RIVER OXYGEN UPTAKE BY BENTHIC MICROORGANISMS

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ABSTRACT

The factors influencing benthic microbial uptake of river dissolved oxygen (DO) and dissolved organic matter (DOM) were investigated in 4 New Zealand rivers receiving organic waste discharges from dairy factories and a pulp and paper company. A chamber system for in situ use was developed and tested against segment oxygen mass balances in 3 rivers. Benthic oxygen uptake rate (BUR) was found to be strongly affected by circulation velocity, electron acceptor (oxygen) concentration and in some instances electron donor (glucose) concentration. Satisfactory agreement between calculated BUR values and those obtained using the chamber was obtained with matching boundary velocities (0.05 m above bed) in the 2 shallow (<0.5 m) rivers, with low chamber values in the deep (>1.5 m) river. Thus chamber devices must adequately simulate the natural flow and turbulence conditions in order to obtain realistic BUR measurements. Maximum BUR was measured for filamentous 'sewage fungus' colonisation at 70.2 g O_2 m^{-2} day^{-1} at 7.0 g O_2 m^{-3}.

Benthic oxygen penetration depths were estimated from chamber and sediment intrinsic activity measurements and ranged from 0.32 to 32 cm, with minimum values for 'sewage fungus' biofilms. Actual measurements of interstitial DO for 2 pumice bed rivers, showed DO present down to at least 10 cm in the Waiotapu River and down to 15 cm in the Tarawera River. The estimated oxygen penetration depths were used to calculate benthic dispersion coefficients. These values ranged from 0.38-16.30 x 10^{-3} cm^2 s^{-1} which are several orders of magnitude higher than previously reported maxima. Such high values imply that a high rate of sediment-water exchange was sustained by turbulent mass transfer processes rather than molecular diffusion.

Benthic biomass values by ATP extraction ranged from 0.67-4.95 µg ATP cm^{-3}, with higher maximum values for the rivers studied than have been reported for sediments in other aquatic systems, and suggesting greater levels of metabolic activity. Benthic biomass involved with sediment-water exchange was shown to be up to 50 times the planktonic biomass present, and generally 5 to 10 times for sites below organic discharges.

Longitudinal and depth profile studies of enzyme activities showed that the predominant benthic metabolic activity changed in relation to distance from the organic discharge and with depth beneath the sediment surface, presumably reflecting changes in organic constituents and concentrations in the overlying flow. Removal of organic wastes from the river water in shallow systems was shown to be largely the net effect of 2 processes: (i) active benthic microbial removal of DOM and (ii) passive settling particulate organic material.

It is concluded that lotic river environments may sustain higher levels of benthic biomass and nutrient transformation than do lentic environments as a result of turbulent sediment-water exchange processes. Substantial differences in the level of activity will occur between rivers with an erosional environment, which promote epilithic metabolic processes, and those with a depositional environment where detrital processes predominate.

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REASONS FOR THE PROJECT

The agricultural sector of the New Zealand economy releases large amounts of organic waste into our river systems. The assimilation of this organic material by the microorganisms present in the receiving water can result in reduced oxygen levels.

The physical river processes of dilution, transport and reaeration are important in determining the river assimilation capacity for organic wastes. Biochemical processes are also of great importance, and of these the rate of exertion of biochemical oxygen demand is often critical. This rate is controlled by the activity of benthic and planktonic bacteria.

Planktonic oxygen demand exertion and physical processes are covered in other projects at the Water Quality Centre, Hamilton. This project addresses the need for detailed research into benthic microbial processes occurring in New Zealand rivers. Through _in situ_ and laboratory investigations, methods are developed and applied to a variety of lotic systems for the assessment of the impact of organic waste discharged to the river.

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Symbol		**Units**
A	biofilm surface area	m^{-2}
A_P	macrophyte photosynthetic rate	$g\ O_2\ g\ DW^{-1}\ h^{-1}$
A_R	macrophyte respiration rate	$g\ O_2\ g\ DW^{-1}\ h^{-1}$
A_{nnn}	absorbance at wavelength nnn	dimensionless
A^*_{nnn}	absorbance after acidification at wavelength nnn	dimensionless
b	pathlength of cuvette	cm
B	BUR rate constant	$g\ O_2\ m^2\ day^{-1}$
C	arbitrary dissolved oxygen (DO) concentration	$g\ m^{-3}$
C_b	DO concentration in bulk liquid	$g\ m^{-3}$
C_o	sediment surface or upstream DO concentration	$g\ m^{-3}$
C_s	saturation DO concentration	$g\ m^{-3}$
D	dilution factor	dimensionless
D_s	benthic dispersion coefficient	$cm^2 s^{-1}$
$\bar{D}$	DO deficit	$g\ m^{-3}$
d	depth from sediment surface to midpoint of uppermost region of the sediment	cm
F	flux from the water to the sediment	$g\ m^{-2}\ day^{-1}$
H	mean depth of water	m
I	intrinsic oxygen uptake rate of sediments or biofilm	$g\ O_2\ m^{-2}\ day^{-1}$
I^*	intrinsic oxygen uptake rate of sediments or biofilm	$g\ O_2\ g^{-1}\ day^{-1}$
K	oxygen mass transfer exponent (OTE)	$m\ day^{-1}$
K'	OTE	$m^3\ g^{-1}$
k	coefficient of sediment/biofilm respiratory decay (base e)	day^{-1}
k_1	a first-order, decreasing specific rate coefficient	$time^{-1}$

k_2	atmospheric reaeration coefficient (base e)	day^{-1}
k_L	mass transfer coefficient (base e)	$m\ day^{-1}$
k_m	half-saturation constant for microbial growth. The Monod constant	$g\ m^{-3}$
k_s	half-saturation constant of an enzymic reaction where the reaction is at half the maximum velocity. The Michaelis-Menten constant	$g\ m^{-3}$
L	ultimate oxygen demand of sample	$g\ m^{-3}$
L_o	total carbon of sample	g
m	coefficient for power law approximation to a logarithmic boundary velocity profile	dimensionless
N	BUR	$g\ O_2\ m^{-2}\ day^{-1}$
P	gross oxygen production due to photosynthesis	$g\ O_2\ m^{-3}\ day^{-1}$
Q	flow	$m^3\ s^{-1}$
Q_{O2}	respiratory quotient	dimensionless
R	planktonic respiration rate	$g\ O_2\ m^{-3}\ day^{-1}$
R_m	macrophyte respiration rate	$g\ O_2\ m^{-3}\ day^{-1}$
R_e	Reynolds number	dimensionless
S	a general symbol for microbial substrate; usual designates an organic carbon source	$g\ m^{-3}$
t	time	h, day
T	temperature	°C
U	mean velocity	$m\ s^{-1}$
v	point velocity	$m\ s^{-1}$
V_T	total volume of chamber	m^3
V_A	volume of chamber exposed to sampling area	m^3
V_E	volume of water filtered for chlorophyll assays	l
V_s	volume of chlorophyll extract	ml
V_e	volume of extract in sample	ml

V_t	total assay volume	ml
W	dry weight of sample extracted	g
x	PNP hydrolysed	μg
X	biomass	$g\ m^{-3}$
X_o	biomass at time t = 0	$g\ m^{-3}$
X_t	biomass at time t = t	$g\ m^{-3}$
X_m	macrophyte biomass	$g\ m^{-3}$
Y	carbon equivalent of oxygen consumed	$g\ C\ m^{-3}$
Y_B	biomass yield; the weight of cells produced per weight of substrate utilised	dimensionless
Y_{o2}	respiratory yield coefficient	dimensionless
Y_s	substrate yield coefficient	dimensionless
z	fixed height above river bed	m
δ	biofilm depth	mm
δ_c	biofilm depth of limiting nutrient penetration	mm
η	effectiveness factor	dimensionless
ρ	bulk density of sediment	$g\ ml^{-1}$
ρ^*	particle density	$g\ ml^{-1}$
μ	specific growth rate (exponential)	h^{-1}
μ'	specific growth rate (linear)	$g\ m^{-3}\ h^{-1}$
ϕ	porosity in volume of pore water per unit volume of total sediment	dimensionless

LIST OF ABBREVIATIONS

ATP	adenosine-5'-triphosphate
BOD	biochemical oxygen demand
BUR	benthic oxygen uptake rate
COD	chemical oxygen demand
DO	dissolved oxygen
DOM	dissolved organic material
EBOD	electrolytic biochemical oxygen demand respirometer
ETS	electron transport system
NUR	nitrogenous oxygen uptake rate
OTE	oxygen mass transfer exponent
PHB	poly-β-hydroxybutrate
PNA	ρ-nitroanaline
PNP	ρ-nitrophenol
POM	particulate organic material
SOD	sediment oxygen demand

GLOSSARY

Allocthonous: Organic matter derived from photosynthesis in the catchment basin and brought into the receiving water in various forms.

Aquatic: Pertaining to organisms living in an environment surrounded by water.

Assimilation capacity: The ability of a body of water that receives waste components to permit the aerobic metabolism of the waste to proceed without limitation due to dissolved oxygen concentration.

Assimilation: The microbial process of uptake of substrate production of new biomass and respiration.

Autochthonous: Organic matter derived from photosynthesis occurring within the water body.

Autotroph: An organism that uses carbon dioxide as its source of carbon for the synthesis of cell material.

Benthic: Pertaining to the bed of a body of water.

Benthos: Organisms living on or in aquatic sediments.

Biofilm respiratory decay kinetics: The decline in respiratory oxygen uptake of a biofilm in the absence of an external supply of organic substrates.

Biomass yield coefficient: The amount of biomass formed per unit of substrate consumed.

Boundary velocity: A flow velocity measured 0.05 m above the benthic substratum.

Constituative enzyme: An enzyme normally present in a microbial cell.

Detrital heterotroph: A heterotrophic microorganism which decomposes particulate organic detirus, requiring exoenzymic breakdown prior to assimilation and respiratory decay.

Effective activity: The metabolic activity of attached organisms as modified by diffusion limitations for supply of nutrients.

Electron acceptor: A chemical entity tht accepts electrons transferred to it from another compound. It is an oxidising agent which, by virtue of its accepting electrons, is itself reduced in the process. Oxygen is the terminal electron acceptor for aerobic metabolism.

Electron donor: A compound or element that supplies electrons for reductive reactions. Reduced organic compounds are the elctron donors for heterotrophic growth. H_2O, H_2S, NH_3 etc are electron donors for autotrophic growth.

Epilithic heterotrophs: A heterotrophic organism which grows attached to a rocksubstratum, and which metabolises dissolved organic matter from the surrounding water.

Heterotroph: An organism that requires organic material as its source of carbon for making cell materials.

Induced enzyme: An enzyme which is producted by a microorganism in response to the presence of a specific substrate.

Intrinsic activity: The metabolic activity of a microorganism or group of microorganisms once diffusion limitations of nutrient supply are removed.

Lentic: Of standing bodies of water.

Lotic: Of flowing waters.

Logarithmic growth: The phase of growth in which the time required for doubling of the cell mass is constant.

Macroscopic rate: The overall metabolic rate measured in the presence of sediment or biofilm structure and natural hydrodynamic conditions.

Microbial biomass: The living material of bacteria, algae and fungal organisms.

Phototroph: An organism that uses light as its primary energy source.

Potential activity: The macroscope rate measured in the presence of excess electron donor (substrate) concentration.

Respiration: The biological process by which organisms pass electrons to a terminal electron acceptor where they oxidise organic or inorganic substrates. During this process, energy is trapped in the form of ATP. Aerobic respiration refers to the use of molecular oxygen as terminal electron acceptor. Anaerobic respiration refers to the use of a compound other than oxygen as terminal electron acceptor, e.g. nitrate in the process of denitrification.

Respiratory quotient: The amount of oxygen consumed per unit of substrate.

Sediment: The material making up the bed of a water body.

Substrata/substratum: The particulate inorganic sediment.

Substrate: The reducing potential (or electronic donor material) used to supply energy for growth.

Sediment respiratory decay kinetics: The decline in respiratory oxygen uptake in the absence an external supply of organic substrates.

Steady state: A condition in which the rate of change of parameters of interest in describing a process is zero.

Chapter 1

INTRODUCTION

1.1 GENERAL INTRODUCTION

The development of steady-state models of dissolved oxygen requires quantitative data pertaining to the sources and sinks of river dissolved oxygen (DO) (Fig. 1.1). Experimental methods to obtain the parameters necessary for model implementation, were required for reaeration measurement ((Wilcock, 1984a, b), aquatic oxygen demand exertion (Hickey and Nagels, 1985), and benthic oxygen uptake rate (BUR) measurement. Additional information concerning the type of biomass present (i.e. autotrophic or heterotrophic) and the nutrient requirements for growth were necessary to relate oxygen consuming processes with nutrient removal processes (e.g. biochemical oxygen demand [BOD] decay).

In shallow rivers, geometric and hydraulic factors combine to make the sessile epilithic, epiphytic and sedimentary bacterial populations as much as 1000 times more numerous than the planktonic bacteria in a given section of the water body (Ladd *et al.*, 1979). If we assume that these sessile bacteria metabolise at the same rate as their planktonic counterparts, we must assume that the heterotrophic activity of the bacteria from the water column would represent only a small fraction of the total heterotrophic bacterial activity that removes dissolved organic matter (DOM) from the system. This is especially so when organic loading results in filamentous microbial growths attached to the streambed, extracting organic material from the water flowing above, and performing in a manner analogous to the trickling filter (Velz, 1970). Evidence indicates that the rate of BOD removal increases with decreasing river depth of flow or increased wetted perimeter (Wright and McDonnell, 1979). The limited data available for New Zealand rivers point in the same direction (Rutherford and O'Sullivan, 1974; McBride, 1982). It appears that removal of BOD by sessile bacterial communities can cause quite substantial losses of river oxygen, particularly if the river is shallow and is subjected to additional inputs of organic material.

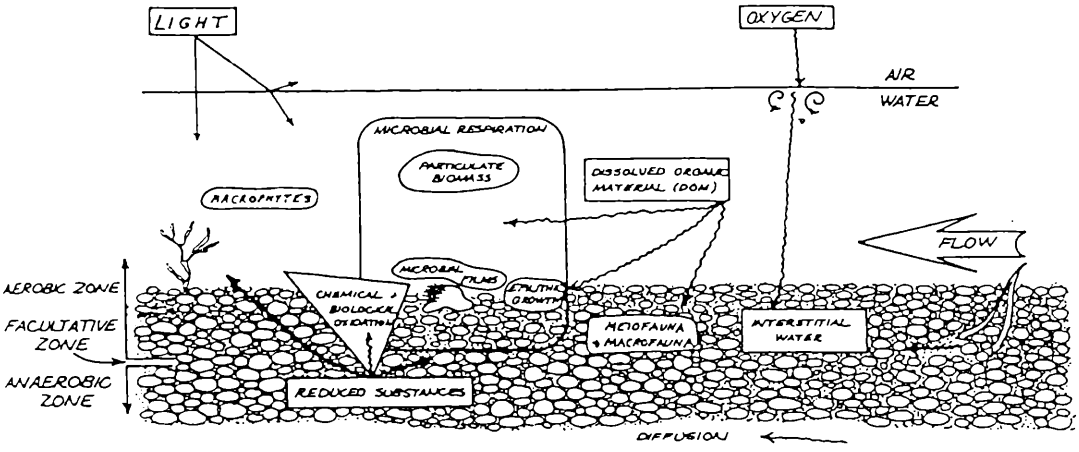


Figure 1.1 : River oxygen: sources and sinks.

The potential for DO removal by attached biomass is high in shallow lotic conditions. Both phototrophs (e.g. algae) and heterotrophs (e.g. bacteria) may be important. At high velocities and turbulence levels an erosional environment exists, with little settling of fine particulate material, and there is a high degree of interaction between interstitial waters and the overlying flow. This enables benthic microbial biomass to metabolise both electron donors (e.g. DOM, ammonium) and electron acceptors (e.g. oxygen, nitrate, sulphate) supplied from the flowing water for respiration and the production of cell biomass. In contrast, a lentic system with reduced water velocities represents a depositional environment, where settled silt, seston and detritus form the sediment, with metabolism of particulate organic material (POM) by microbial biomass and supply of electron acceptors from the overlying flow. The type of microbial biomass present will therefore determine the effect on BUR kinetics of electron donor and electron acceptor concentrations, and hence determine the protocols necessary for performing chamber flux measurements.

1.2 OVERALL AIMS

The ultimate aims, to which this project seeks to contribute are: (i) to apply modelling techniques with a predictive ability for river DO and BOD decay under steady-state conditions; (ii) to develop modelling techniques for application to a priori prediction in relation to environmental perturbation.

Both the location and nature of biological biomass influence the likely effect on the river system. Attached biomass adapts to and influences the overlying flow, whereas, planktonic biomass is advected downstream, with both subjected to continually changing environmental conditions. There are substantial differences between the characteristic time scales for growth and metabolism in each of these communities, and hence the criteria for steady-state approximations. Because the relative importance of attached and planktonic biomass may be anticipated to change between small and large rivers, model construction must account for individual process components. Additionally, the importance of benthic populations may differ considerably between erosional and depositional environments in response to factors such as sediment/water exchange, particle size, and organic carbon content.

To achieve the above aims, studies of processes were necessary in order to identify the relative importance of attached benthic and planktonic microbial populations, the physical, chemical and biological components affecting benthic microbial biomass and activity, and the inter-relationship between these populations and the overlying flow.

1.3 OBJECTIVES OF THE PRESENT STUDY

The objectives were directed towards developing the techniques and methodologies for application to river systems in order to identify the relative importance of physical, chemical and biological processes associated with benthic DO and BOD assimilation processes. Specifically, these objectives were:

i to develop an <u>in situ</u> benthic chamber technique;

ii to investigate the factors influencing BUR (including flow velocity, electron acceptor (e.g. oxygen) and electron donor (e.g. BOD) concentrations, biomass (type and concentration));

iii to develop biochemical methods for the assessment of microbial biomass and activity in relation to organic effluent assimilation;

iv to apply these methods to a variety of rivers receiving a point source of organic effluent to study: (a) the role of upstream and downstream microbial populations; (b) the relative importance of benthic and aquatic biomass to oxygen removal processes.

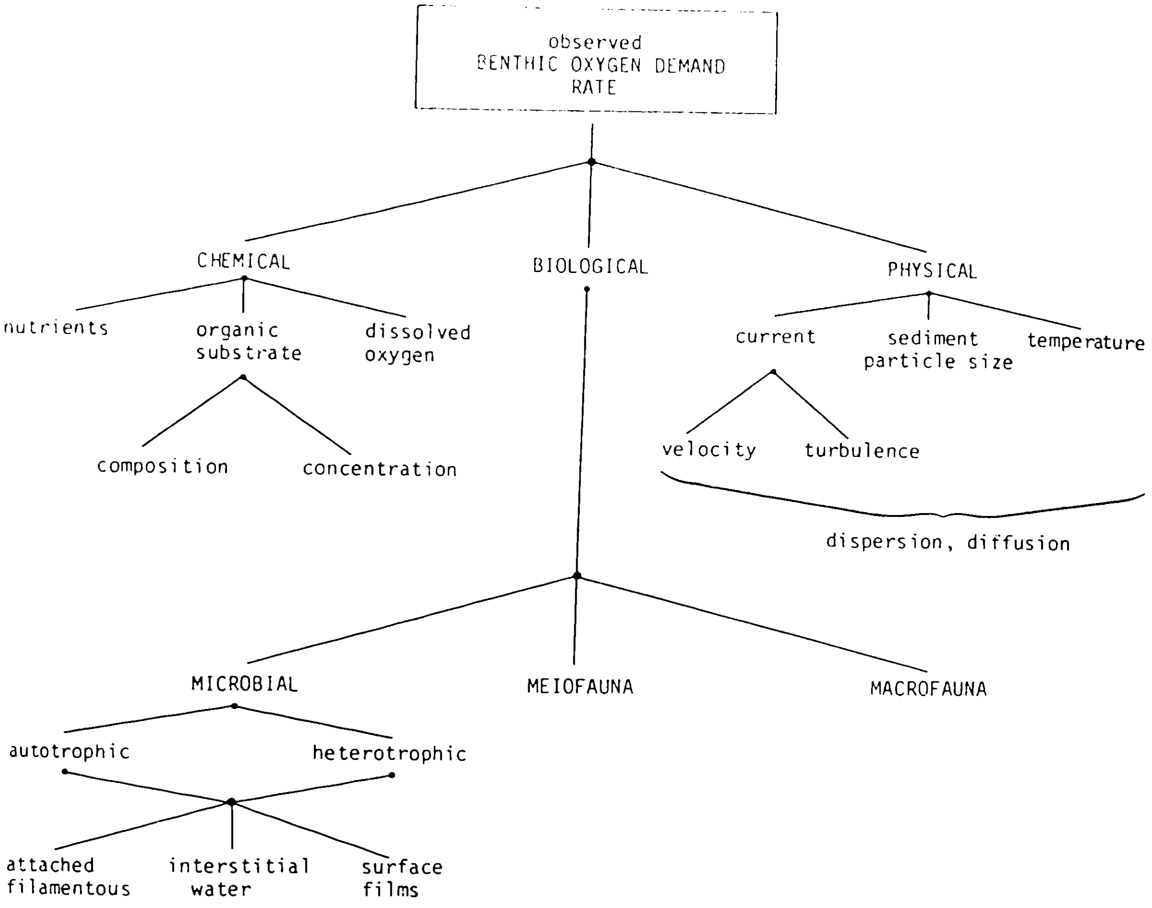


Figure 1.2 : Schematic representation of factors affecting the benthic oxygen uptake rate (BUR) in a lotic system.

Process Description	Biomass		Requirements		Favoured by:
	Type	Example	Electron donor	Electron acceptor	
epilithic[a]	autotrophic	nitrifiers	NH_4^+	O_2	) moderate-) high) velocity
	heterotrohic	bacteria	DOM	O_2, NO_3^-	
detrital[a]	heterotrophic	bacteria protozoa	POM	O_2, NO_3^-	low velocity
phototrophic[a]	phototrophic	algae	hυ	H_2O, O_2	low-high velocity, light

Abbreviations: DOM, dissolved organic matter; POM, particulate organic matter;

[a] See Glossary for additional definition of terms

Table 1.1: A summary of microbial transformation processes associated with sediment-water interactions

1.4 HYPOTHESES

A schematic representation of the interacting factors affecting the benthic oxygen uptake rate is shown in Figure 1.2. The particulate substratum and current are inseparably linked and together greatly influence the biomass which may be present. Antecedent flow conditions may greatly influence bed particle size and the amount of fine debris present, with the larger floods altering even the very nature of the river channel configuration (e.g. pools, riffles and sinuosity). Current at low flow rates determines whether the river zone is depositional (low velocity allowing settling of fine particles), intermediate (moderate velocity with sand-gravel bed), or erosional (high velocity with dunes or coarse bed), with the degree of turbulence present influencing the exchange of nutrients across the sediment-water interface.

The chemical composition of the water together with the light available to the benthos, combine to influence the potential for biomass development (Fig. 1.1). Three situations may be envisaged with regard to microbial processes associated with sediment-water interactions and these are summarised in Table 1.1. All of the processes described remove oxygen from the overlying flow, whereas only the epilithic heterotrophic processes remove DOM and thus result in an active BOD removal. The detrital processes have a requirement for a supply of particulate organic material (POM) and in such situations BOD removal may occur as a result of physical (passive sedimentation), rather than microbial processes. It is, therefore, important to distinguish which process(es) is(are) occurring, in order to enable predictions of the likely effects of perturbations to be made.

The interacting factors (Fig. 1.2) form the basis for the following hypothesis: **The benthic microbial population in shallow rivers may act predominantly as either epilithic heterotrophs (requiring electron donors and electron acceptors from the overlying flow), detrital heterotrophs (requiring only electron acceptors from overlying flow), or a phototrophic population, depending on river chemical and physical conditions.**

Two working hypotheses were formulated to test this hypothesis:

i that simulation of natural hydrodynamic conditions is essential for measurement of the rate of nutrient exchange across the sediment-water interface

ii that the benthic microbial population will develop in response to the sources of energy available for growth, as limited by the prevailing hydrodynamic conditions.

1.5 CONCEPTUAL MODEL

To predict adequately the likely response of a river system to environmental perturbations requires an understanding of the factors influencing the final rate process. Such an outline is shown in Figure 1.3. However, a desire to quantify the magnitude of response necessitates a detailed evaluation of key components within the system. Design of the measurements necessary to achieve these aims in a field experimental programme, is assisted by developing a conceptual model of the system.

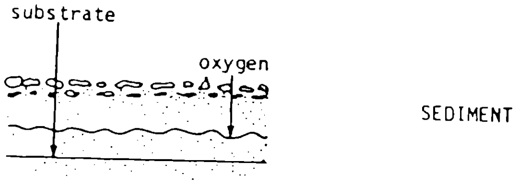
The ability of a given reactant to penetrate into the sediments is a function of the dispersion coefficient (influenced by sediment permeability and river turbulence), and the concentration gradient (influenced by microbial activity, river concentrations and the reaction stoichiometry). Mass transfer limitations are possible if:

i the reactant cannot penetrate through the complete active layer;

ii the microbial activity is so high that the reactant is consumed in the outer layer; or

iii the concentrations of the reactants in the river are so low that insufficient driving gradient is available. In this regard two cases may be postulated (i) the electron donor (e.g. glucose) penetrates deeper than the electron acceptor (e.g. oxygen), (ii) the electron acceptor penetrates deeper than the electron donor (Fig. 1.3).

Case (i) Electron donor (substrate) penetrates deeper than electron acceptor (oxygen)



Case (ii) Electron acceptor (oxygen) penetrates deeper than electron donor (substrate)

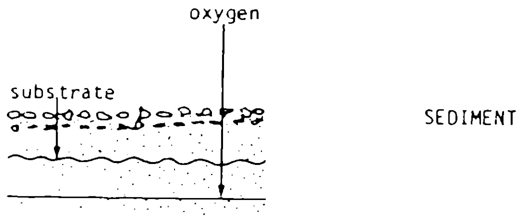


Figure 1.3 : Conceptual model for potential benthic mass transfer limitations in a lotic river system.

Thus measurements were required of the macroscopic rate which refers to the actual flux across the sediment-water interface *in situ*, which may be subjected to numerous constraints (e.g. diffusion, nutrient concentration, temperature). Removal of possible diffusion limitations by homogenisation of the sediment material with overlying flow and measuring the intrinsic activity (I) (which in this context will always refer to the rate of oxygen uptake), facilitates calculation of the minimum depth of sediment oxygen penetration. Macroscopic potential rate measurements may also be made, and refer to the maximum potential rate with excessive electron donor (substrate) concentration but at specified temperature. Electron donor and electron acceptor removal rates are related by the respiratory quotient (Qo_2) which refers to the amount of electron acceptor consumed per mass of electron donor the biomass yield coefficient : Y_B, which refers to the amount of cell biomass formed per mass of electron donor consumed and Yo_2 relative to the mass of electron acceptor consumed.

1.6 RESEARCH APPROACH

Testing of the working hypotheses was performed in 7 steps:

i Designing and testing of an *in situ* chamber system against segment oxygen mass balance determinations to establish credentials. Chamber design features allow for testing of the effects of velocity, electron acceptor and electron donor concentrations.

ii Development of suitable biomass measurement methods and estimation of the minimum depth of oxygen penetration in order to determine the relative importance of planktonic and benthic biomass in oxygen removal.

iii Metabolic studies were made on the whole system in order to determine the relative contribution made by types of biomass present (e.g. bacteria, algae, macrophytes) to river oxygen dynamics, using specific biochemical measurements.

iv Determination of the relationship between the benthic microbial biomass and the chemical characteristics of the river water by using enzyme activities and biomass determinations.

v Determination of the interactions between attached and planktonic populations in terms of river productivity and decay processes, using longitudinal surveys and planktonic biomass measurements.

vi Comparative studies made in different river systems (e.g. sand, pumice beds).

vii Derivation of predictive abilities for BUR from detailed analysis of chamber kinetics and diagenetic modelling.

Chapter 2

LITERATURE REVIEW

2.1 DISTRIBUTION OF BIOMASS IN RIVERS

2.1.1 Introduction

A large number of models, of varying complexity, have been developed to describe river oxygen mass balances since the classical model of Streeter and Phelps (1925). Early applications were restricted to large rivers where the important processes include reaeration, planktonic respiration by microbial communities and possibly photosynthesis. In small rivers with decreasing mean depth comes an increased importance of benthic microbial, invertebrate and macrophyte respiration. In such small rivers, the use of models developed in large rivers may give erroneous predictions because the key processes differ. To be useful as tools for planning and decision-making the key components must be identified. This particularly applies to the need to describe both river oxygen and organic assimilation processes. The latter process is restricted to heterotrophic microorganisms, whereas the former is associated with all biomass present. Although total biomass present may favour macrophytes or invertebrates, the high surface-to-volume ratio of the microbial biomass results in higher metabolic rates (Lassere, 1976). This justifies emphasis on microbial biomass for studies aimed at nutrient transformations in aquatic systems.

2.1.2 Planktonic Biomass

Bacteria in natural aquatic systems do not exist in the balanced, nutrient-rich environment typified by laboratory cultures. Bacteria in the aquatic environment must endure changes in solar inputs, substrate type, concentration of oxidisable substrate, nitrogen and phosphorus availability, and temperature and oxygen saturation. They are washed from the surface of both living and decaying material and they are scoured from the sediments (Stevenson, 1978).

For the survival of heterotrophic bacteria living in a natural aquatic environment a food supply is necessary in order to provide energy for the maintenance of cellular functions, for synthesis of new cellular material and for growth. In an unpolluted stream this food supply would be provided by allochthonous (i.e. decomposed organic matter which has leached into the water body) or autochthonous material (i.e. exudates and breakdown products derived from the photosynthesis of algae and macrophytes present). A waste effluent will greatly increase the concentration of organic substrate present in the aquatic environment and may also introduce a large microbial population. Such effluents may contain small organic molecules (e.g. sugars and amino acids) which may be rapidly assimilated by the bacteria, or larger and more complex molecules, (e.g. cellulose, proteins and fats), which require enzymatic degradation prior to their uptake by the microorganisms, and hence are assimilated more slowly.

Several formulations have been proposed to express the growth dynamics of a microbial population which is limited solely by the concentration of a single substrate. The earliest model of BOD progression was proposed by Phelps (1912) and has since received wide acceptance as a model for organic effluent addition to natural waters and for some waste waters. This is a monomolecular first order decay model that may be expressed as follows:

$$\frac{dS}{dt} = -k' S \qquad 2.1$$

where S is the amount of substrate (e.g. BOD) remaining to be oxidised at time t, and k' is the reaction coefficient with units of time^{-1}.

Monod (1942, 1949) developed a bimolecular model analagous to Michaelis-Menten kinetics which adequately described the relationship between microbial growth rate and substrate concentration. Monod kinetics may be expressed as follows:

$$\mu = \mu_{max} \cdot \frac{S}{k_m + S} \qquad 2.2$$

where $\mu = \frac{dX}{dt} (\frac{1}{X})$ is the specific growth rate, μ_{max} is the maximum specific growth rate, S is the substrate concentration, k_m is the half-saturation constant for growth, and X is the biomass density. If the population density changes, an expression describing the relation between changes in population and concomitant changes in substrate concentration is also necessary. It is generally assumed that the biomass yield coefficient (Y_B) is constant though this need not be so, and Y_B may change in relation to both concentration and biodegradability of substrate (see section 2.2.4). Simkins and Alexander (1984) have shown that 6 models describing mineralisation kinetics (including zero order, first order, logistic, and Monod with growth), may be derived from the general Monod equation with specific initial conditions relating to initial biomass and substrate concentration conditions.

Such models may also be applied to describe the growth characteristics of an attached epilithic heterotrophic population in response to changes in ambient substrate concentration.

2.1.3 Benthic Biomass

The relative importance of planktonic and benthic activities will vary with the mean depth of the river. The factors which control the magnitude of the benthic activity include biological, chemical and physical components (Fig. 1.2), with the natural hydrodynamic conditions influencing sediment-water exchange and habitat suitability for microbial development.

2.1.3.1 Hydrodynamic influences. Hydrodynamic conditions at the sediment-water interface dictate the suitability or otherwise of the environment for settling of organic matter and influence sediment-water exchange of nutrients. Turbulent flows dominate water movement in river systems, with the flow velocity exhibiting a logarithmic velocity profile on approaching a solid surface (Smith, 1975). Immediately adjacent to the surface there exists a thin layer where velocity decreases linearly, termed the laminar or viscous sub-layer. Surfaces whose roughness elements are enclosed entirely within the viscous sub-layer are said to be smooth. When particles project through the sub-layer, they shed small eddies and the surface is termed rough. In water a grain size of about 0.6 mm diameter will be sufficient to cause disruption of a smooth boundary (Leeder, 1982). Table 2.1 shows calculated viscous sub-layer thicknesses in relation to flow velocity. For an algal cell which has settled on the bed, the critical boundary velocity (measured 0.05 m above bed) for the onset of erosion is about 0.15 m s^{-1} (Smith, 1975); below this velocity. resuspension is unlikely and the environment may be considered depositional.

Thus the presence of hydrodynamically rough boundary particulates in river systems may enhance mass transfer processes by localised turbulence and may also alter the settling characteristics of the bed for detrital material. Rivers with boundary velocities of approximately 0.15 m s^{-1} or less may be considered as potentially depositional environments. More detailed discussion of the biological and sedimentary importance of the benthic boundary layer is available in a number of texts (McCave, 1976; Smith, 1975; Leeder, 1982).

U (m s^{-1})	0.01	0.05	0.1	0.5	1.0
δ^* (mm)	27	5.4	2.7	0.54	0.27

For water at 15 °C

Table 2.1: Relation between mean velocity (U) and thickness (δ^*) of the viscous sub-layer (from Smith 1975)

At high flow velocities the microbial biomass must adhere to the solid surface in order to avoid being scoured from the surface. Under favourable conditions growths may extend to smother the bed.

2.1.3.2 Biofilms. Biofilms occur naturally in rivers, forming complex phototrophic/heterotrophic assemblages in oligotrophic systems, where they may represent the bulk of microbial biomass present in the river (Geesey et al., 1978). With increasing organic matter input, the biofilms tend to become dominated by heterotrophic organisms resulting in an increased assimilation rate within the river (Wuhrmann, 1972). Extreme organic pollution results in the visibly obvious development of 'sewage fungus' filamentous growths (i.e. attached benthic growths with a fluffy cotton-wool-like appearance, composed predominantly of either bacteria or fungal organisms). 'Sewage fungus' biofilms are essentially epilithic heterotrophs, extracting both organic matter and dissolved oxygen (DO) from the water flowing above them (Wuhrmann, 1974). Indeed, their activities can be so high as to seriously deplete the river oxygen resource (Velz, 1970).

Research reported, and the models developed for heterotrophic biofilms have generally been process engineering oriented, with emphasis on measurement of dissolved organic material removal rates (electron donor), whereas the environmental emphasis has generally been directed towards oxygen kinetics (electron acceptor). These two components are stoichiometrically related, under steady state conditions, using the respiratory quotient (Qo_2) for the microbially catalysed reaction. Furthermore the research has generally been conducted at low Reynolds number and hence laminar flow conditions (to simulate trickling filter systems) (e.g. Atkinson et al. 1967, Atkinson and Davies 1974) or at very high Reynolds number to eliminate liquid boundary layer diffusional resistance and hence measure a kinetically controlled reaction (e.g. Kornegay and Andrews (1968), La Motta (1976c)). The laboratory studies of Williamson and McCarty (1976b) and Trulear and Characklis (1982), have emphasised the importance of both the thickness of the heterotrophic biofilms and the laminar sub-layer, both of which influence the effective kinetics of the system. Rittman and McCarty (1980a, b, 1981) have constructed models to enable calculation of the minimum concentration of substrate necessary to support a biofilm. To what extent these models may be extended to biofilms in river systems, is limited by our knowledge of both natural biofilms and boundary layer hydrodynamics. Castaldi and Malina (1982) have demonstrated a greatly increased macroscopic oxygen uptake rate for laboratory biofilms with progression from laminar to turbulent flow conditions, thus emphasising the importance of boundary layer hydrodynamics. To predict adequately river dissolved oxygen in such systems, therefore, requires an understanding of the factors affecting the oxygen uptake kinetics and the development of models describing both biofilm growth and reaction kinetics.

In rivers where the bed is mobile, such as pumice bed rivers, development of biofilms is restricted by the sloughing and abrasion. In such rivers, thin biofilms develop throughout the bed with the active zone limited by supply of nutrients from the overlying flow. Under such conditions, the rate of microbial reaction may be limited by either electron donor or electron acceptor concentrations as described in the Conceptual Model (section 1.5).

2.1.3.3 Detritus. Planktonic algal and bacterial biomass may settle to form an organically enriched sediment in low velocity conditions. For lentic environments, such as lakes, large areas of the bed may be mud, consisting entirely of such detritus.

Degradation of cellular organic carbon which has settled on the bed relies on subsequent heterotrophic colonisation and autolysis to provide the DOM necessary for growth and metabolism. Under such conditions of detrital heterotrophs may develop, requiring only the supply of electron acceptors(e.g. oxygen, nitrate) from the overlying waters.

The great majority of research associated with sediment metabolism and sediment-water interactions has been performed in lentic environments containing fine particulate, organically-enriched sediments. The research work described here is directed towards lotic systems, although work on lentic systems is reviewed (sections 2.2, 2.5).

2.2 MICROBIAL PARAMETERS

Biochemically-mediated nutrient transformations result from both planktonic and sediment dwelling living organisms. Comparison between river systems requires measurement of the nutrient transformation process activities and identification of the key biomass component(s).

Methods which have been developed for planktonic communities may not be suitable for direct application to sediment studies. This is of particular concern when the desire is to relate measured rate processes to the active biomass present, since a large proportion of the population may be dormant (Stevenson, 1978). Additionally, microbially-mediated nutrient transformations involve both electron donors and electron acceptors which are related by the reaction stoichiometry. Environmental conditions may vary considerably. resulting in a range of possible transformations. The conditions influencing reaction conditions are therefore of importance in environmental studies.

By far the largest numbers of sediment microbial studies have been made on fine particulate lake or marine sediments. Some smaller lotic environments have been studied (e.g. Winterbourn, 1976; Pennak and Lavelle, 1979; Rounick and Winterbourn, 1983) with greatest interest in primary productivity or the breakdown of allochthonous material (e.g. leaf litter).

2.2.1 Biomass

Recently a number of new techniques have become available for the enumeration of bacterial numbers in aquatic environments. Fluorescence microscopy, using epi-illumination, has been employed to enumerate total numbers of bacteria in a water sample (e.g. Zimmerman and Meyer-Reil, 1974; Daley and Hobbie, 1975). These epifluorescent methods have been applied in conjunction with scanning electron microscopy for the study of bacterial colonisation of marine and sediments by Weise and Rheinheimer (1978). Such methods have been extended to estimate the numbers of respiring organisms using ρ-iodonitrotetrazolium violet (INT) reduction (Zimmerman <u>et</u> <u>al</u>., 1978; Tabor and Neihof, 1982) or to detect specific metabolic activity by the use of labelled compounds and autoradiography (Meyer-Reil, 1978a).

In attempting to estimate biomass in the sediment, a number of complications arise as a result of the large amount of particulate material and organic debris present. A number of studies have used direct microscopic counts using fluorescent labelling (e.g. Meyer-Reil 1978a; Jones et al., 1979). These methods are generally difficult to use because of the need to separate and recognise the bacteria from the particulate matter.

A number of biochemical methods have been applied for enumeration of planktonic micro-organisms. These include methods for ATP (which includes both eucaryotic and procaryotic organisms present), chemiluminescent detection of bacteria (e.g. Miller and Vogelhut, 1978) and Limulus amoebocyte lysate method for bacteria (Watson et al., 1977).

The most widely applied biochemical indicator of sediment biomass has been ATP because of its universal occurrence in living matter. To be useful as a biomass indicator, ATP requires consistent rigorous extraction from the natural substratum and minimisation of the effects of substances that interfere with the luciferin-luciferase reaction (Lundin and Thore, 1975b). Most ATP extractions use : organic solvents (Ausmus, 1973; Dhople and Hanks, 1973; Clarke et al., 1978), boiling or slightly alkaline buffer (Holm-Hansen and Booth, 1966; Lundin and Thore, 1975a; Bancroft et al., 1976; Christian et al., 1975; Bullied, 1978; Jones and Simon, 1977) or cold acid (Lee et al., 1971; Karl and La Rock, 1975; Lundin and Thore, 1975a; Cunningham and Wetzel, 1978; Karl and Craven, 1980). Differences in these extraction techniques can be attributed most directly to their effectiveness in cell lysing, inhibition of ATPase activity, removal of ATP and the degree to which they extract substances which are inhibitory to the luciferin-luciferase bioassay system (Karl, 1980). Comparisons have shown no single method to be the most effective (see Lundin and Thore, 1975a; Cunningham and Wetzel, 1978; Clarke et al., 1978). Difficulties with extraction procedures and the small size of samples generally result in considerable variation in results. The time needed to remove the organic solvent from the ATP extract limits wide applicabilty for environmental studies. Extractions made in boiling buffer provide satisfactory results for planktonic systems where particulate materials are minimal, but for many sediments, result in poor efficiency of extraction compared to the more rigorous chemical extraction methods. However, the methods of Jones and Simon (1977) and Bullied (1978) appear to give better extraction. The extraction with cold sulphuric acid, although potentially superior to using boiling buffers, introduces a high degree of variability (Lundin and Thore, 1975a; Bancroft et al., 1976) and greatly increases concentrations of ionic species that can totally inhibit the bioluminescence reaction of luciferin-luciferase. Acid extraction requires dilution and a means to remove a significant proportion of the cations by cation exchange or chelation (Karl and La Rock, 1975; Cunningham and Wetzel, 1978).

Karl (1980) in reviewing the applications of cellular nucleotide measurements in microbial ecology, where environments may range considerably in terms of physiological suitability for growth, documented a wide range in nucleotide content. Environmental responses may differ between organisms, with the nucleotide ratios reflecting community growth rate. Under favourable conditions the carbon to ATP ratio is fairly constant and may be used as an estimator of biomass. Considerable emphasis on methods was considered justified because of the need for optimal extraction techniques from a diverse range of environments.

In summary, some evaluation of methods would seem necessary prior to adopting a suitable technique for the sampling and extraction of ATP from the particular sediment type being considered. Most important is the need for representative sampling and rigorous extraction.

ATP provides a measure of the total biomass present, but gives no information concerning the contribution made by individual benthic constituents. The major biomass fractions anticipated (bacteria, algae, meiofauna and macrofauna) may be individually estimated as follows :

Bacteria. A specific method for the determination of bacterial biomass in aquatic sediments has been used by a number of authors (King and White, 1977; Moriarty, 1975; 1978). This involves the quantitative extraction of muramic acid, a component of the peptidoglycan found only in the cell walls of bacteria and blue-green algae, which furnishes a measure of detrital or sedimentary procaryotic biomass. The need for gas chromatographic analysis and the degree of analytical complexity, prevented application to this study.

The method considered appropriate involved estimating total microbial biomass by ATP and then subtracting the estimated algal biomass from chlorophyll a, to give the bacterial biomass by difference. Although C/ATP ratios may vary considerably depending on species, environmental and physiological conditions (Karl, 1980), a conversion factor of ATP x 250 (Holm-Hansen and Booth, 1966) was considered appropriate for initial investigations to allow comparison with other biomass and total carbon estimates.

Algae. Chlorophyll a constitutes approximately 1-2% of the dry weight of organic matter in all algae (including cyanobacteria) and provides a useful estimate of algal biomass. Chlorophyll content of cells varies with species or taxonomic groups and is affected by age, growth rate, light and nutrient conditions (Vollenweider, 1974). A conversion factor of chl a x 16 (Paerl et al., 1976) was considered appropriate for initial investigations to provide an algal biomass estimate.

Benthic algal biomass may be extracted using acetone (Jones and Simon, 1980) and spectrophotometrically measured. In sedimentary systems the accumulation of chlorophyll degradation products may cause substantial interference to chlorophyll a assays, necessitating correction for pheophytin and other degradation products (Lorenzen, 1967).

Meiofauna and macrofauna. ATP extraction by boiling buffer without mechanical agitation has been reported to provide poor extraction conditions for metazoans (Karl, et al., 1978; Karl 1980). Therefore, ATP measurements may be considered as predominantly microbial biomass.

In rivers with mobile beds the metazoan habitat suitability may be low. In other rivers their biomass levels may be higher, however, their direct metabolic contribution is expected to be small (Lasserre, 1976). Of greater interest in fine particulate sediments are the bioturbation effects of invertebrates, resulting in mixing and irrigation of the surface layers (Berner, 1980).

2.2.2 Physiological State

In the literature, a number of biochemical parameters and measurements have been proposed as measures of the physiological state of the microbial population. These provide indirect measures of growth rate, nutrient imbalances and carbon flow in the system.

The recovery of poly-β-hydroxybutrate (PHB) from estuarine microflora (Herron et al., 1978) has been shown to be indicative of imbalanced metabolism. This is a carbon storage compound accumulated by micro-organisms in times of nutrient imbalance (Dawes and Senior, 1973). The accumulation of PHB has also been shown to occur when the organisms are subjected to low dissolved oxygen tensions (Sonnleitner et al., 1979), and restricted nutrient supply (Nickels et al., 1979).

The metabolic state of a population may be assessed by measurement of its adenylate charge (Wiebe and Bancroft, 1975; Karl and Holm-Hansen, 1978). The concentration of ATP in organisms is linked to the metabolic state of that organism (Chapman and Atkinson, 1977); when the organism is physiologically inactive the concentrations of adenosine diphosphate (ADP) and adenosine monophosphate (AMP) increase. By equating the relative concentrations of these phosphorulated nucleotides, an energy charge of those organisms present may be calculated, providing a measure of community potential for growth. Although this technique has been applied to phytoplankton studies, serious potential difficulties associated with incomplete extraction of the nucleotides from sediments may limit useful application (Karl, 1980).

The above techniques will not be applied in these studies; however, they may be of use in future studies concerned with nutrient transformations and respiratory yields within the river system.

2.2.3 Metabolic Activity

The location of maximum microbial activity zones may be identified using measurements of metabolic processes common to all living organisms, or more specific assays related to the nutrient transformations of interest. The assay suitability may also be influenced by measurement sensitivity and simulation of natural conditions.

Although oxygen uptake provides a non-specific measure of the total community activity, a number of factors (e.g. sensitivity to diffusion limitations as influenced by water velocities and saturation kinetics for both electron donor and electron acceptor concentrations) can create experimental simulation difficulties. A number of general methods offering increased sensitivity for estimation of metabolic activity have been applied including rates of lipid biosynthesis (White et al., 1977), DNA polymerase activity (Sapienza and Mague, 1979) tritiated thymidine uptake (Tobin and Anthony, 1978), sulphate uptake (Monheimer, 1974) and the activity of the electron transport system (ETS) (Zimmerman, 1975; Jones and Simon, 1979). More specific measures of microbial activity have been made with the use of compounds labelled with radioactive isotopes (e.g. Dietz et al., 1977; Meyer-Reil, 1978b; Wright, 1978). Meyer-Reil et al., (1980) have shown that the bacterial standing crop does not necessarily reflect bacterial activity and that cell numbers, biomass, and uptake rates of glucose in the water overlying the sediments are influenced by sediment properties. Such interactions may be of increased importance in river systems where biomass growing attached to the bed is sloughed off into the overlying flow.

In organically polluted systems the aquatic concentrations increase from the natural $\mu g\ \ell^{-1}$ range to mg ℓ^{-1} concentrations (Williams, 1973). Mineralisation rates using a variety of labelled compounds (e.g. glucose, acetate) may provide valuable insights into maximum rates (e.g. Meyer-Reil, 1978b; Gillespie, 1982), turnover time (e.g. Gocke, 1977b) and carbon cycling by the microbial population (e.g. Kepkay and Novitsky, 1980; Novitsky and Keplay, 1981). However, reports of applications of isotope methods to organic pollution studies in lotic systems are limited. Spencer and Ramsay (1978) found good correlation between V_{max} and other indicators of organic enrichment for 3 New Zealand rivers. Gillespie (1982) reported extremely high V_{max} values associated with pulp mill pollution of the Tarawera River in New Zealand. Wyndham and Costerton (1981) have studied hydrocarbon biodegradation potentials in a Canadian river draining oil sand regions.

The difficulties of relating environmental conditions to metabolic activities for environments widely varying in biomass levels have prompted the suggested use of specific activity indices (Wright, 1978; Goulder, 1980). These studies have demonstrated that the specific activity per bacterium may vary as much as 32-fold between planktonic sampling sites without significant changes in total count. Such a large variation suggests an increase in activity per bacterium and not simply an increase in the percentage of active cells in the ecosystem. This contention is supported by the large variation in activity per bacterium reported by Ladd et al. (1982) for stream bacteria.

Biomass specific activity indices appear to offer potentially useful techniques for relating the metabolic capabilities of the microorganisms present to changes in constituents of the surrounding flow. This technique will be used in conjunction with catabolic enzyme measurements of river sediments (see Section 2.4).

2.2.4 Yield Relationships

For environmental studies involving the addition of organic wastes to natural waters, both the rate of removal of the organic material and the resulting deoxygenation processes may be of interest. The relation between these two components are the yield coefficients for microbial reactions.

Bacteria have been shown to have different efficiencies of metabolism depending on the concentration and type of carbon source present (Wright 1971; Williams, 1973). For planktonic bacteria respiring substrate at concentrations <50 $\mu g\ \ell^{-1}$ the percentage glucose respired is generally found to be between 10 and 30% of the total used, whereas respiration of at least 50% of the substrate occurs if the concentrations are above 500 $\mu g\ \ell^{-1}$ for the same microbial population. Growth of an organism on a single substrate will involve the organisms in extensive metabolic transformations and therefore increase the energy needed for growth. Thus the high concentrations of substrate result in increased respiratory demand.

The nature of the carbonaceous substrate has been shown to result in widely varying respiratory quotient and biomass yield coefficients for microorganisms (Payne, 1970; Bailey and Ollis, 1977). The implication for river deoxygenation is that bottle carbon and BOD relationships may differ substantially from the river values in response to changes in effluent quality, both from the discharge and longitudinally as organic assimilation occurs. This effect increases with a greater benthic metabolic contribution, since these organisms may adapt to optimally metabolise the organic material present in the overlying flow.

Nutrient limitations for oxygen, nitrogen and phosphorus supply have been shown to result in PHB accumulation (Dawes and Senior, 1973; Nickels et al., 1979). Such changes must therefore influence respiratory yield relationships.

As dissolved oxygen becomes depleted, and redox conditions suitably reduced, other electron acceptors are utilised by different physiological types of anaerobic bacteria if they are present. The sequence of use of these electron acceptors can be predicted on thermodynamic grounds according to the amount of energy that will be liberated from a common electron donor as it is oxidised by each of the electron acceptors (Billen, 1982a). Thermodynamic criteria suggest the sequence O_2, Mn^{4+}, NO_3^-, Fe^{3+}, Fe^{2+}, SO_4^{2-}, HCO_3^- as the electron acceptors become less oxidising and less energy is available. The microorganisms which perform NO_3^- respiration are generally facultative in nature and may therefore continue to consume electron donors using NO_3^- when O_2 is exhausted. Microbial SO_4^- and 'CO_2' respiration (the latter may be better looked at as H_2 oxidation using CO_2) requires increasingly negative redox potential, anaerobiosis and specialised groups of bacteria.

Changes in yield coefficients are likely to be of increased importance in systems where benthic biomass predominates, and organic assimilation conditions differ greatly from the planktonic based exertion kinetics on which the majority of mathematical models are based.

2.2.5 Interrelationships Between Parameters

In general poor correlation is shown between total benthos biomass and net primary productivity (e.g. Pennak and Lavelle, 1979) or between measures of heterotrophic activity and microbial biomass (e.g. Bobbie et al., 1978; Meyer-Reil et al., 1978). This reflects the wide range in benthic conditions affecting chemosynthesis and photosynthesis if within the photic zone. Therefore, further work must seek to describe the habitat factors which influence both the standing-stock of biomass present and intrinsic activity. Greater microbial biomass and activities within the sediment as measured by several methods, are readily correlated with the overall degree of eutrophication of lakes and organic inputs into other aquatic systems (e.g. Hargrave, 1972a; Wood and Chua, 1978; Jones et al., 1979; Jones, 1980; Meyer-Reil et al., 1980) though not necesarily with the organic content of the sediment involved. A large number of chemical parameters (e.g. phosphate, ammonia, nitrate and nitrite) show similar variations, indicating that the transformations of dissolved inorganic nutrients may be closely linked (Meyer-Reil et al., 1980). A single chemical component may control reaction rate processes; however the physical factors controlling the establishment, nutrient exchange, and activity of the microbial population may control the magnitude of overall benthic metabolism (e.g. sediment particulate sizes, temperature) (Jones, 1980).

The lack of correlation in sediment systems between activity and biomass measurements is probably indicative of a natural diverse community where there are a large range of organisms of multifarious abilities present, combined with those physical constraints which may be restrictive (Fig. 1.2). It is therefore important to identify the relative importance of components which together control the overall rate process.

2.3 ORGANIC WASTE DISCHARGES

Organic wastes may originate from agricultural, domestic, industrial, urban and rural runoff sources, reaching receiving waters by direct or indirect pathways. The principal components include: particulate organic matter (POM) and debris, which may settle out; dissolved organic matter (DOM), which will promote both planktonic and epithic heterotrophic growth; and inorganic nutrients, which will promote autotrophic growth.

This section covers briefly the nature of organic material content of point source discharges and the methods used for measuring the concentration and characterisation of those discharges.

2.3.1 Characteristics and Composition

Effluents vary widely in terms of biodegradability depending on the origin of the organic waste. Readily degradable wastes originating from factory processing, may contain low molecular weight compounds which are easily assimilated (e.g. sugars or amino acids). More refractory wastes generally contain high molecular weight compounds requiring extracellular enzymatic breakdown prior to microbial assimilation (e.g. protein, fats or cellulose).

The nature of effluents may be substantially modified prior to discharge by a treatment system. Biological treatment (e.g. by oxidation ponds) may increase the content of POM due to microbial cell production. Thus treatment processes are generally likely to reduce the supply of DOM available for epilithic heterotrophic development attached to sediments, favouring an increase in sediment detrital heterotrophs. The former organisms will actively remove DOM and thus enhance the rate of effluent removal, whereas the latter merely metabolise settled POM from the overlying flow affecting no processing enhancement.

2.3.2 Measurement of Waste Characteristics

Various methods are available for measurement of the total carbon content of a waste. Chemical oxygen demand (COD) is frequently used for both waste and receiving water measurements (APHA, 1976); however no indication of the amount of biologically available material present is provided by this test.

Measurement of cumulative oxygen consumption by planktonic microorganisms using the biochemical oxygen demand (BOD) test (APHA, 1976) is widely applied to monitor organic effluents. Frequently limited to a standard 5 day determination, staging of measurements provide the bottle deoxygenation coefficient (k_L), while indices of biodegradability of the carbon materials present are obtained by ratios of BOD_5 to BOD_{20} and BOD to COD. Such measurements may vary greatly between organic effluent types depending on the nature of the DOM and the quantity of viable or otherwise POM present.

Because of the potential for development of epilithic heterotrophs attached to sediments in rivers, it is important to be able to measure accurately the concentration of readily degradable DOM present. For this reason, prefiltered BOD measurements (FBOD) should also be performed to provide a measure of the amount of biodegradable DOM present.

Although the bottle BOD test may be criticised for numerous shortcomings (e.g. quiescent conditions, low organic concentrations, nutrient status, non-differentiation of nitrogenous oxygen demand resulting from the growth of bacterial nitrifiers present) it can still provide a measure of the amount of oxidizable organic material present in the receiving water. To provide detailed measurement of oxygen demand exertion kinetics an electrolytic respirometric system may be used (Hickey and Nagels, 1985).

2.4 CATABOLIC ENZYMES

2.4.1 Introduction

Enzymes are biological catalysts responsible for the regulation of all cellular metabolic processes, and for the breakdown and processing of organic material used by heterotrophic organisms for energy production. Microorganisms produce enzymes which vary enormously in specificity and which are responsible for the breakdown of organic compounds. These enzymes may be either induced in response to the presence of a compound or constitutive in nature (Brock, 1974). Thus enzyme systems present a means of looking at the metabolic activity of the microbial population present in relation to its nutrient sources and environment.

Monitoring of both polluted and unpolluted environments for phosphatase, saccharase, amlyase and lipase activities show seasonal variation and induction with addition of substrate (Verstraete et al., 1976). Similarly, Jones (1971) indicated that numbers of microorganisms producing amalyases, lipases and proteases, respectively correlated well with amylase, lipase and protease activity of lake water samples, with both water and mud samples showing seasonal peaks in activity. Meyer-Reil (1981) attributed accumulation of organic matter in sediments to the breakdown of the algal spring bloom in a marine estuary, with amylase, glucosidase and proteolytic enzyme activity rates paralleling the pattern of seasonal variation in planktonic concentrations of carbohydrates and proteins.

Natural river sediments with low amylase, cellulase, and urease activities have also been reported to show markedly increased activity when appropriate substrates were added (Duddridge and Wainwright, 1981, 1982).

Multiple biochemical assays of microbial biomass and activities were applied to estuarine detrital microbiota colonising polyvinyl chloride needles and pine needles (Bobbie et al., 1978). Enzyme levels appeared to follow the extent of colonization and showed a progression with time in the relative activity of various enzyme classes.

Thus the microbial enzyme activity reflects the nature of the energy yielding substrate, with sediment enzyme activities in flowing waters reflecting the characteristics of the overlying water and surrounding environment.

2.4.2 Enzyme-Metabolism Relationships

A range of enzyme types are potentially useful for study of the response of microbial biomass to milk waste discharged into a river. Activity measurements may be used as an index of microbial response to the environment, with the aim in this application of identifying 2 metabolism categories (see Table 1) :

i epilithic heterotrophs - where both electron donors (DOM) and electron acceptors (e.g. DO) are supplied from the overlying flow

ii detrital heterotrophs - where electron acceptors (e.g. DO) are supplied from the overlying flow but the electron donor supply is largely derived from the degradation of cell material (POM).

The enzymes which are suitable for identifying the metabolism categories are (Brock, 1974) :

β-galactosidase:	induced in response to lactose present in milk wastes released into the receiving water and will indicate epilithic heterotrophic metabolism.
β-glucosidase	a glycosidase which is active in cell wall and polysaccharide breakdown.
Protease	for the breakdown of protein derived from either milk wastes or cell material.
bis-phosphatase:	an enzyme induced for the breakdown of deoxyribonucleic acid (DNA) and therefore indicative of the degradation of cell material and detrital heterotrophic metabolism.
Alkaline phosphatase:	produced in response to low available phosphorus conditions and therefore may be indicative of nutrient limitations.

2.4.3 Summary

A range of enzyme types offer potential to gain insight into the relationships between organic characteristics in the overlying flow and the energy source of the biota. In addition to areal measurements, comparisons on a biomass specific basis (outlined in section 2.2.3) are required to distinguish metabolic changes from those factors which influence the level of biomass present (see Fig. 1.2).

The objective of this study was to compare activities of microbial populations from various locations within the river. As such, a measure of the maximum potential activity was desired. Permeabilisation of the cell membranes of microorganisms using a freeze-thaw cycle in the presence of a low concentration of anionic detergent, has been shown to result in substantially higher enzyme activities than for either whole cell or crude extract preparations (Miozzari et al., 1978). This method offers additional advantages in logistics and ease of sample handling for multiple station surveys with very limited manpower resources.

2.5 MEASUREMENT OF RIVER OXYGEN METABOLISM

2.5.1 Introduction

Measurement of oxygen mass balances for segments of rivers is necessary both to put relative magnitudes of the different respiratory components into perspective and to test BUR measurements against independent mass balance assessments. The importance of individual components will vary greatly between river systems, as will the appropriate study methods. It is therefore, important to assess both the merits of various techniques as well as the assumptions which may be required about experimental methods or the empirical relationships used in estimating river oxygen processes.

2.5.2 Whole River System Metabolism

Because of the effect of photosynthesis, diurnal observations of dissolved oxygen are frequently necessary in order to obtain an adequate description of the oxygen status of a river. A considerable number of publications have appeared since the pioneering work of Odum (1956) aimed largely at documenting primary productivity. Common to all river oxygen studies is the difficulty associated with estimation of the atmospheric reaeration coefficient, either experimentally or using one of the numerous empirical equations available (Wilson and MacLeod, 1974). Direct experimental measurements of the reaeration coefficient (k_2) by using gas tracer techniques (Wilcock, 1984a) have shown that empirical equations may underestimate k_2 by up to 40% in the New Zealand rivers of interest (Wilcock, 1984b). This may lead to erroneous estimates of the BUR in situ. An alternative method for k_2 estimation uses the diurnal curve itself, regressing the rate of change of DO concentration against the DO saturation deficit to obtain k_2 as the slope (Cohen and Church, 1981; Servais et al., 1984). This technique may provide an alternative approach for systems where a substantial diurnal variation occurs.

Segment oxygen mass balances are proposed for checking measurements made using chamber devices in situ.

2.5.3 Benthic Measurements

2.5.3.1 Factors affecting sediment-water interactions. Measurement of benthic oxygen and nutrient mass transfer have largely been achieved by core techniques (e.g. Edwards and Rolley, 1965; Graneli, 1977, Pomroy et al., 1983), benthic chambers operated in situ (e.g. James, 1974; Sonzogni et al., 1977; Bott et al., 1978), chamber systems operated in vitro (e.g. Bowman and Delfino, 1980; Belanger, 1981; Jeppesen, 1982), or more recently with the use of micro-electrodes for oxygen studies (e.g. Revsbech et al., 1980; Revsbech and Ward, 1983). The choice of the appropriate method depends on the environmental substratum and hydrodynamic conditions requiring simulation, together with the practicalities of available equipment and sampling logistics.

Berner (1980) has described the various processes affecting diffusion, such as molecular, invertebrate bioturbation and wave-current stirring, all of which influence mass transfer processes in lentic (standing water) systems. Turbulent dispersion results in mass transfer processes which are orders of magnitude greater than molecular diffusion (Liepmann, 1979). However, research on high velocity lotic (flowing water) systems appears lacking. The high velocity and turbulence of shallow streams may result in high interstitial dissolved oxygen (DO) concentrations below 30 cm depth within the river bed (Williams and Hynes, 1974; Whitman and Clark, 1982), as shown by the development of rich and varied invertebrate psammon communities (Williams and Hynes, 1974). The ability of core and chamber systems to simulate adequately the mass transfer processes actually occurring in river systems, is a major consideration which to date has not been tested. Although microelectrode methods offer great potential for accurate concentration profile measurements and subsequent flux calculation without artificial enclosures, their fragile construction severely limits application with coarse sediments (Revsbech et al., 1981).

Little experimental emphasis has been placed on the role of boundary layer hydrodynamics in mass transfer processes. Although chamber systems have been applied in both lentic environments (e.g. Pamatmat and Banse, 1969; Edberg and Hofsten, 1973) and lotic environments

(James, 1974; Bott. et al., 1978; Pennak and Lavelle, 1979; Jeppesen, 1982), design and operating conditions for chambers in relation to natural flow conditions, have not been established. Chamber design is reviewed in greater detail in Chapter 4.

2.5.3.2 Other Factors Affecting the Benthic Oxygen Uptake Rate. A combination of site-specific biological, chemical and physical factors may influence BUR as illustrated in Figure 1.2.

This sub-section briefly reviews studies of those factors which influence BUR measurement. More comprehensive reviews are available (Belanger, 1981; Bowman and Delfino, 1980) and a recent symposium (Hatcher and Hicks, 1985).

Dissolved oxygen. A number of sediment oxygen demand (SOD) measurements made on mud sediments have reported a DO dependent rate of SOD exertion (Edwards and Rolley, 1965; Hargrave, 1969b; Pamatmat, 1971; Fillos and Molof, 1972; Edberg and Hofsten, 1973; Graneli, 1977; Polak and Haffner, 1978; Chiaro and Burke, 1980; Jeppesen, 1982). More recently Jeppesen (1982) has reported diurnal variation of BUR with an apparent relation to DO using measurements made in vitro over a 24 h period. Chiaro and Burke (1980) have suggested modelling SOD as a function of DO concentration using an oxygen mass transfer coefficient determined from extended chamber measurements. Fillos and Molof (1972) and Martin and Bella (1971) regard the SOD exertion rate as being relatively independent of DO concentration at least above 2 g m^{-3}. Both of these experimental systems, however, involved suspension of large amounts of solids which effectively provide extremely large surface area thus decreasing diffusion limitations into the mud. No reports describing the BUR kinetics for lotic systems have been sighted.

Temperature. Temperature affects the metabolic activity of the sediment organisms and the rate of molecular diffusion of oxygen. Edwards and Rolley (1965), Edberg and Hofsten (1973), Graneli (1977) and Jorgensen (1977) reported the temperature dependency of SOD. The relative change in rate observed over a 10 °C temperature increase (Q_{10}) varied considerably between studies. Jorgensen (1977) reported a Q_{10} of 3.2 for coastal muds, whereas, Edberg and Hofsten (1973) reported decreasing values with increasing temperature

Temperature range (°C)	Q_{10}
5-15	3.4
10-20	2.1
15-25	1.5

Planktonic microorganisms generally show Q_{10} values of 2 (Wright, 1978). No references have been sighted for Q_{10} sediment measurements in lotic systems.

Seasonal variations in water temperatures have been reported to result in increased summer oxygen demands in lotic systems as a result of biomass proliferations (e.g. Naiman and Sedell, 1979).

Particle size. Benthic particle size is important in regulating invertebrate habitat, providing a surface for microbial attachment, and also influences diffusion and dispersive nutrient supply.

In fine sediment systems, supply of nutrients by diffusion decreases with decreasing particle size owing to greater tortuosity and hence increased diffusion path lengths (Berner, 1980). For water movement through the sediment structure by percolation and dispersive mass transfer processes, the permeability is of greatest importance (Leeder, 1982). Porosity measurements give an indication of the amount of fractional space between solids, but may show no relation to the permeability of flow through the substratum, which requires passage between pores to be available. Thus there is a need to identify the importance of active sediment-water flow interactions, and ultimately to quantitatively identify the factors influencing these processes.

The importance of sediment type and particle size has been outlined by Cummins (1974) in terms of the ecological habitat provided within the stream. Particle size and oxygen uptake have both been measured in short-term experiments by Hargrave (1972a) for microbial populations on mud-sand and various types of detritus from lakes. He reported a negative linear correlation on logarithmic axes of substrate particle size with the oxygen uptake of those particles. Detritis consumed up to three orders of magnitude more oxygen per day dry weight than sand, with uptake rates inversely related to particle diameter. In a recent review of oxygen uptake by microbial communities by Hargrave and Philips (1977) they report that a linear relationship appears to exist between community oxygen consumption and substrate surface area for sand, pebble, rock and detrital surfaces under constant conditions of temperature, pH and oxygen concentration.

Thus the role of particle size appears to represent a key component in aquatic systems both with regard to habitat suitability and mass transfer processes.

Organic content. In depositional environments the degradation of settled POM exerts an oxygen demand in aerobic layers, with anaerobic degradation in deeper anoxic regions resulting in the production of reduced substances which diffuse into the upper oxygenated sediment layers (Fig. 1.1).

Some researchers have found SOD to be independent of organic matter content of the sediments (e.g. Edwards and Rolley, 1965) while others report correlations (e.g. Hargrave, 1972b). These correlations are generally complicated by the great variety and complexity of sediment types, age and general environmental conditions of the benthic materials.

The presence of both oxidised and reducing layers in a benthic depth profile may necessitate the use of experimental techniques to identify the nature of the oxygen demand. The release of reduced substances by anaerobic microoganisms and the subsequent diffusion of these reduced compounds into the aerobic zone, can result in a significant oxygen demand. The importance of the reduced zone has been shown by the increased oxygen demand measured in core systems as a result of increases in sludge core depth (e.g. Fillos and Molof, 1972). To some extent these anaerobic diffusion processes have been measured as and expressed as chemical oxygen demand. A number of investigators have used various metabolic poisons to prevent active microbial respiration and so by difference calculated the fraction of total oxygen demand attributable to chemical oxygen demand by reduced compounds (e.g. Teal and Kanwisher, 1961; Fillos and Molof, 1972; Hargrave, 1972b; Wang, 1980; Chiaro and Burke, 1980).

The organic matter content of sediments decreases with increasing flow velocities because of reduced deposition, and one might anticipate a lesser importance of chemical oxygen demand in high velocity lotic systems.

2.5.4 New Zealand Studies on BUR

Some BUR measurements on the pumice sediments of the Waikato River (mean velocity >0.5 m s^{-1}) have been made by Boubeé (1977) using a stirred core technique. Only 60% of the calculated mass balance BUR could be accounted for using this technique. Gillespie (1982) studied benthic biomass and oxygen uptake rate in the pumice of the Tarawera River in vitro and showed substantial increases in all parameters measured for stations located downstream of a pulp and paper discharge. Cooper (1984) using a chamber technique in situ measured BUR values of up to 44 g O_2 m^{-2} day^{-1} in polluted rivers with 'sewage fungus' communities, and showed that benthic associated nitrification may be a significant process affecting river DO, particularly in the winter months. Other microbial metabolism studies were limited to biofilms associated with stone surfaces in small mountain streams draining bush catchments (Rounick and Winterbourn, 1983).

2.5.5 Summary

Chamber enclosure systems in situ offer the best experimental approach for investigation of the range of factors which may influence BUR in lotic systems. Because the majority of chamber systems designed to date have been for lentic environments with low circulation velocities, the design and testing of a chamber capable of high circulation velocities must constitute an important component of this research programme.

A predictive ability for river response to organic effluents, which is necessary for the management of lotic ecosystems may only be developed once the relative importance of planktonic and benthic micobial biomass have been established. Thus planktonic and benthic measurements of specific microbial biomass communities, together with sediment enzyme activity measurements are techniques which can provide valuable insights into the influence of organic effluents on the river systems. Since total benthic metabolism is influenced by numerous factors, including biological, chemical and physical interactions (Fig. 1.2), importance of each of these components must be established for lotic systems prior to the development of suitable predictive techniques. Once developed, the chamber techniques together with specific biomass and metabolic activity methodologies should provide a suite of techniques enabling this goal to be met.

Chapter 3

METHODS

TABLE OF CONTENTS

3.1 INTRODUCTION

The Methods reported in this chapter cover the majority of those used throughout the Thesis. Minor modifications or special techniques used for some studies are reported in the appropriate sections. Since a major objective of this Thesis involved the design and testing of a benthic chamber system, details of this work are covered in a separate chapter (Section 4.1).

3.2 DESCRIPTION OF STUDY RIVERS

3.2.1 Introduction.

The criteria for the selection of the study rivers were that they: (a) received a point source loading of organic effluent; (b) had a mean flow velocity greater than 0.2 m s^{-1}; (c) had a bed composed of sand or gravel substrata; (d) were of less than 1.0 m depth to allow ready wading access and the installation of chamber systems; (e) were within convenient travelling distance (i.e. <100 km).

Rivers differing in their predominant sediment composition were sought to enable comparison of the effects of sand, pebbles and rhyolitic tephra (or 'pumice') substrata on benthic microbial colonisation and activity.

Field Sites. Field measurements were carried out in 6 rivers selected according to the above criteria (Fig. 3.1). Detailed investigations of river processes, including seasonal and longitudinal studies were performed on the Waitoa River. Segment oxygen mass balance determinations together with microbial biomass and chamber BUR measurements were carried out on the Waitoa, Waiotapu and Tarawera Rivers, whereas measurements in the other rivers were largely limited to a single sampling station.

3.2.2 Waitoa River

The Waitoa is a tributary of the Piako River, 40 km east of Hamilton. The river flows in a northerly direction draining a watershed composed entirely of developed pasture of the Waikato region (Fig. 3.1). Rainfall is highest in the winter, although rainfalls in the summer months result in frequent floods as shown in the flow hydrograph (Fig. 3.2). The river characteristics are detailed in Table 3.1.

The river receives untreated effluent from a dairy factory located at Waharoa (Fig. 3.3), which results in a river biochemical oxygen demand (BOD_5) of 10 to 30 g m^{-3}, compared with <2.5 g m^{-3} upstream.

Five sampling stations covering an 11 km reach of the river were selected. These consisted of 1 control station, located above the discharge, and 4 stations below the discharge (Plate 3.1, Fig. 3.3). These were:

Landsdowne Road (L)	1 km above the discharge (-1)
Waharoa (W)	~ 50 m below the discharge (0)
Hockley's Farm (H)	2 km below the discharge (2)
Signal's Farm (S)	5 km below the discharge (5)
Walton Road Bridge (WB)	10.8 km below the discharge (10).

River	Location	Catchment Area km2	Low Flow Discharge $m^3 s^{-1}$	Mean Velocity $m s^{-1}$	Mean Depth m	Width m	Temperature Range Annual °C	Temperature Range Summer Daily[a] °C
Waitoa	175°45'E, 37°45'S	122	0.2-0.5	0.15-0.30	0.3	4-6	6-26[b]	16-22
Waiotapu	176°20'E, 38°25'S	228	2.5	0.4	0.75	6-10	5-25[b]	20-25
Tarawera	176°43'E, 38°05'S	906	25	0.7	1.2-1.8	20-25	6.7-21.7[c]	17-19
Mangapiko	175°14'E, 38°00'S	151	0.2-0.5	~0.2	~0.4	4-6	-	-
Oraka	175°45'E, 37°59'S	136	2	0.4-0.5	0.5	4-6	-	15-17
Manawatu	175°40'E, 40°25'S	3900	20	0.5	1.0	20-30	8.0-23.0[c]	18-21

[a] from diurnal monitoring
[b] from field measurements
[c] from Mosley (1982)

Table 3.1: Characteristics of study rivers

The vegetation bordering the study section was minimal with grazed paddocks extending to include the river bank. Isolated patches of Kahikitea (White Pine) (Podocarpus dacrydioides) and willow (Salix sp.) were present, but these provided minimal shading of the river bed. Extensive colonisation by blackberry occurred along some sections.

Bed material was largely coarse sand and gravel throughout the reach. Particle size sorting had occurred as a result of floods, resulting in a mosaic of gravel and sand patches.

Submerged vegetation at the Landsdowne Road station (Plate 3.2) was, upon initiation of this study in the spring of 1981, confined to

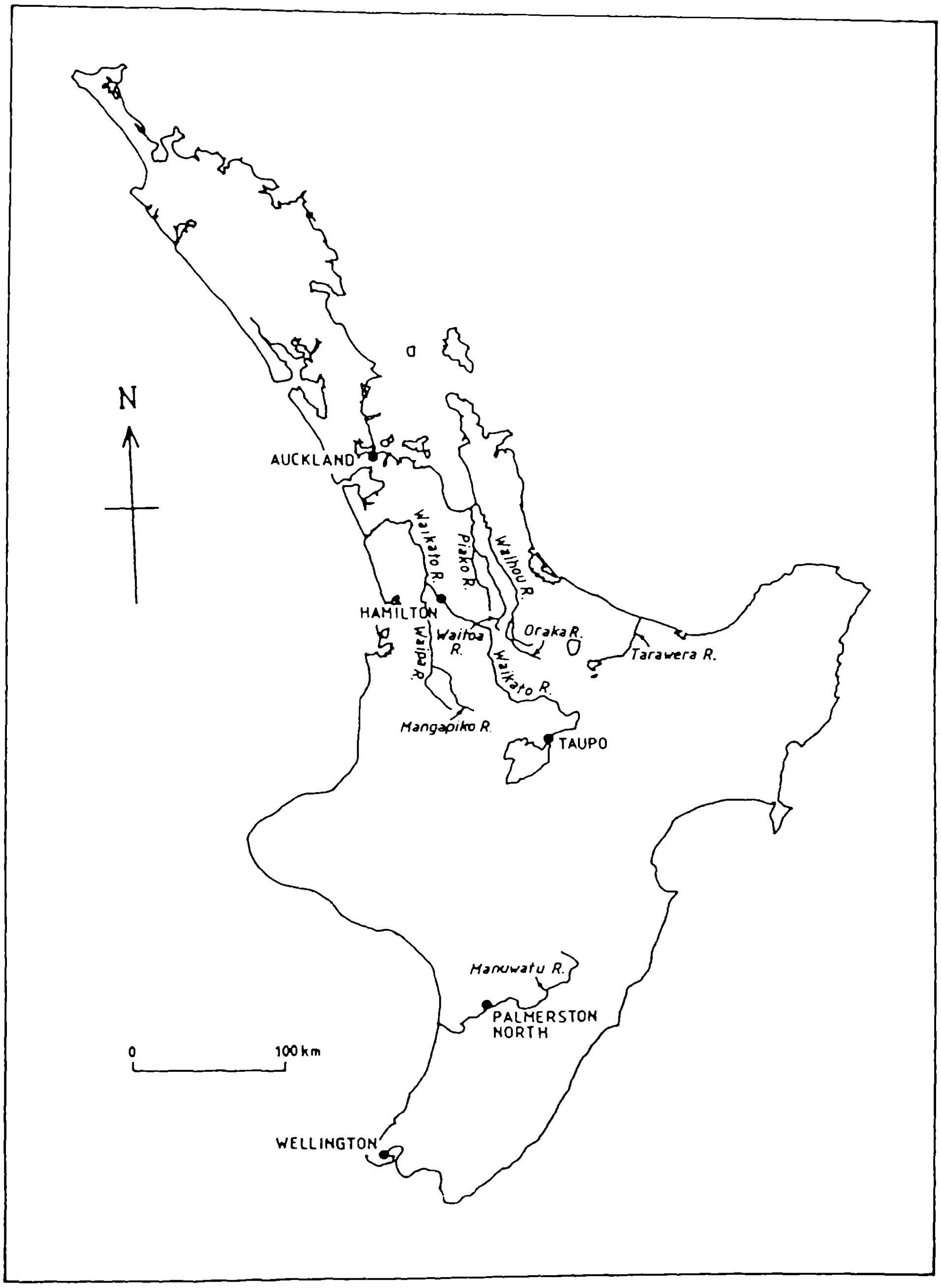


Figure 3.1 : New Zealand location map showing study rivers.

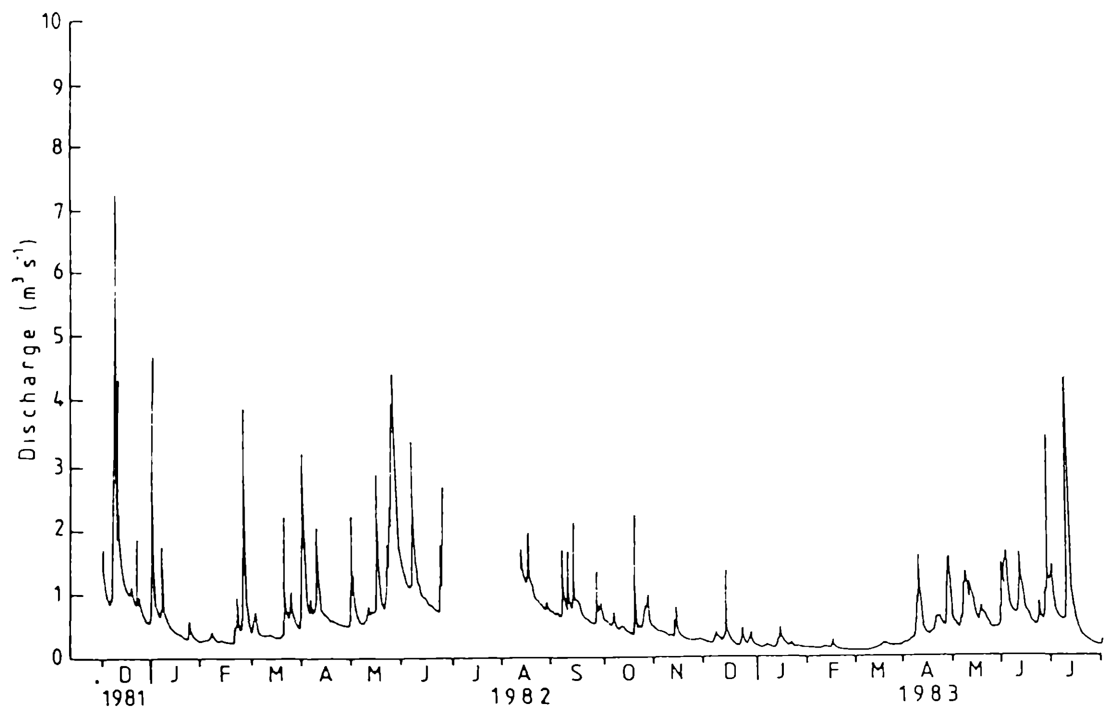


Figure 3.2 : Flow hydrograph for Waitoa River at the Landsdowne Road station for December 1981 to August 1983.

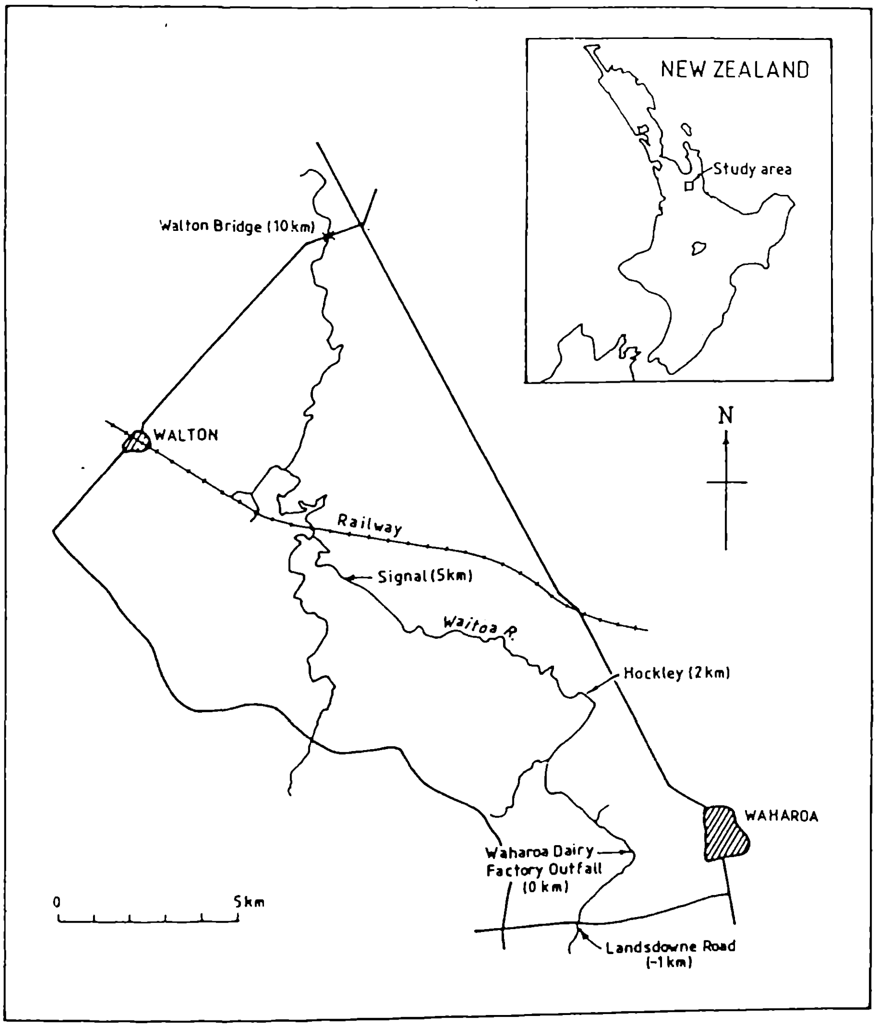


Figure 3.3 : Map showing sampling stations on the Waitoa River.

Plate 3.1 Aerial view of the Waitoa River showing sampling stations. Flown December 1983 with a north facing view.

Plate 3.2 Filamentous Oedogonium sp. algal colonisation of the Waitoa River at Landsdowne Road, February 1982.

Plate 3.3 Prolific Egeria densa macrophyte colonisation of the Waitoa River at Landsdowne Road, February 1983.

Plate 3.4 Waharoa dairy factory effluent discharge to the Waitoa River.

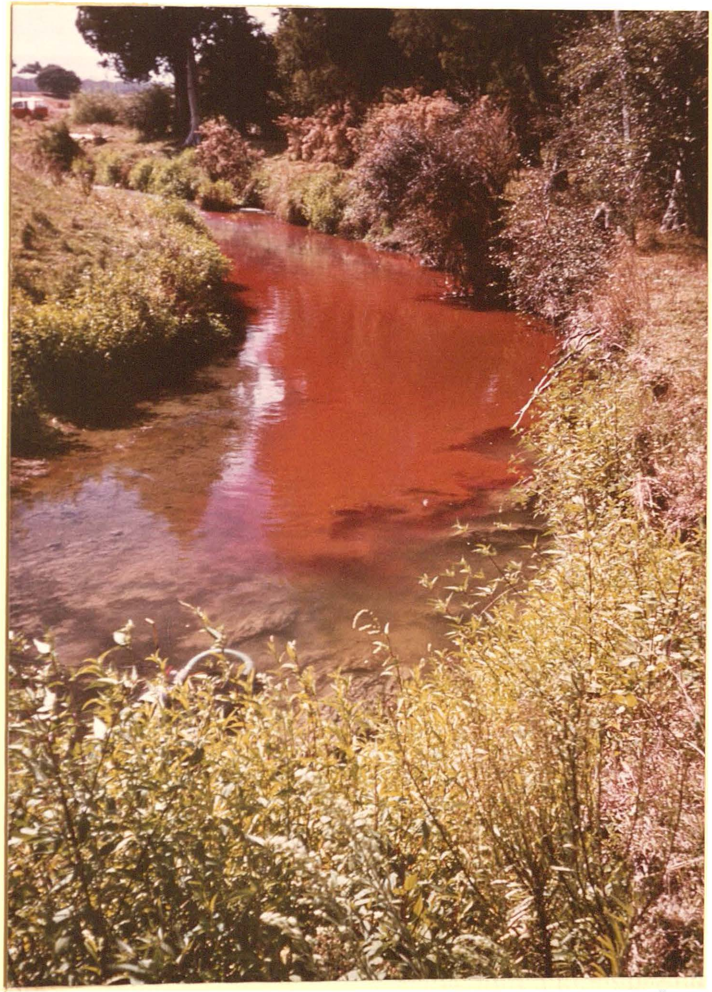


Plate 3.5 Dye study in the Waitoa River 50 m below Waharoa discharge showing 'sewage fungus' proliferation, 16 February 1982.

Plate 3.6 Potamogeton crispus and Oedogonium sp. colonisation of the Waitoa River at Walton Bridge, February 1981.

Plate 3.7 Extensive Potamogeton crispus proliferation of the Waitoa River at Walton Bridge, February 1983.

peripheral macrophytes (*Potamogeton crispus*, *Egeria densa* and *Nitella* sp) and extensive periphyton colonisation by an *Oedogonium* sp. (Plate 3.2), but complete *Egeria densa* domination had occurred by summer 1983 (Plate 3.3). Below the discharge (Plate 3.4) a prolific growth of 'sewage fungus' developed which sometimes extended for up to 2 km downstream (Plate 3.5), and which smothered the very few macrophytes present in this section. At the Walton Bridge, 10 km downstream, macrophyte colonisation by *Potamogeton crispus* and filamentous algae (*Oedogonium* sp.) were present in 1981 (Plate 3.6), which had developed 60% macrophyte coverage of the river bed by summer 1983 (Plate 3.7).

Only one tributary was present in the study section, and this contributed an estimated 30% to the main river flow at 8 km below the discharge. Numerous open drains empty into the river from the surrounding farmland, but they contributed to river flow only after rain. Above the discharge point, the water was clear with little apparent suspended material during summer base flow conditions; however, during flood flows and throughout the winter turbid conditions persisted with the waters having a milky-brown appearance. Below the discharge point the turbidity increased as a result of discharged milk solids and the growth of bacteria (see Plate 3.4).

The 1981/82 summer field programme concentrated on the Waitoa River. However, difficulties were experienced because of floods disrupting low flow sampling. Further experiments planned for the 1982/83 summer were largely prevented by low flow conditions, and the high effluent loading which resulted in anoxic conditions between 2 km and 5 km below the discharge.

3.2.3 Waiotapu River

The Waiotapu is a tributary of the Waikato River, 120 km southeast of Hamilton (Fig. 3.1). The river drains a predominantly agricultural catchment although its headwaters receive some geothermal inputs. Details of river characteristics are given in Table 3.1.

The river receives intermittent loading of untreated effluent from the Reporoa dairy factory, with the majority of waste being disposed of by spray irrigation. The river is also subjected to effluent loadings from numerous dairy sheds and at least two piggeries.

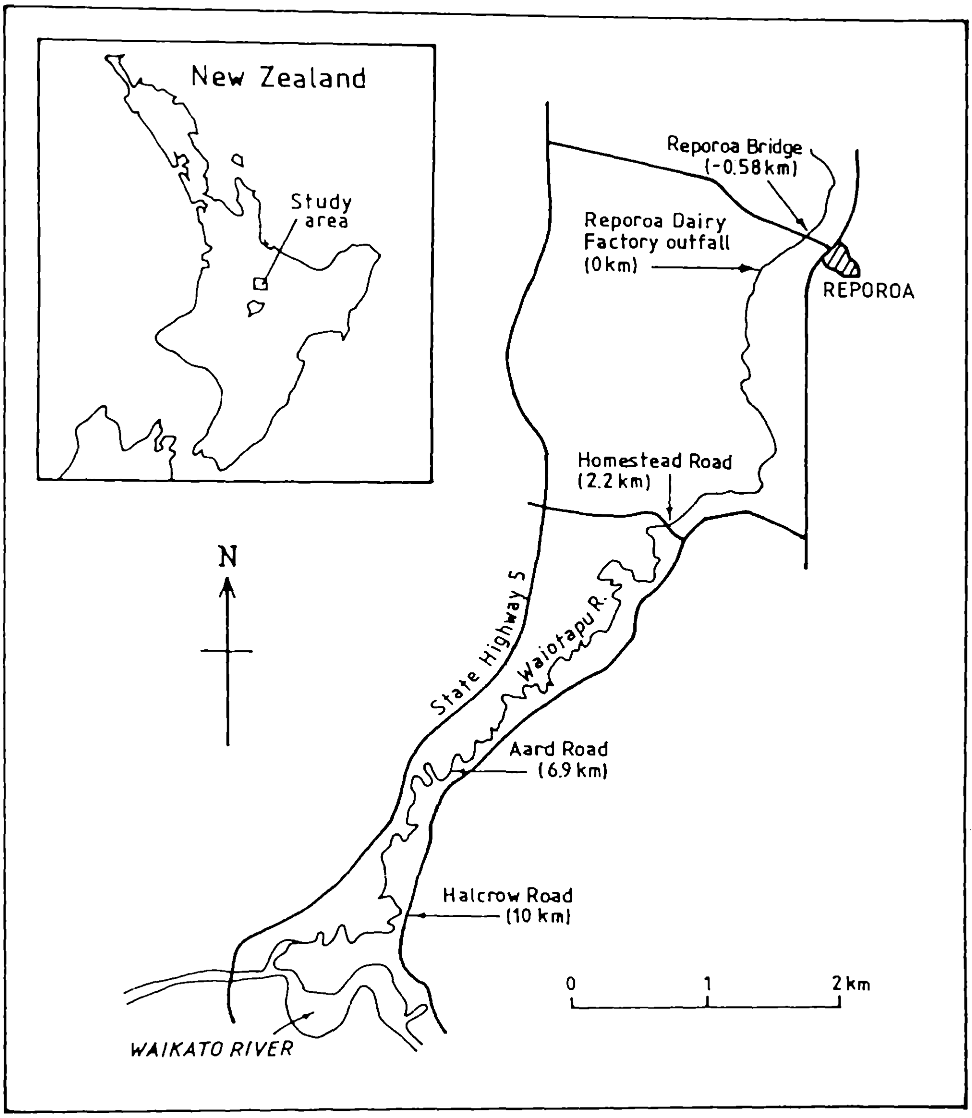


Figure 3.4 : Map showing sampling stations on the Waiotapu River.

Plate 3.8 Reporoa dairy factory discharge to the Waiotapu River, January 1983.

Five sampling stations covering a 10.6 km reach of the river were selected. These consisted of 1 control station, located above the discharge, and 4 stations below the discharge. These are shown on the location map (Fig. 3.4). The reach of 2.78 km including the discharge was subjected to the most intensive studies. Plate 3.8 shows the effluent discharge point from the Reporoa dairy factory.

The river bed was composed mainly of pumice (rhyolitic tephra). This material has a low density, resulting in a mobile bed with downstream dune movement. Particle size sorting caused by dune movement has resulted in mosaics of 'coarse' and 'fine' particulates.

Colonisation by submerged vegetation was minimal as a result of the mobile bed and limited to growth of Comsopogon sp. attached to some rocks and logs which protruded from the bed. Extensive sloughing of this material occurred and numerous drifting filaments were visible.

3.2.4 Tarawera River

The Tarawera River originates from Lake Tarawera and flows north into the Bay of Plenty, 150 km east of Hamilton (Fig. 3.1). The watershed combines developed pasture and forest, consisting predominantly of Pinus radiata. Details of river characteristics are given in Table 3.1.

The river receives primary-treated sewage from the town of Kawerau (pop. 9000) and processing waste from a paper mill (Caxton Paper Mills Limited) and the Tasman Pulp and Paper Company. The latter constitutes the majority of the river effluent load which consists of biologically treated waste, resulting in a river BOD_5 of 8 g m^{-3}.

Seven sampling stations covering a 30 km reach of the river were selected. The location of the effluent discharges are shown in Figure 3.5, with sampling stations listed in Appendix 5.

The riverbed was composed mainly of pumice with mobile dunes.

Submerged vegetation was limited to sites above the discharge as shown in Plate 3.9. Below the discharge point from the pulp mill the river is darkened considerably by the waste (Plate 3.10).

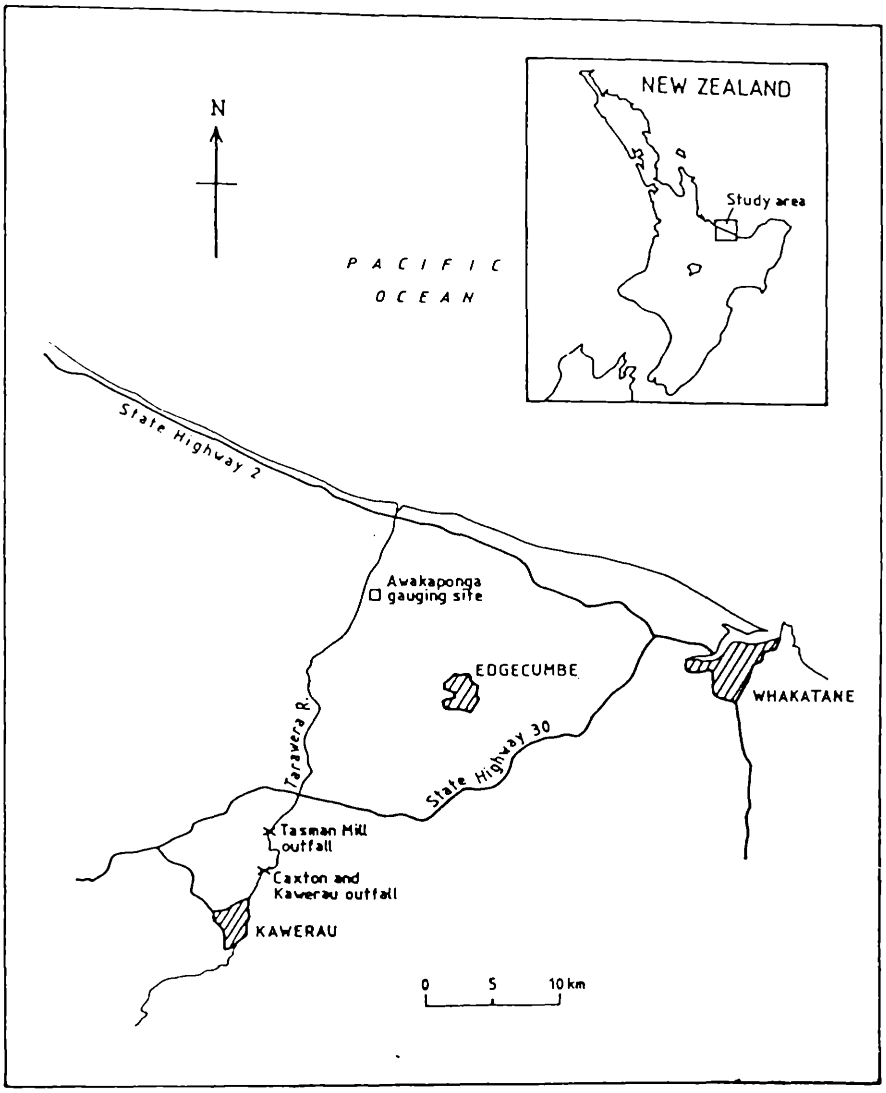


Figure 3.5 : Map showing the location of discharges on the Tarawera River.

