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Treatment of Agricultural Wastewaters and Growth of *Schoenoplectus validus* in Constructed Wetlands

A thesis submitted in partial fulfilment of
the requirements for the degree
of
Doctor of Philosophy
in Biological Sciences
at the
University of Waikato

by

Christopher Charles Tanner

University of Waikato
Hamilton, New Zealand

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A/P 1

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*From underground silos
bulrush rhizomes fire round after round
of emerald tipped shoots*

*— up through the litter of last years
victory celebration*

Abstract

Constructed wetlands have the potential to provide simple, low-cost, low-maintenance, natural treatment systems for agricultural wastewaters.

The aims of the present study were:

1. To evaluate the treatment performance of gravel-bed wetland systems supplied with dairy farm wastewaters and provide basic data for the development of suitable loading criteria.
2. To investigate the effect of wetland plants on treatment performance.
3. To investigate the effect of wastewater loading rates on plant productivity, nutrient uptake, and growth characteristics.

Schoenoplectus validus (soft-stem bulrush) was chosen as the test species, since this is the plant most commonly used in wetland treatment systems in New Zealand, and is also in widespread use in the United States and Australia.

The effect of loading rate and presence of plants on wastewater treatment was investigated over a 20 month period, in four pairs of gravel-bed wetlands operated with different loading rates of dairy shed wastewaters. Mean annual suspended solids (SS) removals of 75-85% were achieved in the wetlands, irrespective of loading rate or the presence of plants. In the planted wetlands, mean mass removal of carbonaceous biochemical oxygen demand (CBOD), during the first 12 months of monitoring, increased from 76 to 90%, total BOD (CBOD + nitrogenous BOD) from 50 to 80%, faecal coliforms from 95 to over 99%, total nitrogen (TN) from 48 to 75%, and total phosphorus (TP) from 37 to 74% as wetland retention time rose from 2 to 7 days (i.e. inversely related to loading rate). The unplanted wetlands generally showed similar performance to the planted wetlands at low loading rates, but poorer BOD, nitrogen and phosphorus removal at the highest loadings tested. Comparison of the performance of the horizontal-flow wetlands with that of up-flow wetland tanks, showed similar reduction of BOD and SS in relation to volumetric loading, but reduced N and P removal, particularly at high loading rates.

Net annual plant production measured in the wetlands was high, rising from ~2.5 to 3.5 kg (dry weight) m⁻² with increasing wastewater loading, during the first growth season. Plant biomass and tissue nutrient levels declined down the wetland channels reflecting fertility gradients in the wetlands. Nutrient accumulation in living and dead plant biomass in mid-summer, after two years of wetland operation, although high compared to levels reported for natural communities, only represented 6 to 10% of the N removal and 6 to 13% of the P removal recorded for the wetlands. Above-ground litter inputs from plants were estimated to contribute significant quantities of readily and slowly

available carbon substrates to the wetland surface, equivalent to between 60 and 125% of the available organic carbon loadings, and 80 to 190% of the CBOD loadings supplied to the wetlands in wastewaters.

In further experiments, above and below-ground plant growth was compared in wetland microcosms batch-fed with a range of agricultural wastewater types and strengths, with and without nutrient additions, and in complete nutrient solutions. *S. validus* was capable of growth over a wide range of wastewater nutrient levels, but showed maximum growth at the highest levels tested. Reduced rooting depths were however noted in the higher strength organic wastewaters. Comparisons with root development in nutrient solutions and nutrient amended wastewaters suggested that factors associated with organic compounds in the wastewaters (e.g. hypoxic stress caused by high BOD levels), rather than increasing nutrient levels, restricted root penetration. Similar reductions in the proportion of deep-growing roots were noted at high loadings in the larger-scale wetlands. These growth responses have important consequences for the design of subsurface-flow wetland systems, where bed depths are matched to plant rooting depth, to maximise contact between wastewaters and the root-zone.

Acknowledgments

Specific acknowledgments, of which there are many, have been given at the end of each paper in the main body of the thesis.

I am especially grateful for the friendship, support, and encouragement of my workplace supervisor, John Clayton. John ensured that all the resources necessary were available to complete the project. His assistance and good counsel in this project, as well as the grounding provided by the 12 years or so of scientific apprenticeship through which he has led me, were an invaluable asset.

I thank Keith Thompson, my university supervisor, for his friendship, fresh ideas, and editorial comments. Keith supported me to do this project in the way I wanted. He was there when I needed him, and allowed me space when I did not.

I thank Professor Silvester for his valuable input during the early design stages of the project.

I am very grateful to my employers, MAF Technology who originally supported this project, and to NIWA—Ecosystems, who have supported its completion. My thesis study would not have happened without me having been given the chance to combine work and study.

I thank James Sukias for his assistance with the wetland research programme during the last 2 years, for taking some of the heat off me at work when necessary, and for his help with the final presentation of this document.

I also thank all my work colleagues (the old Ruakura bunch) for their friendship and assistance in numerous ways, including: hard physical labour during construction of the experimental wetlands, sampling, and proof-reading.

This project has occurred during an eventful time in my life. In the four and a half years since my first registration, my partner Trish and I, have had 3 more children, travelled to the USA, moved house twice and extended our current one. I thank Trish for her love, support and forbearance, and the sacrifices she has made to allow me to follow this path. Trish and my children, Greer (10), Isaac (3 $\frac{1}{2}$), Theo (18 months), and Arden (3 months), have helped sustain me and keep my feet firmly on the ground over this period.

I also thank my parents and many friends, old and new, begged and borrowed, who have helped in many tangible and intangible ways.

Foreword

From conception, the study has set out to be multifariously "applied".

Firstly, the topic is "applied"; covering studies on the treatment of agricultural wastewaters in constructed wetlands, and the growth of wetland plants in these hyper-eutrophic systems.

Secondly, the mode of presentation is "applied". A decision was made at the beginning of the study to present the thesis as a series of papers to ensure that the information became readily accessible, rather than buried in the thesis graveyard. All of the chapters included in the thesis have either been prepared for submission as scientific papers, or as conference proceedings (see status of individual papers below). Each chapter is an individual unit, complete with its own introduction, methodology and discussion. As such, it follows recent Dutch models for D. Phil. thesis presentation, and earlier University of Waikato, School of Science and Technology theses, such as that of Bill Snelgar (1981), who was a pioneer in this regard.

Thirdly, the study has been undertaken within an "applied" work environment. The project was conceived and initiated while employed in MAF Technology (Ministry of Agriculture and Fisheries). Subsequent restructuring of science in New Zealand, with the establishment of Crown Research Institutes, led to the transfer of this research programme to NIWA–Ecosystems (National Institute of Water and Atmospheric Research). The study was carried out within a normal work environment. The broad demands of the project have included preparation of Foundation for Research, Science, and Technology bids for the funding of the study (all of the "chapters" of the thesis have been or are FRST contract objectives), as well as input on many related wetland projects and consultancies, some of which are referred to in the text. The project has derived considerable benefit from the funding, resources and interaction available in this work environment, and the cross-fertilisation between experimental research and its practical application.

Chapter 1 of the thesis is a general introduction to constructed wetlands and their potential role in agriculture. It was presented at Technical Session No. 8 (The Use of Wetlands for Wastewater Treatment), of the New Zealand Land Treatment Collective, held in Whangarei during April 1993, and is published in the proceedings of that meeting.

Chapter 2 includes two papers on the effects of loading rate and plants on treatment of dairy shed wastewaters in constructed wetlands, which have been submitted to *Water Research*. These papers are co-authored by John Clayton, in recognition of his

involvement in the conception and establishment of these trials and his ongoing supervision of the study; and Martin Upsdell for his biometrical input to the study, particularly the application of Bayesian curve fitting and statistical techniques to the data. The versions of the papers included here have been revised after preliminary acceptance for publication.

Chapter 3 compares the treatment of dairy shed wastewaters in horizontal and up-flow wetland systems. It was prepared early in the study (July 1992), soon after the full water quality dataset became available, for presentation at the International Association on Water Quality Conference on "Wetland Systems in Water Pollution Control" held in late November 1992. An earlier version is published in the proceedings of that conference. The version presented is that presently in press; *Water Science and Technology*, 29 (4), and due for publication in early June, 1994.

Chapter 4 presents microcosm studies of the growth and nutrition of *Schoenoplectus validus* in a range of agricultural wastewaters. The paper was revised taking into account reviewers comments and has recently been published; *Aquatic Botany* 47 (2): 131-153 (1994).

Chapter 5 includes two papers on plant productivity and growth responses, and nutrient dynamics in relation to wastewater loading. These have been prepared for submission to the *Journal of Applied Ecology*.

Due to the different requirements of the various journals and conference proceedings the chapters vary in layout and style. Each page is numbered consecutively through the thesis (bottom centre) and also individually for each paper (upper right). Summaries of water quality data for the Ruakura experimental wetlands are included in the appendices.

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*The Oak Tree and the Reed*¹

One day the oak tree said to the reed:
'You have every reason to blame nature.
To you even a wren is a heavy burden,
And the slightest breath of air
That happens to ripple the surface of the water
Forces you to bow your head.
Whereas, like the highest mountain,
I not only give shade from the sun,
But resist the stormy blast.
To you every wind is a gale,
To me it's only a gentle breeze.
If only you grew beneath the shelter of my branches
You wouldn't suffer so much.
I would protect you from the tempest.
But you often take root
On the banks of the kingdoms of the wind.
I think that nature has treated you unjustly.'
'Your pity', replied the slender plant,
'Springs from your natural goodness. But don't worry.
For me the winds are less formidable than they are for you.
I bend and don't break.
So far you have resisted their furious gusts without breaking.
But let us see what happens.'
As these words were spoken
There rushed upon them from the edge of the horizon
The most terrific gale that had ever blown from the north.
The oak tree stood firm; the reed bent double.
The wind blew more fiercely still,
Until with its terrible strength it uprooted the tree
Whose branches had reached up to heaven
And whose roots were among the graves of the dead.

¹ This fable was La Fontaine's favourite.

From: Aesop's Fables, as told by La Fontaine

CHAPTER 1



Alf's

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Wetlands, wastewaters and agriculture.

Chris C. Tanner

*Ecosystems Division, National Institute of Water and Atmospheric Research,
Ruakura Research Centre, Private Bag 3123, Hamilton*

Agricultural development in many areas of New Zealand has been synonymous with wetland drainage. Over 90% of our natural wetlands have now been drained or substantially modified. Continued drainage, both direct and indirect¹, and unmanaged grazing threaten many of our remaining wetlands. As well as destroying the habitat of a diverse range of plants, fish and wildlife, we have lost the functions of these wetlands as moderators of flooding, siltation and nutrient transport. Natural wetlands act to detain, store and regulate runoff; reducing peak flows, and sustaining baseflow to streams during dry periods. They are important buffer zones between land and water; intercepting and trapping sediment and nutrients in surface runoff, and reducing nitrate levels in groundwater seepage zones.

Agricultural land uses have major effects on the quality of New Zealand's inland and coastal waters, and their associated fisheries and wildlife. The restoration and creation of wetlands, detention ponds and riparian zones in agricultural catchments offer considerable potential to mitigate point and diffuse-source pollution from agricultural activities. Other benefits include conservation of wildlife and fisheries, enhanced biodiversity, and more aesthetic rural landscapes.

Agricultural wastewaters.

Farmed livestock in New Zealand have been estimated to produce 40-50 times the wastes of the human population (Dakers and Painter, 1982). Although the majority of these wastes are distributed directly back onto grazed pastures, significant quantities of high-strength wastewater are produced by animals housed permanently (e.g. pigs) or temporarily (e.g. dairy sheds and stock yards). Wastewaters are also generated during silage production and agricultural processing (e.g. dairy factories, abattoirs, wool

¹ Water levels in natural wetlands are often closely linked to the local water table. Water tables may be indirectly lowered by drainage of surrounding farmland, excessive groundwater extraction, or decreased catchment water yield (e.g. as a result of increased interception and canopy evaporation in exotic forest plantations).

scourers). These point sources of wastewater require treatment if discharged to waterways to avoid serious pollution problems.

Oxidation ponds are widely used to treat dairy shed and piggery wastewaters. Their discharges commonly have levels of biochemical oxygen demand (BOD) 4 to 8 and 6 to 12 times greater respectively than those of similarly treated domestic sewage. Recent studies of the performance of dairy farm oxidation ponds (Hickey et al., 1989) have demonstrated the wide variability in the quality of their discharges and focussed concern on their potential environmental impacts in receiving waters. Of particular concern are the high levels of BOD, suspended solids (SS), pathogens and nutrients, particularly ammonia. High levels of ammonia (often above 75 g m^{-3}) are common in agricultural wastewaters. Ammonia may be directly toxic to aquatic organisms² and cause significant deoxygenation (nitrogenous biochemical oxygen demand, NBOD) in receiving waters due to microbial nitrification (e.g. Cooper, 1986). The NBOD of dairy shed wastewaters is often 3 to 5 times that shown by the standard BOD test. Piggery wastewaters may have NBOD values up to 16 times higher than their BOD, where partial waste recycling is practiced. This suggests that the standard BOD test alone is a poor measure of the true oxygen demand characteristics of agricultural wastewater discharges. Total BOD (BOD + calculated NBOD³) or COD with appropriate calibration, are better measures by which to evaluate the impacts of agricultural discharges, and to use as basis for treatment system design.

In light of recent information on the potential environmental impacts of agricultural wastewaters, and the requirement for sustainable management of natural waters under the Resource Management Act, it is likely that higher treatment standards will be required for dairy shed and other agricultural discharges in the near future, particularly in sensitive catchments. Further information is needed on the impacts of present discharges, the environmental tolerances of New Zealand fish and invertebrate species, and the sensitivities of different types of receiving waters. For example, lowland soft-sediment streams are likely to be more tolerant than gravel-bed streams, allowing for less stringent treatment

²Un-ionised ammonia (NH_3) is the principal toxic form. The portion of un-ionised ammonia increases at high pH and temperature. Its relative toxicity to aquatic organisms is also affected by these factors, and by dissolved oxygen and carbon dioxide concentrations, salinity, previous acclimation, fluctuations in exposure, and the presence of other toxicants.

³(NBOD can be calculated as $4.33 \times \text{NH}_4\text{-N}$ (see Cooper, 1986))

requirements. From an industry viewpoint, improved treatment standards and reduced environmental impacts on waterways draining agricultural areas are also important in enhancing the 'clean-green' image of New Zealand's agricultural products both nationally and in overseas export markets.

Constructed wetlands

Constructed wetlands are an emerging technology which offers considerable potential to upgrade oxidation pond treatment systems to meet higher standards. They are a simple, low-cost tertiary treatment option, ideally suited to small-scale on-site applications. Once constructed, they do not rely on machinery, or continual energy or labour inputs.

Constructed wetlands combine saturated substrates, shallow water, and aquatic plants, animals, and microbes for the treatment of wastewaters. They attempt to simulate and optimise processes which operate in natural wetlands. Wastewaters are treated by percolation through aquatic vegetation (surface-flow) or the root-zone of wetland plants (subsurface-flow) cultured in channels or beds (Figure 1)

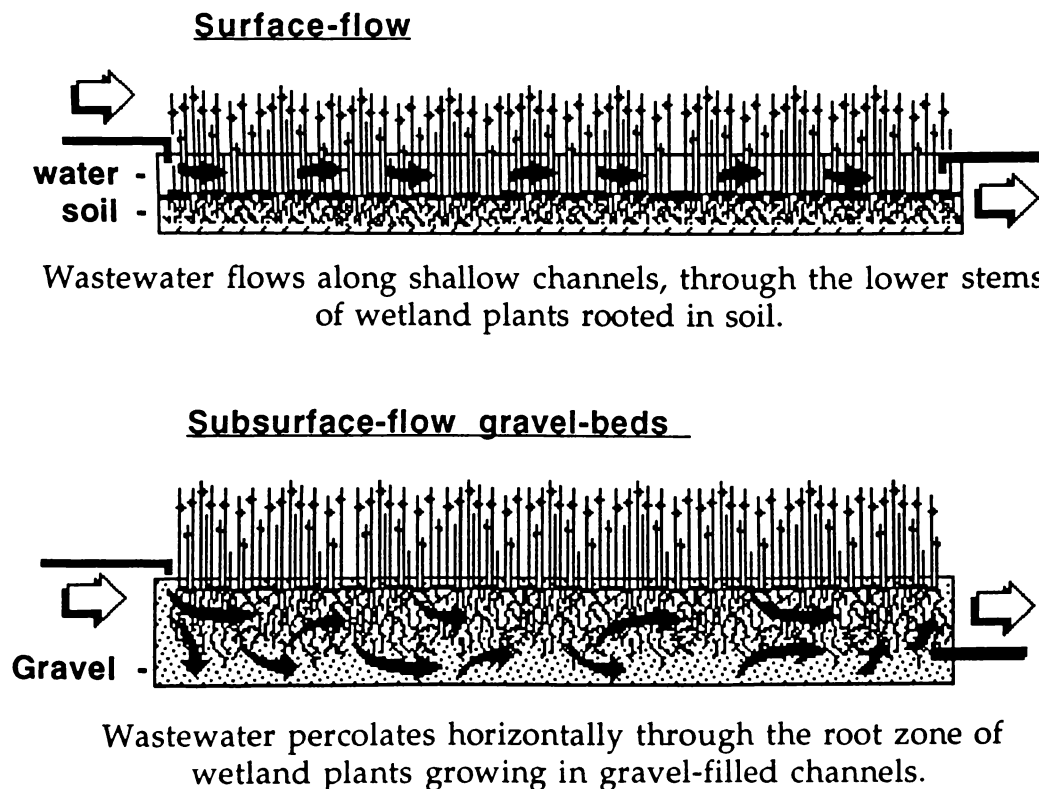


Figure 1. The two most common types of constructed wetland being tested in New Zealand.

Suspended and dissolved organic matter is effectively reduced in artificial wetlands through settling, filtration, and microbial degradation. Long-term phosphorus removal in constructed wetlands is generally limited by the sorption capacity of the sediments and organic matter (Richardson, 1985). This, plus assimilation and storage in plant tissues and litter may initially remove significant proportions (up to 70%) of P loadings. However, removal rates generally drop as sorption sites become saturated, and plant biomass and net nutrient uptake stabilises. Use of substrata with high P sorption capacities, including iron oxides and steel wool, clays such as allophane, and various industrial and mineral processing wastes such as flue ashes, bauxite clays and melter slag may provide increased P uptake (e.g. Wood and Hensman, 1989; Masters, 1989; James et al., 1992; Weiss et al., 1992). Studies of sewage oxidation pond inputs to a natural wetland at Paihia (Cooke, 1992, 1993; Cooke et al., 1992a) identified P complexing and sedimentation processes, which offer the potential to improve P removal in artificial wetlands by linking them to supplies of "reactants" (reduced iron and humic compounds) from natural wetlands.

The predominant nitrogen removal mechanisms in constructed wetlands are microbial conversion to gaseous products and storage in sediments, detritus, and living biomass. Direct volatilisation of ammonia is generally negligible unless pH levels rise above eight. Harvestable plant uptake will generally only remove around 10% of normal influent loadings, and is therefore not a cost effective removal mechanism. Sequential nitrification ($\text{NH}_4 \rightarrow \text{NO}_2 \rightarrow \text{NO}_3$) and denitrification ($\text{NO}_3 \rightarrow \text{N}_2\text{O}$ and N_2) is considered to be the only sustainable N removal process. Being predominantly anaerobic environments, constructed wetlands loaded at appropriate rates can effectively remove nitrate from wastewaters via microbial denitrification, if suitable organic substrates are present (eg. Gersberg et al., 1983; van Oostrom and Russell, 1993). Plant litter production may assist denitrification by supplying degradable carbon substrates.

In high ammonia wastewaters, the rate limiting step is initial nitrification, which requires aerobic conditions. Aerobic micro-zones occur in the surface waters and substrates of constructed wetlands, and in the root-zones of wetland plants. Wetland plants possess special anatomical adaptations for the aeration of their roots and rhizomes, which act as preferential pathways for gas exchange (via diffusion, and thermo-osmotic and venturi-induced mass-flow) between the substrate and

atmosphere. The practical significance of such plant-mediated root-zone oxygenation in wastewater treatment systems is still unclear, with conflicting evidence emerging from a number of studies. Nevertheless, experimental studies which have included unplanted controls have generally shown improved BOD and N removal in the presence of wetland plants (eg. Gersberg et al. ,1983, 1986; Bavor et al. , 1987; Tanner et al., submitted a, b).

Nitrification may be enhanced by physically increasing aeration of the substratum. Davies and Hart (1990) increased nitrogen removal three-fold in gravel-bed wetlands, by using compressed air to create aerated zones in the substratum. Studies presently underway at NIWA-Ecosystems are testing the potential to use water level fluctuations and ammonia-adsorbing substrata such as zeolites (see Cooke, et al., 1992b) to increase nitrogen removal from ammonia-rich wastewaters. Alternatively, Cooper (1993) has shown the benefits of the coupling of land and wetland treatment (e.g. the Rotorua City sewage treatment system) to achieve effective nitrogen and phosphorus removal. In this case, spray irrigation of wastewaters onto pine forest plantations growing on P retentive soils provided major reduction of P. Nitrate in ground waters draining the sloping irrigation area is intercepted by wetlands in the valley bottoms. Here waterlogged anaerobic sediments with high levels of accumulated plant detrital matter provide ideal conditions for denitrifying bacteria, which convert through-flowing nitrate into gaseous forms of N that are vented to the atmosphere.

Sulphur compounds have been shown to be effectively removed in soil-based subsurface-flow artificial wetlands treating sulphur-rich textile industry wastewaters (Winter and Kickuth, 1989). Around 80% removal of incoming sulphur compounds was recorded, with the majority being converted to elemental and organic forms which were strongly retained in the wetland sediments. Heavy metal removal may also be achieved by a complex of physical, chemical and biological mechanisms (Dunbabin and Bowmer, 1992). The mechanisms involved differ depending on the chemistry of the metal involved and the nature of the associated wastewater, but include adsorption, chelation, precipitation, ion exchange, and assimilation; with the sediment again being the main sink. The sustainability of all such sediment based removal mechanisms are dependant on the retention capacity of the substratum, the rate of sediment and organic matter build-up in the wetland, and the rate of co-reactant supply in relation to loading rates and flow patterns.

Maintenance of specific environmental conditions (e.g. redox potentials and pH levels) in the sediment may be required to ensure accumulated elements are not released back into throughflowing waters.

Treatment performance

There is an extensive literature on constructed wetland treatment of domestic sewage wastewaters. Watson et al. (1989), Conley et al. (1991), Reed and Brown (1992), and Brix (1992) have summarised North American and European data, while Kloosterman and Griggs (1989) and Bhamidimarri et al. (1991) have summarised the more limited data available in New Zealand. Kinetic models derived from a number of pilot studies in the USA have led to the development of standard design recommendations for domestic sewage wetlands (Reed et al., 1988; USEPA 1988; WPCF, 1990). Although these provide useful general design guidelines, their direct application to high-ammonia agricultural wastewaters is questionable.

Performance data relevant to the treatment of agricultural and other high-ammonia wastewaters is presently limited. Table 1 summarises available data for BOD and TN removal in a range of wetland systems treating high-ammonia wastewaters. Although loading rates vary considerably, most studies show BOD removals above 70%. TN removals vary between 20 and 91%, with percentage removals showing a general negative relationship to loading rates. Removal rates of 40 to 50% appear to be achievable at economically feasible loading rates ($\sim 1\text{--}2 \text{ g TN m}^{-2} \text{ d}^{-1}$). Most of the studies listed have also reported major reductions ($> 70\%$) in SS and faecal coliforms ($> 90\%$).

Gersberg et al. (1986) recorded mean BOD and TN reductions of 70 to 81% and 30 to 94% respectively, in subsurface-flow gravel-bed wetlands (0.76 m deep) supplied with primary sewage wastewaters. Similar COD removals of 60 to 70%, but markedly lower TN reductions of around 20 % were reported by van Oostrom & Cooper (1990) for meat processing wastewaters treated in shallower (0.45 m) gravel-bed systems during two consecutive periods at higher TN loadings. Similar levels of BOD and TN removal were recorded for these wastewaters supplied at three different stages of pretreatment to surface-flow wetlands. Hammer & Pullin (1992) reported BOD and TKN reductions of 90 and 91% respectively from diluted piggery wastewaters treated in an extensive surface-flow wetland and wet meadow system in Alabama, USA.

Preliminary results of studies undertaken in England (Gray et al., 1990) show BOD and NH_4 reductions of 93 and 69 % respectively at influent levels of 244 and 84 g m^{-3} , in sequential down and horizontal-flow limestone-bed wetlands treating combined septic tank and piggery wastewaters. A number of other experimental wetland systems have been constructed in the USA for the treatment of dairy farm wastewaters (Davis et al., 1992; Holmes et al., 1992), but minimal performance data is presently available. Preliminary results of surface-flow systems in Mississippi (Davis et al., 1992) show BOD removals of around 60 to 70% at design retention times of 5.5 days.

Preliminary results of piggery wastewater treatment trials established at two South Auckland piggeries in association with the Auckland Regional Council, are summarised by Sukias (this meeting). Studies of dairy shed wastewater treatment carried out on the Ruakura Research Farm (Dairy Research Corporation) in Hamilton (Tanner et al., submitted) show mean BOD removal increasing from 76 to 92 % and TN removal increasing from 48 to 75 % as theoretical retention times were increased from 2 to 7.5 days. SS removals of around 70% were recorded at all of the loading rates tested. Similar levels of BOD and SS removal, but lower levels of TN removal per unit volume were found for upflow wetlands treating the same wastewaters (Tanner, 1992).

Investigations of treatment performance and organic solids accumulation rates within wetlands treating dairy shed wastewaters have led to the development of a hybrid wetland design incorporating an upstream surface-flow section followed by a subsurface-flow section (Tanner, 1991). Full-scale wetlands comparing the performance of this design and complete surface-flow systems side by side, have been constructed on a 500 cow farm by the Bay of Plenty Regional Council (see Bruere, this meeting), and on 2 farms in the Waikato in association with Environment Waikato and Anchor Products (NZ Dairy Group). Treatment objectives for these systems are in the order of 60 to 80% BOD & SS removal, and 30 to 50 % TN removal. Field testing of these systems over the next 2 to 3 years should provide valuable information for development of standard industry guidelines for wetland treatment of dairy-shed wastewaters. A number of full-scale wetland systems for the treatment of ammonia-rich wastewaters have been constructed recently, including one at a piggery in Hautapu (near Hamilton), and another treating meat processing wastewaters. Future monitoring of these will provide additional data.

Limitations and advantages

The major advantages of constructed wetlands for the treatment of agricultural wastewaters are their ability to cost-effectively upgrade discharges from existing oxidation pond systems⁴, and their low labour and resource requirements once established.

Recent research here and overseas has generated an information base relevant to treatment of dairy and other agricultural wastewaters in constructed wetlands. Studies to date, have shown constructed wetlands can effectively reduce BOD and SS levels (60 - 80 %), and Faecal Coliforms (> 3 orders of magnitude); but sustainable TN reductions greater than 50 % do not appear practically achievable from high ammonia wastewaters at the present time, using simple wetland designs. These N removal levels may however be adequate in many areas, where large or less sensitive streams and rivers provide sufficient dilution (>45 fold) of discharges.

Initial plant establishment in constructed wetlands requires careful control of water levels; and measures to exclude grazing by pukeko and livestock, and control competing weeds. However, once established they form resilient largely self-maintaining biological systems. Plant toxicity problems have been encountered with some high strength piggery and woolscour wastewaters, attributed to excessive levels of ammonia, fatty acids and salinity. This emphasises the need for adequate pretreatment or dilution of wastewaters before entry to wetlands.

Costs

Construction is relatively inexpensive; The costs for a combined surface/subsurface-flow system for a 120 - 150 cow dairy farm, where suitable clay subsoils are available on site, are estimated to be in the order of \$3-5000-. Operational costs are minimal, being limited to occasional mechanical clearance of influent zones (about every 8 to 10 years), maintenance of inlet and outlet piping, and weed control around the site. Construction costs will be higher where synthetic or other lining materials are required, site access is limited, or suitable land is not available below existing oxidation ponds, thereby requiring the use of a pump.

⁴Current investment in oxidation pond treatment systems is substantial. Dairy farm pond systems alone are estimated to have a replacement cost of over \$25 million.

The future of wetlands in agriculture

Constructed wetlands offer considerable potential to provide cost-effective treatment of agricultural wastewaters. Their low labour requirements and operating costs are likely to be popular with farmers, with advantages similar to those that have led to the increasing move towards oxidation pond treatment systems. Compliance monitoring is also made easier because their treatment performance is not dependant on day to day management. Constructed wetlands utilise the natural cleansing properties of plants, soils and microbes, and are generally compatible with Maori preferences for agricultural waste treatment. However, appropriate education programs may be required to demonstrate treatment concepts and performance capabilities, and gain local support.

Further information is still needed on the long-term performance and sustainability of wetlands treating high strength agricultural wastewaters. It is planned to continue monitoring of the pilot wetlands set up at Ruakura (now in their fourth year of operation) to provide information on organic solid accumulation and degradation rates. Further improvements in the treatment performance of constructed wetlands may be possible in the future by the use of alternative flow configurations such as step-feeding and partial recycling (Tchobanoglous, 1987), and physical methods of increasing oxygen incorporation such as pulsed flow and fluctuating water levels (Brix and Schierup, 1990), possibly in association with substrates with high ammonia binding capacities. Although such modifications show considerable potential to increase the N removal performance of constructed wetlands in the future, small farm-based systems must retain simplicity as a cornerstone of their design.

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CHAPTER 2



EFFECT OF LOADING RATE AND PLANTING ON TREATMENT OF DAIRY FARM WASTEWATERS IN CONSTRUCTED WETLANDS

I. REMOVAL OF ORGANIC POLLUTANTS

CHRIS C. TANNER^{1,2}, JOHN S. CLAYTON¹ AND MARTIN P. UPSDELL³

¹National Institute of Water and Atmospheric Research, P.O. Box 11-115, Hamilton; ²Department of Biological Sciences, University of Waikato, Private Bag 3105, Hamilton, and ³New Zealand Pastoral Agriculture Research Institute, Private Bag 3123, Hamilton, New Zealand.

Abstract— The effect of influent loading rate on mass removal of BOD, SS and faecal coliforms (FC) from dairy parlour wastewaters was compared in four pairs of planted (*Schoenoplectus validus*) and unplanted gravel-bed wetlands (each 19 m²). The wetlands were operated at nominal retention times of 7, 5.5, 3 and 2 days, with in and out-flows sampled fortnightly over a 20 month period. Hydraulic flows were monitored to enable calculation of the mass flows of pollutants. Influent water quality varied markedly over the trial period (CBOD₅, 20-300 g m⁻³; SS, 60-250 g m⁻³; faecal coliforms (FC), 10³-10⁶ MPN (100ml)⁻¹). NBOD was an important component of total BOD, being around 1.5 times higher than the influent CBOD₅, and 2 to 10 times higher than the effluent CBOD₅. Outflow levels of CBOD₅, SS and faecal coliforms rapidly mirrored changes in influent loadings. Mean mass removal of CBOD₅ increased from 60-75 to 85-90%, total BOD (CBOD₅+NBOD) from 50 to 80% and FC from 90-95 to >99% with increasing wetland retention time during the first 12 months of monitoring. Mean annual SS removals of 75-85% were recorded irrespective of loading rate. High levels of dissolved humic colour in the wastewaters were little affected by passage through the wetland at short retention times, but were reduced by up to 40% at longer retentions. Mass removals of CBOD₅, SS and FC showed monotonic relationships to mass loading rates, with little difference between the performance of planted and unplanted wetlands, except for CBOD₅ at high loadings (> 3 g m⁻² d⁻¹). The planted wetlands showed significantly improved removal rates for CBOD₅ at higher loadings, and 1.3 to 2.6 fold higher mass removals of total BOD.

Key words- artificial wetlands, subsurface-flow, root-zone method, agricultural wastewater, BOD, suspended solids, faecal coliforms, dissolved colour, *Schoenoplectus validus*, bulrush.

INTRODUCTION

Point and diffuse-source pollution of waterways draining agricultural land have become an increasing concern in many countries as farming practices intensify and rural land uses diversify. In New Zealand, dairy farming is a major primary industry, with dairy cattle numbers (3.5 million) exceeding that of the human population. About 7,000 million litres of milk are produced annually of which 80-90% is exported in some form, representing around 25% of the world dairy export market and 20 % of New Zealand's overseas earnings (Dept. of Statistics, 1992). Based largely on year-round pastoral grazing, point-source discharges of farm wastewater are generally only produced as a result of twice-daily milking operations (approx 8-10% of total waste production). These dairy parlour wastewaters composed of excreta and mud washed from concrete holding yards with high pressure hoses, waste milk, and milking equipment cleaning water (including detergents, acid and alkalising agents, and sanitisers), are commonly treated in two stage oxidation ponds (MAF, 1985) before discharge to surface waters. A recent study of the performance of dairy farm oxidation ponds in New Zealand (Hickey et al., 1989) demonstrated the wide variability in the quality of these effluents and focussed concern on their potential environmental impacts in sensitive receiving waters.

Constructed wetlands are an emerging 'low-tech' method of wastewater treatment which is particularly suited to small-scale on-site applications (Hammer, 1989; Cooper & Findlater 1990). They are considered to have considerable potential for the further treatment of agricultural oxidation pond discharges, because of their relatively low cost, low operation and maintenance requirements, and lack of reliance on machinery or energy inputs. However, information on wetland treatment of agricultural wastewaters is presently very limited. In gravel-bed constructed wetlands, wastewaters typically flow through shallow gravel-filled channels in which emergent aquatic plants grow hydroponically. The gravel matrix provides an extensive surface area for microbial colonisation and promotes physical settling and filtration (Brix, 1987). In addition to aesthetic and wildlife habitat enhancement, aquatic plants assimilate nutrients, produce plant litter and other organic compounds, and modify the microclimate and hydrology of wetland treatment systems. They may also increase microbial decomposition of organic matter by root-zone oxygen release and venting of gaseous by-products of anaerobic decomposition, via their internal aeration systems (aerenchyma).

This and a companion paper (Tanner et al., submitted) report findings of a program to evaluate constructed wetland technologies for use on farms, and develop appropriate design guidelines. The objectives of the present study were to:

1. Investigate the effect of loading rate on removal of biochemical oxygen demand, suspended solids, dissolved colour, and faecal indicator bacteria from dairy farm wastewaters in subsurface-flow gravel-bed constructed wetlands, and
2. Evaluate the effect of planting with the emergent aquatic plant *Schoenoplectus validus* (M. Vahl.) A. Love et D. Love (soft-stem bulrush) on treatment performance. This species

has been commonly employed in municipal wetland wastewater treatment systems in North America, Australia and New Zealand.

METHODS

Trials were carried out on the Ruakura Research Farm (Dairying Research Corporation, Hamilton, New Zealand) in 8 channels (9.5 x 2 x 0.6 m deep) filled to 0.4 m depth with alluvial rhyolitic gravel (10-30mm diameter, 35-37% effective pore space). The channels were lined with 1 mm thick butyl rubber and water balance tests showed leakage to be negligible. Adjacent pairs of channels were supplied with one of 4 inflow rates of dairy parlour wastewaters after pre-treatment in a two-stage oxidation pond system. The loading rates corresponded to hydraulic rates of approximately 20, 26, 45, and 68 mm d⁻¹; giving nominal retention times of 2, 3, 5.5, & 7 days (actual hydraulic loadings given in Table 1). Wastewater from the secondary pond was first pumped to a primary header tank (1000 L), then gravity-fed to 4 (one per pair of channels) secondary header tanks (150 L) fitted with float valves. Hourly inflows to the head of the wetlands were metered via automatically controlled (Irri Trol model MC12plus irrigation controller; California, USA) solenoid operated hydraulic valves (Richdel model 1225; Nevada, USA). The strength of the influent wastewater was increased from September 1991 (latter 5 months of trial) by reduction of the effective size of the pre-treatment aerobic pond, using a movable polyethylene barrier. Inflow rates to the 2 wetlands operating at 7 days retention time were also readjusted at this time to ~3.8 days retention (37 mm d⁻¹) as part of a concurrent experiment.

Water levels were maintained 20 mm below the gravel surface at the downstream end of the wetlands, by adjusting the height of the outflow pipes. Outflow volumes were monitored by tipping buckets which were read weekly and, as for inflows, cleaned and re-calibrated monthly. Vegetative propagules of *Schoenoplectus validus*, collected from a natural population growing locally, were planted out (8 propagules m⁻²) in one of each pair of channels in early November, 1989 (spring). Unplanted channels were retained as controls with weeds controlled periodically by hand weeding and spot applications of glyphosate herbicide. Plants were allowed to establish in the wetlands over a 3 month period, with periodic applications of NPK fertiliser, before selected inflow rates of wastewaters were introduced. Details of plant sampling in the wetlands during the trial are given in Tanner et al. (submitted).

Inflowing wastewater and the outflow of each channel were sampled fortnightly from June 1990 to January 1992 (20 months) and analysed using standard methods (APHA, 1989) for 5 day carbonaceous biochemical oxygen demand (CBOD₅; unseeded, nitrification inhibited) and suspended solids (SS; GFC filter, gravimetric); and temperature, conductivity, pH (Solomat MPM 2000 meter; Connecticut, USA) and dissolved oxygen recorded (saturated air calibrated Yellow Springs Instruments Model 54A DO meter; Ohio, USA). Faecal

coliforms (FC) were sampled four-weekly using the membrane filter M-FC agar technique (APHA, 1989). To help define the oxygen demand characteristics of the wastewater before and after wetland treatment, supplementary analysis of CBOD₂₀, chemical oxygen demand (COD; closed reflux, titrimetric; APHA 1989) and filtered (GFC) CBOD₅ were performed on 4 occasions during the spring and early summer of 1991, on the influent wastewater and each channel outflow. Potential nitrogenous oxygen demand (NBOD) was calculated as 4.33 times the NH₄-N concentration (as in Cooper, 1986), using nitrogen data from a companion study (Tanner et al., submitted). Dissolved colour due to humic compounds (yellow substance) was monitored in inflows and outflows on 5 occasions during 1991 by measuring the absorbance of filtered samples at 440 nm (10 mm path length, Shimadzu UV190 spectrophotometer), and is expressed as g_{440} (the absorption coefficient at 440 nm; Kirk, 1983).

Water quality and flow data were smoothed to remove day to day variation and reveal underlying trends. Smoothing was performed using Bayesian techniques (Upsdell, 1985; Upsdell and Wheeler, 1992) which allowed analysis of non-normal data distributions and generation of confidence intervals. To allow for the increasing variability of larger values, FC results were analysed using a Poisson error distribution and a log link function. To enable evaluation of true removal rates in the presence of seasonally fluctuating influent strengths, and valid comparison of planted and unplanted wetlands with different hydrological budgets, performance was compared where possible on a mass flow basis by combining smoothed in and out-flow rate curves, and smoothed water quality concentration curves. Adherence to first-order kinetics (assuming plug-flow) was verified and rate constants calculated from mean concentration values for the four wetlands, using graphical methods and linear regression, as outlined in Tchobanoglous and Schroeder (1987).

RESULTS AND DISCUSSION

Hydrology

Rainfall varied markedly on a month to month basis but was evenly spread through the seasons with a mean of 3.4 mm d⁻¹ recorded during the trial period (Ruakura Meteorological Station, 2.5 km west of experimental site). Pan evaporation showed a distinct seasonal pattern increasing from monthly averages of 0.5 mm d⁻¹ in winter to around 4.5 mm d⁻¹ in summer, with an annual mean of 2.7 mm d⁻¹. During the trial there was little difference between hydraulic inflow and outflow rates in the unplanted wetlands, suggesting that overall rainfall additions and evaporation losses were similar. The planted wetlands showed net water losses of around 2 mm d⁻¹, presumably due to evapotranspiration and interception losses from the plant canopy. Comparison of inflows and outflows between 900 and 1700 hours on 2 consecutive clear sunny days in early January 1992 (maximum air temperature 25 °C, pan evaporation 5.5 and 5.8 mm d⁻¹) showed evaporation rates between 0.5 and 1.0 mm hr⁻¹ for the unplanted channels, compared to losses of between 0.9 and 1.6 mm hr⁻¹ in the planted

wetlands. This was similar to maximum midday rates reported previously for planted gravel-bed wetlands operating nearby (van Oostrom and Cooper, 1990).

Temperature, pH, and conductivity

The temperature of the outflows from the wetlands were generally 1-3 °C above mean daily air temperatures at the site and around 0.7 °C below those of the inflowing wastewater, ranging from a minimum of 7-9 °C in winter to a maximum of 22 °C during summer. Planting had little effect on outflow temperatures during winter, but lowered temperatures during summer by 0.5 - 1 °C. Additional in situ measurements during sunny summer days showed plant shading resulted in 2 - 4 °C cooler surface water temperatures (20 mm depth) at midday.

Mean inflow pH values of 7.2 were reduced slightly to between 6.6 and 7.0 in the outflows. Influent conductivity ranged from 500 - 1100 $\mu\text{S cm}^{-1}$ in the first winter to summer period and 700 - 1500 in the second, reflecting both seasonal increases in wastewater loading and increased evaporation losses during the summer. Mean conductivity for the July 1990-91 year was reduced during passage through the wetlands from 793 at the inflow to between 700 and 550 $\mu\text{S cm}^{-1}$ with increasing retention time.

Oxygen demand

Dissolved oxygen levels in the influent wastewater, commonly around 1 - 2.5 g m^{-3} , rose to above 6 in mid-winter when organic loadings and temperatures in the oxidation ponds were lowest. Slightly higher mean outflow oxygen levels of 2 - 3 g m^{-3} were recorded, with a small but consistent rise (0.5 - 1 g m^{-3}) noted with increasing retention time and little difference between planted and unplanted beds.

Mean influent CBOD₅ concentrations of 57 (± 7.5) g m^{-3} during the first year of monitoring, were increased to over 200 g m^{-3} during the final spring-summer period (Fig. 1). During this period of the trial (1991/2), SS accounted for around 40 % of influent CBOD₅. COD and calculated NBOD levels in the influent were 3.2 and 1.5 fold higher respectively than the standard CBOD₅. Despite addition of a standard nitrification inhibitor (APHA, 1989), CBOD₂₀ determinations showed wide fluctuations due to various degrees of nitrification (confirmed by subsequent ammonia and nitrate analyses) occurring during the extended incubation period.

Figure 2 shows mass loadings and removal rates of CBOD₅ for the planted and unplanted channels during the 20 month study period, while Table 1 summarises loading and removal data for the annual period July 1990 to July 1991 (i.e. starting 7 months after planting and 4 months after initiation of wastewater inflows). Between 35 and 40% of outflowing CBOD₅ was attributable to SS during the final spring-summer period. Removal of CBOD₅ was similar for planted and unplanted channels, with mean reductions in the first year increasing from 76 to 92% and 60 to 85% respectively with increasing retention time. At the

highest loading tested, mean annual CBOD₅ removal in the unplanted wetland was significantly ($P < 0.01$) lower than that of the planted systems. Mass removal rates closely followed loading rates with a maximum removal of $\sim 13 \text{ g m}^{-2} \text{ d}^{-1}$ recorded during the period of highest loading (Sept. 1991). Removal of CBOD₅ in relation to retention time was poorly described by first-order (plug flow) kinetic models. Within the range tested, mass removal rates for the planted wetlands showed a near-linear relationship to mass loading during the first year of monitoring (Fig. 3). The relationship, when converted to volumetric rates, was very similar to that derived for 12 comparable wetland systems treating sewage wastewaters by Conley et al. (1991).

Mean mass removal of total BOD (CBOD₅ + NBOD; Fig. 3) rose to nearly $6 \text{ g m}^{-2} \text{ d}^{-1}$ while percentage removals decreased from around 80 to 50% with increasing loading in the planted wetlands. Removal of NBOD is likely to be higher than that calculated from NH₄-N data, due to in situ ammonification of organic forms of N (see Tanner et al., submitted). The planted wetlands showed greater removal of total BOD than the unplanted wetlands, particularly at higher loadings (significant at $P < 0.001$), where in the absence of net ammonia removal, mass reductions of total oxygen demand in the unplanted wetlands declined to $< 2 \text{ g m}^{-2} \text{ d}^{-1}$ (about 20% removal). COD levels in the outflows were 4 to 10 times higher than the CBOD₅ (Fig. 4), and showed a close relationship to the total BOD (Fig. 4). Removal of total BOD (assuming plug-flow) followed first-order kinetics, with a K_t of 0.17 ($r^2 = 0.94$), and a K_{20} of 0.22 d^{-1} (using a van't Hoff-Arrhenius temperature coefficient (θ) of 1.06; Conley et al., 1991).

Suspended solids

High and often variable levels of SS, largely composed of phytoplankton biomass and detritus, are common in discharges from oxidation ponds (Hickey et al., 1989; Wrigley & Toerien, 1990). Influent SS levels varied markedly in the present study ($60 - 250 \text{ g m}^{-3}$), showing an overall mean of 120 g m^{-3} with no apparent seasonal trends (Fig. 1). During the first year of monitoring, SS levels were reduced by over 75% at all loading rates, giving mean effluent concentrations between 14 and 36 g m^{-3} (Table 1). Outflow SS concentrations in the lowest loaded wetlands responded rapidly to increases in wastewater loadings in September 1991, but the mean percentage removal remained relatively stable ($\sim 80\%$) until the end of the trial. Mass removal rates for SS showed a linear relationship to mass loading (Fig. 3), similar to that reported by Conley et al. (1991). There was no discernible difference between the performance of planted and unplanted channels, with both showing similar fluctuations during the monitoring period (Fig. 5). Other gravel-bed wetland studies employing unplanted controls (e.g. Wolverton et al., 1983; Gersberg et al. 1986; Bavor et al., 1987; van Oostrom and Cooper, 1990) have also generally found little difference in their solids removals compared to planted systems, suggesting removal is primarily due to physical processes which are little affected by plant growth.

Excavation of gravel substrates along the channels showed accumulation of organic solids occurred preferentially at the head of the channels, as noted in other studies (Reed and Brown, 1992). These accumulations resulted in partial surface-flow of applied wastewaters in the upstream 1 - 2 m of the highest loaded channels, apparently due to partial clogging of interstitial spaces within the gravel matrix. Further studies are in progress to evaluate the net accumulation rates of organic and inorganic solids, which are likely to be critical factors affecting the flow dynamics, and the long-term functioning and sustainability of subsurface-flow wetland systems.

Faecal coliforms

Mean FC densities in the influent showed a gradual rise during the monitoring period from $< 1 \times 10^3$ to $> 1 \times 10^6$ mpn (100 ml)⁻¹ (Fig 1). FC removal rates closely followed loading rates during the course of the experiment showing an overall linear relationship (Fig 3). Mean removal rates from 90 to over 99% were recorded with increasing retention time during the first year of monitoring (Table 1), with no significant difference ($P < 0.05$) between the planted and unplanted wetlands. FC removals of around 90% were still recorded over the final summer period, at loading of $92 \text{ mpn} \times 10^7 \text{ m}^{-2} \text{ d}^{-1}$. Gersberg et al. (1987) have previously reported improved total coliform removal in gravel-bed wetlands planted with *Schoenoplectus validus* compared to that in unvegetated controls. Mean effluent FC concentrations of between 15 and 40 ($\times 10^3$) mpn (100ml)⁻¹ were recorded at intermediate retention times (Table 1), suggesting livestock drinking water guidelines of 1000 mpn (100ml)⁻¹ would generally be met in receiving waters at moderate levels of dilution (> 15 to 40 fold).

Dissolved colour

The potential effects of dissolved colour on receiving water colour are likely to vary depending on the nature of the existing particulate and dissolved determinants of hue, with blue-coloured waters in particular, showing high sensitivity to small additions of yellow substance (Davies-Colley and Close, 1990). Humic compounds typically account for 85-100% of absorbance in the visible region of the spectrum in natural waters and comprise around 40-60% of the dissolved organic carbon (Thurman, 1985). They are considered to be largely refractory compounds, with residence times in excess of 100 days in natural waters.

The dairy farm wastewaters studied, were highly coloured and strongly light attenuating with mean g_{440} values of $27 \pm 5.9 \text{ m}^{-1}$ recorded. This was considerably higher than values obtained by Davies-Colley et al. (1992) in a study of 11 domestic sewage oxidation ponds in New Zealand (mean 6 m^{-1}), reflecting the more concentrated nature of dairy farm wastewaters and their high levels of organic decomposition products. Outflow g_{440} values were similar to influent levels at short retention times, but showed a gradual decline with increasing retention time (Fig 6). There was no apparent difference between dissolved colour outflows from the

planted and unplanted beds. Moore and Skarda (1992) also reported minimal colour removal (generally <10%) in rock-filter wetlands treating pulp mill wastewaters, with greater reductions apparent in the planted systems operating at 10 days than at 2 days retention.

The potential for photolytic degradation of humic compounds would have been minimal in the subsurface-flow wetlands and likely biological decomposition rates (even in the presence of high nutrient levels) too low to account for the apparent reductions in g₄₄₀ noted with increasing retention time. Adsorption onto the surfaces of the gravel substrates and particulates, incorporation into biofilms, and hydrous metal oxide precipitation are possible removal mechanisms (Steinberg & Muenster, 1985). Such adsorption potential is likely to be limited in gravel-bed constructed wetlands. In the long-term, decomposition of accumulated organic matter and autochthonous production (plant litter) may, as in most natural wetlands (Thurman, 1985; Urban et al., 1989), result in the net export of dissolved humic compounds. Complexation of metals and trace anions (including phosphate) by these humic substances is likely to markedly affect their environmental behaviour both during treatment and in receiving waters (Steinberg & Muenster, 1985; Suffet & MacCarthy, 1989).

CONCLUSIONS

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Constructed wetlands operating at nominal retention times between 2 and 7 days show considerable potential for the removal of BOD, SS and FC levels from dairy farm wastewaters after pre-treatment in oxidation ponds. Dissolved colour was only reduced at the highest retention times tested, and is unlikely to be sustainable in the long term. Monotonic relationships were found between mass loading and removal rates for CBOD₅ (planted wetlands only), and SS and FC (both planted and unplanted wetlands) over the range tested. These were similar to those reported for CBOD and SS removal in comparable systems treating domestic sewage. NBOD in the dairy farm wastewaters tested, was the largest component of total BOD in both inflows and outflows; this should be taken into consideration in the design and performance assessment of wetland systems treating agricultural and other high ammonia wastewaters. Over an annual period, mean mass removals of total BOD (CBOD+NBOD) in the planted wetlands increased with mass loading, while percentage removal decreased from around 80% at the lowest loading tested to 50% in the highest. Mass removal of SS and FC was similar for both planted and unplanted channels, but removal of total BOD and CBOD₅ at high loadings, was significantly improved in the planted wetlands.

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Figures:

Fig. 1. Influent concentrations of CBOD and SS (g m^{-3}), and FC (mpn (100ml)^{-1} , log scale). Fitted curves show mean values (solid lines) and their 95% confidence intervals (fine dashed lines).

Fig. 2. Mean mass loading (solid lines) and removal rates of CBOD₅ recorded for each hydraulic loading rate (expressed as nominal retention time) in the planted (dotted line) and unplanted (dashed line) wetlands over the 20 month monitoring period. The arrow heads show when the influent wastewater strength to all wetlands was increased, and the hydraulic loading to the wetland operating at ~7 day retention was increased, giving a modified retention time of ~3.8 days. The mass loadings achieved in the planted and unplanted wetland pairs differed slightly (Table 1), but showed almost identical patterns during the trials. To aid comparison, the mass removal rates for both the planted and unplanted wetlands have been proportionally standardised to a single mean loading curve. To aid clarity, the individual data points have been omitted and only smoothed mean curves shown for the data. Figure 1 (in which data points are retained) gives an example of how the smoothing operation relates to the data.

Fig. 3. Relationship between mean mass loading and removal rates of CBOD, total BOD, SS, and FC for the 4 planted (Δ) and 4 unplanted ($\bullet$) wetlands for the annual period July 1990 to July 1991. All rates are expressed as $\text{g m}^{-2} \text{d}^{-1}$ except those for FC; $\text{mpn} \times 10^7 \text{ m}^{-2} \text{d}^{-1}$. Smoothed means and their 85% confidence intervals are shown for the planted (solid lines and heavy shading respectively) and unplanted wetlands (heavy and fine dashed lines respectively, no shading). Where confidence intervals overlap, lines are not significantly different at the 95% level of confidence. The lines were forced to go through the origin, where a zero loading corresponds to zero removal. The 1:1 line (dotted) is shown to indicate 100% removal; on the FC graph it is obscured by the data.

Fig. 4. Relationship between concentrations of COD, and carbonaceous and total BOD for the wetland outflows (all g m^{-3}), during the final spring-early summer period. The regression lines shown are logarithmic ($r^2 = 0.84$) and linear ($r^2 = 0.71$) least squares fits respectively ($n = 40$).

Fig. 5. Mean mass loading (solid lines) and removal rates of SS recorded for each hydraulic loading rate (expressed as days retention time) in the planted (dotted line) and unplanted (dashed line) wetlands over the 20 month monitoring period. Other details as for Fig. 2.

Fig. 6. Relationship between effluent dissolved colour expressed as g440 and the nominal retention time of wastewater in the wetlands. Each value is the mean of 5 samples sampled over an annual period (1991). The solid line shown is a least squares linear regression ($r^2 = 0.82$).

