THE PEEL-HARVEY ESTUARINE SYSTEM STUDY (1976 - 1980)

TECHNICAL REPORT

ECOLOGY OF CLADOPHORA

1981

D.M. Gordon, P.B. Birch
and A.J. McComb

DEPARTMENT OF CONSERVATION AND ENVIRONMENT
BULLETIN No. 91

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PUBLICATIONS:
THE PEEL-HARVEY ESTUARINE SYSTEM STUDY (1976-1980)

This report is one of 14 technical reports that were presented to the Environmental Protection Authority's Estuarine and Marine Advisory Committee as part of the Peel-Harvey Estuarine System Study (1976-1980).

The publications arising from the study are listed below and are available from the Department of Conservation and Environment, 1 Mount Street, Perth WA 6000.

- The Peel-Harvey Estuarine System Study (1976-1980). A report to the Estuarine & Marine Advisory Committee December 1980. E.P. Hodgkin, P.B. Birch, R.E. Black, and R.B. Humphries, Department of Conservation and Environment, Report No. 9.

- The Peel-Harvey Estuarine System Study. A report by the Estuarine and Marine Advisory Committee to the Environmental Protection Authority, March 1981. Department of Conservation and Environment, Bulletin No. 88.

TECHNICAL REPORTS

BULLETIN No.

89 The Peel Inlet and Harvey Estuary System Hydrology and Meteorology. R.E. Black and J.E. Rosher. June 1980.

90 Sediments and Organic Detritus in the Peel-Harvey Estuarine System. R.G. Brown, J.M. Treloar and P.M. Clifton. August 1980.

91 The Ecology of *Cladophora* in the Peel-Harvey Estuarine System. D.M. Gordon, P.B. Birch and A.J. McComb. 1981.

92 The Decomposition of *Cladophora*. J.O. Gabrielson, P.B. Birch and K.S. Hamel. October 1980.

93 The Control of Phytoplankton Populations in the Peel-Harvey Estuarine System. R.J. Lukatelich and A.J. McComb. 1981.

94 Cyanobacteria and Nitrogen Fixation in the Peel-Harvey Estuarine System. A.L. Huber. October 1980.

95 Phosphatase Activities in the Peel-Harvey Estuarine System. A.L. Huber. October 1980.

96 The Sediment Contribution to Nutrient Cycling in the Peel-Harvey Estuarine System. J.O. Gabrielson. 1981.

97 Aspects of the Biology of Molluscs in the Peel-Harvey Estuarine System, Western Australia. F.E. Wells, T.J. Threlfall and B.R. Wilson. June 1980.

98 The Fish and Crab Fauna of the Peel-Harvey Estuarine System in Relation to the Presence of *Cladophora*. I.C. Potter, R.C.J. Lenanton, N. Loneragan, P. Chrystal, N. Caputi and C. Grant. 1981.

99 Phosphorus Export from Coastal Plain Catchments into the Peel-Harvey Estuarine System, Western Australia. P.B. Birch. October 1980.

100 Systems Analysis of an Estuary. R.B. Humphries, P.C. Young and T. Beer. 1981.

101 Peel-Harvey Nutrient Budget. R.B. Humphries and R.E. Black. October 1980.

102 Nutrient Relations of the Wetlands Fringing the Peel-Harvey Estuarine System. T.W. Rose and A.J. McComb. August 1980.

PREFACE

This report describes work carried out in the Department of Botany, University of Western Australia, from April 1976 to September 1979 as part of a study on the Peel-Harvey Estuarine System under the auspices of the Estuarine Marine Advisory Committee, Department of Conservation and Environment, Western Australia.

The bulk of this material is the basis of a Ph.D. thesis prepared by David Gordon, whose salary was met by a Research Studentship from the University of Western Australia. Research maintenance and logistical support was provided by the Department of Conservation and Environment. Chapter 8 was largely prepared by Peter Birch of that Department, who collaborated in other aspects of the research.

We are indebted to a number of persons, including R.P. Atkins, K. Hamel, R.J. Lukatelich and F. Salleo for help in the field and laboratory, and E.P. Hodgkin and R.B. Humphries for discussion. J. Kuo provided ultrastructural illustrations.

Appendix 4, a map of the aquatic vegetation, was drawn by the Media Services Centre, University of Western Australia. This appendix is issued separately.

CONTENTS

APPENDICES

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

GENERAL INTRODUCTION

1.1 INTRODUCTION

The following report describes the growth, distribution and nutrient requirements of Cladophora in the Peel-Harvey estuarine system. The work reported was undertaken between April 1976 and September 1979 and is concerned with one particular species causing nuisance problems on beaches surrounding the estuary.

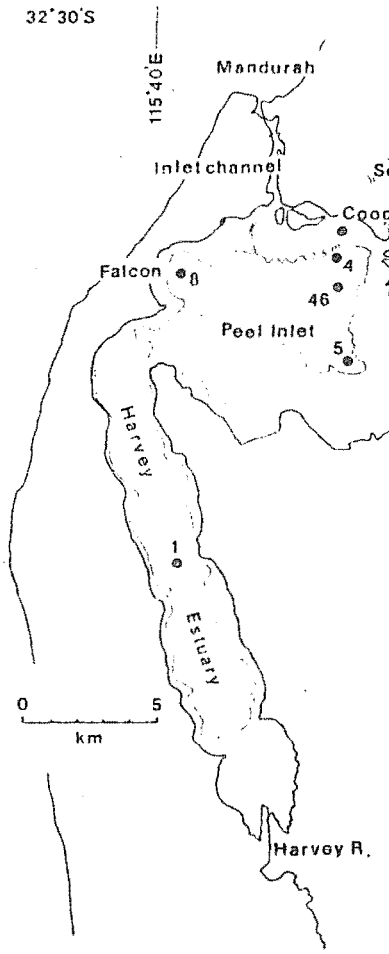
1.2 HISTORICAL ASPECTS

There are no published early descriptions of prolific algal growth in the estuary, although there have been verbal accounts of 'weed', principally accumulations of the aquatic angiosperms Ruppia and Halophila, having been removed by bullock cart from surrounding beaches. These plants are aesthetically more acceptable in the estuary than algae, which have apparently been a nuisance to fishermen netting in the estuary long before the start of the present problem. Frequent descriptions of 'once clean, white beaches' by local residents and fishermen suggests that, despite the early observations of 'weed' on the beaches, the presence of Cladophora (and its relatives, the filamentous species Chaetomorpha and Enteromorpha) in vast amounts is a relatively recent event. A noticeable change in the inlet occurred in the late 1950's with the establishment of several algal species, including Cladophora and Enteromorpha (Cross 1974).

Excessive algal growth does not seem to have caused a noticeable problem for residents until after the mid-1960's, this coinciding with the first complaints of noxious odours resulting from decomposing algae, principally the ball-like Cladophora locally called goat weed.[1] An algal survey by Allender in 1966 showed no sizeable Cladophora populations in Peel Inlet, though he did collect some in the Harvey Estuary.

The alga increased into the 1970's, necessitating clearing and removal from certain beaches, in particular off the Falcon[2] townsite at Novara Beach, on the west shore of Peel Inlet, and at Coodanup on the north shore of Peel Inlet (see Fig. 1.1).

Footnote [1]. The use of this name by residents and fishermen is fairly widespread and raises an interesting etymological point since it is hard to visualize how such an 'appropriate' name became established. The Peel Inlet species resembles, outwardly, the Cladophora which forms 'lake balls' and which was referred to as 'Aegagropila'. The word originates from the Swedish botanist Linnaeus in 1763 who used it as a species name. In translation it means 'goat ball' since Linnaeus likened the alga to the balls which occur in the stomach of the Bezoar goat Capra aegagrus (Newton 1950). Though the species in Peel Inlet is clearly distinct from aegagropiloid lake-balls (see Chapter 2), it would appear that the local name certainly does have some botanical relevance.



DIMENSIONS

Inlet Channel

length : 5 km

Peel Inlet

area : 75 km^2

volume : 60×10^6 m^3

Harvey River

area : 56 km^2

volume : 56×10^6 m^3

Rivers

Serpentine : 24 km (includes Goegrup L.)

Murray : 24 km

Harvey : 4 km

FIGURE 1.1: PEEL-HARVEY ESTUARINE SYSTEM

(showing Cladophora experimental sites and the dimensions of the system)

Weed clearing from the shorelines, using a bulldozer equipped with a rake, was started in 1973 and has continued regularly since then. The resulting 'spoil' is removed by truck. This action is primarily cosmetic, the effective cleared area being the beach itself and the very shallow shoreline margins. Observations on Cladophora growth before the start of the study in 1976 suggest that the alga had reached particularly large proportions by 1974 (Rippingale 1974, 1975). Quantities of Cladophora being removed from the Novara and Coodanup shores at present are far less than those removed in 1974 (Hodgkin et al. 1980).

Both beaches are residential and undoubtedly the most popular for recreation and amateur fishing. This point is rather well demonstrated, albeit indirectly, from the results published by the Peel Inlet Management Authority following a large-scale rubbish removal programme for the estuarine shoreline ("The West Australian" newspaper, 10th January 1981). Included in their "collection" were some 357 pairs of shoes from the waters at Coodanup, a site very popular for crabbing. This provides a useful, if somewhat crude, census of amateur fishing numbers for one particular shoreline!

Recent observations on the system are compatible with the earlier descriptions, with a consistent absence of Cladophora and other macroalgae, in any substantial quantities in the Harvey Estuary, this being characteristically more turbid due to wind stirring and carrying a greater phytoplankton load (Lukatelich and McComb 1981). The importance of phytoplankton to overall production in Peel Inlet was presumed small before quantitative data were available, though its potential as a source of nutrients for the benthic communities there was recognized (Rippingale 1974). The presence of large blooms, principally of diatoms, in Peel Inlet during winter, at time comparable in magnitude to those in the Harvey Estuary (Lukatelich and McComb 1981), suggests a significant contribution by this source to nutrient cycling in Peel Inlet. Recent massive spring blooms of the blue-green 'alga' Nodularia spumigena, mainly in the Harvey Estuary, (Huber 1980; Lukatelich and McComb 1981) are reminiscent of earlier blooms of the blue-green Oscillatoria in the Harvey (Rippingale 1974) and of Nodularia in the Serpentine River in 1970 (Hodgkin et al. 1980).

1.3 Morphometry of the System

The geomorphology of the Peel-Harvey Estuarine System has been described in detail by Brown et al. (1980). The dimensions of the system are shown in Figure 1.1. Located some 70 km south of Perth, the estuary comprises two large, shallow (average depth, 1-2 m), interconnected coastal lagoons, the Peel Inlet and the Harvey Estuary, separated from the Indian Ocean by a dredged, narrow inlet channel. The bathymetry of each lagoon may be broadly differentiated into a deeper central basin surrounded by shallow marginal shelves (Brown et al. 1980).

Footnote[2]. During this study, the bay in the estuary near to the Falcon townsite has been referred to as 'Falcon Bay', as there was no formal name for the area. In fact, this name is used officially to describe the ocean bay to the west of Falcon. Since this work was carried out, the estuarine site, referred to as 'Falcon Bay' in this report, has been formally named 'Cox Bay' after Cox Cottage, a nearby settlers homestead.

1.4 Hydrology and Meteorology

Results of hydrological and meteorological studies have been described in detail by Black and Rosher (1980), the data for the latter having been collected at a permanent weather station situated on the Robert Bay shore, Peel Inlet. The system is fed by three major tributaries - the Murray River and Serpentine River (both of which are tidal some distance upstream), and the Harvey River, entering at the southern extremity of the Harvey Estuary (Fig. 1.1). These rivers drain the surrounding catchment (total area $\sim$ 11,300 km^2) but only the Murray catchment ($\sim$ 7,900 km^2) remains undammed. The Serpentine River flow has been reduced by the construction of a pipehead (1957) and a main (earth fill) dam (1961), while pipehead dams were constructed on the North and South Dandalup rivers, within the catchment area, in the early 1970's, followed by the construction of an earth fill dam on the South Dandalup in 1973. The Harvey River (and a number of minor drains) drain agricultural land on the coastal plain, the hills catchment of the Harvey River having been dammed for irrigation. The total undammed catchment of the estuary is approximately 9,600 km^2.

All rivers feeding the estuary are highly seasonal and are the principal source of freshwater, and consequently of allochthonous nutrients, to the system; over 90% of incoming nutrients are derived from a limited period of about 10 weeks of high freshwater flow in winter (Black and Rosher 1980). Ground water seepage, in contrast, appears to contribute only a small percent to the total (Black and Rosher 1980).

This system, which now remains permanently open to the sea and is therefore subject to marine intrusion, may be classified as a 'permanently open' estuary (Hodgkin and Lenanton 1981). The shallow nature of the lagoonal basins and the restricted tidal exchange result in hypersalinity for about half the year, alleviated by the short period of freshwater input in winter. In this respect, the seasonality reflects similar trends found in other estuaries of south-western Australia (Rochford 1951; Hodgkin and Lenanton 1981). The salinity range in Peel-Harvey estuary is wide, typically less than 10 $^{o}/oo$ in winter and rising to over 50 $^{o}/oo$ in summer. Hypersalinity is strongly influenced by evaporative water loss during the hot summer months (Black and Rosher 1980).

1.5 *Cladophora* in other systems

Nuisance growth of *Cladophora* occurs worldwide; the alga's presence, for example, in the Great Lakes, North America, has been associated with increased eutrophication around heavily populated shorelines (Young 1970, Wezernak *et al.* 1975). *Cladophora* was reported on the increase in polluted western Lake Erie during the late 1940's, presumably exacerbated by nutrients derived from the adjoining Detroit River and from decomposing algal blooms (Taft and Kishler 1973). Both Lakes Ontario and Erie are now considered eutrophic with a large growth of *Cladophora* and a diminished variety of phytoplankton genera (Palmer 1980). Poor species diversity and excessive growth of *Cladophora* and related macroalgae is also a noticeable result of increased eutrophication in the Peel-Harvey estuarine system.

The alga is present as seasonal blooms in many lotic systems (e.g. Bellis and McLarty 1967; Whitton 1970; Wong and Clark 1976), particularly rapidly flowing waters of many North Temperate rivers where it is often the dominant benthic alga (Blum 1956). The sudden appearance of large growths of Cladophora in rivers is usually linked with some input of nutrients and, as such, it has been considered possibly useful as a pollution indicator organism (Whitton 1975). Earliest documented instances of large Cladophora populations have been for those species forming 'lake balls' in freshwater lakes (Acton 1916; Wesenburg-Lund 1903; Nakazawa 1974), notable because of their unusual aegagropiloid growth habit. Similar accounts of aegagropilous Cladophora species exist for the United States of America (Munier and Folger unpublished data). They noted that Cladophora (principally C. holsatica and C. aegagropila) had been recorded in only 4 states up to 1952 (Daily cited Munier and Folger) and reported an extensive population growing in Lake Champlain, Vermont.

Large aegagropiloid populations are apparently not confined to warm climates only. This is well illustrated by Jónasson (1979) who reports extensive growth of Cladophora aegagropila on the floor of Lake Myvatn, a eutrophic, sub-arctic lake in Iceland. This particular species, in outward appearance, resembles the Peel species (see Hunding 1979).

Beside rivers and lakes, Cladophora is present in estuarine and marine waters where pollution is recognized (e.g. Norin and Waern 1973, Klavestad 1978, Bach and Josselyn 1978; 1979 Mathieson 1980). Bach and Josselyn report extensive growth of a free-living, mat-forming aegagropiloid Cladophora which has noticeably increased in shallow bays in Bermuda, presumably as a result of nutrient enrichment of waters derived from sewage and agricultural runoff. Cladophora is now also a problem in San Francisco Bay (Josselyn, pers. comm.).

Cladophora, along with Enteromorpha and Chaetomorpha, frequently becomes readily established in polluted estuarine systems (see Edwards 1972). Both Cladophora sericea and Enteromorpha intestinalis were found to dominate the estuarine flora of a Norwegian fjord, their growth being stimulated by plant-derived nutrients in the water (Holt 1979). Both these genera are amongst the pioneer algae to become established in estuaries associated with a number of Icelandic fjords (Munda 1978).

Examples of prolific growth of Cladophora, particularly of of aegagropilous species, in other Australian estuaries, are limited. Studies are presently under way in the Department of Botany, Monash University, on growth of filamentous species of Cladophora in the Barwon River estuary, Victoria (Narkiewicz, pers. comm.). An aegagropilous Cladophora species has been collected from Moss Ball Lake, Bunga Inlet (Gippsland Lakes, Victoria). This particular population withstands wide changes in salinity and has been tentatively identified as C. echinus (S. Ducker, pers. comm.). Other systems which do not yet have a documented Cladophora problem have the potential to do so. Drift algae, for example, are now a problem affecting

public beaches around Port Philip Bay, Melbourne, (V. Brown, pers. comm.). Of the estuaries of south-western Australia, the Peel-Harvey estuary is, perhaps, the most eutrophic with the most extensive documented cover of benthic algae. This is clearly evident when it is compared with the Blackwood River estuary (Congdon and McComb 1980) which, with its smaller volume, greater tidal exchange and largely forested catchments, has a low nutrient status compared with the Peel-Harvey estuary.

Benthic plants in the Blackwood, consist largely of aquatic angiosperms (principally Ruppia, Zostera and Potamogeton) with a low biomass of macroscopic algae. Occasional blooms of Rhizoclonium (Cladophorales) may form extensive mats during spring and summer (Congdon and McComb 1981).

Macroscopic algae are better represented in the Swan River estuary, close to Perth, where the variety of species is somewhat larger than in either Peel Inlet or the Blackwood River estuary (see Allender 1981). This estuary, like the Blackwood, does not show obvious signs of pollution at present; in neither system is there a large build-up of Cladophora. This was not always the case, however, for the Swan River, where accumulations of nuisance algae and complaints of noxious odours have been recorded from as early as the 1870's up until the late 1960's (Hodgkin and Vicker, unpublished M.S.). The offending algae were largely Cladophora, Chaetomorpha, and Enteromorpha, these comprising the major species of summer drift populations (Royce 1955).

CHAPTER 2

MORPHOLOGY AND LIFE HISTORY

2.1 INTRODUCTION

This chapter provides information on the morphology, reproduction and anatomy of the 'ball-forming' Cladophora from Peel Inlet, including a brief account of its growth habit and fate following deposition on beaches around the shoreline.

Techniques are described here in which the alga was grown in the laboratory in pure culture, so that cell dimensions and morphology of cultured material could be compared with those from specimens collected in the field, and used in attempts to identify the species from published keys.

The following discussion deals only with the 'problem' species, which builds up to large populations and which is the dominant benthic alga growing in the estuary. At least two other Cladophora species have been collected from Peel Inlet during the investigation, and some details of these are given in Appendix 3.

The format and terminology used here to describe the alga follows to a large extent that of van den Hoek (1963) in his review of the European species.

2.2 MATERIALS AND METHODS

2.2.1 Sterilization

Completely defined media have been developed for growing algae (e.g. Provasoli et al. 1957, Smith and Wiedeman 1964, McLachlan 1973, Nichols 1973), but the most difficult step is sterilization. A number of disinfectants have been used in the preparation of pure cultures (e.g. Hamilton 1973). In preliminary experiments small fragments (20-30 cells) of Cladophora were placed for 5 to 10 minutes in 2% solution of sodium hypochlorite in estuary water, rinsed in filter-sterilized estuary water, and plated on agar (see below), but the treatment was lethal, with rapid bleaching of the cells. Lower concentrations also produced cell bleaching (Table 2.1). Antibiotics were therefore investigated. Cladophora fragments were rinsed in sterile estuary water, soaked in sterile estuary water containing antibiotics on an orbital shaker for 4 hours, then rinsed in sterile estuary water before being plated on agar or placed in liquid medium in culture tubes (Pyrex No. 9825). Anti-biotics were prepared by adding 1 ml of stock to 9 ml of sterile estuary water. The stock solution contained (in mg ml^{-1}) Ampicillin 4, Tetracycline 0.1 and Gentamycin 0.1 (Commonwealth Serum Laboratories, Perth).

Table 2.1 Effect of sodium hypochlorite on filaments of Cladophora plated on agar nutrient medium. Results shown are the means of 3 replicates at each treatment concentration. Fragments of alga (20-30 cells) were shaken in sterile estuary water for 2 hours, rinsed, then bathed in the hypochlorite solutions. Following treatment, the fragments were rinsed in sterile estuary water and plated on 1% agar containing estuary water medium. Incubation was for 30 days at 25°C under warm light (Philips, 12 x 40w fluorescent tubes) and incandescent bulbs (Philips 8 x 60w) with 12 hour daylength. Control and blank plates were run with treatments.

Hypochlorite concentration %	Exposure time (mins)	Growth during incubation	Contamination of fragments %	Mean number of bacterial colonies per plate	Comments
2.9	10	Nil	75	4	All cells bleached
	5	"	7	0.3	"
	2.5	"	8	2	"
1.5	10	"	40	2	"
	5	Slight	0	0	"
	2.5	"	30	2	"
0.7	10	"	0	8	"
	5	"	45	5	"
	2.5	"	100	6	"
0.4	10	"	60	3	"
	5	"	90	9	"
	2.5	"	100	8	Cells partially bleached
0.15	10	"	40	2	All cells bleached
	5	"	100	11	"
	2.5	"	100	8	Cells partially bleached
0.07	10	"	80	3	All cells bleached
	5	Moderate	100	7	Cells partially bleached
	2.5	Very good	100	7	No cells bleached - some parts replatable but bacterial contamination present
Controls 0	-	Very good	100	19	-
Blanks 0	-	-	0	0	Plate clean

2.2.2 Nutrient media

Estuary water medium was based on that of Jennings (1968), which was in turn derived from that of Schreiber (1930). Modifications were the addition of nitrogen as NH_4NO_3 rather than $NaNO_3$, and replacement of ferric EDTA by disodium EDTA.

A fully defined artificial seawater medium was also used, based on the ASP_{12} recipe of Provasoli (1964), and is described in detail in Chapter 6. Media used for liquid cultures were filter-sterilized (0.2 µm, Millipore Corp., Massachusetts). Solid media were prepared by autoclaving a solution of 5 g agar in 100 ml distilled,deionised water, and adding to filter-sterilized nutrient media. Where estuary water medium was used, artificial components were included with the agar during autoclaving and the filter-sterilized estuary water added separately. Nutrient media were warmed to 40°C before addition of the hot agar mixture, giving a 1% agar solution which was poured immediately into sterile 5 cm diameter plastic petri dishes. All sterile manipulation was carried out in a laminar flow unit (Model CF43C, Gelman Clemco Pty. Ltd., Australia). Cultures were placed in a growth cabinet at 23°C with a 12 hour photoperiod under warm light (Philips, 12 x 40w fluorescent tubes) and incandescent bulbs (Philips 8 x 60w), giving between 150 and 200 µE m^{-2} sec^{-1} PAR).

2.3 RESULTS AND DISCUSSION

2.3.1 Description

Thallus normally profusely branched, resulting in a dense, spongy, spherical clump which may become very 'ball-like', from 1 to 3 cm in diameter (Fig. 2.1, 2.2). In general, branches radiate from a central point (Fig. 2.1), but organization is not always so obvious and plants may become fragmented in appearance, sometimes giving rise to long, sparsely branched filaments (Fig. 2.3l). Branching is predominantly intercalary with poor acropetal development (Fig. 2.3a-j). Though in outward appearance plants resemble eg. the aegagropiloid balls described frequently from both freshwater and marine habitats, the morphology is clearly distinct from these (see Ecology, this chapter).

Insertion of branches occurs close to the apical pole of the parent cell (Fig. 2.3n,o). Only very rarely has lateral insertion of branches been observed (Fig. 2.3o). Short branches (1 to 2 cells) are straight and relatively rigid, longer branches are usually straight or refract, rather than falcate (Fig. 2.3a-k,n). Rigidity of branches results in a spongy texture to the thallus, which is robust rather than delicate. Older cells may have 2 or 3 (infrequently 5 or 6) branches arising from the same parent cell (Fig. 2.3o). Cells of the main axes, particularly the older cells towards the basal or (when the thallus is distinctly spherical) the central region of the thallus, are somewhat club-shaped (clavate) in appearance (Fig. 2.3o) being wider close to the apex where branches arise. The angle of insertion of

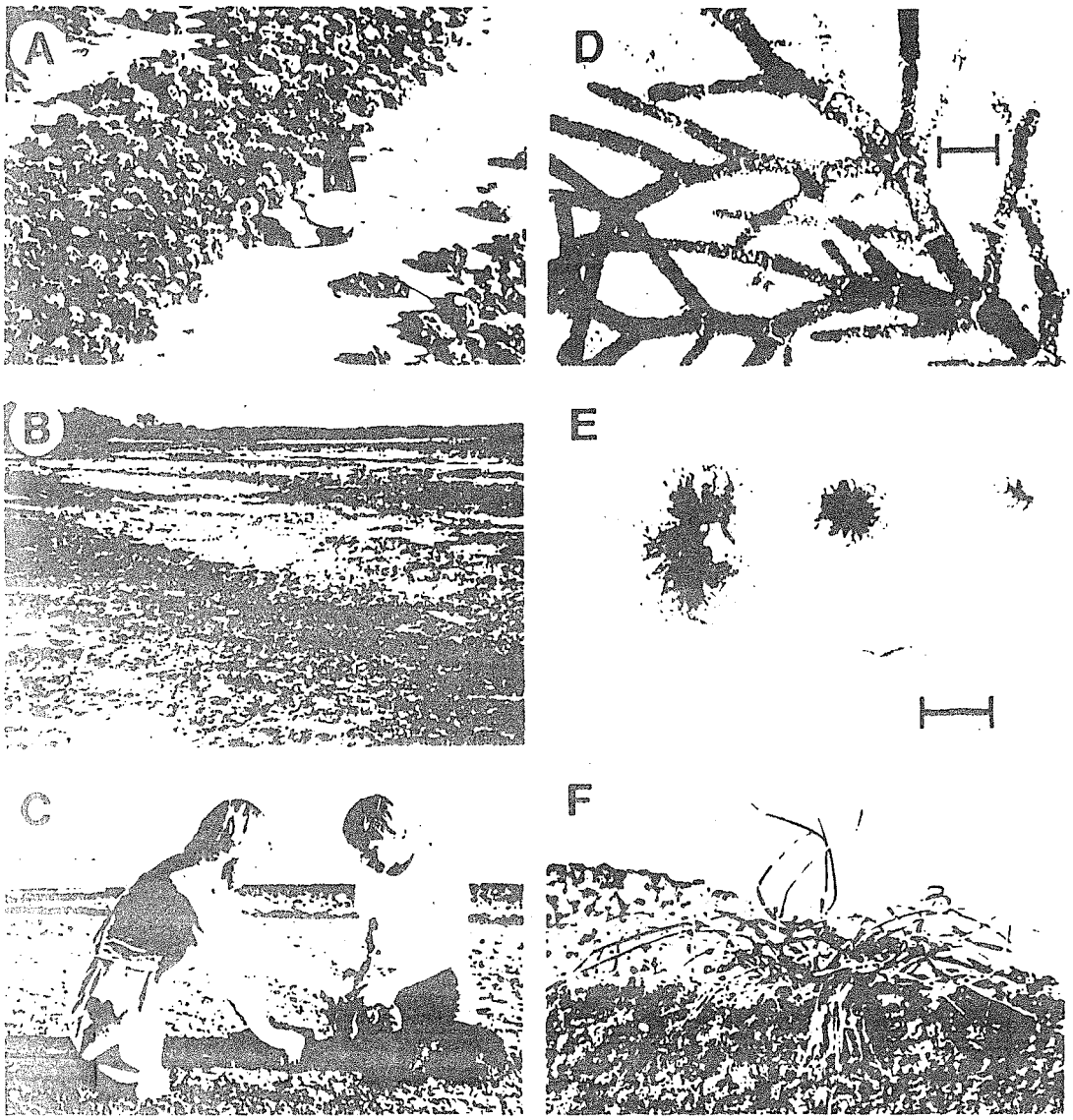


Figure 2.1 Aspects of *Cladophora* growth in Peel Inlet.

A *Cladophora* 'balls' washed onto beach sand.

B Coodanup shoreline, Peel Inlet during low tide showing extensive cover of algae, principally *Cladophora*, overlying *Ruppia* meadows on the shallow, sandy marginal platform.

C Collecting *Cladophora* from an offshore bank accumulated in the shallow water at Coodanup, Peel Inlet. This particular build-up is 140 m long with a dry weight biomass of 75 tonnes.

D Characteristic branching of the thallus; cells have dense chromatophores and thick walls. The scale shown is 100 µ.

E New plants of *Cladophora* grown in culture are much-branched and spherically organized with new branches radiating from a central point. The scale shown is 0.25 mm.

F Terrestrial weed *(Trachyandra divaricata)* growing on a dried mat of adhering *Cladophora* 'balls'. These mats are produced from desiccation of accumulated *Cladophora* 'balls' following their deposition on beaches.

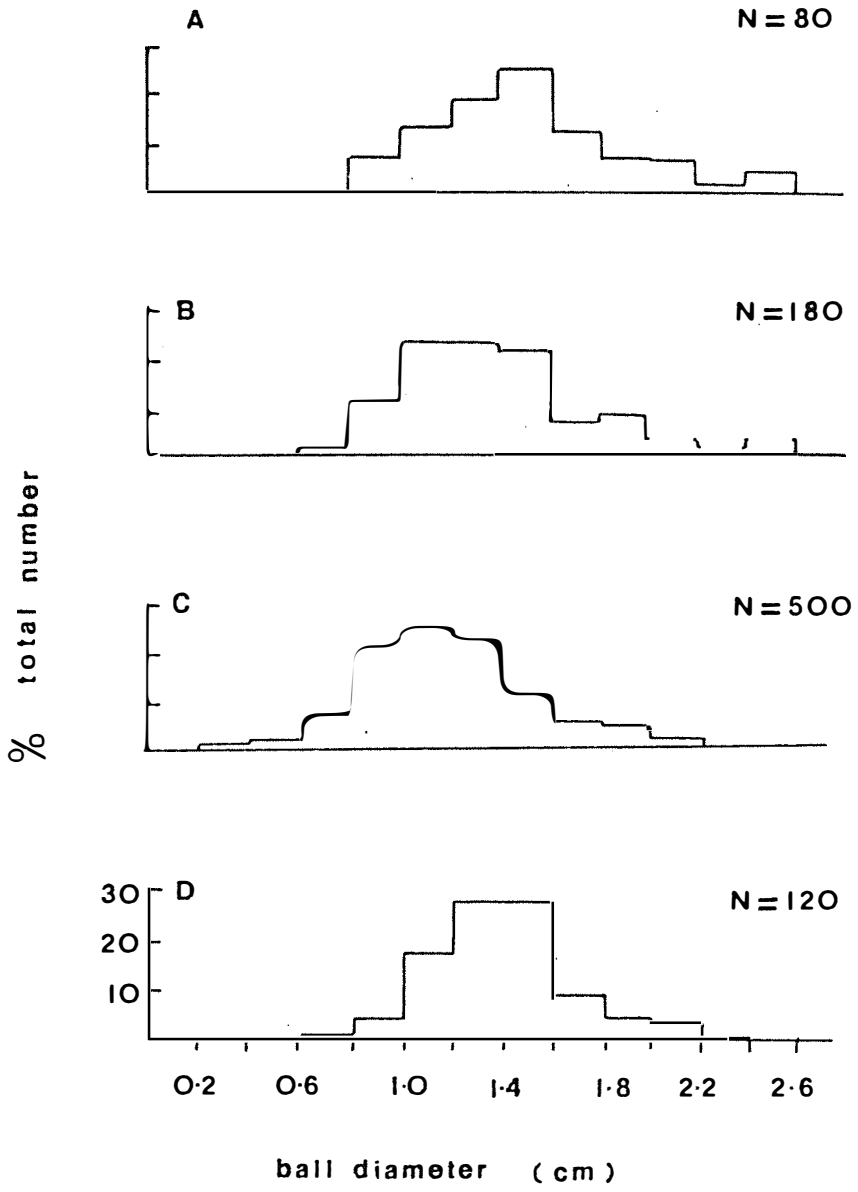
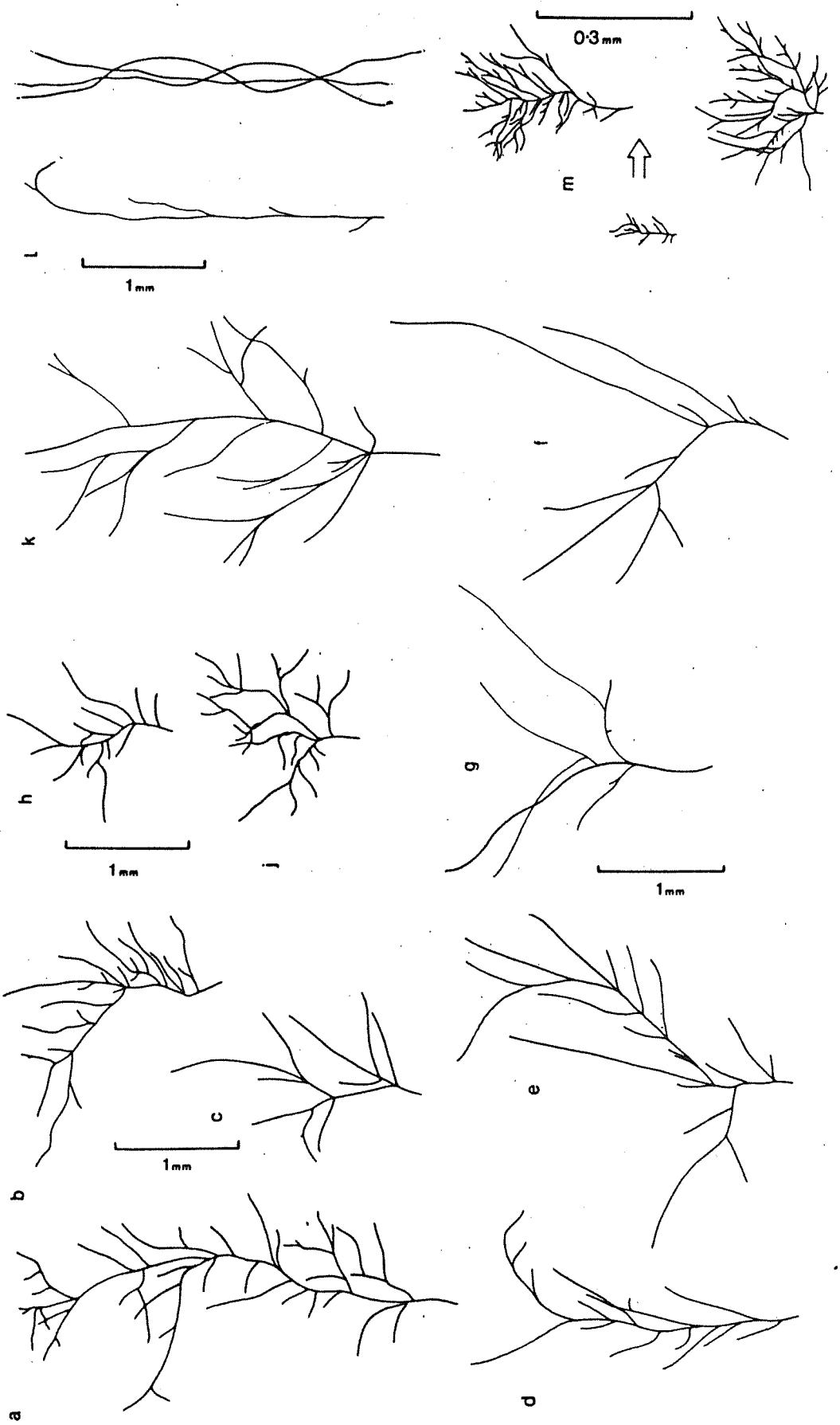


Figure 2.2 Numbers of *Cladophora* balls in different size classes. Samples were drawn from the algal bed at Post 46, Peel Inlet, April to August 1976, (A, B and C), and from a population of balls which had drifted to the mouth of the Serpentine River in November 1976 (D).

Figure 2.3 a-j. Branching patterns of *Cladophora* collected from station 4, Peel Inlet, June 1978, and grown for 4 weeks in the laboratory in estuary water at different salinities. a-c. 30 °/oo. d-f. 8 °/oo. g. 0 °/oo. h, j. 50 °/oo. k. *Cladophora* collected at Post 46, Peel Inlet, November 1977. l. From sample free-floating, Harvey Estuary, August 1978. Filaments are long (∿ 50 cm), very sparsely branched and intertwined. m. Growth on solid medium (1% agar-estuary water) for 7 weeks in culture (150-200 $\mu Em^{-2} sec^{-1}$ PAR; 25°C). n. *Cladophora* collected from Post 46, Peel Inlet, November 1977. Branches are apically inserted, short and relatively rigid. o. Older cells are clavate in appearance with up to 6 branches arising from one cell. p. Growth in static culture may give rise to irregular branching and cell dimensions. q. Variation in appearance of apical cells of ultimate branches. r. Development of zooids. Zooid primordia (polygonally shaped) arise from contraction of the dense chromatophore. Movement within the cell increases as zooids develop. The pore develops through initial rupture of outside wall layers. s. Mature zooids are biflagellated and elliptical in appearance. t-v. Fertile cells may be either terminal or intercalary or whole branches may become fertile. Non-fertile branches are depicted here with their dense chromatophores filling the cells. w-z. Germlings of *Cladophora* grown from spores released into sterile estuary water. New plants are shown after 3 weeks in culture (150-200 $\mu Em^{-2} sec^{-1}$ PAR; 25°C), some already having produced a 1-2 celled branch while others showed incomplete cross-wall development.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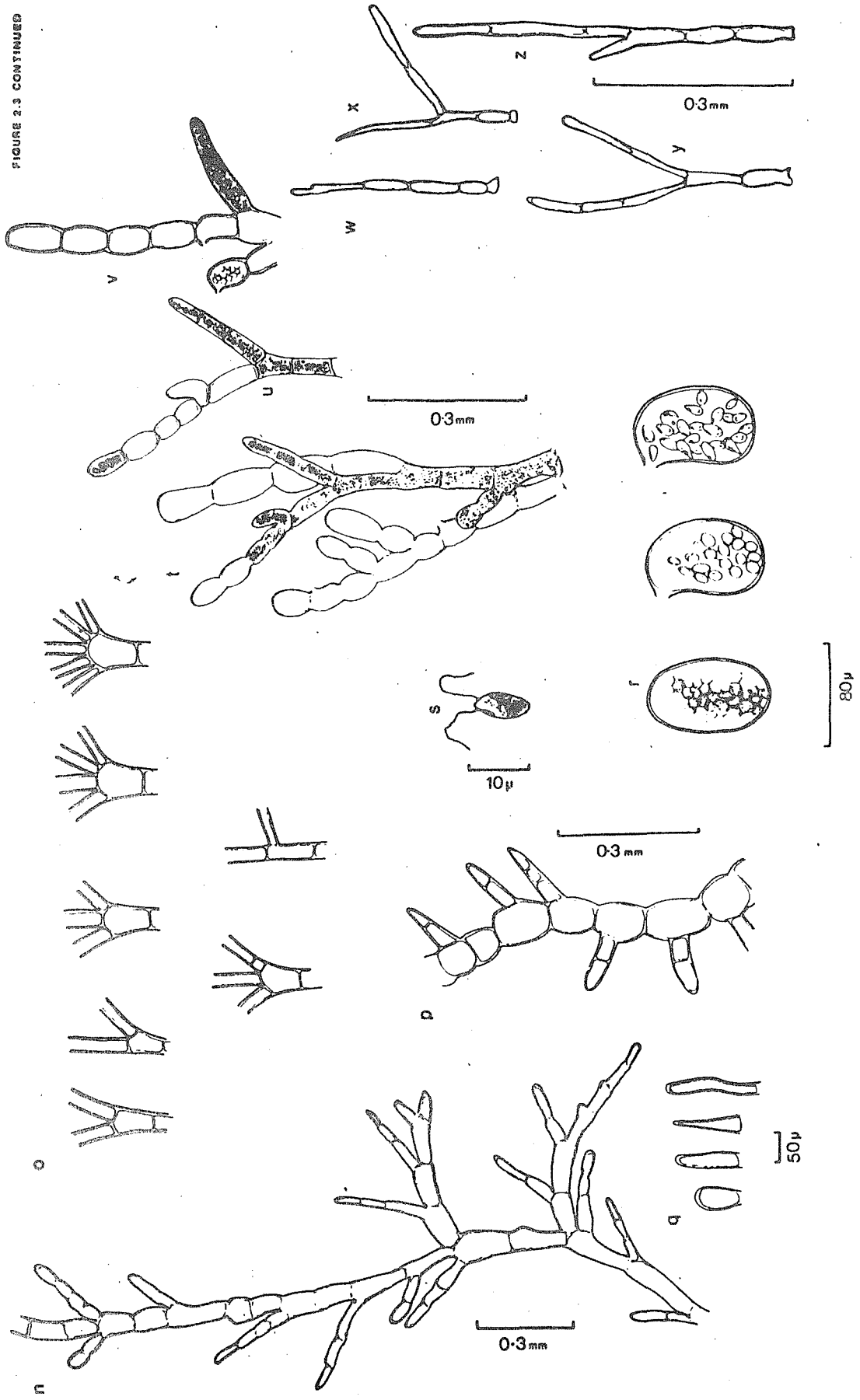

FIGURE 2.3 CONTINUED

branches is wide (approximately 45°); they are never closely appressed to the axis of origin. Negative polarity of cells has not been observed. Rhizoids have not been found. Apical cells are typically cylindrical and taper slightly (Fig. 2.3q), the apex being rounded, rarely pointed. Cell walls are thick (1-2 μm) in older cells but less so in apical cells and cells of ultimate branches.

Whole branches or parts may become fertile (Fig. 2.3t-v). These consist of a number of barrel-shaped zooidangia[1] sometimes with occasional branching and non-branching infertile cells intercalated along their length. Sporulating cells occur on ultimate branches resulting in a disintegration of this part of the thallus basipetally to the parent axis. Zooidangial cells are typically shorter and more stout in appearance than infertile cells (Fig. 2.3t). Apical zooidangia are rounded at the tip (Fig. 2.3r,t,v). A single pore is formed either apically (for apical zooidangia) or sub-apically. No pores have been found far from the apex of the cell.

Plants are generally dark green in colour though this can vary depending on light intensity and nutrient status of the tissue (see Chapter 7). Those algae buried in the mud of the estuary floor for prolonged periods are typically dark green.

Thick-walled cells may be produced, under certain conditions, similar to akinete cells described for other Cladophora species (e.g. Bellis and McLarty 1967). In the species here, when under stress the peripheral radiating filaments may die back, leaving a central core of thick-walled, dark green cells with dense chromatophores. These are presumably resistant to prolonged adverse conditions and regenerate a new thallus when conditions improve.

2.3.2 Ecology

This species is found predominantly free-floating, though specimens have been occasionally found growing attached to rocks. The alga forms beds of loose-lying clumps on the estuary floor in the deeper water of parts of the estuarine basin (1.5-2 m), where it exists in some areas throughout the year (see Chapter 3). The beds are typically 1-10 cm, up to 40 cm deep. A deep (50 cm) bed was, for example, found near the mouth of the Harvey Estuary near Dawesville (station 24) in summer 1977.

Plants grown from small fragments in static culture displayed radial organization of the developing thallus, suggesting that the ball-like habit of this species is largely the result of the branching characteristics of filaments, and not entirely to the physical rolling motion along the estuary floor, as has been

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Footnote[1] Here the terms 'zooid' and 'zooidangium' are used in a general sense only, and refer to flagellated swarmers and the fertile cells from which they arise, irrespective of their position in the life-cycle.

described for 'lake-balls' (Wesenburg-Lund 1903; Acton 1916). However, oscillation and lateral movement of the alga in beds on the estuary floor would presumably increase the spherical appearance of the thallus. Successful artificial globing of fragments has been reported for Cladophora growing in vertically rotated cultures (Nakazawa and Abe 1973, Nakazawa 1976). The latter author found that the spherical organization was lost when plants were returned to static conditions.

Field records during this study have shown larger more spherical plants occasionally present in the bed at Post 46 (see Fig. 2.2), a site in the deeper water of the estuarine basin (see Fig. 1.1), compared with their counterparts further inshore, where the alga appeared more fragmented. The species here, with its aegagropilous habit, resembles in outward appearance the frequently described 'lake-balls' found in freshwater lakes (e.g. Acton 1916, Nakazawa 1976), and resembles also some Cladophora balls reported from marine habitats (e.g. Sakai et al. 1977, Bach and Josselyn 1978). However, it is clearly morphologically distinct from these, particularly the 'lake-balls' which may show development of a hollow centre to the plant and concentric layered filaments within the ball (e.g. Lorenz cited van den Hoek 1963).

Both the species here and the 'lake-balls', whose mode of formation involves some degree of organized branching of the filaments, may be distinguished from the 'ball-like' aggregates of Cladophora repens reported in a marine coastal habitat by Newton (1950). The latter resemble in their development the 'fibre-balls' of the seagrass Posidonia found locally on beaches.

Animal populations

The algal bed supports a large animal population. Besides providing shelter in summer for the blue-swimming crab Portunus pelagicus, filaments of this species are abundant in smaller animals, particularly amphiphods (Amphipods), which presumably feed on the numerous epiphytic diatoms associated with the algal filaments (see Fig. 2.5A, B). Numbers of these animals appear to vary seasonally. The data here are very limited, but high numbers have been observed, in the bed at station 4, in December 1977 and in July 1978. On the latter occasion a count of these animals from sub-samples yielded an estimated 1.4×10^4 animals per square metre, this associated with a biomass in the bed of 460 g dry weight m^{-2}.

Associated decomposing material

Sediments underlying beds are very reduced due to decomposition, which results in a fine black ooze up to 20 cm thick which, when disturbed, rapidly clouds the overlying water releasing a strong H_2S odour.

Occurrence spatially

The alga is not confined to the deeper water. Movement of the bed depends on wind action, with layers of algae

on the floor oscillating to and fro through resulting wave movement. Under strong wind conditions, particularly those with a south-west component during the afternoon, the alga may be transported along the bottom. The alga becomes buoyant through photosynthetically produced oxygen bubbles trapped in the filaments. Windrows of floating spheres of alga can be seen running long distances across the water surface. Vast amounts of Cladophora are transported at certain times of the year from sites of growth onto the surrounding beaches.[1] There, the alga accumulates in large banks, sometimes up to 1 m deep (Fig. 2.1), and decomposes. On arrival at the shallow areas of the marginal shelf, the alga may overlie and eliminate extensive meadows of the aquatic angiosperms Ruppia megacarpa and Halophila ovalis, the latter being predominantly found in deeper water than Ruppia, but not extending much further than the shelf slope. These plants reinvade when the algal bank deteriorates or shifts onto the beach. The alga grows here entangled in the filaments of large masses of other Cladophorales, particularly Chaetomorpha linum, Enteromorpha spp. and Cladophora spp. which at certain times float in large masses above the meadows in the shallow water.

This species tolerates a wide seasonal change in salinity, ranging from less than 10 °/oo in winter to over 50 °/oo in summer, and there is evidence that it can survive for a short time at least in waters 4 times the salinity of seawater (see Chapter 7). The alga is also tolerant of high temperatures (see Chapter 6), persisting throughout the year over a range from 11°C to 27°C in the deeper water, up to 35°C in the shallow water near the shore in summer.

Decomposition appears to be slow (see Appendix 1) in water and presumably slower in air. Following deposition on the beach, the alga may form a crusty mat of adhering balls which may be over 1 m deep. When broken apart, these mats yield a green, moist agglomeration of balls (containing mostly degraded chlorophylls) on which the shoreline vegetation may grow (see Fig. 2.1). These mats smother extensive areas of marsh vegetation fringing the shoreline, particularly the sedges Juncus kraussii and Scirpus maritimus (Rose and McComb 1980, Backshall and Bridgewater 1981).

2.3.3 Culture

The morphology is similar, in culture, to that of plants from the field (Fig. 2.3a-m). This is also reflected in the similarity between the cell dimensions of filaments grown in pure culture and those of field material (Table 2.2).

Footnote[1] The financial value of the alga may be somewhat under-rated in Western Australia. Cladophora "balls" in North America are presently sold for biology classwork for the princely sum of $14 per 100! (Catalog 50, Carolina Biological Supply Co., 1979-1980).

Table 2.2 Cell dimensions (mean and range) of *Cladophora* collected (a) from the algal bed, March to May 1976; and (b) in sterile cultures on 1% agar-estuary water medium for 5 weeks.

a.	length (μ)	width (μ)	l/w ratio
Terminal cells	58 27-101	22 13-31	2.69 1.10-4.42
Sub-terminal cells	60 42-89	27 22-31	2.25 1.75-3.18
Main axis	180 84-313	59 32-80	3.10 1.27-5.05
Branch cells[1]	110 47-199	38 18-53	2.92 1.74-4.86
b.			
Terminal cells	139 92-192	31 24-40	4.59 2.40-6.00
Sub-terminal cells	129 72-186	30 24-34	4.33 2.88-5.47
Main axis	144 112-168	37 36-38	3.93 3.11-4.67
Branch cells	164 100-248	36 22-48	4.75 2.50-7.82

[1] 'Branch cell' here refers to randomly selected intercalary cells cut off from the parent axis.

Growth on solid medium was slow, with some evidence of irregular branching compared with those in liquid culture eg. (Fig. 2.3p). Fragments (20 to 40 cells), plated on agar, grew into colonies 4 mm to 5 mm in diameter after 8 to 12 weeks. Slow growth was also evident in static liquid cultures. For example, an inoculum of 12 cells placed in artificial seawater medium, without agitation, produced a spherically organized plant 8 mm in diameter after 12 months. Growth in aerated, enriched artificial seawater, in contrast, can produce rapid growth (see Chapter 7). New plants are spherically organized with radiating branches similar to those found naturally (Fig. 7.3; Chapter 7).

2.3.4 Reproduction

Occurrence and induction of sporulation

Data on reproductive behaviour are limited. Although sporing material was sought intensively during the first 2 years of the study, and intermittently since then, fertile material has been collected only from specimens attached to rocks at Soldier's Cove, near the estuary mouth, and this in only one period, during September and October 1978. Free-floating specimens were collected at this time from accumulated banks at Coodanup and placed in nutrient-poor seawater in a growth cabinet for over 10 weeks. This material produced fertile branches when transferred for 6 days to enriched artificial seawater (0.25 mg ℓ^{-1} PO_4-P; 2.5 mg ℓ^{-1} NH_4 + NO_3 - N). A population collected from station 5, near Austin Bay, at this time was similarly induced to spore when removed from nutrient-depleted seawater into enriched artificial seawater, at different salinities. Plants produced sporing branches after 3 days in solutions at 28 °/oo, and after 6 days at 35 °/oo. No sporangia were produced at either 3 °/oo or 58 °/oo. These trends are reflected somewhat in the field observations. On the only occasion when sporing material was observed in the field, following the onset of river flow in winter 1978, water nutrients at the site rose to very high levels; total phosphorus 0.4 mg ℓ^{-1}, and total nitrogen 0.85 mg ℓ^{-1}. This nutrient enrichment, which followed a prolonged period with little run-off from the catchments (including winter 1977), offers a likely mechanism for inducing sporulation of the Cladophora species in Peel Inlet.

Liberation of spores from certain other algae has been induced by a number of methods, particularly those involving a change of environmental factors (see Dring 1974). Reports on spore induction on Cladophora are limited. An early study by Cook and Price (1928) suggested that aeration of cultures was significant to zoospore formation. Bellis (1968) noted sporulation in filaments of C. glomerata growing at different temperatures. Successful formation of swarmers in cultures of C. sauterii was reported by Yabu (1975) using artificial seawater containing 16 mg ℓ^{-1} NO_3-N and 4 mg ℓ^{-1} orthophosphate. Whether the response to a replacement with fresh medium observed here is due to a specific nutrient remains unclear.

Zooid development and liberation

Zooid formation began with a contraction of the dense chromatophore filling the cell, giving rise to polygonally shaped zooid primordia (Fig. 2.3r). Movement within the cell at this stage was restricted. Zooids gradually became more rounded in appearance, and eventually separated within the parental cell. Movement became increasingly rapid with maturation of zooids. The pore develops by initial rupture of the outside wall layer, the zooids being retained in the cell by a mucilaginous plug. Expulsion occurs rapidly and in single file, the zooids oriented with either the anterior or posterior end forwards. Before release, zooids may be seen pushing on the plug of the developing pore. A permanent record of these stages has been photographed on video tape.

Zooids were typically elliptical in appearance (Fig. 2.3s) 6 to 9 µm long and 3 to 4 µm wide, though there was variation in the size and shape of developing zooids.

Details of flagellae were not well documented, though zooids appeared biflagellated, with a single eye spot. Flagellae were some 12 µm in length.

Some zooidangia were observed containing only 1 or 2 zooids, sometimes of unequal size, within a cell in which no pore was obvious. There was some evidence, in others, of fusion of zooids before release, these bodies having two eye-spots. Such fusion may be the result of unsuccessful separation of zooids during formation in the zooidangium (see van den Hoek 1963, p. 171).

Discharged zooids, washed into sterile estuary water medium produced large numbers of 5 to 7 celled germlings on the base of the petri dish within 2 weeks (Fig. 2.3w-z). These plants were attached to the base of the dish by cup-shaped holdfasts, some having already formed a 1 or 2 celled branch (Fig. 2.3x,y), while others showed, at this stage, incomplete cross wall development eg. (Fig. 2.3z).

