SALT TOLERANCES IN *CLADOPHORA* (CHLOROPHYCEAE) : A STUDY OF POPULATIONS AND SPECIES.

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy by

David Neville Thomas.

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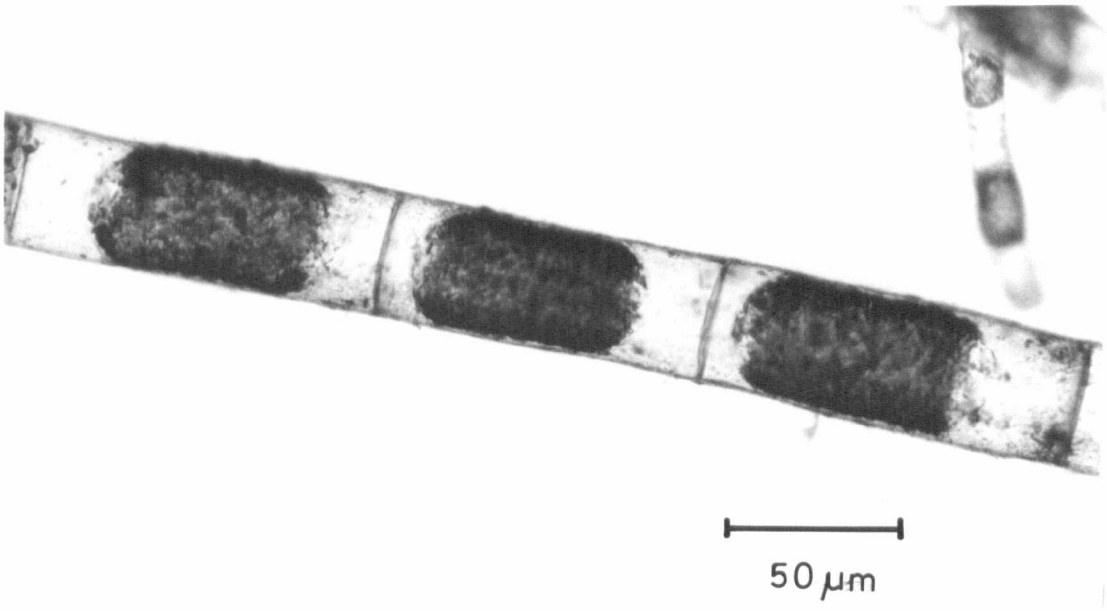

Cladophora glomerata cells following treatment with seawater (34°/oo).

ABSTRACT

DAVID.N.THOMAS.

SALT TOLERANCES IN CLADOPHORA (CHLOROPHYCEAE): A STUDY OF POPULATIONS AND SPECIES.

The effects of changes in external salt concentration upon two species of Cladophora have been studied: C. rupestris, a predominantly marine species, and C. glomerata, a freshwater species. Both species, however may be found growing in brackish-water habitats.

Measurements of salt tolerance were made by recording net photosynthesis and respiration following incubation in salinity treatments (0 to 102^{o}/oo) for periods of time up to 24h. Salt tolerance measurements were also made by comparing the net photosynthetic activity of the plants in normal salinity conditions following treatment with these media.

The salt tolerances of the two species proved to be quite distinctive, Cladophora rupestris tolerating a broad range of salinities, whereas C. glomerata showed little tolerance to even slight increases in salinity. Salt tolerance was also measured at different temperatures (-9 to 30^{o}C). At extreme temperatures, C. rupestris had a reduced salinity-tolerance range. C. glomerata proved better able to tolerate increased salinity at higher temperatures.

The seasonal variation in external salinity and temperature experienced by the plants was measured. The measured tolerances to temperature and salinity of both species exceeded the extremes of these two variables recorded in their natural environment.

The changes that occur in the protoplast and total-cell volume of both species were recorded using photomicroscopic techniques. The cells of both species plasmolysed in hyperosmotic treatments, and did not fully deplasmolyse over a 24h period. There was no evident increase in cell volume of Cladophora rupestris cells in hypo-osmotic media.

Measurements were made of changes that occur in thallus content of Na^{+}, K^{+}, Cl^{-}, Ca^{2+} and Mg^{2+}, during osmotic stress. Cladophora rupestris is able to maintain a fairly constant K^{+}/Na^{+} ratio over a wide range of salinities, whereas this ratio is greatly reduced in C. glomerata plants even in 1.5^{o}/oo treatment media.

The salt tolerances of Baltic populations of these two species were compared with those of U.K. plants. The Baltic species showed a convergence of salt tolerance. There were much more pronounced differences between the tolerances of Baltic and U.K. Cladophora glomerata than between the two C. rupestris populations. Measurements of cell, protoplast and thallus ion changes during salinity stress were also made on Baltic plants. The smaller celled Baltic C. rupestris more maintained constant ionic levels at lower salinities than its U.K. counterpart. Baltic C. glomerata maintained ionic equilibrium at higher salinities than U.K. plants.

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TABLE OF CONTENTS

1. INTRODUCTION.

1.1 THE TAXONOMY AND ECOLOGY OF THE GENUS CLADOPHORA.

The genus Cladophora contains filamentous green algae with thalli that are densely or sparsely branched, and which grow by means of apical and/or intercalary cell divisions. In some species the relative amount of apical and intercalary growth is directly proportional to the degree of water movement, apical cell divisions being more frequent in more turbulent waters. Plants are normally found attached to the substrate by branching rhizoids or by a simple hyaline disciform holdfast, though free-floating forms of certain species are known. The cells are multinucleate, but the number of nuclei present is not species specific. In the species investigated by Wik-Sjöstedt (1970), newly formed cells were generally found to have 5-35 nuclei per cell, whereas older main-filament and basal cells contained up to 200 nuclei per cell. Alternation of generations is almost universal within the genus, although some species multiply only by thallus fragmentation, and a few species reproduce entirely by asexual zoospores. Very detailed descriptions of the morphology, taxonomy and ecology of Cladophora species are given by Hoek (1963 and 1982a), Söderström (1963) and Womersley (1984). Extensive cytogenetic investigations have also been carried out by Wik-Sjöstedt (1970) and Wik-Sjöstedt and Nordqvist (1970).

The taxonomic treatment of the genus has often been very confused and unsatisfactory, primarily due to the

large degree of environmental and age-dependent plasticity shown in many species. The basic chromosome number for all Cladophora species is six, though Wik-Sjöstedt (1970) has identified polyploid series in a number of species, which will contribute to the intraspecific variation recorded in these algae. Hoek (1963) refers to the "900 names given in the course of about 150 years to 37 European species and subspecific taxa of Cladophora", and a similar comment is made by Söderström (1963), "...by the middle of the 19th century more than one hundred names of Cladophora were published and so the confusion began". In their independent attempts to simplify the classification of European species, Hoek (1963) and Söderström (1963) disagreed about the number of species and forms, and in doing so revealed the very real difficulty involved in classifying such a heterogenous assembledge as this. The points of difference between the two authors are discussed by Söderström (1965), but most recent check-lists and floras have followed the classification proposed by Hoek (1963), and therefore species nomenclature and authorities given in this thesis have been also been taken from this manuscript. As a result of experiments on C. glomerata, Dean (1966) has shown that a number of environmental conditions may affect characters used in many of the systematic studies of the genus, therefore illustrating the limitations on their taxonomic value. More detailed cytological studies, such as those by Wik-Sjöstedt (1970) and protein immunological distance ivestigations similar

to those of Olsen-Stojkovich et al. (1986), may do much to clarify the origins of morphological variation within this genus. Olsen-Stojkovich (1987) has shown that morphological characteristics of cladophoroid genera are often conservative, concealing considerable molecular diversity. Molecular data should therefore be a valuable addition to any algal systematic investigation, especially when comprehensive comparative morphological information is difficult to obtain, or when the taxon in question generates few morphological characters (Olsen-Stojkovich 1987).

The genus is cosmopolitan and, unusually among algae, its species are found in fresh, brackish and marine waters. In his study of European Cladophora Hoek (1963) recognises 27 marine species and 11 species occurring in freshwater. Many of these were also reported as being found in brackish-waters, although the range of salinities in which each species may occur is usually quite specific: C. albida, C. sericea and C. vagabunda are found growing over a broad range of salinities, whereas species such as C. prolifera, C. pellucida and C. hutchinsiae have very restricted salinity ranges.

The phytogeographic distribution of 43 marine species of Cladophora in the north Atlantic Ocean have been described by Hoek (1979, 1982b), and eight distinct distribution groups have been described. There is a large number of species restricted to the tropical western Atlantic and warm temperate Mediterranean-Atlantic

regions, which is linked with the prevalence of endeminism in these waters. According to Hoek (1979), the phytogeographic limits are determined by physical barriers, or notably by temperature restrictions on the survival and/or reproduction of the plants. Cambridge et al. (1984) have shown that experimentaly determined temperature limits for growth and survival of five species of Cladophora were consistant with summer and/or winter temperatures at the limits of their geographic distributions. However, much of this phytogeographical analysis is based upon mean temperatures of surface waters, and it is questionable how closely these relate to the fluctuations experienced, especially by intertidal plants in nature.

In this study the responses to salt stress of two species of Cladophora have been investigated, and the variation of salinity tolerance within and between species recorded. The selected species are :

(a) Cladophora rupestris (L.) Kütz : This is one of the few species of Cladophora that is easily recognised, posing few taxonomic difficulties (Hoek 1963). The thallus consists of a pseudodichotomously branching main axis with branches of different lengths. Growth is mainly by means of intercalary cell division. Newly formed cells initiate branches at their apical poles and so give rise to rows of branches of different ages (Hoek 1963). The characteristic stiffness of the plants is held to be due to the cells having particularly thick, multilayered,

complex cell walls. The cell wall structure of this species has been described in great detail by Hanic and Craigie (1969). The size of the plants is directly related to the degree of exposure of wave action (Hoek 1963), plants up to 20cm being found in sheltered locations.

It has the widest geographical distribution of all Cladophora species (Hoek 1963), growing mainly in the intertidal region of marine rocky shores, often as an understorey plant beneath larger fucoid algae. It is found in estuaries and also in the weakly saline waters ($6^{o}/oo$) of the Baltic Sea where it is a sublittoral species (Hällfors et al. 1981, Waern 1952). Behre (1961) also reports Cladophora rupestris growing at the mouth of the river Geeste at Bremerhaven in salinities of $3-6^{o}/oo$.

(b) Cladophora glomerata (L.) Kütz : This is probably the most variable morphologically of all Cladophora species (Hoek 1963, Whitton 1970). Plants may have densely branched intricate thalli or may be extremely poorly branched with all possible intermediate states, which may be attached or free-floating. In general the plants are attached, and have pseudodichotomously branched main axes with acropetally branched systems, growth being mainly apical but intercalary growth increasing basipetally. Intensive sporulation often results in the terminal branch systems disintegrating and reducing the thallus to a single axis growing by intercalary cell divisions. In still waters these axes may become detached and grow up to several metres (Hoek 1963). The seasonal

growth characteristics of this species are discussed by Niiyama (1986), Rosemarin (1985) and Whitton (1970).

Cladophora glomerata is predominantly a freshwater species, with a very widespread distribution, which may become troublesome when its growth is prolific (Whitton 1970). It has been found growing in waters of salinity of 15-17°/oo near Kiel on the Baltic coast (Hoek 1963) and also at other hydrolittoral sites in the Baltic Sea at salinities of 4-6°/oo (Hällfors et al. 1981, Waern 1952, Wallentinus 1975). It was recorded as growing in the eulittoral zone of the River Tweed Estuary by Norton (1976). Söderström (1963) quotes an upper salinity limit of 20°/oo for this species, although Dean (1966) grew C. glomerata for up to 8 months in seawater (34°/oo), and also observed the release and germination of zoospores at this high salinity.

The lack of an isomorphic alternation of generations in Cladophora glomerata, which is usual in other Cladophora species, has been reported by several authors (Hoek 1963, Whitton 1970). These works report the rarity of copulation of gametes, reproduction being effected mainly by asexual zoospores. However Shyam (1980) proposes that there are two lines of life-cycle evolution within this species : one having high ploidy levels and no alternation of generations, and the other low ploidies involving diploid sporophyte and haploid gametophyte stages in a strictly alternating sequence.

1.2 ALGAE AND SALINE ENVIRONMENTS

The global and local distributions of macro-algae are governed by the interaction of abiotic and biotic factors. The former include wave-action, light intensity and quality, photoperiod, salinity and temperature. Biotic factors such as epiphytism, grazing and competition also affect distribution (Lobban et al. 1985). Temperature is widely regarded as the most important determinant of global distribution (Section 1.1), but these other factors may have local importance and so determine small-scale patterns of species distribution. Salinity is likely to operate in this manner.

Except for those living in purely freshwater and subtidal environments, all algae are subjected to brackish water of some form. Brackish water has been defined by Hartog (1970) as ".... water with an unstable salinity, which results from the dilution or concentration of sea salts." This definition has resulted in the author classifying at least nine different types of brackish-water habitat. The factors responsible for such salinity variations are described by Hartog (1967, 1968 and 1970). Although these papers give a very thorough account of the ways in which different saline environments may arise, they do not include the formation of hypersaline conditions in contained water bodies resulting from the freezing out of salt during ice formation (Edelstein and McLachlan 1975, Ganning 1971). It is noteworthy that the ecological significance of salinity variations in certain

brackish habitats is thought by some to be overestimated, following very small salinity fluctuations being recorded in physico-chemical studies on rock pool systems (Daniel and Boyden 1975, Ganning 1971), although c.f. Edelstein and McLachlan (1975).

The frequency and magnitude of fluctuations in external salinity is a major factor governing the ability of a plant to establish itself in a marine or brackish environment. Algae need to maintain cell turgor for cell division and growth to occur, and many cellular metabolic processes can only function properly within limited intracellular osmotic conditions. Under salinity stress the ionic equilibrium of algal cells will be altered, and therefore, plants must possess morphological and/or physiological mechanisms to maintain cell ionic homeostasis under such conditions (Bisson and Gutknecht 1980). There is a number of comprehensive works that describe the osmotic responses of algal cells to salinity stress (Bisson and Gutknecht 1980, Cram 1976, Hellebust 1976, Kauss 1978, Zimmermann 1978). The short term responses of algae to hypo- and hypersaline conditions are summarised in Fig. 1.1.

Cram (1976) suggested that the term 'turgor regulation' should be applied to walled cells, and 'volume regulation' to wall-less cells. However, Zimmermann (1978) has shown that these two processes are interrelated by the elastic properties of the cell wall (if present) and plasmalemma. These relationships are described by the

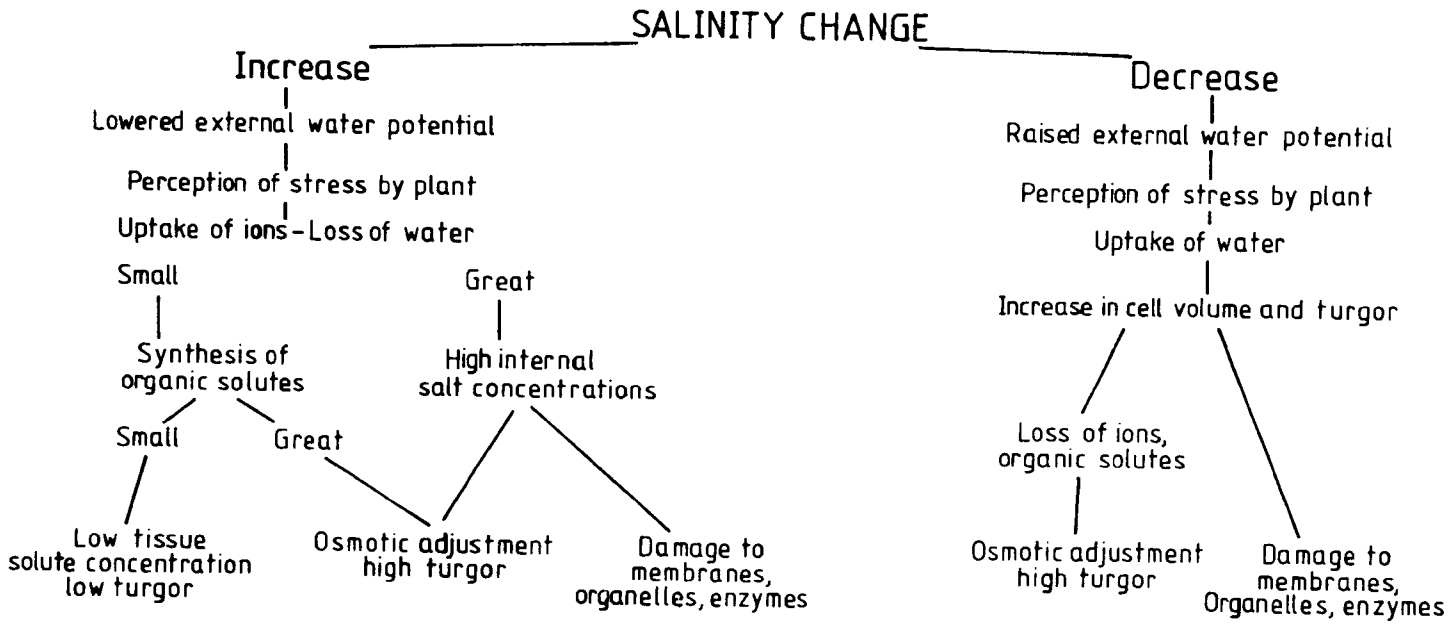


Fig.1.1 Short-term responses of algae to changes in external salinity. (After Russell 1987a).

following equation taken from Reed et al. (1980a):

$$\Delta\psi_o = \Delta P - \Delta\pi_i = (\varepsilon + \pi_i)\ \frac{\Delta v}{v}$$

ψ_o: External water potential. ε : Elastic modulus. π_i: Internal osmotic pressure. v : Volume. P: Hydrostatic (turgor) pressure.

In cells with rigid cell walls, ε is large and so volume changes are small, therefore turgor pressure shows the greatest change in response to altered external osmotic pressure. In wall-less cells, ε and P are very low, and volume changes account for most of the physical response to hypo- and hypersaline conditions (Reed 1984).

When exposed to changes in external salinity, and the subsequent increase or decrease in cell volume/turgor, plant cells alter their intracellular osmotic potential by altering their concentrations of osmotically active particles (inorganic ions and/or organic compounds) so that cell turgor/volume is restored. Very little is known about the mechanisms by which cells detect a need to initiate turgor regulatory processes, although it is thought that membrane deformation may provide the initial signal (Bisson and Gutknecht 1980, Zimmermann 1978). The means of conveying this signal and subsequent control of turgor regulation are also little understood, although Kauss and his colleagues working with the unicell Poterioochromonas malhamensis have described the control and possible mechanisms for the accumulation of isofloridoside during upshock conditions (Kauss 1978, 1983, Kauss and Rausch 1984). A number of studies have shown that in many algae, although they survive an osmotic

shock, recovery of turgor/volume is often incomplete (Dickson et al. 1980, Kirst and Bisson 1979, Reed 1983b, Reed et al. 1980a). These would indicate that the cells of these plants can withstand a range of cell turgor potentials (Reed 1983b).

Table 1.1 shows the wide diversity of inorganic ions and organic compounds used by a few of the numerous algae in which the osmotica used during turgor regulation have been identified. It is energetically less costly to regulate ion concentrations than to synthesise and degrade organic solutes, however, normal enzyme activity generally can only take place within narrowly defined intracellular ionic contents (Bisson and Kirst 1979). There is evidence that in some algae enzyme adaptation has occurred, enabling metabolic pathways to function under normally inhibitory intracellular ionic levels. Studies on the respiratory enzyme pyruvate kinase extracted from Porphyra umbilicalis indicate that it has a considerable tolerance to high concentrations of K^+ (Wiencke 1984) which would be encountered under hypersaline conditions. Kirst (1977a,b) has shown that when changes in external osmotic pressure are small the unicellular alga, Platymonas sudcordiformis alters its intracellular ion levels to regulate turgor pressure. However, when exposed to large changes although there is an ionic response, changes in the concentration of mannitol are more important. The major osmotica of Laminaria digitata varies seasonally, NO_3^- and K^+ being utilised during the winter, but during the summer when the

SPECIES	IDENTIFIED OSMOTICA	REFERENCE
CHLOROPHYTA		
Chaetomorpha linum	K^+ and Cl^-	Zimmermann & Steudle 1971
Codium spp.	K^+, Cl^- and DMSP	Bisson & Gutknecht 1977 and Reed 1983a
Dunaliella spp.	Glycerol, Sucrose	Ben Amotz 1974 and Ginzberg 1978
Enteromorpha intestinalis	K^+, Cl^-, Sucrose, Glutamic acid, DMSP and Proline	Brown & Hellebust 1980, Edwards *et al.* 1987, Reed 1983a and Young *et al.* 1987b
Lamprothamnion spp.	K^+, Cl^-, Na^+ and Sucrose	Bisson & Kirst 1980, 1983, Kirst 1977a and Okazaki *et al.* 1984
Platymonas subcordiformis	K^+, Na^+, Cl^-, DMSP, Mannitol, Hormarine and Glycine Betaine	Kirst 1977a, 1977b
Scenedesmus obliquus	Sucrose	Wetherell 1963
Stichococcus spp.	Proline and Sorbitol	Brown & Hellebust 1980
Ulothrix fimbriata	Sucrose and Glutamic acid	Brown & Hellebust 1980
Ulva lactuca	K^+, Na^+, Cl^- and DMSP	Dickson *et al.* 1980
CHRYSOPHYTA		
Potriochromonas malhamensis	Isofloridoside and K^+	Kauss 1978 and Kauss *et al.* 1975
Monochrysis lutheri	Cyclohexanetetrol	Craigie 1969
Cyclotella cryptica	Proline	Liu & Hellebust 1976
CYANOBACTERIA		
Nostoc muscorum	Sucrose	Blumwald & Tel-Or 1982
Synechococcus spp.	Sucrose	Reed & Stewart 1985
Synechocystis spp.	Glucosyl glycerol	Reed & Stewart 1985
PHAEOPHYTA		
Ascophyllum nodosum	Mannitol	Munda 1967 and Reed *et al.* 1985
Himanthalia elongata	Altritol	Chudek *et al.* 1984
Fucus spp.	Mannitol	Munda 1967 and Reed *et al.* 1985
Laminaria digitata	K^+, NO_3^- and Mannitol	Davison & Reed 1985
Pelvetia canaliculata	Mannitol and Volemitol	Reed *et al.* 1985
Pilayella littoralis	K^+, Na^+, Cl^- and Mannitol	Reed 1980 and Reed & Barron 1983
RHODOPHYTA		
Griffithsia monilis	K^+, Na^+, Cl^- and Digeneaside	Bisson & Kirst 1979
Polysiphonia lanosa	K^+, Cl^- and DMSP	Reed 1983b
Porphyra perforata	Floridoside, Isofloridoside and K^+	Eppley & Cyrus 1960 and Kauss 1968
Porphyra purpurea	Floridoside, K^+, Cl^-, Na^+	Reed *et al.* 1980b, 1981

DMSP :- β dimethylsulphoniopropionate
Floridoside :- α galactosyl-glycerol
Isofloridoside :- O-α-D-galactopyranosyl-(1→1)-glycerol

Table 1.1 Inorganic ions and organic solutes that have been shown to be osmotically active in a small selection of the many algae in which osmotica have been identified.

NO_3^- content of seawater is limited mannitol is accumulated preferentially to generate cell turgor (Davison and Reed 1985).

There have been very few physiological studies conducted to determine the responses of Cladophora species to saline stress and to identify osmotically active ions and/or solutes present within them. This is surprising considering the diversity of saline habitats occupied by this genus.

It is well established that there are significantly different morphological and physiological adaptations within certain species of marine algae growing in different saline environments (Munda 1977, Reed 1983b, Reed and Baron 1983, Russell 1985a,b, Yarish and Edwards 1982). These will be discussed in greater detail in a later section of the thesis. There have been no similar eco-physiological studies conducted on Cladophora species, and very little is known about the incidence and magnitude of variation within these.

1.3 AIMS OF THIS STUDY

Using the two species Cladophora glomerata and C. rupestris the purpose of this study is :

1. To measure salt tolerance, using net photosynthesis and dark respiration as an indirect measure of cell damage caused by salinity stress.

2. To measure the changes in protoplast and total cell volumes that occur when the plants are subject to changed external salinity conditions.

3. To measure the changes in the intracellular concentrations of several major ions, in response to saline change.

4. To investigate eco-physiological variation within species growing in different saline environments.

5. To measure the interactive effect of temperature upon salt tolerance.

7. To correlate the responses measured in the laboratory with the salinity and temperature conditions encountered in the field.

More detailed descriptions of the relevent literature and studies will be given in the sections covering the above, in which the methods employed will also be outlined.

2. MATERIALS.

2.1 PLANT MATERIAL

During this investigation, U.K. and Baltic populations of both Cladophora rupestris and C. glomerata were studied. Experiments on U.K. material were conducted at the Botany Department, University of Liverpool, Liverpool, U.K., and those on Baltic material at Tvärminne Zoological Station, SW Finland.

Cladophora rupestris

U.K. plant material was collected from an exposed shore on the NW side of Hilbre Island which is situated at the mouth of the Dee Estuary, Cheshire, U.K. (Fig.2.1, site 1). Hilbre is an archipelago of three small Bunter sandstone islands connected by low-lying reefs, to form a chain running parallel with the water channel. There is no evidence of salinity stratification at this site in the estuary, water mixing being complete. The water is often turbid with suspended matter showing signs of domestic pollution (Russell 1972, 1973). Plants were taken from a population located in the upper part of the eulittoral zone, where they were growing on a shaded vertical rock face (Plates 2.1 and 2.2). The same population was used throughout the study.

Baltic plants were obtained from two sites in the permanently submerged sublittoral zone from sites in the Tvärminne archipelago (Fig.2.2.). The substrate in this part of the Baltic Sea is mainly bare bedrock (predominantly granite and gneiss) and boulders in exposed

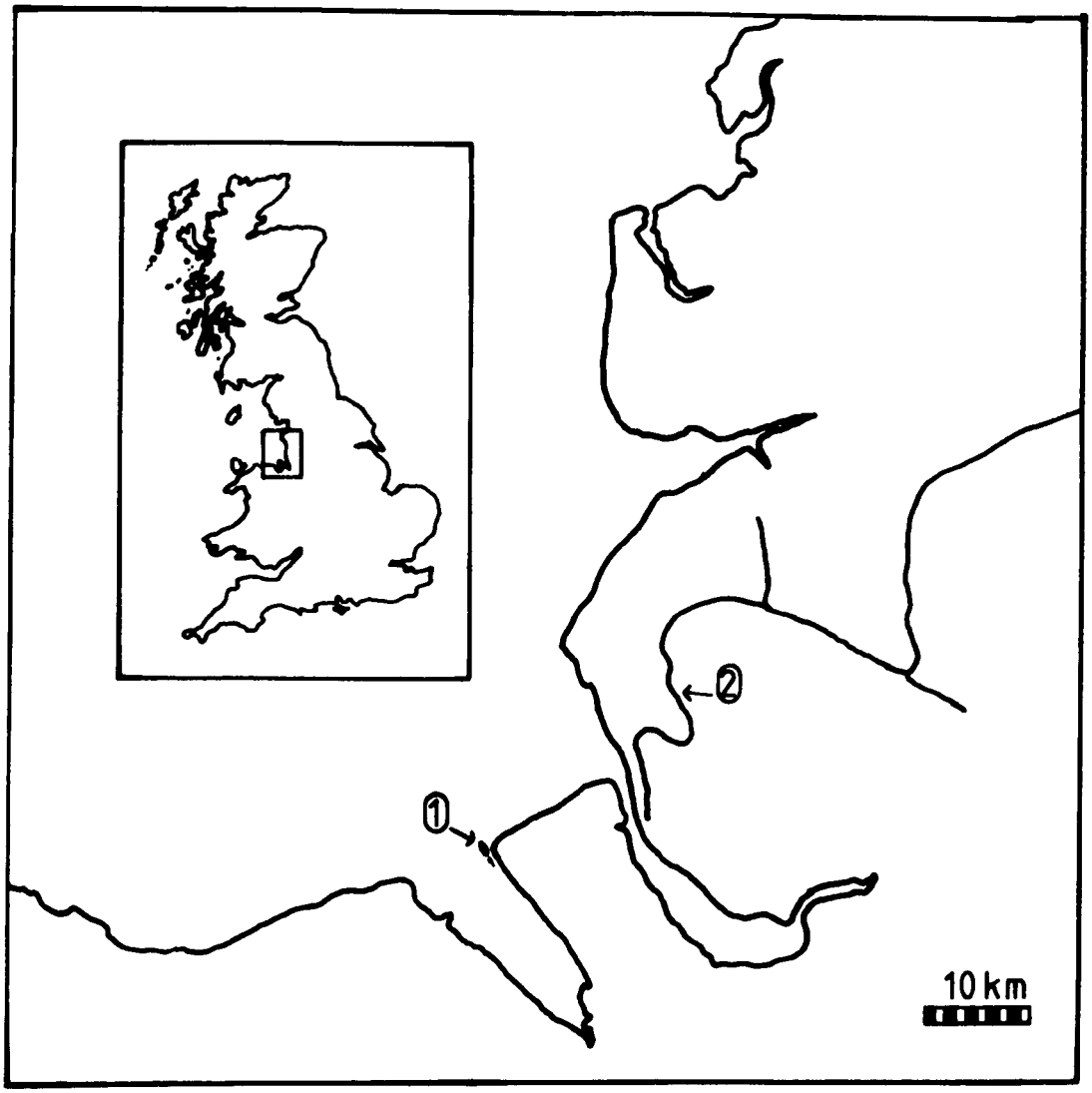


Fig.2.1 A map showing part of the North West coast of England. The course of the western section of the Leeds-Liverpool Canal is also shown. Site (1) Hilbre Island, Cheshire. Site (2) Maghull, Merseyside.

Plate.2.1 The rock face on the NW side of Hilbre Island, Cheshire, U.K. from which Cladophora rupestris was collected. The position of the site used throughout the study is indicated by the arrow.

Plate 2.2 The sampling site on Hilbre Island, taken approximately 2h after high water. The arrow indicates the position of the Cladophora rupestris population used during this study. (Photograph by courtesy of G.Russell)

Plate 2.1

Plate 2.2

locations, with sediment bottoms (mud, sand and gravel) only being found in very sheltered areas (Hällfors et al. 1981, Luther et al. 1975). There is ice cover in this area generally for 4-5 months in the year, and there is thermal and salinity stratification of the water, especially during summer months (Luther et al. 1975). Plants were collected by divers from depths of 2m at site 2, and 1m at site 3 (Fig.2.2). At both sites the Cladophora was growing as understorey plants below Fucus vesiculosus, and at these off-shore sites the plants are often subject to very strong underwater swell.

Cladophora glomerata

U.K. plant material was collected using a grapnel from a benthic population in the Leeds-Liverpool Canal, Maghull, Merseyside (Fig.2.1, site 2). The plants were growing in soft sediments at a depth of approx. 1.5m in an unshaded section of the canal. The water in this part of the canal has a mean annual pH of 8.5 (Howard et al. 1984) and is unpolluted. There is very little water movement, and flow rates are within the range 0.4 to 0.7km day^{-1} (Howard et al. 1984). This section of the canal is subject to ice cover in the winter.

Baltic plants were collected from the periodically submerged hydrolittoral zone (Hällfors et al. 1981), at a depth of 0.5m in a sheltered bay on an inshore skerry (Fig.2.2, site 4). The plants were growing on stable large boulders. Also see Plates 2.3 and 2.4.

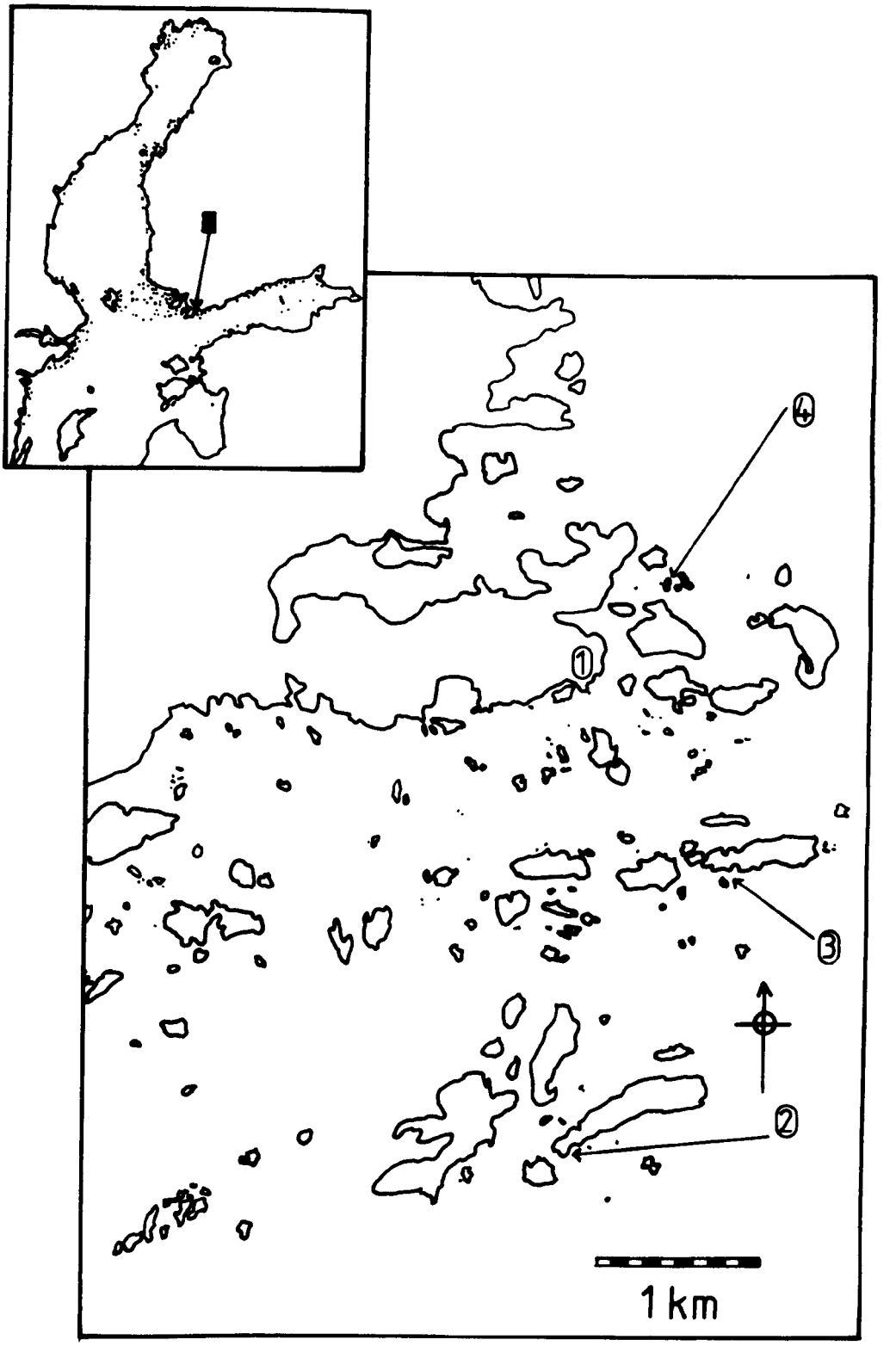


Fig.2.2 A map of the Tvärminne archipelago, SW Finland. (1) Zoological Station. (2) Langskär. (3) Furuskär. (4) Östra Kvarnskärgrundet

Plate 2.3 The sheltered bay on Östra Kvarnskärgrundet from which Baltic Cladophora glomerata was collected. The plant were growing on boulders at a depth of 0.5m.

Plate 2.4 This shows the prolific growth of Baltic Cladophora glomerata in the spring, at a site very close to that used during this present study. (Photograph by courtesy of G.Russell).

Plate 2.3

Plate 2.4

2.2 STORAGE CONDITIONS

U.K. plant material was returned to the laboratory in clean plastic bags and stored at 10°C, under artificial light of approximately 50 $\mu E\ m^{-2}\ s^{-1}$ (8h light : 16h dark). Cladophora rupestris was kept in aerated filtered seawater (34°/oo), and C. glomerata in aerated filtered canal water. During storage and before experimentation water was changed at least every 72h.

Baltic plants were returned to the aquarium of the Zoological Station in seawater-filled plastic buckets, and maintained in aerated running Baltic seawater (6°/oo) at 10°C, under artificial light of approximately 10 $\mu E\ m^{-2}s^{-1}$ (12h light : 12h dark), Experiments with all the species populations were performed within 5 days of collection.

3. ENVIRONMENTAL MEASUREMENTS

3.1 INTRODUCTION

The salinity fluctuations experienced by a plant in the intertidal environment during periods of emergence have been said to be a primary factor influencing the local distribution of algae in the intertidal zone on rocky-shores and in estuaries (Hartog 1968, Wilkinson 1980). However, although the salt tolerances of numerous algal species and the physiological mechanisms underlying such tolerances have been measured (Section 1.2), there have been very few measurements of the actual salinity variation experienced by these plants on the shore, both in the short term (tidal) and seasonally (Davenport 1982). Such measurements are vital, if the results of physiological salt tolerance studies are to be interpreted ecologically.

Temperature has been shown to influence the responses of algae to salinity stress (Dawes et al. 1978, Fralick and Mathieson 1975, Yarish and Edwards 1982, Zavodnik 1975), and therefore it is important that temperature variations in the field are also recorded during investigations into the effects of salinity fluctuations upon plants. In one of the few existing comprehensive studies made, Cawthorne (1979) made monthly measurements of salinity and temperature fluctuations from the lower shore of a well mixed part of the Conwy estuary, N.Wales, and showed there to be markedly different diurnal salinity and temperature profiles due to changing seasonal and

climatic influences (summary by Davenport 1982).

Most other physico-chemical investigations on intertidal rocky shores have been made on rock pool systems (Daniel and Boyden 1975, Edelstein and McLachlan 1975, Ganning 1971). However, due to their unique nature they should be treated as distinct habitat types within the intertidal environment, and the salinity and temperature fluctuations recorded within them should not be identified with those experienced by plants growing in emergent areas within this zone.

The primary purpose of this part of the study was to make measurements of the seasonal variation in salinity and temperature experienced by the Cladophora rupestris plants growing at the Hilbre site. The physiological responses to salinity stress will then be discussed in the context of these measurements in later sections of the thesis. Howard et al. (1984) have already made very detailed measurements of the water chemistry of a number of sites on the Leeds-Liverpool Canal, including one situated very close to that used during this study. Due to the uniformity of water quality in this part of the canal (Howard et al. 1984) the physico-chemical conditions of the site used during this present study are assumed to be the same as those described by these authors. Details of the seasonal changes in salinity and temperature experienced by the Baltic populations at Tvärminne, have been described by Luther et al. (1975).

3.2 METHODS

The temperature and salinity of the surface seawater were recorded at the Hilbre site, for the period October 1985 to May 1987. The temperature and salinity of water retained among emergent Cladophora rupestris plants was also measured over this period. Temperature was measured using a mercury thermometer (accuracy $\pm 0.5^{o}C$), and salinity measured by means of a refractometer (Aquafauna. Bio-Marine inc.). Water was sampled from emergent Cladophora thalli for salinity measurement with a $1.0cm^3$ clean plastic hypodermic syringe.

The content of the major cations, Na^+, K^+, Ca^{2+} and Mg^{2+} present in the water surrounding emergent plants was also measured on several occasions. Pre-weighed glass microfibre filters (7.0cm diameter) were placed onto the plants and samples of thallus-surface water absorbed. Three replicate samples were taken. Each saturated filter was placed in a clean plastic sample jar, sealed and returned to the laboratory. The papers were re-weighed (they were generally found to hold 0.9 to 1.5g of seawater), and then washed in the sample jars in 75 or $100cm^3$ of distilled water for 10-15min. The ionic content of the washes obtained were then measured using flame emission and atomic adsorption spectrophotometry as appropriate (PYE Unicam SP90A). Similar measurements were made occasionally using samples of surface seawater absorbed by filters.

Access to Hilbre Island on foot is restricted to

periods of mid-tide level and below. All visits to Hilbre were made on falling tides and just before low water. The Cladophora rupestris plants at this site are exposed to terrestial climate conditions at approximately 2h after high water (Plate 2.2) and therefore are emergent for periods of up to 8h during a tidal period. At the times of collection therefore, the plants had been emergent for periods of approximately 4h.

The seaonal variations in water temperature were also recorded at Maghull, and on occasions the ionic content of water samples, collected in clean plastic bottles was measured.

3.3 MEASUREMENTS

3.3a Temperature

The seasonal fluctuations in the temperature of the water at Hilbre and at Maghull are shown by Figs.3.1 and 3.2 respectively. The variation in water temperature of emergent Cladophora rupestris is also shown by Fig.3.1. Both sites experience very similar variations in temperature during the year, though the temperature of canal water is slightly higher than that of Hilbre seawater during summer. The surface water of the canal often freezes during winter, though the benthic C. glomerata, under these conditions experiences temperatures of 2-4^{o}C due to higher temperatures of water under the ice. Winter temperatures of the thallus-surface water of emergent C. rupestris were generally higher than those of seawater, and the reverse is true during the warmer

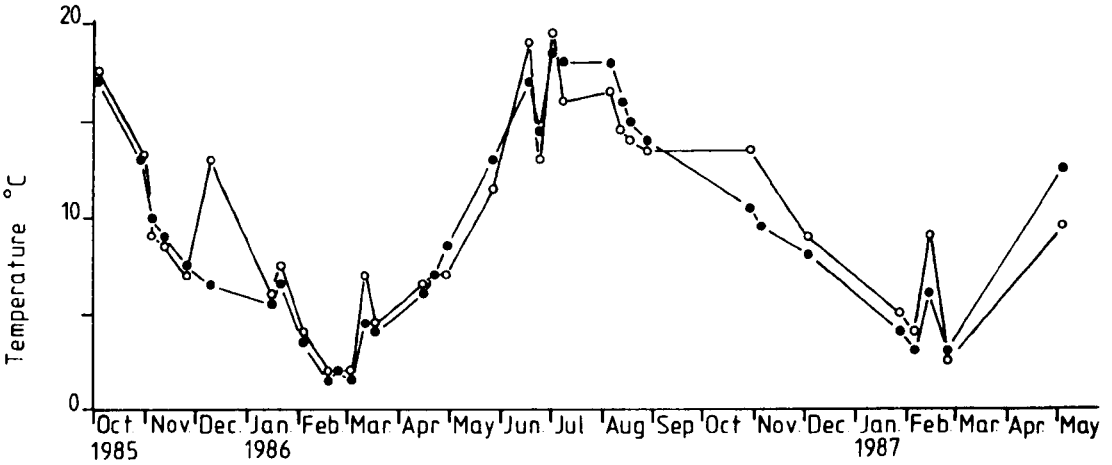


Fig.3.1 The seasonal variation in water temperature recorded at Hilbre Island between October 1985 to May 1987. -●-●- : Surface seawater. -O-O- : Water retained by emergent Cladophora rupestris plants.

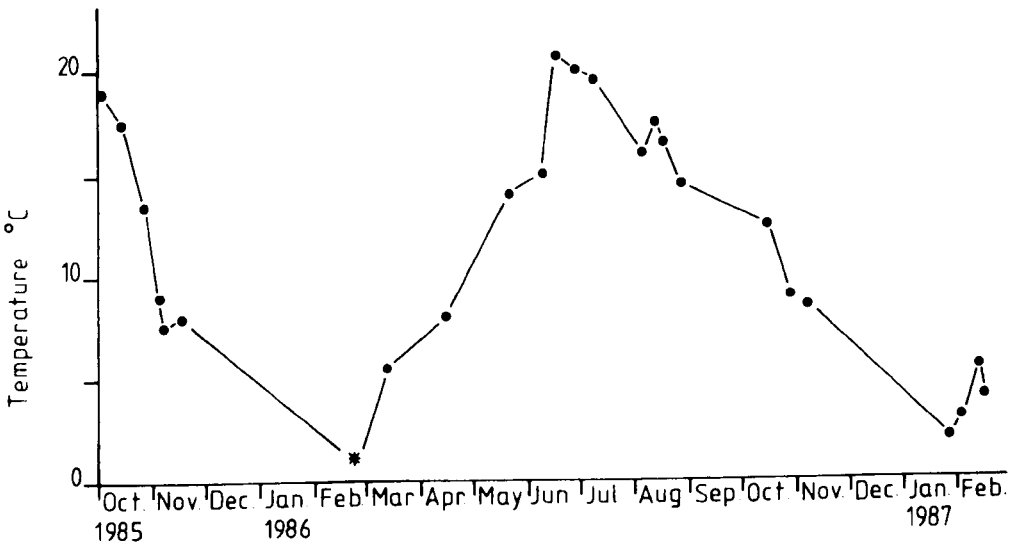


Fig.3.2 The seasonal variation in the water temperature of the Leeds-Liverpool Canal at Maghull between October 1985 to May 1987. * : Thick ice cover.

periods of the year.

3.3b Salinity

Fig.3.3 shows the seasonal variation in salinity of the Hilbre seawater and of the water retained among emergent Cladophora rupestris plants. Except for three occasions the salinities experienced by the exposed plants were equal to or, more frequently, greater than that of seawater. On these three occasions at which lower salinities were recorded, the measurements were made following heavy precipitation, and the values given are means of very variable readings, e.g. on 15.04.86, salinity readings ranging from 2 to 31^{o}/oo were recorded in a 0.5m^{2} area.