Plate 3.9 The Tarawera River Showing the pulp and paper mills.

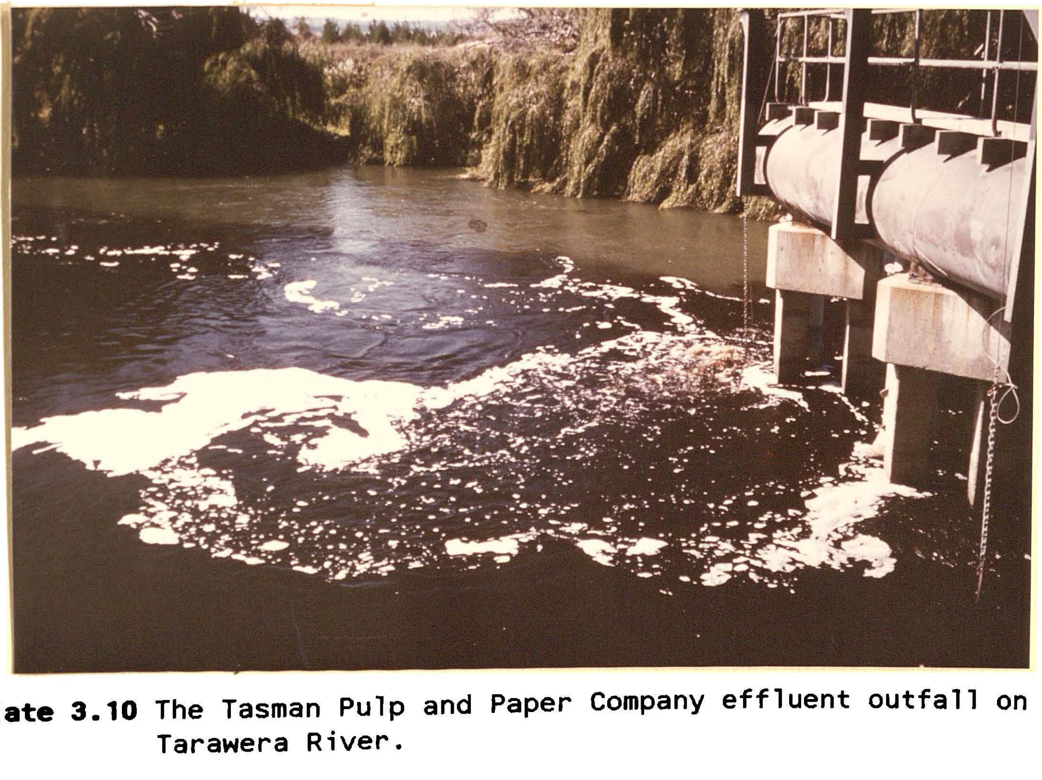


Plate 3.10 The Tasman Pulp and Paper Company effluent outfall on the Tarawera River.

3.2.5 Oraka River

The Oraka River is a tributary of the Waihou River, 50 km east of Hamilton (Fig. 3.1). River characteristics are given in Table 3.1.

The river receives an oxidation pond discharge from the Tirau Dairy Factory, which results in a BOD_5 of 5 to 7 g m^{-3}.

The river was sampled at 2 stations, one 500 m above and the other a similar distance below the dairy discharge point.

The bed material consists of coarse gravel, which developed a 'sewage fungus' biofilm below the discharge.

3.2.6 Manawatu River

The Manawatu River flows west after rising in the Ruahine Range (Fig. 3.1). River characteristics are given in Table 3.1.

The river receives municipal, dairy and meat processing effluents, resulting in BOD_5 values up to 10 g m^{-3}. The bed consists of cobbles (100-150 mm diameter) with an armoured bed during low flow periods.

Only one station was sampled and this was located downstream of all discharges, where extensive 'sewage fungus' colonisation existed.

3.3 FIELD PROCEDURES

3.3.1 Selection of sampling sites

A sampling station on the Waitoa River was chosen based on preliminary surveys indicating the location of the dissolved oxygen (DO) sag curve. Stations were required to be on a straight section of the river and in an area of representative cross-section.

Selection of the sampling sites for benthic sampling at a station were based on either random sites in the central flow region, or the stratified sampling of visually selected areas of 'coarse' and 'fine' particulates.

Similar procedures were followed to select sampling stations and sites in the Waiotapu and Tarawera rivers.

3.3.2 Sampling dates

Monthly longitudinal aquatic sampling surveys of the Waitoa River were undertaken for BOD, biomass (ATP), DO, and nutrients.

Continuous monitoring of DO and temperature were made at the Landsdowne Road station to identify seasonal variation in productivity. Measurements at other stations were undertaken, chiefly during low flow in summer when river DO was minimal.

Three longitudinal surveys of the Waitoa were undertaken to study those river processes which involved microbial metabolism in relation to effluent degradation. Measured were : (a) benthic microbial biomass; (b) enzyme activities; and (c) oxygen uptake rates.

Field sampling and experimental work was undertaken during the summer seasons 1981/82 through 1984/85. Segment oxygen mass balance studies were undertaken on the Waitoa, Waiotapu, and Tarawera rivers. Measurements of the distribution of microbial biomass, activity and DO beneath the sediment-water interface were also made on these rivers. Field measurements on other rivers generally included planktonic and benthic biomass and oxygen uptake kinetics. Benthic samples were also taken on some occasions for extended measurements of oxygen uptake kinetics to establish the rate of sediment carbon turnover in these shallow river systems.

3.3.3 Planktonic sampling

Samples were routinely taken using a 10 ℓ plastic bucket and rope from mid-stream at a given sampling location. Figure 3.6 shows a schematic diagram indicating sampling handling procedures for various analyses. After field measurement of DO and temperature, samples were placed on ice for transportation to a laboratory in 5 ℓ acid-washed plastic containers for processing. Processing was generally started within about 5h after sampling. For these methods see section 3.5.1.

Field measurement of DO and temperature were made using a YSI model 57 meter (Yellow Springs Instrument Company, Yellow Springs, Ohio) calibrated using tap water saturated with air.

Automatic sampling using a Mannings model 4500 set to composite 500 ml sample bottles at 4 x 15 min intervals, was used on one occasion to

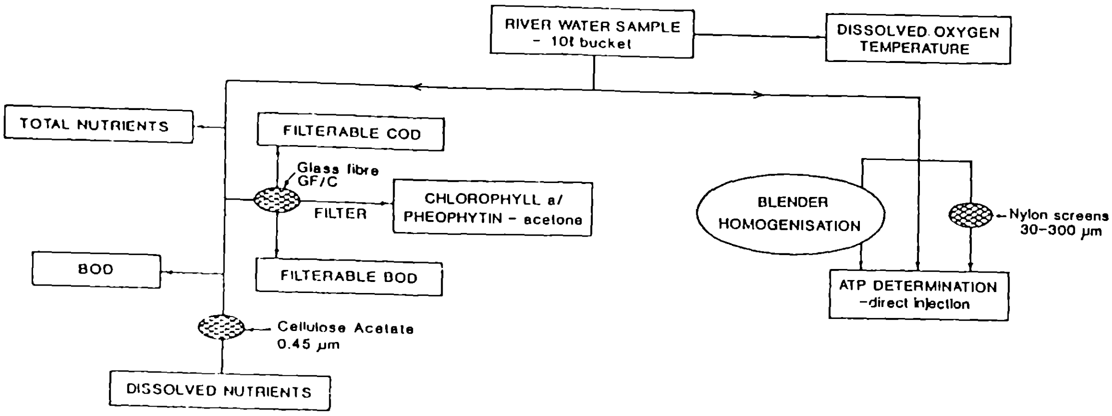


Figure 3.6 : Schematic representation of aquatic sample handling procedures.

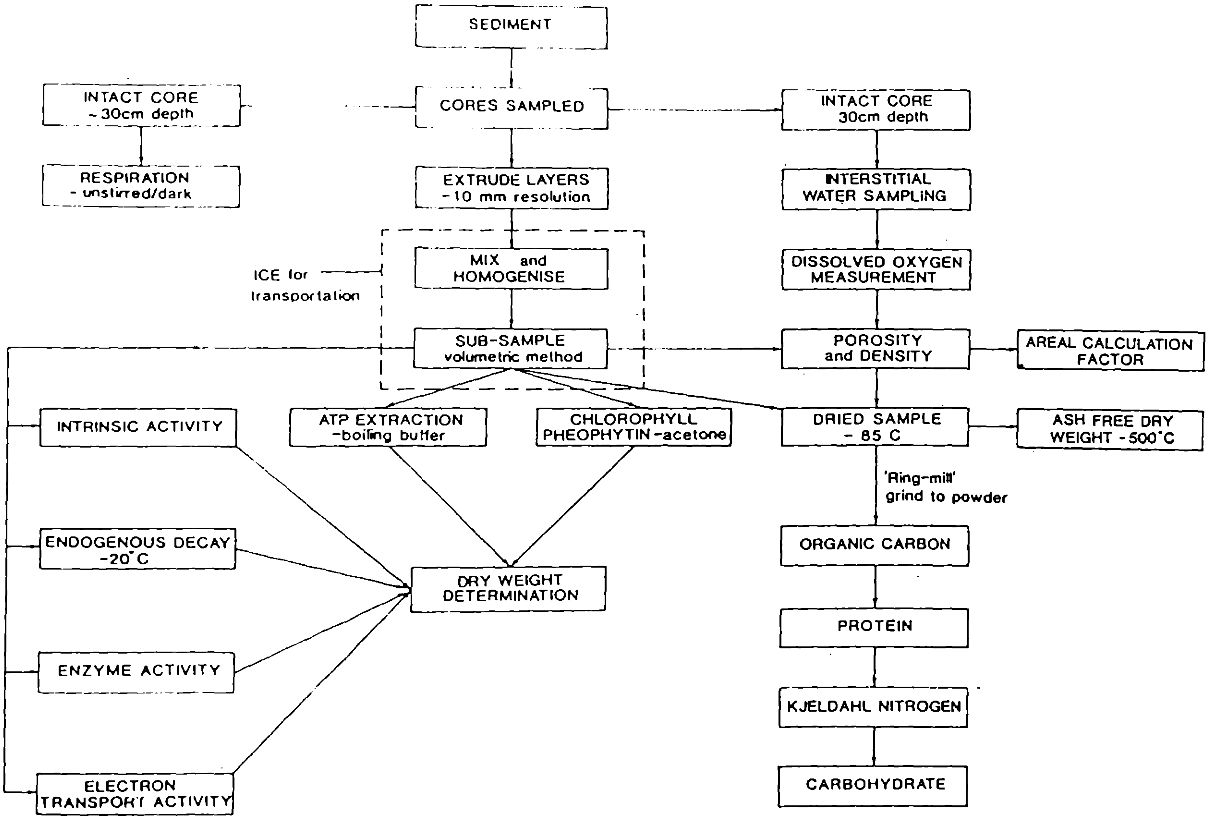


Figure 3.7 : Schematic representation of sediment sample handling procedures.

monitor diurnal variation of the dairy factory discharge. Samples were held on ice for analysis after the 24 h sampling period.

3.3.4 Planktonic monitoring

Continuous records of DO and temperature were obtained using YSI model 56 monitors previously calibrated using tap water saturated with air. These were installed in the river attached to metal stakes driven into the bed, and protected from drifting debris by steel 'finger-rake' screens placed upstream and angled to deflect material away. If the river velocity was <0.1 m s^{-1} a submersible stirring probe was used.

3.3.5 Benthic sampling

Five core samples were taken randomly from the mid-stream sediment, or from each of the chosen sediment types (e.g. 'coarse' or 'fine' particulates), using a 75 mm (internal diameter) 'perspex' core pushed into the riverbed and closed with a bung. The core was removed to the bank, the sediment fractions extruded, sliced into 10 mm sections and transferred to plastic bags. Figure 3.7 shows a schematic diagram indicating the sample handling procedure.

The bulked sediment fractions from each layer were homogenised by vigorous stirring with a spatula in a glass beaker. When sampling biofilms, either the top 10 mm sediment fraction or just the biofilm layer was removed, and vigorously agitated with an equal volume of water from the location, placed in a graduated measuring cylinder and allowed to settle for 5 minutes in order to determine the floc volume. This was used for estimation of the approximate biofilm thickness. The sample was further diluted 5x with river water, and homogenised using a Sorval Omnimix blender set at speed 8 for 30 sec in order to eliminate diffusional resistances (Bailloid and Boyle, 1970).

Intact cores were bunged securely at each end and transported on ice.

3.3.6 Benthic measurements

3.3.6.1 Chamber measurements _in situ_. Full details of chamber design, construction, features, and testing are provided in Chapter 4, together with details of the installation, operation and recording of chamber measurements. Details of methods associated with the testing of the chamber systems are also given in Chapter 4.

The benthic 'nitrogenous oxygen uptake rate' (NUR) was determined by either addition of ally-2-thiourea (ATU) to the chamber system (final concentration 10 g m^{-3}), or by measurement of the net inorganic NH_4^+ flux into the sediments, and a conversion factor (x 4.33 from Wezernak and Gannon (1968)) to estimate NUR. The latter method may underestimate the total NUR since the NH_4^+ production rate from within the sediments will not be accounted for (Cooper, 1984).

3.3.6.2 Core measurements **in vitro.** BUR measurements were made on unstirred 75 mm ID core samples, incubated in the dark at river temperature. Oxygen measurements were generally obtained by insertion of a stirring BOD probe through a port at 30 to 60 min intervals.

3.3.6.3 Measurement of sediment intrinsic oxygen uptake rates (I) in vitro. Routinely, 4 replicate subsamples of mixed sediment were placed in BOD bottles 2 x 8.5 ml volumes in each, 2 bottles were filled only with river water from the site, 2 had glucose added (20 g m^{-3} final concentration). All were incubated in the dark at river temperature. Light incubations were also done in order to estimate potential photosynthetic production. Bottles were vigorously shaken every 15 min to 30 min in order to reduce possible oxygen diffusion limitation, with DO measurements taken every 30 min to 60 min (variable measurement interval depending on activity) for a 2 h to 4 h period. The intrinsic activity (I) was estimated from the first three measurements.

Preliminary experiments on sediments obtained from the Waitoa River indicated that no statistical differences in intrinsic activites were obtained between those samples which were : (a) continuously stirred; (b) shaken at 15 min intervals; or (c) shaken at 30 min intervals. The results of these experiments are given in Appendix 1.1. Subsequent work, however, on pumice sediments showed a large response to stirring. Continuously stirred samples showed a 2.8-fold increase in intrinsic activity over comparable samples shaken at 1 h intervals, reducing to a 1.3 fold increase if samples were agitated at 15 min intervals. Details of these experiments are given in Appendix 1.2. Agitation of large numbers of samples at intervals of 15 min was extremely difficult in the field and hence rate estimates were limited to a minimal number of replicates (usually 3), measured over a short period (usually 45-60 min).

Continuous measurement using a stirring probe was initially found to be impractical due to the long measurement periods necessary to obtain an adequate fall in DO for accurate measurement. The development of a variable-offset amplifier gave a 10-fold resolution, and this instrument was used for studies on the Tarawera River.

3.3.6.4 Calculation of areal benthic oxygen uptake rates. In order to convert the intrinsic activity in sediments (I*) (method 3.3.6.3) from μg oxygen per g sediment per hour to g oxygen per m^2 per day for comparison with chamber BUR measurements, the sediment bulk density (ρ) was measured (see section 3.5.2.11). In order to calculate aerial rates (I) an arbitrary depth of 1 cm was chosen and the following calculation used

$$I \text{ g } O_2 \text{ m}^{-2}\text{day}^{-1} = I^*\mu\text{g } O_2 \text{ g}^{-1} \text{ h}^{-1} \times \rho \text{ g cm}^{-3} \times 1 \text{ cm} \times 10^4 \text{ cm}^2 \text{ m}^{-2} \times 24 \text{ h day}^{-1} \times 10^{-6} \mu\text{g g}^{-1} \qquad 3.1$$

The calculated areal rates were then integrated to the depth of sediment DO penetration to equate with the measurement total flux (i.e. the BUR).

3.3.6.5 Calcuation of the effectiveness factor (η) and depth of oxygen penetration (δ_c). The concept of catalytic effectiveness has been applied to biofilms (La Motta, 1976b). The effectiveness factor is expressed as:

$$\eta = \frac{\delta c}{\delta} \qquad 3.2$$

where δ is the biofilm depth and δ_c is the depth of limiting nutrient penetration.

This equation defines the fraction of total intrinsic activity present which is expressed as the measured or macroscopic rate. For biofilms the measured chamber BUR (N, g O_2 m^{-2} day^{-1}) may be expressed as a function of the intrinsic activity (I, g O_2 m^{-2} day^{-1}) as follows:

$$\eta = \frac{N}{I} \qquad 3.3$$

To estimate the depth of oxygen penetration into the biofilm requires an estimate of biofilm thickness (δ) and using eq 3.2

$$\delta_c = \eta\ \delta \qquad 3.4$$

For sediments the areal intrinsic activity (I*) is calculated on the basis of a 1 cm thickness layer. Therefore, for sediment the depth of oxygen penetration is represented by

$$\delta c = \frac{N}{I} \text{ cm} \qquad 3.5$$

3.3.6.6 DO profiles in benthic interstitial water. For the Waiotapu River, interstitial water was sampled from replicate cores using a long needle attached to a 0.5 ml syringe inserted through a rubber disc covering the core surface (the rubber disc was used to minimise the possible drawing down of surface water along the syringe needle). Once the needle was positioned at the desired depth, 0.4 ml of interstitial water was withdrawn and injected into a calibrated Rank oxygen electrode (Rank Bros., Bottisham, England) previously flushed with nitrogen gas. The DO was measured within 5 min of removal of the core from the river.

For the Tarawera River, cores were collected by divers from the centre of the river using Perspex cylinders having holes drilled at 1 cm intervals along their length. The holes were taped over for collection. Care was taken during collection to avoid disturbance, but some was unavoidable. Samples of the interstitial water (0.8 ml) were taken using 1 ml hypodermic syringes; the needle entered the central core through the taped-over hole. Gas shielding of the electrode was with either nitrogen or propane gas. The small gas void volume within the syringe was not purged with nitrogen for these measurements, and subsequent experiments showed that this was probably a large contributing factor to the failure to locate anoxic conditions within these sediments.

3.3.6.7 Calculation of the benthic dispersion coefficient (D_s). Dispersion coefficients for the mass transfer of dissolved oxygen from the water to the sediment were calculated using two sources of data: (i) from chamber estimates of oxygen flux (i.e. BUR) and from the measurements of pore water oxygen concentrations in the uppermost strata of sediment; and (ii) from the chamber BUR estimates and using the estimated depth of oxygen penetration based on the intrinsic activity measurements. The following rearranged finite difference form of Fick's first law was used:

$$D_s = \frac{-F}{\phi \frac{\Delta c}{\Delta d}} \qquad 3.6$$

Where D is the dispersion coefficient (cm^2 s^{-1}); F is the flux from water to the sediment, in this case the BUR (g O_2 cm^{-2} s^{-1}); ϕ is the porosity (%) (see Section 3.5.2.11) ϕ was assumed to be 0.5 for all calculations; Δc is the difference in concentration between the uppermost region of the sediment and the water above, and Δd is the depth from the sediment surface to the midpoint of the uppermost region of the sediment (Berner 1980; Florek and Rowe, 1983).

Using the chamber and intrinsic activity measurements for (ii) above, the depth of oxygen penetration is calculated (eqn 3.5) which is equivalent to 2d for eqn 3.6.

3.4 Determinations of Segment Oxygen Mass Balance

Details of methods used to determine mass balances are given in the appropriate chapters (Section 6.1 for the Waitoa River, 5.3.2 for the Waiotapu River and 5.4.2 for the Tarawera River).

3.5 LABORATORY PROCEDURES

This section covers the methods used for planktonic and benthic samples together with the calculations necessary. All samples were transported from the field to the laboratory on ice prior to further handling procedures.

3.5.1 Planktonic analyses

An outline of all preparatory procedures for aquatic sample analysis described in this section is shown in Fig. 3.6.

3.5.1.1 Nutrients. All nutrient analyses described in this subsection were performed by the Water Quality Centre Analytical laboratory.

Samples for analysis for dissolved nutrients were filtered using 0.45 µm pore size cellulose acetate membrane filters, and with total nutrient samples, stored at -20 °C for subsequent analysis. Inorganic nutrients were determined using an automated system with dissolved reactive phosphate being analysed by the method of Downes (1978a), nitrate-nitrogen by the method of Downes (1978b), and ammonium-nitrogen by the method of Technicon Corporation (1973). Total phosphorus and total dissolved phosphorus were analysed as described by American Public

Health Association - APHA - (1976) following digestion with sulphuric acid/potassium persulphate. Total Kjeldahl nitrogen was determined by digestion of samples containing less than 20 µg ammonium-nitrogen with a sulphuric acid/potassium sulphate/zirconium sulphate mixture followed by colorimetric analysis for ammonium nitrogen as indophenol blue (following APHA 1976).

3.5.1.2 **Biochemical oxygen demand (BOD).** The routine procedure applied for this test followed APHA (1976) involving a 5 day incubation at 20 °C and yielding the 'BOD_5'. Dilution was with standard dilution water if the anticipated BOD exertion exceeded 8 g m^{-3}.

Additionally a BOD measurement on a pre-filtered sample (designated FBOD) was sometimes determined to estimate the relative proportion of oxygen demand attributable to dissolved organic material (DOM) assimilation (i.e. principally microbial growth) compared with the total BOD which may contain a significant contribution for particulate algal and bacterial respiration (i.e. respiratory decay). Samples were pre-filtered using a Whatman GF/C filter with a 5% reinoculation of the unfiltered sample (prior to any subsequent dilution), to ensure that an adequate microbial seed was present.

3.5.1.3 **Chemical oxygen demand (COD).** Samples for COD analysis were analysed by the Water Quality Centre laboratory using the APHA (1976) method. Samples designated FCOD were pre-filtered using a Whatman GF/C filter prior to determination.

3.5.1.4 **Electrolytic biochemical oxygen demand (EBOD).** For several experiments involving the addition of whole milk and glucose to river water samples, the kinetics of aquatic oxygen demand exertion were measured using a commercial EBOD apparatus modified to increase sensitivity and minimise interferences (Hickey and Nagels, 1985). Routinely, 1 ℓ samples were incubated at 20 °C, and monitored automatically at hourly intervals for 5 days.

3.5.1.5 **Adenosine 5'-triphosphate (ATP).** ATP was assayed as a measure of total microbial biomass present.

Preparation. Water samples were vigorously agitated prior to subsampling for extraction. An experiment to determine the sizes of the particulate biomass present was done using a range of nylon mesh filters (300, 90 and 30 µm). The effect of vigorous homogenisation of the sample was tested using a Sorval blender (speed 8 for 30 s) prior to subsampling. Routinely, subsamples were taken from the population which had passed through a 30 µm filter (i.e. <30 µm) and a vigorously homogenised whole sample to measure total biomass.

Extraction. ATP was extracted by direct injection of 1 ml of sample into 5 ml of boiling McIlvaine buffer (Bullied, 1978) held in a sand bath. After 30 sec the sample was transferred to a boiling water bath and held on the boil for 5 min. The volume was made up to 6 ml and any turbid samples centrifuged prior to storage at -20 °C until analysis. Extractions were performed in triplicate.

Direct injection extraction was preferred to prior concentration by filtering because substantial ATP loss has been shown to occur with the filtration process (Jones and Simon, 1977; Fuhrman and Azam, 1980; Pridmore et al., 1982).

Assay. ATP concentrations were measured using the luciferin-luciferase method with reconstitution according to Jones & Simon (1977) with only minor modifications. 50 mg of crude luciferase (FLE-50, Sigma Chemical Company) were hydrated with 7 ml of reconstitution mixture (bovine serum albumin, 0.1% w/v; EDTA, 1 mM; $MgSO_4$, 10 mM; adjusted to pH 7.5 with 1 M KOH) (Lundin and Thore, 1975a). To this was added 3 ml of D-luciferin solution prepared by dissolving 10 mg of D-luciferin mixture, which was then centrifuged at 4000 x g for 3 minutes and allowed to equilibrate for 1 hour in the dark at room temperature.

Light emission from the enzyme reaction was monitored using a JRB model 3000 ATP photometer (Science Application Inc. San Diego, Ca., USA) and a chart recorder. ATP content was estimated by the peak height integration of the first 3 seconds of light emission after 100 µl of enzyme preparation was injected into 200 µl of extract. Comparison with results obtained from an integrated assay (15 sec delay and a 60 sec assay) were also performed.

Results were derived from a double-logarithmic plot of ATP concentration versus integral light emission, with standards ranging from 0.005-50.0 µg (ATP) l^{-1}. Corrections were then made for dilution or concentration of the field samples during extraction.

Internal standards of pure ATP (50 pg) were added to 1 ml of the extracts to test for inhibition of the bioluminescent reaction. The ATP content of the spiked sample (μg (ATP) l^{-1}) was compared with the sum of the sample and spike determined individually.

Assays were initially performed in triplicate. However, reproducibility was generally found to be better than ±5% (SE) so single assays were performed and the reaction kinetics followed on a chart recorder.

3.5.1.6 Chlorophyll a and pheophytin. Chlorophyll a measurements were made to estimate the algal biomass present.

Extraction. Replicate 200 to 1000 ml samples were vacuum concentrated on 47 mm diameter Whatman GF/C filters, placed in 10 ml of 90% acetone ($MgCO_3$ neutralised) and homogenised in a teflon tissue grinder. Following centrifugation (4000 x g for 5 min) of extracts, spectrophotometric absorbtion at 665 nm, 645 nm, 630 nm and 750 nm was measured with repeat measurement at 665 nm and 750 nm 1 min after acidification (0.1 vol HCl). Acidification enables correction to be made for the chlorophyll a breakdown product pheophytin a which may be present.

Assay. When pheophytin pigments were absent the trichromatic equation of Parsons and Strickland (1963) was used:

$$\text{chl } \underline{a} = [11.6\, A_{665} - 1.31\, A_{645} - 0.14\, A_{630}] \frac{V_s}{V_E \cdot b} \qquad 3.7$$

In the presence of pheophytin pigments, the equations of Lorenzen (1967) were used:

$$\text{Chl } \underline{a} = [26.7\, (A_{665}^{*} - A_{665})] \frac{V_s}{V_E \cdot b} \qquad 3.8$$

$$\text{pheophytin} = [26.7\, (1.7\, A_{665}^{*} - A_{665}) \frac{V_s}{V_E \cdot b} \qquad 3.9$$

where A_{665}, A_{645}, A_{630} and A_{950} = absorbance at respective wavelengths (nm); A^* = absorbance after acidification; V_s = volume of extract (ml); V_E = volume of water filtered for extraction (ℓ); b = pathlength of curvette (cm).

The choice of equation was based on an estimate of pheophytin pigments present. The change in specific absorbance resulting from acidification (corrected for turbidity i.e. A_{750}) may be used as a testing criterion. The lower specific absorption of pheophytin (Lorenzen, 1967) results in a theoretical ratio of $A_{665}/A_{665}*$ of 1.7. The testing equation used was:

$$T = \frac{A_{665} - A_{750}}{A_{665}* - A_{750}*} \qquad 3.10$$

3.5.1.7 Glucose. Glucose measurements were made on subsamples taken from the chamber in experiments in which additional glucose supplemented the natural dissolved organics. Subsamples (10 ml) were immediately membrane-filtered (0.45 µm pore size, cellulose acetate) and stored on ice (<4 h) prior to freezing (-20 °C) for storage prior to assay.

Enzymatic analysis using hexokinase (Bergmeyer et al., 1974) was performed on the samples. Detection limit for this assay was 0.5 gm^{-3}.

3.5.2 Benthic Analyses

An outline of all preparatory procedures for benthic sample analysis described in this section is shown in Fig. 3.7.

3.5.2.1 ATP

Extraction. ATP was extracted (in pentuplicate) by addition of approximately 2 ml of homogenised sediment (using an adapted 5 ml syringe) to 16 ml of boiling McIlvaine buffer (Bullied, 1978) held in a sand bath. After 30 sec the sample was transferred to a boiling water bath for 4.5 min, then transferred to an ice bath for cooling. To two of these samples an internal standard of ATP was added (total amount 5-10 x the anticipated substratum extraction) immediately following sediment addition, to allow correction for extraction efficiency. Cooled samples were transferred to a graduated centrifuge tube, centrifuged (4000 x g, 5 min) and the volume made up to 20 ml with McIlvaine buffer prior to freezing (-20 °C) for storage until assay. Dry weights were determined for individual samples.

Comparisons were performed between the extraction efficiencies of boiling McIlvaine buffer (Bullied, 1978), sulphuric acid (Karl and La Rock, 1975), and trichloroacetic acid (TCA) (Jenkinson and Oades, 1979). The acid extraction was found to produce more variable results with greatly increased inhibition of bioluminescence requiring correction.

Ease of manipulation and consistency favoured the McIlvaine buffer extraction procedure. A summary of the results of these work-up procedures is given in Appendix 1.2.

Assay. The luciferin-luciferase method of Karl and La Rock (1975), was used with peak height integration (0-3 sec) after injection of 200 µl of enzyme preparation into 1000 µl of sample extract. For comparison some integration assays were also performed involving a 15 sec delay and a 60 sec integration. Because of the high degree of coloured humic material extracted, great care was taken with internal spiking of ATP to determine interferences. Routinely only one sample replicate was spiked, however, where visible differences in colour occurred, individual samples were spiked. Correction for extraction efficiency and assay interference in the ATP measurements was using:

$$\mathrm{ATP} = \frac{\text{mean ATP}}{\text{Extraction efficiency x Inhibition factor}}\ \mu\mathrm{g\ g}^{-1} \qquad 3.11$$

where

$$\text{Inhibition factor} = \frac{\text{(Sample + spike) - sample}}{\text{(Blank + spike) - blank}} \qquad 3.12$$

$$\text{Extraction efficiency} = \frac{(\text{mean ATP.g}^{-1} + \text{spike}) - \text{mean ATP.g}^{-1}}{[(\text{Blank+spike})-\text{Blank}]\text{mean g}^{-1} \text{ x Inhibition factor}} \qquad 3.13$$

3.5.2.2 Chlorophyll _a_ and pheophytin.

Extraction. The method used was that of Jones _et al_. (1979). Approximately 2 ml of sediment was placed in 18 ml of acetone ($MgCO_3$ treated, 4 °C) and 1.5 ml of distilled water, in replicate assays. This was left overnight at 4 °C in the dark, then vigorously agitated and centrifuged (4000 x g for 5 min). Dry weights were determined for individual samples.

Assay. Procedures were as for aquatic chlorophyll samples (described in section 3.5.1.6) except that total chlorophyll extracted was expressed on a dry weight basis.

3.5.2.3 EBOD. The electrolytic respirometer, modified for increased sensitivity (Hickey and Nagels, 1985), was used on both intact sediment cores and homogenised subsamples to determine the rate of respiratory decay measured in the absence of added external carbon source.

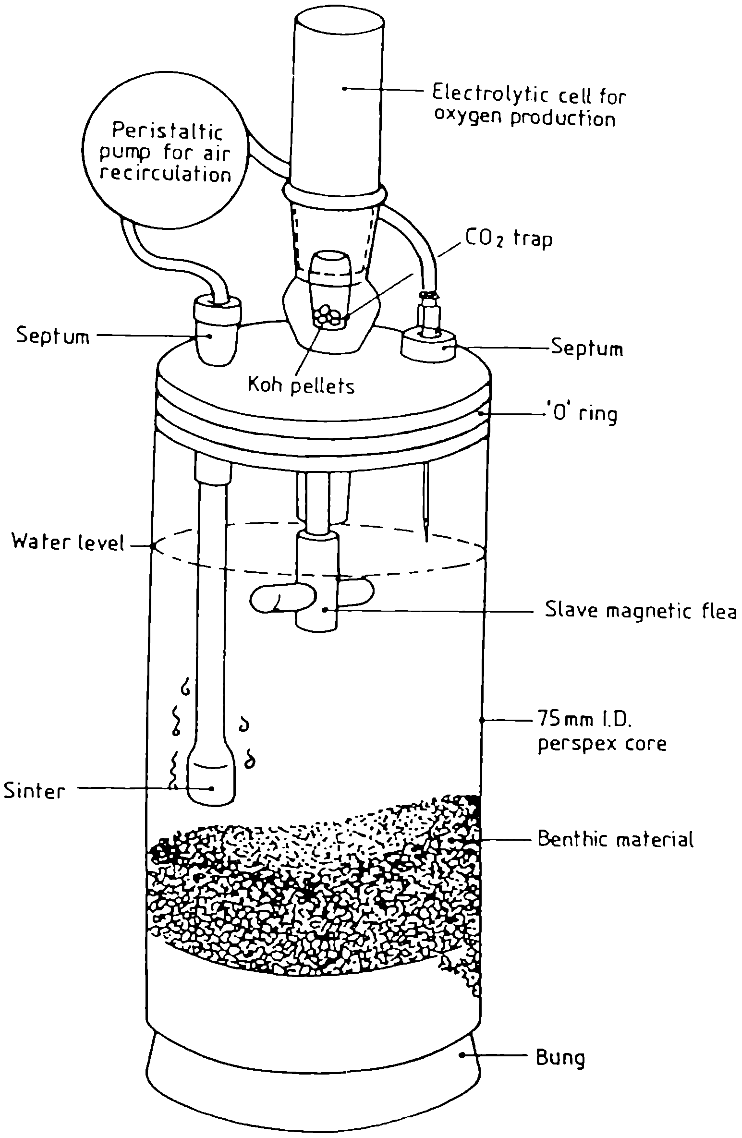


Figure 3.8 : Apparatus used for measurements of the oxygen uptake rate of cores with the EBOD respirometer.

Procedure. Samples of homogenised material (50 g wet weight) were placed in 1200 ml bottles, overlain with 1000 ml of GF/C filtered upstream water (for Waitoa and Waiotapu River samples) supplemented with BOD dilution water nutrients (APHA, 1976), and maintained at 20 °C with vigorous stirring. CO_2 was removed using potassium hydroxide pellets. Automatic monitoring of the oxygen consumed was recorded at 1 h intervals.

Measurements with intact cores used the apparatus shown in Fig. 3.8. This featured a recirculating air sparge system to maintain oxygen saturation, while additional turbulence was provided by a magnetic 'slave' stirrer. This was operated at a level well below the shear stress necessary to scour the sediment surface.

Data handling. A first order decay rate model was fitted to the cumulative respiration progression data expressed in terms of carbon equivalents by stoichiometry. That is:

$$Y = L_o (1 - e^{-kt}) \qquad 3.14$$

where Y = carbon equivalent of oxygen consumed (g C m^{-3}); L_o = total carbon of sample (g); k = first order decay rate coefficient (day^{-1}); and t = time (day).

The total amount of oxidisable material present was determined by measuring the readily oxidisable carbon (see section 3.5.2.8).

Curve fitting was by manual iteration of the variable k. The visual fitting procedure for a first order curve has an estimated accuracy for k of ±0.005 day^{-1}.

The half-life for endogenous respiration was calculated using the equation:

$$t_{\frac{1}{2}} = \frac{\ell n\ 2}{k} \text{ day} \qquad 3.15$$

3.5.2.4 Enzyme activities. Enzyme activities were measured for a range of functional enzyme types to characterise the activity of the microorganisms present. The background describing the choice of enzymes and the objectives for these experiments is given in the Literature Review (section 2.5).

The enzyme activities assayed for were:

	Derivative
β-galactosidase	PNP-β-D-galactoside
β-glucosidase	PNP-β-D-glucoside
protease	azo-casein and α-N-benzoyl-DL-arginine-PNA
alkaline phosphatase	PNP-phosphate disodium
phosphodiesterase	bis-PNP-phosphate.

where PNP refers to ρ-nitrophenol and PNA to the ρ-nitroanaline derivatives.

Extraction. To simplify the logistics of handling multiple samples from several locations necessitated the development of a simplified extraction procedure. A freeze/thaw extraction technique with a non-ionic detergent (Triton X-100) proposed by Miozzari et al. (1978) for enzyme assays, was tested (see work up procedure in Appendix 1.2). Standards for extraction efficiency were not included because the properties of enzyme types naturally present may well differ substantially from commercially available enzymes which would be used. The use of internal standards to test for possible inhibition effects (e.g. by heavy metals) would also be subject to similar difficulties. The sediments of interest are not likely to have significant heavy metal contamination since organic discharges were the only major contamination.

The extraction solution of Jones and Simon (1979) was used except that the Triton X-100 concentration was reduced to 0.2% (w/w), since preliminary experiments indicated lower final activity at the higher 1% concentration (see Appendix 1.3) as recommended for bacterial cells (Miozzari et al., 1978). Triplicate volumetric subsamples (8.5 ml) of homogenised sediment were added to 50 ml of extraction solution. This consisted of:

Triton X-100	3 ml ℓ^{-1}
Polyvinyl pyrrolidone	1.5 g ℓ^{-1}
$MgSO_4$	75 μmole ℓ^{-1}
in TRIS/EDTA	50 mmol ℓ^{-1}/0.2 mmol ℓ^{-1} buffer (pH 7.4).

The samples were frozen (-20 °C) for at least 12 h and assayed within 2 weeks. No measurements of enzyme stability relative to storage period were made. However, Miozzari et al. (1978) reported no loss of their enzyme activities with 2 weeks storage.

Assay. All enzyme assays were performed using PNP derivatives (Sigma Chemical Co., St Louis, USA) except the protease assay which measured hydrolysis of azo-casein, and used a PNA derivative.

Extracts were thawed, centrifuged (4000 x g for 45 min at 0 °C) and the supernatant held on ice.

The assay follows the basic procedure of Browman and Tabatabai (1978) using stock solutions of PNP-substrate (5 mM) incubated with extract at 35 °C for 60 min in triplicate. The reagents comprised of:

2 ml enzyme extract
0.5 ml PNP solution
2 ml STOP

where the STOP was generally TRIS-NaOH buffer (0.1 M, pH 12) but for the phosphatase assay the STOP was phosphate buffer (pH 12) (Tabatabai and Bremner, 1970).

Controls were performed by adding extract solution after the STOP had been performed. The test solutions and controls were read at 410 nm.

Standards were prepared by dissolving 1 g of ρ-nitrophenol (or ρ-nitroanaline) in 1 ℓ of distilled water.

Protease activity determined using hydrolysis of azo-casein (0.1%) used reagents consisting of:

1 ml azo-casein
4 ml TRIS buffer (0.1 M, pH 8.0)
3 ml STOP

where the STOP was 5% trichloroacetic acid (TCA).

Controls were as for the PNP assays with absorbance measurements at 440 nm.

Rate of hydrolysis was reported at $\Delta A_{440} \cdot \text{time}^{-1}$.

Calculation of enzyme activities.

$$\text{Activity} = \frac{x \cdot V_t \cdot D \cdot 0.121}{t \cdot V_e \cdot W} \quad \text{mmol PNP} \cdot g^{-1} \cdot h^{-1} \qquad 3.17$$

where x = PNP hydrolised (μg); V_t = total volume of assay (ml); t = incubation time (h); V_e = volume of enzyme extract in sample (ml); D = dilution factors for the enzyme extract; and W = dry weight of sample extracted (g).

3.5.2.5 Dry weight. Sediment was dried to constant weight at 85 °C (12-24 h depending on sample size), with individual sample weights determined for each subsample.

3.5.2.6 Ash-free dry weight (AFDW). Otherwise known as loss on ignition (LOI) measurements were made to provide a crude estimate of the quantity of organic carbon present (assumed as 50% of LOI after Vollenweider (1974)). Dried sediments were heated to 500 °C for 1 h.

3.5.2.7 Organic carbon. Carbon was analysed on dried material which had been 'ring-milled' to produce a fine powder, using the method of Gaudette et al. (1974). This method, refered to as 'readily oxidisable carbon' involves a ferrous sulphate titration of a sulphuric acid/dichromate mixture to which a sample had been added. Interlaboratory comparison with more rigorous total combustion methods for carbon has shown satisfactory agreement between standard samples (results not shown).

3.5.2.8 Protein. Protein was assayed on powdered samples after Rausch (1981) according to the method of Izhaki (1964). Sediment material was extracted several times in boiling alkali, with protein assay by a copper complex reaction (micro Biuret method). Bovine serum albumin (BSA) was used as standards.

3.5.2.9 Total Kjeldahl nitrogen (TKN). TKN was analysed on powdered samples by the WQC laboratory following APHA (1976) procedure. Intercalibration with the BSA standards used for the protein assay showed the nitrogen content of BSA to be 10% (w/w). This allowed conversion of protein assays to total nitrogen equivalents.

3.5.2.10 Carbohydrate. Duplicate areas of approximately 4 cm^2 were scraped from artificial substrata and transferred into vials containing 5 ml of 2N sulphuric acid. Sediment samples were analysed from powdered material. The hydrolysis and assay procedure followed Geesey et al. (1978) involving 4h at 94°C for hydrolysis, with subsequent analysis using a phenol-sulphuric assay. Glucose was used as a standard.

3.5.2.11 Porosity and Density. A volumetric method was generally applied to the measurement of sediment characteristics. A 250 ml measuring cylinder, approximately 100 ml of sediment and a constant volume of 100 ml of water were used. The dry weight (DW) of sediment was known, and 100 ml of water added to this material in a measuring cylinder. the following were calculated by:

Particle density (ρ*)

$$\rho^* = \text{DW} \div (\text{total volume} - 100)\ \text{g ml}^{-1} \qquad 3.17$$

Bulk density (ρ)

$$\rho = \text{DW} \div (\text{wet sediment volume})\ \text{g ml}^{-1} \qquad 3.18$$

Porosity (ϕ)

$$\phi = \frac{(\text{value added - total volume + wet volume})}{\text{wet volume}} \times \frac{100}{1}\ \% \qquad 3.19$$

3.5.2.12 Particle size. Particle size and descriptions used herein refer to the Wentworth scale shown in Table 3.2 (after Doeglas, 1968).

Five size classes were considered adequate for particle size measurement in the study rivers. A set of copper-wired square-mesh sieves (0.5, 1, 2, 4 and 8 mm) were used to separate fractions.

The sample (~ 200 g) was sieved on an automatic shaker with a standard sieving time of 15 min. per sample. Particles coarser than 4 mm were hand-sieved (8 mm mesh), while larger particles predominating in some rivers were measured individually using a ruler. The contents of each sieve or otherwise determined fractions were weighed to the nearest 0.1 g.

Whenever a reference is made to a certain grain-size fraction, the fraction is referred to by the lower limit, which corresponds with the sieve mesh that retained the fraction (e.g. the 2-4 mm fraction is referred to as the 2 mm fraction).

Measurements were intended to provide preliminary comparisons between stations, sites and rivers. Larger samples would be necessary to adequately describe the sediment characteristics (Leeder, 1982).

Fraction (mm)	Phi value ($-\log_2$ fraction)	Sieve mesh (mm)	Phi index of fraction (Doeglas)	Name of fraction
128-256	-7	ruler	$\underline{8}$	cobbles
64-128	-7	ruler	$\underline{7}$	cobbles
32-64	-5	ruler	$\underline{6}$	large pebbles
16-32	-4	16 (round)	$\underline{5}$	small pebbles
8-16	-3	8 (round)	$\underline{4}$	coarse gravel
4-8	-2	4 (square)	$\underline{3}$	medium gravel
2-4	-1	2 (square)	$\underline{2}$	fine gravel
1-2	0	1 (square)	1	very coarse sand
0.500-1	1	0.500 (square)	1	coarse sand
0.250-0.500*	2	0.250 (square)	2	medium sand
0.125-0.250*	3	0.125 (square)	3	fine sand
0.0625-0.125*	4	0.0625 (square)	4	very fine sand
0-0.0625*	5-10	pipette	0	silt and lutum

*these fractions were not distinguished in these studies

Table 3.2: Grain-size fractions and their definition following the Wentworth classification (Doeglas, 1968)

3.5.3 Artificial substrata

The analytical methods applied to artificial substrata measurements did not differ substantially from those previously described; however, some details of sample preparation necessitate inclusion in this section.

The homogenised biofilm was used for respiration measurement (see section 3.3.3) and ATP determination (section 3.5.1.5). Material was concentrated by filtration (Whatman GF/C) for other analyses (section 3.5.2).

Intact biofilms were used for some respiratory measurements. These were placed in the EBOD apparatus (section 3.5.2.3) overlain with 500 ml of BOD dilution water (APHA, 1976) in a 1500 ml reaction vessel with stirring and recirculated sparged air. Samples were incubated at 20 °C with hourly recording of oxygen consumption.

3.6 CALCULATIONS

3.6.1 Conversion of microbial biomass measurements to carbon equivalents

To enable determination of the relative importance of autotrophic and heterotrophic components of the microbial population, and the amount of living organic matter present, the biomass was expressed in terms of carbon equivalents.

ATP. A conversion factor of 250 x ATP was used to estimate microbial carbon (Hamilton and Holm-Hansen, 1967).

Chlorophyll a. A conversion factor of 16 x chl a was used to estimate autotrophic carbon (Paerl *et al.*, 1976).

3.6.2 Apparent microbial production rates from longitudinal planktonic biomass samples.

The rate of production and decay of planktonic aquatic microbial biomass in the Waitoa River was calculated for 3 longitudinal surveys. Travel time between stations was measured on 1 occasion by Wilcock (1984c) using a tracer dye, and scaled to other discharges using the mean velocities obtained from the rating curve upstream at Landsdowne Road.

Biomass (X) was measured (using ATP converted to carbon equivalent) on 2 size fractions : (i) total (X_{TOT}); (ii) <30 µm ($X_{<30\ \mu m}$). Production was calculated on the basis of both exponential (Eqn 3.20) and linear (Eqn 3.21) microbial production rates for both size fractions.

$$\mu = \frac{\ell n\ X_t - \ell n X_o}{t} \qquad 3.20$$

where μ = specific growth rate (h^{-1}); X_t = biomass at time t (g C m^{-3}); X_o = biomass at time o (g C m^{-3}); and t = travel time between stations (h).

$$\mu' = \frac{X_t - X_o}{t} \qquad 3.21$$

where μ' = specific growth rate ($gm^{-3}\ h^{-1}$).

The >30 µm (termed large particle biomass) was determined by difference from the total and <30 µm (termed small particle biomass) using:

$$\Delta X_{>30\mu m} = X_{TOT} - X_{<30\mu m} \qquad 3.22$$

3.7 SAMPLING DESIGN AND DATA PROCESSING

3.7.1 Introduction

The output of an environmental sampling programme must inevitably be a compromise between an optimal sampling design for high accuracy for a particular determinand at a given location, a need to sample multiple locations, and the precision of measurement techniques. Sampling logistics are greatly compounded when the desired programme requires 'instantaneous' sampling from multiple locations, for several determinands and at reasonably frequent intervals, with extremely

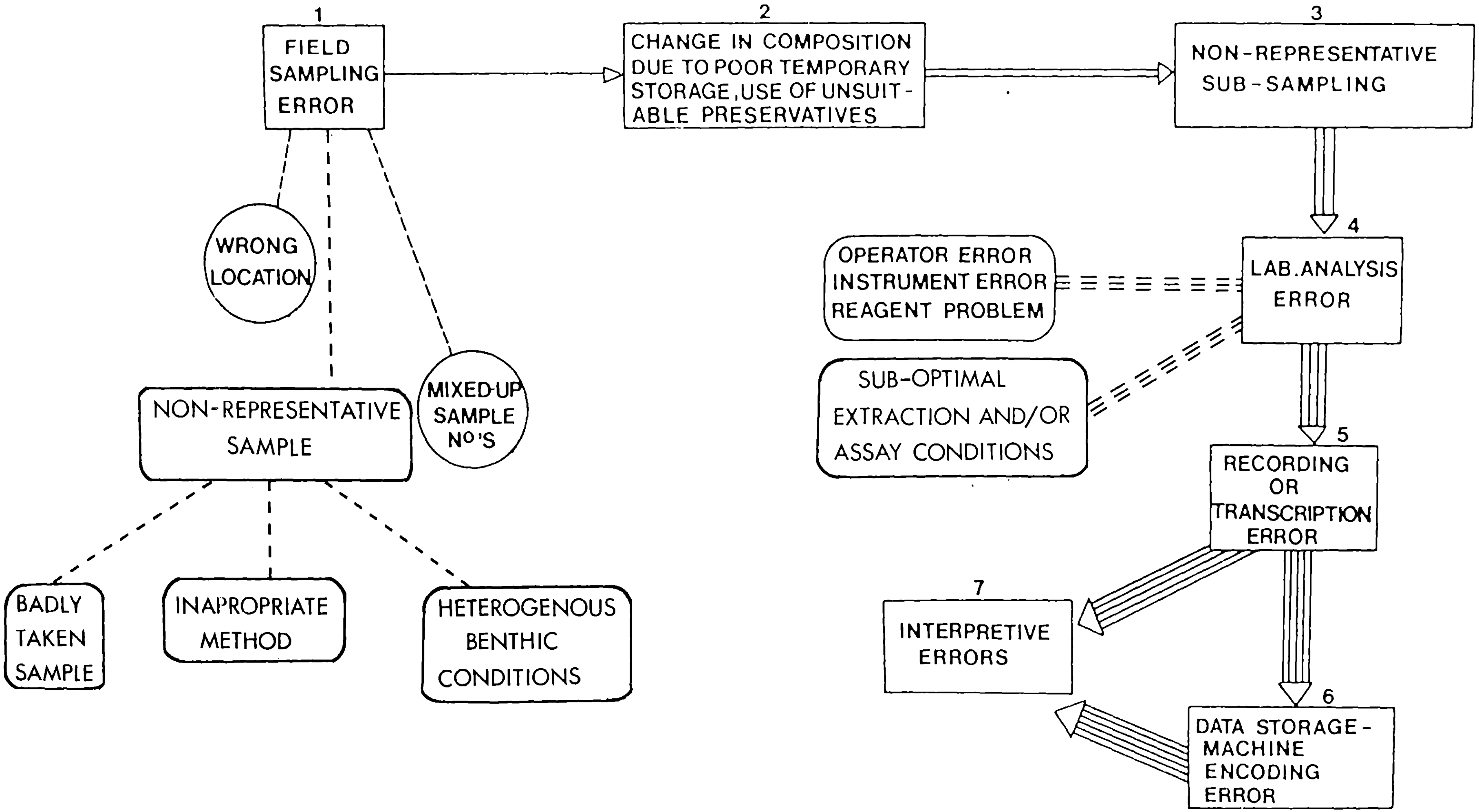


Figure 3.9 : Sources of error in benthic sampling programme (after Golterman et al., 1983).

limited manpower resources. Such was the experimental design for the river process studies described herein, and this section lays out the compromises made in performing the experimental programme.

The techniques deployed in these studies involved measurements made in situ which required simulation of natural hydrodynamic and chemical conditions, together with measurement of potential activities, and biochemical and concentrations present in the sediments. Each variable involved in a measurement made in situ, may to some extent affect the final rate process measurement, with the final data interpretation subject to numerous sources of error which may be summarised as in Figure 3.9.

The potential is for errors to become cumulative, as illustrated in Figure 3.9, and for methodological procedures to greatly influence the final result of a sampling programme.

Each data collection programme is a compromise between what is actually possible and what is ideal. This research programme was developed on the basis of a practically manageable field sampling programme with the development of optimal methodologies for both field and laboratory estimations.

3.7.2 Field Sampling

A major objective of the field research programme was to examine microbial biomass and activity longitudinally above and below an organic waste discharge. Because of both the highly variable nature of the organic discharge and river flows in relation to microbial generation times, sampling surveys required effectively 'instantaneous' measurement of each of the parameters. Major potential sources of error may result from : (i) an inappropriate method, as in using a device in situ which does not simulate natural conditions; (ii) non-allowance for benthic measurement variability, either by use of sampling devices with small enclosed areas compared with the natural substratum particle size or heterogeneity, or by insufficient replication of samples. For river bed sampling these two considerations are of great significance and importance in relation to equipment design and deployment (see Introduction of Chapter 4 regarding chamber design).

3.7.3 Compositional Changes

Since the combined effects of biological, chemical and physical processes are important in establishing sediment biota and chemical gradients, great care must be undertaken in sample handling. It is therefore necessary to minimise possible changes by the use of appropriate handling conditions and preservatives. The rate of biochemical reactions should be reduced by keeping samples cool (<4°C) and in the dark. Anoxic samples must be denied exposure to oxygen. Appropriate chemical preservatives may be necessary to prevent changes to samples taken for analyses.

3.7.4 Sub-sampling

To ensure that sub-samples are representative of the original material, it is essential that the original material is effectively homogenised before sub-sampling. Depending on the analysis required, samples may be manually or mechanically homogenised in either wet or dry conditions. For many chemical analyses it may be desirable to grind sediment material to a powder in order to minimise the numbers of sub-samples necessary.

3.7.5 Laboratory analyses

Both physical and chemical analyses are subject to operator error, instrument error, and reagent problems. Biochemical analyses present additional difficulties concerned with extraction and assay conditions. Reaction rates may be influenced by saturation kinetics, diffusion limitations, cofactors, feedback mechanisms, pH and temperature. Extractions of biochemical components which may be subjected to rapid intracellular turnover, and sensitive to denaturation requires both rigorous and protective conditions. Methodological considerations therefore play an important role especially concerned with identification of rate limiting factors.

Because natural environmental conditions are frequently sub-optimal, care must be taken to differentiate between in situ and potential rates of reaction. Additionally, substances present may actually interfere with the component or assay, either naturally or as a result of extraction conditions. The use of internal standards and control assays are necessary to identify and correct for such interferences.

3.7.6 Data Storage and Statistical Testing

Both field and laboratory measurements were transcribed from working note books to computer storage. Computer programmes were written for computation routines on most analyses and for analysis of field monitoring data (e.g. chamber and diurnal oxygen measurements).

The students t-test was used for the majority of statistical significance testing, with allowance for degrees of freedom. Analysis of variance (ANOVA) packages available upon the initiation of these studies were unable to handle variations in assay numbers and missing values, so were not used. Care was taken to avoid multiple inference using the t-test.

The F-test was applied to significance testing for slope differences in chamber kinetics studies (Chatterjee and Price, 1977).

Chapter 4

CHAMBER DEVELOPMENT AND APPLICATION

TABLE OF CONTENTS

4.1 INTRODUCTION

This chapter describes the design, construction and testing of a chamber system suitable for application to lotic systems. The reason for including together both chamber measurements and segment oxygen mass balance estimates is that together these clearly establish the usefulness of the instrument, its precision, and the relative magnitude of effect attributable to a particular variable in the measurement system. Details of work referred to in this section are provided in later sections of this thesis.

4.2 DESIGN, CONSTRUCTION AND TESTING

That the level of turbulence may affect metabolic activity has been demonstrated for epilithic communities (e.g. McIntyre, 1966; Lock and John, 1979); and several authors have shown that estimates of sediment oxygen demand (SOD) may be increased with stirring of the overlying water (e.g. Edwards and Rolley, 1965; Martin and Bella, 1971; James, 1974). Unfortunately, interpretation of these data in relation to natural conditions is limited by the nature of the agitation provided within devices: with laboratory cores featuring either no stirring (e.g. Bott et al. 1978; Belanger, 1981) or an arbitrary level (e.g. Graneli, 1977; Pomroy et al., 1983); and recirculating systems with arbitrary agitation levels (e.g. Belanger, 1981; Grimm and Fisher, 1984). Chamber agitation levels are based on a variety of criteria including: 'rapid complete mixing' (Chiaro and Burke, 1980); 'continuously mixed... to prevent the formation of concentration gradients' (Hargrave and Phillips, 1981); and 'gently stirred' (Grimm and Fisher, 1984). James (1974) and Jeppesen (1982) present designs featuring unidirectional flow velocities which may be varied (0.1-0.4 m s^{-1} for James and 0-1 m s^{-1} for Jeppesen) to match the lotic environment. No reports documenting the response of river BUR to flow velocity have been sighted. It was therefore considered important to design a chamber featuring variable circulation velocity in order to quantify the importance of river velocity to BUR processes and furthermore, to test the results obtained against an oxygen mass balance. The mean flow velocity of the study rivers was in the range 0.15-0.7 m s^{-1} at summer low flow (see Table 3.1), requiring a large pump recirculation system in order to obtain boundary velocities (0.05 m above bed) comparable with river values. A flume-like chamber construction with upstream baffles and diffusers was considered necessary to obtain an even flow distribution over the sampling area and avoid the vortex turbulence characteristic of cylindrical systems at high circulation velocities.

The internal dimensions and shape of a chamber system are of importance in relation to benthic flux measurements for a number of reasons: (i) in order to obtain flow conditions with homogenous mixing and minimal wall effects; (ii) in order to provide a sampling area which is large relative to the sediment particle size since this will allow some integration of sediment heterogeneity and hence reduce measurement variability; and (iii) for measurement sensitivity, where a lower chamber height allows greater sensitivity for the measurement of metabolic rates. Although the fine sediment present in lentic systems may be sampled or enclosed with a wide range of core or chamber areas (see Bowman and Delfino, 1980), a larger sampling area is generally necessary for measurements in lotic systems because of larger sediment particle size (e.g. Bott et al., 1978; Pennak and Lavelle, 1979). The study rivers (described in Section 3.2) contained sediments of coarse sand and gravel with the pumice bed rivers having similar particle size range (see Appendix 4). The chamber design presented here has sampling dimensions minimally 10 times (i.e. 0.2 m) the dimensions of the large particles present (10-20 mm). The chamber depth of 0.1 m was 5 times the particle dimensions, which should minimise possible hydraulic effects caused by the chamber lid.

A further consideration is that the chamber experiments are essentially a batch system, where an area of water is isolated over the bed and both electron donor and electron acceptor concentrations decrease with time, whereas the river is a continuous flow system, with constant or slowly changing concentrations. Thus tests must be conducted to determine how well the batch system models the prototype by nutrient addition to the chamber water.

The purpose of this section is to describe the relationship between: (i) boundary velocity and measured BUR: (ii) electron acceptor concentration (i.e. DO) and measured BUR: (iii) electron donor concentration (i.e. glucose) and measured BUR; and (iv) to test the suitability of the chamber measurement system and operating criteria by undertaking segment oxygen mass balance estimates of BUR on 3 rivers.

Plate 4.1 Benthic chamber system showing 230 V submersible pump, with oxygen and temperature monitoring equipment.

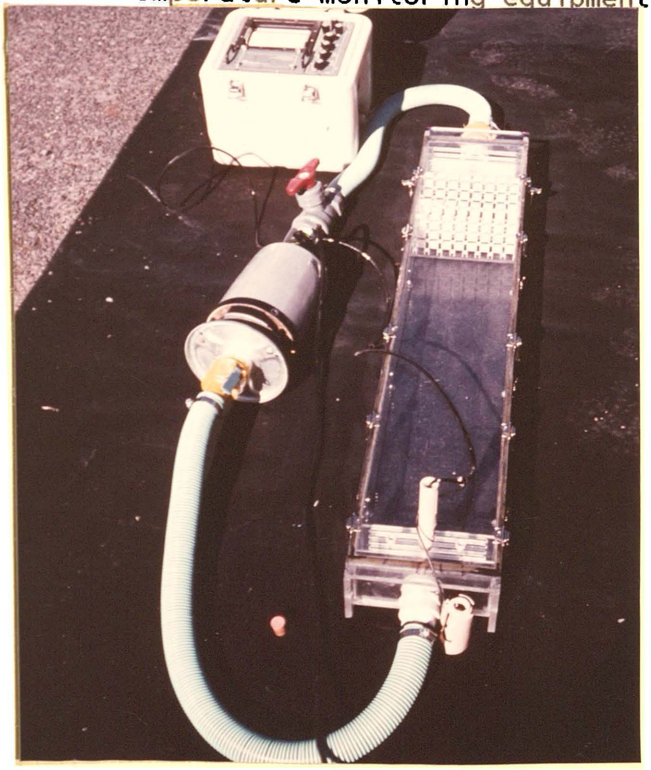


Plate 4.2 Large and small benthic chamber systems installed on the Waitoa River at Walton Bridge, February 1983

Additionally chamber turbulence characteristics in relation to flow velocity were determined by measurement of oxygen mass transfer coefficients (k_L) for the air/water interface. These values are compared with k_L values experimentally measured in the river systems.

4.2.1 PROCEDURES

4.2.1.1 Chamber Design

The chamber, illustrated in Figure 4.1, was rectangular and featured unidirectional flow analogous to lotic systems. A sampling area of 0.16 m^2 minimised the effect of benthic sampling variability in the lotic systems. The chamber was made of clear poly(methylmethacrylate) (Perspex) which enabled us to measure photosynthesis and to make visual observations during operation. Detachable stainless steel flanges were routinely used for rivers with sand and pebble substrata.

Temperature influences both diffusion processes and microbial reaction rates. For chamber systems _in situ_, the temperature is not controlled, but maintained at ambient conditions. Provision for detachable trays would facilitate removal of colonised bed material from the river for experiments _in vitro_ where measurement temperatures may be controlled.

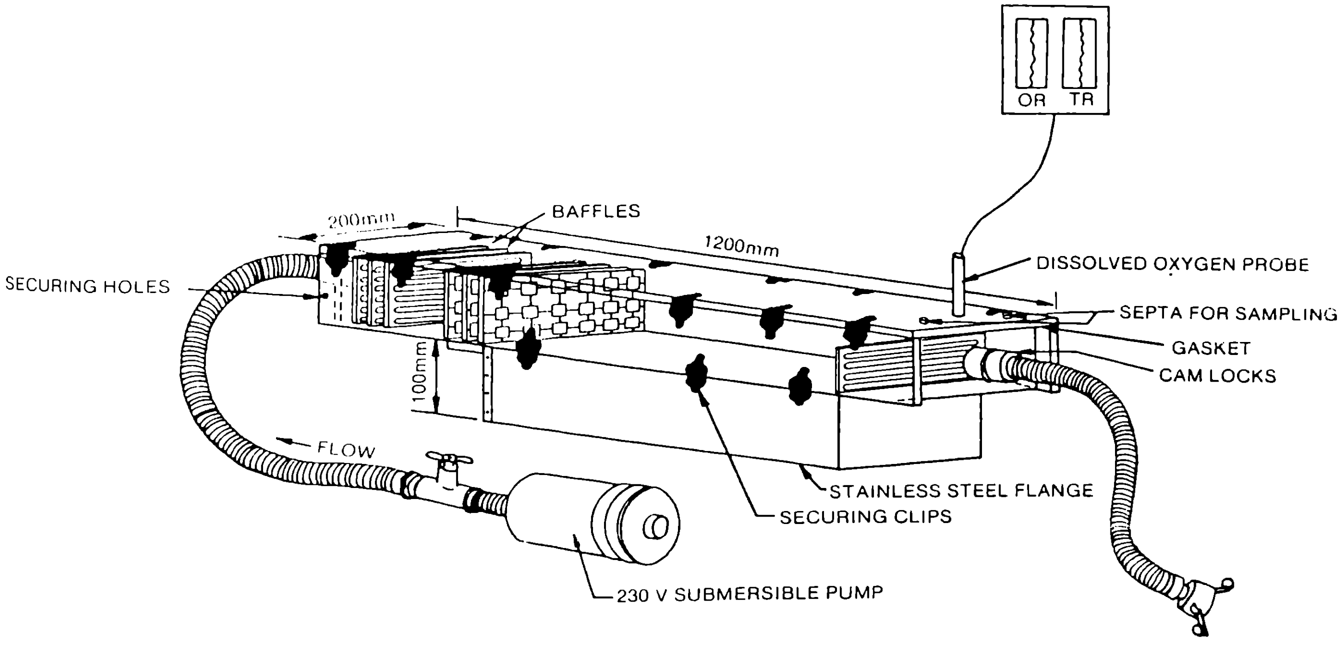


Figure 4.1 : Benthic metabolism chamber constructed of 10 mm 'Perspex' for measurement of benthic oxygen uptake rate and primary production. The area enclosed is 0.16 m^2 with a total chamber volume of 39 1. Circulation is provided by a 230 V submersible pump driven by a portable generator. (OR = oxygen recorder, TR = temperature recorder).

A 230 V submersible pump (Tsurumi model L250) was used to recirculate the water in the chamber. The circulation velocity could be varied from zero to 0.35 m s^{-1}. The greatest disadvantage of the 230 V pump was its heat production, which could cause a rapid 2°C temperature increase within the chamber if used in rivers of low velocity (<0.15 m s^{-1}). Three horizontally-milled diffusers followed by three collimating diffusers ensured nearly uniform velocity distribution (< ± 10% variation) over the sampling area which comprised 2/3 of the total chamber length. Dye injections were used to show that velocity was visually uniform and turbulence homogeneous.

The **circulation velocity** was determined using an Ott meter situated at the mid-point 0.05 m above the substratum in the large chamber. This meter could be left in position for measurement during the operation of the chamber. Comparable measurements made 0.05 m above the bed of the river are referred to here as **boundary velocities**.

A smaller chamber system was also constructed for comparison of BUR kinetics on various substrata, and permitted manipulation of the nutrient concentration in the chamber water under standard conditions. This was similar to the large chamber but had a maximum circulation velocity of 0.21 m s^{-1}, powered by a 12 V submersible pump and covered an area of 0.06 m^2.

Details of the two chamber systems are given in Table 4.1 and shown in Plates 4.1 and 4.2.

Manipulation of concentrations of electron acceptors and electron donors within the chamber system was required in order to determine the response of the BUR kinetics. For this reason a reoxygenation method was developed using hydrogen peroxide and catalase (Hickey, 1985b). This enabled: (a) BUR measurements to be made over a greater oxygen concentration range; (b) the repeatability of measurements to be tested and; (c) extended subsampling of the chamber water to be made. Glucose was added to a final concentration of 20 g m^{-3} to determine the response to electron donor concentrations.

	External Dimensions m	Internal Dimensions m	Area Sampled A_S, m^2	Volume Sampled V_A, m^3	Total Volume V_T, m^3	V_A/V_T %	A_X/V_T m^{-1}	Cross-sectional Area A_X, m^2	Maximum Velocity U, m s^{-1}	Turnover[a] Time (at Umax) s	Reaeration[b] Coefficient (at U max) k_2, day^{-1}, base e	Mass[c] Transfer Coefficient k_L, m.day^{-1}
Large chamber	1.2 x 0.22	0.8 x 0.2 x0.1 depth	1.6×10^{-1}	1.6×10^{-3}	3.9×10^{-2}	41	4.10	2.0×10^{-2}	0.35	6.50	22.5	2.25
Small chamber	0.8 x 0.24	0.38 x 0.16 x0.05 depth	6.08×10^{-2}	3.04×10^{-3}	8.48×10^{-3}	36	7.17	8.0×10^{-3}	0.22	4.82	31.6	1.58

[a] V_T/UA_X

[b] As measured across the area sampled (A_S) and reported at 20°C (converted from 30°C measurement using Equation 4.2).

[c] Calculated from reaeration coefficient using Equation 4.4.

Table 4.1: Large and small chamber characteristics

Chamber DO and temperature were continuously monitored using a YSI model 56 or 57 dissolved oxygen meter (Yellow Springs Instruments, Yellow Springs, Ohio) and recorded with an 8-bit data logger (Tasman, Mini Logger) at 30 sec intervals. A stirring probe was used when the circulation velocity was less than 0.2 m s^{-1}. The DO monitor was calibrated using water saturated with air at the operating temperature. BUR values (g O_2 m^{-3} h^{-1}) were estimated by finite difference calculation of the slope of the DO versus time plots at intervals of approximately 0.4 g O_2 m^{-3} and converted to BUR (g m^{-2} day^{-1}).

River water samples (four replicates) were taken at the time of chamber closure, sealed in unstirred biochemical oxygen demand (BOD) bottles (two of which contained added glucose at 20 g m^{-3} final concentration) and incubated (approximately 2 hours) in the dark at river temperature to allow for correction of chamber estimates of BUR for aquatic oxygen demand.

4.2.1.2 Chamber Operation

The chamber system was routinely installed with the flanges attached and the pumping ports disconnected, either by pushing into the benthic substratum or by attachment to preset bases. To remove detrital particles which may have been disturbed on installation, the pump was attached and run for an initial period of 2 minutes with the inlet port open at low circulation velocity (~ 50% of maximum). High circulation velocities could not be used because the suction of the large pump drew water through the substratum thus displacing the original interstitial water present. The ports were then closed and the circulation velocity increased to the measured river boundary velocity, up to the limit imposed by the maximum chamber circulation velocity.

Repeat runs on the same area of the stream bed were achieved by either disconnection of the circulatory hoses and exchange with the surrounding river flow (termed 'wash through') for about 2 minutes prior to resealing, or by the enzymatic decomposition of hydrogen peroxide using catalase (Hickey, 1985b).

These chamber systems have been successfully used in rivers with mean flow velocities of up to 1 m s^{-1}. This was accomplished by use of a current deflector, constructed of a 1.5 m x 2 m area of butyl rubber sheet (2mm thickness), weighted on three sides using heavy chains, and placed upstream of the chamber and pump system to prevent scour. A similar cover was used to provide a dark environment for BUR measurements.

4.2.1.3 Turbulence Measurement

A measure of chamber turbulence was required which could test the effect of varying circulation velocity and boundary roughness.

The reaeration coefficient (k_2) was used as a measure of turbulence. This parameter was used because empirical equations were available which related k_2 with flow velocity and depth (see Wilcock, 1982). Additionally, experimental techniques had been developed to measure k_2 in the river systems (Wilcock, 1984a,b), thus enabling comparisons to be made between measurements using the chamber and those made in the river itself.

A chamber with lid attached, was inverted and placed in a constant temperature (30 ± 1°C) water bath, with the 'base' open to the atmosphere. The DO concentration in the chamber was lowered to 1 to 2 g m^{-3} by the addition of sodium sulphite and cobalt chloride catalyst (Robertson, 1980). The recovery of DO concentration was recorded on a YSI model 56 oxygen monitor. DO concentration follows the equation (Wilcock, 1984):

$$\ln \left(\frac{C_s - C_1}{C_s - C_2}\right) = k_2 \, (t_2 - t_1) \qquad (4.1)$$

where C_s is the saturated DO concentration at the temperature of the water, C_2 and C_1 are DO concentrations at time t_2 and t_1 respectively and k_2 (base e) is the reaeration coefficient. k_2 was estimated by regressing the ln DO deficit against time.

All measurements were converted from a measurement temperature (T), to a reference temperature of 20°C using the relation of Elmore and West (1961)

$$k_{2(T)} = k_{2(20)} \, [1.0241]^{T-20} \qquad (4.2)$$

Measurements of k_2 were scaled by the ratio of the volume exposed to the atmosphere (V_A) to the total volume (V_T) according to equation (4.3)

$$k_2 = k_2 \text{ (Observed)}.\frac{(V_T)}{V_A} \qquad (4.3)$$

For a given surface area, A and volume V_T, k_2 is related to the mass transfer coefficient, k_L, by the expression (Wilson and Macleod, 1974):

$$k_2 = k_L \cdot \left(\frac{A}{V_T}\right) \qquad (4.6)$$

Reaeration measurements were made using a plastic grill inserted : (a) 5 mm beneath the gas liquid interface and covering the exposed surface area; and (b) at the base of the liquid flow. The grill of 15 mm height was composed of square sections of 17 mm internal dimensions each separated by 7 mm. Material thickness was 1 mm.

Reaeration measurements were compared with values calculated using the O'Connor-Dobbins (1958) formula:

$$k_2 = 3.74\ U^{0.5}/H^{1.5} \qquad (4.5)$$

where U = mean velocity, m s^{-1} and H = mean depth, m.

The river boundary velocity (U_z) at 0.05 m was calculated from the mean velocity assuming a power law approximation to the logarithmic velocity profile equations given by, Smith (1975):

$$U_z = U(m+1)\ \frac{z^m}{H} \qquad (4.6)$$

where m = typically 0.1-0.2, assumed 0.2 here.
z = fixed height above bed, 0.05 m here.

4.2.1.4 Segment Oxygen Mass Balance Determination

Segment oxygen mass balances were calculated in order to estimate the net BUR for comparison with chamber measurements. Three rivers were studied, with mean depths of 0.4 m, 0.8 m and 1.67 m, each with the reaeration coefficient measured by gas tracer. The pebble bed of the Waitoa was extensively colonised with 'sewage fungus' below a dairy discharge point, whereas pumiceous sediments were predominant in the Waiotapu, which received intermittent dairy waste loading, and also in the Tarawera which received biologically treated pulp and paper mill wastes. Details of methods are given in the in the Results (section 6.2.1 for the Waitoa, 5.3.2 for the Waiotapu and 5.4.2 for the Tarawera).

4.2.2 RESULTS AND DISCUSSION

4.2.2.1 Comparison of Chamber and Segment Oxygen Mass Balance BUR

BUR measurements were made with matching chamber and river boundary velocities in 2 out of 3 rivers. For the three rivers studied, chamber BUR estimates ranged from 28.0 to 52.2 g $m^{-2}day^{-1}$ with the maximum value obtained for 'sewage fungus' communities. Aquatic oxygen demand was always small and macrophyte respiration only a significant component in calculation of net BUR for the Waiotapu River with BUR values ranging from 28.3 to 75.3 g $m^{-2}day^{-1}$ (Table 4.2). Comparison of the measured and calculated (ie mass balance) BUR estimates showed overlap of the 95% confidence range for the 2 shallow rivers (Table 4.2). The respiration of filamentous 'sewage fungus' attached to logs and debris in the Waitoa River, and not included in chamber measurements, may have contributed to the lower measured BUR. For the deeper Tarawera River the maximum calculated BUR was 52.4±8.1 g m^{-2} day^{-1}, with chamber measurements amounting to 65% and 47% of the BUR estimates on the 2 sampling occasions (Table 4.2). The 95% confidence levels for calculated and measured BUR did not overlap on either occasion.

4.2.2.2 Mass Transfer

In order to test whether the chamber caused water to percolate down into the sediments and escape past the 100 mm deep flange, dye was injected into the chamber while operating at full velocity (0.35 m s^{-1}) on coarse sand substrata, with visual inspection for exchange with the surrounding water flow. A good seal was generally obtained for mean particle sizes <20 mm. Complete removal of a bung (20 mm dia) at the chamber mid-point showed no exchange, indicating a negligible pressure differential between the chamber and the river. For substrata comprised of larger particles, either a deeper preset flange, or trays were found necessary in order to obtain an adequate seal.

When Rhodamine dye was injected into coarse sand river sediments to depths of 1-5 cm, it was rapidly washed out (typically within 10 minutes) indicating substantial percolation of river water into the sediments. Findings were in agreement with results of Grimm and Fisher (1984) who found that the dye injected at 5-30 cm depth within the gravel sediment of a desert stream, re-emerged downstream. Similar results for dye injections at 1 cm depth were obtained in the chamber system, with diffuse dye emergence 1 to 5 cm downstream but with

River[a], Sampled	Depth	Velocity[b] (mean)	Discharge	Measured[c] k_2	Segment length			Oxygen demand $g\ m^{-2}\ day^{-1}$			Reference
	m	$m\ s^{-1}$	$m^3\ s^{-1}$	day^{-1}	km	Gross	Macrophyte	Aquatic	Net	BUR[d] (measured)	
Waitoa 16 Feb 1982	0.40±0.08	0.19	0.7	10.9±0.6	2.1	75.3±8.7	Nil	<2.0	75.3±8.7	52.3±6.5 [$U=0.25m\ s^{-1}$][e]	Hickey 1985b; Section 6.2
Waiotapu 9 Mar 1983	0.80±0.08	0.40	3.5	7.7±0.6	2.2	35.3±2.5	7.0±2.3	<1.5	28.3±3.4	28.0±2.8 [$U=0.35m\ s^{-1}$][f]	Hickey 1984; Section 5.3.2
Tarawera 9 Mar 1984	30%[1.67±0.13 70%[1.32±0.10 (14)	0.66±0.03] 0.76±0.03]	26.6	7.03±0.24	11.9	49.9±3.8 (7)	Nil	4.8±0.3 (19)	45.1±3.8	29.3±4.2 [$U=0.35m\ s^{-1}$][e] (7)	Section 5.4.2
Tarawera 14 Feb 1985	1.4±0.1 (14)	0.71±0.01	22.3	8.6±0.3	11.9	59.8±8.1 (6)	Nil	7.4±2.8 (4)	52.4±8.1	24.7±4.0 (12)	Section 5.4.2

NOTE: mean ± 1 standard error; value in brackets is number of measurements (n).

[a] Segment location: Waitoa River 175°44'E, 37°45'S; Waiotapu River 176°20'E, 38°25'S; Tarawera River 176°44'E, 38°02'S.

[b] Mean velocity from dye tracer experiments.

[c] Gas tracer technique Wilcock (1984b) and J.C. Rutherford, R.J. Wilcock and C.W. Hickey (unpublished Water Quality Centre data).

[d] All large chamber system measurements, adjusted for aquatic respiration rate.

[e] Boundary layer and velocity for benthic chamber.

[f] Chamber maximum velocity. Measured river boundary layer 0.40 m s^{-1}.

Table 4.2: Segment oxygen demand studies

exchange between the river and the chamber prevented by the flange seals.

Emergence of the dye was affected by the relative positions of the site of injection of the dye and the proximity of dune structures in the river bed. For some locations complete removal of the injected dye occurred, indicating an infiltration zone with river water entering the sediments. Thus the bed morphology and presumably its structural composition may influence exchange process with the overlying flow.

4.2.2.3 Chamber Turbulence

Figure 4.2 shows the variation of chamber k_2 and the effect of inserting surface grills at various chamber velocities. A minimal effect occurred with insertion of grills at the base of flow (to simulate boundary roughness), so data are not shown. Measured k_2 values for the river (Wilcock, 1984b), and k_2 values calculated using the O'Connor-Dobbins (1958) formula, considered appropriate for the New Zealand rivers of interest in this study (Wilcock, 1984b), each plotted against river boundary velocity are also shown.

Both chamber and predicted stream k_2 values showed a similar response to changes in circulation velocity. Regression equations for measurements without the surface grill inserted were : $k_2 = 39.7\ U^{0.54}$ ($r^2=0.940$, $n=8$) for the large chamber, and $k_2 = 69.5\ U^{0.52}$ ($r^2=0.839$, $n=6$) for the small chamber. The exponent of U (the mean velocity) was not significantly different from the value 0.5 in equation (4.5).

An approximately 30% decline in the value of k_2 was observed following insertion of surface grills into chambers when operating at maximum circulation velocity ($P<0.01$, $P<0.05$ for large and small chambers respectively, all $n=3$). At lower circulation velocities the response was not significantly different from replicate determinations at ± 8% (S.E., $n=3$).

It must be stressed that the O'Connor-Dobbins equation can at best be considered only an approximation to natural stream reaeration coefficients. Experimental measurements, based on a gas tracer method (Wilcock 1984a), have been shown to be on average 1.4-fold but may be up to 3-fold greater than predicted by Equation 4.5 (Wilcock, 1984c). Measured k_2 values for the rivers studied (Wilcock, 1984b) are also

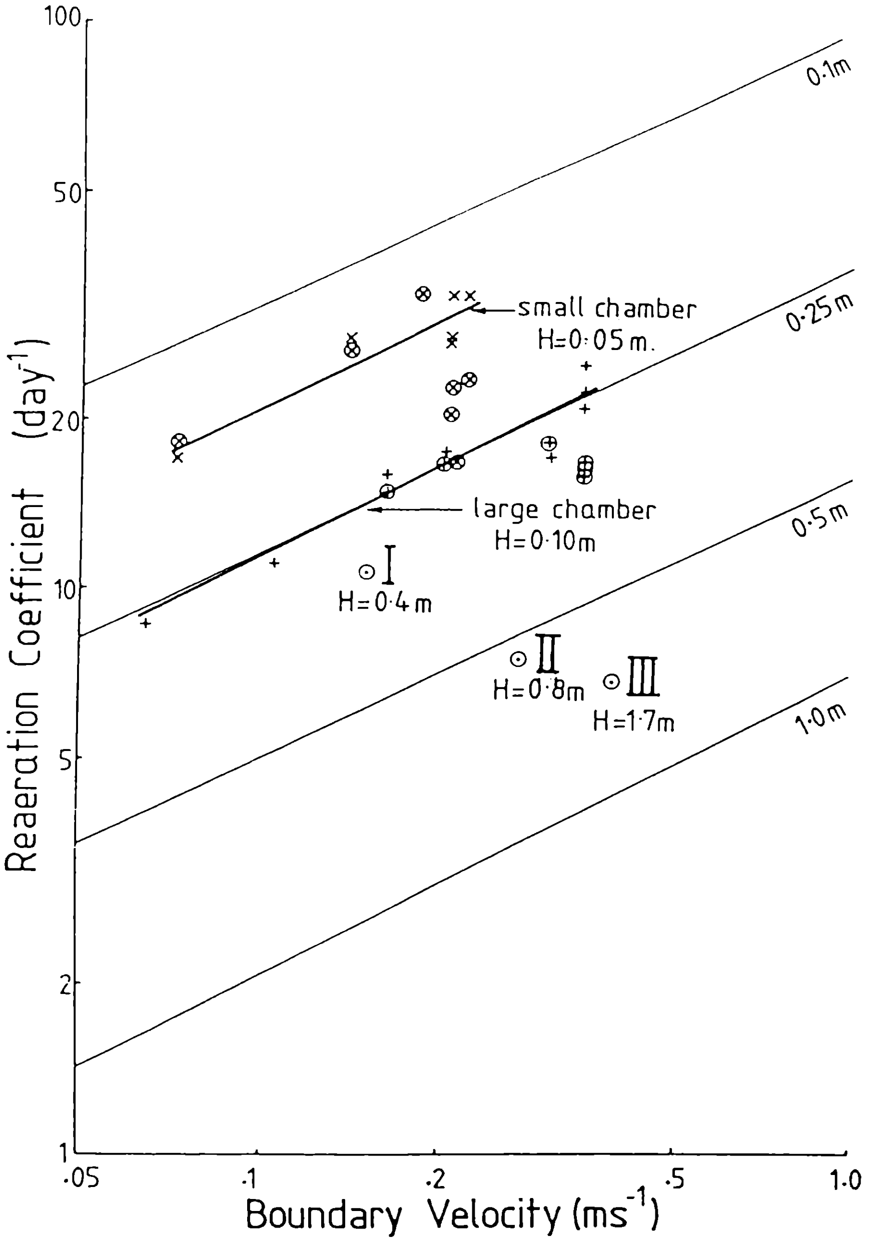


Figure 4.2 : Comparison of chamber and river reaeration coefficients:

a measured in large chamber with ⊕ and without + surface grill

b and in small chamber with ⊗ and without x surface grill

c regression equation for large chamber ——— ($k_2 = 39.7U^{0.54}$ $r^2 = 0.94$ n = 8)

d regression equation for small chamber --- ($k_2 = 69.5U^{0.52}$ $r^2 = 0.84$ n = 6)

e predicted by O'Connor-Dobbins (eqn 4.5), depths 0.1-1.0 m, for boundary velocities calculated using eqn 4.6.

f measured by gas tracer with boundary velocities calculated using eqn 4.6, for :

I Waitoa River.

II Waiotapu River.

III Tarawera River.

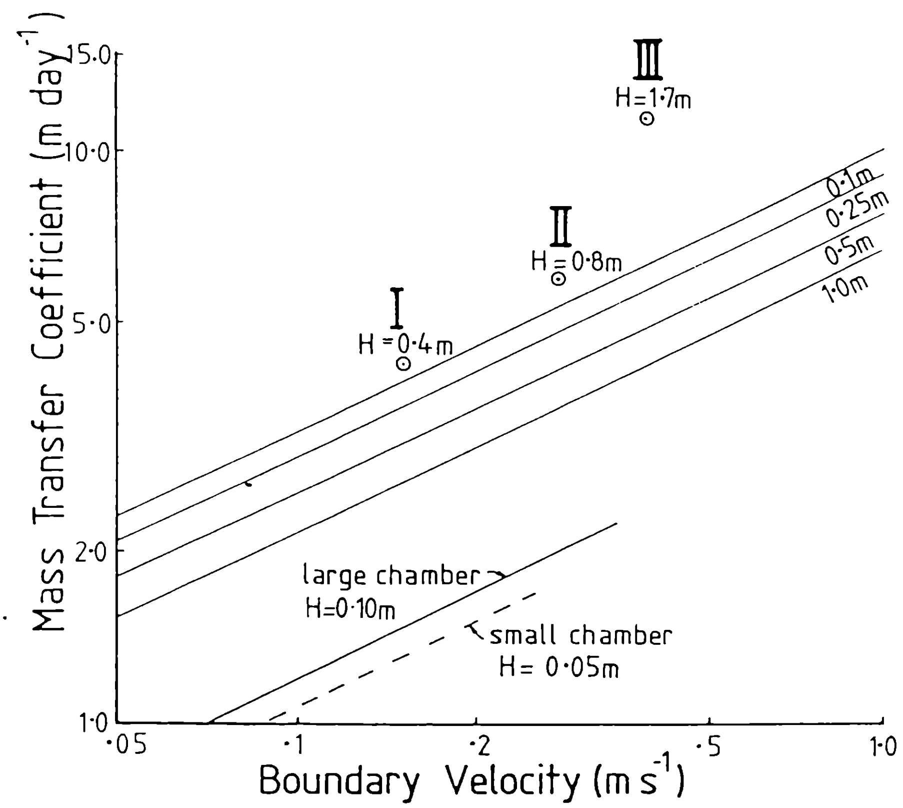


Figure 4.3 : Comparison of chamber and river mass transfer coefficients (k_L) calculated from data shown in Figure 4.2 using eqn 4.4. Gas tracer measured values are:

I Waitoa River.

II Waiotapu River.

III Tarawera River.

O'Connor-Dobbins formula (eqn 4.5) values shown as diagonal lines.

shown on Figure 4.2, relative to a calculated boundary velocity. Figure 4.2 suggests that if chamber velocities were set equal to the river boundary velocity, then the chambers would seriously over-estimate the value of k_2. Indeed to match k_2 values in the Waitoa River, the large chamber would apparently need to be run at 0.08 m s^{-1} as opposed to the measured river boundary velocity of 0.25 m s^{-1} used in the experiments described here.

There are good reasons, however, for not matching k_2 in the chamber and river. Use of the chamber system restricts the maximum turbulent eddy diameter to that of the mean chamber height, whereas for the river the comparable dimension is that of the mean river depth. Turbulent mass transfer is dominated by eddies with the greatest momentum, i.e., those whose eddy diameter approximates the mean depth or chamber height. Consequently a comparison of river and chambers based on the mass transfer coefficient (i.e. normalised relative to the mean depth) would be more appropriate.

Figure 4.3 shows calculated mass transfer coefficients (k_L) (Eqn 4.4) based on: (a) measured k_2 values for rivers (Wilcock 1984b); (b) O'Connor-Dobbins predicted k_2 values for rivers (Eqn 4.5); and (c) measured chamber k_2 values. The mass transfer coefficient of the two chambers are comparable, but their values are less than predicted or measured for river systems. Insertion of a base grill caused a slight decline in k_L for the small chamber ($P<0.10$, $n=3$) with no significant response in the large chamber system. The chamber k_L were approximately 40% (Waitoa) 35% (Waiotapu) and 18% (Tarawera) of the river value.

4.3 GENERAL DISCUSSION

Chamber measurements of benthic oxygen uptake rate (BUR) made in situ for the three rivers studied, ranged from 28.0 to 52.2 g $m^{-2}day^{-1}$. Comparison with segment oxygen mass balances, showed satisfactory agreement with calculated BUR estimates from 28.3 to 75.3 g $m^{-2}day^{-1}$. Chamber conditions proved adequate for the estimation of net BUR for the 2 shallow rivers but substantially underestimated the mass transfer conditions necessary for BUR measurement in the deeper Tarawera River. Confidence in the mass balance calculations was greatly increased by experimental measurement of the reaeration coefficient (95% confidence range, ±10%), since measured values have been shown to be up to 3-fold higher than estimates from empirical equations (Wilcock, 1984 b, c). Greatest uncertainty was derived from mean depth estimation for shallow rivers (see Table 4.2). These results demonstrate the suitability of the chamber design and the operating criterion of matching boundary velocities for the 2 shallow rivers studied, thus providing confidence for the examination of factors affecting BUR kinetics. The substantial underestimation of BUR in the deeper Tarawera River suggests that chamber and environmental conditions were not comparable, necessitating the identification of the key hydrodynamic factors operating in the river, but not present in the chamber system.

Failure to simulate environmental conditions adequately within a chamber system (i.e. poor instrument precision) thus resulting in erroneous estimates of the rate process <u>in situ</u>, may be caused by flow velocity, turbulence or altered nutrient concentrations. Variation of these factors may greatly exceed the inaccuracies of determinations resulting from variability derived from the river bed.

The influence of river bed variability on BUR estimates in the 3 rivers studied gave standard errors ranging from 4.8 to 28.2% for 4 chamber sampling sites at 7 locations, giving a 95% confidence maximum calculated on the mean error of ±20.5% (Table 6.2, 5.1, 5.22 and 5.36). This uncertainty was small when compared with that which may be introduced by low circulation velocity or electron donor or electron acceptor concentrations.

The effect of the circulation velocity used in the chamber has been tested for both 'sewage fungus' and pumice substrata, with each showing a large response, and an approximately linear relationship between BUR and chamber circulation velocity. Doubling of the circulation velocity from 0.15 to 0.30 m s^{-1} showed a 1.8-fold increase in 'sewage fungus' BUR (section 6.3), and a 3.2-fold increase in BUR for pumice substrata in the Waiotapu River (Hickey, 1985a; section 5.3). Unstirred replicate cores of the same pumice (0 m s^{-1} velocity) showed a very low BUR amounting to less than 5% of the chamber estimate. The concomitant increase in BUR with circulation velocity suggested that turbulent diffusion processes were dominating the benthic mass transfer processes within the chamber system.

Repeatable BUR dependence on DO concentration in the overlying water has been shown for pumiceous sediments (Hickey 1985a, section 5.3.5). The effect was large with BUR decreasing from saturation DO, from 38.0 g m^{-2} day^{-1} to 8.5 g m^{-2} day^{-1}, for a decrease in DO concentration from 9 g m^{-3} to 4 g m^{-3} (Hickey, 1984b), with an exponential function providing the best fit to the observed data. A BUR dependence on DO concentration has also been shown for 'sewage fungus' biofilms, with the response affected by biofilm characteristics (section 6.3).

Increased electron donor (glucose) concentration resulted in a rapid BUR increase of up to 109% for the pumice sediments (Hickey, 1984b, Table 5.27), .whereas the 'sewage fungus' biofilms showed no BUR response (Table 6.2). This suggested that the benthic microbial population present in the pumice river systems were epilithic heterotrophs with rate limitation by either electron donor and electron acceptor concentrations in the overlying flow.

The oxygen mass balances demonstrated that the principal sink for river DO was associated with the sediment. This benthic respiration was 7 - 38-times the planktonic oxygen demand, indicating the considerable importance of benthic processes and leading to a need for an understanding of those factors which influence the exchange of oxygen between water and the underlying sediment. Although the chamber BUR measurements reported here were restricted to 'sewage fungus' and pumiceous sediments, other measurements on coarse sand substrata have shown BUR values of 25.0 g $m^{-2}day^{-1}$ (section 5.2.2, Table 5.1). These BUR values were considerably higher than those sediment oxygen demands reported for lentic waters, which are typically <5 g $m^{-2}day^{-1}$ (see Bowman and Delfino, 1980) and the river 'sewage fungus' maximum BUR of 14.5 g $m^{-2}day^{-1}$ reported by Boyle and Scott (1984). The higher BUR may be attributable to the greater boundary velocity present in both the river and chamber systems.

Because of the importance of exchange of nutrients between the sediment and the water in shallow lotic systems, and the discrepancy between the calculated and measured BUR in the Tarawera River (Table 4.2), some consideration of the mechanisms of sediment-water exchange is warranted. This especially applies to the demonstrated need for chamber devices to adequately simulate adequately the hydrodynamic conditions necessary for measurement. Two processes may be of greatest importance for exchange across the sediment-water interface: (i) turbulent momentum exchange; and (ii) percolation driven by shear velocities caused by irregularities protruding through the laminar boundary layer. The chamber design may restrict each of these processes; the former because of the presence of the chamber lid, and the latter because of the sediment flanges. The relative importance of these in natural river flow, has not been determined. In the following paragraphs, the factors contributing to sediment-water exchange are discussed.

Interstitial dissolved oxygen concentrations measured in the Waiotapu and Tarawera rivers (sections 5.3.8, 5.4.6) showed dissolved oxygen present to >10 cm depth. Integration of microbial respiratory activity to that depth was required in order to account for the observed BUR. This implies the presence of considerable exchange between the interstitial and surface waters in these rivers.

In flumes over smooth and rough boundaries McQuivey and Richardson (1969) have shown the maximum turbulence eddy diameter to be about half the total depth. Similar results were reported for turbulence measurements in river systems (McQuivey, 1973). Wave-induced percolation through sandy seashore sediments has been demonstrated with measurements in situ (Webb and Theodor, 1968, 1972; Steele et al, 1970; Riedl and Machan, 1972.) The decrease in pressure fluctuations with depth (z) in the permeable sediment was fitted to a power law with exponent $(-2\pi z/\lambda)$, where λ is the wavelength of surface waters (Riedl et al, 1972). Since turbulent mass transfer is associated with momentum exchange across the boundary layer, restriction of the maximum eddy diameter will limit the maximum momentum components and hence the localised pressure fluctuations driving mass transfer. This will result in a decreased maximum benthic penetration depth for the chamber system, compared to the natural river.

The turbulence present in the chamber system is derived from two origins : (a) turbulence generated by the pump and diffuser baffle system; (b) turbulence derived from shear flow at the bed, lid and walls. The experiments involving insertion of surface grills showed a minimal response, suggesting a minimal turbulence contribution from large eddy diameters approximating the chamber depth (Fig. 4.2).

Insertion of grills at the base of chamber flow, to simulate a large change in boundary roughness, also showed a minimal response. This finding is surprising since Lau (1975) found that in a flume at Reynolds numbers comparable with chamber values (35,500 to 11,200) the k_2 value doubled as the mean particle size increased from 1.0 mm to 6.7 mm and hence both boundary shear stress and turbulence increased. The k_2 value measured in the small chamber was approximately double that measured in the large chamber; significantly less than the 2.8-fold increase predicted for constant U (from Eqn. 4.5) for respective mean depths of 0.05 m and 0.1 m. These results suggested that mixing in the chambers was dominated by turbulence generated at the inlet diffuser, rather than by turbulence generated within the chamber at the bed or walls.

Measurement of the mass transfer coefficient for the air-water interface showed that the chamber k_L values obtained here underestimated by some 60 - 80% those measured in the natural rivers (Fig. 4.3). Whether it is appropriate to compare mass transfer measurements across a gas-liquid interface with those of a solid-liquid boundary is uncertain; however, assuming that the chamber represents a smaller turbulence scale, this smaller scale would result in a decreased sediment penetration compared to that in the natural river. This decreased penetration would in turn be expected to decrease nutrient supply to deep sediments resulting in lower flux measurements. Such effects are likely to be a contributory factor in the moderately deep Tarawera River.

The degree to which interstitial percolation through the dune structure affects sediment-water exchange and is limited by the chamber flanges is unknown. Percolation through dunes has been shown to occur in other rivers (Grimm and Fisher, 1984), with exchange to 30 cm of sediment depth. The depth and extent of interstitial water exchange has not been determined for pumice bed rivers.

