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PERIPHYTON AND WATER QUALITY

IN THE MANAWATU RIVER, NEW ZEALAND

A thesis presented in partial fulfilment of the
requirements for the degree of Doctor of Philosophy in
Biotechnology at Massey University

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ABSTRACT

The factors responsible for the establishment and summer proliferation of attached filamentous algae in the Manawatu River were investigated. The life cycle of the dominant alga *Cladophora* was observed to be closely linked with the seasonal river and climatic changes. The magnitude and frequency of flush events were the major factors responsible for reducing the attached algal biomass. During steady low flow conditions, the results of phosphorus nutrient availability tests demonstrated that phosphorus availability frequently limited the growth rate of the *Cladophora* proliferations. The concentration of dissolved reactive phosphorus during these periods was 3-4 mg P m⁻³. Dissolved inorganic nitrogen concentrations during steady low flow conditions were low, compared to overseas rivers that experienced similar filamentous algal proliferations, and the results of nitrogen nutrient availability tests never indicated nitrogen limitation of the *Cladophora* growth rate.

The water quality effects of these proliferations were also investigated. The two effects monitored were; diurnal fluctuations of Dissolved Oxygen (DO) and pH. These could become quite severe and consequently affect the river's ability to adequately assimilate effluent discharges from Palmerston North and its associated food industries. Of the two algal-induced fluctuations, DO was the more important. Frequently, maximum daily DO deficits (DOD_m) of 3.0 g m⁻³ were observed and these severely limited the river's ability to satisfy the oxygen demands of all discharges while maintaining the minimum desirable DO concentration.

A regression equation was developed using the data from both the 1981/82 and 1982/83 seasons to predict the daily DOD_m . The largest contribution to the total predicted DOD_m was from the total river community respiration followed by a seasonal effect, the river flow, the regression constant and the terrestrial insolation. The regression equation accounted for 72% of the observed variation in the daily DOD_m during the two seasons.

Fluctuations in the pH of the Manawatu River were also important, as a component of the effluent discharges is ammonia, the toxicity of which increases exponentially with a linear rise in pH. However, algal-induced pH fluctuations were reduced downstream of the discharges by bacterial respiration associated with the oxygen-demanding effluents. This phenomenon and the timing of both pH and ammonia fluctuations meant that toxic concentrations were not observed, although the temporal variation of ammonia was often erratic. However, future discharge changes may alter this situation, and continued surveillance of downstream pH and ammonia is warranted.

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TABLE OF CONTENTS

	<u>PAGE</u>
<u>ABSTRACT</u>	i
<u>ACKNOWLEDGEMENTS</u>	iii
<u>TABLE OF CONTENTS</u>	iv
<u>LIST OF FIGURES</u>	viii
<u>LIST OF TABLES</u>	xiii
<u>CHAPTER 1 INTRODUCTION</u>	1
<u>CHAPTER 2 LITERATURE REVIEW</u>	6
2.1 Introduction	6
2.2 Eutrophication and attached filamentous algae in rivers	7
2.3 Water quality effects of algal metabolism	8
2.3.1 Dissolved Oxygen	8
2.3.2 pH	12
2.3.2.1 pH and ammonia	16
2.4 River primary productivity	18
2.5 <i>Cladophora</i>	23
2.5.1 Introduction	23
2.5.2 Identification	23
2.5.3 Environmental parameters affecting the growth of <i>Cladophora</i>	23
2.5.3.1 Temperature	24
2.5.3.2 Light	25
2.5.3.3 Water movements	26
2.5.3.4 Nutrients	28
2.5.3.5 Substrates	32
2.5.3.6 Summary	32
2.5.4 Life cycle	32
2.5.5 Biomass and its measurement	33
2.6 Nutrient Availability Tests	36
2.6.1 Introduction	36
2.6.2 Phosphorus	37
2.6.2.1 Total Tissue Phosphorus	37
2.6.2.2 Extractive Phosphorus	40
2.6.2.3 Phosphorus Uptake Rate	41
2.6.2.4 Alkaline Phosphatase Activity	46
2.6.3 Nitrogen	47
2.6.3.1 Total Tissue Nitrogen	47
2.6.3.2 Ammonium Absorption Rate	47
2.7 Chemical estimations of biologically available nutrients	48
2.7.1 Phosphorus	48
2.7.2 Nitrogen	49
2.8 General conclusions drawn from the literature review	49
<u>CHAPTER 3 METHODS</u>	51
3.1 Algal identification	51
3.2 Algal biomass and distribution	51

	<u>PAGE</u>
3.2.1 Artificial substrates	52
3.2.2 Natural substrates	52
3.2.3 Measurement techniques	52
3.3 River site characteristics	54
3.3.1 Physical characteristics	54
3.3.2 Intersite substrate comparison	56
3.4 Light measurements	57
3.5 River nutrients	57
3.5.1 Phosphorus	58
3.5.2 Nitrogen	59
3.5.3 Quality assurance of chemical methods	60
3.6 Nutrient Availability Tests	61
3.6.1 Phosphorus	61
3.6.2 Nitrogen	62
3.7 Laboratory culturing	63
3.7.1 Introduction	63
3.7.2 Isolation techniques	63
3.7.3 Experimental methods	67
3.8 Dissolved Oxygen dynamics	68
3.8.1 Dissolved Oxygen and temperature	68
3.8.2 Primary productivity	70
3.9 pH and alkalinity	73
3.10 Data analysis	73
<u>CHAPTER 4 LABORATORY CULTURE EXPERIMENTS</u>	74
4.1 Introduction	74
4.2 Results and discussion	75
4.2.1 Transition from a surplus to a limiting nutrient situation	75
4.2.1.1 Phosphorus	75
4.2.1.2 Nitrogen	79
4.2.2 Transition from a limiting to a surplus nutrient situation	81
4.2.2.1 Phosphorus	81
4.2.2.2 Nitrogen	86
4.3 The application of Nutrient Availability Test results achieved in the laboratory, to their use in field studies	89
4.4 Conclusions and recommendations	92
<u>CHAPTER 5 RECONNAISSANCE SURVEY RESULTS 1980/81</u>	94
5.1 Introduction	94
5.2 <i>Cladophora</i> biomass density and distribution	94
5.3 River nutrients	98
5.4 Nutrient Availability Tests	101
5.5 Primary productivity and Dissolved Oxygen fluctuations	104
5.6 Ammonia and pH	105
<u>CHAPTER 6 RESULTS FROM THE 1981/82 AND 1982/83 SEASONS</u>	107
6.1 Introduction	107
6.2 Physical site characteristics	107

	<u>PAGE</u>
6.3 Light and temperature conditions	110
6.4 <i>Cladophora</i> biomass density and periphyton distribution	112
6.5 Nutrients	118
6.5.1 Phosphorus	118
6.5.2 Nitrogen	124
6.6 Nutrient Availability Tests	127
6.6.1 Phosphorus	127
6.6.2 Nitrogen	133
6.7 Primary productivity and Dissolved Oxygen fluctuations	141
6.7.1 Introduction	141
6.7.2 Primary productivity	141
6.7.2.1 Primary productivity during the 1981/82 season	141
6.7.2.2 Primary productivity during the 1982/83 season	154
6.7.2.3 Spatial variation and apportioning primary productivity	162
6.7.3 Dissolved Oxygen Deficits	167
6.7.3.1 The impact of discharges on the Dissolved Oxygen profile	167
6.7.3.2 Upstream Dissolved Oxygen profiles and the maximum daily Dissolved Oxygen Deficits, 1981/82	172
6.7.3.3 Upstream Dissolved Oxygen profiles and the maximum daily Dissolved Oxygen Deficits, 1982/83.	182
6.8 pH and ammonia	195
6.9 Summary of results from the 1981/82 and 1982/83 seasons	205
6.9.1 Light and temperature conditions	205
6.9.2 <i>Cladophora</i> biomass density and distribution	205
6.9.3 River nutrients	206
6.9.4 Nutrient Availability Tests	206
6.9.5 Primary productivity and Dissolved Oxygen fluctuations	207
6.9.6 Maximum daily Dissolved Oxygen Deficits	207
6.9.7 pH and ammonia	208
 <u>CHAPTER 7 THE PRACTICALITIES OF LOTIC <i>CLADOPHORA</i> CONTROL STRATEGIES</u>	 209
7.1 Chemical control	209
7.2 Physical removal	209
7.3 Nutrient reduction	209
7.4 Summary	210
 <u>CHAPTER 8 AREAS THAT WARRANT FURTHER STUDY</u>	 211
 <u>CHAPTER 9 CONCLUSIONS</u>	 213
 <u>ABBREVIATIONS</u>	 217
 <u>REFERENCES</u>	 219

APPENDICES

	<u>PAGE</u>
1. Photographs	240
2. Computer programme for primary productivity analysis	243
3. Computer programme sample output	247
4. Monitoring the effects of attached filamentous algae on Dissolved Oxygen	254
5. Calculations showing the effect of compensating Net Areal Primary Productivity for the variation of k_2 with temperature	263
6. Intersite Phosphorus Nutrient Availability Test comparisons, testing the hypothesis of increased downstream phosphorus limitation occurring during 1982/83.	265
7. <i>Cladophora</i> biomass density, environmental parameters and primary productivity data, 1981/82.	266
8. <i>Cladophora</i> biomass density, environmental parameters and primary productivity data, 1982/83.	270

LIST OF FIGURES

<u>FIGURE</u>	<u>TITLE</u>	<u>PAGE</u>
1.1	Map of New Zealand	2
1.2	The Manawatu River showing sampling sites.	3
1.3	A portion of the flow duration curve for the Manawatu River.	4
2.1	A representation of major sources and sinks of Dissolved Oxygen in the Manawatu River.	9
2.2	Effect of differences in plant density and sunlight on river Dissolved Oxygen profiles at 15 ⁰ C.	10
2.3	The relationship between pH and the proportion of inorganic carbon species in solution.	13
2.4	Concentrations of total ammonia that contain 0.08 g m ⁻³ un-ionized ammonia-N .	17
2.5	The life cycle of <i>Cladophora</i> in the Manawatu River.	34
2.6	The relationship between Total Tissue Phosphorus (TTP) and the specific growth rate.	38
2.7	The relationship between Extractive Phosphorus (EP) and the specific growth rate (μ).	42
2.8	The factors controlling the phosphorus uptake kinetics.	41
2.9	The relationship between the Phosphorus Uptake Rate (PUR) and the Total Tissue Phosphorus (TTP).	44
2.10	The relationship between Phosphorus Uptake Rate (PUR) and the external Phosphorus (P).	44
2.11	The relationship between Extractive Phosphorus (EP) and the Phosphorus Uptake Rate (PUR).	45
3.1	Percentage cover scale for attached filamentous algal distribution.	55
3.2	Diagram of wedge-deflector and probe arrangement.	69
3.3	Schematic diagram of algal chamber.	72
4.1	Response of <i>Cladophora</i> Phosphorus Nutrient Availability Tests to gradual phosphorus depletion.	76

<u>FIGURE</u>	<u>TITLE</u>	<u>PAGE</u>
4.2	Response of <i>Cladophora</i> Phosphorus Nutrient Availability Tests to sudden phosphorus depletion.	78
4.3	Response of <i>Cladophora</i> Nitrogen Nutrient Availability Tests to sudden phosphorus depletion.	80
4.4	Response of <i>Cladophora</i> Phosphorus Nutrient Availability Tests to sudden phosphorus depletion. (Phosphorus Uptake Rate and Extractive Phosphorus).	82
4.5	Response of <i>Cladophora</i> Nitrogen Nutrient Availability Tests to changes in nitrogen availability.	83
4.6	Response of <i>Cladophora</i> Phosphorus Nutrient Availability Tests to changes in nitrogen availability.	84
4.7	Response of <i>Cladophora</i> Phosphorus Nutrient Availability Tests to cessation of phosphorus limitation. (Total Tissue Phosphorus and Extractive Phosphorus).	85
4.8	Response of <i>Cladophora</i> Phosphorus Nutrient Availability Tests to cessation of phosphorus limitation. (Phosphorus Uptake Rate and Alkaline Phosphatase Activity).	87
4.9	Response of <i>Cladophora</i> Nitrogen Nutrient Availability Tests to cessation of phosphorus limitation.	88
5.1	<i>Cladophora</i> biomass fluctuations at Site M, 1980/81.	95
5.2	The Manawatu River flow, 1980/81.	95
5.3	Total Nitrogen and Total Phosphorus fluctuations at site M, 1980/81.	100
6.1	A longitudinal profile of the Manawatu River bed height above sea level.	108
6.2	A comparison of the areas taken up by different stone sizes, at sites T, D and M.	109
6.3	Maximum river temperatures and surface light intensity, 1981/82.	111
6.4	Maximum river temperature and surface light intensity 1982/83.	111
6.5	<i>Cladophora</i> biomass fluctuations at sites T, D and M, 1981/82.	113

<u>FIGURE</u>	<u>TITLE</u>	<u>PAGE</u>
6.6	The Manawatu River flow 1981/82.	113
6.7	<i>Cladophora</i> biomass fluctuations at Sites T, D and M 1982/83.	114
6.8	The Manawatu River flow 1982/83.	114
6.9	An idealized representation of the development of a <i>Cladophora</i> assemblage.	116
6.10	The average (three sites) Total river Phosphorus and river flow, 1981/82.	120
6.11	A diurnal study of Total Nitrogen, Total Phosphorus, Total Tissue Phosphorus and Extractive Phosphorus at Site M, 23 February 1982.	122
6.12	Dissolved Inorganic Nitrogen (DIN), Dissolved Reactive Phosphorus (DRP) and the DIN/DRP fluctuations, 1982/83.	123
6.13	Dissolved Reactive Phosphorus fluctuations at sites T, D and M during a period of <i>Cladophora</i> proliferation 25 January - 7 February 1983.	125
6.14	The average (three sites) Total river Nitrogen fluctuations, 1981/82.	126
6.15	The average (three sites) Total river Phosphorus (TP) fluctuations, 1981/82.	128
6.16	<i>Cladophora</i> Total Tissue Phosphorus (TTP) fluctuations at Site M, 1981/82.	128
6.17	<i>Cladophora</i> Alkaline Phosphatase Activity (APA) at Site M, 1981/82.	129
6.18	<i>Cladophora</i> Extractive Phosphorus (EP) at Site M, 1981/82.	129
6.19	<i>Cladophora</i> Phosphorus Nutrient Availability Tests at Site T, 1981/82.	130
6.20	<i>Cladophora</i> Phosphorus Nutrient Availability Tests at Site D, 1981/82.	131
6.21	<i>Cladophora</i> Phosphorus Nutrient Availability Tests at Site M, 1982/83. (Total Tissue Phosphorus and Extractive Phosphorus).	134
6.22	<i>Cladophora</i> Phosphorus Nutrient Availability Tests at Site M, 1982/83. (Alkaline Phosphatase Activity and Phosphorus Uptake Rate).	134

<u>FIGURE</u>	<u>TITLE</u>	<u>PAGE</u>
6.23	Total Tissue Phosphorus in <i>Cladophora</i> at Sites T, D and M, 1982/83.	135
6.24	Extractive Phosphorus in <i>Cladophora</i> at Sites T, D and M, 1982/83.	136
6.25	Phosphorus Uptake Rate in <i>Cladophora</i> at Sites T, D and M, 1982/83.	137
6.26	Alkaline Phosphatase Activity in <i>Cladophora</i> at Site T, D and M, 1982/83.	138
6.27	<i>Cladophora</i> Nitrogen Nutrient Availability Tests at Site M, 1982/83.	139
6.28	Gross Photosynthesis (GP) and Total Respiration (TR) during 1981/82.	142
6.29	The relationship between Gross Photosynthesis and Total Respiration during 1981/82.	144
6.30	The variation in the P/R ratio during 1981/82.	145
6.31	Net Areal Primary Productivity (NAP) during 1981/82.	147
6.32	Daily Net Primary Productivity profiles for two-day periods, 1981/82.	148
6.33	Community description (Hornberger plot), 1981/82.	151
6.34	The daily Dissolved Oxygen fluctuations (ΔDO), 1981/82.	152
6.35	The relationship between Gross Photosynthesis and the Dissolved Oxygen fluctuation (ΔDO) 1981/82.	153
6.36	Gross Photosynthesis and Total Respiration, 1982/83.	155
6.37	The relationship between Gross Photosynthesis and Total Respiration, 1982/83.	156
6.38	The variation in the P/R ratio during 1982/83.	158
6.39	Net Areal Primary Productivity (NAP) during 1982/83.	160
6.40	Community description (Hornberger plot) 1982/83.	161
6.41	The daily Dissolved Oxygen fluctuations (ΔDO), 1982/83.	163
6.42	The relationship between Gross Photosynthesis and the Dissolved Oxygen fluctuation (ΔDO), 1982/83.	164

<u>FIGURE</u>	<u>TITLE</u>	<u>PAGE</u>
6.43	Comparison of Dissolved Oxygen profiles at sites T and D, 12-13 February 1982.	166
6.44	The Dissolved Oxygen (DO) fluctuations at sites T and K during 30 January 1982 - 4 February 1982.	168 & 169
6.45	Some Dissolved Oxygen profiles at sites T and C, 1982.	170
6.46	Some Dissolved Oxygen profiles at site T, 1981/82.	173
6.47	Average site biomass of <i>Cladophora</i> and observed maximum daily Dissolved Oxygen Deficit 1981/82.	178
6.48	Average site biomass of <i>Cladophora</i> and observed maximum daily Dissolved Oxygen Deficit 1982/83.	185
6.49	The relationship between predictors and the maximum daily Dissolved Oxygen Deficit (DOD_m), and the contribution each predictor makes to the total DOD_m , 1981/82.	190
6.50	The relationship between predictors and the maximum daily Dissolved Oxygen Deficit (DOD_m) and the contribution each predictor makes to the Total DOD_m , 1982/83.	191
6.51	pH fluctuations at various sites during 1981/82.	196
6.52	pH fluctuations at downstream sites during 1981/82.	196
6.53	Total ammonia fluctuations at sites T, D and K, 5-6 February 1982.	197
6.54	Total ammonia fluctuations at site K, 12-13 February 1982.	197
6.55	Daily pH fluctuations during 1982/83.	200
6.56	pH fluctuations at sites M and K during 2 February 1983 - 6 February 1983.	201
6.57	pH and ammonia fluctuations at sites M and K 14-15 January 1983.	202
6.58	pH and ammonia fluctuations at site K 27-28 January 1983.	202
6.59	pH and ammonia fluctuations at site K, 1-2 February 1983.	203

LIST OF TABLES

<u>TABLE</u>	<u>TITLE</u>	<u>PAGE</u>
1.1	Locations of the major dischargers to, and study sites of, the Manawatu River.	1
2.1	A summary of pH fluctuations reported from a variety of rivers.	15
2.2	A comparison of lotic primary productivity data.	22
2,3	Estimates of critical water phosphorus concentrations in various aquatic situations.	30
2.4	Examples of <i>Cladophora</i> biomass density in lakes and rivers.	36
2.5	Values of Phosphorus Nutrient Availability Tests for <i>Cladophora</i> associated with a limiting or surplus situation.	39
2.6	Values of Nitrogen Nutrient Availability Tests for <i>Cladophora</i> associated with a limiting or surplus situation.	48
3.1	Quality assurance of analytical methods, 1981/82.	60
3.2	Accuracy and precision of nitrogen and phosphorus digestion methods.	61
3.3	A comparison of the media used to culture <i>Cladophora</i> .	64
4.1	Values observed for laboratory Nutrient Availability Tests associated with a limiting or surplus situation.	89
4.2	A summary of laboratory Nutrient Availability Test response times.	90
5.1	A distribution survey of periphyton in the tributaries of the Manawatu River, 9 May 1981.	96
5.2	<i>Cladophora</i> biomass density variation on one sampling occasion, at site M, during a proliferation	97
5.3	A summary of nutrient data collected weekly during December and January 1980/81.	99
5.4	<i>Cladophora</i> Nutrient Availability Test results from site T during the 1980/81 season.	101
5.5	Periphyton survey, Phosphorus Nutrient Availability Tests and Total river Phosphorus from the Manawatu River and some of its tributaries, 9 May 1981.	103

<u>TABLE</u>	<u>TITLE</u>	<u>PAGE</u>
5.6	Primary Productivity data at site T during early 1981.	104
5.7	Ammonia concentration at various sites in early 1981.	106
5.8	Ammonia concentrations at site T 22-23 March 1981.	106
5.9	pH fluctuations at site M, 11 April 1981.	106
6.1	The range of average site velocities recorded during low flow periods.	107
6.2	Comparison of primary productivity at sites T and D 12-13 February 1982.	165
6.3	Correlation coefficient matrix of the maximum daily Dissolved Oxygen Deficits and some river and environmental variables, 1981/82.	173
6.4	The parsimonious regression equation and associated statistics for predicting the maximum daily Dissolved Oxygen Deficits, 1981/82.	179
6.5	Correlation coefficient matrix of the maximum daily Dissolved Oxygen Deficits and some river and environmental variables, 1982/83.	183
6.6	The parsimonious regression equation and associated statistics for predicting the maximum daily Dissolved Oxygen Deficits, 1982/83.	184
6.7	Correlation coefficient matrix of the maximum daily Dissolved Oxygen Deficits and associated variables in 1981/82 and 1982/83.	186
6.8	The parsimonious regression equation and associated statistics for predicting the maximum daily Dissolved Oxygen Deficits, 1981/82 and 1982/83.	187
6.9	A t-test comparison of the residuals from the regression equation for each season.	188
6.10	Data illustrating the influence of each term in the regression equation for 1981/82 and 1982/83	193
6.11	Ammonia concentrations and other relevant variables at site K during March 1982.	198
6.12	A comparison of some observed ammonia concentrations with the recommended values at ambient pH and 20°C.	204

CHAPTER 1

INTRODUCTION

1. INTRODUCTION

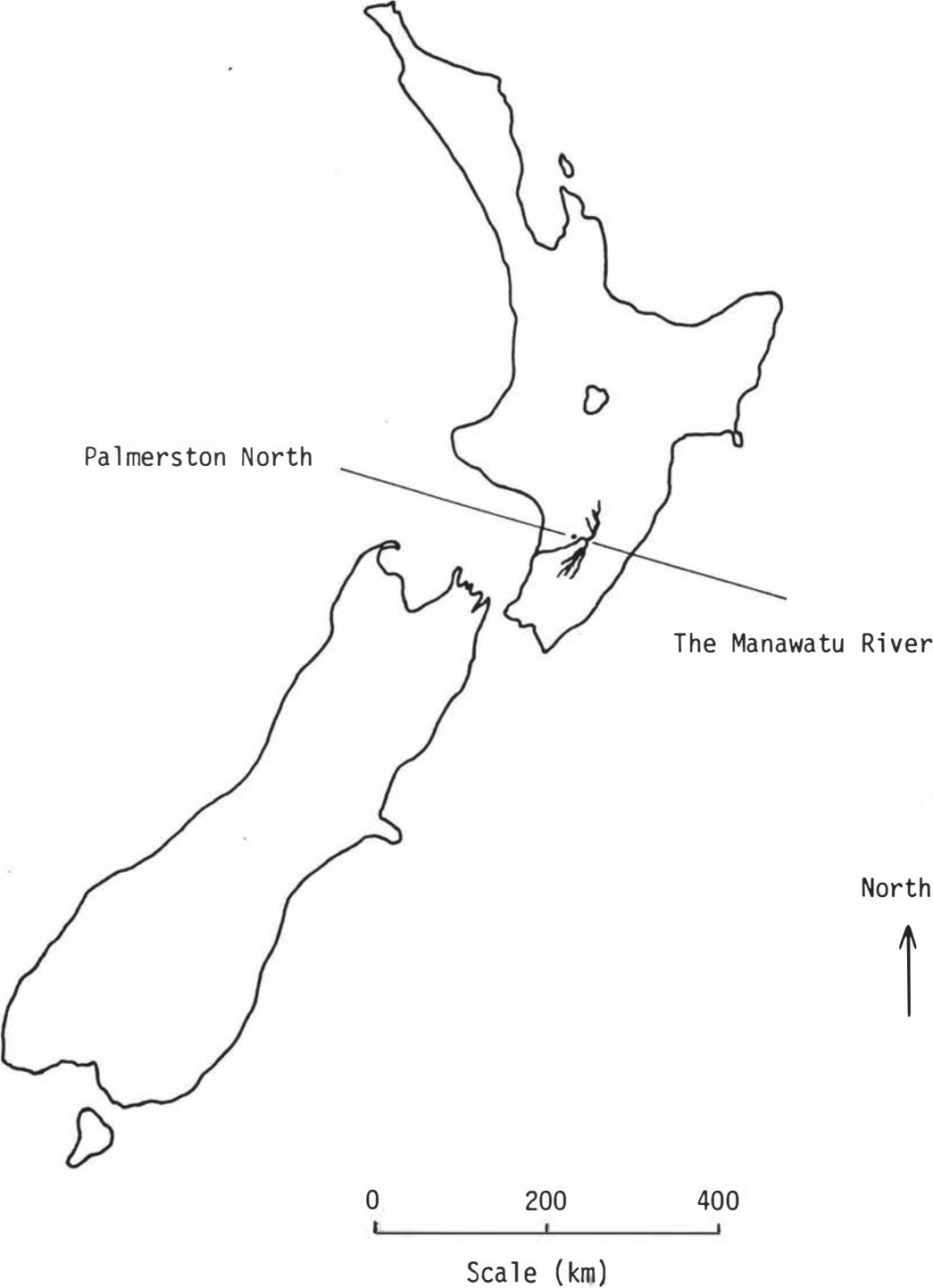
The Manawatu River is situated in the lower half of the North Island of New Zealand. (Figure 1.1) The river is approximately 226 km long and has a catchment area of 4950 km² encompassing parts of both the Tararua and Ruahine ranges. The predominant surrounding land uses are pastoral and agricultural farming.

The mean flow of the river is 54.6 m³ s⁻¹ (period of record 16 December 1971 to 4 May 1976). A portion of the flow duration curve measured at site D (figure 1.2) for the same period is shown in figure 1.3. The 4% low flow (i.e. that flow that is equalled or exceeded 96% of the time) of 12.8 m³ s⁻¹ is used by the Manawatu Regional Water Board (MRWB) as the basis of effluent standards for the major river dischargers (Currie, 1977). Recent flow data (up to 1982) indicate little variation from the above characteristics. The location of the three major dischargers, the Palmerston North City Corporation (PNCC), the Manawatu Co-op Dairy Co. and the Longburn Freezing Co. are given, together with other study sites, in Table 1.1 and are illustrated in figure 1.2.

TABLE 1.1: Locations of the major dischargers to, and study sites of, the Manawatu River

Site/Discharge	Abbreviation	N.Z. Map Coordinate
Te Matai Road	(T)	N149 177372
Depot	(D)	N149 133336
Maxwells Line	(M)	N149 083317
Palmerston North City Corporation		N149 077311
Shirriffs Road	(S)	N149 060303
Walkers Road	(W)	N149 049288
Manawatu Coop Dairy Company		N149 055302
Longburn Freezing Company		N149 048293
Karere Road	(K)	N149 038284
Opiki Bridge	(O)	N148 972257

Figure 1.1 Map of New Zealand



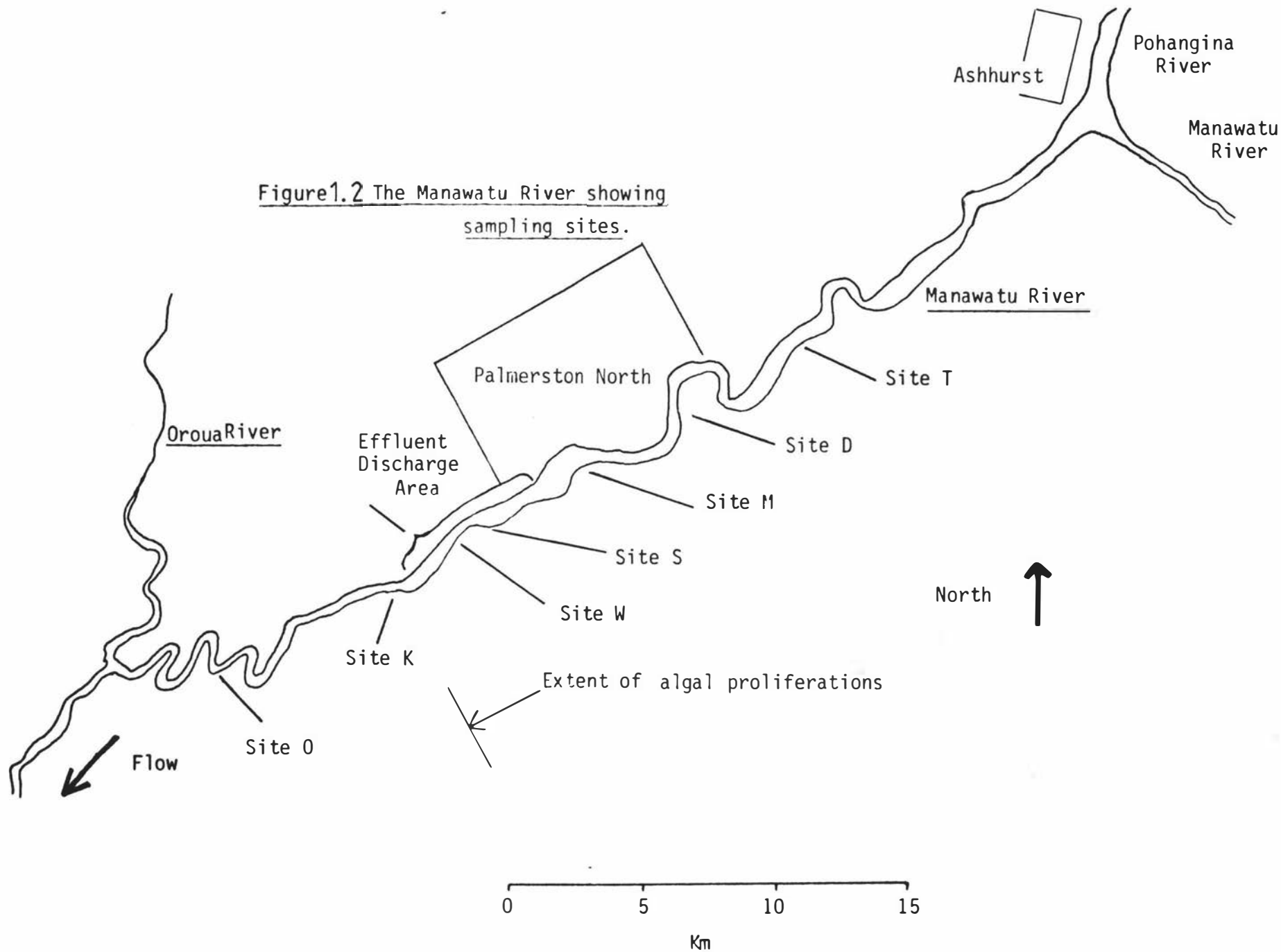
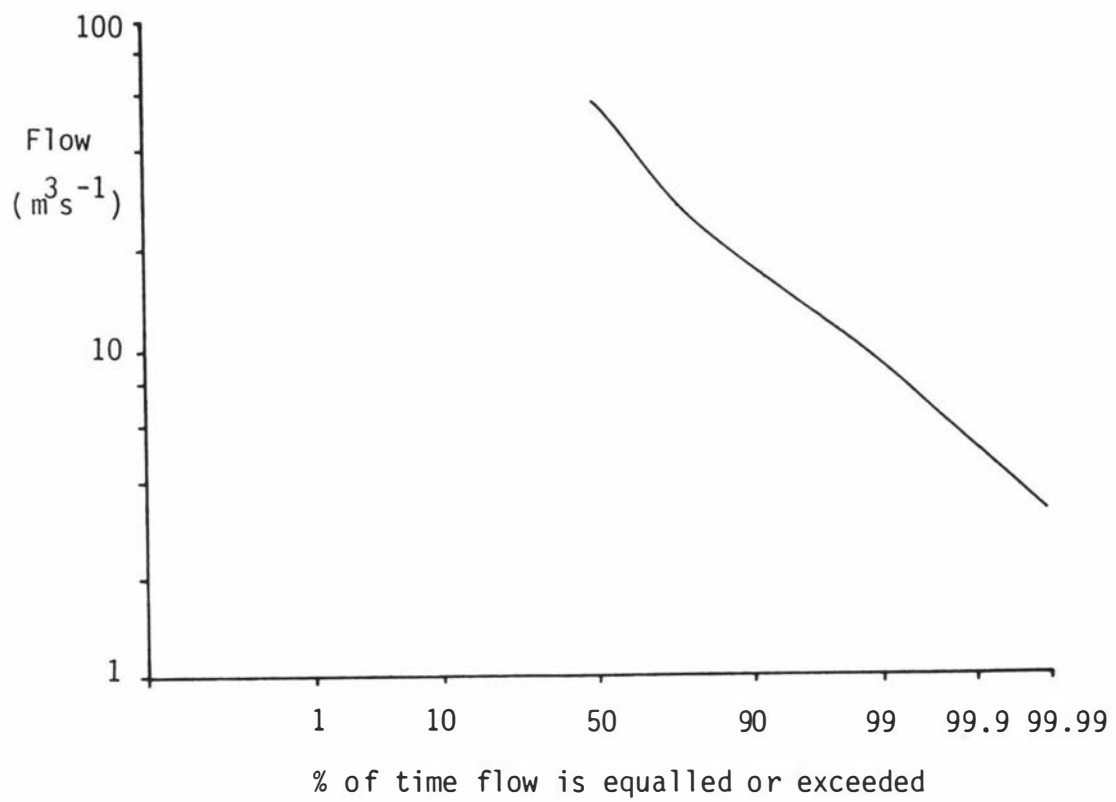


Figure 1.2 The Manawatu River showing sampling sites.

Figure 1.3 A portion of the flow duration curve for the Manawatu River .(At site D , 16/12/71 - 4/5/76)(From Currie,1977)



Extensive proliferations of attached filamentous algae have been observed in the Manawatu River, during summer low flow periods. (Appendix 1, photographs 1-4). These algal proliferations have two major effects:

- (a) The physical presence of thick mats of attached filamentous algae in the river, and often decaying on the river edges, disrupts both the aesthetic appeal and recreational uses of the river.
- (b) Water quality effects of the algal metabolism can produce dramatic fluctuations in dissolved oxygen (DO) and pH. These oscillations can have important consequences further downstream, when the low DO concentrations and high pH levels make it difficult for the river to assimilate discharges containing oxygen-demanding organics and ammoniacal wastes (the toxicity of which increases with higher pH), while maintaining a water quality that is not hazardous to aquatic life.

The purposes of this study were:-

- (a) To investigate the factors responsible for the establishment and development of attached algal proliferations in the Manawatu River.
- (b) To quantify the magnitude of the algal-induced DO and pH fluctuations and identify the factors responsible for the 'worst case' situations.
- (c) To use the information obtained (from (a) and (b)) as a guide to establish whether or not some algal control measures are warranted.

CHAPTER 2

LITERATURE REVIEW

2. LITERATURE REVIEW

2.1 Introduction

The historical documentation of attached filamentous algae in the Manawatu River began in 1956/57, when during the summer, abundant green algae were observed upstream and to 10km downstream of Palmerston North (M.O.W., 1957). The contribution of these growths to the DO profiles of the river was briefly discussed by Johannesson (1958) who noted a marked diurnal fluctuation. There is a lack of published data concerning the algae in the Manawatu River until 1977, when concern about "... vigorous filamentous algal growth ..." was noted (Currie, 1977). This concern centred on two major aspects:-

- (a) The maintenance of water quality for the protection of aquatic life.
- (b) The condition and appearance of the river as it relates to the various river users.

The metabolic activities of the algae, the dominant genus identified as *Cladophora*, are responsible for the major part of the observed DO and pH fluctuations (Section 2.3) (Currie, 1977). Upstream of Palmerston North, DO concentrations have been seen to vary from 6.9 g m^{-3} to 12.0 g m^{-3} and pH has been seen to rise from 7.6 to 9.1 (Currie, 1977). These upstream fluctuations are especially important when they are superimposed on the effects of effluent discharges in the vicinity of Palmerston North. The low DO concentration that can be found during the early morning can severely limit the river's assimilative capacity and its ability to maintain the minimum legislative requirements for DO. In the reach below the discharges the river is classified D under the Water and Soil Conservation Act, 1967. This classification states that the DO should not fall below 5.0 g m^{-3} . (Currie, 1977).

The pH of a water body receiving ammoniacal/proteinaceous effluent is an important water quality consideration, as the toxicity of ammonia is dependent on the pH. The proportion of the more toxic unionized form increasing with elevated pH (Section 2.3.2.1). Downstream concentrations of total ammonia have reached 2.3 g N m^{-3} at pH 8.0 (Currie, 1977). The resulting concentration of unionized ammonia, at 20°C would be approximately 0.15 g N m^{-3} , which can be contrasted with the United States Environmental Protection Agency (EPA) criterion for un-ionized ammonia of $0.02 \text{ g NH}_3 \text{ m}^{-3}$

(0.0165 g NH₃-N m⁻³). (EPA, 1972)

Extensive proliferations of attached filamentous algae can choke the shallow river margins and often extend as thick mats across the whole width of shallow reaches. Filaments often grow to two metres in length and can effect the river users in two ways:-

- (a) Foul-smelling and unsightly beds of rotting algae are deposited on the river margins as the water flows during seasonal dry periods.
- (b) Recreational activities such as canoeing, swimming, jet-boating and fishing are disrupted by algal filaments becoming entangled with any submerged objects.

2.2 Eutrophication and attached filamentous algae in rivers

Eutrophication is the natural or artificial addition of nutrients to water bodies and the effects caused by such additions. Thus, Warren (1971) makes the distinction between:-

- (a) Eutrophication which, in its strictest sense, is the increase in nutrient supply to a water body.
- (b) The effects of eutrophication, which may be expressed in various ways. When these effects are undesirable they may be considered a form of pollution.

Increased stream fertility and the subsequent accelerated growth of aquatic plants (periphyton, phytoplankton, 'higher' macrophytes, excluding fungi) can be a natural progression. However, the blooms of aquatic plants observed in North Western Europe and North America in the 1950's and 1960's were directly linked to the increased use of phosphate builders in detergents and to changing agricultural practises. Hynes (1960) contends " ... therefore, that rivers and streams are now enriched almost everywhere and that they are better places for plant growth than they were a century ago". He also documents some filamentous opportunistic algal species, many of which (*Cladophora*, *Stigeoclonium*, *Ulothrix*) have been observed in the Manawatu River. The problem of attached filamentous algal proliferations, notably *Cladophora*, has also been noted in the adjacent Rangitikei River and Wanganui River catchments (Fowles, 1982).

The factors responsible for both quantitative and qualitative changes in lotic flora vary and are often somewhat mercurial. Various factors have been identified as being involved in the formation of attached filamentous algal proliferations. The following feature prominently in the literature pertaining to *Cladophora*. (Chudyba, 1965; Mantai, 1978; Neil & Owen, 1964; Shear & Konasewich, 1975; Whitton, 1970).

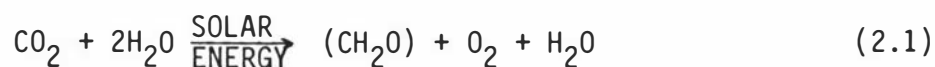
- (a) Temperature
- (b) Light
- (c) Water Movements
- (d) Substrates
- (e) Nutrients

Both the spatial distribution and temporal changes have been linked with one or more of the above. The published data on the influence(s) of these variables on the growth of *Cladophora* will be considered in detail in Section 2.5.3.

2.3 Water quality effects of algal metabolism

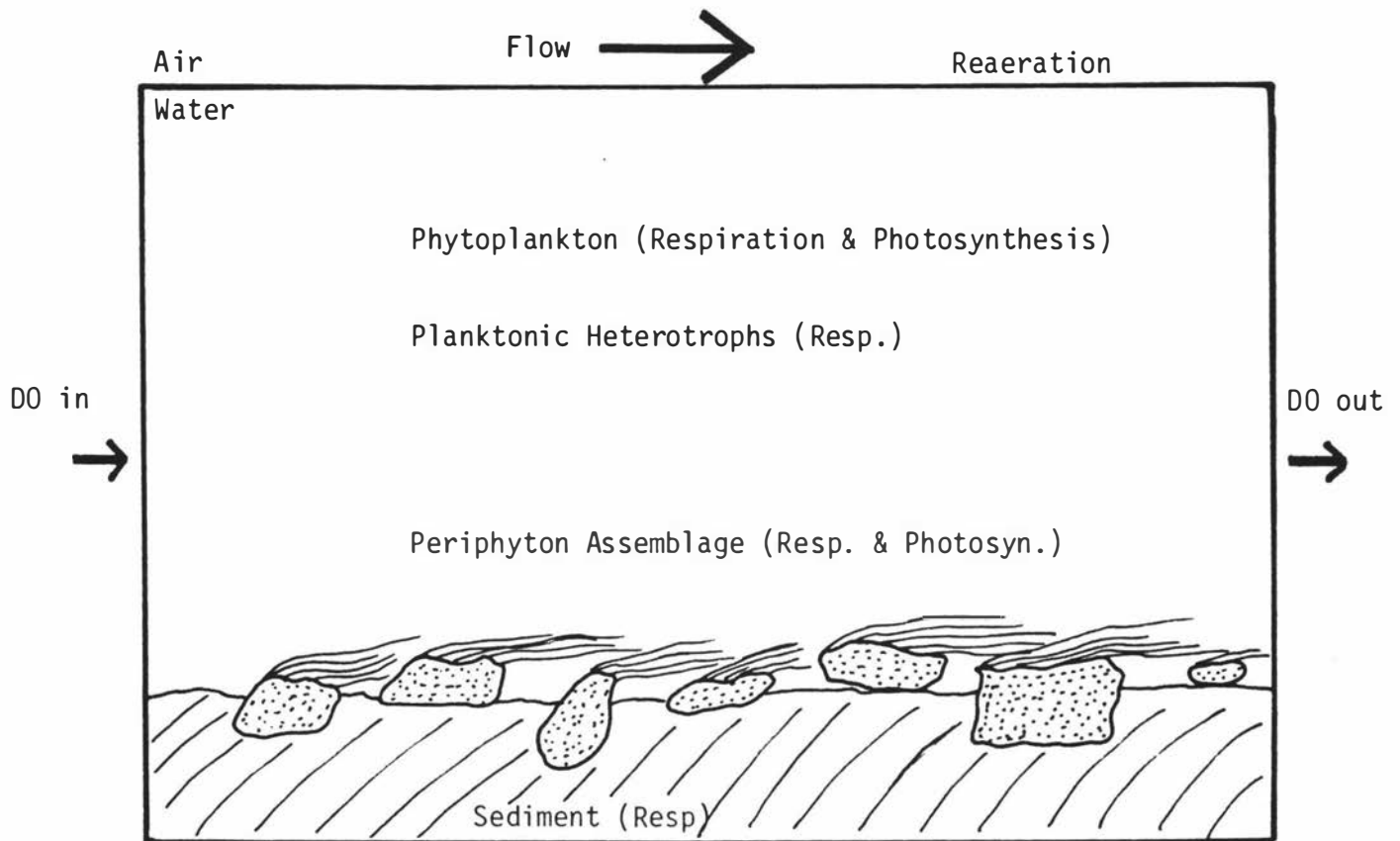
2.3.1 Dissolved Oxygen

The DO and pH affects described in section 2.1 are caused by the photosynthetic and respiratory activities of the algae. In the most basic terms, the former process may be represented by equation (2.1)



Simplistically, respiration may be considered as the reverse of this equation. As each process involves oxygen, the DO of the river can rise or fall, depending on the relative magnitude of the photosynthetic and respiratory processes. A representation of the major sources and sinks of DO involved in a periphyton-dominated river is illustrated in figure 2.1. The net effect will depend on various factors, including the algal community biomass and composition, river flow, season, time of day, temperature and nutrients. Thus, in rivers experiencing aquatic plant growths a variety of DO profiles can occur, as illustrated in figure 2.2

Figure 2.1 A representation of major sources and sinks of dissolved oxygen in the Manawatu River.

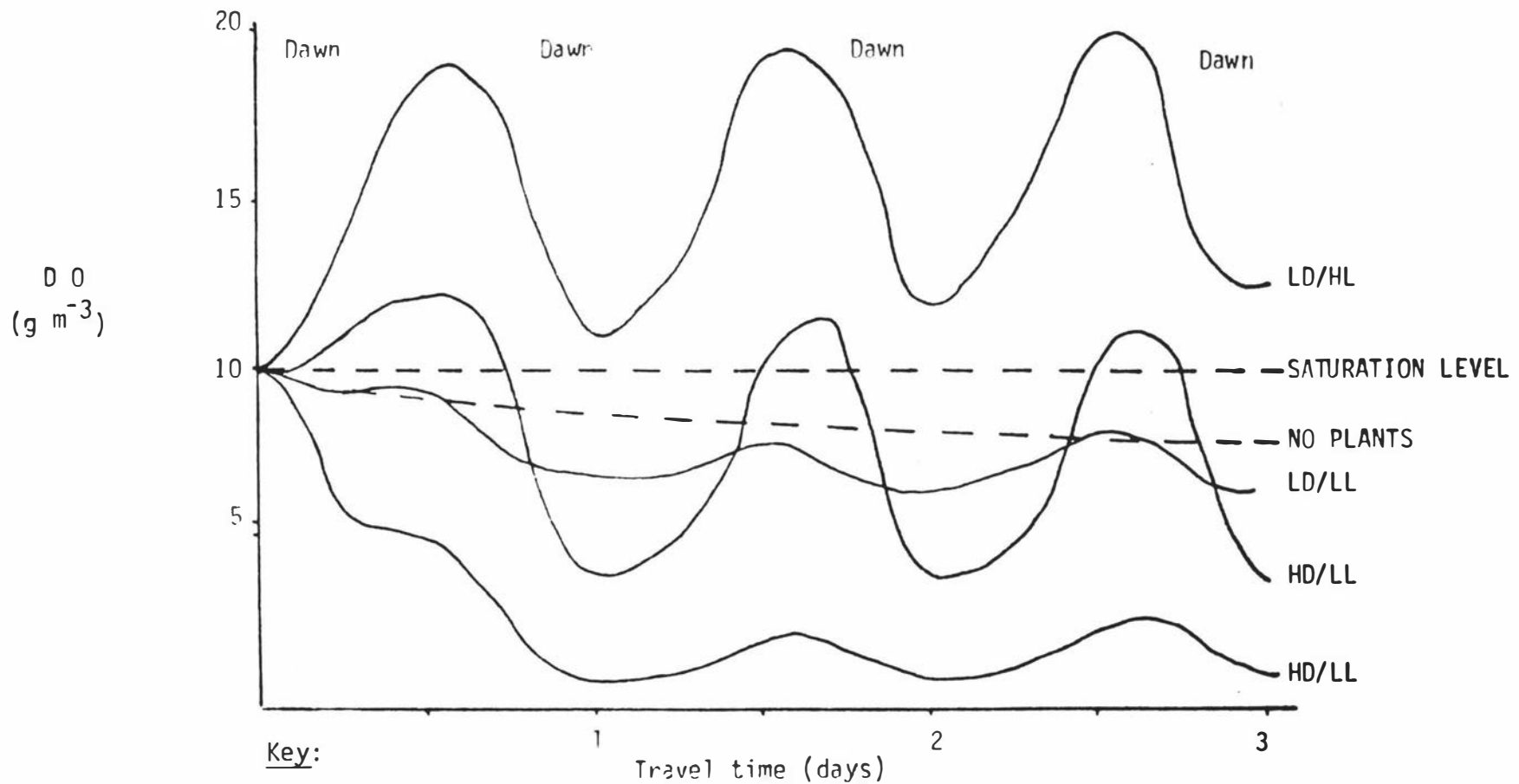


$$DO\ out = DO\ in + Net\ Community\ DO\ Production \pm\ reaeration$$

Oxygen Sources :- Phytoplankton
 Periphyton (Some loss of oxygen bubbles)
 Reaeration (Atmospheric oxygen)

Oxygen Sinks :- Phytoplankton
 Periphyton
 Reaeration
 Planktonic Heterotrophs
 Sediment Heterotrophs

FIGURE 2.2: Effect of differences in plant density and sunlight on river dissolved oxygen profiles at 15°C (after Owens *et al.*, 1969)



This figure illustrates the relationship between the attached filamentous algal biomass and the light available for photosynthesis. At low biomass levels all the filaments will have unrestricted access to the downwelling radiation. The photosynthetically produced oxygen will cause elevated DO concentrations. High metabolic activity will also incur a raised respiratory rate, which will takeover as darkness approaches. Respiration rates will also increase in response to higher DO concentrations (Owens & Mavis 1964; McIntire, 1966). Photorespiration may also be responsible for a part of the total respiration (Section 3.8.2).

As the algal biomass increases, filaments near the water surface can restrict the passage of light to filaments lower down. The biomass can build up to the point that a large portion of the algal assemblage receives very little light and as a consequence acts as an oxygen sink instead of a source.

In a river experiencing aquatic plant-induced DO fluctuations, the effects of oxygen-demanding organic effluents can increase the severity of the sags. If the organic load is constant, then an appreciably lower deficit is seen during the night. Concern centres around the worst case scenario during the summer, when factors can combine to produce a minimum DO. The low flows reduce the total mass of oxygen available in the river, and the higher temperatures enhance respiratory activity as well as decreasing the solubility of oxygen.

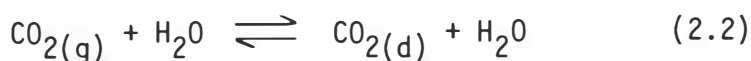
The asset and liability nature of benthic algal proliferations will be dependent on the temporal variation of any discharges. If a large demand is made on the available oxygen in a river at the same time that the algal respiratory effect is dominant then an otherwise acceptable DO minimum may be reduced below legislative levels set to maintain desirable aquatic flora and fauna.

It has been emphasized (O'Connell & Thomas, 1965) that the asset nature of algal photosynthesis should be considered as a safety factor, whereas means for overcoming the adverse effects of algal respiration should be built into any effective and reliable water

water pollution control programme.

2.3.2 pH

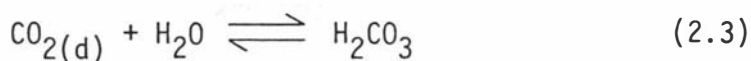
The equilibrium reactions of inorganic carbon are the major factors governing the buffering capacity and pH of natural waters. The distribution of the species of total CO_2 (Free CO_2 , HCO_3^- and CO_3^{2-}) is related to the pH of the water (See figure 2.3). The following equations outline the entry of gaseous CO_2 into a water body and the ensuing equilibrium reactions (Stumm & Morgan, 1981; Wetzel, 1975a; Whitton 1975) :-



where: $\text{CO}_{2(g)} = \text{CO}_2$ gaseous

$\text{CO}_{2(d)} = \text{CO}_2$ dissolved

This dissolved CO_2 hydrates slowly (half-life approximately 15 s) to form carbonic acid:-



The carbonic acid dissociates rapidly in comparison to its rate of formation:-



The equilibrium constants for these equations:-

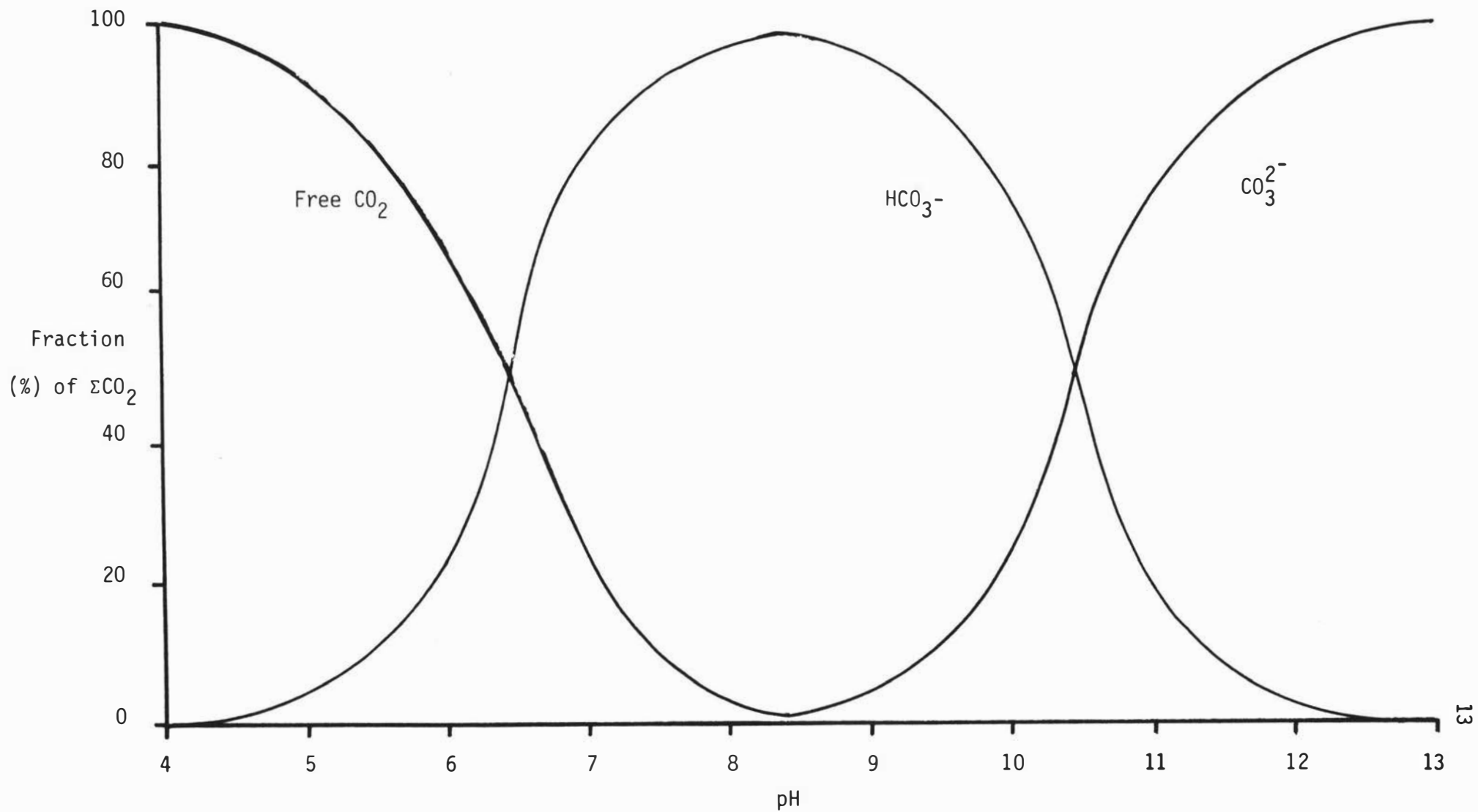
$$k_1 = \frac{(\text{H}^+)(\text{HCO}_3^-)}{(\text{H}_2\text{CO}_3)} \quad (2.6) \quad \text{pk}_1 = 6.43 \text{ at } 15^\circ\text{C}$$

$$k_2 = \frac{(\text{H}^+)(\text{CO}_3^{2-})}{(\text{HCO}_3^-)} \quad (2.7) \quad \text{pk}_2 = 10.43 \text{ at } 15^\circ\text{C}$$

Uptake of CO_2 during photosynthesis results in the removal of H^+ ions and a consequent rise in pH. There will also be a loss of H^+ ions, associated with the uptake of nitrate and phosphate, this is usually minor relative to changes induced by the CO_2 uptake demands placed on the equilibria reactions (Equation 2.3-2.5). The effect of respiration will be the reverse of the above and a depression of pH will occur (Stumm & Morgan, 1981).

Many aquatic plants can utilize bicarbonate which is taken up by

Figure 2.3 The relationship between pH and the proportion of inorganic carbon species in solution. ($\Sigma\text{CO}_2 = \text{CO}_2 + \text{HCO}_3^- + \text{CO}_3^{2-}$) (After Wetzel, 1975a)



active transport and dehydrated in the cell cytoplasm. This is coupled with stoichiometric excretion of hydroxyl ions, the net effect, in terms of the pH, is then similar to the uptake of free CO_2 . (Wetzel, 1975a)

Large diurnal pH fluctuations have been observed in many lotic situations supporting various combinations of aquatic plants. Early examples of pH ranges and magnitudes found in lentic situations are: 7.5-9.6 (Philip, 1927) and 4.0-12.3 (Schütte & Elsworth, 1954). pH dynamics in rivers have received less attention. The available data are summarized in table 2.1.

Fish mortalities observed in the River Tweed during the late afternoon, were attributed to the prolonged exposure to the elevated pH. (TRPB, 1957). There are no reports of damage to aquatic life in the other references in table 2.1.

The production of elevated pH has been cited as a competitive response by aquatic macrophytes and some periphytic algae, attempting to maximize available bicarbonate (See figure 2.3) at the expense of free carbon dioxide, giving them a competitive advantage over phytoplankton only able to utilize free carbon dioxide (Halstead & Tash, 1982; Round, 1973).

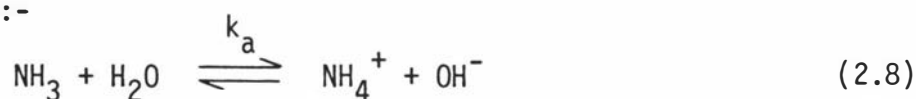
TABLE 2.1: A summary of pH fluctuations reported from a variety of rivers

River & Location	pH		Av. Flow (m ³ s ⁻¹)	Alkalinity (Eq m ⁻³)	Width (m)	Benthic Plants (gDWm ⁻²)	Reference
	Maximum	Minimum					
Havelse Denmark	9.1	7.5	0.6	NA	3.6	Macrophytes 40	Simonsen & Harremoës, 1978
Madison U.S.A.	8.2	7.7	13.0	5.8	42	Macrophytes 200	Wright & Mills, 1967
Bere U.K.	8.5	8.0	0.8	4.6	NA	Periphyton 30*	Marker, 1976; Marker & Gunn, 1977
Raritan U.S.A.	9.4	7.8	0.8	1.4	14	Various Periphyton 15*	Flemer, 1970
Tweed U.K.	10.5	7.8	NA	NA	NA	<i>Cladophora</i> NA	TRPB, 1957

* Chl a data converted to gDWm⁻² using chl a = 1.5% DW (Vollenweider, 1969)
NA = Data not available.

2.3.2.1 pH and Ammonia

pH is important in rivers, such as the Manawatu, that receive ammoniacal wastes, as it affects the toxicity of ammonia (Figure 2.4). Ammonia gas is soluble in water in the form of ammonium hydroxide which dissociates readily into ammonium and hydroxyl ions as follows:-



It is widely accepted that the toxicity of ammonia is directly related to the concentration of un-ionized ammonia (NH_3) (Alabaster & Lloyd, 1980). The percentage of NH_3 present in an ammonia solution can be calculated from the following:- (Alabaster & Lloyd, 1980)

$$\% \text{NH}_3 = 100 / (1 + \text{antilog}(\text{p}K_a - \text{pH})) \quad (2.9)$$

$$\text{p}K_a = 10.05 - 0.032T \quad (2.10)$$

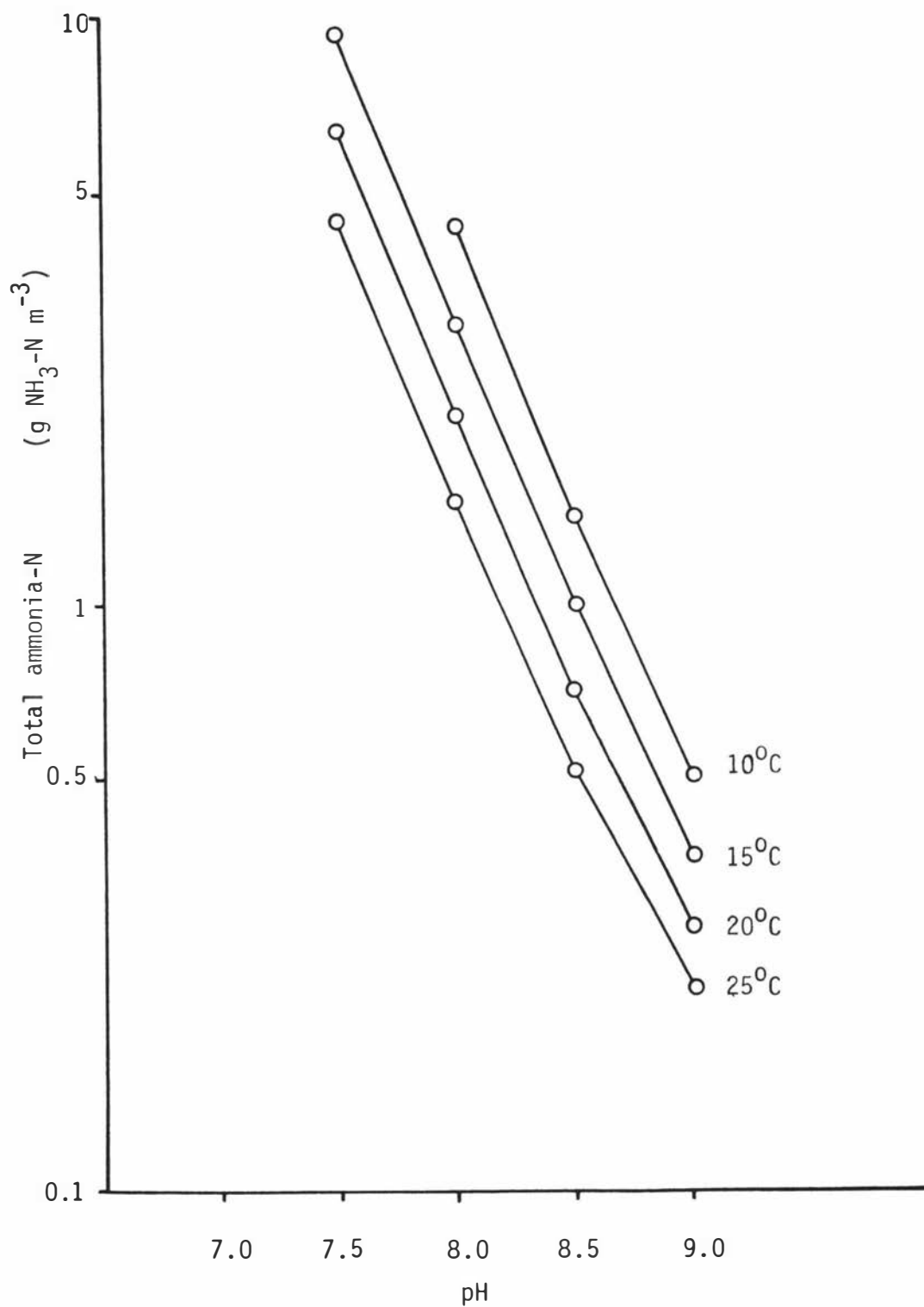
where T = Temperature ($^{\circ}\text{C}$)

The United States Environmental Protection Agency (EPA) criterion is $0.02 \text{ g NH}_3 \text{ m}^{-3}$ (EPA, 1972). However, in any consideration of ammonia toxicity the following relationships should be borne in mind (Alabaster & Lloyd, 1980; Szumski *et al*, 1982; Thurston *et al*, 1981):-

- (a) The proportion of NH_3 increases with increased pH
(See equation 2.9 and figure 2.4.)
- (b) The toxic concentration of NH_3 increases with increased pH and alkalinity. (Szumski *et al*, 1982)
These relationships modify the interpretation of (a), and become increasingly important for pH values above 8.0.
- (c) The toxic concentration of NH_3 decreases with increased temperature (figure 2.4) and decreased DO (Alabaster & Lloyd, 1980; Thurston *et al*, 1981).

The setting of river standards for ammonia was deferred in the last major study carried out by the Manawatu Regional Water Board (Currie, 1977). The only New Zealand water right that has incorporated an ammonia standard used a value of $0.08 \text{ g NH}_3\text{-N m}^{-3}$ ($0.10 \text{ g NH}_3 \text{ m}^{-3}$)

FIGURE 2.4: Concentrations of total ammonia that contain 0.08 g m^{-3} un-ionized ammonia-N



(figure 2.4) as the acceptable medium value (maximum concentration $0.11 \text{ g NH}_3\text{-N m}^{-3}$ ($0.13 \text{ g NH}_3 \text{ m}^{-3}$)) for the Makarewa River, Southland. (Black, 1979).

The US EPA and the European Inland Fisheries Advisory Commission (EIFAC) have criteria of $0.02 \text{ g NH}_3 \text{ m}^{-3}$ and $0.025 \text{ g NH}_3 \text{ m}^{-3}$ respectively. These criteria are based on safety (application) factors of 0.1 and 0.125 used with the lowest lethal concentration of $0.2 \text{ g NH}_3 \text{ m}^{-3}$ reported for rainbow trout fry (Liebmann, 1960, quoted by Alabaster & Lloyd, 1980). Although short-term exposure to concentrations of un-ionized ammonia below $0.15 \text{ g NH}_3 \text{ m}^{-3}$ may not kill a significant proportion of a fish population, adverse physiological or histopathological effects may occur (Lloyd & Orr, 1969). However, the use of a safety factor of 0.1 has been criticised as being not scientifically justified, overprotective, and in terms of the costs of water pollution control, unnecessarily expensive. (Ruffier *et al*, 1981; Szumski *et al*, 1982). The latter authors argue for a lower threshold acute toxic concentration (for cold water fish species) of $0.2 \text{ g NH}_3\text{-N m}^{-3}$ used in conjunction with a safety factor of 0.2 giving a criterion of $0.04 \text{ g NH}_3\text{-N m}^{-3}$. They also advocate taking into account point (b) noted above.

There is still much discussion in the U.S.A. on the various factors that should be incorporated into an ammonia criterion. The EPA are presently soliciting input to a draft document that will supercede the present criterion. (Szumski *et al*, 1982).

2.4 River primary productivity

Lotic primary productivity can be defined as the capacity of a river to build up simple organic compounds from solar or chemical energy. The relative proportion of autochthonous (produced in the river) and allochthonous (originating from outside the river) production depends on numerous factors such as the river order, precipitation, activity in the catchment area and season. The primary productivity of an aquatic ecosystem can be used as a reflection of water quality and community composition (Hornberger *et al*, 1976 & 1977).

Primary productivity can best be determined by measuring changes in

D₀ or Dissolved Carbon (including ¹⁴C) as opposed to gravimetric measurements, which will be confused by losses such as sloughing-off and grazing.

Advantages of oxygen methods over carbon methods include the following:-

- (i) Cheaper and simpler equipment is required to measure D₀ compared to that needed for ¹⁴C methodology.
- (ii) Robust commercially available monitors are available that allow simultaneous and continuous measurements of D₀ and temperature.
- (iii) Estimates of gross, net primary productivity and respiration can be made.
- (iv) They are more applicable than any others in highly productive systems.

Disadvantages include:-

- (i) The accuracy is poor in low productivity situations.
- (ii) They infer that oxygen production is stoichiometrically related to the build up of organic material.
- (iii) In order to partition the components of net primary productivity assumptions must be made about the necessary corrections for respiration.
- (iv) In highly productive situations there can be loss of oxygen as bubbles (Wetzel, 1975b).

Primary productivity measurements involving D₀ can be divided into two categories:-

- (a) Enclosed chambers:- These isolate portions of the community either *in situ* or in a controlled laboratory setting. The design of these have evolved from the simple light and dark BOD (Biochemical Oxygen Demand) bottles to semi-enclosed chambers that can be placed over sections of the riverbed. However, due to the contagious distribution of most lotic periphytic communities the replication needed for accuracy precludes the use of the *in situ* technique in all but the most extensive growth situations (Wylie & Jones, 1981).

- (b) Free-diurnal curve methods:- These techniques involve monitoring the DO dynamics of the whole river. If the DO fluctuations occur simultaneously along the river then single station monitoring will adequately describe the situation. If there is longitudinal development of plant material then 'upstream-downstream' (or two station) monitoring has to be employed. By following both the day and night dynamics, values for respiration, gross and net primary productivity can be calculated (Odum, 1956) (See section 3.8.2).

The primary productivity of a periphytic community will vary as it develops. Consequently, the water quality effects will also change. During the undisturbed development of a periphytic community there will be changes in the overall composition. In laboratory streams temporal changes have been observed in filamentous algal assemblages involving layer of older/decomposing cells, empty diatom frustules and silt. There was also sloughing off of large portions of the community, the amount of which increased as the current velocity increased beyond an optimum. (McIntire, 1968; Horner *et al*, 1982). In a thermal stream (37°C) a build up of layers of senescent cells to form a thick algal mat was observed and the involvement of extracellular products in controlling growth rates hypothesized (Stockner, 1968).

- relationship between periphytic algal density and primary productivity has frequently been described as an inverse one (Eichenburger & Hermann, 1975; Marker, 1976; Pfeifer & McDiffet, 1975) which is expected when the effects of increasing self-shading and prototrophs are considered (Section 2.3.1). However, a linear relationship has been reported to exist between the density of the algae of the Danube River and their primary productivity (Tomajka, 1973). It is important to clarify in such studies the methods used and relevant aspects of the river such as current velocity, as these can have a large influence on the results. Some of the studies do not include all the relevant hydrological data, and as a result their conclusions may need to be reinterpreted.

Factors controlling primary productivity have been discussed by many, notably: Edwards & Owens (1962); Vollenweider (1969); Wetzel (1975b) and Whitton (1975). (See section 2.5.3). Results from some lotic

primary productivity studies are presented in table 2.2.

A wide range of values have been reported, including negative net primary productivity, indicating a dominance of heterotrophic over phototrophic activity. Despite the fact that the community composition is often cited as a major factor determining the net primary productivity, there have been few attempts at partitioning the community metabolism. One study, using chambers and antibiotics found that bacterial respiration contributed 30-35% of the total oxygen demand. (Hargrave, 1969). Both chambers and bottles have been used in attempts to identify the algal contribution to the community primary productivity. However, data derived from bottle studies may seriously underestimate primary productivity due to the importance of flow for maximum nutrient utilization. (Pfeiffer & McDiffett, 1975; Rodgers & Harvey, 1976; Whitford & Schumacher, 1961).

Many of the studies cited in table 2.2 involved the attached filamentous alga *Cladophora*, which is discussed in detail in the following section.

TABLE 2.2: A comparison of lotic primary productivity data

Net Areal Primary Productivity ($\text{gO}_2\text{m}^{-2}\text{d}^{-1}$)	Gross Photosynthesis ($\text{gO}_2\text{m}^{-2}\text{d}^{-1}$)	Respiration ($\text{gO}_2\text{m}^{-2}\text{d}^{-1}$)	Comments	Reference
-1.0→15.0			<i>Cladophora</i> comm. diurnal analysis	Wong <i>et al</i> , 1976
0.9→ 5.5			Periphyton. Light & dark bottles	Ertl & Tomajka, 1973
4.7→11.6			Diurnal analysis. Periphyton & phytoplankton	Flemer, 1970
4.7→16.5	8.1→14	7.3→12.9	<i>Cladophora</i> , comm. Diurnal analysis & chamber	O'Connell & Thomas, 1965
9.4→12.5			Filamentous algae	Pfeiffer & McDiffett, 1975
	0.4→14.0	4.2→20.2	Comm. diurnal analysis	Butcher, 1937 cited by:
	0.6→59		10 Florida Springs	Odum, 1956
	10.1→48		Diurnal analysis	Duffer & Dorris, 1966
	1 →10		Art.channels periphyton.	Eichenberger & Wuhrmann, 1975

2.5 Cladophora

2.5.1 Introduction

Cladophora is a member of the Phylum *Chlorophyta*, Class *Bryopsidophyceae* and Order *Cladophorales* (Round, 1973). Members of the genus have been found in both marine and freshwater environments. In the past fifty years the ubiquitous nature of this algae combined with the accelerated enrichment of many lakes and rivers has led to the worldwide occurrence of *Cladophora* proliferations.

2.5.2 Identification

Precise identification poses a major problem, especially in view of the morphological plasticity of the species (Graham, 1982; Whitton, 1970). The detailed texts by Soderström (1963) and Van den Hoek (1963) need consulting in conjunction with life cycle observations, before species level identification can be established. This aspect has been by-passed by many working with *Cladophora*, and the relevance of some published work is doubtful as the species is often not mentioned, or in some cases identification is contradictory. (Neil & Owen, 1964; Storr & Sweeney, 1971).

Using basic texts (APHA, 1975; Palmer, 1980) and the more detailed studies mentioned above, together with observations of the algae throughout the year, the predominant alga in the Manawatu River was identified as *Cladophora glomerata* (L) Kutz. Comparison with a culture sample (from the Culture Centre of Algae and Protozoa Cambridge, U.K.) confirmed this conclusion, when allowance was made for the morphological changes that occur when *Cladophora glomerata* is artificially maintained (See section 3.7).

2.5.3 Environmental parameters affecting the growth of *Cladophora*

Whitton (1970) summarizes much of the literature on *Cladophora*, especially those species found in eutrophic waters. This review will attempt to update Whitton's survey with recently published material. Whitton (1970) described examples of seasonal fluctuations observed at a variety of world-wide locations. These fluctuations are attributed to a variety of environmental factors which have been

outlined in section 2.2. Each will be discussed in some depth.

2.5.3.1 Temperature

Cladophora glomerata has been reported in a variety of fresh waters occurring either as a well defined summer proliferation (Bolas & Lund, 1974; Eichenberger, 1967 (quoted by Whitton, 1970); Pitcairn & Hawkes, 1973) or as two distinct summer and autumn blooms with an intervening decline (Chudyba, 1965; Bellis & McLarty, 1967; Blum, 1957; Mason, 1965; Storr & Sweeney, 1971; Wong *et al.*, 1978).

In seven Canadian river systems increased *Cladophora* biomass was seen to be associated with a general warming of the waters to 18.5 - 22.0°C (Wong *et al.*, 1978). In Lake Michigan *Cladophora* has been reported as increasing most rapidly when the water temperature ranged from 17-20°C (Herbst, 1969). These field results have been supported by laboratory experiments that obtained maximum biomass production at approximately 18°C. (Storr & Sweeney, 1971). However, *in situ* studies on *Cladophora* photosynthesis have indicated optima at about 25°C (Adams & Stone, 1973; Moore, 1978). These studies can also be supported by laboratory work that show maximum biomass production occurring in the range 25-30°C (Bellis, 1968; Gerloff & Fitzgerald, 1976; Hoffman & Gerloff, 1980; Whitton, 1970; Zuraw, 1969).

It should be noted, when trying to clarify these two seemingly incompatible temperature optima ranges, the studies cited above deal specifically with *Cladophora glomerata* (Whitton's (1970) review refers to *Cladophora* species) and this species has been observed in, and collected from, a variety of locations. Each location may then have a specific ecotype with different environmental optima. Experimental procedures have not been standardised and these will influence the comparability of results. The applicability of some experimental studies to the river situation could be questioned in view of a reported significant expansion of the temperature range when nutrients were added to unenriched lake water. (Storr & Sweeney, 1971).

Experimental evidence has been obtained that indicates that there are two temperature optima, corresponding to the processes of

zoosporogenesis and biomass production. (Hoffman & Gerloff, 1980). Sporogenesis has been noted as occurring in the field in the range 15-20°C. A lowered temperature has been seen to be responsible for morphogenetic effects such as akinete formation and sporogenesis (Whitton, 1970). It has also been demonstrated that a raised temperature caused akinete formation (Mason, 1965). Upper tolerance limits quoted for *C. glomerata* also appear to cover a wide range, from 25°C (Storr & Sweeney, 1971) to greater than 31°C (Gerloff & Fitzgerald, 1976). However, it has been shown that the upper tolerance limit of *C. glomerata* varies according to the water temperature fluctuations (Wong *et al*, 1978). As the seasonal variation increases, so the upper tolerance level decreases. These findings thus throw some doubt on the usefulness of laboratory studies conducted at a constant temperature.

Lower tolerance limits have received less attention, 6°C was observed as the limit for detectable growth in the River Wear (Whitton, 1970). Culture studies have shown that growth ceases at 5°C (Bellis, 1968). Bolas and Lund, (1974) observed that growth reduction of *Cladophora* proliferations could be attributed to winter temperatures of 3°C. Other studies have observed healthy background tufts of *Cladophora* at the time of freeze-up (Chudyba, 1965; Wong *et al*, 1978).

2.5.3.2 Light

The phenomena of twin annual peaks (Spring and Autumn) of *Cladophora* growth has been attributed to, among other factors (cf. section 2.5.4), changes in light availability (Wong *et al*, 1976). The first peak is caused by summer warming and increased insolation. This effect is steadily reduced by shading (in relatively narrow streams) from riparian foliage. As autumn arrives and the shade cover is lost, so the algae are given another boost by the newly available light and the second peak is observed. However, other theories have attempted to explain the twin peak phenomena. (See section 2.5.4).

In the laboratory, light intensities used to achieve maximum photosynthetic response vary from 90-125 μ einsteins (μ E) $m^{-2} s^{-1}$ Photosynthetically Active Radiation (PAR) using a variety of photoperiod regimes

(Gerloff & Fitzgerald, 1976; Hoffmann & Gerloff, 1980; Moore & Traquair, 1976; Storr & Sweeney, 1971; Whitton, 1967). Recent experimental studies have observed an optimum light intensity for net photosynthesis at $400 \mu\text{E m}^{-2} \text{s}^{-1}$ (Graham *et al.*, 1982). This is closer to summer averages of $700\text{-}1000 \mu\text{E m}^{-2} \text{s}^{-1}$ that have been recorded at the plant depth. (Graham *et al.*, 1982; Wong *et al.*, 1976).

The effect of increasing the photoperiod has been shown to cause an exponential increase in biomass production (Storr & Sweeney, 1971). However, recent factorial experiments designed to statistically assess the effect of vitamins, light intensity, temperature and photoperiod on growth and zoosporogenesis, found that while photoperiod variations contributed very little to the dry weight production they did significantly control the magnitude of zoosporogenesis. (Hoffmann & Gerloff, 1980)(See section 2.5.3.1).