3.3c Ionic variation

The measurements made of the ion content of water retained by emergent Cladophora rupestris plants are given in Table 3.1. The ion measurements made on the surface seawater are also given for a number of sampling dates. The measurements show that the emergent plants are subject to relatively stable ionic conditions, as is the surface seawater. The levels obtained are consistent with values obtained from data given by Riley and Chester (1971) : Na^{+} (468mM), K^{+} (10mM), Ca^{2+} (10mM) and Mg^{2+} (53mM). The Na^{+} content recorded on the 28.10.86 is particularly low although the other ions are not as diluted and, since measurements made at the same time did not show any reduction in salinity, this is probably an erroneous

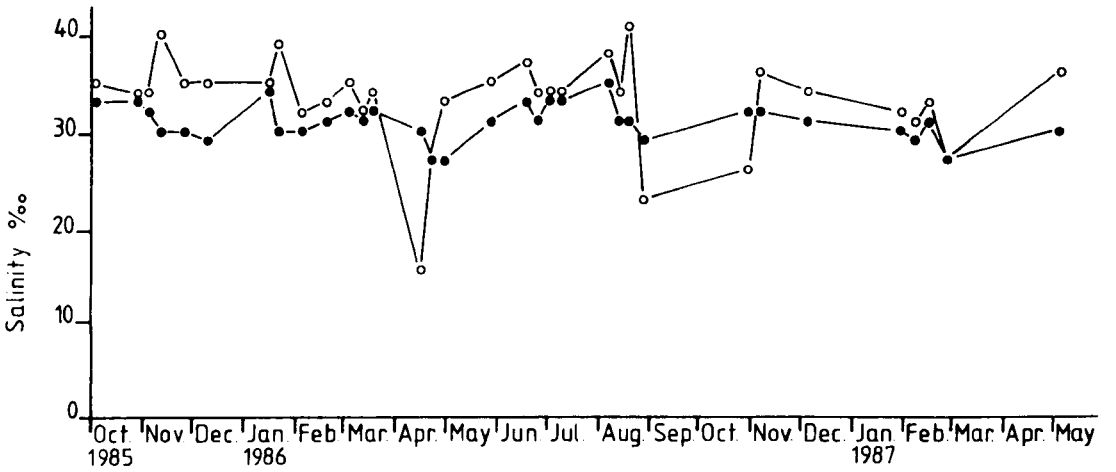


Fig.3.3 The seasonal variation in the salinity of the seawater at Hilbre Island between October 1985 to May 1987. -●-●- : Surface seawater. -O-O- : Water retained by emergent Cladophora rupestris plants.

ENVIRONMENTAL MEASUREMENTS

DATE	Na^+	K^+	Ca^{2+}	Mg^{2+}
12.11.84	A. 445.6 (±9.0) B. 156.7 (±12.1)	11.3 (±0.3) 5.8 (±0.1)	*	*
23.05.86	*	13.5 (±0.3)	10.7 (±0.2)	*
02.10.85	396.9 (±40.6)	17.3 (±0.1)	13.4 (±0.1)	61.2 (±2.4)
28.10.85	235.7 (±3.0)	9.9 (±0.3)	9.2 (±0.1)	47.2 (±0.2)
11.11.85	502.7 (±19.4)	15.7 (±0.9)	11.5 (±0.1)	67.3 (±2.4)
25.11.85	414.6 (±6.5)	13.4 (±0.2)	*	55.6 (±2.4)
03.02.86	406.2 (±1.9) 372.2	11.2 (±0.1) 10.2	10.0 (±0.3) 9.1	48.0 (±0.1) 43.0
03.03.86	373.9 (±46.7) 559.3	13.8 (±1.0) 21.6	6.6 (±1.4) 12.7	43.0 (±5.8) 64.0
16.06.86	506.3 (±6.6) 442.0	15.4 (±0.2) 14.0	7.6 (±0.2) 7.0	54.7 (±2.1) 44.8
07.07.86	456.6 (±9.7) 466.2	15.2 (±0.4) 14.3	8.0 (±0.2) 7.5	46.7 (±1.5) 48.1
04.08.86	474.6 (±7.8) 428.0	28.2 (±0.3) 23.7	12.5 (±0.2) 11.3	47.3 (±1.3) 45.9
16.08.86	450.0 (±5.9)	19.6 (±0.3)	10.9 (±0.03)	49.3 (±1.2)

Table 3.1 The major ion content (mM) of water retained by emergent Cladophora rupestris plants. +/- Standard errors are given in parenthesis. There were 3 replicates. The underlined data are values obtained from single samples of the surface seawater at Hilbre Island.

DATE	Na^+	K^+	Ca^{2+}	Mg^{2+}
17.05.85	1.4	0.2	1.6	1.7
02.10.85	2.7	0.3	3.4	0.9
14.10.85	1.2	0.3	1.2	0.5
11.11.85	1.0	0.2	1.1	0.03
16.12.85	1.6	0.3	3.2	0.6
16.06.86	2.2	0.2	0.9	0.6
07.07.86	0.1	0.2	0.8	0.6
HOWARD ET AL 1984	1.7 (0.6)	0.2 (0.02)	1.3 (0.2)	0.6 (0.1)

Table 3.2 The major ion content (mM) of Leeds-Liverpool Canal water at Maghull. The mean annual values measured by Howard et al. (1984) are also given. Standard deviations are given in parenthesis.

reading.

On 12.11.84 measurements were also taken from a site experiencing substantial freshwater run-off following heavy precipitation (12.11.84b.), and there is an obvious dilution of the ionic content of the water surrounding these Cladophora rupestris plants.

The measurements made of the major ion content of the canal water show that there is little seasonal variation in the levels of these ions (Table 3.2), and they are similar to those obtained in the more extensive investigation of Howard et al. (1984). See Table 3.2 for the mean annual values obtained by these authors.

3.4 DISCUSSION

Fig.3.4 shows temperature-salinity hydroclimographs for the Baltic and U.K. sites used in this study. Data collected from an off-shore site for stenohaline surface-water of the Irish Sea (Slinn and Eastham 1984) are given for comparison. The mean monthly data for the Baltic and Irish Sea are based on readings taken over many years, whereas those for Hilbre are based on measurements made during this study and so are based on only a few readings (in July only 1 reading taken). The plots show that although the Baltic and Hilbre plants are subject to large temperature variation during the year, the salinities at the two sites do not vary much. However both the salinity and temperature variation are considerably greater than recorded for open-Irish Sea water.

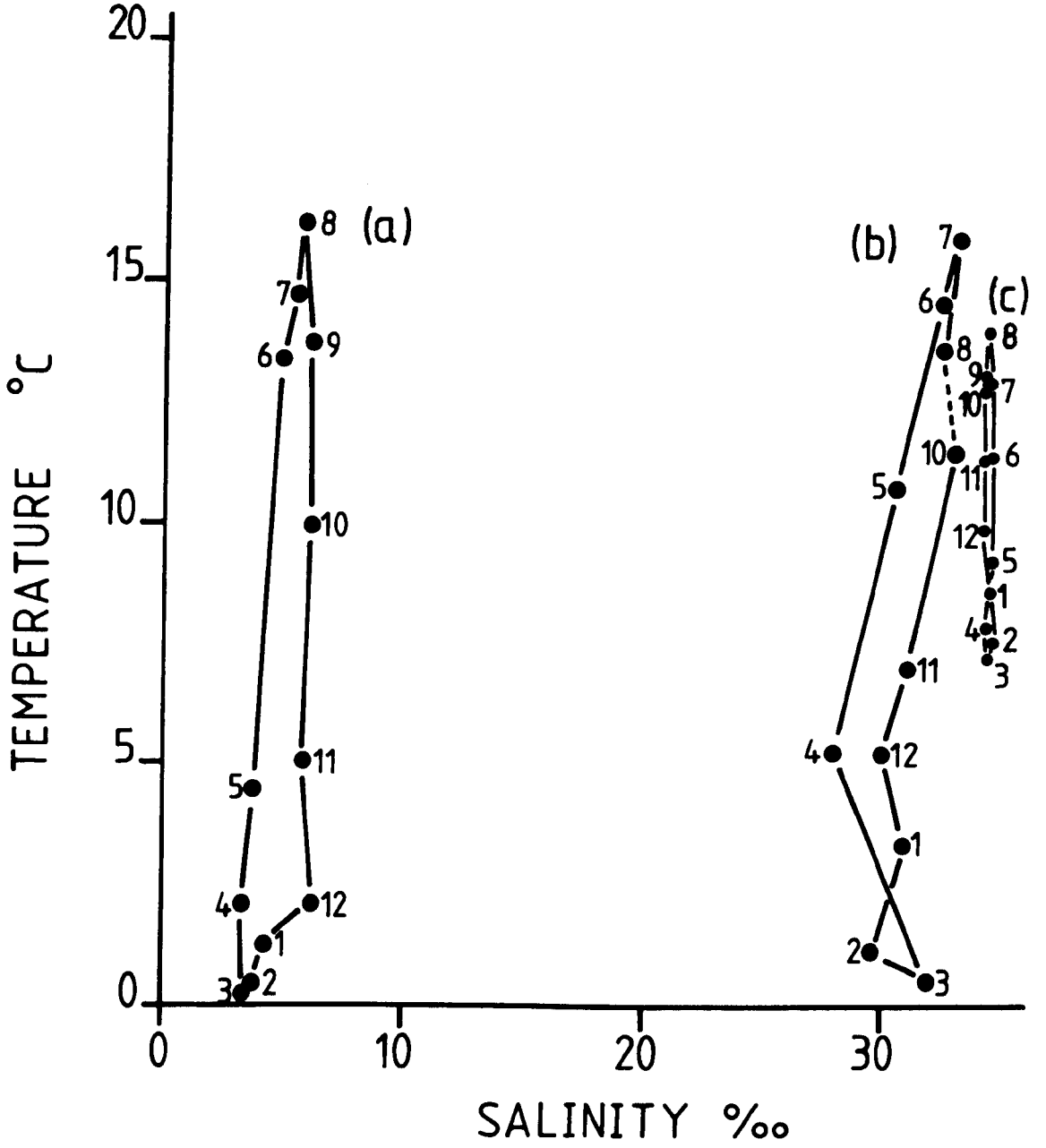


Fig.3.4 Temperature-salinity hydroclimographs for : (a) Surface seawater at Tvärminne (Luther et al. 1975). (b) Surface seawater at Hilbre Island. (c) Surface seawater of an offshore site in the Irish Sea (Slinn and Eastham 1984). Data points are monthly mean temperature and salinity readings. N.B. 1 = January .... 12 = December.

Hilbre Cladophora rupestris during periods of emergence, experiences both hypo- and hypersaline conditions resulting from prevailing climatic conditions. Generally, these salinity changes are not very large, normally 0-5°/oo above that of the seawater. These measurements would suggest that it is rare for the plants growing at this site to experience large fluctuations in external salinity. This is supported by the fairly constant ionic contents measured of the water surrounding emergent plants. These low salinity fluctuations may be due partly to the small tuft-like growth form of the C. rupestris plants retaining water, and not presenting a large surface area for dilution and evaporation of this water to take place. Daniel and Boyden (1975) and Ganning (1971) have reported that the salinities of intertidal rockpools are quite stable, and the results obtained in the present study indicate that certain protected emergent intertidal habitats may be exposed to less extreme salinity fluctuations than has often been supposed.

It is important to note that interstitial thallus water samples were taken from Cladophora rupestris plants that had been exposed for only about 1/2 of their total emergence period. It is probable therefore, that at the times of resubmergence these algae had experienced greater changes in salinity than those recorded when visiting the site. The highest increase in salinity recorded was 10°/oo, and if a linear relationship between salinity and exposure-time is assumed, the plants may infact experience

increases in salinity of approximately 20°/oo over the whole emergent period. However, increases of up to 5°/oo were more usual, which may represent total increases of 10°/oo during low tide periods. The summer of 1986 was also particularly poor, and it is likely that had there been a period of very hot, dry weather higher temperatures and salinities would have been recorded. Higher temperatures would also have been recorded for both the surface seawater and canal water. By taking temperature measurements only during the day, the lowest winter temperatures were likewise unrecorded. Therefore, any further studies would have to be much more detailed, and to include diurnal fluctuations in temperature and salinity, measured at regular intervals over many years. Such investigations should also be made in conjunction with more extensive meteorological measurements, such as air temperature, rain-fall and relative humidity.

4. EFFECT OF SALINITY ON PHOTOSYNTHESIS AND RESPIRATION

4.1 INTRODUCTION

Investigations of cellular damage to macroalgae, caused by changes in salinity, have frequently made use of carbon metabolism as an indirect expression of stress. Much of this work has concentrated on the effects of salinity upon net photosynthesis (Gordon et al. 1980, Ogata and Matsui 1965, Penniman and Mathieson 1985, Reed et al. 1980c, Yarish et al. 1979, Zavodnik 1975). Table 4.1 is a summary of the relatively few salt tolerance studies conducted on green algae that have included measurements of net photosynthesis. There have been considerably more similar investigations made on species from the other major algal groups. The data presented in this table show the oxygen production by the plants over a range of salinities. Where possible the units of seawater concentration and photosynthetic rate have been transformed to $^{o}/oo$ and $mgO_2h^{-1}g^{-1}$(dry weight) respectively. When it has been necessary to estimate the ratio of fresh weight : dry weight, values of the % water content of Enteromorpha prolifera at different salinities (Young et al 1987a) have been used. The data presented in table 4.1, and those from studies conducted on other species indicate that most marine algae show maximum photosynthetic activity in salinities of 30-40$^{o}/oo$, with very reduced activity in extreme hypo- and hypersaline conditions.

A number of salt tolerance investigations have also

SPECIES	METHOD OF OXYGEN DETERMINATION	SALINITY ‰	NET PHOTOSYNTHESIS	REFERENCE	COMMENTS
ENTEROMORPHA INTESTINALIS	W	3 44 90	24 21 13	OHNO 1976	2,3
ENTEROMORPHA LINZA	W	2 44 90	11 21 13	KJELDSON & PHINNEY 1972	1
CLADOPHORA AFF. ALBIDA (C. MONTAGNEANA GORDON ET AL 1985)	E	0 30 60	3 7 6	GORDON ET AL 1980	2,6
CLADOPHORA REPENS	M	0 25 50	0.8* 3.2 1.6	DAWES ET AL 1978	
CLADOPHORA VAGABUNDA	W	46 62 90	20* 25 9	BOLOGA 1979	4
ULVA EXPANSA	M	0 22 33	0.7* 5.0 5.0	KJELDSON & PHINNEY 1972	1
ULVA LACTUCA	M?	31• 35 40	0.11* 0.16 0.23	LEGENDRE 1921	
	W	0% S.W.? 100 200	0.08+ 0.31 0.12	FROMAGEOT 1923	
	?	0% S.W. 100	2.5++ 12.0	GESSNER & HAMMER 1960	
	W	0 20 42	0.4* 2.1 3.5	ZAVODNIK 1975	5,7
ULVA PERTUSA	M	0• 33 63	19* 26 19	OGATA & MATSUI 1965	3

W : WINKLER TITRATION . M : MANOMETRIC . E : OXYGEN ELECTRODE .

+ $mgO_2h^{-1}cm^{-2}$; ++ $cm^3N/100\ Na_2S_2O_3 20min^{-1}$

• : UNITS OF SEAWATER CONCENTRATION HAVE BEEN TRANSFORMED
* : UNITS OF OXYGEN PRODUCTION HAVE BEEN TRANSFORMED
1 : EXPTS. PERFORMED AT VARIOUS TEMPS
2 : EXPTS. PERFORMED AT VARIOUS ACCLIMATION PERIODS
3 : EXPTS. PERFORMED AT VARIOUS BICARBONATE CONCENTRATIONS
4 : COMPARED ATTACHED AND DETACHED MATERIAL
5 : COMPARED MATERIAL FROM DIFFERENT SALINE HABITATS
6 : EXPTS. PERFORMED WITH ARTIFICIAL AND NATURAL SEAWATER
7 : EXPTS. PERFORMED WITH DISTILLED WATER AND SPRING WATER AS DILUENTS

Table 4.1 A summary of salt tolerance studies that have been conducted on members of the Chlorophyceae (macroalgae only) that have used net photosynthesis as an indirect measure of cell damage during or following exposure to changes in external salinity. Photosynthetic rates are expressed as mgO_2h^{-1} g(dry weight)$^{-1}$ unless indicated otherwise.

involved measurements of dark respiration in conjunction with photosynthetic measurements (Dawes et al. 1978, Fralick and Mathieson 1975, Kjeldson and Phinney 1972, Mathieson and Burns 1971, Mathieson and Dawes 1974). The respiratory responses to salinity measured by these authors seem to vary between species. Ogata and Takada (1968) have reported that a number of species show increased respiration in hyposaline water, although hypersaline conditions caused supression of respiratory activity. Dawes et al. (1978) also measured high respiratory rates in several species after exposure to extreme hyposaline conditions. However, Mathieson and Burns (1971) showed that respiration rates of Chondrus crispus increase in both hypo- and hypersaline water. Studies on four Eucheuma species (Mathieson and Dawes 1974) have revealed a tendency for there to be low respiration rates at the salinities which give maximum photosynthesis, and vice versa. The results of many authors have led to Wilkinson (1980) in his review of estuarine algae to generalise that photosynthesis decreases and the rates of respiration increase at low salinities, leading to reductions in net photosynthesis.

The ability of plants to photorespire makes direct measurements of photosynthetic activity complicated. However, Bidwell and McLachlan (1985) have shown photorespiration not to occur in a selection of algae representing the major algal groups, and including Cladophora rupestris. These authors suggest that other

reports of the presence of photorespiration in algae may be due to measurement of light respiration unrelated to photorespiration. Such respiration occurred in actively growing meristematic tissue or in sporulating tissue, or when thallus-surface pH was raised, and also as a result of thallus wounding.

This part of the present investigation was undertaken to record photosynthetic and respiratory responses to salinity in both Cladophora rupestris and C. glomerata as indirect measures of the salt tolerances of these two species. The work was conducted using U.K. material only. Photosynthesis and respiration was measured after different incubation times to determine whether or not length of exposure to a particular salinity affects the ability of a plant to survive. Measurements of photosynthesis were made at light intensities slightly greater than that of saturating light intensity, and so it was necessary to make a preliminary study of the effect of light intensity on photosynthesis for both species.

4.2 SATURATION LIGHT INTENSITY

4.2a METHOD

Clean portions (0.019-0.021g) of plant tissue were weighed after careful blotting and then placed in the chamber of a Clark-type oxygen electrode (Rank) (see Delieu and Walker 1972), containing 2.5cm^3 of oxygen saturated filtered seawater for Cladophora rupestris or filtered canal water for C. glomerata. The chamber was maintained at a temperature of 12 $\pm$1^{o}C, using a

temperature controlled water jacket around the chamber. A foil reflector was placed behind the electrode to reflect any transmitted light back into chamber.The oxygen production in the sealed chamber was recorded on a flat-bed recorder, over a range of light intensities (20-2,000 $\mu E\ m^{-2}\ s^{-1}$) obtained by altering the distance between an external light source (Widioscope projector lamp, 250W 24V Halogen type 72) and the chamber. The oxygen production rate at each light intensity was measured until a constant rate was obtained. Three replicate samples of each species were used.

The light intensity (PAR) incident on the inside of the oxygen electrode chamber at each position of the light source had previously been measured by placing a light probe behind a chamber-unit that had been cut in half. The light intensity was recorded using a light meter (Macam Quantum/Radiometer/Photometer Q101).

The rate of oxygen production was measured from the slope of the recording, and expressed as $mgO_2h^{-1}g^{-1}$ (fresh weight). The electrode was calibrated using oxygen saturated media and media deoxygenated with sodium dithionite. The oxygen concentrations of the saturated media at 12^oC was measured using a modified Winkler titration (Strickland and Parsons 1972).

4.2b RESULTS

The photosynthesis versus light intensity curves at 12^oC for both Cladophora rupestris and C. glomerata are given in Fig.4.1 (see Appendix i). There were very marked

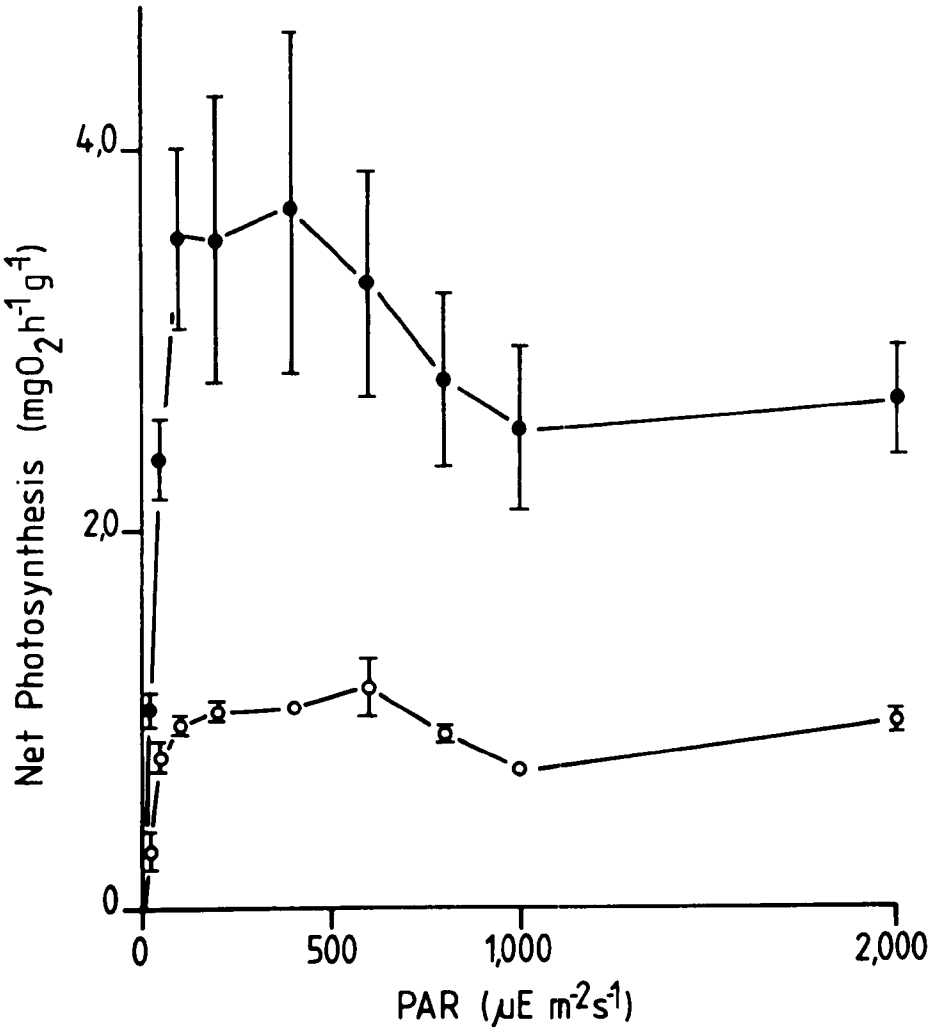


Fig.4.1 The effect of light intensity (at 12°C) upon the net photosynthesis of Cladophora rupestris (-●-●-) and C. glomerata (-O-O-). The Standard Error is shown for each sample.

differences in the rates of oxygen production between the two species at this temperature. The data for C. rupestris are quite varied, though it is apparent that light is saturating at intensities above 100 $\mu E\ m^{-2}s^{-1}$. There was inhibition of photosynthesis at the very high light intensities.

The light saturation curve for Cladophora glomerata is less variable than that obtained for C. rupestris, and light is saturating at 200 $\mu E\ m^{-2}s^{-1}$. At light intensities greater than 800 $\mu E\ m^{-2}s^{-1}$ there was slight inhibition of photosynthesis, although at 2,000 $\mu E\ m^{-2}s^{-1}$ higher rates of oxygen production are obtained.

On the basis of the light saturation curves, a light intensity of 200 $\mu E\ m^{-2}s^{-1}$ was adopted as a suitable intensity at which to make the following measurements of net photosynthesis of both species during saline treatment.

4.3 EFFECTS OF SALINITY TREATMENT

4.3a METHOD

Salinity treatments in these experiments were based upon seawater obtained from the Menai Straits, Anglesey, N.Wales. This water (34^{o}/oo) was diluted to produce a range of hyposaline treatments : 0 to 11^{o}/oo. By evaporating the water at 80^{o}C two hypersaline treatments were made : 68 and 102^{o}/oo. Cladophora rupestris was treated with media of 0, 6, 11, 34, 68 and 102^{o}/oo and C. glomerata with 0, 6, 11 and 34^{o}/oo. The 0^{o}/oo treatment for C. glomerata was filtered canal water. The net

photosynthesis and dark respiration rates were measured following treatment at intervals over a 24h period (1, 2, 4, 6, 12 and 24h).

Samples of clean plant material were blotted carefully, and 0.019-0.021g portions were weighed. Five replicate samples were used at each salinity treatment. These were placed in $25cm^3$ of appropriate treatment media in small beakers, which were aerated and kept at a constant temperature of 11 $\pm 1^{o}C$ in a thermostatically controlled water bath, and under continuous illumination of 45 $\mu E\ m^{-2}s^{-1}$. After incubation, the samples were treated as described above, and net photosynthesis measured in oxygen saturated treatment media, at 13 $\pm 1^{o}C$ at a light intensity incident on the inside of the chamber of 200 $\mu E\ m^{-2}s^{-1}$ (Section 4.2).

Dark respiration rates were then measured after switching off the light source and placing a foil hood over the chamber. Oxygen consumption was recorded until a constant rate was obtained.

4.3b RESULTS

The results for Cladophora rupestris are shown by Figs.4.2 and 4.3, and those for C. glomerata by Figs.4.4 and 4.5 (Appendix i). The values given at each salinity are those obtained from plant material incubated for 24h, since these are the best indications of tolerance by the plant to a particular salinity treatment. The variation in responses with time are illustrated in the smaller figures.

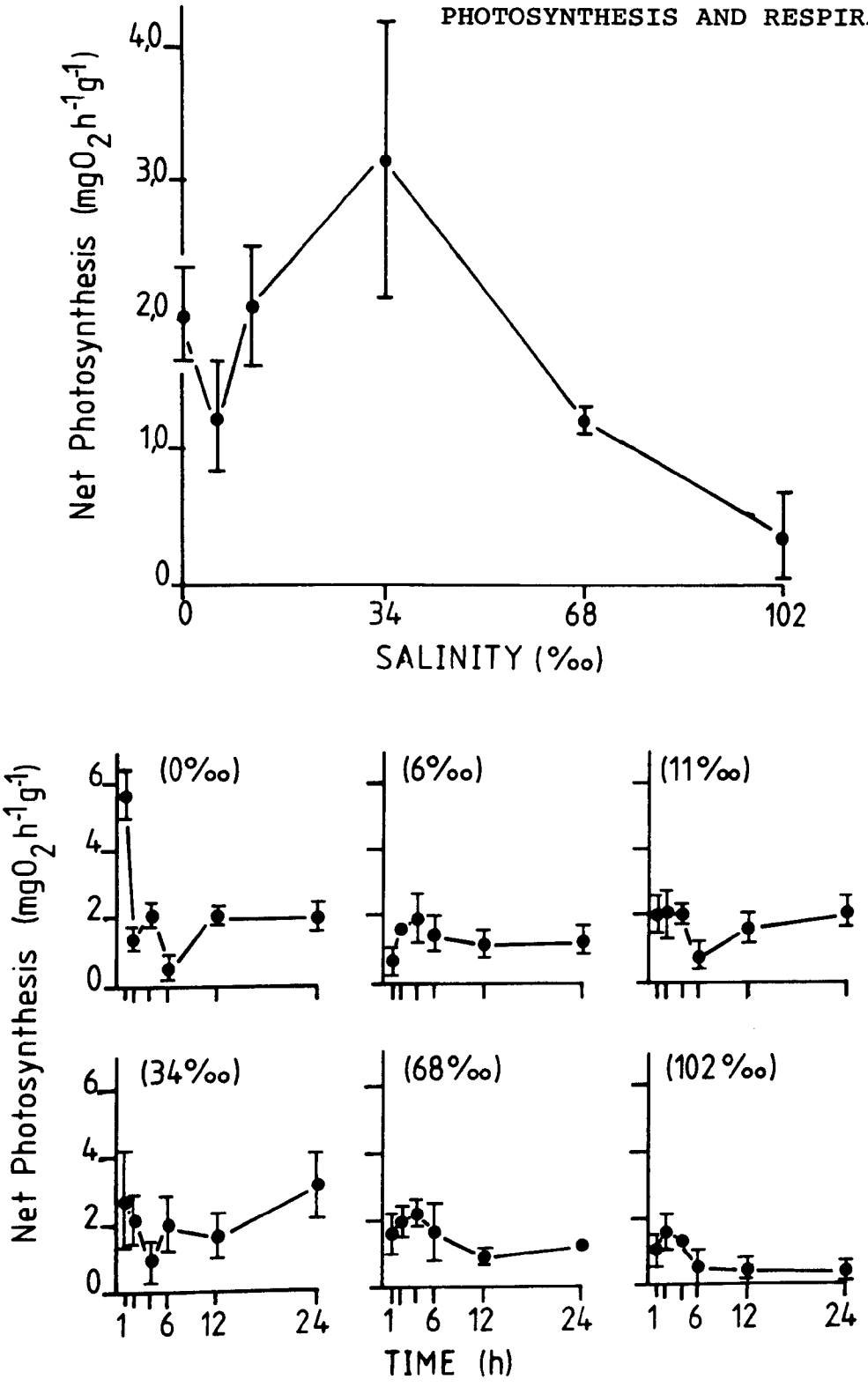


Fig.4.2 The effect of salinity change upon the net photosynthesis of Cladophora rupestris. The smaller diagrams show the effect of incubation time at each salinity treatment. 95% confidence limits are shown for each sample.

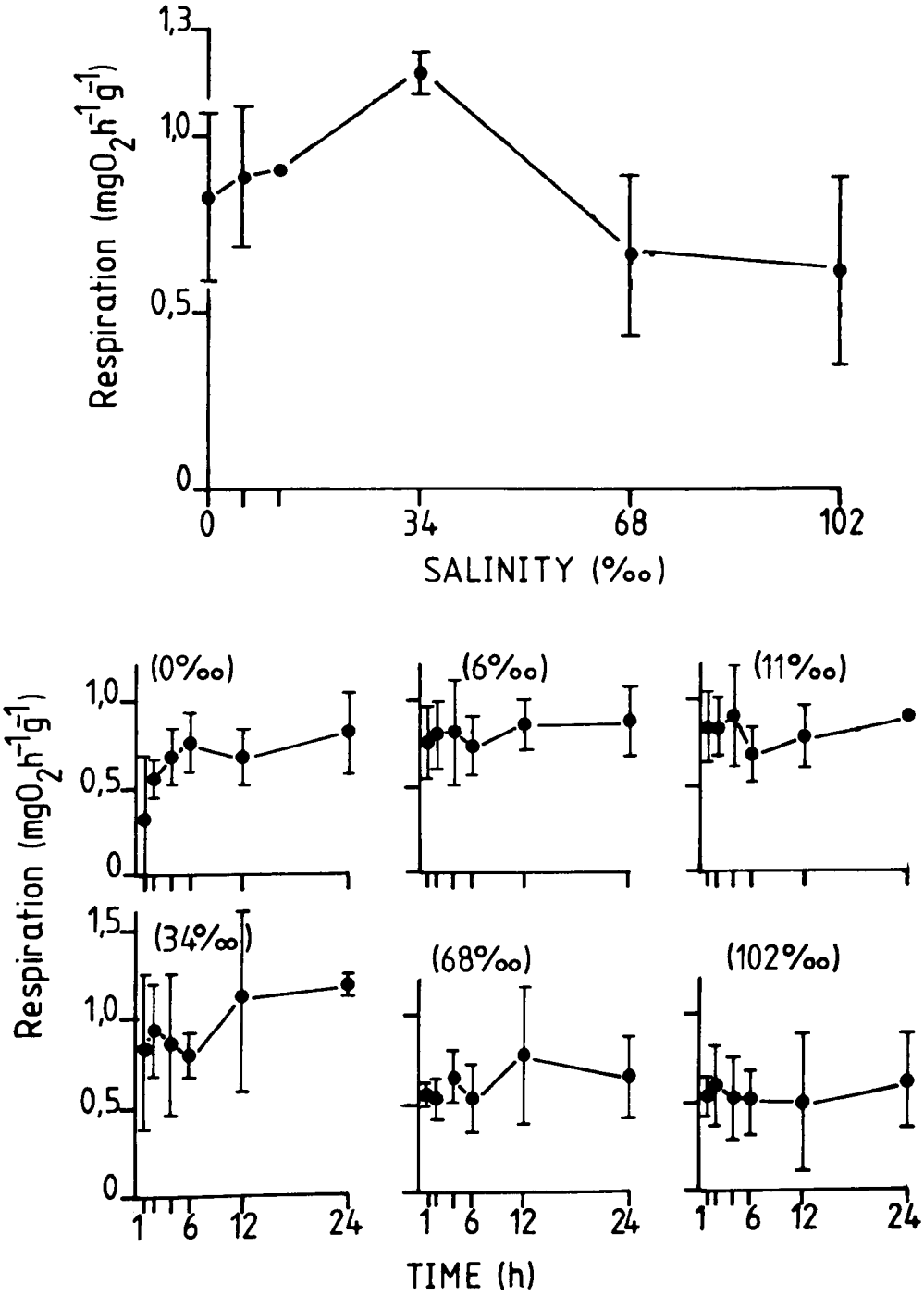


Fig.4.3 The effect of salinity change on the respiration of Cladophora rupestris. The smaller diagrams show the effect of incubation time at each salinity treatment. 95% confidence limits are shown for each sample.

Cladophora rupestris showed the highest net photosynthetic rates in normal seawater (34°/oo). The plants incubated in the 6, 68 and 102°/oo treatment media show greatly reduced rates of oxygen production (Fig.4.2), however, the rate of oxygen production of the algae in 0°/oo surprisingly was higher than for the plants in the 6°/oo treatment. The only significant difference in photosynthetic response with time was shown by plants incubated in the 0°/oo media, much higher values being obtained after 1h than at any other time.

The dark respiration rates recorded for Cladophora rupestris again, were highest in the 34°/oo media (Fig.4.3). There were no differences between the respiratory activities of the plants incubated in 0, 6 and 11°/oo, which is also true for the algae treated with 68 and 102°/oo media. The only variation in respiratory response with time was in the 0°/oo media with greatly reduced rates occuring after 1h.

The net photosynthesis of Cladophora glomerata was greatest at 0°/oo declining with increasing salinity (Fig.4.4), with very low rates being recorded for plants treated with 34°/oo media. Length of incubation time did not affect the photosynthetic rates in any of the salinities.

The only significant reductions in the dark respiration rates of Cladophora glomerata occurred in the plants incubated in the 34°/oo seawater (Fig.4.5). The rates obtained at all the salinities were very variable,

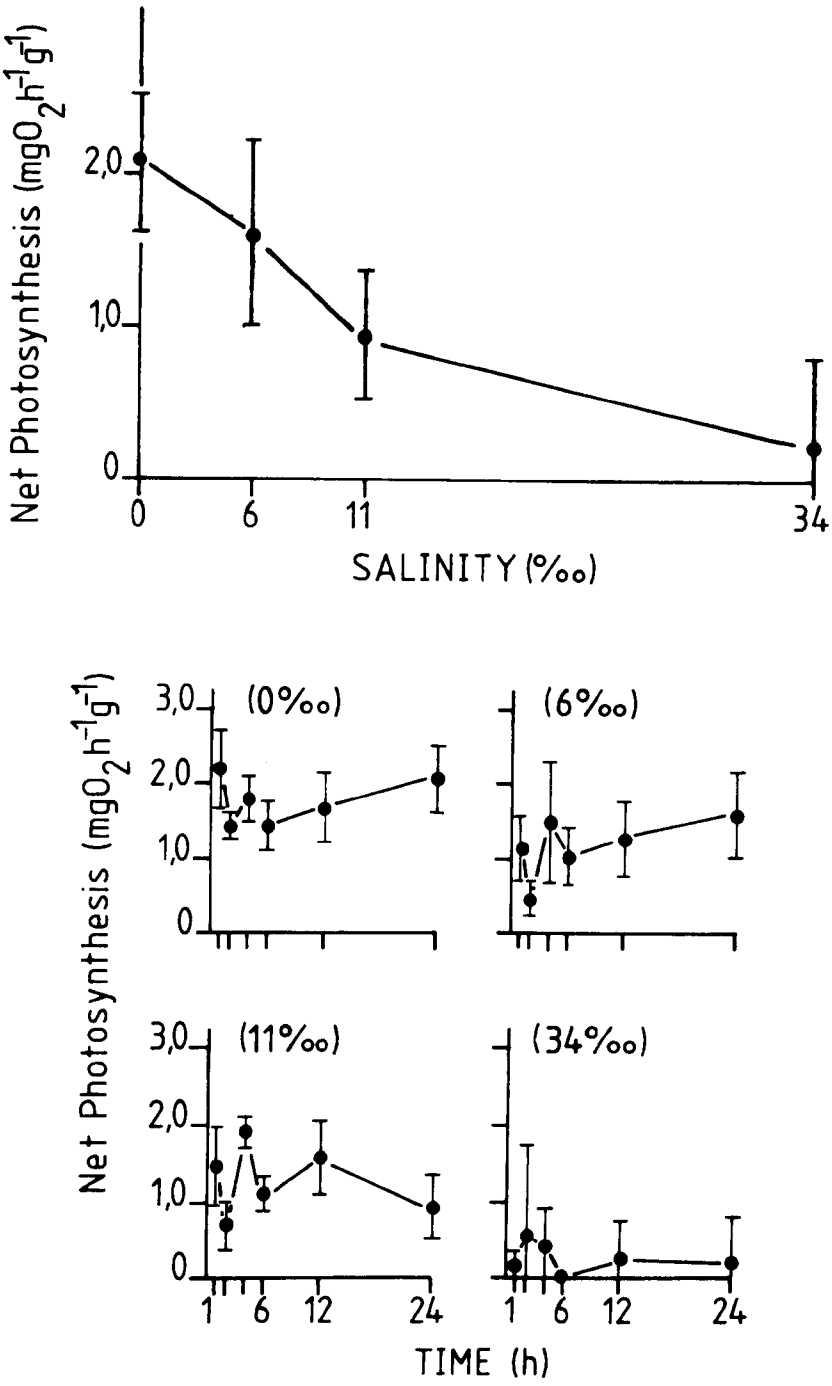


Fig.4.4 The effect of salinity change upon the net photosynthesis of Cladophora glomerata. The smaller diagrams show the effect of incubation time at each salinity treatment. 95% confidence limits are shown for each sample.

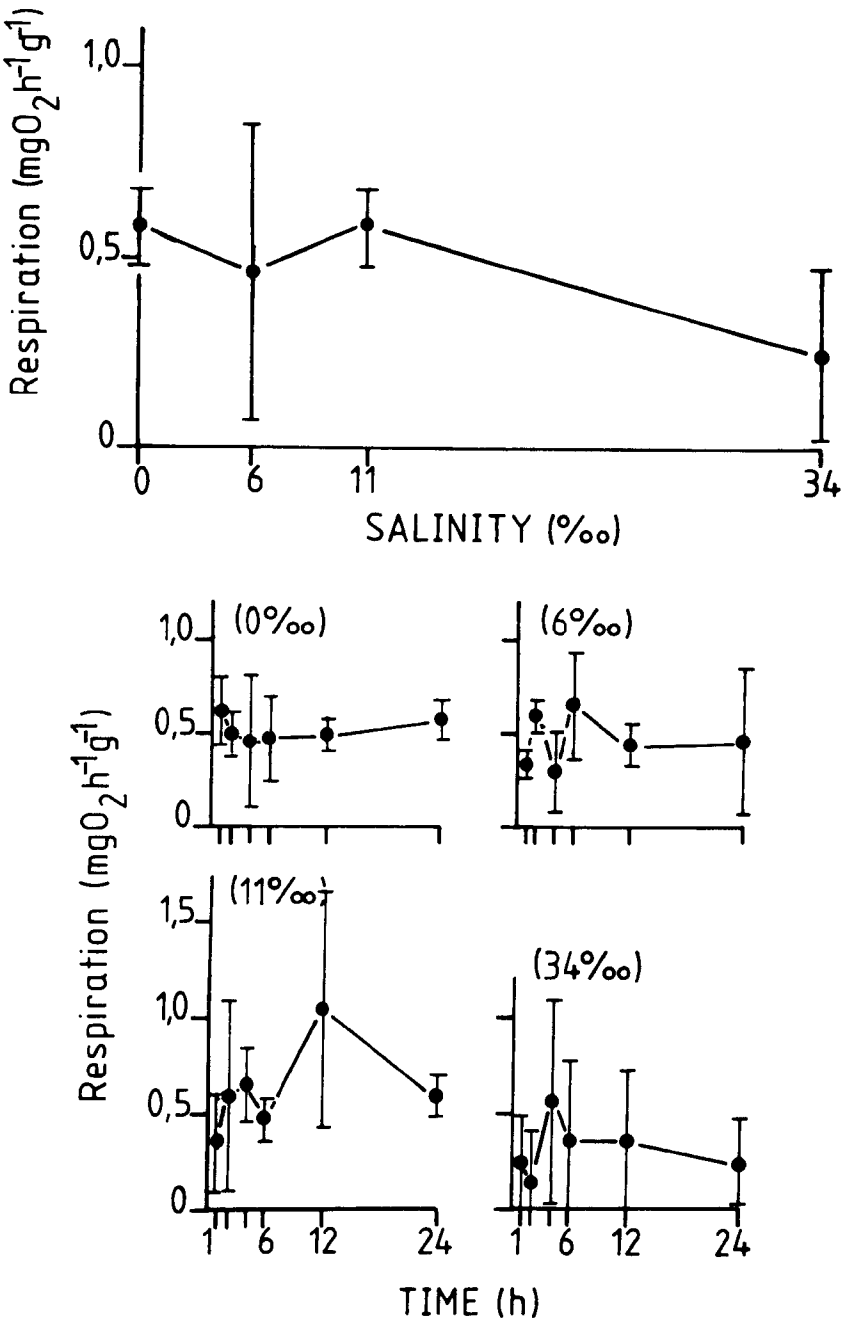


Fig.4.5 The effect of salinity change on the respiration of Cladophora glomerata. The smaller diagrams show the effect of incubation time at each salinity treatment. 95% confidence limits are shown for each sample.

although those at 11 and 34^{o}/oo were prticularly so. There was no significant effect of time upon the response of respiration to salinity treatment.

4.4 DISCUSSION

The inhibition of the net photosynthesis of Cladophora rupestris at high light intensities and the very low light saturating intensity measured, are factors which govern the characteristic distribution of this species, plants growing in shaded habitats and as understory plants (Hoek 1963). The much greater photosynthetic rates of C. rupestris compared to those of C. glomerata is in part a reflection of the greater chlorophyll content of the marine species. Following extraction by DMSO and methanol (Duncan and Harrison 1982), C. rupestris was found to contain 3mg(Chl a) g^{-1} (wet weight), compared with 0.6mg g^{-1} for C. glomerata.

The responses of the two species to salinity change are quite distinct : Cladophora glomerata shows little tolerance to increased salinity, whereas Cladophora rupestris can tolerate both increases and decreases in external salinity, although the plants have a greater tolerance of hyposaline conditions. The higher rates of net photosynthesis in C. rupestris in the 0^{o}/oo treatments than in those in 6^{o}/oo are anomalous. However, these high rates were perhaps caused by changes in rates of oxygen diffusion due to damage to membrane structure following such severe hyposaline shock, and may not accurately express the photosynthetic activities of the plants or

their ecological optima.

Bidwell and McLachlan (1985) have shown that representatives of the three main marine algal groups depend primarily upon HCO_3^- as a carbon supply for photosynthesis. The affinity for HCO_3^- has been shown to be greater in marine than in freshwater macrophytes, the latter using primarily CO_2 (Sand-Jensen and Gordon 1984). The dilution of this carbon supply (CO_2 and HCO_3^-) is thought to be the reason for the decline in photosynthesis of several marine algae in hyposaline conditions (Hammer 1968, Ogata and Matsui 1965). Dawes and McIntosh (1981) and Zavodnik (1975) have also demonstrated that algae exhibit enhanced photosynthetic activity in seawater diluted with nutrient rich spring water compared with plants in seawater diluted with distilled water.

Respiration in both species was less affected by changes in salinity than photosynthesis. Cladophora rupestris showed only slightly reduced respiration rates in hyposaline treatments, though greater reductions occurred in hypersaline conditions. C. glomerta showed little respiratory response to salinity, although the increased variability of respiration rates at higher salinities, and the few very low values obtained at $34^o/oo$, would suggest that disruption to the respiratory mechanisms had occurred. It is interesting to note that the respiration responses recorded for C. rupestris during this study do not follow the trends discussed previously (Section 4.1), in that hyposaline conditions did not cause

increases in respiration, and the highest respiration rates were not associated with the lowest rates of photosynthesis. However, the lower rates obtained in hypersaline waters are similar to those described by other workers.

Yeo (1983) has discussed the energy requirements of the physiological adaptations made by higher plants exposed to increases in external salinity. These regulatory processes bring about an increased demand for energy, which can be supplied either by an increase in respiration rate, or by diverting energy from other cellular processes. The reductions in respiration recorded in the experiments on both species mean that, at certain salinities, the energy requirements for osmotic adjustment can be met only at the cost of other metabolic activity, and subsequent growth.

These results indicate salt tolerances that follow closely the salinity regimes of the two plant populations: Cladophora glomerata living in stable freshwater conditions and C. rupestris being subjected to dilutions of thallus surface water during precipitation, and to a lesser extent, hypersaline conditions produced by evaporation. Although the extent of hypersaline stress experienced by plants on the shore may have been underestimated in Section 3, the salinity tolerance of the C. rupestris population is clearly greater than the salinity fluctuations measured, and the plants could be expected to survive short-term salinity changes of greater amplitude.

5. EFFECT OF SALINITY ON CELL VOLUME

5.1 INTRODUCTION

In general there is a biphasic physical response by wall-less plant cells to altered external salinity (Zimmermann 1978). Within a few seconds of exposure to change, swelling or shrinkage of the cells occurs, which is caused almost exclusively by the flow of water into or from the cell depending on the direction of the osmotic stress. The second phase, during which the cell is restored to (or close to) its original volume, takes place over a longer period of time (minutes or hours). This is brought about by the regulation of intracellular levels of osmotically active particles. A similar biphasic response has been shown in a number of walled cells (Zimmermann 1978). The plasmolysis and subsequent deplasmolysis that can be observed taking place in the cells of many algae under hypersaline stress is an indication of this biphasic response taking place.