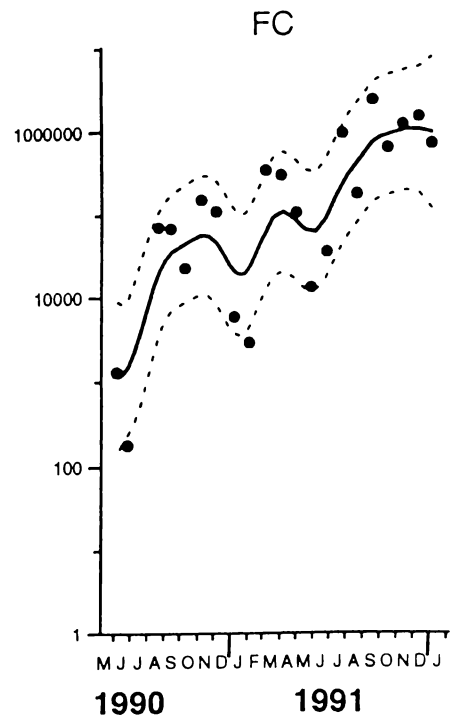
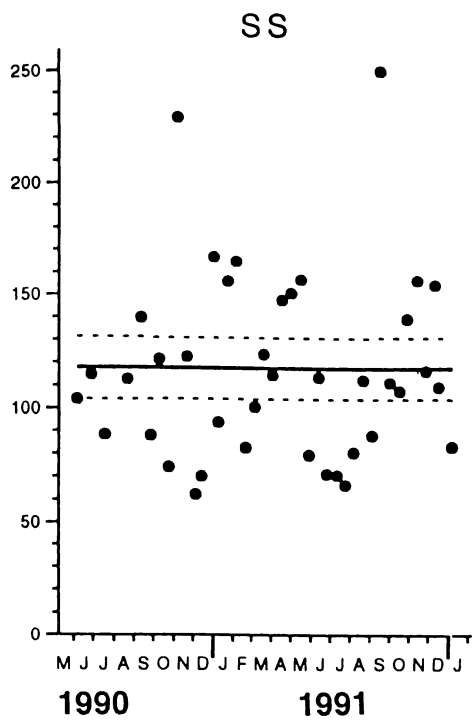
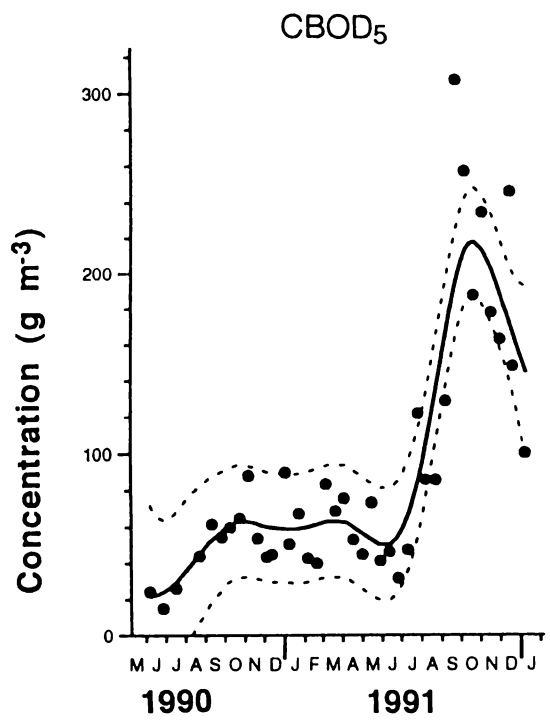


Figure 1

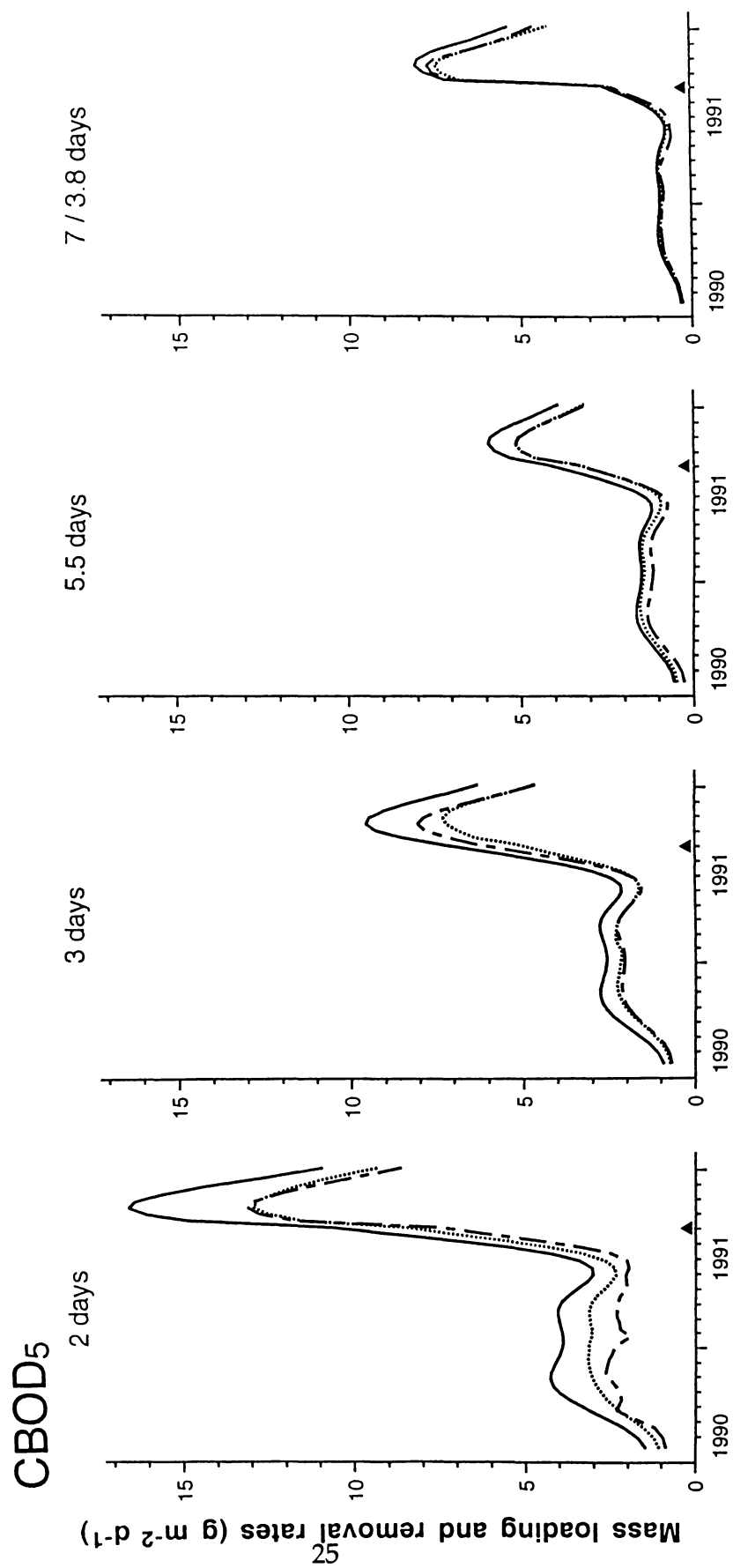


Figure 2

Figure 3

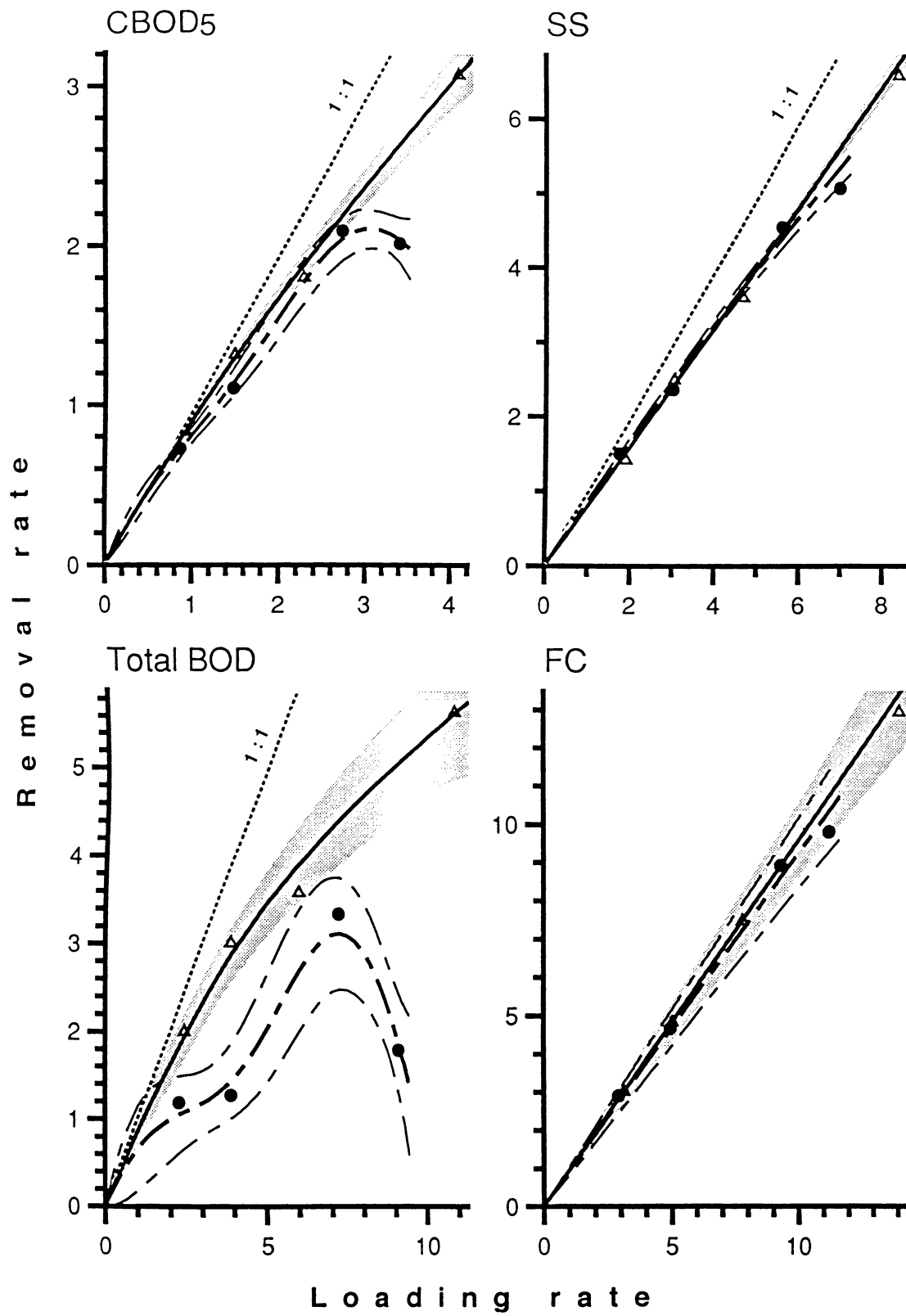
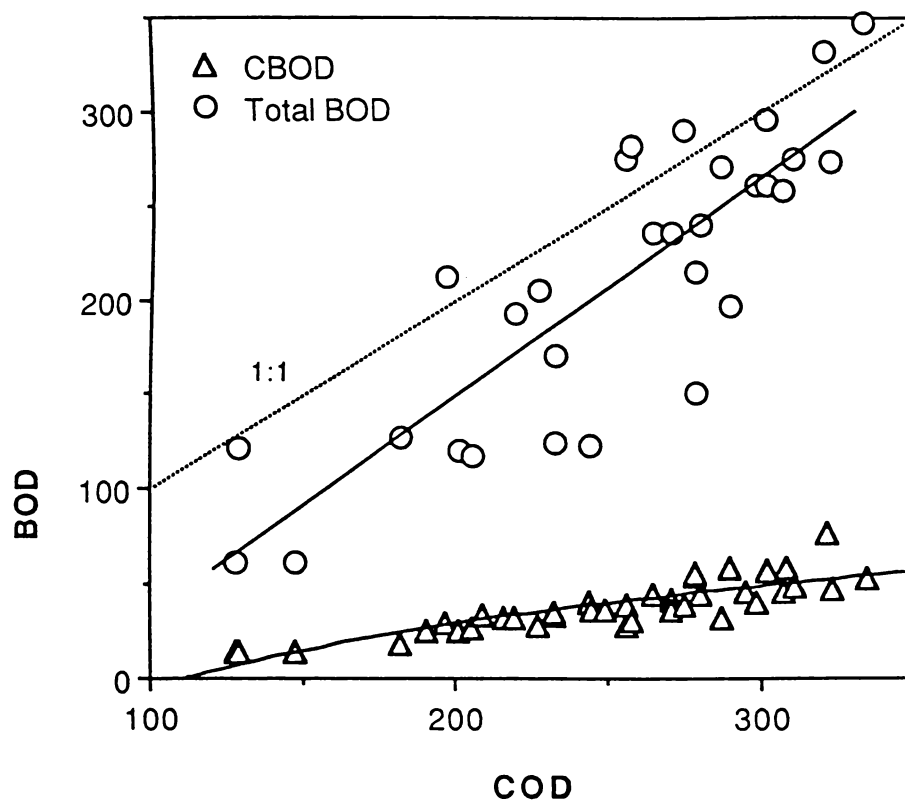


Figure 4



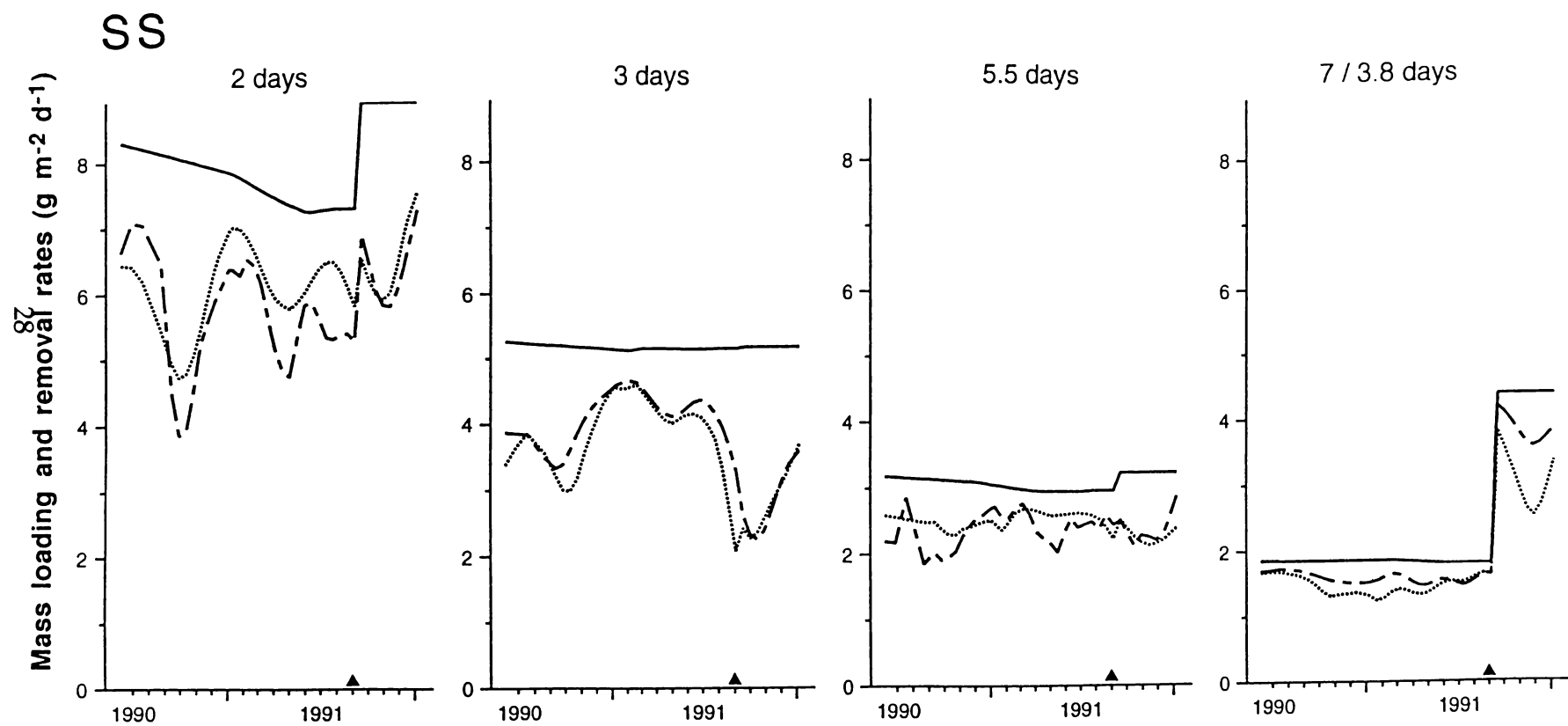


Figure 5

Figure 6

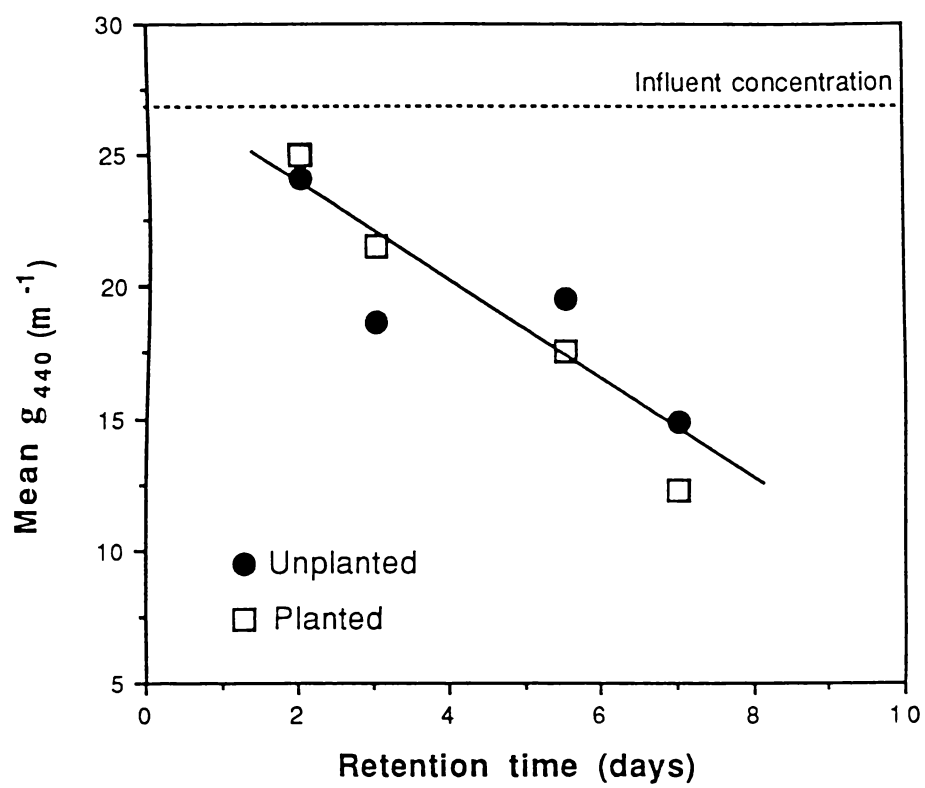


Table 1: Summary of mean annual hydraulic loadings, and mean mass loadings, percentage mass reductions and final effluent concentrations of CBOD₅, SS and FC for the experimental wetlands during the period July 1990 - July 1991. Standard deviations are shown in parentheses.

Nominal retention time (days)	CBOD ₅								SS				FC							
	(influent concentration $57 \pm 7.5 \text{ g m}^{-3}$)								(influent concentration $118 \pm 0.01 \text{ g m}^{-3}$)				(influent concentration $189 \pm 170 \text{ mpn} \times 10^3 (100 \text{ ml})^{-1}$)							
	Hydraulic loading (mm d^{-1})		Mass loading ($\text{g m}^{-2} \text{ d}^{-1}$)		Percentage reduction		Effluent concentration (g m^{-3})		Mass loading ($\text{g m}^{-2} \text{ d}^{-1}$)		Percentage reduction		Effluent concentration (g m^{-3})		Mass loading ($\text{mpn} \times 10^7 \text{ m}^{-2} \text{ d}^{-1}$)		Percentage reduction		Effluent concentration ($\text{mpn} \times 10^3 (100 \text{ ml})^{-1}$)	
	unplanted	planted	unplanted	planted	unplanted	planted	unplanted	planted	unplanted	planted	unplanted	planted	unplanted	planted	unplanted	planted	unplanted	planted	unplanted	planted
2	63.0 (8.7)	72.5 (1.5)	3.4 (0.51)	4.1 (0.54)	60 (5.3)	76 (3.4)	31 (15.4)	27 (16.9)	7.0 (0.59)	8.5 (0.04)	73 (11.3)	78 (10.1)	35 (12.7)	33 (11.9)	10.5 (8.8)	13.6 (12.2)	91 (2.3)	95 (1.4)	63 (67.4)	45 (48.0)
3	45.2 (5.0)	42.5 (5.0)	2.7 (0.36)	2.3 (0.30)	77 (3.7)	80 (4.1)	21 (10.5)	22 (16.5)	5.6 (0.06)	4.7 (0.03)	81 (8.2)	78 (10.0)	34 (16.0)	36 (16.2)	9.0 (8.1)	7.6 (6.8)	94 (3.0)	95 (1.6)	27 (31.5)	39 (42.9)
5.5	26.1 (1.0)	26.4 (0.9)	1.5 (0.20)	1.5 (0.19)	75 (3.4)	90 (6.0)	18 (8.0)	15 (12.2)	3.0 (0.10)	3.1 (0.05)	78 (10.4)	83 (5.6)	26 (11.0)	30 (12.6)	4.8 (4.2)	4.9 (4.3)	92 (5.8)	98.6 (0.7)	16 (11.5)	15 (16.5)
7	20.0 (9.2)	20.9 (9.1)	0.9 (0.11)	0.9 (0.12)	85 (5.4)	92 (1.9)	11 (10.6)	11 (10.2)	1.8 (0.01)	1.9 (0.03)	85 (3.8)	76 (6.2)	14.2 (7.8)	34 (15.8)	2.8 (2.6)	3.0 (2.7)	99.1 (0.6)	99.6 (0.4)	6.9 (12.0)	8.8 (21.0)

EFFECT OF LOADING RATE AND PLANTING ON TREATMENT OF DAIRY FARM WASTEWATERS IN CONSTRUCTED WETLANDS II. REMOVAL OF NITROGEN AND PHOSPHORUS

CHRIS C. TANNER^{1,2}, JOHN S. CLAYTON¹ AND MARTIN P. UPSDELL³

¹National Institute of Water and Atmospheric Research, P.O. Box 11-115, Hamilton; ² Department of Biological Sciences, University of Waikato, Private Bag 3105, Hamilton, and ³ New Zealand Pastoral Agriculture Research Institute, Private Bag 3123, Hamilton, New Zealand.

Abstract— The effect of influent loading rate on mass removal of nitrogen and phosphorus from dairy parlour wastewaters was compared in four pairs of planted (*Schoenoplectus validus*) and unplanted gravel-bed wetlands (each 19 m²). The wetlands were operated at nominal retention times of 7, 5.5, 3 and 2 days, with in and out-flows sampled fortnightly over a 20 month period. Hydraulic flows were monitored to enable calculation of the mass flows of nutrients, and plant biomass and tissue nutrient levels sampled to evaluate plant nutrient uptake. Influent water quality varied markedly during the trial period (TN, 10-110 ; NH₄-N, 5-70 ; and TP 8-18 g m⁻³). As theoretical wastewater retention times decreased from 7 to 2 days, mean reduction of TN decreased from 41 to 12% and 75 to 48%, in the unplanted and planted wetlands respectively, and TP removal decreased from 36 to 12% and 74 to 37% . Maximum TN and TP mass removal rates of 5 and 0.7 g m⁻² d⁻¹ respectively were recorded during the study. Mass removal rates of TN and TP averaged over an annual period, increased gradually with mass loading rates in the planted wetlands, but showed a marked decline at high loadings in the unplanted wetlands. Net storage by plants in the first year of monitoring accounted for between 3 and 20% of the greater N removal and between 3 and 60% of the greater P removal recorded in the planted compared with unplanted wetlands.

Key words- artificial wetlands, subsurface-flow, root-zone method, nitrogen, ammonium, phosphorus, agricultural wastewaters, *Schoenoplectus validus*, bulrush.

INTRODUCTION

Agricultural wastewaters characteristically contain high levels of organic matter and nutrients. The potential of constructed wetlands to reduce organic pollutants from dairy farm wastewaters after oxidation pond pre-treatment has been investigated in a companion study (Tanner et al, submitted). The present study investigates the effect of wastewater loading rates and planting with *Schoenoplectus validus* (M. Vahl.) A. Love et D. Love (soft-stemmed bulrush) on removal of nitrogen (N) and phosphorus (P) in subsurface-flow gravel-bed

wetlands. In addition to the undesirable effects these nutrients may have on aquatic plant proliferation and the eutrophication of water-bodies, the direct toxicity of the high levels of ammoniacal N present in dairy farm wastewater discharges and their high nitrogenous oxygen demand are of concern (Hickey et al., 1989).

The potential for nutrient uptake and storage by aquatic plants, sediments, detritus, microbes and fauna in wetland treatment systems is finite (Howard-Williams, 1985). Ammonia volatilisation losses are likely to be negligible at temperatures below 25 °C, unless the pH of the wastewaters increases above eight (Jayaweera and Mikkelsen, 1991). Microbial transformations to gaseous products (e.g. nitrification-denitrification and sulphate reduction) appear to be the only sustainable means of nutrient removal. Apart from providing additional N and P storage compartments in gravel-bed wetlands, plants growing hydroponically in the substratum may enhance microbial N removal by modifying environmental conditions in the substratum (Hansen and Anderson, 1981) and providing carbon substrates (plant litter and organic exudates) for denitrifying bacteria (Gersberg et al., 1983). The internal aeration system (aerenchyma) of plants adapted to wetland conditions increases sediment-atmosphere gas exchange. Diffusive and mass-flow transport of oxygen via plant shoots and its release in the plant root-zone (Armstrong et al., 1990) may increase redox potentials in the substratum, enhancing microbial decomposition and nitrification rates (Howard-Williams, 1985; Gersberg et al., 1986), and increasing sediment P sorption capacity (Wathugala et al., 1987)

The objectives of the present study were to:

1. Investigate the effect of loading rate on removal of N and P from dairy farm wastewaters in subsurface-flow gravel-bed constructed wetlands, and
2. Evaluate the effect of planting with the emergent aquatic plant *S. validus* on treatment performance.

METHODS

Full details of the design and operation of the 8 experimental wetlands and the organic constituents of the dairy farm wastewaters are given in Tanner et al. (submitted). Briefly, trials were carried out on the Ruakura Research Farm (Hamilton, New Zealand) between January 1990 and 1992 in 8 channels (9.5 x 2 x 0.6 m deep), lined with butyl rubber, and filled to 0.4 m depth with alluvial rhyolitic gravel (10-30mm diameter, 35-37% effective pore space). Adjacent pairs of channels were each supplied with one of 4 selected inflow rates (nominal retention times of 2, 3, 5.5, & 7 days) of dairy parlour wastewaters, after pre-treatment in a two-stage oxidation pond system. In September 1991 (latter 5 months of trial), the strength of the influent wastewater was increased to all wetlands, and the hydraulic inflow rate increased to the lowest loaded wetlands, reducing their retention time from 7 to ~3.8 days.

One of the channel pairs at each of the four hydraulic loading rates tested, was planted with *S. validus* and one maintained plant-free. In the first winter of the trial (August 1990)

senescent above-ground plant biomass was harvested and removed from the wetlands to simplify monitoring of plant production. Thereafter, above (including standing litter) and below-ground growth was sampled at approximately two month intervals during the year to provide estimates of plant nutrient uptake. Care was taken to minimise disturbance of the wetlands during sampling, with removal of plant material limited to less than 2% of the area of each wetland in total during the trial. Using a random coordinate positioning system, twenty-four 60 mm diameter (28 cm²) core samples were obtained from each wetland at each sampling. The uncohesive gravel substrata were removed from the cores in situ, using a commercial wet and dry vacuum cleaner, with root material retained in a customised 2mm mesh filter chamber. Gravel substrata were replaced after sampling. Shoots, roots and rhizomes, litter, and flower heads were washed, oven-dried (80°C) to constant weight, and analysed for N and P using automated methods, after Kjeldahl digestion (salicylic acid modification using selenium catalyst). Plant data was analysed by ANOVA (Genstat 5; Lawes Agricultural Trust).

Wastewater inflows and outflows were sampled approximately fortnightly from June 1990 to January 1992 (20 months) and analysed for ammonium (NH₄-N; Brown, 1973), nitrate and nitrite N (abbreviated to NO₃-N; method 418C, APHA, 1985), TKN (modified micro Kjeldahl digestion using selenium catalyst); TP and DRP (total and dissolved reactive P; NWASCA, 1982). Total nitrogen (TN) was calculated as the sum of TKN and NO₃-N. Water quality and flow data were smoothed to remove day to day variation and reveal underlying trends. Smoothing was performed using Bayesian techniques (Upsdell, 1985; Upsdell and Wheeler, 1992) which allowed analysis of non-normal data distributions and generation of confidence intervals. To allow for the increasing variability of larger values, NO₃-N results were analysed using a Poisson error distribution and a log link function. To enable evaluation of true removal rates in the presence of seasonally fluctuating influent strengths, and valid comparison of planted and unplanted wetlands with different hydrological budgets, performance was compared where possible on a mass flow basis by combining smoothed in and out-flow rate curves, and smoothed water quality concentration curves. Adherence to first-order kinetics (assuming plug-flow) was verified and rate constants calculated from mean concentration values for the four wetlands, using graphical methods and linear regression, as outlined in Tchobanoglous and Schroeder (1987).

RESULTS

Inflows

Influent wastewater concentrations of N and P varied seasonally (Fig. 1) increasing rapidly in the spring (October-November) as seasonal milk and concomitant wastewater production increased following calving. Mean TN and TP concentrations of 38.2 (± 15.7) and 11.2 (± 1.9) g m⁻³ during the annual period July 1990 to July 1991 resulted in mean loading

rates between 0.6 and 2.7 g N m⁻² d⁻¹, and 0.17 and 0.8 g P m⁻² d⁻¹ (Table 1). TN and TP concentrations rose to 110 and 18 g m⁻³ respectively in the second summer (1991/92) increasing mass loadings to between 3 and 8 g N m⁻² d⁻¹, and 0.5 and 1.3 g P m⁻² d⁻¹ (Figs. 2 and 3). DRP comprised around 80% of influent TP, whilst around 57% of TN was in the form of NH₄-N, 9% as NO₃-N, and by difference 34% organic N. NO₃-N fluctuated between < 0.1 and 8 with a mean of around 3 g m⁻³.

Removal rates

TN removal rates closely followed inflow loading patterns (Fig. 2), with maximum apparent removal rates of around 5 g m⁻² d⁻¹ recorded in the final summer when loadings were increased to 8 g m⁻² d⁻¹. TP removal rates (Fig. 3) only loosely followed loading rates, with fluctuations of greatest magnitude noted for the planted wetlands. Peaks of TP removal occurred during the first summer, coincident with seasonal increases in temperature and plant biomass. Periods of net P export from the wetlands were noted, particularly in the unplanted wetland operating at the highest loading, and in the final spring when mass loadings were markedly increased. Mean annual TN and TP mass removal rates in the planted wetlands increased gradually with mass loading (Fig. 4). Mass removal of TN was significantly lower ($P < 0.01$) in the unplanted wetlands. Both N and P removal declined markedly at the higher loading rates tested in the unplanted wetlands, dropping to below one third of that recorded at equivalent loadings in the planted beds. Removal of TN and TP followed first-order kinetics (assuming plug-flow), with reaction rate constants (K_t) of 0.16 d⁻¹ ($r^2 = 0.86$) and 0.14 d⁻¹ ($r^2 = 0.94$) respectively.

DRP comprised between 70 and 80% of outflow TP in both planted and unplanted wetlands (similar to % in inflows); while NH₄-N increased to around 70% of TN in the outflows, with NO₃-N and organic N declining to 5-10% and 25-30% respectively. Percentage NH₄-N removals in the planted wetlands (Table 1) increased from 34 to 71% with increasing retention time, while unplanted channels showed lower more variable NH₄-N removals of between -7 and 19%.

Plant uptake

Seasonal growth of plant shoots began in early September (spring) with maximum plant biomass and flowering occurring during January-February (mid summer). Seasonal maximum total and potentially harvestable (above-ground) plant biomass declined from 3.6 to 2.9 and 2.6 to 1.9 kg m⁻² respectively with decreasing wastewater loadings. Gradients of diminishing plant growth were also apparent down the wetland channels, particularly at the lower loading rates tested, suggesting reduced nutrient availability was limiting plant growth. Further details of plant performance in relation to loading rate will be the subject of a future publication.

Table 2 shows net annual storage of N and P in plant tissues and standing litter in the experimental wetlands. Less than 7 % of the annual loadings and less than 8% of the annual

removals of N and P recorded for the wetlands could be ascribed to such plant storage. During active growth periods (which generally coincided with seasonally high influent loadings) plant uptake could still only account for around 11-26% of the N and 11-29% of the P removal rates recorded over the same period in the wetlands. Net annual plant storage in the first year of monitoring, represented around 3-19% and 3-60% respectively of the greater annual N and P removals recorded for the planted compared to the unplanted wetlands.

DISCUSSION

Planted wetlands showed greater overall removal of N and P from dairy farm wastewaters than unplanted wetlands, with differences most apparent in the higher loaded wetlands. A number of other studies that have included unplanted control wetlands have also found increased N removal from ammonia-rich wastewaters in planted wetlands (Wolverton et al., 1983; Gersberg et al., 1986; Wathugala et al., 1987; Fisher, 1990; and McIntyre and Riha, 1991). In the present study, mean annual mass removal rates of N and P in the planted wetlands rose, and percentage removals decreased as mass loadings were increased. TN removal rates in relation to loading were similar to those derived in studies of two other comparable systems treating high ammonia domestic sewage wastewaters (Fig. 5), but significantly above those reported for systems treating meat processing wastewaters (van Oostrom and Cooper, 1990).

Studies by Breen (1990) and Rogers et al. (1990, 91) have shown plant uptake of both N and P can be a significant removal mechanism in small batch-fed bucket-scale systems, where high surface and edge to volume ratios maximise light interception and root-wastewater contact, and plants are in their active growth phase. However, calculations based on sustainable rates of plant productivity and tissue nutrient levels in full-scale wetlands (e.g. Spangler et al., 1976; Howard-Williams, 1985; Gersberg et al., 1986) suggest that rates of harvestable plant nutrient uptake are unlikely to be significant in wastewater treatment systems operating at realistic loading rates. In the present study, plant nutrient storage compartments were still developing. Estimates of plant nutrient uptake showed net annual storage in plants and standing litter could only account for between 3 and 20% of the improved N and 3 and 60% of the improved P removal observed in the planted wetlands. This suggests that other plant-mediated factors, such as increased storage and sorption potential in plant litter and organic residues, or modifications of the wetland environment (e.g. rhizosphere oxidation ; Armstrong et al., 1990) enhanced N and P removal in planted wetlands. Plant rhizosphere aeration may stimulate aerobic decomposition processes, increasing nitrification and subsequent gaseous losses of N via denitrification (Reddy et al., 1989; Hansen and Anderson, 1981), and decreasing relative levels of dissimilatory nitrate reduction to ammonium (Tiedje, 1988).

P accumulation of 90 g kg^{-1} (125 g m^{-3}) of substrate has been noted previously in wetland microcosms employing the same rhyolitic gravel as that used in the present study (C.C. Tanner, unpubl. data). Spangler et al. (1976), Breen (1990) and Mann (1990) also found substrate P adsorption to be an important removal mechanism in the gravel-bed wetland systems they studied. Walthugala et al. (1987) noted increased P accumulation associated with the aerobic upper root-zone of *Phragmites* growing in sand-bed wetland microcosms. Elevated redox potentials and levels of plant-derived humic substances in the root-zone may have increased sediment P sorption and CO_3^{2-} precipitation with electro-active elements such as iron (Bostrom et al., 1982) or increased P bound in the microbial pool. The sustainability of such substrate sorption and storage processes is likely to be limited at high application rates such as those tested in the present study. Episodes of TP export were recorded for all the experimental wetlands, suggesting that either their sorption capacity was exceeded at these times, or that biological or chemical changes in the substratum (e.g. reduced redox potentials) were resulting in desorption and release from storage compartments in the substratum.

The present study has shown that constructed wetlands, during their first two years of operation, can remove considerable quantities of N and P from ammonia-rich dairy farm wastewaters. Treatment levels recorded for the planted wetlands suggest that the direct NH_3 toxicity (e.g. USEPA, 1986) and nitrogenous oxygen demand (see Tanner et al., submitted) of the discharges would have been reduced 1.5 to 3.5 fold with increasing retention time, and TP levels reduced 1.6 to 3.8 fold. Further studies are required to clearly identify the relative importance and sustainability of different nutrient removal processes, and to evaluate ways these processes may be enhanced to improve the long-term nutrient removal capabilities of constructed wetland systems.

Acknowledgments— This study was funded by the New Zealand Foundation for Research, Science and Technology with assistance from Environment Waikato. It was undertaken as part of the D. Phil. program of the first author (University of Waikato, Hamilton, New Zealand). We are grateful to Keith Thompson (University of Waikato) for design input during the project; and Robert Davies-Colley and James Cooke of NIWA-Ecosystems for valuable comments on the manuscript. Water and plant tissue analyses were performed by the Ruakura Laboratories of AgResearch (NZ), and the experimental site was provided by the Dairying Research Corporation of New Zealand. Technical support during the trials was provided at various times during the trials by James Sukias, Aleki Taumoepeau, Phillip Rogers, Frank Parry, Richard Ford and Adam Fricker.

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Figures:

Fig. 1. Influent concentrations and fitted mean curves for (a) TN (●) and NH₄-N (○), and (b) TP (●) and DRP (○).

Fig. 2. Mean mass loading (solid lines) and removal rates of TN recorded for each hydraulic loading rate (expressed as nominal retention time) in the planted (dotted line) and unplanted (dashed line) wetlands over the 20 month monitoring period. The arrow heads show when the influent wastewater strength to all wetlands was increased, and the hydraulic loading to the wetland operating at ~7 day retention was increased, giving a modified retention time of ~3.8 days. The mass loadings achieved in the planted and unplanted wetland pairs differed slightly (Table 1), but showed almost identical patterns during the trials. To aid comparison, the mass removal rates for both the planted and unplanted wetlands have been proportionally standardised to a single mean loading curve. To aid clarity, the individual data points have been omitted and only smoothed mean curves shown for the data. Figure 1 (in which data points are retained) gives an example of how the smoothing operation relates to the data.

Fig. 3. Mean mass loading (solid lines) and removal rates of TP recorded for each hydraulic loading rate (expressed as days retention time) in the planted (dotted line) and unplanted (dashed line) wetlands over the 20 month trial period. Other details as for Fig. 2.

Fig. 4. Relationship between mass loading and removal rates (both g m⁻² d⁻¹) of TN and TP for the 4 planted (Δ) and 4 unplanted (●) wetlands over the annual period July 1990 to July 1991. Fitted mean curves and their 85% confidence intervals are shown for the planted (solid lines and heavy shading respectively) and unplanted (dashed lines and fine dashed lines without shading) wetlands. Where confidence intervals overlap, lines are not significantly different at the 95% level. The curves were forced to go through the origin, where a zero loading corresponds to zero removal. The 1:1 line (dotted) indicates 100% removal.

Fig. 5. Comparison of annual mass TN loading and removal rates found in the present study (●) with those calculated for two comparable systems (Δ - Gersberg et al., 1986; ○- Fisher, 1990) treating high ammonium domestic wastewaters. A fitted curve (solid line) and its 95% confidence interval (shaded zone) is shown for the combined data. The 1:1 line (dotted) indicates 100% removal. The data from the present study is for 4 wetlands (all planted with *S. validus*) operating side by side at different hydraulic loading rates; that from Gersberg et al. (1986) is for 3 wetlands operating side by side at the same loading rate, each planted with a different species; while that from Fisher (1990) is for 2 wetlands planted with different species, operating over 3 sequential (approximately annual) periods at different loading rates.

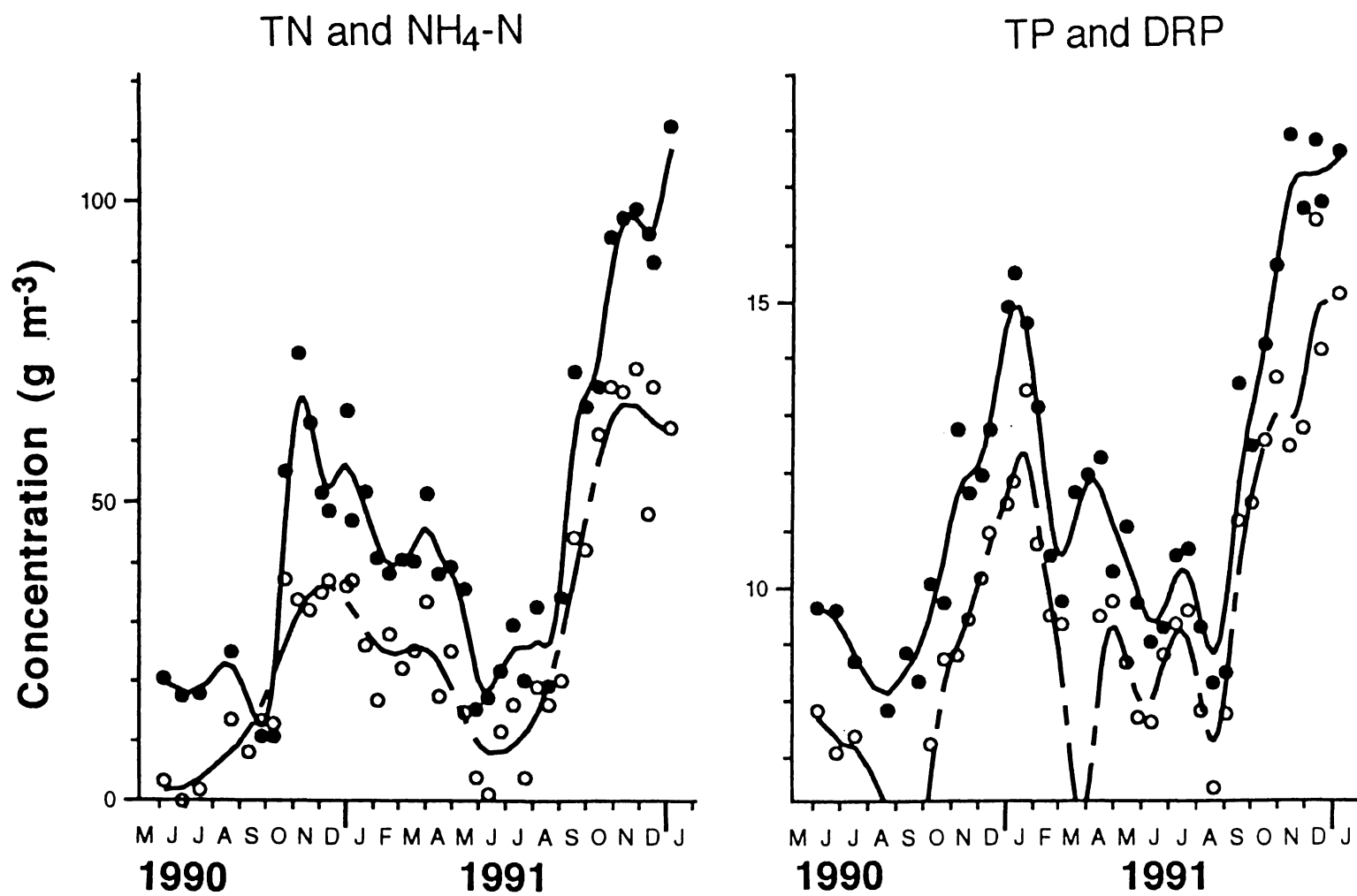


Figure 1

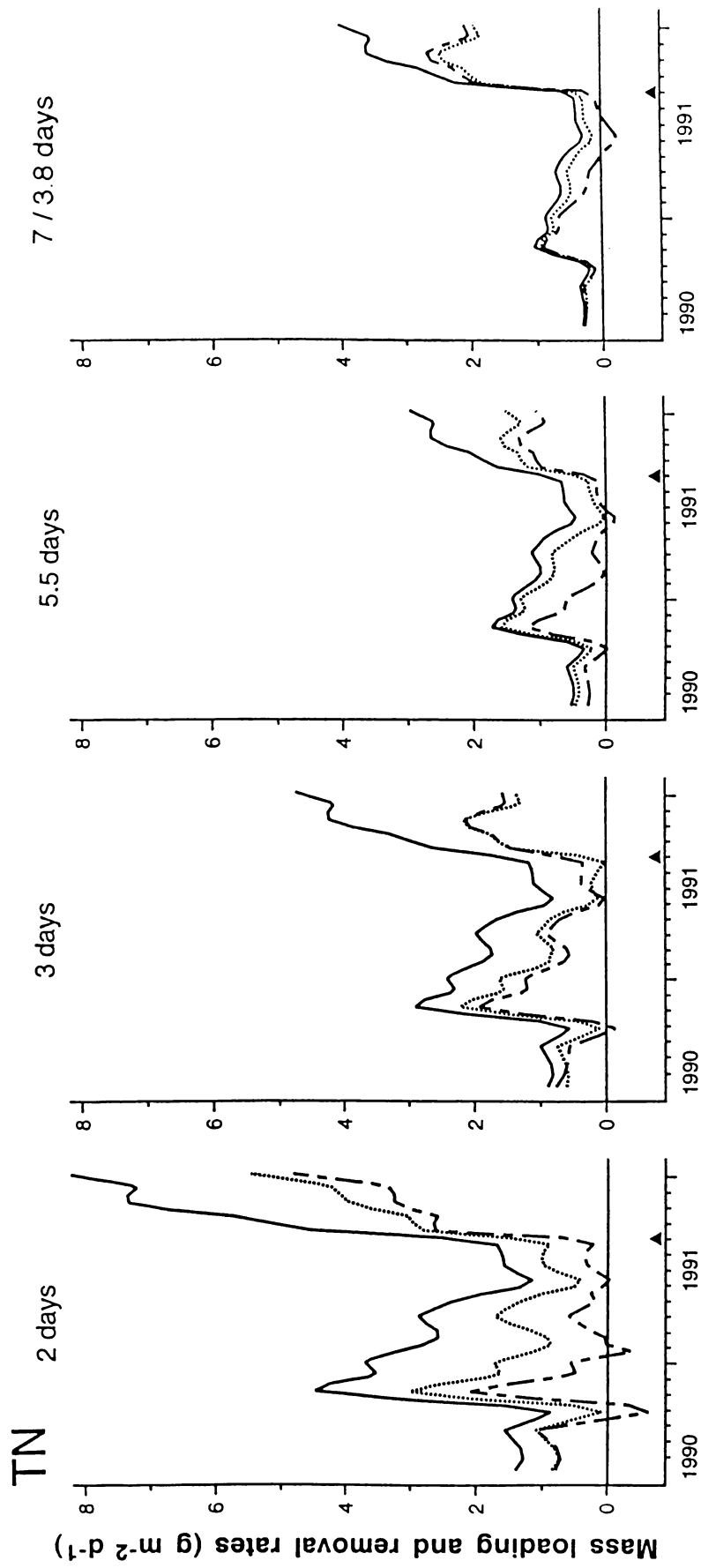


Figure 2

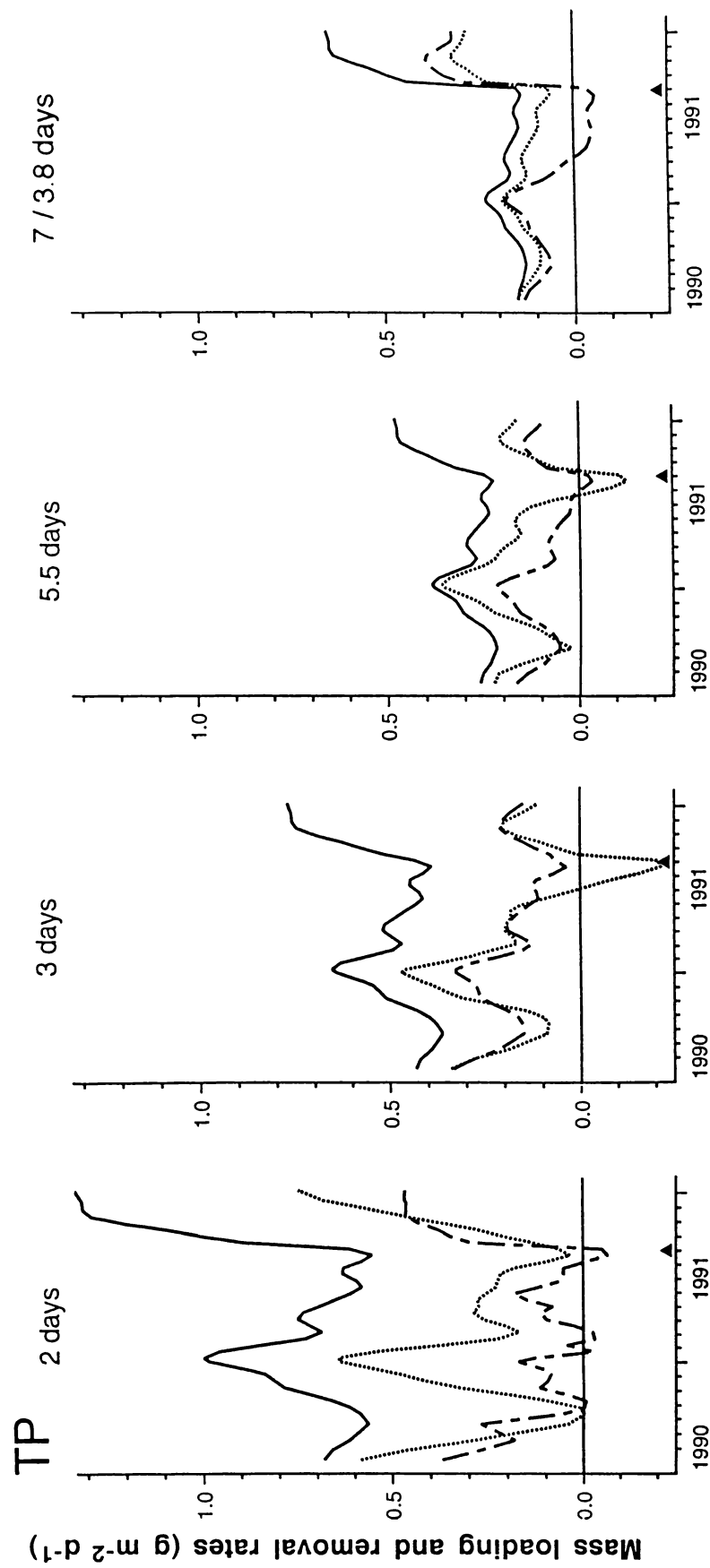


Figure 3

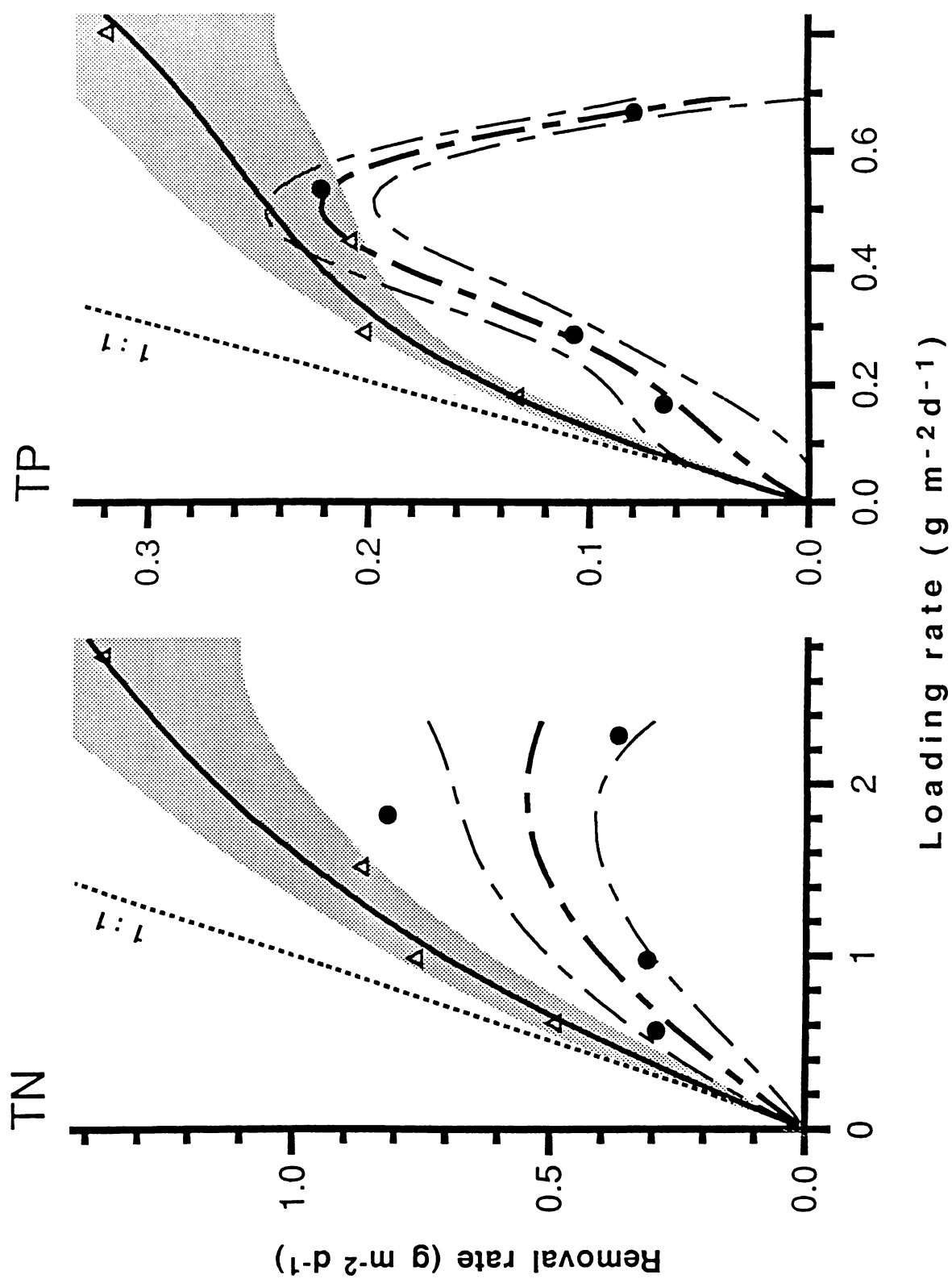


Figure 4

Figure 5

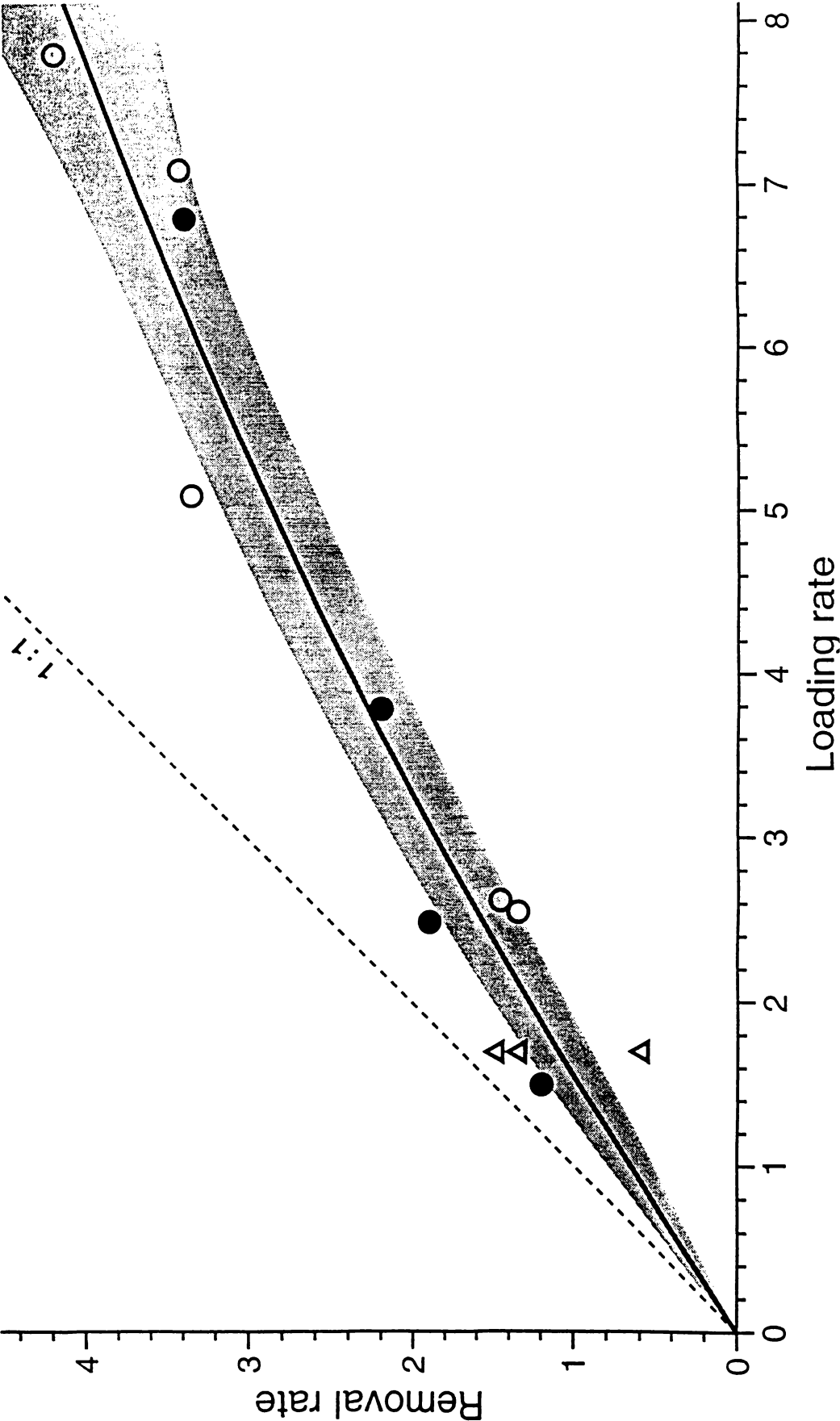


Table 1: Summary of annual mean mass inflows and outflows, and percentage removals of TN, NH₄-N and TP; and outflow concentrations of NH₄-N and DRP in the outflows during the period July 1990 - July 1991. Standard deviations are shown in parentheses.

Retention time (days)	Mass inflow (g m ⁻² d ⁻¹)		Mass outflow (g m ⁻² d ⁻¹)		Percentage TN and NH ₄ -N removal				Outflow NH ₄ -N concentration (g m ⁻³)	
	unplanted	planted	unplanted	planted	unplanted		planted		unplanted	planted
Nitrogen					TN	NH ₄	TN	NH ₄		
2	2.3 (1.0)	2.7 (1.1)	1.9 (0.8)	1.4 (0.6)	12	-7	48	34	26.3 (15.3)	21.6 (14.5)
3	1.8 (0.8)	1.5 (0.6)	1.0 (0.3)	0.65 (0.20)	39	19	52	42	22.8 (14.2)	18.3 (13.0)
5.5	1.0 (0.4)	1.0 (0.4)	0.68 (0.24)	0.23 (0.13)	25	-10	71	59	22.6 (12.8)	14.2 (11.9)
7	0.58 (0.24)	0.62 (0.26)	0.28 (0.18)	0.12 (0.04)	41	6	75	71	13.9 (13.4)	11.4 (12.2)
					Percentage TP removal		Outflow DRP concentration (g m ⁻³)			
					unplanted		planted		unplanted	planted
Phosphorus										
2	0.67 (0.13)	0.80 (0.14)	0.59 (0.15)	0.48 (0.09)	12 (10.9)		37 (18.5)		8.7 (2.7)	8.1 (2.8)
3	0.53 (0.09)	0.44 (0.07)	0.31 (0.05)	0.24 (0.06)	41 (9.0)		45 (17.0)		7.0 (2.4)	6.8 (2.8)
5.5	0.29 (0.05)	0.29 (0.05)	0.18 (0.03)	0.09 (0.05)	35 (16.5)		67 (20.8)		6.5 (2.3)	4.6 (2.7)
7	0.17 (0.03)	0.18 (0.03)	0.10 (0.06)	0.05 (0.007)	36 (39.8)		74 (5.7)		4.5 (2.7)	3.6 (2.2)

Table 2: Plant uptake of nitrogen and phosphorus in the planted experimental wetlands (July 1990-July 1991). Standard errors of the difference are given in parenthesis for plant uptake values.

Wetland retention time (days)	Annual wetland loading (g m ⁻²)	Annual wetland removal (g m ⁻²)	Net annual plant uptake (g m ⁻²)	Percentage of annual wetland loading	Percentage of annual wetland removal	Percentage removal during main growth period*
Nitrogen						
2	1000	500	10.2	1.0	2.0	11
3	555	318	14.1	2.5	4.4	20
5.5	361	277	16.4	4.5	6	18
7	226	179	14.5	6.4	8	26
			(5.9)			
Phosphorus						
2	293	117	2.2	0.8	1.9	11
3	162	77	3.9	2.4	5.1	27
5.5	106	74	3.6	3.4	4.9	21
7	65	49	2.6	3.9	5.3	29
			(1.34)			

* Main growth period defined as November 21, 1990 to January 22, 1991

CHAPTER 3



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TREATMENT OF DAIRY FARM WASTEWATERS IN HORIZONTAL AND UP-FLOW GRAVEL-BED CONSTRUCTED WETLANDS

Chris C. Tanner

*Ecosystems Division, National Institute of Water and Atmospheric Research ,
PO Box 11-115, Hamilton, New Zealand.*

ABSTRACT

The treatment performance of four horizontal and two up-flow pilot-scale constructed wetlands planted with *Schoenoplectus validus* were investigated over a 20 month period (two winters and two summers) treating dairy parlour wastewaters at a range of loading rates. Removal of BOD, TN and TP were positively correlated with retention time. Reduction of BOD (70 - 90%) and SS (40 - 90%) in relation to loading rate were similar in both flow formats. The horizontal-flow wetlands showed 40 - 90 % reduction of TN and 30 - 80 % reduction of TP. The up-flow wetlands showed reduced levels of TN and TP removal, particularly when the loading rates were increased during the latter 5 months of the trial. Results are compared with those of other published studies of horizontal and upflow wetlands treating $\text{NH}_4\text{-N}$ rich organic wastewaters.

KEYWORDS

Wastewater treatment; agricultural wastewater; wetlands; *Schoenoplectus validus*; BOD; suspended solids; nitrogen; ammonia; phosphorus.

INTRODUCTION

Farmed livestock in New Zealand have been estimated to produce 40 - 50 times the wastes of the human population (Dakers & Painter, 1982). The majority of these wastes are voided directly back onto grazed pasture, but significant point-sources of wastewaters are still generated by permanently and temporarily housed animals. The milking of 2.5 million dairy cows produces around 115 million litres of wastewater each day comprising both excreta and mud washed from holding yards using high pressure hoses, and udder and milking equipment wash-water which includes detergents and sanitisers. In New Zealand approximately half of the dairy parlour wastewaters are presently treated using oxidation ponds, the remainder treated variously by oxidation ditches, spray irrigation, ground soakage, or discharged untreated to waterways or natural wetlands.

Dairy farm oxidation ponds (anaerobic and aerobic lagoons) are constructed according to recommended guidelines (MAF, 1985, NZAEI, 1985). Studies of their performance (Hickey et al., 1989) suggest that higher levels of treatment are necessary for safe discharge in sensitive catchments, and where there is less than 250 fold dilution in the receiving stream. The high level of $\text{NH}_4\text{-N}$ in oxidation pond effluents is of particular concern because of both its direct toxicity to fish and invertebrates, and its oxygen demand (NBOD) which is commonly 4-fold higher than the measured CBOD. Because of their relatively low cost, operation and maintenance requirements, and lack of reliance on machinery or energy inputs, constructed wetlands were considered to have excellent potential for the further treatment of dairy farm oxidation pond wastewaters. The

present study is part of a program to evaluate constructed wetland technologies and develop appropriate design guidelines for use in agriculture.

Recent experimental results using 10 - 25 L microcosms (Breen and Chick, 1989; Breen, 1990; Mitchell et al., 1990; Rogers et al., 1990,91) and upscaled designs (e.g. Brett, 1989) suggest that vertical-flow formats may significantly improve wastewater treatment through enhanced contact with the plant root-zone; but there is little information enabling valid comparison of their performance with that of conventional horizontal-flow systems. This paper presents preliminary results of dairy farm wastewater treatment in both horizontal and up-flow gravel-bed constructed wetlands planted with *Schoenoplectus validus* (soft-stemmed bulrush or lake clubrush).

METHODS

Dairy parlour wastewaters pretreated in two-stage oxidation ponds on the Ruakura Research Farm (Dairy Research Corporation, Hamilton, New Zealand) were fed at set rates to 4 horizontal-flow, butyl rubber lined channels (9.5 x 2m) filled to 0.4m depth; and 2 upflow polyethylene tanks (1.5m diameter) filled to 1m depth with prewashed alluvial rhyolitic gravel (10-30mm diameter, 35-37% effective pore space). Figures 1 and 2 show the experimental wetland layouts. Aerobic wastewater was pumped to a primary header tank, then gravity-fed to 150 L. secondary header tanks fitted with float valves. Hourly inflows were metered via automatically controlled hydraulically-assisted solenoid valves, and outflows monitored by weekly readings of calibrated tipping buckets. Vegetative propagules of *S. validus* were collected from a single natural population and planted in mid November, 1989 (spring). Plantings were established over a 3 month period with periodic wastewater and NPK fertiliser applications to encourage deep root growth, before selected inflow rates of wastewaters were gradually introduced (nominal retention times 2, 3, 5.5, & 7 days, horizontal-flow; 2.2 & 6.5 days, up-flow).

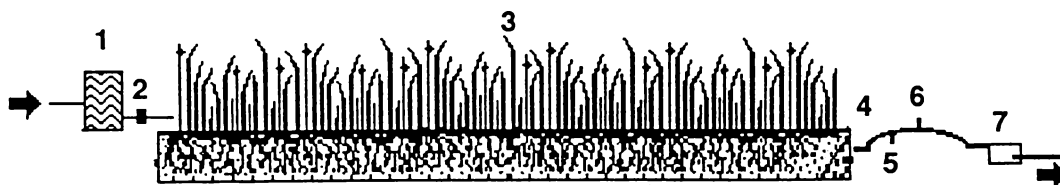


Figure 1. Horizontal-flow butyl rubber lined, gravel-bed channels; 1. 2 γ header tank, 2. Inflow via time-controlled solenoid valve, 3. Wetland plants, 4. Outflow pipe, 5. Sampling valve, 6. Adjustable flexible pipe set to control water level 30 mm below the gravel surface, 7. Tipping bucket outflow recorders with manual counters.