One quantitative field study has reported that the PAR available to *Cladophora* in several Canadian rivers was linearly related to the daily net photosynthesis ($r^2=0.53$) after fluctuations in water turbidity, mean depth and total reflection were accounted for. (Wong *et al.*, 1976)

The growth rate and reproductive ability of aquatic plants are governed by many, often interacting, nutritional and environmental factors. Algal tissue nutrient levels, adaption to seasonal conditions, physiological races (ecotypes) can all affect light and temperature optima. (See section 2.5.3.1) (Maddox & Jones, 1964; Round, 1973; Senft, 1978)

2.5.3.3 Water movements

Two aspects of lotic water movements will be considered:-

- (a) Current velocity effects.
 - (b) Gross water movements or floods.
- (a) An increased water flow past periphyton has been shown to enable a more efficient use of mineral nutrients and promote increased net photosynthesis. (Horner & Welch, 1981;

Whitford & Schumacher, 1961). The effect of increasing current velocity was significant in increasing respiration and phosphorus uptake between 1 or 2 cm s⁻¹ and about 45 cm s⁻¹, and is attributed to an increase in the diffusion gradient at or near the cell surface (Falco *et al*, 1975; Schumacher & Whitford, 1965).

- (b) However, it is also important to note that as current velocities exceed 50 cm s⁻¹, sloughing-off can become increasingly important. The physical growth characteristics, attachment mode and microclimate of the periphyton will all be important in determining the tolerable current velocity range. Laboratory work with non-filamentous periphyton has shown reduced biomass development at current velocities in excess of 50 cm s⁻¹ (Horner & Welch, 1981). Field observations of lotic *Cladophora* have established the velocity range of 25-50 cm s⁻¹ as the most favourable for growth. Increased sloughing-off occurred as velocities increased beyond 100 cm s⁻¹. (Chudyba, 1965).

Field observations on periphyton in rivers resembling the Manawatu affected by spasmodic floods are not common mainly because of difficulties in applying study techniques designed for relatively steady flow conditions, and because the periphyton under study are frequently under threat of being obliterated!

Flow increments and the concurrent velocity increases have been qualitatively linked with biomass fluctuations, larger flow increases or spates have been seen to scour surfaces leaving bare substrate which is then available for colonization (Chudyba 1965; Whitton, 1970). Tett *et al* (1978) note that "Water movement sorts particles by size, but the process is complex and the earlier current regime can result in a substrate and periphyton distribution that only roughly reflects velocities at the time of sampling".

The load, both suspended and dissolved, associated with flood events can have an important influence on both the light climate and nutrient status of any remaining periphyton. The sudden

availability of dissolved nutrients such as phosphorus and nitrogen will lead to saturation of the cell quota. (Beaumont, 1975; Vollenweider, 1969) (See section 2.5.3.4) The increased suspended load will also have major effects on the light climate of any periphyton, prolonged PAR reductions often existing long after the initial flush event. (Tett *et al*, 1978).

2.5.3.4 Nutrients

During the past few decades much work has been published on the ways in which nutrients affect algal, especially phytoplankton, growth. There has also been substantial work carried out on *Cladophora* species. Three points should be noted:

- (a) The importance of species level identification is crucial for truly comparable results.
- (b) Controversy still exists as to whether, at any given time one nutrient, will limit growth (Threshold effect) or multiple nutrients will limit growth (Multiplicative effect) (Ahlgren, 1980; Droop, 1974).
- (c) Limiting nutrient(s) identification in natural systems will be hampered by other uncontrollable and often unmeasured factors.

Phosphorus, and to a lesser extent nitrogen, have been found to be important factors controlling *Cladophora* proliferations. Early work by Neil and Owen (1964) showed that growths of *Cladophora* could be established, where none had previously existed, by artificial loading of phosphate and nitrogen to Lake Huron. In a number of U.K. rivers it was shown that *Cladophora* biomass was correlated with phosphate concentration. Rivers with less than 1 g m^{-3} total inorganic phosphate phosphorus produced only modest annual growths of *Cladophora* (5 g m^{-2} compared to 75 g m^{-2} annual mean dry weights, (See section 2.5.4)) (Pitcairn & Hawkes, 1973). However, the authors acknowledged that "... phosphorus requirements vary according to other water parameters such as hardness, alkalinity, concentration of organic compounds, etc. ... and can thus only be satisfactorily quoted for a named organism in a named body of water". Their field studies were backed up with laboratory

work showing similar critical threshold values, below which, maximum growth rates were reduced (See table 2.3).

Sewage discharges into the Kentish Stour led Bolas and Lund (1974) to conclude that "... there seems no doubt that phosphorus is a major controlling element". These authors observed maximum *Cladophora* biomass in enriched river stretches where the phosphorus (reactive phosphate phosphorus) reached approximately 1.0 g m^{-3} . An upstream phosphorus concentration of about 0.2 g m^{-3} was seen to support only modest growths.

A study of several Canadian rivers identified, on a community photosynthetic basis, as opposed to a dry weight basis, a much lower critical water phosphorus concentration (table 2.3). (Wong & Clark, 1976).

These authors point out that detached or free floating (after the definitions of Wetzel, (1975a)) filaments (FFF) may complicate the identification of any relationship between attached algal biomass and water phosphorus concentration. (see Section 2.5.5)

The general variability of the critical values reported should be considered in the light of the many variables assessed (e.g. the variation in phosphorus species tested for and the analytical method used) and whether the result was derived from *in situ* observations and analysis or from laboratory batch cultures. (Table 2.3). A wide variety of media have been used to cultivate *Cladophora*. Some have been of a defined nature, while others have incorporated supplements from soil, sewage, or any convenient eutrophic water source. Interpretation of the data is thus complicated, and the applicability of some batch experiments to a lotic environment is questionable.

TABLE 2.3: Estimates of critical water phosphorus concentrations in various aquatic situations

Critical Water P (g m ⁻³)	Comments	Reference
1.00	Total inorganic P. River <i>Cladophora</i>	Pitcairn & Hawkes, 1973
0.95	Total inorganic P. Culture <i>Cladophora</i>	Pitcairn & Hawkes, 1973
0.28	Total inorganic P. Culture <i>Cladophora</i>	Gerloff & Fitzgerald, 1976
0.06	Total P. River <i>Cladophora</i>	Wong & Clark, 1976
0.02	Reactive P. River <i>Cladophora</i> Comm.	Henley <i>et al</i> , 1980
0.02	Dissolved Reactive P. Lab.Stream periphyton	Horner <i>et al</i> , 1982
0.01	Total P. Lake & River Phytoplankton	Mackenthum, 1968
0.008	Dissolved reactive P. Art. Stream Periphyton	Wuhrmann & Eichenberger, 1975
0.002	Dissolved reactive P. Lake <i>Cladophora</i>	Auer, 1982
<0.001	Dissolved Reactive P. Lake Phytoplankton	Mackereth, 1953

Other possible nutrients that may influence or limit *Cladophora* growth include various metals and vitamins. Mantai (1978) suspected that these may have been important in Lake Erie and noted that different factors may limit growth at different times during the growing season. Trace metals have been recognized as essential in any media for *Cladophora* cultivation (Gerloff & Fitzgerald, 1976; Whitton, 1970). However, there is no record of a proven case of trace metal limitation in an established proliferation. The need for vitamins, notably B₁ (Thiamine) and B₁₂ (Cyanocobalamin) has been established in culture studies (Gerloff & Fitzgerald, 1976; Hoffman & Gerloff, 1980). However, there have, as yet, been no reported cases of vitamin limited *Cladophora* growth in the field.

The possibility of carbon limiting *Cladophora* growth appears unlikely, as growth is usually only seen in calcareous freshwaters where the total carbon and buffering capacity will be high. pH fluctuations caused by algal metabolism will not usually alter the total carbon available for *Cladophora* (although the proportions of species in the CO₂/HCO₃⁻/CO₃²⁻ equilibrium will alter) unless there is precipitation of CaCO₃. Only in extremely eutrophic systems (or other special cases such as very low alkalinity lakes or extremely hard water lakes) is it likely that carbon could become limiting (Goldman *et al*, 1972). These cases do not apply to the Manawatu River.

Cladophora are not favoured by low nitrogen waters, and a high phosphate to inorganic nitrogen ratio has been shown to be selective for growth (Whitton, 1970). Productivity increases in Lake Michigan are apparently related to increased nutrients including phosphate and nitrogen, with total P varying from 0.067 - 0.095 g m⁻³ and nitrate N from 0.08 - 0.16 g m⁻³ (Adams & Stone, 1973). During the summer period in Lake Erie the nitrate concentration was thought to limit *Cladophora* as it was seen to drop from 3-4 g N m⁻³ to near zero for a two week period. (Mantai, 1978).

In six Canadian rivers the total nitrogen concentration never fell below 0.89 g m⁻³ and no correlation was identified between total tissue nitrogen and total water concentrations. (Wong & Clark, 1976)

Nutrient additions to experimental channels using diverted river water showed significant growth increases on phosphate addition, whereas nitrate increments gave no significant increases. The background levels of these nutrients would corroborate the results, with phosphate varying from 0.2 - 1.0 g PO₄-P m⁻³ and nitrate from 3 - 7 g N m⁻³ (Bolas & Lund, 1974). Similarly in a number of U.K. rivers with high nitrogen levels (NO₃-N 2.7 - 12.5 g m⁻³, NH₃-N 0.1 - 2.8 g m⁻³) no significant correlation was seen between the mean annual dry weight of *Cladophora* and nitrogen (Pitcairn & Hawkes, 1973).

2.5.3.5 Substrates

Cladophora will grow on a wide variety of substrates (Chudyba, 1965; Shear & Konasewich, 1975; SWA, 1981; Whitton, 1970). The most prolific growths are usually associated with rough large stones with fissures, although glass slides have been seen to maintain *Cladophora* growths. (Whitton, 1970). The importance of stone size in relation to flow conditions has been noted (Shear & Konasewich, 1975; Whitton, 1975) (See section 2.5.3.3). The influence of the chemical composition of the substratum on the establishment and growth of benthic algae is as yet poorly understood.

2.5.3.6 Summary

The environmental factors responsible for controlling the growth and distribution of *Cladophora* have been considered. Each specific proliferation of *Cladophora* is only made possible by the correct combination of all these factors, together with the physical attributes of the alga and its unique life cycle (See section 2.5.4).

2.5.4 Life cycle

The historical development of a river site, in terms of flush events, stone size, previous *Cladophora* growth, will have a major bearing on its future algal production. Summer proliferations arise from three major sources (see figure 2.5):

- (a) The overwintering filaments and basal holdfasts that survive in small tributaries and well sheltered spots in the main

- river, provided winter floods are not excessive. (Blum, 1982; Chudyba, 1965; Whitton, 1970).
- (b) Akinetes (modified thick walled cells) formed during a variety of adverse conditions, these can germinate into vegetative cells under favourable circumstances (Mason, 1965; Whitton, 1970). Rapid vegetative growth can arise from both (a) and (b) when conditions allow. In turn, from these growths some cells give rise, throughout the season to:
- (c) Zoospores. These can be produced in massive quantities (See section 2.5.3.1 and 2.5.3.2), and settle on submerged substrates to produce extensive proliferations from which more zoospores may arise.

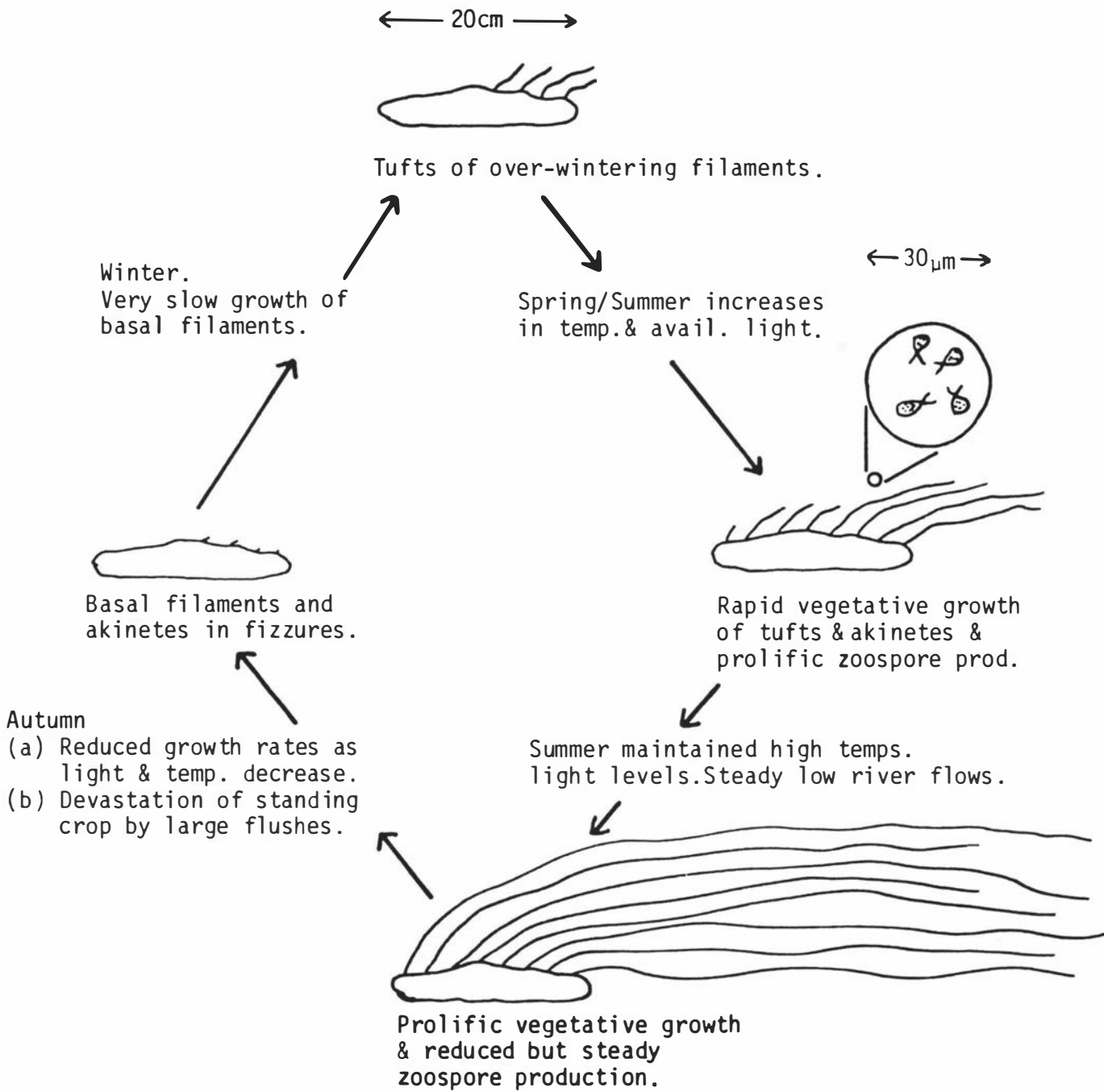
Each of the above stages in the life cycle plays a contributory role to the formation of lotic *Cladophora* proliferations. Upstream overwintering filaments and akinetes deposited on stable rocks and logs in sheltered areas are responsible for initiating spring/summer vegetative growth. The numerous zoospores produced, then begin colonization of those bare surfaces that have been scoured or exposed during past flushes and high winter flows.

This totally asexual life cycle supports the contention by Graham (1982) that *Cladophora glomerata*, in adapting from a marine to a freshwater existence, has lost the capacity for sexual recombination. The reported observations of alternation of generations can be attributed to the difficulty of species taxonomy of *Cladophora* due to morphological plasticity (Shear & Konasewich, 1975; Whitton, 1970). Polyploidy (cells containing more than two complete sets of homologous chromosomes) has been observed in *Cladophora*, and this is thought to partially offset the loss of genetic variability that occurs when sexual recombination is absent. (Graham, 1982)

2.5.5 Biomass and its measurement

Cladophora occurs primarily attached to stable substrate, however a portion of the biomass can become free floating (detached from the substrate), especially during flow increases.

Figure 2.5 The life cycle of *Cladophora* in the Manawatu River.



A variety of methods have been used to estimate the quantity of attached *Cladophora* in lentic and lotic situations, including percentage cover, chlorophyll a, filament length, wet weight, dry weight, ash-free dry weight and bio-volume. (Carnes & Millner, 1980; Mantai, 1978; SWA, 1981; Whitton, 1970).

These numerous techniques often make comparisons difficult and even when one technique, notably dry weight, is considered there is still considerable scope for methodological variations (See appendix 4). The contagious (random clumped) distribution of periphyton has been widely recorded and presents obvious sampling problems for accurately and precisely estimating biomass especially at low densities (Chudyba, 1965; Frietzon, 1980; Mantai *et al*, 1982; Pitcairn & Hawkes, 1973; Wong & Clark, 1979; Wong *et al*, 1979). Some published data on dry weight biomass in lakes and rivers are presented in table 2.4.

The summer prominence of *Cladophora* observed worldwide has been attributed to the combination of the ideal physico-chemical conditions, general warming and increased insolation (Canale *et al*, 1982; Storr & Sweeney, 1971). However, there is still some dispute about the reasons for the reported autumn declines (See section 2.5.3). Higher temperatures that increase daily respiration to rates that are greater than the gross photosynthesis have been cited as a cause of senescence of *Cladophora* (Graham *et al*, 1982; Lorenz & Herdendorf, 1982). Also, diatoms have been suspected of causing enhanced sloughing-off as a consequence of their physical biomass accrual on the *Cladophora* filaments and competition for nutrients (Stevenson & Stoermer, 1982).

The amount of free-floating filaments can constitute a major portion of a total river *Cladophora* population. To date, there have been few studies that quantify this highly variable parameter. In a number of Canadian rivers an average biomass of 3.5 kg fresh weight was found to be trapped by a 1 m² wire screen suspended vertically in the flow for six hours (Wong & Clark, 1976). In Lake Huron a sloughing rate of 0.1 day⁻¹ was commonly observed, compared to a maximum net specific growth rate of 0.7 day⁻¹. (Auer & Canale, 1980).

TABLE 2.4: Examples of *Cladophora* biomass density in lakes and rivers

Biomass g DW m ⁻²	Situation and Location		Reference
5-75	Rivers	U.K.	Pitcairn & Hawkes, 1973
10-100	Rivers	U.S.A.	Pfeifer & McDiffet, 1975
500 max.	Lake Ontario	Can./U.S.A.	Shear & Konasewich, 1975
148 av. 1000 max.	Rivers	U.K.	Butcher, 1937 (cited by Whitton, 1970)
431 av. 982 max.	Lake Erie	U.S.A.	Neil & Jackson, 1982
216 max.	River Wear	U.K.	Whitton, 1970

2.6 Nutrient Availability Tests

2.6.1 Introduction

Nutrient availability tests (NATs) have been used to evaluate the amount of a particular nutrient, or nutrients, available to aquatic plants in a water sample, or to assess the nutritional status of *in situ* plants (APHA, 1975). In the latter case a variety of techniques have been commonly used for different nutrients. (Fitzgerald & Nelson, 1966; Fitzgerald, 1969a; Gerloff & Skoog, 1954; Gerloff & Krombholz, 1966; Healey, 1973a). These differ in their abilities to describe short or long term changes in the nutritional status of algal cells.

The interpretation of these NATs involves two major assumptions:

- (a) The result is not affected by other nutritional and environmental variations: While it has been shown that these NATs are reliable indicators of nutrient limitation and surplus (Healey & Henzel, 1979 & 1980), the magnitude of values can be affected by other factors such as temperature, light intensity and the concentration of other nutrients. (Laws & Bannister, 1980; Maddox & Jones, 1964; Nalewajko & Lee, 1980; Senft, 1978 ; Stewart & Wetzel, 1982) (See section 4 e.g. figures 4.3, 4.6 and 4.9)

- (b) The washed *Cladophora* field sample gives a NAT response indicative of its river situation. The pre-washed macroscopically 'clean' selected samples often had small quantities of microscopic epiphytes and associated debris. (See section 6.4). This investigation concentrated on the condition of the *Cladophora* filaments (in relation to the river nutrients) as opposed to the whole assemblage. The importance of any host-epiphyte interaction will be reduced in an assemblage that consists largely of young, rapidly growing, and thus relatively 'clean', *Cladophora* filaments. (Stevenson & Stoermer, 1982). As the growth rate of the *Cladophora* decreases with the onset of autumn (reduced available light, and lower temperatures) the epiphyte population appeared more able to colonize the filaments and thus can become more important in influencing the nutritional status of the underlying *Cladophora* filaments. (Fitzgerald, 1969b; Stevenson & Stoermer, 1982).

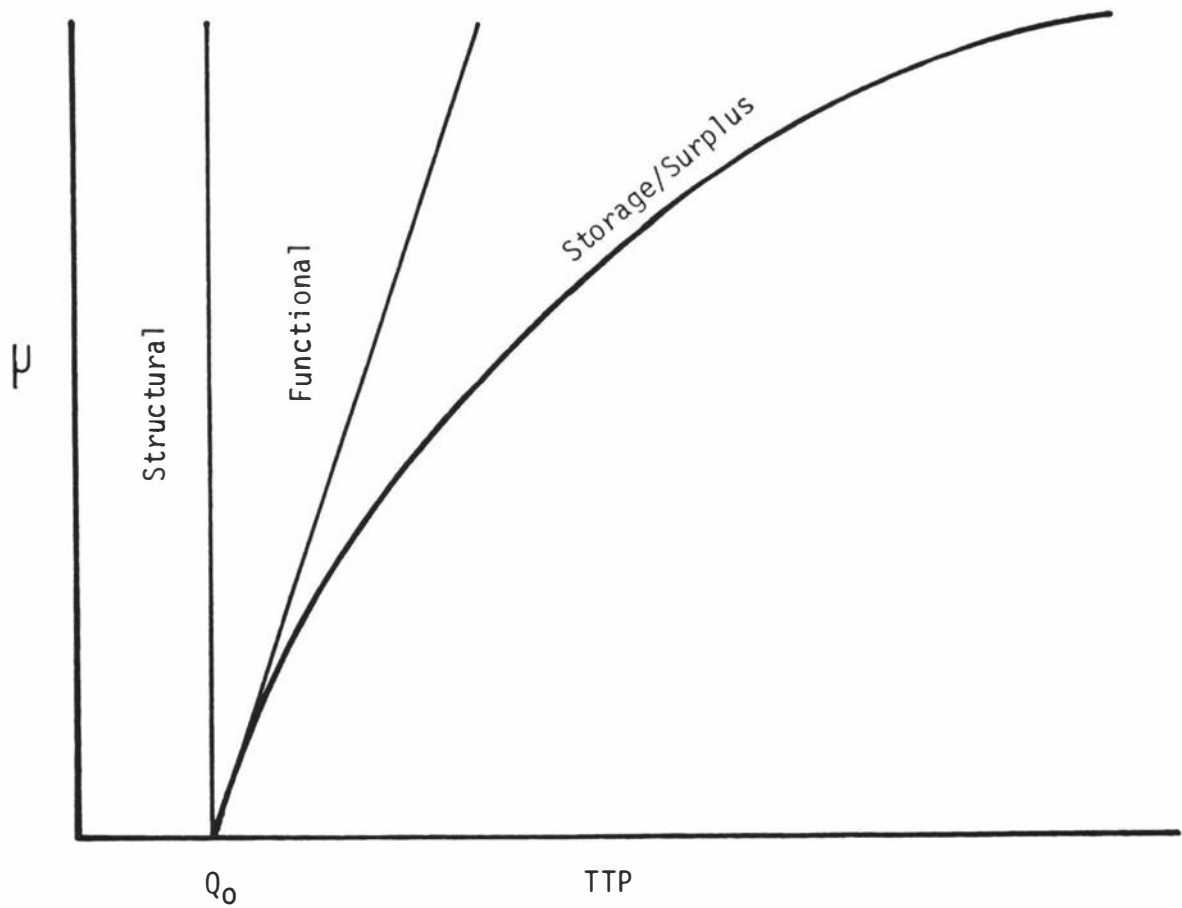
2.6.2 Phosphorus

2.6.2.1 Total Tissue Phosphorus (TTP)

The total phosphorus (P) found in algal tissue is an indication of the external P that has been available over prolonged growth periods. (Gerloff & Krombholz, 1966; Gerloff & Skoog, 1954). The three functional components of TTP are illustrated in figure 2.6 and detailed as follows:-

- (a) The structural components are required to maintain the integrity and viability of the alga and will include DNA-P and membrane lipid-P. When the internal P consists of structural P only, the growth rate will be zero. This concentration is defined as the critical TTP concentration (Q_0 in fig. 2.6). The generally accepted critical TTP concentration for *Cladophora* is 0.05 - 0.06% P (Auer & Canale, 1982a; Gerloff & Fitzgerald, 1976) (See table 2.5)
- (b) The functional components, such as phosphorylated intermediates and RNA - P, are involved in the cells metabolic activities. The size of this functional group

FIGURE 2.6: The relationship between Total Tissue Phosphorus (TTP) and the specific growth rate (μ)* (after Fuhs, 1969)



* Q_0 = minimum or critical value when $\mu = 0$.

TABLE 2.5: Values of Phosphorus Nutrient Availability Tests for Cladophora associated with a limiting or surplus situation

Nutrient Availability Test	Activity or level associated with		Reference
	Limitation	Surplus	
TTP (% P)	<0.06	>0.06	Gerloff & Fitzgerald (1976)
	<0.16	>0.16	Wong & Clark (1976)
	<0.05	>0.05	Auer & Canale (1982a)
EP (% P)	<0.02	>0.02	Mantai <i>et al</i> , (1982)
	<0.02	>0.02	Lin (1971)
PUR ($\mu\text{g P}(10\text{mg})^{-1} \text{hr}^{-1}$)	>6	<3	Auer & Canale (1982b)
	>1	<1	Healey & Hendzel (1979)
APA (Enz.Units mg^{-1})	200	20	Mantai (1978)

KEY:

TTP = Total Tissue Phosphorus

EP = Extractive Phosphorus

PUR = Phosphorus Uptake Rate

APA = Alkaline Phosphatase Activity

will have the major influence on the growth rate (μ).

- (c) The storage compounds, of polyphosphates, are produced when the demand for initial structural and functional compounds has been satisfied.

The relationship between the TTP and the growth rate has received much attention (Ahlgren, 1980; Auer & Canale, 1982b; Droop, 1973; Fuhs, 1969; Gotham & Rhee, 1981) and the hyperbolic curve illustrated in figure 2.6 is usually designated the Droop function (Droop, 1973) and described mathematically as follows:-

$$\mu = \mu_m \left(\frac{Q - Q_0}{Q} \right) \quad (2.11)$$

μ = specific growth rate (day^{-1})

μ_m = Maximum specific growth rate (day^{-1})

Q = Total Tissue Phosphorus (TTP) (% P)

Q_0 = Critical TTP (% P)

Thus, as Q approaches Q_0 then μ will effectively drop to zero, and as Q increased above Q_0 , μ approached μ_m .

The ecological consequence of the hyperbolic shape of figure 2.6 is that a high growth rate is maintained for as long as possible in the face of dwindling TTP caused by a lack of available external P. The influence of external P is discussed in section 2.6.2.3

2.6.2.2 Extractive Phosphorus (EP)

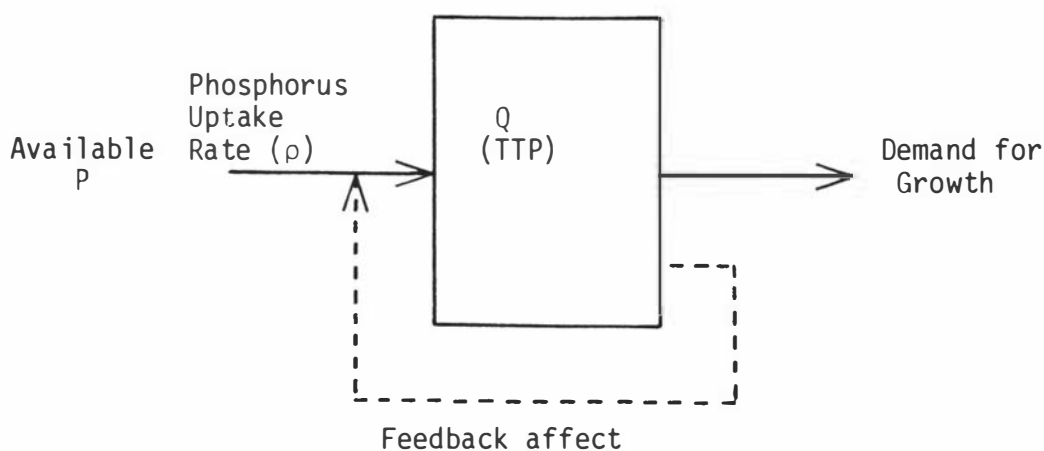
Algae can store P beyond the strict nutritional requirements of the species. This P is stored as polyphosphate compounds which are used as a basic P store during normal metabolic activities as well as being a supply in times of external P scarcity (Kuhl, 1974). This surplus can be estimated by a standard mild extractive technique (See section 3.6.1) (Fitzgerald & Nelson, 1966). It should be remembered that EP is only an estimation of the storage or surplus P, and will include portions of both the functional and structural components. It should not be confused with the actual surplus component illustrated in figure 2.6. However, the EP has been shown to be a reliable quantitative measure for assessing P limitation (Fitzgerald & Nelson, 1966; Gotham & Rhee, 1981; Lin, 1977). The

distinction should be made between the EP and the actual surplus P in the algal cell. The EP does not drop to zero at zero growth rate, due to EP including some of the structural P (see figure 2.6) (Fuhs, 1969; Rhee, 1973). The minimum EP, denoted EP_0 in figure 2.7 is analogous to the critical TTP or Q_0 , and while the theoretical surplus portion of the TTP is zero the EP test still gives the generally accepted minimum value of 0.02 % P (Lin, 1971; Mantai *et al* 1982)(See table 2.5).

2.6.2.3 Phosphorus Uptake Rate (PUR)

If the TTP is depleted algae will compensate by increasing the PUR to restore supplies and maintain high growth rates (See section 2.6.2.1, figure 2.9 and equations 2.11 and 2.12). The PUR will then be controlled by two systems, illustrated below:-

FIGURE 2.8: The factors controlling the phosphorus uptake kinetics (after Auer & Canale, 1982a).



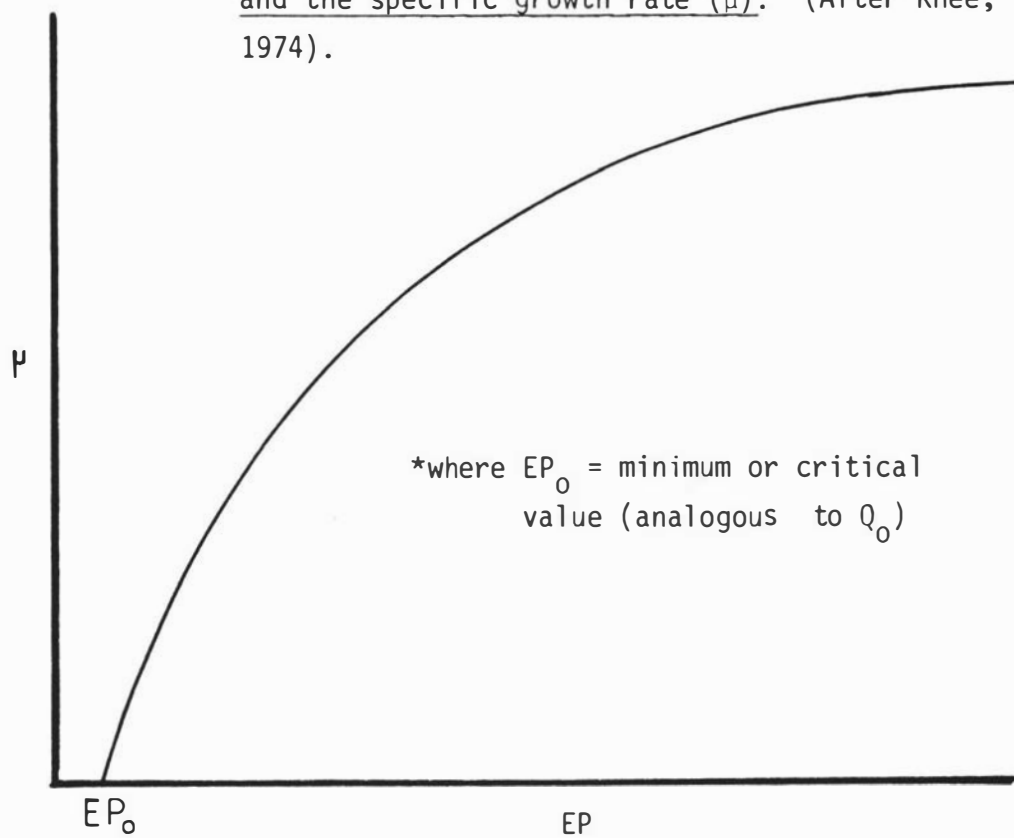
Thus, the PUR (ρ) is governed by two quantities:-

- (a) The TTP (Q) through the following equation:-
(after DiToro, 1980)

$$\rho = \rho_m \left(\frac{K_q}{K_q + (Q - Q_0)} \right) \quad (2.12)$$

K_q = Half-saturation constant for uptake as a function Q
(See figure 2.9)

Figure 2.7 The relationship between Extractive Phosphorus (EP)
and the specific growth rate (μ). (After Rhee,
1974).



Thus, as $Q \rightarrow Q_0$, $\rho \rightarrow \rho_m$

and, as $Q \rightarrow Q_{\max}$, $\rho \rightarrow \rho_{\min}$.

The TTP is more commonly monitored than EP as the TTP, in contrast to the EP, is a well defined biological quantity and there is a large body of comparable historical data.

(b) The available external P through the following equation:-

$$\rho = \rho_m \left(\frac{P}{k_m + P} \right) \quad (2.13)$$

P = External available P

k_m = half-saturation constant for uptake as a function of external available P (See figure 2.10).

Thus, as $P \rightarrow \infty$, $\rho \rightarrow \rho_m$

and, as $P \rightarrow 0$, $\rho \rightarrow 0$

The shape of the curve illustrated in figure 2.10 varies dramatically between algal species. The kinetic coefficients, ρ_m and k_m , will govern interspecies nutrient competition and can alter with the physiological state of the species. (For a more detailed discussion of the ecological consequences of various ρ_m and k_m combinations see:- Auer & Canale, 1982b; Nalewajko & Lean, 1980; Rosemarin, 1982). Combining equations 2.12 and 2.13 the following results:- (Auer & Canale, 1982a)

$$\rho = \rho_m \left[\left(\frac{P}{k_m + P} \right) \left(\frac{k_a}{k_q + (Q - Q_0)} \right) \right] \quad (2.14)$$

Thus the two factors, external available P and TTP are combined. A maximum value of the PUR will be achieved when external available P is at a maximum and TTP approaches the critical concentration. A minimum PUR will occur at high TTP.

The Phosphorus Uptake Rate (PUR) has been shown to be a function of the EP as illustrated in figure 2.11(A similar relationship to that depicted in figure 2.9 for PUR and TTP).

It is not practical to identify a critical PUR, however, values greater than approximately $6 \mu\text{g P}(10\text{mg})^{-1} \cdot \text{hr}^{-1}$ have been identified

Figure 2.9 The relationship between the Phosphorus Uptake Rate (PUR) and the Total Tissue Phosphorus (TTP)
(after Auer & Canale, 1982b)

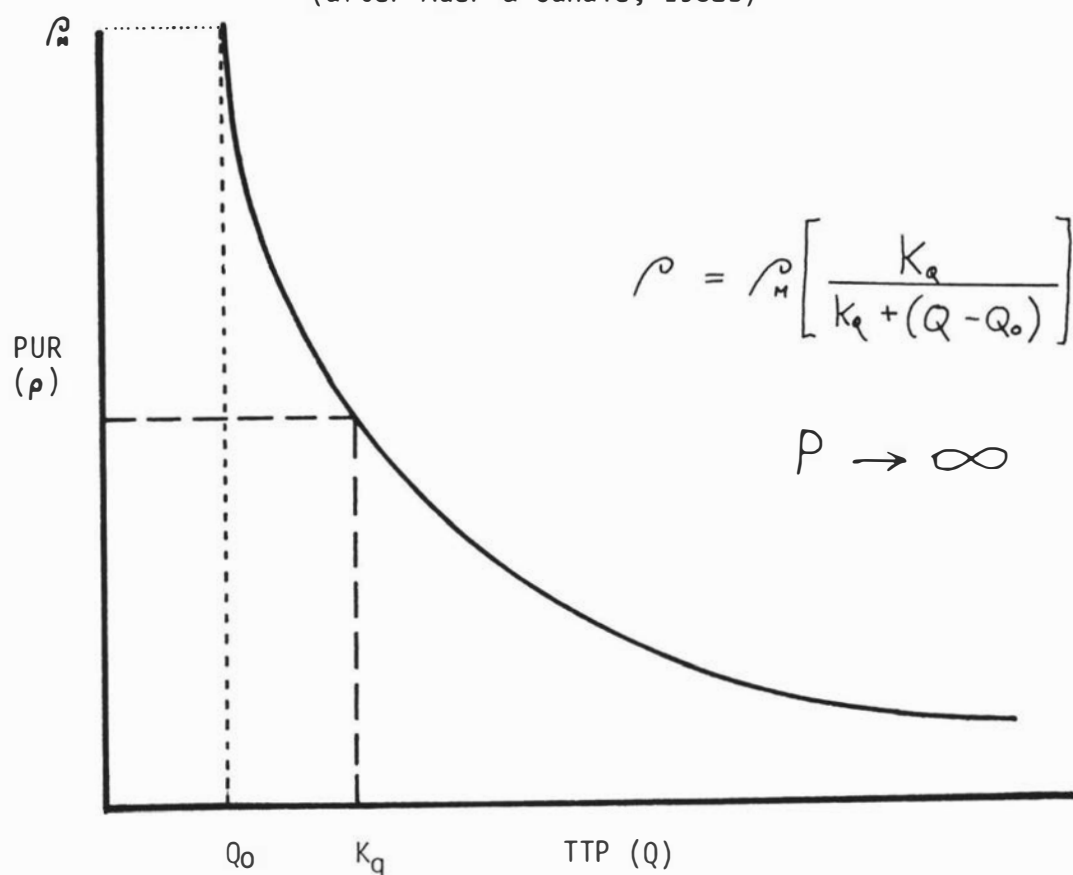


Figure 2.10 The relationship between Phosphorus Uptake Rate (PUR) and the external available Phosphorus (P) (after Auer & Canale, 1982b)

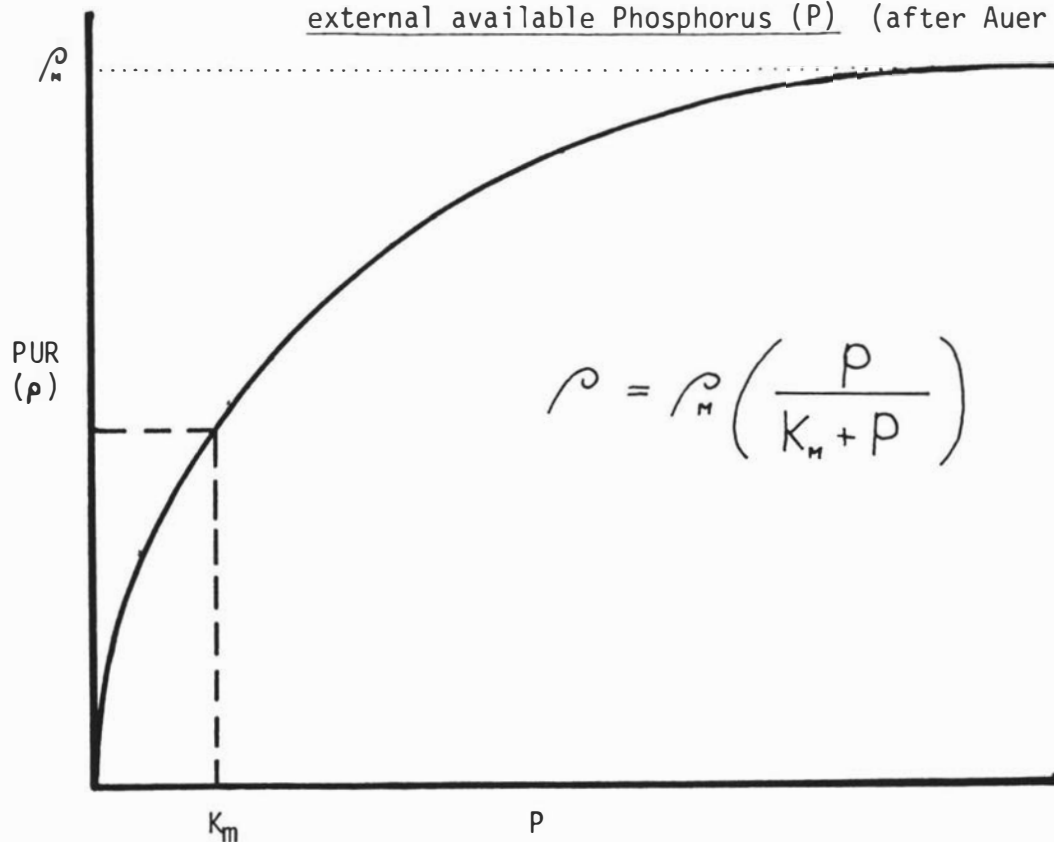
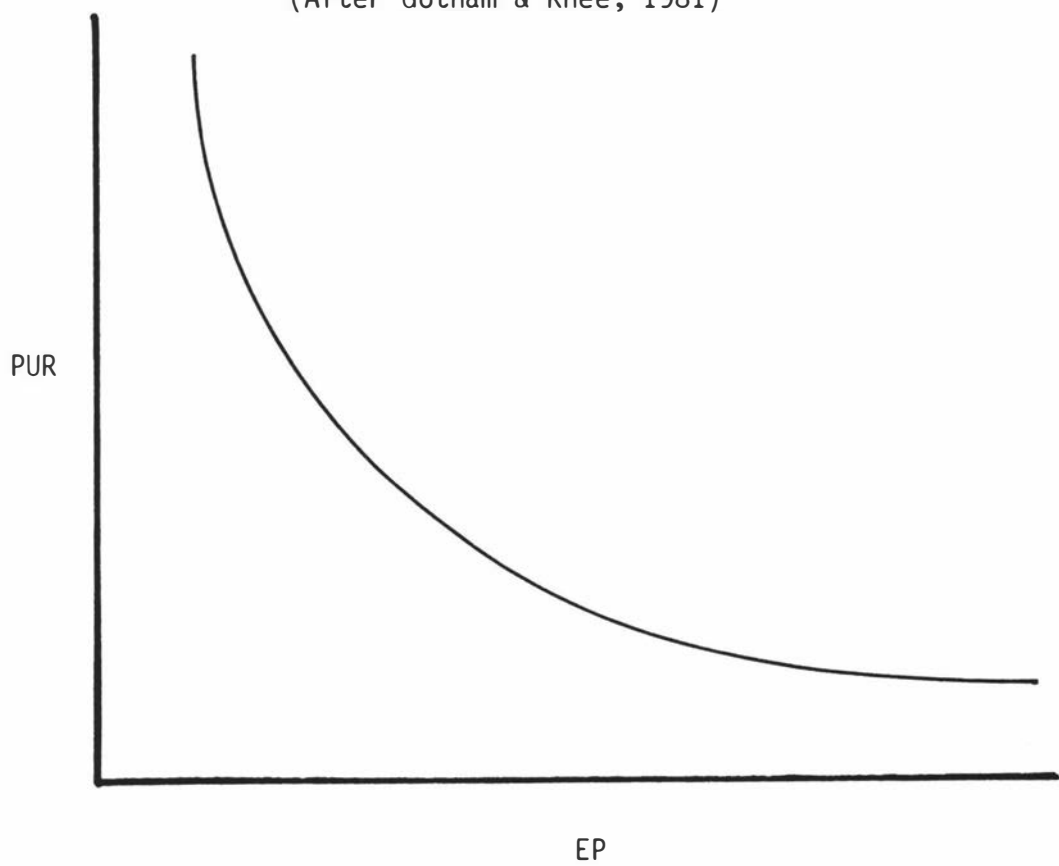


Figure 2.11 The relationship between Extractive Phosphorus (EP) and the Phosphorus Uptake Rate (PUR)
(After Gotham & Rhee, 1981)



as associated with P limitation in *Cladophora* and activities less than $3 \mu\text{g P (10 mg)}^{-1} \text{ hr}^{-1}$ associated with surplus available P. (Auer & Canale, 1982b) (See table 2.5).

In any discussion of algal P uptake dynamics, account should be taken of the excretion (efflux or leakage) of P compounds from the cell. (Brown & Harris, 1978; Law *et al*, 1976). The phenomenon of organic P excretion has been observed in some freshwater and marine phytoplankton. The rate of Dissolved Organic P (DOP) excretion has been observed to be proportional to light intensity and the age of some algae. P limitation was seen to reduce DOP excretion in some species while in others nitrogen limitation caused reductions in DOP excretion. (Kuenzler, 1970; Lean & Nalewajko, 1976). Published studies have concentrated on identifying the phenomena casual relationships, however, it has been noted that the rate of DOP excretion can be as much as 10% of the (net) PUR. No studies on the quantitative relationship between the rate of algal DOP excretion and the TTP appear to have been reported, but two basic relationships are recognized: (Lean & Nalewajko, 1976):-

- (a) When TTP equals Q_0 , excretion of DOP (E) will be zero.
- (b) When TTP increases, E will also increase. A maximum may be reached at the maximum TTP concentration.

2.6.2.4 Alkaline Phosphatase Activity (APA)

Many algae are known to have repressible phosphatase enzymes able to hydrolyse P from exogenous P esters when growth is limited by the external P supply. Algae that are P limited have been observed to have 25-30 times more Alkaline Phosphatase Activity (APA) than algae with surplus external available P (Fitzgerald & Nelson, 1966; Kuenzler & Perras, 1965) (See table 2.5). Thus high APA will be indicative of a past P shortage (See section 4.3). Elevated APA can persist for 1-3 days after a P limiting situation has been relieved. (Healey, 1973b; Mantai, 1978). Levels of APA around $200 \text{ Enz. Units mg}^{-1}$ have been identified in *Cladophora* during P shortage. A surplus P environment usually produces APA of approximately $20 \text{ Enz. Units mg}^{-1}$ (Mantai, 1978; Mantai *et al*, 1982)

An important feature of this P NAT is that as it is not under the control of any internal P component, any relationship between TTP, EP or PUR and APA is only due to all the parameters being linked to the supply of external available P. (cf. Healey, 1973b) (Bone, 1971; Healey & Hendzel, 1979). High APA is not halted by product inhibition. The addition of orthophosphate to a P-limited alga will stop further synthesis but elevated APA will only be lowered as the growth of the alga dilutes the activity (Fitzgerald & Nelson, 1966) (Laboratory continuous cultures will also lose some of the high APA alga).

The slow response of the APA to a relief from a P-limiting situation, relative to the speed of the response to a P-limiting situation, will have important consequences for interpretation of field data. (See section 4.3).

2.6.3 Nitrogen

2.6.3.1 Total Tissue Nitrogen (TTN)

Analogous to TTP, this test indicates the nitrogen (N) that has been available to the alga. There is also a critical concentration below which growth will be limited by the nitrogen supply. (Gerloff & Skoog, 1954) (See table 2.6). In contrast to P, N is not stored in isolated structures, rather it is distributed between free amino acids, and the amino acid composition of algal peptides and protein (Morris, 1974).

During N-limitation the proportion of these two major components changes, free amino acids decreasing to allow more of the N to be involved in structural and functional components. (Maske, 1982) The growth rate is a function of the TTN, and follows the Droop formula as discussed in section 2.6.2.1. (See equation 2.11 and figure 2.6) (Goldman & McCarthy, 1978).

2.6.3.2 Ammonium Absorption Rate (AAR)

The rate at which ammonium N is assimilated is 4-5 times greater in algae that are N-limited than in algae with an adequate

nitrogen supply. (Fitzgerald, 1968) (See table 2.6). The Ammonium Absorption (Uptake) Rate (AAR) is analogous to the PUR and similar relationships between AAR and ammonium N and PUR and external available P have been documented (Wheeler *et al*, 1982). A linear relationship has been observed between AAR and the TTN for a marine diatom (Goldman & McCarthy, 1978) (cf. figure 2.9) whereas, in other algae the relationship appears to follow that outlined in figure 2.9. (Wheeler *et al*, 1982).

TABLE 2.6: Values of Nitrogen Nutrient Available Tests for *Cladophora* associated with a limiting or surplus situation.

NAT	Activity or Level associated with		Reference
	Limitation	Surplus	
TTN (% N)	<1.1	>1.1	Gerloff & Fitzgerald (1976)
AAR ($\mu\text{gN}(10\text{mg})^{-1}\text{hr}^{-1}$)	25	5	Fitzgerald (1969a)

KEY: TTN = Total Tissue Nitrogen
AAR = Ammonium Absorption Rate

2.7 Chemical estimations of biologically available nutrients

2.7.1 Phosphorus

Orthophosphate P ($\text{PO}_4\text{-P}$) is the only P form directly assimilated by algae (Lean, 1973; Sonzogni *et al*, 1982). Dissolved reactive phosphorus (DRP) is commonly used as an estimate of the $\text{PO}_4\text{-P}$. (APHA, 1975; Stainton, 1980) (See section 2.5.3.4) However, there are two major problems involved in relating an algal growth response to the DRP of a water sample.

(a) The DRP analysis:

This involves an acid hydrolysis that releases $\text{PO}_4\text{-P}$ from some organic P compounds. Thus the DRP will often overestimate the immediately available $\text{PO}_4\text{-P}$ in a natural water sample (Downes & Paerl, 1978; Stainton, 1980).

Most DRP analytical methods do not include any recommendations on the sample volume or filtration pressure. Large sample volumes and high filtration pressures may introduce significant errors. (Tarapchak *et al.*, 1982) (See section 3.5)

(b) The transformation of P compounds:

There is a flux between various P forms in natural waters, mediated by chemical and biological mechanisms. The sediments, attached biota and plankton of aquatic systems will all have a role in the uptake, release and transformation P compounds. One potentially important transformation is the hydrolysis of organic-P compounds mediated by repressible algal and bacterial phosphatases produced in response to a lack of $\text{PO}_4\text{-P}$. (See section 2.6.2)

This phenomenon can cause interpretive difficulties when the quantity of biologically available P present in a test medium is being assessed with a standardised bioassay procedure. (APHA, 1975). Some P may only become available following transformations occurring during the test period of the bioassay. (Twinch & Breen, 1982)

2.7.2 Nitrogen

There are similar interpretive difficulties involved in trying to reconcile N species with biologically available nitrogen. The DIN estimates measure the major portion of easily assimilated N compounds in freshwaters. Other nitrogen compounds that can be assimilated by many algae, such as amides, amino acids, purines and pyrimidines are usually quantitatively less important in well oxygenated running water. (McCarthy, 1980; Morris, 1974; Stumm & Morgan, 1981). The transformation of dissolved organic nitrogen compounds can alter the total available N in a similar fashion to the P situation (Morris, 1974; Twinch & Breen, 1982).

2.8 General conclusions drawn from the literature review

The salient features of this survey are noted as follows:-

- (i) *Cladophora* proliferations have been reported worldwide in both lotic and lentic environments, and have been

responsible for many water quality and water use problems.

- (ii) The unique life history and physical attributes of *Cladophora* are unalterable facts that enable it to be a successful opportunist.
- (iii) There are a variety of environmental factors that are involved in establishing proliferations. Of all the nutrients investigated, phosphorus was most frequently cited as blameworthy.
- (iv) Nutrient Availability Tests can be used to directly assess the nutritional status of *Cladophora*. These can be used to test hypotheses made on the basis of river nutrient concentrations.

CHAPTER 3

METHODS

3. METHODS

3.1 Algal identification

The dominant alga in the Manawatu River was identified as *Cladophora glomerata* (L.) Kütz using the following criteria:

- (a) Basic keys of Palmer (1980) and Prescott (1970).
- (b) Detailed keys of Van den Hoek (1963) and Soderstrom (1963).
- (c) Observation of the zoospore morphology (Van den Hoek, 1963).
- (d) Comparison with photographs in the literature (e.g. Bellis, 1968; Chudyba, 1965; Dean, 1966; Moore & Traquair, 1976; Pitcairn & Hawkes, 1973; Scott & Bullock, 1975).
- (e) Comparison with a laboratory cultured sample provided by the culture centre for Algae and Protozoa Cambridge (U.K.)

C. glomerata is a highly branched filamentous green alga usually found growing attached to stable substrate, but filaments can be sloughed-off and become free-floating. The thallus is coenocytic, multinucleate and the chloroplast is present as a reticulate sheet. The cell walls are thick and lamellate without any mucilage covering (Hence their popularity with epiphytes)(Appendix 1, photograph 5). Reproduction is by biflagellate asexual zoospores. Vegetative growth occurs both through apical and intercalary division. Identification to species level, based on morphology, can be difficult because of the highly variable cell dimensions. "The phases in the life history of plants growing in the same locality may show an extremely different morphology, and various environmental conditions may produce their own ecological modifications". (Van den Hoek, 1963).

Other algae were identified only to genus level using the keys of Palmer (1980) and Prescott (1970).

3.2 Algal Biomass and distribution

There are many techniques available for measuring attached algal biomass (Sládecková, 1962; Wetzel & Westlake, 1969; Whitton, 1975). These can be divided into those that sample the natural substrate (Blum, 1957; Carnes & Millner, 1980; Douglas, 1958; Ertl, 1971;

Pitcairn & Hawkes, 1973; Saunders & Eaton, 1976), and those that utilize artificial substrates such as glass, wood, stone or plexiglass (Austin *et al*, 1981; Castenholz, 1961; Cooke, 1956; Grzenda & Brehmer, 1960; Lowe & Gale, 1980; Marshall, 1978; Sládeček & Sládečková 1964; Tippet, 1970). As these two strategies have advantages and disadvantages both were investigated.

3.2.1 Artificial substrates

Two types of artificial substrate were investigated:

- (a) Cut stones: Large river stones were cut to produce slices of approximate standard microscope slide dimensions (75mm x 25mm x 2-5mm). These were fixed to heavy stainless steel sheets (approximately 1m x 0.3m), which were then suspended in the water column or placed on the river bed.
- (b) Concrete plates: (approximately 150mm x 100mm x 15mm) were obtained that had uniformly wrinkled and fizzured surfaces, similar to natural river substrate observed to support prolific *Cladophora* growth. These were distributed in areas known to support heavy proliferations.

3.2.2 Natural substrates

The *Cladophora* were sampled using a random quadrat sampling technique (Blum, 1957; Wood, 1975). Maximum growth areas of about 100m² were identified and these were sampled using ten random throws of a 0.04m² quadrat. The rationale for these choices have been detailed elsewhere. (Freeman & McFarlane, In Press) (Appendix 4)

3.2.3 Measurement techniques

The frequency of sampling was usually weekly, however, when algal growth was rapid this increased to twice weekly. The algal material originating from the quadrat or the artificial substrate was either scraped off *in situ* or, when the biomass was minimal, first transferred to the bank via a plastic bucket to ensure all the algae were collected. The collected algae were rinsed with tap water at the laboratory and any visible non-algal material was removed. A variety of biomass measurement parameters were considered prior to dry weight being selected (See section 2.5.5). No special treatment

of the algae was needed during the brief transport period between sampling and processing (maximum time 2 hours). The drying temperature used was 64°C. A constant dry weight is not achieved at higher temperatures, due to the loss of volatile organics (Carnes & Millner, 1980).

Free floating filaments (FFF) of *Cladophora* were measured in a fashion similar to that outlined by Wong & Clark (1976). A square wire frame (0.25 m²) with a wire or plastic mesh (diameter = 3mm) was positioned vertically in the river flow for a period of 24 hours, after which the entangled filaments were collected and dried at 64°C until constant weight was achieved.

The algal biomass removed from the screen was converted to a river concentration (mg DW m⁻³), using the following assumptions:

- (a) The biomass collected at the one position in the river was an accurate representation of the whole upstream river.
- (b) There was no diurnal variation in the sloughing rate.

A sample calculation using data collected on 27-28/3/83 is presented below to demonstrate the method used:

$$\begin{aligned} \text{Rate of FFF capture} &= 27.3\text{g (0.25 m}^2\text{)}^{-1} \text{ (24 hours)}^{-1} \\ \text{(vertical area)} &= 109.2 \text{ g DW m}^{-2} \text{ (24 hours)}^{-1} \\ &= 1.26 \text{ mg DW m}^{-2} \text{ s}^{-1} \end{aligned}$$

$$\begin{aligned} \text{River cross-sectional area} &= 48\text{m} \times 0.9\text{m} \\ &= 43.2 \text{ m}^2 \end{aligned}$$

$$\begin{aligned} \text{Mass flow of FFF} &= 43.2 \times 1.26 \text{ mg DW s}^{-1} \\ &= 54.4 \text{ mg DW s}^{-1} \end{aligned}$$

$$\text{Average river flow (27-28/3/82)} = 17.2 \text{ m}^3 \text{ s}^{-1}$$

$$\begin{aligned} \text{Therefore average concentration of FFF} &= \frac{54.4}{17.2} \\ &= 3.16 \text{ mg DW m}^{-3} \end{aligned}$$

The amount of river phytoplankton was measured periodically by extracting the GF/C filtered material, from a two litre sample of

river water, in warm methanol for a chlorophyll-a estimation (Golterman *et al*, 1978).

The distribution of *Cladophora* and other periphyton along the Manawatu River and some of its tributaries was investigated periodically on a semi-quantitative basis using a percentage cover scale similar to that of Thomas & Schanz (1976) (Figure 3.1)

3.3 River site characteristics

3.3.1 Physical characteristics

Physical characteristics of the various sites were important for two aspects of the study. Firstly, so that the importance of various lotic physical features to *Cladophora* development could be recognized, and secondly, so that single station productivity analysis could be performed (Section 3.8.2). The characteristics were measured as follows:

- (a) Reach Width: This was initially measured with a line, and stakes were then positioned to determine changes.
- (b) Reach and Site Current Velocity: The average reach velocities were estimated with floats over a distance of 200m, a conversion factor of 0.7 was used to calculate the bulk velocity (Hynes, 1970; Wood, 1975). Specific site current velocities, as well as checks on the average reach velocities, were carried out with pygmy current meter (AOTT Kepton Meter).
- (c) The daily mean riverflow was provided by the Manawatu Regional Water Board. This was measured with a permanent gauging station, the rating curve (i.e. the relationship between gauge height and river flow) of which was checked weekly.
- (d) The average depth was calculated using the data from (a) - (c), using the following equation (Hynes, 1970):-

$$F = W \times H \times U \quad (3.1)$$

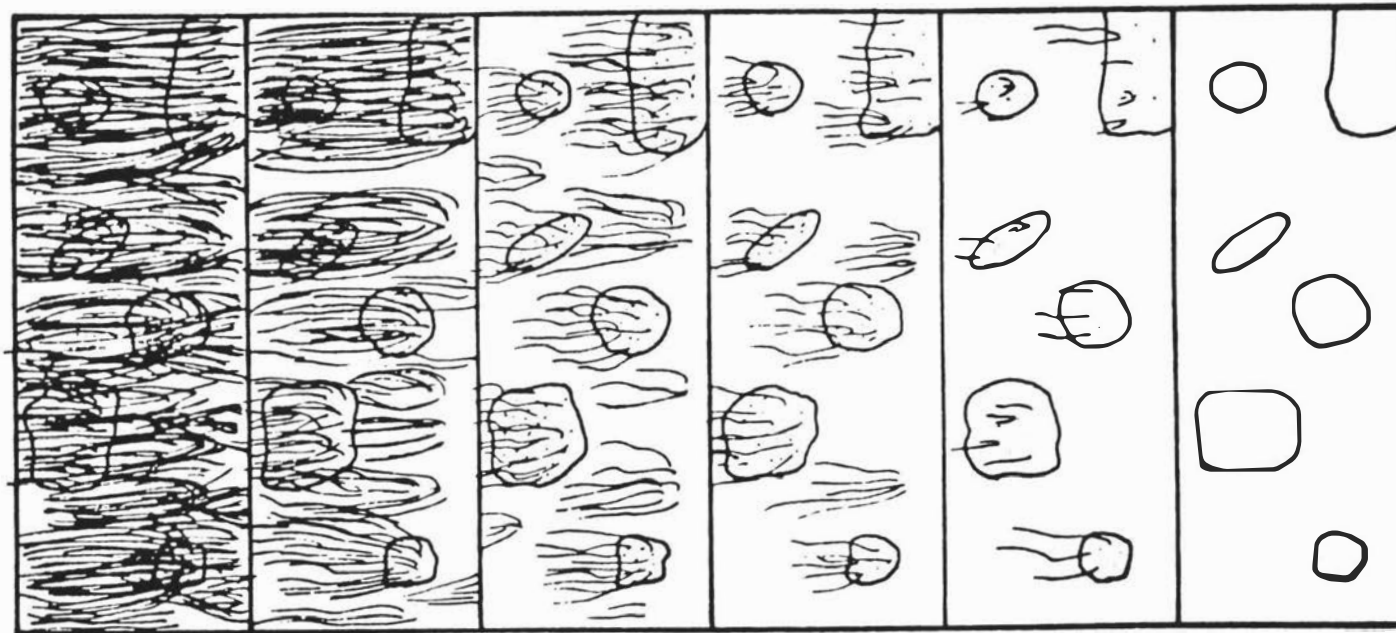
where: $F = \text{River flow (m}^3 \text{ S}^{-1}\text{)}$

$W = \text{Average reach width (m)}$

$H = \text{Average reach depth (m)}$

Figure 3.1 Percentage cover scale for attached filamentous algal distribution.

(Based on Thomas & Schanz , 1976)



% Cover

80-100

60-80

30-60

10-30

0-10

0

Number

5

4

3

2

1

0

U = Average reach current velocity (m s^{-1})

Thus, rearranging equation (3.1):-

$$H = \frac{F}{W \times U} \quad (3.2)$$

- (e) The reaeration coefficient (k_2) was calculated using the simple predictive equation of O'Connor and Dobbins (1958).

$$k_2 = 3.74 U^{0.5} H^{-1.5} \text{ (base e, day}^{-1}\text{)} \quad (3.3)$$

This equation is suitable for a river with the low flow characteristics of the Manawatu. (Wilcock, 1982). No account was made for the variation of k_2 with temperature (see Section 3.8.2).

While there are moves to try and maintain consistency in the use of day^{-1} as the units of expression for k_2 , (Wilcock, 1982) for the purposes of productivity analysis it was expressed as hours^{-1} . Equation (3.3.) was therefore modified to:

$$k_2 = 0.156 U^{0.5} H^{-1.5} \text{ (base e, hours}^{-1}\text{)} \quad (3.4)$$

All sites were situated well away from any bankside trees and throughout the year were free from any shading affects. No significant temperature differences have been observed between the 3 upstream sites, when measured simultaneously.

3.3.2 Intersite substrate comparison

This exercise was designed to explore the distribution of stone sizes at the three main study sites. A random quadrat sampling strategy, similar to that used for algal biomass was used. (See section 3.2.3). Each site was sampled by twelve random throws of a 0.04 m^2 square quadrat. All stones greater than 1cm average diameter found lying on the surface in each throw were collected, their average diameter measured and each stone counted into a specific size class. The arithmetic mean of each class was then used for calculations to assess the total area presented, by each class. The stones were assumed to be flat discs with a radius of half the average diameter and the exposed area was calculated using the

simple equation:-

$$\text{Area} = \pi (\text{radius})^2 \quad (3.5)$$

This is obviously an oversimplification of the real situation, but nevertheless serves as a useful basis for an intersite comparison.

3.4 Light measurements

Light data were obtained from a variety of sources during the course of this study. The location of each source and type of measurement are detailed below:-

- (a) Daily integrals (Langleys d^{-1}) were measured at the Horticultural Research Centre, Ministry of Agriculture & Fisheries, Levin approximately 50km south-west of Palmerston North.
- (b) Daily integrals (kW hours) were measured by the Plant Physiology Division, Department of Scientific and Industrial Research, Palmerston North.
- (c) Daily underwater integrals ($\text{E m}^{-2} \text{d}^{-1}$)(PAR) were measured at 30cm depth, 5m upstream of site M. The output from a quantum meter (LI.COR LI 185B Quantum/Radiometer/Photometer) was continually recorded on a TOA EPR 200A chart recorder. The integral was calculated with the aid of a digital planimeter (Hewlett Packard HP 9825A) made available by the Soil Conservation Science Centre, Ministry of Works & Development, Aokautere.

3.5 River nutrients

These were sampled primarily at the three major sites T, D and M (See figure 1.2) using acid-cleaned 2l plastic containers. Samples were collected at least weekly between 0800 and 1000 hours. Each sample was collected by wading in as close to the centre of the river as possible, and taking a composite sample of four 500 ml sub-samples over a one minute period from a depth of approximately 30 cm.

Storage and preservation techniques varied during the study depending on the specific nutrients being examined. When only total nutrients were being analysed the samples were preserved immediately by acidification with 1ml of concentrated sulphuric acid. When dissolved

nutrients were being monitored the samples were either filtered on-site, using pre-washed 0.45 μm membrane filters, or stored on ice until filtering at the laboratory. The vacuum pressure applied during filtration was always less than 500mm Hg, and the sample volume used was usually 200ml. These quantities are slightly higher than the recommended maximum values of 300mm Hg and 100mls. (Tarapchek *et al*, 1982). The time between taking the first sample and processing at the laboratory was usually two to three hours.

3.5.1 Phosphorus

(a) Total Phosphorus (TP)

This was initially analysed using an ammonium persulphate digestion (APHA, 1975) and the resultant reactive phosphate was estimated using the method of MOWD, (1980a) based on that of Murphy & Riley (1962). During the 1981/82 season, the digestion step was changed to one that allowed simultaneous determination of both total phosphorus and nitrogen (see Section 3.5.2).

(b) Total Dissolved and Dissolved Reactive Phosphorus (TDP, DRP)

These components of the total phosphorus are considered by many to be the best practical indicators of the phosphorus available to algae (Healey, 1973b; Lin, 1977; Wong & Clark, 1976 and see Section 2.7).

The TDP test involves the persulphate digestion step, detailed above, being carried out after filtration. The resultant orthophosphate can be analysed by the molybdenum blue method with or without the addition of hexanol extraction for low phosphorus samples. (MOWD, 1980a). The DRP test simply involves the analysis of the 0.45 μm filtrate for reactive phosphorus.

All glassware associated with the phosphorus tests was cleaned with 50% (v/v) H_2SO_4 and washed repeatedly in distilled-deionised water before use.

3.5.2 Nitrogen

(a) Total Nitrogen (TN)

This was initially analysed by the method of Soloranzo & Sharp, (1980) using an alkaline potassium persulphate to oxidize the vast majority of nitrogen containing compounds to nitrate. Compounds containing N-N bonds, N=N bonds and NH=C groups have been shown to be resistant to persulphate oxidation (Nyda hl, 1978). This method was modified later in the study to be able to allow simultaneous TP and TN determinations. The procedure followed was similar to that detailed by Langner & Hendrix (1982). The process occurs in two stages:-

- (i) Nitrogen is oxidized in the initially alkaline medium. As the digestion proceeds the resulting bisulphate ions lower the pH and permit:
- (ii) Digestion of the phosphorus-containing compounds.

Subsamples are neutralized and analysed for phosphate and nitrate. The resulting nitrate was analysed using two cadmium reduction columns (APHA, 1975)

(b) Nitrate (and Nitrite)

In the 1982/83 season the dissolved inorganic nitrogen species were monitored. Nitrate was analysed as mentioned above, by reduction to nitrite, which was then determined by the sulphanilic acid/N.(1-naphthyl)-ethylenediamine dihydrochloride method. (APHA, 1975)

Preliminary studies found that very low nitrite levels existed upstream of any discharges.

(c) Ammonia

This was determined by a modified alkali-phenol method, sensitive over a wide concentration range (MOWD, 1980b; Scheiner, 1976). Samples collected from upstream of the discharges were collected as described in section 3.5, and downstream samples, collected either 'by hand' or with the aid of a continuous automatic sampler (Manning S4040)(kindly lent by

the M.R.W.B.), were preserved with 1ml conc. $H_2SO_4/21$ sample.

3.5.3 Quality assurance of chemical methods

Regular analyses were carried out to gauge the accuracy and precision of the basic chemical tests.

The results of one season's checks are presented in table 3.1.

TABLE 3.1: Quality assurance of analytical methods, 1981/82

Quality Assurance description	DRP	Nitrate	Ammonia
True (Gravimetric) (τ) Concentration ($\mu g\ l^{-1}$)	10.0	200	100
Number of Measurements (N)	20	20	20
Measured mean concentration (\bar{x}) ($\mu g\ l^{-1}$)	10.4	190	113
Range	8.7-13.0	179-200	95-156
Standard Deviation (s) ($\mu g\ l^{-1}$)	1.1	5.2	16
Variation Coefficient (%)	2.5	0.6	3.2
Relative Error (%)	4.0	5.3	11.5
Limit of Detection $\mu g\ l^{-1}$	1.5	2.0	15

$$\begin{aligned} \text{Variation Coefficient} &= \frac{\text{St. Error}}{\bar{x}} \times 100 \\ &= \frac{(s/\sqrt{N})}{\bar{x}} \times 100 \end{aligned} \quad (3.6)$$

$$\text{Relative Error} = \frac{\sqrt{(\tau - \bar{x})^2}}{\tau} \cdot 100 \quad (3.7)$$

The recovery efficiencies of the total nitrogen and total phosphorus tests were tested using EDTA ($CH_2.N(CH_2.COOH)CH_2.COONa \cdot 2H_2O$) and DL-0-Phosphoserine ($NH_2.CH(COOH).CH_2O.PO_3H_2$) respectively. These are detailed in table 3.2:-

TABLE 3.2: Accuracy and Precision of nitrogen and phosphorus digestion methods

Recovery, accuracy and precision	EDTA Nitrogen	DL-0-Phosphoserine phosphorus
Concentration N or P ($\mu\text{g l}^{-1}$)	200	200
Number of tests	4	4
Mean Recovery (%)	92.6	90.4
Standard Deviation (%)	3.6	4.6
Variation Coefficient (%)	1.9	2.5

3.6 Nutrient Availability Tests (NATs)

Algal material was collected from at least three locations at each site, mixed and washed vigorously in the river before being placed in ice for transport to the laboratory. Samples were usually collected between 0800 and 1000 hours.

The time between taking the first sample and the start of its analysis in the laboratory was about 1 hour. In the laboratory, the algae were vigorously and quickly washed in (room temperature) D11 media (See table 3.3) minus phosphorus (-P) to remove the vast majority of any epiphytes before the NATs were begun. No additions were made to compensate for the loss of K in K_2HPO_4 as sufficient was supplied as KCl.

3.6.1 Phosphorus

(a) Total Tissue Phosphorus (TTP)

Cladophora dried at 64°C , was digested according to the method described in section 3.5.1. The amount of oxidant was increased to 250 mg $\text{K}_2\text{S}_2\text{O}_8$ /mg DW alga to cope with the large quantity of organic material. (Langner & Hendrix, 1982).

(b) Extractive Phosphorus (EP)

The method used was that of Fitzgerald and Nelson (1966). When employed in the laboratory culture experiments, the limited algal material available necessitated a smaller (25 ml) medium volume. D11 (-P) medium was used, and the resultant

reactive phosphate analysed by the method detailed in section 3.5.1.

(c) Phosphorus Uptake Rate (PUR)

The method followed was basically that detailed by Healey (1973b). This was adapted for a filamentous algal situation in which a relatively large amount of algal material was available. Approximately 20-50mg of thoroughly rinsed *Cladophora* were placed in 110ml of 0.5 or 1 mg $\text{PO}_4\text{-P l}^{-1}$ D11 media (depending on the suspected nutritional status of the alga). This was incubated, with occasional shaking, in the dark for two hours. This procedure also allowed simultaneous determination of the Ammonia Absorption Rate (See section 3.6.2). The algal material was collected on pre-washed, dried and weighed GF/C filters and the supernatant analysed for reactive phosphate.

(d) Alkaline Phosphatase Activity (APA)

The method followed was basically that of Fitzgerald and Nelson (1966), with the exception that the supernatant was analysed immediately after incubation without the addition of any orthophosphate, and that the cultures were agitated throughout the incubation period. The results are expressed as Enzyme Units which are defined as the amount of enzyme liberating 1nmole of nitrophenol hr^{-1} . (Fitzgerald & Nelson, 1966).

3.6.2 Nitrogen

(a) Total Tissue Nitrogen (TTN)

Cladophora dried at 64°C , was digested according to the methods described in section 3.5.1. Increased amounts of oxidant were used (See section 3.6.1) to cope with the higher levels of organic material encountered.

(b) Ammonia Absorption Rate (AAR)

The method used was that described by Fitzgerald (1968) adapted to run simultaneously with the PUR procedure. (See section 3.6.1) The initial ammonia concentration was either 0.5 or 1 mg $\text{NH}_3\text{-N l}^{-1}$ depending on the suspected nutritional status of the alga.

Tris buffer was omitted from the D11 media used in this NAT as it interferes with the ammonia determinations. (Healey, 1979).

3.7 Laboratory culturing

3.7.1 Introduction

The aims of these experiments were to establish:-

- (i) The relationship between the NATs and the respective nutrient concentrations.
- (ii) The response times of the NATs to changes from a surplus to a limiting situation and vice versa.

While a continuous culture flowing system would be more similar to a river situation than a batch non-flowing system, the above relationships could be identified initially in batch cultures.

3.7.2 Isolation techniques

Attempts at culturing *Cladophora* began in April, 1981. Preliminary efforts were not successful, due to inadequate sample preparation and the lack of a reliable source of vitamins for which *Cladophora* has a demonstrated need (Gerloff & Fitzgerald, 1976; Hoffman & Gerloff, 1980; Moore & McLarty, 1975).

Initially, cultures were sustained in Gorham's medium (See table 3.3) made up with river water in an attempt to provide vitamins. Other strategies attempted to try and supply essential vitamins, included supplementing Gorham's medium with Millipore filtered settled sewage and filtered liquor from a mixed phytoplankton community.

Cultures were grown under 'super-gro' fluorescent tubes at light intensities of approximately $40-100 \mu\text{E m}^{-2}\text{s}^{-1}$ PAR ($25-50 \text{ W m}^{-2}$ PAR). Air was vigorously pumped through the culture vessels (media volumes of 250 ml, 1 litre, and 4 litres) with sufficient force to cause a circulating current without dislodging the filaments which were attached to the air hoses. Each bottle was

TABLE 3.3: A comparison of the media used to culture *Cladophora*

Gorham's (1)		D11 (2)	
Chemical	mg l ⁻¹	Chemical	mg l ⁻¹
NaNO ₃	496	Mg SO ₄ .7H ₂ O	100
K ₂ HPO ₄	369	KCl	30
MgSO ₄ .7H ₂ O	75	Na ₂ SiO ₃ .9H ₂ O	60
CaCl ₂ .2H ₂ O	36	Ca(NO ₃) ₂ .4H ₂ O	150
Na ₂ SiO ₃ .9H ₂ O	58	Na ₂ EDTA.2H ₂ O	12.5
Na ₂ CO ₃	20	FeSO ₄ .7H ₂ O	5.0
Ferric citrate	6	H ₃ BO ₃	1.0
Citric acid	6	ZnSO ₄ .7H ₂ O	0.1
EDTA	1	MnSO ₄ .H ₂ O	0.2
		Na ₂ MoO ₄ .2H ₂ O	0.025
		CuSO ₄ .5H ₂ O	0.025
		K ₂ HPO ₄	15
		NaHCO ₃	100
		Thiamine HCl (B ₁)	2.0
		Cyanocobalamin (B ₁₂)	0.002
		Biotin	0.002

REFERENCES:

1. Carnes & Millner, 1980
2. Hoffman & Gerloff, 1980

loosely stoppered with cotton wool.

The various culturing strategies used did not successfully maintain healthy specimens over long time periods. Deterioration was recognized by a generally dishevelled appearance, complete encrustation by epiphytes (including blue-greens), loss of pigmentation and other colour changes.

When a satisfactory supply of vitamins became available the D11 medium was employed (Table 3.3). This medium was initially more successful; however, during lengthy culturing periods a variety of epiphytes began to proliferate. Techniques pursued to try and produce uni-algal axenic cultures included:

- (i) Vigorous and multiple washing of filaments in media.
- (ii) Dragging filaments through agar, after which those observed microscopically, to be largely free of epiphytes were isolated as 'starters'.

Neither technique proved successful in the long term. The latter technique offered much promise, however, the starter biomass was insufficient for rapidly building up a large standing crop for nutrient tests. Attempts were made at establishing axenic cultures using:

- (1) Chlorine treatments (Lewin, 1959) which involved exposing the sample to 10 mg l^{-1} chlorine for approximately one hour.
- (2) Antibiotic treatments (Holshaw & Rosowski, 1973; Moore & McLarty, 1975) which involved exposure to 100 mg l^{-1} penicillin and 50 mg l^{-1} streptomycin sulphate.

After treatment the algae were transferred to liquid medium from which samples were taken periodically. Throughout all manipulations standard aseptic techniques were used. Filament and medium samples were tested for bacterial growth by plating out on to TGE (Tryptone glucose extract) agar and agarized (1.5%) D11 media.

Attempts to establish uni-algal axenic cultures of *Cladophora* were unsuccessful. Correspondence with researchers involved with

Cladophora in the Great Lakes area, notably Professor Gerloff (University of Wisconsin at Madison) revealed that successful uni-algal cultures may be possible, providing that work starts with a clean starter such as fresh zoospores. However, Professor Gerloff's staff have been unsuccessful in their attempts to make field samples of *Cladophora* axenic due to bacteria adhering to the cell walls. The inner surfaces of old sporangia act as especially impregnable reservoirs of bacteria.

During the winter of 1982, work was resumed on the laboratory culture techniques. Eventually, methods were developed to enable successful uni-algal growth of winter isolates (from a small tributary stream). Batch experiments were initially carried out in replicate (3-4) 250 ml conical flasks. This method proved unsatisfactory as the difference between replicates often made interpretation of trends impossible. To reduce this problem, the batch volume was increased to 10 litres to enable multiple sampling of one homogeneous population (Appendix 1, photograph 6). Successful growth was obtained by adhering to the following steps:

- (a) Only fresh isolates were used. These were visually free from epiphytes and rinsed vigorously in stream water.
- (b) In the laboratory the filaments were hand shredded into approximately 1cm lengths, washed vigorously and repeatedly (5 times) in distilled water and separated using a fine netting.
- (c) Algal culture vessels were pre-soaked in 10% (v/v) HCl, and washed thoroughly with distilled water before use.
- (d) When preparing the media only deionized water was used.
- (e) Separate EDTA, iron (acidified) and 'other metals' solutions were used.
- (f) Vitamin solutions were replaced monthly and always frozen when not in use.
- (g) Phosphorus levels were maintained below 2 mg P l^{-1} .
- (h) Only aeration was used to agitate and circulate the culture.
- (i) 'Tris' buffer concentrations were restricted to less than, or equal to a 4 mM final concentration, and buffered to pH

8.0 using 1 M HCl.