The conceptual difficulties associated with chamber measurements are not greatly decreased by measurement using tunnel respirometers as proposed by James (1974) and Whittemore (1984), because the presence of the tunnel restricts intrinsic river turbulence, and tunnel surface friction restricts longitudinal flow velocity. However, tunnel measurements do allow percolation through the dune structure and could, therefore, provide an estimate of the importance of this phenomenon.

At present, to test the accuracy of chamber measurements, one faces the difficulties of performing a river segment oxygen mass balance with the additional variables of planktonic respiration, photosynthesis and atmospheric reaeration, together with the variability inherent in natural systems. Because of the uncertainties and difficulties imposed on performing such balances, it is desirable that further research should also be performed in order to determine the importance of various hydrodynamic components of the benthic mass transfer processes applicable to chamber simulation.

4.4 SUMMARY AND CONCLUSIONS

An *in situ* benthic chamber design was developed for use in lotic systems. It features a sampling area of 0.2 m x 0.8 m to minimise the effect of benthic sampling variability; the capability of a circulation velocity up to 0.35 m s^{-1} with recirculating unidirectional flow; construction of a clear 'Perspex' to facilitate measurement of photosynthetic production and benthic oxygen uptake rate (BUR), with an ability to use pre-set flange bases or trays containing substrata.

Comparison of chamber BUR measurements with segment oxygen mass balances determined for 3 rivers, showed satisfactory agreement when the chamber boundary velocity was matched with that of the river (each measured at the same height) for the 2 shallow rivers (<0.8 m) but underestimated BUR by 47% to 65% in the deep river (~1.5 m). BUR was 7- to 38- times the planktonic oxygen demand for these river segments.

The BUR is a function of the flow velocity of the overlying water. The BUR for unstirred (zero velocity) core samples was less than 5% of the maximum chamber BUR measured at 0.35 m s^{-1} velocity. Therefore, predominantly turbulent dispersion processes were influencing the benthic mass transfer processes. Thus, to obtain representative BUR measurements it is essential that chamber devices simulate natural velocity and turbulence characteristics.

Chamber turbulence was determined by measuring the reaeration coefficient (k_2) over a range of circulation velocities. The chamber k_2 response to velocity was similar to that predicted using the O'Connor-Dobbins formula over the velocity range studied (0.06-0.35 m s^{-1}). Results suggested that the eddy size was largely derived from the baffle dimensions of the diffuser system and were not generated within the benthic sampling area of the chamber. The chamber mass transfer coefficients (k_L) were compared with experimentally determined values for the rivers studied, and shown to be 60-80% less than the river values. The present chamber probably underestimates benthic turbulent momentum exchange, resulting in a decreased maximum sediment penetration depth for nutrients. It is, therefore, essential to perform chamber measurements using minimum incubation times to lessen possible hydrodynamic and nutrient depletion effects.

Chapter 5

STUDIES IN SITU ON RIVERS

5.1 GENERAL INTRODUCTION

This chapter presents and discusses data obtained for longitudinal studies *in situ* on 3 river systems, with sampling upstream and downstream of point source organic discharges. The 3 rivers were:

i the Waitoa, receiving untreated effluent from a dairy factory (discussed in section 5.2);

ii the Waiotapu, receiving occasional inputs of untreated effluent from a dairy factory (discussed in section 5.3); and

iii the Tarawera, receiving primary-treated sewage and processing wastes from pulp and paper mills after aerated lagoon treatment (discussed in section 5.4).

The criteria for selection of the study rivers were that they: (a) received a point source loading of organic effluent; (b) had a mean flow velocity of greater than 0.2 m s^{-1}; (c) had a bed composed of sand or gravel substrata; (d) were of less than 1 m depth to allow wading access for the installation of chamber systems; and (e) were within a convenient travelling distance (<100 km). Detailed site descriptions for all study rivers are given in Chapter 3.

The 3 rivers intensively studied were different both with respect to discharge types (i.e. dairy and pulp mill effluents), regimes (i.e. intermittant and continuous), and benthic substrata composition (i.e. sand and pumice) present.

Segment oxygen mass balance estimates of BUR were made on the above 3 river systems and compared with BUR measurements obtained using the benthic chamber system *in situ*. Details of the chamber design and a summary of these results have been presented in Chamber 4. This chapter presents details of the mass balance measurements for a 2.2 km reach of the Waiotapu River, where the relative importance of planktonic, benthic and macrophyte respiration was determined; and planktonic and benthic measurements for a 11.9 km reach of the Tarawera River. Details of the mass balance measurements for the 2.1 km reach in the Waitoa River are presented with the 'sewage fungus' results in Chapter 6.

Longitudinal studies, with sampling upstream and at several sites up to 10 km (20 km in the Tarawera River) downstream of the discharge, were undertaken to establish:

i The composition of planktonic and benthic microbial biomass, using total and phototrophic specific biomass measurements and obtaining heterotrophic biomass by difference.

ii the relative importance of planktonic versus benthic biomass, using chamber BUR and sediment intrinsic activity measurements to obtain minimum estimates of the depth of oxygen penetration into the bed, and hence an estimate of the active benthic biomass.

iii the response of heterotrophic microbial biomass within the river to the organic waste components, using specific biochemical enzyme assays. Metabolism of dissolved organic matter (DOM) was indicated by the activity of an induced enzyme (β-galactosidase in response to lactose present in milk wastes), with the activity of a constituative enzyme (bis-phosphatase which assists breakdown of cellular DNA) indicating metabolism of particulate organic material (POM). These enzyme studies were only made on the Waitoa River, receiving untreated dairy factory wastes, with the intention of repeating and extending this work in the following season once an aerated lagoon treatment system had been installed. Unfortunately, no treatment was installed and a drought caused severe deoxygenation downstream of the discharge, thus limiting ongoing work to measurement of seasonal variation in river oxygen dynamics at the upstream station.

The metabolic activity of the benthic microorganisms was shown to be greater than the planktonic for each of the study rivers. Benthic metabolism of internal and detrital carbon stores will therefore determine the rates of response to decreases in effluent load and the attainment of steady-state conditions. Measurements of benthic carbon and intrinsic activity are used to estimate the carbon turnover time for comparison between sampling stations and rivers. The sediment respiratory decay kinetics were measured in the Waitoa and Waiotapu Rivers in order to determine the benthic respiratory response to effluent cessation.

For this Chapter it was thought more appropriate if the experimental results for each river were presented and discussed in relation to organic waste effects, together with a short summary of major findings and their relevance. A general discussion and overview is presented at the end of the chapter. This section compared the benthic measurements between the study rivers, and the New Zealand rivers with studies from a range of other aquatic environments. A section is also included which overviews the limitations of the study and considers additional work which would have been of value to these studies.

5.2 WAITOA RIVER

5.2.1 Introduction

The Waitoa River studies were designed to investigate the factors influencing the river oxygen dynamics. These included: seasonal hydrology, planktonic and benthic biomass, biomass composition, chamber BUR, sediment intrinsic activity, benthic substatum variation, together with the longitudinal changes associated with a point source discharge of dairy effluent.

Three longitudinal surveys of the Waitoa River were done during the dairy processing season. Detailed surveys were undertaken to coincide with high spring and low summer river flows. An additional survey was undertaken shortly after a summer flood, to facilitate comparison with the low flow survey.

5.2.2 Benthic Oxygen Uptake Rate (BUR) measurements _in situ_, sediment intrinsic activity and calculation of the depth of sediment oxygen penetration

This section outlines the BUR measurements obtained using the chamber and the sediment intrinsic activity (I) measurements made in the Waitoa River during the 1981/92 season when the dairy factory was operating. Together, these measurements facilitate estimation of the depth of oxygen penetration into the sediments (δ_c), and enable calculation of the benthic dispersion coefficient to be made. Additional measurements of intrinsic activity and microbial biomass levels associated with longitudinal surveys are presented in following sections (5.2.4 and 5.2.6); and 'sewage fungus' measurements in section 6.3.

Sampling was undertaken at 5 stations on the Waitoa River, with 1 upstream and 4 downstream of the dairy discharge (Fig. 3.3 and Plate

Date (km)	Temp °C	DO gm^{-3}	Velocity[a] ms^{-1}	BUR $gm^{-2}day^{-1}$	Glucose %	Gross Photosynthesis gm^{-2} day^{-1}	Sediment Intrinsic Activity gm^{-2} day^{-1}	Glucose %	Depth of oxygen Penetration cm	Dispersion Coefficient cm^2 s^{-1} x 10^{-3}
LANDSDOWNE ROAD (-1)										
20 Nov 1981	18.0	7.5	0.25	8.9±2.0(3)	0	-	3.11±0.67(4)*	-	2.9±0.9	5.0±1.9)
			0.15	4.7		-	3.11±0.67(4)*	-	1.5±0.4	1.1±0.3)
12 Jan 1982	22.0	10.0	0.25	17.6	-	9.4	1.47±0.06(3)	-	11.9±0.5	24.3±1.0
25 Jan 1982	17.0	9.3	0.25	24.6	-	33.9	2.23±0.04(2)	-	11.0±0.2	33.8±0.6
8 Mar 1982										
Fine	19.0	7.0	0.25	13.5	0	4.2	0.42 (1)	-	32.1	71.2
Coarse	19.0	7.0	0.25	5.3	0	2.7	0.73 (1)	-	7.2	6.3
20 Oct 1982	14.0	9.5	0.25	10.9	0	0.25	1.41±0.16(3)	0	9.6±1.6	12.8±2.1
8 Dec 1982	23.0	9.5	0.25	15.2	-	0	0.79±0.15(2)	-	19.2±3.6	35.7±6.8
WAHAROA (0)										
9 Oct 1982	20.0	7.5	0.25	21.3±6.0(2)	-	-	61.2±4.2 (3)	-	0.35±0.10	1.1±0.5
19 Nov 1981	20.0	8.0	0.25	13.5±4.1(2)	0	-	4.0±0.63(2)	-	3.4±1.2	6.6±3.0)
	20.0	8.0	0.15	9.9			4.0±0.63(2)	-	2.5±0.4	4.4±0.7)
20 Nov 1981	22.0	8.0	0.25	16.4	0	-	4.0±0.28(3)	-	2.0±0.1	2.3±0.2
12 Jan 1982	24.0	6.5	0.25	12.9	-	0	8.18±0.87(3)	-	1.6±0.2	3.6±0.4
5 Mar 1982										
Fine	24.0	7.0	0.25	14.0	0	0	12.7±4.3(4)	0	1.1±0.4	5.1±1.7
Coarse	24.0	7.0	0.25	9.4	6	0	23.5±3.0(3)	0	0.37±0.05	0.55±0.07
1 Nov 1982	18.5	6.6	0.25	21.6	0	-	28.0±1.5(4)	0	0.77±0.04	29.0±0.2
HOCKLEY (2)										
20 Nov 1981	20.5	5.5	0.25	5.3	-	-	5.88±0.32(4)*	-	0.90±0.06	1.0±0.1
12 Jan 1982	29.0	4.0	0.25	23.4	-	0	12.78±0.01(3)	-	1.83±0.01	12.5±0.1
SIGNALS(5)										
13 Oct 1981	17.0	5.9	0.25	8.6	-	-	2.56±0.30(2)	-	3.4±0.4	5.7±0.7
12 Jan 1982	30.0	2.5	0.25	8.5	-	0	10.34±0 (2)	-	0.82	3.2
WALTON BRIDGE (10)										
13 Oct 1981	17.0	5.0	0.25	4.3	37	-	3.12±0.30(2)	-	1.37±0.1	1.4±0.1
12 Jan 1982	27.5	6.6	0.25	4.9	-	-	11.28±1.35(2)	-	0.43±0.05	0.38±0.05
9 Nov 1982	20.8	3.3	0.25	6.4	-	18.9	16.1±2.1 (2)	0	0.40±0.05	0.92±0.12)
	20.8	3.3	0.15	5.3	-	19.4	16.1±2.1 (2)	0	0.33±0.04	0.63±0.08)

mean ± 1 standard deviation (number of observations)

*indicates values obtained from individual core samples for heterogeneity

[a] river velocity 0.25 ms^{-1}

TABLE 5.1: BUR, sediment intrinsic activity and calculated benthic dispersion coefficients for the Waitoa River

Station (km)	Number of Determinations, n	Benthic Oxygen Uptake Rate $gm^{-2}\ day^{-1}$	Sediment Intrinsic Activity, I $gm^{-2}\ day^{-1}$	Depth of Oxygen Penetration, δ_c cm	Dispersion Coefficient, D_s $cm^2\ s^{-1}$
Landsdowne Road (-1)	7	14.0±6.1	1.45±0.95	13.5±9.5	$27.2\pm22.9\times10^{-3}$
Waharoa (0)	7	15.6±4.5	20.2±20.3	1.4±1.1	$3.2\pm2.1\times10^{-3}$
Hockley (2)	2	14.4±12.8	9.3±4.9	1.4±0.7	$6.8\pm8.1\times10^{-3}$
Signal (5)	2	8.6±0.1	6.5±5.5	2.1±1.8	$4.5\pm1.8\times10^{-3}$
Walton Bridge (10)	3	5.2±1.1	10.2±6.6	0.73±0.55	$0.90\pm0.51\times10^{-3}$

mean ± 1 standard deviation

Table 5.2: Summary statistics for Table 5.1

3.1). Above the discharge, at Landsdowne Road (-0.1 km), extensive growth of the filamentous algae Oedogonium sp gave a maximum upstream BUR of 24.6 g O_2 m^{-2} day^{-1} and maximum photosynthetic oxygen production rate giving a gross productivity of 33.9 g O_2 m^{-2} day^{-1} (Table 5.1). The minimum upstream BUR value recorded was on coarse bed particulates and had a value of 5.3 g m^{-2} day^{-1}, with a mean of 14.0±6.1 g m^{-2} day^{-1} (Table 5.2). The Waharoa station, immediately below the discharge, showed prolific colonisation of 'sewage fungus' which resulted in the highest measured BUR of 70.2 g m^{-2} day^{-1} (Table 6.2). The BUR declined markedly when floods removed the biofilm; this removal resulted in the lowest BUR of 9.9 g m^{-2} day^{-1} (5 March 1982, Table 5.1), with a mean of 15.6±4.5 g m^{2} day^{-1} (Table 5.2). BUR values obtained below the discharge were not significantly different from the upstream station ($P>0.2$). Chamber measurements at stations 2 and 5 km downstream were limited by low concentrations of river DO. Maximum BUR at the Hockley station (2 km) was 23.4 g m^{-2} day^{-1} and that at the Signal station (5 km) was 8.6 g m^{-2} day^{-1} with no significant difference between adjacent stations ($P>0.2$). At the Walton Bridge station (10 km) BUR was low (5.2±1.1 g m^{-2} day^{-1}) (Table 5.2) though blue-green algae present (predominantly Oscillatoria) resulted in a high rate of photosynthetic oxygen production giving a gross productivity of 19.4 g O_2 m^{-2} day^{-1}. BUR was significantly lower at 10 km than both the adjacent upstream station ($P<0.05$) and the Landsdowne Road upstream station ($P<0.05$) (Table 5.2).

Glucose was added to the chamber, to ensure that oxygen concentration was the rate limiting reactant. Glucose addition on numerous occasions showed minimal BUR response (Table 5.1), indicating that environmental conditions were generally oxygen limiting. Lactose addition was also made with minimal BUR responses comparable with glucose results.

River bed particle size heterogeneity (see Appendix 4) and subsequent differences in microbial colonisation, and sediment-water exchange, may result in variability of BUR measurements. Such BUR sampling variabilities associated with river-bed heterogeneity were minimised by use of a large chamber base area. When benthic conditions permitted sampling in areas where distinct particle size sorting had occurred, chamber measurements showed higher BUR values associated with the fine particulates at both the Landsdowne Road (155%, 8 March 1982, Table 5.1) and Waharoa (49%, 5 March 1982, Table 5.1) stations. Microbial biomass and activities associated with sediment areas of fine and coarse particulates were investigated in detail with results described in section 5.2.7. Areal variability of BUR measurement for 'sewage fungus' colonisation was estimated to be ±17% for 95% confidence (n=4 from 24 September 1981, Table 6.2) and for Landsdowne Road sediments the value was estimated to be ±30% (n=3 from 20 November 1981, Table 5.1). Greater uncertainties, however, resulted from the strong apparent dependence of BUR on DO concentration, for both biofilm (see Chapter 6) and sediment measurements. For 'sewage fungus' biofilms, repeatability of the chamber measurements were established (<±15% for 95% range, see Table 6.3) and analysis of the BUR kinetics is discussed in Chapter 6. Detailed discussion of the BUR dependence on DO concentration is presented in Chapter 7 and the results presented here are restricted to the initial BUR measurements.

All chamber measurements were performed at circulation velocities equivalent to measured river boundary velocities. Decreasing the velocity has shown to measure a concomittant decrease in BUR values measured for biofilms (see Chapter 6, Fig 6.1), where adjacent chamber measurements were made at 0.25 $m\ s^{-1}$ and 0.15 $m\ s^{-1}$ circulation velocity (c.f. river 0.25 $m\ s^{-1}$), low velocity BUR values were only 58% at Landsdowne Road (20 November 1981, Table 5.1) and 27% at Waharoa (19 November 1981, Table 5.1), of the adjacent measurements at the river boundary velocity.

Removal of possible diffusion limitations within the sediment matrix provided an estimate of the intrinsic activity (I) of the sediments which was obtained by measuring the rate of oxygen uptake of sediment material obtained from the surface layer of 5 randomly sampled cores (see Methods 3.3.5). Preliminary experiments for the 5 Waitoa sampling stations (detailed in Appendix 1.1) showed that continuous stirring increased the value of I by approximately 50% as compared with hourly intermittent agitation. Addition of glucose, to measure the maximum potential intrinsic activity (Imax) at the river temperature, showed a greater increase in I for stirred than for intermittently agitated (20% c.f. 10% see Appendix 1.1). No significant decrease in I occurred with decreasing oxygen concentration for continuously stirred glucose-supplemented sediment during the measurement periods (approximately 7h from 8 to 0 g O_2 m^{-3}, results not shown). This suggests that when diffusion limitations were removed, approximately zero order kinetics were obtained for respiration of the sediment microbial community. The practicalities of performing field measurements and instrumentation necessitated discrete rather than continuous intrinsic activity measurements. This may have resulted in a slight underestimation of I values. The 95% confidence interval for replicated intrinsic activity measurements varied between stations, but was generally less than ±20% (Table 5.1).

Variability of intrinsic activity measurements between individual core samples from the bed was determined for the upstream and 2 stations below the discharge. At Landsdowne Road (20 November 1981) values of I for single measurements on 4 cores gave a 95% confidence range of ±43%, whereas 3 'sewage fungus' cores with values of I estimated using triplicate measurements at Waharoa (24 September 1981) showed a range of ±13%, and values of I for single measurements on 4 cores at Hockley Station (20 November 1981) showed a range of ±11% (see * values in Table 6.1). Although the degree of benthic heterogeneity and subsequent measurement variability, might be expected to vary considerably between stations in relation to floods and subsequent biomass development, the practicalities of determining the degree of statistical confidence for each sampling occasion, at each station and for each measured parameter, were prohibitive. For this reason 5 random core samples were routinely taken, homogenised together, and all subsequent sub-sampling was from this homogenate (sampling scheme in Fig 3.7). All results reported are based on this sampling regime, which was also applied as a stratified sampling regime to visually 'fine' and 'coarse' areas of bed substratum.

A summary of longitudinal surveys of river stations showed that intrinsic activity increased by an order of magnitude below the organic discharge over the I values obtained upstream. Values obtained were 1.45±0.39 g m^{-2} day^{-1} for the upstream station, and 20.23±8.30 g m^{-2} day^{-1} for downstream station ($P<0.05$) (Table 5.1).

No significant differences in I values were consistently detected between adjacent downstream stations ($P>0.2$). Intrinsic activity at

Walton Bridge (10 km) was 7 -fold higher than that measured at the Landsdowne Road station ($P<0.01$) (Table 5.2 summarises results from Table 5.1).

Seasonal variation in measured I values showed high variability for each of the sampling stations. An estimated 95% confidence range (from Table 5.2) was ±54% for the upstream station, and increased to ±82 -171% for stations below the organic discharge. This substantiates the visual observations of the influence of frequent floods (Fig 3.2) which removed all biofilms and attached growths from the bed subsequent microbial recolonisation of the bed often occurred rapidly once low flows had resumed.

An estimate of the depth of oxygen penetration (δ_c) into the sediments may be made from the chamber BUR and the intrinsic activity of the sediment using Eqn 3.5. Oxygen penetration depths calculated for the Waitoa River are shown in Table 5.1 and summarised in Table 5.2. Above the discharge at Landsdowne Road δ_c varied both seasonally and with sediment type, ranging from 7.2 cm to 32.1 cm, with a mean value of 13.5±3.9 cm. Below the discharge at Waharoa, δ_c significantly ($P<0.01$) declined with a minimum value of 0.35 cm occurring when 'sewage fungus' biofilm was present, and a maximum of 3.4 cm; with a mean 1.37±0.44 cm. Estimates of δ_c at 2 km, 5 km and 10 km downstream are limited in number; however, adjacent stations showed no significant difference between values ($P>0.2$) at 1.37±0.66 cm, 2.10±1.81 cm and 0.73±0.39 cm respectively. The oxygen penetration depth at the Walton Bridge station (10 km) was approximately 1/20 that of the upstream Landsdowne Road station ($P<0.05$).

A benthic dispersion coefficient (D_s) may be used to describe the benthic mass transfer characteristics at individual stations, and so enable comparison between rivers to be made, with calculation of D_s using Eqn 3.6. D_s values estimated for the Waitoa River are shown in Table 5.1 and summarised in Table 5.2. Benthic D_s values were substantially higher at all sampling stations than were molecular diffusion processes. Measured values were in the range 0.4×10^{-3} to 7.12×10^{-3} $cm^2\ s^{-1}$ (c.f. molecular diffusion ~ 10^{-5} $cm^2\ s^{-1}$). The mean D_s value upstream at Landsdowne Road was $27.2 \pm 22.9 \times 10^{-3}$ cm^2

s^{-1} with the downstream Waharoa station value amounting to only 12% of that value at $3.2 \pm 2.1 \times 10^{-3}$ cm^2 s^{-1} ($P<0.05$). D_s estimates at the 2 km and 5 km downstream stations show of no significant differences between adjacent stations ($P>0.2$) with mean values of $6.8 \pm 8.1 \times 10^{-3}$ cm^2 s^{-1} and $4.5 \pm 1.8 \times 10^{-3}$ cm^2 s^{-1} respectively. A further significant decline in D_s occurred on progression to the Walton Bridge (10 km) station, which gave a mean D_s value of $0.90 \pm 0.51 \times 10^{-3}$ cm^2 s^{-1} ($P<0.05$). The D_s value at this station was only 1/30 that of the upstream Landsdowne Road station ($P<0.10$).

5.2.3 The relative importance of planktonic and benthic biomass

Planktonic and benthic biomass carbon estimates were based on ATP assays (Methods section 3.5) and are shown in Table 5.3 for longitudinal surveys in late spring (5 November 1981); summer, subsequent to a flood (12 January 1982); and summer low flow (25 January 1982) conditions. Based on the simplifying assumption of a uniform 1 cm depth of sediment-water interaction at all stations on each sampling occasion, the benthic biomass present was up to an order of magnitude greater than the planktonic biomass (range 1.2 to 10.8 times). Benthic dominance was lowest upstream, increasing below the discharge then decreasing to approximately the upstream value by 10 km. However, this analysis was only approximate since chamber measurements indicated considerable variation in the depth of sediment-water interaction (Table 5.1).

A complete longitudinal survey including chamber BUR and I measurements was made on 12 January 1982, enabling estimates of the oxygen penetration depth (δ_c) to be made for each station. Based on those (δ_c) estimates benthic biomass was up to 20 times the planktonic biomass (range 2.1 to 19.4 times), with comparable high values of benthic dominance above and immediately below the discharge, then increasing to a maximum at 2 km (Table 5.3). A chamber BUR measurement upstream on 25 January 1982 showed that the benthic biomass had increased to be approximately 50 times the planktonic biomass, due to benthic filamentous algal proliferation (Table 5.3).

Chamber BUR and biomass measurements were not always able to be performed on the same day. Therefore only approxiate estimates of the relative importance of planktonic and benthic biomass were possible for

						Biomass Carbon		Benthic Biomass Factor[b]			
Date	Station km	Temperature °C	Discharge $m^3 s^{-1}$	Mean Velocity $m\ s^{-1}$	Mean Depth, H m	Planktonic $mg\ m^{-3}$	Benthic[a] $mg\ m^{-2}$	Benthic[c] Depth, δ_c cm	Factor	Benthic[d] Depth, δ_c cm	Factor
5 Nov 1981[e]	-1	16.5	0.84	0.40	0.40	322.5	167.9	1	1.30	9.6[h]	12.5
	0					365.0	503.8	1	3.45	2.0[h]	6.9
	2					222.5	800.0	1	8.99	0.9[h]	8.1
	5					317.5	1168.8	1	9.20	3.4[i]	31.3
	10		1.68	0.55	0.41	362.5	569.3	1	3.93	1.4[i]	5.5
12 Jan 1982[f]	-1	23.0	0.58	0.30	0.35	805.0	324.0	1	1.15	11.9	13.7
	0					542.5	1742.2	1	9.18	1.6	14.7
	2					1047.5	3960.3	1	10.80	1.8	19.4
	5					902.5	810.8	1	2.57	0.8	2.1
	10		0.70	0.20	0.60	492.5	524.8	1	1.8	0.4	2.1
25 Jan 1982[g]	-1	20.0	0.33	0.19	0.33	[900]	1465.0	1	4.93	11.0	54.3
	0					902.5	2866.9	1	9.63	1.6[j]	15.4
	2					1400.0	3636.6	1	7.87	1.8[j]	14.2
	5					2837.5	2886.9	1	3.08	0.8[j]	2.5
	10		0.50	0.10	0.60	420.0	1022.3	1	4.1	0.4[j]	1.6

a measurement of a 1cm surface layer

b $\frac{\text{Benthic biomass} \times \delta_c}{H \times \text{Planktonic Biomass}}$

c arbitrary depth
d calculated depth from Eq. 3.2.2, Table 5.1
e aquatic sampled 12 Oct 1981
f aquatic sampled 11 Jan 1981
g aquatic sampled 27 Jan 1981
h from 20 Nov 1981, Table 5.1
i From 13 Oct 1981, Table 5.1
j assumed as for 12 Jan 1982

TABLE 5.3: The relative importance of planktonic and benthic biomass in the Waitoa River

some surveys. For a late spring survey (5 November 1981) benthic biomass was up to 30 times the planktonic biomass (range 5.5 to 31.3 times), with a median value upstream (12.5 times) and generally lower values below the discharge with the exception of the maximum value which occurred at 5 km (Table 5.3). The summer low flow survey (25 January 1982), using the sediment oxygen penetration depths estimated from 12 January 1982, showed that a large decrease in benthic dominance occurred below the discharge at Waharoa to approximately 15-fold (c.f. 54-fold upstream) further decreasing downstream to be less than 2-fold at 10 km.

Interpretation of total biomass measurements in terms of river oxygen and organic matter processing requires separation of the type of biomass components present (i.e. whether the organisms are phototrophic or heterotrophic).

5.2.4 Phototrophic and heterotrophic composition of benthic and planktonic biomass

This section describes investigations of the type of microbial biomass present using: ATP determination as a measure of total biomass; chlorophyll a as a measure of the phototrophic component with a correction for pheophytin breakdown pigments (Methods 3.5.1.6); and estimation of the heterotrophic component by difference. All biomass estimates were converted to carbon equivalents in order to provide a common base for comparisons (Methods 3.6.1, using ATP x 250 and chl a x 16 to obtain carbon equivalents). In order to separate benthic and planktonic derived biomass fractions, planktonic samples were subjected to differential filtration prior to biomass determination, (see sampling scheme Fig. 3.6).

Benthic biomass. ATP measurements of total biomass for the surface 1 cm of sediment are shown for 3 longitudinal surveys of the Waitoa River in Figure 5.1A. A late spring survey (5 November 1981) showed a 3-fold increase in biomass below the discharge ($P<0.01$) followed by a further increase to 2 km ($P<0.05$) and 5 km ($P<0.01$) with a biomass decrease to 10 km ($P<0.01$), where biomass present was approximately 3-times the upstream Landsdowne Road station. No discrimination of biomass types present were made on these sediments because attached algal growths were not visible.

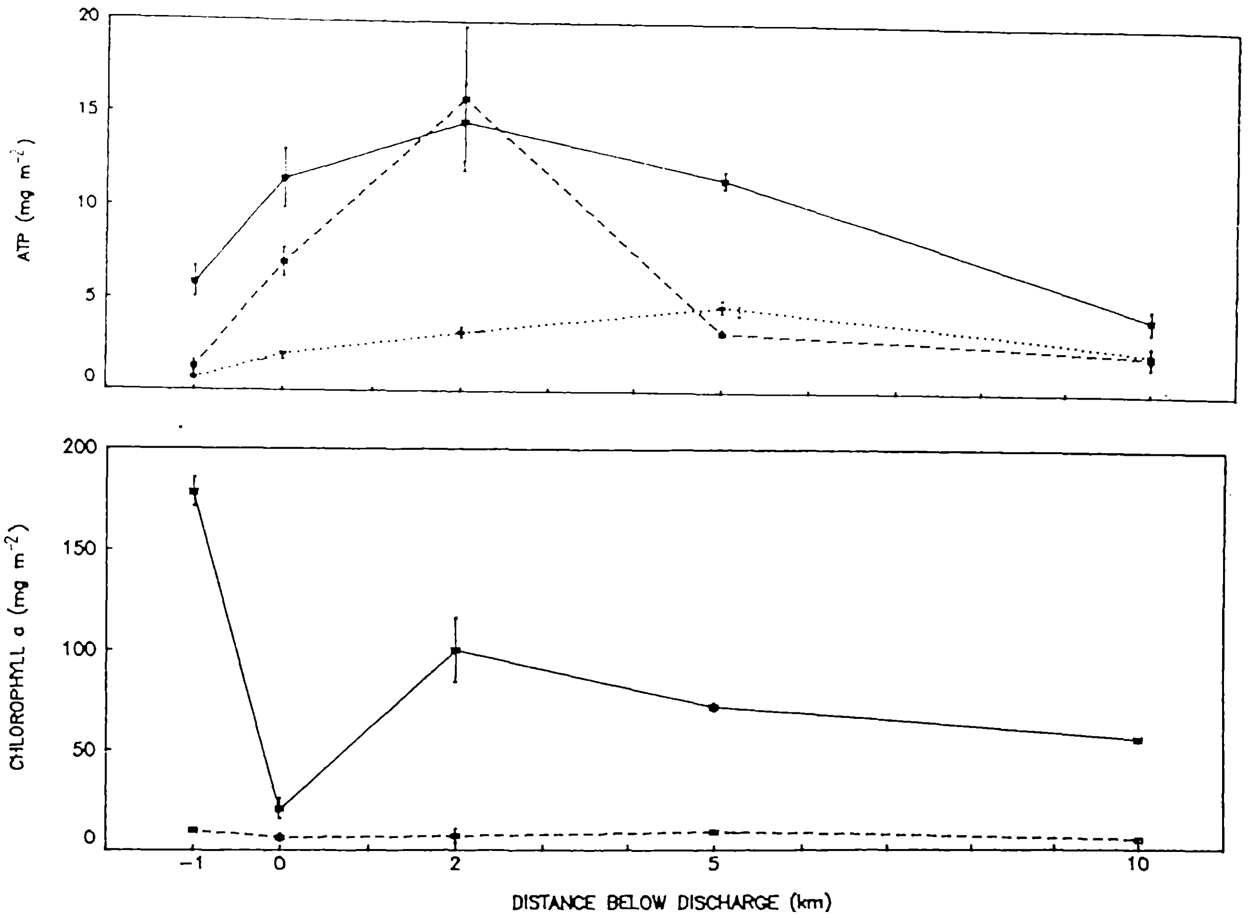


Figure 5.1 : Benthic ATP and chlorophyll a measurements in the Waitoa River
A ATP
B Chlorophyll a
Symbols : benthic
x.....x sampled 5 November 1981
■ - - - ■ sampled 12 January 1982
★———★ Sampled 25 January 1982
Error bars represent 1 standard error range.

A summer low flow survey (25 January 1982), showed a maximum ATP value 3-fold greater than that present during the late spring survey (5 November 1981), occurring at 2 km downstream from below the discharge (Fig. 5.1A). The first summer survey was started when low flow resumed 5 days after a flood which had removed attached algae and 'sewage fungus'. Algal biomass was uniformly low (Fig. 5.1B, 12 January 1982) with high pheophytin present upstream and downstream from the discharge (Table 5.4). The 13 day period prior to the next sampling (25 January 1982) showed a steady decrease in river flow (0.51 m^3 s^{-1} down to 0.40 m^3 s^{-1}) with benthic algal biomass increasing substantially; as shown by the chlorophyll a measurements (Fig. 5.1B). Extensive colonisation by filamentous algae occurred with Oedogonium sp. at Landsdowne Road, and Ulothrix sp. at Hockey (2 km). Blue green algae of Oscillatoria sp. were present at Signal (5 km) and Walton Bridge (10 km) stations. Chlorophyll degradation products were present at the Waharoa and Signal stations with pheophytin amounting to 17.8% and 35.6% of the chlorophyll a respectively (Table 5.4). Thus rapid benthic algal biomass development may occur under favourable environmental conditions, with the predominant species varying between stations.

Comparison of the 2 summer surveys in terms of biomass carbon present is shown in Figure 5.2. The survey following the flood (12 January 1982) showed a uniformly low phototrophic biomass with no significant difference between adjacent stations ($P>0.2$), accounting for 50% of the benthic biomass present upstream at Landsdowne Road but substantially less below the discharge. Total biomass increased below the discharge (5.4-fold, $P<0.001$), with a further increase to 2 km (2.3-fold, $P<0.05$) where autotrophic carbon was 2.9% of total biomass. Total biomass declined to 5 km ($P<0.05$), and showed a further decline to 10 km ($P<0.10$), where the total biomass present was not significantly different from that present at the upstream station ($P>0.1$). A subsequent survey 13 days later (25 January 1982) gave phototrophic biomass estimate which exceeded the total biomass estimate at Landsdowne Road. This result may have been caused either by the use of an inappropriate sampling technique for the filamentous Oedogonium sp. present, since filaments may well have been excluded from the volumetric sub-sampling for ATP; or possibly by use of an inappropriate biomass

Site (km)	Chlorophyll a mg m^{-2} sampling 12 Jan 1982	Pheophytin mg m^{-2} sampling 12 Jan 1982	%	Chlorophyll a mg m^{-2} sampling 25 Jan 1982	Pheophytin mg m^{-2} sampling 25 Jan 1982	%
Landsdowne Road (-1)	9.8	2.2	22.7	178.1±7.1	ND	0
Waharoa (0)	6.5	8.1	124.0	20.9±5.0	3.7	17.8
Hockley (2)	7.2	0.5	6.7	100.1±16.0	1.8	1.8
Signal (5)	9.4	ND	0	71.9±2.2	25.6	35.6
Walton Bridge (10)	5.4	ND	0	55.6±0	ND	0

Table 5.4: Sediment chlorophyll a and pheophytin concentrations in the Waitoa River

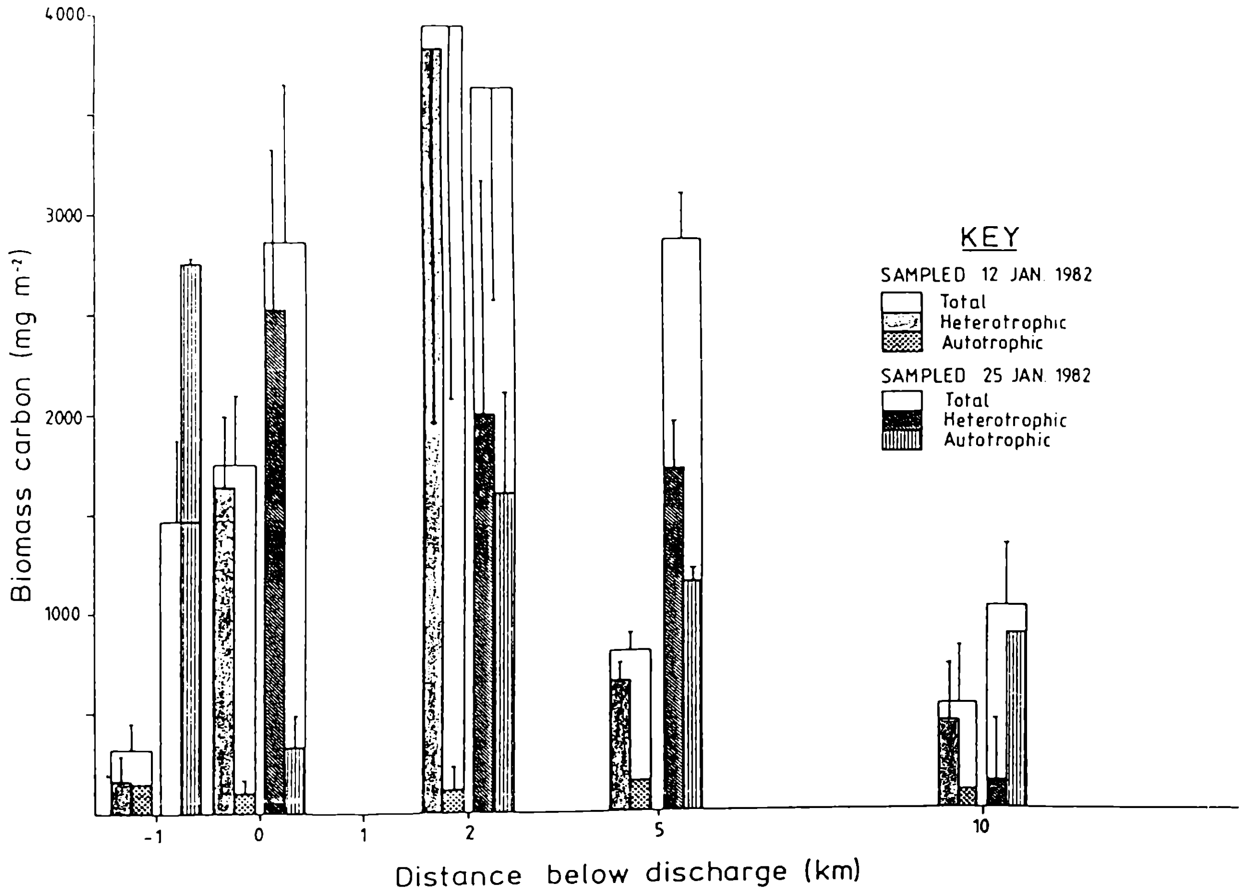


Figure 5.2 : Benthic biomass carbon estimates for longitudinal surveys of the Waitoa River. Errors bars represent 95% confidence range.

carbon conversion factor (Karl, 1980). Downstream of the discharge at Waharoa extensive 'sewage fungus' colonisation had developed with 2.0-times the upstream biomass present, with a heterotrophic portion of 88.4% (Fig. 5.2). There was no significant difference between total or heterotrophic biomass levels present at the 3 stations below the discharge ($P>0.2$; between 0, 2 and 5 km stations). However, phototrophic biomass increased approximately 5-fold to constitute 44% of the total biomass present ($P<0.001$) at the Hockley station (2 km), declining slightly to 40% at Signals (5 km) ($P<0.10$). Total biomass declined between 5 km and 10 km by 65% ($P<0.001$), to a lower value than that present upstream at Landsdowne Road ($P<0.10$ Fig. 5.2). At 10 km, 87% of the total biomass was phototrophic. Phototrophic biomass increased significantly ($P<0.001$) between summer surveys for each of the 2 km, 5 km and 10 km stations, whereas heterotrophic biomass increased only at 5 km ($P<0.001$).

Planktonic biomass. Results obtained for longitudinal planktonic biomass measurements in conjunction with benthic surveys are shown in Table 5.5. The spring survey (12 October 1981) showed no significant differences between adjacent stations ($P>0.2$) (total travel time 6.4 h). The summer survey following a flood (11 January 1982) showed a 2-fold biomass increase from 0 km to a maximum at 2 km ($P<0.01$), followed by a 53% decine to 10 km ($P<0.05$) where the biomass was 61% of the upstream value ($P<0.10$) (total travel time 7.8 h). A summer low flow survey (27 January 1982), showed a 3-fold biomass increase from 0 km to a maximum at 5 km downstream ($P<0.001$), followed by a 85% biomass decline to 10 km ($P<0.001$) where the planktonic biomass was only 47% of the upstream value (total travel time 12.7 h).

Detailed analysis of the type of planktonic biomass present and its size distribution using a 30μm filtration (see Methods section 3.5.1 and Figure 3.6) were performed on 10 February 1982. This survey was preceded by a period of low river flow (13 days subsequent to 27 January survey). Results are shown in Figure 5.3. Biomass at Waharoa (0 km) was 86% less than the 27 January survey ($P<0.001$). Below Waharoa, the downstream biomass showed a substantial increase to the maximum at 5 km (18.6-fold, $P<0.05$), followed by a 92% decline to 10 km ($P<0.05$) where the biomass level was not significantly different from

Date	Station km	Total Biomass Carbon mg m^{-3}	Biomass Carbon <30μm mg m^{-3}	<30 μm/Total %	Significance Level
12 Oct 1981	-1	322.5±116.1	87.5±10.4	27	P<0.05
	0	365.0±262.8	117.5±35.0	32	NS
	2	222.5±37.8	147.5±7.4	66	P<0.05
	5	317.5±9.5	212.5±2.3	67	P<0.001
	10	362.5±25.4	287.5±21.0	79	NS
11 Jan 1982	-1	805.0±153.0	275.0±27.5	34	P<0.01
	0	542.5±65.1	502.5±271.4	93	NS
	2	1047.5±188.6	677.5±61.0	65	P<0.05
	5	902.5±225.6	292.5±67.3	32	P<0.05
	10	492.5±216.7	300.0±72.0	61	NS
27 Jan 1982	-1	-	-	-	-
	0	902.5±45.1	765.0±71.1	85	P<0.05
	2	1400.0±196.0	952.5±303.8	68	NS
	5	2837.5±340.5	1985.0±176.7	70	P<0.05
	10	420.0±138.6	675.0±324.0	-	NS

mean ± 1 standard deviation (all n=3)

TABLE 5.5 : Planktonic biomass measurements for longitudinal surveys made in conjunction with benthic measurements in the Waitoa River.

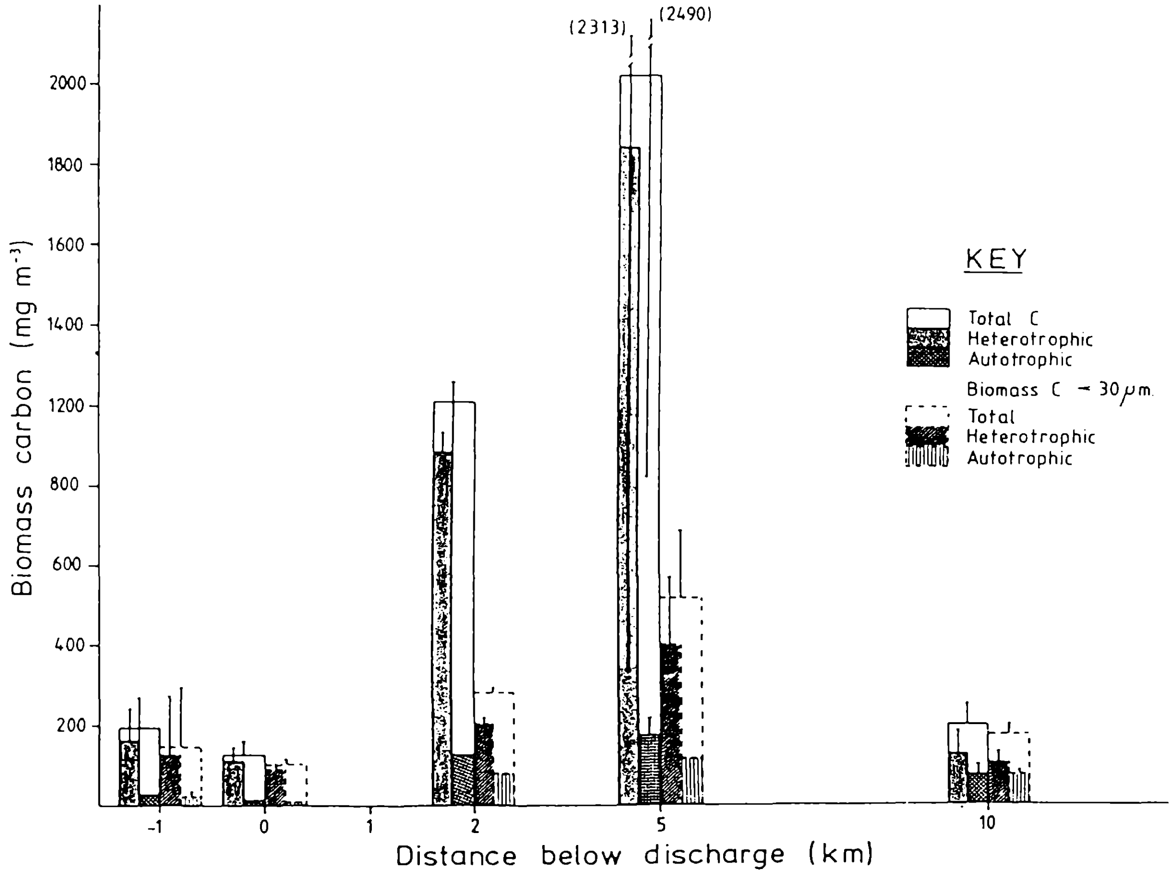


Figure 5.3 : Differential filtration with heterotrophic and autotrophic determination of Waitoa River planktonic biomass. Error bars represent 95% confidence range.

the upstream value ($P>0.2$) (total travel time 15.1 h). Differential size filtration showed 28% of the biomass to be smaller than 30 µm at 2 km downstream ($P<0.001$), with 22% at 5 km ($P<0.05$), and no significant difference at other stations. The <30 µm biomass fraction increased from 0 km to 2 km ($P<0.001$), and from 2 km to 5 km ($P<0.05$), then decreased by 66% by 10 km ($P<0.001$). The phototrophic biomass present in both total and <30 µm fractions increased from 0 km to 2 km distance from the discharge point ($P<0.001$), to be 12.6% and 27.3% of the respective size fractions. No significant difference was detected at the 5 km station ($P>0.2$), whereas a decline occurred by 10km ($P<0.001$), where the phototrophic biomass represented 37.5% and 42.1% of the total and 30 µm fractions respectively. Thus the planktonic biomass had a larger phototrophic component for stations below the discharge than the 15.0% and 14.4% for respective large and small particle microbial biomass size fractions upstream.

Details of chlorophyll a and pheophytin analyses are given in Table 5.6. Pheophytin pigments represented a significant component of total pigments at each of the stations sampled, ranging from 26% to 146% of the chlorophyll a present. Pheopigments appeared to be overrepresented in the total biomass samples compared with the <30 µm fraction. Upstream, the <30 µm size fraction represented 72% of the phototrophic biomass, decreasing to 17% at 2 km downstream, but then increasing to virtually 100% by 10 km.

Because of the high variability of planktonic ATP determinations for samples taken from below the discharge, further studies were initiated to investigate the sizes of the microbial biomass present, together with the effects of homogenisation of the sample (see Methods section 3.5.1.5). Results obtained for a longitudinal survey on 29 October 1982 are shown in Figure 5.4. Homogenisation resulted in an increase in the mean levels of biomass measured for each of the stations, although the difference was only significant at Landsdowne Road (-1 km, $P<0.05$) and Waharoa (0 km, $P<0.05$). Analytical uncertainly was reduced by approximately 50% following homogenisation to between 6 and 10%. The various filtrates were compared with the homogenised samples and showed for the 300 µm filtrate a significant decline only at 5 km ($P<0.05$) and 10 km ($P<0.05$); whereas for a 90 µm filtrate, upstream (-1 km) ($P<0.001$)

Station	TOTAL			<30 µm			Chlorophyll a	Pheophytin
	Chlorophyll a	Pheophytin	Pheo/Chl	Chlorophyll a	Pheophytin	Pheo/Chl	Total/30 µm	Total/30 µm
	mg m^{-3}		%	mg m^{-3}		%	%	%
Landsdowne Road (-1)	1.87±0	1.69±0.53	90	1.35[a]	0.35[a]	26	72	21
Waharoa (0)	-	-	-	-	-	-	-	-
Hockley (2)	8.01±0	11.72±1.18	146	4.81±0.76	4.54±0.23	94	17	39
Signal (5)	11.02±1.42	11.88±1.41	107	7.34±0	6.45±0.33	88	67	54
Walton Bridge (10)	4.68±0.94	3.51±0.62	75	4.67±0	1.87±0	40	99	53

all mean ± 1 standard deviation for n = 2 samples

[a] n=1 samples

TABLE 5.6: Chlorophyll a and pheophytin content of Waitoa River aquatic biomass, sampled 10 Feb 1982

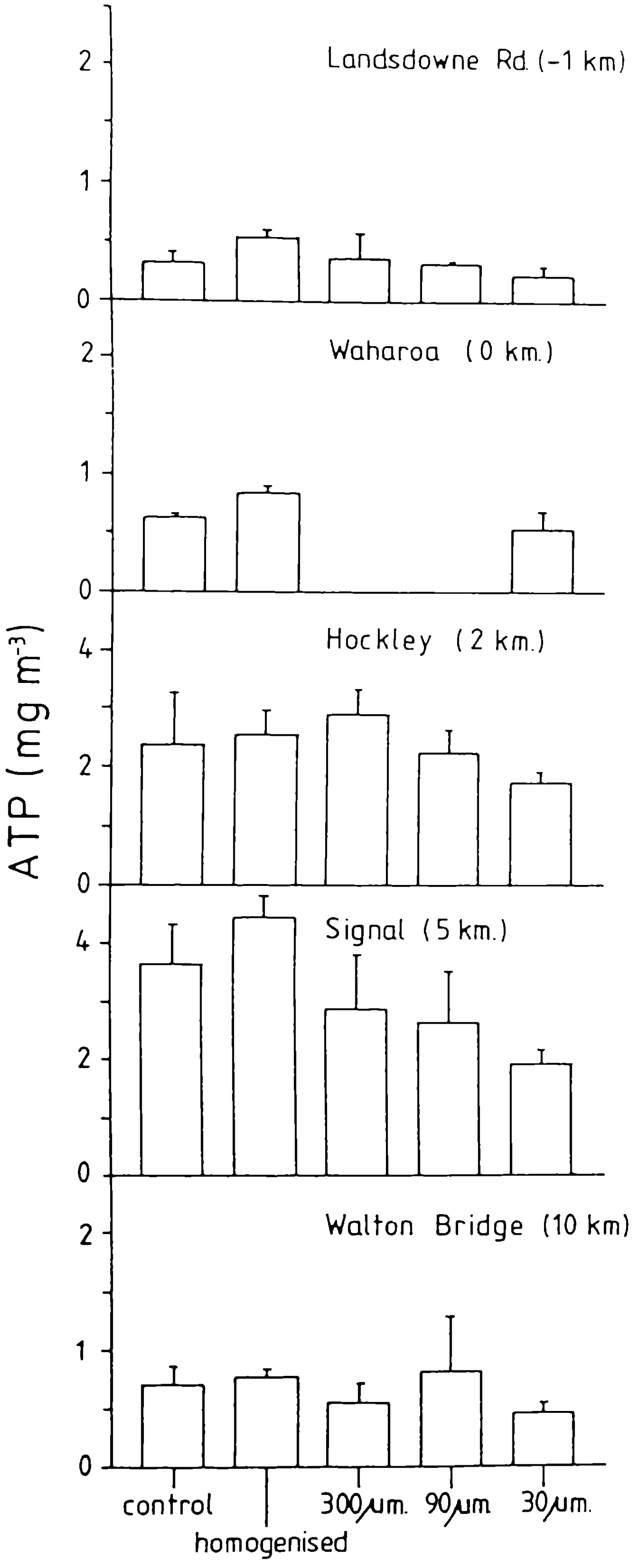


Figure 5.4 : Particle size fractionation and homogenisation of Waitoa River planktonic biomass. Error bars represent 95% confidence range.

and 5 km showed a significant decline in biomass. Each of these filtrate size fractions showed a high analysis uncertainty of up to ±40%. The 30 µm filtrate was significantly lower for each of the 5 stations ($P<0.01$, $P<0.05$, $P<0.05$, $P<0.001$, $P<0.001$ from upstream) with a small error range of 7% to 20%, suggesting that the bulk of filamentous microorganisms were present in the size fractions above 30 µm.

5.2.5 Effect of temperature on intrinsic activity.

The influence of incubation temperature on intrinsic activity was obtained for samples from the 5 longitudinal stations on the Waitoa River over the temperature range encountered in surveys (10-25°C). The effect could be explained by a single Arrhenius equation of form

$$\text{Intrinsic activity at } T°\text{C} = I\ (1.074^{\,T-20})$$

Where I is the measured intrinsic activity at 20°C. Measurements were made at 4 temperatures with replicate samples ($r^2>0.95$ for all stations). Results are detailed in Appendix 1.2. This temperature response is equivalent to a Q_{10} of 2.0. No attempt was made to measure the temperature maxima for I.

5.2.6 Enzymatic measurement of benthic processes

The section describes results obtained from enzyme activity measurements for longitudinal surveys in the Waitoa river. The background to these measurements has been discussed in the Literature Review (section 2.5). Measurements included: (i) total metabolic activity measures of oxygen uptake rate, termed intrinsic activity (I); (ii) heterotrophic metabolism enzymes: galactosidase, glucosidase and protease; and (iii) phosphatase enzymes: bis-phosphatase and alkaline phosphatase.

Microbial populations were compared by the calculation of specific activities relative to measured biomass, which in turn enabled the predominant energy source of the heterotrophic population to be identified.

Areal activity

Intrinsic activity. Intrinsic activity (I), for the 1 cm surface layer of sediment sampled on 3 longitudinal surveys ranged from 1.4 g O_2 m^{-2} day^{-1} to 18.9 g O_2 m^{-2} day^{-1} (Fig. 5.5). Lowest I values were obtained for the upstream station (-1 km) with the maximum values associated with 'sewage fungus' colonisation at Waharoa (0 km). Maximum I values occurred immediately downstream or 2 km below the outfall, and decreased to 10 km downstream, except for the survey following the flood when a negligible downstream activity decrease occurred. The maximum I value for the spring survey was only 32% of the summer survey.

Galactosidase, glucosidase and protease activity. Galactosidase activity proved to be an extremely sensitive indicator of the response to lactose present in dairy effluent. Sediment activities measured ranged from the very low upstream values of 0 to 307.6 mole PNP m^{-2} day^{-1} to a maximum of 241,410 mole PNP m^{-2} day^{-1} measured below the discharge (0 km) (Fig. 5.6A). Maximum areal activity increased by approximately 600-fold between spring and summer surveys. Increased distance downstream of the discharge showed a substantial decrease in activity of approximately 20-fold for the spring survey (5 November 1981); 30-fold for the survey following a summer flood (12 January 1982); and 240-fold for summer low flow survey.

Areal glucosidase activity at Waharoa showed an approximate 30-fold increase in the 13 day interval between summer surveys, indicating a rapid change in response to environmental conditions.

Glucosidase activity in the sediments was (20-fold) higher during summer low flow than during the spring survey. The summer survey showed a significant activity increase below the discharge (5.4-fold) followed by a decline to 10 km (3-fold, (Fig. 5.6B). A similar activity peak and larger downstream decline in activity (up to 12-fold) occurred for other sediment surveys.

Protease activity assays using a casein hydrolysate assay proved particularly difficult. 'Blank' values were not acceptably low, neither was there good reproducibility. Successful assays were obtained, however, using the PNA substrate (methods 3.5.2). Results for the

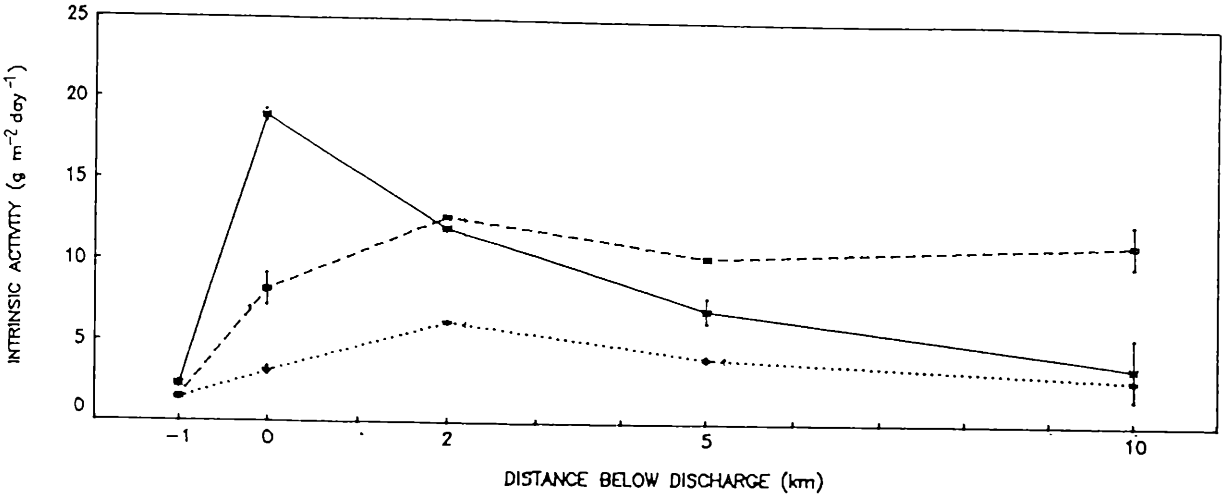


Figure 5.5 : Intrinsic activity of sediments in the Waitoa River. Symbols as for Fig. 5.1.

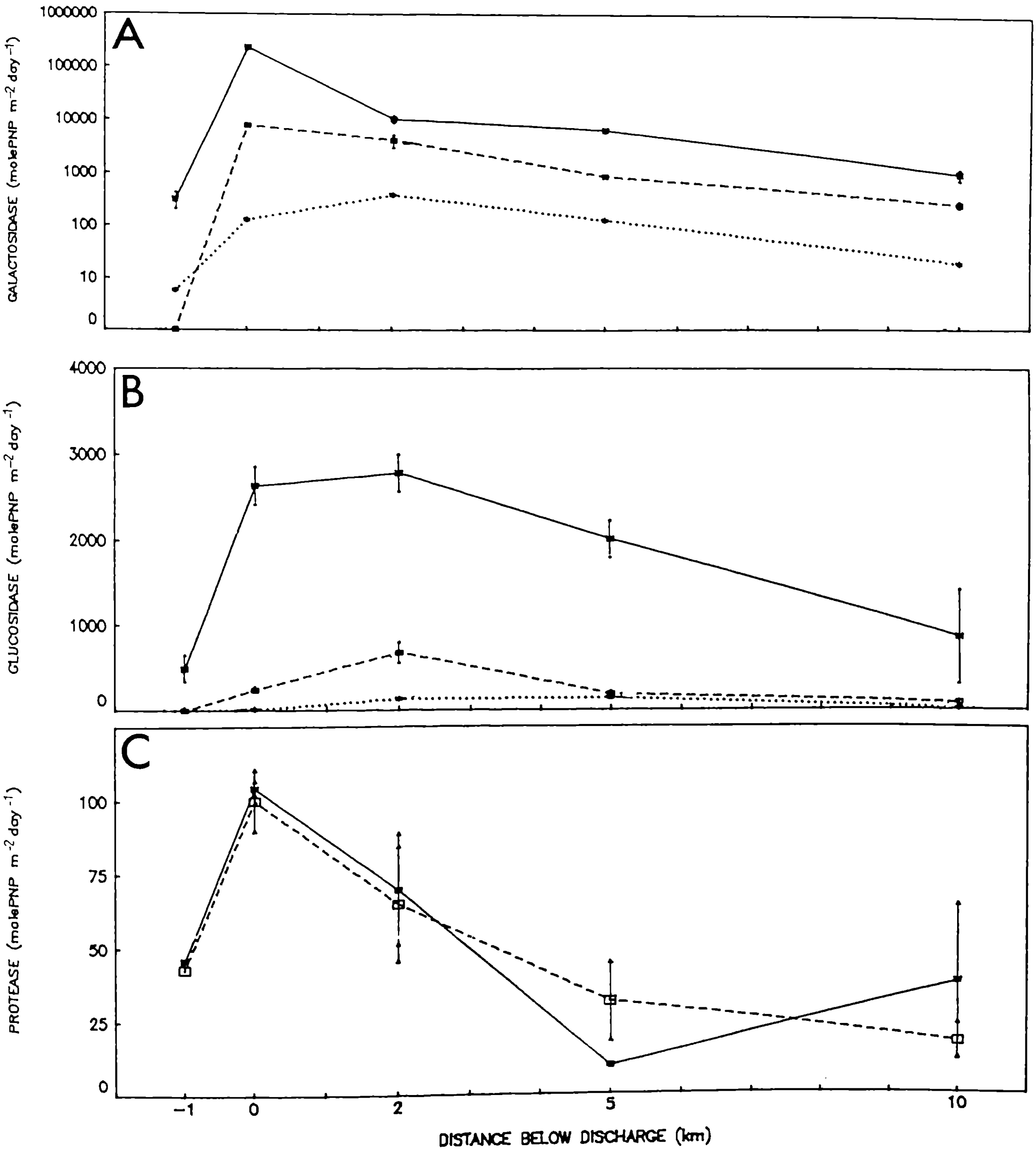


Figure 5.6 : Galactosidase (A), glucosidase (B), and protease (C) activity of sediments in the Waitoa River. Symbols as for Fig. 5.1.

summer surveys of sediments are shown in Figure 5.6C. Activity measurements below the outfall were greater than were upstream values (2.3-fold) on both summer surveys, declining to 20% of the upstream value at 5 km, and then increasing to 10 km in the low flow survey. Notably the upstream station showed high proteotytic activity suggesting the importance of protein metabolism.

Bis-phosphatase and alkaline phosphatase activity. Bis-phosphatase activity was low upstream, and increased downstream for both the spring survey (by 8.5-fold), and for the summer survey (10.0-fold, Fig. 5.7A). Maximum activity was greater for the summer survey (by 3.7-fold). The sampling after the summer flood showed no detectable bis-phosphatase activity for stations at 0 km, 2 km, and 5 km. A large increase in activity occurred, however, in the 13 day period prior to the 25 January 1982 survey, when a uniformly high activity was measured from 0 to 10 km.

Alkaline phosphatase activity showed similar trends to the bis-phosphatase activity with a significant increase occurring between the spring and summer surveys (5.4-fold, $P<0.001$, Fig. 5.7B). The summer survey also showed a increase below the discharge (4.4-fold) declining to 10 km.

Biomass specific activities

Measurements of total biomass (as ATP, Fig. 5.1A) were used to calculate the biomass specific activities for the microbial populations.

Galactosidase, glucosidase and protease. Biomass specific galactosidase activity was always low above the discharge (Fig. 5.8A). Activity peaked at 2 km for the spring survey, decreasing to 10 km (by 14.0-fold). Maximum activity increased by 2 orders of magnitude between the spring and the summer survey (170-fold) with this peak activity associated with 'sewage fungus' at Waharoa (0 km), with a large decrease to 10 km (87.3-fold). The survey after the summer flood showed a relatively even activity distribution downstream of a maximum activity at Waharoa. The maximum specific activity increase occurred at the Waharoa Station in the 13-day period prior to the next survey (25 January 1982).

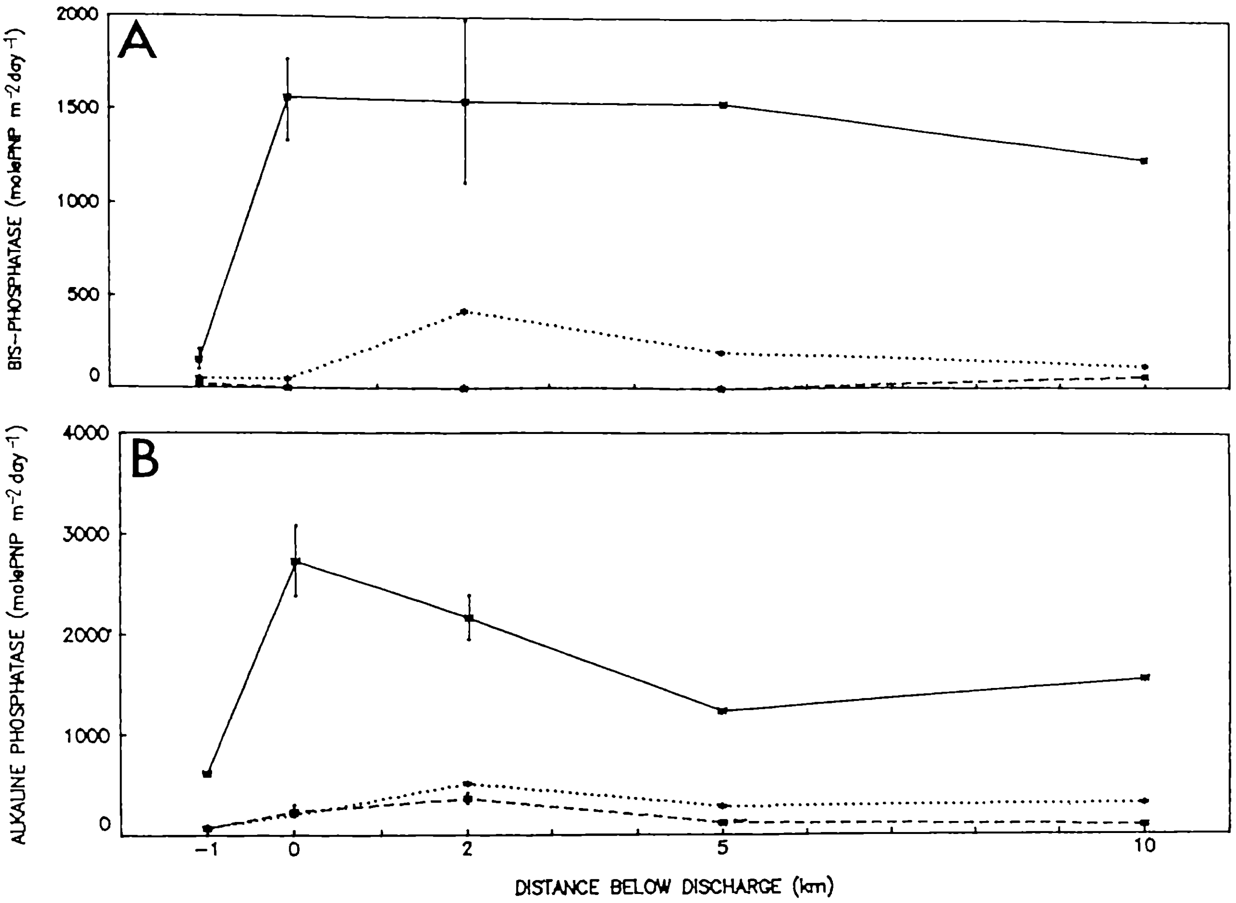


Figure 5.7 : Bis-phosphatase (A) and alkaline phosphatase (B) activity of sediments in the Waitoa River. Symbols as for Fig. 5.1.

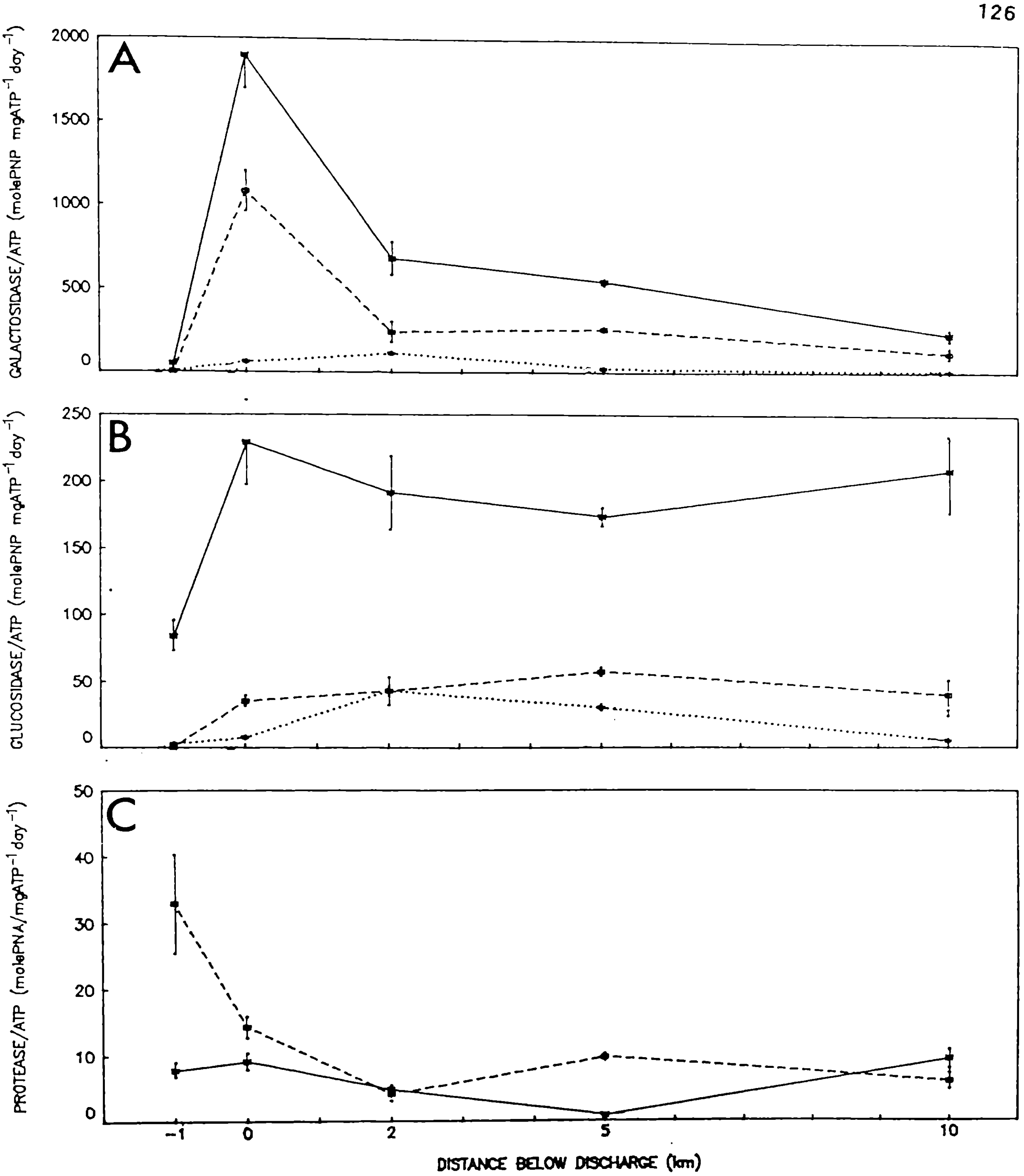


Figure 5.8 : Galactosidase (A), glucosidase (B), and protease (C) biomass specific activities for sediments in the Waitoa River. Symbols as for Fig. 5.1.

Specific glucosidase activity was generally low upstream although this increased for the summer survey (Fig. 5.8B). Activity increased below the discharge for all surveys, peaking at 2 km for the spring survey and decreasing to 10 km (8.4-fold). Maximum activity increased between the spring and the summer survey (5.4-fold), and showed no significant decline in activity downstream of the outfall (P>0.2). The survey after the summer flood showed a uniform low activity with no significant difference between downstream stations (P>0.2). Specific activities increased evenly at each downstream station in the 13-day period prior to the next survey (25 January 1982).

Biomass specific protease activity was greatest, at the upstream Landsdowne Road station after the summer flood (Fig. 5.8C). On this survey, activity decreased below the discharge with a minimum value at 2 km and increasing values to 5 km and 10 km where activity was approximately 30% of the upstream value. In the 13-day period prior to the 25 January 1982, activity significantly decreased at upstream, 0 km and 5 km (P>0.001) stations, with no significant change occurring at 2 km and 10 km (P>0.2).

Bis-phosphatase and alkaline phosphatase. The spring survey showed that biomass bis-phosphatase specific activity was significant above the discharge, decreasing immediately downstream, and peaking at 2km, followed by a further decrease to 10 km, which was not significantly different from the upstream station activity (Fig. 5.9A). Maximum activity increased between the spring and the summer survey (2.3-fold), when Waharoa (0 km) showed a significant increase from upstream (5.1-fold), with no significant difference to 5 km, followed by an increased activity to 10 km, which was an order of magnitude greater than the upstream value (11.5-fold). The survey after the summer flood showed minimal bis-phosphase activity. The maximum specific activity increase occurred at the 10 km on station in the 13-day period prior to the next survey (25 January 1982).

Biomass specific alkaline phosphatase activities showed minimal differences between sampling surveys (Fig. 5.9B). Upstream activity was significant for each survey and increased downstream only for the summer survey to the Waharoa station (2.3-fold), with a maximum value at

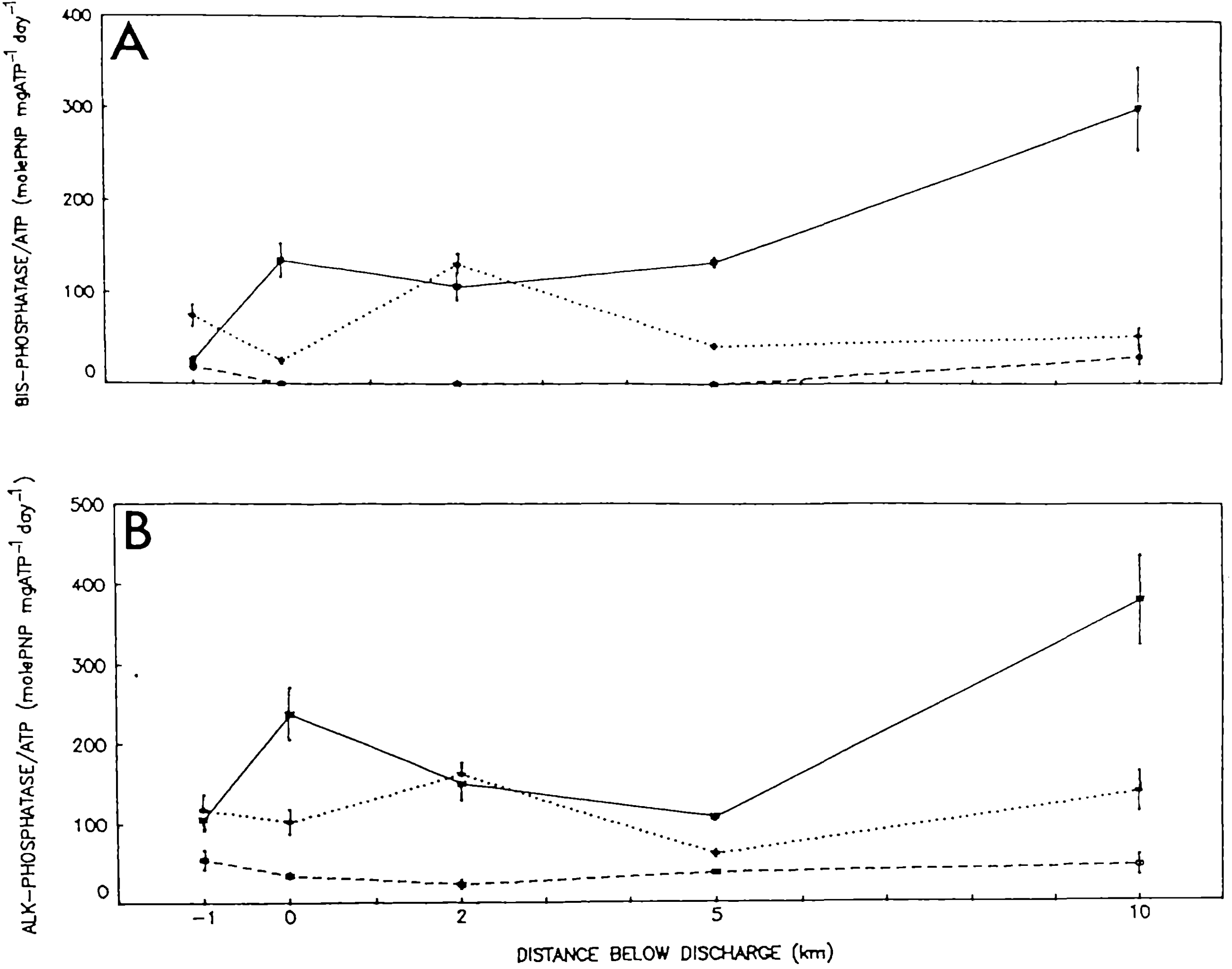


Figure 5.9 : Bis-phosphatase (A) and alkaline phosphatase (B) biomass specific activities for sediments in the Waitoa River. Symbols as for Fig. 5.1.

10 km (11.5-fold). The survey after the summer flood showed uniformly low activities with no significant difference between station values. The maximum specific activity increase occurred at the 10 km station in the 13-day period prior to the next survey (25 January 1982).

5.2.7 Sediment depth profile measurements of biomass and activity

The microbial biomass and activities were measured to 10 cm of sediment depth on 5 and 8 March 1982 for the Waharoa and Landsdowne Road stations on the Waitoa River. Each station was sampled in areas of fine and coarse bed particulates with particle size analysis shown in Appendix 1.4. The bed at Landsdowne Road had no visible attached algae and neither was there a significant 'sewage fungus' biofilm present at Waharoa at the time of sampling.

The purpose of these investigations was to estimate the extent of interaction between the sediment and the overlying flow.

Areal biomass and activity

Redox profiles. Measurements of redox potential for fine and coarse particulates at Landsdowne Road (Fig. 5.10), showed values 200 mV down to 15 cm depth. Both profiles showed a discontinuity in the 5-8 cm region. The fine material showed an even light colour throughout, whereas the coarse material darkened at 8 cm depth.

Redox potential measurements at Waharoa (Fig. 5.11), for fine particulates showed a rapid decline. No measurements could be made in the coarse particulates as the probe broke. The fine material blackened at 2 cm depth, whereas the coarse material blackened at 5 cm depth. Material from black regions smelt strongly of hydrogen sulphide.

At Landsdowne Road, biomass and carbon profiles and ATP levels were comparable on both fine and coarse surface particulates, decreasing by 50 - 60% to 10 cm (Fig. 5.12A). Chlorophyll a measurements showed comparable surface measurements, but a maxima occurred at 2 - 3 cm for the coarse and 5 - 6 cm for the fine, being higher than surface values (2.6-fold, Fig. 5.12B). Levels then declined to 10 cm.

Below the discharge at Waharoa, surface ATP values were higher on coarse than fine particulates, with each showing a rapid decline to 5 - 6 cm

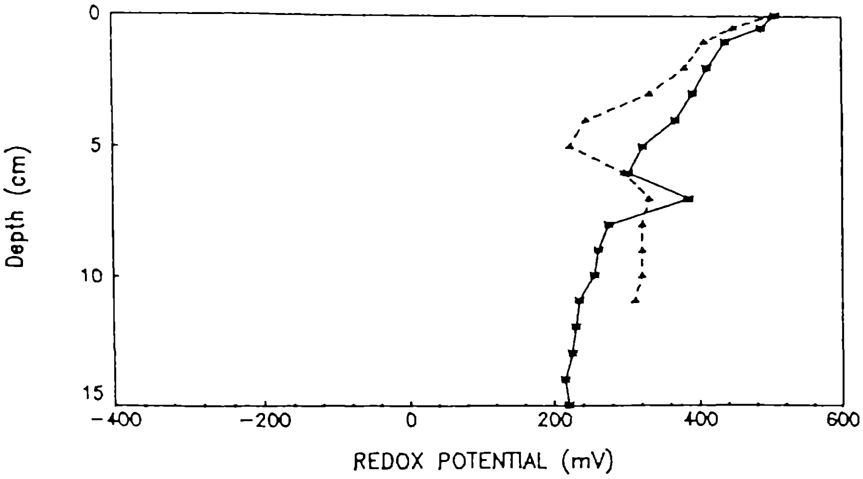


Figure 5.10 : Benthic redox profiles at Landsdowne Road on the Waitoa River sampled on 8 March 1982, for fine (*——*) and coarse (Δ----Δ) profiles. The fine profile showed an even light colour throughout, whereas the coarse profile darkens at 8 cm depth.

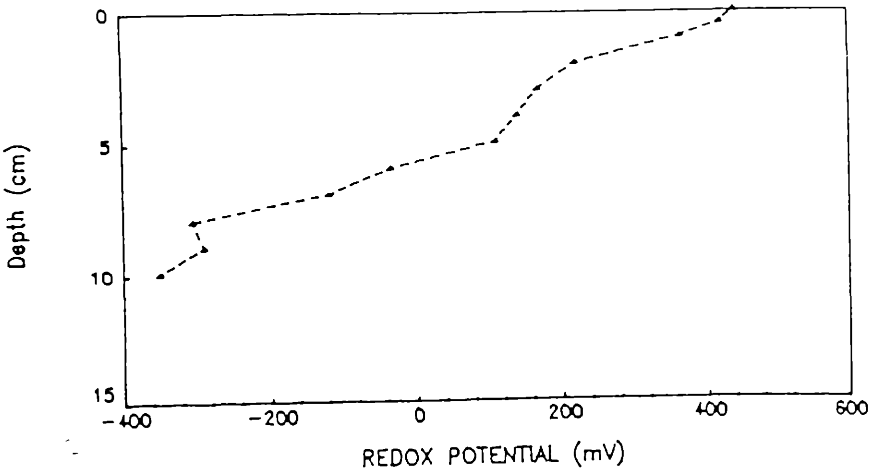


Figure 5.11 : Benthic redox profiles at Waharoa on the Waitoa River sampled on 5 March 1982, for fine (*——*) and coarse (Δ----Δ) profiles. Fine profile blackened at 2 cm (data not shown as probe broke), whereas the coarse profile blackens at 5 cm.

Station and Date	Depth cm	Total Carbon gm^{-2}	Biomass Carbon $mg\ m^{-2}$	Autotrophic Carbon $mg\ m^{-2}$	Biomass C/ Total C %	Hetero C/ Biomass %
LANDSDOWNE ROAD 8 Mar 82						
- fine	0-1	9.36	600.0	314.1	6.4	48
	1-2	10.53	345.0	192.8	3.3	44
	2-3	5.04	335.0	189.9	6.6	43
	5-6	4.83	375.0	745.8	7.8	0
	9-10	12.06	255.0	195.2	2.1	23
- coarse	0-1	10.61	540.0	205.0	5.1	62
	1-2	11.31	612.5	503.4	5.4	18
	2-3	10.08	455.0	295.7	4.5	35
	5-6	3.93	142.5	46.1	3.6	68
	9-10	6.62	162.5	68.3	2.5	58
WAHAROA 5 Mar 82						
- fine	0-1	11.07	1047.5	-	9.5	-
	1-2	6.54	1490.0	-	22.8	-
	2-3	4.87	1110.0	-	22.6	-
	5-6	4.66	185.0	-	4.0	-
	9-10	5.42	210.0	-	3.9	-
- coarse	0-1	18.24	3297.5	-	18.1	-
	1-2	10.43	1962.5	-	18.8	-
	2-3	15.12	1345.0	-	8.9	-
	5-6	11.67	182.5	-	1.6	-
	9-10	22.11	75.0	-	0.33	-

TABLE 5.7 : Sediment depth profiles of microbial biomass and organic carbon measurements in the Waitoa River.

Station, Date	Depth cm	Total Carbon gm^{-2}	Total Nitrogen gm^{-2}	Carbon/ Nitrogen	Total Protein gm^{-2}	Total Carbohydrate gm^{-2}	Protein/ Carbohydrate
LANDSDOWNE ROAD 8 Mar 1982							
- fine	0-1	9.3	0.95	9.8	25.7	6.6	3.9
	1-2	10.5	0.91	11.5	25.9	5.0	5.2
	2-3	5.0	0.71	7.0	25.9	4.0	6.5
	6-6	4.8	0.95	5.1	32.6	5.0	6.5
	9-10	12.1	0.77	15.7	35.5	5.2	6.8
- coarse	0-1	10.6	1.52	7.0	48.3	6.6	7.3
	1-2	11.3	1.34	8.4	49.6	7.2	6.9
	2-3	10.1	1.37	7.4	48.6	8.3	5.9
	5-6	3.9	0.69	5.7	34.6	7.7	4.5
	9-10	6.6	0.82	8.0	-	8.8	-
WAHAROA 5 Mar 1982							
- fine	0-1	11.1	1.91	5.8	39.1	5.3	7.4
	1-2	6.5	1.48	4.4	15.6	4.0	3.9
	2-3	4.8	1.32	3.6	21.6	4.3	5.0
	5-6	4.7	0.96	4.9	23.5	5.8	4.1
	9-10	5.4	1.14	4.7	25.0	5.4	4.6
- coarse	0-1	18.2	3.43	5.3	46.5	11.0	4.2
	1-2	10.4	1.79	5.8	47.4	9.4	5.0
	2-3	15.1	1.57	9.6	28.4	7.7	3.7
	5-6	11.7	1.40	8.4	36.4	7.1	5.1
	9-10	22.1	3.39	6.5	95.4	13.6	7.0

TABLE 5.8 : Sediment depth profiles of carbon, nitrogen, protein and carbohydrate measurements in the Waitoa River depth (Fig. 5.13). Surface values were higher than those at Landsdowne Road (5.5-fold).

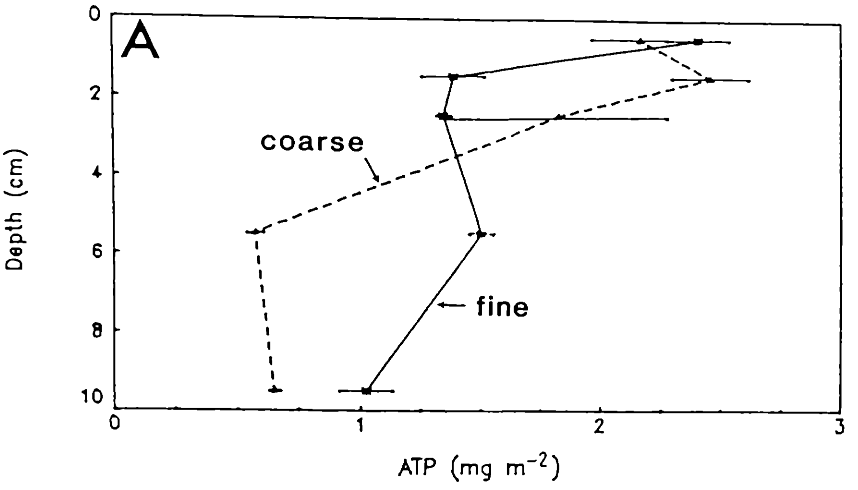
Benthic microbial biomass and total carbon measurements are shown in Table 5.17. At Landsdowne Road the microbial biomass constituted 5 - 6% of the total carbon, decreasing to 2 - 2.5% at 10 cm depth. Heterotrophic biomass varied from 0 to 68% of the total biomass estimate. At Waharoa the biomass carbon increased approximately 2-fold to 1 - 2 cm to peak at 22.8%. Levels declined rapidly to 10 cm.

The characteristics of the benthic carbon material present was investigated using nitrogen, protein and carbohydrate measurements shown in Table 5.8. Differences in C/N and protein/carbohydrate ratios were minimal between both sediment types and the 2 sampling stations. A consistent difference in composition occurred, however; between the fine particulate 0 - 1 cm and 1 - 2 cm layers for each station.

Intrinsic activity. Intrinsic activity at both Landsdowne Road (Fig. 5.14) and Waharoa (Fig. 5.15) was greatest for the coarse particulates. The decline in activity to 3 cm depth was minimal at Landsdowne Road; however, at Waharoa showed a rapid decline from surface values which were approximately 32-fold higher than upstream. Intrinsic activity increased at 5 - 6 cm depth at Waharoa (Fig. 5.15) caused by chemical oxygen demand of the hydrogen sulphide present. This led to poisoning of the electrode and restricted further measurements.

Galactosidase and glucosidase activity. No glycosidase activity was detected for any of the samples taken from Landsdowne Road. At Waharoa (Fig. 5.16) galactosidase activity was greater than glucosidase with each showing distinct maxima for the coarse particulates. All activities decline rapidly with depth with 0 activity below 5 - 6 cm depth. This suggests that the maximum depth sediment penetration from the overlying flow was 5 - 6 cm, inducing galactosidase production in response to lactose from the dairy effluent.

Bis-phosphatase and alkaline phosphates activity. Both enzyme activities declined from comparable fine and coarse particulate surface values at Landsdowne Road (Fig. 5.17), although each showed a sub-surface peak at 5 - 6 cm in the fine particulates. This corresponds



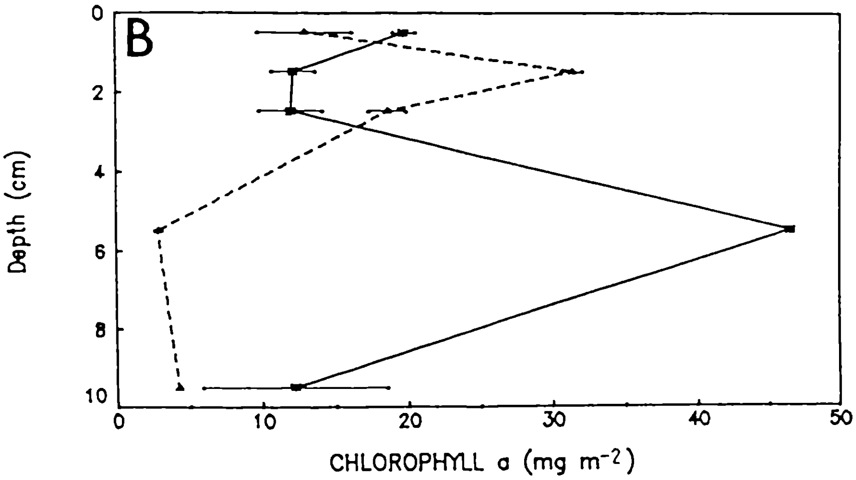


Figure 5.12 : Sediment depth profiles of benthic biomass at Landsdowne Road on the Waitoa River, for ATP (A) and chlorophyll a determinations (B). Symbols as for Fig. 5.10.

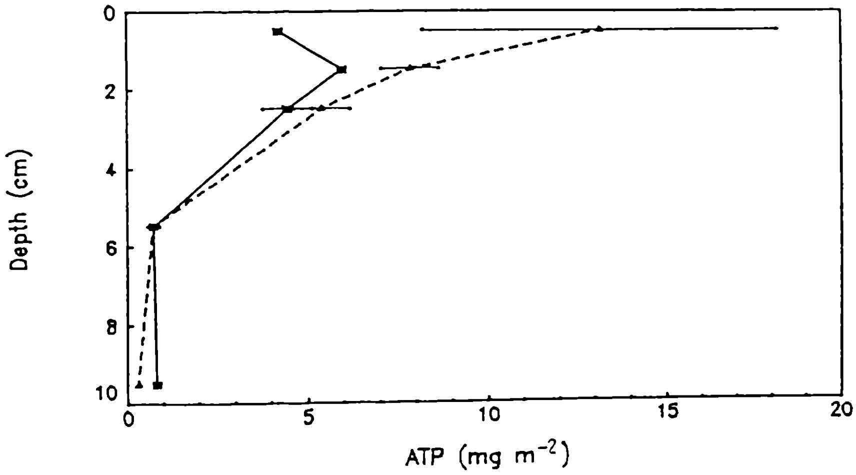


Figure 5.13 : Sediment depth profiles of benthic biomass at Waharoa on the Waitoa River, for ATP determinations. Symbols as for Fig. 5.11.

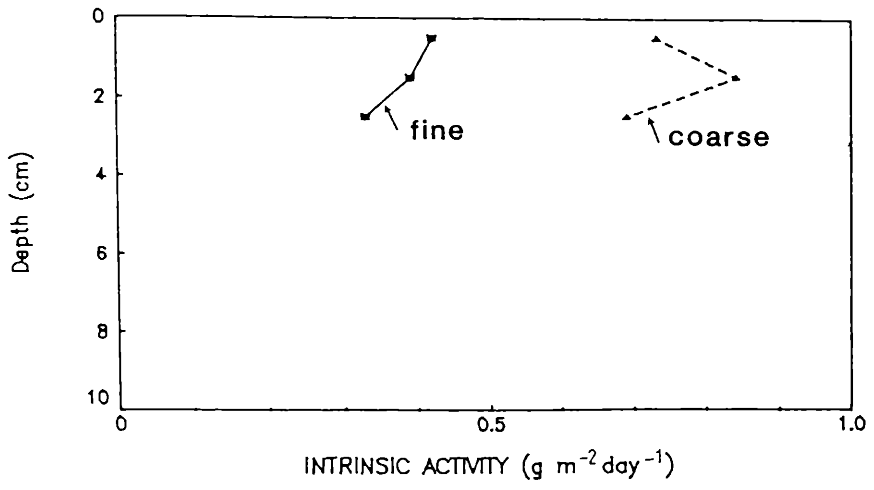


Figure 5.14 : Sediment depth profiles of intrinsic activity at Landsdowne Road on the Waitoa River. Symbols as for Fig. 5.10.

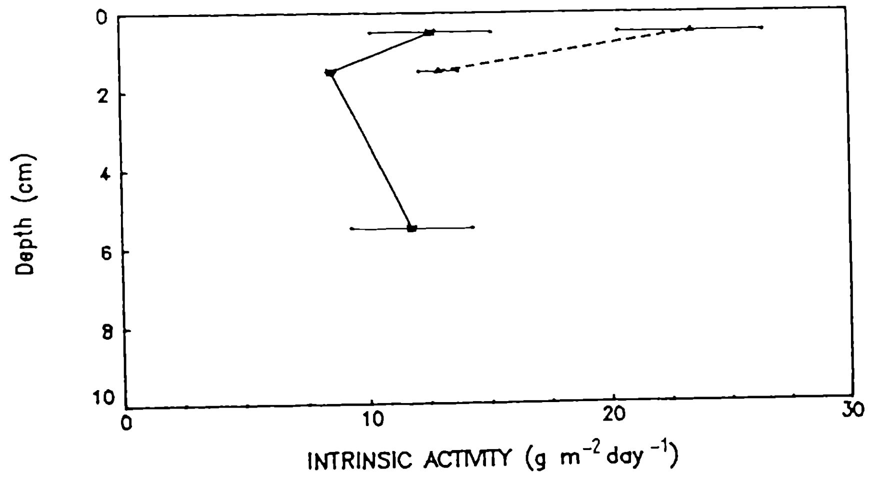


Figure 5.15 : Sediment depth profiles of intrinsic activity at Waharoa on the Waitoa River. Symbols as for Fig. 5.11.

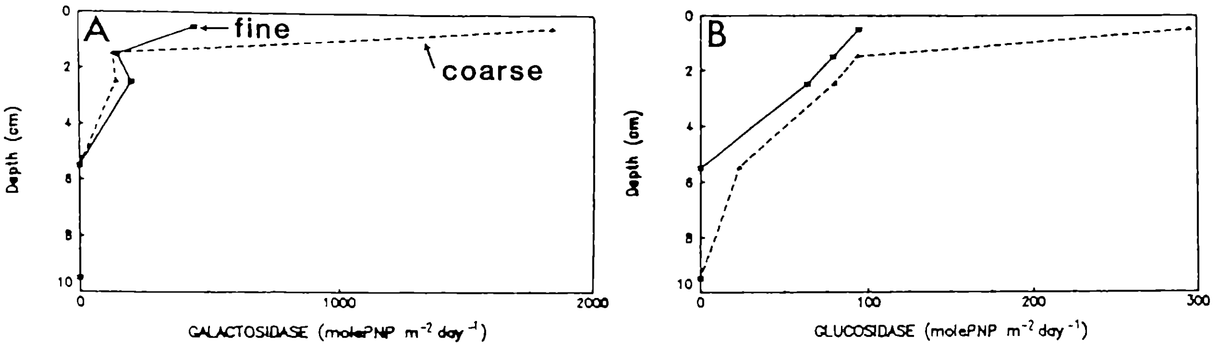


Figure 5.16 : Sediment depth profiles of galactosidase (A) and glucosidase (B) activity at Waharoa on the Waitoa River. Symbols as for Fig. 5.11.

