated with nucleic acids, stabilizing their secondary structures and stimulating their biosynthesis (KNEIFEL, 1979; AGRAWAL, AGRAWAL, LEE, KRAMER and PILLAI, 1992);

2) arginine, a polyamine precursor, is important in carrot cell culture with respect to embryogenesis. There is an increase in polyamine concentration prior to embryogenesis (MINOCHA, 1988; EVANS and MALMBERG, 1989);

3) polyamines are involved in root formation and growth; e.g. *Phaseolus* (EVANS and MALMBERG, 1989);

4) there are increased polyamine levels during floral initiation and development; e.g. tomato flowers, germinating seeds, pollinated ovaries and growth of fruit (MINOCHA, 1988; EVANS and MALMBERG, 1989);

5) there are decreased polyamine levels during senescence linked with ethylene production (GALSTON, 1983; EVANS and MALMBERG, 1989). It is thought that polyamines can control the biosynthesis of ethylene and can block the ethylene synthetic pathway as S-adenosylmethionine (SAM) is a common precursor of both ethylene and polyamines (BRADLEY, EL-FIKI and GILES, 1984; MINOCHA, 1988);

6) polyamines are involved in regulation of transcription and translation (MONTAGUE, KOPPENBRINK and JAWORSKI, 1978) and DNA, RNA, protein synthesis and plasma membrane integrity (MINOCHA, 1988). In the alga *Chlorella vulgaris* Beijerinck, there is an increase in ornithine decarboxylase activity prior to DNA synthesis and autospore release. These effects can be inhibited by DFMO and partially reversed by adding putrescine. Addition of DFMO had little effect on the first division cycle as there is most probably sufficient endogenous polyamine pools. DFMO had a greater effect on the second division cycle as the endogenous polyamine pool was exhausted (COHEN, ARAD, HEIMER and MIZRAHI, 1984); and

7) there are increased polyamine levels in response to various physical and chemical stresses which may help reduce stress-induced damage to the cell (MINOCHA, 1988).

1.10 Aims of study

The work in this thesis can be divided into three broad areas of investigation.

The first component was descriptive, concentrating on the morphology, ultrastructure and reproductive life history of *Pteroceramium*.

The second component of this work had an ecophysiological basis. The morphological plasticity of the pinnae (wings) of *Pteroceramium* were investigated in a range of environmental parameters to test their phenotypic stability. This was important as the wings are the main diagnostic feature of the new genus, separating it from the genus *Ceramium*. The wings are paired lateral expansions of the cortical band. It is proposed that the function of the wings is to increase the surface area of the alga for light interception.

The horizontal and vertical distribution of *Pteroceramium* is not known as it has only been found on three occasions - twice in seaweed drift at Trafalgar Beach on the Natal south coast (AKEN per. comm.) and once in a shaded overhang on the intertidal in the Eastern Cape (STEGENGA and BOLTON, 1992). Growth optima and physiological tolerance limits of *Pteroceramium* when grown under these environmental parameters was also determined as well as its photosynthetic performance under various environmental conditions. It was hoped that the results obtained from these studies would give some indication of the requirements of the alga and where it might be expected to be found in the waters of the east coast of South Africa.

The third component of this thesis had a physiological basis. With algae becoming commercially important as fertilizers, agar producers, food sources and in biomedics, attempts are being made at tissue culturing. However, there is very little known about the chemical controls of growth in algae. This component of work investigated chemical controls of growth and morphogenesis in *Pteroceramium*.

To summarize, the aims of this study were to:

1) describe the morphology and ultrastructure of this new species at the light microscope, SEM and TEM levels to enable a formal description to be written (Chapter 2);

2) describe the reproductive biology of *Pteroceramium* (Chapter 3);

3) test the morphological stability of the wings in a range of environmental conditions and to determine if they are a sufficiently stable character on which to base a new genus (Chapter 4);

4) determine the growth optima and tolerance limits of *Pteroceramium* for various environmental factors to give an indication of its possible geographical distribution (Chapter 4);

5) determine the photosynthetic performance of *Pteroceramium* under various environmental conditions to give an indication of its possible vertical distribution (Chapter 5);

6) determine the function of the wings (Chapters 2, 4 and 5); and

7) determine the chemical growth controls operating in *Pteroceramium* (Chapter 6).

Chapter 2

Vegetative morphology and ultrastructure

2.1 Introduction

Pteroceramium has a distinctive morphology with some specialization between axial cells and cortical cells. *Pteroceramium* displays a growth pattern which appears similar to that of the closely related *Ceramium* species. However, it has been proposed by AKEN and NORRIS (in prep.) to separate *Pteroceramium* from the closely related *Ceramium* species. The main diagnostic character for this new genus would be the paired wings.

One of the aims of this work was to define the type of growth in *Pteroceramium* and to investigate ultrastructural features to show any differences which occur between the various cell types. This will enable a formal description to be written. No previous anatomical studies have been carried out on *Pteroceramium*.

2.2 Materials and Method

2.2.1 Experimental organism

The male gametophyte of *Pteroceramium* was collected in a seaweed drift at Trafalgar Beach on the Natal south coast, South Africa on 28 September 1985 and brought into unialgal culture. The alga has since been kept in unialgal culture in the Department of Botany, University of Natal, Pietermaritzburg, and is maintained in Provasoli's Enriched Seawater (PES; Appendix A.1) at 20°C in a 16:$\bar{8}$ hour light:dark photoregime. It was illuminated by a bank of incandescent and fluorescent lights providing photosynthetically active radiation between 30 μmol m^{-2} s^{-1} and 80 μmol m^{-2} s^{-1}. These are referred to in this study as standard growth conditions. Except for the reproductive study, this clonal material of the male gametophyte was used in all experiments.

Ceramium glanduliferum Kylin was collected at Mkambati, Transkei on 8 September 1991 from the eulittoral region. Since then, it has been maintained in unialgal culture in PES in the same standard growth conditions used for *Pteroceramium.*

2.2.2 Light microscopy

Living material of *Pteroceramium* and *Ceramium glanduliferum* were mounted onto slides with either seawater or glycerine jelly (Appendix B) as the mounting medium and were viewed using a compound photomicroscope (Carl Zeiss, Germany). Photographs were taken using Ilford Pan F 50 ASA film.

2.2.3 Scanning electron microscopy (SEM)

Living material of *Pteroceramium* was fixed for 1 hour in 3% glutaraldehyde in 90% seawater. After two rinses in 90% seawater for 15 minutes each, and two 15 minute washes in 0.05 M phosphate buffer (Appendix C.1), the specimens were placed in the enzyme β-glucuronidase (400 units ml^{-1} in 0.05 M phosphate buffer) for 20 minutes at 35°C to remove the surrounding fibrillar mucilage (MEYER and PIENAAR, 1984). This was followed by two 15 minute rinses in phosphate buffer. Material was then freeze-fractured in liquid nitrogen using a razor blade and small hammer. After staining with osmium tetroxide (OsO_4) for 30 minutes (1:1 OsO_4 and seawater) and two 15 minute rinses in phosphate buffer, material was run through a graded dehydration series with 15 minute washes in 30%, 50%, 70%, 80% 90%, 100% and 100% ethanol. Material was then critical point dried and mounted onto stubs. Specimens were viewed with a Hitachi S-570 scanning electron microscope.

2.2.4 Transmission electron microscopy (TEM)

Material of *Pteroceramium* was cut into small pieces (2-5 mm in length) and fixed in 0.5% glutaraldehyde in 90% seawater (pH 7.0) for 30 minutes. It was then transferred for 1 hour to 3% glutaraldehyde in 0.05 M cacodylate buffer and 0.2 M sucrose made up in 90% seawater (pH 6.5). After rinsing in 0.05 M cacodylate and 0.1 M sucrose for 10 minutes, followed by a 10 minute wash in cacodylate buffer, material was stained for 1 hour in OsO_4 in cacodylate (1:1 solution) and then rinsed twice in cacodylate for 10 minutes each. Material was then run through a graded dehydration series in ethanol with 10 minute rinses in 30%, 50%, 70% (overnight), 90% and three rinses in 100% ethanol. Material was then placed in propylene oxide for 30 minutes for two washes followed by infiltration with Spurr's epoxy resin. This was done by 2 hours in 25% resin and 75% propylene oxide with the last 30 minutes under vacuum, 2 hours in 50% resin and 50% propylene oxide with the last hour under vacuum, 2 hours in 75% resin and 25% propylene oxide under vacuum for the entire period, overnight in 100% resin under vacuum and 6 hours in 100% resin under vacuum. The samples were then polymerized (modified from TRICK and PUESCHEL, 1990; RASCIO, MARIANI, DALLA VECCHIA and TREVISAN, 1991a and b).

Embedded material was mounted on perspex stubs and sections cut using a LKB Ultrotome III with glass knives made on a LKB Type 780 IB knifemaker. Thin gold sections were floated onto water, expanded with chloroform vapours and picked up on copper grids with a 200 hexagonal mesh.

Sections were poststained for 10 minutes in 2% aqueous uranyl acetate, rinsed briefly in distilled water, stained for 10 minutes in lead citrate followed by another brief rinse in distilled water. Sodium hydroxide pellets were used to prevent lead precipitation.

Sections were viewed using a Joel 100CX transmission electron microscope operating at an accelerating voltage 80 kV.

2.3 Results

Pteroceramium has an uniaxial thallus arrangement with distichously arranged, paired wings. There were pseudodichotomous branches with alternate branches becoming dominant. The axial cells were paler red compared with the cortical cells and increased in size away from the apex. There were between 4-12 axial cells per segment, although most commonly, there were between 5-8 axial cells. Segment 1 (seg. 1) refers to the cells in the apical tip before the first pseudodichotomy, seg. 2 to the region between the first and second pseudodichotomy etc. (Plate 2.1 Fig. 1). Rinsing with β-glucuronidase effectively removed the mucilaginous covering to reveal the striated appearance of the cell wall (Plate 2.2 Fig. 1). The morphology of *Pteroceramium* was distinctive from *Ceramium glanduliferum* which lacked wings but had axial cells and nodal cortication (Plate 2.1 Fig. 2).

The dome-shaped apical cell divided horizontally to produce a subapical cell which developed into an axial cell. For branching, the apical cell divided obliquely (Plate 2.1 Fig. 3). A nodal cortical band stilled developed at the pseudodichotomy (Plate 2.1 Fig. 4).

The cortical region was more pigmented than the axial cells. In *Pteroceramium*, there are six pericentral cells making up each cortical node. Pc1, the first formed pericentral cell, was always on the outer face according to the preceding pseudodichotomy. It was the largest pericentral cell and gave rise to the larger of the paired wings. Pc6 was the second largest pericentral cell formed and was always on the inner face, giving rise to the smaller of the paired wings (Plate 2.1 Fig. 5 and 6). This resulted in the characteristic distichous arrangement of the wings.

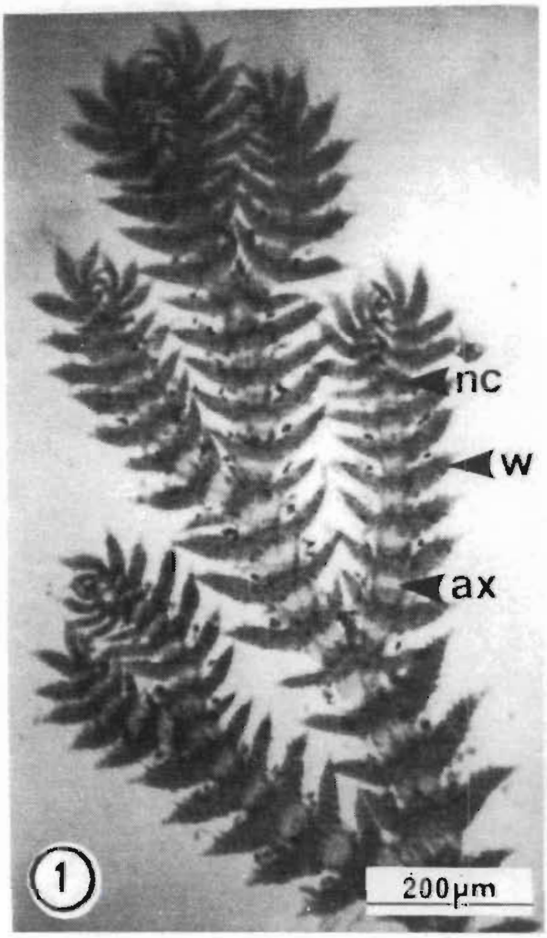
Pc2-Pc5 are smaller and gave rise acropetally to 3-5 celled, uniseriate spines. The pericentral cells also divided further to form the lateral branches of limited growth which make up the cortical band (Plate 2.1 Fig. 5 and 6; Plate 2.2 Fig. 3). Rhizoids also arose from the pericentral cells and were elongated, unpigmented single cells or multicellular and functioned in attachment to the substratum (Plate 2.2 Fig. 1 and 2). The wings had up to 30 cells. Some of the wings had a toothed acropetal edge (Plate 2.2 Fig. 4). There was a thick mucilage layer covering *Pteroceramium* (Plate 2.1 Fig. 3, 5, 6 and 7).

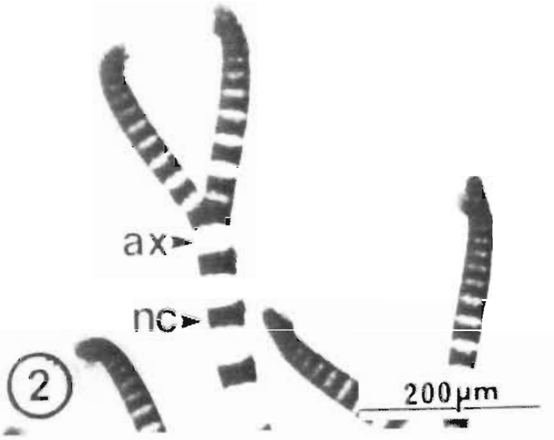
Adventitious branches of unlimited growth formed in the more mature parts of the thallus from the transformation of an apical cell of a lateral branch of limited growth. These had a distinct morphology, with axial cells being smaller than those on the parent branch and the wings being greatly reduced in size. With increasing distance from the parent branch, the morphology resumed its normal appearance with larger axial cells and wings and pseudodichotomous branching (Plate 2.1 Fig. 7).

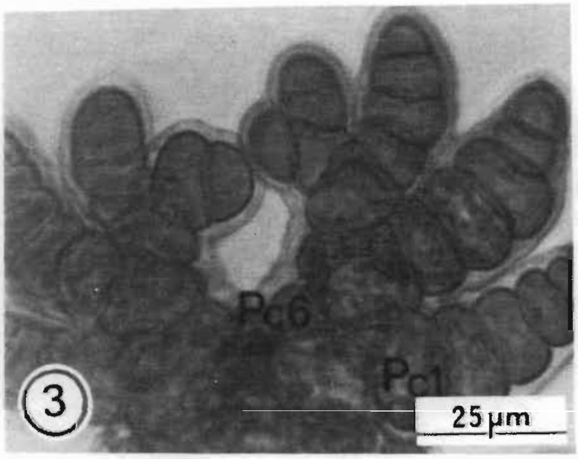
Mature axial cells had a large central vacuole. Cytoplasm was peripheral, consisting mainly of discoid chloroplasts (Plate 2.3 Fig. 1). These had the typical Rhodophyta morphology with single, evenly spaced thylakoids with phycobilisomes attached to their outer surface, giving the thylakoids a granular appearance. Between the chloroplasts there were smaller mitochondria which were similar to those found in higher plants and green algae. There was a double unit membrane with infolding of the inner membrane to form tubular cristae. There were also large floridean starch granules present within the cytoplasm (Plate 2.3 Fig. 2).

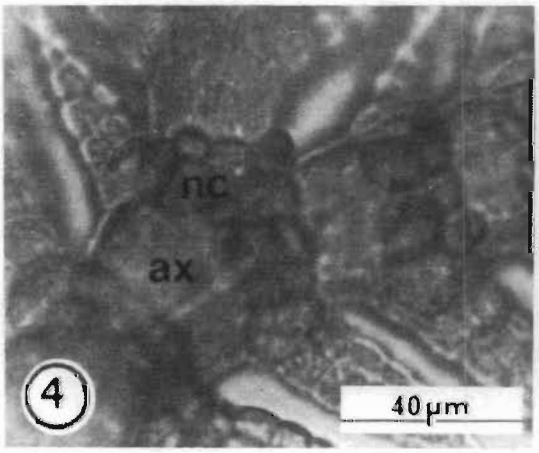
The wings have a denser cytoplasm than axial cells. Chloroplasts were more closely packed together around the periphery of the cell with numerous floridean starch granules and some mitochondria. Vacuoles were few and small or absent from the cytoplasm (Plate 2.3 Fig. 3). The wing cells are smaller than the axial cells (Plate 2.3 Fig. 4).

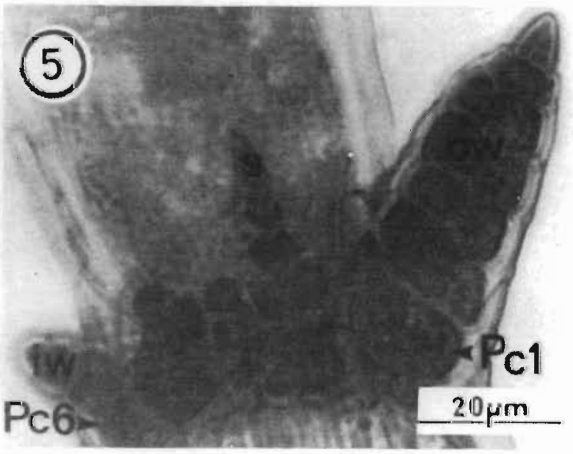
The inner cell wall consisted of a loosely embedded fibrillar network which was not homogeneously distributed but grouped in parallel layers between electron transparent areas. The outer wall had the fibrillar network more closely arranged and so appeared as a homogeneous layer. Pit connections were present in *Pteroceramium*. The pit connections agreed with the ordinal classification of PUESCHEL (1990; Table 1.1), *Pteroceramium* had only a plug core and no cap layers. The plug core was surrounded by a plug cap membrane (Plate 2.3 Fig. 5).

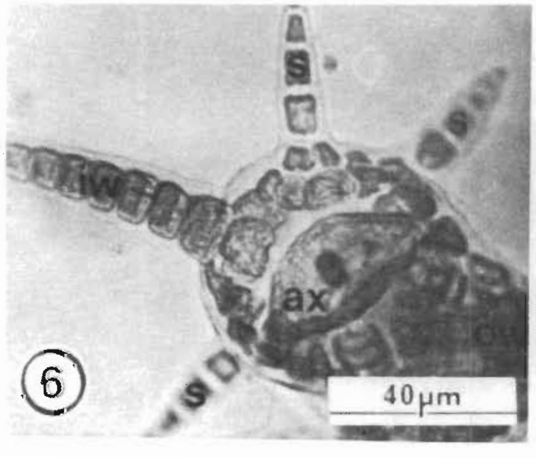

①
nc
w
ax
200µm

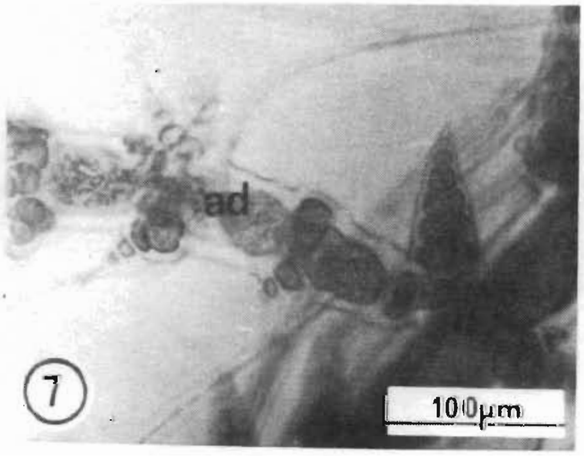

②
ax
nc
200µm

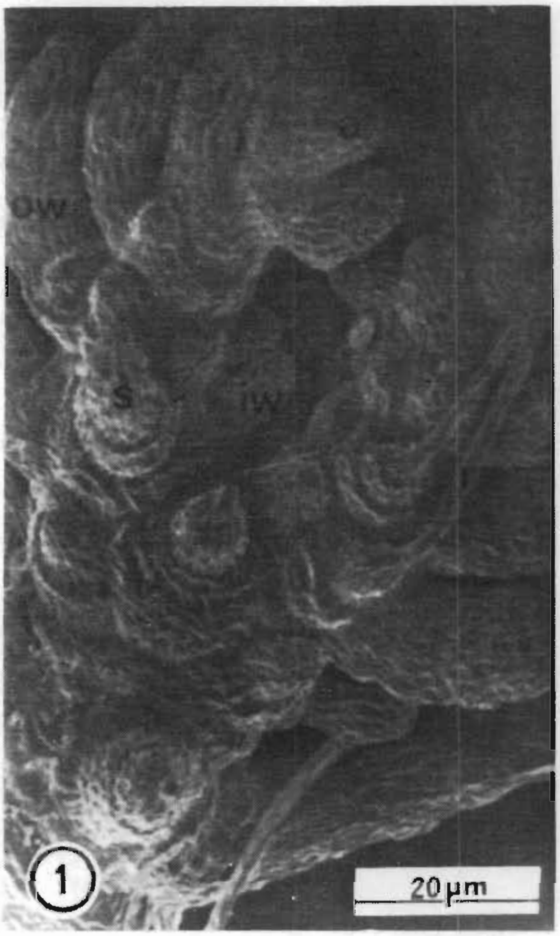

③
Pc6
Pc1
25µm

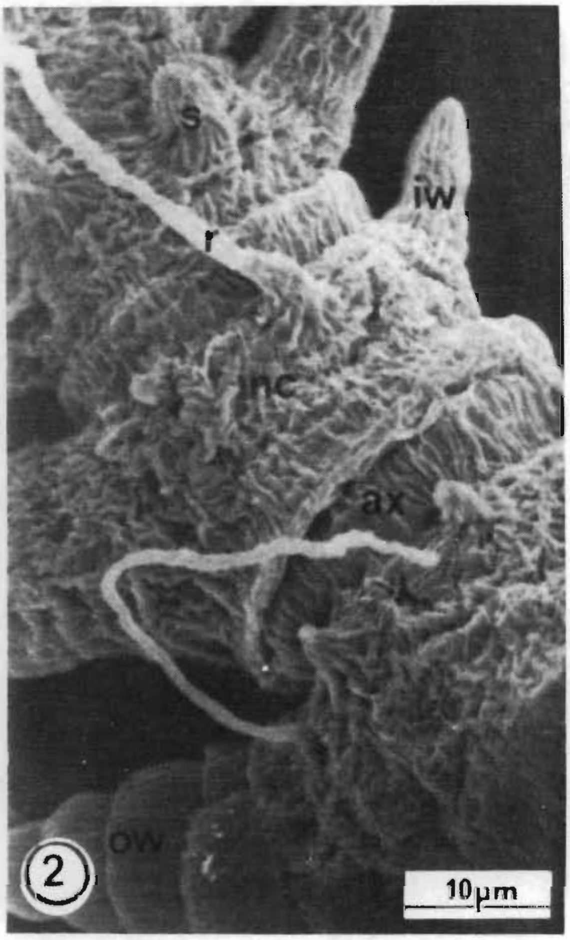

④
nc
ax
40µm


⑤
s
ow
iw
Pc1
Pc6
20µm

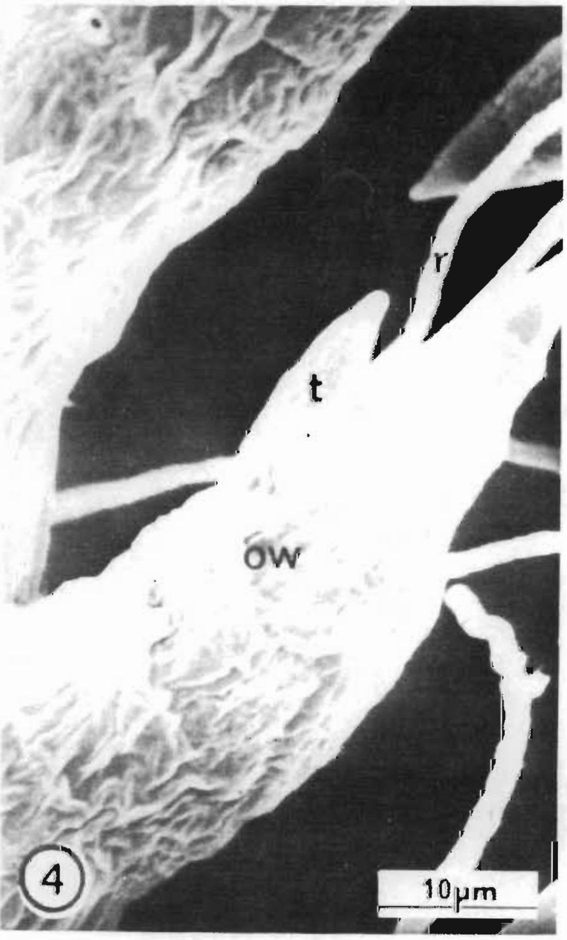

⑥
s
iw
ax
s
40µm


⑦
ad
100µm

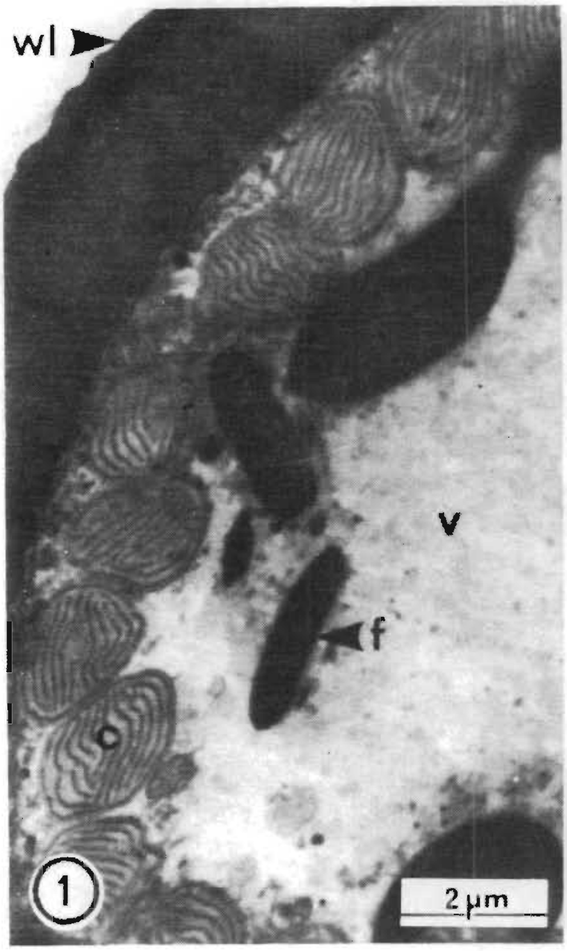

ow
s
iw
20µm
1


s
iw
r
inc
ax
ow
10µm
2


Pc
ax
10µm
3

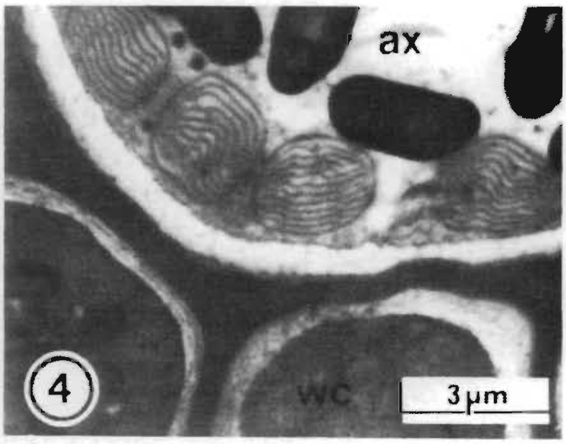

r
t
ow
10µm
4

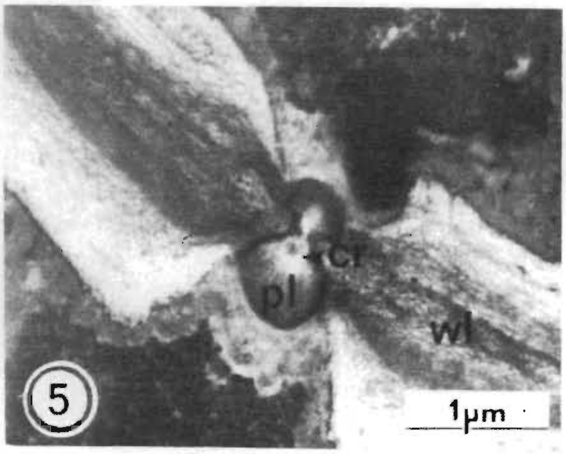
```
SELECT TAG to AMEND, <M>ARC TAGS, <RETURN> for NEXT or <X>it ?
ENTER NUMBER or <X> if NONE of these X

3.6     UNP  CATALOGUE NEW ACQUISITION  TERMINAL 4
BRN 276343 in 19940331  TERMINAL 4 amended 31 MAR 94
000     nam [monograph]
010     $d0.00 $s15
200*10 Aspects of structure, growth and morphogenesis in a new filamentous red
   alga (Ceramiaceae, Rhodophyta) $fby Wendy Ann Stirk $3ASSGMOSTA
210*    Pietermaritzburg $cUniversity of Natal $d1993 $3PGUNATL
300     Thesis (Ph.D. ; Botany) - University of Natal, Pietermaritzburg, 1993
700 1   Stirk, Wendy Ann $3STIRKWA
990     276343 $b94031520 $cIN PROCESS
996     DONATION
```


wl
v
f
c
1
2 µm


m
2
6µm


3
2 µm


ax
wc
4
3µm


cr
pl
wl
5
1µm

2.4 Discussion

By definition, an apical cell always divides to produce a new apical cell with one pit connection and a subapical cell with two pit connections, independent of the position of the dividing wall (PARSONS, 1975). *Pteroceramium* displays sympodial growth which is defined as the development of a main axis from secondary branches, each representing the dominant fork of a dichotomy (NORRIS, WOLLASTON and PARSONS, 1984). This is compared to monopodial growth where the new lateral initial is formed on a subapical cell (PARSONS, 1975).

Two types of sympodial growth have been defined:

1) ramisympodial growth occurs where a succession of determinate axes are formed in an intercalary position on a previously determinate axis; and

2) cellulosympodial growth occurs where the apical cell is continually displaced to one side and the subapical cell takes over the function of producing further axial growth. The displaced apical cell may continue growth to form a lateral branch of determinate growth (PARSONS, 1975; NORRIS, WOLLASTON and PARSONS, 1984). This is the growth pattern found in *Pteroceramium*, resulting in its pseudodichotomous growth form with alternate branches becoming dominant.

Pteroceramium has a high degree of cortication although the axial cells are only partially covered. The cortical band has six pericentral cells which give rise to wings (Pc1 and Pc6) and spines (Pc2-Pc5). Primary spines are modifications of acropetal primordia of lateral branches of limited growth (DIXON, 1960a). Spines also occur in some *Ceramium* species although these "spinous species" do not form a natural group (WOMERSLEY, 1978). The function of these spines is unknown. The wings of *Pteroceramium* are unique, not being found in any other species, and are considered the main diagnostic feature of *Pteroceramium*.

There appears to be some degree of specialization between cell types. Axial cell elongation results in an increase in thallus length and is correlated to an increase in vacuole size (DIXON, 1960a). Cells making up the cortical band (pericentral cells, wings and spines) appear to have a photosynthetic function as the chloroplasts are more densely packed. Possibly the wings are a morphological adaptation to increase the surface area of the alga for light interception. This corresponds with the increased density of chloroplasts found in these cells suggesting that they have a photosynthetic function. Similar specialization is seen in *Pterocladia capillaceae* (Gmelin) Bornet *et* Thuret which has a multiaxial filament arrangement. The cells making up the thallus can be divided into two distinct regions. Cells on the peripheral region of the thallus appear to have a photosynthetic function with large chloroplasts and rare floridean starch while the larger medullary cells have few plastids and several starch grains, suggesting a storage function (RASCIO, MARIANI, DALLA VECCHIA and TREVISAN, 1991b).

Another unusual morphological character of *Pteroceramium* is the difference in size between the six pericentral cells. Pc1 is always the largest, Pc6 the second largest and Pc2-Pc5 the smallest and similar in size. This does not appear to occur in *Ceramium* species.

Chapter 3

Reproduction

3.1 Introduction

Reproduction in the Rhodophyta has already been discussed in Chapter 1. This chapter includes specific references to reproduction in the Ceramiaceae.

Most Ceramiales have a *Polysiphonia*-type life history. There is great uniformity in the female reproductive structure with auxillary cell development occurring after fertilization (BROADWATER and SCOTT, 1982). *Pteroceramium* is closely related to *Ceramium* species and so is expected to have a similar pattern of reproductive development although the final decision to this awaits availability of female material.

Most species of *Ceramium* are dioecious. Unpigmented spermatangia are formed superficially from mother cells derived from apical cells of lateral branches of limited growth and spread from an initial adaxial position to cover the whole cortical node. Each mother cell forms 3-5 spermatangia (DIXON, 1960a and b; WOMERSLEY, 1978). Spermatia have one or two massive mucilage containing vesicles called spermatial apparatus which are derived from endoplasmic reticulum and possibly golgi vesicles and occupy the majority of the cell volume (PUESCHEL, 1990). Spermatial vesicles are composed of mucopolysaccharide (BRAWLEY and WETHERBEE, 1981).

Carpogonial branches are produced intermittently along the axis with pericentral cells functioning as supporting cells. Usually Pc1 becomes the supporting cell, resulting in the four-celled carpogonial branch developing on the outer face relative to the preceding pseudodichotomy (DIXON, 1960a and b; BOLD and WYNNE, 1978). Carpogonial branch cells are smaller than cortical cells but the supporting cell is larger than the normal Pc1. There is also a reduction in the number of lateral branches formed (DIXON, 1960a). Both one and two carpogonial branches per supporting cell with the corresponding number of auxillary cells can be seen on the same thallus; e.g. *Ceramium diaphanum/strictum* complex from Cornwall (DIXON, 1960a).

As in all Ceramiaceae, the auxillary cell in *Ceramium* is cut off from the supporting cell after fertilization (DIXON, 1960a). The young carposporophyte forms a cluster of cells on the surface of the female gametophyte. Once mature, it may be surrounded by a loose, incomplete involucre of adventitious lateral branches of unlimited growth arising from segments below (DIXON, 1960a; SIMONS, 1966; BOLD and WYNNE, 1978), although "the trend in the Ceramiaceae clearly is towards the suppression of the vegetative branches (sterile group) during the development of the procarp" (GORDON and WOMERSLEY, 1966).

The tetrasporangia are produced from lateral branches of limited growth. Evidence suggests that the tetrasporangia are attached to the pericentral cells with no other supporting cell being involved (SIMONS, 1966). The number of tetrasporangia per cortical band varies. The first tetrasporangium always develops on the outer face of the axis relative to the preceding pseudodichotomy, with the rest forming in a whorl (DIXON, 1960a). Cell division is usually tetrahedral although there are some examples of a cruciate division pattern (DIXON, 1960a, WOMERSLEY, 1978). The degree of emergence of the tetrasporangium from the cortical band is determined by the degree of cortication. For example, if there is continuous cortication, the tetrasporangia is completely immersed (DIXON, 1960a; SIMONS, 1966; BOLD and WYNNE, 1978; WOMERSLEY, 1978).

Spore germination of both the carpospores and tetraspores is bipolar. Once the spore has become attached to the substrate by a cushion of mucilage, the spore divides to form a principal apical cell and a rhizoid rudiment. The principal apical cell divides to form the axial cells. The first few cells formed, especially in the cortical band, may be slightly aberrant in development. There may also be up to 18 axial cells before the first pseudodichotomy. The rhizoid rudiment elongates to form the basal rhizoid and then adventitious rhizoids develop (DIXON, 1960a; HAWKINS, 1972). Occasionally spores develop *in situ* and result in an obvious disturbance in the cell arrangement (DIXON, 1960a).

In *Ceramium* species, there are two known types of accessory reproduction:

1) lateral polysporangia usually containing 5-12 spores are formed superficially as opposed to being embedded in the cortical band; e.g. *Ceramium diaphanum* Kylin, *Ceramium strictum*, *Ceramium deslongchampsii* Chauvin and *Ceramium vertebrale* Rosenvinge; and

2) an irregular-shaped mass of parasporangia are formed only in an apical position; e.g. *Ceramium strictum* and *Ceramium diaphanum* (DIXON, 1960a).

Vegetative propagation also occurs with new thalli arising from detached fragments of the axis. Rhizoids form from the cortical band for attachment (DIXON, 1960a).

The aims of the work detailed in this chapter were to describe the reproductive biology of *Pteroceramium*. This will be included in the formal description of the species.

3.2 Materials and Method

Observations were made from specimens collected in drift material washed up at Trafalgar Beach, Natal south coast on 28 September, 1985. All three life-cycle stages were collected. The material was mounted onto microscope slides using Karo Syrup (AKEN, pers. comm.). Since then, these slides have been stored at the Botany Department, University of Natal, Pietermaritzburg.

Observations of these slides were made using a compound photomicroscope (Carl Zeiss, Germany). Photographs were taken with Ilford Pan F 50 ASA film.

3.3 Results

Pteroceramium showed a typical *Polysiphonia*-type life history with isomorphic gametophyte and tetrasporophyte phases. The gametophytes were dioecious.

The colourless and spherical spermatangia formed in clusters on the cortical band of the male gametophyte (Plate 3.1 Fig. 1). Initially they formed in an adaxial position at the base of the wing and then spread along the wing, in many instances covering the entire wing. They also spread over the cortical band but in a less dense cluster than found covering the wings. Spermatangia did not cover the spines. Usually the entire segment was reproductive although spermatangial clusters varied in size, the largest developing from Pc1. Spermatangia were also found on immature segments; e.g. seg. 2.

In *Polysiphonia harveyi* Bailey the spermatangia are borne on a two-celled stalk, the lower cell being smaller and the upper cell elongated and vacuolated (SCOTT, BOSCO, SCHORNSTEIN and THOMAS, 1980). In *Pteroceramium*, there is no stalk cell. Instead, the spermatangia are attached directly to the cortical cells (Plate 3.1 Fig. 2). Each spermatangium had a large basal spermatial vesicle to aid in spermatium dispersal (Plate 3.1 Fig. 3).

Carpogonia were not nearly as abundant as the spermatangia and were scattered throughout the thallus. Pc1 was always the supporting cell for the four-celled carpogonial branch. The carpogonial cells were colourless but similar in size to the cortical cells. The carpogonial branch replaced the outer wing and so was detectable by the disruption in thallus construction (Plate 3.1 Fig. 4). Spines were still present as they do not arise from Pc1. Pc1 appeared to be the same size as usual but then it was normally larger than the other pericentral cells. No trichogynes could be seen.

After fertilization, a spherical carposporophyte developed (Plate 3.2 Fig. 1). At approximately a 10-cell stage, the cells were still colourless (Plate 3.2 Fig. 2) although they became pigmented at some stage after this (Fig. 3.1). The presence of auxillary cells could not be confirmed. The gonimoblast filament cells were larger than cortical cells. Most of the carpogonial cells were square, but towards the periphery they were rectangular with a rounded edge. Even at maturity, there was no involucre of adventitious laterals. Instead, there appeared to be some torsion so that the carposporophyte was found between two branches (Plate 3.2 Fig. 1).

Tetrasporangia were produced in abundance along the entire thallus. There appeared to be no sequence of development with mature and immature tetrasporangia being interspersed. There was a maximum of two tetrasporangia per cortical node, developing from Pc1 and Pc6. Those derived from Pc1 developed first as, in examples where there is only one tetrasporangium on the cortical node, it was always derived from Pc1 (Plate 3.2 Fig. 3).

Tetrasporangia were partially embedded in the acropetal surface of the wing at the node and were surrounded by mucilage. At first, the roughly oval developing tetrasporangium was totally embedded in the wing. It reached approximately full size before the first division. There was successive division with the first division in a median plane. Invaginations appeared from the periphery inwards. These invaginations were slightly unequal, the first appearing on the side farthest away from the axial cell (Fig. 3.2). The orientation of the successive divisions was such that the division on the lower half of the tetrasporangium was always visible. This division was almost 90° to the medium division. This resulted in a decussate cruciate tetraspore arrangement. The tetraspores were connected at the centre by an isthmus (Plate 3.2 Fig. 4).

The life-history of *Pteroceramium* is summarised in Figure 3.2.

3.4 Discussion

To establish a life history, information is needed on the occurrence of each morphological phase in the field, germination of each reproductive body, growth to reproductive maturity and chromosome number of each phase to determine the

Plate 3.1

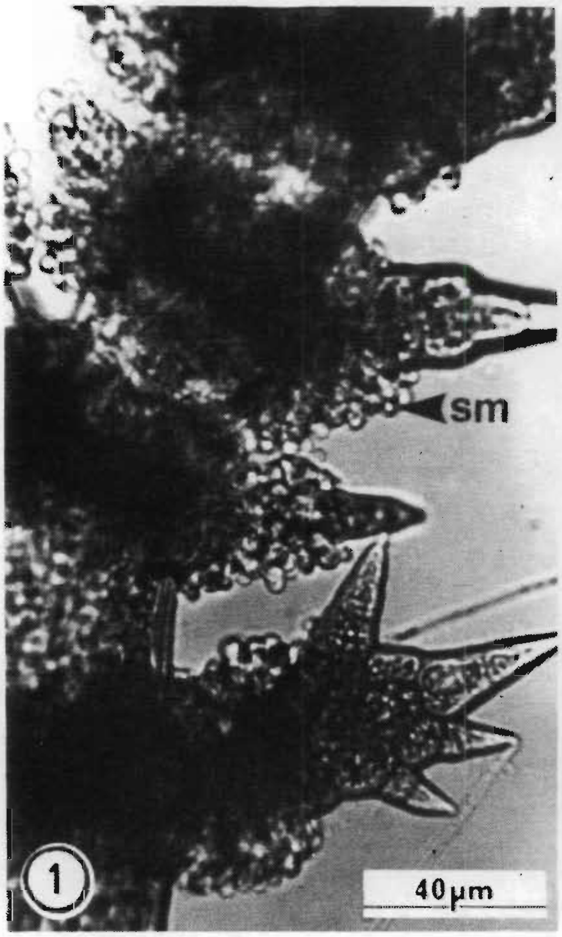
Male and Female gametophyte phases of *Pteroceramium*.

Fig. 1 Male gametophyte showing spermatangia (sm) covering the cortical band and wings.

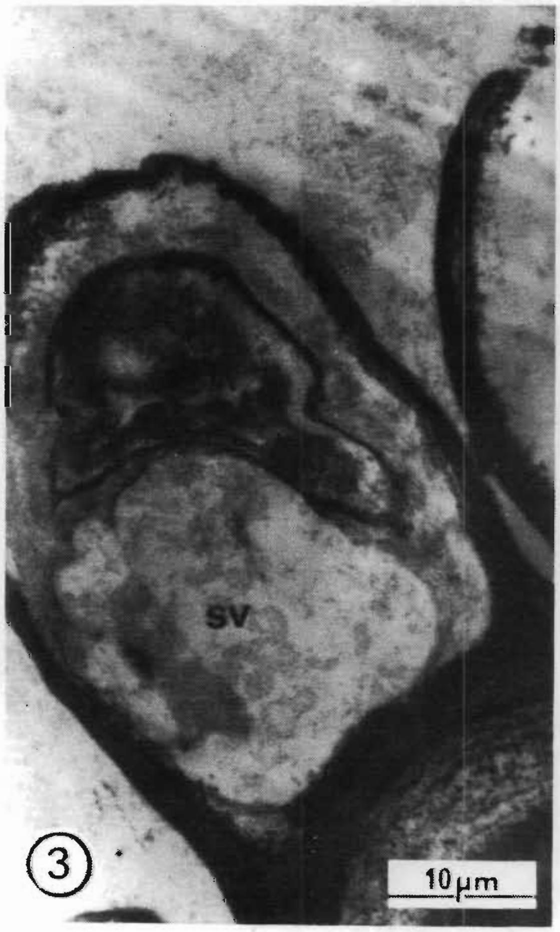
Fig. 2 Attachment of the spermatangia to the cortical band.

Fig. 3 Ultrastructure of a spermatangium showing the large basal spermatial vesicle (sv).

Fig. 4 Female gametophyte with a four-celled carpogonial branch (cb). This branch replaces the outer wing (ow) with Pc1 as the supporting cell.


sm
1
40µm


2
3µm


sv
3
10µm

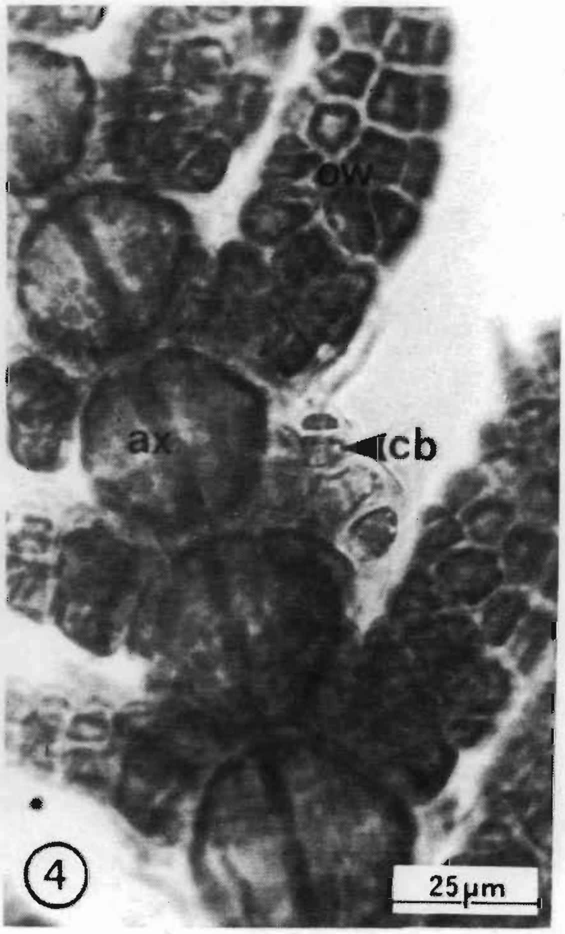

ow
ax
cb
4
25µm

Plate 3.2

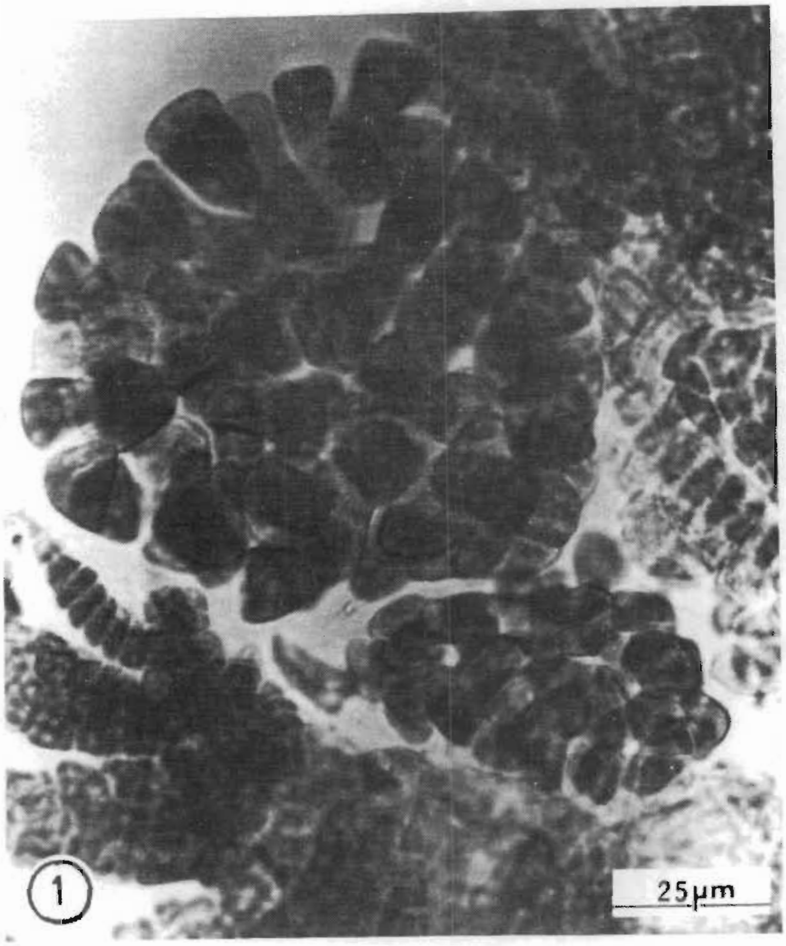
Carposporophyte and tetrasporophyte phases of *Pteroceramium*

Fig. 1 A fully developed carposporophyte found between two branches for protection.

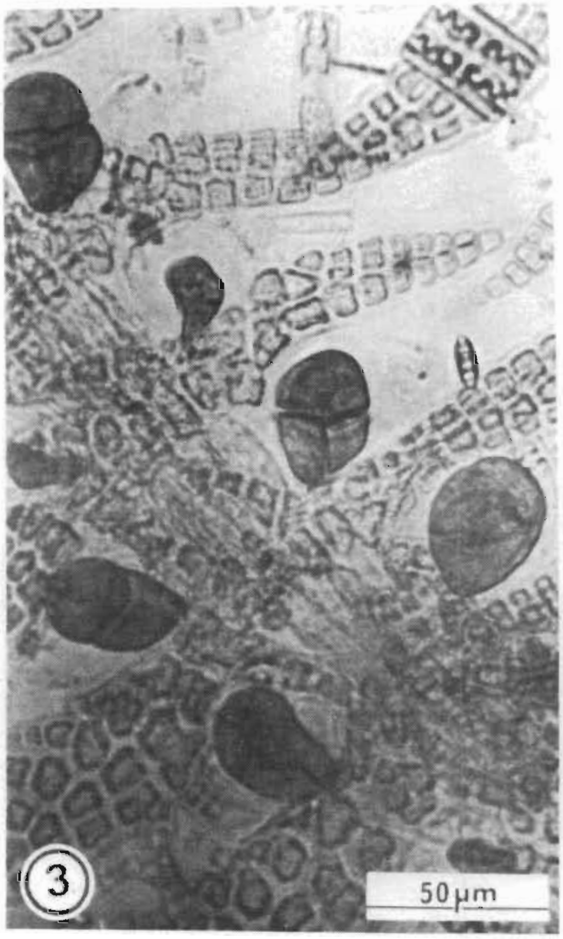
Fig. 2 A developing carposporophyte (➤) at approximately the 10-cell stage. It is still colourless.

Fig. 3 Tetrasporophyte plant showing various stages of tetrasporangia development.

Fig. 4 Decussate cruciate tetrasporangium embedded in a wing and covered by mucilage.


①
25µm


②
20µm


③
50µm


④
20µm

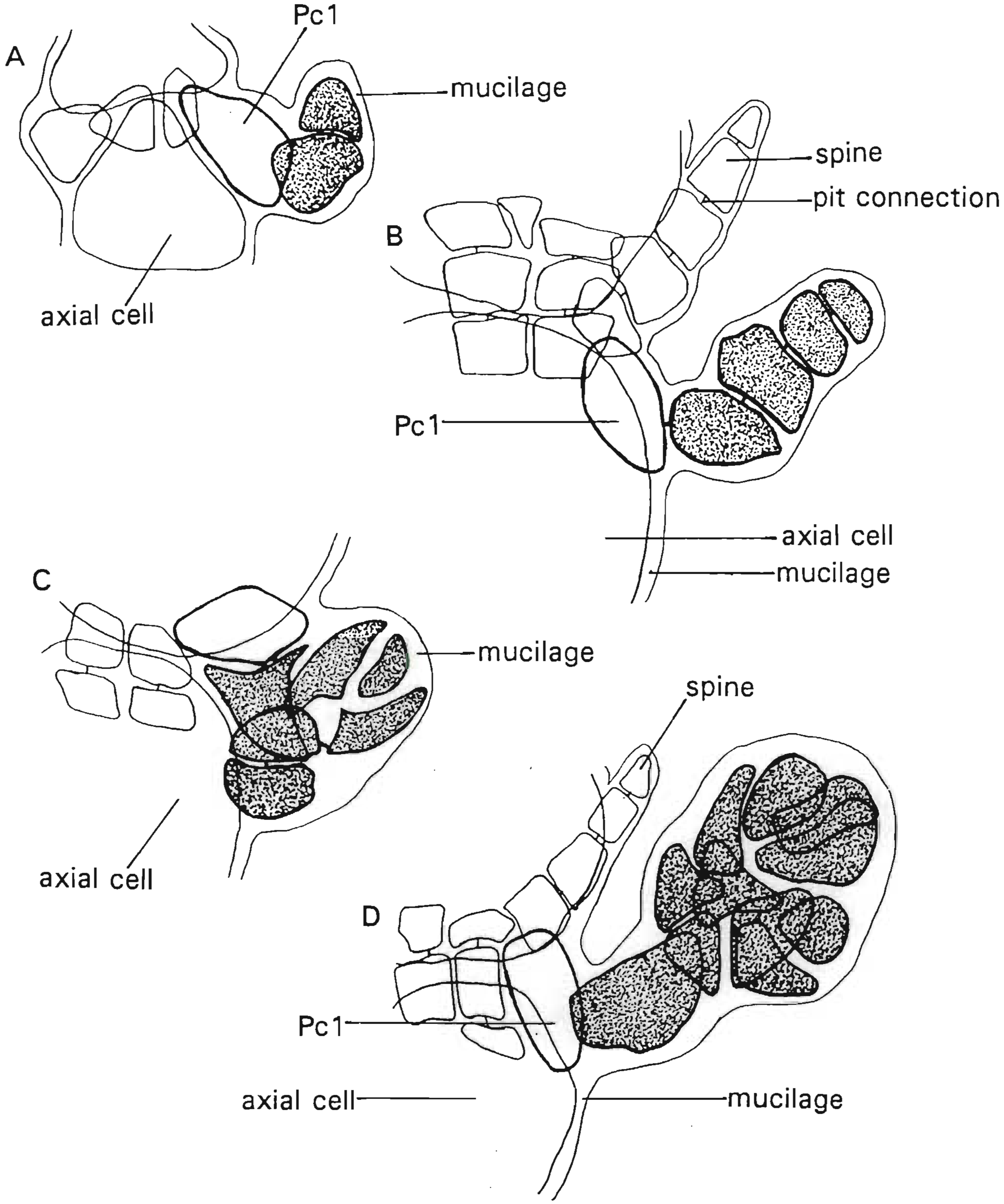


Fig. 3.1 Development of the female carpogonial branch and carposporophyte in *Pteroceramium*. A) Developing carpogonial branch at the two-cell stage. B) Four-celled carpogonial branch. C) and D) Development of the carposporophyte. At this stage, they are still colourless.

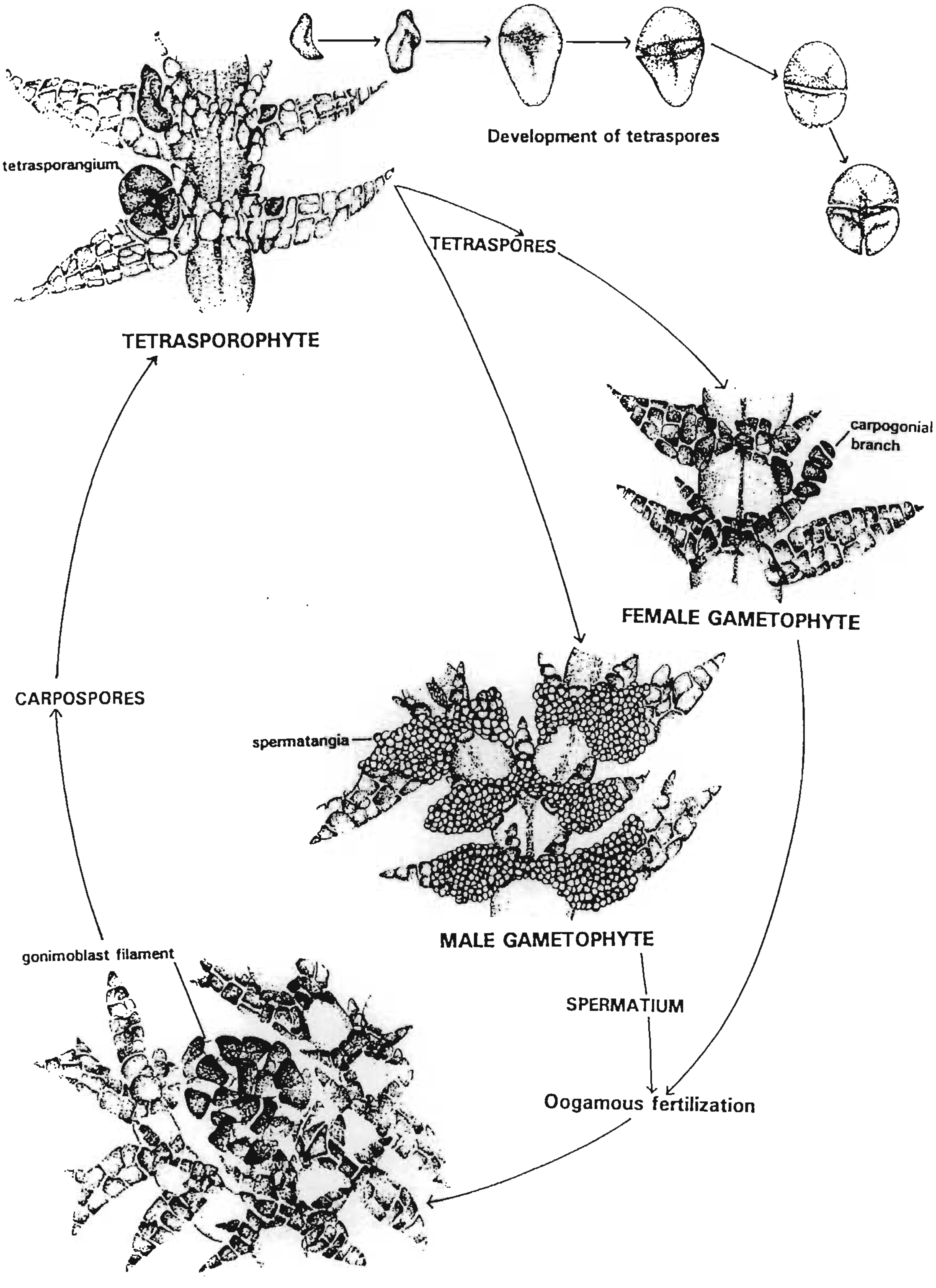


Fig. 3.2 Schematic life history of *Pteroceramium*.

position of syngamy and meiosis (DIXON, 1982). Most life history variations at familial and generic level involve some apomictically produced spores which are often derived from structures occurring in the sexual life history. Thus there is a great need for cytological investigations to determine the site of meiosis (MARTIN, 1969).

Reproductive studies of *Pteroceramium* were limited as only the male gametophyte is established in culture and it is difficult to find living specimens in the field. The full life history of *Pteroceramium* cannot be determined as developmental cycles and chromosome counts cannot be done. However, from the fixed material available, *Pteroceramium* appears to show a typical *Polysiphonia*-type life history. This accords with its classification into the Ceramiaceae. As *Pteroceramium* is so similar to *Ceramium* species, there is little doubt about ploidy and type of life cycle. The main difference is that *Pteroceramium* has decussate cruciate tetrasporangia whereas the majority of *Ceramium* species have tetrahedral tetrasporangia, although there are a few examples of cruciate division patterns (DIXON, 1960a; WOMERSLEY, 1978).

In laboratory based studies, *Ceramium shuttleworthianum* (Kützing) Silva and *Ceramium pedicellatum* Candolle completed their life history in six months and *Ceramium rubrum* (Hudson) C.Agardh in one year (EDWARDS, 1973). Life histories are known to range from one month in *Callithamnion byssoides* Arnott *ex* Harvey in Hooker to three years in *Membranoptera multiramosa* Gardner (EDWARDS, 1973). The period of time needed for *Pteroceramium* to complete its life history could not be determined.

A common assumption is that sex results in genetic recombination and diversity among progeny. SEARLES (1980) proposed that the triphasic life history "maximizes the potential for genetic recombination and genetic diversity from the union of one pair of gametes." It is thought that syngamy in the ancestral red algae was relatively rare owing to non-motile gametes relying on chance delivery by water currents. Evolution would need to favour selection whereby the zygote had a very high survival expectation as well as the ability to produce many diploid cells to compensate for the lack of motile gametes. The production of numerous diploid cells is a common phenomenon in diplohaplobiontic life histories; e.g. all higher plants produce a large number of diploid cells mitotically from the zygote and many of these cells have the potential to divide meiotically (SEARLES, 1980).

The triphasic life history can be viewed as a compensatory adaptation to multiply the diploid genome before meiosis. Also, after fertilization there may be a proliferation of gametophytic tissue to form a protective envelope (cystocarp) which provides nourishment and shelter for the developing carposporophyte. The essential element in this life history is the "retention, nurture and replication of the zygote" (SEARLES, 1980).

Several dozen carposporophytes may be found on a single thallus and this argues against rare syngamy. However, the ratio of number of carposporophytes to relative production of gametangia is high. Carposporophytes may also be formed apomictically; e.g. *Gigartina papillata* (C.Agardh) J.Agardh giving a false indication of the success rate of syngamy (SEARLES, 1980). There are some adaptations to increase the chances of syngamy including:

1) the development of a trichogyne;

2) amoeboid movement of the spermatium over the surface of the female gametophyte to reach the carpogonium; and

3) the release of spermatia in slime strands, allowing for self fertilization and fertilization in closely spaced populations (SEARLES, 1980).

No trichogyne was visible on the carpogonial branch of *Pteroceramium* and so it is not known if this is a possible adaptation to increase the chances of syngamy. *Ceramium* species do have trichogynes. However, it can be assumed that syngamy is fairly successful in *Pteroceramium* as there were numerous developing carposporophytes on a single plant.

The tetrasporophyte appears superfluous in the triphasic life history as meiotic division at the time of carpospore production results in a diversity of haploid spores as seen in the *Liagora tetrasporifera*-type life history. However, a free living diploid individual has a potential pool of recessive alleles, a means of further replication of the diploid genome especially if the genotype is maintained asexually, and if it is phenotypically distinct, an alternative ecological niche (SEARLES, 1980). Another important function of the carposporophyte may be for dispersal (HAWKES, 1990).

The costs of reproduction are a trade-off among different components of an organism's life history with these costs being a fundamental constraint on the evolution of life history patterns. Thus life history evolution can be viewed as "the competitive allocation of resources to growth, maintenance and reproduction" (REZNICK, 1992).

There are two main theories for the widespread occurrence of sexual processes:

1) long term explanation - sexually reproducing populations evolve faster than populations without sex. This is the more generally accepted theory. With asexual reproduction, after the establishment of a favourable mutation at one locus, the descendants of that mutant have to increase in number sufficiently to give a reasonable chance for a second favourable mutant to arise among the descendants of the first, slowing down evolution. In large populations, there are a number of different loci at which favourable mutations could possibly occur. Sexual reproduction is also faster when colonizing a new environment as two merging populations can combine their best genotypes (MAYNARD SMITH, 1971). The main criticism of this theory is that the advantages of sexual reproduction are long term, conferred on a group as a whole; i.e. sexual reproduction requires group selection (MAYNARD SMITH, 1971; TREISMAN, 1976); and

2) immediate explanation - sexual reproduction produces offspring with a wide range of phenotypes, therefore having a greater immediate chance of

> producing some offspring of high fitness. For sexual reproduction to be advantageous, variation in the environment is necessary. If dispersal is not random, sexual reproduction is disadvantageous as an offspring has the best chance of surviving if it is identical to its parent which is already established in the environment. This is achieved by asexual reproduction. However, sexual reproduction is advantageous in random dispersal and in unpredictable environments (MAYNARD SMITH, 1971).

Sexual reproduction is inefficient as half the genotype is lost during meiotic oogenesis, whereas asexual reproduction preserves the whole genotype. The main advantage of sexual reproduction is in an environment where variation is frequent. This affects the individuals and produces results by selection between them. Also, adaptation to major environmental variables is likely to be polygenic rather than depending on a single locus. Thus, sexual reproduction confers more resilience to adapting to major environmental fluctuations, whereas in a constant environment asexual reproduction is better. With a larger polygenic system there is a wider range of potential genotypes. In constantly changing environments, asexual reproduction would be weeded out by changing adaptational demands with sufficient frequency and there would be pressure selecting against pre-adaptation. Species which live in unstable environments have obligatory sexual reproduction. As the environment is always changing this theory is plausible (TREISMAN, 1976).

It is generally accepted that sexual reproduction does not give an immediate advantage and is inefficient, transmitting only part of the genotype but is maintained for the favourable long-term effects on evolution potential and group survival. However, it cannot be true to assume that sexual reproduction has short-term disadvantages as populations exist which produce both sexually and asexually, implying no net disadvantage to sexual processes. By implication, sexual reproduction must have approximately a two-fold advantage to balance the cost of meiosis (WILLIAMS and MITTON, 1973).

Most Rhodophyta have both sexual and asexual reproduction. However, there is variation in the sporophyte: gametophyte ratio and from the 1:1 gametophyte sex ratio (HAWKES, 1990). In many species of the Ceramiales, the tetrasporophytes outnumber the gametophytic plants (EDWARDS, 1973). These variations could result from greater fecundity and survivorship of one phase or from either obligate or facultative asexual processes which allow for persistence of a species when environmental conditions are not favourable to sexual reproduction. There is also a geographical component with asexual reproduction being prevalent at the limits of the population's distribution (HAWKES, 1990). Within the Ceramiales in Europe, the tetrasporophyte usually extends farther north than the gametophytes and at the extreme northern limits, there are only vegetative thalli. This could be a "physiological expression of reproductive capacity" (EDWARDS, 1973).

All the sexual phases of *Pteroceramium* were found together. This could possibly indicate that it was growing in ideal conditions rather than near the limits of its geographical range. The geographical range for each phase is not known.

"Bet-hedging" with divergent morphologies may be an adaptation to different sources of mortality. Crusts are more susceptible to overgrowing by competing organisms while erect axis are more susceptible to grazing pressure and mechanical damage. A species will be able to survive best in unpredictable circumstances if there is continuous production of both phases (MAGGS, 1988). Heteromorphic life histories have evolved under different environmental constraints and so extend the range of habitats and reduce the likelihood of local extinction (SHEPHERD, 1981; LITTLER, LITTLER and TAYLOR, 1987; LUXORO and SANTELICES, 1989).

Within the *Polysiphonia*-type life history there are examples of biological differences between isomorphic phases and this could affect distribution; e.g. in *Iridaea laminarioides* Bory it was found that the gametophytes had a growth optimum of 20°C and long days (16 hours) while the sporophytes preferred 15°C and short days (12 hours). Sporophytes could withstand only one hour emersion while gametophytes three hours. Herbivores also preferred young tetrasporophytes to young gametophytes (LUXORO and SANTELICES, 1989). In *Callithamnion baileyi* there was no significant difference between the growth rates of tetrasporophytes and gametophytes in daylength and light intensity (WHITTICK and WEST, 1979).

Comparisons of tetrasporophytes, male and female gametophytes of *Polycavernosa debilis* (Forsskål) Fredericq *et* J.Norris showed no significant differences in net production, herbivore resistance and calorific values. Thus no short-term ecological or physiological differences could be attributed to ploidy level. It appeared that "a single morphological form represents the best compromise in fitness" (LITTLER, LITTLER and TAYLOR, 1987).

In most organisms, either the haploid or diploid phase dominates. However, these sexual phenotypes may change seasonally. In *Gracilaria verrucosa* there is higher mortality and lower survivorship in haplospores than diplospores. This may be because diplospores have better protection against deleterious effects of somatic mutations owing to complementation in contrast to haplospores. However, the reason for maintaining the haploid-diploid life history may be the different selective advantages presented; i.e. haplospores have a higher dispersal ability and therefore can colonise new sites while diplospores have a higher survivorship and so can expand established populations (DESTOMBE, VALERO, VERNET and COUVET, 1989). This could possibly apply to *Pteroceramium*.

The isomorphic phases of *Pteroceramium* can be presumed to have no biological differences as they were found growing together in the same habitat and all were reproductive. However it is not known how these phases vary seasonally and the specimens could possibly have been collected at a time when there was overlap between the seasonal growth of the various phases. More extensive collections throughout the year need to be made to verify this.

Phylogenetic relationships are important and emphasis is now being placed on morphological and biological species concepts with many hybridization experiments being done. The auxillary cell may have evolved as a site for the introduction of morphogenetic factors that either initiate gonimoblast filaments or transform their mode of development or as an isolating mechanism that operates at a second level to reject incompatible fertilization (HOMMERSAND and FREDERICQ, 1990). Adherence of connecting filaments or cells to an auxillary cell appears to involve cell-specific interactions.

Results of some hybridization experiments are *Palmaria palmata* var. *mollis* from the Pacific was not interfertile with *Palmaria palmata* var. *palmata* from the Atlantic and so was elevated to specific status (HAWKES, 1990). Within the *Ceramium diaphanum-strictum* complex, *Ceramium strictum* from America is morphologically indistinguishable from the Norwegian isolate but they are intersterile, suggesting a taxon within a species complex with a hybrid zone between *Ceramium tenuicorne* Kützing and *Ceramium strictum* (HAWKES, 1990). *Microcladia coulteri* Harvey and *Microcladia californica* Farlow have a similar number of chromosomes but hybridization experiments showed connections between spermatia and trichogynes but no cystocarp development (GONZALEZ and GOFF, 1989). Hybridization experiments between *Polysiphonia boldii* Wynne *et* Edwards from the Gulf of Mexico and *Polysiphonia hemisphaerica* Areschoug from Scandinavia showed that many tetraspores were formed and released from these crosses but had low germination (0.7%) compared to *Polysiphonia boldii* x *Polysiphonia boldii* which showed 70% tetraspore germination. Thus these two species are interfertile but have greatly reduced viability. This may indicate disjunct populations in the process of speciation (RUENESS, 1973). BOO and LEE (in HOMMERSAND and FREDERICQ, 1990) carried out interspecific crosses with *Antithamnion sparsum* Tokida and *Antithamnion defectum* Kylin and found that gonimoblast development and carpospore release occurred when *Antithamnion sparsum* (male) was crossed with *Antithamnion defectum* (female). A cross the other way around resulted in fertilization and a normal auxillary cell and connecting cells being cut off and enlarging. However, the connecting cell failed to fuse with the auxillary cell and further development ceased at this point.

It would be interesting to carry out crosses between *Pteroceramium* and some *Ceramium* species to determine how compatible or closely related these genera are as "where feasible, when making taxonomic decisions, results of crossability tests should always be employed in conjunction with morphological, ecological, karoylogical and genetic data" (HAWKES, 1990).

Chapter 4

Environmental factors affecting growth and morphogenesis

4.1 Introduction

Growth is a response to some trigger, often environmental, with some algae anticipating favourable seasons while other algae respond to seasons (KAIN, 1989). If environmental factors satisfy the primary physiological needs of an organism, they directly govern the onset of activities of the organism at certain times of the year. If a longer preparatory phase for completion of developmental steps is required, these are triggered by a reliable and predictable environmental variable such as ambient temperature or photoperiod (LÜNING and TOM DIECK, 1989; LÜNING, 1990).

A prerequisite for growth is that total energy and carbon fixed by photosynthesis exceed that used by respiration for maintenance metabolism (LOBBAN, HARRISON and DUNCAN, 1985; KIRST, 1990). If an alga is grown over a gradient of a single environmental parameter and there is a significant change in the alga's performance, this parameter is considered limiting. A limiting factor is an essential resource that is present in quantities most closely approaching the critical minimum needed by the organism. If the limiting factor is increased, the population will grow faster until the level of some other essential resource becomes limiting. As growth is also limited by excess, species have an upper and lower tolerance limit to different factors. To survive an alga must be flexible in its requirements and be able to endure less than optimum conditions which are still within its physiological tolerance limits (DARLEY, 1982).

Morphological variation is a widespread phenomenon in many seaweeds (D'ANTONIO and GIBOR, 1985). External form of the thallus is a major criterion for taxonomic discrimination (MURRAY and DIXON, 1973). Phenotypic plasticity correlated with diverse ecological, seasonal and geographical conditions is a major problem in marine algal systematics with many new species being erected on minor morphological criteria (GARBARY, 1979a). There is also morphological plasticity to enhance the acquisition of resources for growth (HANNACH and WAALAND, 1989). To understand morphological variation, something must be known of the effects of diverse ecological conditions on growth (MURRAY and DIXON, 1975).

For the genus *Ceramium*, eight morphological characters have commonly been used to separate species:

1) the number of pericentral cells which may vary from 4-10 but are relatively constant for each species;

2) the development of the cortical band which is the principal diagnostic feature. Little is known about factors controlling the development of the band;

3) the dimensions of the axial cells which are important in determining the dimensions of the frond;

4) the "index of cortication" which is the ratio of the length of the cortical band to the length of the adjacent non-corticated portion of the axial cell;

5) the occurrence of adventitious branches of unlimited growth;

6) the distance between pseudodichotomies;

7) the curvature of the apices which vary between species from being strongly incurved to straight; and

8) the spines which are modified lateral branches of limited growth (DIXON, 1960a).

Of these eight characters, only three are considered to be taxonomically useful; viz. curvature of the apices, development of the cortical band and the morphology of spines. The number of pericentral cells may also be used with caution (DIXON, 1960a).

In field experiments it is difficult to delineate precise causes affecting algal growth and morphology as several factors vary simultaneously, while in the laboratory, each factor can be experimentally controlled (EDWARDS, 1979; D'ANTONIO and GIBOR, 1985; FREDRIKSEN and RUENESS, 1989). Thus laboratory experiments provide valuable information on the potential influence of environmental factors on algal morphology and growth even though the artificial, constant conditions should always be appreciated (KAIN and NORTON, 1990). Unialgal or axenic conditions have no natural interactions with other organisms and higher nutrient levels. Therefore, one must be careful in extrapolations to natural conditions (DARLEY, 1982). Laboratory conditions may differ so widely from those occurring naturally in the sea that the physiological state of algae may change (SCHÖNE and SCHÖNE, 1982).

All factors interact and the effect of any one factor may depend on the status of another factor; e.g. light and nitrogen, nutrient status and water motion (HANNACH and WAALAND, 1989) with the optimum level of one factor under certain conditions being sub-optimum under other conditions (SOEDER and STENGEL, 1974). There is also a diverse response from algae owing to variation in age, reproductive stage, collection site and nutrient status (FREDRIKSEN and RUENESS, 1989). Growth optima and tolerance ranges may vary greatly between developmental stages with growth to maturity depending on survival at microscopic stages (HANNACH and WAALAND, 1989).

The objectives of this study of response of *Pteroceramium* to changing environmental conditions were twofold:

1) to gain an understanding of the physiological tolerance limits of *Pteroceramium* under a range of environmental conditions so as to provide some insight into the ecological requirements of the alga and its possible geographical distribution on the east coast of South Africa; and

2) to determine the morphological stability of the wings of *Pteroceramium* and to assess their usefulness as a diagnostic character in delineating the experimental alga from *Ceramium*.

4.2 Materials and Method

4.2.1 Experimental apparatus

Seawater was collected from the Natal coast and stored in 20 litre polyethylene drums at room temperature. Seawater was double filtered through Whatman's No. 1 filter paper and then diluted to 90% seawater using distilled water to prevent the formation of precipitates during autoclaving. PES nutrients were then added and the enriched seawater autoclaved for 10 minutes at 121°C and 120 kPa. Heat sterilization (autoclaving) removes CO_2 from the medium, thereby raising the pH. The seawater was also allowed to stand for at least 24 hours after autoclaving before use, to allow the pH to return to normal.

Experiments were carried out in pre-packed, sterile plastic repli-dishes (Bibby Sterilin Ltd, United Kingdom). Wherever possible, all equipment and media were autoclaved. Apical tips comprising 2-4 segments were added aspectically to 4 ml PES in separate compartments in the repli-dishes. By using clonal material, genetic variation was greatly reduced (D'ANTONIO and GIBOR, 1985). Eight replicate plants were used per treatment. The repli-dishes were sealed with parafilm. The biomass: culture volume ratio was sufficiently low to prevent other factors; e.g. micronutrients, from becoming limiting (DeBOER, GUIGLI, ISRAEL and D'ELIA, 1978).

4.2.2 Preliminary experiment with various growth media

Pteroceramium was grown in various media to determine which was the most suitable. The growth media tested were:

1) 0.45 μm membrane (Schleicher and Schüll, West Germany) filtered seawater with no additional nutrients;

2) MET 44, a medium based on filtered seawater slightly enriched with essential nutrients, trace metal and vitamins (SCHÖNE and SCHÖNE, 1982). This was filter sterilised through a 0.45 μm membrane filter (Appendix A.2);

3) Erdschreiber Enriched Seawater sterilized by autoclaving for 10 minutes (Appendix A.3); and

4) PES which was also sterilized by autoclaving.

The experiment ran for 18 days. The vigour of the material was noted every third day using a dissecting microscope (Wild Photomicroscope M400, Germany) although growth was not quantified.

4.2.3 Collection and analysis of data

Every second day during each of the experiments listed below (Section 4.2.4), the total number of axial cells and the total thallus length were measured using an inverted microscope (C.K. Olympus, Japan). These measurements were transformed to logarithms (base$_{10}$) and growth curves constructed by plotting the logarithm of axial cell number or thallus length against time (days). The slope of the curve was taken as the relative growth rate (RGR; LAING, CHRISTELLER and TERZAGHI, 1989). The RGR determined for axial cell number was a measure of the rate of apical cell division and the RGR determined for thallus length a measure for the rate of axial cell elongation.

When each experiment was terminated, permanent mounts of the experimental material were made, using glycerine jelly. This mounting medium was suitable as cells retained their natural size. From these slides the length (L), widest width (W) and narrowest width (w) of the axial cells, as well as the length and basal width of the wings and the number of cells in the wings were measured, using a compound microscope (Carl Zeiss, Germany). Wherever possible morphometric data of the fourth segment away from the apical tip (seg. 4) along the dominant axis were used. Five replicate plants were used for each treatment.

Axial cell volume was determined for each cell using the formula described in Figure 4.1. As the axial cells fit no described shape, each cell was viewed as two truncated cones joined base to base. The volume of each truncated cone was based on the average volume of two cylinders, one inside the other, with their diameters being the widest (W) and narrowest widths (w) measured for the axial cell. A barrel shape was the nearest fit but even for this, the curvatures of the axial cells were not as great, being almost straight in the axial cells.

Surface area of the wings was determined, using the formula for the area of a triangle (½ base x height) where the base is the basal width of the wing and the height the length of the wing. This was a measure of the area available for light interception.

All data were analyzed using the Kruskal-Wallis One Way Analysis of Variance by Ranks and significant differences revealed by Multiple Range Testing (Tukey). This was carried out using the Statgraphics 5.0 package (STSC, USA).

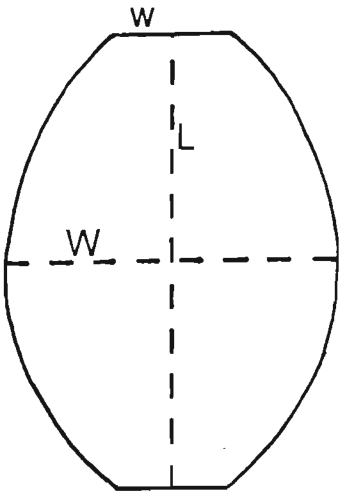
4.2.4 Environmental factors tested

Both physical and chemical factors were investigated. For all experiments, the standard growth conditions as described in Section 2.2.1 were kept constant and only the factor under investigation was varied. The factors tested were:

Physical factors

i) Irradiance - Thalli were grown at four irradiance levels; viz. 193 μmol m^{-2} s^{-1}, 129 μmol m^{-2} s^{-1}, 79 μmol m^{-2} s^{-1} and 30 μmol m^{-2} s^{-1} for 12 days in a Constant Environmental Chamber (Conviron EF7, Controlled Environments Inc, USA).

ii) Spectral quality - Light qualities tested were a control with the white light from fluorescent and incandescent lights, red light (650 nm peak), green light (545 nm peak) and blue light (450 nm peak). Monochromatic filters (Carolina Biological Supply Company, USA) fitted onto light-tight boxes were used to investigate effects of monochromatic light on growth and morphogenesis in

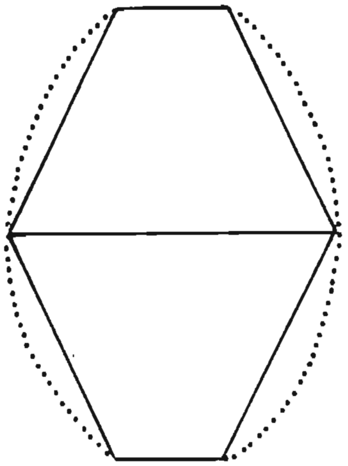


Measurements recorded for axial cell

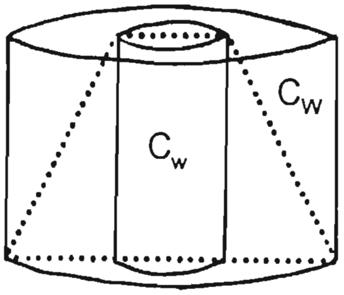
where L = length of cell

W = widest width of cell

w = narrowest width of cell



Axial cell viewed as two truncated cones joined base to base.



Volume (V) of cylinder (C) = $(d^2\pi/4)h$

where d = diameter

h = height

Calculations:-

i) V of $C_w = (w^2\pi/4)L/2$

ii) V of $C_W = (W^2\pi/4)L/2$

iii) V of truncated cone = (V of C_w + V of C_W)/2

iv) V of axial cell = V of truncated cone × 2

Note: The division in equation iii) and the multiplication in equation iv) cancel each other out and so are omitted from the final calculations.

Fig. 4.1 Formula for determining axial cell volume.

Pteroceramium. Even when the boxes were lined with aluminium foil to increase reflection, irradiance levels were very low at 1 μmol m^{-2} s^{-1} and the temperature in the boxes being 18°C. A stronger light source could not be used as this increased the temperature inside the boxes to above 30°C which would have killed the algae. The control replicates were covered with shade cloth so that they were subjected to the same low irradiance. Owing to the low irradiance levels, algal growth was very slow. Measurements were taken every third day with the experiment running for 39 days.

iii) <u>Photoperiod</u> - The five light regimes tested were $16{:}\overline{8}$ hour light:$\overline{\text{dark}}$ cycle, $14{:}\overline{10}$ hour (the longest natural photoperiod occurring on the eastern Cape coast), $12{:}\overline{12}$ hour, $10{:}\overline{14}$ hour (the shortest photoperiod occurring on the eastern Cape coast; STONE, 1988) and $8{:}\overline{16}$ hour. This experiment was carried out in photoperiod cabinets lined with aluminium foil to increase reflection, raising the irradiance levels to 35 μmol m^{-2} s^{-1}. The experiment ran for 10 days.

iv) <u>Temperature</u> - The growth of the alga was monitored at 15°C, 20°C, 25°C and 30°C. The experiment ran for 12 days. Various Convirons were set at the different temperatures.

v) <u>Sand abrasion</u> - Sand collected at Scottburgh on the Natal south coast was washed in slow running tap water for 5 hours and then oven-dried for 48 hours. The sand was separated into four grades using a brass mesh sieve (Endecotts Test Sieves, Endecotts, United Kingdom) shaken on an Endecotts Test Sieve Shaker for 5 minutes. The four grades of sand were:

Coarse - 1 mm to 1.7 mm in diameter
Medium - 0.85 mm to 1 mm in diameter
Fine - 0.5 mm to 0.85 mm in diameter
Very fine - < 0.5 mm in diameter

The sand was sterilized by autoclaving for 20 minutes. Control replicates were grown in seawater without sand. In each experimental treatment, a 1 cm layer of sand was placed into crystallising dishes (18 cm diameter) on top of the plant material and PES was added so that there was a layer of water on top of the sand. Four crystallising dishes, each containing two plants per treatment, were used. The dishes were continuously shaken on a rotary shaker. The thalli would normally float to the surface of the water. To keep the thalli in constant contact with the sand, phytoplankton netting was stretched across the top of the sand and held in place by an inverted watchglass. Each crystallising dish was covered with a petri-dish to reduce the rate of evaporation. Water levels were topped up whenever necessary.

Growth was not monitored during the experiment as the sand hid the plant material, making it difficult to find. After 14 days, the experimental material was removed from the sand and mounted onto slides for analysis.

vi) Smothering by sand - Experimental material was grown in the four grades of PES saturated sand used in the above experiment (Section 4.2.4.v.) and buried at three depths - 0.5 cm, 1 cm and 2 cm. There were two crystallising dishes each containing three plants per treatment. The apical tips of *Pteroceramium* were placed at least 2 cm away from the edge of the dish to prevent lateral illumination and each dish had a base of aluminium foil so that no light could enter from below. The plant material was grown in a Conviron with illumination from above. The experiment ran for 14 days. Growth was not monitored during the experiment but at the end of the experiment, the material was recovered and mounted onto slides for analysis.

vii) Gravity - Experimental material was grown in tumbling flasks secured to a geostat which made one revolution every 90 minutes. Six replicates were used. At the start and end of the experiment, photographs were taken of each plant using a dissecting photomicroscope. These photographs were used to determine the angle of each pseudodichotomy. Morphometric measurements were also recorded.

Chemical factors

viii) Nitrogen and phosphorus concentrations - A defined medium, Müllers Synthetic Seawater (Appendix A.4) was used so that the quantities of nutrients available to the algae were defined. Nitrogen-free, phosphorus-free, nitrogen- and phosphorus-free and normal nutrient stocks of Müllers Synthetic Seawater were prepared. Nitrogen was supplied as nitrate and phosphorus as orthophosphate. The four nutrient stocks were combined to give the required concentrations of both nutrients in the prepared media. There were four concentrations each of nitrogen and phosphorus. There was a log increase with the lowest concentrations lacking either nitrogen or phosphorus and the highest concentration having either full strength nitrogen or phosphorus. At full strength, Müllers Synthetic Seawater has nitrogen concentrations of 1.18 mM $NaNO_3$ and phosphorus concentrations of 140 μM Na_2HPO_4 (McLACHLAN, 1973). The media were filter sterilized using a 0.45 μm membrane filter. Before the experiment algae were grown in nitrogen and phosphorus free Müllers Synthetic Seawater for three days to deplete the nutrients stored in the thallus. The experiment ran for eight days.

ix) Salinity - Thalli were grown at five different salinities; viz. 20°/₀₀, 30°/₀₀, 34°/₀₀ (normal seawater), 40°/₀₀ and 50°/₀₀. Seawater was diluted with distilled water to obtain lower salinities or concentrated by evaporation to give higher salinities. PES was added and all media filter sterilized through a 0.45 μm membrane filter. The experiment ran for 10 days.

x) pH - Plants were grown at pH 5.5, pH 6.5, pH 7.5 and pH 8.5. To maintain the pH levels being tested, two different buffers were used as there was no one ideal buffer for the desired pH range. Tris (Tris-(hydroxymethyl)aminomethane) at a concentration of 0.66 mM was used to maintain the medium at pH 7.5 and pH 8.5. This was already added in the PES medium. MES (2-[N-morpholino]ethanesulfonic acid) was used to maintain the medium at pH 5.5 and pH 6.5. MES, added at 2 mM, replaced Tris in the medium. MES has a PK_a

of 6.15 (GOOD, WINGET, WINTER, CONNOLLY, IZAWA and SINGH, 1966).

At the start of the experiment, the media were adjusted to the correct pH using HCl or NaOH. The media were filter sterilized through a 0.45 μm membrane filter. The experiment ran for 12 days.

4.3 Results

4.3.1 Preliminary experiment with various growth media

All replicates in 0.45 μm membrane filtered seawater died after four days, most probably owing to lack of nutrients. The replicates in MET 44 showed no growth although they maintained their red colour. Growth was observed in all thalli growing in Erdschreiber Enriched Seawater and PES. It was decided to use PES in all further experiments.

4.3.2 Development of axial cells and wings in standard growth conditions

Preliminary measurements of the axial cells and cortical bands of *Pteroceramium* showed that cells vary in size in different parts of the thallus. These variations are not directly related to age but rather to stage of differentiation because the rate of development may differ under various conditions. If meaningful comparisons are to be made between organs or cells, they must be made on fully differentiated tissues in equivalent positions on the thallus (DIXON, 1971).