Once a culture was observed to be growing successfully, as demonstrated by macro and microscopic examination, the filaments were examined at least every two days and any contaminants and/or epiphytes were maintained at insignificant levels by adoption of one or more of the following:-

- (1) Removal of the test alga and vigorous agitation in distilled water.
- (2) Removal of the test alga and media followed by a thorough scrubbing and acid rinsing of the culture vessel walls to remove any attached growth.
- (3) A combination of (1) and (2) with fresh medium added.

The environmental conditions were similar to those detailed previously in this section, with the following modifications:-

Light was provided by three 40 W fluorescent tubes supplemented by two 150 W incandescent bulbs, and run on a 16:8 hr light:dark daily cycle. The light intensity at the culture surface was about $100 \mu\text{E m}^{-2}\text{s}^{-1}$ (PAR). The temperature of the culture was maintained at 20°C ($\pm 2^{\circ}\text{C}$) in a temperaturecontrolled growth room.

3.7.3 Experimental methods

- (i) Small Scale Replicate Batches (See figure 4.1).

One successful stock culture was split into twenty approximately equal inocula (20-50 mg wet weight). Each of these was placed in 200 mls of ($100 \mu\text{g P1}^{-1}$) D11 medium contained in aerated 250 ml conical flasks loosely stoppered with cotton wool (See section 4.2). During the experiment, two flasks were sampled on each occasion and duplicate analyses from each were possible. Thus, each graphical datum point represents the mean of these, and their standard deviation. After day 10 the algal biomass from the remaining flasks was becoming too bulky for the volume of media, consequently the algal biomass was split and placed in a fresh 200 mls of (-P) D11 medium (as above).

(ii) Large Scale Parallel Batch Cultures

These were performed in duplicate 10 l containers (See appendix 1, photograph 6). From each batch enough algal material was available throughout the experiments to carry out at least duplicate analyses for each NAT. Thus, the illustrated data (Figures 4.2-4.9) represent the total mean and standard deviation of the duplicate batches.

Minor evaporative losses were replaced, at least every two days with fresh distilled-deionized water.

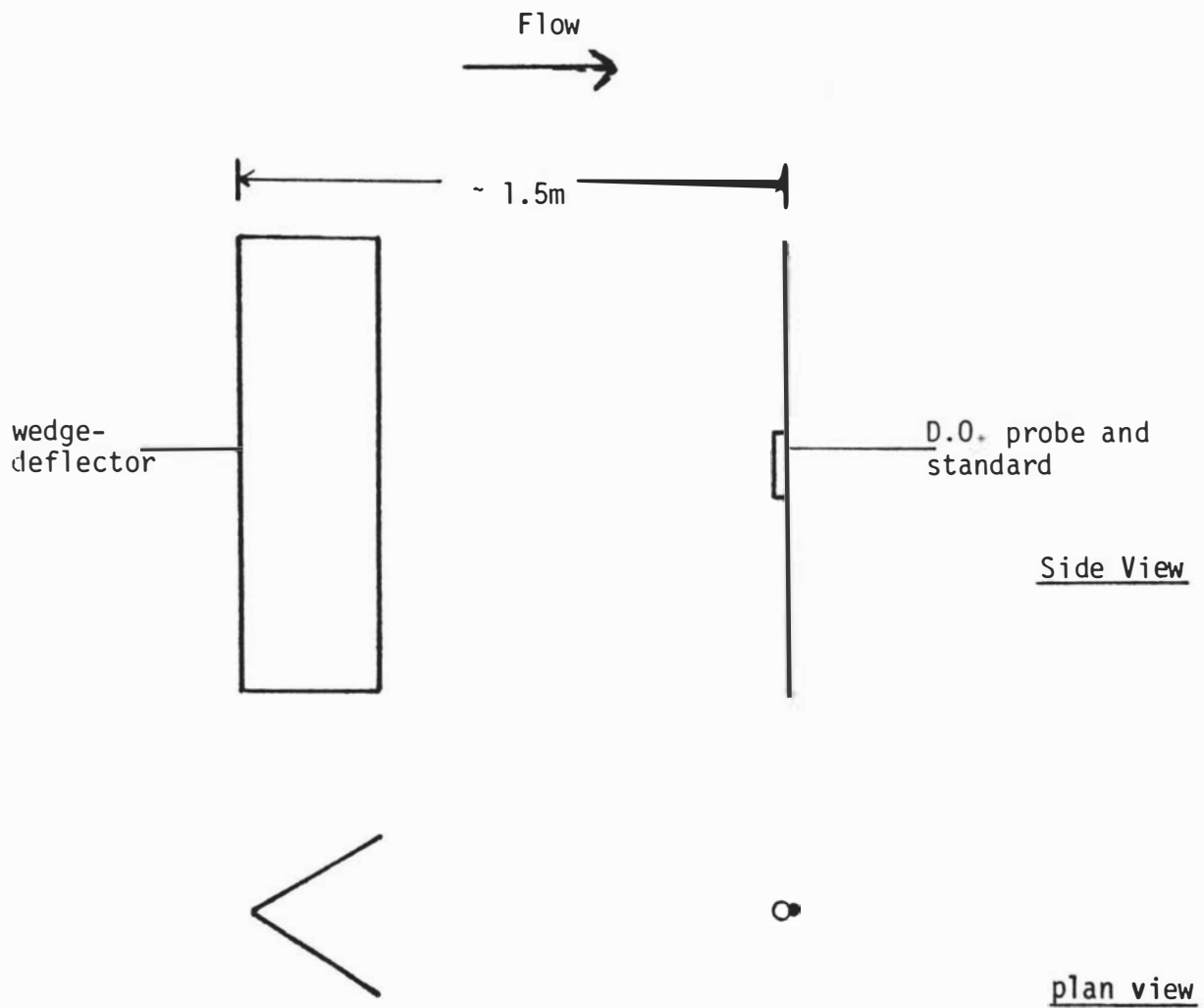
3.8 Dissolved Oxygen dynamics

3.8.1 Dissolved Oxygen and temperature

The changes in DO and temperature were monitored at a number of sites in the Manawatu River. The majority of work during the 1981/82 season was carried out at site T, and that in the 1982/83 season at a site a little upstream of site M. (Figure 2). The basic calibration procedure was carried out at each site, regardless of whether the profiles were destined for productivity analysis or for basic comparative purposes. This involved on site air-calibrating the DO probe of the DO/Temperature monitors (YSI model 56, one kindly loaned by the M.R.W.B.) after initial studies found satisfactory agreement between the monitor and the Winkler method (A.P.H.A., 1975) as well as confirming the accuracy of the thermistor readings compared to a previously calibrated 0-20°C mercury-in-glass thermometer. The DO calibration was checked every two days.

As the *Cladophora* proliferations developed, the amount of free-floating *Cladophora* (See section 3.2.3) increased to the extent that deflecting wedges were used, upstream of the DO probe and its supporting standard, to divert the algae and prevent accumulation on the probe which would interfere with the true readings (See figure 3.2). This arrangement was also used effectively at sites downstream of discharges where floating clumps of sewage fungus caused similar problems. There were also problems at these 'downstream' sites with

FIG. 3.2 Diagram of wedge-deflector and probe arrangement



growth of sewage fungus on the probes. To prevent this, either a shaking probe (YSI5695 Submersible Stirrer) was used or frequent probe cleaning and membrane changes were performed. The wedges were positioned to deflect floating material, yet still maintain the probe in an adequate current velocity (usually greater than 0.4 m.s^{-1}).

3.8.2 Primary productivity

The primary productivity of the river was calculated on the basis of DO dynamics measured at a single station. (See section 2.3.1). The river was treated as a system experiencing a simultaneous rise and fall of oxygen. The factors that govern these fluctuations are depicted in the following equation:-

$$\frac{\Delta C}{\Delta t} = k_2 (C_s - C) + (PR) \quad (3.8)$$

$$\frac{\Delta C}{\Delta t} = \text{Rate of change of DO (g.m}^{-3} \text{ hr}^{-1})$$

$$k_2 = \text{Reaeration Coefficient (base e) (hrs}^{-1})^*$$

$$C_s = \text{Saturation concentration of oxygen at the ambient river temperature during } \Delta t \text{ (g.m}^{-3})$$

$$C = \text{Average river DO during } \Delta t \text{ (g.m}^{-3})$$

$$PR = \text{Net hourly primary productivity (g O}_2 \text{ m}^{-3} \text{ hr}^{-1})$$

*(Reaeration Coefficient converted from day⁻¹ units using equation (3.4), section 3.3.1).

This finite difference method is based on that detailed by O'Connell & Thomas (1965). The continuous DO and temperature data were used to measure the average hourly values. C_s was calculated from the average temperature ($^{\circ}\text{C}$) using a linear equation derived from a graphical construction of the data of Golterman *et al* (1978).

$$C_s = 0.225 T + 13.55 \quad (3.9)$$

This equation is valid from 9°C to 24°C with an accuracy of $\pm 0.2 \text{ gm}^{-3}$.

In order to rapidly calculate the daily primary productivity a computer programme was developed with the assistance of

Dr A. Cleland (Biotechnology Department, Massey University) to rapidly perform the hourly calculations and produce the desired output. This programme and a sample input and output result are contained in appendices 2 and 3.

The processes and assumptions of this programme are outlined as follows:-

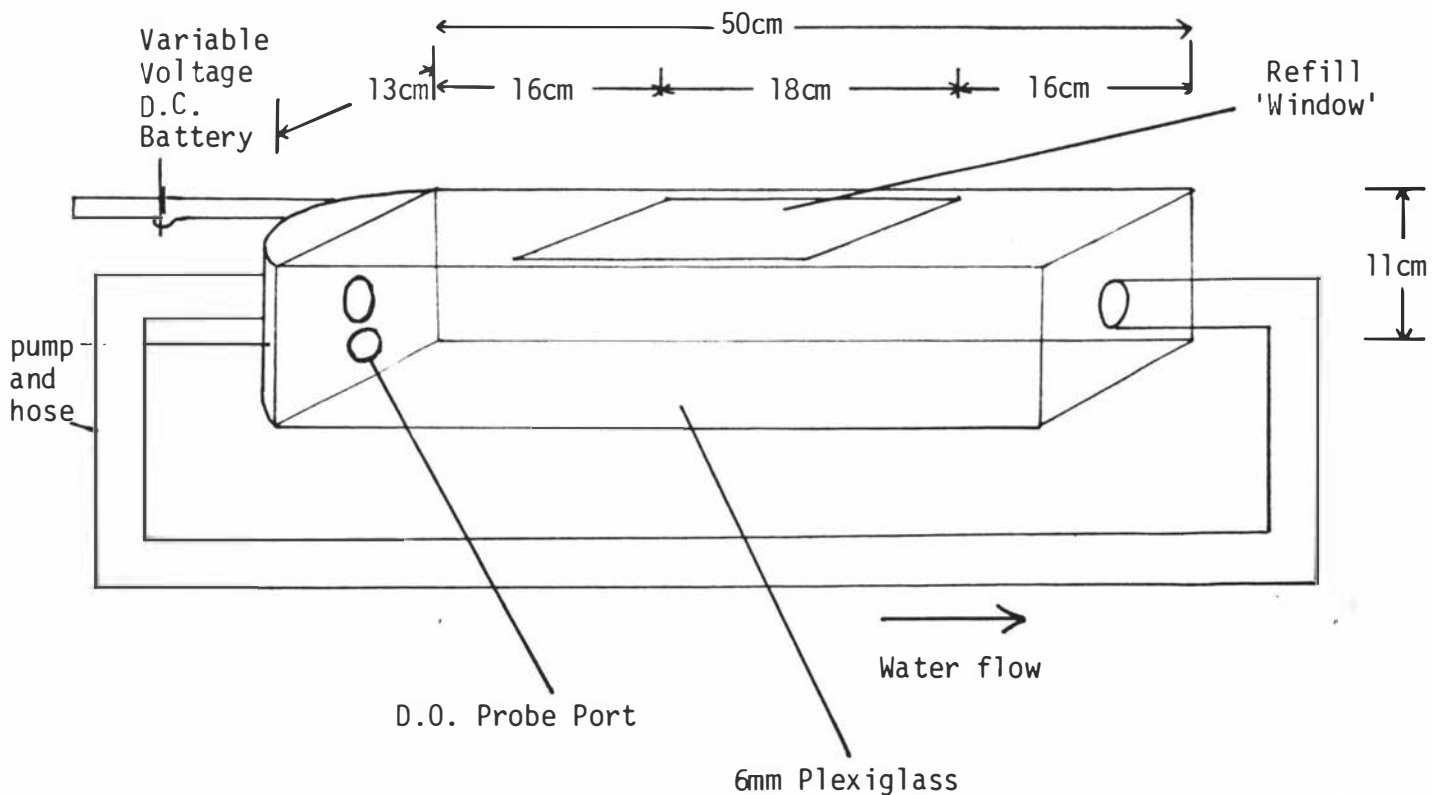
- (a) A day was defined as the time from sunrise to sunrise.
- (b) k_2 was calculated using the daily physical characteristics of the reach. (see Section 3.3.1)
- (c) The hourly productivity was then calculated using equation (3.8).
- (d) Gross production was estimated by assuming a linear increase of respiration through the day from the low pre-dawn rates to the high post-sunrise rates. (Odum & Wilson, 1962). The increase is due to the onset of photorespiration (Birmingham & Colman, 1979; Hough, 1974; Mantai & Haase, 1977) and increases in DO and temperature (McDonnel & Weeter, 1972; McIntire, 1966; Owens & Mavis, 1964). Thus, from the hourly net production and respiration the hourly gross production can be calculated.
- (e) No correction of k_2 for the range of temperatures experienced was done. The relevant equation is presented below. (Elmore & West, 1961):

$$k_2 = k_2(20) (1.0241)^{T-20} \quad (3.10)$$

- (f) Lastly, for each day the following parameters were calculated : (See appendix 3)
 - (i) The Maximum Gross Photosynthesis rate ($\text{g O}_2 \text{ m}^{-3} \text{ hr}^{-1}$)
 - (ii) The Minimum Respiration rate ($\text{g O}_2 \text{ m}^{-3} \text{ hr}^{-1}$)
 - (iii) Gross photosynthesis ($\text{g O}_2 \text{ m}^{-3} \text{ d}^{-1}$)
 - (iv) Total Community Respiration ($\text{g O}_2 \text{ m}^{-3} \text{ d}^{-1}$)
 - (v) P/R Ratio
 - (vi) Net Areal Primary Productivity ($\text{g O}_2 \text{ m}^{-2} \text{ d}^{-1}$)

The failure to correct the productivity for the variation of k_2 with temperature results in slight under and over estimation of the Net Productivity. Uncertainty of approximately 0-2% occurs on an hourly basis and if the temperature fluctuates around 20°C this uncertainty will tend to cancel itself out on a daily basis. An example of the effect of compensating for the variation of k_2 with temperature is illustrated in appendix 5 using data from day four of appendix 2. The apportioning of the productivity between various components of the river community was investigated using a recirculating clear plexiglass chamber. The design was a composite of many concepts. (Marker, 1976; Pamatmat, 1965; Pfeifer & McDuffett, 1975; Thomas & O'Connell, 1966) and is illustrated in figure 3.3

FIGURE 3.3: Schematic diagram of algal chamber



3.9 pH and alkalinity

A number of surveys were carried out in which pH was monitored by spot checks, at a variety of sites. Later in the study a submersible pH probe and continuous recording system were successfully developed.

A standard laboratory electrode with an additional 50m of coaxial cable was made submersible by carefully sealing all joints and electrical connections with 'Araldite' epoxy resin. Other more flexible sealing compounds were tested but were found to leak or interfere with the pH electrode signal. A portable (Cole-Palmer Chemcadet) pH meter was linked to either a TOA EPR 200A or a Cheswell 301E chart recorder.

These studies were usually complimented by ammonia determinations. (See section 3.5.2). Total alkalinity was monitored periodically during these runs. (APHA, 1975).

3.10 Data analysis

Statistical data analyses such as regression, correlation, t-tests and analysis of variance were performed by the Minitab statistical package (Minitab, 1982) using a Prime 1 computer.

CHAPTER 4

LABORATORY CULTURE EXPERIMENTS

4. LABORATORY CULTURE EXPERIMENTS

4.1 Introduction

Much of the literature pertaining to algal NATs is species-specific, and while there has been considerable relevant work carried out with Great Lakes *Cladophora glomerata* the application of these data to the Manawatu River *C. glomerata* can not be done without complementary studies to ascertain the response of the local species to specific nutritional circumstances.

One major aspect of NATs that has received very little attention is the response time (i.e. the time taken for a NAT to respond to a change in the relevant external nutrient supply). The speed of this response has important consequences for the interpretation of data. A result indicative of nutrient limitation could represent a recent situation (hours) or be a relic of some historic event (days).

The objectives of the experiments described in this chapter were to establish the following :-

- (a) The values for each NAT associated with a surplus and a limiting situation.
- (b) The response time of each NAT to transitions between surplus and limiting nutrient situations.

There are two changes in nutritional status that are of interest. They are the transition from a surplus to a limiting nutrient situation and *vice versa*. The problems of supplying sufficient nutrients in batch cultivations mean that nutrient conditions compared to a lotic situation may be very dissimilar. Thus, it should be remembered that transitions made in the laboratory will be much less subtle than any nutrient fluctuations occurring in steady flow river situations.

The major advantage and disadvantage of laboratory batch NATs are respectively as follows :-

- (a) The environmental and nutritional variables can be controlled, so that the effect of varying one or more can be identified.

- (b) The environmental and nutritional conditions used to culture the alga are dissimilar in many aspects to the lotic situation.

4.2 Results and discussion

Time constraints and the availability of inoculum meant that the initial nutritional status of the alga used in otherwise similar experiments varied (e.g. EP in figures 4.1, 4.2 and 4.4). Accordingly, the time needed, for parameters such as TTP and EP to reach their critical values, after being exposed to a (-P) medium, varied. Thus, interpretations of response times need to take cognizance of this.

4.2.1 Transition from a surplus to a limiting nutrient situation

4.2.1.1 Phosphorus

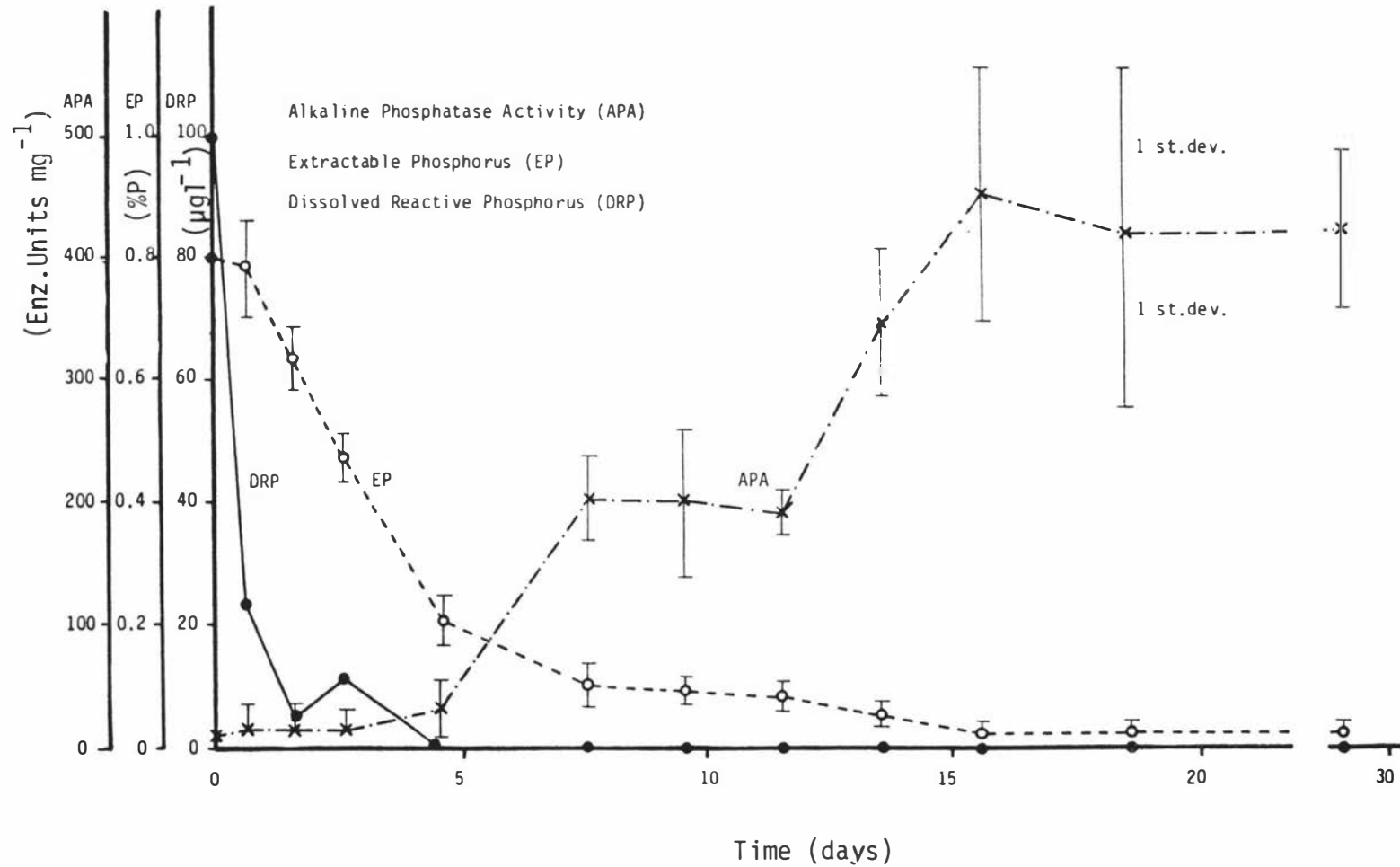
Initial experiments using small scale 250 ml batch replicates (See section 3.7.3) were unsuccessful due to the replicate variation being too large for any trend identification. This was a consequence of having an unavoidable variation in the inoculum biomass resulting, eventually, in the alga of each replicate being in a different state of nutrient depletion. However, when considerable time and effort were devoted to producing equivalent inocula, the range of values from duplicate treatments could be substantially reduced. The results of one such experiment are illustrated in figure 4.1. Cleaned equivalent inocula were added to conical flasks containing 200 mls of $100 \mu\text{g P l}^{-1}$ D11 media. (See section 3.7.3). Three extra inocula were prepared to get an estimate of the initial biomass variation. These gave the following results :

$$\bar{x} = 6.1 \text{ mg DW}; \quad s = 0.6 \text{ mg DW}$$

After five days, the DRP was below the detection limits in the media tested, demonstrating that the algae were actively growing and had utilized all the available phosphorus (P), including some internal stores (as evidenced by the rapid decline of EP). The term DRP was used instead of orthophosphate, as there may be a significant amount of reactive high molecular weight phosphate

Figure 4.1 Response of *Cladophora* Phosphorus Nutrient Availability Tests to gradual Phosphorus depletion.

(Small scale batch replicates, 250 ml , n=4)



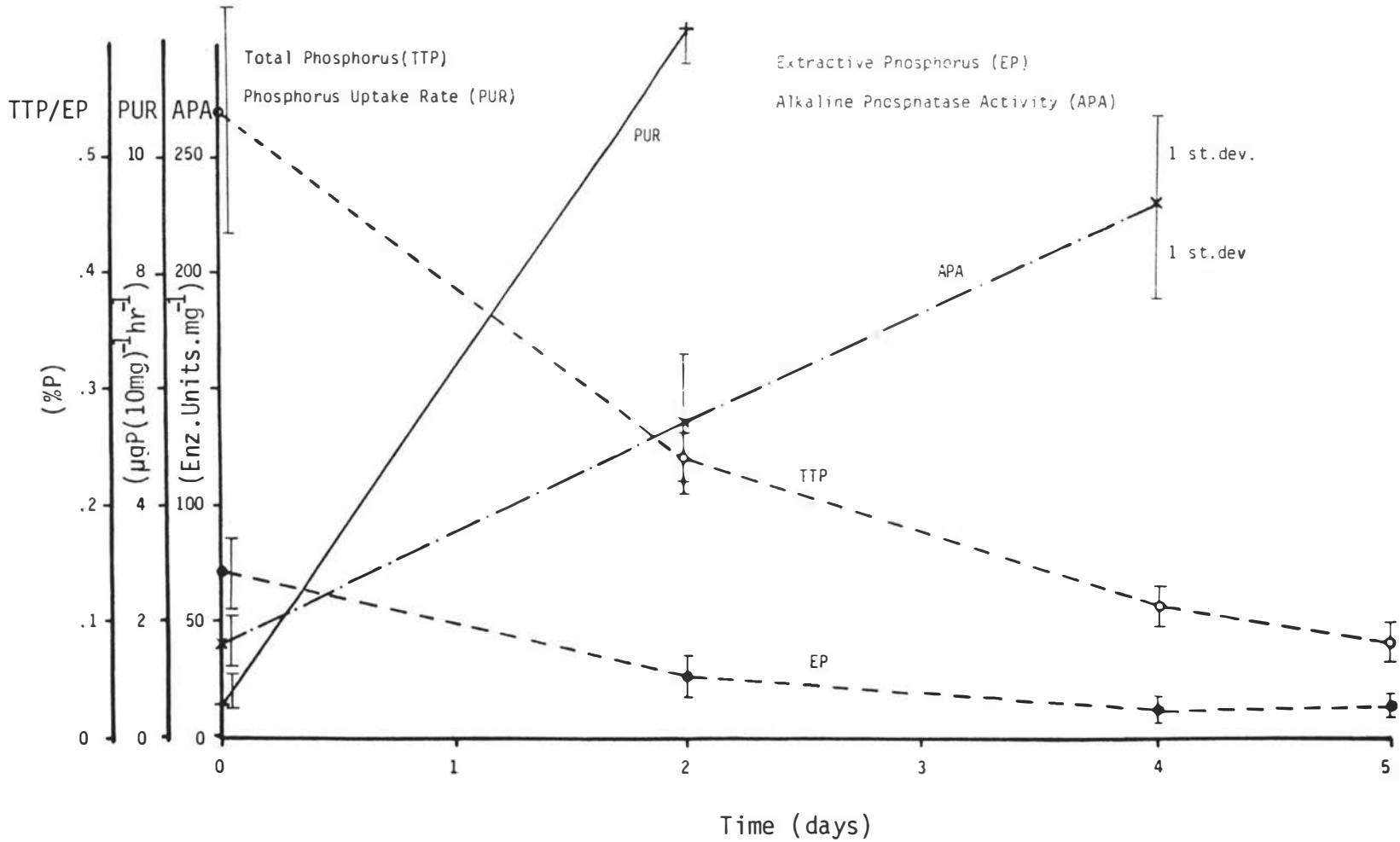
compounds present as a result of algal metabolism. (Nalewajko & Lean, 1980) (See section 2.6.2.3). The APA remained at a low level until the external P supply was depleted, after which it rose over a period of ten days to a peak of approximately 420 Enz. Units mg^{-1} . This experiment was useful in that the external P supply was gradually reduced and the consequences of this could be monitored. However, the small scale replicate design often gave rise to a considerable spread of results for the APA NAT (See section 3.7.3).

Large duplicate batch cultures (See section 3.7.3) were adopted to overcome some of the above problems. Figure 4.2 illustrates one phosphorus starvation experiment. The isolated alga were inoculated directly into (-P) D11 media. At this stage of the seasonal *Cladophora* development there were few clean inocula available for culturing and consequently the sampling frequency had to take account of the limited biomass available in each batch. Thus, by the time of the second sampling on day 2 (Figure 4.2) the PUR had risen dramatically to $12 \mu\text{g P (10 mg)}^{-1}\text{hr}$. Another experiment was performed (See figure 4.4) to get a more accurate response time for the PUR. Time constraints meant that the alga had to be used in a further experiment (Figures 4.7-4.9) and thus APA was not allowed to reach the extreme levels observed in the previous experiment (Figure 4.1). Despite these drawbacks, the experiment did allow good estimates to be made of the critical TTP and EP. The EP had levelled off by day 4 at a minimum of 0.02%. There do not appear to be any published data referring to accepted critical levels of EP in *Cladophora*, however, Lin (1977) observed a minimum of 0.6%, Mantai *et al*, (1982) one of 0.02%, and a minimum of 0.04% has been found in *Cladophora* from the Manawatu River. (See sections 2.6.2 and 6.6.1).

The TTP concentration had dropped to 0.08% by day five, this result together with the outcome of a TTP test carried out on a sample from the previous experiment on day 18 which gave a value of 0.07% indicate a critical TTP of 0.07%. Published critical values of 0.06% are very similar, although the minimum value from any Manawatu River site has not been observed to drop below 1.0% (See sections 2.6.2 and 6.6.1).

Figure 4.2 Response of *Cladophora* Phosphorus Nutrient Availability Tests to sudden Phosphorus depletion.

(Dissolved Reactive Phosphorus = 0)



From these two experiments it is possible to get a good idea of the APA associated with a surplus or limiting P situation. Values greater than 150 Enz. Units mg^{-1} will be found in *Cladophora* experiencing a shortage of available P, whereas levels around 50 Enz. Units mg^{-1} will be indicative of surplus P.

Field studies on *Cladophora* in the Great Lakes have reported APA to range from 40-320 Enz. Units mg^{-1} (Mantai, 1978; Mantai *et al.*, 1982). Values found in the Manawatu River have ranged from 10-450 Enz. Units mg^{-1} . (See section 2.6.2 and 6.6.1).

The APA is a useful parameter as it is independent of the internal P concentration. The response time after exposure to (-P) media was approximately 2-3 days. During this experiment the N NATs were also monitored to see if they were affected by the changes in the P NATs. The results are illustrated in figure 4.3. There was no significant change in either the AAR or the TTN over the course of the experiment.

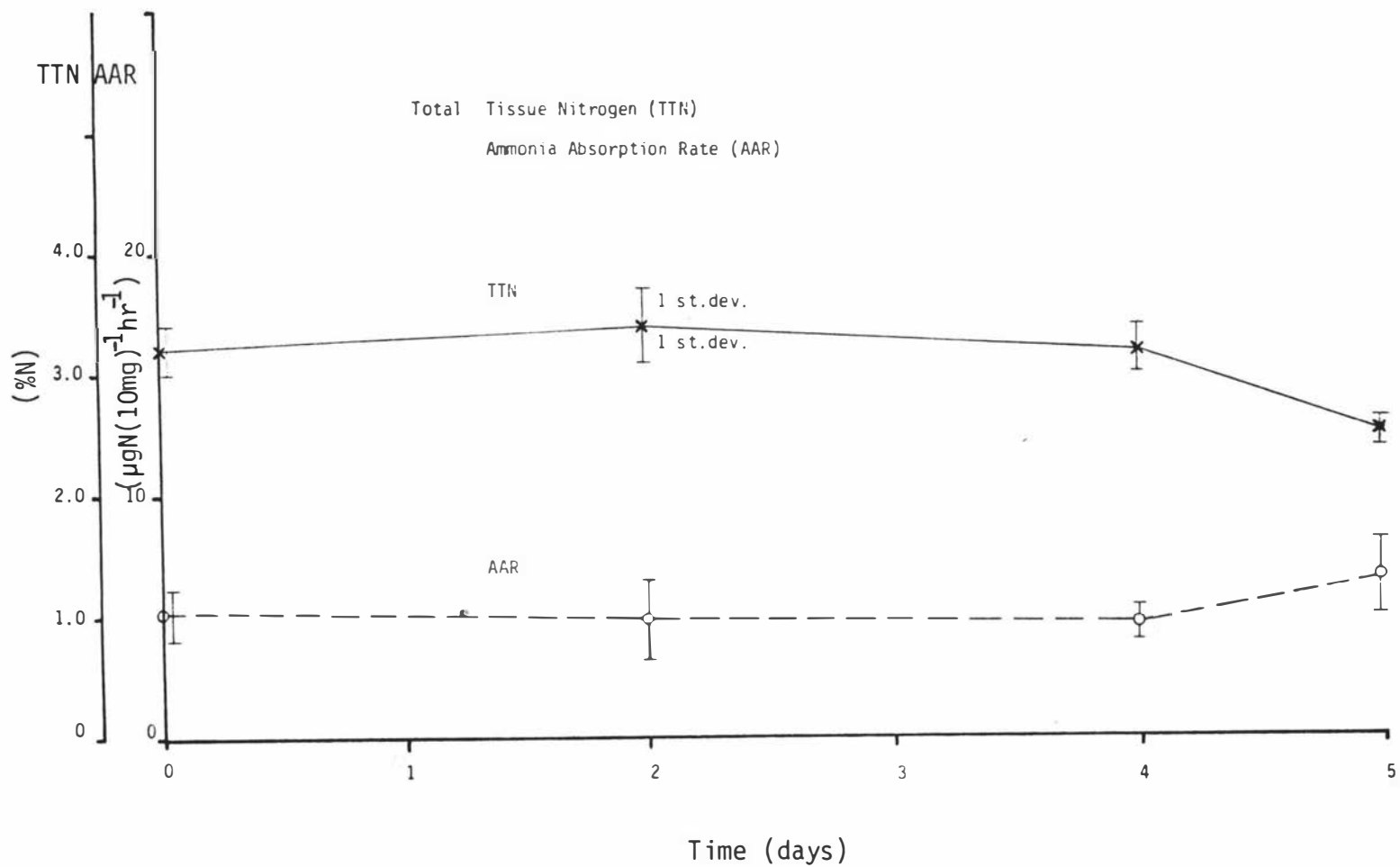
The experiment illustrated in figure 4.4 was carried out to determine more accurately the response time of the PUR to sudden P deprivation. EP was also monitored as a check on the internal algal nutritional status. The PUR rose from a 'surplus value' of around $0.4 \mu\text{g P (10mg)}^{-1} \cdot \text{hr}^{-1}$ to just over $6.0 \mu\text{g P (10 mg)}^{-1} \cdot \text{hr}^{-1}$ in two days. The peak PUR did not reach the high value of around $12 \mu\text{g P (10 mg)}^{-1} \cdot \text{hr}^{-1}$ recorded in the earlier experiment. This may be due to genetic differences (ecotypes), as the inocula were isolated from different rivers at different times during the year, or it may be a consequence of prolonged laboratory culturing. The PUR values are comparable with those recorded for *Cladophora* from the Great Lakes where a range of $0.2 - 18 \mu\text{g P (10 mg)}^{-1} \cdot \text{hr}^{-1}$ has been observed (Auer & Canale, 1980). Maximum values recorded in the Manawatu have been in the vicinity of $8 \mu\text{g P(10 mg)}^{-1} \cdot \text{hr}^{-1}$.

4.2.1.2 Nitrogen

The response of *Cladophora* to sudden deprivation of the nitrogen supply is illustrated in figure 4.5. There was a steady decline in

Figure 4.3 Response of *Cladophora* Nitrogen Nutrient Availability Tests to sudden Phosphorus depletion.

(Dissolved Reactive Phosphorus = 0)



the TTN, however, before the accepted critical concentration of 1.1% N (Section 2.6.3) was reached, the alga began to exhibit symptoms of decay. Microscopic examination revealed pale grey-green and shrunken chloroplasts, which were characteristics observed in some earlier N starvation experiments. One of the duplicate batch cultures lost viability between the 40hr and 60hr period when the AAR dropped to nearly zero and microscopic examination revealed gross symptoms of N starvation. Thus data after 60 hrs were duplicates from just one batch culture.

Field studies of *Cladophora* in the Great Lakes have observed that TTN concentrations range from 1.6-4.6%. (Mantai *et al*, 1982), while Manawatu River data covers concentrations from 1.8-6.6% N (See sections 2.6.3 and 6.6.2). The discrepancy between the field and laboratory results is a possible illustration of the consequences of the different nutritional and environmental conditions. (See section 4.1 and 4.2.1.1).

The AAR responded significantly within 12 hours and peaked at approximately $24 \mu\text{g N (10mg)}^{-1} \cdot \text{hr}^{-1}$ after 40 hours. Laboratory studies on *Cladophora* from the Great Lakes observed an AAR of $3 \mu\text{g N (10mg)}^{-1} \cdot \text{hr}^{-1}$ in complete medium and $18 \mu\text{g N (10mg)}^{-1} \cdot \text{hr}^{-1}$ in -N medium. (Fitzgerald, 1968). The range found in the Manawatu River was from $0.8-4.2 \mu\text{g N (10mg)}^{-1} \cdot \text{hr}^{-1}$. (See sections 2.6.3 and 6.6).

P NATs were monitored throughout the experiment (Figure 4.6). TTP decreased but did not approach the critical region. (See section 2.6.2). There was a surplus of available P at all times.

4.2.2 Transition from a limiting to a surplus nutrient situation

4.2.2.1 Phosphorus

(a) Total Tissue Phosphorus and Extractive Phosphorus

The results of one experiment, (duplicate 10 l batches) in which P-starved *Cladophora* were relieved by a sudden transition to a $1000 \mu\text{g P l}^{-1}$ D11 media, are illustrated in figures 4.7 and 4.8. The P cell quota was rapidly satisfied as TTP rose from 0.08 to 0.92% P within 12 hrs. Similarly the EP

Figure 4.4 Response of *Cladophora* Phosphorus Nutrient Availability Tests to sudden Phosphorus depletion.

(Phosphorus Uptake Rate and Extractive Phosphorus) (Dissolved Reactive Phosphorus = 0)

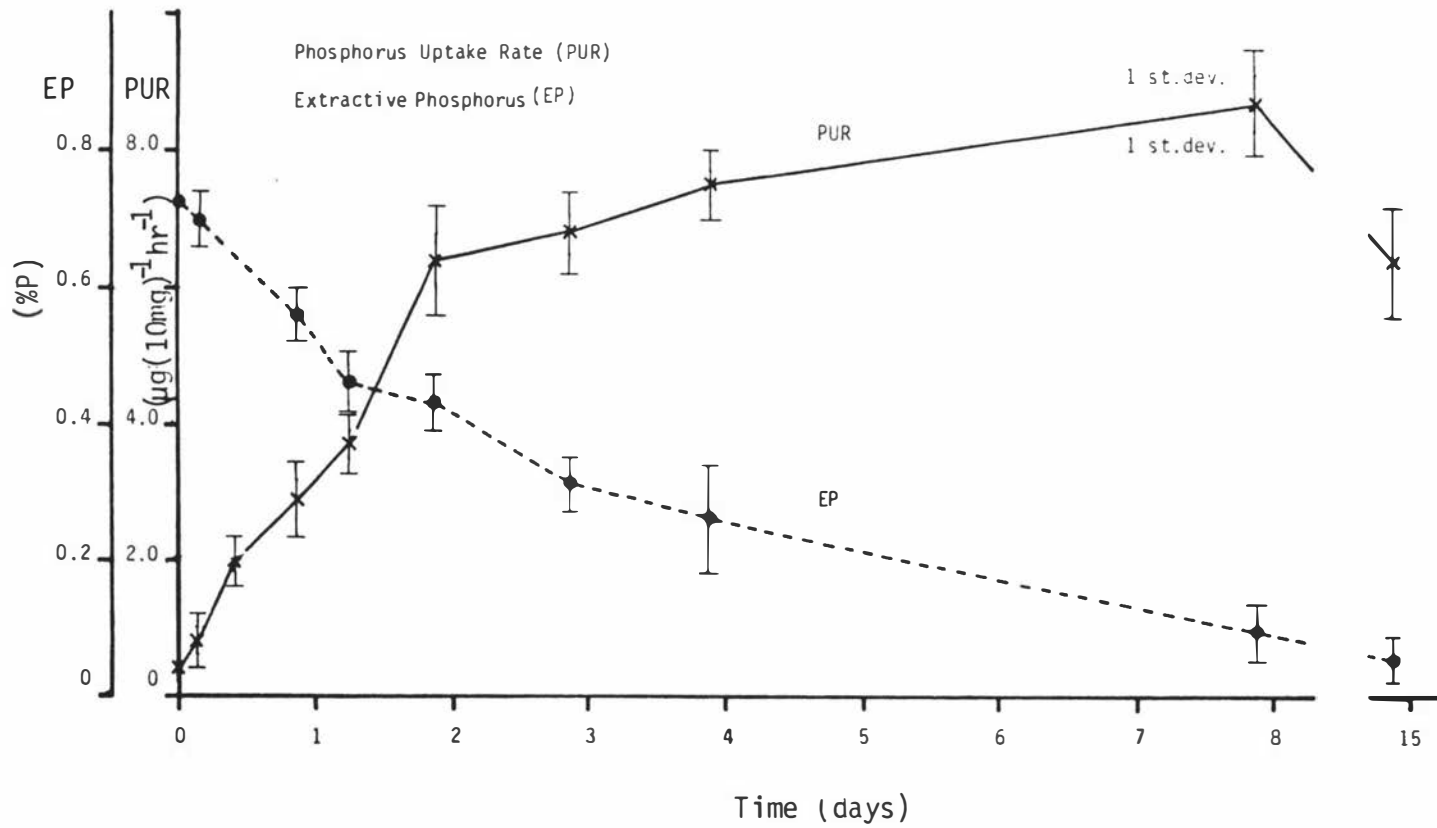


Figure 4.5 Response of *Cladophora* Nitrogen Nutrient Availability Tests to changes in Nitrogen availability.

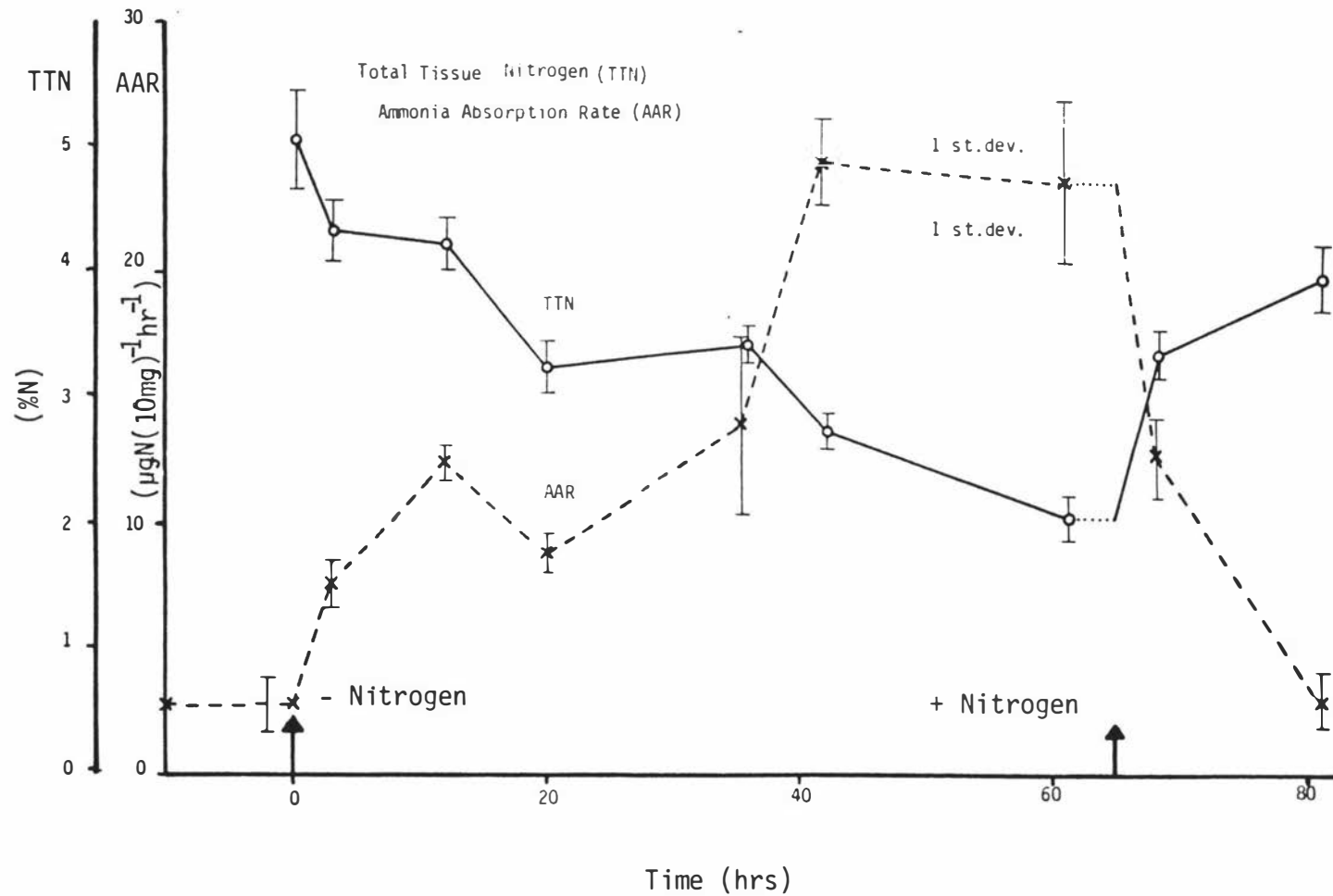


Figure 4.6 Response of *Cladophora* Phosphorus Nutrient Availability Tests to changes in Nitrogen availability.

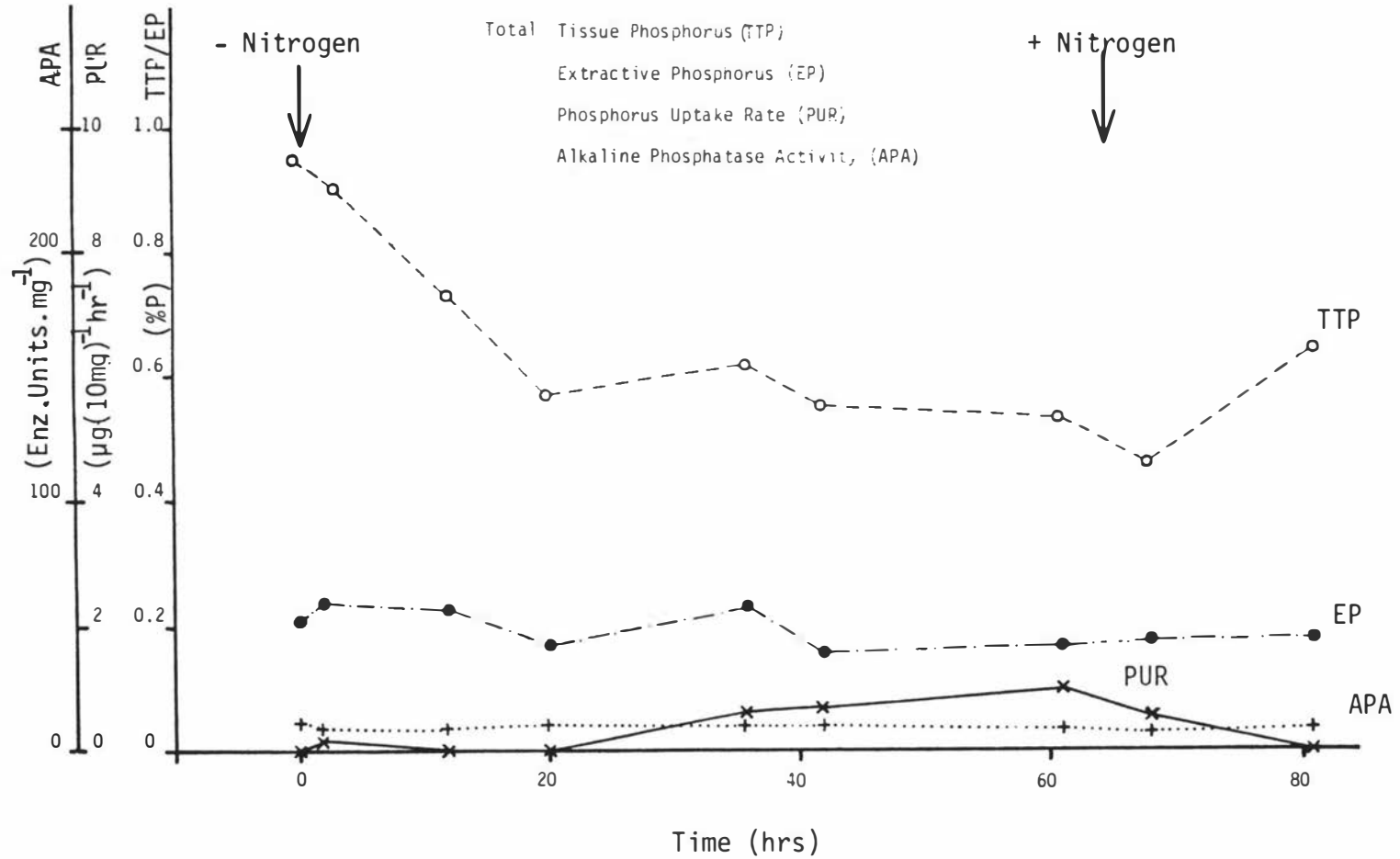
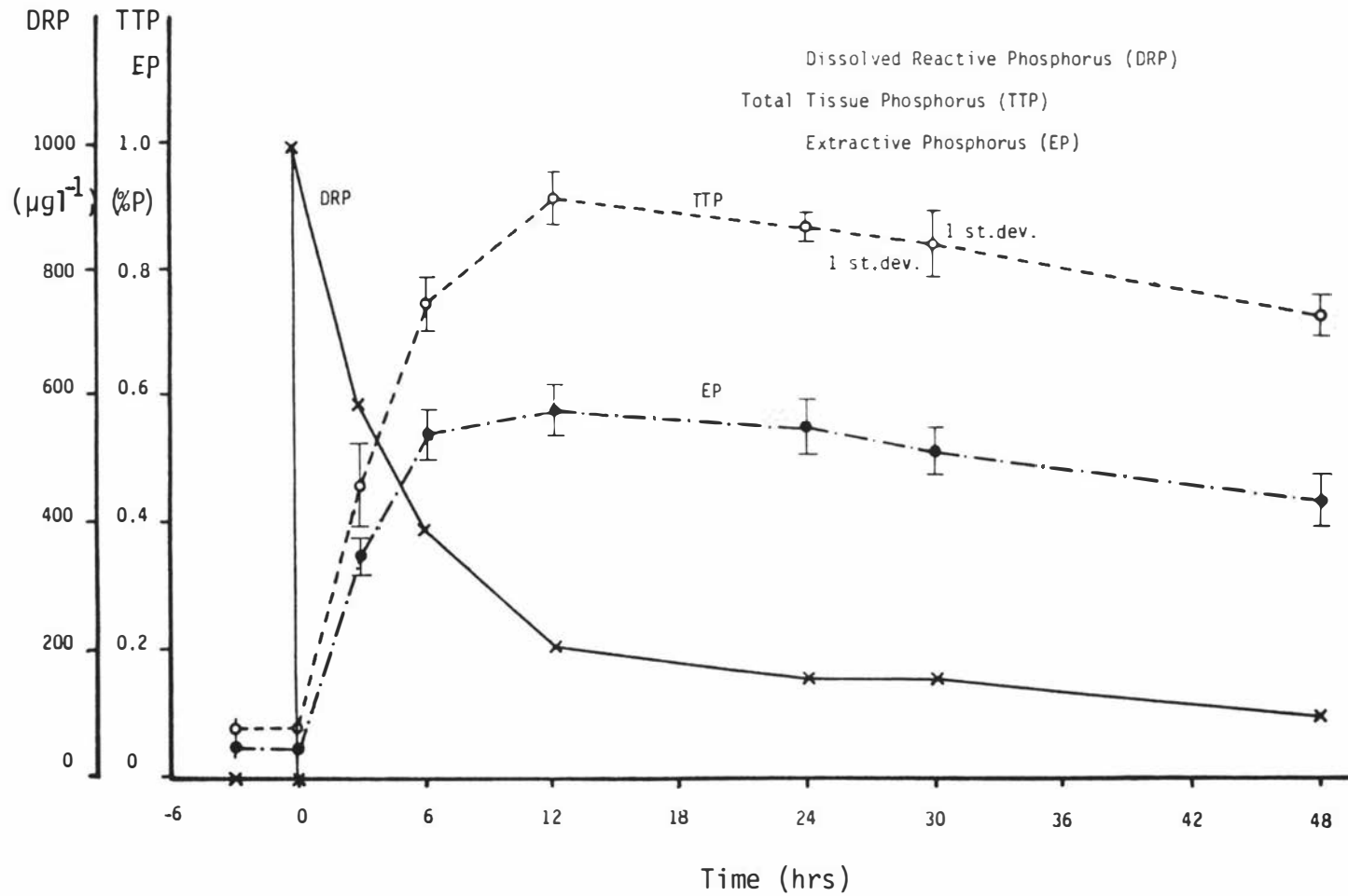


Figure 4.7 Response of *Cladophora* Phosphorus Nutrient Availability Tests to cessation of Phosphorus limitation.
 (Total Tissue Phosphorus and Extractive Phosphorus)



increased over the same time interval from 0.04 to 0.59% P.

(b) Phosphorus Uptake Rate

The relationship between the PUR and the internal P is revealed by the reduction of PUR as the levels of TTP and EP rise. (Figure 4.8). At the peak TTP concentration a net efflux of DRP into the test media was observed. This was the result of a feedback response that enables the alga to avoid excessive accumulation of internal P. It is well known that algae can excrete high molecular weight P compounds (Kuenzler, 1970; Lean & Nalewajko, 1976). Negative PURs have not been reported by researchers working on the nutrition of Great Lakes *Cladophora*. However, Healey (1973b) has reported a drop from an elevated PUR to zero when *Anabaena variabilis* was relieved from P limitation.

(c) APA

Elevated levels were still recorded after 48 hrs (and again when checked at 96 hrs). This is a consequence of the nature of the enzyme involved. (See section 2.6.2). The implications of this will be discussed in section 4.3.

N NATs were also monitored during this experiment TTN showed little variation while there was a slight but inconsequential reduction in the AAR. (Figure 4.9).

4.2.2.2 Nitrogen

Referring back to figure 4.5 the N starvation was broken by a transition of the viable culture to a surplus N D11 medium. Both the TTN and AAR responded within hours to the change quickly approaching values observed previously to be associated with a surplus situation.

Figure 4.8 Response of *Cladophora* Phosphorus Nutrient Availability Tests to cessation of Phosphorus limitation.
 (Phosphorus Uptake Rate and Alkaline Phosphatase Activity)

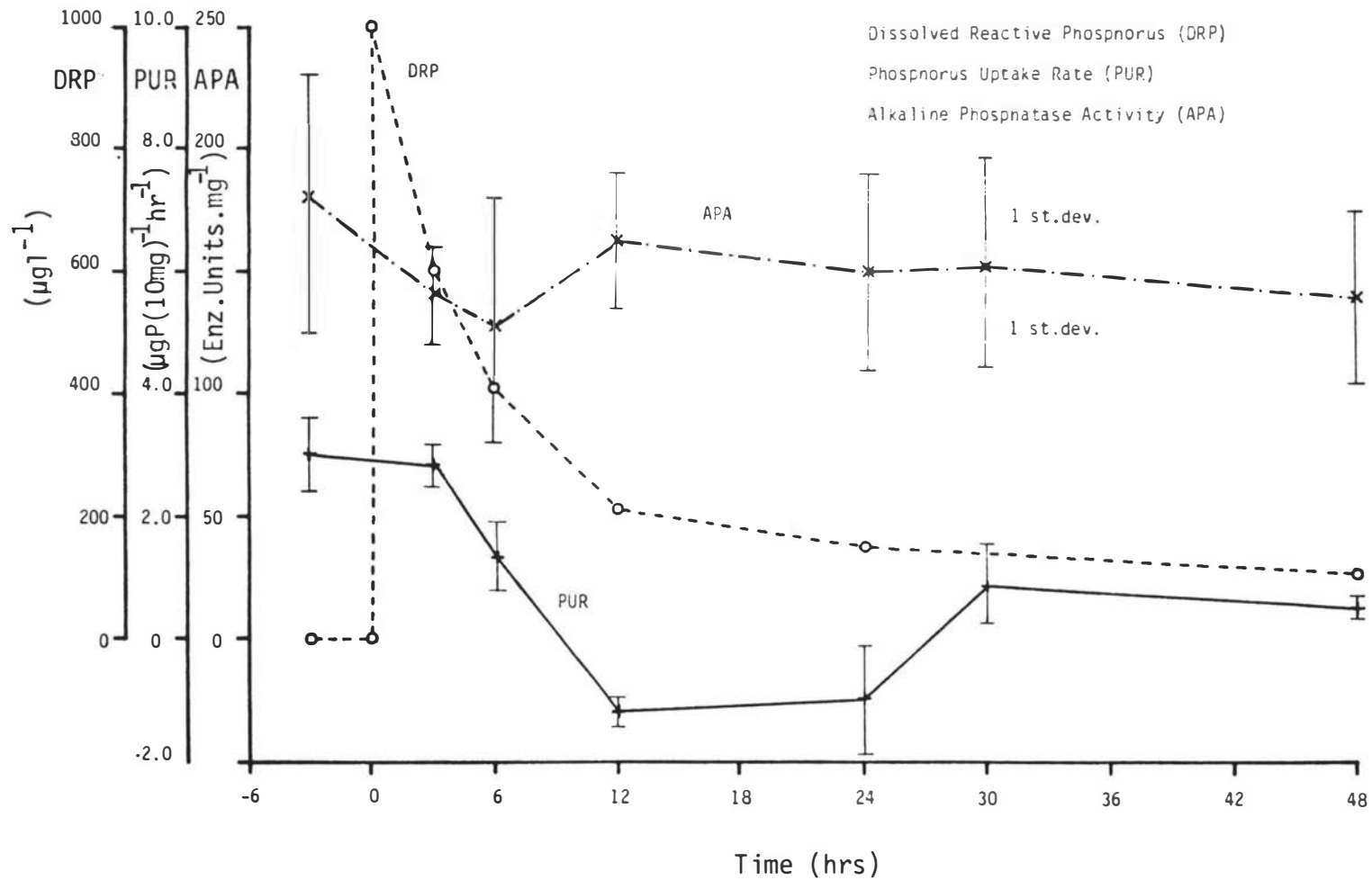
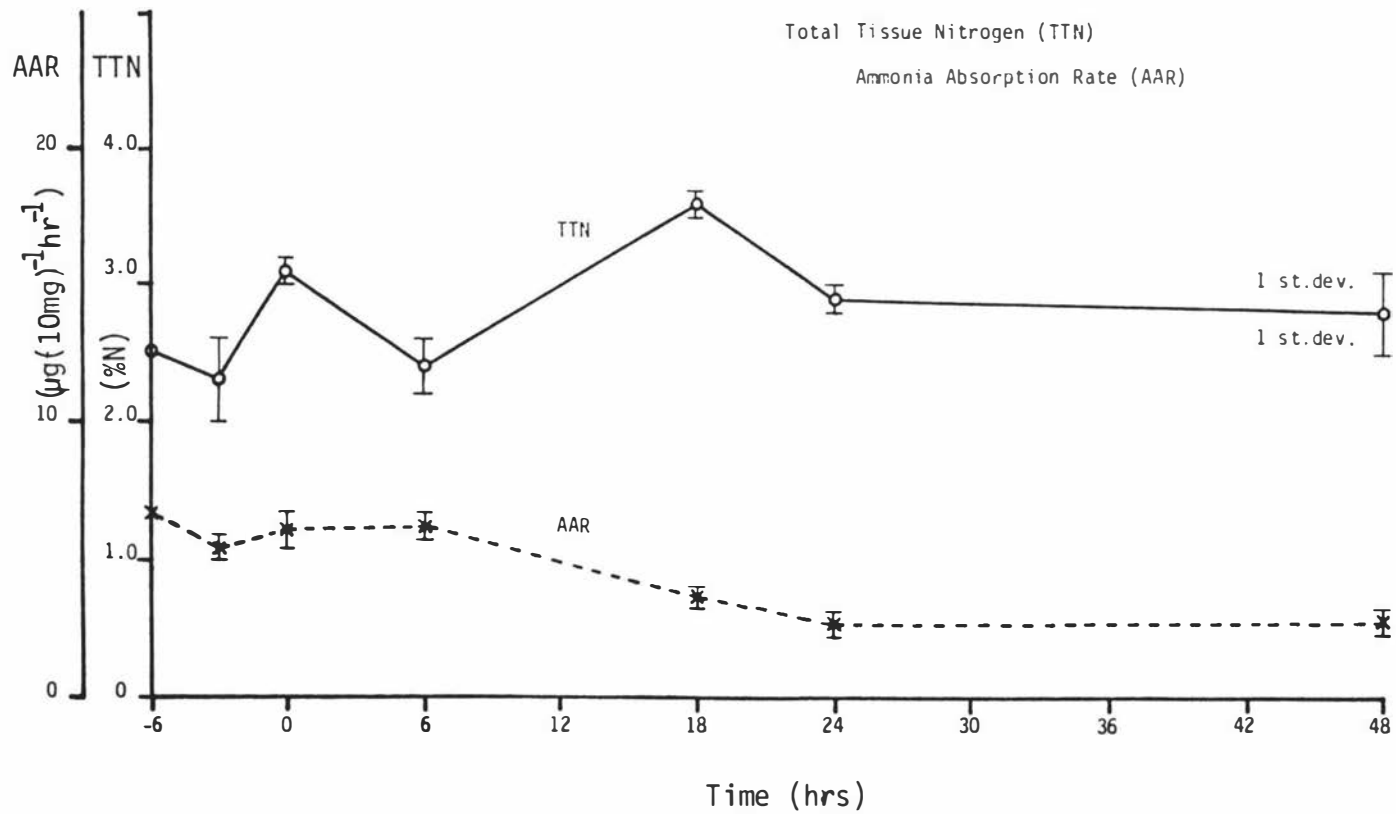


Figure 4.9 Response of *Cladophora* Nitrogen Nutrient Availability Tests to cessation of Phosphorus limitation.



4.3 The application of Nutrient Availability Test results achieved in the laboratory, to their use in field studies

A summary of results obtained from the experiments, illustrated in figures 4.1-4.9, is presented in tables 4.1 and 4.2. The NAT values presented in table 4.1 are broadly similar to literature values detailed earlier in tables 2.5 and 2.6. The distinction between a surplus and a limiting situation for the enzymatic NATs (APA, PUR and AAR) is gradual, as opposed to the precise critical concentrations defined for the tissue components (TTP, EP and TTN). The large differences between NAT response times illustrates the need for these to be considered when attempting to interpret NAT results. There are advantages and disadvantages in having NATs that respond at different rates.

TABLE 4.1: Values Observed for Laboratory Nutrient Availability Tests associated with a limiting or surplus situation

NAT	Activity/concentration associated with	
	Limitation	Surplus
TTP (% P)	<0.07	>0.07
EP (% P)	<0.02	>0.02
PUR ($\mu\text{g P}(10\text{mg})^{-1}.\text{hr}^{-1}$)	>2.0	<1.0
APA ($\text{Enz. Units.mg}^{-1}$)	>150	<50
TTN (% N)	<2.0	>2.0
AAR ($\mu\text{g N}(10\text{mg})^{-1}.\text{hr}^{-1}$)	>6.0	<4.0

TABLE 4.2: A Summary of Laboratory Nutrient Availability Tests
response times

Response to Surplus → Limiting Situation

Nutrient	NAT	
Phosphorus	V.Slow	APA
	Slow	TTP, EP
	Fast	PUR
Nitrogen	Slow	TTN
	Fast	AAR

Response to limiting → Surplus Situation

Nutrient	NAT	
Phosphorus	Slow	APA
	Fast	TTP, EP, PUR
Nitrogen	Fast	TTN, AAR

KEY:

V.slow > 4 days

Slow = 2-4 days

Fast = 2-12 hours

The ecological significance of the slow and fast response times are as follows:

(a) Slow Response Times

The TTN, TTP and EP will only be reduced gradually in response to a lack of external available nutrients. High growth rates (See section 2.6.2 and equation 2.6) will be maintained until internal concentrations near critical values. Any nutrients that become available, while the internal nutrients are becoming depleted, will be rapidly assimilated.

The APA changes very slowly in response to a relief from P limitation, this characteristic does not appear to confer any ecological disadvantage to the alga, and may serve to maintain some ortho-phosphate production if relief from limitation is short-lived. Thus, P may be made continually available in an environment that might otherwise be constantly fluctuating between a limiting and a surplus situation.

(b) Fast Response Times

These appear to be a consequence of the need to rapidly utilize any nutrients that may be only briefly available and/or only available in patches (Quarmby *et al*, 1982).

The transport mechanisms responsible for P and N uptake respond rapidly to a break in nutrient limitation, consequently the tissue components also respond swifly. There also appears to be a net release of reactive P from the alga after the maximum cell quota has been reached.

The control of uptake kinetics by both internal and external nutrients is also evidenced by the relatively rapid response of PUR and AAR to a change from a surplus to a limiting situation. (See section 2.6.2).

Ideally, the NATs should be sampled with a frequency approaching their response times. This approach would enable the distinction to be made between a short-term fluctuation and a long term trend in the river's nutritional status. However, to sample NATs daily would be prohibitive. Instead, a compromise must be reached that allows for each specific situation. During this study (Sections 5.4 and 6.6) NATs were usually sampled at least weekly, and often twice weekly if values indicated nutrient conditions were limiting or changing. The need to sample *in situ* algae frequently enough to have meaningful results, may clash with the time available to carry out all the NATs used in these laboratory experiments. For this reason the advantages and disadvantages of the various tests are briefly discussed below:

A great advantage of the TTN, TTP and EP NATs is that they can be dried and stored for analysis at a later date. All are relatively rapid and should be included in any thorough sampling strategy. However, as TTP and EP give similar responses the TTP can be used in preference as it measures a clearly defined biological quantity and can be compared with the large amount of published material quoting TTP concentrations. The APA test can be very useful because of its independence from either TTP or EP. The AAR test is a valuable 'backup' to the TTN test. (See tables 4.1 and 4.2) All of the above tests have well-defined concentrations or activities that can be associated with situations of nutrient surplus or limitation. (See tables 4.1 and 4.2). However, the PUR test is less than ideal in this respect and if available time was a major consideration in a proposed study, this test would not be as useful as the others.

4.4 Conclusions and recommendations

The results of these experiments indicate that when trying to interpret NAT field results the following points must be considered:

- (a) The activity/concentration compared to the established surplus and limitation levels.
- (b) The response time for transitions from one nutrient situation to another.
- (c) The frequency of sampling, in relation to the response

times.

- (d) The potential intra-site variation found for each NAT on every sampling occasion.

CHAPTER 5

RECONNAISSANCE SURVEY RESULTS 1980/81

5. RECONNAISSANCE SURVEY RESULTS 1980/81

5.1 Introduction

Initially, during the summer season of 1980/81 a reconnaissance survey of the Manawatu River was performed. This helped identify and refine the necessary methodology and site locations for extensive field work in the 1981/82 and 1982/83 seasons.

5.2 *Cladophora* biomass density and distribution

The establishment of *Cladophora* proliferations were hampered during the beginning of the 1980/81 seasons by two very large spates. (Figures 5.1 and 5.2). However, towards the end of January short tufts of *Cladophora* were observed extensively in the Manawatu River, and many of its tributaries, downstream to site S. (Figure 1.2). The *Cladophora* assemblages included some examples of other filamentous algae such as *Ulothrix*, *Stigeoclonium* and *Spirogyra*, as well as a diverse epiphytic population on its own filaments. Downstream of the Manawatu Co-op Dairy discharge, the *Cladophora* was only infrequently observed due to the river substrate changing from one dominated by stones and pebbles (1-15 cm diameter) to one dominated by small pebbles (1-2 cm diameter) and sand (<0.5cm av. diam.) (Figure 1.2). This extremely mobile bed was overrun by a prolific 'sewage fungus' community that dominated by competitively excluding slower growing filamentous algae. The Manawatu River in this region often follows the classical pattern of a phototrophic community being replaced by a heterotrophic one in a river receiving organic discharges. (Hynes, 1960).

The pattern of *Cladophora* build-up is illustrated in figure 5.1. The influence of large flush events in reducing biomass levels and the rapid growth rate that occurred during steady low flow periods were the two most prominent features of figures 5.1 and 5.2. Sustained rainfall, resulting in prolonged flushes in May 1981, caused the cessation of studies in the Manawatu River. Brief surveys before and after these flushes revealed *Cladophora* and many other periphytic algae 'over-wintering' in isolated reaches on stable substrate such as large boulders and embedded logs. The *Cladophora* filaments there were noticeably short, 10-30 cm, as

Figure 5.1 *Cladophora* biomass fluctuations at site M, 1980/81

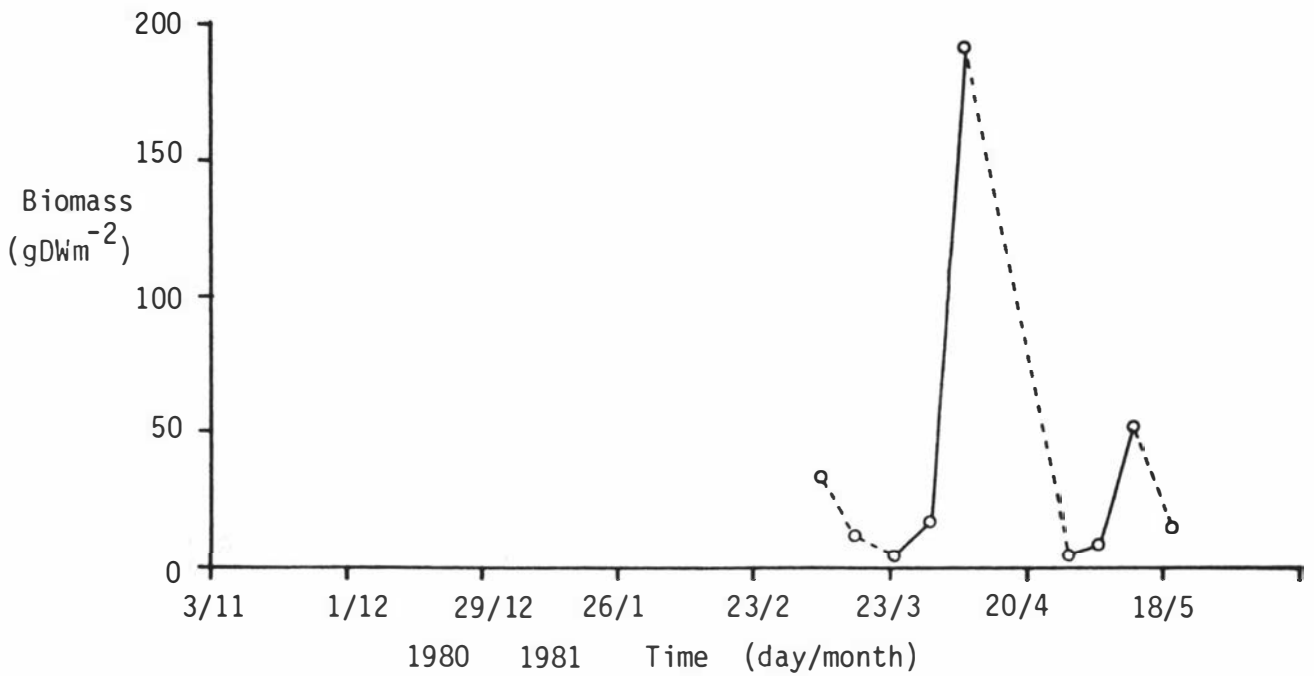
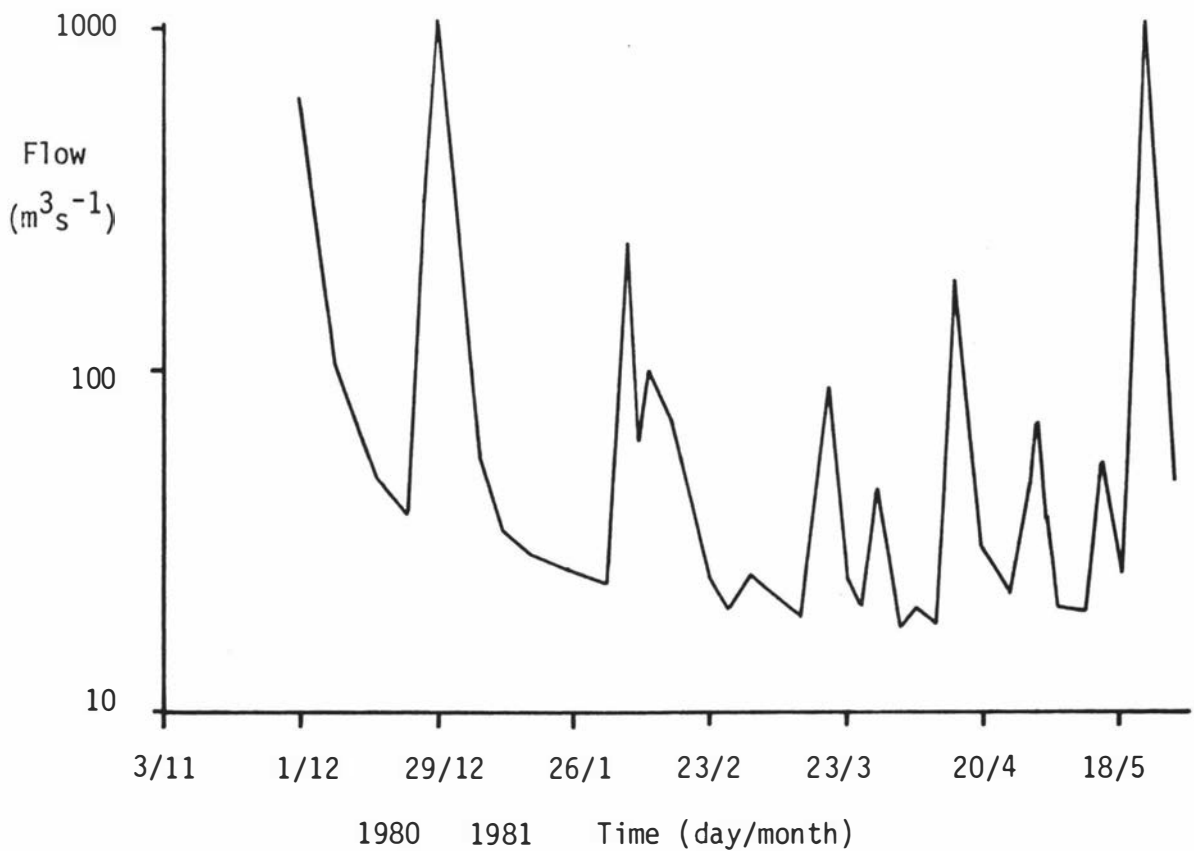


Figure 5.2 The Manawatu River flow 1980/81.



opposed to lengths of greater than 200cm often observed in healthy proliferations in the main river. Each over-wintering filament was usually thickly encrusted with epiphytes (see Section 2.5.5). The results of one semi-quantitative distribution survey carried out prior to the May spates are included in table 5.1 presented below:-

TABLE 5.1: A distribution survey of periphyton in the tributaries of the Manawatu River, 9 May 1981

River	Major Periphyton Species	Total Cover (0-5)*
Mangahao	<i>Spirogyra, Stigeoclonium</i>	2
Makakahi	<i>Cladophora, Ulothrix</i> <i>Anacystis</i>	2
Mangatainoka	<i>Ulothrix</i>	1
Mangatainoka	<i>Ulothrix</i>	3
Makuri	<i>Cladophora</i>	2
Tiramea	<i>Cladophora</i>	2
Upper Manawatu	<i>Cladophora</i>	1
Upper Manawatu	<i>Cladophora</i>	1
Mangatanui	<i>Spirogyra, Ulothrix</i>	1
Upper Manawatu	<i>Cladophora, Anacystis</i>	1
Pohangina	<i>Cladophora</i>	3

* Cover scale: see Section 3.2.3 and figure 3.1

These results illustrate the modest biomass yet diverse nature of the periphyton from the Manawatu River tributaries. The factors responsible for *Cladophora* becoming the dominant species, especially in the main river, are detailed in section 2.5.4. (W.P.S.)

During these initial surveys of the *Cladophora* proliferations, it became obvious that some systematic biomass sampling regime was needed to reduce the error involved in sampling the contagiously distributed algae (Figure 3.1), to an acceptable level. The results of one survey carried out to try and identify a satisfactory level of precision, and the effort needed to achieve it are presented in table 5.2:

TABLE 5.2: *Cladophora* biomass density variation on one sampling occasion, at site M, during a proliferation

Throw	Algal Biomass g(0.04m ⁻²)	Throw	Algal Biomass g(0.04m ⁻²)
1	1.5	11	1.1
2	4.2	12	1.9
3	4.9	13	3.8
4	1.3	14	6.3
5	2.4	15	4.5
6	1.0	16	2.7
7	1.4	17	0.9
8	3.1	18	3.3
9	3.4	19	1.5
10	2.4	20	2.9

$$\bar{x} = 2.8 \text{ g}(.04 \text{ m}^{-2})$$

$$s = 1.5 \text{ g}(.04 \text{ m}^{-2})$$

$$\text{or, } \bar{x} = 70 \text{ g m}^{-2}$$

$$s = 38 \text{ g m}^{-2}$$

For an arbitrarily chosen level for the coefficient of variation (CV) of 10% of the following results occur: (Sokal & Rohlf, 1973):

$$CV = \frac{(s/\sqrt{N})}{\bar{x}} \times 100 \quad (5.1)$$

$$CV = 10 = \frac{(38/\sqrt{N})}{70} \times 100$$

Therefore $N \approx 30$

Since approximately one hour was required to collect twenty samples this level of precision would be an unrealistic goal for regular multi-site monitoring. Reducing the anticipated CV to 20%, the following results occur:

$$CV = 20 = \frac{(38/\sqrt{N})}{70} \times 100$$

Therefore $N \approx 8$

This was considered to be a realistic number of samples able to be collected from each of the three study sites. In practise ten samples were usually collected from each site, bringing the CV to 17%.

Other methodological considerations are presented in Section 3.1.

5.3 River nutrients

Initial work concentrated on partitioning the total phosphorus (TP) and total nitrogen (TN) at various sites, both above and below the discharges. A summary of this exploratory work is presented in Table 5.3. At the upstream sites this serves to illustrate, firstly, the importance of the dissolved reactive portion in the upstream TP and secondly, highlights the dominance of nitrate N in the TN. The influence of the various discharges can be seen by substantial increases in ammonia and nitrite as well as large increases in particulate, and to a lesser extent, DRP.