The nature and extent of plasmolysis and deplasmolysis in the salinity stressed cells of numerous marine macroalgae including several Cladophora species, was used as criterion in many early salt tolerance investigations (Biebl 1937,1939,1952, Hofler 1931). A short summary of these studies is given by Gessner and Schramm (1971). Biebl (1937, 1939), by making osbservations of plasmolysis, deplasmolysis and cell viability over wide ranges of salinity, determined values of 'osmotic resistance' for many species and used these to

distinguish between ecological groups of marine algae.

It has been reported by several authors that the cells of some algae do not plasmolyse under hypersaline stress, but that swelling of the cell walls occurs instead (Fischer 1984, Gessner and Schramm 1971). This is because in normal salinity conditions turgor pressure compresses the cell wall, but when turgor is reduced the microfibrillar components of the cell wall separate and the wall swells. Turgor regulation by these cells is indicated by a shrinking of the swollen cell walls.

This part of the study was undertaken to make quantitative measurements of the changes that occur in cell and protoplast volume during salinity stress in both Cladophora glomerata and C. rupestris, under controlled light and temperature conditions. Such a method would therefore be a refinement of the more qualitative approach used by Biebl and his co-workers. The photomicroscopic technique employed, only allowed for measurements of area changes of the cell and protoplasm to be made, and it was assumed that such changes are directly proportional to any changes in cell volume that occur. Also, because of possible cell wall thickening (see above), total cell areas were measured from the inner surface of the cell wall.

5.2 METHODS

Single clean filaments were separated carefully from plants and then attached to the bottom of cavity slides using a small amount of molten agar. The slides were then

filled with 1.5-2.0cm^3 of seawater for Cladophora rupestris and canal water for C. glomerata, and then transferred to a constant temperature-room at 10°C. This temperature was selected since it is approximately the median of the annual temperature range both plant populations are exposed to. The plant material was examined under a microscope (Zeiss Photomicroscope II) which was also housed in the cold room. For consistancy, it was decided that the responses of the third cell proximal to the apex of C. rupestris filaments, and the fifth cell from the apex of C. glomerata would be measured. Undamaged cells were located that had no or very few epiphytes and these were then photographed. These could be relocated at later stages of the experiment using co-ordinates on the microscope stage. One cell was chosen from each slide, and five slides were used at each treatment salinity.

The water surrounding the plant material was then removed and replaced with 1.5-2.0cm^3 of a treatment media: 1.5, 6, 34, 68 and 102°/oo for Cladophora rupestris. and 0, 1.5, 6 and 34°/oo for C. glomerata. Photographs were taken of the cells at : 5min, 30min, 1h, 4h, 6 and 24h, although photographs were only taken at 5min, 1h, 6h and 24h for C. rupestris in 34°/oo and C. glomerata in 0°/oo treatment media. The plants were kept under constant illumination during the experimental period. A standard graduated 1mm scale was also photographed at this magnification to enable subsequent measurements of cell

and protoplast area.

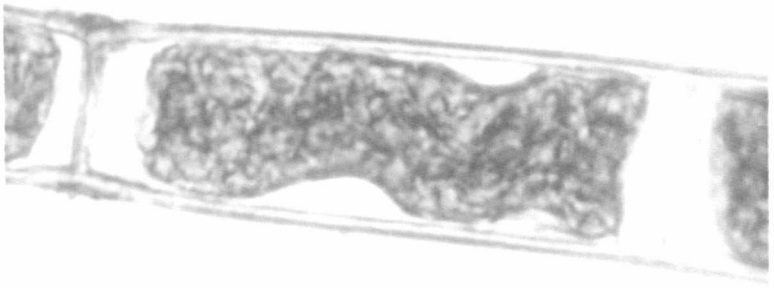
Photomicrographs were produced, and prepared for an analytical procedure (developed in the Computer Laboratory, Liverpool University) that incorporated a computer graphics system to measure areas on the photomicrographs. Images of protoplast and total-cell areas taken with a video camera were digitised, and quantified using a computer programme. The system was calibrated with known areas based on the measurements made using suitably enlarged photomicrographs of the 1mm scale. In order to produce clear video images it was necessary to blacken the areas to be measured and to whiten everything else (see Plate 5.1a,b). However, when the data produced using this technique were analysed it was clear that this system produced an unacceptable amount of error. Unfortunately further refinement of the digitising process and computer software were required which could not be made within the time available.

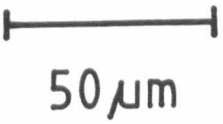
It was decided, therefore to make use of the photomicrographs obtained from the computer study, and quantify the cell area changes by means of a weighing method. Photocopies of the high-contrast photomicrographs were made. The protoplast area (black) was cut out from the total cell area and weighed, and the remaining cell area (white) was weighed also. These weights were transformed to area-measurements by weighing standard areas of paper based on measurements from the micrometer scale. By adding the weights of protoplast area to the

Plate 5.1a Photomicrograph of a Cladophora glomerata cell following 30min incubation in 34°/oo treatment media.

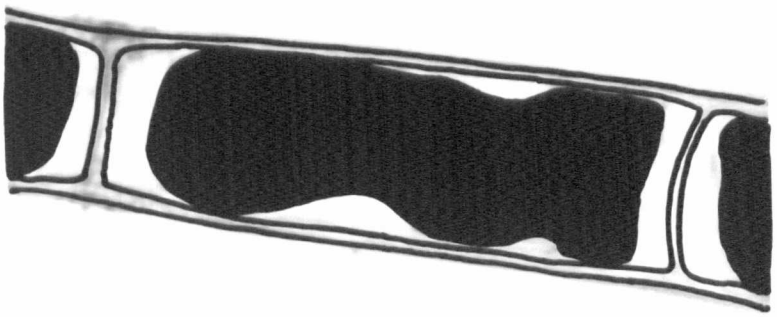
Plate 5.1b A high contrast image produced from the photomicrograph above.

PLATE 5.1a





5.1b



rest of the cell area, a measure of the total cell area was obtained. However, the weight of white photocopied paper was found to be 10% less than for blackened paper, and therefore weights of non-protoplast areas had to be scaled up by 10% to make them comparable with those of protoplast-areas.

5.3 RESULTS

Images produced from the photomicrographs of a Cladophora rupestris cell exposed to $102^{o}/oo$ treatment media are shown in Fig.5.1, which serves to illustrate the way in which the changes in protoplast and total-cell areas were recorded, and also the nature of the response of this species under severe hypersaline stress.

The results for C. rupestris and C. glomerata are shown in Figs.5.2 and 5.3 respectively (also Appendix ii). The calculated total-cell, and protoplast areas for salinity stressed cells have been expressed as percentages of their respective areas before exposure to salinity treatment. The mean percentage changes of protoplast area are presented as well as the mean percentage changes in plasmolysed areas of the cells (total cell area - protoplast area). Since percentage changes have been used it is not statistically valid to calculate the error about mean values, and so the range of values obtained for each sample is shown. Reductions in total-cell area are indicated in the figures when reductions in protoplast area are not associated with an appropriate increase in plasmolysed area. During the experiments some cells were

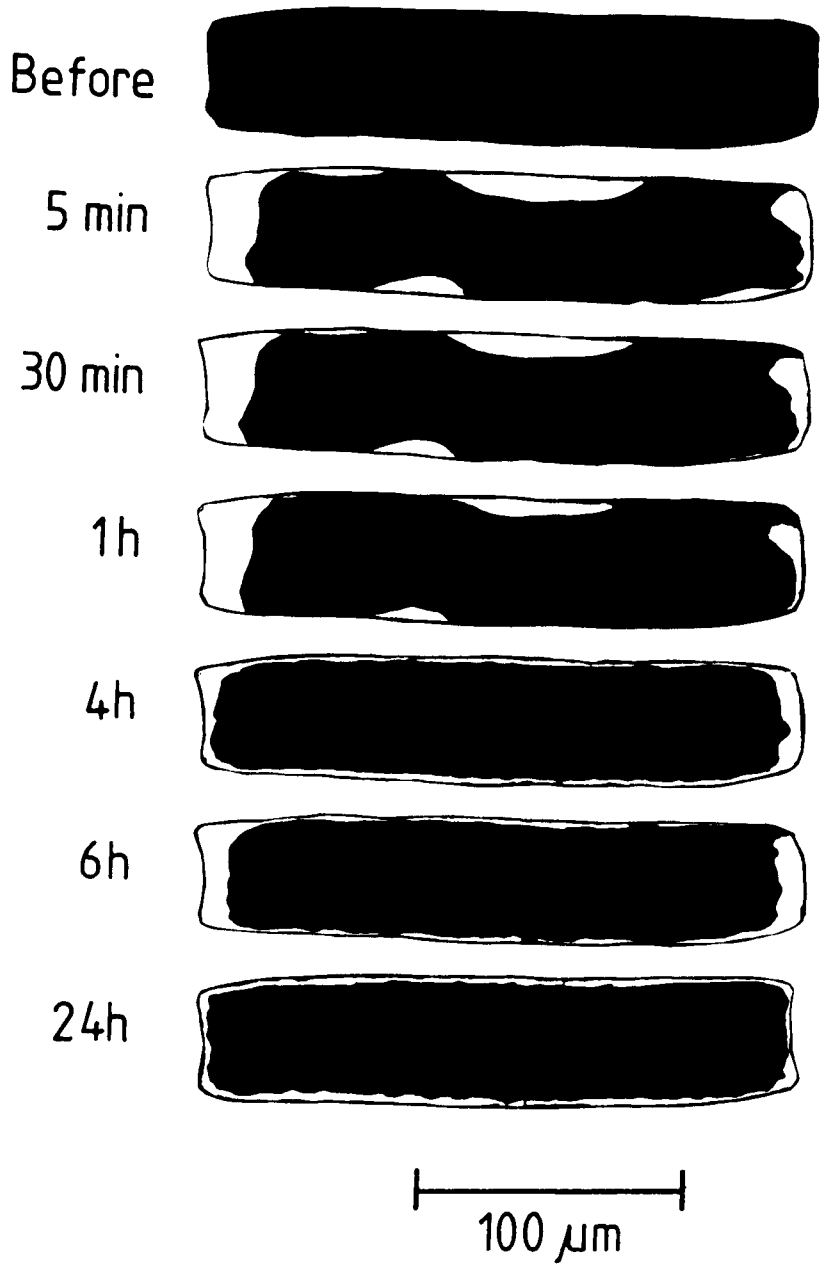


Fig.5.1 The high contrast images produced from photomicrographs of a Cladophora rupestris cell taken after different periods of incubation in treatment media of 102°/oo.

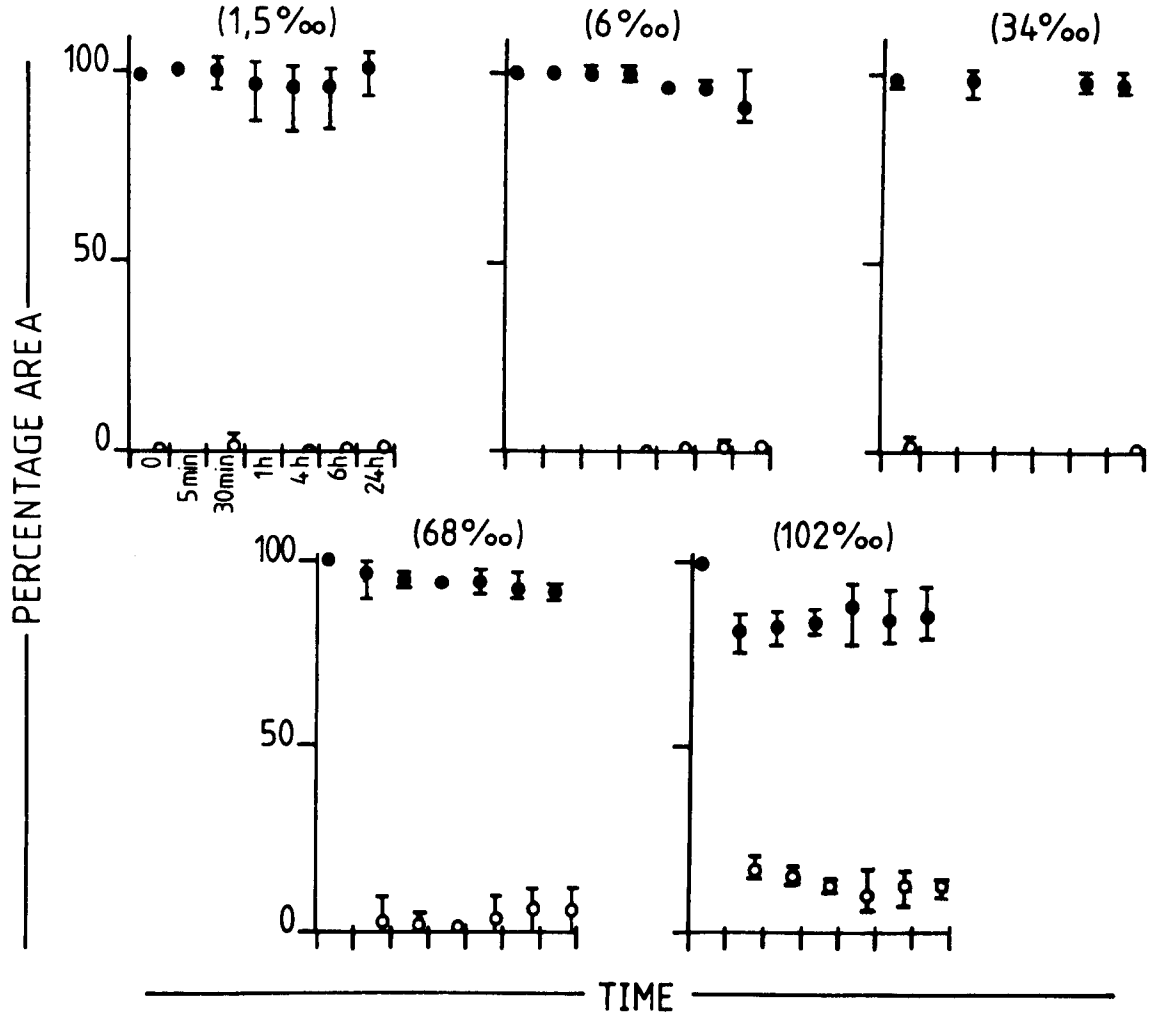


Fig.5.2 The changes in protoplast (-●-●-), and plasmolysed areas (-O-O-) of Cladophora rupestris cells exposed to salinity treatments over a 24h period. The range of values for each sample is indicated.

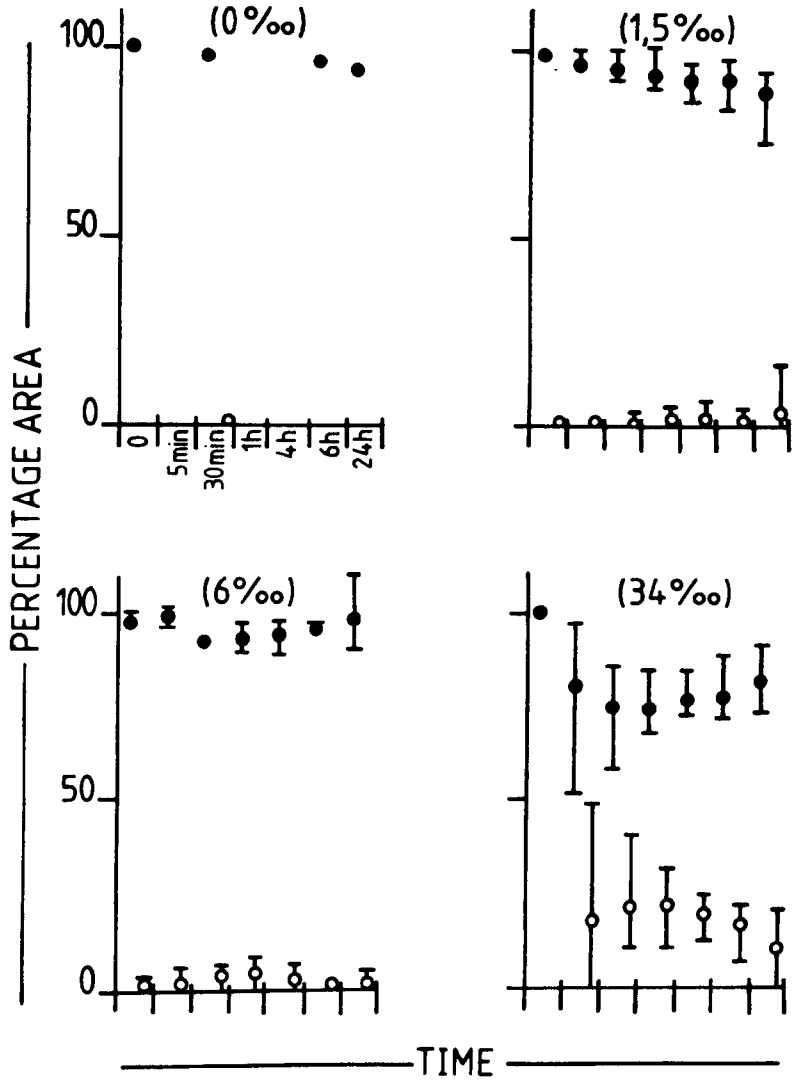


Fig.5.3 The changes in protoplast (-●-●-), and plasmolysed areas (-O-O-) of Cladophora glomerata cells exposed to salinity treatments over a 24h period. The range of values for each sample is indicated.

mis-identified and a few of the mean values are based on 2 to 4 cells only (Appendix ii).

There were fluctuations in the areas measured for Cladophora rupestris cells incubated at 34°/oo, and this variation is attributed to natural fluctuations within the cell and to error introduced by the experimental technique. Salinity-induced changes may therefore be indicated by changes in area that exceed those obtained in the control medium (34°/oo).

Generally the C. rupestris cells (Fig.5.2) in the 1.5 and 6°/oo treatment media maintained a constant protoplast volume. However, a number of cells inexplicably shrank in these hyposaline treatments (1.5°/oo at 30min to 24h, and 6°/oo at 24h). In the 68°/oo treatment there was only slight plasmolysis, and there was no evidence of deplasmolysis occurring. Cells exposed to 102°/oo showed a much greater degree of plasmolysis, again with no obvious deplasmolysis taking place.

There was an inverse relationship between salinity increase and degree of plasmolysis in the cells of Cladophora glomerata (Fig.5.3), although up to 6°/oo there is only slight plasmolysis. Particularly extensive plasmolysis occurs in the 34°/oo treatment and there was also a reduction in total-cell area at 6 and 24h at this salinity, which partly accounts for the apparent deplasmolysis at these incubation times. A reduction in the total-cell area also took place in the 1.5°/oo treatment.

5.4 DISCUSSION

Because of the technical problems encountered with the analytical procedures proposed and the crudity of the method finally adopted, this section of the overall study was not developed to the extent originally planned. The difficulties encountered in developing and conducting a simple quantiative microscopic method for measuring the plasmolytic behaviour of algal cells have clearly been demonstrated. For continuity, cells were used from the same position on the filaments. However, the cells chosen may not show typical responses for the thalli as a whole, since they may have been very recently formed. However cell age is difficult to determine in algae such as these Cladophora species, in which growth occurs by intercalary as well as apical cell division. The responses of several age classes of cell should be measured in any similar future study.

The experiments were performed at a fairly low temperature, and it is questionable whether the plants would experience the very high salinities used combined, with such low temperatures in the field (Section 3.3). The responses of the cells may therefore be different at higher temperatures, because of changes in metabolic rates. Therefore in a more comprehensive study measurements should be taken over a range of temperatures. The effect of light intensity should also be considered for similar reasons. No measurements were made of any swelling of the cell walls, which would be desirable for a

more complete description of the volume changes that occur within the cells.

There were also a number of sources of error associated with the particular method used : The blackening and whitening of the photomicrographs and the cutting out of the areas to be measured relied often on critical judgements of the extent of cell and protoplast boundaries. If the microscope is not focused in exactly the same plane of focus each time a photograph is taken, further variation may be introduced.

It has therefore, proved difficult in practice to make accurate quantitative refinements of the techniques used by Biebl and his co-workers, although with the rapid development of microscope systems with integrated computerised image analysis, it may be possible to make the large number of precise measurements using microscopic techniques that are required to measure the physical responses of cells exposed to osmotic stress.

Even though the data obtained have a lot of associated error, it is still possible to discuss general trends in the physical responses of the cells of these two species. The response of Cladophora rupestris in the hyposaline treatments indicate that the cell walls were able to restrict swelling of the protoplast brought about by the inflow of water. Wiencke (1987) using a pressure probe technique has shown that C. rupestris cells can withstand a wide range of cell turgor pressure (6 to >30 bars) in diluted seawater. He attributes this tolerance to the

distinctive composition and construction of the cell wall, which contains cellulose and a complicated arrangement of microfibrils in numerous lamellae (also see Hanic and Craigie 1969). Further water loss from the cells exposed to the hypersaline treatments was probably prevented by the increase in intracellular osmotica at these salinities (investigated later in Section 6).

The responses of Cladophora rupestris cells therefore show some resemblence to those of the non-rigid walled Porphyra purpurea cells, in which the primary response to dilution is an increase in turgor, and in concentrated water there is a reduction of protoplast volume that is not subsequently restored (Reed et al. 1980a). However, the rigid cell walls of C. rupestris mean that any protoplast volume changes in hypersaline conditions result in plasmolysis taking place, which was not observed in P. purpurea due to the very elastic nature of the cell walls.

There was a similar reponse to hyperosmotic conditions in Cladophora glomerata cells, with no apparent restoration of protoplast volume taking place following a severe saline shock, and it would seem that the complete biphasic response to salinity change described in Section 5.1 may also fail to take place in the cells of this species. As in the case of C. rupestris, it is thought that further intracellular water loss did not occur in the saline treatments because of intracellular regulation of osmotica by the cells.

The visual inspection of cells exposd to saline

stress used by Biebl and his co-workers, clearly does not enable the accurate measurement of volume and turgor changes taking place. More detailed studies using established physiological techniques should be made to measure the physical changes that evidently occur within the cells of these two species under changed salinity conditions.

6. EFFECT OF SALINITY ON THALLUS ION CONTENT

6.1 INTRODUCTION

Algal cells can regulate turgor pressure under changed salinity conditions by altering levels of intracellular inorganic ion and/or organic solutes (Section 1.2). The principal ions involved in turgor regulatory processes are K^+, Na^+ and Cl^-, the internal concentrations of which are governed by the activities of ion pumps or membrane permeability of the plasmalemma and tonoplast (Kauss 1978, Kirst and Bisson 1979, Raven 1976). These ions are regulated to varying degrees depending on the nature of the salinity stress, period of incubation and light conditions (Bisson and Gutknecht 1977, Bisson and Kirst 1979). Adaptatation to hypersaline conditions generally involves a major effect on active ion uptake, whereas, during adjustment to low salinities, passive efflux of pumped ions plays a major role (Raven *et al.* 1980).

Algae with large central vacuoles (notably giant-celled algae) have considerably higher intracellular inorganic ion levels than microalgae which have higher cytoplasm : vacuole ratios. In the former turgor is regulated almost entirely by means of inorganic ions whereas, in the latter, turgor regulation is acheived primarily by accumulation and degradation of low molecular weight organic solutes (Kirst and Bisson 1979). In general, organic solutes and K^+ (plus anion) are accumulated in the cytoplasm of algal cells while

inorganic salts (NaCl and KCl) are mainly restricted to the vacuole (Reed et al. 1981). Vacuoles produced in cells of Porphyra umbilicalis under hyperosmotic stress have been shown to serve as compartments for the accumulation of Na^+ and Cl^-, and K^+, whereas floridoside and isofloridoside levels increase in the cytoplasm (Wiencke and Läuchli 1981, Wiencke et al. 1984). Similar compartmentalised models of osmotic regulation have been described for higher-plant halophytes (Flowers et al. 1977, Wyn Jones et al. 1977).

Kirst and Bisson (1979) working on seventeen species of algae representing the three major algal groups have distinguished three classes as regards the intracellular content of Na^+ and K^+ measured over a range of external salinites ;

1. Plants with high levels of Na^+ and low K^+.
2. Plants with high levels of K^+ and low Na^+.
3. Plants with equal amounts of K^+ and Na^+.

These authors suggest that the Cl^- regulation system is primarily responsible for turgor regulation in the algae studied since the the Cl^- responses always parallel those of the internal osmotic pressure response. Active Cl^- influx is thought to be the major ion pump used by algae for turgor generation, although in some plants active K^+ influx is more important (Raven et al. 1980). Generally Na^+ is actively extruded at the plasmalemma of algal cells and, in some species, it is actively transported into the vacuole which may be associated with cytoplasmic turgor

regulation (Raven 1976).

The distribution of the major divalent cations, Ca^{2+} and Mg^{2+} suggests that their influx into algal cells is passive (Raven 1976). Kauss and Rausch (1984) have shown that transient increases in the cytoplasmic Ca^{2+} content of Poterioochromonas malhamensis may be important in the initiation of isofloridoside production in hyperosmotic conditions. Ca^{2+} is also thought to be important in maintaining membrane selectivity towards K^+ and Na^+ in Porphyra perforata (Eppley and Cyrus 1960). Munns et al. (1983) have suggested that monovalent/divalent cation ratios (K^+/Ca^{2+} and Na^+/Ca^{2+}) and their effects on the stability of biological membranes, may be an important feature of salinity tolerance in plants. It is possible therefore that cells regulate Ca^{2+} levels to maintain these ratios, so that membrane damage is avoided. Transient increases in cytoplasmic Ca^{2+} have also been shown to take place in the internodal cells of Lamprothamnium succinctum during hyposaline stress (Okazaki and Tazawa 1987).

This part of the study was undertaken in order to measure the variation in ion content of the two species exposed to different salinities, to determine whether or not the regulation of intracellular ions has a role in the maintanence of turgor under changed external osmotic conditions in these plants. Measurements were made on plants incubated under the same conditions, and at the same time intervals described in Section 4.3a.

6.2 METHODS

Treatment media were prepared as described previously in Section 4.3a. Cladophora rupestris was treated with media of 1.5, 6, 11, 34, 68 and 102^{o}/oo, and C.glomerata with 0, 1.5, 6, 11 and 34^{o}/oo. The 0^{o}/oo treatment for C. glomerata was filtered canal water. The major-ion content of plant tissue was measured following treatment at intervals over a 24h period : 1, 2, 4, 6, 12 and 24h.

Clean plant material was incubated in 500cm^{3} of treatment media under continuous illumination at approximately 45 μE $m^{-2}s^{-1}$ and a controlled temperature of 12 $\pm$1^{o}C. After incubation samples were removed and blotted carefully using a standard blotting procedure. Five replicate tissue portions were weighed (0.0100-0.0125g), and each washed for 5min in 25cm^{3} of sucrose solution isotonic with the treatment medium (Appendix iii). These were blotted and then ashed in porcelain crucibles at 450^{o}C for 2h in a muffle furnace. The ashed material was dissolved in 10% (v/v) HCl, and the Na^{+}, K^{+}, Ca^{2+} and Mg^{2+} concentrations measured using flame-emission and atomic absorption spectrophotometry as appropriate (PYE Unicham SP90A).

Determination of tissue Cl^{-} concentrations were made on plant material that had been blotted, weighed, washed in isotonic sucrose and finally extracted in hot water (approx. 85^{o}C) for 1h. The concentration of Cl^{-} was measured by electrometric titration with $AgNO_3$.

6.3 RESULTS

The results for Cladophora rupestris are shown in Figs.6.1 to 6.5, and those for C. glomerata in Figs.6.6 to 6.10 (Appendix iii). All of the ion contents are expressed as mmol kg^{-1} (fresh weight). The results have been presented in the same way as the net photosynthesis and respiration rates in Section 4.3b.

There was an increase in Na^+ levels with increased salinity in Cladophora rupestris plants (Fig.6.1). These levels did not alter markedly over the 24h period in any of the salinities. The K^+ levels did not increase in salinities up to $34^o/oo$, but rose with increasing salinity in the hypersaline treatments (Fig.6.2). The levels of K^+ for the first 6h in $102^o/oo$ appear to be very low, especially when compared with values obtained at $68^o/oo$. However, after 6h these increased greatly to levels similar to those in the $68^o/oo$ media. Cl^- levels showed similar responses to those of K^+ (Fig.6.3).

There was no evident effect of salinity upon the Mg^{2+} levels in Cladophora rupestris (Fig.6.4). There were increases in Ca^{2+} in the 0 and $6^o/oo$ treatments with very slight increases in the hypersaline media (Fig.6.5). Length of incubation does not appear to affect the levels of the Ca^{2+} and Mg^{2+} in the tissues.

The Na^+ levels increased with increasing salinity in Cladophora glomerata (Fig.6.6). There was a reduction in K^+ levels in the $34^o/oo$ media (Fig.6.7), and also slight reductions in Cl^- levels in saline treatments (Fig.6.8).

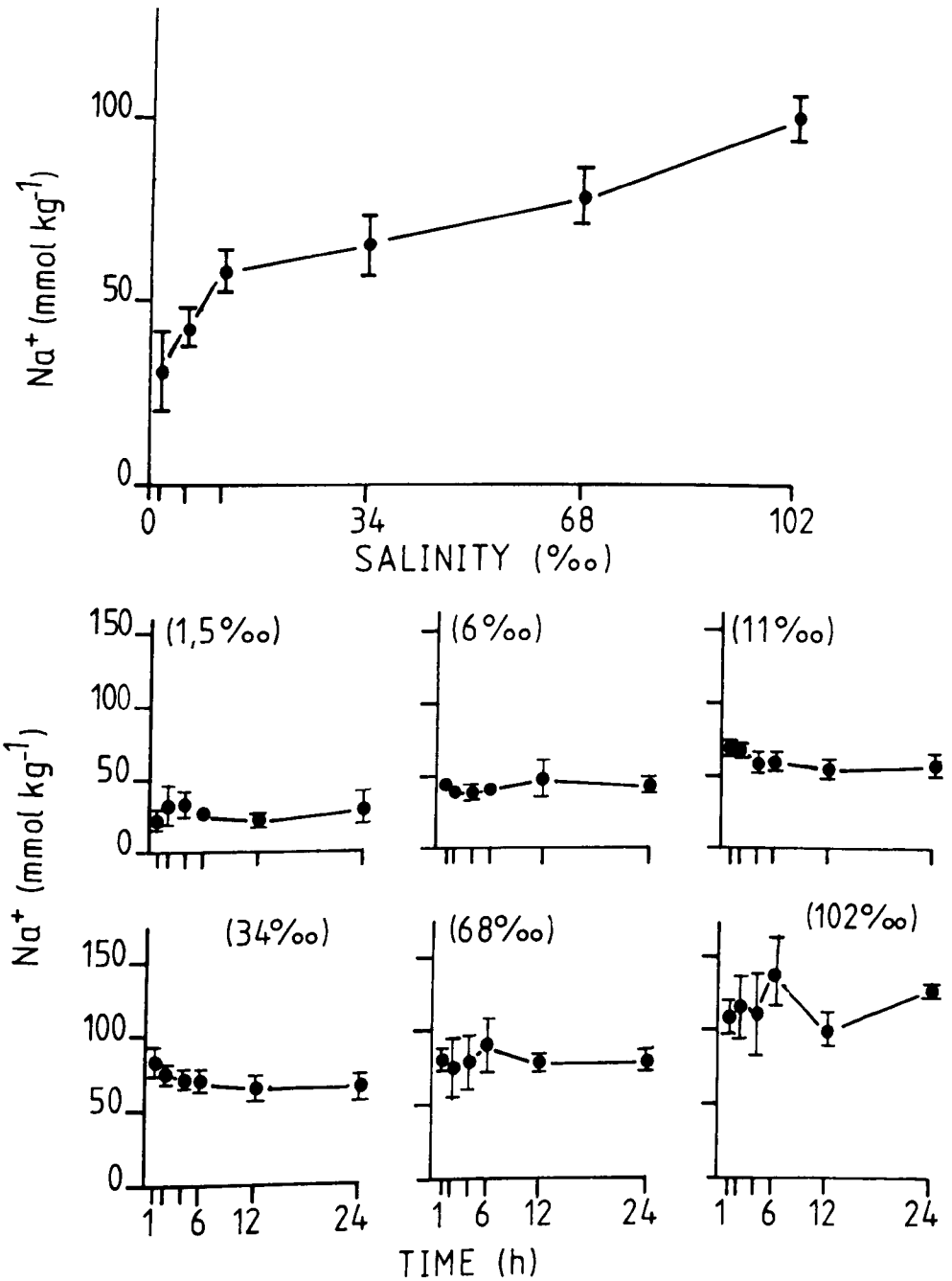


Fig.6.1 The variation in the thallus content of Na^+ of Cladophora rupestris plants exposed to changes in external salinity. The smaller diagrams show the effect of incubation time at each salinity treatment. 95% confidence limits are shown for each sample.

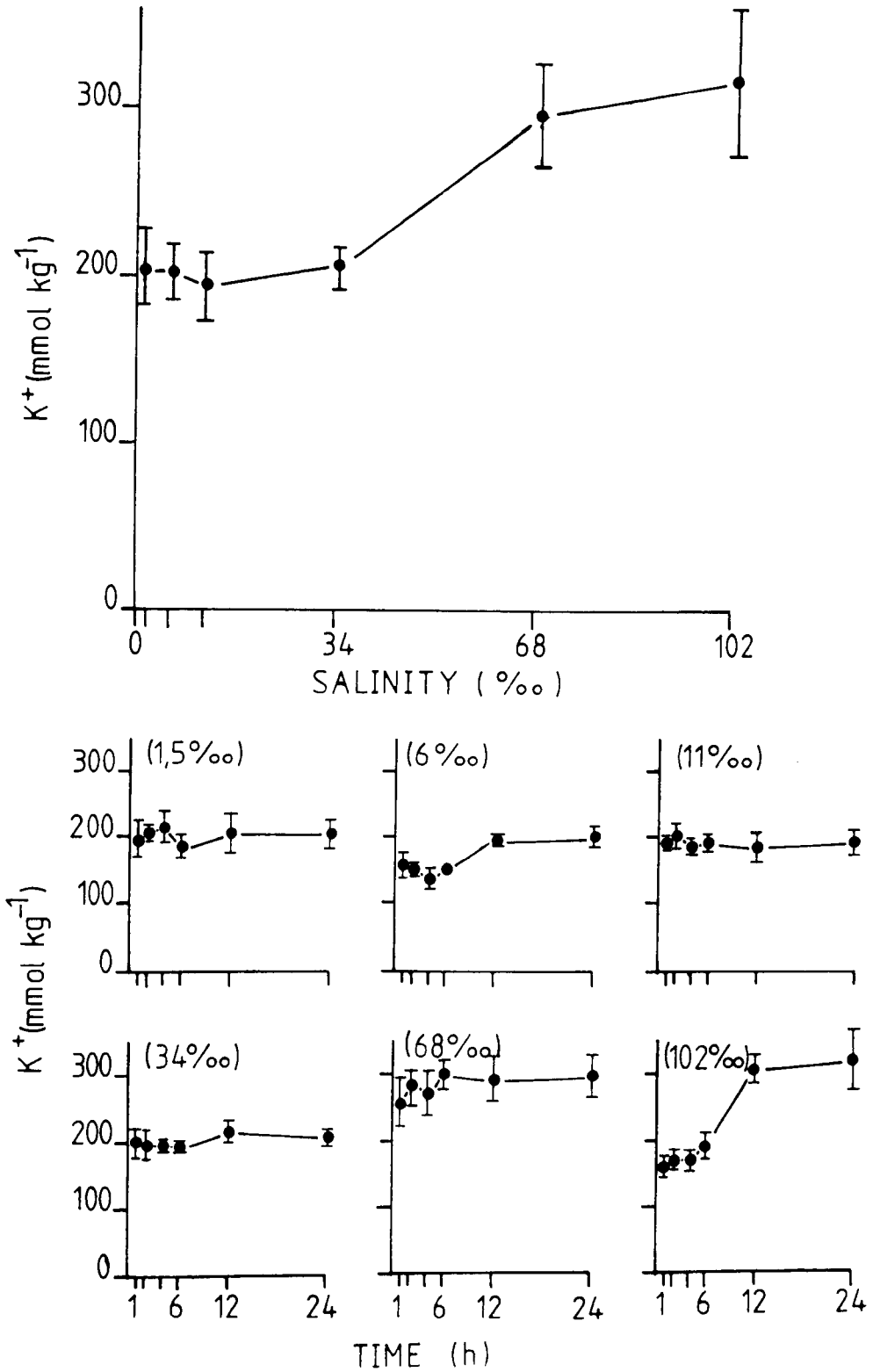


Fig.6.2 The variation in the thallus content of K^+ of Cladophora rupestris plants exposed to changes in external salinity. The smaller diagrams show the effect of incubation time at each salinity. 95% confidence limits are shown for each sample.

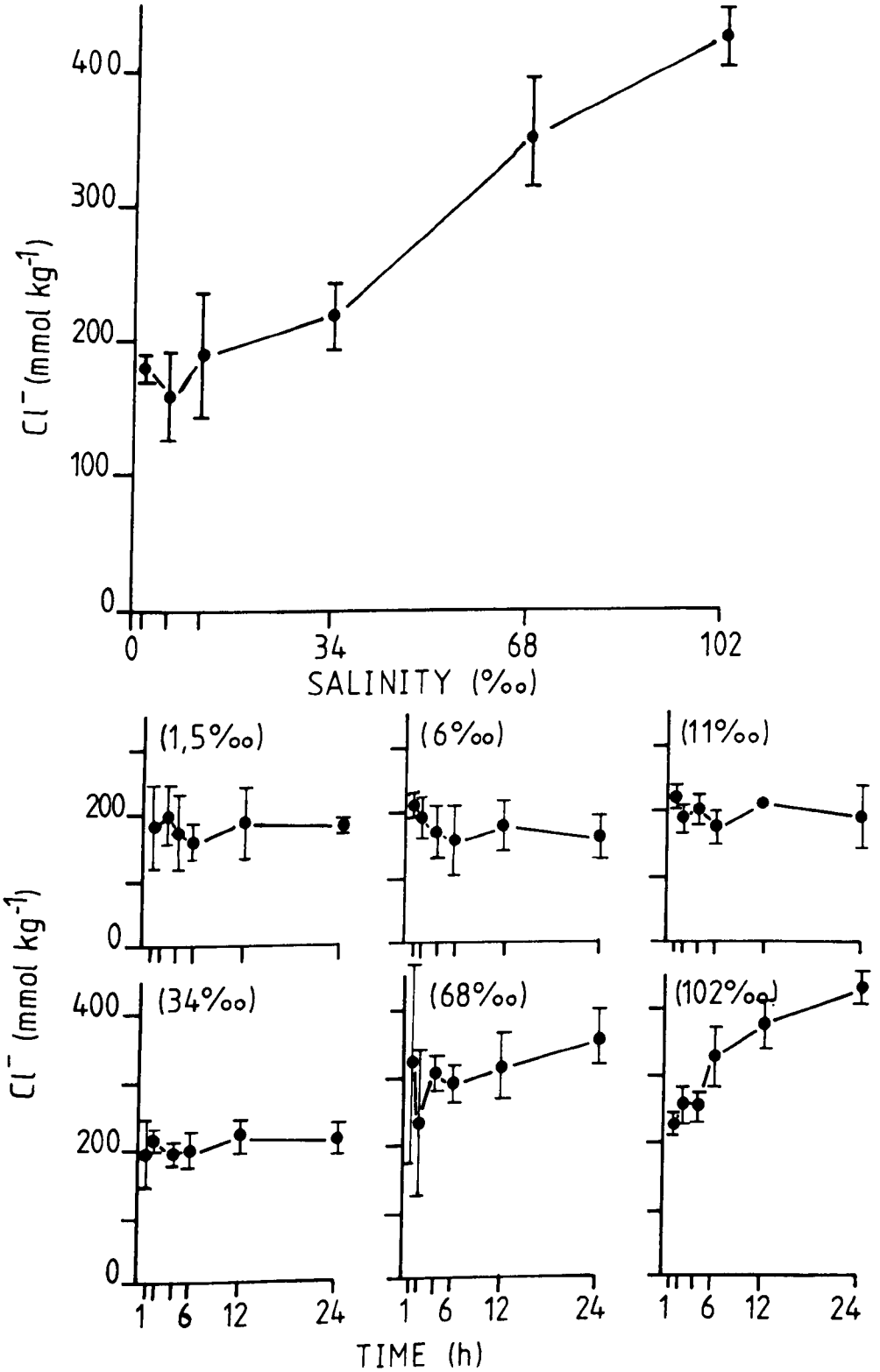


Fig.6.3 The variation in the thallus content of Cl^- of Cladophora rupestris plants exposed to changes in external salinity. The smaller diagrams show the effect of incubation time at each salinity treatment. 95% confidence limits are shown for each sample.

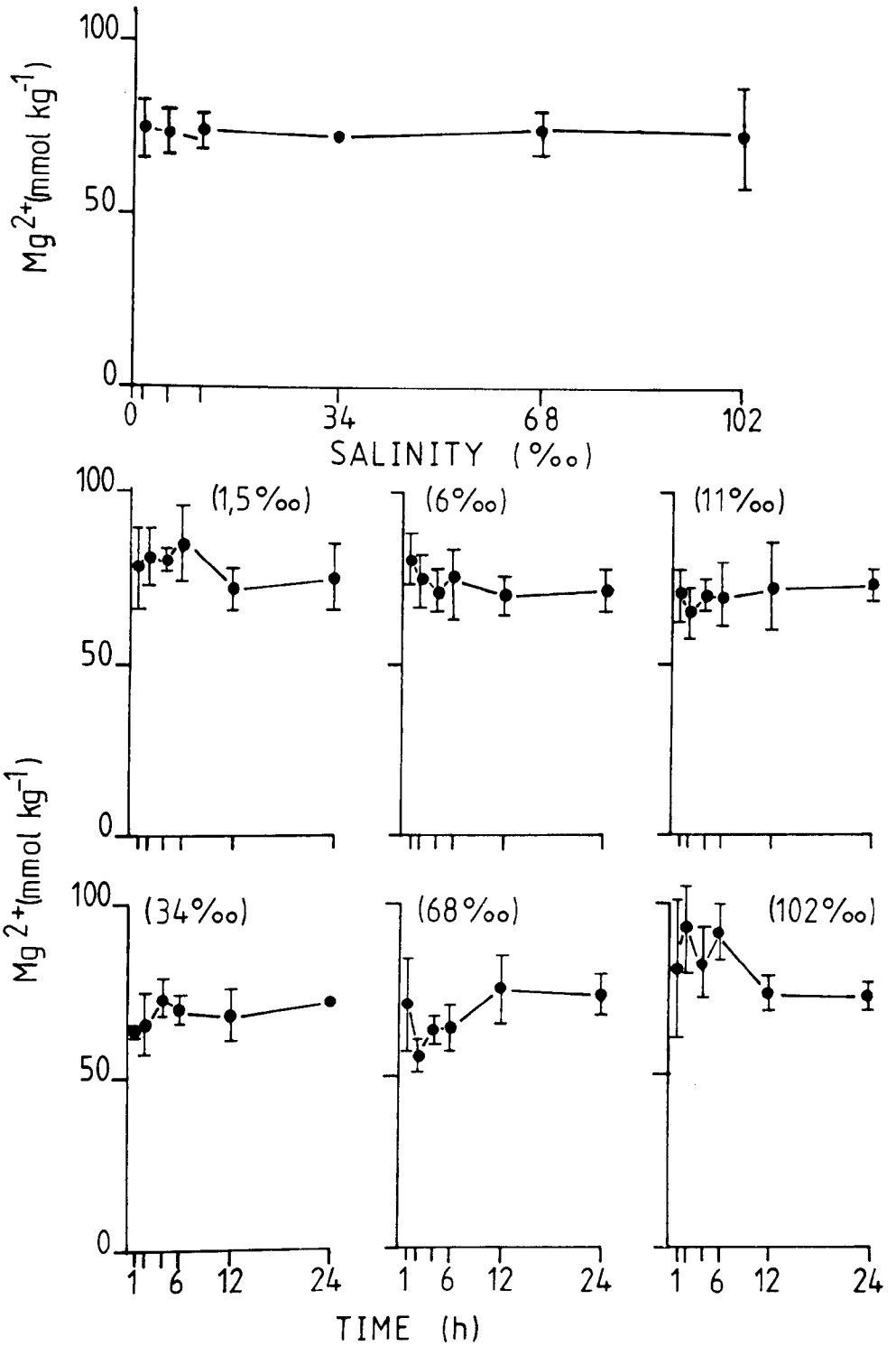


Fig.6.4 The variation in the thallus content of Mg^{2+} of Cladophora rupestris plants exposed to changes in external salinity. The smaller diagrams show the effect of incubation time at each salinity treatment. 95% confidence limits are shown for each sample.