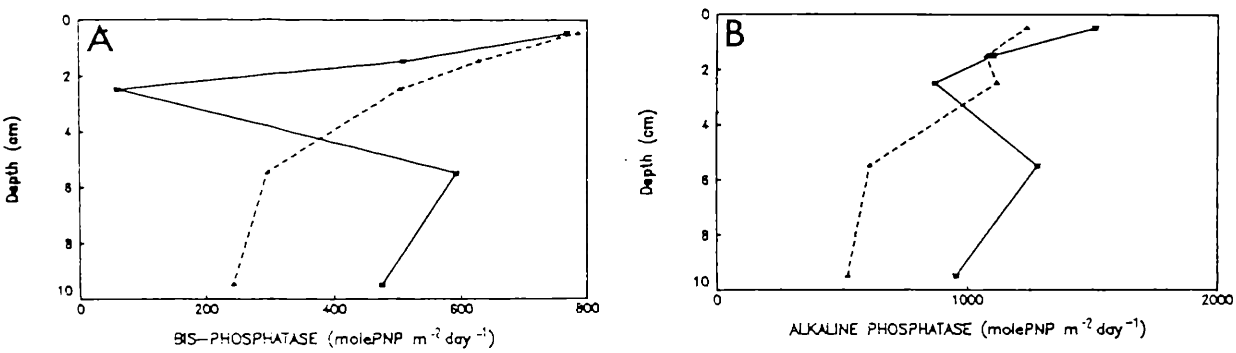


Figure 5.17 : Sediment depth profiles of bis-phosphatase (A) and alkaline phosphatase (B) activity at Landsdowne Road on the Waitoa River. Symbols as for Fig. 5.10.

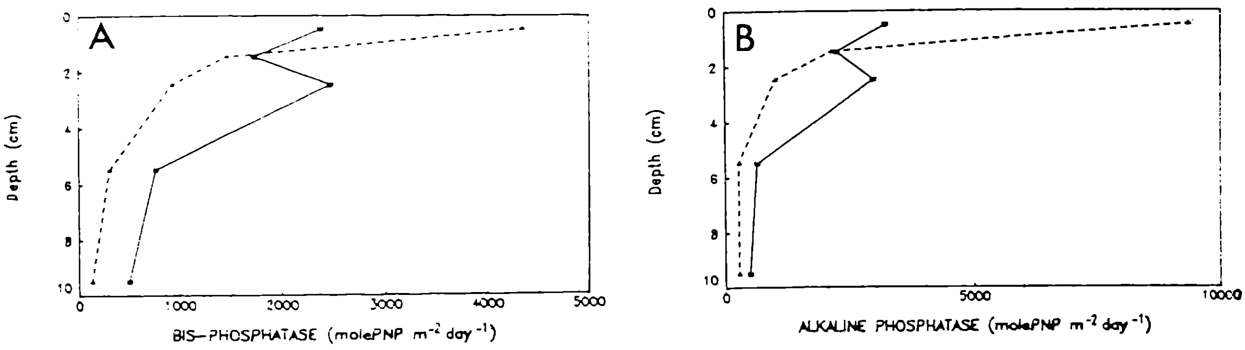


Figure 5.18 : Sediment depth profiles of bis-phosphatase (A) and alkaline phosphatase (B) activity at Waharoa on the Waitoa River. Symbols as for Fig. 5.11.

with a chl a peak measurement (Fig. 5.12B). At Waharoa (Fig. 5.18), peak activities occurred on the coarse particulates, with all activities declining sharply to 5 - 6 cm depth and then showing little further decline. A sub-surface activity peak occurs for each of the enzymes at 2 - 3 cm depth in the fine particulates. This corresponds with the redox discontinuity (Fig. 5.11).

Biomass specific activities

Galactosidase and glucosidase biomass specific activities. Values obtained for Waharoa depth profiles (Fig. 5.19) showed comparable surface activities in relation to bed particulates for each of the enzymes. Activities declined rapidly with depth for galactosidase, with a small sub-surface peak at 3 - 4 cm depth (Fig. 5.19A). Activities for glucosidase declined to 0 by 5 - 6 cm in the fine, with coarse particulates showing peak activity at 5 - 6 cm depth and 0 at 10 cm.

Bis-phosphatase and alkaline phosphatase biomass specific activities. At Landsdowne Road both enzyme activities showed a general increase with increasing depth and minimal differences betweeen particulates (Fig. 5.20). At Waharoa both enzyme activity values decreased slightly sub-surface, then increased with increasing depth (Fig. 5.21). Bis-phosphatase specific activity showed greatest activity in the fine particulates with a maximum value at 5 - 6 cm depth (Fig 5.21A). Alkaline phosphatase specific activity showed greater sub-surface values for the fine particulates.

5.2.8 Apparent production rates for planktonic microbial biomass and benthic carbon turnover

This section uses results from planktonic ATP measurements obtained for two particle size fractions to estimate apparent microbial production and decay rates for the river segments between sampling stations. Additionally, benthic biomass and total carbon measurements are presented, together with sediment respiratory decay kinetics measurements, which provide estimates of the rates of benthic carbon turnover, and hence the benthic response time to effluent cessation.

Apparent production rates for planktonic microbial biomass. ATP biomass measurements for differentially filtered size fractions, and the

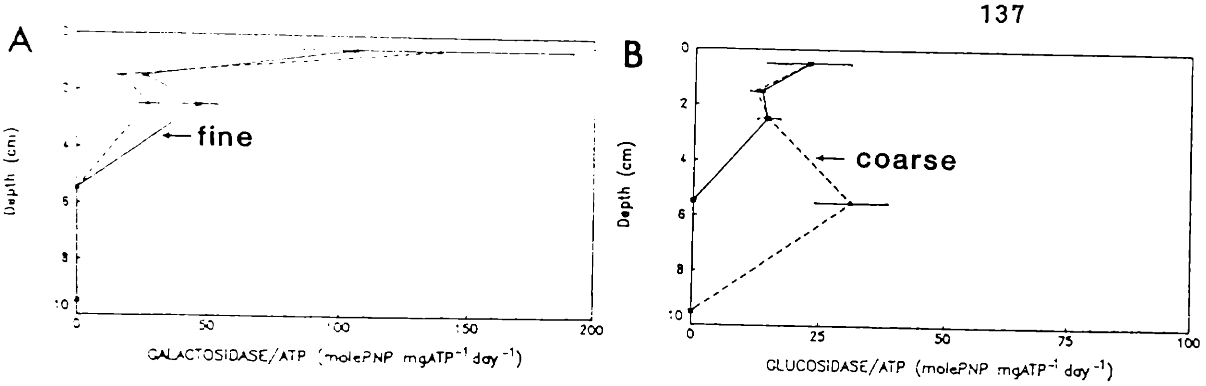


Figure 5.19 : Sediment depth profiles of galactosidase (A) and glucosidase (B) biomass specific activity at Waharoa on the Waitoa River. Symbols as for Fig. 5.11.

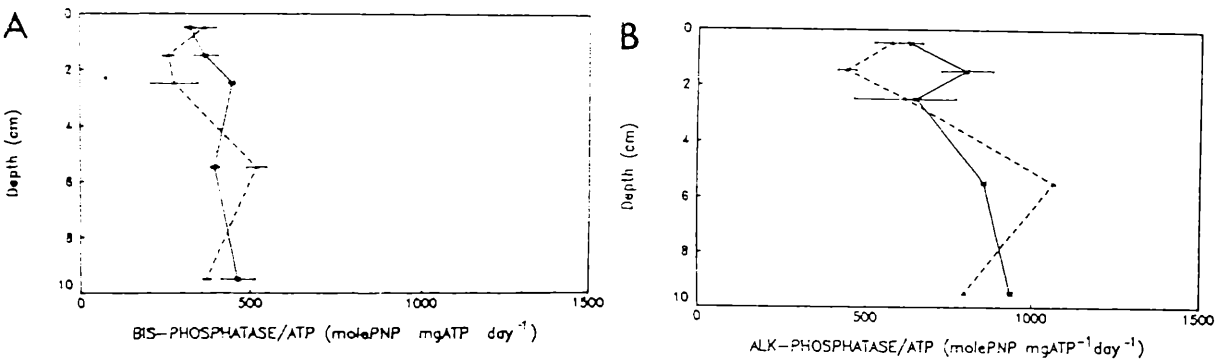


Figure 5.20 : Sediment depth profiles of bis-phosphatase (A) and alkaline phosphatase (B) biomass specific activity at Landsdowne Road on the Waitoa River. Symbols as for Fig. 5.10.

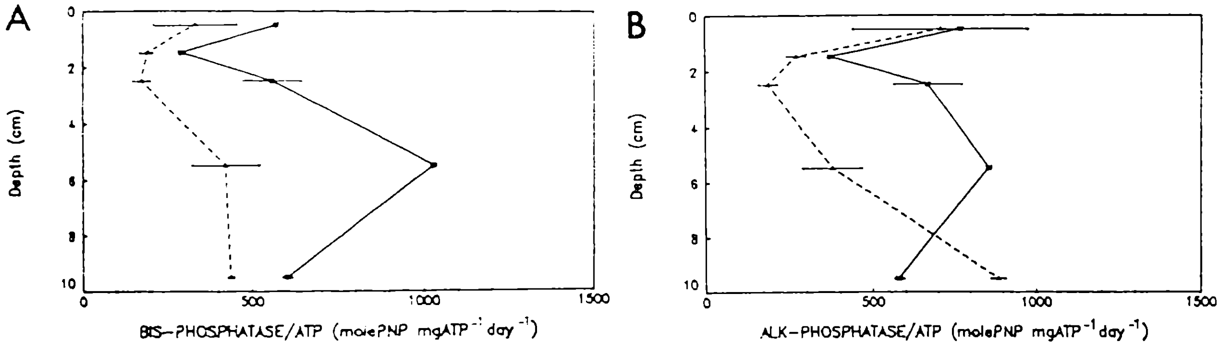


Figure 5.21 : Sediment depth profiles of bis-phosphatase (A) and alkaline phosphatase (B) biomass specific activity at Waharoa on the Waitoa River. Symbols as for Fig. 5.10.

				<30 µm			>30 µm			
Date	Station km	Travel Time[a] h	Total Biomass mg m^{-3}	Biomass mg m^{-3}	Production[b] Rate mg m^{-3} h^{-1}	Growth Rate[c] h^{-1}	Biomass[d] mg m^{-3}	Production[b] Rate mg m^{-3} h^{-1}	Growth Rate[c] h^{-1}	Areal Production Rate mg m^{-2} h^{-}
11 Jan 82[e]	-1		805.0	275.0			530.0			
		-			-	-		-		-
	0		542.5	502.5			40.0			
		1.52			115.1	0.20		217.1	1.46	73.8
	2		1047.5	677.5			370.0			
		2.13			-180.8	-0.39		112.7	0.23	38.3
	5		902.5	292.5			610.0			
		4.17			1.8	0.01		-100.8	-0.28	-34.3
	10	[7.82]	492.5	300.0			192.5			
27 Jan 82[f]	-1		-	-			-			
		-			-	-		-		-
	0		902.5	765.0			137.5			
		2.46			76.2	0.09		126.0	2.00	42.8
	2		1400.0	952.5			447.5			
		3.44			300.2	0.21		117.7	0.19	40.0
	5		2837.5	1985.0			852.5			
		6.76			-193.8	-0.16		-126.1	-1.00	-55.7
	10	[12.66]	420.0	675.0			0			
10 Feb 82[g]	-1		195.8	150.3			45.5			
		-			-	-		-		-
	0		128.3	105.0			23.3			
		2.92			60.4	0.34		243.3	1.08	82.7*
	2		1015.0	281.3			733.7			
		4.09			59.1	0.15		140.3	0.14	47.7
	5		1830.3	522.8			1307.5			
		8.04			-43.8	-0.13		-159.9	-0.51	-54.3
	10	[15.05]	199.5	177.5			22.0			

a Based on the travel time measured by Wilcock (1984) using dye tracer and scaled using calculated mean velocities from the gauging station at Landsdowne Road. A tributary dilution contributing approximately 30% to the flow occurs between the 5 and 10 km stations, for which no allowance is made in these calculations

b Production calculated for linear production rate using Eqn.3.22, $\mu' = \frac{X_B - X_0}{t}$

c Production calculated for exponential growth using Eqn.3.20, $\mu = \frac{\ln X_B - \ln X_0}{t}$

d $\Delta X_{>30\mu m} = X_{TOT} - X_{<30\mu m}$ Eqn. 3.22

e Discharge (Q) = 0.58 m ^{3}s^{-1}, Mean velicity (U) = 0.37 m s^{-1}, Mean depth ($\bar{H}$) = 0.34 m

f Q = 0.33 m^{3}s^{-1}, U = 0.19 m s^{-1}, H = 0.34 m

g Q = 0.27 m^{3}s^{-1}, U = 0.16 m s^{-1}, H = 0.34 m

* maximum calculated areal production rate

[] total travel time

Table 5.9: Apparent microbial production rates for planktonic biomass in the Waitoa River

river travel time between sampling stations were used for calculation of apparent production rates. Sampling during winter months, when no dairy factory effluents were present, showed no significant differences between biomass size fractions or between stations ($P>0.2$), with >90% present as <30 µm (results not shown). Calculated apparent microbial production rates are shown for 3 successive summer surveys during the low flow period (11 January-10 February 1982) in Table 5.9. The surveys showed that production of large particulate biomass (i.e. >30 µm) always exceeded small particulate biomass (i.e. <30 µm) (by up to 5.7-times) adjacent to the discharge (0 km-2 km), then declined in the following reach (2 km-5 km). A dramatic decline in both planktonic biomass size fractions occurred between 5 km and 10 km for all surveys with a generally greater biomass decline for the >30 µm fraction. The calculated rates of decline between 5 km and 10 km often exceeded the production rate in upstream sections.

Because the phototrophic biomass component was relatively low for summer surveys (eg Fig. 5.3), calculation on the heterotrophic basis only, makes little difference to the production rate calculations (results not shown).

Maximum large particulate biomass production amounted to 2.0 g C m^{-2} day^{-1} with an average of about 1.3 g C m^{-2} day^{-1} (Table 5.12).

Benthic biomass and carbon. Microbial biomass carbon and total carbon measurements for 3 longitudinal benthic surveys are shown in Table 5.10. The spring survey showed a low upstream total carbon, which increased (7.5-fold) to 10 km downstream. Biomass % carbon ranged from 6.4% of the total upstream, to a maximum of 8.9% at 5 km, then decreasing to 2.9% at 10 km. The survey after the summer flood showed a higher upstream carbon level higher than the spring survey (by 10.9-fold), further increasing downstream (by 1.3-fold) to peak at 10 km. Biomass carbon ranged from 1.1% of the total carbon upstream, to a maximum of 12.1% at 2 km, then decreased to 1.4% by 10 km. Carbon measurements only were made on 19 January 1982, and showed a substantial decline in upstream, 5 km and 10 km estimates (data shown in Table 5.12). The survey on 25 January 1982 showed an increase in upstream carbon levels compared with the previous survey, which was probably attributable to

Date	Station km	Total Carbon gm^{-2}	Biomass Carbon $mg\ m^{-2}$	Heterotrophic Biomass %	Biomass C/ Total C %	Hetero C/ Total C %
5 Nov 81	-1	2.63	167.9	-	6.4	-
	0	7.44	503.8	-	6.8	-
	2	19.90	800.9	-	4.2	-
	5	13.10	1169.6	-	8.9	-
	10	19.80	569.8	-	2.9	-
12 Jan 82	-1	28.70	324.7	51.8	1.1	0.57
	0	32.90	1741.8	94.0	5.3	5.0
	2	32.60	3962.9	97.1	12.1	11.8
	5	22.20	811.8	81.4	3.7	3.0
	10	36.50	524.8	83.6	1.4	1.2
25 Jan 82	-1	16.80	1465.9	0	8.7	0
	0	39.20	2867.6	88.4	7.3	6.5
	2	25.70	3636.6	56.0	14.1	7.9
	5	17.30	2887.7	60.1	16.7	10.0
	10	13.90	1023.0	13.0	7.3	0.95

Table 5.10 : Sediment microbial biomass and organic carbon measurements for longitudinal surveys of the Waitoa River.

Date	Station km	Total Carbon gm^{-2}	Total Nitrogen gm^{-2}	Carbon/ Nitrogen	Total Protein gm^{-2}	Total Carbohydrate gm^{-2}	Protein/ Carbohydrate
5 Nov 81	-1	2.6	1.1	2.5	24.0	6.8	3.5
	0	7.4	2.1	3.5	27.7	7.3	3.8
	2	19.9	4.3	4.4	49.3	10.2	4.8
	5	13.0	3.2	4.1	35.5	6.5	5.5
	10	19.8	3.0	6.7	37.6	11.5	3.3
12 Jan 82	-1	28.7	2.8	10.3	27.6	5.4	5.1
	0	32.9	4.3	7.6	31.2	8.9	3.5
	2	32.6	4.9	6.7	58.3	10.1	5.8
	5	22.2	3.3	6.8	27.6	6.2	4.5
	10	36.5	4.1	8.9	32.3	9.0	3.6
25 Jan 82	-1	16.9	2.8	6.0	32.4	12.8	2.5
	0	39.2	6.9	5.7	55.6	19.1	2.9
	2	25.7	5.2	5.0	43.8	13.2	3.3
	5	17.3	4.5	3.9	46.8	11.3	4.1
	10	13.9	4.1	3.4	53.3	10.0	5.3

TABLE 5.11 : Sediment carbon, nitrogen, protein and carbohydrate measurements for longitudinal surveys of the Waitoa River surveys.

algal development (see Fig. 5.1B). Carbon levels increased downstream of the outfall (by 2.3-fold), with only 83% of the upstream value present at 10 km. Biomass carbon comprised 8.7% upstream of the total at the point increasing to 16.7% at 5 km, and decreasing to 7.3% at 10 km.

An estimation of heterotrophic biomass present showed maximal values of 10% to 12% of the total carbon at stations below the discharge for each of the summer surveys.

Sediment depth profiles for fine and coarse particulates were performed upstream and downstream of the discharge with estimates of biomass and carbon shown in Table 5.10. All profiles showed greatest carbon concentrations at the surface decreasing to 6 cm and increasing to the 10 cm layer. For each of the upstream sediments percentage of biomass carbon was from 3% to 8% in the surface 6 cm, decreasing to 2% at 10 cm.

Below the discharge the coarse sediments showed a maximal surface percentage of biomass carbon of 18.1% which decreased abruptly to 1.6% at 6 cm. In contrast the fine sediments showed an approximate 2-fold increase in biomass carbon present in the 1-2 cm layer at 22.8%, which decrease to 4.0% at 6 cm.

Tables 5.8 and 5.11 show carbon to nitrogen ratios, and protein to carbohydrate ratios for depth profiles and 4 longitudinal surveys respectively. A longitudinal trend was only evident for the summer low flow survey (25 January 1982), with a decreasing downstream C:N ratio and an increasing protein/carbohydrate ratio (Table 5.11). No trends were evident to 10 cm sediment depth either upstream or below the discharge (Table 5.8).

Benthic carbon turnover and sediment respiratory decay. The benthic carbon turnover rate for the top 1 cm layer of sediments was calculated using intrinsic activity and total carbon measurements. Results obtained for longitudinal surveys are shown in Table 5.12. No distinct seasonal differences were evident between spring (5 November 1981) and summer (January 1982) surveys. Longitudinal trends varied between surveys, but generally showed a minimum value below the discharge, which increased to 10 km downstream. Both the maximum turnover time of 52.1

Date	Station km	Total Carbon gm^{-2}	Intrinsic Activity $g\ O_2\ m^{-2}\ day^{-1}$	Carbon Turnover Time[a] day	Decay Coefficient k day^{-1}	Respiratory half-life[b] day
5 Nov 81	-1	2.63	1.42	4.9	-	-
	0	7.44	3.11	6.4	-	-
	2	19.90	6.27	17.1	-	-
	5	13.10	4.04	8.7	-	-
	10	19.80	2.80	18.9	-	-
12 Jan 82	-1	28.70	1.47	52.1	-	-
	0	32.90	8.18	10.7	-	-
	2	32.60	12.78	6.8	-	-
	5	22.20	10.34	5.7	-	-
	10	36.50	11.28	8.6	-	-
19 Jan 82[c]	-1	6.07	-	-	-	-
	0	43.89	14.54	8.1	0.20	3.5
	2	26.50	6.05	11.7	0.15	4.6
	5	14.48	4.73	8.2	0.10	6.9
	10	16.76	4.24	10.5	0.10	6.9
25 Jan 82	-1	16.80	2.28	19.7	-	-
	0	39.20	18.92	5.5	0.10	6.9
	2	25.70	12.07	5.7	0.125	5.5
	5	17.30	7.07	6.5	0.25	2.8
	10	13.90	3.56	10.4	0.10	6.9

[a] Calculated as $\frac{\text{Total Carbon}}{\text{Intrinsic Activity x 0.375*}}$

[b] $T_{½} = \frac{\ln 2}{k}$

[c] Intrinsic activity measurements obtained from EBOD measurements

*value obtained from stoichiometry ($C:O_2$)

TABLE 5.12: Sediment carbon turnover rate and respiratory decay measurements for the Waitoa River.

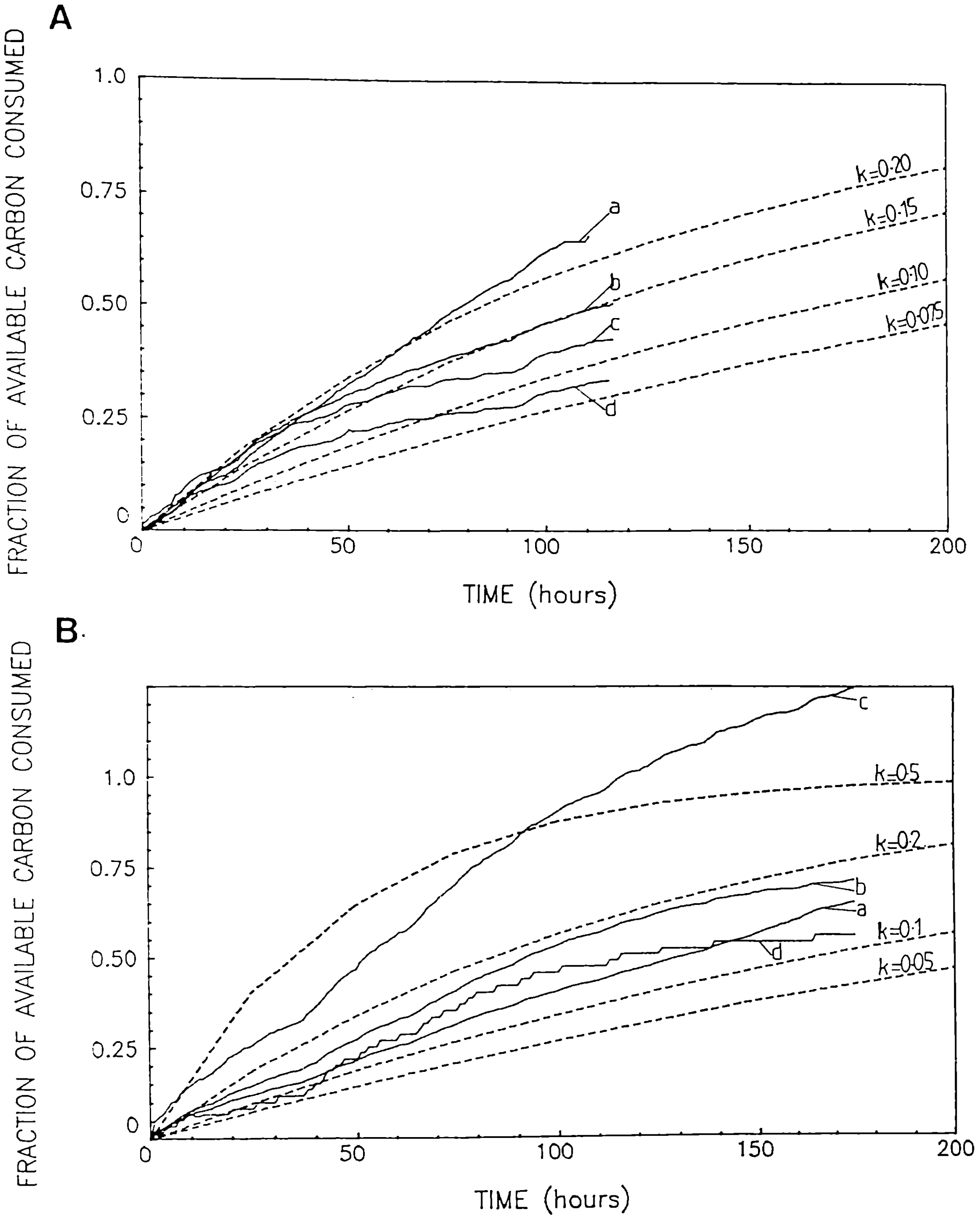


Figure 5.22 : Sediment respiratory decay measurements for longitudinal surveys of the Waitoa River, sampled on 19 January 1982 (A) and 25 January 1982 (B), assuming 75% available carbon.

Key: (distance below discharge)

a	Waharoa (0.1 km)
b	Hockley (2 km)
c	Signal (5 km)
d	Walton Bridge (10 km)

Dashed line denotes first order decay curves with coefficient (k) shown.

days and the minimum value of 4.9 days were obtained for the upstream Landsdowne Road Station.

The sediment respiratory decay rate for the top 1 cm layers was determined using the electrolytic respirometer system (see Methods section 3.5.2.3). Results obtained for the 2 longitudinal surveys are shown in Figure 5.22, together with first order decay model approximations. Respiration measurements were converted to carbon equivalents and the percentage assimilation calculated relative to a total carbon measurements. For all data shown in Figure 5.22 the biodegradable or 'available' carbon present was taken as 75% of the total carbon, as this generally resulted in a better approximation to first order decay. Measurements for the first longitudinal survey (19 January 1982, Fig. 5.22A) showed comparable decay coefficients initially (<20h) but then diverged. The single first order decay coefficients generally showed a poor overall fit. Measurements for a later longitudinal survey (25 January 1982, Fig. 5.25B) also showed multiple component decay kinetics would be more appropriate for some stations. The greatest biodegradability occurred at the 5 km downstream station.

The decay coefficient were used to calculate respiratory hall-lives for the surface sediments, which ranged from 2.8 to 6.9 (Table 5.12).

5.2.9 Dissolved oxygen and BOD measurements

This section summarises the diurnal oxygen monitoring data for Landsdowne Road and longitudinal studies below the dairy discharge. BOD monitoring comprised: (a) single samples taken during longitudinal surveys; (b) a diurnal survey; (c) biodegradability; and (d) differential pre-filtration studies.

Diurnal BOD loading survey - 14/15 December 1981. A 24-hour monitoring of river BOD_5 and COD concentrations at Waharoa (0 km) was undertaken, together with single samplings at downstream stations, and simultaneous continuous DO and temperature monitoring at two stations, 2 km and 5 km below the outfall. Results are shown in Figure 5.23, with BOD_5 ranging from 5 g m^{-3} to 8 g m^{-3} from 1400 to 0200 h when BOD_5 increased to 30 g m^{-3}. Effluent quality varied greatly over the 24 h period with COD/BOD ratios ranging from 1.5 to 50. (Appendix 2). DO monitoring at the 2 km station showed a mean DO of approximately 6.8 g m^{-3} with a diurnal amplitude of variation of 1.2 g m^{-3}. Mean DO at the 5 km station was relatively constant at approximately 6 g m^{-3}. The large BOD increase resulted in a small deficit at 2 km increasing to 1.2 g m^{-3} at 5 km (Fig. 5.23).

Single downstream measurements of BOD_5 and DO showed a minimum DO of 5.1 g m^{-3} occurred at 5 km, and that BOD had declined to 2.2 g m^{-3} by 10 km downstream (Table 5.13). A calculated travel time showed downstream BOD_5 was related to a period with approximately 30 g m^{-3} of BOD_5 at Waharoa (0 km).

Summer 81/82 monitoring. Details of temperature and DO concentration measurements taken during the 3 intensive longitudinal surveys are shown in Table 5.14. Minimum DO (% saturation) values occurred during the spring (5 November 1981) survey. In the period following the summer flood (12 January 1982), the maximum deficit increased and occurred further upstream. Such random sampling may provide a poor indication of the river DO status because of diurnal fluctuations (see following sections).

Because of the variable organic load in the effluent discharge, single samples taken for BOD_5 determinations were of limited use for defining ambient concentrations. All BOD measurements made during this study are presented in Appendix 2 with those taken during January and February 1982 summarised in Table 5.15. Although BOD_5 values were generally low upstream, and increased below the discharge, a high variability limited the detection of longitudinal trends below the discharge.

Biodegradibility and differential filtration of river BOD. River measurements of BOD_5, BOD_{20}, and COD on whole and pre-filtered river water are presented in Appendix 2.

$BOD_{20/5}$ ratio for Landsdowne Road ranged from 1.67 to 3.70 (mean 2.68, n=5) which was not significantly different from values obtained from stations below the discharge which ranged from 1.18 to 4.31 (mean 2.19, n=11) ($P>0.2$).

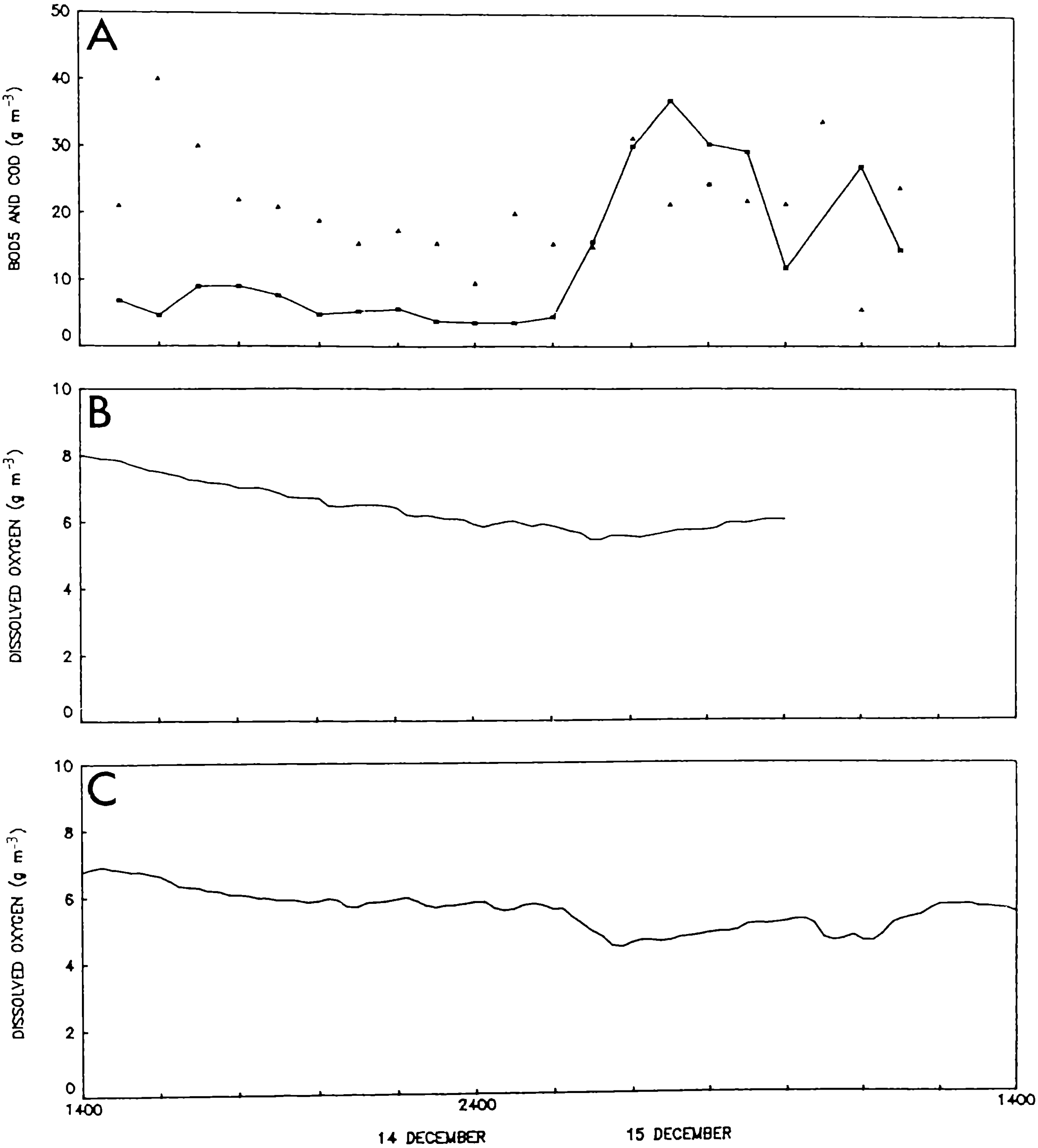


Figure 5.23 : Diurnal BOD$_5$, COD and oxygen monitoring in the Waitoa River for 14-15 December 1981. Discharge was 1.26 m^3 s^{-1}.
A BOD$_5$ (*-*) and COD (Δ) monitored at Waharoa (0 km).
B DO monitored at Hockley (2 km) adjusted for travel time.
C DO monitored at Signal (5 km) adjusted for travel time.

Station (km)	Travel Time h	Sampling Time	Temperature °C	Dissolved Oxygen gm^{-3}	Saturation %	BOD_5 gm^{-3}
Landsdowne Road (-1)	-	10:37	18.5	8.50	91	0.95
Waharoa (0)	0	10:18	19.0	8.10	87	30†
Hockley (2)	0.77	9:56	20.5	6.40	71	-
Signal (5)	1.85	~9:30	20.0	5.10	56	5.60
Walton Bridge (10)	3.97	9:17	20.0	6.30	69	2.20

Discharge 1.14 m^3s^{-1} with calculate velocity of 0.72 ms^{-1}
†Measured from BOD monitoring at Waharoa (Figure 5.23) allowing for travel time to Walton Bridge

TABLE 5.13 : BOD_5 measurements a longitudinal survey in the Waitoa River associated with a diurnal monitoring.

Date	Discharge m^3s^{-1}	Station km	Temperature °C	Dissolved Oxygen gm^{-3}	Saturation %
5 Nov 1981	0.84	-1	16.5	9.50	97
		0	20.8	8.60	96
		2	20.0	5.90	65
		5	19.0	3.20	35
		10	17.0	5.40	56
12 Jan 1982	0.51	-1	23.0	9.95	116
		0	26.0	8.50	105
		2	28.0	4.60	59
		5	30.0	3.60	48
		10	27.5	6.60	84
25 Jan 1982	0.42	-1	17.0	9.30	96
		0	20.0	8.10	89
		2	20.0	5.80	64
		5	20.5	6.10	68
		10	18.0	7.30	77

TABLE 5.14 : Planktonic DO measurements for longitudinal surveys of sediments in the Waitoa River.

Station (km)	BOD_5 gm^{-3}			
	Range	Mean	Standard Deviation	Number
Landsdowne Road (-1)	0.74-2.10	1.14	0.71	4
Waharoa (0)	3.80-20.90	11.18	7.59	4
Hockley (2)	4.70-27.90	13.37	12.66	3
Signal (5)	6.40-13.3	10.20	3.50	3
Walton Bridge (10)	2.80-8.7	4.30	2.93	4

TABLE 5.15: Summary of BOD_5 measurements for longitudinal Waitoa River surveys: January/February 1982

Date	Station km	BOD_5 gm^{-3}	$FBOD_5$ gm^{-3}	FBOD/BOD %	Temperature °C	DO gm^{-3}
12 Oct 1982[a]	-1	2.0	1.1	55	13.5	10.3
	0	28.9	28.2	98	15.5	9.8
	2	28.8	11.6	40	16.0	4.6
	5	25.6	18.6	73	16.5	3.8
	10	3.6	2.1	58	14.8	4.6
10 Nov 1983[b]	-1	1.2	0.8	67	19.8	9.5
	0	>80	38.8	49	24.0	8.3
	2	15.8	7.4	47	24.5	1.7
	5	9.6	4.7	49	22.5	2.4
	10	4.2	1.6	38	20.5	3.5

[a] flow 0.47 m^3 s^{-1}

[b] flow ~0.5 m^3 s^{-1}

TABLE 5.16: Differential filtration and BOD_5 measurements on the Waitoa River

A wide range in biodegradability was also suggested by comparison of filtered COD and BOD_5 determinations (Fig. 5.23A). Upstream measurements tended towards a high refractory carbon component (ie high COD/BOD ratio) whereas other stations, especially those immediately below the outfall showed a widely varying range. (Appendix 2).

Filtered COD accounted for 80% of the total COD at Landsdowne Road (r^2 = 0.969, n = 36). (Appendix 2).

Differential filtration (using GF/C filters) prior to BOD_5 determination was performed for 2 longitudinal surveys (Table 5.16). A dry spring preceded the 82/83 summer season resulted in October and November river flow and benthic biomass conditions visibly resembling the January/February period of the previous summer. Pre-filtration showed that particulate and soluble BOD contributed approximately equal proportions upstream values, whereas effluent loading was composed of 50% to 100% soluble BOD.

Seasonal variation in river oxygen metabolism. A flow hydrograph record for the 1981/82 summer season is shown in Fig. 5.24. Diurnal oxygen measurements obtained from the Waitoa River and a number of other New Zealand rivers studied, are plotted on a lotic metabolism diagram (Fig. 5.25, after Servais et al., 1984), with details provided in Table 5.17. Data obtained from the Waitoa River at the Landsdowne Road station above major point source influences (W series on Fig. 5.25), showed that the trophic state and oxygen characteristics of the river change seasonally. The increased organic loading below the discharge, results in large oxygen deficits as measured at 10 km downstream (WW on Fig. 5.25).

5.2.10 Discussion and Conclusions

The research work described in this chapter attempted to measure the range of BUR and determine the relative importance of planktonic aquatic and benthic biomass influencing the oxygen dynamics in a shallow river system, subjected to a point source organic discharge. Additionally, investigations sought to establish the type of biomass present, and how that biomass was influenced by the organic material in the overlying flow, river flow conditions, and other seasonal influences. Such measurements would contribute to the testing of the working hypotheses

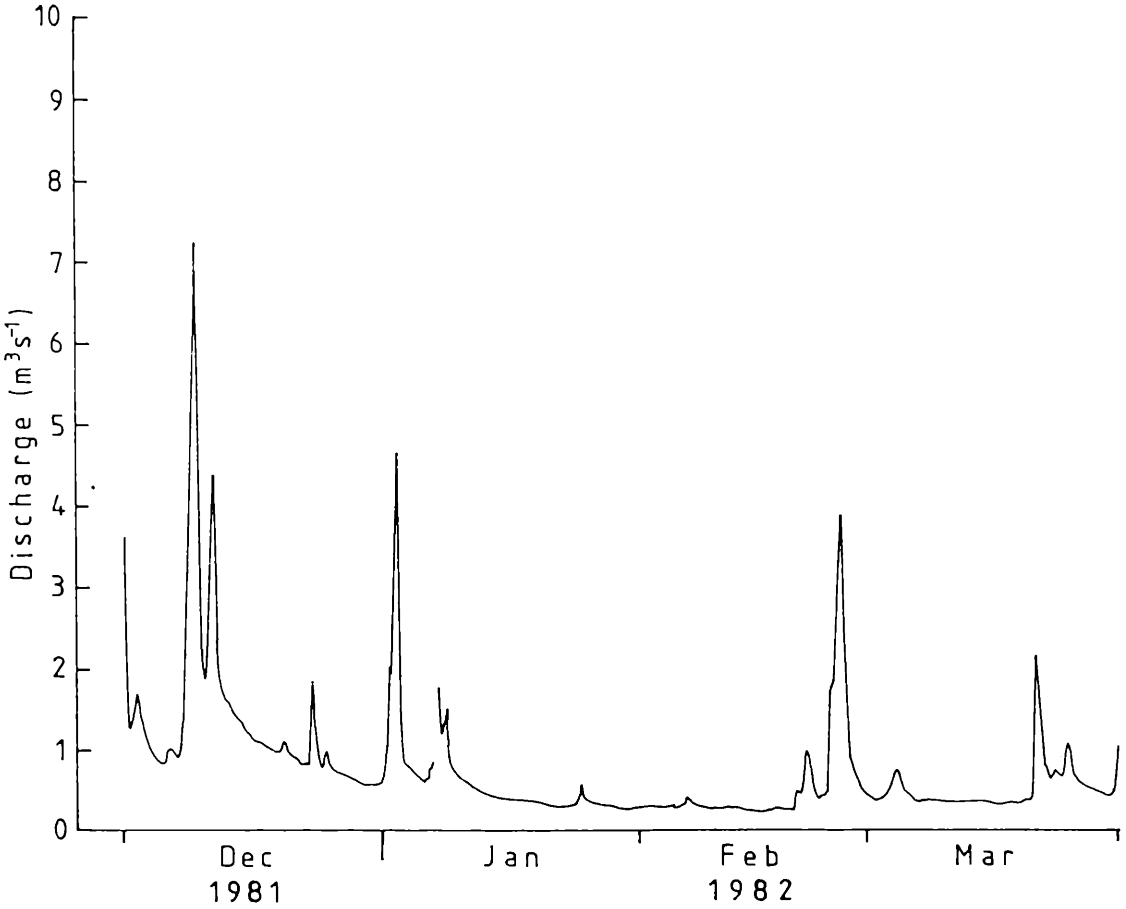


Figure 5.24 : Discharge hydrograph for the Waitoa River at Landsdowne Road for December 1981 to March 1982.

River	Sampled	Code	Flow $m^3 s^{-1}$	Depth m	Velocity $m s^{-1}$	k_2 day^{-1}	(method reference)	Gross Photosynthesis $gm^{-3}day^{-1}$	Respiration	P/R	Notes
Waitoa -Landsowne Rd	25 Jan 1982	W1	0.42	0.34	0.22	6.5	(1)	13.10	15.62	0.84	above discharge
	5 Mar 1982	W2	0.60	0.37	0.30	6.5	(1)	8.85	22.78	0.39	
	9 Jun 1982	W3	1.17	0.50	0.41	6.5	(1†)	0.0	11.38	-	
	14 Jul 1982	W4	1.64	0.63	0.51	6.5	(1†)	0.0	8.45	-	
	20 Oct 1982	W5	0.65	0.52	0.18	6.5	(1)	12.45	26.04	0.49	
	1 Nov 1982	W6	0.43	0.49	0.13	6.5	(1)	13.56	19.53	0.69	
	8 Dec 1982	W7	0.31	0.57	0.08	4.5	(1)	16.60	19.41	0.87	
	2 Feb 1983	W8	0.13	0.51	0.05	4.5	(1)	15.50	30.94	0.50	
Waitoa -Walton Br	5 Nov 1982	WW	0.56	0.40	0.19	11.0	(2)	17.57	93.98	0.19	10 km below discharge
Manawatu	26 Feb 1983	M1	15.1	0.53	0.48	6.4	(4)	8.32	6.17	1.35	above major discharges
Manawatu -Karere Rd	22 Feb 1983	M2	20.0	1.18	0.37	3.2	(2)	7.04	14.53	0.49	below all major discharges
Manawatu -Jackeytown Rd	22 Feb 1983	M3	20.0	1.18	0.37	3.2	(2)	9.23	18.19	0.51	2km below above site
Tarawera -Town Br	23 Nov 1983	T1	-	1.17	0.69	5.54	(3)	1.33	3.14	0.42	above discharge
Tarawera -SH30	23 Nov 1983	T2	-	1.17	0.69	5.54	(3)	1.47	13.05	0.11	3 km below Tasman discharge
Tarawera -Awakaponga	23 Nov 1983	T3	27.9	1.32	0.79	7.45	(3)	2.05	23.18	0.09	15km below Tasman discharge

References:

1 Calculated using night-time respiration after Servais et al (1984); †, k_2 equal to other values when absence of diurnal oxygen fluctuations prevents calculation.

2 Gas tracer method Wilcock (1984b).

3 Gas tracer method.

4 Calculated using O'Connor-Dobbins (1958) formula.

TABLE 5.17 River diurnal oxygen measurements : calculation of gross photosynthesis and respiration rates

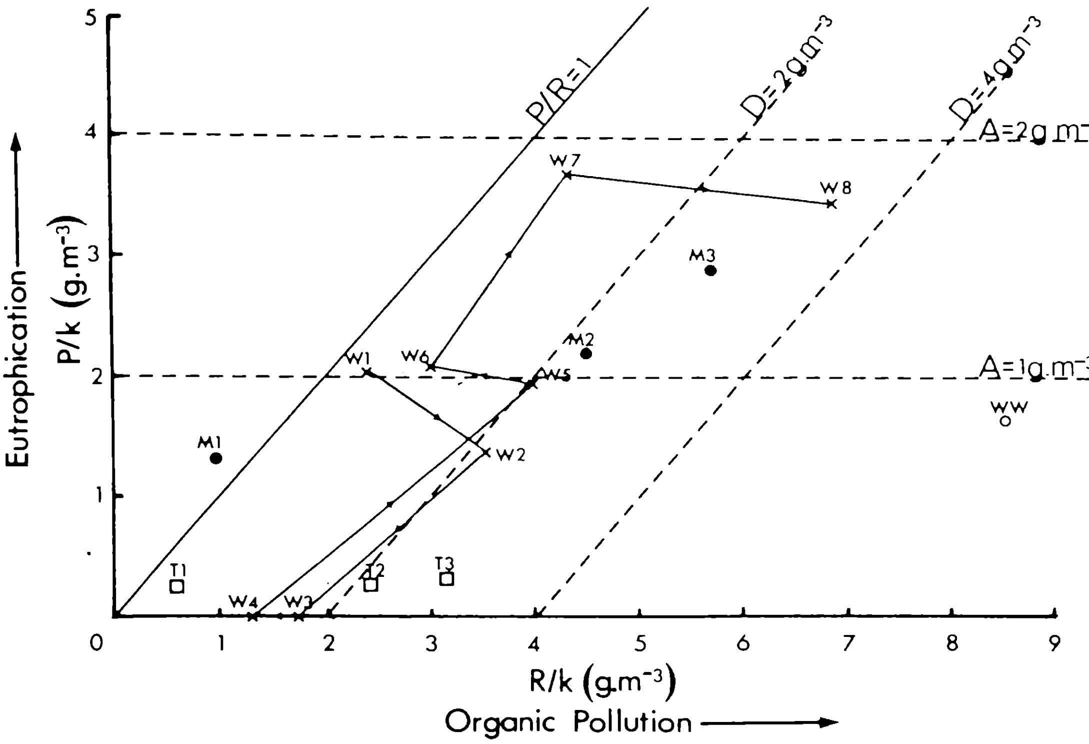


Figure 5.25 : Lotic metabolism diagram : photosynthesis and respiration reduced to the reaeration capacity. Diagonal lines on the diagram represent the position of rivers in relation to their mean oxygen deficit (D), with horizonal lines indicating amplitude (A) of diurnal variations (calculated using a β = 0.5 derived from a 12 hour day length and k_2 about 7 day^{-1} from Simonsen and Harremoës (1978)). Details of data and stations given in Table 5.17.
Symbols:
x W series Waitoa River above Waharoa dairy discharge
o WW Waitoa River 10 km below Waharoa dairy discharge
• M series Manawatu River
☐ T series Tarawera River.

stated in Chapter 1, by determining the benthic biomass and activity responses to environmental changes (ie the effluent), the nature of the energy source using specific enzyme and biomass measurements together with the factors influencing interaction between the benthos and the overlying flow.Interaction between the benthos and the overlying flow provides the basis for a conceptual model of the key components influencing mass transfer processes in shallow river systems.

BUR and sediment oxygen penetration. The maximum chamber BUR measurements in situ of 24.6 g O_2 m^{-2} day^{-1} (Table 5.1) and 70.2 g O_2 m^{-2} day^{-1} (Table 6.2) were associated with the presence of filamentous algae (Oedogonium sp) at the upstream station and filamentous 'sewage fungus' below the outfall, respectively. Comparison of mean BUR values for above and below the outfall showed no significant difference; however the development of heterotrophic biofilms downstream resulted in a 14-fold increase in benthic intrinsic activity (Table 5.2). This in turn resulted in a decrease in the inferred depth of benthic oxygen penetration from 13.5 cm upstream, to 1.4 cm below the discharge. (Table 5.2). Although the mean BUR value had decreased to 37% of the upstream value at 10 km downstream, a high intrinsic activity resulted in a 95% reduction in the depth of benthic oxygen penetration. The values estimated for oxygen penetration depths may well be less than in situ conditions if: (i) the agitation levels are greater than naturally present (i.e. I values overestimated); (ii) microbial activities decrease with depth. A consistent method has been used to allow comparison between measurements, however both of the above factors are generally likely to be significant (see sediment biomass profiles in figs. 5.10 and 5.11) and would warrent greater study in future investigations.

The wide range in BUR values found at both 'clean' and 'polluted' stations, showed no relation to river BOD concentration, (compare Tables 5.1 and 5.15) and emphasised the need for measurement and understanding of those factors which influence the benthic microbial biomass development and sediment-water interactions. To some extent, temporal variability at an individual station may have been related to frequent floods, (Fig. 3.2), which visibly disrupted attached biofilm communities and may have disrupted the bed structure. Variability between stations may additionally be influenced by changes in ambient water nutrient composition and concentration, which may alter the composition of or rate of microbial biomass development.

Benthic dispersion coefficients. Chamber measurements showed an effective benthic dispersion (D_s) of 27.2×10^{-3} cm^2 s^{-1} above the discharge. This value is more than 3 orders of magnitude greater than the molecular diffusion coefficient for oxygen in water ($\sim 2 \times 10^{-5}$ cm^2 s^{-1}). The value of D_s decreased by 88% below the discharge, presumably due to the biofilms present on sediment particles, and by 97% to the 10 km station (Table 5.2). These measurements suggest that the factors which influence sediment-water exchange (as measured by the value of Ds) may vary greatly within a river system. Sediment particle size was generally comparable between sampling stations, although a slight increase in fine material occurred 10 km downstream (see Appendix 4). Substantial differences in D_s values result from the activity of the microbial biomass present, which influences the depth of benthic oxygen penetration. When biofilms develop, which smother the bed, oxygen penetration is minimal and D_s values calculated are low. Comparison of measured and literature D_s values are presented in Section 5.5 with application to descriptive and predictive modelling of BUR in Chapter 7.

Thus the key components outlined in the conceptual model of benthic flux (Chapter 1.5) have been identified. The high flux measurements were the result of 2 factors: (i) the effluent stimulated an increase in epilithic heterotrophic biomass with high sediment intrinsic activity; (ii) turbulent dispersion processes substantially increased the rate of sediment-water exchange, compared with molecular diffusion processes. The BUR _in situ_ was largely rate limited by the electron acceptor (oxygen) concentration, rather than electron donor concentration. Changes in river flow velocity (affecting mass transfer), and effluent loading (affecting sediment biomass type and sediment intrinsic activity), were both responsible for substantial influences on the BUR _in situ_.

The relative importance of benthic and planktonic biomass. Determination of the relative importance of benthic and planktonic microbial populations is made difficult because of the need to establish the active depth of microbial biomass. Use of the estimates for the oxic depth, obtained from benthic chamber and intrinsic activity measurements, showed that the microbial benthos was 2- to 50-fold dominant over the planktonic biomass (Table 5.3). Maximal values of the benthic/planktonic ratio occurred upstream of the discharge where the benthic oxygen penetration was maximal and the planktonic biomass low, decreasing longitudinally below the discharge, where both increased planktonic and benthic biomass levels together with a reduced depth of sediment oxygen penetration, decreased the magnitude of benthic dominance. Both spring and summer surveys showed benthic dominance with the only major differences associated with filamentous algal development at the upstream station.

The benthic biomass contribution means that the rates of microbially mediated nutrient transformations may be enhanced over planktonic rates by up to 50-fold. Additionally, the rate of river oxygen response to changes in effluent loading may be markedly altered if: (i) benthic biomass adapted to the overlying organic composition and showed an increased growth rate (and possibly standing stock) in response to increased concentration; or (ii) uptake and storage of organic carbon from the overlying flow occurred and so decreases in effluent concentration will show minimal microbial activity decline. Benthic biomass levels in the surface 1 cm showed significant increases below the discharge. (Fig. 5.1). Comparison of spring and summer surveys showed 3-fold higher summer biomass maxima. These seasonal differences may be attributed to the flood frequency in the river (Fig. 3.2), with the frequent spring floods limiting filamentous microorganism development and with the visibly high turbidity levels (<10 cm depth of visibility) restricting algal growth. The low flows and decreased flood frequency of summer (Fig. 5.24) together with visibly clearer water (>50 cm depth of visibility), allowed development of filamentous algal communities. Monitoring 5-days and 13-days subsequent to a summer flood showed rapid algal colonisation (Fig. 5.1B), with the filamentous <u>Oedogonium</u> sp upstream (Plate 3.2) and <u>Ulothrix</u> sp at 2 km downstream with the blue-green algae (<u>Oscillatoria</u> sp.) at 5 km and 10 km. Algal biomass contribution to BUR was therefore highly variable with 510%

upstream and ranging from zero near the outfall to 87% at 10 km downstream (Fig. 5.2). The increased algal biomass was accompanied by a reduction in the heterotrophic biomass present. High levels of attached algal biomass will result in night-time DO depletion and high chamber BUR measurements, but do not actively enhance organic effluent removal within the river.

River deoxygenation and carbon removal processes. The heterotrophic biomass present removes river DO in the process of degrading organic material present. It was therefore necessary to determine whether the sediment biomass was epilithic heterotrophs(actively removing DOM and DO from the overlying flow) or a detrital heterotroph (metabolising sediment POM and DO from the overlying flow). To better understand the key energy yielding processes of the microorganisms present, a number of enzyme tests were undertaken. The background to these assays has been outlined in greater detail in the Literature Review (Chapter 2).

Enzyme assays proved a highly sensitive metabolic technique when compared with aspects of general physiology such as the oxygen uptake rate. The latter showed a maximum areal activity of approximately 20 g O_2 m^{-2} day^{-1} (or 0.63 mole O_2 m^{-2} day^{-1}) (Fig. 5.5A). In comparison the enzyme activities showed orders of magnitude higher rates of up to 200 000 mole m^{-2} day^{-1} for β-galactosidase activity. The requirement for specific induction of the β-galactosidase enzyme system (Brock, 1974) and the high activities downstream which were absent above the discharge, indicated that microorganisms did adapt to the nature of the chemical environment. Whether the magnitude and type of enzyme activity present varied in response to environmental conditions, therefore was of fundamental importance in establishing the role of the benthic microorganisms.

Enzyme activities may be compared between longitudinal stations on an areal basis to show overall metabolic trends; however, large differences in microbial biomass present (which may be influenced by physical or nutrient concentration effects) necessitated calculation of the biomass specific activity in order to compare the metabolic capability of microbial populations between stations. To distinguish whether the sediment biomass present were either epilithic or detrital heterotrophs was approached by observing the relative changes in specific activities at a station in the establishment period subsequent to a summer flood - a 'disturbed equilibrium approach'; and relies on the detection of key functional enzyme activity increases at a particular station, which will identify the active energy-yielding metabolism.

Epilithic heterotrophs were identified by measurement of activity of β-galactosidase; an enzyme specifically induced in the presence of lactose, which proved extremely sensitive to the presence of milk wastes. Activities ranged from no detectable activity upstream, and increased by orders of magnitude below the discharge and showed large downstream decreases in activity (Fig. 5.6A).

Other enzymatic measures of metabolism wee provided by proteolytic activity was high upstream suggesting that proteolytic metabolism was either an important energy or nitrogen source (Fig. 5.6C); and glucosidase activity which showed low upstream activity measured at summer low flow, which was possibly associated with cellulose metabolism and downstream activity which showed a rapid increase in activity subsequent to the summer flood and suggested that much of this activity may be associated with organic debris (Fig. 5.6B).

Detrital heterotrophs were identified by bis-phosphatase activity which is associated with both turnover of cell and degradation of DNA released from dead cells. Activity increased substantially below the discharge showing greatly increased activity levels at low river flows following a flood (Fig. 5.7A).

Alkaline phosphatase is induced in response to phosphorus limitation. Low activities were measured at all stations for each survey; however a large activity increase generally occurred during summer low flow conditions (25 January 1982, Fig. 5.7B). Upstream of the discharge, an increased activity occurred associated with <u>Oedogonium</u> proliferation. Higher activities present below the discharge suggested that the biofilms present were experiencing some phosphorus limitation.

Since microbial activities were not restricted to the surface km of sediments, it is important to consider the changes in metabolism which may occur with increased depth.

Depth of sediment-water interaction and particle size differences. The depth of sediment-water interaction and differences between bed particle size areas were investigated using enzyme activity measurements both upstream and immediately downstream of the organic discharge. Since β-galactosidase enzyme activity is induced in response to the presence of lactose (Brock, 1974), the depth to which β-galactosidase activity extends may be used as an indicator of the extent of sediment-water interaction. No sediment glycosidase enzyme activities were detected upstream of the organic discharge in the sediment depth profile survey. Below the discharge, coarse particulate material showed substantially higher galactosidase activity in the surface. 2 cm and no detectable activity below 5-6 cm depth (Fig. 5.16A), with fine particulates having lower surface activities and rapidly declining below 2-3 cm depth (Fig. 5.16A). Blackening of the sediments occurred at 2 cm in the fine and 5 cm in the coarse particulates as a result of anoxic conditions and sulphide deposition. Chamber and intrinsic activity measurements at the time of sampling, gave minimum sediment DO penetration depths of 1.1 cm and 0.37 cm for fine and coarse particulates respectively (Waharoa, 5 March 1982, Table 5.1). The enzyme activity profiles may possibly better represent the natural DO penetration depths present or antecedent conditions rather than actual conditions *in situ* at the time of measurement. Onterpretation of the enzyme data under non-steady-state conditions (i.e. the variability of DO and BOD concentrations with time) is difficult since the turnover time of the galactosidase enzyme is unknown. This is likely to be of the order of 1-2 days however, since sediment respiratory decay measurements gave respiratory half-lives of minimally 2.8 (Table 5.12). As such, the enzyme methods could represent a valuable technique for studying the depth of interaction between sediments and overlying flows.

Sediment bis-phosphatase and alkaline phosphatase depth profiles were also measured. Upstream at Landsdowne Road, the surface activities for both enzymes were comparable and gradually decreased in activity to 10 cm depth (Fig. 5.17). Both activities showed a discontinuity at 5 - 6 cm in the fine particulates which corresponds with a measured chlorophyll *a* peak (Fig. 5.12). Activities were substantially higher below the discharge at Waharoa, than they were upstream, with greater activity on coarse particulates (Fig. 5.18). Both enzyme activities decreased rapidly to 5 - 6 cm depth, with a sub-surface activity peak in the fine particulates. Thus all enzyme activity in anoxic regions was substantially less than the near-surface activities.

Biomass specific enzyme activities. Since the size of microbial populations varied considerably, both longitudinally (Fig. 5.1A) and with sediment depth (Figs. 5.12, 5.13), the specific enzyme activities with respect to biomass were calculated in order to compare the relative importance of metabolic processes, and determine whether environmental adaption occurred.

Biomass specific β-galactosidase activity was greater during summer than during spring (Fig. 5.8A). Subsequent to the summer flood, greatest activity changes occurred close to the discharge, suggesting that lactose-based metabolism was largely limited to this region. Specific protease activities were highest immediately following the summer flood and decreased in activity to the subsequent sampling (Fig. 5.8C). The high activity upstream of the discharge suggested that the activity was associated with material brought in during the flood. A decrease in benthic total carbon and the carbon:nitrogen ratio during the sampling period supported this contention (Table 5.11). The lower specific protease activities below the discharge, suggested that metabolism of soluble protein was not induced to higher levels as a result of the milk wastes. (Fig. 5.8C). Specific glucosidase activities increased upstream and to a greater extent downstream of the discharge, subsequent to the summer flood. Bis-phosphatase measurements, which showed quite high values during the spring and substantially increased activity subsequent to the summer flood (Fig. 5.9A). The largest increase occurred 10 km downstream, indicating that activity at this station was predominantly the degradation of cell material.

Biomass specific alkaline phosphatase activity indicated some phosphorus limitation in the spring and showed increased activities subsequent to the summer flood (Fig. 5.9B). Greatest increases occurred immediately below the discharge and at 10 km, apparently associated with biofilms present.

Biomass levels through the sediment showed a gradual decline to 10 cm depth above the discharge (Fig. 5.12), with higher surface biomass levels downstream showing a rapid decline to 5 - 6 cm depth, and minimal further decline (Fig. 5.13). Coarse particulates at Waharoa showed the highest biomass levels. Despite the large differences of biomass between different particle sizes, the biomass specific β-galactosidase activity values were similar and both showed a rapid activity decline to 5-6 cm depth (Fig. 5.19A). Glucosidase activities also declined rapidly to a depth of 5-6 cm (Fig 5.19B). The decline with depth of biomass specific activity indicates a progressive decrease in importance of a catalytic metabolism supplied from the overlying flow.

Biomass specific activities of bis-phosphatase and alkaline phosphatase showed slight increases with sediment depth at Landsdowne Road (Fig. 5.20). Below the discharge, sub-surface activities were high at either 5-6 cm or 10 cm depth, indicating an increase in decompositioned metabolism importance.

In summary, the sediment depth profile measurements suggest: (i) for upstream a relatively even colonisation of biomass to 10 cm depth, with a decompositional metabolism; (ii) for downstream - a rapid decrease in biomass associated with sediment anoxia between 2-5 cm; with a predominantly epilithic heterotrophic catalytic metabolism present in the surface layers and a detrital heterotrophic metabolism present at greater depth.

The biomass and enzyme data may be summarised, as shown in Figure 5.26, showing the location and type of biomass present and the primary heterotrophic metabolic activity at summer low flow, in relation to the organic discharge. The qualitative picture which emerges is that of the 'classical' diagram of response downstream of an organic discharge to a river, with phototrophic organisms upstream, heterotrophic downstream and a return to phototrophic dominance. The important difference is, however, that these changes occur over 10 km of flow (~8 h travel), with substantial metabolic changes occurring within this distance.

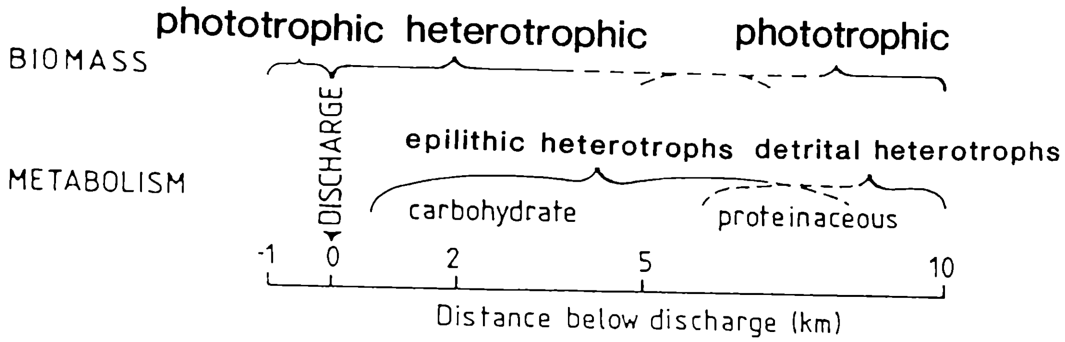


Figure 5.26 : A schematic diagram showing the predominant biomass and heterotrophic metabolic processes present in the Waitoa River.

River BUR upstream was largely the result of filamentous algal biomass and hence not related to BOD in the over-lying flow but to nutrients present. Below the discharge, the BUR was largely in response to carbohydrates present in the over-lying flow and as such could be termed epilithic heterotrophic metabolism, and would result in an enhanced removal rate of DOM. At 10 km downstream there was some increase in epilithic heterotrophic proteolytic activity compared with upstream sites (Fig. 5.8), which may have resulted from a depletion of river carbohydrate concentrations and metabolic shift to protein metabolism. However, the major heterotrophic activity was associated with the degradation of cell material and suggested the predominance of detrital heterotrophs. The major biomass constituent at this station was, however, an algal biofilm. These results qualitatively indicated the key heterotrophic metabolic processes and the development of phototrophic biomass where suitable light and nutrient conditions existed, confirming the hypothesis (Chapter 1) that: 'the benthic microbial population in shallow rivers may act predominantly as either epilithic heterotrophs, detrital heterotrophs, or a phototrophic population, depending on the river chemical and physical conditions'. Furthermore, such differences occur over short reaches of river (< 10 km) below outfalls, making predictive modelling of river processes extremely difficult because of the large number of microbial components.

Thus the type of the benthic microbial biomass has been shown to dynamically interact with both chemical and physical aspects of the aquatic ecosystem.

Interactions Between the Benthos and the Overlying Flow

As discussed in the previous sections, the activity of the benthic microbial biomass predominated over the planktonic, and showed large activity changes in relation to distance from the effluent outfall. This section considers the production and utilisation of carbon and oxygen in the river system, together with the influence of benthic productivity on the overlying flow.

Productivity. Longitudinal monitoring of planktonic ATP above and below the untreated dairy discharge provided an opportunity to measure microbial biomass changes attributable solely to river processes. Terrestrial inputs of microbial biomass were very low, with very little over-hanging vegetation and only one small tributary inflow downstream of the 5 km sampling station. Filtration of planktonic samples (using a 30 µm nylon screen) prior to ATP extraction, provided an arbitrary size fractionation of small particle biomass (<30 µm) and large particle (>30 µm) microbial populations. Longitudinal winter surveys when the factory was inoperative, showed no differences between sampling stations with most of the ATP present in the small particle biomass fraction. Longitudinal summer surveys showed a rapid increase in total biomass followed by a rapid decline. Differential filtration, together with chlorophyll a analysis for phototrophic biomass determination, showed that a substantial biomass increase was associated with the large particle biomass fraction to 5 km and that phototrophs were always <15% of the total biomass estimate (Fig. 5.3). Between the 5 km and 10 km stations the river biomass declined to upstream values, with no significant difference between particle size fractions. Thus river mechanisms were operating which resulted in rapid production and removal of planktonic biomass. A schematic diagram showing the relative contributions of large and small particle biomass to total planktonic biomass is shown in Figure 5.27. Estimates of microbial production rates may be made providing the travel time between stations is known. Dye tracer measurements associated with reaeration studies (Wilcock 1984b) together with the river discharge rating curve, were used for travel time calculations. Microbial production rate was calculated using: (i) a linear apparent production rate and (ii) an exponential growth rate for both size fractions. Results obtained from 3 longitudinal surveys undertaken during the 81/82 summer low flow period (sampling dates Table 5.9, see Fig. 5.24) are shown in Table 5.9. The surveys were started (11 Jan 82) 5-days subsequent to a flood, when minimal 'sewage fungus' biomass was present. Substantial colonisation and development occurred in the following period of decreasing flow. For the reach immediately below the discharge (0-2 km), production of the large particle biomass (>30 μm) fraction always exceeded the small particle biomass (<30 μm), and increased through the period. Thus the bulk of production was from the large particulate fraction.

Microscopic examination of the large particle biomass fraction showed that virtually all of the material present were filamentous 'sewage fungus' organisms, morphologically identical to the organisms growing attached to the river bed. These visual observations provide circumstantial evidence that the differential filtration method may give a measure of the rate of shedding of biomass from biolfilms in aquatic systems. If the organisms attached to the bed are continuously growing and sloughing material into the overlying flow, then an apparent linear rate of biomass increase may be anticipated for a parcel of water flowing downstream. Calculations based on a linear production rate for large particle biomass (>30 μm) are given in Table 5.9. On each sampling occasion the rate of production declined with increasing distance downstream of the discharge, which is consistant with visual observations of the decline in attached 'sewage fungus'.

Apparent microbial production rates were calculated using linear and exponential growth models for both biomass size fractions. Calculation of the specific growth rate (μ) for exponential growth in the reach immediately below the discharge gave values of μ for small particulate biomass which ranged from 0.09 to 0.34 h^{-1}, and for large particulate biomass of 1.08 to 2.00 h^{-1} (Table 5.9). These values may be compared with laboratory derived growth rates measured for river biomass grown on glucose of 0.08 to 0.12 h^{-1} for 10 to 50 $g\ m^{-3}$ glucose concentrations

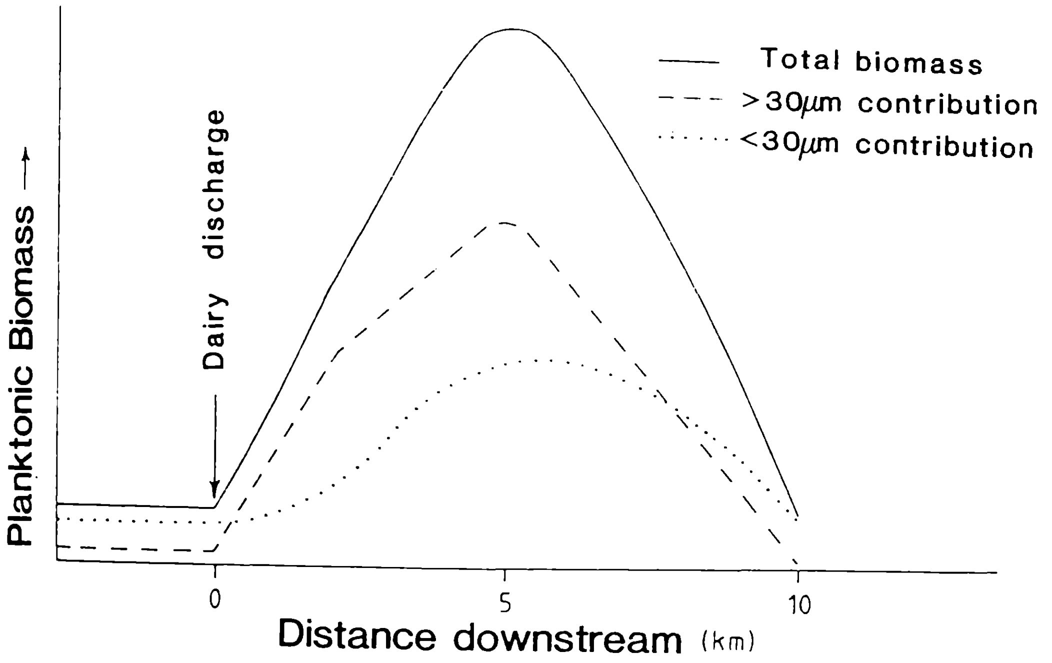


Figure 5.27 : A schematic diagram showing the relative contributions of planktonic and benthic derived biomass to total aquatic biomass present in the Waitoa River.

respectively (C.W. Hickey and J.W. Nagels; unpublished data). Gaudy and Gaudy (1981) report values of μ of up to 0.5 h^{-1} for heterogeneous populations of sewage origin. It seems likely, therefore, that the small particle size fraction does represent a fraction of the population reproducing exponentially in the planktonic phase. However, the extremely high apparent growth rate for the large particle biomass fraction requires further consideration. Unfortunately these techniques have not been applied to other river environments, so comparisons cannot be made. Further experimental work would also be required to define the optimal filter size, which may vary between environments depending on the nature of material present on the bed. Values of areal production of microbial biomass have been calculated (Table 5.9) to allow comparison of respiratory oxygen uptake and production, in order to determine whether steady-state river conditions exist (see following section).

The rates of decline in the river planktonic biomass were observed to be greater than the production rates, and thus resulted in upstream biomass values being obtained by 10 km downstream (Table 5.9). This was surprising and suggested that mechanisms existed which removed biomass carbon from the river flow. The efficiency of removal did not appear to be influenced by the biomass particle size; however the segment location and size fraction showing greatest decline changed during the sampling period. Autodigestion rates represent the cell maintenance energy requirements and are normally at most 1/10 of the maximum growth rate (Pirt, 1975; Gaudy and Gaudy, 1981). The high rate of microbial biomass decline measured in the river suggests that another mechanism was responsible for the rapid removal of river biomass. Macrophytes present below the discharge were silt-laden compared with those upstream suggesting that much of the planktonic biomass was removed by settling. These macrophytes may well act as a biological filter which promotes settling. The implications of this 'assimilation' process are that although particulate carbon was rapidly removed from the river flow, it remains present to be resuspended causing high suspended solids and possibly extensive oxygen depletion during flood events. The potential importance of this process has been recognised and discussed by Harremoes (1982) and documented in the context of stormwater overflows by Hvitved-Jacobsen (1982).

In summary then, it appears that the benthic microorganisms actively contribute to removing DOM from the overlying flow and in so doing produce POM which may be sloughed off the bed. Removal of POM occurs largely because of the passive physical process of settling, which may be enhanced by macrophytes and large debris present. Therefore 2 processes combine to result in the rapid rates of BOD assimilation observed in shallow river systems: (i) active benthic microbial removal; (ii) passive sedimentation.

Whether the river system can be considered in steady-state for mass balance considerations requires evaluation of the carbon and oxygen budgets.

Dissolved Oxygen and Carbon Dynamics in the Waitoa River. This sub-section provides an overview of the measured oxygen dynamics and BOD removal processes operating in the Waitoa River.

Oxygen. River DO, planktonic biomass and chamber BUR measurements obtained during a longitudinal survey (12 January 82) form the basis of the oxygen transfer rate calculations shown in Figure 5.28. This figure should be regarded as showing the relative magnitudes of processes, rather than an accurate and literal portrayal of an oxygen mass balance. Additional diurnal oxygen monitoring data is included to provide an estimate of possible macrophyte (or algal) biomass contributions. The key components influencing the observed river DO were benthic photosynthesis and atmospheric reaeration supplying river DO, and BUR and to a lesser extent macrophyte respiration utilising river DO. Planktonic components were very small both upstream and downstream of the discharge relative to other process contributions. Benthic respiration dominated the oxygen removal processes, both upstream and downstream, and showed decreasing activity with increasing distance below the discharge. The macrophyte biomass contributed significantly to the river oxygen metabolism, to constitute a major component 10 km downstream. The most important point which should be stressed here, is that substantial changes in both the magnitude and nature of component processes occurred over short river distances (~10 km) which equate to very small time intervals (~8 h travel time). An understanding of the factors influencing each of these component processes is therefore essential for predicting river oxygen dynamics in small rivers.

Monitoring of diurnal oxygen above the discharge showed that the common practice to consider only BOD degradation as the most important factor affecting the oxygen concentration in shallow rivers is inadequate, since phototrophic production (and respiration at night) can be major factors affecting river oxygen dynamics. Because of the effect of photosynthesis, diurnal observations of dissolved oxygen are necessary to obtain an adequate description of the oxygen status of a river. Diurnal curves of dissolved oxygen have largely been used in a descriptive role as P/R diagrams that describe the trophic state of an ecosystem (following Odum, 1956). The terms autotrophic, balanced, and heterotrophic have been applied to ecosystems with P/R values of greater than 1, equal to 1, and less than 1, respectively. The use of gross photosynthesis and respiration reduced to reaeration capacity (P/k_2, R/k_2) as diagram coordinates (Servais *et al.* 1984) may be used to

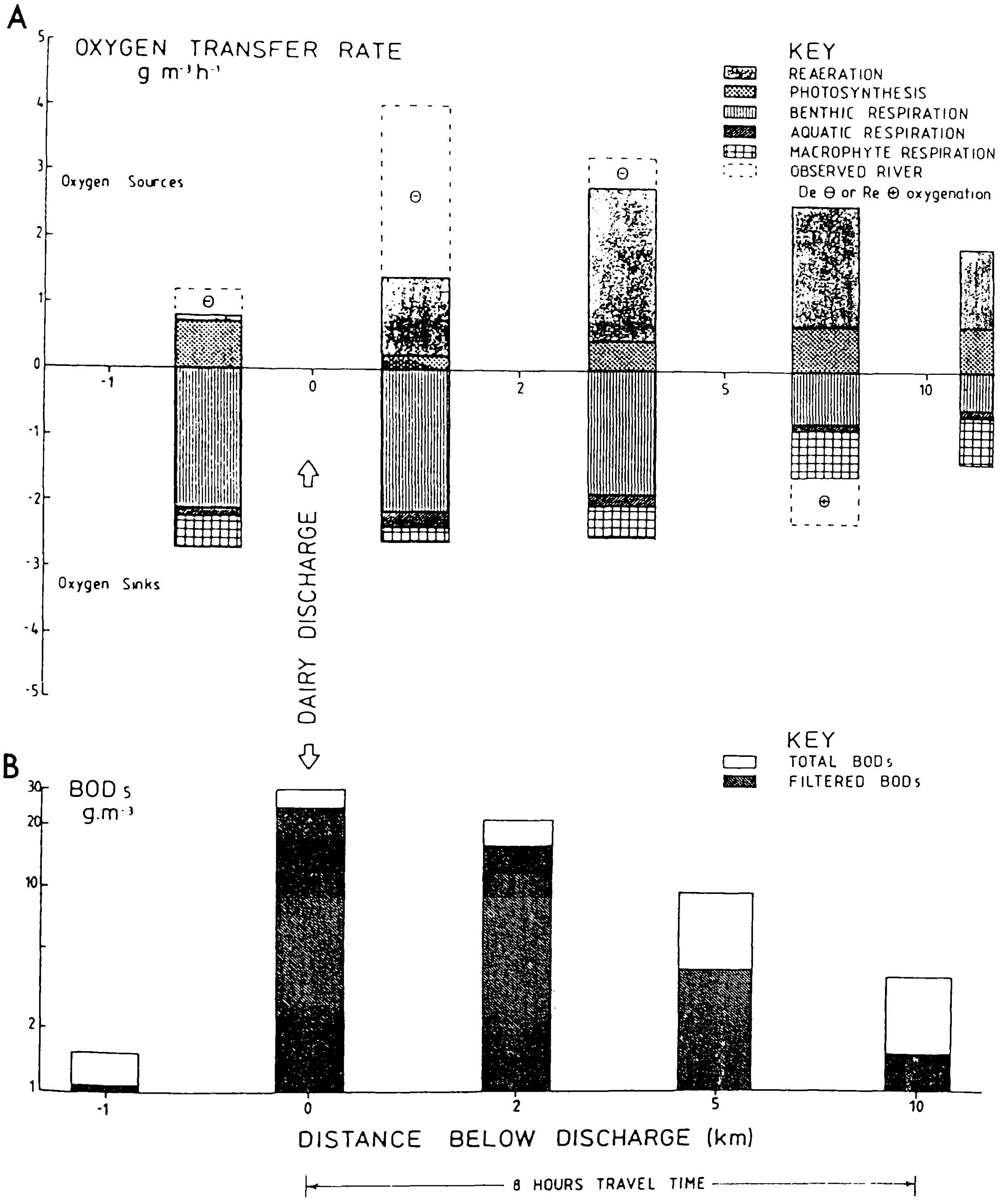


Figure 5.28 : Components contributing to the rates of river oxygen production (sources) and consumption (sinks) (A) and BOD decay (B) in the Waitoa River. Details of data and calculations are given in Appendix 2.

directly relate trophic state to the aeration state of the water. Servais et al. (1984) have shown that the daily mean oxygen deficit ($\bar{D}$ was determined by the value:

$$\bar{D} = \frac{R-P}{k_2} \qquad 5.8$$

where R is the total community respiration (g O_2 m^{-3} day^{-1}); P the gross primary production (g O_2 m^{-3} day^{-1}); and k_2 the reaeration coefficient (day^{-1}, base e). The amplitude (A), however, was only dependent on the value of P and k_2, being given by the relation:

$$A = \frac{P}{k_2}\beta \qquad 5.9$$

where β is a factor dependent only on k_2 and the relative day length (i.e., hours of daylight, which vary seasonally). The value of β can be obtained from the nomograph of Simonsen & Harremoes (1978). Unpolluted rivers display neither significant oxygen deficits, nor substantial diurnal oxygen fluctuations, and are located near the principal axis intercept of a lotic metabolism diagram (Fig. 5.25).

Organically polluted rivers (mean deficits greater than 2 g m^{-3}) are located on the right side in Fig. 5.25, with the value of the mean oxygen deficit located on a straight line parallel to the diagonal, intercepting the R/k_2 axis at this value (Fig. 5.25). Eutrophic rivers are located toward the upper part of the diagram, with the amplitude (A) of the diurnal fluctuation around the mean deficit located on a horizontal line intercepting the P/k_2 axis at $A.\beta^{-1}$ (Fig. 5.25).

Diurnal oxygen metabolism data obtained for a number of New Zealand rivers are plotted on Fig. 5.25 with details provided in Table 5.17. Data obtained from the Waitoa River (W series on Fig. 5.25) at a station above major point source influences, shows how the trophic state and oxygen characteristics of the river change seasonally. When sampling was started in January 1982, the benthos was dominated by the filamentous algae Oedogonium sp., resulting in a large diurnal DO variation (W1). Decreasing light levels and decaying algae in autumn, gave greater oxygen deficits and decreased production (W2), followed by negligible photosynthetic production during the high flows and turbid conditions of winter (W3 and W4). In early summer there was: (a) increased productivity (W5); (b) the influence of flood flows (W5→W6); and (c) the development of a macrophyte dominated community of initially high productivity (W7), followed by extensive decomposing organic material accumulation (W8) associated with the 1982/83 drought (see Fig. 3.2). At this time river oxygen concentrations ranged from 4 to 10 g m^{-3} daily. The reaeration coefficient decreased 44% from 6.5 day^{-1} to 4.5 day^{-1} as a result of decreasing flow velocity and increasing mean depth associated with macrophyte growth (Table 5.17). Such extensive macrophyte dominance might be expected for many lowland streams draining pastoral catchments as a result of diffuse nutrient loads. Both seasonal variation and floods substantially effect the oxygen dynamics of the upstream station.

The monitoring 10 km below the Waharoa dairy factory discharge on the Waitoa River (WW on Fig. 5.25), showed a considerable mean deficit of 7 g m^{-3} resulting from the discharge, with a diurnal amplitude variation amplitude of about 1 g m^{-3}. Comparison of spot river DO and BOD measurements to estimate deoxygenation and assimilation coefficients is complicated by the presence of phototrophic organisms.

Carbon. Quantitative estimates of river BOD assimilation were particularly difficult because of the discontinuous nature of the direct loading of untreated effluent (Plate 3.4). This resulted in the mean BOD value measured 2 km below the outfall being higher than samples taken immediately downstream (Table 5.15). Only 1 continuous monitoring of river BOD was undertaken and this showed a diurnal discharge pattern with values up to 30 g m^{-3} (Fig. 5.23). Changes in the relative BOD and COD values throughout the day suggested substantial changes in effluent biodegradability, probably in relation to factory processes (Fig. 5.23). Longitudinal river sampling in relation to this discharge showed that a 30 g m^{-3} BOD_5 was reduced to 2.2 g m^{-3} in 4 h of river travel time, despite the relatively high river flows at that time (Table 5.13).

If we assume that the combined benthic and aquatic BOD exertion can be modelled by first order kinetics, then the river BOD decay coefficient is:

$$k_1 = \frac{1}{\Delta t} \ln \frac{Lo}{L} \qquad 5.10$$

$$= \frac{24}{4} \ln \frac{30}{2.2}$$

$$k_1 = 15.7 \text{ day}^{-1}$$

A substantially higher river BOD decay coefficient might be expected if sampling had been performed under low flow conditions. A summary of k_1 values reported for other rivers is shown in Table 5.18 and suggests that these rivers may possess a high benthic activity together with particulate BOD removal mechanisms operating in the Waitoa River.

Studies involving pre-filtration of BOD samples supported the contention that there was rapid DOM removal with the production of benthic biomass, which was continually sloughed off the bed resulting in minimal total BOD decline (Table 5.16). Subsequent removal of particulate BOD then occurred by settling processes, and resulted in a rapid overall net BOD 'assimilation'. A diagramatic representation of BOD removal in the Waitoa River is shown in Figure 5.28B. The river BOD composition (ie soluble and particulate BOD) and concentration changed substantially (note the logarithmic scale) over short river distances (~ 10 km) which equate to very small time intervals (~ 8h). The chemical composition, and presumably also the biodegradability of the river DOM, changed significantly in the 10 km below the outfall as indicated by the catabolic enzyme composition of the microorganisms present (Fig. 5.26).

River	Typical low flow $m^3\ s^{-1}$	k_1 day^{-1}	Reference
Mataura	14	2*	McKenzie and McBride (1982)
Manawatu	20	0.7-12	Currie and Rutherford (1982)
Waipa	20	0.7*	McBride and Rutherford (1982)
Tarawera	25	5.2*	Piper (1982), McBride (1982)
Waikato	180	1.2-1.8*	Rutherford (1982)
Waikato	180	0.6-0.8*	Rutherford (1982)

*Estimated by model calibration and verification using river DO and BOD_5

TABLE 5.18: Summary of New Zealand river BOD removal coefficients

This metabolic adaption of attached microorganisms to the composition of the overlying DOM, and the substantial level of active benthic biomass will certainly result in the rapid assimilation observed (Fig. 5.28). Such metabolic adaption of attached river organisms suggests that an optimal assimilation rate for an organic component will develop in a particular river segment. Conventional tests for biodegradability (eg BOD) involve a sequential series of enzyme or species successions necessary to metabolise the organic material. Thus the measured biodegradation rate in a bottle measurement system may be substantially less than that within a shallow river, even if a high initial inoculum of adapted microorganisms were added to the bottle, because the process of metabolic assimilation is fundamentally different. Conditions present within a river, however; are not necessarily static and 'reproducible' since environmental habitat influences (eg floods, diurnal DO and temperature fluctuations) may influence the attached microorganisms, and lead to changes in biomass levels, shifting a zone of specific metabolic activity up or down within the river system. To simply describe and model such a system requires steady-state conditions to exist. For steady-state conditions to exist, the benthic biomass must reach a level where the standing stock is constant, and new biomass produced from DOM assimilation is lost to the overlying flow, or through predation to secondary production.

Export of large particulate biomass which is probably derived from sloughing from the bed into the overlying flow amounted to maximally 82.7 mg C m^{-2} h^{-1} (Table 5.9) for the reach below the discharge. Calculation of a segment oxygen mass balance for the measured station DO concentrations (detailed in section 6.1), and allowing for the small planktonic respiration gave an estimated BUR of 27.2 g O_2 m^{-2} day^{-1} or 10.2 g C m^{-2} day^{-1}. This amounts to 420 mg C m^{-2} h^{-1} respired carbon which, assuming a 50% respiratory yield (Yo_2) should equal the new biomass produced. This calculated value is considerably more than the measured (maximum (82.7 mg C m^{-2} h^{-1}) and suggests that the river is either not in steady-state or cell yields (Yo_2) are extremely low. Visual observation of 'sewage fungus' development would suggest a productivity cycle of growth and decay, rather than long term steady-state conditions. Rapid growth occurs after a flood. This establishes a thick biofilm which decays at the base, to be sloughed off the substratum. Therefore, the attached biomass would actively remove DOM carbon during growth and short term steady-state conditions may exist. The growth and turnover of 'sewage fungus' biofilms will be discussed in Chapter 6. These showed that the substantial carbon storage within the biofilms resulted in respiratory half-lives of 1.85-5.54 days.

Downstream of the 'sewage fungus' colonisation the benthos received a supply of carbon by settling from the overlying flow. The rate of turnover of benthic carbon would dictate the metabolic response times for changes in ambient water quality.

Carbon turnover provides an indication of the amount of stored benthic carbon relative to the rate of metabolic activity. The rate of sediment respiratory decay provides a measure of the biodegradability of carbon present, and would reflect the benthic response to effluent cessation.

The sustained benthic metabolic activity represents the combined effects of microbial growth and decay as influenced by the supply of electron donors (carbon) and electron acceptors (oxygen). Changes to any one of these components could affect the net flux and turnover times. How such changes may affect the measured flux will be considered in detail in Chapter 7. In this sub-section the measurements made on individual components will be discussed.

Carbon input. Above the discharge the carbon supply may be derived from autochthonous sources (by algae and macrophyte proliferation) and allochthonous sources (as organic debris during rainfall and runoff processes). Below the outfall the carbonaceous effluent is added to these other river organic inputs.

To put organic inputs in perspective, estimates of the upstream and downstream carbon inputs to the river are compared. For simplicity only photosynthetic fixation is considered upstream with comparison in terms of oxygen equivalents:

Upstream fixation 12.5 g O_2 m^{-3} day^{-1} (20 Oct 1982 from Table 20 for river flow of 0.65 m^3

m^{-1})

Downstream input from factory at	28 g BOD_5 m^{-3} in river 0.47 m^3s^{-1} river flow (Fig. 3.2)	(value from Table 5.19 for 12 Oct 1982)
	→ 11.4×10^5 g BOD_5 day^{-1}	

$\frac{\text{Factory}}{\text{Fixation}} = 91.2 \times 10^3\ m^3$ i.e. factory production exceeds river photosynthetic fixation to 1 m^3 of water by 90×10^3.

Thus 28 g m^{-3} of BOD_5 is equivalent to a 90-fold increase in river production. Considering that this loading appears to be 'assimilated' in 10 km of river length, this represents a potential 9-fold increased organic loading for the river study reach. An important distinction should be made here, in that the bulk of upstream carbon input is derived from particulate organic material (ie plant debris), whereas, the factory input was DOM (ie milk wastes) with markedly different biodegradabilities and subsequent microbial response.

Benthic carbon. Longitudinal surveys showed that benthic carbon ranged from 2.6 to 39.2 g C m^{-3}, with the lowest value occurring upstream in spring and the highest associated with 'sewage fungus' colonisation below the outfall (Table 5.10). Carbon levels increased substantially below the outfall in spring, whereas a summer flood resulted in high values throughout the river system, declining both upstream and 10 km below the outfall in a subsequent survey. The nature of the carbonaceous material present was determined by measurements of total nitrogen, protein and carbohydrate. Total nitrogen ranged from 1.1 to 6.9 g N m^{-2} with C:N ratios from 2.5 to 10.3 (Table 5.11). The narrow range of these ratios and minimal longitudinal trends suggests that the material present was generally of similar composition. Protein and carbohydrate concentrations each showed substantial increases below the discharge, however, protein:carbohydrate ratios also covered a very small range (Table 5.11) despite considerable variation in the microbial biomass present.

Benthic microbial biomass carbon. Calculation of the benthic biomass carbon as a percentage of total carbon present gave values ranging from 1.1 to 16.7% (Table 5.10). In spring, similar values were obtained throughout the system, with maximum values in summer. The summer flood resulted in an influx and presumably downstream displacement of organic material which had a low living biomass content. In the intervening period prior to the subsequent survey microbial colonisation increased with the net removal of much of the carbon present. The high biomass levels present relative to the total carbon would suggest a high rate of nutrient removal and necessitate a continual supply across the sediment water interface, since reserves would otherwise be rapidly depleted.

The above studies were limited to the surface 1 cm layer. However, chamber studies indicated oxygen penetration to substantially greater depths (Table 5.2). For this reason sediment depth profile measurements were undertaken above and below the discharge. Sediment biomass measurements upstream of the discharge point showed a gradual decrease in percentage living carbon to 10 cm depth (Table 5.7), with a notably high autotrophic component present throughout these profiles. Below the discharge at Waharoa, sediment depth profiles for fine particulates showed a subsurface biomass carbon maximum of 22.8% decreasing to 3.9% at 9-10 cm (Table 5.7). The mobile nature of benthic particles across the fine sand surface possibly restricted surface 'sewage fungus' colonisation, whereas, anoxic conditions at depth probably resulted in reduced biomass levels. Coarse particulates supported a substantial 'sewage fungus' colonisation and showed a rapid decline in biomass where conditions became anoxic.

Thus, upstream of the discharge the concentration of nutrients in the overlying flow and level of sediment-water exchange was sufficient to maintain comparable levels of benthic biomass to 10cm depth. Below the discharge with presumably similar levels of turbulent sediment-water exchange, the increased concentration of DOM resulted in increased growth rates, a higher standing stock, and a restriction in active biomass depth caused by oxygen depletion.

This supports the Conceptual Model proposed in Chapter 1, where the combined effects of electron donor and electron acceptor concentrations in the overlying flow, and benthic microbial activity will influence the depth of active sediment biomass and consequently the observed flux.

Carbon turnover. Under steady-state conditions an estimate of the time scale for carbon turnover may be obtained by measuring the intrinsic activity in relation to the total carbon present. Intrinsic activity measurements represent the potential activity for the surface 1 cm layer when not limited by electron acceptor (oxygen) supply. Since oxygen penetration estimates were generally greater than 1 cm depth (Table 5.1) these measurements may therefore be used to represent rates in situ.

Calculated longitudinal carbon turnover times ranged from 4.9 to 52.1 days. The majority of values, however, were less than 10 days (Table 5.12). A minimum value generally occurred below the discharge, despite the elevated carbon levels present. This reflected the increased intrinsic activity of the sediments below the discharge. There was a slight tendency for an increased turnover time at the 10 km station, which could represent a build up of organic debris.

Benthic depth profiles above the discharge showed uniform activity, biomass and carbon levels to 10 cm depth, which gave comparable turnover time values. Below the discharge, however, uniformly high benthic carbon levels occurred to 10 cm depth, but with microbial biomass ranging from very high at surface levels to very low at 10 cm. Thus a greater turnover time would occur at increasing depth.

The effect of such carbon reserves within the sediment would be to sustain BUR when effluent load declines by providing a supply of presumably metabolisable carbon.

Sediment Respiratory Decay. The rate of degradation and assimilation of the assemblage of benthic carbon compounds present, or biodegradability, was measured using the electrolytic respirometer system to obtain estimates of the sediment respiratory decay coefficient (k). A simple a 1st order model was used and calculated relative to 75% of the measured total organic carbon initially present. Direct comparison of values of k between stations was achieved by assuming a constant proportion of readily biodegradable carbon present (75% in this case), though this compromised the 'goodness-of-fit' to some progressions. Results (shown in Fig. 5.22) gave k values of between 0.10 and 0.20 day^{-1} (Table 5.12). On the first longitudinal survey the biodegradability decreased longitudinally downstream of the discharge, and showed a poor overall fit to the simple mathematical simulations (Fig. 5.22A). An improved fit may have been obtained by assuming a lower percentage of available carbon at the downstream stations; however, adequate initial estimates may be obtained from the approach used. The second longitudinal survey showed comparable biodegradability at several stations with a maximum rate occurring 5 km downstream (Fig. 5.22B). The decay kinetics occurred in multiple steps for some stations, suggesting that complex secondary degradation processes were occurring and which may have included nitrogenous metabolism.

Half-lives of sediment respiratory decay ranged between 2.8 and 6.9 days for the surface layer of the Waitoa sediments. These values are substantially higher than the flushing time appropriate to planktonic biomass (<15 h), and as such one would therefore anticipate a slower response time to decreases in river effluent loading than normally anticipated for large rivers where planktonic biomass predominates.

5.2.11 Summary

Benthic oxygen uptake rate (BUR) was shown to be high both above and below the organic discharge (section 5.2.2). Upstream BUR was associated with phototrophic communities (filamentous algae), whereas downstream heterotrophic communities ('sewage fungus') resulted in the highest BUR values recorded. BUR decreased downstream, caused not by decreasing heterotrophic activity, but presumably by a decreasing depth to which oxygen penetrated the sediment. Wide variability in BUR values occurred at individual stations because of floods removing attached biofilms, and seasonal changes in effluent load.

The minimum depth of oxygen penetration into the sediments was calculated to range from 0.32-32.0 cm, with the minimum associated with 'sewage fungus' colonisation and the maximum occurring upstream of the discharge. A wide range in the calculated depth to which oxygen penetrated the sediments at a given site occurred because of varying microbial colonisation and activity, influenced by seasonal flow and water quality conditions (section 5.2.2), and uncertainties in estimations of sediment activities.

Benthic biomass was shown to be up to 50-times the planktonic biomass influencing river oxygen dynamics (section 5.2.3), with the maximum value obtained above the discharge and up to 20-times downstream. However the type of benthic biomass was variable with a 0 - 87% range in algal contribution (section 5.2.4). Heterotrophic benthic biomass cominated close to the discharge, with a high proportion of algal biomass present by 10 km downstream. Thus greatly enhanced deoxygenation rates occur because of the presence of active microbial biomass.