In mid-February the sampling effort concentrated on the TN and TP at the upstream sites (Figure 5.3 and table 5.3). Complimentary sampling of nitrate and DRP by the MRWB was planned for both the 1980/81 and 1981/82 seasons, however, unforeseen difficulties prevented this being carried out. The data from site M serve to illustrate the TN and TP fluctuations observed (Figure 5.3)

There is a steady decline in TP as the flow (Figure 5.3) drops to a steady low flow concurrent with a build-up of *Cladophora* biomass. This pattern is interrupted by a flush towards the latter part of March and again by a large flush in April. After this spate the concentration of TP began to gradually decline. The decreased P loading to the river as well as algal uptake of P are thought to be the major causes of the trend. In order to ascertain the importance of *Cladophora* uptake on the P concentration, intensive P monitoring would be needed along a substantial length of the river affected by proliferations (see Section 6.5). The TP concentrations recorded were generally low compared to those published as being critical for the growth and proliferation of *Cladophora* (see

**TABLE 5.3: A summary of nutrient data collected weekly during
December and January 1980/81 (mg N or P m⁻³)**
(Refer to figure 1.2 for site locations)

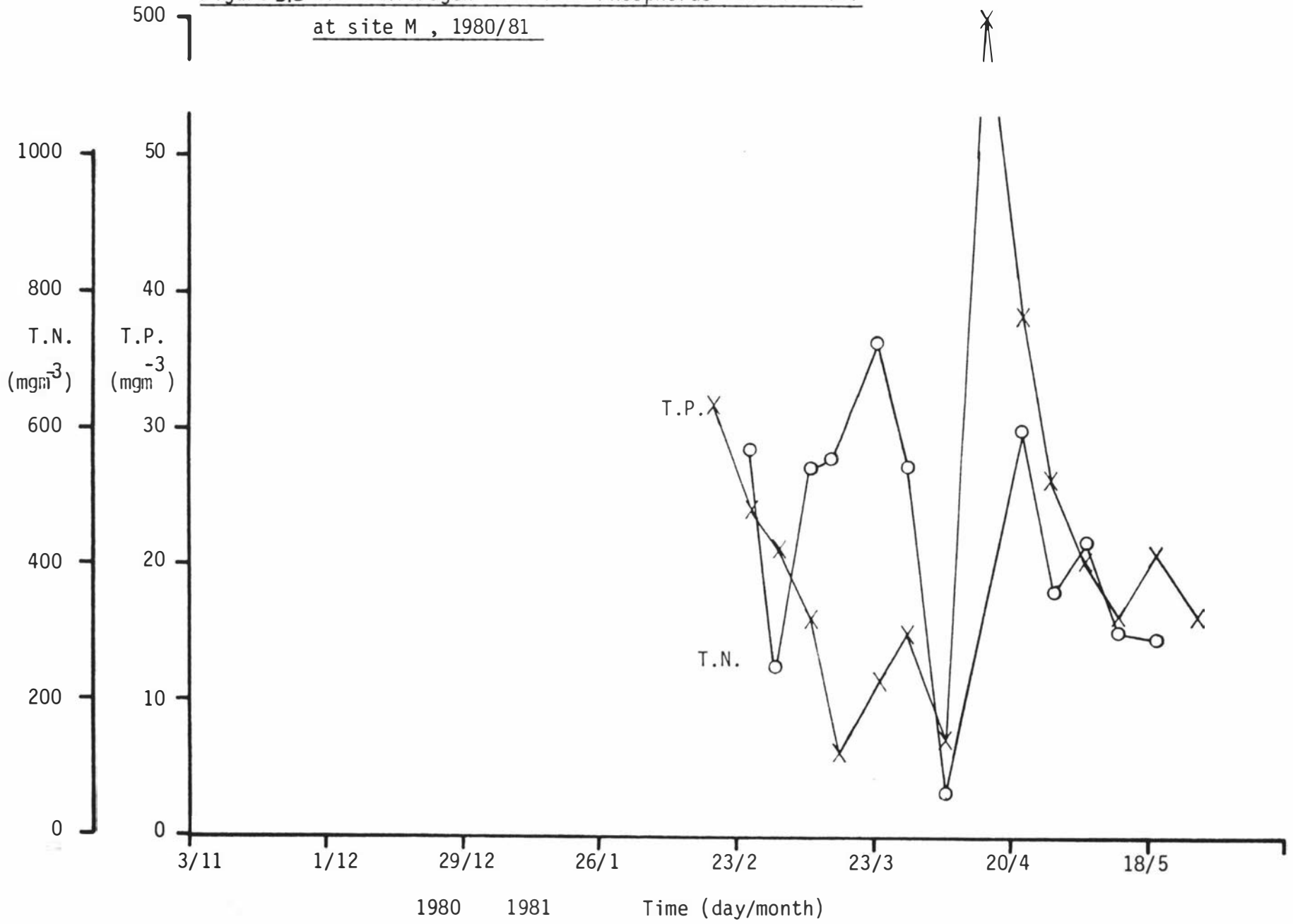
Nutrient		Sites	D	M	S	J	K	O
TP	Maximum		52.4	41.9	72.6	197	148	164
	Average		22.0	21.5	44.0	101	93.3	96.3
	Minimum		5.6	7.3	12.9	31.5	33.1	28.2
DRP	Maximum		29.6	37.1	50.8	122	85.4	86.6
	Average		13.4	14.9	26.1	66.9	58.1	57.0
	Minimum		2.5	3.2	4.6	16.2	11.1	25.4
NH ₃	Maximum		86.0	48.9	271	212	140	275
	Average		50.9	46.5	131	187	98.9	118
	Minimum		14.2	21.7	60.0	103	27.4	50.4
NO ₃	Maximum		659	649	737	653	644	600
	Average		295	275	279	271	269	276
	Minimum		46.2	33.1	32.3	66.1	77.4	63.1
NO ₂	Maximum		8.1	6.7	9.3	13.2	19.9	31.0
	Average		4.1	3.3	5.2	7.8	11.1	15.2
	Minimum		<1.0	<1.0	1.1	<1.0	<1.0	1.9
TN*	Average		551	547	928	1188	982	1006

KEY:

*Average value of four tests

TP = Total Phosphorus
 DRP = Dissolved Reactive Phosphorus
 TN = Total Nitrogen

Figure 5.3 Total Nitrogen and Total Phosphorus fluctuations
at site M, 1980/81



Section 2.5.3.4). The N concentrations observed were usually well in excess of the critical region dictated by either the P concentration or the published critical N values. The relatively low P levels indicated that P may well play an important role in the development of *Cladophora* proliferations. With this in mind Nutrient Availability Tests (Sections 2.6 and 4) were utilized to establish more directly, the nutritional status of the alga.

5.4 Nutrient Availability Tests (NATs)

P NATs and some N NATs were performed on samples from site T. The results from these analyses are presented in table 5.4:

TABLE 5.4: *Cladophora* Nutrient Availability Test results from site T during the 1980/81 season

Date	Extractive Phosphorus (%P)	Total Tissue Phosphorus (%P)	Ammonium Absorption Rate $\mu\text{g N (10mg)}^{-1} \cdot \text{hr}^{-1}$
23.2.81	0.10		
27.2.81	0.10		10.0
28.2.81		3.9	
4.3.81		2.3	
9.3.81		2.4	
13.3.81	0.13	2.8	
22.3.81		2.4	
2.4.81		2.9	6.0
3.4.81	0.10		
7.5.81	0.11	2.0	

The data indicated that surplus P and N were available to the algal population, at site T, prior to the sampling dates. (See section 4.3 for comparisons with levels associated with surplus/limitation.)

The presence of epiphytes throughout the season tended to confirm the hypothesis that there was surplus available N (Fitzgerald, 1968, 1971). However, during the winter of 1982 newly published work appeared to refute the infallibility of this relationship. (Stevenson & Stoermer, 1982).

It was felt that the P NATs needed some test that was complimentary, yet independent of the two tissue P components. The Alkaline Phosphatase Activity (APA) was chosen as a third P NAT for the ensuing season. (See section 6.6 and section 2.6.1 for rationale).

A survey was carried out on 9 April 1981 to sample some tributaries of the Manawatu River for TP and to test any resident periphyton with the P NATs. The results are presented in table 5.5. Generally, low TP concentrations were found. The Pohangina River is noteworthy because of its high TP concentration, relatively large flow and because it joins the Manawatu River near Ashhurst (Figure 1.2). The results from the Total Tissue Phosphorus (TTP) and Extractive Phosphorus (EP) often appear to be contradictory. The TP often indicating little available P while the TTP invariably indicated a surplus.

These results served to highlight the fact that the NATs do not necessarily reflect the nutrient status of the water at the time of sampling, and underlined the need for a better understanding of the P NATs and at least one additional P NAT in the sampling programme. (Section 4.1)

The range of TP indicated that the ultimate TP content of the Manawatu River (below its confluence with the Pohangina (Figure 1.2)) is a composite of many tributaries containing various amounts of TP.

TABLE 5.5: Periphyton survey, Phosphorus Nutrient Availability Tests and Total River Phosphorus from the Manawatu River and some of its tributaries, 9 May 1981

River	Major Periphyton genera	Total River P (mg.m ⁻³)	Extractive P (%)	Total Tissue (P) (%)
Mangahao	<i>Spirogyra</i> * <i>Stigeoclonium</i> *	<5.0	0.03	1.3
Makakahi	<i>Cladophora</i> *, <i>Ulothrix</i> , <i>Anacystis</i>	9.6	0.13	2.1
Mangatainoka	<i>Ulothrix</i> *	<5.0	0.04	2.3
Mangatainoka	<i>Ulothrix</i> *	<5.0	0.11	3.6
Makuri	<i>Cladophora</i> *	<5.0	0.22	5.0
Tiramea	<i>Cladophora</i> *	<5.0	0.06 0.04	2.4 3.1
Upper Manawatu	<i>Cladophora</i> *	<5.0	0.06 0.06	3.4 3.3
Upper Manawatu	<i>Cladophora</i> *	8.9	ND	ND
Mangatanui	<i>Spirogyra</i> * <i>Ulothrix</i> *	43.4	0.04	3.5
Upper Manawatu	<i>Cladophora</i> * <i>Anacystis</i>	<5.0	0.04 0.03	2.5 2.2
Pohangina	<i>Cladophora</i> *	25.7	0.06	4.4

* Species tested

ND - No Data

5.5 Primary productivity and Dissolved Oxygen fluctuations

Initial studies showed that the upstream reaches (from Ashhurst to site M (Figure 1.2)) experienced almost identical diurnal DO fluctuations. Thus, single station diurnal DO analysis was used to estimate the community primary productivity (See section 2.4 and 3.8.2). During this season a limited number of continuous DO monitoring periods were possible. However, they were carried out under a variety of conditions and gave a good indication of the expected magnitude and range of the various primary productivity components. The data obtained are summarised in table 5.6.

TABLE 5.6: Primary productivity data at site T during early 1981

Date	Gross Photosynthesis ($\text{g O}_2 \text{m}^{-3} \text{d}^{-1}$)	Total Respiration ($\text{g O}_2 \text{m}^{-3} \text{d}^{-1}$)	Net primary productivity ($\text{g O}_2 \text{m}^{-3} \text{d}^{-1}$)	P/R	Net Areal primary productivity ($\text{g O}_2 \text{m}^{-2} \text{d}^{-1}$)
23.1.81	4.90	-4.14	0.76	1.18	0.38
3.2.81	7.92	-7.56	0.36	1.05	0.40
2.3.81	1.21	-1.31	-0.10	0.92	-0.09
2.4.81	4.03	-3.93	0.10	1.02	0.08
7.4.81	3.96	-4.70	-0.74	0.84	-0.65
8.4.81	3.70	-4.68	-0.98	0.79	-0.88

The data were not extensive enough to warrant vigorous scrutiny of all the DO deficits (DODs) or minimum DO concentrations. During the one major growth period (mid-March to early April) a DO minimum and maximum of 8.1 g m^{-3} and 11.2 g m^{-3} respectively, were found on 8 April 1981. The water temperature at the minimum DO was 15.5°C giving a DOD of 1.9 g m^{-3} . The relatively large negative primary productivity during this two-day monitoring period was apparently due to overcast conditions restricting the gross photosynthesis of the relatively high *Cladophora* assemblage biomass density. The effects of other factors on productivity and DOD could obviously not be thoroughly investigated. This was to be one of the main aims of investigations in the 1981/82 and 1982/83 seasons (see Section 6.7).

5.6 Ammonia and pH

The total ammonia concentrations found at various sites along the Manawatu River revealed peaks at site K (Table 5.7). This agreed with previous findings that effluent discharges, in particular the freezing works, were responsible for high ammonia concentrations at site K (Figure 1.2 and Section 5.4) (Currie, 1977). Sampling was carried out to estimate spatial and temporal variations rather than to identify maximum concentrations. (Table 5.7)

The temporal variation at site T (Table 5.8) was minimal in comparison to observed downstream fluctuations. Downstream temporal variations were investigated in the 1981/82 season (Section 6.8).

A limited number of studies on pH fluctuations were performed. During higher flows and periods when algal biomass was minimal, pH was relatively stable at approximately 7.5 ± 0.4 . However, during periods of high insolation when algal proliferations were established, pH fluctuations were more dramatic (Table 5.9). At these elevated pH levels, concern about toxic levels of ammonia increases (Section 2.3.2) and to this end, more intensive monitoring of the pH was performed during the following seasons (Section 6.8).

TABLE 5.7: Ammonia concentration at Various Sites in Early 1981
(Refer to figure 1.2 for site locations)

Date Site	Ammonia-N (mg m^{-3})							
	7.1.81	13.1.81	20.1.81	27.1.81	3.2.81	10.2.81	17.2.81	25.2.81
D	25	42	23	14	56	86	76	84
M	48	22	27	75	36	99	41	25
S	127	60	94	116	150	114	271	113
K	380	102	137	183	151	130	198	212
J	123	30	27	121	140	126	84	140
O	81	114	83	98	137	50	60	275

*Sampling time was approximately 10.00 - 11.00 on each date.

TABLE 5.8: Total ammonia concentrations at site T 22-23 March 1981

Time	1300	1515	1800	2130	0730	1115	1315
Ammonia -N(mg m^{-3})	44	52	61	80	36	16	25

TABLE 5.9: pH fluctuations at site M, 11 April 1981

Time	0930	1130	1330	1530	1730	1930	2130
pH	8.1	8.2	8.4	8.5	8.5	8.3	7.8

CHAPTER 6

RESULTS FROM THE 1981/82 AND 1982/83 SEASONS

6. RESULTS FROM THE 1981/82 AND 1982/83 SEASONS

6.1 Introduction

This chapter represents a continuum of both seasons in order to present an intelligible sequence of events from one year to the next. Patterns and hypotheses identified in one season can be tested against data from both. Ideally, continual yearly monitoring would be necessary to discern short and long term changes in water quality parameters.

6.2 Physical site characteristics

The three major sampling sites, T,D and M (See figure 1.2) were investigated in relation to their average current velocities and substrate characteristics. The ranges of current velocities recorded during low flow periods ($12-30 \text{ m}^3 \text{ s}^{-1}$) are presented in table 6.1. This illustrates the nature of each site's slope and exposure to the main river flow. Site T was set apart from a deep

TABLE 6.1: The range of average site velocities recorded during low flow periods ($12-30 \text{ m}^3 \text{ s}^{-1}$)
(Refer to figure 1.2 for site locations)

Site	Current Velocities (m s^{-1})
T	0.6 - 0.9
D	0.6 - 1.0
M	0.4 - 0.7

river channel area and consequently had a relatively stable flow regime, being somewhat protected from minor flush events, the bulk of increased flows being directed down the deep channel area.

Site D experienced a similar range of current velocities during low flow periods. However, it was in the main river channel and consequently more exposed to the full force of the river during flush events.

Site M had generally lower current velocities compared to the other major sites. This was attributed to the decreased river slope.

Figure 6.1 A longitudinal profile of the Manawatu River bed height above sea level.

(After Currie, 1977 & MCB, 1950)

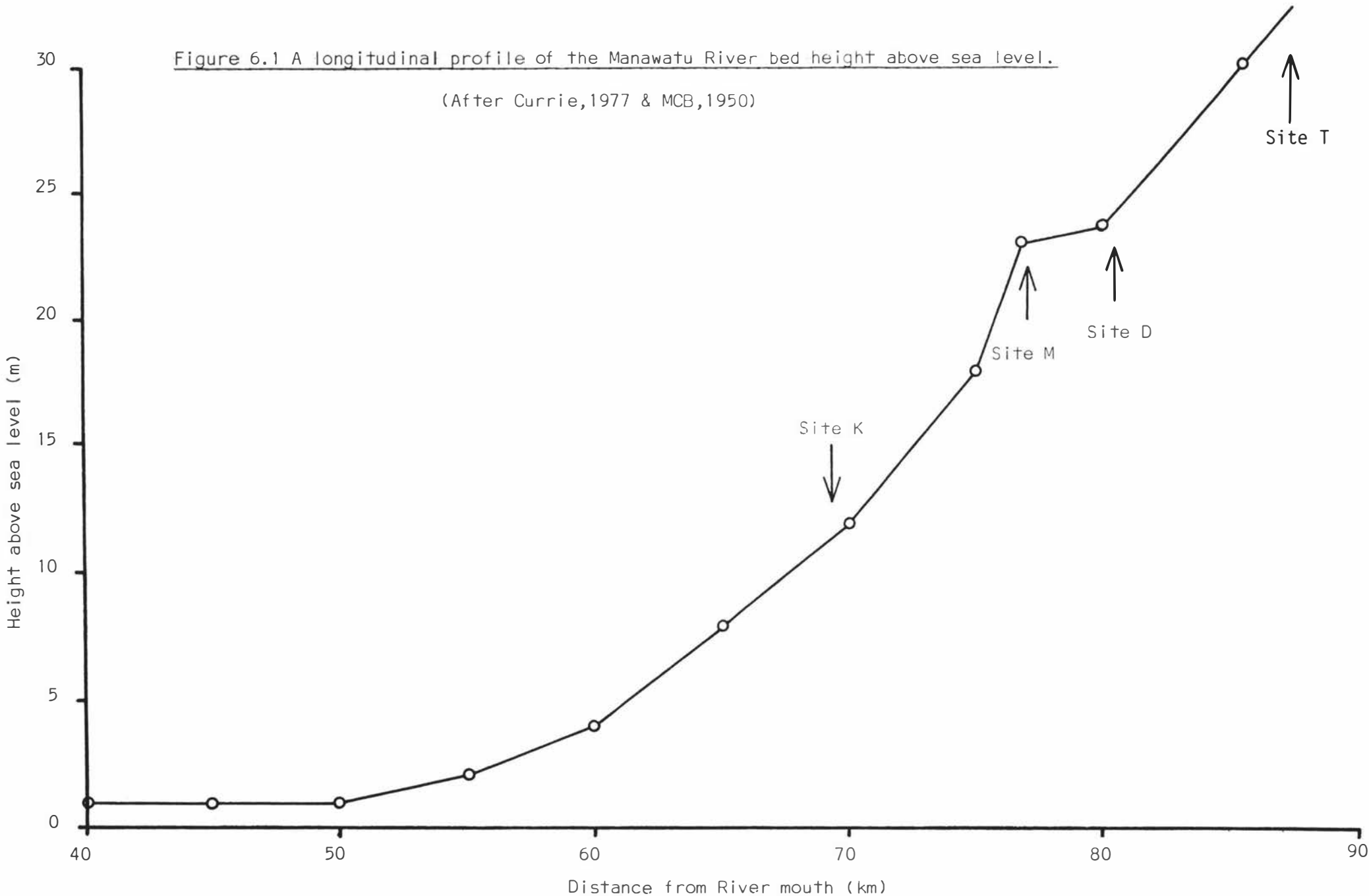
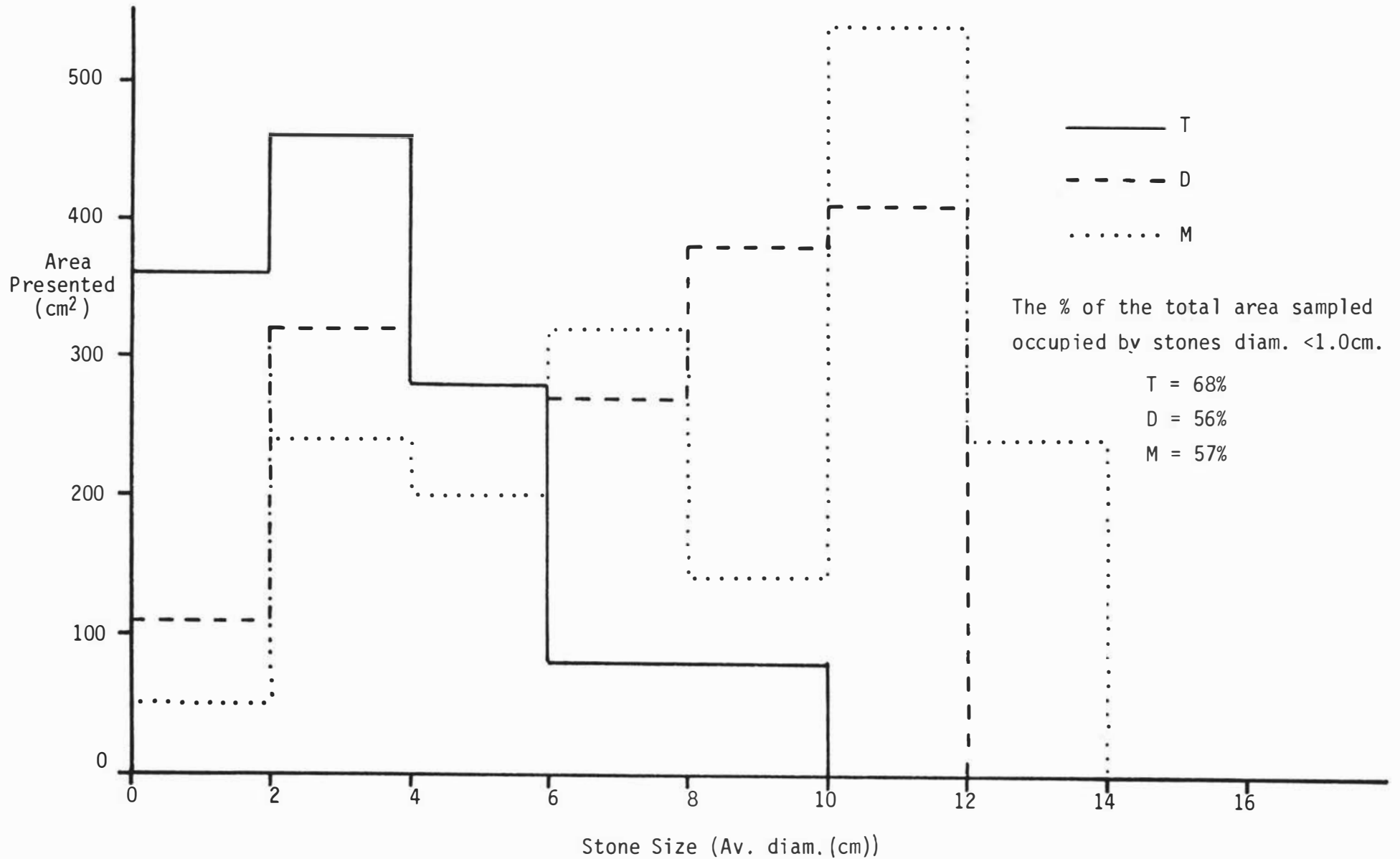


Figure 6.2 A comparison of the areas taken up by different stone sizes, at sites T,D,and M.
 (Total area of river bed sampled = 4800 cm²)



(Figure 6.1). Another feature of this site was the proportion of large stable stones that are relatively immobile in comparison to the substrate at the other sites. (See figure 6.2).

The distribution of stone size at each site has an important bearing on the stability of the substrate and thus its suitability as an area for *Cladophora* proliferation. The distribution of stone size at the various sites is illustrated in figure 6.2 (See section 3.3.2) At site T there was a noticeable dominance of relatively small stones between 1-6 cm average diameter. In contrast, at site D there was a more equal sharing of the total 'flat' presented area (i.e. each stone is treated as a flat disc) between stones in the 2-12 cm diameter size range. There were also more representatives from the larger (6-12 cm diameter) stone sizes. The distribution found at site M illustrated a major reason for generally higher *Cladophora* biomass densities being found there. Site M was usually left with a remnant post-spate population, in contrast to site D, and sometimes site T which were often totally decimated. (See section 6.4).

6.3 Light and temperature conditions

Light and temperature conditions were generally similar over the study periods (Figures 6.3 and 6.4). One noticeable difference can be seen between the November light data, with the monthly average being considerably higher in the 1981/82 season. This, combined with the relatively stable river flow conditions, (See figures 6.5 - 6.8) appears to have produced conditions more suitable for the establishment of *Cladophora* in the 1981/82 season than in the 1982/83 season. The availability of light during the onset of the 1982/83 season was much reduced because of frequent flush events. (Figure 6.8)

The river temperature data are only intended as a guide to the seasonal variation. Daily changes can be large and often rapid, dependant not only on total insolation but also on factors such as the duration of peak insolation and the nighttime cloud cover. (See appendices 7&8) These daily fluctuations, superimposed on

Figure 6.3 Maximum river temperatures and surface light intensity,
 1981/82
 (Light Measured 50km from Palmerston North)

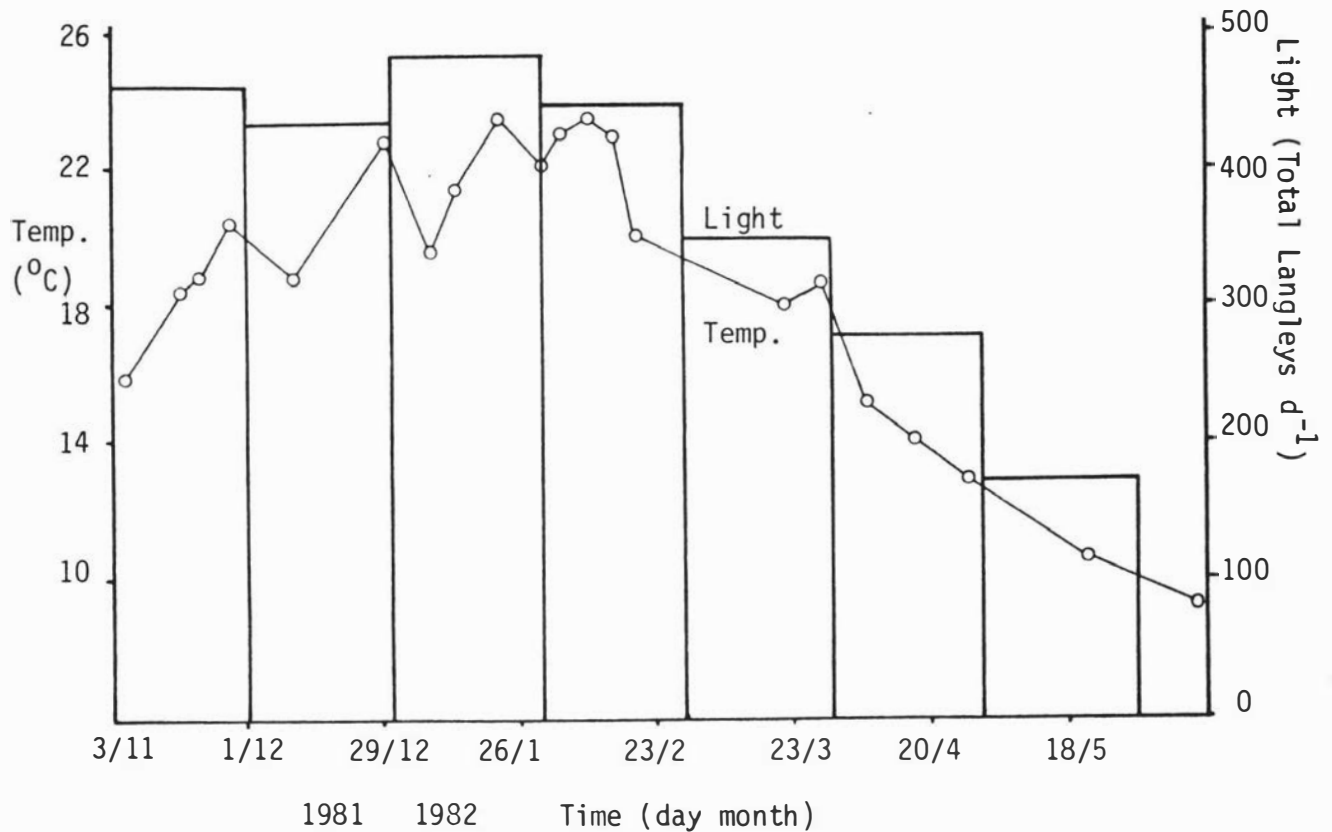
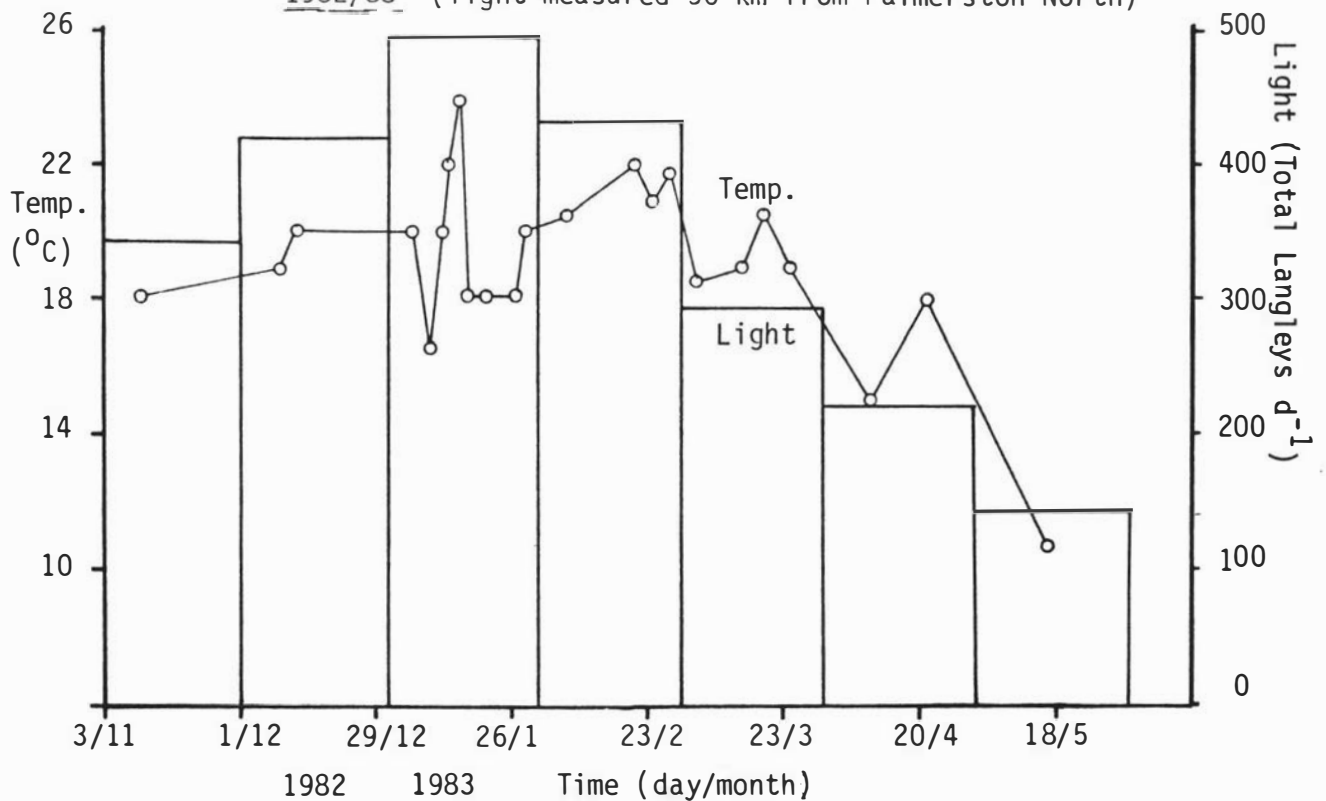


Figure 6.4 Maximum river temperature and surface light intensity,
 1982/83 (light measured 50 km from Palmerston North)



a seasonal trend, could influence the development of the periphyton community structure. (Wong *et al*, 1978). However, some quantitative measure would be needed to account for the magnitude, duration and frequency of the fluctuations before any causal relationships could be identified.

6.4 *Cladophora* biomass density and periphyton distribution

The dominant influence of flush events on both *Cladophora* establishment and proliferation is illustrated in figures 6.5 - 6.8. The biomass of the *Cladophora* assemblage frequently increased from just visible tufts of approximately 1 g DW m^{-2} to quite dense proliferations of $80\text{--}100 \text{ g DW m}^{-2}$. One peak value in early January 1982 of 300 g DW m^{-2} was a little suspect as the drying period was unavoidably cut short due to a breakdown of the 64°C drier. The importance of spates in reducing the standing algal crop is apparent in both seasons. It can also be deduced from the data that there was a need for a relatively steady river flow before *Cladophora* could first establish and then proliferate. During the 1982/83 season there were considerably more flush events in November/December than in the previous season. (cf. figures 6.6 and 6.8). These had three major effects:

- (i) Any established growths were reduced or decimated, depending on the size of the flush.
- (ii) During the larger spates the riverbed substrate was thrown about, ripping off any existing vegetative filaments, and some overwintering basal filaments. Spates could also shift the whole bed substrate, burying existing stones with newly excavated ones.
- (iii) The large flush events can seriously interfere with the life cycle pattern (See section 2.5.4). Disruptions to the sequence of zoospore production and establishment can mean that a large part of the inoculum potential is lost as the high current velocity reduces the number of zoospores available and able to settle on stable, and mobile substrates.

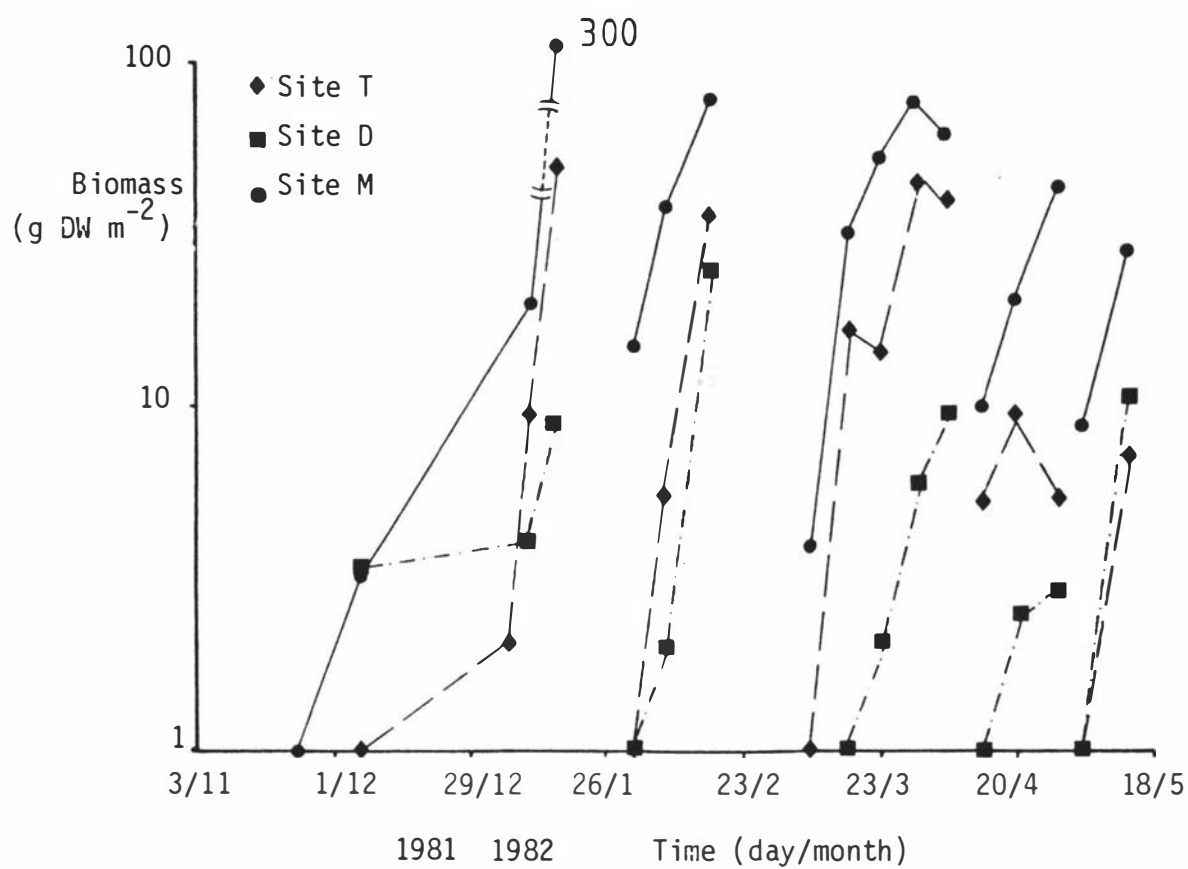
Figure 6.5 *Cladophora* biomass fluctuations at sites T, D and M. 1981/82

Figure 6.6 The Manawatu River flow 1981/82

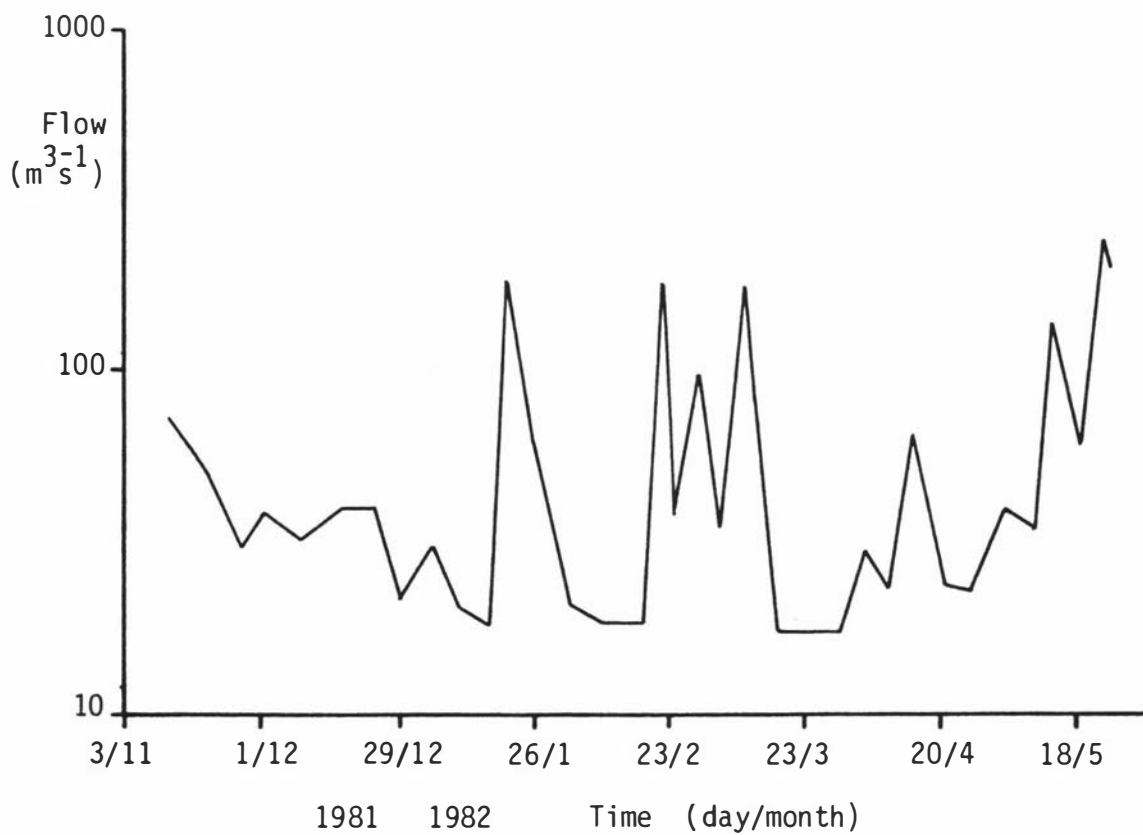


Figure 6.7 *Cladophora* biomass fluctuations at sites T,D and M . 1982/83

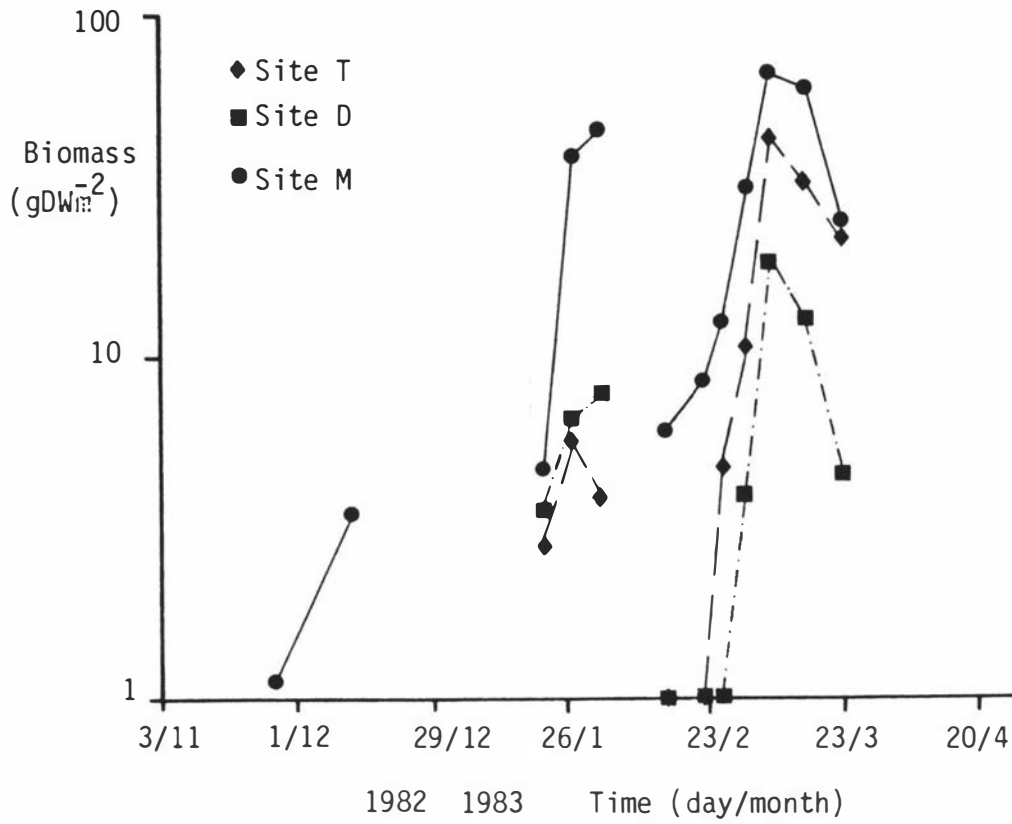
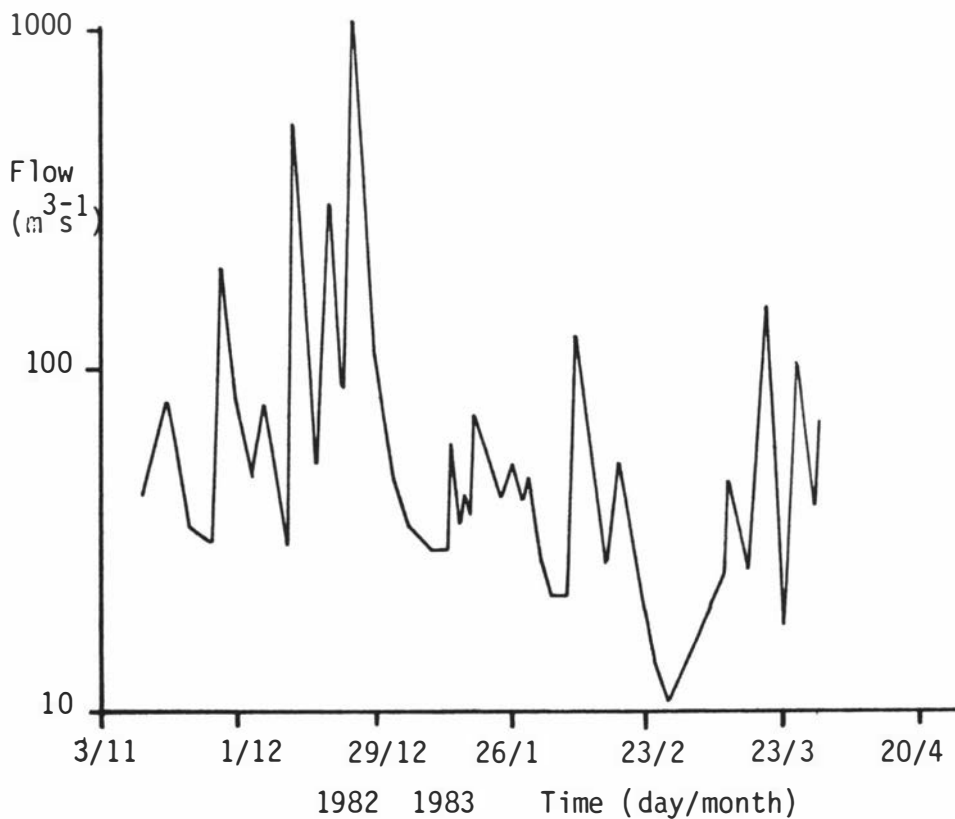


Figure 6.8 The Manawatu River flow 1982/83

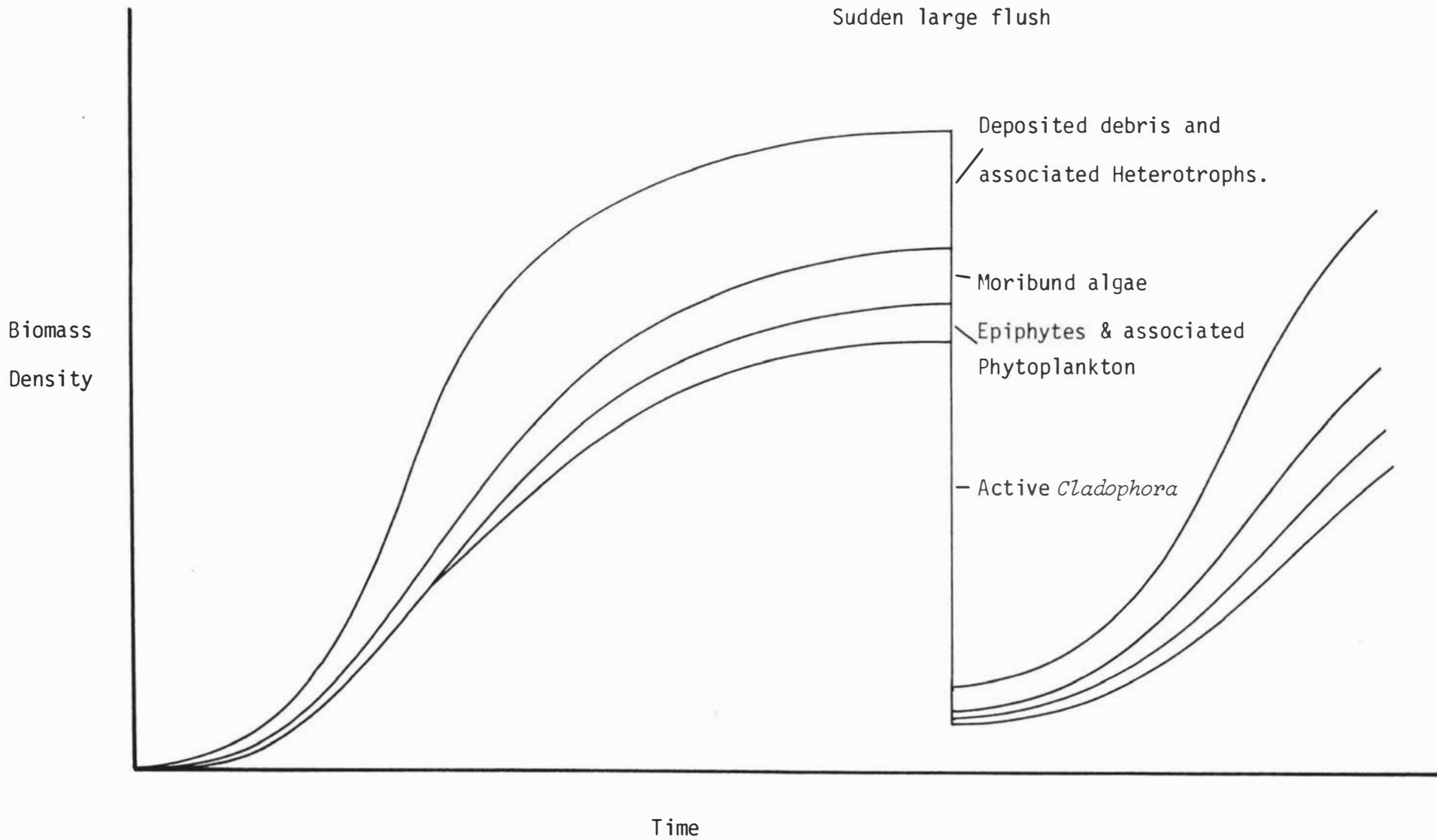


In the early stages of the 1981/82 season there were few flush events of any magnitude, and *Cladophora* proliferations became established throughout the upper river. The basic pattern of *Cladophora* development was seen to be one of exponential growth, interrupted by flush events.

A stylized representation of the exponential development and flush-induced decimation of a *Cladophora* proliferation is presented in figure 6.9. The proportion of each component in the *Cladophora* assemblage varies from site to site. The site velocity and history appear to be important factors involved in the qualitative description. During one *Cladophora* growth period the amount of dry weight debris separated from the active *Cladophora* filaments was found to be 41% at sites T and D and 47% at Site M. This would appear to substantiate the observation that there was invariably more debris associated with the growth at Site M. (See section 6.2 and figure 6.1). The change in river slope between sites D and M (Figure 6.1) together with a broader river width (e.g. 50m at Site M, 42m at Site D, flow = $15 \text{ m}^3 \text{ s}^{-1}$ with an average depth at both sites of 0.8m) was responsible for a generally reduced current velocity at site M. Site M was thus more susceptible to the settling out of particles that would have remained in suspension at higher velocities. The biomass was also consistently greatest at site M and usually least at site D. (Figures 6.5 and 6.7). These intersite differences are thought to be a consequence of site characteristics and their exposure to the force of flushes. (See section 6.2). The intersite nutrient differences are discussed in section 6.5. However, these indicated a less favourable nutrient environment at Site M, which was apparently less important than the physical/hydrological intersite differences that caused site M to be the most favourable for *Cladophora* assemblage proliferations.

Macro and micro observations during *Cladophora* assemblage developments and flush-induced reduction revealed that the post flush composition varied between sites and was also dependant on the magnitude of the flush. There were four major types of response to flush events, dependant on the assemblage composition, flush magnitude and assemblage position in relation to river topography. Extreme assemblage characteristics have been used to illustrate

Figure 6.9 An idealized representation of the development and denudation of a *Cladophora* assemblage.



these points.

Clean *Cladophora* Assemblages (CCA) are identified as those largely devoid of epiphytes and moribund *Cladophora*, and Dirty *Cladophora* Assemblages (DCA) as those with few healthy *Cladophora*. Thus, the effects of 'minor' and 'major' flushes on these two assemblage types are as follows:

(a) 'Clean *Cladophora* Assemblage' (CCA)

- (i) Minor Flush: This would tear off any easily removed and older, filaments and roll some of the small stones. The remnant community will be very similar in composition to the initial one.
- (ii) Major Flush: Because the CCA would be initially in a relatively swiftly flowing region of the river a major flush will have a large impact on the growths, often totally decimating the CCA leaving a bare stone substrate.

(b) 'Dirty *Cladophora* Assemblage' (DCA)

- (i) Minor Flush: Similarly to the above situation, a minor flush event will remove the older and easily dislodged filaments.
- (ii) Major Flush: Because the DCA would be in a relatively 'protected' river stretch the effect of a major flush can often just remove a large proportion of the filamentous material extending out into the river, leaving a closely adhering mat of basal filaments and associated debris. If the flush is massive enough, of course, it will totally decimate the DCA in a similar fashion to the CCA.

As the biomass builds up, the faster sloughing-off rate increases the amount of free floating filaments. These were monitored infrequently at site T and found to range from about 2-4 mg m⁻³ during steady flow periods in the 1981/82 season, at biomass levels of approximately 30-70 g DW m⁻². (See section 3.2.3).

During the following 1982/83 season the frequent spates early in the 'season' (Figure 6.8) apparently restricted the establishment of *Cladophora*, and species of *Gomphonema* and *Stigeoclonium* rapidly became abundant in the cool water following flush events. Quantitative measures of these species were not made, as their morphology and growth characteristics were radically different from those of *Cladophora*. *Stigeoclonium tenue* grows faster (maximum specific growth rate 2.5 times greater) than *Cladophora glomerata* under equivalent conditions (Rosemarin, 1982). However, it does not usually proliferate in flowing rivers (average site velocity $> 0.4 \text{ ms}^{-1}$) such as the Manawatu River, as the substrate only supports a limited tuft size/length before sloughing off occurs (Rosemarin, 1982). The vacated space usually had a background of *Gomphonema* and/or *Cladophora*, which then dominated the niche. *Gomphonema* was dominant for most of January, apparently preferring the cool ($< 17^{\circ}\text{C}$) water that persisted after the major spate of December 26 1982. (Figure 6.8). There were isolated *Cladophora* growths during this period, however, the majority were thickly coated with *Gomphonema*. As the river temperature increased, towards the end of January, the *Gomphonema* mats began to degrade and slough-off. This was possibly due to increased respiration dominating photosynthesis caused by the differential response of the two processes to temperature. A continual dominance of metabolic activities by respiration leads to a loss of phototroph viability (Lorenz & Herdendorf, 1982). This progression left patches of rock exposed from which old basal *Cladophora* filaments began to develop, together with some *Stigeoclonium* colonization. *Cladophora* eventually dominated the available substrate, and was monitored until a large flush at the end of March (Figure 6.8), after which, field work was curtailed. (Appendix 1, photographs 3 & 4)

6.5 Nutrients

6.5.1 Phosphorus

As mentioned in Section 5.3, the average TP concentration was low in comparison to most overseas figures cited as necessary for lotic *Cladophora* proliferations (Section 2.5.3.4). The data presented in figure 6.10 illustrate the effect flush events had on the TP concentration. Monitoring of the DRP and nitrate during the

1981/82 season was again to have been undertaken by the MRWB, however, unforeseen difficulties prevented this, leading to the need to place increasing reliance on the Nutrient Availability Tests (Section 6.6).

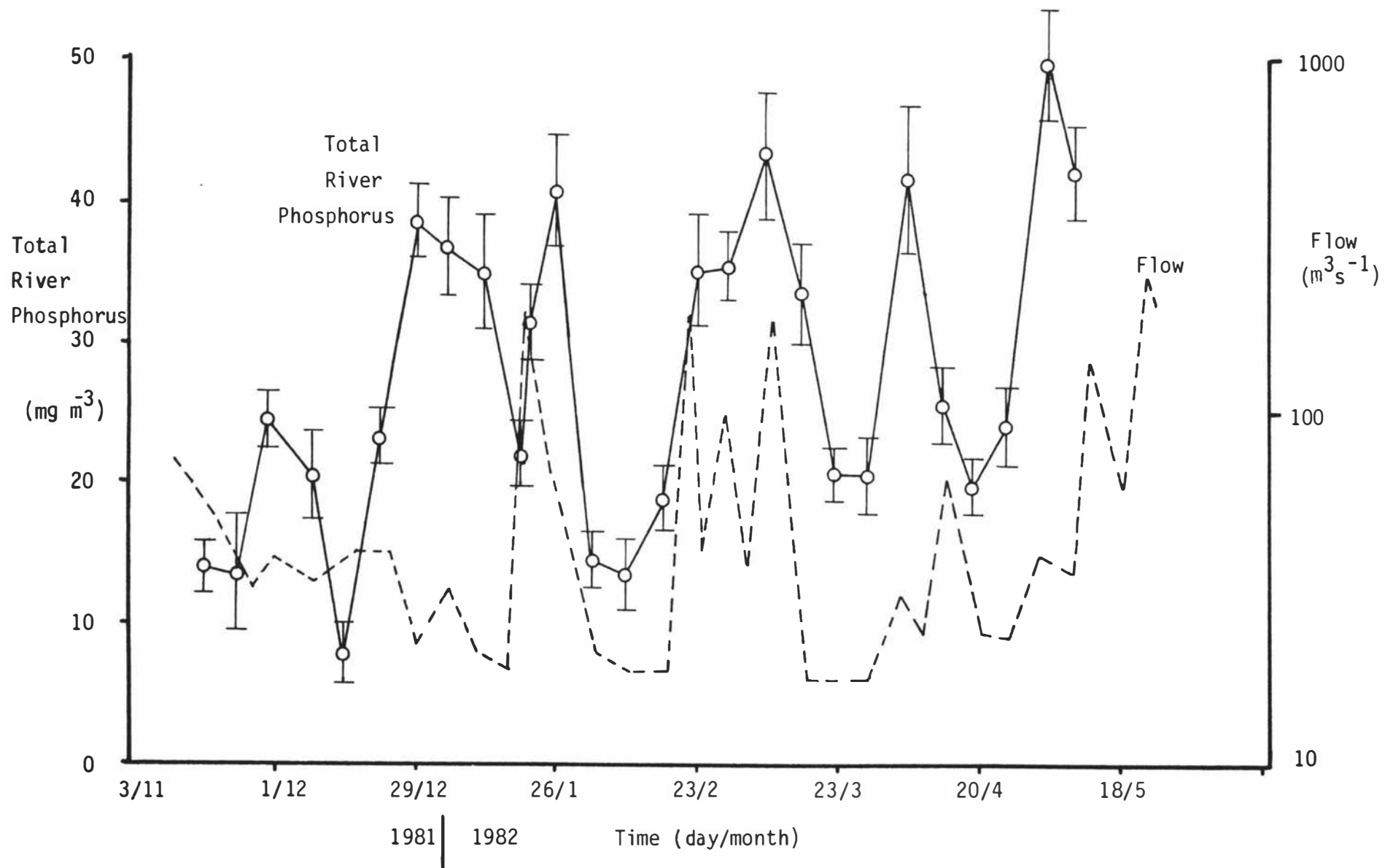
The fluctuations in TP concentrations can be seen to be linked primarily to the variations in river flow. (Figure 6.10) The periodic flushes caused by heavy rainfall can be seen to dramatically increase the P load of the river. The extent of this addition will depend on many factors, including rainfall intensity and duration, and the state of the catchment area in relation to its ability to retain P. Thus, while a relatively minor flush towards the end of December 1981 caused a large increase in TP, a much larger flush towards the end of January 1982 resulted in a very similar TP concentration.

During extended periods of low flow the TP concentration decreased. Whether the reduction was solely attributable to the flow decrease is questionable. The *Cladophora* proliferations, that occur extensively along the whole of the upper Manawatu River and some of the tributaries, will through their P uptake cause some impact on the TP budget of the river system. *Cladophora* has been shown to have high specific P uptake rates in comparison to many other aquatic plants (Auer & Canale, 1980; Howard-Williams, 1981).

It would be possible to make an estimate of the P uptake capacity of the Manawatu River *Cladophora* and thus the impact of this on the river P concentration. However, such an undertaking would have to involve some estimate of the percentage river bottom area covered by an average *Cladophora* biomass. This would necessitate some remote sensing of the river to get a realistic estimate of the average *Cladophora* percentage cover. This information would then be combined with biomass data and the results of P NATs. The limited resources of this study meant that a more synoptic approach was adopted to observe P dynamics.

A diurnal study of TP, together with TN, TTP and EP was carried out during February 1982. The results are presented in figure 6.11.

Figure 6.10 The average (3 sites) Total river Phosphorus and river flow , 1981/82.



The initial TP concentration was high probably due to the after-effects of a recent flush (Figure 6.8), and while a large fluctuation of TP was observed, it was probable that this was the result of stochastic processes rather than a massive photosynthetically induced algal uptake of P (Round, 1973). There was a slight increase in TTP and EP. However, to make any useful inference about the effect of *Cladophora* proliferations on the river P a more intensive temporal, and spatial study would be needed, monitoring both the P NATs and the readily available P, as opposed to the TP. This was investigated in the following 1982/83 season (See section 6.6.1)

In the 1982/83 season it was decided to personally monitor the DRP (Figure 6.12). While this fraction of the TP is not necessarily synonymous with the biologically available P it is considered the next best thing for routine monitoring. (Section 2.7).

Relatively high DRP concentrations were observed during the initial frequent flush period, however, from January 1983 the average DRP concentration was never observed to fall below about 14 mg P m^{-3} . (See figure 6.12). The precision during this season was usually much better than that found for TP in the preceding season. (Figure 6.10). The DRP appears to be less influenced by flush events although this observation may well be a result of the sampling regime used. The river was not usually sampled during flush events and evidence from the P NATs indicated that there was an initial surge of available P which does not linger as long as TP. During the initial stages of a flush event the river apparently contained a large amount of readily available P, after this event the elevated flow contained mainly particulate P and thus sampling at this stage would not indicate the past affluence of available P. The flush on 9 February 1983 significantly raised the DRP concentration from 3 to 9 mg P m^{-3} which was then slowly reduced over a period of about one month. Whether this reduction was attributable to algal uptake or some other removal mechanism could not be determined from the available data. (^{32}P labelled DRP would be needed to assess the relative importance of other P sinks). The DRP concentrations recorded at each site have been examined to see if there was significant DRP removal along the study reach during

Figure 6.11 A diurnal study of Total Nitrogen ,Total Phosphorus
 Total Tissue Phosphorus and Extractive Phosphorus
 at site M , 23/2/82. (Precision as in figures 6.10 ,6.14, 6.16 & 6.17)

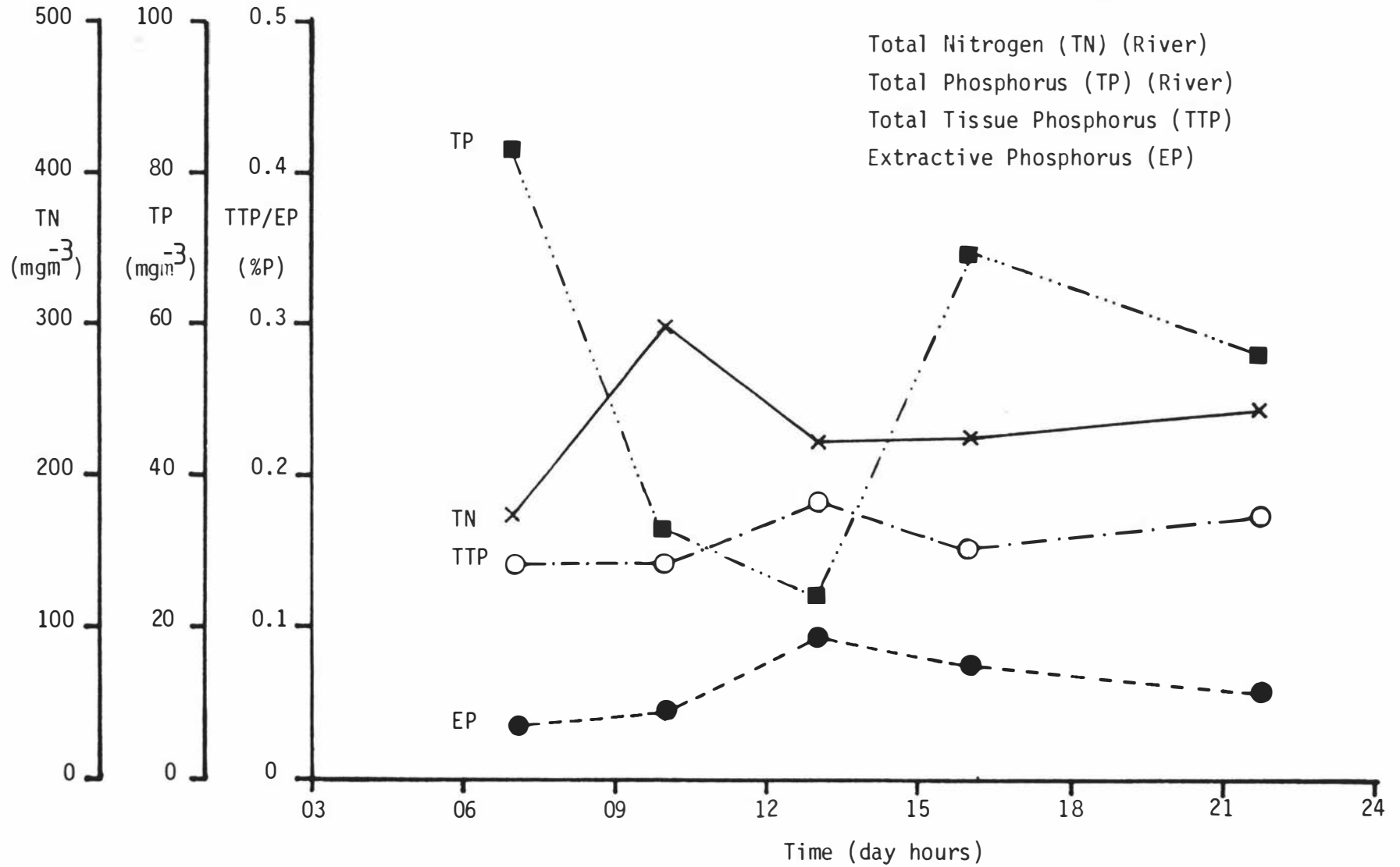
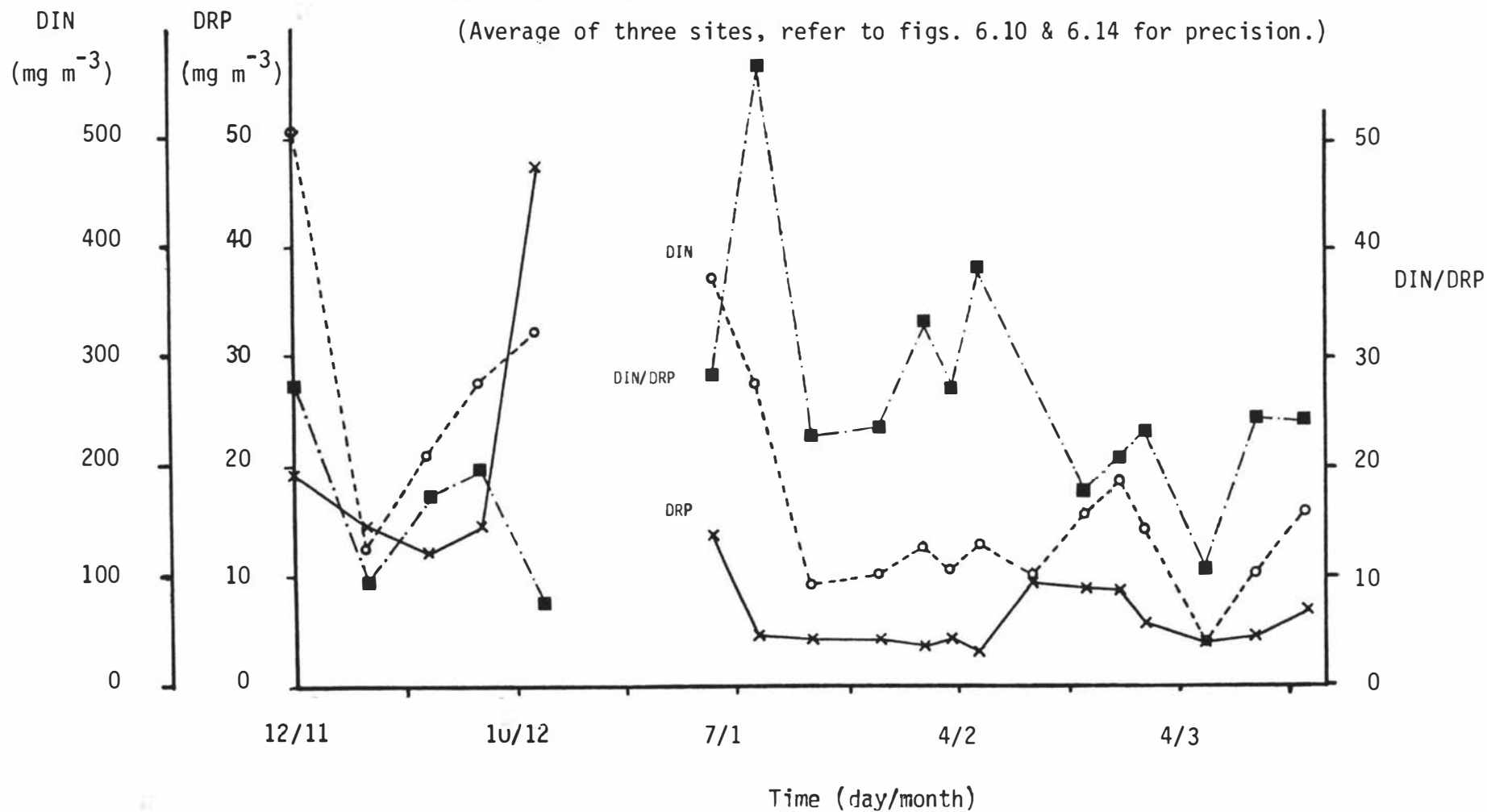


Figure 6.12 Dissolved Inorganic Nitrogen (DIN) , Dissolved Reactive Phosphorus (DRP)
and the DIN/DRP fluctuations 1982/83.



steady flow, *Cladophora* proliferation, conditions. (Figure 6.13). It was not possible to identify a consistent reduction of DRP over the length of the reach under study (13 river kilometres from Site T to Site M). Minor flow variations from the 26 to 29 January may have disturbed a downstream trend that was evident on 26 January and 7 February.

The impact of these very low DRP concentrations (3.3 - 4.7 mg P m⁻³) are discussed further in section 6.6 in relation to the P NAT results obtained during the same period.

The significance of the DIN to DRP ratio is discussed in section 6.5.2.

6.5.2 Nitrogen

The Total river Nitrogen (TN) observed during the 1981/82 season generally fluctuated between 200 and 600 mg N m⁻³ (Figure 6.14). One very large value was obtained after a large flush in early December 1981. Studies of TN and TP during 'off-season' flush events support the view that extremely high TN concentrations are usually found after spates. The fluctuations of TN were not as closely linked to the rainfall and consequently increased flows, as TP was (cf. figures 6.10 and 6.14). In absolute terms there was usually a surplus in relation to the concentration dictated by the algal nutrient demand (See section 6.6) and the TP concentration.

In the 1982/83 season the amount of readily available nitrogen (nitrate-N and ammonia-N) was much less than had been envisaged on the basis of TN concentrations observed in the previous season. (Figure 6.12). The DIN:DRP ratio, which assessed the available river nutrients can be used, together with a knowledge of, the absolute concentrations, *Cladophora* biomass, and flow to get an approximate estimate of the comparative nutritional capability of the river. During the relatively steady flow period that occurred through January and the first week of February 1983, the ratio was consistently high, although when considered in the light of relatively high DRP levels, it should not be taken as evidence for P limitation of the *Cladophora* growth rate. After the flush event on 9 February

FIGURE 6.13: Dissolved Reactive Phosphorus fluctuation at sites T,D and M during a period of *Cladophora* proliferation 25 January 1983 - 7 February 1983
 (Bars indicate on St. dev.)

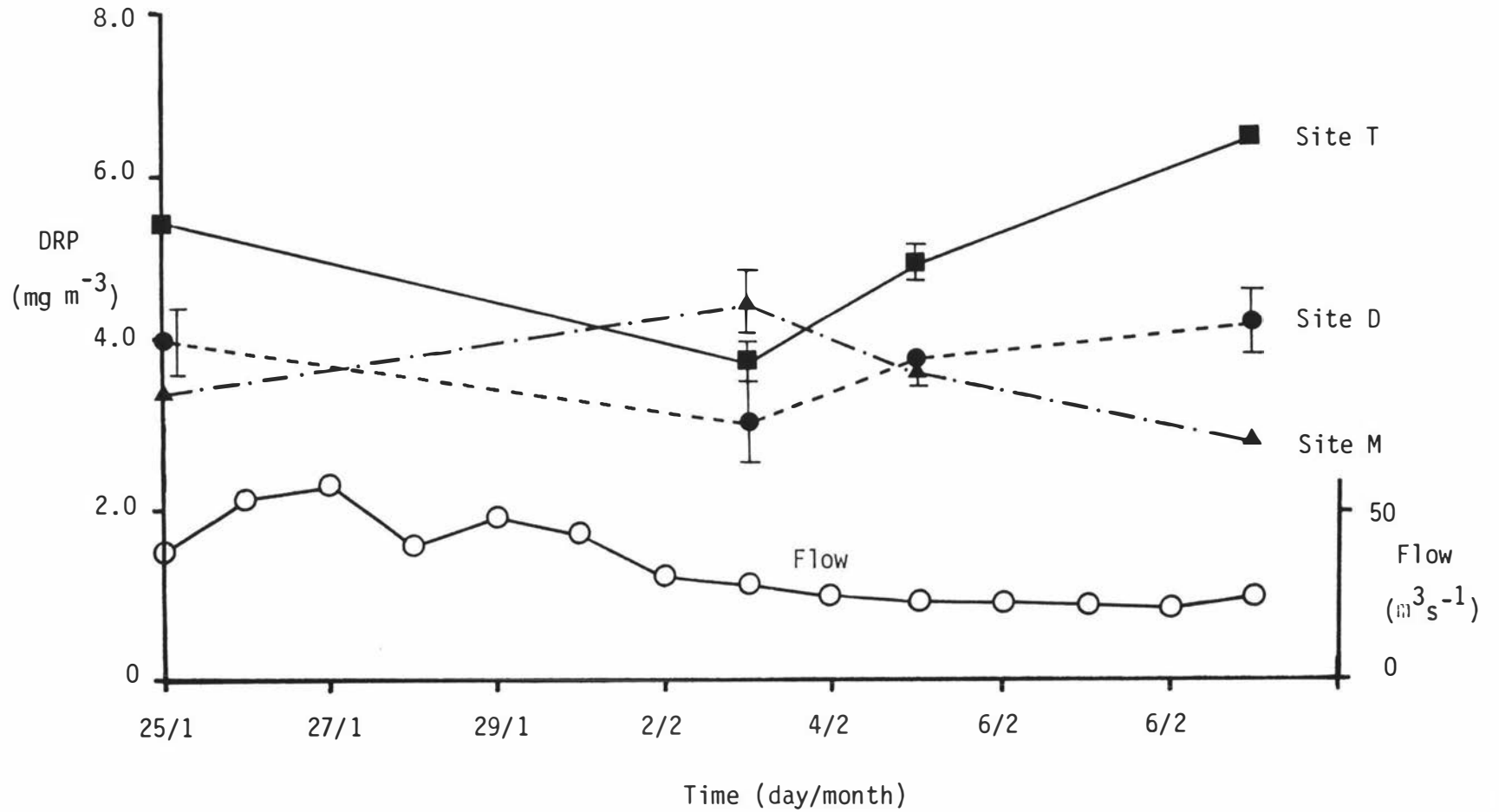
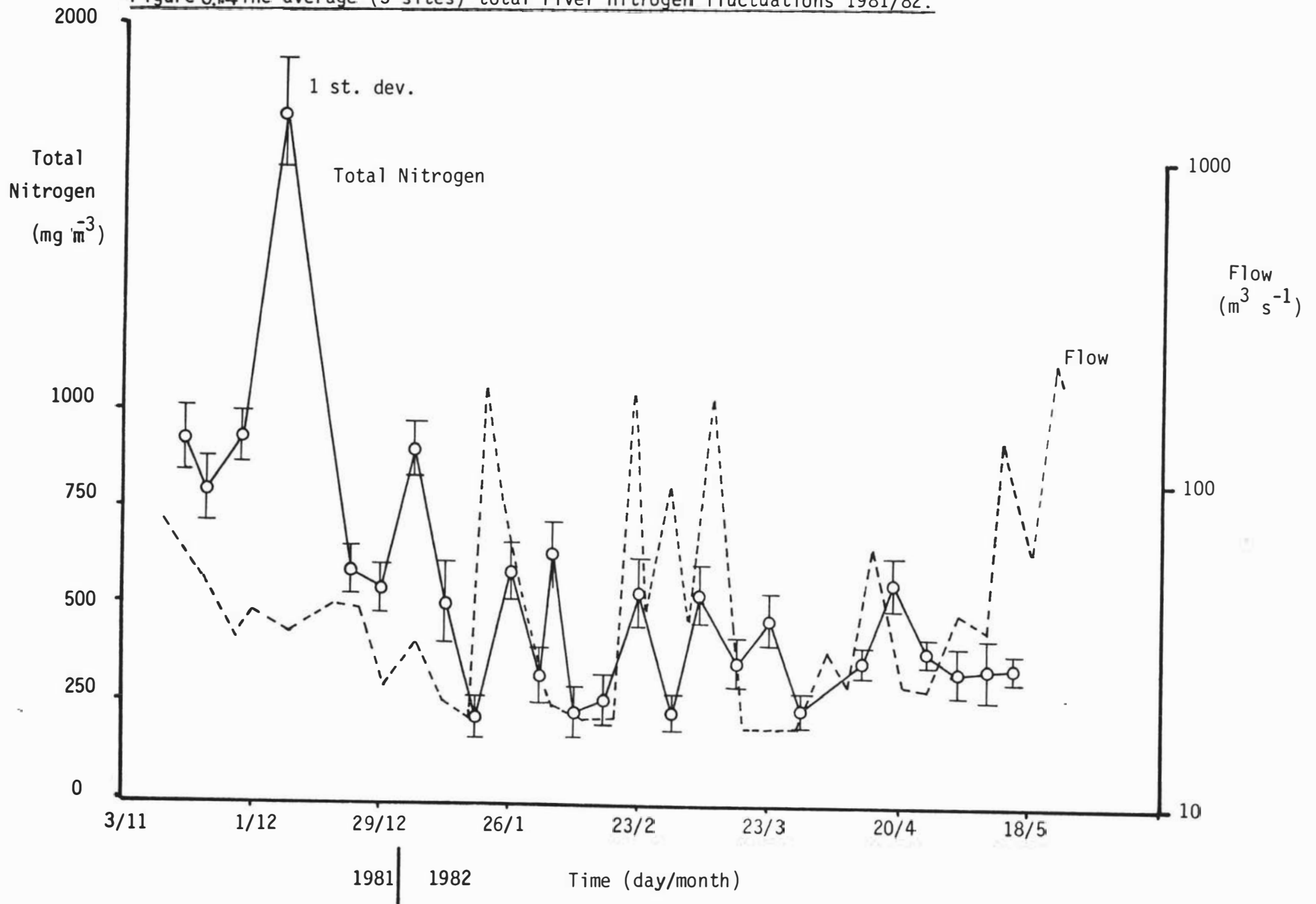


Figure 6.14 The average (3 sites) total river nitrogen fluctuations 1981/82.



the ratio began to increase although this was apparently due to an increase in the available nitrogen rather than a differential decrease of DRP over DIN. Another interesting feature apparent from figure 6.12 was the frequent simultaneous reduction in all the variables illustrated. This was especially conspicuous at the beginning of March. This effect indicated that the available N was possibly becoming limited in relation to the available P. This hypothesis is further explored using the more direct N NATs in section 6.6.2.