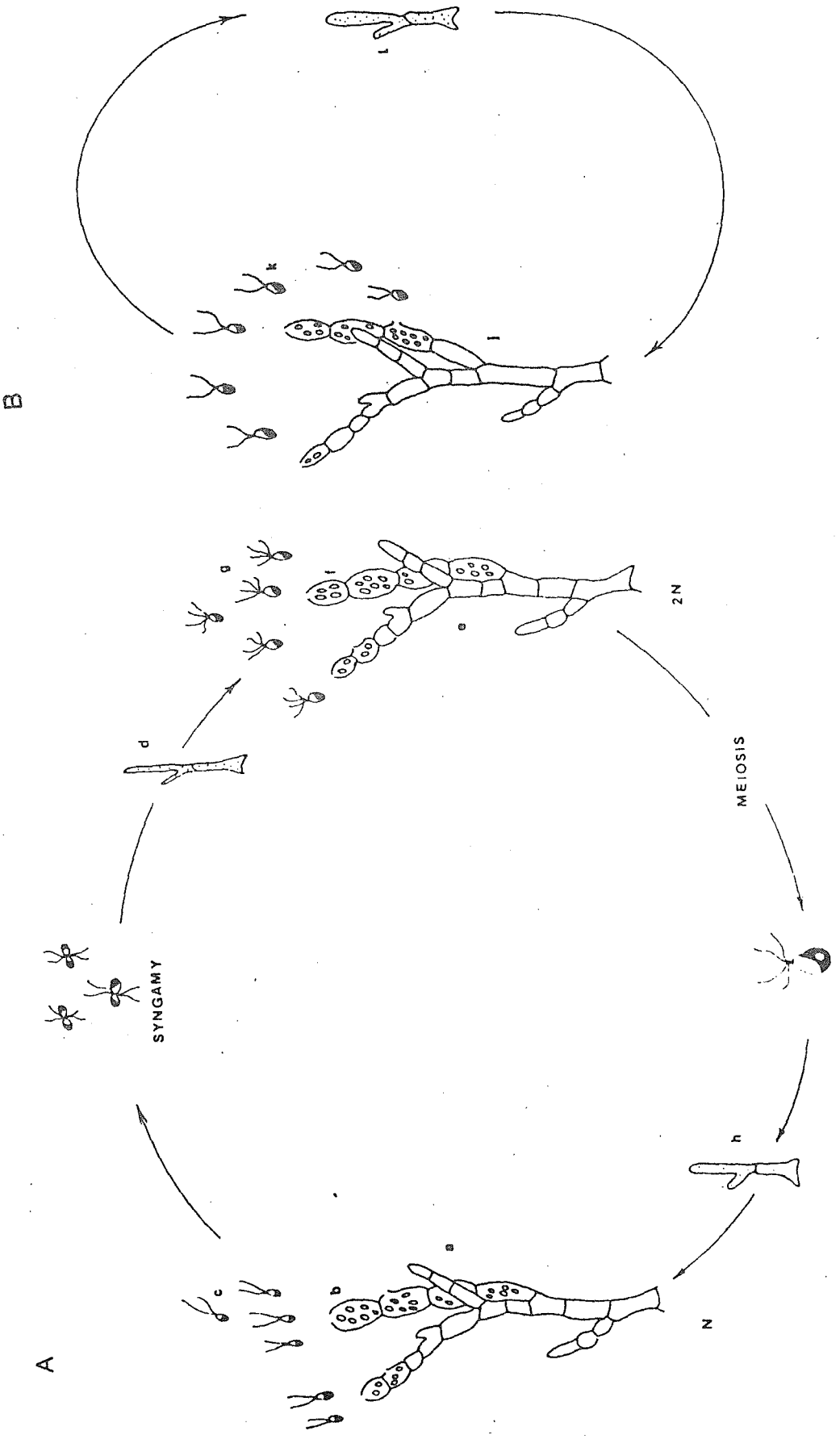
2.3.5 Life-history

The existence of a complete life-cycle in Cladophora is not necessary for successful regeneration (e.g. Mason 1965). However, a number of species show a regular alternation between a biflagellated isogamate producing generation and a quadriflagellate spore-producing generation (Fig. 2.4A), as for example C. rupestris (Schreiber cited van den Hoek (1963) and C. albida var. albida (Bliding cited van den Hoek 1963). Others have only been found reproducing by biflagellate zoospores (Fig. 2.4B), e.g. C. prolifera, C. albida var. albida (van den Hoek 1963). For several species no reproductive behaviour has yet been observed. These include C. echinus, C. aegagropila and C. battersii (van den Hoek 1963).

Figure 2.4 Life-cycles of *Cladophora*.

A. Some species display a regular alternation of isomorphic generations. Haploid plant of coenocytic filaments (a) produces fertile branches with gametangia (b) which rupture to release biflagellated isogametes (c). Following fusion of gametes (syngamy) new plants (d) develop which give rise to a diploid plant of coenocytic filaments (e) identical in appearance to the haploid. Filaments of the diploid develop fertile branches with sporangia (f). These rupture to release quadri-flagellate spores (g) which develop into new plants (h).

B. Certain species display only asexual reproduction. Haploid or diploid plant (i) produces fertile branches with sporangia (j) which rupture releasing biflagellates (or quadri-flagellate) zoospores (k) which develop directly into new plants (l).


B
A
SYNGAMY
MEIOSIS
2N
N
a
b
c
d
e
f
g
h
i
k
l

Though much of the observed growth of the species from Peel Inlet is presumably by vegetative fragmentation, the isolated sporing events are consistent with the life-cycle shown in Fig. 2.4B. No fusion of released spores was observed here and presumably therefore resulting new plants did not arise from the development of a zygote.

2.3.6 Ultrastructure and Cytology

Cells of the filament are coenocytic, typically with a dense chromatophore filling much of the cell and with few vacuolar spaces (see Fig. 2.1). Chloroplasts are discoid and numerous, and associated with them there may be large deposits of starch (Fig. 2.5D). Starch grains here appear somewhat large in comparison to those shown in fine structure photographs of other Cladophora species (e.g. Wik-Sjöstedt and Nordqvist 1970; McDonald and Pickett-Heaps 1976). Large numbers of starch grains, associated with cell stroma, have been observed in both Cladophora and Chaetomorpha (Chan et al. 1978). Changes in starch content of cells during growth may be linked to the ability of the alga to become buoyant. There have been no studies as far as we are aware on such changes in free-floating species, though some work has been reported with members of the Rhodophyta (Ozaki et al. 1967, Ramus 1972, Sheath et al. 1979). Starch contents in natural populations of a species of the red alga Gracilaria have been shown to fall with decreasing light intensities (Tripoli and Beth 1976). A similar response may occur here where, for example, large numbers of balls are exported from the algal bed under conditions of poor light (see Chapter 9), presumably as a result of reduced cell density arising from starch losses. This, and the entrapment of photosynthetically produced oxygen in filaments of the thallus, mentioned earlier, offer the two most likely mechanisms for the alga's buoyancy.

The cell wall of this species appears similar to that described for other Cladophorales, being lamellated with alternating microfibrillar and amorphous layers (Fig. 2.5C). However, the wall is particularly thick. The organization and deposition of cell wall material has been described in detail for a number of Cladophorales by Frei and Preston (1961, 1962).

No cytological data is available for this species though most published reports for a number of different species of Cladophora show a haploid number, from mitosis, of n = 12 (2n = 24) (Koshizaka 1973, Shyam 1980). A similar number has been implied for C. callicoma, where 12 bivalents were observed at diakinesis (Chaudhary 1978).

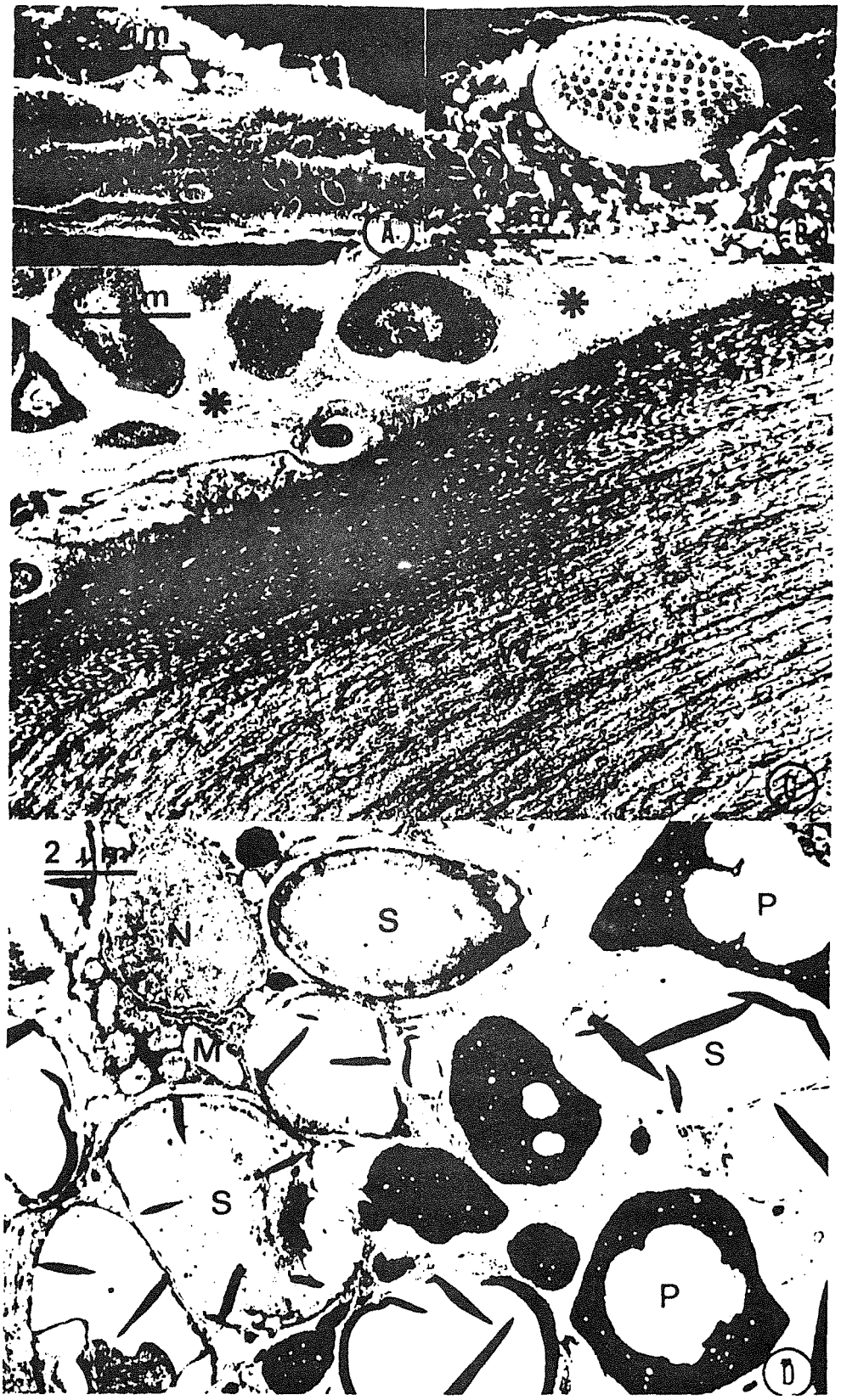
2.3.7 Taxonomy

Confusion surrounding the taxonomy of the genus Cladophora is well known and reflected somewhat in the variety of keys published to identify species growing in diverse habitats

Figure 2.5 A, B. *Cladophora* filament with adhering epiphytic diatoms and bacteria.

C. Section through the cell wall (W) showing bands of amorphous and microfibrillar layers. On the outside surface is a polysaccharide matrix (*) associated with algal and bacterial epiphytes (E).

D. Transverse section through the cell showing starch deposits (S) associated with the chloroplasts, nucleus (N), mitochondria (M) and phenols (P) associated with lipid material.


m
A
m
B
C
2 µm
N
S
P
M
S
S
P
D

(e.g. Chapman 1961, Nizamuddin and Begum 1973, Zaneveld and Willis 1974, Abbott and Hollenberg 1976). A major revision of the European members was undertaken by van den Hoek (1963) and it is this work which has been primarily consulted here.

Specimens of the alga have been formally lodged at the herbarium, Department of Botany, The University of Western Australia (UWA 2806a), and sent to Professor C. van den Hoek, Department of Plant Systematics, Biology Centre, University of Groningen, The Netherlands (UWA 2806b) and to Professor H.B.S. Womersley, Botany Department, University of Adelaide, South Australia (UWA 2806c).

The genus has been divided into 11 taxonomic sections according to van den Hoek (1963) based on a consideration of hypothetical derived or primitive characters. The species here is placed in the section Rupestres based on the following considerations.

1. Branches are apically inserted, growing into pseudodichotomies rather than being laterally inserted (this is a derived feature not found in the sections Affines, Basicladia, Aegagropila, Repentes and some species of Longi-articulatae).

2. No inversion of polarity (this is a derived feature not found in either the sections Basicladia or Aegagropila).

3. Growth predominantly intecalary rather than apical; weak acropetal organization (this is a primitive feature not found in the sections Glomeratae or Longi-articulatae).

4. Cells in general are apically swollen rather than swollen in the middle (this is a derived feature not found in the sections Affines, Chamaethamnion and Basicladia).

5. Sporangia opening through apical pores rather than halfway along the cell (this is a derived feature not found in the sections Cornuta, Affines, Chamaethamnion and Basicladia).

6. No rhizoids have been observed as yet, and certainly not from base to apex. (This eliminates, for the present, the sections Repentes, Aegagropila, Boodleoides and Affines).

Nomenclature

Attempts to identify the species before reproductive data were available suggested some affinity with C. battersii, with its spongy, aegagropiloid habit, lack of rhizoids and lack of sporing stages in the life-cycle. Present understanding suggests a closer affinity with C. albida, and, in particular, C. albida var. biflagellata. There are a number of parallels between C. albida and the species found here. In particular,

1. The similarity between cell dimensions of the filaments and reproducing bodies of both species.

2. Similarity in thallus morphology, though differentiation of species depending on whether they are free-floating or attached, is considered here a poor criterion for separation, particularly in view of the similarity between attached and free-floating specimens of this plant collected in Peel Inlet.

3. Ability to tolerate a wide ecological amplitude including extreme hypersalinity.

4. Growth in shallow reduced muddy habitats subject to H_2S production.

5. Robust thallus which can tolerate desiccation.

6. A phytogeographical distribution in the Northern Hemisphere consistent with the climatic conditions found at Peel Inlet (see van den Hoek 1969).

7. Similarity in behaviour of reproducing plants.

The alga here may be subject to morphological changes as a result of its estuarine habitat (e.g. Allender and Smith 1978). This is highlighted by the following comments of van den Hoek (pers. comm.) on the species from Peel Inlet.

"...Your material certainly much resembles Cl. albida. However, it is undoubtedly a separate species differing from Cl. albida by its aegagropiloid habit, its thick cell-walls, its dark cell-contents, and its 'hesitation' to sporulate. Possibly it is a new species..... There are several interesting parallels of (this) Cladophora. In Europe, Cl. battersii is an aegagropiloid relative of Cl. sericea; Cl. retroflexa an aegagropiloid relative of Cl. prolifera. In tropical and warm temperature Eastern America, Cl. jungiorum is an aegagropiloid relative of Cl. montagneana...., and Cl. blomquistii another aegagropiloid relative of Cl. prolifera. This suggests that lagoonal estuarine conditions may promote the origin of isolated aegagropiloid species... ."

2.4 CONCLUSIONS

1. The species of Cladophora which builds up to large populations in Peel Inlet, occurs predominantly as free-living spheres, from 1 to 3 cm in diameter, which form loose-lying beds covering extensive areas of the estuary floor. These beds may occur throughout the year in certain areas.

2. The species is morphologically distinct both from the frequently described 'lake-balls' of freshwater systems, and from other Cladophora species which cause similar pollution problems elsewhere.

3. Morphology and cell dimensions are similar (though not identical) in field-collected specimens and in those cultured in the laboratory.

4. The spherical habit of the plant can be observed in static cultures, and suggests that the ball-like appearance, at least in part, is a result of an organized radial branching system, rather than from a physical rolling movement in the bed.

5. Growth is predominantly by vegetative fragmentation of the thallus, fertile plants having only been observed once, during a limited period following the onset of river flow.

6. Sporulation was induced in non-fertile plants in the laboratory following their transfer from nutrient-depleted to nutrient-enriched medium. This is consistent with the observations of sporulation in the field. New plants were grown from released spores in laboratory culture.

7. Sporulation stages observed here are consistent with a life-cycle involving reproduction of biflagellated spores which develop directly into new plants. These stages have been photographed on video-tape.

8. The species has thick cell walls, a dense chromatophore with numerous chloroplasts, and typically large deposits of starch.

9. Present understanding of the taxonomy of this species suggests an affinity with Cladophora albida.

CHAPTER 3

THE BIOMASS AND DISTRIBUTION OF CLADOPHORA IN THE ESTUARY

3.1 INTRODUCTION

Mention was made in Chapter 1 that in general terms, macroalgae (including Cladophora) are essentially confined to Peel Inlet, with little contribution from the Harvey Estuary, which is dominated by phytoplankton.

This chapter provides estimates of the total biomass of Cladophora in the system, and on its distribution. The biomass data are then used to estimate the total amount of N and P in the alga. Most of the data come from intensive sampling surveys carried out at 36 sites in the estuary, in the autumn and spring of 1978 and 1979.

Estimates are also included of the biomass of Cladophora accumulated inshore at a site susceptible to large build-ups of the alga, for comparison with estimates from the whole estuary. Attention is also given to the results of a 'drifter-bottle' study, carried out to discover if shore accumulation might be derived only from adjacent parts of the estuary.

3.2 MATERIALS AND METHODS

3.2.1 Biomass and Cover

The main surveys were carried out on 14th-17th March 1978, 15th August 1978, 30th March 1979, and 14th September 1979. Cladophora was collected at each of the 36 sites (Fig. 3.1), using a coring device (64 cm^2 area), 5 replicates per site. Samples were returned to the laboratory, washed of adhering sediments, and sorted into Cladophora and other plants, and oven-dried at 80°C. Resulting dry weights were converted to grams per square metre.

Cover was assessed directly by diving at each site and expressing the contribution of each of the biota present as a percentage of the total visible.

3.2.2 Distribution

The distribution of Cladophora was determined using a computer programme (SYMAP; Dougenik and Sheehan 1977) to produce maps based on the percentage cover data for each survey. The programme uses the data provided for the sampled points and calculates values at intervening points, assuming that the seven nearest data points have an effect which is nearly proportional to the square of their distance from each intervening point. The data set was divided into 6 equal class intervals from 0 - 100 percent cover. Contours of the class boundaries are produced by different symbols and overtyping.

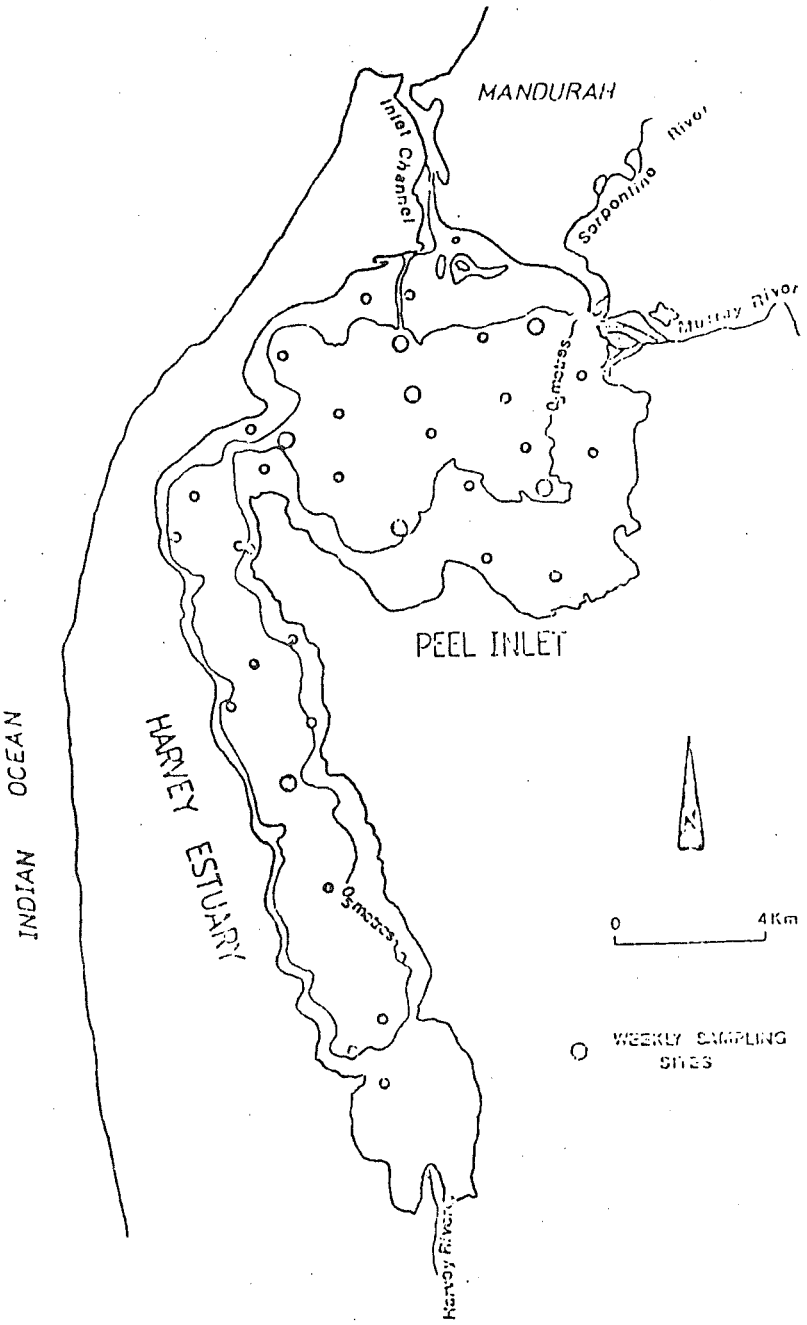


Figure 3.1 The location of the 36 sites used during intensive sampling surveys carried out in March and August 1978, and March and September 1979.

3.2.3 Biomass mapping and estimates of total biomass

Total biomass in the system was estimated from planimetry of computer-drawn maps of the observed biomass. These maps were produced in the same way as those of percentage cover, but using 10 class intervals.

Some sites may be broadly classified as areas of gross accumulation as opposed to 'beds' which may have been formed at the site, and at these areas of accumulation Cladophora biomass can build up to very high levels. This is particularly the case for those sites in the south-east sector of Peel Inlet. This results in a few sites with biomass in a range well above the remaining sites. Size classes for these maps were therefore proportioned accordingly, such that 9 classes were given equal weight and the last, including high biomass sites, was given a range 10 times that of the others. A digitizer (2000 Series, Summagraphics Corp., Connecticut, U.S.A.) was used to measure the areas of each size class interval.

Biomass in each size class was estimated by multiplying each size class area by the mean biomass per unit area for that class interval. The southern part of the Harvey and part of the Peel basin, total area 65 km^2 (about 50% of the whole), were taken as having zero biomass. The biomass estimates for all 10 classes were summed to produce an estimate of total biomass of Cladophora in the estuary.

In 1977, before intensive sampling, an estimate was made of biomass based on general field distribution and a few weighed samples.

3.2.4 Nutrients bound in Cladophora

Biomass of Cladophora for each survey, was converted to amounts of total nitrogen and phosphorus using as a conversion factor the mean concentration of N and P measured in Cladophora tissue from an algal bed at station 4 in Peel Inlet between August 1977 and November 1978. These are 26.3 mg N g^{-1} dry weight and 2.17 mg P g^{-1} dry weight.

3.2.5 Biomass accumulated in inshore beds

The biomass of Cladophora accumulated at the shores around the estuary was estimated for one site of algal accumulation at Coodanup during 1978 and 1979.

Relative dimensions of these beds were measured directly using a tape measure, and the biomass of each estimated from sample cores (area 0.05m^2) obtained from the centre of each bed. The resulting biomass was converted to total nitrogen and phosphorus using the factors desribed in 3.2.4.

3.2.6 Drifter Bottles

Fifty, 250 ml plastic bottles filled with estuary water and capped, were released at each of 6 stations in Peel Inlet on one occasion (15th August 1978). The bottles floated just under the water surface. Each contained a slip recording site of release, and a map on which any finder was invited to mark the site of collection, and return the slip to the Mandurah office of the Department of Fisheries and Wildlife.

3.3 RESULTS

3.3.1 Distribution

Distribution of Cladophora (as % cover) is shown for each sampling occasion in Figures 3.2 to 3.5. The major areas of distribution are confined to Peel Inlet, being virtually absent in the Harvey Estuary; this is consistent with general observations made during the course of the study.

In Peel Inlet, Cladophora is particularly pronounced in the eastern half, and in particular the north-east and south-east sectors. On the western shore, Cladophora is always present in Falcon Bay, and the cover is nearly always complete. In contrast, distribution in the eastern sector of Peel is more variable, there being marked differences between the maps, particularly between March and August 1978. The latter loss occurred at a period of significant (but 'average') river flow, when there was marked decline of biomass from the algal beds in the Inlet and a massive build-up of imported algae on the shoreline.

Cladophora cover was consistently poor in the western part of the central basin of Peel Inlet, an area extending from the entrance channel (Stick's Channel) south-west to the entrance to the Harvey Estuary.

3.3.2 Total Biomass and Nutrients

The total biomass of Cladophora in the system is shown in Table 3.1, along with corresponding amounts of total nitrogen and total phosphorus.

Errors associated with such calculations are necessarily large, and although there appears to be a general increase in biomass until March 1979, it is probably safer to merely conclude an average figure of about 30,000 tonnes of Cladophora over the period between 1976 and March 1979. After this, there was a marked decline to the September 1979 figure, which is only 16% that of the average for the previous 3 years. This corresponds to a decrease in total N from 800,000 to 130,000 kg, and of total P from 65,000 kg to 11,000 kg bound up in biomass.

Figure 3.2 Distribution of *Cladophora* in the Peel-Harvey Estuarine System, March 1978, shown as percentage cover. Data were collected from 36 sites represented by numerals and mapped using the SYMAP programme (Dougenik and Sheehan 1977). The range is from 0 to 100% divided into 6 equal size classes. Darkest shading indicates highest percent cover.

Figure 3.3 Distribution of *Cladophora* in the Peel-Harvey Estuarine System, August 1978, shown as percentage cover. Data were collected from 36 sites represented by numerals and mapped using the SYMAP programme (Dougenik and Sheehan 1977). The range is from 0 to 100% divided into 6 equal size classes. Darkest shading indicates highest percent cover.

Figure 3.4 Distribution of *Cladophora* in the Peel-Harvey Estuarine System, March 1979, shown as percentage cover. Data were collected from 36 sites represented by numerals and mapped using the SYMAP programme (Dougenik and Sheehan 1977). The range is from 0 to 100% divided into 6 equal size classes. Darkest shading indicates highest percent cover.

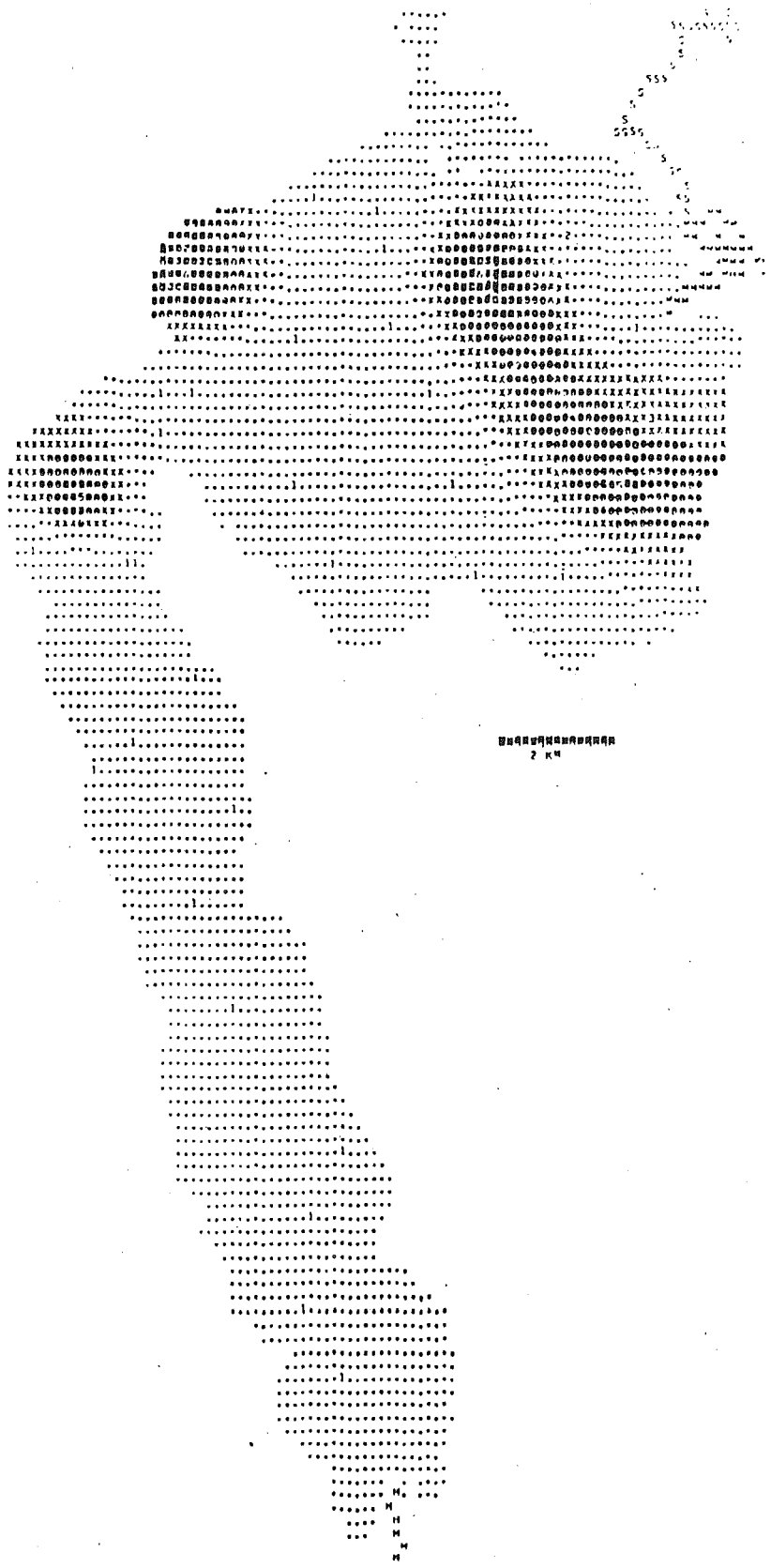


Figure 3.5 Distribution of *Cladophora* in the Peel-Harvey Estuarine System, September 1979, shown as percentage cover. Data were collected from 36 sites represented by numerals and mapped using the SYMAP programme (Dougenik and Sheehan 1977). The range is from 0 to 100% divided into 6 equal size classes. Darkest shading indicates highest percent cover.

Table 3.1 Biomass of *Cladophora* in the Peel-Harvey Estuarine System with corresponding nitrogen and phosphorus in the tissue. Data for 1978 and 1979 were calculated from planimetry of maps (SYMAP; Dougenik and Sheehan 1977) of biomass collected from 36 sites.

	Biomass dry weight (tonnes x 10^3)	Nitrogen (kg x 10^3)	Phosphorus (kg x 10^3)
1976-1977 [1]	16	500	50
March 1978[2]	19	500	41
August 1978	33	870	72
March 1979	46	1200	100
September 1979	5	130	11

[1] Data are preliminary estimates from the first year of the study (Atkins *et al.* 1977).

[2] Nutrients calculated using mean total nitrogen and phosphorus in *Cladophora* at station 4, Peel Inlet between August 1977 and November 1978.

3.3.3 Biomass and Nutrients accumulated inshore

The data for Cladophora accumulated in beds at Coodanup, Peel Inlet, is shown during 1978 and 1979 in Table 3.2.

The accumulated biomass on both occasions is similar, between 140 and 180 tonnes dry weight. Nutrient totals tied up in this biomass averaged 4000-5000 kg total N and 300-400 kg total P. This biomass, though extensively fouling the beach fronts, makes up less than 1% of the total biomass of the estuary as computed in Table 3.1.

3.3.4 Drifter Bottles

The distribution of drifter bottles on the shores following their release from sampling stations in Peel Inlet, is shown in Fig. 3.6. Most retrievals were made on shores susceptible to heavy build-up of Cladophora, particularly on the eastern shores (Coodanup, Yunderup, and on the west shore, Falcon Bay). It is clear that the Cladophora accumulated at these regions might, in principle, be at least in part derived from quite remote Cladophora beds. Bottles were also collected at the northern end of the Harvey Estuary consistent with the presence of Cladophora at this location as shown in Figures 3.2 to 3.5.

The broken lines are speculative, but indicate the simplest explanation of the movement of the bottles, and they result in a general anti-clockwise gyre of wind-driven surface water.

3.4 DISCUSSION

3.4.1 Distribution and Biomass

Consistently, Cladophora is most pronounced in the eastern half of Peel, and at the west near Falcon Bay. The distribution observed in the study was independently confirmed in November 1979, by interpretation and ground truthing of aerial photographs.[1] Algal cover is most variable in the south-east at Austin Bay, which may in part be a major site of accumulation. The most consistent areas of best cover, viz. Falcon Bay and the north-east sector of the Inlet at Coodanup, correspond to those areas where Cladophora forms extensive beds in the deeper water of the basin. Not surprisingly, the areas of heavy cover suggested in the maps generally lie close to the shores most susceptible to accumulation, particularly Coodanup, South Yunderup, Austin Bay, Falcon and Robert Bay. Drifter bottle results suggest that floating algae may be transported to a number of shores, in some instances far removed from their sites of origin, and may even be driven into the Harvey Estuary, presumably by wind-driven, surface water movement. Collection sites correspond relatively well with the shorelines most susceptible to algal accumulation.

Changes between sampling occasions in cover of the alga were not reflected in changes in the biomass. For example,

1. Footnote : see Appendix 4, this Report.

Table 3.2 Biomass of *Cladophora* accumulated in beds at Coodanup, Peel Inlet during 1978 and 1979 with corresponding nitrogen and phosphorus in the tissue.

Date	Site	Distance from shore (m)	Area of bed (m^2)	Biomass (tonnes dry weight)	Nitrogen (kg)	Phosphorus[2] (kg)
August 1978	West Coodanup	200	3350	50	1300	109
	" "	320	2856	72	1980	160
	East Coodanup	400	150	2	42	3
	" "	400	1470	16	413	34
				140	3735	306
September 1979	West Coodanup	100-300	18200[1]	176[1]	4600	380
	East Coodanup	300	400	4	112	9
				180	4712	389

[1] Sum of 26 separate beds at this site.

[2] Nutrients calculated using mean total nitrogen and phosphorus in *Cladophora* at station 4, Peel Inlet between August 1977 and November 1978.

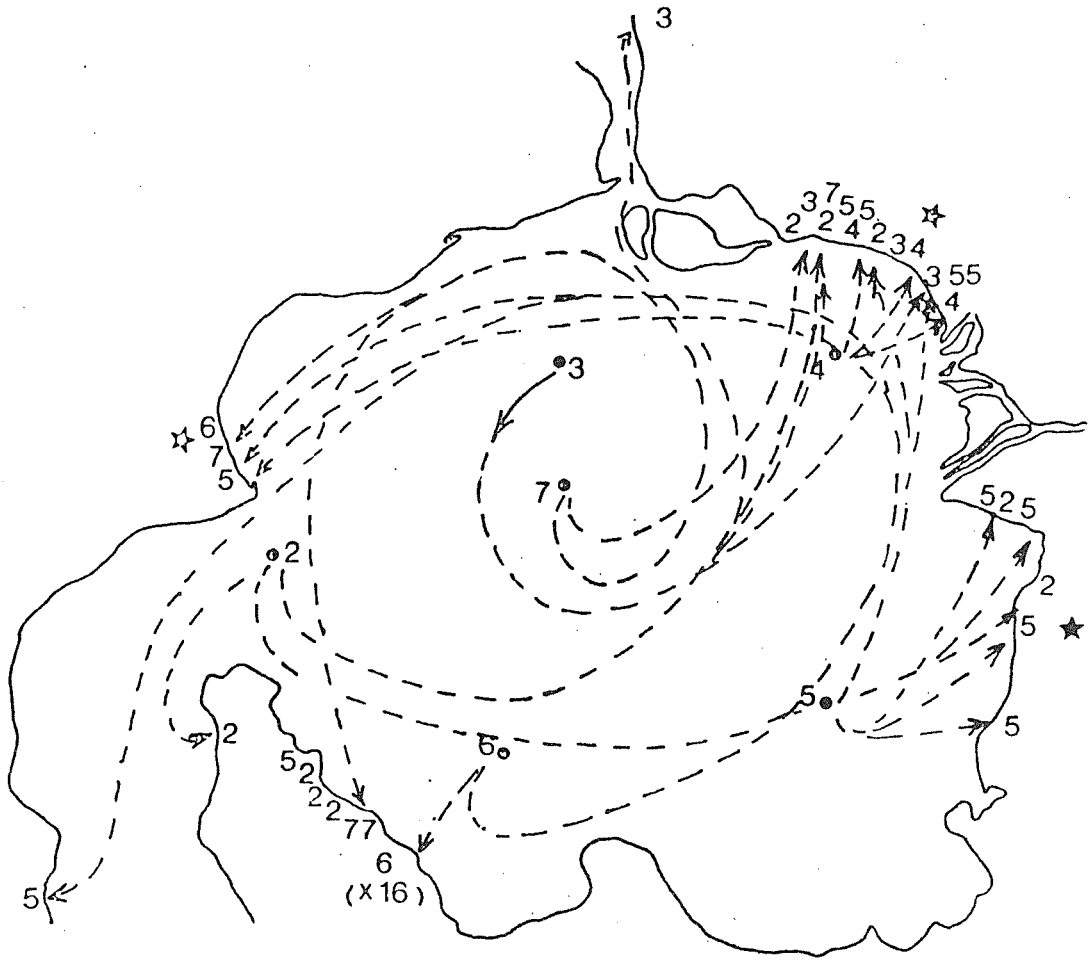


Figure 3.6 The distribution of drifter bottles released at sampling stations 2 to 7 in Peel Inlet, 15th August 1978.

Numbers at the shore represent locations of collection and indicate the sites at which the bottles had been released.

Asterisks indicate the general shore areas most susceptible to accumulation of *Cladophora*.

The line to the south-west of station 3 shows the observed path of drifters from that station. All other lines (broken) are speculative, but represent the simplest, reasonably consistent explanation for bottle distribution. They suggest a general anti-clockwise gyre of wind-driven, surface water movement.

some sites which had a low % cover of Cladophora gave relatively high biomass in coring, the Cladophora having been covered by a thin layer of sediment. Maps based on visible cover only reflect distribution at the surface.

It might be noted in passing that in a later Chapter evidence is presented that light limits photosynthesis immediately below the surface of an algal bed, and so the % cover may convey a valuable impression of the extent of the productive algal bed area.

The total biomass in the estuary offers some indication of the amount of Cladophora potentially available for growth under suitable conditions, particularly since the alga is tolerant to long periods of burial in the sediments (Gabrielson et al. 1980). The marked reduction in total biomass after March 1979 (Table 3.1) suggests that the presence of Cladophora in the Inlet is declining.

While the sampling problems and the method used to estimate total biomass must be surrounded by considerable, ill-defined errors, the conclusion seems inescapable that the amount present in beach drifts at Coodanup represented a small proportion of the total Cladophora biomass in the estuary. Biomass of alga imported to the beaches is small in comparison with total amounts in the estuary, as reflected by the data for Coodanup which were less than 1% of the total in the estuary during 1978.

Assuming these figures are typical for import to the beaches, accumulation of the magnitude observed in 1978 at Coodanup at even 10 other sites around the shores would only account for about 5-6 % of the total biomass present at this time. Nevertheless, such a small percentage of the biomass produces effective beach fouling.

The loss of some 20,000 tonnes between March 1979 and September 1979 can not be readily explained. Losses to the beach are not of sufficient magnitude. In March 1979, some burial was seen in the field (sites with low cover and high biomass) and it is possible that much of the loss in biomass is accounted for by decomposition.

In September 1979, when Cladophora biomass had fallen, there were marked increases in other filamentous algae, particularly Chaetomorpha and Enteromorpha, and this was also noted during regular sampling trips in the estuary. However, because of the nature of the corer used, they are poorly represented in the samples taken.

3.5 CONCLUSIONS

1. Cladophora is confined to Peel Inlet and the northern section of the Harvey Estuary.

2. Cover is consistently poor in the central basin of Peel Inlet between Stick's Channel and south-west to the entrance to the Harvey Estuary. Most distribution is confined to the eastern sector of the Inlet and to the west at Falcon Bay.

3. Results of a drifter bottle study suggest that floating Cladophora may be distributed to a number of shores around the Inlet, in some cases far removed from their site of origin. The location of release of bottles on the shores is consistent with transport by a wind-driven anti-clockwise gyre of surface water movement.

4. Total biomass Cladophora in the estuary between March 1978 and March 1979 averaged 30,000 tonnes dry weight corresponding to 800,000 kg N (total N) and 70,000 kg P (total P).

5. Total biomass of Cladophora declined to 5000 tonnes dry weight in September 1979 corresponding to 130,000 kg N and 11,000 kg P.

6. Measurement of Cladophora biomass accumulated in beds at Coodanup on two occasions in 1978 and 1979 averaged 160 tonnes dry weight, corresponding to 4000 kg of N and 400 kg of P. This biomass accounts for less than 1% of the total biomass of Cladophora in the estuary.

7. The marked decline in biomass between March 1979 and September 1979 cannot be readily explained by loss to the beaches.

CHAPTER 4

SEASONAL CHANGES IN ENVIRONMENTAL FACTORS AND NUTRIENTS

4.1 INTRODUCTION

This chapter deals with the seasonal variation in environmental factors and nutrients, particularly nitrogen and phosphorus, associated with a site in the Peel estuarine basin where there have been extensive and persistent beds of Cladophora. The data reported here were collected along with biomass measurements from 1976 to 1979, in order to relate observed changes in biomass to seasonal variation in these factors.

The first year of the study, 1976, was one of inventory aimed at defining the major physico-chemical parameters of the system (Atkins et al. 1977). Water samples above the algal bed were collected monthly, along with biomass, at Harbour and Lights Post 46 (Fig. 5.1).

These results suggested that a shorter-term sampling programme might be appropriate, and from August 1977 water samples were collected at weekly intervals from 7 stations, including the Harvey Estuary. This decision was fortuitous since the following 2 years, 1978 and 1979, included periods of substantial river flow, necessitating a more frequent sampling collection.

The results obtained for one of these sites and Post 46 are presented here to provide a continuous record over the 4 year period. The results of short-term (24 hour) studies on particular occasions are discussed briefly and the variation in nutrients and environmental factors compared with corresponding weekly temporal changes.

Concentrations of nutrients in the overlying water are compared with those present within the algal bed, and their possible role in cycling of P and N in the bed discussed briefly.

Finally, nutrient concentrations observed here are compared with those reported for estuarine and freshwater systems elsewhere, and the degree of eutrophication of waters associated with the Cladophora growth in this study, assessed from water quality criteria reported in the literature.

4.2 MATERIALS AND METHODS

4.2.1 Sampling Sites

From April 1976 water samples were collected at approximately monthly intervals at Post 46 (see Figure 1.1) a site in the estuarine basin situated in the north-east section of Peel Inlet close to the mouths of the Murray and Serpentine Rivers. This site was chosen for seasonal biomass measurements (see Chapter 5) but because of its proximity to a navigation channel was unsuitable for demarkation as an experimental plot. For this reason a similar site (station 4) was chosen a few hundred metres north (Figure 1.1) which was clear of boating traffic yet in other respects essentially the same as the previous site. This latter station was one of 7 located around the Peel-Harvey estuarine system from which water quality was monitored at weekly intervals from August 1977 to September 1979.

4.2.2 Sample Collection

Surface water samples were initially collected by filling high density polyethylene bottles (Duranol, Melbourne) from 10 cm under the surface. Bottom samples were taken using a Hales Water Sampler (Welch 1948) which effectively collected water from 30 cm above the algal bed. Samples were stored in high density polyethylene bottles on ice in the field and frozen on return to the laboratory. Samples for ortho-phosphate determination were stored in low density polythylene bottles (Trojan, Joyce Bros. West. Aust.) which were previously iodine-impregnated (APHA 1971). With the start of the weekly sampling programme in August 1977, water samples were collected in 150 ml sealable polyethylene bags ("Whirlpak", Nasco, Kansas, USA) on ice in the field and frozen on return to the laboratory.

Water samples from within the algal bed ("interalgal water") were collected by diving and removing by suction, the interstitial water from between algal balls with a 50 ml syringe. These samples were then released into Whirlpaks and treated identically to water-column samples.

4.2.3 Water Depth and Secchi Depth

Water depth was measured with a graduated plumb-line. Secchi readings were recorded with an 8 cm disc painted with black and white quadrats. Readings were always taken on the unshaded side of the boat.

4.2.4 Light Attenuation and PAR

Light profiles were obtained at weekly intervals from August, 1977 at station 4 and from August 1978 at Post 46 using an underwater sensor (LiCor, Lincoln Instrument Company, Nebraska, USA) to measure photosynthetically active radiation (PAR). The slope of the line from a regression of log PAR with depth was used to calculate the attenuation coefficient, E, through the water column on each occasion. Bottom readings were obtained by extrapolating the regression line to the depth of the algal bed. Light readings for both sites were usually recorded in the mid-afternoon. Attenuation coefficients are reported here as positive values, following the convention of Kirk (1977).

4.2.5 Temperature and Salinity

Profiles of salinity and temperature were measured using a model 602 Hamon Salinity-Temperature Bridge (Autolab, Sydney) calibrated with standard seawater (Standard Seawater Service, Charlottenlund, Denmark). During the early stage of the study (April to August 1976) salinity was estimated from chlorinity measurements of surface and bottom waters using a chloride titrator (Aminco, American Instrument Co. Inc. Maryland, USA) and expressed as $^{o}/oo$ using conversion tables (Strickland and Parsons 1972). Water temperatures on these occasions were measured with a mercury thermometer.

4.2.6 Dissolved oxygen

Dissolved oxygen was measured by Winkler titration (APHA 1971) in the first year of the study, and subsequently with a portable oxygen meter (Model 2110, Delta Scientific, Lindenhurst, New York). Readings were taken in the mid-afternoon. Percentage saturation was calculated using temperature-salinity records for the site and an oxygen solubility nomograph (Strickland and Parsons 1968).

4.2.7 pH and Alkalinity

pH was measured directly using a portable pH meter (Metrohm E488, Herisau, Switzerland). Total alkalinity was measured on surface and bottom samples using the indicator method (APHA 1971) and expressed as mg ℓ^{-1} $CaCO_3$.

4.2.8 Phosphorus

Total phosphorus was measured by digestion of water samples with a 1 : 1 mixture of concentrated nitric and perchloric acid and analysis of the resulting total orthophosphate using the single solution method (Major et al. 1972). During the early stages of this study, digestion was

carried out in 150 ml conical flasks placed on electric frypans, but this step was modified after August 1977 with the use of a programmable block digester (Windrift Instruments, Welshpool (West. Aust.) which allowed unsupervised simultaneous digestion.

Orthophosphate was measured colorimetrically by the single solution method (Major et al. 1972), based on the technique of Murphy and Riley (1962). Samples collected weekly were filtered in the field through 0.45 µm filters (Millipore Corp., Massachusetts). 'Organic' phosphorus was calculated from the difference between total phosphorus and orthophosphate present in undigested samples.

4.2.9 Nitrogen

Ammonia-nitrogen was measured on unfiltered water samples by the cyanurate method (Dal Pont et al. 1974). Nitrate plus nitrite-nitrogen was determined on unfiltered samples during the first year of the study using large volume cadmium reduction columns (APHA 1971). Analyses after October 1977 were undertaken using a cadmium reduction column built into an autoanalyser (Technicon Industrial Method 158-71 lW, Technicon Industrial Systems, Tarrytown, New York).

Organic nitrogen was determined by Kjeldahl digestion. During the first year of the study, samples were digested with concentrated sulphuric acid using mercury catalyst tablets (BDH, Poole, England) and treatment with phosphate buffer to remove ammonia. Analyses were carried out by distillation on a Markham still and titration with HCl, using boric acid to detect the end port (APHA 1971). After 1977, digestion of samples was carried out using a programmable block digester (Windrift Instruments, Welshpool, West. Aust.; Industrial Method 376-75W/B, Technicon Industrial Systems, Tarrytown, New York) and the resulting ammonia subsequently analysed with an autoanalyzer (Industrial method 329-73 W/B, Technicon Industrial Systems, Tarrytown, New York). The ammonia level of the sample was then subtracted from this figure to give 'organic' nitrogen. Total nitrogen was determined from the sum of organic, ammonia-, nitrate- and nitrite-nitrogen.

4.2.10 Diel Variation

On two occasions during winter and in late summer-early autumn, water samples were collected above the Cladophora bed at station 4, every 2-3 hours over a 24 hour period. The first diel study was conducted on 16th and 17th August 1978 when rivers were flowing, the water was stratified, and nutrient concentrations at the growth site were high. The second was run over 9th and 10th March 1979 under typical summer conditions, when river flow was negligible, water temperatures high, and the water hypersaline and not

stratified. Changes in nitrogen and phosphorus were recorded along with temperature, salinity, dissolved oxygen and chlorophyll 'a'. Because of the importance of effects of water movement and wind action to an interpretation of observed changes, a current meter was used to measure water changes. Wind data were obtained from the Robert Bay weather station (Black and Rosher 1980). On these occasions parallel data sets were collected at station 1, a site in the Harvey Estuary where Cladophora does not occur. No attempt has been made here to interpret in detail the results of these diel studies, this being reported more fully elsewhere (Lukatelich and McComb 1981), however, the extent of variation in nutrients observed here over a short-term sampling interval, are compared statistically with the seasonal data collected regularly over a longer period, from weekly sampling, using the approach outlined by Naiman and Sibert (1977).

4.2.11 Sulphate-Sulphur

Sulphate was measured on composite surface and bottom water samples by the barium sulphate precipitation technique (Allen et al. 1974).

4.3 RESULTS

4.3.1 Water Depth/Secchi Depth

Both Post 46 and station 4 are located in the estuarine basin where water depth is typically greater than 1.5 m. Between April 1976 and September 1979 water depth averaged 1.60 ± 0.02 m S.E. at Post 46 and 1.86 ± 0.01 m S.E. at station 4 between August 1977 and September 1979. Average Secchi depth for these sites over the corresponding time intervals were 1.52 ± .03 S.E. and 1.63 ± .05 m S.E. respectively.

During 1976 Secchi depth remained greater than or equal to water depth except in August, when there was a short period of river flow. Water clarity remained high throughout 1977 at Post 46, with no Secchi readings less than water depth. In contrast, during 1978 when there was substantial river flow in winter, water clarity was reduced markedly for a number of weeks, and consequently Secchi readings were lower; for example at station 4 in September 1978 Secchi depth was only 0.45 m.

4.3.2 Light Attenuation and PAR

Light profiles were measured at station 4 from August 1977 to September 1979 and at Post 46 from August 1978 to September 1979. Resulting attenuation coefficients averaged $0.46 \pm 0.02\ m^{-1}$ and $0.48 \pm 0.04 m^{-1}$ (S.E.) for both sites respectively. The minimum recorded attenuation coefficient was $0.15\ m^{-1}$, in December 1977, when water clarity was

extremely good above the Cladophora bed. The maximum recorded was 1.60 m^{-1} during July 1978 when river flow had effectively removed light reaching the estuary floor.

These coefficients are high compared with the range reported for nearby coastal waters and in Cockburn Sound, the latter being subject to the development of large phytoplankton blooms with resulting increase in turbidity (Chiffings 1979).

No light attenuation data were available during the first year of the study, water clarity at this time being gauged from Secchi depth readings. These were continued when light profiles were begun so that they might provide some estimate of attenuation during the first year of the study. The relation between attenuation of PAR from the profiles with corresponding Secchi readings is shown for station 4 and Post 46 data together in Figure 4.1. Only those Secchi readings less than water depth are shown. The relationship is significant ($r = 0.74$; $n = 54$). Substituting the lowest Secchi depth measured in August 1976, at 1.5 m, into this equation results in an estimated attenuation coefficient for this period of about $0.5m^{-1}$. PAR reaching the algal bed averaged 108 ± 91 (S.D.) $\mu E\ m^{-2}\ sec^{-1}$ between August 1977 and September 1979, the maximum occurring during summer (December 1977) at about 400 $\mu Em^{-2}\ sec^{-1}$. Minimum PAR was recorded in winter (July 1978; July 1979) when bottom readings were effectively zero.

4.3.3 Temperature and Salinity

Changes in salinity and temperature above the Cladophora bed are shown in Fig. 4.2. Only surface water temperatures have been plotted since these did not differ from bottom readings by more than 1°C. Temperatures ranged from 27°C in summer to 12°C in winter. Because of the shallow nature of the estuary, changes in water temperatures follow changes in air temperatures fairly closely (Atkins et al. 1977).

Seasonal changes in salinity were distinct, with hypersalinity occurring each summer, reaching a maximum of over 50°/oo in 1978. During summer the water column is well mixed as suggested by the lack of difference between surface and bottom salinity. In contrast, during winter, salinity levels fell to as little as 2°/oo (surface water; July 1978), and there was a distinct stratification with the onset of river flow in winter. Not unexpectedly, the lowest salinity recorded in the water column above the bed over the 4 years of the study occurred in July 1978, when there was the most significant and prolonged river flow of the entire study. In contrast, during 1977, the driest year of the study, the highest winter salinities were recorded; these did not fall below 15°/oo.

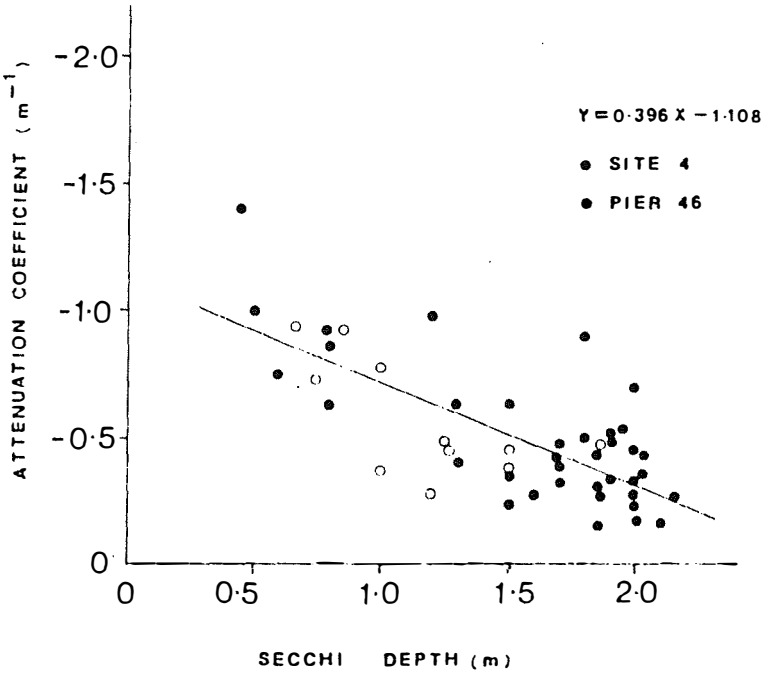
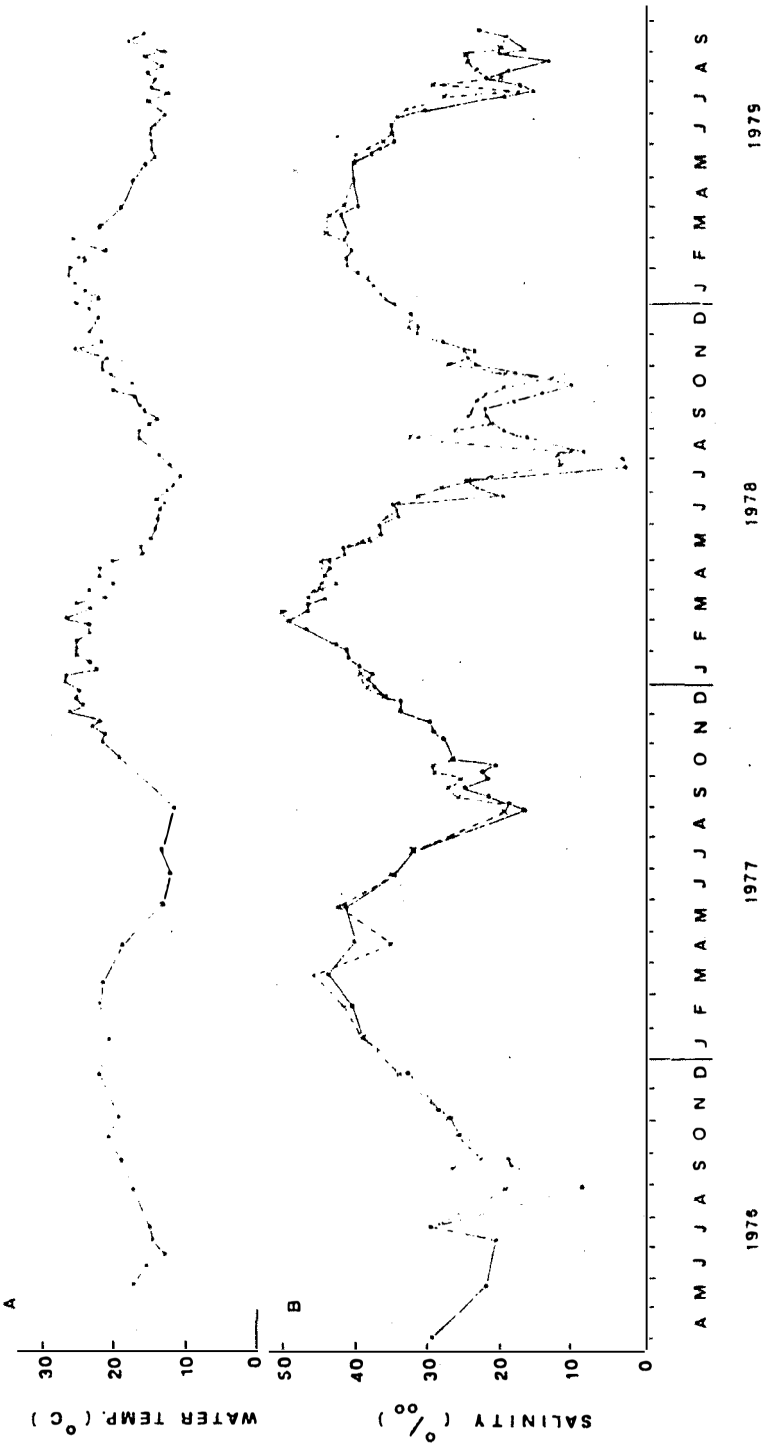


Figure 4.1 Light attenuation coefficients (m^{-1}) as a function of corresponding Secchi depth (m). Data were collected weekly at station 4 and Post 46, Peel Inlet.