In *Pteroceramium* there was a general rapid increase in both axial cell volume (Fig. 4.2) and wing size (cell number and surface area; Fig. 4.3A and B) with increasing distance from the apical tip. By seg. 4 both the axial cell and wing development had almost reached maximum size for the given growth conditions. There was a drop in size of both axial cells and wings in seg. 6 as these cells were damaged at the start of the experiment when the thalli were cut. Wherever possible, all further comparisons on axial cell volume and wing size were made using measurements from seg. 4 as the cells appear to be almost fully differentiated at this stage.

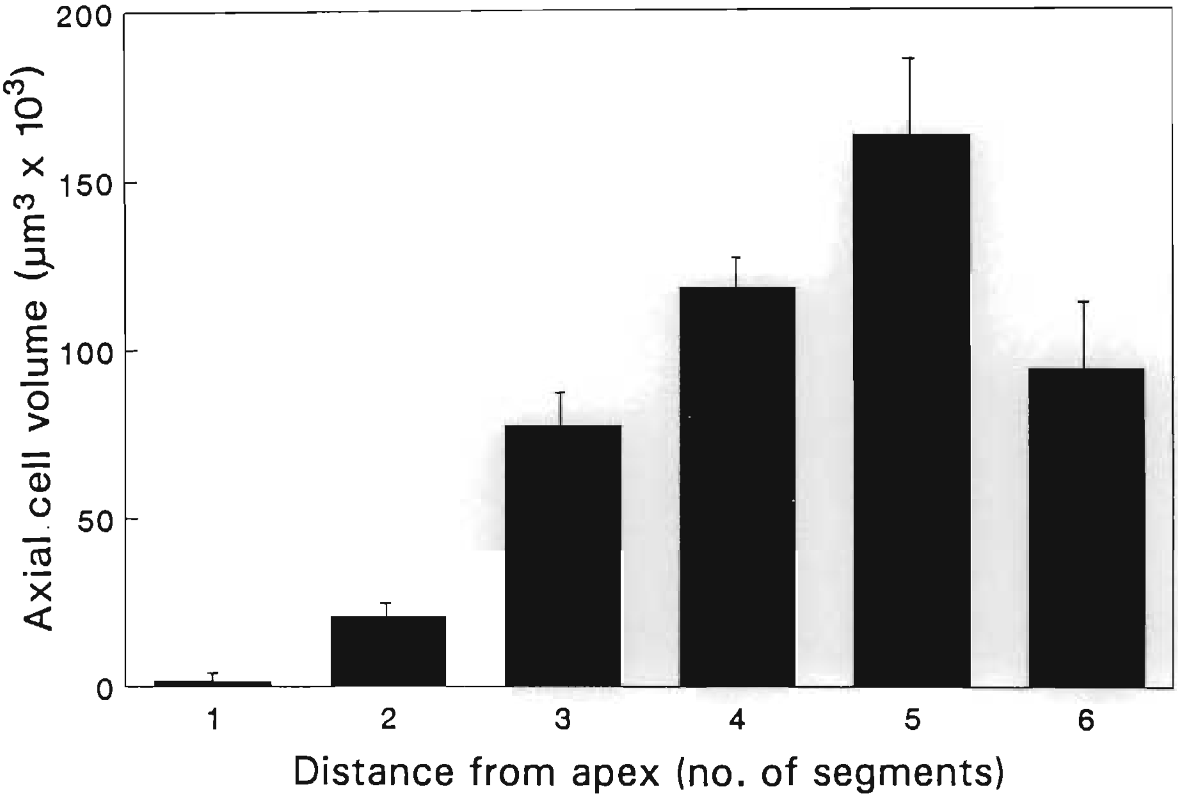


Fig. 4.2 Development of axial cells in *Pteroceramium* when grown in standard growth conditions. The average axial cell volume for each segment is shown.

4.3.3 Environmental factors

i) Irradiance - The RGRs of *Pteroceramium* determined by increase in axial cell number and thallus length, were similar for plants grown at the three highest irradiances while the RGRs of the material growing at 30 μmol m^{-2} s^{-1} was significantly lower ($P<0.01$; Fig. 4.4A and B; Table 4.1).

Plants grown in 79 μmol m^{-2} s^{-1} had significantly larger axial cells at seg. 4 along the dominant axis ($P<0.0001$) and thalli growing at 129 μmol m^{-2} s^{-1} had significantly

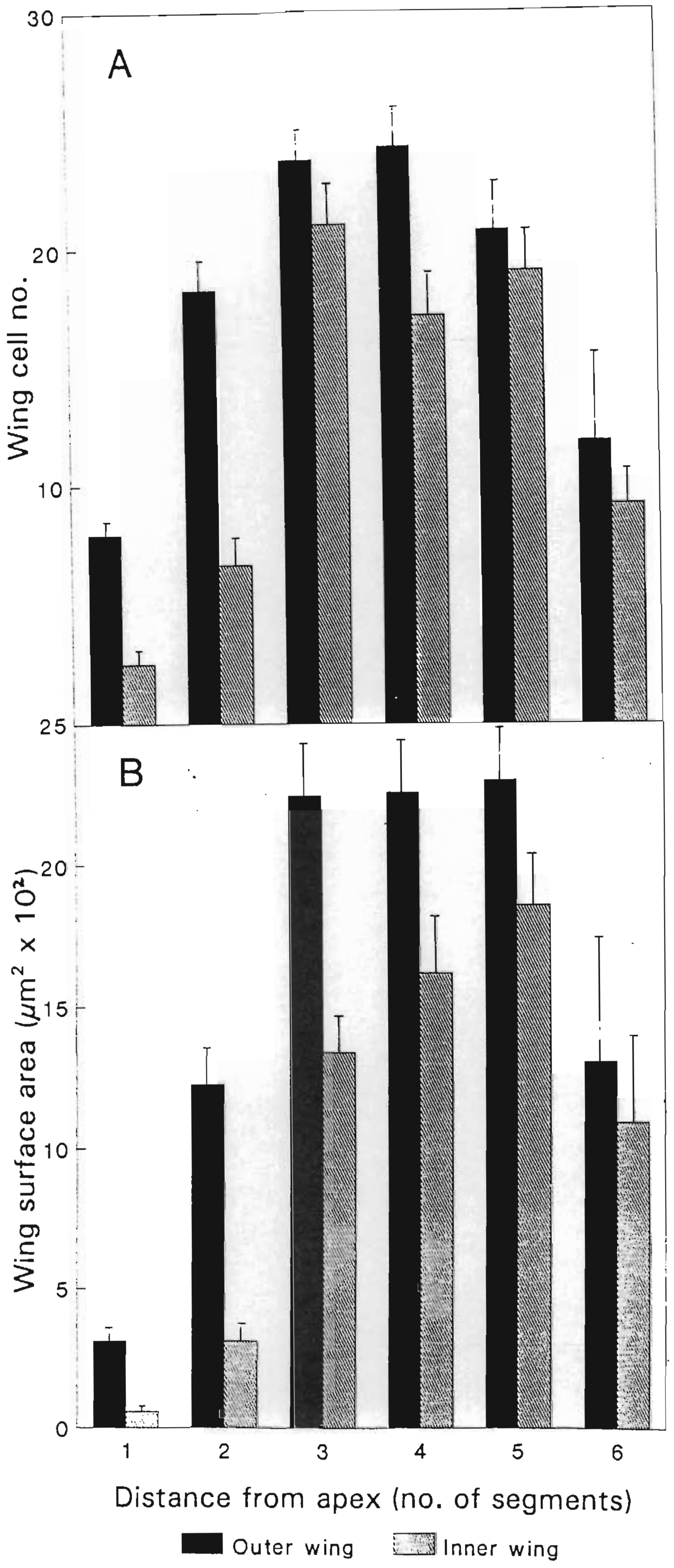


Fig. 4.3 Development of wings in *Pteroceramium* when grown in standard growth conditions. The average wing size for each segment is shown. A) Number of cells in the wing. B) Surface area of the wings.

smaller axial cells than the other treatments. Plants grown in the three lowest irradiances had similar sized wings with the wings of the material grown at the highest irradiance, being significantly smaller (outer wing cell number and surface area $P<0.0001$; inner wing cell number and surface area $P<0.01$; Fig. 4.5; Table 4.1).

The average number of axial cells in seg. 4 was similar for all treatments ($P=0.8$). It was also observed that material growing in the highest irradiance was a paler red colour, suggesting either photodamage to the pigments or adjustment in pigment content (see Chapter 5).

Table 4.1 Growth and morphometric results taken at seg. 4 of *Pteroceramium* when grown at different irradiance levels. Results are shown as mean ± standard error. RGR = relative growth rate, ax cell = axial cell, vol = volume, OW = outer wing, IW = inner wing, SA = surface area.

		Irradiance	(μmol m^{-2} s^{-1})	
Parameters measured	193	129	79	30
RGR (ax cells)	0.11 ± 0.01	0.10 ± 0.001	0.10 ± 0.001	0.07 ± 0.01
RGR (length; μm)	0.11 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.06 ± 0.01
Ax cell vol (μm^3 x 10^3)	57 ± 5	118 ± 9	197 ± 28	96 ± 11
OW cell no.	13 ± 1	24 ± 2	24 ± 1	19 ± 2
IW cell no.	10 ± 1	17 ± 2	17 ± 2	17 ± 2
OW SA (μm^2 x 10^3)	1.3 ± 0.1	2.3 ± 0.2	2.4 ± 0.1	1.7 ± 0.2
IW SA (μm^2 x 10^3)	0.9 ± 0.1	1.6 ± 0.2	1.7 ± 0.2	1.6 ± 0.4
Ax cell no. seg. 4^{-1}	6 ± 1	5 ± 1	5 ± 1	5 ± 0.4

ii) Spectral quality - The control replicates had a significantly faster RGR when calculated using both axial cell number and thallus length ($P<0.01$) while the RGR for all replicates grown in monochromatic light were similar (Fig. 4.6A and B; Table 4.2).

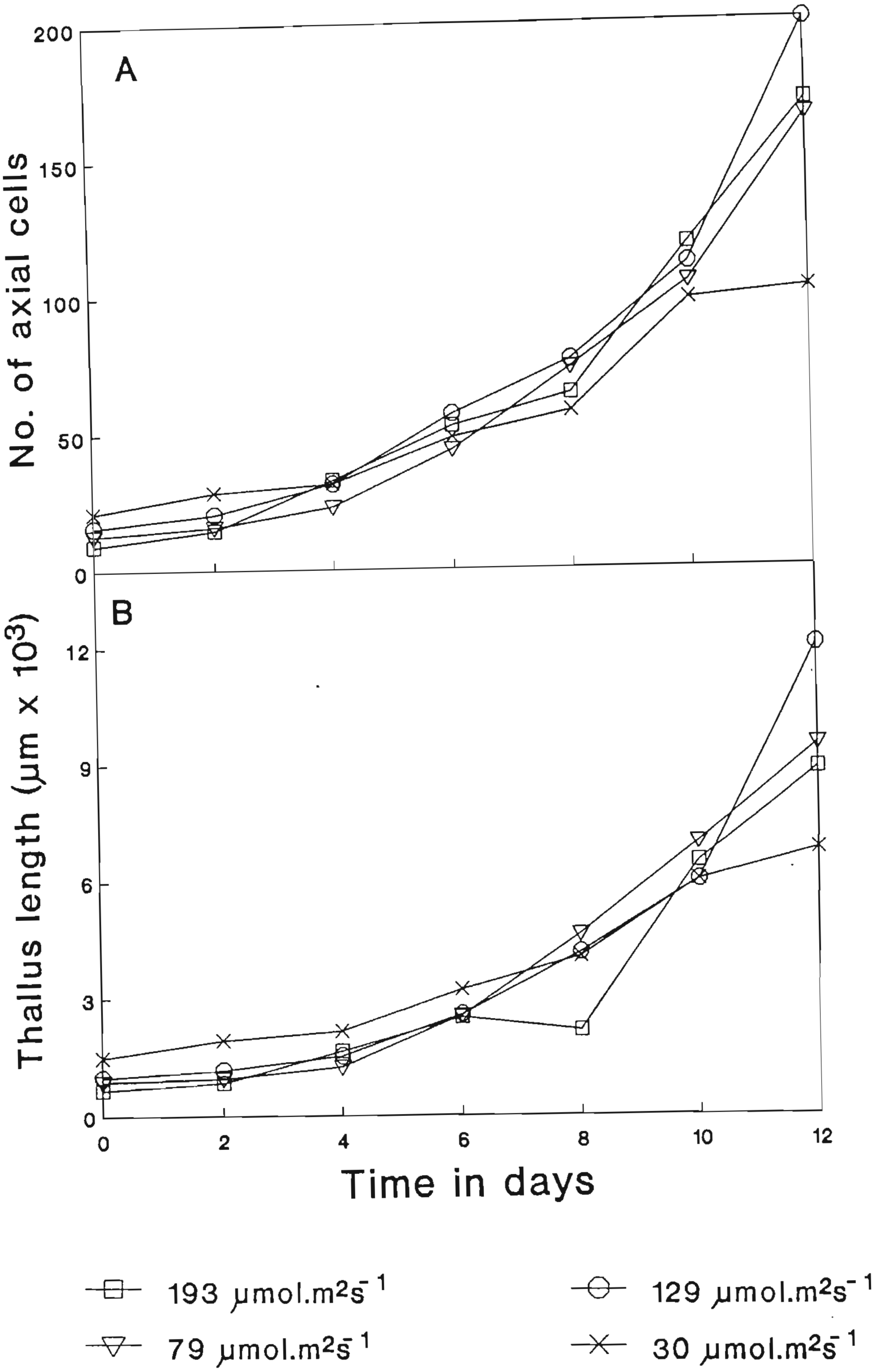


Fig. 4.4 Growth of *Pteroceramium* when grown at different irradiance levels. A) Change in number of axial cells. B) Change in thallus length.

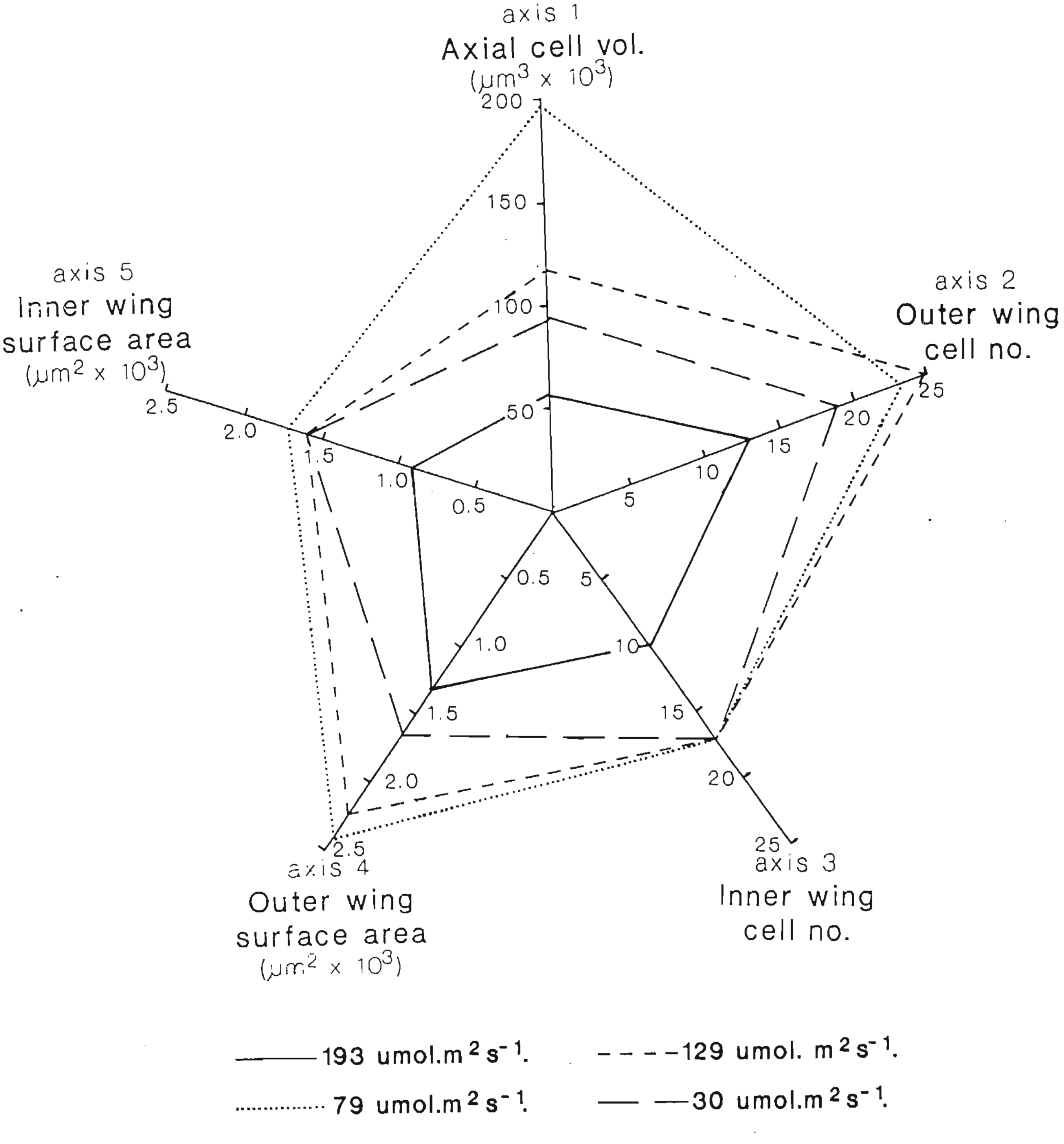


Fig. 4.5 Effect of irradiance on axial cell volume (axis 1), wing cell number (axes 2 and 3) and wing surface area (axes 4 and 5) in *Pteroceramium*. Measurements were taken at seg. 4 along the dominant axis.

Because of the slow RGRs, morphometric measurements were taken on seg. 3 rather than seg. 4. There were no significant differences ($P=0.1$) between axial cell volume for the four treatments. The outer wings of the control treatment had significantly more cells ($P<0.05$) than the other treatments. However, the outer wing surface areas were all similar ($P=0.3$). The inner wings were also similar in cell number ($P=0.4$) and surface area ($P=0.7$). There were no significant differences ($P=0.6$) in the number of axial cells in seg. 3 (Fig. 4.7; Table 4.2).

Table 4.2 Growth and morphometric results taken at seg. 3 of *Pteroceramium* when grown in different spectral qualities . Results are shown as mean ± standard error. RGR = relative growth rate, ax cell = axial cell, vol = volume, OW = outer wing, IW = inner wing, SA = surface area.

	Spectral quality			
Parameters measured	Control	Red	Green	Blue
RGR (ax cells)	0.01 ± 0.001	0.004 ± 0.001	0.005 ± 0.001	0.002 ± 0.001
RGR (length; μm)	0.01 ± 0.001	0.002 ± 0.000	0.004 ± 0.001	0.002 ± 0.001
Ax cell vol ($\mu m^3 \times 10^3$)	73 ± 12	46 ± 5	48 ± 6	86 ± 17
OW cell no.	10 ± 1	7 ± 1	6 ± 1	8 ± 2
IW cell no.	5 ± 1	4 ± 0.3	4 ± 1	6 ± 2
OW SA ($\mu m^2 \times 10^3$)	0.6 ± 0.1	0.7 ± 0.1	0.5 ± 0.1	0.7 ± 0.1
IW SA ($\mu m^2 \times 10^3$)	0.3 ± 0.03	0.3 ± 0.004	0.3 ± 0.1	0.5 ± 0.2
Ax cell no. seg. 3^{-1}	6 ± 1	6 ± 1	4 ± 2	4 ± 1

iii) Photoperiod - There were no significant differences in the RGRs of *Pteroceramium* ($P=0.05$) between any treatments when using axial cell number as a measure of growth. However, material grown in the shortest light period ($8:\overline{16}$ hour) had a significantly lower RGR for thallus length than material growing in the longer light periods ($P<0.01$). There were no significant differences between the RGRs for thallus length at longer light exposures (Fig. 4.8A and B; Table 4.3).

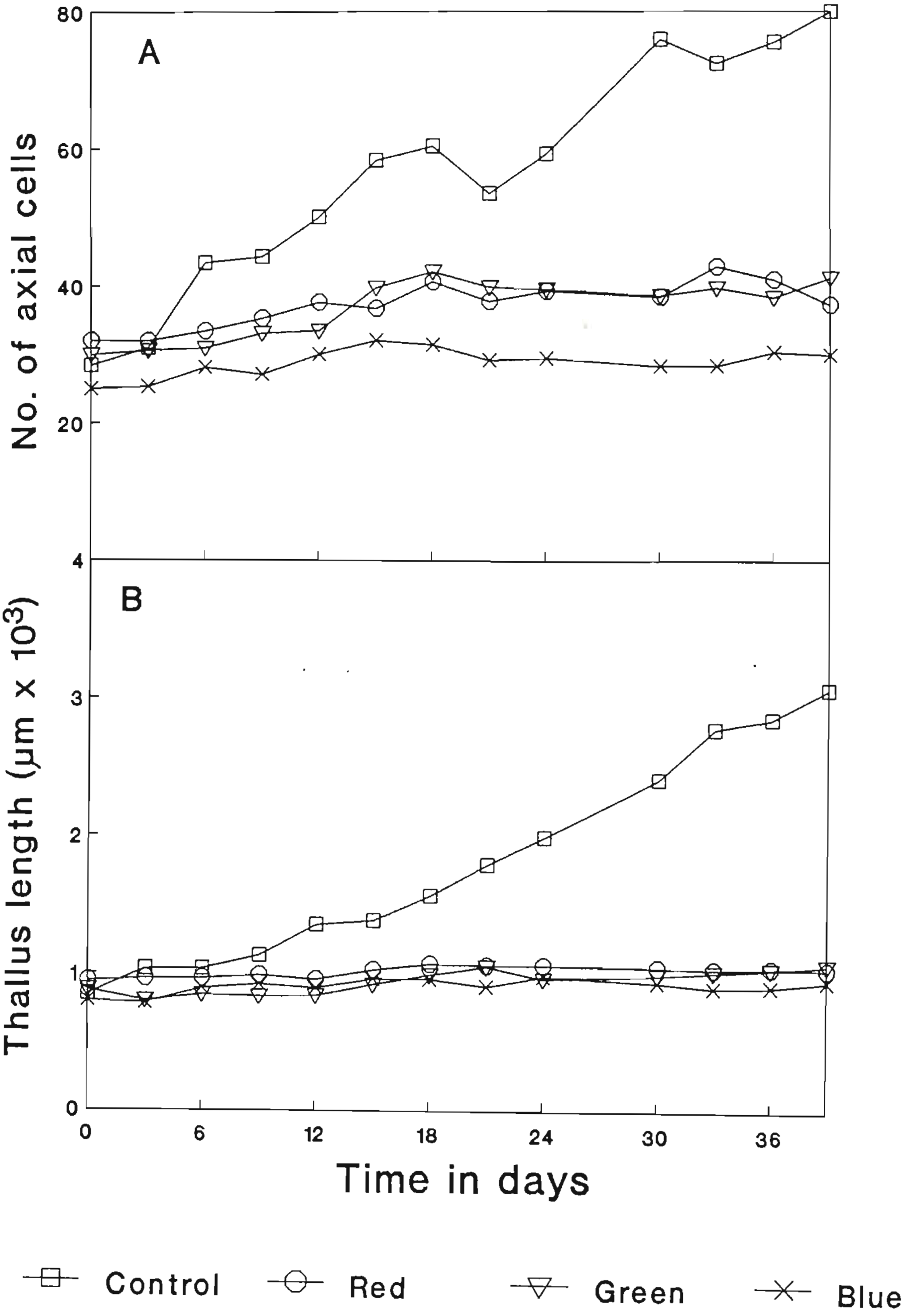


Fig. 4.6 Growth of *Pteroceramium* when grown in various monochromatic light fields. A) Change in number of axial cells. B) Change in thallus length.

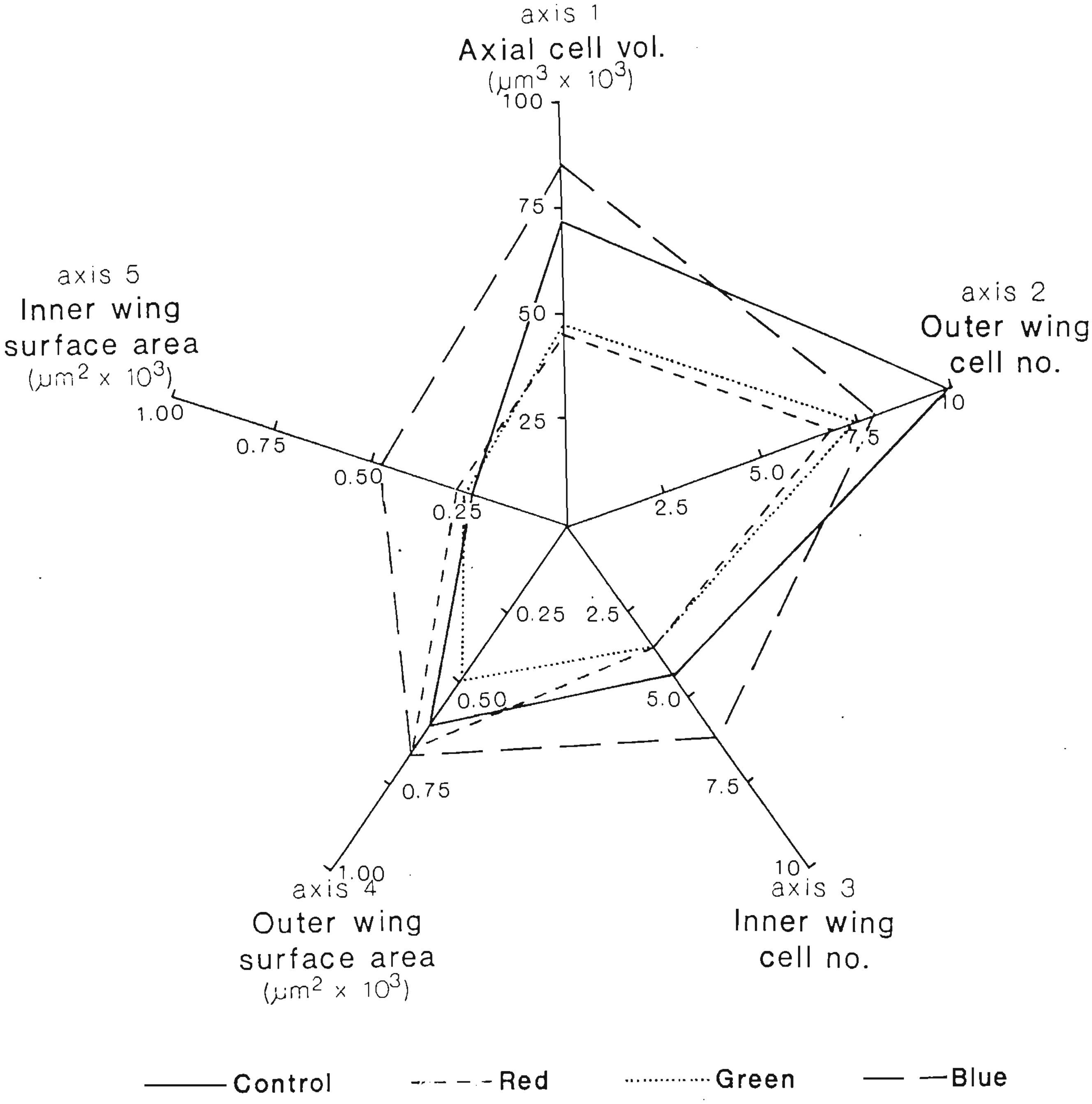


Fig. 4.7 Effect of monochromatic light on axial cell volume (axis 1), wing cell number (axes 2 and 3) and wing surface area (axes 4 and 5) in *Pteroceramium*. Measurements were taken at seg. 3 along the dominant axis.

Material growing in the longest daylength had significantly smaller axial cells at seg. 4 (P<0.0001) than all other treatments with shorter light periods. Thalli grown in 14:$\overline{10}$ hour had significantly larger outer wings (cell number P<0.01; surface area P<0.05) while material growing in 16:$\overline{8}$ hour had significantly smaller inner wings (cell number P<0.0001; surface area P<0.001) than the other replicates. Photoperiod had no significant effect on the number of axial cells in seg. 4 between any treatments (P=0.8; Fig. 4.9; Table 4.3).

Table 4.3 Growth and morphometric results taken at seg. 4 of *Pteroceramium* when grown in different photoperiods. Results are shown as mean ± standard error. RGR = relative growth rate, ax cell = axial cell, vol = volume, OW = outer wing, IW = inner wing, SA = surface area.

Parameters measured	Photoperiod (L:$\overline{D}$ hour)				
	16:$\overline{8}$	14:$\overline{10}$	12:$\overline{12}$	10:$\overline{14}$	8:$\overline{16}$
RGR (ax cells)	0.07 ± 0.01	0.05 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.04 ± 0.01
RGR (length; μm)	0.06 ± 0.01	0.04 ± 0.01	0.05 ± 0.003	0.05 ± 0.004	0.02 ± 0.001
Ax cell vol (μm^3 x 10^3)	47 ± 6	116 ± 12	78 ± 13	67 ± 18	98 ± 13
OW cell no.	9 ± 1	16 ± 1	12 ± 2	11 ± 2	8 ± 1
IW cell no.	4 ± 1	11 ± 1	8 ± 2	6 ± 1	11 ± 2
OW SA (μm^2 x 10^3)	0.8 ± 0.01	1.2 ± 0.2	1.2 ± 0.2	0.7 ± 0.1	0.8 ± 0.2
IW SA (μm^2 x 10^3)	0.3 ± 0.1	1.1 ± 0.2	0.7 ± 0.1	0.5 ± 0.1	1.0 ± 0.2
Ax cell no. seg. 4^{-1}	5 ± 1	5 ± 1	5 ± 0.3	4 ± 1	4 ± 1

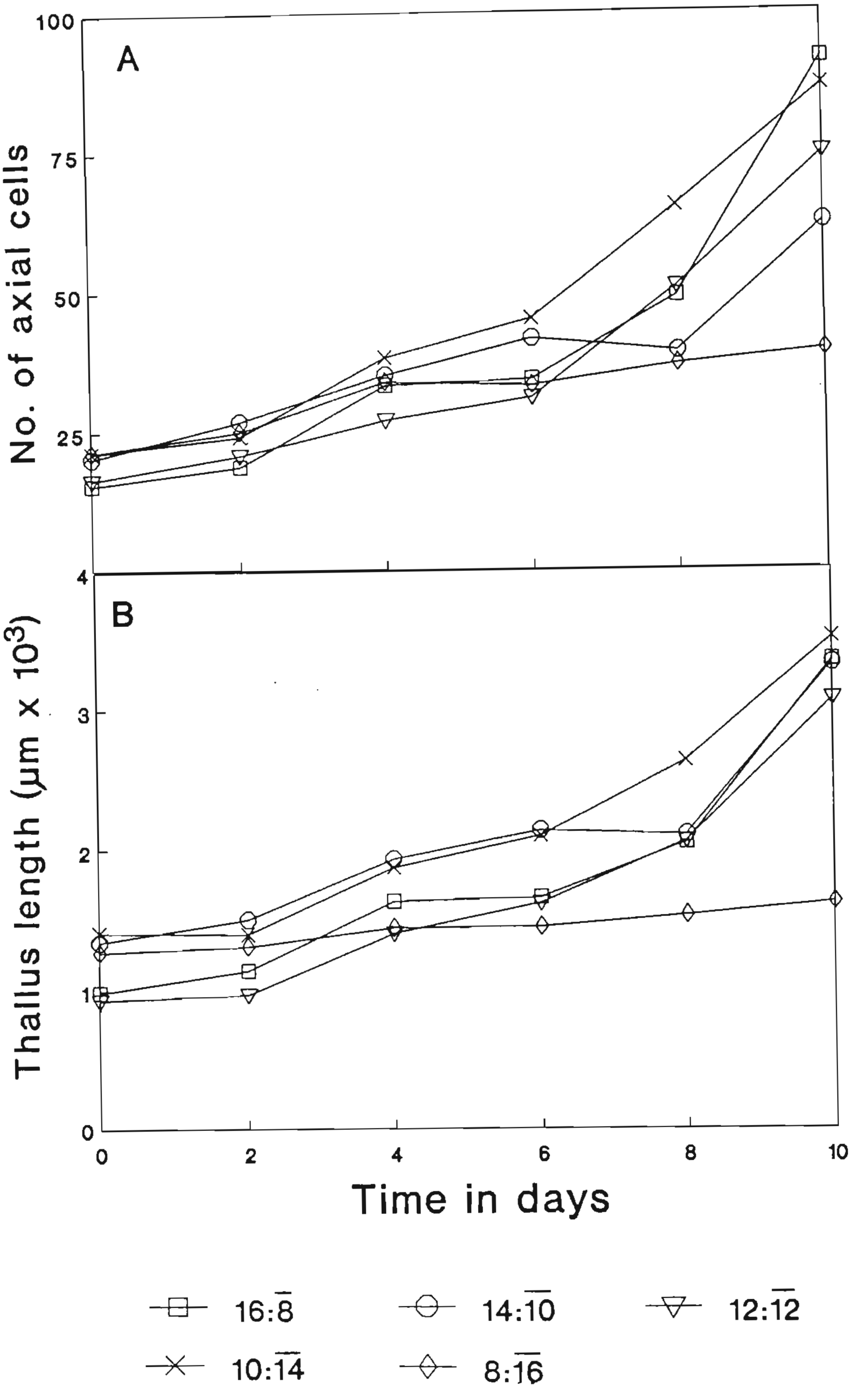


Fig. 4.8 Growth of *Pteroceramium* when grown in various photoregimes. A) Change in number of axial cells. B) Change in thallus length.

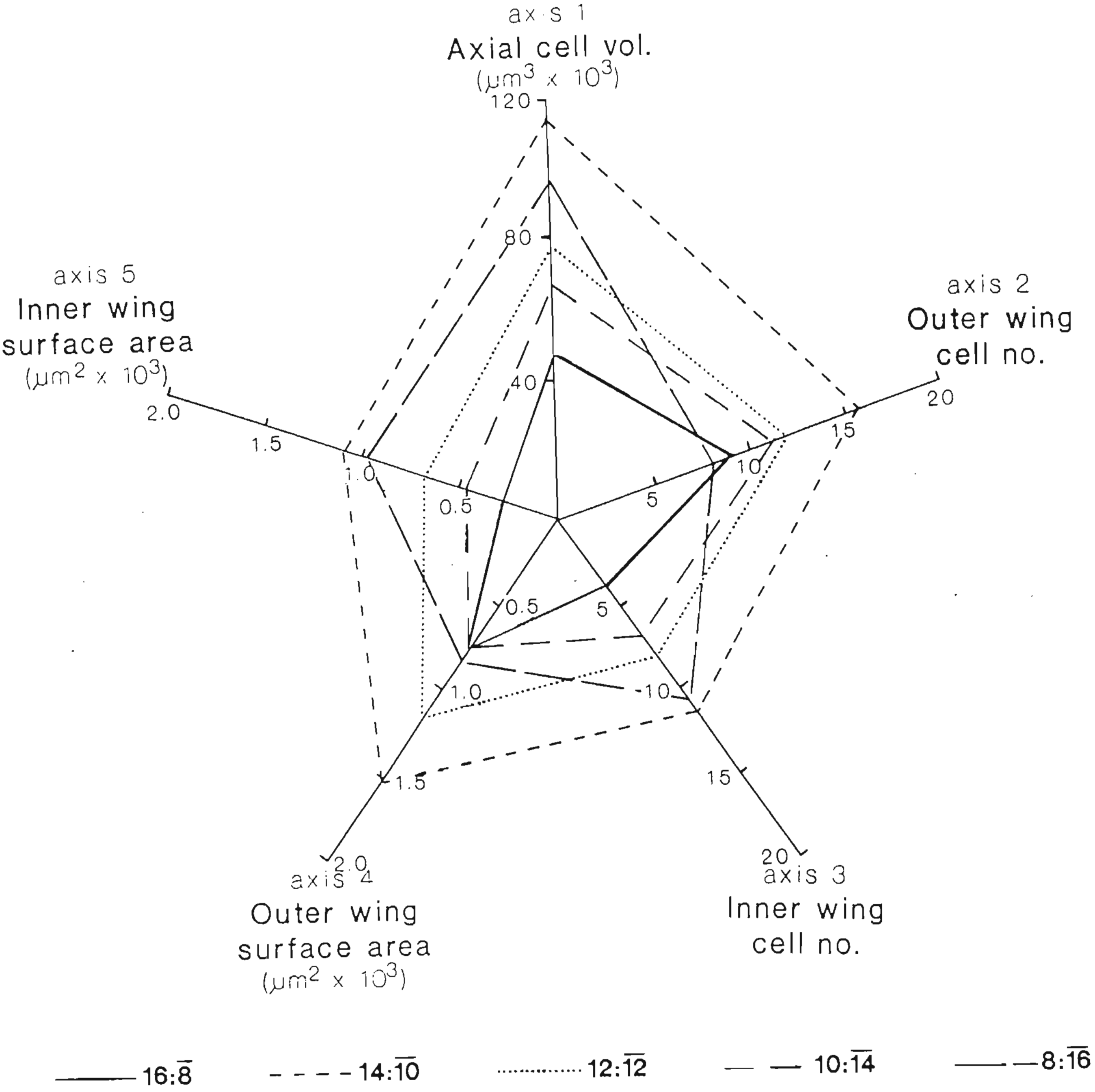


Fig. 4.9 Effect of photoperiod on axial cell volume (axis 1), wing cell number (axes 2 and 3) and wing surface area (axes 4 and 5) in *Pteroceramium*. Measurements were taken at seg. 4 along the dominant axis.

v) Temperature - The highest RGRs for both axial cell number and thallus length were recorded in the thalli grown at 20°C and 25°C. Significantly lower RGRs ($P<0.05$) were measured in the material grown at 15°C and 30°C compared to thalli growing at 20°C (Fig. 4.10A and B; Table 4.4).

No morphometric measurements were obtained for the material grown at 30°C as there was insufficient new growth. Axial cells were significantly larger at seg. 4 ($P<0.0001$) when grown at 25°C. Temperature had no significant effect on the wing cell number (outer wing $P=0.5$; inner wing $P=0.1$) and surface area (outer wing $P=0.6$; inner wing $P=0.4$). The number of axial cells per segment were not significantly different ($P=0.9$) between treatments (Fig. 4.11; Table 4.4).

Table 4.4 Growth and morphometric results taken at seg. 4 of *Pteroceramium* when grown in different temperatures. Results are shown as mean ± standard error. RGR = relative growth rate, ax cell = axial cell, vol = volume, OW = outer wing, IW = inner wing, SA = surface area.

Parameters measured	Temperature (°C)			
	15	20	25	30
RGR (ax cells)	0.01 ± 0.01	0.04 ± 0.01	0.02 ± 0.01	0.00 ± 0.00
RGR (length; μm)	0.01 ± 0.003	0.03 ± 0.01	0.02 ± 0.01	0.00 ± 0.002
Ax. cell vol (μm^3 x 10^3)	60 ± 0.2	63 ± 0.9	198 ± 4.1	-
OW cell no.	16 ± 2	13 ± 1	14 ± 2	-
IW cell no.	9 ± 2	8 ± 1	6 ± 1	-
OW SA (μm^2 x 10^3)	1.5 ± 0.3	1.1 ± 0.1	1.2 ± 0.2	-
IW SA (μm^2 x 10^3)	0.9 ± 0.2	0.7 ± 0.1	0.5 ± 0.1	-
Ax cell no. seg. 4^{-1}	5 ± 0.3	5 ± 0.3	6 ± 2	-

v) Sand abrasion - No morphometric results could be shown for the material growing in the coarse and very fine sand as there was insufficient new growth. Control

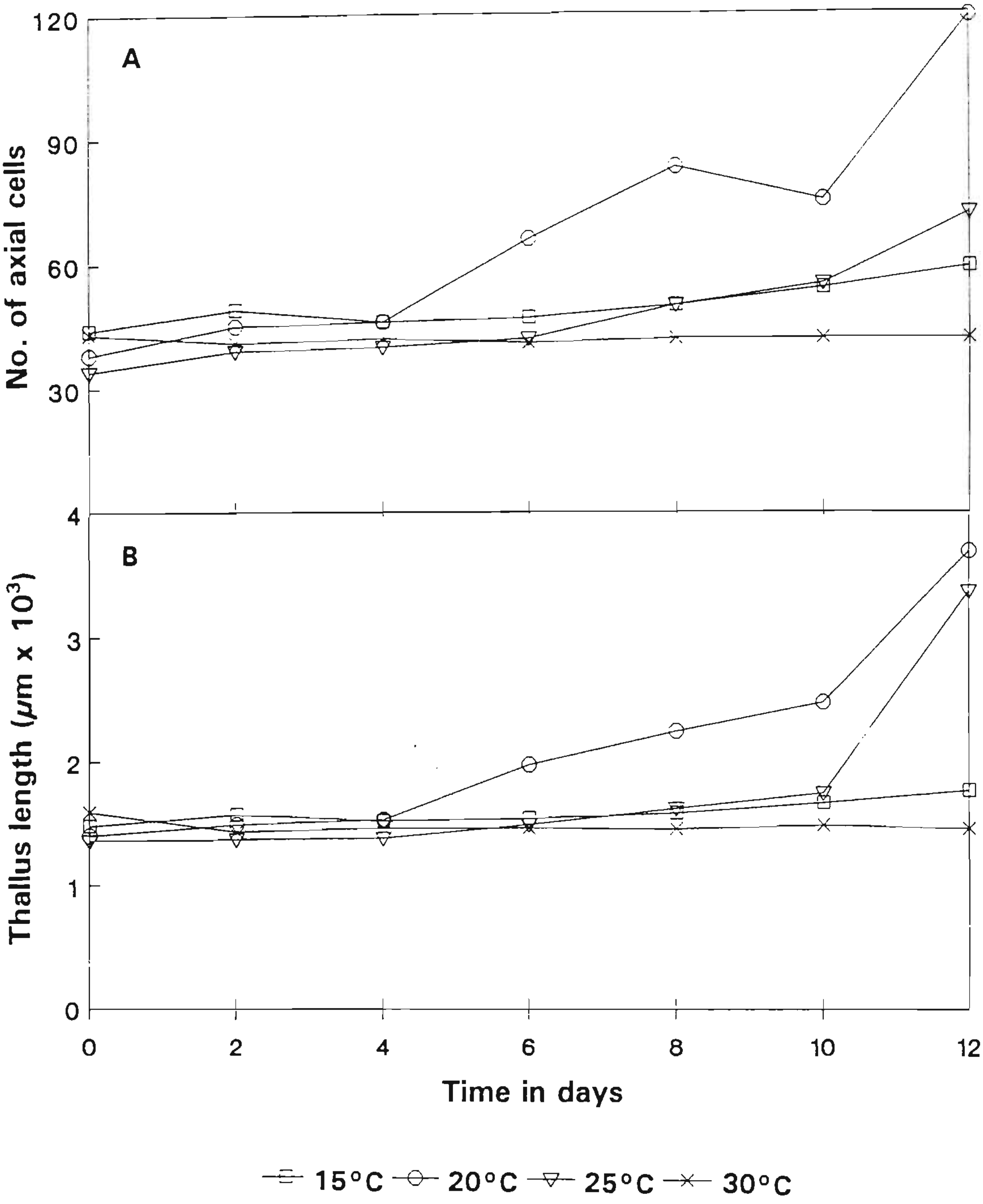
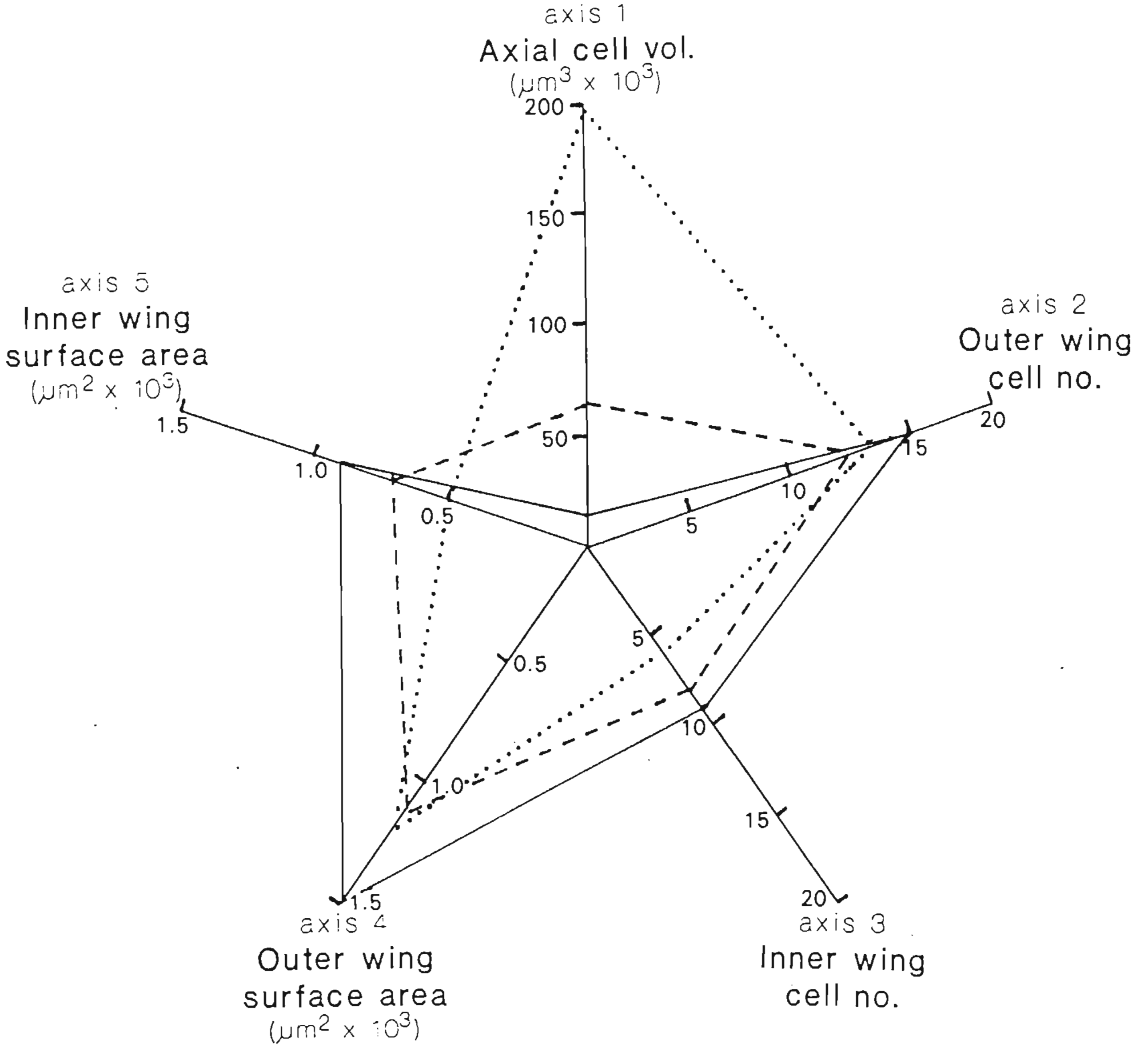


Fig. 4.10 Growth of *Pteroceramium* when grown in different temperatures. A) Change in number of axial cells. B) Change in thallus length.



——— 15°C - - - - 20°C ········ 25°C

Fig. 4.11 Effect of temperature on axial cell volume (axis 1), wing cell number (axes 2 and 3) and wing surface area (axes 4 and 5) in *Pteroceramium*. Measurements were taken at seg. 4 along the dominant axis.

replicates had significantly larger ($P < 0.05$) axial cells at seg. 3 along the dominant axis than other treatments. There were no significant differences between outer wing size (cell number $P=0.2$; surface area $P=0.1$) and inner wing size (cell number $P=0.6$; surface area $P=0.1$) between treatments. There was also no significant difference in the number of axial cells per segment ($P=1$; Fig. 4.12; Table 4.5).

Table 4.5 Morphometric results taken at seg. 3 of *Pteroceramium* when abraded by different grades of sand. Results are shown as mean ± standard error. ax cell = axial cell, vol = volume, OW = outer wing, IW = inner wing, SA = surface area.

			Grade of	sand	
Parameters measured	Water	Coarse	Medium	Fine	Very fine
Ax cell vol (μm^3 x 10^3)	180 ± 39	-	72 ± 12	63 ± 14	-
OW cell no.	17 ± 2	-	12 ± 2	13 ± 2	-
IW cell no.	10 ± 2	-	6 ± 1	8 ± 2	-
OW SA (μm^2 x 10^3)	1.4 ± 0.2	-	0.8 ± 0.1	1.0 ± 0.2	-
IW SA (μm^2 x 10^3)	0.5 ± 0.1	-	0.5 ± 0.1	0.6 ± 0.2	-
Ax cell no. seg. 3^{-1}	3 ± 0.4	-	3 ± 0	3 ± 1	-

vi) Smothering by sand - Control plants had significantly larger axial cells at seg. 4 ($P=0$) than any of the plants that were buried under sand. A similar trend was seen for the wings at seg. 4 ($P=0$). In the plants buried under sand, either the wing did not develop and had only a pericentral cell or differentiation was arrested after only one or two wing cells had formed. The number of axial cells per segment was not significantly different between treatments ($P=0.3$; Table 4.6).

vii) Gravity - Gravity apparently has no significant effect on axial cell volume ($P=0.4$), outer wing size (cell number $P=0.7$; surface area $P=0.2$) and inner wing size (cell number $P=0.7$; surface area $P=0.5$) at seg. 4. There was also no significant difference in number of axial cells in seg. 4 between treatments ($P=0.2$; Fig. 4.13; Table 4.7).

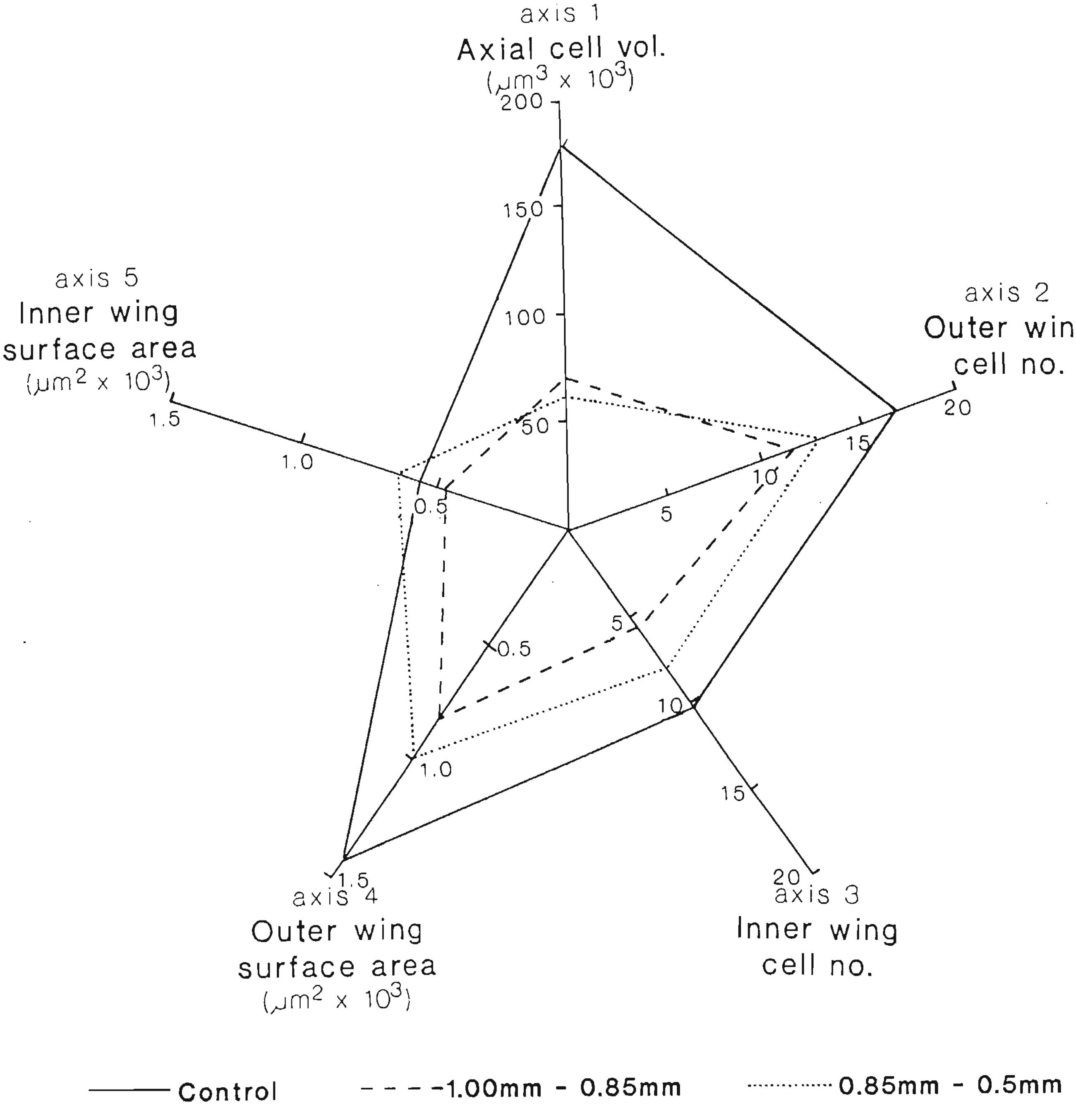


Fig. 4.12 Effect of sand abrasion on axial cell volume (axis 1), wing cell number (axes 2 and 3) and wing surface area (axes 4 and 5) in *Pteroceramium*. Measurements were taken at seg. 3 along the dominant axis.

Table 4.6 Morphometric results taken at seg. 4 of *Pteroceramium* when buried by different grades of sand at different depths. Results are shown as mean ± standard error. ax cell = axial cell, vol = volume, OW = outer wing, IW = inner wing, SA = surface area.

Treatment		Parameters measured					
Grade of sand	Depth	Ax cell vol (μm^3 x 10^3)	OW cell no.	IW cell no.	OW SA (μm^2 x 10^3)	IW SA (μm^2 x 10^3)	Ax cell no. seg. 4^{-1}
Water	-	75 ± 7	16 ± 1	10 ± 1	1.3 ± 0.1	0.9 ± 0.1	6 ± 0.2
Coarse	0.5 cm	23 ± 3	6 ± 1	3 ± 0.4	0.4 ± 0.1	0.1 ± 0.02	5 ± 1
	1 cm	15 ± 4	7 ± 1	3 ± 1	0.4 ± 0.1	0.2 ± 0.1	4 ± 1
	2 cm	11 ± 1	3 ± 1	3 ± 0.4	0.2 ± 0.1	0.1 ± 0.03	5 ± 1
Medium	0.5 cm	12 ± 2	5 ± 1	2 ± 0.4	0.2 ± 0.1	0.1 ± 0.03	5 ± 0.4
	1 cm	34 ± 4	9 ± 1	5 ± 1	0.8 ± 0.1	0.4 ± 0.1	4 ± 1
	2 cm	6 ± 1	5 ± 1	2 ± 0.4	0.3 ± 0.1	0.1 ± 0.02	5 ± 1
Fine	0.5 cm	39 ± 5	10 ± 1	6 ± 0.3	0.8 ± 0.1	0.4 ± 0.04	4 ± 0.2
	1 cm	19 ± 3	6 ± 1	4 ± 1	0.4 ± 0.1	0.2 ± 0.1	5 ± 1
	2 cm	20 ± 3	6 ± 1	4 ± 1	0.5 ± 0.1	0.2 ± 0.03	5 ± 1
Very fine	0.5 cm	24 ± 3	9 ± 1	4 ± 0.4	0.6 ± 0.1	0.3 ± 0.1	4 ± 0.4
	1 cm	15 ± 3	6 ± 1	3 ± 1	0.3 ± 0.1	0.1 ± 0.04	5 ± 0.3
	2 cm	25 ± 4	8 ± 1	3 ± 1	0.5 ± 0.1	0.2 ± 0.04	4 ± 1

Continual tumbling did not alter the angle of branching of each pseudodichotomy (P=0.8; Table 4.7).

viii) Nitrogen and phosphorus concentrations - There was a general increase in the RGRs of *Pteroceramium* when determined on both axial cell number and thallus length with increasing concentrations of nitrogen and phosphorus (Fig 4.14A and B).

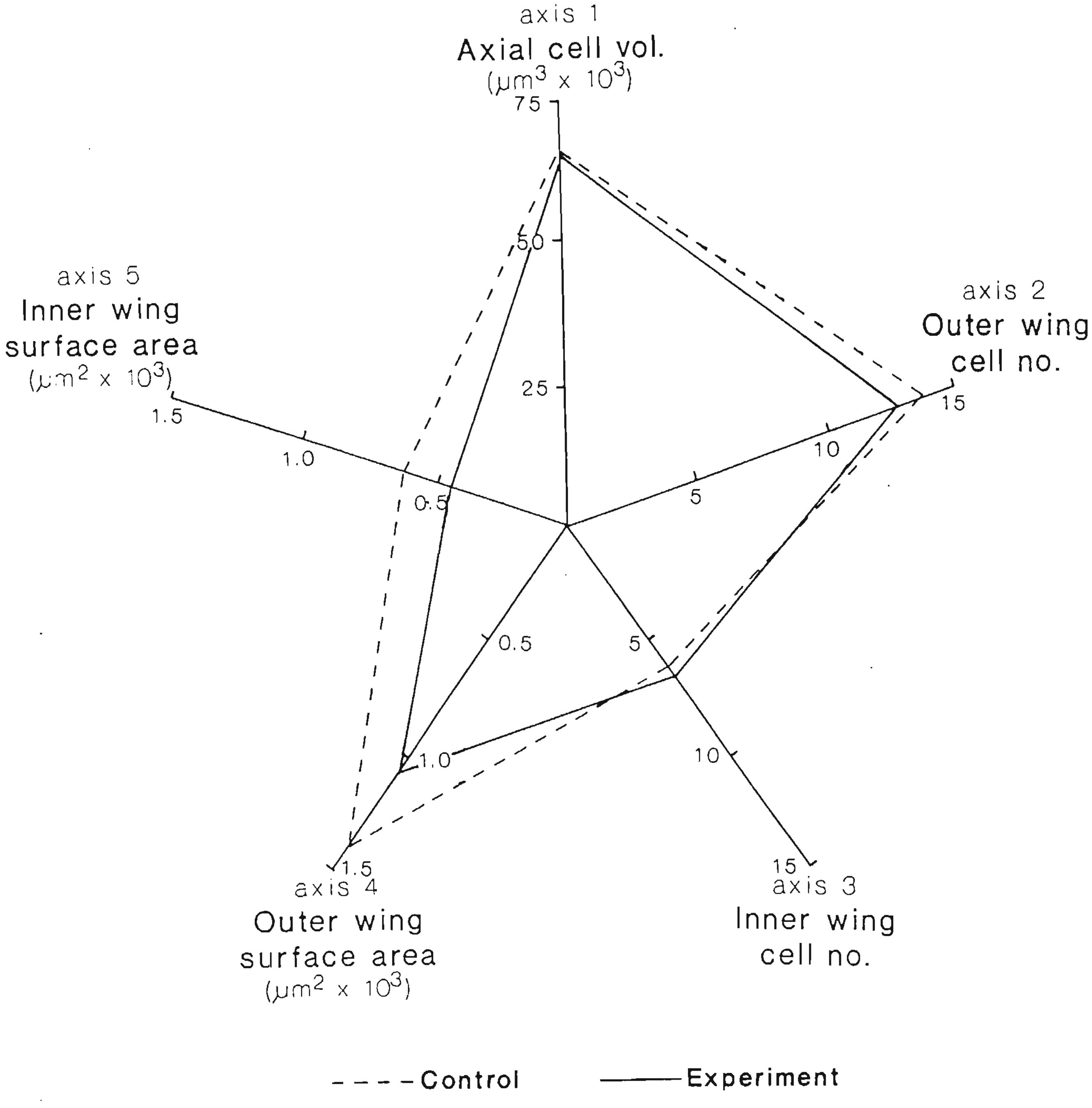


Fig. 4.13 Effect of continual tumbling on axial cell volume (axis 1), wing cell number (axes 2 and 3) and wing surface area (axes 4 and 5) in *Pteroceramium*. Measurements were taken at seg. 4 along the dominant axis.

Table 4.7 Morphometric results taken at seg. 4 of *Pteroceramium* when continually tumbled. Results are shown as mean ± standard error. ax cell = axial cell, vol = volume, OW = outer wing, IW = inner wing, SA = surface area.

	Treatment	
Parameters measured	Control	Experiment
Ax cell vol (μm^3 x 10^3)	66 ± 11	65 ± 7
OW cell no.	14 ± 1	13 ± 1
IW cell no.	6 ± 1	7 ± 1
OW SA (μm^2 x 10^3)	1.4 ± 0.2	1.1 ± 0.1
IW SA (μm^2 x 10^3)	0.7 ± 0.1	0.5 ± 0.1
Ax cell no. seg. 4^{-1}	6 ± 1	4 ± 0.4
Angle of branching	54° ± 3	53° ± 3

Morphometric results were taken using cells in seg. 3. Nitrogen and phosphorus concentrations had less effect on axial cell volumes (Fig. 4.14C) and wing development (Fig. 4.15A-D) than it did on the RGRs. The sharp rise in all morphometric measurements at 0 nitrogen and phosphorus is due to slow growth and so cells formed in old, more favourable culture conditions were measured.

ix) Salinity - Algae grown at $34^{\circ}/_{00}$ had significantly higher RGRs for both axial cell number and thallus length ($P<0.01$) than material grown at the other salinities. Plants grown at $30^{\circ}/_{00}$ and $40^{\circ}/_{00}$ showed very little growth but maintained their red colour, while material grown at salinities above and below this lost their pigments and died (Fig. 4.16A and B; Table 4.8).

No morphometric measurements could be made for plants grown at $20^{\circ}/_{00}$, $30^{\circ}/_{00}$ and $50^{\circ}/_{00}$ as there was insufficient new growth. The material grown at $34^{\circ}/_{00}$ had significantly larger axial cells ($P<0.0001$) and outer wings (cell number and surface area $P<0.001$) at seg. 4 than the material grown at $40^{\circ}/_{00}$ but the inner wings were similar in both cell number and surface area ($P=0.05$). Salinity had no effect ($P=0.4$) on the number of axial cells in seg. 4 between treatments (Fig. 4.17; Table 4.8).

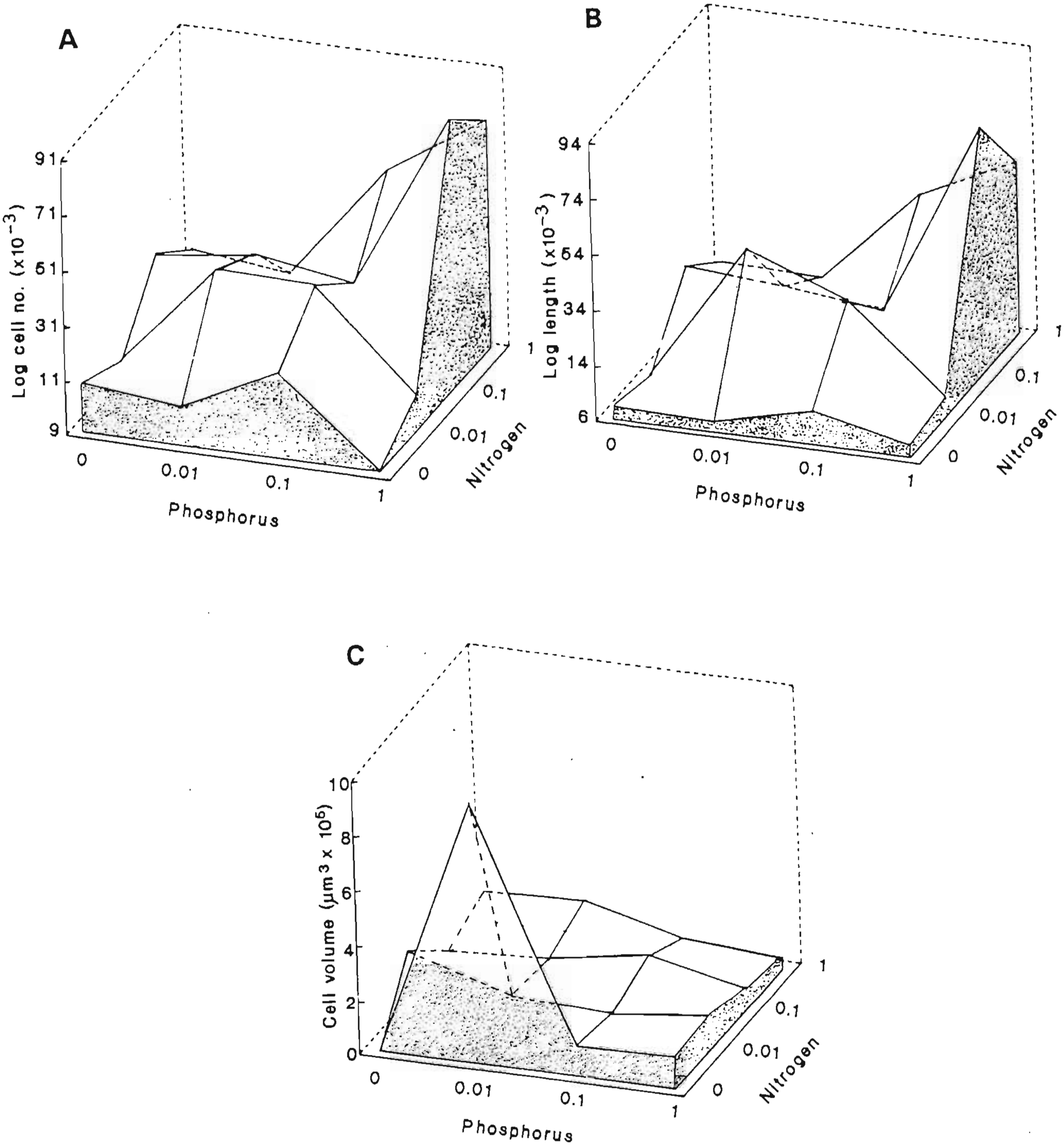


Fig. 4.14 Growth and morphometric results of *Pteroceramium* when grown in various nitrogen and phosphorus concentrations. A) RGRs determined on change in number of axial cells. B) RGRs determined on change in thallus length. C) Average axial cell volume at seg. 3 along the dominant axis.

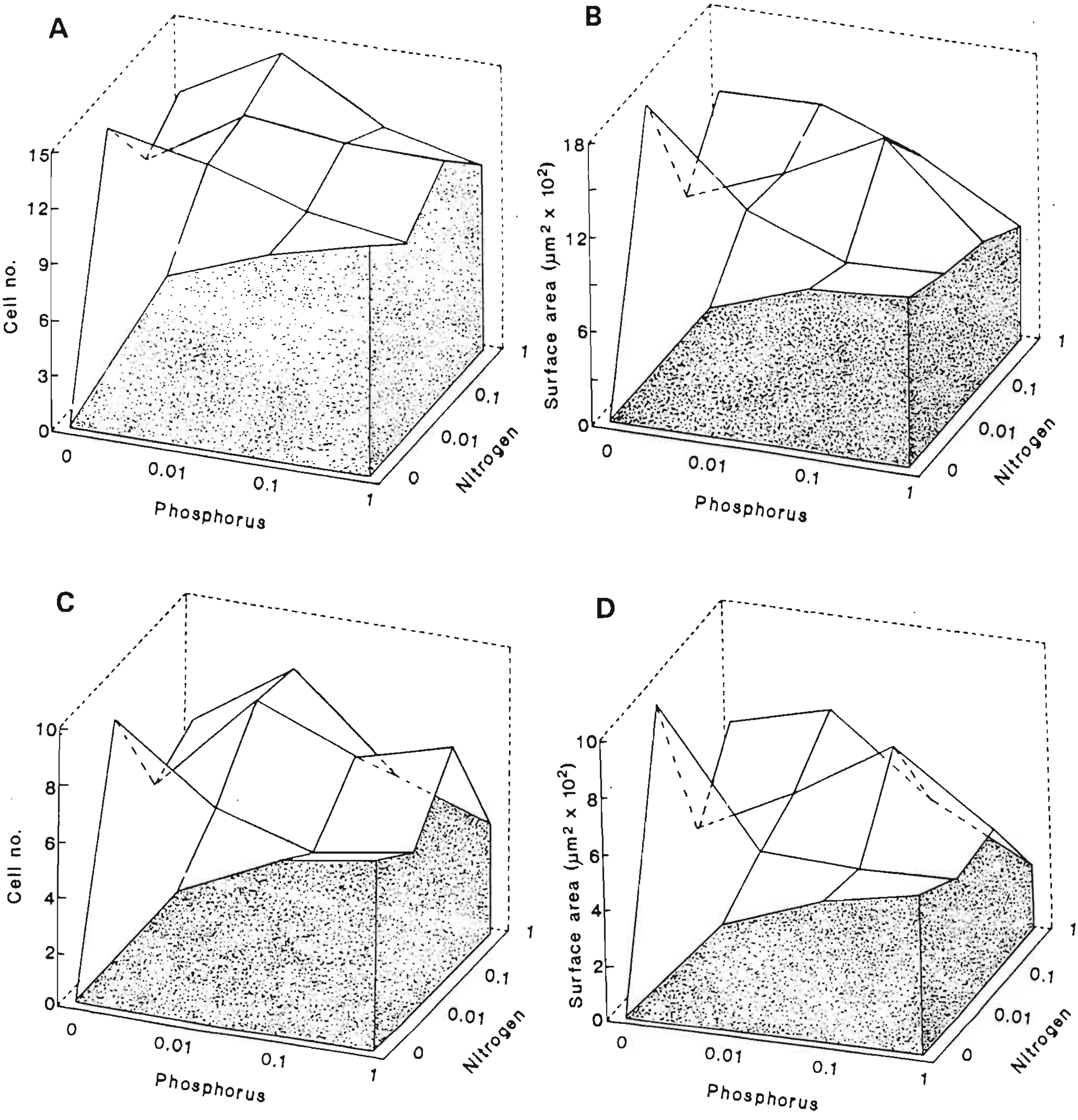


Fig. 4.15 Effect of various nitrogen and phosphorus concentrations on *Pteroceramium*'s wing size at seg. 3 along the dominant axis. A) Outer wing cell number. B) Outer wing surface area. C) Inner wing cell number. D) Inner wing surface area.

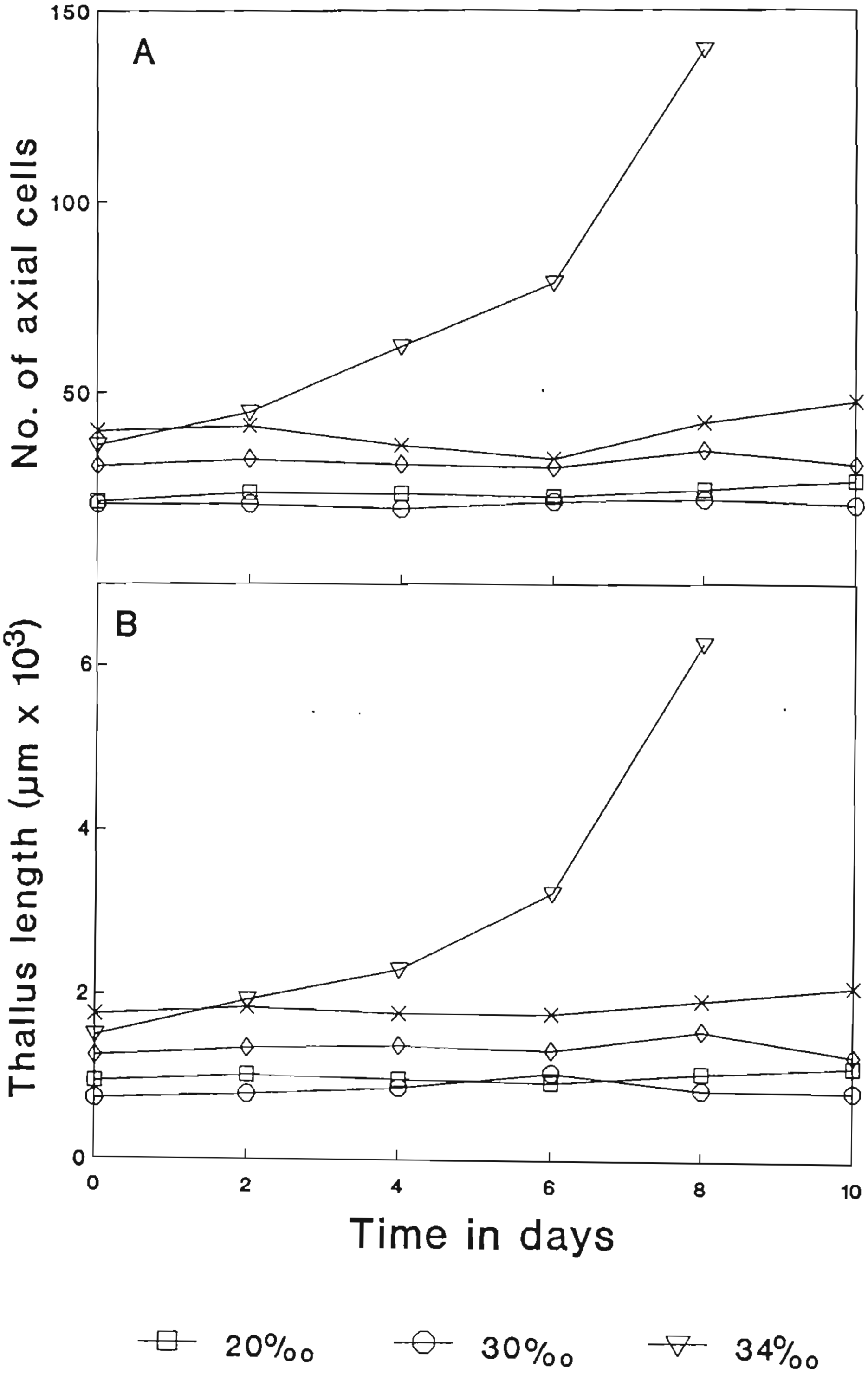


Fig. 4.16 Growth of *Pteroceramium* when grown in different salinities. A) Change in number of axial cells. B) Change in thallus length.

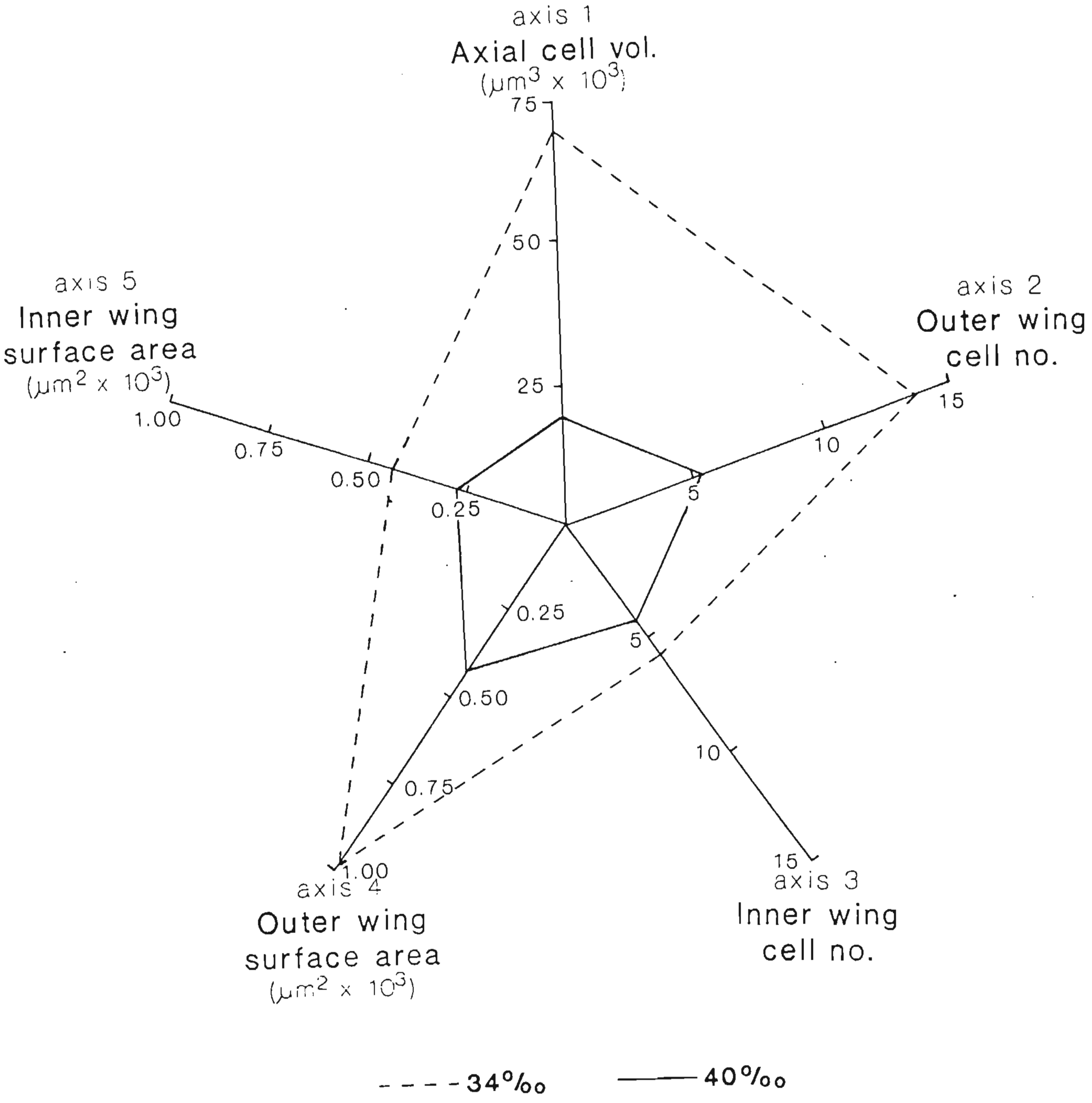


Fig. 4.17 Effect of salinity on axial cell volume (axis 1), wing cell number (axes 2 and 3) and wing surface area (axes 4 and 5) in *Pteroceramium*. Measurements were taken at seg. 4 along the dominant axis.

Table 4.8 Growth and morphometric results taken at seg. 4 of *Pteroceramium* when grown in different salinities. Results are shown as mean ± standard error. RGR = relative growth rate, ax cell = axial cell, vol = volume, OW = outer wing, IW = inner wing, SA = surface area.

Parameters measured	Salinity 20	(°/$_{oo}$) 30	34	40	50
RGR (ax cells)	0.01 ±0.01	-0.00 ±0.01	0.10 ±0.01	0.01 ±0.01	-0.01 ±0.01
RGR (length; μm)	0.01 ±0.003	0.01 ±0.01	0.08 ±0.01	0.03 ±0.02	0.01 ±0.01
Ax cell vol (μm^3 x 10^3)	-	-	70 ± 8	19 ± 4	-
OW cell no.	-	-	14 ± 1	5 ± 1	-
IW cell no.	-	-	6 ± 1	4 ± 1	-
OW SA (μm^2 x 10^3)	-	-	1.0 ± 0.1	0.4 ± 0.1	-
IW SA (μm^2 x 10^3)	-	-	0.4 ± 0.1	0.3 ± 0.1	-
Ax cell no. seg. 4^{-1}	-	-	4 ± 1	7 ± 3	-

x) pH - RGRs for both axial cell number and thallus length were significantly higher ($P<0.001$) in the material grown at pH 7.5 and pH 8.5 while the material grown at the two lower pHs, died (Fig. 4.18A and B; Table 4.9).

No morphometric measurements were made on the material grown at the two lowest pHs because there was insufficient new growth. Material growing at pH 7.5 had significantly larger axial cells at seg. 4 along the dominant axis than the material growing at pH 8.5 ($P<0.05$). No significant differences were detected in the wing cell number (outer wing $P=1$; inner wing $P=0.5$), wing surface area (outer wing $P=0.6$; inner wing $P=0.5$) and number of axial cells in seg. 4 ($P=0.1$) for the algae grown at the two highest pHs (Fig. 4.19 and Table 4.9).

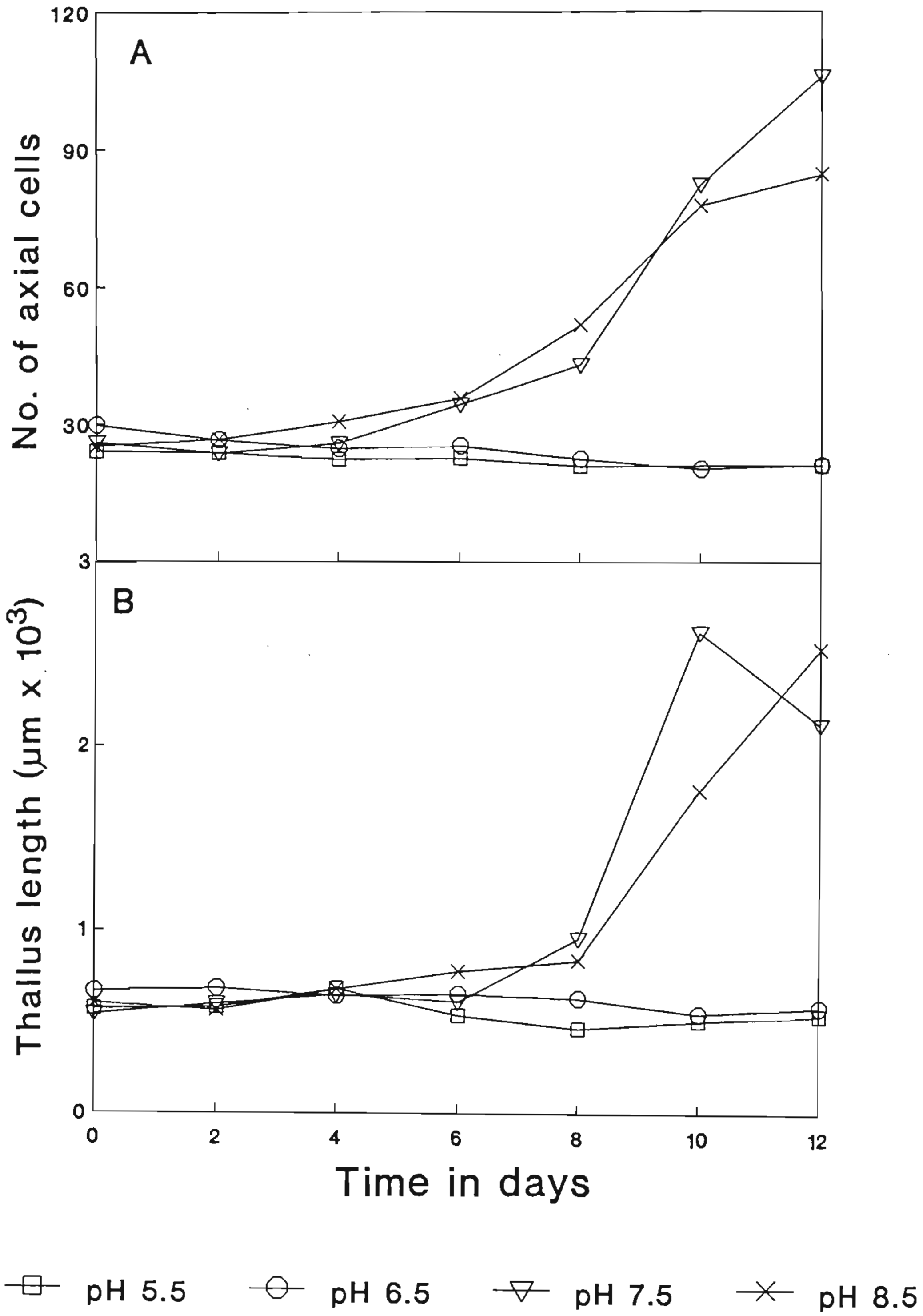


Fig. 4.18 Growth of *Pteroceramium* when grown in different pHs. A) Change in number of axial cells. B) Change in thallus length.

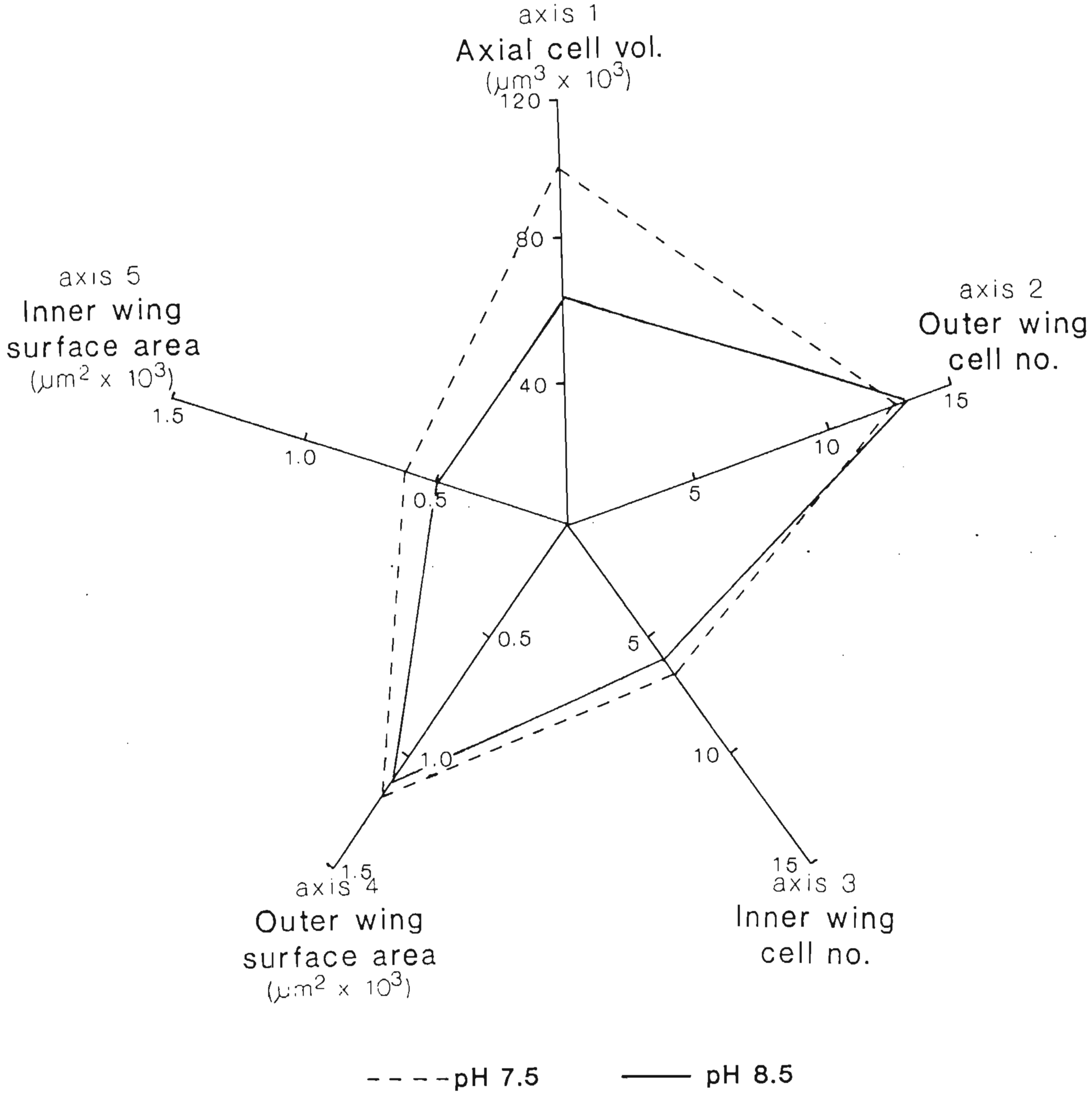


Fig. 4.19 Effect of pH on axial cell volume (axis 1), wing cell number (axes 2 and 3) and wing surface area (axes 4 and 5) in *Pteroceramium*. Measurements were taken at seg. 4 along the dominant axis.