6.6 Nutrient Availability Tests

6.6.1 Phosphorus

During 1981/82, fluctuations of TP, TTP and EP followed similar trends and an inverse relationship was observed between these variables and the Alkaline Phosphatase Activity (APA) (Figures 6.15 - 6.18). The usefulness of comparing the results of Phosphorus Nutrient Availability Tests (P NATs) with correlation coefficients is reduced because of the following:

- (a) The variable response times (See section 4.3) of the P NATs will mean that TTP and EP will show recovery from limitation faster than APA.
- (b) The natural biological variation and changes caused by other factors (e.g. see section 2.6) during times of surplus P will tend to hinder the identification of specific relationships.

Bearing these interpretive difficulties in mind it is possible to follow the three P NATs through the 1981/82 season. On figures 6.16-6.18 the critical concentrations or regions have been indicated. Where a significant difference occurred between the literature values (Section 2.6.2) and the laboratory culturing results (Section 4.3) the latter were chosen. The P NATs results for sites T and D show similar, but not as pronounced, fluctuations to site M, these are illustrated in figures 6.19 and 6.20. The responses illustrate a temporal consistence and a downstream trend of increasing P limitation.

Figure 6.15 The average (3 sites) Total River Phosphorus (TP)

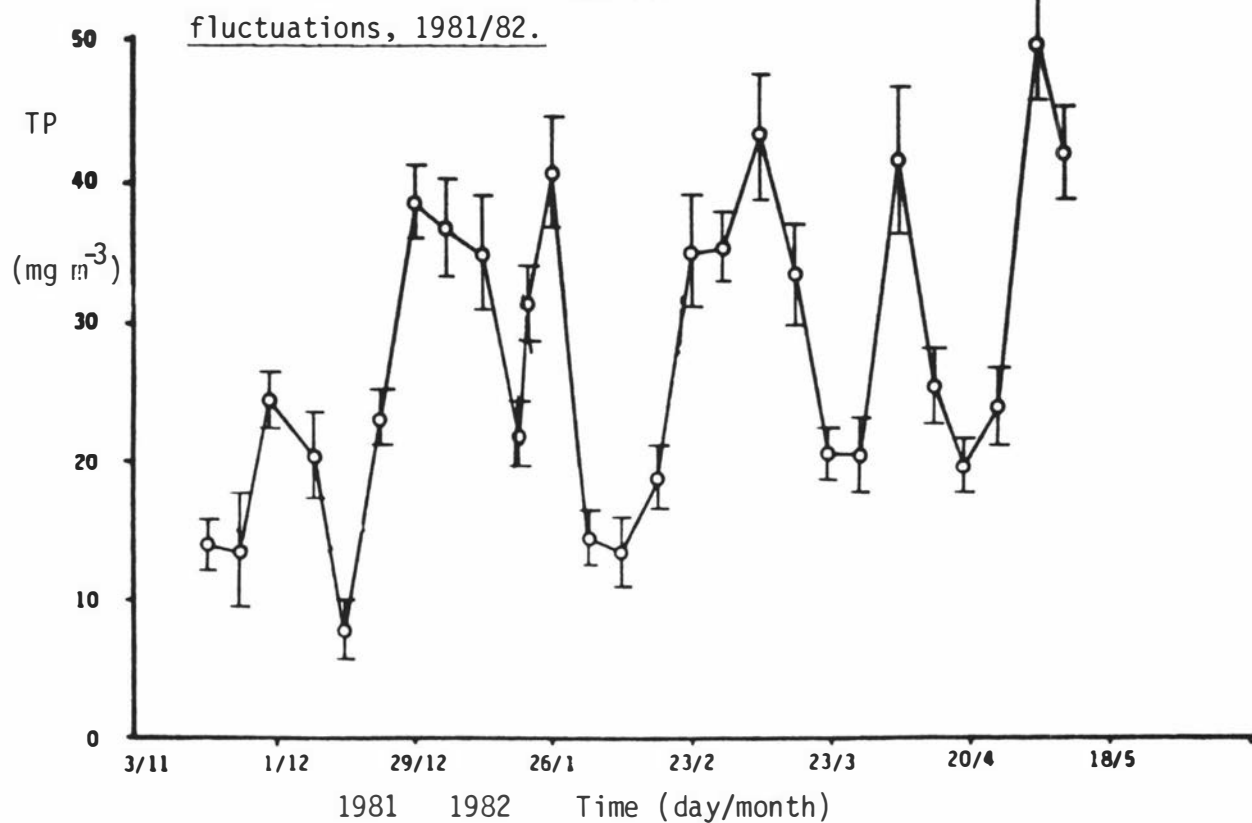
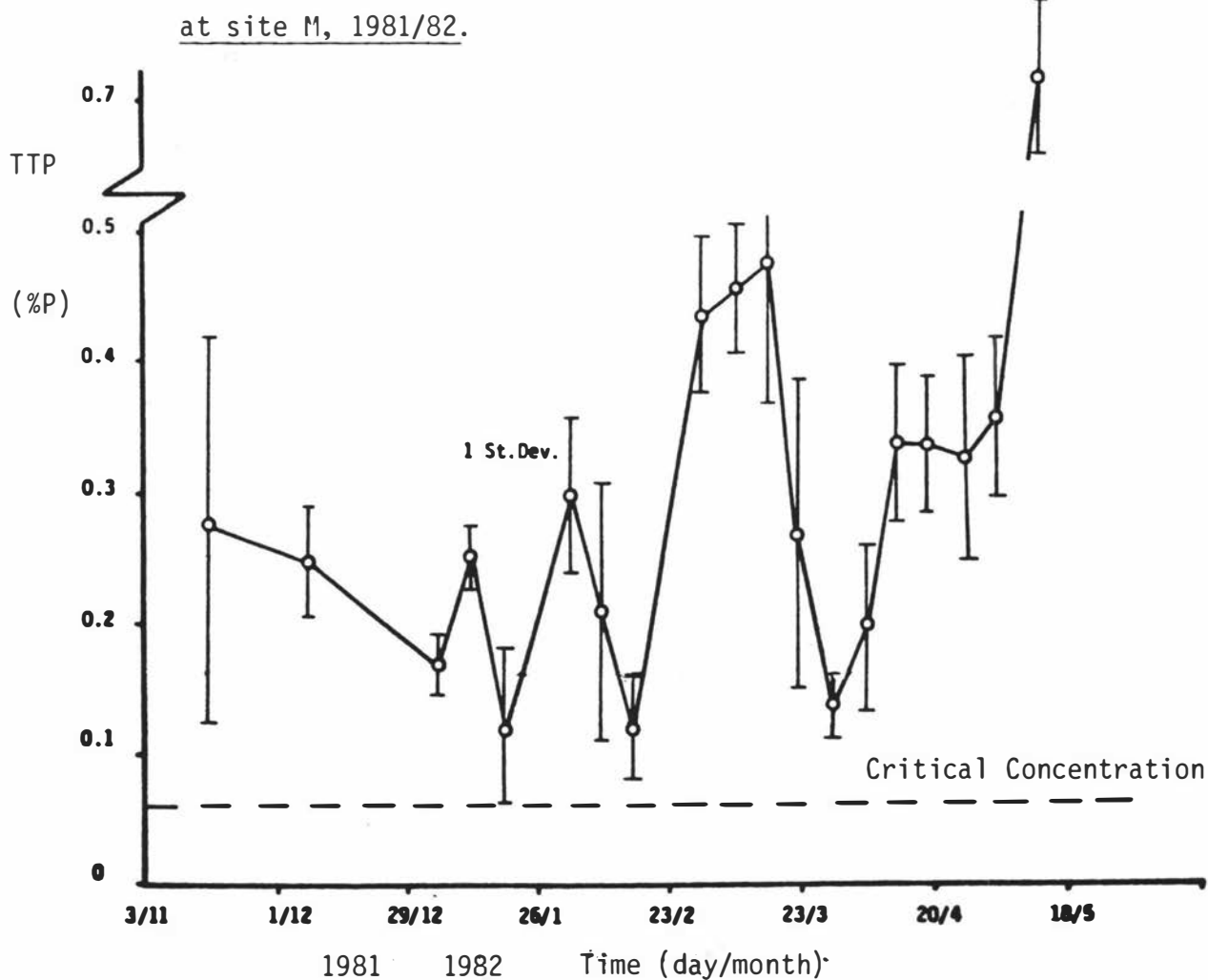
Figure 6.16 *Cladophora* Total Tissue Phosphorus (TTP) fluctuations

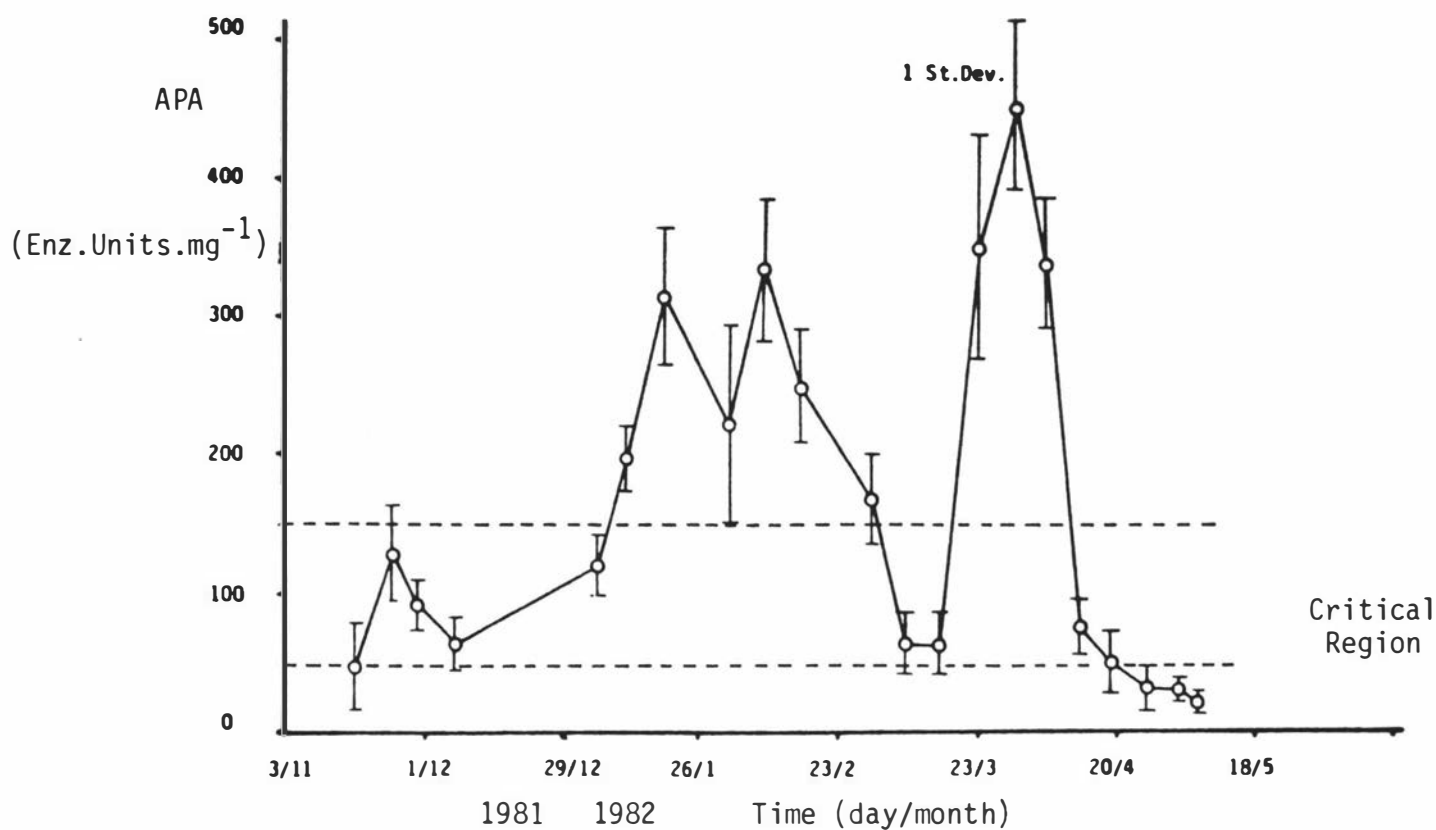
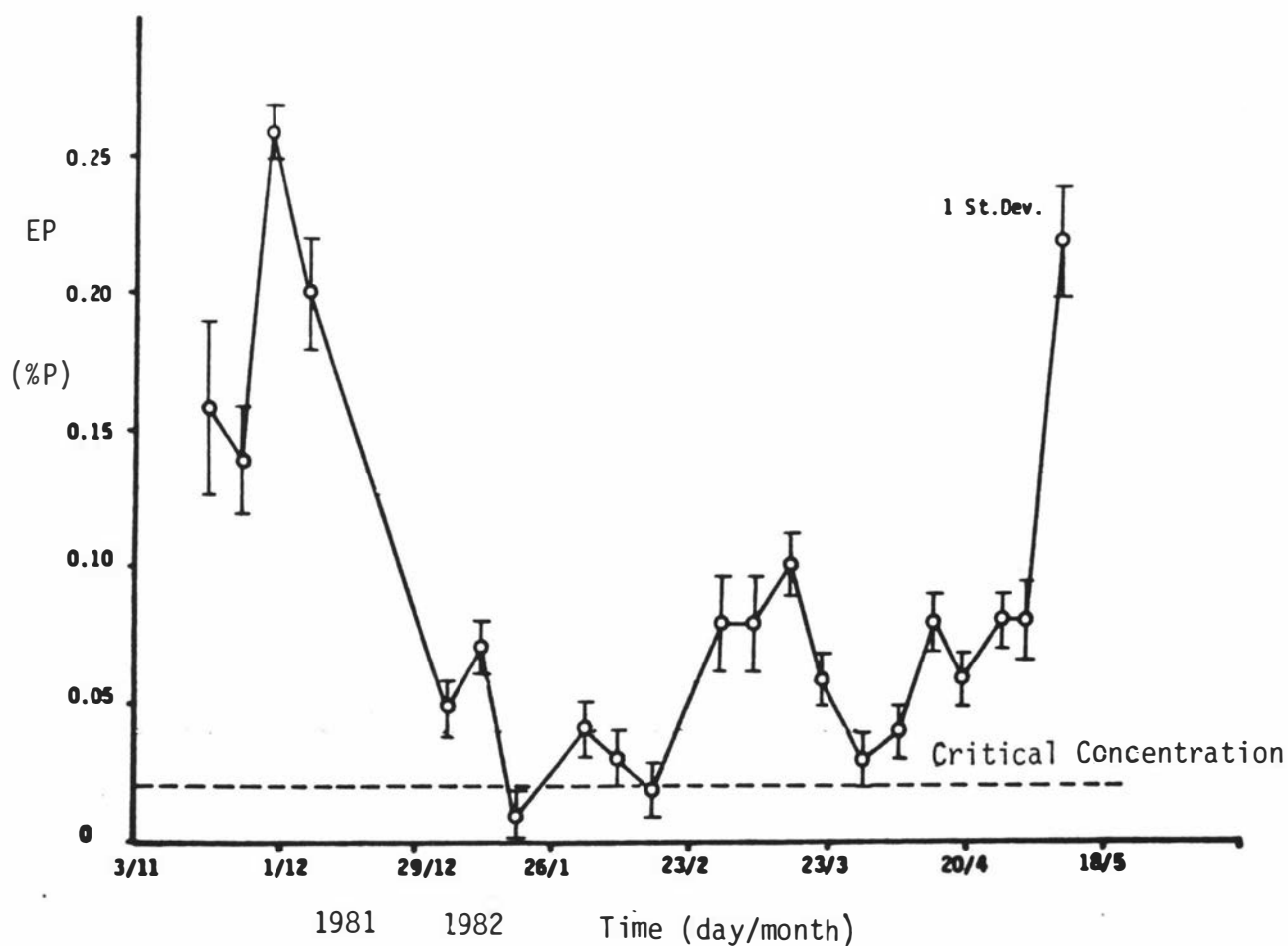
Figure 6.17 *Cladophora* Alkaline Phosphatase Activity (APA) at site M, 1981/82.Figure 6.18 *Cladophora* Extractive Phosphorus (EP) at site M, 1981/82.

Figure 6.19 *Cladophora* Phosphorus Nutrient Availability Tests at site T, 1981/82
(Precision as in figures 6.15 -6.18)

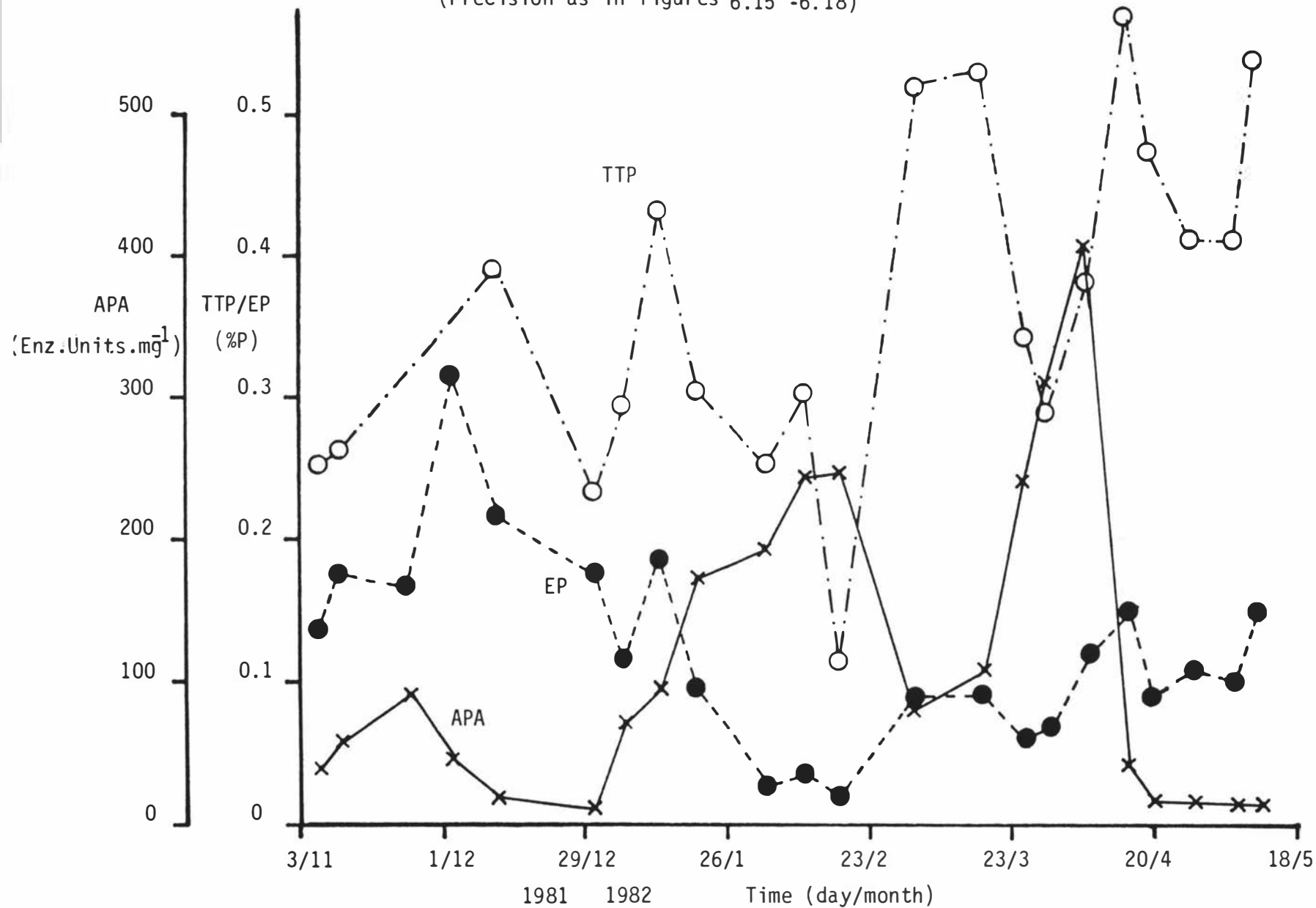
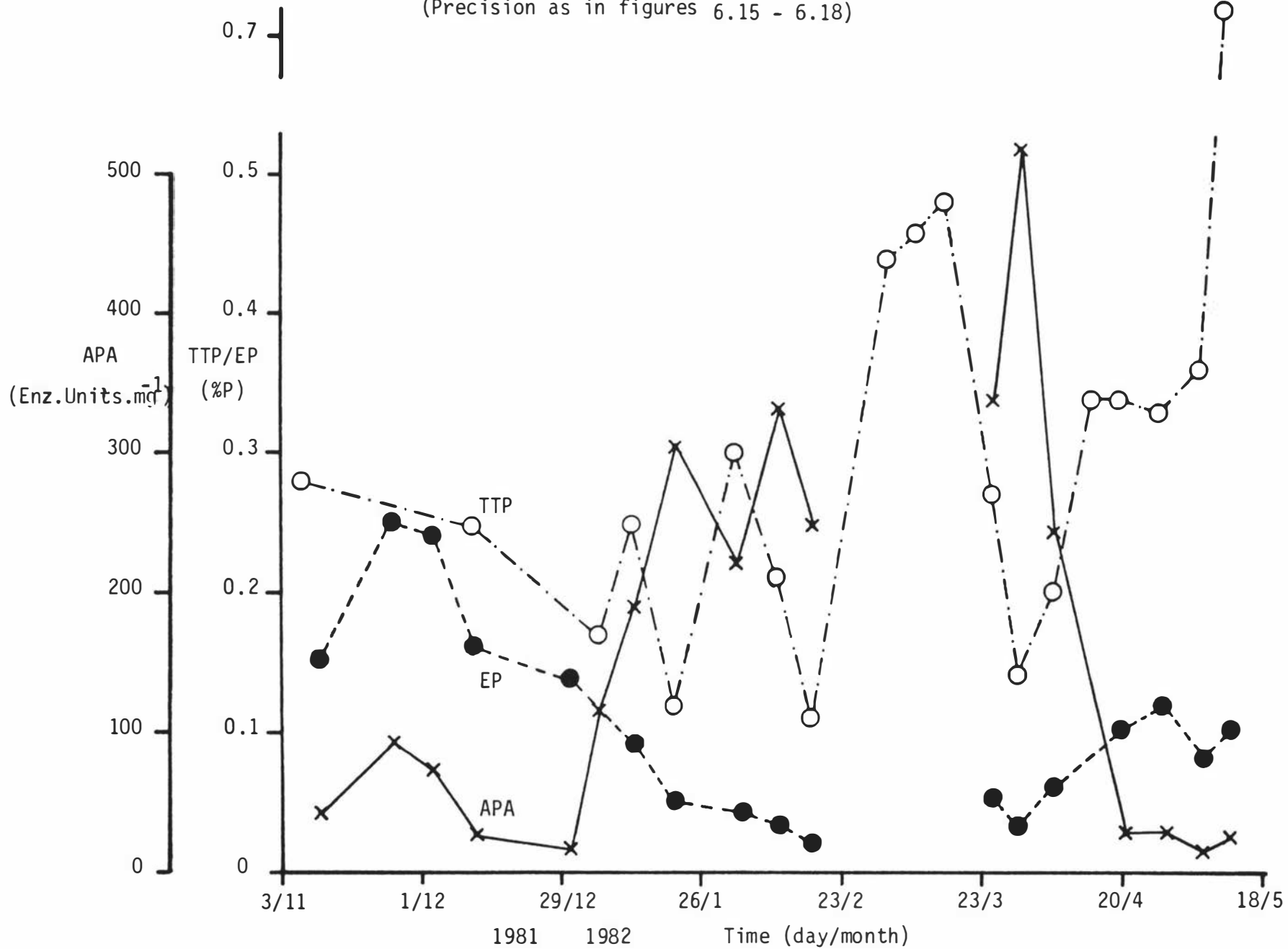


Figure 6.20 *Cladophora* Phosphorus Nutrient Availability Tests at site D, 1981/82

(Precision as in figures 6.15 - 6.18)



The identification of periods of P limitation by two different types of P NATs substantiates the hypothesis that the growth rate of *Cladophora* proliferations were at times limited by low concentrations of available P during the 1981/82 season.

The P NATs for *Cladophora* collected from site M during the 1982/83 season are presented in figures 6.21 and 6.22. These data indicate that surplus P was available to the minimal *Cladophora* growth present before the period of erratic spates during December and January (See section 6.4). One sustained depression of TTP and EP concentrations occurred during the beginning of February 1983. This was mirrored by an elevated Phosphorus Uptake Rate (PUR) and APA, indicating that the low DRP concentration of about 4 mg P m^{-3} (Section 6.5.1) was limiting the *Cladophora* growth rate.

After the large flush of 9 February had passed the DRP concentration had increased to 10 mg P m^{-3} (Figure 6.12) and all the P NATs indicated surplus P was available. Towards the end of February a period of reduced P availability was indicated by a drop in the EP concentration. However, the TTP remained high, the APA very low, while the PUR response was not decisive.

During March the data from the four NATs were often contradictory, APA and PUR frequently indicated P limitation whereas TTP remained consistently high and EP, while low, didn't drop to a critical level. The DRP dropped from approximately 10 to 4 mg P m^{-3} at the beginning of this period and subsequently remained between 4 and 7 mg P m^{-3} (Figure 6.12). Thus, fluctuations around the hypothesised critical DRP concentration of 4 mg P m^{-3} could have enabled the rapidly responding PUR to frequently indicate P surplus. The high APA was probably a remnant of a brief P limitation period which wasn't prolonged enough to register on the TTP and EP tests (Tables 4.1 and 4.2).

The intersite variation of the four P NATs are presented in figures 6.23 - 6.26. (Precision is similar to that presented in figures 6.21-6.22). These data indicated a general increase in P limitation moving downstream along the 13km study reach. This was

more pronounced between the results of P NATs at site T and those recorded for sites D and M. The intersite differences were compared using simple t-tests performed on the data from each site on every sampling date. (Appendix 6). The downstream trend was most apparent for TTP between site T and sites D and M. There was also a significant ($P < 0.05$) difference between site T and sites D and M for EP and APA. However, the PUR test did not show any significant trend. This was probably due to its relative insensitivity to small differences in river available P concentrations.

6.6.2 Nitrogen

During the 1981/82 season no quantitative Nitrogen NATs were undertaken, because the continual presence of epiphytes and consistently high TN concentration indicated surplus available N (Section 2.6.3). However, the initiation of monitoring the more available DIN together with the recently published criticisms of the infallible nature of epiphytes as indicators of surplus available N (Section 2.6.3) lead to the re-establishment of monitoring N NATs.

The TTN test can be used in conjunction with the TTP test to give the TTN:TTP ratio which has often been used to assign a limiting role to either N or P. Ratios range from 5:1 to 15:1 for 'normal' algae (Rhyther & Dunstan, 1971). A ratio greater than 30:1 is usually taken as evidence of P limitation and one less than 10:1 indicative of N limitation (Atkinson & Smith, 1983). However, due to the differential cycling rates of N and P the ratio should only be used as a rough guide and should only be interpreted with a knowledge of the absolute TTN and TTP concentrations.

The N NAT data for the 1982/83 season are presented in figure 6.27 together with the TTN:TTP ratio. The TTN exhibited one pronounced depression at the beginning of February 1983, approaching the critical region of approximately 2% identified in section 4.3 but still above the 1.1% cited in the literature (Section 2.6.3). However, this depression does not produce manifestations of N limitation in the AAR test, which never rose above $5 \mu\text{g N (10 mg)}^{-1} \text{ hr}^{-1}$ indicating surplus N was present throughout the season (Section 4.3). The TTN:TTP ratio usually remained between the

FIGURE 6.21 *Cladophora* Phosphorus Nutrient Availability Tests at site M, 1982/83 (Total Tissue Phosphorus and Extractive Phosphorus)

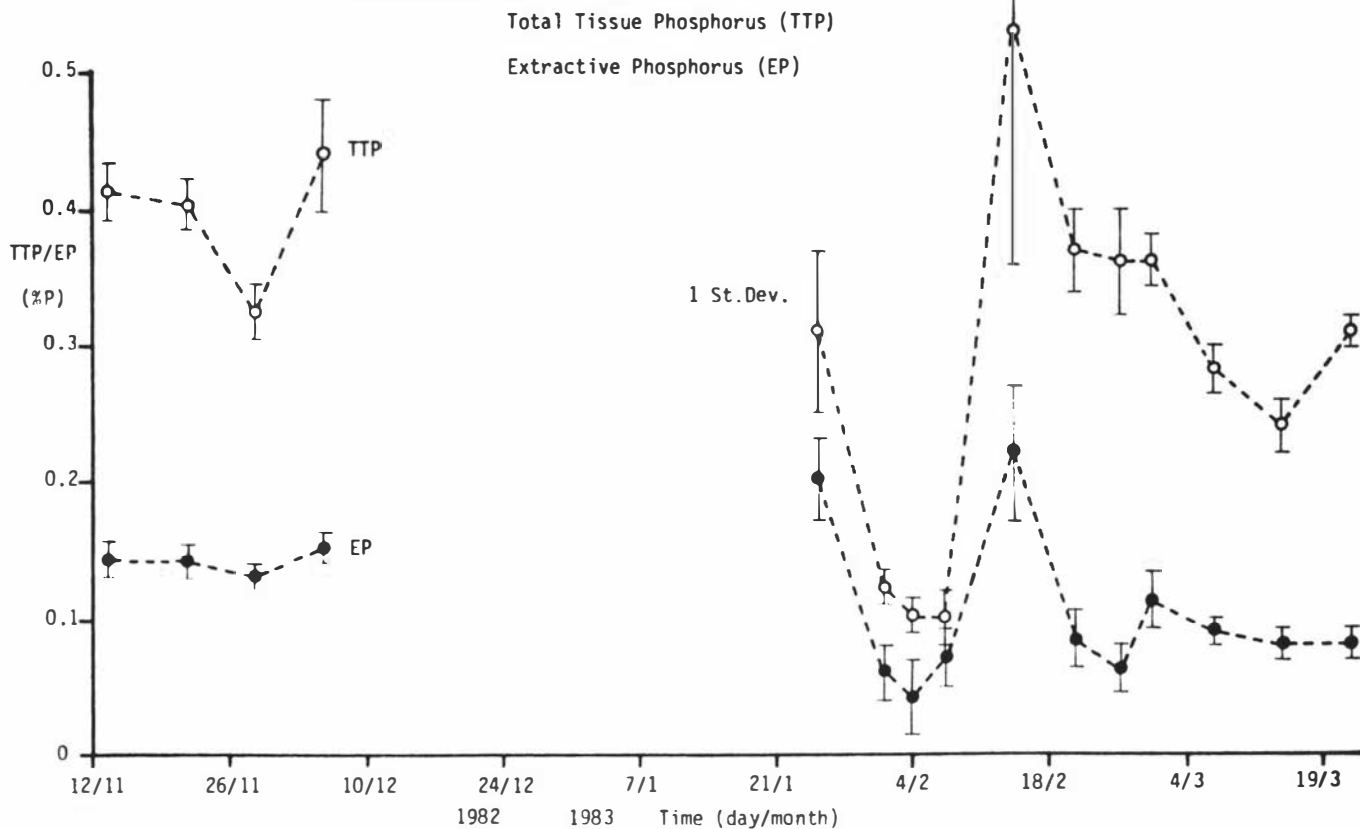


FIGURE 6.22 *Cladophora* Phosphorus Nutrient Availability Tests at site M, 1982/82 (Alkaline Phosphatase activity and Phosphorus Uptake Rate)

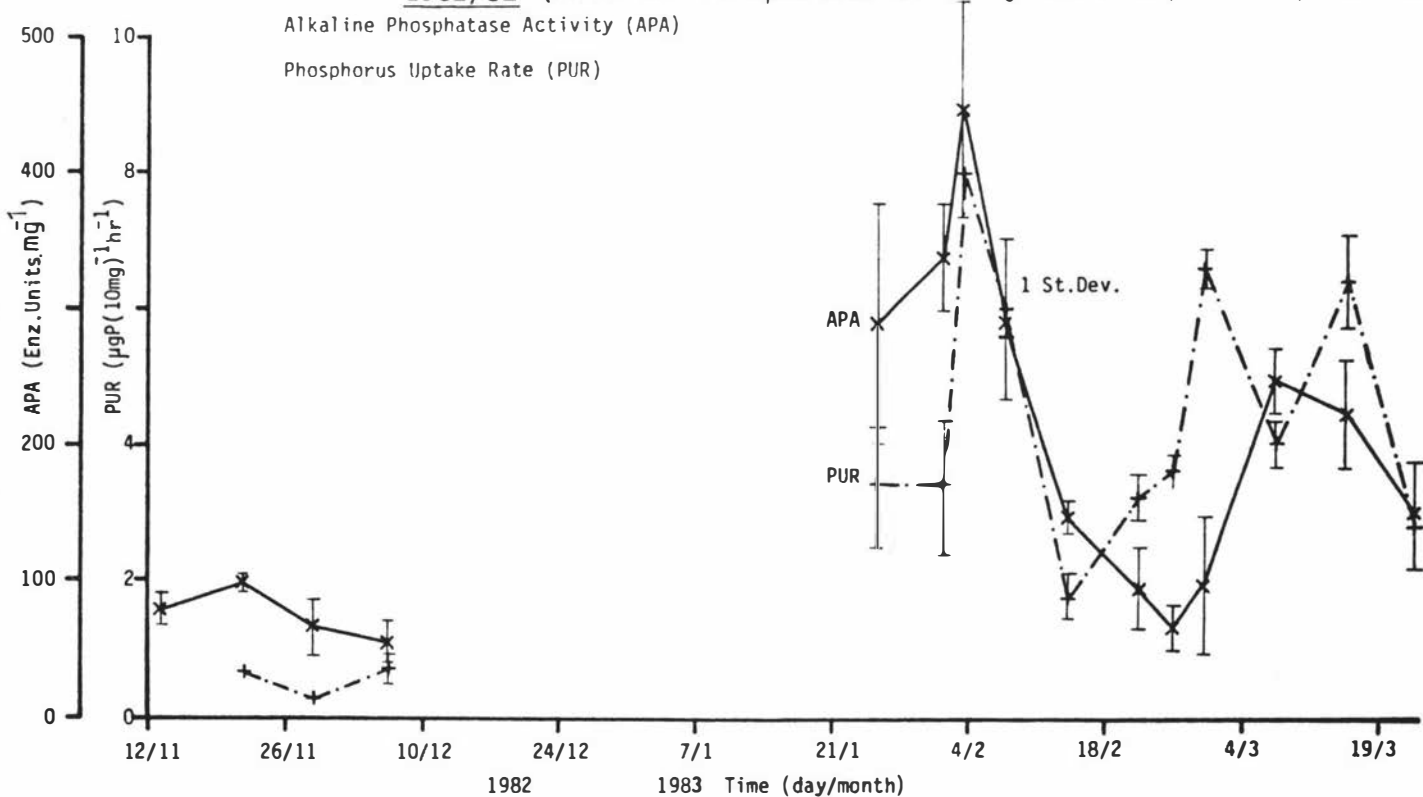


Figure 6.23 Total Tissue Phosphorus in *Cladophora* at sites T , D and M , 1982/83

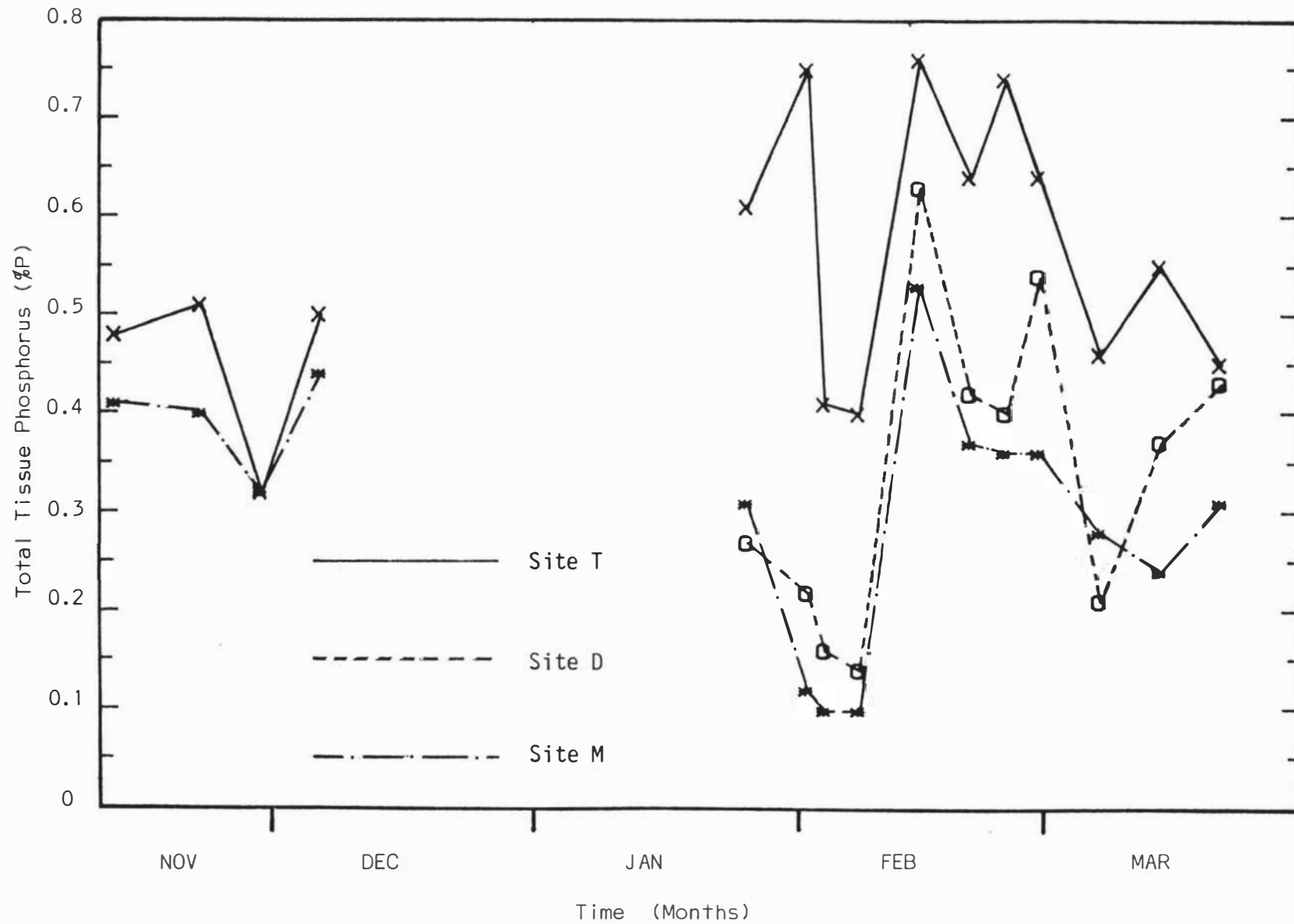


Figure 6.24 Extractive Phosphorus in *Cladophora* at sites T , D and M , 1982/83.

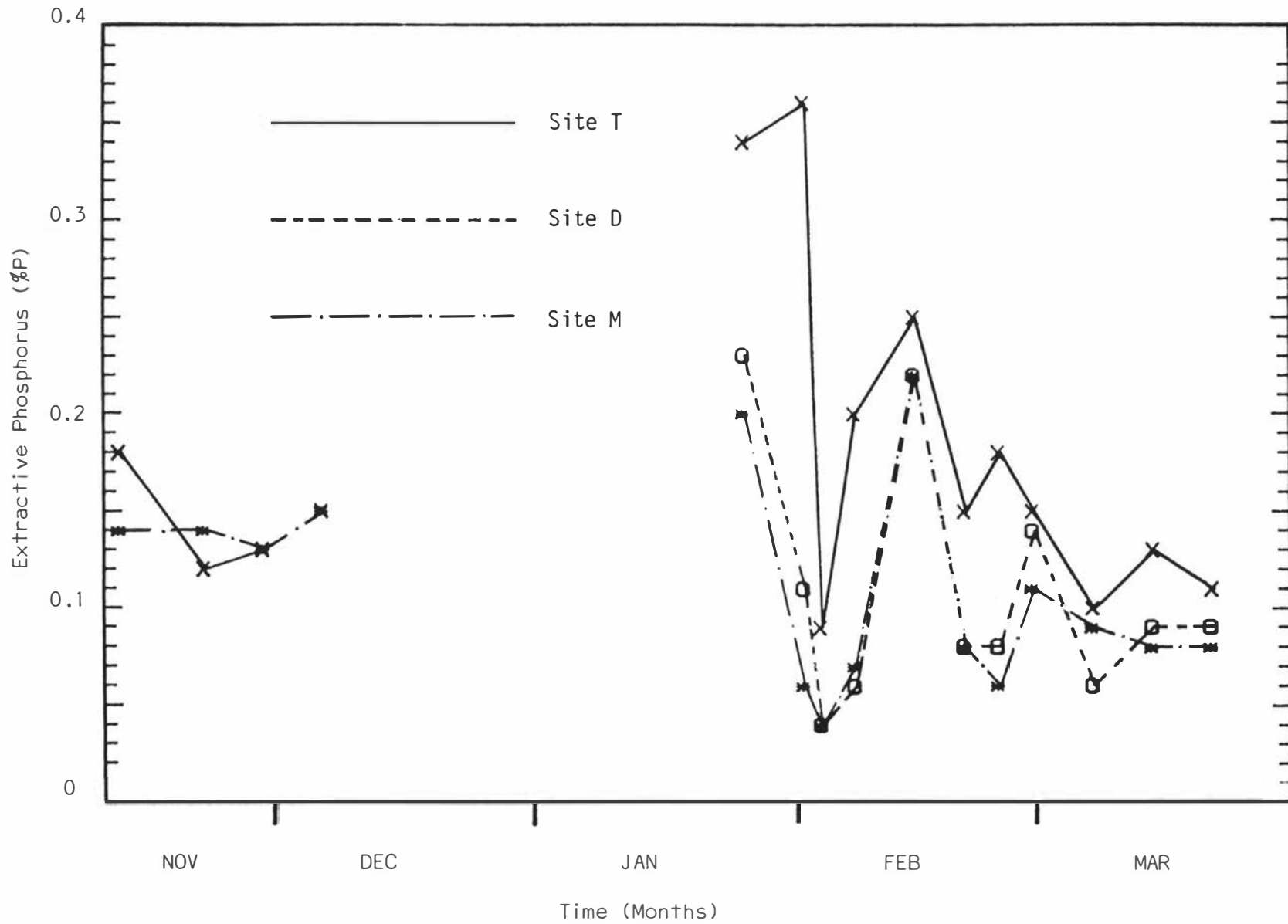


Figure 6.25 Phosphorus Uptake Rate in *Cladophora* at sites T, D and M, 1982/83.

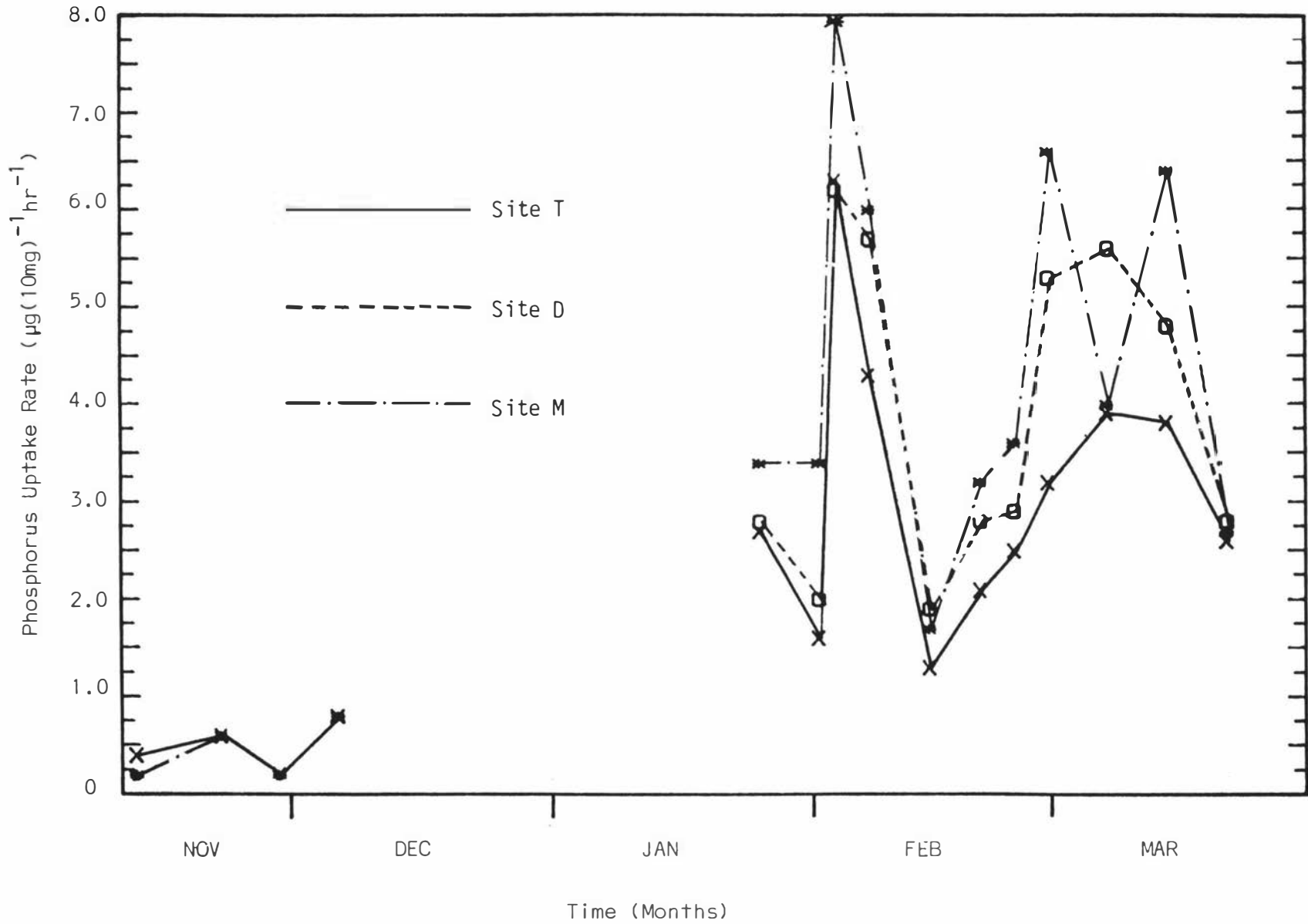


Figure 6.26 Alkaline Phosphatase Activity in *Cladophora* at sites T, D and M, 1982/83.

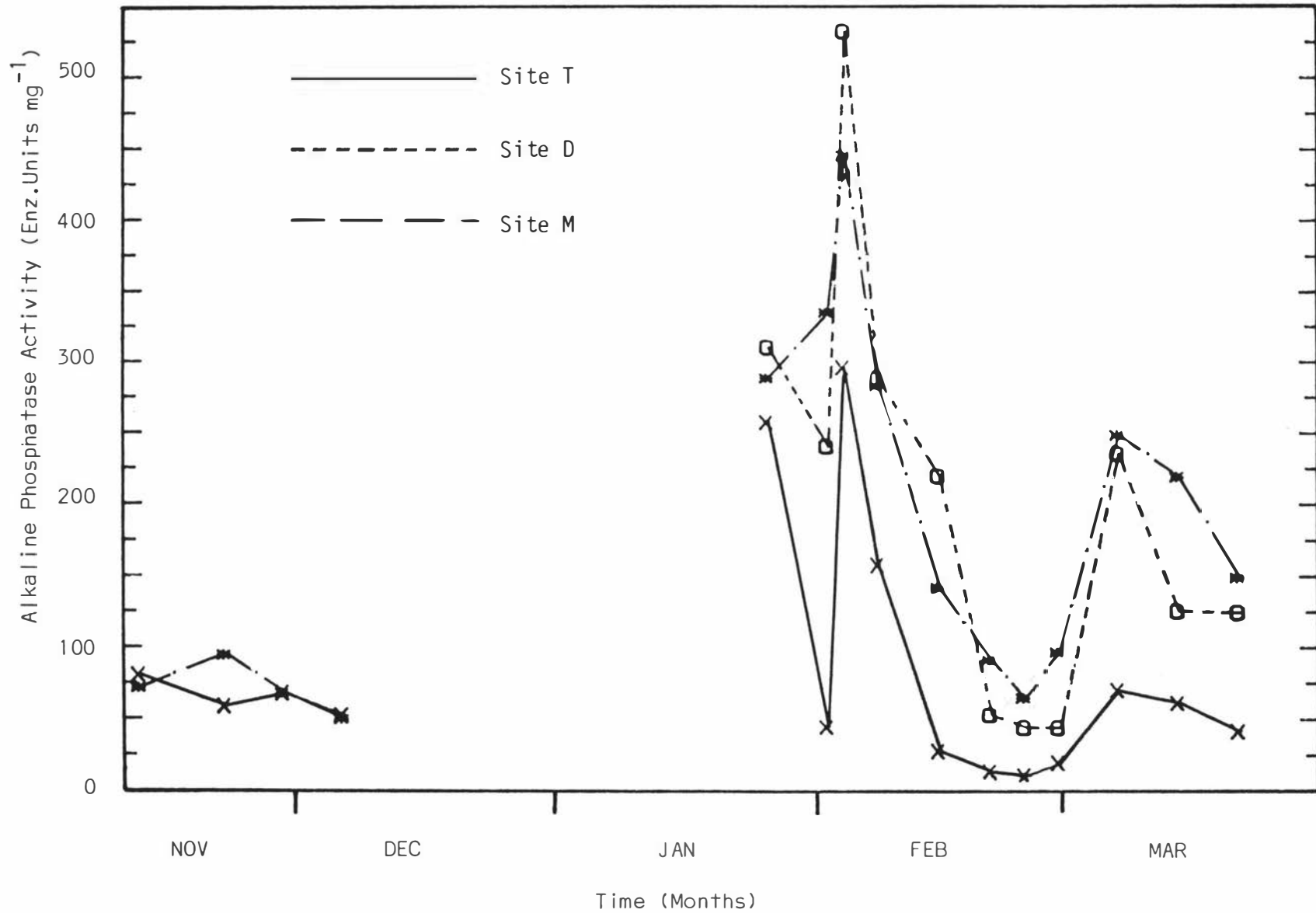
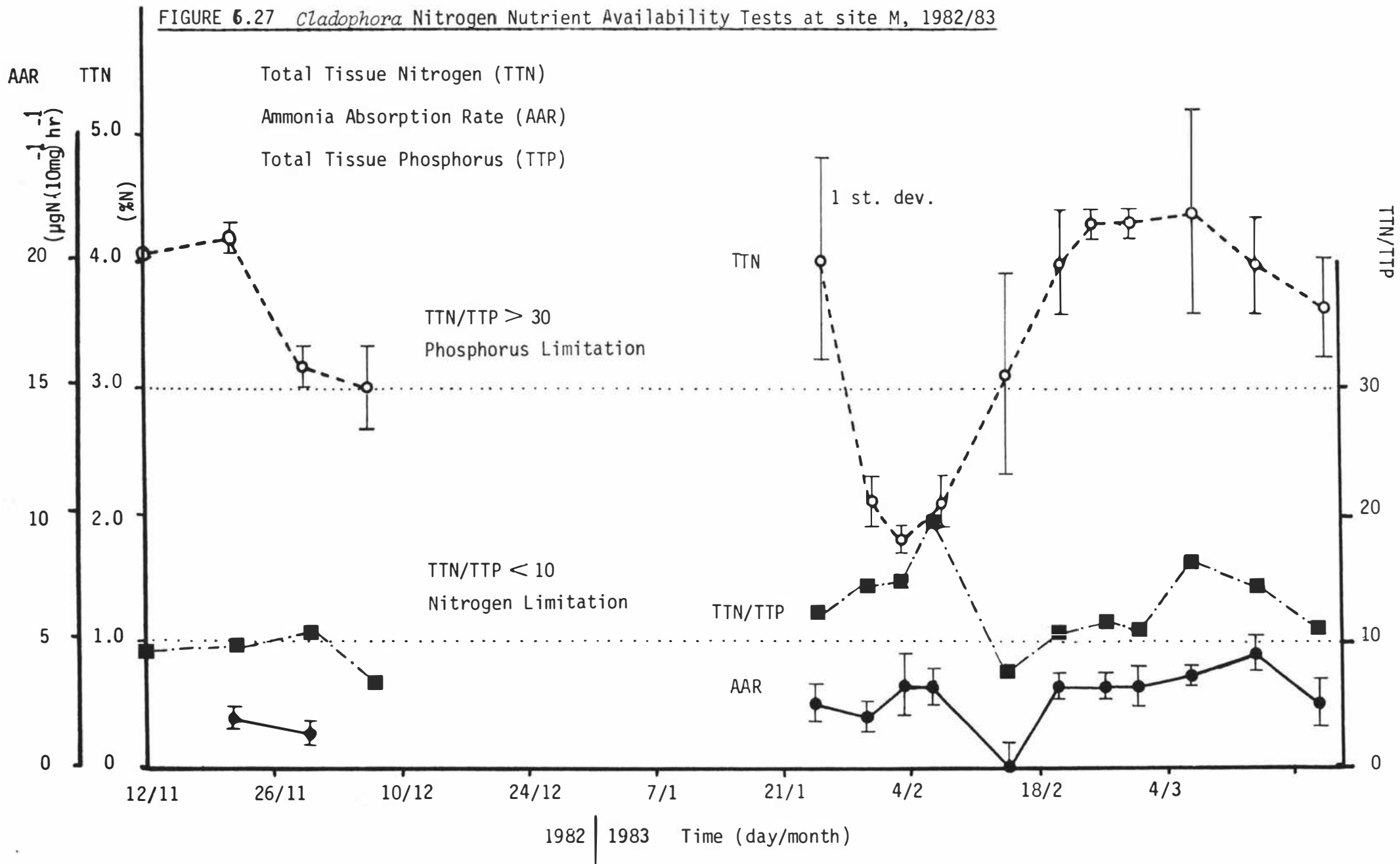


FIGURE 6.27 *Cladophora* Nitrogen Nutrient Availability Tests at site M, 1982/83



limits indicating N or P limitation. Once during mid-February the ratio dropped below 10 but as the TTN concentration was 3.1% this result was not indicative of N limitation.

These N NATs results agree with the generally high DIN concentrations and high DIN:DRP ratios found during the season (Section 6.5 and figure 6.12). The differential reduction of DIN over DRP that occurred during early March, referred to in section 6.5.2, does not appear to be due to any N limitation, as indicated by the TTN and AAR. Thus it can be concluded that the *Cladophora* growth rate was never limited by N availability during the 1982/83 season.

6.7 Primary Productivity and Dissolved Oxygen fluctuations

6.7.1 Introduction

This section has been divided into two major parts:

(6.7.2) Primary productivity (and river community description.)

(6.7.3) Dissolved Oxygen Deficits (DODs).

While the two sections are intimately associated, they have important conceptual differences. Net community primary productivity is a measure of the river's ability to produce new organic material, however, interpretation of productivity data is very dependant on the measurement technique used. In this study, productivity was estimated by monitoring the river's DO fluctuations. Such an approach involves a number of assumptions (See section 2.4).

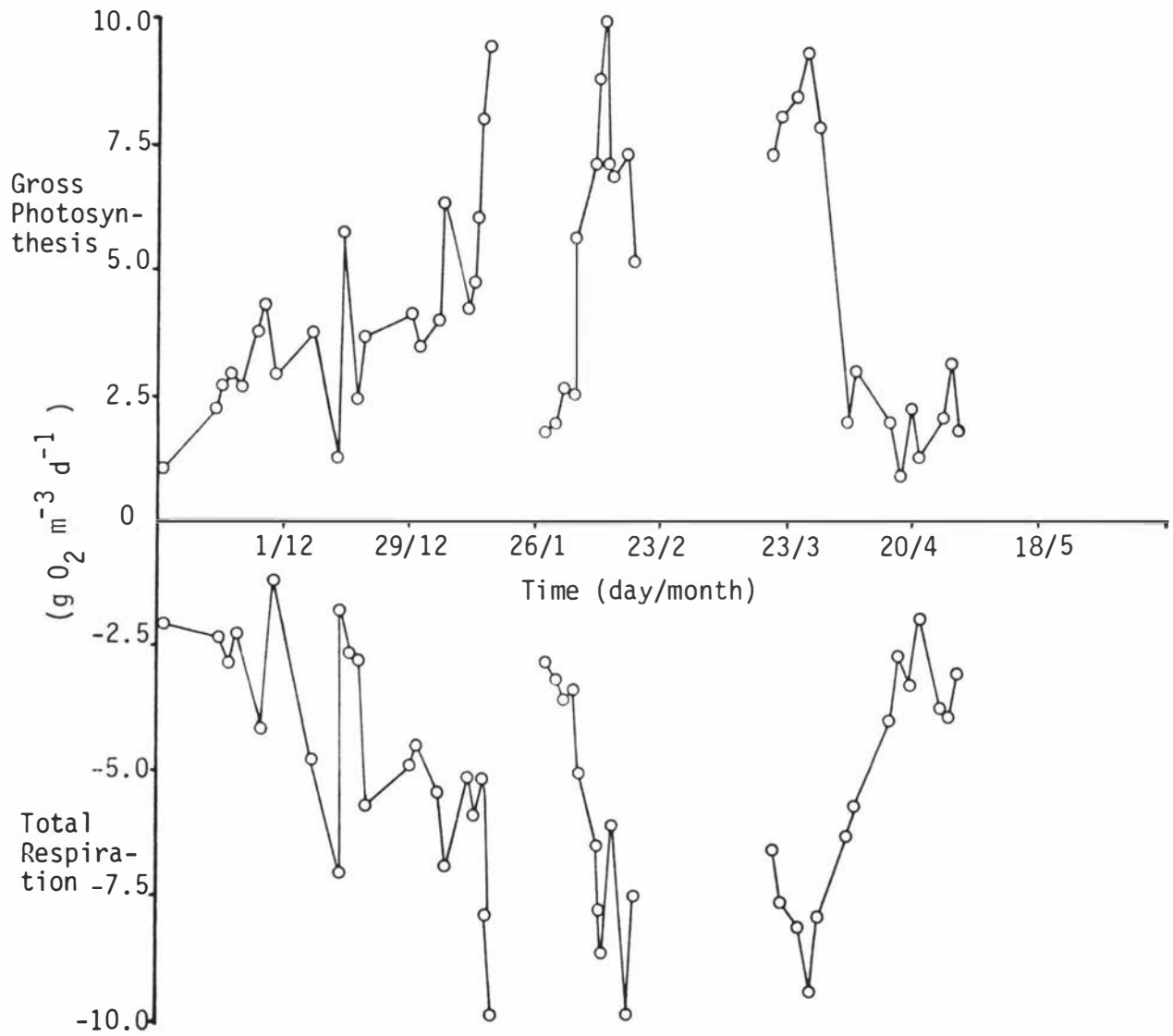
Very different DO profiles may result from the net production of equivalent dry weight biomass periphyton assemblages. (Section 6.4). The DODs are a consequence of the net community primary productivity and as they measure the depression of DO below saturation, they are parameters of prime importance for a river such as the Manawatu that has to withstand the effects of both organic discharges and *Cladophora* proliferations (See section 2.3).

6.7.2 Primary productivity

6.7.2.1 Primary productivity during the 1981/82 season

Monitoring of primary production began in early November 1981 during relatively modest flows of about $50 \text{ m}^3 \text{ s}^{-1}$. At this time there were no significant amounts of periphyton visible in the river. During December, *Cladophora* became established, albeit at a low biomass density (approximately 3 g DW m^{-2}). The metabolic activity of the *Cladophora* assemblage caused gradual increases in both Gross Photosynthesis (GP) and Total Respiration (TR) (Figure 6.28). The mid-January acceleration in *Cladophora* growth rate (Figure 6.5) was mirrored by the sharp increases in both GP and TR. After interruption by a major flush event (Figure 6.6) this pattern of rapid increases in GP and TR was repeated. However, during mid-February a sudden halt was observed in the increase of GP. There

Figure 6.28 Gross Photosynthesis (GP) and Total Respiration (TR)
during 1981/82.



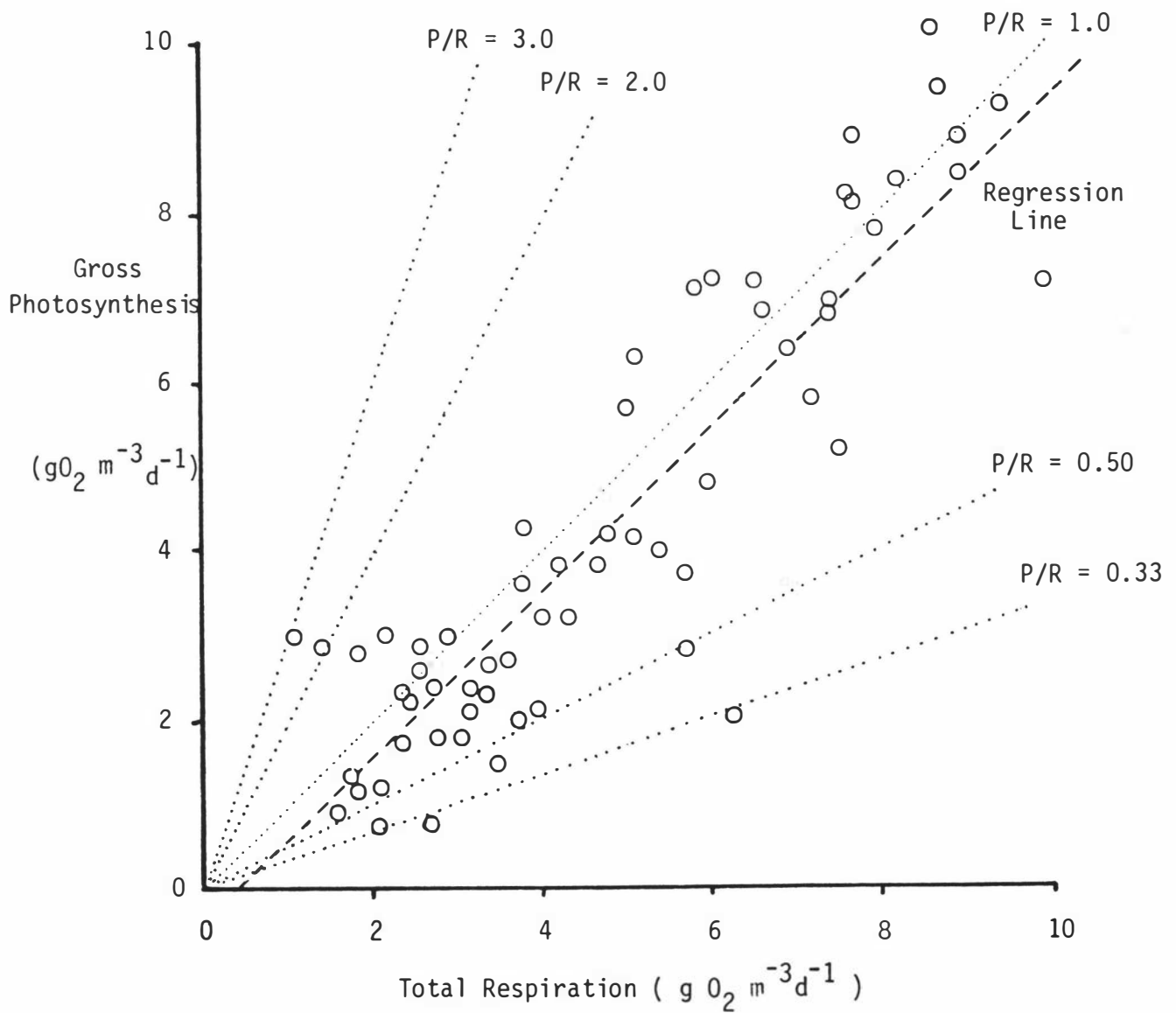
was no flow variation or major reduction of daily insolation during this period. (See appendix 7). The reason for the marked decrease in GP was most likely attributable to two causes:-

- (a) The average river temperature dropped from 21⁰C to 16⁰C over a four day period, most likely due to heat losses during a spell of very clear nights.
- (b) P NATs during the period indicated that metabolic activities, and hence the growth rate, were being limited by the availability of P (Figures 6.15 - 6.18).

After a series of major flushes (Figure 6.6) the *Cladophora* became re-established and high values of both GP and TR were observed. The proliferations were then affected by a number of small flushes which eventually reduced biomass levels and maintained a high level of turbidity in the river resulting in the 'tailing off' of both parameters observed towards the end of April.

The data in figure 6.28 were used to construct figure 6.29. The linear nature of the relationship ($r^2 = 0.826$) with a regression slope of 0.992 illustrated that over the whole season the river 'average' indicated that the periphyton community was phototrophic. The scatter of the data in figure 6.29 around the P/R=1.0 line, reveals many examples of dominance by both photosynthetic and respiratory activity. The regression intercept at zero Gross Photosynthesis indicates an average background respiration of $0.42 \text{ g O}_2 \text{ m}^{-3} \text{ d}^{-1}$ in the absence of any photosynthesis. A clearer picture of the changing trophic status of the river can be seen in figure 6.30. The dramatic changes observed during November and December are a result of small changes in GP and especially TR which are manifest as large variations in the P/R ratio but in absolute terms, the net result (Figure 6.31) is much less dramatic. After this period there were three notable intervals that had P/R greater than one, indicating the autotrophic nature of the assemblage. The environmental features common during these periods were either increased insolation or a drop in river flow, both having the same net effect of increasing the available light. (Figure 6.6 and appendix 7). It should be noted however, that

Figure 6.29 The relationship between Gross Photosynthesis and Total Respiration during 1981/82.*



*Regression Equation

$$GP = 0.418 + 0.992 TR$$

Slope = 0.992

$$r^2 = 0.806$$

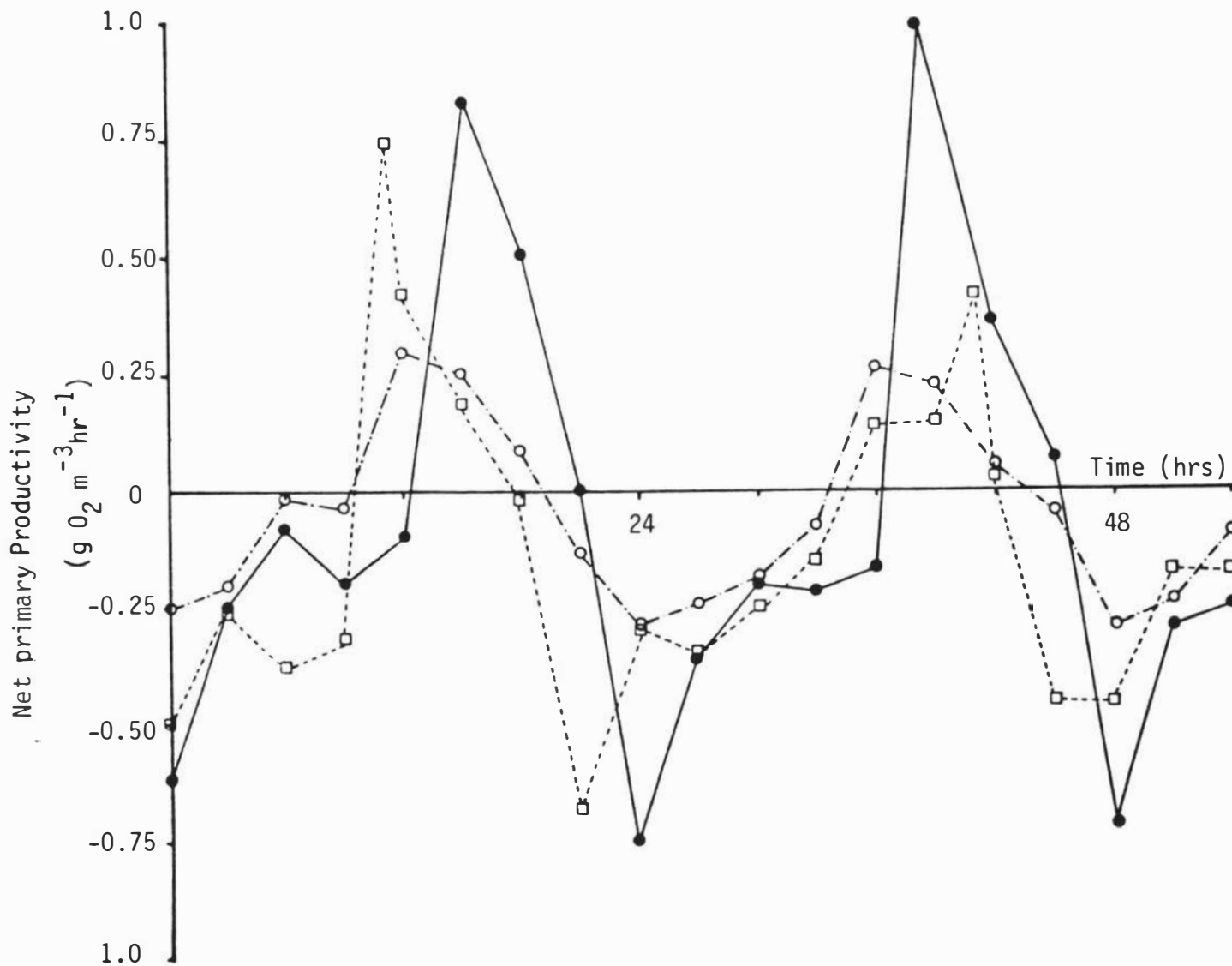
during the course of the day there can be rapid (in terms of minutes) changes in the GP profile caused by variations in the light climate. (Gallegos, 1977; Kelly *et al*, 1983).

The low P/R ratios recorded during April were indicative of some of the points made earlier (Section 6.4) in reference to the 'clean' and 'dirty' *Cladophora* assemblages. (CCA and DCA). The series of small flush events (Figure 6.6 and appendix 7) left a DCA and very turbid waters often persisted. The net result was that while the amount of potential phototrophs was high, GP was minimal compared to the DCA TR.

The data in figure 6.28 can also be used (together with the average river depth) to determine the Net Areal daily Primary Productivity (NAP) which is the basic term for describing the build up of organic material in an aquatic system (Section 2.4) (Figure 6.31). The majority of the data indicate a river of relatively modest productivity (Section 2.4). However, there are two instances of large negative NAP values which illustrate the dramatically different effects that can result from just one *Cladophora* assemblage. The first radical departure happened in mid-February and was the result of a sudden period of extremely overcast conditions occurring after a long spell of fine weather when *Cladophora* proliferations were extensive (the average site biomass density had reached 65 g DW m^{-2}) (See appendix 7). The second major depression occurred in early April and was the result of increased turbidity and reduced biomass caused by small flush events. (Figure 6.6). The minor variations in NAP do not appear to be easily explained by changes in available light, temperature available P, or flow. Note, however, the approximate estimation of the available light (Section 3.4). During the three periods of extensive *Cladophora* proliferations the P NATs indicated that there was some limitation of the growth rate. The subject of factors that affect the *Cladophora* assemblages metabolic activities will be discussed in detail in Section 6.7.3.

One aspect of NAP data that deserves greater attention is the daily Net Primary Productivity (PR) profile which can illustrate a number of

Figure 6.32: Daily Net primary productivity
for two-day periods 1981/82.



KEY:

	<i>Cladophora</i> Biomass (g DW m ⁻²)	Light (Day 1, Day 2) (Langleys d ⁻¹)	
○	27-28/11/81	2	617,465
□	17-18/2/82	65	291,36
●	27-28/3/82	25	447,360

important points. The data in figure 6.32 represent PR profiles obtained during various environmental conditions and *Cladophora* biomass densities. The profile obtained during the absence of any extensive *Cladophora* growths had a modest regular fluctuation while that recorded just prior to the February flush (Figure 6.6) illustrated the situation that can occur at high biomass densities when the available light is abruptly reduced. The photosynthetic electron transport system reacts rapidly to changes in available light and thus the metabolic reactions involved in the production of DO are also fairly rapidly affected (Gallegos, 1977; Kelly *et al.*, 1983; Round, 1973). However, the assemblage respiration does not appear to be reduced to the same extent, resulting in a large negative NAP (Figure 6.31). The profile obtained during 27-28 March 1982 indicated the relatively high levels of both positive and negative PR that can result from a modest *Cladophora* biomass when exposed to moderate insolation. No apportioning of the respiration between community components was made because the whole assemblage primary productivity was being monitored.

The low pre-dawn and high post-sunset hourly NAP values observed during proliferation periods are indicative of photorespiration (light dependant DO uptake and dissolved CO₂ release) occurring in the *Cladophora* assemblage. (Sections 2.4 and 3.8.2).

The components of net community primary productivity can be used to describe the eutrophication potential and degree of organic pollution of a river. (Hornberger *et al.*, 1977). Such a description is presented in figure 6.33 using the data from the 1981/82 season. The abscissa may be considered as a respiration index and the ordinate as a eutrophication index.

The areas marked on the graph have been empirically derived and are similar to those described by Hornberger *et al.*, (1977). They should, however, only be taken as guidelines. Over the whole season, most of the possible combinations were observed. The data can be divided up into three sections based on the periphyton development.

- (a) The three data points in the upper left-hand section, were all derived from the November pre-proliferation period when

there was little periphyton present in the river. (See figure 6.5).

- (b) The data points observed below the $\sqrt{(P/R)} = 0.7$ line were all from the April period during which there was a low biomass DCA and a number of small flushes. The data point in the lower right-hand side was obtained on 6 April. This was the first monitoring day after the major flushes. (See figure 6.6). The water was markedly turbid and apparently manifest as a condition more usually associated with an organically polluted river, i.e. one that would have a fairly high TR but comparatively little GP. The turbid water restricts the photosynthetic activity of the periphyton, and will usually contain large numbers of heterotrophic bacteria that have been swept into the river along with a surplus of readily available nutrients.
- (c) The third identifiable section of the diagram is the broad sweep running diagonally across of the graph from left to right. This illustrates the trend of increasing eutrophication that occurs as the *Cladophora* became established throughout the river. Examples on the graph show the gradation that can occur, from a very low density of *Cladophora* ($<1\text{g DW m}^{-2}$) (squares) to results obtained on one 6-day monitoring period when there was a modest biomass density ($12\text{-}24\text{ g DW m}^{-2}$) (full circles).

The magnitude of the diurnal DO fluctuations (ΔDO) that result from the metabolic activities of the *Cladophora* assemblages can be used as an indication of the river productivity (Figure 6.34). The ΔDO does not, take any account of temperature and reaeration but nevertheless is a simple, easily interpretable presentation of data from many DO profiles. The trends observed in figure 6.34 closely follow those observed earlier in figure 6.28.

Another useful periphyton community description can be found by comparing daily GP and the ΔDO (Figure 6.35).

If the ΔDO was caused by a heterotrophic community the ratio (GP/ ΔDO) would be markedly less than 2. The data illustrated in figure 6.35 are clustered around the regression slope of 1.98

Figure 6.33 Community description (Hornberger plot) 1981/82.

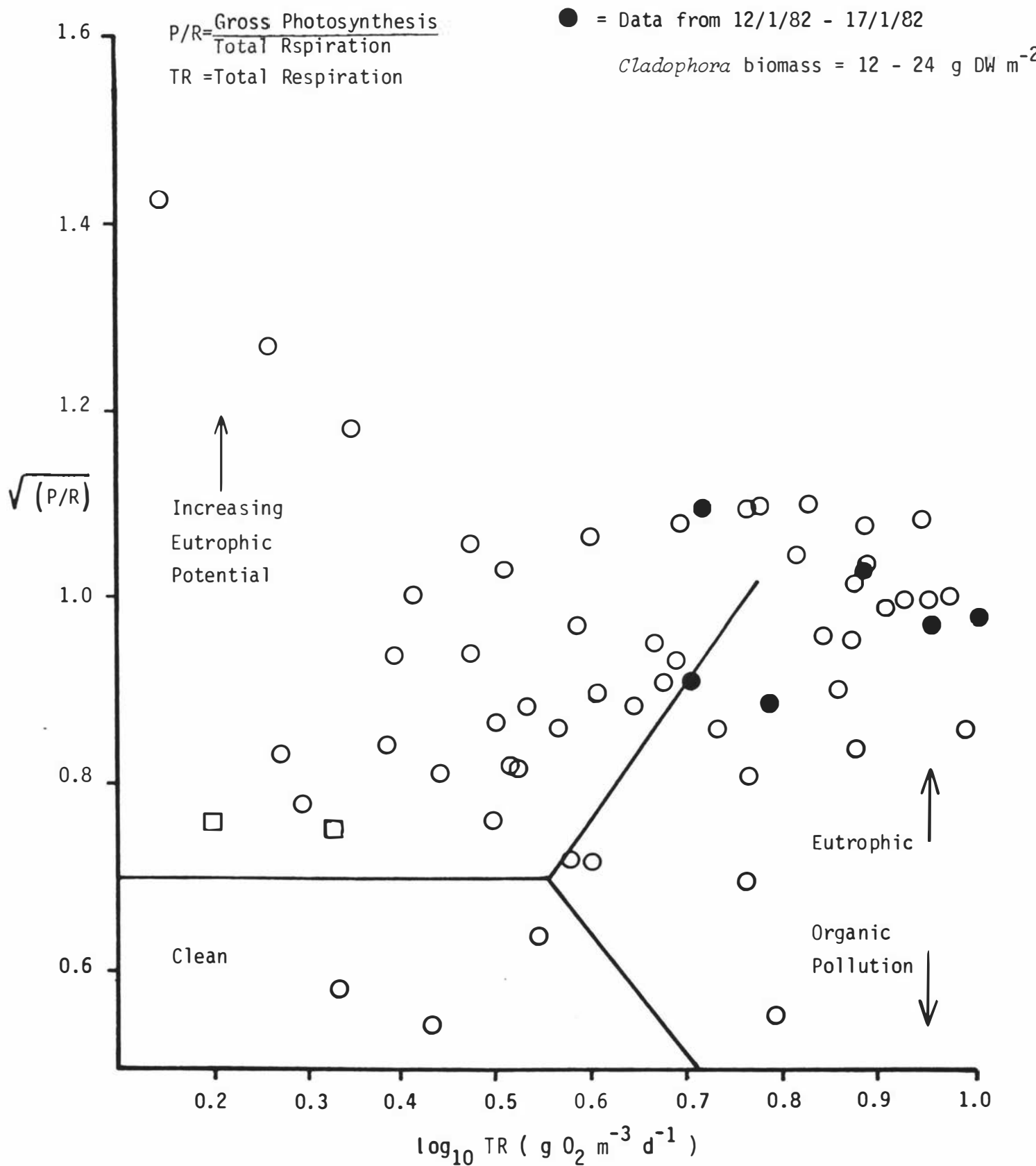


Figure 6.34 The daily dissolved oxygen fluctuations (ΔDO), 1981/82.