Figure 4.2 Change in A) water temperature (^{o}C) and B) surface (full line) and bottom (dashed line) salinity ($^{o}/oo$) above the *Cladophora* bed, Post 46, Peel Inlet from April 1976 to September 1979. Data up to August 1977 were collected monthly, and weekly thereafter.


A
B
WATER TEMP. (°C)
SALINITY (‰)
30
20
10
0
50
40
30
20
10
0
A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S
1976
1977
1978
1979

4.3.4 Dissolved Oxygen

Dissolved oxygen averaged 8.02 ± 0.17 (S.E.) mg ℓ^{-1} at the surface, and 7.23 ± 0.25(S.E.) mg ℓ^{-1} above the algal bed. These data combine monthly oxygen readings at Post 46 from June 1976 to July 1977 with those readings obtained weekly from August 1977 to September 1979.

Dissolved oxygen in the water was typically well above a concentration considered to be critical to aquatic life (Water Authority Criteria cited Welch 1980) with highest values occurring on the bottom immediately above the algal bed during fine conditions, when the algal bed was actively photosynthesising.

Surface dissolved oxygen ranged from 4.2 mg ℓ^{-1} (68% saturation) in March 1978 to 14.2 mg ℓ^{-1} (167% saturation) in August 1979. Bottom oxygen levels were from 1.3 mg ℓ^{-1} (16% saturation) in August 1978 to 18.0 mgℓ^{-1} (230% saturation) in September 1977. The latter value was recorded during fine weather when field records showed substantial amounts of floating Cladophora in the estuary, particularly at station 4.

Bottom readings were typically highest during the summer months, particularly November-December 1978, when oxygen was consistently greater than 11 mg ℓ^{-1} over 3 consecutive weeks. Low bottom oxygen levels were observed in September and October 1978, the latter coinciding with high turbidity and poor light penetration which reduced photosynthesis within the algal bed.

4.3.5 pH and Alkalinity

The pH of estuary water above the Cladophora bed averaged 8.18 ± 0.03 (S.E.) and 8.20 ± 0.03 (S.E.) for surface and bottom water respectively (all data combined). The range was 7.10 to 8.57 at the surface and 7.70 to 8.60 at the bottom. pH was typically lowest during winter each year, when estuary water was diluted by incoming river water. The values here are within the range of those reported for other estuarine systems (e.g. Spencer 1956).

Changes in alkalinity from November 1978 to September 1979 at station 4 averaged 104 ± 3 (S.E.) and 107 ± 2 (S.E.) mg ℓ^{-1} $CaCO_3$ for surface and bottom water respectively, with a range during this time interval, of 64 to 122 and 78 to 120 for surface and bottom water respectively. Lowest alkalinities were observed during winter when there was an input of river water to the site and not unexpectedly coincided with lowest measured pH, as for example in July 1979.

4.3.6 Phosphorus and Nitrogen

Nutrient levels are presented in Figures 4.3 and 4.4. Concentrations of nitrogen and phosphorus over the 4 year period were averaged excluding the winter data (June to August inclusive) and are shown in Table 4.1. The winter data were averaged separately and are given in Table 4.2.

Neglecting winter concentrations, orthophosphate levels were below 10 $\mu g\ \ell^{-1}$ each year in both surface and bottom water, giving corresponding average total phosphorus concentrations of 52 and 46 $\mu g\ \ell^{-1}$ respectively. Ammonia dominated the inorganic nitrogen components of the water column in both surface and bottom water. Corresponding nitrate+nitrite levels were low (less than 5 $\mu g\ \ell^{-1}$). These data, in combination with organic nitrogen, result in mean total nitrogen concentrations of $\sim$590 $\mu g\ \ell^{-1}$ for both surface and bottom water.

Water column nutrients remained low each year except during winter following the onset of river flow. This was particularly the case in 1978, a year in which there was the greatest and most prolonged freshwater input of nutrients in the entire study, and to a lesser extent in 1979, where the Murray River showed the highest volume flow, but over a much shorter time interval than the previous year (Fig. 4.5).

The inorganic nitrogen component above the Cladophora bed on these occasions was dominated by nitrate+nitrite at the surface, reaching a maximum of nearly 3.5 mg ℓ^{-1} (Figure 4.4), while bottom water, close to the algal bed, was relatively higher in ammonia.

Orthophosphate levels in winter were also high during 1978 and 1979, the mean concentration being up to four times greater than for a corresponding period in previous 'dry' years. Highest contributions for bottom water occurred in 1978; the observed maximum in 1979 was lower, corresponding to a less prolonged river flow.

There was a close relationship between increased nutrient concentrations derived from river flow and increased light attenuation above the Cladophora bed, as shown in Fig. 4.2 for orthophosphate. The attenuation coefficient remained low (0.5 m^{-1}) throughout the non-flow period of the year, reaching a maximum when nutrient input to the site was highest. At this time there was effectively no light reaching the algal bed. During the first two years, particularly 1977, when both river flow and nutrient concentrations were very much reduced compared to subsequent years, light reaching the algal bed would have been little affected by increased turbidity resulting from river flow. This is borne out to some extent by the high Secchi disc readings recorded throughout most of 1976 and 1977.

Figure 4.3 A) Light attenuation coefficients (m^{-1}) collected weekly at Post 46, Peel Inlet from August 1977 to September 1979. B) Change in surface (full line) and bottom (dashed line) orthophosphate concentration ($\mu g\ \ell^{-1}$) and C) change in surface (full line) and bottom (dashed line) 'organic' phosphorus concentration ($\mu g\ \ell^{-1}$) in the water above the *Cladophora* bed. Nutrient data shown were collected monthly between April 1976 and August 1977 and weekly from August 1977 to September 1979.

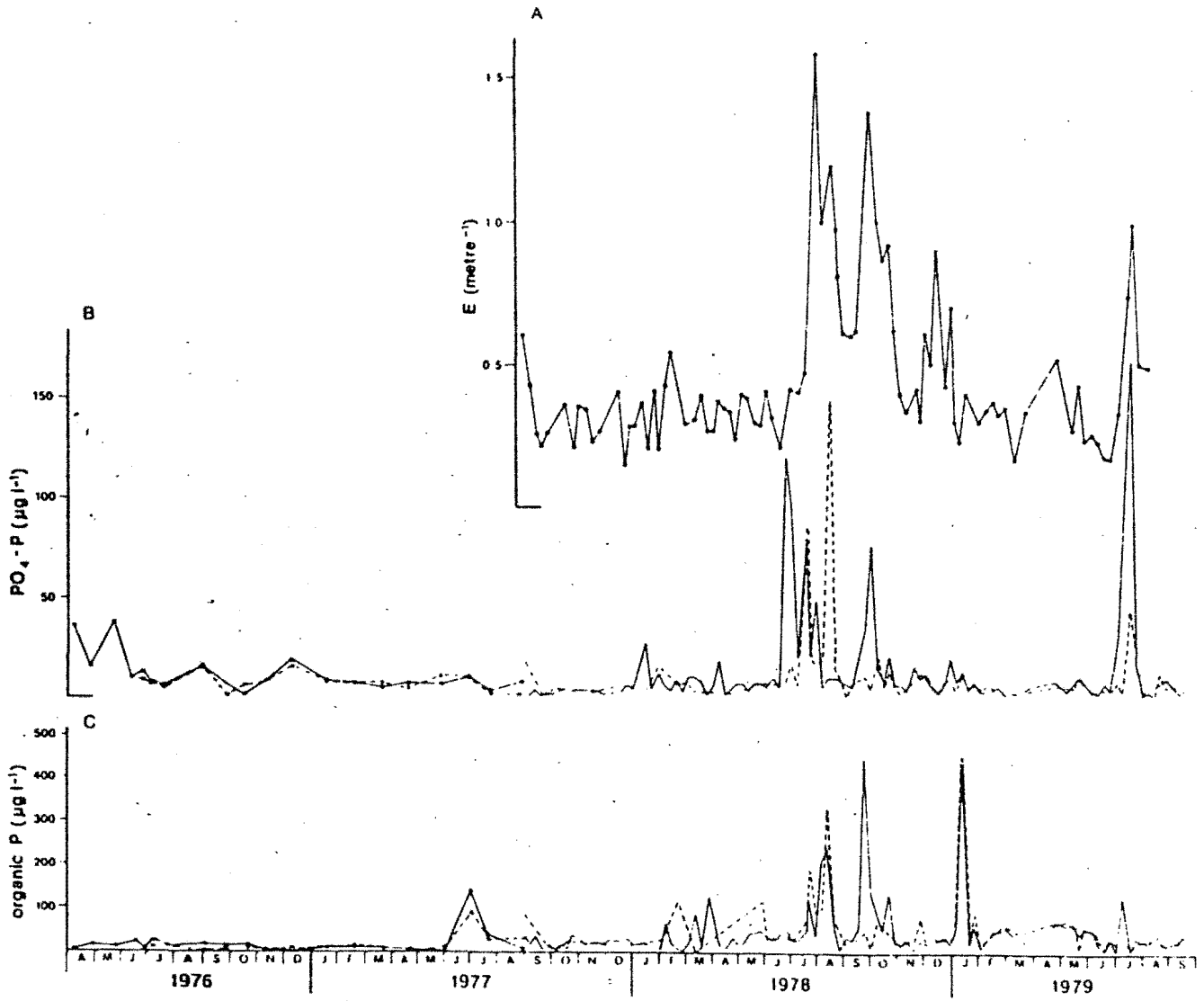

A
E (metre⁻¹)
1·5
1·0
0·5
B
PO4 - P (µg l⁻¹)
150
100
50
C
organic P (µg l⁻¹)
500
400
300
200
100
A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S
1976
1977
1978
1979

Figure 4.4 A) Change in ammonia-nitrogen concentration (μg ℓ^{-1}), B) nitrate+nitrite-nitrogen concentration (μg ℓ^{-1}) and C) 'organic' nitrogen concentration in the water above the *Cladophora* bed. Data were collected monthly from April 1976 to August 1979 and weekly thereafter. Surface values are shown with a full line; bottom values with a dashed line.

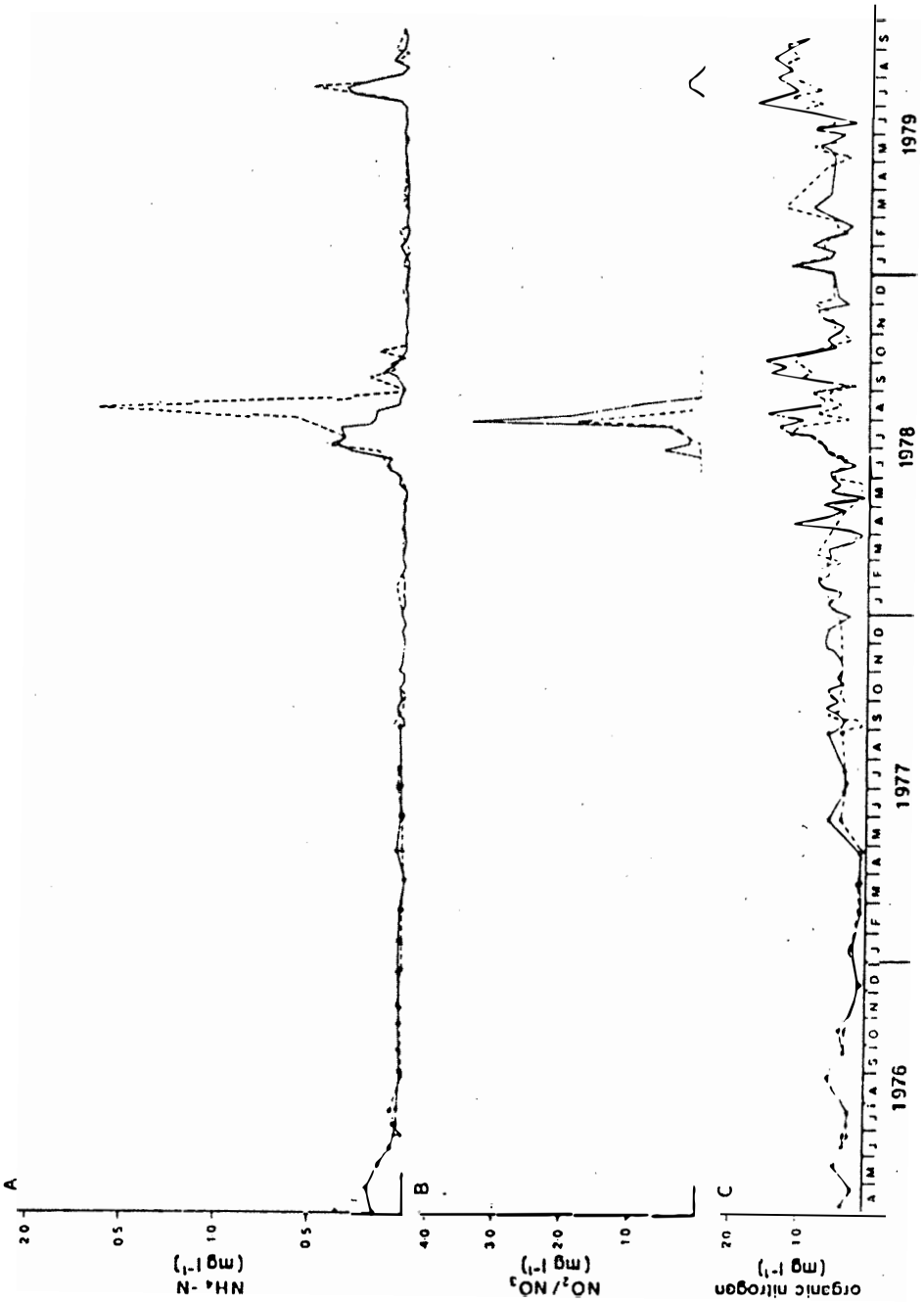

A
B
C
NH_4 -N (mg l^{-1})
NO_2/NO_3 (mg l^{-1})
organic nitrogen (mg l^{-1})
1976
1977
1978
1979

Table 4.1 Concentrations of nitrogen and phosphorus (µg litre^{-1}) in the water column above the *Cladophora* bed. Data shown are the means ± S.E. of surface and bottom water measured between April 1976 and September 1979, excluding the winter period (June to August) of each year. Data from April 1976 to August 1977 were obtained from monthly measurements at Post 46 while those from August 1977 to September 1979 were obtained from weekly records at station 4, Peel Inlet.

Phosphorus		$\bar{x}$ ± S.E.	Nitrogen		$\bar{x}$ ± S.E.
PO_4-P	surface	7.5 ± 1.0	NH_4-N	surface	24.8 ± 3.0
	bottom	6.0 ± 0.5		bottom	28.2 ± 3.9
Organic-P	surface	44.7 ± 7.9	NO_3-N	surface	4.3 ± 0.6
	bottom	38.8 ± 6.3		bottom	3.3 ± 0.2
			Organic-N	surface	558 ± 36
				bottom	556 ± 30

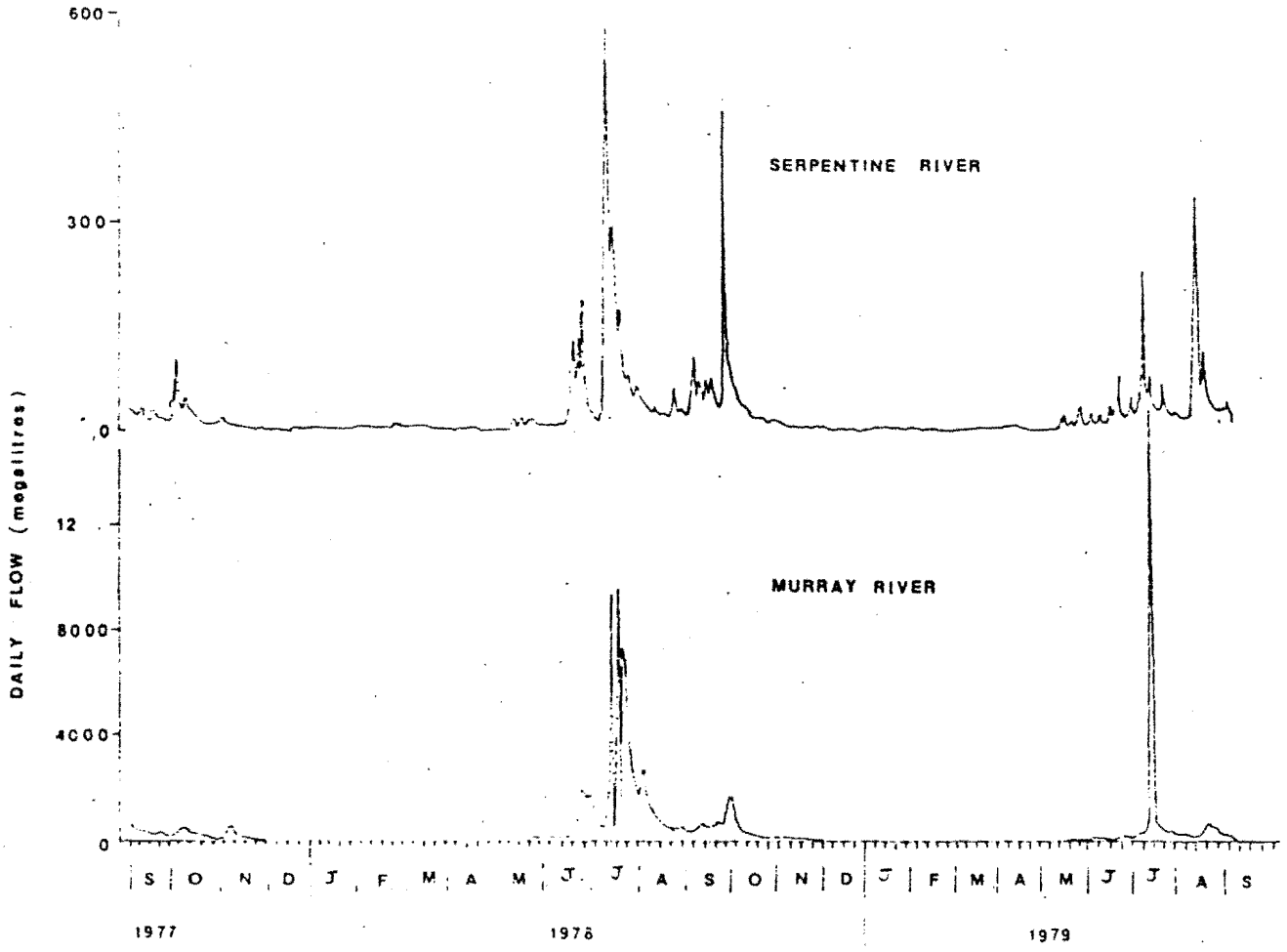
Table 4.2 Concentrations of nitrogen and phosphorus ($\mu g\ litre^{-1}$) of surface (S) and bottom (B) water for each winter (June to August inclusive) of the study. Data shown are means ± standard error of monthly readings at Post 46 and weekly readings at station 4. The maximum value shown refers to the highest concentration recorded from each data set.

		1976*		1977*		1978		1979	
		$\bar{x}$ ± S.E.	Max. value	$\bar{x}$ ± S.E.	Max. value	$\bar{x}$ ± S.E.	Max. value	$\bar{x}$ ± S.E.	Max. value
PO_4-P	S	14 ± 5	35	7 ± 2	11	30 ± 10	116	31 ± 14	165
	B	13 ± 5	-**	6 ± 2	11	25 ± 10	143	9 ± 3	42
Organic-P	S	14 ± 3	23	48 ± 27	150	74 ± 20	256	42 ± 10	136
	B	11 ± 2	15	42 ± 13	92	92 ± 23	344	34 ± 4	57
NH_4-N	S	49 ± 14	108	14 ± 2	19	165 ± 34	387	94 ± 31	332
	B	45 ± 16	-**	15 ± 2	20	427 ± 123	1656	94 ± 40	518
NO_3-N	S	3 ± 1	8	8 ± 5	28	658 ± 269	3450	58 ± 25	263
	B	3 ± 1	-**	8 ± 5	-	270 ± 135	1830	17 ± 5	63
Organic-N	S	345 ± 48	530	422 ± 43	575	827 ± 151	2449	1028 ± 122	1724
	B	350 ± 46	-**	430 ± 52	625	705 ± 162	2549	888 ± 81	1240

*Post 46 data.

**No maximum recorded value since a composite surface and bottom sample was included in the data set.

Figure 4.5 Record of daily water flow (megalitres) for Serpentine and Murray Rivers from September 1977 to September 1979. Data courtesy of R.E. Black.


600
300
0
12
8000
4000
0
DAILY FLOW (megalitres)
SERPENTINE RIVER
MURRAY RIVER
S O N D J F M A M J J A S O N D J F M A M J J A S
1977
1978
1979

4.3.7 Nutrients within the algal bed

Concentrations of nitrogen and phosphorus in water surrounding the algae in the Cladophora beds ('inter-algal' water) are shown in Table 4.3 and are compared with corresponding concentrations of these nutrients in the overlying water column. These data are the means of samples collected monthly between November 1977 and June 1978. Clearly water nutrient concentrations are higher in water surrounding the alga, as evident from the higher ratios, particularly of orthophosphate. Inorganic nitrogen in the algal bed is dominated by ammonia, nitrate+nitrite remaining nearly as low here as in the overlying water column.

4.3.8 Diel Variation

Seasonal changes in salinity each year were dramatic. In contrast, short-term changes measured over 24 hours in August and March 1978 were small and were largely attributable to movement of water past the site on these occasions. The winter range for surface and bottom water was 8.3 to 12°/oo and 30 to 31.5°/oo, respectively in August and 40.5 to 42°/oo and 41.8 to 44°/oo in March.

Water temperature did not vary by more than 3°C in either diel study, the range for surface and bottom water near the algal bed being 15.1 to 18.2 and 16.2 to 16.7 in winter, 24.1 to 26.3 and 24.1 to 27.0 in summer. Water was warmest near the bed in the mid-afternoon period.

Diel changes in dissolved oxygen showed no distinct pattern. During 24 hours in winter, dissolved oxygen ranged from 14.1 to 16.0 mg ℓ^{-1} (149% to 175% saturation) at the surface, and 1.3 to 5.4 mg ℓ^{-1} (16% to 67% saturation) on the bottom, the low values being predominantly the result of light attenuation reducing photosynthesis of the algal bed. No stratification of dissolved oxygen was observed over 24 hours in summer, where surface values ranged from 6.5 to 9.5 mg ℓ^{-1} (98% to 149% saturation) and bottom values ranged between 6.0 to 10 mg ℓ^{-1} (93% to 156% saturation).

Nitrogen and phosphorus concentrations of the water column on these occasions are shown in Figures 4.6 and 4.7. These varied erractically over each 24 hour period, the winter diel study having typically higher concentrations than those of the following summer since this coincided with input of nutrient-rich river water at the growth site. During the winter study, when bottom oxygen concentrations showed the algal bed to be essentially anaerobic, both ammonia and orthophosphate were at high concentrations in the water immediately above the algal bed. The range of concentrations over 24 hours, was also very wide for these nutrients in August compared with those in March.

Table 4.3 Concentrations of nutrients in the water between *Cladophora* balls, compared with the water above the algal bed[1].

	Water $\mu g.\ell^{-1}$	Interalgal $\mu g.\ell^{-1}$	Ratio. Inter-algal water : water column
NH_4-N	43 (16)	186 (52)	4.3
NO_3-N	3 (0.6)	7 (1.3)	2.3
N organic	828 (260)	1845 (352)	2.2
N total	874 (253)	2038 (352)	2.3
PO_4-P	6 (1)	93 (25)	15.5
P organic	27 (11)	145 (67)	5.4
P total	33 (10)	238 (69)	7.2

[1]Each datum is the mean of 5 determinations, (with standard error) taken at monthly intervals in summer and autumn of 1978.

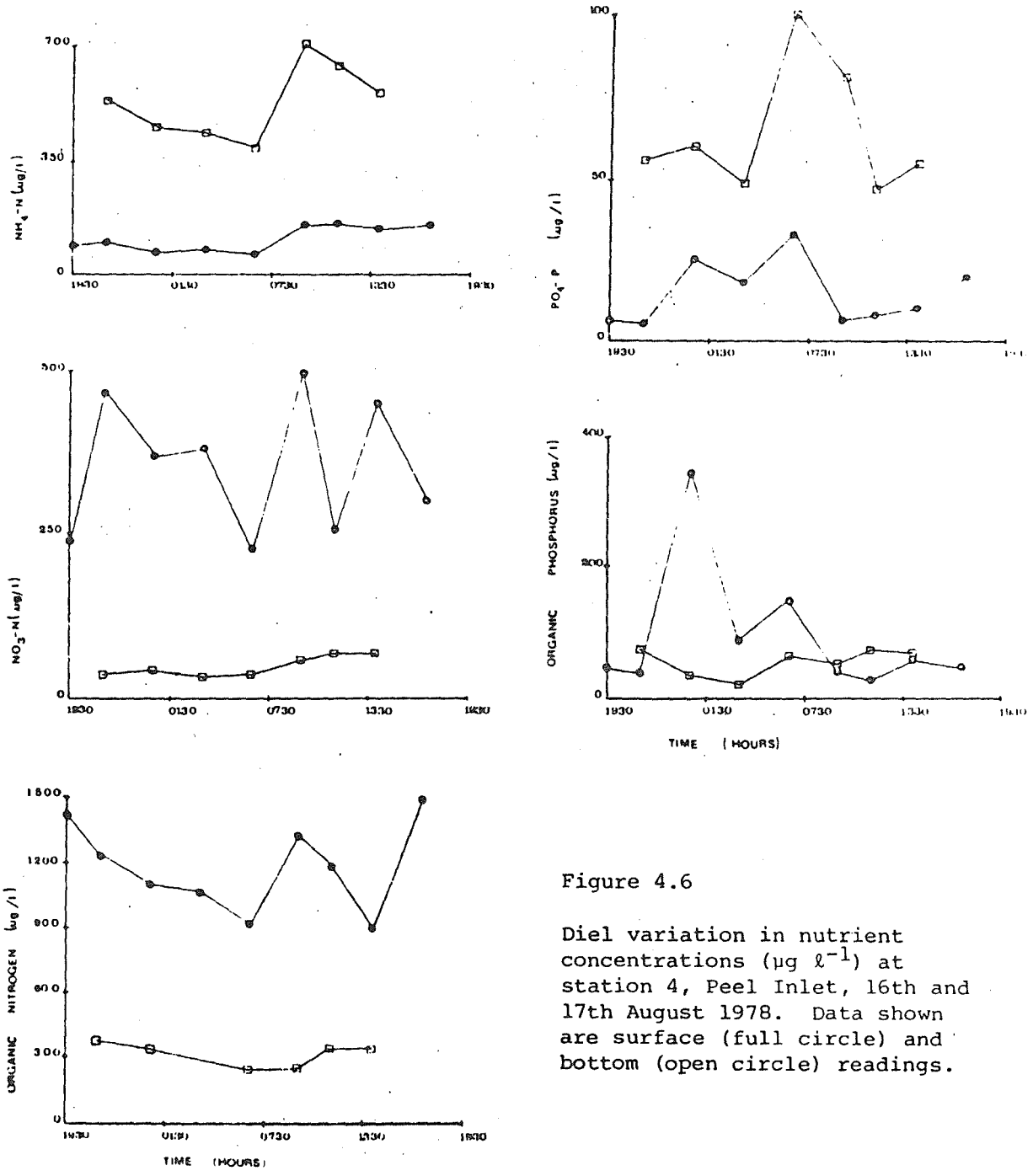


Figure 4.6

Diel variation in nutrient concentrations (μg ℓ^{-1}) at station 4, Peel Inlet, 16th and 17th August 1978. Data shown are surface (full circle) and bottom (open circle) readings.

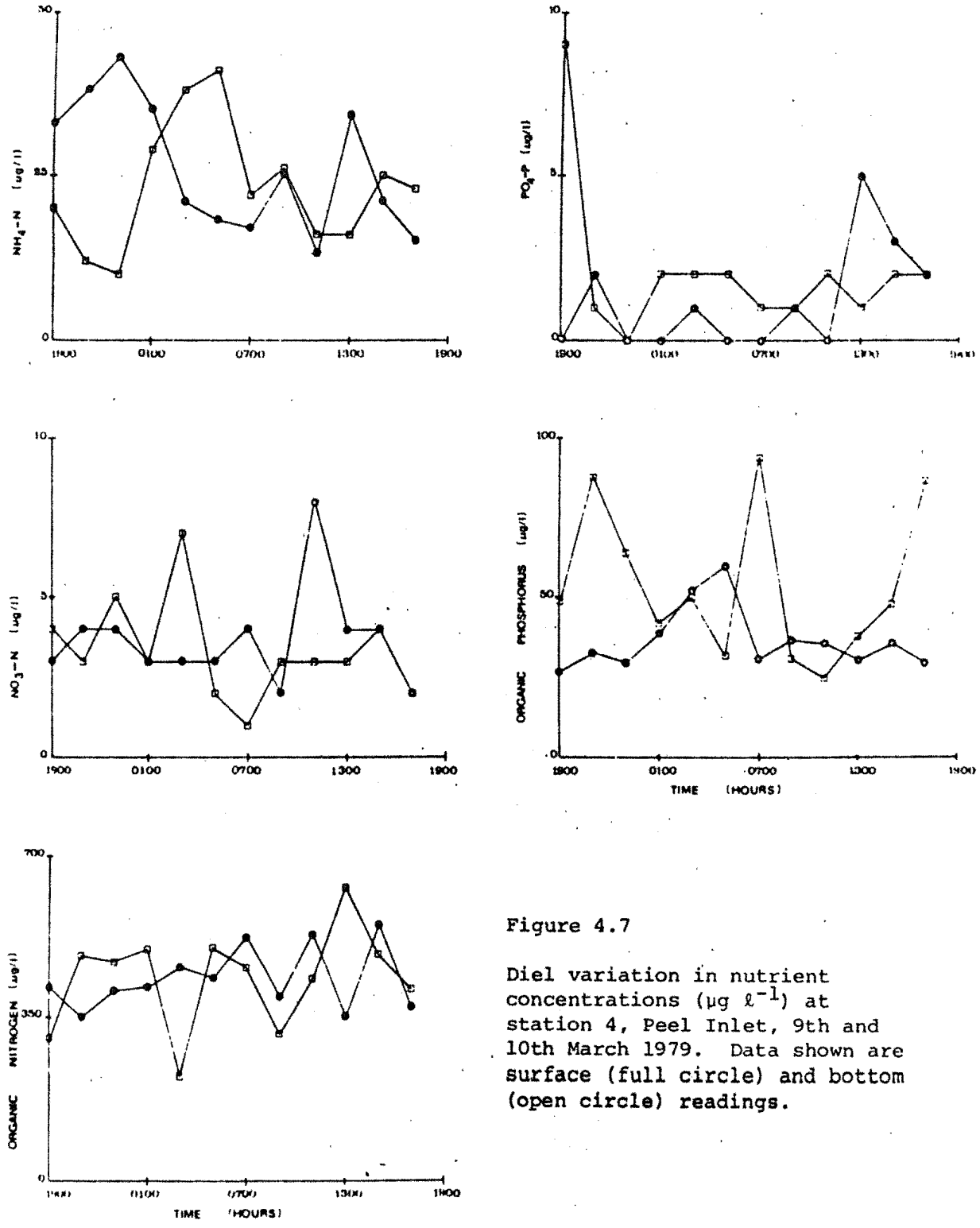


Figure 4.7

Diel variation in nutrient concentrations (µg ℓ^{-1}) at station 4, Peel Inlet, 9th and 10th March 1979. Data shown are surface (full circle) and bottom (open circle) readings.

Similarly, nitrate+nitrite concentrations ranged widely over 24 hours in both surface and bottom water in August compared to those for the summer study, when levels were consistently low throughout the water column.

Variation in nutrient concentrations and environmental variables are compared for diel studies and regular weekly sampling in Table 4.4 where the coefficients of variation are shown and expressed on a percentage basis. With the exception of the diel changes in surface phosphate during winter, surface nitrate in summer and organic nitrogen of bottom water in winter, the variation in nutrients and environmental factors over each diel study was less than the variation in the corresponding season's weekly data. This would suggest that, in so far as the diel changes here offer a true reflection of 'typical' summer and winter short-term variation, weekly sampling may be sufficiently sensitive to detect most temporal changes, even during winter, the season where levels of nutrients in the water column are most erratic.

4.3.9 Sulphate sulphur

Monthly changes of composite surface and bottom samples at Post 46 between April 1976 and April 1977 were between 400 mg ℓ^{-1} and 1100 mg ℓ^{-1} (mean 750 mg ℓ^{-1}) and followed closely the observed changes in chloride during this period (Atkins et. al. 1977). Like chloride, sulphate reflects the contribution of marine water to the estuarine environment. The range of concentration observed here is not unusually great, though the presence of hydrogen sulphide in the chemically reduced lower layers of the algal bed prompted collection of these data during the early stage of the study.

4.4 DISCUSSION

The seasonal changes in nutrient concentrations reported here reflect the seasonal changes expected in estuaries of southwestern Australia, which are characterised by a poorly defined tidal fluctuation and a limited period of freshwater discharge governed by the winter rainfall regime (Rochford 1951).

The 4-year records of environmental factors and nutrients in the water column above the Cladophora bed show a regular seasonal pattern, with light, temperature and salinity each reaching its maximum in summer and falling to low levels in winter. In contrast, for nutrients the reverse is true; concentrations are typically highest in winter, largely as a result of the significant input of nutrient-rich river water form the Serpentine and Murray Rivers to the estuary (see Fig. 4.5). Freshwater discharge as a source of allochthonous material, including nutrients, to watersheds has been reported frequently (e.g. Kidd and Sander 1979, Seki et al. 1979, Sharpley and Syers 1979, Sholkovitz 1979).

Table 4.4 Coefficients of variation (100 x S.D./$\bar{x}$) of physico-chemical data collected from station 4 over a 24 hour period during winter and summer compared with those from data collected during regular weekly sampling at the same site. Figures shown are for diel studies run on 16th and 17th August 1978 (winter) and on 9th and 10th March 1979 (summer). Coefficients for weekly data are calculated for the periods 27th June 1978 to 31st October 1978 inclusive (winter) and 2nd January 1979 to 20th March 1979 inclusive (summer), the winter weekly data including those dates where there was significant river flow.

	Diel Variation				Weekly Variation			
	winter		summer		winter		summer	
	surface	bottom	surface	bottom	surface	bottom	surface	bottom
PO_4-P	71	53	140	105	113	161	86	54
Organic P	107	33	26	44	113	103	151	132
NH_4-N	34	33	38	40	98	127	97	63
NO_3-N	30	71	35	46	184	223	27	52
Organic N	18	86	16	24	53	65	40	39
Salinity	12	1	1	2	52	28	6	7
Temperature	7	1	3	4	22	19	11	10
Dissolved oxygen	5	34	14	20	16	40	16	24

The significance of catchment soils in governing the levels of river nutrients which are supplied to the estuary can be gauged by comparing the results of this study with those of other systems, for example the Nanaimo River estuary, Canada (Naimen and Sibert 1978). These authors observed a distinct seasonal variation in inorganic N and P which peaked during winter. However, because high precipitation resulted in heavily leached soils, river nutrient concentrations derived from catchments remained low. In contrast, the river discharge into Peel Inlet strongly influenced seasonal changes in nutrient concentrations in the estuarine basin, these high concentrations to a large extent being the result of surface runoff from fertilised soils of surrounding catchments (Birch 1980).

The seasonal difference in nutrient concentrations in Peel Inlet, where background concentrations of inorganic N and P remain relatively consistent and low throughout the no-flow period, contrasts with seasonal variation displayed by some shallow estuaries in the U.S.A. where peak phosphate, for example, coincides with minimum river flow, and river water acts by diluting, rather than enriching, the estuarine water (Pomeroy et al. 1972).

In this study, input of river water to the growth site is therefore considered a major factor in governing seasonality of nitrogen and phosphorus. Indirectly it contributes to the seasonal change in light available to the algal bed since coloured water derived from the rivers results in increased turbidity and high light attenuation through the water column. It also imposes salinity stratification and reduced mixing during the winter period.

Both 1976 and 1977, particularly the latter, were relatively 'dry' compared with the following two years of the study. This is clear from the long-term (40 years) flow records for the Murray River (PWD data), which reflect changing patterns of rain falling on the estuary, and is also suggested from the high Secchi depth readings during the early stages of the study. The absence of any substantial nutrient peaks in either the winter of 1976 or 1977, and the relatively high value of bottom salinities at the growth site on these occasions, particularly 1977, reinforce this view.

4.4.1 Nutrient cycling

Above the Cladophora bed, where inorganic N and P remain consistently low during periods of no-flow, sources of N and P for maintaining the algal bed on the estuary floor are derived predominantly from sediments, which are richer in nutrients than the overlying water (Gabrielson 1981). Low phosphate concentrations in

the water column similar to those found here when no external input of nutrients occurs, have been reported in waters of a Florida estuary as a result of the existence of an equilibrium between dissolved and adsorbed phosphates at the sediment-water interface (Moshiri and Crumpton 1978).

A similar mechanism is likely to operate in this system where the sediments underlying the bed are typically anaerobic and reduced. Release of phosphates under such conditions may be high; as much as 50 mg P m^{-2} day^{-1} has been reported for a hard-water lake sediment (Bengtsson cited Welch 1980). Release rates up to nearly 4 mg P m^{-2} day^{-1} have been reported from field experiments at station 4 in this study (Gabrielson 1981) and these represent a potentially large source of phosphorus to the water column. Nutrients in the water surrounding algal filaments in the bed (interalgal water) are at very much higher concentrations than those of the overlying water throughout the year, (Table 4.3), and the bed may act to some extent as a trap for upwardly moving nutrients released by sediments. High interalgal concentrations relative to those of overlying water are consistent with the suggestion that there is diffusion of nutrients away from the decomposing lower layers of the bed to the water column above (McComb et al. 1981).

Local mixing may produce small increases in concentrations in the water column as suggested by the 'noise' present in background nutrient concentrations (Figs. 4.3 and 4.4). The most obvious causal factor is wind-stirring and to a lesser extent disturbance by motorcraft. This latter factor has been reported to produce significant increases in ortho- and total P in lakes (Yousef et al. 1980) and may well be significant in this study in view of the large number of registered motorcraft. Physical effects of disturbance by motorcraft have been observed in this study, where the algal bed may become disrupted, both algal and underlying reduced sediments being mixed into the water column. Clouding of the water persists for a considerable time after disturbance.

Inorganic nitrogen is dominated in this system by ammonia rather than nitrate, in both the overlying water or interalgal water in the algal bed. This has also been reported for a similar system in Bermuda (Bach and Josselyn 1978) where there is an extensive growth of a mat-forming Cladophora. The nature of the algal growth in both their study and ours favours an ammonia-dominated nitrogen regime since the reduced, anaerobic muds underlying healthy algae at the bed surface are associated with a bacterial environment biologically capable of ammonification. Low nitrate+ nitrite levels in no-flow periods are most likely the result of anaerobic conditions in the sediments inhibiting nitrification, this requiring favourable redox conditions (Billen 1975).

Cladophora beds and underlying sediments in Peel Inlet offer a ready source of ammonia to overlying waters through remineralization of plant nitrogen and decaying plant material in the sediments. This contribution may be quite high, as suggested by the work of Owens *et al.* (1979) on an estuary dominated by prolific growth of *Enteromorpha*. These authors reported that 14% of nitrogen released by the alga could be detected as ammonia in the overlying water after 28 days. The remaining ammonia detected originated from decaying *Enteromorpha* in the sediments. This nitrogen pathway is compatable with the observed high ammonia concentrations found in interalgal water of the algal bed in this study (Table 4.3)

Much of the ammonia present in the water column, derived from the estuary floor, will be cycled through phytoplankton and zooplankton. There is some evidence that the amount of ammonia regenerated from the latter is small compared with that accumulated by phytoplankton (Smith 1978), though significant amounts of ammonia have been reported to be regenerated by zooplankton in coastal waters (Conway and Whitledge 1979).

No estimates of nitrite are available in this study, being included with nitrate. However, low concentrations of the latter imply levels of nitrite are similarly low. This form has been found to be photochemically unstable in seawater (Zafirou and True 1979) and its presence in large amounts in the water column would be unexpected here.

Much of the organic P and N, which dominated the water column nutrients, particularly in no-flow periods (Figs. 4.3 and 4.4) was present as detrital and resuspended material. The exceptions are the winters of 1978 and 1979 where peaks of organic N and P, following the input of nutrient rich river water to the site, are predominantly present in living plant material, these periods coinciding with large blooms of phytoplankton (Lukatelich and McComb 1981).

Bacteria associated with detrital material may be important to P cycling (Rhee 1972). A likely pathway in estuarine systems has been outlined by Correll *et al.* (1975) where orthophosphate is taken up by bacteria on the surface of suspended sediments or detritus, passed on to filter feeders and released as organic phosphorus.

Sedimenting phytoplankton blooms, such as those during the winter of 1978 in the study, offer a further source of reusable nutrient which can be released into the water column (McComb *et al.* 1981).

4.4.2 Comparison with other systems

Concentrations of nitrogen and phosphorus in the water column here conform in many respects to those measured above and within a similar Cladophora mat in Bermuda (Bach and Josselyn 1978). These authors reported orthophosphate in undetectable levels in overlying waters, while corresponding nitrate-, nitrite- and ammonia-nitrogen concentrations were typically 4, 10 and 28 μg ℓ^{-1} respectively. This is close to the mean concentrations of each measured during the no-flow period in this study (Table 4.1). Nutrients present in areas associated with Cladophora growth can differ markedly, as for example in Lake Erie where growth of C. glomerata was associated with consistently low orthophosphate levels (10-15 μg ℓ^{-1}) but very high nitrate-nitrogen, reaching as much as 1 - 2 mg ℓ^{-1} throughout the summer (Mantai 1978).

Concentrations of nitrogen and phosphorus in Peel Inlet are relatively high compared with measurements in other estuaries of south-western Australia (e.g. Spencer 1956, Congdon and McComb 1980). The latter authors reported a maximum orthophosphate concentration of only 14 μgℓ^{-1} from two years' data collected in the Blackwood River estuary. Peak concentrations of nutrients here are also higher than those of estuaries in eastern USA (e.g. Maurer et al. 1978). This is particularly the case if the winter nutrient inputs in this study are included. Areas were there is excessive growth of Cladophora in the Lower Great Lakes are associated with very high nutrients in the water, far in excess of the estimated critical concentrations of 30 μgℓ^{-1} orthophosphate, and 300 μgℓ^{-1} inorganic nitrogen (Herbst 1969).

4.4.3 Water quality

Water quality at the growth site in Peel Inlet can be gauged to some degree from the trophic tables produced for lake systems. As a basis of comparison we can use Table 4.1, which neglects the high concentrations resulting from river input and is therefore a conservative representation of typical concentrations. Total P concentrations are within the range considered by Vollenweider (1971) to occur in eu-polytrophic lakes, his range being between 0.03 and 0.10 mgℓ^{-1}; inorganic nitrogen levels would be classified as oligotrophic. Levels here, however, do equate with the eutrophic classification outlined by Sakamoto for Japanese waters (Sakamoto cited Vollenweider 1971). A more recent classification of trophic status for lake systems has been proposed (Vollenweider and Kerekes 1980), eutrophic water being designated as having total P between 16 and 386 (mean 84) μgℓ^{-1} and total N between 393 and 6100 (mean 1875 μgℓ^{-1}). These updated figures suggest that water overlying the algal bed is eutrophic, particularly in the case of phosphorus, though the total nitrogen figures here would better fit the "mesotrophic" range.

Critical nutrient concentrations for estuarine waters have been established by some workers and are discussed by Jaworski et al. (1972). Recommended limits for inorganic P and N of 20 and 350 µg ℓ^{-1} have been reported for the Potomac Estuary in the U.S.A., while the upper limit for total phosphorus, recommended for estuarine waters by the Committee on Water Quality Criteria, was 50 µg ℓ^{-1}. This is close to that found above the algal bed here during the no-flow period. Pritchard (cited Jaworski et al. 1972) considered total P levels below 30 µg ℓ^{-1} in estuarine waters as low enough to maintain a healthy biological environment. A statistical comparison of water quality in Peel Inlet using the early records of CSIRO Fisheries and Oceanography between 1949 and 1956, the Peel Inlet Management Authority between 1972 and the present and the nutrient data from this study suggests that nutrient concentrations have increased markedly (Humphries et al. 1981), and this may largely be the result of increased nutrient loads into the estuary, particularly of total phosphorus, derived from surrounding catchments.

Considering the very high concentrations of nitrogen and phosphorus in the water each winter as a result of river flow, and the enormous banks of nutrients which build up in algal beds around the Inlet, it is clear that this system is eutrophic.

4.5 CONCLUSIONS

1. Long-term regular sampling of salinity, temperature and light above the Cladophora bed shows that all these factors are highest during summer and fall to a minimum in winter.

2. Above the algal bed there is a marked seasonal difference in salinity, from as low as 2°/oo to over 50°/oo.

3. Hypersalinity persists for 7 months of the year, from December to June.

4. The annual range of water temperatures is typically between 12°C and 27°C, while a 3°C difference may be observed at the site over 24 hours. There is no marked stratification, temperature differences between surface and bottom being rarely more than 1°C.

5. Light attenuation through the water column is low throughout most of the year except in winter when coloured water, derived from river flow, enters the growth site and results in increased turbidity. Secchi depths correspond closely to calculated attenuation coefficients at the growth site, being greater than water depth through the year except in winter, corresponding to the period of river flow.

6. Photosynthetically active radiation at the surface of the algal bed ranges seasonally from ∿400 $\mu E\ m^{-2}\ sec^{-1}$ to effectively zero during winter, the low values coinciding with the period of winter river-flow.

7. Seasonal changes in dissolved oxygen are not marked and are rarely at levels critical to aquatic life. Water near the algal bed is typically supersaturated with oxygen in summer, as a result of photosynthesis, but with little diel variation. In winter, deoxygenation occurs near the algal bed coinciding with the period of poorest light penetration.

8. pH ranged seasonally from 7.70 to 8.60 near the algal bed, while the range for alkalinities was 78 to 120 mg ℓ^{-1} $CaCO_3$. Lowest alkalinities and highest pH occurred in winter when there was input of freshwater to the growth site.

9. Of the 4 years of the study, 1978 and 1979 produced the greatest seasonal change in nutrients as a result of significant freshwater river discharge during both winters. The previous 2 years were 'dry' in comparison and nutrient concentrations in the water column remained low.

10. During no-flow periods, inorganic phosphorus remained low in the water. During river flow, concentrations rose to as much as 150 $\mu g\ \ell^{-1}$, with high levels persisting in the water column for a number of weeks.

11. Inorganic nitrogen, in no-flow periods, is dominated by ammonia-nitrogen, nitrate+nitrite-nitrogen remaining typically at very low levels (∿5 $\mu g\ \ell^{-1}$).

12. During river flow most freshwater input of nitrogen to the growth site occurs as nitrate, and to a lesser extent as ammonia. Following significant flow, as in 1978, nitrate-nitrogen can reach as much as 3.4 mg ℓ^{-1}.

13. Organic nitrogen and phosphorus dominate total N and P of the water column throughout the no-flow period of the year. Observed peaks in these nutrients from July to October 1978 and for a similar period in 1979, correspond to diatom blooms in the overlying water. Peaks in organic N and P observed on other occasions during the study, to levels close to those which were associated with blooms, are most likely the result of local mixing and resuspension of detrital material.

14. Concentrations of inorganic nutrients were consistently higher in the interstitial water surrounding individual algal fragments ('interalgal' water) compared with corresponding nutrients of the overlying water.

15. Coefficients of variation of samples collected over 24 hours on 2 occasions during summer and winter are generally lower than those measured on corresponding weekly samples, suggesting that the weekly sampling interval is sufficiently sensitive to detect marked temporal nutrient changes.

16. A comparison of mean concentrations of nitrogen and phosphorus in the water overlying the algal bed with those reported in the literature and in trophic classification tables indicate that the waters in this study are eutrophic.

CHAPTER 5

BIOMASS AND GROWTH RATES IN THE FIELD

5.1 INTRODUCTION

As noted in the previous chapters, Cladophora can be collected at all times of the year from several regions in the estuary, and also occurs in banks around the shore, where it is driven by wind-induced currents. A life cycle involving spores does not have to be completed each year, and so it appears that most of the alga observed in the estuary reproduces vegetatively. It seemed important to work out the season of the year when growth occurs at a maximum rate, and at the same time to document environmental variables which might be expected to control growth. Attention was concentrated initially on one site where the alga was known to occur throughout the year, and the algal biomass occurring per unit area at the site was measured at different times. As shown below, although changes in biomass were observed, it was difficult to disentangle the amount of growth which may have occurred at the site, from the physical import or export of alga in the region. The observations were therefore supplemented by enclosing populations of known amounts of alga in perforated flasks and submerging them in the same region of the estuary. The flasks could be recovered and the contents weighed, so that the change in biomass could be documented. The change could then be related to observed fluctuations in measured environmental variables.

Once the field programme was established, attention was also directed to the laboratory studies described in the three succeeding chapters. In the general discussion at the end of this report, the changes in biomass described in this chapter are assessed in relation to the laboratory studies.

5.2 MATERIALS AND METHODS

5.2.1 Biomass

Biomass of the algal bed was measured monthly, along with environmental variables and nutrients in the overlying water (see Chapter 4) between April 1976 and May 1979 at a site where the alga grows essentially permanently (Harbour and Lights Post 46; Fig. 5.1). Twenty replicate samples were collected each month using a grab sampler (area 0.05m^2) to remove the alga from the bed, including that which was decomposing in the underlying layers. Samples were washed, dried at 80°C to constant weight, and biomass expressed as grams per square metre.

5.2.2 Growth Rates

Growth rates of Cladophora were measured in the field from change in dry weight of imprisoned populations, over approximately monthly intervals at station 4 (Fig. 5.1) between May 1977 and June 1979. At the start of each incubation, algae were collected from the bed by diving and

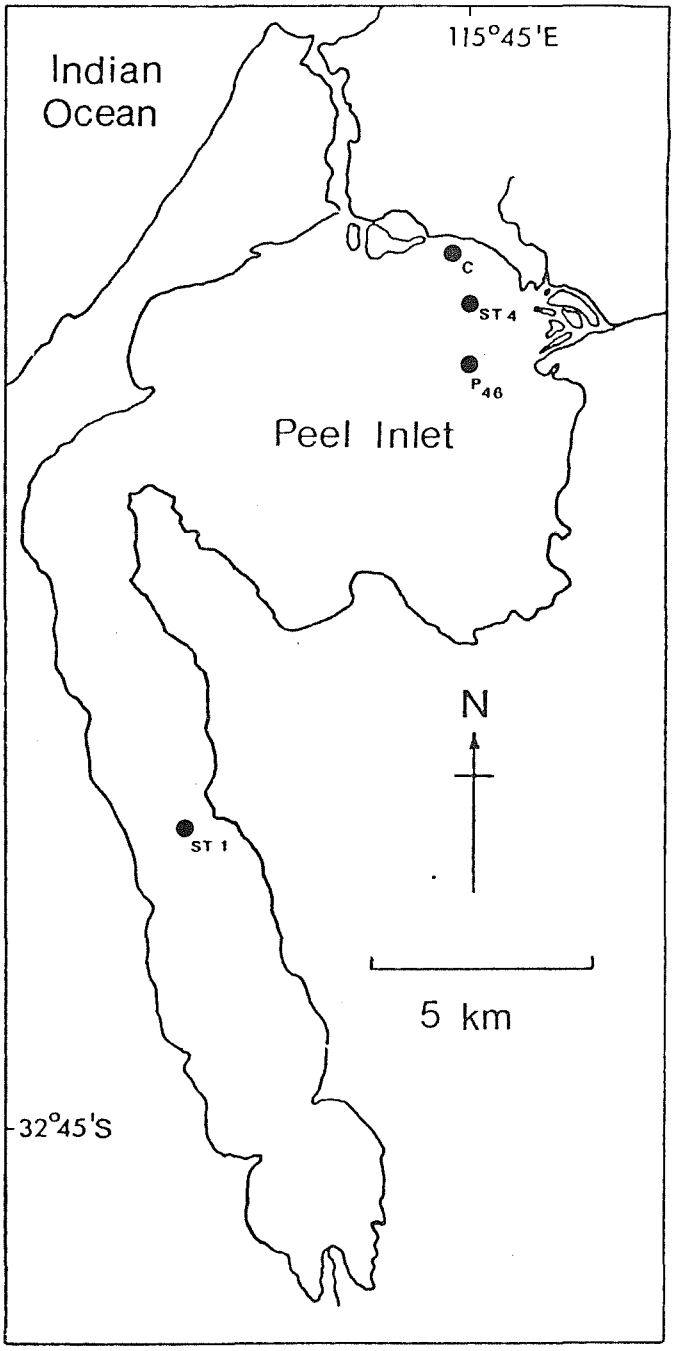


Figure 5.1 Showing location of experimental sites; station 4 (st 4), station 1 (st 1), Coodanup shallows (C), and Harbour and Lights Post 46 (P46) from which biomass and growth data were obtained.

scooping the uppermost layers into a diver's bag. Sub-samples (3-5 g fresh weight) of the bulked sample were then placed into each of 20, 250 ml transparent, perspex culture flasks (Costar, Cambridge, Mass., U.S.A.) previously perforated (25 x 0.4 mm diameter) to allow nutrient exchange with the surrounding bed. Choice of number and size of perforations in the flasks was made after testing their efficiency to exchange rhodamine dye with their surrounds. The flasks were then attached to a transparent acrylic perspex support (1.9 m x 6.3 cm x 9.5 mm), anchored onto the estuary floor so that the contents of each flask was associated with the top layer of the algal bed. Initial weights each month were determined by randomly selecting 20 prepared inocula and returning these to the laboratory immediately for dry weighing. From summer 1978, identical sets of flasks were prepared each month and transported to two other sites - station 1 in the Harvey Estuary (Fig. 5.1) where water clarity is typically much reduced relative to station 4, with no significant benthic algal growth and no record of Cladophora growth, and on the marginal shelf at Coodanup, about 500 m inshore from station 4 where water depth is only some 50 cm. This latter site, dominated by growth of aquatic angiosperms, notably Ruppia megacarpa and Halophila ovalis, is subject to large accumulations of Cladophora which may drift shorewards covering extensive areas of the seagrass beds.