Table 4.9 Growth and morphometric results taken at seg. 4 of *Pteroceramium* when grown in different pHs . Results are shown as mean ± standard error. RGR = relative growth rate, ax cell = axial cell, vol = volume, OW = outer wing, IW = inner wing, SA = surface area.

	pH			
Parameters measured	5.5	6.5	7.5	8.5
RGR (ax cells)	-0.02 ± 0.00	-0.02 ± 0.00	0.05 ± 0.01	0.05 ± 0.01
RGR (length; μm)	-0.002 ± 0.03	-0.007 ± 0.002	0.07 ± 0.01	0.06 ± 0.01
Ax cell vol ($\mu m^3 \times 10^3$)	-	-	101 ± 14	65 ± 9
OW cell no.	-	-	13 ± 2	13 ± 2
IW cell no.	-	-	7 ± 1	6 ± 1
OW SA ($\mu m^2 \times 10^3$)	-	-	1.2 ± 0.2	1.1 ± 0.2
IW SA ($\mu m^2 \times 10^3$)	-	-	0.6 ± 0.1	0.5 ± 0.1
Ax cell no. seg. 4^{-1}	-	-	3.8 ± 1.4	5.6 ± 0.7

4.4 Discussion

The growth rate *per se* does not influence the ultimate size of the plant but rather the time taken to achieve that size (NORTON, MATHIESON and NEUSHUL, 1981). Especially when studying plants over a time course where growth is at suboptimal conditions, morphometric comparisons are often made on plants which have not reached equivalent developmental stages (GARBARY, 1979b). For this reason, wherever possible, morphometric measurements for *Pteroceramium* were made using seg. 4, as at this point cells have appeared to have almost reached maximum size for their growth conditions, regardless of the time taken to reach that size. It was not practical to make measurements further back along the thallus as experiments would have had to run for longer to allow for sufficient new growth and then other factors may have become limiting.

4.4.1 Physical factors

Light (irradiance, spectral quality and photoperiod) is one of the major environmental variables affecting growth and development in red algae (CHARNOFSKY, TOWILL and SOMMERFELD, 1982). Most algae are found within the photic zone with their vertical distribution influenced by the relationship between irradiance levels, light quality and photosynthesis (BIRD, CHEN and McLACHLAN, 1979; TSENG, ZHOU and PAN, 1981). In the marine environment, irradiance can often limit growth (LAPOINTE and DUKE, 1984), each particular alga having critical lower irradiance levels although other factors such as competition and grazing pressure also play a role in determining an alga's lower distribution (LÜNING, 1981a).

Irradiance directly controls growth rates in nature (LAPOINTE, 1981), its primary importance being to provide energy for photosynthesis. The relationship between growth and irradiance is a hyperbolic function with inhibition of growth occurring at supraoptimal irradiances (LOBBAN, HARRISON and DUNCAN, 1985). The growth of *Pteroceramium* was irradiance limited at 30 μmol m^{-2} s^{-1}, as seen by the reduced growth rates. The upper saturation limit for maximum growth was obtained in the three highest irradiances as there was no increase in the growth rate with increasing irradiance, showing that a plateau had been reached. The material growing in the highest irradiance (193 μmol m^{-2} s^{-1}) was a paler red colour, indicating either photodamage or adjustment in pigment content (see Chapter 5). This indicates that irradiance levels were becoming supraoptimal.

The depths to which seaweed can grow are determined by available irradiance and the efficiency of the alga in utilising the light. The irradiances that *Pteroceramium* was exposed to in the present study were all relatively low, considering that the average irradiance at the ocean surface is approximately 2000 μmol m^{-2} s^{-1} (HODGESON, 1981). The growth rates of *Pteroceramium* at the different irradiances suggest that the alga is restricted to low light climates typical of sublittoral reef environments and in shaded niches in tide pools (STIRK and AKEN, 1993). This belief is supported by the fact that *Pteroceramium* was collected as an epiphyte on sublittoral reef species; e.g. *Haloplegma africanun* Kützing and *Callophycus densus* (Sonder) Kraft on the Natal south coast (AKEN, pers. comm.) and in rocky overhangs in the intertidal on the eastern Cape coast (STEGENGA and BOLTON, 1992).

Similar results were obtained for *Callithamnion hookeri* (Dillwyn) S.F.Gray which grew best in lower irradiances (592 lux which converts to 10 μE m^{-2} s^{-1}; LÜNING, 1981a) with less growth at higher irradiances (7532 lux = 127 μE m^{-2} s^{-1}). This species is able to grow in poorly illuminated conditions such as rocky overhangs and crevices as growth is saturated at low irradiances (EDWARDS, 1979). Growth was also saturated at relatively low irradiances in *Callithamnion byssoides*, restricting it to the lower eulittoral and shaded areas under overhanging rocks and vertical substrata in the upper sublittoral (KAPRAUN, 1978). *Porphyra* sporelings have a lower irradiance requirement than adult plants, reflecting irradiance conditions of emerging sporelings (HANNACH and WAALAND, 1989).

Irradiances needed to saturate growth are often linked to temperature; e.g. increased irradiance is necessary to saturate growth at temperatures below 24°C in *Codium fragile* (Suringar) Hariot while inhibitory high irradiances are more pronounced at high temperatures and longer photoperiods (DARLEY, 1982); in *Porphyra abbottae* Krishnamurthy irradiance levels are important only at temperatures above 12°C (HANNACH and WAALAND, 1989).

Irradiance levels may affect morphology; e.g. there is increased lateral branch production in *Gelidium latifolium* (Greville) Bornet *et* Thuret when grown in high irradiances (300 μmol m^{-2} s^{-1}) which increased self shading (FREDRIKSEN and RUENESS, 1989); when germlings of *Gelidium robustum* (Gardner) Hollenberg *et* Abbott are grown in 40 μE m^{-2} s^{-1}, the plants are smaller and more stunted but with greater development of rhizoidal cell clusters than plants growing at higher irradiances. High irradiance (110 μE m^{-2} s^{-1}) facilitated branch initiation (D'ANTONIO and GIBOR, 1985).

Irradiance levels also affected the morphology of *Pteroceramium*, there being a decrease in both axial cell volume and wing size when grown in the highest irradiance, even though the growth rates were unaffected, implying that the thalli were under stress at near supraoptimal conditions. The largest axial cell volumes were obtained from material growing at 79 μmol m^{-2} s^{-1}, indicating that this is near the optimum irradiance level for cell development and maturation. The wings of *Pteroceramium* at the three lowest irradiances were a similar size. They appear to have reached their full potential size as determined by the alga's genotype, even if this was attained at different rates. The wings being smaller at the highest irradiance shows a phenotypic response to irradiance levels. The decrease in wing surface area at the highest irradiance suggests that the function of the wings is to increase the surface area of the alga for light interception. Where irradiance levels are already saturating, a large surface area for light interception is not necessary (STIRK and AKEN, 1993).

Blue light appears to be a general requirement for normal development in seaweeds and promotes the synthesis of proteins, RNA and DNA. Red light promotes the synthesis of carbohydrates (SOEDER and STENGEL, 1974; LOBBAN, HARRISON and DUNCAN, 1985; LÜNING, 1990). In the freshwater alga *Lemanea* Bory, there is lowest primary productivity under green light, intermediate productivity under red light and highest productivity under blue light (THIRB and BENSON-EVANS, 1983).

When *Pteroceramium* was grown in monochromatic light conditions, there was a decrease in growth regardless of the wavelength. However, as the thalli were grown in a very low irradiance (1 μmol m^{-2} s^{-1}) which was growth limiting, these effects may have been exaggerated. Monochromatic light had very little effect on morphogenesis in *Pteroceramium*, with axial cell volume and wing size (excluding outer wing cell number) being similar to the control.

Removal of green light had no effect on the growth of *Callithamnion hookeri* (Edwards, 1979). In *Griffithsia pacifica* Kylin shoots are positively phototropic and rhizoids negatively phototropic. The phototropic response for the rhizoids is initiated in blue and green light but there is no response to yellow, orange or red light (WAALAND, NEHLSEN and WAALAND, 1977).

Spectral quality is thought to control mainly reproductive processes. These were not studied for *Pteroceramium* as only the male gametophyte was grown in culture. In

Lemanea there is higher germination of carpospores under red and yellow light, lower germination under green light and no germination under blue light. A transfer to white light reverses the inhibitory effects (THIRB and BENSON-EVANS, 1983). The light requirement for monospore germination in *Bangia atropurpurea* (Roth) C.Agardh (as *Bangia fuscopurpurea* (Dillwyn) Lyngbye) is 10 μmol m^{-2} s^{-1} for 50% germination with the highest germination (81%) at 35 μmol m^{-2} s^{-1}. Green light was the most effective, followed by red and then blue light. Germination is under the "photosynthetic effect", showing a relationship to total quanta received (CHARNOFSKY, TOWILL and SOMMERFELD, 1982).

The rôle chromatic photoreceptors play is still unclear (FALKOWSKI and LA ROCHE, 1991). Blue and red wavelengths are primarily responsible for activating the photoreceptors which control the alga's response to light (DRING, 1986). The red light photoreceptor is the phytochrome system with red and far-red reversibility. There are also many photomorphogenetic responses to blue and ultraviolet light. The chemical nature of this photoreceptor is not known but is speculated to be either a flavin or pterin (RÜDIGER and LÓPEZ-FIGUEROA, 1992) and is thought to control chlorophyll synthesis (LÓPEZ-FIGUEROA and NIELL, 1990).

Daylength is important in determining daily supply of quanta and the total time available for photosynthesis. Many algae are sensitive to daylength which acts as a means of predicting seasons (KAIN and NORTON, 1990); e.g. *Porphyra tenera* Kjellman forms conchosporangia from the *Conchocelis* phase in autumn when the critical daylength is 12.5 hours or less. The conchospores grow in winter and disappear in summer (DARLEY, 1982). *Laminaria hyperborea* Foslie forms a new frond in early winter and *Bonnemaisonia hamifera* forms tetraspores in late autumn. Most responses are short day responses with there only being a few long day responses in algae. Critical daylengths generally increase for algae growing in the higher latitudes (LÜNING, 1990).

Ceramium isolates show extensive phenotypic plasticity when grown in different daylengths (GARBARY, 1988); e.g. in *Ceramium echoinotum* J.Agardh, *Ceramium rubrum*, *Ceramium shuttleworthianum* and as well as in *Antithamnion spirographidis*

Schiffner there is an increase in the rate of apical cell division with increasing light periods with the rate at 8 hours almost half that at 16 hours. In all these species, daylength also affects cell length with cells receiving a greater number of hours light per day being larger. This indicates that photoperiod is the main controlling factor in growth (GARBARY, GRUND and McLACHLAN, 1978; GARBARY, 1979a).

Unlike the effect of photoperiod on *Ceramium* species, photoperiod had no effect on the RGR when determined by an increase in axial cell number in *Pteroceramium* (= rate of apical cell division) but it did effect the RGR when determined by thallus length (= axial cell elongation), decreasing the rate at $8{:}\overline{16}$ hour. This indicates that the critical daylength for maximum rates of elongation is between 8-10 hours. This is relatively short compared with *Ceramium* species which had a 16 hour critical light period (GARBARY, GRUND and McLACHLAN, 1978). Often the reason for reduced growth in short daylengths is increased periods of dark respiration causing a reduction in the amount of available energy for growth (BIRD, CHEN and McLACHLAN, 1979).

On the South African coast the shortest daylength in winter is $10{:}\overline{14}$ hour (STONE, 1988). The length of the dark period would be extended in deeper waters owing to rapid attenuation of irradiance by water. Perhaps daylength may play a role in determining the lower limits of distribution for *Pteroceramium*, especially in winter when all irradiance levels are lower (STIRK and AKEN, 1993).

Photoperiod also affects the final size of the axial cells in *Pteroceramium*. There is a decrease in axial cell size in the longest light period ($16{:}\overline{8}$ hour). There could possibly have been some inhibition from too much light. In these experiments, the mean daily illumination differed (M.D.I. = irradiance x length of light period (hour)/24 hour); MURRAY and DIXON, 1973) and refers to the total amount of irradiance received for growth (GARBARY, 1979b) and so material in the longest photoperiod may have received inhibitory amounts of irradiance.

Dumontia contorta (Gmelin) Ruprecht has a northern hemisphere distribution and shows maximum development of macrothalli in winter and spring when there are low temperatures and high nutrient concentrations. Basal crusts, called microthalli, are the summer form. In experimental conditions, macrothalli are formed only in short day ($8:\overline{16}$ hour) conditions at low temperatures (8°C-16°C) in all irradiance conditions. A night break at $8:\overline{16}$ hour suppresses macrothalli development. This is one of the few examples in which alternation of phases is triggered by photoperiod (RIETEMA and KLEIN, 1981; RIETEMA, 1982).

Temperature is a critical ecological factor as it is fundamentally important to all aspects of metabolism with the internal temperature of algae usually being near the external temperature. Temperature is an important factor determining the general geographical distribution of algae with each species having its own particular geographical limit. Stenothermal species tolerate a 10°C range in temperature and eurythermal species tolerate a temperature range greater than 10°C. The four critical temperatures in determining the geographical range are minimum and maximum temperature for survival and minimum and maximum temperature for reproduction which are narrower than those for growth and survival. Unusually hot or cold spells may wipe out a species on the edge of its range (LOBBAN, HARRISON and DUNCAN, 1985). Eurythermal algae have the advantage of a wide geographical distribution but are not as efficient as stenothermal species which may develop optimally adapted enzymes for a narrow temperature range (LÜNING, 1990).

Optimum temperatures for species vary, the more widely distributed algae reacting less strongly to temperature changes than species with a more restricted geographical range (KAIN and NORTON, 1990). Temperature optima of species grown in culture may be narrower than normal as temperatures are kept constant. The optimum temperature may also vary with age and heteromorphic life history stages (LOBBAN, HARRISON and DUNCAN, 1985). Resting stages of many algae possess a greater tolerance to extreme temperatures than do vegetative stages (SOEDER and STENGEL, 1974; KAIN and NORTON, 1990).

Stable environments generally produce stenothermal responses. Eulittoral species tend to exhibit a wider tolerance than sublittoral species (LÜNING, 1990). Temperature is also important in seasonal variation for algae (KAIN and NORTON, 1990). There are many examples of temperature optima and extremes correlating with mean monthly seawater temperatures. *Ceramium shuttleworthianum*, a species found in the Norwegian flora, has a culture range of 5°C-23°C with an optimum of 15°C-18°C. In nature, its inability to survive colder winter temperatures would limit its northward extension (RUENESS, JACOBSEN and ASEN, 1990). *Gracilaria verrucosa* has a laboratory temperature optimum of 25°C-30°C which coincides with temperature conditions in the known areas of its distribution (HURTADO-PONCE and UMEZAKI, 1987). Similarly, the growth of *Gigartina pectina* Dawson is inhibited below 14°C and above 26°C in the laboratory and this corresponds to temperatures encountered in the field, there being a decrease in abundance in summer when water temperatures are high (PACHECO-RUIZ, ZERTUCHE-GONZALEZ, CABELLO-PASINI and BRINKHUIS, 1992). Geographic distribution is usually correlated to mean monthly temperatures of seawater. However, air temperatures may be more critical than seawater temperatures for eulittoral species (EDWARDS, 1979).

The highest RGRs for *Pteroceramium* were obtained when it grew at 20°C and 25°C, correlating with the average sea temperatures in the area where it was collected. The temperatures on the Natal south coast are highest between January and March (22.9°C) and lowest in August and September (19.4°C) determined from average sea surface temperatures collected daily from 1978 to 1991 by the Natal Sharks Board. At low tide the temperature in rock pools on the intertidal region in Natal may rise to over 30°C which *Pteroceramium* cannot tolerate. This suggests that *Pteroceramium* is suited to sublittoral waters where temperatures are lower or in tide pools in the lower eulittoral region where fluctuations in water temperature are not great (STIRK and AKEN, 1993). This corresponds with where *Pteroceramium* has previously been found growing.

At higher temperatures, molecules have more energy and so metabolic reactions proceed faster. The temperature coefficient Q_{10} usually doubles with every 10°C increase ($Q_{10} = 2$) although its range in algae is between 1.1 and 5.3. Higher temperatures cause thermal denaturation of enzymes, and damage to other proteins and membranes (LÜNING, 1990). Once cooled, these enzymes may become active again or be permanently damaged (LOBBAN, HARRISON and DUNCAN, 1985). Heat hardiness is due to resistance of enzymes to denaturing. At low temperatures, lipids and proteins of the cellular membranes are destroyed due to the formation of intracellular ice crystals (LÜNING, 1990). Cold hardiness is achieved by the accumulation of organic solutes such as free sugars and oligosaccharides which lower the freezing point and so prevent ice crystal formation (SOEDER and STENGEL, 1974; DARLEY, 1982). Heat and cold hardiness increase from the littoral fringe to the sublittoral zone. Generally, tropical species can survive up to 40°C and polar algae withstand temperatures less than 0°C.

Acclimation to temperature is achieved by constant turnover of proteins with heat stability related to the temperature at which the enzyme was formed (LOBBAN, HARRISON and DUNCAN, 1985). Adaptation to temperature involves the production of more enzymes in lower temperatures. Higher temperatures cause a reduction in enzyme concentrations (SOEDER and STENGEL, 1974). Poikilotherms cannot regulate their internal temperature but can become acclimatised if the rate of temperature change is slow; e.g. seasonal changes, and so can minimise the effect of temperature fluctuations. This acclimation is a passive process (LOBBAN, HARRISON and DUNCAN, 1985).

Temperature also affects morphology in size, external and internal structures and branching patterns in algae. Generally plants growing in colder temperatures have a larger final size (GARBARY, GRUND and McLACHLAN, 1978); e.g. maximum plant size and cell length decrease along a temperature gradient in some *Audouinella* species and *Ceramium rubrum* (GARBARY, GRUND and McLACHLAN, 1978; GARBARY, 1979a). Temperature also affects the morphology of *Chondrus crispus* with the thalli being more compact and heavily branched at higher temperatures than at lower temperatures (SIMPSON and SHACKLOCK, 1979).

Temperature had little effect on the morphology of *Pteroceramium* but morphometric measurements could be taken only at near-optimum temperatures and so the effects of more extreme temperature could not be assessed.

There are many examples of the effects of interactions between temperature and photoperiod on algal growth and morphology as these two factors are the main seasonal triggers. In *Callithamnion byssoides* maximum growth was obtained at 8 hour and 14 hour daylengths with temperatures between 20°C and 25°C. In longer daylengths, increased irradiance rather than direct photoperiod effects operate (KAPRAUN, 1978).

Longer daylengths ($8:\overline{16}$ hour) resulted in more rapid growth and maturation with longer filament length and diameter in *Bangia atropurpurea* with an optimum temperature of 15°C. At 22°C only monospores are produced, at 15°C both monospores and carpospores are produced which develop into the *Conchocelis* phase and at 9°C, carpospores are exclusively produced. Thus the alternation between phases is governed by both temperature and photoperiod (SOMMERFELD and NICHOLS, 1973).

There is growth between 10°C and 24°C in *Gigartina acicularis* (Roth) Lamouroux with cystocarps being formed only between 14° and 18°C. There is growth in photoperiods of $16:\overline{8}$ hour and $8:\overline{16}$ hour but only cystocarp development in 12 hours or shorter light periods which can be blocked by night breaks. This shows a seasonal effect with gametangia formation limited to autumn (GUIRY and CUNNINGHAM, 1984).

Using three different strains of *Asparagopis armata* Harvey, it was found that reproduction was induced at 8-9 hour daylength at temperatures between 17°C-21°C in the Italian strain, 8 hour daylength at 15°C-21°C in the Irish strain and 9 hour daylength and 13°C-17°C in the Australian strain. This corresponds with reproduction in autumn being controlled by daylength and temperature (GUIRY and DAWES, 1992).

The development of erect axes in *Nemalion helminthoides* is a long day photoperiodic response modified by temperature. Erect axes develop at temperatures between 7°C-13°C. At these temperatures, there is an increase in the number of axes with increasingly longer light periods until 15 hour light which is the critical daylength (CUNNINGHAM and GUIRY, 1989).

Sediments settling on top of algae shade them as well as smothering and scouring them. Smothering by sand cuts off light and nutrients and changes the chemical nature of the microenvironment by causing a reduction in O_2 concentrations and an increase in CO_2 and hydrogen sulfide (H_2S) concentrations. In areas of high water motion, sediments can also cause mechanical damage by scouring action (DEVINNY and VOLSE, 1978). Mucilage may reduce abrasion damage by lubrication (KAIN and NORTON, 1990).

Sandy beaches have fewer algae than rocky beaches. Psammophilic (sand loving) algae are resistant to burial for months at a time; e.g. *Gymnogongrus linearis* (C.Agardh) J.Agardh, *Laminaria sinclairii* (Harvey) Farlow, Anderson *et* Eaton and *Phaeostrophion irregulare* Setchell *et* Gardner. These generally have tough, thick walled cells with a great ability to regenerate and they have asexual reproduction. They are physiologically adapted to withstand darkness, nutrient deprivation, anaerobic conditions and high H_2S concentrations (LOBBAN, HARRISON and DUNCAN, 1985). Sediments may also interfere with gametophyte development by preventing spore attachment; e.g. *Macrocystis pyrifera* (Linnaeus) C.Agardh (DEVINNY and VOLSE, 1978). Sand movement is usually seasonal with a build up of sand in spring which is washed away in autumn (LOBBAN, HARRISON and DUNCAN, 1985).

When *Pteroceramium* was continually scoured by sand, the axial cells were smaller, indicating that these conditions were stressful. However, *Pteroceramium* can withstand smothering for a least two weeks in shallow sand as the experimental material was able to grow, although at a reduced rate. The wings were greatly reduced when *Pteroceramium* was grown under sand, most probably owing to lack of space and as a means to conserve energy for upward growth out of the sand. There was no trend between particle size (size of interstices) and wing size, indicating that any smothering by sand reduces wing size. Replicates which grew out of the sand resumed their normal appearance with wings. This indicates phenotypic plasticity but the constancy of the wings under most conditions makes them a reliable diagnostic character.

There are certain privileged angles; viz. 42° and 53°, often found in plants (LE GUYADER and FERRE, 1988). *Pteroceramium* was typical of this where the angle of each pseuodichotomous branch was 53°. Continuous tumbling had no effect on the morphology of *Pteroceramium*. This was not unexpected as in natural environments where there is constant water motion, plants are continually moved, disrupting the gravitational pull to which the plant is exposed.

Water motion is important in determining abundance, distribution and productivity of algae; e.g. *Kappaphycus alvarezii* DOTY (DOTY), *Kappaphycus striatum* SCHMITZ (DOTY) and *Eucheuma denticulatum* (Burman) Collins *et* Hervey showed faster growth in areas with higher water motion and lower growth rates in areas with reduced water motion. Thicker thalli have a greater diffusion resistance for entry and exit of materials into the centre of the thallus and therefore need greater water motion (GLENN and DOTY, 1992).

Water is in continual motion from large-scale ocean currents, tidal currents, waves generated by local wind conditions to small scale circulation patterns caused by density changes (CARRINGTON, 1990). Moderate water motion is beneficial as it facilitates nutrient uptake by decreasing and renewing the boundary layer, therefore reducing gradients of nutrients adjacent to the thallus. However, if local water motion is too large it may cause mechanical damage and interfere with growth processes. In *Porphyra abbottae* increased water motion, especially at low nutrient concentrations, caused an increase in blade size which was used as a measure of growth (DARLEY, 1982; HANNACH and WAALAND, 1989; LÜNING, 1990; GLENN and DOTY, 1992) as well as a means of supplying gases, removing waste products and preventing settling of silts (DARLEY, 1982). Water motion also affects light penetration, temperature and salinity changes (LOBBAN, HARRISON and DUNCAN, 1985).

4.4.2 Chemical factors

The availability of nutrients is one of the primary factors regulating growth, reproduction and biochemistry of seaweeds. These effects are more marked in areas of high algal densities such as semitropical and tropical regions. Normal biochemistry of algae require 14-21 specific elements with 56 elements having been reported in algae, although their presence is not evidence that they are utilised (DeBOER, 1981). Essential elements are those which when deficient, limit algal growth and cannot be replaced by another element. These include carbon, hydrogen, oxygen, nitrogen, phosphorus, magnesium, iron, copper, manganese, zinc and molybdenum (LOBBAN, HARRISON and DUNCAN, 1985).

The nutrient available in the smallest quantity will limit growth when all other factors are favourable. Nutrient supply rate and turnover times are more important than the nutrient concentration (LOBBAN, HARRISON and DUNCAN, 1985). The macronutrients nitrogen and phosphorus and the micronutrients iron, manganese and zinc are thought to be the most likely limiting for growth in macroalgae and plankton as their concentrations vary the most in seawater due to biological processes (DeBOER, 1981; CHOPIN, HOURMANT, FLOC'H and PENOT, 1990; KAIN and NORTON, 1990; LÜNING, 1990; REED, 1990).

Under conditions of nutrient limitation, plants exhibit symptoms which are specific according to the element that is limiting depending on the cellular role fulfilled by that compound. Nitrogen limitation in algae causes a decrease in enzyme activity associated with nitrogen metabolism; e.g. nitrate reductase, glutamine synthase and glutamate synthase. Phosphorus limitation causes a decrease in phosphorylated metabolites as well as the internal structure becoming highly disorganised. Nitrogen and phosphorus are closely associated in plant biochemistry, particularly through protein synthesis; e.g. ATP. ADP, NADP and nucleic acids and so are important potentially limiting factors for plant growth (DUARTE, 1992). Plants respond promptly and specifically to nutrient resupply (VANO, RIGANO, ESPOSITO, MARTINO and RIGANO, 1992).

Nitrogen is an important component in all amino acids and hence enzymes, other proteins, nucleotides and some pigments (LOBBAN, HARRISON and DUNCAN, 1985). The most important inorganic nitrogen forms in seawater are ammonia (NH_4^+), which is assimilated the most rapidly, and nitrate (NO_3^-). These occur in concentrations up to 40 mmol m^{-3} and 4 mmol m^{-3}, respectively, in oceanic waters and in concentrations 10 times this in coastal waters. Another source of inorganic nitrogen is nitrite (NO_2^-) but this occurs in lower concentrations and is generally considered to be a more toxic form. Dissolved nitrogen gas is the most abundant but is in an unusable form (LOBBAN, HARRISON and DUNCAN, 1985). Other forms of nitrogen include urea and amino acids.

Ammonia is already in a reduced form and can therefore be directly incorporated into amino acids. Ammonia has dual phase uptake with a high affinity system operating at low substrate concentrations which is saturating, and a non-saturating linear diffusive component operating at high substrate concentrations which is less selective (DeBOER, 1981; DARLEY, 1982); e.g. *Ceramium rubrum* has a high affinity uptake system operating when concentrations are below 10 mmol m^{-3} and a linear diffusion component at higher concentrations (REED, 1990).

Nitrate is the next favoured form of nitrogen supply. Uptake exhibits saturation kinetics, is inhibited by metabolic inhibitors, and is stimulated by light indicating that it is an active process. Nitrate needs to be reduced before utilization. Eight electrons are required to reduced nitrate to nitrite and then to ammonium (KAIN and NORTON, 1990). These involve the enzymes nitrate reductase and nitrite reductase, respectively. Ammonium is incorporated into the carbon skeleton by the glutamine synthetase-glutamate synthase cycle (GALVÀN, CÓRDOBA, CÀRDENAS and FERNÀNDEZ, 1991).

Growth response to nitrogen shows saturation type kinetics with both limitation and toxicity. High nitrogen concentrations will be toxic as ion pumps are needed to control influx. This will cause a reduction in the growth rate (LAING, CHRISTELLER and TERZAGHI, 1989).

Algae have a great storage capacity, usually in vacuoles, for both nitrogen and phosphorus; e.g. *Ceramium rubrum* has internal tissue concentrations 3-4 times higher than the concentration necessary to sustain maximum growth. Sometimes internal levels as high as 8-18 times have been found (LYNGBY, 1990). Nitrogen is often limiting in summer when higher temperatures and increased irradiance cause rapid growth and so more nitrogen is required. Algae may use what nitrogen they have stored to sustain growth (LOBBAN, HARRISON and DUNCAN, 1985; LYNGBY, 1990); e.g. *Gracilaria tikvahiae* McLachlan can accumulate sufficient nitrogen in six hours to support growth for 14 days. Other organic nitrogen reserves include amino acids and other unspecified cell proteins (LOBBAN, HARRISON and DUNCAN, 1985; KAIN and NORTON, 1990; REED, 1990).

Gracilaria species have increased pigment and protein levels when grown in enriched seawater. They have the ability to store nitrogen, mainly incorporated in phycoerythrin, and so continue production after nutrients are no longer available (DAWES and KOCH, 1990). Growth in high light in *Gracilaria* species is often limited by nitrogen and then it has the ability to utilize its pigments. In low light conditions, the pigments act as a nitrogen sink as well as to increase the light harvesting "antennae". Phycobilisomes make up to 60% of the total cellular protein and so represent a large nitrogen reserve (LAPOINTE, 1981; LAING, CHRISTELLER and TERZAGHI, 1989). Similarly *Gelidium latifolium* uses phycobiliproteins as a nitrogen source when external nitrogen is limiting. As phycoerythrin is arranged on the periphery of the phycobilisome, it can be utilised with little effect on energy transfer (FREDRIKSEN and RUENESS, 1989).

Phosphorus is an important component in nucleic acids, co-enzymes, proteins and phospholipids and in energy transfer (ATP; LOBBAN, HARRISON and DUNCAN, 1985). Phosphorus exists as free inorganic orthophosphate ($H_2PO_4^-$ and HPO_4^{2-}) and as metallophosphate complexes with Mg^{2+}, Ca^{2+} and Na^+ and as organic phosphate compounds. Orthophosphate is the principal form used by algae but is less than a third of the total phosphorus in seawater. It has high affinity uptake at low concentrations and linear uptake at higher concentrations. The other two phosphorus forms rely on extracellular enzymatic hydrolysis before uptake. Excess phosphorus is stored as vacuolar phosphate or cytoplasmic polyphosphate granules (DeBOER, 1981; LOBBAN, HARRISON and DUNCAN, 1985; REED, 1990).

Phosphorus is incorporated into organic phosphorylated compounds; e.g. in *Chondrus crispus* Stackhouse, over 50% is hexose monophosphate and 11% ATP. When the nutrients in the seawater are exhausted in spring, the alga can live on the compounds stored in winter (CHOPIN, HOURMANT, FLOC'H and PENOT, 1990).

Phosphorus can inhibit $CaCO_3$ precipitation. This can inhibit growth in coralline algae *Corallina officinalis* Linnaeus, *Cheilosporum sagittatum* (Lamouroux) Areschoug and *Jania rubrum* (Linnaeus) Lamouroux by preventing calcite formation which is necessary for the alga's development when growing in full strength phosphorus of Grunds medium. Coralline algae can be used as indicator organisms for relative levels of phosphorus in seawater (BROWN, DUCKER and ROWAN, 1977). When *Agardhiella subulata* (C. Agardh) Kraft *et* Wynne is grown in low phosphorus concentrations, there is a decrease in the floridean starch content, indicating that it can utilize stored carbohydrates when there is no surplus phosphorus uptake (CHOPIN, HANISAK and KOEHN, 1991).

Pteroceramium shows a general increase in growth rate with increasing levels of nitrogen and phosphorus. The critical concentrations of nitrogen and phosphorus for *Pteroceramium* in laboratory based experiments where the medium is not continually renewed, appears to be approximately full strength Müllers Synthetic Seawater. Toxic concentrations were not reached as there was no drop in growth rates at higher concentrations. There was little effect on morphogenesis showing that nitrogen and phosphorus affect growth rates and not cell development and maturation.

The surface area:volume ratio is important with there being faster uptake in filamentous species than in those with more fleshy thalli; e.g. development of hyaline hairs in *Ceramium rubrum* in reaction to nutrient deficient conditions increase surface area (KAIN and NORTON, 1990; REED, 1990). In *Pteroceramium*, the wings may also function to increase the surface area for nutrient uptake and so could also explain the higher floridean starch granules present in the wing cells compared to the axial cells (see Chapter 2).

Plants have different assimilation preferences for ammonia and nitrate. For *Gracilaria foliifera* (Forsskål) Børgesen and *Neoagardhiella baileyi* (Harvey *ex* Kützing) Wynne *et* Taylor, the specific growth rate increased with increasing nitrogen enrichment. There was also higher growth when supplied with ammonia than nitrate and a lower growth rate when supplied with urea. For macroalgae the optimum nitrogen range is generally between 120 μM-214 μM and as most media have a far higher concentration, growth in culture should not be nutrient limiting (DeBOER, GUIGLI, ISRAEL and D'ELIA, 1978). *Gelidium nudifrons* Gardner has similar rates for simultaneous uptake of ammonia and nitrate although irradiance stimulates nitrate reduction (BIRD, 1976).

In ice algal communities, early growth has predominantly nitrate metabolism and in declining populations there is predominantly ammonia metabolism. Nitrate is provided by physical processes while ammonia is derived *in situ* from biological metabolism and so would accumulate in the latter part of the growth season. Uptake is light independent for both nitrogen species but is substrate dependent. However, it does not appear that nitrogen is limiting in these populations with silicon more likely to limit growth (HARRISON, COTA and SMITH, 1990).

Ion absorption is across a boundary layer and a plasmalemma. The rate of ion uptake depends on the rate of diffusion across the boundary layer (LOBBAN, HARRISON and DUNCAN, 1985). There are three types of uptake:

1) passive diffusion along an electrochemical gradient requiring no metabolic energy. As cellular pH is usually anionic, up to 30 times increased cation concentrations can be found inside cells than in the medium surrounding them;

2) facilitated diffusion along the electrochemical gradient. This involves a carrier concept (permeases) which binds with the ion, diffuses across to the other side of the membrane and discharges the bound ion. This is faster than passive diffusion and has ion selectivity and displays saturation kinetics. This has no direct involvement with energy; and

3) active transport against the electrochemical gradient is an exergonic reaction (energy requiring). It is selective, involving carriers and displays saturation kinetics. It is sensitive to metabolic inhibitors. Transport proceeds at a greater rate than passive diffusion. Algae have electrogenic pumps which involve Cl^- influx and/or Na^+ efflux (DeBOER, 1981; LOBBAN, HARRISON and DUNCAN, 1985; REED, 1990).

Nutrient uptake is influenced by a number of other factors including:

1) irradiance which provides energy via photosynthesis and stimulates growth thereby increasing nutrient uptake and protein synthesis;

2) temperature increases the rate of carrier mediated uptake and affects the rates of enzymes involved in cellular metabolism;

3) water motion increases nutrient uptake by decreasing the boundary layer;

4) salinity and pH affect the concentration and availability of nutrients; and

5) biological factors such as type and age of tissue, nutritional past history and interplant variability (DeBOER, 1981; LOBBAN, HARRISON and DUNCAN, 1985).

Salinity is measured as grams salt kg^{-1} solution ($^{\circ}/_{\circ\circ}$). Salinity affects seawater density, light refraction and electrical conductivity. Its biological significance is its ion concentration, density and osmotic pressure. There are altogether 74 naturally occurring elements in seawater with Cl^- making up 55% and Na^+ 30.6% by weight of dissolved salts. As there are more cations than anions, seawater is slightly alkaline around pH 8.0-pH 8.3. Major elements have a concentration of more than 1 mg l^{-1} and trace elements are found in smaller quantities. These elements proportions are relatively constant except for nitrogen and phosphorus which are in the greatest demand (LOBBAN, HARRISON and DUNCAN, 1985).

There is usually a decrease in species diversity with dilution or concentration of seawater. There may be a stepwise acclimation of populations to stressful salinities which can result in local salinity ecotypes. Salinity varies spatially and temporally and the distribution of a species reflects its preferred or tolerated salinity range and its ability to withstand change over time (BIRD and McLACHLAN, 1986).

All metabolic processes tend to have an optimum salinity and a decrease in salinity causes stunted growth. Eulittoral seaweeds generally tolerate between $10^{\circ}/_{oo}$-$100^{\circ}/_{oo}$ while sublittoral seaweeds are less tolerant between $18^{\circ}/_{oo}$-$52^{\circ}/_{oo}$. There is also phenotypic acclimation (LOBBAN, HARRISON and DUNCAN, 1985; KAIN and NORTON, 1990); e.g. diatoms growing in estuarine regions tolerate a wider salinity range than marine diatoms (MIZUNO, 1992).

Change in salinity can effect an organism in three ways:

1) cause osmotic stress with direct impact on cellular water potential;

2) ion (salt) stress caused by inevitable uptake or loss of ions (part of the acclimation process); and

3) change of cellular ionic ratios due to selective ion permeability of the membrane (KIRST, 1990).

Intertidal algae need to be able to tolerate variations in external osmotic pressure and external ion concentrations. This is not subject to internal regulation as water permeability of the plasmalemma is large (REED, 1990). If the alga is placed in a hypersaline solution, water flows out of the cell. The reduction in turgor pressure causes the cytoplasm and vacuoles to shrink in proportion to the external osmotic pressure and the plasmalemma tears away from the cell wall. This damage is irreparable. The ability to withstand a high salinity is due to the elasticity of the cell wall, allowing it to collapse so that the plasmalemma will not tear away (LOBBAN, HARRISON and DUNCAN, 1985; REED, 1990). If the alga is placed in a hyposaline solution, water flows into the cell causing it to swell and burst which is also fatal. Algae with rigid cell walls are able to restrict cell volume increase (REED, 1990). The ability to tolerate salinity chance by shrinking and swelling is important in tidal cycles where changes are short term. As this is a passive process it is less energy expensive (KIRST, 1990).

There are two physiological processes behind salinity tolerance; viz. osmoregulation and turgor regulation. Osmoregulation maintains a constant internal osmotic potential and is found mainly in animals. Turgor regulation adjusts the cellular osmotic potential to keep cell turgor constant. This is typical of marine algae (KIRST, 1990).

Marine red algae are fairly sensitive to salinity with few species being recorded growing below $15^{\circ}/_{\infty}$. Estuarine algae typically tolerate lower salinities better than oceanic species and supralittoral algae have a broader tolerance range than eulittoral algae with sublittoral species having the narrowest tolerance limit (REED, 1990). Eulittoral and supralittoral algae need a wider tolerance range than sublittoral species due to precipitation and evaporation causing salinity fluctuations. If the alga is not growing in optimal conditions, there are slower growth rates which make it harder to compete with other species (BIRD and McLACHLAN, 1986; LÜNING, 1990).

Growth at low salinities may be governed by availability of certain ions; e.g. Ca^{2+} is needed in cell wall formation. Algae can survive at an even broader range than those required for growth. At near tolerance limits growth is sacrificed in order to maintain osmotic adjustment (KIRST, 1990).

Under extreme hypo- and hyperosmotic stress, photosynthesis and respiration are inhibited. Generally microalgae recover quicker, in a few minutes, than thalloid species which take up to several hours. Generally Rhodophyceae and Chlorophyceae are more sensitive to hypoosmotic treatment than Phaeophyceae. In brackish waters there is often a reduction in size and marked change in morphology (KIRST, 1990).

Adaptation to salinity involves adjustment of the total number of osmotically active intracellular solute molecules. There is an increase in ions with increasing salinity (REED, 1990). The main ions involved are K^+, Na^+ and Cl^- and to a lesser extent, sulphates (SO_4^-; DARLEY, 1982; LÜNING, 1990). Other cations involved include Ca^{2+} and Mg^{2+} and the anion are NO_3^-. These vary widely in algae with all or a combination of these ions operable; e.g. *Griffithsia monilis* Harvey when in downshock has a loss of K^+ and in upshock an accumulation of Na^+. There is no correlation between ion used and taxonomic classes or thallus morphology. Usually the vacuole dominates osmotic relations during salinity stress as it has the largest volume. Ion-selective carriers and facilitated diffusion regulate ion concentrations. The energy requirement for these is approximately 10%-30% of energy gained from respiration (KIRST, 1990).

Organic osmolytes are low molecular weight organic solutes which are accumulated or degraded in response to changing salinities. These fit into taxonomic classes as they are mainly photosynthetic products. They are polyols; e.g. glycerol in *Dunaliella*, mannitol in most Phaeophyceae and Prasinophyceae, sorbitol and proline in most diatoms and Chlorophyceae, sucrose in Chlorophyceae and Charophyceae, and floridoside, isofloridoside and digeneaside in Rhodophyceae. There are also other compounds such as ammonium compounds; e.g. glycinebetaine which occur in some algae. Osmolytes have a high energy cost but are "compatible solutes" which at high concentrations cause minimal inhibition of metabolism and membrane-dependent processes. Algae may have more than one type of osmolyte (KIRST, 1990; LÜNING, 1990; REED, 1990).

The response to changes in salinity is a well regulated, biphasic process:

Phase 1 has a rapid change in turgor pressure caused by massive fluxes in or out of an organism following the osmotic gradient. In microalgae these fluxes last 5-10 seconds and in macroalgae, they last from minutes to hours. There is an influx of water in hypoosmotic treatments and an efflux in hyperosmotic conditions. This is a passive process and depends on physicochemical properties of the cell wall-membrane complex; e.g. elasticity and water permeability.

Phase 2 has osmotic adjustment (osmoacclimation) where the cellular concentrations of osmotically active solutes (osmolytes) changes until a steady state is reached. This is a slower process than phase 1 lasting 40-120 minutes in microalgae and 2-3 days in macroalgae. The change in ion concentration and organic osmolytes is under direct metabolic control (KIRST, 1990; LÜNING, 1990).

These events are co-ordinated usually with ionic contents preceding organic osmolytes. Once a steady state is reached, there are more compatible osmolytes than metabolically adverse ions (KIRST, 1990).

Pteroceramium is stenohaline with a narrow tolerance range for growth (between 30°/$_{oo}$ and 40°/$_{oo}$). This is a smaller tolerance range than generally found in algae but this may be due to being grown in culture for a long period where the salinity had been kept constant and so the algae may have lost some of its ability to adjust to osmotic changes. Nevertheless, the stenohaline response of *Pteroceramium* suggests it is better suited for sublittoral environments where the salinity is more constant (STIRK and AKEN, 1993). *Callithamnion hookeri* has a narrow salinity optimum of 30°/$_{oo}$-40°/$_{oo}$, making it relatively stenohaline. As this species is an open coast species and is not found in estuaries, this corresponds to its stenohaline response (EDWARDS, 1979).

Similarly, some *Gracilaria* species gradually die when in salinities over 35°/$_{oo}$ (GUANZON and DE CASTRO, 1992). The effect of salinity on a number of other *Gracilaria* species was tested and they were shown to have a broad tolerance range of 19°/$_{oo}$-60°/$_{oo}$. In unfavourable conditions there was discolouration and necrosis while at higher salinities there was complete bleaching and death within one week. The apical tissue was the most sensitive. These species showed little genetic adaptation to native salinities (BIRD and McLACHLAN, 1986).

When *Asterocytis ornata* (C.Agardh) Hamel is grown in seawater it grows in tufts with branched filaments with axially compressed cells. When grown in diluted seawater, the cells become oval and are more or less separated but attached by strands of mucilage (LEWIN and ROBERTSON, 1971). Growth and development of the coralline algae *Pneophyllum caulerpae* (Foslie) Jones *et* Woelkerling and *Fosliella cymodoceae* (Foslie) Jones *et* Woelkerling is adversely affected by high salinity with there being a decrease in cover, density and fertility. This may be attributed to a decrease in the photosynthetic rate, metabolic interference by ion concentrations, competition for carbon skeletons with an osmoticum or limitation of essential elements (HARLIN, WOELKERLING and WALKER, 1985). There is variation in growth of euryhaline *Bostrychia radicans* Montagne and *Caloglossa leprieurii* (Montagne) J.Agardh as a function of different salinities. These are correlated to a large extent by the salinity regimes in natural environments where they were collected (YARISH, EDWARDS and CASEY, 1979).

Euryhaline species may either have:

1) ecophenic/non-genetic adaptation/acclimation. This is important in estuarine organisms; e.g. *Delesseria sanguinea* Lamouroux tolerates decreased salinities by gradual acclimation; or

2) ecotypic/genetic differentiation resulting in noticeable differences between populations (YARISH, EDWARDS and CASEY, 1979).

The pH affects the availability of many nutrients due to their dissociation rates and the ionic state of polar inorganic and organic compounds. The pH also effects the electrical charge of the cell wall surface, ion transport systems at the plasmalemma and the associated membrane potentials. There is also a more direct effect of pH on growth as metabolic processes are controlled by enzymes which are sensitive to pH (SOEDER and STENGEL, 1974; GORDON and SAND-JENSEN, 1990). There was a decrease in net photosynthesis of *Gracilaria tikvahiae* when in a pH above pH 8.0. This shows that it is unable to utilize bicarbonate as a carbon source (see Chapter 5). Yields are reduced in dense seaweed beds where poor water mixing creates microenvironments with high pHs (DeBUSK and RYTHER, 1984).

Pteroceramium has a small pH tolerance range for growth suggesting that it has evolved in conditions where pH remains more or less constant. It is not known if this effect of pH was direct or indirect (STIRK and AKEN, 1993). In a closed system such as rock pools and in dense stands of algae, pH is continually altered due to various metabolic processes. In open waters the pH is generally more stable as seawater acts as a buffer. These results suggest that *Pteroceramium* prefers open waters where the pH is more constant.

The different RGRs of *Pteroceramium* may also have been due to the different buffers which were used to maintain the various pHs although this seems unlikely. MES and HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid), another zwitterionic N-substituted aminosulfonic acid buffer, are chemically similar and are regarded as useful physiological buffers. Comparative photosynthetic work by GOOD, WINGET, WINTER, CONNOLLY, IZAWA and SINGH (1966) using Tris and HEPES buffers showed that HEPES has fewer toxic side effects and produced better results than Tris. Tris and MES could not be compared as they have different buffering ranges. McFADDEN and MELKONIAN (1986) also found that HEPES was a particularly successful buffer for algal culture media involving five classes of microalgae.

The length of the pseudodichotomies is not considered to be of any taxonomic importance in *Ceramium* as there is considerable variation in this character (DIXON, 1960a). In *Pteroceramium* the number of axial cells in seg. 4 was highly variable (3-8 cells per segment) within treatments. This confirms that axial cell number per segment in the seaweed investigated is not a useful taxonomic character (STIRK and AKEN, 1993).

Chapter 5

Regulation of photosynthesis in *Pteroceramium*

5.1 Introduction

Much interest has been placed in the photobiology of marine algae as:

1) macroalgae have a greater diversity of photosynthetic pigments than higher plants which means greater physiological variation in macroalgae;

2) greater diversity of natural light conditions (both spectral and irradiance levels) existing underwater than in terrestrial habitats means greater variation in photobiological responses; and

3) early evolutionary stages began in the sea and so knowledge of physiological mechanisms in present marine algae may advance understanding of mechanisms operating in higher plants (LÓPEZ-FIGUEROA and NIELL, 1989).

There are many environmental factors affecting both photosynthetic and respiration rates of organisms within the marine habitat. Algae need continually to acclimatize to optimize photosynthesis under new conditions (LAPOINTE and DUKE, 1984). "Acclimation" is the short-term adjustment that an organism can make to its environment within the limits of its genotype compared to "adaptation" which is a long-term alteration in the genotype, reflecting an evolutionary process (LAPOINTE and DUKE, 1984; LEVY and GANTT, 1988; HANNACH, 1989; GANTT, 1990; FALKOWSKI and LA ROCHE, 1991; RÜDIGER and LÓPEZ-FIGUEROA, 1992). The degree of acclimation which a cell can undergo depends on its prior life history (PAHL-WOSTL and IMBODEN, 1990).

Most work has investigated physiological acclimation of seaweeds to changing irradiance conditions as this is the most dynamic factor in marine environments (LOBBAN, HARRISON and DUNCAN, 1985). Irradiance varies regularly (diurnally and seasonally) and unpredictably (localised shading phenomena and atmospheric changes). High frequency fluctuations owing to wave action cause focusing of light (GREENE and GERARD, 1990) and low frequency fluctuations may be expected on a seasonal cycle.

The best documented photoacclimation response is at the cellular level; viz. a change in the light harvesting pigments. When there is a shift from high to low irradiance, the alga undergoes an energy crisis (FALKOWSKI and LA ROCHE, 1991) and responds by increasing the pigment concentration per unit weight. This may be accompanied by an increase in the ratio of accessory pigment to chlorophyll *a*. The overall result is a higher photosynthetic efficiency (LÜNING, 1981b; BREEMAN and TEN HOOPEN, 1984; LAPOINTE and DUKE, 1984; HANNACH, 1989; REISKIND, BEER and BOWES, 1989; PAHL-WOSTL and IMBODEN, 1990; FALKOWSKI and LA ROCHE, 1991). Photoacclimation plays an important role in buffering the photosynthetic performance in the face of low frequency variations in irradiance (FALKOWSKI and LA ROCHE, 1991). To change the pigment content, there may either be an increase in size of the photosynthetic units (total chlorophyll to reaction centres) or a change in number of the photosynthetic units (GANTT, 1990). Both strategies may occur together (FALKOWSKI and LA ROCHE, 1991).

Other photoacclimation responses to low irradiance include:

1) morphological changes such as a change in cell volume to increase the surface area to enhance light capture, gaseous exchange and nutrient absorption (REISKIND, BEERS and BOWES, 1989). Other examples of morphological change may be an increase in number and density of thylakoid membranes, an increase in the size of pyrenoids and other storage bodies and a change in the number of plastids per cell (FALKOWSKI and LA ROCHE, 1991); and

2) physiological changes which minimize the irradiance required for photosynthesis (FALKOWSKI and LA ROCHE, 1991); e.g. RUBISCO activity changes to maximise photosynthesis (LAPOINTE and DUKE, 1984). There is often a slower growth rate accompanied by a build up of reserves enabling the alga to survive during winter when light is most often limiting. This results in many perennial deep water algal forms (LÜNING, 1981a and b).

The Theory of Chromatic Adaptation proposed by ENGELMAN (1883 in BOGORAD, 1975), is that algae produce pigments that maximally absorb the prevailing light. As green light usually penetrates the deepest, red algae which occur at depth are thought to have evolved pigments for growth in deep water. This infers that the colour of the submarine light field is a mechanism structuring seaweed communities and that the most effective mechanism for filling the "green window" in the visible spectrum is absorption by phycoerythrin (RAMUS and VAN DER MEER, 1983). However, it has been found that green and brown algae are equally efficient in deep waters, having evolved other mechanisms for light absorption in the "green window" (SAFFO, 1987). These include different growth rates, changes to instantaneous photosynthetic parameters by the regulation of the number of photosynthetic units and their intrinsic turnover time, activity of RUBISCO (RAMUS, 1983), different pigment densities and other accessory pigments; e.g. brown algae have fucoxanthin and chlorophyll *c* and many siphonous greens have siphonaxanthin (RAMUS, 1981). As pigment concentrations and their ratios change in response to irradiance, it was concluded that "chromatic adaptation in red seaweeds is the accidental by-product of their physiological adaptation to low irradiances" (RAMUS and VAN DER MEER, 1983). Additional evidence against this theory is that red algae are found in high proportions in both deep and shallow waters (LÜNING, 1981b; SAFFO, 1987).

Algae show typical C_3 photosynthesis but have relatively reduced photosynthetic rates when compared with terrestrial plants (REISKIND, BEER and BOWES, 1989; GORDON and SAND-JENSEN, 1990) as the diffusion resistance of CO_2 is 10^{-4} times greater in water than in air. Increased water flow can reduce this resistance barrier by decreasing the thickness of the unstirred aqueous layer around the algae (REISKIND, BEER and BOWES, 1989).

Algae growing in the upper photic zone (eulittoral and upper sublittoral zones) typically show sun-type photosynthetic features while algae growing in the deeper zones (lower sublittoral) show shade-type features (LÜNING, 1981a; TSENG, ZHOU and PAN, 1981). These "shade-adapted" features include slow growth rates, longevity of the thallus, low photosynthetic rates at light saturation, high pigment content, high accessory pigment:chlorophyll *a* content, and low compensation and saturation points (REISKIND, BEER and BOWES, 1989; LÜNING, 1990). Saturation points of only 5%-20% surface irradiance are not uncommon in the Chlorophyta, Phaeophyta and Rhodophyta (Table 5.1). Irradiance levels play an important role in determining the lower limits at which an alga can grow by limiting the maximum net photosynthesis (REISKIND, BEER and BOWES, 1989).

Table 5.1 Lower irradiance limits for saturating photosynthesis in macroalgae.

Species	Lower irradiance limit	Depth	% surface irradiance	Reference
Laminaria hyperborea (single plant)	71 μmol m^{-2} s^{-1}	8 m	1%	LÜNING, 1981b
Laminaria hyperborea (group)	400 μmol m^{-2} s^{-1}	4 m	4%	LÜNING, 1981b
Crustose red	6 μmol m^{-2} s^{-1}	15 m	0.05%	LÜNING, 1981b
Crustose red*	0.008 μmol m^{-2} s^{-1}	268 m	-	LITTLER, LITTLER and TAYLOR, 1983
Bangia atropurpurea	1 μmol m^{-2} s^{-1}	-	-	ORFANIDIS, 1992
Halimeda	-	130-140 m	>0.01%	PECKOL and RAMUS, 1992

* deepest reported algae

To date, *Pteroceramium* has been found in only seaweed drift growing as an epiphyte on deep water species; e.g. *Haloplegma africanum* and *Callophycus densus*. This suggests that *Pteroceramium* is a sublittoral species and in such habitats, irradiance is often limiting. It is proposed that *Pteroceramium* has become adapted to light attenuated environments by growing wings to increase the surface area of the alga for light interception. As irradiance levels are a critical environmental parameter affecting photosynthetic rates in marine habitats, the aim of this work was to determine the photosynthetic responses of *Pteroceramium* to various irradiance conditions. As temperature and pH also play a fundamental role in regulating the dark phase reactions of photosynthesis, their effects on photosynthesis in *Pteroceramium* were also investigated. This would give an indication of *Pteroceramium*'s possible vertical distribution on the shore.

5.2 Materials and Methods

5.2.1 Experimental material

The male gametophyte of *Pteroceramium*, collected at Trafalgar Beach and *Ceramium glanduliferum*, collected at Mkambati from the eulittoral region, were used in all experiments.

5.2.2 Experimental apparatus

Photosynthetic rates can be measured either by CO_2 consumption or O_2 liberation. Respiration rates are independent of photosynthesis but are measured simultaneously to show net photosynthesis (RAMUS, 1981). In the experiments described below, O_2 liberation was determined polargraphically using a Clark Type Oxygen Electrode (Hansatech D.W., Hansatech Limited, United Kingdom). This method allows for continuous readings and has a reported accuracy of 2% (RAMUS, 1981).

The electrode disc was located in the floor of the reaction chamber. Cigarette paper (Rizzla medium weight) moistened with saturated KCl (AnalaR, BDH Chemicals, United Kingdom) was placed over the platinum cathode located in the dome of the electrode disc. The same KCl solution was used to flood the well where the silver anode was located. The KCl solution acts as an electrolyte and the cigarette paper as a bridge providing electrical continuity between the two electrodes. An O_2 permeable membrane (YSI single membrane, YSI Incorporated, USA) was placed over the electrode disc and secured with an O ring applied at the base of the dome so that the membrane was pulled smooth and all air bubbles removed. The cigarette paper and membrane were trimmed to size. The electrode disc was inserted in the bottom of the reaction chamber and sealed using another O ring.

The platinum cathode was negative in respect to the silver anode. The platinum cathode surface discharged the electrons generated by the reduction of O_2 to the silver anode electron acceptor. The electrical current generated was converted to a voltage output signal which was large enough to be monitored in the 1 volt range (WALKER, 1990).

The reaction chamber was made from borosilicate glass to maximise heat exchange between the reaction mixture and the water jacket (internal volume 40 ml). The water jacket was connected to a cooler which maintained the required temperature with ±1°C accuracy. The reaction chamber was placed on a magnetic stirrer and a magnetic follower inside the reaction chamber stirred directly above the electrode, displacing any diffusion resistance barrier. When required, N_2 was bubbled into the reaction chamber via a pasteur pipette to lower O_2 concentrations.

A slide projector (Pradovit CA 1500, Leitz, Canada) was used as the light source. The levels of irradiance were controlled using seven screens made from shade cloth sewn onto wire frames (16 cm x 26 cm) which were placed directly in front of the slide projector. Irradiance levels reaching the alga in the reaction chamber would be slightly lower than the recorded values owing to reflection from the water jacket.

5.2.3 Calibration of oxygen electrode

The calibration of the oxygen electrode was carried out as described by DELIEU and WALKER (1972). A known volume (2 ml) of O_2 saturated distilled water, obtained by vigourous agitation by hand, was placed in the reaction chamber. Once the output reading had stabilized, the temperature and mV output were recorded. This reading was referred to as the air-line. The air-line and mV output were set at approximately 700 mV-750 mV (70% of total output).

A few grains of sodium dithionite ($Na_2S_2O_4$; BDH Chemicals, United Kingdom) were added to the distilled water and the following reaction occurred:

$$Na_2S_2O_4 + O_2 + H_2O \longrightarrow NaHSO_4 + NaHSO_3$$

As the O_2 was removed from the water, the mV output dropped rapidly until the reaction reached virtual completion and the reading stabilized. The mV output was recorded. This was called the N_2-line as the same result could be achieved more slowly by sparging the medium with N_2. The reaction chamber was then thoroughly rinsed with distilled water.

The mV span was determined by subtracting the N_2-line from the air-line to determine the maximum dissolved O_2 concentration in distilled water at a given temperature and atmospheric pressure and converted to μg O_2. As temperature and atmospheric pressure affect the solubility of O_2 and the saturation capacity of water, a temperature correction factor (TCF) and altitude correction factor (ACF) were used. These values were obtained from GOLTERMAN, CLYMO and OHNSTAD (1978). The altitude of Pietermaritzburg is 680 meters. The following equation was used:

$$1 \text{ mV} = (\text{TCF}/(\text{mV span x ACF})) \text{ x } 1000 = \mu g \ O_2$$

Calibration of the electrode was checked after every 5-8 sets of readings. The membrane was replaced daily.

5.2.4 O_2 liberation measurements

Plant material was subcultured 2-4 weeks prior to each experiment so that actively growing cultures were used, as there was photooxidation of the phycobiliproteins and a lack of new synthesis of pigments in old material (GANTT, 1990). A single "pompom" of *Pteroceramium* with a fresh weight between 0.04 g-0.15 g was placed in the reaction chamber with 2 ml PES and allowed to equilibrate for 5 minutes.

Respiration rates were then measured by covering the reaction chamber with a black cloth. As O_2 was consumed, there was a net drop in the mV output. Once there was a steady decrease, the drop was measured over a 3-5 minute period. The medium was then N_2 sparged to lower the O_2 concentration to near the N_2-line and the reading allowed to stabilize before the lights were switched on. Readings were then taken at progressively higher irradiance levels. Prior to each reading, a one minute equilibration period was allowed at the new irradiance level, although the experimental material appeared to respond immediately to changes in irradiance. Once a steady state equilibrium was reached, readings were taken for 3-5 minute period. All readings were taken at 20°C unless otherwise stated. Whenever the mV output rose to approximately 70% 0_2 saturation, the medium was N_2- sparged, as near O_2 saturation levels can cause a reduction in O_2 liberation owing to increased photorespiration and so cause erroneous results.

After the O_2 liberation measurements, *Pteroceramium* was left overnight in an oven set at 80°C and the dry weight determined the following morning. Five replicate plants were used per treatment.

5.2.5 O_2 liberation experiments

i) Irradiance experiment - Photosynthetic rates were determined for *Pteroceramium* when preconditioned for a week prior to experimentation at three different irradiances; viz. 30 μmol m^{-2} s^{-1}, 80 μmol m^{-2} s^{-1} and 150 μmol m^{-2} s^{-1}.

ii) Comparison with *Ceramium glanduliferum* - Photosynthetic rates were determined for *Pteroceramium* and *Ceramium glanduliferum*. Both species had been grown under standard growth conditions prior to experimentation.

iii) Exposure to high irradiance - *Pteroceramium* was grown in standard growth conditions prior to the experiment. Experimental material was placed in the reaction chamber and exposed to a high irradiance (3114 μmol m^{-2} s^{-1}) for either 1 minute, 5 minutes, 10 minutes, 30 minutes, 60 minutes or 120 minutes. The control replicates were not exposed to this high irradiance. O_2 liberation measurements were taken immediately after exposure.

iv) Exposure to lower irradiance - The above experiment was repeated (Section 5.2.5.iii) but plants were exposed to a lower irradiance (819 μmol m^{-2} s^{-1}).

v) Recovery after exposure to high irradiance - Material was grown in the standard growth conditions prior to experimentation. Experimental material was placed in the reaction chamber and exposed to high irradiance (2752 μmol m^{-2} s^{-1}) for 2 hours. The thallus was then placed in 17 ml sterilised PES in McCartney bottles and returned to standard growth conditions. Photosynthetic readings were taken either 0 hours, 6 hours, 12 hours or 24 hours after exposure. Individual plants were used for each treatment. The control replicates were not exposed to this high irradiance but were otherwise treated similarly.

vi) Acclimation to temperature - *Pteroceramium* was grown at three different temperatures; viz. 15°C, 20°C and 25°C for a week prior to the experiment. All other growth conditions were kept constant. O_2 liberation measurements were taken not only at the temperature at which the material was preconditioned but also at the other two temperatures.

vii) pH - *Pteroceramium* was grown in the standard growth conditions. O_2 liberation measurements were taken at pH 5.93, pH 6.70, pH 7.92, pH 8.84 and pH 9.71. The pH was adjusted using 0.1 M HCl or 0.1 M NaOH immediately prior to the readings. Buffers were not used as seawater was a sufficiently good buffer for these experiments. It was not possible to precondition the material in these pHs as the material died under the more acidic conditions (STIRK and AKEN, 1993; Chapter 4).

5.2.6 Determining photosynthetic parameters

The rate of O_2 liberation was determined as $\mu g\ O_2\ min^{-1}\ mg^{-1}$ dry weight. Photosynthetic/irradiance curves were plotted showing net photosynthesis. Five physiological expressions defining photosynthesis were determined from these curves (Fig. 5.1):

1) α showed the apparent photosynthetic efficiency at subsaturating irradiances (LEDERMAN and TETT, 1981). It was determined by the initial steepest part of the curve. The slope was proportional to the number of reaction centres and their size (RAMUS, 1981) and showed the photochemical rate (COUTINHO and ZINGMARK, 1987). The first four points of the curve were used to determine α for *Pteroceramium* replicates. The slope was determined using regression analysis (Statgraphics 5.0, STSC, USA).

2) P_{max} was the irradiance specific uptake rate showing the maximum photosynthetic rate of the species at saturating irradiances (SICKMAN and MELACK, 1992). This rate was dependent on temperature (LEDERMAN and TETT, 1981). P_{max} is proportional to the number of reaction centres and their turnover time (RAMUS, 1981). It was often difficult to read the P_{max} value off the photosynthetic/irradiance curve as the asymptote was reached very slowly. This was due to a gradual increase in light penetration to the shaded side of the thallus (LÜNING, 1981a), the upper surface of the thallus becoming light saturated before the lower surface (WALKER, 1990). An average of the last four points was used to determine P_{max} for *Pteroceramium*.

3) I_k was the minimum irradiance needed for saturating photosynthesis. It was used as an index of irradiance adaptation with lower I_k values indicating adaptation to low irradiance levels (SICKMAN and MELACK, 1992). I_k for *Pteroceramium* was determined by P_{max}/α.

4) I_c was the compensation point where the photosynthetic O_2 liberation equalled respiratory O_2 consumption (LÜNING, 1990). This was read off the photosynthetic/irradiance curve.

5) Rsp is the respiration rate showing O_2 consumption in the dark.

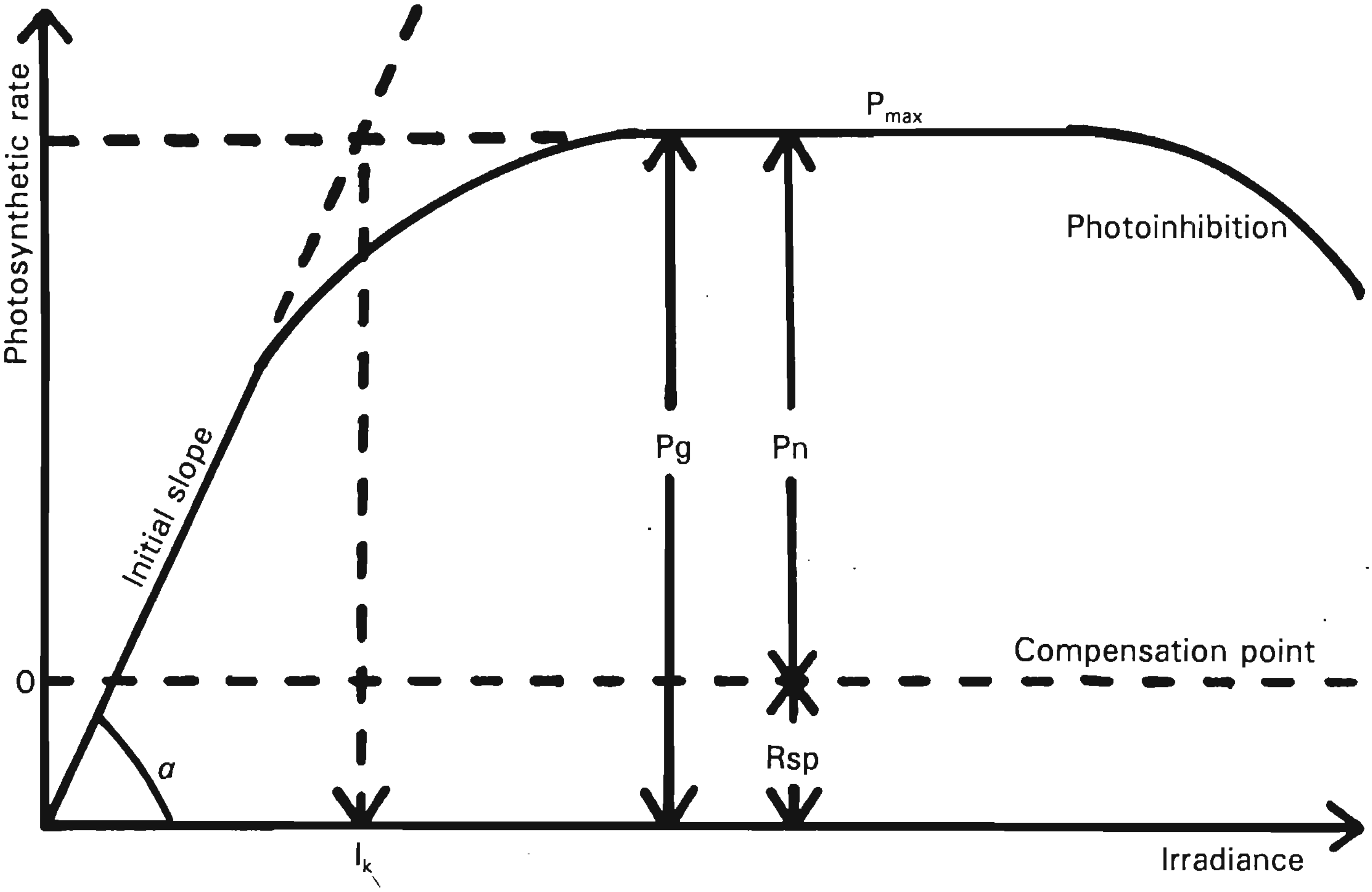


Fig. 5.1 Generalized photosynthetic/irradiance curve where Pg = gross photosynthesis, Pn = net photosynthesis, α = initial slope of curve, P_{max} = maximum photosynthetic rate, I_k = saturation irradiance, Rsp = respiration rate and I_c = compensation point (RAMUS, 1981).

For some experiments these parameters were also determined on a total pigment basis (chlorophyll *a* + carotene + phycoerythrin + phycocyanin) rather than a dry weight basis. The parameters are then indicated with a superscript B (^{B}; SICKMAN and MELACK, 1992).

These photosynthetic parameters were determined for each replicate. The data were analyzed statistically (Statgraphics 5.0) to reveal any significant differences between treatments. All data were first analyzed using the non-parametric Kruskal Wallis One-Way Analysis by Rank. If this showed significant differences between treatments, the parametric One-Way Analysis of Variance was performed and a multiple range test (Tukey) used to reveal the differences.

5.2.7 Correlation between wet and dry mass

Pill vials were oven dried overnight at 80°C. After being cooled for 1 hour in a desiccator, their mass was recorded. Pieces of *Pteroceramium* of various sizes (50 replicates in total) were blotted dry, placed in the vials and the mass of fresh *Pteroceramium* and the vial recorded. These were oven dried overnight at 80°C, cooled in a desiccator for 1 hour and the mass of the vial and dried *Pteroceramium* recorded. From these measurements, the wet and dry mass of *Pteroceramium* could be determined. Linear regression analysis was carried out using Statgraphics 5.0 to determine the correlation between the wet mass and dry mass for *Pteroceramium*.

This procedure was repeated for *Ceramium glanduliferum* using 31 replicates.

5.2.8 Extraction of pigments

Pteroceramium was grown at 30 μmol m^{-2} s^{-1}, 80 μmol m^{-2} s^{-1} and 150 μmol m^{-2} s^{-1} respectively with the other standard growth conditions remaining unchanged. *Ceramium glanduliferum* was grown at 80 μmol m^{-2} s^{-1}. There were four replicates per treatment.

i) Chlorophyll *a* and carotene extraction - As chlorophyll and carotenoids are easily light degraded (JENSEN, 1978), all work was carried out in dim light. Experimental material was rinsed in distilled water to remove excess salt, blotted dry and weighed. After being ground in a mortar and pestle with acid washed sand (40-100 mesh size) in 2 ml freshly made 90% acetone, the material was centrifuged for 5 minutes at 5.5 x g. The supernant was removed and the pellet reground in 2 ml acetone and centrifuged for a further 5 minutes. The two supernants were combined and centrifuged for a further 5 minutes. This supernant was removed and temporarily stored in McCartney bottles wrapped in aluminium foil at 4°C (JENSEN, 1978).

ii) <u>Phycobiliprotein extraction</u> - This extraction was also carried out in dim light. Experimental material was washed in distilled water, blotted dry and weighed. It was ground up in a mortar and pestle using acid washed sand in 5 ml 0.05 M phosphate buffer (Appendix C.1). The material was centrifuged for 5 minutes at 5.5 x g. The supernant was removed and the pellet reground in 5 ml phosphate buffer and centrifuged for a further 5 minutes. This resulting supernant was added to the previous supernant and centrifuged for a further 5 minutes. This supernant was temporarily stored in aluminium wrapped McCartney bottles at 4°C (BEER and ESHEL, 1985).

For both the extracts, absorption spectrum readings were immediately taken on a spectrophotometer (Varian DMS 90 Ultraviolet-Visible Spectrophotometer, Varian Techtron Pty. Ltd, Australia) between 750 nm-250 nm (slit width 2 nm).

Pigment concentrations were determined using the following formulae:

chlorophyll *a* (mg l^{-1}): $[(12.7 \times A_{663}) - (2.69 \times A_{645})]$ (MEEKS, 1974)
carotene (g l^{-1}): $[(A_{480} - (0.033 \times A_{661}))/193]$ (EVANS, 1988)
phycoerythrin (mg ml^{-1}): $[(A_{564} - A_{730})0.12]$ (BEER and ESHEL, 1985)
phycocyanin (mg ml-1): $[(A_{618} - A_{730})0.15]$ (BEER and ESHEL, 1985)

The 730 nm absorption reading is used as a correction factor for scattering and background turbidity (SIEGLMAN and KYCIA, 1978). Results are shown as μg pigment mg^{-1} dry weight with the dry weight being determined using the correlation factor previously determined in Section 5.2.7.

5.3 Results

5.3.1 O_2 liberation results

All results are given as gross photosynthesis.

i) <u>Irradiance experiment</u> - The largest photosynthetic response was obtained from material preconditioned at 80 μmol m^{-2} s^{-1} and the smallest response from material preconditioned at 150 μmol m^{-2} s^{-1} (Fig. 5.2), although there were no significant differences between Rsp, P_{max} and α values for these treatments (Table 5.2). However, there was a significant difference between I_k values ($P<0.05$) with the material preconditioned at 150 μmol m^{-2} s^{-1} having the smallest value. This material also had a significantly higher I_c value ($P<0.05$).

Table 5.2 Photosynthetic parameters of *Pteroceramium* when grown at different irradiance levels. All results are given as mean ± standard error. DW = dry weight.

Treatment μmol m^{-2} s^{-1}	Rsp μg O_2 min^{-1} mg^{-1} DW	I_c μmol m^{-2} s^{-1}	α	P_{max} μg O_2 min^{-1} mg^{-1} DW	I_k μmol m^{-2} s^{-1}
30	-6.0 ± 1.5	14.6 ± 3.2	0.27 ± 0.07	50.85 ± 13.1	192 ± 9
80	-8.3 ± 2.5	17.2 ± 3.1	0.29 ± 0.06	67.66 ± 15.8	228 ± 17
150	-8.4 ± 0.9	40.0 ± 8.5	0.18 ± 0.03	31.78 ± 5.8	175 ± 12

Figure 5.3 and Table 5.3 show the photosynthetic/irradiance curves and photosynthetic parameters when determined using total pigment content rather than dry weight. Although there were no significant differences between P_{max}^B and α^B for the treatments, the largest photosynthetic response was still obtained from the material preconditioned at 80 μmol m^{-2} s^{-1}. The lowest photosynthetic response was obtained for the material growing at 30 μmol m^{-2} s^{-1}. I_K^B was significantly lower ($P<0.05$) for material

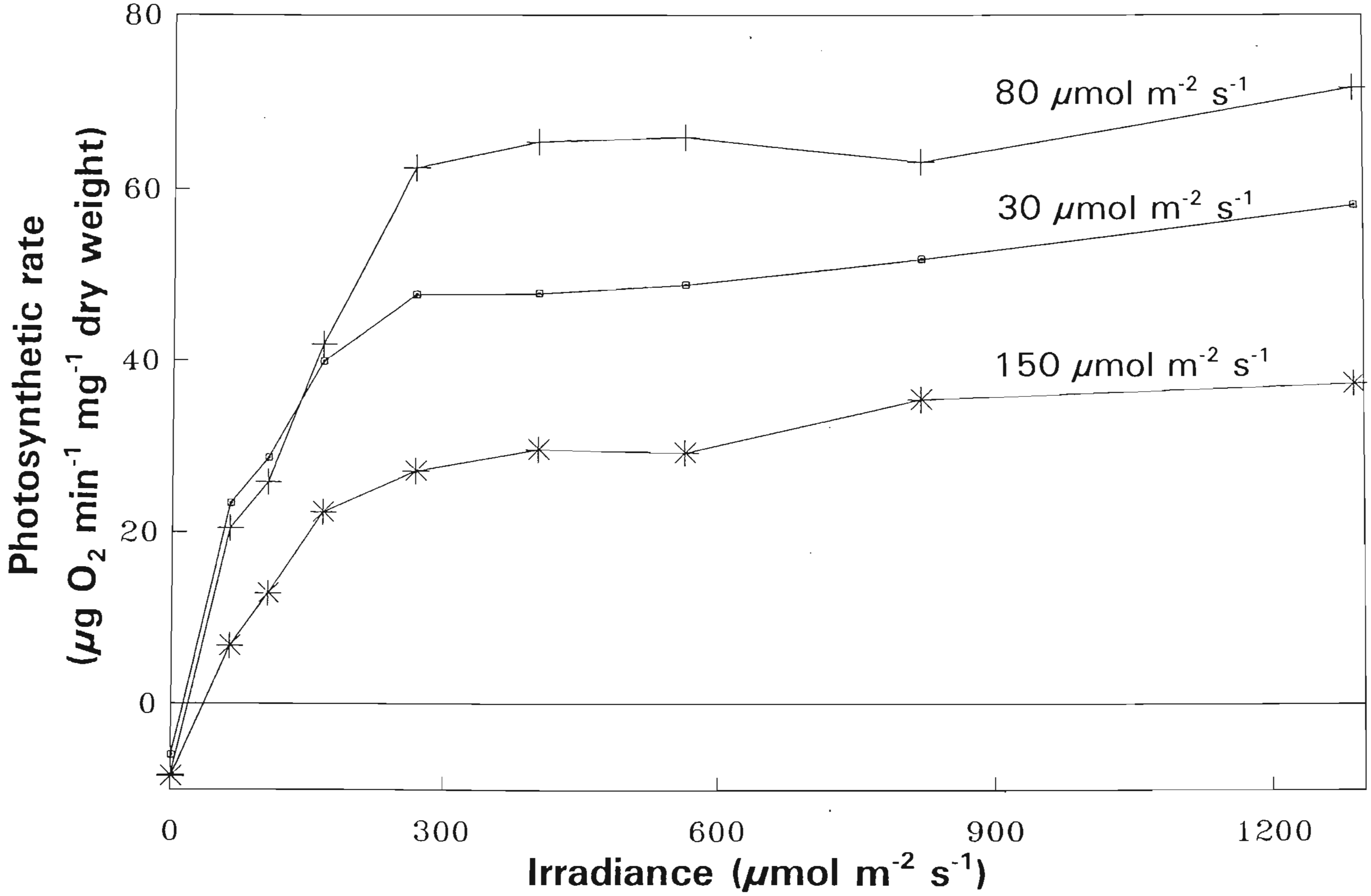


Fig. 5.2 Photosynthetic/irradiance curve for *Pteroceramium* when preconditioned at three irradiance levels.

preconditioned at 150 μmol m^{-2} s^{-1} while Rsp^B ($P<0.05$) and I_c^B ($P<0.05$) were significantly higher.

Table 5.3 Photosynthetic parameters of *Pteroceramium* based on total pigment content when grown at different irradiance levels. All results are given as mean ± standard error. PG = pigment.

Treatment μmol m^{-2} s^{-1}	Rsp^B μg O$_2$ min^{-1} μg^{-1} PG	I_c^B μmol m^{-2} s^{-1}	α^B	P_{max}^B μg O$_2$ min^{-1} μg^{-1} PG	I_k^B μmol m^{-2} s^{-1}
30	-0.6±0.2	14.8±3.6	0.03±0.01	5.53±1.48	193±10
80	-1.1±0.3	17.2±2.7	0.04±0.01	9.16±2.07	235±19
150	-1.7±0.2	41.4±7.6	0.04±0.01	6.76±1.24	181±12

ii) <u>Comparison with *Ceramium glanduliferum*</u> - *Pteroceramium* had a significantly higher I_c value ($P<0.01$) than *Ceramium glanduliferum.* P_{max} ($P<0.05$) and I_k ($P<0.01$) were significantly higher for *Ceramium glanduliferum*. There was no significant difference between Rsp and α values (Fig. 5.4 and Table 5.4).

Table 5.4 Photosynthetic parameters of *Pteroceramium* and *Ceramium glanduliferum* when grown at 80 μmol m^{-2} s^{-1}. All results are given as mean ± standard error. DW = dry weight.

Treatment	Rsp μg O$_2$ min^{-1} mg^{-1} DW	I_c μmol m^{-2} s^{-1}	α	P_{max} μg O$_2$ min^{-1} mg^{-1} DW	I_k μmol m^{-2} s^{-1}
Ptero-ceramium	-8.3±2.5	17.2±3.1	0.29±0.06	67.66±15.8	228±17
Ceramium glanduliferum	-11.1±5.7	3.8±0.7	0.22±0.03	116.90±12.6	550±38

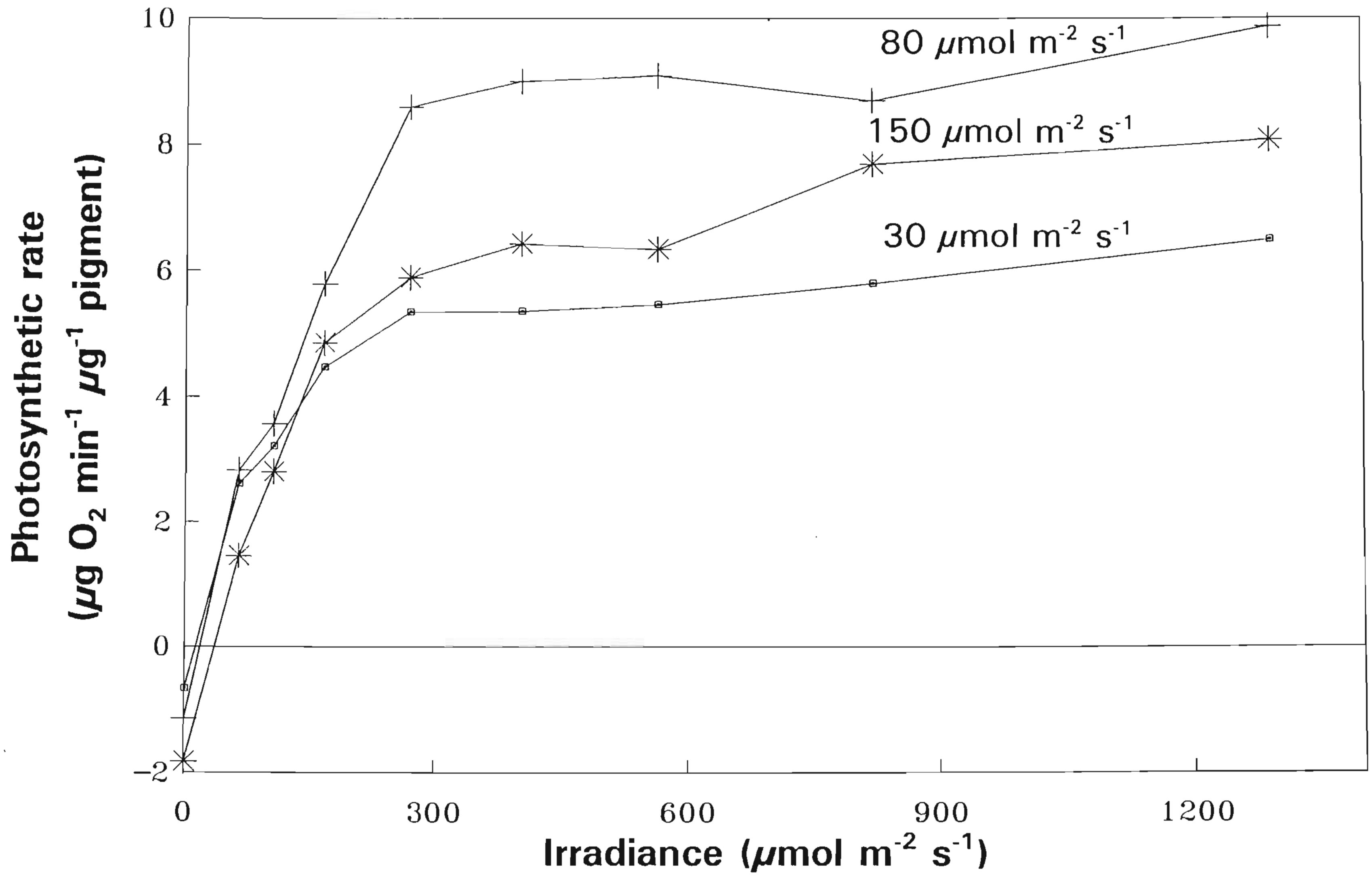


Fig. 5.3 Photosynthetic/irradiance curve for *Pteroceramium* when preconditioned at three irradiance levels. Results are determined on a total pigment content basis.

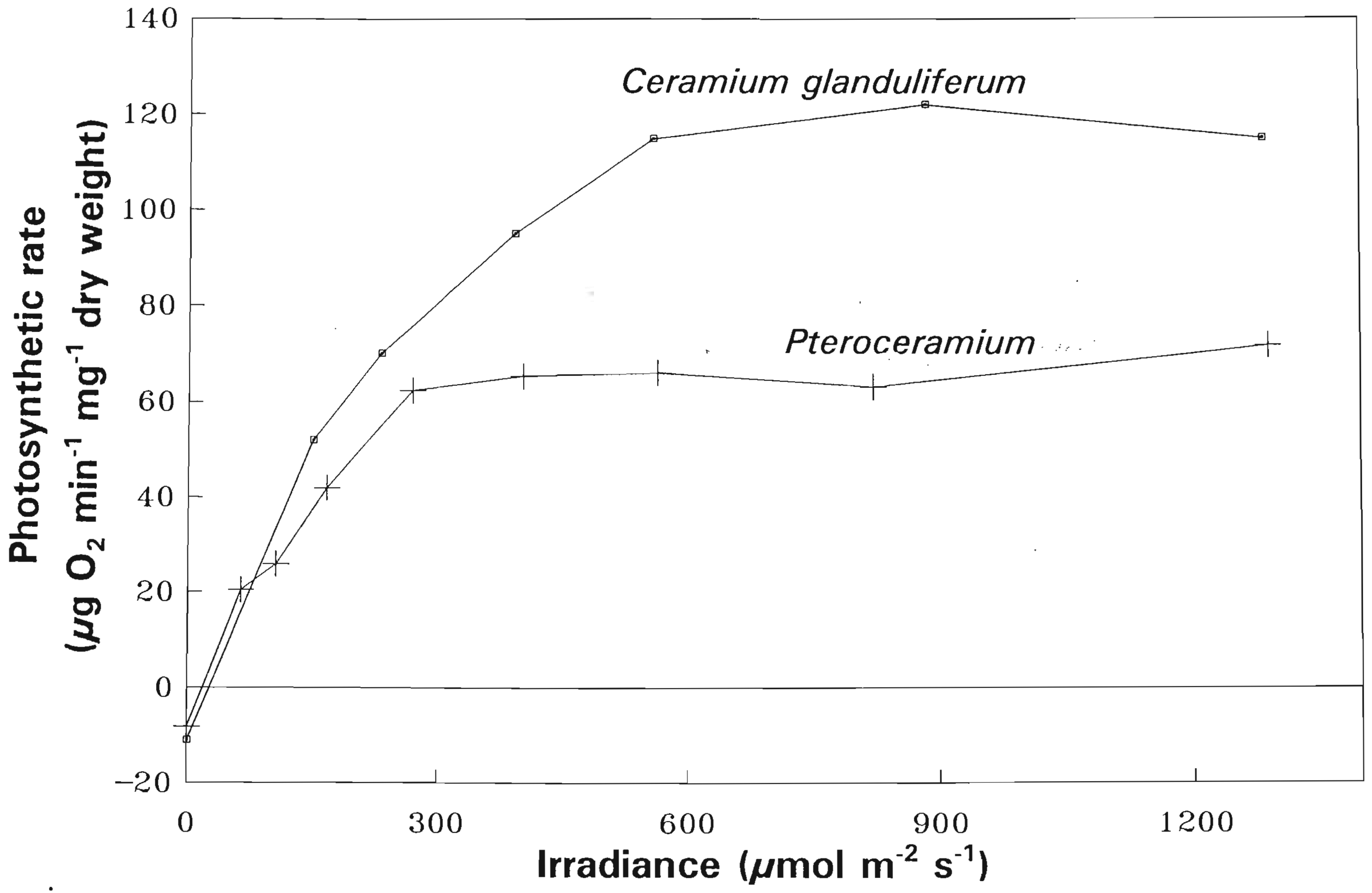


Fig. 5.4 Photosynthetic/irradiance curves of *Pteroceramium* and *Ceramium glanduliferum* when grown at 80 μmol m^{-2} s^{-1}.

When the photosynthetic irradiance curves and photosynthetic parameters were determined using total pigment content rather than dry weight, there was a reversal in the photosynthetic performances of *Pteroceramium* and *Ceramium glanduliferum* (Fig. 5.5 and Table 5.5). There was no significant difference in Rsp^B, I_c^B and P_{max}^B values. However, *Pteroceramium* had a significantly higher α^B ($P<0.05$) value and a significantly lower I_k^B ($P<0.01$) value than *Ceramium glanduliferum.*

Table 5.5 Photosynthetic parameters determined on a pigment content basis for *Pteroceramium* and *Ceramium glanduliferum* when grown at 80 μmol m^{-2} s^{-1}. All results are given as mean ± standard error. PG = pigment.