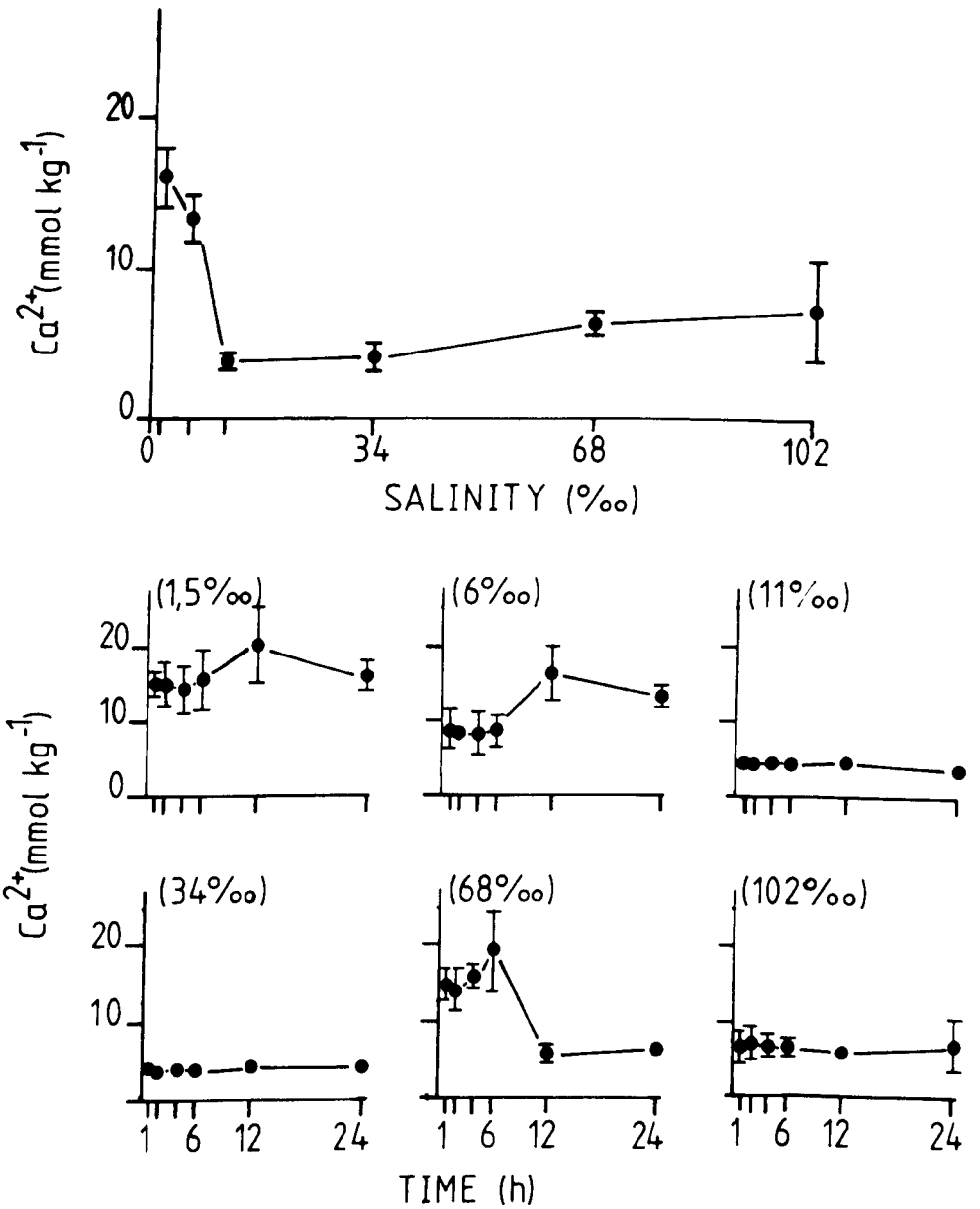


Fig.6.5 The variation of the thallus content of Ca^{2+} of Cladophora rupestris plants exposed to changes in external salinity. The smaller diagrams show the effect of incubation time at each salinity treatment. 95% confidence limits are shown for each sample.

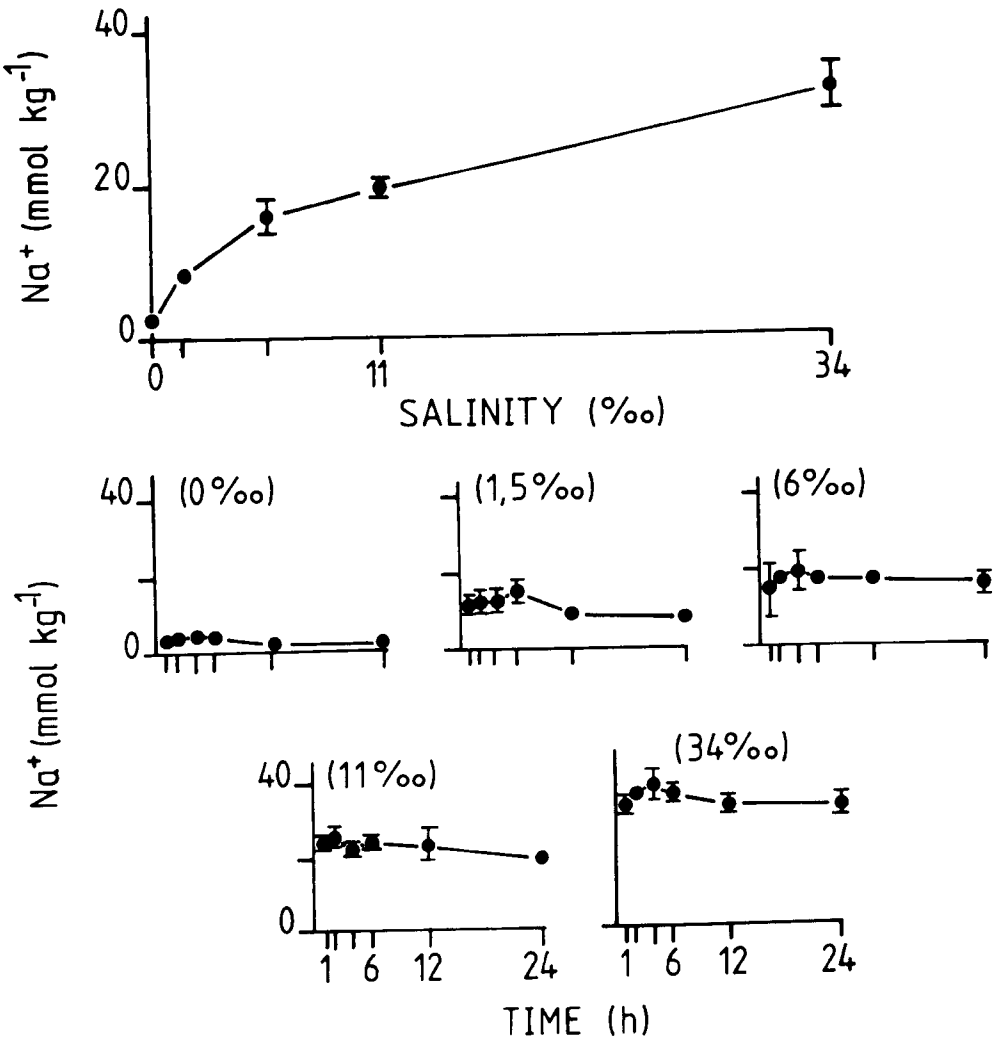


Fig.6.6 The variation in the thallus content of Na^+ of Cladophora glomerata plants exposed to increase in external salinity. The smaller diagrams show the effect of incubation time at each salinity treatment. 95% confidence limits are shown for each sample.

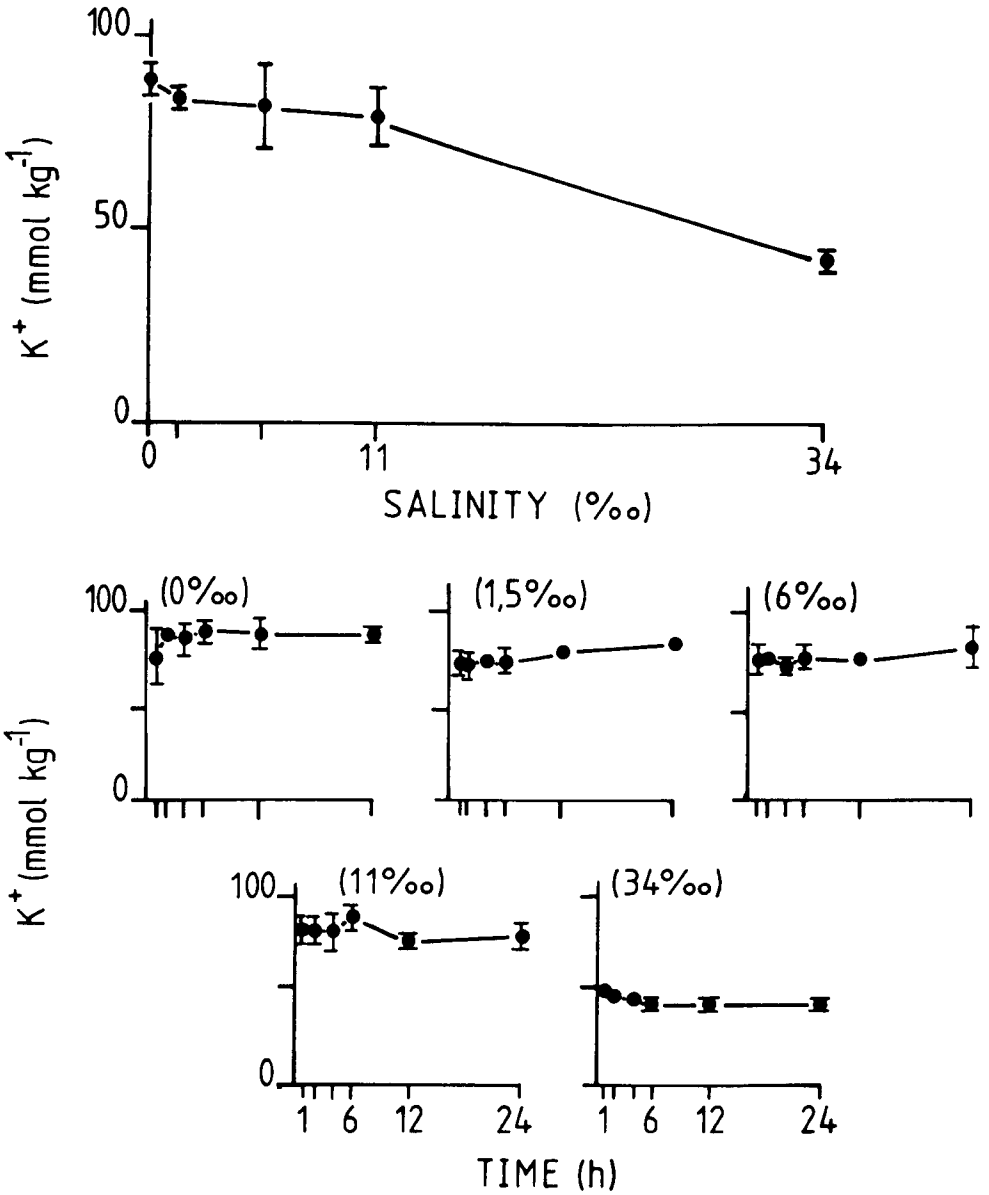


Fig.6.7 The variation in the thallus content of K^+ of Cladophora glomerata plants exposed to increases in external salinity. The smaller diagrams show the effect of incubation time at each salinity treatment. 95% confidence limits are shown for each sample.

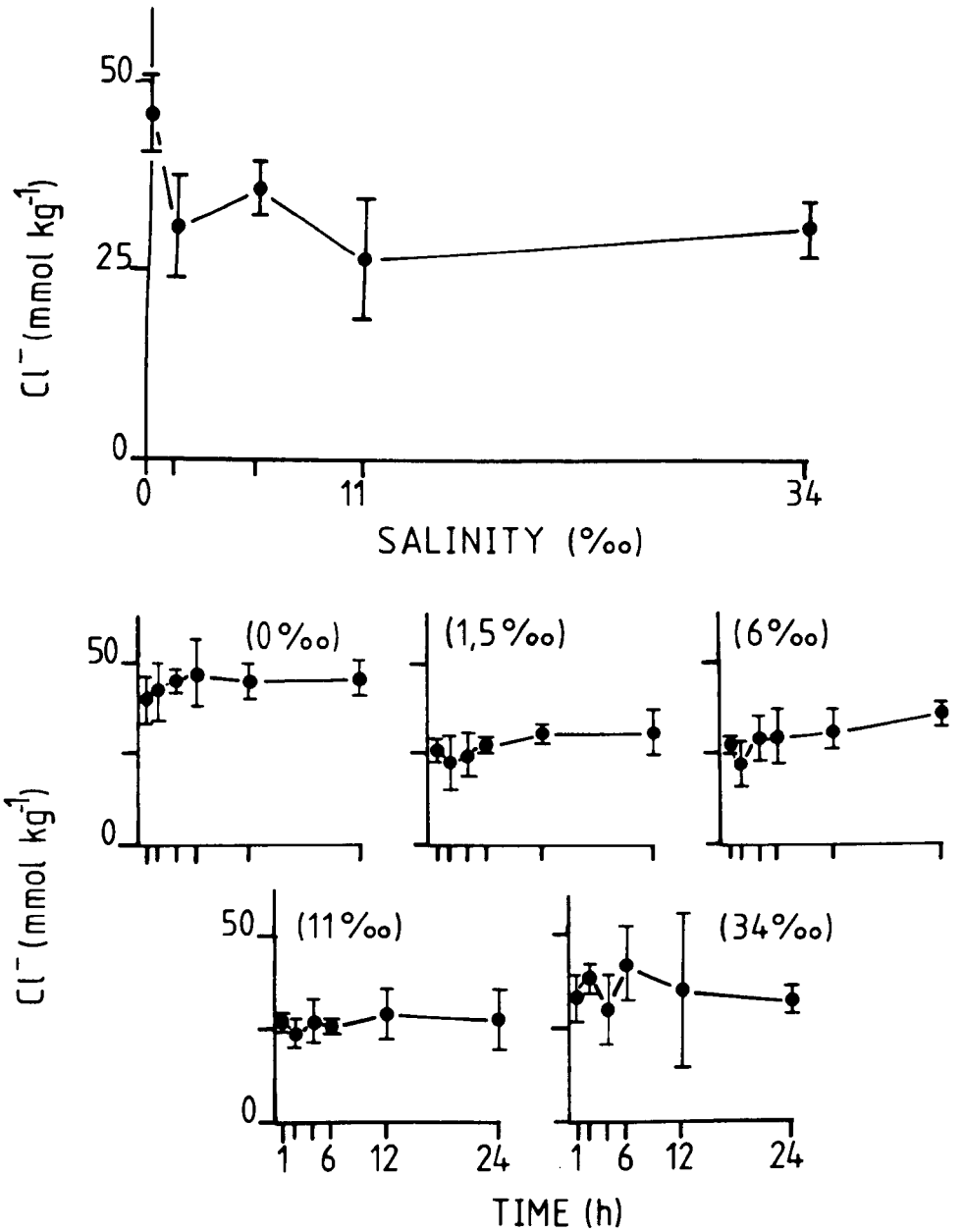


Fig.6.8 The variation in the thallus content of Cl^- of Cladophora glomerata plants exposed to increases in external salinity. The smaller diagrams show the effect of incubation time at each salinity treatment. 95% confidence limits are shown for each sample.

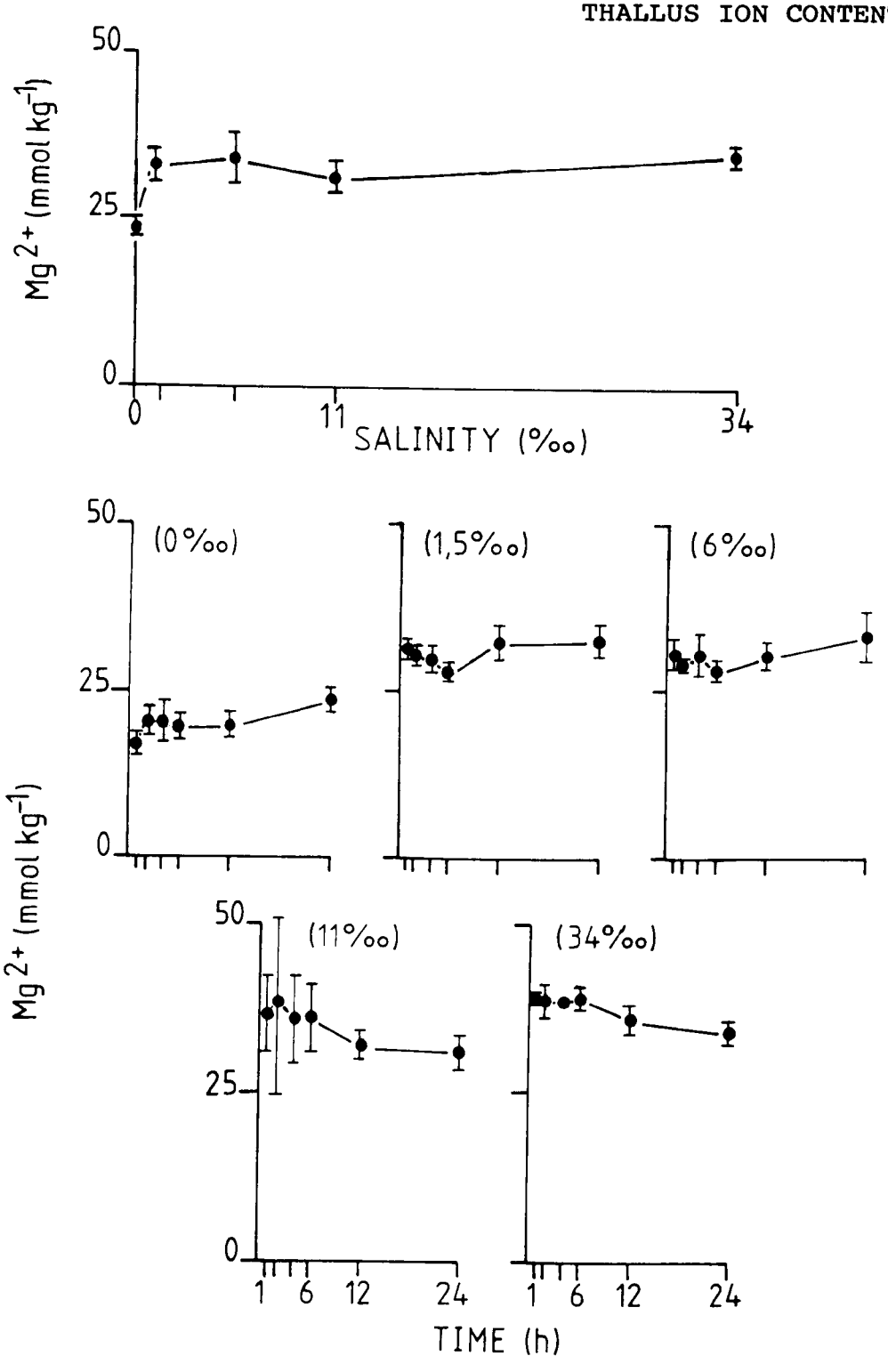


Fig.6.9 The variation in the thallus content of Mg^{2+} of Cladophora glomerata plants exposed to increases in external salinity. The smaller diagrams show the effect of incubation time at each salinity treatment. 95% confidence limits are shown for each sample.

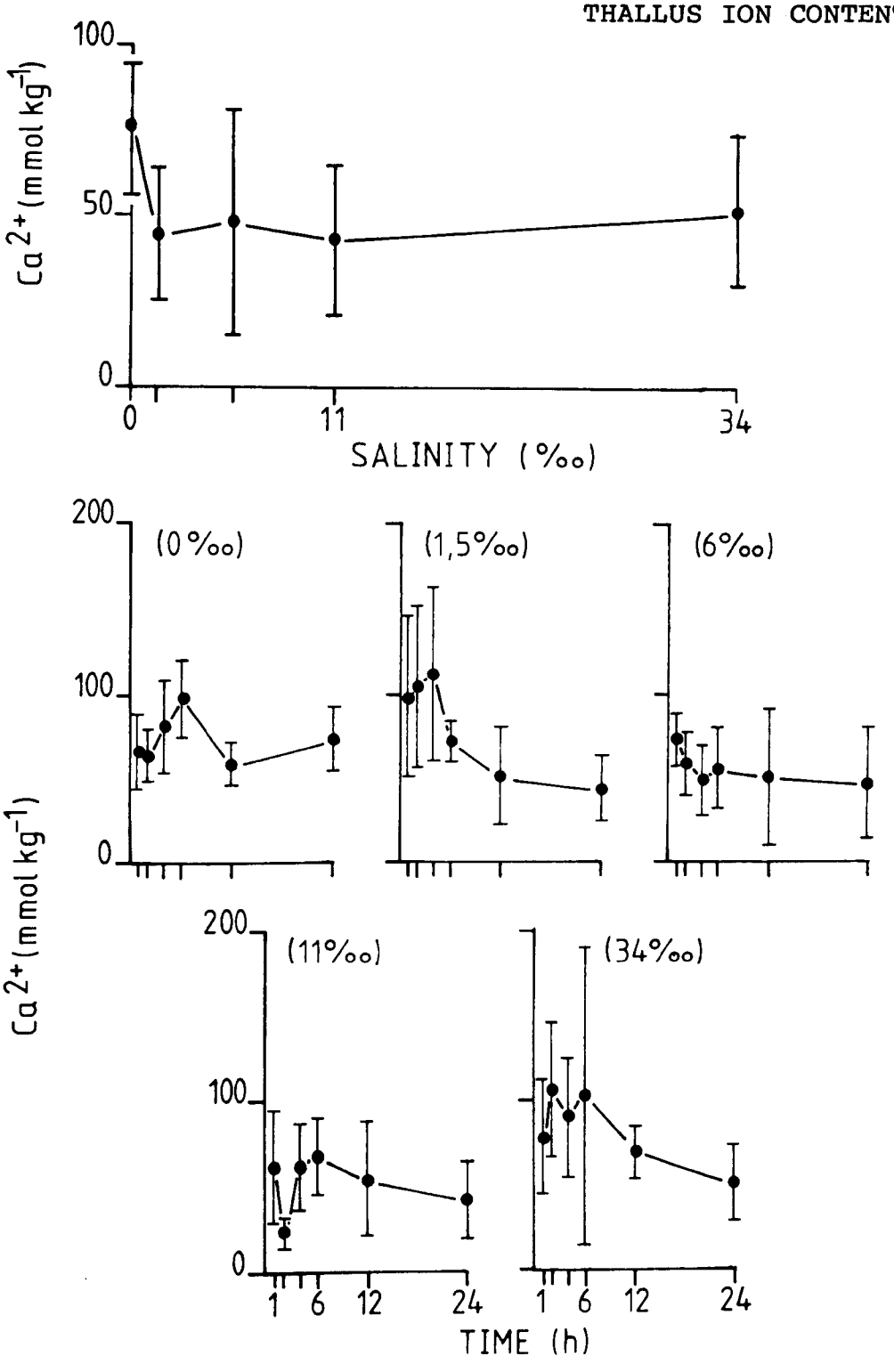


Fig.6.10 The variation in the thallus content of Ca^{2+} of Cladophora glomerata plants exposed to increases in external salinity. The smaller diagrams show the effect of incubation time at each salinity treatment. 95% confidence limits are shown for each sample.

There were slightly lower levels of Mg^{2+} in Cladophora glomerata plants at $0^{o}/oo$ than at the other salinities (Fig.6.9). The Ca^{2+} values obtained were particularly varied (Fig.6.10), and there does not appear to be any change in Ca^{2+} in response to salinity. Length of incubation time did not greatly effect any of the ion levels measured in C. glomerata.

6.4 DISCUSSION

The limitations of using isotonic sucrose as an agent for removing extracellular ions in plant tissue have been discussed by Ritchie and Larkum (1984). Sucrose, a non-electrolyte will not remove ions bound by fixed negative charges on the cell wall (cation exchange capacity), and so may lead to over-estimates of intracellular ion content, especially of Na^{+}, Ca^{2+} and Mg^{2+}. However, other available washing agents have additional disadvantages, e.g. $Ca(NO_3)_2$ is not suitable when Ca^{2+} content is being measured.

Adjustment of the levels of K^{+}, Na^{+} and Cl^{-} in general occurs within the first hour of treatment. However, the adjustment of K^{+} and Cl^{-} in Cladophora rupestris at $102^{o}/oo$ happens only after 6h, and may indicate that during this period there is some inhibition of regulatory processes.

In both Cladophora glomerata and C. rupestris the levels of K^{+} are higher than those of Na^{+} in all salinities. The Cl^{-} levels are of a similar magnitude to those of K^{+} in C. rupestris, but are lower in the C.

glomerata plants. In both species the levels of Cl^- vary in a similar way to those of K^+. The combined K^+ and Na^+ content of both species is not balanced by Cl^-, this imbalance of charge is likely to be accounted for by the excess negative charges associated with cellular proteins or anions such as SO_4^{2-} and NO_3^- (Reed 1983b).

The maintenance of a steady equilibrium between K^+ and Na^+ is an indication that ionic regulation has occurred within cells during the restoration of turgor pressure following osmotic shock. Figs.6.11 and 6.12 show how the K^+/Na^+ ratios vary during these experiments in Cladophora rupestris and C. glomerata respectively (see Appendix iii). Any reductions in very low levels of Na^+, will cause there to be dramatic increases in the K^+/Na^+ ratio, which are not a true reflection of the variation of the individual ions. This is the reason for the greatly increased values at 12 and 24h in $0^o/oo$ and to a lesser extent in the $1.5^o/oo$ media for the C. glomerata plants (c.f. Figs.6.6 and 6.7). However, the K^+/Na^+ ratio of C.glomerata is clearly reduced even in the $1.5^o/oo$ treatment media, and falls further with increasing salinity. C. rupestris is able to maintain a fairly constant K^+/Na^+ ratio over a wide range of salinities (11 to $102^o/oo$), significant increases only occuring in 0 and $6^o/oo$. The increases in the ratio after 6h in $102^o/oo$, are due to the higher levels of K^+ measured which have been discussed above. It should be noted that these ratios will be underestimates of actual intracellular equilibria,

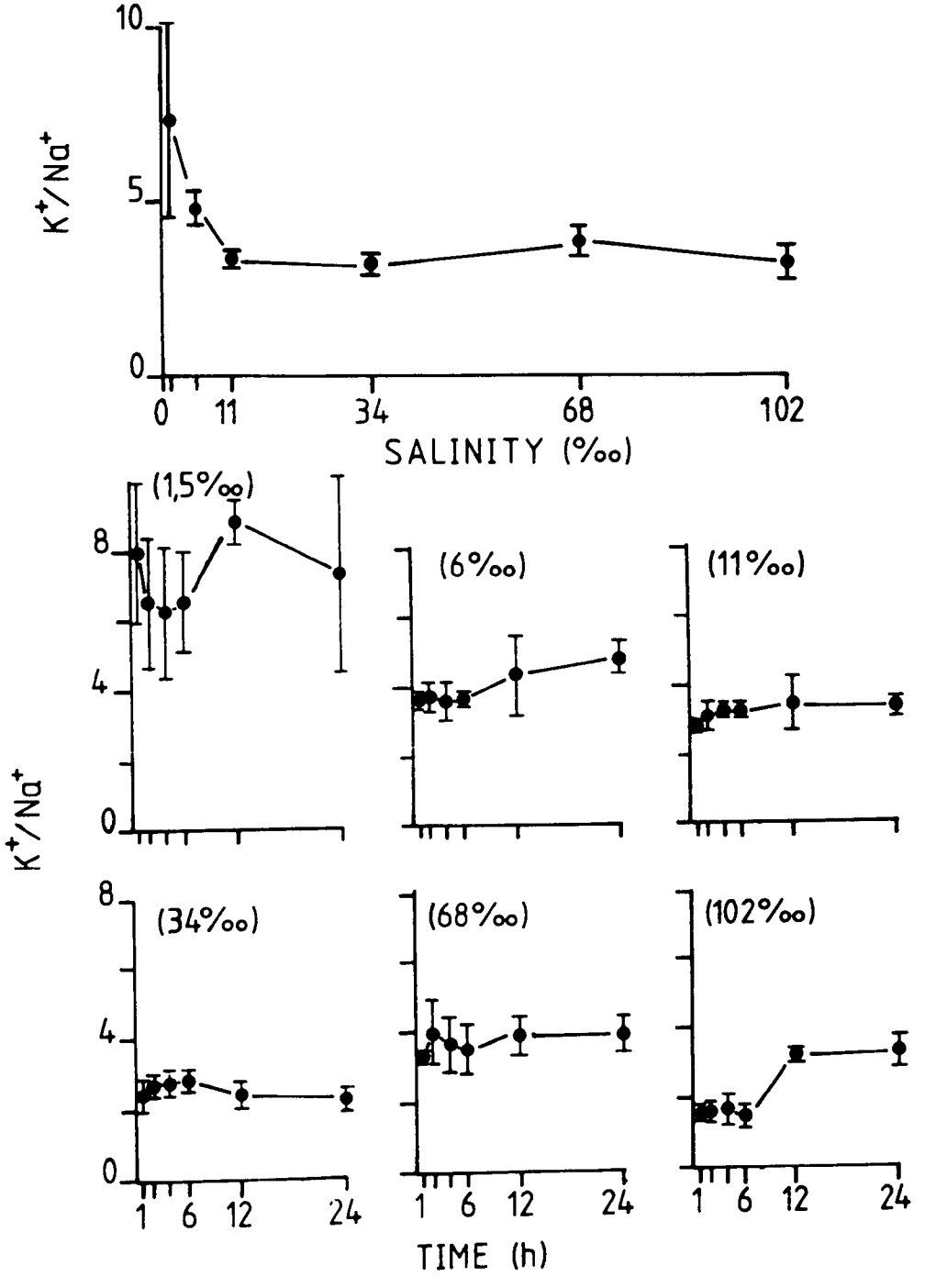


Fig.6.11 The variation due to salinity change of the K^+/Na^+ ratio in Cladophora rupestris plants. The smaller diagrams show the effect of incubation time at each salinity treatment. 95% confidence limits are shown for each sample.

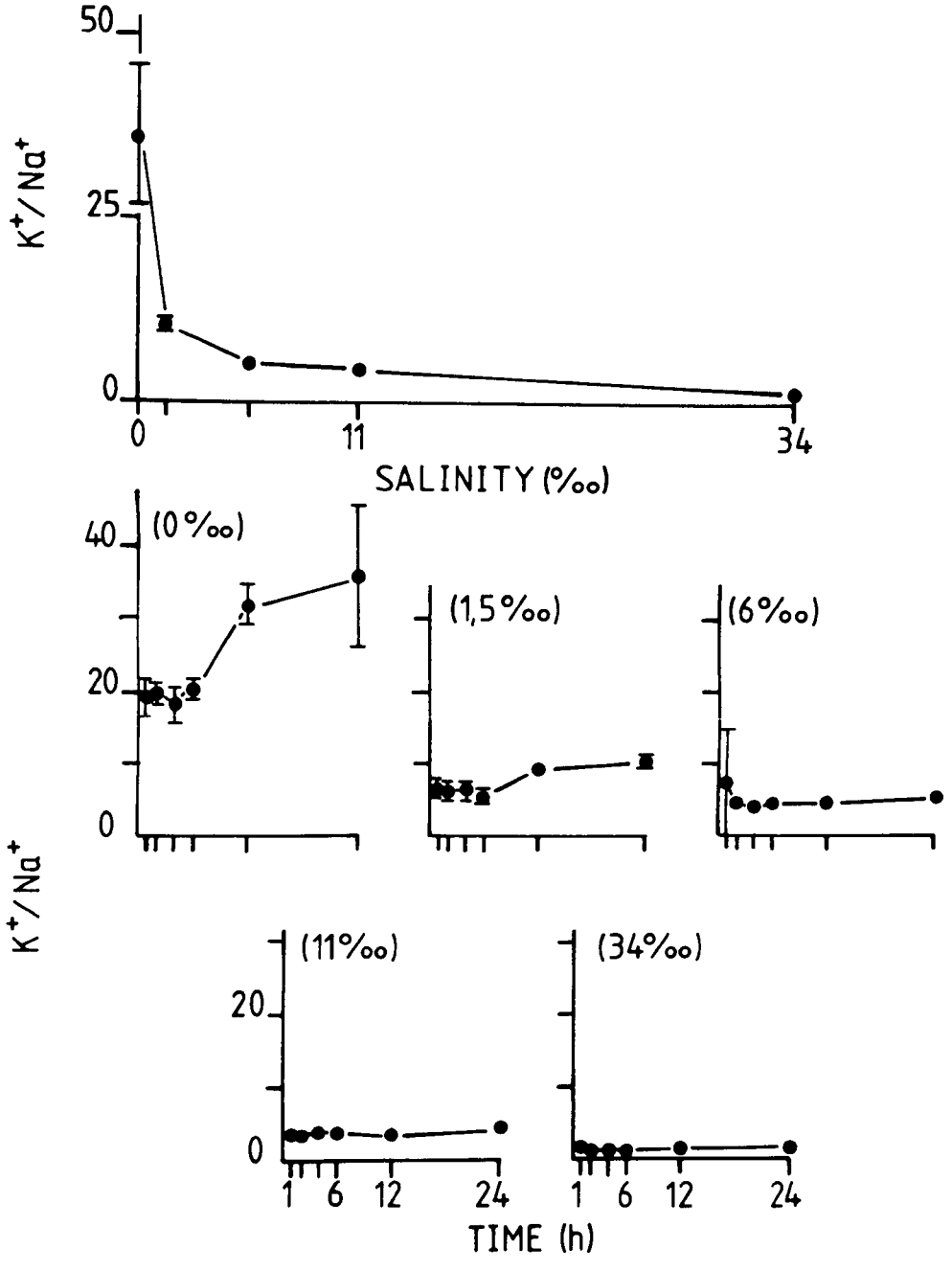


Fig.6.12 The variation due to increasing salinity in the K^+/Na^+ ratio of Cladophora glomerata. The smaller diagrams show the effect of incubation time at each salinity treatment. 95% confidence limits are shown for each sample.

especially at the higher salinities due to the overestimation of Na^+ (see above) and possible underestimation of K^+ through loss from the cytoplasm.

The differences in Ca^{2+} and Mg^{2+} levels in both species are probably largely accounted for by adsorption onto the cell walls. There are large increases in the Ca^{2+} levels in C. rupestris in 0 and 6°/oo treatment media. It is possible that Ca^{2+} may be involved in some capacity in the initiation of a turgor regulatory process in these conditions, as has been reported in Poterioochromonas malhamensis in hyperosmotic conditions (Kauss and Rausch 1984).

This work has shown, that C. rupestris is able to regulate its intracellular ionic levels over a wide range of salinities, whereas C. glomerata does not appear to be able to maintain ionic homeostasis when exposed to even slight changes in external salinity. However, much more detailed studies are needed, to determine precise intracellular levels of these and other osmotically active components, and to measure the transport of these across the plasmalemma and tonoplast under modified salinity conditions.

7. COMBINED EFFECTS OF SALINITY AND TEMPERATURE

7.1 INTRODUCTION

Detailed investigations have been made of the independent effects of temperature and salinity upon the rates of net photosynthesis of many macro-algae. Often, such measurements are made as separate parts of the same study (Chock and Mathieson 1979, Gordon et al. 1980, Penniman and Mathieson 1985), but the interactive effects of the two external factors are not discussed. As mentioned in Section 3.1, other studies in which the influence of temperature upon the photosynthetic activity and growth of algae subject to saline stress have been measured, indicate that algae may have markedly different abilities to withstand changes in their external osmotic conditions when treated at different temperatures (Dawes et al. 1978, Fralick and Mathieson 1975, Kjeldson and Phinney 1971, Yarish and Edwards 1982, Zavodnik 1975). Dawes et al. (1976) and Lehnberg (1978) have also demonstrated the complex interactive effects of temperature, salinity and light intensity upon the photosynthetic activity of Hypnea musciformis and Delessaria sanguinea respectively. All of these authors have demonstrated that external pressures such as salinity should not be treated as distinct and unrelated in their influence upon the growth and/or reproduction, and ultimately the distribution of a particular species or ecotype within a species.

This part of the study was therefore undertaken in

order to measure the effects of temperature upon the responses to saline stress of net photosynthesis in both Cladophora rupestris and C. glomerata. The measured responses of the plants under laboratory conditions could then be correlated to the environmental conditions experienced in the field (Section 3). A method was used which measured the net photosynthesis of plants in recovery conditions following a period of saline stress, since it was felt that this gives a more realistic measure of the damage resulting from the stress (Section 4.4). This approach is similar to that used by Gessner (1969), Dring and Brown (1982) and Russell (1987b).

7.2 METHODS

A range of salinity treatments was prepared as previously described in Section 4.3a. Cladophora rupestris was treated with : 0, 6, 11, 34, 68 and 102°/oo and C. glomerata with 0, 1.5, 6, 11 and 34°/oo. Experiments were performed at the following temperatures : $-9\pm3^{o}C$, $4\pm1^{o}C$, $10\pm1^{o}C$, $15\pm1^{o}C$, $20\pm1^{o}C$, $25\pm1^{o}C$ and $30\pm1^{o}C$.

Similar-sized clean portions of thallus were incubated in $150cm^3$ of the treatment media at the experimental temperature for 48h. Due to the freezing of media at $-9^{o}C$, and the subsequent need for thawing out of plants before further stages of of the experiment, this material was incubated in smaller volumes (15 to $20cm^3$) contained in shallow Petri dishes. Three replicates of each treatment were used in a random block design. Following treatment at the various experimental

temperatures, all treatment flasks were allowed to stabilise at 10°C for approximately 2h before experimentation proceeded.

The plant material was then removed from the treatments and blotted carefully to remove any residual surface water. The plants were then placed into 125cm^3 recovery vessels filled with water natural to the locality in which the plants normally grow. Thus Cladophora rupestris recovered in sawater (34°/oo) and C.glomerata in filtered canal water. These waters had first been boiled to remove oxygen, and then enriched with $NaHCO_3$ as a carbon source. These flasks were then stoppered without introducing any air and incubated at 10°C under artificial light (60 $\mu E\ m^{-2}s^{-1}$) for 2h in a random block design. Three flasks containing only de-oxygenated water were incorporated as controls. Flasks containing dead Cladophora thalli would have been better controls, although in practice no measurable amounts of oxygen were introduced into the flasks containing salinity treatments that killed plant material.

Plants were removed from the flasks without introducing any air, and the oxygen that had been produced by the samples was determined using a modified Winkler titration method (Strickland and Parsons 1972). The mean oxygen concentration of the three control flasks was subtracted from the values obtained from the treatment flasks to give the net amount of oxygen produced by the plant tissue. The plant material was oven-dryed at 50°C to

constant weight and weighed so that the net photosynthetic rate could be expressed as : $mgO_2h^{-1}g^{-1}$ (dry weight). This method is similar to that described by Russell (1987b).

7.3 RESULTS

The combined effects of temperature and salinity upon Cladophora rupestris and C. glomerata are shown in Fig.7.1 and Fig. 7.2 respectively (also Appendix iv). The data were analysed by one-way ANOVA to test for a significant response to salinity at each temperature (see Table 7.1). A two-way ANOVA showed the responses of both species to salinity and to temperature to be significant (Table 7.2). The interaction between salinity and temperature was also found to be significantly different in both species (Table 7.2).

In the case of Cladophora rupestris, the most marked inhibition of net photosynthesis was obtained with a combination of low salinity ($0^{o}/oo$) and high temperature (25 and $30^{o}C$). At the highest temperature, this species was also damaged greatly by the highest salinity ($102^{o}/oo$). The lowest temperature ($-9^{o}C$) proved to be more damaging when associated with low salinities (0, 6 and $11^{o}/oo$). At all other combinations of salinity and temperature, the plants were capable of oxygen production, though this was usually significantly reduced at the saline extremes (0 and $102^{o}/oo$).

After treatment at $-9^{o}C$ Cladophora glomerata produced no oxygen at any salinity except at $0^{o}/oo$ in which extremely low levels were obtained (Fig.7.2). At all other

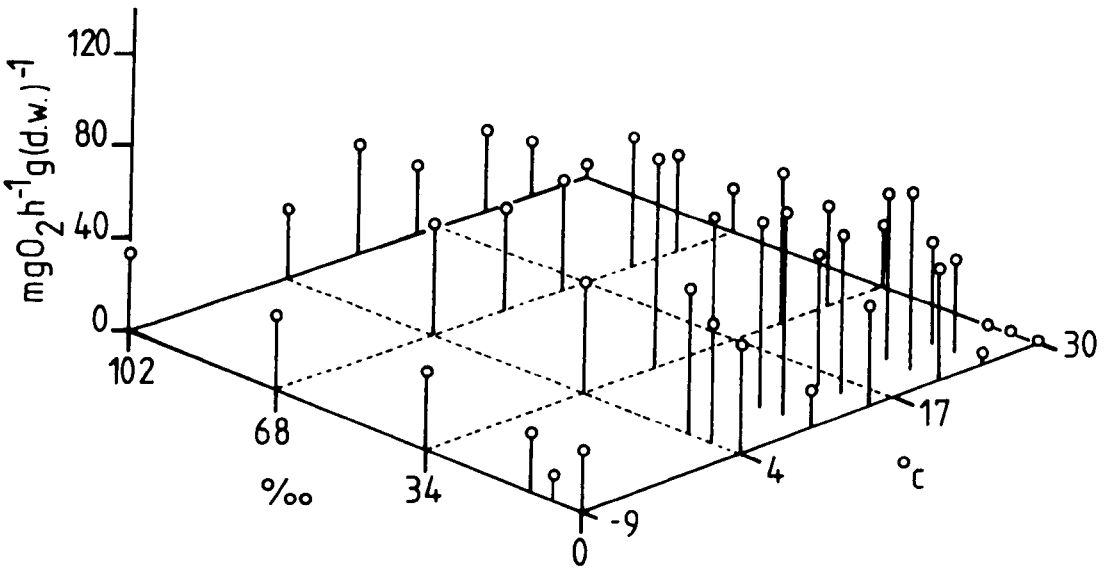


Fig.7.1 The combined effect of salinity and temperature upon the net photosynthesis of Cladophora rupestris.

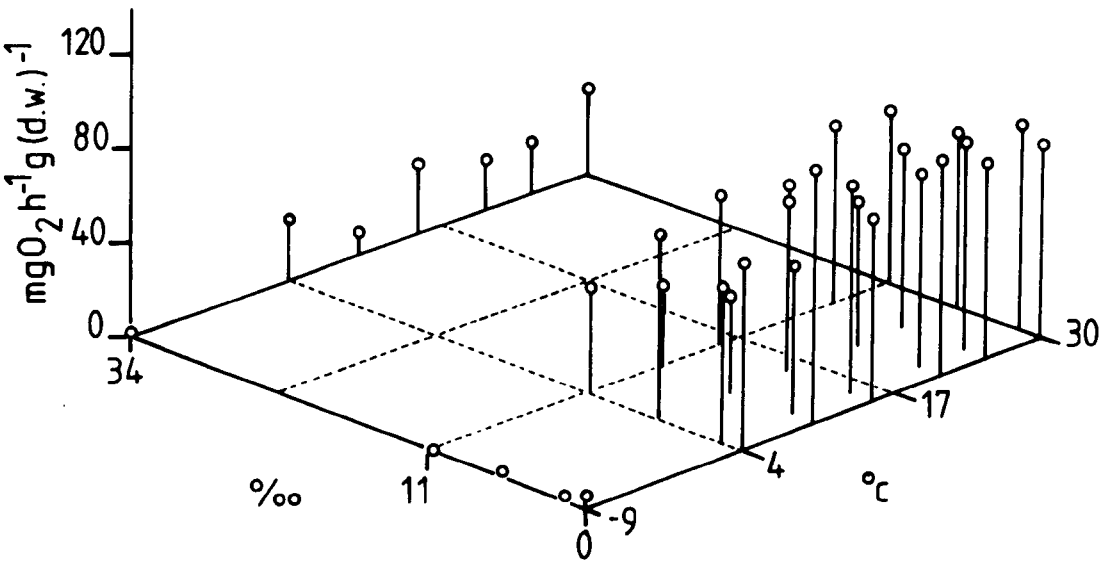


Fig.7.2 The combined effect of temperature and salinity upon the net photosynthesis of Cladophora glomerata.
N.B. The salinity scale is different to that used in Fig.7.1.

SPECIES	TEMPERATURE °C						
	-9	4	10	15	20	25	30
C. rupestris	***	N.S	**-	**-	N.S	***	***
C. glomerata	N.S	N.S	***	*--	***	***	*--

Table 7.1 The level of significance of the net photosynthetic responses to salinty treatment shown by Cladophora rupestris and C. glomerata at different experimental temperatures, following one-way ANOVA.
(N.S $P>0.05$, *-- $P<0.05$, **- $P<0.01$, *** $P<0.001$).

	C. rupestris	C. glomerata
(A) SALINITY	***	***
(B) TEMPERATURE	***	***
(C) INTERACTION OF AxB	***	***

Table 7.2 The level of significance of the net photosynthetic responses to the combined effects of salinity and temperature of Cladophora rupestris and C. glomerata following two-way ANOVA.
(see Table 7.1 for notation used).

temperatures, treatment in 34°/oo significantly reduced oxygen production. The most productive sector of Fig.7.2 is clearly that determined by the salinities 0 to 11°/oo and temperatures 17 to 30°C. The levels of oxygen production under normal salinity conditions (0°/oo) showed no significant response to temperature increase, although the net photosynthesis of the plants in the 34°/oo treatments increased with temperature.

7.4 DISSCUSION

From investigations of Cladophora rupestris in growth culture, Cambridge et al. (1984) have concluded that this species is tolerant of temperatures from below -5 up to 25-30°C. The results obtained during this study indicate a higher survival tolerance limit, the plants not being killed at 30°C under normal salinity conditions (34°/oo). Biebl (1959) also recorded C. rupestris surviving temperatures between -8 and 30°C for at least 12h. However, the activity of the plants was significantly reduced at this high temperature, and longer periods of incubation might cause greater cell damage and subsequent plant mortality. The temperature tolerance shown by C. glomerata agrees with that reported by Whitton (1970), the plants being most active photosynthetically at the high temperatures, but not tolerating the very low temperature of -9°C. The temperature extremes measured at both sampling sites (Section 3.3a) fall well within the tolerance limits for the two species, and clearly C. rupestris can survive at lower temperatures, and C.

glomerata considerably higher temperatures than were recorded in the field. However, it is important to remember that the field temperature extremes may have been underestimated due to the sampling procedure adopted and also the climatic conditions during 1984 - 1986 (Section 3.4).

By combining the values of net photosynthesis obtained at each salinity for all seven temperatures it is possible to produce generalised salt tolerance curves for both species (Fig.7.3). These curves suggest that Cladophora glomerata tolerates only slightly saline waters, net photosynthesis being significantly reduced in salinities of 11°/oo and above. C.rupestris is more euryhaline, oxygen production being significantly depressed only in the extremes of 0 and 102°/oo. The results show a similar salt tolerance for C. glomerata to that obtained using an oxygen electrode method to measure net photosynthesis (Section 4). However, the measurements made previously on C. rupestris using the oxygen electrode showed a narrower range of salinity tolerance.