5.2.3 Growth rates and environmental variables

Growth rates at each site were compared with environmental data collected at weekly intervals during the study (see Chapter 4). These data were averaged over the period of incubation of each set of flasks, and correlations sought between growth and environmental variables using the programmes of Nie et al. (1975). Light energy reaching these imprisoned populations was calculated from daily visible global radiation data, integrated over the incubation period, corrected for surface reflectance and attenuated to the depth of incubation from coefficients obtained on a weekly basis at each site (see Chapter 4).

5.2.4 Effect of water movement on bottom disturbance

In order to assess the effects of water movement on disturbance of Cladophora populations anchored in flasks on the estuary floor, sets of plaster blocks similar to those described by Doty (1971) were placed at different depths through the water column and allowed to "dissolve" for a number of hours. The results were reported as percent weight loss over the time period of immersion, after correction for losses of control blocks placed in 'still' water over a similar time period.

Two sets of plaster blocks were set out above the Cladophora bed at station 4 and in the Harvey Estuary at station 1 on 9th March 1979 and a further set placed at station 4 on 10th March 1979 to include the period with an afternoon sea breeze.

Blocks were attached to perspex bases and tied in pairs by a nylon line which was anchored to the estuary floor under tension using a subsurface buoy. Those blocks nearest the bottom were tied approximately 1 cm clear of the estuary floor or the algal bed, depending on the site. This experiment was repeated on 13th - 14th July 1979 at these sites, each being run at approximately the same time for ∿20 hours, from 10.15 a.m. to 7.15 p.m. and from 10.40 a.m. to 7.45 p.m. at station 4 and station 1,respectively.

5.3 RESULTS

5.3.1 Change of biomass

Change in biomass of the Cladophora bed at Post 46 is shown in Figure 5.2 for a 3 year period. These ranged from 30 to 1200 g dry wt m^{-2} (average 700 ± 70 S.E.). There was a marked increase from July to September 1976, followed by a further increase over the following summer, reaching a maximum in February 1977. A similar change was not observed in subsequent years, the biomass remaining essentially unchanged for the remainder of 1977 and up to April 1978, after which there was a dramatic decline, over the following 4 months, to levels some 15% of the maximum. The algal bed did not subsequently recover, there being only a relatively slight increase in biomass during the following summer.

The relation between monthly biomass and environmental factors, in particular, salinity, water temperature, light and nutrient concentrations of the overlying water, is shown in Table 5.1. This includes analyses of the complete data set and also, separately, for the periods of marked biomass change, viz. the increase from July 1976 to March 1977, and the decrease from April 1978 to October 1978.

From Table 5.1 it can be seen that monthly biomass of Cladophora was not well correlated with environmental variables, when the complete data set was analyzed. This was also the case with the decreasing portion of the curve, from April 1978 to October 1978. For that period there was a positive correlation between salinity and biomass, because the alga was lost from the bed at the onset of river flow, but it is not thought that there was any effect of salinity on algal growth; this possibility is taken up in succeeding chapters. In contrast, from July 1976 to February 1977, the ascending portion of the curve, biomass was better correlated with light and temperature, and to a lesser degree with ammonia-nitrogen and salinity.

5.3.2 Growth of imprisoned populations

Growth rates of Cladophora were determined for imprisoned populations in the algal bed at station 4, (Fig. 5.3) on the estuary floor at Coodanup (Fig. 5.4) and in the Harvey Estuary (Fig. 5.5).

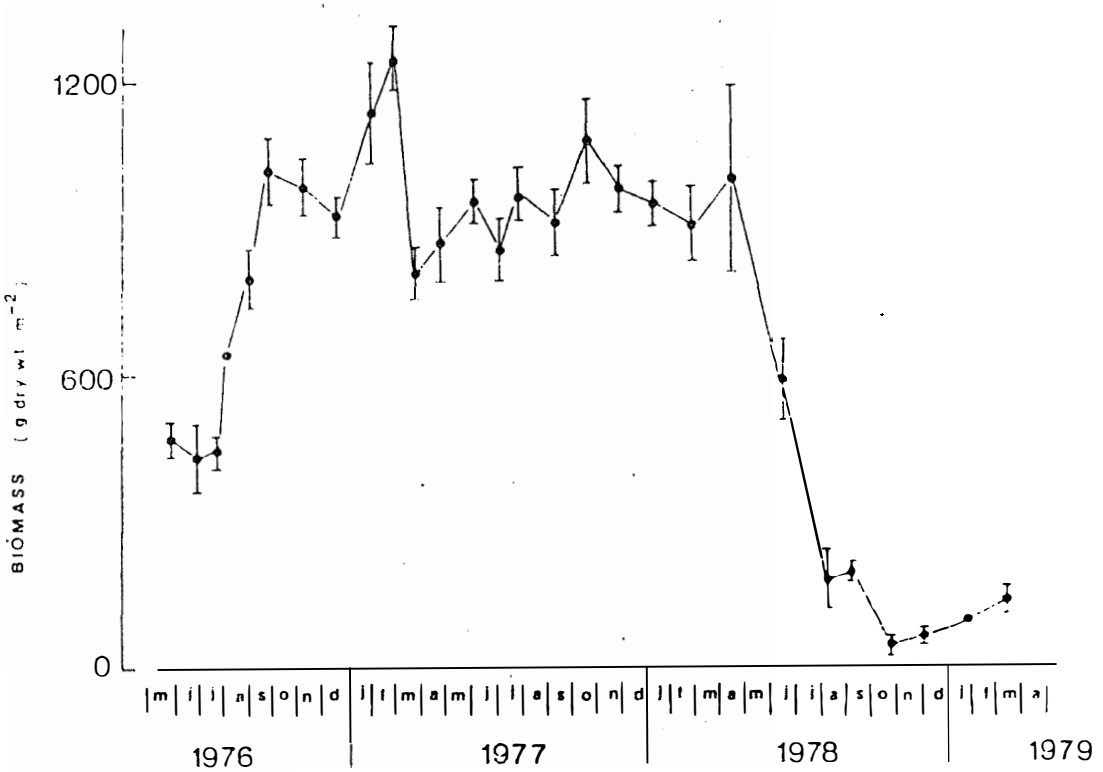


Figure 5.2 Biomass (g dry wt m^{-2}) of *Cladophora* at Post 46, Peel Inlet between May 1976 and March 1979. Each point is the mean of 20 replicates ± S.E.

Table 5.1 The relation between biomass of *Cladophora* (grams dry wt. m^{-2}) and environmental variables for all data (May 1976 to May 1979), period of initial increase (July 1976 to February 1977), and period of decrease (April 1978 to October 1978). Independent linear correlations were calculated and the environmental variables arranged in order of decreasing correlation, with a line at $p>0.05$. Data are bottom readings.

All data (May 1976 to May 1979)			Period of increase (July 1976 to February 1977)			Period of decrease (April 1978 to October 1978)		
Order	r	P	Order	r	P	Order	r	P
P phosphate	-.357	.034	Temperature	.811	.013	Salinity	.935	.001
Salinity	.355	.035	Radiation	.763	.023	P phosphate	-.715	.037
P total	-.306	.061	N ammonia	-.756	.025	N organic	.450	.224
N total	-.304	.061	Salinity	.455	.152	Radiation	-.404	.251
N ammonia	-.300	.065	P organic	.434	.165	N nitrate	-.306	.309
N nitrate	-.283	.076	N total	-.396	.189	N ammonia	-.289	.319
N organic	-.231	.124	N organic	-.328	.236	Temperature	.278	.333
P organic	-.226	.128	P total	.286	.270	P total	-.159	.399
Radiation	.096	.318	N nitrate	.085	.428	N total	.052	.467
Temperature	-.0004	.499	P phosphate	-.083	.430	P organic	-.014	.491
(n = 28)			(n = 7)			(n = 5)		

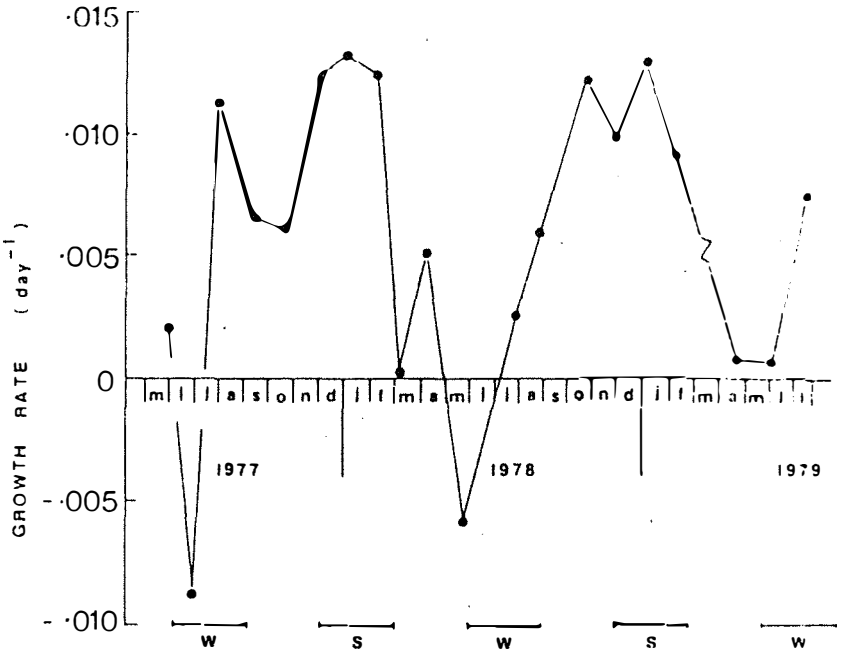


Figure 5.3 Growth rate (day^{-1}) of imprisoned populations of *Cladophora* at station 4, Peel Inlet, May 1977 to August 1979. Winter and summer are depicted as W and S respectively.

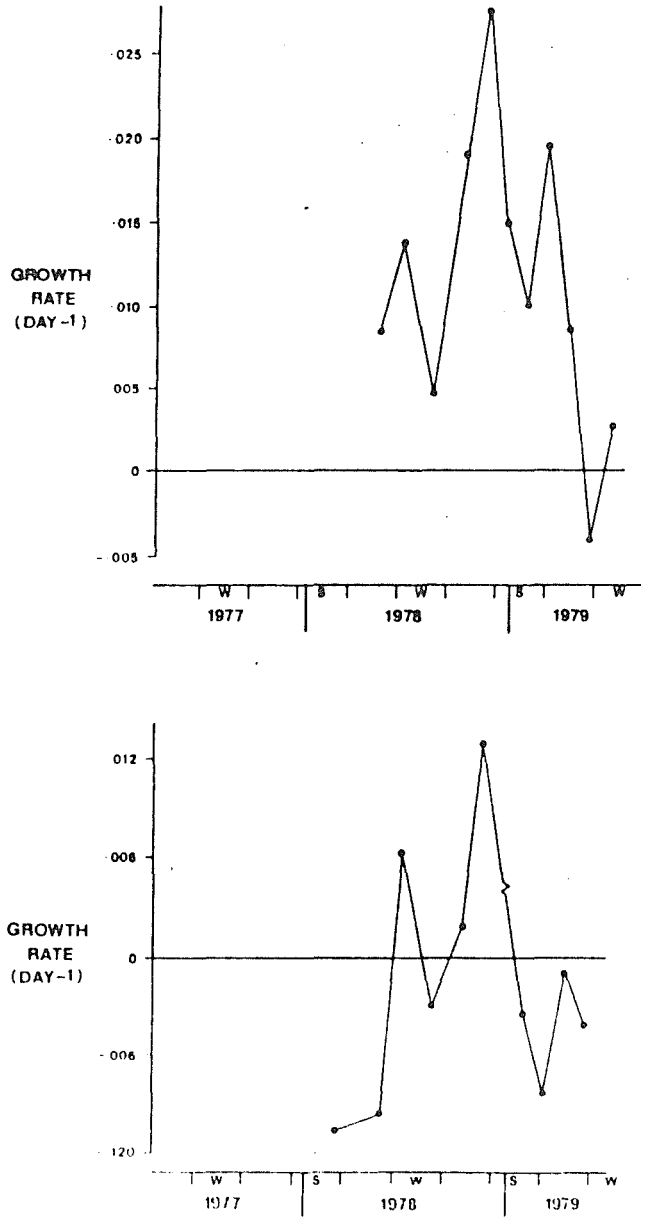


Figure 5.4 Growth rate (day^{-1}) of imprisoned populations of *Cladophora* transferred to shallow water at Coodanup, Peel Inlet, May 1978 to August 1979.

Figure 5.5 Growth rate (day^{-1}) of imprisoned populations of *Cladophora* transferred to station 1, Harvey Estuary, February 1978 to June 1979.

A distinct seasonal change in growth occurred at station 4 with maximum rates occurring over the summer periods and minimum rates in winter. In the winter periods there were weight losses in the flasks, presumably as a result of decomposition and respiratory loss. The maximum growth rates (dry weight increase) recorded at station 4 over the 2 years was 0.013 mg per mg per day, with an average of 0.006 day $^{-1}$.

At Coodanup, inshore from station 4, growth rates were consistently higher than at station 4, with a maximum 0.027 day $^{-1}$ during the summer. Again, lowest growth rates were obtained in winter, though these did not fall to levels as low as those in the deeper site, during winter 1979; winter growth rates were generally around 0.010 day $^{-1}$, in the shallower site.

Populations transferred to the Harvey Estuary showed erratic growth, with the winter rates typified again by losses of weight in the flasks. These data give an average growth rate of - 0.002 day $^{-1}$ between February 1978 and February 1979.

The relationships between growth rates and their environmental variables is given in Table 5.2, where independent correlations are shown in decreasing order of significance.

Clearly for those populations in the Peel Inlet, light energy on the algal bed and water temperature were the factors most strongly related to the observed seasonal fluctuations in growth rate. The importance of light to growth rates is further highlighted in the combined data from station 4 and Coodanup.

For both Peel sites, nutrient concentrations in overlying water, particularly nitrate, ammonia and phosphate, were not significantly correlated with growth rate. These results contrast with those given by the data for the Harvey Estuary, where strongest correlations were observed with organic nitrogen in the water column, and the attenuation coefficient, factors which reflect the often high turbidities of this site. Combining the data from all sites resulted in highest overall correlations of growth with light and temperature.

The amount of variance in the data accounted for by environmental variables at each site was also assessed using multiple linear regression analyses, and is shown in Tables 5.3 to 5.6, for station 4, station 1, Coodanup and station 4 combined, and all sites.

Over 80% of the variance in growth rate at station 4 was accounted for by the environmental factors, of which over 30% was contributed by water temperature fluctuations, and a further 30% by salinity, maximum growth rates occurring in summer when the water is hypersaline. For station 4 data alone, the contribution to variance by light was small compared to temperature, though the inclusion of data from Coodanup with those from station 4 resulted in nearly 40% of variation accounted for by light alone. A further 20% was due to salinity and temperature.

Table 5.2 Relationships between *Cladophora* growth rates (day^{-1}) and environmental variables. Independent linear correlations were calculated and the environmental variables arranged in order of decreasing correlation, with a line at $p > 0.05$. Data are bottom readings.

All sites combined			Station 4 and Coodanup combined			Station 4			Coodanup			Station 1 Harvey Estuary		
Order	r	P	Order	r	P	Order	r	P	Order	r	P	Order	r	P
Radiation	.652	.00001	Radiation	.631	.0002	Temperature	.555	.005	Radiation	.607	.023	N organic	-.784	.004
Depth	-.569	.00009	Temperature	.474	.005	Radiation	.514	.017	Temperature	.591	.028	Attenuation coeff	-.772	.004
Temperature	.373	.008	Depth	-.417	.014	Depth	-.502	.020	Depth	-.516	.051	Salinity	-.666	.018
Dissolved O_2 % sat	.417	.015	Dissolved O_2 % sat	.282	.136	Dissolved O_2 % sat	.282	.136	N ammonia	-.276	.205	N total	.640	.023
P organic	-.344	.017	N organic	.209	.143	N organic	.280	.137	P phosphate	-.265	.215	Radiation	-.539	.054
P total	-.333	.020	N ammonia	-.185	.173	N nitrate	-.199	.222	N total	-.234	.243	P organic	-.346	.164
Salinity	-.305	.031	P phosphate	-.184	.175	Salinity	-.185	.238	N nitrate	-.196	.281	N nitrate	-.287	.210
N nitrate	-.281	.043	N nitrate	-.174	.187	N ammonia	-.165	.263	Salinity	-.165	.314	P total	-.287	.211
N ammonia	-.262	.056	Salinity	-.171	.191	P phosphate	-.153	.279	Attenuation coeff	-.149	.331	P phosphate	.243	.249
N total	-.207	.106	Attenuation coeff	-.132	.251	Attenuation coeff	-.075	.387	P total	-.124	.358	N ammonia	-.216	.274
P phosphate	-.106	.264	N total	-.053	.393	P total	-.053	.419	P organic	-.067	.422	Depth	.137	.353
N organic	-.063	.354	P total	-.035	.430	P organic	-.023	.465	N organic	.035	.458	Dissolved O_2 % sat	.125	.365
Attenuation coeff	-.029	.432	P organic	.010	.479	N total	-.014	.479				Temperature	-.077	.416
(n = 49)			(n = 29)			(n = 18)			(n = 11)			(n = 11)		

Table 5.3 The amount of variance in growth rates of *Cladophora* at Station 4 accounted for by different environmental variables. Data calculated by multiple linear regression analysis.

Variable	% Variance (cumulative)
temperature	32.7
salinity	69.4
N organic	75.1
depth	81.0
P organic	81.8
radiation	82.9
N nitrate	83.9
P phosphate	84.0
N ammonia	84.6

Table 5.4 The amount of variance in growth rates of *Cladophora* at Station 1, Harvey Estuary, accounted for by different environmental variables. Data calculated from multiple linear regression.

Variable	% Variance (cumulative)
N organic	78.4
depth	91.3
P organic	97.8
P phosphate	100.0

Table 5.5 The amount of variance in growth rates of *Cladophora* (station 4 and Coodanup sites combined) accounted for by different environmental variables. Data calculated by multiple linear regression.

Variable	% Variance (cumulative)
radiation	39.9
salinity	49.9
temperature	59.9
depth	61.0
P phosphate	61.9
N ammonia	65.3
N organic	67.7
N nitrate	67.9

Table 5.6 The amount of variance in growth rates of *Cladophora* (all sites combined) accounted for by different environmental variables. Data obtained from multiple linear regression.

Variable	% Variance (cumulative)
radiation	42.5
salinity	60.3
temperature	65.3
P organic	69.3
N organic	69.9
depth	70.0
P inorganic	70.3
N ammonia	70.4

5.3.3 Radiation

Light energy reaching the imprisoned populations is shown in Figure 5.6 where daily visible radiation reaching the flasks has been averaged over each monthly incubation period. Light available for growth was greatest at the shallower inshore site at Coodanup and least for the Harvey Estuary site.

5.3.4 Water Movement

The results of two plaster block experiments in March and July 1979 are shown in Figure 5.7 where loss of weight of blocks during immersion at stations 1 and 4 are given for different depths through the water column. Rates of dissolution reflect, somewhat crudely, the degree of water movement at different depths on the occasions under the different wind and wave conditions.

Weight losses at the Harvey Estuary site in March 1979 were consistently greater than those above the Cladophora bed at station 4 on this occasion. Water movement at the bottom near the estuary floor at station 1 was as great as that at the surface, suggested by the similar degree of dissolution of the blocks. In contrast, weight losses at station 4 decreased with increasing depth, even with the presence of a sea breeze when wave action at the site is usually enhanced. Losses at the sites in July 1979 produced similar results to those of the previous March at station 4 but not station 1, where weight losses were reduced near the estuary floor compared with the water surface, though the losses were again consistently greater than those of station 4 over a corresponding time period. Data for wind speed and directions on these occasions are not yet available though it is likely that much of the observed differences, particularly at station 1 on each occasion will be largely the result of different wind and wave regimes.

5.4 DISCUSSION

Levels of biomass of Cladophora in the algal bed resemble those reported for an unattached Cladophora which causes similar environmental problems in Bermuda (Bach and Josselyn, 1978).

The high biomass reported for mat-forming Cladophora species in this and the Bermuda growth site contrasts with those reported for stands of attached Cladophora in marine and estuarine habitats (Jansson 1974; Munda 1978; Hallsfors cited Wallentinus 1979) or for those growing in rivers (Whitton 1970; Wetzel 1975). The area over which Bach and Josselyn reported extensive development of Cladophora beds, some 189 x 10^3 m^2, is small compared with the area of distribution for this study (see Chapter 3) reflecting the extent of the Cladophora "problem" in Peel Inlet.

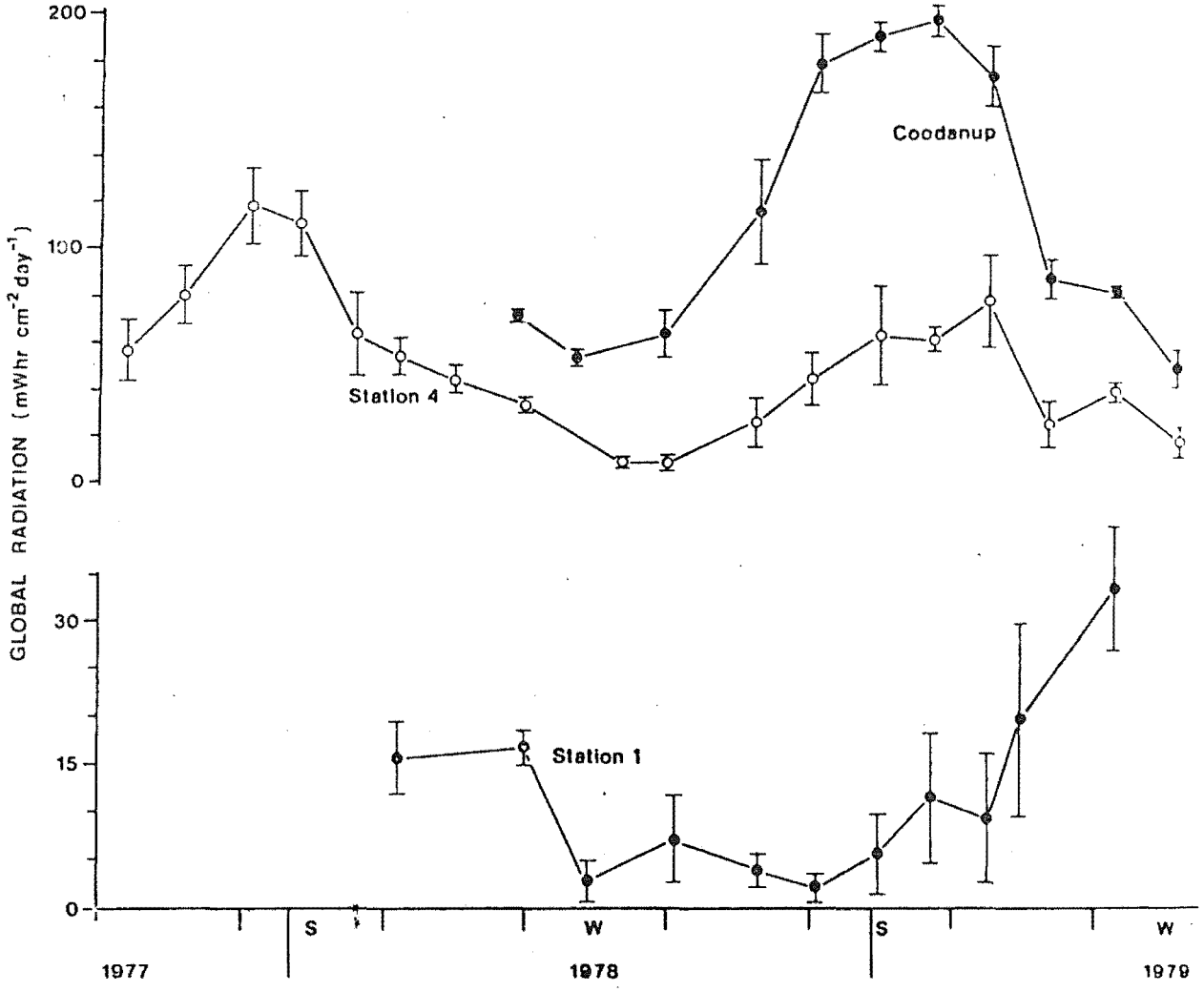


Figure 5.6 Light (mW hr cm^{-2} day^{-1}) reaching imprisoned populations of *Cladophora* on the estuary floor at each growth site. Each point shown is the mean ± S.E. for each monthly growth interval. Note the change of scale for Harvey Estuary data (station 1).

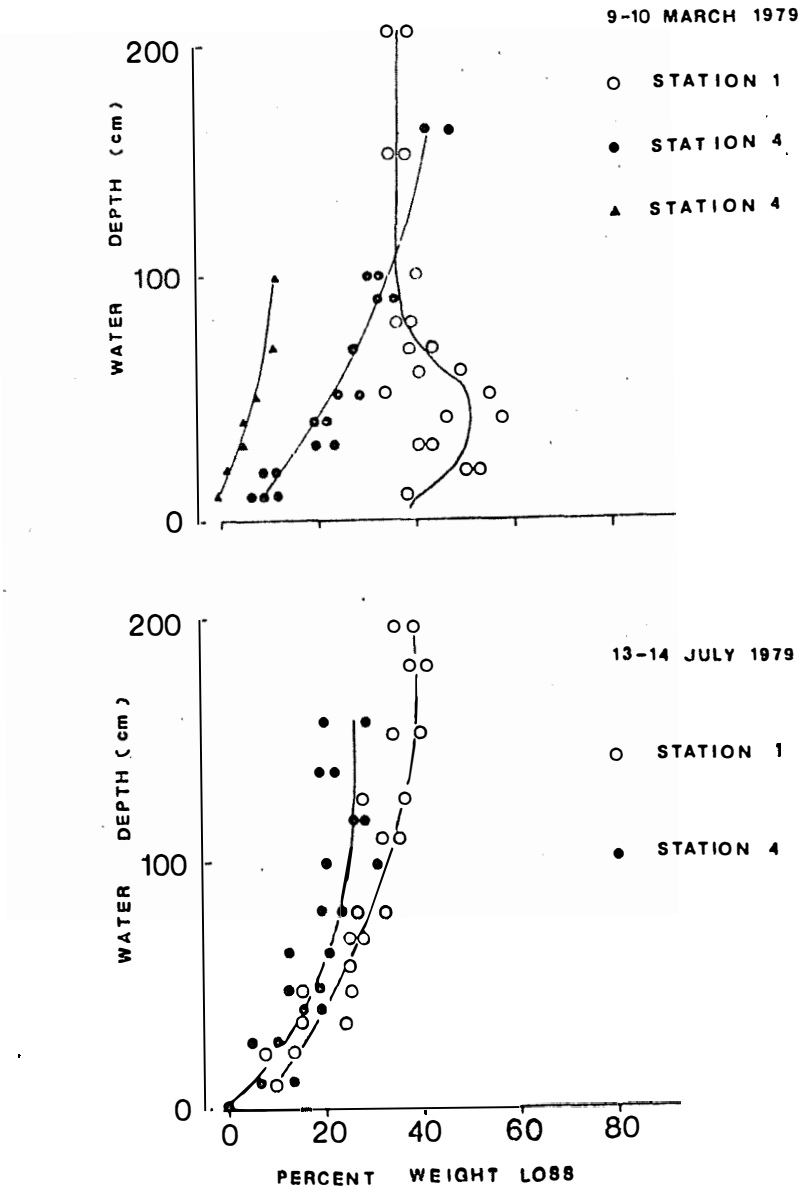


Figure 5.7 Percent weight loss of plaster blocks in the water column at station 4, Peel Inlet and station 1 in the Harvey Estuary. Results shown are from two experiments on 9th and 10th March 1979 and 13th and 14th July 1979. Data for station 1 in March (○) were collected over 16 hours between 2400 hours and 1600 hours. Those at station 4 in March were collected over 14.5 hours between 1900 and 0830 hours (●) and again over a further 5 hours between 1200 and 1655 hours (▲). Results in July at station 4 (●) and station 1 (○) were each collected over 21 hours, between 1015 and 0715 hours and 1040 and 0735 hours respectively.

Biomass measured during the spring of 1976, reaching a maximum in February 1977, was well correlated with some environmental factors, in particular, temperature and light (Table 5.1). However, neither monthly incremental changes nor the relative rates of biomass change between each sampling period, were significantly correlated with the environmental variables, suggesting that the observed increase in 1976 was due, not only to growth, but also, in part, to import and export of alga from the site. This aspect is discussed more fully in Chapter 9.

Monthly biomass was also poorly correlated with environmental variables when the complete data set was analysed, and also when analyses were carried out for the 7 months beginning April 1978, when there was a marked loss of Cladophora from the beds. The decline in biomass was associated with several weeks of substantial flow to the estuary from the Murray and Serpentine Rivers, located close to the site of growth, which resulted in decreased light reaching the algal bed (Fig. 5.6).

The extent to which growth estimated from measurements of biomass, was masked by changes due to import and export of alga can be gauged by comparing the growth rates obtained from imprisoned populations (Fig. 5.3) with the biomass data (Fig. 5.2). Growth rates exhibited a distinct seasonal pattern, with maxima in summer and minima in winter. These fluctuations were not reflected in the biomass data, particularly during 1977, when biomass was relatively static. It is interesting that the period of almost unchanged biomass was associated with a relatively dry year, with poor river flows compared with the following year (see Black and Rosher 1980). Growth rates in the algal bed at the start of winter 1977 were higher than those of the corresponding period in 1978.

The significant correlations obtained in the Peel between growth rates and light and temperature (Table 5.2) suggest the importance of these variables in controlling the growth of Cladophora. Light in particular is highlighted by the correlation obtained when both station 4 and Coodanup data were combined. Growth rates in the shallower, inshore site at Coodanup during winter were more than twice those measured in the algal bed (Figs. 5.3 and 5.4). In the shallows, growth rates in winter were generally higher than at station 4, and were almost certainly the result of better light conditions. At both sites however, nutrients in the overlying water were not strongly correlated with growth. Their relatively insignificant contribution to the observed growth changes is further highlighted by the somewhat poor nutrient status at Coodanup compared with the deeper site; in the shallows at Coodanup there is no algal bed and the sediment is a relatively coarse sand.

The poor contribution of overlying water nutrients to growth rates measured in the algal bed are further emphasised in the multiple regression analyses for the combined Peel sites (Table 5.5) where most variance (60%) was accounted for by radiation, temperature and salinity. The role of nutrients, nevertheless, for growth in the algal bed is

likely to be significant, despite the apparently small effects of nutrient levels in the water column. Concentrations of nitrogen and phosphorus in the "inter-algal" water surrounding the algal balls in the bed are typically very much higher than those of the overlying water (see Chapter 4; table 4.3). Their contribution to growth rates within the algal bed will be explored further in Chapter 9.

In contrast to the growth rates measured in Peel, those from the site in Harvey Estuary were not well correlated with light and temperature. Growth rates were erratic, displaying little seasonal trend, with marked weight losses on frequent occasions. Correlations with environmental variables suggest that Peel and Harvey sites are very different. Better correlation was obtained with organic nitrogen and salinity, than with temperature and light. Radiation reaching the flasks in the Harvey site was very low compared with the Peel (Fig. 5.6), being frequently below the compensation point (see Chapter 6). The algae were therefore severely light-limited for much of the time. Growth rates were strongly correlated with attenuation coefficient, this being a reflection of the turbidity of the water column.

The rapid increase in growth rates to a maximum of 0.012 mg mg $^{-1}$ day $^{-1}$ in December 1978 is difficult to explain since it occurred with the onset of a blue-green "algal" bloom which developed in November 1978 and which led to a marked reduction of light reaching the estuary floor, the lowest recorded during this experiment (Fig. 5.6).

Growth rates at this site were also likely to be affected by the action of wind, disturbing sediments and thus altering water chemistry. In the Harvey Estuary, winds of between 15 and 25 km hr $^{-1}$ can produce waves which are capable of moving mud at a depth of 1.5 to 2.5 m (Robertson 1972), suggesting that much of the mud in the basin can be reworked. This is compatible with the observation in this study where on some collection dates, flasks firmly anchored to the estuary floor were found filled with sediment, effectively blocking light. Winds, particularly those from a south-westerly direction, may be sufficient to move sediments on the estuary floor, increasing the turbidity of overlying water.

Results from plaster block experiments (Fig. 5.7) reinforce this view, where, at very much lower wind speeds then the figures quoted above, water movement at the bottom, measured from dissolution of the blocks, can be as high as that measured at the surface at station 1, in the Harvey estuarine basin. This is not the case at station 4 where water movement is very much reduced close to the algal bed. Major differences in controlling factors such as fetch distance, wind deviation and strength are apparent between these sites. The action of wind and resulting wave forces on disturbance of bottom sediments may be a deciding factor in the successful colonization of the estuary floor by Cladophora.

5.5 CONCLUSIONS

1. Biomass changes in the algal bed over 3 years ranged from 30 to 1200 grams dry weight m^{-2} with an average of 700 ± 70 grams dry weight m^{-2}.

2. A marked increase in biomass between July and September 1976 continued over the following summer reaching the maximum in February 1977. Biomass then remained relatively static until April 1978, when there was a marked decline to low levels in October 1978. The algal bed did not subsequently recover, there being only a small rise over the following summer.

3. Monthly biomass in the algal bed was generally poorly correlated with environmental variables. The increase observed in 1976 was correlated with light and temperature and can, in part, be attributed to growth. Biomass expressed either as incremental changes each month or as relative rates of increase each month were not correlated with environmental factors.

4. Loss of algae from the bed in winter 1978 is associated with the onset of river flow, resulting in increased water turbidity. This loss from previously high levels is unlikely to be due to decomposition but rather to physical removal from the site to the beaches.

5. Measurement of imprisoned populations in the algal bed show that maximum growth occurs in summer and minimum growth in winter, the latter associated with loss of weight of algae in the flasks, presumably from decomposition and respiration. The extent of this loss is similar, in magnitude, to typical growth rates.

6. The seasonal changes in growth rates in the algal bed are strongly correlated with light and temperature rather than nutrients of the overlying water.

7. Algae "transplanted" to a shallow site, where there is more light available for growth, gave growth rates over twice those at the algal bed in the basin. Unlike station 4, this site has no algal bed nor an obvious source of nutrients on the estuary floor, emphasising the significance of light and temperature producing such high growth rates.

8. Growth rates in the shallow site at Coodanup were strongly correlated with temperature and light, particularly the latter.

9. Light, salinity and temperature account for nearly 60% of variation in growth rates observed in the Peel, with only a further 7% explained by water nutrients.

10. Growth rates in those algae "transplanted" to a site in the Harvey Estuary were erratic, giving poor correlations with either temperature or light. Most variation was explained by water nutrients, contrasting with the observations in Peel Inlet.

11. The significant correlation between attenuation coefficient and growth rates suggests that water turbidity may be important to the lack of successful growth in the Harvey Estuary.

12. Observations of sediment accumulation into the flasks at the Harvey Estuary site suggest that physical sediment movement, presumably through the action of strong winds, offers a further limitation to successful growth.

13. Results from losses of weight of plaster blocks immersed in the water column at station 4 and station 1 reinforce the view that the latter site is susceptible to sediment disturbance through the action of significant water movement close to the estuary floor.

14. The rapid decline in growth rates in the Harvey during summer 1978 are presumably due to the formation of a blue-green phytoplankton bloom in the estuary in November, resulting in a loss of available light for growth.

15. Growth of Cladophora in the field is closely related to light, temperature, and to a lesser extent, salinity.

Chapter 6

EFFECTS OF LIGHT, TEMPERATURE AND SALINITY ON PHOTOSYNTHESIS IN *CLADOPHORA*

6.1 INTRODUCTION

The last chapter provided evidence that growth of *Cladophora* occurs mainly in the summer when light and temperature are high, but, as shown elsewhere, concentrations of inorganic nutrients in the water column are low. Further, we have seen that the plants are subjected to a wide seasonal range of salinities, from as low as 2 parts per thousand in winter to some 50 parts per thousand in summer. Using field data alone, it is clearly difficult to disentangle the possible importance of these various environmental factors in controlling *Cladophora* growth, and for this reason a series of laboratory experiments was undertaken, in which the various factors could be specifically examined.

In this chapter are described experiments on the effects of light, temperature and salinity on the photosynthesis of *Cladophora*. Photosynthesis was studied because it is the mechanism which underlies growth, but also because in contrast to increase in dry weight, it can be measured sensitively and may be expected to respond quite quickly to changes in light and temperature. An oxygen electrode was used to provide a rapid and sensitive measure of oxygen evolution during photosynthesis (e.g. Griffiths *et al.* 1978), and is well suited to investigation with small pieces of algae such as those of the *Cladophora* under study here. The data obtained are then used in examining the possible effects on photosynthesis at different temperatures, salinities, and light intensities encountered in the field.

6.2 MATERIALS AND METHODS

6.2.1 Plant Material and Media

Material was collected from essentially permanent algal beds at two sites, off Coodanup (site 4) and Falcon Bay (site 8), and placed in either estuary water or artificial seawater supplemented with high levels of inorganic nitrogen and phosphorus (Table 6.1). The artificial media were modified from the ASP_{12} recipe described by Provasoli (1964).

TABLE 6.1

Modified artificial seawater medium used for growing Cladophora.

The medium is adapted from the ASP_{12} recipe of Provasoli 1964.

	chemical	amount (gl^{-1})
Salt block	NaCl	28.0
	KCl	0.7
	$MgSO_4.7H_2O$	7.0
	$MgCl_2.6H_2O$	4.0
	$Ca(asCl^-)$	0.4
Nutrient block	NH_4NO_3	1.430×10^{-3} (= 5,000 µg N_2^{-1})
	K_2HPO_4	1.145×10^{-4} (= 250 µg P_i^{-1})
	$NaHCO_3$[a]	0.2
	$Na_2SiO_3.9H_2O$	1.5×10^{-2}
Vitamin block	B_{12}	2×10^{-7}
	Biotin	1×10^{-6}
	Thiamine - HCl	1×10^{-4}
Buffer	TRIS	1.0
Trace metals	P_{II}[b]	10 ml
	S_{II}[c]	10 ml
pH	8.0	
Salinity	33.7°/oo	

[a] Bicarbonate added at levels similar to those indicated from alkalinity measurements from estuary water in Peel Inlet.

[b] 1 ml of P_{II} contains : EDTA (Na_2), 1 mg; Fe ($asCl^-$), 0.01 mg; $B(H_3BO_3)$, 0.2 mg; Mn (as Cl^-), 0.04 mg; Zn (as Cl^-), 5 µg; Co(as Cl^-), 1 µg.

[c] 1 ml. of S_{II} metals contains: Br(as Na), 1 mg; Sr(as Cl^-), 0.2 mg; Rb (as Cl^-), 0.02 mg; Li (as Cl^-), 0.02 mg; Mo (as Na salt), 0.05 mg; I (kI), 1 µg.

Solutions of different salinities were prepared by partially evaporating estuary water and reconstituting with appropriate additions of distilled, de-ionised water. These were supplemented with vitamins, trace elements, phosphate-phosphorus at 155 $\mu g\ \ell^{-1}$, nitrate-nitrogen at 310 $\mu g\ \ell^{-1}$ and $NaHCO_3$ at seawater concentration (200 $mg\ \ell^{-1}$). Artificial seawater solutions of varying salinity were also prepared, by manipulating the concentrations of major salts (Table 1), while maintaining their ratios. The artificial media were supplemented with phosphate-phosphorus at 250 $\mu g\ \ell^{-1}$ and ammonia + nitrate-nitrogen at 2,500 $\mu g\ \ell^{-1}$.

Cladophora was placed in estuary water of different salinities over the range 10-59°/oo. Some of those at 10°/oo were transferred to solutions at 0.2°/oo after 3 days. Oxygen production (see below) was determined after 6 days in a growth cabinet at 25°C, 12 hour photoperiod, at a light intensity of 200 $\mu E\ m^{-2}\ sec^{-1}$ from a mixture of fluorescent tubes (warm white, Phillips) and incandescent bulbs. A similar set was prepared in artificial seawater in the range 2.8 to 60°/oo, and rates of photosynthesis determined immediately, and again after 19 days in the growth cabinet, as described above.

6.2.2 Oxygen Production

This was monitored using a Clark-type oxygen electrode (Rank Bros., Bottisham, England) consisting of a platinum cathode and silver anode separated from the stirred reaction chamber by a thin teflon membrane. The concentration of O_2 was recorded on a chart recorder, and rate of O_2 production calculated from the slope of a 5-10 min. chart record. The electrode was calibrated with air-saturated medium at the start of each run, and checked for drift frequently. Solubilities of oxygen were calculated using a standard nomogram which allowed for temperature and salinity (Strickland and Parsons 1972). These table values were periodically checked with Winkler O_2 titrations; variation between the two was never more than 10%.

Fragments of Cladophora (1-5 mg fresh weight) were introduced into the reaction chamber and equilibrated with 8 ml. of medium, which had been previously de-oxygenated by bubbling for 2 mins. with nitrogen containing 0.03% CO_2.

A quartz-halogen lamp (Philips, 150W, 15 V) attached to a fibre optic arm (L150B, Schott, Mainz, West Germany) provided a source of cold illumination for the electrode. The working wavelength of the lamp was 400-850 nm. Intensity was varied with the control of the instrument

and by mesh screen filters of varying density. Light was measured as photosynthetically-active radiation (PAR) with an underwater light sensor (Licor, Lambda Instrument Company, Nebraska, U.S.A.) placed at the base of a reaction chamber from which the electrode had been removed. The source provided up to 2,150 $\mu E\ m^{-2}\ sec^{-1}$ quantum flux.

A water jacket surrounding the reaction chamber and fed from a thermostatically-controlled water bath provided temperature regulation.

6.2.3 Field measurements

Field measurements of PAR (using the instrument described above), temperature and salinity were monitored on a weekly basis for over a year at the two sites. PAR at the surface of the algal bed at both sites was calculated from the regression obtained from a vertical profile through the water column, and extrapolated to the depth of the bed. Data for Falcon Bay were collected at about mid-day, and from Coodanup in mid-afternoon.

6.2.4 Algal bed measurements

The amount of photosynthetically-active radiation which could pass through successively deeper layers of a bed of Cladophora was determined. A transparent, Perspex jar, basal area 314 cm^2, was illuminated from above with a source (500 W Photolita, Philips) which provided 1,800 $\mu E\ m^{-2}\ sec^{-1}$ over the base of the jar. A quantum sensor was placed beneath the jar, which contained water. Increasing amounts of Cladophora were added to form a successively deeper "algal bed", and PAR through the bed was recorded. Twenty readings were taken across the base of the jar at each chosen depth.

6.3 RESULTS

6.3.1 Light and rate of photosynthesis

The dependence of photosynthesis on PAR is shown in Figure 6.1. There are two distinct portions of these curves - a light-dependent phase, where the photosynthetic rate depends on intensity, and a light-independent phase, where light is not limiting.

The compensation point, where net photosynthesis balances dark respiration, occurred at 20 $\mu E\ m^{-2}\ sec^{-1}$ (approximately 1% full sunlight PAR). Saturating light levels were reached between 200 and 300 $\mu E\ m^{-2}\ sec^{-1}$ for both estuary water and artificial seawater, approximating 15% full sunlight PAR.

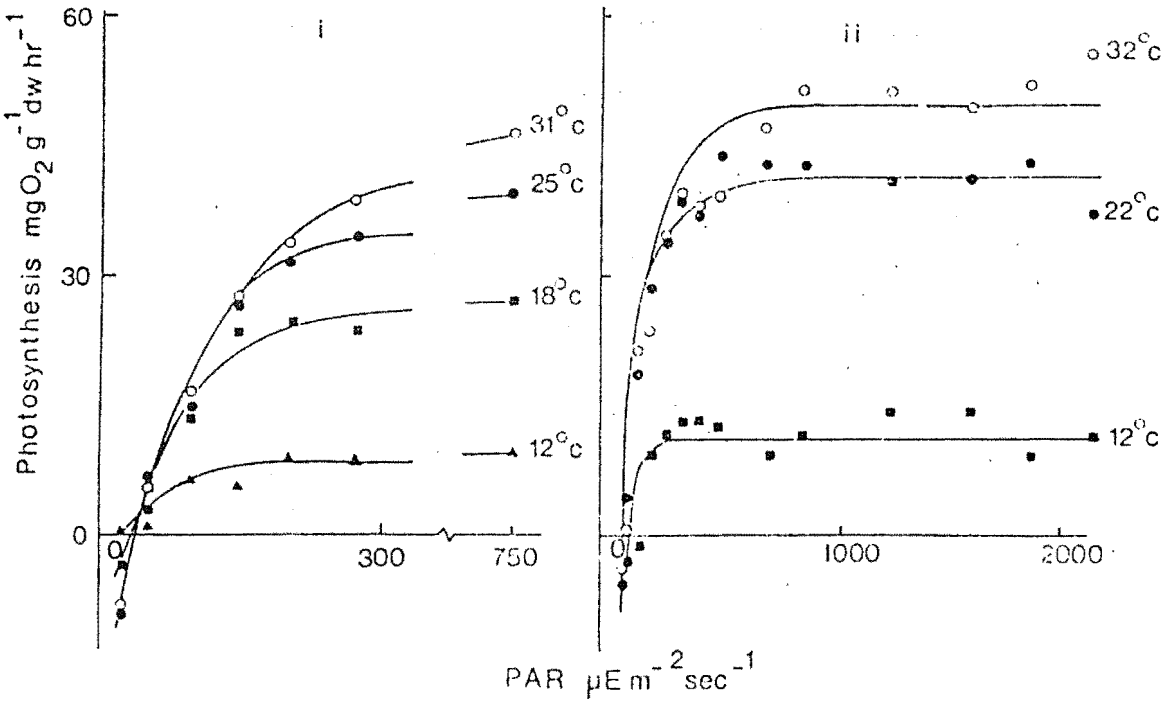


Figure 6.1. The dependence of rate of photosynthesis of *Cladophora* on photosynthetically-active radiation (PAR) at different temperatures in (i) estuary water and (ii) artificial seawater media.

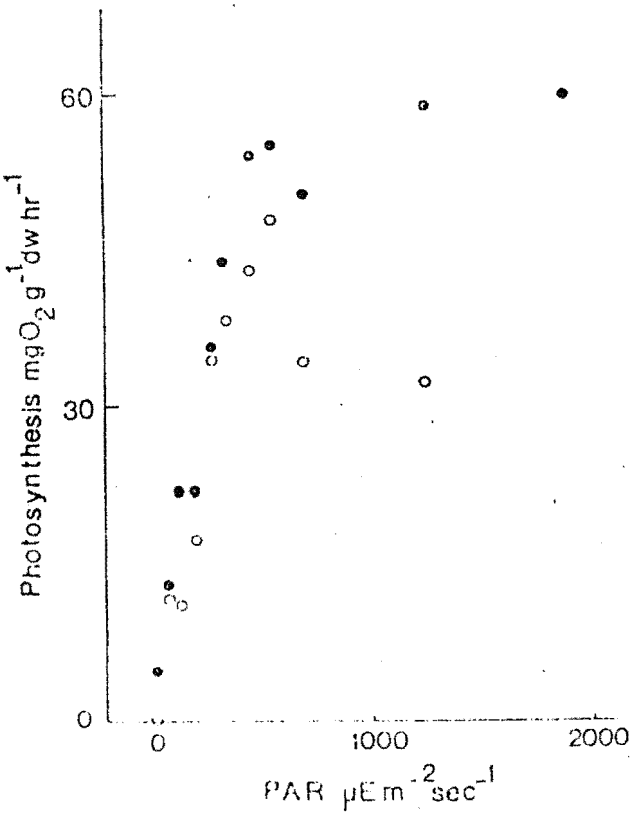


Figure 6.2. Rates of photosynthesis of *Cladophora* produced from successively increasing light intensities (closed circles) to saturating levels, followed by subsequent stepwise return to low levels (open circles).

There appeared to be no photosynthetic inhibition at high light intensities. However, at intermediate intensities, higher rates were obtained as intensities were increased towards that of full sunlight, when compared with those obtained when stepwise decrease from high levels were used (Fig. 6.2). The effect was not observed when plants at low levels (200 $\mu E\ m^{-2}\ sec^{-1}$) were subjected to short (2 min.) exposures to high levels (2,000 $\mu E\ m^{-2}\ sec^{-1}$) and returned to low levels.

6.3.2 Light reaching the surface of the algal bed

The light intensity at the surface of the Cladophora bed, on the floor of the estuary, is given in Figure 6.3 where saturating light levels are indicated by the broken line. At the surface of the bed PAR is often below compensation in winter, partly because of cloud cover and partly due to the high turbidity of the estuary water when the rivers flow.

6.3.3 Attenuation of light within an algal bed

Attenuation of light through the Cladophora bed is shown in Figure 6.4; levels decreased markedly with increasing bed depths, the compensation point, taken as 1% of incident PAR, being reached within 1 cm of the surface.

6.3.4 Temperature

Photosynthetic-light curves at different temperatures are included in Figure 6.2. Maximum rates occurred at 32°C for short exposure times, falling away sharply above 42°C (Fig. 6.5). Increasing the exposure time to 30 minutes at each temperature tended to lower the optimum. When Cladophora was maintained in enriched artificial seawater at 32°C the rate fell up to 40% in 8 hours, but had returned to the original rate when tested after 27 hours at 32°C.

6.3.5 Salinity

The effects of altered salinity on photosynthetic rates are shown in Figure 6.6 for enriched estuary water and artificial seawater. In both cases there was little effect over the range 10-40°/oo, though there was some suppression at 0.2°/oo and at 60°/oo.

6.4 DISCUSSION

6.4.1 Light

The level of light saturation for the species in this study, 15% of full sunlight PAR, resembles that reported for Cladophora glomerata in the Pamlico River estuary,

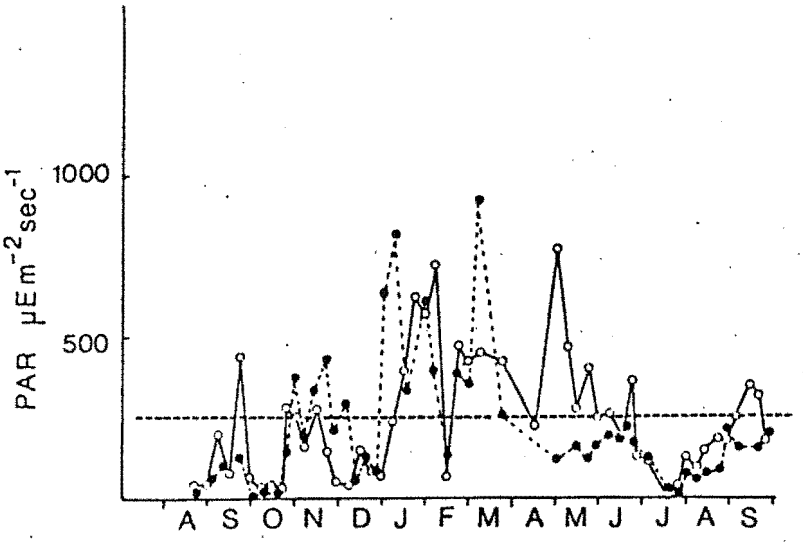


Figure 6.3 Photosynthetically-active radiation (PAR) reaching the surface of the *Cladophora* beds at site A (open circle) and site B (closed circle). Data shown are readings taken weekly between August 1978 and September 1979. The dashed line represents the level of saturating light (250 $\mu E\ m^{-2}\ sec^{-1}$ PAR) obtained experimentally for *Cladophora* at 22°C.

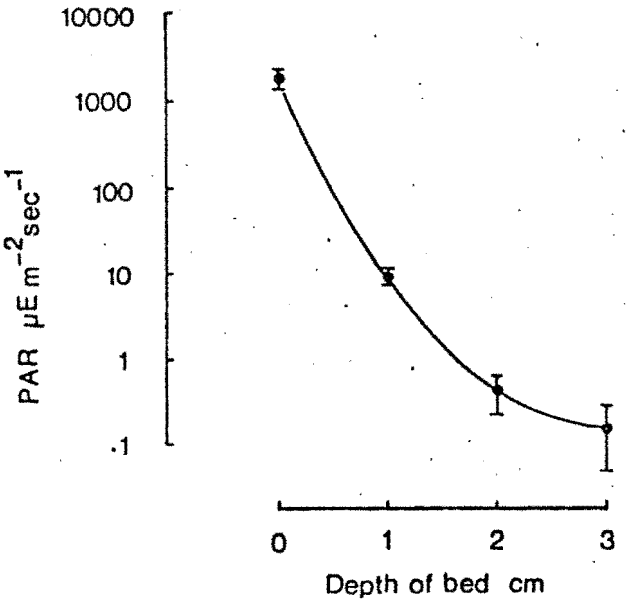


Figure 6.4 Attenuation of light as photosynthetically-active radiation (PAR) through different depths of *Cladophora* in an algal bed. Each point is the average of 20 readings along with its standard error.

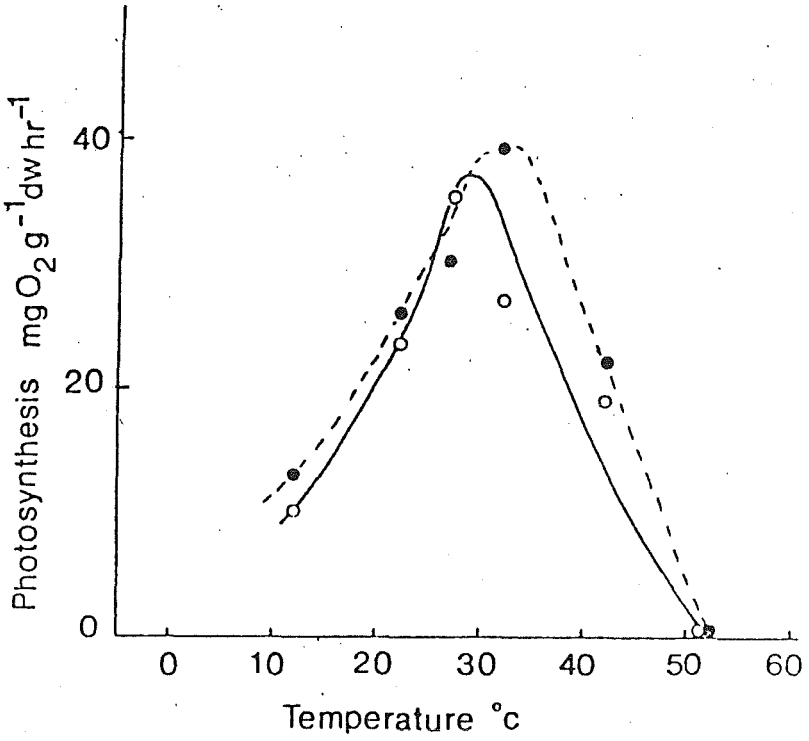


Figure 6.5 Rates of photosynthesis of *Cladophora* over a range of temperatures. The algae were subjected to either 5 minutes (closed circles) or 30 minutes (open circles) exposure at each temperature before readings were taken.