Treatment	Rsp^B μg O_2 min^{-1} μg^{-1} PG	I_c^B μmol m^{-2} s^{-1}	α^B	P_{max}^B μg O_2 min^{-1} μg^{-1} PG	I_k^B μmol m^{-2} s^{-1}
Pteroceramium	-1.1 ±0.3	17.2 ± 2.7	0.04 ± 0.01	9.16 ± 2.1	235 ± 19
Ceramium glanduliferum	-0.7 ± 0.4	23.6 ± 8.3	0.01 ± 0.00	7.35 ± 0.8	551 ± 38

iii) Exposure to high irradiance - Short exposure (1 minute, 5 minutes and 10 minutes) to high irradiance had no significant effect on *Pteroceramium*'s photosynthetic performance (Fig. 5.6 and Table 5.6). Exposure for over 30 minutes caused a significant decrease in P_{max} ($P<0.0001$) and α ($P<0.001$) and exposure for over 60 minutes caused a significant decrease in I_k ($P<0.1$). Rsp was not affected by exposure to high irradiances. There was a significant increase in I_c ($P<0.05$), the control and 1 minute exposure having lowest I_c values and material subjected to 120 minutes exposure having the highest I_c values.

iv) Exposure to lower irradiance - Exposure to 819 μmol m^{-2} s^{-1} had little effect on the photosynthetic response of *Pteroceramium* apart from causing a significant decrease in P_{max} ($P<0.1$) after exposure for 120 minutes (Fig. 5.7 and Table 5.7).

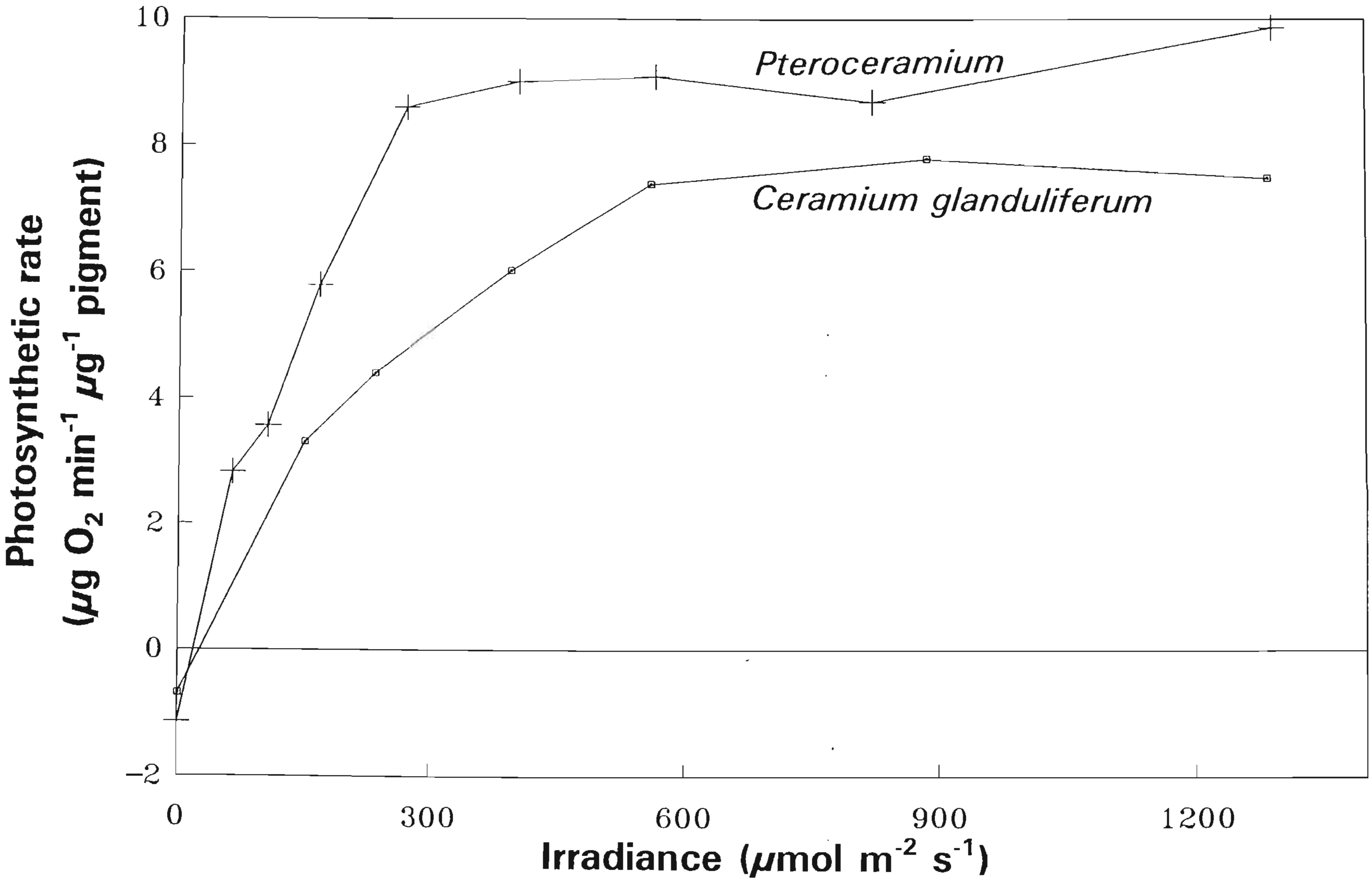


Fig. 5.5 Photosynthetic/irradiance curves of *Pteroceramium* and *Ceramium glanduliferum* when grown at 80 μmol m^{-2} s^{-1}. Results are determined on a total pigment content basis.

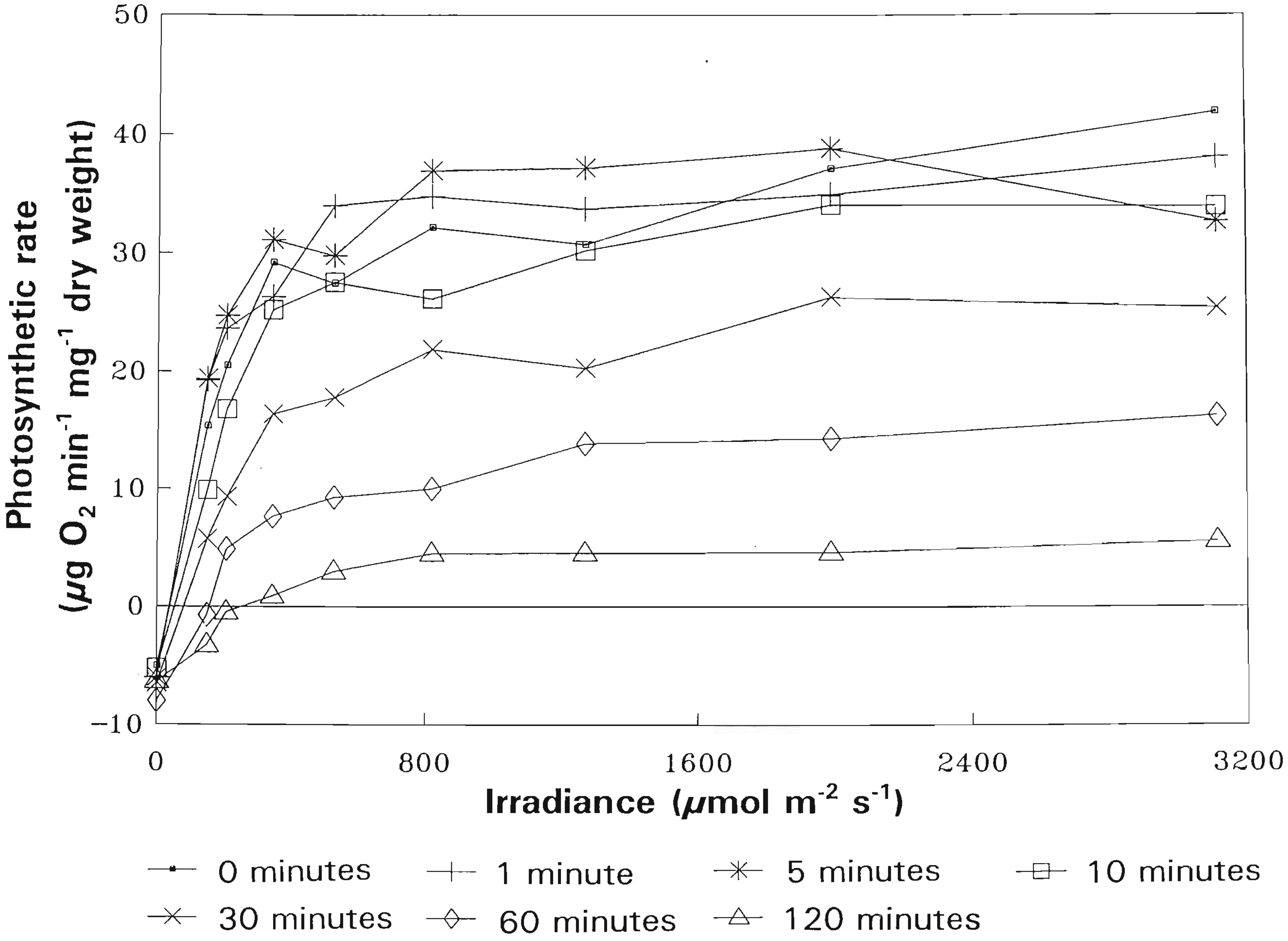


Fig. 5.6 Photosynthetic/irradiance curve of *Pteroceramium* when exposed to 3114 μmol m^{-2} s^{-1} for various lengths of time.

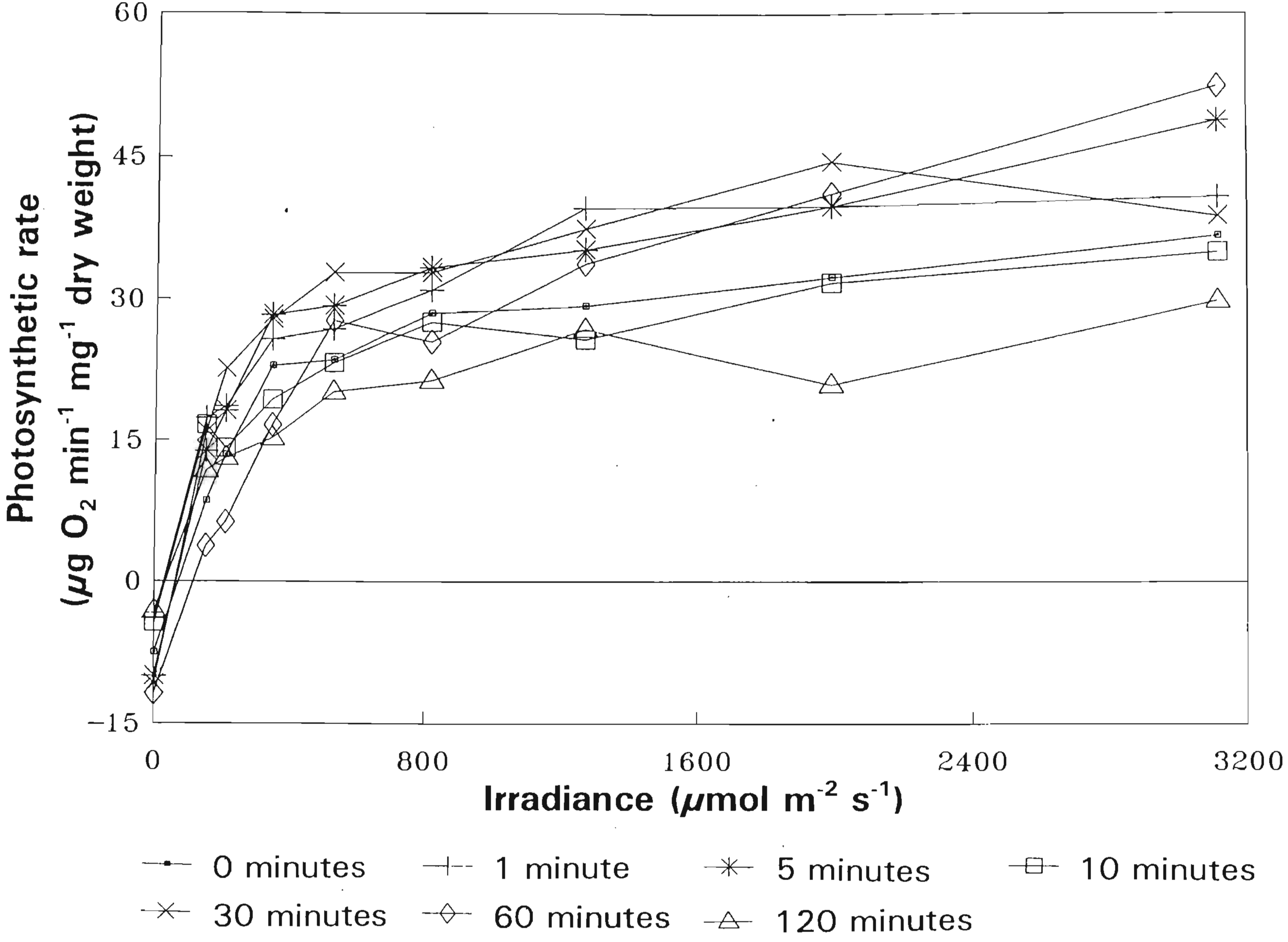


Fig. 5.7 Photosynthetic/irradiance curve of *Pteroceramium* when exposed to 819 μmol m^{-2} s^{-1} for various lengths of time.

Table 5.6 Photosynthetic parameters of *Pteroceramium* exposed to 3114 μmol m^{-2} s^{-1} for various lengths of time. All results are given as mean ± standard error. DW = dry weight.

Exposure time	Rsp	I_c	α	P_{max}	I_k
	μg O$_2$ min^{-1} mg^{-1} DW	μmol m^{-2} s^{-1}		μg O$_2$ min^{-1} mg^{-1} DW	μmol m^{-2} s^{-1}
Control	-5.1 ± 2.0	43.8 ± 10.4	0.16 ± 0.02	69.37 ± 4.81	431 ± 26
1 min	-6.0 ± 1.6	34.2 ± 5.2	0.13 ± 0.01	49.63 ± 2.44	388 ± 26
5 min	-6.0 ± 3.8	48.8 ± 4.3	0.16 ± 0.02	53.65 ± 3.42	345 ± 44
10 min	-5.2 ± 2.1	80.0 ± 28.9	0.12 ± 0.01	42.64 ± 1.84	365 ± 38
30 min	-6.5 ± 2.8	89.2 ± 43.4	0.09 ± 0.01	31.71 ± 2.54	358 ± 20
60 min	-8.0 ± 1.9	131.5 ± 20.8	0.06 ± 0.00	17.58 ± 2.83	286 ± 41
120 min	-6.3 ± 1.1	237.5 ± 47.2	0.03 ± 0.07	6.81 ± 0.71	256 ± 56

Table 5.7 Photosynthetic parameters of *Pteroceramium* exposed to 819 μmol m^{-2} s^{-1} for various lengths of time. All results are shown as mean ± standard error. DW = dry weight.

Exposure time	Rsp	I_c	α	P_{max}	I_k
	μg O$_2$ min^{-1} mg^{-1} DW	μmol m^{-2} s^{-1}		μg O$_2$ min^{-1} mg^{-1} DW	μmol m^{-2} s^{-1}
Control	-7.5 ± 4.6	57.8 ± 14.6	0.09 ± 0.02	31.58 ± 3.01	408 ± 105
1 min	-3.8 ± 1.8	28.3 ± 12.8	0.08 ± 0.01	37.79 ± 4.60	455 ± 49
5 min	-10.0 ± 4.7	58.8 ± 25.6	0.11 ± 0.02	38.03 ± 3.51	352 ± 37
10 min	-4.4 ± 3.0	23.8 ± 12.8	0.07 ± 0.01	30.01 ± 2.48	453 ± 16
30 min	-16.4 ± 8.3	62.3 ± 34.7	0.11 ± 0.02	41.22 ± 1.77	425 ± 116
60 min	-11.8 ± 9.2	104.8 ± 37.8	0.08 ± 0.04	38.19 ± 5.94	474 ± 92
120 min	-3.2 ± 2.2	31.3 ± 19.3	0.05 ± 0.01	24.78 ± 4.81	485 ± 54

v) Recovery after exposure to high irradiance - There was a significant decrease in P_{max} ($P<0.1$), α ($P<0.1$) and I_k ($P<0.05$) and a significant increase in Rsp ($P<0.05$) and I_c ($P<0.1$) immediately after exposure (0 hours) to high irradiance when compared with the control (Fig. 5.8 and Table 5.8). P_{max} and α values for 6 hours, 12 hours and 24 hours after exposure were lower but not significantly different from the control.

Table 5.8 Photosynthetic parameters of *Pteroceramium* showing recovery after exposure to 2752 μmol m^{-2} s^{-1} for 2 hours. All results are shown as mean ± standard error. DW = dry weight.

Recovery time	Rsp	I_c	α	P_{max}	I_k
	$\mu g\ O_2\ min^{-1}\ mg^{-1}$ DW	$\mu mol\ m^{-2}\ s^{-1}$		$\mu g\ O_2\ min^{-1}\ mg^{-1}$ DW	$\mu mol\ m^{-2}\ s^{-1}$
Control	-10.4±1.3	29.7±4.9	0.26±0.05	87.76±16.4	338±10
0 hours	-25.4±5.9	359.1±23.5	0.11±0.03	29.88±9.02	251±26
6 hours	-10.4±1.9	48.0±17.7	0.21±0.04	60.42±13.2	297±13
12 hours	-9.7±3.8	28.2±5.3	0.21±0.05	70.74±15.5	338±13
24 hours	-5.9±0.7	45.6±19.8	0.14±0.03	54.00±3.65	409±60

vi) Acclimation to temperature - Photosynthetic/irradiance curves and photosynthetic parameters are shown in Figure 5.9 and Table 5.9 for *Pteroceramium* when preconditioned at three temperatures and O_2 liberation measurements taken at these temperatures. The range of temperatures investigated had no significant effect on photosynthetic responses in *Pteroceramium*.

Photosynthetic/irradiance curves and photosynthetic parameters for *Pteroceramium* when acclimatised at 15°C and then photosynthetic measurements taken at 15°C, 20°C and 25°C are shown in Figure 5.10 and Table 5.10. A rapid 5°C increase to 20°C caused a significant increase in P_{max} ($P<0.001$). However, a rapid 10°C increase to 25°C caused a significant decrease in the P_{max} value. A 5°C increase caused a significant increase in α ($P<0.05$) but a 10°C increase had no effect. A 5°C increase

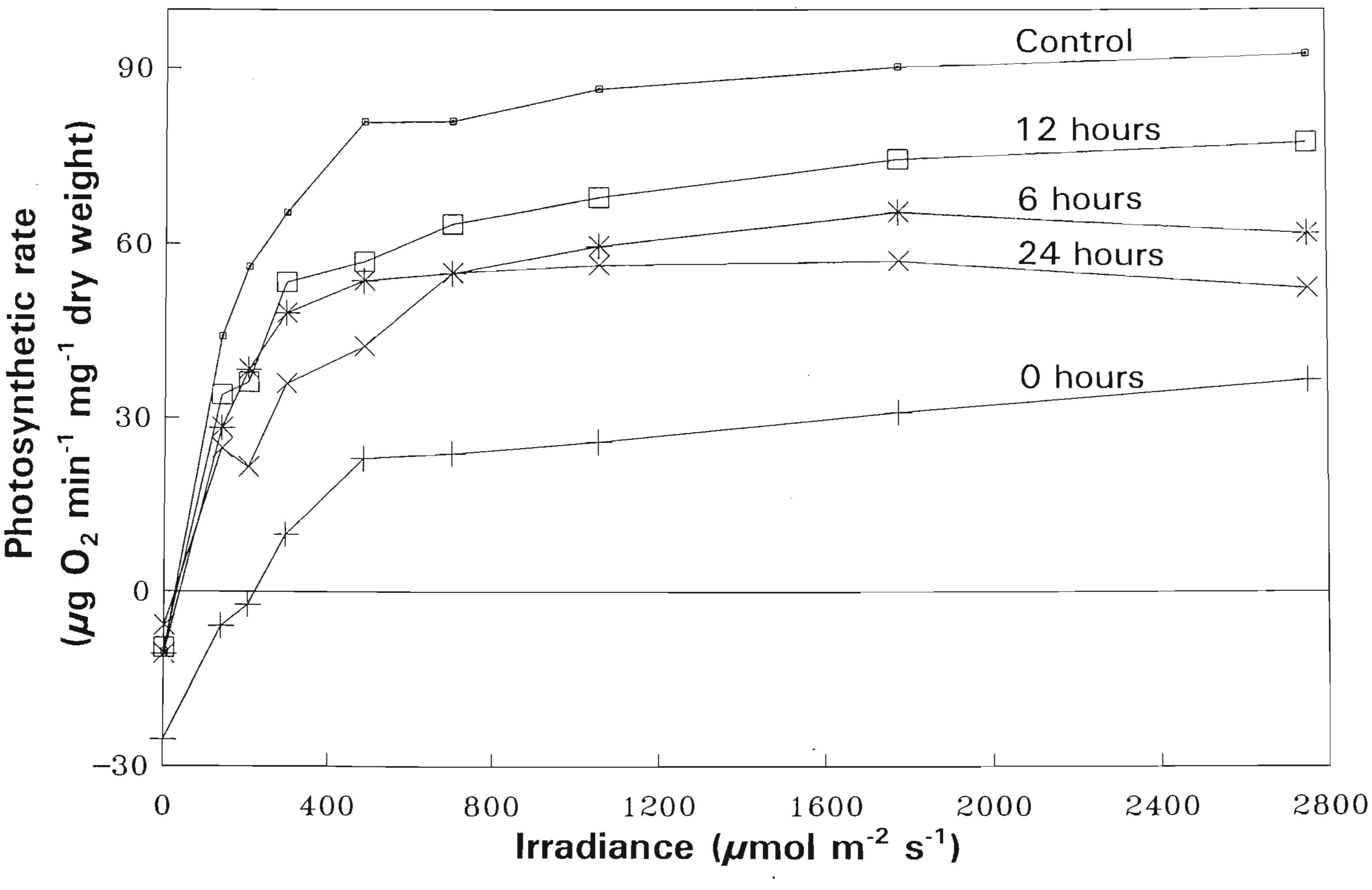


Fig. 5.8 Photosynthetic/irradiance curve of *Pteroceramium* showing the rate of recovery after exposure to 2752 μmol m^{-2} s^{-1} for two hours.

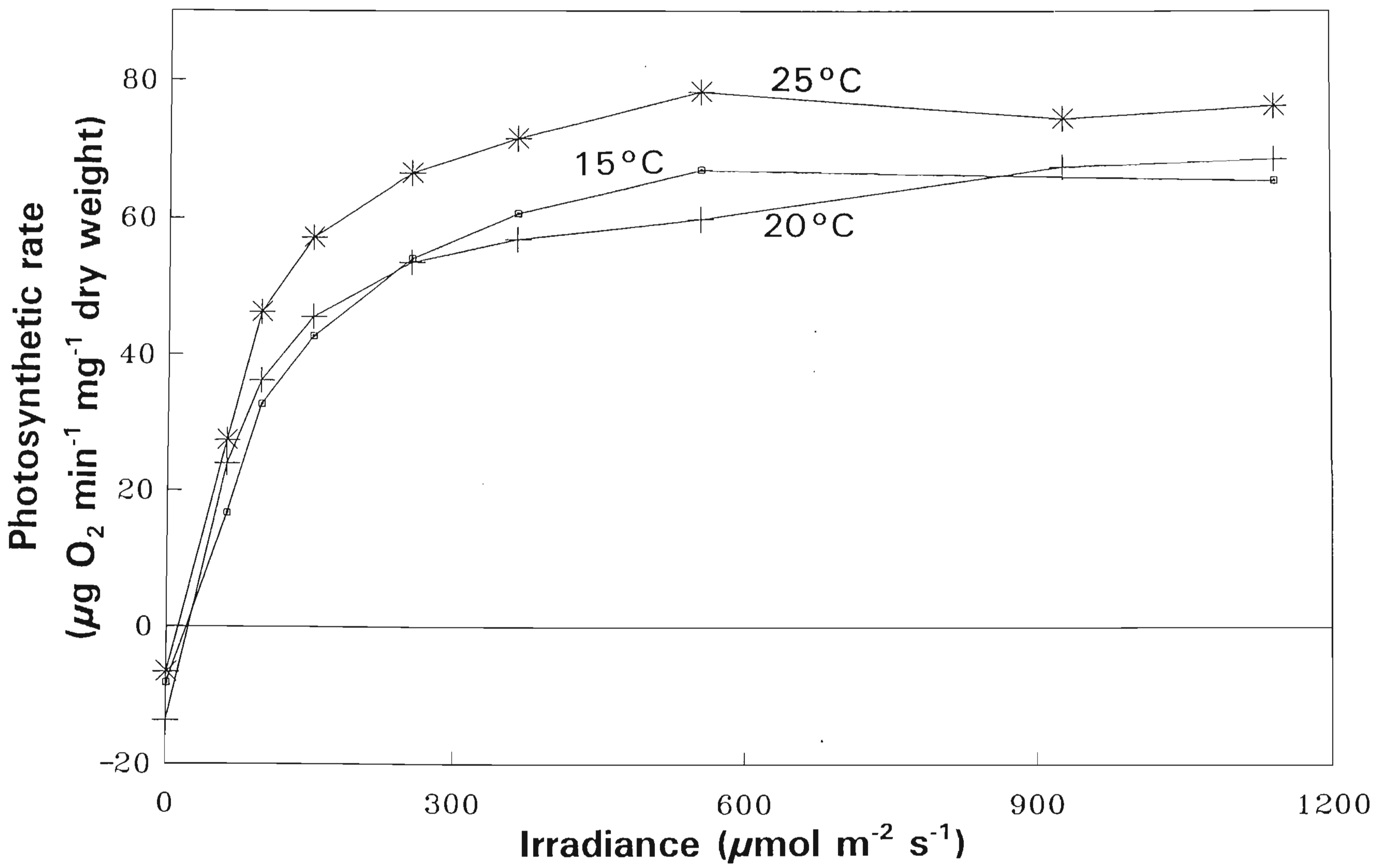


Fig. 5.9 Photosynthetic/irradiance curve of *Pteroceramium* when preconditioned at three temperatures. Measurements were taken at the same temperature at which the material was preconditioned.

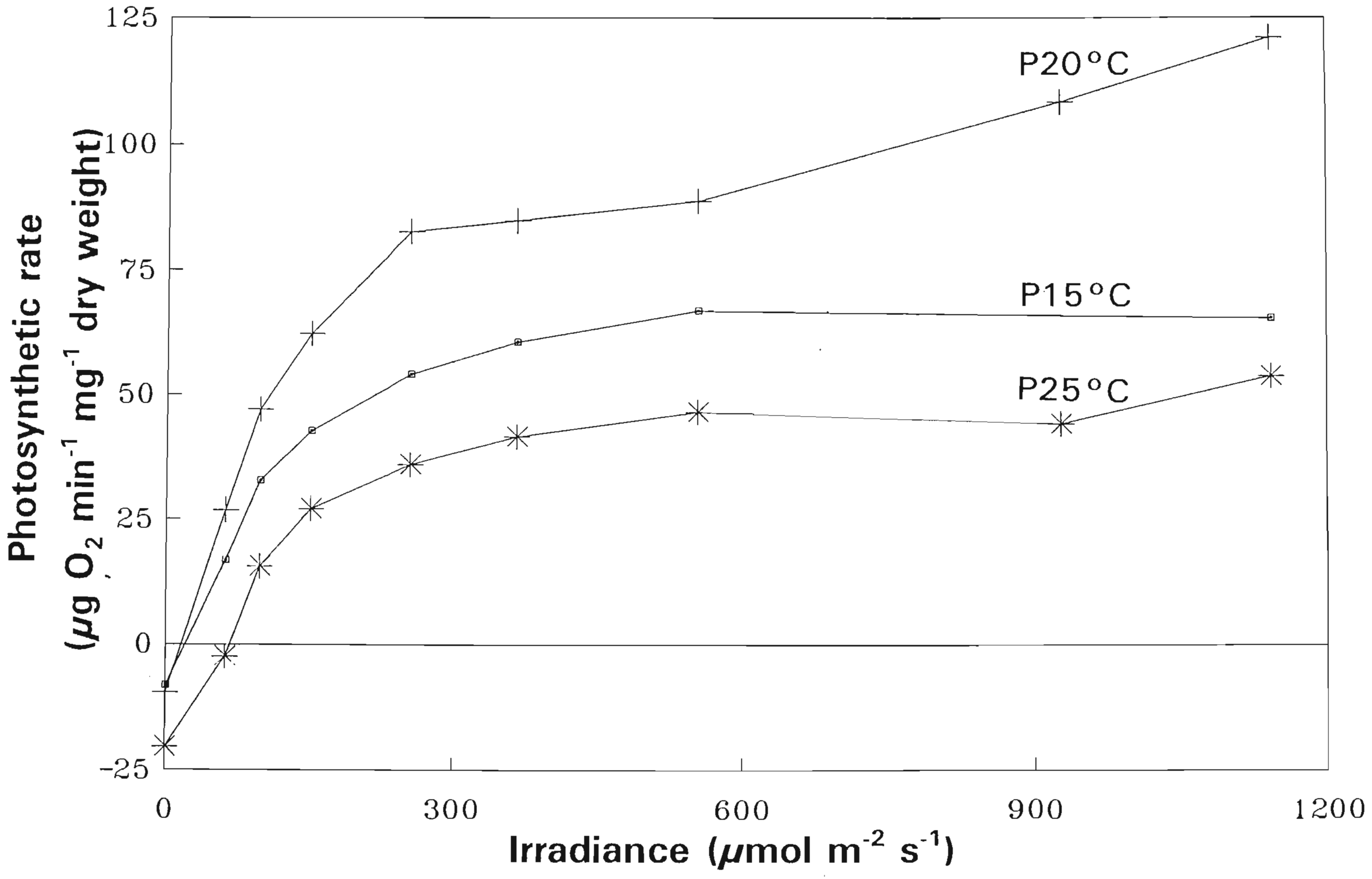


Fig. 5.10 Photosynthetic/irradiance curves of *Pteroceramium* when preconditioned at three temperatures. For example, P/20°C indicates that the material was preconditioned at 20°C. Measurements were taken at 15°C.

Table 5.9 Photosynthetic parameters of *Pterocerämium* showing effect of temperature. Photosynthetic measurements were taken at the temperature at which material was grown. All results are given as mean ± standard error. DW = dry weight.

Temperature	Rsp μg O_2 min^{-1} mg^{-1} DW	I_c μmol m^{-2} s^{-1}	α	P_{max} μg O_2 min^{-1} mg^{-1} DW	I_k μmol m^{-2} s^{-1}
15°C	-8.3 ± 2.1	19.4 ± 3.7	0.34 ± 0.02	66.94 ± 4.23	196 ± 11
20°C	-13.7 ± 8.3	23.8 ± 6.9	0.39 ± 0.07	63.21 ± 5.71	173 ± 20
25°C	-6.7 ± 1.3	14.6 ± 3.9	0.43 ± 0.05	75.04 ± 6.20	178 ± 7

had no effect on I_k but a 10°C increase caused a significant decrease in I_k ($P<0.05$) and a significant increase in Rsp ($P<0.05$) and I_c ($P<0.05$).

Table 5.10 Photosynthetic parameters of *Pteroceramium* after growing at 15°C and measurements taken at 15°C, 20°C and 25°C. All results are given as mean ± standard error. DW = dry weight.

Temperature of readings	Rsp μg O_2 min^{-1} mg^{-1} DW	I_c μmol m^{-2} s^{-1}	α	P_{max} μg O_2 min^{-1} mg^{-1} DW	I_k μmol m^{-2} s^{-1}
15°C	-8.3 ± 2.1	19.4 ± 3.7	0.34 ± 0.02	66.94 ± 4.23	196 ± 11
20°C	-9.7 ± 3.8	15.3 ± 4.5	0.48 ± 0.04	100.81 ± 11.8	211 ± 24
25°C	-20.4 ± 4.4	69.0 ± 11.3	0.32 ± 0.08	47.72 ± 10.19	156 ± 14

Photosynthetic/irradiance curves and photosynthetic parameters for *Pteroceramium* when acclimatised at 25°C and photosynthetic measurements taken at 15°C, 20°C and 25°C are shown in Figure 5.11 and Table 5.11. A rapid 5°C decrease to 20°C had no significant effect on the photosynthetic processes. A rapid 10°C decrease to 15°C caused a significant increase in Rsp ($P<0.05$) and I_c ($P<0.05$) and a significant reduction in P_{max} ($P<0.001$), α ($P<0.05$) and I_k ($P<0.05$).

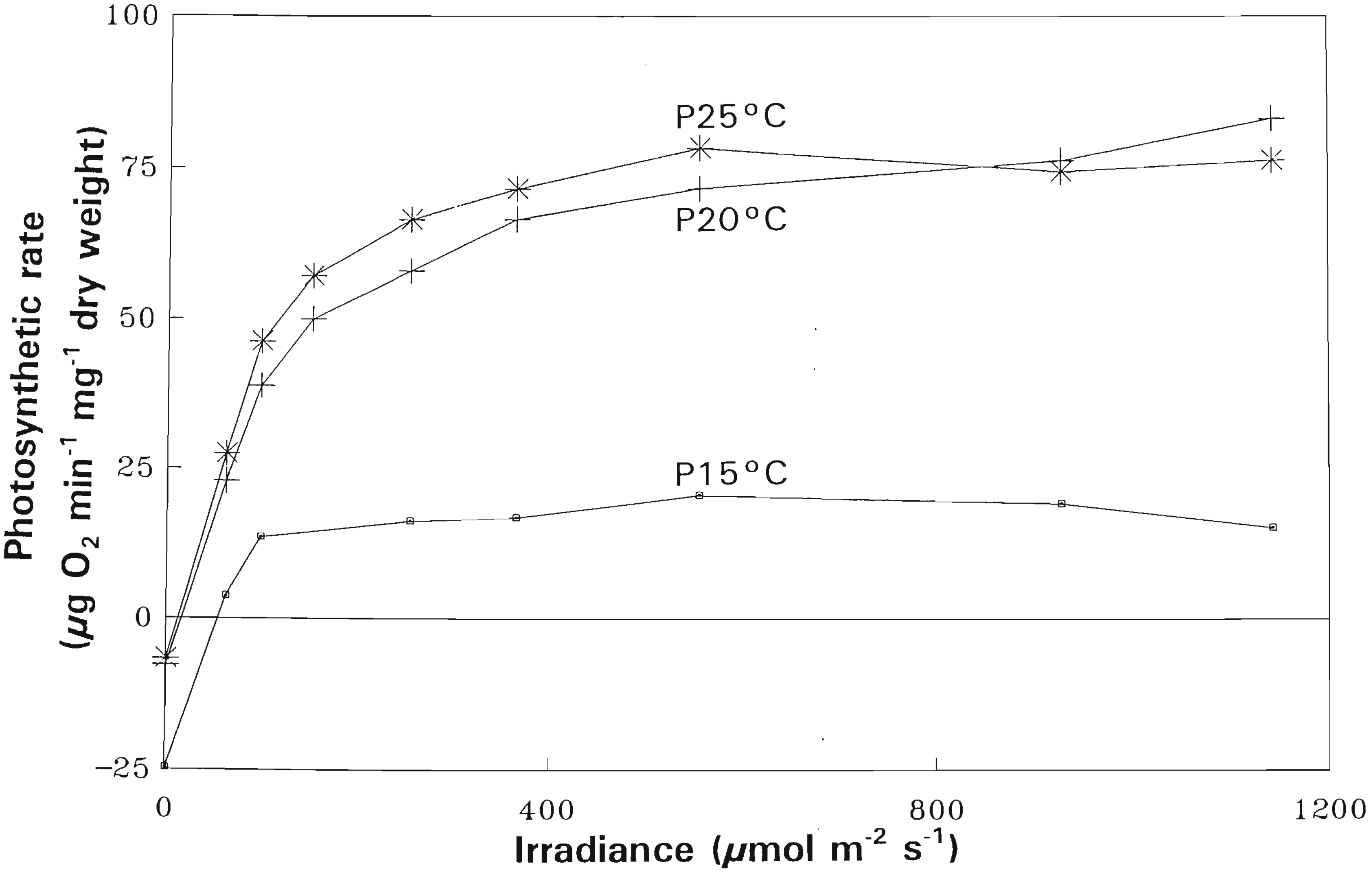


Fig. 5.11 Photosynthetic/irradiance curves of *Pteroceramium* when preconditioned at three temperatures. For example, P/20°C indicates that the material was preconditioned at 20°C. Measurements were taken at 25°C.

Table 5.11 Photosynthetic parameters of *Pteroceramium* after growing at 25°C and measurements taken at 15°C, 20°C and 25°C. All results are given as mean ± standard error. DW = dry weight.

Temperature of readings	Rsp $\mu g\ O_2\ min^{-1}\ mg^{-1}$ DW	I_c $\mu mol\ m^{-2}\ s^{-1}$	α	P_{max} $\mu g\ O_2\ min^{-1}\ mg^{-1}$ DW	I_k $\mu mol\ m^{-2}\ s^{-1}$
15°C	-24.8±2.9	45.0±5.6	0.25±0.02	17.94±2.74	74±14
20°C	-7.8±3.6	13.8±2.4	0.39±0.05	74.44±5.46	200±18
25°C	-6.7±1.3	14.6±3.9	0.43±0.05	75.04±6.20	178±7

vii) pH - An increase in pH above that of the control (pH 7.92) caused a significant decrease in P_{max} ($P<0.001$) and α ($P<0.01$), and a significant increase in I_c ($P<0.05$) with increasing pH. A decrease in pH below the control caused a significant increase in P_{max} and I_k ($P<0.1$; Fig. 5.12 and Table 5.12).

Table 5.12 Photosynthetic parameters of *Pteroceramium* taken at different pHs. All results are given as mean ± standard error. DW = dry weight.

pH	Rsp $\mu g\ O_2\ min^{-1}\ mg^{-1}$ DW	I_c $\mu mol\ m^{-2}\ s^{-1}$	α	P_{max} $\mu g\ O_2\ min^{-1}\ mg^{-1}$ DW	I_k $\mu mol\ m^{-2}\ s^{-1}$
pH 9.71	-1.3±0.6	194.7±52.0	0.03±0.02	5.23±2.44	241±41
pH 8.84	-9.8±2.2	145.9±23.2	0.09±0.02	24.01±7.88	253±55
pH 7.92	-6.3±2.5	62.5±35.9	0.18±0.01	44.94±3.90	255±30
pH 6.70	-10.4±3.6	32.5±8.3	0.16±0.05	67.34±5.25	342±14
pH 5.93	-6.4±1.3	26.0±4.4	0.17±0.02	63.52±8.06	368±28

5.3.2 Correlation between wet and dry mass

There was a strong correlation (0.968) at the 99% confidence level between the wet and dry mass of *Pteroceramium*. The slope of the linear regression was 0.158 and the

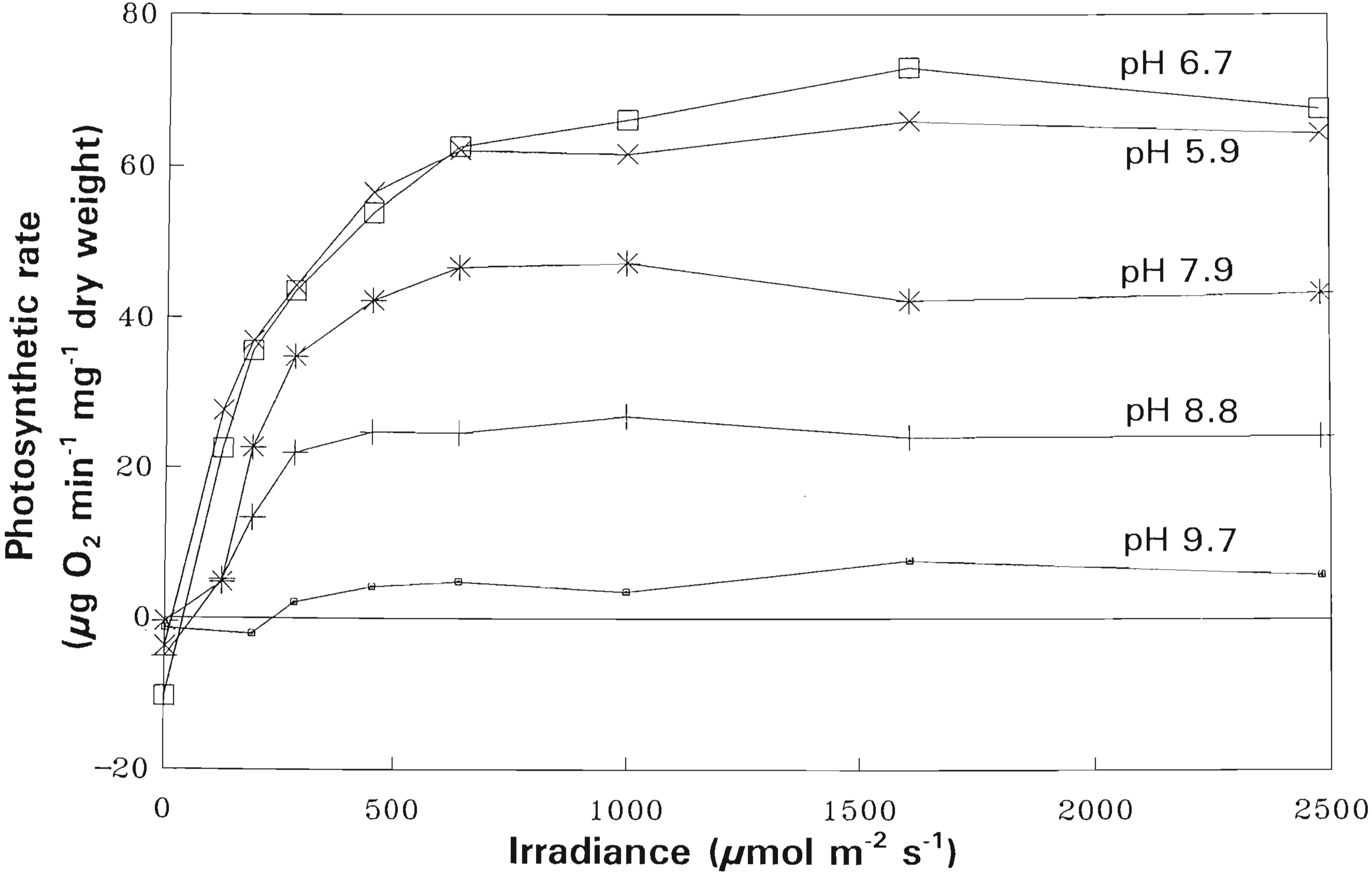


Fig. 5.12 Photosynthetic/irradiance curves of *Pteroceramium* when measurements were taken at different pHs.

Y-intercept was 2.885^{-4}. This shows that the dry mass makes up 15.8% of the wet mass of *Pteroceramium*.

There was also a strong correlation (0.939) at the 99% confidence level between wet and dry mass of *Ceramium glanduliferum*. The slope of the linear regression was 0.061 and the Y-intercept was 4.213^{-4}. This shows that the dry mass makes up 6.1% of the wet mass of *Ceramium glanduliferum*.

The good correlation between wet and dry mass makes conversions between the wet mass and dry mass accurate.

5.3.3 Pigment content

This method only gave a total crude extract and was used as a rough estimate of the pigment content of the alga (JENSEN, 1978). No purification techniques were attempted and so there could be mutual interference between the biliproteins and other substances (TALARICO and KOSOVEL, 1980).

There was a general decrease in the pigment content of *Pteroceramium* with increasing preconditioning irradiance although these concentrations were not significantly different (Table 5.13). The dominant pigment was phycoerythrin, giving the alga its red colour. In all Rhodophyta, phycoerythrin is found in the highest concentrations (KOSOVEL and TALARICO, 1979; HONSELL, KOSOVEL and TALARICO, 1984).

Table 5.13 Pigment content of *Pteroceramium* when grown at different irradiances. All results are given as mean ± standard error.

Irradiance	Chlorophyll *a*	Carotene	Phycoerythrin	Phycocyanin
μmol m^{-2} s^{-1}	μg pigment mg^{-1} dry weight			
30	0.87 ± 0.13	0.39 ± 0.06	5.86 ± 0.60	2.22 ± 0.34
80	0.65 ± 0.16	0.27 ± 0.05	5.12 ± 0.35	1.50 ± 0.52
150	0.46 ± 0.10	0.26 ± 0.05	3.50 ± 0.93	0.98 ± 0.32

For all pigments, *Ceramium glanduliferum* had a significantly higher ($P < 0.05$) pigment content than *Pteroceramium* when growing at 80 μmol m^{-2} s^{-1} (Table 5.14).

Table 5.14 Pigment content of *Pteroceramium* and *Ceramium glanduliferum* when grown at 80 μmol m^{-2} s^{-1}. All results are given as mean ± standard error.

	Chlorophyll *a*	Carotene	Phycoerythrin	Phycocyanin
		μg pigment mg^{-1} dry weight		
Pteroceramium	0.65 ± 0.16	0.27 ± 0.05	5.12 ± 0.35	1.50 ± 0.52
Ceramium glanduliferum	1.77 ± 0.10	0.79 ± 0.09	8.85 ± 1.55	4.50 ± 1.07

5.4 Discussion

Photosynthetic/irradiance curves are static, assuming a fixed relationship between the photosynthetic rate and actual irradiance level. There is also the assumption that the physiological parameters are fixed properties for individuals of a given species rather than relating these parameters to the nutrient status of the medium, temperature, prior irradiance history, pH and developmental stage (RAMUS, 1981; LOBBAN, HARRISON and DUNCAN, 1985; COUTINHO and ZINGMARK, 1987; PAHL-WOSTL and IMBODEN, 1990).

The photosynthetic values recorded for *Pteroceramium* are not absolute but indicate likely trends and responses to various stimuli. There are many factors which are listed below that influence the readings and complicate interpretation of the results. Where possible, the effect of these factors have been minimised.

High O_2 concentrations are known to inhibit net photosynthetic O_2 liberation (REISKIND, BEER and BOWES, 1989). For example, *Ulva lactuca* Linnaeus., *Enteromorpha* sp., *Ceramium strictum*, *Fucus serratus* Linnaeus, *Fucus vesiculosus* Linnaeus, *Codium* sp., *Udotea* sp. and *Sargassum* sp. showed between 3%-30% reduced photosynthetic rates with increasing O_2 concentrations. This inhibition is caused by competition between O_2 and CO_2 for reaction sites on the bifunctional enzyme RUBISCO and can be alleviated by efficient use of external HCO_3^- (GORDON and SAND-JENSEN, 1990). To overcome this problem in the experiments with *Pteroceramium*, O_2 levels were not allowed to rise above 70% saturation during the O_2 liberation readings.

Apart from the O_2 concentration build up around the plant, there is pH alteration and dissolved inorganic carbon depletion (GORDON and SAND-JENSEN, 1990). To prevent a diffusion barrier building up around the thallus of *Pteroceramium*, the medium in the reaction chamber of the O_2 electrode was continually stirred.

Wounding by cutting is known to increase dark respiration which causes a decrease in net photosynthetic rates; e.g. *Delsea flaccida* (Suhr) Papenfuss showed a drop in photosynthesis after cutting, taking two hours to recover fully (LOBBAN, HARRISON and DUNCAN, 1985). This may be due to a release of a secondary metabolite which is toxic and would be especially marked in a small volume of water (KNOOP and BATE, 1988a). To overcome this potential problem, whole plants of *Pteroceramium* were used.

Photosynthetic/irradiance curves cannot be used to predict primary productivity as there are diurnal fluctuations (FALKOWSKI and LA ROCHE, 1991). In many marine algae there is an early afternoon depression followed by recovery in the late afternoon. This is not full recovery as the morning maximum is not reached (RAMUS, 1981; KNOOP and BATE, 1988b). This depression may be due to photoinhibition, photorespiration or endogenous rhythms (HANELT, HUPPERTZ and NULTSCH, 1992). However, these endogenous rhythms are not present in all algae. For example, *Gracilaria tikvahiae* showed no differences when readings were taken over the whole day (PENNIMAN and MATHIESON, 1985). No such endogenous depressions were observed in *Pteroceramium*.

5.4.1 Effect of irradiance

"The ability to adjust the composition of the photosynthetic apparatus to achieve a more efficient harvesting of light energy is expected to be of significant advantage to organisms which are subjected to long-term variations in the light environment" (CUNNINGHAM, DENNENBERG, MUSTARDY, JURSINIC and GANTT, 1989). Algae acclimatize to the light environment by changing their light harvesting pigments. Pigment content is a dynamic factor closely correlated with environmental factors such as irradiance and changes in seasonal factors (HONSELL, KOSOVEL and TALARICO, 1984). For example, *Gracilaria verrucosa* showed variable pigment concentrations over the year with chlorophylls and carotenes peaking in summer and the phycobiliproteins in winter. However, the ratios of the various biliproteins remained constant, indicating a stoichiometric ratio between pigments localized in the same phycobilisome (KOSOVEL and TALARICO, 1979).

Pteroceramium had a decrease in pigment content when grown in higher irradiances as seen in the pigment extractions. This indicates its ability to acclimatize to changes in irradiance levels although the exact mechanism is not known. Another morphological acclimation to lower irradiance are the wings which are smaller at the higher irradiances (STIRK and AKEN, 1993; Chapter 4).

Photoacclimation confers some photobiological advantages such as maximal photosynthetic rates, improved net growth efficiency by adjusting respiration rates to increase gross photosynthesis and by improving the quantum yield for photosynthesis. When grown in low light, a physiological advantage to lower I_c would allow net carbon gain under severely limiting irradiance conditions while acclimation to high irradiances results in a much greater photosynthetic capacity. I_c is expected to coincide with winter irradiances at lower light limits (KAIN and NORTON, 1990). The quantum yield for photosynthesis is the ratio of photochemical reaction to the rate of irradiance absorption. This is shown by the equation:

$$R = ICa^*$$

where R = rate of light absorption
I = spectral irradiance
C = cellular concentration of absorbing pigments
a^* = *in vivo* optical absorption cross section of pigments as applied mainly to phytoplankton (BERNER, DUBINSKY, WYMAN and FALKOWSKI, 1989)

The optical absorption cross-section is affected by:

1) changes in cell shape, size and morphology;

2) changes in chloroplast size, shape, number, morphology and distribution within the cell;

3) changes in the stacking of thylakoid membranes; and

4) changes in optical properties of thylakoid membranes (BERNER, DUBINSKY, WYMAN and FALKOWSKI, 1989).

These changes contribute to the "package effect". When algae acclimate to low irradiance, there is an increase in C accompanied by a non-linear increase in R and decrease in a^* owing to the "package effect". With decreasing irradiance, there is an increase in the number of thylakoid membranes per stack within the chloroplasts. Thus those in the centre of the stack are subject to self-shading and an increase in the number of absorbing molecules per surface area. Thus acclimation to low light is a self-limiting process as an increase in pigment concentration results in a decrease in their efficiency (FALKOWSKI and LA ROCHE, 1991). The same effect is noticeable in *Pteroceramium*. Material grown at 30 μmol m^{-2} s^{-1} had the highest pigment content but still had α rates which showed a decrease in efficiency of light absorption owing to both internal and external self-shading.

Looking at the total pigment content to determine whole thallus irradiance absorption and efficiency can be misleading as the morphology and cellular architecture affect the internal distribution of irradiance (HANNACH, 1989). It was found with *Porphyra abbottae* that there was highest absorption of intact blades in material growing in the lowest irradiance (17.5 $\mu mol.m^{-2}.s^{-1}$) and there is a non-linear correlation between pigment content and whole thallus absorption owing to self-shading (HANNACH, 1989). The decrease in absorption efficiency of the pigments as their concentration increases in the thallus is due to the "package effect" which can reduce the efficiency of light capture. However, in the field, light is diffuse and so this "package effect" may actually enhance light capture (HANNACH, 1989).

The concentration of the antennae pigments and the ratio of accessory pigment to primary antennae pigment is inversely correlated with irradiance levels. There are two strategies in qualitative adjustment and once these are known, accurate predictions of photosynthetic performance can be made (RAMUS, 1981). The two acclimation strategies to low irradiance are to increase the number of photosynthetic units (reaction centres) or to increase the size (cross sectional area) of each reaction centre. P_{max} is proportional to the number of traps and their intrinsic turnover time and as the number of traps increase, more irradiance is needed to saturate photosynthesis, hence I_k will increase. α is proportional to the number of traps and their cross sectional area. As the size of the photosynthetic units increase, less light will be needed to saturate photosynthesis as the photosynthetic units become more efficient (RAMUS, 1981; LOBBAN, HARRISON and DUNCAN, 1985).

Thus, when photosynthetic rates are determined on biomass:

1) a change in the number of traps with a fixed antennae size in response to low irradiance will not affect α values but will increase P_{max} values (Fig. 5.13A); and

2) a fixed number of traps with an increase in antennae size in response to low irradiance will have higher α values while P_{max} values remain unchanged, being equal to those in high irradiance conditions (Fig. 5.13B).

Both these strategies will improve photosynthetic performance at subsaturating irradiances but only strategy 2 will show enhanced photosynthesis as irradiances approach compensation point.

When *Pteroceramium* was grown in low irradiance conditions, α increased. This indicates that photosynthetic efficiency was increased by increasing the cross-sectional area of the photosynthetic units (strategy 2). Also, the decrease in I_k at the highest

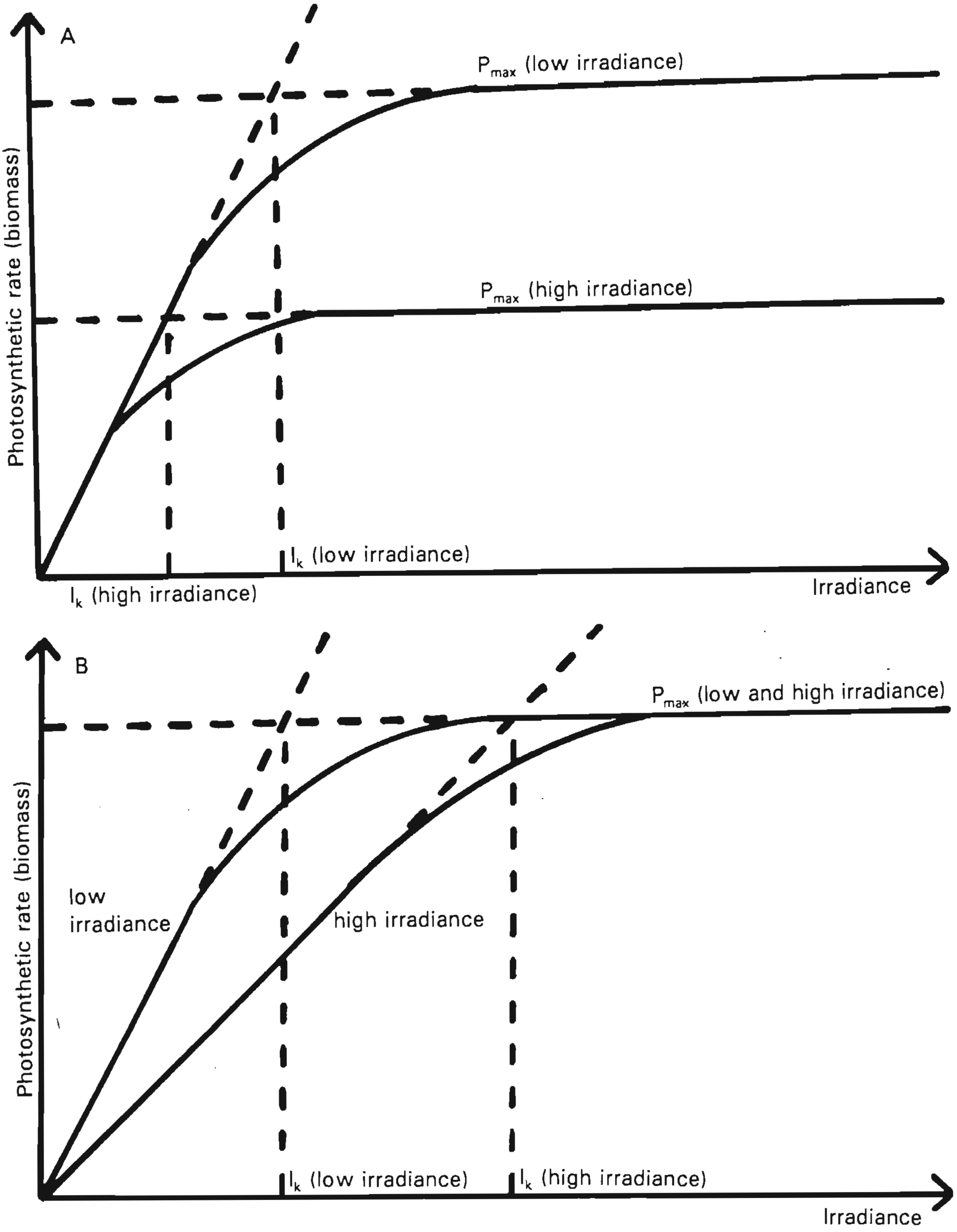


Fig. 5.13 Model photosynthetic/irradiance curves, normalizing photosynthetic rates to biomass, comparing two strategies for the adjustment of photosynthetic units to extreme high and low irradiance environments. A) Strategy 1 where there is a change in the number of traps with a fixed antennae size. B) Strategy 2 where there are a fixed number of traps with a change in antennae size (RAMUS, 1981).

irradiance indicates that strategy 1 was in operation. Thus *Pteroceramium* appears to photoacclimatize by a combination of increasing both the size and number of light traps.

When photosynthetic rates are determined on a pigment basis to show pigment efficiency:

1) a change in number of traps with a fixed antennae size will not change either α or P_{max} values in response to low irradiance (Fig. 5.14A); and

2) a fixed number of traps with a change in antennae size will result in decreased α and P_{max} values in response to low irradiance (Fig. 5.14B).

The net effect of these strategies is increased quantum capture to compensate for diminishing quantum flux density.

When the photosynthetic rates of *Pteroceramium* were plotted on a total pigment content rather than a dry weight basis, it was found that the pigments of the material growing at 150 μmol m^{-2} s^{-1} were more efficient in the overall process of photosynthesis even though they had a lower pigment content than the material growing at 30 μmol m^{-2} s^{-1}. This may be due to the "package effect". However, the best photosynthetic response was obtained from the material grown at 80 μmol m^{-2} s^{-1}. This material had a higher I_k^B value, indicating that more irradiance is needed to saturate photosynthesis owing to an increase in the number of traps. Other physiological strategies which may have been operational in *Pteroceramium* were not investigated.

When *Porphyridium cruentum* (S.F.Gray) Naegeli (as *Porphyridium purpureum* (Bory) Drew *et* Ross) was allowed to acclimatize for four weeks at four irradiance levels between 6-280 μE m^{-2} s^{-1}, there was a decrease in the number of major light harvesting complexes with increasing irradiance. This was accompanied by an increase in the amount of ATPase, RUBISCO and Fd-NADP^{+} until light saturating growth was achieved. Thus there was a change in the number of light harvesting antenna pigments

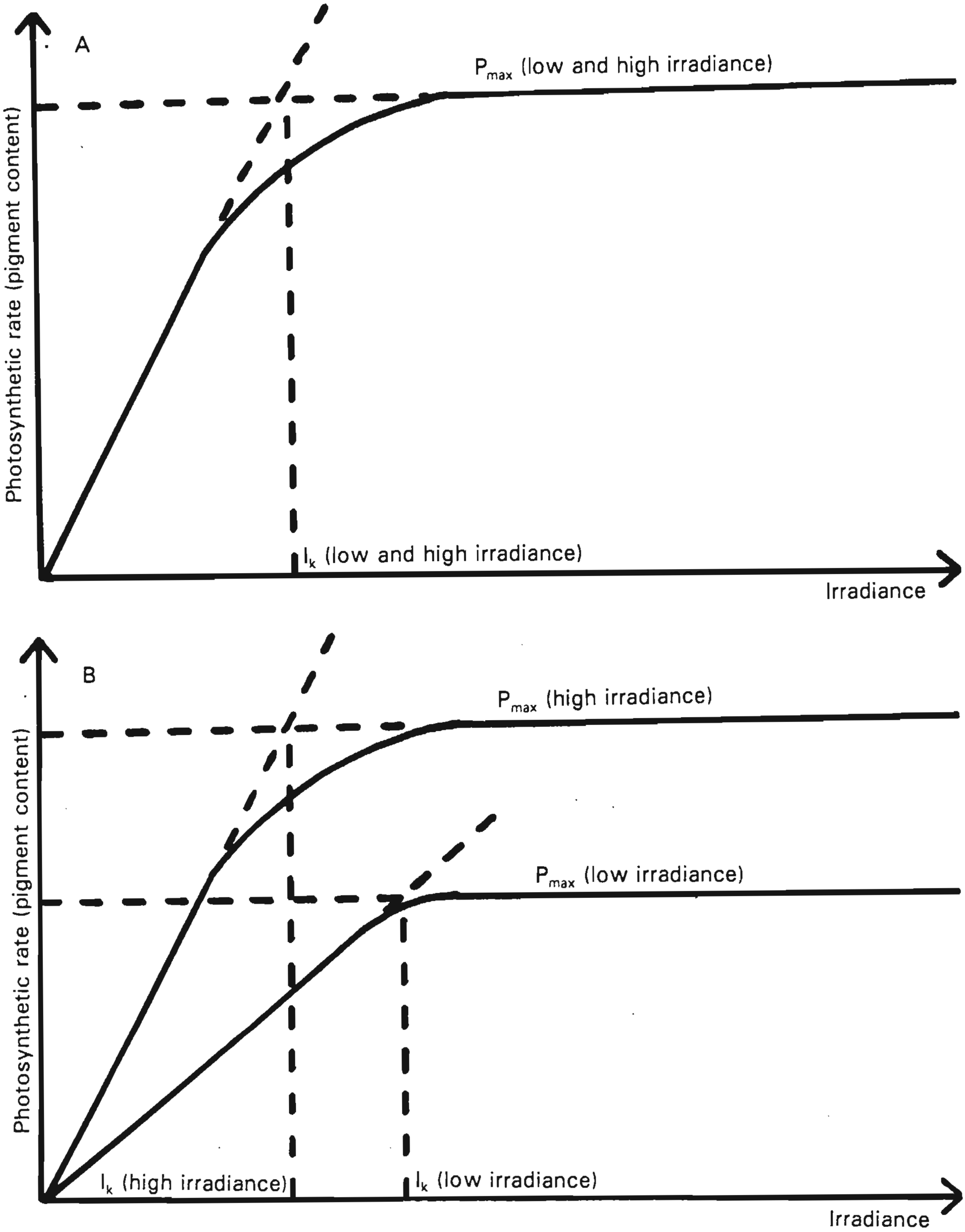


Fig. 5.14 Model photosynthetic/irradiance curves, normalizing photosynthetic rates to pigment content, comparing two strategies for the adjustment of photosynthetic units to extreme high and low irradiance environments. A) Strategy 1 where there is a change in the number of traps with a fixed antennae size. B) Strategy 2 where there are a fixed number of traps with a change in antennae size (RAMUS, 1981).

and reaction centres relative to the number of enzymes and electron carriers relative to membrane area (CUNNINGHAM, VONSHAK and GANTT, 1992).

Another strategy is illustrated by *Acrosymphyton purpuriferum* (J.Agardh) Sjostedt which showed no change in pigment content with increasing irradiance. Rather, the change in photosynthetic rate is due to a change in the enzymatic rate. This response is similar to that in higher plants and green algae. Perhaps changing pigment content is energetically too high in this alga (BREEMAN and TEN HOOPEN, 1984).

Irradiance levels needed for saturating photosynthesis are approximately double those needed for saturating growth (LÜNING, 1981b). Irradiance levels needed to saturate a species (I_k) show some correlation with habitat:

1) eulittoral species are saturated at 400-600 μE m^{-2} s^{-1};

2) upper- and mid-sublittoral species are saturated at 150-250 μE m^{-2} s^{-1}; and

3) deep sublittoral algae require less than 100 μE m^{-2} s^{-1} to saturate photosynthesis (LOBBAN, HARRISON and DUNCAN, 1985; LÜNING, 1990).

Ceramium glanduliferum with an I_k of 550 μmol m^{-2} s^{-1}, falls into the eulittoral category. This corresponds with where it was found growing at Mkambati. Following this scheme, *Pteroceramium* fits into the upper- and mid-sublittoral group with an I_k of 228 μmol m^{-2} s^{-1} implying that it is suited to low irradiance conditions.

When the photosynthetic/irradiance curves of *Pteroceramium* and *Ceramium glanduliferum* were replotted on a pigment content basis, *Pteroceramium* was found to have a significantly higher α value than *Ceramium glanduliferum*. This indicates that the pigments of *Pteroceramium* are more efficient at capturing and converting irradiance into chemical energy than those of *Ceramium glanduliferum*. This optimizing of the photosynthetic potential would be a great advantage when growing in light attenuated conditions. Also, if the wings of *Pteroceramium* increase the surface area of the plant for light interception, this would be a morphological advantage in low irradiance conditions.

There is great diversity in algal morphology. However, even amongst different Divisions, certain morphological forms are continually repeated. This indicates convergent adaptation to factors that regulate survival and reproduction (LITTLER, LITTLER and TAYLOR, 1983). These reflect different survival strategies, with a cost versus benefit trade off between capacities for rapid growth, reproduction, environmental tolerance, resistance to predation and competition for nutrients, space and irradiance (LITTLER and LITTLER, 1980), all playing a critical role in the fitness of an individual (LITTLER, LITTLER and TAYLOR, 1983).

LITTLER, LITTLER and TAYLOR (1983) have recognised six functional-form groups:

1) sheet group: characterised by thin, tubular and sheet-like (foliose) external morphology with a soft texture. The internal anatomy is uncorticated and one to several cell layers thick; e.g. *Ulva*, *Enteromorpha*, and *Dictyota*;

2) filamentous group: delicately branched and uniseriate, multiseriate or lightly corticated with a soft texture; e.g. *Centroceros*, *Spyridia*, *Chaetomorpha* and *Ceramium*;

3) coarsely branched group: having an upright morphology, corticated with a fleshy-wiry texture; e.g. *Laurencia* and *Liagora*;

4) thick leathery group: having blades and branches with a differentiated internal anatomy, are heavily corticated and thick-walled with a leathery or rubbery texture; e.g. *Sargassum*, *Gracilaria* and *Padina*;

5) jointed calcareous group: are articulated, calcareous and upright with a stony texture, calcified genicula and flexible intergenicula with parallel cell rows; e.g. *Halimeda*, *Galaxaura*, *Jania* and *Amphiroa*; and

6) crustose group: having an epilithic, prostrate and encrusting external morphology. They have calcified or uncalcified parallel rows of cells and a stony or tough texture; e.g. *Porolithon*, *Hydrolithon*, *Neogoniolithon* and *Peyssonnelia*.

Productivity studies show there is a trend of decreasing productivity from the sheet group to the crustose group. The relative abilities of the various morphologies to utilize light and obtain nutrients would appear to account for these differences. The sheet and filamentous groups have been selected for high net primary productivity with the thallus form being adapted to obtaining nutrients (high surface: volume ratios) and utilizing light energy with thin thallus construction minimising internal self-shading of the photosynthetic apparatus. Thicker calcareous algae have a low photosynthetic rate owing to differential allocation of resources between photosynthetic and structural tissues with selection pressure for tougher morphologies, resulting in persistence in time but slower growth rates (LITTLER and LITTLER, 1980; LITTLER and ARNOLD, 1982). This confers resistance against herbivory as well as the ability to withstand abrasion and wave shearing forces (LITTLER, LITTLER and TAYLOR, 1983). Some deep water Phaeophyta (*Dictyota*, *Lobophora* and *Stypopodium*) off Bermuda showed relatively high *in situ* photosynthetic rates. All these Phaeophyta tested had thin, flat thalli, allowing a high surface to volume ratio. This shows the adaptive nature of the morphology to deep water, nutrient poor environments (PECKOL and RAMUS, 1992). *Bangia atropurpurea* growing in the minimal irradiance of 1 μmol.m^{-2}.s^{-1}, has an extremely thin thallus which reduces self-shading, resulting in a lowering of I_k. This is opposed to thick and structurally complex thalli, as found in the Fucales, where there is a higher I_k owing to low light transmission to the lower photosynthetic layers (ORFANIDIS, 1992).

The proportion of non-photosynthetic tissue is also important in determining photosynthetic efficiency. Mature plants generally have a higher light requirement owing to the higher proportion of non-photosynthetic tissue (LÜNING, 1981a). The proportion of non-photosynthetic tissue also affects the biomass of pigments when determined on a weight basis (HANNACH, 1989).

Pteroceramium can be classified into the filamentous group with an adaptive morphology to low irradiance as there is no structural material and little non-photosynthetic material. There is a high surface to volume ratio owing to the wings, which are also only one cell layer thick and so there is little self-shading and enhanced uptake rates.

5.4.2 Photoinhibition and recovery

Photoinhibition is the reduction in photosynthetic capacity induced by strong irradiance conditions and ultraviolet light. The main site of photoinhibition is in photosystem II (LÜNING, 1990; NULTSCH, PFAU and HUPPERTZ, 1990), causing disruption of electron transport to photosystem I. The decline in efficiency of photosystem II is shown by a loss of fluorescence yield (POWLES, 1984). The reduced energy flow from photosystem II to photosystem I means a decrease in NADPH concentrations needed for CO_2 fixation (GANTT, 1990).

Photoinhibition is a common phenomenon in algae (Table 5.15). Sublittoral algal species often need a shorter exposure to full daylight than do intertidal species for photoinhibition to set in (LÜNING, 1981a). It is a dynamic process depending on prior irradiance history, duration of exposure and irradiance levels (NULTSCH, PFAU and HUPPERTZ, 1990; PAHL-WOSTL and IMBODEN, 1990). *Pteroceramium* showed increased photoinhibition with both increasing lengths of exposure and higher irradiance levels. This decay in photosynthetic response with time is known as "accumulated inhibition" (PAHL-WOSTL and IMBODEN, 1990). The decrease in α in *Pteroceramium* indicated a loss of efficiency of light capture resulting in a decrease of P_{max}. The extent of photoinhibition depends on the efficiency of repair mechanisms and protective measures against high irradiance and ultraviolet light (LÜNING, 1990).

Photoinhibition is regarded as a light adaptation phenomenon protecting the photosynthetic apparatus from photodamage (NULTSCH, PFAU and HUPPERTZ, 1990) by funnelling excess excitation energy away from the photosystem II reaction sites (HANELT, HUPPERTZ and NULTSCH, 1992). This process is reversible. Carotenoid pigments have a protective function, absorbing irradiance but not

Table 5.15 Photoinhibition in algae.

Species	Irradiance (μE m^{-2} s^{-1})	Occurrence	Reference
Chlorophyta			
Enteromorpha prolifera (Müller) J.Agardh	1800	No	COUTINHO and ZINGMARK, 1987
Enteromorpha linza (Linnaeus) J.Agardh	500	Yes	COUTINHO and ZINGMARK, 1987
Bryopsis plumosa (Hudson) J.Agardh	1800	No (February)	COUTINHO and ZINGMARK, 1987
Bryopsis plumosa	1000	Yes (March)	COUTINHO and ZINGMARK, 1987
Ulva rotundata Bliding	850	Yes	FRANKLIN, LEVAVASSEUR, OSMOND, HENLEY and RAMUS, 1992
Phaeophyta			
Ectocarpus siliculiosus (Dillwyn) Lyngbye	500	Yes	COUTINHO and ZINGMARK, 1987
Laminaria hyperborea	1500	Yes	LÜNING, 1981b
Fucus spp. (eulittoral)	2520*	No	LÜNING, 1981b
Rhodophyta			
Eucheuma isiforme (C.Agardh) J.Agardh	1200	Yes	DAWES and KOCH, 1988
Gastroclonium coulteri	1400	No	HODGSON, 1981
Polyneura hilliae Kylin	168*	50% drop	NULTSCH, PFAU and HUPPERTZ, 1990
Polyneura hilliae	336*	78% drop	NULTSCH, PFAU and HUPPERTZ, 1990
Gracilaria tikvahiae	1600	No	GANTT, 1990
Porphyra yezoensis Ueda	1600	No	GANTT, 1990
Porphyridium purpureum	2300*	No (15°C)	GANTT, 1990
Porphyridium purpureum	2300*	Yes (35°C)	GANTT, 1990

Species	Irradiance (μE m^{-2} s^{-1})	Occurrence	Reference
Porphyra perforata J.Agardh (eulittoral)	-	25% drop	BOSE, HERBERT and FORK, 1988
Porphyra nereocystis Anderson (sublittoral)	-	55% drop	BOSE, HERBERT and FORK, 1988
Phycodrys sinuosa (Good *et* Woodward) Kützing (sublittoral)	420*	Yes	LÜNING, 1981b
Delesseria sanguinea (sublittoral)	420*	Yes	LÜNING, 1981b

* converted from W m^{-1} (LÜNING, 1981a)

transferring excitation energy to the reaction centres (LÜNING, 1990; FALKOWSKI and LA ROCHE, 1991). For example, *Petroglossum nicaeensa* (Duby) Schotter had 90% more carotene when growing in the mouth of a cave compared with samples growing inside the cave (CALABRESE, 1972). Photorespiration may counter photoinhibition by maintaining electron transport between the photosystems and allowing the generation of energy for CO_2 fixation. With an increase in irradiance levels, photorespiration is known to increase (GANTT, 1990). *Ceramium glanduliferum* had a higher carotene content than did *Pteroceramium*. This may be due to *Ceramium glanduliferum* growing in the eulittoral where more protection is necessary from high irradiances.

When excitation energy exceeds the capacity for photoprotection, photodamage occurs, resulting in bulk pigment destruction (POWLES, 1984; FRANKLIN, LEVAVASSEUR, OSMOND, HENLEY and RAMUS, 1992) by photosensitized oxidation of excited chlorophyll *a* (LÜNING, 1981a). This damage is irreversible. Photooxidation is a secondary phenomenon occurring after a distinct lag phase when there is a decline in photosynthesis due to photoinhibition.

Recovery also depends on the duration and intensity of exposure (Table 5.16). Generally algae should recover quickly if photoinhibition is part of their regulatory process in photosynthesis. There is usually full recovery if no photooxidation has occurred (LÜNING, 1981a). Recovery has two distinct phases with a rapid initial phase which is irradiance independent, followed by a slower phase which is irradiance dependent, usually needing weak light (NULTSCH, PFAU and HUPPERTZ, 1990). Recovery in *Polyneura hilliae* was found to be temperature dependent, there being faster recovery at higher temperatures (10°C-20°C). This is because recovery is an enzymatic process. There were the typical two phases of recovery in *Polyneura hilliae* with phase one taking approximately six hours (HANELT, HUPPERTZ and NULTSCH, 1992).

Table 5.16 Recovery rates in some algae after photoinhibition.

Species	Irradiance $\mu E\ m^{-2}\ s^{-1}$	Length of exposure	Recovery time	Full recovery	Reference
Ulva rotundata	850	1 hour	20 minutes	No	FRANKLIN, LEVAVASSEUR, OSMOND, HENLEY and RAMUS, 1992
		5 hours	1 hour	No	
	1700	1 hour	2 hours	No	
		5 hours	6 hours	No	
Porphyra nereocystis (sublittoral)	-	1 hour	35 minutes	55% recovery	BOSE, HERBERT and FORK, 1988
Porphyra perforata (eulittoral)	-	1 hour	30 minutes	83% recovery	BOSS, HERBERT and FORK, 1988
Polyneura hilliae	840*	1 hour	1-2 days	Yes	HANELT, HUPPERTZ and NULTSCH, 1992

* converted from W m^{-2} (LÜNING, 1981a)

Pteroceramium had not regained its full photosynthetic potential 24 hours after exposure to high irradiance. As the results do not show any improvement between six hours and

24 hours, it can be assumed that no further recovery is likely to occur. This indicates that photodamage had occurred and was irreversible in *Pteroceramium*.

It is not unlikely that irradiance levels in the field could reach as high as 3000 μmol.m^{-2}.s^{-1} on the lower Natal south coast owing to the higher latitude (31°03'S; 30°14'E) compared to those readings in Table 5.17. The geographic variations in yearly integral irradiance above the water surface show a threefold difference in magnitude between the tropics and poles (LÜNING, 1981a). Apart from irradiance levels increasing in the lower latitudes, there is also an increase in daylength (KAIN and NORTON, 1990) which would increase the mean daily illumination. This would increase the effects of accumulated inhibition.

Table 5.17 Variations in maximum irradiance with changing latitudes.

Irradiance	Latitude	Reference
1750 μE m^{-1} s^{-1}	43°05'N	PENNIMAN and MATHIESON, 1985
2000 μE m^{-1} s^{-1}	36°37'N	HODGESON, 1981
2400 μE m^{-1} s^{-1} (highly reflective clouds)	36°37'N	HODGESON, 1981

It is likely that *Pteroceramium* would be regularly exposed to such high irradiances if it were growing in the upper eulittoral region. The ecological implications are that the inability of *Pteroceramium* to recover fully after exposure to such high irradiances could possibly limit its upper distribution by reducing net growth as it would not be an advantage to be exposed to high irradiance levels at frequent intervals. It is therefore likely that *Pteroceramium*'s distribution could be limited to deep water or shaded habitats.

5.4.3. Effect of temperature

Temperature is another fundamental environmental factor controlling photosynthetic rates as the dark reactions of photosynthesis are enzymatically controlled. Carbon fixation is controlled by RUBISCO. Sucrose synthesis, which regulates the amount of inorganic phosphorus for ATP regeneration, is also enzyme controlled. Temperature also affects other enzymatic and physical processes including respiration. Thus, temperature effects are shown mainly by P_{max} (COUTINHO and ZINGMARK, 1987). Initial photochemical reactions (as shown by α) are independent of temperature although dark respiration usually increases with increasing temperature and therefore I_c increases (DAVISON, 1991).

Generally with an increase in temperature, there is an increase in I_k in many algae (KAIN and NORTON, 1990). There is also a greater potential for photorespiration as the Michaelis Menten constant (k_m) of RUBISCO for O_2 increases more slowly with increasing temperature than for CO_2 (DAVISON, 1991). Temperature has little effect on photosynthetic rates so long as irradiance is below saturation since the photochemical processes are then limiting the overall rate rather than the enzymatic processes. This is important ecologically as most populations grow at subsaturating irradiances (DAVISON, 1991).

A photosynthetic-temperature curve shows a typical enzymatic response. With an increase in temperature, there is an exponential increase in photosynthesis from the biological zero to the optimum temperature resulting in maximum photosynthesis. With further increase in temperature, there is a sharp drop in photosynthesis to zero, indicating the lethal upper temperature limit for photosynthesis (KNOOP and BATE, 1990). The upper thermal tolerance depends on the stability of photosystem II which is thought to be linked to the degree of saturation of the thylakoid membranes by fatty acids (DAVISON, 1991). At high temperatures, there is a disruption of energy transfer between the phycobilisomes and photosystem II.

There are three classes of temperature responses:

1) a short term response when plants are suddenly exposed to a change in temperature; e.g. tidal displacement of thermocline or tidal immersion/emersion (LOBBAN, HARRISON and DUNCAN, 1985). The typical response is that P_{max} increases progressively with increasing temperature with a Q_{10} (change over 10°C) of

approximately 2.0. This increases until the optimum temperature is reached and then there is a rapid drop in P_{max}. For example, *Gracialaria tikvahiae* showed an increase in photosynthetic rates from 5°C upwards. At 37.5°C the photosynthetic rate dramatically decreased (PENNIMAN and MATHIESON, 1985). In macroalgae, there is usually a broad temperature optimum ranging over several degrees (DAVISON, 1991);

2) photosynthetic acclimation (phenotypic change) owing to seasonal changes. Temperature affects the rate of photosynthesis, but changes to which the alga is normally exposed in the field does not eliminate photosynthesis (HODGESON, 1981). When the alga is grown in low temperatures, there is an increase in P_{max}, reduced sensitivity to changes in temperature, lower optimum temperature for photosynthesis and reduced tolerance to high temperature. Enzymes also increase in concentration in low temperatures to compensate for their reduction in activity (DAVISON, 1991); and

3) photosynthetic adaptation (genetic differences) are long-term changes owing to natural climatic cycles and human influences; e.g. "greenhouse effect". There are also species which grow in different thermal ecotypes (DAVISON, 1991). Arctic algae are an extreme example of temperature adapted algae. They are able to photosynthesize at 0°C in very low irradiances of 3-10 μmol m^{-2} s^{-1} with their photosynthetic rates being within the same order of magnitude as temperate algal species (THOMAS and WIENCKE, 1991).

The first two types of responses were investigated for *Pteroceramium*. *Pteroceramium* is able to acclimatize to slow changes in temperature with photosynthetic acclimation taking less than a week. This was not unexpected as *Pteroceramium* needs to be able to adjust to seasonal changes in water temperature. In the Natal coastal waters around St. Michael's and Port Edward, the summer maximum is 22.9°C and the winter minimum is 19.4°C (temperature data supplied by the Natal Sharks Board). The results indicate that the seasonal temperature changes which *Pteroceramium* would be exposed to in natural conditions would not affect photosynthesis. The growth rates of *Pteroceramium* are lower at 30°C (STIRK and AKEN, 1993; Chapter 4) and could suggest a dropping off of photosynthetic rates. A broader spectrum of temperatures still needs to be investigated to determine the photosynthetic/temperature response curve of *Pteroceramium.*

Rapid changes in temperature had more effect on the photosynthetic performance of *Pteroceramium. Pteroceramium* shows the typical response to increased temperature by having a significant increase in P_{max} with a 5°C rise in temperature. However, P_{max} drops with a 10°C increase. As this is accompanied by an increase in respiration (and hence I_c), it indicates that the plant is possibly under some stress. Similarly, *Pteroceramium* is able to accommodate a 5°C decrease in temperature but not a 10°C decrease. This is again accompanied by an increase in respiration, indicating that such a large rapid decrease in temperature is stressful to *Pteroceramium.* Such rapid changes in temperature do occur in tidepools in Natal. There is an increase in temperature at low tide owing to heating by the sun and a decrease in temperature with the incoming tide. These photosynthetic results of *Pteroceramium* suggest that such conditions as found in the eulittoral region could be detrimental to *Pteroceramium.* The rate of acclimation under new temperature conditions would add useful knowledge of the dynamics of temperature effects of photosynthesis in *Pteroceramium.*

Temperature also affects the solubility of gases. With an increase in temperature, there is a decrease in solubility. In a closed system at equilibrium, a temperature increase from 23°C to 30°C results in a decrease of 46.3 μM to 38.3 μM CO_2 and 215.9 μM to 194.3 μM O_2 (REISKIND, BEER and BOWE, 1989). This would also be more marked in tidal pools. This may have a direct effect on the net photosynthetic rate.

5.4.4.Effect of pH

The marked effect of pH on the rate of photosynthesis in *Pteroceramium* could be a result of dissolved inorganic carbon concentrations which are directly influenced by the pH (REISKIND, BEER and BOWES, 1989). When seawater is in equilibrium with the atmosphere, it has a pH between pH 8.0-pH 8.2 and a dissolved inorganic carbon content of between 2.0 mM-2.5 mM (LIGNELL and PEDERSÉN, 1989; REISKIND, BEER and BOWES, 1989). At this stable pH, the dominant form of carbon is HCO_3^-

with less than 200 μM CO_3^{2-} and 10 μM CO_2 present (LIGNELL and PEDERSÉN, 1989). At pH 10, the dominant form of carbon is CO_3^{2-}. As the pH is lowered, the proportion of HCO_3^- increases at the expense of CO_3^{2-}. At more acidic pHs, CO_2 becomes the dominant carbon species. By pH 6, it is the major form and by pH 4, 99% of the dissolved inorganic carbon is CO_2 (REISKIND, BEER and BOWES, 1989).

The availability of various dissolved inorganic carbon species may influence the carbon form used in macroalgal photosynthetic carbon fixation. CO_3^{2-} is an unusable form of carbon (REISKIND, BEER and BOWES, 1989) and could account for the significantly decreasing photosynthetic rates of *Pteroceramium* when placed in increasingly more alkaline pHs.

Photosynthesis in submerged algae generates an increase in the O_2 concentration, a rise in pH and a decrease in the dissolved inorganic carbon concentration around them. Dense stands of algae in sheltered habitats with reduced physical exchange with open water masses; e.g. rockpools, can alter the water conditions (GORDON and SAND-JENSEN, 1990). Such conditions would limit photosynthesis in the more pH sensitive algae (REISKIND, BEER and BOWES, 1989). As *Pteroceramium* shows marked sensitivity to changes in pH, this implies that it is not likely to be found in rockpools, especially high up in the eulittoral region where water exchange occurs less frequently but is more likely to be found in open waters.

Owing to the high concentration of HCO_3^- at pH 8, it has been proposed that many seaweeds are able to utilize HCO_3^- as their carbon source in photosynthesis in conjunction with CO_2. For example, some Gracilariaceae and *Chondus crispus* have been shown to be HCO_3^- utilizers (LIGNELL and PEDERSÉN, 1989). The affinity of the alga for CO_2 generally remains higher than for HCO_3^-. Exceptions to this are *Ceramium rubrum* and *Fucus vesiculosus* which have been found to have higher affinities for HCO_3^- than many freshwater plants even though they have similar CO_2 affinities (REISKIND, BEER and BOWES, 1989). Lowering the pH should produce a dramatic increase in photosynthesis if CO_2 is the major external force. If there is not a large change, this is an indication that the plant can effectively use HCO_3^- (HOLBROOK, BEER, SPENCER, REISKIND, DAVIS and BOWES, 1988). The increase in the photosynthetic rate of *Pteroceramium* in the more acidic pHs suggests that external CO_2 concentrations are important and could possibly limit photosynthesis.

RUBISCO uses CO_2 as a substrate and so HCO_3^- must first be dehydrated. This is done by the enzyme carbonic anhydrase which has been found in seaweeds although it is not localised as in unicellular algae (UUSITALO, AXELSSON, CARLBERG, LARSSON and RYBERG, 1990).

In the past it has been tacitly assumed that dissolved inorganic carbon concentrations were not limiting for photosynthesis. However, the ambient levels of the preferred dissolved inorganic carbon species may actually be restricting to the full photosynthetic potential. The diffusion rate of CO_2 is several thousand times slower in water than in air. This is made worse in marine habitats as a high salinity means 10% less CO_2 than in freshwater (11 μm CO_2 at 25°C), although HCO_3^- concentrations are relatively high (2.0 mM-2.5 mM) because of the high pH (REISKIND, BEER and BOWES, 1989). Four out of the five algae tested by HOLBROOK, BEER, SPENCER, REISKIND, DAVIS and BOWES (1988), showed that photosynthesis was CO_2 limited until levels were substantially raised above 2.5 mM, indicating that natural CO_2 levels are insufficient to saturate photosynthesis. This supports the view that there are mechanisms to concentrate CO_2 at RUBISCO carboxylation sites. Further investigations with *Pteroceramium* where the pH is lowered without altering dissolved inorganic carbon availability could prove interesting.

Microalgae have been found to have CO_2 concentrating mechanisms which are activated when transferred to low CO_2 concentrations (CARLBERG, AXELSSON, LARSSON, RYBERG and UUSITALO, 1990). The green macroalgae *Enteromorpha compressa* (Linnaeus) Greville, *Enteromorpha linza* and *Monostroma grevillei* (Thuret) Wittrock have been shown to be capable of enhancing HCO_3^- utilization at high pH (pH 9.85), taking approximately 24 hours at 6°C-7°C to complete activation of their mechanisms (CARLBERG, AXELSSON, LARSSON, RYBERG and UUSITALO, 1990). Deactivation of these mechanisms is much slower. The amount of activation also varied depending on the site of collection. For example, material collected from wave exposed sites already had 30% activation, material from calm waters had 40%-70%

activation and material from tide pools 100% activation indicating that important factors are water motion, irradiation and nutrient status (LARSSON, AXELSSON, CARLBERG, RYBERG and UUSITALO, 1990). There are two possible mechanisms explaining efficient HCO_3^- utilization in macroalgae:

1) CO_2 concentrating mechanisms as found in microalgae. This involves absorption of respiratory CO_2 by phosphoenol pyruvic acid and requires close association between mitochondria and chloroplasts (AXELSSON, CARLBERG, LARSSON, RYBERG and UUSITALO, 1990); and

2) modification of HCO_3^- utilization by the regulation of carbonic anhydrase activity in cell walls (CARLBERG, AXELSSON, LARSSON, RYBERG and UUSITALO, 1990).

These can be seen as adaptations which take active advantage of eulittoral environmental conditions (AXELSSON, CARLBERG and RYBERG, 1989). Red algae are less efficient at HCO_3^- utilization than green and brown algae. This may be due to their relying on carbonic anhydrase rather than CO_2 concentrating mechanisms as the green and brown algae rely on (UUSITALO, AXELSSON, CARLBERG, LARSSON and RYBERG, 1990).