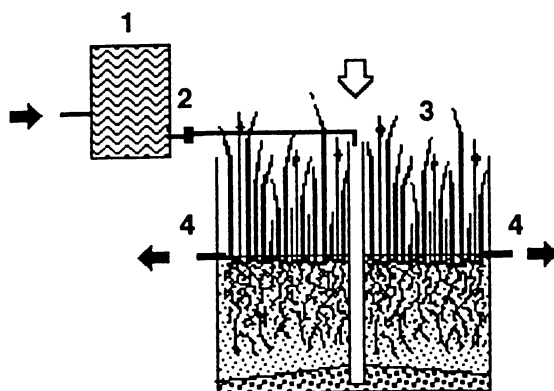


Figure 2. Upflow tanks showing central inflow pipe to basal cone of coarse gravel and surface outflow pipes (3 equidistantly spaced around tank, set 30 mm below the gravel surface). Numeric key as for Fig 1; tipping buckets not shown.

Inflows and outflows were sampled fortnightly from June 1990 to January 1992 (20 months) and analysed for BOD, SS, $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$ (APHA, 1985); TKN (modified Kjeldahl digestion using selenium catalyst); TP, DRP (NWASCO, 1982); pH, temperature (Solomat MPM 2000 pH meter); and dissolved oxygen (Yellow Springs Instruments, Model 54A DO meter). Total nitrogen (TN) was calculated as the sum of TKN and $\text{NO}_3\text{-N}$. Inflow and tipping bucket volumes were calibrated monthly. Water quality and flow data were smoothed to remove week to week fluctuations and reveal the underlying trends. Smoothing was performed using Bayesian techniques (Upsdell and Wheeler, 1992), which allowed analysis of non-normal data distributions and generation of confidence intervals.

RESULTS

Inflows

Inflow volumes were maintained at constant levels during the trial, but the strength of the influent wastewater was increased from September 1991 (latter 5 months) by reduction of the effective size of the pretreatment aerobic pond, using a movable polyethylene barrier. Inflow BOD levels rose from around 60 g m^{-3} during the first year to over 200 g m^{-3} (Fig 3). Although inflow SS varied markedly from week to week ($60 - 250 \text{ g m}^{-3}$) it showed no significant seasonal or overall trends, with a mean of 120 g m^{-3} . TN and TP showed marked increases during both spring-summer periods as seasonal milk production (and hence yard holding times and wastewater production) increased after calving. TN (mean composition: 57 % $\text{NH}_4\text{-N}$, 9% $\text{NO}_3\text{-N}$, 34% organic N) levels of around $50 - 70 \text{ g m}^{-3}$ during the 1990/91 summer rose to over 100 g m^{-3} during the 1991/92 summer, while TP (80% DRP) rose to maximum levels of 18 g m^{-3} .

Outflows

SS concentrations in the wetland outflows commonly ranged between 10 and 60 g m^{-3} with little obvious relation to retention time or flow format. Marked reductions in BOD were recorded for both flow formats, with outflow levels inversely related to retention time. Outflow TN (62 - 73% $\text{NH}_4\text{-N}$, 2.3 - 9.5% $\text{NO}_3\text{-N}$, 22 - 36% organic N) showed a strong relationship to inflow concentration and retention time. During the 1990/91 summer occasional peaks of $\text{NO}_3\text{-N}$ up to $6 - 8 \text{ g m}^{-3}$ were recorded, but levels remained consistently below 1 g m^{-3} during the remainder of the study period. Levels of TP (over 90% DRP) varied widely but showed an overall reduction with increasing retention time. During the period July to November, 1991 outflow concentrations exceeded inflow levels in all but the longest retention time horizontal-flow wetland.

Outflow temperatures were similar to those of the inflowing wastewater, varying from 9 and 7°C respectively in mid winter 1990 and 1991 (July) to 22°C in both summers. The pH of the horizontal-flow wetland outflows was reduced to between 6.4 and 6.9 with increasing retention time, but remained similar to the inflow ($7.1 - 7.2$) in the up-flow tanks. Dissolved oxygen levels were generally low ($2 - 3.5 \text{ g m}^{-3}$ in the horizontal-flow and $2.5 - 5 \text{ g m}^{-3}$ in the up-flow wetlands), and showed a small but consistent rise ($0.5 - 1 \text{ g m}^{-3}$) with increasing retention time.

Treatment Performance

Figure 4 shows mean percentage reduction values calculated from mass balance data at each loading rate. Reduction of SS varied between 40 and 90% with little apparent relationship to retention time. There was a general positive relationship between retention time and percent reduction of BOD and TN. Comparison between the two flow formats shows greater levels of BOD and TN reduction in relation to retention time for the horizontal-flow wetlands. Percentage TN reduction in the the upflow wetlands showed a marked decline during the latter spring-summer period as wastewater strength increased. Percentage TP reduction varied markedly over time in both flow formats (-50 to $+90\%$), except in the longest retention time horizontal-flow channel which showed a gradual decline in effectiveness from 90 to 40% reduction during the period of the trial. Relatively greater % reductions in relation to loading were recorded for the horizontal-flow wetlands, but marked declines in % TP reduction were apparent for both flow formats in spring of each year (September) as seasonal wastewater loadings rapidly increased.

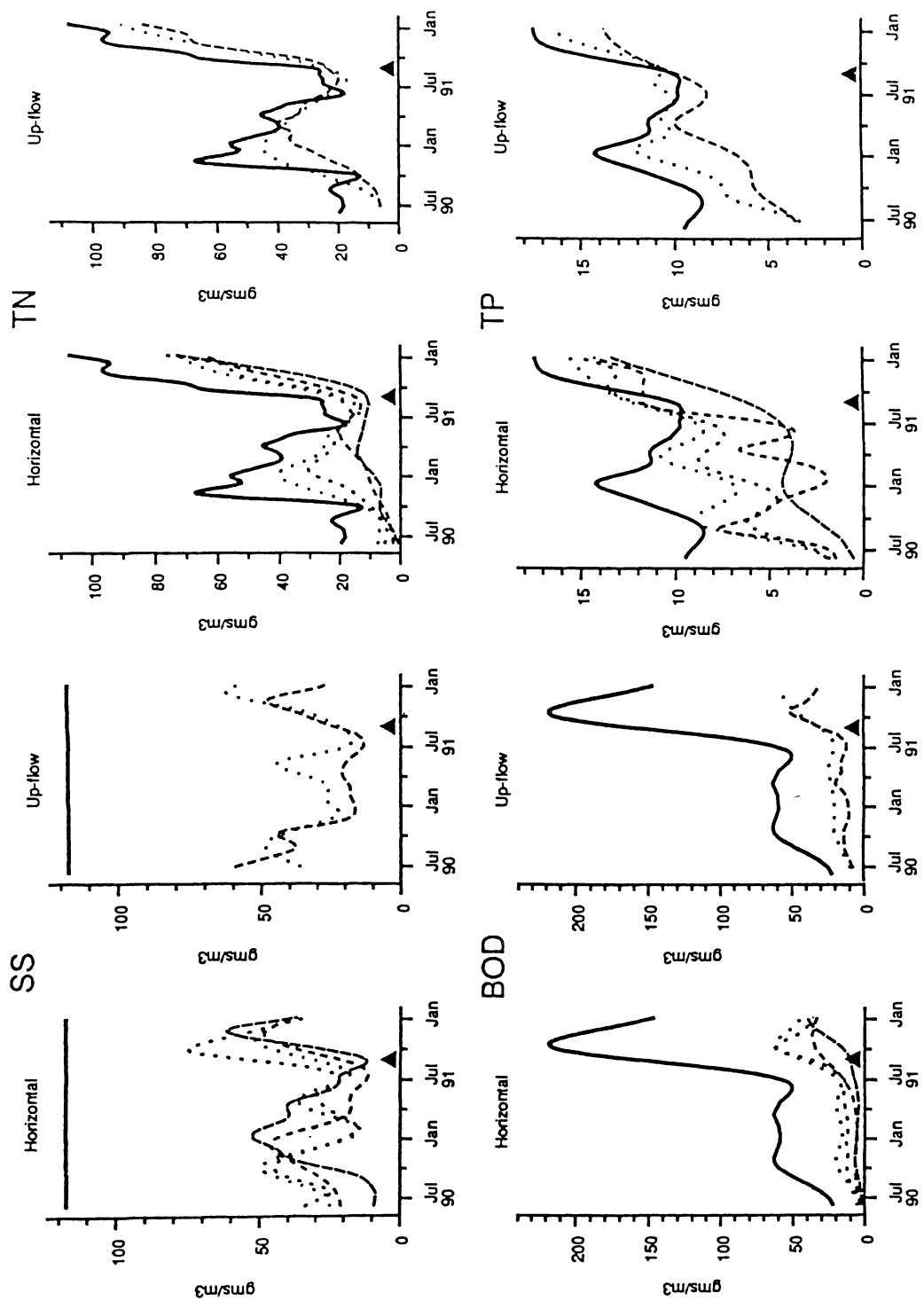


Figure 3. Mean Influent (solid line) and effluent (dashed lines) concentrations (g m^{-3}) of SS, BOD, TN and TP in horizontal and upflow wetlands. Dash length is proportional to the retention time (2, 3, 5.5 & 7 days, horizontal-flow; 2.2 & 6.5 days, up-flow). Arrow heads show timing of influent wastewater strength increase.

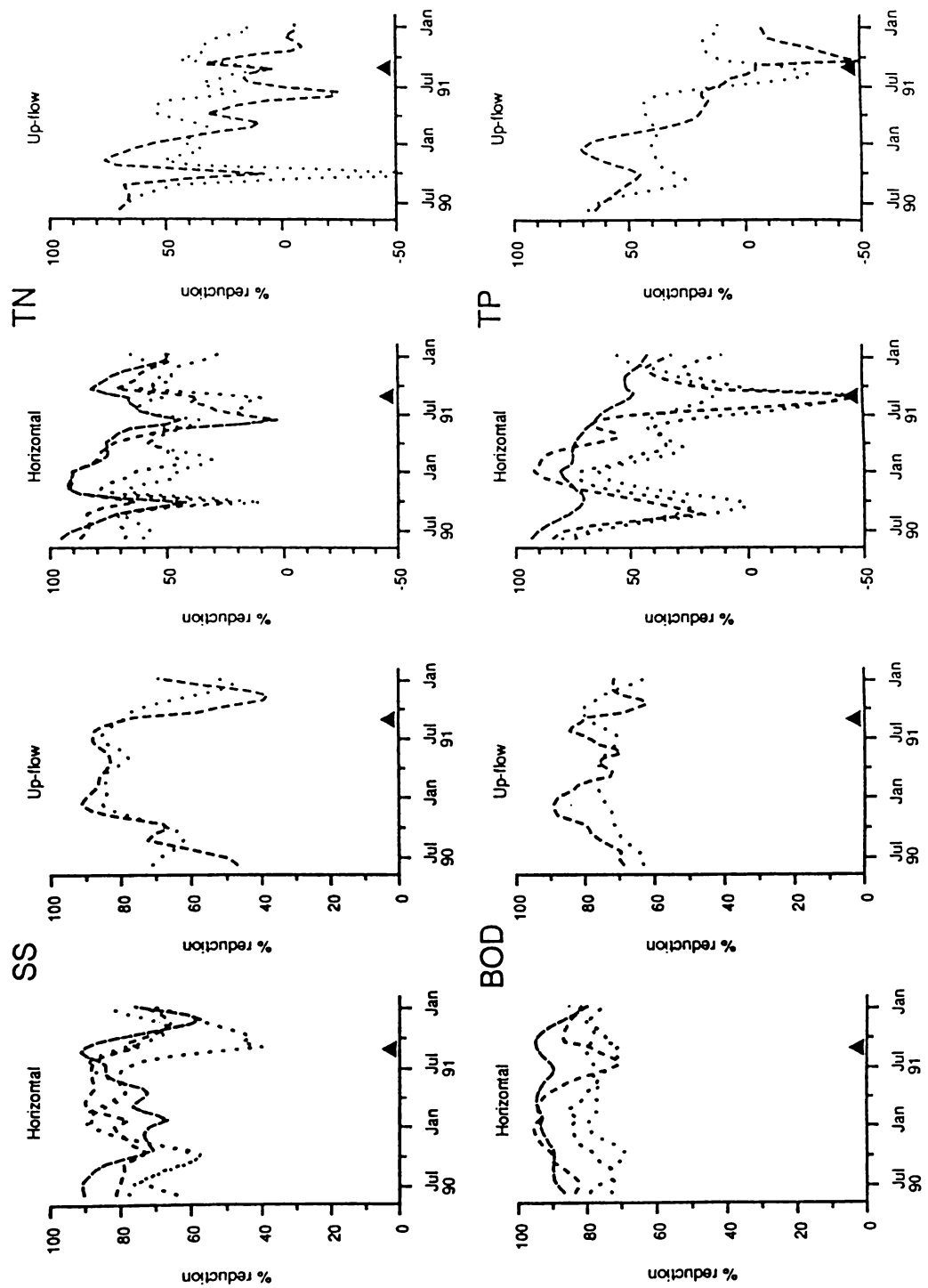


Figure 4. Mean percentage reduction in SS, BOD, TN and TP over time, in relation to retention time, for the horizontal and up-flow wetlands (symbols as for Fig 3).

Pollutant reduction rates per unit treatment volume showed a general positive correlation with mass loading rates. Figure 5 shows reduction rates representative of the two consecutive spring-summer periods sampled. Horizontal and up-flow wetlands showed similar performances for SS and BOD. Reduction rates for TN and TP in relation to loading were similar during the initial spring-summer period, but were considerably lower in the up-flow wetlands during the latter spring-summer period. During this period mass TN reduction rates in the up-flow wetlands fell to below half those of the horizontal-flow wetlands; that is to levels even on an areal basis, below those of the (2.5 times shallower) horizontal wetlands.

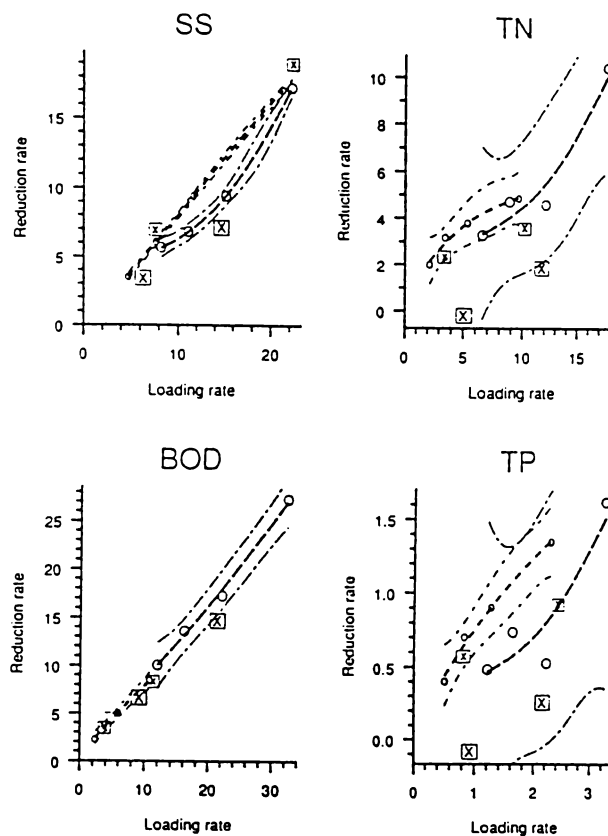


Figure 5. Relationship between mass reduction and loading rates (both $\text{g m}^{-3} \text{d}^{-1}$) for SS, BOD, TN and TP in the horizontal (O) and up-flow (X) wetlands for December 1990 (small symbols) and 1991 (large symbols). Fitted curves and their 95% confidence intervals (fine dashed lines) are shown for the horizontal-flow data.

DISCUSSION

The wastewaters treated in the present study were characteristic of partially treated agricultural and sewage wastewaters in which ammonium is the predominant nitrogen form present. Preliminary results presented here demonstrate the potential of appropriately designed gravel-bed constructed wetlands to significantly reduce SS, BOD and TN in dairy farm wastewaters. Removal of BOD, TN and TP were positively correlated with retention time. Reduction of TP was minimal except at long retention times, showing wide fluctuations and a general decline over the course of the trial. This suggests that plant uptake and storage, and sediment adsorption capacities of the wetlands were exceeded at the loading rates tested and that such levels of P reduction will not be sustainable in the long term.

Large-scale up-flow format constructed wetland designs are still in development (eg. Brett, 1989) and that used in the present study is but one interpretation. Overall performance of these up-flow wetlands in relation to loading rate, when considered on a treatment volume or retention time basis, was similar to that of the horizontal-flow wetlands for SS and BOD. The up-flow wetlands showed poorer TN and TP reduction,

TABLE 1: COMPARISON OF SYSTEM SPECIFICATIONS, AND MEAN TN LOADING AND REDUCTION LEVELS FOR SELECTED HORIZONTAL AND UP-FLOW GRAVEL-BED WETLANDS TREATING ORGANIC WASTEWATERS WITH HIGH LEVELS OF AMMONIACAL NITROGEN. THE WETLANDS IN THESE STUDIES WERE PLANTED WITH *SCHOENOPLECTUS VALIDUS*, *TYPHA* SPECIES, OR *PHRAGMITES AUSTRALIS*.

Study	Wetland area (m ²)	Wetland volume (m ³)	Inflow BOD or *COD (g m ⁻³)	Inflow conc. (g m ⁻³)	NH ₄ -N (%)	Total Nitrogen		Loading rate (g m ⁻³ d ⁻¹)	Removal rate (g m ⁻³ d ⁻¹)	Reduction (%)		
						Area					Volume	
						Loading rate (g m ⁻² d ⁻¹)	Removal rate (g m ⁻² d ⁻¹)				Loading rate (g m ⁻³ d ⁻¹)	Removal rate (g m ⁻³ d ⁻¹)
<u>Horizontal-flow</u>												
Gersberg et al. (1986) ^{1,2}	65	49	118	28	89	1.3	1.2	1.7	1.5	91		
						1.3	1.0	1.7	1.3	79		
						1.3	0.5	1.7	0.6	36		
Bavor et al. (1987) and Fisher (1990) ²	400	200	42	52	86	3.5,3.2	1.9,1.5	7.8,7.1	4.2,3.4	54,48		
			61	36	86	1.2	0.6	2.6,2.6	1.5,1.4	56,53		
			235	72	69	1.9	1.2	4.3	2.6	60		
van Oostrom and Cooper (1990)	18	7.2	*260, 100 ³	133	95	8.1	1.6	20	4.0	20		
			*240, 100 ³	129	95	4.1	0.8	10	2.0	20		
Present study (July 1990 -1991)	19	7.6	57 (±7.5)	38 (± 15.7)	57	0.6	0.5	1.5	1.2	74		
						1.0	0.8	2.5	1.9	70		
						1.5	0.9	3.8	2.2	51		
						2.7	1.4	6.8	3.4	47		
<u>Up-flow</u>												
Breen et al. (1990)	0.043	0.01	*227	32	61	0.44	0.4	2.3	2.2	95		
Mitchell et al. (1990)	0.043	0.01	*227	32	61	0.44	0.4	2.3	2.1	91		
Rogers et al. (1991)	0.08	0.022	-	27	51	0.7	0.7	2.5	2.5	98		
				55	60	1.4	1.3	5	4.9	98		
				55	60	2.8	2.5	10	9.1	91		
Present study (July 1990 - 1991)	1.77	1.77	57 (±7.5)	38 (± 15.7)	57	2.5	1.0	2.5	1.0	32		
						7.4	2.8	7.4	2.8	34		

¹ Data for wetlands planted with *Schoenoplectus*, *Phragmites* and *Typha* species respectively; Assumes all nitrogen in outflows was in dissolved inorganic forms

² Mass removals calculated assuming outflow volume equivalent to inflow, all other mass data as given in studies cited.

³ Estimated mean BOD value (Albert van Oostrom, personal communication)

particularly during the latter period of the trial when loading rates were increased. However, when considered in terms of land area, the deeper upflow wetlands received 2.5 times greater areal wastewater loadings for a given retention time, thereby achieving similar SS and BOD removal on less than half the land area. Conversely they incorporated 2.5 times less surface area and planted area per unit treatment volume. The consequent reduced potential for oxygen diffusion from the atmosphere, plant nutrient uptake and storage, and plant mediated nitrification-denitrification potential via rhizosphere oxygenation, may account for the poorer TN removal observed in the up-flow wetlands. Experimental studies by Reddy et al. (1989) have shown increased ammonium loss via sequential nitrification-denitrification in the presence of wetland plants, and planted gravel-bed wetlands have generally shown improved N removal compared to unplanted wetlands (eg Wolverton et al., 1983; Gersberg et al., 1986; Rogers et al., 1991).

The performance of the up-flow wetlands in removal of N and P was less than that expected from the results of previous microcosm experiments (see Table 1). The levels of TN removal achieved in the horizontal-flow wetlands (47 - 74%) were within the range of values reported by Gersberg et al. (1986) and Fisher (1990) for primary and secondary sewage wastewaters containing high levels of $\text{NH}_4\text{-N}$, but considerably above values reported by van Oostrom & Cooper (1990) for abattoir wastewaters at higher loading rates in similar gravel-bed channels (less than 10 km away).

Although full mass balance partitioning of N and P sinks is not available at this time, preliminary analysis of above-ground plant biomass and tissue N and P levels suggests negligible difference in harvestable plant nutrient storage (per unit area) between the horizontal and up-flow wetlands. Plant uptake was the major mechanism of N and P removal reported for the upflow wetland microcosms used by Breen & Chick (1989), Breen (1990), Mitchell et al. (1990), and Rogers et al. (1990,91). The present study differed from these microcosm experiments in the use of a different wastewater type, was carried out outdoors under different climatic conditions (ie. reduced temperatures and evapotranspiration rates, and without exclusion of rainfall), used deeper larger-scale tanks (mesocosms) with reduced surface to volume and edge to surface area ratios, and employed hourly rather than once (or twice; Rogers et al., 1991) daily wastewater applications (ie. essentially continuous cf. semi-batch flow respectively). Dye studies reported for the "up-flow" microcosm studies (Breen, 1990; Rogers et al., 1990), in fact show that wastewaters mixed completely between additions. Similar dye studies carried out in the tanks used in the present study showed them to be essentially plug-flow (i.e. truly upflow).

Care is needed when comparing or extrapolating results from experiments such as these carried out at different scales. For example, three to five times greater mean above-ground plant biomass was noted within 200 mm of the edges of the experimental channels and tanks in the present study, in association with increased densities of rhizomes and roots. This was likely to be due to the greater light availability along the edge of the wetlands and ramification of rhizomes and roots confined by the container walls. This may lead to atypical plant nutrient uptake and rhizosphere oxygenation potentials in small-scale experimental systems, resulting in apparent plant-mediated treatment levels which are not practically achievable in up-scaled systems. The presence of such edge effects is particularly relevant to the interpretation of the results of bucket-scale microcosm experiments.

The results of the present study suggest that vertical flow in itself is not likely to increase treatment efficiency per unit of wetland volume. It emphasises the importance of surface to depth ratios in determining the supply of atmospheric oxygen to wetland treatment systems and the relative importance of plant mediated nutrient uptake and oxygen release. Further studies are required to evaluate the treatment characteristics and practical utility of different hydraulic loading strategies (e.g. batch, continuous, intermittent) for both horizontal and vertical-flow wetlands.

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Plate 1. View of the experimental wetlands in February 1990 showing rapid establishment of *Schoenoplectus validus* (3½ months growth since planting).

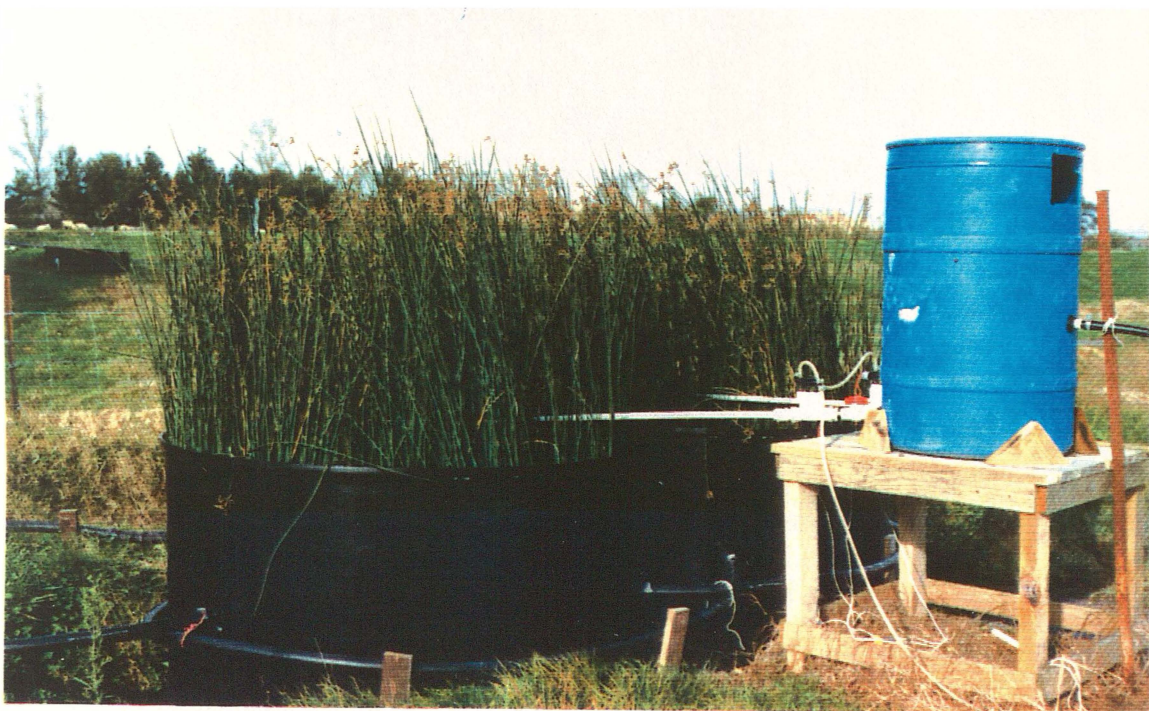


Plate 2. The experimental upflow wetlands after 14 months growth.



Plates 3 & 4. Two views of the experimental wetlands (photographed from the top of the blue header tanks) showing seasonal changes in the wetland vegetation.
 (a) = February 1991 (late summer).
 (b) = July 1991 (mid-winter).



Plate 5. Mature growths of *Schoenoplectus validus* (maximum height ~1.8m) in the experimental wetlands in the second summer (early January, 1992) showing dense understory of litter.



Plate 6. Regrowth of new culms through the previous years litter, early October 1991.



Plate 7. Longitudinal views of the experimental wetlands in January 1991, showing gradients of plant growth along the channels (inflows on right side of pictures).

(a) = L1 - lowest loading rate

(b) = L2

(c) = L4 - highest loading rate



Plate 8. The hard work of driving large corers (18 per wetland) into the gravel substratum to extract roots and rhizomes - Final sampling, January 1992.



Plate 9. View of the fully inserted core. The root mass was cut up with a knife to allow excavation in 10cm layers using the small, flat bottomed scoop shown.



Plate 10. View of the wetland microcosms (Expt 2) showing *Schoenoplectus validus* after ~ 90 days culture in different dilutions of dairy wastewater, with and without full nutrient addition.

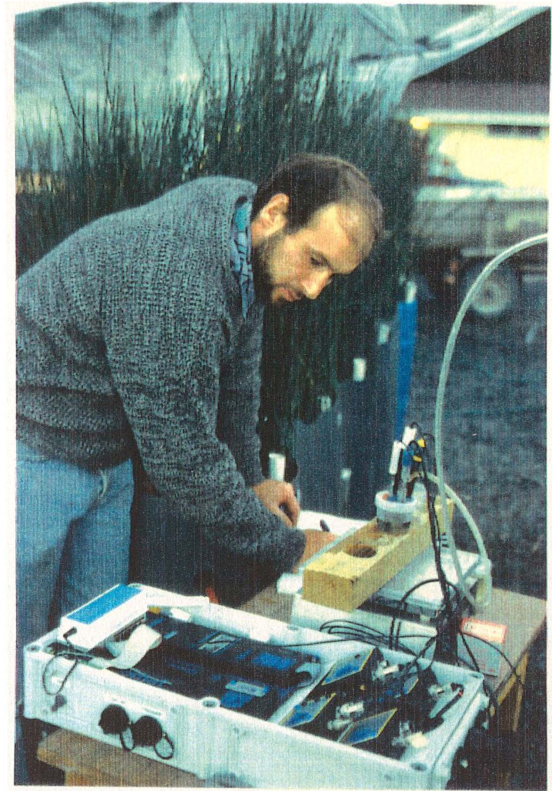


Plate 11. Monitoring environmental conditions in the microcosms. Redox potential, pH, dissolved oxygen and temperature being measured in water samples drawn from different depths in the microcosms. The sample chamber was maintained anaerobic by constant flushing with N₂ gas. (The author sporting a "holiday season" beard).

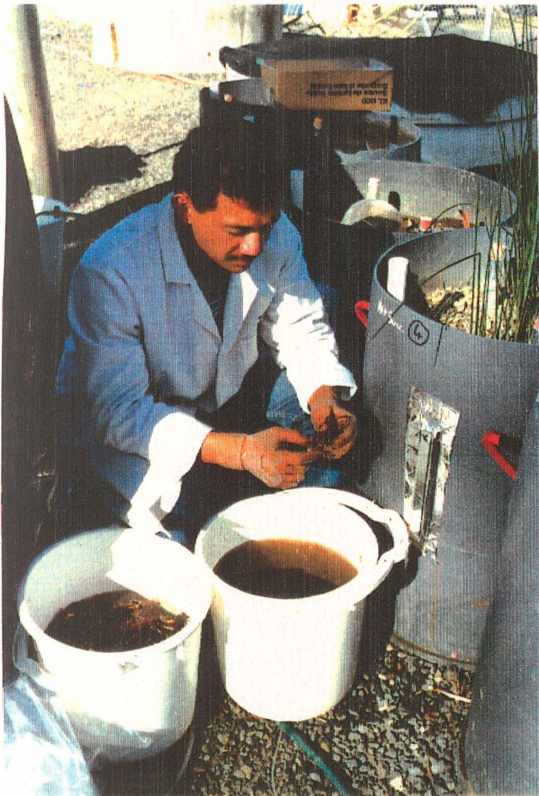


Plate 12. The arduous task of sorting and washing rhizome and root material from the microcosms (Aleki Taumoepeau assisting).



Plate 13. Final harvesting of the microcosms showing sorting of culms into different length classes. (Adam Fricker assisting).

CHAPTER 4



Alc 1

C.T. 10/91

Growth and nutrition of *Schoenoplectus validus* in agricultural wastewaters

Chris C. Tanner

*Ecosystems Division, National Institute of Water and Atmospheric Research, P.O. Box 11-115, and
Department of Biological Sciences, University of Waikato, Private Bag 1305, Hamilton, New Zealand*

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Abstract

The above- and below-ground growth of *Schoenoplectus validus* (Vahl) A. Löve & D. Löve was investigated in piggery and dairy farm wastewaters. Experiments were carried out in batch-fed gravel-bed wetland microcosms (190 l), fitted with root-observation panels. Growth in wastewaters was compared with that in diluted and nutrient-amended wastewaters, a complete inorganic nutrient solution and tap water. *S. validus* was capable of growth over a wide range of nutrient and organic matter strengths. Above-ground growth was up to two times greater in the higher-strength wastewaters than in nutrient solution alone, despite similar or higher nutrient levels in the latter. Even dilute additions of wastewater ($N < 3 \text{ g m}^{-3}$ and $P < 10 \text{ g m}^{-3}$) to the nutrient solution ($N > 200 \text{ g m}^{-3}$ and $P > 45 \text{ g m}^{-3}$) resulted in significant growth enhancement. Mean above- and below-ground biomass of around $2.0 \text{ kg dry weight (DW) m}^{-2}$ and $1.25 \text{ kg DW m}^{-2}$, respectively, were recorded in primary dairy wastewater alone, rising to 3.3 kg DW m^{-2} and 1.5 kg DW m^{-2} , respectively, with nutrient amendment. Maximum crop growth rates of up to $29 \text{ g m}^{-2} \text{ day}^{-1}$, and demographic growth rates of up to $25 \text{ m (culm length) m}^{-2} \text{ day}^{-1}$, and $24 \text{ culms m}^{-2} \text{ day}^{-1}$ were recorded over the 116 day culture periods. Plant uptake of N and P decreased from 43% and 93%, respectively, at low nutrient concentrations to 6% and 7% as the nutrient content of the culture solutions was increased, and below-ground:above-ground biomass allocation decreased from around 3 to 0.45. Rhizomes commonly made up around 80% of the below-ground biomass. Rooting depths in the higher-strength organic wastewaters tested were reduced compared with those in nutrient solutions with similar or higher nutrient levels, and also with increasing wastewater addition to nutrient solution, suggesting that factors associated with organic compounds present in the wastewater (e.g. biological oxygen demand), rather than increasing nutrients, led to restricted root penetration. Root death was noted in plants growing in piggery wastewaters, where high ammonia concentrations (mean 222 g m^{-3}) were at potentially phytotoxic levels.

Introduction

Vascular plants adapted to growth in waterlogged soils and shallow waters are an integral part of a range of aquatic systems being developed for treatment of wastewaters (e.g. US Environmental Protection Agency (USEPA), 1988; Task Force on Natural Systems, 1990). Gravel-bed constructed wet-

lands (also known as vegetated submerged beds and root-zone systems) are one of the more common wetland design formats employed for wastewater treatment (Reed and Brown, 1992). Wastewaters are treated by flow through gravel-filled channels in which emergent aquatic plants grow hydroponically.

The aim of the present study was to investigate plant responses to culture in the coarse gravel substrates and hypereutrophic conditions characteristic of these wetland treatment systems. The study investigates above- and below-ground productivity, tissue nutrient levels and root growth responses of *Schoenoplectus validus* (Vahl) A. Löve & D. Löve (soft-stemmed bulrush or lake clubrush), in relation to nutrient and environmental conditions provided by a range of agricultural wastewaters. *S. validus* has been commonly employed in constructed wetlands treating sewage wastewaters in North America, Australia and New Zealand.

The objectives of the study were to determine whether agricultural wastewaters provide suitable macro- and micronutrient sources and environmental conditions for the growth of *S. validus* in gravel-bed constructed wetlands, and to evaluate the effects of wastewater 'strength', both in terms of nutrients and oxygen demand, on productivity, nutrient uptake and root system development.

Methods

Wetland microcosms

Plants were grown in 190 l open-top polyethylene tanks, each fitted with a subsurface root-viewing panel fabricated from clear acrylic, two interstitial water sampling tubes, a basal inflow (30 mm i.d.) and two surface outflow (15 mm i.d.) pipes (Fig. 1). Each tank was filled to 650 mm depth with prewashed alluvial rhyolitic gravel of 10–30 mm diameter with an effective interstitial space of 39%, giving a free-space volume of approximately 56.5 l. The root-viewing panels were positioned 80 mm below the gravel surface to allow unimpeded growth of rhizomes above them.

Experiments were performed outdoors at the Ruakura Research Centre, Hamilton (37°47'S, 175°19' E; 40 m above sea-level) during two consecutive summer–autumn periods; 16 January–10 May 1990 (Experiment 1) and 7 December 1990–2 April 1991 (Experiment 2). Climatic conditions during these periods are summarised in Table 1. Treatments were assigned randomly to tanks arranged in two east–west rows 1.5 m apart. For Experiment 2, tanks were covered by a clear horticultural plastic overhead shelter (about 2 m above the tank tops) to exclude rainfall. This reduced light levels (photosynthetically active radiation) by about 10%, but made negligible difference to air temperatures.

Vegetative propagules of *S. validus* (30–40 mm rhizome sections with 3–4

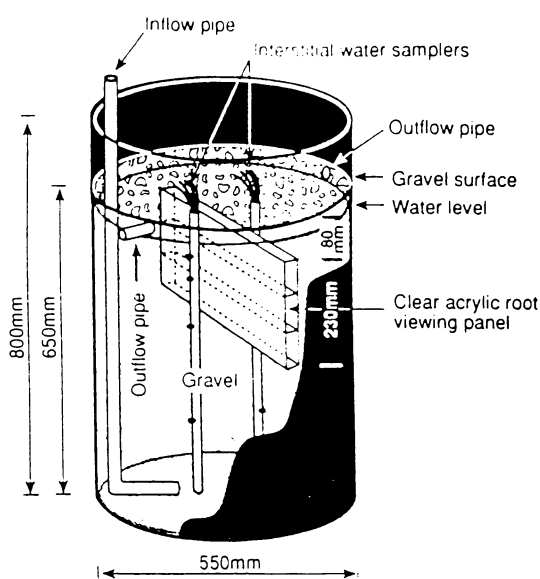


Fig. 1. Plan showing layout of experimental tanks (plants not shown).

Table 1
Summary of mean climatic data for Experiments 1 and 2¹

	Experiment 1	Experiment 2
Minimum air temperature (°C)	12.7	11.9
Maximum air temperature (°C)	23.2	22.8
Rainfall (mm day ⁻¹)	2.6	na ²
Sun hours (day ⁻¹)	6.8	8.1
Solar radiation (MJ m ⁻² day ⁻¹)	15.8	20.5

¹All data from the Ruakura Meteorological Station, situated about 150 m south of the experimental site.

²Rainfall excluded by overhead shelter.

mature culms trimmed to 200 mm length) were collected from a natural population on the shore of the Waikato River at Hamilton. These were initially propagated outdoors for 4 weeks in water of 100 mm depth under 75% shade cloth. Propagules showing growth of new shoots and roots were then selected for uniformity of size and condition, and nine (Experiment 1) or 13 (Experiment 2) were planted in each experimental tank.

Wastewater and nutrient solutions

In Experiment 1 plant growth was compared in oxidation-pond pretreated piggery wastewater, anaerobic pond (primary) and aerobic pond (secondary) dairy parlour wastewaters, a complete hydroponic nutrient solution alone and in combination with secondary dairy wastewater, and tap water. Each treatment was replicated three times. Treatment means and standard errors

(SED) were calculated using one-way ANOVA (Genstat 5, 1988).

Experiment 2 compared growth over a range of five dilutions of primary dairy wastewater (2, 10, 30, 60 and 100%) with and without nutrient solution addition, and in nutrient solution alone. Addition of nutrient solution was designed to overcome the differences in nutrient levels of diluted wastewaters, thus allowing comparison of the effects of other wastewater factors (e.g. biological oxygen demand; BOD) on plant growth. The resulting concentration–response relationships were compared using Bayesian smoothing techniques (Upsdell and Wheeler, 1992).

Tanks were initially filled with tap water and then, after a 10 day establishment period, batch-fed weekly (60 l per tank) with new culture solutions. These were added slowly to the base of the tanks via the inflow pipe, displacing the previous solution through the surface outflow pipes. Dye studies showed water movement to be essentially plug-flow using this procedure. Water levels were checked every 2 days and adjusted by addition of tap water to compensate for evapotranspiration losses.

Piggery wastewater was collected weekly from an aerobic lagoon at Johnson's Piggery, Hautapu, and primary and secondary dairy wastewaters were collected, respectively, from anaerobic and aerobic treatment ponds on the Ruakura Research Centre Farm. Nutrient solution (Bioplus professional growers mix, Biotissue Products, Hamilton) was prepared fresh from stock solutions, with Fe supplied as an EDTA chelate. Hamilton City tap water, a treated supply from the Waikato River (mean pH 7.4, conductivity $210 \mu\text{S cm}^{-2}$; CaCO_3 alkalinity 41 g m^{-3} , $\text{NO}_3\text{-N}$ and dissolved reactive phosphorus (DRP) less than 1 g m^{-3} , S 6.7 g m^{-3} , Mg 2.7 g m^{-3} , Ca 9.3 g m^{-3} , Na 20 g m^{-3} , K 3.4 g m^{-3} , Mn less than 0.02 g m^{-3} , Zn 0.010 g m^{-3} , Cu less than 0.002 g m^{-3} , Fe less than 0.04 g m^{-3} , Cl^- 23 g m^{-3} , F^- 1.08 g m^{-3}) was used to dilute wastewaters and prepare nutrient solutions.

Water sampling and analysis

Samples of added wastewaters and nutrient solutions were taken periodically during trials and analysed for common wastewater parameters and nutrients (see Table 1) using standard methods (American Public Health Association (APHA), 1985; except $\text{NO}_3\text{-N}$, total phosphorus (TP) and DRP, National Water and Soil Conservation Organisation (NWASCO), 1982). During the last 3 weeks of Experiment 2, tank outflows were also sampled and analysed as a measure of the changes in culture solutions between batch loadings. Dissolved oxygen was measured using a sodium sulphite solution zeroed, saturated-air calibrated YSI model 54A DO meter (Yellow Springs Instruments, Yellow Springs, OH, USA), and electrical conductivity, pH and redox potential (interstitial water only) using a Solomat MPM 2000 minilab

system (Solomat, Stamford, CT, USA). Each wastewater was analysed on two occasions for acetic, propionic, butyric and valeric fatty acids by partition gas chromatography using a Chromosorb 101 column.

Three weeks before the end of each experiment, subsurface interstitial water samples were drawn from the experimental tanks using in situ sampling tubes. Samplers were constructed of 500 mm lengths of acrylic pipe (15 mm i.d.), encasing four flexible polyethylene tubes (1.5 mm i.d.) which extended from the gravel surface to depths of 50, 100, 200 and 400 mm. Each tube protruded 10 mm from the pipe into the surrounding solution. Care was taken to ensure that samples remained anaerobic during collection. The standing water in the tubes was initially drawn into a syringe via a dual manifold and then the sample (50–60 ml) drawn up into another. This was immediately injected into a continuously N₂ flushed acrylic chamber (120 ml) fitted with temperature, redox, dissolved oxygen and pH probes (detailed above). Redox potentials were measured on duplicate samples, as a semi-quantitative indication of subsurface oxidation status (Gambrell and Patrick, 1978), using a combination platinum and Ag/AgCl reference electrode. The platinum electrode was cleaned between readings using fine pumice, and standardised using ferrous–ferric solution (Light, 1972), with readings taken after 2 min equilibration. Values are expressed relative to the standard hydrogen electrode (E_h).

Plant sampling

The simple structure of *S. validus* (a horizontal subsurface rhizome, with roots and tapering cylindrical culms produced at nodes, leaves reduced to basal sheaths and inflorescences borne terminally) facilitated the use of simple nondestructive demographic techniques to measure growth during trials, in addition to final biomass determinations. Culm growth was monitored periodically during experiments using a removable multi-tiered quadrat clamped to the tanks. The number of culm tips was counted in each 150 mm tier of the sampler. The total culm length for each tank was calculated by multiplying the mid-point value of each length class by the number of shoots in that class. At the end of each trial culms were cut at the gravel surface and their bases washed in tap water to remove any adhering sediments. Culms were sorted into 150 mm length classes using a compartmentalised measuring tray, then counted, dried for 3 days at 80°C in a forced air oven and weighed.

Root growth was monitored during trials using the prefitted root-viewing panels and a small purpose-designed inclined rectangular mirror with etched horizontal sampling lines. Root intersections were counted along both sides of the central 300 mm section of the tanks, at six depths between 100 and 300 mm. A twin fibre-optic light source was used to aid observation. Only primary roots were counted, not the fine lateral roots. The proportion of deep roots (200–300 mm depth) was compared with those at 100–200 mm depth.

At the end of trials, the tanks were excavated and the gravel sorted and wet-sieved (2 mm mesh) to recover below-ground plant material. The total rhizome length was measured. After further washing and soaking in tap water overnight at 4°C (Experiment 1) or 2 h (Experiment 2) to release loosely bound nutrients, three representative subsamples were removed and sorted to determine the relative biomass of rhizomes (including below-ground culm bases) and roots, then all below-ground tissues were dried as described for above-ground samples and weighed.

Dried plant tissue samples were ground and analysed for macro- and micronutrients as described by Cornforth (1981). N and P were determined simultaneously using automated methods after Kjeldahl digestion (salicylic acid modification using selenium catalyst). All other analyses were performed on nitric–perchloric acid digests: Na, K, Ca and Mg by four-channel flame photometer; Mn, Zn, Cu and Fe by atomic absorption spectroscopy; S by a turbidimetric method; B by the Curcumin method.

Results

Experiment 1

Culture conditions

Levels of total N and P were similar in the piggery wastewater and nutrient solution (Table 2). In the piggery wastewater, N was present predominantly as NH_4 and organic forms, and nearly half of the P was in particulate forms, whereas in nutrient solution all the nutrients were in soluble forms, with the majority of N supplied as NO_3 . Other nutrient levels were broadly similar. Nutrient and degradable organic matter (BOD) concentrations in the primary dairy wastewater were around 30–50% of those in piggery wastewater. Similar levels of BOD but higher suspended solids (SS) levels (both largely owing to phytoplankton), and considerably lower N (mainly as NO_3) and other nutrient levels were present in secondary dairy wastewater. Combining secondary dairy wastewater and nutrient solution resulted in minimal increase in nutrient levels above those in nutrient solution alone, but added organic constituents.

The redox potentials (E_h) of the wastewaters and nutrient solution before addition were all above 500 mV. Four days after culture solution exchange mean interstitial water E_h had reduced by about 100 mV from initial levels, except in secondary dairy treatments, which showed lower mean E_h values of 354 ± 42 mV. Little change in E_h was noted with depth in the culture tanks (100–400 mm). Dissolved oxygen levels measured in interstitial waters were between 0.2 and 1 g m⁻², and showed no consistent correlation with treatment or depth. Nutrient solution treatments showed reductions of around 0.8 pH units (i.e. to pH about 5.5) between exchanges, whereas pH remained

Table 2

Composition of wastewaters and nutrient solutions (NS). Values given are means, with standard deviations in parenthesis; units are all g m^{-3} , except conductivity ($\mu\text{S cm}^{-1}$) and pH

Parameter	Experiment 1				Experiment 2	
	Piggery ($n=9$)	Primary dairy ($n=9$)	Secondary dairy ($n=9$)	Nutrient solution ($n=3$)	Primary dairy ($n=5$)	Nutrient solution ($n=3$)
Suspended solids	706 (133)	76 (25.7)	218 (60)	–	103 (7.2)	–
BOD	321 (45)	59 (10.0)	40 (9)	–	95 (16.5)	–
TKN ¹	302 (65)	100 (35.5)	25 (6)	–	130 (73)	–
NH ₄ -N	222 (19)	97 (6.7)	0.52 (0.58)	7.2 (2.55)	91 (20.4)	13.3 (6.7)
NO ₃ -N	0.007 (0.010)	0.007 (0.008)	2.1 (1.24)	230 (17.3)	2.6 (0.73)	281 (24)
TP	55.4 (12.6)	17.2 (1.2)	9.0 (1.33)	–	14.8 (2.1)	–
DRP	27.2 (16.0)	13.6 (3.2)	5.8 (2.03)	46 (1.2)	14.0 (1.7)	61 (13.5)
Total S	–	–	–	66 (1.0)	9.1 (3.2)	80 (13)
Sulphide	0.023 (0.040)	0.88 (1.62)	0.021 (0.026)	–	–	–
Ca	–	–	–	193 (12)	40 (2.5)	263 (6)
Mg	–	–	–	47 (2.1)	14 (1.1)	61 (4.6)
K	119 (30)	106 (16)	63 (17)	283 (6)	120 (7.1)	385 (5)
Na	77 (3)	33 (2)	27 (1)	28 (1.5)	31 (2)	25 (0.0)
Mn	–	–	–	1.8 (0.1)	0.50 (0.10)	2.4 (0.04)
Zn	–	–	–	0.08 (0.03)	2.27 (0.70)	0.11 (0.01)
Cu	0.21 (0.24)	0.03 (0.01)	0.00 (0.01)	0.02 (0.01)	0.03 (0.03)	0.08 (0.01)
Fe	–	–	–	1.06 (0.61)	1.19 (0.20)	1.03 (0.79)
B	–	–	–	0.78 (0.01)	0.36 (0.11)	0.9 (0.06)
Mo	–	–	–	0.22 (0.17)	0.03 (0.02)	0.46 (0.03)
Conductivity	2640	1600 (70)	590 (42)	2500 (34)	1330 (52)	2890 (22)
pH	7.9 (0.27)	6.9 (0.14)	8.0 (0.71)	6.3 (0.06)	6.8 (0.11)	6.2 (0.07)
Dissolved oxygen	0.76 (0.75)	1.08 (0.81)	6.9 (0.06)	–	1.03 (0.25)	–

¹Total Kjeldahl nitrogen.

similar to initial levels (see Table 2) in all other treatments. Volatile fatty acid analyses of wastewaters showed less than 0.1 mmol l^{-1} of acetic, butyric or valeric acids in any samples, and less than 0.1 mmol l^{-1} , 0.9 mmol l^{-1} and 0.2 mmol l^{-1} of propionic acid in piggery, primary and secondary dairy wastewaters, respectively.

Plant response

Maximum above-ground growth of *S. validus* occurred in piggery, primary dairy and secondary dairy plus nutrient treatments with biomass values of between 1450 and 1700 g m^{-2} (Fig. 2a), culm densities of over 2000 m^{-2} , culm heights up to 1.5 m and total culm lengths around 1700 m m^{-2} (Fig. 3) recorded. Mean crop growth rates (CGR) of 12.7 – $14.6 \text{ g m}^{-2} \text{ day}^{-1}$ were recorded in these treatments, with approximately linear increases in total shoot length between 60 and 116 days (Fig. 3), corresponding to mean demographic crop growth rates (DCGR) of 21 – $25 \text{ m (culm length) m}^{-2} \text{ day}^{-1}$ and 18 – $20 \text{ culms m}^{-2} \text{ day}^{-1}$. Above-ground growth in secondary dairy and nutrient solution treatments individually were about half of that recorded in combination, whereas growth in tap water alone was minimal. Negligible culm mortality was recorded except in the water treatment where growth of new culms was associated with death of older culms so that each plant maintained only 2–4 live culms, all less than 0.5 m tall.

Below-ground:above-ground biomass allocation decreased from around

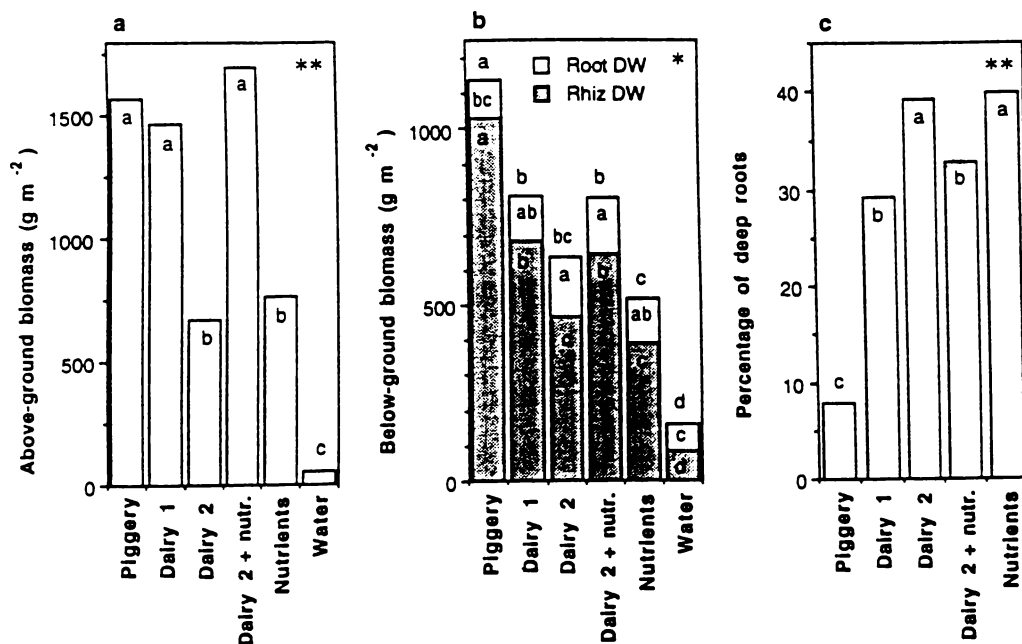


Fig. 2. (a) Above- and (b) below-ground biomass, and (c) proportion of deep roots (200–300 mm depth) within the observed depth range (100–300 mm), after 116 days culture in wastewaters, nutrient solution and tap water (Experiment 1). Dairy 1, primary; Dairy 2, secondary. Values with different letters are significantly different at $*P < 0.05$, or $**P < 0.01$.

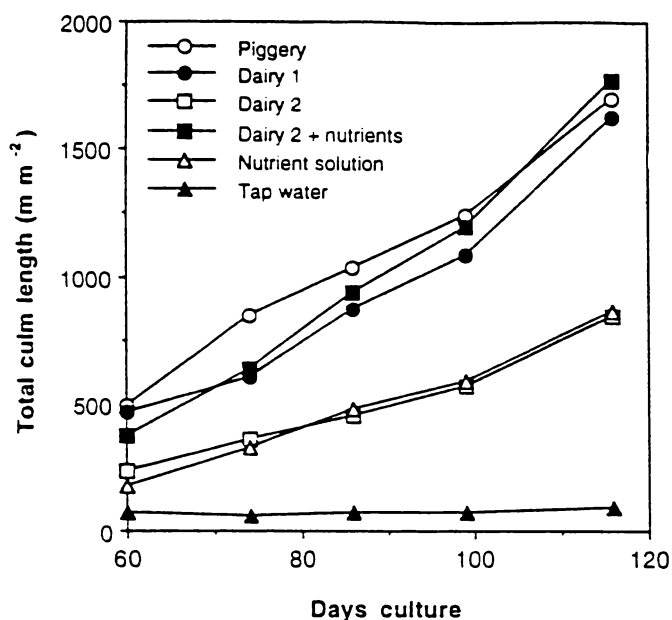


Fig. 3. Above-ground growth curves derived from demographic measurements of total culm length for Experiment 1 (SED = 60).

three in tap water to 0.9 in low-strength secondary dairy wastewater and 0.5 in the higher-strength and nutrient-amended wastewaters. Below-ground growth showed a gradual rise with increasing wastewater nutrient strength (Fig. 2b). Maximum biomass was recorded in piggery wastewater; this consisted of 85–95% rhizomes and culm bases with a mean rhizome length of 78 m m^{-2} . Rhizomes made up 73–84% of below-ground biomass in the other treatments. Root biomass showed high variability, but root density per metre of rhizome was significantly ($P < 0.05$) lower for piggery wastewater than for primary dairy and secondary dairy plus nutrient solution treatments, despite similar above-ground biomass.

Individual roots were clearly visible through the viewing panels, with no apparent precipitation of iron or sulphides on the outer panel surfaces. Observations in the rhizosphere zone showed clear downward growth of primary roots, with numerous fine lateral roots (up to 20 mm long) produced, starting 10–40 mm behind the root tip. Comparison of root profiles shows poor root penetration below depths greater than 210 mm in the piggery treatments (Fig. 4). Net root growth over the latter half of the culture period was minimal in piggery wastewater, with browning and apparent death of fine lateral roots noted near the end of the culture period. Numerous detached dead lateral roots were recovered during final below-ground sampling. This contrasts with the gradual increase in root numbers recorded for other treatments without noticeable root death. The maximum depth of root penetration was also restricted in primary dairy wastewater (300–350 mm) compared with other treatments (400–450 mm), but, as for secondary dairy wastewater plus nutrients, around 30% of roots were still recorded in the deepest half (200–300

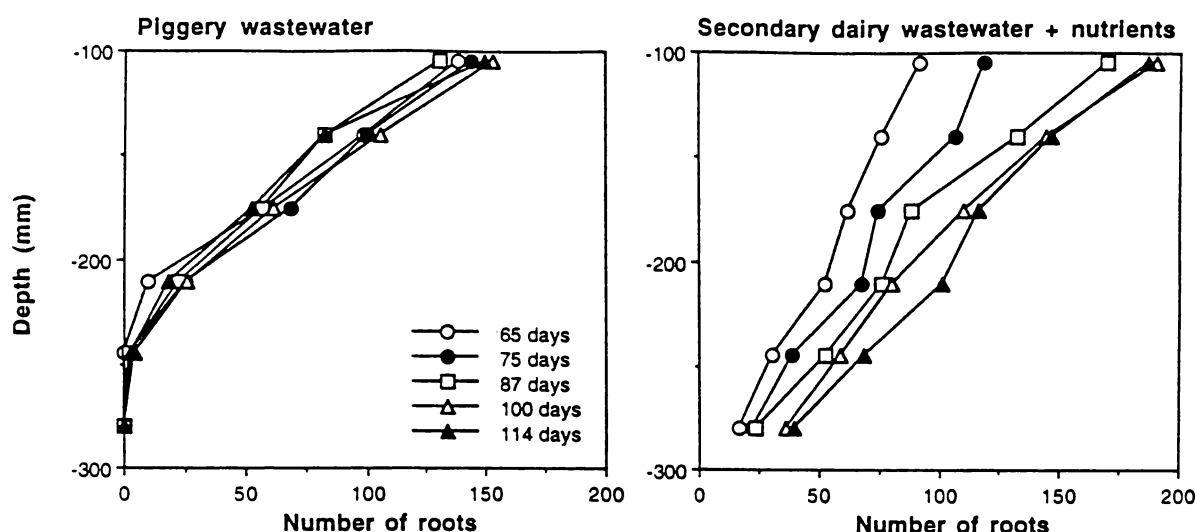


Fig. 4. Comparison of root growth profiles (100–300 mm depth) in representative tanks (Experiment 1) supplied with high-BOD piggery wastewater, and low-BOD secondary dairy wastewater amended with nutrients to give similar nutrient levels.

mm) of the root-viewing panels (Fig. 2c). The proportion of deep roots increased to around 40% in secondary dairy wastewater and nutrient solution separately.

Maximum above-ground (25 g kg^{-1}) and below-ground (22 g kg^{-1}) tissue N was recorded in piggery wastewater. Levels of around $18\text{--}20 \text{ g kg}^{-1}$ N were recorded for all other treatments except water ($5\text{--}6 \text{ g kg}^{-1}$) and secondary dairy wastewater (below-ground; 12.4 g kg^{-1}). N levels were 15–45% higher in young than mature culms and 20–50% greater in rhizomes than roots. Tissue levels of P ranged from 3.2 to 3.8 g kg^{-1} (above-ground) and from 2.9 to 3.8 g kg^{-1} (below-ground) for all treatments except water ($0.9\text{--}1.2 \text{ g kg}^{-1}$). Mature culms had around 30–60% less P than young culms, but overall levels were similar for culms, roots and rhizomes. Despite these differences, the mean atomic ratio of N:P in above-ground tissues remained between 11.2 and 14.7 for all treatments.

Plants grown with nutrient solution showed higher S, Ca and Cu levels than those grown in wastewaters. Although higher concentrations of Na and Cu (used as pig food additive) were present in the piggery wastewaters, tissue levels of Cu were not elevated and the higher Na tissue levels recorded (7.5 g kg^{-1}) were still within the range of levels recorded locally in natural populations ($2.7\text{--}20.2 \text{ g kg}^{-1}$, C.C. Tanner, unpublished data, 1991).

Experiment 2

Culture conditions

The mean composition of the primary dairy wastewater and nutrient solution from which culture solutions were prepared is summarised in Table 2.

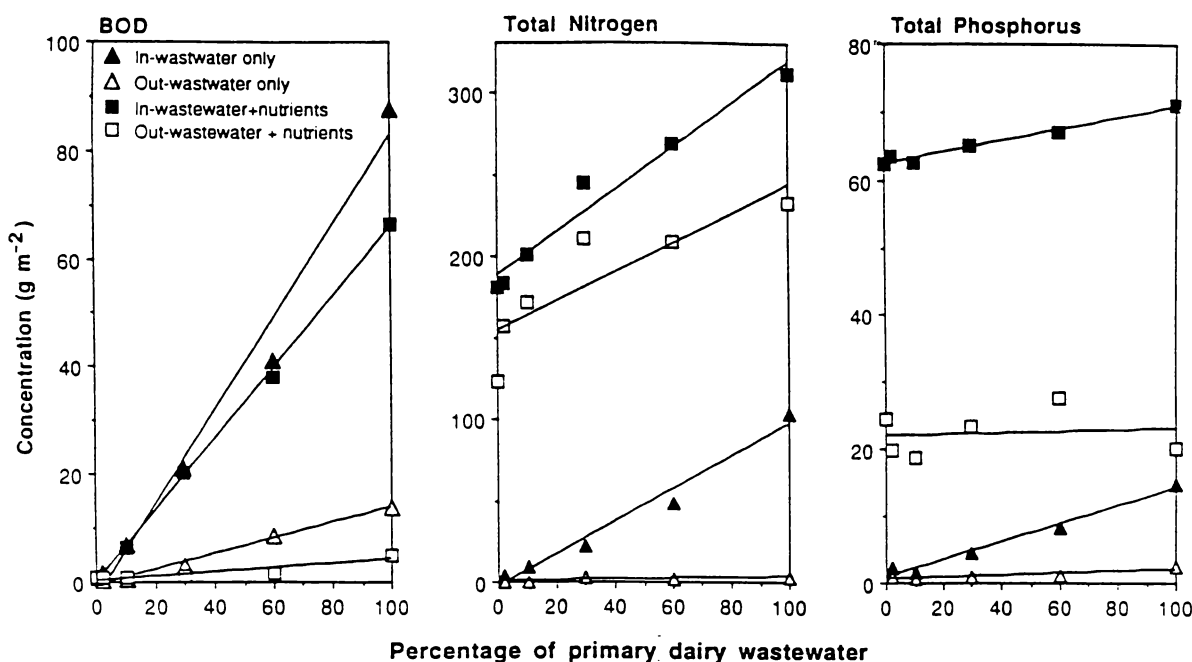


Fig. 5. Changes in BOD, TN and TP concentrations of culture solutions between batch additions (In, initial; Out, final). Each value is the mean of two measurements taken in the last 3 weeks of Experiment 2. Lines shown are linear regressions.