Benthic metabolism was shown to respond to temperature with a Q_{10} of 2.0 (section 5.2.5). Microbial enzyme activities were used to identify the active metabolic processes in the sediments (section 5.2.6). Studies showed that the benthic metabolism varied substantially with increasing distance from the discharge. Epilithic heterotrophs predominated immediately downstream of the discharge (indicated by β-galactosidase activity), with an increasing downstream contribution from detrital heterotrophs (indicated by bis-phosphatase activity). Studies of the sediment profile showed epilithic heterotrophs at the surface 1-4 cm with detrital heterotrophs at increasing depth (section 5.2.7). Measurements of planktonic biomass levels showed river process resulted in rapid development of planktonic biomass followed by equally rapid removal processes (section 5.2.8). Thus active benthic DOM removal processes were occurring near surface adjacent to the discharge, with passive settling of POM supplying carbon at downstream stations. Such processes will require further investigation because of their potential importance for BOD removal from the river water.

Diurnal oxygen monitoring showed a considerable seasonal range in river trophic status at the upstream station (section 5.2.9). This occurred because of algal periphyton and macrophyte metabolism with a productivity maxima associated with the summer low river flow. Below the discharge oxygen deficits were high with minimal diurnal variation immediately downstream, but an increasing effect at 10 km. River organic loading from the dairy factory was estimated to be 90-fold higher than the natural photosynthetic carbon fixation processes. The downstream organic carbon levels were increased as a result of loading, though up to 16% of this was attributable to living biomass (section

5.2.8). Because of the benthic dominance, metabolic response times to decrease in effluent loading will increase in magnitude from the flushing time (several hours) to the sediment respiratory decay time (several days).

5.3 WAIOTAPU RIVER

5.3.1 Introduction

The Waiotapu River studies were designed to investigate river oxygen dynamics in a river having a pumice bed, and to produce a segment oxygen mass balance with which the chamber oxygen measurements could be compared. Investigations included: planktonic and benthic biomass measurements; biomass composition; chamber BUR; sediment intrinsic activity; benthic substratum variation; together with the longitudinal changes associated with a point source dairy effluent discharge.

The dairy effluent consisted of untreated milk wastes, discharged when the land disposal system became overloaded or when factory spillages occurred. One longitudinal survey was performed during the dairy season, together with sediment sub-surface depth profile measurements. The segment oxygen mass balance was performed shortly after cessation of effluent discharges.

5.3.2 Segment Oxygen Mass Balance

Introduction

The chamber was used in situ in order to compare the river segment oxygen mass balance, with the measurements of BUR values obtained using the chamber. Values obtained were used in order to determine the relative importance of aquatic, benthic and macrophyte respiration in this river system.

The time of sampling was late summer, shortly after the shut-down of the dairy factory, which previously had subjected the river to intermittent effluent loading. This avoided the necessity to monitor the concentration and flow of the effluent.

Data Collection

The Sampling Programme. The following measurements were made in order to determine a river segment oxygen mass balance:

a Continuous monitoring of river dissolved oxygen and temperature were made at 2 stations; Reporoa and Homestead Road (2.8 km downstream). The sampling was done on 8 and 9 March 1983.

b Surface photosynthetically active radiation (PAR) was monitored at a site 10 km distant (Forest Research Institute, Rotorua). River PAR extinction was determined in relation to water depth to equate the light received by the aquatic macrophytes with surface measurements. This sampling was done on 8 and 9 March 1983.

c The river oxygen reaeration coefficient had previously been determined for this river segment at equivalent flow by Wilcock (1984b) using a gas tracer method. Travel time (2.0 h) was also measured at that time by using a tracer dye.

d Four BUR measurements _in situ_ were made using the large chamber at the Homestead Road station in order to determine the BUR kinetics. These values were subsequently used for comparison with the oxygen mass balance BUR estimate. The sampling was done on 9 March 1983.

e One BUR measurement was made _in situ_ using the large chamber in order to determine the response to circulation velocity within the chamber. The sampling was done on 9 March 1983.

f Six BUR measurements were made _in situ_ using the large chamber at 2 stations in the river segment in order to estimate benthic BUR sampling variability. The sampling was done on 18 January 1983.

g Respiration and photosynthetic rates of aquatic macrophytes were made in the laboratory. These experiments were done in order to establish a biomass specific respiration and biomass specific photosynthesis/PAR relationship. This would enable macrophyte biomass estimates to be made from the segment photosynthesis/PAR relationship. Laboratory experiments were done on 20 January 1984.

Procedures

Methods used in the field

a **Monitoring.** River DO and temperature were monitored continuously at each end of the reach. PAR was estimated as 45% of the hourly value of the Kipps photometer record, located 10 km distant, and obtained from the Forest Research Institute, Rotorua. An attenuation coefficient of 2.19 m^{-1} for the river water was measured using a LiCor meter and a 4π sensor.

b **Chamber measurements.** Measurements of BUR made <u>in situ</u> were obtained using the large benthic chamber. Details of design, installation and operation of the chamber system are given in Chapter 4.

Repeat determinations of BUR kinetics on the same area of the stream bed were achieved by reoxygenation to saturation accomplished by addition of a catalase suspension to the chamber (final concentration 80 units/ml), followed by addition of a calculated volume of diluted hydrogen peroxide (10 ml of 0.5 M H_2O_2 releases 9.0 g m^{-3} of O_2) (Hickey, 1985b).

The circulation velocity was measured using an Ott propeller meter mounted at the mid-point of the chamber 50 mm above the bed, and which could be left in position for measurement during chamber operation.

River water samples (four replicates) were taken at the time of chamber closure, sealed in unstirred BOD bottles (two with glucose added at 20 g m^{-3} final concentration) and incubated for approximately 2 hours in the dark at river temperature. This allowed for the correction of chamber measurements for aquatic oxygen demand in order to determine the BUR. In all cases however, the aquatic oxygen demand was low (<0.2 g $m^{-3}h^{-1}$) and no correction was made. The river water samples were also used for determination of BOD_5 (APHA, 1976).

c **Measurements of drifting macrophytes.** The biomass of drifting filaments of macrophyte was estimated using a net ($1.68 \times 10^{-2} m^2$ area, 2 mm mesh) held 0.2 m beneath the surface in mid-stream for 3 min. The measured flow velocity was used to calculate the total volume sampled. Macrophyte biomass was determined by overnight drying at 85 °C.

Measurements Used in the Laboratory

Photosynthesis/PAR experiments. Macrophytes were collected, transported to Hamilton and held overnight in aerated river water. Photosynthesis and respiration measurements were made in the chamber fitted with a perspex base and top, and held in a constant-temperature (20±1 °C) tank with a white internal lining. Oxygen was monitored using a YSI meter connected to a scale expanding amplifier (1 g m^{-3} full scale).

Lighting was provided by a bank of cool white fluorescent tubes, augmented by natural diffuse sunlight for the single maximum light measurement (480 $\mu E\ m^{-2}s^{-1}$). PAR was measured using a LiCor meter and a

4π sensor, located inside perspex of the same thickness as that which was used in the chamber system.

Calculations

Diurnal curve analysis. Monitored diurnal oxygen concentration data obtained for the upstream station was analysed using a single station finite difference analysis method, with a single two station monitoring analyses to provide the segment photosynthesis and respiration estimates. Both diurnal curve analyses follow the method of Odum (1956). The river reaeration rate coefficient was measured in the Waiotapu River for equivalent hydrological conditions by a colleague, Dr R.J. Wilcock, using a gas tracer (methyl chloride) technique (Wilcock, 1984a, b). The hourly net productivity was then calculated from the volumetric rate of change of DO concentration and the product of the reaeration coefficient and the DO concentration deficit using the formula:

$$\frac{\Delta C}{\Delta t} = k_2(C_s - C) + (P - R) \qquad 5.1$$

where $\frac{\Delta C}{\Delta t}$ = rate of change of DO, g $m^{-3}h^{-1}$; k_2 = reaeration rate coefficient h^{-1}; C_s = saturation DO at the river temperature, g m^{-3}; C = mean DO during period Δt, g m^{-3}; Δt = time between measurements or time of travel between stations, h; P = oxygen production due to photosynthesis, g O_2 $m^{-3}h^{-1}$; R = oxygen removal due to respiration, g O_2 $m^{-3}h^{-1}$.

The reaeration coefficient was assumed to be constant with no variation of k_2 with temperature.

C_s was calculated from the measured river temperature using a fitted equation derived from the data of Wilhelm et al. (1977).

$$C_s = 14.0437 + T(0.003148T - 0.3106) \qquad 5.2$$

This equation was derived and found to be valid from 12 °C to 31 °C with an accuracy of ±0.01 g m^{-3}.

Total respiration was assumed constant and calculated from the mean of the night-time values and converted to a daily basis.

Photosynthetic rates were calculated using the mean respiration rate established from night-time measurements, and gross photosynthesis from the integral of the net photosynthetic rates during daylight hours.

Conversion of volumetric data to benthic areal estimates was made using the mean depth estimated by Wilcock (1984b).

Mass balance. The rate of change of oxygen concentration with time $(\frac{\Delta C}{\Delta t})$ is given by:

$$\frac{\Delta C}{\Delta t} = k_2\bar{D} - Raq - (BUR + R_m - P_m)\frac{1}{H} \qquad 5.3$$

where $\bar{D}$ = mean oxygen deficit below saturation (C_s), g m^{-3}; R_{aq} = aquatic respiration rate, g O_2 $m^{-3}h^{-1}$; BUR = benthic oxygen uptake rate, g O_2 $m^{-2}h^{-1}$; R_m = respiration rate of the submerged macrophytes, g O_2 m^{-2}(consumed)h^{-1}; P_m = the gross macrophyte photosynthetic rate, g O_2 m^{-2}(produced)h^{-1}; and H = mean depth, (0.75 m).

River macrophyte respiration was estimated from a biomass specific photosynthesis/PAR relationship. These values were obtained using chamber measurements made on isolated macrophytes in the laboratory. Laboratory and river measurements were compared at equivalent PAR inputs. Calculations were made, first, to estimate the river macrophyte biomass (X_m):

$$X_m = \frac{P_m}{A_p} \quad \text{g DW m}^{-3} \qquad 5.4$$

and, second, the macrophyte respiration rate (R_m):

$$R_m = X_m \cdot A_R \quad \text{g } O_2 \text{ m}^{-3}\text{h}^{-1} \qquad 5.5$$

where X_m = macrophyte biomass, g DW m^{-3} and DW = dry weight; P_m = gross photosynthesis of river macrophytes (at a standard PAR allowing for the river PAR extinction and the mean macrophyte depth), g O_2 $m^{-3}h^{-1}$; A_p = gross macrophyte photosynthetic activity coefficient (at standard PAR) as determined in the laboratory, g O_2 g $DW^{-1}h^{-1}$; A_R = macrophyte respiratory activity coefficient, (determined in the laboratory), g O_2 g $DW^{-1}h^{-1}$.

RESULTS

Diurnal DO concentration at the upstream Reporoa station was from 5.5-11.0 g m^{-3} (64-130% saturation), the mean temperature was 23.2 °C with a range of 2.3 °C. At the downstream Homestead Road station DO concentration range was from 4.3-8.8 g m^{-3} (50-102% saturation), the mean temperature was 22.3 °C with a range 3.3 °C.

Diurnal oxygen concentration measurements were analysed using a single station method for upstream at Reporoa and a two station method for the segment below the dairy discharge point, to estimate daily gross photosynthesis and respiration. Table 5.19 shows (a) the gross photosynthesis (P) expressed as g O_2 m^{-2} day^{-1}; (b) gross respiration (R) expressed as g O_2 m^{-2} day^{-1}, and ; (c) the ratio P/R for both the Reporoa station and Reporoa/Homestead Road reach of the river. For the same 24 h period photosynthetic production was 15.4% less and respiration 56% greater for the segment compared with the upstream conditions. This was reflected in a decrease in the P/R ratio from 0.67 upstream to 0.35 in the segment.

Mean segment DO concentrations are shown in Figure 5.29 with the calculated oxygen exchange rate (P-R). The night-time exchange rate (P-R), which is equivalent to the respiration rate in the absence of photosynthesis showed a significant decline from -2.0 to -1.6 g $m^{-3}h^{-1}$ or 20%, which was associated with a river DO concentration decrease from 8.0 to 5.2 g m^{-3} ($P<0.001$, $r^2 = 0.544$, $n = 17$) and a temperature decline of 0.2 °C. Night-time respiration rate calculated using single station measurements also decreased in relation to DO concentration at both Reporoa ($P<0.20$, $r^2 = 0.20$, $n = 11$) and Homestead Road ($P<0.05$, $r^2 = 0.57$, $n = 9$). The rate of respiratory decline in response to decreasing DO concentration (i.e. regression slopes) were -0.15, -0.013, and -0.90 h^{-1} for the segment, Reporoa and Homestead Road respectively.

Photosynthetic production rate in relation to surface PAR was calculated using the mean night-time respiration rate, acknowledging that this may introduce an estimation error because of an apparent relationship between respiration and DO concentration established from diurnal curve analyses (previous paragraph). Figure 5.30A shows the recorded PAR record and gross photosynthetic production rate. Photosynthetic rate/PAR relationships are shown for Reporoa, Homestead Road and the

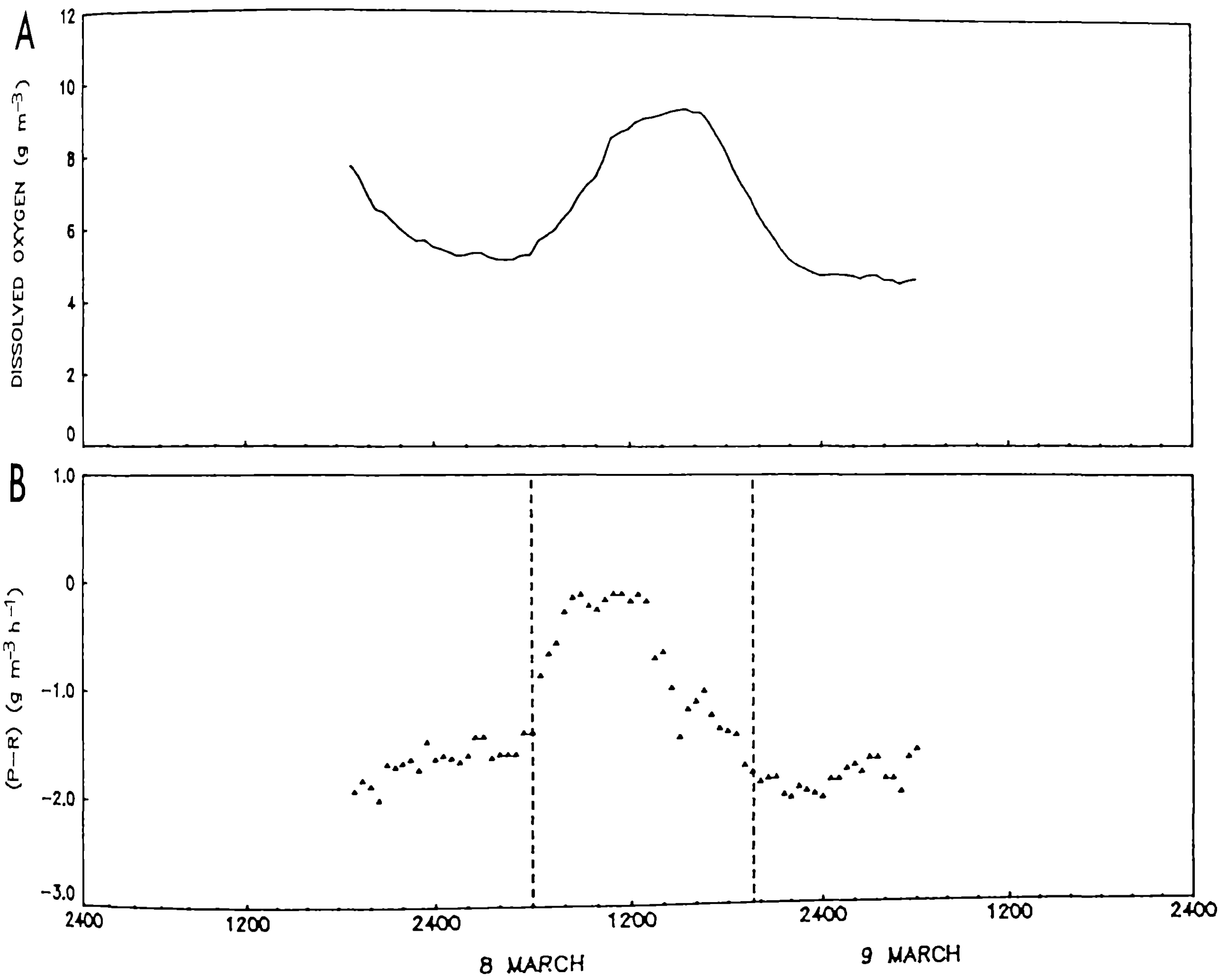


Figure 5.29 : Segment mean DO (A) and oxygen exchange rate (B) for the Waiotapu River sampled 8-9 March 1983.

	Photosynthesis g O_2 m^{-2}day^{-1}	Respiration g O_2 m^{-2}day^{-1}	P/R
Reporoa station	13.0	19.9	0.67
Reporoa-Homestead Road Reach	11.0	31.1	0.35

[a] Sampled on 8-9 March 1983 with a water temperature of 23.0 °C, and calculated using a single station method for Reporoa (upstream) and a 2 station method for the reach below the discharge; the reaeration coefficient was k_2 = 7.7±1.1 day^{-1}; and the mean depth 0.75±0.08 m (mean ±1 standard error) (Wilcock, 1984c).

TABLE 5.19: Summary of gross photosynthesis and respiration calculated from diurnal oxygen measurements in the Waiotapu River.

segment measurements in Figure 5.31. The values obtained in the morning were used for regression analysis because there was a tendency for hysteresis between increasing and decreasing light conditions. A geometric relationship (on logarithmic axes) showed greatest explained variances ($P<0.001$, $r^2 = 0.98$, $n = 6$) ($P<0.001$, $r^2 = 0.95$ $n = 6$) and ($P<0.001$, $r^2 = 0.97$ $n = 12$) respectively, with exponents of 0.77, 0.78 and 0.40.

The photosynthesis/PAR relationship for isolated Comsopogan sp, the dominant macrophyte present, was measured in the circulating chamber system and showed a hyperbolic relationship for the (P-R) versus PAR measurements (Fig. 5.32). Measurements were limited, however, to relatively low light intensities available from the fluorescent light sources, and a single maximum light measurement which was obtained with the assistance of the afternoon sun. The maximum laboratory light level was approximately 60% of the river maxima, after allowing for river water extinction. A geometric relationship for all data gave an exponent of 0.26 ($P<0.01$, $r^2 = 0.84$, $n = 7$) whereas excluding the high PAR measurement gave an exponent of 0.36 ($P<0.05$, $r^2 = 0.78$, $n = 6$).

The biomass of macrophytes present in the river was calculated using the river photosynthesis/PAR regressions (Fig. 5.31) and laboratory biomass specific photosynthetic rate coefficients with each measured for equivalent PAR conditions (Eqn. 5.4). Biomass estimates and laboratory biomass specific respiration rate coefficients were used to estimate the respiratory contribution of the macrophyte population (Eqn. 5.5) Table 5.20 summarises these results and shows a 2-fold greater biomass estimated from the production at 200 $\mu Em^{-2}s^{-1}$ for macrophytes below the discharge. In comparison with the results shown in Table 5.19, macrophyte respiration represents 35.2% at Reporoa and 22.5% of the total segment respiration. Although the daily gross photosynthetic production was less in the segment, the higher production at 200 $\mu Em^{-2}s^{-1}$ reflected the different PAR saturation kinetics observed (Fig. 5.32).

Drifting filaments of Comsopogan sp rapidly collected on objects placed in the river. The net captured biomass was 0.585 g DW m^{-3}. Measured ash free dry weight (AFDW) and assuming 50% carbon gives 0.216 g C m^{-3}. For the measured discharge of 2.5 m^3s^{-1} the total macrophyte export was

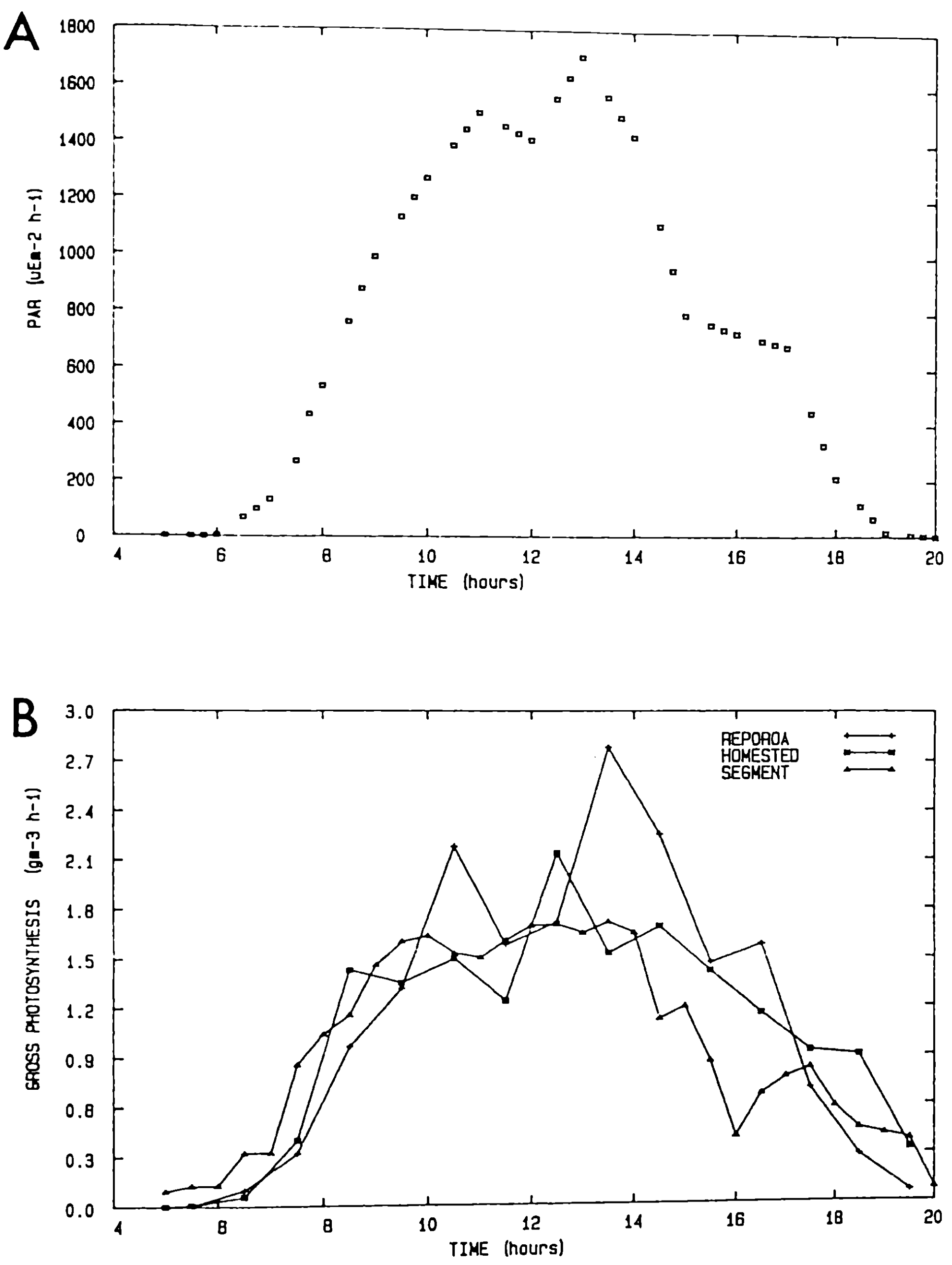


Figure 5.30 : PAR (A) and gross photosynthetic production (B) for the Waiotapu River sampled 8-9 March 1983. PAR was calculated as 45% of the Kipps surface measurement located 10 km distant and provided by the Forest Research Institute, Rotorua.

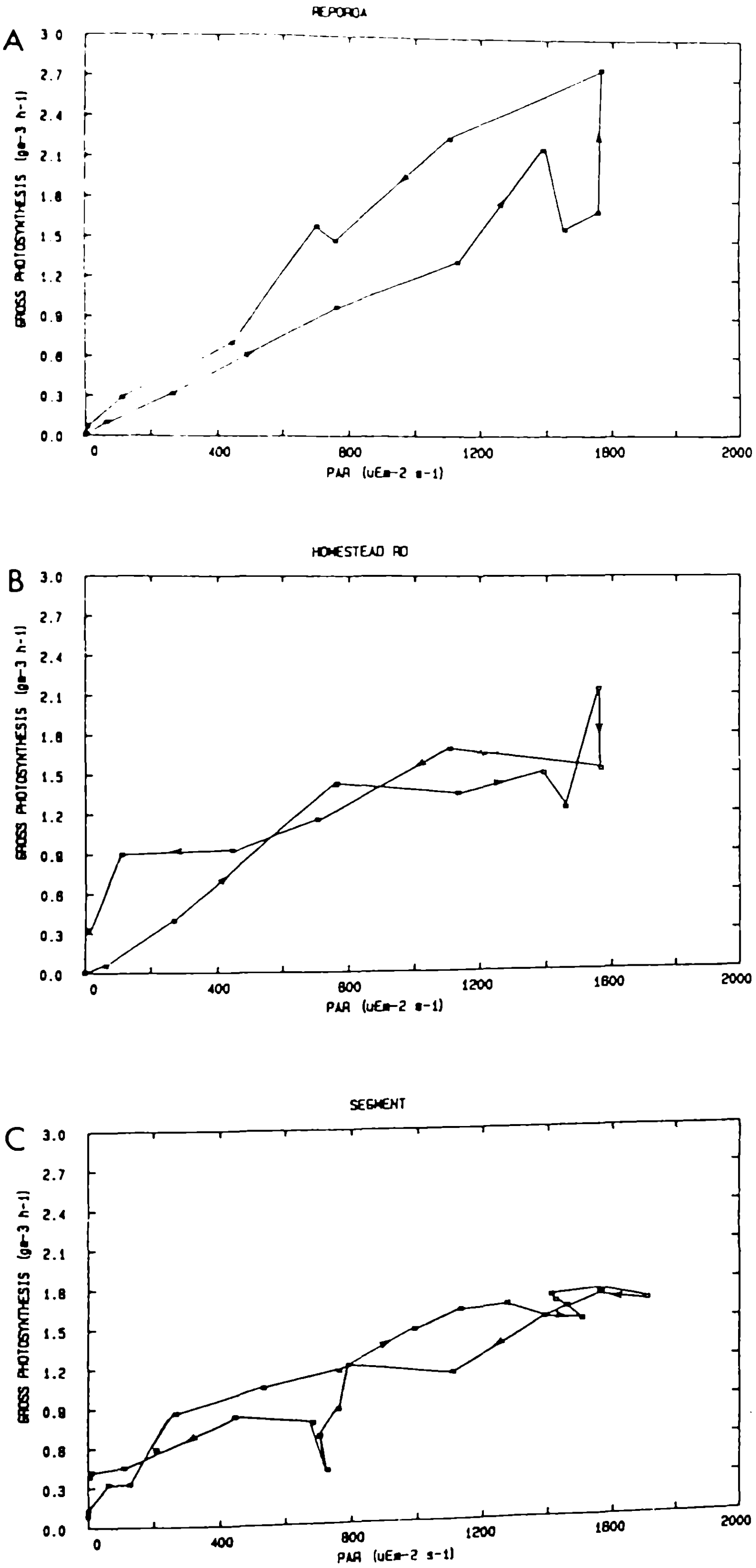


Figure 5.31 : Gross photosynthetic production efficiency for the Waiotapu River.
A Reporoa (upstream) station
B Homestead Road (downstream) station
C Segment (two station) calculation.

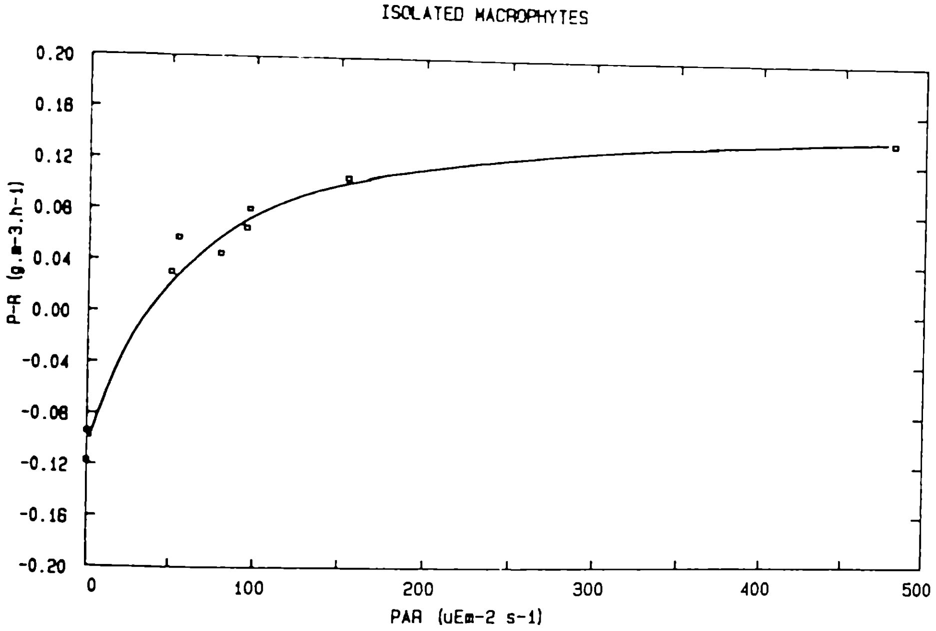


Figure 5.32 : Net photosynthetic production efficiency for isolated Comsopogan sp. macrophytes. Experiments were performed using the chamber under constant temperature laboratory conditions (20 °C) using cool white fluorescent lighting with sunlight assistance for the high light value. Curve represents a visual fit through the data points.

approximately 46.7 kg C day^{-1}, this weight was regarded as being derived from release from the river substratum.

Factors Affecting Chamber BUR Measurements

Several factors may affect the BUR values obtained from benthic chamber measurements. The latter may be influenced by (a) the natural heterogeneity of the bed and subsequent microbial colonisation resulting in variability of BUR measurements; (b) the measurement conditions within the chamber, including circulation velocity, electron acceptor (e.g. oxygen) and electron donor (e.g. glucose) concentrations.

Variability. Previous chamber measurements of BUR at 2 stations with visually similar bed characteristics, using equivalent circulation velocities, had shown the apparent benthic variability to be low at <10% (standard deviation) (Reporoa: sampled 17 January 1983, BUR = 22.8±2.2 g m^{-2}day^{-1} (n = 4), DO = 8.2 g m^{-3}; Homestead Road: sampled 18 January 1983, BUR = 14.8±0.7 g m^{-2} day^{-1} (n = 4, 2 sites with repeated measurements on each), DO = 5.0 g m^{-3}.)

This provided some confidence that under the conditions present in this river, the area sampled by the chamber system was sufficient to minimise the effects of heterogeneity of bed colonisation which might lead to variation in BUR measurement. Investigations involving other factors which influence the chamber BUR measurement, therefore concentrated on detailed measurements at single sampling sites.

Circulation velocity effect on BUR. An approximately linear relationship between BUR and velocity was observed over the velocity range 0.12-0.35 m s^{-1} (Fig. 5.33). Lowering velocity from 0.33 m s^{-1} to 0.12 m s^{-1}, decreased BUR from 27.5 to 6.0 g m^{-2} day^{-1}, with a slope of 115.0 g s m^{-3} day^{-1}. This difference in slope resulted from BUR decrease attributable to lower oxygen concentrations, rather than a physical effect of increasing or decreasing velocity (see following section). An approximate doubling of the circulation velocity resulted in a 3-fold increase in BUR. Unstirred replicate cores (0 m s^{-1} velocity) showed very low BUR at 0.30±0.03 g m^{-2} day^{-1} and 1.29±0.02 g m^{-2} day^{-1} for fine and coarse substrata respectively.

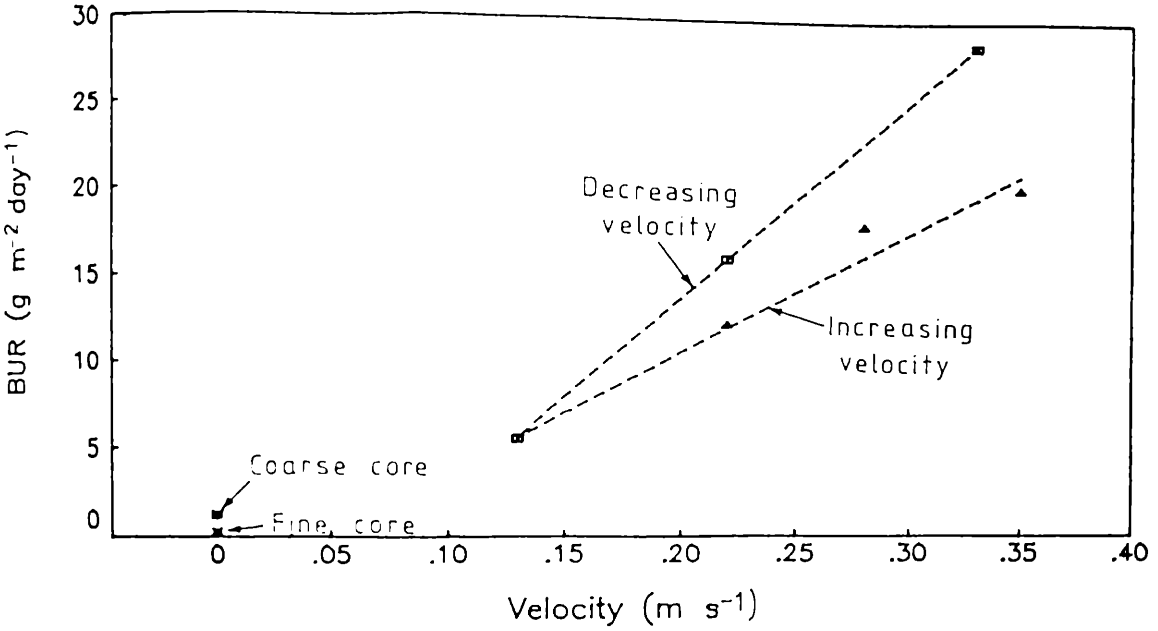


Figure 5.33 : Effect of chamber circulation velocity on initial BUR with glucose added (20 gm^{-3}). Measurement using large chamber and unstirred cores on fine and coarse particulates at Homestead Road site on Waiotapu River, sampled 9 March 1982, at river temperature 21 °C. River velocity 50 mm above bed was 0.40 ms^{-1}.

	Photosynthesis[a]	A_P,[b]	A_R,[b]	X_m,[c]	R_m,[d]	
	g O_2 m^{-3}h^{-1}	g O_2 g DW h^{-1}		g DW m^{-3}	g O_2 m^{-3}h^{-1}	g O_2 m^{-2}day^{-1}
Reporoa station	0.336	10.79x10^{-4}	6.19x10^{-4}	311.4	0.391	3.47
Reporoa-Homestead Road Segment	0.681			631.1	0.391	7.03

[a] river gross production measured at 200 μEm^{-2} s^{-1} from regressions for Fig. 5.31.

[b] biomass specific photosynthesis (A_P) at 200 μEm^{-2}s^{-1} and respiration (A_R) coefficients

[c] X_m refers to aquatic macrophyte biomass

[d] R_m refers to aquatic macrophyte respiration rate

TABLE 5.20: Macrophyte biomass and respiration rate calculated for the Waiotapu River

The equation relating the measured chamber BUR ($g\ m^{-2}\ day^{-1}$) to circulation velocity was found to be:

$$BUR = 115\ (U) - 9.41 \qquad 5.6$$

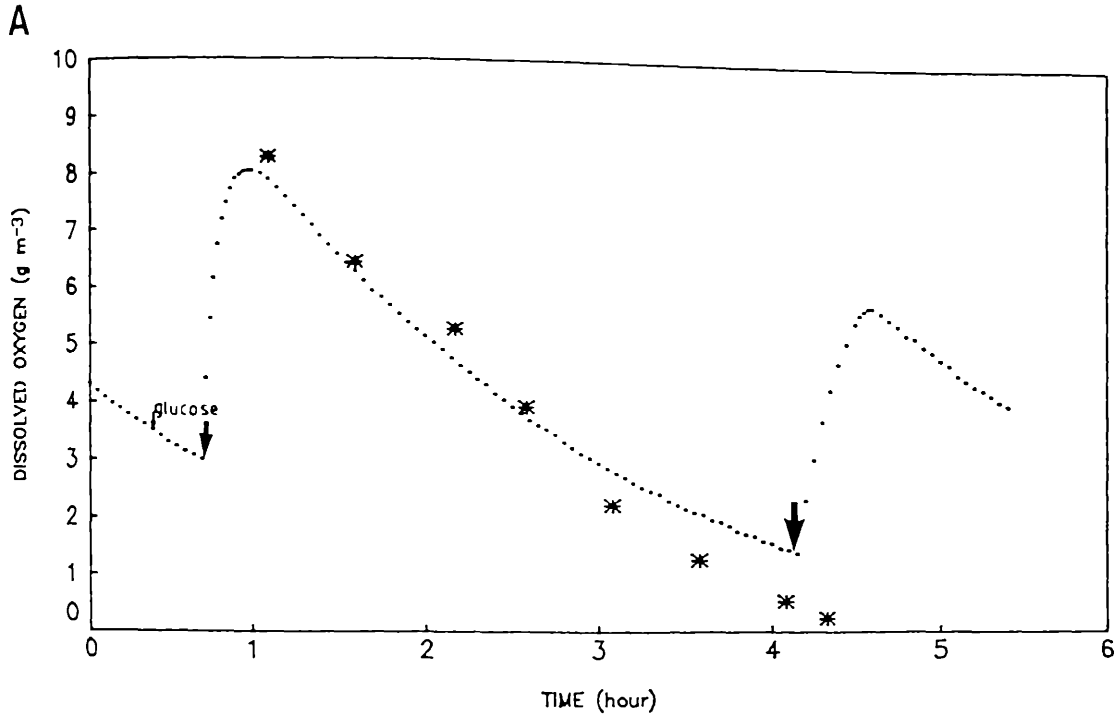
where U is the velocity >0.1 $m\ s^{-1}$; as measured for the decreasing velocity.

Oxygen and glucose effect on BUR. Figure 5.34 shows the changes in dissolved oxygen concentration with time as measured using the chamber. The fall in DO concentration proceeded at a decreasing rate and was repeatable following reoxygenation. Following glucose addition, to ensure that only the electron acceptor (oxygen) was rate-limiting, the decreasing rate of fall of DO concentration was still present, indicating that this decreasing rate of oxygen removal was not caused by electron donor depletion. Rather there is strong evidence that BUR (the slope of DO vs time plot) is influenced by the chamber DO concentration present at any one time.

The maximum measured BUR was 41.5 $g\ m^{-2}\ day^{-1}$ at 9.8 $g\ m^{-3}$ initial DO concentration, which had declined to 13.5 $g\ m^{-2}\ day^{-1}$ at 6.0 $g\ m^{-3}$ DO. Addition of glucose increased BUR by 35% and was rapidly depleted (Fig. 5.34B). The respiratory quotient (Qo_2) was estimated at 42% by linear regression of g O_2 consumed versus g glucose consumed ($P<0.05$, $r^2 = 0.989$, $n = 4$). Thus the BUR could be limited by either the electron acceptor or electron donor concentration present in the chamber system. The rapid depletion of electron donor could result in a change in rate limiting nutrient during the measurement period.

Because of the apparent BUR dependence on DO concentration, regression equations were required to facilitate BUR prediction and comparison between runs.

Analysis procedure. An example of the analysis procedure used to determine BUR kinetics is shown in Fig. 5.35. Figure 5.35 shows the DO vs time series used to calculate a slope, which is plotted against the mean DO value between the adjacent points, as shown in Figure 5.35B. As a mean DO decreased from 9 $g\ m^{-3}$ the BUR decreased from 38.0 $g\ m^{-2}\ day^{-1}$ to 8.5 $g\ m^{-2}\ day^{-1}$. A linear regression through these results showed an explained variance (r^2) of 0.843 but, as a model this was not considered satisfactory because of curvature shown by the data and the prediction of negative BUR values as DO approached zero. An improved



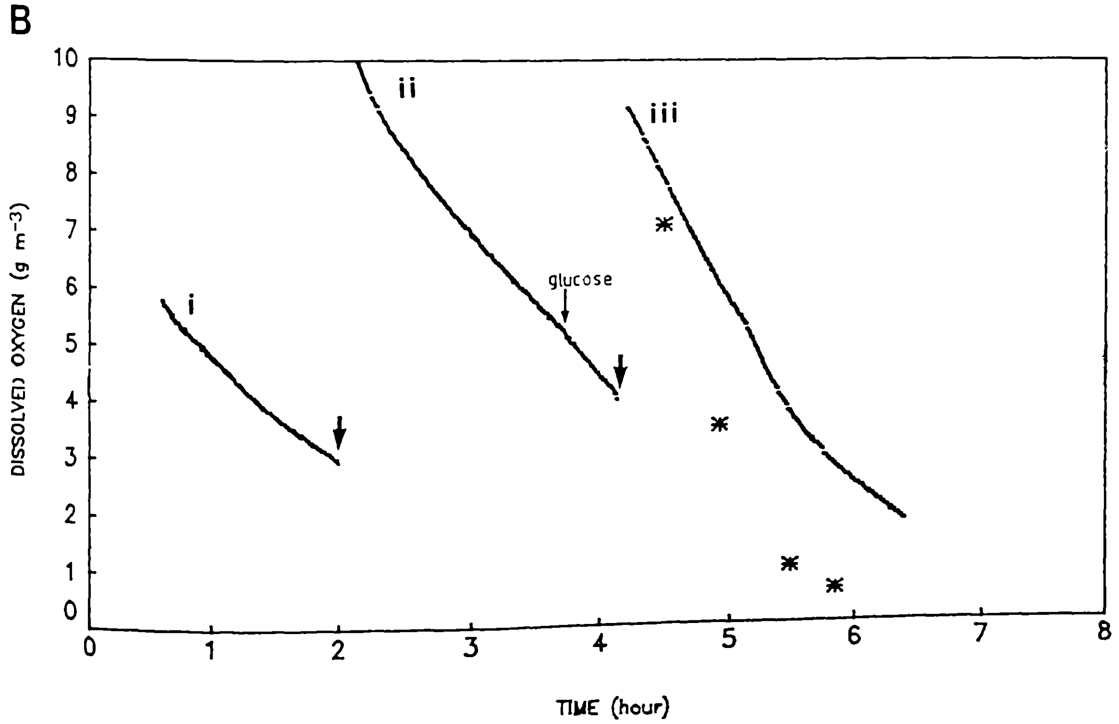


Figure 5.34 : Measurement of <u>in situ</u> chamber dissolved oxygen on fine particulates in the Waiotapu River, sampled 9 February 1983 (A) and 9 March 1983 (B). Heavy arrows denote time of reoxygenation using catalase/peroxide. Glucose addition to a final concentration of 20 gm^{-3} with concentrations x 0.5 shown (*).

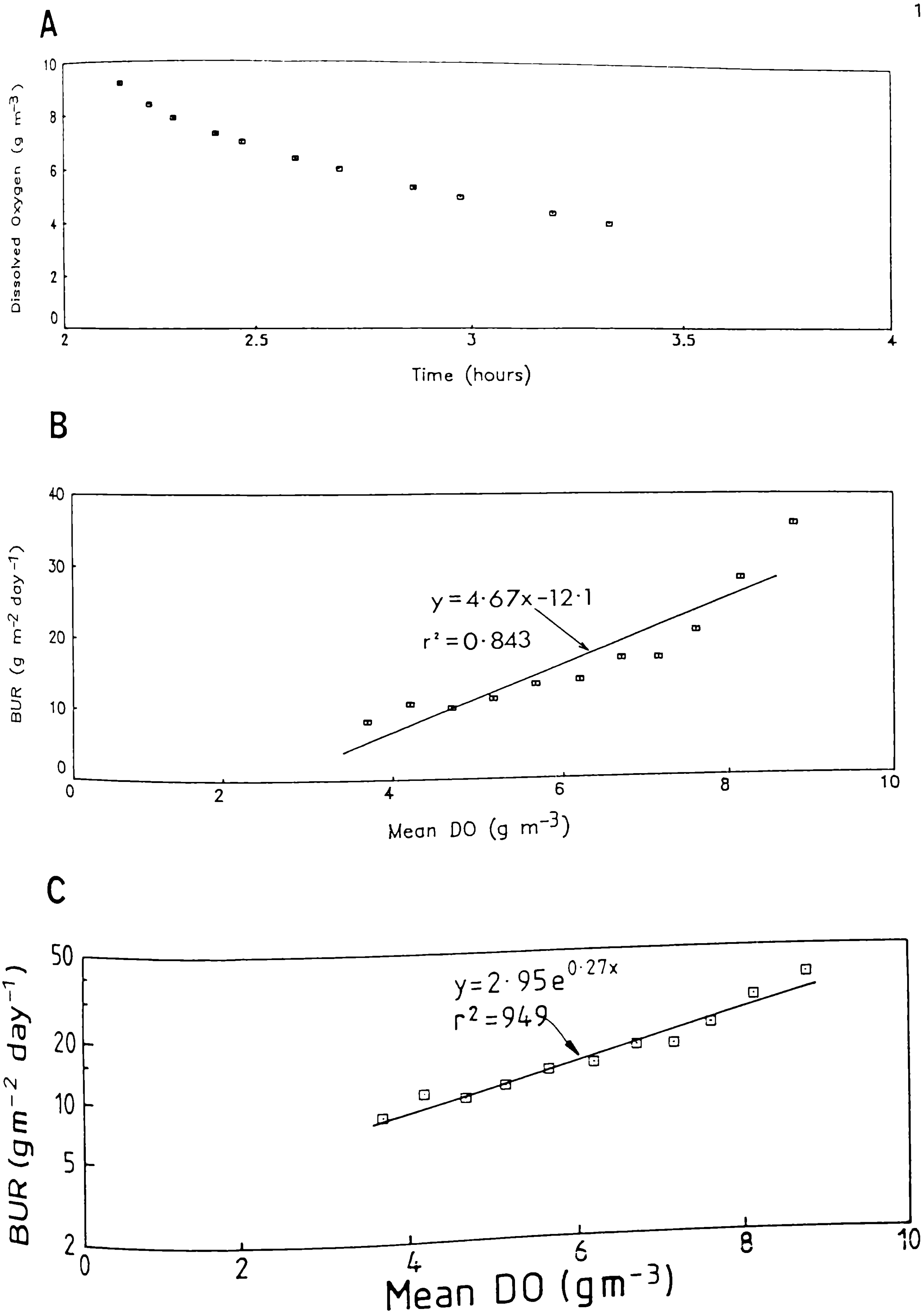


Figure 5.35 : Analytical procedure for BUR kinetics, small chamber on coarse particulates (B(ii) from Fig. 5.36).
A DO vs time
B BUR vs mean DO
C log BUR vs mean DO.

fit ($r^2 = 0.949$) was found for a semi-log regression (log BUR versus DO). This procedure was applied to all runs as shown in Figure 5.36 and detailed in Table 5.27. These results fit an exponential relationship of the form

$$N = Ae^{C_b K'} \qquad 5.7$$

where N is the BUR, g O_2 m^{-2} day^{-1}; K' is the chamber oxygen mass transfer exponent (OTE), $m^3 g^{-1}$; C_b is the dissolved oxygen concentration in the bulk liquid, g m^{-3}; and A is a constant for a given test, g O_2 m^{-2} day^{-1}. K' is thus an index of the degree to which river DO concentration affects the transfer of oxygen into the benthic layer. Following addition of glucose the value of K' significantly decreased (F-test, $P<0.01$), indicating a lesser response to decreasing oxygen concentrations.

Both the segment oxygen mass balance and the chamber measurements showed a decreasing BUR in response to decreasing DO concentration. Night-time segment mean DO concentration decreased from 8.0 to 5.2 g m^{-3} with a 20% segment BUR decline (Fig. 5.29B). Chamber BUR decreased from 19.0 to 11.2 g $m^{-2}day^{-1}$ (down 41%) under 'natural' conditions and from 26.9 to 19.5 g $m^{-2}day^{-1}$ (down 28%) with glucose addition (Fig. 5.34B and Table 5.21). Because the electron donor (glucose) was rapidly removed from the chamber system (see Fig. 5.34), the concentration naturally present might also be expected to be depleted rapidly within the chamber system. For this reason the experiment where glucose was added to the chamber was used for comparison with the segment oxygen mass balance.

Segment Oxygen Mass Balance

Estimates of the mass balance components outlined in Eqn 5.3 were made at a DO of 8.0 g m^{-3} and are shown in Table 5.22. Benthic respiration dominated in this segment with a negligible contribution from aquatic respiration and a 19.8% component attributable to macrophyte biomass.

The chamber BUR value and the calculated net BUR, showed substantial overlap of the 95% confidence intervals and provides confidence in the chamber measurements.

Comparison with the segment oxygen mass balance BUR (Table 5.22) using a chamber circulation velocity of 0.20 m s^{-1}, 50% lower than that of the river boundary velocity, gives an estimate of the BUR of 13.5±2.3 g $m^{-2}day^{-1}$ at 7.5 g m^{-3} DO (from Fig. 5.34); this is significantly below the calculated BUR of 28.3±3.4 g $m^{-2}day^{-1}$.

Chamber and sediment type	Velocity ms^{-1}	Expt[a]	K',[b] m^3g^{-1}	A,[b] g O_2 $m^{-2}day^{-1}$	r,[c]	n,[d]
large, fine	0.35	ii	0.211	3.74	0.921	10
		iii	0.129	9.97	0.891	8[e]

[a] corresponds to run numbers on Fig. 5.37B; only 2 experiments yielded sufficient values to allow calculation of the regression equations

[b] coefficients of Eqn 5.7 $BUR = Ae^{C_b K'}$

[c] linear regression correlation coefficient

[d] number of points used in determinations

[e] glucose added between runs (ii) and (iii) (20 gm^{-3} final concentration)

[f] values not determined as too few points

TABLE 5.21: Regression equation coefficients for large chamber DO uptake measurements in the Waiotapu River for 9 March 1983

Dissolved oxygen gm^{-3}	Segment oxygen demand			Chamber BUR
	Total[a]	Macrophyte g O_2 $m^{-2}day^{-1}$	BUR	g O_2 $m^{-2}day^{-1}$
8.0	35.3±2.5	7.0±2.3	28.3±3.4	28.0±2.8

[a] The data are for 8-9 March 1983, with water temperature 23.0 °C, segment length 2.8 km; travel time 2.0 h; reaeration coefficient, 7.7±1.1 day^{-1}; and mean depth, 0.75±0.08 m (Wilcock, 1984c).

[b] BUR = Total-Macrophyte (mean ± 1 standard error)

TABLE 5.22: Summary of chamber and night-time segment oxygen mass balance BUR estimates for the Waiotapu River

Macrophyte productivity (from Table 5.19) exceeded respiration by 57%, suggesting that a net input of macrophyte carbon was occurring within the segment.

Discussion

The benthic chamber was designed to provide a unidirectional, recirculating system with homogeneous turbulence and velocity comparable with the river benthic boundary velocity. Concomitant changes in velocity and BUR were observed in the chamber system, with unstirred core measurements, which would approximate BUR attributable to molecular diffusion processes, of about 5% of the maximum velocity chamber BUR. The approximate 3-fold increase in BUR resulting from a doubling of chamber circulation velocity, would indicate that turbulent dispersion processes were dominating the benthic mass transfer processes within the chamber system.

The influence of the chamber hydrodynamics, principally in terms of potential mass transfer limitation (or exaggeration), was a major concern regarding the suitability of the chamber technique. Chamber BUR measurements were found to fall within the 95% confidence range calculated from a segment oxygen balance (see Table 5.22), provided that boundary velocities were comparable. A chamber velocity half that of the river (0.40 m s^{-1}), resulted in a BUR estimate outside the calculated segment BUR range. Thus, failure to match the hydrodynamic conditions occurring in the river may result in considerable error in BUR estimation.

The segment oxygen mass balance showed a significant 20% decline in night-time respiration rate associated with a DO decline of 2.5 g m^{-3} which could not be attributed to a 0.2°C temperature decease. Chamber BUR values at comparable DO concentrations, decreased by 41% with river water and by 28% with glucose addition in the chamber system. The filamentous algae, Comsopogan sp, may have contributed to the observed decline in segment night-time respiration rate. Owens and Maris (1964) have reported respiration rate increases of up to 200% for aquatic macrophytes for DO changes in the range 1.2-17 g m^{-3} at 20°C. Since calculated macrophyte respiration accounts for less than 20% of the total (Table 5.22), a decline of the above magnitude would at most result in a 6.5% decrease in respiration rate. Macroinvertebrates are reported to show a respiration rate dependence on DO (Lasserre, 1976; Nagell, 1973). The mobile river bed, however, resulted in very low numbers of aquatic invertebrates present. Assuming ½ order diffusion kinetics for BUR (Baillod and Boyle, 1970; Atkinson and Davies, 1974; La Motta, 1976b; Williamson and McCarty, 1976a; Harremoes, 1976, 1977), a decrease in DO from 8.0 to 5.2 g m^{-3} would result in a 19.5% BUR decline, providing an explanation for this observation.

Calculated net macrophyte carbon fixation for Reporoa was 750 kg C day^{-1}, and for the segment 125 kg C day^{-1} (calculated from Tables 5.19 and 5.20). Based on a discharge of 2.5 m^{3}s^{-1}, measured export of macrophyte biomass amounted to 46.7 kg C day^{-1}, an order of magnitude less than the daily amount fixed. This suggests that the river macrophytes were not in steady state but were increasing net segment biomass.

The catalase/peroxide reoxygenation method made it easy to obtain reproducible measurements over an area of bed. Reoxygenation showed a reproducible decreasing chamber BUR from saturation DO. Methodological artefacts which might cause this type of result were not responsible. Sediment particles which may have been disturbed on chamber installation were removed by operation for an initial period in open cycle with the surrounding river water. Possible chamber leaks to the surrounding flow were investigated by visual inspection and by dye injection. The possibility that electron donors became depleted during the measurement period, was investigated by first adding glucose then reoxygenating the system (see Fig. 5.34). Although the BUR increased following glucose addition, a decreasing rate occurred from saturation DO following reoxygenation, indicating that although the concentration of electron donor (glucose) may affect the measured BUR, the kinetics were strongly influenced by the electron acceptor (oxygen) concentration.

The relationship between sediment oxygen demand and oxygen concentration in the overlying water was studied by Edwards and Rolley (1965) and Hargrave (1969b) using undisturbed cores. They proposed an exponential relationship, but the scatter in their values was high. Edberg and Hofsten (1973) showed that both exponential and linear relationships were applicable for high oxygen concentrations, whereas Graneli (1977) reported that a linear expression provided the best fit, independent of incubation temperature. I have found that for lotic systems, exponential functions best explain the dependence of BUR on DO concentration. The form of relationship may, however, be influenced by the effects of non steady-state dispersion kinetics occurring within the chamber system. These factors are discussed in a later section dealing with BUR modelling in these sediment systems (Chapter 7).

The measurements made in the chamber clearly showed a marked response to circulation velocity and glucose addition, factors which support the conceptual model proposed for lotic benthic systems (Chapter 1). Thus the BUR may be limited by either electron acceptor or electron donor concentrations, with natural turbulence levels greatly influencing sediment-water exchange processes.

The average segment submerged macrophyte biomass of 0.32 kg C m^{-3} (assuming 50% of dry weight as carbon) produced 0.05 kg C $m^{-3}day^{-1}$ giving a daily activity coefficient of 0.013 kg C assimilated for each kg of biomass C day^{-1}. For Reporoa this activity coefficient was 0.056 kg C kg C^{-1} day^{-1}. These values are low compared with those obtained with planktonic algae which range from 0.01-11.3 kg C kg C^{-1} day^{-1} (Berman and Pollinger, 1974).

The inverse of the activity coefficient (i.e. biomass ÷ primary productivity = turnover time) gives a measure of the time required to renew the carbon of the standing crop. For Reporoa the turnover time was 18.0 days whereas the segment value was estimated at 76.5 days. This difference was largely derived from the photosynthesis/PAR saturation response of the two analyses since these were used for biomass estimation. Thus, because of macrophyte dominance of river photosynthetic production, response to environmental changes would most likely be slower than would a system with benthic or planktonic algal dominance.

Both the river and laboratory studies showed the photosynthesis/PAR response was best fitted by geometric functions. The Reporoa station values showed minimal deviation from a linear relationship (with exponent 0.77) whereas the segment values showed a stronger hyperbolic response (with exponent 0.40). The laboratory measurements showed greater saturation response (with exponent 0.26) and showing a slight decrease with the single high PAR value excluded (exponent 0.36). The reason for these differences are unknown. CO_2 depletion caused by prolonged incubation or insufficient pH buffering (Matai and Haase, 1977) was not responsible because of the short time periods used for incubations. Better definition of photosynthesis/PAR relationships, and the factors affecting them, would certainly be necessary for application of these methods to a similar river situation where macrophytes were more dominant.

A combination of field and laboratory measurements as used for this river system provide a suitable technique for estimating the respiratory contribution of aquatic macrophytes to the river system, provided that rivers are shallow and self-shading effects are minimal. When dense macrophyte beds occur, or grow throughout a considerable depth range, estimation of an average or adequate description of the environmental light climate becomes difficult. This then severely limits the ease of application of laboratory biomass specific photosynthesis/PAR and respiration relationships.

5.3.3 Benthic oxygen uptake rate (BUR) measurements in situ, sediment intrinsic activity, and calculation of the depth of sediment oxygen penetration

This section outlines the chamber BUR and sediment intrinsic activity (I) measurements performed in the Waiotapu River, which were used to estimate the depth of penetration of oxygen into the sediments, and calculate the effective dispersion coefficient.

The mobile pumice bed of the Waiotapu River effectively prevented the colonisation of attached filamentous microorganisms. The highest BUR measured for in situ conditions was 39.0 g O_2 m^{-2} day^{-1} occurring 2.2 km below the outfall, with a maximum benthic production of 32.2 g O_2 m^{-2} day^{-1} above the outfall (Table 5.23). A minimum BUR 8.8 g m^{-2} day^{-1}, was measured upstream (for 0.35 m s^{-1} measurements).

The longitudinal survey (17 January 1983) showed a BUR increase of 2.6-fold below the outfall together with an increase of 3.3-fold; $P<0.001$) in intrinsic activity (Table 5.23). BUR values were comparable at 0 and 2.2 km downstream, but intrinsic activity had declined to 52% of the value at 0 km ($P<0.05$). The calculated minimum depth of benthic oxygen penetration was 4.3 cm at the upstream station, decreasing below

Date Station (km)	Temp °C	DO gm^{-3}	Velocity[a] ms^{-1}	BUR $gm^{-2}day^{-1}$	Glucose %	Gross Photosynthesis gm^{-2} day^{-1}	Sediment Intrinsic Activity gm^{-2} day^{-1}	Glucose %	Depth of Oxygen Penetration cm	Dispersion Coefficient cm^2 s^{-1} × 10^{-3}	Particulate Description
REPOROA BRIDGE **(-0.55)**											
17 Jan 1983	22.0	7.5	0.35	8.8	0	32.2	2.05±0.20 (2)	64	4.3±0.4	5.8±0.6	
REPOROA OUTFALL **(0)**											
17 Jan 1983	24.0	8.5	0.35	22.8±2.2 (4)	33	15.8	6.81±0.09 (2)	0	3.3±0.3	10.4±1.0)	
			0.20	12.8		5.6			1.9±0.1	3.2±0.1)	
HOMESTEAD ROAD **(2.2)**											
29 Nov 1982	21.0	6.5	0.35	39.0	0		6.10±0.60 (2)	31	6.4±0.6	44.5±4.5)	
		7.5	0.20	16.3	0				2.6±0.3	7.8±0.8)	
17 Jan 1982	24.0	5.0	0.35	20.5	12	0	3.53±0.16 (2)	54	5.8±0.3	27.6±1.3)	fine
			0.20	20.2	7	0	3.53	54	5.7	26.8)	
		5.0	0.20	28.8	109	0	3.5^b		7.6	47.2	coarse
9 Feb 1983	21.0	6.2	0.35	21.3	-	-	3.53±0.53 (2)	0	6.0±0.9	24.0±3.6)	fine
	21.0	6.0	0.20	8.5	-	-			2.4±0.4	4.0±0.6)	
	21.0	5.0	0.20	5.4	-		3.18±0.85 (2)	35	1.7±0.5	2.1±0.6	coarse
10 Feb 1983	21.0	4.0	0.20	5.0	24		3.45±0.02 (2)	58	1.5±0	2.1±0	coarse
	21.0	5.0	0.20	4.2	-		2.59±0.07 (2)	110	1.6±0.1	1.6±0.1)	fine
	21.0	4.4	0.35	11.9	0				4.6±0.1	14.4±0.4)	
9 Mar 1983	25.5	6.0	0.20	16.3	64	-	2.72±0.35 (2)	77	6.0±0.8	19.0±2.5	coarse
	25.5	5.4	0.20	15.9	10	-	4.49±0.81 (2)	61	3.5±0.6	13.3±2.4)	fine
	25.5	5.6	0.35	17.5	35	-			3.9±0.7	14.2±2.6)	
AARO Road **(6.9)**											
17 Jan 1983	24.0	4.0	0.35	12.6	-	-	4.97±0.56 (2)	30	2.5±0.3	9.3±1.1	

mean ± 1 standard deviation (number of observations)

[a] comparable river, velocity 0.4 ms^{-1}

[b] assumed value equal to fine particulates

TABLE 5.23: BUR, sediment intrinsic activity and benthic dispersion coefficients for the Waiotapu River the outfall and increasing to 5.8 cm penetration at 2.2 km downstream. No photosynthetic production was detected at the 2.2 km and 6.9 km stations.

BUR values in situ were frequently limited by river electron donor concentrations, with BUR increases ranging from 0% to 109% in response to glucose additions (Table 5.23). Sediment intrinsic activities generally showed a greater rate increase than the corresponding chamber measurement (Table 5.23).

Estimates of the chamber respiratory quotient (Qo_2) were made on 2 occasions, based on glucose and oxygen concentration changes. Results are shown on Figure 5.34A for 10 February 1983 where the initial glucose uptake rate was 81.1 g glucose m^{-2} day^{-1} ($P<0.05$, $n=4$) and Qo_2 was 23% ($P<0.001$, $r^2=0.981$, $n=7$). For 9 March 1983 (Fig. 5.37B), the initial glucose uptake was 56.1 g glucose m^{-2} day^{-1} ($P<0.05$, $n=4$) and Qo_2 was 43% ($P<0.05$, $r^2=0.994$, $n=4$).

Intrinsic activity measurements on pumice substrata were shown to be strongly affected by the degree of agitation. Continuous stirring gave I values 2.8-fold greater than 1 hourly measurements, 1.7-fold greater than ½ hourly measurements, and 1.3-fold greater than samples agitated at 15 min intervals (results shown in Appendix 1). For technical reasons measurements could only be made using agitation at 15 min intervals and may therefore have underestimated the 'true' intrinsic rate. Measurements ranged from 2.05 g m^{-2} day^{-1} above the discharge to 6.81 g m^{-2} day^{-1} below the discharge, with a maximum value of 7.24 g m^{-2} day^{-1} with glucose supplementation (Table 5.26).

Continuously stirred I value measurements with small quantities of sediment material, showed no apparent decline in activity with decreasing DO concentration. These results are presented in Chapter 7.

The effective dispersion coefficient calculated from chamber measurements, comparable with river conditions (i.e. 0.35 m s^{-1} circulation velocity), ranged from 5.8×10^{-3} cm^2 s^{-1} upstream to a maximum of 47.2×10^{-3} cm^2 s^{-1} on a coarse particulate section (Table 5.23).

A summary of the results shown in Table 5.23 is provided in Table 5.24.

Station (km)	n	BUR gm^{-2} day^{-1}	Intrinsic Activity gm^{-2} day^{-1}	Depth cm	Dispersion Coefficient cm^2 s^{-1} x 10^{-3}
Reporoa Bridge (-0.55)	1	8.8	2.05	4.3	5.8
Reporoa outfall (0)	1	22.8	6.81	3.3	10.4
Homestead Road (2.2)	5	22.0±10.2	4.56±1.56	5.7±1.5	24.9±12.4
Aard Road (6.9)	1	12.6	4.97	2.5	9.3

[a] large chamber (0.35 ms^{-1}) measurements only

TABLE 5.24: Summary statistics for Table 5.23[a]

5.3.4 Distribution of microbial biomass and activity in relation to benthic particle size

The moving dunes present in the Waiotapu River created areas of fine and coarse particulates, the latter generally associated with the leading edge of the dune. Comparisons of BUR values for these two types of bed material were made using two small chambers (see section 4.2). Additionally, intrinsic activity and biomass measurements were made on fine and coarse particulates and wet-seived size fractions from surface particulates.

Chamber BUR measurements. The small chamber BUR measurements (velocity 0.21 m s^{-1}) generally gave lower BUR values ($P<0.1$, 59%, $n=6$) than did those from the large chamber (velocity 0.35 m s^{-1}) on comparable substrata. Comparison of fine and coarse particulate BUR measurements on 3 occasions showed substantially differing BUR on only 1 occasion, with higher values for the fine particulates (February 1983, Table 5.23). The maximum calculated depth of sediment oxygen penetration and dispersion coefficient were not consistent in relation to the sediment fraction or magnitude, with D_s values varying by an order of magnitude for the coarse particulates between surveys. This may have been caused by varying thickness of microbial biofilms which would affect the dispersion coefficient estimates.

The large chamber was applied to different particulate size areas on 1 occasion. BUR was greater for the coarse particulates (1.3-fold) with a greater depth of oxygen penetration and D (1.7-fold) (17 January 1983, Table 5.23).

ATP

Particulate fraction mm	ATP µg g^{-1}	∑ATP fraction µg	∑ATP/Total %
>4	0.50±0.16 (4)	15.79	6.9*
1.7-4	1.12±0.08 (4)	86.91	37.8
<1.7	1.44±0.17 (4)	125.70	**54.7**
interstitial water	-	1.24	2.0

INTRINSIC OXYGEN UPTAKE RATE

Particulate fraction mm	I µg 02 g DW^{-1} ha^{-1}	∑I fraction µg O_2 h^{-1}	∑I/Total %
>4	12.5	0.33	3.7*
1.7-4	46.3	3.57	39.4
<1.7	59.0	5.15	**56.9**

DRY WEIGHT

Particulate fraction mm	Fraction/Total %
>4	13.9
1.7-4	40.4
<1.7	45.7

*under-representation; bold face indicates over-representation

TABLE 5.25: Biomass and intrinsic activity for sediment particle size fractionation from the Waiotapu River

Intrinsic activity and biomass in relation to benthic particle size. This section presents the results of measurements made on sieved sediment particulate fractions from Homestead Road surface sediments (sampled 19 January 1983).

Results of biomass and intrinsic activity measurements on 3 sieved size fractions of surface (0-1 cm) sediments are shown in Table 5.25. The 3 fractions >4 mm, 1.7-4 mm and <1.7 mm represented 13.9%, 40.4% and 45.7% of the dry weight respectively. ATP measurements of biomass were significantly lower (~50%) for the >4 mm fraction ($P<0.001$), with this size class being under-represented in relation to total biomass, whereas, the <1.7 mm fraction was over-represented. Interstitial water present was estimated to contain only 2% of the total biomass present. However, this figure would be expected to be highly variable depending on handling and water content.

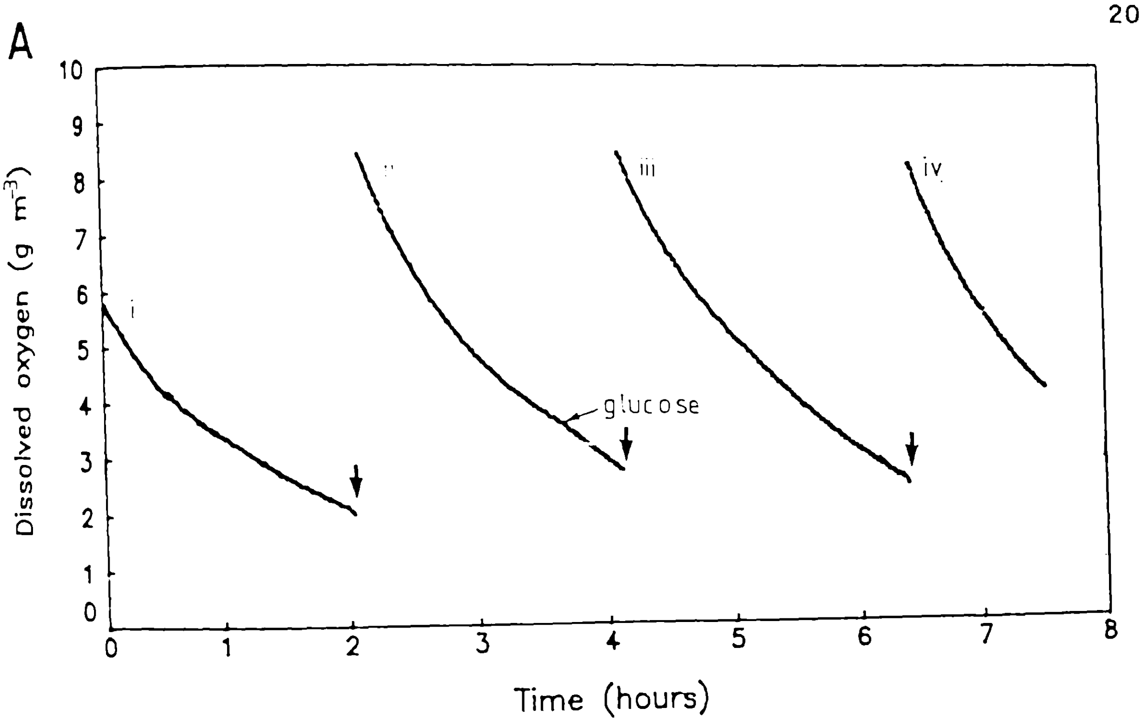
Intrinsic activity measurements increased with decreasing particle size from 12.5 $\mu g\ O_2\ g\ DW^{-1}\ h^{-1}$ to 59.0 $\mu g\ O_2\ g\ DW^{-1}\ h^{-1}$ resulting in under-representation and over-representation of size classes respectively.

5.3.5 The kinetics of chamber BUR measurements

This section presents data obtained for chamber BUR kinetics and details response to electron donor concentration (as glucose), and electron acceptor concentration (as oxygen).

Effect of oxygen on BUR

Figures 5.36A and B show dissolved oxygen concentration changes with time as measured using the small chambers on fine and coarse particulates respectively. The fall in DO concentration proceeded at a decreasing rate, an effect which was repeatable following reoxygenation. After adding glucose in order to ensure that only the electron acceptor (oxygen) was rate-limiting this decreasing rate of fall of DO concentration was still present, indicating that the decreasing rate of oxygen removal was not caused by electron donor depletion. Rather there was strong evidence that BUR (the slope of DO vs time plot) was influenced by the chamber DO concentration present at any one time. This dependence can be satisfactorily modelled by an exponential relationship [eqn (5.7)] (Fig. 5.37).



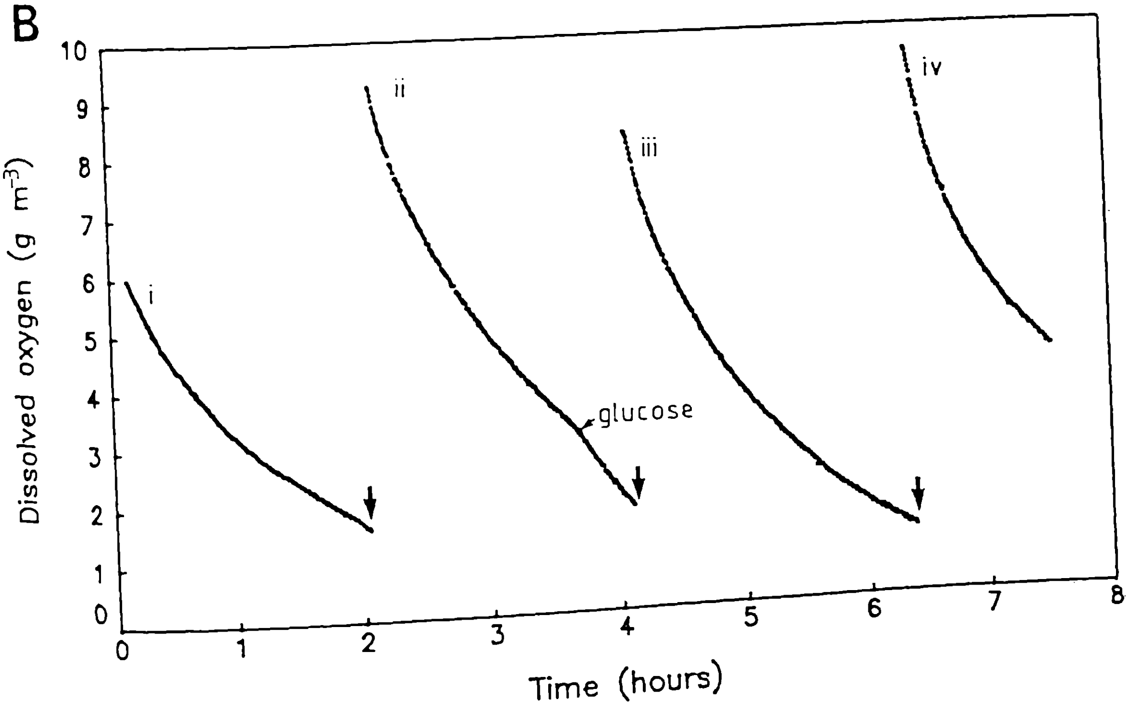


Figure 5.36 : Measurement of in-situ chamber dissolved oxygen concentration progressions for coarse (A) and fine (B) particulates. Homestead Road station on Waiotapu River, sampled 9 March 1983 at river temperature 21 °C, depth 0.4 m, velocity 50 mm above bed 0.40 ms^{-1}. Heavy arrows denote time of reoxygenation using catalase/peroxidase. Glucose addition to final concentration 20 gm^{-3}. Identification of runs (i-iv) adjacent to curves.

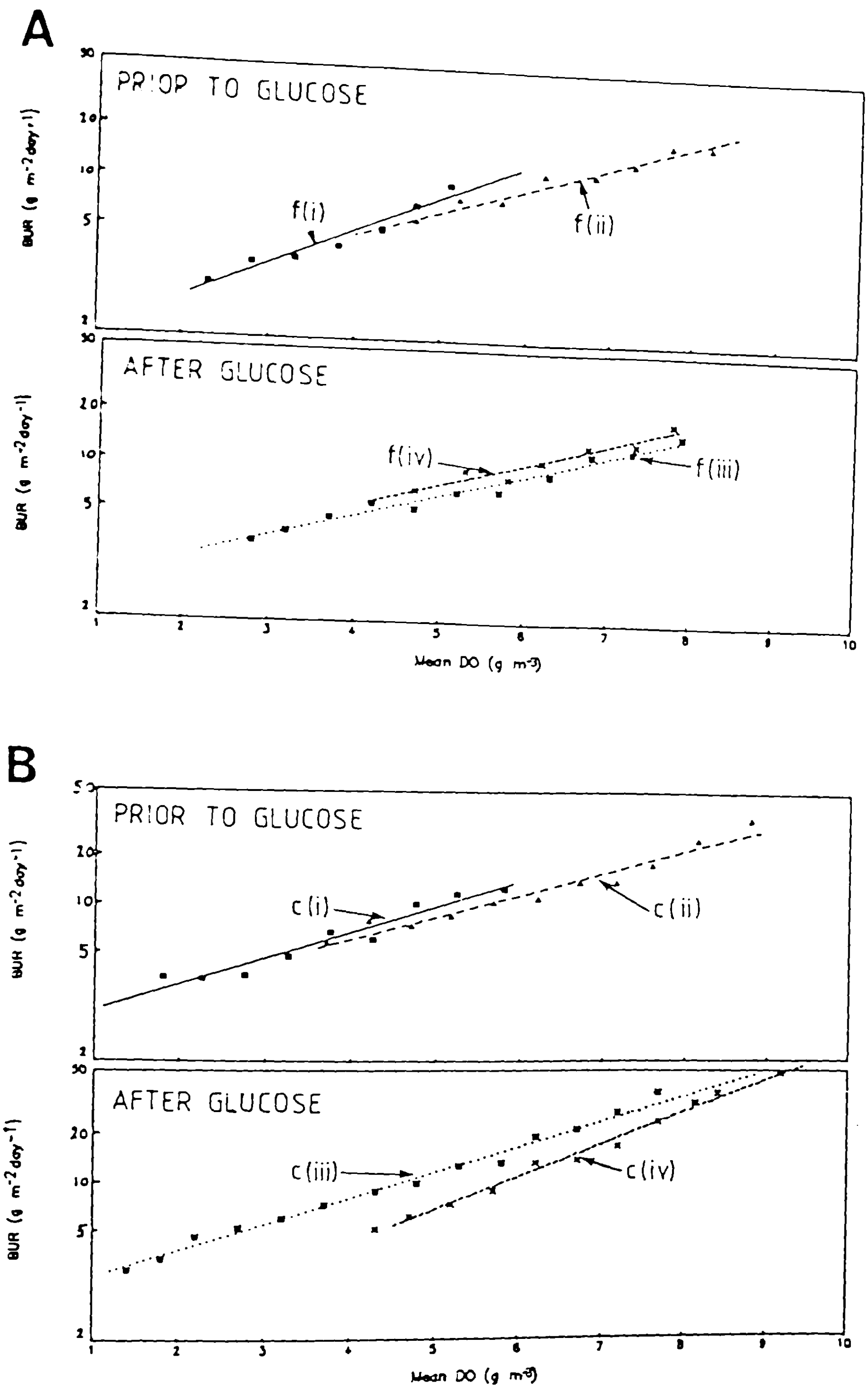


Figure 5.37 : Plots of log BUR vs DO for small chambers from (Fig. 5.36). All sampled 9 March 1983.

A Small chamber, fine particulates (f), velocity 0.20 ms^{-1}

B Small chamber, coarse particulates (c), velocity 0.20 ms^{-1}.

Symbols:

Prior to glucose addition (BOD_5 1.0 gm^{-3})

■——■ run (i)

Δ----Δ run (ii)

glucose added (20 gm^{-3})

★····★ run (iii)

x----x run (iv)

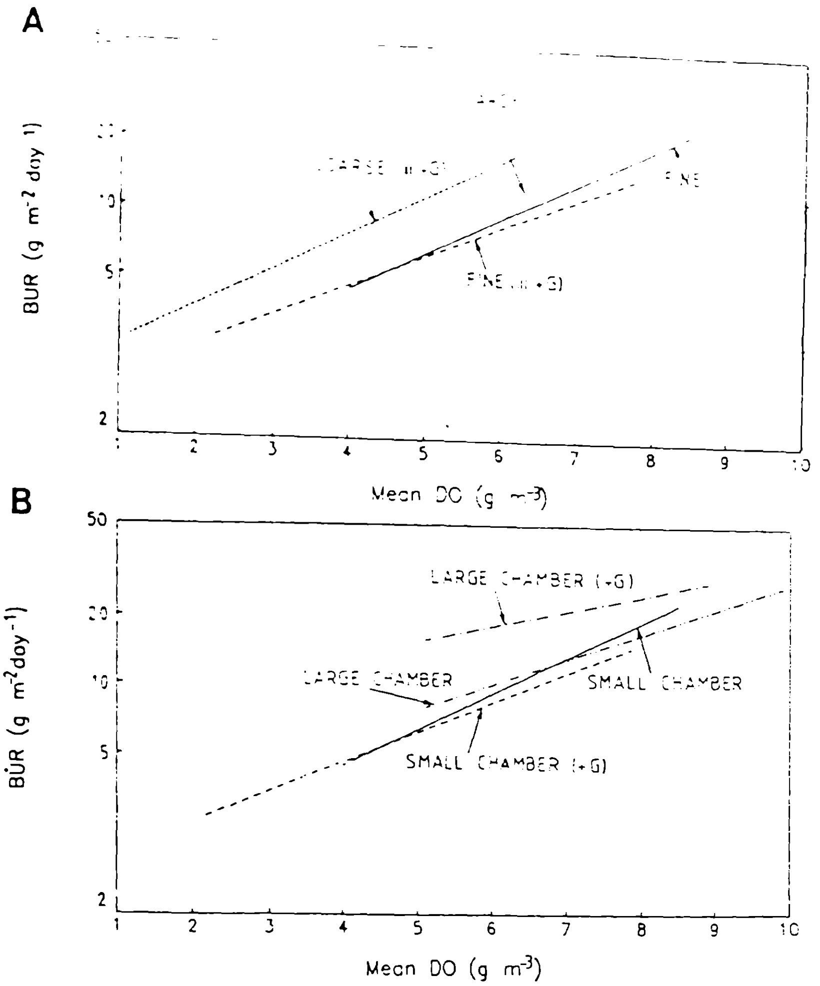


Figure 5.38 : Overlay plots (regression lines only) of log BUR vs DO for runs prior to and after glucose addition(+G).
A Small chamber (circulation velocity 0.20 ms^{-1}) on fine (f) and coarse (c) particulates.
B Comparison of the effect of circulation velocity on fine particulates, small chamber (velocity 0.20 ms^{-1}) and large (LC) (velocity 0.35 ms^{-1}).

Note (a) labels on graphs refer to run number for small chambers in Fig. 5.36.
(b) glucose added to 20 gm^{-3} between runs (ii) and (iii).

Chamber and Sediment type	Circulation Velocity, $m\ s^{-1}$	DO, $g\ m^{-3}$	BUR (run number),[a] $g\ m^{-2}\ day^{-1}$ (i)	(ii)	(iii)	(iv)	Glucose response,[b] %
large, fine	0.35	8	-	20.3	28.0[b]	-	38
		4	9.2	8.7	16.7[b]	-	92
small, fine	0.20	8	-	22.6	18.2[b]	21.9	-11
		4	7.3	6.9	7.0[b]	8.0	2
small, coarse	0.20	8	-	24.7	36.3[b]	30.1	47
		4	9.4	8.5	10.8[b]	6.4	27
intrinsic rate, fine (0-1 cm)	-	8	4.5 ± 0.8		7.2 ± 0.6[c]		61
intrinsic rate, coarse (0-1 cm)	-	8	2.7 ± 0.4		4.8 ± 0.2[c]		78

[a] refers to labels on Figure 5.36.
[b] glucose (20 gm^{-3}) added between runs (ii) and (iii)
[c] glucose (20 gm^{-3}) added

TABLE 5.26: Comparison of BUR for large and small chambers, sampled at Homestead Road, on Waiotapu River, 9 March 1983

Effect of Glucose on BUR

Prior to glucose addition, replicate runs at 4 g m^{-3} of DO (i) and (ii) varied by less than ±7%, Table 5.26. The BUR at equivalent DO consistently showed a slight decrease with reoxygenation (i.e. runs (ii) < (iii) in Table 5.26) and suggested that rapid electron donor depletion may be affecting the BUR (see also Fig. 5.37). Table 5.26 indicates, however, a substantial BUR increase followed glucose addition for the large chamber.

The maximum BUR value recorded was for glucose supplemented measurements on coarse substrata with the small chamber, at 49.0 g m^{-2} day^{-1} at 9 g m^{-3} DO, which had declined to 11.0 g m^{-2} day^{-1} and 4 g m^{-3} DO (see slopes of Fig. 5.36A). Results obtained for the small chamber showed that over the complete range of DO studied, that glucose addition did not cause a significant change in BUR for the fine particulates ($P>0.2$, $F^2 = 0.65$, $n = 34$), but significantly increased BUR for coarse particulates ($P<0.01$; $F^2 = 5.5$, $n = 44$) see Figure 5.38A. Using the large chamber, fine sediments showed a response to glucose addition, see Figure 5.38. Regression equations are summarised in Table 5.27.

Details of curve fitting coefficients to linear, exponential and geometric relationships are given in Appendix 3.

The reasons for this difference in BUR response to glucose addition may be attributed to circulation velocity and chamber turbulence influencing mass transfer, and the influence of transient state diffusion kinetics. These effects are discussed in Chapter 7.

5.3.6 The relative importance of planktonic and benthic biomass

Planktonic and benthic biomass carbon estimates based on ATP assays are shown in Table 5.28 for late spring and summer surveys. Planktonic biomass showed a progressive decline downstream of 36% ($P<0.05$) to the 10 km station. Benthic biomass measurements, together with chamber BUR estimates of the depth of benthic oxygen penetration, showed that benthic biomass was approximately 30-fold greater than planktonic biomass on each sampling occasion. Because of the relatively small longitudinal changes in planktonic biomass, only one station was sampled on the summer survey and this measurement was used for calculations over

Chamber and sediment type	Velocity, ms^{-1}	Run[a]	K,[b] m^3g^{-1}	A,[b] gm^{-2} day^{-1}	r[c]	n[d]
small, fine	0.20	i	0.407	1.43	0.976	7
		ii	0.297	2.10	0.983	9
		iii	0.247	2.61	0.988	11)
		iv	0.252	2.92	0.964	7)[e]
Small, coarse	0.20	i	0.310	2.71	0.970	9
		ii	0.266	2.94	0.974	11
		iii	0.304	3.19	0.993	15)
		iv	0.388	1.35	0.995	10)[e]
large, fine	0.35	i	-[f]	-	-	-
		ii	0.211	3.74	0.921	10
		iii	0.129	9.97	0.891	8[e]

[a] corresponds to run numbers on Figure 5.36. (large chamber data not shown)

[b] Coefficients of Eqn 5.7 BUR = Ae^{C_bK}, where C_b is the dissolved oxygen concentration (gm^{-3})

[c] linear regression correlation coefficient

[d] number of points used in determinations

[e] glucose (20 gm^{-3}) added between runs (ii) and (iii)

[f] values not determined as too few points

TABLE 5.27A: Coefficients for exponential equations describing BUR

	SC/c+G	SC/c	SC/f+G	SC/f	LC/f+G	LC/f
LC/f	N.A.	N.A.	**	**	**	-
LC/f+G	N.A.	N.A.	**	**	-	
SC/f	**	*	N.S.	-		
SC/f+G	**	**	-			
SC/c	**	-				
Sc/c+G	-					

Abbreviations SC = small chamber, LC = large chamber,
/f = fine substrata, /c = coarse substrata,
+G = glucose added,
F-test ** = $p<0.01$, * = $p<0.05$
N.S. = not significant ($P>0.2$); N.A. = not applicable.

TABLE 5.27B: Correlation matrix to determine confidence that regression equations are different

TABLE 5.27: Regression equation coefficients for chamber dissolved oxygen uptake measurements. Comparison between sediment particle size and chambers with different circulation velocities. Measurements at Homestead Road site on Waiotapu River, sampled 9 March 1983

DATE	Station km	Temperature °C	Discharge $m^3 s^{-1}$	Mean Depth, H m	Biomass Carbon Planktonic $mg\ m^{-3}$	Biomass Carbon Benthic[b] $mg\ m^{-2}$	Benthic Biomass Factor[a] Benthic Depth[c], δ_c cm	Benthic Biomass Factor[a] Factor	Benthic Biomass Factor[a] Benthic Depth[d], δ_c cm	Benthic Biomass Factor[a] Factor
29 Nov 1982	-0.55	21.0	2.4	1	496.3±77.9 (3)	-		-	-	-
	0				467.3±12.6 (3)	1992.5	1	4.3	6.4	27.3
	2.2				392.5±30.3 (3)	-	-		-	-
	6.9				317.5±17.5 (3)	-	-	-	-	
17 Jan 1982[e]	-0.55	22.0		0.75	385.0[f]	290.0	1	1.0	4.3	4.3
	0	24.0			385.0[f]	1220.0	1	4.2	3.3	13.9
	2.2	24.0			385.0±85.0 (3)	1527.5	1	5.3	5.8	30.7
	6.9	24.0			385.0[f]	2740.0	1	9.5	2.5	23.7
	10.0	24.0			385.0[f]	1045.0	1	3.6	2.5	9.1

[a] $\frac{\text{Benthic biomass} \times \delta}{H \times \text{Planktonic Biomass}}$

[b] measurement representing a 1 cm surface layer

[c] arbitrary depth

[d] calculated depth from Eq. 3.5, Table 5.26

[e] aquatic sample from 10 Feb 1982

[f] values are assumed based on 2.2 km measurement

TABLE 5.28: The relative importance of planktonic and benthic biomass in the Waiotapu River

Station (km)	ATP mg m^{-2}	Chl a mg m^{-2}	Total mg m^{-2}	Biomass Carbon Phototrophic	 Heterotrophic mg m^{-2}	Photo/Total %
Reporoa Bridge (-0.55)	1.16±0.05 (4)	3.55 (1)	290.0	56.8	233.2	20.0
Reporoa outfall (0)	4.88±1.76 (4)	2.64 (1)	1220.0	42.2	1177.8	3.5
Homestead Road (2.2)	6.11±0.80 (4)	1.58 (1)	1527.5	25.3	1502.2	1.7
Aard Road (6.9)	10.96±3.55 (4)	1.86 (1)	2740.0	29.8	2710.2	1.1
Halcrow Road (10.0)	4.18±0.83 (4)	2.77 (1)	1045.0	44.3	1000.7	4.2

Mean ± 1 standard error
No pheophytin detected

TABLE 5.29: Benthic biomass composition for a longitudinal survey of the Waiotapu River, sampled 17 January 1983

the complete river reach. Benthic biomass dominance ranged from 4.3-fold upstream, peaked at 30.7-fold 1.6 km downstream and then declined to an estimated 9-fold at 10 km.

5.3.7 Autotrophic and heterotrophic composition of benthic particulates

This section presents results of total biomass estimates by ATP determination, chlorophyll a for phototrophic autotrophs, and the use of a nitrification inhibitor (allyl-thiourea, ATU) to estimate the importance of chemosynthetic autotrophs. Estimates of inorganic nitrogen flux, were obtained from concentration changes of NH_4^+ and NO_3^- in the chamber waters during measurements in situ.

Planktonic biomass. Planktonic biomass measurements of total and 30 µm filtrates showed no significant difference (P>0.1) at each of the stations sampled (Table 5.28). Planktonic chlorophyll a measurements were made on 1 occasion (11 February 1983) and showed 12.9±1.3 mg m^{-3} to be present, 59% of which was associated with the <30 µm particle size (P<0.01). Phototrophic biomass carbon represented an estimated 54% of the total biomass estimate.

Benthic biomass. Total biomass ATP and phototrophic chlorophyll a measurements for the surface 1 cm sediments for the longitudinal survey (11 February 1983) are shown in Table 5.29. Benthic biomass showed an increase immediately below the discharge (4.2-fold, $P<0.01$) with a maximum of 10.96 mg m^{-2} 6.9 km downstream of the discharge, representing an order of magnitude increase (9.5-fold) on the upstream biomass.

Above the discharge, phototrophic biomass constituted 20% of the total biomass present, declining to <5% below the discharge. No pheophytin was detected in chlorophyll assays.

Benthic nitrogenous oxygen uptake rate (NUR) was estimated by addition of allyl-thiourea (ATU, final concentration 10 gm^{-3}, see Methods Section 3.3.6.1) to the benthic chamber. At Homestead Road (1.6 km) on the spring survey (29 November 1982), addition of both NH_4^+ and ATU showed no effect, whereas addition of ATU alone showed a 27% decline in intrinsic activity measurements ($P<0.05$). Results obtained for the longitudinal summer survey (17 January 1983) showed a response to ATU addition with NUR ranging from 2.1 g O_2 m^{-2} day^{-1} to 4.6 g O_2 m^{-2} day^{-1} or 10% to 30% of the BUR (Table 5.30). No intrinsic activity NUR values were determined.

Estimates of inorganic nitrogen flux. Chamber nutrients were measured on 2 occasions at the Homestead Road station. The river sediments removed both nitrogen species for the overlying flow on each occasion. NH_4^+ and NO_3^- fluxes of 0.19 g N m^{-2} day^{-1} ($P<0.05$, $r^2 = 0.937$, $n = 5$) and 0.56 g N m^{-2} day^{-1} ($P<0.01$, $r^2 = 0.964$, $n = 5$) respectively for sampling 10 February 1983 (Fig. 5.33A, nutrient data not shown; initial concentrations of 95 mg N m^{-3} for NH_4^+ and 618 mg N m^{-3} for NO_3^-). Ammonium flux was zero ($P>0.2$, $n = 4$, NH_4^+) and NO_3^- flux was 0.32 g n m^{-2} day^{-1} ($P<0.1$, $r^2 = 0.854$, $n = 4$, NO_3^-) for sampling 9 March 1983 (Fig. 5.33B, nutrient data not shown; initial concentrations 31 mg N m^{-3} for NH_4^+ and 460 mg N m^{-3} for NO_3^-). In terms of potential NUR, the maximum flux of NH_4^+ from the overlying flow would be 0.8 g O_2 m^{-2} day^{-1} (or 7% of the BUR, see Table 5.30).

Station (km)	BUR Total	Nitrifiers	Nitrifiers/Total
	gm^{-2} day^{-1}		%
Reporoa Bridge (-0.55)	8.8	2.2	25
Reporoa outfall (0)	22.8	4.6	20
Homestead Road (2.2)	20.5	2.1	10
Aard Road (6.9)	12.6	3.8	30

TABLE 5.30: Benthic nitrogenous oxygen uptake rate in the Waiotapu River, sampled 17 January 1983

5.3.8 Distribution of microbial biomass, intrinsic activity and dissolved oxygen with sediment depth

This section presents results obtained from sediment depth profiles of fine and coarse particulates for 1 station, Homestead Road (see Plate 5.1), sampled for microbial biomass and intrinsic activity (sampled 9 February 1983) and DO profiles (9 March 1983). Details from sediment particle size analyses are shown in Appendix 4.

Intrinsic activity profiles are shown in Figure 5.39. On this occasion there was no significant difference ($P>0.2$) between surface activities for either fine or coarse particulates at 3.5 g O_2 m^{-2} day^{-1}, or between activities to 10 cm depth. Addition of glucose produced no response in the surface layers but showed a 55% to 70% increase in intrinsic activity for the 1-2 cm and 5-6 cm layers for each of the particulates ($P<0.001$).

Microbial biomass profiles are shown in Figure 5.40. ATP concentration was 9.43 mg m^{-2} for surface, fine particulates which had declined by 29% at 10 cm depth ($P<0.1$). Considerable variation in estimates (~±25%) resulted in low confidence levels for differences between layer biomass estimates. ATP concentration was 6.92 mg m^{-2} for the coarse particulate surface layer which was not significantly different from the fine ($P>0.1$). Biomass increased at the 1-2 cm depth (7.2-fold, $P<0.01$), with comparable levels present to 10 cm depth.

Calculation of microbial biomass composition for the Homestead Road depth profiles showed that the phototrophic biomass (as chlorophyll a) accounted for less than 2% of the total microbial biomass present in each of the particulate profiles (Table 5.31).

Plate 5.1 Coarse and fine particulate cores from the Waiotapu River at Homestead Road, 10 March 1983. External diameter of cores are 75 mm.