The increased photosynthetic rates of *Pteroceramium* in the more acidic media may be due to the higher CO_2 levels. This may not be ecologically important as *Pteroceramium* is unable to grow in media with acidic pHs (STIRK and AKEN, 1993; Chapter 4). However, this may indicate that *Pteroceramium* is not an efficient HCO_3^- utilizer.

Chapter 6

Morphogenesis in *Pteroceramium*

6.1 Introduction

Most seaweeds arise from a single undifferentiated cell, usually a zygote or spore. This cell has the potential to form a whole organism by expression of the genotype modified by environmental cues to produce the phenotype. Most cells are totipotent, only a few cells in algal thalli being irreversibly differentiated (LOBBAN, HARRISON and DUNCAN, 1985). Development is a coordinated series of morphological, physiological and biochemical events which produce differentiated cells and tissues with distinctive characteristics. For example, rhizoids which are specialised for anchorage, are elongated, adhesive and pale in colour, while axial cells which are broader and more deeply pigmented, have a photosynthetic function (WAALAND, 1990). Morphology is the final expression of development and is the result of interaction between:

1) deposition of axes as determined by position, number and manner of formation of filaments of unlimited growth;

2) shape of axes as determined by growth in length, breadth and depth; and

3) longevity of erect thallus.

The ultimate form depends on interactions between cell division, enlargement and differentiation (WAALAND and CLELAND, 1972; DIXON, 1973; GARBARY, 1979b).

Morphogenesis is an orientated process with cells and plants having polarities, especially apico-basal polarities. As plant cells are not able to move, they have the ability to shift their polar axes in response to external cues (LE GUYADER and

FERRE, 1988). The rate of apical cell division, frequency, position and timing of branch initiation on both the main axis and lateral determinate and indeterminate branches is important in establishing the final size and morphology of a plant (WAALAND, 1990). The position of the branches may be unilateral, alternate or irregular. This pattern may be genetically predetermined or subjected to environmental control. In *Callithamnion hookeri* the length of time between formation of a subapical cell and the division of that cell to form a branch is genetically determined, and in *Griffithsia pacifica* the number of branches per plant is correlated with total daily irradiation (WAALAND, 1990).

The geometry of the outer growth form is a projection of inner geometrical relationships. The inner geometry of an organism can be viewed as a communication matrix determined by distance, allowing for efficient transport of substances and transfer of information (NEUBAUER and KURKA, 1991). POETHIG (1984 in LE GUYADER and FERRE, 1988) states "...in plants the expression of pattern at a cellular and morphological level probably coincides with, and is critical to the pattern generating mechanisms operating at subcellular level". No one part can be changed without influencing the rest of the structure.

A form emerges as a structural discontinuity as morphogenesis is a gradual construction (LE GUYADER and FERRE, 1988). An explanation of cell morphogenesis must account for succession of form rather than form *per se* as the course of development is integral through space and time (GREEN, 1969).

Genes are a key factor in morphogenesis and there are two extreme views on their role:

1) Genes tightly control growth and determine directly the shape of the organism throughout its whole development; i.e. morphogenesis is under direct control of the nucleus. Differentiation or appearances of inhomogeneties in shape is due to non-uniform control of the nucleus; or

2) Genes are involved in the formation of proteins and the regulation of biochemical machinery with self-organisation being a physicochemical process under

cytoplasmic control. Inhomogeneties in shape are due to instability of this self-organisation (PELCE and POCHEAU, 1992).

When morphogenesis is studied under controlled conditions, patterns of development can be defined and the modifications induced by environmental cues can be determined. To understand the development of form, it is necessary to have information on those environmental factors which exert control over gross aspects of growth and development (DIXON, 1971). There are many examples of environmental modification on the genotype. Rhizoid orientation in *Caulerpa prolifera* (Forsskål) Lamouroux is influenced by gravity with rhizoid initials containing many amyloplasts (LOBBAN, HARRISON and DUNCAN, 1985). Pelagic forms lack polarity and grow in all directions; e.g. *Sargassum natans* (Linnaeus) J.Meyen and *Sargassum fluitans* Børgesen in the Sargasso Sea (LOBBAN, HARRISON and DUNCAN, 1985). When *Callithamnion* grows in wave exposed areas, it is robust and heavily corticated with short axial cells and when it grows in a sheltered habitat, it has longer axial cells and little or no cortication (LOBBAN, HARRISON and DUNCAN, 1985). In some *Audouinella* species, cell length gradually decreases with increasing temperature and high temperatures reduce lateral branch formation (GARBARY, 1979a). Cortical cell division in some *Ceramium* species when grown under short day conditions, keep pace with axial cell enlargement, resulting in plants with continuous cortication. When incubated in over 16 hours light these species have distinct cortical bands and longer, uncorticated internodes as cortical cell division could not keep pace with increased axial cell enlargement. After about three weeks, these cortical bands become less distinct as they grow more. Maximum axial cell length was around 600 μm (GARBARY, GRUND and McLACHLAN, 1978).

Although results can show typical patterns of development, there must be caution in interpreting morphological results as even clonally derived material shows great individual variation in morphology (RUENESS and RUENESS, 1982); e.g. an isolate of *Callithamnion hookeri* has an average of eight cells in an apical row before the first lateral branch is formed from the third or fourth cell below the apical cell, while another isolate has on average 17 cells per apical row with the first lateral branch arising from the fifth cell (RUENESS and RUENESS, 1982).

Filamentous red algae show complex patterns of cell elongation but little is known about internal mechanisms modifying vegetative development and cell growth. Some studies have shown that cell dimensions and elongation in parts of the thallus are influenced by the apices while external factors such as temperature and daylength can also modify developmental patterns to some extent (GARBARY, BELLIVEAU and IRWIN, 1988).

Elaborate patterns of symmetry in the Rhodophyceae are a reflection of rigid control of growth and development (DIXON, 1971). Growth of a filament is by an increase in cell number and size; i.e. rate of apical cell division and subsequent enlargement of subapical cells. The degree of enlargement is variable and is subject to environmental modification (DIXON, 1973). Filamentous red algae such as the genera *Griffithsia*, *Antithamnion* and *Callithamnion* are excellent organisms in which to study cellular control of morphogenesis as:

1) they have a simple branched morphology which allows ready access to individual cells for observation and manipulation;

2) new axial cells are added only at the apex, resulting in an apico-basal gradient in cell age along any thallus. Subapical cells may divide to produce lateral branches and adventitious rhizoids;

3) single cells are totipotent, allowing for the developmental pattern from a single cell to be observed;

4) they have a relatively simple morphology but with at least two distinct types of somatic cells; and

5) many species grow relatively fast under controlled laboratory conditions (DIXON, 1971; WAALAND, 1989 and 1990).

Pteroceramium is a suitable alga in which to study morphogenesis as it has a regular form and growth pattern with an apical cell lineage pattern, a high capacity to regenerate and a remarkable degree of differentiation and organization in form with axial cells, cortical cells, wings and rhizoids.

The aims of the work described in this chapter were to define patterns of growth for *Pteroceramium* when grown in controlled conditions and to establish some internal mechanisms which are operational in *Pteroceramium*.

6.2 Materials and Methods

6.2.1 Experimental material

The same clone of male gametophytic material as used in the preceding chapters was used. It was grown in the same standard growth conditions unless otherwise stated.

6.2.2 Morphometric analysis

Measurements were taken at the fourth segment (seg. 4) away from the apical tip along the main axis as cells are almost fully expanded at this point. The first axial cell in the segment and its derivatives; i.e. those nearest the apical tip, were not included as the branching disrupted normal development. The axial cell volume, and both outer and inner wing cell number and surface area, were determined as described in Chapter 4. Pericentral cell volume was determined using the equation for the volume of a sphere ($^4/_3\, r^3\pi$). Fifty replicates plants were used with a total of 246 sets of cell measurements. Comparisons were made between the various measurements and Linear Regression Analysis (Statgraphics 5.0) carried out. Comparisons were made between:

1) axial cell volume: Pc1 volume
2) axial cell volume: Pc6 volume
3) axial cell volume: combined volume of all pericentral cells
4) axial cell volume: outer and inner wing surface area
5) axial cell volume: outer and inner wing cell number
6) Pc1 volume: Pc6 volume
7) Pc1 volume: Pc2 + Pc3 + Pc4 + Pc5 volume

8) Pc6 volume: Pc2 + Pc3 + Pc4 + Pc5 volume
9) outer wing surface area: inner wing surface area
10) outer wing cell number: inner wing cell number
11) Pc1 volume: outer wing surface area
12) Pc1 volume: outer wing cell number
13) Pc6 volume: inner wing surface area
14) Pc6 volume: inner wing cell number
15) outer wing surface area: outer wing cell number
16) inner wing surface area: inner wing cell number

6.2.3 Developmental study

Well-slides were made using glass cover slips (22 mm x 50 mm) as a base and gluing on glass tubes (diameter 15 mm) with Bostik Marine Sealant (silicone adhesive marine glue). Once dry, these were thoroughly rinsed in running water. Coverslips were used as a base as these were thinner than commercially available well-slides and allowed the specimen to be focused better when viewing with an inverted microscope. These modified well-slides also held larger volumes and so material could be grown for longer periods of time without having to replenish the medium.

Actively growing material with 2-3 segments was placed in 1.5 ml PES and the well-slides sealed with a glass cover slip (18 mm x 18 mm) and vaseline. The replicates were left for two days to allow for growth to resume.

On the third day, the material was examined using an inverted microscope (Zeiss IM 35, West Germany) and diagrams were made of the first few cells in the apical tip. This was called Day 0. The material was examined every day and diagrams showing the sequence of cortical cell development drawn. The experiment was terminated after Day 4 as the material became too profusely branched to see detail accurately. There were five replicates.

6.2.4 Dorsiventrality study - effect of unidirectional irradiance

The experiment described above (Section 6.2.3) was repeated taking care that the light source was only from above. After Day 3 the direction of the illumination was altered so that it shone from below. The slides were covered with a dark cloth so that no light could reach the plants from above. Observations were made for a further four days (Day 7). Six replicates were used.

6.2.5 Dorsiventrality study - effect of gravity

The experiment described above (Section 6.2.3) was repeated but with the thalli being manually turned over after Day 3 or Day 4. Observations were made until Day 6. Four replicate plants were used in this experiment.

6.2.6 Regions of axial cell elongation

The vital stain Calcofluor White M2R (disodium salt of 4,4'-bis[4-anilo-bis(β-hydroxyethyl)amino-s-triazine-2-ylamino]-2,2'-stilbene disulfonic acid; Sigma Chemicals, USA) was made up in a 0.01% aqueous solution with PES and filter sterilized through a 0.45 μm membrane. Small pieces (5-6 segments) of actively growing material were placed in this solution for 30 minutes, washed in sterilized PES for 1 minute (WAALAND and WAALAND, 1975; GARBARY and BELLIVEAU, 1990) and then placed in sterilized repli-dishes with 4 ml PES.

Photographs were taken at Day 0, Day 1, Day 2 and Day 4 using an inverted fluorescent microscope with a combination of exciter and barrier filters (HBO BP 395-440/FT 460/LP 470). Photographs were taken using Pan F 50 ASA film with the shutters being held open manually for 18 seconds.

Good fluorescent brighteners have a high intensity of induced fluorescence, good chemical stability and low toxicity (FRANK, 1991). These diamino stilbene disulfonate stains specifically bind to cellulose and chitin and possibly other β-linked polymers in cell walls by hydrogen bonding. They are useful stains owing to their low toxicity

(HUGHES and McCULLY, 1975; RONCERO and DURÁN, 1985; BELLIVEAU, GARBARY and McLACHLAN, 1990). The toxic effects of these brighteners are not known but they may affect polysaccharide deposition (BELLIVEAU, GARBARY and McCULLY, 1990).

6.2.7 Ca^{2+} concentrations in *Pteroceramium*

Actively growing pieces of *Pteroceramium* (5-6 segments) were incubated in 400 units/ml β-glucuronidase (Type IX, Sigma, USA) made up in half-strength PES for 20 minutes at 30°C (MEYER and PIENAAR, 1984). This removed the external mucilaginous layer to allow for easier penetration of the stain. Material was then rinsed twice in half-strength PES and incubated in the dark for 20-30 minutes in 0.5 mg ml^{-1} chlorotetracycline (Sigma Chemicals, USA) made up at pH 6.98. Thalli were rinsed in half-strength PES and then mounted onto glass slides. Material was observed within 10 minutes using an inverted fluorescent microscope with an interference filter combination of HBO BP 450-490/FT 510/LP 470 (REISS and HERTH, 1979). Photographs were taken using Pan F 50 ASA film with a shutter speed of 18 seconds.

Chlorotetracycline is a lipophilic chelate probe for divalent metals; eg. Ca^{2+} and Mg^{2+}. These two ions have different excitation and emission spectra and the use of the right filters can separate the two ion responses (SAUNDERS and HEPLER, 1981). Chlorotetracycline fluoresces when bound to Ca^{2+} and this fluorescence is enhanced when the chlorotetracycline-Ca^{2+} complex is associated with the membrane (KROPF and QUATRANO, 1987).

6.2.8 Effect of exogenous application of plant hormones on *Pteroceramium*

The different hormones tested were:

i) Auxin (Indole-3-acetic acid; IAA; Sigma Chemicals, USA) with concentrations ranging from 10^{-9} M to 10^{-5} M being used. The experiment ran for 12 days.

ii) Gibberellic acid (GA_3; Sigma Chemicals, USA) with concentrations ranging from 10^{-9} M to 10^{-5} M being used. The experiment ran for 12 days.

iii) Cytokinins (Kinetin; Sigma Chemicals, USA) with concentrations ranging from 10^{-9} M to 10^{-5} M being used. The experiment ran for 9 days.

The above listed hormones were added to PES and filter sterilized through a 0.45 μm membrane. Apical tips of *Pteroceramium* with 2-4 segments were added aseptically to 4 ml of medium in separate compartments in repli-dishes. Eight replicate plants were used per treatment. Growth and morphometric measurements as described in Chapter 4, were taken for these replicates, although growth measurements were taken only every third day rather than every second day as described earlier.

6.2.9 Mung bean bioassay

Experimental material (fresh mass 4.06 g) was extracted in 80% methanol and left overnight at 10°C. The extract was filtered and dried down in a flash evaporator at -40°C whereafter 100 ml 0.2 M phosphate buffer (pH 8) was added (Appendix C.2). The extract was partitioned with 100 ml ethyl acetate, the layers separating after about 15 minutes. The upper phase (pH 8) was kept. The separation was repeated three times. After separation, the pH was lowered to pH 3 and another separation carried out by adding ethyl acetate. All three phases (phosphate buffer, ethyl acetate at pH 8 and pH 3) were kept overnight at 10°C. Each phase was dried down in a flash evaporator and the residue made up to 50 ml with distilled water and sonicated to dissolve the extract. From each of these three solutions, various concentrations were made up to test the effect of root initiation in Mung Beans:

1) 30 ml extract
2) 15 ml extract and 15 ml distilled water
3) 5 ml extract and 25 ml distilled water

Mung beans, grown in vermiculite with stems approximately 8 cm in length, were cut off at the base. Seven plants were placed in each solution and left overnight at 20°C.

The following morning the mung beans were placed in distilled water, which was topped up as required. After seven days, the number of roots which grew from the Mung beans was counted. Data was analyzed using Kruskal Wallis One-Way Analysis of Variance and if there were significant differences, One-Way Analysis and a Multiple Range Test (Tukey) were done to reveal these differences (Statgraphics 5.0).

6.2.10 Effect of exogenous application of polyamines, their precursors and inhibitors on *Pteroceramium*

The same experimental procedure was followed as described in Chapter 4. However, growth was monitored only on Day 0, Day 6 and Day 12. The chemicals applied were:

i) Arginine (L-arginine free base; Sigma Chemicals, USA), a polyamine precursor, in the concentration range of 10^{-6} M to 10^{-2} M.

ii) Ornithine ((D-2,5-diaminopentanoic acid) hydrochloride; Sigma Chemicals, USA), a polyamine precursor, in the concentration range of 10^{-6} M to 10^{-3} M.

iii) Agmatine (1-amino-4-guanidinobutane; Sigma Chemicals, USA), a polyamine precursor, in the concentration range of 10^{-6} M to 10^{-2} M.

iv) Methionine (L-methionine-DL-sulfoximine; Sigma Chemicals, USA), a polyamine precursor, in the concentration range of 10^{-6} M to 10^{-3} M.

v) Putrescine (1,4-diaminobutane;tetramethyl-enediamine; Sigma Chemicals, USA), a diamine, in the concentration range of 10^{-6} M to 10^{-2} M.

vi) Spermidine (N-[3-aminopropyl]-1,4-butanediamine; Sigma Chemicals, USA), a triamine, in the concentration range of 10^{-6} M to 10^{-2} M.

vii) Spermine (N,N'-bis[3-aminopropyl]-1,4-butanediamine; Sigma Chemicals, USA), a tetramine, in the concentration range of 10^{-6} M to 10^{-2} M.

viii) DFMO (α-difluoromethyl ornithine; Nunclon, Denmark), an inhibitor of ornithine decarboxylase, in the concentration range of 10^{-6} M to 10^{-2} M.

ix) CHAP (cyclohexamine hydrobromide; Sigma Chemicals, USA), an inhibitor of spermidine synthase, in the concentration range of 10^{-6} M to 10^{-2} M.

x) MGBG (methylglyoxal bis-(guanyl-hydrazone) dihydrochloride; Sigma Chemicals, USA), an inhibitor of s-adenosylmethionine decarboxylase, in the concentration range of 10^{-6} M to 10^{-3} M.

6.2.11 HPLC separation of polyamines

Bulk cultures of *Pteroceramium* were grown in 250 ml erlenmeyer flasks containing 150 ml PES with air bubbled through them. Polyamines were extracted from the material following the method described in UPFOLD and VAN STADEN (1991). Experimental tissue was weighed, ground in liquid nitrogen and freeze-dried overnight. It was then reground to a fine powder using liquid nitrogen. Material was extracted at 4°C for 1 hour in 0.2 M $HClO_4$ (1 ml 100 mg^{-1} material). The extract was filtered through a 0.45 μm membrane and 2 ml of 2 M NaOH and 10 μl benzoyl chloride added to 1 ml of extract. After vigorous shaking, the extract was left overnight at room temperature. The extract was fractionated by adding 2 ml chloroform and was centrifuged for 20 minutes in a clinical centrifuge (Hettich Universal 2S, Germany) at 2000 rpm. After removing and discarding the upper layer, 2 ml deionised distilled water was added to wash the extract. The extract was centrifuged at 2000 rpm for 10 minutes. The upper aqueous layer was discarded and the denser chloroform layer dried under nitrogen. After resuspending in 300 μl of 80% HPLC-grade methanol, 100 μl extracts were fractionated using HPLC.

The extraction procedure was repeated for *Pteroceramium* but at 25 times more concentrated level; i.e. after overnight extraction in 0.2 M $HClO_4$, 25 ml of *Pteroceramium* extract was used. Other chemicals were added at 25 times more than described in the above method to keep the ratios of each chemical constant. Three replicates were used.

Separation of the polyamines was achieved using a gradient elution programme on a Beckman System Gold HPLC (USA) with an Ultrasphere 5 μM ODS column at a flow rate of 1 ml min^{-1}. Gradient elution was programmed from 30% methanol:70% 0.2 M acetic acid buffered to pH 3.5 using triethylamine to 100% methanol over 50 minutes. Three replicates were used.

Standard extraction curves for putrescine, spermidine and spermine were done using 1 mg ml^{-1} of the polyamine dissolved in distilled water. The same procedure as described above was followed using 1 ml of extract, except that the washing with deionised distilled water was omitted. Various amounts of the standards were injected into the HPLC ranging from 10 μl to 40 μl. Standard curves were drawn using peak area. The retention times for each polyamine were also determined (UPFOLD and VAN STADEN, 1991).

6.2.12 Morphological differences between dominant and subordinate branches

The number of axial cells and the length of seg. 4 along the dominant axis were recorded in actively growing material of *Pteroceramium*. The number of axial cells and the length of the corresponding subordinate branch were also recorded. The branch number (number of branch segments from the apex) was also noted. In this experiment, 100 replicates were used.

6.2.13 Apical dominance

The apical segment of the dominant branch was surgically removed under sterile conditions using fine sewing needles with sharpened points. These points were sharpened to a cutting edge using a diamond and fixed onto dissecting needle handles. The excised thalli were transferred to a watch glass, rinsed twice in sterilised PES to remove any extruded cytoplasm so as to reduce bacterial infection and then placed in 4 ml PES in repli-dishes.

Axial cell number and the length of each segment, and the number of true and adventitious branches, were recorded for the next 10 days. Twenty-five experimental replicates were used in this experiment as there was a high frequency of bacterial infection which killed the thalli. At the end of the experiment, there were eight uninfected thalli with excised apical tips and seven control replicates.

The ratio of axial cell number to number of adventitious branches was determined. Kruskal Wallis One-Way Analysis of Variance was done to reveal significant differences between the treatments and these differences revealed by One-Way Analysis and a Multiple Range Test (Tukey; Statgraphics 5.0).

The above experiment was repeated but the apical tips of subordinate branches were removed rather than apical tips of dominant branches. The thalli grew for seven days. Four replicates remained at the end of the experiment. No statistical analyses were done on this data.

6.2.14 Apical dominance in the presence of exogenous IAA

The apical dominance experiment described above (Section 6.2.13) was repeated but with various concentrations of IAA (10^{-9} M to 10^{-4} M) added to the filter sterilized medium. The number of replicates ranged from 5-9 depending on bacterial contamination. There were two control treatments, one without the apical tips removed and one with the tips excised but grown only in PES. Observations were made on Day 0 and Day 10. The ratio of axial cells to number of adventitious branches was determined.

6.3 Results

6.3.1 Morphometric analysis

There was a significant correlation for all comparisons made (Table 6.1). The strongest correlations occurred between the outer and inner wings, and the size of the pericentral cells to the wing size.

Table 6.1 Correlation between various mature cell types taken at seg. 4 along the dominant axis in *Pteroceramium*. ax = axial cell, vol = volume, Pc = pericentral cell, OW = outer wing, IW = inner wing, SA = surface area.

Comparison pairs	Correlation coefficient	Standard error
ax cell vol:Pc1 vol	0.346	P<0.001
ax cell vol:Pc6 vol	0.408	P<0.001
ax cell vol:combined vol of all Pc's	0.462	P<0.001
ax cell vol: OW SA + IW SA	0.544	P<0.001
ax cell vol: OW cell no. + IW cell no.	0.451	P<0.001
Pc1 vol:Pc6 vol	0.508	P<0.001
Pc1 vol:Pc2 + Pc3 + Pc 4 + Pc5 vol	0.282	P<0.01
Pc6 vol:Pc2 + Pc3 + Pc4 + Pc5 vol	0.488	P<0.001
OW SA:IW SA	0.557	P<0.001
OW cell no:IW cell no.	0.456	P<0.001
Pc1 vol:OW SA	0.610	P<0.001
Pc1 vol:OW cell no.	0.528	P<0.001
Pc6 vol:IW SA	0.560	P<0.001
Pc6 vol:IW cell no.	0.491	P<0.001
OW SA:OW cell no.	0.872	P<0.001
IW SA:IW cell no.	0.921	P<0.001

6.3.2 Developmental study

From the sequence of development of apical tips (Fig. 6.1A-E), it can be noted that the division of the apical cell was transverse to produce new subapical cells and oblique to produce pseudodichotomous branching.

The patterns of development were constant:

Pc1 always developed on the outer face; e.g. Rep 1-Day 0, Rep 2-Day 0, Rep 3-Day 0 and 1, Rep 4-Day 0 and 2, Rep 5-Day 0;

Pc2 always developed on the lower surface; e.g. Rep 1-Day 0, 2 and 3, Rep 2-Day 2, Rep 3-Day 0 and 3, Rep 4-Day 2, Rep 5-Day 2;

Pc3 developed on the upper surface; e.g. Rep 2-Day 4, Rep 4-Day 3; and

Pc4 developed on the lower surface; e.g. Rep 4-Day 3.

The development of Pc5 and Pc6 was not observed.

The first division of the pericentral cells was usually apically; e.g. Rep 1-Day 4, Rep 3-Day 3, Rep 4-Day 2 and 4 and Rep 5-Day 3 although it was sometimes basal; e.g. Rep 3-Day 3, Rep 4-Day 4 and Rep 5-Day 2. Spines were also seen; e.g. Rep 4-Day 4. The outer wing developed quickly from Pc1, often before the other pericentral cells were cut off; e.g. Rep 2-Day 1,2 and 4, Rep 3-Day 3, Rep 4-Day 2,3 and 4 and Rep 5-Day 2 and 3.

6.3.3 Dorsiventrality study - effect of unidirectional irradiance

The same development was noted in the first few days (Fig. 6.2A-F) as seen in the above experiment:

Pc1 always developed on the outer face; Rep 1-Day 1 and 3, Rep 2-Day 0 and 1, Rep 5-Day 1;

Pc2 always developed on the lower surface; e.g. Rep 1-Day 3, Rep 2-Day 1 and 2, Rep 3-Day 1, Rep 4-Day 1, Rep 5-Day 0 and 3 and Rep 6-Day 1;

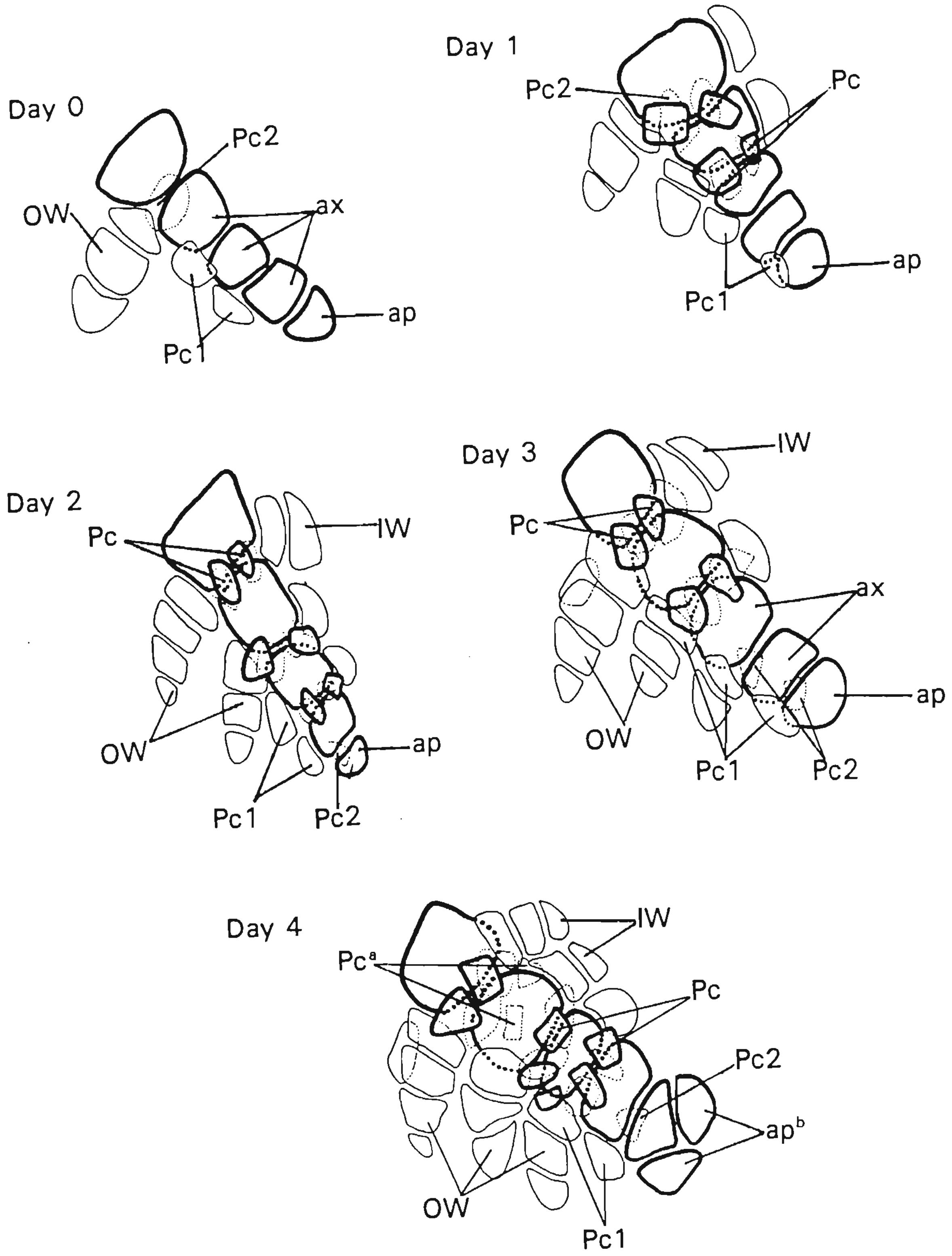


Fig. 6.1A Diagram illustrating the development of the cortical band region in actively growing tips of *Pteroceramium* (Rep 1). ap = apical cell, ap^b = branching apical cell, ax = axial cell, Pc = pericentral cell, OW = outer wing, IW = inner wing, Pc^a = apical derivative of a pericentral cell,—— = upper surface, ······· = lower surface.

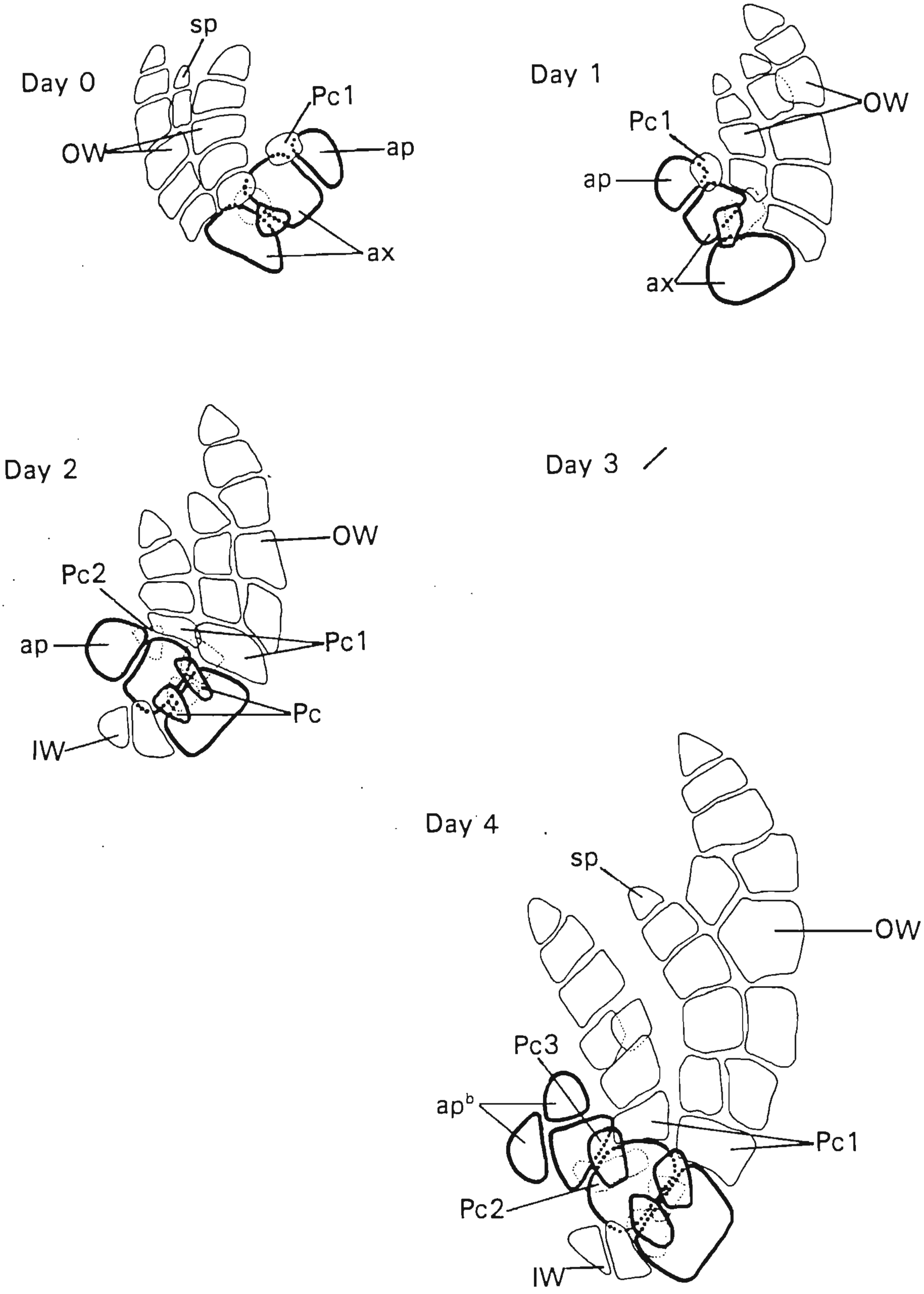


Fig. 6.1B Diagram illustrating the development of the cortical band region in actively growing tips of *Pteroceramium* (Rep 2). ap = apical cell, ap^b = branching apical cell, ax = axial cell, Pc = pericentral cell, OW = outer wing, IW = inner wing, sp = spine, —— = upper surface, ………… = lower surface.

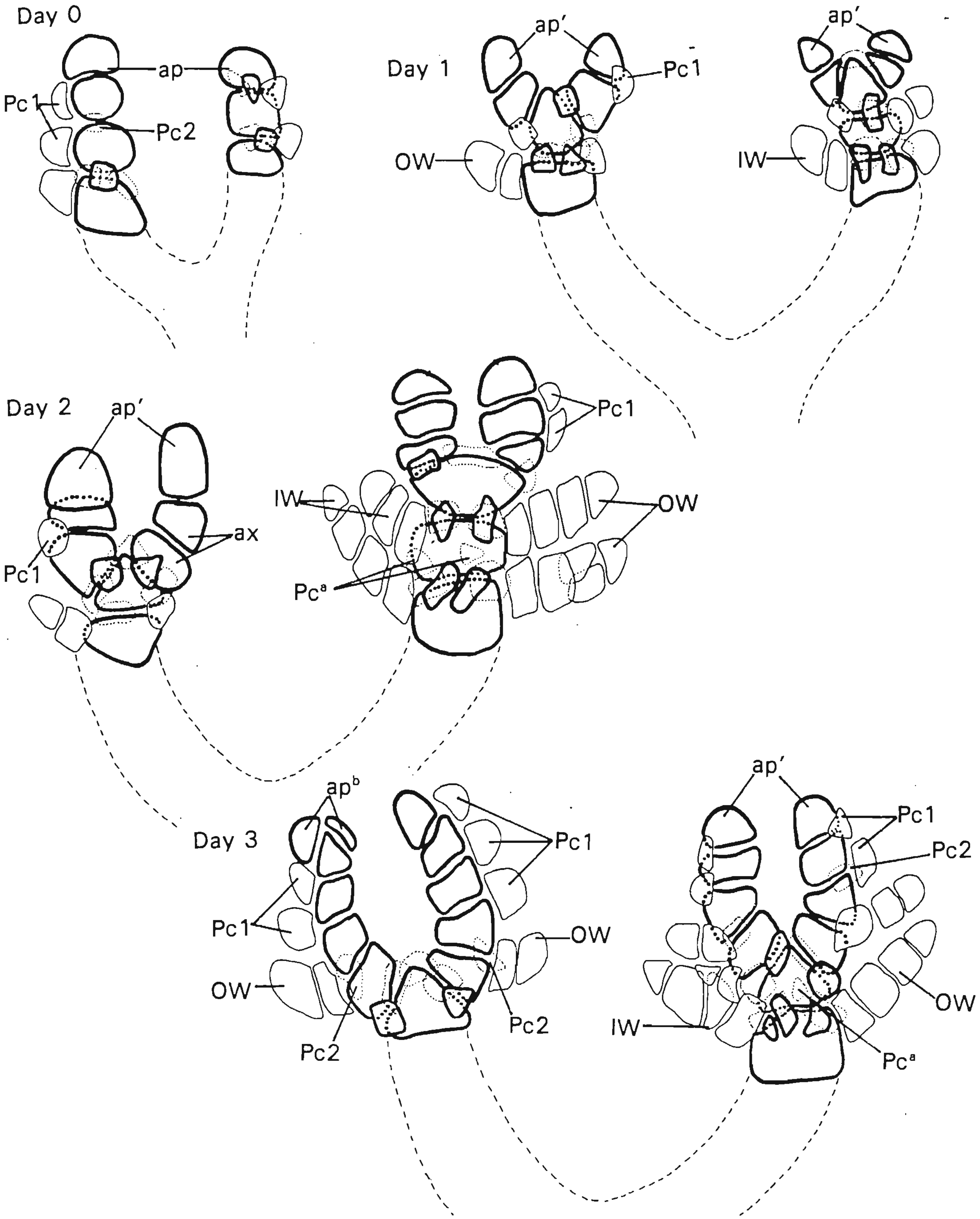


Fig. 6.1C Diagram illustrating the development of the cortical band region in actively growing tips of *Pteroceramium* (Rep 3). ap = apical cell, ap' = new apical cell, ap^b = branching apical cell, ax = axial cell, Pc = pericentral cell, OW = outer wing, IW = inner wing, Pc^a apical derivative of a pericentral cell, —— = upper surface, ········ = lower surface.

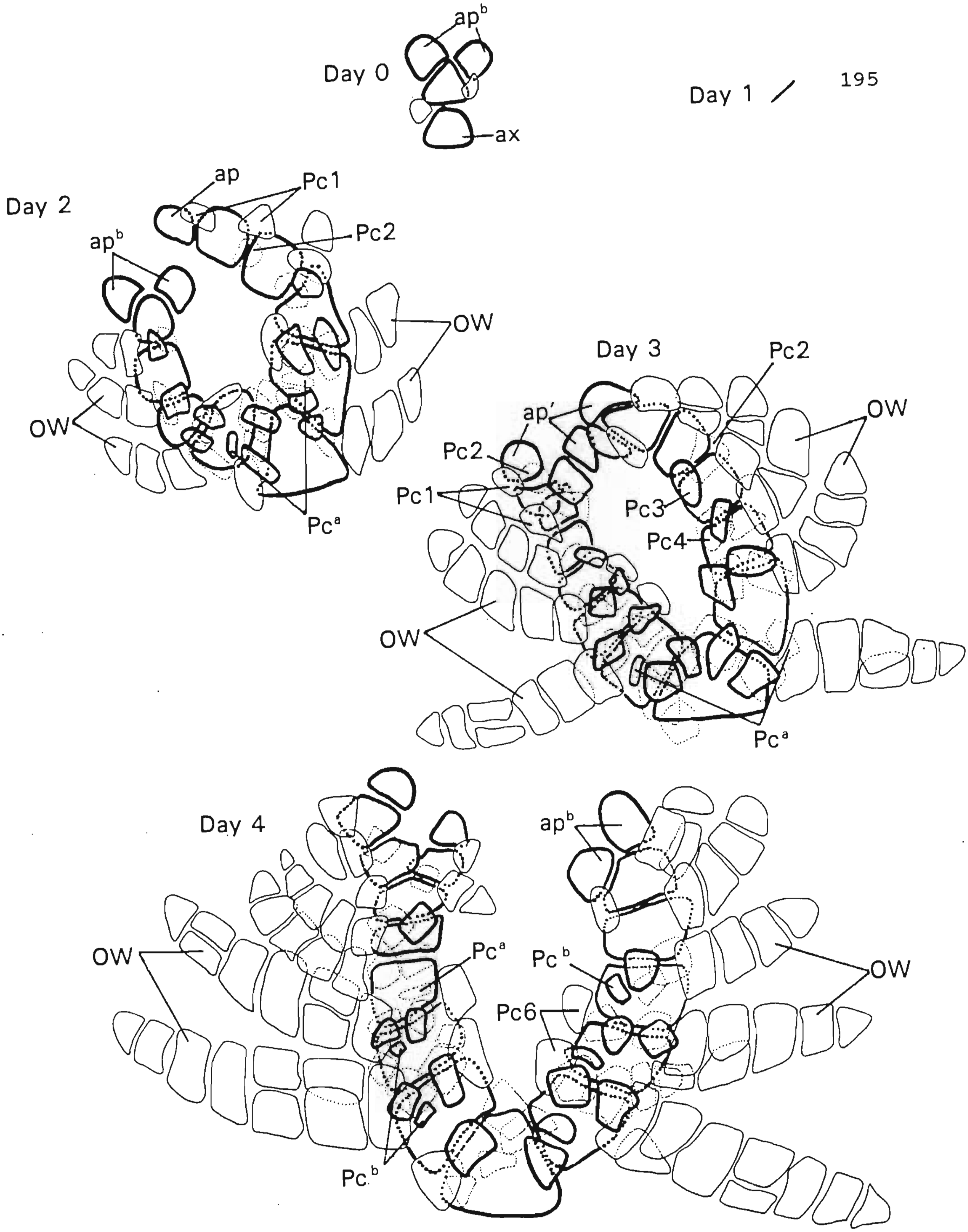


Fig. 6.1D Diagram illustrating the development of the cortical band region in actively growing tips of *Pteroceramium* (Rep 4). ap = apical cell, ap' = new apical cell, ap^b = branching apical cell, ax = axial cell, Pc = pericentral cell, OW = outer wing, Pc^a = apical derivative of a pericentral cell, Pc^b = basal derivative of a pericentral cell, ——— = upper surface, ·········· = lower surface.

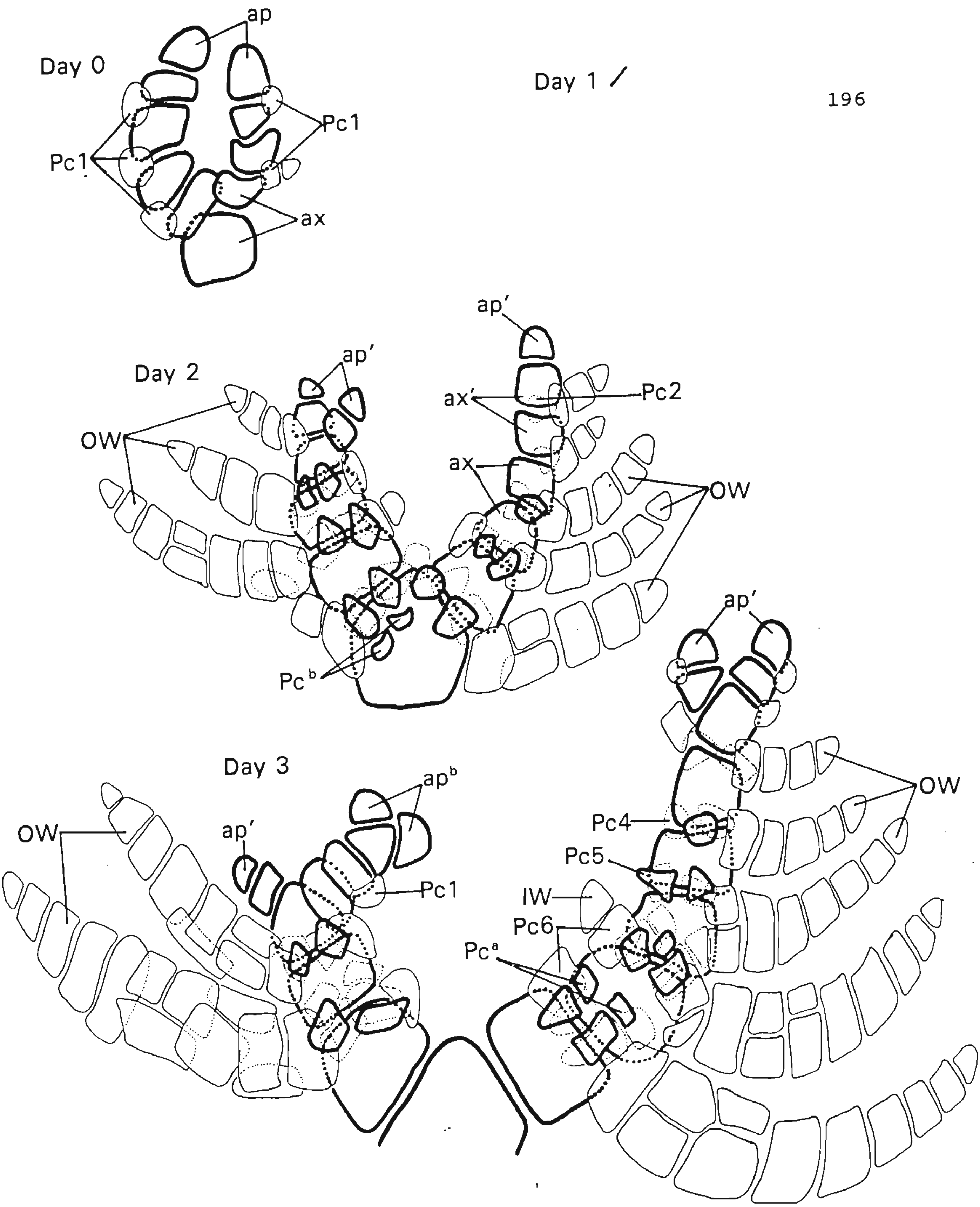


Fig. 6.1E Diagram illustrating the development of the cortical band region in actively growing tips of *Pteroceramium* (Rep 5). ap = apical cell, ap' = new apical cell, ap^{b} = branching apical cell, ax = axial cell, ax' = new axial cell, Pc = pericentral cell, OW = outer wing, IW = inner wing, Pc^{a} = apical derivative of a pericentral cell, Pc^{b} = basal derivative of a pericentral cell, ——— = upper surface, ·········· = lower surface.

Pc3 developed on the upper surface; e.g. Rep 2-Day 2, Rep 3-Day 1 and 2, Rep 4-Day 2 and Rep 5-Day 1; and

Pc4 developed on the lower surface; e.g. Rep 3-Day 3.

The development of Pc5 and Pc6 were not observed.

The first division of the pericentral cells in this experiment was also usually apical; e.g. Rep 1-Day 3.

Cortical band development was not altered after a change in the direction of illumination. The same pattern as before was seen in the new growth which formed after the change in the direction of light:

Pc1 developed on the outer face e.g. Rep 1-Day 5 and 6, Rep 2-Day 6, Rep 3-Day 4 and Rep 5-Day 5 and 6;

Pc2 developed on the lower surface; e.g. Rep 2-Day 6, Rep 3-Day 5, Rep 5- Day 6 and Rep 6-Day 6; and

Pc3 developed on the upper surface; e.g. Rep 3-Day 6 and Rep 5- Day 7.

The development of Pc4, Pc5 and Pc6 was not observed.

6.3.4 Dorsiventrality study - effect of gravity

The same development was noted in the first few days as in the above two experiments (Fig. 6.3A-D) where:

Pc1 developed on the outer face; Rep 1-Day 1 and 2, Rep 3-Day 1, 2 and 3 and Rep 4-Day 2 and 3;

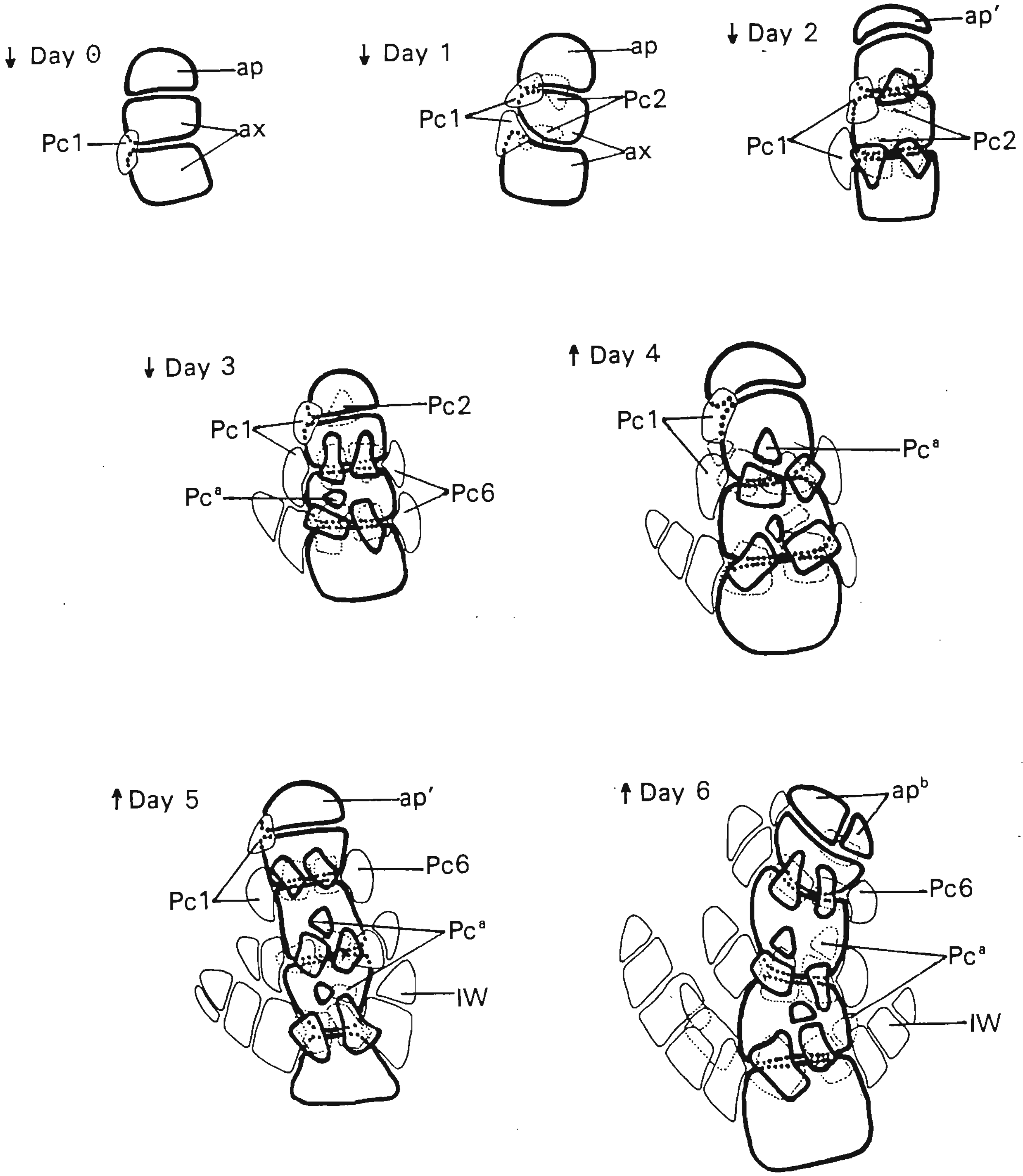


Fig. 6.2A Diagram illustrating the development of the cortical band region in actively growing tips of *Pteroceramium* (Rep 1) when there is a change in the direction illumination. ap = apical cell, ap' = new apical cell, ap^b = branching apical cell, ax = axial cell, Pc = pericentral cell, OW = outer wing, IW = inner wing, Pc^a = apical derivative of a pericentral cell, —— = upper surface, ········ = lower surface, ↑ = direction of illumination.

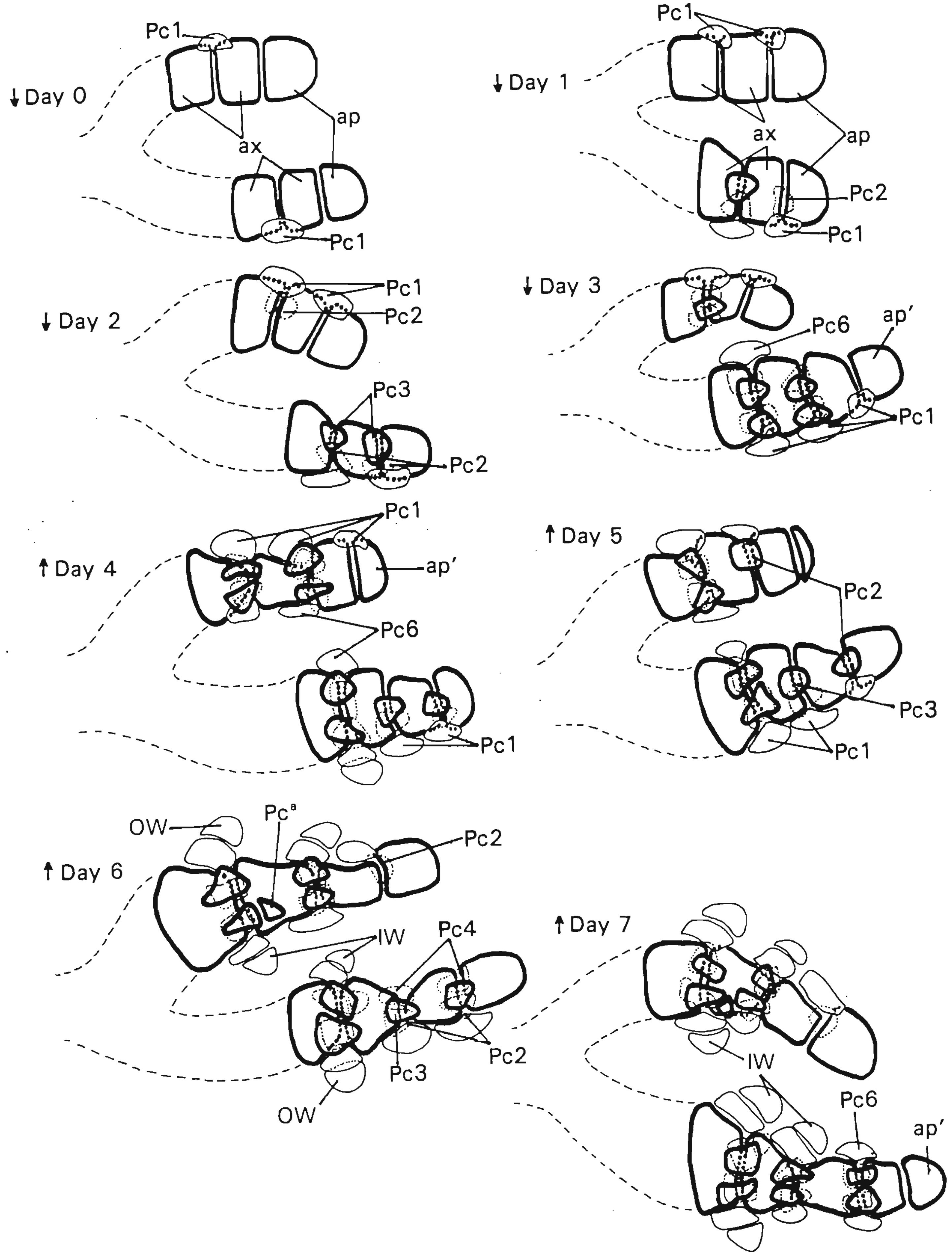


Fig. 6.2B Diagram illustrating the development of the cortical band region in actively growing tips of *Pteroceramium* (Rep 2) when there is a change in the direction of illumination. ap = apical cell, ap' = new apical cell, ax = axial cell, Pc = pericentral cell, OW = outer wing, IW = inner wing, Pc^{a} = apical derivative of a pericentral cell —— = upper surface ····· = lower surface, ↑ =

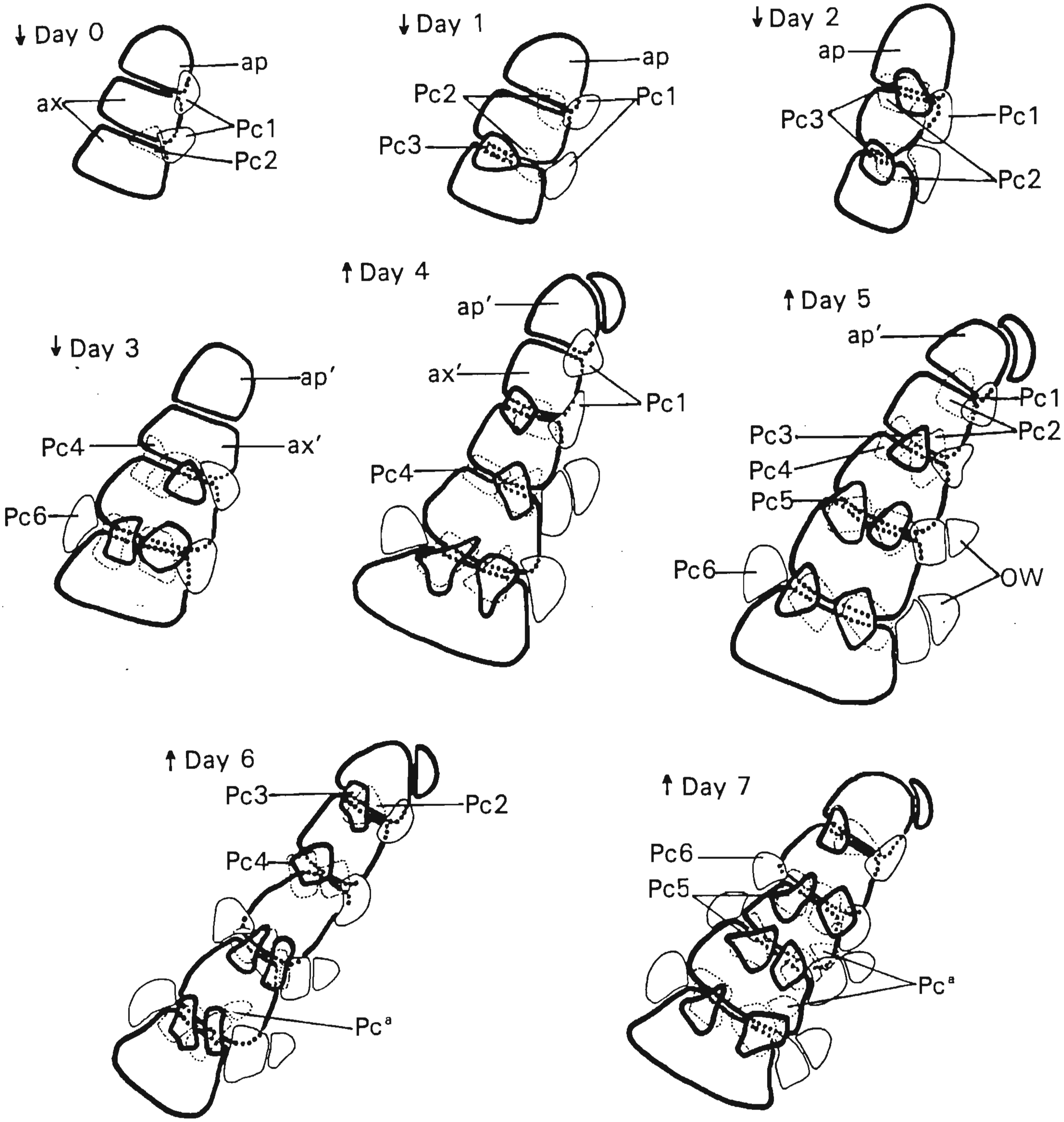


Fig. 6.2C Diagram illustrating the development of the cortical band region in actively growing tips of *Pteroceramium* (Rep 3) when there is a change in the direction of illumination. ap = apical cell, ap' = new apical cell, ax = axial cell, ax' = new axial cell, Pc = pericentral cell, OW = outer wing, Pc^a = apical derivative of a pericentral cell, ——— = upper surface, ········ = lower surface, ↑ = direction of illumination.

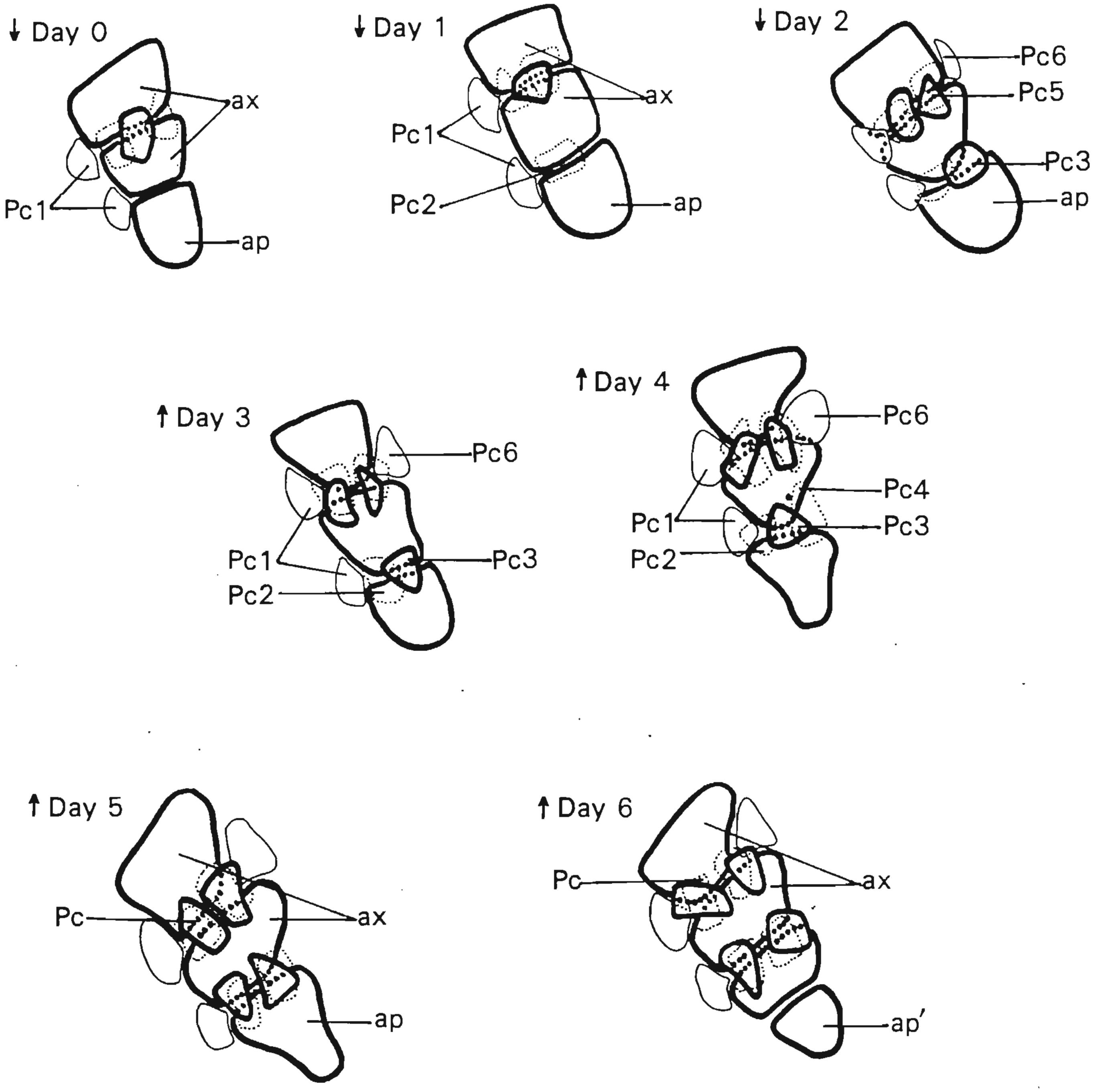


Fig. 6.2D Diagram illustrating the development of the cortical band region in actively growing tips of *Pteroceramium* (Rep 4) when there is a change in the direction of illumination. ap = apical cell, ap' = new apical cell, ax = axial cell, Pc = pericentral cell,——— = upper surface,········ = lower surface, ↑ = direction of illumination.

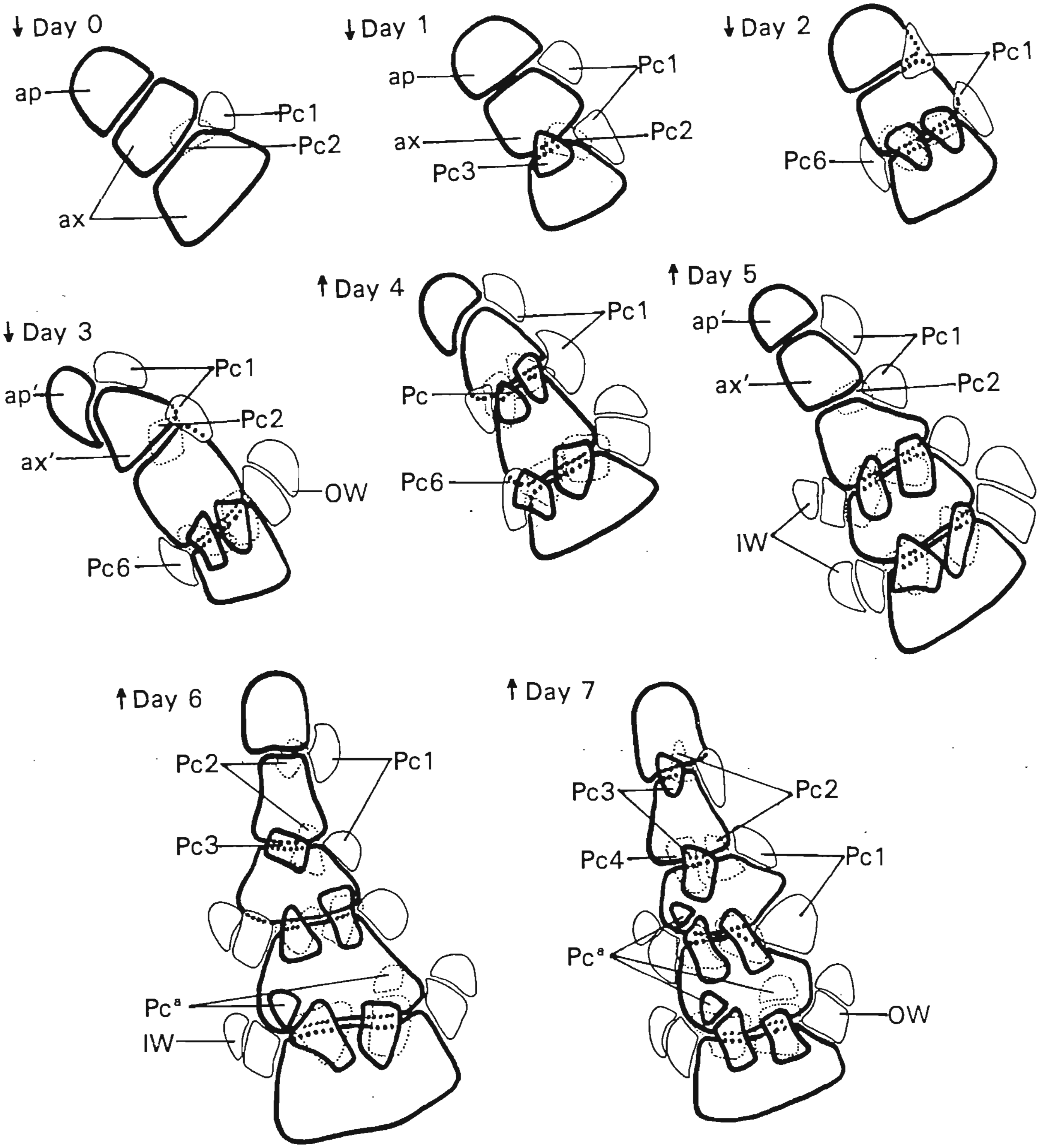


Fig. 6.2E Diagram illustrating the development of the cortical band region in actively growing tips of *Pteroceramium* (Rep 5) when there is a change in the direction of illumination. ap = apical cell, ap' = new apical cell, ax = axial cell, ax' = new axial cell, Pc = pericentral cell, OW = outer wing, IW = inner wing, Pc^a = apical derivative of a pericentral cell, —— = upper surface, ·········= lower surface, ↑ = direction of illumination.

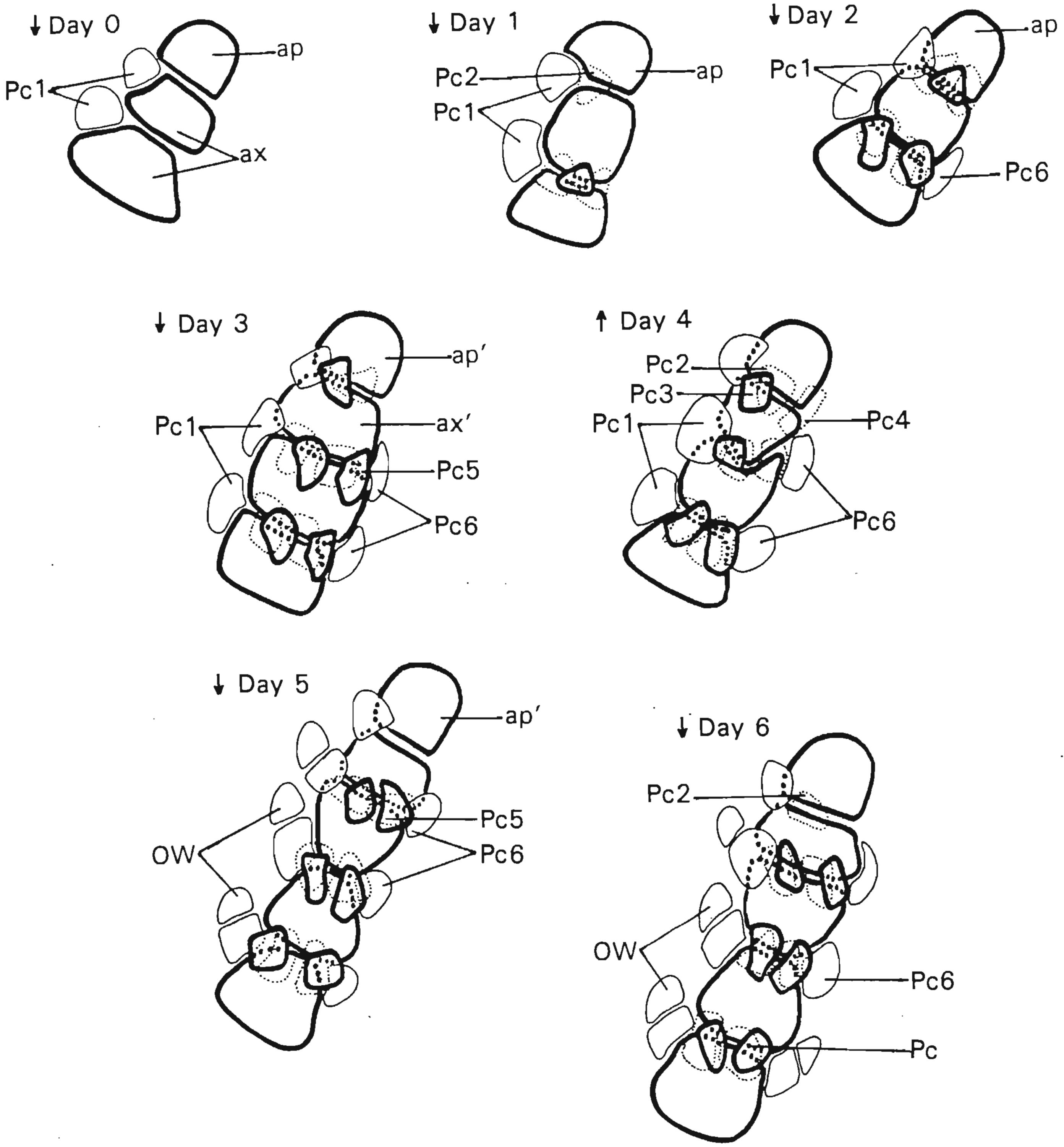


Fig. 6.2F Diagram illustrating the development of the cortical band region in actively growing tips of *Pteroceramium* (Rep 6) when there is a change in the direction of illumination. ap = apical cell, ap' = new apical cell, ax = axial cell, ax' = new axial cell, Pc = pericentral cell, OW = outer wing, ——— = upper surface, ... = lower surface, ↑ = direction of illumination.

Pc2 developed on the lower surface; e.g. Rep 1-Day 1, Rep 2-Day 0, Rep 3-Day 1, Rep 4-Day 2 and 3 and Rep 4-Day 2 and 3; and

Pc3 developed on the upper surface; e.g. Rep 2-Day 1 and Rep 4-Day 3.

The material was manually turned over before further pericentral cells were cut off.

Looking at the new axial cells formed after the change in the orientation, (the upper and lower surface are still referred to as upper and lower even though they are now actually reversed), it could be seen that cortical band development was not affected by gravity as:

Pc1 developed on the outer face; Rep 1-Day 5 and 7, Rep 2-Day 6 and 7, Rep 3-Day 5 and 6 and Rep 4-Day 6;

Pc2 developed on the lower surface; e.g. Rep 1-Day 5, Rep 2-Day 6, Rep 3-Day 5, Rep 4-Day 6; and

Pc3 developed on the upper surface; e.g. Rep 1-Day 6 and Rep 4- Day 6.

The development of Pc4, Pc5 and Pc6 were not observed.

6.3.5 Regions of axial cell elongation

Distinct non-fluorescing bands formed basally after Day 2, indicating that a single basal band is deposited during axial cell elongation (Plate 6.1 Fig. 1-5).

6.3.6 Ca^{2+} concentrations in *Pteroceramium*

The rhizoids show clear Ca^{2+} concentrations as is common in tip elongation. There also appeared to be Ca^{2+} concentrations in the wing tips and apical segments and in a few examples, in the cortical band (Plate 6.1 Fig. 6).

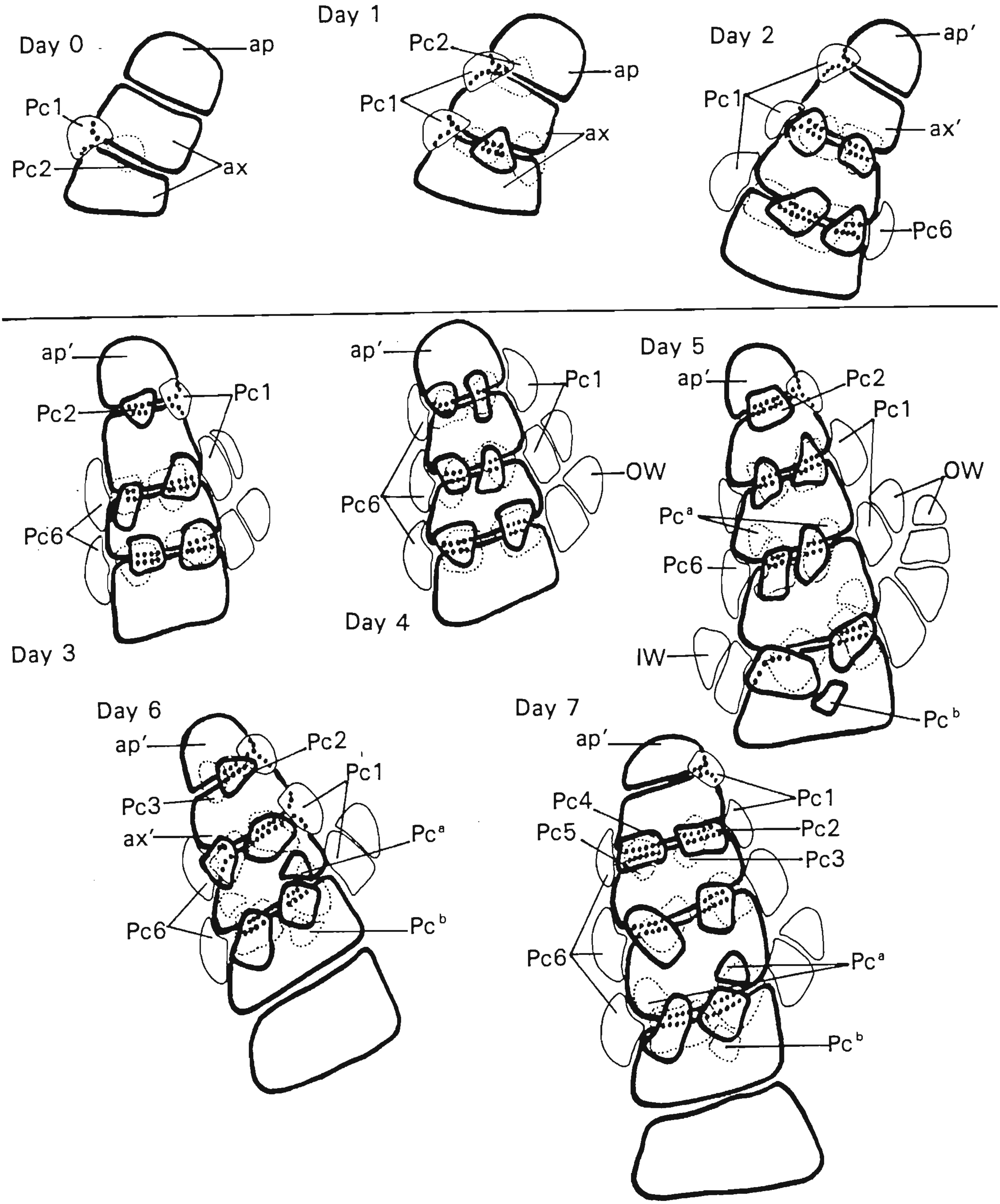


Fig. 6.3A Diagram illustrating the development of the cortical band region in actively growing tips of *Pteroceramium* (Rep 1) when the direction of gravity is altered. ap = apical cell, ap' = new apical cell, ax = axial cell, ax' = new axial cell, Pc = pericentral cell, OW = outer wing, Pc^a = apical derivative of a pericentral cell, Pc^b = basal derivative of a pericentral cell, ——— = upper surface, ... = lower surface, —— = plant turned over to change gravity.

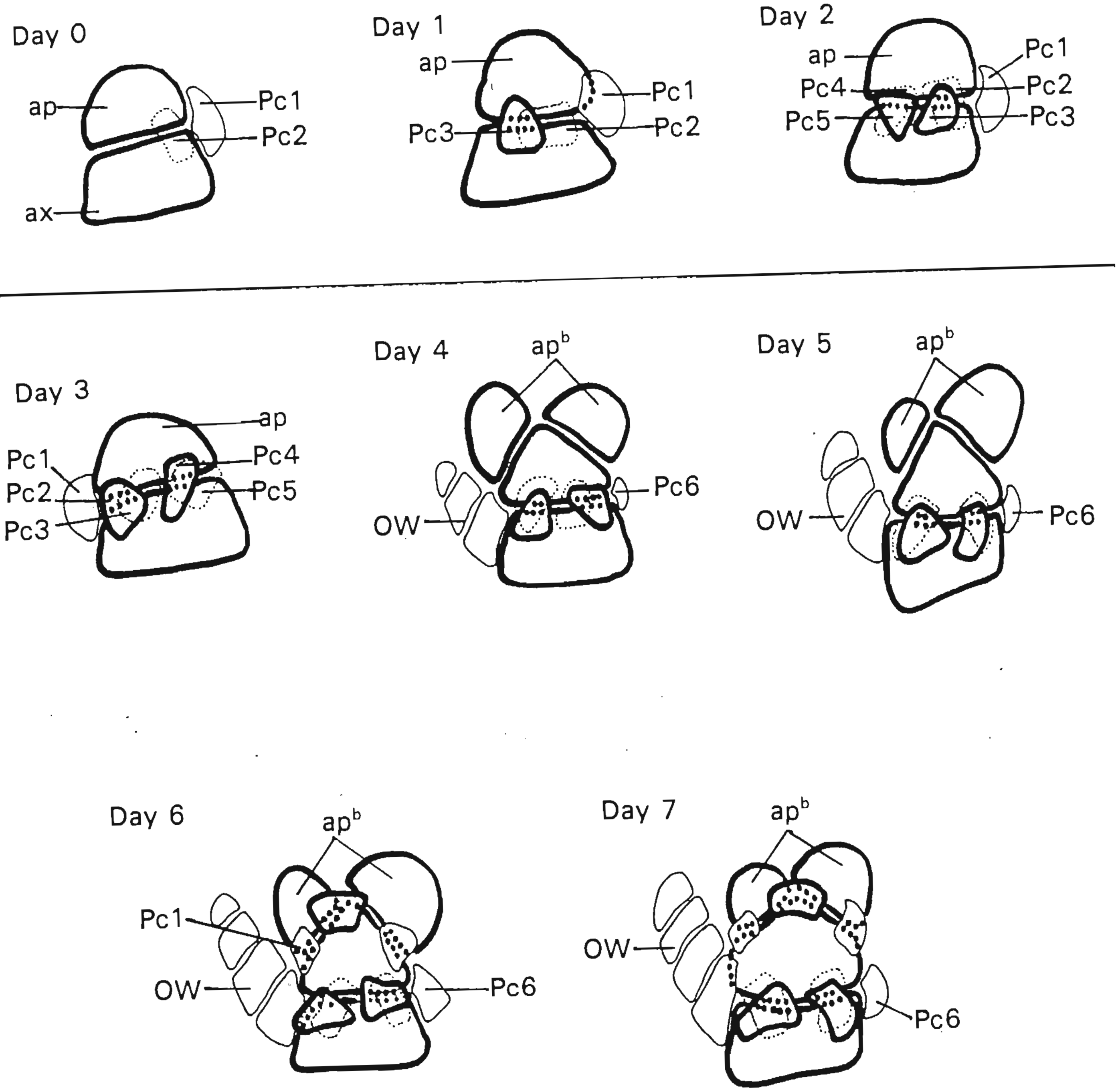


Fig. 6.3B Diagram illustrating the development of the cortical band region in actively growing tips of *Pteroceramium* (Rep 2) when the direction of gravity is altered. ap = apical cell, ap^b = branching apical cell, ax = axial cell, Pc = pericentral cell, OW = outer wing, ——— = upper surface, ········ = lower surface, —— = plant turned over to change gravity.

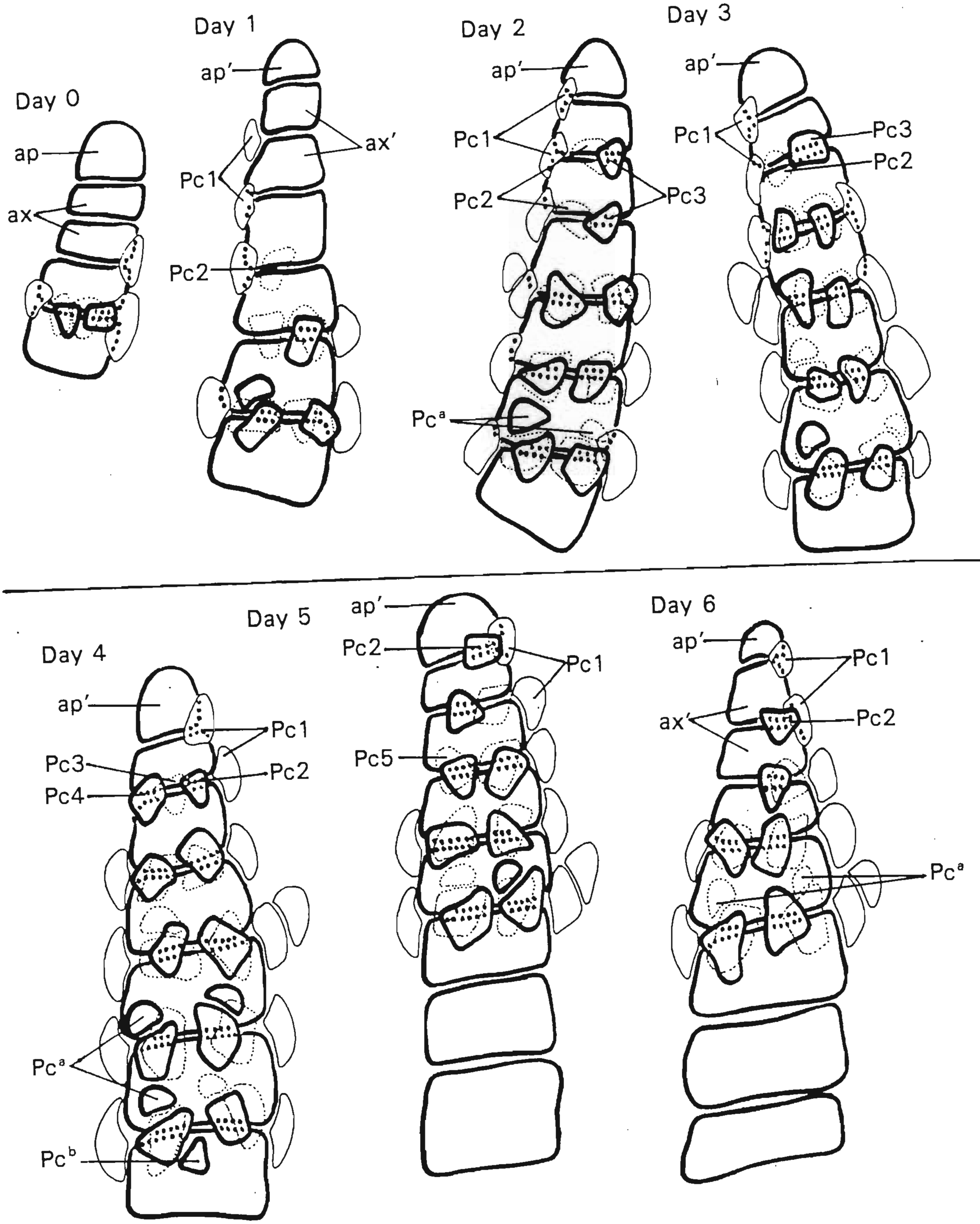


Fig. 6.3C Diagram illustrating the development of the cortical band region in actively growing tips of *Pteroceramium* (Rep 3) when the direction of gravity is altered. ap = apical cell, ap' = new apical cell, ax = axial cell, ax' = new axial cell, Pc = pericentral cell, Pc^a = apical derivative of a pericentral cell, Pc^b = basal derivative of a pericentral cell,—— = upper surface,......... = lower surface, —— = plant turned over to change gravity.