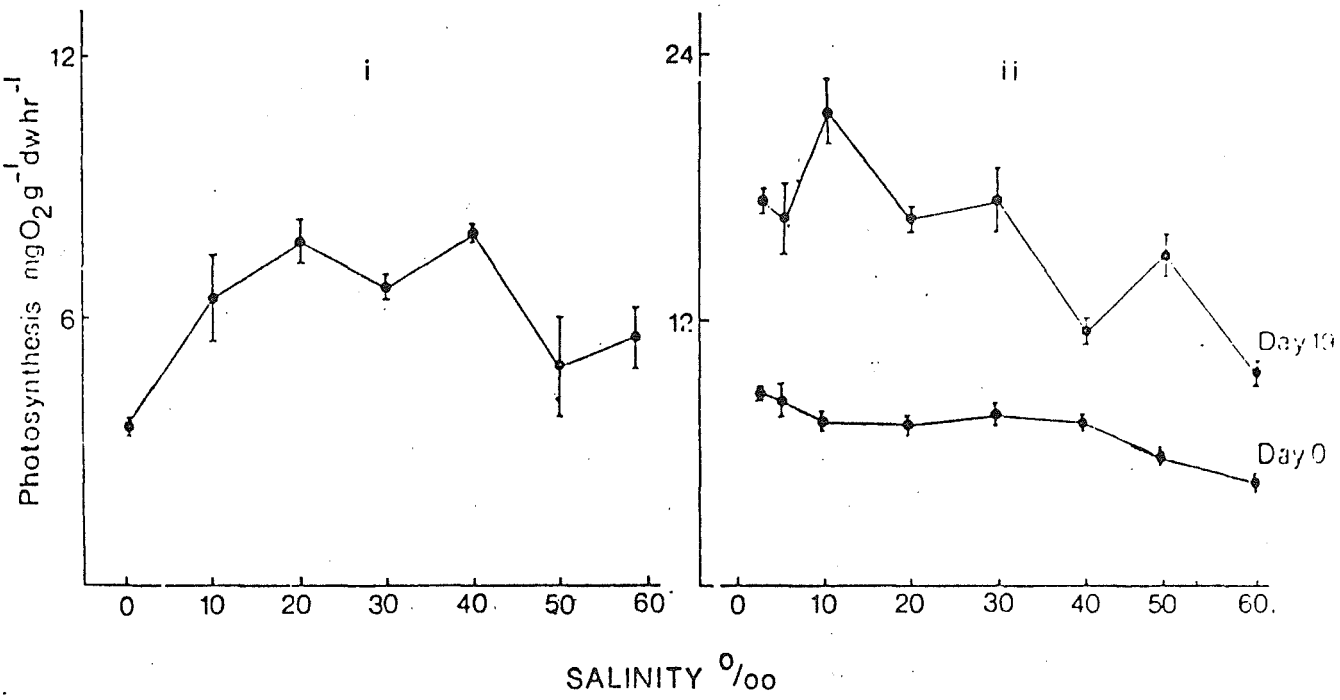


Figure 6.6 Rates of photosynthesis of *Cladophora* over a range of salinities in (i) estuary water, and (ii) artificial seawater medium. Rates in (ii) are shown at day 0 and after 19 days at their respective salinities. Each point is the average of 3 replicates along with its standard error.

North Carolina (Holmes 1976). The light-productivity curves in this study are also similar to that produced for Cladophora glomerata growing in the littoral zone of the Baltic Sea (Wallentinus 1978). Wallentinus considered this particular species to require high light intensities and reported a good correlation between productivity and insolation. The data for C. glomerata growing in the intertidal zone of the Baltic (King and Schramm 1976) was estimated by us to be light-saturated above 17% full sunlight, assuming full sunlight as 1,160 W m^{-2} (Jerlov 1970). Whether Cladophora favours low or high light intensities has been the subject of some debate (Adams and Stone 1973; Mantai 1974). Despite the existing discrepancies in saturation levels, which are partially a result of morphological differences, these figures suggest that Cladophora species, in general, utilise light at levels fairly typical of submerged aquatic higher plants (Lloyd et al. 1977).

Light reaching the estuary floor at the sites of collection in summer ranged from 46 to 798 with an average of 333 µE m^{-2} sec^{-1}, while winter values were between 3 and 353 with an average of 140 µE m^{-2} sec^{-1} (Fig. 6.3). During peak river flow, which occurs in winter, increased turbidity can reduce light at the bed to nearly undetectable levels. Comparing experimentally-obtained saturating light values with these field data suggests that light often limits photosynthesis at the surface of the bed in winter, but not in summer. However, reduction in PAR within the algal bed, through self-shading, is dramatic (Fig. 6.4); the uppermost 1 cm very efficiently excludes light from lower layers, with over 99% of light being removed at this depth. Net photosynthesis would cease at the compensation depth, just under 1 cm, even if full sunlight reached the surface of the beds. As these are often 10 cm, and up to 40 cm deep, it must be concluded that productivity in the beds is light limited. Indeed, the lower layers of such a bed are typically decaying. The significance of self-shading to productivity rates has also been shown for a bed of Cladophora prolifera in in Bermuda, where there is a 90% reduction in productivity below a single layer of the ball-like alga, each sphere being about 3 cm in diameter (Bach and Josselyn 1978). Nevertheless, plants lower in the bed do receive light if wave action produces movement of the algae. Further, oxygen bubbles trapped within individual plants result in their becoming buoyant and drifting away, exposing previously shaded, underlying layers.

Although there is no photo- inhibition at high intensities, lower rates were obtained on return from high to lower light levels. A similar response has been reported for several phytoplankton species by Harris and Lott (1973),

who found the effect to be more pronounced the higher the light intensity reached above saturating levels. In our work the effect was not observed after short (2 min.) exposures to high levels, suggesting some impairment of photosynthesis by prolonged periods at high light intensity. Prolonged exposures (1 hr) to high light intensity (about 100 Klux; almost 2,000 $\mu E\ m^{-2}\ sec^{-1}$) have been shown to decrease photosynthesis of Cladophora insignis (Steemann-Nielsen 1952), resulting in reduced rates on return to low levels (3 Klux; about 60 $\mu E\ m^{-2}\ sec^{-1}$). This effect, put down to photo-oxidation of the photo-chemical mechanism, was reversible, with re-establishment to normal rates after a number of hours. Such an effect might be expected to occur with the species in this study following prolonged exposure, but is probably of little ecological significance in the algal beds, in view of the range of intensities shown in Fig. 6.4. However, at shallow sites in summer where intensities can be sometimes well above saturation over much of the day, prolonged periods of high light intensity may well reduce the productivity of the alga.

6.4.2 Temperature

The temperatures used here are within the range ecountered in the estuary. Water above the algal bed may reach 27°C in summer, while shallow, inshore bays can reach 35°C on a hot day. Winter temperatures are typically between 11° and 15°C (McComb et al. 1981).

The high rates observed above 30°C (Fig. 6.5) indicate that, for short exposure times at least, Cladophora may efficiently photosynthesise in the warmer, inshore areas during summer. Q_{10} measurements of 3.55 between 12° and 22°C, and 1.19 between 22° and 32°C indicate a greater sensitivity to temperature change over the lower range. The reduced rates at 12°C at saturating light levels (Fig. 6.1) suggest that low water temperatures may contribute significantly to a reduction in photosynthetic activity in winter.

Most reports on limits of temperature tolerance have been related to growth data rather than the shorter-term phenomenon of rate of photosynthesis. The distinction between the two is clear; nevertheless it is interesting to compare our data with those obtained in growth experiments on other Cladophora species. Bellis (1968) working with C. glomerata reported vigorous growth in culture up to 30°C, though the alga was killed after 24 hours at 35°C. Riverine Cladophora in temperate habitats appear to survive best between 15° and 25°C, though cell damage occurs above 33°C (Whitton 1967). This contrasts with Cladophora prolifera which has been shown to survive 46°C for up to 2 hours, following a gradual warm-up to this temperature (Vilhelm cited in Gessner 1970).

It is clear from this study that Cladophora photosynthesises actively over a wide range of temperatures; however there is some evidence for this range being extended further if more prolonged exposure times were used.

6.4.3 Salinity

Varying the salinity did not markedly affect the photosynthetic rates in either estuary water or artificial seawater over a wide salinity range (Fig. 6.6). Rates were relatively high in artificial seawater after 19 days due to a gradual response to the high level of available nutrients. A study of the rates of photosynthesis of Cladophora repens, growing in the intertidal zone of a mangrove community, showed a similar broad tolerance to salinity variations, between 10 and 40°/oo (Dawes et al. 1978). This ability to tolerate such a wide range is presumably an adaptive advantage in an environment with strongly fluctuating salinities.

6.5 CONCLUSIONS

1. Cladophora is remarkably tolerant of changes in salinity, which are unlikely to control growth rates in the field.

2. Temperatures fall to limiting levels in winter.

3. Light intensities reaching the surface of the algal bed are often at limiting levels in winter, but they exceed these levels in summer.

4. Light is so rapidly attenuated in an algal bed that algae more than about 1 cm below the surface are below the compensation point (about 1% of full light) even under high incident light intensities.

5. Light presumably limits the rate of algal growth within the beds, even in summer.

CHAPTER 7

EFFECTS OF PHOSPHORUS, NITROGEN AND SALINITY ON GROWTH OF *CLADOPHORA* IN CULTURE

7.1 INTRODUCTION

Although as shown in the previous chapter, light must limit the production of algal beds, even in summer, nevertheless the problem of algal growth in the estuary is essentially one of eutrophication,or nutrient enrichment. It becomes important therefore to examine the response of the alga to known levels of nutrients, which can then be related to levels found in the field. It might be borne in mind from the outset that nutrient levels are high in the water column in winter, while growth occurs essentially in summer. For this reason some attention is given here to the levels of nutrients accumulated by the tissues, which might be redeployed in growth at a later stage, and this matter is addressed more directly in Chapter 8.

Because a growth increase in response to nutrient addition occurs more slowly and can be detected less sensitively than an increase in photosynthesis,in response to light and temperature, the alga was cultured in the laboratory and its growth measured by change in dry weight. The general strategy was to raise the plants under adequate (non-limiting) light and temperature conditions, and then examine the effects on growth of nitrogen and phosphorus separately and together. Salinity effects were investigated in another experiment. Short term photosynthetic measurements suggested little effect (Chapter 6), but here the possibility of a longer-term growth response was examined.

7.2 MATERIALS AND METHODS

7.2.1 Plant Material

Cladophora was collected from Falcon Bay, Peel Inlet in February 1979 and grown in artificial seawater (see below) at 25°C for two weeks before the start of this study. Known fresh weights (20 mg) of algae were initially placed into 2 litre conical flasks filled with 1.5 litres of medium.

These cultures were essentially "unialgal", but there were always some epiphytes associated with the filaments. Where epiphyte loads were high, the alga was shaken in N- and P-free artificial seawater and examined under the microscope.

7.2.2 Medium

The background medium was the same artificial seawater described in the previous chapter (Table 6.1). The medium

has a wide spectrum of nutrients, and has been used successfully in cultures of marine macroalgae (e.g. Iwasaki 1967) including Cladophora (Wik-Sjöstedt and Nordqvist 1970). Modifications were the addition of an inorganic carbon source, as $NaHCO_3$, giving alkalinities similar to those of the estuary. Inorganic nitrogen was added as NH_4NO_3, as both N species are present in the estuary. Inorganic phosphorus was added as K_2HPO_4. No glycerophosphate and hence no nitrilotriacetic acid buffer was included. This buffer was deleted because its presence interfered with colour development during ammonia determinations; however, the pH of the medium during these experiments did not vary by more than 0.3 of a unit (8.0 ± 0.3). Two experiments were run, one in which inorganic P was varied and the second in which inorganic N was varied, in both cases maintaining all other nutrients at levels unlikely to be limiting. For logistical reasons, both experiments were conducted essentially as batch cultures, making possible the measurement of rates of uptake of inorganic N and P. Concentrations of N and P were measured daily, and regular solution changes were required, usually every third day. Where the treatment concentration required was low, only the particular nutrient required was added at intermediate times. Uptake rates of PO_4 - P, NH_4 - N and NO_3 - N were calculated as loss from solution.

7.2.3 Nutrient Analyses

Orthophosphate was analysed by the single solution method (Major et al. 1972). Ammonia-N was determined using the phenolnitroprusside technique (Dal Pont et al. 1974), and nitrate-nitrogen using an auto analyser (Technicon Industrial Method No. 100-70W, Technicon Industrial Systems, Tarrytown, New York). pH and alkalinity were recorded initially and when solutions were changed, the latter by titration (APHA 1971). Total N and P in algal tissue was measured on acid digested samples (100% HNO_3 followed by 50% $HClO_4$); nitrogen was measured colorimetrically (Technicon Industrial Method No. 334 - 74 W/B, Technicon Industrial Systems, Tarrytown, New York) and phosphorus by the single solution method (Major et al. 1972).

Chlorophyll a was measured on 30 mg (fresh weight) samples ground with acid-washed sand containing basic $MgCO_3$, washed into centrifuge tubes to a volume of 8 ml with 90% w/w acetone, and kept in the dark for 24 hours. Solutions were centrifuged (3000 x g; 15 min) and read on a spectrophotometer at 665 and 770 nm using 1 cm cells (Series 634, Varian Techtron Pty. Ltd., Australia). Chlorophyll a concentrations were calculated (Parsons and Strickland 1963) and expressed as mg g^{-1} dry weight. Phaeophytins were measured in these samples following acidification with 0.1N HCl (Lorenzen 1967).

7.2.4 Experimental design

Each experiment was run in three stages: growth in N or P depleted media ("pre-conditioning") was followed by growth in complete media, and finally a return to depleted media. Pre-conditioning was for 7 days during which algae to be grown under different P regimes were placed in P-free medium with 5.0 mg l^{-1} inorganic N, while those to be grown under different N regimes were supplied with 0.5 mg l^{-1} P, but no inorganic N. Three replicate flasks of

of inorganic P and N concentrations similar to those at sites of growth in the estuary (McComb et al. 1981). Phosphorus treatments were from 0 to 0.25 mg ℓ^{-1} and nitrogen from 0 to 5.0 mg ℓ^{-1}. Because nutrient levels fell between solution changes, concentrations used in calculations were the average of intermediate concentrations observed during each period. The levels were, for initial and averaged concentrations (in brackets), 0 (0); 0.010 (0.006); 0.025 (0.015); 0.050 (0.032); 0.250 (0.203) mg ℓ^{-1}P, each at 5.0 mg ℓ^{-1}N, and 0 (0.007); 0.10 (0.08); 0.50 (0.43); 1.0 (0.83); and 5.0 (4.56) mg ℓ^{-1}N each at 0.5 mg ℓ^{-1}P. P treatments were run for 3 weeks and N for 2 weeks, after which the algae were returned for 3-4 weeks to solutions identicial to those in which they had been pre-conditioned.

7.2.5 Culture apparatus

Algae were grown in acid-washed, 2 litre Erlemeyer flasks supported over a bank of 4 x 110 watt fluorescent lamps (Sylvania, Cool White, VHO 48"), with a day length of 12 hours, at 23 ± 1°C. Photosynthetically-active radiation (PAR) was measured with a quantum sensor (Licor, Lincoln, Nebraska, USA). Readings were taken with the sensor immersed in a flask containing 1.5 litres of medium. The light ranged from 270 to 450 $\mu E\ m^{-2}\ sec^{-1}$ which is above saturation for this species (Gordon et al. 1980). All solutions were aerated continuously, the air bubbling through 25%, H_2SO_4 into de-ionised, distilled water and finally into each flask.

7.2.6 Growth measurements

Growth was measured as change in fresh weight over approximately weekly intervals and converted to dry weight from previously determined fresh weight-dry weight ratios (2.56 ± 0.10).

7.2.7 Calculations

Ralative growth rates were determined for each interval using the equation $\overline{RGR} = (\ln W_2 - \ln W_1)/(t_2 - t_1)$ where W_1 and W_2 are dry weight at times t_1 and t_2 respectively.

The Monod growth expression $\mu = \mu_{max}\ (s/K_s + s)$ was used to calculate equations relating the growth rate, μ, to the external nutrient concentration, s. The half-saturation constant, K_s, and the maximum growth rate, μ_{max}, were estimated from a linear regression of μ on s/μ. Similarly, uptake rates, v, were modelled using a linear transform of the Michaelis-Menten equation where $s/v = K/v_{max} + 1/v_{max} \cdot s$. This transform was chosen instead of the frequently used Lineweaver-Burk plot since the latter has been shown to give more biased results (Dowd and Riggs 1965). Transforms of this sort are usually performed on very short time-course depletion curves (e.g. D'Elia et al. 1978), where substrate concentration remains relatively unaffected. In the present study, where uptake was measured from depletion of nutrients over longer periods (up to 24 hours), substrate concentrations fell appreciably, and here 's' represents the average concentration of substrate during this time.

7.2.8 Salinity and growth

The effects of salinity on growth were investigated by growing the alga for 14 days using the medium described in Chapter 6.

7.3 RESULTS

7.3.1 Growth

Increases in dry weight are shown in Figures 7.1 and 7.2.

The greatest response for P-treated plants was obtained with the highest substrate concentration, representing some 17-fold increase in dry weight over 3 weeks. During the third week of treatment, yields were proportional to the substrate concentration supplied. Growth occurred by fragmentation of algal material, and the formation of new ball-like plants similar to those observed in the estuary (Fig. 7.3).

For nitrogen, there was no significant different after 2 weeks amongst final weights attained by plants given 0.5, 1.0 or 5.0 mg ℓ^{-1}, which all gave more than a 5-fold increase in dry weight over the controls.

Relative growth rates (RGR) for each weekly interval, are shown in Tables 7.1 and 7.2 for P and N trials, respectively, along with the estimated doubling times over each interval. A regression of the logarithm of growth increments against time showed that algae at each substrate concentration in these experiments were growing exponentially in complete medium. The lag phase of the growth curve was most obvious during the first week, with lower RGR values, particularly in the N treatments (Table 7.2).

Relative growth rates for both experiments during 7 days of pre-conditioning in depleted media suggest that the inocula were growing well even before treatments were started. During this period the algae in P-free media doubled their weight in 5 days (Table 7.1) and in 8 days for those growing in N-free media (Table 7.2). Growth rates fell steadily in control flasks during these experiments to 20% of rates measured over the week of pre-conditioning. These give estimated doubling times of 28 and 34 days after 4 and 3 weeks without P and N, respectively. While these treatments showed steadily decreasing rates, those algae in higher substrates had increased their rates up to 0.17 day^{-1} by the end of the growth period in complete medium, this representing a doubling time of 4 days (Table 7.2).

Equations relating growth during this final week of treatment in complete medium to the external substrate concentrations are shown in Figures 7.4 and 7.5. Concentrations below about 0.02 and 0.03 mg ℓ^{-1}P strongly influenced the growing rate, and above these levels the effect was much reduced. For nitrogen, growth rates were saturated above 0.4 mg ℓ^{-1}, with the greatest response below 0.1 mg ℓ^{-1}.

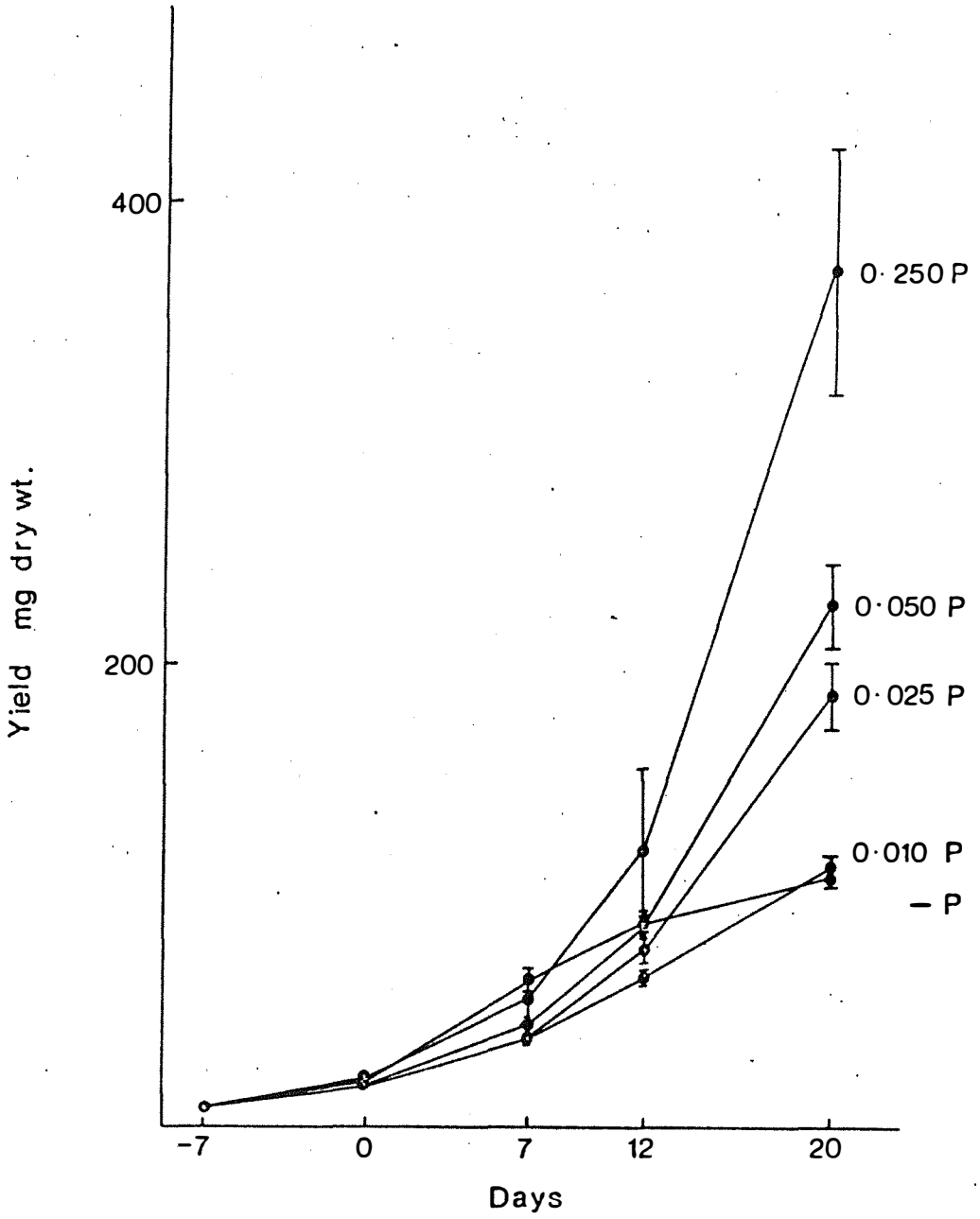


Figure 7.1 Yields of *Cladophora* grown with different inorganic phosphorus substrate concentrations (0 to 0.25 mg ℓ^{-1} P) each in the presence of 5.0 mg ℓ^{-1} inorganic nitrogen. Data shown include pre-conditioning in P-depleted medium for 7 days (-7 to 0) followed by 20 days in complete medium. Each point is the mean of 3 replicates ± S.E.

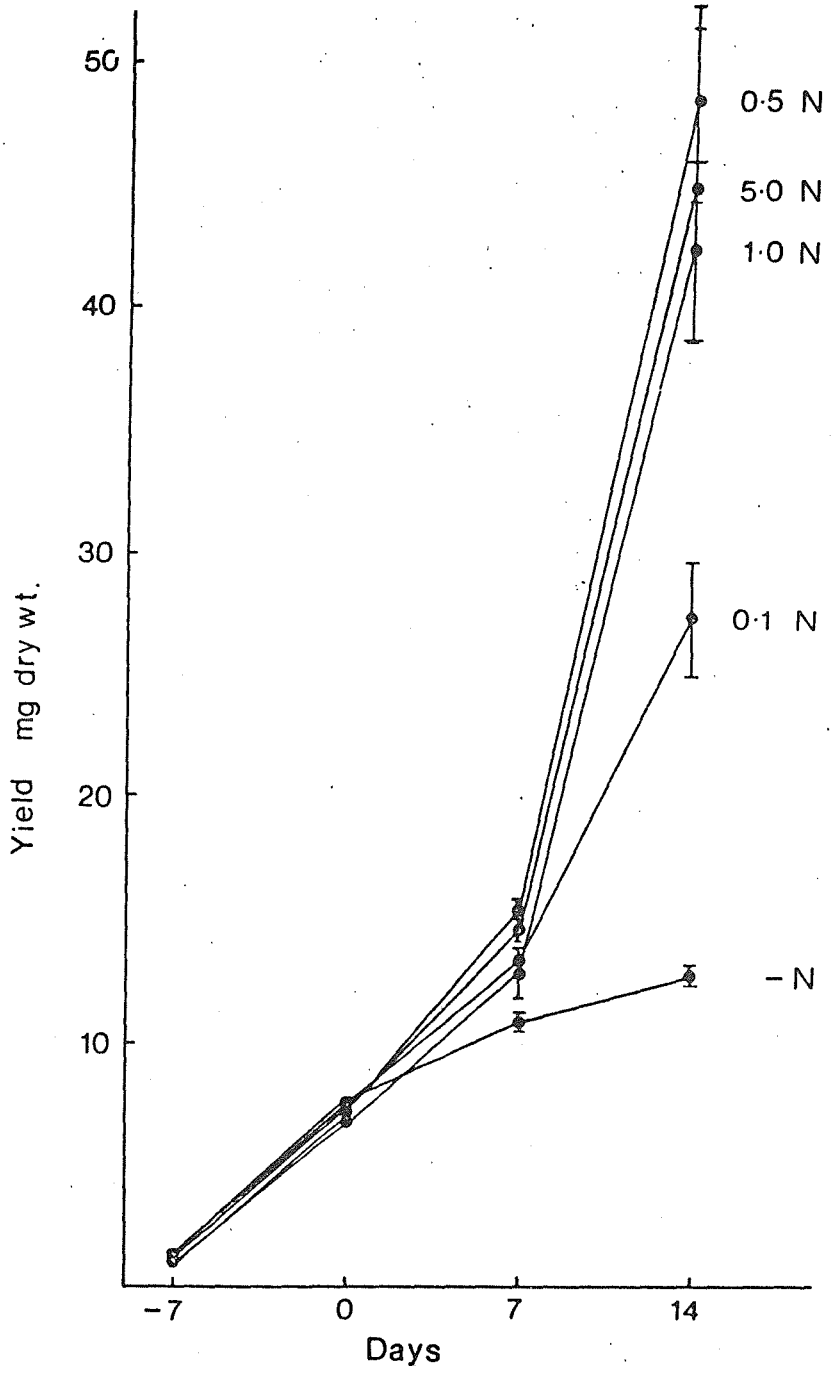


Figure 7.2 Yields of *Cladophora* grown with different inorganic nitrogen substrate concentrations (0 to 5 mg ℓ^{-1}N) each in the presence of 0.5 mg ℓ^{-1} inorganic phosphorus. Data shown include pre-conditioning in N-depleted medium for 7 days (-7 to 0) followed by 14 days in complete medium. Each point is the mean of 3 replicates ± S.E.

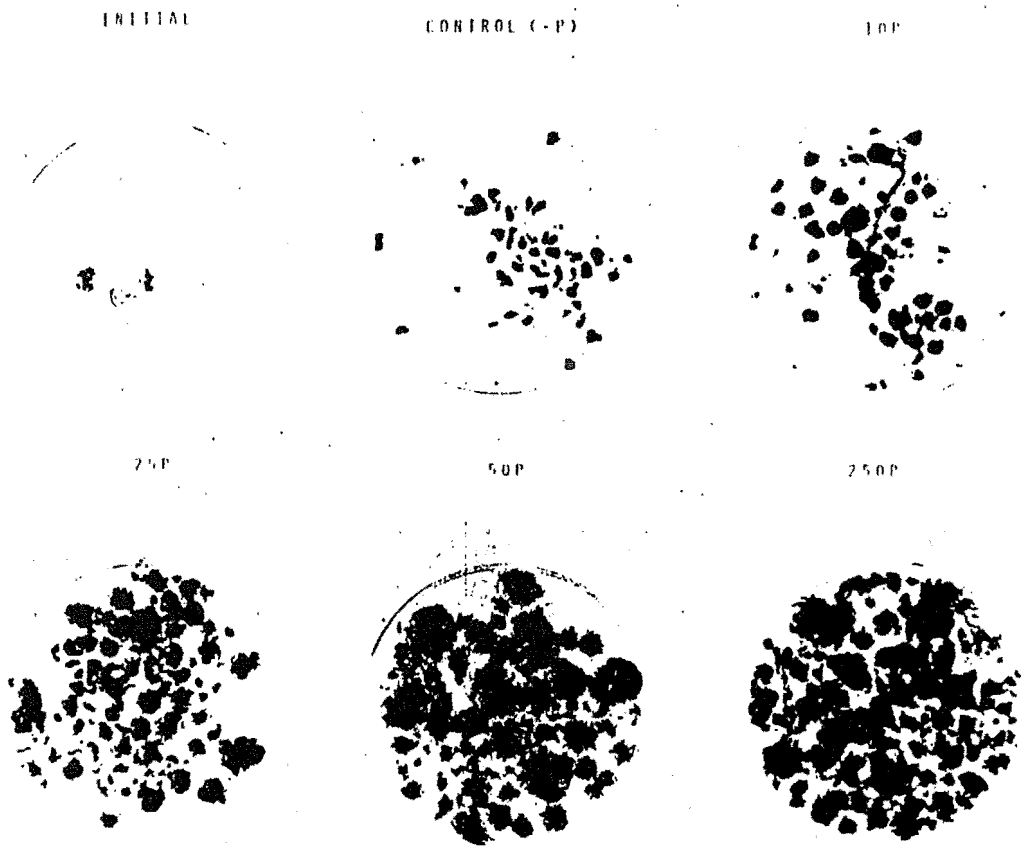


Figure 7.3 Yields of *Cladophora* after 20 days growth in complete medium with control (P-free), 10P (0.010 mg ℓ^{-1}P), 25P (0.025 mg ℓ^{-1}P), 50P (0.050 mg ℓ^{-1}P) and 250P (0.250 mg ℓ^{-1}P), each in the presence of 5.0 mg ℓ^{-1}N, compared with initial inoculum. New plants are ball-like, densely branched, radiating filaments similar to those in beds in the estuary.

TABLE 7.1

THE EFFECT OF PHOSPHORUS ON GROWTH OF CLADOPHORA IN THE PRESENCE OF ADEQUATE NITROGEN

Growth is shown both as relative growth rates (RGR; g dry weight of alga, i.e. day^{-1}) and as doubling times (D), the time taken for a doubling in dry weight. RGR is mean ± S.E. for three replicates in each treatment. Nitrogen was provided at 5.0 mg ℓ^{-1}

Substrate P (mg ℓ^{-1})		Pre-conditioning in depleted medium (-P)	Treatment with complete medium			Growth on return to depleted medium (-P) at the end of the experiment			
		Days 7 - 0	0 - 7	7 - 12	12 - 20	0 - 6	6 - 18	18 - 23	23 - 30
0	RGR	0.130 ± 0.001	0.165 ± 0.009	0.065 ± 0.001	0.026 ± 0.002	0.027 ± 0.002	0.039 ± 0.002[1]	0.025 ± 0.004	0.018 ± 0.002
	D	5.3 - 5.4	4.0 - 4.4	10.5 - 10.8	24.7 - 28.9	23.9 - 27.7	16.9 - 18.7	23.9 - 33.0	34.6 - 43.3
0.010	RGR	0.109 ± 0.008	0.111 ± 0.001	0.102 ± 0.005	0.068 ± 0.001	0.092 ± 0.011	0.032 ± 0.002	0.073 ± 0.002[2]	0.028 ± 0.009
	D	5.9 - 6.7	6.2 - 6.3	6.5 - 7.1	10.0 - 10.3	6.7 - 8.5	20.4 - 23.1	9.2 - 9.8	18.7 - 36.5
0.025	RGR	0.112 ± 0.001	0.110 ± 0.001	0.138 ± 0.008	0.110 ± 0.001	0.152 ± 0.001	0.051 ± 0.001	0.079 ± 0.003	0.037 ± 0.001
	D	6.1 - 6.2	6.2 - 6.3	4.7 - 5.3	6.2 - 6.5	4.5 - 4.6	13.3 - 15.9	8.4 - 9.1	18.2 - 19.2
0.050	RGR	0.126 ± 0.004	0.118 ± 0.003	0.137 ± 0.006	0.119 ± 0.001	0.215 ± 0.001	0.078 ± 0.001	0.055 ± 0.001	0.042 ± 0.001
	D	5.3 - 5.7	5.7 - 6.0	4.8 - 5.3	5.8 - 5.9	3.2 - 3.3	8.8 - 9.0	12.4 - 12.8	16.1 - 16.9
0.250	RGR	0.134 ± 0.009	0.145 ± 0.016	0.151 ± 0.012	0.141 ± 0.015	0.219 ± 0.020	0.061 ± 0.004	0.047 ± 0.010	0.024 ± 0.005
	D	4.8 - 5.5	4.3 - 5.3	4.2 - 5.0	4.4 - 5.5	2.9 - 3.5	10.7 - 12.2	12.2 - 18.7	23.9 - 36.5

[1] [2] Higher rates, particularly in the low treatments, are presumably due to P contamination

TABLE 7.2

The effect of nitrogen on growth of Cladophora in the presence of adequate phosphorus.

Growths given as RGR (day^{-1}) and doubling times D (days), as detailed in the caption for Table 7.1. Phosphorus was provided at 0.5 mg l^{-1}.

Substrate N (mg l^{-1})		Pre-conditioning in depleted medium (-N)	Treatment with complete medium		Growth on medium for depleted medium (-N) at the end of the experiment		
	Days	-7 -0	0 - 7	7 - 14	0 - 6	6 - 14	14 - 20
0	RGR	0.087 ± 0.001	0.049 ± 0.005	0.022 ± 0.002	0.016 ± 0.005	0.008 ± 0.002	
	D	7.9 - 8.1	12.8 - 15.7	28.9 - 34.6	33.0 - 63.0	69.3 - 115.5	
0.1	RGR	0.083 ± 0.013	0.078 ± 0.003	0.101 ± 0.004	0.038 ± 0.002	0.024 ± 0.007	
	D	7.2 - 9.9	8.5 - 9.2	6.6 - 7.1	17.3 - 19.2	22.3 - 40.8	
0.5	RGR	0.078 ± 0.003	0.108 ± 0.001	0.163 ± 0.009	0.120 ± 0.007	0.032 ± 0.002	0.013 ± 0.006
	D	8.5 - 9.2	6.3 - 6.5	4.0 - 4.5	5.4 - 6.1	20.4 - 23.1	36.5 - 99.0
1.0	RGR	0.078 ± 0.006	0.093 ± 0.007	0.170 ± 0.001	0.111 ± 0.002	0.043 ± 0.001	0.011 ± 0.001
	D	8.2 - 9.6	6.9 - 8.1	4.0 - 4.1	6.1 - 6.3	15.7 - 16.5	57.8 - 69.3
5.0	RGR	0.085 ± 0.008	0.099 ± 0.-01	0.161 ± 0.014	0.083 ± 0.005	0.070 ± 0.02	-0.007 ± 0.001
	D	7.4 - 9.0	6.9 - 7.1	4.0 - 4.7	7.9 - 8.9	9.6 - 10.2	-

[1] Rates low and erratic; data averaged for last 14 days

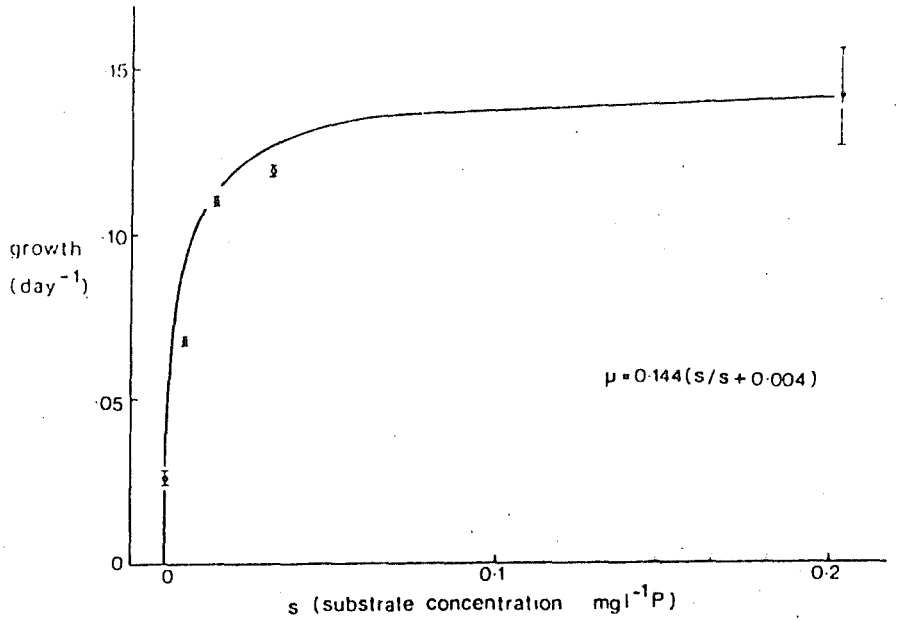


Figure 7.4 Relative growth rates (day^{-1}) of *Cladophora* during last week of growth in complete medium (0 to 0.25 mg ℓ^{-1}P; 5.0 mg ℓ^{-1}N) as a function of the substrate P concentration. Each point is the mean of 3 replicates ± S.E.

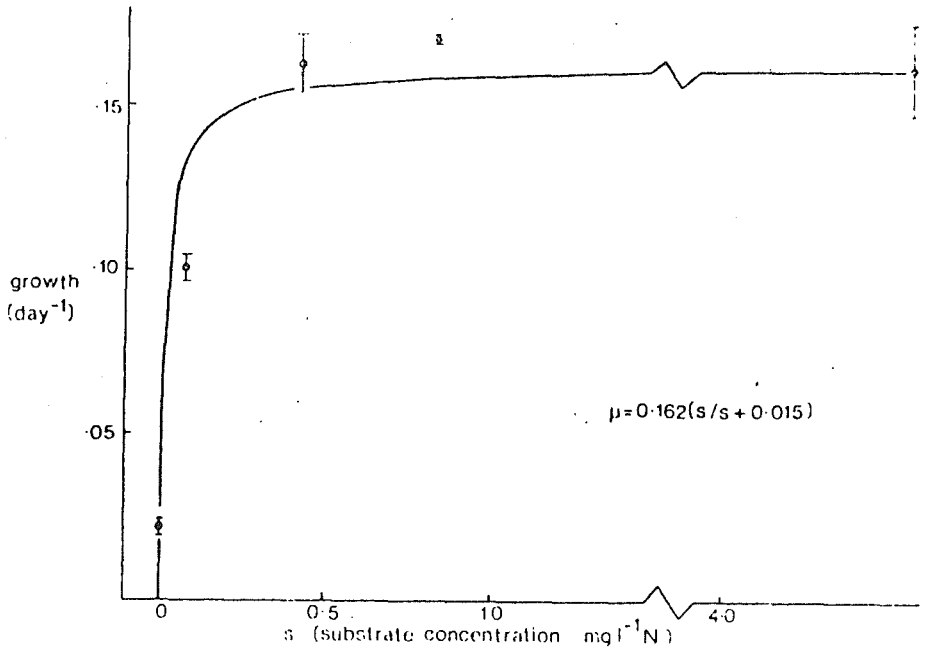


Figure 7.5 Relative growth rates (day^{-1}) of *Cladophora* during the last week of growth in complete medium (0 to 5.0 mg ℓ^{-1}N; 0.5 mg ℓ^{-1}P) as a function of the substrate N concentration. Each point is the mean of 3 replicates ± S.E.

7.3.2 Growth on return to minimal medium

Rates of growth following a return to either P-free or N-free media are shown in Tables 7.1 and 7.2, and yields are given in Figs. 7.6 and 7.7. For both nutrients, better growth was obtained on return to depleted media, if the algae had been growing in high concentrations of nutrients. Though it was very difficult to entirely remove inorganic P from the P-free media, relative growth rates fell to 0.018 day $^{-1}$ after 50 days without added P (though with N in high concentration, (Table 7.1). Similarly, those algae in N-free media (though with P in high concentration) showed little growth after 40 days (Table 7.2). In contrast, those previously given high concentrations of N or P continued growing at high rates for a longer period. For example, following 3 weeks in high nutrients, Cladophora returned to P-free media was still growing well (up to 0.042 day^{-1}) 4 weeks later (Table 7.1) with a doubling time up to 16 days. Those returned to N-free media for 3 weeks, after 2 weeks in high nutrients, showed lower growth rates (up to 0.013 day^{-1}) with a doubling time greater than 36 days (Table 7.2).

7.3.3 Nutrient uptake

Rates of uptake of P for each substrate concentration are shown in Table 7.3. As noted above (see Materials and Methods), these data were fitted to a Michaelis-Menten transform and the resulting maximum uptake rates (v_{max}) and half-saturation constants (K_s) are shown at the beginning and near the end of growth in complete medium (Table 7.4).

Uptake rates increased with increasing P substrate concentrations, particularly during the final week in complete medium. The affinity for P was more obvious in cultures supplied with the nutrient soon after pre-conditioning in P-free medium, with higher rates during the first week. This is also evident from the lower K_s values for the first day in complete medium compared with those measured 18 days later. Uptake rates were typically lower when calculated over a combined light and dark period compared to those measured over shorter periods in the light.

The average weekly uptake rates of ammonia and nitrate-nitrogen are shown in Table 7.5. Cladophora was able to use both N species simultaneously in all treatments, though at the highest substrate concentration, uptake of nitrate was negligible compared to ammonia. Ammonia uptake rates were higher than those for nitrate at all treatment concentrations. Preferential uptake of ammonia during the last week of growth in complete medium was demonstrated here, where rates of simultaneous uptake of both species in the light and in the dark are compared (Table 7.6). Uptake rates were clearly lower in the dark compared with those in the light, though there was still preferential uptake of ammonia.

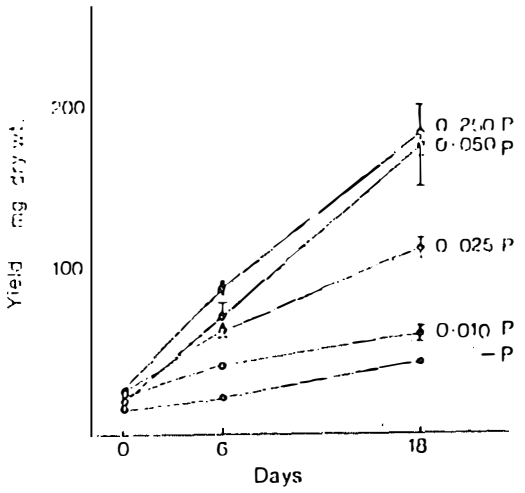


Figure 7.6 Yields of *Cladophora* on return to medium without added P in the presence of 5.0 mg ℓ^{-1}N, following growth for 3 weeks in complete medium (0 to 0.25 mg ℓ^{-1}P; 5.0 mg ℓ^{-1}N). Each point is the mean of 3 replicates ± S.E.

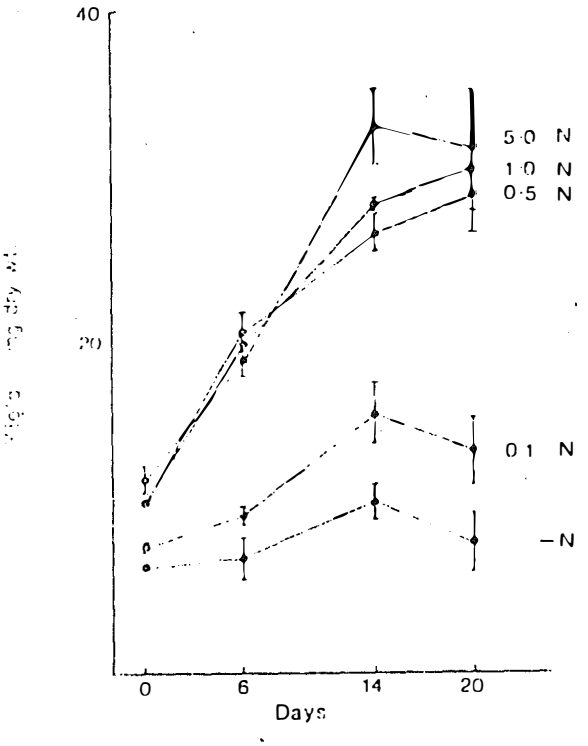


Figure 7.7 Yields of *Cladophora* on return to medium without added N in the presence of 0.5 mg ℓ^{-1}P, following growth for 2 weeks in complete medium (0 to 5.0 mg ℓ^{-1}N; 0.5 mg ℓ^{-1}P). Each point is the mean of 3 replicates ± S.E.

TABLE 7.3

Uptake rates of phosphate-phosphorus by Cladophora growing in complete medium with different levels of phosphate.

Data are weekly means (± S.E.) from daily uptake rates. There were 3 replicates of each treatment. The daily rates were calculated from measurements made over light + dark periods of 13, 22 and 20 hours for weeks 1, 2 and 3 respectively.

Substrate P ($mg\ l^{-1}$)	P uptake ($mg\ g^{-1}$ dry weight hr^{-1})		
	Week 1	Week 2	Week 3
0.010	0.036 ± 0.009	0.006 ± 0.001	0.008 ± 0.002
0.025	0.071 ± 0.015	0.017 ± 0.003	0.015 ± 0.004
0.050	0.072 ± 0.013	0.031 ± 0.002	0.025 ± 0.007
0.250	0.078 ± 0.019	0.034 ± 0.006	0.056 ± 0.002

TABLE 7.4

Phosphorus uptake rates for Cladophora maximum uptake rate (V_{max}) and half-saturation (K_s) for phosphate phosphorus is shown for different substrate concentrations (0 - 0.25 mg l^{-1}P). Nitrogen was present at 5.0 mg ℓ^{-1} inorganic nitrogen. Data shown are at the start (day 1) and near the end (day 18) of growth in complete medium. They were calculated from the mean of 3 replicates at each of 5 substrates concentrations in the light (L) and over a combined light and dark period (L + D).

	Illumination	Time (hrs)	V_{max} (mg g^{-1} dry wt. hr^{-1})	K_s (mg l^{-1})
Day 1	L	4.5	0.127 ± 0.003	0.002 ± 0.003
Day 1	L & D	17.0	0.121 ± 0.001	0.011 ± 0.001
Day 18	L	3.5	0.112 ± 0.019	0.015 ± 0.001
Day 18	L & D	22.5	0.058 ± 0.012	0.055 ± 0.010

TABLE 7.5

Inorganic nitrogen uptake by *Cladophora*

Uptake rates of ammonia and nitrate-nitrogen were measured for *Cladophora* growing in otherwise complete medium, with different N concentrations. Data are weekly means (± S.E.) based on daily uptake rates; 3 replicates were used at each concentration.

Rates were measured over combined light and dark periods of 28 ± 6 and 23 ± 5 hours for weeks 1 and 2 respectively.

	N uptake (mg g^{-1} dry weight hr^{-1})			
	Week 1		Week 2	
Substrate N ($mg\ \ell^{-1}$)	NH_4-N	NO_3-N	NH_4-N	NO_3-N
0.1	0.15 ± 0.06	0.09 ± 0.03	0.15 ± 0.05	0.11 ± 0.04
0.5	0.45 ± 0.12	0.15 ± 0.05	0.32 ± 0.06	0.23 ± 0.05
1.0	0.88 ± 0.18	0.15 ± 0.07	0.54 ± 0.06	0.26 ± 0.29
5.0	1.76 ± 0.46	0.22 ± 0.14	0.63 ± 0.17	0.05 ± 0.03

TABLE 7.6

Uptake of ammonia and nitrate by Cladophora

Rates were measured after 2 weeks growth in complete medium with different levels of ammonium nitrate. The experimental period was 5 hours in the light and 17 hours in the dark. Each datum is the mean of 3 replicates ± S.E.

Substrate N ($mg\ \ell^{-1}$)	N uptake ($mg\ g^{-1}$ dry weight hr^{-1})			
	NH_4-N		NO_3-N	
	Light	Dark	Light	Dark
0.1	0.42 ± 0.03	0.10 ± 0.01	0.33 ± 0.02	0.11 ± 0.01
0.5	0.64 ± 0.12	0.12 ± 0.05	0.47 ± 0.04	0.26 ± 0.12
1.0	0.53 ± 0.06	0.23 ± 0.02	0.65 ± 0.13	0.07 ± 0.03
5.0	1.67 ± 0.49	0.33 ± 0.17	0	0.04 ± 0.03

These data give v_{max} and K_s values, in the light, of 1.82 ±0.31 mg g $^{-1}$ dry weight h^{-1}; 0.29 ± 0.16 mg ℓ^{-1} for ammonia-nitrogen, and 0.59 0.08 mg g^{-1} dry weight hr^{-1}; 0.02 ± 0.01 mg ℓ^{-1} for nitrate-nitrogen. The transform for NO_3- N was calculated excluding rates at the highest substrate N concentration, these having fallen close to zero. Over the substrates used, however, the fit is significant (r = 0.99).

7.3.4 Nutrients in the algal tissue

The relationship between the total amount of nutrient taken up from solution over the period of treatment and the corresponding change in total P and N in the tissue is shown for both experiments in Fig. 7.8. There is almost a 1:1 (r = 0.99) relationship between these for the P treatments. Those grown in the highest P concentration, however, have exaggerated tissue P concentrations, which suggests some contamination at these higher levels. In contrast, the relationship was not linear with nitrogen, where there was little further increase in nitrogen concentration in the tissue in the presence of large amounts of this nutrient. This has also been reported for aquatic angiosperms (Gerloff and Krombholz 1966) where most N added to the medium was recovered in the tissue, except in those plants growing with high external N concentrations (> 21 mg ℓ^{-1}). Nitrogen taken up but not incorporated may have been released back to solution in organic form at these high levels. At the time of the last harvest, organic, nitrogen in the water was high (0.78 ± 0.01 to 1.03 ± 0.01 mg ℓ^{-1}), particularly in the two highest N treatments. Nevertheless, the relationship between the amount of nutrient taken up and corresponding incorporation into the tissue is linear for substrate P and N supplied at concentrations similar to those in estuary water.

Concentrations of nitrogen and phosphorus in the tissue and their corresponding N: P ratios during each phase of the experiment are summarised in Tables 7.7 and 7.8, where increasing substrate concentrations result in increases in tissue N and P in complete medium. Concentrations of nitrogen and phosphorus in the tissue following the period of growth in complete medium are shown as a function of P and N substrate concentrations in Fig. 7.9. Saturation of the tissue is evident when high concentrations of inorganic N are supplied, though this is not the case with phosphorus, where the high substrate concentration supplied did not saturate the tissue; higher concentrations of P may well have resulted in even higher tissue P levels. Similar trends have been observed with Cladophora in the field (Wallentinus 1979), where tissue nitrogen concentrations were saturated above 50 mg g^{-1} dry weight (c.f. 76 mg g^{-1} in culture in this study), these levels corresponding to growth with the highest external nitrogen concentration. For the alga growing without added P (controls) there was a reduction in tissue P of over 50% after 4 weeks (Table 7.7). N in this tissue was initially high, though within the range encountered in the field, but was reduced by a

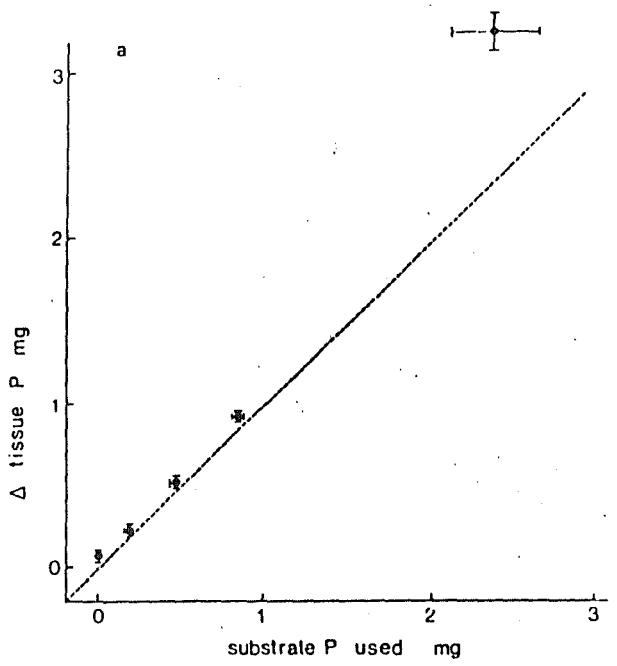


Figure 7.8a Increase in total phosphorus (mg) present in the tissue of *Cladophora* after 3 weeks growth in complete medium (0 to 0.25 mg ℓ^{-1}P; 5.0 mg ℓ^{-1}N) as a function of the amount of inorganic phosphorus (mg) taken up over this time.

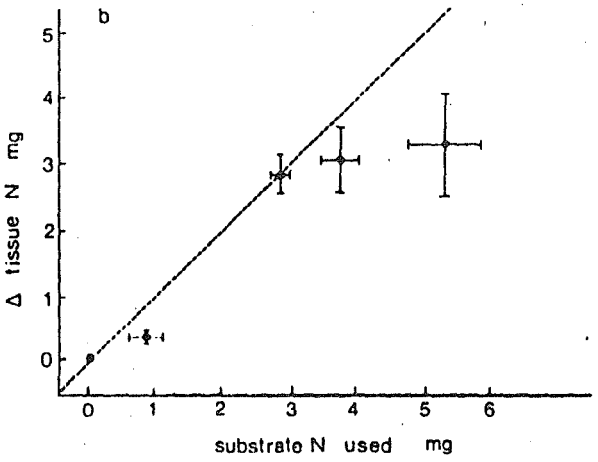


Figure 7.8b Increase in total nitrogen (mg) present in the tissue of *Cladophora* after 2 weeks growth in complete medium (0 to 5.0 mg ℓ^{-1}N; 0.5 mg ℓ^{-1}P) as a function of the amount of inorganic nitrogen (mg; NH_4-N + NO_3-N) taken up over this time.

TABLE 7.7

Effect of substrate on tissue concentration of phosphorus and nitrogen in Cladophora. The concentration of P and N in the tissue are shown for different concentrations of P in the presence of 5.0 mg l^{-1} N.

Each figure is the mean ± S.E. of 3 replicates.

Substrate P ($mg\ l^{-1}$)	Concentration in tissue ($mg\ g^{-1}$ dry weight)								
	Initially			After 3 weeks growth in complete Medium			Four weeks after return of Medium without added P		
	P	N	N:P	P	N	N:P	P	N	N:P
0	2.04 ± 0.15	43.0 ± 0	21:1	0.99 ±0.16	21.8 ± 1.5	22:1	0.53 ± 0.09	18.6 ± 1.8	35:1
0.010	2.04 ± 0.15	43.0 ± 0	21:1	2.21 ±0.16	41.5 ± 1.6	19:1	0.86 ± 0.02	29.5 ± 1.6	34:1
0.025	2.04 ± 0.15	43.0 ± 0	21:1	2.92 ±0.18	47.5 ± 2.2	16:1	0.74 ± 0.03	28.0 ± 1.9	38:1
0.050	2.04 ± 0.15	43.0 ± 0	21:1	4.24 ±0.07	56.7 ± 2.6	13:1	0.61 ± 0.02	26.0 ± 0.7	42:1
0.250	2.04 ± 0.15	43.0 ± 0	21:1	8.88 ±0.31	50.0 ± 2.1	6:1	0.76 ± 0.04	28.6 ± 0.6	38:1

TABLE 7.8

Effects of substrate nitrogen on tissue concentrations of phosphoros and nitrogen in *Cladophora*. Concentrations of P and N are shown, for different concentrations of N in the presence of 5.0 mg l^{-1} P.