The ability of Cladophora rupestris to withstand much lower temperatures than C. glomerata, is possibly due to the former having a much higher intracellular ionic content (Section 5) and possibly higher levels of organic solutes, which may confer a greater degree of protection against damage caused by freezing (Russell 1987b). The greater oxygen production of C.rupestris following higher salinity treatment at -9°C, may also be a result of the

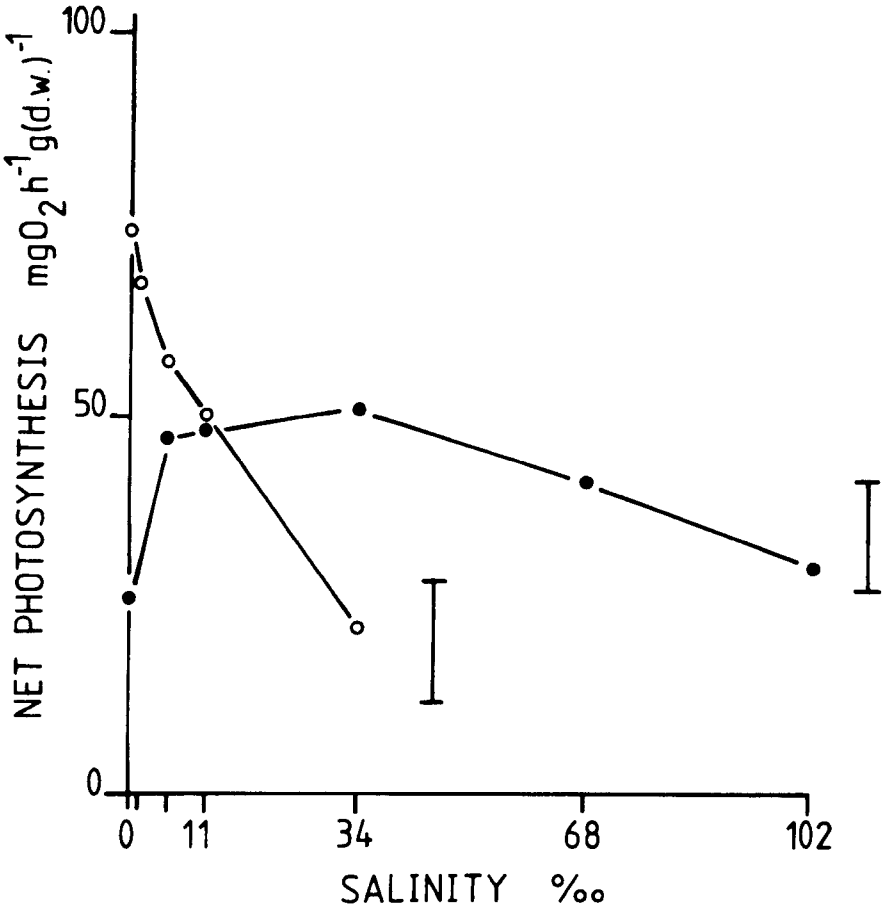


Fig.7.3 Generalised salt tolerance curves for Cladophora rupestris (-●-●-) and C. glomerata (-O-O-). The bars represent the Least Significant Difference for each species.

increases in intracellular osmotica at these salinities, protecting the cells from freezing effects.

The damage caused to Cladophora rupestris by a combination of high temperature and salinity extremes, implies that its cell physiology is susceptable to osmotic imbalance at temperatures close to the upper temperature limits of the plant. On the other hand, optimum oxygen production by C. glomerata in 34°/oo was obtained at higher temperatures, which would suggest that its cells are better able to make osmotic adjustments in warm water. However, Russell (1987b) discusses problems involved in extrapolating the results obtained from short-term experiments, to conditions experienced in nature. It is thought that the 48h incubation periods used in this study are sufficient to show trends in the responses of these two species, but caution should be used in interpreting such results, and more long-term experiments are desirable.

Temperature is evidently an important factor governing the ability of both species of Cladophora to withstand salinity stress, and the results therefore confirm the importance of interactions between environmental variables in determining the distribution of macroalgae in nature.

Cladophora rupestris results resemble those otained by Russell (1987b) from Fucus vesiculosus. Both these algae have similar geographical distributions (Hoek 1982b, Powell 1963, Russell 1987b), and they are ecologically

similar in that they occupy the same eulittoral zone of tidal Atlantic shores, C. rupestris often growing as an understorey plant below F. vesiculosus.

8. POPULATION DIFFERENCES IN SALINITY RESPONSES

8.1 INTRODUCTION

A number of salt tolerance studies have led to the identification of genetically distinct ecotypes within several species of algae. These ecotypes have evolved in osmotically stressed environments in response to strong selection pressure. This work has been confined largely to compared responses of algae to changes in external salinity by marine and brackish water populations (Reed 1983b, Russell 1987a). The distinction between physiological (plastic) and genetic (ecotypic) differences in the responses of these plants has been emphasised (Reed 1984), although it has been argued that such distinctions may prove difficult to make in practice (Russell 1985b).

Reed (1983b) and Reed and Barron (1983) showed that there are differences in the osmotically active components in the cells of marine and estuarine isolates of Polysiphonia lanosa and Pilayella littoralis, which, coupled with differences in cell morphology, explain the variation in salt tolerance between the two isolates. Young et al. (1987b), have also shown there to be morphological differences between marine, rockpool and estuarine populations of Enteromorpha intestinalis, and also differences in the content of K^+ (the major osmotically active ion), Na^+ and Cl^-. Such investigations of the physiological processes underlying intraspecific variation in salt tolerance in algae are important in the understanding of the evolutionary adaptations that have

occurred in conspecific populations growing in different saline habitats.

Nellen (1966) and Russell (1985a,b) have shown that the salt tolerances of a number of Baltic algae have diverged considerably from those of North Atlantic shores. The Baltic Sea, with its water of very reduced salinity (Section 3.4), is reported to contain populations with greater tolerance to low salinities coupled with an increased susceptibility to higher salinities in comparison with U.K. populations (op.cit.). There are few similar comparative studies on salt tolerances of the Baltic flora, and how these differ from conspecific marine and freshwater populations from Western Europe.

The aim of this work was to measure ways in which Baltic and U.K. populations of Cladophora glomerata and C. rupestris differ in their salt tolerances, and to relate any differences to intraspecific variation in physiological responses to salinity change. Most of the results for U.K. populations have been presented and discussed in previous sections of this study, however, for ease of comparison, data collected for U.K. plants has been reproduced in this section.

8.2 METHODS

Because of time limitations on work at Tvärminne Zoological Station, some economies in experiment design were necessary, which are listed below. Since incubation conditions for Baltic plants were determined by those available at the Baltic laboratory, some of the

experiments were conducted under different conditions than those used in U.K. experiments. These differences are also given.

8.2a MORPHOLOGICAL VARIATION

Measurements of the cell length and width of both Baltic and U.K. species populations were made using plant material that had been preserved in 5% (v/v) formalin in seawater of the appropriate salinity. U.K. C. glomerata was preserved in a modified Transeau's solution (Prescott 1951). The cellular dimensions were measured by direct microscopy (magnification : x100) using a calibrated eye-piece scale. Measurements were made of ten randomly chosen cells from each of five different plants to give a total sample of 50 cells for both populations of the two species. Herbarium specimens of each species population were also prepared, which have been deposited in Liverpool City Museum.

8.2b VARIATION IN PHOTOSYNTHETIC ACTIVITY

The method used in this part of the study was the same as that employed in Section 7.2, although experiments were conducted at 10^{o}C only. During treatment with saline media Baltic plants were kept at a light intensity of approximately 10 μE $m^{-2}s^{-1}$ (12h light : 12h dark) which is different to that used in experiments with U.K. material (Section 7.2). Both Baltic species recovered in de-oxygenated Baltic water (6^{o}/oo) enriched with $NaHCO_3$, under lighting conditions that were also different to

those used in U.K. experiments (C. rupestris : 50 $\mu Em^{-2}s^{-1}$ and C.glomerata : 95 $\mu E\ m^{-2}s^{-1}$).

8.2c VARIATION IN CELL VOLUME RESPONSE

The method used is outlined in Section 5.2. However, photographs were taken of Baltic plants at longer time intervals, i.e at 30min, 1, 6 and 24h of treatment, and at 30min, 6 and 24h only for control plant material (incubated at $6^{o}/oo$).

8.2d VARIATION IN IONIC RESPONSE

Plants were treated in the same way as described in Section 6.2, but in experiments on Baltic species the $11^{o}/oo$ treatment medium was not used, and samples were only taken after 2, 6 and 24h incubation. The Baltic experiments were conducted at a lower light intensity than used in the U.K. (Baltic at 10 $\mu E\ m^{-2}s^{-1}$ and U.K. at 45 $\mu Em^{-2}s^{-1}$), and also at a slightly lower temperature (Baltic at 10 $\pm 1^{o}C$ and U.K. at 12 $\pm 1^{o}C$). Due to restrictions on time and resources the ion contents of the Baltic samples were determined on returning to the U.K., using the same techniques outlined in Section 6.2. Therefore, the samples were blotted following washing in isotonic sucrose and then dryed to constant dryness at $50^{o}C$ before being stored in sealed containers for return to Liverpool.

8.3 RESULTS

Baltic Cladophora rupestris plants are generally larger than Hilbre plants (Plates 8.1a,b), which are

unusually small compared with other U.K. populations. The appearance the thalli of both populations are very similar. The two populations of C. glomerata show quite different overall morphologies (Plates 8.2a,b). Baltic plants grow as long richly-branched filaments, whereas the U.K. material showed two morphological types : Plants were branched only when young and subsequently developed into long unbranched filaments. These two forms and intermediate stages are shown in Plate 8.2a. The younger plants were used in U.K. experiments during this study.

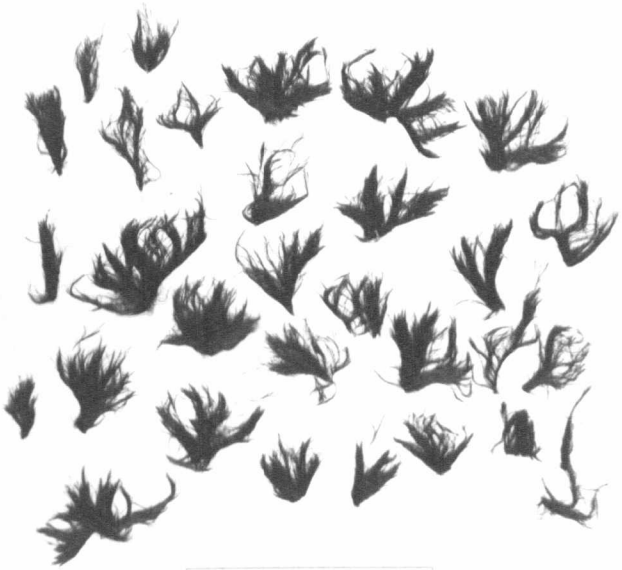
There are also morphological differences at the cellular level between Baltic and U.K. populations of both species. The cells of U.K. Cladophora rupestris are generally bigger than the cells of Baltic plants (Fig.8.1). This difference is emphasised if a crude estimate of cell volume is made using the measurements made and assuming cell volume is equivalent to the volume of a cylinder (Fig.8.3). Baltic C. glomerata has a greater proportion of small cells compared with U.K. material, but the Baltic plants also have a number of cells that are much larger than any cells from the U.K. population (Fig.8.2). This difference is clearly shown by the estimates of cell volume given in Fig.8.3.

The effect of salinity upon the net photosynthesis of the two populations of both Cladophora rupestris and C. glomerata are shown in Figs.8.4 and 8.5 respectively. The responses were shown to be significant ($P \leqslant 0.05$) following one-way ANOVA, and the Least Significant Difference is

Plate 8.1a Herbarium specimens of the U.K. Cladophora rupestris. The scale is the same as that in Plate 8.1b.

Plate 8.1b Herbarium specimens of the Baltic Cladophora rupestris population.

PLATE 8.1a



8.1b

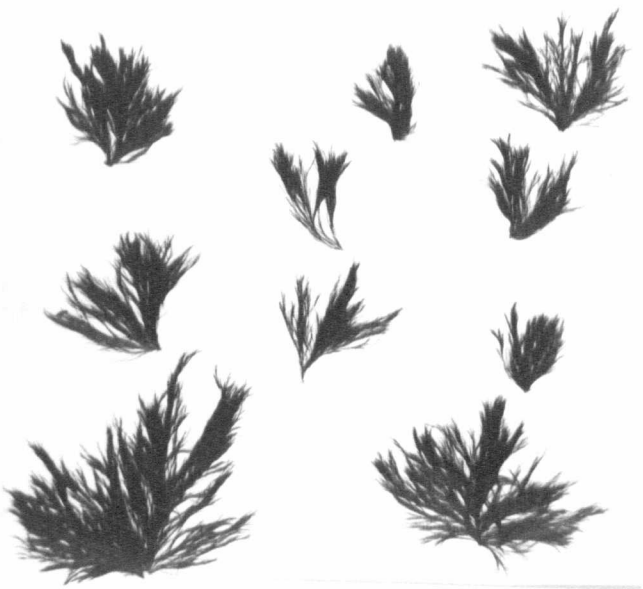
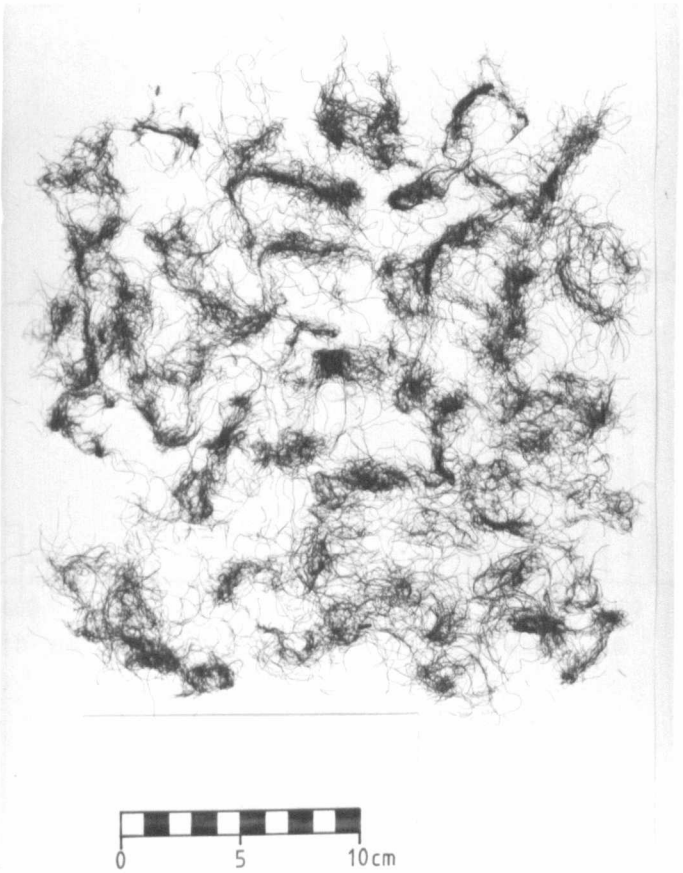


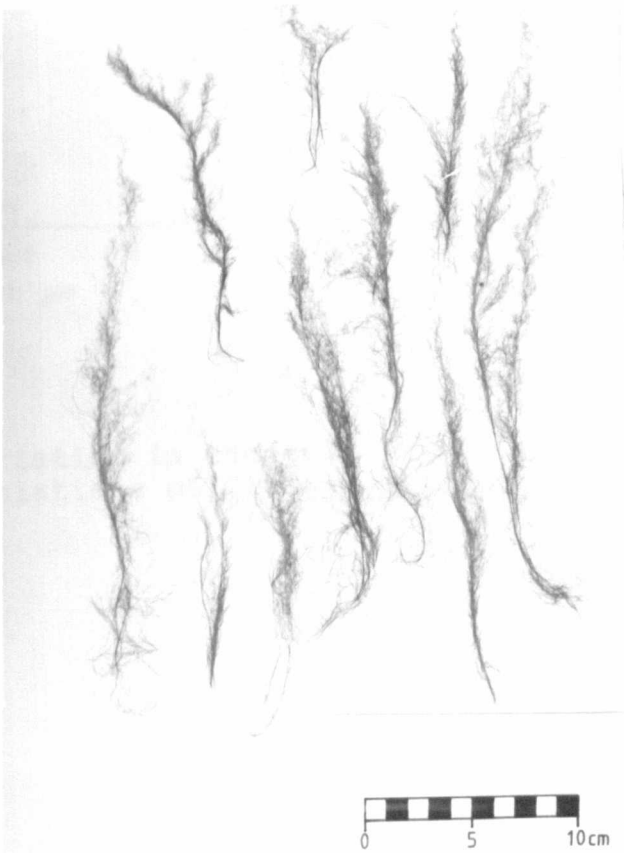
Plate 8.2a Herbarium specimens of the U.K. Cladophora glomerata population. Shows branched and unbranched forms of this species.

Plate 8.2b Herbarium specimens of the Baltic Cladophora glomerata population.

PLATE 8.2a



8.2b



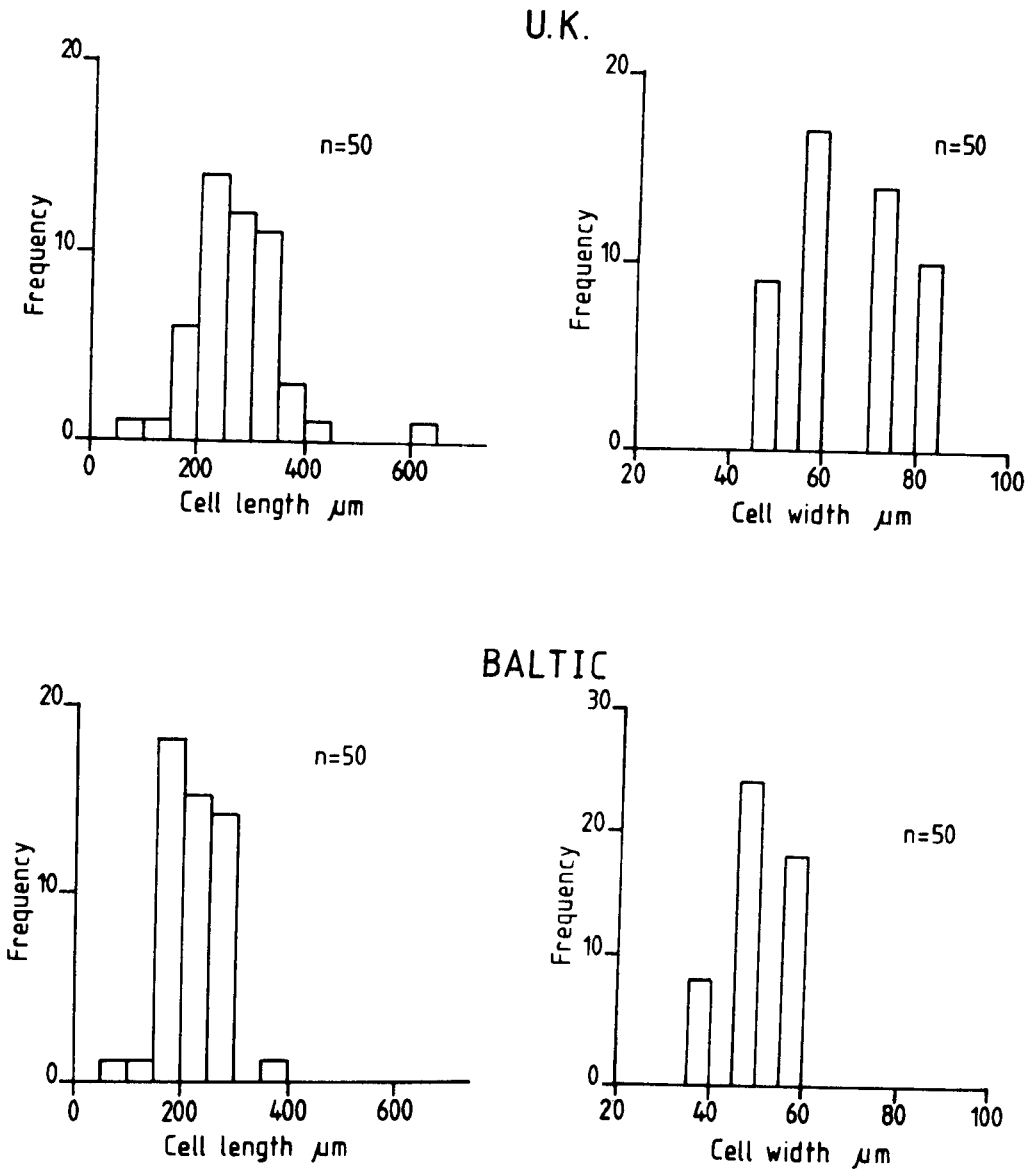


Fig.8.1 The variation in the cell length and width of U.K. and Baltic populations of Cladophora rupestris. (n = the sample size).

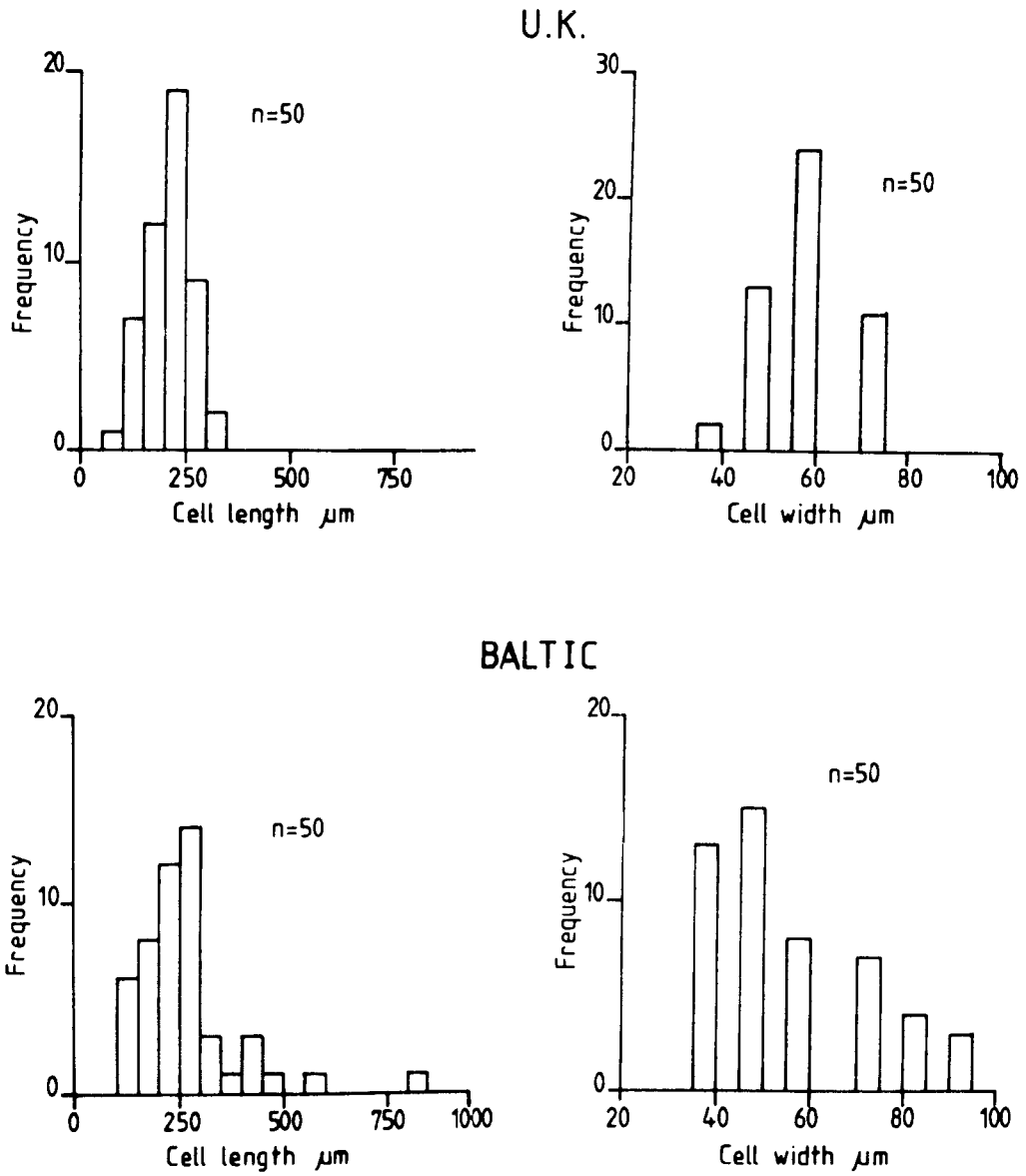


Fig.8.2 The variation in the cell length and width of U.K. and Baltic populations of Cladophora glomerata. (n = the sample size).

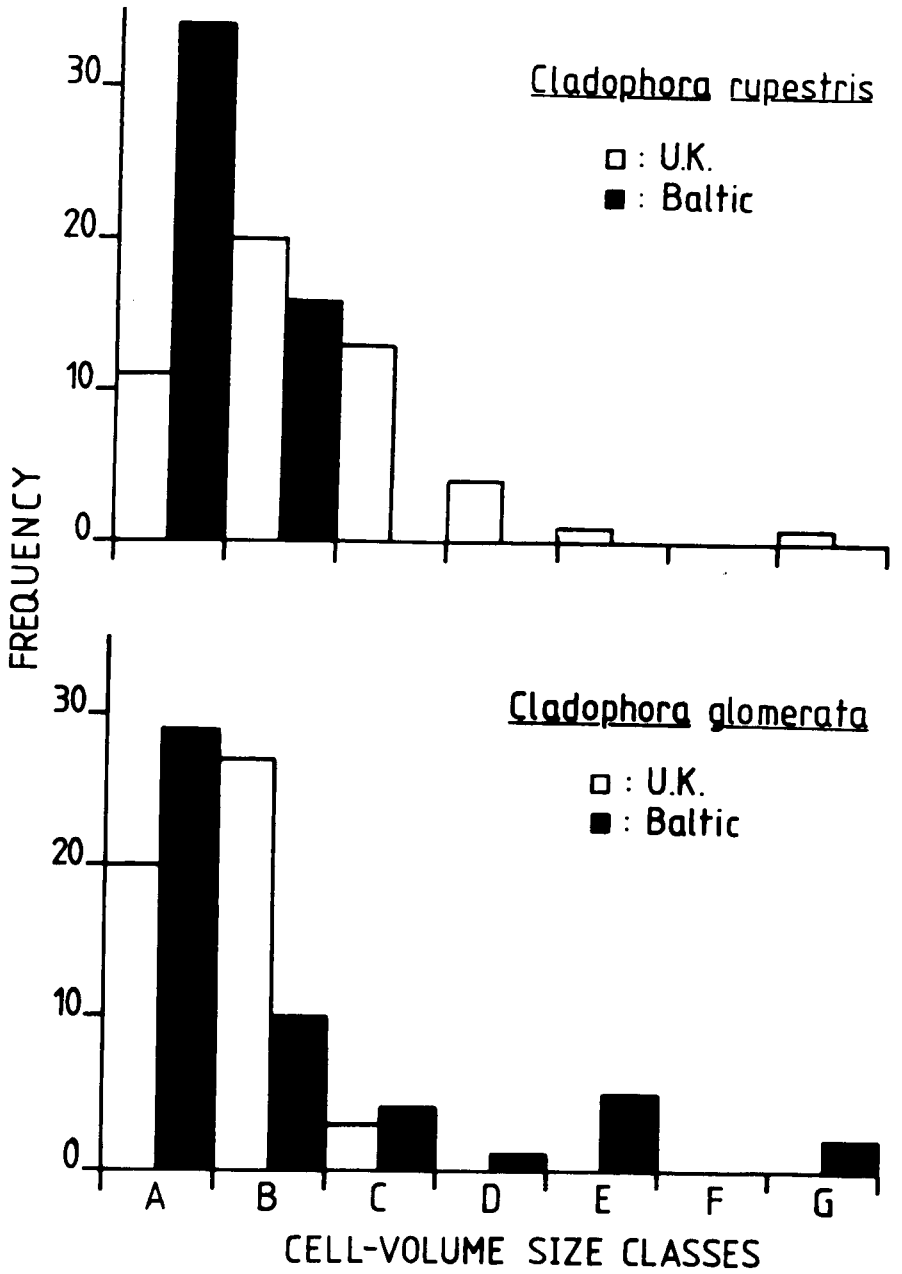


Fig.8.3 The variation in the cell volumes (μm^3) of U.K. and Baltic populations of Cladophora rupestris and C. glomerata. The size classes are :

A = 0 to $5x10^5$; B = $5x10^5$ to $10x10^5$; C = $10x10^5$ to $15x10^5$;

D = $15x10^5$ to $20x10^5$; E = $20x10^5$ to $25x10^5$;

F = $25x10^5$ to $30x10^5$; G = $30x10^5$ to $35x10^5$.

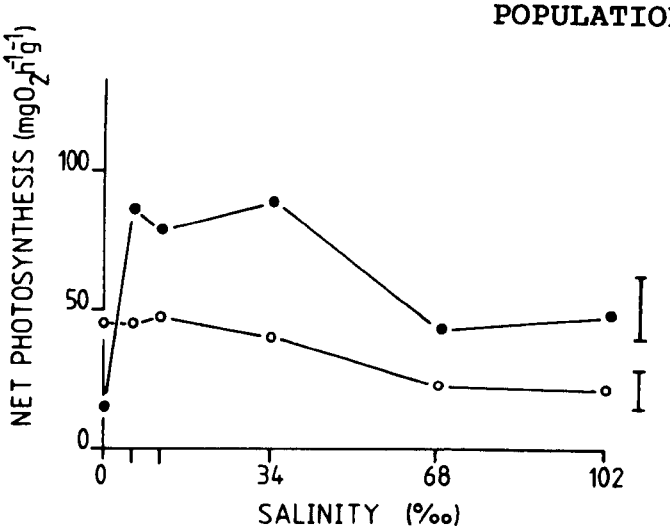


Fig.8.4 Effect of salinity upon the net photosynthetic rate of Cladophora rupestris. -●-●- : U.K. plants. -O-O- : Baltic plants. The Least Significant Difference is shown for both populations.

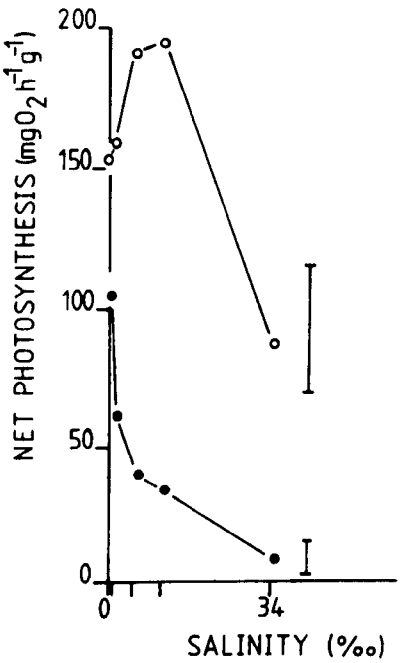


Fig.8.5 Effect of salinity upon the net photosynthetic rate of Cladophora glomerata. -●-●- : U.K. plants. -O-O- : Baltic plants. The Least Significant Difference is shown for both populations.

given for each of the populations.

The U.K. Cladophora rupestris was tolerant over a broad range of salinities (6 to 34°/oo), though it was less tolerant of severe hypo- and hypersaline treatments. Baltic C. rupestris showed a slightly wider salt tolerance (0 to 34°/oo), only having significant reductions in oxygen production in the extreme hypersaline media.

U.K. Cladophora glomerata proved to have very little salt tolerance, showing a marked decrease in activity even in the 1.5°/oo treatment, whereas its Baltic counterpart was tolerant of salinities up to 11°/oo, although these plants were unable to tolerate normal seawater of 34°/oo.

There were very large differences in the rates of production of the two populations of Cladophora glomerata, which were probably due to the very different morphologies of the plants. The more intricate thalli of the Baltic plants (see above) tend to have a greater surface area exposed to the incident light. However, strict comparisons of the rates of photosynthesis are not feasible, since it was not possible to obtain exactly the same light conditions during the experiments in both the Finnish and Liverpool laboratories.

The effect of salinity change upon the cell and protoplast volume of Cladophora rupestris was similar in both populations (Fig.8.6), with marked changes occurring in the 68 and 102°/oo treatments only. The cells of U.K. plants showed a greater degree of plasmolysis in the 68°/oo medium than the Baltic plants. There was, however,

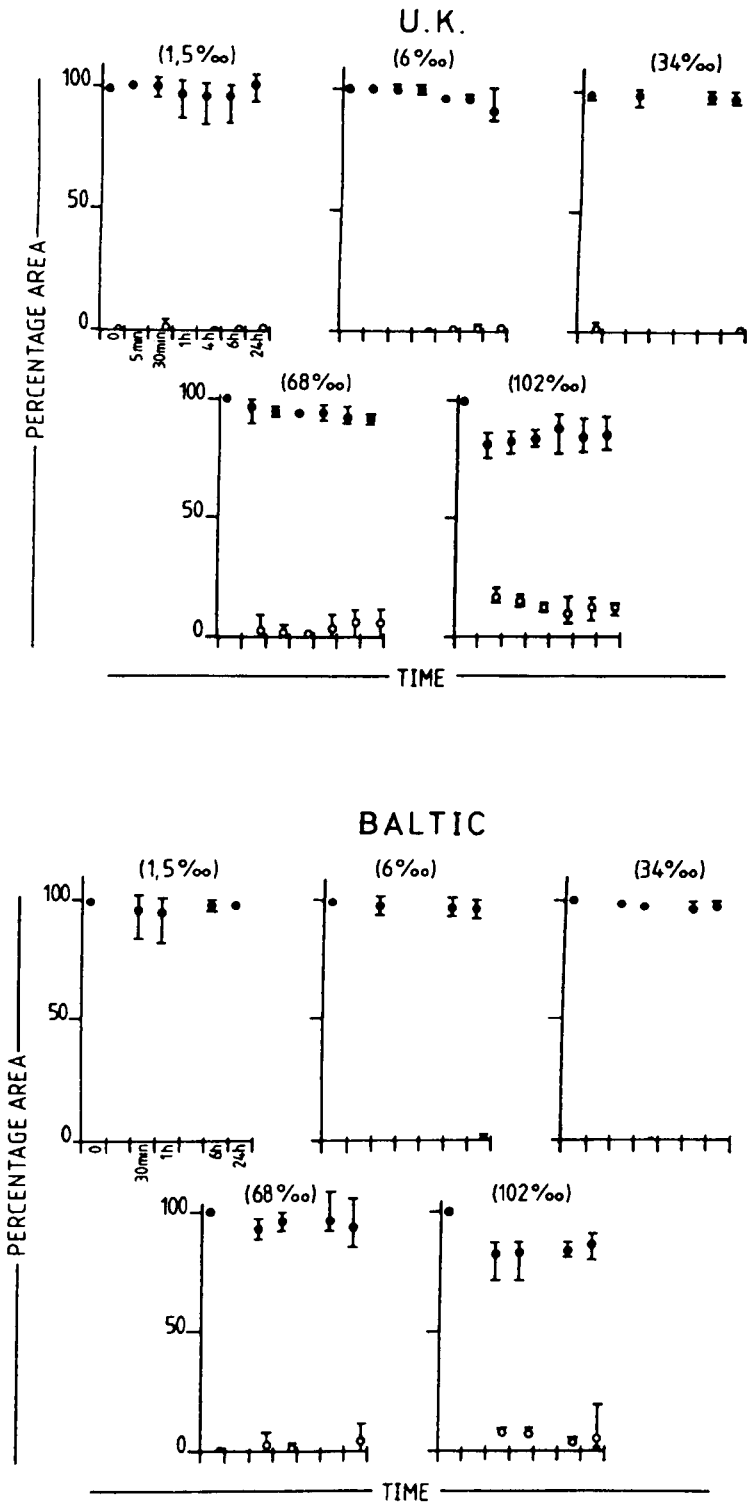


Fig.8.6 The changes in protoplast (-●-●-), and plasmolysed areas (-O-O-) of U.K. and Baltic populations of Cladophora rupestris exposed to salinity treatments over a 24h period. The range of values for each sample is indicated.

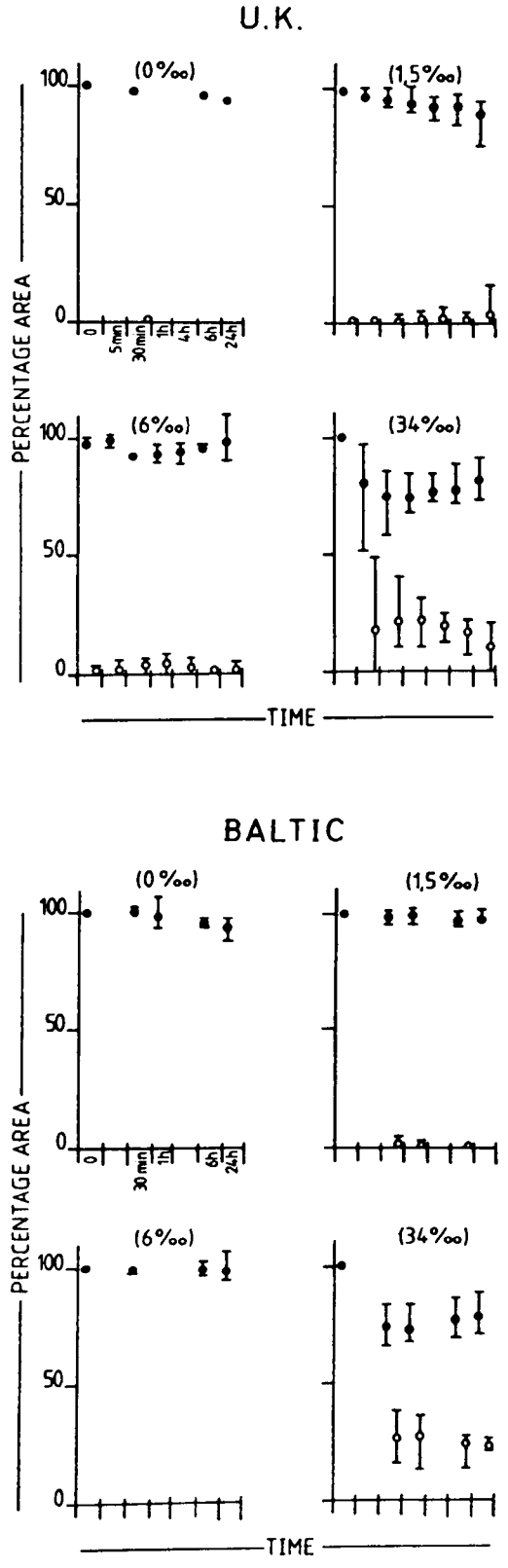


Fig.8.7 The changes in protoplast (-●-●-), and plasmolysed areas (-O-O-) of U.K. and Baltic populations of Cladophora glomerata cells exposed to increases in salinity over a 24h period. The range of values for each sample is indicated.

a great reduction in the total-cell volume of Baltic plant cells in the 102°/oo treatment, which did not occur in U.K. cells.

Baltic Cladophora glomerata showed very little change in protoplast volume, except at 34°/oo in which there was very marked plasmolysis, although total-cell volume remained quite constant over the whole salinity range (Fig.8.7). In contrast, the cells of U.K. material plasmolysed at 1.5°/oo and the degree of plasmolysis rose with increased salinity. The total-cell volume was also reduced in the U.K. cells in the 1.5 and 34°/oo treatment media.

In both Cladophora rupestris populations there was an increase in Na^+ levels with increased salinity (Fig.8.8). K^+ levels did not vary greatly in treatments up to 34°/oo, and then rose in the 68°/oo salinity (Fig.8.9). There was a reduction in the K^+ content of Baltic plants in the 102°/oo media. Low levels of K^+ were recorded for U.K. plants in 102°/oo and Baltic plants in both 68 and 102°/oo during the first 6h of treatment. Cl^- levels showed a similar response in both populations to those of K^+ (Fig.8.10), although there was no great reduction of Cl^- levels after 24h in Baltic material. The levels of K^+ and Cl^- were higher in the U.K. plants over the whole range of salinity treatments, whereas levels of Na^+ were higher in the Baltic plants.

Levels of Mg^{2+} reach slightly higher values in U.K. Cladophora rupestris than in Baltic plants (Fig.8.11), and

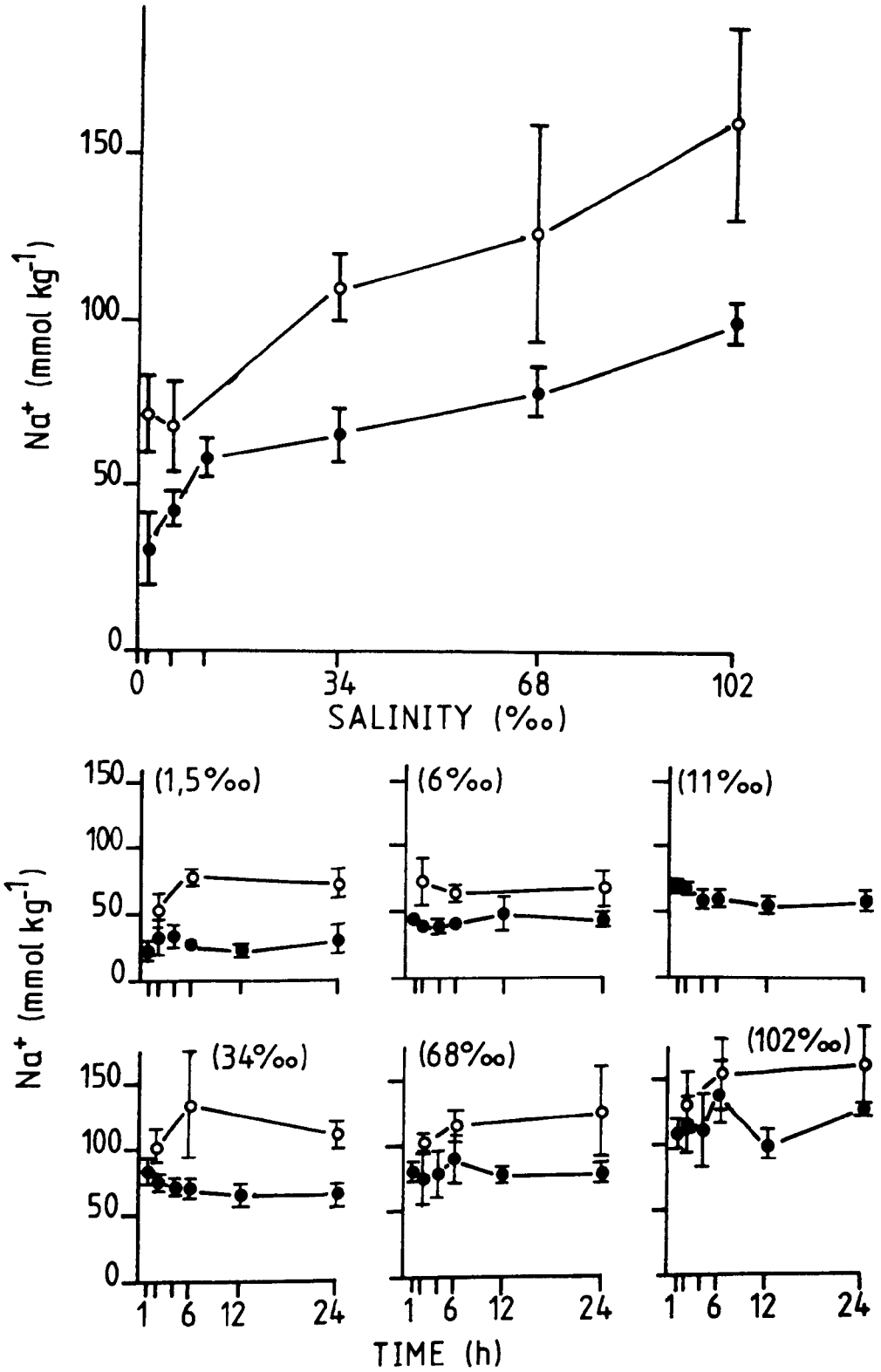


Fig.8.8 The effect of salinity on the thallus content of Na^+ of U.K. (-●-●-), and Baltic (-O-O-) populations of Cladophora rupestris. The smaller diagrams show the effect of incubation time at each salinity treatment. 95% confidence limits are shown for each sample.

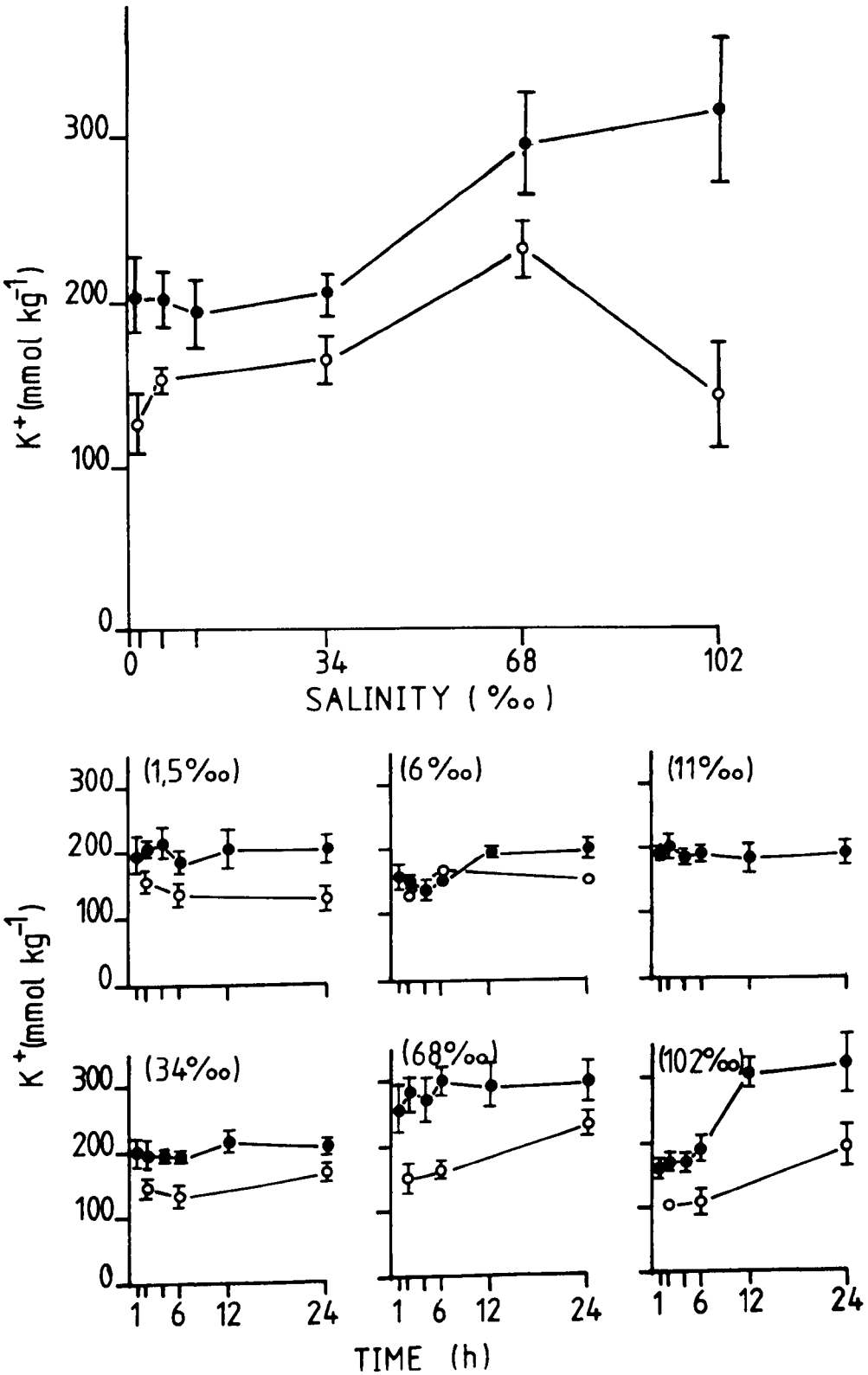


Fig.8.9 The effect of salinity on the thallus content of K^+ of U.K. (-●-●-), and Baltic (-O-O-) populations of Cladophora rupestris. The smaller diagrams show the effect of incubation time at each salinity treatment. 95% confidence limits are shown for each sample.