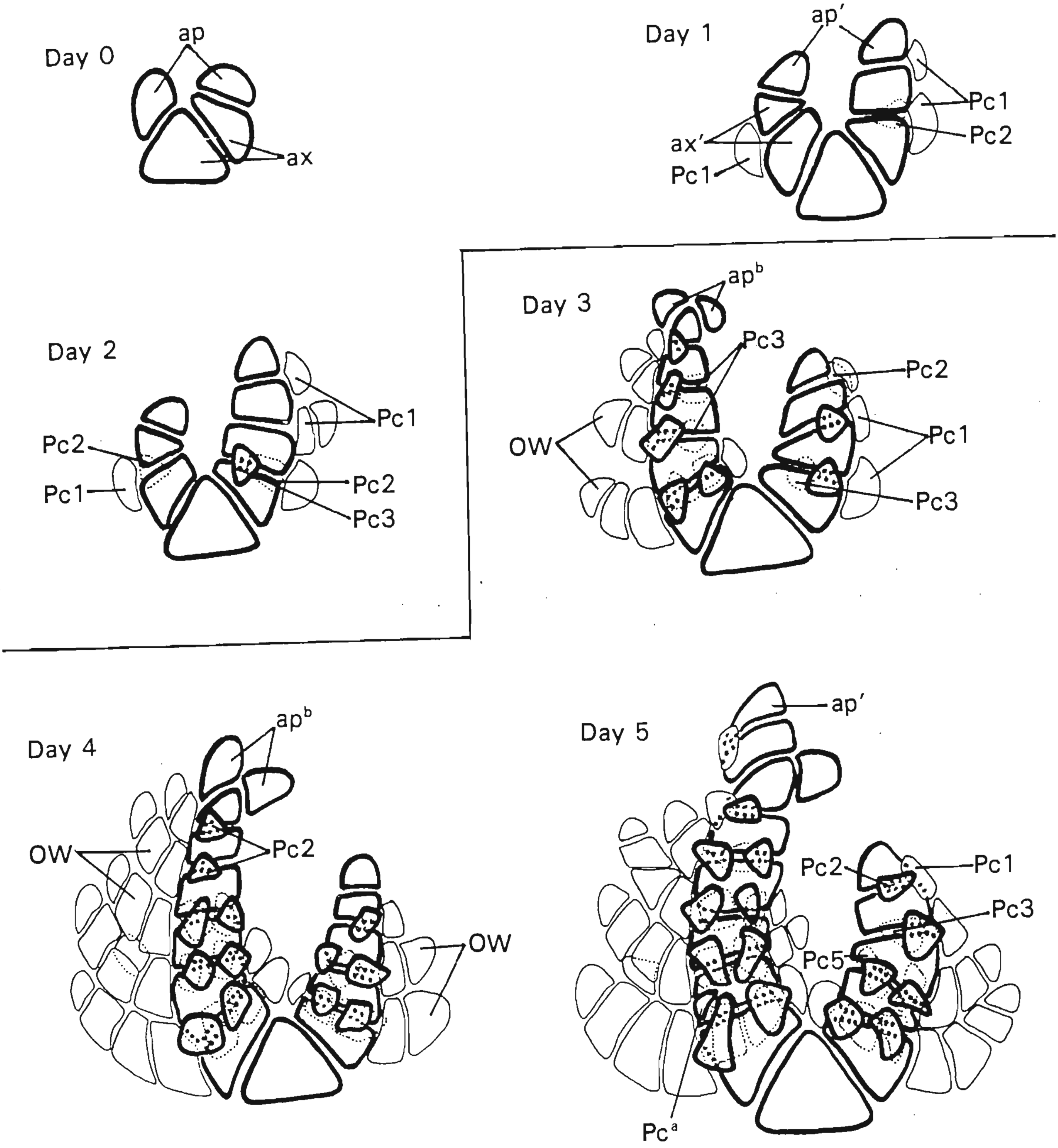
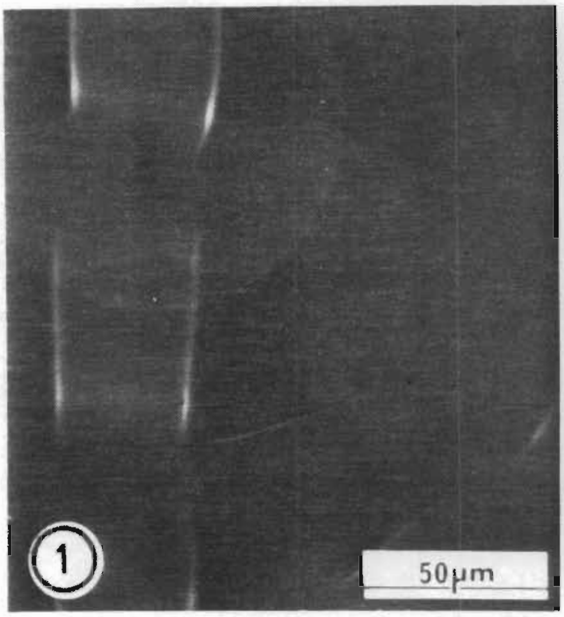
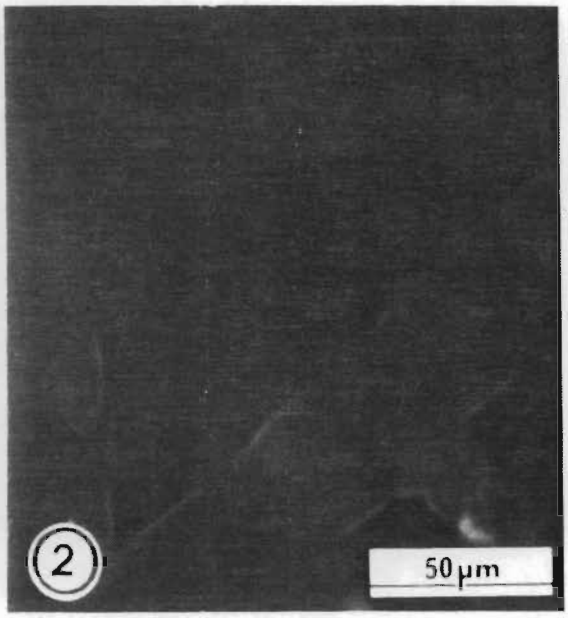


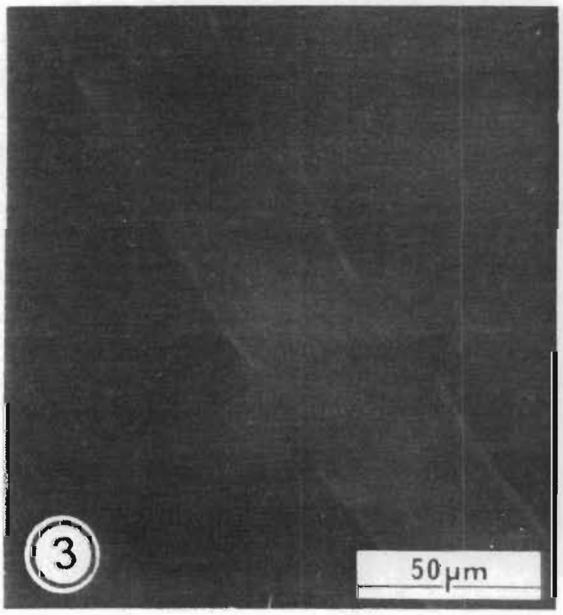
Fig. 6.3D Diagram illustrating the development of the cortical band region in actively growing tips of *Pteroceramium* (Rep 4) when the direction of gravity is altered. ap = apical cell, ap’ = new apical cell, ap^b = branching apical cell, ax = axial cell, ax’ = new axial cell, Pc = pericentral cell, OW = outer wing, Pc^a = apical derivative of a pericentral cell, ——— = upper surface, ········ = lower surface, —— = plant turned over to change gravity.



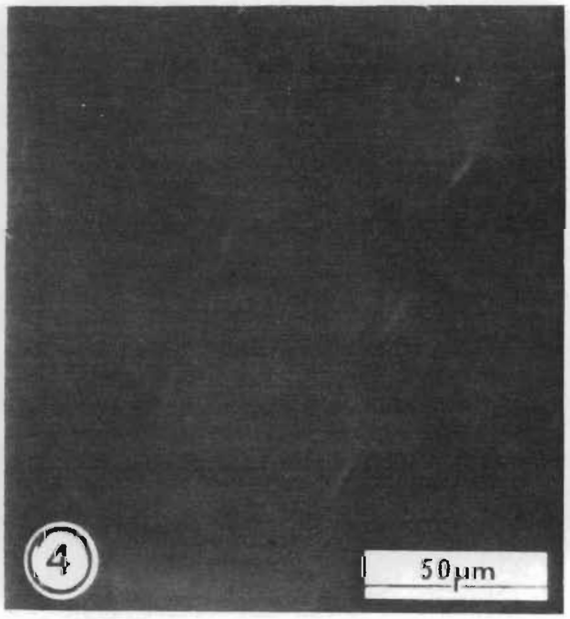
①



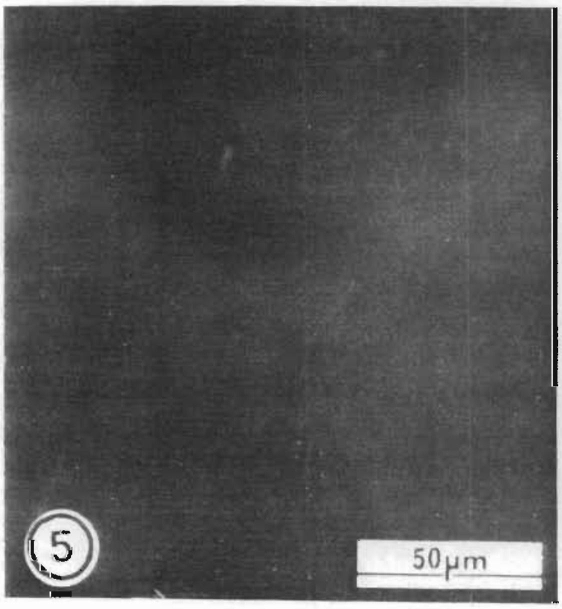
②



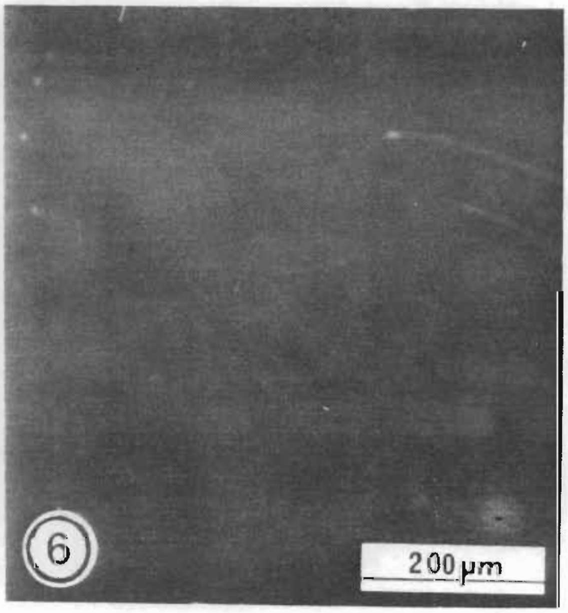
③



④



⑤



⑥

6.3.7 Effect of exogenous application of plant hormones on *Pteroceramium*

i) IAA - There were no significant differences between the RGRs when determined using both axial cell number and thallus length between any of the treatments (Fig. 6.4A and B; Table 6.2). Similarly, there were no significant differences between outer and inner wing surface area and cell number in seg. 4 along the dominant axis of *Pteroceramium* for any of the treatments. Axial cell volume was significantly different between control and 10^{-8} M replicates ($P<0.01$; Fig. 6.5; Table 6.2).

Table 6.2 Growth and morphometric results taken at seg. 4 of *Pteroceramium* when grown in different IAA concentrations. Results are shown as mean ± standard error. RGR = relative growth rate, ax cell = axial cell, vol = volume, OW = outer wing, IW = inner wing, SA = surface area.

	IAA concentration (M)					
Parameters measured	Control	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}
RGR (ax cells)	0.06 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01
RGR (length; μm)	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.07 ± 0.01
Ax cell vol ($\mu m^3 \times 10^3$)	87 ± 8	121 ± 16	197 ± 46	136 ± 12	133 ± 12	141 ± 20
OW cell no.	14 ± 1	15 ± 1	15 ± 1	15 ± 2	14 ± 2	11 ± 2
IW cell no.	9 ± 1	11 ± 1	8 ± 2	9 ± 2	9 ± 1	6 ± 1
OW SA ($\mu m^2 \times 10^3$)	1.2 ± 0.2	1.4 ± 0.2	1.4 ± 0.2	1.3 ± 0.2	1.4 ± 0.2	1.0 ± 0.2
IW SA ($\mu m^2 \times 10^3$)	0.7 ± 0.1	0.8 ± 0.1	0.7 ± 0.2	0.8 ± 0.2	0.9 ± 0.1	0.4 ± 0.1

ii) GA_3 - There were no significant differences between the RGRs for both axial cell number and thallus length between any of the treatments (Fig. 6.6A and B; Table 6.3). There was a significant difference in axial cell volume at seg. 4 ($P<0.01$), material growing in 10^{-5} M GA_3 having larger cells. Similarly, the outer and inner wings at

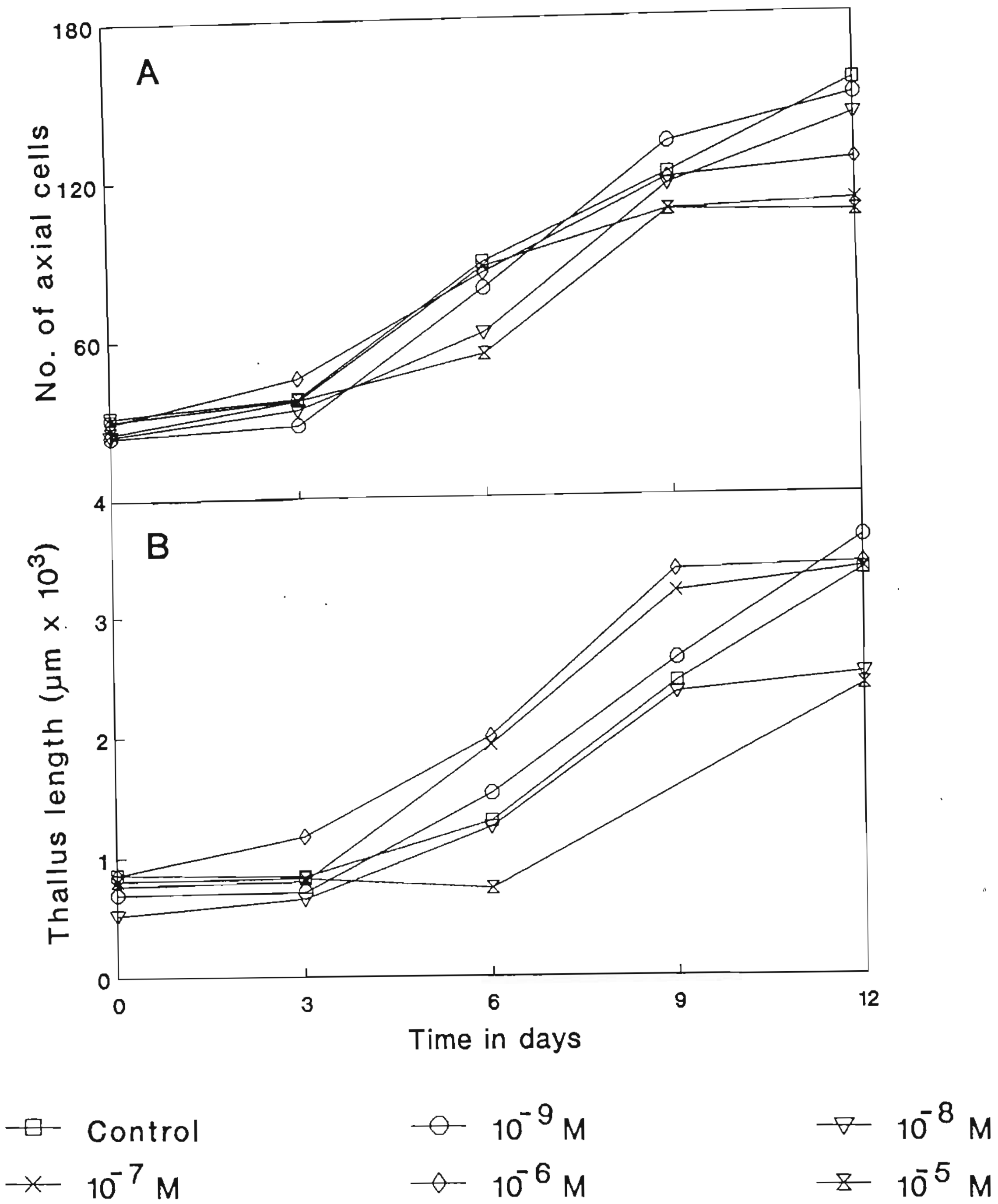


Fig. 6.4 Growth of *Pteroceramium* in varying concentrations of IAA. A) Change in number of axial cells. B) Change in thallus length.

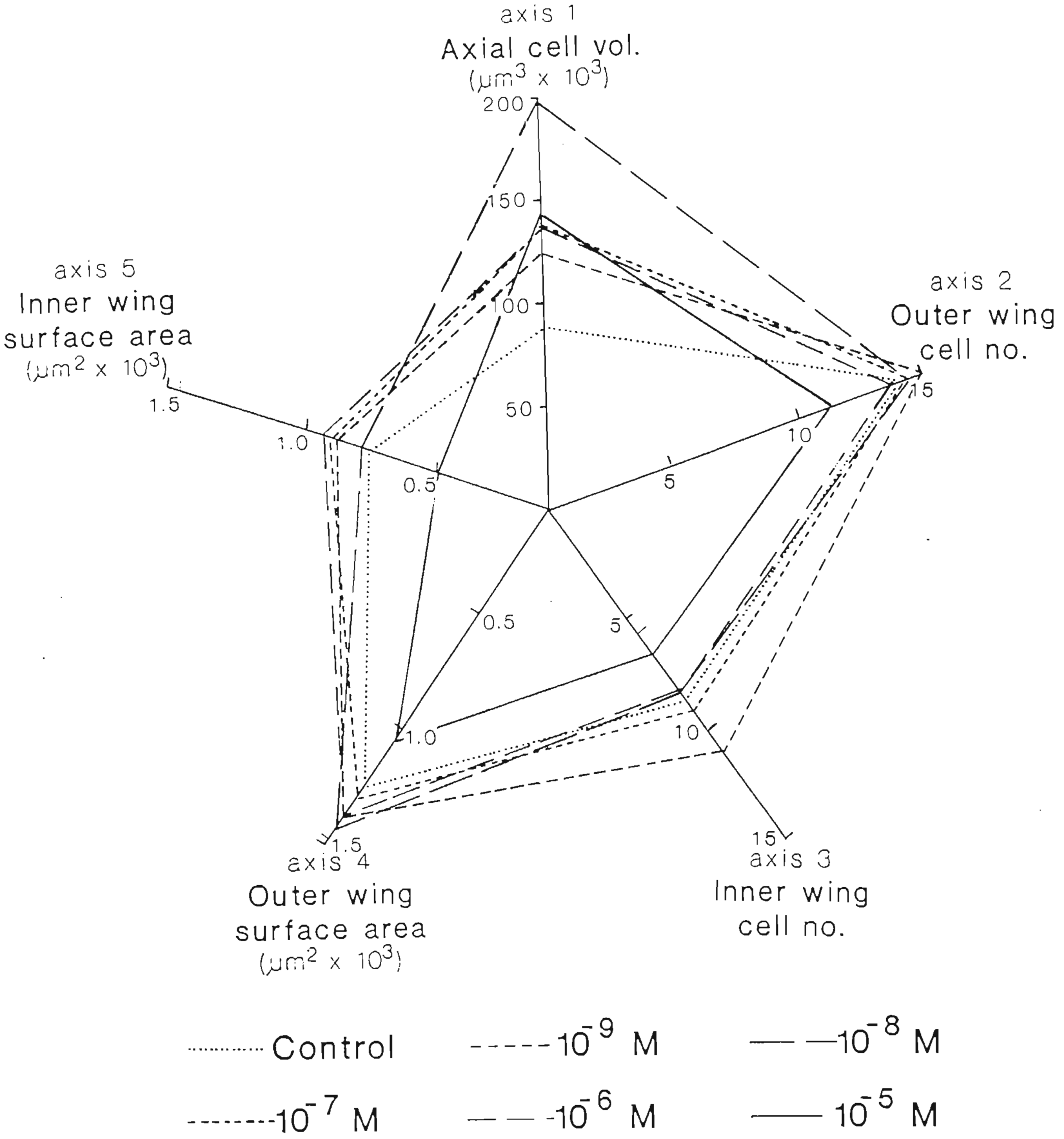


Fig. 6.5 Effect of IAA on axial cell volume (axis 1), wing cell number (axes 2 and 3) and wing surface area (axes 4 and 5) in *Pteroceramium*. Measurements were taken at seg. 4 along the dominant axis.

seg. 4 along the dominant axis had significantly more cells ($P < 0.001$) when grown in 10^{-5} M GA_3, although the wing surface areas were similar (Fig. 6.7; Table 6.3).

Table 6.3 Growth and morphometric results taken at seg. 4 of *Pteroceramium* when grown in different GA_3 concentrations. Results are shown as mean ± standard error. RGR = relative growth rate, ax cell = axial cell, vol = volume, OW = outer wing, IW = inner wing, SA = surface area.

	GA_3 concentration (M)					
Parameters measured	Control	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}
RGR (ax cells)	0.05 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.07 ± 0.01	0.09 ± 0.01
RGR (length; μm)	0.06 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.07 ± 0.01	0.08 ± 0.02
Ax cell vol ($\mu m^3 \times 10^3$)	54 ± 13	63 ± 4	60 ± 9	68 ± 7	84 ± 9	105 ± 13
OW cell no.	12 ± 2	13 ± 1	8 ± 1	11 ± 1	9 ± 1	15 ± 2
IW cell no.	7 ± 2	8 ± 1	5 ± 1	7 ± 1	6 ± 1	12 ± 2
OW SA ($\mu m^2 \times 10^3$)	1.3 ± 0.2	1.2 ± 0.1	0.8 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	1.4 ± 0.2
IW SA ($\mu m^2 \times 10^3$)	0.5 ± 0.1	0.7 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	1.0 ± 0.2

iii) Kinetin - There were no significant differences between the RGRs when determined using both axial cell number and thallus length between any of the treatments (Fig. 6.8A and B; Table 6.4). Similarly, there were no significant differences between axial cell volume, outer and inner wing surface area and cell number in seg. 4 when *Pteroceramium* was grown in different kinetin treatments (Fig. 6.9; Table 6.4).

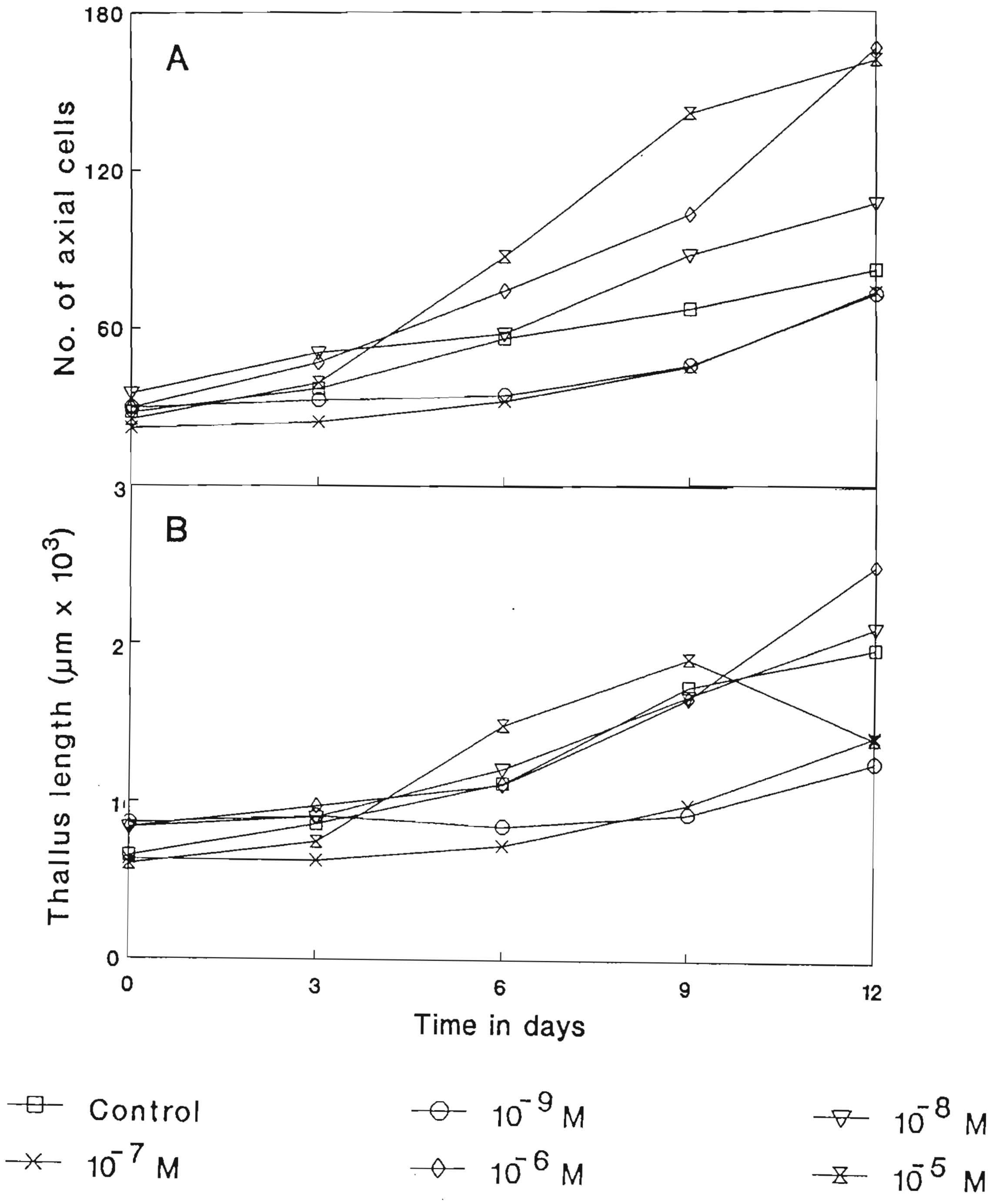


Fig. 6.6 Growth of *Pteroceramium* in varying concentrations of GA_3. A) Change in number of axial cells. B) Change in thallus length.

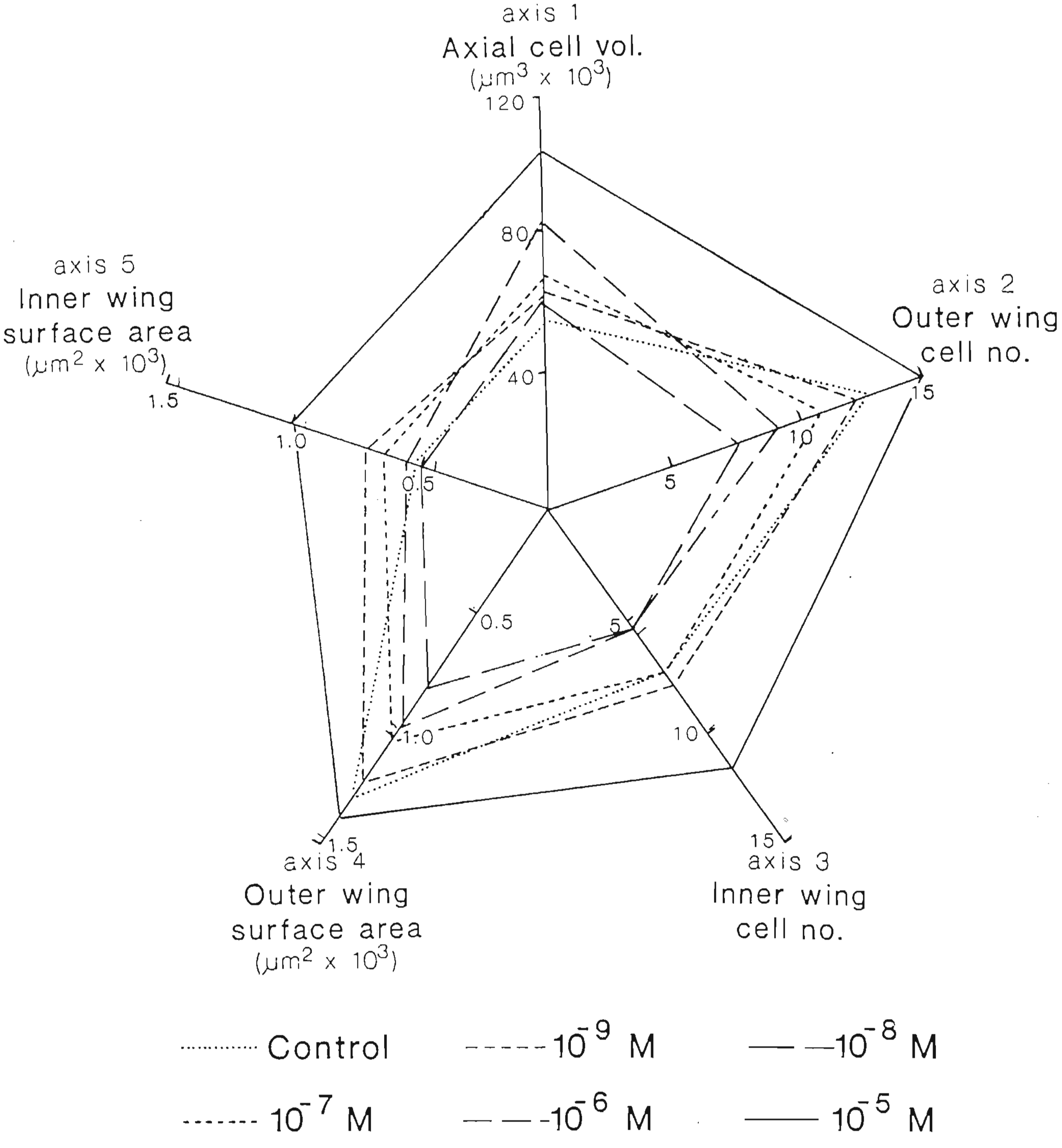


Fig. 6.7 Effect of GA_3 on axial cell volume (axis 1), wing cell number (axes 2 and 3) and wing surface area (axes 4 and 5) in *Pteroceramium*. Measurements were taken at seg. 4 along the dominant axis.

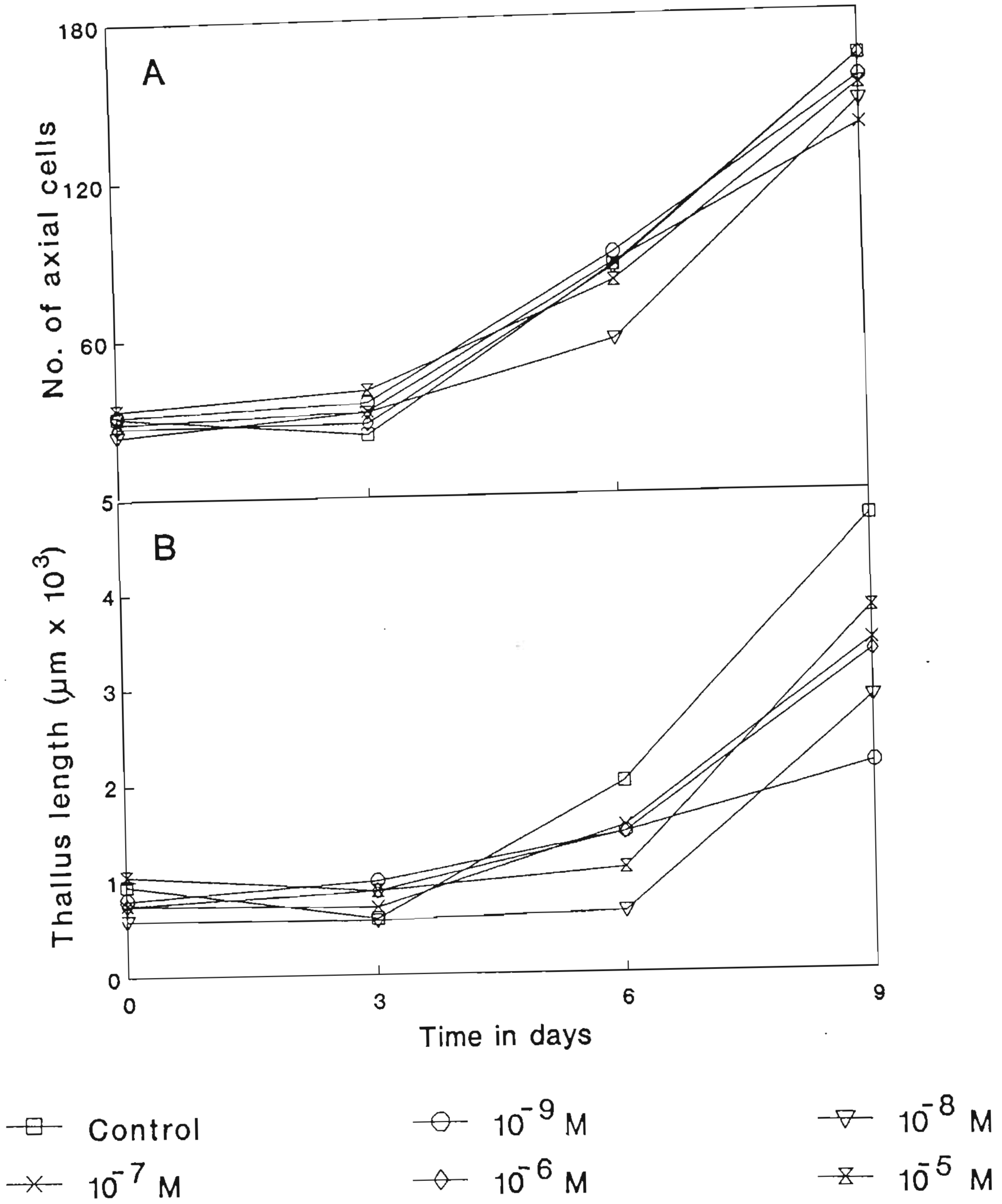


Fig. 6.8 Growth of *Pteroceramium* in varying concentrations of kinetin. A) Change in number of axial cells. B) Change in thallus length.

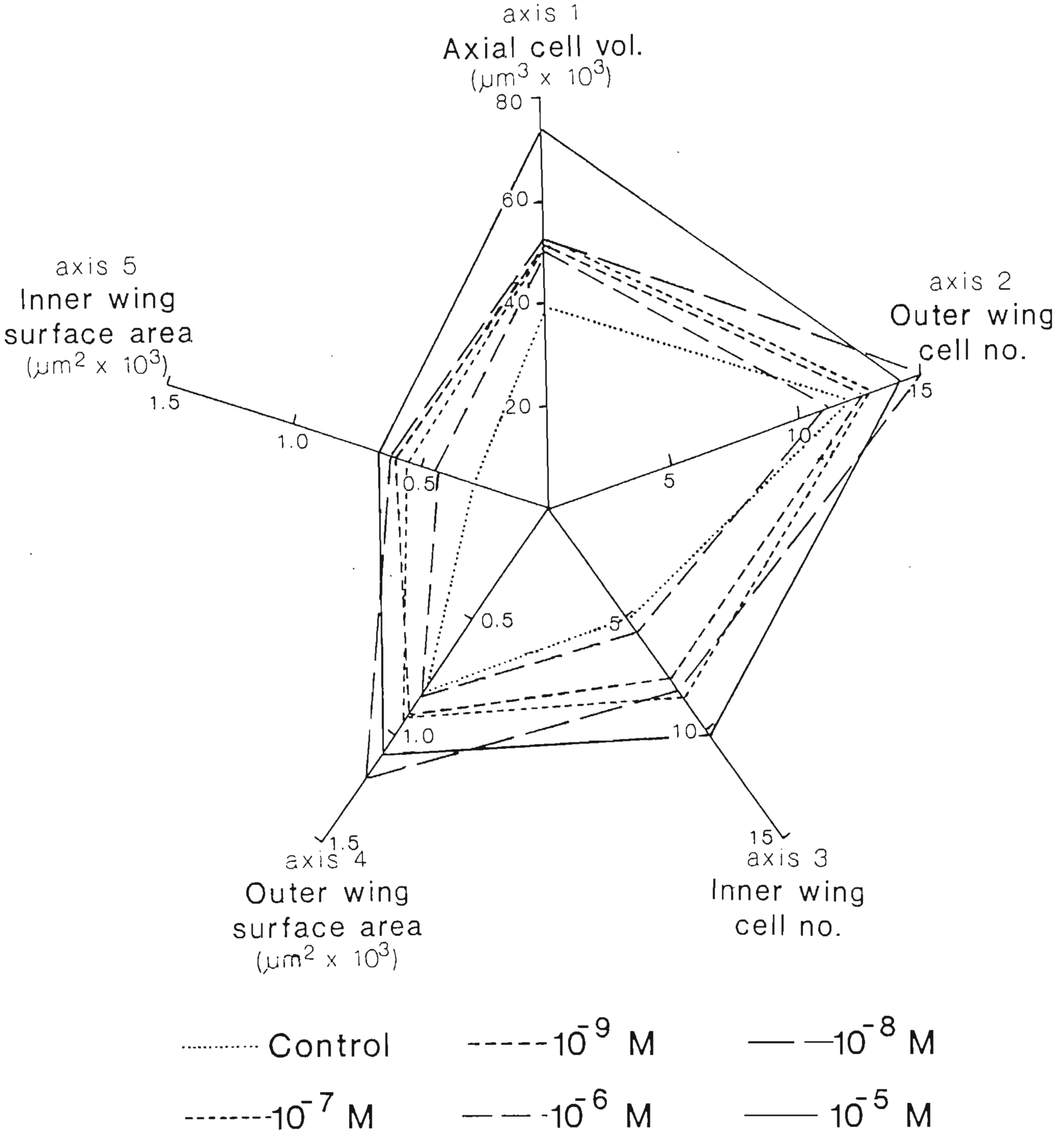


Fig. 6.9 Effect of kinetin on axial cell volume (axis 1), wing cell number (axes 2 and 3) and wing surface area (axes 4 and 5) in *Pteroceramium*. Measurements were taken at seg. 4 along the dominant axis.

Table 6.4 Growth and morphometric results taken at seg. 4 of *Pteroceramium* when grown in different kinetin concentrations. Results are shown as mean ± standard error. RGR = relative growth rate, ax cell = axial cell, vol = volume, OW = outer wing, IW = inner wing, SA = surface area.

Parameters measured	Kinetin concentration (M)					
	Control	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}
RGR (ax cells)	0.09 ± 0.01	0.08 ± 0.12	0.10 ± 0.01	0.10 ± 0.02	0.09 ± 0.01	0.08 ± 0.01
RGR (length; μm)	0.08 ± 0.004	0.06 ± 0.01	0.09 ± 0.01	0.09 ± 0.004	0.10 ± 0.01	0.08 ± 0.01
Ax cell vol (μm^3 x 10^3)	62 ± 14	-	101 ± 18	65 ± 6	82 ± 14	-
OW cell no.	10 ± 2	-	11 ± 2	10 ± 2	12 ± 4	-
IW cell. no.	8 ± 1	-	5 ± 1	7 ± 3	7 ± 2	-
OW SA (μm^2 x 10^3)	0.8 ± 0.2	-	0.8 ± 0.1	0.8 ± 0.2	1.1 ± 0.4	-
IW SA (μm^2 x 10^3)	0.5 ± 0.1	-	0.4 ± 0.2	0.5 ± 0.2	0.7 ± 0.2	-

6.3.8 Mung bean bioassay

The material placed in the phosphate buffer died after four hours and no roots were initiated. There were no significant differences in root initiation between the other treatments (Table 6.5).

6.3.9 Effect of exogenous application of polyamines, their precursors and inhibitors on *Pteroceramium*

i) Arginine - There was a significant difference ($P<0.0001$) in the RGRs of plants using both axial cell number and thallus length as criteria for growth, with the treatments 10^{-5} M and 10^{-6} M arginine showing the fastest growth compared to the

Table 6.5 Number of roots initiated by mung beans following application of *Pteroceramium* extract. Results are shown as mean ± standard error.

Treatment	No. of roots
Control	9.1 ± 1.9
PO_4 -30 ml extract	0 ± 0
PO_4 - 15 ml extract	0 ± 0
PO_4 - 5 ml extract	0 ± 0
pH 8 - 30 ml extract	11.4 ± 1.7
pH 8 - 15 ml extract	9.3 ± 1.4
pH 8 - 5 ml extract	10.4 ± 2.0
pH 3 - 30 ml extract	10.3 ± 1.3
pH 3 - 15 ml extract	7.1 ± 1.6
pH 3 - 5 ml extract	7.3 ± 1.6

control replicates. With increasing arginine concentrations, there was a decrease in the RGRs until at 10^{-3} M and 10^{-2} M there were negative growth rates (Fig. 6.10A and B; Table 6.6).

Morphometric measurements could not be carried out on the replicates growing in the three highest concentrations of arginine as there was insufficient new growth. Material growing at 10^{-5} M and the control had significantly larger ($P<0.0001$) axial cells at seg. 4 of the dominant axis than the other treatments. The outer ($P<0.01$) and inner ($P<0.0001$) wings of the control replicates had significantly more cells at seg. 4 than the other treatments. However, the surface areas were similar for the control, 10^{-5} M and 10^{-6} M arginine, and significantly smaller ($P<0.01$) at 10^{-4} M (Fig. 6.11; Table 6.6).

ii) Ornithine - The RGRs using both axial cell number and thallus length as a measure of growth, for replicates growing in 10^{-6} M and 10^{-5} M ornithine were similar to the control. There was a significant decrease in the RGRs for replicates growing in higher concentrations (Fig. 6.12A and B; Table 6.7).

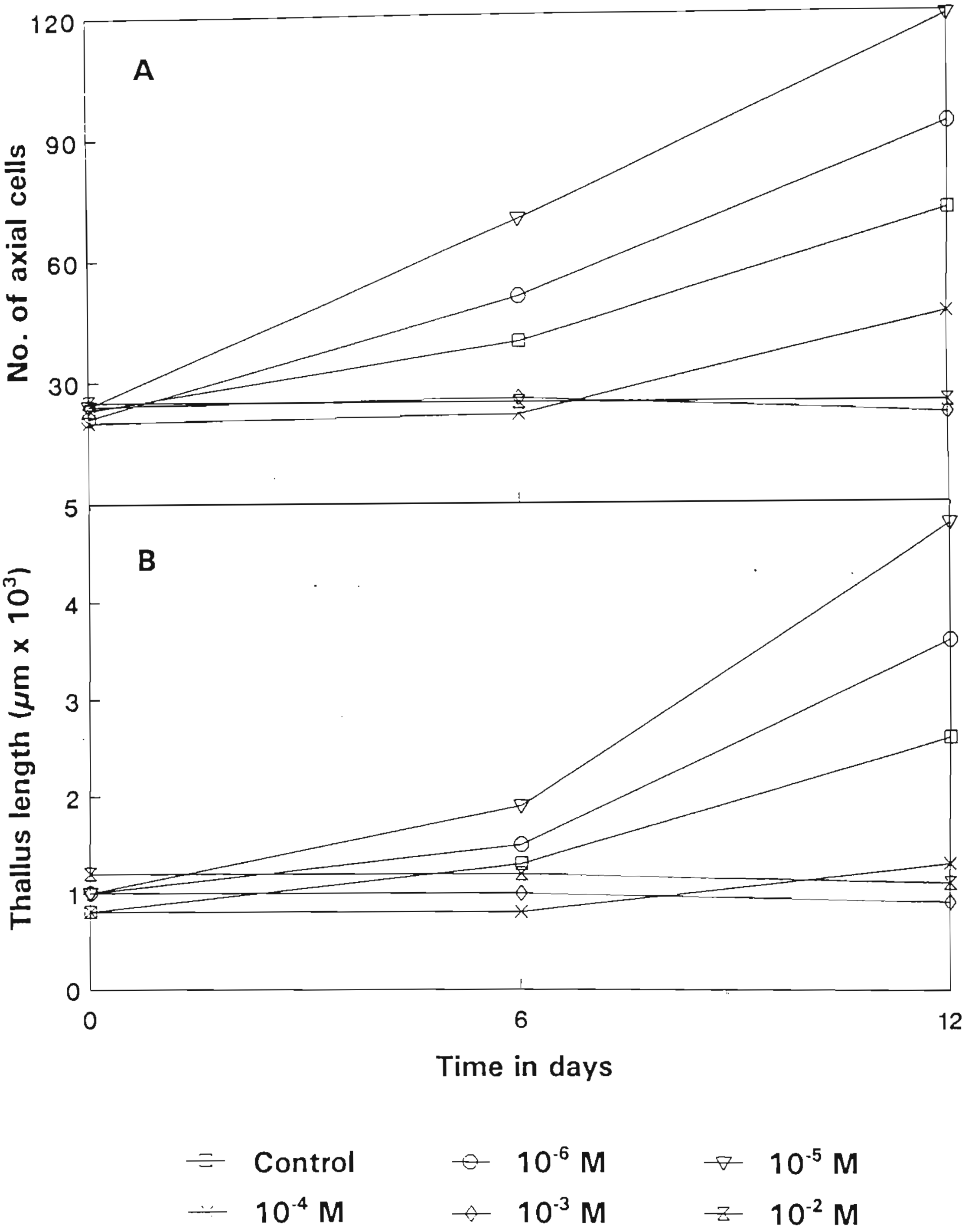


Fig. 6.10 Growth of *Pteroceramium* in varying concentrations of arginine. A) Change in number of axial cells. B) Change in thallus length.

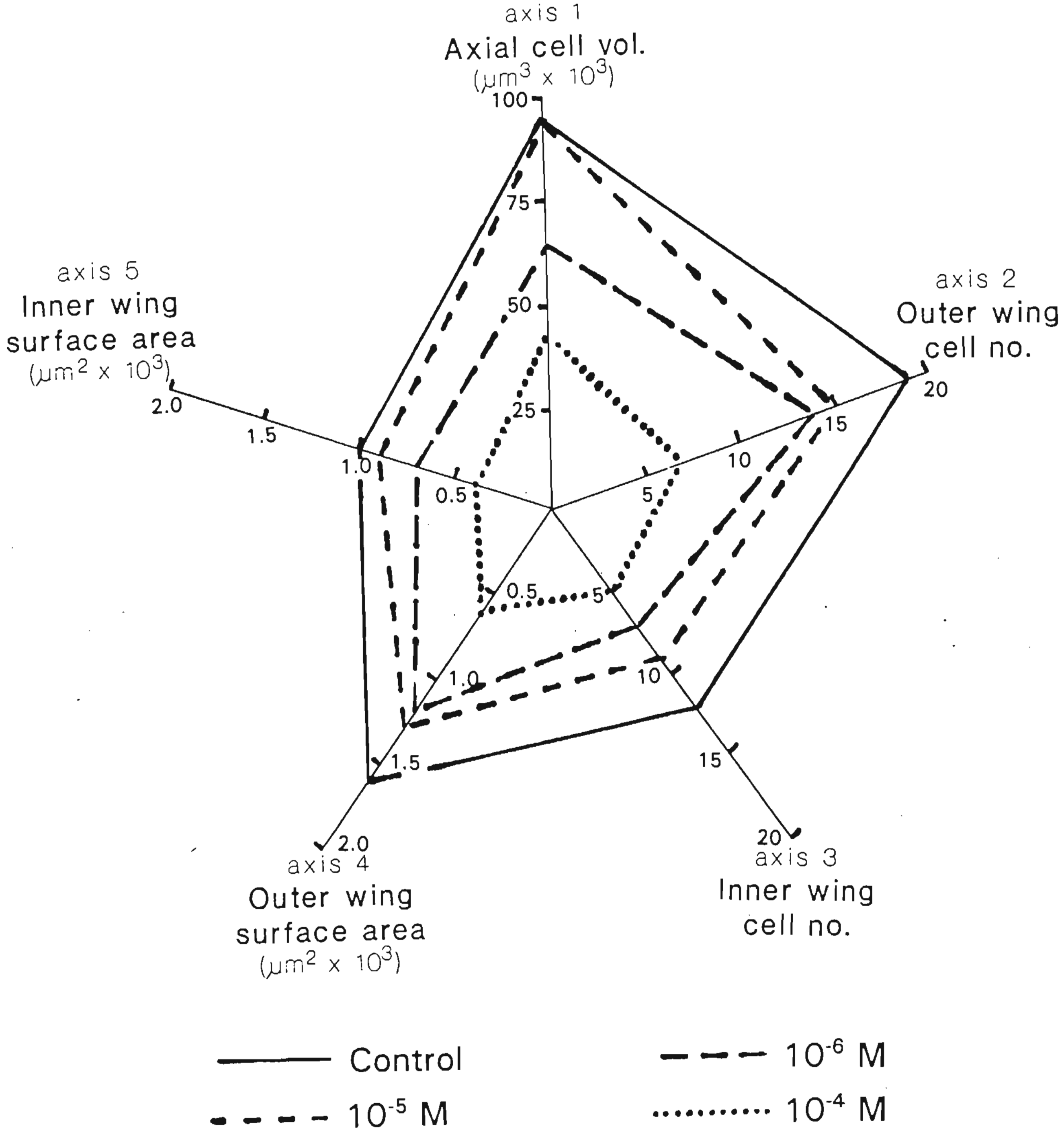


Fig. 6.11 Effect of arginine on axial cell volume (axis 1), wing cell number (axes 2 and 3) and wing surface area (axes 4 and 5) in *Pteroceramium*. Measurements were taken at seg. 4 along the dominant axis.

Table 6.6 Growth and morphometric results taken at seg. 4 of *Pteroceramium* when grown in different arginine concentrations. Results are shown as mean ± standard error. RGR = relative growth rate, ax cell = axial cell, vol = volume, OW = outer wing, IW = inner wing, SA = surface area.

	Arginine concentration (M)					
Parameters measured	Control	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}
RGR (ax cells)	0.04 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.02 ± 0.01	-0.01 ± 0.002	-0.001 ± 0.003
RGR (length; μm)	0.04 ± 0.01	0.04 ± 0.01	0.06 ± 0.004	0.02 ± 0.01	-0.002 ± 0.001	-0.007 ± 0.01
Ax cell vol (μm^3 x 10^3)	95 ± 6	64 ± 5	93 ± 7	37 ± 10	-	-
OW cell no.	19 ± 1	14 ± 1	15 ± 2	7 ± 3	-	-
IW cell no.	12 ± 1	7 ± 1	9 ± 1	4 ± 1	-	-
OW SA (μm^2 x 10^3)	1.6 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	0.6 ± 0.2	-	-
IW SA (μm^2 x 10^3)	1.0 ± 0.1	0.7 ± 0.1	0.9 ± 0.1	0.4 ± 0.1	-	-

Morphometric measurements were not taken for material grown at 10^{-3} M as there was insufficient new growth. The axial cells were significantly larger at seg. 4 ($P<0.0001$) in replicates grown in 10^{-6} M and much smaller in the 10^{-4} M treatment. Both the outer and inner wings showed a significant decrease ($P<0.0001$) in cell number at seg. 4 along the dominant axis compared to the control, those growing at 10^{-4} M ornithine being much smaller. The control and replicates grown at 10^{-6} M had similar wing surface areas and there was a significant decrease in surface area ($P<0001$) for replicates grown in higher concentrations (Fig. 6.13; Table 6.7).

iii) Agmatine - The RGRs, using both axial cell number and thallus length, were similar to the control when material was grown in 10^{-6} M and 10^{-5} M agmatine. Increasingly

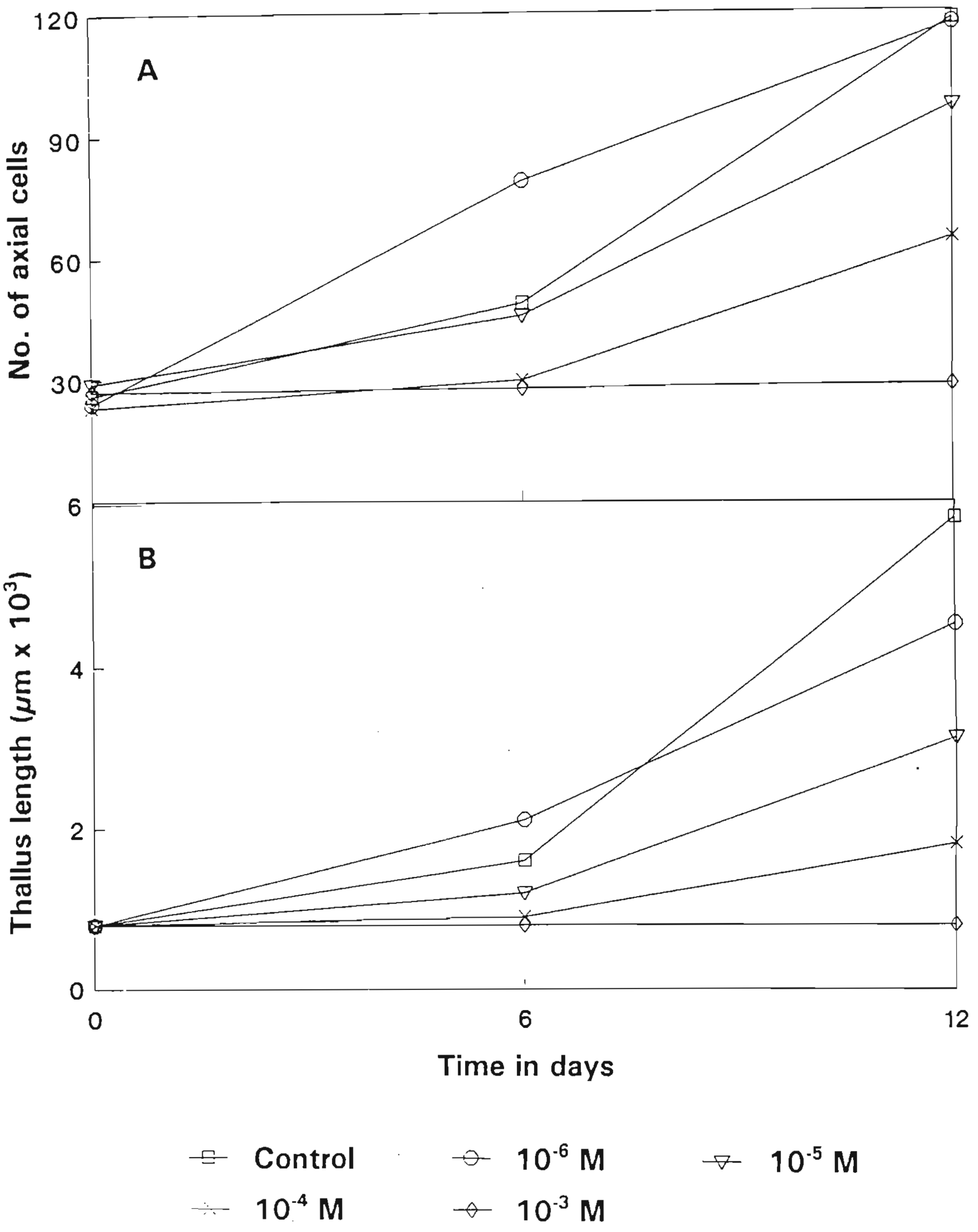


Fig. 6.12 Growth of *Pteroceramium* in varying concentrations of ornithine. A) Change in number of axial cells. B) Change in thallus length.

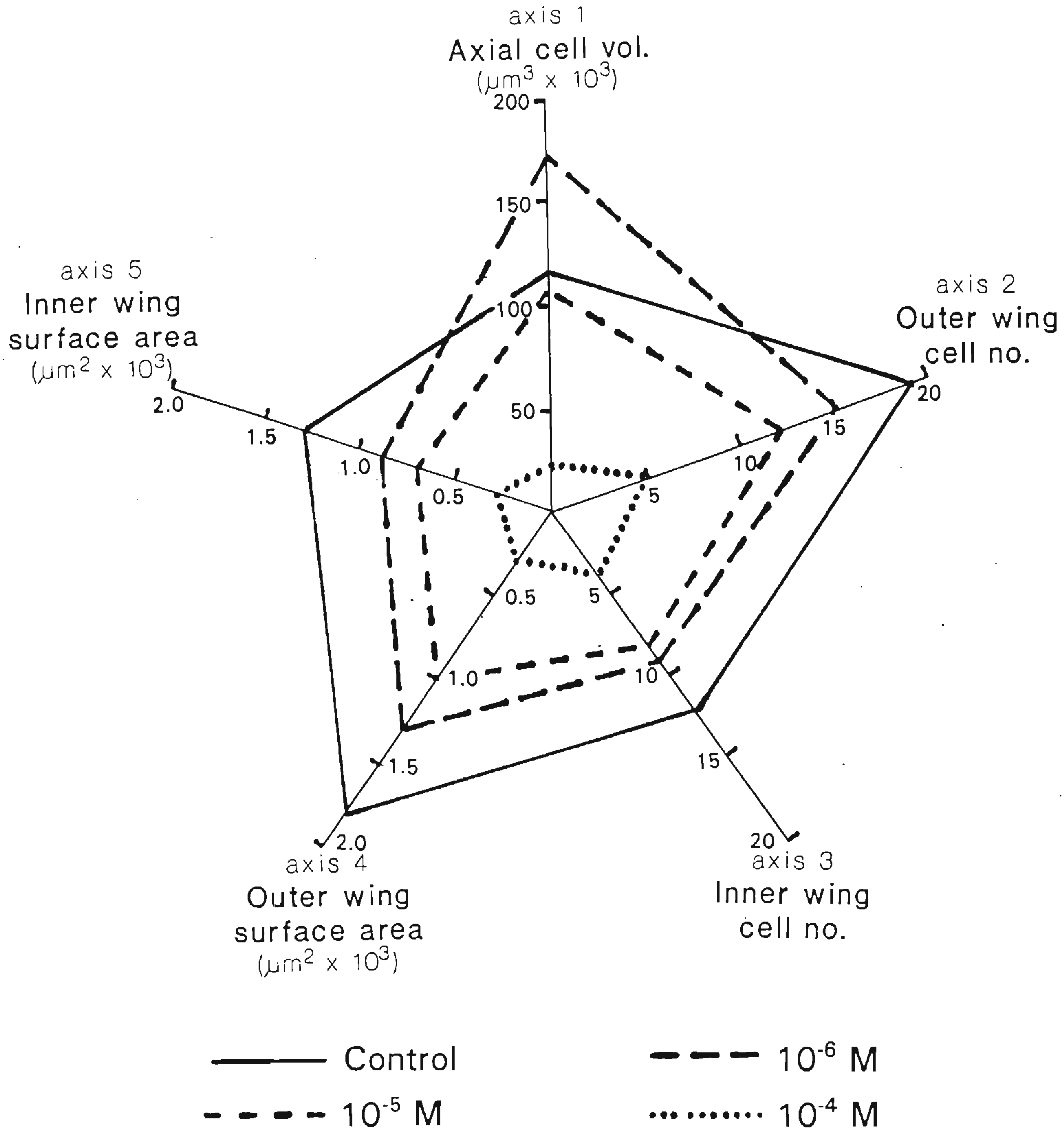


Fig. 6.13 Effect of ornithine on axial cell volume (axis 1), wing cell number (axes 2 and 3) and wing surface area (axes 4 and 5) in *Pteroceramium*. Measurements were taken at seg. 4 along the dominant axis.

Table 6.7 Growth and morphometric results taken at seg. 4 of *Pteroceramium* when grown in different ornithine concentrations. Results are shown as mean ± standard error. RGR = relative growth rate, ax cell = axial cell, vol = volume, OW = outer wing, IW = inner wing, SA = surface area.

Parameters measured	Ornithine concentration (M)				
	Control	10^{-6}	10^{-5}	10^{-4}	10^{-3}
RGR (ax cells)	0.06 ± 0.01	0.07 ± 0.02	0.05 ± 0.01	0.03 ± 0.01	0.002 ± 0.001
RGR (length; μm)	0.06 ± 0.01	0.07 ± 0.01	0.05 ± 0.01	0.03 ± 0.01	-0.0003 ± 0.001
Ax cell vol (μm^3 x 10^3)	116 ± 6	170 ± 14	105 ± 9	23 ± 4	-
OW cell no.	19 ± 1	15 ± 1	12 ± 1	5 ± 1	-
IW cell no.	12 ± 1	9 ± 1	8 ± 1	4 ± 1	-
OW SA (μm^2 x 10^3)	1.8 ± 0.1	1.3 ± 0.2	1.0 ± 0.2	0.3 ± 0.1	-
IW SA (μm^2 x 10^3)	1.3 ± 0.1	0.9 ± 0.1	0.7 ± 0.1	0.3 ± 0.1	-

higher concentrations of agmatine resulted a significant decrease ($P<0.0001$) in the RGRs (Fig. 6.14A and B; Table 6.8).

Morphometric measurements were not taken for material grown in the two highest agmatine concentrations as there was insufficient new growth. Axial cells were significantly smaller at seg. 4 along the dominant axis ($P<0.0001$) when grown in 10^{-4} M agmatine. Similarly, both the outer ($P<0.01$) and inner ($P<0.0001$) wings had significantly fewer cells and a smaller surface area at this high concentration (Fig. 6.15; Table 6.8).

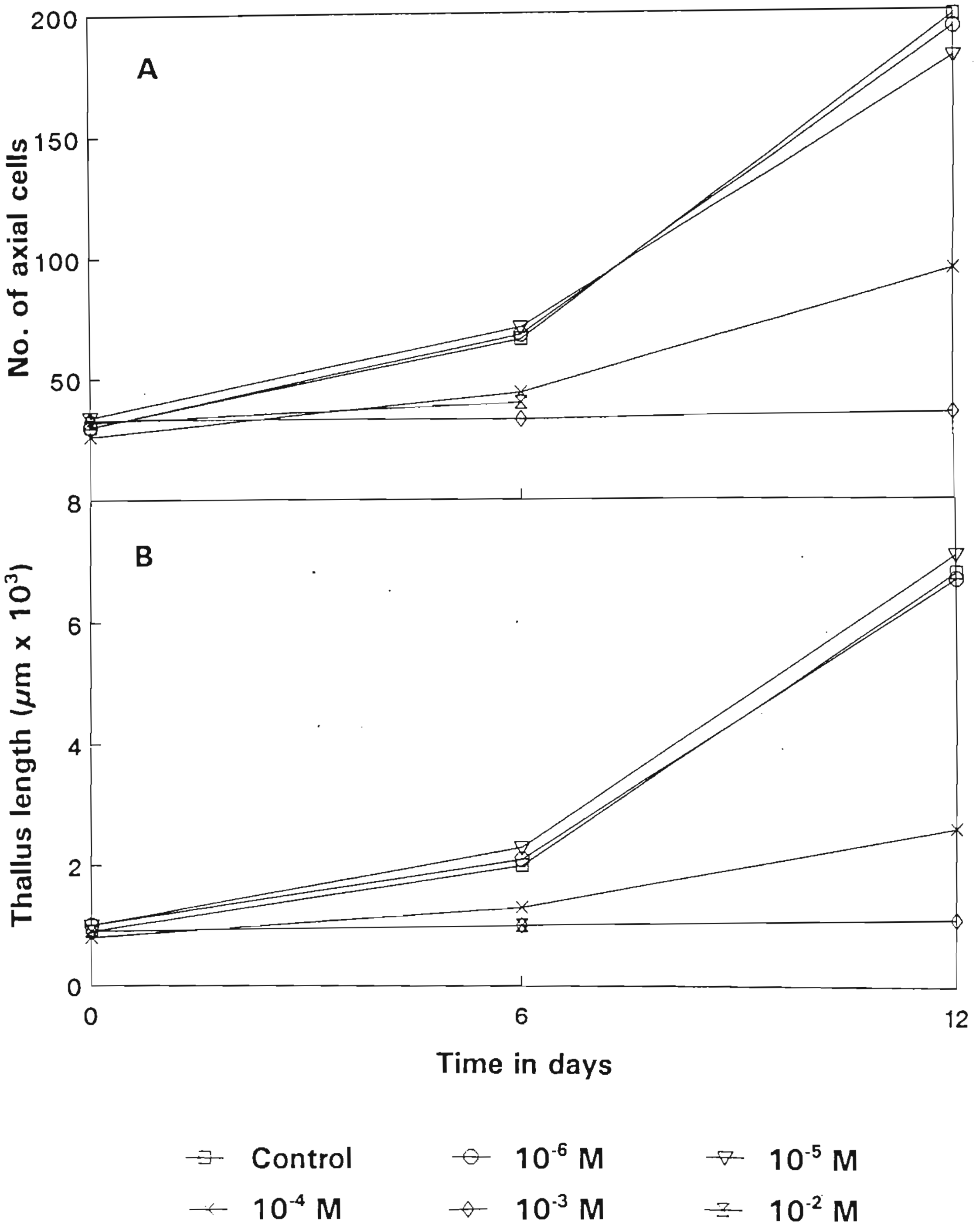


Fig. 6.14 Growth of *Pteroceramium* in varying concentrations of agmatine. A) Change in number of axial cells. B) Change in thallus length.

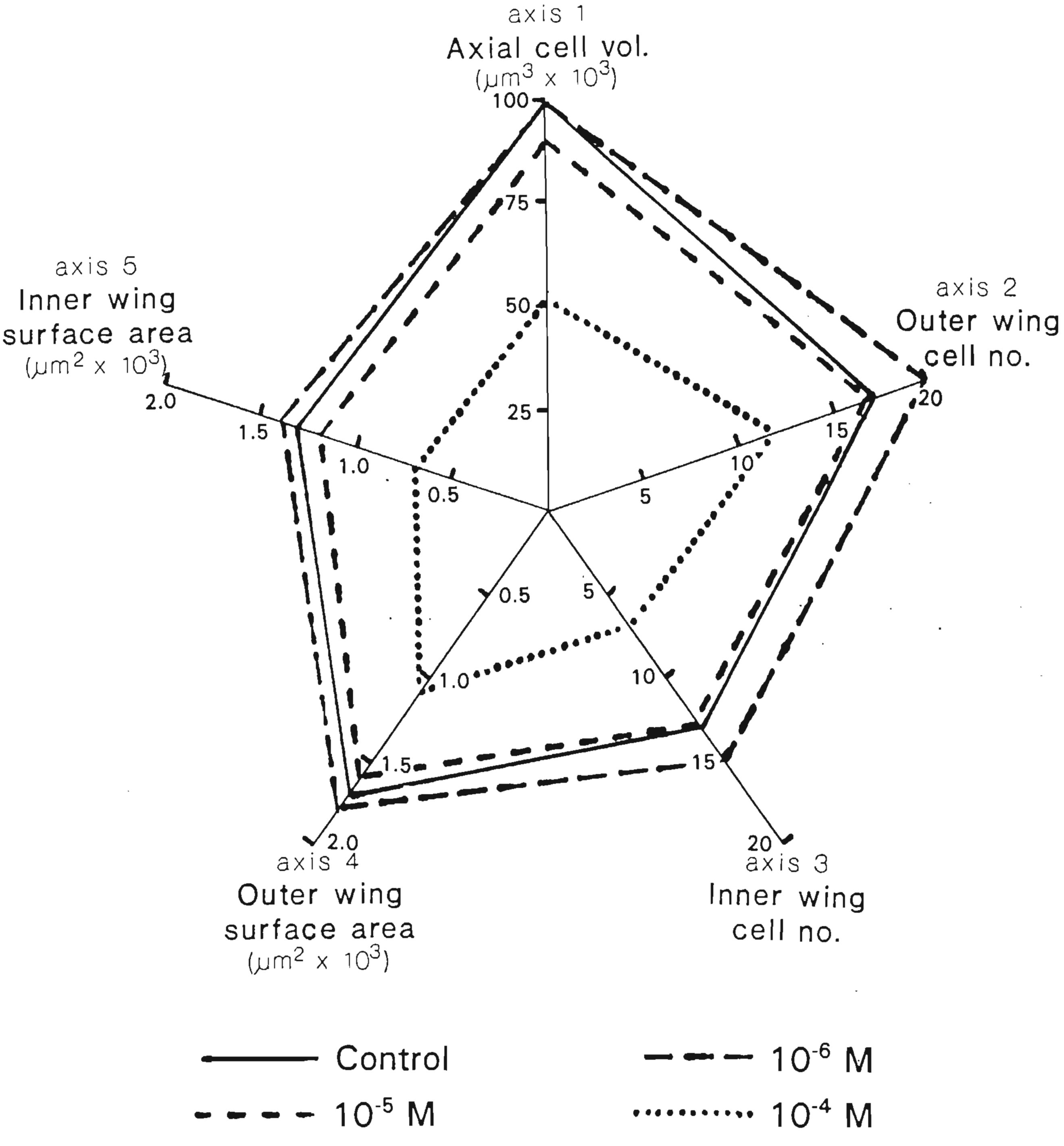


Fig. 6.15 Effect of agmatine on axial cell volume (axis 1), wing cell number (axes 2 and 3) and wing surface area (axes 4 and 5) in *Pteroceramium*. Measurements were taken at seg. 4 along the dominant axis.

Table 6.8 Growth and morphometric results taken at seg. 4 of *Pteroceramium* when grown in different agmatine concentrations. Results are shown as mean ± standard error. RGR = relative growth rate, ax cell = axial cell, vol = volume, OW = outer wing, IW = inner wing, SA = surface area.

Parameters measured	Agmatine concentration (M)					
	Control	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}
RGR (ax cells)	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.002 ± 0.003	0.01 ± 0.01
RGR (length; μm)	0.07 ± 0.01	0.07 ± 0.003	0.08 ± 0.01	0.05 ± 0.01	0.01 ± 0.002	0.004 ± 0.003
Ax cell vol (μm^3 x 10^3)	99 ± 8	98 ± 13	90 ± 7	51 ± 5	-	-
OW cell no.	17 ± 1	20 ± 2	17 ± 1	12 ± 1	-	-
IW cell no.	13 ± 1	15 ± 1	13 ± 1	7 ± 1	-	-
OW SA (μm^2 x 10^3)	1.7 ± 0.1	1.8 ± 0.2	1.6 ± 0.1	1.1 ± 0.1	-	-
IW SA (μm^2 x 10^3)	1.3 ± 0.1	1.4 ± 0.1	1.2 ± 0.1	0.7 ± 0.1	-	-

iv) Methionine - The RGR of replicates using both axial cell number and total thallus length, were significantly lower ($P<0.001$) when grown in the two highest methionine concentrations (10^{-4} M and 10^{-3} M). The RGRs for material at 10^{-5} M and 10^{-6} M were similar to the control (Fig. 6.16A and B; Table 6.9).

Morphometric measurements could not be taken for the material grown in the two highest concentrations as there was insufficient new growth. Axial cell volume at seg. 4 along the dominant axis was significantly greater ($P<0.05$) for the material growing in 10^{-6} M methionine while material growing at 10^{-5} M was similar to the control. The outer wing size (both cell number and surface area) was unaffected by methionine application. However, both the inner wing cell number and surface area were significantly larger ($P<0.05$) when grown in 10^{-6} M (Fig. 6.17; Table 6.9).

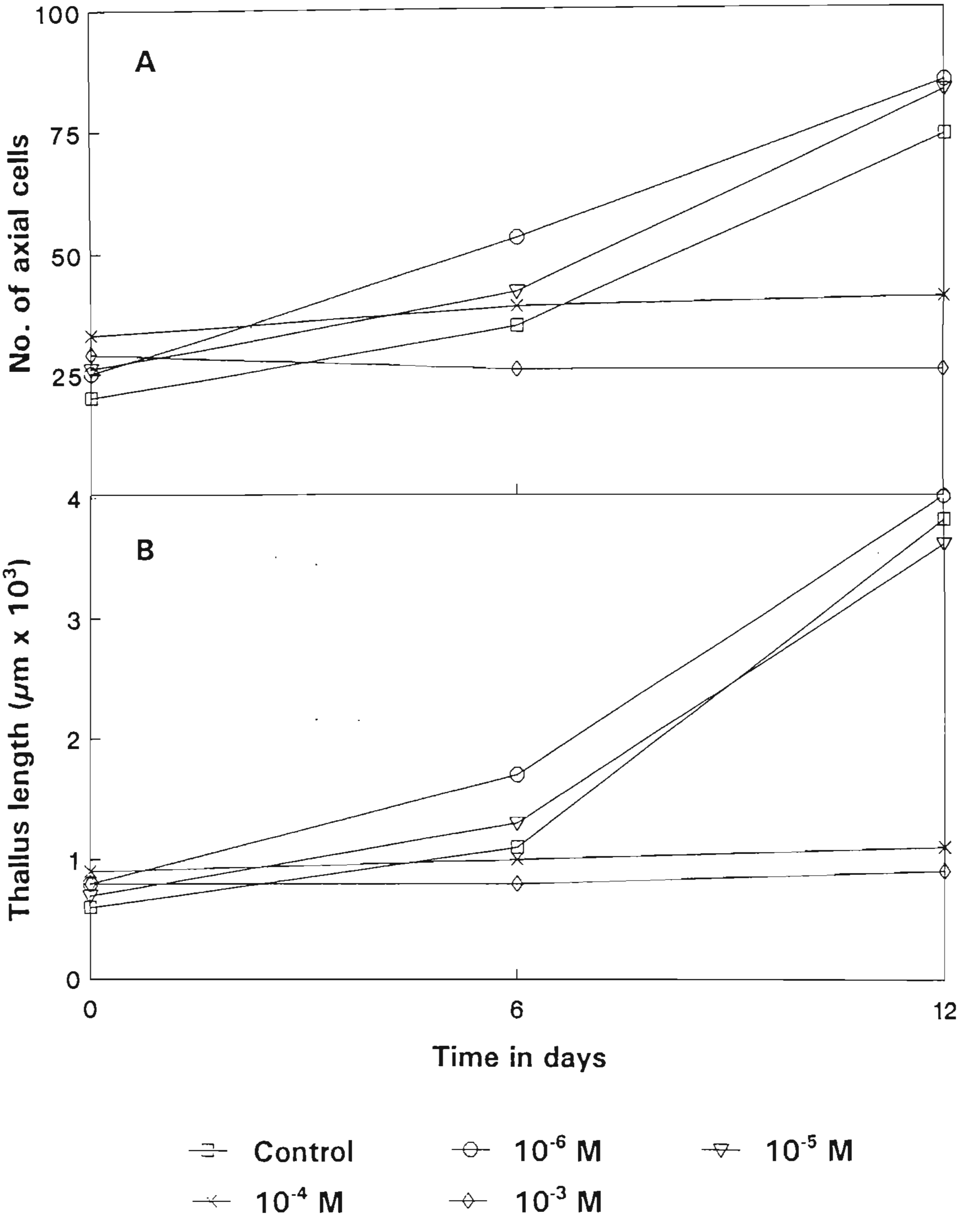


Fig. 6.16 Growth of *Pteroceramium* in varying concentrations of methionine. A) Change in number of axial cells. B) Change in thallus length.

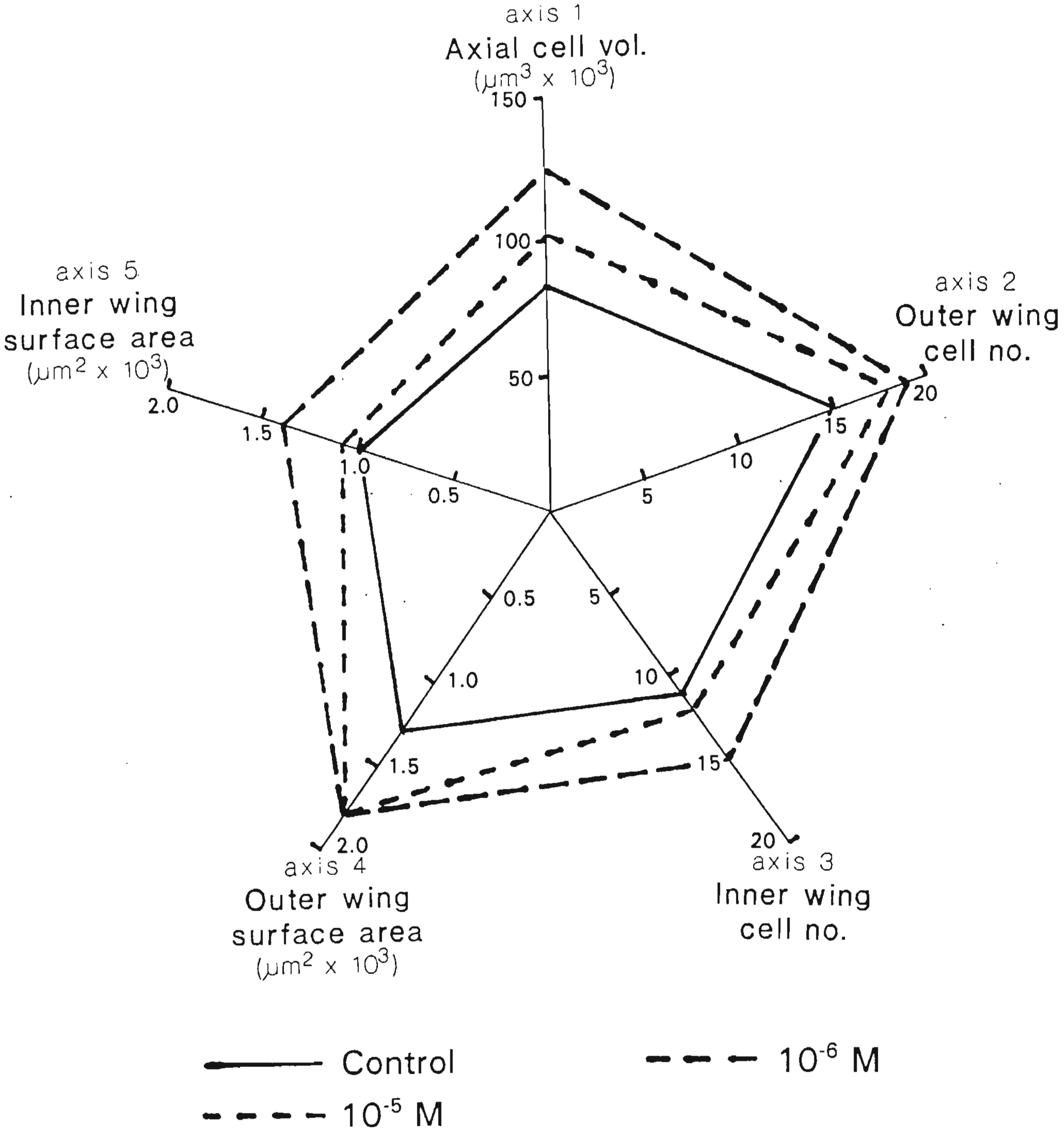


Fig. 6.17 Effect of methionine on axial cell volume (axis 1), wing cell number (axes 2 and 3) and wing surface area (axes 4 and 5) in *Pteroceramium*. Measurements were taken at seg. 4 along the dominant axis.

Table 6.9 Growth and morphometric results taken at seg. 4 of *Pteroceramium* when grown in different methionine concentrations. Results are shown as mean ± standard error. RGR = relative growth rate, ax cell = axial cell, vol = volume, OW = outer wing, IW = inner wing, SA = surface area.

Parameters measured	Methionine concentration (M)				
	Control	10^{-6}	10^{-5}	10^{-4}	10^{-3}
RGR (ax cells)	0.05 ± 0.004	0.05 ± 0.01	0.05 ± 0.01	0.01 ± 0.003	-0.004 ± 0.01
RGR (length; μm)	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.004	0.01 ± 0.002	0.0004 ± 0.003
Ax cell vol ($\mu m^3 \times 10^3$)	82 ± 10	124 ± 10	101 ± 15	-	-
OW cell no.	15 ± 2	19 ± 2	18 ± 2	-	-
IW cell no.	11 ± 1	15 ± 2	12 ± 2	-	-
OW SA ($\mu m^2 \times 10^3$)	1.3 ± 0.2	1.8 ± 0.2	1.8 ± 0.2	-	-
IW SA ($\mu m^2 \times 10^3$)	1.0 ± 0.1	1.4 ± 0.2	1.1 ± 0.2	-	-

v) Putrescine - The lowest concentrations of applied putrescine (10^{-6} M and 10^{-5} M) had no effect on the RGRs compared with the control. Increasingly higher concentrations of putrescine caused a significant decrease ($P<0.0001$) in the RGRs using both axial cell number and thallus length (Fig. 6.18A and B; Table 6.10).

Morphometric measurements were not taken for the material growing in the two highest putrescine concentrations as there was insufficient new growth. Material grown at 10^{-6} M had similar sized axial cells at seg. 4 to the control but increasingly higher concentrations of putrescine caused a significant decrease ($P<0.0001$) in size. Similarly, all the wing measurements at seg. 4 for the material grown in 10^{-4} M putrescine were significantly lower than the control replicates ($P<0.0001$; Fig. 6.19; Table 6.10).

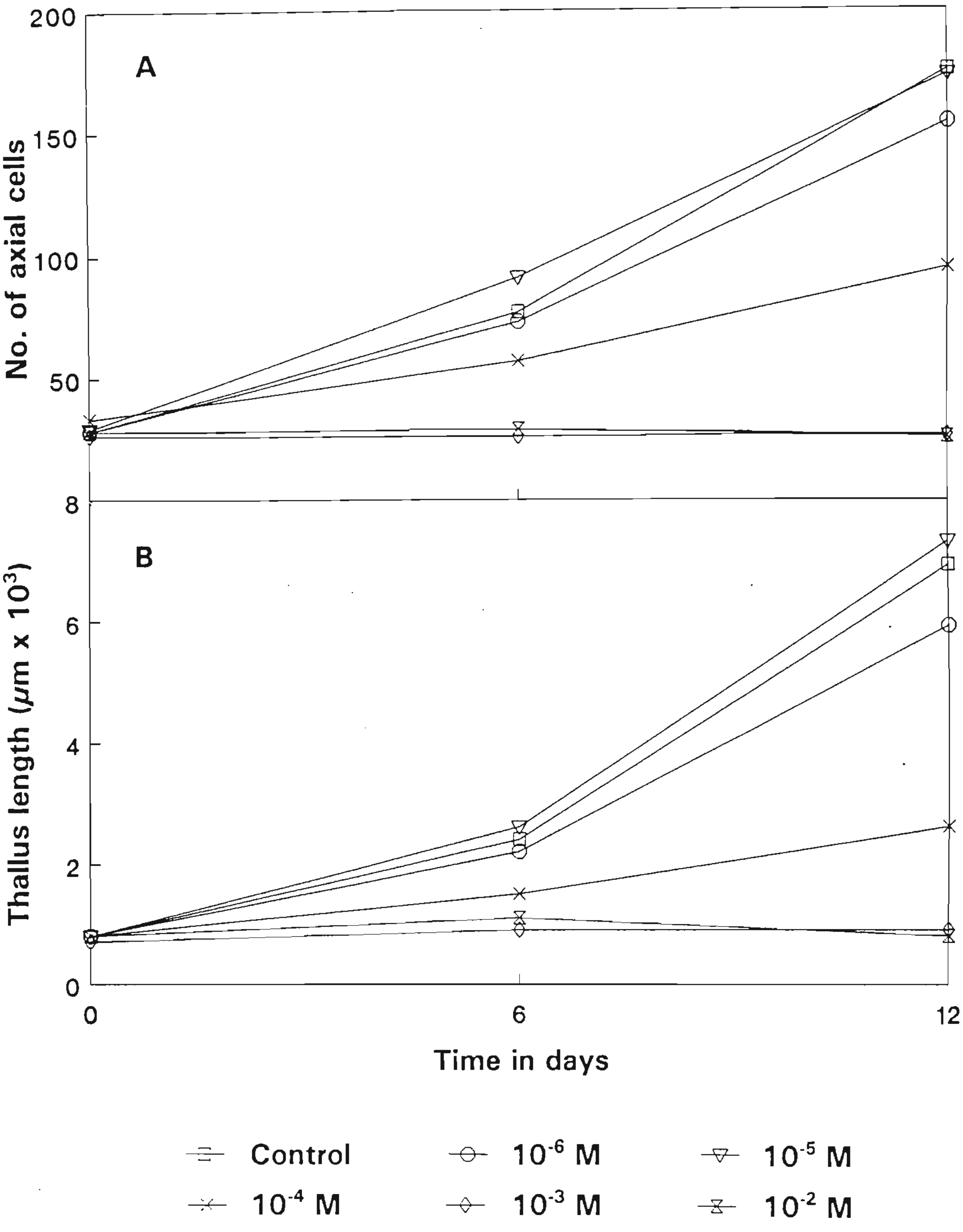


Fig. 6.18 Growth of *Pteroceramium* in varying concentrations of putrescine. A) Change in number of axial cells. B) Change in thallus length.

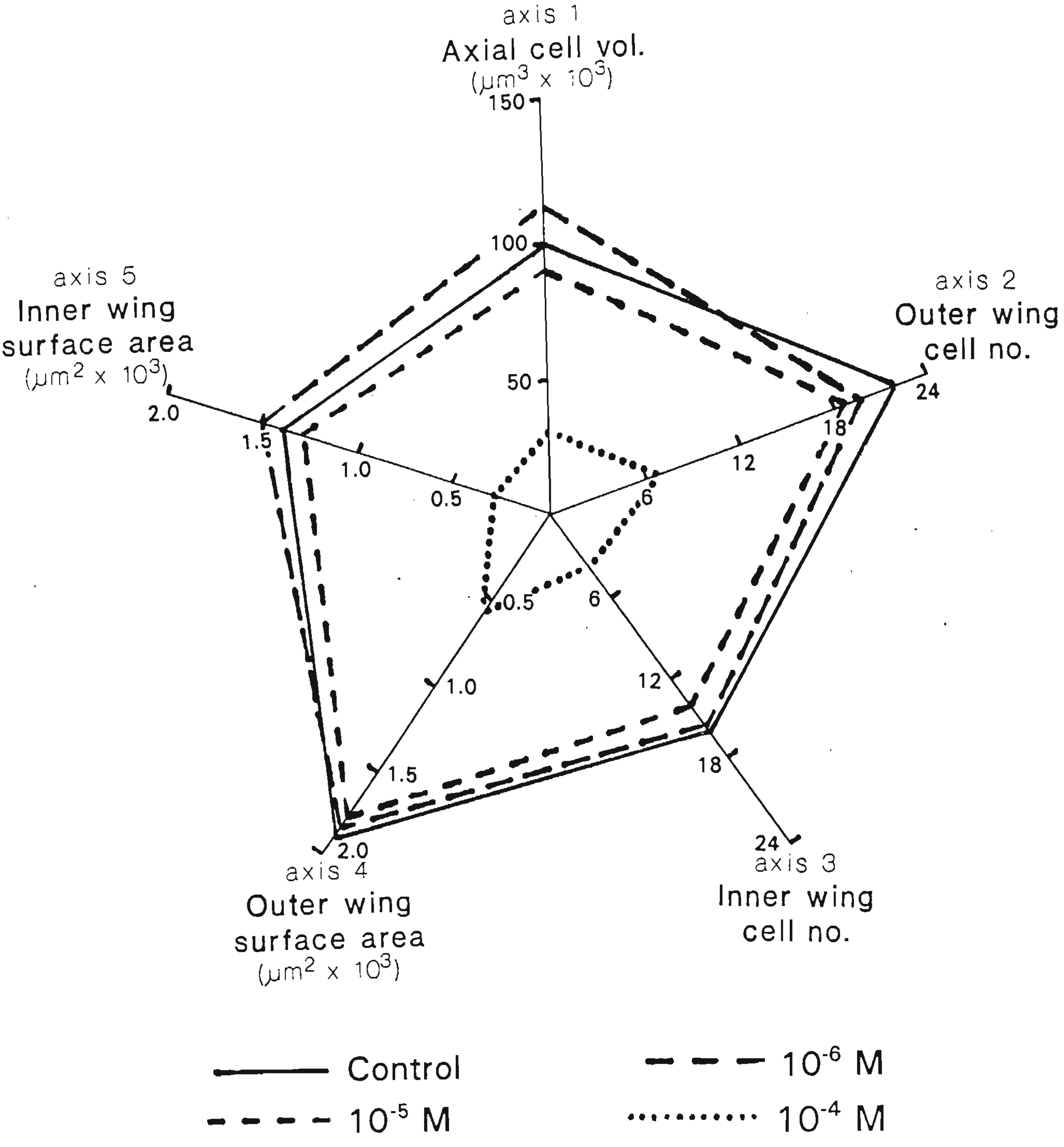


Fig. 6.19 Effect of putrescine on axial cell volume (axis 1), wing cell number (axes 2 and 3) and wing surface area (axes 4 and 5) in *Pteroceramium*. Measurements were taken at seg. 4 along the dominant axis.

Table 6.10 Growth and morphometric results taken at seg. 4 of *Pteroceramium* when grown in different putrescine concentrations. Results are shown as mean ± standard error. RGR = relative growth rate, ax cell = axial cell, vol = volume, OW = outer wing, IW = inner wing, SA = surface area.

	Putrescine concentration (M)					
Parameters measured	Control	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}
RGR (ax cells)	0.07 ± 0.003	0.08 ± 0.02	0.07 ± 0.01	0.03 ± 0.01	0.00 ± 0.002	0.002 ± 0.003
RGR (length; μm)	0.09 ± 0.004	0.08 ± 0.01	0.09 ± 0.01	0.04 ± 0.01	0.01 ± 0.002	0.001 ± 0.002
Ax cell vol (μm^3 x 10^3)	96 ± 5	112 ± 8	89 ± 7	31 ± 3	-	-
OW cell no.	22 ± 1	20 ± 1	19 ± 1	7 ± 1	-	-
IW cell no.	16 ± 1	16 ± 1	14 ± 1	4 ± 0.4	-	-
OW SA (μm^2 x 10^3)	1.9 ± 0.1	1.9 ± 0.2	1.8 ± 0.2	0.6 ± 0.1	-	-
IW SA (μm^2 x 10^3)	1.4 ± 0.1	1.5 ± 0.1	1.3 ± 0.1	0.3 ± 0.04	-	-

vi) Spermidine - The RGR determined using axial cell number was significantly higher ($P<0.0001$) in the control replicates. There was a significant decrease in the RGR with increasingly higher concentrations of spermidine applied to the medium. A similar trend was seen for the RGR determined using thallus length, except that material growing in 10^{-6} M and 10^{-5} M spermidine had similar rates to the control replicates ($P<0.001$; Fig. 6.20A and B; Table 6.11).

Morphometric measurements were not taken for the material growing in the two highest concentrations as there was insufficient new growth. Axial cell volume at seg. 4 was unaffected by spermidine at the lower concentrations (10^{-6} M and 10^{-5} M). There was a significant decrease ($P<0.00.1$) in axial cell volume when *Pteroceramium* was grown in 10^{-4} M spermidine. The material in all treatments had similar sized wings at seg. 4

(cell number and surface area) except for those grown in 10^{-4} M where the wings were significantly smaller ($P < 0.001$; Fig. 6.21; Table 6.11).