The concentrations of N and P supplied to the culture tanks rose from 2.6 g m^{-3} and 0.3 g m^{-3} , respectively, to 133 g m^{-3} and 15 g m^{-3} , respectively, as the proportion of wastewater applied increased from 2 to 100%. Addition of nutrient solution to the wastewater increased concentrations of total nitrogen (TN) by around 200 g m^{-3} (predominantly in the form of NO_3), and TP by around 40 g m^{-3} . Between batch additions, culture solutions underwent large fluctuations in BOD, TN and TP (Fig. 5). After 7 days culture, BOD was reduced by around 90% in all treatments. TN and TP were reduced to low levels (less than 1 g m^{-3} and less than 3 g m^{-3} , respectively) in unamended primary dairy wastewater. Although greater reductions of $30\text{--}60 \text{ g m}^{-3}$ TN and around 40 g m^{-3} TP were recorded in nutrient solution amended treatments, levels still remained above the initial concentrations in wastewater alone. Comparison of mass inflow and outflows of TN and TP between batch additions shows maximum removal rates of $2.0 \text{ g m}^{-2} \text{ day}^{-1}$ and $1.2 \text{ g m}^{-2} \text{ day}^{-1}$, at inflow rates equivalent to $10.2 \text{ g m}^{-2} \text{ day}^{-1}$ and $2.0 \text{ g m}^{-2} \text{ day}^{-1}$, respectively.

The E_h of the culture solutions before addition were all around 500 mV. Interstitial water E_h in the culture tanks, measured 24 h after solution replacement, showed oxidised conditions (more than 300 mV) in the upper 200 mm of the experimental tanks, with a general decline of around 100 mV at 400 mm depth. Values of 400–500 mV were recorded at 400 mm depth at wastewater levels below 30%, reducing to around 300 mV at 60% and 200 mV in

full-strength wastewater. This pattern was similar for both nutrient solution amended and unamended treatments, with an overall rise of between 50 and 100 mV recorded 5 days after culture solution replacement. Measured interstitial dissolved oxygen concentrations ranged between 0.2 and 1 g m⁻³, showing little apparent relation to effluent strength, depth or time. Culture solutions maintained pH levels of 6.0–6.5 between exchanges, except for nutrient solution alone and in combination with wastewater at dilutions of less than 30%, in which pH dropped to levels of 5.0–5.5.

Plant response

Above- and below-ground biomass and total length of culms increased as the proportion of primary dairy wastewater addition increased (Fig. 6). Growth of nutrient-amended treatments was greater than corresponding unamended treatments, except for below-ground growth in full-strength wastewater, which approached that of the amended treatment. This relationship was similar for both root and rhizome components of below-ground growth. Only minimal mortality of old culms occurred during the trial (less than 3% of culms at final harvest). Mean culm density, length and biomass values at final harvest rose to over 3000 m⁻², 0.96 m and 1.1 g, respectively, in the highest-strength nutrient-amended wastewater treatments. This corre-

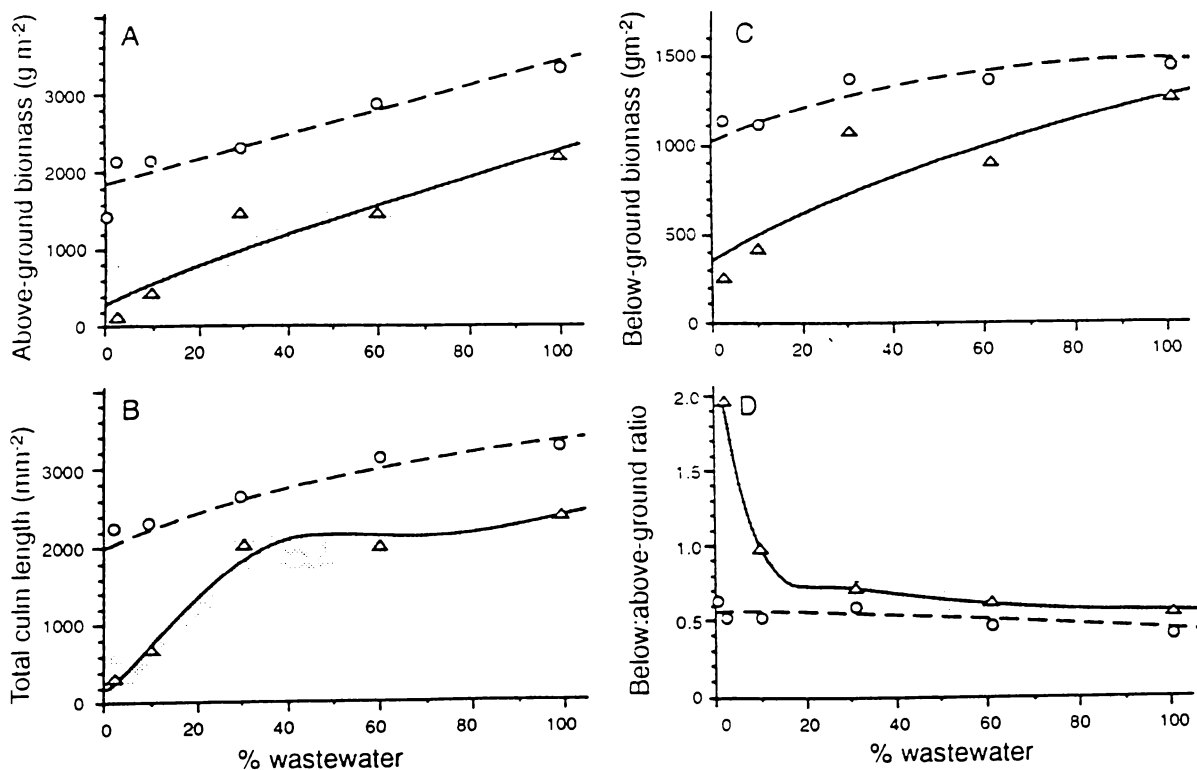


Fig. 6. Above- and below-ground growth of *S. validus* after 116 days culture (Experiment 2) in selected dilutions of primary dairy wastewater alone (Δ) and with nutrient amendment (○). The shaded areas show 85% confidence intervals for the fitted curves; where these do not overlap, values are significantly different at the 95% level.

sponded to a maximum total culm length of nearly 3 km m^{-2} and a biomass of $3.3 \text{ kg dry weight (DW) m}^{-2}$.

Initial above-ground growth was relatively slow, with DCGR (0–44 days) values of less than $5 \text{ m (culm length) m}^{-2} \text{ day}^{-1}$, but rose progressively with increasing nutrient supply (Fig. 7). Increases in total culm length were essentially linear after 40 days culture, with a slight increase in growth rate recorded between 94 and 116 days (maximum DCGR; $51 \text{ m (culm length) m}^{-2} \text{ day}^{-1}$, $46 \text{ culms m}^{-2} \text{ day}^{-1}$). Mean overall CGR values up to $29 \text{ g (above-ground DW) m}^{-2} \text{ day}^{-1}$, and DCGR up to $25 \text{ m (culm length) m}^{-2} \text{ day}^{-1}$ and $20\text{--}24 \text{ culms m}^{-2} \text{ day}^{-1}$ were recorded at the higher nutrient levels tested.

Below-ground:above-ground biomass ratios of nearly two recorded in the

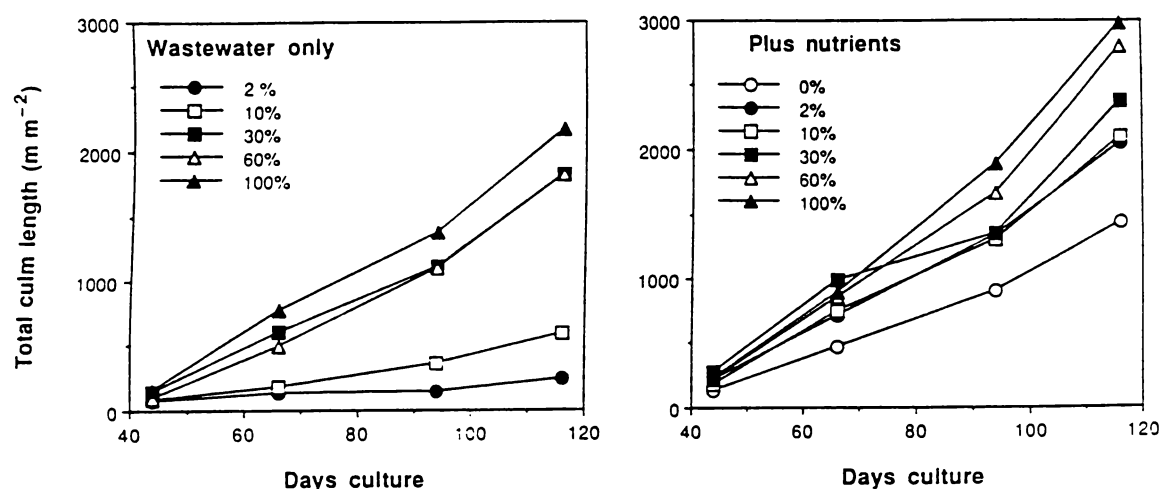


Fig. 7. Above-ground growth curves at five dilutions of primary dairy wastewater alone and with nutrient amendment, derived from demographic analysis of culm lengths (Experiment 2).

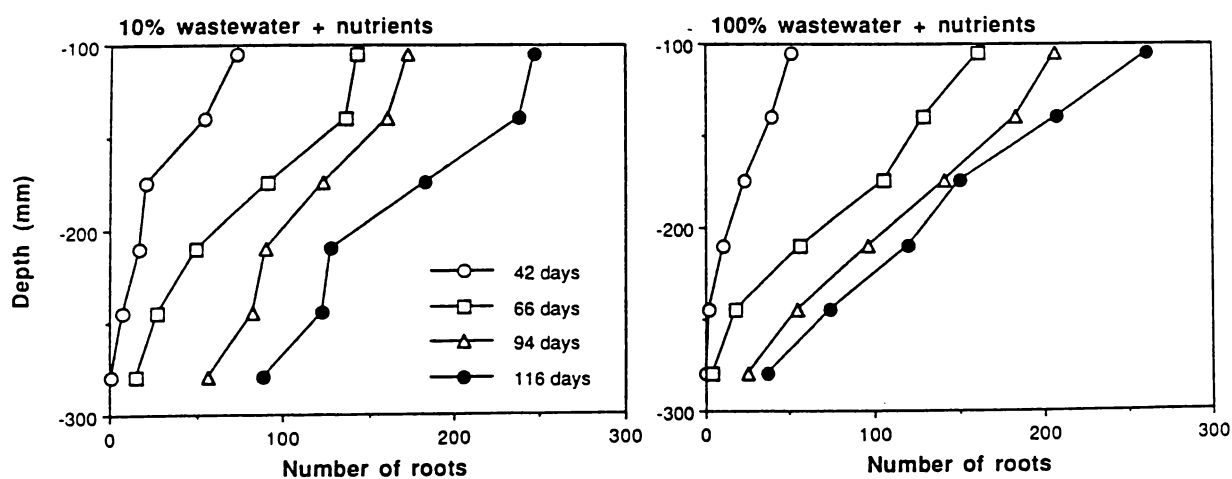


Fig. 8. Comparison of root development profiles (100–300 mm depth) in representative tanks (Experiment 2) supplied with low (10%) and high (100%) concentrations of primary dairy wastewater amended with nutrient solution.

unamended 2% wastewater treatment declined markedly to levels around 0.65 in full-strength wastewater, declining further to around 0.45 with nutrient solution amendment (Fig. 6). Rhizomes and below-ground culm bases made up 80–85% of the below-ground biomass at final harvest, except in the unamended 2% and 10% treatments where they represented 87% and 89%, respectively. Rhizomes reached maximum lengths of around 100 m m^{-2} with biomass values (including culm bases) up to 1.24 kg m^{-2} . Around 50% more roots were recorded in the root-viewing zone (100–300 mm depth) in nutrient solution amended treatments, with a greater proportion of roots (27–40% compared with 20–30%) recorded at greater depths (200–300 mm). The proportion of these deep roots was highest for nutrient solution alone (58%) and showed a general decline with increasing concentration of wastewater (Fig. 8).

Tissue nutrients

Tissue nutrient levels are summarised in Fig. 9. Above- and below-ground

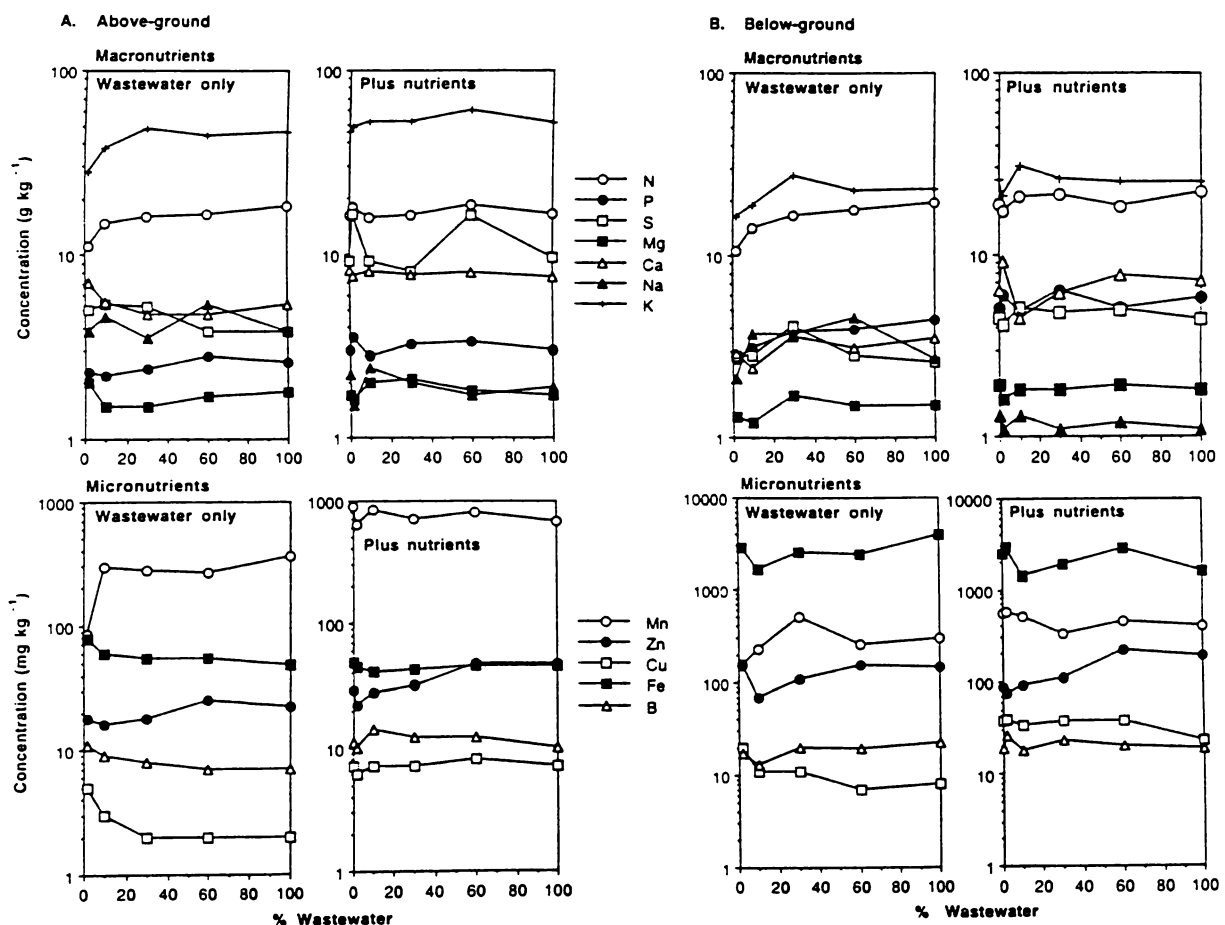


Fig. 9. Effect of increasing concentrations of primary dairy wastewater alone and with nutrient amendment on above- and below-ground tissue nutrient levels of *S. validus* (Experiment 2). (Note the log scale on vertical axes, and the different units used for macro- and micronutrients.)

Table 3
Plant uptake of nitrogen and phosphorus in relation to levels applied during Experiment 2 (116 days culture)

Treatment (Proportion of primary dairy wastewater)	Nitrogen				Phosphorus			
	Total applied (g m ⁻²)	Net plant uptake (g m ⁻²)		Plant uptake as a percentage of total applied	Total applied (g m ⁻²)	Net plant uptake (g m ⁻²)		Plant uptake as a percentage of total applied
		Above- ground	Below- ground			Above- ground	Below- ground	
2%	9	1.3	2.5	43	1.0	0.3	0.6	93
10%	44	6.3	5.7	27	5.0	0.9	1.2	42
30%	133	24	18	32	15	3.5	4.1	49
60%	266	24	16	15	30	4.1	3.5	25
100%	448	41	24	14	50	5.8	5.5	23
Nutrient solution	988	23	16	4	205	4.2	4.4	4
2%+nutrients	997	39	19	6	206	7.6	6.7	7
10%+nutrients	1032	34	23	6	210	6.0	5.5	5
30%+nutrients	1121	38	29	6	220	7.4	8.8	7
60%+nutrients	1253	53	25	6	235	9.5	7.1	7
100%+nutrients	1432	55	32	6	255	10	8.4	7

levels of N and below-ground levels of P increased gradually with increasing proportion of wastewater in unamended treatments, but were relatively similar for all nutrient solution amended treatments, as were atomic ratios of N and P (11.3–12.5). Atomic ratios in wastewater alone showed a greater range, from 10.7 in 2% to 15.7 in full-strength wastewater. Levels of K showed a general rise with increasing percentage of wastewater, both with and without nutrient solution addition, whereas tissue levels of other macronutrients were similar for all treatments. In nutrient solution amended treatments, S, Ca and Mn were generally higher, and Na and Fe lower than unamended treatments, whereas levels of Zn increased with increasing proportion of wastewater addition.

Plant uptake during Experiment 2 accounted for up to 40% and 90%, respectively, of the N and P inputs in diluted wastewaters (Table 3), reducing to around 15% and 25%, respectively, of those in full-strength wastewater, and 6% and 7%, respectively, of those in nutrient-amended wastewater. Plant storage of N and P during the trials increased from less than 5 g m^{-2} and 1 g m^{-2} , respectively, in the lowest-strength wastewaters tested to 87 g m^{-2} and 18 g m^{-2} , respectively, at the highest nutrient loadings tested.

Discussion

Growth conditions

Agricultural wastewaters from a range of sources and stages of treatment were able to support growth of *S. validus* in gravel-bed wetland microcosms. Apart from the piggery wastewater, nutrient levels were below those commonly employed in standard-strength artificial nutrient solutions (e.g. Smith et al., 1983). Atomic ratios of N:P ranged between 7 and 20 in the wastewaters compared with 11 in the nutrient solution. N was present predominantly as ammonium in the piggery and primary dairy wastewaters, and as nitrate (95%) in the nutrient solution. Considerable reductions in BOD, TN and TP were recorded in the culture solutions between weekly batch exchanges, resulting, particularly in low-strength wastewaters, in fluctuating nutrient and subsurface environmental conditions during the growth trials.

Redox conditions in the root zone of the experimental tanks were considerably less reduced than those commonly reported for natural wetland soils (e.g. Gambrell and Patrick, 1978). Although they declined with increasing wastewater BOD, the interstitial water E_h recorded were generally above 350 mV (the approximate boundary between oxidising and reducing conditions; Gambrell and Patrick, 1978). This and the presence of low levels of dissolved oxygen measured in the upper two-thirds of the culture tanks suggest that conditions did not become strongly anaerobic in the plant root zone, except intermittently in the highest-strength wastewaters and in localised zones within organic particles and biofilms. The ability of a range of wetland plants to

transport oxygen into the root zone via aerenchyma is well-established (Armstrong et al., 1991). Experimental studies by Dunbabin et al. (1988), Reddy et al. (1989) and Rogers et al. (1991), using synthetic and sewage wastewaters, have shown increased oxygen levels and E_h in the presence of wetland plants, with concomitant enhancement of BOD reduction and nitrification rates.

Plant productivity

Maximum above-ground productivities of around 3.3 kg DW m^{-2} for the 116-day trials were recorded at the highest nutrient levels tested. This is in the high range of annual above-ground productivities recorded in temperate climates for emergent marsh and swamp species in natural and fertilised stands (e.g. Bradbury and Grace, 1983; Neill, 1990), or in experimental culture (Dykyjova et al., 1971; Barko and Smart, 1978; Urlich and Burton, 1988). The high productivity recorded for *S. validus* in the present study is likely to be due, in part, to edge effects arising from the relatively small size of the experimental tanks. Although culm growth remained essentially erect in the tanks, culms were subject to lateral as well as downward ambient light, and to greater evapotranspiration potential (e.g. 'oasis' and 'clothes-line' effects; Linacre, 1976). Additionally, confinement by the container walls led to ramification of rhizomes with subsequent increased densities of culms and roots along the tank edges. Such edge effects, also noted in similar microcosm studies by Dykyjova et al. (1971), and Urlich and Burton (1988), caution against direct extrapolation of productivity and nutrient uptake potentials to larger-scale wetland systems.

Demographic analysis showed near-linear increases in total culm length over the latter two-thirds of the culture periods. Comparison of biomass and demographic measures of above-ground growth at the end of Experiment 2 shows that sensitivity to changes in culture nutrient levels decreased in the order biomass > culm length > culm density. Plants growing at moderate to high nutrient levels allocated resources to increasing individual culm height rather than density. As culm height increases, the structural constraints of the simple cylindrical culms of *S. validus* result in increasing culm diameters, and hence a relative increase in biomass per unit of culm length.

A complete inorganic nutrient solution was included in growth trials as a baseline from which to compare plant growth and nutrition. However, growth in relation to the nutrient levels supplied was greater in plants cultured in wastewaters or with wastewater addition. Higher biomass production was recorded in piggery and primary dairy wastewaters than in the nutrient solution, which respectively contained similar levels and approximately three times higher levels of macronutrients. Addition of relatively dilute secondary dairy wastewater to the nutrient solution (Experiment 1) resulted in an ap-

proximate doubling of above-ground biomass and a significant increase in below-ground biomass. Similarly, addition of 2, 10 or 30% of primary dairy wastewater (Experiment 2), despite representing at most a 12% increase in N and a 7% increase in P, also resulted in significant increases in growth compared with full-strength nutrient solution alone.

Although a range of artificial nutrient solutions have been used in experimental studies of aquatic plants, little published information exists on their comparative performance for wetland species. Preliminary replicated trials (C.C. Tanner, unpublished data, 1991) had shown excellent growth of *S. validus* in the Bioplus nutrient solution used in the present study; similar to that in Ruakura solution (Smith et al., 1983), and greater than that in either nitrate- or ammonium-based Long Ashton solution (Hewitt and Smith, 1975), or in the modified Setlik and Simmer solution used extensively by Dykyjova et al. (1971) in hydroponic culture of helophytes.

The large increases in plant growth noted as a result of relatively small and dilute wastewater additions to the nutrient solution in the present study, and the results of monitoring data collected during trials, suggest that neither modification of environmental conditions such as pH, E_h or oxygen concentrations, nor provision of additional nutrients (except as noted below) or changes in relative levels of NH_4 and NO_3 (Marschner, 1986) are alone likely to be the mechanisms involved. Organic constituents in the wastewater may have accounted for the improved growth observed. The wastewaters used showed strong yellow–brown coloration caused by the presence of humic substances (absorption coefficient at 440 nm greater than 25 m^{-1}), which have been shown to modify nutrient availability and toxicity to plants via complexation, chelation and ion exchange (Vaughan and Malcolm, 1985; Stevenson and Fitch, 1986) and to affect plant physiological processes via growth regulation (Gross, 1991).

Tissue nutrient levels

Plant tissue levels of N and P showed a general relationship to their concentration in the culture solutions. As hypothesised by Shaver and Melillo (1984) plant nutrient uptake efficiency was negatively correlated, and absolute nutrient uptake per unit area positively correlated with nutrient supply in the culture solutions. Plant uptake during Experiment 2 accounted for around 15% of the N and 25% of the P inputs to tanks supplied with primary dairy wastewater, reducing to 6% and 7%, respectively, with nutrient addition. The proportion of N and P taken up by plants rose to 43% and 93%, respectively, as dilution of the wastewater solutions was increased.

Comparison of tissue nutrient levels with those obtained in other studies are complicated by seasonal and age-related variations in different plant tissues. Maximum above-ground tissue N and P levels above 20 g kg^{-1} and 3.5

g kg⁻¹, respectively, in the higher-strength wastewaters were in the upper range for emergent wetland species generally. They were similar to maximum values reported for *S. validus* cultured in silty clay substrata by Barko and Smart (1978), but two times and three times greater, respectively, than those recorded for mature natural populations in New Zealand (10.5–13.3 g kg⁻¹ and 1.1–2.7 g kg⁻¹, respectively, $n=7$; C.C. Tanner, unpublished data, 1990–1992) and in Quebec, Canada (mean 13.5 g kg⁻¹ and 2.4 g kg⁻¹, respectively; Auclair, 1979).

Addition of increasing proportions of primary dairy wastewater to the nutrient solution, despite resulting in an overall doubling of above-ground growth in Experiment 2, did not cause any obvious changes to tissue levels of macro- or micronutrients, except for moderate increases in Zn. Concentrations of Zn supplied in the wastewater were around 20 times higher than in nutrient solution, but tissue levels in plants grown in wastewater were similar to or lower than those in plants grown in nutrient solution. Interpretation of the significance of such elevated supply of Zn is hindered by the lack of information on the specific micronutrient requirements of wetland plants, and the complexity of their interactions both inside and outside the plant.

Below-ground growth responses

The coarse gravel substrata commonly used to maintain high hydraulic conductivities in subsurface-flow wetlands provide considerable mechanical impedance to root and rhizome growth. Dykyjova et al. (1971) noted irregular rhizome growth patterns for a range of wetland species when cultured in coarse sands and gravels, and Parr (1990) has shown significantly reduced rhizome penetration for *Phragmites* growing in gravel-bed wastewater treatment wetlands compared with soil-based systems. Impedance can be expected to vary in relation to the proportion and size of interstitial spaces, which is a function of the size distribution, shape and surface roughness of particles. In the present study, rhizomes became distorted by their tortuous growth through the gravel substratum. Their growth was primarily horizontal and limited to the upper 60 mm of the substratum, except at the tank edges where rhizome growth was sometimes deflected deeper. Roots were observed to ramify freely within the interstitial spaces, but even in low-BOD culture solutions their density declined markedly at depths greater than 400 mm. Their basic structure was similar to that described for a number of other emergent species (e.g. Conlin and Crowder, 1989), with numerous fine laterals up to 20 mm in length, growing from the primary roots. These fine subapical laterals have been identified as the major zones of oxygen release in the roots of several wetland plant species (Conlin and Crowder, 1989; Armstrong et al., 1991).

Although below-ground:above-ground allocation showed a general decline

with increasing nutrient level of the culture media, as commonly observed for other plant species (Marschner, 1986), absolute levels of below-ground growth showed a gradual increase. Below-ground:above-ground ratios reached a minimum of 0.45 at the highest nutrient levels tested (N, 388 g m^{-3} ; P, 76 g m^{-3} ; K, 505 g m^{-3}). Roots generally made up only around 20% of the below-ground biomass, giving true root:shoot ratios (i.e. nutrient- compared with carbon-assimilating tissues) as low as 0.12.

The depth penetration of roots and the proportion of deep-growing roots (200–300 mm compared with 100–200 mm) was reduced in the higher-strength wastewaters tested (BOD levels above about 60, TN above 80 g m^{-2}). High N levels have been reported to suppress root growth, with greatest effects noted when N is supplied as ammonium (Marschner, 1986). Cizkova-Koncalova et al. (1992) have reported depressed root length, biomass and aerenchyma formation for *Carex* spp. cultured in nutrient-amended organic wastewaters compared with nutrient-only controls. Their results suggest that anaerobiosis (causing increased hypoxic stress) and high N levels (modifying carbon allocation patterns) may act synergistically to depress root growth. Moog and Janiesch (1990) found varying growth responses for three *Carex* spp. cultured in anaerobic conditions. Root growth responses ranged from suppression to enhancement of root biomass, with more compact dense root systems noted under anaerobic conditions in the flood-tolerant species.

In the present study, inhibition of root growth was most marked in piggery wastewater, where roots were restricted to depths less than 280 mm and showed negligible growth during the latter half of the trial. During this period, browning and death of many of the fine lateral roots was noted in this wastewater, the strongest tested in terms of organic content (BOD) and ammonia levels. Phytotoxicity has been reported previously in gravel-bed constructed wetlands treating considerably higher-strength piggery wastewaters (Finlayson et al., 1987), attributed primarily to high concentrations of volatile fatty acids (Bowmer, 1985). Fatty acid concentrations measured in the wastewaters used in the present study were negligible compared with those shown to be phytotoxic to *Typha domingensis* Pers. (Bowmer, 1985), and below levels found to be detrimental to rice growth (Chandraskaran and Yoshida, 1973). However, ammonium-N concentrations in the piggery wastewater (mean 222 g m^{-3}) exceeded levels reported to be inhibitory to rice (100 g m^{-3} ; Tanaka et al., 1958) and water hyacinth (200 g m^{-3} , de Casabianca-Chassany et al. 1992).

General design criteria have recently been developed for subsurface-flow gravel-bed constructed wetlands (e.g. USEPA, 1988; Task Force on Natural Systems, 1990), and the practical application and performance of these systems have been reviewed (Conley et al., 1991; Reed and Brown, 1992). Plant-mediated oxygen transport into the root zone is generally considered to be important in the promotion of aerobic decomposition processes and nitrogen

transformations. Plant oxygen fluxes of between 5 and 45 (mean 20) g m⁻² day⁻¹ have been suggested for design purposes. The rooting depth of wetland plants is one of the main factors used to determine bed depth, thus attempting to maximise contact between wastewaters and the root zone. Rooting depths of 0.3 m, 0.6 m and 0.76 m for *Typha*, *Phragmites* and *Scirpus* (= *Schoenoplectus*) spp., respectively, have been reported by Gersberg et al. (1986) and widely adopted as guidelines to species performance. However, observations of a range of operational gravel-bed systems (Parr, 1990; Reed and Brown, 1992; C.C. Tanner, unpublished observations, 1990–1993) show that plant roots seldom penetrate deeper than 0.3 m regardless of the species utilised, suggesting that current bed-depth design guidelines should be reviewed. The results of the present study show that the rooting depth and density of *S. validus* may vary in response to the oxygen demand and nutrient characteristics of the wastewaters, and be affected by the presence of phytotoxic concentrations of compounds such as ammonia. A gradient of plant response is likely along the length of treatment channels as 'treatment processes' modify the oxygen demand and nutrient characteristics of the wastewater. Optimisation of root–wastewater contact in wetland treatment systems requires consideration of plant growth responses to such wastewater characteristics, which may limit the concentrations, loading rates and types of wastewaters that can be effectively treated in these systems.

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CHAPTER 5



Growth of *Schoenoplectus validus* in wastewater treatment wetlands

1. Productivity and growth responses to wastewater loading.

CHRIS C. TANNER

NIWA–Ecosystems, National Institute of Water and Atmospheric Research, P.O. Box 11-115, Hamilton, and Department of Biological Sciences University of Waikato, Private bag, 3105 Hamilton, New Zealand.

Summary

1. The above and below-ground growth of *Schoenoplectus validus* was investigated over two growth seasons in four gravel-bed constructed wetlands supplied with different hydraulic loading rates of dairy farm wastewaters. The selected loading rates spanned a 3 to 4-fold range of biochemical oxygen demand, N and P supply. Seasonal patterns of growth, biomass allocation, reserve storage, and net annual productivity, and longitudinal and vertical gradients of growth down the wetland channels were determined using a combination of harvest and demographic techniques.
2. Marked seasonal patterns of spring emergence, summer growth and autumn senescence were observed, with little overwintering of live above-ground biomass. Starch, the dominant long-term storage substance, comprised ~20% of rhizome dry weight in autumn. Mobilisation during spring reduced starch levels by around half, with depletion greatest in the fastest growing, highest loaded wetlands.
3. Above and below-ground biomass, culm height and turnover, and allocation to seed production increased with wastewater loading rates. Gradients of decreasing culm and rhizome biomass down the wetland channels reflected the effects of wastewater treatment processes on nutrient supply. Mean culm densities between 1000 and 1400 m⁻² during summer occupied between 2.3 and 5.3% of the wetland surface, giving estimated specific gas transport capacities of 12 to 24 mm² (lacunal area) g⁻¹ of below-ground biomass
4. Net annual primary productivity (NAPP), including above-ground mortality, during the first growth season rose from ~2.5 to 3.5 kg m⁻² with increasing wastewater loading. Above-ground litter inputs from plants were estimated to contribute between 100 and 140 g m⁻² of readily available carbon and between 270 and 375 g m⁻² of slowly degradable carbon substrates to the surface of the wetlands. This was equivalent to between 75 and 30% respectively of the available carbon supplied to these wetlands in wastewaters. Readily available plant C inputs in the first year were estimated to exert a theoretical oxygen demand equivalent to between 80 and 25% respectively of their wastewater CBOD loadings.

5. Mean mid-summer below : above-ground biomass ratios were similar for all wetlands at between 0.6 and 0.7, with below-ground growth accounting for between 10 and 23% of NAPP. Rhizomes, which comprised around 80% of the below-ground biomass, were generally restricted to the upper 10 cm of the substratum. Over half the root biomass also occurred in the upper 10 cm, with negligible root penetration recorded below 30cm depth.

Introduction

Constructed wetland wastewater treatment systems combine elements of natural wetland systems (saturated substrates, shallow water, aquatic plants and microbes) for the purification of wastewaters. In subsurface-flow wetlands (also known as vegetated submerged beds or root-zone systems), wastewaters flow through the root-zone of wetland plants growing in soil or gravel substrata. Essentially fixed-film biological reactors, treatment is achieved by a complex of physical, chemical and biological processes (Brix, 1987; Reed and Brown, 1992). Planted wetlands, when compared to unplanted controls, commonly show enhanced removal of nitrogen and pathogens (e.g. Gersberg et al., 1983, 1986, 1987; Tanner et al., in press a, b). The potential roles of plants in treatment processes include: nutrient and heavy metal uptake, cycling and storage; modification of environmental conditions in the bed by rhizosphere oxygenation and gas venting; the production of exudates, leachates and litter as carbon sources for microbial processes such as denitrification, and as sources of biologically and chemically active substances such as humic acids (Brix, 1987; Gersberg et al., 1983, 1986; Howard-Williams, 1985; Seidel, 1976; Wetzel, 1990). The presence of a plant cover and associated litter, may also affect wastewater treatment processes by modifying the microclimate and hydrology of wetland treatment systems (e.g. via transpiration, rainfall interception, shading, and insulation).

Although there is considerable information on the plant dynamics and productivity of natural and fertilised wetlands, most studies of constructed wetlands receiving wastewaters have only addressed general aspects of plant growth and nutrient content. Intensive studies of the growth of helophyte species in wastewaters have generally been short-term and small-scale (eg. Spangler et al., 1976; Koncalova et al. 1993; Tanner, 1994). Recently, Parr (1990) has documented a range of factors affecting *Phragmites australis* growth in wetland wastewater treatment systems constructed in the United Kingdom, and aspects of the growth and nutrient dynamics of this species (Gries and Garbe, 1989; Surface et al., 1993) and *Glyceria maxima* (Sundblad and Wittgren, 1989) have been studied in wetland treatment systems.

Schoenoplectus validus (Vahl) A. Love and D. Love (soft-stem bulrush) and related morphologically similar species such as *S. lacustris* and *S. acutus* (all previously placed within the genus *Scirpus* sens. lat.) have been employed widely in wetland wastewater treatment systems. The present study focuses on the growth of *S. validus* in subsurface-flow, gravel-bed constructed wetlands treating ammonia-rich dairy farm wastewaters. The objectives of the study were:

1. to measure net annual primary production (NAPP) over a range of commonly applied wastewater loading rates, and
2. to investigate the effects of wastewater loading rates and wetland fertility gradients on seasonal plant growth patterns; allocation to above and below-ground tissues and storage compounds; and root depth distribution.

Concurrent studies of plant nutrient accumulation and cycling are dealt with in a companion paper (Tanner, submitted.).

Methods

WETLAND SYSTEMS

Experiments were carried out on the Ruakura Research Farm, Hamilton, New Zealand (lat. 37° 47' S, long. 175° 19' E, alt. 40 m ASL) between November 1989 and January 1992. The climate of the area is characterised as warm wet (c. 1200 mm yr⁻¹) temperate. The mean daily temperature range rose from about 3 - 13 °C in July (mid-winter) to 12.5 - 23 °C in January (mid summer) during both of the growth periods studied, with mean daily solar radiation increasing seasonally from about 6 to 23 MJ m⁻² respectively (Ruakura Meteorological Station records, AgResearch, Hamilton). The 4 pilot-scale wetlands studied (described fully in Tanner (1992) and Tanner et al. (submitted a)) were rectangular channels (surface dimensions 9.8 by 2.3 m), lined with butyl rubber and filled to 0.4 m depth with a local alluvial rhyolitic gravel (10-30 mm diameter, 35% effective porosity). Dairy farm wastewaters pretreated in a two-stage oxidation pond system were applied at different rates to the end of each channel, exiting from the opposite end. The loading rates (designated L1 to L4) correspond to mean hydraulic application rates of 16, 26, 40 and 72 mm d⁻¹, giving nominal retention times of 7.5, 5.5, 3 and 2 days respectively. The strength of the influent wastewater was increased in the second growth season (from Sept 5, 1991) by manipulation of the pretreatment pond size. At the same time, inflows to L1 were increased to 37 mm d⁻¹ (i.e. above that of L2) as part of a concurrent experiment. Water levels in the wetlands were maintained approximately 20 mm below the gravel surface throughout the trials.

Influent wastewater strength fluctuated seasonally in response to variations in waste production and oxidation pond performance, with highest levels occurring in spring and summer when plants were actively growing (Table 1). Mean mass loading rates of carbonaceous biochemical oxygen demand (CBOD) were around 3 fold higher and total BOD (CBOD + calculated nitrogenous BOD; $4.33 \times \text{NH}_4\text{-N}$), N and P levels 2 fold higher during the second growth season monitored. Around 57% of N loadings were in the ammonium form, 9% as nitrate, and 34% in organic forms, while 80% of P loadings were in dissolved reactive forms. Further details of wastewater constituents, loading rates, and treatment performance in the wetlands are documented in Tanner et al. (submitted a, b). Figure 1 shows predicted gradients of major wastewater constituents along the wetland channels, for each growth season, assuming plug flow and first-order removal (Bavor et al., 1989; USEPA, 1988).

PLANT SAMPLING

The wetlands were planted (8 propagules m^{-2}) in early November 1989 (spring) with rhizome cuttings of *S. validus* collected from a natural population growing nearby on the shores of the Waikato River at Hamilton. After an 11 month period of establishment, growth was monitored over two spring-summer growth periods and the intervening autumn and winter (October 1990 to January 1992). Weeds were removed manually at 2-3 month intervals to maintain monospecific stands. Senescent above-ground biomass was harvested and removed from the wetlands, near the end of the first winter (mid-August, 1990), to simplify demographic monitoring of growth.

Sampling was restricted to the central 1.5m of the wetlands to avoid edge effects. Culm densities were monitored at one to two month intervals along 5 permanent transects in each wetland (see Fig. 2). Each 5 cm wide belt transect was delineated by 2 parallel high-tensile stainless steel rods (4.75 mm diameter) set 20 cm above the substratum using wooden block supports at the midpoint and each end. Culm numbers were counted in fourteen graduated 10 cm sections (i.e. 50 cm^2 quadrats) across each transect. Each culm was also marked at 20 cm height using a nontoxic vinyl paint. Culm demography was then monitored by repeat censuses of culm cohorts (age classes) differentiated by the colour of their first paint mark. Culms were considered to be dead when more than half of their length was necrotic. Cell extension in the simple tapering cylindrical culms, which grow from basal meristems in the rhizome, was restricted to the lower leaf-sheathed zone (< 20 cm above ground level). Therefore, short-term culm extension rates were able to be monitored by measuring increases in the height of the paint markings over 9 or 10 day periods. Canopy heights ($\approx$ 75 percentile culm height) were estimated to within 10 cm, with the aid of a 2 m rule, by sighting across the wetland above each transect.

Above and below-ground biomass was sampled sparingly at 1-3 month intervals between August 1990 and June 1991. Care was taken to minimise disturbance of the wetlands, with harvesting of plant material from less than 2% of the area of each wetland in total during the trial, restricted to 4 zones intermediate between the transects (see Fig. 2). Twenty-four 6 cm diameter (28 cm²) core samples (6 per zone) were obtained from each wetland using random coordinates. The uncohesive gravel substate were removed from the cores on site, using a commercial vacuum cleaner; with culm bases, rhizomes, roots and gravel retained in a customised 2mm mesh filter chamber. Excavated cores were replaced with fresh gravel after sampling. After washing in tap water; culms, standing litter, flower heads, roots and rhizomes (including attached culm bases) were separated and oven-dried (80°C) before weighing. Biomass data was analysed using ANOVA (Genstat 5, Lawes Agricultural Trust, UK)

Annual culm turnovers were calculated on the basis of live culm losses using life tables (Verhoven et al., 1988), with cohort life expectancies calculated as outlined in Krebs (1972), and the mean above-ground biomass duration (biomass time integral) as in Kvet and Ondok (1971). Net primary production was estimated for the first season (1990/91), both as the difference between the maximum and minimum biomass of live and standing dead tissues (Milner and Hughes, 1968), and as the sum of positive increments in live above-ground biomass until maximum biomass was reached corrected for losses of above-ground tissues due to mortality and grazing (similar to Houghton, 1985), plus increases in below-ground biomass (assumes below-ground biomass increase results primarily from translocation of photosynthate, rather than remobilisation of cellular carbon during senescence; see discussion). Losses of above-ground biomass were calculated for each sampling as the product of the reduction in the density of previously marked live culms and the mean mass of live and standing dead culms at the previous sampling.

At the end of the trial (January 1992) above and below ground biomass gradients and root depth profiles were sampled along the length of the wetland channels. Sixteen 21 cm diameter (346 cm²) cores, and the live and standing dead plant material directly above them, were systematically sampled in each wetland (see fig 2 for sampling regime). After draining of the wetlands, graduated sharp-edged steel cylinders were driven 35 cm into the substratum and excavated in three 10 cm layers. Samples were stored in plastic bags under refrigeration (4°C) before recovery (generally within 48 hours) of live and dead rhizomes (including culm bases), and roots by wet sieving (2 mm mesh). Marked culms were also harvested from the permanent transects at the end of the trial, and sorted into age classes on the basis of their earliest paint mark. After careful washing and sorting, above and below-ground tissues were dried and weighed as described above. Biomass data on gradients within the wetlands were

smoothed using Bayesian curve fitting techniques (FLEXI; Upsdell and Wheeler, 1992), with mean values calculated for each wetland by integration of the smoothed biomass-distance curve.

NON-STRUCTURAL CARBOHYDRATE ANALYSIS

Subsamples of live rhizome were washed and freeze-dried within 2 hours of collection. Non-structural carbohydrates excluding starch, were extracted using 80% ethanol (Wilson and Bailey, 1971). After removal of ethanol, the extract was hydrolysed for 1 hour using 0.5 N sulphuric acid, then cooled, 0.2 g m⁻³ inositol added as an internal standard, and the solution neutralised using calcium carbonate. Soluble sugars were then determined by gas chromatography (Hewlett Packard model 5880A with high performance cross-linked methyl silicone 25 m capillary column; 0.17 µm film thickness, 0.31 mm internal diameter) after trimethylsilation (Sweeley et al., 1963) using Tri-Sil reagent (Pierce, Rockford IL., USA). Starch was analysed in the residue of the ethanol extractions. After autoclaving at 121 °C for 1 hour, the residue was cooled to below 60 °C before incubation for 1 hour at 55 °C in acetate buffered (pH 4) 0.05% amyloglucosidase solution. Starch concentrations were then determined using the Trinder's colorimetric reaction (Barham and Trinder, 1972).

Results

Marked seasonal patterns of plant growth and senescence were observed in the wetlands. Few culms emerged or showed active growth during the winter period. New culm growth began in the first month of spring (early September). During the first growth season culm numbers increased most rapidly and reached highest densities (around 1400 m⁻²) at the highest wastewater loadings (Fig. 3). Growth rates and maximum seasonal biomass were highest for L3, which showed the highest rhizome biomass. Seasonal increases in culm density were similar for the second season when organic matter and nutrient loadings were markedly higher, except in the highest loaded wetland (L4), which produced a taller lower-density stand. Figure 3 shows mean culm production and cohort turnover for each wetland. Mean life expectancies for the cohort of culms produced between October 2 and November 21 1990 were similar in all wetlands at between 110 and 120 days, but mean annual culm turnover rates showed gradual increases with increasing wastewater loading; from 2.8 to 3.6 (for the period October 1990 to 1991) and from 2.6 to 3.8 (January 1991 to 1992). Analysis of marked culms harvested at the end of the trial (January 1992) showed that between 40 and 55% of the above-ground live biomass had been produced in the previous 28 days, and 70 to 80% in the previous 50 days, with less than 10% produced before November of that season (i.e. >78 days old).

Culms produced early in the season grew to heights of less than 0.5 m. These were quickly over-topped, with successive cohorts increasing to maximum heights of over 2 m. Mean canopy heights recorded during mid-summer (mid January) in the first growth season, were between 1.2 and 1.7 m. Strong winds followed by heavy rain storms at the end of January (wind run 382 km, 23.1.91; rainfall 66 mm, 26.1.91; Ruakura Meteorological Station records) caused partial collapse of the canopy (Fig 4). Lodging was most severe in the taller stands growing at the head of each wetland and in the higher loaded wetlands (L3 & 4). During the first season, mean mid-summer canopy heights were positively correlated with wastewater loading rates, while in the second season (when overall mass loading rates were markedly higher) they were similar for all wetlands. Over 95% of culms were actively growing ($> 1 \text{ mm d}^{-1}$) in October 1990, with mean extension rates of between 7 and 17.5 mm d^{-1} recorded along the sampling transects, declining with increasing distance from the wastewater source (Fig 5). Mean extension rates of actively growing culms had increased to between 15 and 30 mm d^{-1} by December 1990, but 40 to 60% of live culms were no longer growing.

Figure 6 shows seasonal changes in plant biomass components recorded during the first season. Live culm biomass showed a similar seasonal pattern to culm density, with maxima ($1700 - 2200 \text{ g m}^{-2}$) reached earlier in the wetlands with higher wastewater loadings. Despite these differences, live above-ground biomass duration was similar at around 240 kg d m^{-2} for all loadings except L3, where a higher value of 302 kg d m^{-2} was recorded. Flower initiation began in November. By mid summer flower biomass accounted for between 7 and 17% of above-ground biomass, increasing with wastewater loading. Standing dead culm biomass showed large increases during February and March as senescence proceeded. Senescing culm material often collapsed in clumps, resulting in patchy distribution of fallen and standing litter within the wetland. Mean over-wintering live culm biomass was generally below 200 g m^{-2} .

Mean below-ground biomass at the start of the monitoring period was around 700 g m^{-2} , except for L3 where it approached 1200 g m^{-2} . Significant below-ground biomass increases ($P < 0.05$) were recorded during the latter part of the first growth season (after the peak of flowering) in all but the highest loaded wetland (L4), where final biomass remained below 1000 g m^{-2} . Observations of harvested rhizome material suggested minimal mortality had occurred up to the end of the first growth season of monitoring (18 months after planting).

Rhizome soluble sugar content remained relatively constant in all treatments during the sampling period, at between 0.2 and 1.0 % of dry weight. Starch content, initially around 20% of rhizome dry weight, showed significant depletion during the spring

growth period (Fig. 7), rising again to initial levels or higher by early autumn (March). The amplitude of seasonal fluctuations increased with observed rates of plant growth; rising gradually with wetland wastewater loading and highest near wastewater inflows to the wetlands.

Net annual primary production (NAPP) estimates for the wetlands ranged from around 2800 to 3500 g m⁻², when above-ground mortality was taken into account (Table 2). Above-ground values calculated in this manner were around 1.2 times higher and total NAPP 1.2 to 1.4 times higher, than those based on maximum-minimum biomass of live and dead tissues. Standing dead plant material at maximum above-ground biomass in the first season represented between 16 and 60% (highest in faster growing stands) of the culm mortality recorded, while that present at the end of the growing season (June) represented between 40 and 60% of estimated mortality. This suggests that around half of dying above-ground production had either been decomposed while standing, removed by grazers, or deposited on the wetland surface by mid winter.

January total biomass values rose slightly in the second season, but with greater contribution from standing litter and dead rhizome biomass (Fig. 8). Live above and below-ground biomass respectively (Fig. 8), were 30 to 50% and 6 to 40% lower than those recorded at the same time in the first season. Comparison of mean plant biomass for the second season (Fig. 8) shows a general increase in all components, except dead rhizomes, with increased wastewater loading. Live culm biomass generally decreased down the length of the wetland channels, whilst live rhizome biomass remained relatively constant (Fig. 9).

Mean live below : above-ground biomass ratios in the wetlands were similar for all wetlands, at between 0.6 and 0.7. Roots comprised between 10 and 20% of live below-ground biomass, giving true root : shoot ratios (nutrient cf. carbon assimilating tissues) of around 0.1 in all wetlands. Root biomass showed high spatial variability, with no clear biomass gradients discernable down the length of the wetland channels, but mean wetland values rose gradually with increasing wastewater loading (Fig. 10). Root density decreased markedly with depth, with little or no roots observed below 30 cm depth. Figure 11 shows the mean proportion of deep-growing roots (>10 cm depth) in relation to wetland loading rates, at 1, 4, and 8 m distances down the wetland channels. At 1 m (the inflow end) deep roots comprised around 40% of root biomass at all loadings. With progression down the wetland towards the outflow (4 then 8 m), there was a trend of increasing proportion of deep roots in the lower loaded wetlands (rising to 60-70% deep roots in L1 and L2). Increased wastewater loadings to L1 during the second growth season (total BOD loadings increased to 17.2 g m⁻² d⁻¹) did not result in death of the deep growing roots in this wetland, however these deep roots may not have been sustainable over the longer-term, at these higher loadings.

DISCUSSION

SEASONAL DYNAMICS

The seasonal pattern of growth recorded for *S. validus* in the wastewater treatment wetlands was similar to those reported for natural populations on the west coast of North America (Hoffnagle, 1980; Hall and Yesaki, 1987), with strongly pulsed spring and early summer growth followed by senescence of above-ground tissues in the autumn. Mobilisation of starch reserves in the over-wintering rhizomes, was associated with rapid growth of above-ground culms in early spring, with above-ground growth rates increasing gradually with wastewater loading. Although achievement of maximum biomass occurred earlier in the higher loaded wetlands, overall biomass duration was similar for all loading rates. Flowering was initiated in early summer, with final seed development in late summer overlapping with a period of rapid rhizome growth and carbohydrate storage. As in natural communities growing nearby on the shores of the Waikato River (author's unpublished observations), very little culm emergence or growth was observed over the winter period.

Significant reductions in rhizome biomass in association with spring culm growth (e.g. Steinmann and Brandle, 1984) were not discernable in the below-ground samples collected, but major reductions in nonstructural carbohydrate reserves were noted at this time. Concentrations of starch, the dominant storage substance, were reduced to less than half their initial levels during late spring, with mobilisation greatest at high wastewater loadings, where plant growth rates were highest. Mean autumn starch concentrations of around 24% of rhizome biomass, were somewhat lower than those reported for wild populations of *S. lacustris* (~30%; Steinmann and Brandle, 1984), *Typha latifolia* (45%; Kausch et al., 1981), and *Phragmites australis* (~38%; Best and Dassen, 1987).

Although around half of the measured starch reserves were still present in the rhizomes after the spring growth period, considerable proportions of those remaining may have been inaccessible to the plant (Chapin et al., 1990). Starch levels remained depleted for longer periods in the higher-loaded, faster-growing stands, but recovered to similar levels during early autumn, and showed broadly similar rates of decline over the first half of winter. Cizkova-Koncalova et al. (1992) have hypothesised that reduced autumn storage, and greater depletion of starch reserves during the winter period may be a predisposing factor in the decline of reedbeds subject to elevated nutrient and organic matter loadings. Plants are likely to be more vulnerable to physical disturbances, such as grazing, trampling, harvesting and periods of high water levels, during periods of rhizome starch depletion. High N concentrations may also increase susceptibility to physical damage, by reducing the development of structural

tissues. Increased lodging of the taller, higher nutrient loaded stands was noted in the present study, but levels of culm structural tissues were not investigated.

NET PRODUCTIVITY

S. validus was able to flourish and achieve high levels of productivity in the high nutrient, anaerobic conditions of the wastewater treatment wetlands. Mean NAPP values increasing from 2.8 to 3.5 kg m⁻² (including above-ground mortality before maximum biomass) with wastewater loading, were in the upper range recorded for helophytes in temperate regions (Bradbury and Grace, 1983). Very few new culms emerged after February, and mean culm extension rates were negligible, suggesting little additional above-ground production occurred after maximum biomass. Recycling of carbon compounds from senescing above-ground tissues was assumed to be minor, with autumn rhizome biomass increases fuelled predominantly by translocation of current photosynthate. Lytle and Hull (1980) studying the fate of photoassimilated ¹⁴CO₂ in *Spartina alterniflora*, found that basal organs continued to be a major sink during autumn, accounting for 30 to 40% of assimilated carbon within two days. Subsequent losses of the ¹⁴C label retained in the culms, did not result in significant increases in labelling of basal plant tissues during above-ground senescence, suggesting most was lost via litterfall, leaching and microbial respiration (see Newell et al., 1989). In a recent review of plant storage, Chapin et al. (1990) concluded that, N and P containing organic compounds (mainly proteins and polyphosphate respectively) are in general, the only quantitatively important carbon compounds recycled from senescing leaves (unless specific storage compounds are actively accumulated, e.g. starch or fructans). Carbon compounds associated with N and P are estimated to have accounted for less than 10% of the above-ground biomass of mature culms of *S. validus*, based on conversion of tissue N concentrations to crude protein equivalents (N x 6.25; max ~8%), with less than half lost via retranslocation and leaching during senescence (Tanner, submitted).

Maximum above-ground biomass values recorded in the present study (over 2.5 kg m⁻²) were similar to those found for *S. validus* in 116 day microcosm studies utilising wastewater from the same source (0.24 m² batch-fed tanks; Expt. 2; Tanner, 1994). They were considerably higher than those that have been reported for natural populations of *S. validus* in North America; Polinski and Boyd (1972) recorded mean standing crops of 1.38 kg m⁻² at Par Pond, South Carolina; Hoffnagle (1980) 0.80 kg m⁻² (estimated NAPP 1.2 kg m⁻²) at Coos Bay, Oregon; and Hall and Yesaki (1987) maxima of 0.91 kg m⁻² (assuming ash content of 7.5%; Auclair, 1979) in the Frazer River Estuary, British Columbia. Standing crops of *S. validus* in the present study were also higher than those reported for *Phragmites australis* growing in soil and gravel-based wetlands treating sewage (< 1 kg m⁻²; Gries and Garbe, 1989; Parr, 1990), or

gravel and sand wetlands treating landfill leachates ($0.7 - 1.5 \text{ kg m}^{-2}$; Surface et al., 1993), but considerably less than mean levels recorded for *S. californicus* in a gravel-bed wetland treating piggery wastewaters (4.3 kg m^{-2} ; Sukias and Tanner, 1993).

As hypothesised by Grime (1979) and Chapin (1980) for plants adapted to growth in fertile environments, turnover rates in the wetlands rose with increasing wastewater nutrient supply. Estimated above-ground biomass turnover recorded in the period up to maximum biomass, rose from 14 to 21% in the wastewater wetlands, corresponding with turnover of between 30 and 40% of culms during the same period. Biomass allocation to flowering and seed production also rose with wastewater loading. Neill (1992) reported similar turnover, and flowering responses (the latter only at deep water sites) for *Scolochloa festuacea* growing in prairie marshes, after nitrogen fertilisation. In contrast, Jordan et al. (1990) reported reduced flowering of *Typha augustifolia* stands fertilised with N and P.

Below-ground tissues comprised around 23% of the total NAPP recorded at the two lower wastewater loadings tested, decreasing to 13 and 10% of total production with increasing loading. Although very little dead rhizome material was observed during the first monitoring season when productivity estimates were made, it is likely that below-ground production was underestimated as mortality, grazing and exudation losses of roots and rhizomes were not accounted for. By January of the following season, dead rhizomes made up around half of the below-ground biomass, showing considerable turnover had occurred. This suggests a rhizome lifetime of between 18 and 24 months for *S. validus* in the wastewater treatment wetlands, considerably shorter than the 3 to 4 years reported for natural and hydroponically cultured *Phragmites communis* (= *P. australis*; Dykyjova and Kvet, 1978). Root turnover is likely to be greater than that of rhizomes. Previous microcosm studies of *S. validus* growth in similar wastewaters, suggested root lifetimes of around 4 months (Tanner, 1994).

Dense covers (90-100%) of standing and partially fallen plant litter remained after the second winter, becoming a major component (40-50%) of above-ground biomass in the second season. Removal of five 0.1 m^2 plots of surface litter from one of the wetlands during the second winter, to enable nutrient analysis (author's unpublished data), resulted in an approximate threefold increase in early spring culm emergence in these plots compared to uncleared areas of the wetland. Jordan et al. (1990) found that high covers of litter reduced shoot densities and biomass in *Typha augustifolia* stands by more than 50%. Similar effects noted for real and artificial (plastic strips) litter, suggested this was predominantly a response to physical shading. Light levels measured in moderate-density standing litter (~1 m tall) in the wastewater treatment wetlands prior to spring culm emergence, using a multi-sensor integrating canopy meter (author's unpublished data; 12-2 pm, under a clear sky), showed

photosynthetically active radiation at 200 mm above the substratum was reduced to between 1 and 15% of incident levels.

POTENTIAL PLANT CARBON INPUTS

Plant litter fall in constructed wetlands returns a proportion of stored nutrients, and contributes both readily and slowly degradable carbon substrates to the benthic microbial community. During litter decay, nutrients will be temporarily immobilised within the microbial community. In low-carbon nitrified wastewaters, such litter inputs may provide a valuable source of carbon substrates promoting denitrifying activity (e.g. Gersberg et al., 1983, van Oostrom & Russell, 1992), but in organic ammonium-rich wastewaters such as those being treated in the present study, they will add to the oxygen demand already exerted on the treatment system by wastewater inputs.

Not all plant litter production will be deposited on the wetland surface, and much which does may not become available to benthic denitrifiers. Lodge (1991) has convincingly refuted the dogma that living macrophyte tissues are rarely consumed. Plants growing in high nitrogen conditions, such as wastewater treatment wetlands, are likely to suffer higher levels of grazing than natural populations (Tschardtke., 1990; Jordan et al., 1990; Price, 1991). In the present study, grazing of succulent young culm tips by local populations of swamp hen (*Porphyrio porphyrio*) was observed, and high densities of culm-boring moth larvae (identified as two *Bactra* spp; John Dugdale pers comm.) were noted during plant sampling, particularly in late summer and early autumn. Of the ungrazed above-ground tissues, around 30% are readily degradable (Polinski and Boyd, 1972; Godshalk and Wetzel, 1978), the remainder being composed of more resistant fibrous constituents (cellulose, hemicellulose, and lignin), of which significant proportions are refractory (~18-23% of original dry weight for perennial species; Morris & Lajtha, 1986).