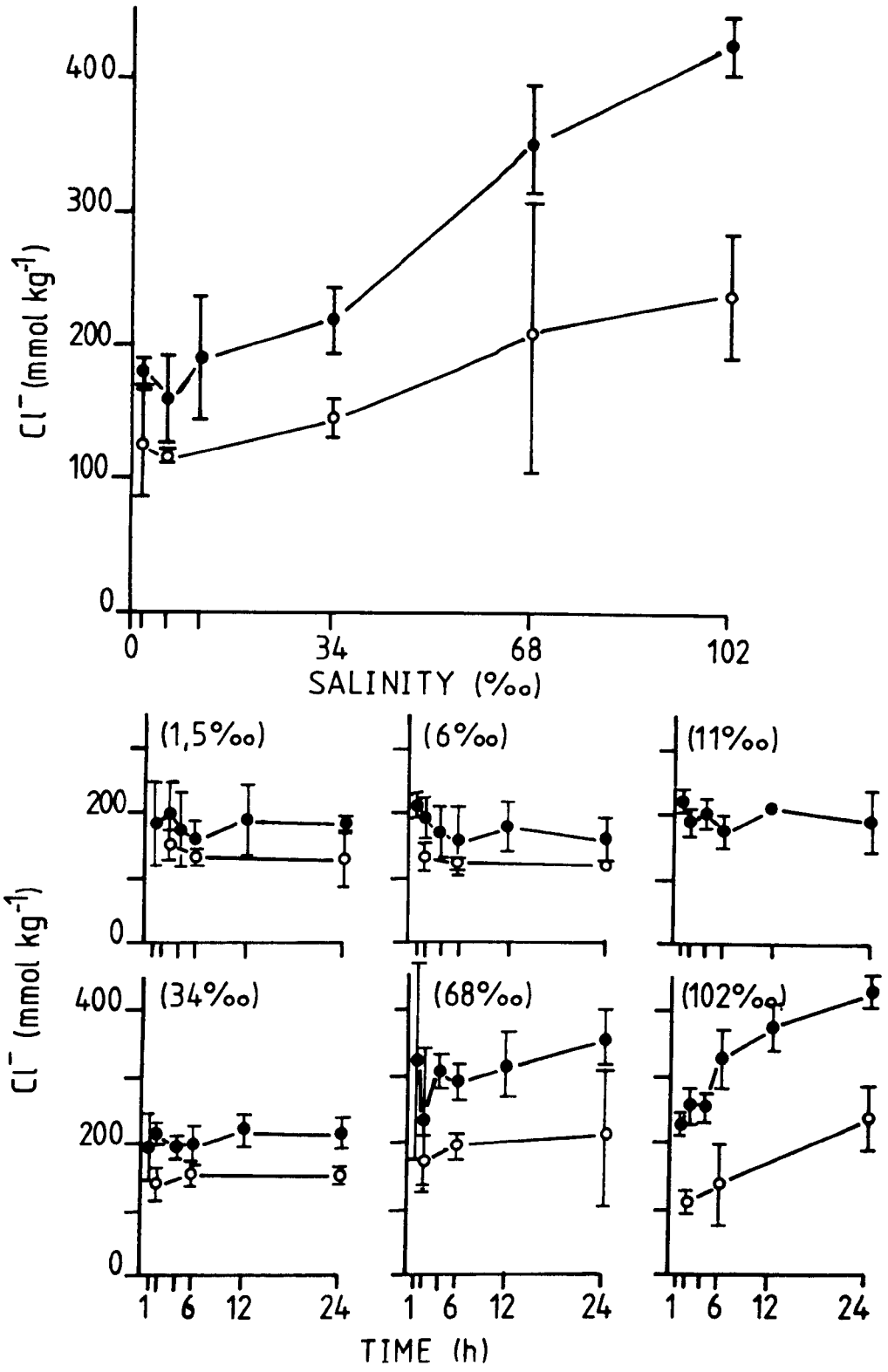


Fig.8.10 The effect of salinity on the thallus content of Cl^- of U.K. (-●-●-), and Baltic (-O-O-) populations of Cladophora rupestris. The smaller diagrams show the effect of incubation time at each salinity treatment. 95% confidence limits are shown for each sample.

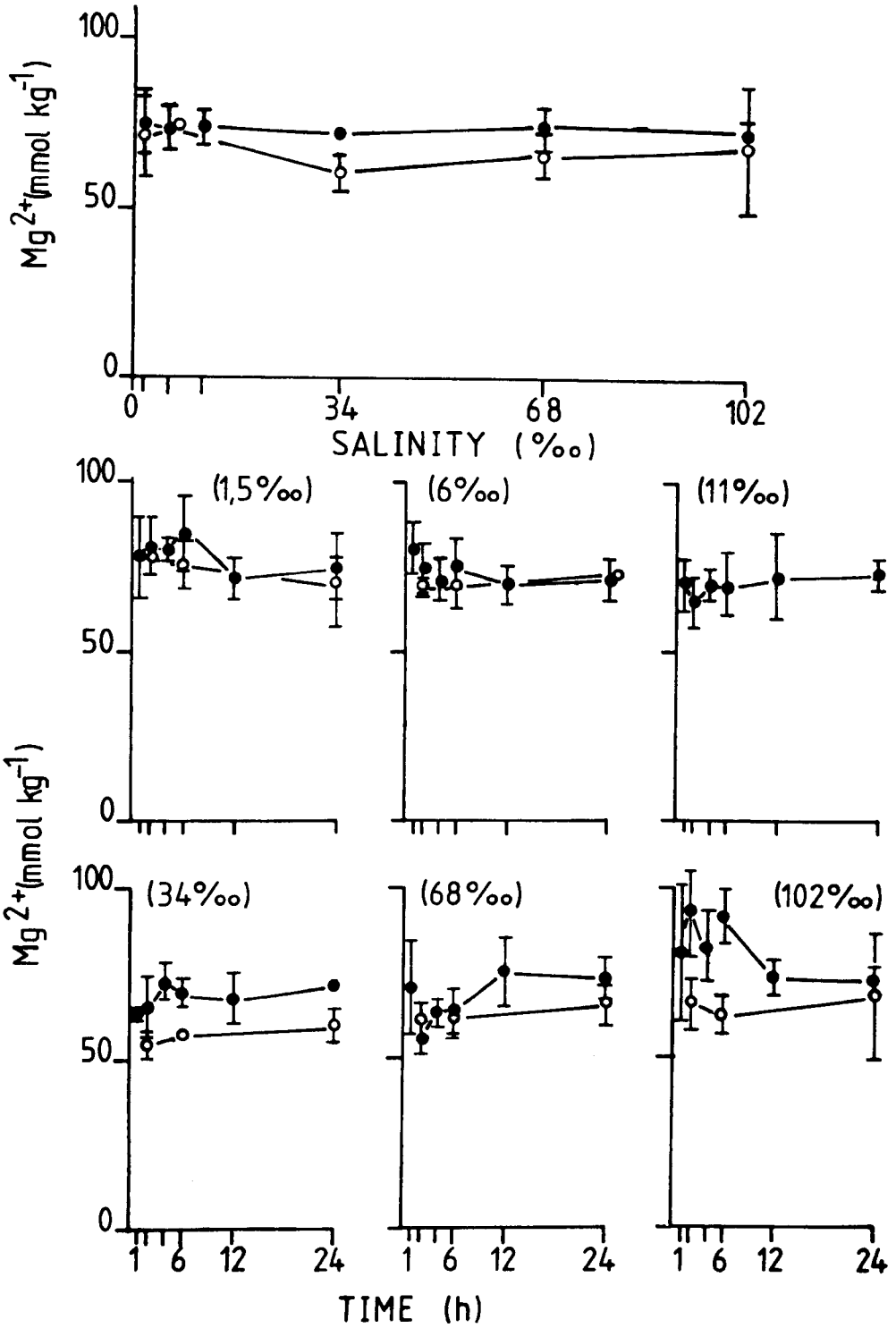


Fig.8.11 The effect of salinity on the thallus content of Mg^{2+} of U.K. (-●-●-), and Baltic (-O-O-) populations of Cladophora rupestris. The smaller diagrams show the effect of incubation time at each salinity treatment. 95% confidence limits are shown for each sample.

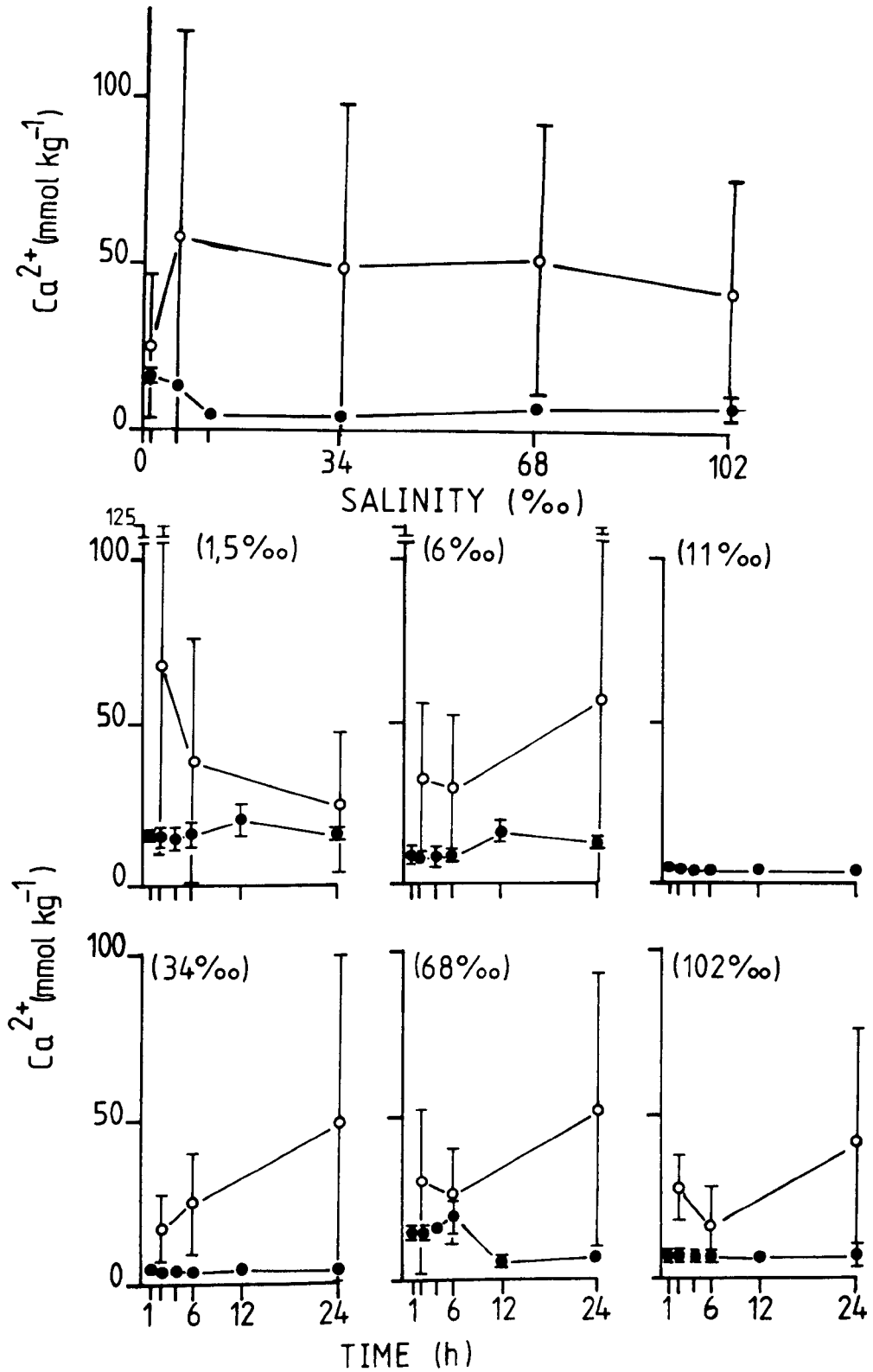


Fig.8.12 The effect of salinity on the thallus content of Ca^{2+} of U.K. (-●-●-), and Baltic (-O-O-) populations of Cladophora rupestris. The smaller diagrams show the effect of incubation time at each salinity treatment. 95% confidence limits are shown for each sample.

also increase following treatment at 102°/oo. There were higher levels of Ca^{2+} in the Baltic population (Fig.8.12), although the importance of this difference is not clear due to the great variation in values obtained from the Baltic samples.

Length of incubation appears not to have an effect on the Na^{+}, Ca^{2+} and Mg^{2+} in the tissue in both populations of Cladophora rupestris. However, there was variation in the K^{+} and Cl^{-} levels with time in both Baltic and U.K. material subjected to hypersaline stress.

The levels of Na^{+}, K^{+} and Cl^{-} were higher in Baltic Cladophora glomerata than in U.K. plants (Figs.8.13 to 8.15). The tissue content of Na^{+} rose in both populations with increasing salinity (Fig.8.13), with incubation time having no effect. The levels of K^{+} increased slightly with salinity in the Baltic plants, which compares with the slight decrease in the U.K. material (Fig.8.14). There was no significant change in Cl^{-} levels in Baltic plants (Fig.8.15), though there was considerable variation in these values. U.K. plants showed only a slight decrease in levels of Cl^{-} with increasing salinity.

The amount of Mg^{2+} recorded in both populations of Cladophora glomerata was very similar, and both had lower levels of this ion in the 0°/oo treatment (Fig.8.16). Very high levels of Ca^{2+} were recorded in the U.K. population (Fig.8.17), although these values were very varied. There was no apparent change in Ca^{2+} levels in response to salinity change. The length of incubation does not have

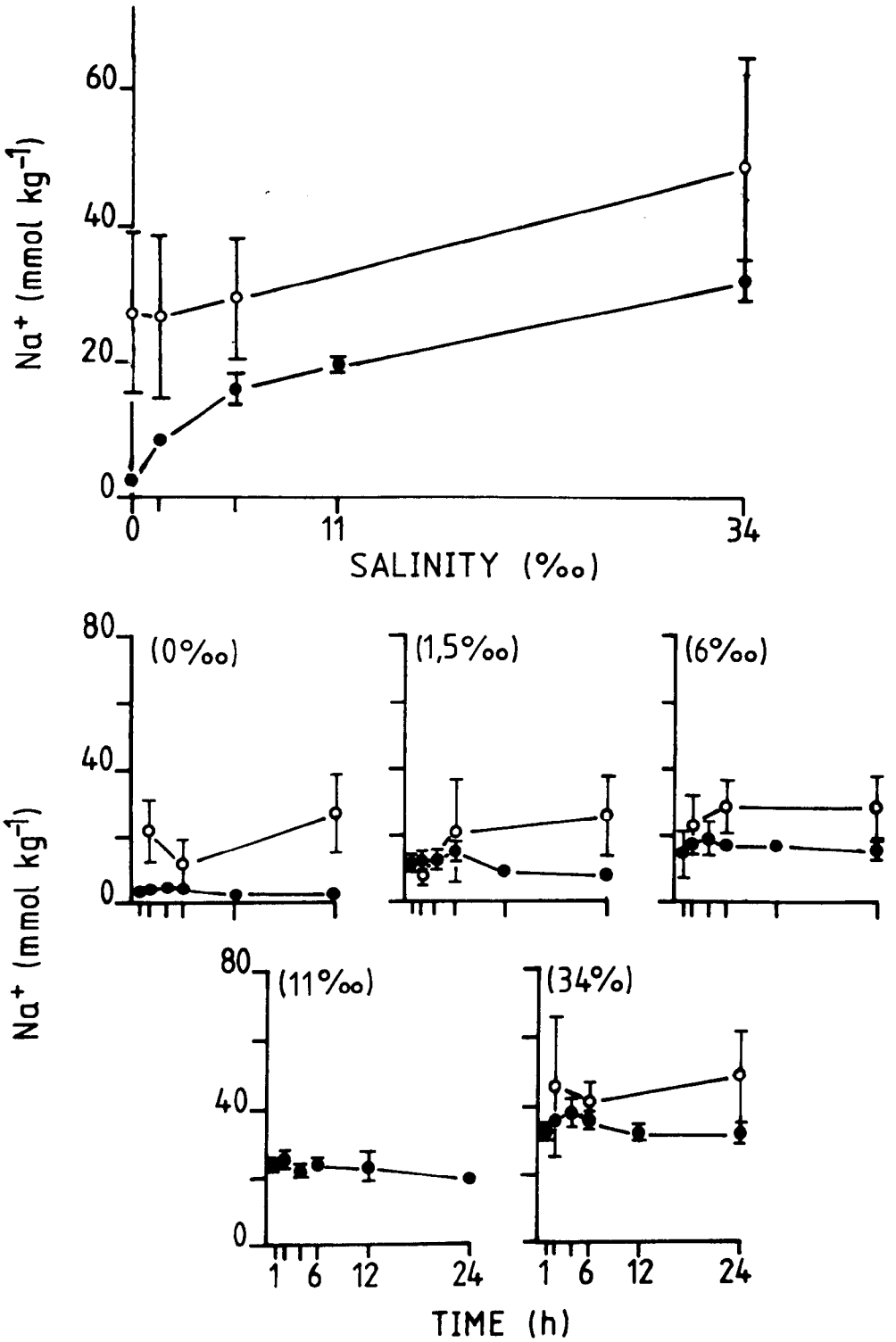


Fig.8.13 The effect of salinity on the thallus content of Na^+ of U.K. (-●-●-), and Baltic (-O-O-) populations of Cladophora glomerata. The smaller diagrams show the effect of incubation time at each salinity treatment. 95% confidence limits are shown for each sample.

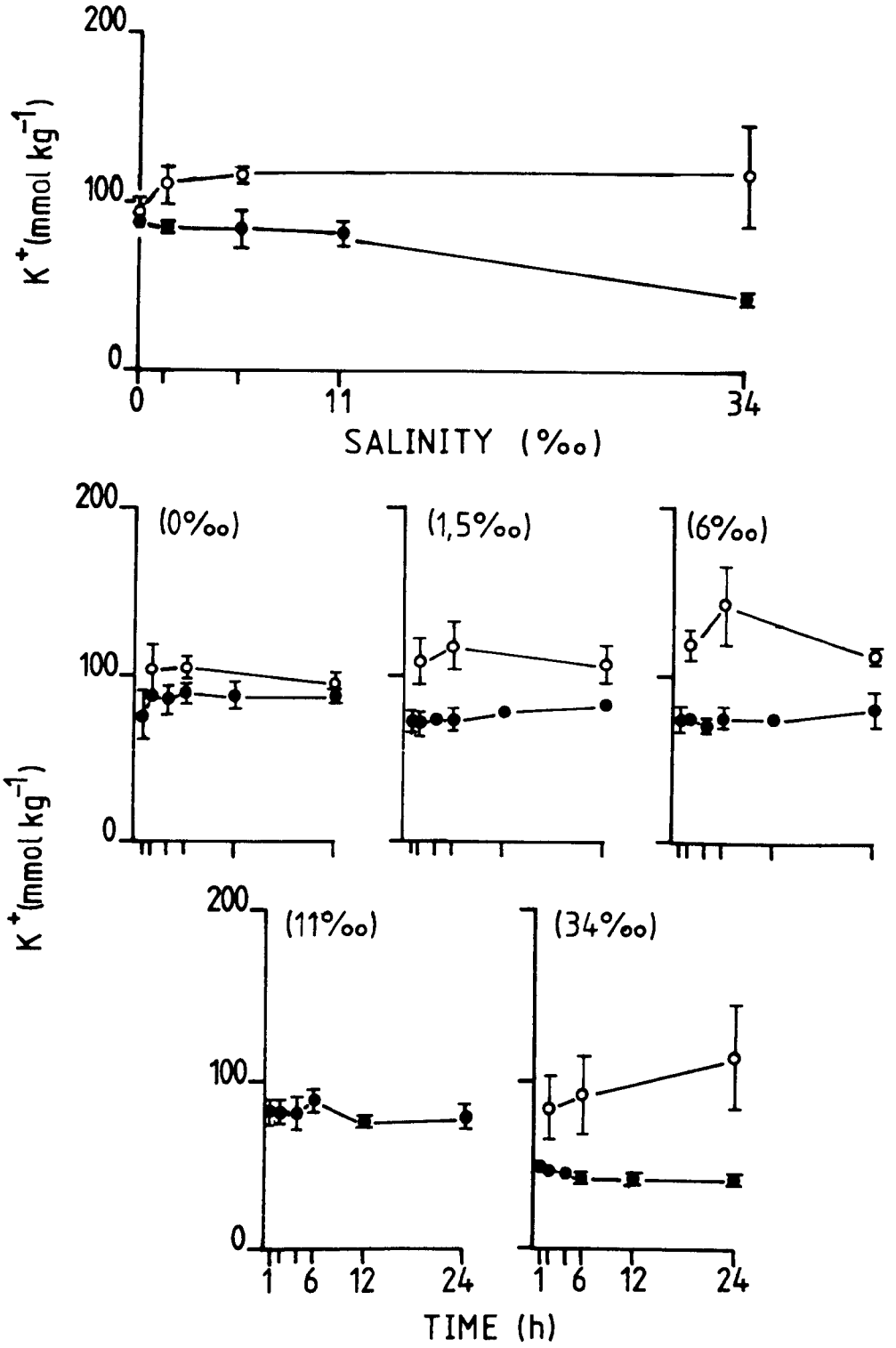


Fig.8.14 The effect of salinity on the thallus content of K^+ of U.K. (-●-●-), and Baltic (-O-O-) populations of Cladophora glomerata. The smaller diagrams show the effect of incubation time at each salinity. 95% confidence limits are shown for each sample.

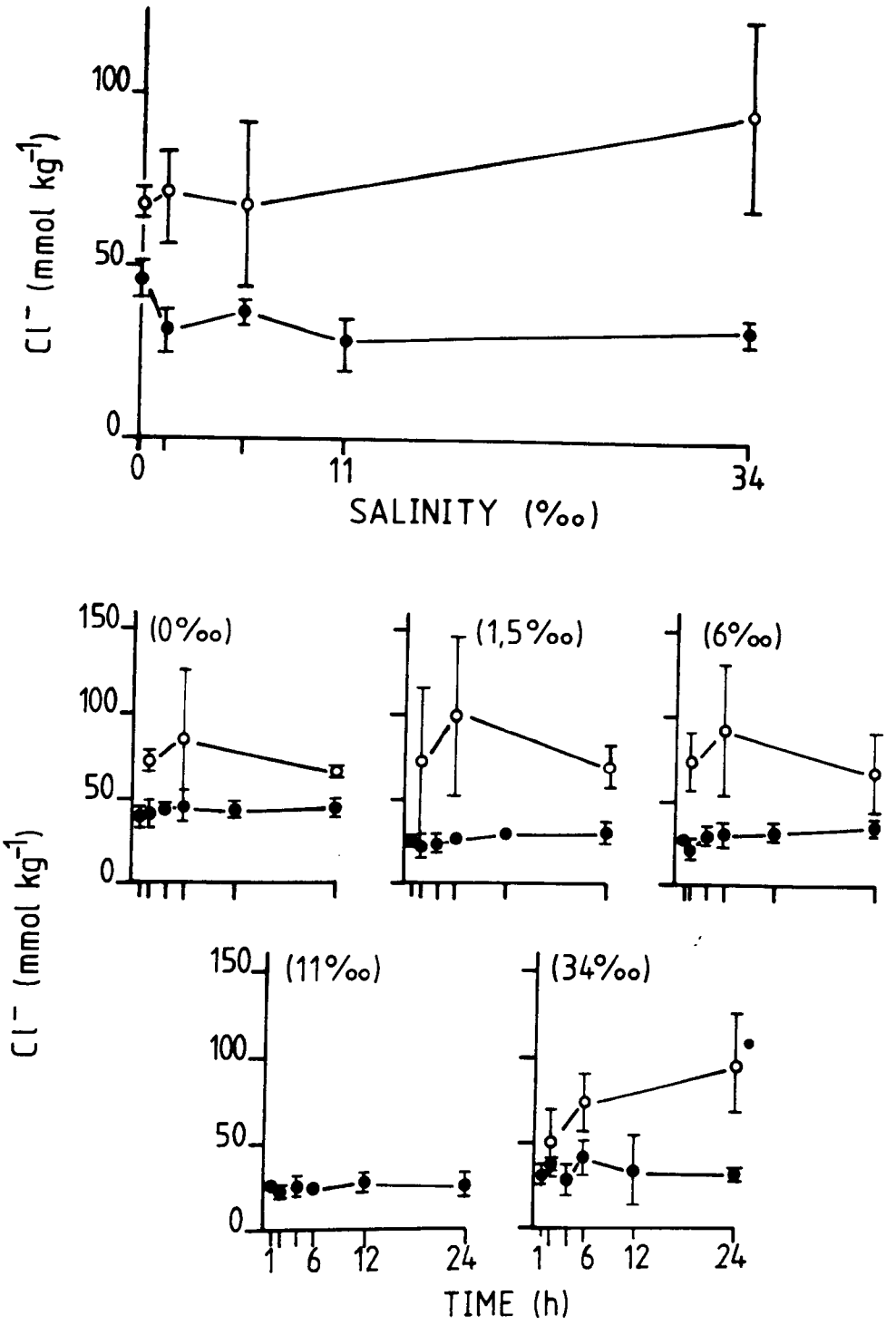


Fig.8.15 The effect of salinity on the thallus content of Cl^- of U.K. (-●-●-), and Baltic (-O-O-) populations of Cladophora glomerata. The smaller diagrams show the effect of incubation time at each salinity treatment. 95% confidence limits are shown for each sample.

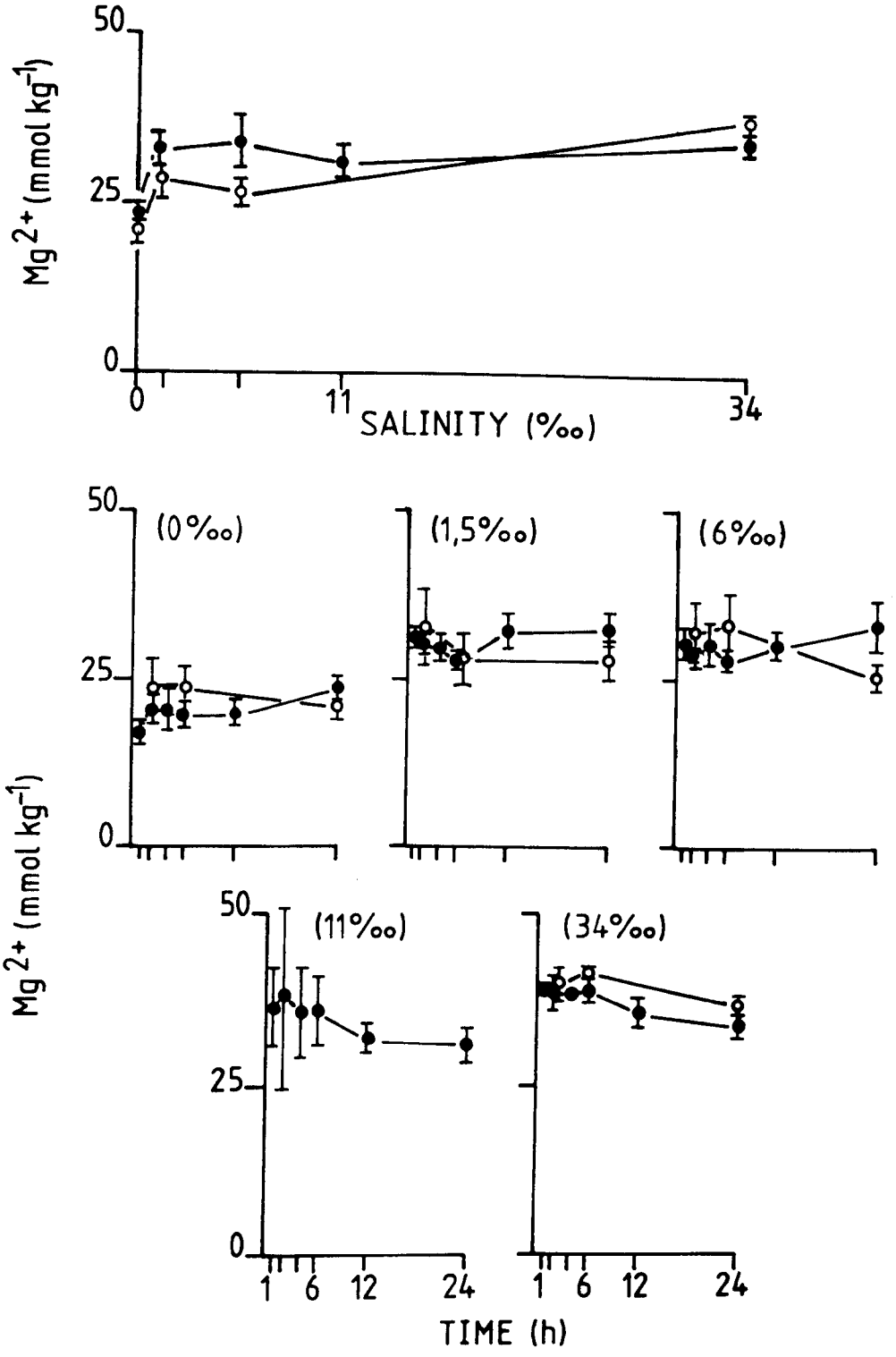


Fig.8.16 The effect of salinity on the thallus content of Mg^{2+} of U.K. (-●-●-), and Baltic (-O-O-) populations of Cladophora glomerata. The smaller diagrams show the effect of incubation time at each salinity treatment. 95% confidence limits are shown for each sample.

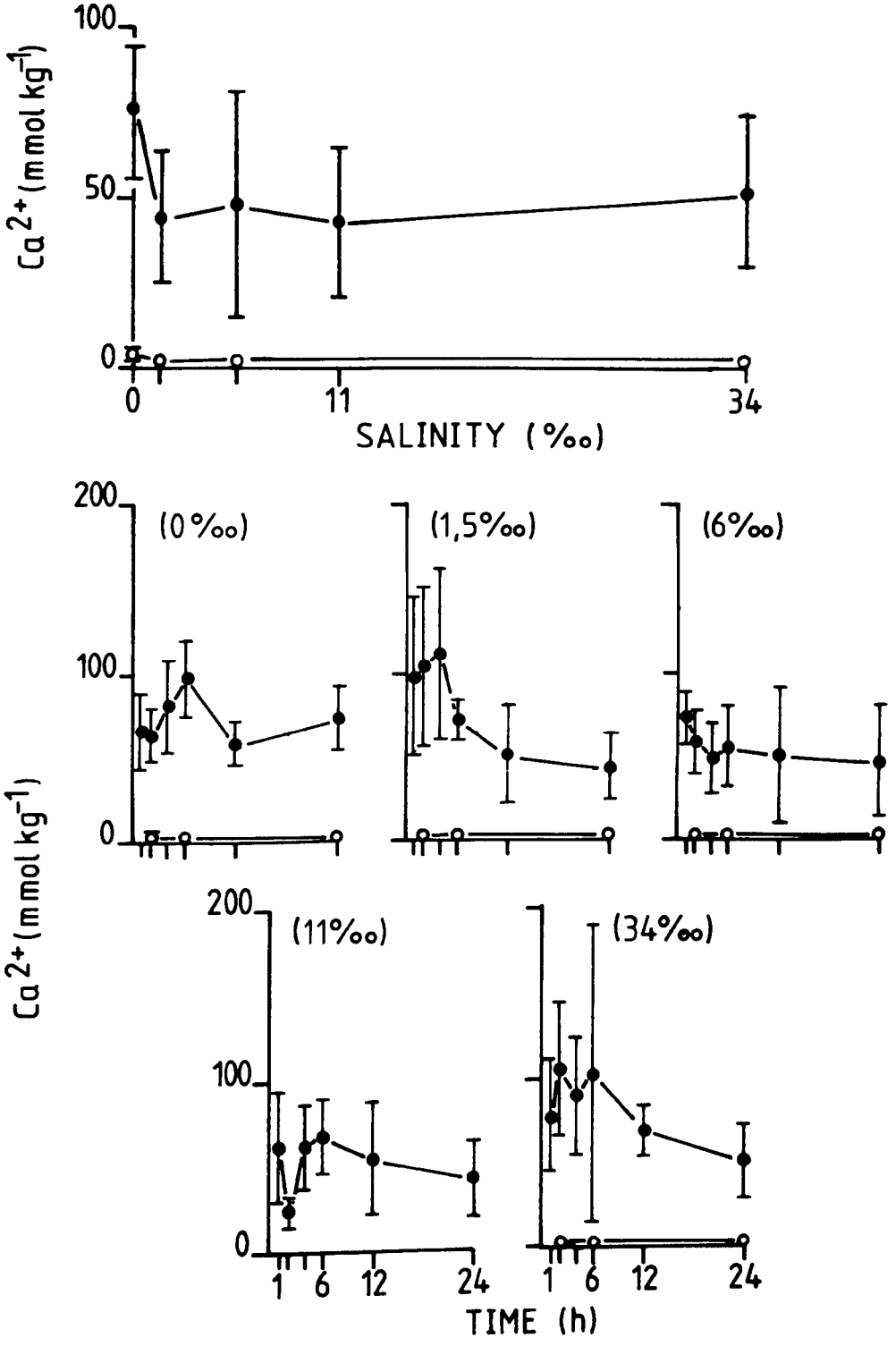


Fig.8.17 The effect of salinity on the thallus content of Ca^{2+} of U.K. (-●-●-), and Baltic (-O-O-) populations of Cladophora glomerata. The smaller diagrams show the effect of incubation time at each salinity treatment. 95% confidence limits are shown for each sample.

any effect on the levels of Mg^{2+} and Ca^{2+} in both C. glomerata populations.

The results for the experiments conducted using Baltic plants are given in Appendix v, and those for U.K. plants in Appendices ii to iv.

8.4 DISCUSSION

The net photosynthetic responses to changes in salinity, of the two U.K. species were very distinctive : Cladophora rupestris was tolerant over a wide band of salinities, whereas C. glomerata showed little tolerance to salinity. The responses of the two Baltic species were less distinct. Except when treated with freshwater the two populations of C. rupestris showed similar tolerance over the range of saline treatments. The responses of Baltic C. glomerata were very different from those of U.K. material. It is possible that selection pressures imposed by the brackish waters of this part of the Baltic Sea have led to a convergence of salt tolerance in these two species.

Although the measurements of cell and protoplast volume changes have a lot of associated error because of the experimental method used (Section 5), population differences in the responses of the cells were observed. The changes in protoplast and cell volume reflect quite closely the salinity tolerances described above: the cells of both Cladophora rupestris populations plasmolysed in hypersaline conditions, and reductions in the total-cell volumes of Baltic cells also took place in the $102^{o}/oo$ treatment. The greater reduction of phtosynthetic activity

in U.K. plants than in Baltic material at 68°/oo, was also reflected by a greater degree of plasmolysis that occurred in U.K. cells compared with the responses of Baltic cells in this salinity. Plasmolysis of Baltic C. glomerata cells only occurred in the 34°/oo treatment, which was the only salinity to cause a significant reduction in photosynthetic activity in this population. Plasmolysis occurred in all salinity treatments in U.K. plants, which corresponded with a loss of photosynthetic activities after exposure to these treatments.

Fig.8.18 shows the ratio of K^+/Na^+ for the two populations of C. rupestris. The Baltic plants appear to have maintained a fairly constant ratio of approximately 1.5 between the salinities of 1.5 to 68°/oo with a reduction only occurring at 102°/oo. U.K. plants, however, maintained a higher ratio (approx. 3.0) consistently over the range 11 to 102°/oo, there being a slight increase in 6°/oo and an even larger increase in the 1.5°/oo treatment. There were lower K^+/Na^+ ratios in Baltic C. glomerata plants (approx. 5.0) compared with U.K. material (10.0 - 20.0), in 0 and 1.5 salinity treatments (Fig.8.19), although during the first 6h of incubation at these salinities this ratio was very varied in Baltic plants. The K^+/Na^+ ratios of both populations fall slightly with increases in salinity above 6°/oo, larger reductions taking place in the U.K. plant material.

There appear to be no marked population differences in the Mg^{2+} content in either of the two species. The

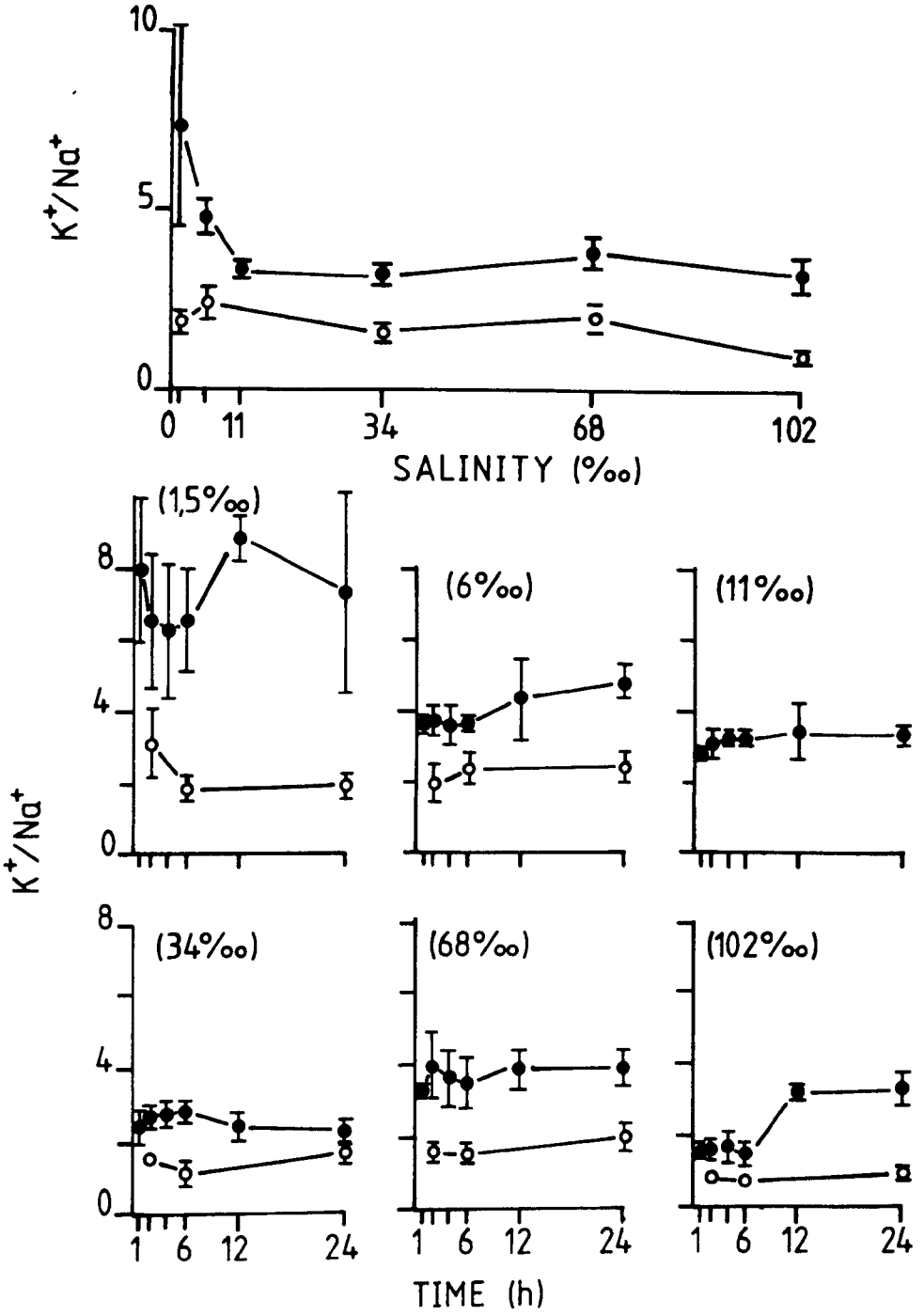


Fig.8.18 The effect of salinity on the K^+/Na^+ ratio of U.K. (-●-●-), and Baltic (-O-O-) populations of Cladophora rupestris. The smaller diagrams show the effect of incubation time at each salinity treatment. 95% confidence limits are shown for each sample.

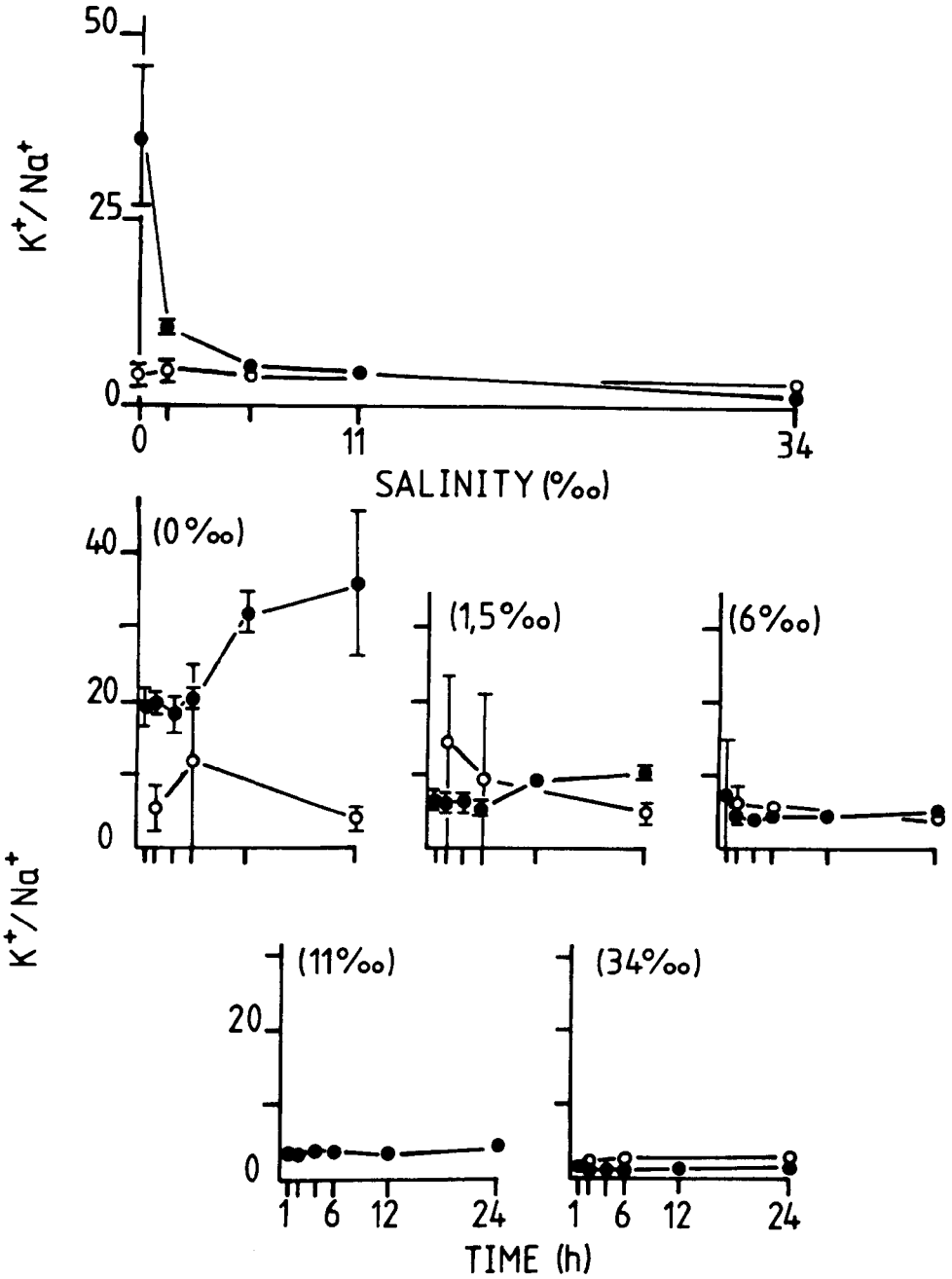


Fig.8.19 The effect of salinity on the K^+/Na^+ ratio of U.K. (-●-●-), and Baltic (-O-O-) populations of Cladophora glomerata. The smaller diagrams show the effect of incubation time at each salinity. 95% confidence limits are shown for each sample.

variation in Ca^{2+} levels within and between species may be a reflection of the different Ca^{2+} contents of the three water bodies studied, and differences in cation adsorption properties between the two species.