Each figure is the mean ± S.E. of 3 replicates.

Substrate P (mg l^{-1})	Concentration in tissue (mg g^{-1} dry weight)								
	Initially			After 2 weeks growth in complete medium			Three weeks after return to medium without added P		
	P	N	N:P	P	N	N:P	P	N	N:P
0	2.64 ± 0.22	36.6 ± 2.9	14:1	2.25 ± 0.08	12.8 ± 0.9	6:1	-	14.5 ± 0.2	
0.1	2.64 ± 0.22	36.6 ± 2.9	14:1	4.07 ± 0.07	17.1 ± 0.6	4:1	4.27 ± 0.13	16.9 ± 4.2	4:1
0.5	2.64 ± 0.22	36.6 ± 2.9	14:1	5.67 ± 0.90	61.4 ± 3.4	11:1	4.27[1]	18.0 ± 2.2	4:1
1.0	2.64 ± 0.22	36.6 ± 2.9	14:1	5.62 ± 0.22	75.9 ± 4.2	13:1	5.19 ± 0.15	22.3 ± 2.8	8:1
5.0	2.64 ± 0.22	36.6 ± 2.9	14:1	6.95 ± 0.83	78.4 ± 7.5	11:1	12.75 ± 1.27	25.6 ± 2.4	2:1

[1] One value only

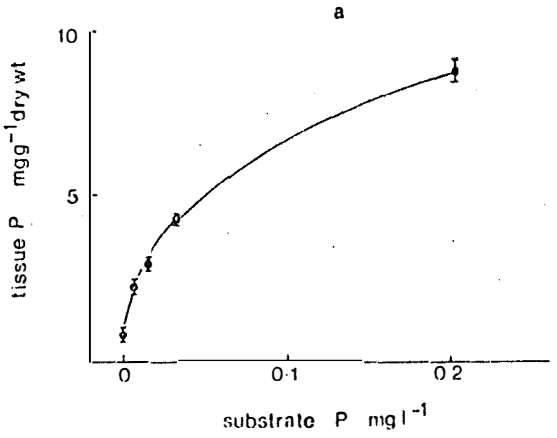


Figure 7.9a Concentration of total P in *Cladophora* tissue (mg g^{-1} dry wt) during the last week of growth in complete medium (0 to 0.25 mg ℓ^{-1}P; 5.0 mg ℓ^{-1}N) as a function of the substrate P concentration (mg ℓ^{-1}) supplied.

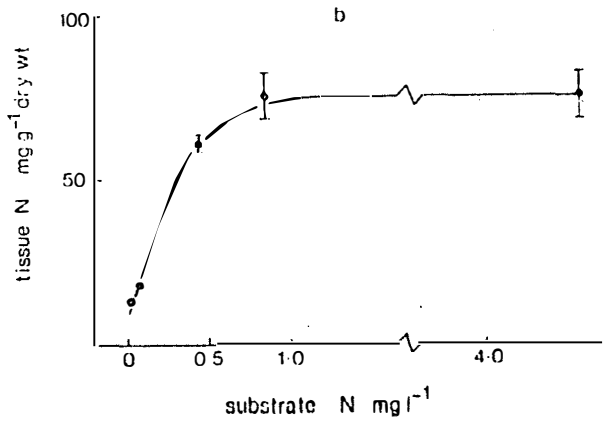


Figure 7.9b Concentration of total N in *Cladophora* tissue (mg g^{-1} dry wt) during the last week of growth in complete medium (0 to 5.0 mg ℓ^{-1}N; 0.5 mg ℓ^{-1}P) as a function of the substrate N concentration (mg ℓ^{-1}) supplied.

similar amount over this period. At P substrate concentrations above 0.01 mg ℓ^{-1}, phosphorus in the tissue was maintained above 2 mg g^{-1}, dry weight, (Table 7.7), and increased with corresponding increases in the substrate concentrations. In these P treatments (with N in high supply), some incorporation of N into the tissue was evident after 3 weeks, particularly the two highest P treatments, with over 50 mg g^{-1} dry weight, this representing an increase over initials of up to 32%.

Nitrogen concentrations fell in tissue to which no N was supplied; the fall was almost 47% during the week of pre-conditioning, and up to 34% over the following 2 weeks when tissue N concentrations were then about 12 mg g^{-1} dry weight (Table 7.8). Tissue N levels increased in those plants given a substrate concentration above 0.1 mg ℓ^{-1} N, reaching a plateau at high substrate concentrations, (Table 7.8) the maximum being 76 mg g^{-1} dry weight, which corresponds with an increase of almost 300% in internal nitrogen over 2 weeks. Some incorporation of P into these tissues was also evident, being most pronounced in the alga grown with the highest N treatments.

Concentrations of tissue P in these plants without added N did not fall markedly during pre-conditioning, though there was an 18% decrease over the following 2 weeks to 2.25 mg g^{-1} dry weight.

7.3.5 Nutrient levels on return to depleted medium

The change in tissue P and N on return to depleted medium is also shown in Tables 7.7 and 7.8. There was a significant reduction in final concentrations after 3-4 weeks, particularly those previously growing in high substrate concentrations of P or N. For example, those algae whose growth in high nutrients resulted in elevated internal P concentrations up to nearly 9 mg g^{-1} dry weight (Table 7.7), were reduced by over 90% to 0.76 mg g^{-1} after 4 weeks in P-free medium. Similarly, those which had accumulated very high N levels (76 mg g^{-1} dry weight) were reduced by over 66% following return to N-free media for 3 weeks.

The loss of stored nitrogen in N-treated algae on return to depleted medium for 3 weeks was proportional to the concentration of substrate N under which they had been previously growing.

Prolonging this period of growth in depleted medium beyond 3 weeks may have reduced tissue nitrogen concentrations in those algae which were previously growing in high N substrates to levels as low as those of the controls. The extent of losses of stored P on return to P-free medium for 4 weeks was similar for all treatments regardless of the substrate concentrations under which they had been previously growing.

7.3.6 Growth rates and nutrient levels

Growth rates during the last week of treatment in complete medium are shown as a function of the corresponding concentration of nitrogen and phosphorus in the tissue in Fig. 7.10. Extrapolation of the "limiting" portion of these curves, to the growth rate at saturating nutrient levels results in a "critical" tissue nutrient concentration of 3.3 mg g^{-1} and 21 mg g^{-1} dry weight for P and N respectively. Here, "critical" tissue content refers to the minimum concentration of nitrogen or phosphorus in the tissue associated with maximum growth (Gerloff and Krombholz 1966). Extrapolating growth rates in Fig. 7.10 to zero give some estimate of the "minimum viable" tissue content for *Cladophora*, below which the growth rate is negligible. For nitrogen this is about 12 mg g^{-1} dry weight, and 0.5 mg g^{-1} dry weight for phosphorus.

7.3.7 Chlorophyll *a*

There was a strong relationship between growth rates in these experiments during the final week of growth in complete medium and corresponding concentrations of chlorophyll *a* in the tissue (Fig. 7.11), these ranging from 3.6 to 12.0 and 0.6 to 9.1 mg g^{-1} dry weight in P and N experiments, respectively. A close relationship between chlorophyll *a* and nitrogen was evident from the strong correlation between changes in tissue chlorophyll *a* and corresponding changes in tissue nitrogen (Fig. 7.12) though the relationship was not significant with phosphorus. This has been reported previously with *Cladophora* (Wallentinus 1975). Those algae growing in high substrate N concentrations were noticeably darker in colour, in contrast to controls where removal of nitrogen resulted in a marked reduction in chlorophyll *a* in the tissue, with a concomitant rise in the levels of phaeophytins. For example those algae grown without N (controls) for 3 weeks had reduced their chlorophyll *a* from 3.73 mg g^{-1} to 0.69 mg g^{-1} dry weight, the latter associated with 3.82 mg g^{-1} phaeophytins. Following a further 3 weeks in N-depleted medium, the controls had obviously senesced, with no measureable chlorophyll *a*, nitrogen starvation producing chlorosis in these cells (Wallentinus 1975).

Chlorophyll *a* in the remaining treatments had similarly fallen at this time, the loss being less marked in those previously growing with high substrate nitrogen, though it is likely these would have fallen to low levels soon after this experiment was terminated.

7.3.8 Salinity

Growth of *Cladophora* after 2 weeks in different substrates is shown in Fig. 7.13; there was essentially no effect, confirming the earlier shorter-term photosynthetic results over the salinity range 2.8-60°/oo (Chapter 6).

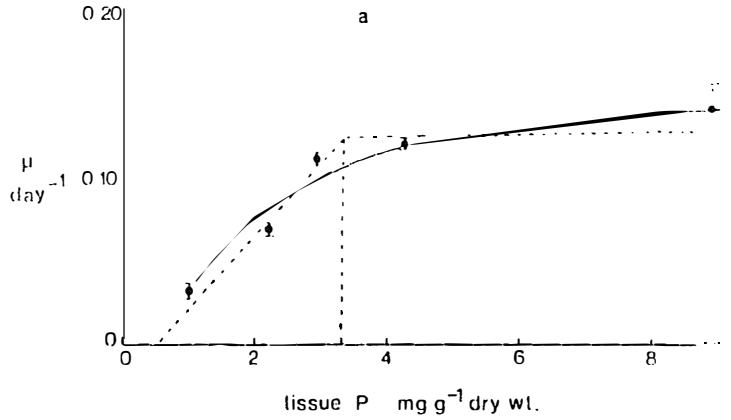


Figure 7.10a Relative growth rate (day^{-1}) of *Cladophora* during the last week in complete medium (0 to 0.25 mg ℓ^{-1}P; 5.0 mg ℓ^{-1}N) as a function of the total P concentration in the tissue (mg g^{-1} dry wt) during this time.

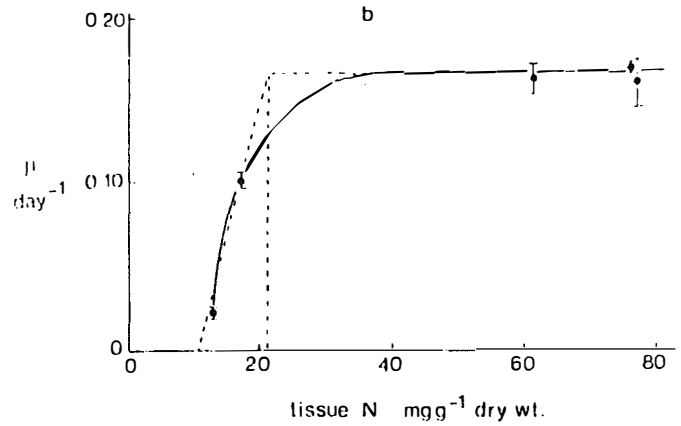


Figure 7.10b Relative growth rate (day^{-1}) of *Cladophora* during the last week in complete medium (0 to 5.0 mg ℓ^{-1}N; 0.5 mg ℓ^{-1}P) as a function of the total N concentration in the tissue (mg g^{-1} dry wt) during this time.

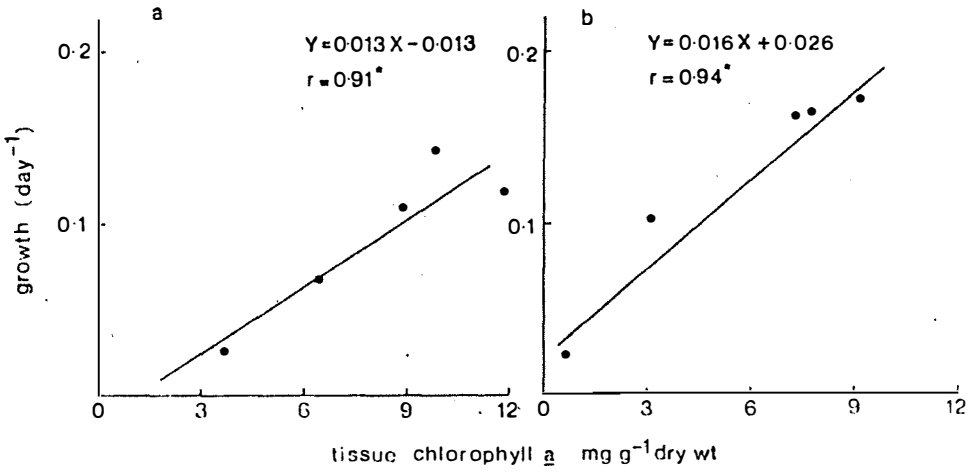


Figure 7.11a Regression of relative growth rate (day^{-1}) of *Cladophora* during the last week in complete medium (0 to 0.25 mg ℓ^{-1}P; 5.0 mg ℓ^{-1}N) with corresponding concentrations of chlorophyll *a* in the tissue (mg g^{-1} dry wt).

Figure 7.11b Regression of relative growth rate (day^{-1}) of *Cladophora* during the last week in complete medium (0 to 5.0 mg ℓ^{-1}N; 0.5 mg ℓ^{-1}P) with corresponding concentrations of chlorophyll *a* in the tissue (mg g^{-1} dry wt).

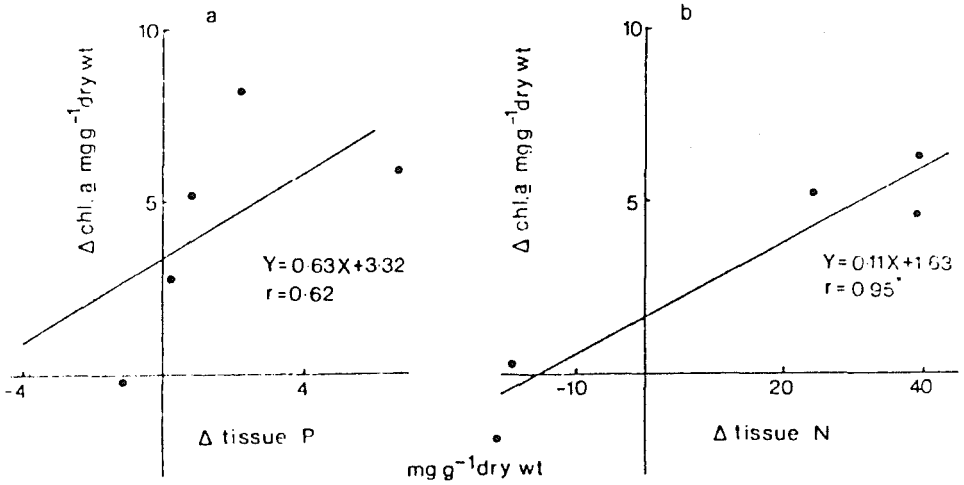


Figure 7.12a Regression of change in concentration of chlorophyll *a* in the tissue (mg g^{-1} dry wt) with corresponding change in tissue P concentration (mg g^{-1} dry wt) following growth in complete medium (0 to 0.25 mg ℓ^{-1}P; 5.0 mg ℓ^{-1}N).

Figure 7.12b Regression of change in concentration of chlorophyll *a* in the tissue (mg g^{-1} dry wt) with corresponding change in tissue N concentration (mg g^{-1} dry wt) following growth in complete medium (0 to 0.50 mg ℓ^{-1}N; 0.5 mg ℓ^{-1}P).

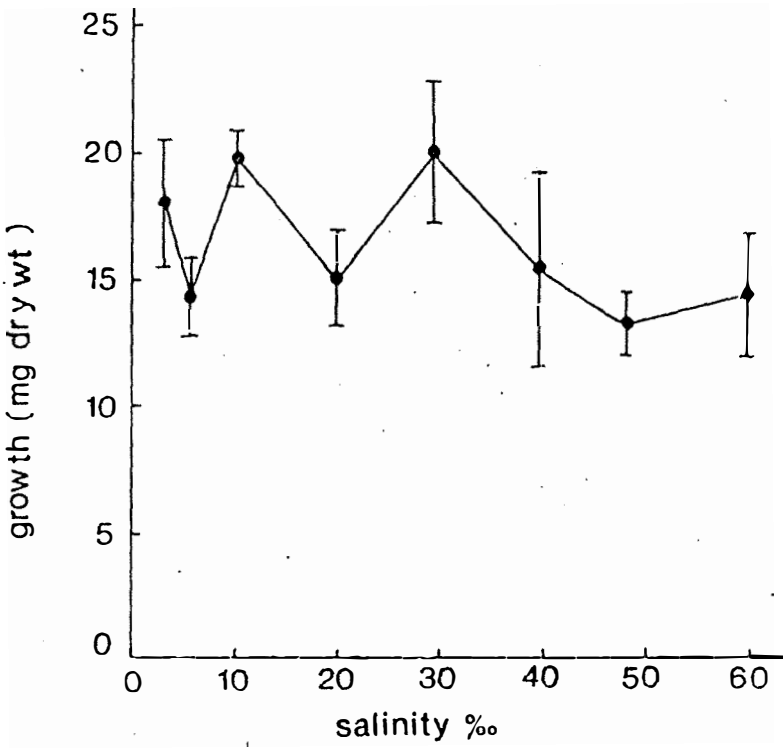


Figure 7.13 Growth of *Cladophora* for 14 days at different salinities in complete medium (0.25 mg ℓ^{-1}P, 2.5 mg ℓ^{-1}N).

7.4 DISCUSSION

7.4.1 Growth

Though there are few data available for comparison, yields of Cladophora in this study appear high when compared with culture studies of other Cladophora species (e.g. Pitcairn and Hawkes 1973). In contrast, reports of cellular doubling times of 1-2 days for a freshwater Cladophora growing exponentially under stagnant conditions in synthetic medium (Zuraw 1969), are higher than the maximum of 3-4 days obtained for whole plants in this study, with high concentrations of N and P supplied (Tables 7.1 and 7.2). A similar doubling time, between 5 and 6 days, has been recorded for the brown alga Ectocarpus growing under near-exponential conditions in medium supplemented with 3 mg ℓ^{-1}P and 7 mg ℓ^{-1}N (Boalch 1961). Comparisons of this sort are of limited use, however, because of the differing responses which may be obtained with different background media (e.g. Oza and Sreenivasa Rao 1977). Aeration and agitation would certainly have promoted growth in the present study by precluding depleted zones around static algae (Conover 1968: Whitford and Schumacher 1961).

It is clear from Fig. 7.2 that under conditions where the P supply is plantiful, solution N:P ratios (by weight) of 10:1, 2:1 and 1:1 result in very similar yields. The response falls with higher ratios if N is limiting, as exemplified with yields obtained with either no added N (controls) or 0.1 mg ℓ^{-1}N.

If N is at high concentration the response is greatest with an N to P supply of 20:1 (by weight), this being the lowest tested in the P trials. Higher ratios produced successively poorer yields as P levels became lower (Fig. 7.1). At sites of growth within the algal bed, Cladophora is intimately associated with nutrient-rich water, resulting from mineralization of decomposing, underlying layers of the bed; evidence for release of nutrients from rotting Cladophora is provided in Appendix 1, and is pursued in greater detail by Gabrielson et al. 1980. Concentrations of ammonia, nitrate and phosphate in this "interalgal water" are in fact some 4, 2 and 15 times, respectively, those of the overlying water, the resulting inorganic N:P ratio being typically 2:1 (by weight) (Table 4.3). Comparing these concentrations with those producing the best yields in culture would suggest that N and P in the algal bed are unlikely to limit growth. In contrast, changes in the concentrations of N and P in the overlying water may be significant to growth of Cladophora at the bed surface and for balls not associated with an algal bed. During the few weeks in winter when river flow results in a considerable input of phosphorus and nitrogen, particularly nitrate; (McComb et al. 1981), water column nutrients are very high, inorganic nitrogen reaching nearly 2 mg ℓ^{-1} at the bottom, while PO_4-P may reach 0.15 mg ℓ^{-1}. In the non-flow period, however, PO_4-P remains low (< 0.01 mg ℓ^{-1}), and could limit the growth rate (Fig. 7.4), while nitrogen, predominantly as ammonia, is rarely above the concentration likely to

saturate growth rates (Fig. 7.5). In winter, therefore, nutrients are unlikely to limit growth, either in the water column or in the bed itself, controlling factors at this time being light and temperature, as discussed in the previous chapter.

7.4.2 Uptake rates

The algae in these cultures were continuously agitated, in contrast to those in the algal bed which remain relatively static. Maximum uptake rates of P observed in culture provide a basis for estimating the alga's best utilization of P under non-limiting conditions, and it is unlikely that rates in the field would exceed this, at least where nitrogen is not limiting.

Uptake rates of ammonia and nitrate, added together in nearly equal proportions, increased with all substrate concentrations except the highest (Table 7.5) where there was suppression of nitrate assimilation, presumably because of the inactivation of nitrate reductase in the presence of high levels of ammonia (Conway 1977; Eppley et. al. 1977). Ammonia was taken up more rapidly than nitrate in this study though both were removed from solution simultaneously (D'Elia and De Boer 1978; Haines and Wheeler 1978; Hanisak and Harlin 1978). The ability of Cladophora to assimilate both ammonia and nitrate is significant during the winter river flush when most allochthonous inorganic nitrogen is present as nitrate. However, recalling that inorganic nitrogen of "interalgal" water within the algal beds is present predominantly as ammonia throughout the year, and in view of the rapid uptake of ammonia-nitrogen in culture, this N species is the most likely major N source for Cladophora growth in the estuary.

Uptake rates in the dark, though lower than those in the light (Table 7.6), provide evidence that Cladophora in the underlying layers of the algal bed (where light is unavailable for growth) utilizes available nutrients when cellular energy reserves permit.

7.4.3 Tissue nutrients and growth rates

In this study increasing substrate concentrations resulted in successively higher final tissue nutrient concentrations (Fig. 7.9), to levels which are well above those recorded in the field. For example, for station 4, a growth site near the river mouth, internal N ranges typically between 16 and 40 mg g^{-1}N dry weight (mean 26 ± 5) and phosphorus between 0.8 and 3.0 mg g^{-1}P dry weight (mean 2.2 ± 0.6), which are similar to levels of 8.to 52 mg g^{-1}N and 0.73 to 5.42 mg g^{-1} reported by Wallentinus (1979) for Cladophora growing in the Baltic.

Tissue N and P levels range, therefore, from above "critical" down to nearly "minimum viable" concentrations, and the higher concentrations suggest luxury uptake. The high tissue concentration found experimentally here, to

levels well above those measured in the field, would suggest that the alga has the capacity under non-limiting conditions to take up nutrients at concentrations well above those considered "critical".

"Critical" concentrations in culture here are high compared with those reported for other aquatic plants. For example, concentrations above 13 mg g^{-1}N dry weight and 1.3 mg g^{-1}P dry weight have been considered indicative of luxury tissue concentrations for aquatic argrosperms (Gerloff and Krombholz 1966), while a value of 1.6 to 1.7 mg g^{-1} P dry weight was reported for a riverine Cladophora, as the tissue P concentration above which there was little increase in photosynthetic rates(Wong and Clark 1976). An even lower "critical" value of 0.6 mg g^{-1}P has been reported for Cladophora in culture (Gerloff and Fitzgerald cited in Lin 1977) which is close to the typical "starved" level found for algae in this study following their growth for a number of weeks without phosphorus (Table 7.7). Tissue N:P ratios during exponential growth in culture here ranged from 4:1 to 22:1 (by weight) (Tables 7.7 and 7.8). At the highest substrate concentrations, with the best growth rates, these ranged between 6:1 and 13:1 (by weight), rising to as much as 42:1 (by weight) following a return to depleted medium for 4 weeks. These high ratios, associated with low growth rates, particularly in those algae given no P for a number of weeks, clearly reflect P limitation and senescence of the algal. Ratios in those algae producing maximum growth rates in this study are similar to the range of 6:1 to 9:1 (by weight) reported for Cladophora glomerata producing best photosynthetic rates (Wallentinus 1979). A ratio of 7:1 (by weight) has been reported typical for growth of marine phytoplankton (Harris and Riley cited in Redfield et. al. 1963), and this is close to the ratio of "critical" tissue N and P for Cladophora (6.4:1) calculated here from two independent experiments.

It might be noted here that measurement of concentrations of N and P in algal tissue, and their relation to external supply of these nutrients, may well be useful for estimating the degree of nutrient deficiency of the alga (e.g. Healey 1978), particularly in view of the marked reduction in internal N and P which followed removal of either nutrient from solution. The data are consistent with the view that Cladophora has the ability to utilize its stored reserves of nitrogen and phosphorus when external supplies become depleted (Chapman and Craigie 1977; Hanisak 1979), and this matter is pursued in the next chapter.

7.4.4 Salinity and Growth

Results of growing Cladophora for 2 weeks at different salinities were similar to short-term responses to salinity differences discussed earlier (Chapter 6), with no significant difference in the dry weights obtained. This reinforces the suggestion that salinity changes observed in the estuary are unlikely to directly control Cladophora growth in Peel Inlet.

7.5 CONCLUSIONS

1. Treatment with phosphorus in the range 0 - 0.25 mg ℓ^{-1} (with non-limiting N) and nitrogen over the range 0-5 mg ℓ^{-1}P (with non-limiting P) produced yields proportional to increasing concentrations.

2. Growth rates were saturated above 0.4 mg ℓ^{-1}N and 0.2 mg ℓ^{-1}P, and the response was most dramatic in the range 0 - 0.1 mg ℓ^{-1}N and 0 - 0.03 mg ℓ^{-1}P.

3. Cladophora takes up nitrate and ammonia simultaneously with a preference for ammonia. Uptake rate of phosphate, nitrate and ammonia are all reduced in the dark.

4. "Critical" tissue concentrations, above which growth is relatively little affected by further change in concentration, are about 21 mg g^{-1}N and 3.3 mg g^{-1}P.

5. The "minimum viable" tissue content below which growth is essentially precluded, are 12.0 mg g^{-1}N and 0.5 mg g^{-1}P dry weight.

6. There was good correlation between tissue nitrogen levels and chlorophyll a levels.

7. A comparison with tissue levels measured on field grown material, and observations on growth rates after return to media lacking nutrients, provide evidence for "luxury uptake" of nutrients.

8. In the field, Cladophora growing in otherwise non-limiting conditions may be limited by P and N concentrations in the water column above the alga.

9. Between the algal balls, in the bed, N and P concentrations are relatively high and generally above levels which would be limiting if other factors (especially light) were adequate.

10. Yields obtained from growing Cladophora over a wide salinity range, 2 - 60°/oo for 2 weeks were not significantly different, suggesting that the salinity changes observed in the estuary are unlikely to control Cladophora growth.

CHAPTER 8

LEVELS OF NITROGEN AND PHOSPHORUS IN THE TISSUES OF CLADOPHORA FROM THE FIELD AND LABORATORY

8.1 INTRODUCTION

In the experiments described in the last chapter it was found that the levels of nitrogen and phosphorus in the tissues of Cladophora vary according to the nutrient status of the medium in which it was growing, and the suggestion was made that Cladophora may be able to store nitrogen and phosphorus in the tissue for later deployment in growth ("luxury uptake"). In the work described here this possibility was explored more fully in culture experiments, and then attention was directed to the levels of nitrogen and phosphorus in tissue of plants harvested from the field, to find out if these provided any evidence that the levels of one or other nutrient might be critical to growth in the Peel-Harvey system. This work with tissue levels from the field was compared with previously determined critical concentrations of these elements (Chapter 7).

Evidence was also sought for possible changes in tissue levels of nitrogen and phosphorus in the field at different stages of the growth life cycle. It is relevant to recall that in Chapter 5 it was found that Cladophora grows best in summer when light and temperature are highest, but that high nutrient concentrations are only found in the inter-algal water of the bed (which is in darkness below 1 cm due to self-shading) or transiently, in the overlying water in winter. One means of overcoming such temporal or spatial nutritional problems is that nitrogen and phosphorus might be taken up under low light and/or temperature, and utilised later when conditions are more favourable for growth.

8.2 MATERIALS AND METHODS

8.2.1 Tissue N and P contents of Cladophora from the estuary

Cladophora samples were collected at major growth sites (See Figure 1.1) at weekly intervals, along with light, temperature and water nutrients from September 1977 to September 1979. Five core samples of Cladophora, each 50 cm^2 in surface area, were collected from the algal bed at each site. In addition, 20 samples were also collected monthly at site 4 by diving and carefully scooping algae from the surface layer of the bed. Samples were washed in tap water, dried at 70°C, milled and 120 mg subsamples assayed for total tissue P (TP) (Strickland and Parsons 1972) following digestion in $HClO_4$ and HNO_3 (Jackson 1958). Total tissue N (TN) was measured using the autoanalyzer (Technicon Corp, Tarrytown, N.Y., method 334-74 W/B) after digestion in Na_2SO_4 with an Hg catylist. Duplicates did not vary by more than 10%. Where sample size of the 5 combined

replicates was less than 800 mg dry weight, samples from 2 or more weeks were composited.

8.2.2 N and P Uptake

Rates of TN and TP accumulation by Cladophora under different levels of light and temperatures were determined in the laboratory. The algal material was first incubated for 12 days in modified ASP_{12} artificial seawater (see Chapters 6 and 7), with no added N or P so as to reduce tissue levels to a little below those typically found in the field. About 15 mg equivalent dry weight of fresh algae were weighed into each of 72, one litre Erlenmeyer flasks containing 1 litre of medium and incubated at 25 ± 1°C or 16 ± 1°C, approximating summer and winter water temperatures. At each temperature, one third of the flasks was placed in darkness, one third at 50 ± 10 $\mu E\ m^{-2}\ sec^{-1}$ (low light) and the remainder at 400 ± 70 $\mu E\ m^{-2}\ sec^{-1}$ (saturating light) (Chapter 5). Each flask was bubbled with air which had been scrubbed through 25% H_2SO_4, then distilled water, and finally filtered through tintered glass. N and P were added to the medium at 2 mg ℓ^{-1} (NH_4 + NO_3) - N, and 0.2 mg ℓ^{-1} PO_4 - P. These levels approximated the highest observed during the winter of 1978.

Solutions were replaced every 1-4 days to maintain the desired concentrations, more frequent changes being necessary for 25° in high light. Three replicates of each treatment were harvested on days 2, 4, 7 and 12, after which samples were dried and analysed for TN and TP. Due to small sample size, samples of about 2 mg were weighed to ± 0.02 mg, placed in 20 ml de-ionised water and digested and analyzed using standard methods developed for water samples (Strickland and Parsons 1972; Technicon Autoanalyzer methods 376-74 W/B). Variability between replicates was usually less than 5%. A comparative test between this method and that used for the field samples showed good agreement.

8.3 RESULTS

8.3.1 N and P contents of Cladophora from the estuary

The levels of TN and TP in Cladophora at sites 4, 5 and 8 are summarized in Figure 8.1. Sites 4 and 5 show seasonal changes in tissue concentrations, with TN and TP lowest in summer and autumn and highest in winter and spring. Data from site 8, which was collected for only one year, did not exhibit these seasonal trends, although values were generally highest in winter. While the seasonal trends were similar at sites 4 and 5, the weekly collected data (e.g. site 5) displayed marked week to week variations, sometimes as great as the seasonal range.

Figure 8.1 also illustrates the relationship between tissue concentrations and critical concentrations of N and P. (The critical concentration of a nutrient element is the minimum tissue content in a particular species that is necessary for maximum growth; Gerloff and Krombholz 1966).

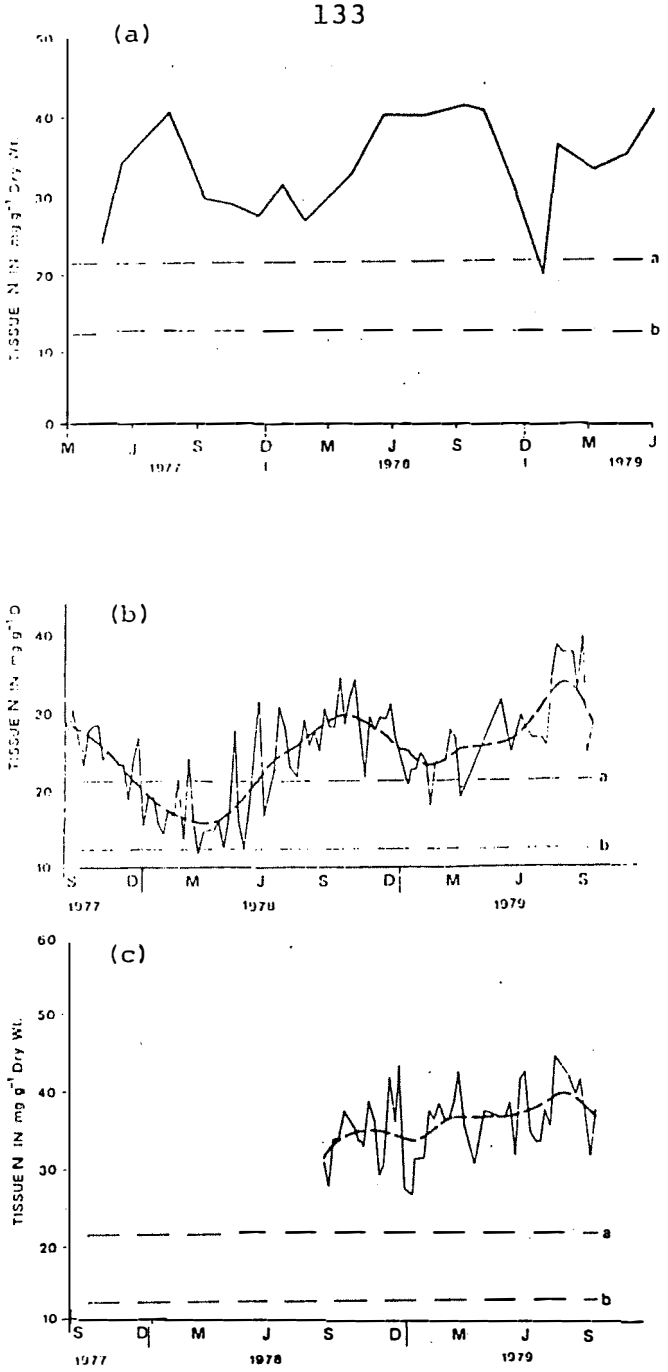


Figure 8.1a Concentrations of tissue N (mg g^{-1} dry wt) in *Cladophora* at (a) site 4, (b) site 5 and (c) site 8 over the period 1977-79. The lines through the weekly collected data (sites 5 and 8) represent the local mean at any point calculated by computer using a recursive least squares algorithm (Young 1980). Line (a) represents the 'critical' concentration of N (21 mg g^{-1} dry wt). Line (b) represents the 'minimum viable' concentration of N (12 mg g^{-1} dry wt, Gordon *et al.* 1981).

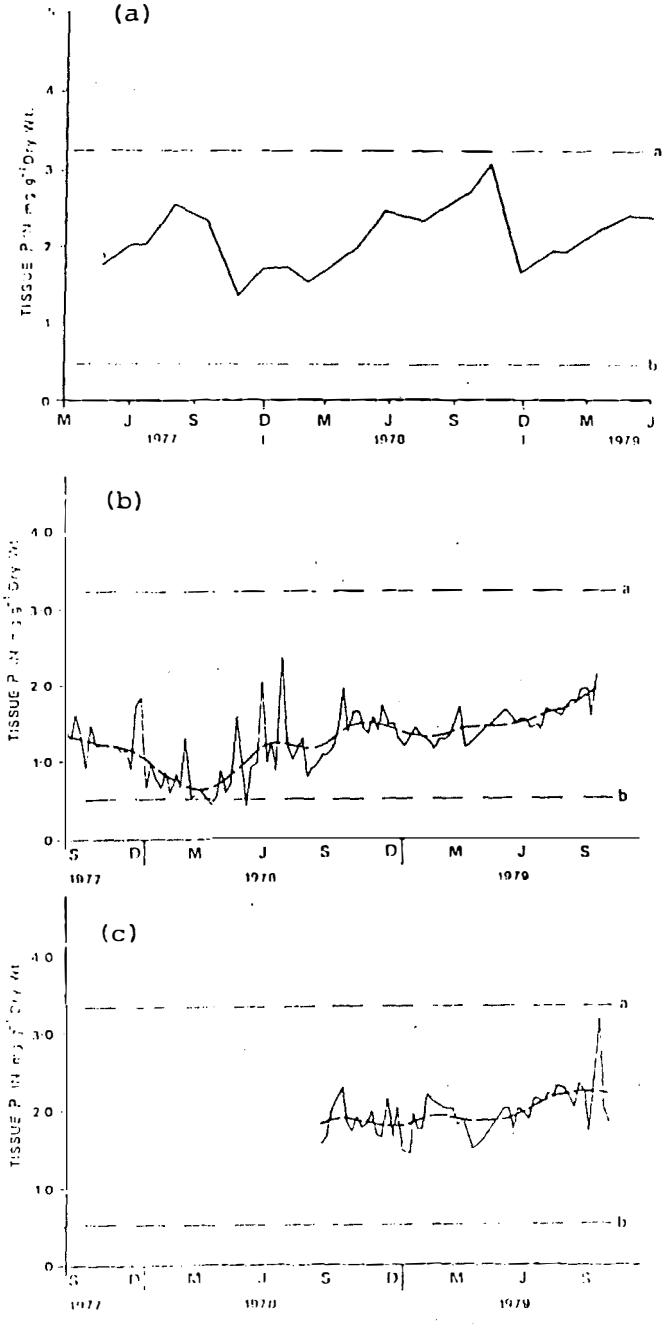


Figure 8.1b Concentrations of tissue P (mg g^{-1} dry wt) in *Cladophora* at (a) site 4, (b) site 5 and (c) site 8 over the period 1977-79. The lines through the weekly collected data (sites 5 and 8) represent the local mean at any point calculated by computer using a recursive least squares algorithm (Young 1980). Line (a) represents the 'critical' concentration of P (3.3 mg g^{-1} dry wt). Line (b) represents the 'minimum viable' concentration of P (0.5 mg g^{-1} dry wt, Gordon *et al.* 1981).

At most times, TN values are above the critical concentration except for early 1978 at site 5, in contrast to TP which was always below the critical concentration. During early 1978 values at site 5 approached levels similar to those considered minimum for growth (i.e. the "minimum viable" concentration, Chapter 7).

Annual means of tissue concentrations for all sites are given in Table 8.1. These were quite similar at sites 4 and 8, both being higher than those recorded for site 5, especially for TP. At all sites, average TP values were below the critical concentration (3.3 mg P g^{-1} dry weight), though this was not the case with TN. This is reflected in the high N : P ratios at the sites (13 - 19 : 1, by weight).

8.3.2 N and P Uptake

Uptake of N and P under different light and temperature conditions are shown in Figures 8.2 and 8.3. In the light, tissue concentrations continued to increase during the 12 days of incubation, but in the dark there were increases of 40-60% in the first week after which these treatments levelled off.

Uptake rates of N and P into the tissue are compared with their corresponding growth rates in Table 8.2. Rates of uptake of N and P for each treatment were similar, except at 16°C low light where N uptake was 1.6 times that for P. At high light, the Q_{10} between 15° and 25° for uptake was about 2. For growth, the Q_{10} was 3.7 - in good agreement with the photosynthetic rate Q_{10} of 3.6 determined over a similar temperature range (see Chapter 6). At each temperature, reduction of light to a low level decreased growth and uptake by approximately 60% except at 16° where N uptake was reduced by only 20% by lowering the light (Table 8.2).

Maximum TP concentrations attained here (4.2 mg P g^{-1} dry weight) exceeded the highest recorded from the field (3.3 mg P g^{-1} dry weight) while the maximum experimental N concentration (38 mg N g^{-1} dry weight) was slightly less than the maximum observed in the field (43 mg N g^{-1} dry weight).

8.4 DISCUSSION

8.4.1 N and P limitation in the estuary

An important finding of this study is that TP concentrations were always lower than the "critical" concentration, in contrast to TN levels which were typically higher than the "critical" concentration. This indicates that Cladophora can generally acquire sufficient N but insufficient P for maximum growth rates and suggests that P could limit growth, depending on other factors such as light and temperature. Earlier studies (Chapter 5) showed that light and temperature are generally sufficient for maximal growth in the photosynthesizing surface (∿ 0.5 cm) of Cladophora beds during summer. Thus summer is the time when P could most likely limit growth. At other times of the year, and at depth in the bed, light and temperature are the most important factors controlling growth.

TABLE 8.1

Concentrations of nitrogen and phosphorus in water, sediment and Cladophora at sites 4, 5 and 8.

	Site 4	Site 5	Site 8
Cladophora			
Tissue N (mg g^{-1} dry weight)	32 ± 1.0	28 ± 0.8	36 ± 0.6
Tissue P (mg g^{-1} dry weight)	2.3 ± 0.08	1.5 ± 0.04	2.0 ± 0.03
TN/TP (by weight)	13:1	19:1	18:1
Water			
$NH_4 + NO_3$ - N (µg l^{-1})	67 ± 15	84 ± 16	109 ± 28
PO_4 - P (µg l^{-1})	7.3 ± 1.0	5.3 ± 0.6	8.0 ± 1.4
Surface Sediments[2]			
Total N (mg g^{-1} dry weight)	7.1	1.3	6.3
Total P (mg g^{-1} dry weight)	0.73	0.09	0.72

[1] Means ± standard errors. Tissue and water nutrient data collected weekly from August 22 1978 - September 4, 1979.

[2] Includes decaying plant material ("black ooze")

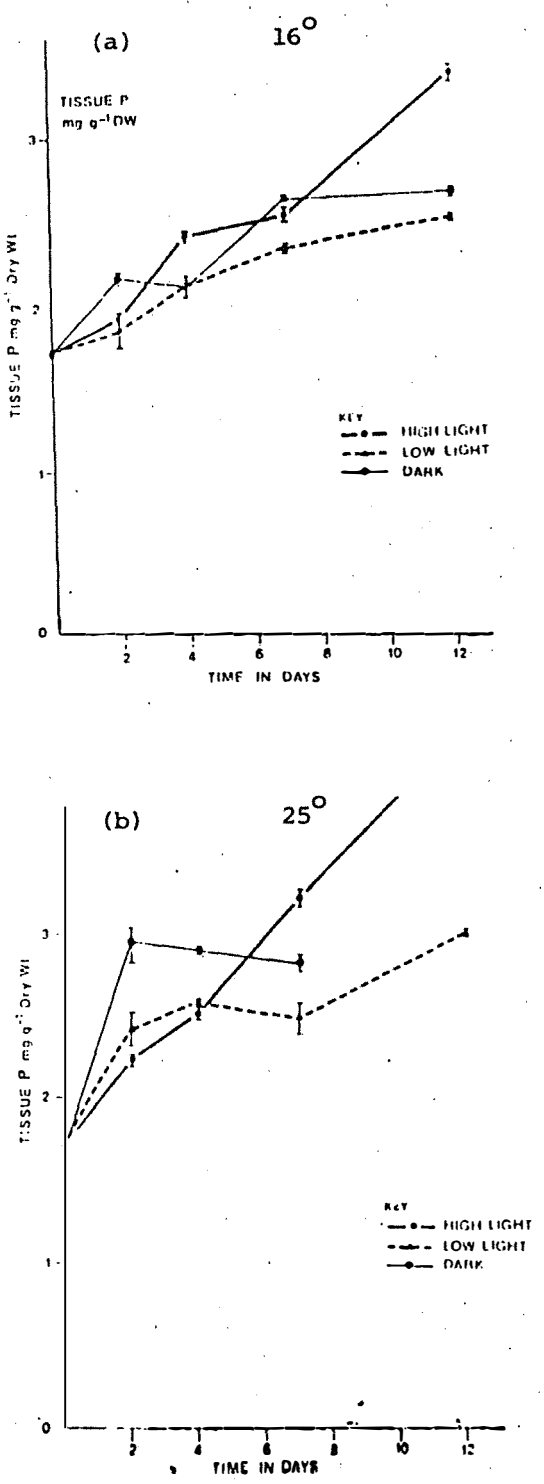


Figure 8.2 Concentration of tissue P (mg g^{-1} dry wt) for *Cladophora* growing in artificial seawater containing 0.2 mg ℓ^{-1} PO_4-P and 2 mg ℓ^{-1} NH_4+NO_3-N at (a) 16 ± 1°C and (b) 25 ± 1°C at light levels of 0 (dark), 50 ± 10 (low light) and 400 ± 70 µE m^{-2} sec^{-1} (high light), for 12 days.

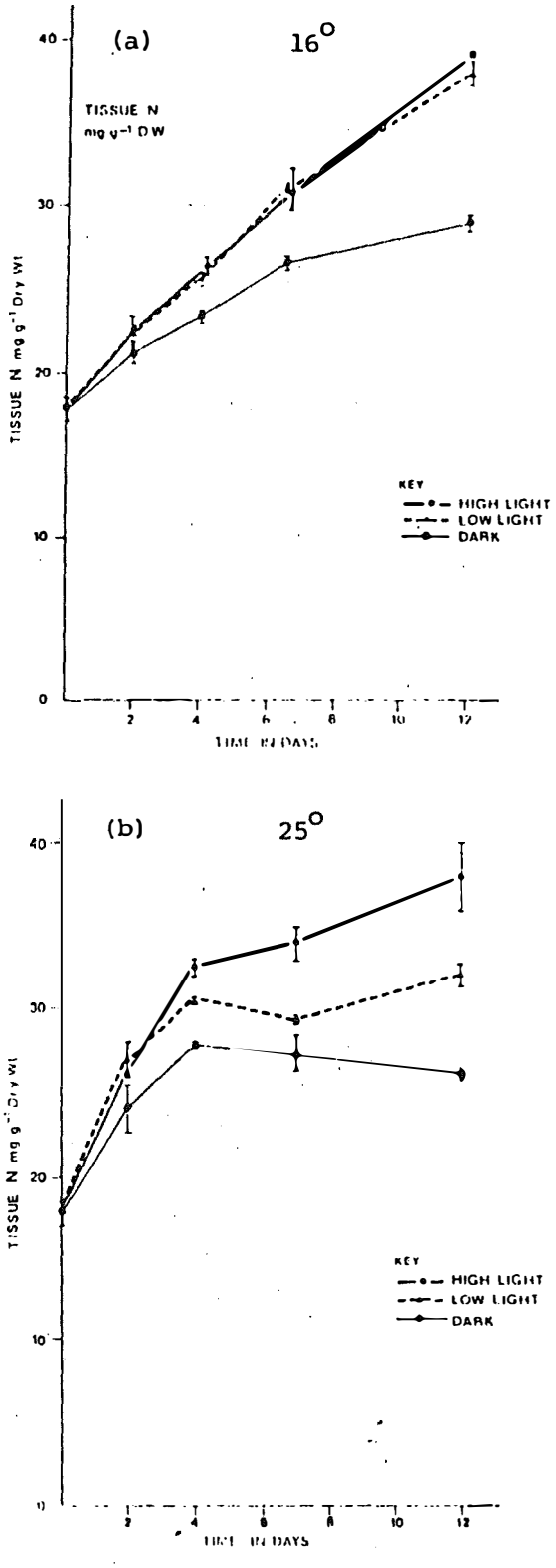


Figure 8.3 Concentration of tissue N (mg g^{-1} dry wt) for *Cladophora* growing in artificial seawater containing 0.2 mg ℓ^{-1} PO_4-P and 2 mg ℓ^{-1} NH_4+NO_3-N at (a) 16 ± 1°C and (b) 25 ± 1°C at light levels of 0 (dark), 50 ± 10 (low light) and 400 ± 70 µE m^{-2} sec^{-1} (high light) for 12 days.

TABLE 8.2

Effects of light and temperature on rate of increase of Cladophora tissue nitrogen, phosphorus and dry weight.[1,2]

Light and Temperature regime [2]	Dry Weight day^{-1}	Tissue N day^{-1}	Tissue P day^{-1}
25 ± 1°C			
high light	0.130 ± 0.015	0.20 ± 0.008	0.210 ± 0.006
low light	0.037 ± 0.008	0.087 ± 0.004	0.085 ± 0.004
dark	0.001 ± 0.012	0.033 ± 0.006	0.032 ± 0.010
16 ± 1°C			
high light	0.035 ± 0.001	0.100 ± 0.002	0.090 ± 0.001
low light	0.015 ± 0.002	0.078 ± 0.001	0.045 ± 0.001
dark	-0.008 ± 0.005	0.032 ± 0.003	0.026 ± 0.002

1 Light levels were 400 ± 70, 50 ± 10 and 0 µE m^{-2} sec^{-1} (high light, low light and dark, respectively).

2 Data are means ± standard errors. The experiment was for 12 days.

8.4.2 N and P uptake and storage

Tissue concentrations of N and P in the field are typically lowest in summer and autumn and highest in winter and spring, though there are some differences between the years and the sites. (See Fig. 8.1). There are also marked weekly variations, which are in part attributed to the small sample size of the weekly data compared with those taken monthly. Small core samples would be affected by including different proportions of senescing tissue with consequently different nutrient levels. Shifts in the surface of the Cladophora bed which continually expose or cover underlying algal layers would have also contributed to this variation. Nevertheless, we can deduce that rates of N and P uptake and accumulation tend to be greater than growth rates during winter/spring, and less during summer/autumn. Presumably some of the summer growth takes place at the expense of stored reserves, suggesting that the various nutrient sources (most importantly, the decaying algal material and sediments) are unable to keep up with the relatively high growth rates of Cladophora at this time, at least at sites 4 and 5.

Tissue N and P values tend to increase from about mid autumn, and this is apparently because growth rates fall more quickly than uptake rates, growth rates being more sensitive to temperature (Table 8.2). Since significant river inputs do not begin until winter, the most obvious major source for the autumn increases is release from the sediment and decaying algae.

In winter, although the rivers contribute large amounts of N and P to the system, the full storage potential of Cladophora is not reached, due to the few weeks in which there is river flow. Maximum tissue concentrations, which are generally recorded in July-September, are well below those achieved in culture (Chapter 7). In 1978, concentrations of N and P in the water rose to levels similar to those used in the present laboratory experiments (2 mg ℓ^{-1} $NH_4 + NO_3$ - N and 0.2 mg ℓ^{-1} PO_4 - P), but only for about one week. For most of the remaining winter period PO_4 - P was typically less than 0.02 mg ℓ^{-1}, although inorganic N remained above 0.50 mg $^{-1}$ for 6 weeks. In the laboratory,tissue increases of 0.8 - 1.6 mg g^{-1} dry weight (depending on light conditions) are possible after 12 days incubation at 16^{o} in 0.20 mg $^{-1}$ PO_4 - P and this is similar to increases observed in the field populations in winter. By extrapolating the data in Figure 8.2, incubation periods of about 7-14 weeks under winter light and temperature would be required to reach the maximum levels achieved in earlier experiments ($\sim$ 9 mg P g^{-1} dry weight; Chapter 7). This is much longer than the observed period of high P concentrations in the overlying water. In the "interalgal" water within the bed, however, high concentrations are maintained throughout the year, but in this case uptake by these shaded algae is probably limited by cellular energy reserves, as is indicated by lower uptake rates in the dark (Figs. 8.2 and 8.3).

Nitrogen uptake into the tissue in the algal beds during winter may also be limited by the few weeks of river flow, and, in addition, the low inorganic P concentrations in the water. In the laboratory,increases from about 20 mg

N g^{-1} dry weight to 40 mg N g^{-1} dry weight (the maximum field concentration) were achieved after 12 days incubation at 2.0 mg NO_3 + NH_3 - N ℓ^{-1} (Fig. 8.3). Since winter inorganic N concentrations were between 1.0 and 2.0 mg ℓ^{-1} for up to 4 weeks in the estuary, higher tissue concentrations may well have been expected in the estuary. However, PO_4 - P concentrations at this time were typically 0.02 mg ℓ^{-1}. Work described in Chapter 7 suggests that the rate of N uptake may be greatly reduced below a concentration of about 0.05 mg $^{-1}$P.

During September and October, following river flow, continued increases in tissue concentrations are presumably due, in part, to recycling of nutrients from decomposing phytoplankton. In winter, a phytoplankton bloom immediately follows the increase in nutrient concentrations but rapidly declines after a few weeks and sediments out onto the algal beds, where decomposition takes place. However, the amount of nutrients actually made available to Cladophora by this mechanism has yet to be quantified. The importance of a particulate source of nutrients, especially P, has also been emphasized in the modelling studies of this system (e.g. Hornberger and Spear 1980, Spear and Hornberger 1980).

8.4.3 Differences between sites

Variations in mean TN and TP, particularly the latter, at site 5 compared to sites 4 and 8 appear related to the mean concentrations of inorganic N and P in the water and total N and P in the surface sediments (Table 8.1). Higher concentrations of P in the water and sediments at sites 4 and 8 are probably due to their proximity to sources of P; site 4 is close to the mouth of the Murray and Serpentine Rivers while site 8 is situated close to outflow from the Harvey Estuary, whose mean total P concentration is about double that of Peel Inlet.

Lack of seasonality noted earlier in the data for site 8 relative to the other two sites may be due, in part, to sedimented phytoplankton from the massive bloom of Nodularia which occured in the Harvey Estuary in November 1978 (Huber 1980; Lukatelich and McComb 1981). Part of this bloom was carried by tidal exchange into the Peel Inlet, and high levels of chlorophyll a were recorded at site 8, due to its proximity to the Harvey Estuary. As this phytoplankton bloom decomposed during December 1978 and January 1979 it may have released significant quantities of N and P for uptake by Cladophora at site 8 and possibly offset the seasonal decline in TN and TP which appeared to be occurring in early summer.

8.5 CONCLUSIONS

1. Tissue analyses show that although nitrogen and phosphorus contents vary seasonally their relation to previously determined "critical" concentrations show that the alga is rarely deficient in nitrogen but is relatively deficient in phosphorus.

2. Tissue concentrations are highest in winter and spring, lowest in summer to autumn.

3. In summer/autumn the low concentrations show that the alga relies to some extent on stored N and P presumably because uptake rates from low nutrient concentration estuary water and sediment and black-ooze release are insufficient to keep pace with high growth rates favoured by summer light and temperature.

4. In winter and spring there is accumulation of nitrogen and phosphorus in the tissue because of high nutrient input from the rivers, in addition to release from surface sediments, black ooze and sedimented phytoplankton. Even so, the full storage potential of Cladophora is not reached, presumably at least in part, because high nutrient concentrations in the estuary, particularly inorganic phosphorus, only last for a few weeks.

5. The data suggest that phosphorus may be an important factor in controlling Cladophora biomass in the estuary.

CHAPTER 9

GENERAL DISCUSSION

9.1 INTRODUCTION

The previous chapters have provided evidence that where Cladophora occurs in beds, which account for most of the biomass, the growth of those beds must be light-limited, partly because of water turbidity (especially in winter), but especially because of self-shading. In winter, temperatures may also drop to limiting levels. Nevertheless, the problem in a more general sense is an increase in biomass due to nutrient enrichment, and so attention must also be directed towards the nutrient requirements of algae at the surface of the beds, and of individual pieces of algae, such as those present before the beds were established. Laboratory experiments served to define the responses of Cladophora under controlled conditions to nutrient, light and temperature conditions and provided evidence that the levels of nutrients in tissues of algae collected from the field are consistent with nutrient (especially phosphorus) limitation at the bed surface.

The first part of the following discussion is particularly concerned with aspects of growth of Cladophora within beds on the estuary floor and develops the concept of a photosynthetically 'producing' biomass at the surface of the bed, as suggested from the observed reduction in photosynthetic rates within a few millimetres of the bed surface (Chapter 6). Field growth rates and biomass changes described earlier (see Chapter 5) have been integrated here to provide some estimate of the contribution to the observed changes from physical import and export, this being potentially significant to biomass measurements of a free-living alga such as this species. The field growth rates in combination with the 'producing' biomass are used here to provide an estimate of net production per unit area within the bed and results are compared with similar data from other ecosystems.