Table 6.11 Growth and morphometric results taken at seg. 4 of *Pteroceramium* when grown in different spermidine concentrations. Results are shown as mean ± standard error. RGR = relative growth rate, ax cell = axial cell, vol = volume, OW = outer wing, IW = inner wing, SA = surface area.

Parameters measured	Spermidine concentration (M)					
	Control	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}
RGR (ax cells)	0.1 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.01 ± 0.004	0.00 ± 0.01
RGR (length; μm)	0.08 ± 0.01	0.08 ± 0.02	0.06 ± 0.01	0.03 ± 0.01	0.01 ± 0.001	0.01 ± 0.003
Ax cell vol ($\mu m^3 \times 10^3$)	80 ± 6	91 ± 9	82 ± 5	40 ± 6	-	-
OW cell no.	18 ± 1	17 ± 1	16 ± 1	6 ± 1	-	-
IW cell no.	12 ± 1	13 ± 1	10 ± 1	5 ± 1	-	-
OW SA ($\mu m^2 \times 10^3$)	1.7 ± 0.2	1.5 ± 0.1	1.3 ± 0.1	0.5 ± 0.1	-	-
IW SA ($\mu m^2 \times 10^3$)	1.1 ± 0.1	1.0 ± 0.1	0.9 ± 0.1	0.4 ± 0.1	-	-

vii) Spermine - The material growing in 10^{-6} M, 10^{-5} M and 10^{-4} M spermine had similar RGRs (both axial cell number and thallus length) to the control. At increasingly higher concentrations, there was a significant decrease ($P < 0.0001$) in the RGRs (Fig. 6.22A and B; Table 6.12).

Morphometric measurements were not taken for the material growing in the two highest concentrations as there was insufficient new growth. The material growing at 10^{-4} M spermine had similar sized axial cells to the control but the material grown at 10^{-5} M

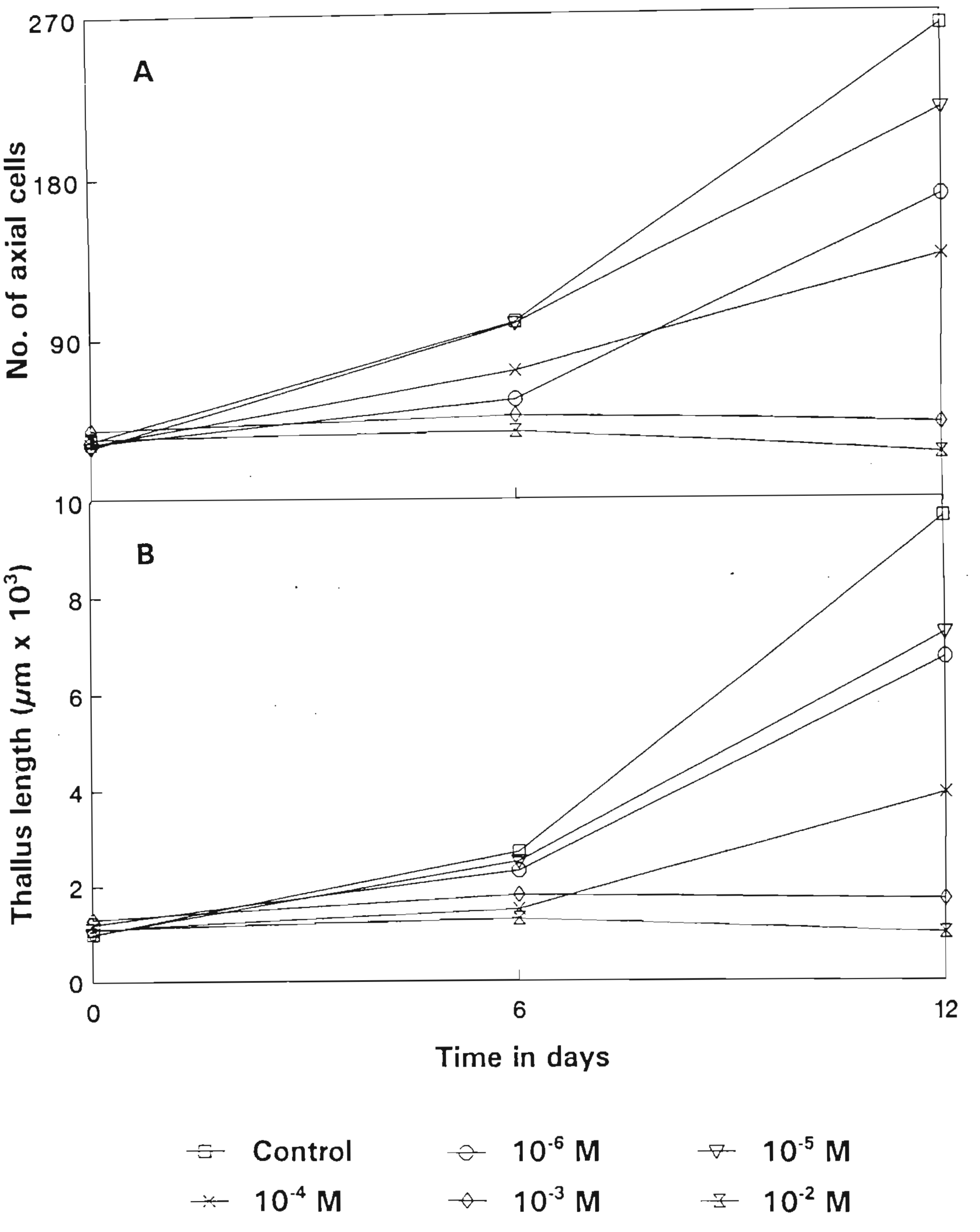


Fig. 6.20 Growth of *Pteroceramium* in varying concentrations of spermidine. A) Change in number of axial cells. B) Change in thallus length.

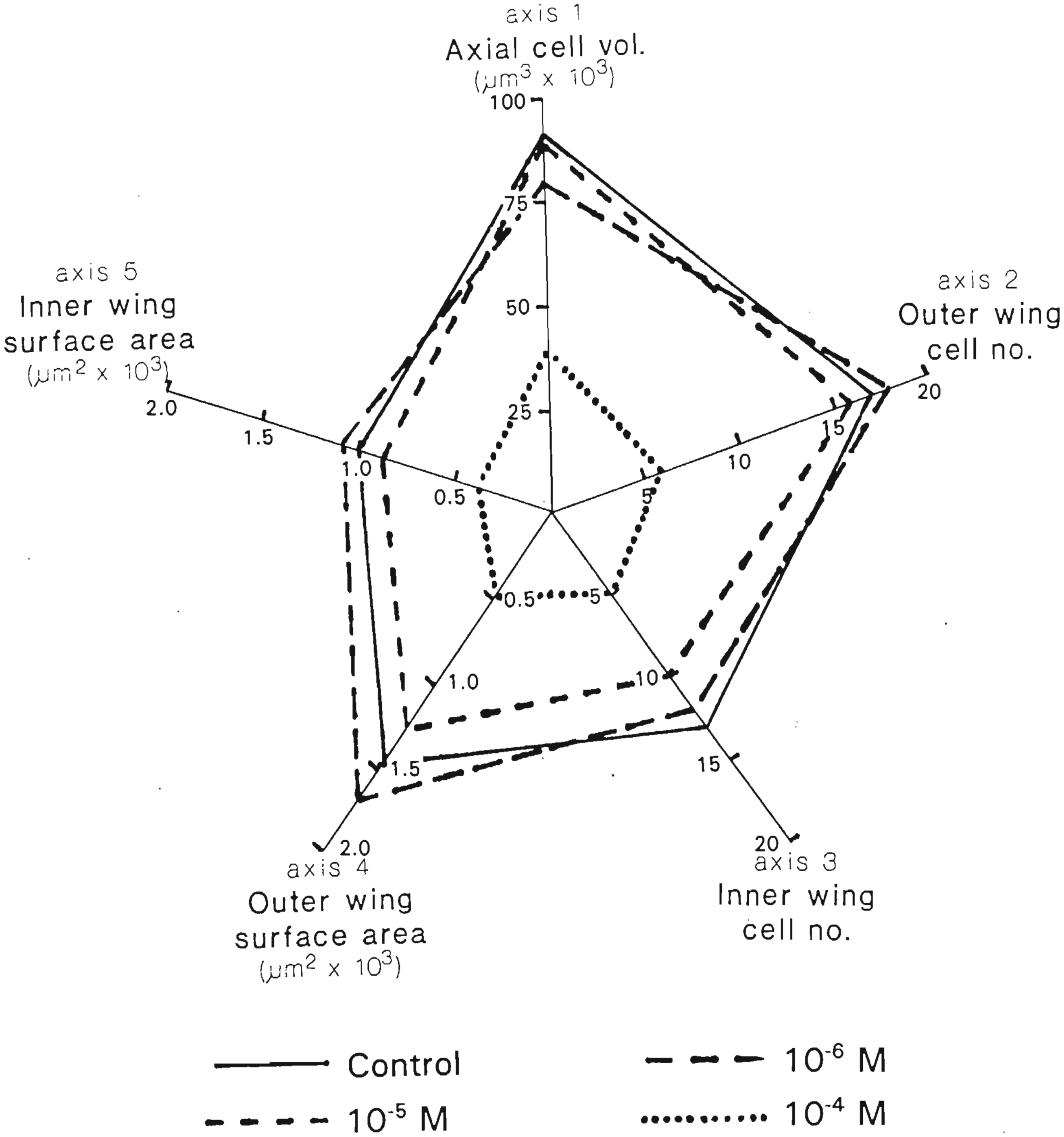


Fig. 6.21 Effect of spermidine on axial cell volume (axis 1), wing cell number (axes 2 and 3) and wing surface area (axes 4 and 5) in *Pteroceramium*. Measurements were taken at seg. 4 along the dominant axis.

and 10^{-6} M had significantly smaller ($P<0.001$) axial cells. Spermine had no apparent effect on wing size at seg. 4 ($P=0.3$; Fig. 6.23; Table 6.12).

Table 6.12 Growth and morphometric results taken at seg. 4 of *Pteroceramium* when grown in different spermine concentrations. Results are shown as mean ± standard error. RGR = relative growth rate, ax cell = axial cell, vol = volume, OW = outer wing, IW = inner wing, SA = surface area.

	Spermine concentration (M)					
Parameters measured	Control	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}
RGR (ax cells)	0.07 ± 0.02	0.05 ± 0.01	0.09 ± 0.01	0.07 ± 0.01	-0.001 ± 0.001	0.0004 ± 0.001
RGR (length; μm)	0.06 ± 0.01	0.05 ± 0.01	0.07 ± 0.01	0.05 ± 0.01	0.004 ± 0.001	0.001 ± 0.001
Ax cell vol (μm^3 x 10^3)	117 ± 8	75 ± 9	75 ± 7	109 ± 11	-	-
OW cell no.	19 ± 1	18 ± 2	15 ± 2	16 ± 2	-	-
IW cell no.	14 ± 1	12 ± 1	12 ± 1	10 ± 1	-	-
OW SA (μm^2 x 10^3)	1.8 ± 0.2	1.6 ± 0.2	1.3 ± 0.2	1.5 ± 0.2	-	-
IW SA (μm^2 x 10^3)	1.3 ± 0.1	1.3 ± 0.1	1.1 ± 0.1	0.9 ± 0.1	-	-

viii) <u>DFMO</u> - Material grown at 10^{-6} M DFMO had significantly fewer axial cells ($P<0.05$) and material grown at 10^{-6} M and 10^{-5} M had significantly smaller ($P<0.01$) thallus lengths compared to the control replicates (Fig. 6.24A and B; Table 6.13).

Morphometric readings were not taken for material growing at 10^{-2} M as there was no new growth. Material growing at 10^{-5} M DFMO had significantly smaller ($P<0.0001$) axial cells at seg. 4 along the dominant axis when compared with the other treatments which were similar to the control. There was a significant decrease in outer wing cell number ($P<0.001$) when grown in 10^{-4} M DFMO and a significant decrease in outer

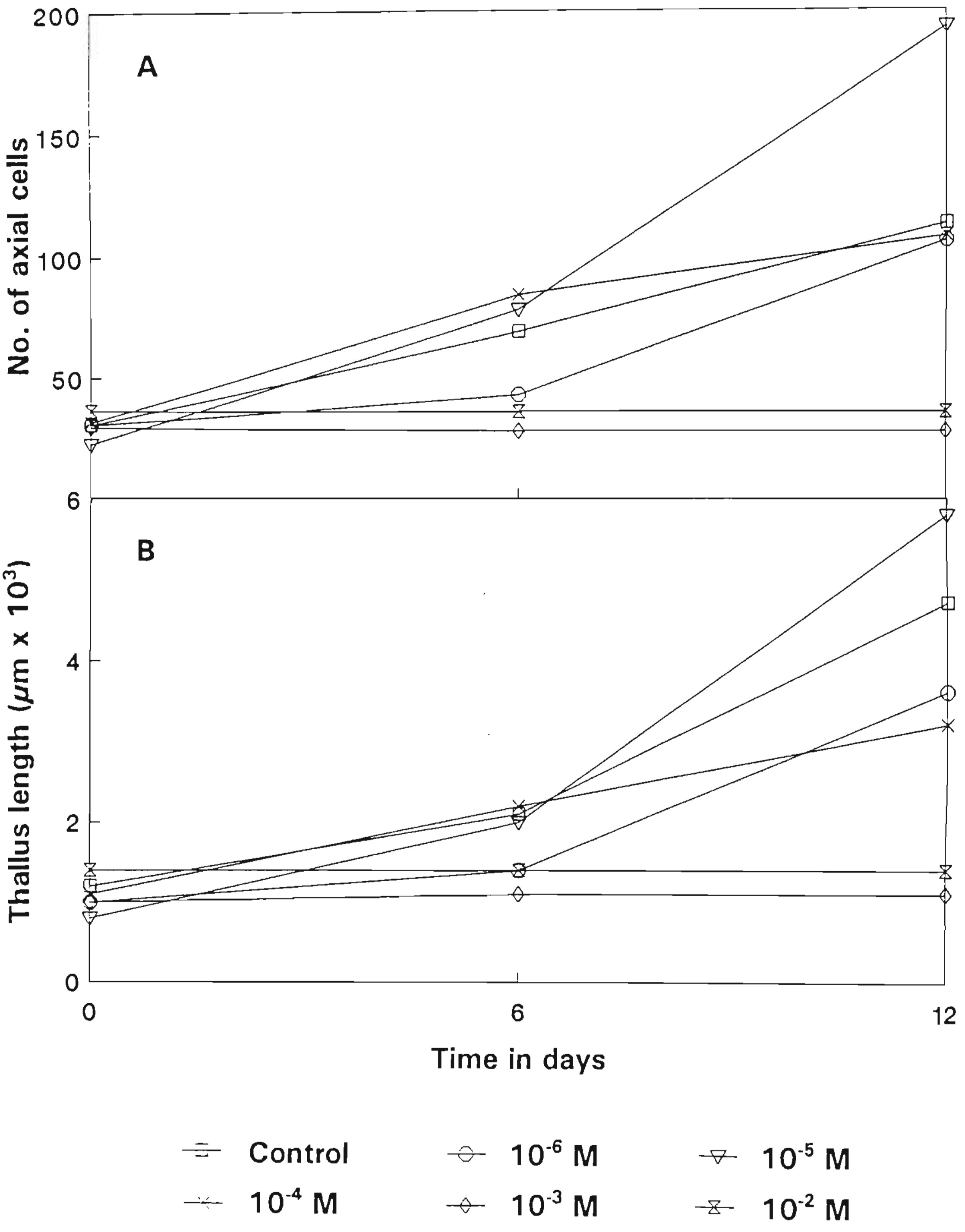


Fig. 6.22 Growth of *Pteroceramium* in varying concentrations of spermine. A) Change in number of axial cells. B) Change in thallus length.

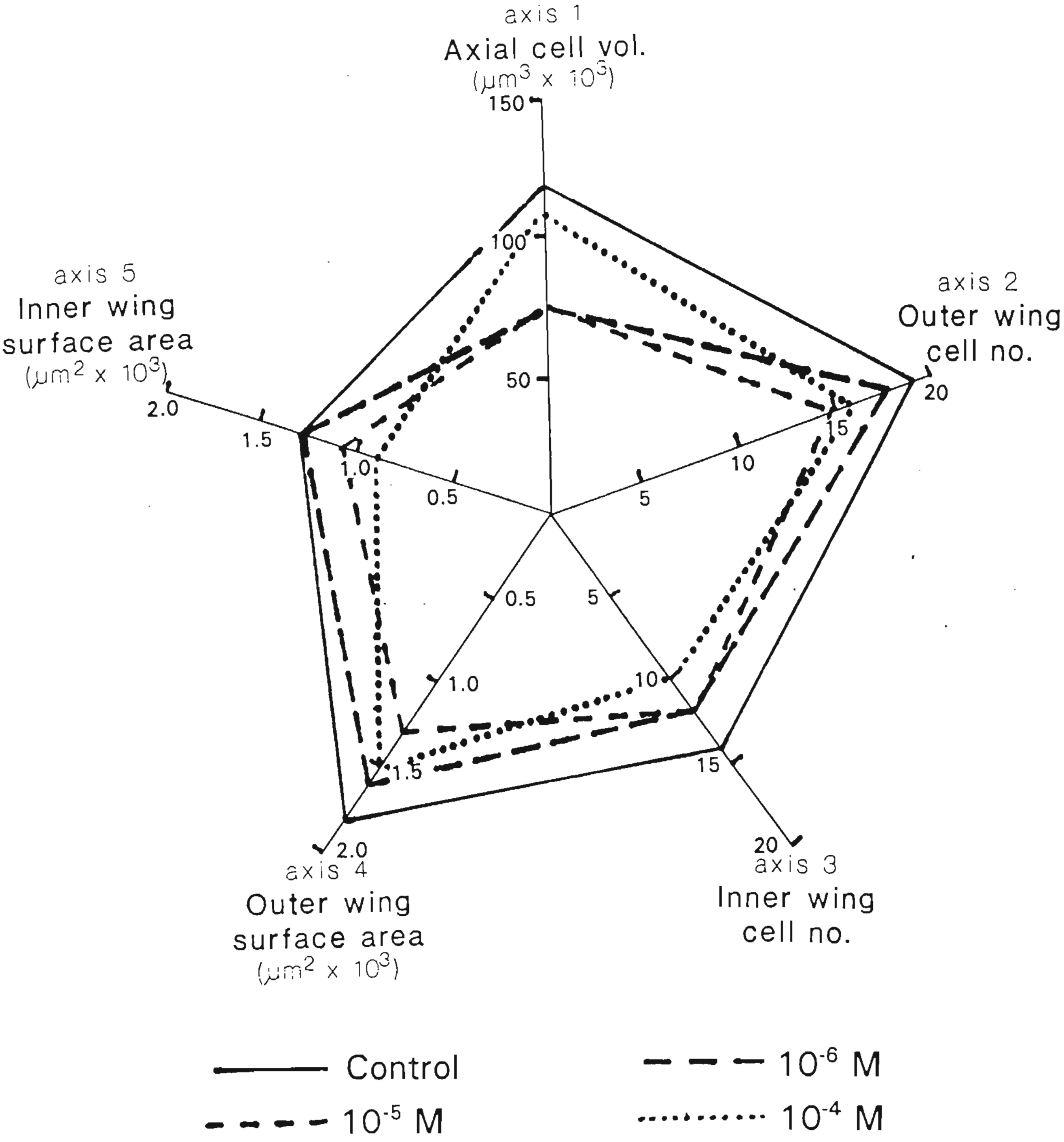


Fig. 6.23 Effect of spermine on axial cell volume (axis 1), wing cell number (axes 2 and 3) and wing surface area (axes 4 and 5) in *Pteroceramium*. Measurements were taken at seg. 4 along the dominant axis.

wing surface area ($P<0.001$) for material grown at 10^{-5} M. For the inner wing, material growing at 10^{-6} M DFMO had significantly higher ($P<0.0001$) cell numbers and material grown at 10^{-3} M significantly fewer cells than the control. The inner wing surface area was significantly larger ($P<0.0001$) than the control when grown at concentrations between 10^{-6} M and 10^{-4} M DFMO and significantly smaller when grown in 10^{-3} M (Fig. 6.25; Table 6.13).

Table 6.13 Growth and morphometric results taken at seg. 4 of *Pteroceramium* when grown in different DFMO concentrations. Results are shown as mean ± standard error. RGR = relative growth rate, ax cell = axial cell, vol = volume, OW = outer wing, IW = inner wing, SA = surface area.

	DFMO concentration (M)					
Parameters measured	Control	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}
RGR (ax cells)	0.04 ± 0.01	0.05 ± 0.01	0.05 ± 0.02	0.05 ± 0.01	0.04 ± 0.01	0.002 ± 0.002
RGR (length; μm)	0.04 ± 0.01	0.07 ± 0.01	0.04 ± 0.02	0.05 ± 0.01	0.04 ± 0.01	0.002 ± 0.01
Ax cell vol ($\mu m^3 \times 10^3$)	134 ± 12	136 ± 9	141 ± 20	161 ± 25	42 ± 6	-
OW cell no.	18 ± 2	23 ± 2	22 ± 2	17 ± 2	11 ± 2	-
IW cell no.	13 ± 2	17 ± 1	16 ± 1	15 ± 1	6 ± 1	-
OW SA ($\mu m^2 \times 10^3$)	1.6 ± 0.3	2.2 ± 0.2	2.0 ± 0.2	1.6 ± 0.2	0.7 ± 0.1	-
IW SA ($\mu m^2 \times 10^3$)	0.9 ± 0.1	1.5 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	0.4 ± 0.1	-

ix) CHAP - The RGRs (using both axial cell number and thallus length) were similar for the control replicates and material grown in 10^{-6} M CHAP. At increasingly higher concentrations there is a significant decrease ($P<0.0001$) of the RGRs (Fig. 6.26A and B; Table 6.14).

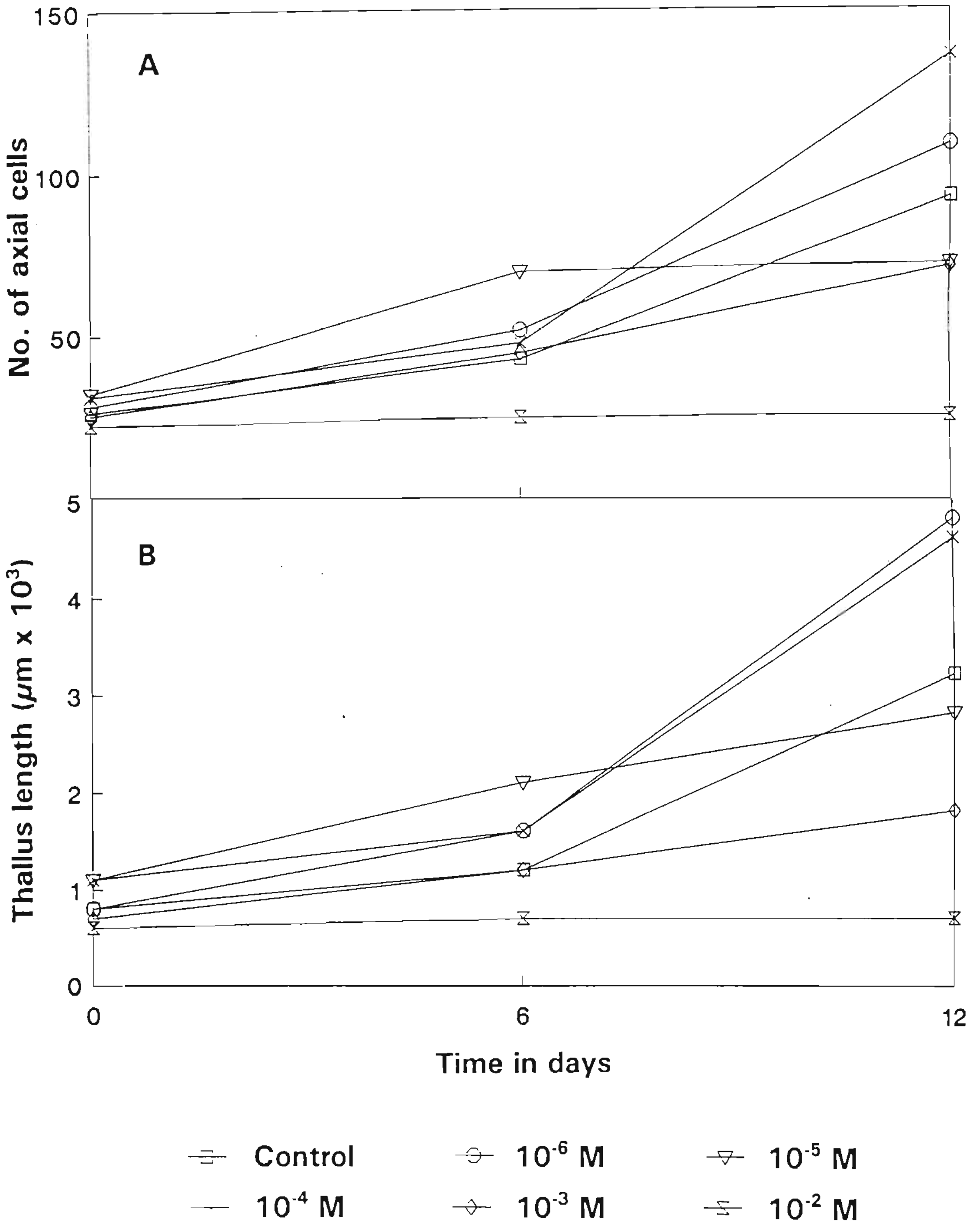


Fig. 6.24 Growth of *Pteroceramium* in varying concentrations of DFMO. A) Change in number of axial cells. B) Change in thallus length.

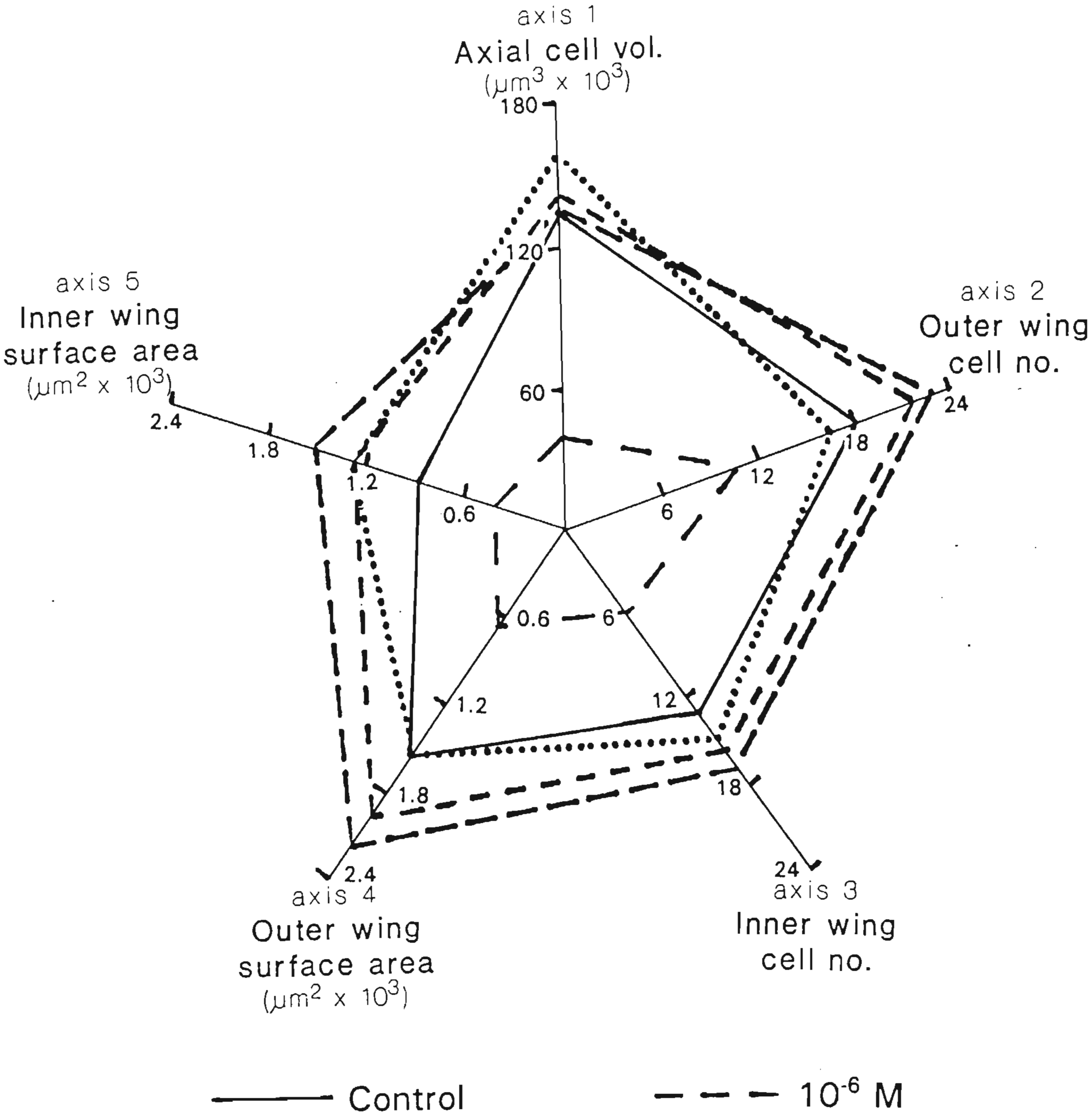


Fig. 6.25 Effect of DFMO on axial cell volume (axis 1), wing cell number (axes 2 and 3) and wing surface area (axes 4 and 5) in *Pteroceramium*. Measurements were taken at seg. 4 along the dominant axis.

Morphometric comparisons could only be made between the control and material grown in 10^{-6} M CHAP as the other treatments had insufficient growth. There were no significant differences between axial cell volume and both outer and inner wing cell number and surface area at seg. 4 between these two treatments (Fig. 6.27; Table 6.14).

Table 6.14 Growth and morphometric results taken at seg. 4 of *Pteroceramium* when grown in different CHAP concentrations. Results are shown as mean ± standard error. RGR = relative growth rate, ax cell = axial cell, vol = volume, OW = outer wing, IW = inner wing, SA = surface area.

Parameters measured	CHAP concentration (M)					
	Control	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}
RGR (ax cells)	0.06 ± 0.01	0.06 ± 0.01	0.02 ± 0.01	-0.004 ± 0.003	-0.005 ± 0.002	-0.004 ± 0.003
RGR (length; μm)	0.06 ± 0.01	0.06 ± 0.01	0.01 ± 0.01	0.004 ± 0.002	-0.002 ± 0.001	-0.006 ± 0.004
Ax cell vol ($\mu m^3 \times 10^3$)	137 ± 8	145 ± 21	-	-	-	-
OW cell no.	22 ± 2	21 ± 2	-	-	-	-
IW cell no.	16 ± 1	16 ± 1	-	-	-	-
OW SA ($\mu m^2 \times 10^3$)	2.0 ± 0.2	2.0 ± 0.2	-	-	-	-
IW SA ($\mu m^2 \times 10^3$)	1.3 ± 0.1	1.3 ± 0.1	-	-	-	-

x) <u>MGBG</u> - The RGRs using both axial cell number and thallus length, were similar to the control when material was grown in 10^{-6} M and 10^{-5} M MGBG. With increasingly higher concentrations, there was a significant decrease in both the axial cell number ($P<0.001$) and thallus length ($P<0.0001$; Fig. 6.28A and B; Table 6.15).

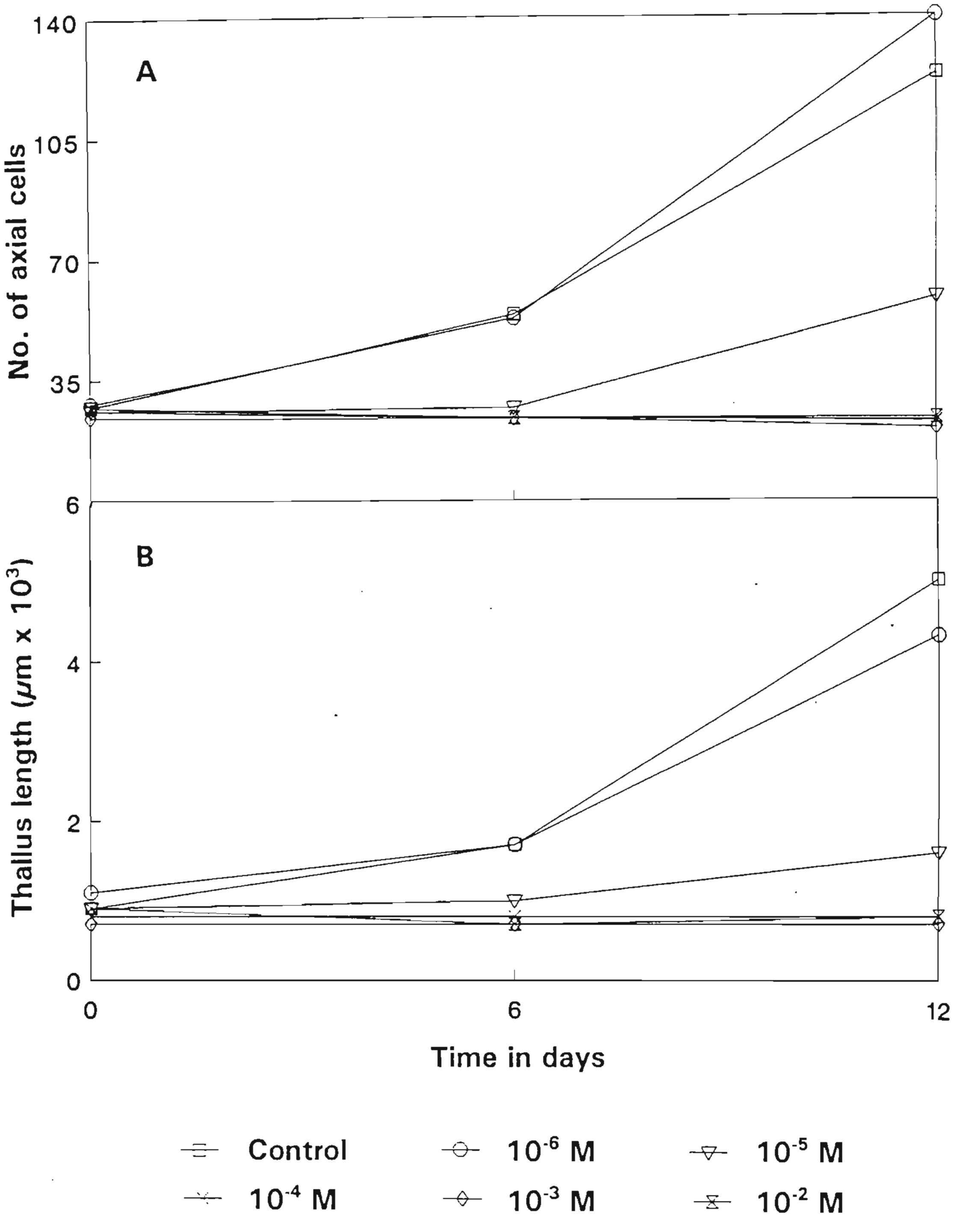


Fig. 6.26 Growth of *Pteroceramium* in varying concentrations of CHAP. A) Change in number of axial cells. B) Change in thallus length.

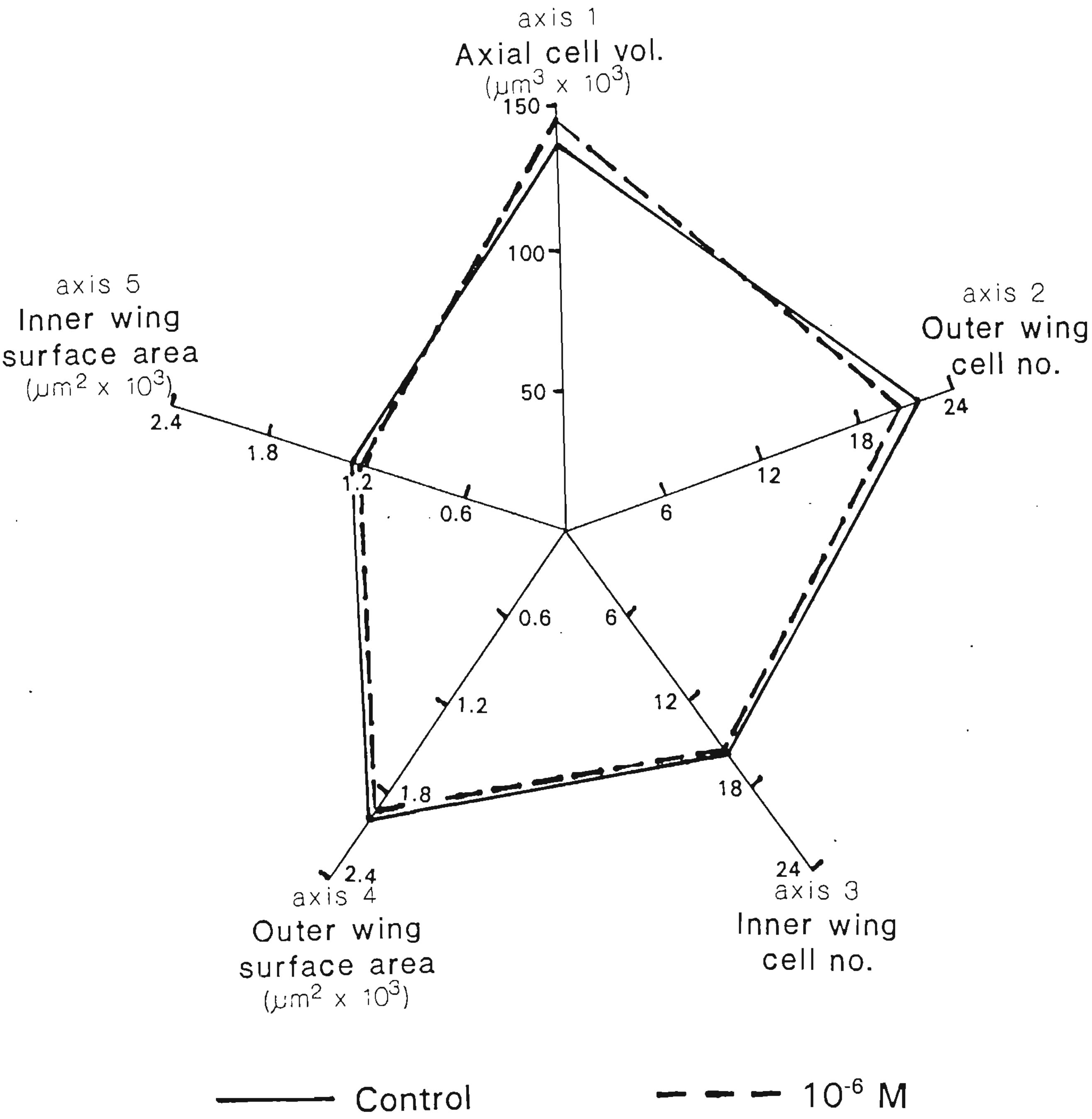


Fig. 6.27 Effect of CHAP on axial cell volume (axis 1), wing cell number (axes 2 and 3) and wing surface area (axes 4 and 5) in *Pteroceramium*. Measurements were taken at seg. 4 along the dominant axis.

Morphometric measurements were not taken for the replicates growing in 10^{-3} M MGBG as there was insufficient growth. Axial cell volumes at seg. 4 along the dominant axis for material growing in 10^{-6} M were similar to the control replicates and with increasingly higher concentrations, there was a significant decrease ($P<0.0001$) in axial cell volume. The outer wings of material growing in 10^{-4} M MGBG had significantly smaller surface areas ($P<0.0001$) with fewer cells ($P<0.01$) than the other treatments which were similar to the control. The surface area of the inner wings were significantly larger ($P<0.0001$) when grown in 10^{-6} M and decreased in size with increasing concentrations of MGBG. However, the inner wing cell number for replicates growing in 10^{-6} M MGBG was similar to the control and decreased significantly ($P<0.0001$) with increasing concentrations (Fig. 6.29; Table 6.15).

Table 6.15 Growth and morphometric results taken at seg. 4 of *Pteroceramium* when grown in different MGBG concentrations. Results are shown as mean ± standard error. RGR = relative growth rate, ax cell = axial cell, vol = volume, OW = outer wing, IW = inner wing, SA = surface area.

	MGBG concentration (M)				
Parameters measured	Control	10^{-6}	10^{-5}	10^{-4}	10^{-3}
RGR (ax cells)	0.05 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.04 ± 0.01	-0.01 ± 0.004
RGR (length; μm)	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.003	0.05 ± 0.01	0.001 ± 0.002
Ax cell vol ($\mu m^3 \times 10^3$)	109 ± 5	107 ± 5	85 ± 5	51 ± 3	-
OW cell no.	23 ± 1	22 ± 1	22 ± 2	16 ± 1	-
IW cell no.	16 ± 1	17 ± 1	14 ± 1	10 ± 1	-
OW SA ($\mu m^2 \times 10^3$)	2.0 ± 0.2	2.2 ± 0.1	2.3 ± 0.2	1.1 ± 0.1	-
IW SA ($\mu m^2 \times 10^3$)	1.3 ± 0.1	1.7 ± 0.1	1.4 ± 0.1	0.7 ± 0.01	-

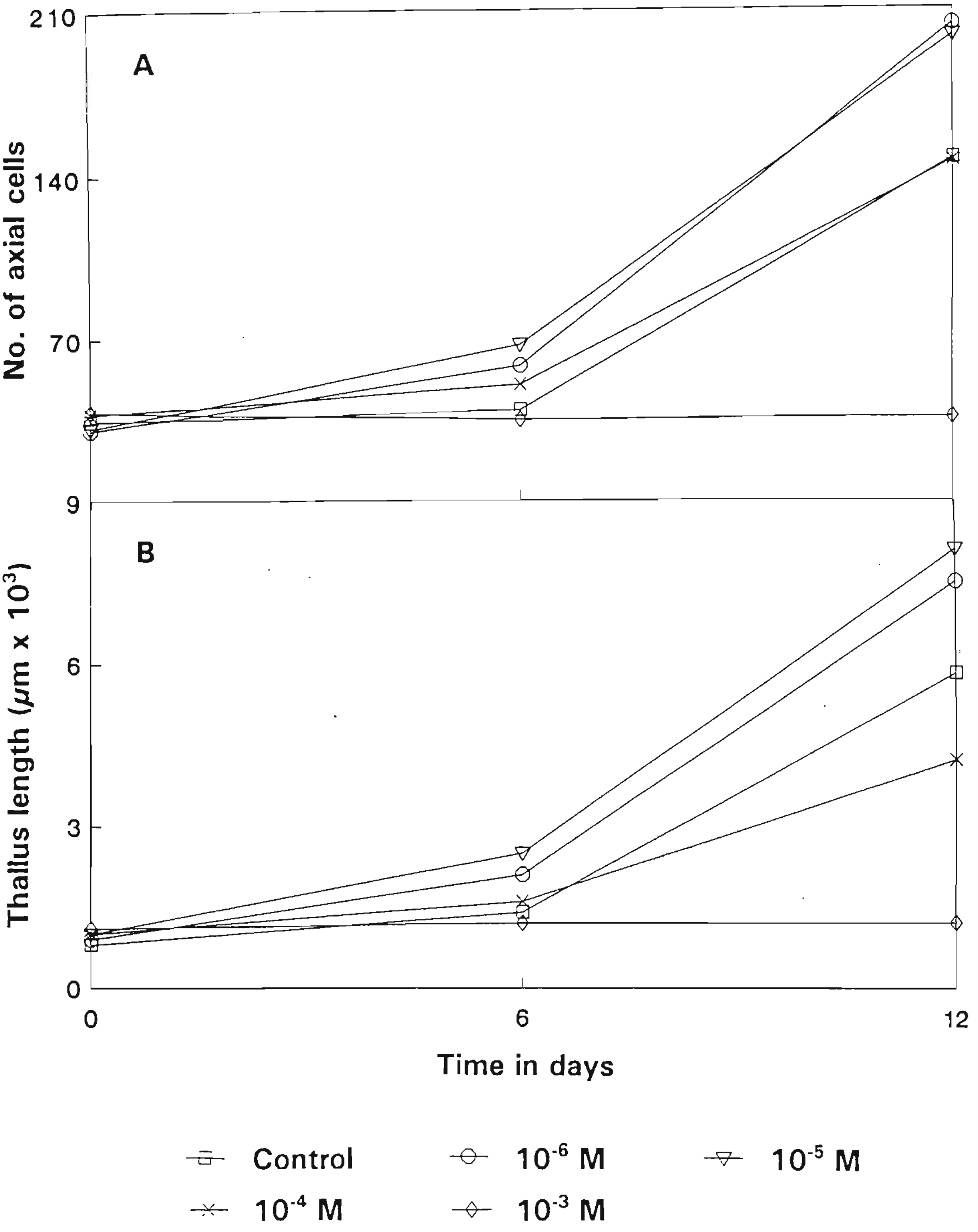


Fig. 6.28 Growth of *Pteroceramium* in varying concentrations of MGBG. A) Change in number of axial cells. B) Change in thallus length.

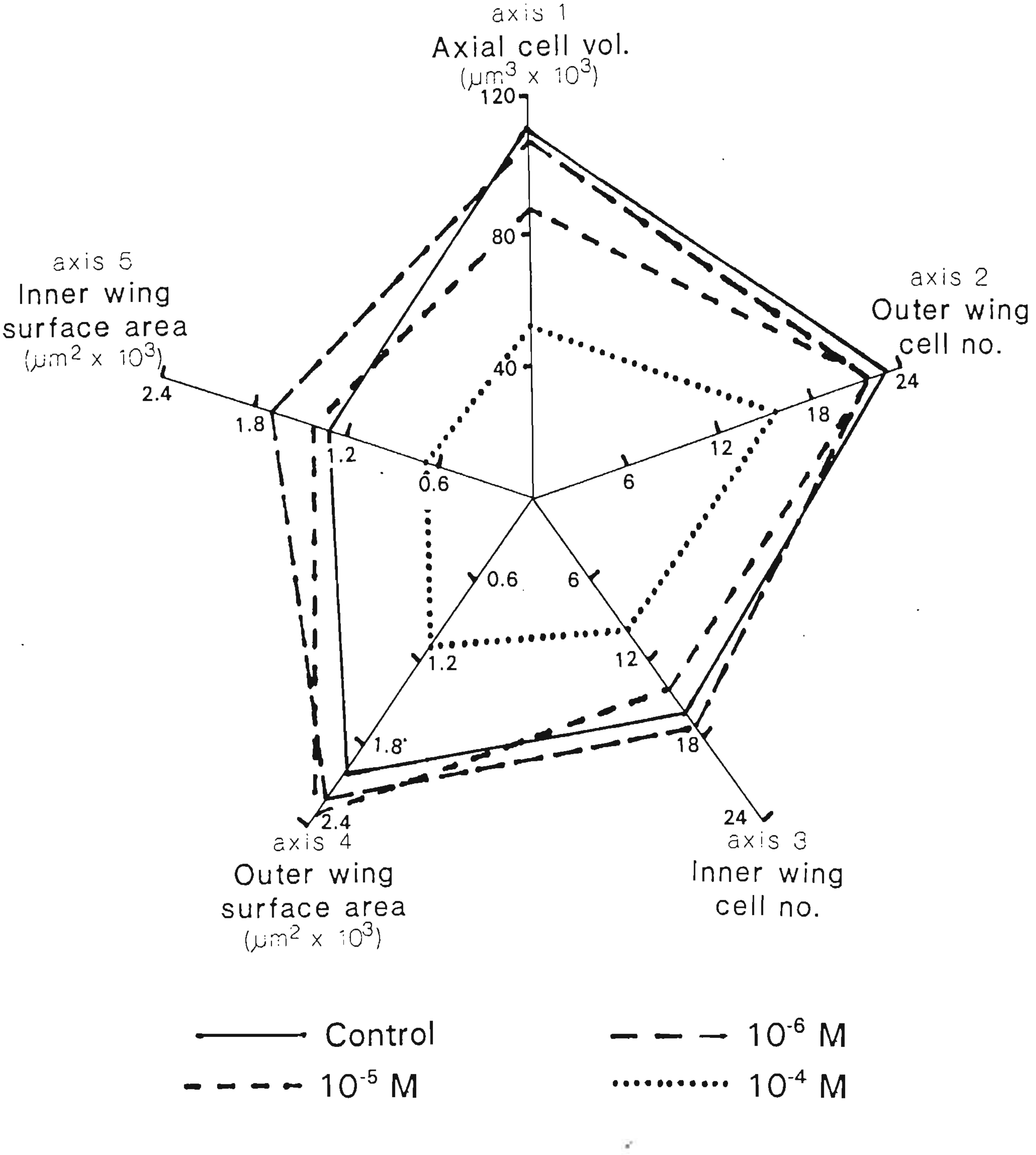


Fig. 6.29 Effect of MGBG on axial cell volume (axis 1), wing cell number (axes 2 and 3) and wing surface area (axes 4 and 5) in *Pteroceramium*. Measurements were taken at seg. 4 along the dominant axis.

6.3.10 HPLC separation of polyamines

The retention times of the polyamines are putrescine 19 minutes, spermidine 24 minutes and spermine 28 minutes as determined by the standard curves.

In the first run using 1 ml of *Pteroceramium* extract, no peaks were detected corresponding with the polyamine standards, indicating that either there were no polyamines present or that they were present in concentrations too low for detection.

In the 25 times more concentrated extracts of *Pteroceramium*, there were peaks at 19 minutes and 24 minutes, suggesting that putrescine and spermidine were present respectively. The concentration as determined from the standard curves using peak area, was 38 μg spermidine g^{-1} fresh weight. The peak for putrescine was too small for concentrations to be accurately determined.

6.3.11 Morphological differences of dominant and subordinate branches

The dominant axis at seg. 4 had fewer cells than the corresponding subordinate branch although the subordinate branch was longer. This indicates that the axial cells in the subordinate segment do not elongate as much as those in the dominant segment. Results show that the majority of the subordinate branches had developed as far as seg. 3, fewer to seg. 2 and a couple to seg. 1 and seg. 4 (Table 6.16).

6.3.12 Apical dominance

There was a significant difference ($P<0.001$) between the control and excised apical tip replicates when comparing the ratio of axial cell number to the number of adventitious branches produced after 10 days growth (Table 6.17). More adventitious branches were produced further away from the apical region where the axial cells were more mature than nearer the apical tip (Fig. 6.30).

Table 6.16 Dominant and subordinate segment measurements in *Pteroceramium.* Results are shown as mean ± standard error.

Branch	Segment measured	Average length (μm)	Average axial cell no.
Dominant	seg. 4	567 ± 23	6 ± 0.1
Subordinate	total for all subordinate segments	403 ± 20	7 ± 0.2
	seg. 1 (17%)	249 ± 45	10 ± 1
	seg. 2 (25%)	293 ± 28	7 ± 0.4
	seg. 3 (41%)	461 ± 26	7 ± 0.2
	seg. 4 (17%)	577 ± 45	7 ± 0.4

Comparisons could not be done with the excised subordinate tip replicates as different plant material was used. However, removal of the subordinate branch did not appear to increase the number of adventitious branches produced (Fig. 6.31).

Table 6.17 Ratio of axial cell number to number of adventitious branches produced after 10 days growth in *Pteroceramium* once the apical tip of the dominant branch was excised. Results are shown as mean ± standard error.

Treatment	Axial cell number:adventitious branches
Control	0.014 ± 0.004
Excised apical tip	0.493 ± 0.071

6.3.13 Apical dominance in the presence of exogenous IAA

The control replicates with intact apical tips had significantly fewer adventitious branches ($P<0.001$) than the other treatments as seen by the lower ratio, confirming the previous apical dominance results. All other treatments had similar ratios showing that exogenously applied IAA does not suppress adventitious branch development after excision of the dominant apical tip (Table 6.18).

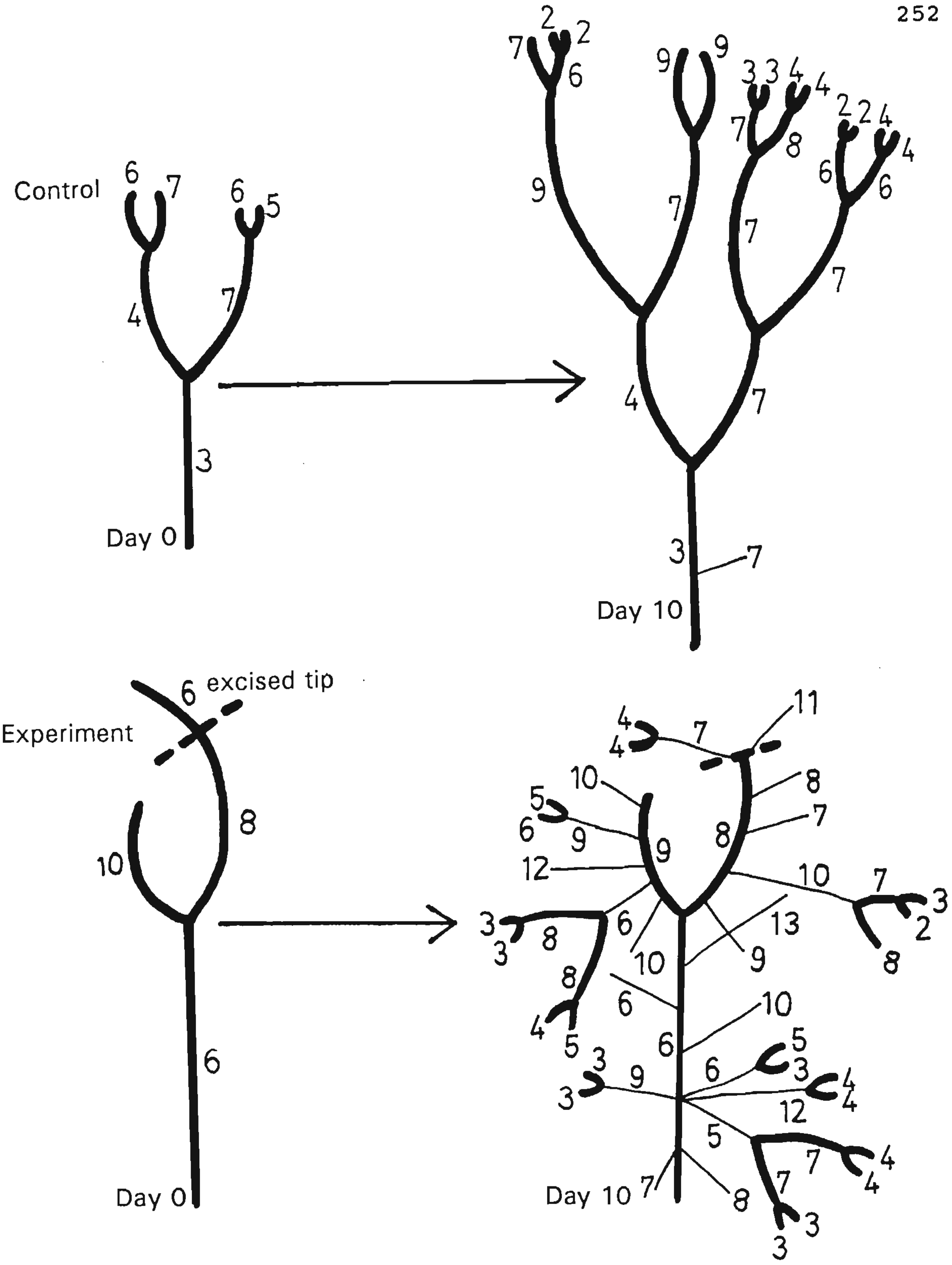


Fig. 6.30 Effect of the removal of the dominant apical tip on growth in *Pteroceramium*. Diagrams are drawn to scale and show the number of axial cells per segment at Day 10. ━ = pseudodichotomous branches arising from divisions of the apical cell, — = adventitious branches arising from pericentral cells.

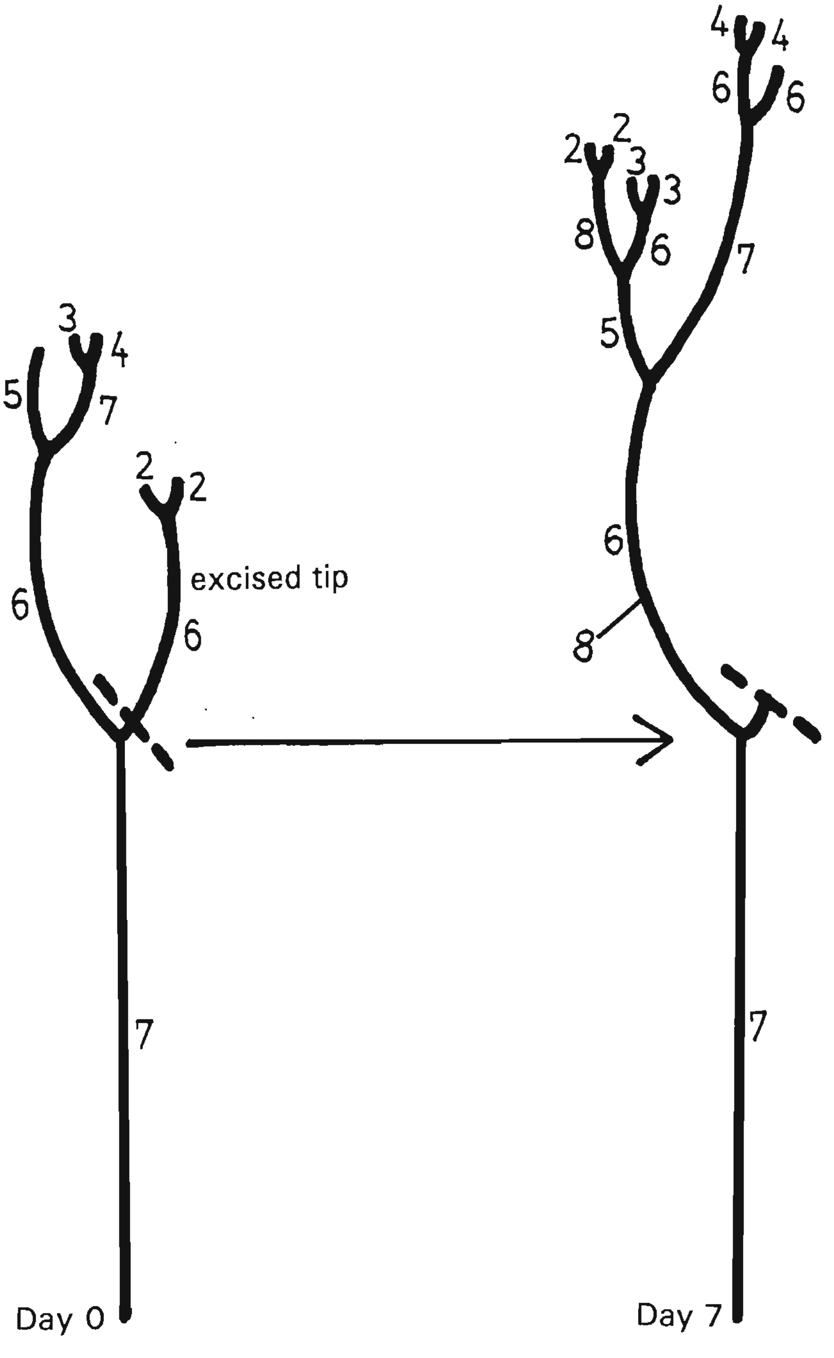


Fig. 6.31 Effect of the removal of the subordinate apical tip on growth in *Pteroceramium*. Diagrams are drawn to scale and show the number of axial cells per segment at Day 7. ▬ = pseudodichotomous branches arising from divisions of the apical cell, —• = adventitious branches arising from pericentral cells.

Table 6.18 Ratio of axial cell number to number of adventitious branches in the presence of exogenous IAA after 10 days growth in *Pteroceramium* plants with excised apical tips. Results are shown as mean ± standard error.

Treatment	Axial cell number: Adventitious branches
Control + tips	0.035 ± 0.008
Control - tips	0.154 ± 0.042
10^{-9} M IAA	0.230 ± 0.036
10^{-8} M IAA	0.456 ± 0.256
10^{-7} M IAA	0.201 ± 0.065
10^{-6} M IAA	0.224 ± 0.072
10^{-5} M IAA	0.464 ± 0.131
10^{-4} M IAA	0.281 ± 0.059

6.4 Discussion

6.4.1 Growth forms

There are three types of meristematic cells in the filamentous Rhodophyceae:

1) apical initials forming indeterminate axes;

2) pericentral cell initials forming adventitious branches; and

3) apical initials forming lateral filaments of determinate growth (cortical band; AGHAJANIAN and HOMMERSAND, 1980).

The apical cell forming the indeterminate axes in the Ceramiaceae is usually dome- or thimble-shaped and divides to produce axial cells. There are four recognised types of apical cell divisions in the Ceramiaceae. They are:

1) transverse cell division;

2) oblique division with the high sides of segments arranged on the same side of the axis;

3) oblique division with the high sides of the segment arranged alternately; and

4) oblique division with the high sides of the segment arranged in a spiral, usually with three or more divisions per 360° rotation (DIXON, 1973; MOE and SILVA, 1979).

These are correlated with patterns of branching and subsequent thallus development with the oblique septa related to structural imbalance (MOE and SILVA, 1979). An oblique cross-walled cell is a pre-requisite for the initiation of apical branching although there are examples in the Ceramiaceae where there are oblique apical cell division resulting in normal growth (DIXON, 1971; HAWKINS, 1972; LOBBAN, HARRISON and DUNCAN, 1985). The apical cell has cyclic variation in size through enlargement and segmentation. This applies mainly to length, the diameter of the apical cell remaining a more constant size (DIXON, 1973). In the filamentous red algae, the number of cells in a filament is a direct indication of the number of times the apical cell has divided (EDWARDS, 1977). Cell enlargement is gradual with age. Smaller apical cells are associated with filaments of limited growth (DIXON, 1973).

Pteroceramium has a typical dome-shaped axial cell which divides by transverse septa. Pseudodichotomous branching is initiated by oblique divisions of the apical cell.

The derivative cells in most algae do not divide again in the plane of cell division in which they originated, although there are a few exceptions in the Corallinaceae and Delesseriaceae. However, derivative cells can divide in other planes to form primordia, giving rise to pericentral cells and apical cells of filaments of limited growth. These usually divide just behind the apical cell, although, if the thallus is damaged, there can be regeneration in older thalli (DIXON, 1973).

Pteroceramium has pericentral cell formation at about two or three cells behind the apical cell. The six pericentral cells develop in a fixed sequence with Pc1 forming on the outer face according to the preceding pseudodichotomy, Pc2 and Pc3 on the lower and upper surface respectively nearest to Pc1, Pc4 and Pc5 on the upper and lower surface respectively furthest from Pc1 and Pc6 forming on the inner face according to the preceding pseudodichotomy. The axial cell elongates at the same time as the pericentral cells are being cut off. Immediately after a pericentral cell is cut off, it begins to divide further to form the cortical band, wings and spines.

There are other patterns of pericentral cell development. *Dasyphila preissii* Sonder has six whorled branchlets at each node, each branchlet being initiated by a pericentral cell. The Pc1 forms on a lateral branch in an adaxial position relative to the main axis. Pc2 forms to the right and Pc3 to the left of Pc1, close to the base of Pc1. Pc4 forms opposite Pc1 with Pc5 and Pc6 forming on the right and left respectively of Pc4. This is similar to pericentral cell initiation in the Rhodomelaceae (WOLLASTON, 1977). In *Cottoniella* sp. Børgesen, the axial cell begins dividing only once it is about six cells behind the apical cell. Pc1 is dorsal and is formed by a periclinal division of the axial cell. The next two pericentral cells are formed laterally by two successive anticlinal divisions. Pc4 is cut off on the ventral side by another periclinal division (CORMACI, FURNARI and SCAMMACCA, 1978). In *Batrachospermum sirodotii* Skuja there are 4-5 pericentral cells. Only after all the pericentral cells are formed does the axial cell elongate (AGHAJANIAN and HOMMERSAND, 1980).

Increase in length of regenerating fragments is due to both cell enlargement and cell division, cell enlargement being more important. That overall growth is primarily a function of cell enlargement is hardly surprising as cell division occurs only at the apex while cell enlargement proceeds in all cells in an axis. The apical region in *Ceramium* can be defined as "that part of the axis in which axial cell diameter is greater than cell length" (GARBARY, 1979b). The length of the apical region can be influenced by light, plants growing under long days having a smaller apical region. The development of fragments proceeds through a number of stages:

1) a lag phase when there is no growth as fragments recover from excision;

2) rhizoid production occurs and the apices resume division. There is little increase in average cell length; and

3) a rapid increase in plant length and an increase in axial cell size. The average cell size stabilises as greater numbers of cells reach their maximum dimensions as controlled by culture conditions (GARBARY, 1979b).

Dramatic enlargement occurs in the subapical cells, there being an increase in cell size moving away from the apex along the axis. Maturation of axial cells shows a great increase in volume owing to increase in size in both length and breadth. In angiosperms, cells may increase in volume 200 times but in Rhodophyta the extent of volume increase is much larger (Table 6.19; DIXON, 1971). A similar trend was seen for *Pteroceramium* which also had a large increase in axial cell volume, usually over 1000 times and in some cases, as much as 3000 times although this is not as great as in some of the Rhodophyceae. In *Pteroceramium* there is an initial rapid axial cell enlargement to the size measured in seg. 4. After this the axial cells may increase a bit more in size but at a much slower rate.

Table 6.19 Enlargement of axial cells in some Rhodophyceae (from DIXON, 1971).

Species	Increase in volume
Batrachospermum vagum (Roth) C.Agardh	815 x
Ceramium rubrum	35 000 x
Ceramium echionotum	14 000 x
Antithamnion plumula (Ellis) Thuret	48 000 x
Lemanea fluviatilis (Linnaeus) C.Agardh	44 000 x

Subordinate branches have cells which are smaller at the base compared to younger axial cells further along the filament. The same phenomenon was observed in *Pteroceramium* with the subordinate branches having more axial cells than the dominant branch but being shorter in length, indicating that axial cell enlargement is suppressed. It appears that the parent filament has some effect on cell enlargement within the lateral filament; e.g. *Callithamnion corymbosum* (J.E.Smith) C.Agardh. This suggests that the size of a cell is dependent on its position rather than what it is (DIXON, 1973).

In the Ceramiaceae, adventitious axes can also affect axial cell enlargement although not so dramatically, as the axial cells are almost fully developed at the time of initiation (DIXON, 1973).

Cell division in *Griffithsia pacifica* is at first rapid with 5-6 cell divisions day^{-1} apex^{-1}. After one or two days, the cell division rate drops to 1 cell day^{-1} apex^{-1}. Cells continue to elongate for 6-8 days with the bulk of elongation occurring after the cell has become subapical. Subapical cells never normally divide but can be induced to divide transversely by removing abutting cells. This shows that differentiation of these cells is not irreversible, even once fully elongated and developed (DUFFIELD, WAALAND and CLELAND, 1972).

Griffithsia pacifica shows diurnal rhythms of cell division and elongation when grown in constant conditions. Cell division is low during the day and peaks at night and has been shown to be a truly endogenous, circadian rhythm with the number of cells produced dependent on irradiance. Subapical cell division is the most affected by irradiance levels while apical cell division and lateral branch formation decreases if the photoperiod is less than 8 hours light. Thus there is an overall effect on morphology with plants growing in short days and low irradiance being sparsely branched and those growing in long days and high irradiance being much branched. Elongation is highest during the earliest part of the day and decreases towards the end of the light period and is the lowest at night. This is not a true endogenous rhythm but is controlled by some environmental factor such as darkness and is little affected by light intensity and photoperiod (WAALAND and CLELAND, 1972).

Regeneration of excised cells and filaments is usually in a polar fashion, producing shoot cells from their exposed apices and rhizoid cells from the newly exposed bases. Cellular polarity is quite strong during regeneration and is not changed by unidirectional light or gravity (WAALAND, 1990). For example, positioning of "leaf" and rhizoid primordia is determined by gravity in *Caulerpa prolifera* rather than direction of irradiance (BUGGELN, 1981). Unidirectional light may affect elongation. Generally, filaments and blades are positively phototropic and rhizoids are negatively phototropic (WAALAND, 1989 and 1990). However, the fixed sequence of pericentral cell development in *Pteroceramium* is not altered by changing the direction of unidirectional light and gravity.

Expansion of cells is a secondary mechanism owing to the generation of turgor pressure. The cell wall is elastic and determines the future shape of the cell with most cells being constrained in one or more directions. Cell shape and direction of growth are controlled by the way the microfibrils are arranged in the cell wall. The pattern of stress in a cylindrical cell favours lateral expansion rather than elongation, but transverse deposition relative to the filamentous axis of the microfibrils in the primary wall restricts lateral expansion and so growth is rather by elongation (BUGGELN, 1981). As the cell elongates, the fibrils are stretched along the axis (LOBBAN, HARRISON and DUNCAN, 1985) but the wall does not become progressively thinner indicating that new cell wall material is added during enlargement (DIXON, 1973). There are two modes of cell wall deposition:

1) apposition by which new material is added only at the inner wall surface; and

2) intusception by which new material is added throughout the wall's thickness (GREEN, 1962; DIXON, 1973).

Cellulose microfibrils are synthesized by enzyme complexes located in the plasmamembrane. It has been implicated that cortical microtubules orientate the direction of microfibril deposition (GALWAY and HARDHAM, 1986). There are three basic patterns of cell wall deposition and elongation:

1) diffuse growth with extension of cell walls by new cell wall material being deposited at random in pre-existing material; e.g. higher plants and certain green algae. The microfibrils are laid down transversely and are passively reorientated

longitudinally during elongation (GREEN, 1962; MÉtRAUX, RICHMOND and TAIZ, 1980);

2) band growth where new cell wall material is deposited in transverse bands at upper and/or lower parts of the cell. Microfibril orientation is random. This type of elongation is restricted to Rhodophyta and *Nitella* (WAALAND, WAALAND and CLELAND, 1972; WAALAND and WAALAND, 1975; GARBARY and BELLIVEAU, 1990); and

3) tip growth where expansion is confined to the apical dome with deposition of new material at growing tips; e.g. fungal hyphae, pollen tubes and root hairs. The microfibrils are randomly arranged.

Diffuse and band growth is due to mechanical properties of the cell wall while tip growth is a physicochemical phenomena involving ionic currents owing to heterogeneous Ca^{2+} concentrations in the cytoplasm which create ion channels or pumps in the plasma membranes; i.e. expansion is under cytoplasmic control (PELCE and POCHEAU, 1992).

Diffuse and band growth patterns are found in intercalary cells and can be observed by staining with Calcofluor White. If growth is by extension of the existing cell wall, the stain will be uniformly diluted. Localized deposition results in bands of unlabelled wall (WAALAND and WAALAND, 1975). Regions of elongation are constant within species but vary between species. *Griffithsia pacifica* has bipolar band growth, the apical band being more active than the basal band in each cell and *Griffithsia tenuis* C.Agardh has two bands with the basal band being more active. *Griffithsia globulifera* Harvey has unipolar band growth with the band located in the apical region of the cell. *Callithamnion* spp. and *Antithamnion kylinii* Gardner have unipolar band growth with a basal band (LOBBAN, HARRISON and DUNCAN, 1985).

Band growth is the most common form of elongation in red algae but there are exceptions to this e.g. *Audouinella dasyae* (Collin) Woelkerling, *Spermothamnion repens* (Dillwyn) Rosenvinge and *Tiffaniella synderae* (Farlow) Abbott show diffuse growth rather than localized wall deposition. Where band growth is present, it accounts for all elongation. This shows that band growth and diffuse growth are mutually exclusive processes in red algal thalli (GARBARY and BELLIVEAU, 1990).

Elongation of axial cells in *Pteroceramium* was by band growth, a single basal band being deposited in each axial cell. The high fluorescence at the base of the axial cells could possibly indicate a high chloroplast concentration with chloroplasts also fluorescing under ultraviolet light (WAALAND and WAALAND, 1975).

Elongating shoot filaments and rhizoids elongate by localised tip growth. Elongation is confined to an apical dome-shaped growth zone and the side walls of the cell are rigid. Elongation is maintained by localised deposition of wall-loosening enzymes and new cell wall material. The shape of the cell is regulated by stiffening of the lateral walls so that the apical dome yields to turgor pressure (WAALAND, 1990). Elongation by tip growth results from the interaction of the shape of the cell's growth zone, the gradient in growth rate within the growth zone and the direction of expansion (BUGGELN, 1981). This generates a cylindrical cell surface at its base. Most of the major cell organelles are absent from the growing tip, there being mainly vesicles derived from Golgi complexes in the growth zone (GREEN, 1962).

There are two contrasting views about the organisation of growing tips:

1) The pole of the tip is structurally and biochemically different from the rest of the cell. This is the more conventional approach and seeks the cause of a large scale phenomenon in behaviour of smaller parts of the system; or

2) The organisation of the tip is viewed as a whole with its pole having a special significance only in the way that the existence of an entire circle conveys some special significance to its centre. This more holistic approach explains the developing shape in terms of control of differentiated growth on the whole system scale as a consequence of mechanical forces acting over distance; e.g. mechanical properties of cell wall or diffusional spread of morphogenetically active substances (LACALLI and HARRISON, 1987).

The maintenance of tip growth involves the perpetuation of certain types of gradients down the growth zone (GREEN, 1962). It has been found that Ca^{2+} is essential in tip growth, affecting both the rate of vesicle fusion and the state of contraction of microfilaments (rigidity) at the tube tip. Localised fusion of secretory vesicles with the apical plasma membrane supplies carbohydrates necessary for the growing tube. The microfilament network at the tip must be sufficiently relaxed to allow the tip to be pushed forward by turgor pressure of the cytoplasm (PICTON and STEER, 1983). Tip to base Ca^{2+} gradients have been found in growing pollen tubes, root hairs, moss caulonema, fungal hyphae and *Acetabularia* (REISS and HERTH, 1979; ROBSON, WIEBE and TRINCI, 1991).

An extensively worked example is in fucoid zygotes which show structural and biochemical polarity established at the time of axis formation, an electrical current having influx at the presumptive rhizoid pole and net efflux at the presumptive thallus pole. Ca^{2+} channels localised at the presumptive rhizoid pole cause a net Ca^{2+} influx as seen by chlorotetracycline fluorescence being restricted to the rhizoid hemisphere. This helps maintain tip growth but plays no role in polarization. Rather, microfilaments are important in axis formation (BRAWLEY and ROBINSON, 1985; KROPF and QUATRANO, 1987; QUATRANO and KROPF, 1989).

Tip growth was observed in *Pteroceramium* in the rhizoids as seen by the fluorescent stains with chlorotetracycline. This shows that a Ca^{2+} gradient is operating in rhizoid growth.

6.4.2 Chemical controls on growth

Studies show that levels of endogenous hormones in seaweeds are similar to levels measured for higher plants (BRADLEY and CHENEY, 1991). Their concentration range is usually between 0.001-10.0 mg l^{-1} (BRADLEY and CHENEY, 1990); e.g. application of concentrations above 10^{-3} M auxin to *Alaria esculenta* (Linnaeus) Greville inhibited growth. IAA has been shown to be present in *Caulerpa paspaloides* (Bory) Greville using bioassays, HPLC and GC-MS. The concentration was approximately 1 mg IAA kg^{-1} fresh weight. This is similar to concentrations found in angiosperms.

However, this IAA may have come from bacteria around the thallus (JACOBS, FALKENSTEIN and HAMILTON, 1985).

There are many examples of higher plant hormones being found in algae (Table 6.20); e.g. *Codium fragile* subsp. *tomentosoides* showed a seasonal IAA response with a broad summer optimum between 10^{-9} M to 10^{-6} M and a winter optimum of 10^{-5} M. Seawater from the same study site also showed a distinct seasonal cycle in its ability to support growth, suggesting the presence in seawater of a growth promoter in summer and a growth inhibitor in winter (HANISAK, 1979).

Table 6.20 Some examples of plant hormones found in algae.

Species	Plant Hormone	Response	Reference
Caulerpa sertularioides (Gmelin) Howe	auxin	-	MISHRA and KEFFORD, 1969
Caulerpa prolifera	auxin	elongation of rhizoids and proliferation of blades	DAWES, 1971
Acetabularia	auxin	promotes stalk elongation, inhibits rhizoid growth	EVANS and TREWAVAS, 1991
Sargassum heterophyllum	auxin	-	EVANS and TREWAVAS, 1991
Porphyra umbilicalis (Linnaeus) J.Agardh	auxin, cytokinin	increase callus production	LIU and KLOAREG, 1991
Agardhiella subulata	auxin, cytokinin	increase cell division and callus formation	BRADLEY and CHENEY, 1991
Chondrus crispus	auxin, cytokinin	improve growth in tissue culture	CHEN and TAYLOR, 1978
Caulerpa taxifolia (Vahl) C.Agardh	cytokinin	-	BRADLEY, 1991
Porphyra perforata	cytokinin	-	BRADLEY, 1991

Table 6.20 Continued

Species	Plant hormone	Response	Reference
Hypnea musciformis (Wulfen) Lamouroux	cytokinin	stimulates phycoerythrin synthesis	JENNINGS, BROUGHTON and McCOMB, 1972
Ecklonia maxima	cytokinin	-	EVANS and TREWAVAS, 1991
Durvillea potatorum (Labillardière) Areschoug)	cytokinin	-	EVANS and TREWAVAS, 1991
Fucus vesiculosus	cytokinin	promotes branching	EVANS and TREWAVAS, 1991
Sargassum muticum (Yendo) Fensholt	cytokinin	-	BRADLEY, 1991
Ulothrix	gibberellin	promotes cell division	EVANS and TREWAVAS, 1991
Ulva	gibberellin	promotes cell division	EVANS and TREWAVAS, 1991
Ecklonia radiata (C.Agardh) J.Agardh	gibberellin	-	BRADLEY, 1991
Gracilaria	gibberellin	improves growth	BRADLEY and CHENEY, 1990
Enteromorpha prolifera	gibberellin	-	BRADLEY, 1991
Ecklonia	absisic acid	-	EVANS and TREWAVAS, 1991
Ulva lactuca	absisic acid	-	EVANS and TREWAVAS, 1991
Petroglossum	absisic acid	inhibits morphogenesis	EVANS and TREWAVAS, 1991
Ascophyllum nodosum (Linnaeus) LeJolis	auxin, cytokinin, absisic acid	-	EVANS and TREWAVAS, 1991

Regeneration of severed filaments of *Griffithsia* has a very regular polarity with rhizoids always forming at the base and shoot cells regenerating from the apex piece. During cell repair, a morphogenetic substance termed rhodomorphin is produced in the rhizoids, which grow to link the two severed cells. Rhodomorphin has a carbohydrate and protein component and is the first endogenous morphogenetic hormone characterised from red algae. There is evidence that it is produced by a number of other red algae (WAALAND, 1989).

An iso-pentenyl type cytokinin and high levels of zeatin and their derivatives have been found in algal extracts (MOONEY and VAN STADEN, 1987). There are many seaweed extracts used as fertilizers and their use result in a general improvement to crops by supplying both cytokinins and nutrients as organic compounds; e.g. amino acids and vitamins (CROUCH and VAN STADEN, 1993). The common seaweeds used in South Africa as fertilizers are *Ecklonia maxima* (Osbeck) Papenfuss (FEATONBY-SMITH and VAN STADEN, 1984) and *Sargassum heterophyllum* (Turner) J.Agardh (MOONEY and VAN STADEN, 1987).

Zeatin and an as yet unknown cytokinin-like compound have been identified in *Macrocystis pyrifera*. The concentrations of these compounds are the highest in young actively growing blades. This is similar to higher plants where levels are also the highest in meristematic regions. The concentrations also vary throughout the year and this correlates to growth. Application of exogenous zeatin stimulates growth (De NYS, JAMESON, CHIN, BROWN and SANDERSON, 1990; De NYS, JAMESON and BROWN, 1991). *Sargassum heterophyllum* has lunar periodicity of spore release. This coincides with increased cytokinin activity in the reproductive laterals (MOONEY and VAN STADEN, 1984).

There are two approaches used to establish the presence of a plant growth regulator:

1) extraction techniques and bioassays; and

2) study of physiological response of algae to exogenous application of growth substance (MOONEY and VAN STADEN, 1986).

Exogenous applications of the hormones IAA, GA_3 and kinetin did not elicit any growth or morphogenetic response from *Pteroceramium* and the Mung Bean Bioassay to test for auxin produced negative results. However, this work is inconclusive as there are many other possible reasons, listed below, for this lack of response.

When one interprets results of exogenously applied plant growth regulators, there are many considerations: the substance may not have been taken up or, if it was absorbed and a negative response obtained, this may indicate that there were already optimal concentrations present in the plant. If results are positive, this does not necessarily prove that the compound is a native regulator in the plant. The response may be due to the plant already containing sufficient endogenously produced plant growth regulator or the compound may have been absorbed from the seawater or epiphytic bacteria and that addition of more compound increases the endogenous levels to inhibitory or supraoptimal levels (BUGGELN, 1976).

Algae growing in optimum conditions are unlikely to have growth promoted further by addition of growth substances (EVANS and TREWAVAS, 1991). Another problem with exogenously applied plant growth regulators is that there is uncertainty in the interpretation of results as unknown amounts are absorbed by the plant and its distribution and extent to which the endogenous levels are actually altered by the treatment is unknown (TREWAVAS, 1991). Exogenous effects are not evidence that the test organism normally uses the compound to regulate processes (PROVASOLI and CARLUCCI, 1974).

Bioassays can detect physiological activity of a substance. They detect certain kinds of hormones but will miss others and results may be complicated by other factors such as penetration or metabolism (BANDURSKI and NONHEBEL, 1984). Bioassays need large quantities of seaweed because plant growth regulators are usually present in exceedingly small quantities. Standard bioassays are also unreliable for seaweeds, as the chemical composition of seaweeds may be different from angiosperms (BUGGELN, 1976). Results obtained from bioassays need to be confirmed by other tests such as GC-MS. GC-MS analyses were not attempted for *Pteroceramium* as all the other hormone results were negative.

Another problem with bioassays and extraction techniques is that sterile material must be used. Microorganisms are known to synthesise all of the five plant hormones as secondary metabolites which they may release into the seawater and so affect algal growth (MOONEY and VAN STADEN, 1986; BRADLEY, 1991; BRADLEY and CHENEY, 1991; EVANS and TREWAVAS, 1991). Seawater has also been shown to contain some active compounds (HANISAK, 1979; BRADLEY, 1991).

The use of bioassays also has uncertainties in interpretation owing to the lack of specificity in responding tissue. On the other hand, GC-MS allows for the identification of hormones but gives no indication of the potential presence of other unknown plant growth regulators and no indication of their function (BANDURSKI and NONHEBEL, 1984; EVANS and TREWAVAS, 1991). Low concentrations of endogenous plant growth regulators is not necessarily an indication of the scarcity of a substance but can also indicate that they are being utilized rapidly (MOONEY and VAN STADEN, 1984).

Any of the above reasons may have contributed to the negative results achieved for *Pteroceramium* when grown in the various hormone treatments, although there is also the possibility of hormones not playing any role in its growth.

Polyamines are found in a wide range of cells. Polyamine synthesis is regulated by feedback control mechanisms involving key biosynthetic enzymes with short half lives. The half life of ornithine decarboxylase is 10 minutes, arginine decarboxylase is 60 minutes-120 minutes and s-adenosylmethionine (SAM) decarboxylase is between 30 minutes-60 minutes. This allows for rapid modulation of polyamine levels in response to various stimuli (PEGG and McCANN, 1982; MINOCHA, 1988).

Fungi and animals synthesize putrescine via the ornithine decarboxylase pathway whereas plants have duel synthesis pathways of ornithine decarboxylase and arginine decarboxylase pathways (Fig. 6.32; PEGG and McCANN, 1982; BRADLEY, EL-FIKI and GILES, 1984; COHEN, ARAD, HEIMER and MIZRAHI, 1984; ADIGA and PRASAD, 1985; SMITH, 1985). These two pathways are interconvertible in plants

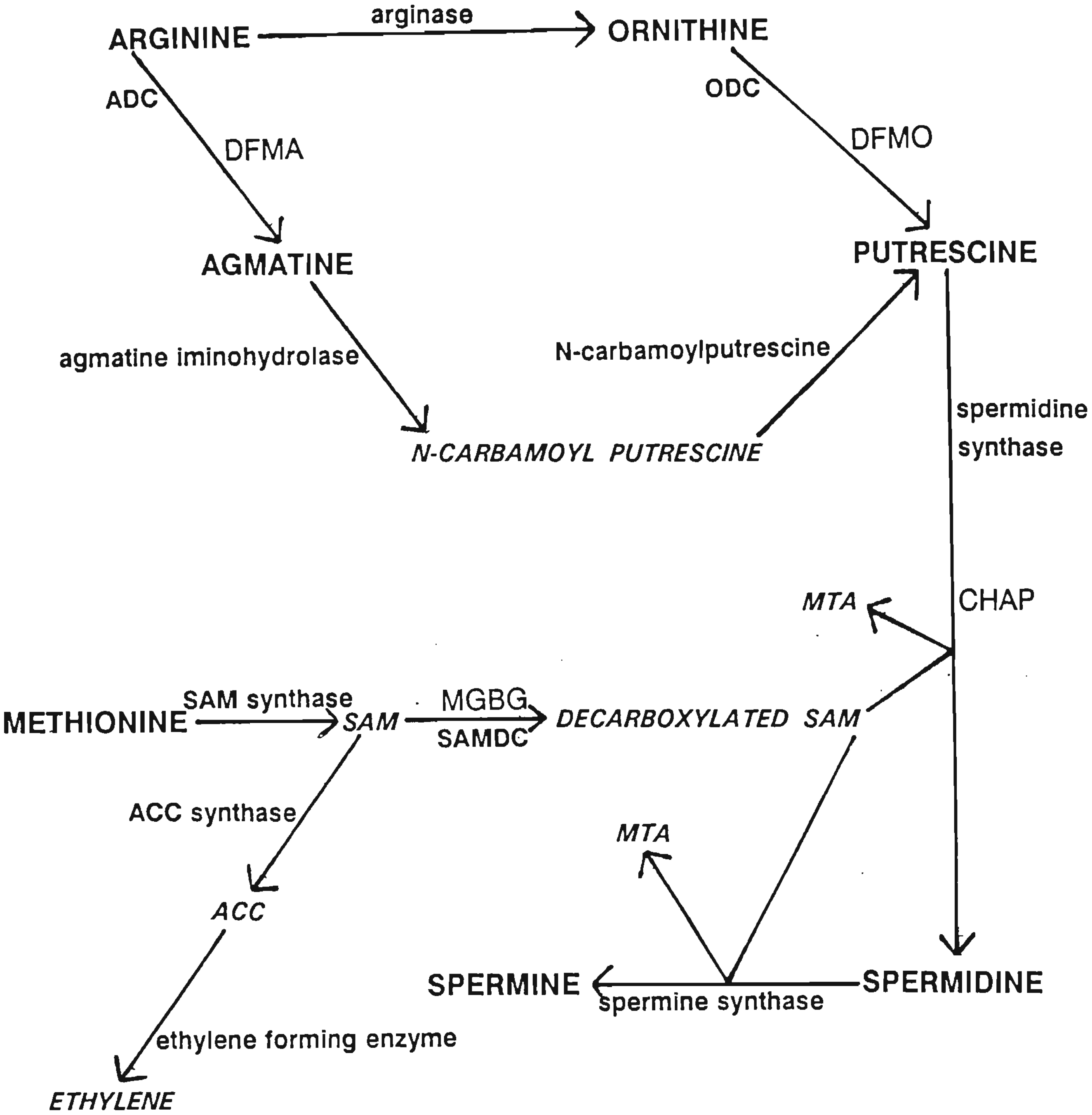


Fig. 6.32 Major biosynthetic pathway of polyamines in plants. SAM = s-adenosylmethionine, ACC = 1-aminocyclopropane-1-carboxylic acid, MTA = methylthioadenosine, DFMA = difluoromethlarginase, DFMO = difluoromethlyornithine, CHAP = cyclohexamine hydrobromine, MGBG = methylglyoxal-bis (guanylhydrazone), ADC = arginine decarboxylase, ODC = ornithine decarboxylase, SAMDC = s-adenosylmethionine decarboxylase.

(GALSTON, 1983). The ratio of arginine decarboxylase to ornithine decarboxylase varies between species, tissue type and the physiological state of the tissue.