Reed and Barron (1983), have shown that estuarine Pilayella littoralis has smaller cells than marine plants. The subsequent increase in surface area : volume in the estuarine population, is advantageous if turgor is regulated mainly by ion fluxes at the plasmalemma. These authors also showed there to be population differences in cell wall thickness, estuarine cells having thinner walls which enabled them to accomodate greater changes in cell volume. Thus the smaller celled Baltic Cladophora rupestris similarly may have been selected for by the brackish Baltic Sea. However, the salinity conditions of this part of the Baltic Sea are fairly stable, unlike those of the estuaries from which the P. littoralis was collected. Measurements of cell wall thicknesses should be made in future studies of these conspecific populations. The differences in cell sizes of the two C. glomerata populations were not as distinct as those of C. rupestris, although the Baltic population did have a number of considerably larger cells than its U.K. counterpart. This may be a result of the cell size measurements being made on the branched forms of U.K. plants, and larger cells are probably more numerous on the older unbranched forms of this population. There were clear differences in the general morphologies of the two C. glomerata populations,

which was not true of the C. rupestris plants. Hilbre C. rupestris plants are probably so small because of the very exposed nature of the site from which they were collected.

It does seem, therefore, that there are morphological and physiological differences between Baltic and U.K. populations of these two Cladophora species, accompanying the differences in salt tolerance recorded by measurements of net photosynthesis and cell volume change. At risk of over-simplification, these differences may be summarised as follows :

1. The smaller-celled Baltic Cladophora rupestris is able to maintain constant ionic levels at lower salinities than its U.K. counterpart.

2. Baltic Cladophora glomerata appears to maintain ionic equilibrium at higher salinities than U.K. plants.

It is probable that some of the observed differences within these species have resulted from acclimation (plastic) responses. However, the greatly increased ability of the Baltic Cladophora glomerata to withstand changes in external osmotic conditions would imply that ecotypic evolution may have occurred within this population. Further experiments are needed to measure the acclimation capacity of these populations in order to deduce the extent of genotypic differentiation (Russell and Bolton 1975, Yarish et al 1979).

9. GENERAL DISCUSSION

The aims of this investigation were to measure the salt tolerances of two Cladophora species, and to identify physiological processes involved in the maintenance of cell homeostasis under changed external salinity conditions. C. rupestris and C. glomerata were selected because of their normally quite distinct distributions, which converge only in certain brackish water habitats. It has been shown that the salinity tolerancess of these two species follow the salinity regimes experienced in the field, the intertidal C. rupestris tolerating a broad band of salinities, and the freshwater C. glomerata showing little tolerance to salinity change. Interspecific differences in tolerance were measured in populations of both species growing in the brackish Baltic Sea. Ideally this study should be developed further to include other Cladophora species, especially as several of these are able to grow in a range of saline habitats (Hoek 1963, 1982a).

In the field, algae are exposed to a multitude of environmental factors that together exert complex interactive effects upon the plants. Plants living in estuarine and intertidal sites are exposed particularly to complicated interactions of physical and chemical conditions, both diurnally and seasonally (Hartog 1967, 1968 and 1970). It is unlikely that the ability of a plant to survive in a particular habitat is limited solely by its response to changes in a single factor, and several

experimental studies have shown that factors such as salinity, light and temperature have interactive effects on the physiology of algae (Dawes et al. 1976 and Lehnberg 1978). The effects of temperature upon the salinity responses of the two Cladophora species clearly indicate the importance of considering environmental interactions in ecophysiological studies. However, there are many other variables that need to be incorporated in the experiment designs of such investigations, including photoperiod, exposure to wave action, nutrient status, dehydration, length of exposure to particular stresses. Of particular importance in studies of estuarine and intertidal algae, is the accurate simulation of the diurnal fluctuations in salinity (Davenport 1982 and Reed et al. 1980c). Obviously experimental procedures required to carry out such comprehensive investigations would be complicated, but results based on responses to variations of an individual variable should be treated with caution, especially if such measurements are to be interpreted ecologically.

If studies like those described above are to be undertaken, it is a prerequesite that the natural environmental influences exerted on the plants are known. It was for this reason that the environmental measurements were made during the course of this study, in particular those at Hilbre Island. However, it is quite clear that this study was not sufficiently extensive to describe fully the magnitude and frequency of the environmental variation experienced by the plants. More

detailed measurements of the changes that occur over longer periods of time are needed, and should include diurnal and seasonal measurements of fluctuations of light intensity, nutrient status, pH, temperature, salinity and exposure to wave action. The differences recorded in salinity and temperature experienced by exposed plants from that of the surface seawater were of interest, although these were under-estimated because of restricted access to Hilbre Island. Further studies would have to record the changes that occur over complete periods of emergence under different types of climatic conditions. These observations should be related to metereological conditions prevalent at the sampling sites and should include measurements of rain fall, air temperature, relative humidity and wind speed. Information about the nature of tidal fluctuations at a particular site should also be used in conjunction with such studies. Because of the heterogenous nature of intertidal shorelines, comprehensive studies would require measurements to be taken at a number of positions on the shore. Again investigations of this nature clearly require a lot of resources and time, but these are certainly necessary if the responses measured under laboratory conditions are to be related meaningfully to the experiences of the plants in nature.

Two different approaches were used to measure salt tolerance in these algae: the first recorded photosynthetic activity while the plants were exposed to

salinity stress, and the second measured photosynthesis under recovery conditions following incubation with a particular salinity treatment. Strict comparison of the two approaches is not possible because of the different techniques used to measure oxygen production and the slightly different incubation times and conditions used. However, the first approach gave more narrow tolerance limits than were evident from the results obtained from plants in recovery conditions, especially in the case of Cladophora rupestris. The second experimental approach probably gives a better measure of plant viability following salinity stress, since the effects of transitory physiological traumas caused by a salinity change are not recorded. It would seem that future investigations may obtain more reliable indications of the actual salinity tolerance of a particular species if the measurement of plant survival is made under normal salinity conditions following salinity treatment. A similar rationale for experimental measurement of desiccation damage has been argued by Dring and Brown (1982).

There were changes in intracellular ionic levels in response to changed external salinities in both Cladophora rupestris and C. glomerata. The techniques used to measure these changes did not provide precise measures of the ion movements taking place, and due to the washing procedure used, the levels of certain ions were probably overestimated. Future studies should concentrate on more precise measurements of the intracellular concentrations

of ions, especially Na^+, K^+ and Cl^-, and the distribution of these within the cell. Radioisotope techniques could be used for the measurement of ion fluxes across cell membranes at different salinities (Reed et al. 1981, Young et al. 1987a,b), and intracellular ion compartmentation could be measured using using electron probe X-ray microanalysis (Wiencke et al. 1984). Further studies also require the measurement of any variation occurring in levels of other ions such as NO_3^- and SO_4^{2-}, as well as in amino acid levels and in levels of organic osmotica such as mannitol, sucrose, DMSP and glycine betaine. These compounds have been shown to be involved in the osmotic responses of algae (Table 1.1), and one of more of them may play a role in the regulation of cell turgor in Cladophora species. Recent work has shown that certain amino acids and glycine betaine may be osmoticaly active when Cladophora rupestris plants are exposed to hyposaline stress (C.Wiencke. pers.comm.).

More accurate measurements of cell volume changes than were possible during this present study should also be included as part of the more detailed physiological investigations. These would include more precise photomicroscopic measurements, and also the use of radioisotope labelling experiments to partition the total tissue water into cellular and extracellular fractions so giving estimates of the apparent osmotic volume (Reed et al. 1980a). Experimental procedures similar to those described by Reed et al. 1980a would be used.

There were clear morphological and physiological differences associated with differences in salt tolerance in Baltic and U.K. populations of both species. However, the experiments on which these observations were based were performed over only short periods of time, and the extent to which the observed differences can be removed by more prolonged acclimation is not clear, nor is it possible to state with certainty that ecotypic variation is involved. The distinction between genotypic and phenotypic differences is not possible using short term studies of the kind described in these investigations. Long-term laboratory growth experiments performed in conjunction with field transplant studies are desirable, if the extent to which ecotypic differentiation has taken place is to be found. Investigations of this nature, and especially the transplant experiments, would expose the plants to seasonal fluctuations in environmental conditions encountered at the Baltic and U.K. sites, so that the plants would be exposed to the full range of selection pressures encountered in nature. Given sophisticated and carefully controlled culture facilities it should be possible to simulate these environmental variations during long term growth experimentation.

In algal population studies, it is important to note the fact that the degree of phenotypic plasticity exhibited within a plant population is ultimately governed by the genotype of those plants. It is also possible that the plastic responses of a plant are limited to only

certain characteristics, and that other morphological and physiological features may be more stable. The suitability of the widely used terms ecotype and ecophene (ecad), first proposed by Turesson (1922), is more questionable in the light of considerations such as these, although they will continue to be useful descriptive terms for population ecologists. Turesson regarded ecotypes as sharply distinguishable entities, although he identified ecotypes only from few, and very contrasting environments, and therefore, could not adequately consider the situation that occurrs in transitory habitats (Faegri 1937). Bolton (1983) has shown there to be a clinal variation in the temperature tolerance of Ectocarpus siliculosus associated with similar rates of change in ambient temperature in a range of isolates from Arctic to warm temperate sites. There may be similar variation in salt tolerance of algal populations found along the salinity gradient between the low salinities of the Baltic proper through to the North Atlantic.

Although the Baltic Sea is brackish in nature, and its surface waters are subject to dilution during ice melt in the spring, the plants living in the Baltic generally experience quite stable salinities. It is probable that the Baltic populations will show quite different adaptations from those exhibited by the algae from a tidal estuarine population, since the latter are subject to diurnal and seasonal changes in salinity of very considerable magnitude. It would be valuable to compare

the responses of estuarine isolates of these two species which are subject to regular salinity fluctuations with isolates from the Baltic. Since the Baltic is not strictly comparable with tidal estuaries, its population may be characterised by tolerances which are not identical with those of estuarine ecotypes, as reported by Reed (1983b), Reed and Barron (1983) and Young et al. (1987b).

It is possible that some of the experimental variation obtained during these studies is due to different life history stages or plants of different ploidy being used : there is a high degree of polyploidy in Cladophora glomerata which is thought to account for the large variation in morphology within the species (Wik-Sjöstedt 1970). Also the sporophytes and gametophytes of Cladophora rupestris are not distinguishable morphologically (Wik-Sjöstedt 1970). Yarish et al. (1986), in experiments designed to show the relationships between global distributional boundaries and temperature responses of algae, exposed several red algal species to different combinations of temperature, light intensity and photoperiod. They showed that the tetrasporophyte stage of Polyneura hilliae is able to survive under conditions similar to those of the northern limit for this species, whereas the gametophyte stage is not. Dixon (1965) also reports that for many red algae it is usually the tetrasporic plants which are reported from further north than sexual stages, whilst at extreme northern limits completely sterile plants only are found. Whittick (1977),

has also shown that only triploid parasporangial Plumaria elegans plants are found at the northern limits of this species in N.America. Therefore there is some evidence to suggest that differences in ploidy may be linked with differences in physical tolerances, and although temperature is thought to be the predominant regulatory factor, I feel that future salinity tolerance investigations need to take ploidy as well as natural selection pressures into account. It would be of interest to conduct cytological studies on Baltic and U.K. populations of both Cladophora species, to look for differences in ploidy associated with the marked variation in salt tolerance of the two species populations.

Burrows (1964) and Khfaji and Norton (1979) have demonstrated that algal distribution is not necessarily governed by the tolerances of the adult plants to environmental conditions. Both of these studies reveal that the earliest stages of development are more vulnerable to salinity stress than mature tissue. Therefore, it is important that the tolerances of all life history stages to changes in salinity and other external fluctuations, are measured.

It follows from this discussion that, if algal ecophysiology is to illuminate algal distribution patterns in nature, then detailed physico-chemical measurements of the conditions in which the algae grow are needed. In the past such measurements have been made of few factors in a restricted number of habitat types. Physiological studies

have been similarly narrowly based. Future studies will have to to involve measurements of responses to many different stress factors, and not just single parameters. It will also become necessary to measure interactive responses of these. There is a need for more knowledge of algal genetics to compliment the physiological investigations. The factors that influence the distribution of algae are many and varied, as are the physiological responses. Together they present a complex and intriguing challenge for the algal ecophysiologist.

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11. APPENDICES

For reference the data presented and discussed in the experimental sections of this study are listed in the following appendices. The corresponding appendix for each section is given below.

Appendix i :- Section 4. Effect of salinity on photosynthesis and respiration.

Appendix ii :- Section 5. Effect of salinity on cell volume.

Appendix iii :- Section 6. Effect of salinity on thallus ion content.

Appendix iv :- Section 7. Combined effects of salinity and temperature.

Appendix v :- Section 8. Population differences in salinity responses.

APPENDIX i

LIGHT INTENSITY ($\mu E\ m^{-2}s^{-1}$)	NET PHOTOSYNTHESIS ($mgO_2h^{-1}g^{-1}$)	
	C.RUPESTRIS	C.GLOMERATA
20	1.05 (±0.09)	0.31 (±0.10)
50	1.62 (±0.07)	0.80 (±0.08)
100	3.53 (±0.47)	0.97 (±0.05)
200	3.52 (±0.75)	1.04 (±0.05)
400	3.69 (±0.87)	1.06 (±0.03)
600	3.30 (±0.58)	1.17 (±0.16)
800	2.79 (±0.45)	0.92 (±0.03)
1000	2.54 (±0.43)	0.73 (±0.01)
2000	2.69 (±0.29)	0.99 (±0.06)

Table Ai.1 Net photosynthetic rates of Cladophora rupestris and C. glomerata at different light intensities, at 12°C.

SALINITY (‰)	TIME (H)	NUMBER OF REPLICATES	MEAN NET PHOTOSYNTESIS	95% CONFIDENCE LIMITS
0	1	4	5.70	4.98 - 6.42
	2	5	1.42	1.15 - 1.69
	4	5	2.06	1.70 - 2.42
	6	5	0.52	0.14 - 0.90
	12	5	2.02	1.75 - 2.29
	24	5	2.00	1.65 - 2.35
6	1	5	0.68	0.20 - 1.16
	2	5	1.60	1.42 - 1.78
	4	5	1.86	1.14 - 2.58
	6	5	1.44	0.89 - 1.98
	12	5	1.12	0.70 - 1.55
	24	5	1.24	0.82 - 1.66
11	1	5	2.00	1.36 - 2.64
	2	5	2.02	1.33 - 2.71
	4	5	2.05	1.72 - 2.32
	6	5	0.78	0.37 - 1.19
	12	5	1.56	1.12 - 2.00
	24	5	2.08	1.64 - 2.52
34	1	5	2.78	1.33 - 4.23
	2	5	2.14	1.37 - 2.91
	4	5	0.88	0.24 - 1.52
	6	5	1.98	1.20 - 2.76
	12	5	1.64	0.98 - 2.30
	24	5	3.16	2.13 - 4.19
68	1	5	1.60	0.99 - 2.21
	2	5	1.92	1.45 - 2.39
	4	5	2.18	1.79 - 2.57
	6	5	1.62	0.77 - 2.47
	12	5	0.90	0.67 - 1.13
	24	5	1.22	1.11 - 1.32
102	1	5	1.02	0.52 - 1.51
	2	5	1.54	1.02 - 2.06
	4	4	1.30	1.17 - 1.43
	6	5	0.52	0.00 - 1.04
	12	5	0.40	0.02 - 0.78
	24	5	0.34	0.00 - 0.68

Table Ai.2 The net photosynthesis ($mgO_2h^{-1}g^{-1}$) of Cladophora rupestris exposed to salinity treatments for different incubation periods.

SALINITY (‰)	TIME (H)	NUMBER OF REPLICATES	MEAN RESPIRATION	95% CONFIDENCE LIMITS
0	1	5	0.32	-0.05 - 0.69
	2	5	0.56	0.45 - 0.67
	4	5	0.68	0.52 - 0.84
	6	5	0.76	0.58 - 0.93
	12	5	0.68	0.52 - 0.84
	24	5	0.82	0.58 - 1.06
6	1	5	0.76	0.55 - 0.96
	2	5	0.80	0.60 - 0.99
	4	5	0.82	0.51 - 1.12
	6	5	0.74	0.57 - 0.91
	12	5	0.86	0.72 - 1.00
	24	5	0.88	0.68 - 1.08
11	1	5	0.84	0.63 - 1.05
	2	5	0.84	0.67 - 1.01
	4	5	0.90	0.61 - 1.19
	6	5	0.68	0.52 - 0.84
	12	5	0.78	0.60 - 1.96
	24	5	0.90	0.90 - 0.90
34	1	5	0.82	0.38 - 1.25
	2	5	0.94	0.68 - 1.20
	4	5	0.86	0.47 - 1.25
	6	5	0.80	0.68 - 0.92
	12	5	1.12	0.60 - 1.64
	24	5	1.18	1.12 - 1.24
68	1	5	0.56	0.49 - 0.63
	2	5	0.54	0.43 - 0.65
	4	5	0.66	0.52 - 0.80
	6	5	0.54	0.35 - 0.73
	12	5	0.78	0.39 - 1.17
	24	5	0.66	0.43 - 0.89
102	1	5	0.54	0.43 - 0.65
	2	5	0.60	0.38 - 0.82
	4	4	0.53	0.29 - 0.76
	6	5	0.52	0.36 - 0.68
	12	5	0.50	0.12 - 0.88
	24	5	0.62	0.35 - 0.89

Table Ai.3 The dark respiration rates ($mgO_2h^{-1}g^{-1}$) of Cladophora rupestris exposed to salinity treatments for different incubation periods.

SALINITY (‰)	TIME (H)	NUMBER OF REPLICATES	MEAN NET PHOTOSYNTHESIS	95% CONFIDENCE LIMITS
0	1	5	2.20	1.70 - 2.71
	2	5	1.44	1.25 - 1.63
	4	5	1.80	1.50 - 2.10
	6	5	1.44	1.11 - 1.76
	12	5	1.68	1.22 - 2.14
	24	5	2.08	1.63 - 2.53
6	1	5	1.14	0.70 - 1.58
	2	5	0.48	0.25 - 0.70
	4	5	1.50	0.70 - 2.31
	6	5	1.04	0.70 - 1.43
	12	5	1.28	0.79 - 1.77
	24	5	1.60	1.01 - 2.19
11	1	5	1.48	0.97 - 2.00
	2	5	0.72	0.40 - 1.04
	4	5	1.92	1.72 - 2.12
	6	5	1.12	0.88 - 1.36
	12	5	1.60	1.13 - 2.07
	24	5	0.94	0.50 - 1.38
34	1	5	0.18	-0.02 - 0.38
	2	5	0.58	-0.58 - 1.75
	4	5	0.44	-0.03 - 0.91
	6	5	0.00	0.00 - 0.00
	12	5	0.28	-0.20 - 0.76
	24	5	0.22	-0.39 - 0.83

Table Ai.4 The net photosynthetic rates ($mgO_2h^{-1}g^{-1}$) of Cladophora glomerata exposed to salinity treatments for different incubation periods.

SALINITY (‰)	TIME (H)	NUMBER OF REPLICATES	MEAN RESPIRATION	95% CONFIDENCE LIMITS
0	1	5	0.62	0.46 - 0.80
	2	5	0.50	0.38 - 0.62
	4	5	0.46	0.10 - 0.82
	6	5	0.48	0.26 - 0.70
	12	5	0.50	0.41 - 0.59
	24	5	0.58	0.48 - 0.68
6	1	5	0.34	0.27 - 0.41
	2	5	0.60	0.51 - 0.69
	4	5	0.30	0.08 - 0.52
	6	5	0.66	0.37 - 0.95
	12	5	0.44	0.33 - 0.55
	24	5	0.46	0.07 - 0.85
11	1	5	0.34	0.08 - 0.60
	2	5	0.58	0.09 - 1.07
	4	5	0.64	0.45 - 0.83
	6	5	0.46	0.35 - 0.57
	12	4	1.03	0.41 - 1.64
	24	5	0.58	0.48 - 0.68
34	1	5	0.22	-0.05 - 0.49
	2	5	0.14	-0.13 - 0.41
	4	5	0.56	0.03 - 1.09
	6	5	0.36	-0.09 - 0.78
	12	5	0.36	-0.01 - 0.73
	24	5	0.24	0.01 - 0.47

Table Ai.5 The dark respiration rates ($mgO_2h^{-1}g^{-1}$) of Cladophora glomerata exposed to salinity treatments for different incubation periods.

APPENDIX ii

SALINITY (‰)	TIME	NUMBER OF REPLICATES	MEAN % PROTOPLASM AREA	RANGE OF % VALUES
1.5	0	5	99.6	98.4 - 100.0
	5MIN	5	101.2	98.9 - 102.5
	30MIN	5	100.8	95.7 - 104.0
	1H	5	97.1	87.4 - 102.4
	4H	4	96.6	84.7 - 101.4
	6H	4	96.5	85.2 - 100.8
	24H	4	101.2	94.1 - 105.1
6	0	5	100.0	100.0 - 100.0
	5MIN	4	100.2	99.7 - 100.6
	30MIN	4	99.7	98.5 - 101.7
	1H	5	100.2	97.9 - 101.7
	4H	4	96.5	95.4 - 97.9
	6H	4	96.4	94.4 - 98.0
	24H	4	91.3	87.5 - 100.5
34	0	4	99.2	96.7 - 100.0
	5MIN	*	*	*
	30MIN	4	98.4	94.0 - 100.4
	1H	*	*	*
	4H	*	*	*
	6H	4	98.0	95.4 - 100.4
	24H	4	97.1	95.0 - 100.0
68	0	5	100.0	100.0 - 100.0
	5MIN	5	96.5	89.5 - 99.0
	30MIN	5	94.9	92.7 - 96.8
	1H	5	94.0	93.2 - 94.6
	4H	5	94.0	90.5 - 97.0
	6H	5	92.1	89.6 - 96.5
	24H	5	91.3	89.3 - 93.1
102	0	5	99.9	99.5 - 100.0
	5MIN	5	81.4	75.8 - 85.7
	30MIN	5	82.4	77.6 - 86.5
	1H	5	83.7	80.3 - 87.9
	4H	4	86.2	77.9 - 94.3
	6H	5	84.7	78.4 - 92.2
	24H	5	85.7	79.8 - 93.6

Table Aii.1 The percentage change in the protoplast area of Cladophora rupestris cells incubated at different salinities over a 24h period. * :- measurements not made.

SALINITY (‰)	TIME	NUMBER OF REPLICATES	MEAN % TOTAL-CELL AREA	RANGE OF % VALUES
1.5	0	5	100.0	100.0 - 100.0
	5MIN	5	101.2	98.9 - 102.5
	30MIN	5	101.6	95.7 - 108.2
	1H	5	97.1	87.4 - 102.4
	2H	4	96.6	84.7 - 101.6
	6H	4	97.1	85.2 - 100.8
	24H	4	101.8	94.1 - 105.6
6	0	5	100.0	100.0 - 100.0
	5MIN	4	100.2	99.7 - 100.6
	30MIN	5	99.7	98.5 - 101.7
	1H	5	100.3	97.9 - 101.7
	2H	4	97.0	95.4 - 97.9
	6H	4	97.0	94.4 - 98.0
	24H	4	91.8	87.5 - 100.5
34	0	4	100.0	100.0 - 100.0
	5MIN	*	*	*
	30MIN	4	98.4	94.0 - 100.4
	1H	*	*	*
	2H	*	*	*
	6H	4	98.3	95.4 - 100.4
	24H	4	97.1	95.0 - 100.0
68	0	5	100.0	100.0 - 100.0
	5MIN	5	98.8	97.4 - 101.2
	30MIN	5	96.1	93.9 - 97.1
	1H	5	94.7	94.0 - 96.2
	2H	5	97.3	95.6 - 99.7
	6H	5	97.3	92.2 - 101.5
	24H	5	96.7	92.3 - 101.2
102	0	5	100.0	100.0 - 100.0
	5MIN	5	98.0	95.8 - 100.2
	30MIN	5	97.0	92.4 - 100.1
	1H	5	95.9	92.9 - 98.9
	2H	4	95.6	89.7 - 99.4
	6H	5	96.9	94.3 - 100.9
	24H	5	98.1	92.9 - 106.6

Table Aii.2 The percentage change in the total-cell area of Cladophora rupestris cells incubated at different salinities over a 24h period. * : measurements not made.

SALINITY (‰)	TIME	NUMBER OF REPLICATES	MEAN % PROTOPLASM AREA	RANGE OF % VALUES
0	0	2	100.0	100.0 - 100.0
	5MIN	*	*	*
	30MIN	2	97.9	97.7 - 98.1
	1H	*	*	*
	2H	*	*	*
	6H	2	96.2	96.2 - 96.2
	24H	2	94.3	93.7 - 94.8
1.5	0	5	99.3	97.7 - 100.0
	5MIN	5	96.4	94.3 - 100.4
	30MIN	5	93.8	92.2 - 100.5
	1H	5	93.8	90.0 - 101.1
	2H	5	92.5	86.4 - 97.0
	6H	5	92.7	84.7 - 97.4
	24H	5	89.6	75.9 - 94.5
6	0	5	97.3	96.1 - 100.0
	5MIN	5	99.5	96.3 - 101.9
	30MIN	5	93.0	90.8 - 93.8
	1H	5	93.2	89.7 - 97.2
	2H	5	94.2	88.9 - 97.8
	6H	5	95.8	94.3 - 97.5
	24H	5	98.6	90.6 - 110.6
34	0	4	100.0	100.0 - 100.0
	5MIN	4	80.0	51.3 - 97.5
	30MIN	4	74.5	57.8 - 85.1
	1H	4	74.1	67.2 - 84.5
	2H	4	76.6	72.4 - 84.3
	6H	4	77.0	71.8 - 88.7
	24H	4	81.7	73.1 - 91.1

Table Aii.3 The percentage change in the proplast area of Cladophora glomerata cells incubated at different salinities over a 24h period. * : measurements not made.

SALINITY (‰)	TIME	NUMBER OF REPLICATES	MEAN % TOTAL-CELL AREA	RANGE OF % VALUES
0	0	2	100.0	100.0 - 100.0
	5MIN	*	*	*
	30MIN	2	98.9	98.8 - 99.0
	1H	*	*	*
	2H	*	*	*
	6H	2	96.2	96.2 - 96.2
	24H	2	94.3	93.7 - 94.8
1.5	0	5	100.0	100.0 - 100.0
	5MIN	5	97.1	94.3 - 100.4
	30MIN	5	96.6	94.1 - 100.5
	1H	5	95.8	93.7 - 101.1
	2H	5	94.5	92.0 - 97.0
	6H	5	94.0	89.1 - 97.4
	24H	5	93.2	91.4 - 94.5
6	0	5	100.0	100.0 - 100.0
	5MIN	5	101.4	96.3 - 107.8
	30MIN	5	97.0	93.5 - 100.2
	1H	5	97.4	95.1 - 98.9
	2H	5	96.6	94.7 - 98.8
	6H	5	97.1	94.3 - 99.3
	24H	5	99.9	94.2 - 110.6
34	0	4	100.0	100.0 - 100.0
	5MIN	4	97.5	95.8 - 99.9
	30MIN	4	95.5	92.6 - 97.9
	1H	4	95.5	92.9 - 98.1
	2H	4	95.6	90.9 - 100.1
	6H	4	93.8	90.9 - 96.1
	24H	4	92.0	89.7 - 93.9

Table Aii.4 The percentage change in the total-cell areas of Cladophora glomerata cells incubated at differnt salinities over a 24h period. * : measurements not made.

APPENDIX iii

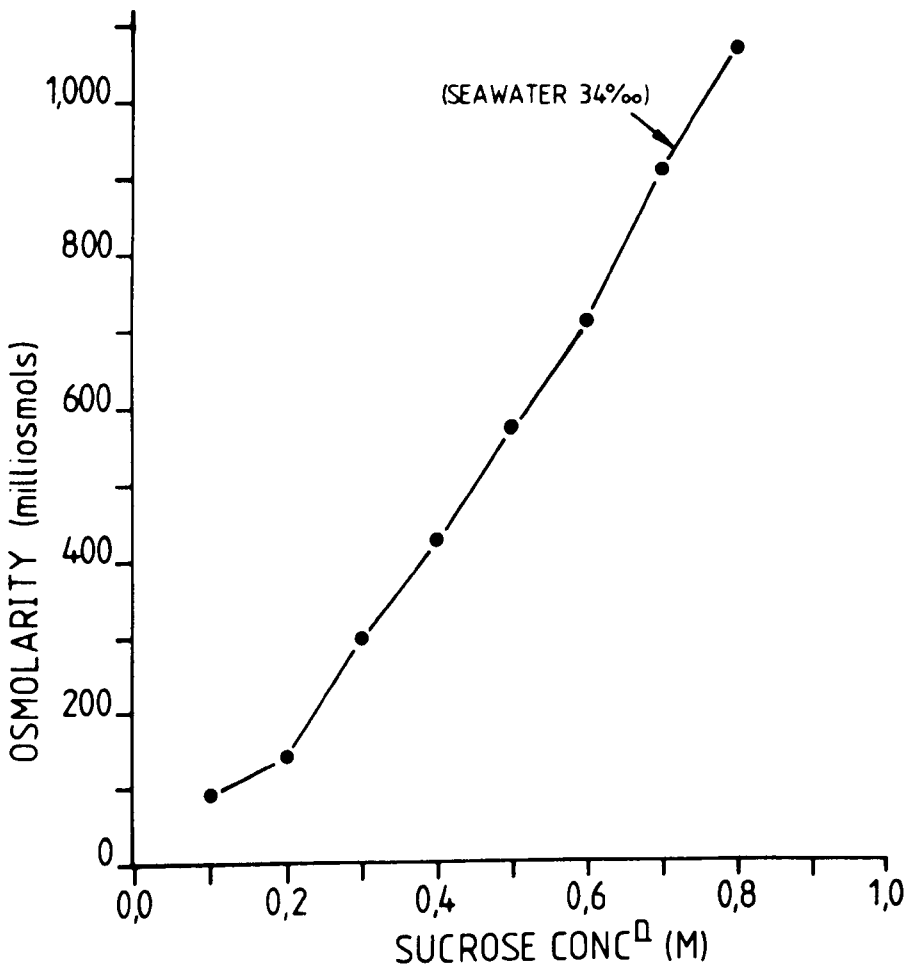

Fig.Aiii.1 The increase in osmolarity with increasing concentration of sucrose solutions (M). The osmolarity of normal seawater (34°/oo) was also measured and is indicated. These data were used to produce isotonic sucrose washes for plants prior to thallus-ion analysis, following salinity treatment.

SALINITY ‰	TIME H	NUMBER OF REPLICATES	MEAN Na^+ LEVELS	95% CONFIDENCE LIMITS
1.5	1	5	25.6	19.2 - 32.0
	2	5	33.6	20.4 - 46.9
	4	5	32.4	28.0 - 42.7
	6	5	28.8	24.8 - 32.8
	12	5	23.5	20.3 - 26.6
	24	5	30.3	19.4 - 41.2
6	1	5	43.8	38.7 - 48.9
	2	5	41.1	38.8 - 43.4
	4	5	38.7	35.3 - 42.1
	6	5	41.8	39.0 - 44.5
	12	5	47.2	35.0 - 59.4
	24	5	42.4	37.2 - 47.6
11	1	5	70.1	65.8 - 74.3
	2	5	66.4	61.4 - 71.4
	4	5	57.8	51.6 - 64.0
	6	5	59.4	53.3 - 65.5
	12	5	54.6	48.1 - 61.1
	24	5	58.0	52.2 - 63.9
34	1	5	83.4	74.7 - 92.1
	2	5	74.2	67.8 - 80.6
	4	5	71.5	64.2 - 78.3
	6	5	69.6	62.3 - 76.9
	12	5	64.4	56.4 - 72.4
	24	5	64.7	56.1 - 73.3
68	1	5	79.3	72.0 - 86.7
	2	5	73.5	53.1 - 93.9
	4	5	77.6	59.4 - 95.9
	6	5	89.0	70.5 - 107.5
	12	5	76.8	70.3 - 83.4
	24	5	78.1	70.7 - 85.5
102	1	5	106.7	95.4 - 118.1
	2	5	113.4	91.5 - 135.2
	4	4	108.6	79.3 - 137.9
	6	5	137.9	114.8 - 161.0
	12	5	98.0	87.5 - 108.6
	24	5	99.9	93.7 - 106.2

Table Aiii.1 The thallus content of Na^+ (mmol kg^{-1}) of Cladophora rupestris incubated for periods up to 24h at differnt salinities.

SALINITY ‰	TIME (H)	NUMBER OF REPLICATES	MEAN K^+ LEVELS	95% CONFIDENCE LIMITS
1.5	1	5	198.0	169.3 - 226.6
	2	5	206.7	193.7 - 219.8
	4	5	214.3	189.8 - 238.8
	6	5	185.5	168.1 - 203.5
	12	5	206.4	175.9 - 236.8
	24	5	205.9	183.1 - 228.6
6	1	5	158.2	138.8 - 177.6
	2	5	151.6	140.4 - 162.9
	4	5	137.4	121.8 - 153.0
	6	5	151.6	145.2 - 158.0
	12	5	195.9	187.6 - 204.2
	24	5	202.1	185.0 - 219.3
11	1	5	194.7	180.7 - 208.6
	2	5	202.2	182.9 - 221.6
	4	5	186.2	174.1 - 198.2
	6	5	192.4	179.5 - 205.2
	12	5	185.8	162.2 - 209.4
	24	5	194.2	173.6 - 214.8
34	1	5	199.7	178.4 - 220.9
	2	5	197.8	175.4 - 220.2
	4	5	196.5	186.5 - 206.5
	6	5	194.3	186.3 - 202.2
	12	5	216.4	199.5 - 233.3
	24	5	206.1	193.5 - 218.7
68	1	5	258.8	221.8 - 295.8
	2	5	262.9	219.2 - 306.7
	4	5	273.0	239.4 - 306.7
	6	5	299.6	278.6 - 320.5
	12	5	292.3	259.5 - 325.0
	24	5	298.4	267.7 - 329.1
102	1	5	161.5	145.7 - 177.3
	2	5	173.0	157.6 - 188.4
	4	4	170.7	155.4 - 186.0
	6	5	191.6	171.1 - 212.1
	12	5	306.6	285.3 - 327.9
	24	5	320.3	275.3 - 365.3

Table Aiii.2 The thallus content of K^+ (mmol kg^{-1}) of Cladophora rupestris incubated for periods up to 24h at different salinities.

SALINITY (‰)	TIME (H)	NUMBER OF REPLICATES	MEAN Cl^- LEVELS	95% CONFIDENCE LIMITS
1.5	1	5	177.5	112.8 - 242.3
	2	4	192.7	145.2 - 240.1
	4	5	169.3	109.6 - 228.9
	6	5	153.4	123.4 - 183.4
	12	5	188.0	134.3 - 241.8
	24	5	180.2	170.1 - 190.3
6	1	5	211.6	190.4 - 232.8
	2	5	191.6	158.9 - 224.3
	4	5	168.1	125.4 - 210.8
	6	5	156.4	102.0 - 210.7
	12	5	178.0	137.9 - 218.2
	24	5	160.0	127.0 - 193.1
11	1	5	220.5	201.1 - 240.0
	2	5	187.4	165.8 - 208.9
	4	5	200.6	175.9 - 225.4
	6	5	174.2	149.6 - 198.9
	12	5	210.3	204.7 - 215.8
	24	4	190.2	142.9 - 237.5
34	1	5	197.2	146.0 - 248.4
	2	5	216.7	200.4 - 233.0
	4	5	197.6	178.0 - 215.2
	6	5	200.9	170.7 - 231.1
	12	5	223.4	197.5 - 249.3
	24	5	219.7	194.8 - 244.5
68	1	3	321.2	172.5 - 469.8
	2	4	234.2	126.6 - 341.8
	4	5	308.3	281.8 - 334.9
	6	5	293.3	265.7 - 320.8
	12	5	315.1	268.4 - 361.8
	24	5	352.2	307.2 - 397.3
102	1	5	229.3	213.5 - 245.1
	2	5	256.0	227.2 - 284.8
	4	5	255.4	234.5 - 276.4
	6	5	327.0	282.0 - 371.9
	12	4	374.6	339.1 - 410.1
	24	5	429.8	405.1 - 454.5

Table Aiii.3 The thallus content of Cl^- (mmol kg^{-1}) of Cladophora rupestris incubated for periods up to 24h at different salinities.

SALINITY (‰)	TIME (H)	NUMBER OF REPLICATES	MEAN Mg^{2+} LEVELS	95% CONFIDENCE LIMITS
1.5	1	5	77.4	65.9 - 88.9
	2	5	80.5	72.2 - 88.8
	4	5	79.3	76.0 - 82.7
	6	5	84.1	72.8 - 95.5
	12	5	71.0	64.7 - 77.3
	24	5	74.6	65.3 - 84.0
6	1	5	79.9	72.6 - 87.2
	2	5	74.1	66.5 - 81.7
	4	5	71.5	65.5 - 77.6
	6	5	75.8	68.5 - 83.2
	12	5	69.6	63.7 - 75.5
	24	5	71.1	64.8 - 77.3
11	1	5	70.0	62.4 - 77.6
	2	5	64.9	56.9 - 72.8
	4	5	69.4	64.7 - 74.2
	6	5	69.4	60.3 - 78.6
	12	5	72.5	60.1 - 85.0
	24	5	73.0	68.2 - 77.8
34	1	5	64.2	62.8 - 65.6
	2	5	66.4	57.6 - 75.2
	4	5	73.1	67.5 - 78.8
	6	5	70.4	66.2 - 74.7
	12	5	68.4	61.1 - 75.8
	24	5	72.0	70.8 - 73.2
68	1	5	70.7	56.9 - 84.6
	2	5	56.4	51.5 - 61.4
	4	5	63.4	59.7 - 67.0
	6	5	63.5	56.8 - 70.3
	12	5	74.7	64.5 - 84.9
	24	5	73.3	67.4 - 79.3
102	1	5	80.5	60.1 - 101.0
	2	5	92.6	79.3 - 105.9
	4	4	82.5	71.6 - 93.4
	6	5	91.3	83.5 - 99.2
	12	5	73.2	68.3 - 78.2
	24	5	71.7	67.8 - 75.7

Table Aiii.4 The thallus content of Mg^{2+} (mmol kg^{-1}) of Cladophora rupestris incubated for periods up to 24h at different salinities

SALINITY (‰)	TIME (H)	NUMBER OF REPLICATES	MEAN Ca^{2+} LEVELS	95% CONFIDENCE LIMITS
1.5	1	5	15.4	13.9 - 16.9
	2	5	15.2	12.1 - 18.3
	4	5	14.5	11.2 - 17.8
	6	5	15.6	11.6 - 19.6
	12	5	20.3	15.3 - 25.4
	24	5	16.1	14.1 - 18.1
6	1	5	8.9	6.2 - 11.7
	2	5	8.2	7.4 - 8.9
	4	5	8.2	5.3 - 11.2
	6	5	8.7	6.6 - 10.8
	12	5	16.4	12.8 - 20.0
	24	5	13.3	11.8 - 14.8
11	1	5	4.1	3.6 - 4.7
	2	5	4.2	3.6 - 4.8
	4	5	4.4	3.7 - 5.1
	6	5	4.3	3.6 - 5.0
	12	5	4.4	4.2 - 4.5
	24	5	4.0	3.5 - 4.5
34	1	5	4.3	3.6 - 4.9
	2	5	3.7	3.4 - 4.0
	4	5	4.1	3.5 - 4.7
	6	5	4.1	3.5 - 4.7
	12	5	4.5	2.8 - 6.2
	24	5	4.1	3.2 - 5.0
68	1	5	14.8	12.7 - 16.9
	2	5	13.9	11.1 - 16.8
	4	5	15.7	14.3 - 17.1
	6	5	19.1	13.8 - 24.4
	12	5	5.8	4.6 - 7.0
	24	5	6.4	5.8 - 7.0
102	1	5	6.8	4.8 - 8.7
	2	5	7.2	5.1 - 9.3
	4	4	6.9	5.4 - 8.4
	6	5	6.8	5.5 - 8.1
	12	5	6.2	5.8 - 6.7
	24	5	7.2	3.8 - 10.5

Table Aiii.5 The thallus content of Ca^{2+} (mmol kg^{-1}) of Cladophora rupestris incubated for periods up to 24h at different salinities.

SALINITY (‰)	TIME (H)	NUMBER OF REPLICATES	MEAN K^+/Na^+ RATIOS	95% CONFIDENCE LIMITS
1.5	1	5	7.95	5.93 - 9.96
	2	5	6.52	4.66 - 8.38
	4	5	6.24	4.39 - 8.10
	6	5	6.54	5.10 - 7.99
	12	5	8.80	8.17 - 9.42
	24	5	7.30	4.52 - 10.10
6	1	5	3.61	3.37 - 3.86
	2	5	3.70	3.30 - 4.11
	4	5	3.57	3.02 - 4.11
	6	5	3.63	3.41 - 3.82
	12	5	4.30	3.16 - 5.44
	24	5	4.79	4.32 - 5.30
11	1	5	2.78	2.57 - 2.99
	2	5	3.05	2.68 - 3.43
	4	5	3.23	3.01 - 3.45
	6	5	3.25	3.03 - 3.46
	12	5	3.45	2.69 - 4.20
	24	5	3.35	3.13 - 3.60
34	1	5	2.42	1.98 - 2.85
	2	5	2.67	2.33 - 2.99
	4	5	2.77	2.43 - 3.12
	6	5	2.81	2.51 - 3.10
	12	5	3.38	3.02 - 3.73
	24	5	3.20	2.93 - 3.50
68	1	5	3.25	3.01 - 3.50
	2	5	3.98	3.07 - 4.89
	4	5	3.60	2.84 - 4.36
	6	5	3.43	2.75 - 4.12
	12	5	3.82	3.21 - 4.34
	24	5	3.83	3.40 - 4.30
102	1	5	1.52	1.30 - 1.75
	2	5	1.55	1.27 - 1.83
	4	4	1.61	1.16 - 2.06
	6	5	1.42	1.09 - 1.74
	12	5	3.13	2.91 - 3.35
	24	5	3.21	2.76 - 3.70

Table Aiii.6 The K^+/Na^+ ratio of Cladophora rupestris incubated for up to 24h at different salinities.

SALINITY (‰)	TIME (H)	NUMBER OF REPLICATES	MEAN Na^+ LEVELS	95% CONFIDENCE LIMITS
0	1	5	4.0	3.3 - 4.6
	2	5	4.4	4.1 - 4.7
	4	5	4.8	4.1 - 5.4
	6	5	4.4	4.2 - 4.6
	12	5	2.5	1.7 - 3.3
	24	5	2.3	1.5 - 3.1
1.5	1	5	11.5	9.3 - 13.8
	2	5	12.2	9.5 - 14.9
	4	5	12.5	9.7 - 15.2
	6	5	14.7	11.7 - 17.8
	12	5	8.7	8.2 - 9.2
	24	5	8.2	7.5 - 8.9
6	1	5	14.2	7.2 - 21.2
	2	5	17.5	16.7 - 18.3
	4	5	19.4	14.4 - 24.3
	6	5	17.7	17.0 - 18.5
	12	5	17.0	16.2 - 17.8
	24	5	15.5	13.4 - 17.6
11	1	5	24.1	22.1 - 26.1
	2	5	25.7	23.2 - 28.2
	4	5	22.1	20.7 - 23.6
	6	5	24.1	22.1 - 26.1
	12	5	23.0	18.6 - 27.5
	24	5	19.3	18.1 - 20.5
34	1	5	32.8	30.9 - 34.7
	2	5	36.2	35.0 - 37.5
	4	5	38.2	34.2 - 42.2
	6	5	35.6	33.1 - 38.2
	12	5	32.3	30.0 - 34.6
	24	5	31.6	28.5 - 34.7

Table Aiii.7 The thallus content of Na^+ (mmol kg^{-1}) of Cladophora glomerata incubated for periods up to 24h at different salinities.

SALINITY (‰)	TIME (H)	NUMBER OF REPLICATES	MEAN K^+ LEVELS	95% CONFIDENCE LIMITS
0	1	5	76.3	61.6 - 90.9
	2	5	87.5	83.6 - 91.4
	4	5	85.7	77.7 - 93.8
	6	5	89.4	83.3 - 95.4
	12	5	88.4	81.2 - 95.6
	24	5	88.3	84.3 - 92.3
1.5	1	5	73.1	67.1 - 79.2
	2	5	71.5	64.2 - 78.8
	4	5	73.6	71.7 - 75.6
	6	5	74.5	68.2 - 80.8
	12	5	78.9	75.9 - 81.9
	24	5	83.8	80.7 - 87.0
6	1	5	74.7	66.8 - 82.7
	2	5	75.2	72.6 - 77.8
	4	5	71.2	66.6 - 75.8
	6	5	76.6	70.3 - 82.9
	12	5	75.5	73.3 - 77.7
	24	5	82.0	71.0 - 93.1
11	1	4	80.5	73.0 - 87.9
	2	5	82.9	76.0 - 89.9
	4	5	82.0	72.5 - 91.6
	6	5	89.0	81.7 - 96.2
	12	5	76.9	73.6 - 80.2
	24	5	79.6	72.1 - 87.1
34	1	5	50.0	46.3 - 53.7
	2	5	48.2	46.4 - 50.0
	4	5	46.6	43.8 - 49.4
	6	5	43.9	40.2 - 47.5
	12	5	42.9	39.7 - 46.1
	24	5	42.5	39.8 - 45.2

Table Aiii.8 The thallus content of K_+ (mmol kg^{-1}) of Cladophora glomerata incubated for periods up to 24h at different salinities.