In an attempt to relate the laboratory-derived data with field observations, two simple Fortran programmes have been developed (Appendix 2). The first of these (Programme A) takes specified environmental parameters for a given time of year and a particular depth in the estuary, and suggests which environmental parameter, of those nominated, might be limiting, and, for a fragment of Cladophora growing at the point nominated, expresses growth rate as a percentage of the maximum growth rate observed in culture. Thus the programme can be used to explore the interactions of environmental variables. The second programme (Programme B) also accepts nominated environmental variables and estimates a 'per day' growth rate, taking into account changes in light

intensity which occur during the day, and respiratory loss which takes place during the night. This programme has been used in a general way to attempt to simulate the observed changes in biomass at the site studied and described in Chapter 5.

9.1.1 EFFECT OF IMPORT AND EXPORT ON BIOMASS CHANGES

Monthly changes in biomass of the Cladophora bed at Post 46, (Fig. 5.2; Chapter 5) include gains and losses due to transport of alga through wind induced water movement, and increased buoyancy as a result of photosynthetic oxygen production. Losses also occur from decomposition of the non-photosynthesising deeper layers within the bed.

That proportion of the change in biomass which is due to factors other than growth can be estimated by computing an 'expected' increase in biomass due to growth and comparing this with the change actually observed. It is necessary to envisage the algal bed as two components, an actively-photosynthesising surface layer, and a respiring and decomposing lower layer. The compensation depth lies within 1 cm of the bed surface (Chapter 6), and for the purpose of calculation the surface layer is taken as 1 cm deep (Chapter 6).

From the relationship between biomass and bed depth measured at the growth site, a 'photosynthetic biomass' was calcualted for a square metre, and used as the biomass initially available for growth each month. The rate of change in this biomass was estimated using data of monthly growth rates of Cladophora imprisoned at the surface of the bed (Chapter 5). These data, collected over two years, were superimposed to provide growth rates for each month of a complete year (Fig. 9.1).

The effect of superimposing results of growth rates from 2 years is shown in Figure 9.1 with a curve tracing a running mean of 3 successive points. This gives a yearly range of -0.001 to 0.013 day^{-1} for the surface photosynthetic layer of the bed, which includes respiratory weight losses. These points lying in each discrete monthly interval were averaged to produce a 'typical' growth rate for each month of the year.

'New' growth by photosynthesis during each month was then calculated from the expression

$$\overline{RGR} = \ell n\ (B_1/B_0) / (t_1 - t_0)$$

where $\overline{RGR}$ = the mean relative growth rate (day^{-1}) for the specified month, B_1 is the expected biomass of the surface layer from the initial photosynthetic biomass, B_0, and $t_1 - t_0$ the time over which the biomass change was measured (days).

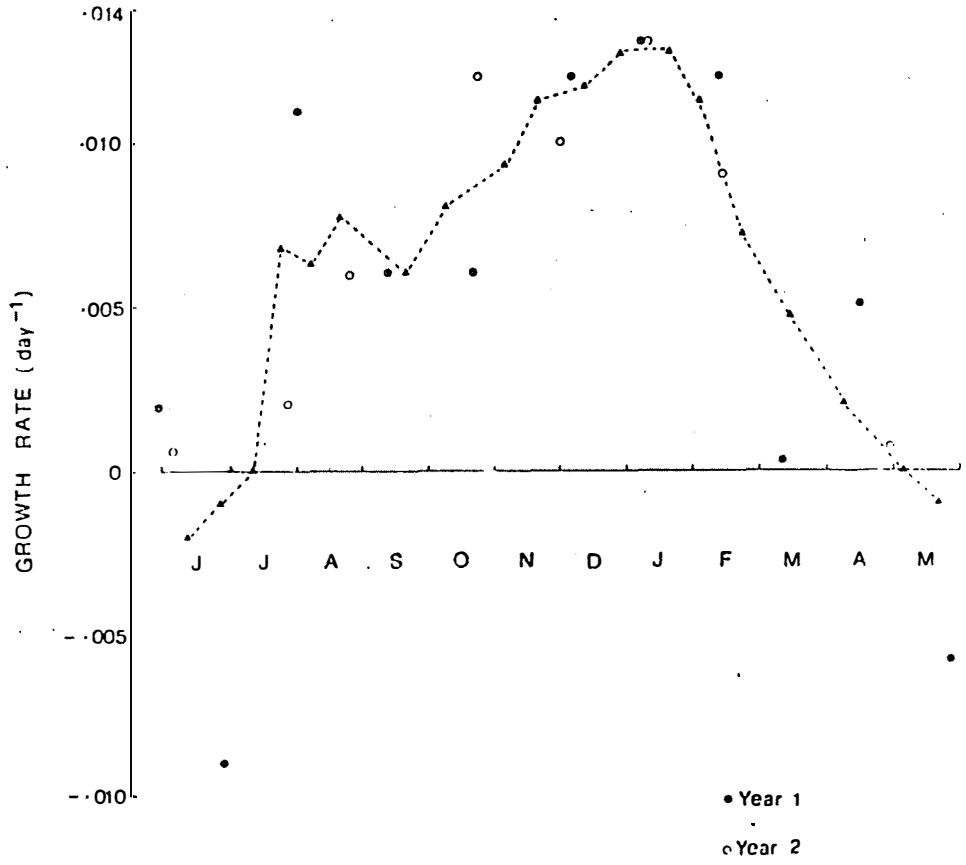


Figure 9.1 Growth rate of *Cladophora* (day^{-1}) imprisoned in flasks in the algal bed at station 4, Peel Inlet. Data were collected at approximately monthly intervals for more than 2 years from May 1977, but are here superimposed over a single year. The dashed line follows a running mean of 3 successive points.

Assuming no losses, the expected total biomass at the end of each month is the sum of the non-photosynthetic biomass of the preceding month and the biomass generated from the initial photosynthetic biomass, B_0. Subsequent monthly increments are calculated in the same way using the growth rate for the month corresponding to the initial observed biomass on each occasion.

That portion of the biomass change attributable to import or loss (including decomposition) is then estimated from the difference between expected biomass and that actually observed.

9.2 RESULTS AND DISCUSSION

Figure 9.1 shows that growth rates throughout the year are not symmetrical, with lowest rates occurring at the end of autumn and in early winter followed by a rise throughout the spring, and a rapid decline after the summer maximum. Over most of the year the surface layer is actively photosynthesising, with only 2 months where there is no growth Thus, if we neglect decomposition and export there will be an increase in biomass. The relationship between biomass and bed depth is shown in Fig. 9.2 The correlation coefficient for these points is $r = 0.93$, suggesting that the relationship is linear. A surface layer of 1 cm corresponds to a biomass of $\sim$ 260g dry wt m^{-2}, and this figure was used to represent the available photosynthetic biomass. Since individual Cladophora balls are often about 1 cm in diameter, any bed thickness shown as less than 1 cm represents incomplete cover, so that the corresponding biomass estimates are subject to large variability.

The contribution to biomass changes in the bed from import and export is shown in Fig. 9.3 along with the observed monthly changes measured at the site, as given previously (Chapter 5). Greatest disturbance of the bed was obvious in summer 1977 and throughout 1978, as suggested by the consistent large deviations from the zero axis.

Differences between observed and expected biomass were as much as $500gm^{-2}$ in a month (February 1977). Though the dynamics of such losses are not known - for example, this loss may have occurred all on one occassion during the month - they may be expressed, on average, on a per day basis. The above difference would then correspond to a rate of loss of 0.018 day^{-1}. Losses and gains for the whole data set were between 0 and 0.021 day^{-1} with a mean of 0.008 day^{-1}.

The contribution of decomposition in the non-photosynthetic layer, to these figures is difficult to assess. Laboratory and field experiments suggest that decomposition may make a very significant contribution to losses (-0.008 ± 0.004 day^{-1}; Gabrielson et al. 1980); this rate is comparable in magnitude to field growth rates.

The large increase in biomass between July and September 1976 (Fig. 9.3) is apparently largely attributable to physical movement rather than growth. Field records for this period show Cladophora floating on the water surface at the time. The relatively static biomass in October and November 1976 occurs when growth rates should be high, suggesting that there

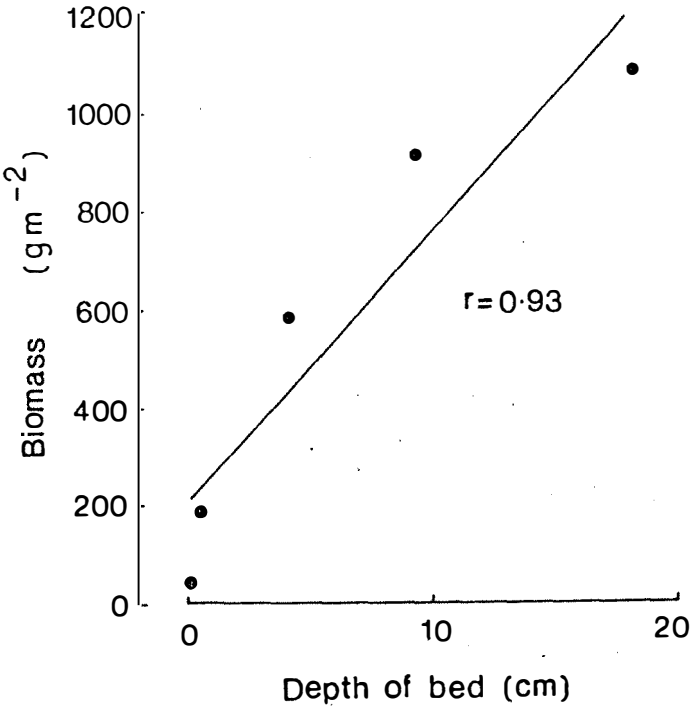


Figure 9.2 Biomass of *Cladophora* (g dry wt m^{-2}) at Post 46, Peel Inlet as a function of the corresponding depth of the algal bed (cm). Each point is the mean of 20 replicate measurements collected between October 1977 and October 1978.

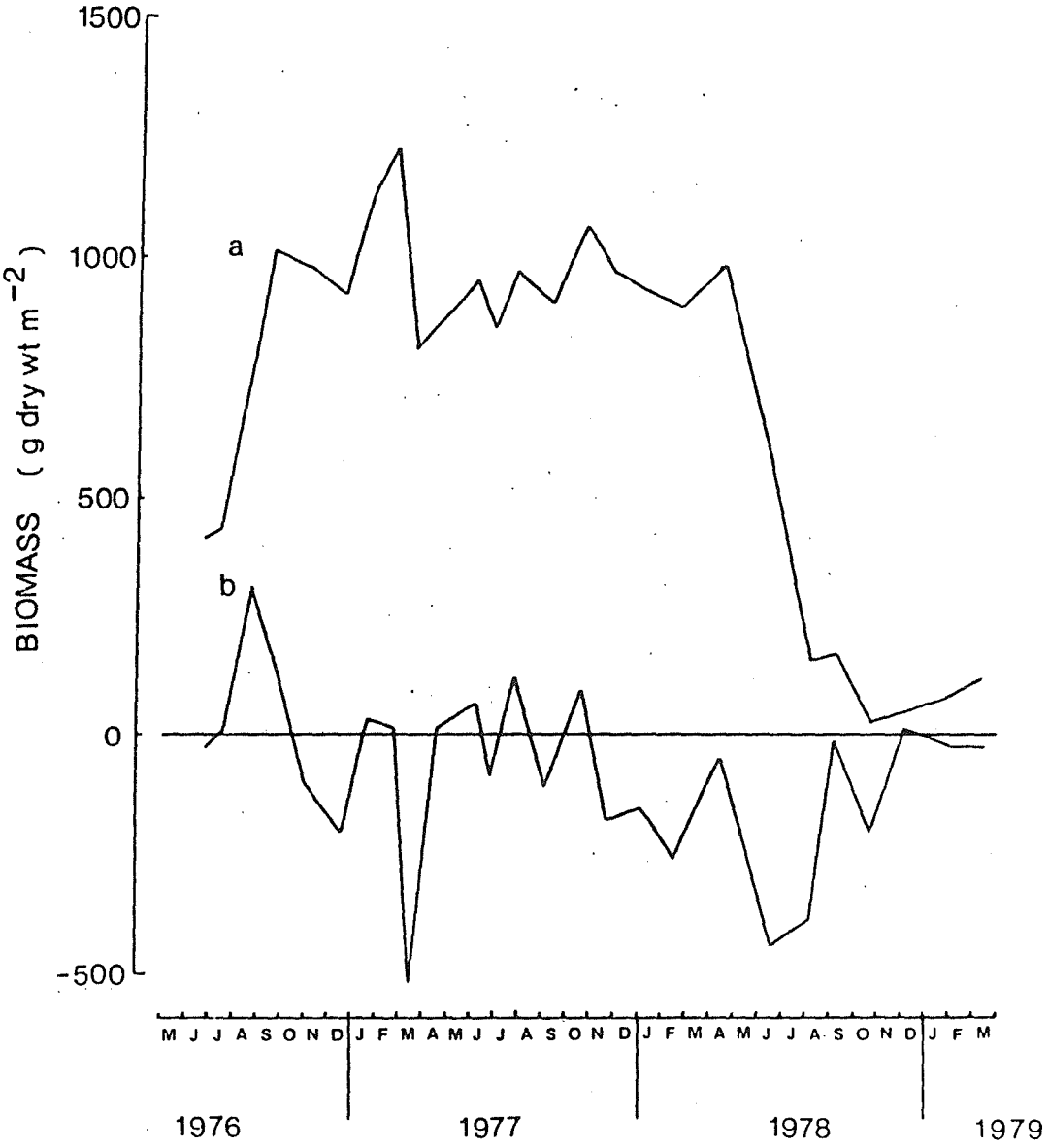


Figure 9.3 Observed biomass changes in the algal bed at Post 46, April 1976 to March 1979 (a) shown with estimated contribution from import and export at the site (b).

is export from the site. Export is more significant during the marked decline in biomass between February and March 1977, when growth rates could still be expected to be high. Field records show water clarity was good at this time, water temperature high (25^0C) and the algal bed very uneven, with Cladophora breaking away from the bed and floating to the surface. These observations are consistent with losses as a result of buoyancy during photosynthesis.

During the following winter observed changes were close to those expected from growth. There was relatively little loss or gain from the site, and biomass did not return to the levels of the previous winter. This can be partially explained by the unsymmetrical growth curve for the year, where growth does not occur in only 2 months of the year (Fig. 9.1).

The decrease in biomass in October 1977 again corresponds to a period when the alga was breaking away from the bed and floating to the surface. Such losses of surface material, however, expose layers of the bed which themselves might be more readily shifted by water surface movement. In fact, at this time a massive accumulation of the alga, some 2 m deep and over 200 m long, was observed near Post 46, consisting largely of decomposed material.

The lack of increase in biomass during summer 1977-1978 when growth rates would be highest again suggests export. Field records are consistent with this interpretation where, particularly in November 1977, the algal bed was extremely variable (from 1 to 20 cm deep) and there were many individual balls floating to the water surface.

During autumn, particularly March 1978, conditions for growth were good, notably light and temperature, and the alga was observed floating in large masses on the water surface. The dramatic decline in biomass before June 1978 occurs before river flow. Interestingly, the high biomass in April was measured some 8 days after a cyclone (Cyclone Alby) passed through the area.

Little loss was observed from the site as a result of such a short but strong wind, but water clarity was markedly reduced.

The change between June and August 1978 includes the onset of river flow when water clarity was reduced drastically, and must be accounted for by export. Export under low light in winter may result from respiratory starch loss reducing cell density (see chapter 2). A three-fold difference in density was found, using specific gravity bottles, between Cladophora maintained in nutrient-enriched media for 3 weeks in either saturating light or darkness. Iodine-staining showed much lower starch levels in the darkened plants.

At this time there were masses of Cladophora accumulated inshore around the beaches (see Chapter 5, table derived from beds in the deeper water. During the following spring and summer the biomass at the growth site did not recover to that of previous years, though the low but increasing biomass changes

probably reflect growth, since there was little evidence of import and export at this time. In contrast to 1976, by the summer of 1979, following the decline of biomass in winter 1978, the algal bed had altered considerably, being dominated by red algae (mostly Chondria sp.) and the green alga Chaetomorpha linum, both of which were prominent throughout 1979. In contrast to previous years, where the Cladophora bed overlay a very reduced fine mud, sediments had become oxidised to a considerable depth ($\sim$ 5 cm), suggesting that the reduced black mud or 'ooze' is susceptible to removal when the alga is lost from the site. This observation reinforces the view that 'ooze' is a product of Cladophora decomposition. The binding effect of algae on such estuarine muds has been reported previously (Frostick and McCave 1979).

9.2.1 NET PRODUCTION

Provided there are no losses of plant material other than through respiration, net production can be calculated most simply from change in biomass (Westlake 1965). In the Cladophora beds in this study, net production, as a measure of photosynthetically-fixed energy, is estimated from the product of the photosynthetically active biomass of the surface of the bed and the growth rate of this layer, over a specified time interval, expressed on a per unit area basis.

Growth rates for each month of the year are obtained from the curve of superimposed rates measured in the field (Fig. 9.1). Because of the nature of the flasks imprisoning these field populations, the amount of alga in each flask (see Chapter 5), and the effects of physical movement of the bed on the estuary floor, it is unlikely that the biomass changes occurring in the flasks would reflect corresponding changes in the surface of the surrounding bed any shallower than 1 cm. This figure is therefore chosen as the depth constituting the active biomass in subsequent calculations of production, though, as suggested later in this chapter, this depth may be considerably less because of self-shading. Growth rates in the flasks are therefore an average of those from the surface in each flask, where high rates might be expected, to the bottom of the flask where the alga is most likely respiring.

The expected daily net production (g dry wt m^{-2} day^{-1}) for each month of a complete year (Table 9.1) was converted to monthly net production by assuming a 30 day month. These monthly figures were then summed to produce the yearly net production and an average for each season of the year in Table 9.1.

Production rates were lowest for the autumn months, falling to some 18% of the summer average. Summing the monthly rates gives a net yearly estimate of $\sim$ 570 g m^{-2} $year^{-1}$. This approach can be used to compare yearly net production rates at Post 46 before and after the decline of the beds in mid-1978. Using the time interval between individual biomass measurements and field growth rates as

Table 9.1 Net production rates of Cladophora for 1 year estimated for a 'photosynthetic' biomass of 1 cm depth and field growth rates.

Month	Net Production		Season mean
	$g\ m^{-2}\ day^{-1}$	$g\ m^{-2}$ month	$\bar{x} \pm$ S.E. $g\ m^{-2}$ month
December	3.15	94.5	
January	3.28	98.4	87.9 ± 8.5
February	2.37	71.1	
March	1.21	36.3	
April	0.52	15.6	16.5 ± 11.2
May	-0.08	-2.31	
June	-0.33	-9.9	
July	0.88	26.4	23.6 ± 18.6
August	1.81	54.3	
September	1.55	46.5	
October	2.06	61.8	62.7 ± 9.6
November	2.66	79.8	

$\Sigma = 572\ g\ m^{-2}\ year^{-1}$

described before, net production rates were calculated for each interval and summed from June to June for the first 2 years, and from June to May of the last year (1978-1979). This gives a yearly net production of 582 and 583 for the first 2 years respectively, which, as expected, is almost identical with estimated yearly net production in Table 9.1. Since the last year included the onset of a marked decline in the algal bed and subsequent reduction in the photosynthetic biomass, estimated net production fell to 119 g m^{-2} $year^{-1}$. This represents a 65% reduction from the previous 2 years.

9.2.2 COMPARISON WITH OTHER SYSTEMS

These productivities compare well with rates calculated elsewhere during summer growth. For example an average net production of 70 g m^{-2} $month^{-1}$ was reported for growth of Cladophora glomerata in the Baltic Sea (Jansson 1974). This figure is higher than the average of 47 g m^{-2} $month^{-1}$ found here (Table 9.1). However, the former is a mean of measurements taken in July and August (summer) only and would be better compared with the summer mean value in Peel Inlet, viz. 88 g m^{-2} $month^{-1}$. Nevertheless, the maximum overall daily net production estimated from field data by Jansson (1974), viz. 3.8 to 10 g m^{-2} day^{-1}, is higher than the daily production maximum in the present study. Rates for summer growth of a free-living ball-forming species, C. prolifera, in Bermuda (Bach and Josselyn 1979) are also high compared to the present study, viz. 7.6 g m^{-2} day^{-1}. This figure was calculated by the authors using a growth rate of 0.018 day^{-1} and a photosynthetic producing biomass of 424 g dry wt m^{-2}. Comparison with the present study, where the corresponding figures are 0.013 day^{-1} and 260 g dry wt m^{-2}, suggests that much of the difference in production between these species is largely a function of the magnitude of the photosynthesising biomass. Individual C. prolifera balls are some 3 times the diameter of those in this study and in their calculations the upper 3-4 cm of the bed is considered photosynthetically active.

Net production calculated here suggests that $\sim$ 0.57 kg dry weight m^{-2} $year^{-1}$ may be a reasonable annual estimate for the Cladophora bed. This is lower than net productivities which can be achieved by large seaweeds in closed experimental systems (see Jackson 1977) but is comparable to the range reported for natural stands of salt marsh plants (Gallagher 1978). Such production comparisons must obviously be viewed cautiously, particularly among different systems, as much of the apparent difference, as outlined above for the mat-forming Cladophora species, is due, not to vastly differing growth rates, but differences in plant density. Daily production rates here are within the range measured for submerged aquatic macrophytes (Westlake 1975) and, interestingly, similar to those calculated for phytoplankton in eutrophic warm lakes (Talling cited Stengel and Soeder 1965).

9.3 NOTES ON THE DEVELOPMENT OF THE PROGRAMMES

9.3.1 General

Both models draw heavily on Blackman's early concept of 'limiting factors', which embodies the concept that when photosynthesis or growth is affected by a number of independent variables, then at one time only one of those variables will be limiting the overall rate. For example, if light and nutrient levels are adequate, temperature may be limiting the rate of plant production; if temperature is raised, the level of phosphorus may become the limiting factor. Much of the early plant physiological literature is concerned with whether or not the principle can be applied rigidly, or whether the factors interact at critical levels and should not be thought of as strictly independent. Recently there has been interest in limitation of growth by more than one nutrient (Droop 1973; Ahlgren 1980). Generally, however, the early concept of Blackman remains as a useful way of disentangling the complex inter-relationships between environmental variables which may each contribute to controlling the growth of the plant. It should also be pointed out that in our experimental work we have assumed for convenience a minimal interaction between environmental variables; for example, to investigate phosphorus concentrations we supplied nitrogen at high levels under non-limiting light and temperature conditions. There is little doubt that more subtle interactions would be revealed by repeating experiments at different levels of nitrogen, or at different light intensities. Nevertheless, for our purposes the only useful approach is to treat the variables independently, and we have done this in the following programmes, in order to effect some synthesis between experimental and field data, as a guide to understanding growth rates under field conditions.

Another problem which becomes apparent in any programme which uses data from different sources, is that when photosynthetic rates, determined in short-term experiments, are multiplied up to growth rates, using assumptions about the relationships between rate of oxygen evolution, carbohydrate production, and increase in dry weight, the resulting derived growth rates are higher than those obtained from longer term culture experiments. For example in the culture experiments (Chapter 7), algal balls were much larger than the fragments used in studies on photosynthesis, and so would be affected by self-shading. Further, cultures were not grown at saturating light intensities for long periods, because in preliminary work, Cladophora began to bleach and grow poorly under continuous high-light conditions. For this reason, alternating light and dark periods were used with consequent periods of respiratory loss at night. We have therefore converted the laboratory data to a percentage basis using the maximum rate (for photosynthetic experiments the maximum rate of photosynthesis; for growth experiments the maximum growth rate obtained in a particular experiment) as 100%.

Fig. 9.4 shows the data derived from Chapters 5 and 6, together with mathematical formulae describing curves which approximate those data, and which were used in the programmes.

In each programme light is an important input. The mean radiation recorded at Perth Airport for four years (1975-1978) was plotted against day number, and a function derived which describes the shape of the resulting curve (Fig. 9.5).

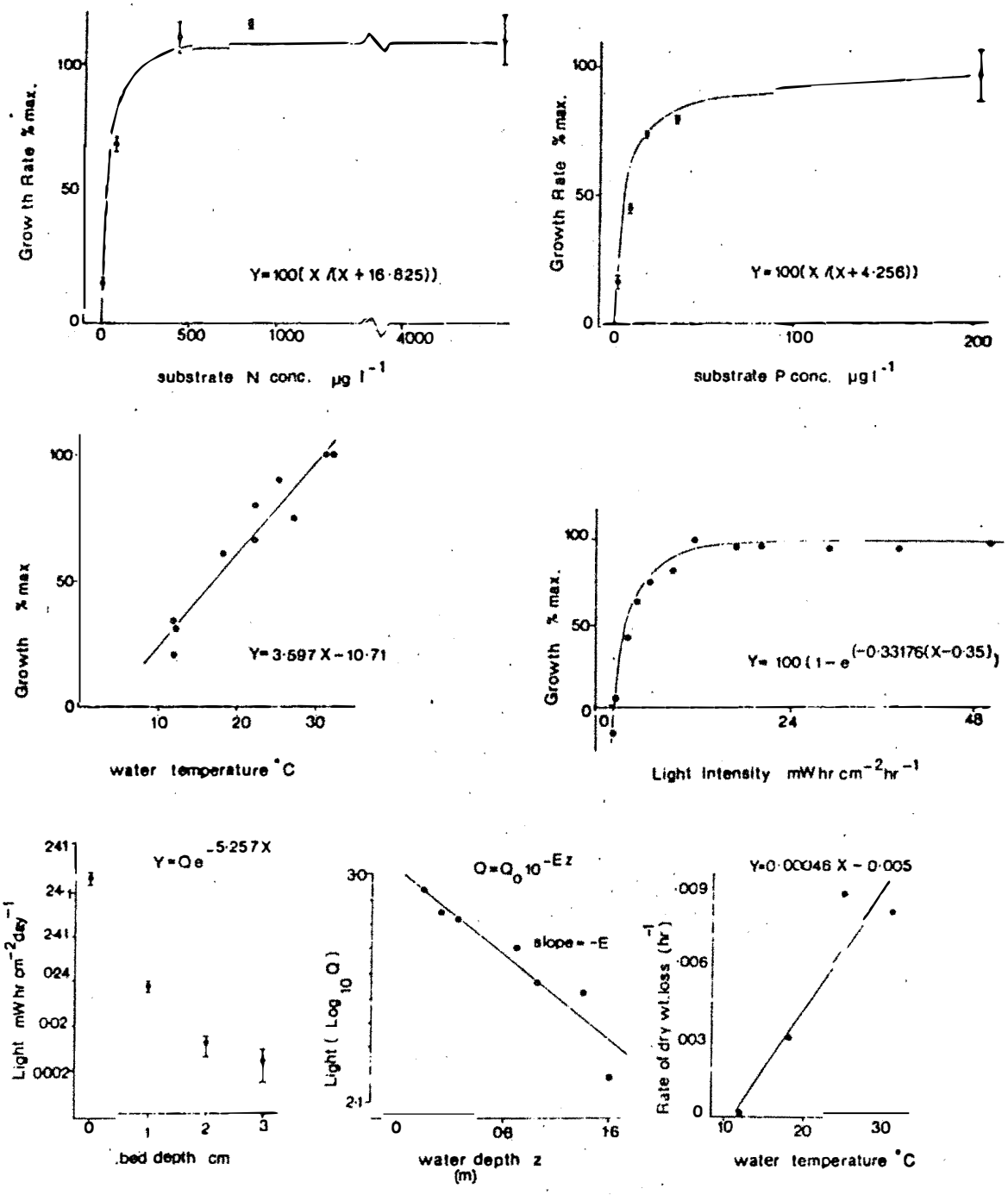
A function was also derived to calculate day length using day number (Fig. 9.5). The radiation derived for each programme (see below) was reduced to that portion of the radiation encompassed by the visible spectrum which is used for photosynthesis, reduced again by 15% to allow for surface scatter, reduced again by a factor which takes into account the nominated attenuation coefficient through the water column and the nominated water depth, and reduced again by the depth within an algal bed, using the formula in Fig. 9.4. This then described the light reaching a fragment of alga presumed to be present at a nominated point in the estuary. The details of these calcualtions are given in Appendix 2.

9.3.2 Programme A

In the simpler of the two programmes, light intensity derived from Perth Airport data is divided by the day length to give an average rate of energy input for the nominated day. Of course, radiation input during the day is not constant, being at a maximum at midday, and the rate of energy input as calculated in this simple way occurs on average at about mid-morning and mid-afternoon; it is for these times of the day to which the model applies. Towards the middle of the day the intensity will be somewhat higher; in the morning and evening, lower. The programme calculates a growth rate for that particular energy input, assuming that all other environmental variables are adequate, then another rate based on nitrogen, another on phosphorus, and another for temperature. These independently calculated growth rates are then compared, and the minimum one selected as the rate which is achieved on that particular occasion. The programme also points out the variables responsible for limiting the growth rate.

9.3.3 Programme B

This programme is concerned not with the growth rate at an instant in time, but rather with total growth rate for the whole day, expressed in grams per gram per day. It therefore deals with light intensity in a more complex way than programme A.



FUNCTIONAL FITS TO EXPERIMENTAL DATA AS USED IN CLADOPHORA SIMULATION MODELS

Figure 9.4 Experimental data used in computer programmes, with the mathematical function describing the fitted curve for each.

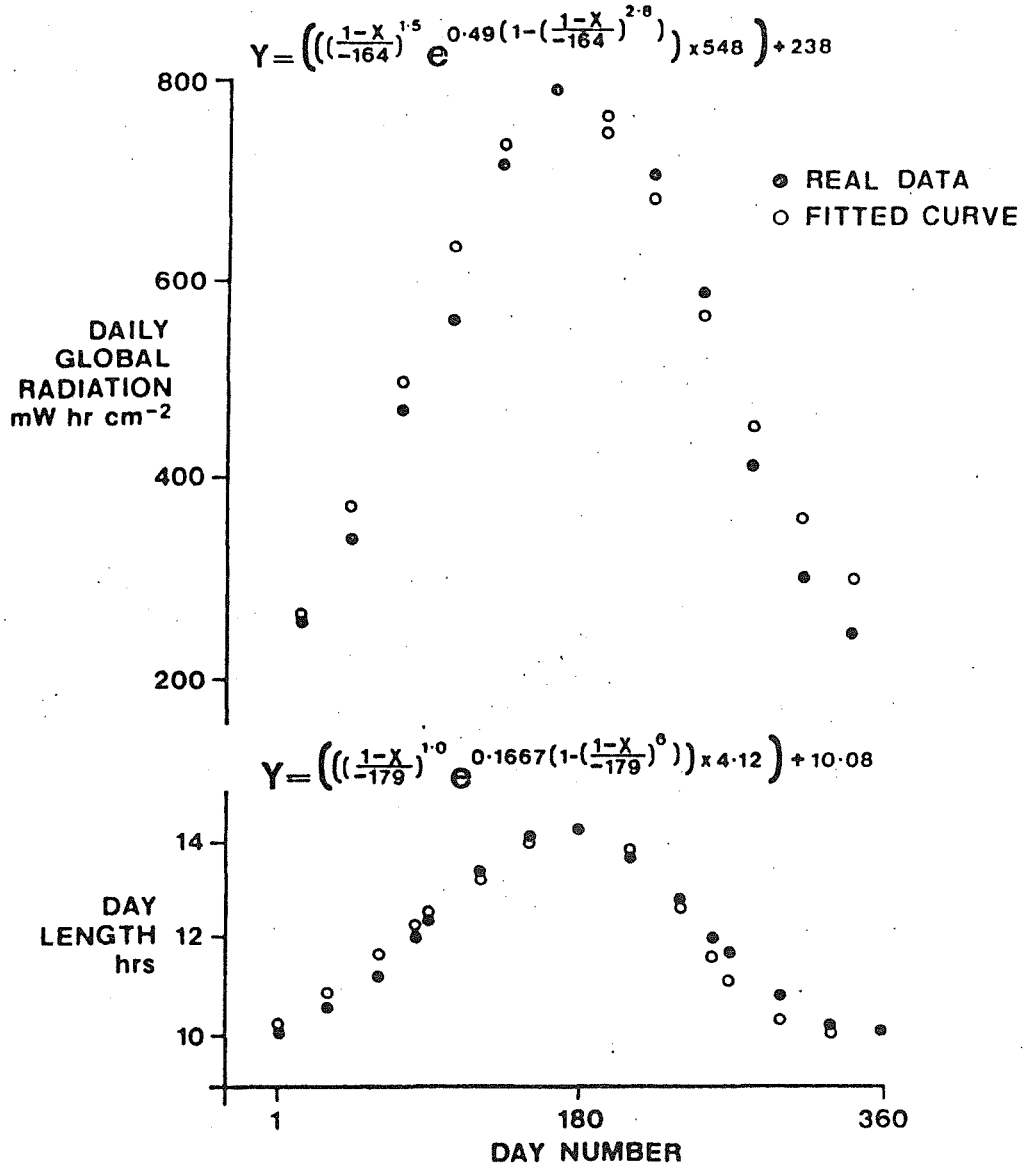


Figure 9.5a Mean daily global radiation, mW hr cm^{-2} at Perth Airport. Data are averaged over 4 years and are shown with a mathematical function describing the fitted curve.

b Mean daylight hours at different times of the year shown with a mathematical function describing the fitted curve. Day 1 represents July 1.

The distribution of light energy arriving at a surface during the day is shown in Fig. 9.6. It is derived from the literature and it is assumed that the shape of this curve is the same for any day in the year. That is, the amount of energy reaching the earth over the year is different, and day length changes, but the percentage distribution of the energy about mid-day is assumed to be constant. In fact, the curves are very similar (see Withrow, 1959). The energy contained in each 20th of the day length was expressed as a percentage. Using the function for energy arriving on a nominated day and the function for day length (Fig. 9.5), the amount of energy was calculated and reduced by the same factors described above. The percentage of that energy arriving in each 20th of the day length was then calculated, and for each of these segments of the day the growth rates determined by light, nitrogen, phosphorus, and temperature were computed and compared. The minimum rate was obtained and converted to biomass increase for the period between dawn and mid-day, and summed. As the curve of light energy is symmetrical about mid-day, calculations were based on the period between dawn and mid-day and simply doubled. The amount of respiration which took place during the night was computed using the function for respiration given in Fig. 9.4 and the day-length data, and subtracted from the increase in biomass. The output of the programme thus gives the calculated growth rate per day.

9.4 LIMITING FACTORS ON PARTICULAR OCCASIONS

We can consider four aspects of the alga's growth characteristics in the estuary: (1) an isolated fragment floating on or in the water column; (2) a fragment of Cladophora on the estuary floor but not associated with an algal bed; (3) a fragment of Cladophora associated with the surface layer of an algal bed; and (4) a fragment of Cladophora at some specified depth within the algal bed.

These cases have been summarised in Table 9.2, where they are looked at for two occasions during the year - in mid-summer and mid-winter (these being the times at which growth rates are maximum and minimum; Chapter 5.). For these calculations, using Programme A, temperature, light attenuation and depth in the water column have been averaged for summer and winter periods at station 4, while concentrations of nitrate-nitrogen, ammonia-nitrogen and phosphate are the means of measurement, either from the water column or within the algal bed (Chapter 5; Fig. 5.8). The situation has been further simplified by considering only a fragment, rather than a "clump" of the alga, thus removing, for the moment, the complexity of self-shading.

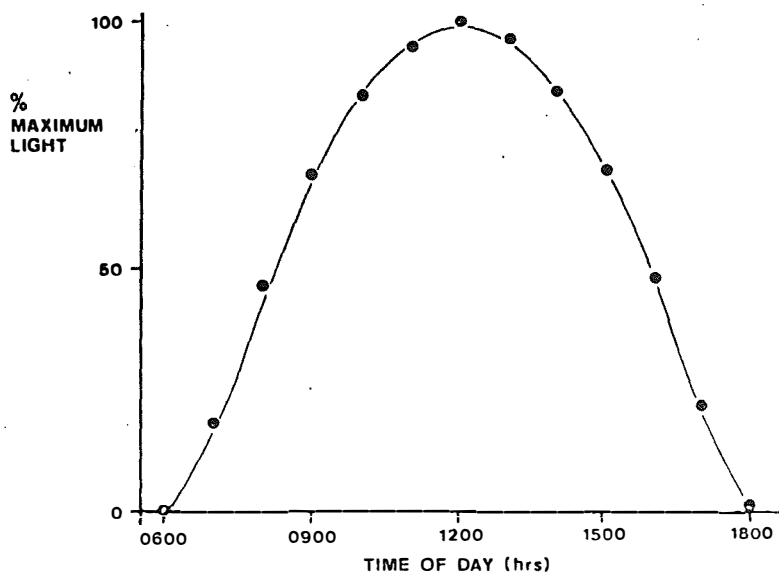


Figure 9.6 Distribution of light energy arriving at water surface during the day (derived from Withrow 1959).

Table 9.2 Analysis from Programme A showing percentage of maximum growth and limiting factors for a *Cladophora* fragment growing in different seasons. Cases considered are:- (i) fragment floating at surface of the water, (ii) fragment on the estuary floor but not in an algal bed, (iii) fragment associated with the surface of an algal bed, (iv) fragment growing 2 mm below surface of algal bed. Data are averages of mid-summer and mid-winter values collected at station 4, Peel Inlet.

	Depth (m)	Temp.(^{o}C)	Attenuation coeff. (m^{-1})	Nutrient source	Growth (% max.)	Limiting factor	Conditions of growth
Summer	0 (surface)	25	-0.35	water column	58.5	phosphate	fragment floating in water column, not associated with an algal bed
Winter	0	14	-0.43	" "	39.6	temperature	
Summer	1.8 (bottom)	25	-0.35	water column	58.5	phosphate	fragment lying on the estuary floor not associated with an algal bed. Winter data are for the period before and during river flows
Winter (no river flows)	1.8	14	-0.43	" "	32.2	light	
Winter (river flowing)	1.8	14	-0.81	" "	-1.2	light	
Summer	1.8 (algal bed (surface)	25	-0.35	interalgal	78.2	light	fragment associated with the surface of an algal bed. Winter data are for the period before and during river flows.
Winter (no river flow)	1.8	14	-0.43	"	32.3	light	
Winter (river flowing)	1.8	14	-.81	"	-1.2	light	
Summer	1.8 (within algal bed)	25	-0.35	interalgal	36.7	light	fragment at 2 mm depth in the algal bed. Winter data are shown for the period before and during river flows.
Winter	1.8	14	-.043	"	5.8	light	
Winter (river flowing)	1.8	14	-0.81		-1.2	light	

For a floating fragment in summer, phosphate concentration is the limiting factor for growth, in contrast to the winter situation where the low water temperature is the deciding factor under the conditions specified. For the winter we are considering the case where there has been no river flow with resulting increased turbidity, which would presumably produce a consequent light limitation.

The second case, identical to the first with the exception of the alga's position in the water column, (now lying on the estuary floor), shows phosphate is once again the limiting factor in summer while light, before and during river flow, is the operating factor in winter. This case presumably resembles the initial establishment of an algal bed with no underlying nutrient bank of sediment or decomposing algae. Light under such conditions is the primary controlling factor during winter. From Table 9.2 the added complexity of river flow, in winter, under otherwise identical conditions to the no-flow period, is enough to increase turbidity to a level which produces negative growth.

Cases 1 and 2 highlight the importance of phosphate supply for the growth, during summer, of algae relying on the water column as a nutrient source, and not associated with an algal bed. The negative growth produced with light limitation, during river flow in winter, is consistent with the weight losses observed in growth experiments during winter (Chapter 5).

The third case is particularly significant, since it considers a fragment growing on the surface of the algal bed. For this simulation we assume that the alga is associated with the nutrient rich "interalgal" water within the bed, an assumption which may not be entirely valid since the boundary between the algal bed and the water column lies within an ill-defined continuum from high to low nutrient concentration. Under these conditions, summer growth is light limited. (This contrasts with the case where the algae is not associated with the "nutrient rich" bed, and phosphate is the controlling factor). In the winter, results are similar, the alga producing a "negative" growth. The cases outlined above are consistent with the view that nutrients in the algal bed are unlikely to limit growth, either in summer or winter, the controlling factor here being light. In contrast, algae relying on water column nutrients are likely to be nutrient limited in summer. Field data (Chapter 8) are consistent with nutrient limitation of Cladophora growth at the surface of the algal bed, and so overall one may suggest that at the immediate bed surface the algal balls rely on water column nutrients.

So far, we have considered an isolated fragment, minimising self-shading effects. As noted earlier, self-shading within the bed is dramatic and results in significant loss of available light for photosynthesis. Results for the final case, for a fragment at some depth within the bed, in this case 0.2 cm, show that light is limiting in both summer and winter. We are, of course, assuming that the bed remains "static", though, in fact, underlying layers do become exposed when the bed shifts.

Self-shading within the algal bed is examined in more detail in Table 9.3 where Programme A has been used to choose which factor is limiting growth at different depths in the algal bed. For these calculations the alga at the bed surface is assumed to be associated with water column nutrients only. The results again suggest that light is the controlling factor within the bed either in summer or winter, though under the conditions imposed here, phosphate is limiting in summer at the bed surface. The summer and winter situations are contrasted in the growth percent shown, where fragments 0.5 cm below the surface can photosynthesise in summer while below 0.2 cm in winter, photosynthesis ceases.

The data so far suggest that P may well be the nutrient which is most important in limiting growth. In view of this, Programme A has been used to manipulate the phosphate concentration under otherwise identical nutrient conditions and predict the concentration above which Cladophora, associated with the water column, will not be P-limited. The results are shown in Table 9.4 for a fragment of Cladophora on the estuary floor and not lying in an algal bed. There, phosphate is limiting growth in summer and, assuming that other variables remain unchanged, the results show that above a concentration of 10 $\mu g\ \ell^{-1}P$ in the water column, P would cease to be limiting, some other factor operating, in this case inorganic nitrogen. Conversely, in winter, where temperature is limiting in this situation, concentrations below 3 $\mu g\ \ell^{-1}P$ in the water column effectively result in P limitation, superceding temperature in controlling growth.

Similar predictions can be made for P in the algal bed, where concentrations are generally higher (Chapter 5, Fig. 5.8). For a fragment 0.2 cm below the surface of the bed, light limits growth throughout the year (Table 9.3). From Table 9.5 it can be seen that P concentrations in "interalgal" water, udner otherwise constant conditions, would have to fall to below 2 $\mu g\ \ell^{-1}P$ to supercede light as the limiting factor either in summer or winter. This, of course, is a most unrealistic situation.

Table 9.3 Analyses from Programme A showing the effect of self-shading on growth of *Cladophora* at different depths in the algal bed in summer and winter. Growth is given as a percentage of maximum along with the corresponding limiting factor. Data used are typical average values collected at station 4 during mid-summer and mid-winter.

	Summer (day 180)			Winter (day 1)		
Depth in Algal Bed (mm)	Nutrient source	Growth (% max.)	Limiting factor	Nutrient source	Growth (% max.)	Limiting factor
0 (surface)	water column	41.3	phosphate	water column	15.1	light
1	interalgal	74.2	light	interalgal	4.6	"
2	"	52.9	"	"	-1.69	"
4	"	17.1	"	"	-8.5	"
5*	"	6.2	"	"	-10.1	"

*This depth is approximately equal to the radius of an individual algal sphere.

Table 7.4 Output from Programme A predicting limiting concentration of P for growth of a fragment of *Cladophora* on the estuary floor, not associated with an algal bed and in the presence of water column nutrient concentrations. Data used are mid-summer values from station 4, Peel Inlet. Nutrient concentrations used are the mean concentrations in the water calculated over a number of months (see Fig. 5.8, Chapter 5).

Season	Day No.	Atten. Coeff. (m^{-1})	Temp. ^{o}C	Water Depth (m)	Depth in Algal Bed (cm)	NO_3-N $\mu g\ \ell^{-1}$	NH_4-N $\mu g\ \ell^{-1}$	PO_4-P $\mu g\ \ell^{-1}$	Limiting factor
Mid-summer	180	-0.35	25	1.8	0	3	43	6	phosphate
"	"	"	"	"	"	"	"	8	phosphate
"	"	"	"	"	"	"	"	10	phosphate
"	"	"	"	"	"	"	"	12	inorganic nitrogen
"	"	"	"	"	"	"	"	14	inorganic nitrogen
"	"	"	"	"	"	"	"	16	inorganic nitrogen
Mid-winter	1	-0.43	14	1.8	0	3	43	6	temperature
"	"	"	"	"	"	"	"	5	temperature
"	"	"	"	"	"	"	"	4	temperature
"	"	"	"	"	"	"	"	3	temperature
"	"	"	"	"	"	"	"	1	phosphate

Table 9.5 Output from Programme A predicting the concentration of P which would be limiting for a fragment of *Cladophora* growing 0.2 cm below the surface of the algal bed. Data used are mid-summer and mid-winter values from station 4, Peel Inlet. Nutrient concentrations are the means for "interalgal" water (see Fig. 5.8, Chapter 5).

Season	Day No.	Atten. Coeff. (m^{-1})	Temp. ^{o}C	Water Depth (m)	Depth in Algal Bed (cm)	NO_3-N $\mu g\ l^{-1}$	NH_4-N $\mu g\ l^{-1}$	PO_4-P $\mu g\ l^{-1}$	Limiting factor
Mid-summer	180	-0.35	25	1.8	0.2	7	186	93	light
	"	"	"	"	"	"	"	80	light
	"	"	"	"	"	"	"	40	light
	"	"	"	"	"	"	"	2	light
	"	"	"	"	"	"	"	0	phosphate
Mid-winter	1	-0.43	14	1.8	0.2	7	186	93	light
	"	"	"	"	"	"	"	80	light
	"	"	"	"	"	"	"	40	light
	"	"	"	"	"	"	"	20	light
	"	"	"	"	"	"	"	0	phosphate

9.5 SEASONAL CHANGES IN GROWTH - SIMULATIONS OF FIELD DATA

The following discussion is concerned with comparing growth rates measured in the field with those produced in a simulation using Programme B. Environmental data used in these simulations were collected weekly at growth sites and integrated over the monthly growth periods. Nutrient data for station 4 simulations were the mean "interalgal" water concentrations collected at the growth site (Chapter 4; Table 4.3). For those sites not associated with an algal bed, that it, station 1 and Coodanup (see Fig. 5.1; Chapter 5), nutrient concentrations used in simulations are the means of the weekly data collected from the water column at station 1 and station 4 respectively. These have been integrated over each approximately monthly growth interval.

Seasonal change in growth at station 4 between August 1977 and August 1979 is shown in Fig. 9.7 along with the simulations produced using Programme B. These include rates at the surface, and at different depths in the algal bed.

Both real and simulated data give distinct seasonal trends with growth rates rising in spring to a maximum in summer, declining once again in autumn to a minimum in winter. The absolute rates, however, differ considerably, the simulated rates at 0 and 0.2 cm generally exceed observed rates. For the remaining seasons each year, simulated rates were mostly negative, suggesting that the observed changes in the field are best reflected by growth at about 0.3 cm depth in the bed. The discrepancy between actual and simulated rates is presumably largely due to the sometimes heavy periphyton growth on the inside and outside of the flasks. In the top layer of the bed, it is likely that the real field growth rates could exceed the observed rates considerably.

It is generally conceded that such high rates as those obtained on small fragments of algae in culture (Chapter 7) are unlikely in natural stands where selfshading and changing environmental factors are operating. For example, maximum growth rates reported for larger seaweeds growing in culture (0.2 day^{-1}; De Boer and La Pointe cited Jackson 1977) are close to those obtained in culture here. Field growth rates, in contrast, of the kelp *Macrocystis*, ranged between 0.006 and 0.011 day^{-1} (Jackson 1977) which is within the range of growth rates obtained in the field for *Cladophora* in this study.

Simulated rates, for those algae grown inshore from station 4, at Coodanup, are shown along with the actual rates, in Fig. 9.8. These reflect the seasonal changes well, but the absolute rates were very much higher than those actually observed. For example, the observed summer maximum of 0.027 day^{-1}, gave a corresponding simulated rate of 0.081 day^{-1}, the difference due, in part, to shading of flasks by

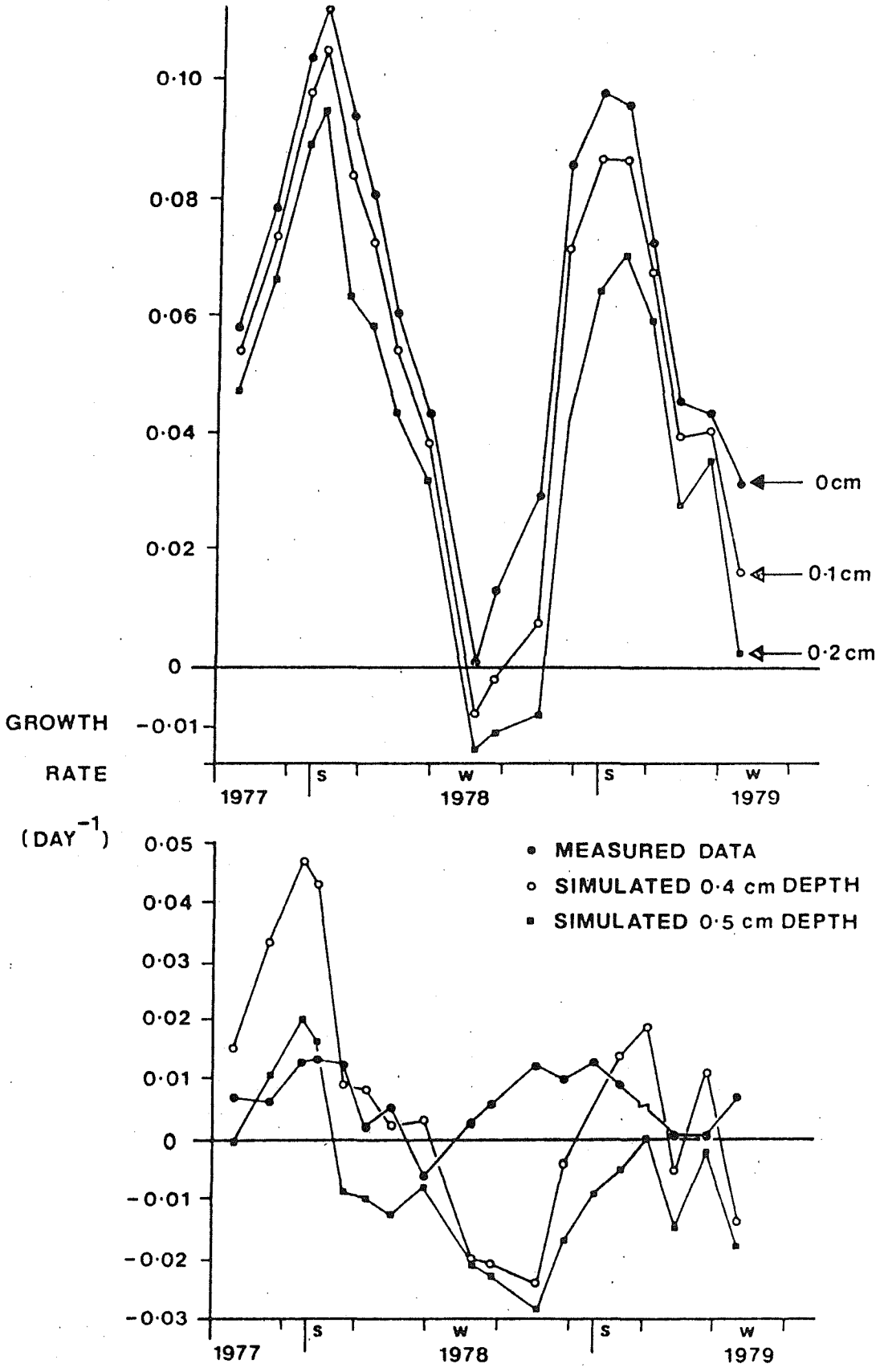


Figure 9.7a Simulated growth rate (day^{-1}) of *Cladophora* at the surface, 0.1 cm and 0.2 cm depth of the algal bed at station 4, Peel Inlet.

b Measured growth rate (day^{-1}) at station 4, Peel Inlet shown with corresponding simulated rates, at 0.4 and 0.5 cm depth in the algal bed.

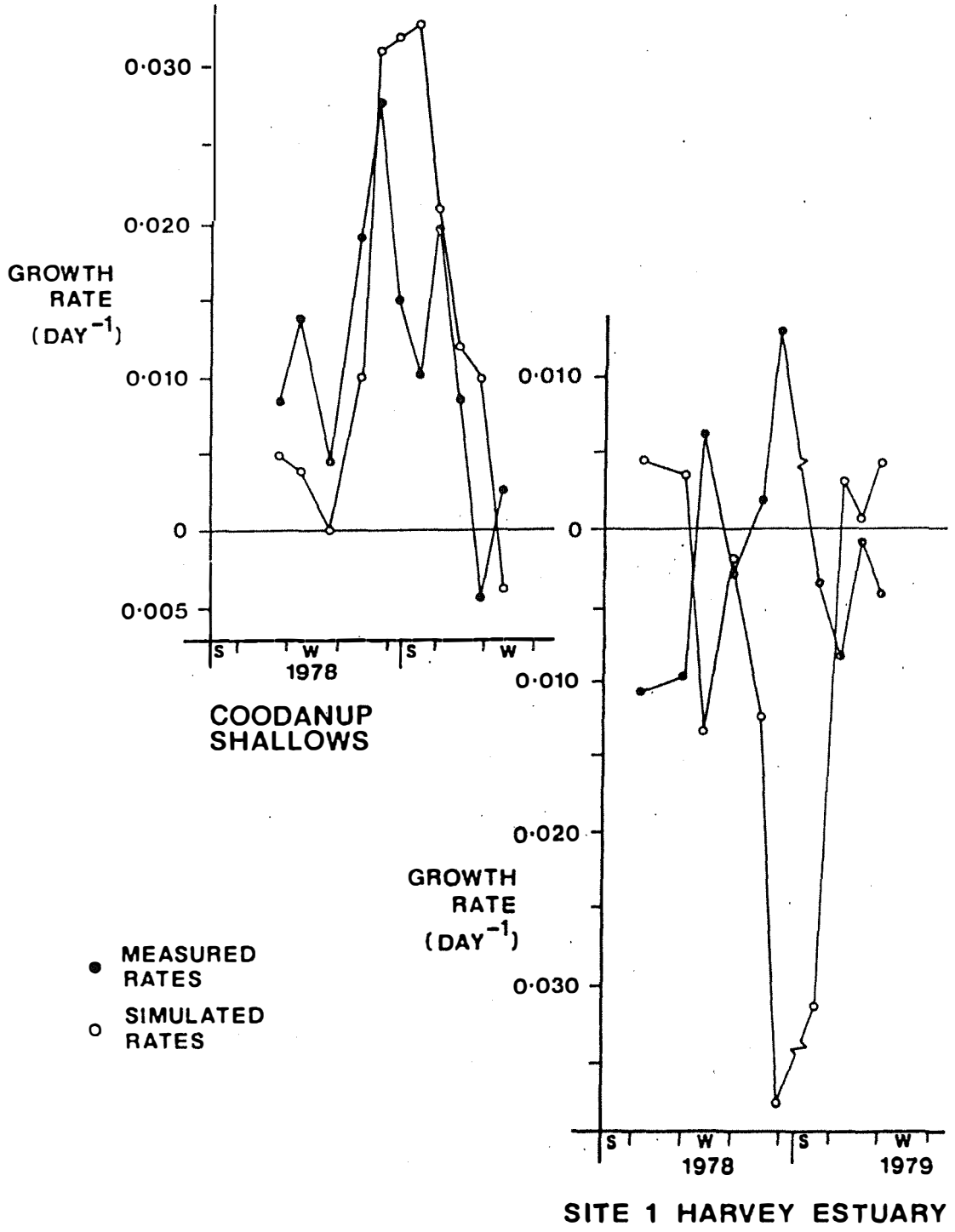


Figure 9.8a Measured growth rate of *Cladophora* (day^{-1}) at Coodanup, Peel Inlet with corresponding simulated rates.

b Measured growth rate of *Cladophora* (day^{-1}) at station 1, Harvey Estuary with corresponding simulated rates.

epiphytes or deposition of algae over the flasks. The simulated curve produced for the Coodanup site was calculated by assuming shading equivalent to burial to a depth of 0.55 cm in an algal bed. Shading of the flasks by overlying filamentous algae may have been a significant factor in producing decreased growth rates between November, 1978 and February, 1979 at this site, these not being reflected in the computer simulation curve corresponding to this period. Field records for this site at this time show a massive bloom of filamentous Cladophorales, particularly Enteromorpha spp. and Chaetomorpha linum, floating on the water surface and covering extensive areas of the shallows. The negative rate in June 1979, not observed the previous winter, occurred when water clarity was very high, but when there was again an increase in Chaetomorpha at the site, presumably shading the flasks.