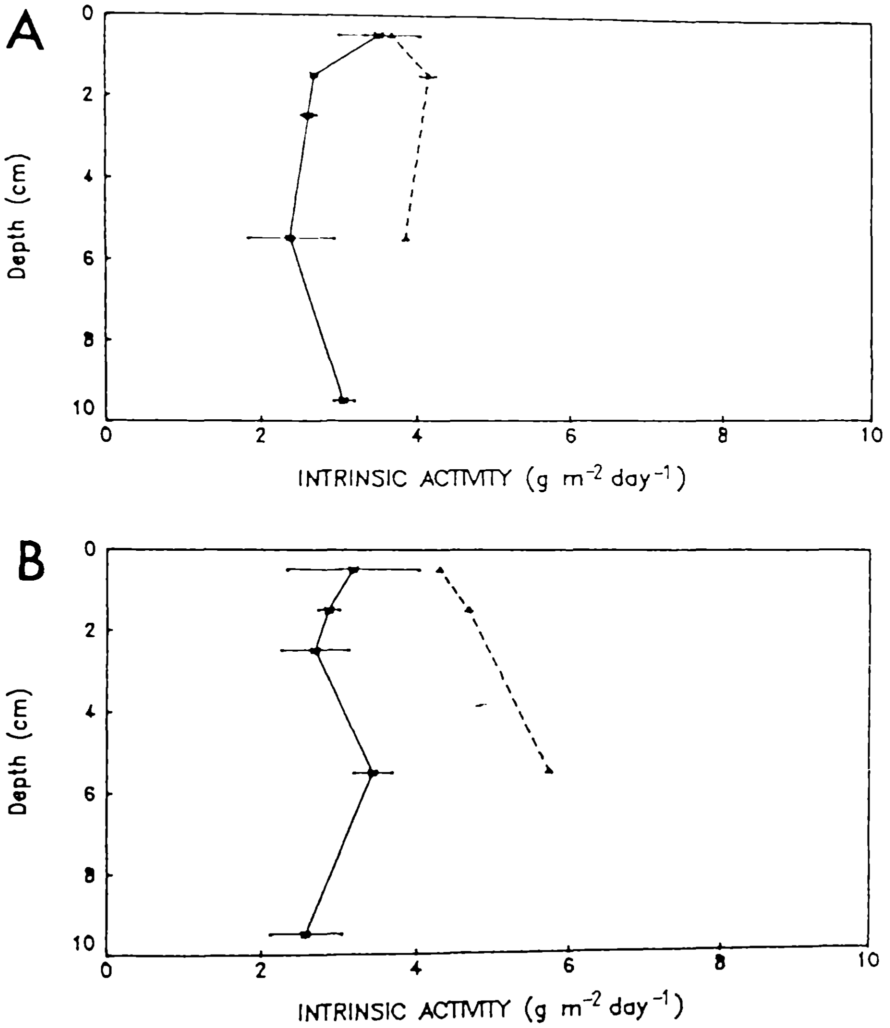


Figure 5.39 : Sediment depth profiles for intrinsic activity in the Waiotapu River sampled 9 February 1983, for fine particulates (A) and coarse particulates (B). Dashed line represents activity with glucose added (final concentration 20 g m^{-3}). Measurement temperature was 21 °C.

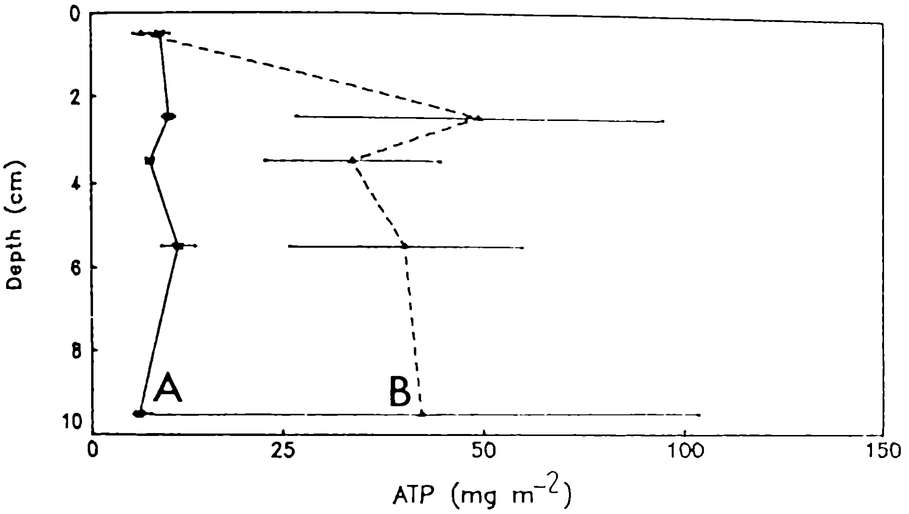


Figure 5.40 : Benthic depth profiles for microbial biomass in the Waiotapu River sampled 9 February 1983, for fine particulates (A) and coarse particulates (B).

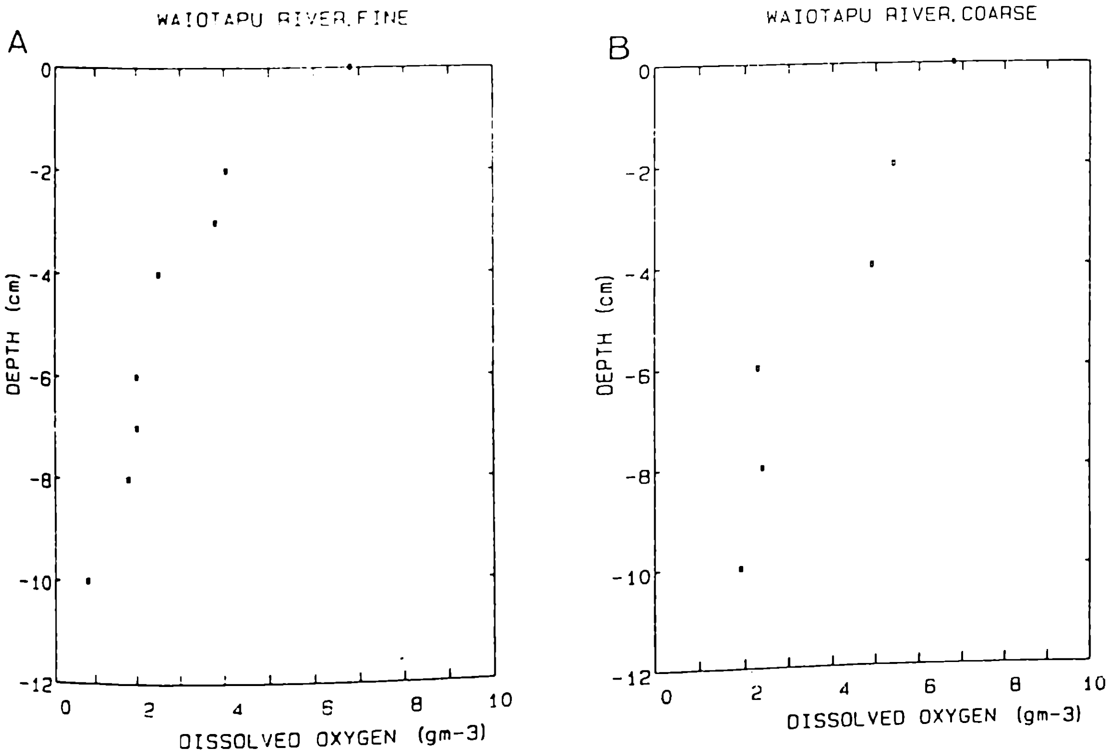


Figure 5.41 : Sediment DO profiles in the Waiotapu River sampled 9 March 1983, for fine particulates (A) and coarse particulates (B).

DO profiles for both fine and coarse particulates were measured on 9 March 1983 and are shown in Figure 5.41. DO was present at 10 cm for each of the profiles, though a higher concentration was recorded at depth in the coarse profile.

Intrinsic activity measurements for 9 March 1983 were 4.49±0.81 g m^{-2} day^{-1} and 2.72±0.35 g m^{-2} day^{-1} for fine and coarse particulates respectively. Neither of these measurements was significantly different from those measured on 9 February 1983 ($P>0.2$). Addition of glucose resulted in an activity increase for fine (1.6-fold, $P<0.01$) and coarse particulates (1.8-fold, $P<0.001$), resulting in significant differences from the 9 February 1983 survey ($P<0.05$, Fig. 5.39).

Microbial biomass present showed no significant difference with 9 Feb 1983 at 8.94±0.53 mg ATP m^{-2} and 6.88 mg ATP m^{-2} for fine and coarse particulates respectively.

5.3.9 Benthic carbon turnover

This section presents measurements from longitudinal and sediment depth profile surveys of benthic biomass and total carbon measurements together with carbon turnover estimates and sediment respiratory decay rate measurements.

Total and biomass carbon of sediments. The longitudinal survey showed that the total sediment carbon increased below the discharge to 24.4 g C m^{-2} at the station 6.9 km downstream. This is about twice that present at the station immediately below the discharge. The total benthic carbon then decreases to upstream levels at 10 km. Microbial biomass carbon estimates increased from 2.4% of the total carbon upstream to 8.1% below the discharge, with a maximum of 11.3% 6.9 km below the discharge (Table 5.32).

Sediment depth profiles showed higher surface carbon content for coarse than fine particulates (1.4-fold), with sub-surface values from 16-30 g C m^{-2}. Biomass carbon estimates ranged from 8.2% to 13.8% of the total carbon to 10 cm depth in the fine particulates (Table 5.33). The proportion of biomass carbon in the coarse particulates increased from 5.1% at the surface to 73.3% at 2-3 cm, with 49.3% living biomass to 10 cm depth.

Particulates Depth cm	ATP mg m^{-2}	Chl a mg m^{-2}	Total C mg m^{-2}	Biomass Carbon Phototrophic mg m^{-2}	Biomass Carbon Heterotrophic mg m^{-2}	Photo/Total %
Fine						
0-1	9.43±1.70	1.64±0.19 (2)	2357.5	26.2	2331.3	1.1
1-2	10.61±1.16	1.37±0.18 (2)	2652.5	21.9	2630.6	0.8
2-3	8.19±0.53	1.37±0.45 (2)	2047.5	21.9	2025.6	1.1
5-6	11.85±2.99	-	2962.5	-	-	-
9-10	6.65±1.20	-	1662.5	-	-	-
Coarse						
0-1	6.92±1.60	2.09±0.15 (2)	1730.0	33.4	1696.6	1.9
1-2	49.54±17.62	2.38±0.55 (2)	12385.0	38.1	12346.9	0.3
2-3	33.78±8.45	2.85±0.46 (2)	8445.0	45.6	8399.4	0.5
5-6	40.04±11.18	-	10010.0	-	-	-
9-10	42.43±26.57	-	10607.5	-	-	-

mean ± 1 standard deviation

TABLE 5.31: Microbial composition of benthic depth profiles, sampled at Homestead Road, on the Waiotapu River, sampled 9 February 1983

Station (km)		Total Carbon g m^{-2}	Total Nitrogen g m^{-2}	Biomass Carbon mg m^{-2}	Biomass C/ Total C %	C:N ratio
Reporoa Bridge	(-1)	12.0	0.85	290.0	2.4	14.1
Reporoa outfall	(0)	15.0	0.69	1220.0	8.1	21.7
Homestead Road	(2.2)	18.8	0.66	1527.5	8.1	28.3
Aard Road	(6.9)	24.4	0.90	2740.0	1.3	27.0
Halcrow Road	(10.0)	12.6	0.40	1045.0	8.3	31.9

TABLE 5.32: Sediment total carbon and nitrogen measurements, and biomass carbon estimates for a longitudinal survey of the Waiotapu River, sampled 17 January 1983

Sediment Depth cm	Total Carbon g m^{-2}	Total Nitrogen g m^{-2}	Biomass Carbon mg m^{-2}	Biomass C/ Total C %	C:N ratio
Fine					
0-1	25.02	0.77	2357.5	9.4	32.3
1-2	26.67	0.64	2652.5	0.0	41.7
2-3	25.10	0.66	2047.5	8.2	38.1
5-6	21.53	0.52	2962.5	3.8	41.3
9-10	19.29	0.60	1662.5	8.6	32.0
Coarse					
0-1	33.74	0.50	1730.0	5.1	67.7
1-2	16.90	0.43	12385.0	3.3	39.1
2-3	20.21	0.34	8445.0	1.8	59.1
5-6	30.62	0.40	10010.0	2.7	76.2
9-10	21.53	0.57	10607.5	9.3	37.8

TABLE 5.33: Sediment depth profile measurements of total carbon and nitrogen, and biomass carbon estimates in Waiotapu River, sampled 9 February 1983

Station (km)	Total Carbon g m^{-2}	Intrinsic Activity g O_2 m^{-2} day^{-1}	Carbon Turnover Time[a] day	Decay Coefficient,[b] k day $^{-1}$	Respiratory half-life[c] day
Reporoa Bridge (-0.58)	12.0	2.05 [3.08][d]	15.6 [10.4]	0.8	0.9
Reporoa Outfall (0)	15.0	6.81 [6.95]	5.9 [5.8]	0.8	0.9
Homestead Road (2.2)	18.8	3.53 [5.42]	14.2 [9.2]	-	-
Aard Road (6.9)	24.4	4.97 [6.48]	13.1 [10.0]	0.5	1.4
Halcrow Road (10.1)	12.6	2.75 [3.69]	12.2 [9.1]	0.4	1.7

[a] Calculated as $\frac{\text{Total Carbon}}{\text{Intrinsic activity} \times 0.375^{*}}$

[b] Assming 20% of the carbon as biodegradable

[c] $t_{1/2} = \frac{\ln 2}{k}$

[d] Bracketed rates are for glucose activities

*Value obtained from stoichiometry ($C:O_2$)

TABLE 5.34: Sediment carbon turnover rate and respiratory decay measurements from the Waiotapu River, sampled 17 January 1983

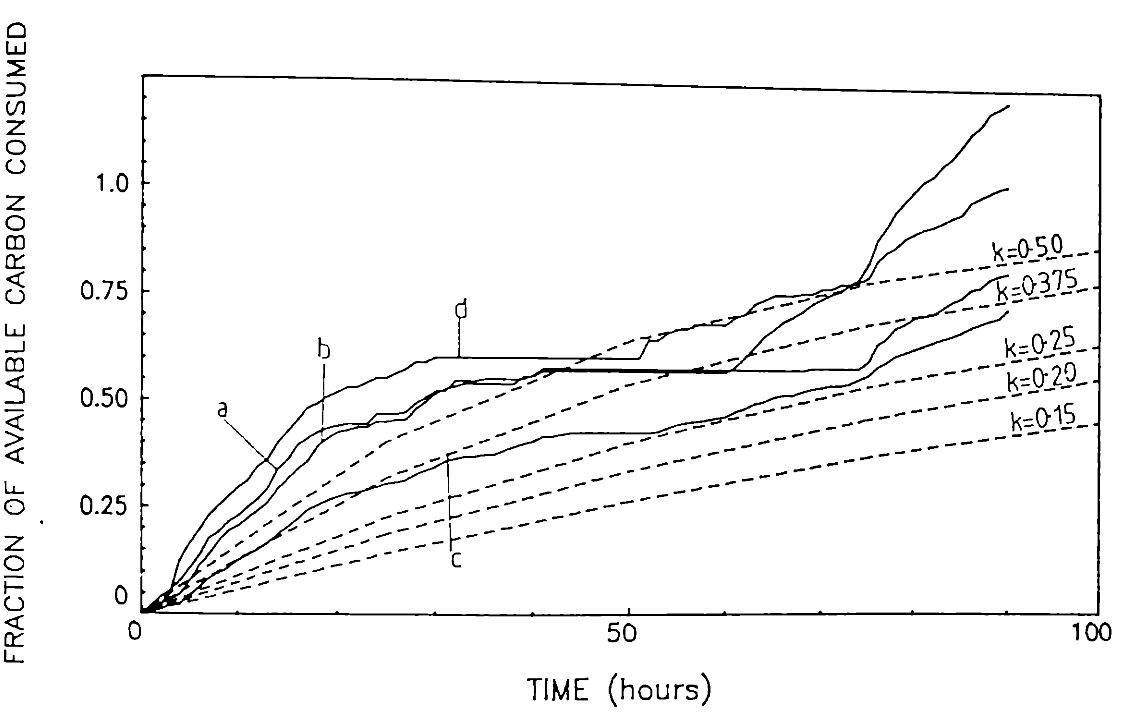


Figure 5.42 : Sediment respiratory decay measurements for the Waiotapu River, sampled 17 January 1983, assuming 20% available carbon.

Key: (distance below discharge)

- a Reporoa (-0.58 km)
- b Outfall (0 km)
- c Aard Road (6.9 km)
- d Halcrow Road (10.0 km)

Dashed line denotes first order decay curves with coefficient (k) shown.

Carbon turnover. The longitudinal survey showed an increasing trend in C:N ratio from 14.1 upstream to 31.9 at the 10 km station (Table 5.32).

Depth profiles at Homestead Road showed a higher C:N ratio only for the surface 0-1 cm and 5-6 cm layers of the coarse particulates (Table 5.33). All other particulate layers show minimal differences indicating similar organic matter composition.

Calculated values of sediment carbon turnover time, based on total carbon and intrinsic activity measurements (converted to carbon equivalents), were in the range 12 to 15 days for all stations, except immediately below the outfall at 5.9 days (Table 5.34). A subsequent sampling for depth profile measurements (Fig. 5.39 and Table 5.33) gave an increased turnover time of up to 30 days, but relatively uniform with depth.

Measurements of sediment respiratory decay rate showed a rapid decay coefficient (k) corresponding to 20% of the total carbon present (Table 5.34 and Fig. 5.42), resulting in respiratory half-lives of 0.9 to 1.7 days. After approximately 60 h incubation the abrasive conditions resulted in a breakdown of the pumice, presumably resulting in increased availability of organic material giving rise to a secondary increase in oxygen uptake (Fig. 5.42). This portion of the curve was not considered for decay coefficient determination.

5.3.10 Discussion and Conclusions

Chamber measurements of BUR made in situ in the Waiotapu River, ranged from 8.8 to 39.0 g m^{-2} day^{-1} (Table 5.23). Longitudinal surveys showed substantial BUR and sediment intrinsic activity increases below the dairy discharge, with a mean sediment oxygen penetration depth of 5.7 cm (Table 5.24). This contrasts with the Waitoa River (Section 5.2.2) where the oxygen penetration depth decreased sharply below the discharge. A number of factors may have contributed to this difference including a lower river effluent concentration. However, the lower density of the pumice bed material (see Table 5.25) together with the higher shear velocity in the Waiotapu (0.4 m s^{-1} c.f. 0.25 m s^{-1} for Waitoa) resulted in mobile dunes which may greatly increase sediment-water exchange. This is reflected in the higher benthic dispersion coefficients calculated for the Waiotapu at 24.9 x 10^{-3} cm^2 s^{-1} (Table 5.24, c.f. Waitoa in Table 5.2).

Glucose addition to the chambers in situ showed that electron donor concentration may be limiting the BUR, with responses ranging from 0 to 109% increase (Table 5.23). Similar responses were also obtained for intrinsic activity measured. Extended subsampling of glucose removal from the chamber showed respiratory quotient (Qo_2) of 0.23 in February (Fig. 5.34A) and 0.43 in March (Fig. 5.34B), suggesting that antecedent effluent loading conditions may influence the relationship between the BOD removal rate and the river deoxygenation rate. The river BUR may be limited by either electron donor or electron acceptor concentrations.

The chamber BUR kinetics were intensively monitored with the aim of providing predictive BUR estimates under: (i) varying DO conditions (as previously applied and discussed, section 5.3.2); (ii) for comparison of measurements on areas differing in bed particle size composition; and (iii) in water with differing nutrient regimes (e.g. with glucose addition). To facilitate these studies, 2 small chambers were constructed (velocity 0.2 m s^{-1}, see Table 4.1 for specifications) and installed in the river. BUR measurements amounted to only 59% of the large chamber (0.35 m s^{-1}) estimates. (Table 5.23). Comparison of BUR values between areas of fine and coarse particulates, showed relatively small differences, which were not consistent. Comparison of particulates at 0.35 m s^{-1} (1 occasion only, 17 January 1982, Table 5.27) favoured the coarse substrata for higher BUR and D_s values. Substantial differences in BUR kinetics were also measured. Thus the major sediment-water exchange differences were related to adequate simulation of natural flow conditions rather than to variability resulting from heterogeneous regions of bed particle size.

All chamber measurements showed repeatable BUR dependence on DO concentration (slope in Figs. 5.33 and 5.36), with addition of glucose confirming the response to be caused by electron acceptor depletion. Manipulation of chamber DO concentration in a small chamber on coarse substrata using catalase and hydrogen peroxide (Hickey, 1985b) and glucose concentration, gave the maximum BUR recorded at 49.0 g m^{-2} day^{-1} at 9.0 g m^{-3} DO (see Fig. 5.36A), and showed significant differences in kinetic response (Figs. 5.37 and 5.38). The reasons for these large DO responses and differences between chambers have been attributed to turbulent diffusion processes and a possible effect of an exponential decay of the turbulent dispersion coefficient with depth

(Hickey, 1985a). However, transient state dispersion kinetics may be responsible. This possibility will be discussed in Chapter 7.

Longitudinal planktonic and benthic biomass surveys, in conjunction with chamber estimates of the depth of benthic oxygen transfer, showed that benthic biomass was 4- to 31-fold greater than was planktonic biomass (Table 5.28), increasing below the discharge. The importance of BUR justifies further examination of the contributary components.

Chamber measurements made in situ showed that substantial photosynthetic production may occur upstream but decreased below the discharge (Table 5.23). Longitudinal biomass measurements showed substantial increases in heterotrophic biomass below the discharge, with the phototrophic component decreasing from 20% upstream to less than 5% (Table 5.29). Thus the discharge significantly altered the algal biomass present. This effect may have been caused by competition for surface area available for growth limitation, since light levels were probably minimally influenced by the intermittant nature of the discharge.

The utilization of NH_4^+ may result in the uptake of DO either by chemotrophs (e.g. nitrifiers) to supply their energy requirements, or by heterotrophs in the course of cell synthesis. Direct measurements of NH_4^+ uptake rate include both of the above processes, whereas the use of a specific inhibitor (ATU) measures only the contribution of heterotrophs in its presence. This latter procedure was used to measure the 'Nitrogenous Oxygen Uptake Rate (NUR)' (section 3.3.6) and hence estimate the metabolic contribution of nitrifiers present. NUR constituted 10 to 30% of the total BUR (Table 5.30). These measurements suggested that NUR was a significant component of the summer oxygen dynamics of the Waiotapu River. The magnitude of these values were comparable with those obtained by Cooper (1984) for this river in the previous year. Measured NH_4^+ uptake from the chamber water could account for a BUR of 0.8 g O_2 m^{-2} day^{-1} suggesting that benthic production of NH_4^+ accounted for most of the NUR. Flux of NH_4^+ and NO_3^- were both into the sediment with the magnitude of the latter comparable with rates measured by Cooper (1984) at the same station the previous summer. In terms of glucose removal by heterotrophs, the NO_3^- consumption rate could lead to an erroneous estimate in Qo_2, with the maximum rate amounting to 15% of the measured BUR.

Detailed investigations of activity and biomass profiles with sediment depth, showed comparable activities from the surface down to 10 cm depth for both of the particulates (Fig. 5.40). Biomass levels were similar to 10 cm in the fine particulates, but increased substantially below 0-1 cm in the coarse particulates (Table 5.31). This increased biomass was not reflected in activity (Fig. 5.39) and may represent a microbial population present (e.g. nitrifiers), but substrate limited under assay conditions. Sub-surface populations did show a substantial response to glucose addition, indicating that electron donor supply was limiting the heterotrophic biomass component at the time of sampling (Fig. 5.39). Phototrophic biomass (as algae) represented less than 2% of the total biomass through each of the profiles (Table 5.31). The overall picture obtained is one of a potentially active heterotrophic microbial population, present to a depth of at least 10 cm, which will be principally limited by electron acceptor penetration (oxygen) but also limited by electron donor supply (glucose).

DO profiles measured in both fine and coarse particulates showed that DO penetration occurred to at least 10 cm for each (Fig. 5.44), and suggested that intrinsic activity to this depth was contributing to the measured BUR. Indeed, the integration of I to this depth was necessary in order to account for measured BUR at high DO concentrations (see Table 5.26). These DO profile data were used in conjunction with chamber flux measurements in order to calibrate the diagenetic model developed in Chapter 7.

Calculation of the interstitial turnover time for DO gave values decreasing from a surface value of 15 min to less than 2 min at 10 cm (Table 5.35). Similar values were obtained for both the fine and coarse particulates. The high rate of turnover for deep sediments implies an active supply of oxygen from the overlying flow, otherwise anoxic conditions would rapidly develop. Estimates of the dispersion coefficient are discussed in Chapter 7.

The mobile nature of the river bed was, by visual assessment, dominated by downstream movement of the larger pumice particles. To some extent this potentially abrasive process may have accounted for the lower surface biomass levels measured on the coarse particulates (Tables 5.25 and 5.31). Sieving prior to measuring intrinsic activity and biomass

(1) Sediment Description	(2) Depth cm	(3) Density[a], ρ g ml^{-1}	(4) Porosity[a], ϕ	(5) Intrinsic Activity $\mu g\ O_2\ g^{-1}\ h^{-1}$	(6) Dissolved Oxygen gm^{-3}	(7) $\mu g\ ml^{-1}$	(8) Turnover Time min
Fine	0-1	1.36	0.50	13.9 [14.6]	6.8	3.4	10.8 [10.3]
	1-2	1.29	0.48	10.7 [16.6]	4.1	2.0	8.6 [5.5]
	2-3	0.94	0.63	10.4 [-]	3.8	2.4	14.7 [-]
	3-4	1.06	0.53	(10)	2.5	1.3	7.5
	4-5	1.21	0.51	(10)	2.0	1.0	6.0
	5-6	1.17	0.54	9.5 [15.6]	2.0	2.2	5.8 [3.6]
	9-10	1.09	0.50	11.8 [-]	0.8	0.4	1.9 [-]
Coarse	0-1	0.54	0.66	34.2 [45.9]	6.8	4.5	14.6 [10.9]
	1-2	1.00	0.53	24.8 [40.6]	5.4	2.9	6.9 [4.2]
	2-3	1.04	0.57	27.0 [-]	(5)	2.7	6.2 [3.8]
	3-4	1.09	0.58	(20)	4.9	2.8	7.8
	4-5	1.03	0.53			-	-
	5-6	1.05	0.50	22.3 [37.0]	2.3	1.2	2.9 [1.8]
	9-10	1.03	0.50	10.4 [-]	1.9	1.0	5.3 [-]

[a] measured 9 March 1982
[b] measured 9 Feb 1982
(7) = (6) x (4) (note: converts to ml of total sediment volume)
(8) = ((7) ÷ ((5) x (3))) x 60
[] measurements with glucose addition
() interpolated values

TABLE 5.35: Calculated interstitial DO turnover time for the Waiotapu River showed that particles >4 mm were under-represented whereas those <1.7 mm were over-represented with respect to dry weight contribution (Table 5.25). This observation may provide some tentative support for the contention that the large particle fractions are affected by the mobility of the bed. This effect may ultimately be extremely important in mobile bed systems, since the maximum intrinsic activity will be affected by the maximum amount of biomass which may colonise the substratum. Estimates of this maximum biomass value have not been obtained in this study; however, the relative magnitude of intrinsic activity as it effects BUR are tested in Chapter 7.

Sediment carbon turnover. Microbial biomass estimates showed that up to 11% of the total surface layer carbon (Table 5.32) and up to 73% of the sub-surface carbon (Table 5.33). That these organisms were limited by the relatively low organic loadings to the Waiotapu, would be supported by the rapid carbon turnover measured immediately below the outfall (Table 5.34), which had a value comparable to that found in the Waitoa during low flow conditions adjacent to the discharge (Table 5.12). Increasing values at downstream stations (Table 5.34), suggested a decreased growth rate, possibly limited by the availability of reducing potential.

Carbon reserves were minimal, with a short respiratory half-life of less than 2 days; the bulk of this material probably composed of mucilagenous sheath material (Lock et al., 1984). C:N ratios increased substantially below the discharge (Table 5.32) and were high throughout the sediment profiles, which would be consistent with biofilm sheath formation (Lock et al. 1984).

To what extent increased organic concentrations (i.e. BOD) would result in increased microbial growth rates, metabolic activities and higher standing crops is uncertain. However, because of the bulk of the benthic biomass are epilithic heterotrophs, modelling of responses in terms of Michaelis-Menten enzyme kinetics for activites, and Monod saturation kinetics for growth rate, would be applicable. The magnitude of response would then depend on the relative values of half-saturation coefficients and river nutrient concentrations, which in turn would dictate whether BOD changes would result in no effect (zero order assumption) or a marked effect (first order asumption). The importance of these effects on predictive modelling of benthic mass transfer processes will be discussed in Chapter 8.

5.3.11 Summary

A segment oxygen mass balance for a 2.8 km reach below the dairy effluent discharge showed that chamber BUR measurements were comparable with segment estimates. Measured BUR was 28.0 g O_2 m^{-2} day^{-1}, up to 30-fold greater than was planktonic respiration (estimated from biomass measurements), and 4-fold greater than macrophyte respiration (section 5.3.2).

The BUR, both in the natural system and in the chamber, appeared to be a function of the velocity of the overlying water. The BUR for unstirred (zero velocity) core measurements was only about 5% of the maximum BUR measured at 0.35 m s^{-1} velocity. Thus, in order to obtain representative BUR measurements, it is important that measurement devices simulate the natural velocity patterns (section 5.3.5).

The BUR declined over time in the chamber in situ as DO concentrations declined. A site-specific exponential relationship between BUR and DO was demonstrated. It was influenced by the distribution of microbial activity with depth and mass transfer processes from the overlying flow (section 5.3.5). Since the BUR is a function of DO concentration in the overlying flow, a relationship should be included (in equation form) in water quality models.

The BUR responded to glucose addition, suggesting that the river BUR may be limited by either electron acceptor (oxygen) or electron donor (glucose) concentrations. The BUR decined over time in the chamber in situ as glucose concentrations declined during the measurement period (section 5.3.5). The combined effects of rapid depletion of both electron donor and electron acceptor suggests that serious underestimation of the natural rate process may result unless the range of natural conditions is simulated.

Longitudinal studies showed higher BUR values below the organic discharge with no reduction in the depth of sediment oxygen penetration. Measured sediment benthic dispersion coefficients were 3 orders of magnitude higher than molecular diffusion suggesting that the degree of sediment-water interaction was high and increased rates of river nutrient transformation should be anticipated (section 5.3.3).

Phototrophic organisms made up 20% of the sediment microbial biomass upstream; this proportion decreased below the discharge (section 5.3.7). Surface biomass levels were less than sub-surface values which were high to at least 10 cm depth, with DO penetration to at least 10 cm (section 5.3.8). Movement of surface particles probably restricted surface levels by abrasion (section 5.3.4). Microbial biomass carbon estimates suggested that up to 11% of the surface and 73% of the sub-surface sediment carbon. High values measured for sediment carbon turnover and respiratory decay rates suggested that the microbial biomass present essentially epilithic heterotrophs, receiving both electron donors and electron acceptors from the overlying flow (section 5.3.9). Thus the high rates of BUR measured in this river were sustained by a large mass flow of nutrients (possibly at low concentration) facilitated by the rapid sediment-water exchange (i.e. high dispersion coefficient).

5.4 TARAWERA RIVER

5.4.1 Introduction

The Tarawera River studies were designed to identify the major component of river DO depletion, and perform a segment oxygen mass balance with which the chamber oxygen measurements could be compared. Investigations included: planktonic and benthic biomass measurements, chamber BUR, sediment intrinsic activity for longitudinal surveys.

The river is a pumice bed system, receiving the organic effluent loading from aerated lagoon treated mechanical pulp and paper mill wastes.

5.4.2 Segment oxygen mass balances

Segment oxygen mass balances were undertaken on 2 occasions (7 March 1984 and 12 February 1985) involving a team of researchers from the Water Quality Centre. Atmospheric reaeration estimates were obtained by Dr R.J. Wilcock using a gas tracer technique (Wilcock 1984a), which together with DO measurements (from approximately 10 stations) was used to calculate the gross oxygen uptake rate using a computer programme written by Dr J.C. Rutherford which incorporated oxygen additions from tributary stream in-flows. Results are detailed in Appendix 5 and shown in Table 4.3 in relation to chamber measurements.

Calculated gross oxygen uptake rate showed no significant decline on either sampling occasion in the 20 km reach to the river mouth

Date	Temp °C	DO gm^{-3}	BUR $gm^{-2}day^{-1}$	Glucose %	Gross Photosynthesis gm^{-2} day^{-1}	Sediment Intrinsic Activity gm^{-2} day^{-1}	Glucose %	Depth of Oxygen Penetration cm	Dispersion Coefficient cm^2 s^{-1} x 10^{-3}
Town Bridge									
10 Mar 1984		10.0	4.10±0.83(2)	-	-	1.18±0.05(2)	-	3.5±0.7	1.7±0.4
Onepu Springs									
12 Feb 1985	16.0	9.1	10.5±3.5(3)	-	8.9±1.8(2)	1.65±0.33(6)	-	6.4±2.5	8.5±3.3
SH30									
10 Mar 1984		6	30.5±11.7(4	-	-	6.71 (1)	11.8	4.6±1.8	26.8±10.3
12 Feb 1985	18.5	7.1	28.8±4.9(4)	-	-	10.18±1.30(6)	-	2.9±0.6	13.5±2.9
Johnston Road									
12 Feb 1985	18.5	6.0	25.0±4.6(4)	0	-	8.65±0.83(10)	0	2.9±0.6	14.0±2.9
Awakaponga									
10 Mar 1984		4	28.2±13.6(4)	-	-	2.83±0.68(3)	-	10.0±5.4	163.0±87.8
12 Feb 1985	19.0	4.8	20.2±11.4(4)	-	-	3.75±0.92(6)	-	5.3±3.3	25.8±15.9
SH2									
12 Feb 1985	20.0	3.8	14.8±1.8(3)	-	-	3.10±0.34(6)		4.8±0.8	22.2±3.6

All chamber velocities 0.35 ms^{-1}

mean ± 1 standard deviation

Table 5.36: BUR, sediment intrinsic activity and calculated benthic diffusion coefficients for the Tarawera River

Chamber BUR measurements on these two occasions underestimated by 35% and by 53% the magnitude of the calculated BUR values. The factors which may have contributed to the low chamber measurements will be discussed in Chapter 7. This likely magnitude of BUR underestimation should be born in mind with respect to the results presented in this section.

5.4.3 Benthic oxygen uptake rate (BUR) measurements *in situ*, sediment intrinsic activity and calculation of the depth of sediment oxygen penetration

This section outlines the chamber BUR and sediment intrinsic activity (I) measurements performed in the Tarawera River. The depth of penetration of oxygen into the sediments, and benthic dispersion coefficient are calculated for longitudinal surveys which included stations above and below the organic outfalls.

The mobile pumice bed of the Tarawera River effectively prevented the colonisation of attached filamentous microorganisms. The highest BUR measured *in situ* was 47.4 g O_2 m^{-2} day^{-1}, measured at a station 3.0 km below the Tasman outfall, with a minimum BUR of 3.5 g m^{-2} day^{-1} at the Town Bridge station, above all effluent discharges (survey 10 March 1984). Results for 2 longitudinal surveys are summarised in Table 5.36, and showed a large BUR increase comparing above and below all discharges (7.4-fold, $P<0.001$), with no significant decline to the Awakaponga station (14.9 km below Tasman outfall) for the 10 March 1984 survey. A more recent survey, incorporating improved measurement techniques and more stations, sampled on 12 February 1985 showed a smaller BUR increase between Onepu Springs and SH30 (2.7-fold, $P<0.01$), with no significant decline to stations 5.9 km and 14.9 km below the discharge, but a 48.6% decline ($P<0.01$) to SH2 (20.4 km downstream). A high productivity was measured at Onepu Springs. (Table 5.36).

Although chamber BUR kinetics did show that the fall in DO concentration proceeded at a decreasing rate, no attempt was made to mathematically describe the curves and only initial rates were recorded (see Chapter 7 for a possible explanation of the causes and discussion).

Glucose addition, made to order in test for electron donor limitation, gave a small intrinsic activity increase and no increase in chamber BUR increase (Table 5.36), with similar sub-surface increases in I (data not shown).

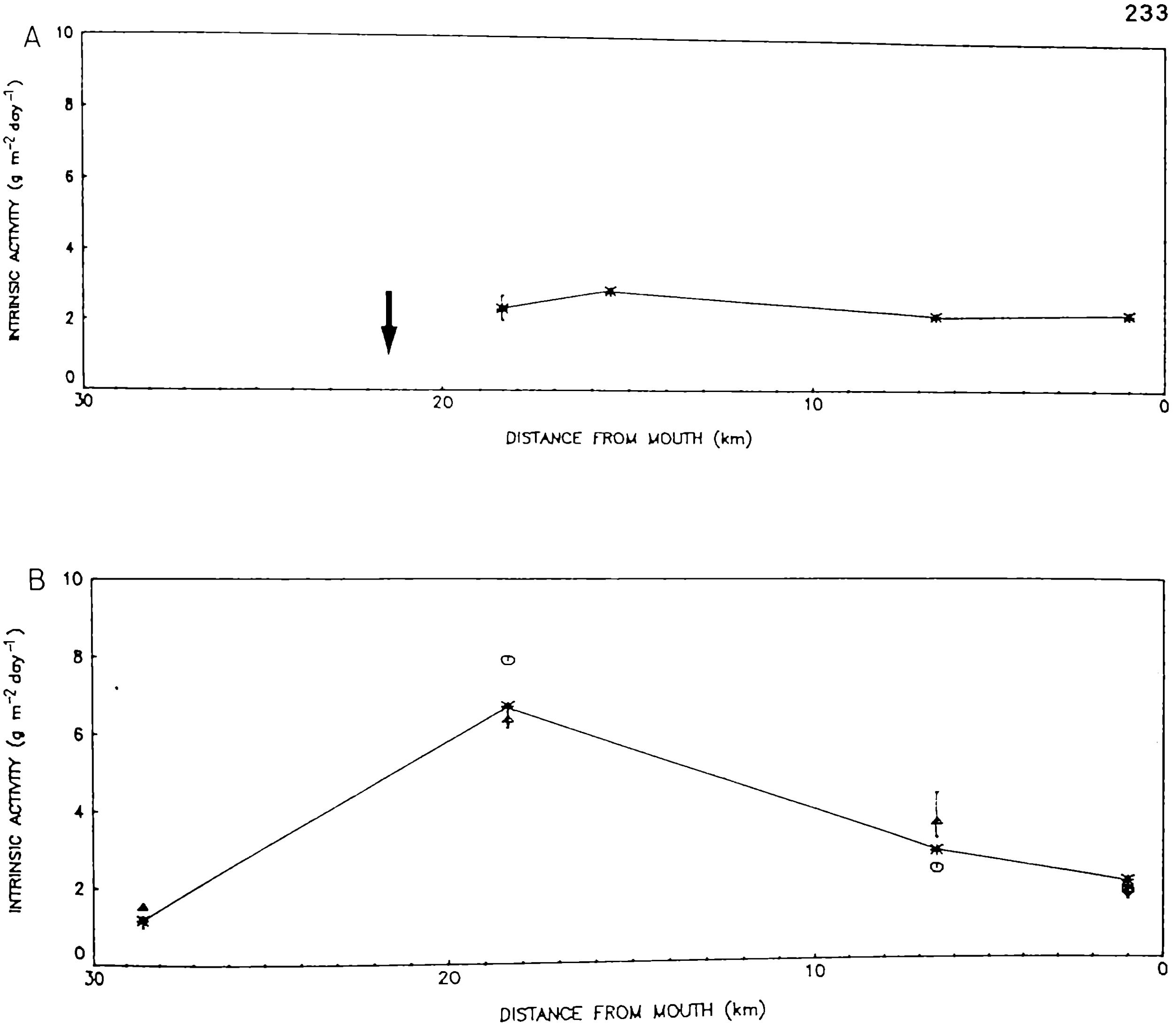


Figure 5.43 : Benthic intrinsic activity measurements (continuously stirred) in the Tarawera River, sampled 30 November 1983 (A) and 7 March (1984) (B). Arrow locates Tasman outfall. All 2-4 replicate samples with ±1 standard error shown, others are single measurements.

Symbols:
★ surface 0-2 cm sample
Δ 4-6 cm sample
O 10-12 cm sample (except 6.5 km station at 20-22 cm in 23 November).

Intrinsic activity measurements with the pumice substrata were found to be strongly affected by the degree of agitation (results not shown), with >2-fold increase for continuous compared with 0.5 h agitation. All measurements reported here are for continuously-stirred samples. Measurements ranged from 1.18 g O_2 m^{-2} day^{-1} above the discharge to a maximum of 10.18 g O_2 m^{-2} day^{-1} below the discharges (Fig. 5.43). For the March 1984 survey an increase occurred above and below the discharges (5.7-fold, $P<0.001$), with a comparable increase occurring for February 1985 between Onepu Springs and SH30 (6.2-fold, $P<0.001$) (Table 5.36), with a progressive downstream decrease in activity to 31% of the maximum at SH2. Sub-surface values were comparable to surface activities to 20 cm depth (Fig. 5.43).

The calculated depth of DO penetration may be compared for the February 1985 longitudinal survey (Table 5.36). The aerobic zone decreased from 6.4±2.5 cm upstream to 2.9±0.6 cm downstream ($P<0.05$), increasing again to 4.8±0.8 cm at SH2 ($P<0.05$).

The calculated dispersion coefficients ranged from 1.7×10^{-3} cm^2s^{-1} to 163.0 cm^2s^{-1}. However, substantial uncertainty was associated with the latter measurement which when excluded from a mean calculation gave 16.1±9.3 cm^2s^{-1} (N=7) (Table 5.36). The February 1985 survey showed an increase between Onepu Springs and SH30 (1.6-fold, ($P<0.1$) and a further increase to SH2 (1.6-fold, $P<0.05$).

5.4.4 The relative importance of planktonic and benthic biomass

This sub-section presents results obtained from the 2 longitudinal surveys in late spring (November 1983) and summer (March 1984), where planktonic and benthic ATP measurements were made.

Planktonic ATP measurements are shown in Figure 5.44. For the spring survey there was no increase in planktonic biomass above the Tasman discharge, with an order of magnitude increase to SH30 (18.5 km) (20.6-fold, $P<0.001$) at 2.90 mg ATP m^{-3} (Figure 5.44A). An intermediate station sampled between the Tasman outfall and SH30 showed a lesser biomass increase (1.4-fold, $P<0.001$), however, this may have been caused by insufficient mixing of the effluent. There was no decline in biomass in the 20 km reach to the sea. The summer survey (Figure 5.44B) showed a biomass increase in the reach above the Tasman outfall (2.3-fold, $P<0.01$) which caused an order of magnitude increase to SH30 at 7.15 mg

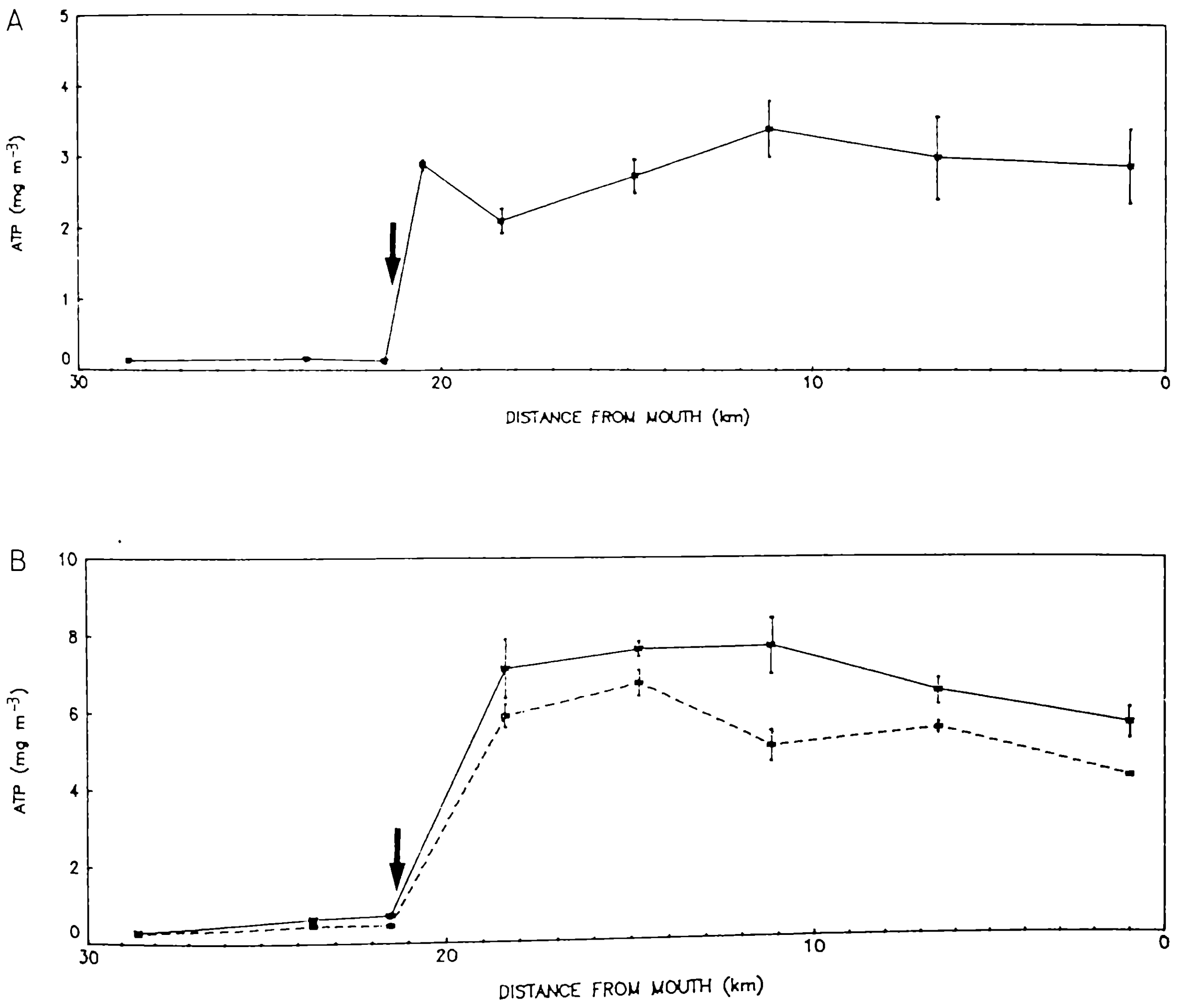


Figure 5.44 : Planktonic ATP measurements from longitudinal surveys in the Tarawera River, sampled 30 November 1983 (A) and 7 March 1984 (B). Arrow locates Tasman outfall. All 3 replicate samples and ±1 standard error.
Symbols:
★——★ Total ATP
■----■ 30 μm pre-filtered ATP.

ATP m^{-3}, (9.8-fold, $P<0.001$). Planktonic biomass gradually declined by 22% from SH30 to SH2 (1 km) ($P<0.1$) or by 27% from Mangaone (14.5 km) to SH2 ($P<0.01$).

Differential filtration prior to ATP determinations (using a 30 µm nylon filter) was used in the summer survey. Above the discharge, 64% to 84% of the biomass was <30 µm (Figure 5.44B). ATP measured in the aerated lagoons of the Tasman Pulp and Paper Co was 63.1±6.3 mg ATP m^{-3} with no significant difference for the 30 µm filtered sample. In the river there was no significant difference at SH30 (18.5 km); however, all downstream stations showed 66% to 88% <30 µm (all $P<0.05$ or greater), but with no longitudinal trend.

Benthic biomass ATP measurements are shown for the spring and summer surveys in Figure 5.45. The maximum benthic ATP value for the surface 0-2 cm depth was 29.5 mg ATP m^{-2} (based on a 1 cm layer) at Johnston Road (Nov 1983), with a minimum of 0.81 mg ATP m^{-2} at Town Bridge in the same survey. Longitudinal changes in spring showed a increase between Town Bridge (26.5 km) and Onepu Springs (23.5 km) (1.9 fold, $P<0.001$), with an order of magnitude increase to SH30 (18.5 km) (18.5-fold, $P<0.001$), and further increase to the maximum at Otakiri (13.0 km) (1.6-fold, $P<0.001$). A 65% decline occurred to Awakaponga (6.5 km). In summer the biomass was greater below the discharge (9.2-fold, $P<0.001$), declining by 59% to Awakaponga. Comparison of the 2 surveys showed a higher upstream biomass in spring (1.2-fold, $P<0.001$), no significant difference at SH30, and 39% decline at Awakaponga. Matata results show either no significant difference or a 62% decline ($P<0.001$) depending on which November results are used (Figure 5.45A).

Sediment depth profile samples showed higher sub-surface biomass levels. For the spring survey, Johnston Road (15.5 km) 10-12 cm depth biomass was greater than surface values (1.7-fold, $P<0.001$) and also at Awakaponga (6.5 km) 20-22 cm biomass was greater than surface values (1.5-fold, $P<0.001$) (Fig. 5.45A). Only SH30 showed higher biomass at 4-6 cm (1.5-fold, $P<0.001$), and at 10-12 cm (1.7-fold, $P<0.001$), in the summer survey, whereas, other stations showed comparable biomass to 10-12 cm depth (Fig. 5.45B). Comparison of spring and summer surveys considering sub-surface biomass showed significantly lower levels in the summer survey (Fig. 5.45).

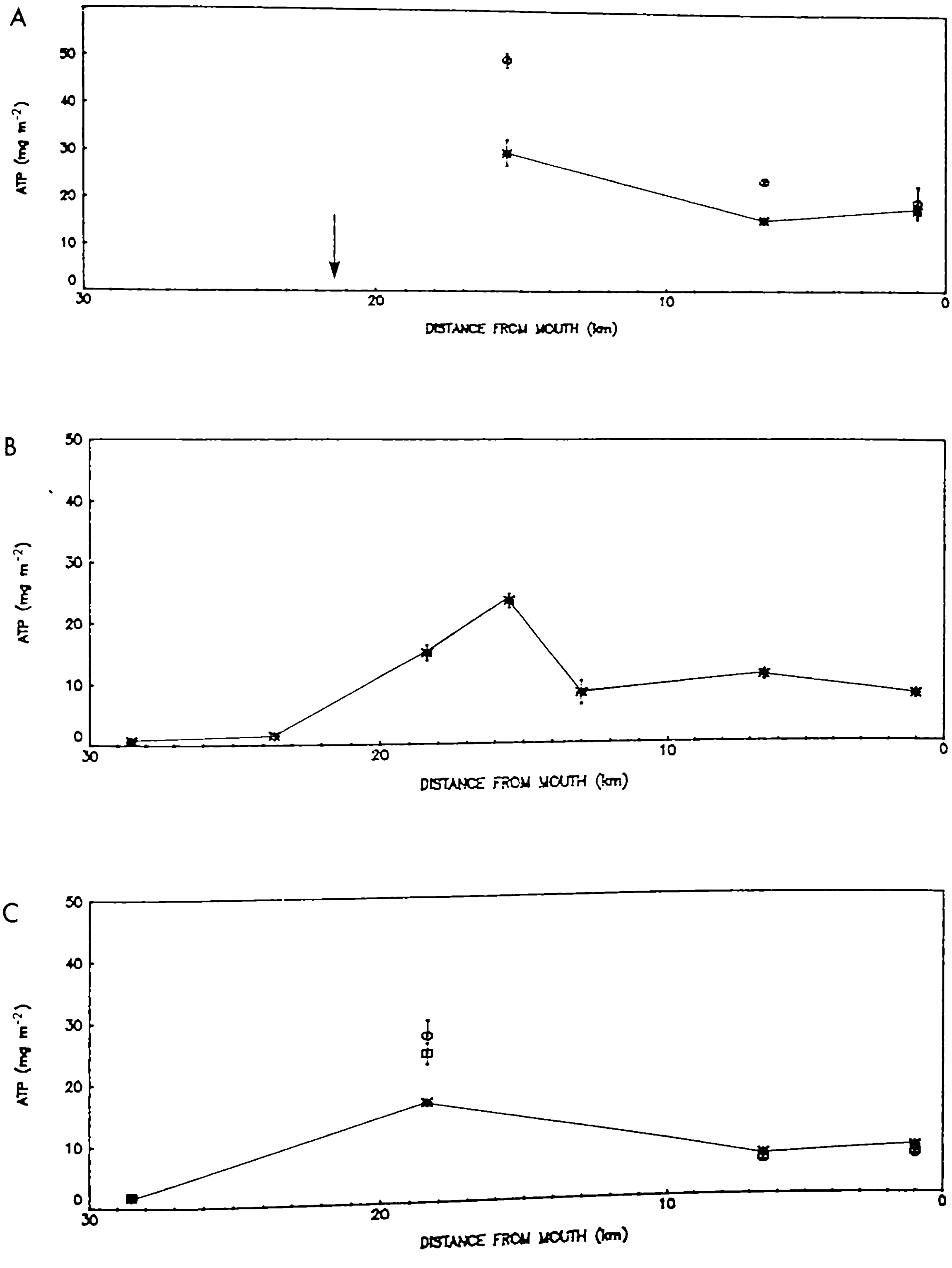


Figure 5.45 : Sediment ATP measurements from longitudinal measurements in the Tarawera River, sampled 23 November 1983 (A), 29 November 1983 (B), and 7 March 1984 (C). Arrow locates Tasman outfall. All 3 replicate samples and ±1 standard error. Symbols: as for Fig. 5.43.

Date	Station	Temperature	Discharge	Mean Depth H	Biomass Carbon		Benthic Biomass Factor[a]			
					Planktonic	Benthic[b]	Benthic[c] Depth, δ	Factor	Benthic[d] Depth, δ	Factor
	km	°C	$m^3 s^{-1}$	m	$mg\ m^{-3}$	$mg\ m^{-2}$	cm		cm	
30 Nov 1983	26.5	16.0	24.3	1.71±0.16	38.0	202.5	1	3.12	-	-
	23.5	-			43.0	392.5	1	5.34	-	-
	→ 18.5	18.0			725.0	3740.0	1	3.02	-	-
	15.5	-			692.5	5880.0	1	4.97	-	-
	13.0	-		1.32±0.10	865.0	2070.0	1	1.82	-	-
	6.5	-	27.9		765.0	2760.0	1	2.73	-	-
	1	20.0			737.0	1945.0	1	2.00	-	-
10 Mar 1984	26.5	16.0	22.6	1.67±0.13	80.2	447.5	1	3.34	6.4	21.4
	→ 18.5	19.2			1787.3	4100.0	1	1.38	2.9	4.0
	6.5	20.3	26.6	1.23±0.10	1609.5	1687.5	1	0.85	5.3	4.5
	1	20.3			1395.5	1942.5	1	1.13	4.8	5.4

arrow indicates discharge location

[a] $\dfrac{\text{Benthic biomass} \times \delta}{\text{H} \times \text{Planktonic Biomass}}$

[b] measurement representing a 1 cm surface layer

[c] arbitrary depth

[d] calculated depth from Eqn. 3.5, Table 5.36

TABLE 5.37: Relative importance of planktonic and benthic biomass in the Tarawera River.

The relative importance of planktonic and benthic biomass is shown in Table 5.37. Comparison of spring and summer surveys using an arbitrary 1 cm depth of benthic interaction, shows greater benthic dominance in the spring (1.8- to 5.3-fold). This results largely from low planktonic biomass and higher benthic biomass in the spring. Using the estimated depth of benthic oxygen penetration (from Table 5.36), benthic biomass was an order of magnitude greater than planktonic biomass above the outfall (21-fold). Values decreased below the outfall (4.0- to 5.4-fold) (Table 5.37).

No attempts were made to differentiate phototrophic and heterotrophic biomass, largely because the highly coloured pulp mill effluents severely limit potential algal growth by reduction of light levels.

5.4.5 Benthic carbon turnover

Benthic biomass carbon estimates, total carbon measurements and carbon turnover calculations are presented. Together, these provide indications of relative growth rate of the microbial populations.

At Town Bridge the biomass carbon estimates were low at 6.4 g C m^{-2} increasing to 16.8 g m^{-2} at SH30 (2.6-fold), with comparable levels at Awakaponga and Matata (Table 5.38). Comparable values were present to a depth of 10-12 cm, with the exception of a high value at Matata.

Calculation of the percentage of living biomass present gave 7.0% at Town Bridge, increasing to 24.5% at SH30 and then decreasing to approximately 11% at Awakaponga and Matata (Table 5.38). A sub-surface peak of 41.9% was present at SH30, whereas other stations showed comparable values or a slight decline.

Carbon turnover time for the surface sediments was estimated as 14.5 days upstream, decreasing to 6.7 days immediately below the discharge and then increasing downstream to 22.5 days at Matata (Table 5.38).

5.4.6 Benthic oxygen depth profiles

Measurement of the oxygen content of the interstitial water sampled from sediment cores removed from the river was attempted (see Methods section 3.3.6.6). Considerable difficulties caused by both the swift current and the non-cohesive nature of the pumice were experienced by divers in removing cores from the river bed. Results shown in Figure

Station	Distance from Mouth km	Sediment Depth cm	Total Carbon g m^{-2}	Biomass Carbon mg m^{-2}	Biomass C/ Total C %	Carbon Turnover Time[a] day
Town Bridge	26.5	0-2	6.4	447.3	7.0	14.5
		4-6	5.0	476.3	9.6	8.8
SH30	18.5	0-2	16.8	4100.5	24.5	6.7
		4-6	18.1	6110.5	33.8	7.6
		8-10	16.5	6795.8	41.9	5.6
Awakaponga	6.5	0-2	14.3	1685.3	11.8	13.5
		4-6	17.9	1509.7	8.5	13.3
		8-10	15.8	1467.3	9.3	17.8
Matata	1	0-2	17.1	1910.6	11.2	22.5
		4-6	14.9	1745.0	11.7	22.5
		8-10	50.2	1616.6	3.2	75.6

[a] Calculated as $\frac{\text{Total Carbon}}{\text{Intrinsic activity x 0.375}}$ (note: intrinsic activity from Fig. 5.43)

*Value obtained from stoichiometry ($C:O_2$)

TABLE 5.38: Sediment carbon measurements and biomass carbon estimates for the Tarawera River, sampled 10 March 1984

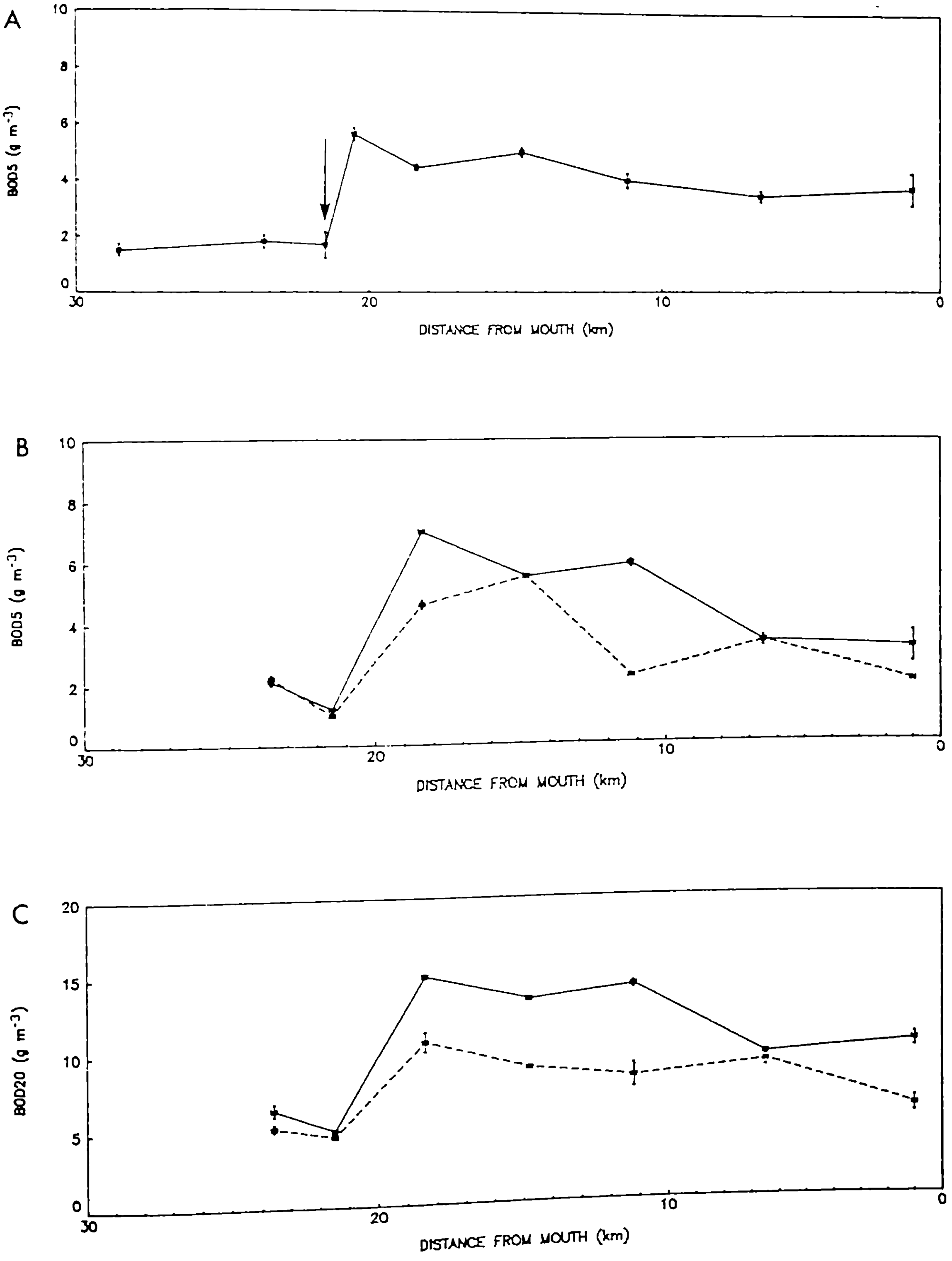


Figure 5.46 : BOD measurements for longitudinal surveys in the Tarawera River, sampled 30 November 1983 for BOD$_5$ (A), and 7 March 1984 for filtered and unfiltered BOD$_5$ (B) and BOD$_{20}$ (C). Arrow locates Tasman outfall. All 2 replicate samples and ±1 standard error.

Symbols:

★——★ total BOD

■----■ 0.45 µm filtered BOD

7.7 indicate a steep DO gradient present for the 2 stations measured from below the discharge; however, no anoxic region could be measured, despite blackening of material below 20 cm depth and sulpurous odours.

Two reasons may have contributed to the DO presence: (i) river water intrusion during sampling procedures; (ii) the sampling method used. It was found subsequently that air present in the needle of the sampling syringe could reoxygenate anoxic water by up to 1 g m^{-3}. This could be prevented by purging of the syringe with an inert gas, or by prior priming of the syringe with O_2-free water, or by removing the offending air.

These DO profile results are used in Chapter 7 for diagenetic modelling of DO flux.

5.4.7 Diurnal oxygen and BOD decay

Diurnal oxygen measurements showed small fluctuations above the discharges. Monitoring results are shown in Table 5.17 and summarised in Figure 5.25.

Longitudinal survey results for BOD sampling are shown in Figure 5.46. In the spring survey the BOD_5 at SH30 (18.5 km) was 4.75 g m^{-3} decreasing to 3.75 g m^{-3} at Awakaponga (6.5 km) (Figure 5.46A). In the summer survey a more comprehensive set of analyses was undertaken. River BOD_5 was 7.2 g m^{-3} at SH30, decreasing to 3.3 g m^{-3} at Matata (1 km) (Figure 5.46B). Filtrate BOD_5, as determined by 0.45 µm filtration, accounted for 67% of the BOD_5 at SH30, showed a downstream decrease and was 65% of the total BOD_5 at Matata. BOD_{20} determinations are shown in Figure 5.46C and show a similar trend to the BOD_5 results.

DO measurements are shown in Appendix 5.

5.4.8 Discussion and conclusions

The Tarawera River was subjected to a continuous loading of biologically-treated organic effluent, in contrast to the other study rivers which were intermittently loaded. The loading consisted of DOM and particulate organic material (POM), with the latter composed of wood fibre and microbial cells, as determined by microscopic examination.

The maximum chamber BUR measurement made <u>in situ</u> was 47.4 g O_2 m^{-2} day^{-1} which occurred downstream of Tasman Pulp and Paper Company at SH30 (18.5 km), where mean BUR values were in the range 20 to 30 g m^{-2} day^{-1} (Table 5.36), substantially higher than the 4.1 g m^{-2} day^{-1} upstream. Considerable variability of chamber BUR values were experienced below the discharge (Table 5.36). The sampling regime attempted to stratify measurements in relation to the dune structure during the most recent survey. Preliminary indications were that rates were higher at the leading edge of the dune, where there is a greater proportion of large particle size material, than near the base where fine material tends to accumulate. Sample numbers were insufficient to test this hypothesis, however, such work would constitute an important component of future research aimed at understanding the benthic mass transfer process. A high rate of photosynthetic production measured at Onepu Springs (Table 5.36) upstream of the major discharge, suggested a significant benthic oxygen production may occur with favourable clear water conditions.

Intrinsic activity measurements for surface sediments showed substantial increases below the Tasman discharge, with a progressive downstream decline (Fig. 5.43). The activity of sub-surface sediments showed minimal difference from surface values to 20 cm depth. The degree of activity increase below the Tasman discharge appeared to vary seasonally (Fig. 5.43), an effect which cannot be attributed to river temperature (Table 5.37), suggesting a response to effluent load or characteristics.

The depth of benthic oxygen penetration (δ_c) and dispersion coefficient (D_s) were calculated using the measured intrinsic activity (I). The rate of intrinsic activity measured for Tarawera pumice material was greatly affected by the amount of agitation, as found for the Waiotapu pumice. This necessitated continuous rather than intermittent agitation for measurement in order to decrease potential diffusion limitations. Sediment intrinsic activity increased substantially below the Tasman discharge, although δ_c values showed minimal decline, ranging from 3 to 10 cm (Table 5.36). Calculated values of Ds were high, with a maximum of 163.0 x 10^{-3} cm^2 s^{-1} and similar in range to measurements made in the Waiotapu River (Table 5.23). Both the depth of oxygen penetration and the dispersion coefficient were calculated from I and, for pumice sediments, I has been shown to be a function of the degree of agitation (i.e. turbulence). An arbitrary degree of turbulence compared to that in the sediments, attempted to minimise possible diffusion limitations around the sediment particles. As such the effective activity of the sediment microorganisms in situ is probably significantly less because of lower tubulence levels. Therefore, estimates of δ_c and D_s obtained by this method represent minimum values applicable to these sediments. Estimates of I are used for diagenetic modelling of BUR in Chapter 7.

Detailed analyses of chamber BUR kinetics were not undertaken, largely because of the postulate that non-steady-state kinetics dominate chamber measurements. Evidence supporting this contention is based on detailed analysis of BUR kinetics obtained from the Waiotapu River and discussed in Chapter 7. Tarawera BUR kinetics did show curvature, which suggested that results were probably consistent with previous kinetic studies on the Waiotapu River.

Benthic and planktonic biomass measurements, in conjunction with estimates of benthic oxygen penetration, showed that benthic biomass was about 20-fold greater than was planktonic biomass; this decreased to 4- to 5-fold below the major discharge, where the effluent added greatly to the planktonic biomass (Table 5.37). Based on the segment oxygen mass balance estimates for the February 1985 sampling (Table 4.3), benthic respiration was 7-fold dominant over planktonic processes. This establishes the importance of benthic biomass and the need to develop an understanding of how the aquatic environment influences benthic biomass development and nutrient exchange processes with the overlying flow.

Above the Tasman discharge the benthic biomass showed significant photosynthetic production (Table 5.36) indicating attached algal biomass. Downstream of the highly coloured discharge, algal biomass was assumed to be limited by the greatly reduced light levels although no biomass measurements were made. Surveys on 4 occasions of stations below the Tasman pond discharge showed that the 1% PAR level was 0.7-1.0 m depth (R.J. Davies-Colley, pers. comm.), which is significantly less than the mean depth of 1.5 m (Table 4.2). Total benthic biomass was estimated by ATP measurement with a maximum of 29.5 mg ATP m^{-2} which declined downstream for both surveys (Fig. 5.45). This value was substantially higher than the maximum Waiotapu surface biomass of 11.0 mg ATP m^{-2} (from Table 5.29). Tarawera biomass levels measured in this study were comparable with those measured by Gillespie (1982), who reported a decreasing level of biomass with increasing distance below the Tasman discharge.

Although both biomass and activity measurements of the surface sediment layers declined with increasing distance below the discharge, the mass balance gross oxygen uptake calculations showed no respiratory decline (Appendix 5). Chamber BUR measurements also declined; however, calculated δ_c and D_s values were higher downstream (Table 5.36). Sediment particle size data showed increased >4 mm fractions for Matata (SH2) but not for Awakaponga (Appendix 4). Thus sediment surface layer properties alone, are probably insufficient to explain differences in mass transfer to sediment depths in the region of 10 to 20 cm necessary to satisfy the mass balance. Crude estimates of dune heights (obtained by wading with ruler attached to waders) showed no significant differences between stations (all ~20 cm). Increased mass transfer may have occurred because of a greater shear stress resulting from a downstream decrease in river mean depth and increased velocity. This increasing shear stress may have been sufficient to increase penetration through the bed structure, thus increasing effective mass transfer, but not greatly influencing dune structure. Further experimental work remains to be done to determine the factors influencing turbulent sediment-water exchange.

Planktonic biomass was measured on 2 longitudinal surveys, and showed no decline in November 1983, but a 22% decline over 20 km from a 2-fold higher maximum level in March 1984 (Fig. 5.44). For a travel time of 8 h this could correspond to a decay coefficient of 0.75 day^{-1}, substantially higher than the 0.1 day^{-1} considered appropriate for cell maintenance processes (Pirt, 1975). Furthermore, differential size filtration of samples consistently showed that about 66% of the particles were less than 30 μm in size. This suggested that the removal of particles by the river did not discriminate at this size range. Potential sites for particulate organic material appear limited in the Tarawera River with a mean velocity >0.5 m s^{-1} and a rectangular cross section supporting only emergent vegetation. Although this littoral area has larger amounts of soft mud entrained, presumably originating from biomass carried by the river, the capacity of this region as a potential site for settling of organic matter is unknown.

Biomass carbon estimates showed that up to 25% of the total benthic carbon for 0-2 cm, and a maximum of 42% at 10-12 cm (Table 5.38). Values were highest at SH30 and comparable at the 2 downstream stations

(Table 5.38). Carbon turnover time ranged from 6.7 to 22.5 days for surface sediments, increasing downstream from a minimum below the discharge, and indicating a longer response time for changes in benthic carbon. The high proportion of benthic biomass carbon and high BUR, together with the downstream removal of DOM (estimated as filtered BOD, see (Fig. 5.46), implied an epilithic heterotrophic population growing attached to the benthic particulates. A major question arises relating to the fate of microbial biomass produced. Assuming steady-state river conditions, a BUR of 30 g O_2 m^{-2} day^{-1} and a low 20% substrate yield (i.e. Y = 20% of carbon to new biomass); an estimated 3 mg ATP m^{-3} planktonic ATP increase (for 1 m deep and 8 h travel time) would be anticipated. Actual river measurements showed a decline in planktonic biomass (Fig. 5.44) suggesting that either: (i) biomass was not sloughed off as living cells; or (ii) that cells were rapidly removed from the system. A possible mechanism for this latter process could be by protozoal predation. Microscopic examination of the pumice showed numerous vorticellid protozoa. Although no quantitative estimates of biomass were made, this population may consume bacterial biomass, and in so doing, constitute a substantial component of the river BUR.

Reports detailing the role of attached protozoal populations in rivers are limited. Lock et al. (1984) report that large numbers of attached protozoa of the Vorticella sp., Vaginicola sp. and Carchesium sp. were present in a Canadian river, though their distribution was restricted to the under-surfaces of rocks. Similar growth of Vorticella sp. restricted to the under-surface of rocks occurs in the Oroua River, New Zealand, downstream of a biologically treated meat works discharge (J.M. Quinn, personal communication). The presence of these protozoal populations raises questions as to their quantitative significance in river carbon pathways.

To enable predictive modelling of this system a better understanding of the factors influencing benthic respiratory yield and respiratory quotients, together with the constituent components of BUR will be required. Predictive modelling of BUR will be further discussed in Chapter 7.

5.4.9 Summary

Chamber BUR measurements were less than (~50%) the BUR values calculated from segment oxygen mass balances for the Tarawera River, which suggested, that the chamber did not adequately simulate the natural hydrodynamic conditions present in the deeper river (section 4.2 and 5.4.2).

Longitudinal studies showed a low BUR values with photosynthetic production upstream, with a large BUR increase below the organic pulp mill discharge. Benthic intrinsic activities and biomass levels also increased substantially below the discharge. The calculated depth of penetration of oxygen into the sediment depth showed minimal decline below the discharge at 3-10 cm. This oxygen supply was sustained by high benthic dispersion coefficients, approximately 3 orders of magnitude greater than molecular diffusion, facilitating the greatly enhanced benthic activities present (sections 5.4.3). This would result in rapid nutrient transformations occurring within the river system.

Planktonic biomass levels were high below the pulp mill oxidation and discharge. However, the benthic biomass was more than 5-times the planktonic contribution. Planktonic biomass levels declined downstream during the ~8 h travel time to the river mouth, which suggested that river mechanisms were operating which removed the biomass produced (section 5.4.4).

Benthic biomass levels remained relatively constant below the organic outfall, with biomass carbon levels constituting up to 24% of the surface sediment carbon, and up to 42% of the sub-surface carbon levels (section 5.4.5). Sediment DO was measured to 15 cm depth at sites below the organic discharge (section 5.4.6). The high measured sediment carbon turnover rate and level of biomass carbon present, suggest that the microbial population present were epilithic heterotrophs, metabolising both electron donors and electron acceptors from the overlying flow. Thus the high rates of BUR measured in this river were sustained by a large mass flow of nutrients, facilitated by the rapid sediment-water exchange.

5.6 GENERAL DISCUSSION

Comparison of sediment biomass and metabolic activity in the New Zealand rivers studied with other aquatic environments. The basis for comparisons cover major factors outlined in the Introduction (Section 1), including those physical, chemical and biological factors which

BUR $\text{gm}^{-2}\ \text{day}^{-1}$	Environment Sampled	Sediment Type	Temperature °C	Measurement Device	Source
A	**Sediments without biofilms**				
0.6-4.8	English rivers	mud	10-20	stirred cores	Edwards and Rolley (1965)
0.92-3.0	Baltic sea	-	13-17	*in situ* chamber	Edberg and Hofsten (1973)
0.43-2.6	Lakes	-	5-18	*in situ* chamber	
0.31-1.44	Streams	-	0-10	*in situ* chamber	
3.60-22.08	Streams	-	-	stirred cores	James (1974)
3.84-27.12	Streams	-	-	*in situ* chamber	
5.28-38.16	Streams	-	-	respiration tunnel	
1.68-9.36	Lakes	-	-	stirred core	
3.12-9.60	Lakes	-	-	*in situ* chamber	
0.27-0.67	Lakes	mud	10	stirred cores	Graneli (1977)
0.22-28.8	Estuary	mud	-	stirred cores	James (1980)
1.61	Lake	mud	-	*in vitro* chamber	Belanger (1981)
0.10-3.0	Estuary	sand	2-16	*in situ* chamber	Hargrave and Philips (1981)
0.21-0.67	Carmarthen Bay, England	sand	8-17	stirred cores	Pomroy *et al.* (1983)
5.0-15.8	New Zealand rivers	sand	8-25	*in situ* chamber	Cooper (1984)
1.8-4.2	Choptank River, USA	mud	18-27	*in situ* chamber	Smith and Fisher (1984)
4.3-23.4	New Zealand rivers	sand		*in situ* chamber	This study
3.5-47.4	New Zealand rivers	pumice		*in situ* chamber	
B	**Biofilms**				
0.7-14.9	River Culm, England	rock	4-21	*in situ* chamber	Boyle and Scott (1984)
8.7-45.2	New Zealand rivers	cobbles	8-25	*in situ* chamber	Cooper (1984)
3.8-70.2	New Zealand rivers	sand-cobbles	16-25	*in situ* chamber	This study

TABLE 5.39: Comparison of BUR measurements from a variety of aquatic environments

ATP Concentration	Environment Sampled	Method of Extraction	Source
A ATP concentrations reported in units of μg g dry sediment $^{-1}$			
1.5-9.5	Surface sediment, temperate lake	Cold sulphuric acid	Lee *et al* (1971)
7.9-9.1	Top 1 cm muddy and sandy mud - 3 stations, Norwegian fjord, bottom was not anoxic	Boiling Tris-buffer	Pamatmat and Skjoldal (1974)
1.2-9.1	Top 1 cm, all seasons, *Spartina alterniflora* march, Georgia, USA	Boiling bicarbonate	Christian *et al*. (1975)
1.09-7.64	Top 1 cm, muddy sediment, Long Island Sound, USA, 14 m depth	Boiling bicarbonate	Yingst (1978)
0.12-0.45	Top 1 cm, sandy beaches (low tide), Southern California	Sulphuric acid	Karl (1979b)
4.61-56.0	Surface sediment, English lakes	Boiling Tris-buffer	Jones (1980)
0.31-6.34	Surface mud, aquiculture ponds, Hawaii	Phosphoric acid	Karl and Craven (1980)
0.77-62.15	Surface sediment, Antarctic and temperate lakes	Boiling phosphate	Simmons *et al* (1983)
0.045-1.10	Top 1 cm, sand in New Zealand rivers	Boiling Phosphate	This study
0.070-2.66	Top 1 cm, pumice in New Zealand rivers	Boiling Phosphate	This study
B ATP concentrations reported in units of μg cm sediment volume $^{-3}$			
0.14-1.18	Top 1 cm, shallow estuary, Beaufort, North Carolina, USA	Boiling Tris-buffer	Ferguson and Murdoch (1975)
0.13-0.67	Surface sediment, 28-345 m depths, North Sea	Boiling Tris-buffer	Ernst (1970)
0.36-3.87	Top 1 cm, muddy sediment, Long Island Sound, USA	Boiling Bicarbonate	Yingst (1976)
0.64-2.19	Surface sediment, English lakes	Boiling Tris-buffer	Jones (1980)
0.67-14.55	Top 1 cm, sand in New Zealand rivers	Boiling Phosphate	This study
0.81-29.51	Top 1 cm, pumice in New Zealand rivers	Boiling Phosphate	This study
C ATP concentrations reported in units of μg cm biofilm $^{-2}$			
0.011-0.014	Stone surface colonisation in bush and tussock streams in New Zealand	Chloroform-Tris and Bicarbonate Buffer	Rounick and Winterbourn (1983)
0.008-0.338	Stone surfaces in Canadian rivers	Boiling Tris-buffer	Lock *et al*. (1984)
0.68-7.70	'Sewage fungus' on natural and artificial substrata in New Zealand rivers	Boiling Phosphate Buffer	This study

TABLE 5.40: Comparison of ATP concentrations of sediments from a variety of aquatic environments

interact, to control the BUR (Fig. 1.2). Furthermore, the functional implications in terms of river processes, of the degree of benthic dominance are discussed as they affect time scales and magnitude of rate processes.

As summarised in Table 5.39 the chamber measurements made in situ for the New Zealand rivers studied showed that the BUR ranged over an order of magnitude, with the lowest rates equating to the highest rates reported for other environments. Both sediment and biofilm BUR maxima reported here are substantially higher than other reported values. With additional measurements demonstrating up to 50-fold benthic dominance in the river systems studied here (subsections 5.2.3, 5.3.6, 5.4.5, 5.5.3), it becomes important to identify the factors contributing to the high BUR.

The physical characteristics of the river systems studied may differ considerably in some but not all components as compared with the aquatic environments studied by other workers. Principal amongst these differences would be the unidirectional flow velocity, which combined with floods and turbulence levels, greatly influence the bed particle size, amount of detritus present and supply of nutrients to the organisms present. Additional differences, such as the relative shallowness of rivers and their turbidity may influence the light penetration and hence the potential for phototrophic development. Microbial biomass may well constitute a substantial component of benthic respiratory demand in all aquatic ecosystems. A comparison of ATP estimations showed that on a dry weight basis the ATP levels found in the rivers studied here were lower than most reported for predominantly silty or muddy environments (Table 5.40A). Considered on a volumetric basis, however, maximum ATP values were substantially higher greater in the rivers studied, than for other systems (Table 5.40B). An order of magnitude higher ATP values were observed for biofilms on an areal basis in this study (Table 5.40C). Assuming that the ATP measurements represent the level of active biomass present, then substantially higher biomass densities are present in the rivers studied here. To sustain this biomass level requires an increased supply of nutrients in order to support metabolic processes and growth. This implies higher benthic dispersion coefficients and a greater degree of sediment-water interaction.

The depth of oxygen penetration into coastal marine sediments as measured using microelectrodes varied from 1 to 5.5 millimeters (Revsbech et al., 1980) and up to 40 cm in estuarine mud sediments (Kepkay and Novitsky, 1980). High interstitial DO concentrations may be present below 30 cm depth within river beds (Williams and Hynes, 1974; Whitman and Clark, 1982), sustaining rich and varied invertebrate psammon communities to that depth (Williams and Hynes, 1974). The depths to which O_2 penetrates the sediments of the rivers studied here have been estimated from both chamber and sediment intrinsic activity measurements and ranged from 0.35 to 32 cm, with the minimum values for 'sewage fungus' biofilms (Table 5.1). Actual measurements of interstitial DO for 2 pumice bed rivers, showed DO present to at least 10 cm in the Waiotapu River (Fig. 5.41) and to 15 cm for the Tarawera River (Subsection 5.4.6 and Fig. 7.7). These estimated oxygen penetration depths were used to calculate benthic dispersion coefficients.

Values of benthic dispersion coefficients obtained in this study are compared with estimates obtained from other aquatic environments in Table 5.41. Values obtained in this study were maximally several orders of magnitude higher than previously reported maxima. Values estimated using benthic oxygen measurements were of comparable magnitude to river estimates obtained by Cooper (1984) using ammonium ion flux and concentration measurements. Based on oxygen mass balance estimates, however, the values estimated from the chamber BUR for pumice rivers may be 50% lower than the actual mean river values. This difference may be caused by two different aspects of the chamber devices: (i) failure to match adequately the turbulent mass transfer conditions present in the river; or (ii) restricted percollation within the sediments caused by the chamber flanges. These factors have been discussed in Section 4.2.3.

The higher BUR values obtained in this study (Table 5.39), when compared with lake or marine environments, may result from enhanced mass transfer phenomena associated with the sediment-water interface. In essentially quiescent environments, the exchange processes are principally controlled by molecular diffusion processes, as evidenced by the

D_s cm^2s^{-1}	Environment Sampled	Sediment Type	Depth cm	Measured Constituent	Source
> 2.8×10^{-5}	Long Island Sound, USA	mud	0-8	sulphate	Goldhaber _et_ _al_. (1977)
10^{-4}	Coastal North Sea	mud	0-3.5	silicate	Vanderborght _et_ _al_. (1977)
$0.5\text{-}2 \times 10^{-4}$	Coastal North Sea	sand	0->15		Billen (1978)
4×10^{-5}	Narrangansett Bay, USA	mud	0-25		McCaffrey _et_ _al_. (1980)
0.88×10^{-5}	Randers Fjord estuary, Denmark	sand	0-1	oxygen	Revsbech _et_ _al_. (1980)
$0.02\text{-}1.3 \times 10^{-3}$	New Zealand rivers	sand	0-2	ammonium	Cooper (1984)
$3.7\text{-}11.1 \times 10^{-3}$	New Zealand rivers	pumice	0-2	ammonium	Cooper (1984)
$0.38\text{-}71.2 \times 10^{-3}$	New Zealand rivers	sand	0-5	oxygen	This study
$1.7\text{-}163.0 \times 10^{-3}$	New Zealand rivers	pumice	0-5	oxygen	This study

TABLE 5.41 : Comparison of benthic dispersion coefficients for sediment interstitial waters from a variety of aquatic environments.

similarity of in situ dispersion coefficients and those for molecular diffusion (Berner 1980; Table 5.41). In systems where the sediment-water interface is disturbed by currents, apparent dispersion coefficients are markedly higher (Vanderborght et al, 1977; Billen, 1978; see Table 5.41). In those New Zealand rivers studied with even higher apparent dispersion coefficients, the role of turbulent mass transfer must be of even greater significance.

High rates of oxygen supply may facilitate the high levels of microbial biomass and activity. However, the sustenance of metabolism and growth requires a continued availability of reducing potential. If we restrict this discussion to heterotrophic metabolism, then the supply of reduced carbon material forms the basis for microbial growth. In quiescent systems the settling of silt and seston forms the basis of carbon metabolism, with a requirement for exo-enzymic breakdown prior to uptake and undertaken by detrital heterotrophs. In turbulent systems the potential exists for supply of DOM to sustain growth as attached biofilms or epilithic growth may extend throughout the sediments or be restricted to surface growths (e.g. 'sewage fungus' as discussed in Section 6.3). The relative importance of these 2 avenues of carbon supply influence both the timescales and factors such as electron donor concentration which may control the overall rate process.

In the rivers studied the carbon turnover time for the top 1 cm (assuming steady-state conditions) ranged from 5 to 52 days for sandy beds (Table 5.12), and 5.6 to 22.5 days for pumice beds (Table 5.34, 5.38). This contrasts with marine estimates of 1.5 years for the top 1 cm, increasing to 17.7 years at 12 cm depth (Hargrave and Philips, 1981). Turnover times in depositional systems will vary greatly between areas of widely differing productivity (see Zeitzschel, (1979) for a marine review); however, the point to be made here relates to the potential magnitude of time scale differences. Additionally, the actual standing stock of biomass in relation to carbon present may differ greatly between systems.

Sandy river sediments showed biomass carbon ranging from 1.1 to 16.7% of the total carbon present (Table 5.10), whereas in pumice bed rivers it ranged from 2.4 to 24.5% for the top 1 cm (Tables 5.32 and 5.45). Estimates for lake muds range from 1.4 to 9.6% (Jones, 1980), with 1.5%

estimated for bacteria attached to marine sands (Meyer-Reil et al. 1980). Thus, not only was the storage reserve of carbon lower for the river sediments studied here, but generally higher proportions of biomass carbon were present.

Interactions between sediments and the overlying flow. Measurements of planktonic biomass production in the Tarawera River were not high enough to account for microbial production rates estimated from the measured BUR values, showing an actual downstream decline. These results have been discussed in Section 5.4.8, with the suggestion that secondary production may be accounted for by protozoal metabolism. Attached protozoa were present attached to pumice granules and their presence may significantly alter respiratory quotients and river response to changes in effluent characteristics, since their food supply is POC rather than the DOM component. Their relative importance is unknown at this time but may constitute a major component of this system.

Longitudinal and depth profile studies of enzyme activities in the Waitoa River showed that the predominant activity changed in relation to distance from the discharge or surface, presumably reflecting changes in organic constituents and concentrations in the overlying flow. These results have been discussed in Section 5.2.9, and summarized in a process diagram (Fig. 5.26). The implications of this adaption for optimal organic metabolism are that yield coefficients will vary with distance downstream in relation to the nature of the carbonaceous substrate (Payne, 1970; Bailey and Ollis, 1977). This means that the relationship between river DOM (or soluble BOD) and oxygen removal (Qo_2) will not be constant throughout the river system. Additional stresses on yield relationships may also be caused by nutrient limitation.

Enzyme activities of alkaline phosphatase were undertaken to indicate possible regions which may be limited by phosphorus availability. The results of longitudinal surveys showed some high specific activities of phosphatase (Fig. 5.9), as did increasing benthic (Fig. 5.20, 5.21) depths. This suggested that PO_4^{3-} supply was limiting, since activity of this enzyme system would otherwise be strongly repressed (Aaronson et al., 1976). Under carbon sufficient conditions but if limited by some other nutrient essential for growth, be it oxygen, phosphorus or nitrogen, bacterial monocultures show substantial accumulation of the carbon storage compound poly-β-hydroxybutrate (PHB) (Dawes and Senior, 1973). Such accumulations have also been shown to occur for estuarine detrital microflora, where PHB accumulation can be stimulated 2- to 300-fold by addition of either sodium phosphate or ammonium nitrate with glucose (Nickels et al., 1979). Adding glucose and nutrients (NH_4^+ and PO_4^{3-}) sharply reduced PHB accumulation. The implication from these field measurements is that under times of other nutrient limitation, but carbon sufficiency, yield relationships may differ considerably from otherwise identical carbon and oxygen conditions. Yield coefficients and respiratory response may also vary in relation to antecedent loading conditions.

Bacteria subjected to discontinuous or varying environmental concentrations of a growth-limiting nutrient may exhibit enhanced uptake mechanisms and variable respiratory quotients. Evidence from 2 Qo_2 estimations for the intermittently-loaded Waiotapu River gave Qo_2 estimates of 48% and 20%. Laboratory reports suggest that higher rates of oxygen consumption may result for discontinuously-fed glucose-limited cultures (Neijssel and Tempest, 1976), with experimental evidence further suggesting that both glucose uptake and growth rate are established by the maximum concentration present (La Motta, 1976a; Gaudy and Gaudy, 1981). Substantial metabolic rate increases were shown for the Waiotapu River with glucose supplementation (Table 5.23). The magnitude of such responses has been shown to vary with microbial growth rate for continuous culture experiments, increasing by up to 5-fold under nutrient-sufficient conditions (Tempest and Neijssel, 1978). How such laboratory experiments relate to the river environmental conditions is unknown; however, the potential for large and rapid metabolic responses to environmental nutrient concentration changes exists and may well be realised in river systems where significant benthic populations are present and growing on DOM supplied.

Diurnal monitoring of upstream stations showed maximal fluctuations between 4 and 10 g m^{-3}. Chamber studies showed BUR to be related to DO concentration. This has been discussed in relation to biofilms in Chapter 6, and will be discussed in relation to sediment systems in Chapter 7. Discussion in this section will be restricted to overviewing the implications for river processes.

The fluctuating DO concentration causes both BUR and DOM removal rate to vary with time. Additionally, a benthic zone experiencing intermittent deoxygenation followed by reoxygenation will be present, with the possibility for optimal conditions to exist for both nitrification and denitrification processes to occur. Cooper (1984) has shown benthic nitrification to be a significant factor in oxygen removal during the winter months in several New Zealand rivers. A substantial denitrification component was inferred during the summer. Ventullo and Rowe (1982) report that the epilithic communities showed denitrification potential under both aerobic and anaerobic conditions. Thus the benthic microorganisms may be responsible for substantial nutrient modifications to the overlying flow.

Review of experimental approach and methods used. This section evaluates the experimental methods desirable for study of the response of swift, shallow rivers to organic effluent discharges.

For each of the rivers studied, benthic processes generally represented 60-90% of the total metabolic potential. Because of this high level of total biomass interacting with the flowing water, the rate of removal of organic waste components may therefore be rapid and result in large changes in river DO and BOD or nutrient concentrations over short distances (<10 km). Thus there is a need to have an accurate knowledge of the river waste concentrations at all river sampling stations, in order to relate benthic biomass levels and metabolism to characteristics of the overlying flow. Unfortunately the work on the Waitoa River was limited in this regard because of the fluctuating nature of the untreated discharge. Studies were planned for the following season after the planned installation of an aerated lagoon treatment system; however, this plant was not installed. If studies are to be performed on a shallow river with an unsteady effluent discharge, then several continuous river monitoring stations, with accurate estimates of the flow time between stations, would be required to assess the extent of effluent effects. Additionally, benthic metabolism would be expected to respond more slowly to effluent changes because of carbon material stored in the sediments; and be sensitive to flood flows which scour off biofilms, increasing both the turbidity and the depth of flow which will limit light penetration to the bed. This may greatly affect attached algal growth and result in the large benthic population changes found in the Waitoa River in the weeks following a flood. Thus the rivers should be at a steady base flow rate for a period of more than 2 weeks prior to sampling. Ideally base flow studies are preferable prior to opportunistic sampling.

Benthic metabolism and biomass measurements are each essential in order to relate river deoxygenation with waste loadings. Benthic chamber systems must simulate natural flow conditions, with a matching of the boundary velocity (0.05 m above bed) providing an adequate criteria. Deep pumice bed rivers (>1.5 m) with dunes were found to give low chamber measurements, possibly due to either (i) restricted interstitial percolation caused by chamber flanges; or (ii) reduced maximum turbulent eddy diameter caused by the chamber lid. Further experimental work is required to better define this problem. Because the chamber BUR values were also affected by DO and sometimes glucose concentrations, measurements should be made as rapidly as possible in order to minimise such effects. Sediment intrinsic activity measurements were made at high levels of agitation in this study in order to minimise diffusion gradients around particles and hence measure the maximum metabolic rate. Therefore, these values obtained for depths of oxygen penetration into the sediments, calculated from intrinsic activity and chamber BUR values (see Method 3.3.6.5), represent minimum estimates. Natural interstitial agitation levels are likely to be less than those in the experimental conditions used here resulting in lower metabolic levels in situ, as suggested by the experiments showing sensitivity of intrinsic activity measurement to agitation levels. Thus the depth of oxygen penetration into natural sediments would be greater than that estimated here. Techniques are necessary which measure the actual depth of sediment DO penetration (micro-electrode or interstitial water sampling) combined with interstitial flow velocity measurements in the field, together with laboratory measurements of sediment metabolic activity at different DO and interstitial flow velocities (e.g. in a tube packed with sediment). Further experimental work is required to develop these techniques.

Enzyme activity measurements appear to offer a promising technique for field investigations, particularly with the use of induced enzymes where activity occurs in response to a specific waste component. Further work is required on the use of the freeze/thaw extraction technique used in this study, in order to document: (i) efficiency of extraction from various sediment types; (ii) stability of various enzymes to frozen storage conditions; and (iii) the effects of inhibitory compounds which may be present (e.g. heavy metals).

Chlorophyll and ATP were used as mesures of phototrophic and total microbial biomass respectively. Care must be taken for each of these methods when analysing sediments which require pheo-pigment correction to chlorophyll extracts, and also when corrections for extraction efficiency and interference have to be made to ATP estimates. The heterotrophic microbial population was estimated by difference after conversion of chlorophyll and ATP values to carbon equivalents. The conversion factors used are derived from studies on planktonic organisms which have been shown to vary in response to environmental conditions (Karl, 1980). While these conversion factors are of considerable use for preliminary investigations, more detailed studies on individual systems would ideally require the determination of conversion factors appropriate to the benthic communities present. Specific biomass measurements are necessary in order to determine the major biomass type, and hence determine whether rates of organic or inorganic nutrient transformation will be increased because of the benthic organisms.

The great importance of benthic microorganisms has been established in these studies. Further studies must identify the factors which determine the maximum attached biomass levels and the requirements for nutrient supply. The level of metabolic activity present is sustained by a high mass flow of nutrients and therefore both concentration and flow velocity components must be considered.

Chapter 6

RIVER OXYGEN UPTAKE AND CARBON TURNOVER IN 'SEWAGE FUNGUS' BIOFILMS

6.1 INTRODUCTION

Organic effluent discharges into 3 of the study rivers resulted in extensive downstream 'sewage fungus' growths. These resulted in rapid deoxygenation of the overlying river water.

Work on heterotrophic biofilms in these rivers is assembled together because it illustrates an application of chamber BUR to river management problems. Extensive biofilm formation on the river bed may be considered separately from other sediment measurements as it is restricted to growth attached to a solid substratum, which becomes smothered with living biomass, greatly restricting the supply of oxygen to the underlying substratum.

This chapter describes the results of chamber BUR measurements in situ and the longer term kinetics of chamber oxygen depletion. BUR was calculated using a segment oxygen mass balance on the Waitoa River for comparison with chamber measurements.

The biofilm respiratory decay rate was measured using the EBOD apparatus in order to estimate the likely effects of decreasing BOD levels on biofilm activity.

6.2 PROCEDURES

6.2.1 Segment oxygen mass balance

This was performed on the Waitoa River over a 2.2 km reach immediately below the dairy effluent discharge, in order to enable comparison of calculated and measured estimates of the BUR. This reach was chosen because of: (i) the minimal amounts of aquatic macrophytes present; (ii) a regular cross-section; and (iii) a large DO depletion caused by the 'sewage fungus'. The longitudinal or time-varying distribution of dissolved oxygen concentration under steady-state conditions requires evaluation of the processes which produce or utilise oxygen and are described by:

$$\frac{dC}{dt} = k_2 (C_s - C) - R \qquad (6.1)$$

which with integration and rearrangement is:

$$R = \left\{k_2\left[C_s - \frac{[C - C_0 e^{-k_2 t}]}{1 - e^{-k_2 t}}\right]\right\}H \qquad \text{g } O_2 \text{ m}^{-2} \text{ day}^{-1} \qquad (6.2)$$

where C_s = dissolved oxygen (DO) at river temperature, concentration at saturation in g O_2 m^{-3}; C_o = upstream DO concentration, in g O_2 m^{-3}; C = downstream DO concentration, in g O_2 m^{-3}; k_2 = reaeration rate coefficient, expressed day^{-1} (base e); t = segment travel time, in h; H = mean depth, in m; and R = total respiration rate, in g O_2 m^{-2} (consumed) day^{-1}.