Culms of helophytes such as *S. validus* are not naturally abscised. Studies of *Typha* and *Spartina* (Davis and van der Valk, 1983; Newell et al., 1989) show that significant decomposition of senescing tissues occurs while they are still standing. Newell et al. (1989) estimated that 15 to 20% of the above-ground carbon (presumably a high proportion of the more labile fractions) in *Spartina alterniflora* stands was lost as CO₂ via aerobic decomposition of standing dead tissues over 3 to 5 month periods. Around 10% of the non-CO₂ losses recorded from dying leaves appear to have been as dissolved leachates, the remainder mainly falling to the substratum as particulate litter and associated microbial biomass. Assuming similar processes operate during senescence of *S. validus* and that herbivory losses account for around 10% of net production, I estimate that around 10% of the senescing above-ground carbon would have been likely to reach the wetland surface in readily available forms. At the above-

ground NAPP levels recorded in the first season of the present study, this would be equivalent to readily available organic carbon additions of between 100 and 140 g m⁻² yr⁻¹ (carbon 45% of dry weight; Hall and Yesaki, 1987). A further 270 to 375 g m⁻² of carbon (~28%) is likely to be released as a result of longer-term decomposition processes, and 180 to 250 g m⁻² of refractory carbon likely to accumulate in the wetland (assuming 20% of tissue carbon is refractory; Morris & Lajtha, 1986). Carbon released via death, sloughing, exudation and secretion of below-ground tissues will be subject to fewer losses, as it is released directly into the wetland substratum, however these pathways of carbon release have been little studied in wetland plants. Sherr & Payne (1978) found that sediment nitrate reductase activity, an indication of denitrification potential, was closely correlated with root and rhizome production in short *Spartina alterniflora* marshes. Their data suggested that below-ground biomass turnover was a major source of organic substrates for denitrifiers in these marshes.

Mean carbon additions to the wetlands via wastewater inputs during the trial are estimated to have ranged between 500 and 1700 g m⁻² yr⁻¹ for the 4 loading rates tested, based on conversion of CBOD and volatile SS (assuming 50% C, 20% refractory) loadings to carbon equivalents. Above-ground plant litter inputs are therefore estimated to have increased the "potentially available" carbon supply to the lowest loaded wetlands by ~75%, reducing to ~30% addition in the highest loaded wetlands. Readily available plant litter C inputs would exert an additional CBOD loading to the wetland of between 260 and 370 g m⁻² yr⁻¹. This was equivalent to between 80 and 25% respectively, of the CBOD loadings to the wetlands in wastewaters over the first year. A high proportion of the leached soluble C compounds and litter deposited on the surface are likely to be intercepted and aerobically decomposed by heterotrophs at the surface of the wetland (Gersberg et al., 1983). As organic acids produced by anaerobic fermentation of cellulose and hemicellulose are considered to be the most likely carbon source for denitrifiers provided by plant residue additions (Beauchamp et al., 1989), shredding and incorporation of slowly degradable residues into deeper anaerobic layers of the wetland substratum by invertebrates, is likely to be important for supplying carbon substrates to these organisms.

PLANT GROWTH RESPONSES AND POTENTIAL GAS EXCHANGE

Preferential settling of particulate organics near the inlet zone and the cumulative effects of microbial decomposition processes create longitudinal and vertical gradients of nutrient availability and physiochemical conditions (e.g. benthic oxygen demand and redox potentials) along wetland wastewater treatment systems (Bavor et al., 1989; Williams et al., 1992). In the present study, above-ground plant biomass and culm height showed consistent reductions along the length of the wetland channels, with no

growth suppression detected even at the inlet end of the wetlands, where signs of toxicity might have been expected. Edwards et al. (1993) have also reported significant gradients in the cover and biomass of *S. validus* plants growing in a two year old gravel-bed constructed wetland treating sewage at Hardin, Kentucky. Reduced plant biomass and NAPP recorded for L4 compared to L3 during the first growth season of the present study, appears to be a relic of poorer initial establishment (resulting in lower rhizome densities at the start of the season) in the higher loaded wetland. Relative growth rates recorded for L4 were not depressed and little difference in tissue nutrient levels were apparent (Tanner, submitted), and by the end of the second season there was little difference in total above and below-ground biomass between L3 and L4.

Many plants adapted to growth in waterlogged wetland soils have the potential to modify environmental conditions in their root-zone by oxygen release (e.g. Hansen and Anderson, 1981) and venting of gaseous products of decomposition (e.g. Morris and Whiting, 1986). Plant mediated oxygen release (optimistically estimated to be in the range of 5 to 45 g m⁻² d⁻¹, USEPA, 1988) is considered to be crucial, both for plant survival and to maximise BOD and ammonium removal (the latter via sequential nitrification-denitrification) in the essentially anaerobic conditions of subsurface-flow constructed wetlands.

Studies by Arenovski and Howes (1992) and Morris and Whiting (1986) have shown significant relationships between the capacity of *Spartina alterniflora* plants to transport gases between the sediment and atmosphere, and their basal culm cross-sectional area and lacunal porosity. The ratio of plant culm lacunal area to below-ground biomass (oxygen transport area per gram of below-ground respiratory demand) has been proposed as a measure of their specific gas transport capacity (Arenovski and Howes, 1992). At mean culm densities and canopy heights recorded in January 1991 for *S. validus* in the present study, the area occupied by culms is estimated to have increased with wastewater loading from 2.3 to 5.3% of the wetland surface (culm diameter (mm) = 1.55 + 3.17 x culm height (m), $r^2 = 0.64$, $n = 115$; authors unpublished data). This would give estimated lacunal areas of between 1 and 2.4% of the wetland surface (assuming a mean lacunal porosity of 45%; Brix et al., 1992), and specific gas transport capacities of between 12 and 24 mm² g⁻¹ below-ground biomass. These capacities are 2 to 4 fold higher than maximum values reported for natural communities of *Spartina alterniflora* by Arenovski and Howes (1992). Actual gas flux rates via plant culms will however be dependant on a complex of other plant and environmental factors (e.g. Armstrong et al 1991; Brix et al, 1992; Konacalova et al., 1993). During winter dormancy, standing dead culms are likely, as shown for *Typha* and *Phragmites* (Jordan & Whigham, 1988; Brix, 1989) to remain active as conduits for gas exchange, helping to maintain aerobic conditions in below-ground tissues.

The zone of plant oxygen release in the wetland substratum will be affected by root density and depth distribution. In the present study, below-ground biomass increased with wastewater loading in the experimental wetlands, maintaining below : above-ground biomass ratios between 0.6 and 0.7. These were similar to the ratios recorded previously for this species in microcosm studies utilising the same wastewater type (Expt. 2; Tanner, 1994), but were low compared to ratios of between 1 and 3 commonly reported for natural communities of tall-growing helophytes (Bradbury and Grace, 1983). Rhizomes, which comprised around 80% of below-ground biomass, and roots grew predominantly in the upper 10 cm of the substratum. Practically no roots were found to penetrate deeper than 30 cm into the gravel substrata, and the proportion of deep growing roots declined with increasing wastewater loading. These rooting depths, similar to those reported for gravel-bed wastewater treatment wetlands by Reed and Brown (1992), Bavor et al. (1988), and Sukias and Tanner, 1993) are much shallower than depths of 76 cm reported for *S. validus* by Gersberg et al. (1986), and widely adopted as a basis for wetland design (e.g. USEPA, 1988). Mechanical impedance may reduce root depth penetration in the gravel-bed wetlands. Parr (1990) reported deeper rhizome penetration in soil than gravel-based wetland treatment systems. Previous studies comparing *S. validus* root growth in gravel-bed microcosms, over a range of dilutions of dairy wastewater with and without complete nutrient solution additions (Tanner, 1994), found that increasing concentrations of organic constituents (BOD) rather than nutrient levels per se, limited root penetration. Models of below-ground plant aeration (see Armstrong et al., 1991), predict that the maximum depth of root penetration will be controlled by the intensity of root respiration and rhizospheric oxygen consumption (hypoxic stress), in addition to anatomical properties of roots such as tissue porosity and wall permeability. Cizkova-Koncalova et al., (1992) and Koncalova et al., (1993) have reported structural changes, including inhibition of aerenchyma and endodermis formation, in the roots of *Phragmites australis* and three *Carex* sp. supplied with high levels of organic matter and nitrogen. These and other responses which affect the passage of oxygen from the atmosphere and its release in the root-zone, require further investigation to enable identification of species and genotypes able to sustain high oxygen transport capacities in the hypereutrophic conditions of wastewater treatment systems.

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Figure captions.

1. Predicted longitudinal gradients of total BOD, N and P loading down the four wetland channels, assuming plug flow and first order reaction kinetics. Mean values for each wetland loading (1-4) during the first season are shown by solid lines and those for the second season by dashed lines. Note: log scale on vertical axis.
2. Plan of wetland channels showing plant sampling zones. T1-5 = permanent transverse belt transects (nondestructive sampling); shaded areas = zones of small core sampling during the first growth season (28 cm² cores, ~5.5% of shaded zones sampled in total, < 2% of total wetland area); black circles = final core (346 cm²) samples, January 1992.
3. Demography of live culms of *S. validus* growing at four wastewater loading rates. Open symbols show mean culm density in the wetlands; closed symbols show production and mortality of individual cohorts.
4. Seasonal changes in mean canopy height for *S. validus* growing at four wastewater loading rates.
5. Mean culm extension rates recorded along the wetland channels in October (spring; SE = 1.23) and December (early summer; SE = 4.12) of the first growth season (1990).
6. Changes in above and below-ground components of biomass at four wastewater loading rates during the first growth season. Values are means for each wetland. Pooled standard errors were dead culms, 197; flowers 93; live culms, 280; rhizomes and roots, 147 ; and total biomass, 384.
7. Changes in mean rhizome starch concentrations for *S. validus* during the first growth season, at four wastewater loading rates; L1, Δ ; L2, $\blacktriangle$; L3, $\bullet$; L4, $\circ$. Each value is the mean of three duplicate samples.
8. Mean biomass (and their standard errors) for live and dead plant tissues in January 1992 (mid-summer), after 2 years growth at four wastewater loading rates.
9. Longitudinal gradients of culm (circles) and rhizome (triangles) biomass along the wetland channels at four wastewater loading rates in January 1992 (mid-summer). Fitted curves and their 85% confidence intervals are shown for each wetland. Lines are significantly different at the 95% level where their confidence intervals do not overlap
10. Depth distribution of mean root biomass at four wastewater loading rates in January 1992 (mid-summer). Bars show standard errors.
11. Mean proportion of deep-growing roots (>10 cm) in relation to wastewater loading, at three distances along the wetland channels at the end of the trial; upstream 1 m, middle 4 m, and downstream 8 m. The shaded zones show 95% confidence intervals for the fitted curves.

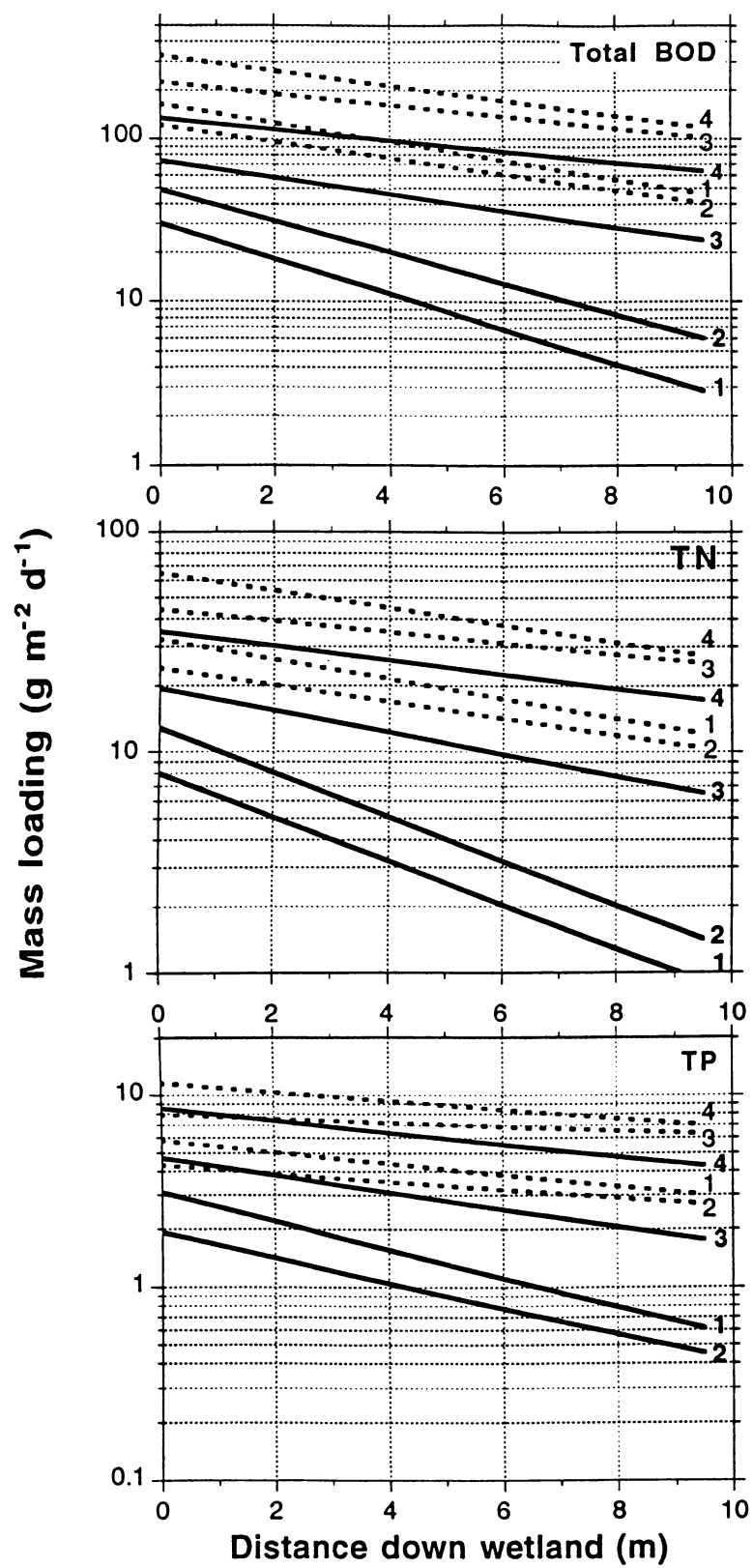
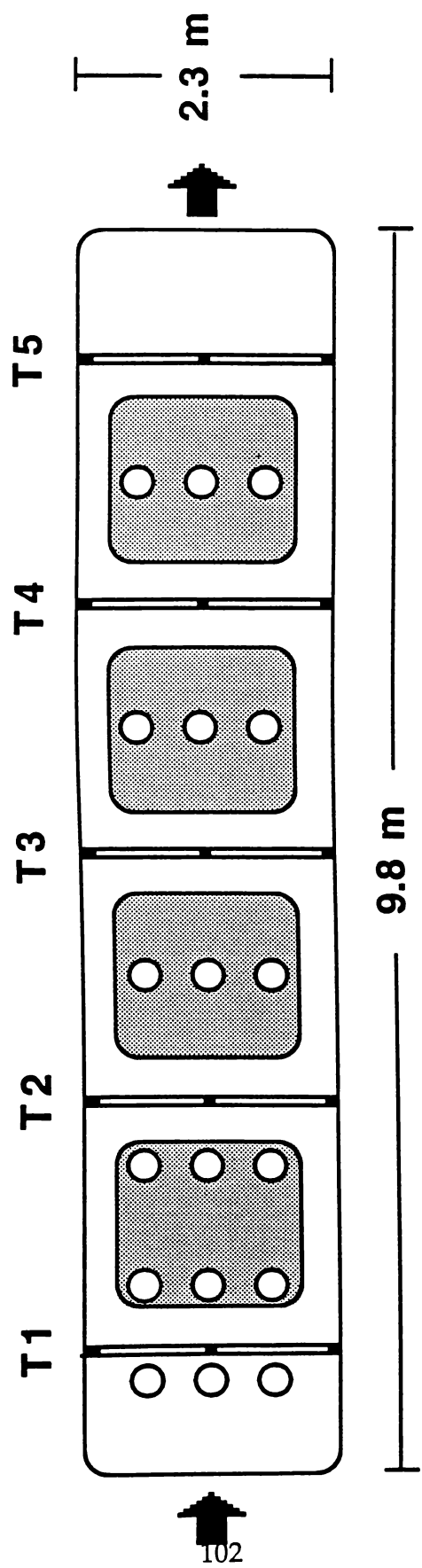


Figure 1

Figure 2



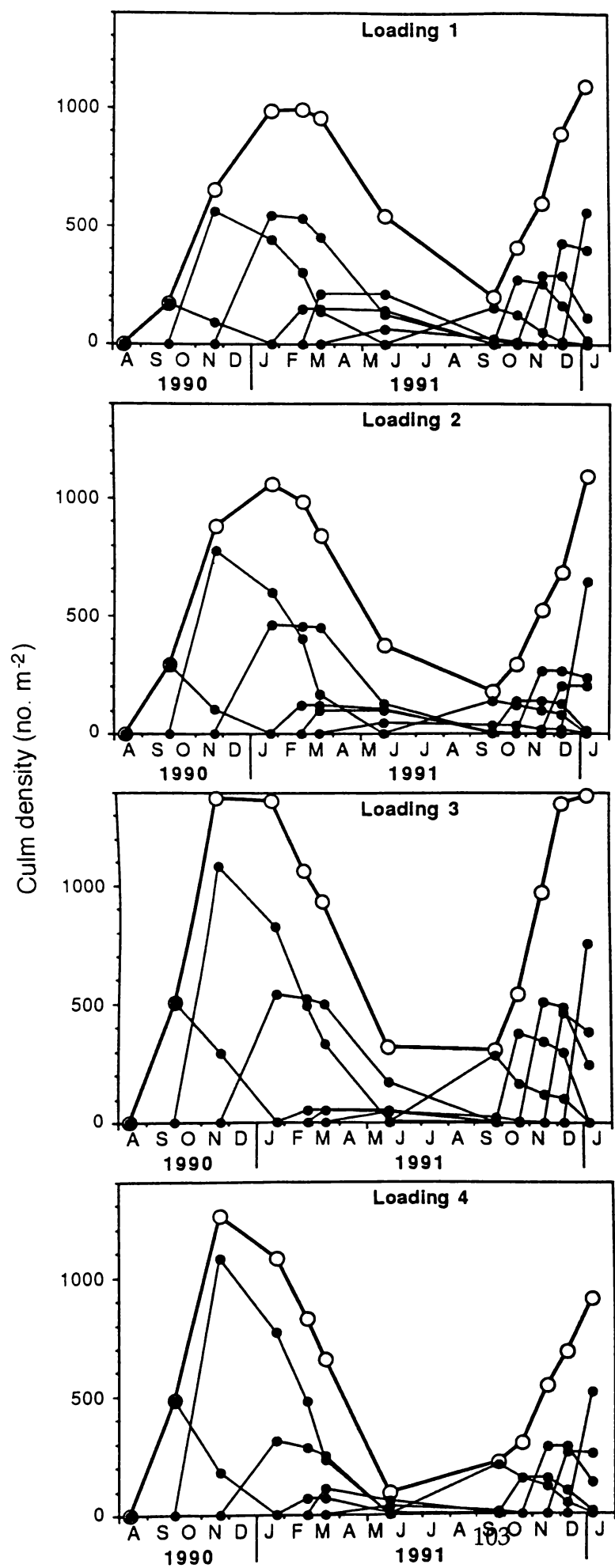
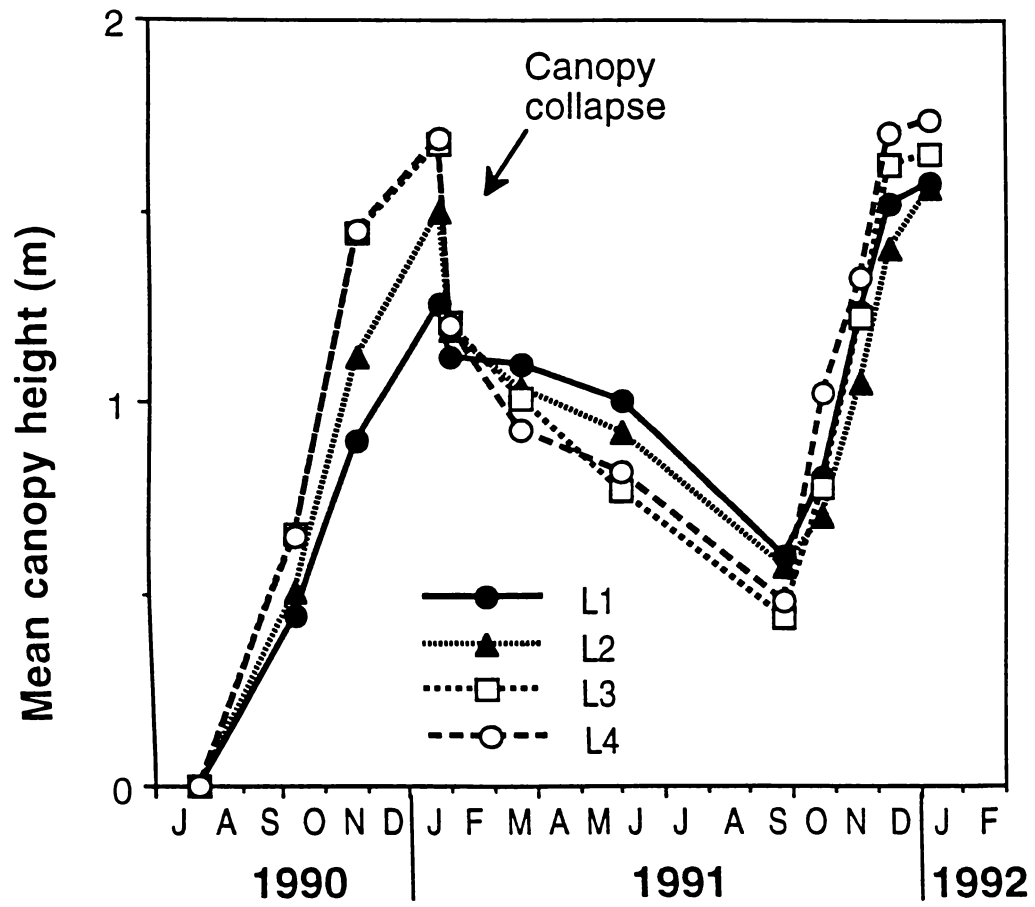


Figure 3

Figure 4



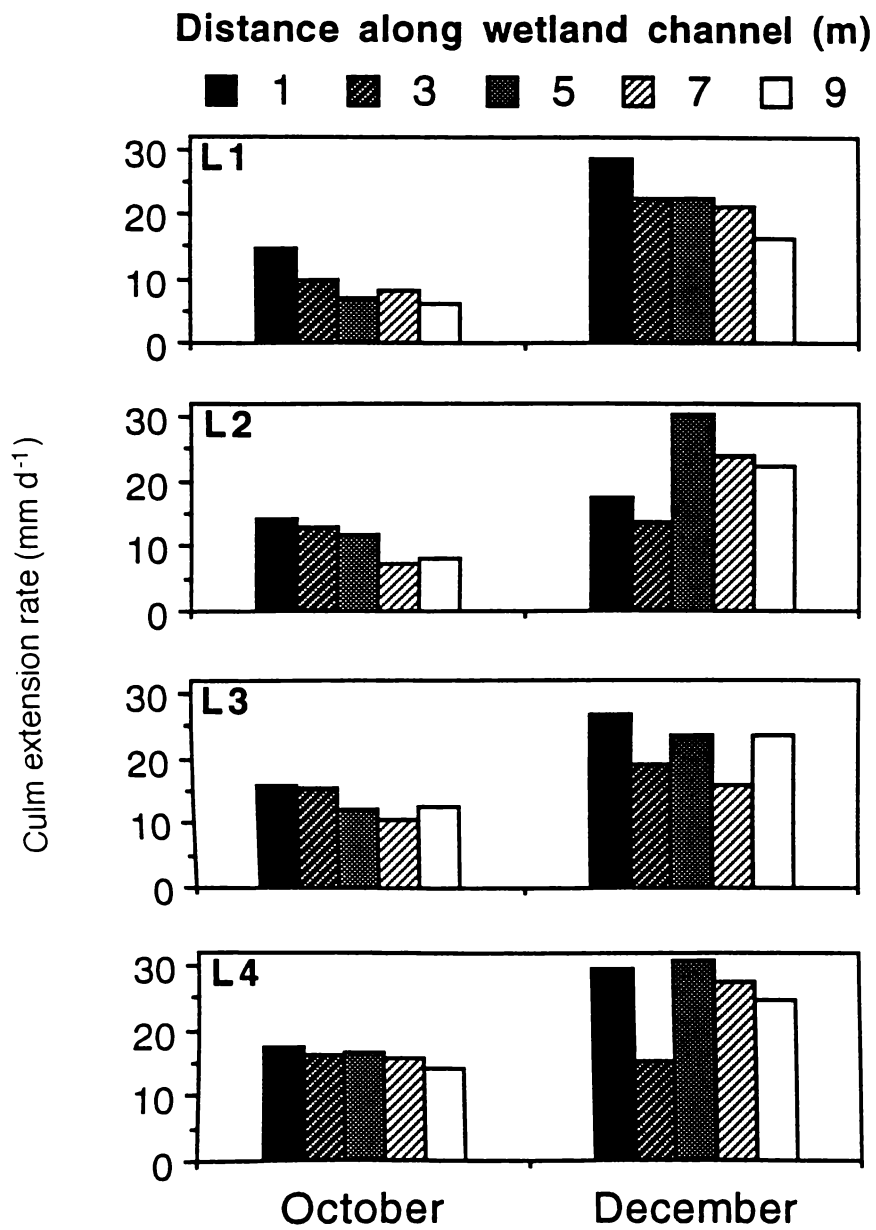


Figure 5

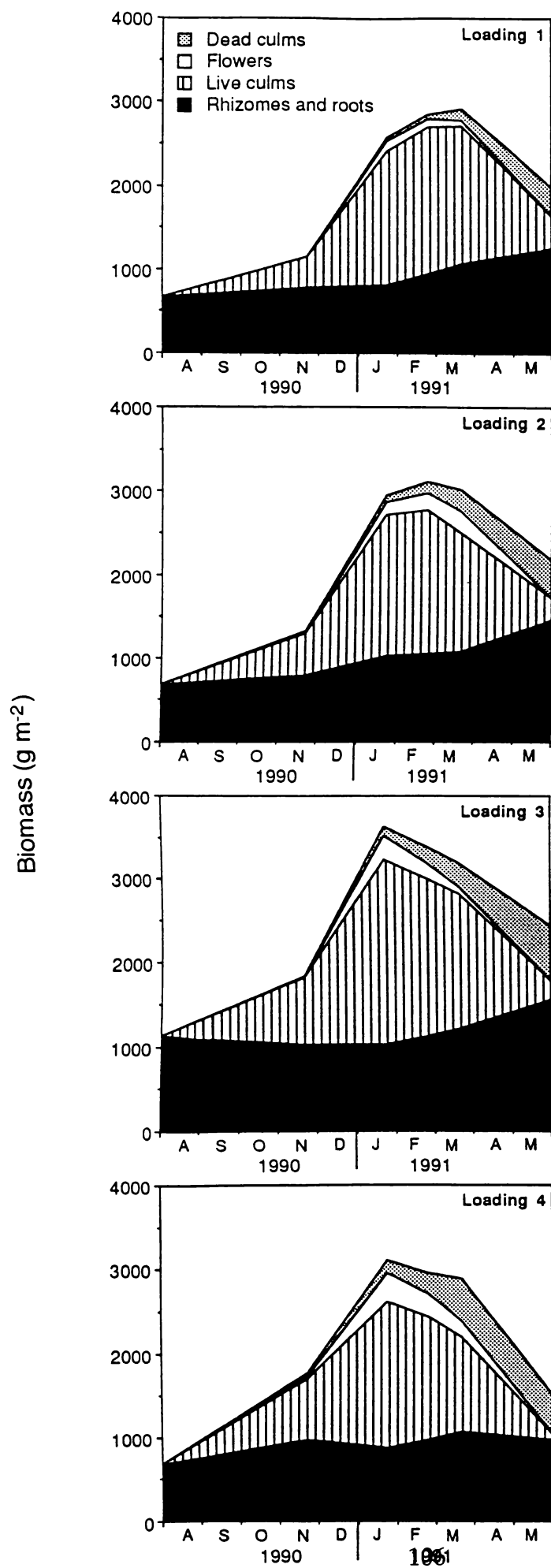


Figure 6

Figure 7

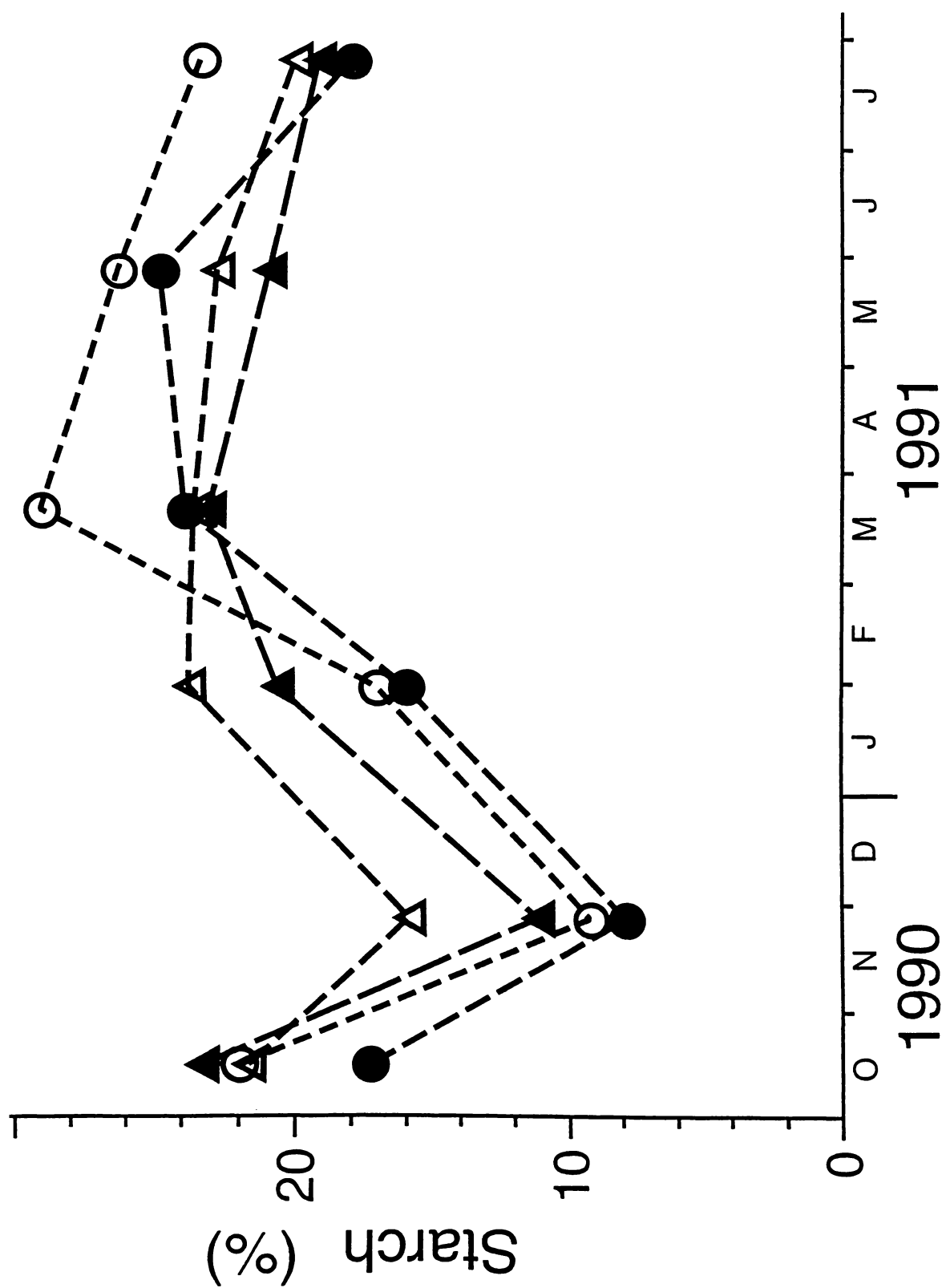


Figure 8

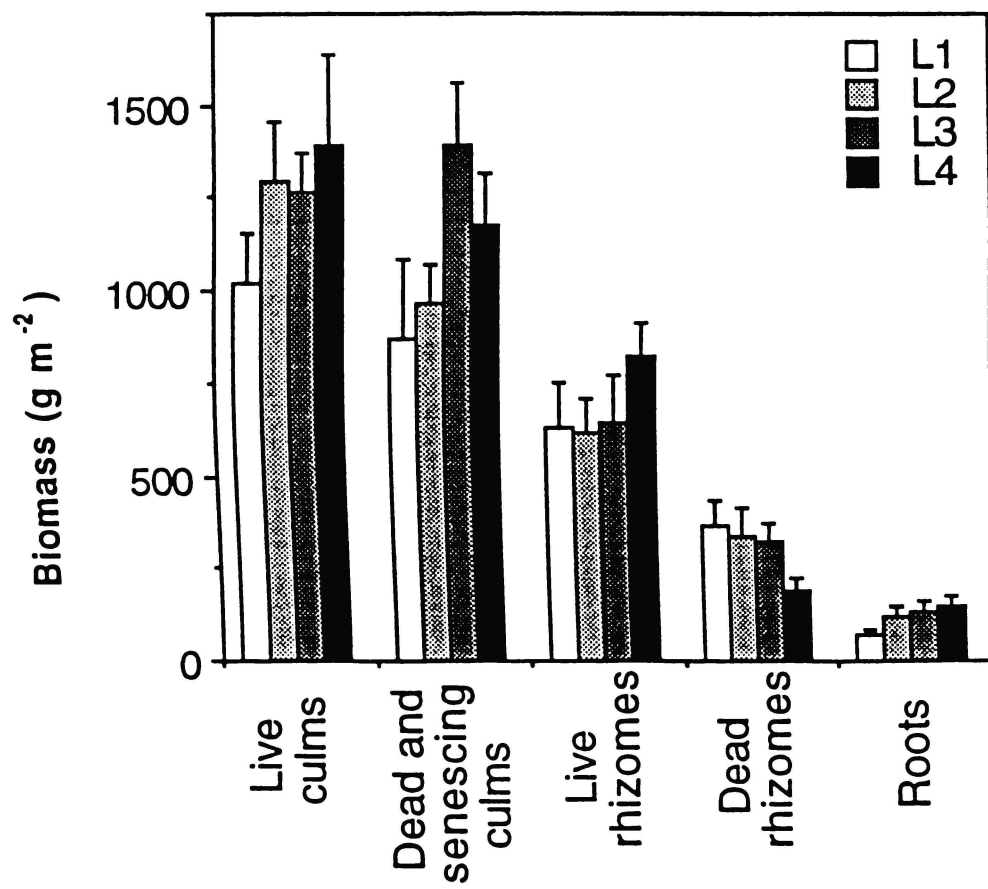


Figure 9

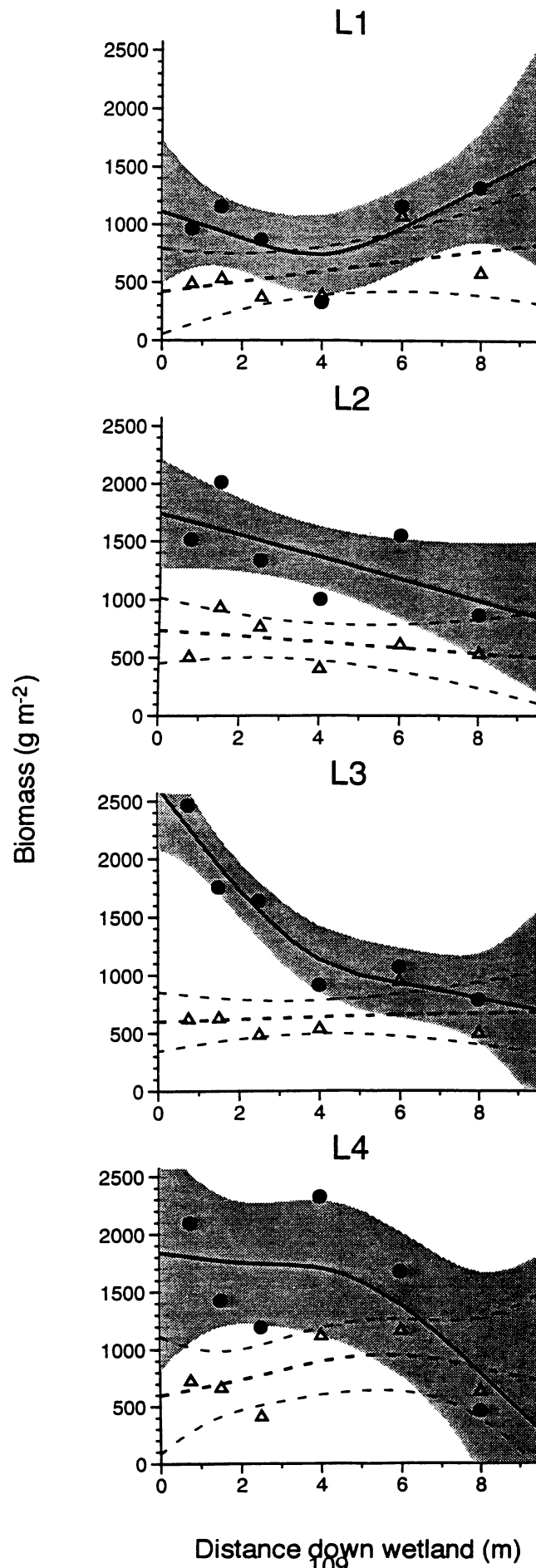


Figure 10

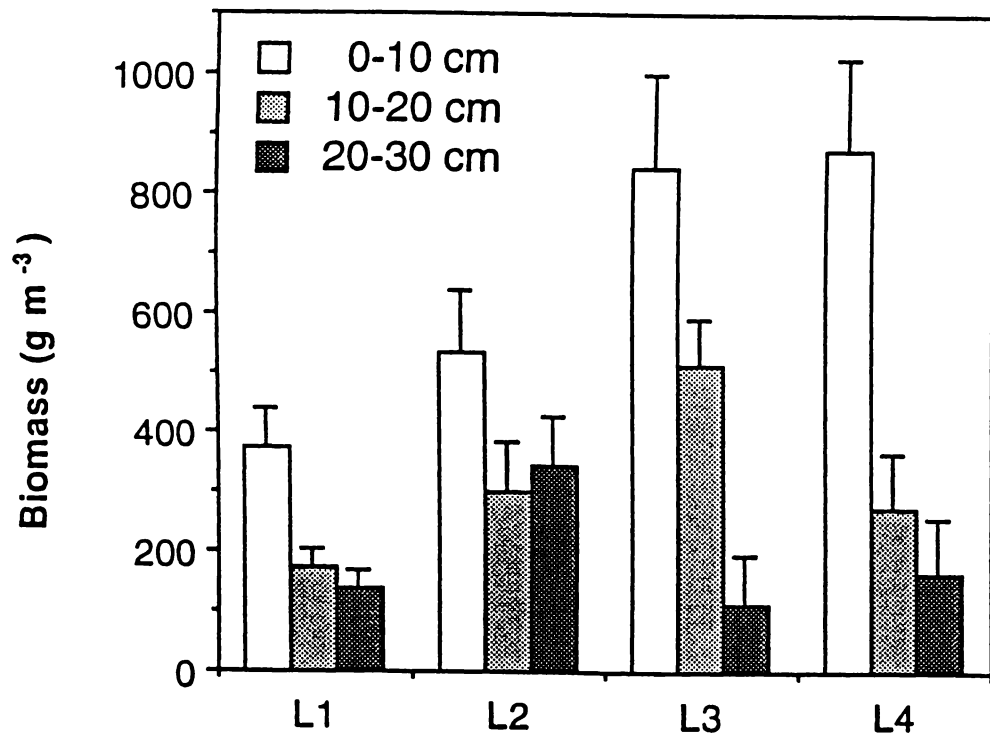


Figure 11

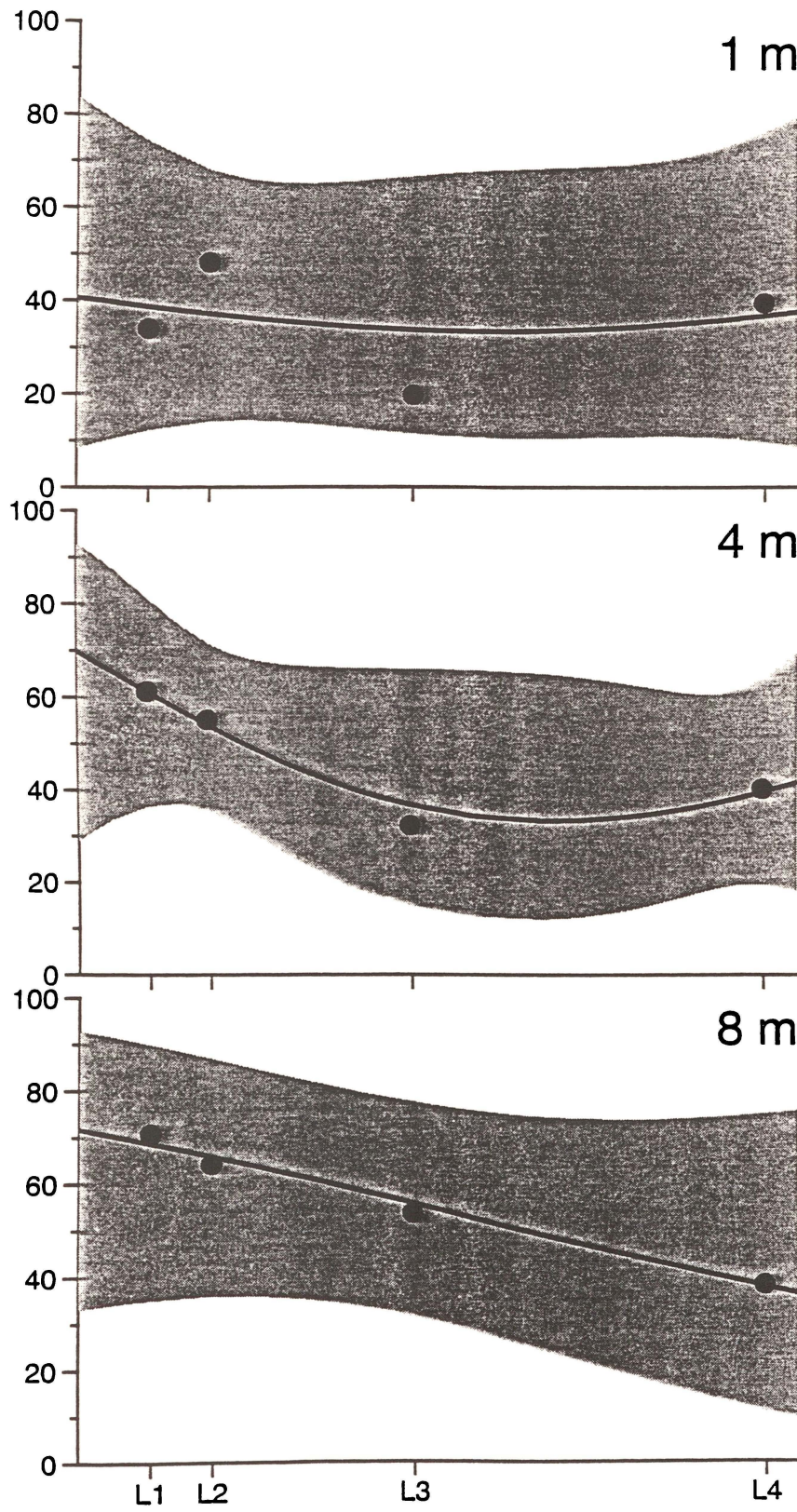


Table 1. Summary of mean wastewater oxygen demand and nutrient loadings to the experimental wetlands. Data for the two plant growth seasons covers the spring early summer period October 1, to January 31. Standard errors are given in parenthesis.

	Loading rate (g m ⁻² d ⁻¹)			
	L1	L2	L3	L4
CBOD *				
mean for trial	2.4 (2.7)	2.5 (1.7)	4.2 (3.3)	6.8 (4.6)
growth season 1	0.98 (0.03)	1.58 (0.05)	2.40 (0.07)	4.36 (0.12)
growth season 2	7.15 (1.00)	5.3 (0.74)	9.8 (1.37)	14.3 (2.01)
Total BOD**				
mean for trial	5.6 (6.2)	5.8 (3.9)	9.8 (7.6)	15.8 (10.6)
growth season 1	3.2 (0.31)	5.1 (0.49)	7.7 (0.75)	14.0 (1.4)
growth season 2	17.2 (1.3)	12.7 (0.97)	23.5 (1.8)	34.3 (2.6)
Total Nitrogen				
mean for trial	1.18 (1.19)	1.26 (0.75)	2.10 (1.44)	3.44 (2.01)
growth season 1	0.84(0.20)	1.35 (0.32)	2.04 (0.49)	3.71 (0.89)
growth season 2	3.4 (0.49)	2.52 (0.36)	4.65 (0.67)	6.80 (0.98)
Total Phosphorus				
mean for trial	0.27 (0.18)	0.32 (0.08)	0.52 (0.17)	0.87 (0.21)
growth season 1	0.20 (0.03)	0.32 (0.04)	0.49 (0.06)	0.90 (0.12)
growth season 2	0.61 (0.06)	0.45 (0.05)	0.83 (0.09)	1.21 (0.13)

Analytical methods given in Tanner et al. (submitted a,b)

* CBOD = Carbonaceous biochemical oxygen demand

** Total BOD= CBOD plus calculated nitrogenous oxygen demand (i.e. due to nitrification of ammonium)

Table 2. Above-ground and total NAPP estimates (first growth season) and mean mid-summer biomass (January 1991 and 1992) for *S. validus* in the experimental wetlands. All values are g (dry weight) m⁻², with biomass standard errors shown in parenthesis.

Wastewater loading	Net annual primary production (1990/91)				Mid-summer biomass (live and dead)			
	Above-ground		Total		Above-ground		Total	
	max-min	including mortality	max-min	including mortality	1991	1992	1991	1992
L1	1902	2209	2257	2807	1757	1878	2580	3051
L2	2058	2453	2324	3225	1925	2262	2948	3360
L3	2582	3032	2496	3468	2582	2659	3626	3775
L4	2223	2652	2261	2936	2223	2558	3099	3715
					(355)	(288)	(384)	(416)

Growth of *Schoenoplectus validus* in wastewater treatment wetlands.

2. Nutrient accumulation and dynamics

CHRIS C. TANNER

NIWA–Ecosystems, National Institute of Water and Atmospheric Research, P.O. Box 11-115, Hamilton, and Department of Biological Sciences, University of Waikato, Private Bag 3105, Hamilton, New Zealand.

Summary

1. Tissue concentrations, and mass fluxes and accumulation of nutrients were investigated over two growth seasons in *Schoenoplectus validus* (soft-stem bulrush) growing in gravel-bed constructed wetlands supplied with four different hydraulic loading rates of dairy farm wastewaters. The selected loading rates spanned a 3 to 4-fold range of biochemical oxygen demand, N and P supply.
2. High culm concentrations of N, P, Mg and Zn in spring declined markedly over the first growth season, while S and Ca showed general increases, and K, Fe and Cu remained relatively stable during the season. Corresponding increases in both plant biomass and overall tissue nutrient levels with increasing wastewater loading, were interpreted as multiple element limitation. Gradients of decreasing tissue concentrations were noted for most macronutrients down the wetland channels, with partial substitution of K by Na observed at low wastewater loading rates.
3. Seasonal patterns of N and P accumulation and loss, and their allocation in plant tissues largely reflected concomitant biomass changes, but with increased seasonal amplitude at high wastewater loadings. During the first year, plant accumulation of N rose by 20 to 35 g m⁻² and P by 4 to 9 g m⁻² with seasonal growth of culms. Net plant N and P uptake rates rose to maximum values of 0.3 g N m⁻² d⁻¹ and 0.1 g P m⁻² d⁻¹ in summer, declining markedly during late summer and autumn. Internal plant nutrient recycling estimated by relative changes in above and below-ground accumulation during autumn, were similar for N, P, and Mg, to mass losses from senescing culms, but showed considerable variation for other nutrients.
4. Mass balance assessments of N and P accumulation in plants after two years of wetland operation (mid-summer), showed that only 6 to 10% of the N removal and 6 to 13% of the P removal recorded from wastewaters passing through the wetlands could be ascribed to plant uptake and accumulation.

Introduction

Quantification of major storage compartments in constructed wetlands (i.e. vegetation, detritus, fauna, microorganisms, sediments, and interstitial water) and the exchange rates between them, is fundamental to understanding their capacity for sustainable nutrient and organic matter removal (Sloey et al., 1978; Howard-Williams, 1985). Most of the studies of nutrient removal in constructed wetlands published to date have been performed on relatively young systems, in which storage compartments are still filling, but few have attempted to measure the relative importance of such storage processes. Studies such as those of Gersberg et al. (1986) and Herskowitz (1986) have compared overall removal rates of N and P with estimates of plant uptake, finding the latter to be relatively small at normal loading rates (generally less than 10-15% of wetland removal). Gries and Garbe (1989) have studied seasonal changes in tissue nutrient and heavy metal levels for *Phragmites australis* growing in a root-zone wetland treatment system in northern Germany, and Surface et al. (1993) have reported above-ground nutrient and heavy metal concentrations, and biomass for this species growing in gravel-bed wetlands treating landfill leachates in New York State. Nutrient concentrations and biomass have also been reported for *Schoenoplectus californicus*, after 2 years growth in gravel-bed wetlands treating piggery wastewaters near Auckland, New Zealand (Sukias and Tanner, 1993). Recent mass balance studies of the fate of nutrients in 10 and 25 L wetland microcosms supplied with daily additions of wastewater (Breen, 1990; Rogers et al., 1990, 1991), found plant uptake could be a major pathway for N and P removal. However, the relevance of these results to the functioning of full-scale constructed wetlands has been questioned (Crites and Tchobanoglous, 1991; Tanner, 1992).

The present study focuses on the nutrient dynamics of *Schoenoplectus validus* (Vahl) A. Love and D. Love (soft-stem bulrush) growing in subsurface-flow, gravel-bed constructed wetlands treating ammonia-rich dairy farm wastewaters. It builds on the results of concurrent studies of seasonal plant growth (Tanner, submitted) and wastewater treatment (Tanner et al., submitted a,b) carried out in the same pilot-scale systems. The objectives of the study were:

1. to determine the extent and seasonal pattern of plant nutrient accumulation and loss over a range of wastewater loading rates.
2. to investigate plant nutritional responses to wastewater loading and fertility gradients.

Methods

WETLAND SYSTEMS

Full descriptions of the pilot-scale wetland systems studied, composition of the applied wastewaters and plant sampling techniques are given in Tanner (submitted) and references cited therein. Briefly, tissue nutrient levels were studied in *S. validus* growing in four gravel-bed wetland channels (surface dimensions; 9.8 by 2.3 m) each supplied with different hydraulic loading rates (16, 26, 40, and 72, mm d⁻¹; designated L1 to L4) of oxidation pond pre-treated dairy farm wastewaters. Influent wastewater strength fluctuated seasonally, with highest levels of oxygen demand and nutrients in spring and summer, when plants were actively growing. Increased wastewater strength in the second season resulted in a 3 to 4-fold increase in mean mass loadings of carbonaceous biochemical oxygen demand (CBOD) and total BOD (CBOD + calculated nitrogenous BOD), and a ~2-fold increase in N and P levels in L2-L4. Increased hydraulic loadings to L1 at the beginning of the second season (as part of a concurrent experiment), resulted in 5 to 7-fold increase in BOD and a 3 to 4-fold increase in nutrient levels. Mean mass loadings and predicted gradients of major wastewater constituents along the wetland channels, assuming plug flow and first-order removal, are shown for each growth season in Table 1 and Figure 1 of Tanner (submitted).

PLANT SAMPLING

After a 11 month period of establishment, growth was monitored over two spring-summer growth periods and the intervening autumn and winter (October 1990 to January 1992). Senescent above-ground biomass was harvested and removed from the wetlands in mid-August, 1990 (winter) before growth and nutrient sampling began. Analyses of macro and micro-nutrients were performed on above and below-ground plant samples after washing in tap water, subdivision into plant components, oven drying (80 °C) and biomass determination. Plant material collected from small cores (28 cm²) sampled at 1 to 3 month intervals between August 1990 and June 1991 were combined (6 core samples) in each of the 4 sampling zones positioned down the length of the wetlands (see Fig. 2, Tanner submitted). Nutrient data was analysed using ANOVA (Genstat 5, Lawes Agricultural Trust, UK), with nutrient masses calculated for each plant component in each zone of the wetlands, as the product of the mean nutrient concentration and the mean biomass.

Above and below-ground plant components harvested from large cores (346 cm²) at the end of the trial (January 1992), were combined at each position (distance) down the wetlands giving 6 samples of each plant component for each wetland (see Fig. 2, Tanner, submitted). Tissue nutrient concentration data along the length of the

wetlands was smoothed using Bayesian curve fitting techniques (FLEXI; Upsdell and Wheeler, 1992), with tissue nutrient masses calculated by combining the smoothed concentration and smoothed biomass values (latter from Tanner, submitted). Mean nutrient concentration and mass values for each wetland were calculated, by integration of the smoothed concentration-distance and mass-distance curves respectively.

Marked cohorts of culms of known age from within permanent belt transects (5 per channel; see Tanner, submitted) were harvested at the end of the trial (January, 1992), sorted into age classes, and analysed to provide indicative data on nutrient concentration changes with age. Samples from within each transect (14 quadrats) were combined for nutrient analysis giving 5 samples per age class for each wetland, and data analysed by ANOVA.

Live culms were also sampled from wild communities of *S. validus* growing at various locations around the North Island of New Zealand, to provide comparative data on the nutrient status of natural communities. Approximately 30 culms were sampled at random from 5 m square areas of monospecific growths: 1. on the shores of the Waikato River at Hamilton (37° 49' S, 175° 17' E); 2. on the shore and in the outlet stream of Lake Waiparera, a sand dune lake in Northland (34° 57' S, 173° 11' E); and from tall and short-growing stands on the shores of Slippery Creek, a tidal stream near Drury, Auckland (37° 7' S, 174° 57' E). Culms were cut just above the sediment surface, then washed, dried and analysed for nutrients in the same way as those from the wastewater treatment wetlands.

NUTRIENT ANALYSES

Dried plant tissue samples were ground and analysed for macro and micro-nutrients. N and P were determined simultaneously using automated methods (adapted from Gehrke et al., 1972 and Basson, 1976) after Kjeldahl digestion (salicylic acid modification, using selenium catalyst). All other analyses were performed on nitric-perchloric acid digests: Na, K, Ca and Mg by 4 channel flame photometer (Clinton, 1967); Mn, Zn, Cu and Fe by atomic absorption spectroscopy; S by a turbidimetric method (Mottershead, 1971); and B by the Curcumin method (Clinton, 1974).

Results and discussion

TISSUE NUTRIENT CONCENTRATIONS

Mean mid-summer (January) above-ground tissue concentrations of N, P, and S showed a general rise with wastewater loading (Table. 1, Fig. 1), with higher levels of N and P recorded during the second growth season, associated with an approximate doubling of their mass loading rates. During the first growth season, culm K levels were significantly lower and Na levels significantly higher ($P < 0.05$) in the lowest loaded wetland (L1). When wastewater strength was increased in the second season, culm K levels rose in L1 and Na levels declined at all loadings. Such substitution of K by Na when K supply is limited ("sparing"), is common for species that are not active Na excluders (Marschner, 1986).

High culm concentrations of N, P, Mg and Zn recorded in the wetlands during spring (November), showed marked declines as the growth season progressed (Fig. 1). Similar patterns have commonly been observed for a range of temperate helophyte species (e.g. Boyd, 1970; Dykyjova and Kvet, 1978). Analysis of marked cohorts of culms in the second summer (January 1992), showed tissue N and P in culms less than 28 days old were similar to mean culm concentrations recorded in spring. Around 20% lower N and 30% lower P concentrations were present in 50-77 day old culms (Fig. 2). Standing dead culms showed 25 to 55% less N and 25 to 60% less P than mature culms (50-77 days old); the percentage reduction declining as loading rates increased. Similar seasonal patterns for N, P, Zn and Mn, but contrasting patterns for S, K, Mg, Fe and Cu, have been reported for wild stands of helophyte species by Hocking (1989a,b), Boyd (1970; only macronutrients sampled), and Dykyjova and Kvet (1978; N, P, Ca, Mg sampled). Seasonal increase in S (Fig. 1), decrease in Mg, and maintenance of stable K, Fe and Cu levels in *S. validus* in the present study may reflect inherent differences in its uptake or allocation patterns, or the higher availability of these nutrients in the wastewaters supplied, leading to reduced levels of internal retranslocation and/or sequestering of excess nutrients in senescing tissues.

Mid-summer culm tissue levels of N, K, S, Ca, Mg, Zn and B in the second season were in general, similar to levels previously reported for *S. validus* grown for 116 days in microcosms supplied with similar dairy farm wastewaters (Tanner, 1994). Above-ground P, Mn and Cu reached higher tissue concentrations in the present study, approaching maximum values recorded for nutrient solution amended dairy wastewaters. Tanner (1994) recorded higher N levels than those in the present study (25 cf. 20 mg g⁻¹) for *S. validus* growing in piggery wastewaters, while Small and Gaynor (1975) have reported levels as high as 33.2 mg g⁻¹ for this species in late-

summer, when growing in sewage sludge deposits. Comparison of above-ground tissue nutrient concentrations recorded in the wastewater treatment wetlands during the second summer, with those of wild populations of *S. validus* (Table 2) shows higher levels of N (except for Ottawa River samples), P and K, but lower levels of S in the wetlands treating wastewaters. Other tissue nutrient levels in the wastewater treatment wetlands were all within the ranges recorded for wild populations.

Mean below-ground tissue concentrations of N and P (Fig. 3) showed a general rise with wastewater loading rate. Below-ground N concentrations remained constant during spring, but dropped markedly in the middle of the first summer (January) in the two highest loaded wetlands, rising again by early autumn (March). P levels in below-ground tissues showed a general decline over the growth season in the lowest loaded wetlands, but remained relatively stable in the two highest loaded wetlands. Below-ground P concentrations showed a consistent rise at all wastewater loading rates during late autumn.

Culm concentrations of N, P, K, S and Zn declined, and Na increased down the wetland channels during the first growth season ($P < 0.05$). Despite large increases in the nutrient loadings entering the wetlands in the second growth season, marked gradients of N, P, K, and S concentration were still apparent (particularly at lower loading rates) in live culm and rhizome tissues at the final mid-summer sampling (Fig. 4). Rhizome Mn and Zn showed contrasting gradients, increasing and decreasing in concentration respectively, down the wetlands (e.g. Fig. 5). Above-ground tissue Zn levels also rose significantly ($P < 0.05$) with loading during the first season, and were significantly higher at the highest wastewater loading rate (L4) in the second season. Studies undertaken under controlled redox and pH conditions have shown that at neutral pH (as in the experimental wetlands, Tanner et al., submitted a) plant available Mn increases and plant available Zn decreases with level of anaerobiosis (Sims and Patrick, 1978). Corresponding changes in plant uptake of Mn and Zn have been demonstrated for rice (Jugsujinda and Patrick, 1977). Differences in Mn and Zn uptake by *S. validus* in the experimental wetlands, are likely to reflect gradients of redox potential created down the experimental wetlands by preferential settling of organic solids in the inlet zone and gradual attenuation of wastewater oxygen demand along the length of the channels (see Tanner et al., submitted a; Fig. 1, Tanner submitted).