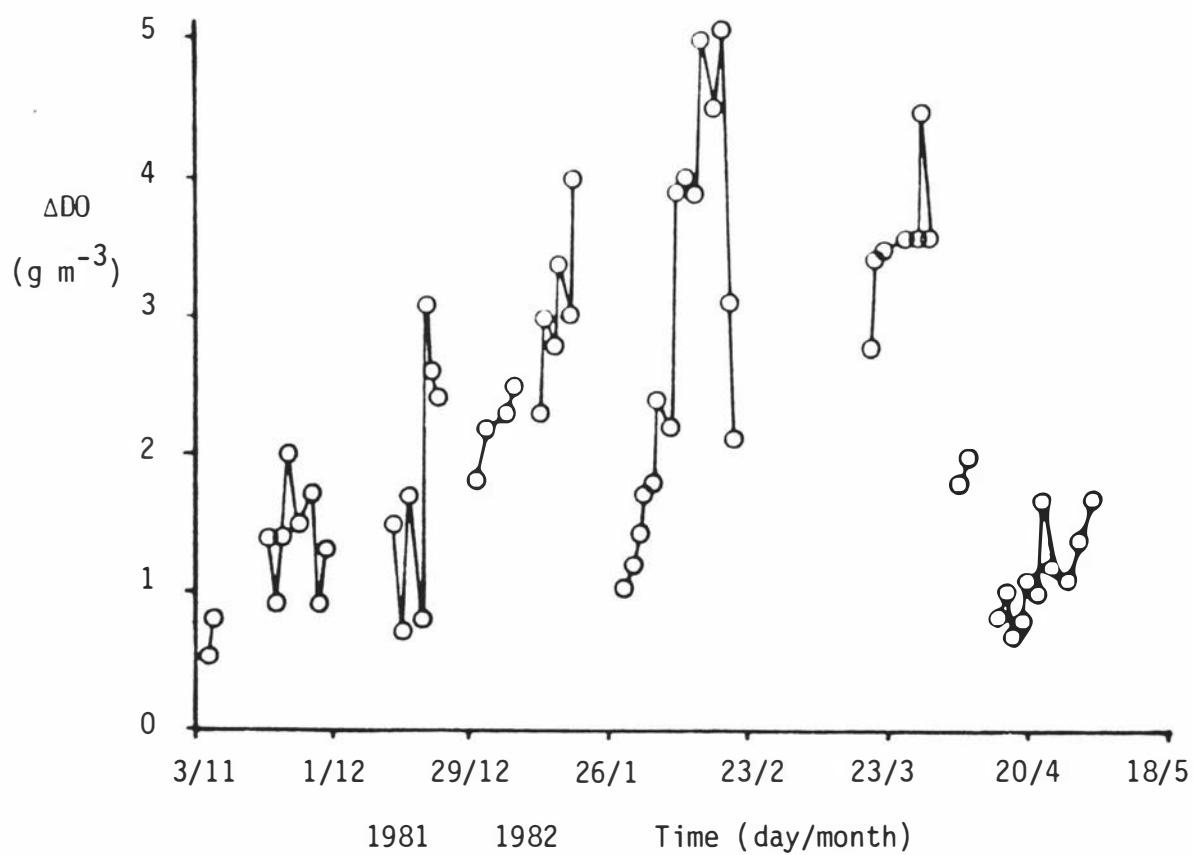
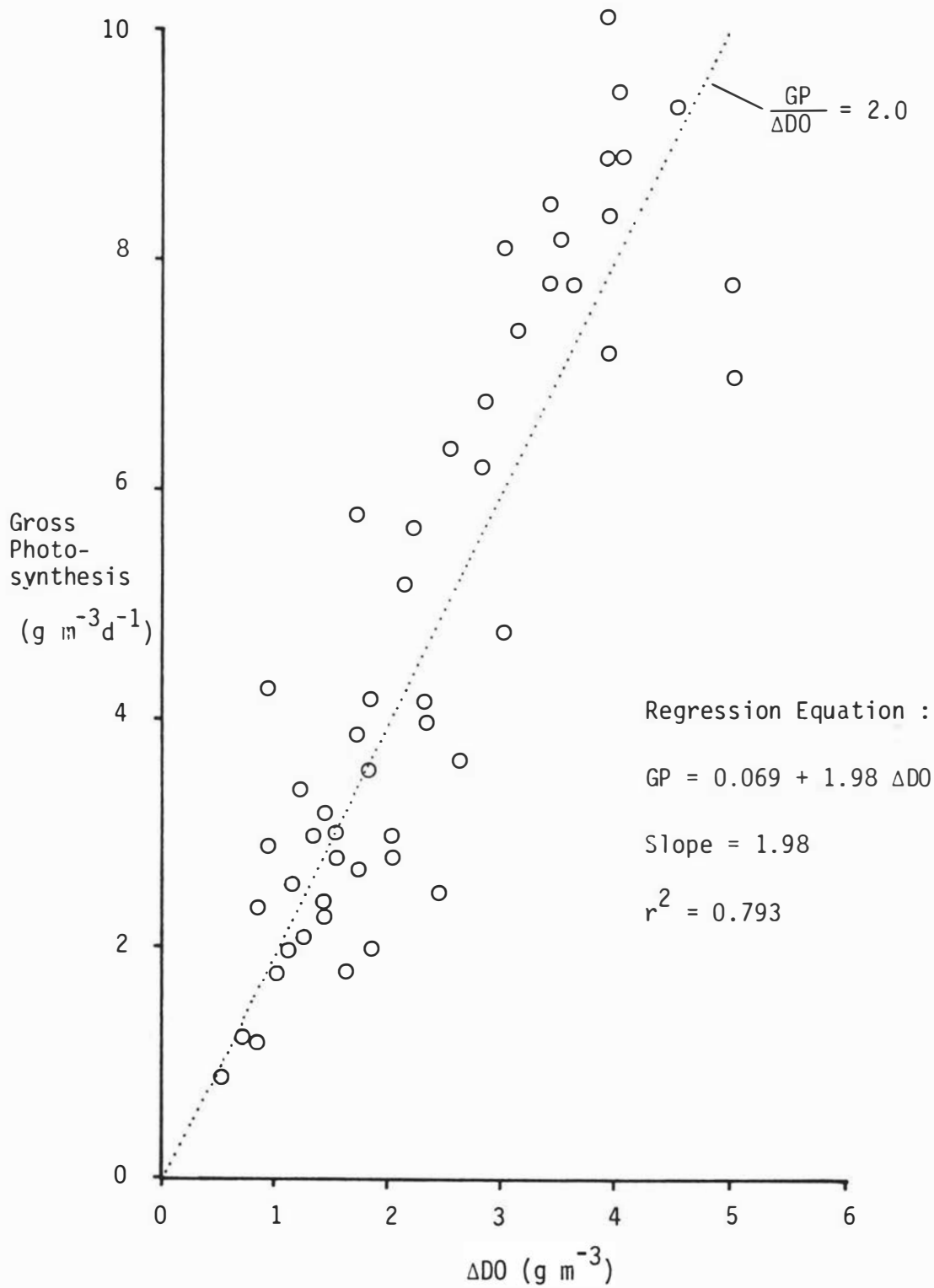


Figure 6.35 The relationship between Gross Photosynthesis and the Dissolved Oxygen fluctuation (ΔDO) 1981/82.



($r^2 = 0.793$) indicative of a phototroph-dominated periphyton community. The deviations away from the relationship do not appear to be easily explained in terms of environmental changes. However, this may be more indicative of the environmental variables being measured rather than their involvement in algal metabolism. Also these values are daily comparisons and are thus integrals of often rapidly changing metabolic activity.

6.7.2.2 Primary productivity during the 1982/83 season

Cladophora did not proliferate during the first few summer months (Section 6.4). *Gomphonema* initially dominated the available substrate and was gradually replaced by *Cladophora* during late January.

The data presented in figure 6.36 illustrate a number of differences between this season and the previous one. During early December the high TR compared to the GP indicated the effects of the *Gomphonema*-dominated periphyton. The suddenly increased TR at this time appears to be a result of slight flow increases (resulting in turbidity increases and reduced insolation) and over-cast conditions. (Appendix 8). The general pattern of figure 6.36 was similar to that observed in the previous season. That is, increases in both GP and TR, with minor fluctuations, were observed as periphyton (*Gomphonema* and *Cladophora*) biomass increased. (See figures 6.7 and 6.8 and appendix 1). Other monitored parameters appeared to have little affect on the GP and TR (See appendices 7 & 8). This could be attributed to the highly variable composition of the periphyton community during the 1982/83 season in contrast to the 1981/82 situation (See section 6.4). The absolute GP and TR values are generally a little higher, and may have been due to the proliferations being more extensive throughout the river.

The P/R data presented in figure 6.37 illustrate a wide range of values. There are many examples of conditions away from, especially below, the P/R = 1 line. However, the range of P/R values observed during both seasons were very similar (1981/82:0.32-2.64; 1982/83: 0.35-2.28). The scattered nature of the data illustrated a

Figure 6.36 Gross Photosynthesis and Total Respiration, 1982/83.

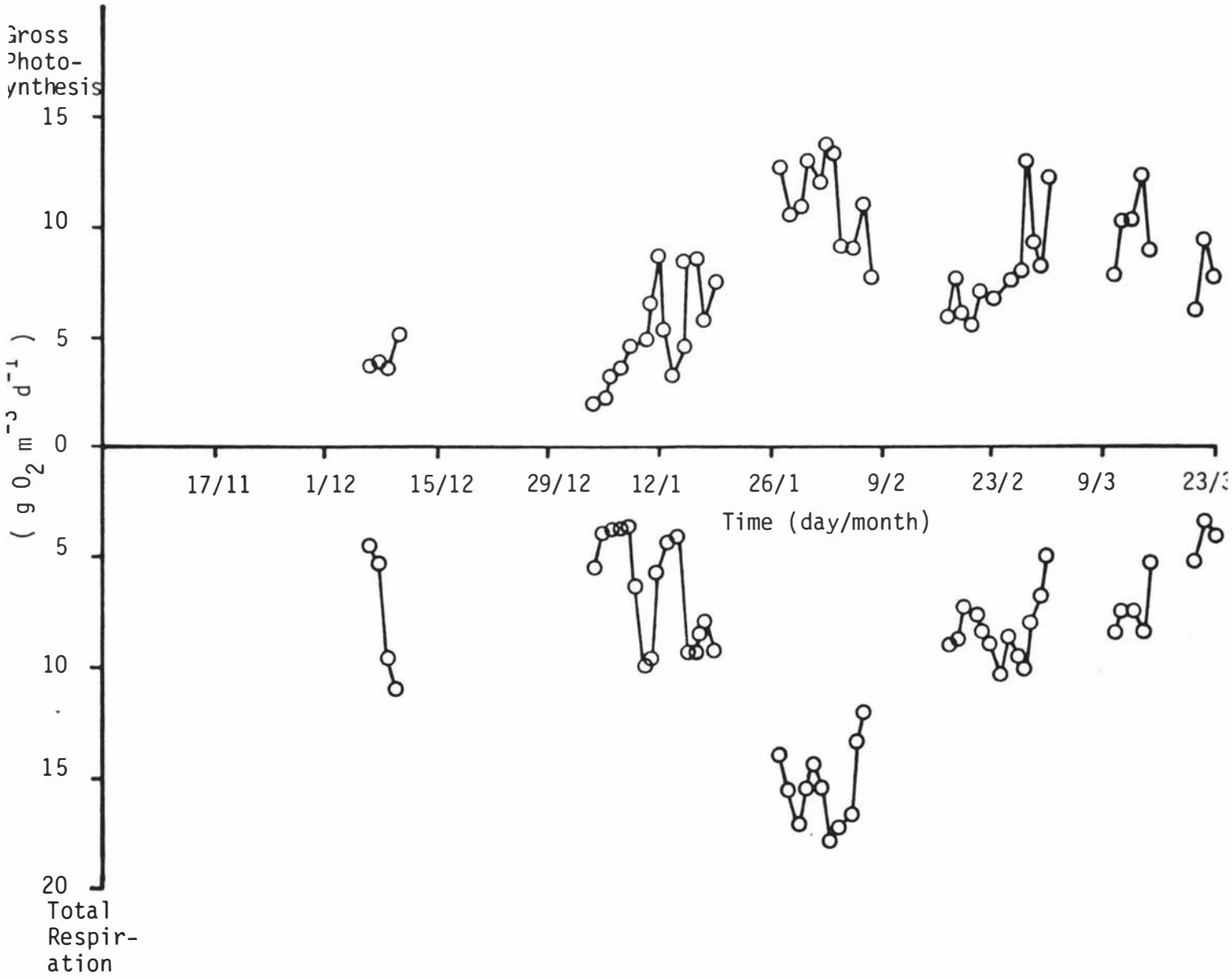
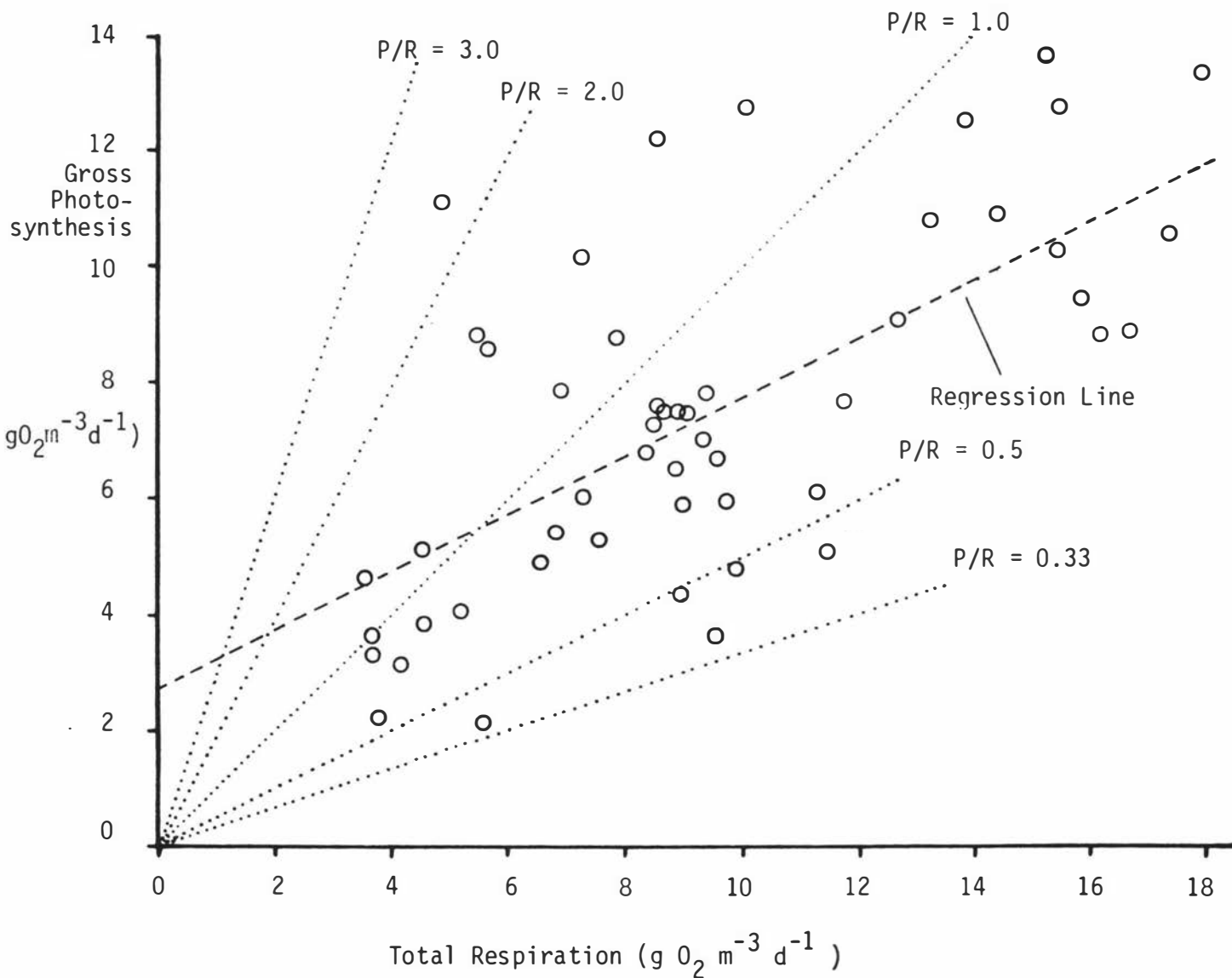


Figure 6.37 The relationship between Gross Photosynthesis and Total Respiration, 1982/83.*



* Regression Equation

$$GP = 2.75 + 0.513 TR$$

Slope = 0.513

$$r^2 = 0.416$$

consequence of the different periphyton communities that developed during the season. There are few examples of TR less than $4 \text{ g O}_2 \text{ m}^{-3} \text{ d}^{-1}$ compared to the previous season. This was probably due to background growths of either *Gomphonema* or *Cladophora* being present at the start of monitoring periods.

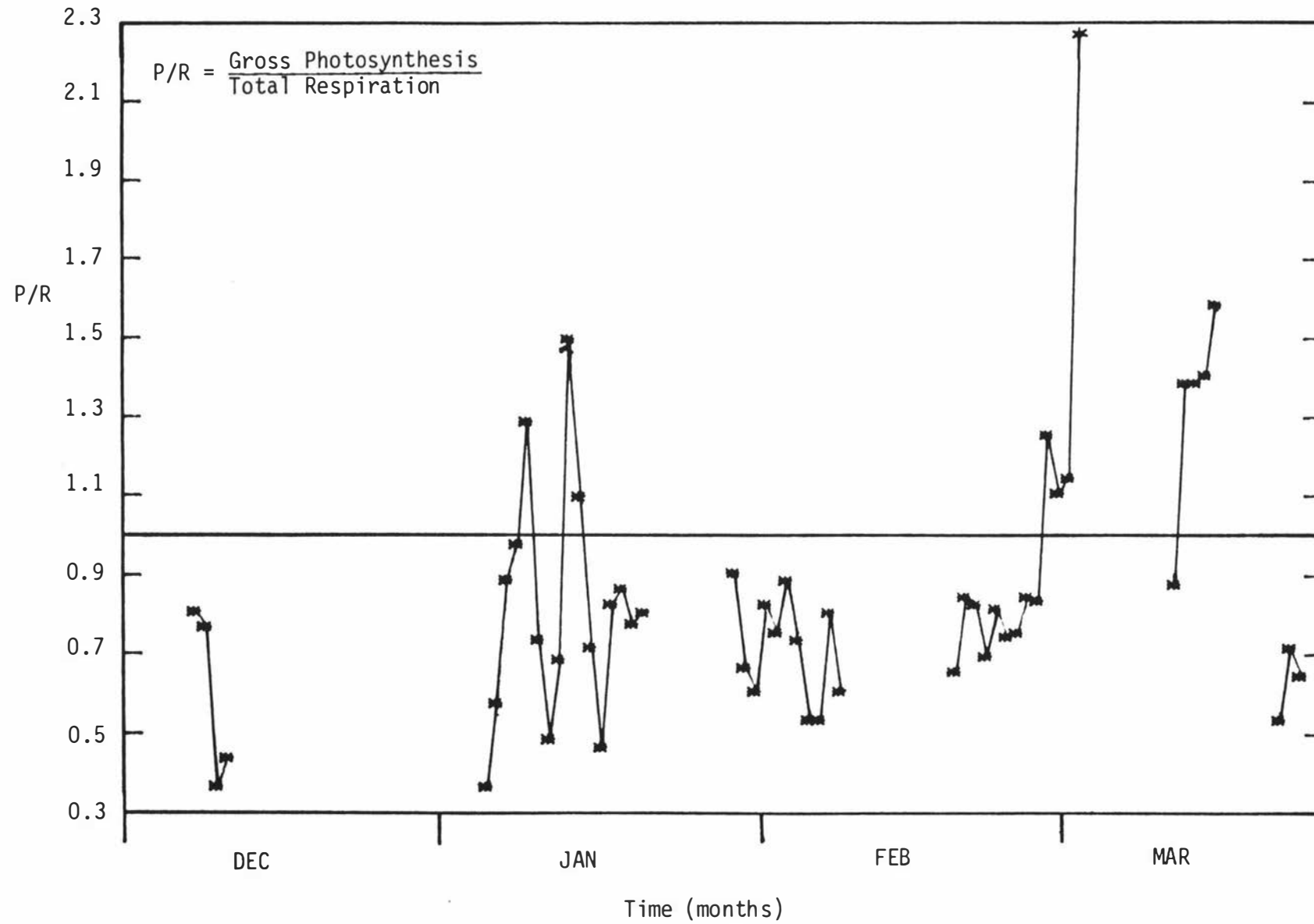
The temporal changes of P/R presented in figure 6.38 demonstrated the nature of the various periphyton assemblages. Most of the monitoring periods indicated that respiratory activities dominated photosynthesis. The first peak in January was attributed to increasing available light due to a decreasing flow (and hence decreasing turbidity) and increased insolation. (See appendix 8). The following trough was due to a sudden small flow increase (Figure 6.8) which, as it rapidly subsided, allowed a new peak to occur. Further minor flushes increased water turbidity and reduced P/R below one. A part of the P/R reduction may be attributable to P limitation which was apparent during this period. (Section 6.6).

The data during early February were a reflection of the relatively low *Cladophora* biomass density and the continued presence of some *Gomphonema* (see Section 6.4). The *Gomphonema* growths were reduced to trace amounts by early March. The prominent P/R peak on 2 March may be attributed to a sudden increase in light available to a relatively high CCA biomass after a period of overcast conditions. (See appendix 8). The availability of P was variable during this period and the peak P/R may have been, in part, due to a release from P limitation. (Figures 6.15 - 6.18).

The P/R data obtained during March were primarily affected by flush events (Figure 6.8) which can leave algal communities, both quantitatively and qualitatively different from the initial assemblage. (Section 6.4). These communities will in turn exhibit very different photo- or heterotrophic characteristics.

The Net Areal Primary Productivity (NAP) data illustrated in figure 6.39 demonstrated both periods of *Gomphonema* and *Cladophora* dominance. The major feature of the data in figure 6.39 was the range of the

Figure 6.38 The variation in the P/R ratio during 1982/83.



NAP in comparison to the values recorded in the previous season, (Figure 6.31). Comparatively high levels of both negative and positive NAP were observed. The NAP was predominantly negative, until the large flush in February 1983, presumably due to the *Gomphonema* mats acting as largely heterotrophic communities. The large maximum DOD reported in Section 6.7.3.3 was a result of this activity. As the *Cladophora* began to become established, after the February flush the frequency and magnitude of positive NAP values increased. The negative NAP values recorded towards the end of March indicate the results of flush events. (See figures 6.7 and 6.8, and above). The importance of the NAP as compared to the P/R ratio is that the magnitude of the productivity can be assessed.

The Hornberger Plot (Figure 6.40) provides some very useful information when compared to that obtained during the previous season. (Figure 6.33). Generally, the two graphs are similar, with the majority of points indicating substantial eutrophication and at times organic pollution. One difference between the two plots was the increased number of examples of potential eutrophication (i.e. high $\sqrt{P/R}$). Another important difference was that there were also more examples of data with high $\log_{10} TR$ values, demonstrating again that a situation of 'organic pollution' can arise usually as a consequence of the periphyton community composition, river turbidity, associated microbial activity and environmental conditions.

The datum point in the top left-hand section was from 2 February and the probable reasons, for its very high P/R value, were outlined earlier.

There was a noticeable absence of data points indicating 'Clean' conditions. This was due to the presence of *Gomphonema* growths during all the monitoring periods prior to February. Thus, there was no monitoring carried out when stone surfaces were free from periphyton growth. The temporal changes in ΔDO are presented in figure 6.41, these reflect the changes seen above (Figure 6.36). As in the previous season the data illustrate the manifestations of periphyton metabolic activities. The amplitude of the

Figure 6.39 Net Areal Primary Productivity (NAP) during 1982/83

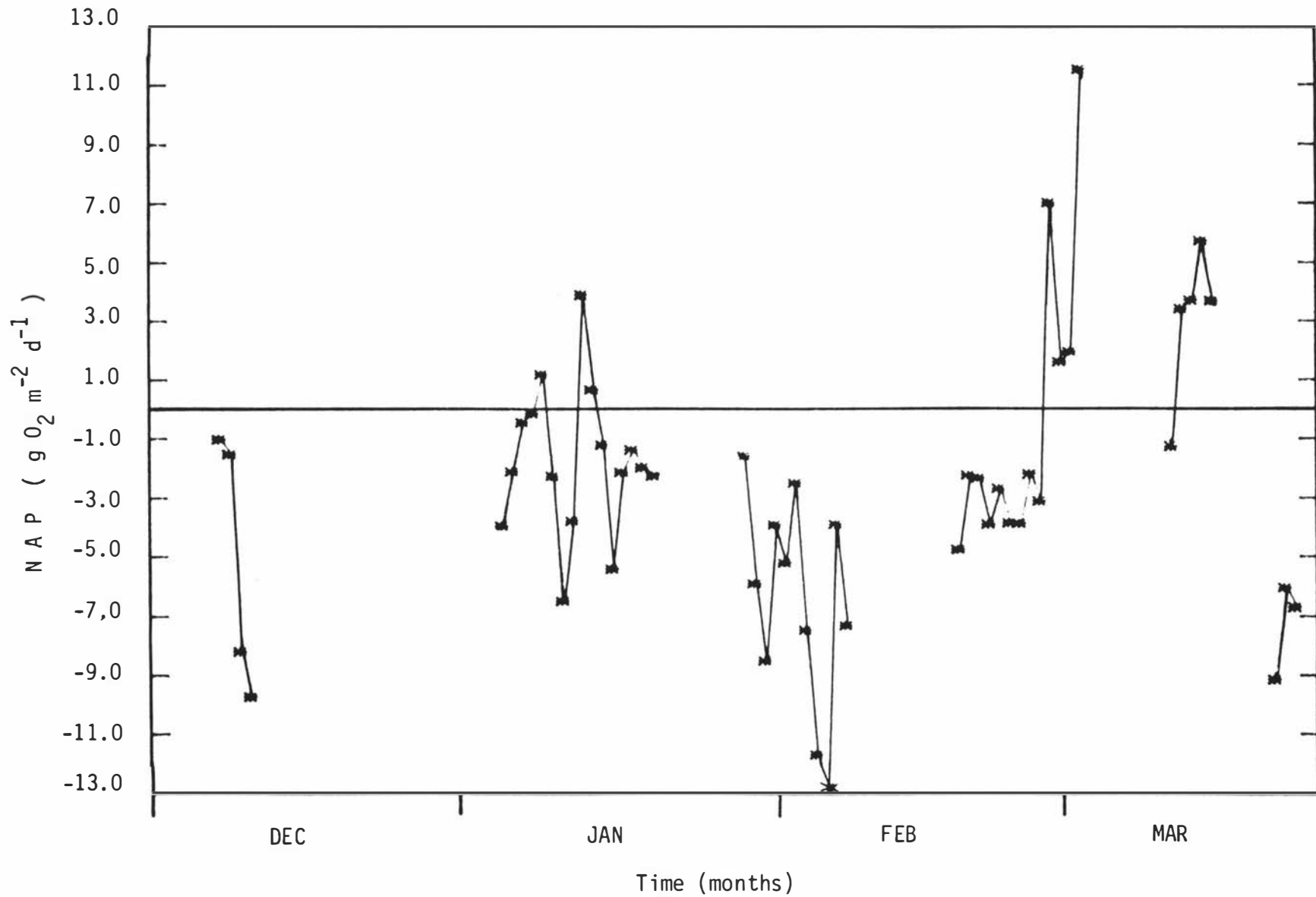
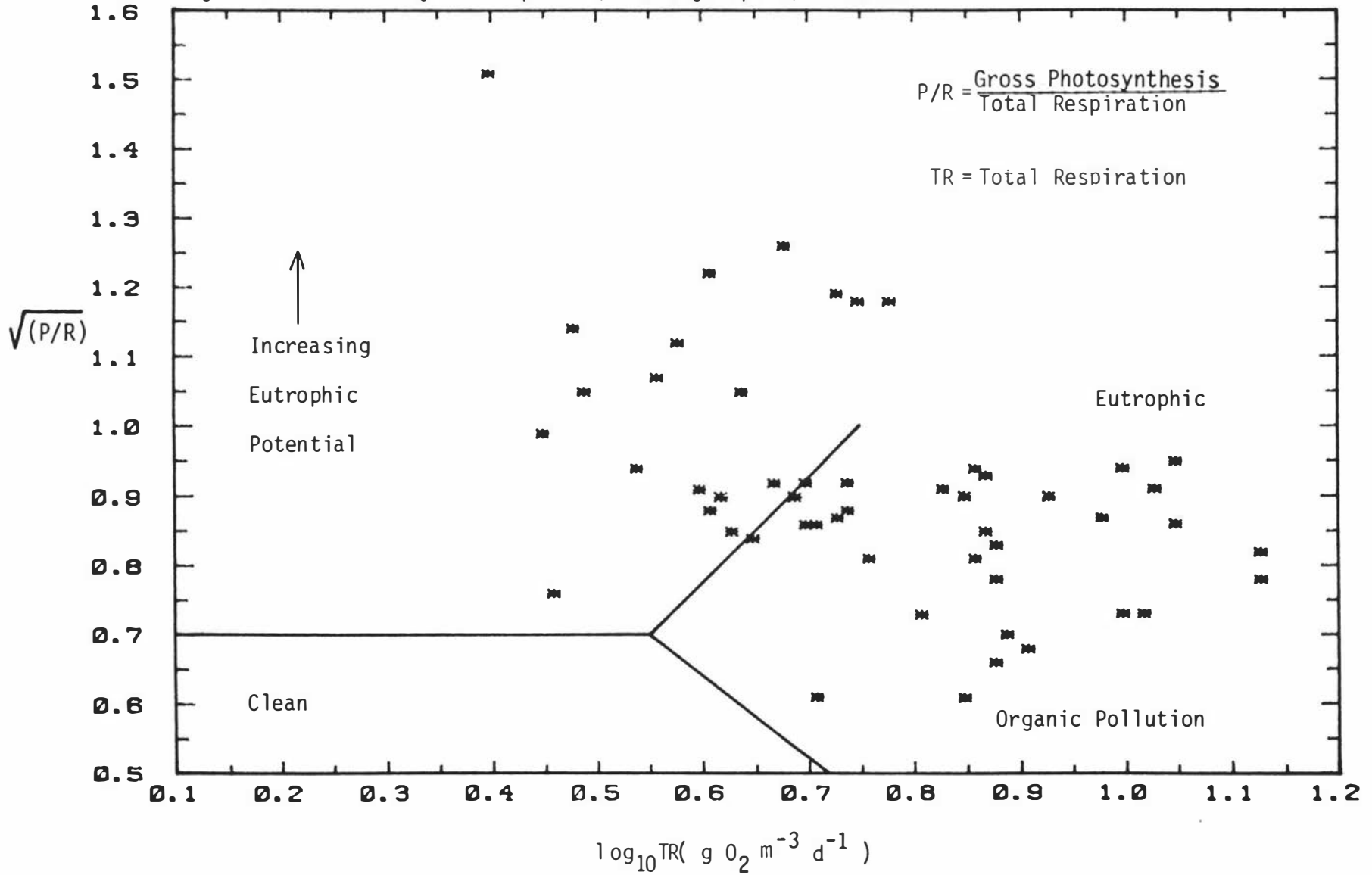


Figure 6.40 Community description (Hornberger plot) 1982/83.



fluctuation being an indication of the combined effects of GP and TR. The magnitude of fluctuations were similar to those observed in the previous season. (Figure 6.34). The ability of a large part of the TR to remain after the GP has been reduced will be discussed in relation to the maximum DOD in Section 6.7.3

The relationship between the GP and the ΔDO can serve as another useful community description. The data in figure 6.42 illustrate a number of differences from the data of the previous season (Figure 6.35). The slope of the regression line is greater (2.14 compared with 1.98), the scatter of points about the line is greater ($r^2 = 0.568$) and there are more examples of GP greater than $10 \text{ g O}_2 \text{ m}^{-3} \text{ d}^{-1}$. The most probable cause of these high values was the concurrently high TR values. A high GP produced from a brightly lit CCA with a low TR would enable a large ΔDO to be achieved. However, if the periphyton was a DCA with a consequently higher TR the DO input to the river would be offset, to some degree, by the relatively higher daytime uptake due to the community respiration.

The ΔDO can be a very useful parameter relating the primary productivity and the DO profile, however in terms of water quality effects, the DOD provides more information. The maximum DOD (DOD_m) describes the worst case situation and warrants careful study in rivers that have yet to assimilate the oxygen demands of organic discharges. (Section 6.7.3).

6.7.2.3 Spatial variation and apportioning primary productivity

During the 1981/82 season, spasmodic shingle extraction throughout the Manawatu River upstream of site M (Figure 1.2) produced marked changes in the river water transparency. In an effort to check the consequences of this on the spatial DO variation and NAP, the primary productivity between site T and site D was investigated over the period 12-13 February 1982. The \bar{DO} profiles obtained are shown in figure 6.43 and the primary productivity data are presented in table 6.2.

Figure 6.41 The daily dissolved oxygen fluctuations (ΔDO), 1982/83.

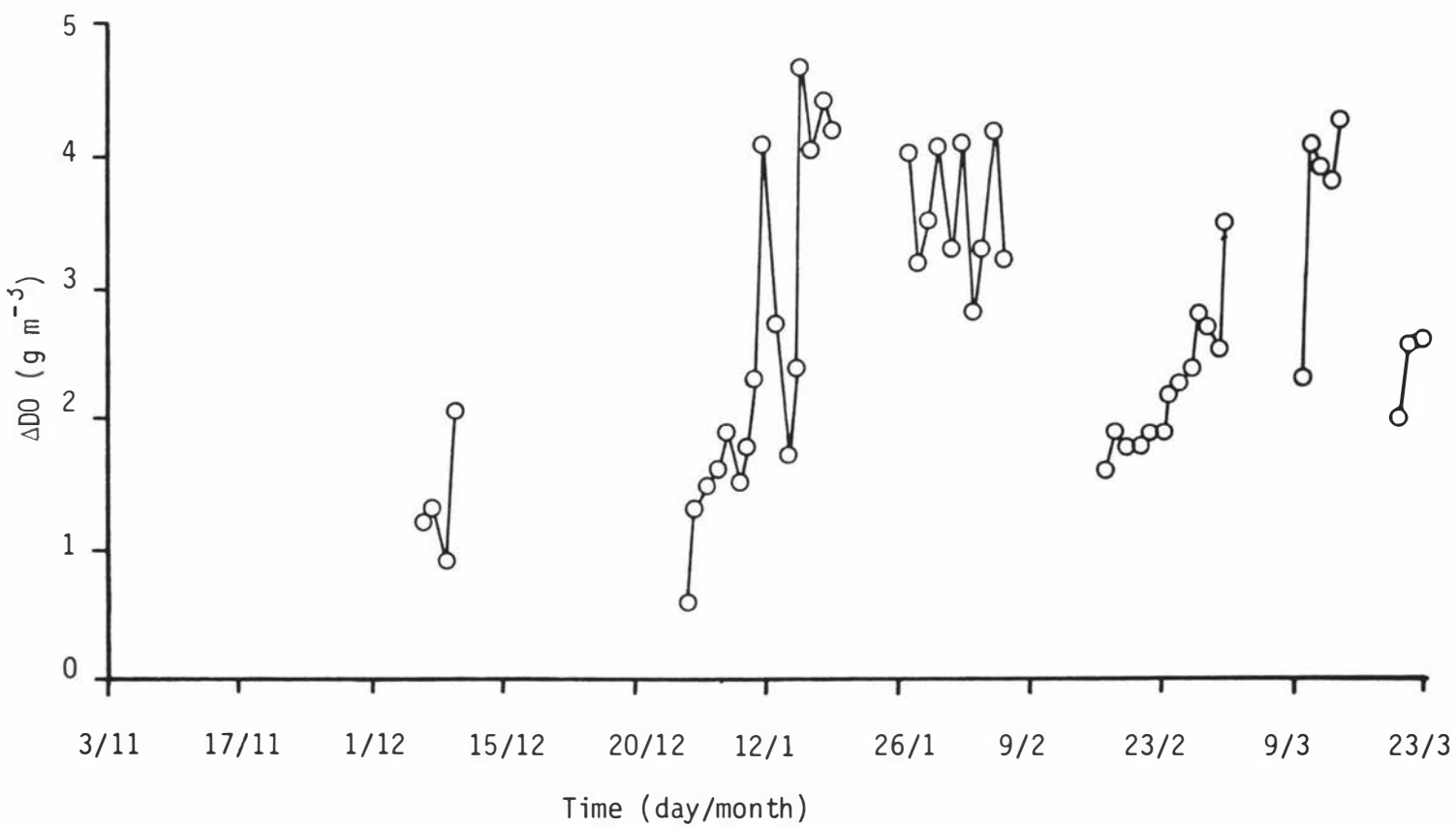


Figure 6.42 The relationship between Gross Photosynthesis and the Dissolved Oxygen fluctuation (ΔDO) 1982/83.

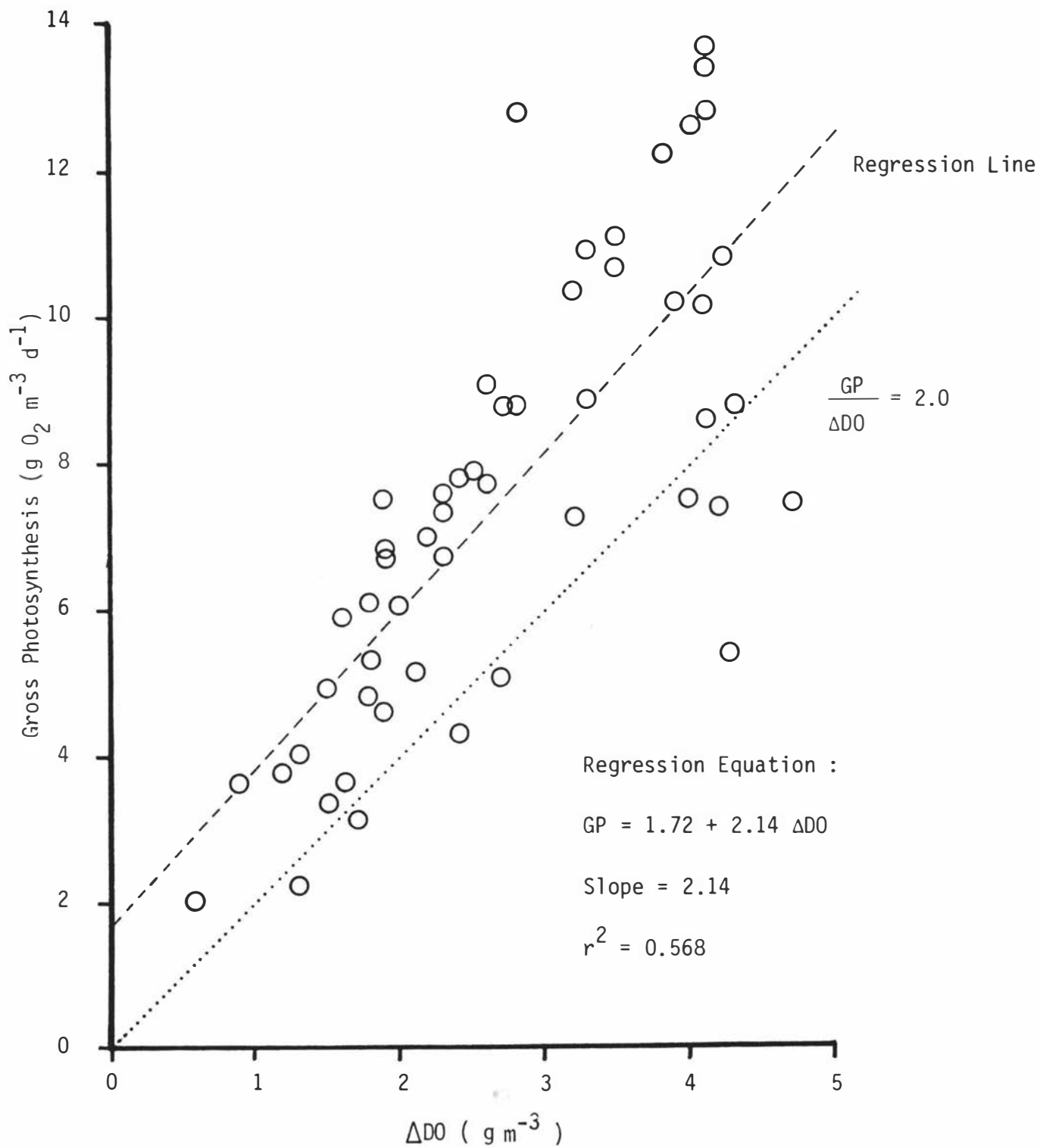


TABLE 6.2: Comparison of primary productivity at sites T and D 12-13/2/82

Site	Gross Photosynthesis (g O ₂ m ⁻³ d ⁻¹)	Total Respiration (g O ₂ m ⁻³ d ⁻¹)	Net Areal Primary Productivity (g O ₂ m ⁻² d ⁻¹)
T			
12.2.82	7.8	-6.5	+1.4
13.2.82	6.7	-5.3	+1.5
D			
12.2.82	8.8	-4.9	+2.7
13.2.82	7.4	-6.8	+0.4

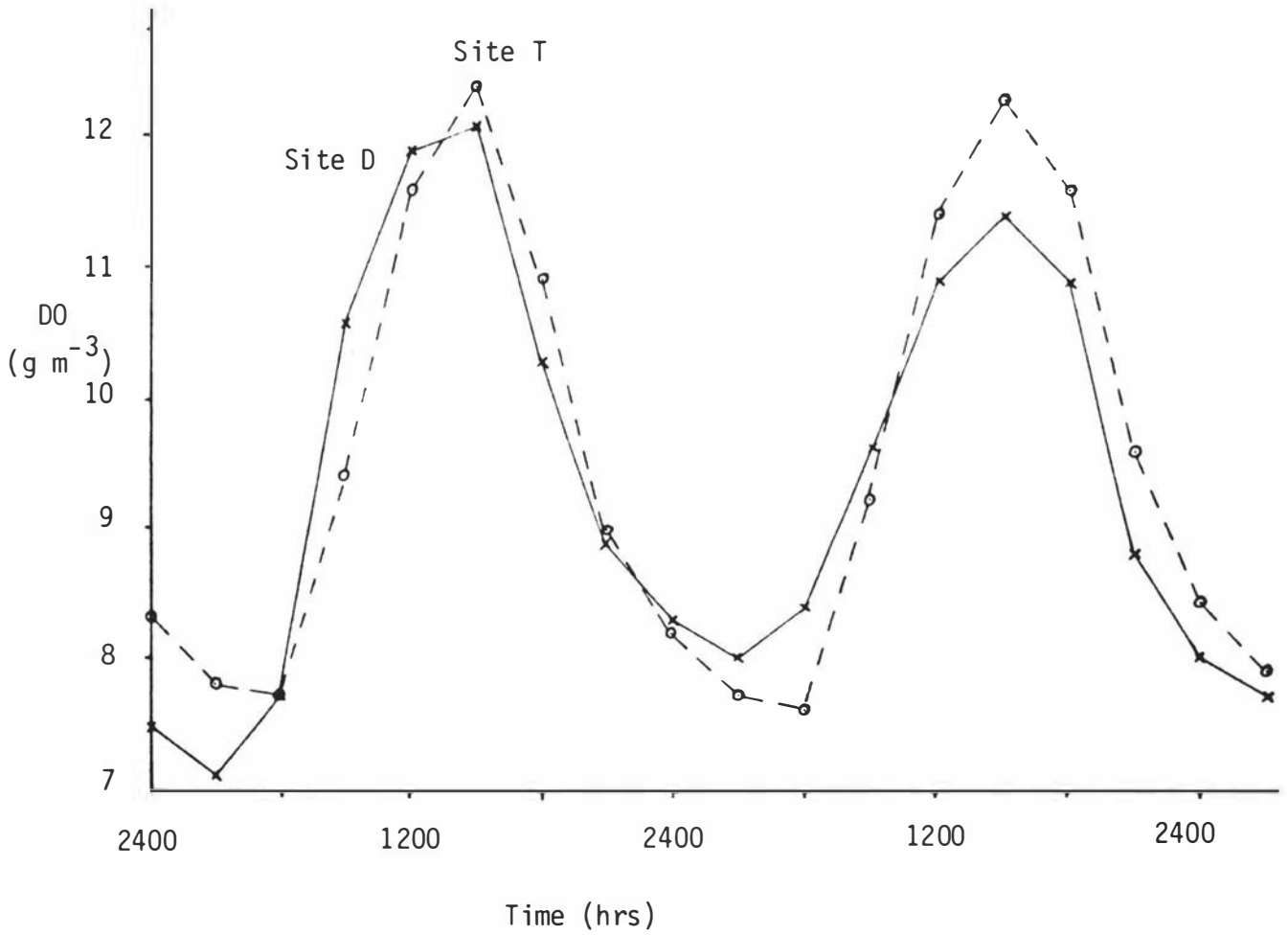
While the profiles in figure 6.43 appear very similar, small differences can be attributed to large variations in algal metabolic activity. The difference between peak heights on 13 February was the result of major differences in metabolic activity (See table 6.2). There are a variety of factors that could contribute to these intersite variations. Qualitative observations of *Cladophora* above site T and along the 7km stretch between the sites, did not reveal any gross differences. The most probable culprit was between site, metal extraction activities which can cause variations in water transparency and nutrients. There are no point discharges, or significant tributary inflows along this river stretch.

These results also demonstrated the need to establish an upstream monitoring site nearer to the point of the first discharge, in order to get a clearer picture of algal water quality effects immediately prior to the impact of discharges.

The apportioning of photosynthetic components of the river community envisaged by the use of chambers did not proceed beyond preliminary field investigations, due to difficulties encountered in estimating the average *Cladophora* biomass (Section 3.2). Sporadic investigations carried out during low/flow periods in January and February 1982, showed the free river phytoplankton levels to vary between 1-4 mg Chl-a equivalent m⁻³, and chamber experiments during this period did not reveal any significant

Figure 6.43 Comparison of dissolved oxygen profiles at sites T and D

12 - 13/2/82 (Temperature fluctuations equivalent).



production attributable to phytoplankton. Thus, over the total river, phytoplankton were assumed to make a negligible contribution to the NAP.

6.7.3 Dissolved Oxygen deficits

6.7.3.1 The impact of discharges on the Dissolved Oxygen profile

Before embarking on a detailed examination of the DO profiles observed above the discharge area, it is instructive to compare the DO profiles of the river above and below the discharges. (See figure 6.44).

The upstream profiles (Site T) illustrate the effects of a fairly modest *Cladophora* proliferation. The increased DO fluctuation on the last two days (3 and 4 February 1982) was evidence of the increased *Cladophora* biomass that occurred during the monitoring period (13-19 g DW m⁻², see figure 6.5 and appendix 7), and the decreasing water turbidity as the river recovered from a recent flush. The DO profiles observed at site K below the discharges were always lower than those at site T, often considerably so. This was attributed to the sum of the effluent BOD exertion and upstream algal respiration. The maximum DO at site K usually occurred at night as a result of the river travel times for each of the discharges and the diurnal fluctuations of the upstream DO. In order to quantify the proportion of each process, intensive temporal and spatial investigations which could follow 'slugs' of water from upstream stretches to the same area below the discharges would be required. (A discussion of the downstream changes in periphyton composition is presented in section 6.4).

The extent of the impact on the river can be determined by the DO deficits (DODs) at site O. During 17 February (Figure 6.45) a DO deficit of 8.4 g m⁻³ was gradually reduced to 1.1 g m⁻³ by late afternoon. The severity and duration of these depressed DO concentrations cause concern about the ability of this section of the Manawatu River to maintain desirable aquatic life in the light of the generally accepted minimum desirable DO concentration of 5.0 g m⁻³ (Train, 1979). The section of the Manawatu River below the discharges is

Figure 6.44 The Dissolved Oxygen(DO) fluctuations at sites T and K

during 30/1/82 - 4/2/82. (Site T *Cladophora* biomass = 15 g m^{-2})

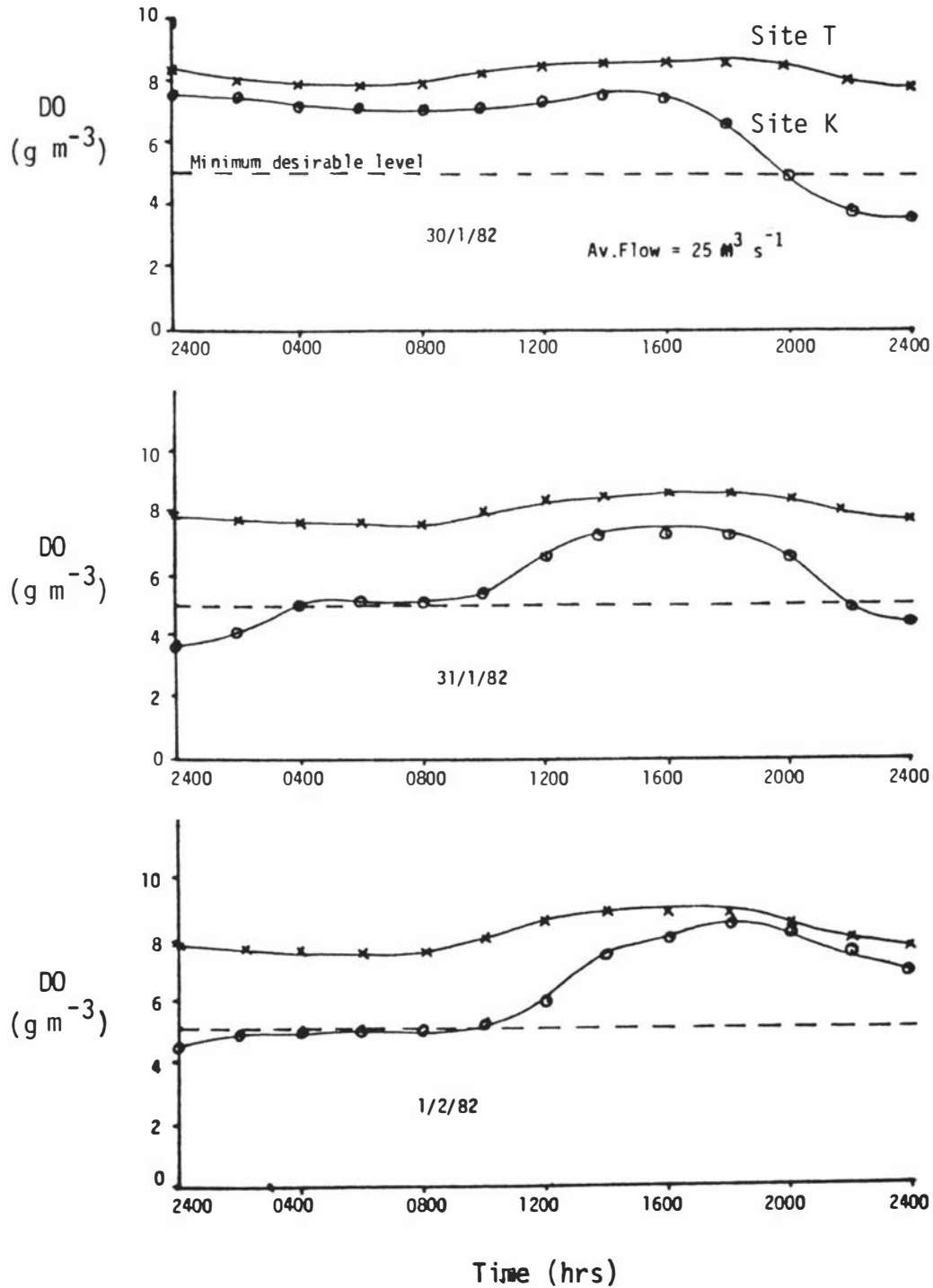


Figure 6.44 Continued.

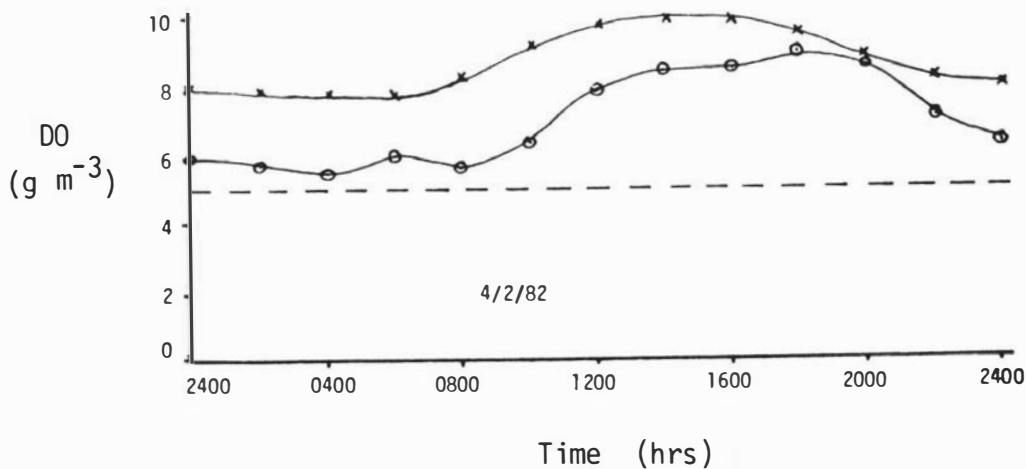
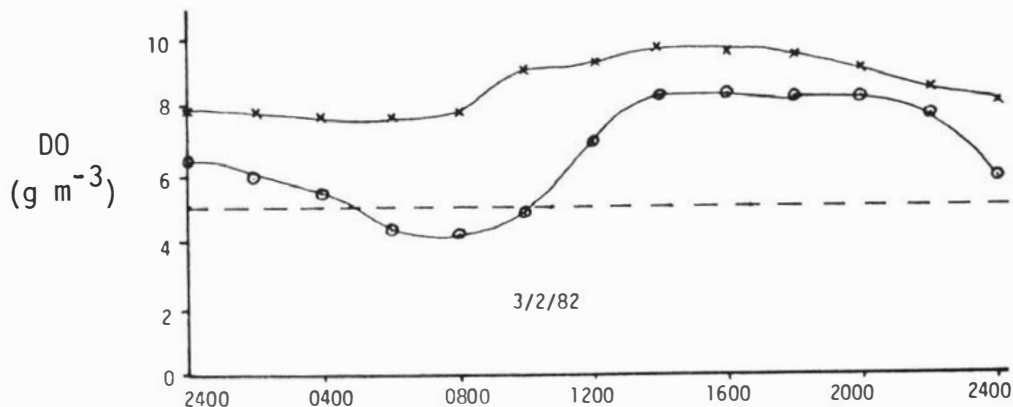
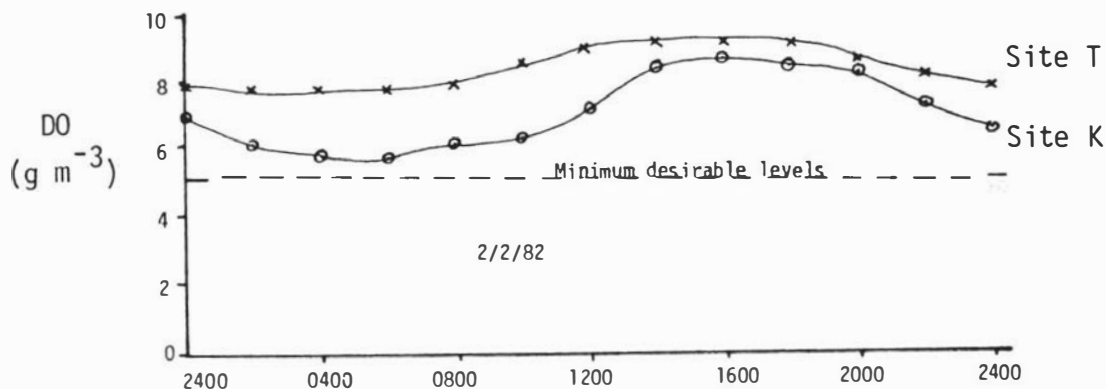
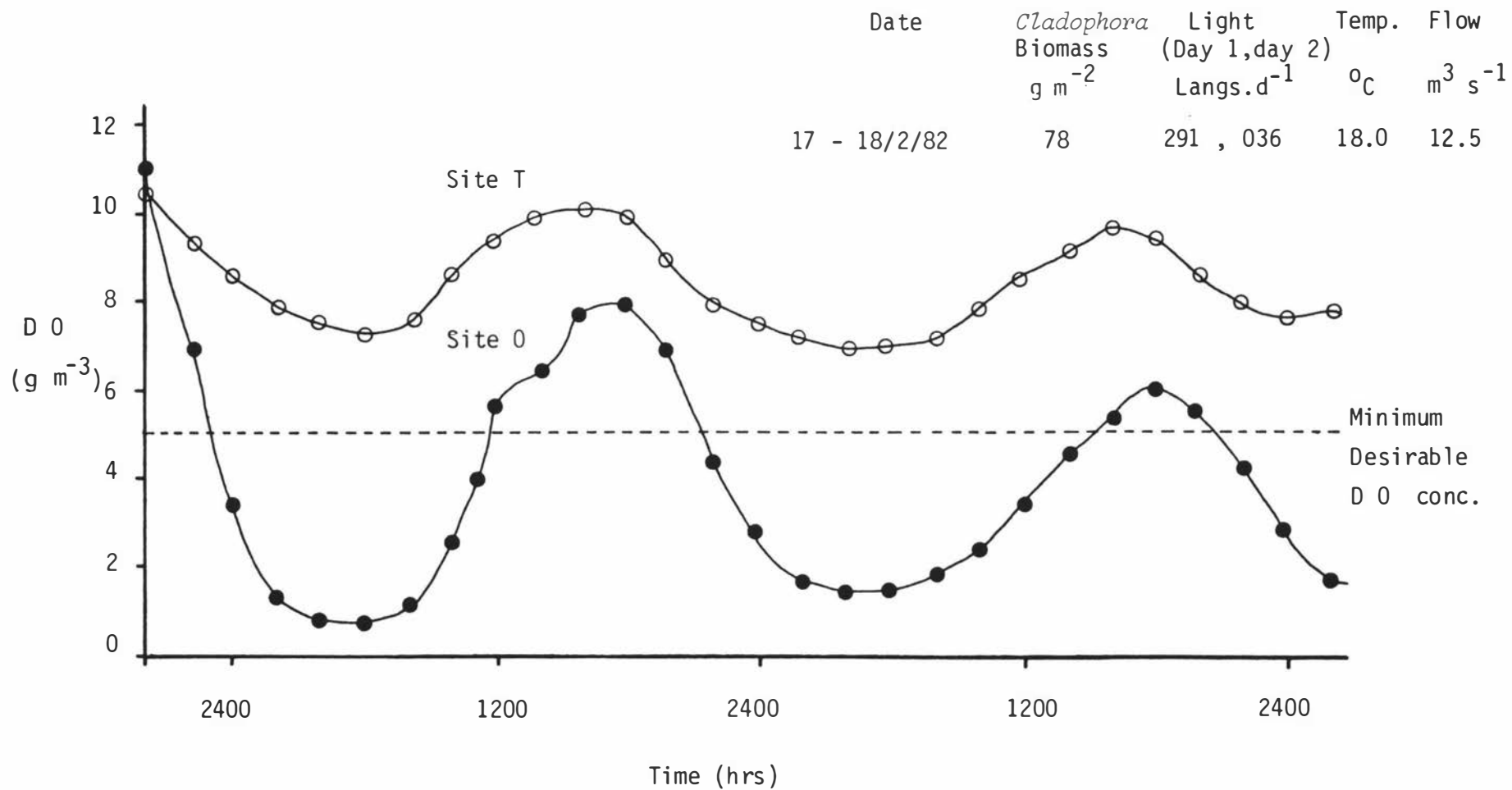


Figure 6,45 Some Dissolved Oxygen profiles at sites T and O, 1982.



classified 'D' under the Water and Soil Conservation Act, 1967, and has a minimum DO concentration standard set at 5.0 g m^{-3} (Currie, 1977). This period of low flow ($12.5 \text{ m}^3 \text{ s}^{-1}$) was interrupted by a major flush on 19 February, and thus the 'stress' was relieved. However, depressions of a similar magnitude were recorded during March 1982.

The data presented for site 0 may be a slight exaggeration of actual events as the current velocity at the site was approximately 0.3 m s^{-1} . The importance of maintaining the DO probe in rapidly flowing ($>0.4 \text{ m s}^{-1}$) water only became apparent later in this study. An error of up to 2 g m^{-3} DO could occur if the probe was incorrectly sited.

Algal respiratory activities detract from the ability of downstream river stretches to maintain a desirable DO concentration. There may therefore be a case for varying discharge schedules to coincide with algal induced DO peaks. However, the occurrence of these peaks (Figure 6.46) should not be relied upon to occur consistently. They may well be utilised as an interim measure before extended treatment facilities for the discharges become operational, and should be borne in mind during any summer low flow period in which less than desirable DO levels exist for any length of time.

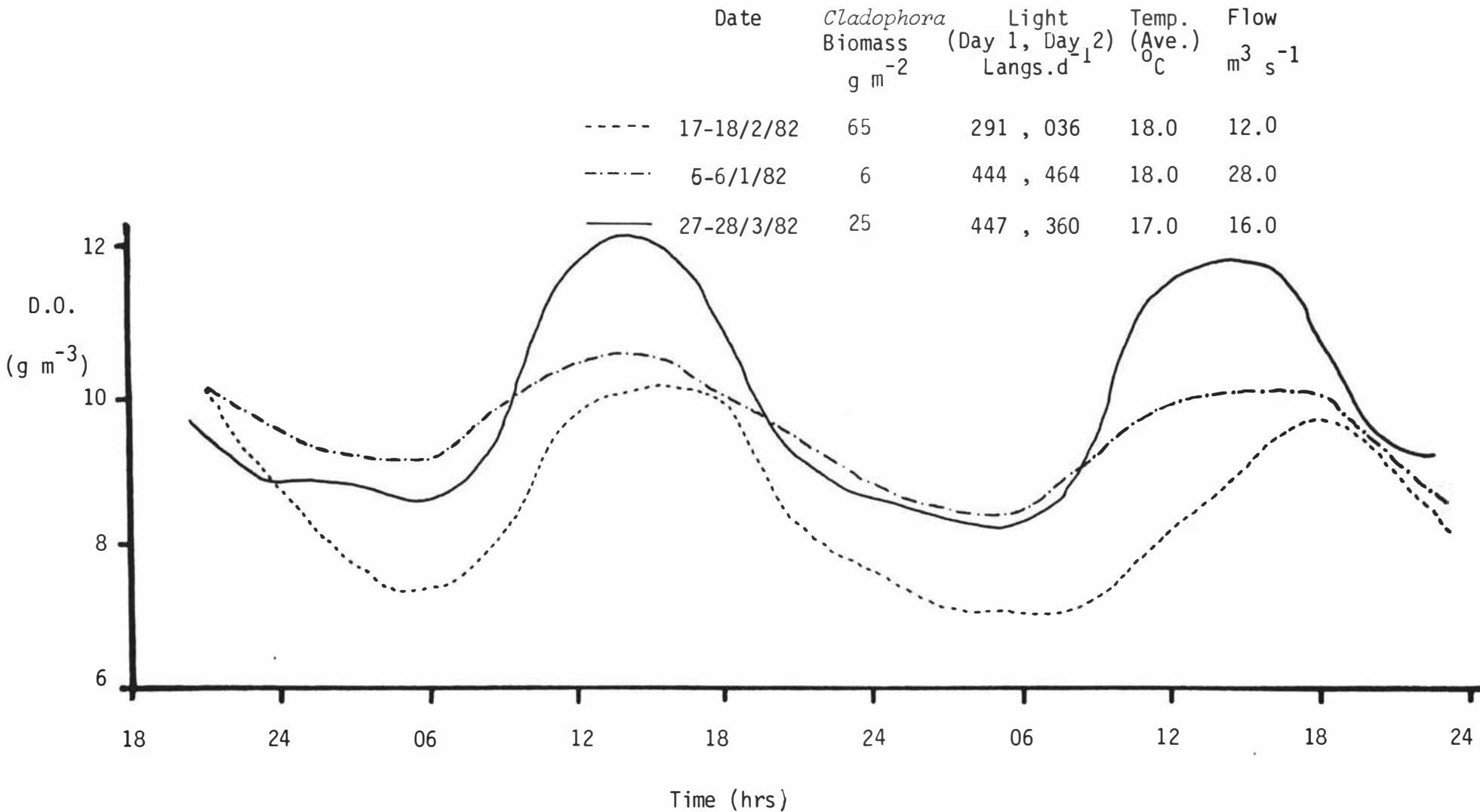
6.7.3.2 Upstream Dissolved Oxygen profiles and the maximum Daily Dissolved Oxygen Deficit 1981/82

The data presented above indicate, that during *Cladophora* proliferations the Manawatu River upstream of the discharges can have DODs that severely reduce the river's ability to assimilate the oxygen-demanding discharges while maintaining a minimum desirable DO concentration. The upstream ΔDO can vary dramatically, from less than 0.5 g m^{-3} , occurring in the absence of any algal growth, to fluctuations of 5.0 g m^{-3} when *Cladophora* proliferations are brightly illuminated. Some of the DO fluctuations that occurred during *Cladophora* development in the Manawatu River are illustrated in figure 6.46. The very low DO profile observed during 17-18 February was attributed to the combination of a relatively high *Cladophora* biomass density and a prior period of high insolation. (Appendix 7). The high light levels raised the rate of metabolic activities in the *Cladophora* proliferation, so that on 17-18 February when light levels were reduced, elevated respiration rates together with the background community respiration were manifest as generally low DO concentrations. Consequently DOD_m s were high, 2.5 & 1.7 g m^{-3} respectively. The decrease over the two days was probably due to the photorespiration component being reduced on the first day of reduced insolation. The other DO profiles, illustrated in figure 6.46, depict a low, and a brightly lit high *Cladophora* biomass density situation .

The link between *Cladophora* photosynthesis and the magnitude of the ΔDO is illustrated by the data presented in figures 6.35 and 6.42.

From the DO profiles and their respective temperature fluctuations it is possible to identify the minimum DO and the maximum daily DOD

Figure 6.46 Some Dissolved Oxygen profiles at site T, 1981/82.



(DOD_m) (usually these occur at the same point in time). The DOD_m is a reflection of the respiratory activity of the river community in that it is the departure from the saturated DO concentration. Many factors will combine to produce a specific DOD_m including physical characteristics of the river, as well as those that affect the metabolism of the algal assemblage. The main factors are as follows:

- (a) Biomass and composition of the *Cladophora* assemblage.
- (b) Light
- (c) Temperature
- (d) Nutrients
- (e) River Flow
- (f) Oxygen-demanding discharges.

Before these factors are compared to the DOD_m the assumptions and limitations of each measurement technique will be discussed briefly:

- (a) Biomass: The average of the three main study sites was used. This should not be confused with a whole river average, which is not easily directly measured. (See sections 2.5.5, 3.2 and Wong & Clark (1979) and Wong *et al*, (1979)).

The theoretical disadvantage of this parameter is that it is a purely quantitative term and does not take account of the assemblage composition. Equivalent biomass values have been obtained from assemblages of visibly different composition. (Section 6.4). Dissimilar algal communities will give various responses to changes in environmental parameters, such that resultant DO profiles can be very different.

Cladophora biomass was sampled once or twice weekly. To obtain a value for the *Cladophora* biomass density between sampling dates a straight line interpolation was usually employed, provided there was relatively little flow variation.

- (b) Light: This was measured as total Langleys day⁻¹ in Levin (50 km South-West of Palmerston North). Thus, these data are both quantitatively and qualitatively different from the photosynthetically active radiation available to the algae (Other light parameters detailed in section 3.4 were not continuously monitored during the whole 1981/82 season).

(c) Temperature: This was measured at the time of the DOD_m . One value is used to describe the daily temperature climate. This does not take any account of diurnal fluctuations, which have been observed to range from $1^{\circ}C$ to $4^{\circ}C$. Such water temperature fluctuations can alter the metabolism of algal communities (Wong *et al*, 1978). The processes of respiration are more responsive to temperature changes than are those of photosynthesis. (McDonnell & Weeter, 1972; McIntire, 1966). Thus, temperature increases, especially those occurring during night time, in response to daytime insolation, will favour the production of large DOD_m s.

(d) Nutrients: While both nutrients and NATs were monitored during the 1981/82 season, there are a number of reasons that reduce the usefulness of incorporating these measurements into formal statistical tests, such as multiple regression analysis. These reasons are detailed as follows:-

Nutrients: The only data available for the 1981/82 season are TP and TN, and as discussed in section 2.7 these parameters do not necessarily describe the physiologically most readily available N and P species.

Sampling Regime: The stochastic nature of river nutrient concentrations works against the feasibility of extrapolating data from one weekly sample to the next, for use on a daily basis for the intervening period.

NAT response times: While data are available, there are difficulties involved with trying to combine both the level and response times of NATs in a formal statistical analysis. (See section 4.3). The drawbacks of extrapolating, noted above, also apply to NATs.

(e) Flow: Daily mean values were measured adjacent to site D. (See section 3.3)

(f) Oxygen-demanding discharges: While these would obviously add to the TR, there are no significant oxygen-demanding discharges to the Manawatu River above Palmerston North.

Bearing the above interpretive difficulties in mind and the fact that any conclusions from the study are valid only for the summer low flow ($\approx <65 \text{ m}^3 \text{ s}^{-1}$) period from approximately November to April, a correlation coefficient matrix (Table 6.3) was constructed with 45 data sets. (See appendix 7)

TABLE 6.3: Correlation coefficient matrix of the maximum daily Dissolved Oxygen Deficits and some river and environmental variables, 1981/82 (Using post 28 November 1981 data, when *Cladophora* biomass density was above the detection limit, see appendix 7)

	DOD _m	BM	L _T	F	T _{av.}	GP	TR	R _m	P/R
BM	0.591								
L _T	0.262	-0.051							
F	-0.174	-0.613	-0.083						
T _{av.}	0.324	0.182	0.579	-0.213					
GP	0.423	0.468	0.267	-0.686	0.418				
TR	0.630	0.594	0.134	-0.554	0.337	0.903			
R _m	0.551	0.544	0.147	-0.570	0.285	0.898	0.909		
P/R	-0.256	-0.008	0.216	-0.416	0.367	0.472	0.147	0.235	
NAP	-0.208	-0.146	0.377	-0.389	0.272	0.497	0.085	0.258	0.719

KEY:

- DOD_m = Maximum Dissolved Oxygen Deficit (g m^{-3})
 BM = Average *Cladophora* site biomass density (g DW m^{-2})
 L_T = Daily terrestrial light (Langleys d^{-1})
 F = Average daily river flow ($\text{m}^3 \text{ s}^{-1}$)
 T_{av.} = Average daily river temperature ($^{\circ}\text{C}$)
 GP = Daily Gross Photosynthesis ($\text{g O}_2 \text{ m}^{-3} \text{ d}^{-1}$)
 TR = Daily Total Respiration ($\text{g O}_2 \text{ m}^{-3} \text{ d}^{-1}$)
 R_m = Maximum hourly Respiration rate ($\text{g O}_2 \text{ m}^3 \text{ hr}^{-1}$)
 P/R = GP:TR ratio
 NAP = Daily Net Areal Primary Productivity ($\text{g O}_2 \text{ m}^{-2} \text{ d}^{-1}$)

The variables with the highest correlation to the DOD_m were Total Respiration (TR) ($r = 0.630$), *Cladophora* biomass density ($r = 0.591$) and the maximum hourly Respiration rate (R_m) ($r = 0.572$).

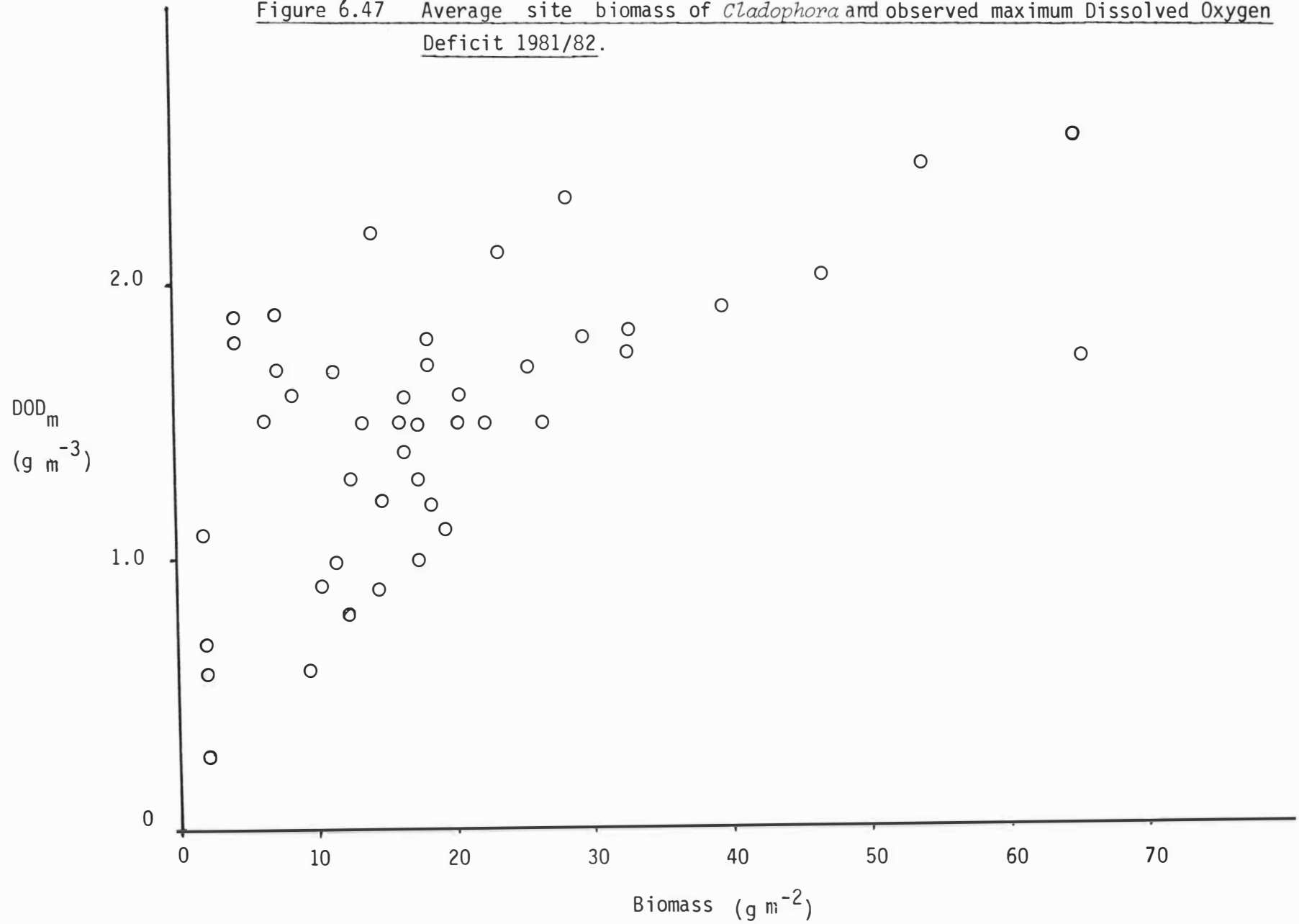
All these parameters were highly significant at $p < 0.001$. The TR acted as a descriptor of the whole river community, as opposed to the *Cladophora* biomass which only accounted for one (albeit major) component. A more detailed study of the influence of TR on the DOD_m will be discussed later when both seasons are considered (Section 6.7.3.3).

The relationship between the *Cladophora* biomass density and the DOD_m was examined more closely, with the aim of identifying possible relationships by plotting these two variables (Figure 6.47). The relationship was an approximate hyperbolic curve. The DOD_m could increase rapidly with only small increases in *Cladophora* biomass. The large scatter at biomass densities less than 30 g DW m^{-2} was attributed to the variable assemblage composition. At higher biomass densities the *Cladophora* community was usually dominated by healthy *Cladophora* filaments. (Sections 5.2 and 6.4).

An upper DOD_m limit of approximately 2.8 g m^{-3} was apparent in figure 6.47. This was due to there being a maximum TR for any specific *Cladophora* assemblage together with the reaeration rate increasing as the DOD rose. (Section 6.7.3.3).

The river flow was negatively correlated ($r=0.613$) with the *Cladophora* biomass density, demonstrating the sloughing-off effect of high flows. The link between the *Cladophora* biomass and the basic primary productivity terms was evident by the positive correlations with GP ($r=0.468$), TR ($r=0.594$) and R_m ($r=0.544$). The usefulness of scrutinizing all the significant correlation coefficients was reduced because of the multiplicity of inter-relationships that occurred making interpretations difficult and often masking or falsely creating relationships. The influence of 'independent' predictors on a dependent variable, such as the DOD_m can be assessed more accurately with multiple linear regression analysis (MLRA) (Minitab, 1982; Sokal and Rolf, 1973). This technique also allows the identification of the relative importance or contribution of each variable to a predicted DOD_m . Variables that have very little effect on the DOD_m can be discarded until only those that account for a large part of the observed DOD_m remain. Two drawbacks involved in using MLRA are:-

Figure 6.47 Average site biomass of *Cladophora* and observed maximum Dissolved Oxygen Deficit 1981/82.