The terms describing production processes of river DO in eqn 6.1 are limited to atmospheric reaeration because submerged macrophytes were largely absent from the study reach.

Measurements of atmospheric reaeration, river oxygen concentration, and chamber measurements were made on 16 Feb 1982. Dr R.J. Wilcock, provided his measurements of k_2 = 10.9 ± 0.6 day^{-1} (mean ±1 standard error), t = 1.44 h, and H = 0.4 ± 0.08 m, some of which have been published (Wilcock 1984b).

Previous monitoring (25-28 Jan 1982) of diurnal oxygen variation above the segment, where macrophytes were prolific, had shown that DO concentration ranged from 6.5 to 12.5 g m^{-3}. Calculations were required to describe the oxygen concentration variation of the water entering the segment prior to the time of measurement for balance calculations (1000 h). For the period monitored, during the early morning (0700-1000 h) the average increase in DO was consistently +0.6 g m^{-3} h^{-1}. The measured upstream DO concentration of 9.5 ± 0.1 g m^{-3} was corrected using this rate and the segment travel time, in order to provide a mean DO concentration entering the reach of C_o = 9.1 ± 0.2 g m^{-3} at 19 °C.

Monitoring at the downstream station (8-15 Feb 1982) had shown that the DO concentration ranged from 0 to 0.9 g m^{-3}. The measured DO used for the balance calculations was C = 0.9 ±0.1 g m^{-3} at 19 °c.

Chamber measurements were performed at one station only, (~100 m below the discharge) with 3 repeated measurements over one site.

The variability in BUR values determined from a previous sampling occasion, with visibly similar 'sewage fungus' colonisation, was included with estimates of the uncertainty in measurement. Because the BUR was shown to be affected by DO concentration (see later Results section), the BUR appropriate to various locations within the segment was calculated by substituting several DO values into the chamber BUR equation. The segment BUR was determined as the arithmetic mean for 4 DO concentrations (9, 7, 5, 3 g m^{-3}), representing a linear decrease in oxygen concentration within the segment.

Calculations were as follows:

1 site, 3 reoxygenations

equation BUR = $32.6\ C^{0.28}$ ($P<0.001$, $r^2 = 0.470$, $n = 25$)

Arithmetic mean for DO = 9, 7, 5, 3 g m^{-3}

BUR = 52.3 ± 5.2 g m^{-2} day^{-1}

Error contribution from BUR variability (from 24 Sept 1981) for 4 sites

43.5 ± 3.2 g m^{-2} day^{-1} or 7.4% (S.E.)

Measurement and Total error

BUR = 52.3 ± 6.5 g m^{-2} day^{-1} (12.4% total error.)

Two artificial substrata surfaces were also used. These consisted of 3 mm polypropylene sheet of appropriate chamber dimensions, one scored with #150 abrasive and the other with 10 mm thickness polyurethane foam glued to the base (after Cairns _et_ _al_. 1979). These artificial substrata were held 0.1 m above the bed with the study surface facing down and allowed to colonise with 'sewage fungus'. After 4 days they were inverted, and placed in the chamber for BUR measurement, then transported to the laboratory for subsequent analyses.

6.3 RESULTS

6.3.1 Factors affecting the measurement of BUR kinetics

Segment oxygen mass balance. To determine whether the chamber system adequately simulated the river hydrodynamics, an oxygen mass balance study was undertaken for the Waitoa River. The chamber and river velocities were matched at 0.25 m s^{-1}. Net BUR estimates and the chamber measurements (Table 6.1) did show an overlap at the 95% confidence interval, though the chamber measurement was somewhat less than the net BUR calculated. Aquatic oxygen demand was not included in these estimates since short term measurements indicated a negligible contribution. Filamentous organisms attached to debris (e.g. logs) and not included in the chamber system may have contributed to the difference. The magnitude of the chamber measurement was, however,

River	Sampling date	Segment Length km	Segment Travel time h	Segment BUR[a] g O_2 m^{-2} day^{-1}	Chamber BUR[b] g O_2 m^{-2} day^{-1}
Waitoa	16 Feb 1982	2.2	1.4	75.3±8.7	52.3±6.5

[a] Segment BUR calculated using a measured k_2, travel time, and mean depth (Wilcock, 1984b) for monitored DO conditions.

[b] Measured chamber BUR obtained for measurement 50 m below discharge only. A mean BUR was calculated using the measured mass transfer equation (see Table 6.3) and four dissolved oxygen calculations (9, 7, 5, 3 gm^{-3}). The standard error estimate was obtained from adjacent site measurements of 24 September 1981 (Table 6.2).

Table 6.1: Comparison of chamber and calculated segment oxygen mass balance BUR estimates

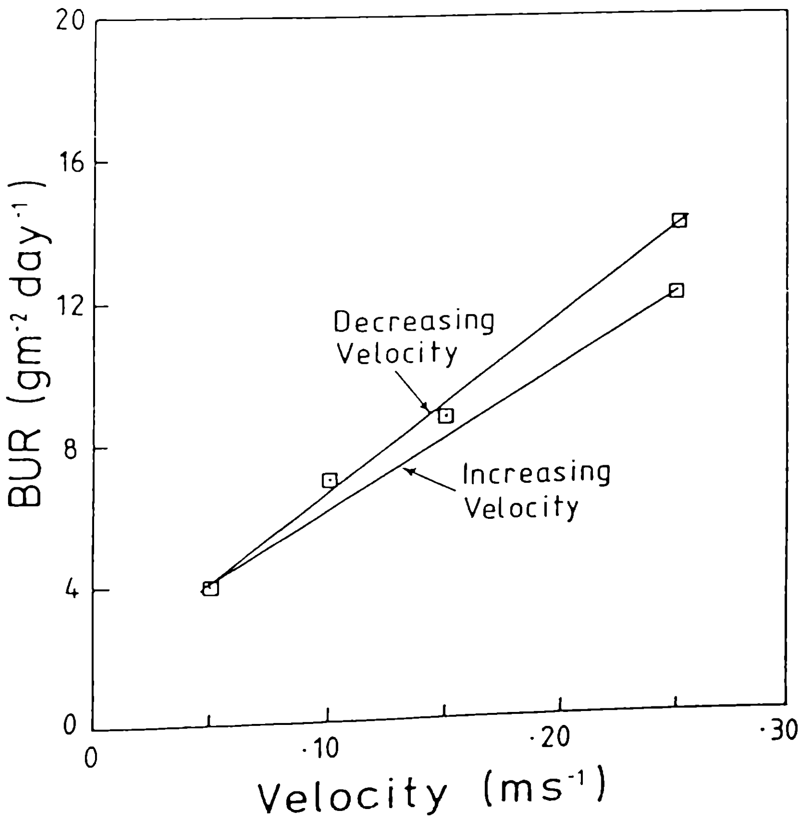


Figure 6.1 : Effect of chamber circulation velocity on BUR. Oraka River biofilm sampled on 25 January 1983.

sufficient to provide confidence in the kinetics of measurement obtained in the chamber system.

Operation of a lower chamber circulation velocity of 0.05 m s^{-1} would result in a BUR of 15.0 g m^{-2} day^{-1} (calculated using Fig. 6.1). This would substantially underestimate river BUR, leading to erroneous conclusions regarding the relative contribution of benthic respiration in this system.

Effect of circulation velocity on BUR. An approximately linear relationship between BUR and velocity was observed over the range 0.05-0.25 m s^{-1}, (see Fig. 6.1) with a 72% decrease in BUR resulting from lowering of the circulation velocity. A subsequent increase of circulation velocity to 0.25 m s^{-1} resulted in an increased BUR within 14% of the initial value. The regression equation was

$$\text{BUR} = 49.4\ U + 1.6\ (r^2 = 0.99,\ n = 4).$$

Chamber BUR measurements at 0.25 and 0.15 m s^{-1} (sampled 24 September 1981, Table 4.4) showed a 41% lower initial rate (both at DO 7.5 g m^{-3}) at 0.15 m s^{-1}, which compares with a 36% lower BUR at equivalent velocities from Figure 6.1.

Effect of dissolved oxygen on BUR. Field measurements using the benthic chamber for the estimation of BUR values *in situ* were made over a wide range of 'sewage fungus' morphologies found in local rivers. These ranged from thin (~1-3 mm) to thick (3-10 mm) films (some with black anoxic interiors), filamentous free-streaming organisms, and smooth films on artificial substrata (polypropylene sheet and polyurethane foam). Microscopic examination of these flocs showed them to be predominantly composed of sheathed filamentous microorganisms, having a morphology consistent with *Sphaerotilus natans* (Dondero, 1961). The thicker films also contained large numbers of protozoa and nematodes.

For all 'sewage fungus' river biofilms measured, the plots of DO concentration versus time showed a pattern similar to Figure 6.2. Shortly after the initiation of the run, glucose was added to the system at a final concentration of 20 g m^{-3} in order to ensure that substrate (electron donor) was not limiting. This appeared not to increase significantly the observed BUR. Thereafter BUR decreased with time

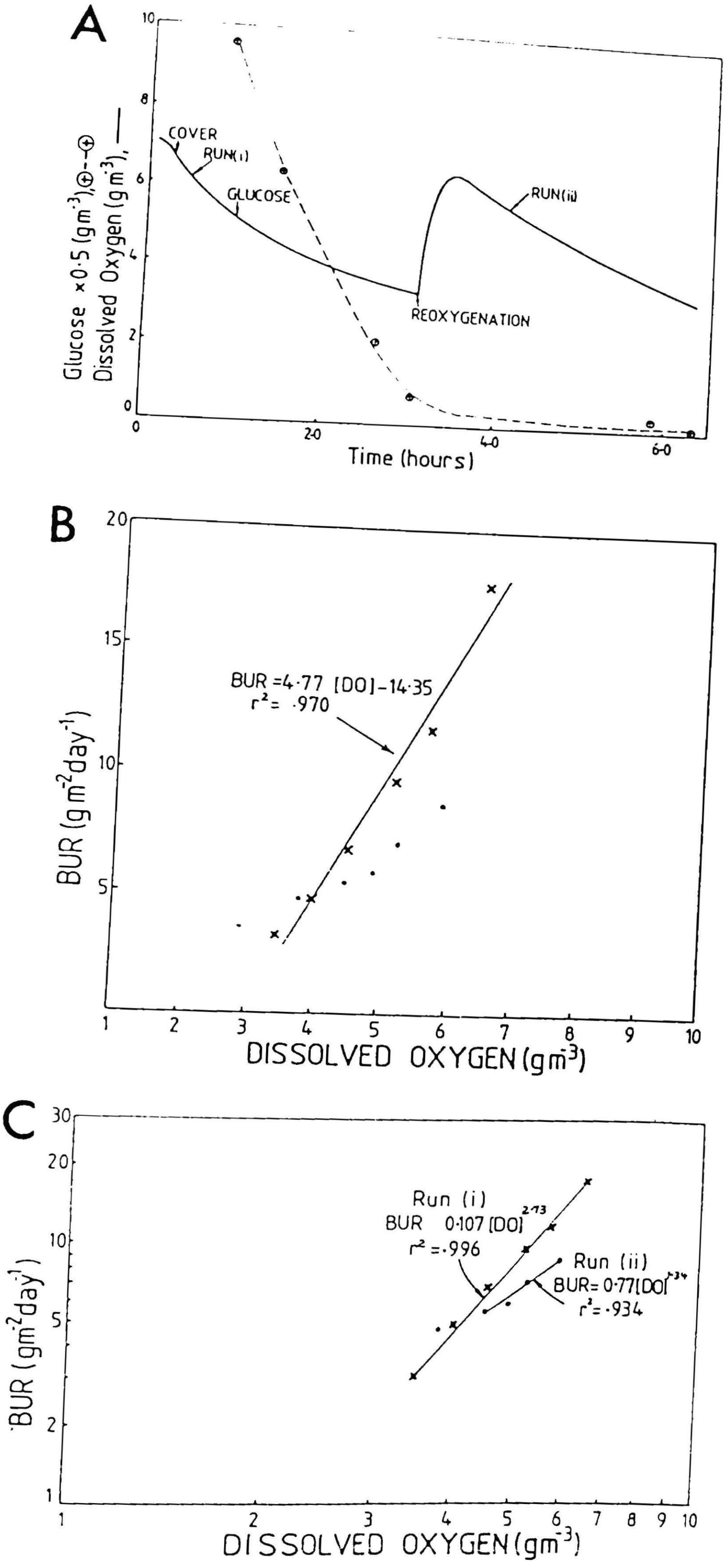


Figure 6.2 : Benthic oxygen uptake rate (BUR) calculation procedure. Manawatu River, sampled 22 February 1983.

A Dissolved oxygen and glucose concentrations versus time. Arrow indicates reoxygenation using catalase/hydrogen peroxide.
Symbols: —— dissolved oxygen, ·---· glucose.

B BUR versus mean DO. Run annotation after A;
x——x Run (i); ·——· Run (ii).

C log BUR versus log DO. Run annotation after A (as above).

simultaneously with both DO and glucose concentration. However, after reoxygenation (using catalase and hydrogen peroxide, (Hickey, 1985b)) BUR values increased to previous levels, which indicated that the decline in BUR was not a result of reduced substrate supply (electron donor), but rather a result of reduced oxygen supply (electron acceptor). Thus the results fit an empirical geometric relationship of the form

$$N = BC_b^K \tag{6.3}$$

where N is the BUR, g O_2 m^{-2} day^{-1}; K is the oxygen transfer exponent (OTE), m day^{-1}; C_b is the dissolved oxygen concentration in the bulk liquid, g O_2 m^{-3}; and B is a BUR constant, g O_2 m^{-2} day^{-1}. The OTE has been defined as the ratio of BUR (g m^{-2} day^{-1}) to DO concentration (g m^{-3}), and was estimated from the slope of a log-log plot of BUR versus DO. The OTE (K) is thus an index of the degree to which river DO concentration affects the transfer of oxygen into the biofilm.

Linearisation of the BUR and DO relationships facilitates both the testing of the reproducibility of chamber BUR kinetics, and the comparison of various biofilms with the variability associated with sampling the river bed. Figure 6.3A shows the chamber BUR progression for filamentous free-streaming 'sewage fungus' growth, with 3 successive exchanges of river water and for a biofilm growing on a coarse pebble bed (each with glucose added). Reproducibility and errors in slope determination for filamentous BUR measurements for the 3 runs was maximally ±10% (±1 S.E.) over the DO concentration range; corresponding measurements for the biofilm were ±14% (Fig. 6.3B). The greater uncertainty associated with the biofilm measurement reflects the decreased slope and hence smaller concentration changes leading to larger errors. The filamentous growth possessed the highest BUR recorded (70.2 g m^{-2} day^{-1} at 7.0 g DO m^{-3}) and showed a minimal effect of DO depletion on BUR. By comparison, the BUR of the biofilm was lower (26.9 g m^{-2} day^{-1} at 7.0 g DO m^{-3}) and showed a greater response to decreasing DO concentration (Fig. 6.3B). Thus a reproducible dependence on DO could be shown, although the magnitude of the concentration affect appeared influenced by the morphology of the biofilm present.

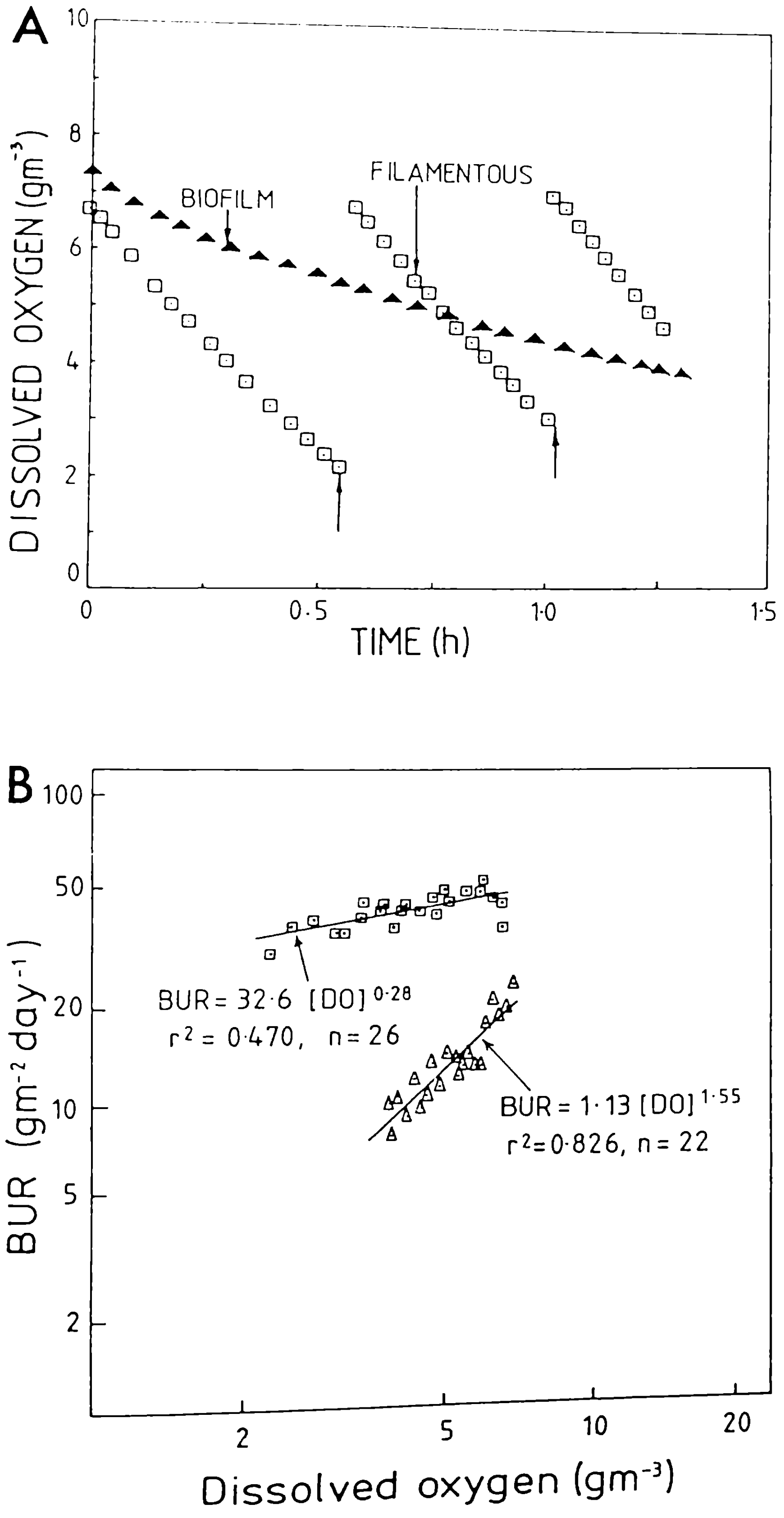


Figure 6.3 : Chamber oxygen uptake progression (A) and log BUR versus log DO concentration (B) for :
□ filamentous 'sewage fungus', sampled 16 February 1982.
▲ biofilm 'sewage fungus', sampled 1 November 1982.
Arrow indicates wash through for reoxygenation.

Areal variability of the chamber BUR measurements from sample area to sample area was estimated when a visibly homogeneous colonisation of biofilm had established 4 days after a flood had occurred (24 Sept 1981). For 4 randomly chosen sites the standard error for the initial rates was 7.4% (from Table 6.2).

Total error associated with a BUR value results from (a) measurement uncertainties (±14%) and (b) sampling variability (± 8%) giving an overall estimate of ±16% or a 95% confidence range of ±32%.

Effect of 'sewage fungus' morphology on BUR. Chamber BUR kinetics measurements were undertaken on natural substrata colonised by various thicknesses of 'sewage fungus' biofilms. Two artificial substrata were incubated in the river in order to enable BUR measurements to be made for biofilms colonising a smooth surface.

The oxygen uptake rate of homogenised biofilm suspensions was used to measure the intrinsic activity (I) of the biomass present, and hence to calculate the effectiveness factor (η, which indicates the fraction of total biofilm depth penetrated by the river DO) (Eqn. 3.2) and the effective depth of chamber oxygen penetration into the biofilm, (δ_c) (Eqn. 3.5).

Additional measurements of viable microbial biomass present by ATP determination and organic carbon were made.

Results are summarised in Table 6.2. The regression equation coefficients for BUR kinetics are shown in Table 6.3. The response of the filamentous organism to decreasing oxygen concentration, (as measured by the oxygen transfer exponent (OTE)) was low, at 0.28 m day-1, and the effectiveness factor high, 0.94. Biofilms showed a greater response to DO (OTE values from 1.66 to 4.12 m day^{-1}). The median OTE value of 2.0 for biofilms would result in a 75% decline in BUR for a DO decrease from 10.0 to 5.0 g m^{-3}. For biofilms the effectiveness factors ranged from 0.61 to 0.25 indicating that the effective depth of oxygen penetration was 0.8 to 3.5 mm (Table 6.2).

The biofilms grown on polypropylene and the polyurethane artificial substrata both showed low BUR values of 3.8 and 8.2 g m^{-2} day^{-1} respectively. Although biofilm thicknesses were comparable with river

Description of Biofilm	Site No.	River	Sampling Date	River Temp. °C	Dissolved Oxygen $g\ m^{-3}$	Estimated Film Thickness mm	Chamber Velocity $m\ s^{-1}$	Oxygen Uptake Rates (BUR) $g\ m^{-2}\ day^{-1}$ Chamber[a]	Homogenate	ATP $mg\ m^{-2}$	Carbon $g\ m^{-2}$	Effectiveness Factor (η) (eqn 3.2)	Depth of Oxygen Penetration (δ_c) (eqn 3.5) mm
Filamentous	1	Waitoa	16 Feb 1982	22	7.0	-	0.25	70.2 (max)	60.4±9.7(3)	-	8.5	0.9	-
					6.5	-	0.25	56.8±2.1(3)					
	2	Waitoa	27 Jan 1985	25	9.0	-	-	-	146.6±2.2(2)	71.0±12.1(3)	7.0 9.6(b)	-	-
Thin	3	Waitoa	2 Mar 1982	20	9.1	2	-	[3.83±0.1(2)](d)	-	-	25.4	-	-
Pebble	4	Waitoa	5 Mar 1982	24	7.0	2	0.25	9.4	23.5±4.2(3)	13.2±5.0(3)	15.5	0.40	0.80
	5	Waitoa	1 Nov 1982	24	7.0	3	0.25	17.0	28.0±3.0(4)	-	16.1(b)	0.61	1.80
	6	Oraka	25 Jan 1983	16	7.0	2	0.25	13.9	-	-	10.8	-	-
Cobble	7	Manawatu	22 Jan 1983	20	7.0	3.1±1.6(4)	0.35	18.0	-	-	13.7±6.1	-	-
Thick	8	Waitoa	24 Sept 1981	16	8.5	8-10	0.25	66.4	(c)	-	-	-	-
					7.5	8-10	0.25	43.5±6.4(4)*					
	9	Waitoa(e)	24 Sept 1981	16	9.0	8-10	0.15	25.9	(c)	-	-	-	-
	10	Waitoa	9 Oct 1981	20	7.5	10	0.25	21.3±6.0(2)	61.2±5.9(3)	47.5±22.1(3)	85.5 (b)	0.35	3.5
	11	Waitoa	19 Jan 1982	20	9.1	8	-	[5.35](d)	21.1(1)(e)	-	42.0	0.25	2.0
	12	Waitoa	25 Jan 1982	25	8.1	9	-	-	18.9±0.1(2)	11.5±2.8(4)	56.0	-	-
<u>Artificial Substrata</u>													
Polypropylene sheet		Waitoa	5 Mar 1982	24	7.0	3	0.25	3.8	18.1±1.3(4)	6.8±1.2(3)	9.8	0.25	0.75
Polyurethane foam		Waitoa	5 Mar 1982	24	7.0	5	0.25	8.2 [5.4±0.8(2)](f)	117.0±16.3(4)	77.0±4.3(3)	32.5 51.6(c)	0.7	0.35

a Maximum BUR measured in repeated runs at the same location as summarised for kinetics (Table 6.3); and replicate determinations (±1 standard deviation (SD), with numbers of determinations in brackets), all by graphical measurement at DO given).

b Estimated as 50% of loss on ignition at 500 °C.

c Results not usable because of sulphide poisoning of oxygen electrode.

d Core samples: bracketed value [] refers to the oxygen uptake rate of an undisturbed core, overlayed with 500 ml of BOD dilution water, stirred with recirculated enclosed air, with measurement using the electrolytic respirometer (20 °C).

e Electrolytic respirometer measurement (20 °C).

f Areal BUR as measured in electrolytic respirometer.

* Indicates measurements at adjacent locations (mean ±1 standard deviation).

Table 6.2: Chamber BUR measurements <u>in situ</u> and intrinsic activity of river 'sewage fungus' colonies.

Description of Biofilm	Site Number[a]	Oxygen Mass Transfer Coefficient,[b] K m day^{-1}	Rate Constant,[b] B g O_2 m^{-2} day^{-1}	Number of Runs[c]	Number of Points[d]	Explained variance[e]
Filamentous	1	0.28	32.64	3	26	0.490
	2	-	-	-	-	-
Thin	3	-	-	-	-	-
	4	-	-	-	-	-
	5	1.55	1.13	1	22	0.826
	6	-	-	-	-	-
	7(i)	2.74	0.107	1	6	0.996
	(ii)	1.84	0.771	1	5	0.934
Thick	8	4.12	0.0985	2	24	0.194
	9	1.66	0.832	1	20	0.908
	10	2.80	0.071	1	6	0.993
	11	-	-	-	-	-
	12	-	-	-	-	-

[a] Site numbers are identified in Table 6.2

[b] Calculated from eqn 6.3; BUR = BC_b^K, where C_b is the oxygen concentration in the bulk fluid

[c] The number of repeated runs at a site

[d] The number of points used for rate determination

[e] (Correlation coefficient)^2

Table 6.3: Regression equation coefficients for chamber BUR kinetics of river 'sewage fungus' colonies.

Description of Sewage Fungus Biota	Station number[a]	Homogenised Specific Respiration Rates				EBOD k day^{-1}	t½ days	C:N[b]
		mg O_2 g C $^{-1}$ h^{-1}		mg O_2 mg ATP $^{-1}$ h^{-1}				
		Endogenous	+ glucose	Endogenous	+ glucose			
Filamentous	2	241.4±3.6(2)	490.0±7.4(2)	42.2±4.2	85.7±8.5	-	-	27.8
Thin	3	[6.29±0(2)][d]	-	-	-	0.075±0.005[f]	9.25	-
	4	53.6±9.9(3)	-	74.2±17.8(3)	-	-	-	5.3
	5	64.9±6.9(3)	72.7±7.6(3)	-	-	-	-	-
	5	31.4(1)[c]	-	-	-	0.375±0.005	1.85	-
	6	100.5(1)[c]	-	-	-	0.125±0.005	5.54	-
	10	29.89±3.0(3)	-	58.1±14.7	-	-	-	-
Thick	11	20.8(1)[c]	-	-	-	0.175±0.005	3.96	4.83
	11	[5.3][d]	-	-	-	0.050±0.005[f]	13.90	4.83
	12	20.3±0.4(2)	-	68.7±8.4	-	-	-	5.66
	12	11.2(1)[c]	-	51.4	-	0.125±0.005	5.54	5.66
<u>Artificial Substrata</u>								
Polypropylene sheet		65.8±5.1(4)	76.7±6.0(4)	94.5±10.3	110.3±11.9	-	-	45.6[b]
Polyurethane foam		111.5±21.0(4) [10.5][e]	165.4±23.0(4)	47.1±3.6	69.8±5.4	0.075±0.005[f]	9.24	16.6[b]

[a] Station numbers identified in Table 6.2

[b] Nitrogen (N) obtained by protein analysis and a conversion factor of N:TKN = 10% protein.

[c] Electrolytic respirometer measurement (20 °C).

[d] Undisturbed core. Carbon measurement from top 10 mm of benthic substrata.

[e] Bracketed value refers to the oxygen uptake rate of an undisturbed film grown on polyurethane foam and held in a stirred 1500 ml reactor containing BOD dilution water, using the electrolytic respirometer (20 °C).

[f] All other decay coefficients are for homogenised biofims except these intact biofilms.

± 1 standard deviation (number of samples).

- Not determined.

Table 6.4: Specific respiration rates and biofilm respiratory decay for homogenised 'sewage fungus' colonies.

biofilms possessing a much higher BUR, biofilms on neither of these substrata showed a response to decreasing oxygen concentrations. Calculated oxygen penetration depths were quite low at 0.75 and 0.35 mm respectively.

Repeated BUR measurements with adjustment of the chamber circulation velocity (performed 24 September 1981, Table 6.2), showed that decreasing the circulation velocity from 0.25 to 0.15 m s^{-1}, resulted in a lower BUR (initially by 41%) and also a lower OTE value (i.e. a decreased response to oxygen concentration).

Intrinsic activity measurements showed no dependence on DO concentrations above 1.0 g m^{-3} (data not shown). The maximum areal intrinsic activity measured was 146.6 g m^{-2} day^{-1}. When I was calculated relative to carbon present (Table 6.4), the thicker (older) biofilms had substantially lower carbon-specific rate than filamentous 'sewage fungus'. The intrinsic and potential activities were comparable for the homogenised biofilms tested, with minimal response to glucose addition, except for the filamentous material (Table 6.4).

6.3.2 Viable biomass measurements

Measurement of ATP concentrations were made in order to estimate the amount of metabolically active biomass present. Mean ATP estimates ranged from 6.8 mg m^{-2} to 71.0 mg m^{-2}. These bore no relation to the estimated biofilm thickness or to the amount of carbon present ($P>0.10$, Table 6.2).

The specific respiration rates of the biomass were largely in the range 60-110 mg O_2 mg ATP^{-1} h^{-1} despite the large ranges of carbon present and of biofilm thicknesses. Values of 68.7 and 58.1 mg O_2 $mgATP^{-1}$ h^{-1} were associated with thick decaying (sulphurous interior) biofilms at an advanced stage of decay (see Tables 6.2 and 6.4). The correlation of endogenous respiration and ATP (r^2 = 0.914, n = 6; Table 6.2), suggests that living biomass constitutes the bulk of the intrinsic activity measured in these biofilms, rather than chemical oxygen demand resulting from reduced substances present.

Carbon to nitrogen (C:N) ratios indicated a decreasing ratio with increasing biofilm thickness (Table 6.4). This presumably reflected the progression in composition from that consisting entirely of Sphaerotilus sp. embedded in a polysaccharide matrix, to a more complex community containing also other bacteria, algae, protozoa and nematodes. Increasing quantities of internal carbon storage compounds may occur with increasing film thickness caused by nutrient limitation (Dawes and Senior, 1973; Nickels et al., 1979), which would tend to offset the increase of the C:N ratio with increased film community complexity.

6.3.3 Carbon turnover

For the management of an aquatic ecosystem, it is particularly important to identify the key biological process components and the respective turnover times. The turnover time determines the time scale necessary for decreases in nutrient loading to be reflected in the metabolic rate processes.

Management of the receiving water may be either process engineering oriented, with emphasis on the BOD removal rate, or environmentally oriented, with emphasis on DO concentration. For environmental management, a knowledge of the reaction stoichiometry is necessary, since it is this which determines the respiratory quotient (Qo_2) of the processing system.

Respiratory quotient. Glucose was routinely added to the chamber system (to ensure electron acceptor concentration was the rate limiting component) and resulted in negligible BUR increases. Monitoring glucose removal rate from the chamber system for the Manawatu River biofilm (Fig. 6.2A), showed rapid assimilation at 8.30 g glucose m^{-3} h^{-1}, which equates to 48.5 g glucose m^{-2} day^{-1}, of which only 9.5% was respired and measured as oxygen demand. This suggests that a large proportion of the glucose was taken up and stored within the biofilm.

Biofilm respiratory decay. Measurements of both intact and homogenised biofilms were made over an extended period, in the absence of added organic nutrients. For the undisturbed biofilm, grown on polyurethane foam, the initial specific respiration rate of carbon compounds as measured in the electrolytic respirometer was an order of magnitude lower than those for the homogenised biofilm (see Table 6.4), but within 15% of the values obtained using the chamber in situ (see Table 6.2). The oxygen demand progression was followed for 13 days, with the visually intact appearance being maintained for the initial 8 days, after which patches sloughed off the surface. The respiratory half-life

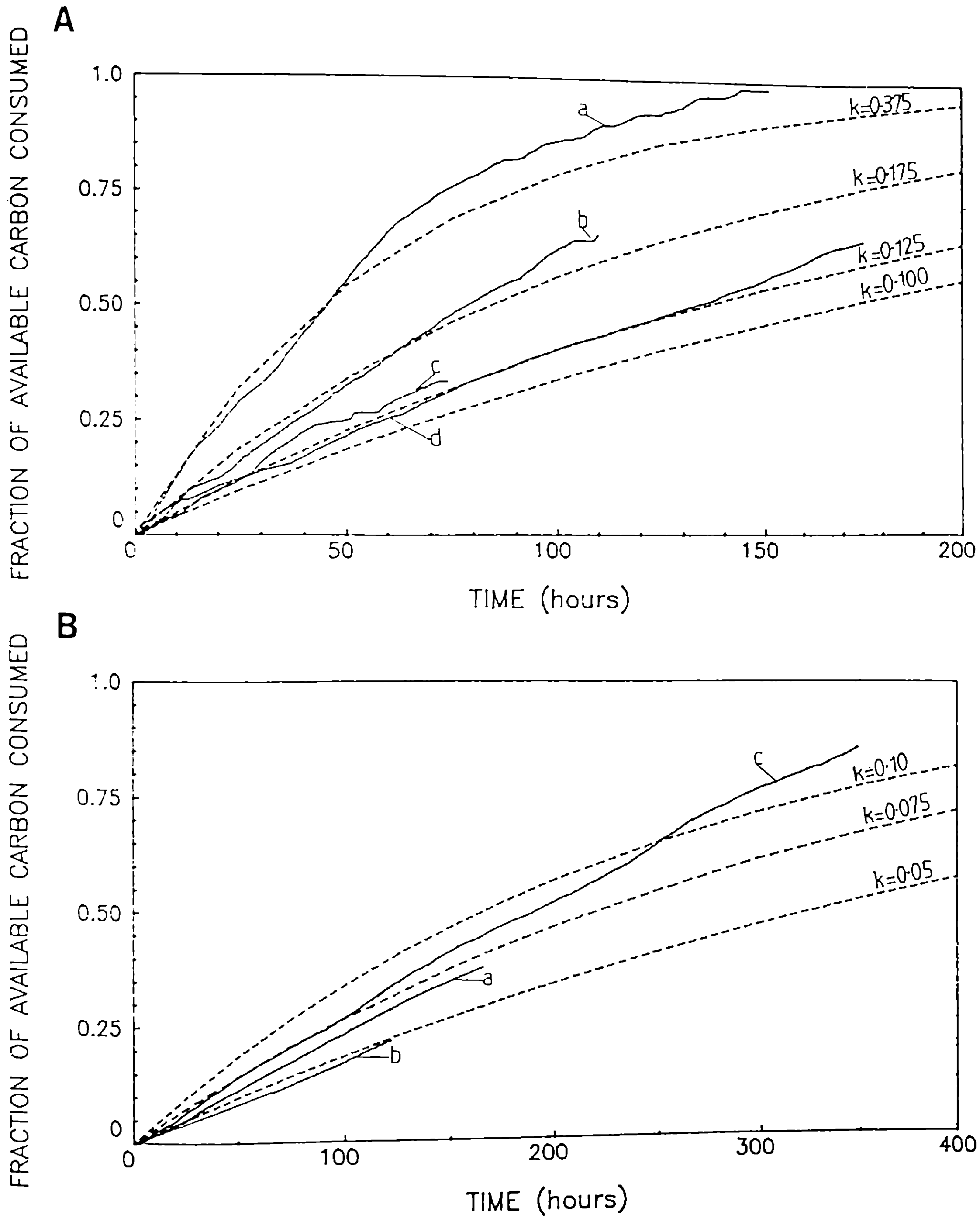


Figure 6.4 : Homogenised (A) and intact (B) endogenous respiratory decay kinetics for 'sewage fungus'

A **Homogenised biofilm, assuming 75% available carbon: key**

a thin biofilm sampled, 1 November 1982
b thick biofilm sampled, 19 January 1982
c thin biofilm sampled, 25 January 1983
d thick biofilm sampled, 25 January 1982.

B **Intact biofilms, assuming 100% available carbon: key**

a undisturbed core sampled, 2 March 1982
b undisturbed cores (2), sampled 19 January 1982
c biofilm grown on polyurethane foam, sampled 5 March 1982

Dashed line denotes fitted first order decay curve with coefficient (k) shown.

was 9.2 days (decay coefficient (k), 0.075 day^{-1}) (Fig. 6.4), although the decay coefficient showed a poor fit after 100h, where an increase in decay rate occurred, followed by a further rate increase at 200h. These could represent sequential carbonaceous degradations or nitrogenous oxygen demand, and no attempt was made to fit these sections of the progression with model decay coefficients.

Undisturbed biofilms incubated in stirred aerated cores gave lower BUR estimates than chamber measurements made in situ. Sampling a thin biofilm (~2 mm, March, 1982) gave a BUR in the core of 3,8 g m^{-2} day^{-1} amounting to only 34% of the chamber estimate, and a thick biofilm (8-10 mm, Jan 1982 core, c.f. October 1981 chamber) gave a BUR in the core of 5.4 g m^{-2} day^{-1}; only about 25% of chamber estimates for similar thickness biofilms (See Table 6.2).

Incubation of these undisturbed cores showed a negligible decline in BUR over a 5-7 day period. Respiratory half-lives of 9.2 and 13.9 days (decay coefficients of 0.075 day^{-1} and 0.05 day^{-1}) would best approximate the data (Table 6.4 and Fig. 6.4). Because the values obtained with stirred cores did not correspond closely with the values for BUR obtained with the chamber, it was considered to be inappropriate to use the endogenous decay coefficient so obtained for intact core biofilms. Rather the decay coefficient of the homogenised biofilm may be considered a more appropriate measure.

The intrinsic activity of the homogenised biofilm substantially declined in the period immediately following homogenisation. Comparison of field and electrolytic respirometer measurements showed decreases of 52% (for 1 November 1982) and 45% (for 25 January, 1982) for Waitoa biofilm samplings (Table 6.4). This may represent the rapid oxidation of reduced chemical species present in the biofilm, or depletion of organic nutrients. The latter appears more likely because of the high correlation of respiration rate with the amount of ATP present.

The biofilm respiratory decay rate of the homogenised biofilms showed the highest value for a thin biofilm, with a half-life of 1.85 days (k = 0.375 day^{-1}, Table 6.3); however, no consistent relationship with biofilm thickness was evident. Half lives ranged from 1.85-6.93 days (k = 0.375-0.100 day^{-1}, n = 3, Table 6.4), indicating that a slow response to effluent cessation would be expected in a river with 'sewage fungus'.

6.4 DISCUSSION

The design criteria of the benthic chamber system, were to provide a undirectional, recirculating system with homogeneous turbulence, and a velocity comparable with that of the sediment boundary velocity (Section 4.2). Concomitant changes in velocity and BUR were observed with the chamber system. This would indicate that turbulent dispersion dominates the mass transfer processes. Thus we might anticipate that the physical characteristics of the river bed will influence turbulent dispersion and the resulting BUR.

The influence of chamber hydrodynamics, and potential mass transfer limitation (or exaggeration), was investigated, as this could have been a weakness in the method. Chamber BUR measurements were less than those estimated for actual river BUR by a segment oxygen mass balance (Table 6.1). However, the magnitude of the chamber BUR was sufficient to provide some confidence in examining biofilm kinetics. The exclusion of filamentous biomass attached to logs and debris from the chamber measurement may account for the higher BUR calculated for the river.

The maximum biofilm BUR values measured were 60-70 g m^{-2} day^{-1}, compared to a maximum potential BUR of greater than 140 g m^{-2} day^{-1}, where diffusion limitations were removed. Other reports of field measurements of BUR for 'sewage fungus' biofilms using chamber systems are limited. Traaen (1975), for indoor channels, reported BUR values ranging from 0.5-4.0 g m^{-2} day^{-1}, whereas Boyle and Scott (1984) have reported values up to 14.9 g m^{-2} day^{-1}. A BUR comparison with environmental engineering studies for a range of channel and laboratory studies are shown in Table 6.5. Maximum BUR values (assuming a respiratory quotient (Qo_2) of 50%) were found in channels fed sucrose at rates of 30.1 g O_2 m^{-2} day^{-1} (Wuhrmann, 1974), and for the laboratory biofilm measurements of Kornegay and Andrews (1968) at 45.0 g O_2 m^{-2} day^{-1}. Calculated laboratory biofilm BUR values were generally very low, despite the presence of biofilms considerably deeper than the 60 µm suggested by Sanders (1966) as the maximum depth for oxygen penetration. Their measured values would appear to be so much lower than the values measured in this study, as to call into question the conceptual basis and assumptions made in the extrapolation of the engineering based models to environmental situations.

Description	Substrate	Temp 0 °C	Subst conc mg.l^{-1}	Substrate removal rate	Oxygen Demand[a] g m^{-2} day^{-1}	Film Thickness µm	Reference
Indoor channels	sucrose	12	5	1.0-3.2x10^{-2} µmole gC^{-1} s^{-1}	0.62-8.95	-	Traaen *et al*. (1972)
Outdoor channels	sewage	10-4	5^b	-	0.5-3.96	-	Traaen (1975)
Indoor channels	glucose	20	20	-	~10	-	Curtis *et al*. (1971)
Outdoor channels	sucrose			0.34-1.5x10^{-2} µmole gC^{-1} s^{-1}	0.41-7.33	-	Wuhrmann *et al*. (1967)
Outdoor channels	glucose	15	0.59	1.4x10^{-2} µmole gC^{-1} s^{-1}	6.95	-	Wuhrmann (1974)
	sucrose	15	1.20	2.6x10^{-2} µmole gC^{-1} s^{-1}	30.12	-	Wuhrmann (1974)
Artificial substrates		-	-	-	1.25-12.5	-	Capblancq & Cassan (1979)
Continuous flow system	nutrient broth	20	20	-	0.88	21.2	Sanders (1966)
		30		-	1.14	21.2	Sanders (1966)
Film reactor	glucose	20	50	11.3x10^{-2} mg cm^{-2} h^{-1}	13.56	150	Onuma & Omura (1982)
Film reactor	glucose	25	135	37.5x10^{-2} mg cm^{-2} h^{-1}	45.0	100	Kornegay & Andrews (1968)
Film reactor	glucose	25	200	8.03x10^{-8} mole s^{-1} cm^{-3}	8.32	320	La Motta (1976a)
Film reactor	glucose	25	50	-	1.96	96	Lee *et al*. (1976)

[a] calculated from the substrate removal rate assuming 50% assimilation if not reported as an areal respiration rate

[b] as BOD_5

Table 6.5: A summary of literature reporting substrate removal rates for microbial films in laboratory reactors and artificial channels. Calculation of data in terms of areal oxygen consumption rate has been made for comparative purposes.

ATP estimates (Table 6.2) showed maximal values at 71.0 mg m^{-2} which were comparable with both the outdoor channel ATP measurements of Traaen (1975) at 75 and 62 mg m^{-2} and the laboratory reactor studies of La Motta (1976a) at 60 mg m^{-2} for a 320 µm biofilm. This suggests that active biomass levels were comparable for each of these studies, despite widely varying environmental and analytical conditions.

Engineering studies are generally based on hydraulically smooth biofilms. High fluid velocities of 0.8 m s^{-1} with biofilm thickness of 60 to 70 µm are reported necessary to eliminate the laminar boundary layer and to enable a kinetically-controlled reaction mechanism to occur (Sanders, 1966; La Motta, 1976b). At lower shear velocities, Atkinson et al. (1967) reported increased glucose removal rates as a result of uneven biofilm growth, which Characklis (1973) has suggested could be due to micro-turbulence caused by surface irregularities. Zelver (1979) has shown that different biofilm densities develop in relation to shear stress, and thus the effective diffusivity may vary greatly around bed protrusions.

In this study the chamber BUR values measured with the smooth artificial substrata were low, with very low effectiveness factors as compared with natural sediments (Table 6.2). This would indicate the presence of a laminar boundary layer exerting considerable diffusional resistance. The rough boundary layer of the natural river substratum gave high chamber BUR values. Calculated oxygen penetration depth ranged from 0.35 to 3.5 mm, 5.8- to 58-fold greater than the 60 µm 'maximum' (Sanders, 1966). The biomass specific respiration rates indicated the presence of an active microbial population in these deep biofilms. Both the thick biofilm development and high metabolic activity of river 'sewage fungus' biofilms would indicate that greater exchange occurs between the overlying flow and biofilm microorganisms in rivers, than in laboratory reactors.

Castaldi and Malina (1982) reported that for their biofilm tube reactor, the macroscopic oxygen uptake rate varied with the magnitude of the Reynolds Number (i.e. circulation velocity), supporting my chamber observations in situ (Fig. 6.1). Since the level of chamber turbulence was shown to vary predictably with circulation velocity ($U^{0.5}$, Chapter 4), the sources of turbulence in the river environment (ie, bulk fluid and bed shear velocity generated turbulence), might be expected to greatly influence mass transfer process at the boundary layer and therefore the rate of metabolism, growth and biomass of the biofilm. Therefore, both the magnitude and kinetics of flux measurements may be greatly affected by the ability of chamber devices to adequately simulate natural hydrodynamic conditions.

A number of factors may influence the value obtained for the biofilm reaction kinetics. Several authors have shown that for incomplete penetration of the biofilm matrix, with zero order reaction kinetics, the apparent reaction order would be half order (Baillod and Boyle, 1970; Atkinson and Davies, 1974; La Motta, 1976b; Williamson and McCarty, 1976a; Harremoes, 1976, 1977). I have found that for the lotic biofilms studied, geometric functions best explain the dependence of BUR on DO concentrations. All biofilms could be considered as deep (i.e. incomplete oxygen penetration), with effectiveness factors less than 1, and therefore have their reaction rates limited by the depth to which oxygen penetrates. Reaction order varied with the morphology of the biofilm and the conditions of measurement, with OTE values from 0.28 m day^{-1} for filamentous 'sewage fungus' to 4.12 m day^{-1} for a thick decaying biofilm. Decreasing the circulation velocity in the chamber significantly decreased both A and K in equation 6.3 (see Table 6.3). The decrease in A indicates that the effective depth of biofilm penetration is decreased, which may be expected with decreased chamber turbulence (Section 4.25). The decrease of K, however, indicates a lesser response to decreasing oxygen concentration, which suggests that the intrinsic activity of the biofilm was not uniform within the matrix.

The BUR values of the smooth biofilms grown on artificial substrata were apparently independent of DO concentration. Castaldi and Malina (1982) showed in a laboratory reactor, that the magnitude of response to changes in DO concentration was dependent on the circulation velocity. Uptake rates were lower at low Reynolds Number (R_e) and a function of DO concentration up to 8 g m^{-3}, whereas, at high R_e a DO rate response occurred at lower DO concentrations. Calculations of the OTE value from their lowest turbulence level (R_e = 32) gave a value of 0.83 (r^2 = 0.988, n = 9), considerably higher than the 0.5 calculated for biofilm matrix diffusion, but less than observed for river biofilms. Thus it appears that the diffusion characteristics across the boundary layer may greatly affect the apparent kinetics. The higher reaction orders measured for river biofilms suggests a different effective reaction mechanism. The observed rate response may have been caused by : (a) reactant or essential nutrient exhaustion; (b) non-homogeneous biomass distribution (e.g. differing growth rates, possible layer of nitrifiers present); (c) non-uniform biofilm density; (d) variable reaction stoichiometry. Reactant (i.e. electron donor) exhaustion was probably not a large contributing factor since glucose had been added to the chamber system, although rapid removal may have caused some effect with extended incubation periods (see Fig. 6.2). The major contributor was probably biofilm inhomogeneity, with the presence of decaying material at depth, and the possibility of distinct species layers. The characteristics of the biofilm matrix are reported to vary as influenced by shear stress (Zelver, 1979), glucose loading (Trulear and Characklis, 1982), carbon to nitrogen ratio of the effluent and biofilm age (Matson and Characklis, 1976). Jorgensen et al. (1983) demonsrated microzonation of photosynthetic organisms and substantial gradients of O_2, H_2S and pH in microbial mats. Thus the river biofilms may well exhibit considerable areal heterogeneity, both of their surfaces and through their depths.

Such morphological diversity may well account for much of the variation in reported biomass yield and respiratory quotients between investigators. The yield coefficient may vary during a typical laboratory experimental period (30-50 hours; see Trulear and Characklis, 1982). Curtis et al. (1971) reported that only 20% of the organic material removed from their experimental channels could be accounted for as 'sewage fungus' biomass, suggesting that 80% was respired. This makes comparison between data sets difficult and suggests that major discrepancies may exist between BOD decay and deoxygenation rate where studies have been made on environments with high benthic activity. Measurement of the rate of glucose removal for the Manawatu River biofilm (Fig. 6.2) showed that only 9.5% of the glucose was respired, suggesting uptake and storage for subsequent oxidation. The variable respiratory quotient may severely limit the application of successful models to the study of environmental biofilms.

Carbon turnover

Calculation of the specific respiration rate for the 'sewage fungus' biomass provided an indication of the growth rate of the biomass present. A high value corresponds to rapid growth and metabolism, while lower values indicate progressive stages of metabolic decline and endogenous decay. The majority of values were within 50-100 mg O_2 mg $(ATP)^{-1}$ h^{-1} with a minimum of 11 mg O_2 mg ATP^{-1} h^{-1} for a thick decaying (sulphurous interior) biofilm (Table 6.4). Thus despite a wide range in thickness and apparent age of biofilms, the biomass specific activity showed little variation, suggesting that the metabolically active zone was probably confined to a narrow surface layer. Considerable differences in specific respiration rate in relation to total carbon were evident, with a general decrease occurring with increased age (Table 6.4). Biofilm respiratory decay measurements were, however, may be markedly affected by the quantity of microbial carbon storage compounds present.

A number of authors (e.g. McKeown (1963), Amberg and Elder (1975)) have suggested the possible use of large discontinuous BOD loadings to the river and in so doing prevent 'sewage fungus' growth and lessen the oxygen deficit. While this system may well be an effective control measure if no 'sewage fungus' is present, it is unlikely to be effective once the biofilms are well established. Measurement of biofilm respiratory decay rates for homogenised biofilms showed respiratory half-lives from 1.85 to 6.93 days (Table 6.4), indicating that a slow response to effluent cessation would be expected. The curve-fitting procedure was optimised by assuming that 75% of the total carbon present was 'available' (this gave an improved fit over using total carbon). This suggests that the actual turnover time for total carbon is greater than estimated value.

Decay and sloughing of filaments and biofilm patches, together with scour caused by floods appear to form the major mechanisms of biofilm removal in lotic systems. Such events may be infrequent, particularly during periods of low flow when oxygen deficits will be greatest. The use of BOD load manipulation can only be effectively used to prevent or limit 'sewage fungus' development in rivers.

6.5 SUMMARY AND CONCLUSIONS

An *in situ* benthic chamber, featuring recirculating flow and controllable velocity (0 to 0.35 m s^{-1}), was used to measure the benthic oxygen uptake rate (BUR) of 'sewage fungus' biofilms. BUR showed a large and rapid response to chamber circulation velocity, indicating that turbulent dispersion processes were dominating the sediment-water exchange processes. Comparison with a segment oxygen mass balance showed that matching of the boundary velocity of the river and chamber was necessary to obtain adequate chamber BUR estimates with maximum BUR values measured of 60 to 70 g m^{-2} day^{-1}. Core system measurements greatly underestimated the BUR in situ, for 'sewage fungus' biofilms. Therefore, in order to obtain realistic estimates of lotic mass transfer processes, chamber systems must simulate river hydrodynamic conditions.

BUR dependence on oxygen concentration in the overlying water was shown for biofilms on a range of coarse benthic substrata. A geometric expression best described the relationship with the slope termed the oxygen mass transfer exponent (OTE). The OTE value was experimentally shown to be influenced by chamber circulation velocity, electron donor concentration (glucose), the age and morphology of the biofilm. The rapid depletion of nutrients during chamber measurements may result in a serious underestimate of natural rate processes. Chamber measurements must, therefore, be made over the range of river environmental conditions present. Subsequent modelling efforts would require the use of an oxygen mass transfer exponent (OTE) to relate BUR to the river DO concentration.

Studies using chamber systems which adequately simulate natural hydrodynamic conditions are necessary to identify the physical and chemical factors affecting the sediment-water exchange processes. Such studies, however, can only identify short term changes in rate processes whereas long term changes in microbial biomass and activity may result from changes in electron donor and electron acceptor concentrations in the overlying flow.

Measurement of biofilm respiratory decay kinetics showed respiratory half-lives of 1.85-5.54 days. This questions the potential effectiveness of intermittent effluent loading of rivers as a measure taken with the aim of controlling established biofilms.

Chapter 7

MODELLING OF BENTHIC OXYGEN FLUX IN SHALLOW TURBULENT RIVERS

7.1 INTRODUCTION

A major objective of this work was to infer what the BUR would be under different concentrations of electron acceptor (oxygen) and electron donor (DOM or BOD) in the overlying river water. Two approaches were available to provide this predictive ability: (i) calculation of the flux using concentration gradients of constituents of the interstitial water; (ii) measurement of the flux directly with _in situ_ devices or laboratory experiments. Such methods have to date been independently applied, with the former using arbitrary or experimentally fitted effective diffusion coefficients to obtain flux estimates (Berner, 1980). Direct flux measurements using devices _in situ_ provide the advantage in that natural environmental conditions are present in terms of chemical composition and concentrations; these may also be manipulated to study effects on flux estimates. An area where serious difficulties arise, however, relates to the nature of mixing turbulence within the device compared with those in the environment. Chamber systems have been shown to minimally require matching of the boundary layer velocity with that of the river system, in order to obtain satisfactory estimates of sediments/water exchange processes. This has been discussed in Section 4.2.

This Chapter uses the sediment profiles of DO concentration in the interstitial water and intrinsic activity measurements of sediments, together with chamber flux measurements to estimate benthic dispersion coefficients and calibrate a diagenetic model for 2 rivers. Flux estimates predicted from the model for different DO concentrations are compared with the chamber measurements. Because the chamber flux measurements were performed under conditions of continually declining DO concentrations, a non-stationary model was also developed and run for step and linear DO concentration changes to obtain transient profiles and flux estimates.

Ultimately, predictions of BUR under conditions widely varying from calibration conditions are desirable. Since changes in effluent composition and concentration may effect both the level of biomass and activity of microbial biofilms attached to pumice sediments, a range of simulations were tested for flux prediction.

The original intention of the work described here was to obtain predictive flux estimates using a simple finite difference model approximation to Fickian diffusion (section 3.3.6.7). Consultation with a mathematical modeller highlighted the desirability of constructing computer programs for the accurate simulation of BUR and sediment nutrient concentration profiles. Such a model was developed by McBride (1985), specifying surface flux (i.e. chamber measurements) for calibration, and surface concentration for confirmation of inferred dispersion coefficients. This model was applied to predictive simulations shown in this Chapter.

7.2 STEADY-STATE SIMULATION OF BUR IN THE WAIOTAPU RIVER

7.2.1 Sensitivity Analysis

The accuracy of the benthic dispersion coefficient (D_s) estimation greatly influences the flux estimate. The field measurements for this analysis were limited, and the results were greatly influenced by both sampling and measurement methodological details as previously discussed (sections 5.3.8 and 5.4.6). As such these analyses may only be used in an exploratory nature. Detailed field experiments of flux and interstitial concentration of NH_4^+ suggested that D_s declined as an exponential function of sediment depth (A.B. Cooper, personal communication). For this reason, D_s profiles were modelled with an exponential decline, with simulation results shown in Figure 7.1 and illustrating the BUR sensitivity and the degree of subjective estimation necessary. A surface D_s of 40×10^{-3} cm^2s^{-1} declining to 27×10^{-3} cm^2s^{-1} at 20 cm depth was chosen as providing a reasonable fit. This D_s value is higher than the 14×10^{-3} cm^2 s^{-1} estimated for chamber installation (Table 5.23). This difference may have arisen for a number of reasons: (i) because of the changing river DO caused by macrophyte metabolism, leading to different surface DO concentrations for core and chamber samples (see Fig. 7.1 and Table 5.23); (ii) a higher than natural agitation level used for the sediment intrinsic activity estimates, resulting in low D_s estimates for the chamber; or (iii) natural heterogeneity of D_s values caused by variability of sediment structure. The relative importance of these components cannot be stated at this time but should be investigated in future studies. At present it is a valuable exercise to use model predictions to evaluate the relative importance of contributing components, and hence to better design future studies.

Figure 7.2 illustrates the sensitivity of both BUR and oxygen profile measurements to changes in the half-saturation constant (k_s) for oxygen

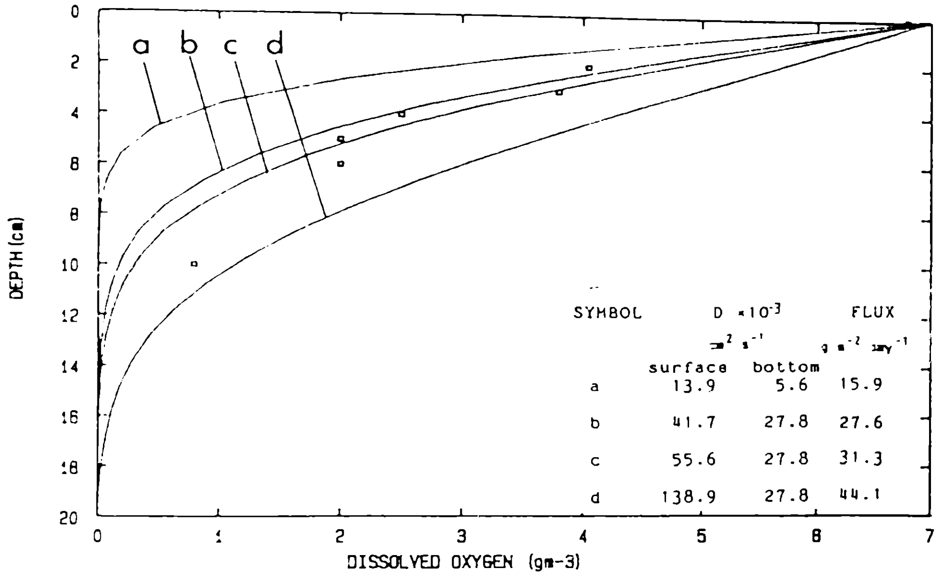


Figure 7.1 : The response of BUR flux estimates to variation in dispersion coefficient (Ds). Data was obtained for fine particulates on the Waiotapu River (9 March 1983; see section 5.3.8). Measured were: BUR flux = 21.4 gm^{-2} day^{-1} at 7.0 gm^{-3} DO concentration (from Fig. 5.34, run (ii) and Table 5.21; corrected for rate response with glucose addition (i.e. 1.35x measured)); Intrinsic activity = 4.6 gm^{-2} day^{-1} (Fig. 5.34); and interstitial DO concentrations ( ) gm^{-3}. Additional model parameters were: porosity (ϕ) = 0.5; oxygen affinity half-saturation constant (k_s) = 1 gm^{-3}.

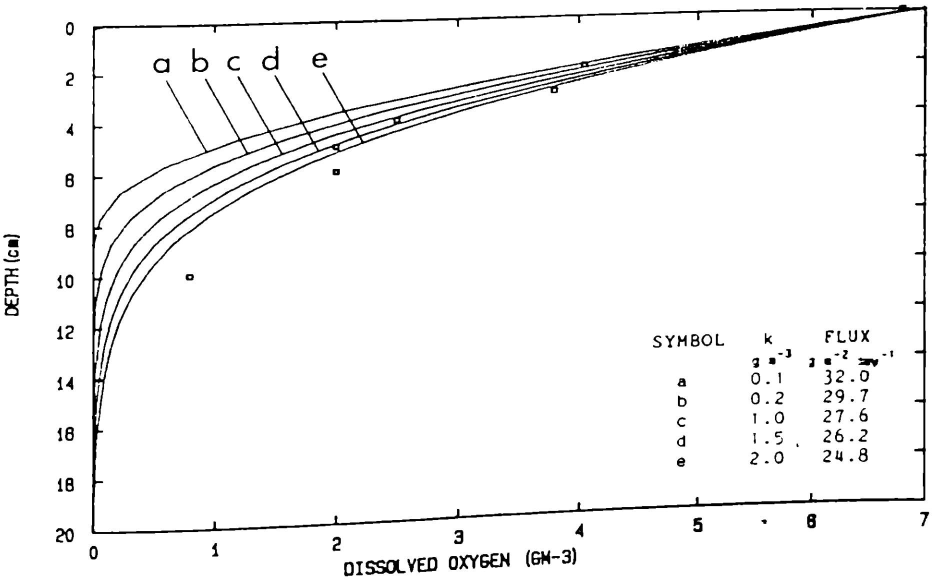


Figure 7.2 : The response of BUR flux estimates to variation in the oxygen affinity half-saturation constant (k_s). Model parameters are as for Fig. 7.1 and using an exponential dispersion coefficient decline from 40 x 10^{-3} cm^2 s^{-1} at the top to 27×10^{-3} cm^2 s^{-1} at the bottom.

affinity. BUR showed a 23% decline for a k_s increase from 0.1 g m^{-3} to 2 g m^{-3} with the depth of sediment oxygen penetration increasing from 9 cm to 19 cm. Although bacterial k_s values have generally been shown to have high affinity (<0.2 g m^{-3}) in well-mixed pure culture experiments (Pirt, 1975), the presence of diffusional resistance caused by aggregation and the resulting concentration gradients may increase the apparent k_s (Matson and Characklis, 1976). Such a situation may well occur in pumice sediments, with the degree of agitation influencing the diffusion kinetics. Matching of natural agitation levels is therefore a key component affecting sediment DO penetration. A k_s value of 1 g m^{-3} was chosen for subsequent simulations.

Steady-state simulations for a range of DO concentrations showed an apparent ½ order reaction for a k_s of 0.1 g m^{-3}, which agrees with the predictions of other workers (see section 4.2 for discussion). Increasing k_s values gave a substantial increase in apparent DO dependence (Fig. 7.3) with a 0.82 reaction order for a k_s of 2 g m^{-3}. Chamber measurements on pumice substrata all showed repeatable BUR dependence on DO (as discussed in section 5.3.5) with apparent reaction orders ranging from 0.92 to 2.5 (Appendix 3).

An experiment was performed to follow the kinetics of oxygen exertion by pumice using different volumes of pre-sieved (<2 mm) material incubated in continuously stirred (using a BOD probe) and monitored BOD bottles. The small volume (17 ml) showed no rate decline as DO approached zero, suggesting a k_s <0.2 g m^{-3} was applicable (Fig. 7.4). A large sediment volume (68 ml), which experienced only surface movement of the upper 5 mm of particles, showed a declining uptake rate from saturation DO concentrations. Analysis of the rate of change of slope showed an optimal fit to a geometric (semi-log) relationship ($P<0.001$, $r^2 = 0.983$, $n = 9$); however, expression as an exponential (log-log) form is desirable to allow comparison with others measurements. The reaction order (exponent) obtained was 0.78 ($P<0.001$, $r^2 = 0.874$, $n = 9$). This experiment demonstrated that nature of the agitation of the sediment particles may not only affect the maximal flux, but also the kinetics of exertion in relation to changing DO concentration. Thus unless chamber flow patterns are closely matched with the natural conditions, erroneous kinetics measurements may result.

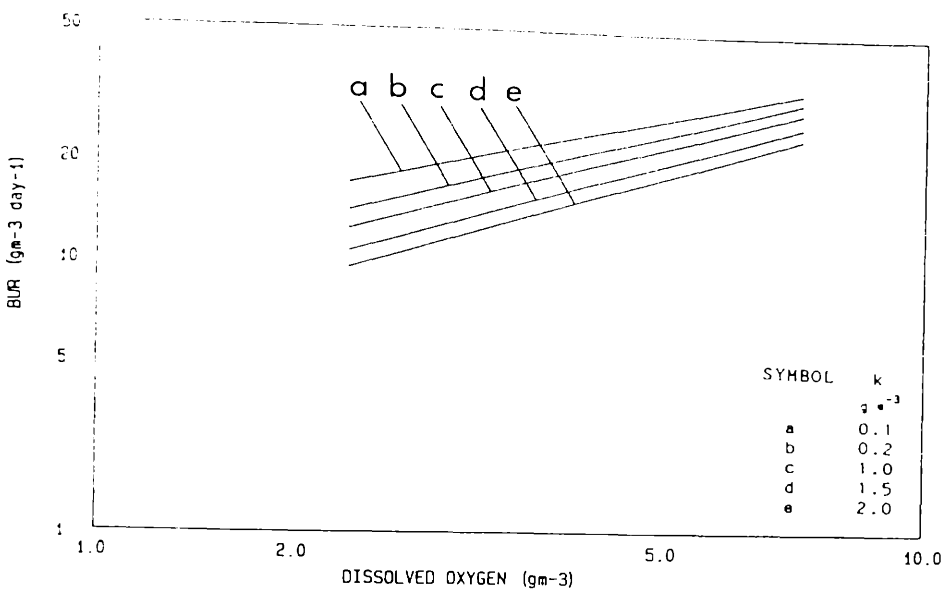


Figure 7.3 : The response of BUR flux estimates to DO concentration for various k_s values with an exponential decline in D_S (as for Fig. 7.2). Other model parameters are as for Fig. 7.1.

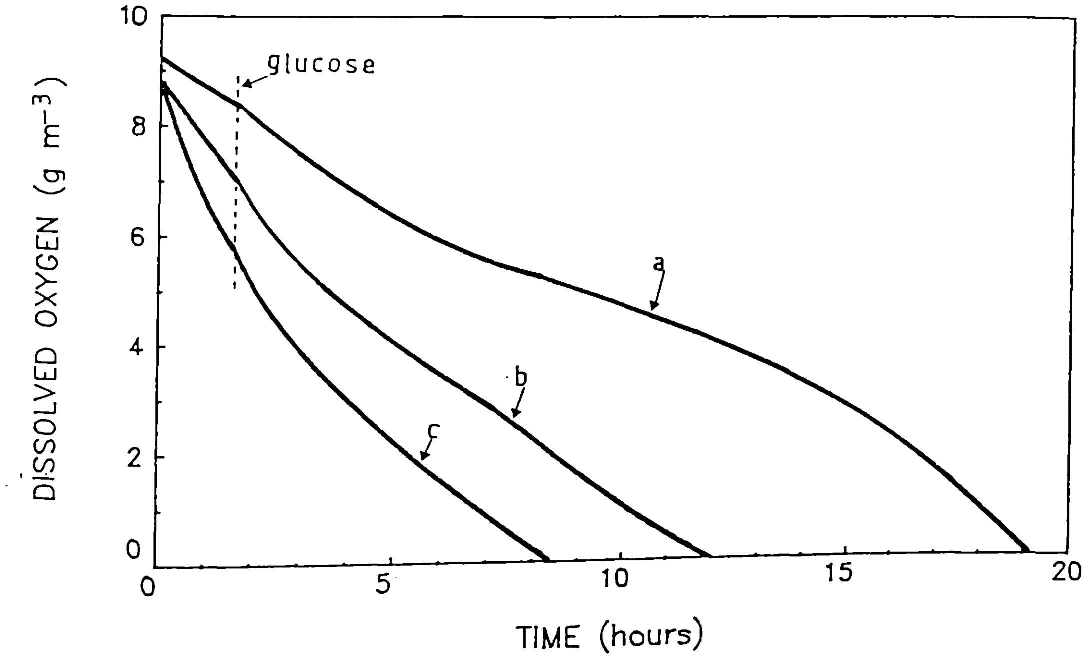


Figure 7.4 : Measurement of dissolved oxygen uptake progressions for pre-sieved (<2 mm) pumice material from the Waiotapu River. Material was continuously stirred (using a BOD probe) and monitored in BOD bottles, containing BOD dilution water (APHA, 1976) and supplemented glucose (20 gm^{-3} final concentration) with incubation at 20 °C. Sediment volumes were: (a) 17 ml; (b) 34 ml; (c) 68 ml.

Although high sediment k_s values may to some extent explain the observed chamber kinetics, the possibility also exists that the rapidly changing chamber DO concentrations may create conditions within the sediments which are not steady-state.

7.2.2 BUR kinetics : a non-stationary model for sediment flux

The assumption of steady-state kinetics is only valid if the characteristic time of variation of the parameters is longer than the turnover time of the various compartments (Billen, 1982b). Thus the concern with the chamber systems is that the rate of change of DO concentration may exceed the rate of dispersive supply of oxygen to deep sediment layers. This may cause a hysteresis in the measurement system, resulting in a pseudo-kinetic order. Two situations are of interest with respect to chamber measurements: (i) the effect of various rates of DO removal on flux estimates; (ii) the effect of step changes in DO concentration.

An estimation of the characteristic diffusion time scale may be obtained using the Einstein-Smoluchowski relation (Jorgensen and Revsbech, 1983)

$$t = \frac{d^2}{2D_s} \qquad 7.1$$

where t = diffusion time(s); d = diffusion path length (cm); Ds = dispersion coefficient (cm^2s^{-1}). Using the value of D_s estimated from Figure 7.1 the diffusion time to 10 cm depth would be 18 min. If molecular diffusion was the sole driving force ($D_s = 2.1 \times 10^{-5}\ cm^2s^{-1}$) the diffusion time would be 28 days.

Model simulations for linear rates of decline in DO concentration, comparable with measured chamber rates, showed that flux estimates were lower than steady-state values with the difference decreasing as DO concentration declined (Fig. 7.5A). A simulation where the value of D_s was halved, together with a steady rate of DO concentration decline, showed a rapid BUR decline followed by a more gradual process (Fig. 7.5 A(d)). This latter simulation gave BUR values closer to those obtained using small chamber measurements (Fig. 7.5B). Model simulations showed reasonably good agreement with chamber measurements as compared with steady-state values (Fig. 7.5). Further detailed simulations are required however, for the initial period of transient flux estimation.

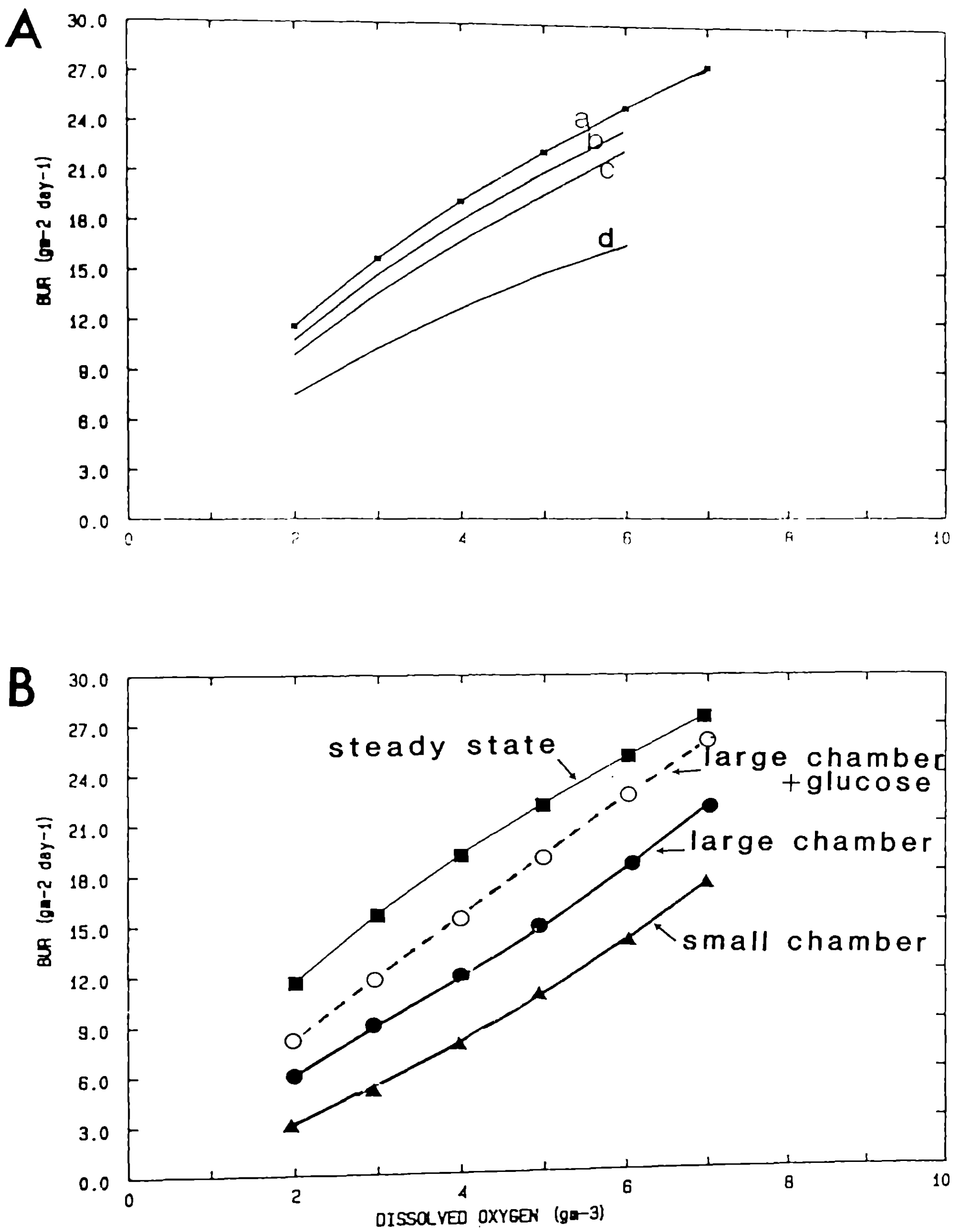


Figure 7.5 : Comparison of model simulations and chamber measurements *in situ* of the apparent BUR flux relationship with DO concentration. Model parameters: $\phi = 0.5$; $I = 4.6$ gm^{-2} day^{-1}; $k_s = 1$ gm^{-3}; Depth = 40 cm; $D_s = 40 \times 10^{-3}$ cm^2 s^{-1} (surface), 27×10^{-3} cm^2 s^{-1} (bottom).

A Model Simulations
a steady state
b steady 5 gm^{-3} h^{-1} DO decline; constant D_s
c steady 10 gm^{-3} h^{-1} DO decline; constant D_s
d steady 5 gm^{-3} h^{-1} DO decline; Ds halved after obtaining steady-state at 7 gm^{-3} DO.
B Steady-state model simulation compared with chamber measurements
a steady-state model predictions
b small chamber with circulation velocity 0.2 ms^{-1} (▲—▲)
c large chamber with circulation velocity 0.35 ms^{-1}
(●—●) are initial chamber measurements,
(○--○) are reoxygenated with glucose added for 9 March 1983 on Waiotapu River (Figs. 5.34 and 5.36).

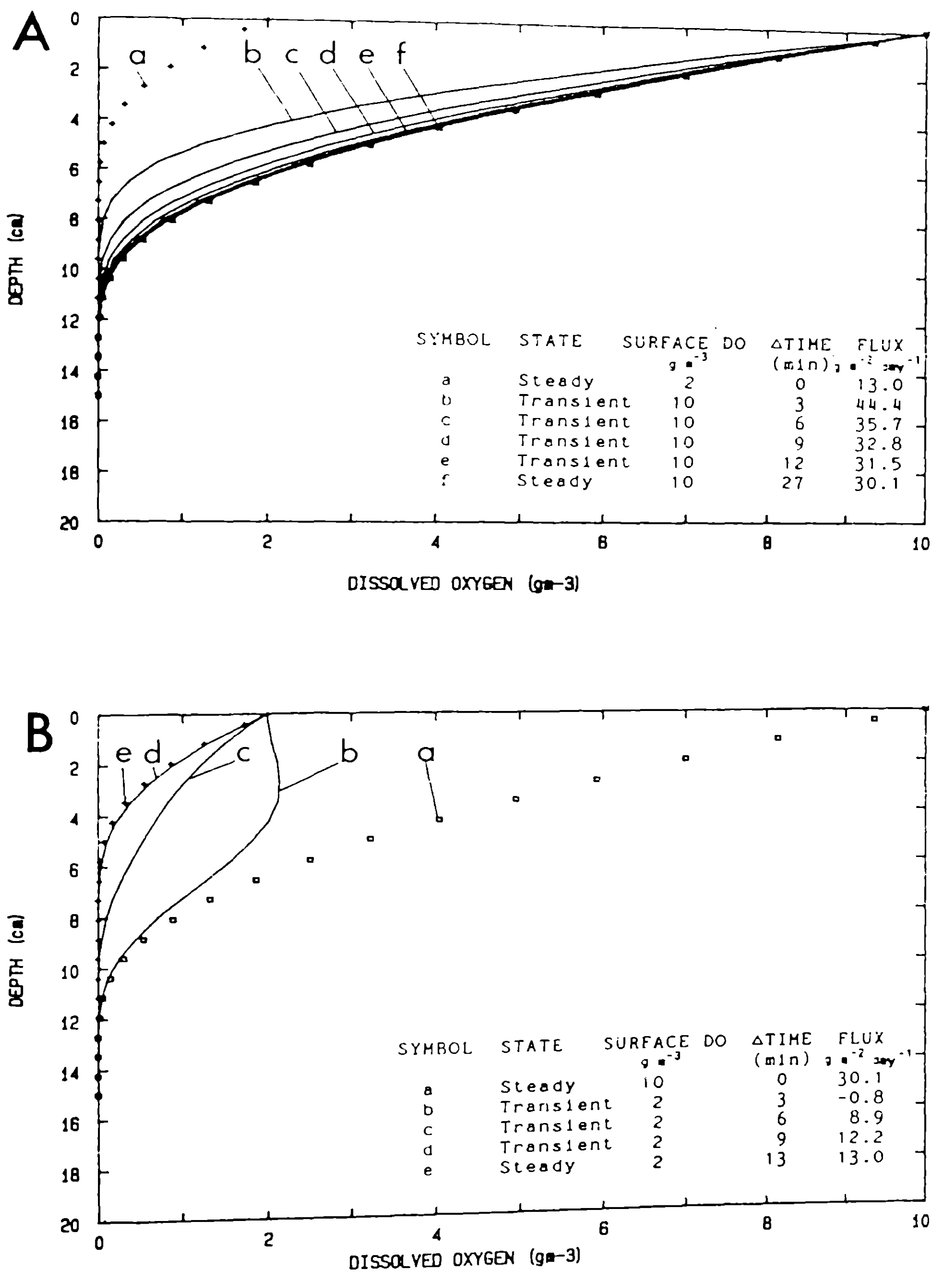


Figure 7.6 : Model simulations of interstitial DO profiles and flux for step changes in overlying DO concentrations. Model parameters: $\phi = 0.5$; $I = 4.6$ gm^{-2} day^{-1}; $k_s = 1$ gm^{-3}. Depth = 20 cm; $D = 40 \times 10^{-3}$ cm^2 s^{-1} (surface), 27×10^{-3} cm^2 s^{-1} (bottom)

A step change up from 2 to 10 gm^{-3}

B step change down from 10 to 2 gm^{-3}.

Symbols represent steady state profiles and each solid line a transitional profile at 3 min intervals.

Model simulation of rapid step changes in DO concentration were considered important for 2 reasons: (i) with regard to reoxygenation of the chamber following oxygen depletion; (ii) for the application of chamber devices to photosynthetically active populations. Rapid reoxygenations either by exchange with the surrounding water or by some other means (e.g. Hickey, 1985b), resulted in oxygen-deficient sediment overlain with oxygen-rich water. As such, a period of time following reoxygenation represents a BUR flux into the sediments in order to satisfy a deficit, rather than a flux driven by respiratory or other oxygen demanding process present *in situ*. For the Waiotapu sediments, a step change from 2 to 10 g O_2 m^{-3} showed a requirement for 12 min equilibration prior to obtaining a value within 5% of the true flux *in situ* (Fig. 7.6A). The implications of these simulations are considerable, in that maximum flux values measured (section 5.3.5, Hickey 1985b) are in error, and all measurements should be disregarded if they are made over a time scale less than the equilibration time following reoxygenation.

If photosynthesis is occurring within the sediments then high concentrations of DO may be produced. Sufficient time must therefore be allowed to obtain a dark-equilibrated profile at a given DO. Figure 7.6B shows steady-state and transient profiles for a step-down from 10 to 2 g m^{-3}. Transition time was estimated as 13 min in order to obtain a new steady-state with initial negative flux predictions, indicating a continued sediment source of oxygen to the overlying flow.

Revsbech *et al*. (1980) measured oxygen profiles in sandy estuarine sediments and estimated a benthic dispersion coefficient (D_s) of 5.2 x 10^{-6} cm^2s^{-1}. Transient profiles obtained for light to dark and dark to light conditions at 18 °C, showed that at least 1 h was required to achieve steady-state for each transition. This implies that chamber flux measurements performed within 1 h of environmental light/dark perturbations would lead to erroneous estimates.

7.3 STEADY-STATE SIMULATION OF BUR IN RELATION TO DO AND INTRINSIC MICROBIAL ACTIVITY FOR THE WAIOTAPU AND TARAWERA RIVERS

Attempts at fitting data obtained from the Tarawera River proved difficult, caused by uncertainties associated with measurement of interstitial DO. A surface D_s values of 111 x 10^{-3} cm^2s^{-1} may be appropriate for Johnston Road when sampled on 23 November 1983 (Fig. 7.7A), whereas, a D_s value of 42×10^{-3} cm^2s^{-1} may be appropriate for SH30 sampled 8 March 1984 (Fig. 7.7B).

Simulation of steady-state DO profiles for a range of sediment intrinsic activities showed the flux to be a function of $I^{0.5}$, with DO penetration increasing from 10 cm for an I of 9.6 g m^{-2} day^{-1}, to 25 cm for an I of 1.2 g m^{-2} day^{-1} (Fig. 7.8).

BUR flux simulations for the Tarawera River were calculated using the measured surface sediment ATP concentrations converted into potential activities, and assuming the D values used for I activity simulation (from Fig. 7.7). Maximum ATP measured was 29.5 mg m^{-2} (Fig. 5.44) which using an estimated specific activity of 26 mg O_2 mg $ATP^{-1}h^{-1}$ (calculated intrinsic activity and ATP measurements Figs. 5.45 and 5.44) as ATP turnover rates in Fig. 5.51) would give a BUR of 55.8 g m^{-2} day^{-1} (profile not shown). Assuming the pulp mill waste was replaced by a more readily degradable waste (e.g. dairy effluent) then the specific metabolic activities and growth rate of the attached biomass would increase. Using a value of 100 mg O_2 mg $ATP^{-1}h^{-1}$ (C.W. Hickey and J.W. Nagels unpublished results for glucose and whole milk measurements) gives an estimated BUR of 110 g m^{-2} day^{-1}. Thus the potential for benthic oxygen removal by attached microbial biomass appears to be extremely high even under present microbial colonisation levels. Considering that higher microbial colonisation levels may result from changes in effluent concentrations or biodegradability characteristics, even higher BUR values may result.

7.4 DISCUSSION AND CONCLUSIONS

Transient flux simulation values obtained under non-stationary DO concentration conditions and for step-wise changes suggest that enclosing devices may result in erroneous flux estimates if process time scales are not allowed for. Model simulations for step-wise increases or decreases in chamber DO concentratons (Figs. 7.6A and B respectively), showed that an equilibration period is required in order to obtain equilibrium concentration conditions within the sediments, and hence accurate flux measurements. The equilibration period under these conditions was approximately 13 min, however, the period would be expected to increase as D_s values decreased. Revsbech _et al_. (1980)

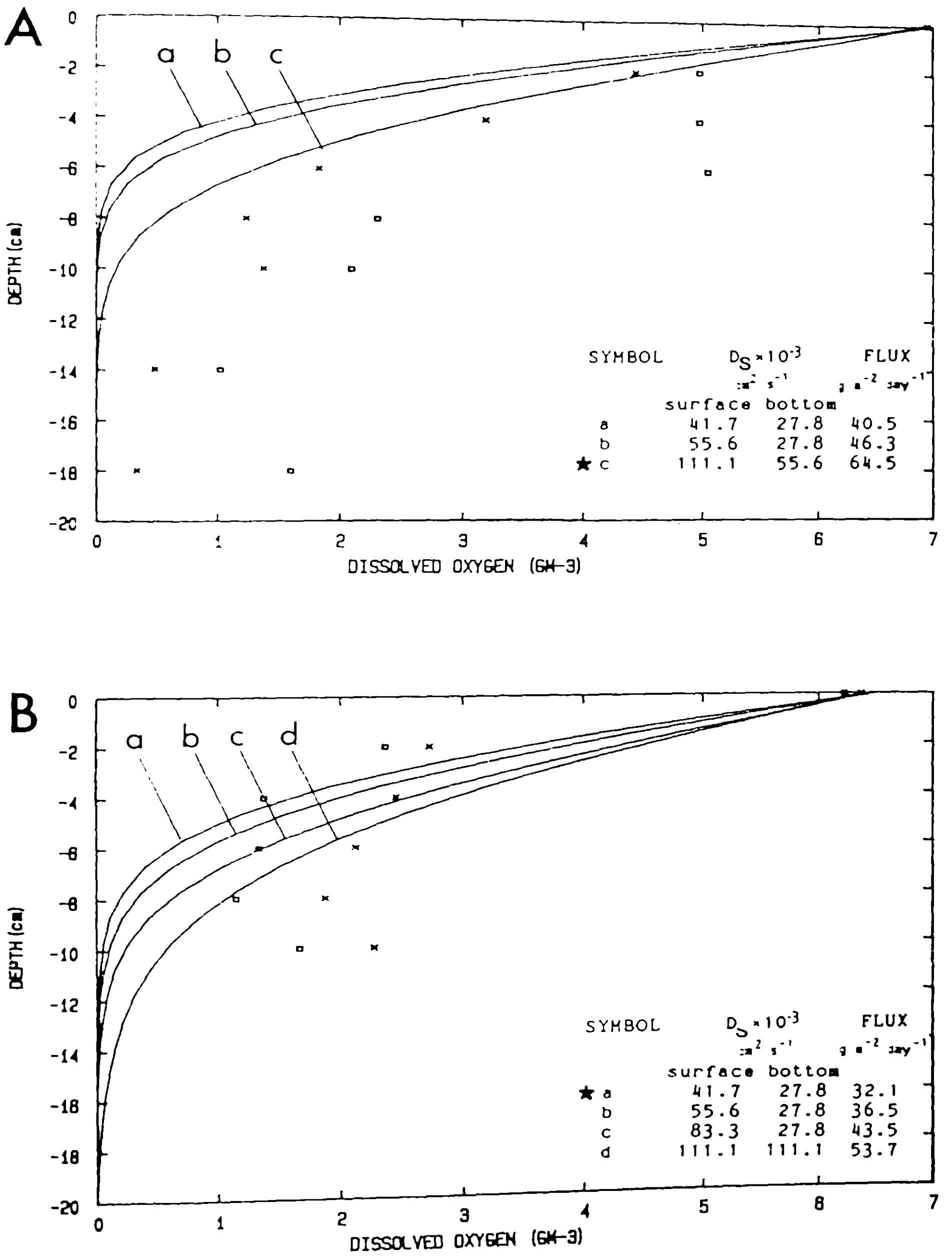


Figure 7.7 : The response of BUR flux estimates to variation in the dispersion coefficient (D_S) for measurements at 2 stations on the Tarawera River.

A Johnston Road (sampled 23 November, 1983). Measured were: I = 8.4 gm^{-2} day^{-1} (Fig. 5.43); and interstitial DO concentrations for core 4 (□) and core 5 (x). Other model parameters: $\phi = 0.5$; $k_s = 1$ gm^{-3}; Depth = 20 cm.

B SH30 (sampled 8 March 1984). Measured were: I = 6.7 gm^{-2} day^{-1} (Fig. 5.43); and interstitial DO concentrations for core 1 (x) and core 2 (□). Other model assumptions: as for A.

★indicates Ds values chosen for simulations.

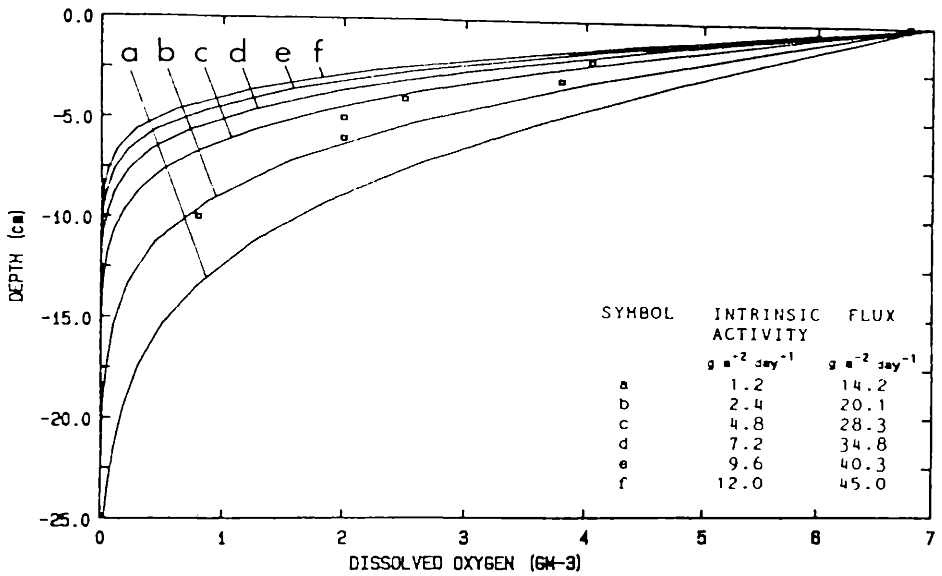


Figure 7.8 : Steady-state model simulations of interstitial DO profiles and flux for variation in sediment intrinsic activity (I). Model parameters: $\phi = 0.5$; $k_s = 1$ gm^{-3}; Depth = 20 cm; Ds = 40 x 10^{-3} cm^2 s^{-1} (surface), 27 x 10^{-3} cm^{-2} s^{-1} (bottom). DO measurements shown (□) are for the Waiotapu River, fine particulates (Fig. 7.1); I = 4.6 gm^{-2} day^{-1}.

measured equilibration periods of at least 1 h for marine sands ($D_s \approx 10^{-6}$ cm^2s^{-1}). Model simulations of flux values for steady rates of DO decline showed lower transient flux values than steady-state conditions (Fig. 7.5A). The magnitude of transient underestimation increased at higher rates of DO removal and amounted to about 20% lower estimates under the simulated conditions (Fig. 7.5 A). A transient simulation at 20 gm^{-3} h^{-1} (which may occur in the small chamber) showed a 38% underestimation of the steady-state flux (results not shown), indicating that serious estimation errors may occur with small chambers and rapid depletion rates. Differences between steady-state model predicted flux values and measured chamber values at comparable DO concentrations amounted to >50% (Fig. 7.5 B), with greater rates of flux decline occurring in the small chamber (having more rapid deoxygenation) than the large chamber. Transient flux simulations did not, however, accurately match the observed chamber conditions (Fig. 7.5B), suggesting that an assumption made in the model construction may require further modification.

Fluxes were calculated by using a model in which all mixing processes were treated in terms of a dispersion coefficient. This coefficient represented the sum of advective and diffusive components, which were then used to calculate flux estimates based on the near surface concentration gradients. This approach has been applied in the past under conditions of wave current mixing (Vanderborght *et al.*, 1977a, b) and with enhanced mixing caused by bioturbation (Berner, 1980). Although fluxes driven by concentration gradients may accurately reflect diffusion processes, dispersive processes (which involve wave current mixing and momentum exchange) may be such that exchange of mass acts independently of the concentration gradient. The implications of this are that although steady-state predictions as modelled may accurately predict the balance between supply and demand, and hence the flux, in the case of non-stationary conditions predictions will be affected by the concentration gradient. Thus there is a need to develop and test a momentum-exchange model for application to non-stationary conditions.

Steady-state modelling provides the best predictive tool, and thus the means of testing our conceptual understanding of benthic mass transfer processes. The BUR has been shown to be dependent on: (i) the sediment-water exchange or dispersion coefficient; (ii) the level of microbial colonisation; and (iii) the concentration of electron donors and electron acceptors in the overlying flow. Simulations have shown that: (a) increases in microbial colonisation of the pumice (i.e., intrinsic activity) will result in a ½ order response for increasing flux (i.e. BUR): (b) the kinetics of BUR will show ½ order dependence on DO concentration, which may increase to first order depending on the sediment affinity for DO (i.e. k_s value). Since the value of k_s is affected by diffusion resistance within the sediment matrix (i.e. internal agitation), further work will be required to define the conditions suitable for measurement of intrinsic activities. Further work is also required on the factors influencing sediment-water exchange processes.

Chapter 8

GENERAL CONCLUSIONS, IMPLICATIONS FOR PREDICTIVE MATHEMATICAL MODELLING AND THE NEEDS FOR FUTURE RESEARCH

8.1 INTRODUCTION

The data presented in the previous chapters provide a sufficient basis for accepting the 2 working hypotheses concerning (i) the influence of hydrodynamic conditions on exchange across the sediment-water interface and (ii) the development of benthic microbial populations in response to available energy sources for growth (Chapter 1, Section 1.4). Thus the microbial population present in a river bed is affected by both chemical and physical aspects of the environment. The interaction between these 3 components (biologial, chemical and physical; as shown in Fig. 1.2) controls the amount of active biomass present, and the rate of nutrient transformations. A Conceptual Model (Chapter 1, Section 1.5) was used to test our understanding of the factors influencing the final rate process.

This chapter reviews how the findings presented in this work relate to the modelling of river processes, and aims to identify the key components and thus facilitate a priori predictions in relations to environmental perturbations.

This discussion will be separated into 3 categories: (i) physical (hydrologic) aspects; (ii) biological; and (iii) chemical (after Fig. 1.2).

8.2 PHYSICAL (HYDROLOGIC)

It has been demonstrated that the river hydrodynamics are important in 3 respects of benthic microbial activity: (i) seasonal flow variation; (ii) intermittent floods; and (iii) base flow conditions. The high flows of winter (Fig. 3.2) transport fine sediments and particulate organic matter (POM) downstream and create the mosaic distributions of medium and coarse particulates present in the summer months. The combined effects of frequent floods and high base flows contribute to the low levels of sediment biomass and activity present in spring (Section 5.2.3). Seasonal diurnal oxygen monitoring at a station above a point source of significant discharge demonstrated substantial changes in river oxygen dynamics in response to seasonal flows and floods which disrupted the biological communities (Section 5.2.3). A flood occurring in summer was shown to substantially alter the biomass levels, with changes in biomass type and metabolic capabilities demonstrated in the recovery period (Section 5.2.4). Thus, the dynamic nature of river flow tends to 'reset' the system, resulting in considerable periods at base flow where growth under non-steady-state conditions may occur. Additionally both the baseflow velocity and the amount of suspended sediment are important in determining the nature and permeability of sediments present, and hence the degree of sediment/water exchange.

The base flow conditions separate rivers into 2 types based on mean velocity. At mean boundary velocities below about 0.15 m s^{-1} a depositional environment exists and particulate organic matter tends to settle on the river bed (Smith, 1975; see Section 2.1). In high velocity rivers an erosional environment exists, which results in a larger benthic particle size, and a habitat suitable for microbial biofilm development. Distinguishing such differences is important in determining the processes affecting the river BOD assimilation capacity.

BOD removal in a river is the net result of 3 major processes:

i the uptake of DOM and conversion to POM;
ii the settling of POM;
iii the respiratory decay of POM.