SALINITY (‰)	TIME (H)	NUMBER OF REPLICATES	MEAN Cl^- LEVELS	95% CONFIDENCE LIMITS
0	1	5	39.6	33.2 - 46.0
	2	5	41.6	33.3 - 49.8
	4	5	44.4	40.9 - 47.9
	6	5	46.4	37.3 - 55.5
	12	5	44.4	39.4 - 49.3
	24	5	45.2	40.3 - 50.1
1.5	1	5	25.7	22.5 - 28.9
	2	5	21.9	14.4 - 29.5
	4	5	24.4	18.5 - 30.4
	6	5	27.1	25.0 - 29.1
	12	5	30.3	27.7 - 32.9
	24	5	30.7	24.0 - 37.5
6	1	5	27.5	25.0 - 30.0
	2	5	22.0	15.8 - 28.2
	4	5	29.2	23.0 - 35.4
	6	5	29.5	22.0 - 37.1
	12	5	31.4	26.0 - 36.7
	24	5	35.8	32.3 - 39.3
11	1	4	26.4	24.0 - 28.7
	2	5	23.0	19.4 - 26.7
	4	5	26.1	20.2 - 32.0
	6	5	25.0	23.4 - 26.6
	12	5	28.4	22.1 - 34.7
	24	5	26.7	18.7 - 34.7
34	1	5	32.2	26.0 - 38.4
	2	5	37.5	33.5 - 41.6
	4	5	29.0	19.9 - 38.1
	6	5	41.6	31.4 - 51.9
	12	5	34.4	13.6 - 55.3
	24	5	31.6	27.9 - 35.4

Table Aiii.9 The thallus content of Cl^- (mmol kg^{-1}) of Cladophora glomerata incubated for periods up to 24h at different salinities.

SALINITY (‰)	TIME (H)	NUMBER OF REPLICATES	MEAN Mg^{2+} LEVELS	95% CONFIDENCE LIMITS
0	1	5	17.1	15.3 - 19.0
	2	5	20.5	18.4 - 22.6
	4	5	20.6	17.5 - 23.6
	6	5	19.9	18.1 - 21.6
	12	5	19.9	17.9 - 21.8
	24	5	23.5	21.8 - 25.1
1.5	1	5	31.3	29.8 - 32.8
	2	5	30.5	29.1 - 31.8
	4	5	29.9	28.0 - 31.8
	6	5	28.2	27.1 - 29.3
	12	5	32.2	29.6 - 34.8
	24	5	32.6	30.2 - 35.0
6	1	5	30.9	28.5 - 33.3
	2	5	29.2	28.0 - 30.5
	4	5	30.7	27.5 - 33.9
	6	5	28.4	26.9 - 30.0
	12	5	30.5	28.4 - 32.5
	24	5	33.5	29.7 - 37.3
11	1	5	36.4	30.6 - 42.1
	2	5	37.7	24.5 - 50.9
	4	5	35.5	29.1 - 42.0
	6	5	35.8	30.9 - 40.7
	12	5	31.7	29.6 - 33.8
	24	5	30.7	28.3 - 33.2
34	1	5	38.8	37.9 - 39.6
	2	5	38.3	35.6 - 40.9
	4	5	38.0	37.2 - 38.9
	6	5	38.6	36.9 - 40.4
	12	5	35.4	33.1 - 37.7
	24	5	33.6	31.7 - 35.4

Table Aiii.10 The thallus content of Mg^{2+} (mmol kg^{-1}) of Cladophora glomerata incubated for periods up to 24h at different salinities.

SALINITY (‰)	TIME (H)	NUMBER OF REPLICATES	MEAN Ca^{2+} LEVELS	95% CONFIDENCE LIMITS
0	1	5	67.6	45.1 - 90.2
	2	5	65.3	49.6 - 81.0
	4	5	82.2	55.0 - 109.5
	6	5	98.5	75.8 - 121.2
	12	5	59.9	47.0 - 72.9
	24	5	75.3	56.2 - 94.4
1.5	1	5	99.0	51.6 - 146.4
	2	5	105.1	57.8 - 152.5
	4	5	112.1	61.1 - 163.1
	6	5	72.6	60.1 - 85.1
	12	5	52.3	22.7 - 81.8
	24	5	44.1	24.6 - 63.5
6	1	5	73.6	57.8 - 89.5
	2	5	58.1	39.9 - 48.3
	4	5	49.5	28.5 - 70.5
	6	5	56.4	32.0 - 80.8
	12	5	51.1	10.3 - 91.8
	24	5	47.3	14.4 - 80.3
11	1	5	62.9	30.1 - 95.8
	2	5	53.8	14.6 - 93.1
	4	5	63.2	38.1 - 88.2
	6	5	68.4	46.1 - 90.8
	12	5	55.6	21.8 - 89.4
	24	5	42.5	20.3 - 64.7
34	1	5	78.9	46.2 - 111.6
	2	5	106.0	66.8 - 145.2
	4	4	90.5	55.7 - 125.3
	6	4	102.9	15.7 - 190.2
	12	5	70.0	54.6 - 85.2
	24	5	51.7	29.3 - 74.2

Table Aiii.11 The thallus content of Ca^{2+} (mmol kg^{-1}) of Cladophora glomerata incubated for periods up to 24h at different salinities.

SALINITY (‰)	TIME (H)	NUMBER OF REPLICATES	MEAN K^+/Na^+ RATIOS	95% CONFIDENCE LIMITS
0	1	5	19.18	16.59 - 21.78
	2	5	19.80	18.15 - 21.45
	4	5	18.13	15.75 - 20.52
	6	5	20.32	18.82 - 21.84
	12	4	31.80	29.09 - 34.50
	24	4	35.88	26.13 - 45.63
1.5	1	5	6.47	5.01 - 7.93
	2	5	6.01	4.58 - 7.45
	4	5	6.05	4.81 - 7.29
	6	5	5.16	4.07 - 6.26
	12	5	9.01	8.69 - 9.51
	24	5	10.22	9.04 - 11.40
6	1	5	7.17	-0.35 - 14.70
	2	5	4.31	4.08 - 4.54
	4	5	3.79	2.94 - 4.63
	6	5	4.32	3.99 - 4.66
	12	5	4.45	4.28 - 4.61
	24	5	5.30	4.88 - 5.71
11	1	4	3.36	2.70 - 4.03
	2	5	3.24	2.84 - 3.65
	4	5	3.71	3.25 - 4.17
	6	5	3.70	3.49 - 3.91
	12	5	3.34	2.78 - 4.01
	24	5	4.13	3.80 - 4.47
34	1	5	1.53	1.35 - 1.71
	2	5	1.33	1.25 - 1.41
	4	5	1.22	1.08 - 1.36
	6	5	1.23	1.10 - 1.37
	12	5	1.32	1.28 - 1.38
	24	5	1.33	1.28 - 1.43

Table Aiii.12 The K^+/Na^+ ratio for Cladophora glomerata incubated for differnt periods up to 24h at different salinities.

APPENDIX iv

TEMPERATURE (°C)	SALINITY (‰)	MEAN NET PHOTOSYNTHESIS	STANDARD ERROR (+/-)
-9	0	25.3	2.0
	6	10.7	3.3
	11	24.2	1.9
	34	32.4	0.7
	68	31.8	1.8
	102	34.1	4.6
4	0	45.2	2.3
	6	50.2	7.0
	11	61.1	10.2
	34	46.8	3.7
	68	47.8	10.7
	102	30.7	5.3
10	0	15.4	9.3
	6	86.4	17.5
	11	78.6	10.1
	34	89.3	10.5
	68	44.4	19.6
	102	48.7	4.0
15	0	42.2	2.4
	6	67.6	3.0
	11	56.0	7.8
	34	55.7	4.9
	68	47.8	3.1
	102	31.1	1.5
20	0	47.4	9.3
	6	75.6	12.2
	11	72.2	15.0
	34	65.0	3.7
	68	57.4	7.2
	102	37.3	8.9
25	0	4.9	2.4
	6	40.1	5.4
	11	44.1	3.6
	34	43.8	1.4
	68	42.9	1.8
	102	25.2	2.8
30	0	1.3	1.3
	6	1.1	0.7
	11	0.0	0.0
	34	27.0	7.8
	68	19.0	2.4
	102	6.4	1.4

Table Aiv.1 The net photosynthetic rates ($mgO_2h^{-1}g^{-1}$) of Cladophora rupestris following incubation in salinity treatments at different temperatures.

TEMPERATURE (°C)	SALINITY (‰)	MEAN NET PHOTOSYNTHESIS	STANDARD ERROR (+/-)
-9	0	5.4	1.59
	1.5	2.0	1.00
	6	2.2	0.05
	11	0.7	0.70
	34	2.1	1.31
4	0	77.6	18.80
	1.5	64.4	9.21
	6	76.8	19.90
	11	44.8	9.19
	34	26.7	3.32
10	0	105.7	3.0
	1.5	62.2	6.1
	6	41.0	5.3
	11	35.0	5.0
	34	9.9	3.1
15	0	76.7	9.0
	1.5	87.2	9.3
	6	71.4	15.1
	11	63.9	6.2
	34	30.8	5.6
20	0	89.8	10.5
	1.5	80.9	5.3
	6	61.1	5.8
	11	57.3	1.8
	34	21.8	2.8
25	0	81.6	1.0
	1.5	87.8	0.8
	6	75.4	6.6
	11	76.4	6.8
	34	22.5	6.5
30	0	81.1	5.5
	1.5	86.9	9.7
	6	74.1	13.2
	11	74.3	4.7
	34	38.5	6.5

Table Aiv.2 Net photosynthetic rates ($mgO_2h^{-1}g^{-1}$) of Cladophora glomerata following incubation in salinity treatments at different temperatures.

APPENDIX v

(A)

CELL LENGTH µM	FREQUENCY U.K.	BALTIC
0 - 49	0	0
50 - 99	1	1
100 - 149	1	1
150 - 199	6	18
200 - 249	14	13
250 - 299	12	14
300 - 349	11	0
350 - 399	3	1
400 - 449	1	0
450 - 499	0	0
500 - 549	0	0
550 - 599	0	0
600 - 649	1	0

(B)

CELL WIDTH µM	FREQUENCY U.K.	BALTIC
20 - 24	0	0
25 - 29	0	0
30 - 34	0	0
35 - 39	0	8
40 - 44	0	0
45 - 49	9	24
50 - 54	0	0
55 - 59	17	18
60 - 64	0	0
65 - 69	0	0
70 - 74	14	0
75 - 79	0	0
80 - 84	10	0

Table v.1 The size class frequency of cell length (A) and width (B) of U.K. and Baltic populations of Cladophora rupestris.

(A)

CELL LENGTH µM	FREQUENCY U.K.	BALTIC
0 - 49	0	0
50 - 99	1	0
100 - 149	7	6
150 - 199	12	8
200 - 249	19	12
250 - 299	9	14
300 - 349	2	3
350 - 399	0	1
400 - 449	0	3
450 - 499	0	1
500 - 549	0	0
550 - 599	0	1
600 - 649	0	0
650 - 699	0	0
700 - 749	0	0
750 - 799	0	0
800 - 849	0	1

(B)

CELL WIDTH µM	FREQUENCY U.K.	BALTIC
20 - 24	0	0
25 - 29	0	0
30 - 34	0	0
35 - 39	2	13
40 - 44	0	0
45 - 49	13	15
50 - 54	0	0
55 - 59	24	8
60 - 64	0	0
65 - 69	0	0
70 - 74	11	7
75 - 79	0	0
80 - 84	0	4

Table A v.2 The size class frequency of cell length (A) and width (B) of U.K. and Baltic populations of Cladophora glomerata.

CELL VOLUME (μm^3)	FREQUENCY	
	U.K	BALTIC
0 - 499999	11	34
500000 - 999999	20	16
1000000 - 1499999	13	0
1500000 - 1999999	4	0
2000000 - 2499999	1	0
2500000 - 2999999	0	0
3000000 - 3499999	1	0

Table A v.3 The size class frequency of cell volume estimates of U.K. and Baltic populations of Cladophora rupestris.

CELL VOLUME (μm^3)	FREQUENCY	
	U.K.	BALTIC
0 - 499999	20	29
500000 - 999999	27	10
1000000 - 1499999	3	4
1500000 - 1999999	0	1
2000000 - 2499999	0	5
2500000 - 2999999	0	0
3000000 - 3499999	0	2

Table A v.4 The size class frequency of cell volume estimates of U.K. and Baltic populations of Cladophora glomerata.

SALINITY (‰)	MEAN NET PHOTOSYNTHESIS	STANDARD ERROR (+/-)
0	45.0	7.34
6	44.8	4.52
11	47.5	2.08
34	40.4	6.03
68	21.9	0.66
102	21.4	2.40

Table A v.5 The net photosynthetic rates ($mgO_2h^{-1}g^{-1}$) of Baltic *Cladophora rupestris* following incubation in salinity treatments at 10°C.

SALINITY (‰)	MEAN NET PHOTOSYNTHESIS	STANDARD ERROR (+/-)
0	151.6	13.00
1.5	158.4	10.40
6	190.5	20.60
11	194.0	14.30
34	86.4	10.00

Table A v.6 The net photosynthetic rates ($mgO_2h^{-1}g^{-1}$) of Baltic *Cladophora glomerata* following incubation in salinity treatments at 10°C.

SALINITY (‰)	TIME	NUMBER OF REPLICATES	MEAN % PROTOPLASM AREA	RANGE OF % VALUES
1.5	0	5	100.0	100.0 - 100.0
	30MIN	5	96.8	84.3 - 103.0
	1H	5	95.8	82.5 - 101.8
	6H	4	98.9	96.2 - 101.0
	24H	4	98.4	97.1 - 100.1
6	0	5	100.0	100.0 - 100.0
	30MIN	5	98.5	95.7 - 102.8
	1H	*	*	*
	6H	5	97.4	94.4 - 102.5
	24H	5	97.4	93.4 - 101.1
34	0	5	100.0	100.0 - 100.0
	30MIN	5	98.6	98.0 - 99.1
	1H	5	97.9	96.8 - 98.5
	6H	5	96.0	94.4 - 97.6
	24H	4	97.5	96.3 - 100.0
68	0	5	100.0	99.5 - 100.0
	30MIN	5	93.4	88.9 - 97.3
	1H	5	96.0	92.6 - 99.2
	6H	4	96.6	92.3 - 108.3
	24H	5	93.4	85.8 - 105.9
102	0	5	100.0	100.0 - 100.0
	30MIN	5	82.8	71.2 - 87.8
	1H	5	83.1	71.3 - 87.8
	6H	5	84.1	80.7 - 87.2
	24H	4	86.8	80.2 - 91.1

Table A v.7 The percentage change in the cell protoplast area of Baltic Cladophora rupestris incubated at different salinities over a 24h period. * : measurements not made.

SALINITY (‰)	TIME	NUMBER OF REPLICATES	MEAN % TOTAL-CELL AREA	RANGE OF % VALUES
1.5	0	5	100.0	100.0 - 100.0
	30MIN	5	96.8	84.3 - 103.0
	1H	5	95.8	82.5 - 101.8
	6H	4	98.9	96.2 - 101.0
	24H	4	98.4	97.1 - 100.1
6	0	5	100.0	100.0 - 100.0
	30MIN	5	98.5	95.7 - 102.8
	1H	*	*	*
	6H	5	97.4	94.4 - 102.5
	24H	5	97.7	93.4 - 101.3
34	0	5	100.0	100.0 - 100.0
	30MIN	5	98.6	98.0 - 99.1
	1H	5	97.9	96.9 - 98.8
	6H	5	96.0	94.4 - 99.6
	24H	5	94.2	96.3 - 100.0
68	0	5	100.0	100.0 - 100.0
	30MIN	5	95.5	92.7 - 99.0
	1H	5	96.8	95.1 - 99.7
	6H	4	96.6	91.3 - 108.3
	24H	5	96.2	89.9 - 106.6
102	0	5	100.0	100.0 - 100.0
	30MIN	5	90.4	78.8 - 94.4
	1H	5	90.7	80.5 - 95.2
	6H	5	88.8	85.1 - 93.4
	24H	4	92.8	84.9 - 100.3

Table A v.8 The percentage change in the total-cell area of Baltic Cladophora rupestris incubated at different salinities over a 24h period. * : measurements not made.

SALINITY (‰)	TIME	NUMBER OF REPLICATES	MEAN % PROTOPLASM AREA	RANGE OF % VALUES
0	0	5	100.0	100.0 - 100.0
	30MIN	5	100.5	98.2 - 102.9
	1H	5	98.6	93.1 - 107.3
	6H	5	95.9	93.7 - 97.2
	24H	4	93.9	87.6 - 97.7
1.5	0	4	100.0	100.0 - 100.0
	30MIN	4	98.9	95.0 - 101.7
	1H	4	99.1	95.2 - 102.2
	6H	4	97.2	94.5 - 101.3
	24H	4	97.9	97.2 - 102.0
6	0	5	100.0	100.0 - 100.0
	30MIN	5	99.2	97.7 - 100.8
	1H	*	*	*
	6H	4	99.1	96.6 - 102.4
	24H	5	98.4	94.2 - 106.1
34	0	5	100.0	100.0 - 100.0
	30MIN	4	73.8	65.8 - 83.6
	1H	5	72.5	67.4 - 83.2
	6H	5	76.3	68.8 - 85.7
	24H	5	78.1	70.6 - 88.7

Table A v.9 The percentage change in cell protoplast area of Baltic Cladophora glomerata incubated at different salinities over a 24h period. * : measurements not made.

SALINITY (‰)	TIME	NUMBER OF REPLICATES	MEAN % TOTAL-CELL AREA	RANGE OF % VALUES
0	0	5	100.0	100.0 - 100.0
	30MIN	5	100.5	98.2 - 102.9
	1H	5	98.6	93.1 - 107.3
	6H	5	95.9	93.7 - 97.2
	24H	4	93.9	87.6 - 97.7
1.5	0	4	100.0	100.0 - 100.0
	30MIN	4	100.0	98.2 - 101.7
	1H	4	100.0	97.8 - 102.2
	6H	4	97.7	94.8 - 101.3
	24H	4	97.9	94.4 - 102.0
6	0	5	100.0	100.0 - 100.0
	30MIN	5	99.2	97.7 - 100.8
	1H	*	*	*
	6H	4	99.1	96.6 - 102.4
	24H	5	98.4	94.2 - 106.1
34	0	5	100.0	100.0 - 100.0
	30MIN	4	98.6	97.9 - 103.9
	1H	5	99.0	96.0 - 102.5
	6H	5	99.5	95.0 - 103.5
	24H	5	100.7	94.4 - 111.7

Table A v.10 The percentage change in the total-cell area of Baltic Cladophora glomerata incubated at different salinities over a 24h period. * : measurements not made.

SALINITY (‰)	TIME (H)	NUMBER OF REPLICATES	MEAN Na^+ LEVELS	95% CONFIDENCE LIMITS
1.5	2	5	52.8	40.0 - 65.6
	6	5	76.2	69.3 - 83.2
	24	5	70.7	58.6 - 82.7
6	2	5	72.3	54.2 - 90.4
	6	5	63.1	56.9 - 69.4
	24	5	67.3	53.1 - 81.5
34	2	5	102.5	89.3 - 115.8
	6	5	135.1	91.8 - 172.5
	24	5	109.3	98.9 - 119.7
68	2	5	101.4	95.3 - 107.5
	6	5	114.4	102.1 - 126.7
	24	5	126.0	93.0 - 159.0
102	2	5	132.5	110.0 - 154.9
	6	5	152.6	126.4 - 178.9
	24	5	160.1	130.8 - 189.5

Table A v.11 The thallus content of Na^+ (mmol kg^{-1}) of Baltic Cladophora rupestris incubated for periods up to 24h at different salinities.

SALINITY (‰)	TIME (H)	NUMBER OF REPLICATES	MEAN K^+ LEVELS	95% CONFIDENCE LIMITS
1.5	2	5	155.7	138.9 - 172.5
	6	5	135.6	119.2 - 152.1
	24	5	127.3	109.2 - 145.5
6	2	5	131.3	120.0 - 142.6
	6	5	156.3	148.1 - 164.5
	24	5	153.8	145.9 - 161.8
34	2	5	143.6	128.2 - 159.0
	6	5	131.6	114.1 - 148.6
	24	5	166.5	151.8 - 181.2
68	2	5	152.1	129.4 - 174.8
	6	5	164.5	151.7 - 177.3
	24	5	234.7	217.0 - 252.5
102	2	5	105.3	100.4 - 110.2
	6	5	110.3	89.8 - 130.8
	24	5	145.8	113.1 - 178.4

Table A v.12 The thallus content of K^+ (mmol kg^{-1}) of Baltic Cladophora rupestris incubated for periods up to 24h at different salinities.

SALINITY (‰)	TIME (H)	NUMBER OF REPLICATES	MEAN Cl^- LEVELS	95% CONFIDENCE LIMITS
1.5	2	5	146.8	123.7 - 170.0
	6	5	127.6	115.8 - 139.3
	24	5	125.8	85.2 - 166.3
6	2	5	127.3	107.0 - 147.6
	6	5	119.0	110.7 - 127.3
	24	5	116.4	111.4 - 121.3
34	2	5	133.0	106.8 - 159.2
	6	5	147.2	126.0 - 168.4
	24	5	145.5	131.0 - 160.1
68	2	5	169.7	130.9 - 208.6
	6	5	193.1	173.6 - 212.5
	24	4	210.4	104.3 - 316.5
102	2	5	109.2	92.0 - 126.3
	6	5	137.7	77.6 - 197.9
	24	4	240.2	192.8 - 287.5

Table A v.13 The thallus content of Cl^- (mmol kg^{-1}) of Baltic Cladophora rupestris incubated for periods up to 24h at different salinities.

SALINITY (‰)	TIME (H)	NUMBER OF REPLICATES	MEAN Mg^{2+} LEVELS	95% CONFIDENCE LIMITS
1.5	2	5	78.3	73.0 - 83.6
	6	5	76.2	69.3 - 83.2
	24	5	70.7	58.6 - 82.7
6	2	5	69.6	67.1 - 72.0
	6	5	70.2	62.8 - 77.5
	24	5	73.3	66.7 - 80.0
34	2	5	55.1	51.1 - 59.0
	6	5	57.6	56.6 - 58.5
	24	5	60.3	55.0 - 65.6
68	2	5	60.8	56.7 - 64.8
	6	5	61.5	56.1 - 67.0
	24	5	65.5	59.0 - 72.0
102	2	5	65.6	57.7 - 73.5
	6	5	62.3	56.5 - 68.0
	24	5	67.7	48.8 - 86.5

Table A v.14 The thallus content of Mg^{2+} (mmol kg^{-1}) of Baltic Cladophora rupestris incubated for periods up to 24h at different salinities.

SALINITY (‰)	TIME (H)	NUMBER OF REPLICATES	MEAN Ca^{2+} LEVELS	95% CONFIDENCE LIMITS
1.5	2	5	67.6	9.8 - 125.4
	6	5	38.3	0.5 - 76.2
	24	5	25.2	3.3 - 47.2
6	2	5	32.8	9.5 - 56.2
	6	5	29.8	7.8 - 51.8
	24	5	57.1	-7.2 - 121.5
34	2	5	16.9	7.1 - 26.7
	6	5	24.9	9.9 - 39.8
	24	5	49.4	0.0 - 98.8
68	2	5	27.0	1.6 - 52.3
	6	5	25.6	11.3 - 39.9
	24	5	51.9	10.4 - 93.3
102	2	5	27.6	17.7 - 37.6
	6	5	15.8	4.0 - 27.5
	24	5	42.3	8.1 - 76.6

Table A v.15 The thallus content of Ca^{2+} (mmol kg^{-1}) of Baltic Cladophora rupestris incubated for periods up to 24h at different salinities.

SALINITY (‰)	TIME (H)	NUMBER OF REPLICATES	MEAN K^+/Na^+ RATIOS	95% CONFIDENCE LIMITS
1.5	2	5	3.06	2.10 - 4.02
	6	5	1.79	1.47 - 2.12
	24	5	1.82	1.49 - 2.16
6	2	5	1.89	1.35 - 2.43
	6	5	2.49	2.17 - 2.81
	24	5	2.33	1.89 - 2.77
34	2	5	1.41	1.32 - 1.49
	6	5	1.04	0.68 - 1.40
	24	5	1.53	1.28 - 1.79
68	2	5	1.51	1.22 - 1.79
	6	5	1.45	1.27 - 1.62
	24	5	1.92	1.50 - 2.33
102	2	5	0.81	0.66 - 0.96
	6	5	0.72	0.67 - 0.77
	24	5	0.92	0.73 - 1.11

Table A v.16 The K^+/Na^+ ratio of Baltic Cladophora rupestris incubated for periods up to 24h at different salinities.

SALINITY (‰)	TIME (H)	NUMBER OF REPLICATES	MEAN Na^+ LEVELS	95% CONFIDENCE LIMITS
0	2	4	21.8	12.2 - 31.4
	6	4	11.6	3.6 - 19.5
	24	5	26.8	14.9 - 38.7
1.5	2	4	8.2	4.5 - 11.9
	6	5	21.3	5.8 - 36.7
	24	5	26.2	14.3 - 38.1
6	2	5	22.7	13.7 - 31.7
	6	5	29.3	21.2 - 37.4
	24	5	28.9	19.9 - 37.9
34	2	5	45.6	25.3 - 65.9
	6	5	41.0	35.0 - 47.1
	24	4	48.7	34.8 - 62.5

Table A v.17 The thallus content of Na^+ (mmol kg^{-1}) of Baltic Cladophora glomerata incubated for periods up to 24h at different salinities.

SALINITY (‰)	TIME (H)	NUMBER OF REPLICATES	MEAN K^+ LEVELS	95% CONFIDENCE LIMITS
0	2	5	104.3	89.5 - 119.1
	6	5	105.0	98.5 - 111.6
	24	5	92.9	83.7 - 102.1
1.5	2	5	109.2	94.6 - 123.9
	6	5	118.8	104.5 - 133.1
	24	5	108.1	96.3 - 119.8
6	2	5	120.4	111.6 - 129.2
	6	5	143.6	120.1 - 167.0
	24	5	113.8	109.3 - 118.3
34	2	5	85.3	66.6 - 104.0
	6	5	93.1	69.6 - 116.7
	24	5	115.7	85.1 - 146.3

Table A v.18 The thallus content of K^+ (mmol kg^{-1}) of Baltic Cladophora glomerata incubated for periods up to 24h at different salinities.

SALINITY (‰)	TIME (H)	NUMBER OF REPLICATES	MEAN Cl^- LEVELS	95% CONFIDENCE LIMITS
0	2	5	72.9	67.3 - 78.6
	6	5	86.7	45.9 - 127.5
	24	5	67.0	63.3 - 70.8
1.5	2	5	73.0	29.6 - 116.4
	6	5	100.8	52.6 - 149.1
	24	5	70.0	57.6 - 82.4
6	2	5	73.1	56.2 - 90.1
	6	5	92.1	53.5 - 130.7
	24	5	67.6	43.7 - 91.5
34	2	5	50.5	30.5 - 70.6
	6	5	74.6	53.5 - 91.7
	24	5	95.6	68.1 - 123.2

Table A v.19 The thallus content of Cl^- (mmol kg^{-1}) of Baltic Cladophora glomerata incubated for periods up to 24h at different salinities.

SALINITY (‰)	TIME (H)	NUMBER OF REPLICATES	MEAN Ca^{2+} LEVELS	95% CONFIDENCE LIMITS
0	2	5	4.8	2.2 - 7.4
	6	5	3.3	2.9 - 3.7
	24	5	4.3	2.9 - 5.7
1.5	2	5	2.5	2.0 - 3.0
	6	5	2.0	1.8 - 2.3
	24	5	2.2	1.8 - 2.7
6	2	5	2.0	1.5 - 2.4
	6	5	2.3	2.2 - 2.4
	24	5	1.8	1.6 - 2.0
34	2	5	2.4	1.9 - 3.0
	6	5	2.6	1.8 - 3.5
	24	5	2.4	2.1 - 2.7

Table A v.20 The thallus content of Ca^{2+} (mmol kg^{-1}) of Baltic Cladophora glomerata incubated for periods up to 24h at different salinities.

SALINITY (‰)	TIME (H)	NUMBER OF REPLICATES	MEAN Mg^{2+} LEVELS	95% CONFIDENCE LIMITS
0	2	5	23.3	18.7 - 27.9
	6	5	23.6	20.4 - 26.8
	24	5	21.4	18.9 - 24.0
1.5	2	5	33.0	27.2 - 38.8
	6	5	28.3	24.3 - 32.3
	24	5	28.2	25.3 - 31.0
6	2	5	32.3	27.6 - 36.9
	6	5	33.6	29.1 - 38.2
	24	5	26.1	24.1 - 28.1
34	2	5	39.1	36.6 - 41.6
	6	5	40.9	39.2 - 42.7
	24	5	36.7	35.0 - 38.4

Table A v.21 The thallus content of Mg^{2+} (mmol kg^{-1}) of Baltic Cladophora glomerata incubated for periods up to 24h at different salinities.

SALINITY (‰)	TIME (H)	NUMBER OF REPLICATES	MEAN K^+/Na^+ RATIOS	95% CONFIDENCE LIMITS
0	2	4	5.23	2.16 - 8.29
	6	4	11.65	-1.59 - 24.90
	24	5	3.80	2.21 - 5.39
1.5	2	4	14.25	4.69 - 23.81
	6	5	9.15	-2.33 - 20.63
	24	5	4.45	2.96 - 5.95
6	2	5	5.82	3.28 - 8.36
	6	5	4.97	4.39 - 5.54
	24	5	4.14	2.85 - 5.42
34	2	5	1.94	1.57 - 2.31
	6	5	2.28	1.70 - 2.86
	24	4	2.63	1.53 - 3.73

Table A v.22 The K^+/Na^+ ratio of Baltic Cladophora glomerata incubated for periods up to 24h at different salinities.

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Interactive effects of temperature and salinity upon net photosynthesis of *Cladophora glomerata* (L.) Kütz. and *C. rupestris* (L.) Kütz.

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Abstract

Rates of net photosynthesis of Cladophora glomerata and C. rupestris have been measured following treatment for 48h in media of different salinities (0 to 102°/oo) and at different temperatures (-9 to 30°C). At extreme temperatures, C. rupestris had a reduced salinity-tolerance range. C. glomerata proved better able to tolerate increased salinity at higher temperatures. The measured tolerances to temperature and salinity of both species exceeded the extremes of these two variables recorded in their natural environment.

Introduction

Detailed investigations have been made of the independent effects of temperature and salinity upon the rates of net photosynthesis of many macro-algae. Often, such measurements are made as separate parts of the same study (Chock and Mathieson 1979, Gordon *et al.* 1980, Penniman and Mathieson 1985), but the interactive effects of the two external factors are not discussed. Other studies in which the influence of temperature upon the photosynthetic activity and growth of algae subject to saline stress have been measured, indicate that algae may have markedly different abilities to withstand changes in their external osmotic conditions when treated at different temperatures (Dawes *et al.* 1978, Fralick and Mathieson 1975, Kjeldson and Phinney 1972, Yarish and Edwards 1982, Zavodnik 1975). Dawes *et al.* (1976) and Lehnberg (1978) have also demonstrated the complex interactive effects of temperature, salinity and light intensity upon the photosynthetic activity of *Hypnea musciformis* (Wulfen) Lamour. and *Delessaria sanguinea* (Huds.) Lamour. respectively. All of these authors have demonstrated that external pressures such as salinity and temperature should not be treated as distinct and unrelated in their influence upon the growth and/or reproduction, and ultimately the distribution of a particular species or ecotype within a species.

The purpose of the present study was to measure the effects of temperature upon the reponses to saline stress of *Cladophora rupestris* and *C. glomerata*, the former being predominantly a marine species and the latter being mostly restricted to freshwater. Both species are also found in brackish water

conditions (Hoek 1963), and therefore, measurements of salt tolerance and the effects of temperature upon salinity responses are of interest. Net photosynthesis was used as an indirect measure of the extent of physiological damage following salinity treatment at various temperatures.

Materials and Methods

Cladophora rupestris was collected from an exposed site in the upper eulittoral zone on Hilbre Island, Cheshire, U.K., which is located at the mouth of the Dee estuary (Russell 1973). C. glomerata was obtained from a benthic population in the Leeds-Liverpool Canal at Maghull, Merseyside, U.K., which is a freshwater site. Plant material was returned to the laboratory and maintained at 10^{o}C in aerated filtered seawater (C.rupestris) and filtered canal water (C. glomerata), which was changed at least every 72h. The plants were kept under artificial light at a light intensity of approximately 50 μE m^{-2} s^{-1} (8h light : 16h dark). All experimental material was used within 5 days of collection.

The temperature and salinity of the surface seawater were recorded at the Hilbre site, for the period October 1985 - May 1987. The temperature and salinity of water retained among emergent Cladophora rupestris plants was also measured over this period. Temperature was measured using a mercury thermometer and salinity measured by means of a refractometer (Aquafauna. Bio-Marine inc.). Water was sampled from emergent Cladophora thalli with a 1.0cm^3 clean plastic syringe for salinity measurement. Access to Hilbre Island by foot is restricted to periods of mid-tide level and below, and all visits were made on falling tides and just before low water. The C. rupestris plants at this site

are exposed to terrestial climate conditions at approximately 2h after high water, and are emergent for periods of up to 8h during a tidal period. At the times of sampling therefore, the plants had been emergent for periods of approximately 4h. Seasonal variations in water temperature were also measured at the C. glomerata site. There is no significant seasonal variation in the salinity conditions at this freshwater site (Howard et al. 1984).

A range of salinity treatments (0 - 102°/oo) was prepared by dilution or evaporation of seawater (34°/oo) collected from the Menai Straits, Anglesey, N.Wales, U.K. Cladophora rupestris was treated with the following media : 0, 6, 11, 34, 68 and 102°/oo. C. glomerata was treated with : 0, 1.5, 6, 11 and 34°/oo. Experiments were performed at the following temperatures:-9±3°c, 4±1°c, 10±1°c, 15±1°c, 20±1°c, 25±1°c and 30±1°c.

Similar-sized clean portions of thallus were incubated in 150cm^3 of the treatment media at the experimental period for 48h. Due to the freezing of the media at -9°c, and the subsequent need for thawing out of plants before further stages of the experiment, this material was incubated in smaller volumes (15 - 20cm^3) contained in shallow Petri dishes. Three replicates of each treatment were used in a random block design. Following treatment in at the various experimental temperatures, all treatment flasks were allowed to stabilise at 10°c for approximately 2h before experimentation proceeded.

The plant material was then removed from the treatments and blotted carefully to remove any residual surface water. The plants were then placed into 125cm^3 recovery vessels filled with water natural to the locality in which the plants normally grow. Thus Cladophora rupestris recovered in seawater (34°/oo) and C.

glomerata in filtered canal water. These waters had first been boiled to remove oxygen, and then enriched with $NaHCO_3$ ($0.42gl^{-1}$) as a carbon source. These flasks were then stoppered without introducing any air and incubated at $10^{o}C$ under artificial light ($60\ \mu E\ m^{-2}\ s^{-1}$) for 2h in a random block design. Three flasks containing only de-oxygenated water were incorporated as controls. Flasks containing dead Cladophora plants would have been better controls, although in practice no measurable amounts of oxygen were introduced into the flasks containing salinity treatments that killed plant material. The experimental light intensities used were lower than the saturating light intensities of both species, however, the plants were collected from very light-limited sites ; C. rupestris from the roof of a large crevice on a south facing rock face, and C. glomerata from a submerged population (1.5m) in the canal.

Plants were removed from the flasks without introducing any air, and the oxygen that had been produced by the samples was was determined using a modified Winkler titration technique (Strickland and Parsons 1972). The mean oxygen concentration of the three control flasks was subtracted from the values obtained from the treatment flasks to give the net amount of oxygen produced by the plant tissue. The plant material was oven-dryed at $50^{o}C$ to constant weight and weighed so that the net photosynthetic rate could be expressed as: $mgO_2h^{-1}g^{-1}$(dry weight). This method is similar to that described by Russell 1987).

Results

The combined effects of temperature and salinity upon Cladophora glomerata and C. rupestris are shown in Figure 1. and Figure 2. respectively. The data were analysed by one-way ANOVA to test for a significant response to salinity at each temperature (Table I.). A two-way ANOVA showed the responses of both species to salinity and to temperature to be significant (Table II.). The interaction between salinity and temperature was also found to be significantly different in both species (Table II.).

After treatment at -9°C Cladophora glomerata produced no oxygen at any salinity except at 0°/oo in which extremely low levels were obtained (Fig.1.). At all other temperatures, treatment in 34°/oo salinity significantly reduced oxygen production. The most productive sector of Figure 3 is clearly that determined by salinities 0-11°/oo and temperatures 17-30°C. The levels of oxygen production under normal salinity conditions (0°/oo) showed no significant response to temperature increase, although the net photosynthesis of the plants in the 34°/oo treatments increased with temperature.

In the case of Cladophora rupestris, the most marked inhibition of net photosynthesis was obtained with a combination of low salinity (0°/oo) and high temperature (25-30°C). At the highest temperature, this species was also greatly damaged by the highest salinity (102°/oo). The lowest temperature (-9°C) proved to be more damaging when associated with low salinities (6 and 11°/oo). At all other combinations of salinity and temperature, the plants were capable of oxygen production, though this was usually significantlly reduced at the saline extremes (0 and

Table I. The level of significance of the responses to salinity treatment shown by Cladophora rupestris and C. glomerata at different experimental temperatures, following one-way ANOVA.
(N.S.= P> 0.05 ; *-- = P< 0.05 ; **- = P< 0.01 ; *** = P< 0.001.)

	Temperature °c						
	-9	4	10	15	20	25	30
Cladophora rupestris	***	N.S.	**-	**-	N.S.	***	***
Cladophora glomerata	N.S.	N.S.	***	*--	***	***	*--

Table II. The level of significance of the net photosynthetic responses to the combined effects of salinity and temperature of Cladophora rupestris and C. glomerata following two-way ANOVA. (see Table I. for notation used).

	C. rupestris	C. glomerata
(a). Salinity.	***	***
(b). Temperature.	***	***
(c).Interaction of axb.	***	***

$102^{o}/oo$).

Discussion and Conclusions

Growth measurements of Cladophora rupestris in culture have led Cambridge et al. (1984) to conclude that this species is tolerent of temperatures from below -5 up to 25-30°C. The results obtained during this study indicate a higher upper temperature limit, the plants not being killed at 30°C under normal salinity conditions ($34^{o}/oo$). Biebl (1959) also, recorded C. rupestris surviving temperatures between -8 and 30°C for at least 12h. However, the activity of the plants was significantly reduced at this high temperature, and longer periods of incubation might cause greater cell damage and subsequent plant mortality. The temperature tolerance shown by C. glomerata agrees with that reported by Whitton (1970), the plants being most active photosynthetically at the high temperatures but not tolerating the very low temperature of $-9^{o}c$. Russell (1987) discusses problems involved in extrapolating the results obtained from short-term experiments, to conditions experienced in nature. It is thought that the 48h incubation periods used in this study are sufficient to show trends in the responses of these two species, but caution should be used in interpreting such results, and further long-term experiments are desirable.

By combining the values of net photosynthesis obtained at each salinity for all seven temperatures it is possible to produce generalised salt tolerance curves for both species (Fig.3.). These curves suggest that Cladophora glomerata is only tolerant of very slightly saline waters, net photosynthesis being significantly reduced in salinities of $11^{o}/oo$ and above. C. rupestris is tolerant of a wide salinity range, oxygen production

being significantly lower only in the extremes of 0 and 102°/oo.

The ability of Cladophora rupestris to withstand much lower temperatures than C. glomerata, is possibly due to the former having a higher intracellular ionic content and possibly higher levels of organic solutes, which may confer a greater degree of protection against damage caused by freezing (see Russell 1987). The greater oxygen production of C. rupestris following higher salinity treatment at -9°C, may also be a result of the increases in intracellular osmotica at these salinities, protecting the cells from freezing effects.

The damage caused to Cladophora rupestris by a combination of high temperature salinity extremes (0 and 102°/oo), implies that its cell physiology is susceptible to osmotic imbalance at temperatures close to the upper temperature limits of the plant. On the other hand, optimum oxygen production by C. glomerata in 34°/oo was obtained at higher temperatures, which would suggest that its cells are better able to make osmotic adjustments in warm water.

The salinity of onshore Hilbre seawater was quite constant over the period readings were taken (27 to 35°/oo). Cladophora rupestris during periods of emergence, experiences both hypo- and hypersaline conditions resulting from prevailing climatic conditions. Generally these salinity changes have proved to be small (5°/oo above and below that of the seawater), although salinities as low as 2°/oo were recorded on a few occasions following very heavy precipitation. These observations would suggest that it is rare for the plants growing at this site to experience large fluctuations in external salinity, although it is important to note that samples were taken from C. rupestris

plants that had been exposed for only about 1/2 of their total emergence period. It is probable therefore, that at times of resubmergence these algae had experienced greater changes in salinity than those recorded when visited the site. The temperature of the seawater varied seasonally between 2 and 20°C. Winter temperatures of emergent C. rupestris were generally higher (1 to 5°C) than those of seawater, and the reverse was true of the warmer periods of the year. There was no period of very hot, dry weather during the summer 1986, which may have led to underestimation of the upper temperature and salinity extremes. Also by taking temperature measurements only during the day, the lowest winter temperatures were likewise unrecorded. The canal water experiences similar variations in temperature during the year, though the maximum recorded temperature of canal water was slightly higher than that of Hilbre seawater during the summer (22°C).

The measured salt tolerances therefore follow closely the salinity regimes of the two plant populations: Cladophora glomerata living in stable freshwater conditions and C. rupestris being subjected to dilutions of thallus surface water during precipitation, and to a lesser extent, hypersaline conditions produced by evaporation. The temperature extremes recorded at both sampling sites fall well within the tolerance limits measured for the two species. The salinity tolerances of both species, although clearly affected by temperature, are not significantly altered over the range of temperatures encountered in the field.

Evidently, temperature is an important factor governing the ability of both species of Cladophora to withstand salinity

stress, and the results emphasize the importance of considering interactions between environmental variables in studies concerned with the distribution of macro-algae in nature.

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Fig.1. The effect of temperature and salinity upon the net photosynthesis of Cladophora glomerata.

Fig.2. The effect of temperature and salinity upon the net photosynthesis of Cladophora rupestris. N.B. The scale used is different than that used in Fig.1.

Fig. 3. Salt tolerance curves for Cladophora glomerata (-0-0-) and C. rupestris (-●-●-). Obtained by adding the responses at each salinity together for all 7 temperatures. The bars represent the Least Significant Difference for each data set.

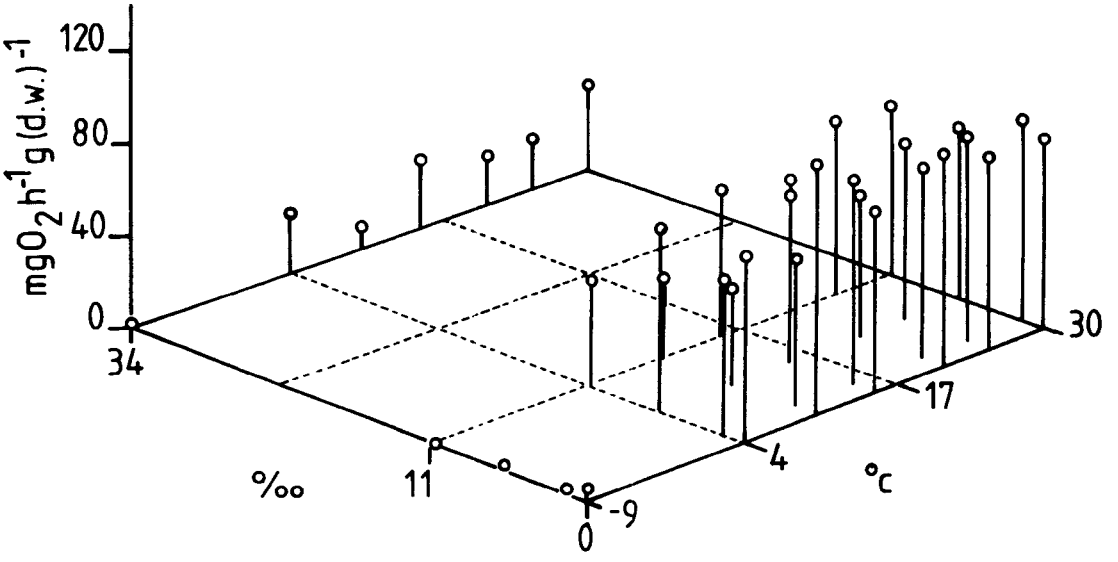

Fig 1

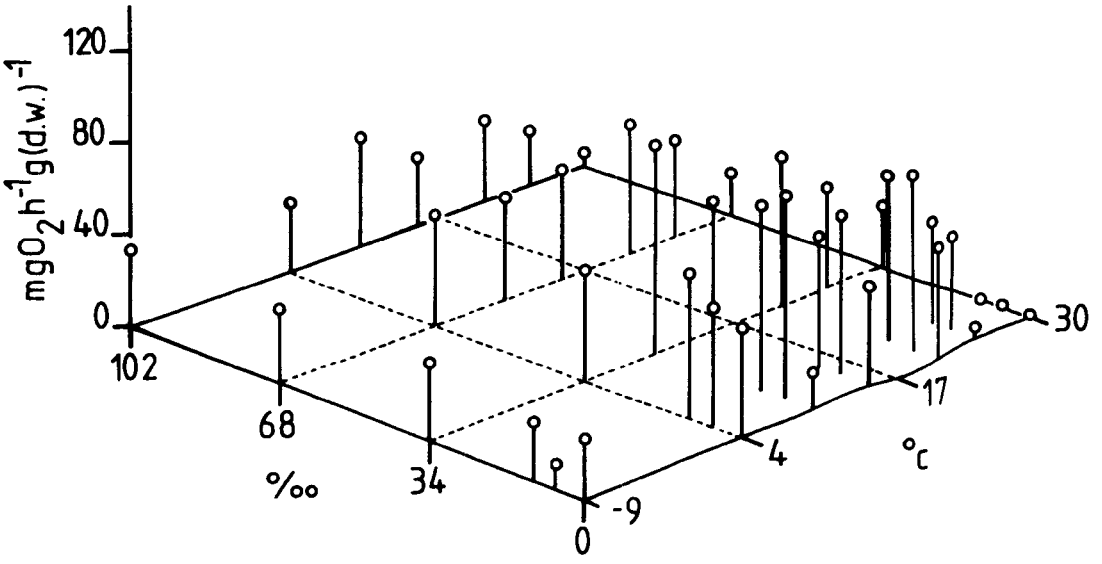

Fig. 2.