- (a) Only linear relationships can be identified, more complex higher order relationships can only be identified by introducing transformations, based on 'scatter plot' analysis and mechanistic rationale, or by using more advanced non-linear analyses.
- (b) The resultant regression equation is only reliable when using data within the numerical bounds of those data used in the initial construction. (See appendices 7 & 8)

This procedure was followed with the 1981/82 data used to construct table 6.3 (Appendix 7). The result is presented in table 6.4 below:-

TABLE 6.4: The parsimonious regression equation and associated statistics for predicting the maximum daily Dissolved Oxygen Deficit, 1981/82

The parsimonious equation:

$$DOD_m = -0.757 + 0.0219 \text{ BM} + 8.29 \times 10^{-3} L_T + 0.0424 F + 0.109 \text{ TR}$$

The importance of predictors:

Predictor	Coefficient	Standard deviation of coefficient	t-ratio = Coeff./St.dev.
	-0.757	0.334	-2.27
BM	0.0219	4.58×10^{-3}	4.77
L_T	8.29×10^{-3}	2.93×10^{-4}	2.83
F	0.0424	0.0102	4.14
TR	0.109	0.0259	4.23

r^2 (adjusted for 40 degrees of freedom) = 63.1%

Standard deviation of regression, $s = 0.312$

Application range of the regression equation:

BM = Average *Cladophora* site biomass density (1-65 g DW m⁻²)

L_T = Daily terrestrial light (36 - 723 Langleys d⁻¹)

F = Average daily river flow (11.9 - 33.8 m³ s⁻¹).

TR = Daily Total Respiration (1.12 - 9.93 g O₂ m⁻³ d⁻¹)

The t-ratios describe the contribution each variable makes to the total predicted DOD_m . Higher numerical values indicate a larger contribution. Thus of the four predictors, light was the least important. The regression equation accounted for 63% of the observed daily DOD_m variation. This result was considered to be of practical predictive value for water quality management. While the parsimonious regression equation has been derived empirically, the importance of the predictors may be explained in mechanistic terms. This exercise, however, will be postponed until the two seasons data are considered together. (Section 6.7.3.3)

Advanced treatment of the available data such as further transformations involving weighted data and the use of higher order equations, was not warranted because of the limitations of the predictors.

This data analysis served to highlight those areas that lacked all the relevant information. These were as follows:

- (a) Light:
 - (i) The transparency of the river water was highly variable and the light available to the algae should theoretically be more accurately measured in the river, rather than using surface light intensity data.
 - (ii) Equivalent daily integrals of available light can result from very different profiles. The identification of the speed of DO response to light would entail further examination of DO profiles together with continuous underwater PAR monitoring. (Assuming no other factors affected the DO profile).
- (b) Biomass: A major disadvantage of algal biomass density estimates was that they only measured a portion of the *Cladophora* assemblage. This community develops, during steady flow periods, to form a complex, in which the active *Cladophora* proportion is only a part. (See section 6.4 and figure 6.9). The influence of flush events on the biomass and composition of the assemblage will depend on the severity

of the flush and the position of the assemblage in the river in relation to the channel topography.

(c) Other Factors:

- (i) Flush events have a variety of effects on the biomass/DO relationship. As well as reducing the assemblage biomass, the flush introduces a large volume of oxygen-saturated water with a high suspended solids load. As the river flow recovers from the impact of the flush, the remaining suspended load can become of increasing importance. The increased turbidity reduces light available to the algae, consequently respiratory processes will dominate the DO profile (depending on the water volume available to satisfy the demand). The suspended load, containing large numbers of heterotrophic bacteria and available nutrients, will also exert an oxygen demand on the river, which can become considerable if still exerted when the river has returned to a low-flow situation.

The suspended load usually also carries a large initial supply of available nutrients. These can be rapidly assimilated by the algae to satisfy any shortages that may have arisen during previous low-flow periods. Nutrients such as phosphorus can be taken up and stored in gross excess of present needs, to be available in case of future shortages. The algae remaining after a flush would usually only be limited by the available light.

- (ii) Planktonic algae are present in the river; however, biomass concentrations ($1-4 \text{ mg m}^{-3}$ Chlorophyll-a) recorded during low-flow periods indicated that they would have little effect on the DO profiles. (See section 6.7.2.3 and Vollenweider, 1969). During these low flow periods there would also be an increased tendency for any phytoplankton to become entrapped in the *Cladophora* proliferations. (Hynes, 1970).

6.7.3.3 Upstream Dissolved Oxygen profiles and the maximum daily Dissolved Oxygen Deficit, 1982/83

The continuation of the studies commenced in the 1981/82 season were somewhat limited by the initial absence of *Cladophora* proliferations. (See section 6.4). However the *Gomphonema* growths meant that large DOD_m s were still observed, notably 3.3 g m^{-3} ($DO = 6.3 \text{ g m}^{-3}$ at 17.5°C) compared to the largest DOD_m of 2.5 g m^{-3} observed in the previous season. However, predictive modelling such as that carried out in section 6.7.3.2 was not attempted in the first part of the season as one of the potentially most important independent variables, algal (*Gomphonema*) biomass, could not be monitored using the established techniques. When *Cladophora* began to re-establish itself in the river during late January 1983, it was possible to restart biomass sampling. Empirical modelling of the DOD_m was carried out in a manner similar to that used for the 1981/82 data. A correlation coefficient matrix, of relevant parameters, is presented in table 6.5. The random nature of the DOD_m - biomass (BM) relationship was apparent ($r=.041$) and illustrated in figure 6.48. The reason for the dramatic difference in the DOD_m - BM relationship between seasons was attributed to the influence of *Gomphonema* during late January and February 1983 (Section 6.4). The *Gomphonema* biomass could not be assessed using the existing sampling technique. High DOD_m s could still be recorded at low *Cladophora* biomass densities because of a high TR due to extensive *Gomphonema* growths. The low DOD_m values observed in figure 6.48 occurred during late February when *Cladophora* biomass density was modest and *Gomphonema* was disappearing from the river.

Many of the variables associated with primary productivity were closely correlated with the DOD_m , MLRA was used to select those variables that were the most parsimonious predictors of the DOD_m variations. Of the productivity variables, TR ($r=0.804$) was chosen, from the two most closely correlated productivity parameters (TR and R_m), to give continuity with the previous season and because its measurement involved less uncertainty than R_m ($r=0.863$). This exercise yielded the results presented in table 6.6.

TABLE 6.5: Correlation coefficient matrix of the maximum daily Dissolved Oxygen Deficits and some river and environmental variables, 1982/83.

(Using only data collected when *Cladophora* biomass was above the detection limit, see appendix 8)
 (Abbreviations explained in table 6.3)

	DOD _m	BM	L _T	L _u	F	T _{max.}	T _{min.}	GP	TR	R _m	P/R
BM	0.041										
L _T	0.167	-0.303									
L _u	0.037	-0.240	0.615								
F	0.308	-0.108	0.262	0.012							
T _{max.}	-0.306	-0.086	0.076	0.385	-0.454						
T _{min.}	-0.365	-0.245	-0.064	0.195	-0.518	0.835					
GP	0.269	0.655	-0.118	-0.041	-0.262	0.093	-0.009				
TR	0.804	0.299	-0.090	-0.008	-0.105	-0.098	-0.156	-0.585			
R _m	0.863	0.130	0.036	-0.121	0.091	-0.335	-0.355	0.350	0.767		
P/R	-0.576	0.433	-0.100	-0.100	-0.187	0.204	0.125	0.404	-0.449	-0.445	
NAP	-0.671	0.194	0.035	-0.024	-0.061	0.189	0.170	0.225	-0.645	-0.575	0.903

KEY:

See table 6.3

L_u = Daily Underwater PAR light (E m⁻² d⁻¹)

T_{min.} = Daily minimum temperature (°C)

T_{max.} = Daily maximum temperature (°C)

TABLE 6.6: The parsimonious regression equation and associated statistics for predicting the maximum daily Dissolved Oxygen Deficit, 1982/83

The parsimonious equation:

$$DOD_m = -0.493 + 0.180 TR - 0.00674 BM + 0.0238 F$$

The importance of predictors:

Predictor	Coefficient	Standard deviation of coefficient	t-ratio = Coeff./St.dev.
	-0.493	0.195	-2.53
TR	0.180	0.0137	13.15
BM	-6.74×10^{-3}	2.46×10^{-3}	-2.74
F	0.0238	4.11×10^{-3}	5.81

r^2 (adjusted for 39 degrees of freedom) = 82.2%

Standard deviation of regression, $s = 0.314$.

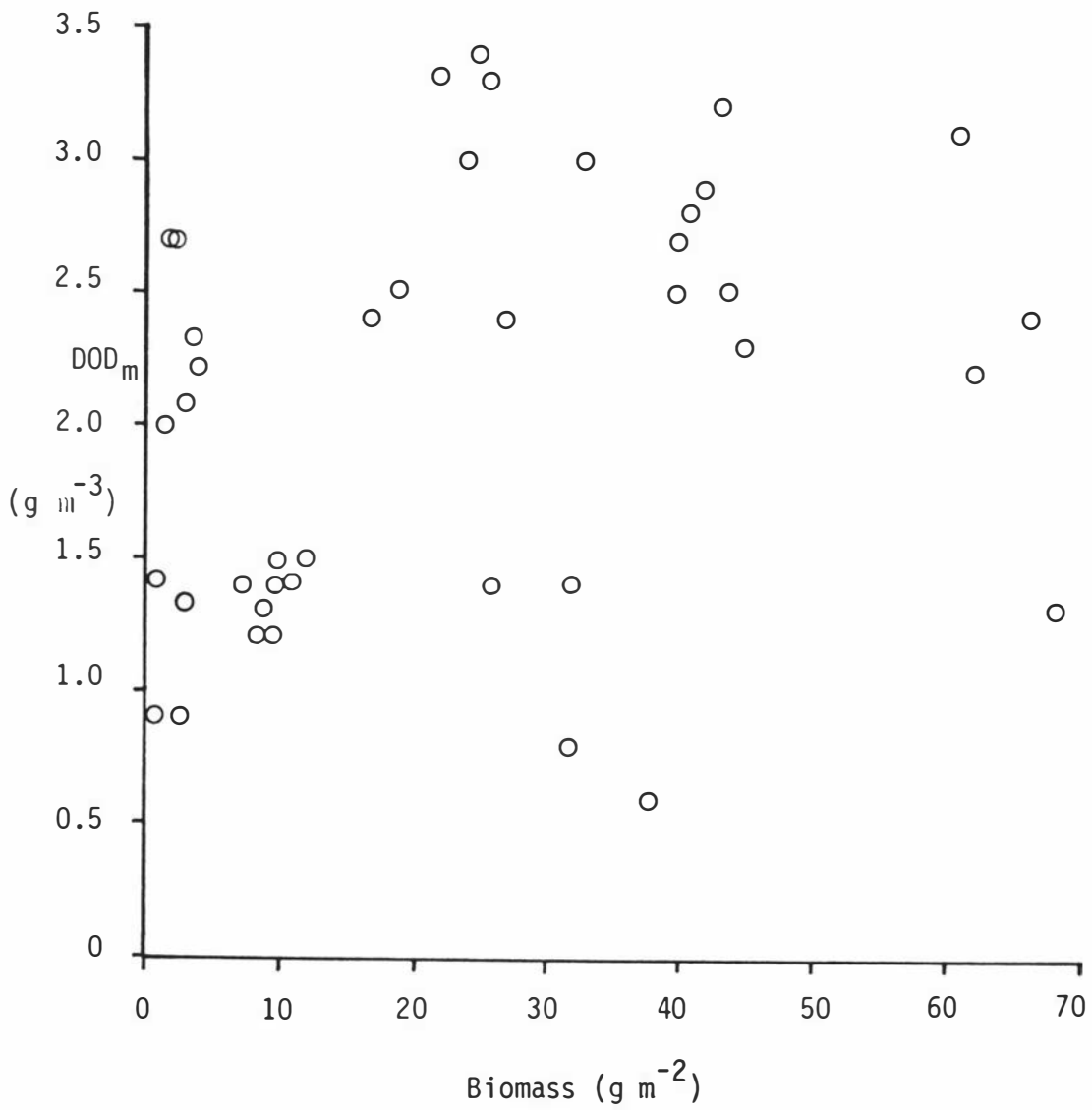
Application range of the regression equation:

TR = Daily Total Respiration (4.23 - 17.95 g O₂ m⁻³ d⁻¹)

BM = Average *Cladophora* site biomass density (1.1 - 68 g DW m⁻²)

F = Daily average river flow (11.0 - 64.5 m³ s⁻¹)

Figure 648 Average site biomass of *Cladophora* and
observed maximum daily dissolved Oxygen Deficit
(DOD_m) 1982/83



Thus, compared to the previous season the predictive ability of the equation has risen to 82.2% with the use of fewer predictors. The standard deviation of the regression equation was very similar; 0.314 as compared with 0.312 in 1981/82. The t-ratios describe the relative importance of the predictors. TR was the most influential followed by F, BM and the constant were much less important. The negative coefficient for BM was attributed to the influence of *Gomphonema* growths during low *Cladophora* biomass densities resulting in a large number of high DOD_m s at low and modest *Cladophora* biomass densities. It is interesting to note that no light predictor featured in this equation. This may be a consequence of the more heterotrophic nature of the *Gomphonema* growths or the complex interaction between shingle extraction and general/localized river turbidity.

In an effort to utilize all the available data from both seasons, the relevant comparable data were pooled, and the resultant correlation coefficient matrix is presented in table 6.7.

TABLE 6.7: Correlation coefficient matrix of the maximum daily Dissolved Oxygen Deficit and associated variables, 1981/82 and 1982/83
(Abbreviations as in tables 6.3 and 6.4)

	DOD_m	BM	L_T	F	TR
BM	0.240				
L_T	0.199	-0.192			
F	0.275	-0.174	0.125		
TR	0.741	0.369	0.008	0.115	
S	0.273	0.091	0.012	0.457	0.596

KEY:

BM = Average *Cladophora* site biomass density ($g\ DW\ m^{-2}$)

L_T = Daily terrestrial light (Langleys d^{-1})

F = Daily average river flow ($m^3\ s^{-1}$)

TR = Daily Total Respiration ($g\ O_2\ m^{-3}\ d^{-1}$)

S = Season coded as 1 for 1981/82, 2 for 1982/83

Both seasons data were then empirically modelled to produce one parsimonious equation that could be used for both seasons. This equation, together with its relevant statistics is presented in table 6.8 below:-

TABLE 6.8: The parsimonious regression equation and associated statistics for predicting the maximum daily Dissolved Oxygen Deficit, 1981/82 and 1982/83

The parsimonious equation:

$$DOD_m = 0.495 + 6.08 \times 10^{-4} L_T + 0.0227 F + 0.168 TR - 0.629 S$$

The importance of predictors:

Predictor	Coefficient	Standard deviation of coefficient	t-ratio coeff./St. dev.
	0.495	0.156	3.17
L_T	6.08×10^{-4}	2.32×10^{-4}	2.63
F	0.0227	4.17×10^{-4}	5.43
TR	0.168	0.0125	13.46
S	-0.629	0.107	-5.88

r^2 (adjusted for 83 degrees of freedom) = 71.5%

Standard deviation of regression, $s = 0.351$

S = season, coded 1 for 1981/82, 2 for 1982/83

Application range of the regression equation.

	Season 1	Season 2
L_T = Daily terrestrial light (Langleys d^{-1})	36-723,	124-731
F = Average daily river flow ($m^3 s^{-1}$)	11.9-33.8,	11.0-64.5
TR = Daily Total Respiration ($g O_2 m^{-3} d^{-1}$)	1.12-9.93,	4.23-17.95

Compared to tables 6.6 and 6.7 presented for the individual seasons, the predictive ability of this regression is still usefully high. One noticeable feature of this equation was that it contained no *Cladophora* biomass term. Instead, the TR acts as a biomass respiration descriptor for the total river community. An

increase in flow produces higher DOD_m , due to an increased suspended load in the river bringing in large numbers of heterotrophic bacteria and nutrients (Sections 5.2 and 6.4). Light increases will raise the total metabolic activity of phototrophs, the respiration portion of this being most noticeable after sunset. The elevated respiration rate can continue on to the following day when, if not 'balanced' by high photosynthetic rates, it may produce very large DOD_m s. This type of time lag may be responsible for some of the unexplained variation of the DOD_m . (Sokal & Rolf, 1973).

The importance of predictors listed in table 6.8 illustrates the contribution each term in the regression equation makes to the DOD_m . Thus, within the application range the TR can have the largest contribution to the DOD_m , followed by the Season and the F. The L_T and the Constant will usually be less important than the other three variables in predicting the total DOD_m .

It was important to establish the accuracy of the total regression equation for each season. This was achieved by comparing the residuals remaining after applying the regression equation to each season. The results are presented below:-

TABLE 6.9 : A t-test comparison of the residuals from the regression equation for each season

Season	N	Mean	Standard Deviation	Standard Error
1981/82	45	0.000	0.374	0.056
1982/83	43	0.000	0.312	0.048

t-test $t=0.00$, $P=1.00$, 84.5 degrees of freedom

Therefore, there was no significant differences between the samples

Thus, the regression equation was applicable, with equal accuracy, to each year. In order to apply the equation in subsequent seasons it would be necessary to identify the periphyton community either as type (I): typical of the 1981/82 season (i.e. a *Cladophora*-dominated assemblage, throughout the season) or type (II): like

the one that occurred in the 1982/83 season (i.e. with *Gomphonema* present for a large part of the season). There would then be two predictive equations to choose from :-

Type (I)

$$DOD_m = 6.08 \times 10^{-4} L_T + 0.0227 F + 0.168 TR - 0.134 \quad (6.4)$$

Type (II)

$$DOD_m = 6.08 \times 10^{-4} L_T + 0.0227 F + 0.168 TR - 0.763 \quad (6.5)$$

At this stage the assumption must be made that these will be the only significant periphyton communities that will develop in the Manawatu River. In practise, the data from future seasons should be continually compared with the regression equation(s), and if necessary the equation(s) adjusted to cater for different periphyton communities.

The relationship and contribution of each predictor variable to the DOD_m are presented in figures 6.49 and 6.50. The scatter plots show the observed relationship between the data collected for each variable, during specific seasons, and the DOD_m . These points therefore correspond to the data used in the individual season correlation coefficient matrices (Tables 6.3 and 6.5). The change in the 1981/82 data (Figure 6.49) from the highly correlated TR ($r=0.630$) to F ($r= -0.174$) and L_T ($r = 0.262$) can be seen in the scatter and direction of the points. The weak negative correlation coefficient of F was forced into a positive contribution to the total regression equation because of the influence of other predictors (As in table 6.4). The scatter plots for the 1982/83 season also illustrate the differences between the highly correlated TR ($r = 0.804$), and F ($r = 0.308$) and L_T ($r = 0.167$). The regression contribution lines (RCLs) were constructed using the regression equation detailed in table 6.8. Average seasonal values were used for two variables and thus the effect of varying the third using data from within the application range, could be observed. The gradient of the RCL indicates the relative importance of each predictor. The differential importance of predictors was especially apparent in figure 6.50, depicting the 1982/83 season. The gradient of the TR RCL was the steepest followed by the F RCL and then

FIGURE 6.49: The relationship between predictors and the maximum daily Dissolved Oxygen Deficit, (DOD_m) and the contribution each predictor makes to the total DOD_m , 1981/82

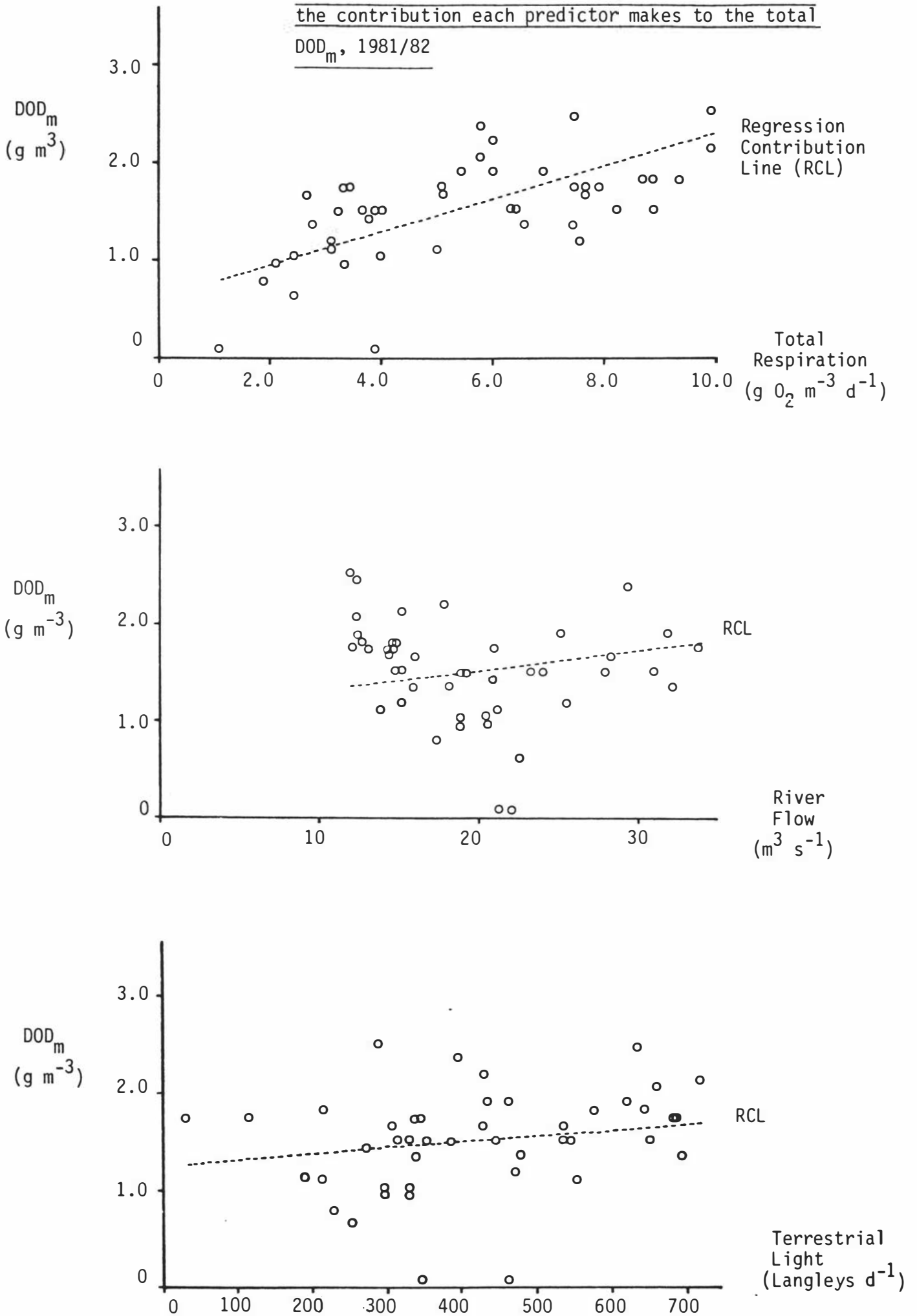
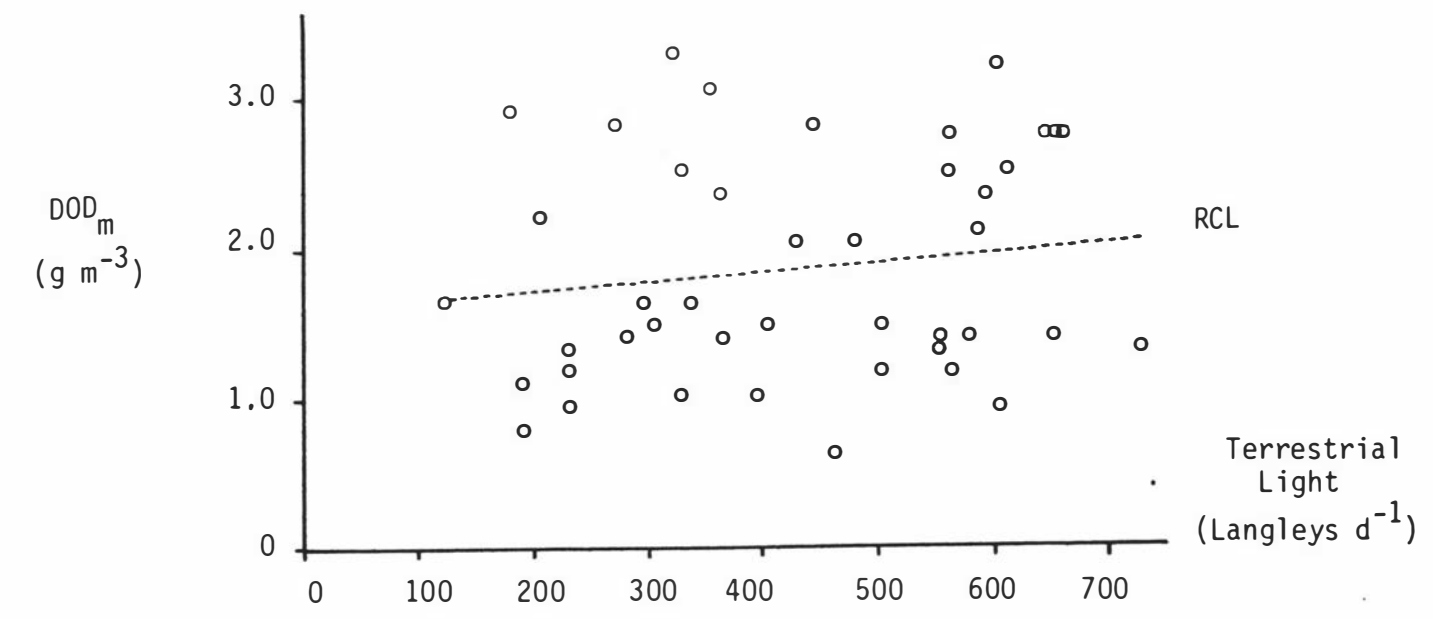
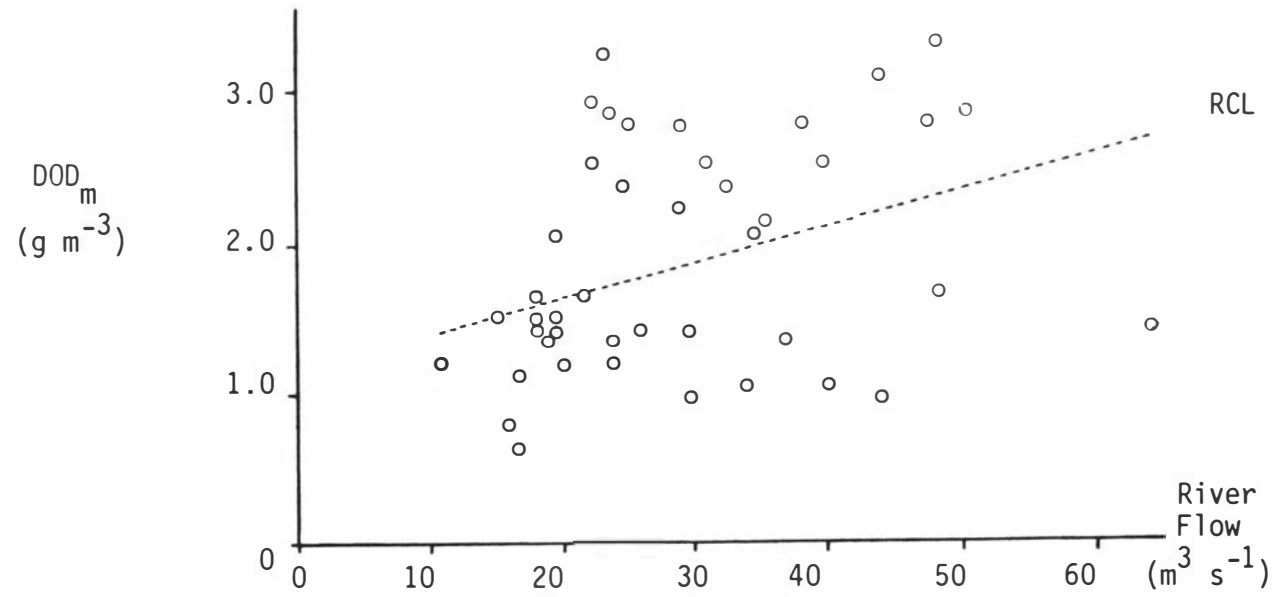
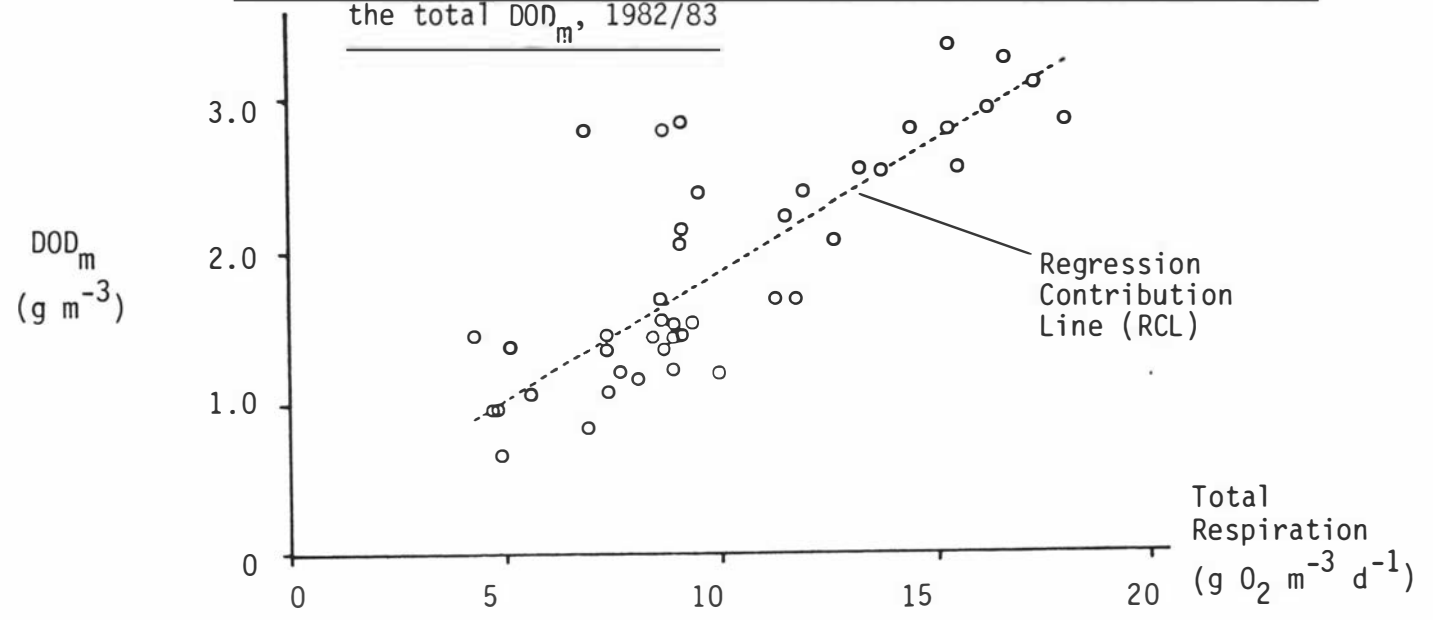


FIGURE 6.50: The relationship between predictors and the maximum daily Dissolved Oxygen Deficit (DOD_m), and the contribution each predictor makes to the total DOD_m , 1982/83



the light RCL. The same basic pattern can be seen in figure 6.49 for the 1981/82 data. Interseason comparisons are difficult because the ranges and averages for all variables, except light were significantly different. (Table 6.8, appendices 7 & 8)

To further illustrate the above points, data from each year (typical 'highs and lows') are presented in table 6.9. The actual contribution each predictor makes to the eventual total predicted DOD_m under various combinations can be seen to vary considerably. The data presented in table 6.9 demonstrate one important difference between seasons; the 1982/83 season had typically much higher TR values, and consequently often higher DOD_m s than 1981/82. (Average TRs 1981/82 = $5.4 \text{ g O}_2 \text{ m}^{-3} \text{ d}^{-1}$, 1982/83 = $10.0 \text{ g O}_2 \text{ m}^{-3} \text{ d}^{-1}$) (Figures 6.49 and 6.50). These were attributed to the *Gomphonema* assemblages having a relatively larger respiratory component than the 'cleaner' *Cladophora* assemblages. There were also more examples of monitoring primary productivity during higher in 1982/83 than in 1981/82. (Average flow for the 1981/82 data = $19.6 \text{ m}^3 \text{ s}^{-1}$, 1982/83 = $29.1 \text{ m}^3 \text{ s}^{-1}$). Thus, during 1982/83 there was most likely higher levels of heterotrophic activity present in the suspended load. The importance of TR and the absence of a BM term in the regression equation illustrates how DOD_m s may be predicted regardless of the periphyton assemblage composition.

Table 6.10 demonstrates the approach that would be taken when predicting DOD_m s in the Manawatu River. For a type II season (1982/83), the worst case combination depicted, predicts a $DOD_m = 3.26 \pm 0.70 \text{ g m}^{-3}$. The predicted DOD_m s are very similar to those observed (See figures 6.49 and 6.50, and appendices 7 & 8) in the field. The regression equation can be a useful tool for water quality management in the Manawatu River. The general approach may be adopted for other water quality parameters, such as pH_{max} , that are affected by the metabolic activities of periphyton assemblages.

While the specific equation derived here would not be applicable to another river, the approach outlined would be. The river and environmental variables that have been demonstrated to be important in the Manawatu River could be monitored. The light data would be available from meteorological stations. The flow monitoring

TABLE 6.10 Data illustrating the influence of each term in the regression equation for 1981/82 and 1982/83
(Equation 6.3)

1981/82							
Light (Terrestrial)		Flow		Total Respiration		Constant + Season Contributed DOD _m (g m ⁻³)	Predicted Total DOD _m (g m ⁻³)
Value (Langleys d ⁻¹)	Contributed DOD _m (g m ⁻³)	Value (m ³ s ⁻¹)	Contributed DOD _m (g m ⁻³)	Value (g O ₂ m ⁻³ d ⁻¹)	Contributed DOD _m (g m ⁻³)		
100	0.06	20	0.45	2	0.34)	-0.134	0.72
100	0.06	20	0.45	5	0.84)		1.22
600	0.36	20	0.45	2	0.34)		1.02
600	0.36	20	0.45	5	0.84)		1.52
600	0.36	50	1.14	2	0.34)		1.71
600	0.36	50	1.14	5	0.84)		2.21
1982/83							
100	0.06	20	0.45	5	0.84)	-0.763	0.59
100	0.06	20	0.45	15	2.52)		2.27
600	0.36	20	0.45	5	0.84)		0.89
600	0.36	20	0.45	15	2.52)		2.57
600	0.36	50	1.14	5	0.84)		1.58
600	0.36	50	1.14	15	2.52)		3.26

95% confidence limits for the predicted DOD_m 2 x standard deviation
= ± 0.70 g m⁻³

equipment would most likely already be in place if the river is near a major urban area. The Total Respiration monitoring may require extra equipment but most Regional Water Boards have DO monitoring/recording equipment and access to computer facilities. Direct input to a computer, of the productivity data via a data logger would greatly increase the efficiency of the whole operation. It would also be prudent to monitor other variables that appear important in specific rivers. Eventually a unique predictive equation could then be developed to enable rapid assessment of any periphyton assemblage impact on the assimilative capacity of a river.

6.8 pH and ammonia

Despite some difficulty in assembling the necessary equipment for the continuous monitoring of pH, a limited number of successful surveys were accomplished during the 1981/82 season with the aid of equipment kindly lent by B. Gilliland, Assistant Water Resources Officer, M.R.W.B. (Figures 6.51 and 6.52). These data illustrate the pH fluctuations that can occur during periods of *Cladophora* growth (Figure 6.7), a peak of pH = 8.8 being seen at site W which is situated downstream of the Palmerston North City discharge, and upstream of the Dairy Company and Freezing Works discharges. Similar peaks were observed in 'spot checks' at sites T and D. The pH fluctuations at site K, where peak ammonia concentrations have been identified, were often observed to be less than the upstream photosynthetically-induced fluctuations. (Sections 2.3.2 and 5.6). This was a result of increased in-river respiration, caused by the organic discharges which imparted sufficient quantities of CO₂ to alter the pH-CO₂-HCO₃⁻-CO₃²⁻ relationship.

The new treatment facilities of the Longburn Freezing Co. (the discharger directly upstream of site K) will involve anaerobic lagoons. These can be expected to increase the ammonia concentration of the discharge (Cooper, 1982). Thus, there is cause for concern if the anticipated reductions in organic effluent to the river also result in larger downstream ammonia concentrations and pH fluctuations.

The results of some ammonia surveys are shown in figures 6.53 and 6.54. The upstream ammonia concentrations are low compared to those seen at site K and agree with concentrations seen in the 1980/1981 season (Section 5.6). The general pattern at site K was that the peak ammonia concentration occurred during the late evening hours, which would reinforce the contention that the Freezing Company is the main contributor to the peak concentrations. (Cooke *et al*, 1980; Currie, 1977).

By the time ammonia concentration began to peak, the pH levels were usually decreasing. (Figures 6.51 and 6.54). Thus, peak pH values

FIG.6.51 pH fluctuations at various sites during 1981/82.

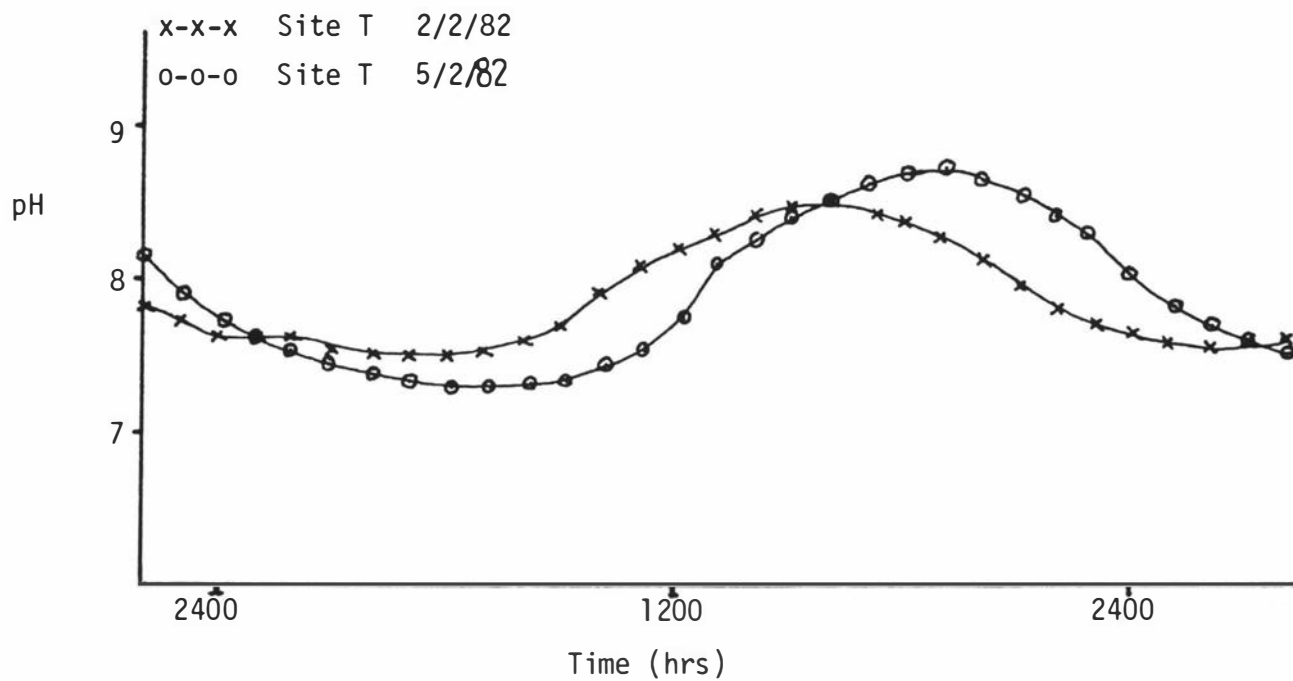


Figure 6.52 pH fluctuations at downstream sites during 1981/82.

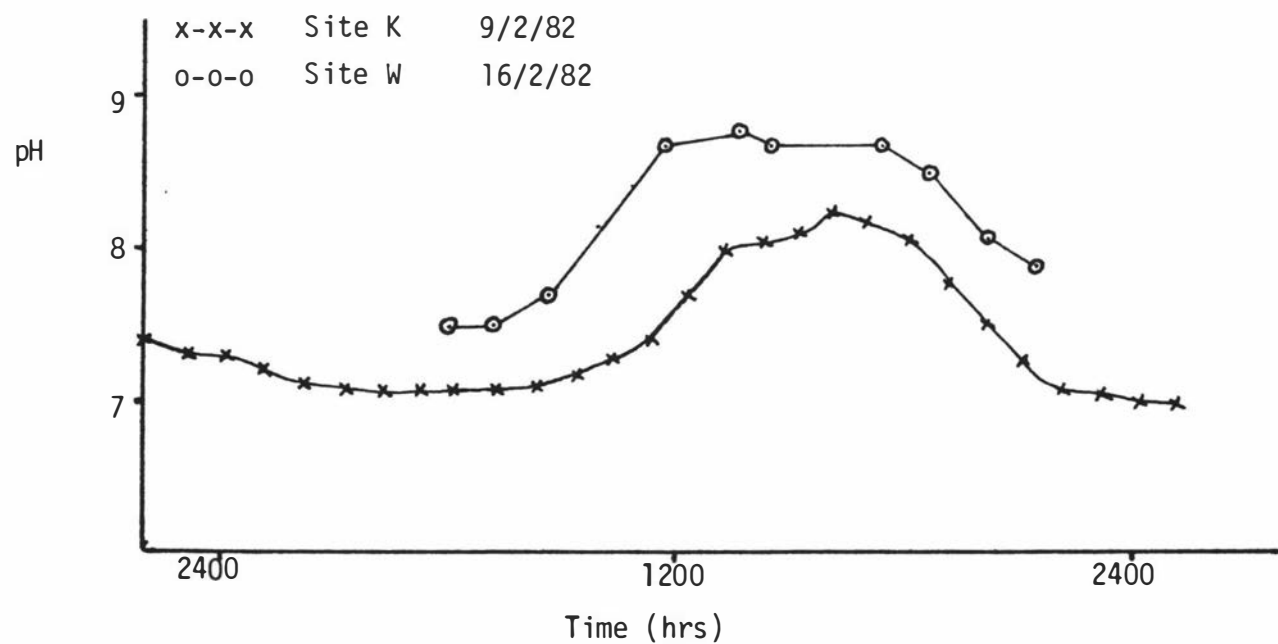


Figure 6.53 Total Ammonia fluctuations at sites T,D & K , 5-6/2/82.

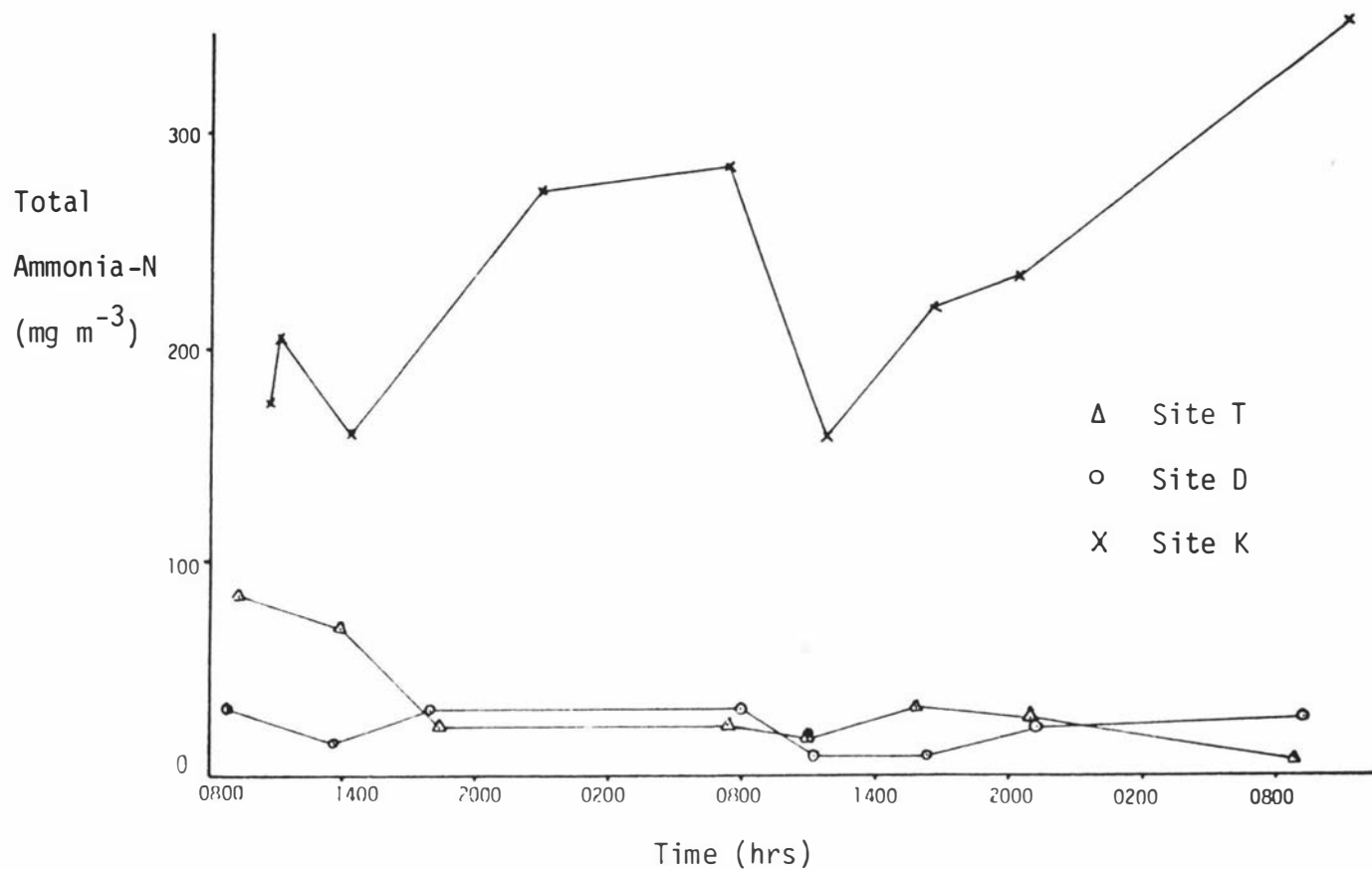
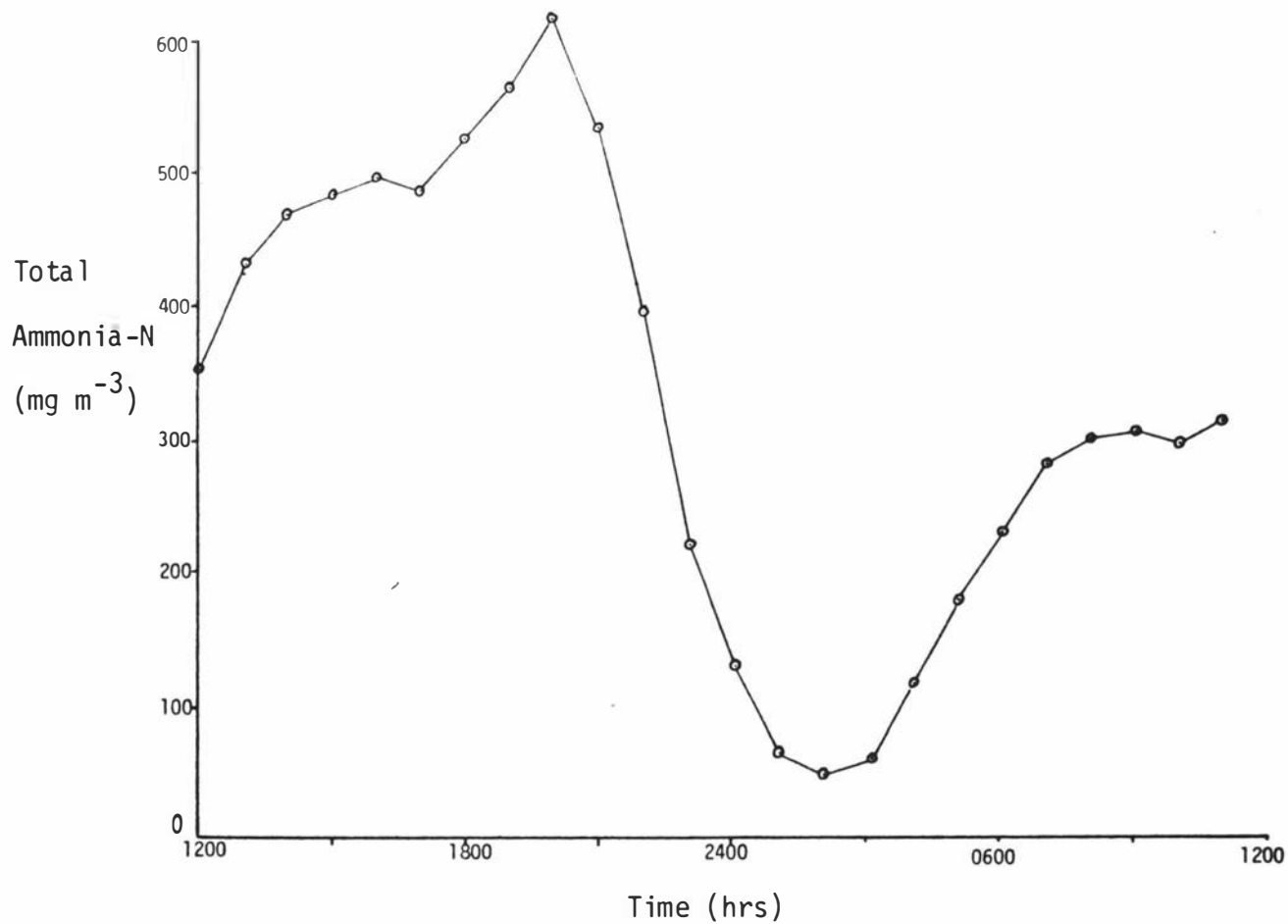


Figure 6.54 Total Ammonia fluctuations at site K , 12-13/2/82.



seen at site K were unlikely to coincide with the peak ammonia concentration. However, this relationship was investigated with during the 1982/83 season with equipment capable of continuous monitoring of pH and ammonia.

The peaks recorded in figures 6.53 and 6.54 are relatively low in comparison to concentrations recorded later in the season during late afternoon when the pH fluctuations are most likely to peak (Table 6.11).

TABLE 6.11: Ammonia concentrations and other relevant variables at site K during March 1982

Site Parameters	Sampling date (day/month) 1982					
	4/3	15/3	16/3	18/3	19/3	22/3
Time (hrs)	1800	1700	1700	1800	1800	1500
Temp. (°C)	17.0	17.0	18.0	17.0	18.0	18.5
pH	7.4	7.5	7.9	7.6	7.5	7.8
Total Ammonia-N(g m ⁻³)	0.30	1.30	1.25	1.06	1.19	1.20

Concern exists about those ammonia concentrations recorded at high pH values. On 16 March, the threshold desirable ammonia-N concentration was approximately 1.7 g m⁻³ and on 22 March, approximately 1.9 g m⁻³ (Section 2.3.2.1) (Black, 1979; Train, 1979). While the observed values are below the recommended criterion the differences are small enough to warrant concern and further investigation. The results from the 1981/82 season indicated that further work was needed in three specific areas to establish:

- (a) The magnitude of, and influence of environmental factors on, pH fluctuations upstream of the discharges.
- (b) The consistency of the reduced pH fluctuations at site K.
- (c) The timing of the pH and ammonia peaks at site K.

During the 1982/83 season, these aspects were closely investigated. The magnitude of the pH fluctuation was seen to increase as the *Cladophora* biomass developed during late January and February 1983

(Figure 6.55). Some reductions in peak values recorded during proliferations were seen to be linked to days that experienced reduced insolation. Modest alkalinities in the range 65-85 g $(\text{CaCO}_3)\text{m}^{-3}$ (1.3-1.7 Eq m^{-3}) were observed during weekly sampling in the 1982/83 season.

Rigorous statistical analysis of pH fluctuations and river and environmental parameters suffered from both the theoretical considerations discussed in the previous two sections, and the few data points available. After the mid-February flush the *Cladophora* recovery (Section 6.4) was indicated by the expansion of the pH fluctuations. The maximum recorded pH was 9.2 and during periods of algal proliferation the peak was regularly greater than 8.5.

Concern centred about whether very alkaline waters travelled down to, were maintained, and coincided with the high ammonia concentrations associated with the organic discharges. The differences between the pH profiles observed above the discharges and those observed at the area of maximum ammonia concentration is illustrated by the data presented in figure 6.56. The maximum pH at site M occurred between 1500 and 2100 hrs, and apart from some minor reversals at low pH the pattern was one of consistently reduced peak pH levels at site K compared to site D. The extent of the difference varied considerably, even during the five-day 'run' illustrated in figure 6.56. The timing and magnitude of the three major dischargers, will have large affects on this intersite pH difference.

Experience from the 1980/81 and 1981/82 seasons led to the expectation of peak ammonia concentrations during the late afternoon/evening (1500-2100hrs). However, during the 1982/83 season when some automated continuous sampling was employed, this pattern did not appear to be consistent. Data presented in figures 6.57-6.59 and especially figure 6.58 indicate that the timing and magnitude of the ammonia peak(s) can be highly variable. The ammonia concentrations were never greater than the criterion discussed in section 2.3.2.1. The difference between the peak river ammonia concentration and the recommended value is illustrated in table 6.12. While it is reassuring to note the absence of hazardous (as defined by the criterion set out in section 2.3.2.1 and figure 2.4) ammonia concentrations during

FIGURE 6.55: Daily pH fluctuations during 1982/83

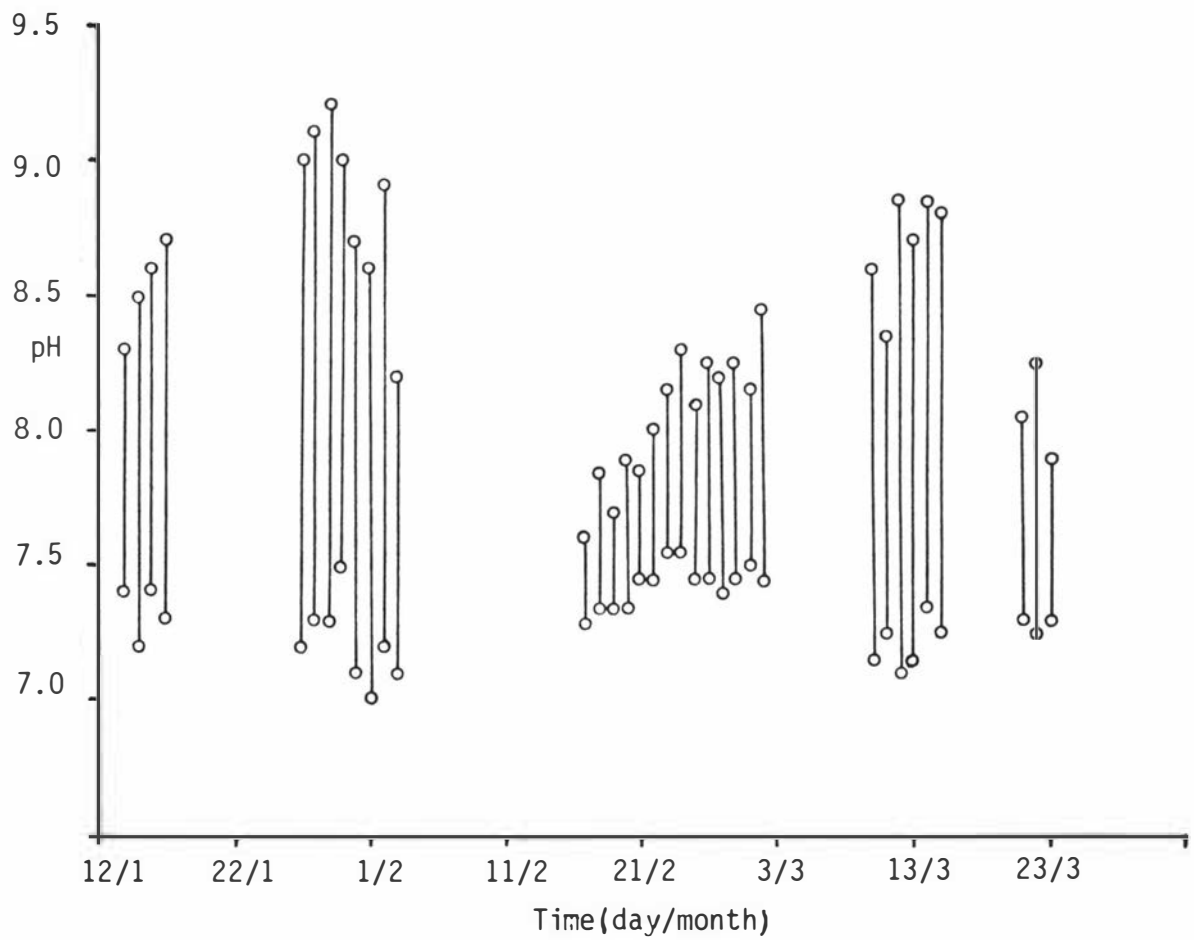
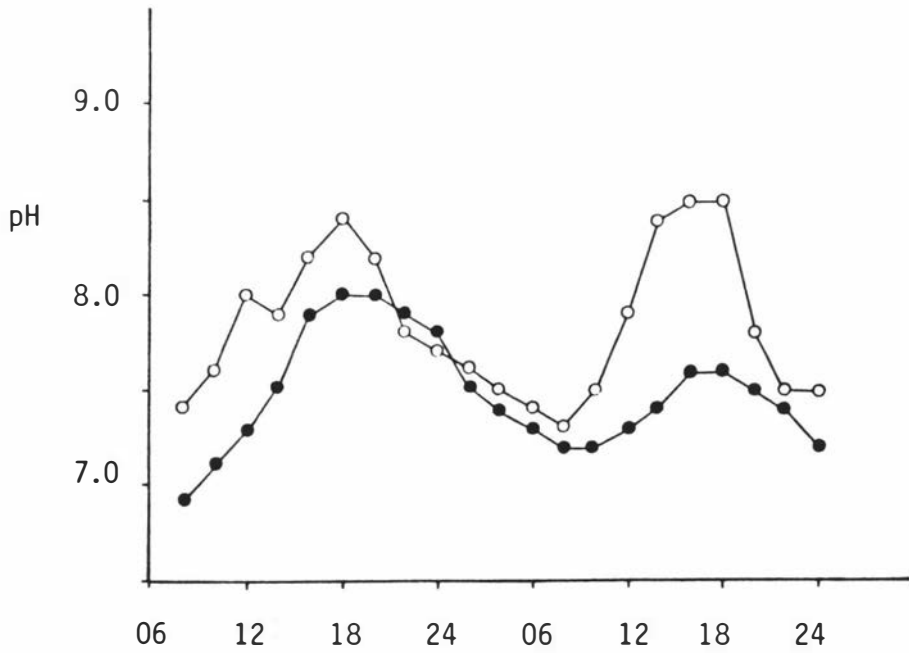
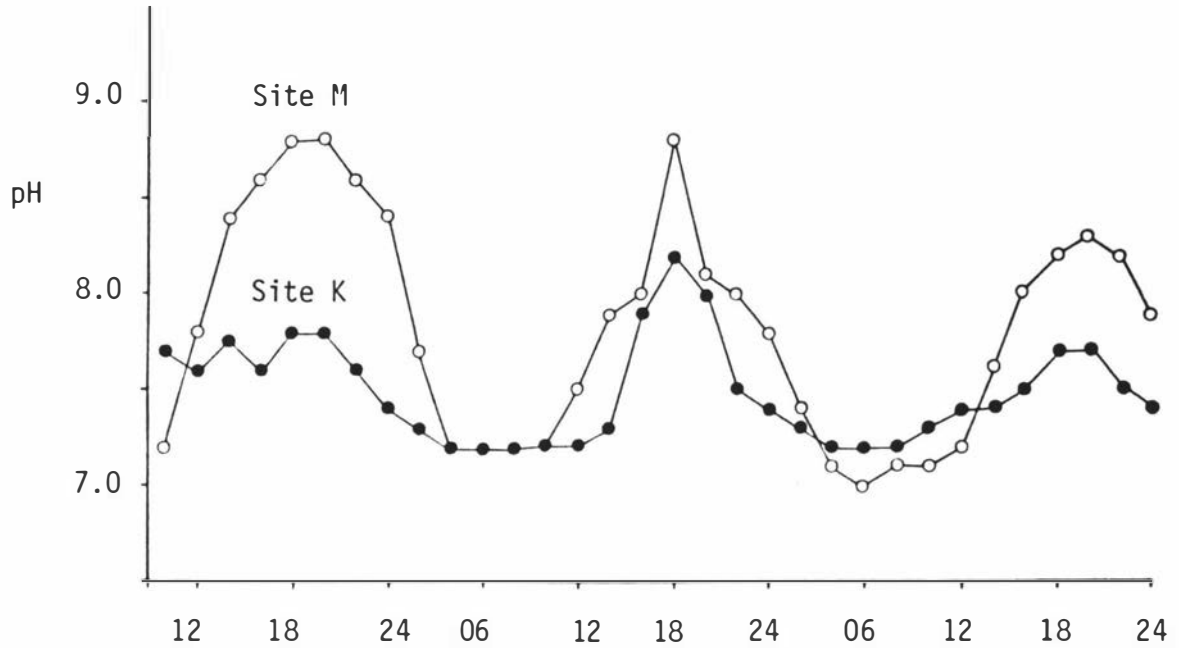


Figure:6.56 pH fluctuations at sites M and K during 2/2/83 - 6/2/83



Time (hrs)

Figure 6.57: pH and ammonia fluctuations at sites M and K

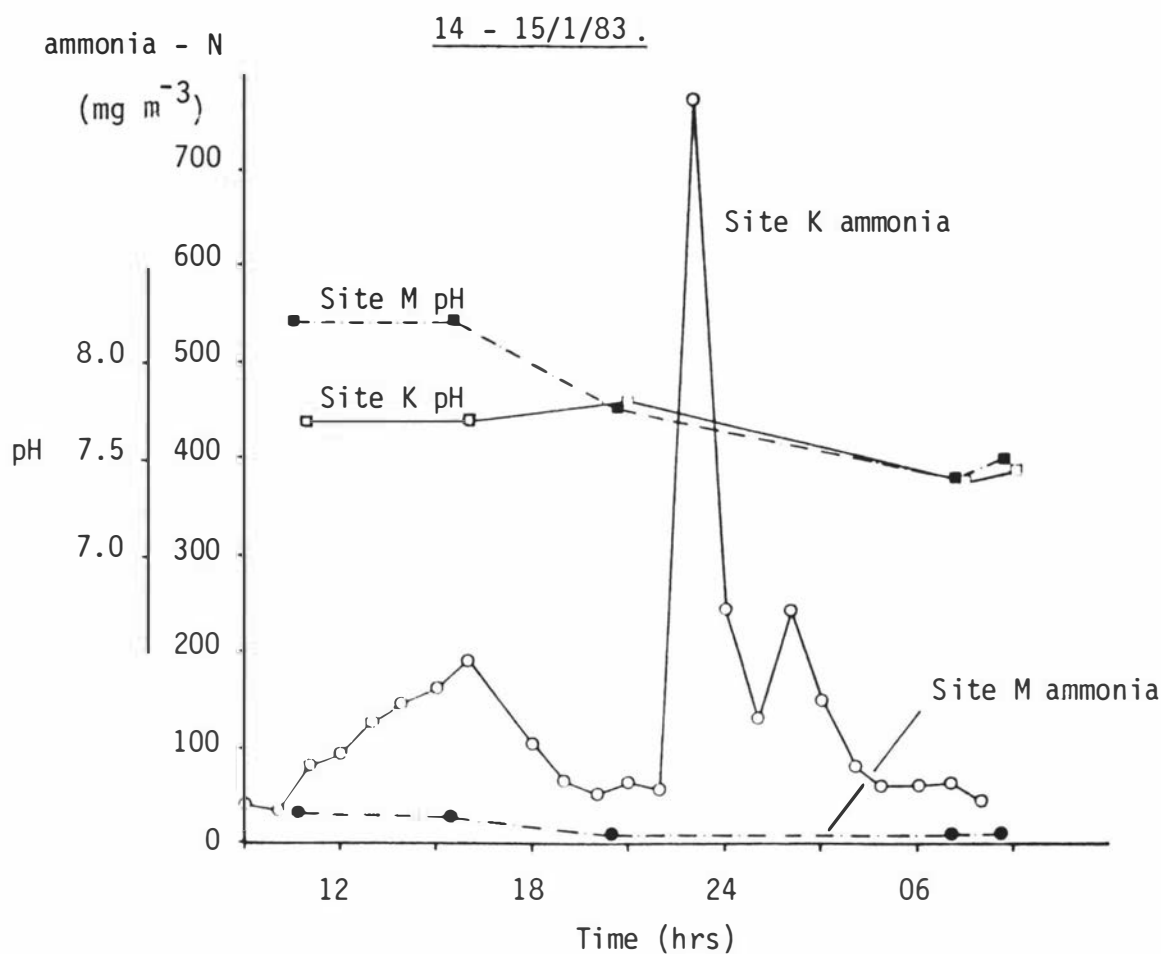


Figure 6.58: pH and ammonia fluctuations at site K 27-28/1/83.

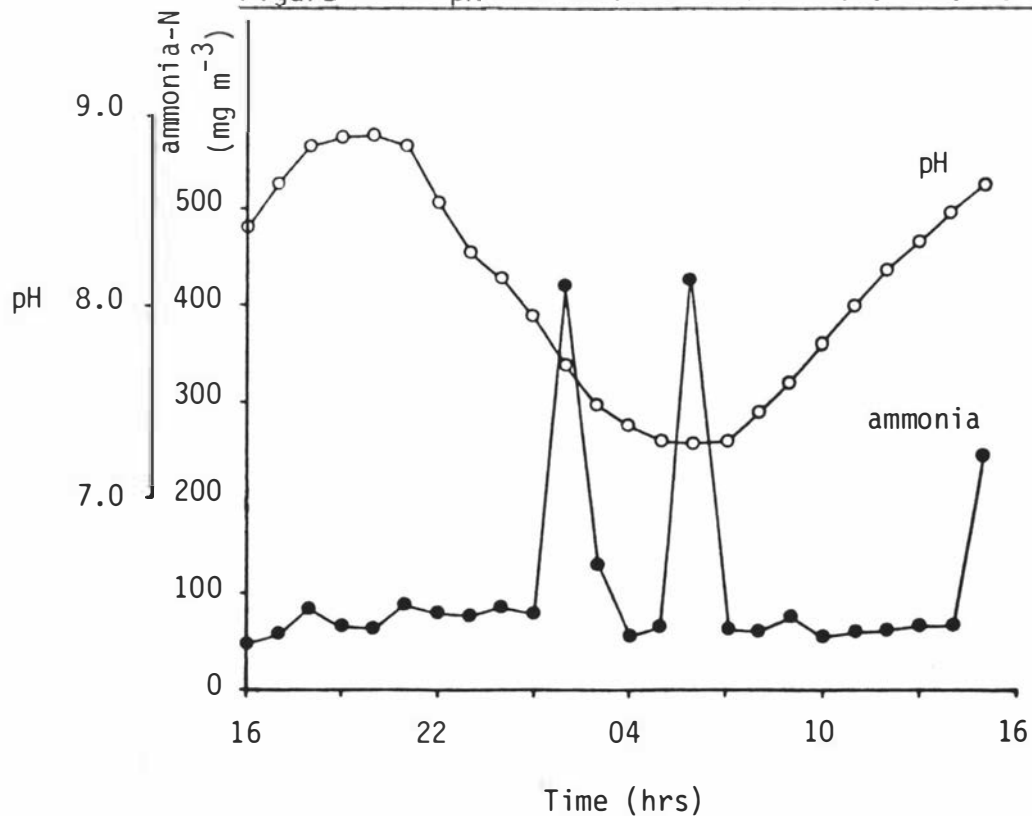
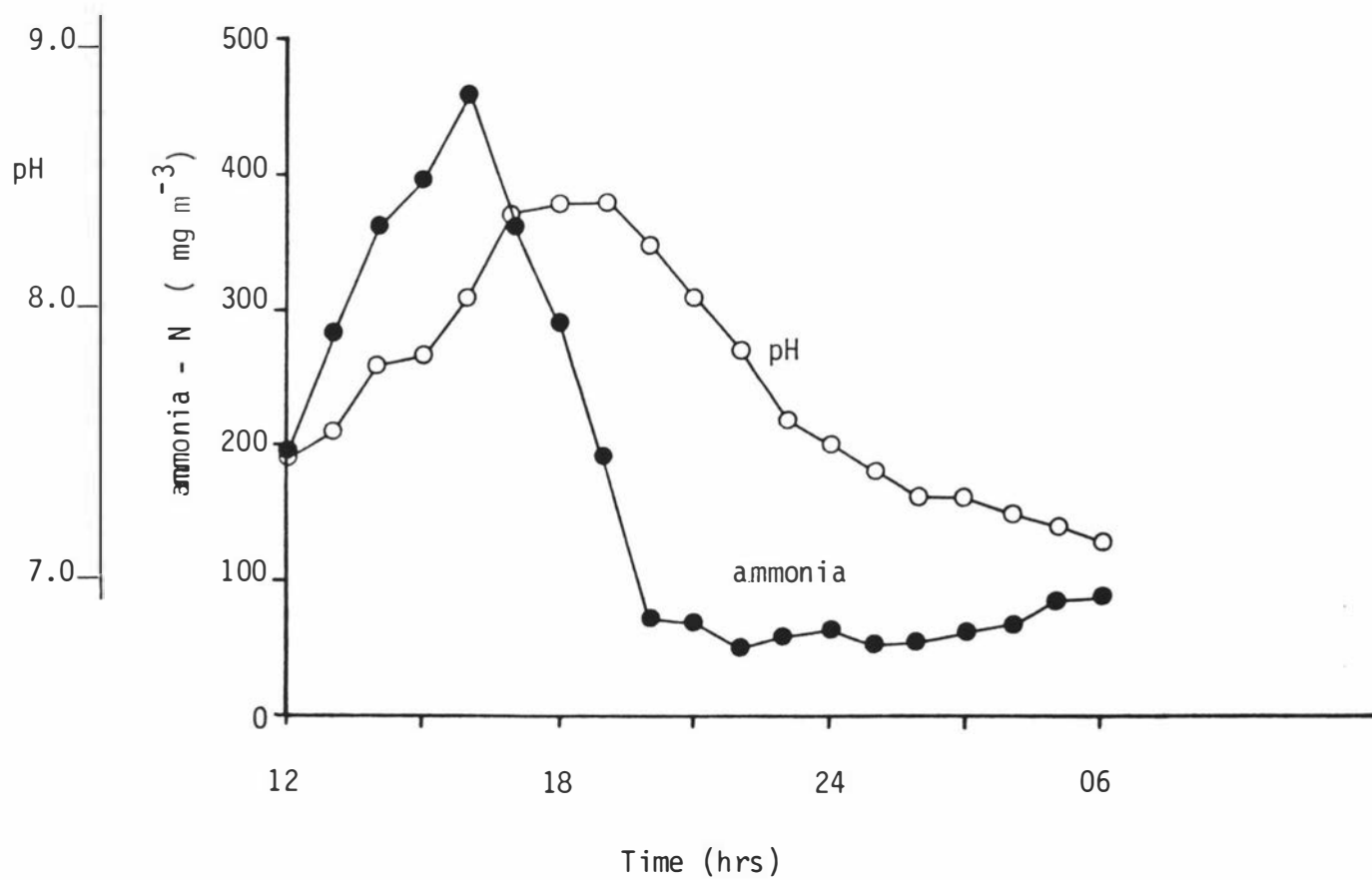


Figure 6.59: pH and ammonia fluctuations at site K, 1-2/2/83.



the surveys, it should also be apparent from figures 6.58 and 6.59 that high pH values have been recorded at site K and the possibility

TABLE 6.12: A comparison of some observed ammonia concentrations with the recommended values at ambient pH and 20°C

Date	28/1/83	28/1/83	14/1/83	1/2/82	1/2/83	1/2/83	27/1/83
River pH	7.70	7.30	7.70	8.05	8.35	8.40	8.90
Observed total ammonia-N (g m^{-3})	0.420	0.423	0.775	0.460	0.365	0.290	0.070
Recommended total ammonia-N (g m^{-3})	3.00	4.60	3.00	2.00	0.84	0.80	0.30

of such maxima coinciding with peak ammonia concentrations should not be discounted. The respiration of the organic effluent components may consistently reduce pH peaks concurrent with the rise of ammonia concentration. However, the reliability of such a relationship will be obscured by the often apparently erratic discharge patterns of the Freezing Co., and the activity of upstream dischargers.

6.9 Summary of results from the 1981/82 and 1982/83 seasons

6.9.1 Light and temperature conditions

The combination, during spring, of increased daily available insolation, longer photoperiod and warmer waters were believed to be the primary mechanisms responsible for initiating *Cladophora* zoosporogenesis and akinete growth. Over both seasons, light and temperature were generally similar. However, relatively short term departures from seasonal trends had important consequences for the type of periphyton community that developed. The dominance by *Gomphonema* growths during January 1983 was partly attributed to the generally cool river waters observed after the 26 December spate. (See section 6.9.2).

The photo- or heterotrophic nature of a periphyton community can be influenced by light and temperature. Given one periphyton community, largely dominated by phototrophs, it may respond as a phototropic community during periods of high insolation, or conversely if river temperatures are high and daytime light availability is reduced, the periphyton community would then give a heterotrophic response. The daily terrestrial light has been shown to constitute a useful predictor for the total daily DOD_m . Conceptually some other light parameter such as a portion of the daily intergral or the previous day's light climate should explain more of the daily DOD_m . However, the identification of the most influential light parameter will always be hampered by the localized and general fluctuations of river transparency.

6.9.2 *Cladophora* biomass density and distribution

Summer proliferations of *Cladophora* were initiated by zoosporogenesis from over-wintering filaments in protected tributaries and germinating akinetes found in both the main river and many of its tributaries. The establishment of zoospores and the development of proliferations required relatively stable flow conditions. The numerous large flushes during December 1982 were the probable reason for the failure of *Cladophora* proliferations to develop until late January. These flushes, and the cool waters that followed in January were also probably

responsible for the *Gomphonema* growths that dominated until late January and didn't disappear from the river until late February/March.

The frequency and magnitude of flush events were important factors governing the composition of established periphyton assemblages. The position of a periphyton community in relation to river current velocity and the force of flush events determined its photo- heterotrophic nature. The stability of the river substrate to flush events was also an important factor in determining both the quantity and quality of periphyton assemblages.

6.9.3 River Nutrients

P concentration was influenced by flush events, with heavy precipitation usually bringing large amounts of readily available P into the river. During steady low flow periods in late January/February, 1983 when *Cladophora* proliferations were extensive, DRP was observed to fall to 4 mg P m^{-3} . Concurrently, P NATs indicated that there was growth rate limitation by P. During the previous season TP concentrations of approximately $14\text{-}16 \text{ mg P m}^{-3}$ were also associated with P NAT responses that indicated growth rate limitation by P.

N concentration was not linked to the flush events in the same fashion as P, although during large flush events in the 1981/82 season both TN and $\text{NO}_3 - \text{N}$ usually increased dramatically (TN up to 2000 mg N m^{-3} from an average of approximately 300 mg N m^{-3} , $\text{NO}_3 - \text{N}$ was usually 50-70% of the TN). In the 1982/83 season the more readily available DIN ($\text{NO}_3 - \text{N}$, $\text{NO}_2 - \text{N}$ and $\text{NH}_3 - \text{N}$) were monitored but at no time did the concentration drop low enough to give a response of N limitation in the N NATs.

6.9.4 Nutrient Availability Tests

During both seasons, the P NATs often indicated that during steady low flow conditions the growth rate of *Cladophora* proliferations was P limited. The degree of limitation increased from sites T

and D to site M. The TTP, EP and APA NATs usually agreed with one another. In some circumstances interpretation was difficult because of the varying response times of the P NATs. The PUR test did not always provide as conclusive evidence for or against P-limitation occurring as the other P NATs did.

The N NATs never gave conclusive evidence for any N limitation of the *Cladophora's* growth rate.

6.9.5 Primary Productivity and Dissolved Oxygen fluctuations

The Primary Productivity of the Manawatu River, during the summer steady flow periods, was observed to be highly variable. Depending on the quantity and quality of periphyton biomass, environmental and river characteristics, the NAP indicated that the river could act as a photo- or heterotrophic community. (e.g. 12 to -13 g O₂ m⁻² d⁻¹). The insolation and river conditions associated with flush events were the main factors that produced the negative NAP values more usually associated with an organically polluted river. The moderately high GP values (e.g. 12 g O₂ m⁻³ d⁻¹) observed were indicative of a river with mild eutrophication.

The DO fluctuations were often quite dramatic, peaks of greater than 12.2 g m⁻³ and troughs of less than 7.0 g m⁻³ were frequently observed. The latter were usually concurrent with high DODs, and often meant that the river's assimilative capacity was reduced.

6.9.6 Maximum daily Dissolved Oxygen Deficits

The daily DOD_ms were examined together with various periphyton, environmental and river parameters, using multiple linear regression analysis to construct an empirical parsimonious regression equation that would explain the variation in the DOD_m. Data from the 1981/82 and 1982/83 seasons were pooled and the resultant predictive equation isolated the TR as the most influential contributor to the final predicted DOD_m followed by the season and the river flow, the least influential predictors were the terrestrial light and the regression constant. This equation accounted for 72% of the DOD_m variation and had 95%

confidence limits of approximately 0.7 g m^{-3} (2 x Standard Deviation).