Ornithine decarboxylase is involved in cell division; e.g. there are elevated ornithine decarboxylase activity associated with intense mitotic division in meristems of tomato and potato and a gradient decreasing down the stem. This reflects a need for high levels of polyamines for intensive synthesis of DNA, RNA and proteins with ornithine decarboxylase activity associated with cell division. Arginine decarboxylase activity showed only a slight gradient down the stem indicating that it has a different role (COHEN, ARAD, HEIMER and MIZRAHI, 1982a; COHEN, HEIMER and MIZRAHI, 1982b).

Arginine decarboxylase concentrations are altered in response to various stresses; e.g. osmotic shock, K^+ deficiency, Mg^{2+} deficiency, acidification, high temperatures and high salinity (GALSTON, 1983; SMITH, 1985; EVANS and MALMBERG, 1989). There are also increases in polyamine concentrations to overcome toxic effects of heavy metals in algae. *Chlorogonium elongatum* (Dangeard) France increases putrescine and spermidine concentrations when treated with mercury. This was from increased arginine decarboxylase activity rather than ornithine decarboxylase activity (AGRAWAL, AGRAWAL, LEE, KRAMER and PILLAI, 1992).

There are other possible minor routes for polyamine synthesis; e.g. from cadaverine or citrulline (ADIGA and PRASAD, 1985). Other minor polyamines found in algae and higher plants may contribute to cellular pools but are not significant sources (MINOCHA, 1988).

Putrescine is a diamine, spermidine a triamine and spermine a tetramine. Primary amines have more than two amine groups (EVANS and MALMBERG, 1989). Free polyamines are positively charged at intracellular pH (GALSTON, 1983; EVANS and MALMBERG, 1989). To convert putrescine to spermidine, an aminopropyl group is added. This is derived from methionine via SAM which is decarboxylated (Fig. 6.32). This step is often rate limiting in spermidine formation. To convert spermidine to spermine, another aminopropyl group also derived from SAM is needed (PEGG and

McCANN, 1982). Another pathway contributing to polyamine biosynthesis is converting methionine through SAM production to spermidine and spermine. SAM is also a precursor of ethylene and suggests some feedback control as competition arises for the common precursor (GALSTON, 1983; EVANS and MALMBERG, 1989). Ethylene is produced in response to stress, tissue damage and senescence. Exogenous application of polyamines delay senescence, indicating some competition for SAM which is a source for spermidine, spermine and ethylene synthesis (ROBERTS, WALKER, THOMPSON and DUMBROFF, 1984).

Use of inhibitors reverses the process and the effect of inhibitors should be overcome by the addition of the appropriate polyamine (EVANS and MALMBERG, 1989). MGBG is a highly specific inhibitor of SAM decarboxylase (PEGG and McCANN, 1982). The inhibitor DFMO is highly specific and can significantly reduce ornithine decarboxylase activity *in vivo* (HAMANA and MATSUZAKI, 1982).

Little is known about other structurally related polyamines. Norspermidine and norspermine have been found in some thermophilic and halophilic bacteria. Norspermine being the most widely distributed in the eukaryotic algae, being found in seven phyla (HAMANA and MATSUZAKI, 1982). Homospermidine is known in some algae, bacteria and higher plants. Macroalgae are also rich in cadaverine (diamine) and agmatine is also present (Table 6.21; KNEIFEL, 1979; HAMANA and MATSUZAKI, 1982; AGRAWAL, AGRAWAL, LEE, KRAMER and PILLAI, 1992).

Various monoamines; e.g trimethylamine, choline and ethanolamine, derived from amino acids by decarboxylation, are also found in algae with the highest concentrations in the Rhodophyta. The other polyamines are found in the highest concentrations in the green microalgae (KNEIFEL, 1979). Acetylcholine is commonly found in animals, fungal hyphae, bryophytes and higher plants, stimulating germination. It is localised within membranes and increases ATP levels. The lack of effect with exogenous application shows poor penetration into plant tissues (TRETYN and KENDRICK, 1991).

Table 6.21 Cellular concentrations of polyamines in Rhodophyta. Put = putrescine, Spd = spermidine, Spm = spermine, Nspd = norspermidine, Nspm = norspermine, Cad = cadaverine, Hspd = homospermidine and Agm = agmatine (from HAMANA and MATSUZAKI, 1982).

	Polyamine (μmol g^{-1})							
Species	Put	Spd	Spm	Nspd	Nspm	Cad	Hspd	Agm
Nemalion pulviratum	1.266	0.004	0.001	0.008	0.004	0.416	0.00	0.008
Chondrococcus japonicus	1.319	0.002	0.000	0.007	0.001	0.333	0.000	0.005
Meristotheca papulosa (Montagne) J.Agardh	0.158	0.001	0.000	0.000	0.001	0.023	0.000	0.001
Gelidium amansii Lamouroux	0.147	0.001	0.001	0.001	0.000	0.033	0.000	0.000
Amphiroa dilatata	0.004	0.001	0.001	0.000	0.001	0.002	0.000	0.001
Porphyra tenera	0.01	0.01	0.12	0.00	0.00	0.00	0.00	0.00

During screening algae for polyamines, the best results are obtained using HPLC although these results are not conclusive as there are many homologies. Norspermidine has been detected in various algae and is homologous with spermidine, while norspermine is relatively rare in algae and is homologous with spermine. To separate these, GC-MS is necessary (KNEIFEL, 1979).

When using HPLC, putrescine and spermidine peaks were detected for *Pteroceramium*. *Scenedesmus acutus* has 0.5 mg-1.1 mg spermidine g^{-1} dry mass and *Chlorella* 2 mg-2.5 mg spermidine g^{-1} dry mass. For these two algae there was a decrease in spermidine concentration with age and decreasing temperature (KNEIFEL, 1979). Freshwater algae always have putrescine and spermidine while spermine is sometimes not detectable. When it is, it is usually in the lowest concentration (KNEIFEL, 1979). Spermine is detected in a limited number of species although species where it is not found are rich in putrescine and spermidine (HAMANA and MATSUZAKI, 1982). Only spermidine occurring in measurable quantities in *Pteroceramium*, may indicate an alternate pathway of biosynthesis, possibly starting with arginine which had a pronounced effect on *Pteroceramium*'s rate of growth.

Exogenous applications of arginine to *Pteroceramium* caused an increased growth rate. However, this may have been due to providing a nitrogen source (SMITH, 1985), although this is unlikely as the medium used should provide sufficient nitrogen. The higher applications of polyamines to *Pteroceramium* generally inhibited both growth and the size of the wings. This can be interpreted as a positive indication that some uptake did occur.

If polyamines are produced in adequate quantities by an organism, their role in growth is not manifested. Some block is necessary to reveal the need for the metabolite (GALSTON, 1983). More promising results were obtained with application of specific polyamine inhibitors to *Pteroceramium*. Even when these were applied at low concentrations (10^{-5} M), growth and wing expansion were inhibited, indicating that polyamines do play some fundamental role in the development of *Pteroceramium* although their exact role could not be determined.

The apex is a self-determining region controlling differentiation (HILLMAN, 1984). Apical dominance is the control exerted by apical portions of the shoot over outgrowths of lateral buds. Thus the branching pattern and hence plant form is determined to a large extent by apical control (CLINE, 1991). Apical dominance can be viewed as a survival mechanism when the apical shoot is damaged; e.g. by foraging animals or wind, providing a reservoir of meristems (PHILLIPS, 1975; CLINE, 1991).

Each apex may influence the development and positioning of lateral structures derived from the same or different apices. Complete or partial inhibition of positioning and development of lateral buds, auxiliary shoots, leaves etc. by an actively growing apical region on the same or different shoot is termed "correlative inhibition". Correlative control in higher plants can be regarded as a form of positional signalling. Excision of the apical portion is a simple method of eliminating correlative inhibition (HILLMAN, 1984). If the apical dominance effect is strong, there is little or no lateral bud outgrowth. However, if apical dominance is weak, the more basal lateral shoots grow to a greater length than the more apically situated lateral shoots (CLINE, 1991).

Basic apical dominance control is at the gene level but environmental and physical factors can override these genetic features. Increased irradiance can weaken the apical dominance effect and altered gravity can overcome apical dominance with plants displaying gravimorphism (PHILLIPS, 1975; CLINE, 1991).

Pteroceramium shows a definite apical dominance effect, the dominant apex controlling the number of adventitious branches produced by the pericentral cells. It is interesting to note that more adventitious branches are produced further away from the apex than at the tip. This may be that more mature pericentral cells have a better ability to produce adventitious branches. Removal of the apex of subordinate branches had no effect on adventitious branch formation, implying that the dominant apex exerts total control. The dominant apex may also exert some control over subordinate branch axial cell elongation as the subordinate branches are all shorter even though they have more axial cells.

Apical dominance has been reported in a number of other algae. In *Apoglossum ruscifolium* Kylin, *Antithamnion plumula* and *Ascophyllum nodosum* where removal of the apical cell induces lateral bud (branch) formation while lateral branch elongation is accelerated with removal of the apical cell as in *Sphacelaria cirrhosa* C.Agardh (BUGGELN, 1981). There is increasing potential to produce new shoot cells the further the original position of the axial cell is from the filament axis in *Griffithsia pacifica* (DUFFIELD, WAALAND and CLELAND, 1972).

An apical cell in *Fucus vesiculosus* inhibits regeneration of branches from a cut surface and once the apical cell is removed, there is branch regeneration (MOSS, 1965). At a distance of 2 mm from the apex, no branches are initiated. A few branches are produced 5 mm away and more branches 15 mm away from the tip. Thus in *Fucus* *vesiculosus*, apical dominance is restricted to only a short distance behind the apex. Germinating oospores of *Fucus vesiculosus* also have apical cells which prevent development of new meristems in its vicinity (MOSS, 1966).

Surgical experiments with *Sargassum muticum* show that there is a zone of inhibition in a limited distance behind the apex with a gradation of dominance effect. Primary lateral apices inhibit extension of secondary laterals and secondary lateral apices inhibit extension of tertiary laterals (CHAMBERLAIN, GORHAM, KANE and LEWEY, 1979).

Experiments with *Antithamnion defectum* staining with Calcofluor White, showed that each axial cell has two bands of elongation - one at the top and one at the bottom. The band forming at the bottom expands first and remains dominant. When all the apices are removed, three or more poorly defined transverse bands are formed. When the main apex is removed, the elongation zone moves towards the upper end of the cell. This shows that regulation of cell wall deposition bands is under apical control. The farther removed a cell is from the apex, the more likely it is to have disrupted bands. There is a hierarchy of control, the apical cell of the main axis being dominant and less dominance being shown by the apical cells of indeterminate laterals. This is similar to apical dominance in higher plants (GARBARY, BELLIVEAU and IRWIN, 1988).

Developmental events of one cell can be influenced by cells at a distance. Decapitation of a main axis in *Antithamnion* induces the formation of new indeterminate axes from the basal cells of determinate branches. Thus the apical cell of an indeterminate axis seems to exert an inhibitory effect on the initiation of new axes of indeterminate growth. The rate of elongation of whole filaments is also altered by excising these filaments from the plant or removing the apex of the main axis. This suggests some form of intercalary communication with both growth-promoting and -inhibiting substances moving along filaments, controlling elongation in lateral branches (WAALAND, 1990).

The most common theory for control of apical dominance in higher plants is the direct effects of auxin. Auxin is produced in the terminal shoot and migrates down the stem, suppressing lateral bud outgrowth. Although evidence for the direct role auxin plays in apical control is inconclusive, it suggests that auxin plays a fundamental role in control of apical dominance (PHILLIPS, 1975; BRADLEY, 1991; CLINE, 1991). Cytokinins also have some effect in being able to stimulate lateral bud outgrowth. Evidence suggests that cytokinins play a secondary role, promoting lateral bud outgrowth after the removal of auxin (PHILLIPS, 1975; CLINE, 1991).

Another possible theory for apical dominance is the nutritional effect. The primary requirement of lateral bud outgrowth is nutrients in its vicinity. The terminal bud acts as a sink for mineral nutrients, sucrose and water and so deprives the lateral buds (PHILLIPS, 1975; CLINE, 1991). Another possible theory is that the nutrients are directed towards the highest concentrations of auxin (PHILLIPS, 1975; BUGGELN, 1981; CLINE, 1991).

Although it has not yet been shown that endogenously produced growth substances provide the actual control mechanism for apical dominance, "regardless of how this is controlled at the biochemical level, apical control may be a key to understanding morphogenesis and differentiation of red algal cells and thalli." (GARBARY, BELLIVEAU and IRWIN, 1988). Application of auxin to *Fucus vesiculosus* appears to inhibit lateral bud formation (MOSS, 1965) while addition of IAA to *Sargassum muticum* on primary apical sections does decrease growth of the primary laterals (CHAMBERLAIN, GORHAM, KANE and LEWEY, 1979).

Experimental guidelines for the presence of hormones are that if the site of hormone production is excised, the response should stop. This response should be restored with the addition of a chemical known to be an appropriate hormone; i.e. should show a specific responses (BRADLEY, 1991).

Exogenous application of IAA to *Pteroceramium* did not reverse the effect of removal of the dominant apex, implying that apical dominance is not controlled by IAA but some other factor, as yet unknown, possibly a polyamine. However, the experimental setup was not ideal as IAA was not applied directly to the excised region but instead the whole plant was emersed. Algae take up nutrients directly from seawater through all parts of their thallus and can therefore accumulate in the tissue elements which are in trace amounts in seawater (MOONEY and VAN STADEN, 1986). This also means that it is difficult to make localized applications.

Chapter 7

Conclusions

7.1 Taxonomy of *Pteroceramium*

Pteroceramium has a distinct morphology with a cellulosympodial growth pattern. Its main diagnostic feature is the distichously arranged paired wings arising from Pc1 and Pc6. Similar wings are not found in any of the *Ceramium* species although some do have spines. It is for this reason that AKEN and NORRIS (in prep.) propose to separate *Pteroceramium* into a new genus.

The wings of *Pteroceramium* do show some phenotypic plasticity, decreasing in size when grown in higher irradiances (193 μmol m^{-2} s^{-1}). However, they are still an obvious character. The size of the wings are constant when grown in different photoperiods, temperatures, nitrogen and phosphorus levels, salinities and pHs. The only environmental conditions which caused a total reduction of the wings, was burial in sand. This could confuse the taxonomy of the species if such thalli were collected. However, as soon as the material had grown out of the sand, the characteristic morphology with distinct wings was resumed. The distinctiveness and persistence of the wings under a wide range of culture conditions suggest that they will make a useful and diagnostic taxonomic character for the new genus.

Other distinguishing features of *Pteroceramium* are:

1) the six pericentral cells with Pc1 being the largest formed and Pc6 the second largest formed;

2) the pericentral cells form in a set sequence with Pc1 forming on the outer face of the segment according to the preceding pseudodichotomy, Pc2 and Pc3 forming on the lower and upper surface respectively nearest to Pc1, Pc4 and Pc5 forming

on the lower and upper surface respectively farthest from Pc1 and Pc6 forming on the inner face. This sequence is set, being unaffected by the direction of illumination and gravity;

3) uniseriate spines consisting of 3-5 cells forming from Pc2, Pc3, Pc4 and Pc5;

4) pit connections with a plug core but no cap layers. This is common in the Ceramiales;

5) transverse divisions of the dome-shaped apical cell to produce subapical cells and oblique divisions to form pseudodichotomous branches;

6) axial cell elongation by the deposition of new cell wall material in a single basal band; and

7) the typical *Polysiphonia*-type life history with spermatangia covering the entire cortical band and wings, and a carpogonial branch which develops in place of the outer wing with Pc1 as its supporting cell. The tetraspores are cruciate rather than the typical tetrahedral arrangement found in *Ceramium* species.

The proposal by AKEN and NORRIS (in prep.) to place *Pteroceramium* in a new genus separated from *Ceramium* has been supported by this study. Further hybridization experiments crossing the male gametophyte of *Pteroceramium* with fertile female gametophytes of some heavily corticated species of *Ceramium* need to be performed to test the biological species concept.

7.2 Distribution of *Pteroceramium*

The physiological tolerance limits determined in culture do provide some insight into the fundamental niche of a species, although the actual realised niche in nature is likely to be much smaller because of competition with other species. Nevertheless, the results obtained in this study allow insight into the possible distribution of *Pteroceramium*.

7.2.1 Vertical distribution

Supralittoral and upper eulittoral algae generally have a greater capacity to tolerate wider ranges of temperature, salinity, desiccation stress and higher irradiances than lower eulittoral and sublittoral algae.

Pteroceramium grew best at lower irradiances (83 μmol m^{-2} s^{-1}) with near inhibitory levels at 193 μmol m^{-2} s^{-1} as seen by a decrease in pigment content at this higher irradiance. These results are supported by the photosynthetic performance of *Pteroceramium* which was best when preconditioned at 80 μmol m^{-2} s^{-1}. Photosynthesis in *Pteroceramium* was inhibited when it was exposed to a high irradiance (3000 μmol m^{-2} s^{-1}) similar to full sunlight for over 30 minutes and the alga was not able to recover fully from this exposure, as seen by reduced photosynthetic rates.

If *Pteroceramium* were growing in the eulittoral region, it would be regularly exposed to such high irradiances during ebb tide. Its inability to recover fully would greatly reduce its growth rate. Ecological implications are that *Pteroceramium* would be outcompeted in the eulittoral region and is more likely to survive in the less physiologically stressful sublittoral conditions.

Pteroceramium showed typical shade-type features. Morphological adaptations to low irradiance conditions were a thin thallus with little non-photosynthetic material and a large surface area. Physiological adaptations included a low I_k value and an ability to adjust the pigment content. These results suggest that *Pteroceramium* is adapted to light attenuated environments such as can be found on the shallow reefs off the Natal south coast. This corresponds to where it has been found as an epiphyte growing on known deep water algal species and in shaded rocky overhangs on the lower eulittoral region.

Pteroceramium also showed a narrow tolerance limit to salinity and pH. The inability of *Pteroceramium*'s photosynthetic apparatus to adjust to rapid fluctuations in temperature and pH suggests that it grows in the sublittoral region where such conditions are more stable than in the eulittoral region where such temperature and pH fluxes occur with each tidal cycle.

The photosynthetic work done on *Pteroceramium* has laid a foundation for future work. This work may include:

1) to test a broader range of irradiance levels between 819 μmol m^{-2} s^{-1} and 3114 μmol m^{-2} s^{-1} to determine at which levels photoinhibition sets in;

2) to investigate the length of time for the two phases of recovery after photoinhibition to determine how rapid recovery is;

3) to find the length of time required for temperature acclimation, which is presently known to be under one week; and

4) to determine whether CO_2 is limiting to photosynthesis.

7.2.2 Horizontal distribution

Based on *Pteroceramium*'s growth response to temperature, it can be concluded that it is a warm temperate/subtropical east-coast species extending from the cooler Cape south coast around Port Alfred to southern Natal. This distribution falls within the critical daylengths (10 hours) required for maximum growth rates of *Pteroceramium*. *Pteroceramium* is able to photosynthetically acclimatize to slow changes in temperature as would be found seasonally in this area.

Future work would include experiments to test a broader temperature range to find the photosynthetic/temperature curve for *Pteroceramium*. This would help in defining the potential geographic distribution of the species.

7.3 Function of the wings

Pteroceramium appears to grow in low irradiance conditions typical of sublittoral reef environments and shaded habitats on the eulittoral region and the wings appear to have evolved to increase the surface area of the alga for light interception. In the highest irradiance tested (193 μmol m^{-2} s^{-1}), there was a decrease in the wing size, suggesting that where irradiances are saturating for growth, it is not necessary to have a large surface area for light capture, although the wings were persistent.

When comparing the photosynthetic rates on a pigment content basis of *Pteroceramium* and the wingless *Ceramium glanduliferum*, *Pteroceramium* had the higher α value, indicating that its pigments were more efficient at capturing irradiance than those of *Ceramium glanduliferum*. This may be due to the wings providing a larger surface area to volume ratio.

This theory is backed up by the ultrastructural study. The axial cells are dominated by a large central vacuole which correlates to the large percentage increase in size with only a few peripheral chloroplasts, mitochondria and floridean starch granules. The main function of the axial cells would be to increase plant height. The cortical and wing cells have a much denser cytoplasm with a few small vacuoles and numerous closely packed chloroplasts and a higher proportion of floridean starch granules than the axial cells. This indicates that the main function of the cortical cells and wing cells is photosynthesis.

7.4 Growth regulation in *Pteroceramium*

Pteroceramium exhibits well defined growth patterns. There is a single basal band laid down during the elongation of each axial cell and the rhizoids exhibit tip growth controlled by Ca^{2+} concentrations. The cortical band is formed in a set sequence which was not altered by the direction of the light or gravity. This shows that there is rigid internal control of growth and patterns of development in *Pteroceramium*.

Exogenous application of higher plant hormones had no effect on the growth and morphogenesis of *Pteroceramium* but more promising results were achieved with exogenous application of polyamines, their precursors, especially arginine which improved growth when applied in low concentrations, and their inhibitors which caused a reduction in growth rates. Spermidine concentrations were also quantified in *Pteroceramium* tissue at concentrations of approximately 38 μg spermidine g^{-1} fresh weight although further research needs to be carried out to identify conclusively that spermidine was present. This would be done using a GC-MS. To verify that polyamine uptake does actually occur, radioactive polyamines precursors, especially arginine, need to be applied to *Pteroceramium*. The exact role that the polyamines play in the development of *Pteroceramium* was not elucidated and needs further investigation. These results hold promise for future tissue culturing of algae whereby growth could possibly be enhanced by addition of polyamines to the growth media.

Pteroceramium also has some form of internal communication with the dominant apical tip suppressing the development of the subordinate branches, mainly by reducing axial cell elongation rather than apical cell division. With the removal of the dominant apical tip, there was an increase in the production of adventitious lateral branches while the subordinate tips did not take over growth. Removal of subordinate tips had no similar response, indicating that the dominant apical tip influences growth. This is similar to apical dominance in higher plants and provides circumstantial evidence for chemical regulation of growth and morphogenesis in *Pteroceramium*.

If such apical dominance mechanisms operate in other algae, this may be a way of increasing the production in culture. The numerous adventitious branches formed each have the potential to form a separate individual plant if removed; i.e. there is an increase in the number of viable apical cells produced. Although on such a small alga as *Pteroceramium*, a process such as this would be time consuming, on larger species this could be a method of enhancing production in culture.

References

ADIGA, P.R. and PRASAD, G.L. 1985. Biosynthesis and regulation of polyamines in higher plants. *Plant Growth Regulation*, 3: 205-226

AGHAJANIAN, J.G. and HOMMERSAND, M.H. 1980. Growth and differentiation of axial and lateral filaments in *Batrachospermum sirodotii* (Rhodophyta). *Journal of Phycology*, 16: 15-28

AGRAWAL, S.B., AGRAWAL, M., LEE, E.H., KRAMER, G.F. and PILLAI, P. 1992. Changes in polyamine and glutathione contents of a green alga, *Chlorogonium elongatum* (Dang.) France exposed to mercury. *Environmental and Experimental Botany*, 32: 145-151

AKEN, M.E. and NORRIS, R.E. in prep.

ALGARRA, P., THOMAS, J-C. and MOUSSEAU, A. 1990. Phycobilisome heterogeneity in the red alga *Porphyra umbilicalis*. *Plant Physiology*, 92: 570-576

ALTMAN, A. 1989. Polyamines and plant hormones. *IN*: BACHRACH, U. and HEIMER, Y.M. (eds). *The Physiology of Polyamines Vol II*. CRC Press, Boca Raton: 121-145

ANG, P.O. and DE WREEDE, R.E. 1990. Matrix models for algal life history stages. *Marine Ecology Progress Series*, 59: 171-181

AXELSSON, L., CARLBERG, S. and RYBERG, H. 1989. Adaptations by macroalgae to low carbon availability. II. Ultrastructral specializations related to the function of a photosynthetic buffer system in the Fucaceae. *Plant, Cell and Environment*, 12: 771-778

AXELSSON. L., CARLBERG, S., LARSSON, C., RYBERG, H. and UUSITALO, J. 1990. CO_2 storage and CO_2 concentrating in brown seaweeds III. A tentative working model. *Current Research in Photosynthesis*, 4: 525-528

BANDURSKI, R.S. and NONHEBEL, H.M. 1984. Auxins. *IN*: WILKINS, M.B. (ed.). *Advanced Plant Physiology*. Longman Scientific and Technical, New York: 1-20

BEER, S. and ESHEL, A. 1985. Determining phycoerythrin and phycocyanin concentrations in aqueous crude extracts of red algae. *Australian Journal of Marine and Freshwater Research*, 36: 785-792

BELLIVEAU, D.J., GARBARY, D.J. and McLACHLAN, J.L. 1990. Effects of fluorescent brighteners on growth and morphology of the red alga *Antithamnion kylinii*. *Stain Technology*, 65: 303-311

BERNER, T., DUBINSKY, Z., WYMAN, K. and FALKOWSKI, P.G. 1989. Photoadaptation and the "package" effect in *Dunaliella tertiolecta* (Chlorophyceae). *Journal of Phycology*, 25: 70-78

BIRD, C.J. and McLACHLAN, J. 1986. The effect of salinity on distribution of species of *Gracilaria* Grev. (Rhodophyta, Gigartinales): An experimental assessment. *Botanica Marina*, 29: 231-238

BIRD, K.T. 1976. Simultaneous assimilation of ammonium and nitrate by *Gelidium nudifrons* (Gelidiales: Rhodophyta). *Journal of Phycology*, 12: 238-241

BIRD, N.L., CHEN, L.C-M. and McLACHLAN, J. 1979. Effects of temperature, light and salinity on growth in culture of *Chondrus crispus*, *Furcellaria lumbricalis*, *Gracilaria tikvahiae* (Gigartinales, Rhodophyta), and *Fucus serratus* (Fucales, Phaeophyta). *Botanica Marina*, 22: 521-527

BLACKWELL, J.R. and GILMOUR, D.J. 1991. Stress tolerance of the tidal pool Chlorophyte, *Chlorococcum submarinum. British Phycological Journal*, 26: 141-147

BOGORAD, L. 1975. Phycobiliproteins and complementary chromatic adaptation. *Annual Review of Plant Physiology*, 26: 369-401

BOLD, H.C. and WYNNE, M.J. 1978. *Introduction to the Algae: Structure and Reproduction*. Prentice-Hall Inc., New Jersey, 706pp

BOO, S.M., FREDRIKSEN, S., RUENESS, J. and LEE, I.K. 1991. Field and culture studies on the life history of *Campylaephora crassa* (Okamura) Nakamura (Ceramiaceae, Rhodophyta). *Botanica Marina*, 34: 437-445

BOSE, S., HERBERT, S.K. and FORK, D.C. 1988. Fluorescence characteristics of photoinhibition and recovery in a sun and a shade species of the red algal genus *Porphyra. Plant Physiology*, 86: 946-950

BRADLEY, P.M., EL-FIKI, F. and GILES, K.L. 1984. Polyamines and arginine affect somatic embryogenesis of *Daucus carota*, 34: 397-401

BRADLEY, P.M. and CHENEY, D.P. 1990. Some effects of plant growth regulators on tissue cultures of the marine red alga *Agardhiella subulata* (Gigartinales, Rhodophyta). *Hydrobiologia*, 204/205: 353-360

BRADLEY, P.M. 1991. Plant hormones do have a role in controlling growth and development of algae. *Journal of Phycology*, 27: 317-321

BRADLEY, P.M. and CHENEY, D.P. 1991. Beneficial applications of plant growth regulators to economically important seaweeds. *Proceedings of the Plant Growth Regulator Society of America*, 18: 72-77

BRAWLEY, S.H. and WETHERBEE, R. 1981. Cytology and Ultrastructure. *IN*: LOBBAN, C.S. and WYNNE, M.J. (eds). *The Biology of Seaweeds*. Blackwell Scientific Publications, Oxford: 248-299

BRAWLEY, S.H. and ROBINSON, K.R. 1985. Cytochalasin treatment disrupts the endogenous currents associated with cell polarization in Fucoid zygotes: Studies of the role of F-Actin in embryogenesis. *The Journal of Cell Biology*, 100: 1173-1184

BREEMAN, A.M. and TEN HOOPEN, A. 1984. Adaptation of photosynthesis to irradiance and daylength in the red alga *Acrosymphyton purpuriferum* (J.Ag.) Sjöst. (Rhodophyceae, Cryptonemiales), a species from the deep sublittoral region. *Phycologia*, 23: 223-231

BROADWATER, S.T. and SCOTT, J. 1982. Ultrastructure of early development in the female reproductive system of *Polysiphonia harveyi* Bailey (Ceramiales, Rhodophyta). *Journal of Phycology*, 18: 427-441

BROADWATER, S.T., SCOTT, J.L. and WEST, J.A. 1991. Spermatial appendages of *Spyridia filamentosa* (Ceramiaceae, Rhodophyta). *Phycologia*, 30: 189-195

BROWN, V., DUCKER, S.C. and ROWAN, K.S. 1977. The effect of orthophosphate concentration on the growth of articulated coralline algae (Rhodophyta). *Phycologia*, 16: 125-131

BUCHANAN, B.B. 1992. Carbon dioxide assimilation in oxygenic and anoxygenic photosynthesis. *Photosynthesis Research*, 33: 147-162

BUGGELN, R.G. 1976. Auxin, an endogenous regulator of growth in algae? *Journal of Phycology*, 12: 355-358

BUGGELN, R.G. 1981. Morphogenesis and growth regulators. *IN*: LOBBAN, C.S. and WYNNE, M.J. (eds). *The Biology of Seaweeds*. Blackwell Scientific Publications, Oxford: 627-660

CALABRESE, G. 1972. Research on red algal pigments. 2. Pigments of *Petroglossum nicaeense* (Duby) Schotter (Rhodophyceae, Gigartinales) and their seasonal variations at different light intensities. *Phycologia*, 11: 141-146

CARLBERG, S., AXELSSON, L., LARSSON, C., RYBERG, H. and UUSITALO, J. 1990. Inducible CO_2 concentrating mechanisms in green seaweeds I. Taxonomical and physiological aspects. *Current Research in Photosynthesis*, 4: 529-532

CARRINGTON, E. 1990. Drag and dislodgment of an intertidal macroalga: Consequences of morphological variation in *Mastocarpus papillatus* Kützing. *Journal of Experimental Marine Biological Ecology*, 139: 185-200

CHAMBERLAIN, A.H.L. and EVANS, L.V. 1973. Aspects of spore production in the red alga *Ceramium*. *Protoplasma*, 76: 139-159

CHAMBERLAIN, A.H.L., GORHAM, J., KANE, D.F. and LEWEY, S.A. 1979. Laboratory growth studies on *Sargassum muticum* (Yendo) Fensholt. II. Apical dominance. *Botanica Marina*, 22: 11-19

CHARNOFSKY, K., TOWILL, L.R. and SOMMERFELD, M.R. 1982. Light requirements for monospore germination in *Bangia atropurpurea* (Rhodophyta). *Journal of Phycology*, 18: 417-422

CHEN, L.C-M. and TAYLOR, A.R.A. 1978. Medullary tissue culture of the red alga *Chondrus crispus*. *Canadian Journal of Botany*, 56: 883-886

CHEUNG, K.W., LEE, K.Y. and HODGKISS, I.J. 1984. The occurrence of tetrasporangia and cystocarps on the same thalli in *Polysiphonia harlandii* (Rhodophyta: Ceramiales). *Botanica Marina*, 27: 571-572

CHOPIN, T., HOURMANT, A., FLOC'H, J-Y. and PENOT, M. 1990. Seasonal variations of growth in the red alga *Chondrus crispus* on the Atlantic French coasts. II. Relations with phosphorus concentration in seawater and internal phosphorylated fractions. *Canadian Journal of Botany*, 68: 512-517

CHOPIN, T., HANISAK, M.D. and KOEHN, F.E. 1991. Effects of seawater phosphorus concentration on floridean starch content in *Agardhiella subulata* (C.Agardh) Kraft *et* Wynne (Rhhodophyceae). *Botanica Marina*, 34: 369-373

CLINE, M.G. 1991. Apical dominance. *The Botanical Review*, 57: 318-358

COHEN, E., ARAD, S., HEIMER, Y.M. and MIZRAHI, Y. 1982a. Participation of ornithine decarboxylase in early stages of tomato fruit development. *Plant Physiology*, 70: 540-543

COHEN, E., HEIMER, Y.M. and MIZRAHI, Y. 1982b. Ornithine decarboxylase and arginine decarboxylase activities in meristematic tissues of tomato and potato plants. *Plant Physiology*, 70: 544-546

COHEN, E., ARAD, S., HEIMER, Y.M. and MIZRAHI, Y. 1984. Polyamine biosynthetic enzymes in cell cycle of *Chlorella*. *Plant Physiology*, 74: 385-388

CONRAD, H.M. and SALTMAN, P. 1962. Growth substances. *IN*: LEWIN, R.A. (ed.). *Physiology and Biochemistry of Algae*. Academic Press, New York: 663-671

CORMACI, M., FURNARI, G. and SCAMMACCA, B. 1978. On the fertile tetrasporic phase of *Cottoniella* Børgesen (Ceramiales, Rhodomelaceae, Sarcomenioideae). *Phycologia*, 17: 251-256

COUTINHO, R. and ZINGMARK, R. 1987. Diurnal photosynthetic responses to light by macroalgae. *Journal of Phycology*, 23: 336-343

CROUCH, I.J. and VAN STADEN, J. 1993. Evidence for the presence of plant growth regulators in commercial seaweed products. *Plant Growth Regulation*, 13: 21-29

CUNNINGHAM, E.M. and GUIRY, M.D. 1989. A circadian rhythm in the long-day photoperiodic induction of erect axis development in the marine red alga *Nemalion helminthoides*. *Journal of Phycology*, 25: 705-712

CUNNINGHAM, F.X., DENNENBERG, R.J., MUSTARDY, L., JURSINIC, P.A. and GANTT, E. 1989. Stoichiometry of photosystem I, photosystem II, and phycobilisomes in the red alga *Porphyridium cruentum* as a function of growth irradiance. *Plant Physiology*, 91: 1179-1187

CUNNINGHAM, F.X., VONSHAK, A. and GANTT, E. 1992. Photoacclimation in the red alga *Porphyridium cruentum*. *Plant Physiology*, 100: 1142-1149

D'ANTONIO, C.M. and GIBOR, A. 1985. A note on some influences of photon flux density on the morphology of germlings of *Gelidium robustum* (Geldiales, Rhodophyta) in culture. *Botanica Marina*, 28: 313-316

DARLEY, W.M. 1982. *Algal Biology: a Physiological Approach*. Blackwell Scientific Publications, Oxford: 163pp

DAVISON, I.R. 1991. Environmental effects on algal photosynthesis: Temperature. *Journal of Phycology*, 27: 2-8

DAWES, C.J. 1971. Indole-3-acetic acid in the green algal coenocyte *Caulerpa prolifera* (Chlorophyceae, Siphonales). *Phycologia*, 10: 375-379

DAWES, C.J. and KOCH, E.W. 1988. Physiological acclimation of the caribbean seaweeds *Eucheuma isiforme* and *Solieria filiformis* (Rhodophyta, Gigartinales) in culture. *Caribbean Journal of Science*, 24: 89-94

DAWES, C.J. and KOCH, E.W. 1990. Physiological responses of the red algae *Gracilaria verrucosa* and *G. tikvahiae* before and after nutrient enrichment. *Bulletin of Marine Science*, 46: 335-344

DeBOER, J.A., GUIGLI, H.J., ISRAEL, T.L. and D'ELIA, C.F. 1978. Nutritional studies of two red algae. I. Growth rate as a function of nitrogen source and concentration. *Journal of Phycology*, 14: 261-266

DeBOER, J.A. 1981. Nutrients. *IN*: LOBBAN, C.S. and WYNNE, M.J. (eds). *The Biology of Seaweeds*. Blackwell Scientific Publications, Oxford: 356-392

DeBUSK, T.A. and RYTHER, J.H. 1984. Effects of seawater exchange, pH and carbon supply on the growth of *Gracilaria tikvahiae* (Rhodophyceae) in large-scale cultures. *Botanica Marina*, 27: 357-362

DELIEU, T. and WALKER, D.A. 1972 . An improved cathode for the measurement of photosynthetic oxygen evolution by isolated chloroplasts. *New Phytologist*, 71: 201-225

DELIVOPOULOS, S.G. 1990. Ultrastructure of the fusion cell in *Ceramium strictum* (Rhodophyta, Ceramiales). *Cytobios*, 63: 177-184

De NYS, R., JAMESON, P.E., CHIN, N., BROWN, M.T. and SANDERSON, K.J. 1990. The cytokinins as endogenous growth regulators in *Macrocystis pyrifera* (L.) C.Ag. (Phaeophyceae). *Botanica Marina*, 33: 467-475

De NYS, R., JAMESON, P.E. and BROWN, M.T. 1991. The influence of cytokinins on the growth of *Macrocystis pyrifera*. *Botanica Marina*, 34: 465-467

DESTOMBE, C., VALERO, M., VERNET, P. and COUVET, D. 1989. What controls haploid-diploid ratio in the red alga, *Gracilaria verrucosa*? *Journal of Evolutionary Biology*, 2: 317-338

DEVINNY, J.S. and VOLSE, L.A. 1978. Effects of sediments on the development of *Macrocystis pyrifera* gametophytes. *Marine Biology*, 48: 343-348

DIXON, P.S. 1960a. Studies on marine algae of the British Isles: The genus *Ceramium*. *Journal of Marine Biological Association of the United Kingdom*. 39: 331-374

DIXON, P.S. 1960b. Studies on marine algae of the British Isles: *Ceramium shuttleworthianum* (Kütz.) Silva. *Journal of Marine Biological Association of the United Kingdom*. 39: 375-390

DIXON, P.S. 1971. Cell enlargement in relation to the development of thallus form in Florideophyceae. *British Phycological Journal*, 6: 195-205

DIXON, P.S. 1973. *Biology of the Rhodophyta*. Hafner Press, New York: 285pp

DIXON, P.S. 1982. Life histories in the Florideophyceae with particular reference to the Nemaliales *sensu lato*. *Botanica Marina*, 25: 611-621

DRING, M.J. 1986. Pigment composition and photosynthetic action spectra of sporophytes of *Laminaria* (Phaeophyta) grown in different light qualities and irradiances. *British Phycological Journal*, 21: 199-207

DUARTE, C.M. 1992. Nutrient concentration of aquatic plants: Patterns across species. *Limnology and Oceanography*, 37: 882-889

DUCKETT, J.G. and PEEL, M.C. 1978. The role of transmission electron microscopy in elucidating the taxonomy and phylogeny of the Rhodophyta. *IN*: IRVINE, D.E.G. and PRICE, J.H. (eds). *Modern Approaches to the Taxonomy of Red and Brown Algae*. Academic Press, London: 157-204

DUFFIELD, E.C.S., WAALAND, S.D. and CLELAND, R. 1972. Morphogenesis in the red alga, *Griffithsia pacifica*: Regeneration from single cells. *Planta*, 105: 185-195

EDELSTEIN, T. and McLACHLAN, J. 1967. Cystocarps and tetrasporangia on the same thallus in *Membranoptera alata* and *Polysiphonia urceolata. British Phycological Bulletin*, 3: 185-187

EDWARDS, P. 1973. Life history studies of selected British *Ceramium* species. *Journal of Phycology*, 9: 181-184

EDWARDS, P. 1977. An analysis of the pattern and rate of cell division, and morphogenesis of sporelings of *Callithamnion hookeri* (Dillw.) S.F.Gray (Rhodophyta, Ceramiales). *Phycologia*, 16: 189-196

EDWARDS, P. 1979. A cultural assessment of the distribution of *Callithamnion hookeri* (Dillw.) S.F.Gray (Rhodophyta, Ceramiales) in nature. *Phycologia*, 18: 251-263

EVANS, L.V. 1988. The effects of spectral composition and irradiance level on pigment levels in seaweeds. *IN*: LOBBAN, C.S., CHAPMAN, D.J. and KREMER, B.P. (eds). *Experimental Phycology: A Laboratory Manual.* Cambridge University Press, Cambridge: 123-133

EVANS, L.V. and TREWAVAS, A.J. 1991. Is algal development controlled by plant growth substances? *Journal of Phycology*, 27: 322-326

EVANS, P.T. and MALMBERG, R.L. 1989. Do polyamines have roles in plant development? *Annual Review of Plant Physiology and Plant Molecular Biology*, 40: 235-269

FALKOWSKI, P.G. and LA ROCHE, J. 1991. Acclimation to spectral irradiance in algae. *Journal of Phycology*, 27: 8-14

FEATONBY-SMITH, B.C. and VAN STADEN, J. 1984. Identification and seasonal variation of endogenous cytokinins in *Ecklonia maxima* (Osbeck) Papenf. *Botanica Marina*, 27: 527-531

FIENBERG, A.A., CHOI, J.H., LUBICH, W.P. and SUNG, Z.R. 1984. Developmental regulation of polyamine metabolism in growth and differentiation of carrot culture. *Planta*, 162: 532-539

FRANK, V. 1991. The use of some fluorescent stains for studying morphogenesis of Micromycetes. *Folia Microbiology*, 36: 92-96

FRANKLIN, L.A., LEVAVASSEUR, G., OSMOND, C.B., HENLEY, W.J. and RAMUS, J. 1992. Two components of onset and recovery during photoinhibition of *Ulva rotundata*. *Planta*, 186: 399-408

FREDRIKSEN, S. and RUENESS, J. 1989. Culture studies of *Gelidium latifolium* (Grev.) Born *et* Thur. (Rhodophyta) from Norway. Growth and nitrogen storage in response to varying photon flux density, temperature and nitrogen availability. *Botanica Marina*, 32: 539-546

GABRIELSON, P.W., GARBARY, D.J. and SCAGEL, R.F. 1985. The nature of the ancestral red alga: Inferences from a cladistic analysis. *Biosystems*, 18: 335-346

GABRIELSON, P.W. and GARBARY, D.J. 1987. A cladistic analysis of Rhodophyta: Florideophycidean orders. *British Phycological Journal*, 22: 125-138

GALSTON, A.W. 1983. Polyamines as modulators of plant development. *Bioscience*, 33: 382-388

GALVÀN, A., CÓRDOBA, F., CÀRDENAS, J. and FERNÀNDEZ, E. 1991. Regulation of nitrite uptake and nitrite reductase expression in *Chlamydomonas reinhardtii*. *Biochemica et Biophysica Acta*, 1074: 6-11

GALWAY, M.E. and HARDHAM, A.R. 1986. Microtubule reorganization, cell wall synthesis and establishment of the axis of elongation in regenerating protoplasts of the alga *Mougeotia*. *Protoplasma*, 135: 130-143

GANTT, E. 1990. Pigmentation and photoacclimation. *IN*: COLE, K.M. and SHEATH, R.G. (eds). *Biology of the Red Algae*, Cambridge University Press, Cambridge: 203-219

GARBARY, D.J., GRUND, D. and McLACHLAN, J. 1978. The taxonomic status of *Ceramium rubrum* (Huds.) C.Ag. (Ceramiales, Rhodophyceae) based on culture experiments. *Phycologia*, 17: 85-94

GARBARY, D. 1979a. The effects of temperature on the growth and morphology of some *Audouinella* spp. (Acrochaetiaceae, Rhodophyta). *Botanica Marina*, 22: 493-498

GARBARY, D. 1979b. Daylength and development in four species of Ceramiaceae (Rhodophyta). *Helgoländer wiss. Meeresunters*, 32: 213-227

GARBARY, D. 1988. Interoceanic hybridization in fully corticated *Ceramium* isolates (Rhodophyta) from Nova Scotia and Washington. *The Korean Journal of Phycology*, 3: 89-93

GARBARY, D., BELLIVEAU, D. and IRWIN, R. 1988. Apical control of band elongation in *Antithamnion defectum* (Ceramiaceae, Rhodophyta). *Canadian Journal of Botany*, 66: 1308-1315

GARBARY, D.J. and BELLIVEAU, D.J. 1990. Diffuse growth, a new pattern of cell wall deposition for the Rhodophyta. *Phycologia*, 29: 98-102

GARBARY, D.J. and GABRIELSON, P.W. 1990. Taxonomy and evolution. *IN*: COLE, K.M. and SHEATH, R.G. (eds). *Biology of the Red Algae*. Cambridge University Press, Cambridge: 477-498

GLENN, E.P. and DOTY, M.S. 1992. Water motion affects the growth rates of *Kappaphycus alvarezii* and related red seaweeds. *Aquaculture*, 108: 233-246

GOLTERMAN, H.L., CLYMO, R.S. and OHNSTAD, M.A.M. 1978. *Methods for Physical and Chemical Analysis of Fresh Waters*. Blackwell Scientific Publications, Oxford: 260pp

GONZALEZ, M.A. and GOFF, L.J. 1989. The red algal epiphytes *Microcladia coulteri* and *M. californica* (Rhodophyceae, Ceramiaceae) I. Taxonomy, life history and phenology. *Journal of Phycology*, 25: 545-558

GOOD, N.E., WINGET, G.D., WINTER, W., CONNOLLY, T.N., IZAWA, S. and SINGH, R.M.M. 1966. Hydrogen ion buffers for biological research. *Biochemistry*, 5: 467-477

GORDON, D.M. and SAND-JENSEN, K. 1990. Effects of O_2, pH and DIC on photosynthetic net-O_2 evolution by marine macroalgae. *Marine Biology*, 106: 445-451

GORDON, E.M. and WOMERSLEY, H.B.S. 1966. The morphology and reproduction of *Sphondylothamnion multifidum* (Hudson) Naegeli (Ceramiaceae). *British Phycological Bulletin*, 3: 23-30

GREEN, P.B. 1962. Cell expansion. *IN*: LEWIN, R.A. (ed.). *Physiology and Biochemistry of Algae*. Academic Press, New York: 625-632

GREEN, P.B. 1969. Cell morphogenesis. *Annual Review of Plant Physiology*, 20: 365-394

GREENE, R.M. and GERARD, V.A. 1990. Effects of high-frequency light fluctuations on growth and photoacclimation of the red alga *Chondrus crispus*. *Marine Biology*, 105: 337-344

GUANZON, N.G. and DE CASTRO, T.R. 1992. The effects of different stocking densities and some abiotic factors on cage culture of *Gracilaria* sp. (Rhodophyta, Gigartinales). *Botanica Marina*, 35: 239-243

GUIRY, M.D. 1978. The importance of sporangia in the classification of the Florideophyceae. *IN*: IRVINE, D.E.G. and PRICE, J.H. (eds). *Modern Approaches to the Taxonomy of Red and Brown Algae*. Academic Press, London: 111-144

GUIRY, M.D. and CUNNINGHAM, E.M. 1984. Photoperiodic and temperature responses in the reproduction of north-eastern Atlantic *Gigartina acicularis* (Rhodophyta: Gigartinales). *Phycologia*, 23: 357-367

GUIRY, M.D. 1987. The evolution of life history types in Rhodophyta: An appraisal. *Cryptogamie, Algologie*, 8: 1-12

GUIRY, M.D. 1990. Sporangia and spores. *IN*: COLE, K.M. and SHEATH, R.G. (eds). *Biology of the Red Algae*. Cambridge University Press, Cambridge: 347-376

GUIRY, M.D. and DAWES, C.J. 1992. Daylength, temperature and nutrient control of tetrasporogenesis in *Asparagopis armata* (Rhodophyta). *Journal of Experimental Marine Biological Ecology*, 158: 197-217

HAMANA, K. and MATSUZAKI, S. 1982. Widespread occurrence of norspermidine and norspermine in eukaryotic algae. *Journal of Biochemistry*. 91: 1321-1328

HANELT, D., HUPPERTZ, K. and NULTSCH, W. 1992. Photoinhibition of photosynthesis and its recovery in red algae. *Botanica Acta*, 105: 227-342

HANISAK, M.D. 1979. Effect of indole-3-acetic acid on growth of *Codium fragile* subsp. *tomentosoides* (Chlorophyceae) in culture. *Journal of Phycology*, 15: 124-127

HANNACH, G. 1989. Spectral light absorption by intact blades of *Porphyra abbottae* (Rhodophyta): Effects of environmental factors in culture. *Journal of Phycology*, 25: 522-529

HANNACH, G. and WAALAND, J.R. 1989. Growth and morphology of young gametophytes of *Porphyra abbottae* (Rhodophyta): Effects of environmental factors in culture. *Journal of Phycology*, 25: 247-254

HARLIN, M.M., WOELKERLING, W.J. and WALKER, D.I. 1985. Effects of a hypersalinity gradient on epiphytic Corallinaceae (Rhodophyta) in Shark Bay, Western Australia. *Phycologia*, 24: 389-402

HARRISON, W.G., COTA, G.F. and SMITH, R.E.H. 1990. Nitrogen utilization in ice algal communities of Barrow Strait, Northwest Territories, Canada. *Marine Ecology Progress Series*, 67: 275-283

HAWKES, M.W. 1990 Reproductive strategies. *IN*: COLE, K.M. and SHEATH, R.G. (eds). *Biology of the Red Algae*. Cambridge University Press, Cambridge: 455-476

HAWKINS, E.K. 1972. Cell differentiation in tetrasporophytes of *Callithamnion roseum* Harvey (Rhodophyceae, Ceramiales). *Phycologia*, 11: 37-41

HILLMAN, J.R. 1984. Apical dominance. *IN*: WILKINS, M.B. (ed.). *Advanced Plant Physiology*. Longman Scientific and Technical, New York: 127-148

HODGSON, L.M. 1981. Photosynthesis of the red alga *Gastroclonium coulteri* (Rhodophyta) in response to changes in temperature, light intensity, and desiccation. *Journal of Phycology*, 17: 37-42

HOLBROOK, G.P., BEER, S., SPENCER, W.E., REISKIND, J.B., DAVIS, J.S. and BOWES, G. 1988. Photosynthesis in marine macroalgae: Evidence for carbon limitation. *Canadian Journal of Botany*, 66: 577-582

HOLZWARTH, A.R. 1991. Structure-function relationships and energy transfer in phycobiliprotein antennae. *Physiologia Plantarum*, 83: 518-528

HOMMERSAND, M.H. and FREDERICQ, S. 1990. Sexual reproduction and cystocarp development. *IN*: COLE, K.M. and SHEATH, R.G. (eds). *Biology of the Red Algae*. Cambridge University Press, Cambridge: 305-345

HONSELL, E., KOSOVEL, V. and TALARICO, L. 1984. Phycobiliprotein distribution in Rhodophyta: Studies and interpretations on the basis of their absorption spectra. *Botanica Marina*, 27: 1-16

HORGAN, R. 1984. Cytokinins. *IN*: WILKINS, M.B. (ed.). *Advanced Plant Physiology*. Longman Scientific and Technical, New York: 53-75

HUGHES, J. and McCULLY, M.E. 1975. The use of an optical brightener in the study of plant structure. *Stain Technology*, 50: 319-329

HURTADO-PONCE, A.Q. and UMEZAKI, I. 1987. Growth rate studies of *Gracilaria verrucosa* (Gigartinales, Rhodophyta). *Botanica Marina*, 30: 223-226

JACOBS, W.P., FALKENSTEIN, K. and HAMILTON, R.H. 1985. Nature and amount of auxin in algae. *Plant Physiology*, 78: 844-848

JENNINGS, R.C., BROUGHTON, W.J. and McCOMB, A.J. 1972. Effect of kinetin on the phycoerythrin and chlorophyll content of a red alga. *Phytochemistry*, 11: 1937-1943

JENSEN, A. 1978. Chlorophylls and carotenoids. *IN*: HELLEBUST, J.A. and CRAIGIE, J.S. (eds). *Handbook of Phycological Methods: Physiological and Biochemical Methods*. Cambridge University Press, Cambridge: 59-70

JONES, R.L. and MacMILLAN, J. 1984. Gibberellins. *IN*: WILKINS, M.B. (ed.). *Advanced Plant Physiology*. Longman Scientific and Technical, New York: 21-52

KAIN (JONES), J.M. 1989. The seasons in the subtidal. *British Phycological Journal*, 24: 203-215

KAIN, J.M. and NORTON, T.A. 1990. Marine ecology *IN*: COLE, K.M. and SHEATH, R.G. (eds). *Biology of the Red Algae*. Cambridge University Press, Cambridge: 377-422

KAPRAUN, D.F. 1978. Field and culture studies on growth and reproduction of *Callithamnion byssoides* (Rhodophyta, Ceramiales) in North Carolina. *Journal of Phycology*, 14: 21-24

KIRK, J.T.O. 1979. Spectral distribution of photosynthetically active radiation in some South-eastern Australian waters. *Australian Journal of Marine and Freshwater Research*, 30: 81-91

KIRST, G.O. 1990. Salinity tolerance of eukaryotic marine algae. *Annual Review of Plant Physiology and Plant Molecular Biology*, 41: 21-53

KNEIFEL, H. 1979. Amines in algae. *IN*: HOPPE, H.A., LEVRING, T. and TANAKA, Y. (eds). *Marine Algae in Pharmaceutical Science*. Walter de Gruyter, New York: 365-401

KNOOP, W.T. and BATE, G.C. 1988a. The effect of wounding on the photosynthetic rates of three subtidal Rhodophytes. *Botanica Marina*, 31: 149-153

KNOOP, W.T. and BATE, G.C. 1988b. Diurnal patterns in the photosynthesis of three subtidal macroalgal Rhodophytes. *Botanica Marina*, 31: 243-249

KNOOP, W.T. and BATE, G.C. 1990. A model for the description of photosynthesis-temperature responses by subtidal Rhodophyta. *Botanica Marina*, 33: 165-171

KOSOVEL, V. and TALARICO, L. 1979. Seasonal variations of photosynthetic pigments in *Gracilaria verrucosa* (Huds.) Papenfuss (Florideophyceae-Gigartinales). *Bollettino Della Società Adriatica di Scienze*, 63: 5-15

KOSOVEL, V. and TALARICO, L. 1982. Preliminary ultrastructural characterization of R-phycocyanin from the red alga *Gracilaria verrucosa* (Huds.) Papenfuss. *Photosynthetica*, 16: 373-374

KRAFT, G.T. 1981. Rhodophyta: Morphology and classification. *IN*: LOBBAN, C.S. and WYNNE, M.J. (eds). *The Biology of Seaweeds*. Blackwell Scientific Publications, Oxford: 6-51

KROPF, D.L. and QUATRANO, R.S. 1987. Localization of membrane-associated calcium during development of fucoid algae using chlorotetracycline. *Planta*, 171: 158-170

KURSAR, T.A. and ALBERTE, R.S. 1983. Photosynthetic unit organization in a red alga. Relationships between light harvesting pigments and reaction centers. *Plant Physiology*, 72: 409-414

LACALLI, T.C. and HARRISON, L.G. 1987. Turning's model and branching tip growth: Relation of time and spatial scales in morphogenesis, with applications to *Micrasterias*. *Canadian Journal of Botany*, 65: 1308-1319

LAING, W.A., CHRISTELLER, J.T. and TERZAGHI, B.E. 1989. The effect of temperature, photon flux density and nitrogen on growth of *Gracilaria sordida* Nelson (Rhodophyta). *Botanica Marina*, 32: 439-445

LAPOINTE, B.E. 1981. The effects of light and nitrogen on growth, pigment content, and biochemical composition of *Gracilaria foliifera* v. *angustissima* (Gigartinales, Rhodophyta). *Journal of Phycology*, 17: 90-95

LAPOINTE, B.E. and DUKE, C.S. 1984. Biochemical strategies for growth of *Gracilaria tikvahiae* (Rhodophyta) in relation to light intensity and nitrogen availability. *Journal of Phycology*, 20: 488-495

LARSSON, C., AXELSSON, L., CARLBERG, S., RYBERG, H. and UUSITALO, J. 1990. Inducible CO_2 concentrating mechanisms in green seaweeds II. Ecology and field observations. *Current Research in Photosynthesis*, 4: 533-536

LAWSON, R.P. and RUSSELL, G. 1967. Simultaneous occurrence of carposporophytes and tetrasporangia in *Polysiphonia urceolata*. *British Phycological Bulletin*, 3: 249-250

LEDERMAN, T.C. and TETT, P. 1981. Problems in modelling the photosynthesis-light relationship for phytoplankton. *Botanica Marina*, 24: 125-134

LEE, R.E. 1980. *Phycology*. Cambridge University Press, Cambridge: 478pp

LE GUYADER, H. and FERRE, M. 1988. Organogenesis at the shoot apex: An attempt at modelization. *Acta Biotheoretica*, 37: 37-86

LEVRING, T. 1969. Light conditions, photosynthesis and growth of marine algae in coastal and clear oceanic water. *Proceedings of the International Seaweed Symposium*, 6: 235-244

LEVY, I. and GANTT, E. 1988. Light acclimation in *Porphyridium purpureum* (Rhodophyta): Growth, photosynthesis, and phycobilisomes. *Journal of Phycology*, 24: 452-458

LEWIN, R.A. and ROBERTSON, J.A. 1971. Influence of salinity on the form of *Asterocytis* in pure culture. *Journal of Phycology*, 7: 236-238

L'HARDY-HALOS, M-Th. and MAGGS, C.A. 1991. A novel life history in *Aglaothamnion diaphanum* sp. nov. (Ceramiaceae, Rhodophyta) from Brittany and the British Isles. *Phycologia*, 30: 467-479

LIGNELL, A. and PEDERSÈN, M. 1989. Effects of pH and inorganic carbon concentration on growth of *Gracilaria secundata*. *British Phycological Journal*, 24: 83-89

LITTLER, M.M. and LITTLER, D.S. 1980. The evolution of thallus form and survival strategies in benthic marine macroalgae: Field and laboratory tests of a functional form model. *The American Naturalist*, 116: 25-44

LITTLER, M.M. and ARNOLD, K.E. 1982. Primary productivity of marine macroalgal functional-form groups from Southwestern North America. *Journal of Phycology*, 18: 307-311

LITTLER, M.M., LITTLER, D.S. and TAYLOR, P.R. 1983. Evolutionary strategies in a tropical barrier reef system: Functional-form groups of marine macroalgae. *Journal of Phycology*, 19: 229-237

LITTLER, M.M., LITTLER, D.S. and TAYLOR, P.R. 1987. Functional similarity among isomorphic life-history phases of *Polycavernosa debilis* (Rhodophyta, Gracilariaceae). *Journal of Phycology*, 23: 501-505

LIU, X-W. and KLOAREG, B. 1991. Tissue culture of *Porphyra umbilicalis* (Bangiales, Rhodophyta). I. The effects of plant hormones on callus induction from tissue explants. *Plant Physiology*, 312: 517-522

LOBBAN, C.S., HARRISON, P.J. and DUNCAN, M.J. 1985. *The Physiological Ecology of Seaweeds*. Cambridge University Press, Cambridge: 242pp

LÓPEZ-FIGUEROA, F. and NIELL, F.X. 1989. Red-light and blue-light photoreceptors controlling chlorophyll *a* synthesis in the red alga *Porphyra umbilicalis* and in the green alga *Ulva rigida*. *Physiologia Plantarum*, 76: 391-397

LÓPEZ-FIGUEROA, F. and NIELL, F.X. 1990. Effects of light quality on chlorophyll and biliprotein accumulation in seaweeds. *Marine Biology*, 104: 321-327

LÜNING, K. 1981a. Light. *IN*: LOBBAN, C.S. and WYNNE, M.J. (eds). *The Biology of Seaweeds*. Blackwell Scientific Publications, Oxford: 326-355

LÜNING, K. 1981b. Photobiology of seaweeds: Ecophysiological aspects. *Proceedings of the International Seaweed Symposium*, 10: 35-55

LÜNING, K. and TOM DIECK, I. 1989. Environmental triggers in algal seasonality. *Botanica Marina*, 32: 389-397

LÜNING, K. 1990. *Seaweeds: Their Environment, Biogeography and Ecophysiology*. John Wiley and Sons Inc., New York: 527 pp

LUXORO, C. and SANTELICES, B. 1989. Additional evidence for ecological differences among isomorphic reproductive phases of *Iridaea laminarioides* (Rhodophyta: Gigartinales). *Journal of Phycology*, 25: 206-212

LYNGBY, J.E. 1990. Monitoring of nutrient availability and limitation using the marine macroalga *Ceramium rubrum* (Huds.) C.Ag. *Aquatic Botany*, 38: 153-161

MAGGS, C.A. 1988. Intraspecific life history variability in the Florideophycidae (Rhodophyta). *Botanica Marina*, 31: 465-490

MAGRUDER, W.H. 1984. Specialized appendages on spermatia from the red alga *Aglaothamnion neglectum* (Ceramiales, Ceramiaceae) specifically bind with trichogynes. *Journal of Phycology*, 20: 436-440

MARTIN, M.T. 1969. A review of life histories in the Nemalionales and some allied genera. *British Phycological Journal*, 4: 145-158

MAYNARD SMITH, J. 1971. What use is sex? *Journal of Theoretical Biology*, 30: 319-335

McLEAN, R.C. and IVIMEY-COOK, W.R. 1952. *Textbook of Practical Botany*. Longmans, Green and Co., London: 476 pp

McFADDEN, G.I. and MELKONIAN, M. 1986. Use of HEPES buffer for microalgal culture media and fixation for electron microscopy. *Phycologia*, 25: 551-557

McKAY, R.M. and GIBBS, S.P. 1990. Phycoerythrin is absent from the pyrenoid of *Porphyridium cruentum*: Photosynthetic implications. *Planta*, 180: 249-256

McLACHLAN, J. 1973. Growth media - Marine. *IN*: STEIN, J.R. (ed.). *Handbook of Phycological Methods: Culture Methods and Growth Measurements*. Cambridge, Cambridge University Press: 25-51

MEEKS, J.C. 1974. Chlorophylls. *IN*: STEWART, W.D.P. (ed.). *Algal Physiology and Biochemistry*. Blackwell Scientific Publications, Oxford: 161-175

MÉTRAUX, J-P., RICHMOND, P.A. and TAIZ, L. 1980. Control of cell elongation in *Nitella* by endogenous cell wall pH gradients. *Plant Physiology*, 65: 204-210

MEYER, S.R. and PIENAAR, R.N. 1984. The microanatomy of *Chroomonas africana* sp. nov. (Cryptophyceae). *South African Journal of Botany*, 3: 306-319

MINOCHA, S.C. 1988. Relationship between polyamine and ethylene biosynthesis in plants and its significance for morphogenesis in cell cultures. *IN*: ZAPPIA, V. and PEGG, A.E. (eds). *Progress in Polyamine Research*. Plenum Publishing Corporation, New York: 601-616

MISHRA, A.R. and KEFFORD, N.P. 1969. Development studies on the coenocytic alga, *Caulerpa sertularioides*. *Journal of Phycology*, 5: 103-109

MIZUNO, M. 1992. Influence of salinity on the growth of marine and estuarine benthic diatoms. *The Japanese Journal of Phycology*, 40: 33-37

MOE, R.L. and SILVA, P.C. 1979. Morphological and taxonomic studies in Antarctic Ceramiaceae (Rhodophyceae). I. *Antarcticothamnion polysporum* gen. *et* sp. nov. *British Phycological Journal*, 14: 385-405

MONTAGUE, M.J., KOPPENBRINK, J.W. and JAWORSKI, E.G. 1978. Polyamine metabolism in embryogenic cells of *Daucus carota*. *Plant Physiology*, 62: 430-433

MOONEY, P.A. and VAN STADEN, J. 1984. Lunar periodicity of the levels of endogenous cytokinins in *Sargassum heterophyllum* (Phaeophyceae). *Botanica Marina*, 27: 467-472

MOONEY, P.A. and VAN STADEN, J. 1986. Algae and cytokinins. *Journal of Plant Physiology*, 123: 1-21

MOONEY, P.A. and VAN STADEN, J. 1987. Tentative identification of cytokinins in *Sargassum heterophyllum* (Phaeophyceae). *Botanica Marina*, 30: 323-325

MOSS, B. 1965. Apical dominance in *Fucus vesiculosus*. *The New Phytologist*, 64: 387-392

MOSS, B. 1966. Polarity and apical dominance in *Fucus vesiculosus*. *British Phycological Bulletin*, 3: 31-35

MURRAY, S.N. and DIXON, P.S. 1973. The effect of light intensity and light period on the development of thallus form in the marine red alga *Pleonosporium squarrulosum* (Harvey) Abbott (Rhodophyta: Ceramiales). I. Apical cell division - main axis. *Journal of Experimental Marine Biological Ecology*, 13: 15-27

MURRAY, S.N. and DIXON, P.S. 1975. The effects of light intensity and light period on the development of thallus form in the marine red alga *Pleonosporium squarrulosum* (Harvey) Abbott (Rhodophyta: Ceramiales). II. Cell enlargement. *Journal of Experimental Marine Biological Ecology*, 19: 165-176

NEUBAUER, Z. and KURKA, P. 1991. Geometry and morphogenesis. *Biology Forum*, 84: 91-100

NGAN, Y. and PRICE, I.R. 1983. Periodicity of spore discharge in tropical Florideophyceae (Rhodophyta). *British Phycological Journal*, 18: 83-95

NORRIS, R.E., WOLLASTON, E.M. and PARSONS, M.J. 1984. New terminology for sympodial growth in the Ceramiales (Rhodophyta). *Phycologia*, 23: 233-237

NORTON, T.A., MATHIESON, A.C. and NEUSHEL, M. 1981. Morphology and environment. *IN*: LOBBAN, C.S. and WYNNE, M.J. (eds). *The Biology of Seaweeds*. Blackwell Scientific Publications, Oxford: 421-451

NULTSCH, W., PFAU, J. and HUPPERTZ, K. 1990. Photoinhibition of photosynthetic oxygen production and its recovery in the subtidal red alga *Polyneura hilliae*. *Botanica Acta*, 103: 62-67

OATES, B.R. and COLE, K.M. 1989. Organelle associations in developing trichoblasts of *Polysiphonia hendryi* (Rhodophyta, Ceramiales). *British Phycological Journal*, 24: 339-345

ORFANIDIS, S. 1992. Light requirements for growth of six shade-acclimated Mediterranean macroalgae. *Marine Biology*, 112: 511-515

PACHECO-RUIZ, I., ZERTUCHE-GONZALEZ, J.A., CABELLO-PASINI, A. and BRINKHUIS, B.H. 1992. Growth responses and seasonal biomass variation of *Gigartina pectinata* Dawson (Rhodophyta) in the Gulf of California. *Journal of Experimental Marine Biological Ecology*, 157: 263-274

PAHL-WOSTL, C. and IMBODEN, D.M. 1990. DYPHORA - a dynamic model for the rate of photosynthesis of algae. *Journal of Plankton Research*, 12: 1207-1221

PAPENFUSS, G.F. 1966. A review of the present system of classification of the Florideophycidae. *Phycologia*, 5: 247-255

PARSONS, M.J. 1975. Morphology and taxonomy of the Dasyaceae and the Lophothalieae (Rhodomelaceae) of the Rhodophyta. *Australian Journal of Botany*, 23: 549-713

PECKOL, P. and RAMUS, J. 1992. Photosynthetic performance of deep-water macroalgae (Phaeophyta, Dictyotales) off Bermuda. *Hydrobiologia*, 231: 93-98

PEGG, A.E. and McCANN, P.P. 1982. Polyamine metabolism and function. *American Journal of Physiology*, 243: C212-C221

PELCE, P. and POCHEAU, A. 1992. Geometrical approach to the morphogenesis of unicellular algae. *Journal of Theoretical Biology*, 156: 197-214

PENNIMAN, C.A. and MATHIESON, A.C. 1985. Photosynthesis of *Gracilaria tikvahiae* McLachlan (Gigartinales, Rhodophyta) from the Great Bay Estuary, New Hampshire. *Botanica Marina*, 28: 427-435

PHILLIPS, I.D.J. 1975. Apical dominance. *Annual Review of Plant Physiology*, 26: 341-67

PICTON, J.M. and STEER, M.W. 1983. Evidence for the role of Ca^{2+} ions in tip extension in pollen tubes. *Protoplasma*, 115: 11-17

PISTOCCHI, R., BAGNI, N. and CREUS, J.A. 1987. Polyamine uptake in carrot cell cultures. *Plant Physiology*, 84: 374-380

POWLES, S.B. 1984. Photoinhibition of photosynthesis induced by visible light. *Annual Review of Plant Physiology*, 35: 15-44

PROVASOLI, L. and CARLUCCI, A.F. 1974. Vitamins and growth regulators. *IN*: STEWART, W.D.P. (ed.). *Algal Physiology and Biochemistry*. Blackwell Scientific Publications, Oxford: 741-787

PUESCHEL, C.M. and COLE, K.M. 1982. Rhodophycean pit plugs: An ultrastructural survey with taxonomic implications. *American Journal of Botany*, 69: 703-720

PUESCHEL, C.M. 1988. Secondary pit connections in *Hildenbrandia* (Rhodophyta, Hildenbrandiales). *British Phycological Journal*, 23: 25-32

PUESCHEL, C.M. 1989. An expanded survey of the ultrastructure of red algal pit plugs. *Journal of phycology*, 25: 625-636

PUESCHEL, C.M. 1990. Cell structure. *IN*: COLE, K.M. and SHEATH, R.G. (eds). *Biology of the Red Algae*. Cambridge University Press, Cambridge: 7-41

QUATRANO, R.S. and KROPF, D.L. 1989. Polarization in *Fucus* (Phaeophyceae) zygotes: Investigations of the role of calcium, microfilaments and cell wall. *IN*: COLEMAN, W.A., GOFF, L.J. and STEIN-TAYLOR, J.R. (eds). *Algae as Experimental Systems*. Allan R. Liss Inc, New York: 111-119

RAMUS, J. 1981. The capture and transduction of light energy. *IN*: LOBBAN, C.S. and WYNNE, M.J. (eds). *The Biology of Seaweeds*. Blackwell Scientific Publications, Oxford: 458-492

RAMUS, J. 1983. A physiological test of the Theory of Complementary Chromatic Adaptation II. Brown, green and red seaweeds. *Journal of Phycology*, 19: 173-178

RAMUS, J. and VAN DER MEER, J.P. 1983. A physiological test of the Theory of Complementary Chromatic Adaptation I. Color mutants of a red seaweed. *Journal of Phycology*, 19: 86-91

RASCIO, N., MARIANI, P., DALLA VECCHIA, F. and TREVISAN, R. 1991a. The vegetative thallus of *Pterocladia capillacea* (Gelidiales, Rhodophyta). II. Pit connections. *Botanica Marina*, 34: 187-194

RASCIO, N., MARIANI, P., DALLA VECCHIA, F. and TREVISAN, R. 1991b. The vegetative thallus of *Pterocladia capillacea* (Gelidiales, Rhodophyta). I. An ultrastructural and cytochemical study. *Botanica Marina*, 34: 177-185

REED, R.H. 1990. Solute accumulation and osmotic adjustment. *IN*: COLE, K.M. and SHEATH, R.G. (eds). *Biology of the Red Algae*. Cambridge University Press, Cambridge: 147-170

REHM, A.M., GÜLZOW, M., MARQUARDT, J. and RIED, A. 1990. Changes in the photosynthetic apparatus of red algae induced by spectral alteration of the light field. II. Further characterization of the light-dependent regulation of the apparent quantum yield of PS I. *Biochemica et Biophysica Acta*, 1016: 127-135

REISKIND, J.B., BEER, S. and BOWES, G. 1989. Photosynthesis, photorespiration and ecophysiological interactions in marine macroalgae. *Aquatic Botany*, 34: 131-152

REISS, H.D. and HERTH, W. 1979. Calcium gradients in tip growing plant cells visualized by chlorotetracycline fluorescence. *Planta*, 146: 615-621

REZNICK, D. 1992. Measuring the costs of reproduction. *Tree*, 7: 42-45

RIETEMA, H. and KLEIN, A.W.O. 1981. Environmental control of the life cycle of *Dumontia contorta* (Rhodophyta) kept in culture. *Marine Ecology Progress Series*, 4: 23-29

RIETEMA, H. 1982. Effects of photoperiod and temperature on macrothallus initiation in *Dumontia contorta* (Rhodophyta). *Marine Ecology Progress Series*, 8: 187-196

ROBERTS, D.R., WALKER, M.A., THOMPSON, J.E. and DUMBROFF, E.B. 1984. The effects of inhibitors of polyamine and ethylene biosynthesis on senescence, ethylene production and polyamine levels in cut carnation flowers. *Plant and Cell Physiology*, 25: 315-322

ROBSON, G.D., WIEBE, M.G. and TRINCI, A.P.J. 1991. Involvement of Ca^{2+} in the regulation of hyphal extension and branching in *Fusarium graminearum* A3/5. *Experimental Mycology*, 15: 263-272

RONCERO, C. and DURÁN, A. 1985. Effect of Calcofluor White and Congo Red on fungal cell wall morphogenesis: *In vivo* activation of chitin polymerization. *Journal of Bacteriology*, 163: 1180-1185

RÜDIGER, W. and LÓPEZ-FIGUEROA, F. 1992. Photoreceptors in algae. *Photochemistry and Photobiology*, 55: 949-954

RUENESS, J. 1973. Speciation in *Polysiphonia* (Rhodophyceae, Ceramiales) in view of hybridization experiments: *P. hemisphaerica* and *P. boldii*. *Phycologia*, 12: 107-109

RUENESS, J. and RUENESS, M. 1985. Regular and irregular sequences in the life history of *Callithamnion tetragonum* (Rhodophyta, Ceramiales). *British Phycological Journal*, 20: 329-333

RUENESS, J., JACOBSEN, T. and ASEN, P.A. 1990. Trekk ved marine benthosalgers utbredelse i Norge belyst ved undersokelser av blant andre rodalgen *Ceramium shuttleworthianum* (pigget rekeklo). *Blyttia*, 48: 21-26

RUENESS, M. and RUENESS, J. 1982. Hybridization and morphogenesis in *Callithamnion hookeri* (Dillw.) S.F.Gray (Rhodophyceae, Ceramiales) from disjunct north-eastern Atlantic populations. *Phycologia*, 21: 137-144

SAFFO, M.B. 1987. New light on seaweeds. *Bioscience*, 37: 654-664

SAUNDERS, M.J. and HEPLER, P.K. 1981. Localization of membrane-associated calcium following cytokinin treatment in *Funaria* using chlorotetracycline. *Planta*, 152: 272-281

SCHÖNE, H.K. and SCHÖNE, A. 1982. MET44: A weakly enriched sea-water medium for ecological studies on marine plankton algae, and some examples of its application. *Botanica Marina*, 25: 117-122

SCOTT, J., BOSCO, C., SCHORNSTEIN, K. and THOMAS, J. 1980. Ultrastructure of cell division and reproductive differentiation of male plants in the Florideophyceae (Rhodophyta): Cell division in *Polysiphonia*. *Journal of Phycology*, 16: 507-524

SCOTT, J. and BROADWATER, S. 1989. Ultrastructure of vegetative organization and cell division in the freshwater red alga *Compsopogon*. *Protoplasma*, 152: 112-122

SEARLES, R.B. 1980. The strategy of the red algal life history. *The American Naturalist*, 115: 113-120

SHEPHERD, S.A. 1981. Ecological strategies in a deep water red algal community. *Botanica Marina*, 24: 457-463

SICKMAN, J.O. and MELACK, J.M. 1992. Photosynthetic activity of phytoplankton in a high altitude lake (Emerald Lake, Sierra Nevada, California). *Hydrobiologia*, 230: 37-48

SIEGELMAN, H.W. and KYCIA, H.K. 1978. Algal biliproteins. *IN*: HELLEBUST, J.A. and CRAIGIE, J.S. (eds). *Handbook of Phycological Methods: Physiological and Biochemical Methods*. Cambridge University Press, Cambridge: 71-79

SIMONS, R.H. 1966. The genus *Ceramium* in South Africa. *Bothalia*. 9: 153-168

SIMPSON, F.J. and SHACKLOCK, P.F. 1979. The cultivation of *Chondrus crispus*. Effects of temperature on growth and carrageenin production. *Botanica Marina*, 22: 295-298

SMITH, T.A. 1985. Polyamines. *Annual Review of Plant Physiology*, 36: 117-143

SOEDER, C. and STENGEL, E. 1974. Physico-chemical factors affecting metabolism and growth rate. *IN*: STEWART, W.D.P. (ed.). *Algal Physiology and Biochemistry*. Blackwell Scientific Publications, Oxford: 714-740

SOMMERFELD, M.R. and NICHOLS, H.W. 1973. The life cycle of *Bangia fuscopurpurea* in culture. I. Effects of temperature and photoperiod on the morphology and reproduction of the *Bangia* phase. *Journal of Phycology*, 9: 205-210

STEGENGA, H. and BOLTON, J.J. 1992. Ceramiaceae (Rhodophyta) of the Cape Province, South Africa: Distribution in relation to concepts of marine provinces. *Botanica Marina*, 35: 99-107

STIRK, W.A. and AKEN, M.E. 1993. Growth and morphogenesis of a new red alga in the Ceramiaceae (Rhodophyta) from Natal Coastal waters. *South African Journal of Botany*, 59: 575-580

STONE, A.W. 1988. Climate and Weather. *IN*: LUBKE, R., GESS, F. and BRUTON, M. (eds.) *A Field Guide to the Eastern Cape Coast*. The Grahamstown Centre of the Wildlife Society of Southern Africa, Grahamstowm: 19-30

TALARICO, L. and KOSOVEL, V. 1978. Properties and ultrastructure of R-phycoerythrin from *Gracilaria verrucosa* (Gigartinales, Florideae) (Huds.) Papenfuss. *Photosynthetica*, 12: 369-374

TALARICO, L. and KOSOVEL, V. 1980. Qualitative interpretation of the absorption spectra of total biliproteic extracts from some Rhodophyta (Florideophyceae). *Bollettino Della Societa Adriatica di Scienze*, 64: 85-97

THIRB, H.H. and BENSON-EVANS, K. 1983. The effect of different light intensities and wavelengths on carpospore germination and the apical tips of the red alga *Lemanea* Bory (1808). *Nova Hedwigia*, 37: 669-682

VANO, V., RIGANO, V.D.M., ESPOSITO, S., MARTINO, C.D. and RIGANO, C. 1992. Growth, photosynthesis, respiration, and intracellular free amino acid profiles in the unicellular alga *Cyanidium caldarium*. Effect of nutrient limitation and resupply. *Physiologia Plantrum*, 85: 652-658

VENIS, M.A. and NAPIER, R.M. 1991. Auxin receptors: Recent developments. *Plant Growth Regulation*, 10: 329-340

WAALAND, S.D. and CLELAND, R. 1972. Development in the red alga, *Griffithsia pacifica*: Control by internal and external factors. *Planta*, 105: 196-204

WAALAND, S.D, WAALAND, J.R. and CLELAND, R. 1972. A new pattern of plant cell elongation: Bipolar band growth. *The Journal of Cell Biology*, 54: 184-190

WAALAND, S.D. and WAALAND, J.R. 1975. Analysis of cell elongation in red algae by fluorescent labelling. *Planta*, 126: 127-138

WAALAND, S.D., NEHLSEN, W. and WAALAND, J.R. 1977. Phototropism in a red alga, *Griffithsia pacifica*. *Plant and Cell Physiology*, 18: 603-612

WAALAND, S.D. 1989. Cellular morphogenesis in the filamentous red alga *Griffithsia*. *IN*: COLEMAN, W.A., GOFF, L.J. and STEIN-TAYLOR, J.R. (eds). *Algae as Experimental Systems*. Allan R. Liss Inc., New York: 121-134

WAALAND, S.D. 1990. Development. *IN*: COLE, K.M. and SHEATH, R.G. (eds). *Biology of the Red Algae*. Cambridge University Press, Cambridge: 259-273

WALKER, D. 1990. *The Use of the Oxygen Electrode and Fluorescence Probes in Simple Measurements of Photosynthesis*. Oxygraphics Limited, Sheffield: 203pp

WHITTICK, A. and WEST, J.A. 1979. The life history of a monoecious species of *Callithamnion* (Rhodophyta, Ceramiaceae) in culture. *Phycologia*, 18: 30-37

WILLIAMS, G.C. and MITTON, J.B. 1973. Why reproduce sexually? *Journal of Theoretical Biology*, 39: 545-554

WOLLASTON, E.M. 1977. Morphology and life history of *Dasyphila preissii* Sonder with notes on the taxonomic position of *Muellerana wattsii* (Harvey) Schmitz (Rhodophyta, Ceramiaceae). *Phycologia*, 16: 443-450

WOMERSLEY, H.B.S. 1978. Southern Australia species of *Ceramium* Roth (Rhodophyta). *Australian Journal of Marine and Freshwater Research*. 29: 205-257

YAMANOUCHI, S. 1906. The life history of *Polysiphonia violacea*. *Botanical Gazette*, 42: 401-449

YARISH, C., EDWARDS, P. and CASEY, S. 1979. A culture study of salinity responses in ecotypes of two estuarine red algae. *Journal of Phycology*, 15: 341-346

Appendix

Appendix A Culture media

Appendix A.1 Provosali's Enriched Seawater (PES)

(McLACHLAN, 1973)

PES is prepared as three separate stock solutions. All PES stock solutions are adjusted to pH 7.5 using either 1N HCl or NaOH and then are filter sterilized through a 0.45 μm membrane filter. Stocks are stored in the dark at 4°C.

Stock 1 - Major salt stock prepared at 100 times the working concentration and made to 1 l with distilled water.

Compound	Mol. wt	Concentration
$NaNo_3$	85.0	0.66 mM
Na_2.glycerolphosphate	315.1	25 μM
NaFe.EDTA	367.1	7.2 μM
Tris buffer	121.1	0.66 mM

Stock 2 - Trace element stock prepared at 100 times the working concentration and made up with 1 l distilled water.

Compound	Mol. wt	Concentration
$ZnSO_4.7H_2O$	287.6	0.8 μM
$MnSO_2.4H_2O$	223.1	7.3 μM
$CoSO_2.7H_2O$	281.1	0.17 μM
Fe.citrate	299.0	1.8 μM
Na_2.EDTA	372.2	26.9 μM
$H_3.BO_3$	61.8	185 μM

The trace elements are chelated by boiling them individually in a small volume of distilled water with Na_2EDTA supplied at an approximate molar ratio of 1:3 until they dissolved. These are cooled, combined and made up to volume.

Stock 3 - Vitamin stock prepared at 1000 times the working concentration and made up to 500 ml with distilled water.

Compound	Concentration
Cyanocobalamin (B_{12})	1.6 μg l^{-1}
Biotin	0.8 μg l^{-1}
Thiamine.HCl	20 μg l^{-1}

To make 1 litre of enriched seawater, 10 ml of stock 1, 10 ml of stock 2 and 1 ml of stock 3 are added to 1 litre of seawater.

Appendix A.2 MET 44

(SCHÖNE and SCHÖNE, 1982)

MET 44 is made up as two stock solutions. Each stock solution is filter sterilized using a 0.45 m filter. Stocks are stored in the dark at 4 C.

Stock 1 - Major salt stock prepared at 100 times the working concentration and made up to 1 litre with distilled water.

Compound	Concentration
$NaNO_3$	3.4 mg l^{-1}
$Na_2HPO_4.12H_2O$	0.925 mg l^{-1}
$Na_2SiO_3.9H_2O$	10.14 mg l^{-1}
$Na_2EDTA.2H_2O$	0.803 mg l^{-1}
$FeSO_4.7H_2O$	60 μg l^{-1}
$MnCl_2.4H_2O$	14.4 μg l^{-1}

Stock 2 - Vitamin stock made up at 1000 times the working concentration.

Compound	Concentration
Cyanocobalamin (B_{12})	0.5 $\mu g\ l^{-1}$
Biotin	0.5 $\mu g\ l^{-1}$
Thiamine.HCl (B_1)	0.5 $\mu g\ l^{-1}$

To make up 1 litre of enriched seawater, 10 ml of stock 1 and 1 ml of stock 2 were added to 1 litre seawater.

Appendix A.3 Erdschreiber Enriched Seawater

(McLACHLAN, 1973)

Erdschreiber Enriched Seawater is prepared as two stocks. All stocks are adjusted to pH 7.5 using 1N HCl or NaOH.

Stock 1 - Major salt stock made up at 100 times the working concentration.

Compound	Mol. wt	Concentration
$NaNO_3$	85.0	2.35 mM
Na_2HPO_3	142.0	149 μM

Stock 2 - Soil extract

Compound	Concentration
Soil extract	50 ml

The soil extract is made by mixing 1 volume virgin soil with 2 volumes distilled water 2 and steaming it for 1 hour. Once cooled, the liquid is cleared by centrifugation and stored in McCartney bottles. The soil used was collected from Ferncliffe, an indigenous forest on the outskirts of Pietermaritzburg, Natal.

To make 1 litre of enriched seawater, 10 ml of stock 1 and 50 ml of soil extract are added to 1 litre seawater.

Appendix A.4 Müllers Synthetic Medium

(McLACHLAN, 1973)

Müllers Synthetic Medium is prepared as five stock solutions. The pH of each stock is adjusted to pH 7.5 using either 1N HCl or NaOH and are stored in the dark at 4°C.

Stock 1 - Major salt stock (1) made up at 100 times the working concentration.

Compound	Mol. wt	Concentration
$NaNO_3$	85.0	1.18 mM
Na_2HPO_4	142.0	140 μM

This stock is altered to make up Müllers Synthetic Medium with complete buffer stock, nitrogen-free, phosphorus-free and nitrogen- and phosphorus-free buffer stocks. These four stocks were used to make up the concentrations needed in the nitrogen and phosphorus experiment.

Stock 2 - Major salt stock (2) made up at 100 times the working concentration.

Compound	Mol. wt	Concentration
NaCl	58.4	26.71g l^{-1}
KCl	74.6	0.73g l^{-1}
$MgSO_4.7\ H_2O$	246.5	6.56g l^{-1}
$MgCl_2.6H_2O$	203.3	23 mM
$CaCl_2.2H_2O$	147.0	13.5 mM
$NaHCO_3$	84.0	2.4 mM

Stock 3 M-I trace element stock prepared at 1000 times the working concentration.

Compound	Mol. wt	Concentration
$ZnSO_4$	287.5	14 μM
$MnSO_4.4H_2O$	223.1	3.8 μm
$Na_2MoO_4.2H_2O$	241.9	0.83 μM
$CoSO_4.7H_2O$	281.1	0.04 μM
$CuSO_4.5H_2O$	249.7	0.008 μM
NaFeEDTA	367.1	1.2 μM
Na_2EDTA	372.2	52.5 μM
H_3BO_3	61.8	32 μM

The trace elements are chelated by boiling them separately with Na_2EDTA added in a molar ratio of approximately 3:1. After these have cooled, they are mixed and made up to volume.

Stock 4 - M-II trace element stock was prepared at 1000 times the working concentration.

Compound	Mol. wt	Concentration
NaBr	102.9	185 μM
$SrCl_2.6H_2O$	266.6	14 μM
$AlCl_3$	133.3	0.21 μM
RbCl	120.9	0.13 μM
LiCl	42.4	0.14 μM
KI	166.0	1.2 μM

Stock 5 - Vitamin stock prepared at 1000 times the working concentration.

Compound	Concentration
Cyanocobalamin (B_{12})	0.5 µg l^{-1}
Biotin	0.5 µg l^{-1}
Thiamine.HCl (B_1)	100 µg l^{-1}

To make up Müllers Synthetic Seawater, combine 10 ml each of Stock 1 and Stock 2 and 1 ml each of Stock 3, Stock 4 and Stock 5 and make up to 1 litre with deionised distilled water.

Appendix B

Glycerine jelly

(McLEAN and IVIMEY-COOK, 1952)

Compound	Ratio	Concentration
Gelatine	1 part	5.08 g
Glycerol	7 parts	35 ml
Water	6 parts	30 ml

The gelatine and water were warmed for 2 hours and then glycerol added. This mixture was warmed and stirred for a further 15 minutes.

Appendix C Phosphate buffers

Appendix C.1 Phosphate buffer (0.05 M)

(SIEGELMAN and KYCIA, 1978)

A) 0.1 M KH_2PO_4

B) 0.1 M K_2HPO_4

The salts were dissolved in distilled water, mixed in equal volumes and the pH adjusted to pH 6.7 to give a 0.05 M phosphate buffer.

Appendix C.2 Phosphate buffer (0.2 M)

A) 0.2 M NaH_2PO_4

B) 0.2 M Na_2HPO_4

The two salt solutions were added in the ratio 5.3 ml of solution A to 94.7 ml of Solution B and then diluted to 200 ml to make a 0.2 M phosphate buffer.