NUTRITIONAL STATUS OF *S. VALIDUS* IN THE WETLANDS

Previous studies of *S. validus* growth in similar dairy farm wastewaters (Experiment 2; Tanner, 1994), have shown that they are nutritionally well balanced, containing macro and micro-nutrients in similar ratios to those employed in artificial nutrient solutions. The concentrations of N and P in wastewaters applied to the wetlands during the

second growth season were about a third of those commonly used in full strength nutrient solutions (e.g. Smith et al., 1983), but mass application rates were very high by agronomic standards, with for example N loadings during the growth season (Table 1, Tanner submitted), ranging from 18 to 50 times maximum fertiliser recommendations for high yielding irrigated rice (160 kg ha^{-1} Stangel, 1979). N and more rarely P, are the elements which have generally been implicated in growth limitation of helophytes in natural communities (Morris, 1991). N in particular, is prone to depletion in anaerobic wetland soils because it is subject to gaseous losses via microbial transformations (nitrification-denitrification). Apart from plant uptake, sediment and organic matter adsorption and coprecipitation are the main means of P removal in wetlands (Richardson, 1985; Cooke, 1992), although the practical significance of possible gaseous losses as phosphane and diphosphane have yet to be resolved (Devai et al., 1988; Gassmann & Glindemann, 1993).

Removal processes in the wetlands collectively reduced the atomic ratios of N and P in the two lowest loaded wetlands, from 9 at the inflow to around 4.5 at the outflow during the first growth season. Much smaller changes in N:P ratios occurred at higher loading rates, but ratios in all of the wetlands were still reduced from 12.3 (inlet) to around 8.8 (outlet) during the second growth season, despite markedly higher nutrient loadings. Mean atomic N:P ratios recorded in the plants in mid-summer of the first growth season, showed a reverse trend to those of the wastewaters passing through them, declining from 9.9 to 8.3 with increasing loading. During the second season mean plant atomic N:P ratios ranged between 11.4 and 12; similar to the upper range recorded for *S. validus* cultured in nutrient amended dairy wastewaters (Tanner, 1994). Much higher values (up to 25) have been reported in wild populations (Table 2), but ratios below 10 in above-ground tissues of this species have not been recorded before; either in microcosm studies encompassing a wide range of nutrient compositions and strengths, including tap water (Tanner, 1994), or in data compiled for wild stands growing in diverse conditions (Table 2). Comparison of mid-summer atomic ratios of other nutrients in relation to tissue N, shows N uptake slowly increasing relative to P, K, S, Ca, Mg, Na, and Mn with increasing wastewater loading. Increased leaf N content has been found to strongly correlate with maximum photosynthetic capacity in a wide range of wild plants (Field and Mooney, 1986). Increased concentrations of N-rich photosynthetic enzymes (e.g. Rubisco) and associated biochemical "machinery" may be of particular importance to *S. validus* productivity, because of the constraints imposed by its very simple (essentially "leafless") morphology, which allows minimal adjustment of its canopy architecture. Alternatively, increasing culm N contents may increased accumulation of short-term N storage compounds (e.g. amides; Marschner, 1986). Uptake of Zn and Cu increased relative to both N and P as loading increased, probably reflecting their greater availability at higher wastewater oxygen demands

(increasingly anaerobic conditions; Sims and Patrick, 1978), while Fe and B ratios showed no consistent trends.

Most wild plants are able to compensate for considerable variations in environmental resource availability, suggesting they possess well developed internal feedback mechanisms allowing them to adjust (within limits) their relative uptake capacities, levels of internal conservation and growth rates to achieve an "optimal" ratio of essential nutrients for growth and reproduction (Bloom et al., 1985). As in previous microcosm experiments (Experiment 2; Tanner, 1994), *S. validus* responded to increased wastewater loadings by gradually increasing both its net productivity and its overall tissue nutrient levels. Levels of K may have been limiting to some extent in the lowest loaded wetlands during the first growth season. However, partial substitution by Na (see above) is likely to have compensated for some, if not all of the plants requirement at such low levels of K "insufficiency" (Marschner, 1986). The responses to nutrient supply seen here for *S. validus* are perhaps best considered as multiple element limitation. The nutrient conditions in the experimental wetlands appear to be analogous to the extreme situation of concentration-limited (in contrast to replenishment rate-limited) nutrient availability presented in the conceptual model of Rastetter and Shaver (1992). That is, where nutrient levels are so strongly buffered, or are subject to such high rates of through-flux that they are essentially non-depletable by the plant. It is hypothesised that in such cases "growth and biomass are simultaneously controlled by the concentration of all elements that have not saturated the plants uptake mechanisms" (Rastetter and Shaver, 1992, p. 1172). The "concentration-limited" model simulations of Rastetter and Shaver (1992) suggest that an increase in any nutrient will always result in some sustainable increase in biomass and the uptake of all elements for which uptake is not saturated. Responses will become smaller as the rate of uptake of any manipulated nutrient approaches saturation point.

NUTRIENT ACCUMULATION AND FLUX

Seasonal patterns of N and P accumulation and loss, and allocation in plant tissues (Fig. 6) largely reflect concomitant biomass changes (see Tanner submitted). However, increased nutrient concentrations at higher wastewater loadings resulted in a rise in the seasonal amplitude of N and P mass changes with wastewater loading. Plant accumulation of N rose by 20 to 35 g m⁻² and P by 4 to 9 g m⁻² as culms grew and flowered in the first growth season. Net fluxes of above and below-ground N and P (Fig. 7) suggest little mobilisation of below-ground reserves occurred during spring regrowth. However, decreases in below-ground N and P were recorded in the highest loaded wetland during early summer, associated with rapid accumulation in developing flowers and seeds. Net N and P uptake rates rose with wastewater loading, with

maximum values of nearly $0.3 \text{ g N m}^{-2} \text{ d}^{-1}$ and $0.1 \text{ g P m}^{-2} \text{ d}^{-1}$ recorded during the late spring early summer period of most rapid above-ground growth (November-January). Net plant uptake over this period was equivalent to (in order of increasing wastewater loading) 26, 18, 20 and 11% of the N removal rate and 29, 21, 27, and 11% of the P removal rate recorded in the wetlands at this time (wetland performance data from Tanner et al., submitted b). Fluxes declined markedly during late summer becoming strongly negative as above-ground senescence proceeded. Net plant N losses were equivalent to between 30 and 100% and net plant P losses equivalent to between 15 and 45% (both inversely related to loading rate) of the mass outflow rates recorded in the wetlands at this time. Much of the N and P in fallen litter would however be released slowly during decomposition, rather than as rapid pulses. As such, seasonal plant uptake of nutrients, temporary storage in plant tissues, and later return in slowly-released forms (plant litter) acts to smooth temporal fluctuations in nutrient fluxes through the wetland.

Below-ground N and P reserves showed net increases over the first growth season, with positive fluxes peaking at between 0.05 and $0.2 \text{ g N m}^{-2} \text{ d}^{-1}$ and 0.01 and $0.02 \text{ g P m}^{-2} \text{ d}^{-1}$ during early autumn (Fig 8). Net below-ground accumulation during above-ground senescence was equivalent to 25 to 40% of above-ground N losses, and 13 to 39% of P, 4 to 48% of S, 1 to 26% of Ca, 0 to 18 % of Mg and 6 to 27% of Mn losses (Table 3), with the percentage of above-ground losses required to account for below-ground accumulation generally showing an inverse relationship to wastewater loading. Comparison of these values with changes in mean culm nutrient concentrations during senescence (corrected for biomass changes), shows losses of N, P and Mg in standing dead culms (i.e. losses attributable to retranslocation and/or leaching) were generally similar to below-ground gains. However, tissue concentrations of S rose in standing dead culms at the lowest wastewater loading, but declined by up to 24% at higher loadings. Ca and Mn levels showed an opposite pattern, with standing dead tissue concentrations rising at moderate to high wastewater loadings. Zn uptake into below-ground tissues during senescence was between 2 and 4 times greater than above-ground losses, suggesting considerable root uptake occurred during autumn. Net below-ground uptake of K and Na was negligible despite large losses from standing dead culms. These highly mobile univalent cations are not generally bound in structural components of plants, and are likely to have been leached from senescing culms (Marschner, 1986; Gaudet, 1977).

The most reliable estimates of nutrient translocation and retranslocation in helophytes are likely to be those which are based on comparisons of relative changes in above and below-ground nutrient mass; rather than those based on relative tissue concentration changes; where concurrent biomass transfers may underestimate or

mask nutrient fluxes (Smith et al., 1988). Seasonal phases of net nutrient translocation in perennial helophytes generally occur over periods of months and may not be synchronous in tissues at different developmental stages. Estimates based on relative mass changes have generally assumed concurrent root uptake to be negligible. Such assumptions may be reasonable for plants growing in nutrient poor environments where the potential for root uptake is limited, but are dubious where, as in the present study, nutrients are readily available in the root-zone. High rates of spring N and P uptake were recorded for *S. validus* in the wastewater treatment wetlands, without apparent decreases in below-ground reserves. Our sampling regime was restrained to avoid significant impacts on the functioning of the wetlands, and may have been too infrequent to pick up short-term decreases in below-ground nutrient reserves. However, the data suggests that any mobilisation of below-ground N and P reserves which did occur was rapidly replaced by root uptake.

Nutrient budget studies such as those of Prentki et al. (1978), Davis and van der Valk (1983), and Smith et al. (1988) for *Typha* spp.; Van der Linden (1980) and Hocking (1989a,b) for *Phragmites australis*; and Hopkinson & Schubauer (1984) for *Spartina alterniflora* suggest that seasonal translocation of a range of macronutrients to and from rhizomes is an important means of nutrient conservation in wild helophyte populations, accounting in the case of N and P, for up to half of the annual flux through these species. In contrast, Klopatek (1978) estimated translocation of N and P into and out of rhizomes accounted for only around 10% of seasonal uptake in a riverine stand of *Scirpus fluviatilis*. Our observations show that significant autumn retranslocation of N, P and Mg from senescing culms to the rhizomes is feasible (see above), but must be tempered by the knowledge that considerable direct root uptake of Zn and elements such as Ca which are normally considered immobile in plants (Hill, 1980) was recorded over the same time frame, and that apparent synchrony of above and below-ground plant fluxes of other nutrients were not always reflected in nutrient concentration changes in senescing culms.

As pointed out by Prentki et al. (1978), Boyd's (1969) study of nutrient uptake by *Justicia americana* (a plant with negligible perennial below-ground storage potential) shows that root uptake rates of 0.54 g N and 0.034 g P m⁻² d⁻¹ are feasible. These are higher than mean autumn uptake rates recorded for *S. validus* rhizomes in the experimental wetlands; and for N, even above plant uptake rates recorded in spring and summer (Fig 7). Isotopic labelling studies would be required to unequivocally determine the extent of internal and external nutrient fluxes associated with seasonal plant growth and the response of different species to varying trophic conditions. Studies of the efficiency of nutrient recovery in relation to nutrient availability for three marsh species (Shaver and Melillo, 1984), suggest that plants growing in high nutrient

conditions such as those in the wastewater treatment wetlands, are likely to show decreased recovery efficiencies (retranslocation) of nutrients during above-ground senescence. However, evidence compiled for a range of plants by Hill (1980) suggests that the physiological, developmental and environmental factors affecting nutrient mobilisation are more complex than suggested by Shaver and Melillo's study, with remobilisation (particularly of variably mobile elements) sometimes greater under nutrient-rich than nutrient-poor conditions.

Plant accumulation of most nutrients rose during the second season (Table 1). L1, which had received increased wastewater loadings ($> L2$) since the beginning of the season, showed the largest increases in above-ground tissue nutrient concentrations, but still showed the lowest accumulation of most nutrients, reflecting its lower below-ground reserves at the start of the season and lower carry-over of standing litter.

Accumulation of N and P was divided roughly equally between above and below-ground tissues; while K, S, Ca, Mg, Mn, and Na was mainly above-ground; and Fe, Zn, Cu, and B below-ground. Nutrient accumulation in dead above and below-ground tissues rose gradually as the wetlands matured, with N, P and S in attached dead tissues comprising between 20 and 30% of total mid-summer plant storage in the second season (Fig. 8). Total plant accumulation of N and P in the wastewater wetlands at this time was around twice that recorded for *Phragmites australis* at maximum biomass, in its third year of growth in a soil-based root-zone wetland treating sewage (32.5 g N and 4.6 g P m⁻²; Gries and Garbe, 1989). Above-ground N accumulation was similar and P, 5 to 11-fold higher than maximum values recorded for similar aged *Phragmites australis* stands (Surface et al., 1993) growing in gravel-bed wetlands treating landfill leachates (very low tissue P contents of 0.5 mg g⁻¹ suggest severe P deficiency in these systems). Mean above-ground N accumulations 1.6-fold higher and P accumulations 3-fold higher, have been recorded for higher biomass (4300 g m⁻²) stands of *S. californicus* growing in similar aged gravel-bed wetlands treating piggery wastewaters (Sukias and Tanner, 1993). Above-ground N, P and K accumulations in the present study were in the high range reported for wild helophyte stands in temperate regions (e.g. data compiled by Prentki et al., 1978). Total biomass accumulation of N, P and K by *S. validus* in the wastewater treatment wetlands was around 2-fold higher, and Mn and Cu 4-fold higher, Zn and Fe 10-fold higher, and Mg, Ca and Na similar to values recorded at maximum seasonal biomass, for *Typha latifolia* growing in eutrophic Lake Mendota marshes, Wisconsin (Smith et al., 1988). However, considerably greater accumulation of nutrients has been reported for extremely high biomass stands of *Phragmites australis* (total biomass >29 kg m⁻²) growing in inland Australia (Hocking, 1989a), and for *Cyperus papyrus* in tropical Africa (Gaudet, 1977).

The present study has quantified seasonal fluxes and accumulation of nutrients, in relation to wastewater loading, for constructed wetlands in their first 2 years of development, when storage compartments are actively filling. Plant accumulation of most nutrients continued to increase during the second growth season, but at reduced rates, and with increasing convergence between the wetlands operating at different wastewater loading rates. Mass balance assessments of nutrient removal in the wetlands (summarised in Table 4) show that over the duration of the trial, between 40 and 70% of the N and 30 and 60% of the P (both inversely related to wastewater loading) were removed from wastewaters during their passage through the wetlands. Only 6 to 10% of the N and 6 to 13% of the P removal could be ascribed to plant accumulation at the end of the trial, with accumulation in sediments and fallen litter, and denitrification presumed to account for the remainder of N and P losses recorded in the wetlands. Plant nutrient accumulations were near their seasonal maxima at this time, with about half of the N and P present in above-ground tissues. Nutrients not internally recycled would be released back into the wetland by leaching or deposited on the surface as litter during the subsequent autumn and winter. Observations of the effects of harvesting of above-ground tissues of *S. validus* growing in the same wastewater treatment systems (author's unpublished data), suggest that harvesting more frequently than once per year detrimentally affects rhizome carbohydrate reserves and the subsequent productivity of stands. Similar conclusions have been reached in harvesting studies of *Typha* crops (Dubbe et al., 1988) and for natural stands of *Glyceria maxima* (Sundblad, 1990), suggesting that the potential to markedly increase sustainable plant uptake by this means is limited.

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Figure captions.

1. Seasonal changes in mean concentrations of macronutrients and Zn in live culms growing at four wastewater loading rates (L1-4), during the first growth season. Pooled standard errors are given in Table 1.
2. Comparison of mean N and P concentrations for different age classes of culms grown at four wastewater loading rates (L1-4), at the end of the trial (January 1992). The pooled standard errors were 0.5 mg g⁻¹ for both N and P.
3. Seasonal changes in mean rhizome N and P concentrations for *S. validus* growing at four wastewater loading rates (L1-4), during the first growth season. Pooled standard errors were: N, 1.22, and P, 0.33 mg g⁻¹.
4. Gradients of live culm (●, solid lines) and rhizome (Δ, dashed lines) N, P, K, and S concentration down each wetland (L1-4), at the end of the trial (January 1992). The points shown are mean values. Smoothed mean curves are shown for each dataset, with 85% confidence intervals delineated by shading or fine dashed lines respectively. Where the confidence intervals of lines do not overlap, they are significantly different at the 95% level.
5. Gradients of rhizome Mn (●) and Zn (○) concentration down the L2 wetland channel at the end of the trial (January 1992).
6. Comparison of N and P accumulation in above and below-ground plant biomass for each loading rate (L1-4), during the first growth season. Values for each component of biomass are plotted cumulatively, so that the top line shows the total mass. Pooled standard errors for N and P respectively were; rhizomes and roots, 2.06, 0.59; live culms, 2.95, 0.73; flowers and seeds, 0.69, 0.16; dead culms 1.10, 0.31; total mass, 3.83, 1.00.
7. Seasonal fluxes of above and below-ground, and total plant N and P accumulation shown for each loading rate (L1-4), during the first growth season.
8. Comparison of mean nutrient accumulation in different components of plant biomass for each wetland (L1-4) at the end of the trial (January 1992; mid-summer)

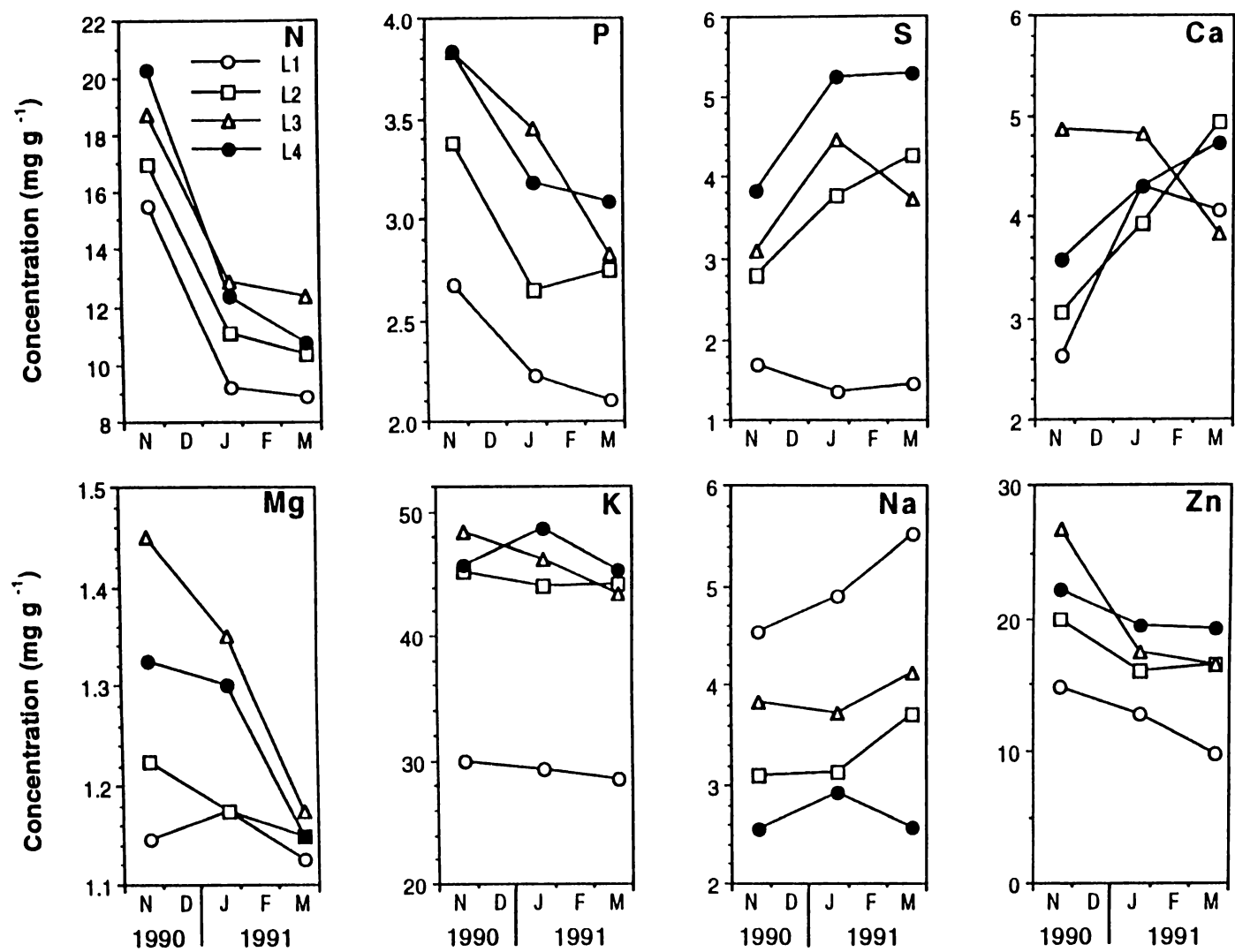


Figure 1

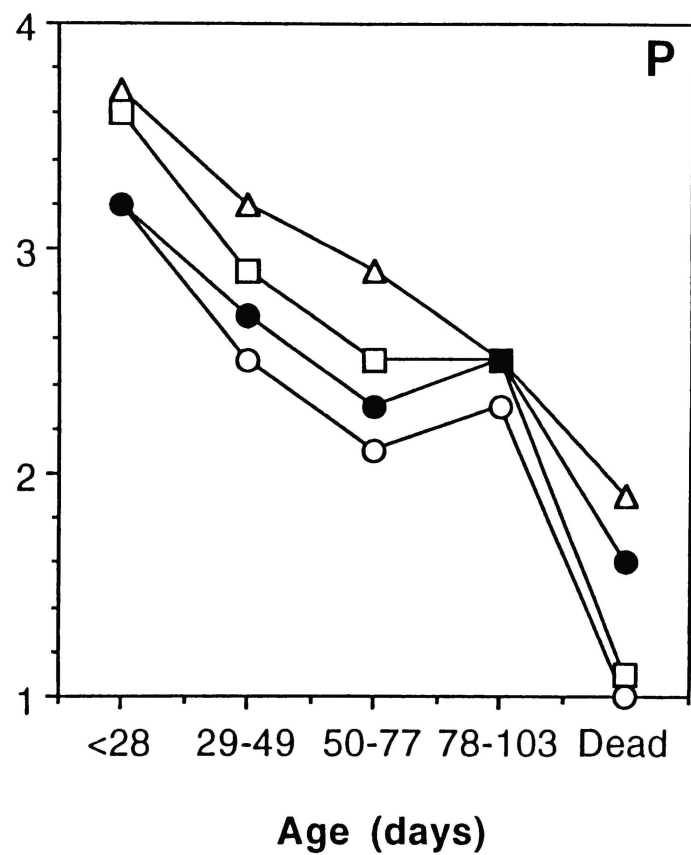
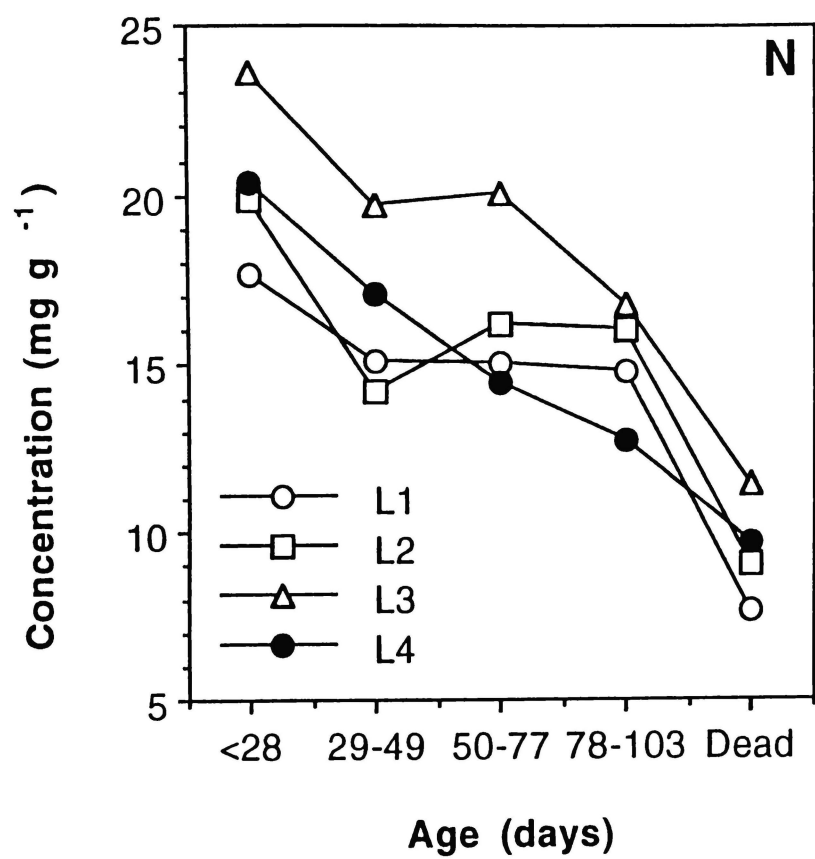


Figure 2

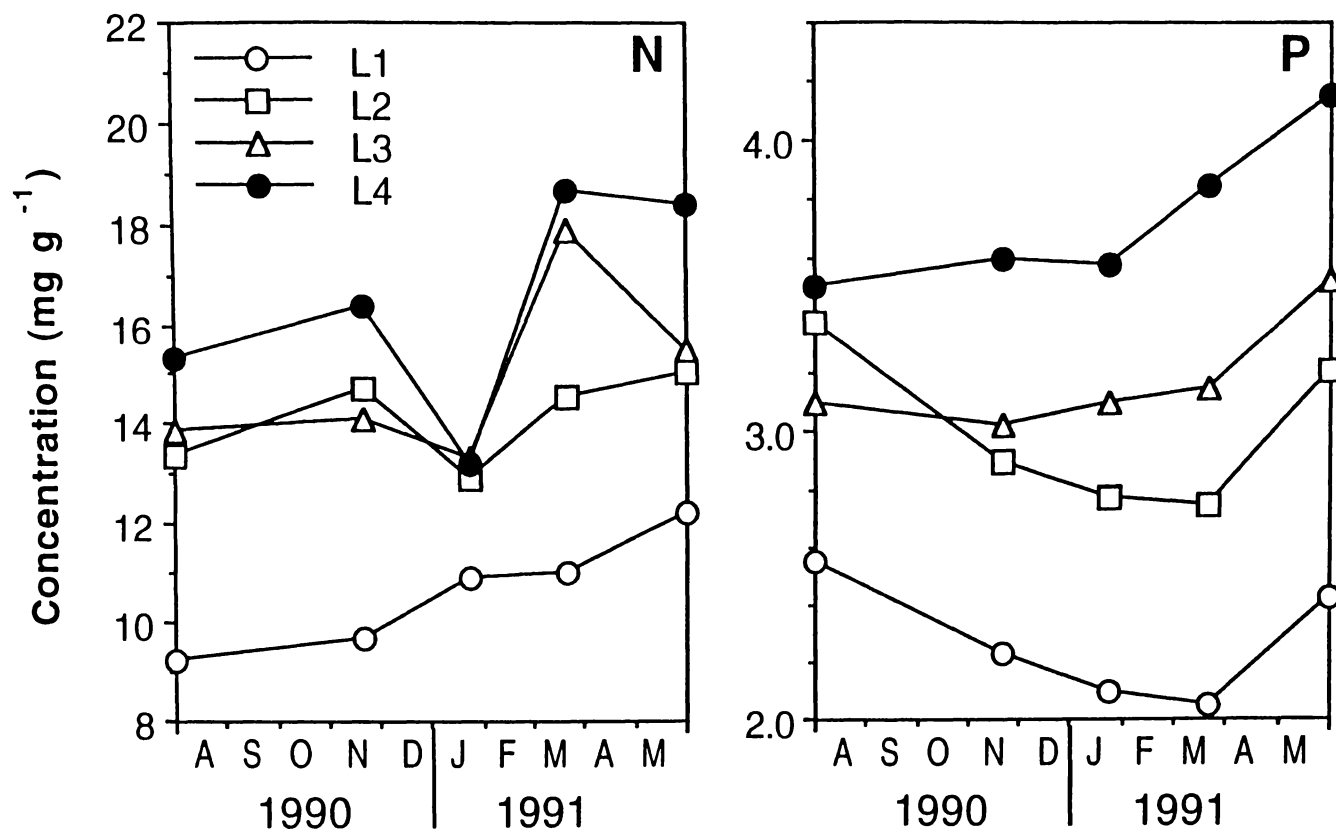


Figure 3

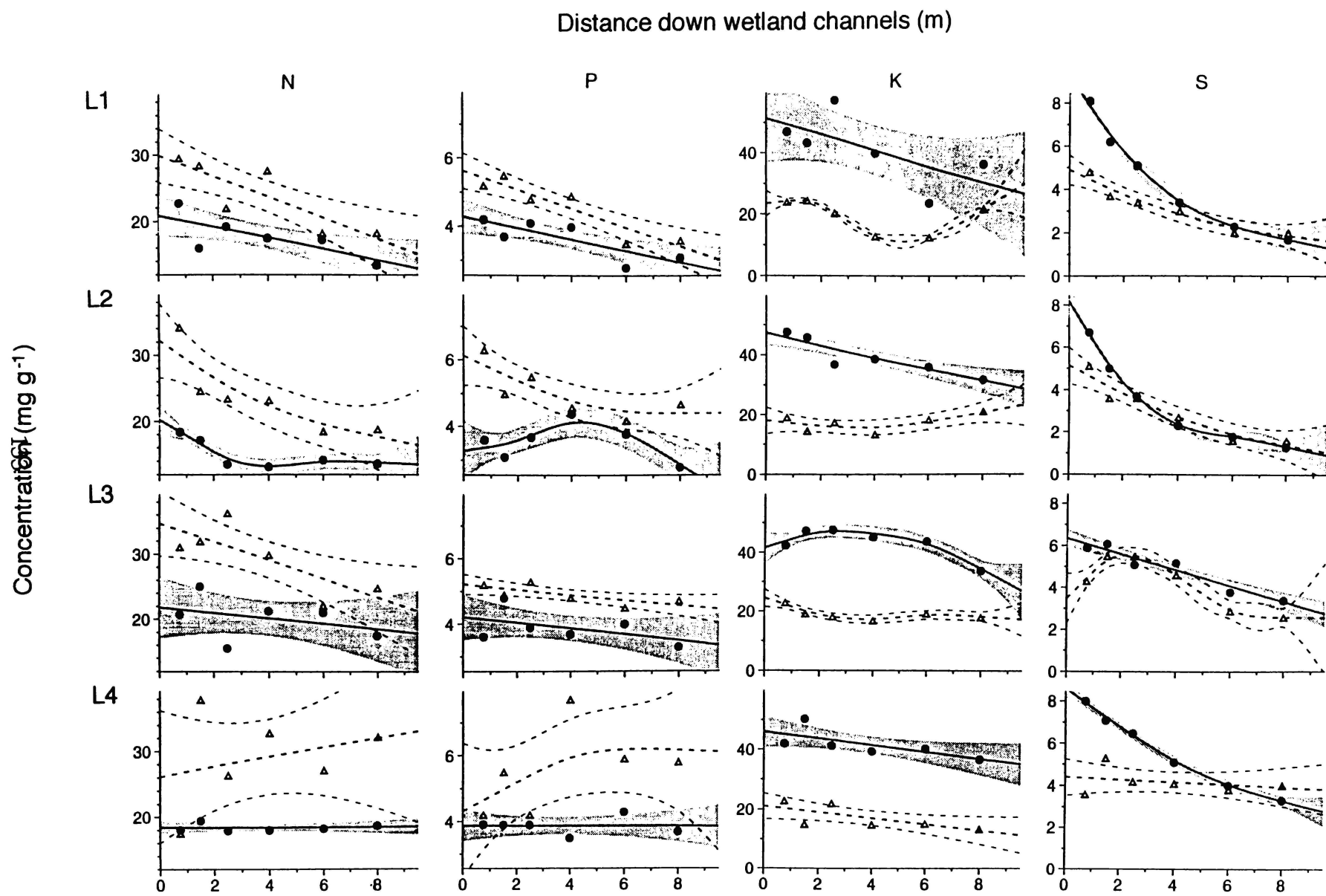


Figure 4

Figure 5

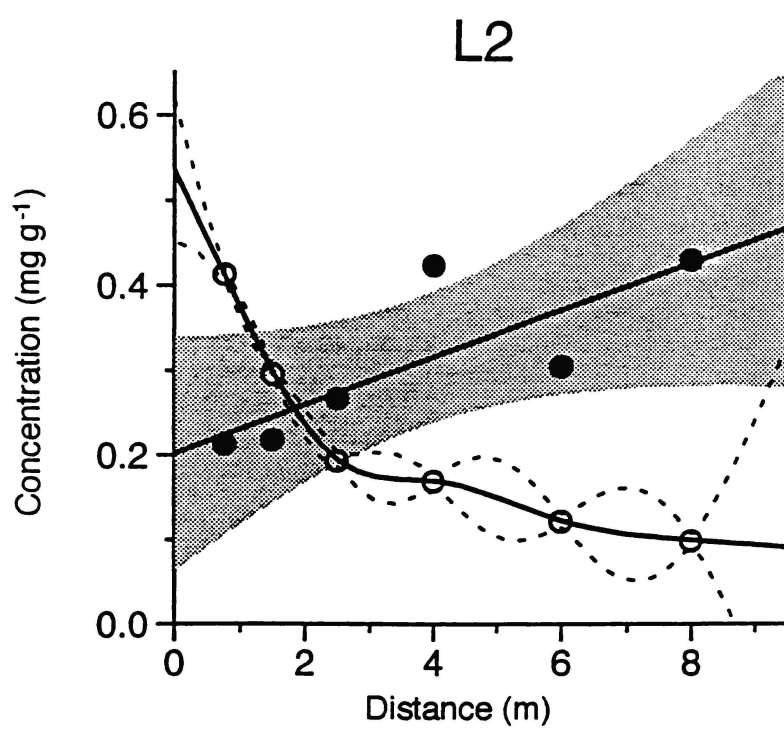
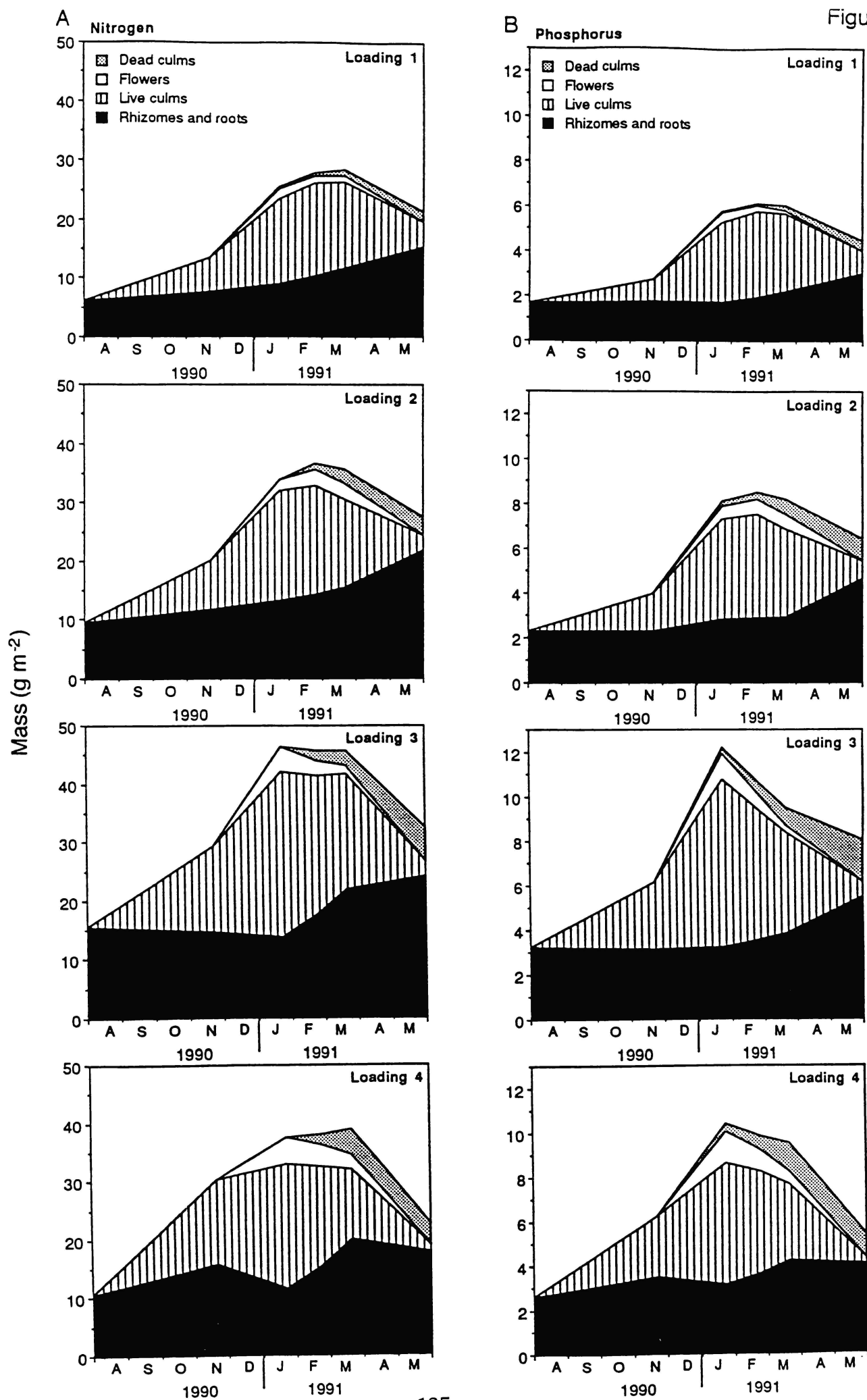


Figure 6



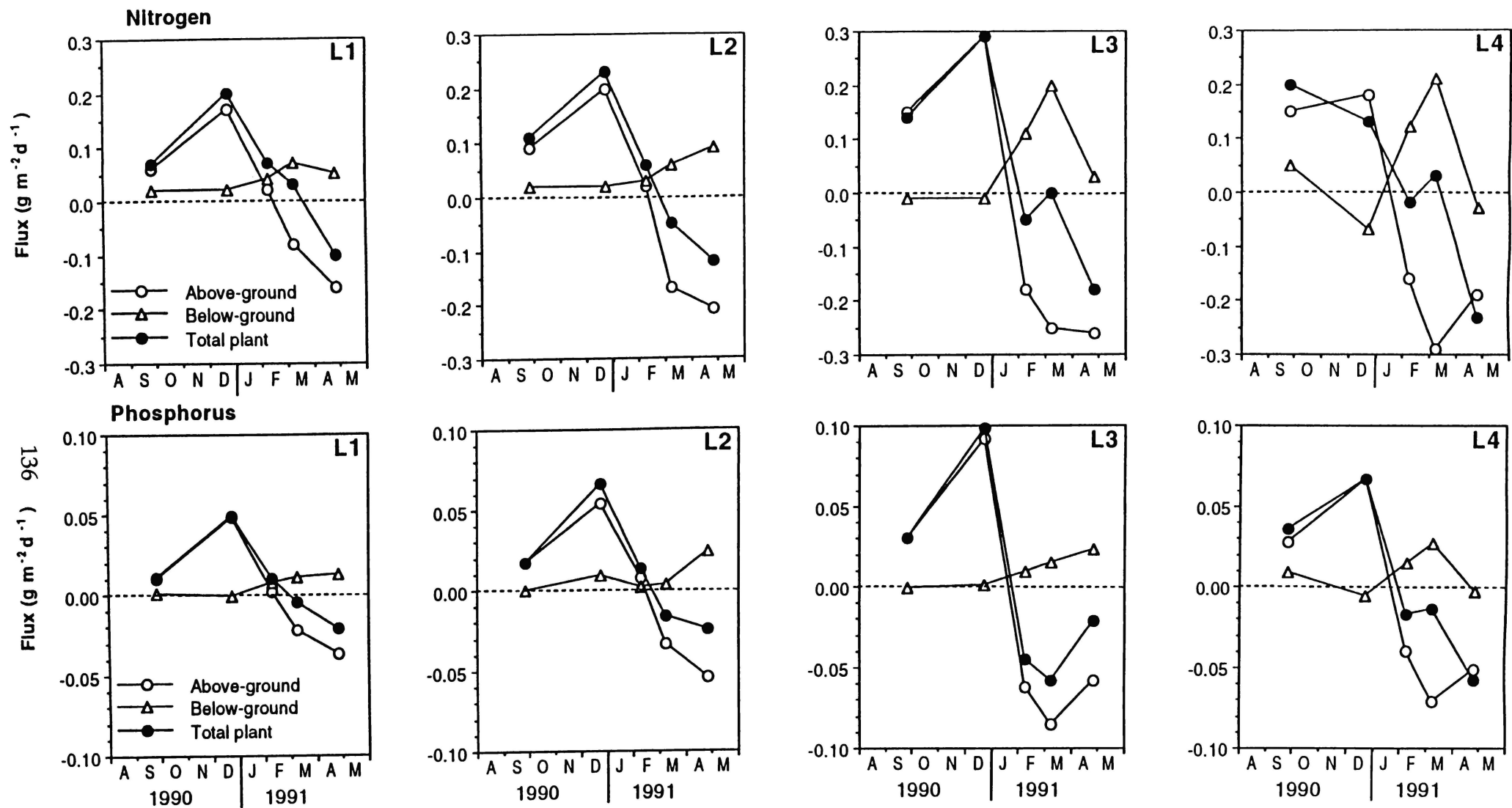


Figure 7

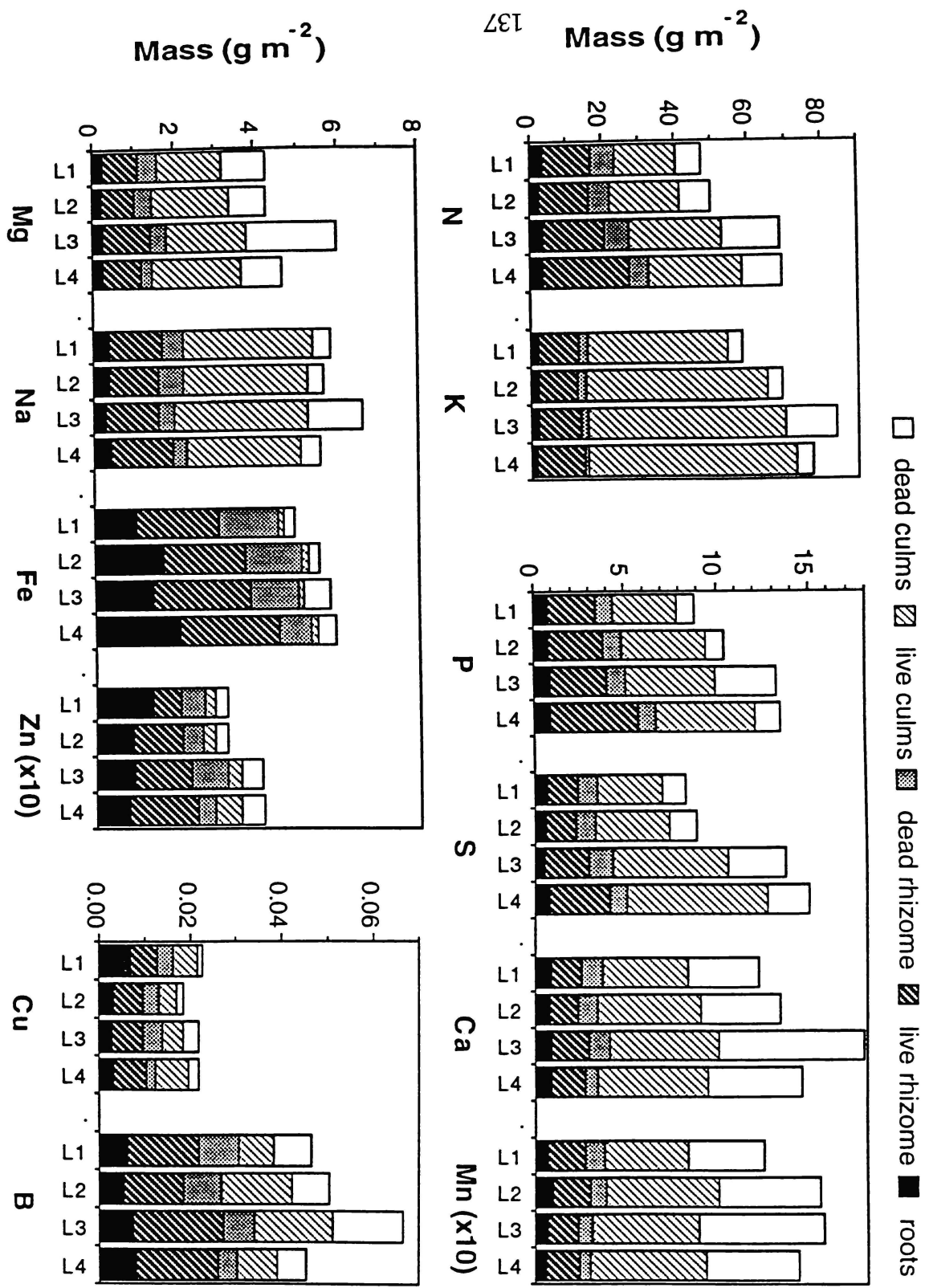


Figure 8

Table 1. Comparison of mid-summer (January) above-ground tissue nutrient concentrations and total plant accumulation (including standing dead culms and dead rhizomes) at four wastewater loading rates in the experimental wetlands during the first two growth seasons. All values are means with pooled standard errors given in parenthesis.

Nutrient	Wastewater loading	Above-ground concentration (mg g ⁻¹)		Total plant accumulation (g m ⁻²)		Nutrient	Above-ground concentration (mg g ⁻¹)		Total plant accumulation (g m ⁻²)	
		1991	1992	1991	1992		1991	1992	1991	1992
N	L1	9.2	16.9	26	48	Na	4.9	3.0	10.0	5.8
	L2	11.1	14.6	35	50		3.1	2.4	8.6	5.6
	L3	12.9	19.8	47	69		3.7	2.6	11.0	6.6
	L4	12.4	18.5	39	69		2.9	2.1	7.9	5.6
		(1.3)	(0.98)	(3.8)	(5.4)		(0.29)	(0.07)	(1.24)	(0.63)
P	L1	2.2	3.5	5.8	8.8	Mn	0.43	0.44	0.72	1.23
	L2	2.7	3.5	8.1	10.3		0.48	0.50	0.91	1.55
	L3	3.5	3.8	12.2	13.2		0.43	0.48	1.10	1.58
	L4	3.2	3.9	10.4	13.4		0.46	0.43	0.98	1.38
		(0.2)	(0.2)	(1.00)	(1.55)		(0.074)	(0.031)	(0.20)	(0.18)
K	L1	29	39	56	58	Zn	0.013	0.025	0.072	0.33
	L2	44	38	93	69		0.016	0.022	0.120	0.32
	L3	46	42	119	78		0.018	0.025	0.161	0.41
	L4	49	41	109	84		0.020	0.042	0.144	0.42
		(1.9)	(2.4)	(13.1)	(8.9)		(0.0024)	(0.0024)	(0.057)	(0.112)
S	L1	1.4	3.7	4.0	8.1	Cu	0.0020	0.0045	-	0.022
	L2	3.8	2.9	9.4	8.9		0.0023	0.0035	-	0.018
	L3	4.5	4.6	13.5	13.6		0.0028	0.0030	-	0.022
	L4	5.3	5.1	13.2	14.9		0.0020	0.0048	-	0.022
		(0.4)	(1.17)	(1.41)	(1.34)		(0.0003)	(0.00072)		(0.0128)
Ca	L1	4.3	4.5	10.1	12.1	Fe	0.081	0.112	-	4.8
	L2	3.9	4.4	11.8	13.3		0.068	0.118	-	5.5
	L3	4.8	5.0	16.6	17.8		0.083	0.100	-	5.8
	L4	4.3	4.6	13.2	14.6		0.073	0.118	-	6.0
		(0.37)	(0.19)	(1.69)	(2.23)		(0.014)	(0.023)		(1.01)
Mg	L1	1.18	1.55	3.10	4.2	B	0.006	0.008	-	0.046
	L2	1.18	1.46	3.75	4.3		0.006	0.012	-	0.050
	L3	1.35	1.63	4.85	6.0		0.007	0.013	-	0.066
	L4	1.32	1.54	4.25	4.6		0.007	0.007	-	0.045
		(0.09)	(0.056)	(0.46)	(0.63)		(0.0021)	(0.0010)		(0.0146)

Table 2: Above-ground tissue nutrient levels and atomic ratio of N and P recorded for wild populations of *S. validus* . All values are expressed on a dry weight basis.

Provenance	sampling time	atomic N:P ratio	Macronutrients (mg g ⁻¹)							Micronutrients (µg g ⁻¹)					source ¹
			N	P	S	Mg	Ca	K	Na	Mn	Zn	Cu	Fe	B	
New Zealand															
Waikato River, Hamilton	Dec 90	10.8	13.3	2.7	5.1	1.6	3.9	35.2	9.5	393	19	6	211	5	1
Lake Waiparera, Northland -shore	Feb 89	21	10.5	1.1	8.7	3.9	6.0	14.6	3.6	629	19	1	67	-	1
-outlet		25	12.4	1.1	6.2	2.1	4.0	13.2	2.7	394	16	1	60	-	
Slippery Creek ² , Drury -tall stands	Feb 91	10.6	10.6	2.2	5.6	3.1	3.8	15.5	11.7	1076	18	4	451	39	1
-short stands		11.5	12.4	2.4	5.8	3.3	2.2	16.4	20.2	771	30	4	634	8	
Canada															
Ottawa River and surrounds,Ontario ³	Aug 73	24.5	27.0 (1.3)	2.44 (0.1)	-	1.47 (0.08)	5.94 (0.36)	17.7 (0.6)	1.1 (0.13)	360 (87.8)	16.9 (1.6)	7.4 (0.70)	242 (19.1)	-	2
Lake St. Francis wetlands, Quebec ⁴	Jun-Oct 70	12.2	13.5	2.45	-	0.76	3.84	15.6	3.2	267	532	8	225	-	3
Frazer River Estuary, Brit. Columbia ⁵	Jun-Sept 81	13	14.7	2.5	-	-	-	-	-	-	-	-	-	-	4

¹ Sources of data: 1. From the present study; 2. Small and Gaynor (1975); 3. Auclair (1979); 4. Hall and Yesaki (1987)

² A tidal stream with saline influences

³ Mean of 19 samples with standard errors given in parenthesis

⁴ Mean of 20 samples

⁵ Mean value for mature culms, converted from ash-free dry weight assuming a mean ash content of 7.5% (Auclair, 1979)

Table 3: Comparison of apparent retranslocation of nutrients to below-ground tissues during autumn senescence of above-ground tissues. Net increases in below-ground (BG) accumulation after maximum biomass are compared to net losses from live above-ground (AG) tissues over the same period (bold type) and to differences in the mean nutrient concentration (corrected for biomass differences) of live and dead culms (italised). Different types have been used to facilitate visual comparisons between values at different wastewater loading rates (L1-L4)

	L 1			L 2			L 3			L 4		
Nutrient	Gain in BG mass (g m ⁻²)	% of AG mass loss	% loss in dying culms*	Gain in BG mass (g m ⁻²)	% of AG mass loss	% loss in dying culms*	Gain in BG mass (g m ⁻²)	% of AG mass loss	% loss in dying culms*	Gain in BG mass (g m ⁻²)	% of AG mass loss	% loss in dying culms*
N	5.1	40	<i>41</i>	7.5	40	<i>40</i>	11.1	35	<i>43</i>	6.2	25	<i>48</i>
P	1.1	36	<i>42</i>	1.8	39	<i>23</i>	2.3	28	<i>34</i>	0.9	13	<i>44</i>
S	0.75	48	<i>-18</i>	1.2	23	<i>14</i>	2.1	22	<i>21</i>	0.4	4	<i>24</i>
Ca	1.4	26	<i>32</i>	0.7	12	<i>-2</i>	1.1	11	<i>-24</i>	0.1	1	<i>-2</i>
Mg	0.25	18	<i>19</i>	0.35	26	<i>11</i>	0.40	13	<i>13</i>	-0.13	-	<i>11</i>
	(mg m ⁻²)			(mg m ⁻²)			(mg m ⁻²)			(mg m ⁻²)		
Mn	197	27	<i>59</i>	171	26	<i>-42</i>	60	6	<i>-43</i>	83	9	<i>-39</i>
Zn	54	490	<i>55</i>	78	430	<i>8</i>	186	460	<i>15</i>	97	235	<i>12</i>

* Mean percentage losses recorded for standing dead tissues were relatively stable for each wetland over the summer-early autumn period. Values given are for mid summer (end of January); negative values = gains

Table 4. Plant accumulation of N and P as a percentage of total loading and removal rates (both g m⁻²) recorded for the wastewater treatment wetlands.

	Wetland loading ¹		% accumulated in plants		Total wetland removal ^{1,2}		% accumulated in plants	
	to Jan 1991	to Jan 1992	Jan 1991	Jan 1992	to Jan 1991	to Jan 1992	Jan 1991	Jan 1992
Nitrogen								
L1	181	755	14.4	6.4	143 (79%)	525 (70%)	18.2	9.1
L2	289	806	12.1	6.2	222 (77%)	531 (66%)	15.8	9.4
L3	444	1344	10.6	5.1	254 (57%)	646 (41%)	18.5	10.7
L4	800	2202	4.9	3.1	400 (50%)	1197 (54%)	9.7	5.8
Phosphorus								
L1	53	173	11.0	5.1	39 (73%)	103 (60%)	14.8	8.5
L2	85	205	9.6	5.0	60 (71%)	103 (50%)	13.6	10.0
L3	130	333	9.4	4.0	61 (47%)	101 (30%)	19.9	13.1
L4	235	557	4.4	2.4	93 (40%)	215 (45%)	11.1	6.2

¹ Mass loading and removal rates have been calculated from water quality and flow data in Tanner et al. (submitted b).

² Percentage removals relative to mass loadings are given in parenthesis

APPENDICES

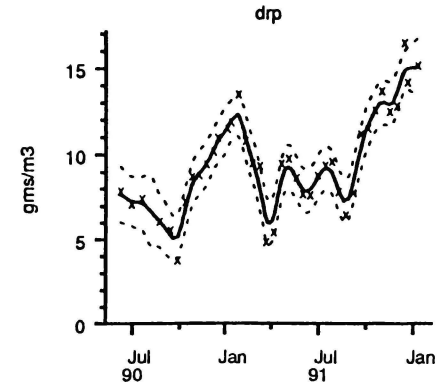
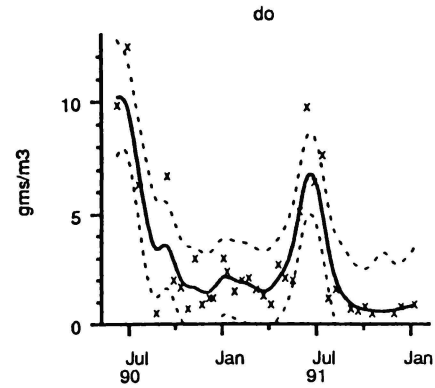
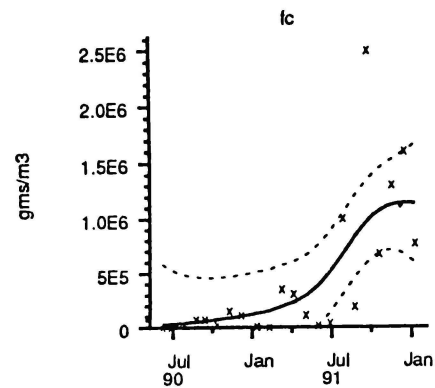
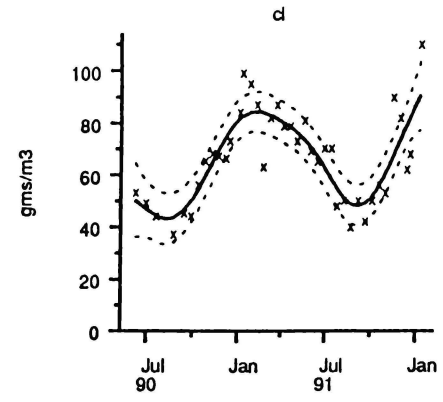
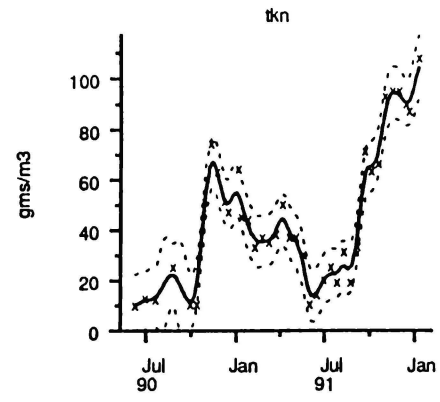
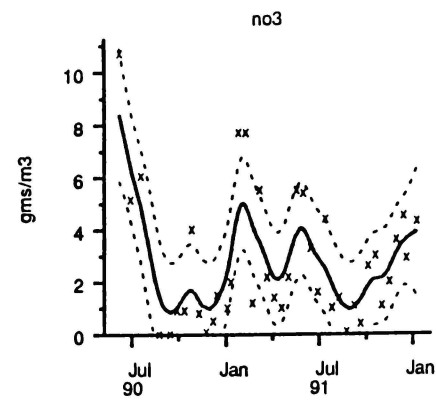
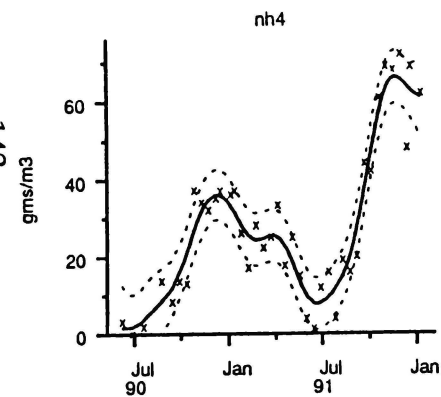
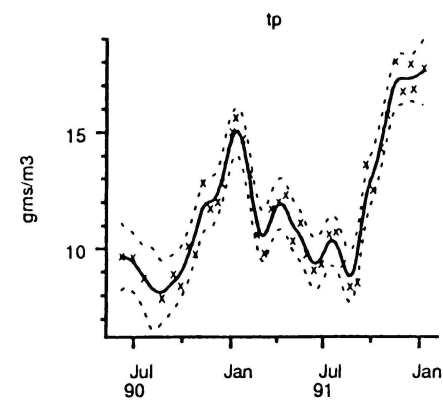
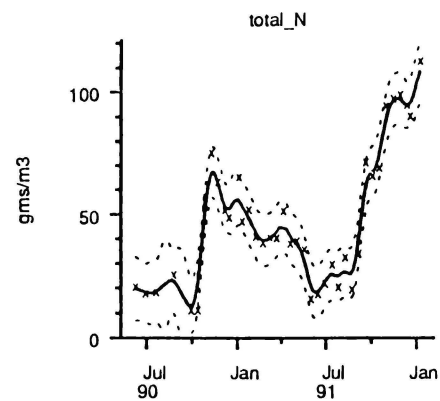
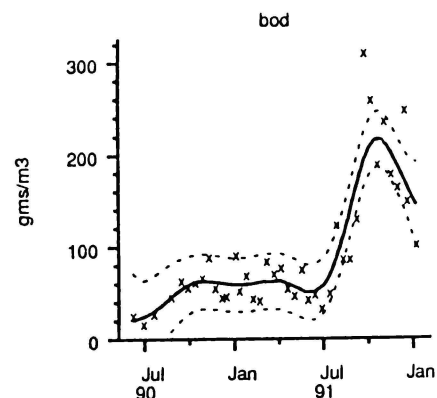
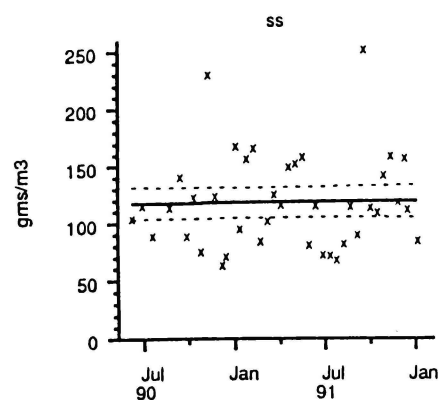


Alf. 1

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Appendix 1 - Inflow wastewater concentrations vs time (g m^{-3})

Each pair of wetlands received a different hydraulic loading of dairy shed wastewater. These graphs show changes in concentration of major parameters during the period of the study. Bayesian curves have been fitted to the data points (x). The solid line represents the mean, and the fine dashed line the 85% confidence intervals of the mean.