In rivers with boundary velocities greater than 0.15 m s^{-1} epilithic heterotrophs may grow as biofilms attach to benthic particulate and actively removing DOM and DO from the overlying flow. Epilithic heterotrophic processes were identified by enzyme activity measurements below the dairy discharge in the Waitoa River (Section 5.2.6), with detrital heterotrophs present further downstream. Thus, both epilithic and detrital heterotrophic process were present in the same reach of the river system. The epilithic heterotrophs resulted in a rapid production of biomass measured in the overlying flow, which showed a comparable rate of decline on progression downstream (Section 5.2.8). Thus the river mechanisms which promote rapid removal of POM will also need to be identified in order to accurately predict river BOD assimilation capacities. In the Waitoa River, the macrophytes showed considerable mud accumulation but the composition of the mud was not tested to determine its origin. Bed particle size and the presence of large debris (e.g. logs) may also affect the settling and retention of POM. Such factors require further research to quantify their importance.

The microoganisms growing attached to the bed must, under steady-state conditions, be subjected to processes which remove biomass from the system. There are 3 mechanisms by which microbial biomass may be lost from surfaces; all have been identified in these studies:

i sloughing;
ii secondary predation;
iii respiratory decay.

Loss of mass by periodic sloughing or continuous shearing has been shown to be very significant for 'sewage fungus' populations (Section 6.2). Such effects appear to be poorly understood, and the location within a biofilm at which detachment will occur cannot be predicted at this time. This is important in the case of sloughing because the composition and subsequent activity of the biofilm may differ substantially from the original biofilm. In the Tarawera River the rate of increase of planktonic biomass was substantially less than might have been anticipated for the rate of benthic activity under steady-state conditions (Section 5.4.5). The presence of protozoal populations attached to the pumice substratum suggested that secondary predation may account for this discrepancy. However, the importance of protozoal activity remains to be determined. Sediment respiratory decay was measured in the Waitoa (sand/gravel substrata, Section 5.2.8) and Waiotapu (pumice substrata, Section 5.3.9) rivers. The respiratory decay was rapid, with carbon turnover occurring within periods of less than a month, which compares with time scales of years for sedimentary environments (Hargrave and Phillips, 1981). In the rivers studied, biomass carbon constituted a large proportion of the total sediment carbon (Sections 5.2.4, 5.3.7, 5.4.4), thus storage reserves were small and without a high rate of exchange with the overlying flow, metabolic activities could not be maintained and would rapidly decline.

The rate of sediment-water exchange in the rivers studied was shown to be several orders of magnitude greater than the predominantly molecular diffusion exchange occurring in lentic environments (Section 5.6). The high level of exchange in the rivers studied was shown to sustain greater levels of active biomass per volume of sediment than were sustained in other aquatic environments (Section 5.6). Thus the high measured rates of nutrient transformations mediated by river sediment may to a large extent be sustained by the magnitude of the dispersion coefficient. Therefore factors which affect mass transfer at the sediment-water interface are likely to greatly influence the flux across the boundary (e.g. flow velocity, depth, fine sediments). A further implication is that the other components mentioned in the Conceptual Model (Section 1.5), such as concentrations in the overlying flow and biomass levels, are likely to be of decreased importance in determining the flux.

Chamber measurements showed concomittant flux increases with circulation velocity, suggesting that mass-transfer processes were turbulent (Section 4.2). The failure of the chamber to simulate adequately the natural conditions present in a deep river (the Tarawera, Section 5.4.2) suggests that other mechanisms of sediment-water exchange may be operating. Further work is required to identify the relative importance of (i) turbulent mass transfer, in order to determine the role of eddy size and turbulence generated near the bed by boundary roughness; and (ii) percolation, or subsurface flow through dune structures which may be present and restricted by the chamber flanges. Such work should include sediment depth profiles for nutrients in situ (e.g. using micro-DO electrodes) and within the simulated conditions of chamber devices. Additional field measurements of sediment interstitial flow velocities (using hot wire anemometers) and pressure fluctuations within the sediments (using electronic pressure transducers), could be used to determine the relative importance of percolation and turbulent exchange. Differences in sediment particle density (e.g. sand and pumice) and size may substantially alter the onset of bed movement. This will influence not only sediment-water exchange processes but also the suitability of the river bed for development of substantial biofilms. Further research is required to determine the role of river hydrologic conditions as it affects particle settling, the onset of particle movement, sediment-water exchange and chamber simulation.

Chamber kinetic measurements may give erroneous respiration rate estimates if steady-state diffusion conditions for sediment-water exchange do not exist, because of rapidly changing concentrations within the chamber (Chapter 7). Step concentration changes also require an equilibration period prior to measurements. The equilibration period required will be dependent on the depth of sediment interaction with the over-lying flow and the magnitude of the benthic dispersion coefficient, both of which vary with environmental conditions (section 5.3.2).

Further work is required to determine the importance of possible non-steady-state conditions to chamber measurements.

8.3 BIOLOGICAL

In the shallow river systems studied the active benthic biomass was generally 10-times (up to 50-times) greater than the over-lying planktonic biomass (Sections 5.2.3, 5.3.6, 5.4.4), meaning that over 90% of the metabolic activity resided on the bed. This dominance of benthic metabolic processes results in greatly enhanced nutrient transformation rates in such rivers.

The level of and predominant type of microbial biomass present varied both longitudinally in relation to the discharge, and in response to hydrologic conditions (section 5.2.4). Above the discharge, phototrophic filamentous algal biomass developed under low flow conditions, whereas downstream a heterotrophic bacterial biomass predominated.

The magnitude of the benthic respiration rate was determined by the level of benthic biomass which could develop and the degree of sediment-water interaction. The activities ranged from a maximum with filamentous bacterial (sewage fungus) communities (maximum 70 g O_2 m^{-2} day^{-1}), and decreased with decreasing particle size (<5 g O_2 m^{-2} day^{-1} on fine sand). These results are summarised in Table 8.1.

Table 8.1 A summary of benthic respiratory activity in relation to benthic conditions

Activity	Organisms/substratum		Effluent Biodegradability	Environmental conditions
maximum	filamentous	bacterial algal	readily	erosional (riffles)
↕	biofilms	large substrata pumice sand (coarse) sand (fine)	↕	↕
minimum	mud		refractory	depositional (pools)

Substantial differences in the level of biomass colonisation may ocur between particle sizes at a station under comparable water quality conditions (sections 5.2.7, 5.3.8). Thus both water quality and physical factors influence the level of microbial biomass present. Further work is required to define the conditions favouring filamentous microbial development (both heterotrophic and phototrophic), since these exert maximum benthic effects on the overlying flow.

The development of predictive models for application to such systems, covering the development of phototrophic, heterotrophic, and autotrophic biomass development, and benthic mass transfer flux, could form an important tool in the management of river ecosystems. Such models should be developed to a stage where the magnitude of management manipulations necessary to affect such populations could be established. To date only conceptual models have been proposed (e.g. Escher and Characklis, 1982; Lock _et al._, 1984). Under conditions such as the mobile pumice bed rivers, the maximum level of attached biomass may be controlled by abrasive sloughing, which could be modelled using a logistic relationship. The sediment metabolic activity would then be determined by the growth rate of the heterotrophic biomass, which is affected by the concentration and biodegradability of the organic wastes present, in relation to an abrasive loss rate.

8.4 CHEMICAL

The respiratory activity of the benthic heterotrophic biomass may result from both epilithic and detrital metablic processes. Biomass-specific enzyme activity methods were developed to determine the proportion of the epilithic heterotrophs present. This showed that the proportion of heterotrophic biomass which was actively removing DOM from the over-lying flow, decreased with increasing distance below the discharge. The proportion of detrital heterotrophs, indicating a depositional environment, increased with increasing distance below the discharge.

Thus the process of 'assimilation' of organic matter by a river system is the net effect of 2 processes: (i) active uptake of DOM; and (ii) the settling of, or passive removal of POM. Mathematical models should account for each of these processes separately, since the processes involved differ greatly and will be influenced by both the degree of waste treatment and river hydrologic conditions.

The epilithic heterotrophic biomass relies on supply of both electron donors and electron acceptors from the over-lying flow. Activity may therefore be rate limited by the concentraton of either of these components (Section 5.3.3). Because the sediment-water exchange is high, with dispersion coefficients up to 1000x molecular diffusion coefficient (Section 5.2.2, 5.3.3), a high level of benthic activity may be maintained by a high mass flow of nutrients rather than by high concentrations.

The implications for mathematical modelling involving such a benthic system are considerable in that changes in electron donor (i.e. waste) concentration may not alter the rate of benthic activity, and hence possibly the level of deoxygenation, but rather the yield (or downstream extent) of effects. Quantification of such metabolic effects will require measurement of the saturation kinetics for metabolism (i.e. Michaelis-Menten) of both electron donors and electron acceptors.

More complex models would be required to be useful for predictive management. These would require the inclusion of saturation growth kinetics (i.e. Monod) for complex organic wastes and the identification and measurement of the predominant loss mechanisms under various river conditions (i.e. sloughing, secondary predation, respiratory decay). Techniques are available to measure the microbial growth rates on complex wastes at environmental concentrations, using pre-filtered samples incubated in an electrolytic respirometer system (Hickey and Nagels, 1985), by determining the rate of increase in respiration during the initial exponential growth phase region. This system has also been used to measure rates of sediment respiratory decay (Section 5.2.8, 5.3.9). Further work is required to assess the importance of other factors affecting benthic microbial biomass.

Several models have been developed, calibrated and verified on laboratory biofilm reactors for heterotrophic and autotrophic biomass, and may be suitable for application to lotic environments, either for the prediction of benthic nutrient flux or biofilm biomass development. Williamson and McCarty (1976a) modelled nutrient flux in deep biofilms, which, as extended by Rittman and McCarty (1981) predicts the minimum level of organic nutrient necessary to support the existence of a heterotrophic biofilm. A more comprehensive model has been proposed by

Kissel _et al_. (1984) to predict competitive heterotrophic and autotrophic (nitrifier) growth, biofilm development (including decayed inert material) and nutrient flux. A predicted BUR of 1.45 g m^{-2} day^{-1} differs greatly from the measured results in this study. However this model awaits experimental verification. The turbulent mass transfer processes present in river systems, would necessitate the use of higher dispersion coefficients to avoid serious nutrient flux underestimation. The development of dynamic models describing benthic microbial growth and activity in river systems would be of great value in management and in testing our understanding of such systems. Because the erosional benthic environment represents an ideal habitat for microbial attachment and growth, even extensive conventional effluent treatment may not reduce the level of benthic metabolism, but rather cause a shift in the predominant type of biomass. Heterotrophic biomass may be replaced by either autotrophic nitrifiers or phototrophic algae, depending on the relative level of DOM or POM, ammonium and light.

REFERENCES

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Aaronson S., Dhawale S.W. and Patni N.J. (1976) Phosphatase synthesis and secretion of Chlamydomonas reinhardi. Its regulation by exogenous phosphate. J. Phycol. 12 : 30.

Amberg H.R. and Elder R. (1975) Intermittent discharge of spent sulphite liquor. Trans. Am. Soc. Civ. Engrs. 122 : 1179-1186.

APHA (1976) Standard methods for the examination of water and wastewater, 14th Edition. APHA, Washington D.C.

Atkinson B. and Davies I.J. (1974) The overall rate of substrate uptake (reaction) by microbial films Part I - A biological rate equation. Trans. Instn Chem. Engrs, 52 : 248-259.

Atkinson B., Swilley E.L., Busch A.W. and Williams D.A. (1967) Kinetics, mass transfer and organism growth in a biological film reactor. Trans. Instn Chem. Engrs. 45 : T257-T264.

Ausmus B.S. (1973) The use of ATP assay in terrestrial decomposition studies. Bull. Ecol. Res. Commun. (Stockholm) 17 : 223-234.

Bailey J.E. and Ollis O.F. (1977) Biochemical Engineering Fundamentals. In McGraw-Hill series in water resources and environmental engineering. McGraw-Hill, New York.

Baillod C.R. and Boyle W.C. (1970) Mass transfer limitations in substrate removal. J. San. Engng Div., Am. Soc. Civ. Engrs 96 (SA2) : 525-545.

Bancroft K., Paul E.A. and Wiebe W.J. (1976) The extraction and measurement of adenosine triphosphate from marine sediments. Limnol. Oceanogr. 21 : 473-480.

Belanger T.W. (1981) Benthic oxygen demand in Lake Apoka, Florida. Water Res. 15: 267-274.

Bergmeyer H.U., Bernt E., Schmidt F. and Stork H. (1974) In: Methods of Enzymatic Analysis (Bergmeyer, H.U., ed.) Vol. 3, pp. 1196-1201; Verlag Chemie, Weinheim/Academic Press, Inc. New York and London.

Berman T.A. and Pollinger U. (1974) Annual and seasonal variations of phytoplankton, chlorophyll, and photosynthesis in Lake Kinneret. Limnol. and Oceanogr. 19 : 31-54.

Berner R.A. (1980) Early diagenesis : a theoretical approach. Princeton, New Jersey.

Billen G. (1978) A budget of nitrogen recycling in North Sea sediments of the Belgian coast. Estuarine and Coastal Marine

Science 7 : 127-246.

Billen G. (1982a) Modelling the processes of organic matter degradation and nutrient recycling in sedimentary systems. In : Sediment Microbiology (Special Publications of the Society for General Microbiology, no. 7). Nedwell D.B. and Brown C.M. (Eds) Academic Press, New York.

Billen G. (1982b) An idealized model of nitrogen recycling in marine sediments. Am. J. Sci. 282 : 512-541.

Bobbie R.J., Morrison S.J. and White D.C. (1978) Effects of substrate biodegradability on the mass and activity of the associated estuarine microbiota. Appl. Environ. Microbiol. 35 179-184.

Bott T.L., Brock J.T., Cushing C.E., Gregory S.V., King D., and Peterson R.C. (1978) A comparison of methods for measuring primary productivity and community respiration in streams. Hydrobiologia 60 : 3-12.

Boubee J.A.T. (1977) Benthic Studies on the Waikato River in Hamilton, N.Z. M.Sc. Thesis, University of Waikato.

Bowman G.T. and Delfino J.J. (1980) Sediment oxygen demand techniques: A review and comparison of laboratory and in situ systems. Water Res. 14 : 491-499.

Boyle J.D. and Scott J.A. (1984) The Role of benthic films in the oxygen balance of an East Devon River. Water Res. 18 1089-1099.

Brock T.D. (1974) Biology of microorganisms. Prentice Hall, New Jersey.

Browman M.G. and Tabatabai M.A. (1978) Phosphodiesterase activity in soils. Soil Sci. Soc. Am. J. 42 : 284-290.

Bullied, N.C. (1978) An improved method for the extraction of adenosine triphosphate from marine sediment and seawater. Limnol. Oceanogr. 23 : 174-178.

Cairns J. Jnr, Kuhn D.L. and Plafkin J.L. (1979) Protozoan colonization of Artificial Substrates. In : Methods and Measurements of Periphyton Communities : A Review. R.L. Weitzel, ed. ASTM STP Pub 690, pp 34-57.

Capblancq J. and Cassan M. (1979) Study of the periphyton of a polluted river (Agout). 11 Metabolism and dynamics of growth on artificial substrates. Annals of Limnology 15 : 211-222.

Castaldi F.J. and Malina J.F. (1982) Velocity-dependent reaction rates in a slime reactor. Jour. Water Poll. Control Fed. 54 : 261-269.

Chapman A.G. and Atkinson D.E. (1977) Adenine nucleotide concentration and turnover rates. Their correlation with biological activity in bacteria and yeast. Adv. Microb. Phyiol. 15 : 253-306.

Characklis W.G. (1973) Attached microbial growths - I. Attachment and growth. Water Res. 7 : 1113-1127.

Characklis W.G. and Cooksey K.E. (1983) Biofilms and Microbial Fouling. Adv. Appl. Micro. 29 : 93-138.

Characklis, W.G., Trulear, M.G. Bryers, J.D. and Zelver N. (1982) Dynamics of biofilm processes:methods. Water Res. 16 : 1207-1216.

Chatterjee S. and Price B. (1977) Regression analysis by example. Wiley, New York.

Chiaro P.S. and Burke D.A. (1980) Sediment Oxygen demand and nutrient release. J. Environ. Eng. Div. Proc. Am. Soc. Civ. Eng. 106 : 177-195.

Christensen J.P. and Packard T.T. (1979) Respiratory electron transport activities in Phytoplankton and bacteria. Comparison of methods. Limnol. Oceanogr. 24 : 576-583.

Christian R.R., Bancroft K. and Wiebe W.J. (1975) Distribution of microbial adenosine triphosphate in salt marsh sediments at Sapelo Island, Georgia. Soil Sci. 119 : 89-97.

Clarke J.R., Messenger D.I., Dickson K.L., and Cairns J. Jr. (1978) Extraction of ATP from Aufwuchs communities. Limnol. Oceanogr. 23 : 1055-1059.

Cohen R.R.H. and Church M.R. (1981) A method for studying primary productivity and photosynthesis in unenclosed batch cultures and field samples. Arch. Hydrobiol. 91 : 231-241.

Collins G.B. and Weber C.I. (1978) Phycoperiphyton (algae) as indicators of water quality. Trans. Am. Microsc. Soc. 97 : 36-43.

Cooper A.B. (1984) Activities of benthic nitrifiers in streams and their role in oxygen consumption. Microb. Ecol. 10 : 317-334.

Cummins K.W. (1974) Structure and function of stream ecosystems. Bio. Sci. 24 : 631-641.

Cunningham H.W. and Wetzel R.G. (1978) Fluvic acid interferences on ATP determinations in sediments. Limnol. Oceanogr. 23 : 166-173.

Currie K.J. and Rutherford J.C. (1982) Management of BOD in the lower Manawatu River. In 'Aquatic oxygen seminar proceedings, Hamilton, November 1980'. Water and Soil Misc. Pub. No. 29. pp. 110-114.

Curtis E.J.C., Delves-Broughton J. and Harrington D.W. (1971) Sewage fungus studies of Sphaerotilus slimes using laboratory recirculating channels. Water Res. 5 : 267-279.

Daley R.J. and Hobbie J.E. (1975) Direct counts of aquatic bacteria by a modified epifluorescence technique. Limnol. Oceanogr. 20 : 875-882.

Dawes E.A. and Senior P.J. (1973) Energy Reserve Polymers in Micro-organisms. Adv. Microbial Physiology 10 : 135-266.

Dhople A.M. and Hanks J.H. (1973) Quantitative extraction of adenosine triphosphate from cultivable and host grown microbes calculation of adenosine triphosphate pools. Appl. Microbiol. 26 : 399-403.

Dietz A.S., Albright L.J. and Tuominen T. (1977) Alternative Model and Approach for Determining Microbial Heterotrophic Activities in Aquatic Systems. Appl. Environ. Microbiol. 33 : 817-823.

Doeglas D.J. (1968) Grain-size indices, classification and environment. Sedimentology 10 : 83-100.

Dondero N.C. (1961) Sphaerotilus, its nature and economic significance. Adv. Appl. Micro. 3 : 77-107.

Downes M.T. (1978a) An automated determination of low reactive phosphorus concentrations in natural waters in the presence of arsenic, silicon, and mercuric chloride. Water Res. 12 : 743-745.

Downes M.T. (1978b) An improved hydrazine reduction technique for the determination of low nitrate levels in fresh water. Water Res. 12 : 673-675.

Duddridge J.E. and Wainwright M. (1981) Enzyme activity measurements as a means of assessing a river sediment's capacity to degrade organic pollutants. Environ. Technol. Letters 2 : 75-80.

Duddridge J.E. and Wainwright M. (1982) Enzyme activity and kinetics in substrate-amended river sediments. Water Res. 16 : 329-334.

Edberg N. and Hofsten B. (1973) Oxygen uptake of bottom sediments Studied in situ and in the laboratory. Water Res. 7 : 1285-1294.

Edwards R.W. and Rolley H.L.J. (1965) Oxygen consumption of river muds. J. of Ecol. 53 : 1-19.

Elmore H.L. and West W.F. (1961) Effect of water temperature on stream reaeration. J. sanit. Engng Div., Am. Soc. civ. Engrs 87, SA6 : 59-71.

Ernst W. (1970) ATP als Indikator fur die Biomasse mariner Sedimente. Oecologia (Berl.) 5 : 56-60.

Escher A. and Characklis W.G. (1982) Algal-bacterial interactions within aggregates. Biotechnol. Bioeng. 24 : 2283-2290.

Ferguson R.L. and Murdoch M.B. (1975) Microbial ATP and organic carbon in sediments of the Newport river estuary, North Carolina. In : Estuarine Research, Vol. 1. pp. 229-250. L.E. Cronin (Ed.). New York : Academic Press.

Fillos J. and Molof A.H. (1972) Effect of benthal deposits on oxygen and nutrient economy of flowing waters. Journal WPCF 44 : 641-662.

Florek R.J. and Rowe G.T. (1983) Oxygen consumption and dissolved inorganic nutrient in marine coastal and shelf sediments of the Middle Atlantic Bight. Int. Rev. Ges. Hydrobiol. 68 : 73-112.

Fuhrman J.A. and Azam F. (1980) Bacterioplankton secondary production estimates for coastal waters of British Columbia, Antarctica and California. Appl. Environ. Microbiol. 39 : 1085-1095.

Gaudette H.E., Flight W.R., Toner L. and Folger D.W. (1974) An inexpensive titration method for the determination of organic carbon in recent sediments. J. Sed. Petrol. 44 : 249-253.

Gaudy A.F. and Gaudy E.T. (1981) Microbiology for Environmental Scientists and Engineers. McGraw-Hill pp. 736.

Geesey G.G., Mutch R., Costerton J.W. and Greene R.B. (1978) Sessile Bacteria : An important component of the microbial population in small mountain streams. Limnol. Oceanogr. 23 : 1214-1223.

Gillespie P.A. (1982) Investigation of the mechanism of oxygen depletion in the Tarawera River. "Aquatic Oxygen Seminar, Proceedings November 1980, Hamilton." Water and Soil Misc. Pub. 29, Ministry of Works and Development, Wellington, New Zealand.

Gocke K. (1977a) Heterotrophic Activity. In : Microbial Ecology of a Brackish Water Environment. Ed. G. Rheinheimer. Springer-Verlag Berlin.

Gocke K. (1977b) Comparison of methods for determining the turnover times of dissolved organic compounds. Mar. Biol. 42 : 131-141.

Goldhaber M.B., Aller R.C., Cochran J.K., Rosenfeld J.F., Martens C.S. and Berner R.A. (1977) Sulphate reduction, diffusion and bioturbation in Long Island Sound sediments : report of the FOAM Group. Am. J. Sci. 227 : 193-237.

Golterman H.T.; Sly, P.G. and Thomas, R.L. (1983) Study of the relationship between water quality and sediment transport. UNESCO, France.

Goulder R. (1980) Seasonal variation in heterotrophic activity and population density of planktonic bacteria in a clean river. J. Ecol. 68 : 349-363.

Graneli W. (1977) Measurement of sediment oxygen uptake in the laboratory using undisturbed sediment cores. Vatten 3 : 251-265.

Grimm N.B. and Fisher S.G. (1984) Exchange between interstitial and surface water: implications for stream metabolism and nutrient cycling. Hydrobiologia 111 : 219-228.

Hamilton R.D. and Holm-Hansen O. (1967) Adenosine triphosphate content of marine bacteria. Limnol. Oceanogr. 12 : 319-324.

Hargrave B.T. (1969a) Similarity of oxygen uptake by benthic communities. Limnol. Oceanogr. 14 : 801-805.

Hargrave B.T. (1969b) Epibenthic algal production and community respiration in the sediments of Marion Lake. J. Fish. Res. Bd. Canada 26 : 2003-2026.

Hargrave B.T. (1972a) A comparison of sediment oxygen uptake, hypolimnetic oxygen deficit and primary production in Lake Esrom, Denmark. Verh. Int. Verein. Limnol. 18 : 134-139.

Hargrave B.T. (1972b) Oxidation-reduction potentials, oxygen concentration and oxygen uptake of profundal sediments of a eutrophic lake. Oikos 23 : 167-177.

Hargrave B.T. and Phillips B.A. (1977) Oxygen uptake of microbial communities on solid surfaces. Aquatic Microbial Communities Cairns, J. Jr (ed.) Garland Publishing, New York.

Hargrave B.T. and Phillips G.A. (1981) Annual *in situ* carbon dioxide and oxygen flux across a subtidal marine sediment. Estuarine, Coastal and Shelf Science 12 : 725-737.

Harremoes P. (1976) The significance of pore diffusion to filter denitrification. J. Wat. Polut. Control Fed. 48 : 377-388.

Harremoes P. (1977) Half-order reactions in biofilm and filter kinetics. Vatten 2 : 122-143.

Harremoes P. (1982) Immediate and delayed oxygen depletion in rivers. Water Res. 16 : 1093-1098.

Hatcher K.J. and Hicks D. (Eds.) (1985) Sediment oxygen demand : Processes, Modelling, and Measurement. U.S. EPA Washington D.C. *in press*.

Herron J.S., King J.D. and White D.C. (1978) Recovery of poly-β-hydroxybutrate from estuarine microflora. *Appl. Environ. Microbiol*. 35 251-257.

Hickey C.W. (1985a) Chamber studies of benthic oxygen uptake kinetics in the Waiotapu River, New Zealand. In *Sediment oxygen demand : Processes, Modelling and Measurement* (K.J. Hatcher ed.) U.S. EPA, in press.

Hickey C.W. (1985b) Quantitative addition of dissolved oxygen to *in situ* benthic chamber systems by use of catalase and hydrogen peroxide. *Appl. Environ. Microbiol.* 49 : 462-464.

Hickey C.W. and Nagels J.W. (1985) Modifications to electrolytic respirometer systems for precise determination of BOD exertion kinetics in receiving waters. *Water Res*. 19 : 463-470.

Holm-Hansen O. and Booth C.R. (1966) The measurement of adenosine triphosphate in the ocean and its ecological significance. *Limnol. Oceanogr.* 11 : 510-519.

Holms W.H., Hamilton I.D. and Robertson A.G. (1972) The rate of turnover of the adenosine triphosphate pool of *Escherichia coli* growing aerobically in simple defined media. *Arch. Mikrobiol.* 83 : 95-109.

Hvitved-Jacobsen T. (1982) The impact of combined sewer overflows on the dissolved oxygen concentration of a river. *Water Res*. 16 : 1099-1105.

Itzhaki R.F. and Gill D.M. (1964) A micro-biuret method for estimating proteins. *Anal. Biochem.* 9 : 401-410.

James A. (1974) The measurement of benthal respiration. *Water Res*. 8 : 955-959.

James A. (1980) The role of bottom deposits in the oxygen regime of a polluted estuary. *Prog. Wat. Tech.* 12 : 491-498.

Jenkinson D.S. and Oades J.M. (1979) A method for measuring adenosine triphosphate in soil. *Soil Biol. Biochem*. 11 : 193-199.

Jeppesen E. (1982) Diurnal variation in the oxygen uptake of river sediments *in vitro* by use of continuous flow through systems. *Hydrobiologia* 91 : 189-195.

Jones J.G. (1971) Studies on freshwater bacteria: factors which influence the population and its activity. *J. Ecol.* 59 : 593-613.

Jones J.G. (1980) Some differences in the microbiology of profundal and littoral lake sediments. *J. Gen. Microbiol.* 117 : 285-292.

Jones J.G. and Simon B.M. (1977) Increased sensitivity in the measurement of ATP in freshwater samples with a comment on the adverse effect of membrane filtration. Freshwater Biology 7 253-260.

Jones J.G. and Simon B.M. (1979) The Measurement of Electron transport system activity in Freshwater benthic and planktonic samples. J. of Appl. Bact. 46 : 305-315.

Jones J.G. and Simon B.M. (1980) Decomposition processes in the profundal region of Btelham Tarn and the Lund tubes. J. Ecol. 68 : 493-512.

Jones J.G., Orlandi M.J.L.G. and Simon B.M. (1979) A microbiological study of sediments from the Cumbrian lakes. J. Gen. Microbiol. 115: 37-48.

Jorgensen B.B. (1977) The sulphur cycle of a coastal marine sediment. (Limfjorden, Denmark). Limnol. Oceanogr. 22 : 814-832.

Jorgensen B.B. and Revsbech, N.P. (1983) Colorless sulphur bacteria, Beggiatoa spp. and Thiovulum spp., in O_2 and H_2S microgradients. Appl. Environ. Microbiol. 45 : 1261-1270.

Jorgensen, B.B., Revsbech, N.P. and Cohen, Y. Photosynthesis and structure of benthic microbial mats: Microelectrode and SEM studies of four cyanobacterial communities. Limnol. Oceanogr. 28 : 1075-1093.

Karl D.M. (1978) Occurrence and ecological significance of GTP in the ocean and in microbial cells. Appl. Environ. Microbiol. 36 : 349-355.

Karl D.M. (1979a) Comments on a paper by J.G. Jones and B.M. Simon, 'Increased sensitivity in the measurement of ATP in freshwater samples with a comment on the adverse effect of membrane filtration'. Freshwater Biology 9 : 281-284.

Karl D.M. (1979b) Adenosine triphosphate and guanosine triphosphate determinations in intertidal sediments. In Methodology for Biomass Determinations and microbial activities in sediments. ASTM STP 673, Litchfield C.D. and Seyfried P.L. Eds.

Karl D.M. (1980) Cellular nucleotide measurements and applications in microbial ecology. Microbiological Reviews 44 : 739-796.

Karl D.M. and Craven D.B. (1980) Effects of alkaline phosphatase activity on nucleotide measurements in aquatic microbial communities. Appl. Environ. Microbiol. 40 : 549-561.

Karl D.M. and Holm-Hansen O. (1978) Methodology and measurement of adenylate energy charge ratios in environmental samples. Mar. Biol. 48 : 185-197.

Karl D.M. and La Rock P.A. (1975) Adenosine triphosphate measurement in soil and marine sediments. J. Fish. Res. Bd. Can. 32 : 599-607.

Karl D.M., Haugsness J.A., Campbell L. and Holm-Hansen, O. (1978) Adenine nucleotide extraction from multicellular organisms and beach sand ATP recovery, energy charge ratios and determination of carbon/ATP ratios. J. Exp. Mar. Biol. Ecol. 34 : 163-181.

Kepkay P.E. and Novitsky J.A. (1980) Microbial control of organic carbon in marine sediments coupled chemoautotrophy and heterotrophy. Mar. Biol. 55 : 261-266.

King J.D. and White D.C. (1977) Muramic acid as a measure of microbial biomass in estuarine and marine samples. Appl. Environ. Microbiol. 33 : 777-783.

Kissel J.C., McCarty P.L., and Street R.L. (1984) Numerical simulation of mixed-culture biofilm. J. envir. Engng Div., Am. Soc. Civ. Engrs. 110 EE2 : 393-411.

Kornegay B.H. and Andrews J.F. (1968) Kinetics of fixed-film biological reactors. J. Wat. Polut. Control Fed. 40 : R460-R468.

Ladd T.I., Costerton J.W. and Geesey G.G. (1979) Determination of the heterotrophic activity of epilithic microbial populations. In : 'Native Aquatic Bacteria : Enumeration, Activity and Ecology'. ASTM STP 695. J.W. Costerton and R.R. Colwell Eds., pp. 180-195.

Ladd T.I., Ventullo R.M., Wallis P.M. and Costerton J.W. (1982) Heterotrophic activity and biodegradation of labile and refractory compounds by groundwater and stream microbial populations. Appl. Environ. Microbiol. 44 : 321-329.

La Motta E. (1976a) Kinetics of growth and substrate uptake in a biological film system. Appl. Environ. Microbiol. 31 : 286-293.

La Motta E.J. (1976b) Internal diffusion and reaction in biological films. Environ. Sci. & Technol. 10 : 765-769.

La Motta E. (1976c) External mass transfer in a biological film reactor. Biotechnol. Bioeng. 18 : 1359-1370.

Lasserre P. (1976) Metabolic activities of benthic microfauna and meiofauna. In The Benthic Boundary Layer. McCave I.N. ed. Plenurn Publishing, New York.

Lau Y.L. (1975) An experimental investigation of reaeration in open-channel flow. Prog. Water Technol. 7 : 519-530.

Lee C.C., Harris R.F., Williams J.D., Armstrong D.E. and Syers J.K. (1971) Adenosine triphosphate in lake sediments : 1. Determinations. Soil Sci. Am. Proc. 35 : 82-86.

Lee E.J., De Witt K.J., Bennett G.F. and Brockwell J.L. (1976) Investigation of oxygen transfer to slime as a surface reaction. Water Res. 10 1011-1017.

Leeder M.R. (1982) Sedimentology : processes and product. Allan and Unwin, London.

Liepmann H.W. (1979) The rise and fall of ideas in turbulence. American Scientist 67 : 221-228.

Lock M.A. and John P.H. (1979) The effect of flow patterns on uptake of phosphorus by river periphyton. Limnol. Oceanogr. 24 : 376-383.

Lock M.A., Wallace R.R., Costerton J.W., Ventullo R.M. and Charlton S.E. (1984) River epilithon : toward a structural-functional model. Oikos 42 : 10-22.

Lorenzen C.J. (1967) Determination of chlorophyll and pheo-pigments : spectrophotometric equations. Limnol. Oceanogr. 12 : 343-346.

Lundin A. and Thore A. (1975a) Comparison of methods for extraction of bacterial adenine nucleotides determined by firefly assay. Appl. Microbiol. 30 : 713-721.

Lundin A. and Thore A. (1975b) Analytical information obtainable by evaluation of the time course of firefly bioluminescence in the assay of ATP. Anal. Biochem. 66 : 47-63.

MacCaffrey R.J., Myers A.C., Davey E., Morrisson G., Bender M., Luedtke N., Cullen D., Froelich P. and Klinkhammer G. (1980) The relation between pore water chemistry and benthic fluxes of nutrients and manganese in Narragansett Bay, Rhode Island. Limnol. Oceanogr. 25 : 31-44.

Martin D.C. and Bella D.A. (1971) Effect of mixing on oxygen uptake rate of estuarine bottom deposits. J. Water Pollut. Control Fed. 43 : 1865-1876.

Matai K.E. and Haase C. (1977) Some factors affecting photosynthesis and respiration measurements in Cladophora glomerata. J. Great Lakes Res. 3: 191-195.

Matson J.V. and Characklis W.G. (1976) Diffusion into microbial aggregates. Water Res. 10 : 877-885.

McBride G.B. (1982) Tarawera River DO Models. In "Aquatic oxygen seminar proceedings, Hamilton, November 1980". Water and Soil Misc. Pub. No. 29. pp. 129-133.

McBride G.B. (1985) Prediction of river benthic oxygen uptake rate. Water Quality Centre, Hamilton. Internal Report No. 85/

McBride G.B. and Rutherford J.C. (1982) Waipa River. In "Aquatic oxygen seminar proceedings, Hamilton, November 1980". Water and Soil Misc. Pub. No. 29. pp. 144-152.

McCave I.N. (Ed) (1976) The Benthic Boundary Layer. Plenurn Publishing, New York.

McIntyre C.D. (1966) Some effects of current velocity on periphyton communities in laboratory systems. Hydrobiologia 27 : 559-570.

McKenzie L.R. and McBride G.B. (1982) Mataura River. In "Aquatic oxygen seminar proceedings, Hamilton, November 1980". Water and Soil Misc. Pub. No. 29. pp. 99-109.

McKeown J.J. (1963) The control of Sphaerotilus natans by a Southern Kraft mill. Part 2 - Intermittent discharge of strong wastes. Industrial Water and Wastes 8 : 30-33.

McQuivey R.S. (1973) Summary of turbulence data from rivers, conveyance channels, and laboratory flumes. U.S. Geol. Surv. Prof. Pap. 802-B.

McQuivey R.S. and Richardson E.V. (1969) Some turbulence measurements in open channel flow. J. hydr. Engng Div., Am. Soc. civ. Engrs 95 : 209-223.

Meyer-Reil L.A. (1978a) Autoradiography and epifluorescence microscopy combined for the determination of number and spectrum of actively metabolizing bacteria in natural waters. Appl. Environ. Microbiol. 36 : 506-512.

Meyer-Reil L.A. (1978b) Uptake of glucose by bacteria in the sediment. Mar. Biol. 44 : 293-298.

Meyer-Reil L.A. (1981) Enzymatic decomposition of proteins and carbohydrates in marine sediments : methodology and field observations during spring. Kieler Meeresforsch., Sonderh. 5 : 311-317.

Meyer-Reil L.A., Bolter M., Liebezeit G. and Schramm W. (1979) Short-term variations in microbiological and chemical parameters. Mar. Ecol. Prog. Ser. 1 : 1-6.

Meyer-Reil L.A., Bolter M., Dawson R., Liebezeit G., Szerinski H. and Wolter K. (1980) Interrelationships between microbial and chemical parameters of sandy beach sediments, a summer aspect. Appl. Environ. Microbiol. 39 : 797-802.

Meyer-Reil L.A., Dawson R., Liebezeit G., and Tiedge H. (1978) Fluctuations and interactions of bacterial activity in sandy sediments and overlying waters. Mar. Biol. 48 : 161-171.

Miller C.A., and Vogelhut P.O. (1978) Chemiluminescent Detection of Bacteria Experimental and Theoretical Limits. Appl. Environ. Microbiol. 35 : 813-816.

Miozzari G.F., Niederberger P. and Hutter R. (1978) Permeabilization of Microorganisms by Triton X-100. Anal. Biochem. 90 : 220-233.

Monheimer R.H. (1974) Sulphate Uptake as a measure of planktonic microbial production in freshwater ecosystems. Can. J. Microbiol. 20 : 825-831.

Monod J. (1942) Recherches sur la Croissance des Cultures Bacteriennes. Hermann and Gie Paris.

Monod J. (1949) Growth of bacteria cultures. Ann. Rev. Microbiol. 3 : 371.

Moriarty D.J.W. (1975) A method for estimating the biomass of bacteria in aquatic sediments and its application in trophic studies. Oecologia (Berlin) 20 : 219-229.

Moriarty D.J.W. (1978) Estimation of bacterial biomass in water and sediments using muramic acid. In Microbial Ecology. Ed. Loutit M.W. and Miles J.A.R., Springer Verlag, Berlin.

Mountfort D.O., Asher R.A., Mays E.I., and Tiedje J.M. (1980) Carbon and electron flow in mud and sandflat intertidal sediments at Delaware Inlet, Nelson, New Zealand. Appl. Environ. Microbiol. 39 : 686-694.

Nagell B. (1973) The oxygen consumption of mayfly (Ephemeroptera) and stonefly (Plecoptera) larvae at different oxygen concentration. Hydrobiologia 42 : 461-489.

Naiman R.J. and Sedell J.R. (1979) Benthic organic matter as a function of stream order in Oregon. Arch. Hydrobiol. 87 : 404-422.

Neijssel O.M. and Tempest D.W. (1976) The role of energy-spilling reactions in the growth of Klebsiella aerogenes NCTC 418 in aerobic chemostat culture. Arch. Microbiol. 110 : 305-311.

Nickels J.S., King J.D. and White D.C. (1979) Poly-B-hydroxybutrate accumulation as a measure of unbalanced growth of the estuarine detrital microbiota. Appl. Environ. Microbiol. 37 : 459-465.

Novitsky J.A. and Kepkay P.E. (1981) Patterns of microbial heterotrophy through changing environments in a marine sediment. Mar. Ecol. Prog. Ser. 4 : 1-7.

O'Connor D.J. and Dobbins W.E. (1958) Mechanism of reaeration in natural streams. Trans. Am. Soc. Civ. Engrs 123 : 641-684.

Odum H.T. (1956) Primary production in flowing waters. Limnol. Oceanogr. 1 102-117.

Onuma M. and Omura T. (1982) Mass-transfer characteristics within microbial systems. Water Science and Technology 14 : 553-568.

Owens M. and Maris P.J. (1964) Some factors affecting the respiration of some aquatic plants. Hydrobiologia 23 : 533-543.

Paerl H.W., Tilzer M.M. and Goldman C.R. (1976) Chlorophyll A versus adenosine triphosphate as algal biomass indicators in Lakes. J. Phycol. 12 : 242-246.

Pamatmat M.M. and Banse K. (1969) Oxygen consumption by the sea bed - II. In situ measurements to a depth of 180 m. Limnol. Oceanogr. 14 : 250-259.

Pamatmat M.M. (1971) Oxygen consumption by the sea bed 4. Shipboard and laboratory measurements. Limnol. Oceanogr. 16 : 536-550.

Pamatmat M.M. and Skjoldal H.R. (1974) Dehydrogenase activity and adenosine triphosphate concentration of marine sediments in Lindapollene, Norway. Sarsia 56 : 1-11.

Parsons T.R., and Strickland J.D.H. (1963) Discussion of spectrophotometric determination of marine plant pigments, with revised equations for ascertaining chlorophylls and carotenoids. J. Mar. Res. 21 : 155-163.

Payne W.J. (1970) : Energy yields and growth of heterotrophs. Ann. Rev. Microbiol. 24 : 17-52.

Pennak R.W. and Lavelle J.W. (1979) In situ measurements of net primary production in a Colorado mountain streams. Hydrobiologia 66 : 227-235.

Phelps E.B. (1912) Biochemistry of Sewage. V International Congress of Applied Chemistry 26 : 251.

Piper M. (1982) Dissolved oxygen depletion of the Tarawera River In "Aquatic oxygen seminar proceedings, Hamilton, November 1980". Water and Soil Misc. Pub. No. 29. pp. 123-128.

Pirt S.J. (1975) Principles of Microbe and Cell Cultivation. Blackwell Scientific Publications, Oxford.

Polak J. and Haffner G.D. (1978) Oxygen depletion of Hamilton Harbour. Water Res. 12 : 205-215.

Pomroy A.J., Joint I.R., and Clarke K.R. (1983) Benthic nutrient flux in a shallow coastal environment. Oecologia (Berlin) 60 : 306-312.

Pridmore R.D., Cooper A.B. and Hewitt J.E. (1984) ATP as a biomass indicator in eight North Island lakes, New Zealand. Freshwater Biology 14 : 73-78.

Pridmore R.D., Hickey C.W. and Hewitt J.E. (1982) The effect of membrane filtration on estimates of microbial ATP in New Zealand freshwaters. N.Z. Jl. Mar. Freshwat. Res. 16 : 311-315.

Rausch T. (1981) The estimation of micro-algal protein content and its meaning to the evaluation of algal biomass I : Comparison of methods for extracting protein. Hydrobiologia 78 : 237-251.

Revsbech N.P., and Ward D.M. (1983) Oxygen electrode that is insensitive to medium chemical composition : use in an acid microbial mat dominated by Cyanidium caldarium. Appl. Environ. Microbiol. 45, 755-759.

Revsbech N.P., Jorgensen B.B., and Blackburn T.H. (1980) Oxygen in the seabottom measured with microelectrode. Science 207 : 1355-1356.

Revsbech N.P., Jorgensen B.B., and Brix O. (1981) Primary production of microalgae in sediments measured by oxygen microprofile, $H^{14}CO_3^-$ fixation and oxygen exchange methods. Limnol. Oceanogr. 26 : 717-730.

Revsbech N.P., Sorensen J., Blackburn T.H. and Lomholt J.P. (1980) Distribution of oxygen in marine sediments measured with micro electrodes. Limnol. Oceanogr. 25 : 403-411.

Riedl R.J., and Machan R. (1972) Hydrodynamic patterns in lotic intertidal sands and their bioclimatological implications. Mar. Biol. 13 : 179-209.

Riedl R.J., Huang N., and Machan R. (1972) The subtidal pump : a mechanism of interstitial water exchange by wave action. Mar. Biol. 13 : 210-221.

Rittmann B.E. and McCarty P.L. (1980a) Model of Steady-State-Biofilm Kinetics. Biotechnol. Bioeng. 22 : 2343-2357.

Rittmann B.E. and McCarty P.L. (1980b) Evaluation of Steady-state-Biofilm Kinetics. Biotechnol. Bioeng. 22 : 2359-2373.

Rittmann B.E. and McCarty P.L. (1981) Substrate flux into Biofilms of any thickness. J. envir. Engng Div., Am. Soc. Civ. Engrs 107 (EE4) : 831-849.

Robertson W.S. (1980) The role of the cobalt catalyst in the unsteady state test. J. Wat. Pollut. Control 51 : 487-495.

Rogers J.H. and Harvey R.S. (1976) The effect of current on periphytic productivity as determined using carbon-14. Wat. Res. Bull 12 : 1109-1118.

Rounick J.S. and Winterbourn M.J. (1983) The formation, structure and utilization of stone surface organic layers in two New Zealand streams. Freshwater Biology 13 : 57-72.

Rutherford J.C. (1982) Waikato River DO simulations. In 'Aquatic oxygen seminar proceedings, Hamilton, November 1980'. Water and Soil Misc. Pub. No. 29. pp. 115-122.

Rutherford J.C. and O'Sullivan M.J. (1974) Simulation of water quality in the Tarawera River. J. envir. Engng Div., Am. Soc. Civ. Engrs, ASCE, Vol. 100, No. EE2, Proc. pp. 369-390.

Sanders W.M. (1966) Oxygen utilization by slime organisms in continuous cultures. Air & Water Poll. Intl. Jour. (G.B.) 10 : 253.

Sapienza C. and Mague T.H. (1979) DNA polymerase activity and growth rates in Artemia salina nauplii. Limnol. Oceanogr. 24 : 572-576.

Servais P., Debecker E. and Billen G. (1984) Annual cycle of gross primary production and respiration in the Viroin River (Belgium). Hydrobiologia 111 : 57-63.

Simkins S. and Alexander M. (1984) Models for mineralization kinetics with the variables of substrate concentration and population density. Appl. Environ. Microbiol. 47 : 1299-1306.

Simmons G.M. Jr., Wharton R.A. Jr., Parker B.C. and Andersen D. (1983) Chlorophyll a and adenosine triphosphate levels in antarctic and temperate lake sediments. Microb. Ecol. 9 : 123-135.

Simonsen J.F. and Harremoes P. (1978) Oxygen and pH fluctuations in rivers. Water Res. 12 : 477-489.

Smith I.R. (1975) Turbulence in Lakes and Rivers. Freshwater Biological Association, Scientific Publication No. 29.

Smith L.K. and Fisher T.R. (1984) Sediment oxygen demand and nutrient fluxes associated with the sediment-water interface. In : Sediment oxygen demand : processes, modelling, and measurement. Hatcher K.J. and Hicks D. (Eds) EPA, Washington D.C.

Sonnleitner B., Heinzle E., Braunegg G. and Lafferty R.M. (1979) Formal Kinetics of Poly-B-Hydroxybutyric acid (PHB) production in Alcaligenes eutrophus H and Mycoplana ruba R14 with respect to the dissolved oxygen tension in ammonium-limited batch cultures. Eur. J. Appl. Microbiol. Biotech. 7 : 1-10.

Sonzogni W.C., Larson D.P., Malueg K.W. and Schuldt M.D. (1977) Use of large submerged chambers to measure sediment-water interactions. Water Res. 11 : 461-464.

Spencer M.J. and Ramsay A.J. (1978) Bacterial populations, heterotrophic potentials, and water quality in three New Zealand rivers. N.Z. Jl. Mar. Freshwat. Res. 12 : 415-427.

Steele J.H., Munro A.L.S., and Giese G.S. (1970) Environmental factors controlling the episammic flora on beach and sublittoral sands. J. Mar. Biol. Ass. U.K. 50 : 907-918.

Stevenson L.H. (1978) A case for bacterial dormancy in aquatic systems. Microbial Ecology 4 : 127-133.

Streeter H.W. and Phelps E.B. (1925) A study of the pollution and natural purification of the Ohio River. Bulletin No. 146. U.S. Public Health Service.

Tabatabai M.A. and Bremner J.M. (1970) Arylsulphatase activity in soils. Soil Sci. Soc. Amer. Proc. 34 : 225-229.

Tabor P.S. and Neihof R.A. (1982) Improved method for determination of respiring individual microorganisms in natural waters. Appl. Environ. Microbiol. 43 : 1249-1255.

Teal T.M. and Kanwisher J. (1961) Gas exchange in a Georgia salt marsh. Limnol. Oceanogr. 6 : 388-399.

Technicon Corporation (1973) Ammonia in water and seawater. Industrial Method No. 154-71W/Tentative.

Tempest D.W. and Neijssel O.M. (1978) Eco-physiological aspects of microbial growth in nutrient-limited environments. Advances in Microbiological Ecology 2 : 105-153.

Tobin R.S. and Anthony D.H.J. (1978) Tritiated thymidine incorporation as a measure of microbial activity in lake sediments. Limnol. Oceanogr. 23 : 161-165.

Traaen T.S. (1975) Biological effects of primary, secondary and tertiary sewage treatment in lotic analog recipients. Verh. Int. Verin. Limnol. 19 : 2064-2069.

Traaen T.S., Ormerod K.S. and Efraimsen H. (1972) Heterotrophic growth in indoor research channels; self-purification and production. Verh. Int. Verin. Limnol. 18 : 891-895.

Trulear M.G. and Characklis W.G. (1982) Dynamics of biofilm processes. J. Wat. Polut. Control Fed., 54 : 1288-1301.

Vanderborght J.P., Wollast R., and Billen G. (1977a) Kinetic models of diagenesis in disturbed sediments. Part 1. Mass transfer properties and silica diagenesis. Limnol. Oceanogr. 22 : 787-793.

Vanderborght J.P., Wallast R., and Billen G. (1977b) Kinetic models of diagenesis in disturbed sediments. Part 2. Nitrogen diagenesis. Limnol. Oceanogr. 22 : 794-803.

Velz C.J. (1970) Applied Stream Sanitation. Wiley-Interscience, New York.

Ventullo R.M. and Rowe J.J. (1982) Denitrification potential of epilithic communities in a lotic environment. Current Microbiology 7 : 29-33.

Verstraete W., Voets J.P., and van Lancker P. (1976) Evaluation of some enzymatic methods to measure the bio-activity of aquatic environments. Hydrobiologia 49 : 257- 266.

Vollenweider R.A. (Ed.) (1974) A Manual on Methods for Measuring Primary Production in Aquatic Environments. IBP Handbook No. 12 Blackwell Scientific Publications, London.

Wang W. (1980) Fractionation of sediment oxygen demand. Water Res. 14 : 603-612.

Watson S.W., Noitsky T.J., Quinby H.L. and Valais F.W. (1977) Detection of bacterial numbers and biomass in the marine environment. Appl. Environ. Microbiol. 33 : 940-946.

Webb J.E. and Theodor J.L. (1968) Wave induced circulation submerged sands through wave action. Nature 220 : 682-683.

Webb J.E. and Theodor J.L. (1972) Wave induced circulation in submerged sands. J. Mar. Biol. Ass. U.K. 52 : 903-914.

Weise W. and Rheinheimer G. (1978) Scanning Electron Microscopy and Epifluorescence Investigation of Bacterial Colonization of Marine Sand Sediments. Microbiol. Ecol. 4 : 175-188.

Wezernak C.T. and Gannon J.J. (1968) Evaluation of nitrification in streams. J. San. Eng. Div. (ASCE) 94 : 883-895.

White D.C., Bobbie R.J., Morrison S.J., Oosterhof D.K., Taylor C.W. and Meeter D.A. (1977) Determination of microbial activity of estuarine detritus by relative rates of lipid biosynthesis. Limnol. Oceanogr. 22 : 1089-1099.

Whitman R.L. and Clark W.J. (1982) Availability of dissolved oxygen in interstitial waters of sandy creek. Hydrobiologia 92 : 651-656.

Whittemore R.C. (1984) The significance of interfacial water velocity n the exertion of sediment oxygen demand. In Sediment Oxygen Demand : Processes, Modelling and Measurement. Hatcher K.J. and Hicks D. (Eds) U.S. EPA, in press.

Wiebe W.J. and Bancroft K. (1975) Use of adenylate energy charge ratio to measure growth state of natural microbial communities. Proc. Natl. Acad. Sci. USA 72 :2112-2115.

Wilcock R.J. (1982) Simple predictive equations for calculating stream reaeration rate coefficients. N.Z. Jl Sci. 25 : 53-56.

Wilcock R.J. (1984a) Methyl chloride as a gas tracer for measuring stream reaeration coefficients. I. Laboratory studies. Water Res. 18 : 47-52.

Wilcock R.J. (1984b) Methyl chloride as a gas tracer for measuring stream reaeration. II. Stream studies. Water Res. 18 : 53-57.

Wilcock R.J. (1984c) Reaeration studies on some New Zealand rivers using methyl chloride as a gas tracer. In Gas Transfer at Water Surfaces (W. Brutsaert and G.H. Jirka eds) : 413-420. D. Reidel Publishing Company.

Wilhelm E., Battino R., and Wilcock R.J. (1977) Low-pressure solubility of gases in liquid water. Chem. Rev. 77 : 219-262.

Williams D.D. and Hynes H.B.N. (1974) The occurrence of benthos deep in the substratum of a stream. Freshwat. Biol. 4 : 233-251.

Williams P.J. (1973) The validity of application of simple kinetic analysis to heterogenous microbial populations. Limnol. Oceanogr. 18 : 159-165.

Williamson K. and McCarty, P.L. (1976a) A model of substrate utilization by bacterial films. J. Wat. Polut. Control Fed. 49 : 9-24.

Williamson K. and McCarty P.L. (1976b) Verification studies of the biofilm model for bacterial substrate utilization. J. Wat. Polut. Control Fed. 48 : 281-296.

Wilson G.T. and Macleod N. (1974) A critical appraisal of empirical equations and models for the prediction of the coefficient of reaeration of deoxygenated water. Water Res. 8 : 341-366.

Winterbourn M.J. (1976) Fluxes of litter falling into a small beech forest stream. N.Z. Jl. Mar. Freshwat. Res. 10 : 399-416.

Wood L.W., and Chua K.E. (1978) Method for studying amino acid flux at the mud-water interface. J. Great Lakes Res. 31 : 29-37.

Wright R.T. (1971) Some difficulties in using 14 C-organic solutes to measure heterotrophic bacterial activity. In *Estuarine Microbial Ecology*. Ed. Stevenson L.H. and Colwell R.R. University of South Carolina Press.

Wright R.T. (1978) Measurement and significance of specific activity in the heterotrophic bacteria of natural waters. *Appl. Environ. Microbiol*. 36 : 297-305.

Wright R.M. and McDonnell A.J. (1979) In-stream deoxygenation rate prediction. *J. Environ. Eng. Div., Proc. Am. Soc. Civ. Eng., ASCE* 105 : 323-335.

Wuhrmann K. (1972) Stream purification. In *Water Pollution Microbiology* (R. Mitchell, ed.) Wiley-Interscience, New York.

Wuhrmann K. (1974) Some problems and perspectives in applied limnology. *Verh. Int. Verin. Limnol*. 20 : 324-402.

Wuhrmann K., Ruchti J. and Eichenberger E. (1967) Quantitative experiments on self-purification with pure organic compounds. *Advances in Water Pollution Research*. Proceedings 3rd International Conference 1 p 229.

Wyndham R.C. and Costerton J.W. (1981) Heterotrophic potentials and hydrocarbon biodegradation potentials of sediment microorganisms within the Athabasca Oil sands deposit. *Appl. Environ. Microbiol.* 41 : 783-790.

Yingst J.Y. (1978) Patterns of micro- and meiofaunal abundance in marine sediments, measured with the adenosine triphosphate assay. *Mar. Biol*. 47 : 41-54.

Zeitzschel B. (1979) Sediment-water interactions in nutrient dynamics. In : *Marine Benthic Dynamics* Tenore K.R. and Coull B.C. (Eds.) University of South Carolina Press.

Zelver N. (1979) Quoted in Characklis *et al.* (1982).

Zimmerman A.P. (1975) Electron transport analysis as an indicator of biological oxidations in freshwater sediments. *Verh. Int. Verein. Limnol.* 19 : 1518-1523.

Zimmerman R. and Meyer-Reil L. (1974) A new method for fluorescence staining of bacterial populations on membrane filters. *Kiel. Meeresforsch* 30 : 24-27.

Zimmermann R., Hurriaga R. and Becker-Birck J. (1978) Simultaneous Determination of the Total number of aquatic bacteria and the number thereof involved in respiration. *Appl. Environ. Microbiol*. 36 : 926-935.

APPENDICES

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Appendix 1: Method Development

1.1 Waitoa River sediments: effect of stirring on intrinsic activity

Station: Landsdowne Road (above discharge)
Date: 16 December 1981
Method: Replicate sediment samples incubated at 20 °C in dark with GF/C filtered water from the station in a 300 ml BOD bottle, for 2 h. 'Unstirred' was measured at ½ h intervals. Glucose was added to a final concentration of 20 g m^{-3}. All results shown are significant ($P<0.05$).

Sediment (DW)	Intrinsic Activity $\mu g\ O_2\ g\ DW^{-1}\ h^{-1}$		
	Unstirred	Stirred	Δ% with stirring
12 g	10.0	15.3	+53
12 g (glucose)	11.7	18.1	+55
Δ% with glucose	+15	+19	
24 g	10.6	17.2	+61
24 g (glucose)	11.8	22.9	+51
Δ% with glucose	+11	+25	

1.2 Waitoa River sediments: effect of temperature on intrinsic activity

Station:
i Landsdowne Road (-1 km, above discharge)
ii Waharoa (0 km)
iii Hockley (2 km below discharge)
iv Signals (5 km)
v Walton (10 km)

Methods: Replicate sediment samples (12 g DW) were incubated in the dark with BOD dilution water supplemented with glucose (20 g m^{-3}) in BOD bottles, and continuously stirred for 1 h. Samples were allowed 0.5 h temperature incubation prior to measurement (mean ± 1 SD).

Temperature °C	Intrinsic Activity $\mu g\ O_2\ g\ DW^{-1}\ h^{-1}$				
	Landsdowne	Waharoa	Hockley	Signals	Walton
10	1.56±0.42	3.21±0.60	6.52±0.20	5.86±1.20	2.46±1.0
15	2.01±0.45	4.94±0.41	11.19±1.40	7.97±0.90	4.66±0.91
20	2.78±0.05	7.16±0.90	12.40±2.10	13.42±0.92	7.67±0.41
25	4.04±0.31	10.73±1.13	20.78±0.11	17.60±0.27	10.67±0
Arrhenius coefficient	1.030	1.082	1.074	1.079	1.103
r^2	0.992	0.999	0.946	0.985	0.981

Mean Arrhenius Coefficient of 1.074

1.3 Waiotapu River sediments: effect of stirring on intrinsic activity

Station: Homestead Road
Date: 24 June 1983
Method: Various volumes (8.5 ml caps) and stirring treatments used to obtain intrinsic activity measurements on replicate samples. Regression slopes on first 3 points or for 2 g m^{-3} DO consumed. Error generally less than ±15%.

Agitation	Intrinsic Activity $\mu g\ O_2\ g\ DW^{-1}\ h^{-1}$		
	2 caps	4 caps	8 caps
Continuous	16.1	16.0	17.8
¼ hourly	12.3	10.7	13.9
½ hourly	9.5	11.5	7.5
1 hourly	5.7	7.9	6.0

Summary: Very sensitive to agitation with even ¼ hourly agitation resulting in a 24% decline in measured activity (2 caps)

1.4 ATP extraction techniques for sediments

Three techniques for sediment ATP exraction were evaluated: (i) trichloroacetic acid (TCA) (Jenkinson and Oades, 1979); (ii) cold sulphuric acid (Karl and LaRock, 1975); and (iii) boiling McIlaine buffer (Bullied, 1978).

The two former methods were found to give more variable extractions with greater interferences to assays than the boiling buffer technique as illustrated below. The boiling buffer technique was only found to be effective if maintained at boiling point using a very hot sand bath for extractions.

Extract	$\bar{x}$ $\mu g\ g^{-1}$	s	n	%	Measured and corrected inhibition (%)
Fine sand					
McIlvaine	0.165	0.020	4	12	0
Acid	0.173	0.010	4	6	13
Coarse sand					
McIlvaine	0.220	0.042	7	19	0
Acid	0.116	0.033	8	29	0

APPENDIX 2: WAITOA RIVER

2.1 Planktonic BOD and COD measurements

DATE	SITE	FLOW	TEMP	BOD5	BOD20	COD	CODF	BOD:COD
810901.00	-1.00	2.34	UNDEF	UNDEF	UNDEF	UNDEF	UNDEF	UNDEF
810901.00	0.0	2.34	UNDEF	UNDEF	UNDEF	UNDEF	UNDEF	UNDEF
810901.00	2.00	2.34	UNDEF	UNDEF	UNDEF	UNDEF	UNDEF	UNDEF
810901.00	5.00	2.34	UNDEF	UNDEF	UNDEF	UNDEF	UNDEF	UNDEF
810901.00	10.00	2.34	UNDEF	UNDEF	UNDEF	UNDEF	UNDEF	UNDEF
810901.00	15.00	2.34	UNDEF	UNDEF	UNDEF	UNDEF	UNDEF	UNDEF
811012.00	1.00	1.11	16.50	UNDEF	UNDEF	UNDEF	9.20	UNDEF
811012.00	0.0	1.11	20.00	UNDEF	UNDEF	UNDEF	13.80	UNDEF
811012.00	2.00	1.11	17.50	UNDEF	UNDEF	UNDEF	9.90	UNDEF
811012.00	5.00	1.11	19.00	UNDEF	UNDEF	UNDEF	21.10	UNDEF
811012.00	10.00	1.11	17.50	UNDEF	UNDEF	UNDEF	14.60	UNDEF
811012.00	15.00	1.11	UNDEF	UNDEF	UNDEF	UNDEF	12.60	UNDEF
811112.00	-1.00	0.84	17.00	1.30	3.60	UNDEF	29.50	UNDEF
811112.00	0.0	0.84	18.70	20.80	35.70	UNDEF	20.00	UNDEF
811112.00	2.00	0.84	18.20	31.00	45.50	UNDEF	40.50	UNDEF
811116.00	-1.00	5.07	UNDEF	8.60	14.30	UNDEF	UNDEF	UNDEF
811116.00	0.0	5.09	UNDEF	20.00	UNDEF	UNDEF	UNDEF	UNDEF
811116.00	2.00	5.09	UNDEF	11.30	22.70	UNDEF	UNDEF	UNDEF
811116.00	5.00	5.09	UNDEF	14.80	26.50	UNDEF	UNDEF	UNDEF
811116.00	10.00	5.09	UNDEF	11.60	18.30	UNDEF	UNDEF	UNDEF
811120.00	-1.00	5.09	UNDEF	3.10	UNDEF	UNDEF	UNDEF	UNDEF
811120.00	0.0	5.09	UNDEF	5.40	UNDEF	UNDEF	UNDEF	UNDEF
811120.00	2.00	5.09	UNDEF	12.40	UNDEF	UNDEF	UNDEF	UNDEF
811202.00	-1.00	1.56	UNDEF	1.87	6.45	UNDEF	UNDEF	UNDEF
811202.00	0.0	1.56	UNDEF	1.93	7.88	UNDEF	UNDEF	UNDEF
811202.00	10.00	1.56	UNDEF	2.10	7.30	UNDEF	UNDEF	UNDEF
811214.00	-1.00	1.56	UNDEF	0.90	UNDEF	UNDEF	UNDEF	UNDEF
811215.00	-1.00	1.26	18.50	0.90	UNDEF	UNDEF	UNDEF	UNDEF
811215.00	0.0	1.26	19.00	UNDEF	UNDEF	UNDEF	UNDEF	UNDEF
811215.00	2.00	1.26	20.50	UNDEF	UNDEF	UNDEF	UNDEF	UNDEF
811215.00	5.00	1.26	UNDEF	5.60	UNDEF	UNDEF	UNDEF	UNDEF
811215.00	10.00	1.26	20.00	2.20	UNDEF	UNDEF	UNDEF	UNDEF
811216.00	-1.00	1.13	20.50	1.60	2.90	UNDEF	32.50	UNDEF
811216.00	0.0	1.13	21.00	5.15	6.75	UNDEF	11.50	UNDEF
811216.00	10.00	1.13	21.00	4.25	5.00	UNDEF	18.50	UNDEF
820111.00	-1.00	0.58	21.00	0.74	UNDEF	UNDEF	13.00	0.06
820111.00	0.0	0.58	26.00	20.90	UNDEF	UNDEF	29.50	0.71
820111.00	2.00	0.58	24.00	7.50	UNDEF	UNDEF	23.00	0.33
820111.00	5.00	0.58	24.00	6.40	UNDEF	UNDEF	14.50	0.44
820111.00	10.00	0.58	24.00	8.70	UNDEF	UNDEF	23.50	0.37
820113.00	-1.00	0.45	23.20	0.50	1.85	UNDEF	UNDEF	UNDEF
820113.00	0.0	0.45	23.50	3.90	7.60	UNDEF	5.00	UNDEF
820113.00	10.00	0.45	23.20	2.90	7.90	UNDEF	12.50	UNDEF
820119.00	-1.00	0.36	UNDEF	1.20	UNDEF	UNDEF	18.00	UNDEF
820127.00	-1.00	0.33	20.00	UNDEF	UNDEF	UNDEF	UNDEF	UNDEF
820127.00	0.0	0.33	26.50	6.60	UNDEF	UNDEF	58.80	0.11
820127.00	2.00	0.33	20.50	4.70	UNDEF	UNDEF	50.00	0.09
820127.00	5.00	0.33	22.00	13.30	UNDEF	UNDEF	63.20	0.21
820127.00	10.00	0.33	21.00	2.80	UNDEF	UNDEF	46.70	0.06
820210.00	-1.00	0.26	19.50	2.10	UNDEF	UNDEF	33.80	0.06
820210.00	0.0	0.26	20.50	13.30	UNDEF	UNDEF	41.10	0.32
820210.00	2.00	0.26	20.00	27.90	UNDEF	UNDEF	52.40	0.53
820210.00	5.00	0.26	23.00	10.95	UNDEF	UNDEF	43.60	0.25
820210.00	10.00	0.26	21.25	2.85	UNDEF	UNDEF	44.40	0.06
820422.00	-1.00	0.56	13.50	0.20	UNDEF	UNDEF	11.40	0.02
820422.00	0.0	0.56	13.50	1.40	UNDEF	UNDEF	15.50	0.09
820422.00	2.00	0.56	14.00	1.13	UNDEF	UNDEF	12.50	0.09
820422.00	5.00	0.56	15.00	0.35	UNDEF	UNDEF	9.50	0.04
820422.00	10.00	0.56	14.50	0.20	UNDEF	UNDEF	11.70	0.02
820609.00	-1.00	1.17	6.50	1.71	UNDEF	14.20	9.90	UNDEF
820609.00	0.0	1.17	7.00	6.66	UNDEF	40.50	34.80	UNDEF
820609.00	2.00	1.17	7.50	1.33	UNDEF	16.10	9.20	UNDEF
820609.00	5.00	1.17	7.50	1.41	UNDEF	11.50	6.20	UNDEF
820609.00	10.00	1.17	7.00	1.18	UNDEF	7.30	7.00	UNDEF
820714.00	-1.00	0.88	8.00	1.85	UNDEF	9.90	7.20	0.19
820714.00	0.0	0.88	8.50	1.95	UNDEF	11.20	10.20	0.17
820714.00	2.00	0.89	9.00	2.50	UNDEF	12.00	11.30	0.21
820714.00	5.00	0.88	9.00	2.70	UNDEF	12.90	12.00	0.21
820714.00	10.00	0.88	8.00	1.95	UNDEF	9.90	12.00	0.20
820811.00	-1.00	1.39	10.50	1.05	UNDEF	15.60	8.50	0.13
820811.00	0.0	1.39	10.50	1.20	UNDEF	16.40	9.70	0.14
820811.00	2.00	1.39	10.50	1.14	UNDEF	13.10	5.50	0.21
820811.00	5.00	1.39	11.00	0.93	UNDEF	16.80	5.50	0.17
820811.00	10.00	1.39	11.00	0.93	UNDEF	14.70	13.10	0.07
820908.00	-1.00	0.88	11.50	1.25	UNDEF	10.50	3.40	0.37
820908.00	0.0	0.88	14.50	12.50	UNDEF	33.00	27.00	0.46
820908.00	2.00	0.98	14.80	12.13	UNDEF	29.00	25.00	0.48
820908.00	5.00	0.88	14.00	6.15	UNDEF	16.00	16.00	0.38
820908.00	10.00	0.89	12.80	2.42	UNDEF	10.10	10.10	0.24
821012.00	-1.00	0.47	13.50	2.00	UNDEF	21.30	19.20	0.09
821012.00	0.0	0.47	15.50	28.93	UNDEF	83.70	81.60	0.35
821012.00	2.00	0.47	16.00	28.77	UNDEF	42.20	30.10	0.68
821012.00	5.00	0.47	16.50	25.57	UNDEF	48.60	26.90	0.53
821012.00	10.00	0.47	14.80	3.60	UNDEF	23.20	19.60	0.16
821020.00	-1.00	0.65	UNDEF	5.12	UNDEF	UNDEF	UNDEF	UNDEF
821110.00	-1.00	0.32	19.80	1.15	UNDEF	19.20	14.70	0.08
821110.00	0.0	0.32	24.00	80.00	UNDEF	192.10	149.70	UNDEF
821110.00	2.00	0.32	24.50	15.75	UNDEF	34.10	26.50	0.46
821110.00	5.00	0.32	22.50	9.56	UNDEF	31.70	24.50	0.30
821110.00	10.00	0.32	20.80	4.21	UNDEF	22.50	20.40	0.21
821208.00	-1.00	0.31	UNDEF	0.60	UNDEF	21.70	17.70	0.03
821210.00	-1.00	0.25	19.20	6.75	UNDEF	40.60	40.60	0.17
830114.00	-1.00	0.30	18.00	UNDEF	UNDEF	UNDEF	UNDEF	UNDEF
830202.00	-1.00	0.13	16.50	UNDEF	UNDEF	11.00	10.00	UNDEF
830302.00	-1.00	0.11	18.00	0.65	UNDEF	12.00	12.00	UNDEF
830413.00	-1.00	0.51	13.20	UNDEF	UNDEF	10.00	12.00	UNDEF
830511.00	-1.00	0.94	12.20	0.23	UNDEF	21.00	19.00	0.01

2.2 Calculation details for Figure 5.28

A Macrophyte respiration and photosynthetic rate estimates from diurnal oxygen monitoring.

Station (km)	Date	k_2 day^{-1}	Photosynthetic Production g O_2 m^{-3} day^{-1}	Macrophyte Respiration g O_2 m^{-3} h^{-1}
Landsdowne Road (-1)	25 Jan 1982	6.5	17.6	0.48[a]
Hockley (2)	9 Feb 1982	11.0	~5.0	0.21[b]
Signals (5)	5 Mar 1982	11.0	11.4	0.48[b]
Walton (10)	5 Nov 1981	11.0	17.2	0.72[b]

[a] assuming respiration ≡ 0.66 production (i.e. a growing population)

[b] assuming respiration ≡ production (i.e. a stationary population)

Station (km)	Travel time h	DO[a] g m^{-3}	ΔDO g m^{-3} h^{-1}	$\bar{D}$ g m^{-3}	k_2 day^{-1}	$k_2\bar{D}$ g m^{-3} h^{-1}	ΔDO-$k_2\bar{D}$ g m^{-3} h^{-1}	BUR[b] g m^{-3} h^{-1}	R_{aq}[c] g m^{-3} h^{-1}	R_m[d] g m^{-3} h^{-1}	P_m[d] g m^{-3} h^{-1}
Landsdowne (-1)	-	9.95	-	0.39	6.5	0.11	-	2.1	0.11	0.48	0.72
Waharoa (0)		8.50						1.5	0.20	0.48	
	1.5		-2.57	2.65	11	+1.22	-3.78	2.2	0.24	0.35	0.35
Hockley (2)		4.60						2.8	0.27	0.21	
	2.1		-0.47	5.10	11	+2.35	-2.82	1.9	0.20	0.35	0.35
Signal (5)		3.60						1.0	0.12	0.48	
	4.2		+0.72	4.1	11	+1.88	-1.17	0.8	0.12	0.60	0.60
Walton (10)		6.60		2.6	11	+1.19		0.58	0.12	0.72	0.72

[a] surveyed 12 Jan 1982

[b] measured using chamber system 12 Jan 1982 except for Landsdown Road which is for 25 Jan 1982

[c] calculated using measured ATP (mg m^{-3}) x 100 mg O_2 mg ATP^{-1} h^{-1} (from Table 6.2)

[d] from Table A above

B Calculation of respiratory components of river dissolved oxygen on the Waitoa River

APPENDIX 3: REGRESSION EQUATION COEFFICIENTS FOR BUR KINETICS IN THE WAIOTAPU RIVER

Station: Homestead Road (2.2 km below discharge)

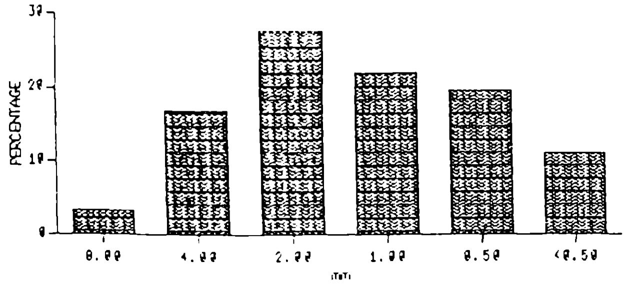
Date: 9 March 1983

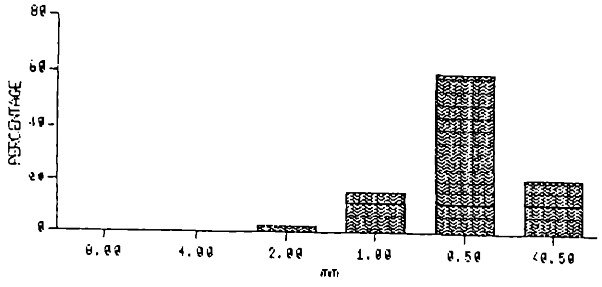
Methods: 2 chambers (large, 0.35 ms^{-1}; and small 0.2 ms^{-1}) on fine (f) or coarse (c) substrata. Repeat reoxygenations using catalase/peroxide (Hickey, 1985b) designated (i)→(iv) with glucose added between (ii) and (iii).
Regressions are for BUR (Y-axis) and DO (X-axis) and use natural logarithms

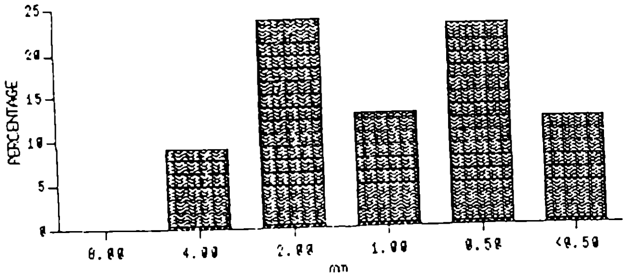
Chamber	Run number	Regression BUR vs DO	ln BUR vs DO	ln BUR vs ln DO
Large (f)	i	2.91x+0.22 n=6, r^2=0.939	0.258x+1.43 n=6, r^2=0.907	1.03x+1.05 n=6, r^2=0.939
	ii	4.52x-14.6 n=10, r^2=0.757	0.211x+1.32 n=10, r^2=0.848	1.51x-0.12 n=10, r^2=0.797
	iii	3.10x+3.17 n=8, r^2=0.805	0.129x+2.30 n=8, r^2=0.794	0.923x+1.42 n=8, r^2=0.830
Small (f)	i	2.93x-3.88 n=7, r^2=0.872	0.407x+0.36 n=7, r^2=0.953	1.41x+0.072 n=7, r^2=0.904
	ii	4.04x-10.9 n=9, r^2=0.952	0.297x+0.74 n=9, r^2=0.966	1.80x-0.67 n=9, r^2=0.968
	iii	2.50x-3.70 n=11, r^2=0.929	0.247x+0.96 n=11, r^2=0.976	1.20x+0.32 n=11, r^2=0.949
	iv	3.73x-8.70 n=7, r^2=0.896	0.252x+1.07 n=7, r^2=0.929	1.53x-1.41 n=7, r^2=0.912
Small (c)	i	2.91x-1.48 n=9, r^2=0.914	0.310x+0.99 n=9, r^2=0.941	1.03x+0.86 n=9, r^2=0.884
	ii	4.67x-12.1 n=11, r^2=0.843	0.266+1.08 n=11, r^2=0.949	1.53x-0.11 n=11, r^2=0.899
	iii	4.64x-5.48 n=15, r^2=0.901	0.304x+1.16 n=15, r^2=0.986	1.18x+0.91 n=15, r^2=0.939
	iv	8.03x-32.0 n=10, r^2=0.903	0.388x+0.30 n=10, r^2=0.912	2.46x-1.72 n=10, r^2=0.966

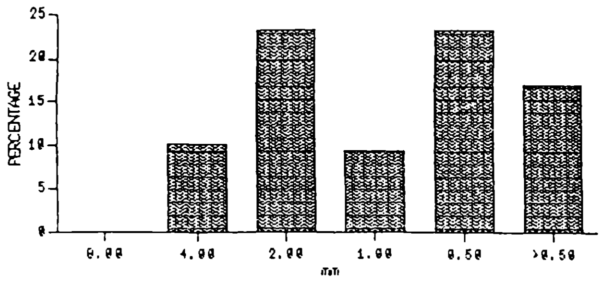
APPENDIX 4: PARTICLE SIZE ANALYSIS

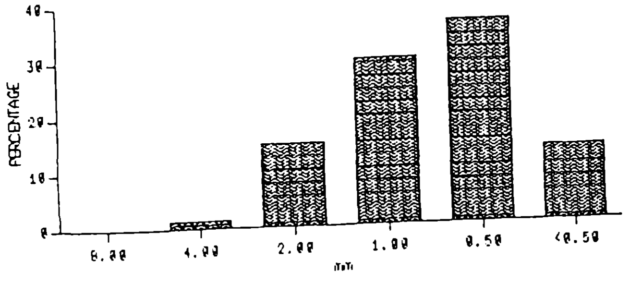
4.1 Waitoa River

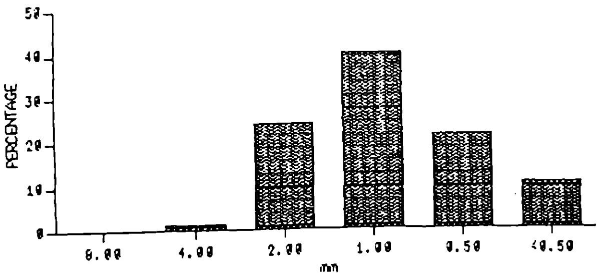

LANDSDOWNE RD COARSE PARTICLE SIZE (0-1cm) 5/3/82

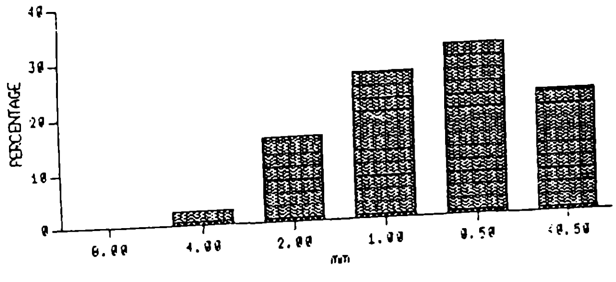

WAHAROA FINE PARTICLE SIZE (0-1cm) 8/3/82


LANDSDOWN RD PARTICLE SIZE 25/1/82

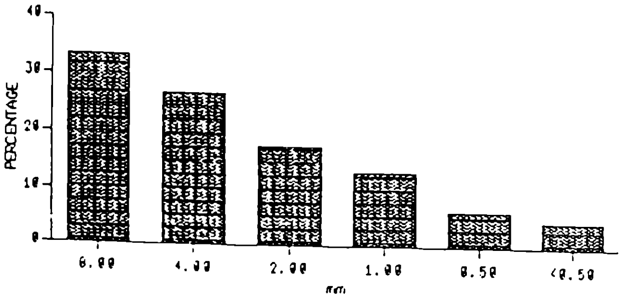

WAHAROA PARTICLE SIZE 25/1/82


HOCKLETS PARTICLE SIZE 25/1/82

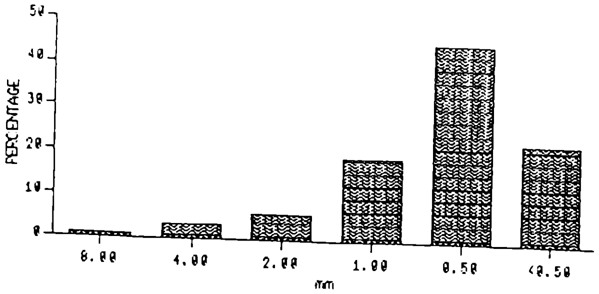

SIGNALS PARTICLE SIZE 25/1/82


WALTON BRIDGE PARTICLE SIZE 25/1/82

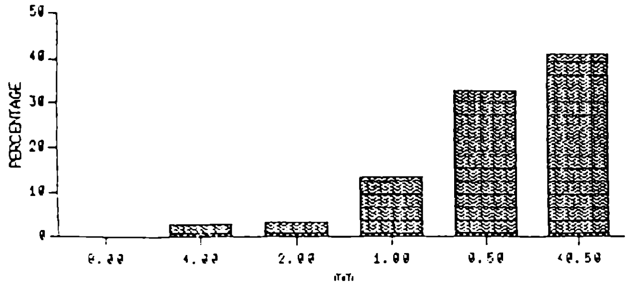
HOMESTEAD RD PARTICLE SIZE COARSE(0-1cm) 9/2/83



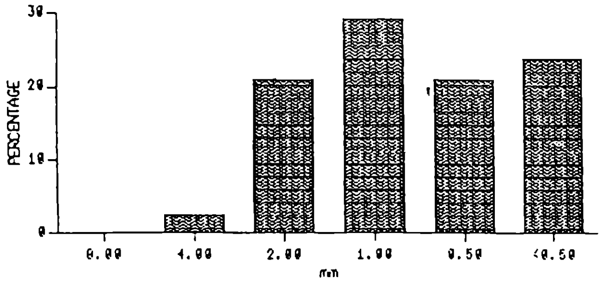
HOMESTEAD RD PARTICLE SIZE FINE(0-1cm) 9/2/83



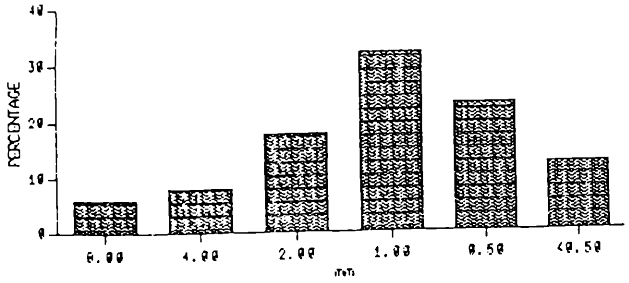
ABOVE DISCHARGE PARTICLE SIZE 17/1/83



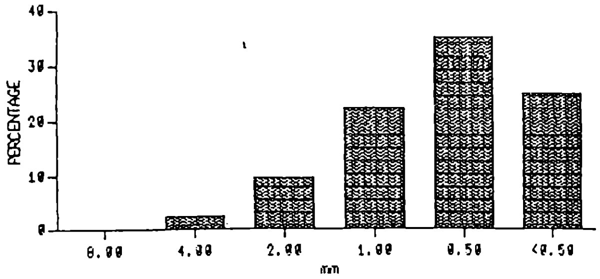
50M BELOW DISCHARGE PARTICLE SIZE 17/1/83



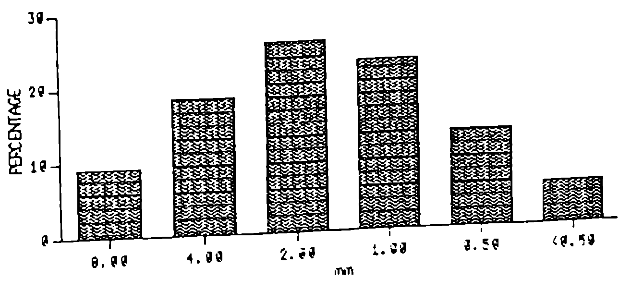
HOMESTEAD RD PARTICLE SIZE 17/1/83



AARD RD PARTICLE SIZE 17/1/83

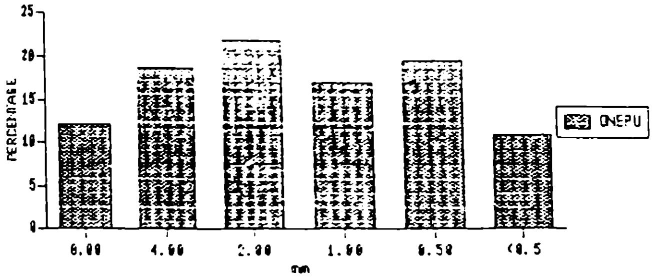


HALCROW RD PARTICLE SIZE 17/1/83

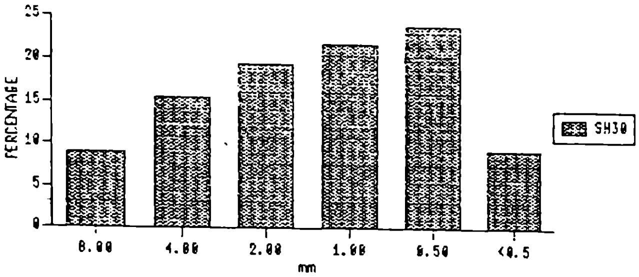


4.3 Tarawera River

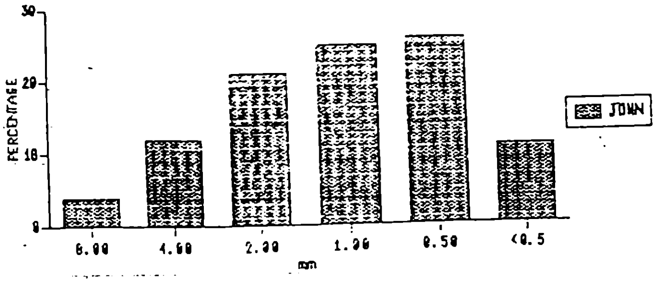
Particle Size Tarawera R Feb 65



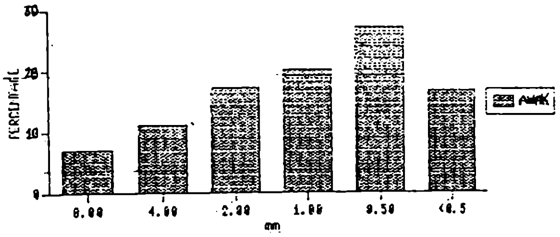
Particle Size Tarawera R Feb 65



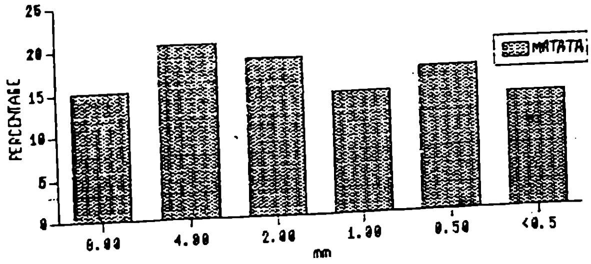
Particle Size Tarawera R Feb 85



Particle Size Tarawera R Feb 85



Particle Size Tarawera R Feb 85



APPENDIX 5: SEGMENT OXYGEN MASS BALANCE CALCULATIONS FOR THE TARAWERA RIVER

LIST OF SAMPLING SITES

Site	Description	km from sea
12	Town bridge	28.5
11B	Water intakes	27.4
-	Caxton outfall	27.1
11C	Foot bridge	26.9
-	Sewage outfall	26.3
11A	Ruruanga confluence	25.7
11	Pipe bridge	25.6
10D	Onepu ramp	23.6
10C		22.8
10B	Above Tasman	21.5
10A	Tasman outfall	21.4
10	Below Tasman	20.5
9B		20.2
9A		18.9
9	SH30	18.4
8	Whites farm	16.4
8A	Johnston Road	15.5
7A	Mangaone confluence	14.8
7	Below 7A	14.5
6	Otakiri	13.0
5	Gillette confluence	11.2
	Braemar spring	10.0
4	Waikamihi confluence	9.2
3	Awakaponga	6.5
2	Island	3.9
1	Awakaponga canal	1.0
1A	SH2 bridge	0.6

```
$
$ CALCULATES GROSS OXYGEN UPTAKE & REAERATION RATE FROM RIVER AND
$ TRIBUATRY SURVEY DATA
$
$ INPUT    'KM' 'U' (M/S) OR 'TOT' (DAYS)
$          'TEMP' (OR 'SAT') 'DO' 'Q' 'QT' 'DOT' 'K2'
$ NOTE U(I),TOT(I),K2(I) REFER TO REACH I-I+1
$      Q(I) IS FLOW AT TOP OF REACH I-I+1
$      QT(I) IS INFLOW NEAR TOP OF REACH I-I+1
$ ALSO NOTE THAT THERE SHOULD BE A LINE OF UNDEFINED VALUES
$ INSERTED AFTER THE BOTTOM SITE
$
$ COMPUTE TIME OF TRAVEL (DAYS) REACH I=I+1
CYC UP 1 'KM' 'KM1' ; COM 'TOT=(KM-KM1)*1000/U/3600/24'
$ COMPUTE REAERATION RATE
COM 'SAT=10**(1.159-0.011394*TEMP+0.00006695*TEMP**2)'
COM 'DEF=SAT-DO' ; CYC UP 1 'DEF' 'DED' ; COM 'REAR=K2*(DEF+DED)/2'
$ COMPUTE GROSS UPTAKE
CYC UP 1 'Q' 'QD' ; CYC UP 1 'DO' 'DOD'
COM 'NET=(DO*Q/QD+DOT*QT/QD-DOD)/TOT' ; COM 'GROSS=NET+REAR'
```

```
LINES        KM        TIME         U        DEGC        SAT         DO          Q          QT         DOT   Site
  1       27.40      702.00      0.66      16.00       9.90       9.40      20.71      -2.34       9.40   11B
  2       26.90      728.00      0.66      16.50       9.80       9.70      18.37       1.25      10.00   11C
  3       23.60      754.00      0.66      17.20       9.60       9.20      19.62       0.0        0.0    10D
  4       21.50      937.00      0.66      17.80       9.50       9.20      19.62       2.58       0.0    10B
  5       18.40     1031.00      0.66      19.20       9.30       7.60      22.20       0.0        0.0     9
  6       16.40     1145.00      0.66      19.70       9.10       7.20      22.20       0.0        0.0     8
  7       14.80     1222.00      0.75      19.80       9.10       6.50      22.20       1.87       9.70   7A
  8       13.00     1258.00      0.75      19.80       9.10       6.45      24.07       0.0        0.0     6
  9       11.20     1345.00      0.75      20.00       9.10       5.90      24.07       2.10       8.00    5
 10        9.20     1423.00      0.75      20.20       9.00       5.70      25.67       0.40      10.20    4
 11        6.50     1506.00      0.75      20.30       9.00       5.40      26.57       0.0        0.0     3
 12        3.90     1615.00      0.75      20.30       9.00       4.95      26.57       0.0        0.0     2
 13        1.00     1707.00      0.75      20.30       9.00       4.70      26.57       0.0        0.0     1
 14       UNDEF       UNDEF     UNDEF      UNDEF      UNDEF      UNDEF      UNDEF      UNDEF      UNDEF
 PRI DATA 'KM' 'DT' 'TOT' 'K2' 'U' 'REAR' 'NET' 'GROSS' LAST

 LINES         KM         DT        TOT         K2          U       REAR        NET      GROSS
   1        27.40         —          —         7.00       0.66        —          —          —
   2        26.90       0.06       0.07       7.00       0.66       1.75       8.97      10.72
   3        23.60       0.04       0.10       7.00       0.66       2.45       0.0        2.45
   4        21.50       0.05       0.16       7.00       0.66       7.00      20.45      27.45
   5        18.40       0.04       0.19       7.00       0.66      12.60      11.40      24.00
   6        16.40       0.03       0.22       7.00       0.66      15.75      24.95      40.70
   7        14.80       0.03       0.25       7.00       0.75      18.37      10.75      29.12
   8        13.00       0.03       0.28       7.00       0.75      20.47      19.80      40.27
   9        11.20       0.03       0.31       7.00       0.75      22.75      15.77      38.52
  10         9.20       0.04       0.35       7.00       0.75      24.15       6.25      30.40
  11         6.50       0.04       0.39       7.00       0.75      26.77      11.22      37.99
  12         3.90       0.04       0.43       7.00       0.75      29.22       5.59      34.81
  13         1.00      UNDEF      UNDEF       7.00       0.75      UNDEF      UNDEF      UNDEF
  14        UNDEF      UNDEF      UNDEF       7.00      UNDEF      UNDEF      UNDEF      UNDEF
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LINES      DATE           KM        TIME       TEMP       DO1       DO2      SITE        Q         QT
  1     850214.00       23.60      850.00     16.00      9.30      9.30     10.40     17.66      0.0
  2     850214.00       20.20      904.00     18.00      6.60      6.60      9.20     19.90      2.24
  3     850214.00       18.40      925.00     18.00      6.60      6.70      9.00     19.90      0.0
  4     850214.00       16.40      957.00     18.20      5.80      5.90      8.00     19.90      0.0
  5     850214.00       14.80     1040.00     18.40      5.60      5.70      7.10     21.40      1.50
  6     850214.00       13.00     1115.00     18.40      5.60      5.70      6.00     21.40      0.0
  7     850214.00       11.20     1155.00     18.50      5.50      5.30      5.00     22.01      0.61
  8     850214.00        9.20     1300.00     19.00      5.20      5.20      4.00     22.33      0.32
  9     850214.00        6.50     1350.00     19.40      5.00      4.80      3.00     22.33      0.0
 10     850214.00        3.90     1450.00     19.70      4.70      4.70      2.00     22.33      0.0
 11     850214.00        1.00     1555.00     20.00      4.30      4.25      1.00     22.33      0.0

LINES       DOT            U         DO        KM1        TOT       SAT       DEF       DED        QD
  1         0.0         0.75       9.30      20.20       0.05      9.86      0.56      2.85     19.90
  2         0.0         0.71       6.60      18.40       0.03      9.45      2.85      2.85     19.90
  3         0.0         0.71       6.60      16.40       0.03      9.45      2.85      3.61     19.90
  4         0.0         0.71       5.80      14.80       0.03      9.41      3.61      3.78     21.40
  5        10.10        0.71       5.60      13.00       0.03      9.38      3.78      3.78     21.40
  6         0.0         0.71       5.60      11.20       0.03      9.38      3.78      3.86     22.01
  7        11.00        0.71       5.50       9.20       0.03      9.36      3.86      4.06     22.33
  8        10.40        0.71       5.20       6.50       0.04      9.26      4.06      4.19     22.33
  9         0.0         0.71       5.00       3.90       0.04      9.19      4.19      4.43     22.33
 10         0.0         0.71       4.70       1.00       0.05      9.13      4.43      4.78     22.33
 11         0.0         0.71       4.30      23.60      -0.37      9.08      4.78      0.56     17.66

LINES       DOD          NET         K2       REAR      GROSS
  1         6.60        31.51       8.60      14.67      46.18
  2         6.60         0.0        8.60      24.54      24.54
  3         5.80        24.54       8.60      27.81      52.35
  4         5.60        -7.92       8.60      31.78      23.86
  5         5.60        24.13       8.60      32.47      56.60
  6         5.50        -1.88       8.60      32.82      30.94
  7         5.20        16.00       8.60      34.05      50.05
  8         5.00         7.93       8.60      35.47      43.40
  9         4.70         7.08       8.60      37.06      44.13

 10         4.30         8.46       8.60      39.59      48.05
 11         9.30        10.49       8.60      22.94      33.43
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14 February 1985

APPENDIX 6: PUBLICATIONS FROM THE THESIS

i Hickey; C.W. (1985) Chamber studies of benthic oxygen uptake kinetics in the Waiotapu River, New Zealand. In Sediment oxygen demand: processes, modelling and measurement (Hatcher, K.J. and Hicks, D. eds) U.S. EPA Washington DC.

ii Hickey, C.W. (1985) Quantitative addition of dissolved oxygen to *in situ* benthic chamber systems by use of catalase and hydrogen peroxide. Applied and Environmental Microbiology 49: 462-464.

iii Hickey, C.W. and Nagels, J.W. (1985) Modifications to electrolytic respirometer systems for precise determination of BOD exertion kinetics in receiving waters. Water Research 19: 463-470.