In contrast to Peel sites, simulations using Harvey data gave mostly negative growth rates (Fig. 9.8). Growth rates at this site have already been found to be better correlated with nutrients and water clarity rather than light and temperature (Chapter 5). However, there appears to be no obvious relationship between simulated and observed data. The simulated curve shows a very negative growth in November and December 1978, in agreement with reduced light reaching the estuary bottom during the development of the blue-green phytoplankton bloom at this time.

Actual growth rates reached a maximum in December 1978 and this is difficult to explain. Presumably enough periods of sufficient light intensity occurred between sampling occasions to allow the alga in the flasks to grow. Water clarity improved markedly from April and into June 1979, though the real growth rates at station 1 remained negative and erratic throughout this period. This may be the result of wind-driven sediments entering the flasks (see Chapter 5), leading to shading and subsequent partial decomposition.

The simulations using data from each site, reinforce the observations that growth of Cladophora in Peel Inlet is seasonal, with maxima in summer and minima in winter. Light is particularly important, as suggested by the higher growth rate obtained with better light conditions at an inshore site, with neither an algal bed nor reduced sediments to provide a source of nutrients. It is quite conceivable that under nearly ideal conditions a fragment of alga lying on the surface of an imprisoned population described here could experience growth rates of the magnitude of those observed in culture (Chapter 7) in contrast to a fragment associated with the same population but lying at the bottom of the flask, effectively below the compensation depth. Growth rates in the flasks, therefore, at best, provide an estimate of the mean growth of the 'photosynthetic

producing biomass' and, in view of the similarity between the magnitude of changes in monthly biomass measurements and those calculated from these field rates, it suggests that observed rates are reasonable, despite the unavoidable reduction in productivity imposed by epiphytic growth on the flasks.

CHAPTER 10

SUMMARY AND CONCLUSIONS

The following remarks summarise the main observations from this study and outline briefly the major reasons for the successful establishment of Cladophora in the Peel-Harvey estuarine system.

1. This species has an affinity with the species albida described for European waters (van den Hoek pers. comm.) but may be a new species. It is visually akin to free-floating aegagropiloid forms (e.g. Hunding 1979) but is not the same as the Cladophora species which have caused problems in Europe, North America and Bermuda, and as far as we know,has not been collected from other estuaries, either in Australia or elsewhere.

2. The alga spores rarely, and sporelings have occasionally been seen attached to objects in the estuary, but most of what we see is the result of vegetative growth - the fragmentation of algal balls, and their growth into new balls; this is readily reproduced under laboratory conditions. Thus the alga is present throughout the year, and does not have to pass through an organised life-cycle.

3. The alga forms 'beds' in water about 1-2 m deep, especially in the eastern half of the Peel, and at Falcon Bay in the west. These beds are loose accumulations of balls, typically 10 cm thick, and can be readily dislodged; the upper layer oscillates back and forth with water movement. However, the beds are sufficiently stable to accumulate a lower layer, a 'black ooze' of decaying Cladophora and detrital particles, and a brownish surface layer of sediment, including diatoms. The alga decomposes relatively slowly, and there appears to be little significant grazing of living plants, though the beds support large populations of amphipods.

4. The amount of alga present in these beds varies in an erratic way. High light, which brings about the evolution of oxygen through photosynthesis, causes the balls to float. These are transported in the surface water, where they may be seen as windrows. The alga can be transported over long distances before meeting an obstruction in the shallows.

5. Losses from the beds also occur in winter, low light leading to destarching and a fall in density; presumably the balls are then more readily dislodged by bottom-water movement, allowing transport in water currents. On occasions, the alga can accumulate in a bed at a rate which is higher than can be attributed to growth, and must be due to the importation of the alga from another region.

6. Shoreward-drifting algae, some small part moving straight to the beach, mostly accumulate in large banks offshore, overlying sites normally covered by the seagrass, Halophila, and Ruppia. These are eliminated, even the rhizomes being killed. The plants re-invade when Cladophora banks shift. The Cladophora directly smothers, and may indirectly compete with, these prominent seagrasses, viewed as more desirable aesthetically than the alga. The macroalgae are also driven into the fringing marsh, eliminating the marsh locally, and presumably altering its nutrient status in other regions.

7. Though this accumulated biomass is sufficient to cause serious beach fouling, it represents only a small proportion of the alga present in beds in deeper water. An estimate for the shoreline at Coodanup, perhaps the most well used area for recreational amateur fishing, was 150 tonnes and for the whole shoreline about ten times this figure. These banks decompose in situ, releasing nutrients into the water column.

8. Despite uncertainties in the calculations as to the exact amounts, the amount of Cladophora removed by 'cosmetic' techniques from the beaches is small in relation to the amount in the estuary.

9. It is difficult to estimate the biomass present. About 30,000 tonnes dry weight was apparently present until September 1979, representing 2-4 times the amount of nitrogen and phosphorus present in the water column on a particular occasion. The alga was not obvious in 1966. (If we assume 10 kg may have been present at that time, a biomass of 30,000 t can be generated by 1976, assuming a growth rate, during six months each year, of 0.009 day^{-1}, which is less than the maximum observed for populations enclosed in perforated flasks during this study. Of course this rough estimate takes no account of losses).

10. The amount of Cladophora appears to have fallen significantly during the last year of the study, 1979, to a quarter or so of that present earlier. This is not accounted for by beach accumulation, and is at least in part due to covering with sediment and decay. There has been a marked increase in other macroalgae recently, particularly of Enteromorpha and Chaetomorpha.

11. It is difficult to work out growth rates of the alga through direct observations on change in biomass at a site, but experiments in the field, in which samples of the alga have been enclosed in perforated flasks, and laboratory experiments concerned with effects of light, temperature, salinity and nutrients on growth, enable the following conclusions to be drawn.

12. Growth is seasonal. It is high in summer and low in winter, and is correlated with changes in temperature and light, rather than changing salinity. The tolerance of this alga to the wide salinity range observed in the field is confirmed in laboratory culture experiments and provides some evidence for the ready adaptability of this species to its habitat.

13. Because of self-shading, only the surface 1 cm of the algal bed can fix more carbon than that lost in respiration; that is, is above the compensation point; thus light limits the overall rate of biomass increase. Photosynthetic rates are high at the bed surface in summer, and of course for individual balls in shallow water, though their residence time there is low; emergent banks accumulated in the shallows desiccate at the surface when exposed, and most biomass in them is below the compensation point.

14. Rates of growth shown by small fragments of algae in laboratory culture are much higher than those obtained when algae are imprisoned in flasks in the field. However, the imposition of 4 mm of self-shading into the laboratory results brings estimated growth rates down to those observed in the field.

15. Despite the importance of light, nutrient supply has allowed the accumulation of large masses of algae. Water column nutrient levels are high in winter if the rivers flow, but fall in summer to levels which, if light is abundant, would limit the growth of the plant.

16. At the surface of the algal bed, under high light, the rate of uptake of nutrients falls behind the rate of growth - by comparison with laboratory studies, tissue levels of nutrients are sufficiently low, particularly in late summer, to suggest severe deficiency, especially of phosphorus. (This conclusion is less true for Falcon Bay, which is close to a possible source of nutrients in the Harvey Estuary, where nutrient levels are higher throughout the year than in Peel Inlet).

17. One mechanism by which this system might trap nutrients in winter and redeploy these for growth during summer, is from stored reserves in the tissue. In winter, the rate of nutrient uptake exceeds the growth rate, and so the plant takes up in 'luxury amounts' more nutrients than it can immediately use. Laboratory studies show that such nutrients can be redeployed, and used to sustain growth for a time in a medium deficient in nutrients. This offers one mechanism for sustaining growth of Cladophora in nutrient-poor, summer water.

18. Another source of nutrients for summer growth is the lower decaying 'black ooze' of the bed; this is nutrient-rich in contrast to overlying waters. This source could not provide nutrients indefinitely without their replacement.

19. Sedimenting phytoplankton may provide a source for replacement of nutrients lost from the bed. Nutrients arriving in the Peel in winter are in part sedimented to the floor of the estuary by phytoplankton blooms, which may be grazed or decayed, and the nutrients recycled. This provides a mechanism for replenishing sediment nutrient stores, so again setting the scene for Cladophora growth in summer.

20. Cladophora is absent from the Harvey Estuary, except at the extreme north, despite ample opportunity for invasion. Imprisoned populations there give erratic, low growth rates. Predictions of growth rates based on laboratory experiments also show low growth rates when known Harvey light attenuation figures are used.

21. The Harvey Estuary is often wind-stirred. High turbidity keeps light at the bottom low, and maintains the nutrient status of the water. The Harvey is phytoplankton-dominated, with a low density of benthic plants; seagrasses occur at low density and are confined to the eastern shore where light penetration is best.

22. In contrast, much of the Peel is less wind-stirred especially in those areas which coincide with Cladophora. Apart from the winter flow period, light penetration is good and water nutrient concentrations and phytoplankton concentrations are low. The system is more dominated by benthic plants, including microscopic algae. On a per metre square basis these microscopic algae often have ten times the amount of chlorophyll when compared with phytoplankton in the water column.

23. Cladophora appears to have been especially successful in exploiting high nutrient supplies at the floor of the estuary, building up an anaerobic bed of decomposing material which helps buffer the living material against periods of nutrient depletion. It is able to grow rapidly in response to light, the cells being typically densely packed with chloroplasts resulting in a high density of chlorophyll per square metre.

24. Of the nutrients, phosphorus is of particular importance. As in most aquatic systems, in the presence of relatively high phosphorus, N-fixing organisms will proliferate and add nitrogen from the atmosphere, as we see in the Harvey Estuary. Further, when the Murray River flows, nitrate levels are so very much higher than phosphate that the amount of phosphorus must operate more critically to control levels of biomass attained in the Peel system as a result of river flow. And more directly, the concentrations of phosphorus in the tissue of Cladophora fall to levels known to be critical for growth, especially during the summer high-growth period; levels of nitrogen are less critical.

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APPENDIX 1

DECOMPOSITION OF CLADOPHORA UNDER CONTROLLED CONDITIONS; PRELIMINARY EXPERIMENT

This experiment was designed to look at the rate of decomposition of Cladophora and, because of the problem involved in doing this in the field, populations were placed in glass tanks which were maintained either in the dark or the light near the laboratory. Dry-weight loss and the nitrogen and phosphorus content of the tissue were measured at the end of the experiment. The levels of nitrogen and phosphorus in the water column above the alga were checked from time to time.

MATERIALS AND METHODS

Fresh Cladophora was collected at Post 46 during late August, 1976, along with estuary water. Ten glass aquaria (30 cm x 40 cm x 30 cm) were placed on a bench under a Sarlon-mesh screen. Into each tank was placed 1659 g fresh weight of Cladophora, which represents 160 g of dry weight. Each of the tanks was then filled with 28 of estuary water, and aerated at a uniform rate. Five of the tanks were wrapped in black plastic to exclude light. Each tank was partly covered with a glass top to reduce evaporation, allowing about 5 cm of air space between the water surface and the cover. The tanks were maintained for 285 days, aeration being discontinued after 70 days when it was realised that decomposition was not proceeding rapidly.

The following parameters were monitored during the study - pH, salinity, dissolved oxygen, temperature, and photosynthetically-active radiation (PAR) above the tank and above the algal bed. Water samples were withdrawn at 1, 2 and 5 months, and at the conclusion of the experiment. The contents of each tank were then passed through a strainer in the same way as had been used to harvest the alga from the estuary initially, the material drained, dry weights taken, and dried material ground for N and P analyses.

RESULTS

Appearance of Plant Material and Change in Dry Weight

The experiment was terminated at 285 days. Cladophora in the tanks in the light appeared brown on the surface in most cases, but was otherwise a normal green colour. In most cases the water had a scum, probably bacterial, at the surface, and in one tank there was blackened algal material beneath the green Cladophora balls.

The water surface in the dark tanks was covered with a thick bacterial (and possibly fungal) scum. All of the Cladophora balls were blackened, although some had green material in the centres of the balls. Under the microscope the cell walls of blackened material were still prominent.

Dry weight changes are given in Table 1. In the light the algal material still represented 84% of that placed in the tanks; in the dark it represented 93%. There is probably no significant difference between these figures, so that in light or dark the algae lost only about 7% to 16% of their initial dry weight. Biomass at the start of the experiment corresponded to 1337 g m^{-2}, which is comparable with that present at Post 46 in summer (e.g. February 1977). The loss of 16% represents some 200 g dry weight m^{-2} over 10 months, i.e. on average 20 g per month.

Light Intensity

The surface of the algal material in the light received during the day 120 ± 15 $\mu E.m^{-2}.sec^{-1}$ PAR (based on 14 measurements taken at different times of the day during the experiment). This represents some 6% of full sunlight. (The light intensity at the water surface was 220 ± 28). Studies with the Clark electrode, and on the attenuation of light in the algal bed (described in Chapter 6) show that light must have been limiting at the surface of the algal bed in these experiments and, as the bed was some 10 cm deep, most of the alga must have been below the compensation point. Presumably this explains why there was no increase in biomass in the light, and, at least in part, why losses in the light resembled those in the dark.

Nutrients in Plant Material

At the end of the experiment the plant material was dried, milled and nutrient analyses carried out (Table 1). In the light the concentration of phosphorus had fallen only slightly, but in the dark, the concentration had fallen markedly. Similarly, nitrogen in the tissue had fallen by nearly 20% over the time in the dark, though there was a very small increase in concentration in the light. This information suggests, along with the blackening of the alga, that the alga had sensced, although the levels of phosphorus present still exceed those obtained in starved living material in subsequent experiments.

Salinity

At the time of setting up the experiment the salinity of the water was 29‰. There was relatively little loss due to evaporation and deficits were made up with distilled water from time to time. There was a small increase during the later part of the experiment (Figure 1). However, as shown in Chapters 6 and 7, these changes would have been without effect on the alga.

Table 1

Changes in weight and phosphorus and nitrogen content of *Cladophora* during incubation for 285 days.

	Initial	Final	
		Light	Dark
dry weight (g)[1]	160	135 ± 8.8	150 ± 4.6
phosphorus concentration $mg.g^{-1}$ dry wt.[1]	2.90 ± 0.03	2.80 ± 0.05	1.70 ± 0.04
nitrogen concentration $mg.g^{-1}$ dry wt.[1]	26.05 ± 0.65	29.37 ± 0.55	21.00 ± 0.71

[1]mean of five replicates accompanied by standard errors

pH

Although this fluctuated somewhat during the experiment there was no dramatic change, though the light tanks were on the whole more acidic than those in the dark (Figure 1).

Temperature

The tank temperatures are given in Figure 2. The light tanks had higher temperatures than the shaded tanks. Temperatures at Post 46 in the estuary range from 12 to 26°C, but in the shallows temperatures may reach 35°C on a hot day.

Dissolved Oxygen

During aeration dissolved oxygen levels were similar in light and dark tanks. After aeration had been discontinued, the dark tanks became anaerobic (Figure 3).

Water nutrients

These are shown in Figures 4 to 7. In the light there was some tendency for nutrient release during the experiment, especially total phosphorus and ammonium nitrogen. However, in the dark there were massive releases of nutrients after about 100 days. Particulate phosphorus represented between 8 and 40% of the total phosphorus in the water. As these systems were rich in micro-organisms there would have been much recycling of the nutrients, and so the release from decomposing material would have been very much greater than these experiments imply.

GENERAL

The overall impression is that *Cladophora* senesces and decays slowly. After ten months in the dark the contents of most cells would have been disorganised, but the very resistant cell walls held the material together. One can only assume that much of the nitrogen and phosphorus of this black material was in fact due to the presence of micro-organisms, rather than to the persistence of cellular organisation. Nevertheless, some small parts of the alga was green and organised at the end of this considerable period. A small amount of the material from one of the dark tanks, consisting of black ooze and a few fragments of green material, was placed in a glass tube in the dark, and more than two years later there was still a small amount of green material amongst the black ooze; when placed in a Clark electrode this photosynthesised perfectly well. Presumably part of the biomass can exist heterotrophically for considerable periods.

The small dry weight losses which took place over the considerable time of the experiment - 10 months - suggest that sudden large losses of biomass from sites in the field could not be accounted for by *in situ* decomposition.

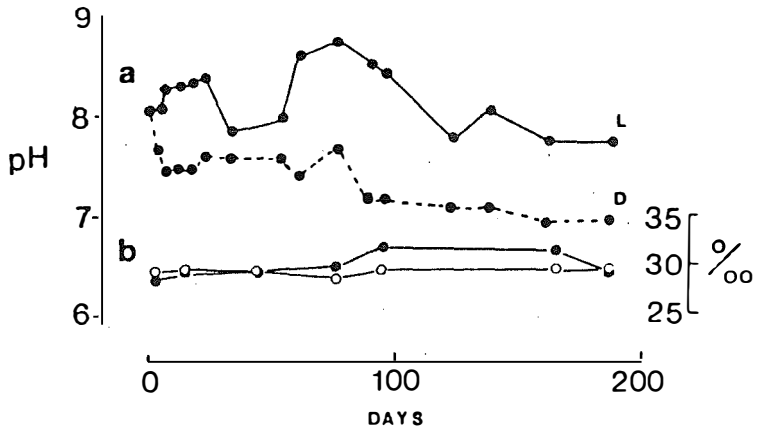

Figure 1a Change in pH of water in the light (L) and in the dark (D) with time.

b Change in salinity of water in the light (o) and in the dark (o).

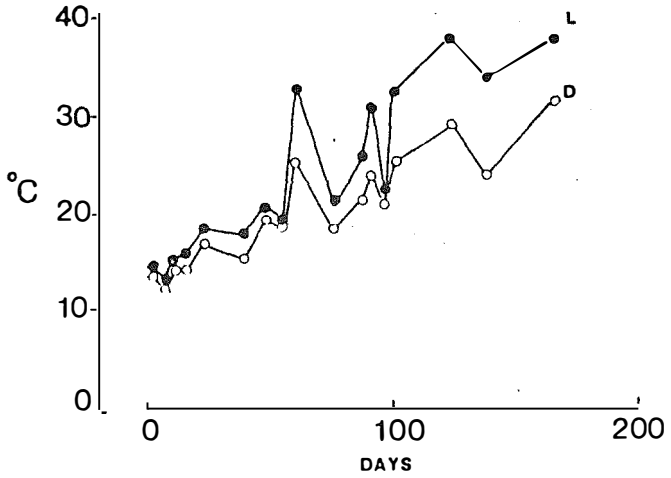

Figure 2 Changes in water temperature with time.

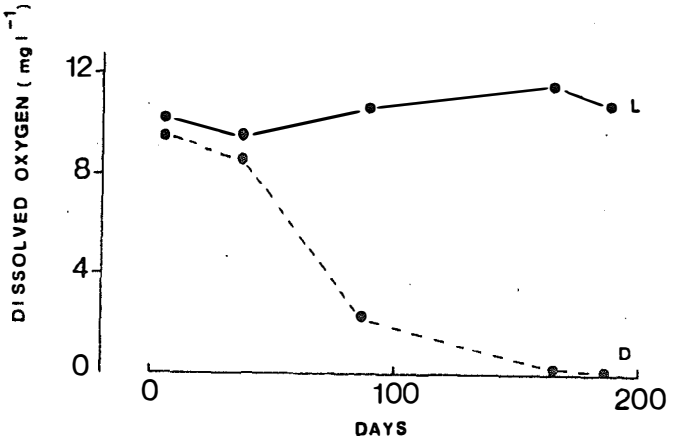

Figure 3 Change in dissolved oxygen concentration in the water with time in the light (L) and in the dark (D).

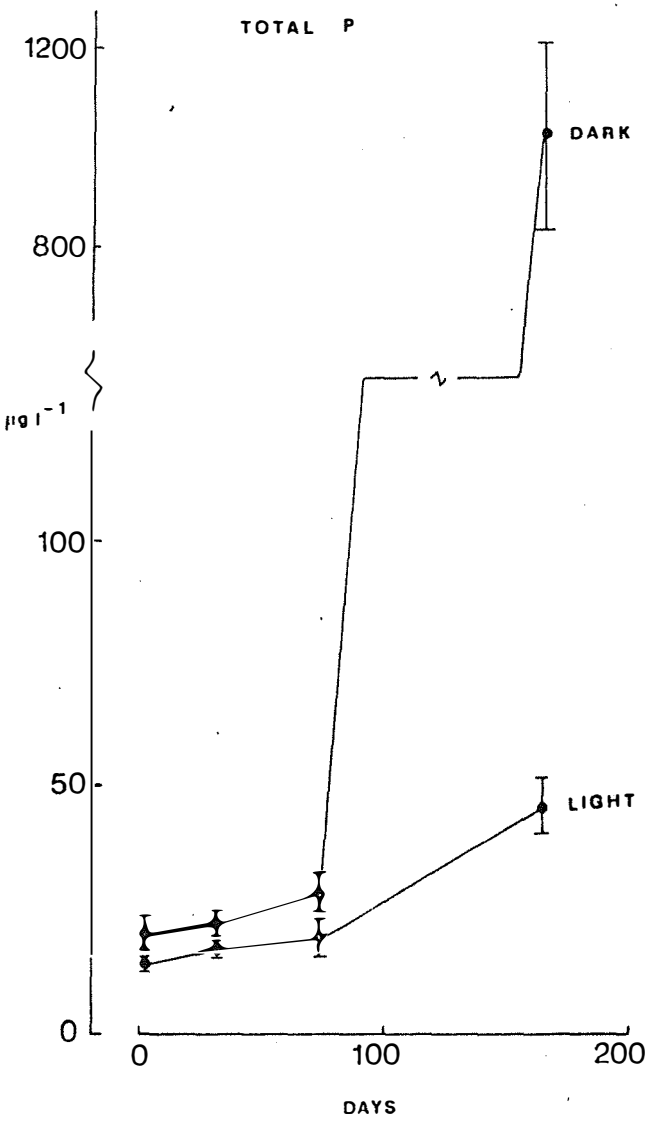

Figure 4 Change in concentration of total phosphorus in the water with time. Each point represents the mean of 5 replicates ± standard error.

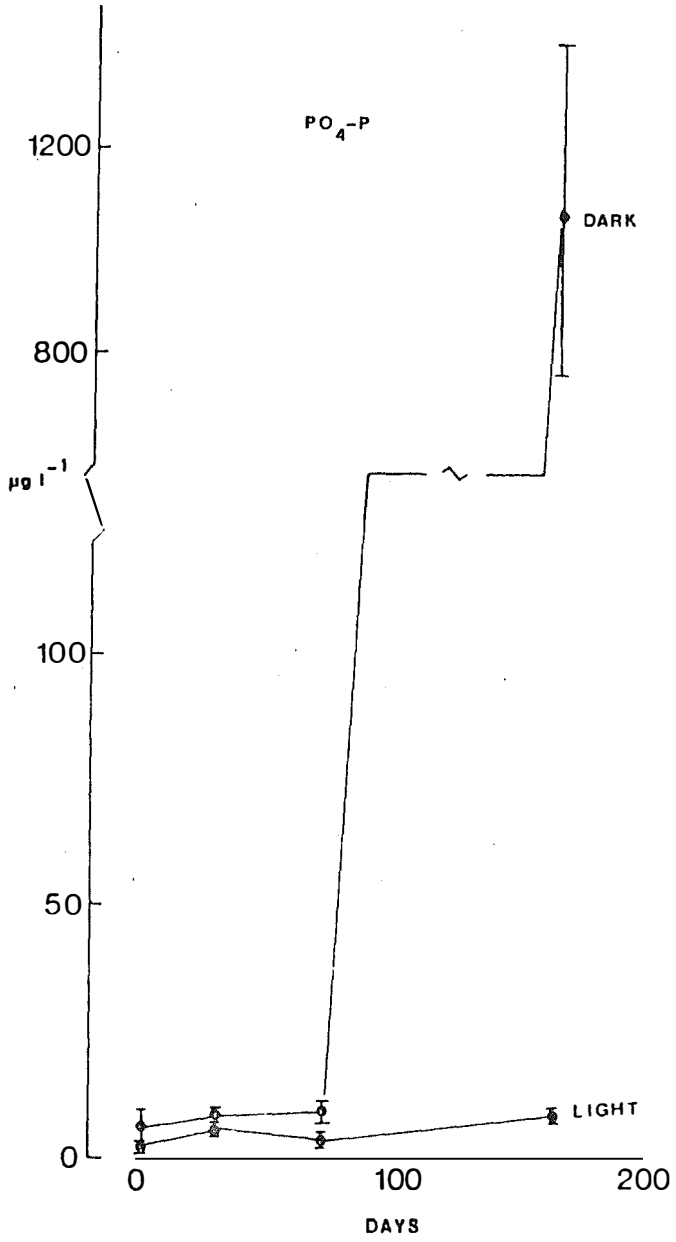

Figure 5 Change in concentration of phosphate - phosphorus in the water with time. Each point represents the mean of 5 replicates ± standard error.

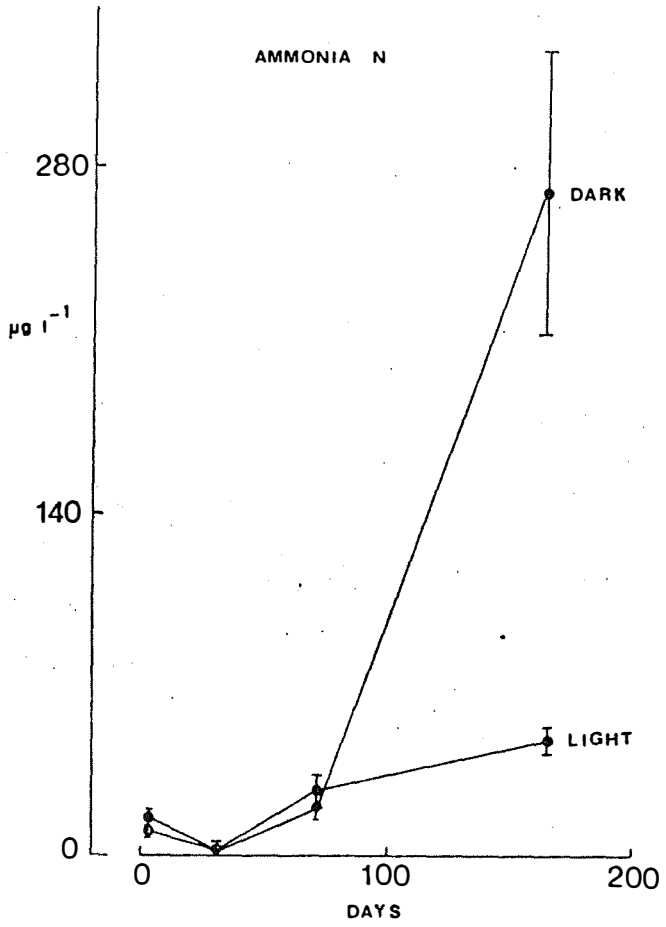

Figure 6 Change in concentration of ammonia - nitrogen in the water with time. Each point represents the mean of 5 replicates ± standard error.

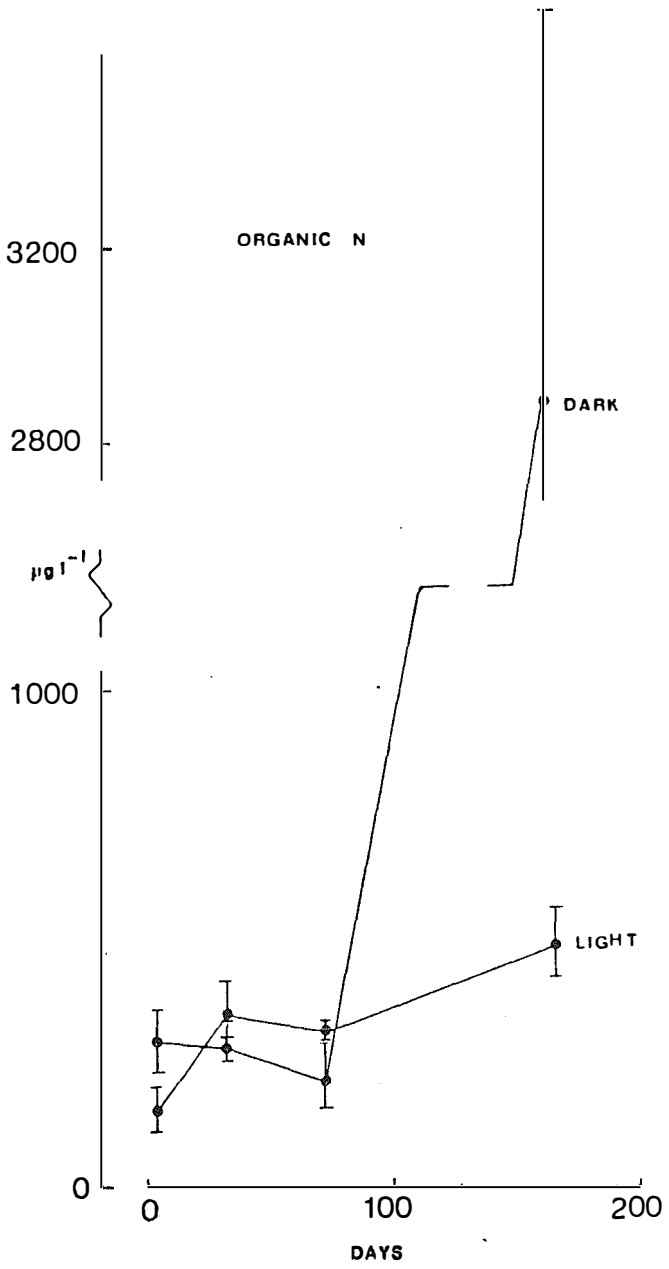

Figure 7 Change in concentration of organic nitrogen in the water with time. Each point represents the mean of 5 replicates ± standard error.

The decomposition of Cladophora has been subsequently studied in greater detail by Gabrielson et al. 1980.

APPENDIX 2

PROGRAMMES FOR CALCULATING CLADOPHORA GROWTH

A. Dependence of instantaneous growth rate on specified environmental variables.

Output shows growth rate as per cent, and selects limiting variable.

```
      COMPUTING CLADOPHORA GROWTH FROM ENVIRONMENTAL VARIABLES.OUTPUT
      IS GROWTH RATE AS A PERCENTAGE OF MAXIMUM EXPERIMENTAL VALUES.
      ENVIRONMENTAL VARIABLES REQUIRED ARE IDY=DAY NUMBER(DAY1=JULY1)
      ,AC=ATTENUATION COEFFICIENT(PER METRE),DW=DEPTH IN WATER
      COLUMN(M),DB=DEPTH IN ALGAL BED (CM),TEMP=WATER TEMPERATURE,
      VNO=NITRATE PLUS NITRITE NITROGEN,VNH=AMMONIA NITROGEN,
      VPO=PHOSPHATE PHOSPHORUS (THESE NUTRIENTS ALL IN
      MICROGRAMS PER LITRE)
    TYPE 1
  1 FORMAT(1X,'ENTER FILE NUMBER: ',$)
    READ (5,2) INUM
  2 FORMAT (I)
    TYPE 301
301 FORMAT('1',50X,'CLADOPHORA GROWTH'/)
    TYPE 302
302 FORMAT(' ',17X,'ENVIRONMENTAL',23X,'VARIABLES',/)
    TYPE 304
304 FORMAT(' ',1X,'DAY',4X,'ATTEN.',3X,'WATER',3X,'DEPTH',6X,'DEPTH ,
   *4X,'NITRATE',3X,'AMMONIA',3X,'PHOSPHATE',19X,'GROWTH',
   *5X,'LIMITING')
    TYPE 305
305 FORMAT(' ',1X,'NO.',4X,'COEFF.',3X,'TEMP .4X,'IN WATER',3X
   *,'IN BED',51X,'PERCENT',4X,'VARIABLE')
    TYPE 306
306 FORMAT('0',7X,'(PER M)',4X, (C)',5X,'(M) ,7X, '(CM)',6X,'(UG/L)'
   *4X,'(UG/L)',5X,'(UG/L)',/)
  3 READ(INUM,101) (IDY,AC,DW,DB,TEMP,VNO,VNH,VPO)
    IF(IDY.EQ.0.00) GO TO 161
101 FORMAT(1X,I3,2X,F5.2,2X,F5.2,2X,F6.2,2X,F4.1,2X,F7.1,2X,F7.1,
   *2X,F7.1)
      CALCULATED GROWTH RATES DUE TO VARIABLES A=PHOSPHATE,B=
      INORGANIC NITROGEN,C=TEMPERATURE,D=LIGHT,USING PERCENT
      OF MAXIMUM RESPONSES OBTAINED IN LABORATORY EXPERIMENTS
      GROWTH RATE A DUE TO PHOSPHATE
    A=100*(VPO/(VPO+4.256))
      GROWTH RATE B DUE TO INORGANIC NITROGEN
    B=100*((VNO+VNH)/((VNO+VNH)+16.825))
      GROWTH RATE C DUE TO TEMPERATURE OF WATER
    C=(3.597*TEMP)-10.710
      GROWTH RATE D DUE TO LIGHT THROUGH THE FOLLOWING STEPS
      LIGHT ENERGY RE AT WATER SURFACE AS GLOBAL RADIATION
      UNITS ARE MW HOURS PER SQ CM DATA FROM GUILDFORD U.A.
      FOLLOWING CALCULATIONS ASSUME AVAILABLE P.A.R.
      TO BE 0.45 OF TOTAL RADIATION AND A LOSS FROM
      SURFACE SCATTER OF 15 PERCENT
    RIDY=IDY
    RX=EXP(0.49*(1-(((1-RIDY)/-164.0)**2.8)))
    RY=((1-RIDY)/-164.0)**1.5
    RE=(((RX*RY*548.0)+238.0)*0.45)*0.85
      DAYLENGTH RL MEAN NUMBER OF DAYLIGHT HOURS AT PERTH W.A.
    RZ=EXP(0.1667*(1-(((1-RIDY)/-179.0)**6)))
    RW=((1-RIDY)/-179.0)**1
    RL=(RW*RZ*4.12)+10.08
      RATE OF LIGHT REACHING THE WATER SURFACE PER HOUR RS
    RS=RE/RL
      ALLOW FOR DEPTH IN WATER RSDW
    RSDW=(RS)*(10**(AC*DW))
      ALLOW FOR DEPTH IN ALGAL BED
    RSDB=RSDW*((EXP(DB*(-5.257))))
      CALCULATION OF D GROWTH RATE DUE TO LIGHT
    D=100*(1-(EXP(-0.33176*(RSDB-0.35))))
    NOW COMPARE A,B,C AND D TO SEE WHICH IS LIMITING
    ROW=150
    IF(A.LT.ROW)ROW=A
    IF(B.LT.ROW)ROW=B
    IF(C.LT.ROW)ROW=C
    IF(D.LT.ROW)ROW=D
    IF(ROW.EQ.A)TYPE 151,IDY,AC,TEMP,DW,DB,VNO,VNH,VPO,A
151 FORMAT(' ',1X,I3,4X,F5.2,4X,F4.1,4X,F5.2,5X,F6.2,3X,F7.1,3X,F7.1,3
   *X,F7.1,22X,F6.2,5X,'PHOSPHATE')
    IF(ROW.EQ.B)TYPE 152,IDY,AC,TEMP,DW,DB,VNO,VNH,VPO,B
152 FORMAT(' ',1X,I3,4X,F5.2,4X,F4.1,4X,F5.2,5X,F6.2,3X,F7.1,3X,F7.1,3
   *X,F7.1,22X,F6.2,5X,'INORGANIC NITROGEN')
    IF(ROW.EQ.C)TYPE 153,IDY,AC,TEMP,DW,DB,VNO,VNH,VPO,C
153 FORMAT(' ',1X,I3,4X,F5.2,4X,F4.1,4X,F5.2,5X,F6.2,3X,F7.1,3X,F7.1,3
   *X,F7.1,22X,F6.2,5X,'TEMPERATURE')
    IF(ROW.EQ.D)TYPE 154,IDY,AC,TEMP,DW,DB,VNO,VNH,VPO,D
154 FORMAT(' ',1X,I3,4X,F5.2,4X,F4.1,4X,F5.2,5X,F6.2,3X,F7.1,3X,F7.1,3
   *X,F7.1,22X,F6.2,5X,'LIGHT')
    GO TO 3
161 STOP
    END
```

B. Dependence of daily growth rate on specified environmental variables.

Output shows total daily growth rate, taking into account changing light intensities during the day, and respiratory losses at night.

```
      COMPUTING CLADOPHORA GROWTH FROM ENVIRONMENTAL VARIABLES.OUTPUT
      IS GROWTH RATE AS GRAMS OF ALGA PRODUCED PER GRAM PER DAY
      ENVIRONMENTAL VARIABLES REQUIRED ARE IDY=DAY NUMBER(DAY1=JULY1),
      AC=ATTENUATION COEFFICIENT(PER METRE),DW=DEPTH IN WATER
      COLUMN (M),DB=DEPTH IN ALGAL BED (CM),TEMP=WATER
      TEMPERATURE.VNO=NITRATE PLUS NITRITE NITROGEN,VNH=
      AMMONIA NITROGEN,VPO=PHOSPHATE PHOSPHORUS(THESE
      NUTRIENTS ALL IN MICROGRAMS PER LITRE)
    TYPE 1
  1 FORMAT(1X,'ENTER FILE NUMBER: ',$)
    READ (5,2) INUM
  2 FORMAT (I)
    TYPE 301
301 FORMAT('1',50X,'CLADOPHORA GROWTH'/)
    TYPE 302
302 FORMAT(' ',17X,'ENVIRONMENTAL',23X,'VARIABLES'./)
    TYPE 304
304 FORMAT(' ',1X,'DAY',4X,'ATTEN.',3X,'WATER',3X,'DEPTH',
   *6X,'DEPTH',4X,'NITRATE',3X,'AMMONIA',3X,'PHOSPHATE',
   *19X,'GROWTH')
    TYPE 305
305 FORMAT(' ',1X,'NO.',4X,'COEFF.',3X,'TEMP',4X,'IN WATER',
   *3X,'IN BED',52X,'RATE')
    TYPE 306
306 FORMAT('0',7X,'(PER M)',4X,'(C)',5X,'(M)',7X,'(CM)',6X,'(UG/L)',
   *4X,'(UG/L)',5X,'(UG/L)',10X,'(G/G/DAY)',/)
  3 READ(INUM,101) (IDY,AC,DW,DB,TEMP,VNO,VNH,VPO)
    IF(IDY.EQ.0.00) GO TO 161
101 FORMAT(1X,I3,2X,F5.2,2X,F5.2,2X,F6.2,2X,F4.1,2X,F7.1,2X,F7.1,
   *2X,F7.1)
      CALCULATE GROWTH RATES DUE TO VARIABLES A=PHOSPHATE,B=
      INORGANIC NITROGEN,C=TEMPERATURE,D=LIGHT, USING PERCENT
      OF MAXIMUM RESPONSES OBTAINED IN LABORATORY EXPERIMENTS.
      GROWTH RATE A DUE TO PHOSPHATE
    A=100*(VPO/(VPO+4.256))
      GROWTH RATE B DUE TO INORGANIC NITROGEN
    B=100*((VNO+VNH)/((VNO+VNH)+16.825))
      GROWTH RATE C DUE TO TEMPERATURE OF WATER
    C=(3.597*TEMP)-10.710
      GROWTH RATE D DUE TO LIGHT IS CALCULATED IN SEVERAL STEPS
      LIGHT ENERGY RE AT WATER SURFACE AS GLOBAL RADIATION
      UNITS ARE MW HOURS PER SQ CM DATA FROM GUILDFORD,W.A.
      FOLLOWING CALCULATIONS ASSUME AVAILABLE P.A.R.
      TO BE 0.45 OF TOTAL RADIATION AND A LOSS FROM
      SURFACE SCATTER OF 15 PERCENT
    RIDY=IDY
    RX=EXP(0.49*(1-((((1-RIDY)/-164.0)**2.8)))
    RY=((1-RIDY)/-164.0)**1.5
    RE=(((RX*RY*548.0)+238.0)*0.45)*0.85
    RZ=EXP(0.1667*(1-((((1-RIDY)/-179.0)**6)))
    RW=((1-RIDY)/-179.0)**1
      DAYLENGTH RL MEAN NUMBER OF DAYLIGHT HOURS AT PERTH W.A.
    RL=(RW*RZ*4.12)+10.08
      ALLOW FOR DEPTH IN WATER RSDW
    RSDW=(RE)*(10**(AC*DW))
      ALLOW FOR DEPTH IN ALGAL BED
    RSDB=RSDW*((EXP(DB*(-5.257))))
    RSDB IS THE LIGHT ENERGY REACHING ALGA EACH DAY,BUT IT FALLS
    AT DIFFERENT RATES AT DIFFERENT TIMES OF THE DAY.
    CONSIDER DAY TO BE DIVIDED INTO EQUAL PARTS
    T IS TIME INTERVAL(HR) IN ARBITRARILY CHOSEN 20TH
    OF DAYLENGTH RL.
    T=RL/20
    ASSUME PROPORTION OF TOTAL ENERGY REACHING ALGA IN EACH
    INTERVAL T IS THE SAME ON ANY DAY.LET
    TA,TB,...TK REPRESENT ENERGY PROPORTIONS FOR THE 10
    INTERVALS FROM SUNRISE TO SOLAR NOON.PROPORTION OF TOTAL
    ENERGY IN EACH INTERVAL CALCULATED FROM AREA UNDER SOLAR
    RADIATION CURVE.
    TA=RSDB*0.008
    RATE AT WHICH LIGHT ENERGY IS RECEIVED IN 1ST INTERVAL IS EA
    EA=TA/T
    RATE OF GROWTH (D PERCENT) AT EA IS NEXT COMPUTED
    D=100*(1-(EXP(-0.33176*(EA-0.35))))
    NOW COMPARE RATE DUE TO LIGHT DURING FIRST INTERVAL
    WITH RATES CALCULATED FOR OTHER VARIABLES A,B AND C
    AND FIND WHICH IS LIMITING
```

```
ROW=150
IF(A.LT.ROW)ROW=A
IF(B.LT.ROW)ROW=B
IF(C.LT.ROW)ROW=C
IF(D.LT.ROW)ROW=D
CONVERT RATE OF GROWTH TO AMOUNT PRODUCED DURING INTERVAL T
MAXIMUM POSSIBLE GROWTH RATE IS SET AT 0.15 PER DAY,
FOUND IN CULTURES WITH A 12 HOUR PHOTOPERIOD.
0.15=0.0125 PER HR OF LIGHT.DESIGNATE AS TOTL.THIS CAN BE
CHANGED IF NECESSARY.
TOTL=0.0125
GRA=(ROW/100)*TOTL*T
ROW=0.0
D=0.0
SEQUENCE ABOVE CONTINUES BELOW FOR OTHER 9 INTERVALS
TB=RSDB*0.027
EB=TB/T
D=100*(1-(EXP(-0.33176*(EB-0.35))))
ROW=150
IF(A.LT.ROW)ROW=A
IF(B.LT.ROW)ROW=B
IF(C.LT.ROW)ROW=C
IF(D.LT.ROW)ROW=D
GRB=(ROW/100)*TOTL*T
ROW=0.0
D=0.0
TC=RSDB*0.050
EC=TC/T
D=100*(1-(EXP(-0.33176*(EC-0.35))))
ROW=150
IF(A.LT.ROW)ROW=A
IF(B.LT.ROW)ROW=B
IF(C.LT.ROW)ROW=C
IF(D.LT.ROW)ROW=D
GRC=(ROW/100)*TOTL*T
ROW=0.0
D=0.0
TD=RSDB*0.081
ED=TD/T
D=100*(1-(EXP(-0.33176*(ED-0.35))))
ROW=150
IF(A.LT.ROW)ROW=A
IF(B.LT.ROW)ROW=B
IF(C.LT.ROW)ROW=C
IF(D.LT.ROW)ROW=D
GRD=(ROW/100)*TOTL*T
ROW=0.0
D=0.0
TE=RSDB*0.106
EE=TE/T
D=100*(1-(EXP(-0.33176*(EE-0.35))))
ROW=150
IF(A.LT.ROW)ROW=A
IF(B.LT.ROW)ROW=B
IF(C.LT.ROW)ROW=C
IF(D.LT.ROW)ROW=D
GRE=(ROW/100)*TOTL*T
ROW=0.0
D=0.0
TF=RSDB*0.125
EF=TF/T
D=100*(1-(EXP(-0.33176*(EF-0.35))))
ROW=150
IF(A.LT.ROW)ROW=A
IF(B.LT.ROW)ROW=B
IF(C.LT.ROW)ROW=C
IF(D.LT.ROW)ROW=D
GRF=(ROW/100)*TOTL*T
ROW=0.0
D=0.0
TG=RSDB*0.137
EG=TG/T
D=100*(1-(EXP(-0.33176*(EG-0.35))))
```

```
      ROW=150
      IF(A.LT.ROW)ROW=A
      IF(B.LT.ROW)ROW=B
      IF(C.LT.ROW)ROW=C
      IF(D.LT.ROW)ROW=D
      GRG=(ROW/100)*TOTL*T
      ROW=0.0
      D=0.0
      TH=RSDB*0.149
      EH=TH/T
      D=100*(1-(EXP(-0.33176*(EH-0.35))))
      ROW=150
      IF(A.LT.ROW)ROW=A
      IF(B.LT.ROW)ROW=B
      IF(C.LT.ROW)ROW=C
      IF(D.LT.ROW)ROW=D
      GRH=(ROW/100)*TOTL*T
      ROW=0.0
      D=0.0
      TJ=RSDB*0.155
      EJ=TJ/T
      D=100*(1-(EXP(-0.33176*(EJ-0.35))))
      ROW=150
      IF(A.LT.ROW)ROW=A
      IF(B.LT.ROW)ROW=B
      IF(C.LT.ROW)ROW=C
      IF(D.LT.ROW)ROW=D
      GRJ=(ROW/100)*TOTL*T
      ROW=0.0
      D=0.0
      TK=RSDB*0.162
      EK=TK/T
      D=100*(1-(EXP(-0.33176*(EK-0.35))))
      ROW=150
      IF(A.LT.ROW)ROW=A
      IF(B.LT.ROW)ROW=B
      IF(C.LT.ROW)ROW=C
      IF(D.LT.ROW)ROW=D
      GRK=(ROW/100)*TOTL*T
      ROW=0.0
      D=0.0
      TOTAL GROWTH FOR GIVEN DAY R=TWICE THE SUM OF
      INDIVIDUAL 10 INTERVALS,ASSUMING ENERGY CURVE IS
      SYMMETRICAL ABOUT SOLAR NOON
      R=(GRA+GRB+GRC+GRD+GRE+GRF+GRG+GRH+GRJ+GRK)*2
      CORRECT THE DRY WEIGHT INCREASE FOR RESPIRATORY LOSS
      DURING THE NIGHT,RESP
      EFFECT OF TEMPERATURE ON RATE OF DRY WEIGHT LOSS = Q
      Q=(TEMP*0.00046)-0.005
      Q IS PER HOUR,ASSUMING MAXIMUM RESPIRATION AT TEMP OF
      31 DEGREES OF 0.009.ASSUME THAT MAXIMUM RESPIRATION
      IS 15 PERCENT OF MAXIMUM POSSIBLE DRY WEIGHT
      INCREASE IN THE LIGHT.AT 0.15 PER DAY,NIGHT RESP. RATE
      =0.0027 PER HOUR
      OX IS ADJUSTED DRY WEIGHT LOSS DUE TO RESPIRATION
      OX=(Q/0.009)*0.0027
      RESP=OX*(24-RL)
      RESULTING GROWTH RATE GR IN UNITS PER DAY
      GR=R-RESP
      TYPE 160,IDY,AC,TEMP,DW,DB,VNO,VNH,VPO,GR
  160 FORMAT(' ',1X,I3,4X,F5.2,4X,F4.1,4X,F5.2,5X,F6.2,3X,F7.1,3X,F7.1,3
     *X,F7.1,22X,F6.3)
      GO TO 3
  161 STOP
      END
```

APPENDIX 3

SPECIMENS OF CLADOPHORA COLLECTED IN PEEL INLET, WESTERN AUSTRALIA

INTRODUCTION

A number of specimens of Cladophora have been collected from Peel Inlet during this investigation which are, in outward appearance, very different to the ball-like species forming the subject of this report.

Descriptions of the other 'species' for which permanent records exist are given below, along with details of cell dimensions to help in their identification.

Specimens have been lodged in the Herbarium, Department of Botany, The University of Western Australia (UWA) and are identified here by their reference numbers.

DESCRIPTION

Specimen No. UWA 2987

Plant consists of fine, pseudodichotomously branching filaments giving rise to a delicate, fine-textured thallus. Branches arise at the apex of the parent cells and continue the direction of the axis. New branches themselves branch to form a further pseudo-dichotomy. This gives rise to typically falcate branched systems. Lateral insertion of branches has not been observed. Older cells may have branches with basal cells partly fused with the adjacent parent cell. Filaments give off typically one (up to three) branches at one axis node. Older branches not intercalated with younger ones. Apical cells typically long and cylindrical with rounded tips. Cell walls thin, in apical cells and alternate branches, thicker in older cells towards base. Chromatophores typically few, cells with much vacuolar space. Inversion of polarity and rhizoids not observed. Fertile plants not found. Length of apical cells 78 - 353µ (mean 243), width 27 - 51µ (mean 38), length-to-width ratio 2.68 - 10.38 (mean 6.52). Length of branch cells 118 - 735µ (mean 357), width 34 - 147µ (mean 78), length-to-width ratio 1.84 - 6.47 (mean 4.63). This specimen found free-floating in large masses overlying Ruppia in shallow water at Coodanup, Peel Inlet, October 1977. Thallus pale green and delicate.

Specimen No. UWA 2988

This specimen similar to UWA 2987 in mode of branching and appearance of cells. Typically one branch (up to four) arising from parent cell, resulting in a fine-textured thallus. Length of apical cells 118 - 235µ (mean 177), width 20 - 39µ (mean 33), length-to-width ratio 3.47 - 9.79 (mean 5.52). Length of branch cells 167 - 784µ (mean 353), width 29 - 127µ (mean 60), length-to-width ratio 3.79 - 9.14 (mean 6.13). This specimen free-floating in shallow water at Coodanup, Peel Inlet, September 1978, overlying beds of Ruppia and intertwined with other free-floating Cladophorales (Enteromorpha spp, Chaetomorpha linum). Thallus pale green to yellow, delicate with a 'slippery' texture.

Specimen No. UWA 2989

Pseudodichotomously branching filaments. Branches arise at apex of parent cell but not continuing the original direction of axis. Angle of branching is wide, up to 90°, giving rise to a widely ramifying thallus with poor organization. Older cells clavate shaped. Cell walls thick. Chromatophores in cells typically dense. Apical cells long, cylindrical with rounded tips. Inversion of polarity and rhizoids not observed. Fertile material not found. Length of apical cells 108 - 255µ (mean 175), width 24 - 39µ (mean 34), length-to-width ratio 2.76 - 7.75 (mean 5.30). Length of branch cells 98 - 598µ (mean 264), width 29 -69µ (mean 51), length-to-width ratio 1.91 - 10.31 (mean 5.54).

This specimen free-floating entangled in other free-floating Cladophorales (Enteromorpha spp.) in shallow water at Robert Bay, Peel Inlet, September 1978. Thallus is dark-green and coarse. Cells of branches resemble, somewhat, those of ball-like species (UWA 2806).

Specimen No. UWA 2990

Cells similar in appearance and mode of branching to UWA 2987 with thin cell walls, few chromatophores and apically inserted branches arising singly to give straight, falcate or occasionally refract branches. The angle of ramification is wide, branches not continuing the original direction of the parent axis. Apical cells long and thin, with rounded tips. Negative polarity and rhizoids not observed. No fertile material found. Length of apical cells 118 - 441µ (mean 249), width 20 - 29µ (mean 26), length-to-width ratio 5.88 - 17.64 (mean 9.64). Length of branch cells 225 - 588µ (mean 357), width 29 - 49µ (mean 38), length-to-width ratio 6.37 - 12.64 (mean 9.45).

This specimen growing free-floating entangled with other Cladophorales (Enteromorpha spp., Chaetomorpha linum) in shallow water, Robert Bay, Peel Inlet, September 1978. Thallus pale green-yellow, delicate, 'slippery' texture.

Specimen No. UWA 2991

Filaments with long, slender cells. Cell walls thick in older cells. Branches arising mostly from apex of parent cell, not continuing the original direction, some at right angles to axis of parent cell. Insertion of branches variable, mostly apical but some sub-apical and less frequently by lateral insertion. Cell contents dense. Apical cells long, cylindrical sometimes with a club-shaped apex, rounded at the tip. Inversion of polarity and rhizoids not observed. No fertile material found. Variable width of branches arising from parent filaments. Length of apical cells 127 - 250µ (mean 185), width 20 - 39µ (mean 27), length-to-width ratio 4.02 - 12.25 (mean 6.95). Length of branch cells 127 - 372µ (mean 234), width 19 - 73µ (mean 35), length-to-width ratio 2.68 - 12.17 (mean 7.16).

This specimen collected at Coodanup, Peel Inlet, September 1979 growing free-floating, extensively overlying beds of Ruppia. Thallus pale green-yellow, delicate with 'slippery' texture.

Specimen No. UWA 2992

Attached species. Cells large and stout in appearance with few chromatophores. Branching pseudodichotomous by apical insertion of one (up to three) branches giving rise to straight or falcate branches, usually continuing the direction of axis of the parent cell. Apical cells large and tapering, rounded at the tip. Inversion of polarity and rhizoids not observed. Fertile material not found. Specimen distinctive due to large cell sizes. Length of apical cells 108 - 363μ (mean 241), width 39 - 78μ (mean 56), length-to-width ratio 2.29 - 7.20 (mean 4.37). Length of branch cells 216 - 706μ (mean 477), width 61 - 255μ (mean 147), length-to-width ratio 1.87 - 5.08 (mean 3.40).

This specimen found growing attached to rocks in shallow water, Soldier's Cove, Peel Inlet, November 1978. Subject to tidal desiccation. Thallus dark green and coarse textured.

APPENDIX 4

AQUATIC VEGETATION MAP OF PEEL INLET, WESTERN AUSTRALIA. INTERPRETATION FROM AERIAL PHOTOGRAPHY AND GROUND TRUTHING, NOVEMBER 1979.

This map is issued separately to this report and is available through the Botany Department, University of Western Australia.