6.9.7 pH and ammonia

Upstream daily pH fluctuations were often observed to be quite dramatic, frequently rising from around 7.2 to above 9.0. In the river reaches, downstream of the discharges, that experienced high ammonia concentrations, upstream pH fluctuations were reduced due to the respiratory input of CO_2 associated with bacterial degradation of the organic effluents. Although toxic concentrations of ammonia were not observed, temporal variations, and peak concentrations were erratic and the absence of toxic situations may well have been simply fortuitous. The implementation of new treatment facilities (designed to reduce the organic loading) may well increase the ammonia concentrations and pH peaks in the downstream reaches.

CHAPTER 7

THE PRACTICALITIES OF LOTIC *CLADOPHORA* CONTROL STRATEGIES

7. THE PRACTICALITIES OF LOTIC *CLADOPHORA* CONTROL STRATEGIES

This chapter will briefly consider the various strategies that could be utilized to reduce the *Cladophora* (and other periphyton) biomass during periods of proliferation. The implicit rationale is that if the total biomass can be reduced then the water quality and river use problems will subsequently be ameliorated (Freeman & McFarlane, 1982).

7.1 Chemical control

An extremely wide array of compounds have been used in the past for controlling algal growths. However, tests overseas have shown *Cladophora* to be particularly hardy when exposed to classic algicides like copper sulphate. Other aquatic life usually suffers before any harm befalls *Cladophora* (Palmer, 1980; Whitton, 1967). However, organic herbicides that have selective toxicity, short half-lives and harmless residues, together with modern application techniques such as gel-impregnation that allow aquatic herbicides to be applied directly to specific sites, may well prove to be efficient and environmentally acceptable for the control of lotic periphyton proliferations. (Clayton & Tanner, 1983).

7.2 Physical removal

The algal biomass could be physically removed when it reaches a pre-determined site density. The identification of this level and the extent of cropping or harvesting needed would, by necessity, be a compromise between the impact of specific biomass densities and the costs involved in removal. Techniques used with lake macrophyte problems in New Zealand may be adapted together with suitable jet-boats for use in the Manawatu River.

One major disadvantage of this strategy is that after algal biomass densities are reduced they may rapidly return to their former status.

7.3 Nutrient reduction

P Nutrient Availability Tests have shown that the growth rate

of *Cladophora* was frequently limited, during low steady flow periods, by the availability of P. If P inputs to the river could be reduced, the frequency and duration of these P limitation periods may be increased. If successful, this strategy would then increase the time needed to produce high biomass densities of *Cladophora*, and consequently increase the possibility of washout occurring before excessive levels had been reached.

A generally lower P concentration in the river could also enable a different periphyton species to dominate. The possibility of other periphyton dominating the river, was realized when flush events and cold river temperatures retarded *Cladophora* development allowing *Gomphonema* to dominate.

7.4 Summary

Before any control strategy could be attempted on a large scale, small scale laboratory and field studies would have to be investigated.

One potentially fruitful approach would involve combining two or more of the above strategies. Firstly, the effects of reducing DRP on the composition and growth rate of *Cladophora* (or other periphyton) could be investigated. Secondly, potential aquatic herbicides could be rigorously investigated to assess their toxicity to the periphyton of concern and other biota of, and involved with, the river. Thirdly, basic investigations could be carried out to test the feasibility of physical removal techniques. The overall aim of a combined strategy would be to reduce the magnitude and frequency of the *Cladophora* proliferations and, in the event of a prolonged steady low flow situation that enabled excessive *Cladophora* to build up, to remove it quickly and cheaply before any major problems developed.

CHAPTER 8

AREAS THAT WARRANT FURTHER STUDY

8. AREAS THAT WARRANT FURTHER STUDY

During this study various avenues of research have appeared that warrant more detailed study. Many have been followed, but others have been beyond the scope of the research. Some of these are detailed below:

(a) Average river *Cladophora* (Periphyton) biomass density:

The use of remote sensing combined with ground calibrations could provide estimates of average periphyton biomass density (As opposed to average site figures). Potentially, this would provide a quantitative parameter, which together with a qualitative description, could be used as a predictor for the DOD_m or maximum pH. (See (e) below). The average river periphyton biomass density could also be used in conjunction with studies designed to elucidate the P pathways in the river. (See (c) below).

(b) *Cladophora* (Periphyton) assemblage composition:

There is a need to have some simple quick methods that could be used to give a qualitative and quantitative description of contagiously distributed periphyton community biomass. Variations on existing techniques (Appendix 4), such as multiple subsampling of large blended composite quadrat samples, could be used to quantify various periphyton assemblage components. (e.g. Chlorophyll-a, dry weight, ash-free dry weight, ATP, carbon, nitrogen and phosphorus). The results would be assessed in terms of effort outlayed and the amount of useful description achieved.

(c) Phosphorus culture studies:

In order to study the effects of specific nutrients on lotic periphyton it is desirable to have a controlled laboratory system that closely resembles the river situation. The 'once through' flow system is ideal in this respect, the exact concentration of a particular nutrient can be measured, adjusted and periphyton responses monitored. Such a system

could help refine many of the relationships between DRP (or some other more readily available P form such as a low molecular weight reactive P component) and P NATs (White & Payne, 1982).

(d) Phosphorus sinks in the Manawatu River :

In order to ascertain the impact of *Cladophora* proliferations (and other periphyton growths) on the P concentration in the river, it would be necessary to follow the fate of a background orthophosphate ^{32}P spike to the river. Specific components of the river system could be analysed for their ^{32}P content and major sinks and pathways could be identified. More detailed tracer experiments could be needed to establish all the components and pathways involved in P cycling.

(e) The relationship between DOD_m and periphyton environmental and river variables:

This could be continued along the lines detailed in section 6.7.3. Many of the predictor variables could be more useful if they were more accurately described (e.g. (a)-(d) above). The basic regression equation could be continually updated in the light of additional data.

(f) pH and ammonia monitoring:

This should be continued, both upstream and downstream of the discharges. Changes (both chemical and biological) that occur due to the implementation of additional waste treatment facilities should be closely examined. It would be worthwhile to monitor upstream daily pH fluctuations in an attempt to construct a predictive equation for maximum pH in a fashion similar to the DOD_m predictive equation.

(g) *Cladophora* control strategies:

The possibilities of the various control strategies outlined in chapter 7 warrant preliminary investigations.

CHAPTER 9
CONCLUSIONS

9. CONCLUSIONS

Seasonal factors play a major role in controlling the development of *Cladophora* proliferations. With the onset of the spring/summer steady low flow periods in the Manawatu River, the benthic substrate becomes available for periphyton colonization. The filamentous alga *Cladophora* has taken advantage of this regular occurrence and become established as the dominant recurrent species, proliferating throughout the upper Manawatu River as the summer progresses. However, the life cycle of *Cladophora* is very dependent on the normal sequence of climatic and river conditions occurring. When these progressions are markedly disturbed the *Cladophora* proliferation development can be interrupted leaving the benthic substrate available for colonization by other opportunistic periphyton.

Providing the environmental conditions have combined to allow the development of a *Cladophora* proliferation (or another periphyton growth), the major factors affecting the biomass are the frequency and magnitude of flush events. Large spates were seen to totally decimate any periphyton and small frequent flushes continually arrested the development.

During steady low flow periods when *Cladophora* had become extensive throughout the river the dissolved phosphorus content of the river water was often low enough to reduce the internal algal phosphorus to values that restricted the maximum growth rate. A downstream trend of increased phosphorus limitation was demonstrated by phosphorus nutrient availability tests, along the major upstream study reach (13 km). Limitation was usually alleviated by nutrient inputs associated with spates.

The dissolved inorganic nitrogen concentration of the river during steady low flow periods was comparatively low, although it never caused the nitrogen nutrient availability tests to give responses indicative of nitrogen limitation.

The *Cladophora* (and other periphyton) caused significant water quality effects, such as large diurnal DO fluctuations. The minimum DO concentration that occurs will reduce the river's ability to assimilate the oxygen-demanding organic discharges that occur further downstream, while maintaining the minimum desirable DO concentration. Many of the factors influencing the algal-induced DO minimum or more specifically the maximum daily DO deficit (DOD_m) have been examined in an effort to understand the processes involved in producing the range of DOD_m . An empirical parsimonious regression equation was developed, using data from both the 1981/82 and 1982/83 seasons, which explained 71.5% of the DOD_m variation. The total Respiration (TR) of the river community contributed the most to the total predicted DOD_m followed by the influence of the different periphyton or season and the average daily river flow (F). The average daily terrestrial light (L_T) and a regression constant also contributed to the total predicted DOD_m . The TR proved to be the most important predictor because it accounted for various periphyton and planktonic community changes that occurred between, and during, seasons.

The DOD_m could be predicted daily by monitoring the relevant parameters, TR, F and L_T , and substituting the data for the variables in the equation. The periphyton proliferation type is identified and the appropriate code used. The magnitude of the predicted DOD_m then enables the water quality manager to identify a potentially hazardous situation and take any necessary remedial action.

The regression equation could only be applied to either of the two specific periphyton situations that developed in the Manawatu River. In future seasons, new data could be used to continually modify and increase the applicability of the regression equation.

The other major water quality effect of periphyton proliferations can be severe diurnal pH oscillations. These upstream algal-induced diurnal pH fluctuations have been seen to be maintained, although somewhat reduced, downstream of the discharges. The bacterial respiration occurring in the region of the discharges was responsible for the amelioration of the pH fluctuations. Using the

presently accepted New Zealand ammonia criterion there were no recorded instances of toxic ammonia concentrations. However, two points should be made:-

- (i) The timing of the pH and ammonia peaks did not usually coincide, however, potentially toxic concentrations have often only been narrowly separated.
- (ii) Additional pollution control facilities designed to reduce the load of oxygen-demanding discharges to the river may concurrently increase the amount of ammonia and also reduce the amelioration of pH fluctuations.

The periphyton proliferations have a number of water quality management implications. These need to take account of answers to the following questions:-

- (i) Is the impact of the algal-induced diurnal DO and pH fluctuations sufficient to warrant either algal control measures or the adoption of stricter controls on the organic/ammonia discharges?
- (ii) Is there a realistic algal control method that could be employed to reduce the biomass of algal proliferations?

The results of this study indicate that during steady low flow periods when algal proliferations are extensive throughout the upper river some control measures may need to be adopted to ensure adverse water quality conditions do not arise. At present there are a number of additional treatment facilities nearing completion that are designed to reduce the organic loads from the three major dischargers. These measures may well increase the minimum DO concentrations but their effect on the pH/ammonia profiles is a subject for speculation.

In the event that the new treatment facilities still leave the river with some severe water quality problems, it may well be prudent to investigate the practicality of some algal control measures. A method of *in situ* biomass reduction would appear

to be a feasible approach either with a 'safe' algicide application or via a physical removal programme.

ABBREVIATIONS

AAR	: Ammonium Absorption Rate ($\mu\text{g N (10 mg)}^{-1} \text{ hr}^{-1}$)
APA	: Alkaline Phosphatase Activity (Enzyme units mg^{-1})
Bd	: Below detection
BM	: <i>Cladophora</i> average site biomass density (g DW m^{-2})
BOD	: Biochemical Oxygen Demand (g m^{-3})
C	: Average dissolved oxygen concentration during Δt (g m^{-3})
C_s	: Saturated dissolved oxygen concentration at the average river temperature during Δt (g m^{-3})
CCA	: Clean <i>Cladophora</i> Assemblage
CV	: Coefficient of Variation
$\Delta C/\Delta t$: Rate of change of dissolved oxygen ($\text{g m}^{-3} \text{ hr}^{-1}$)
ΔDO	: Daily Dissolved Oxygen fluctuation (g m^{-3})
DCA	: Dirty <i>Cladophora</i> Assemblage
DIN	: Dissolved Inorganic Nitrogen (g m^{-3})
DO	: Dissolved Oxygen (g m^{-3})
DOD	: Dissolved Oxygen Deficit (g m^{-3})
DOD_m	: Maximum daily Dissolved Oxygen Deficit (g m^{-3})
DRP	: Dissolved Reactive Phosphorus (g m^{-3})
DW	: Dry Weight
EPA	: Environmental Protection Agency (USA)
EP	: Extractive Phosphorus (% P)
EP_o	: Critical Extractive Phosphorus (% P)
F	: Daily average river flow ($\text{m}^3 \text{ s}^{-1}$)
FFF	: Free Floating Filaments
GP	: Gross Photosynthesis ($\text{g O}_2 \text{ m}^{-3} \text{ d}^{-1}$)
H	: Average river depth (m)
k_m	: Half-saturation constant for uptake as a function of external P (% P)
k_q	: Half-saturation constant for uptake as a function of Q (% P)
k_2	: Reaeration coefficient (hrs^{-1})
L_T	: Daily terrestrial light (Langleys d^{-1})
L_u	: Daily underwater (30 cm depth) light ($\text{E m}^{-2} \text{ d}^{-1}$)
MRWB	: Manawatu Regional Water Board
μ	: Specific growth rate (d^{-1})
μ_m	: Maximum specific growth rate (d^{-1})
N	: Nitrogen

Nd	: No data/not determined
NAP	: Net Areal Primary Productivity ($\text{g O}_2 \text{ m}^{-2} \text{ d}^{-1}$)
NAT	: Nutrient Availability Test
P	: Phosphorus
PAR	: Photosynthetically Available Radiation
PR	: Net Primary Productivity ($\text{g O}_2 \text{ m}^{-3} \text{ hr}^{-1}$)
P/R	: GP/TR ratio
PUR	: Phosphorus Uptake Rate ($\mu\text{g P (10 mg)}^{-1} \text{ hr}^{-1}$)
Q	: Phosphorus cell quota (TTP) (% P)
Q_0	: Critical phosphorus cell quota (% P)
R_m	: Maximum respiration rate ($\text{g O}_2 \text{ m}^{-3} \text{ hr}^{-1}$)
RCL	: Regression Contribution Line
ρ	: PUR ($\mu\text{g P (10 mg)}^{-1} \text{ hr}^{-1}$)
$\rho_{\text{max.}}$: Maximum PUR ($\mu\text{g P (10 mg)}^{-1} \text{ hr}^{-1}$)
$\rho_{\text{min.}}$: Minimum PUR ($\mu\text{g P (10 mg)}^{-1} \text{ hr}^{-1}$)
S	: Season (code 1 for 1981/82, 2 for 1982/83)
$T_{\text{av.}}$: Average daily river temperature ($^{\circ}\text{C}$)
$T_{\text{max.}}$: Maximum river temperature ($^{\circ}\text{C}$)
$T_{\text{min.}}$: Minimum river temperature ($^{\circ}\text{C}$)
TN	: Total river nitrogen (g m^{-3})
TP	: Total river phosphorus (g m^{-3})
TR	: Total Respiration ($\text{g O}_2 \text{ m}^{-3} \text{ d}^{-1}$)
TTN	: Total Tissue Nitrogen (% N)
TTP	: Total Tissue Phosphorus (% P)
U	: Average river current velocity (m s^{-1})
W	: Average river width (m)

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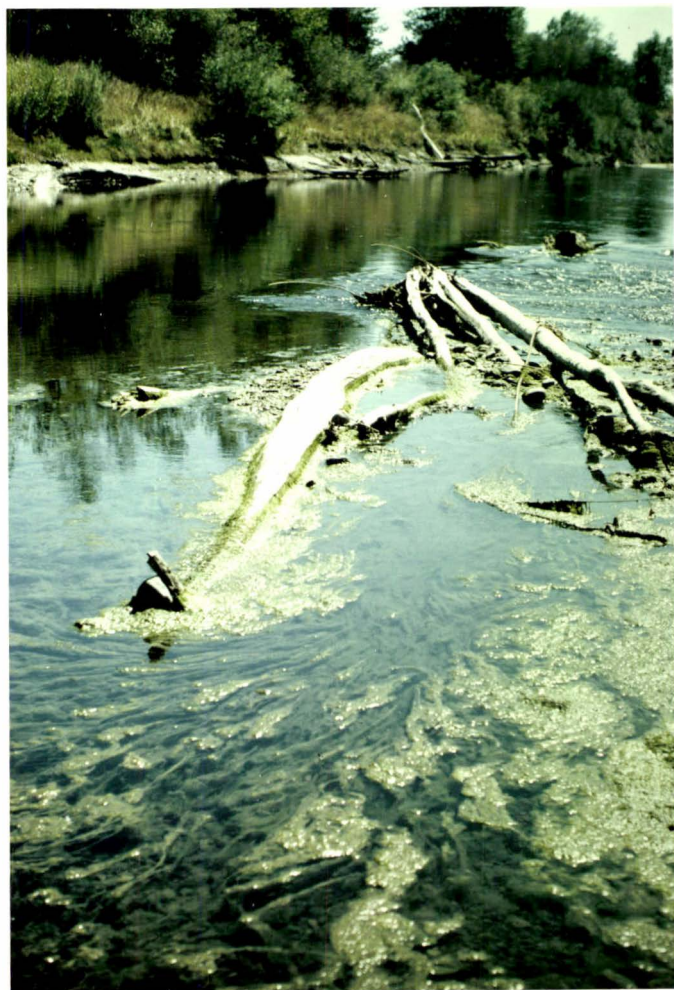
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APPENDIX 1
PHOTOGRAPHS



Photograph 1

Cladophora proliferations near Site M, summer 1981.



Photograph 2

Cladophora proliferations near Site T, summer 1983.



Photograph 3

Close-up of a *Cladophora* assemblage.



Photograph 4

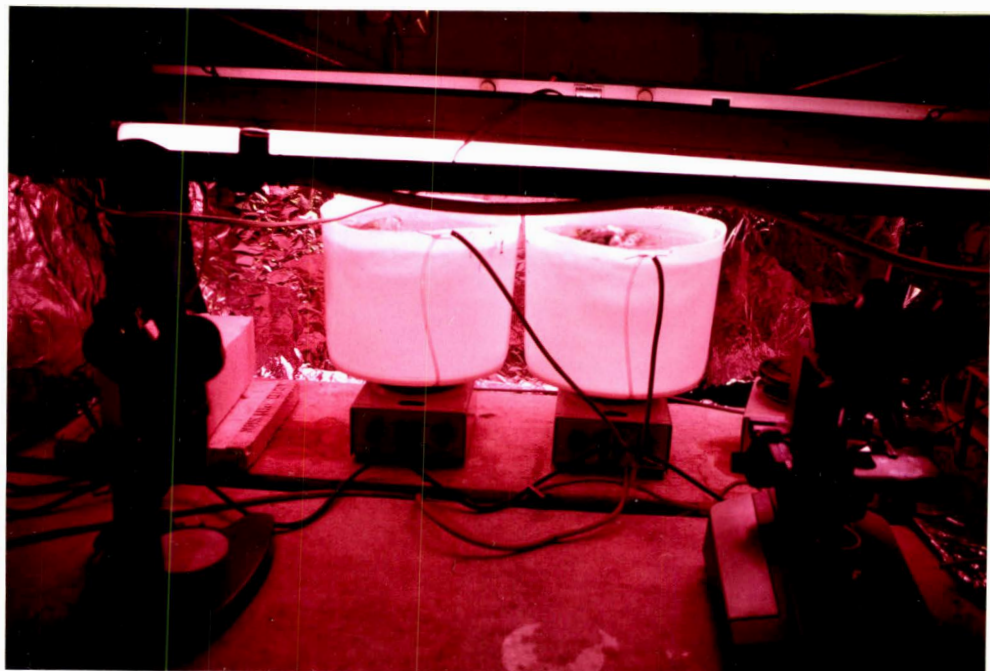
Close-up of a *Cladophora-Gomphonema* assemblage.



Photograph 5

Young rapidly growing
Cladophora filament, bar
equals 100 μm .

Photograph 6
Laboratory culturing
vessels.



APPENDIX 2

COMPUTER PROGRAMME FOR PRIMARY PRODUCTIVITY ANALYSIS

APPENDIX 2

USER: BT0245RIVER

RIVTEST1.F77

COMPUTER PROGRAMME FOR PRIMARY PRODUCTIVITY ANALYSIS

00000	000000	0000	0000	0	000000	00000	0000	0	0	000000	00000
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
00000	0	0	0	0	000000	0	00000	0	0	0	00000
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
00000	0	0000	000000	0	0000	0	0	0000	0	0	0

00000	0000	0	0	000000	000000	0000	0000000	0	0000000	0000000	0000000
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
00000	0	0	0	0	000000	00000	0	0	000000	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0000	0	0	0000000	0000	0	0000	0	0	0

LABEL: PRT002 -FORM CARBON

SPOOLED: 82-03-31.13:08

STARTED: 81-08-03.18:26, ON: AMLC BY: DIABLO

Massey Computer Centre, Diablo Printer.


```

600  FORMAT (4F8.2)
650  CONTINUE
C
C
C
C  THE NEXT SECTION CALCULATES THE RESPIRATION DURING THE DAY
C  BY USING THE VALUES OBTAINED BY EXTRAPOLATING BACK FROM
C  THE EVENING MAX RESPIRATION VALUE TO THE AVERAGE OF THE
C  LAST FOUR MORNING READINGS.
C
C
C
DO 1150 L=1,NDAY
WRITE (7,710)
710  FORMAT('    TIME      P      R  ')
LX=24*(L-1)
RMAX=0
DO 750 I1=20+LX,32+LX
IF ( PR(I1).LT. PR(I1+1) ) GOTO 712
712  IF ( PR(I1).LT. PR(I1+2) ) GOTO 714
714  IF ( PR(I1).LT. PR(I1+3) ) GOTO 716
716  IF ( PR(I1).LT. PR(I1+4) ) GOTO 718
718  IF ( PR(I1).LT. PR(I1+5) ) GOTO 720
720  IF ( PR(I1).LT. PR(I1+6) ) GOTO 722
722  IF ( PR(I1).LT. PR(I1+7) ) GOTO 760
750  CONTINUE
760  RMAX=PR(I1)
HOUR=0
HOUR=( I1)
SRAVM=0
DO 780 I=(NIGHT-3+LX),(NIGHT+LX)
SRAVM=SRAVM+PR(I)
780  CONTINUE
RAVM=0

RAVM=SRAVM/4
DO 800 I= NIGHT+LX, HOUR
R(I)=RAVM+((RMAX-RAVM)/FLOAT(HOUR-(NIGHT+LX)))*
*FLOAT((I)-(NIGHT+LX))
800  P(I)=PR(I)-R(I)
DO 810 I=NIGHT+LX, HOUR
IF(P(I)) 815,820,820
810  CONTINUE
815  R(I)=PR(I)
P(I)=0
820  DO 840 I=HOUR+1,NIGHT+23+LX
R(I)=PR(I)
P(I)=0
840  CONTINUE
PMAX=0
DO 890 I=NIGHT+LX, HOUR
IF (PMAX.LT.P(I)) PMAX=P(I)
890  CONTINUE
RMIN=0
DO 891 I=HOUR,NIGHT+24+LX
IF(R(I).GT.R(I+1)) GOTO 892
891  CONTINUE
892  RMIN=R(I)
DO 900 I=NIGHT+LX,NIGHT+23+LX
900  WRITE (7,1000) TIM(I),P(I),R(I)
1000 FORMAT (3F8.2)

DIMENSION C(200),TIM(200),T(200), PR(200),R(200),P(200)

```

```
SUMP=0
SUMR=0
SUMPR=0
SUMAPR=0
RATIO=0
DO 1100 I=NIGHT+LX,NIGHT+23+LX
SUMP=SUMP+P(I)
SUMR=SUMR+R(I)
SUMPR=SUMPR+PR(I)
1100 RATIO=(SUMP)/(-SUMR)
SUMAPR=SUMPR/(H(L))
WRITE (7,1101) PMAX
1101 FORMAT(17X'MAX PHOTOSYNTHESIS= ',F6.2, ' GMS/CU METRE/HR')
WRITE (7,1102) RMIN
1102 FORMAT(17X' MIN RESPIRATION= ',F6.2, ' GMS/CU METRE/HR')
WRITE (7,1103) SUMP
1103 FORMAT(15X'GROSS PHOTOSYNTHESIS= ',F6.2,' GMS/CU METRE/DAY')
WRITE (7,1104) SUMR
1104 FORMAT(18X'TOTAL RESPIRATION= ',F6.2,' GMS/CU METRE/DAY')
WRITE (7,1105) SUMPR
.105 FORMAT(17X'NET PHOTOSYNTHESIS= ',F6.2,' GMS/CU METRE/DAY')
WRITE (7,1106) RATIO
1106 FORMAT(26X'P/R RATIO= ',F6.2)
WRITE (7,1107) SUMAPR
1107 FORMAT(11X'NET AREAL PHOTOSYNTHESIS= ',F6.2, ' GMS/SQ METRE/DAY')
1150 CONTINUE
```

```
CLOSE(7)
CLOSE(5)
CALL EXIT
END
```

APPENDIX 3

COMPUTER PROGRAMME SAMPLE OUTPUT

APPENDIX 3

USER: BT0245RIVER

JUN. 9. 2. 82

COMPUTER PROGRAMME SAMPLE OUTPUT


```

00000 000000 0000 0000 0 000000 00000 0000 0 0 000000 00000
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 00000 0 0 0 0 0 0 0 0
00000 0 0 0 0 0 000000 0 00000 0 0 0 00000 00000
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
00000 0 0000 000000 0 0000 0 0 0000 0 000000 0 0
    
```

```

0000 0 0 000000 0000 0000 0000 0000 0000
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0000 0000 0 00 0000 00 000000 00 0000 000000
    
```


LABEL: PRT003 -FORM CARBON

SPOOLED: 82-03-31.13:08

STARTED: 82-03-31.14:24, ON: AMLC BY: DIABLO

Gassey Computer Centre, Diablo Printer.

- Time = time, beginning at sunset (hrs)
- D.O. = Dissolved oxygen ($g.m^{-3}$)
- Temp = Temperature ($^{\circ}C$)
- PR = Net Production ($gO_2m^{-3}.hr^{-1}$)
- Q = Flow (m^3s^{-1})
- V = Velocity ($m s^{-1}$)
- H = Depth (m)
- Ak = Reaation Coefficient (hrs^{-1})

TIME	D.O.	TEMP	PR	
0.50	10.40	21.00	-0.40	
1.50	9.90	21.00	-0.23	
2.50	9.60	20.50	-0.37	
3.50	9.20	20.00	-0.61	
4.50	8.60	20.00	-0.35	
5.50	8.30	20.00	-0.16	
6.50	8.20	20.00	-0.17	
7.50	8.10	20.00	-0.18	
8.50	8.00	20.00	-0.19	
9.50	7.90	20.00	-0.20	
10.50	7.80	19.50	-0.11	
Q	V	W	H	AK
14.60	0.29	48.00	1.05	0.08
TIME	D.O.	TEMP	PR	
11.50	7.80	19.50	0.10	
12.50	8.00	19.50	0.74	
13.50	8.80	19.50	0.60	
14.50	9.40	20.00	0.55	
15.50	9.90	20.50	0.70	
16.50	10.50	21.00	0.66	
17.50	11.00	22.00	0.40	
18.50	11.20	22.50	0.32	
19.50	11.30	23.00	0.23	
20.50	11.30	23.00	0.12	
21.50	11.20	23.00	0.01	
22.50	11.00	23.00	-0.11	
23.50	10.70	23.00	-0.24	
24.50	10.30	22.50	-0.38	
25.50	9.80	22.00	-0.53	
26.50	9.20	21.50	-0.49	
27.50	8.70	21.00	-0.43	
28.50	8.30	21.00	-0.35	
29.50	8.00	21.00	-0.27	
30.50	7.80	21.00	-0.18	
31.50	7.70	21.00	-0.30	
32.50	7.50	21.00	-0.21	
33.50	7.40	21.00	-0.11	
34.50	7.40	21.00	-0.11	
Q	V	W	H	AK
13.10	0.29	48.00	0.94	0.09
TIME	D.O.	TEMP	PR	
35.50	7.40	21.00	0.08	
36.50	7.60	21.00	0.52	
37.50	8.20	21.00	0.99	
38.50	9.20	21.50	0.67	
39.50	9.80	21.50	0.73	
40.50	10.40	22.00	0.69	
41.50	10.90	22.50	0.54	
42.50	11.20	23.00	0.47	
43.50	11.40	23.00	0.28	
44.50	11.40	23.50	0.29	
45.50	11.40	23.50	0.07	
46.50	11.20	23.00	-0.05	
47.50	10.90	23.00	-0.40	
48.50	10.30	22.50	-0.57	
49.50	9.60	22.00	-0.54	
50.50	9.00	21.50	-0.40	
51.50	8.60	21.00	-0.44	
52.50	8.20	21.00	-0.38	
53.50	7.90	20.50	-0.30	

54.50	7.70	20.50	-0.22	
55.50	7.60	20.00	-0.24	
56.50	7.50	20.00	-0.25	
57.50	7.40	20.00	-0.15	
58.50	7.40	20.00	-0.15	
Q	V	W	H	AK
12.70	0.29	47.00	0.93	0.09
TIME	D.O.	TEMP	PR	
59.50	7.40	20.00	0.47	
60.50	8.00	19.50	0.73	
61.50	8.80	19.50	0.81	
62.50	9.60	20.00	0.79	
63.50	10.30	20.50	0.66	
64.50	10.80	21.00	0.61	
65.50	11.20	21.50	0.55	
66.50	11.50	22.00	0.37	
67.50	11.60	22.00	0.28	
68.50	11.60	22.00	0.17	
69.50	11.50	22.00	0.37	
70.50	11.60	22.00	-0.04	
71.50	11.30	21.50	-0.39	
72.50	10.70	21.00	-0.77	
73.50	9.80	20.50	-0.55	
74.50	9.20	20.00	-0.52	
75.50	8.70	19.00	-0.47	
76.50	8.30	19.00	-0.31	
77.50	8.10	18.50	-0.33	
78.50	7.90	18.50	-0.25	
79.50	7.80	18.00	-0.26	
80.50	7.70	18.00	-0.17	
81.50	7.70	18.00	-0.17	
82.50	7.70	18.00	-0.06	
Q	V	W	H	AK
12.60	0.29	47.00	0.92	0.09
TIME	D.O.	TEMP	PR	
83.50	7.80	18.00	0.57	
84.50	8.50	18.00	0.85	
85.50	9.40	18.50	0.85	
86.50	10.20	19.50	0.73	
87.50	10.80	20.00	1.01	
88.50	11.60	20.50	0.89	
89.50	12.20	21.50	0.75	
90.50	12.60	22.00	0.17	
91.50	12.40	22.50	0.26	
92.50	12.30	22.50	-0.48	
93.50	11.50	22.50	-0.35	
94.50	10.90	22.00	-0.53	
95.50	10.20	21.00	-0.61	
96.50	9.50	20.00	-0.49	
97.50	9.00	19.50	-0.44	
98.50	8.60	19.00	-0.27	
99.50	8.40	19.00	-0.30	
100.50	8.20	18.50	-0.43	
101.50	7.90	18.00	-0.26	
102.50	7.80	18.00	-0.26	
103.50	7.70	18.00	-0.27	
104.50	7.60	18.00	-0.18	
105.50	7.60	18.00	-0.18	
106.50	7.60	18.00	0.24	
Q	V	W	H	AK
12.40	0.29	47.00	0.91	0.10
TIME	D.O.	TEMP	PR	

TIME	D.O.	TEMP	PR
107.50	8.00	18.00	0.59
108.50	8.70	18.00	0.45
109.50	9.20	18.00	1.02
110.50	10.20	18.50	0.82
111.50	10.90	19.00	0.69
112.50	11.40	19.50	0.85
113.50	12.00	20.00	0.50
114.50	12.20	20.50	0.43
115.50	12.30	21.00	0.23
116.50	12.20	21.00	0.12
117.50	12.00	21.00	-0.11
118.50	11.60	21.00	-0.37
119.50	11.00	20.50	-0.64
120.50	10.20	20.50	-0.52
121.50	9.60	19.00	-0.60
122.50	9.00	18.50	-0.46
123.50	8.60	18.00	-0.30
124.50	8.40	17.50	-0.44
125.50	8.10	17.00	-0.26
126.50	8.00	17.00	-0.27
127.50	7.90	17.00	-0.29
128.50	7.80	16.50	-0.20
129.50	7.80	16.50	0.01
130.50	8.00	16.50	0.14
Q	V	W	H
12.40	0.29	47.00	0.91
TIME	D.O.	TEMP	PR
131.50	8.30	16.50	0.70
132.50	9.10	17.00	0.79
133.50	9.90	18.00	0.78
134.50	10.60	18.50	0.65
135.50	11.10	19.00	0.71
136.50	11.60	20.00	0.68
137.50	12.00	21.00	0.42
138.50	12.10	21.50	0.33
139.50	12.10	22.00	0.23
140.50	12.00	22.00	0.12
141.50	11.80	22.00	-0.33
142.50	11.20	21.50	-0.71
143.50	10.30	21.00	-0.81
144.50	9.40	20.50	-0.69
145.50	8.70	20.00	-0.56
146.50	8.20	20.00	-0.40
147.50	7.90	19.50	-0.44
148.50	7.60	19.00	-0.38
149.50	7.40	18.50	-0.30
150.50	7.30	18.50	-0.31
151.50	7.20	18.00	-0.32
152.50	7.10	18.50	-0.33
153.50	7.00	18.50	-0.23
154.50	7.00	18.50	-0.23
TIME	P	R	
10.00	0.22	-0.12	
12.00	0.87	-0.13	
13.00	0.74	-0.14	
14.00	0.70	-0.15	
15.00	0.86	-0.16	
16.00	0.83	-0.17	
17.00	0.58	-0.18	
18.00	0.51	-0.19	
TIME	D.O.	TEMP	PR

AK
0.10

TIME D.O. TEMP PR

19.00	0.43	-0.20
20.00	0.33	-0.21
21.00	0.23	-0.22
22.00	0.12	-0.23
23.00	0.00	-0.24
24.00	0.00	-0.38
1.00	0.00	-0.53
2.00	0.00	-0.49
3.00	0.00	-0.43
4.00	0.00	-0.35
5.00	0.00	-0.27
6.00	0.00	-0.18
7.00	0.00	-0.30
8.00	0.00	-0.21
9.00	0.00	-0.11
10.00	0.00	-0.11

MAX PHOTOSYNTHESIS=	0.87	GMS/CU	METRE/HR
MIN RESPIRATION=	-0.24	GMS/CU	METRE/HR
GROSS PHOTOSYNTHESIS=	6.39	GMS/CU	METRE/DAY
TOTAL RESPIRATION=	-5.66	GMS/CU	METRE/DAY
NET PHOTOSYNTHESIS=	0.73	GMS/CU	METRE/DAY
P/R RATIO=	1.13		
NET AREAL PHOTOSYNTHESIS=	0.70	GMS/SQ	METRE/DAY

TIME P R

11.00	0.17	-0.09
12.00	0.63	-0.11
13.00	1.13	-0.14
14.00	0.84	-0.17
15.00	0.92	-0.19
16.00	0.91	-0.22
17.00	0.78	-0.24
18.00	0.74	-0.27
19.00	0.58	-0.30
20.00	0.61	-0.32
21.00	0.42	-0.35
22.00	0.32	-0.37
23.00	0.00	-0.40
24.00	0.00	-0.57
1.00	0.00	-0.54
2.00	0.00	-0.40
3.00	0.00	-0.44
4.00	0.00	-0.38
5.00	0.00	-0.30
6.00	0.00	-0.22
7.00	0.00	-0.24
8.00	0.00	-0.25
9.00	0.00	-0.15
10.00	0.00	-0.15

MAX PHOTOSYNTHESIS=	1.13	GMS/CU	METRE/HR
MIN RESPIRATION=	-0.40	GMS/CU	METRE/HR
GROSS PHOTOSYNTHESIS=	8.06	GMS/CU	METRE/DAY
TOTAL RESPIRATION=	-6.82	GMS/CU	METRE/DAY
NET PHOTOSYNTHESIS=	1.24	GMS/CU	METRE/DAY
P/R RATIO=	1.18		
NET AREAL PHOTOSYNTHESIS=	1.32	GMS/SQ	METRE/DAY

TIME P R

11.00	0.49	-0.02
12.00	0.78	-0.05
13.00	0.89	-0.08
14.00	0.90	-0.11
15.00	0.80	-0.14

TIME D.O. TEMP PR

TIME D.O. TEMP PR

16.00 0.78 -0.18
 17.00 0.76 -0.21
 18.00 0.61 -0.24
 19.00 0.55 -0.27
 20.00 0.47 -0.30
 21.00 0.70 -0.33
 22.00 0.32 -0.36
 23.00 0.00 -0.39
 24.00 0.00 -0.77
 1.00 0.00 -0.55
 2.00 0.00 -0.52
 3.00 0.00 -0.47
 4.00 0.00 -0.31
 5.00 0.00 -0.33
 6.00 0.00 -0.25
 7.00 0.00 -0.26
 8.00 0.00 -0.17
 9.00 0.00 -0.17
 10.00 0.00 -0.06

MAX PHOTOSYNTHESIS= 0.90 GMS/CU METRE/HR

MIN RESPIRATION= -0.39 GMS/CU METRE/HR

GROSS PHOTOSYNTHESIS= 8.06 GMS/CU METRE/DAY

TOTAL RESPIRATION= -6.54 GMS/CU METRE/DAY

NET PHOTOSYNTHESIS= 1.52 GMS/CU METRE/DAY

P/R RATIO= 1.23

NET AREAL PHOTOSYNTHESIS= 1.63 GMS/SQ METRE/DAY

TIME P R
 11.00 0.53 0.04
 12.00 0.87 -0.01
 13.00 0.92 -0.07
 14.00 0.86 -0.13
 15.00 1.20 -0.19
 16.00 1.14 -0.25
 17.00 1.06 -0.30
 18.00 0.54 -0.36
 19.00 0.68 -0.42
 20.00 0.00 -0.48
 21.00 0.00 -0.35
 22.00 0.00 -0.53
 23.00 0.00 -0.61
 24.00 0.00 -0.49
 1.00 0.00 -0.44
 2.00 0.00 -0.27
 3.00 0.00 -0.30
 4.00 0.00 -0.43
 5.00 0.00 -0.26
 6.00 0.00 -0.26
 7.00 0.00 -0.27
 8.00 0.00 -0.18
 9.00 0.00 -0.18
 10.00 0.00 0.24

MAX PHOTOSYNTHESIS= 1.20 GMS/CU METRE/HR

MIN RESPIRATION= -0.35 GMS/CU METRE/HR

GROSS PHOTOSYNTHESIS= 7.79 GMS/CU METRE/DAY

TOTAL RESPIRATION= -6.50 GMS/CU METRE/DAY

NET PHOTOSYNTHESIS= 1.29 GMS/CU METRE/DAY

P/R RATIO= 1.20

NET AREAL PHOTOSYNTHESIS= 1.39 GMS/SQ METRE/DAY

TIME P R
 11.00 0.47 0.12
 12.00 0.37 0.07

TIME D.O. TEMP PR

TIME D.O. TEMP PR

13.00	1.00	0.03
14.00	0.83	-0.01
15.00	0.74	-0.06
16.00	0.95	-0.10
17.00	0.65	-0.15
18.00	0.62	-0.19
19.00	0.47	-0.23
20.00	0.39	-0.28
21.00	0.21	-0.32
22.00	0.00	-0.37
23.00	0.00	-0.64
24.00	0.00	-0.52
1.00	0.00	-0.60
2.00	0.00	-0.46
3.00	0.00	-0.30
4.00	0.00	-0.44
5.00	0.00	-0.26
6.00	0.00	-0.27
7.00	0.00	-0.29
8.00	0.00	-0.20
9.00	0.00	0.01
10.00	0.00	0.14

MAX PHOTOSYNTHESIS=	1.00	GMS/CU	METRE/HR
MIN RESPIRATION=	-0.37	GMS/CU	METRE/HR
GROSS PHOTOSYNTHESIS=	6.70	GMS/CU	METRE/DAY
TOTAL RESPIRATION=	-5.33	GMS/CU	METRE/DAY
NET PHOTOSYNTHESIS=	1.37	GMS/CU	METRE/DAY
P/R RATIO=	1.26		
NET AREAL PHOTOSYNTHESIS=	1.51	GMS/SQ	METRE/DAY

TIME	P	R
11.00	0.53	0.16
12.00	0.71	0.08
13.00	0.77	0.00
14.00	0.72	-0.08
15.00	0.87	-0.15
16.00	0.91	-0.23
17.00	0.73	-0.31
18.00	0.72	-0.39
19.00	0.70	-0.47
20.00	0.67	-0.55
21.00	0.30	-0.63
22.00	0.00	-0.71
23.00	0.00	-0.81
24.00	0.00	-0.69
1.00	0.00	-0.56
2.00	0.00	-0.40
3.00	0.00	-0.44
4.00	0.00	-0.38
5.00	0.00	-0.30
6.00	0.00	-0.31
7.00	0.00	-0.32
8.00	0.00	-0.33
9.00	0.00	-0.23
10.00	0.00	-0.23

MAX PHOTOSYNTHESIS=	0.91	GMS/CU	METRE/HR
MIN RESPIRATION=	-0.71	GMS/CU	METRE/HR
GROSS PHOTOSYNTHESIS=	7.64	GMS/CU	METRE/DAY
TOTAL RESPIRATION=	-8.28	GMS/CU	METRE/DAY
NET PHOTOSYNTHESIS=	-0.63	GMS/CU	METRE/DAY
P/R RATIO=	0.92		
NET AREAL PHOTOSYNTHESIS=	-0.70	GMS/SQ	METRE/DAY

APPENDIX 4

MONITORING THE EFFECTS OF ATTACHED FILAMENTOUS ALGAE
ON DISSOLVED OXYGEN

APPENDIX 4

**MONITORING THE EFFECTS OF ATTACHED FILAMENTOUS ALGAE
ON DISSOLVED OXYGEN****M.C. Freeman and P.N. McFarlane**

Biotechnology Department, Massey University

ABSTRACT

Methods used to measure attached filamentous algae in the Manawatu River are discussed. The dissolved oxygen deficits (DOD) that can result from these growths are then examined in an attempt to identify the variables responsible for producing large DOD. Multiple linear regression analysis led to a simple equation that accounts for much of the DOD variation.

INTRODUCTION

Proliferations of attached filamentous algae occur during summer low flow periods in the Manawatu River. These growths are dominated by the alga *Cladophora* and cause two kinds of problems:-

- (1) Reduction in the user values of the river, as the algae interfere both physically and aesthetically with activities centred on the river.
- (2) Water quality effects, which may impair the river's ability to assimilate sewage and food industry discharges.

These problems lead to the following questions:-

- (1) Do the water quality effects of the algae warrant their control?
- (2) How can these algal proliferations be controlled?
- (3) Can the water quality effects caused by the algae be predicted, to allow suitable management strategies to be developed?

Questions 1 and 2 have been considered elsewhere (Freeman and McFarlane,

Design of water quality surveys : proceedings of a symposium. Ed. R.A. Hoare. Water & Soil Division, Ministry of Works & Development for the National Water & Soil Conservation Organisation, Wellington, 1983. (Water & Soil Miscellaneous Publication No XX.)

(1982). This paper considers question 3 by examining the relationship between the attached filamentous algae and the dissolved oxygen (D.O.) in the river. The night time respiratory demands of the algae cause the D.O. level to become depressed below saturation, the difference between the saturation and actual level being known as the D.O. deficit (D.O.D.). Knowledge of the maximum D.O.D. due to algal respiration, and the conditions under which it occurs, could help the Manawatu Regional Water Board co-ordinate water quality management strategies aimed at maintaining the water quality of downstream reaches receiving oxygen demanding discharges.

ALGAL BIOMASS ESTIMATION

A major characteristic of the algal proliferations in the Manawatu River is the patchy or clumped distribution, which results from the combination of the reproductive strategies of the algae and the physical characteristics of the river. To quantify the average algal biomass directly would involve considerable time and a variety of techniques. However, discrete areas of intensive growth recur each year, and these can be used as indicators of the many others that occur throughout the upper reaches. In this study a number of these maximum growth areas were identified at various locations along the river.

Biomass variables commonly used to estimate attached filamentous algal biomass include dry weight, ash free dry weight, chlorophyll and A.T.P. The advantages and disadvantages of these parameters have received much discussion (e.g. Vollenweider, 1969; McIntire, 1975). A.T.P. estimations do not discriminate between phototrophs and heterotrophs. Chlorophyll measurements can be easily affected by short-term environmental factors. Ash free dry weight measurements are usually only of importance when diatom dominated populations are being considered. These disadvantages leave dry weight measurement as a technique that involves few interpretive difficulties, gives estimates which can be easily compared with other studies, and with which a large number of replicates may be analysed.

Quadrat throw sampling was chosen to sample the natural river substrate (Wood, 1975). (The use of a number of artificial substrates was investigated, but algal development was poor and did not match the natural substrate.) This technique could be used in all stages of algal growth and

was practical in a variety of river conditions. The magnitude of the following variables had to be determined:-

- (1) The quadrat size
- (2) The number of throws
- (3) The area to be sampled
- (4) The number of sites
- (5) The frequency of sampling

1. A quadrat size of 0.04 m^2 was chosen to allow the measurement techniques to cope with biomass expected (from less than 1 g/m^2 to greater than 500 g/m^2) throughout the season.

2. The number of throws taken at each site must be a compromise to attain a specific level of accuracy. Test runs of 20 throws were taken to estimate the mean and standard deviation of the measurements - a typical result was a mean of 12.3 and a standard deviation of 6.3 ($\text{g}/0.04 \text{ m}^2$). It was decided that the precision of the estimate of the mean would be adequate if the standard deviation of that estimate were 20% of the mean. This means that

$$\frac{6.3/\sqrt{N}}{12.3} = 0.2$$

i.e. $N = 7$. A safety factor of three extra throws was chosen, which resulted in ten throws on each sampling occasion.

3. The sites chosen must be large enough to enable sampling to continue without significantly affecting the test population. If the total area sampled is $10 \times 0.04 \text{ m}^2$, then the site area must be related, in some manner, to this value. A maximum impact of 1% of the total population was chosen as an ideal, resulting in a sampling site of 40 m^2 . In practice, it is easier to delineate square sampling sites and it was found that growth areas of $10 \times 10 \text{ m}$ occurred quite frequently. This size was chosen, resulting in an impact of only 0.4% at every sampling occasion.

4. Three major sites were chosen for study, on the basis of their differing physical characteristics, which were representative of the many growth areas observed along the upper river (Fig. 1).

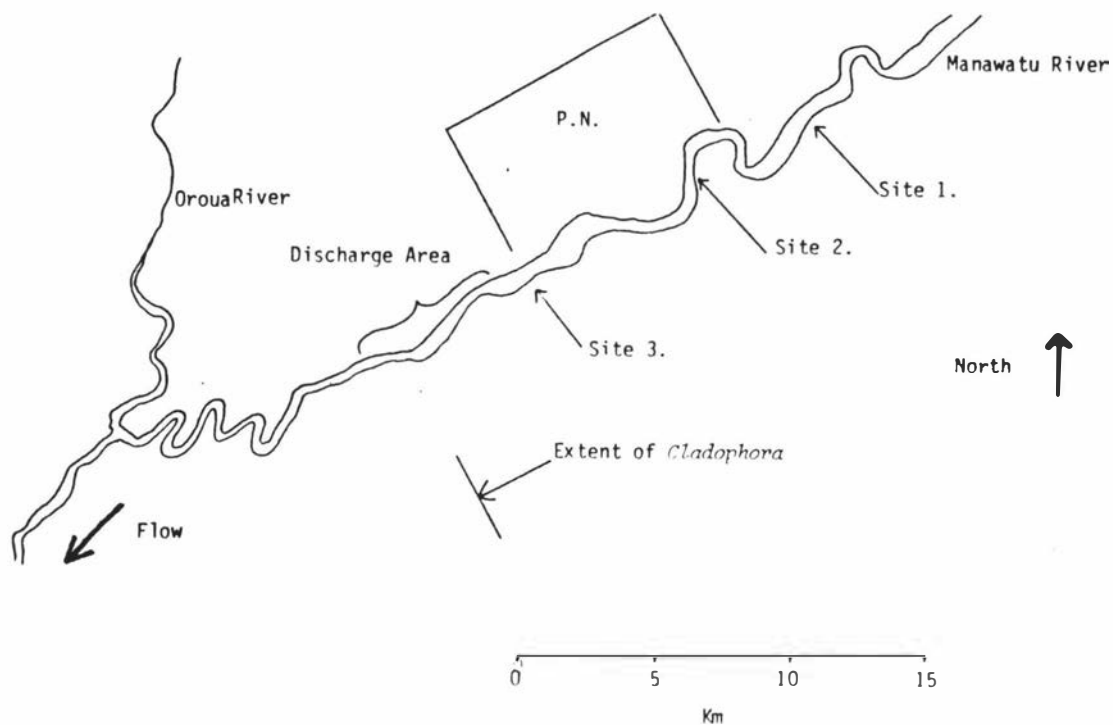


FIG. 1 : THE MANAWATU RIVER, SHOWING SAMPLING SITES

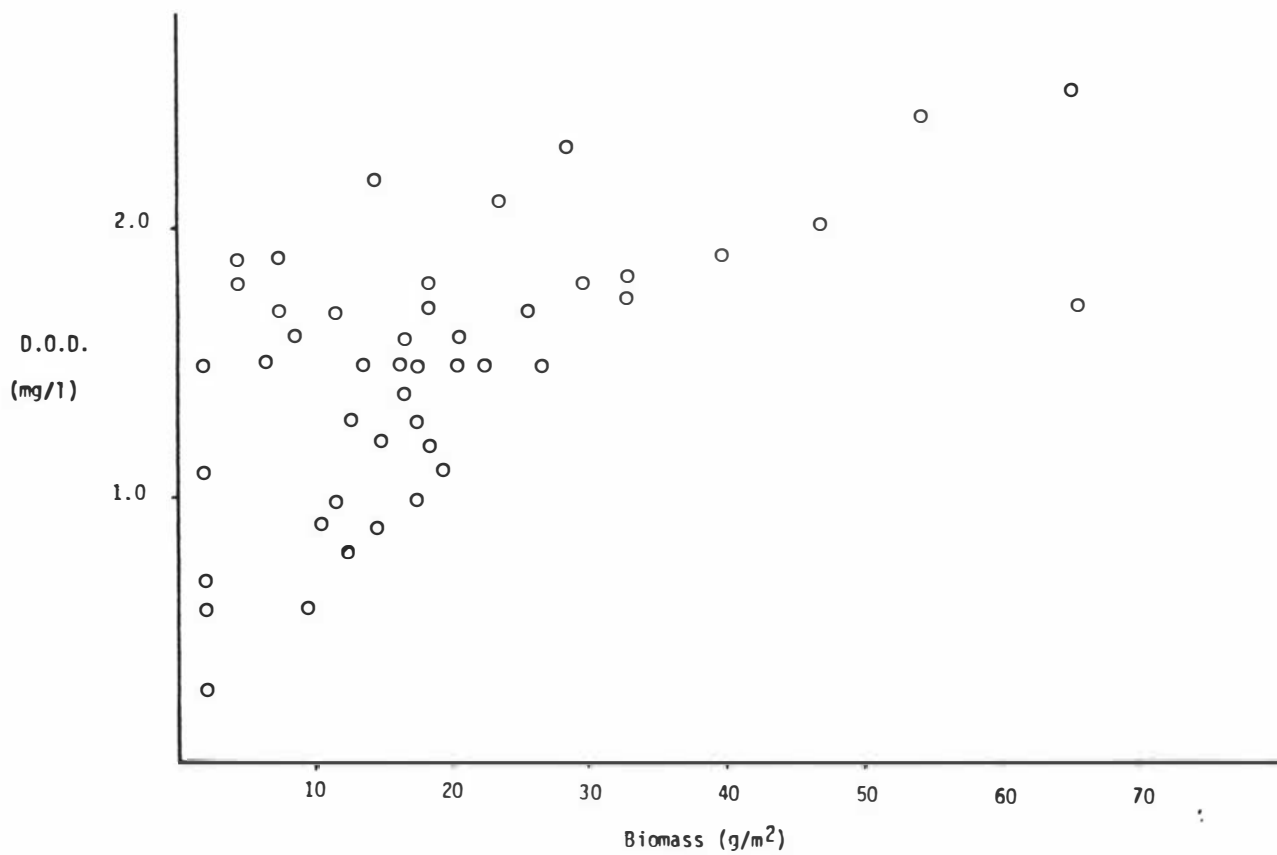


FIG. 2 : AVERAGE (SITE) BIOMASS OF CLADOPHORA VERSUS OBSERVED MAXIMUM DOD

5. The frequency of sampling depends on the river conditions, and the growth rate of the proliferation.

D.O. MONITORING

D.O. was monitored continuously using a YSI model 56 D.O. monitor. D.O. was initially calibrated using the Winkler technique, and air calibrations were performed every two days during each run. Temperature was measured simultaneously with a YSI thermistor calibrated against a mercury-in-glass thermometer. The D.O. variation was measured at one site which was shown to be representative of the upper reaches. The total error in the measurement of D.O. was approximately ± 0.3 mg/l. (The sum of the instrument component errors, non-ideal probe behaviour and the probe calibration uncertainty) (YSI, 1980).

When attached algae are present in the river, the night time respiratory activities cause a pre-dawn sag in the D.O. values. The maximum D.O.D. was calculated by comparing this minimum D.O. value with the expected saturation level that would result from a consideration of temperature alone.

Some of this D.O.D. can be attributed to the oxygen demand of the river sediments, but this effect was thought to be relatively small.

BIOMASS AND D.O. DEFICITS

The maximum D.O.D. was measured on forty-eight occasions during the 1981/82 season. Concurrently, a number of variables expected to influence the oxygen demand of *Cladophora* were measured.

- (1) Light : measured as total Langleys/day in Levin (about 50 km south-west of Palmerston North).
- (2) Temperature : measured at the time of the maximum D.O.D.
- (3) Flow : measured adjacent to site 2 and given as a daily mean.
- (4) Biomass : average of three sites.

As a preliminary exercise, the relationship between the algal biomass and the maximum D.O. deficit was examined (Fig. 2).

This simple comparison illustrated the need for further information if all

the factors influencing the maximum D.O.D. are to be identified. One simple technique which may identify relationships is the use of a correlation coefficient matrix. The matrix for the variables mentioned above is presented in Table 1.

Table 1 : Correlation coefficient matrix of variables

	Max D.O.D.	Biomass	Temp	Flow
Biomass	0.610			
Temp	0.268	0.084		
Flow	-0.435	-0.678	-0.041	
Light	0.329	-0.007	0.508	-0.201

This table illustrates the degree of linear correlation between the variables and the maximum D.O.D. The variable that is most closely related to the D.O.D. is the biomass (Fig. 2). Temperature and light are both positively correlated with D.O.D. (although the temperature correlation coefficient is below the significance level at $p = 0.05$ with 46 d.f.). Flow is seen to be negatively related to D.O.D. This is mainly attributed to the reduced biomass that results from flow increases, as illustrated by the high negative correlation between flows and biomass. The matrix also shows that light and temperature are closely related.

The next simple technique that may be utilised is a multiple regression analysis using all of the above factors, with less important variables being omitted until the maximum correlation is achieved. This shows that biomass and light are the major influences on the regression. If only these factors are considered in a regression analysis the following equation is obtained:

$$\text{D.O.D. (g/m}^3\text{)} = 0.655 + 0.0206 \text{ Biomass (g/m}^2\text{)} + 0.009 \text{ light (Langleys/day)}$$

(St. Dev. = 0.359, $r^2 = 0.46$, 45 d.f.)

This allows a water manager to calculate (to a given confidence level) the expected D.O.D. from any combination of biomass and light.

This analysis shows that about half of the variance in D.O.D. is explained by these factors alone ($r^2 = 0.46$). If a more complete picture is desired

then the determining factors need to be more fully described and other possible influences need to be examined.

The following aspects have been identified as deserving further description.

Light :

(a) The transparency of the river water is highly variable, so the light available to the algae would be more accurately measured in the river, rather than by using surface light intensity data.

(b) Equivalent daily integrals of available light can give very different D.O.-time graphs. The identification of the most important light parameter will entail further examination of D.O. graphs together with a more complete data base for light.

Nutrients : A limited amount of data has been collected regarding total phosphorus, total nitrogen and algal nutrient availability. On the basis of this information it is not possible to identify specific relationships. In the future, it may be possible to carry out some intensive studies involving the above, in order to assess their involvement.

Biomass : A major disadvantage of algal biomass estimates is that they only measure a portion of the *Cladophora* assemblage. This community develops, during steady flow periods, to form a complex in which the active *Cladophora* proportion is only a part. The rest is composed of active epiphytic and associated algae, moribund algae, and silt and debris. The influence of flush events on the biomass and composition of the assemblage will depend on the severity of the flush and the position of the assemblage in the river in relation to the channel topography. Thus, two equal algal biomass measurements may be taken from very different assemblages which will have very different effects on the D.O. dynamics.

Other Factors :

(a) Flush events have a variety of effects on the biomass/D.O. relationship. As well as reducing the assemblage biomass, the flush introduces a large volume of oxygen saturated water with a high suspended solids load. As the river recovers from the impact of the flush, the remaining suspended load can become of increasing importance. The increased turbidity reduces light available to the algae, allowing

respiratory processes to dominate the D.O. dynamics (depending on the water volume available to satisfy the demand). The suspended load can also exert an oxygen demand on the river, the importance of which can become significant if it is still exerted when the river has returned to a low-flow situation.

The suspended load also carries a large supply of nutrients. These can be rapidly assimilated by the algae to satisfy any shortages that may have arisen during previous low flow periods. Nutrients such as phosphorus can be taken up and stored in excess of present needs to be available in case of shortages in the future. The algae remaining after a flush would then usually only be limited by the available light.

(b) Planktonic algae are present in the river; however, levels recorded to date indicate that they have a minor affect on the D.O.D.

CONCLUSIONS

Some exploratory attempts at determining the factors which influence the effects of attached algae on maximum D.O.D.'s. have been presented. The results from these surveys have tentatively identified the relative importance of factors such as flow, temperature and light. The results also indicate that in order to fully describe events a comprehensive set of data is needed. However, from a management viewpoint the critical variables have been identified, and used to develop a maximum D.O.D. prediction equation which explains about half of the observed variance.

ACKNOWLEDGEMENT

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Mr M.C. Freeman is a Commonwealth Postgraduate Scholar.

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DISCUSSION

C.W. Hickey

Have you tried relating max. day DO - min day DO vs biomass present?

Author

Yes, but I think a water quality manager is more concerned about the worst case DO profile i.e. the maximum DOD.

C.W. Hickey

Do you agree that the option used by yourself (dry weight) for measurement of 'algal biomass' is really only suitable for filamentous algae and not for algal mats or other benthic algal populations?

Author

Yes, although the parameter used will depend on what you hope to do with the end result. In my case I think the predictive ability of my 'model' (equation) could be improved by using a parameter(s) that can describe both qualitative and quantitative changes.

J.C. Rutherford

In your Figure 3, you show large variation of DOD at low biomass, and low variation at high biomass, which I find surprising. Can you explain why this occurs?

Author

I would attribute these variations to the different composition of communities that may give equal biomass estimates. Also, the light climate experienced by the algal proliferation is not measured, and this may vary

considerably depending on river events and environmental variations. Consequently the effects on the oxygen dynamics will change.

APPENDIX 5

CALCULATIONS SHOWING THE EFFECT OF COMPENSATING NET AREAL
PRIMARY PRODUCTIVITY FOR THE VARIATION OF K_2 WITH TEMPERATURE

APPENDIX 5 Calculations showing the effect of compensating Net Areal Primary Productivity for the variation of K_2 with temperature
 (See day 4, Appendix 3)

C	$\frac{\Delta C}{\Delta t}$	Average Temp ($^{\circ}\text{C}$)	C_s	Average C	$C_s - C$	$K_{20}(C_s - C)$	K_T	$K_T(C_s - C)$	PR (K_{20})	PR (K_T)
7.8										
8.5	0.7	18.00	9.5	8.2	1.3	0.12	0.086	0.11	0.58	0.59
9.4	0.9	18.25	9.4	9.0	0.4	0.04	0.086	0.03	0.86	0.87
10.2	0.8	19.00	9.3	8.8	0.5	0.05	0.088	0.04	0.75	0.76
10.8	0.6	19.75	9.1	10.5	-1.4	-0.13	0.089	-0.12	0.73	0.72
11.6	0.8	20.25	9.0	11.2	-2.2	-0.21	0.090	-0.20	1.01	1.00
12.2	0.6	21.00	8.8	11.9	-3.1	-0.29	0.092	-0.28	0.89	0.88
12.6	0.4	21.75	8.6	12.4	-3.8	-0.36	0.094	-0.36	0.76	0.76
12.4	-0.2	22.25	8.5	12.5	-4.0	-0.38	0.095	-0.38	0.18	0.18
12.3	-0.1	22.50	8.5	12.4	-3.9	-0.37	0.096	-0.37	0.27	0.27
11.5	-0.8	22.50	8.5	11.9	-3.4	-0.32	0.096	-0.33	-0.48	-0.47
10.9	-0.6	22.25	8.5	11.2	-2.7	-0.26	0.095	-0.26	-0.34	-0.34

Key: $C = DO$ (g m^{-3})

$\Delta C/\Delta T =$ hourly change in C ($\text{g m}^{-3} \text{ hr}^{-1}$)

$K_{20} = K_2$, assuming no variation with temperature ($K_{20} = 0.095 \text{ hr}^{-1}$)

$K_T = K_2$ at the ambient temperature

using $K_T = K_{20} (1.0241)^{T-20}$ ($T = ^\circ\text{C}$)

Average Temp. + average $C =$ Calculated from the data (Appendix 3)

for the time period over which $\Delta C/\Delta t$ was computed

$PR =$ Net productivity ($\text{g O}_2 \text{ m}^{-3} \text{ hr}^{-1}$)

calculated using equation (3.8) :-

$$PR = \frac{\Delta C}{\Delta t} - K_2 (C_s - C)$$

APPENDIX 6

INTERSITE PHOSPHORUS NUTRIENT AVAILABILITY TESTS COMPARISONS,
TESTING THE HYPOTHESIS OF INCREASED DOWNSTREAM PHOSPHORUS
LIMITATION OCCURRING DURING 1982/83 (†-TESTS)

APPENDIX 6 Intersite Phosphorus Nutrient Availability Tests comparisons,
testing the hypothesis of increased downstream Phosphorus
limitation occurring during 1982/83 (t-tests)

	Comparing Sites T-D	Comparing Sites T-M	Comparing Sites D-M
TTP	Sig. at $P < 0.01$	Sig. at $P < 0.01$	No sig. diff.
EP	Sig. at $P < 0.05$	Sig. at $P < 0.02$	No sig. diff.
APA	Sig. at $P < 0.05$	Sig. at $P < 0.05$	No sig. diff.
PUR	No sig. diff.	No sig. diff.	No sig. diff.

KEY:

TTP = Total Tissue Phosphorus

EP = Extractive Phosphorus

APA = Alkaline Phosphatase Activity

PUR = Phosphorus Uptake Rate

APPENDIX 7

CLADOPHORA BIOMASS DENSITY, ENVIRONMENTAL PARAMETERS AND
PRIMARY PRODUCTIVITY DATA, 1981/82

APPENDIX 7 *Cladophora* biomass density, environmental parameters and primary productivity data, 1981/82

Date	BM ₂ (g m ⁻²)	Light (Langs.d ⁻¹)	Flow (m ³ s ⁻¹)	Av.Temp. (°C)	GP (gO ₂ m ⁻³ d ⁻¹)	TR (gO ₂ m ⁻³ d ⁻¹)	P/R	NAP (gO ₂ m ⁻² d ⁻¹)	DOD _m (g m ⁻³)	ΔDO (g m ⁻³)
4/11/81	b.d	295	56.3	15.0	0.87	-1.55	0.56	-0.36	Nd	0.5
5/11/81	b.d	241	49.9	15.0	1.18	-2.09	0.56	-0.48	Nd	0.8
18/11/81	b.d	565	38.1	17.5	2.28	-2.38	0.96	-0.05	Nd	1.4
19/11/81	b.d	680	48.5	17.0	2.93	-1.42	2.07	0.82	Nd	0.9
20/11/81	b.d.	350	39.1	17.0	2.88	-2.58	1.11	0.16	Nd	1.5
21/11/81	b.d.	726	37.0	18.0	3.02	-2.87	1.05	0.08	Nd	2.0
22/11/81	b.d.	738	31.7	17.0	2.96	-2.20	1.35	0.42	Nd	1.5
23/11/81	b.d.	653	29.2	18.0	2.75	-1.76	1.56	0.59	Nd	1.5
27/11/81	1.0	617	22.8	18.0	3.85	-4.21	0.91	-0.27	1.1	1.7
28/11/81	1.0	465	22.3	18.0	4.31	-3.84	1.12	0.36	0.1	0.9
29/11/81	1.0	353	21.2	18.0	2.96	-1.12	2.64	1.49	0.1	1.3
7/12/81	2.5	403	32.8	16.0	3.79	-4.72	0.80	-1.49	1.8	1.5
15/12/81	b.d.	261	38.8	18.5	1.26	-1.81	0.70	-0.30	-0.5	0.7
16/12/81	b.d.	387	33.8	18.5	5.81	-7.15	0.81	-0.70	0.3	1.7
17/12/81	b.d.	185	32.5	18.0	2.59	-2.60	1.00	0.01	-0.3	1.1
18/12/81	b.d.	421	32.7	17.5	2.37	-2.74	0.86	-0.20	-0.7	0.8
19/12/81	b.d.	546	33.2	18.5	Nd	Nd	Nd	Nd	Nd	Nd
20/12/81	b.d.	202	35.2	19.0	3.74	-5.74	0.65	-1.09	0.7	2.2
21/12/81	b.d.	109	41.2	19.0	Nd	Nd	Nd	Nd	Nd	Nd
30/12/81	b.d.	592	23.2	21.0	4.21	-4.76	0.88	-0.41	1.5	1.8
31/12/81	b.d.	108	24.2	19.5	3.41	-4.26	0.80	-0.61	1.8	2.2

5/1/82	5.0	444	31.8	18.0	3.96	-5.39	0.74	-0.90	1.9	2.3
6/1/82	7.0	464	25.2	18.0	6.41	-6.89	0.93	-0.33	1.9	2.5
12/1/82	12	690	20.9	20.0	4.22	-5.06	0.83	-0.69	1.7	2.3
13/1/82	15	437	17.8	21.5	4.80	-6.02	0.80	-1.12	2.2	3.0
14/1/82	17	433	15.8	18.5	6.25	-5.12	1.22	1.08	1.6	2.8
15/1/82	19	581	14.9	19.5	8.48	-8.90	0.95	-0.41	1.8	3.4
16/1/82	21	541	14.4	18.0	8.14	-7.66	1.06	0.48	1.6	3.0
17/1/82	24	723	15.2	19.0	9.52	-9.93	0.96	-0.41	2.1	4.0
30/1/82	13	698	32.2	21.0	1.82	-2.78	0.65	-0.59	1.3	1.0
31/1/82	14	539	28.1	20.5	2.14	-3.22	0.67	-0.76	1.5	1.2
1/2/82	15	474	25.5	20.5	2.36	-3.15	0.75	-0.58	1.2	1.4
2/2/82	17	552	23.2	20.5	2.70	-3.63	0.74	-0.77	1.5	1.7
3/2/82	18	388	19.5	18.5	3.62	-3.85	0.94	-0.19	1.5	1.8
4/2/82	19	688	14.8	18.0	2.62	-3.38	0.78	-0.71	1.7	2.4
5/2/82	20	559	13.9	19.0	5.72	-4.95	1.16	0.77	1.1	2.2
9/2/82	22	657	14.6	21.0	7.19	-6.46	1.11	0.70	1.5	3.9
10/2/82	26	344	13.1	20.0	8.92	-7.68	1.16	1.32	1.7	4.0
11/2/82	33	650	12.7	18.0	10.16	-8.64	1.18	1.63	1.8	3.9
12/2/82	40	624	12.6	18.0	7.18	-5.95	1.21	1.35	1.9	5.0
13/2/82	47	664	12.4	16.5	7.06	-5.83	1.21	1.36	2.0	4.5
14/2/82	54	643	12.4	18.5	6.92	-7.44	0.93	-0.57	2.4	5.1
17/2/82	65	291	12.0	18.0	7.42	-9.87	0.75	-2.78	2.5	3.1
18/2/82	65	036	11.9	19.0	5.23	-7.46	0.70	-2.56	1.7	2.1
20/3/82	17	341	18.2	15.0	6.75	-6.55	1.03	0.21	1.3	2.8
21/3/82	18	482	16.0	15.5	7.78	-7.43	1.05	0.40	1.3	3.4

22/3/82	19	467	15.1	17.0	8.17	-7.56	1.08	0.73	1.2	3.5
27/3/82	23	447	19.2	16.5	8.36	-8.24	1.01	0.11	1.5	3.9
28/3/82	27	360	15.3	17.0	8.88	-8.92	1.00	-0.04	1.5	3.9
29/3/82	30	214	14.8	16.0	9.34	-9.37	1.00	-0.04	1.8	4.5
30/3/82	33	113	14.4	16.0	7.83	-7.88	0.99	-0.06	1.7	3.6
6/4/82	27	331	24.1	15.0	2.00	-6.30	0.32	-5.28	1.5	1.8
7/4/82	29	402	29.6	15.0	2.81	-5.74	0.49	-2.42	2.3	2.0
16/4/82	7	315	31.0	13.0	2.08	-3.96	0.53	-1.32	1.5	0.8
17/4/82	8	352	33.8	13.0	1.46	-3.47	0.42	-1.30	1.7	1.0
18/4/82	9	311	28.5	13.5	0.79	-2.69	0.29	-1.43	1.6	0.7
19/4/82	10	261	22.6	13.5	2.20	-2.48	0.89	-0.24	0.6	0.8
20/4/82	11	331	20.7	13.0	2.25	-3.29	0.68	-0.96	0.9	1.1
21/4/82	12	334	18.9	12.0	1.70	-2.43	0.70	-0.74	1.0	1.0
22/4/82	13	230	17.5	12.5	1.21	-1.94	0.62	-0.67	0.8	1.7
23/4/82	15	303	19.0	13.0	0.72	-2.12	0.34	-1.18	0.9	1.2
27/4/82	17	279	21.1	11.5	1.98	-3.79	0.52	-1.51	1.4	1.1
28/4/82	18	302	20.7	12.0	3.22	-3.95	0.82	-0.62	1.0	1.4
20/4/82	19	213	21.3	12.0	1.79	-3.10	0.58	-1.08	1.1	1.6

b.d. below detection

Nd no data/not determined

Key:

BM = Average *Cladophora* site biomass

Light = Daily terrestrial light

Flow = Average daily river flow

Av. temp. = Average daily river temperature

GP = Gross Photosynthesis

TR = Total Respiration

P/R = The ratio GP/TR

NAP = Net Areal Primary Productivity

DODm = Daily Maximum Dissolved Oxygen Deficit

Δ DO = Daily Dissolved Oxygen Fluctuation

APPENDIX 8

CLADOPHORA BIOMASS DENSITY, ENVIRONMENTAL PARAMETERS AND
PRIMARY PRODUCTIVITY DATA, 1982/83

APPENDIX 8 *Cladophora* biomass density, environmental parameters and primary productivity data, 1982/83

Date	BM g m ⁻²	Light Terr. (Langs.d ⁻¹)	Light U/W (E m ⁻² d ⁻¹)	Flow m ³ s ⁻¹	Temp. Min. (°C)	Temp. Max. (°C)	GP (g O ₂ m ⁻³ d ⁻¹)	TR (g O ₂ m ⁻³ d ⁻¹)	P/R	NAP (g O ₂ m ⁻² d ⁻¹)	DOD _m (g m ⁻³)	ΔDO (g m ⁻³)
7/12/82	2.9	610	25.2	44.0	17.0	19.5	3.77	-4.65	0.81	-0.99	0.9	1.2
8/12/82	3.1	731	Nd	37.0	17.0	20.0	3.97	-5.15	0.77	-1.49	1.3	1.3
9/12/82	3.3	599	29.9	32.6	18.0	20.5	3.62	-9.65	0.37	-8.17	2.3	0.9
10/12/82	3.6	208	11.7	29.1	18.5	20.0	5.13	-11.52	0.44	-9.70	2.2	2.1
4/1/83	bd	439	Nd	51.0	18.0	20.5	2.07	-5.62	0.37	-3.91	Nd	0.6
5/1/83	bd	613	Nd	42.3	18.0	20.0	2.23	-3.82	0.58	-2.08	Nd	1.3
6/1/83	bd	249	Nd	52.0	16.0	17.0	3.30	-3.69	0.89	-0.42	Nd	1.5
7/1/83	bd	371	Nd	42.7	15.0	16.5	3.59	-3.66	0.98	-0.10	Nd	1.6
8/1/83	bd	473	Nd	47.0	15.0	16.5	4.59	-3.55	1.29	1.22	Nd	1.9
9/1/83	bd	502	Nd	42.1	14.5	17.0	4.90	-6.59	0.74	-2.22	Nd	1.5
10/1/83	bd	172	Nd	39.3	16.0	17.0	4.82	-9.93	0.49	-6.46	Nd	1.8
11/1/83	bd	396	Nd	34.8	17.0	21.0	6.67	-9.60	0.69	-3.73	Nd	2.3
12/1/83	bd	430	Nd	32.0	17.0	20.5	8.57	-5.72	1.50	3.93	Nd	4.1
13/1/83	0.8	234	Nd	29.8	17.0	20.0	5.11	-4.64	1.10	0.70	0.9	2.7
14/1/83	1.1	661	19.3	64.5	16.5	18.5	3.06	-4.23	0.72	-1.17	1.4	1.7
15/1/83	1.4	454	14.1	50.4	17.0	18.5	4.30	-9.12	0.47	-5.37	2.8	2.4
16/1/83	1.8	482	12.3	34.5	15.5	17.5	7.51	-9.09	0.83	-2.10	2.0	4.7
17/1/83	2.2	570	14.1	47.8	16.5	18.0	7.52	-8.67	0.87	-1.35	2.7	4.0
18/1/83	2.6	657	Nd	38.5	17.5	19.0	5.39	-6.93	0.78	-1.95	2.7	4.3
19/1/83	2.9	588	Nd	35.5	17.0	19.0	7.43	-9.14	0.81	-2.22	2.1	4.2

28/1/83	19	330	18.2	39.8	16.5	18.5	12.63	-13.88	0.91	-1.54	2.5	4.0
29/1/83	26	323	15.2	48.7	17.5	20.0	10.32	-15.41	0.67	-5.88	3.3	3.2
30/1/82	32	361	9.4	44.3	16.5	18.0	10.56	-17.24	0.61	-8.47	3.0	3.5
31/1/83	38	613	28.7	31.0	16.5	20.0	12.76	-15.47	0.83	-3.85	2.5	4.1
1/2/83	40	650	22.3	29.1	17.0	20.0	10.94	-14.39	0.76	-5.16	2.7	3.3
2/2/83	40	665	25.8	25.5	18.5	21.5	13.68	-15.28	0.89	-2.45	2.7	4.1
3/2/83	41	273	12.3	23.6	18.5	21.0	13.37	-17.95	0.74	-7.44	2.8	4.1
4/2/83	42	184	14.6	22.5	17.5	20.5	8.76	-16.22	0.54	-11.64	2.9	2.8
5/2/83	43	610	17.0	23.1	16.0	19.0	8.93	-16.69	0.54	-12.86	3.2	3.3
6/2/83	44	566	7.0	22.5	15.5	18.0	10.83	-13.30	0.81	-3.86	2.5	4.2
7/2/83	45	364	Nd	24.5	15.0	17.0	7.33	-11.99	0.61	-7.29	2.3	3.2
18/2/83	7.5	559	15.8	26.0	20.5	22.0	5.92	- 8.97	0.66	-4.72	1.4	1.6
19/2/83	8.3	511	17.0	23.5	20.0	22.0	7.49	- 8.87	0.85	-2.20	1.2	1.9
20/2/83	9.0	560	19.3	18.7	20.0	22.0	6.05	- 7.32	0.83	-2.30	1.3	1.8
21/2/83	9.5	236	10.0	20.0	17.5	20.5	5.28	- 7.57	0.70	-3.87	1.2	1.8
22/2/83	10	585	22.8	19.4	19.0	20.5	6.84	- 8.39	0.82	-2.66	1.4	1.9
23/2/83	10	306	21.7	19.4	19.0	21.5	6.71	- 8.94	0.75	-3.82	1.5	1.9
24/2/83	11	365	17.0	18.3	19.0	20.5	6.99	- 9.22	0.76	-3.83	1.4	2.2
25/2/83	12	507	17.6	18.3	19.0	21.5	7.38	- 8.63	0.85	-2.16	1.5	2.3
26/2/83	16	410	14.1	15.1	18.5	21.0	7.83	- 9.37	0.84	-3.09	1.5	2.4
27/2/83	21	567	19.3	11.0	19.0	21.0	12.77	- 10.10	1.26	7.07	1.2	2.8
28/2/83	26	195	14.1	16.5	18.0	20.0	8.80	- 7.92	1.11	1.62	1.1	2.7
1/3/83	32	193	8.2	15.6	18.0	18.0	7.92	- 6.90	1.15	1.98	0.8	2.5
2/3/83	38	469	Nd	16.3	18.5	21.5	11.09	- 4.86	2.28	11.56	0.6	3.5
11/3/83	68	340	7.6	48.5	15.5	19.0	7.63	- 8.65	0.88	-1.23	1.6	2.3

12/3/83	66	399	14.1	40.5	17.0	19.0	10.16	7.31	1.39	3.44	1.0	4.1
13/3/83	64	281	14.1	29.5	18.0	20.0	10.17	7.33	1.39	3.77	1.4	3.9
14/3/83	62	236	12.9	23.7	18.5	20.5	12.17	8.60	1.41	5.76	1.3	3.8
15/3/83	61	331	18.2	33.9	17.0	20.0	8.77	5.53	1.59	3.71	1.0	4.3
21/3/83	24	298	17.6	22.0	16.5	18.5	6.07	11.32	0.54	-9.13	1.6	2.0
22/3/83	25	436	19.9	19.6	18.0	19.0	9.14	12.69	0.72	-6.02	2.0	2.6
23/3/83	26	124	8.2	18.0	18.5	19.0	7.70	11.80	0.65	-6.69	1.6	2.6

bd = below detection limits

Nd = no data/not determined

KEY:

Abbreviations as in appendix

Light U/W = light (PAR) at 30cm depth.

Temp. min. = The minimum daily temperature

Temp. max. = The maximum daily temperature