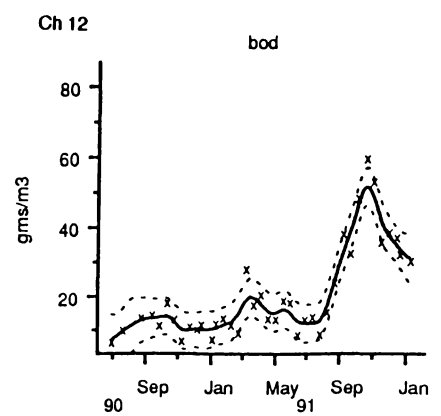
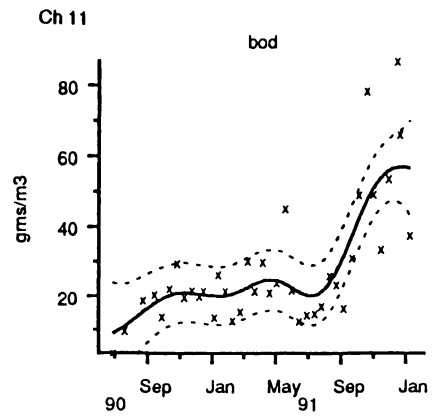
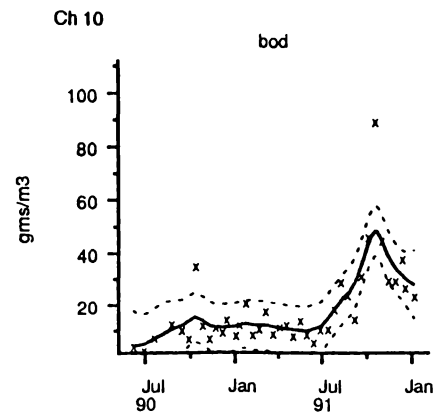
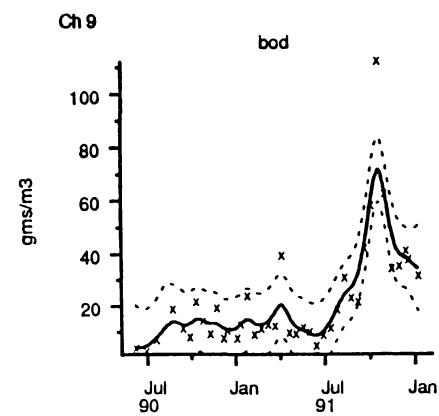
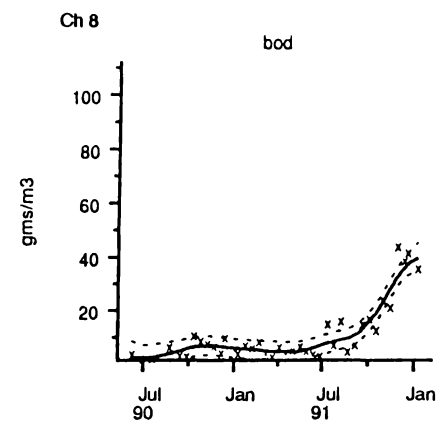
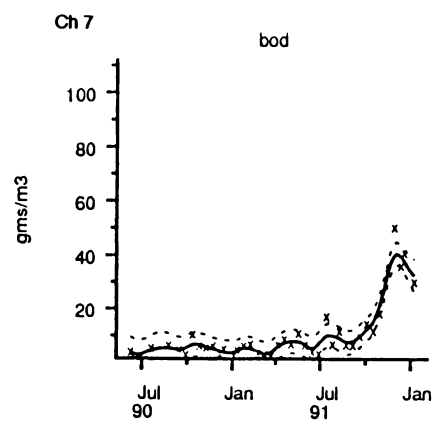
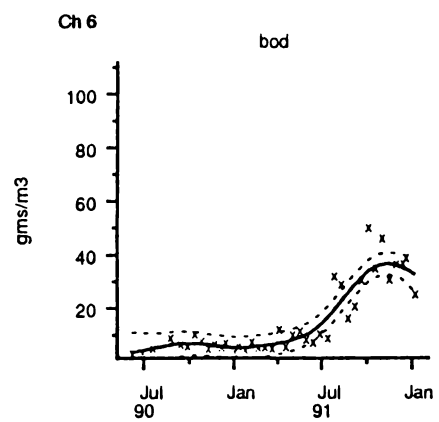
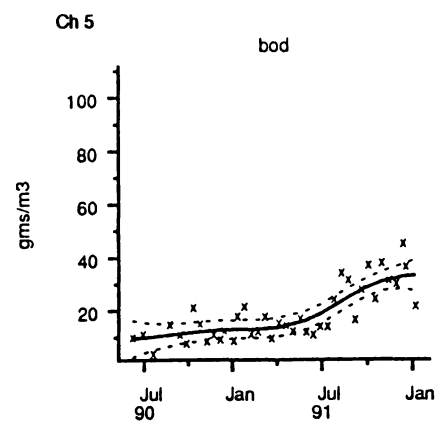
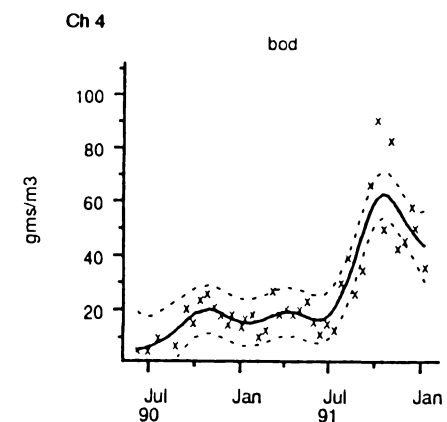
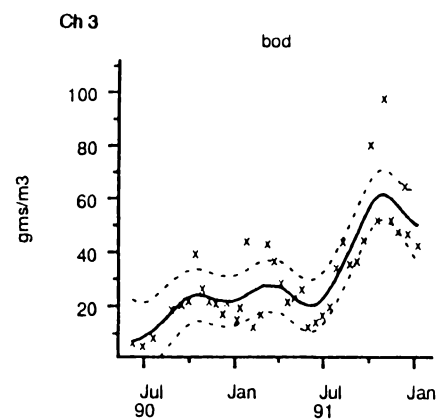
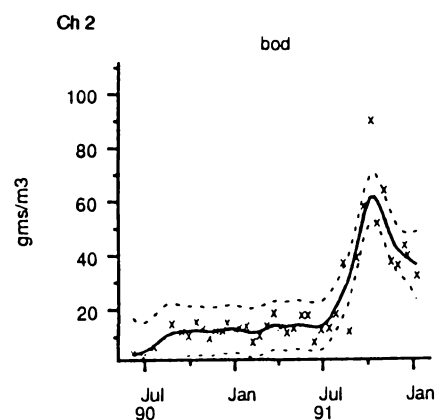
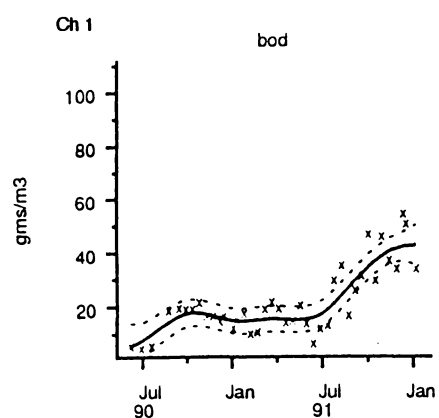
Appendix 2 - Outflow concentrations (g m⁻³) vs time for water quality parameters

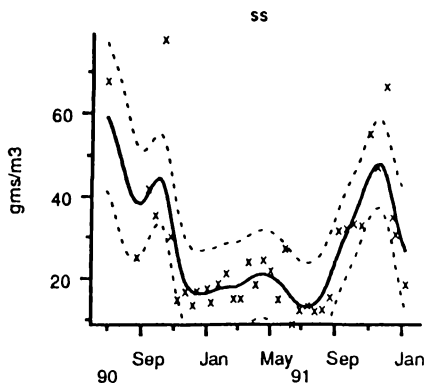
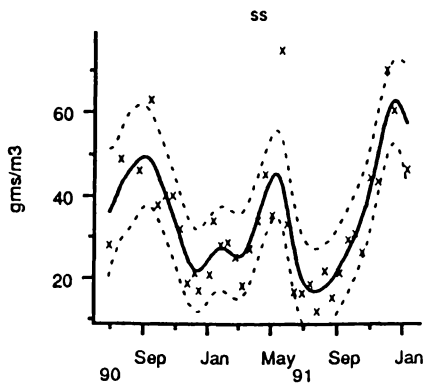
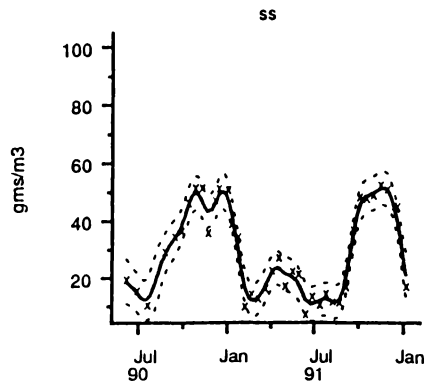
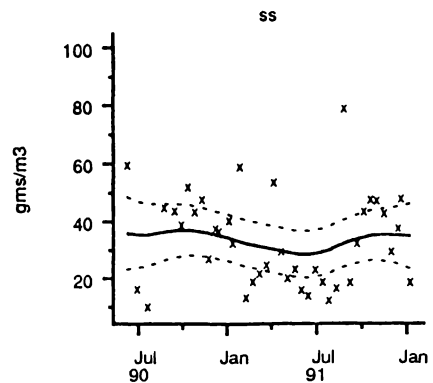
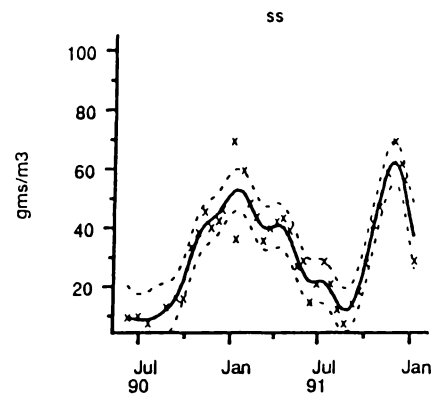
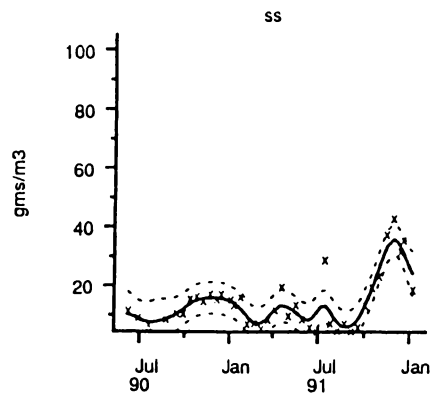
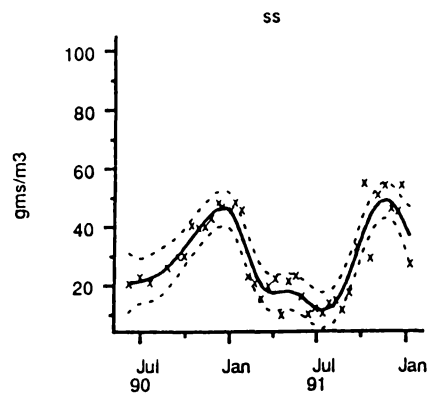
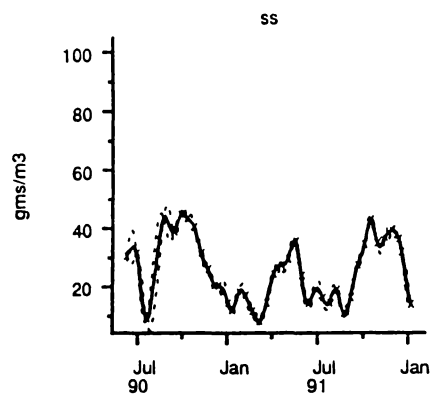
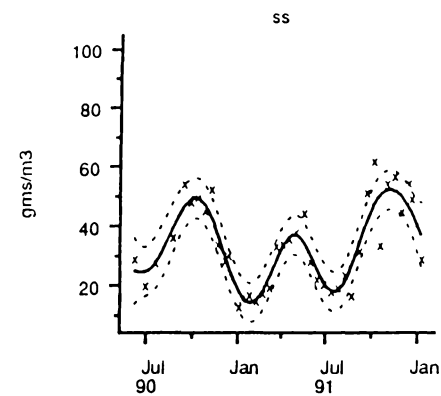
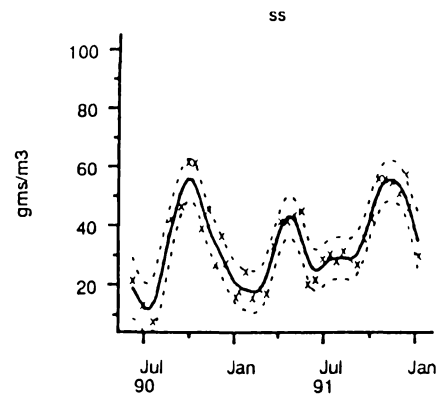
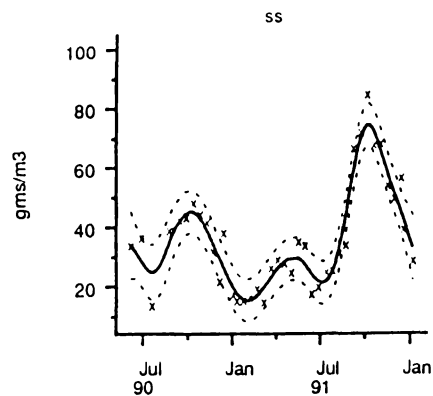
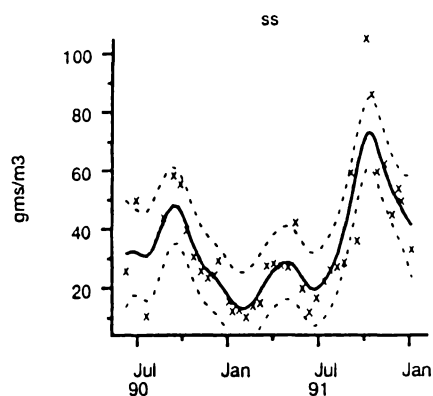
Each graph (1 - 10) refers to a different horizontal-flow wetland. The final two graphs (11 & 12) are for the up-flow wetlands. Each pair of wetlands received a different hydraulic loading of dairy shed wastewater. These graphs show changes in concentration of major parameters during the period of the study. Bayesian curves have been fitted to the data points (x). The solid line represents the mean, and the fine dashed line the 85% confidence intervals of the mean. Where the confidence intervals of two superimposed curves do not overlap, they are significantly different at the 95% level.

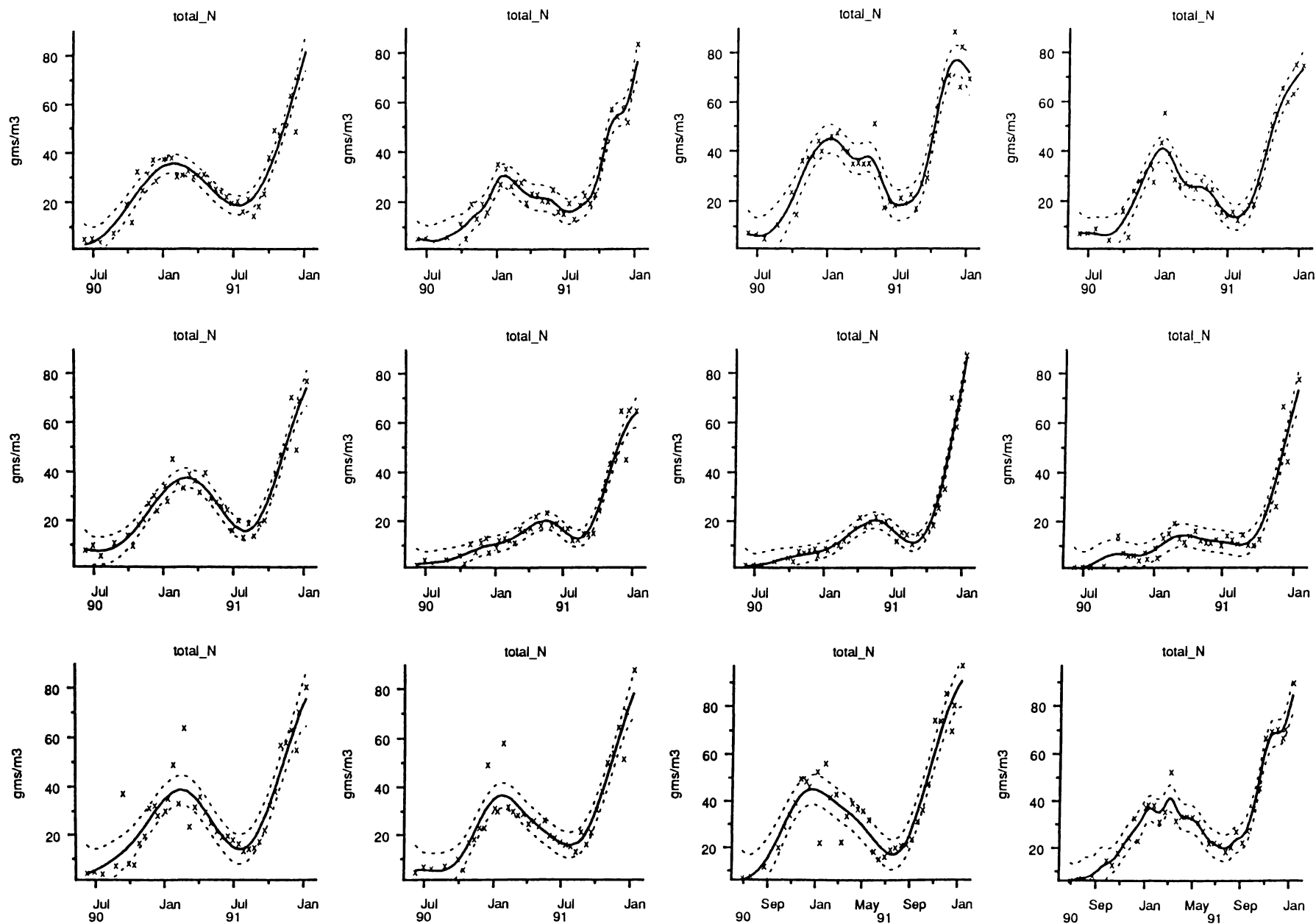
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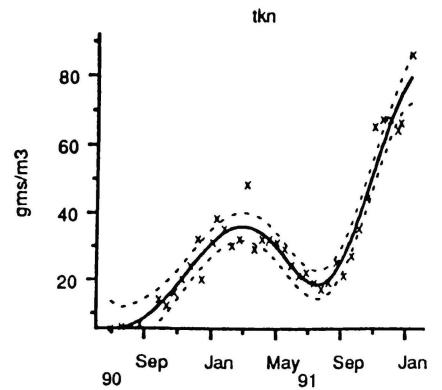
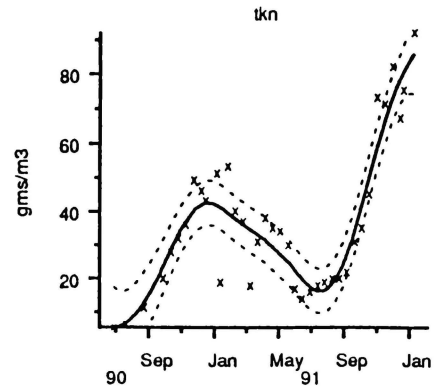
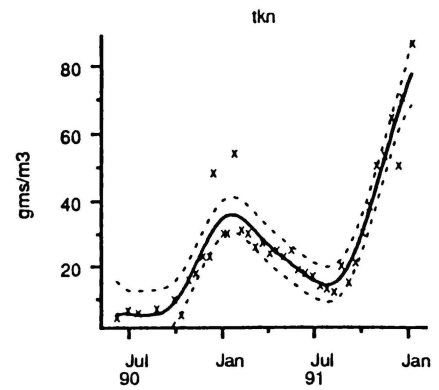
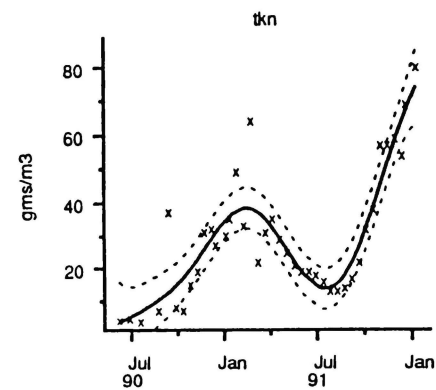
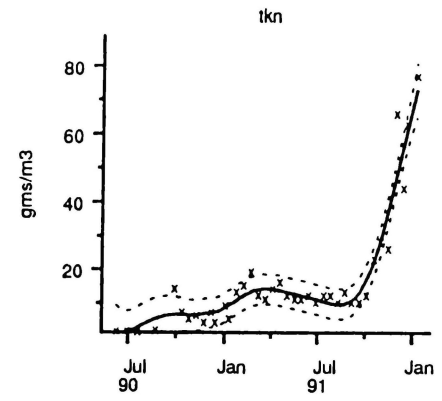
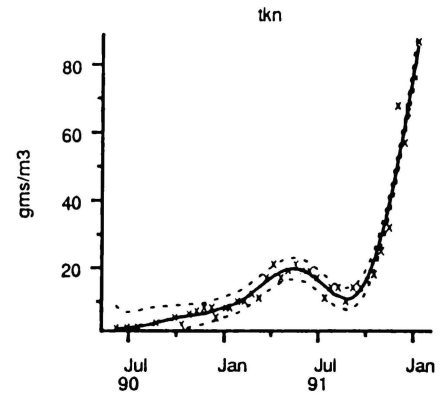
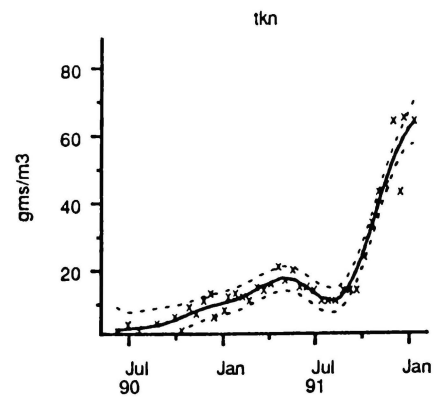
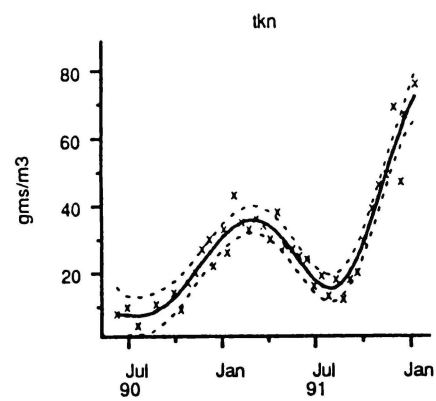
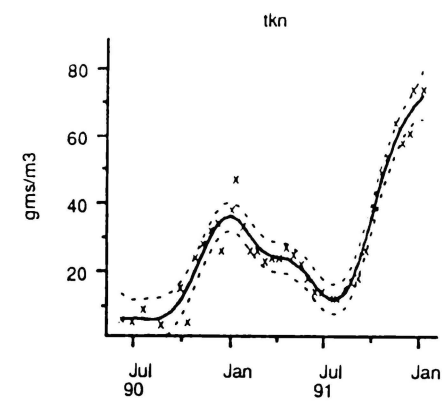
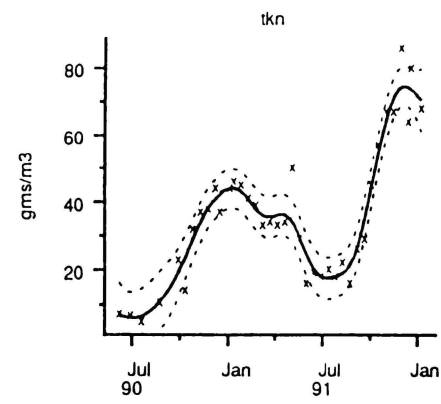
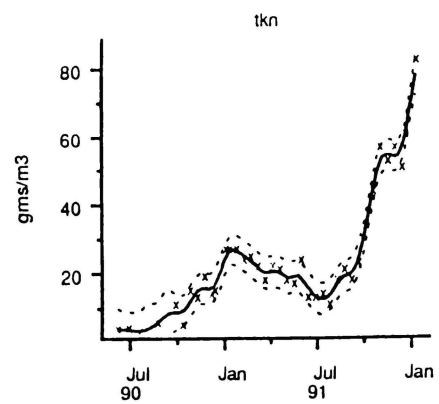
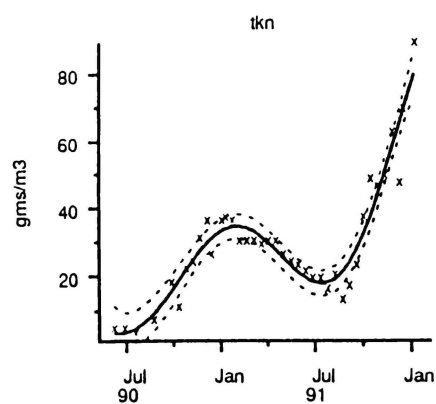
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4	Horizontal flow	<i>S. validus</i>	2
5	Horizontal flow	-	5.5
6	Horizontal flow	<i>S. validus</i>	5.5
7	Horizontal flow	-	7
8	Horizontal flow	<i>S. validus</i>	7
9	Horizontal flow	Mixed species	3
10	Horizontal flow	<i>S. validus</i> harvested	3
11	Up-flow	<i>S. validus</i>	2.2
12	Up-flow	<i>S. validus</i>	6.5

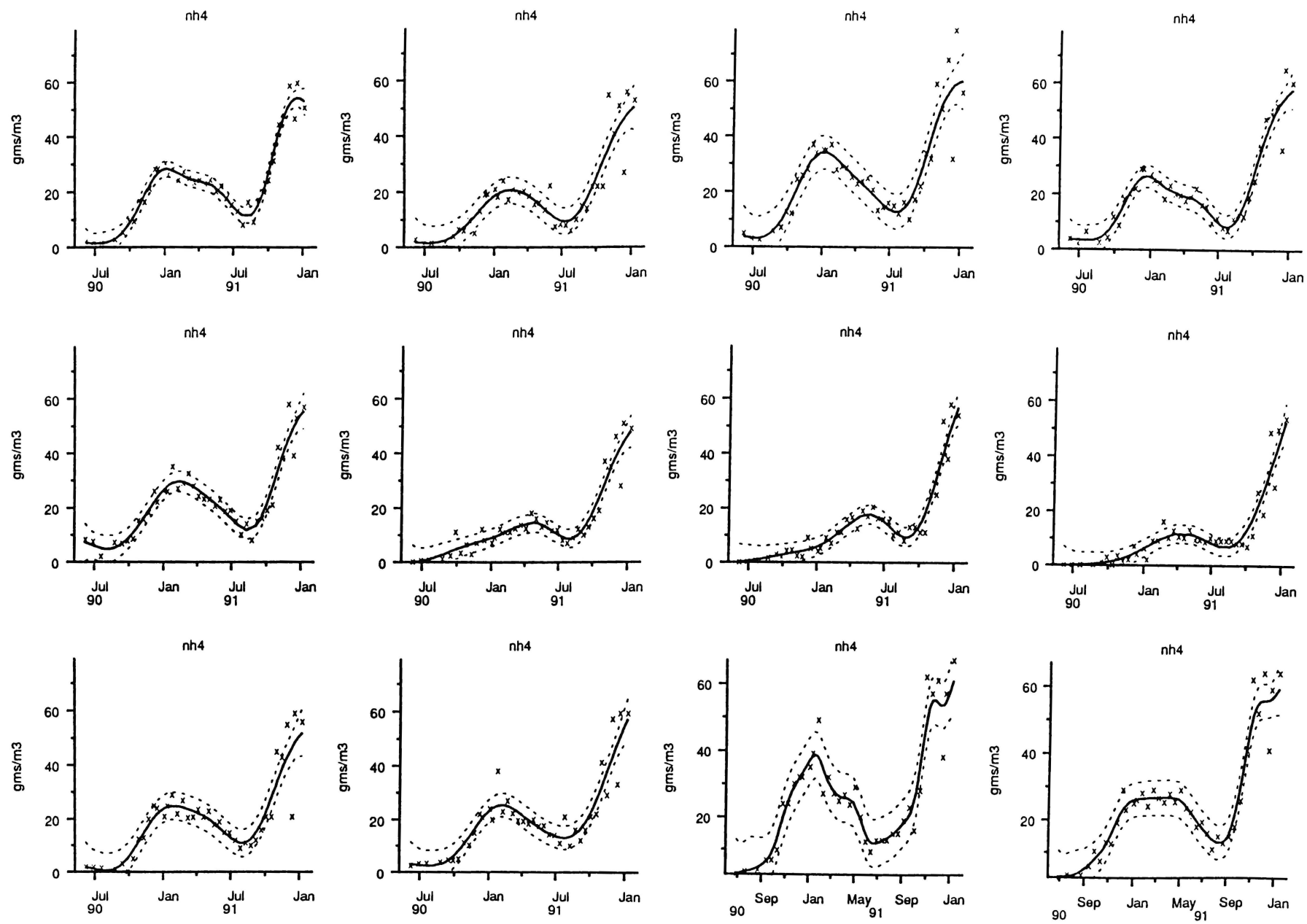
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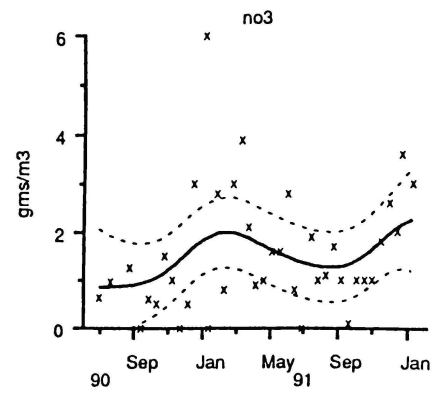
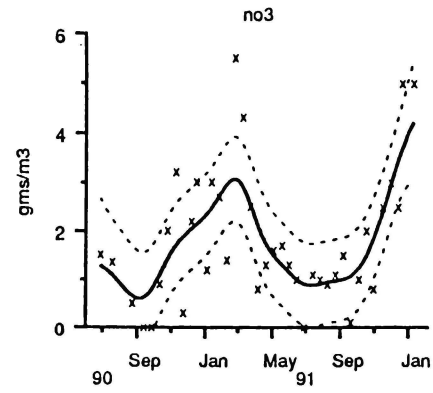
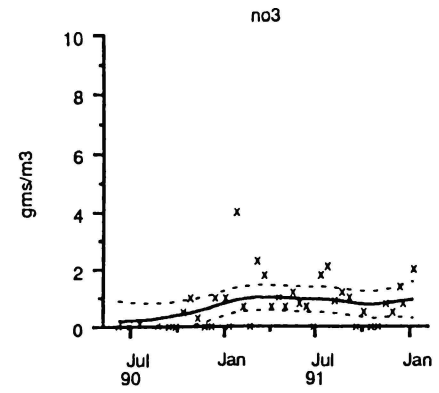
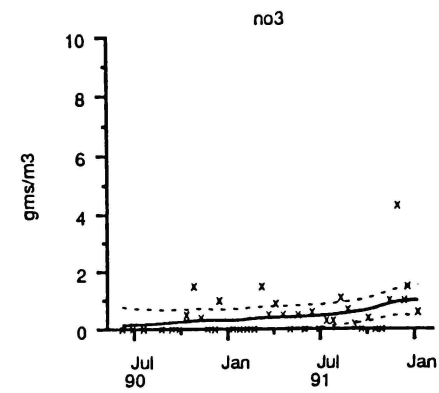
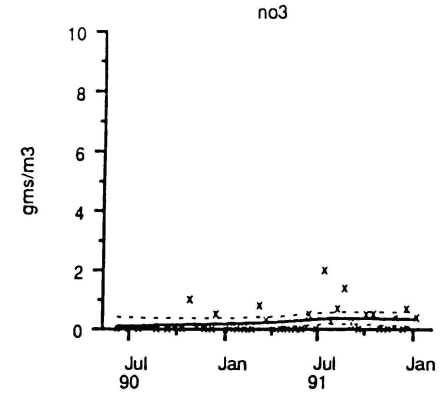
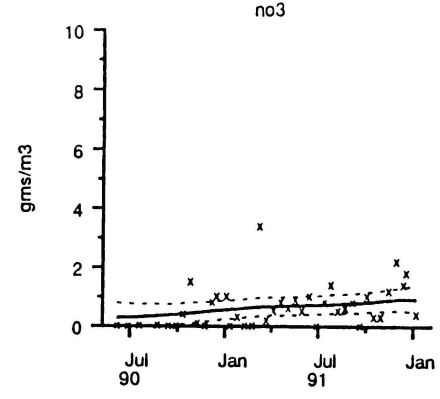
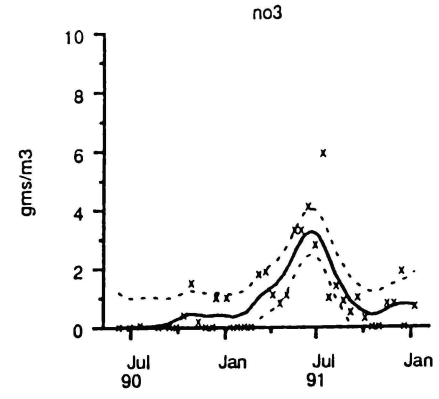
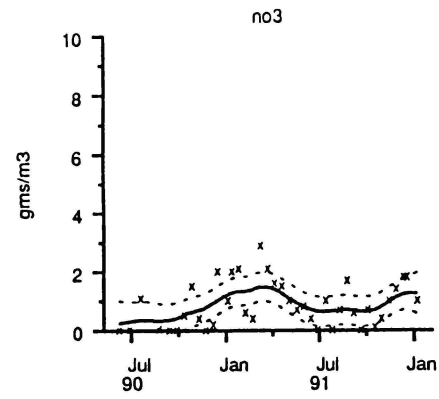
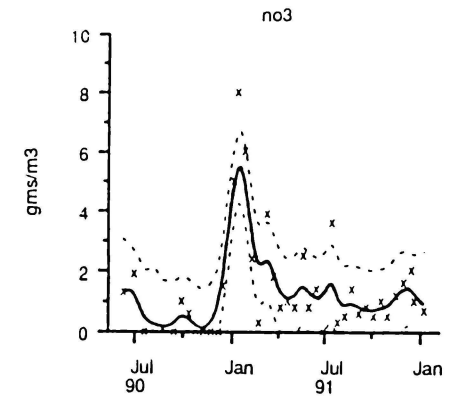
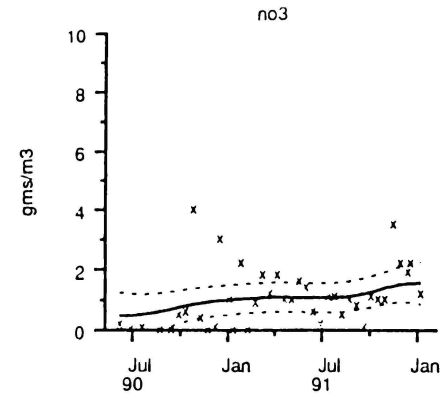
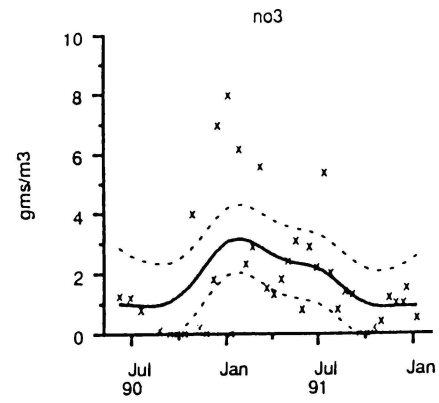
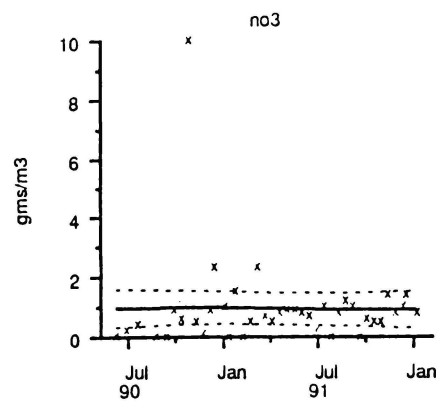


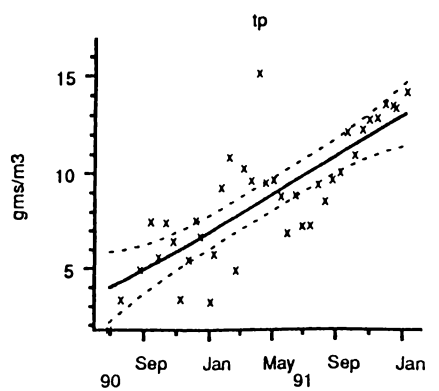
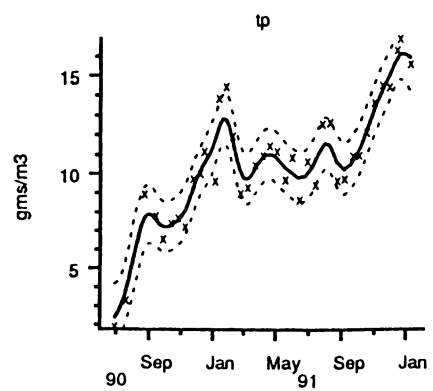
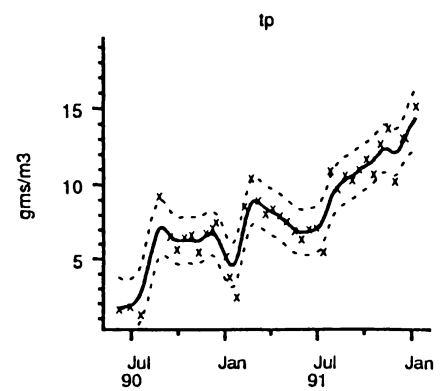
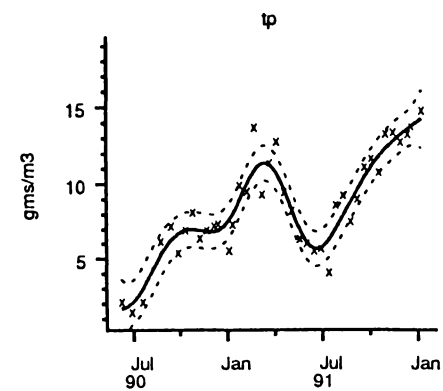
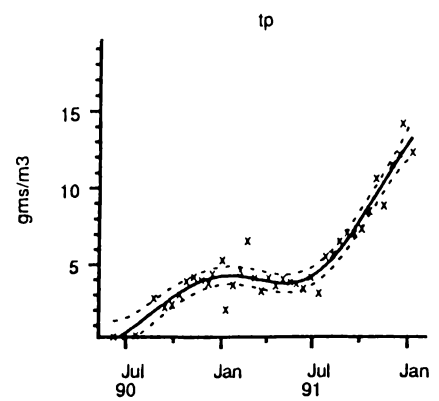
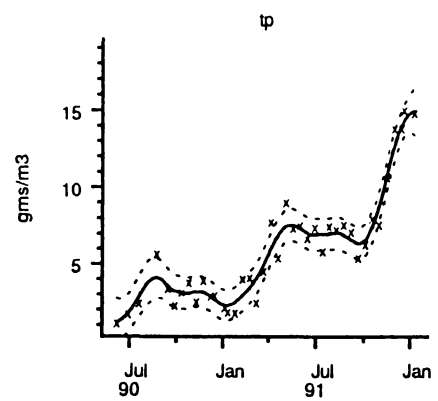
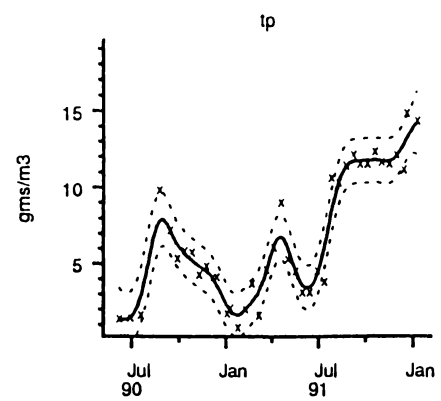
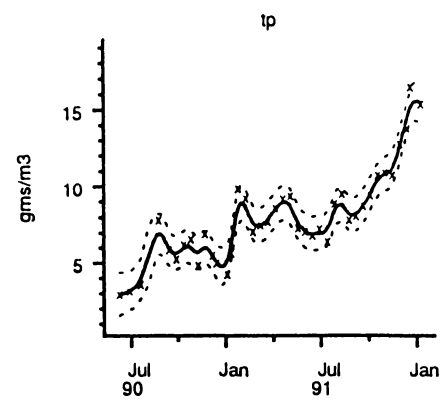
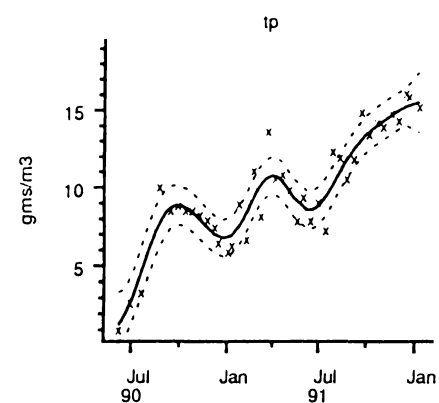
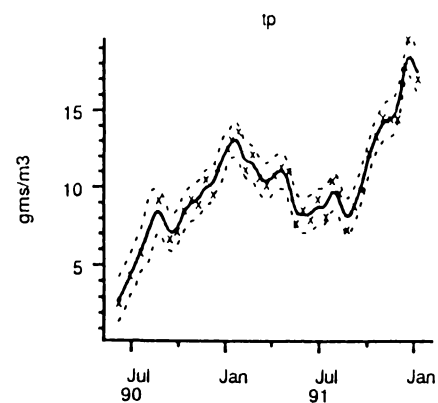
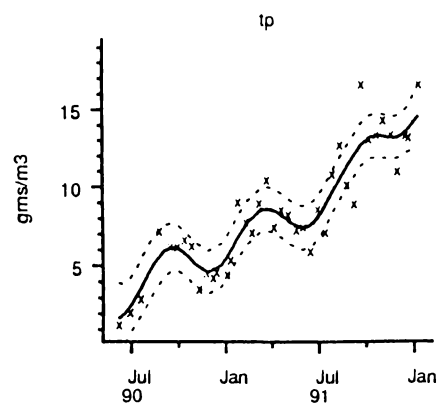
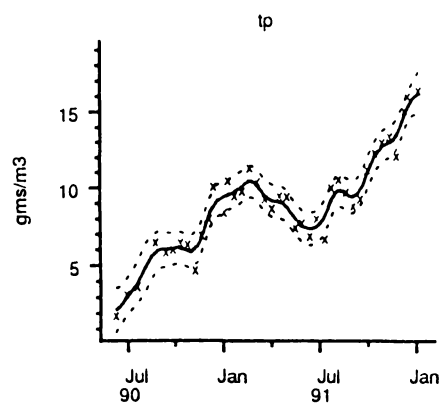


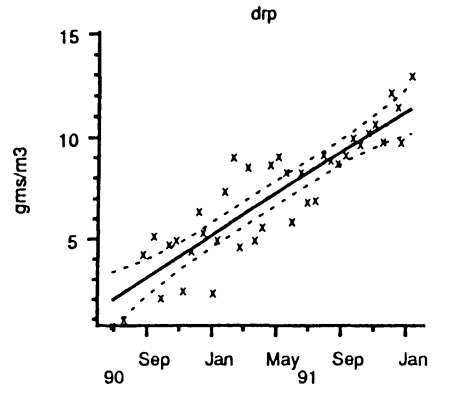
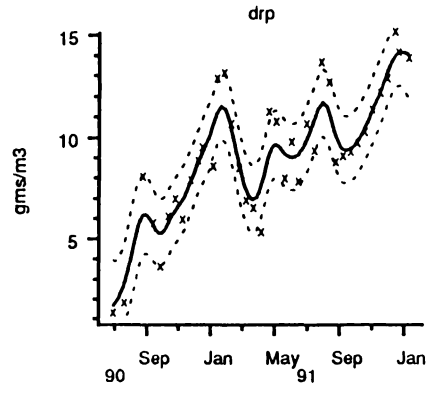
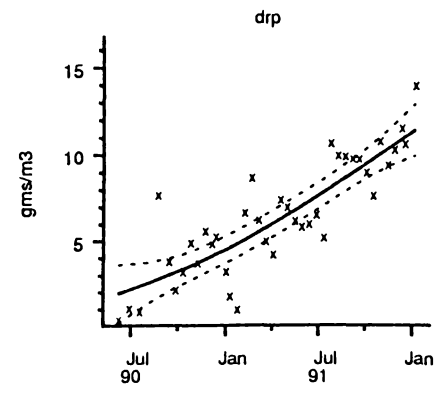
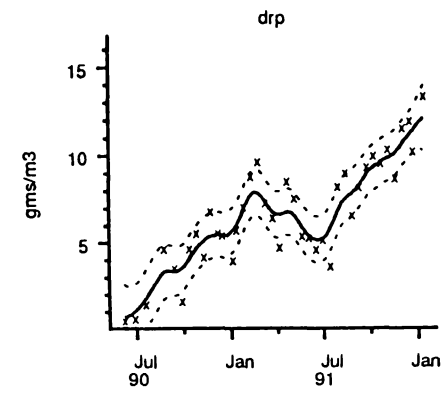
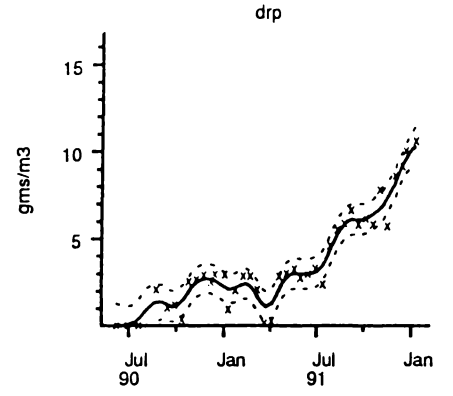
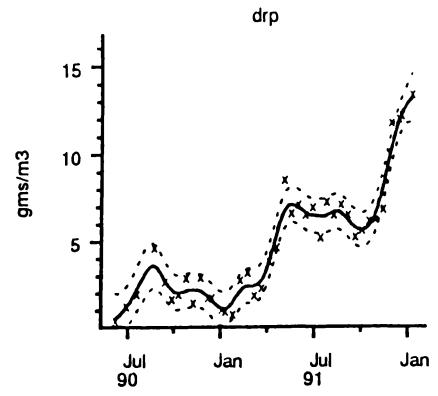
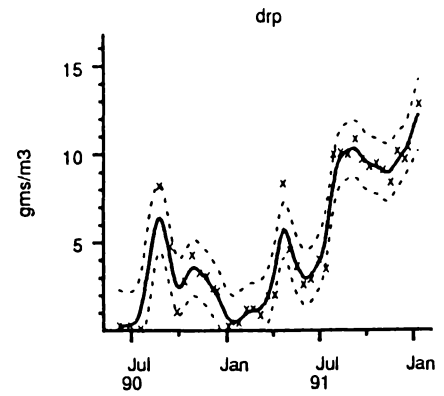
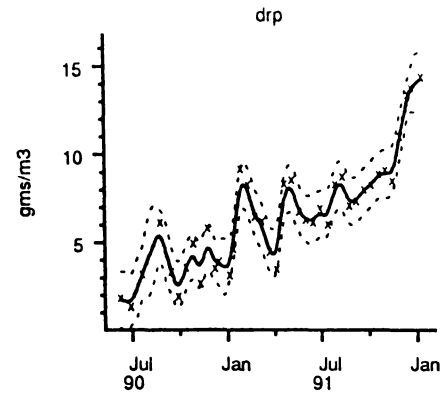
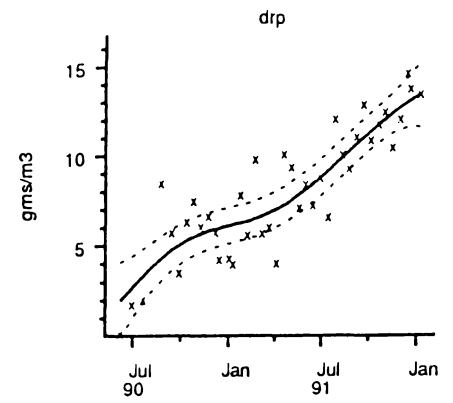
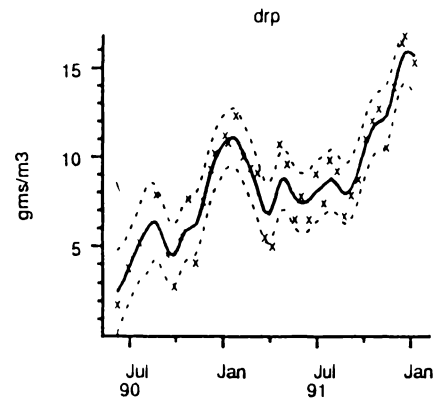
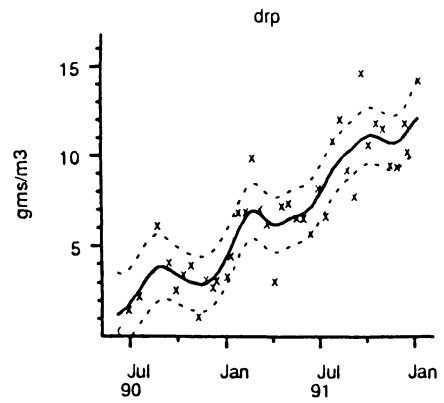
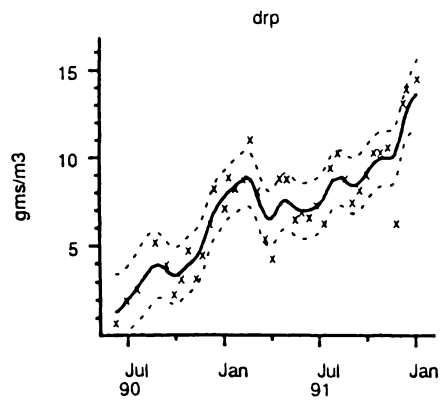


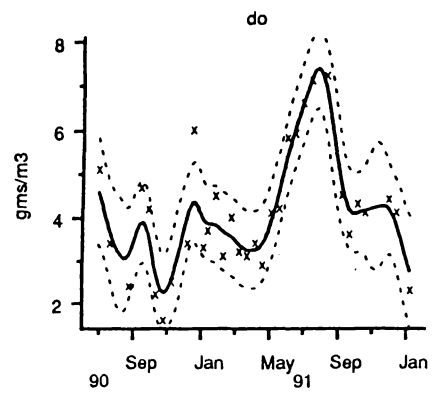
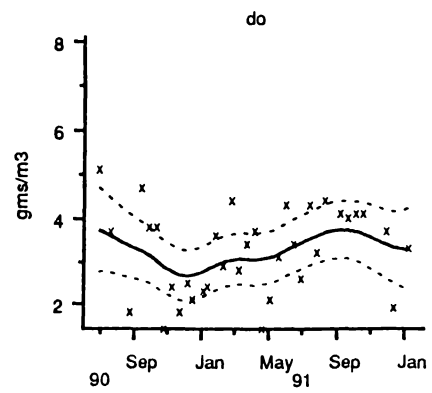
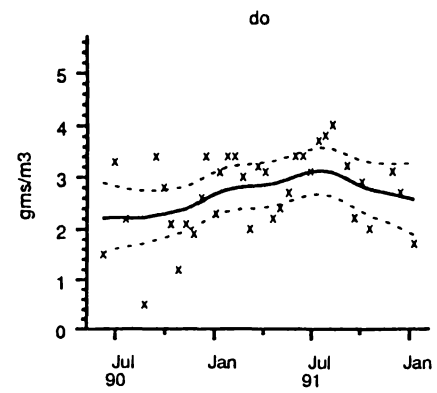
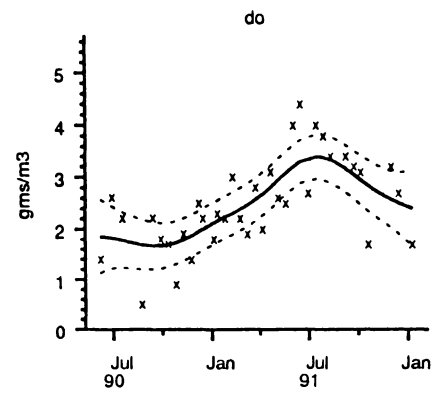
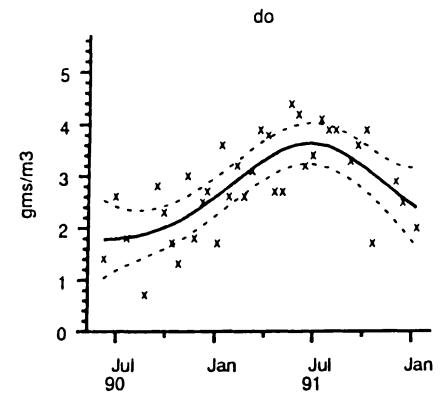
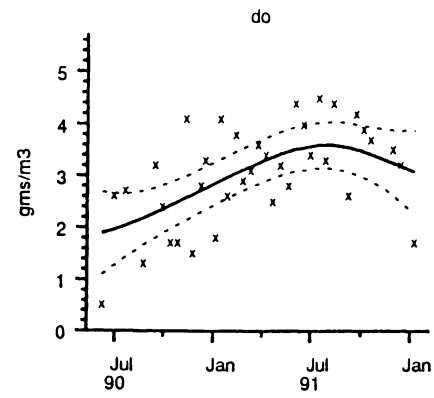
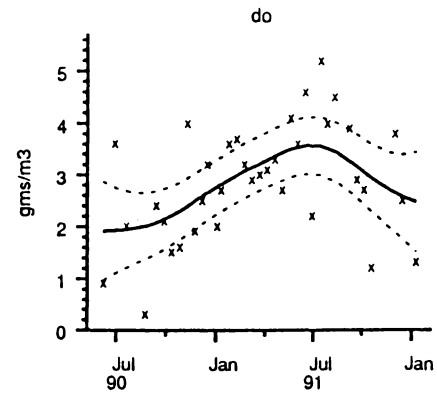
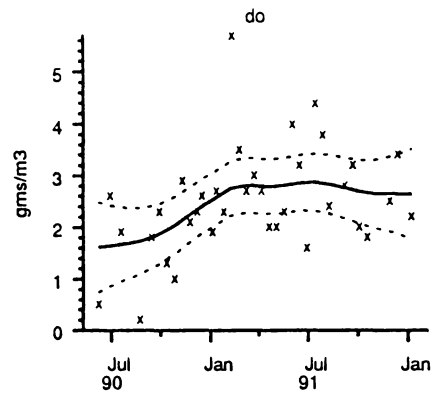
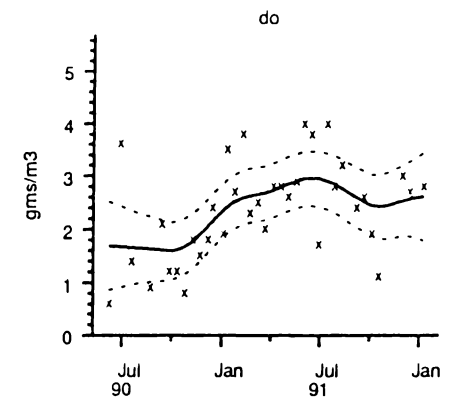
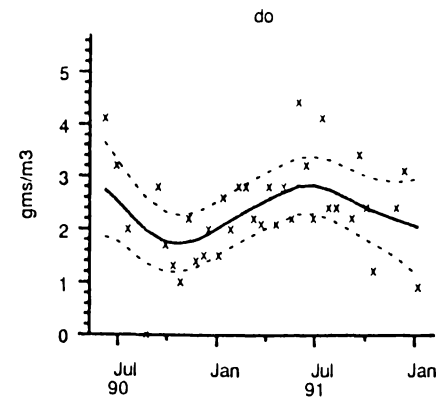
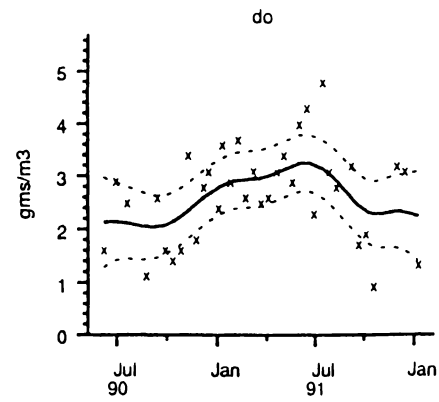
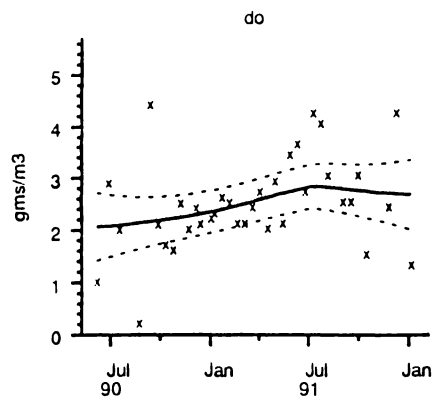


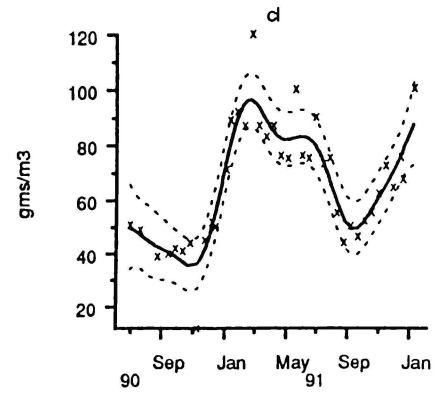
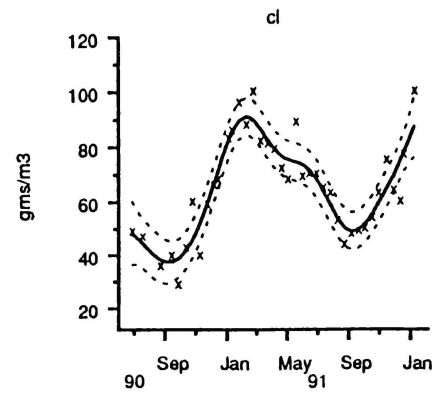
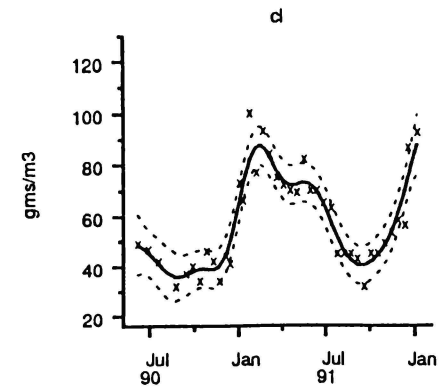
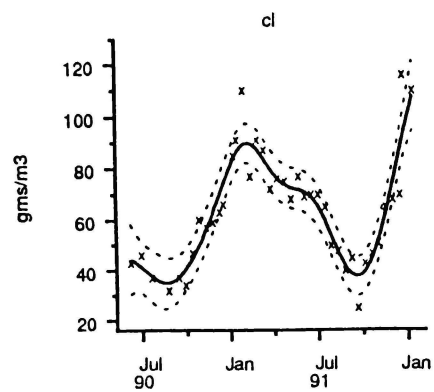
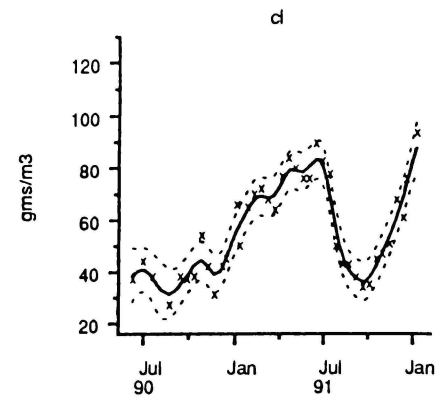
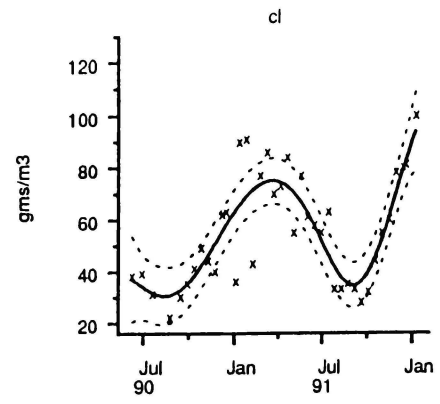
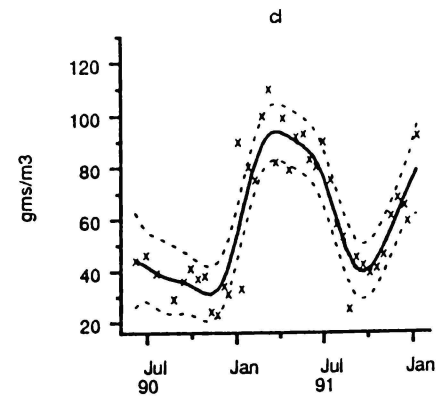
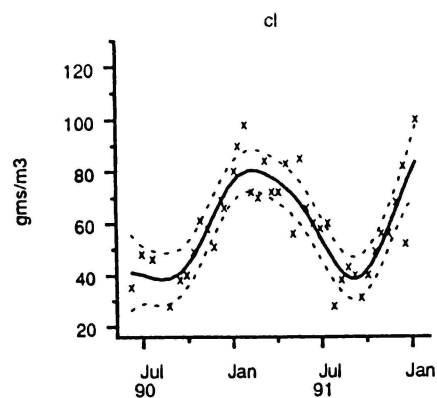
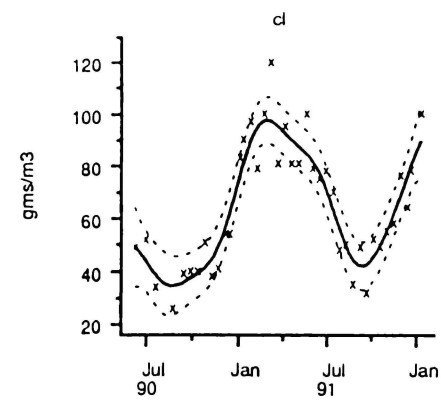
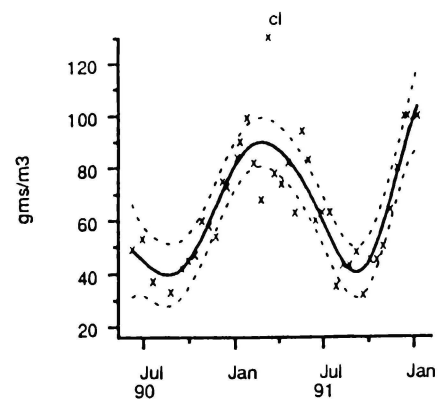
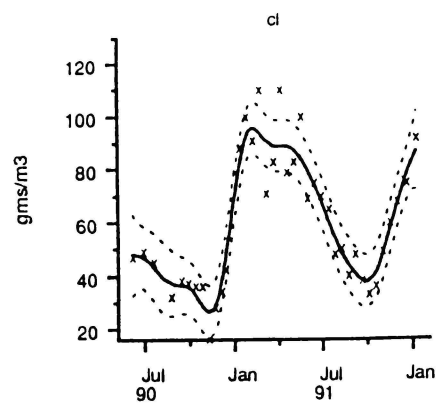
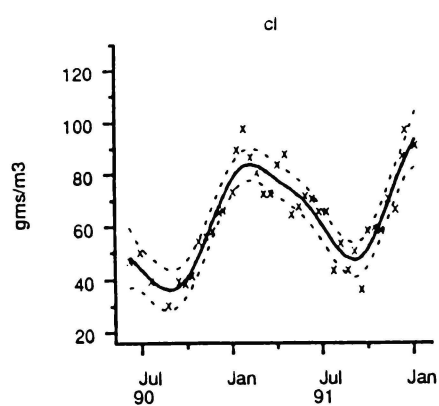


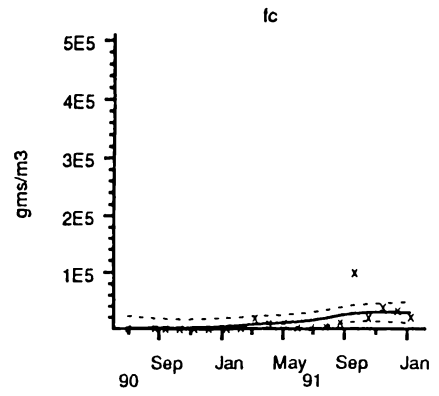
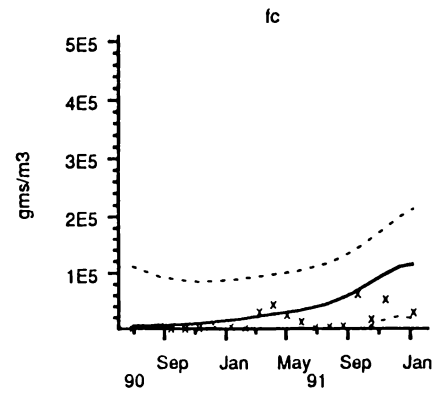
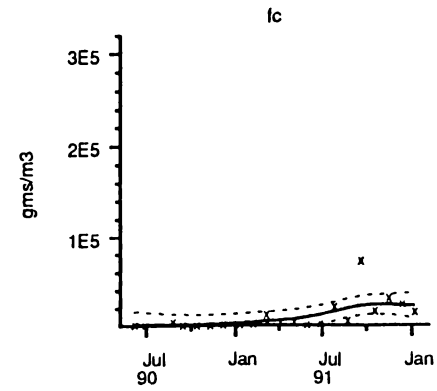
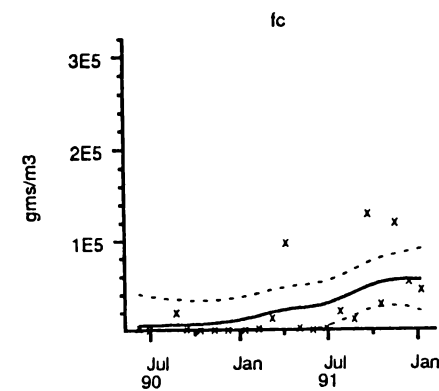
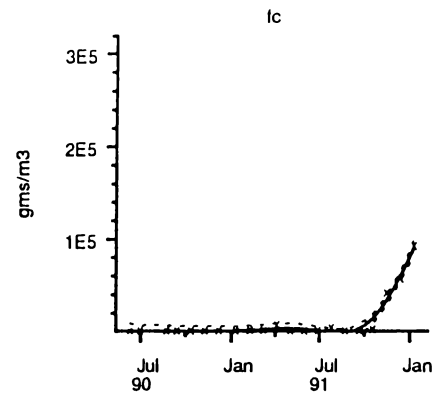
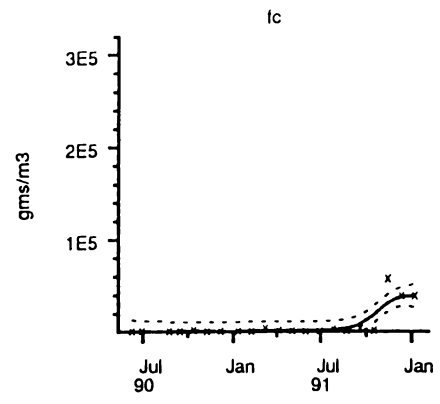
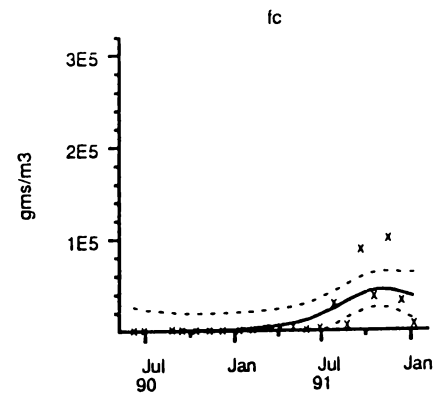
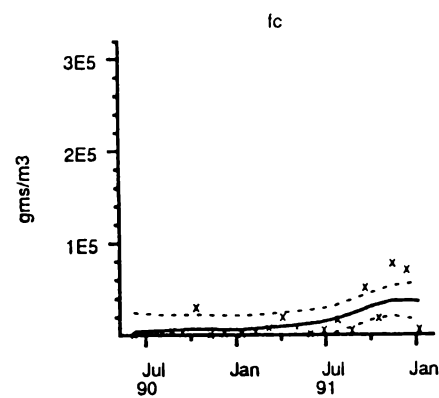
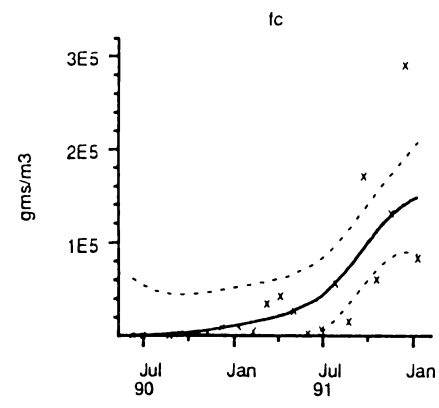
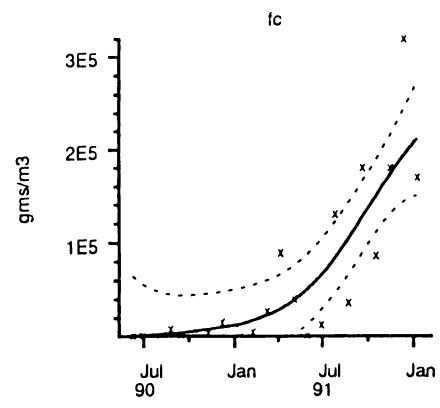
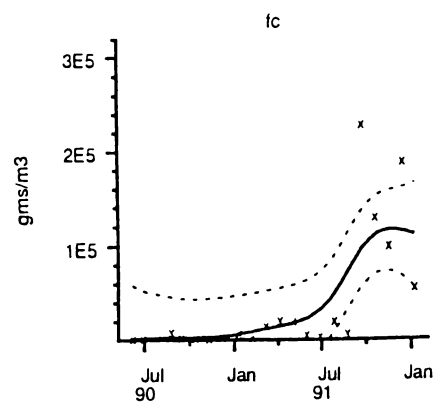
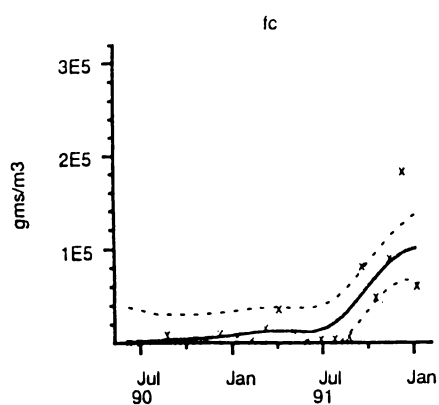












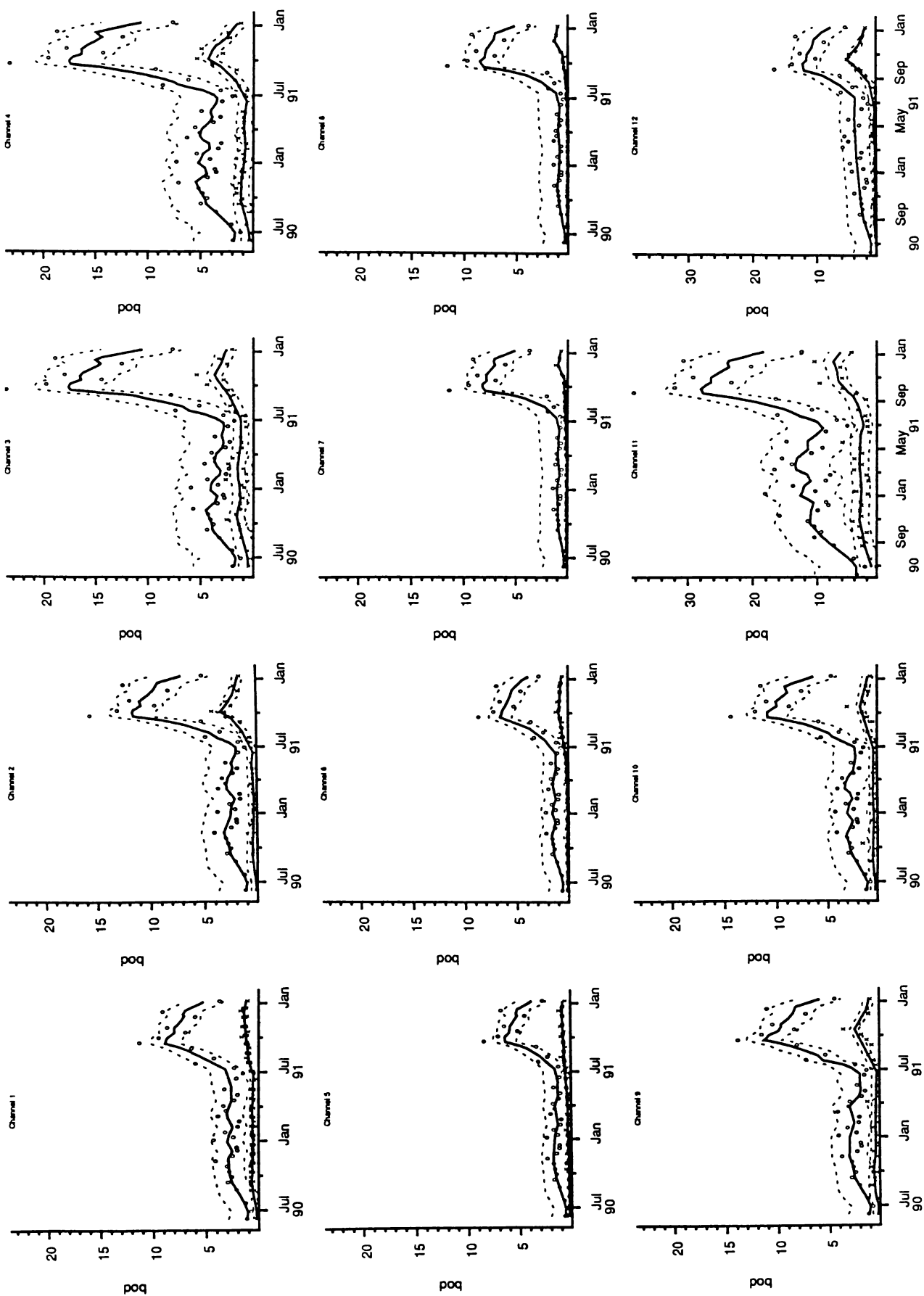
Appendix 3 - Mass inflow and outflow rates ($\text{g m}^{-2} \text{ d}^{-1}$) vs time for selected water quality parameters.

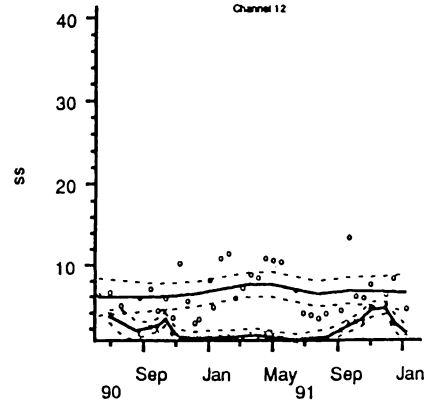
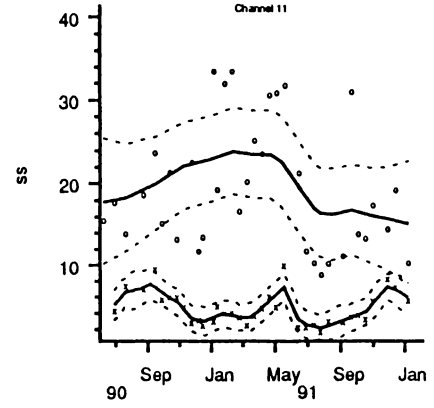
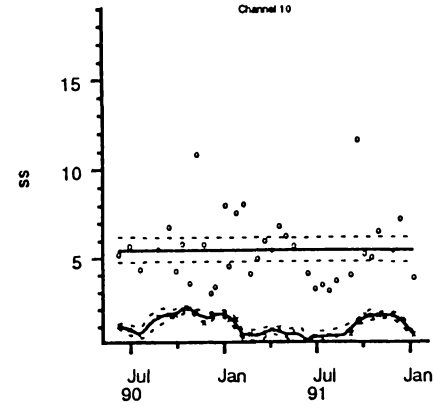
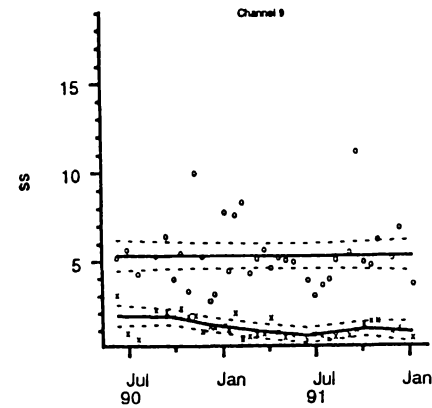
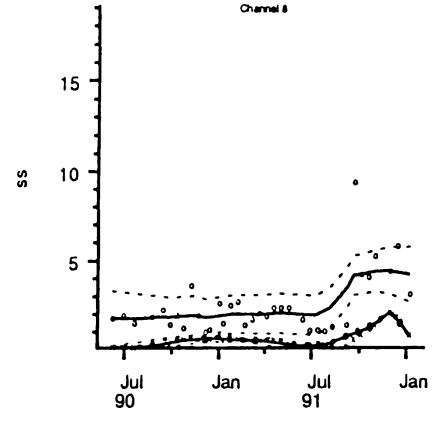
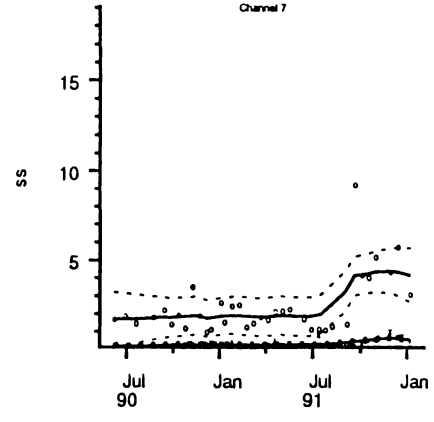
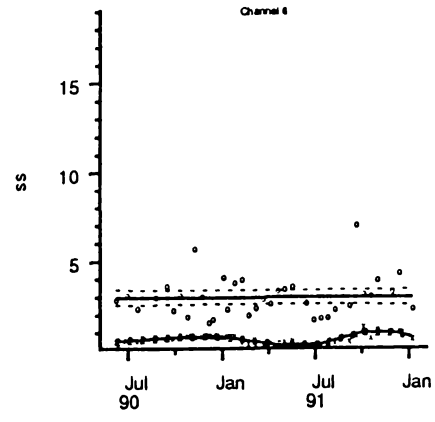
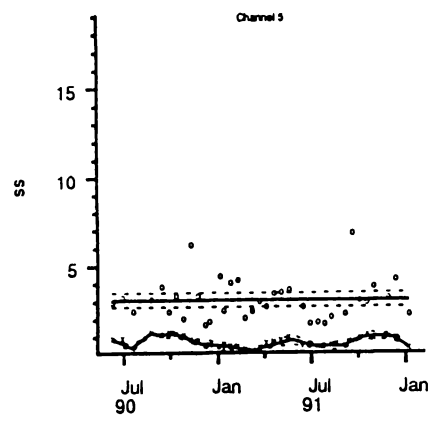
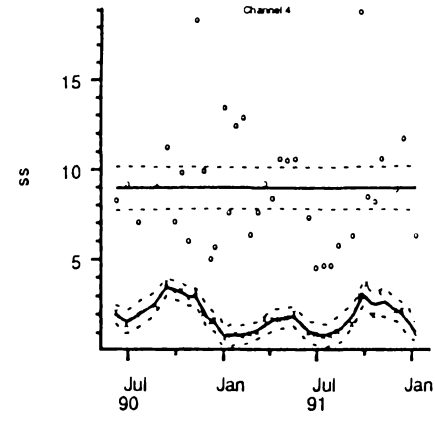
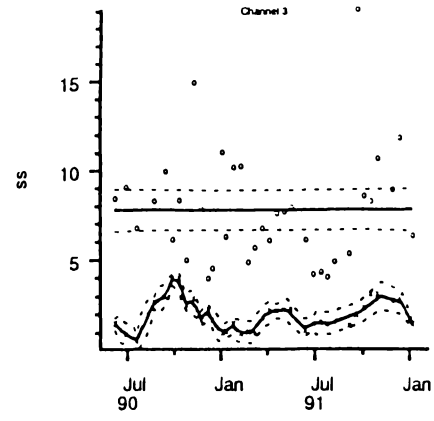
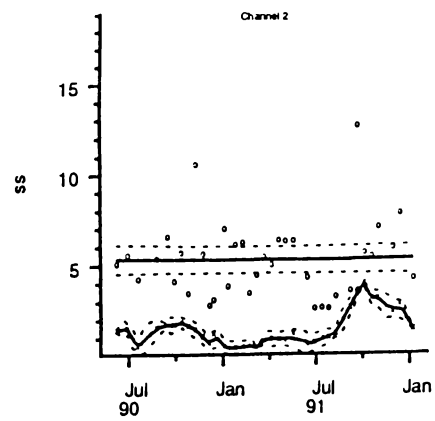
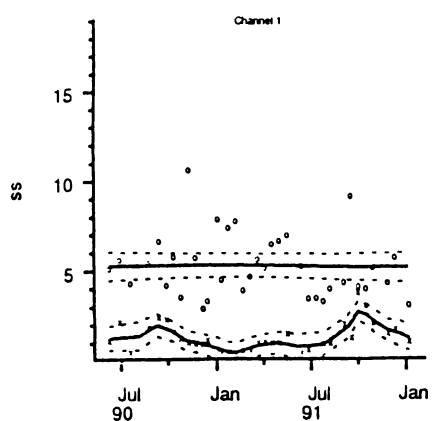
Each graph (1 - 10) refers to a different horizontal-flow wetland. The final two graphs (11 & 12) are for the up-flow wetlands. Each pair of wetlands received a different hydraulic loading of dairy shed wastewater. These graphs show changes in concentration of major parameters during the period of the study. Bayesian curves have been fitted to the data points (x). The solid line represents the mean, and the fine dashed line the 85% confidence intervals of the mean. Where the confidence intervals of two superimposed curves do not overlap, they are significantly different at the 95% level.

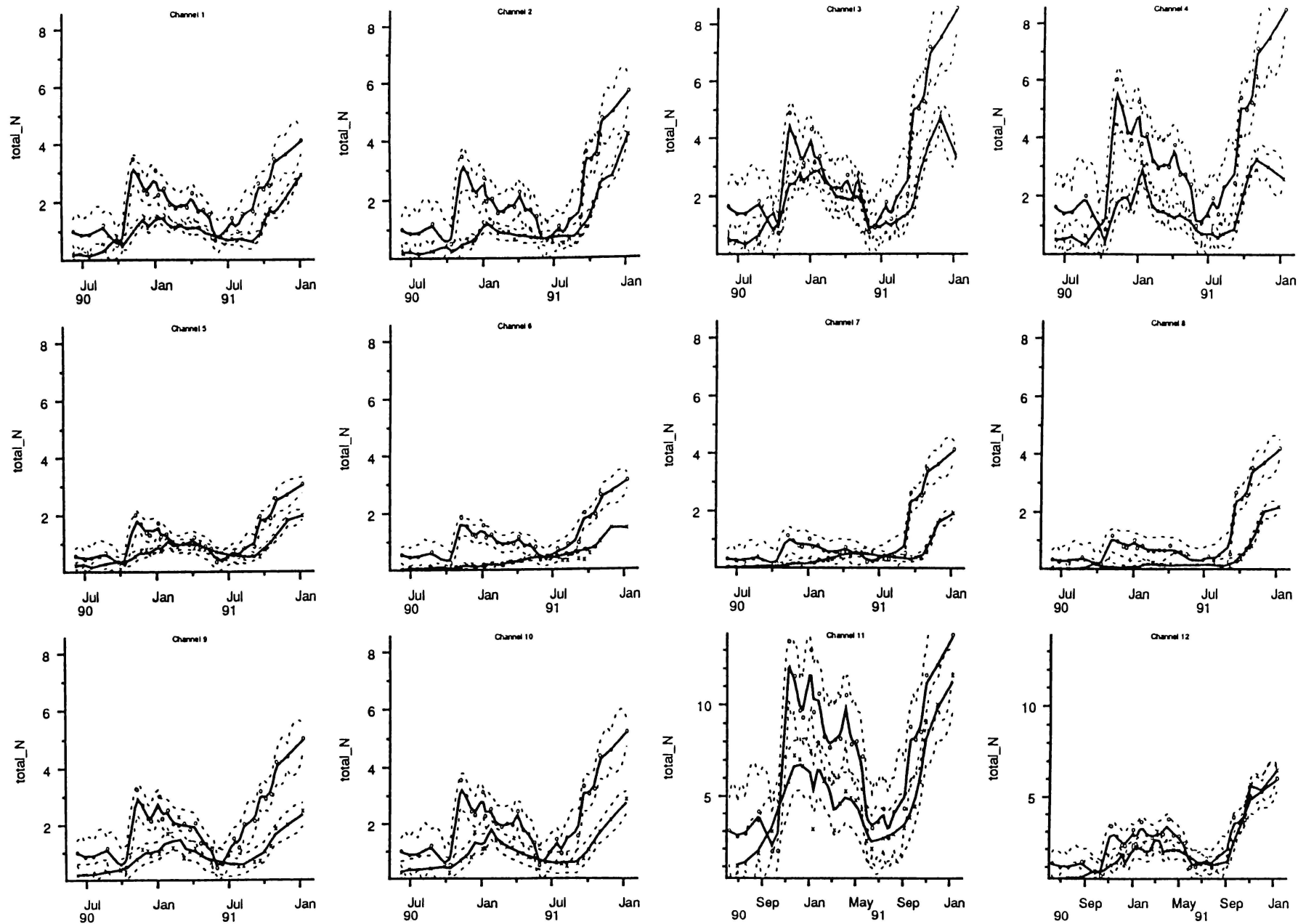
The treatments which correspond to each channel are:

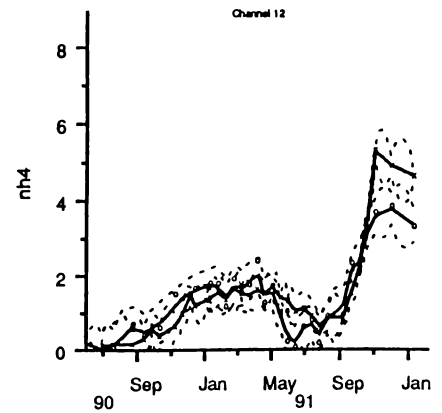
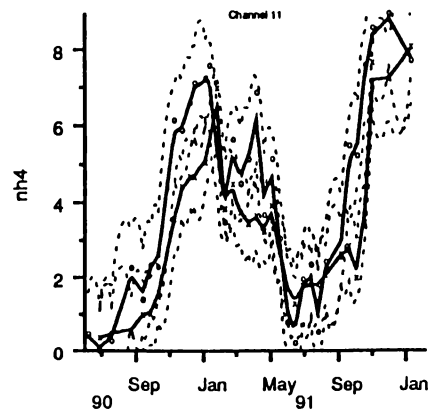
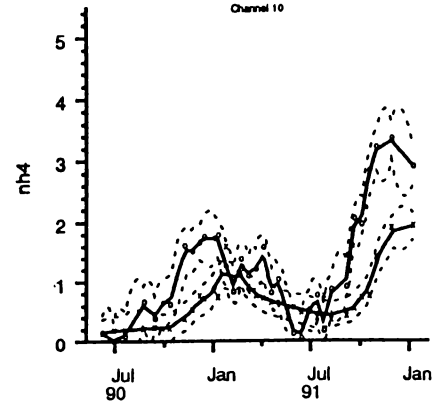
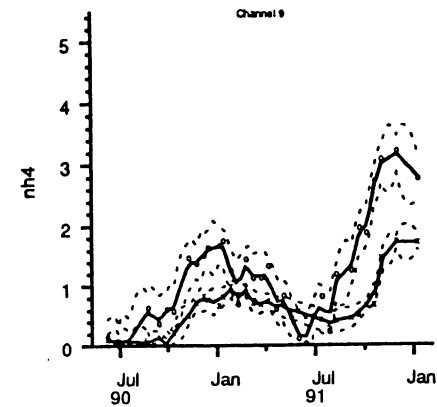
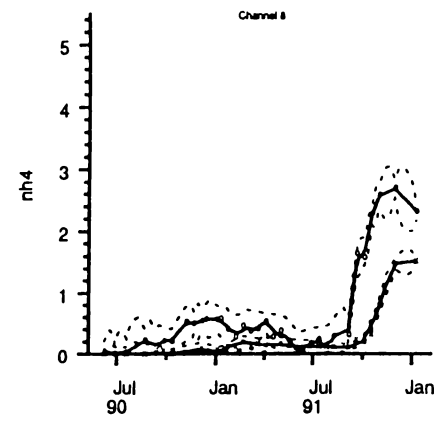
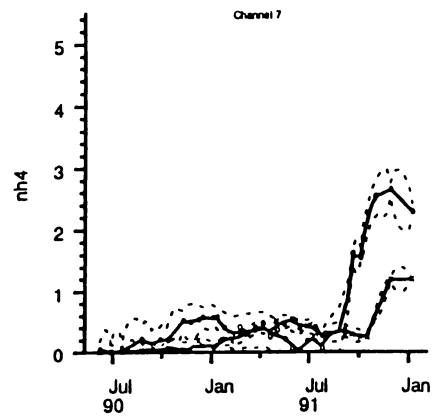
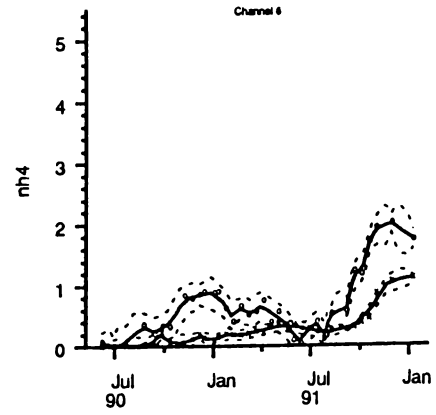
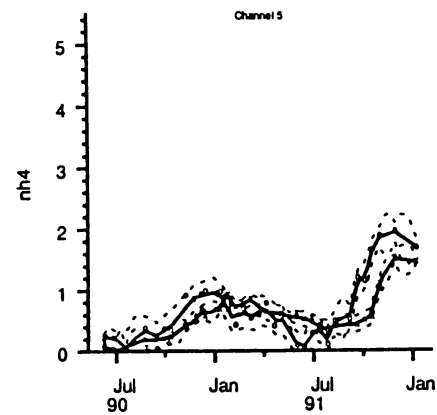
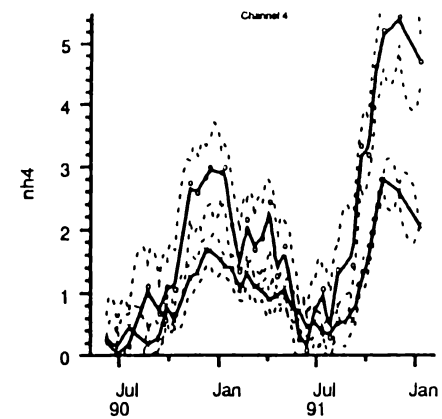
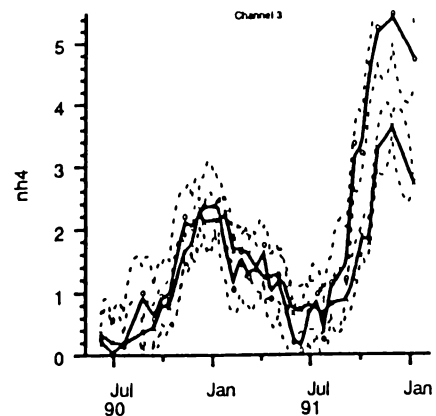
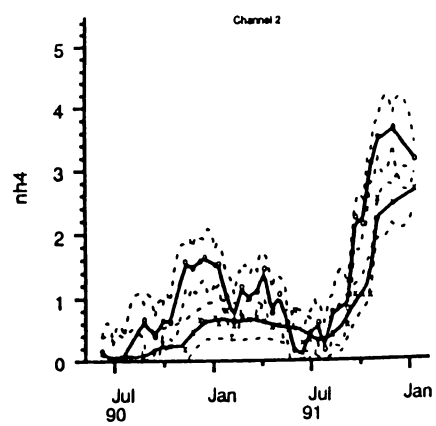
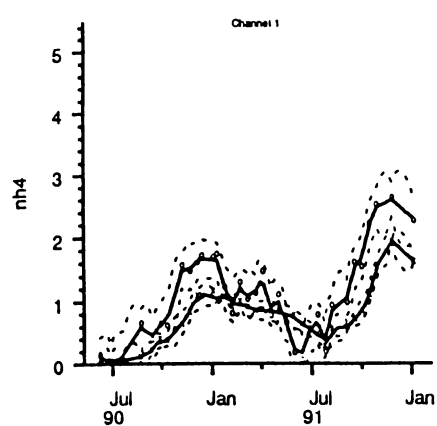
Channel	Type	Planted	Nominal Retention time (d)
1	Horizontal flow	-	3
2	Horizontal flow	<i>S. validus</i>	3
3	Horizontal flow	-	2
4	Horizontal flow	<i>S. validus</i>	2
5	Horizontal flow	-	5.5
6	Horizontal flow	<i>S. validus</i>	5.5
7	Horizontal flow	-	7
8	Horizontal flow	<i>S. validus</i>	7
9	Horizontal flow	Mixed species	3
10	Horizontal flow	<i>S. validus</i> harvested	3
11	Up-flow	<i>S. validus</i>	2.2
12	Up-flow	<i>S. validus</i>	6.5

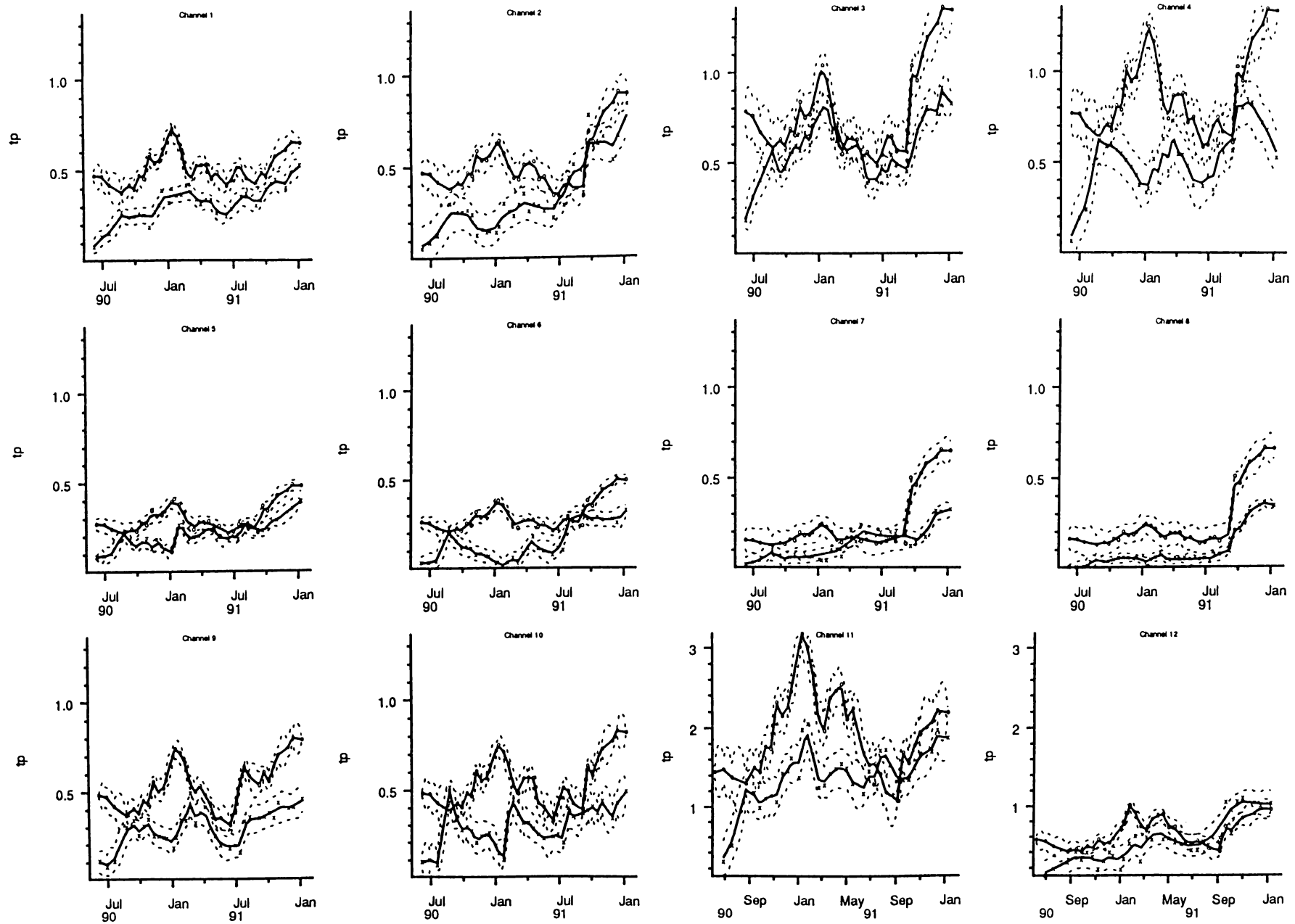
Results from Channel 9 & 10 are not dealt with in this thesis.











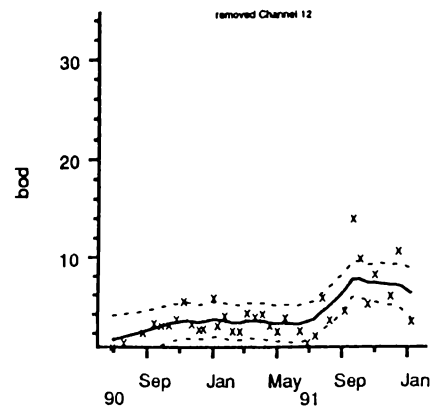
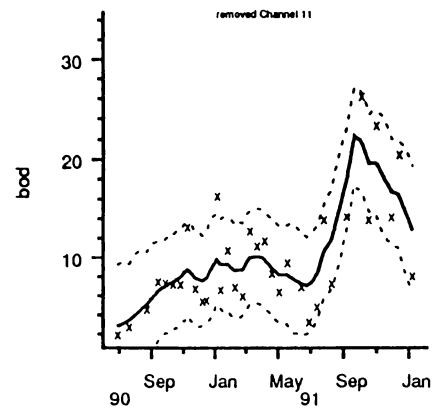
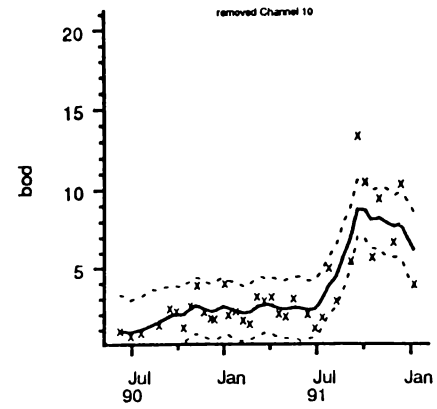
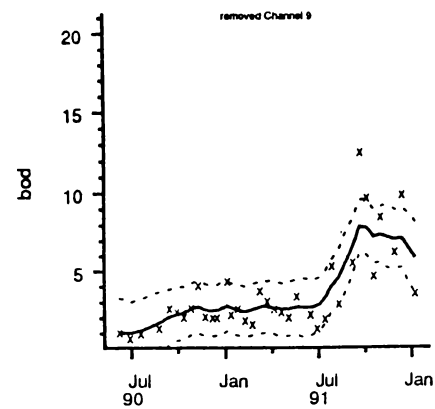
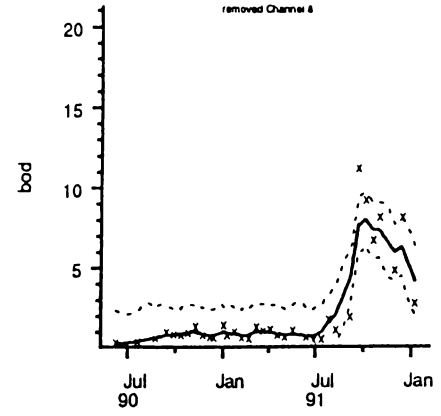
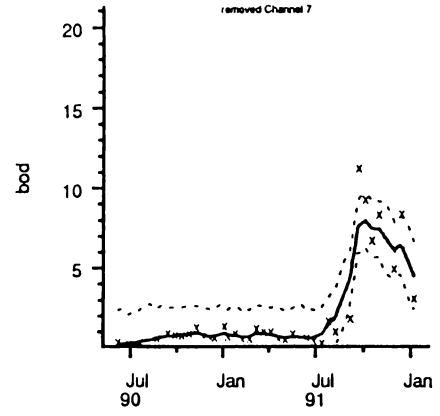
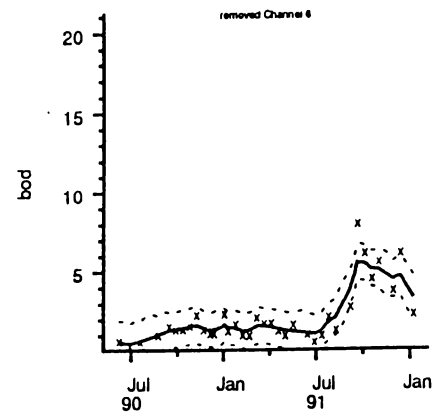
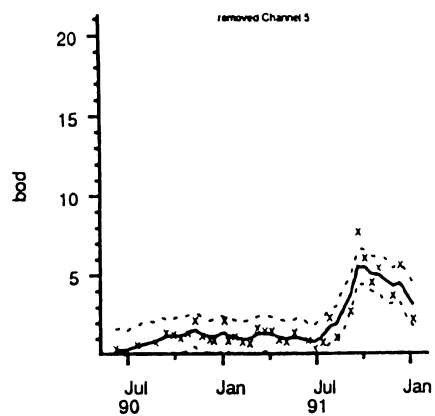
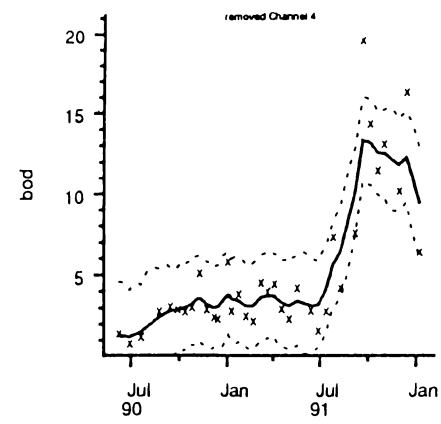
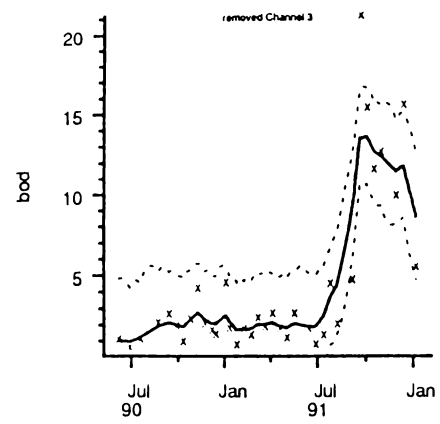
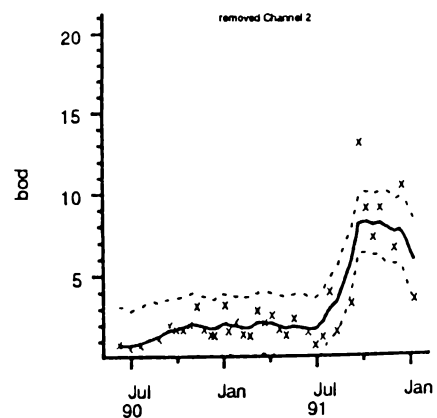
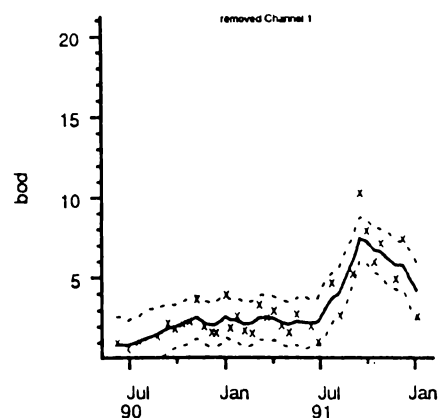
Appendix 4 - Mass removal rates ($\text{g m}^{-2} \text{d}^{-1}$) vs time for selected water quality parameters

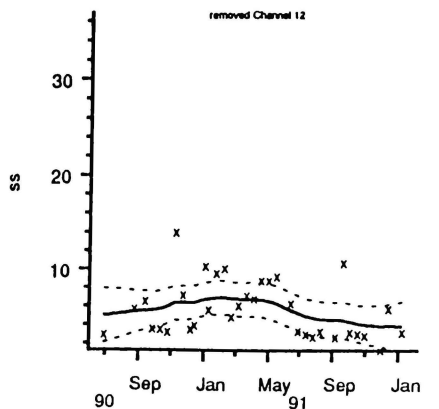
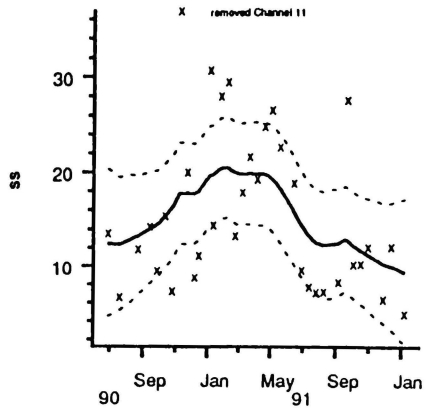
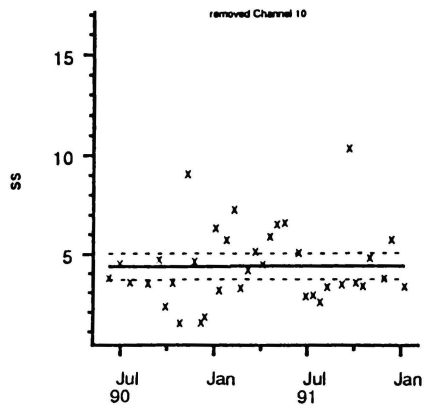
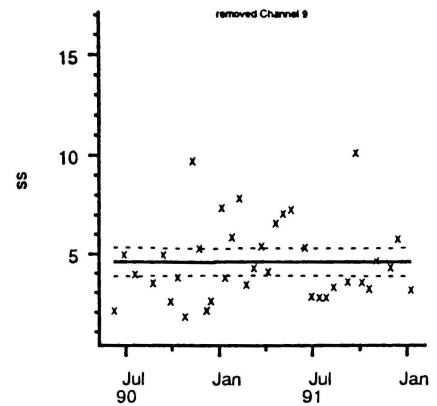
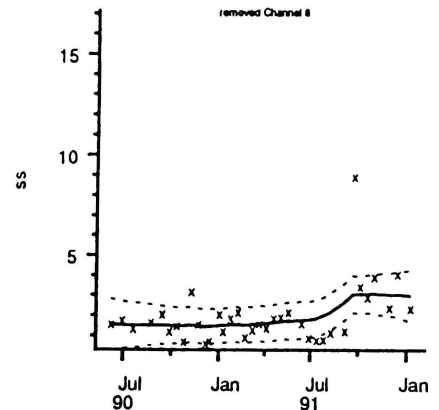
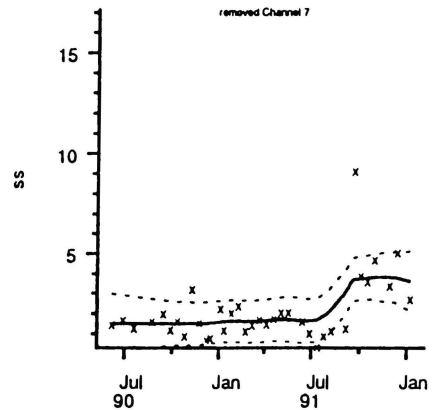
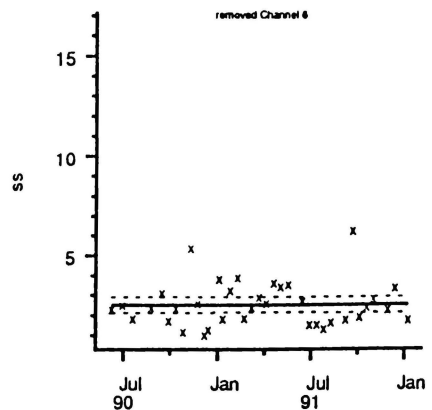
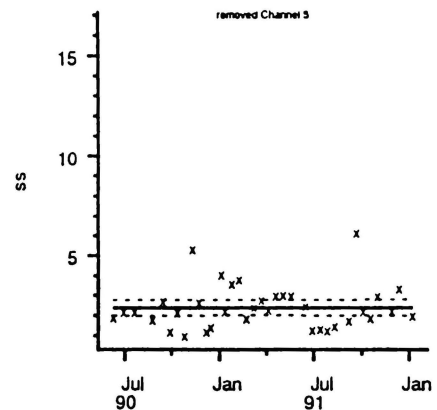
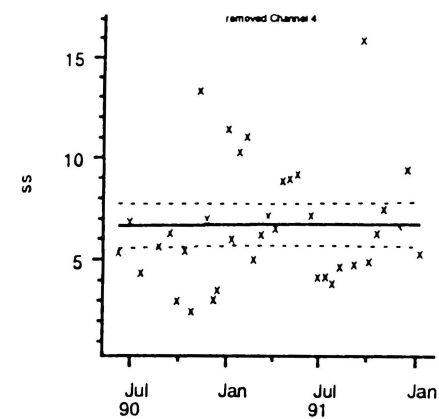
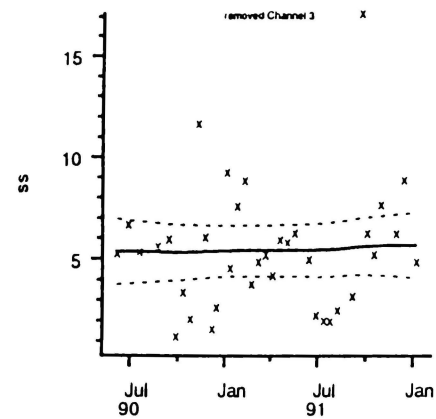
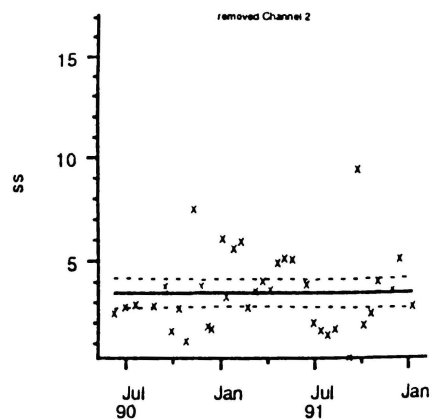
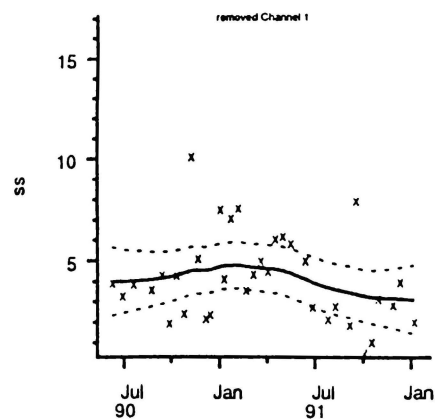
Each graph (1 - 10) refers to a different horizontal-flow wetland. The final two graphs (11 & 12) are for the up-flow wetlands. Each pair of wetlands received a different hydraulic loading of dairy shed wastewater. These graphs show changes in concentration of major parameters during the period of the study. Bayesian curves have been fitted to the data points (x). The solid line represents the mean, and the fine dashed line the 85% confidence intervals of the mean. Where the confidence intervals of two superimposed curves do not overlap, they are significantly different at the 95% level.

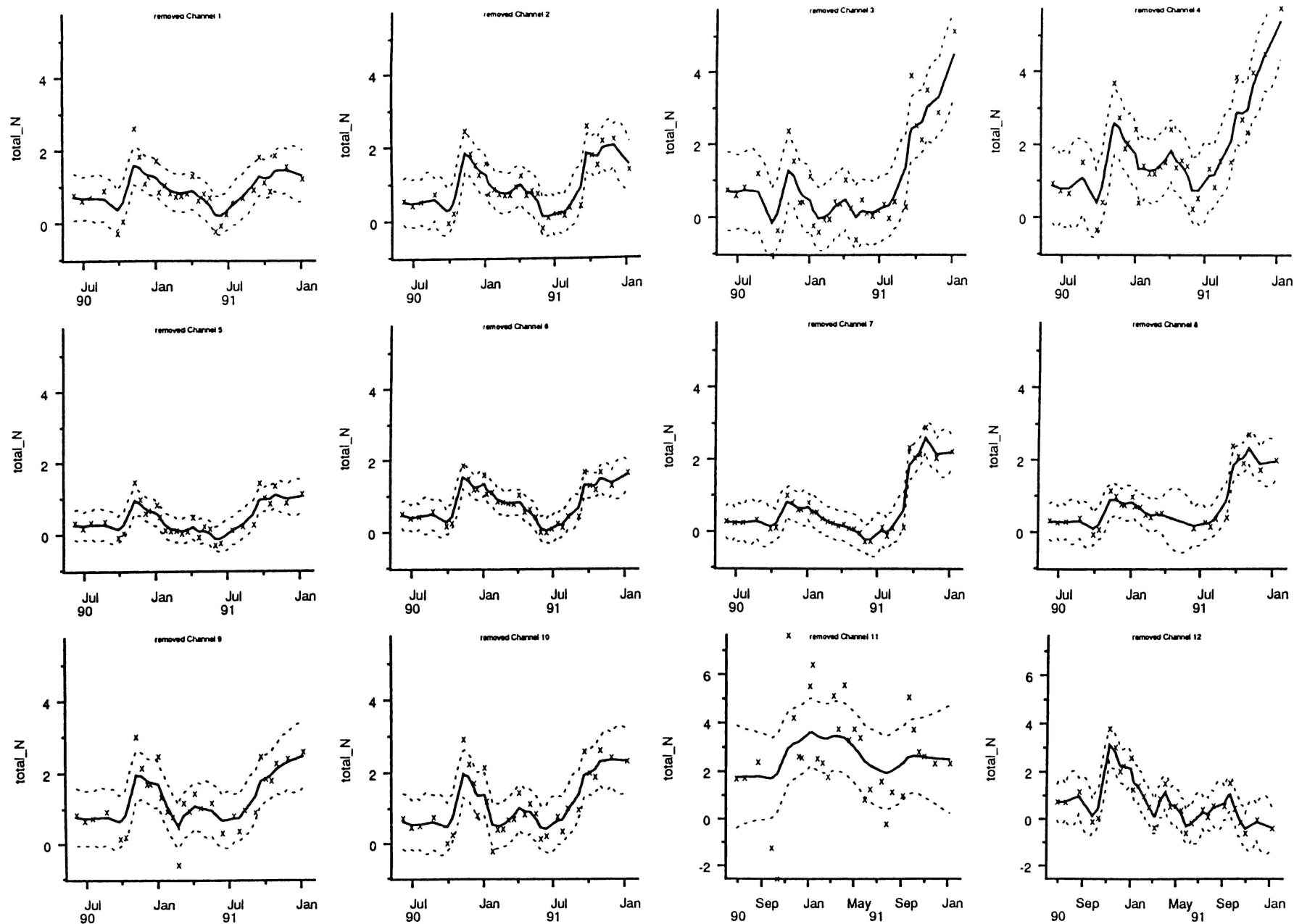
The treatments which correspond to each channel are:

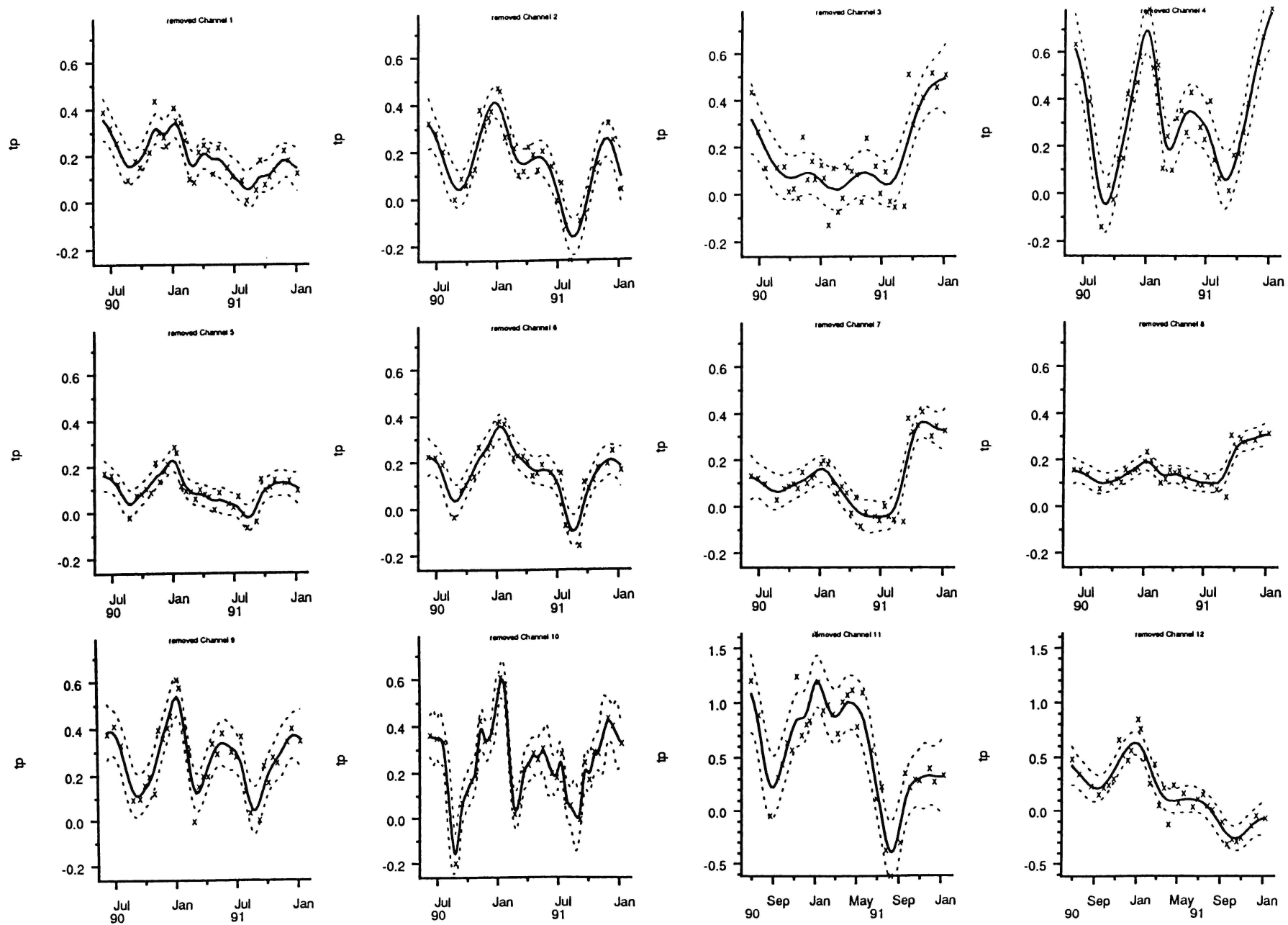
Channel	Type	Planted	Nominal Retention time (d)
1	Horizontal flow	-	3
2	Horizontal flow	<i>S. validus</i>	3
3	Horizontal flow	-	2
4	Horizontal flow	<i>S. validus</i>	2
5	Horizontal flow	-	5.5
6	Horizontal flow	<i>S. validus</i>	5.5
7	Horizontal flow	-	7
8	Horizontal flow	<i>S. validus</i>	7
9	Horizontal flow	Mixed species	3
10	Horizontal flow	<i>S. validus</i> harvested	3
11	Up-flow	<i>S. validus</i>	2.2
12	Up-flow	<i>S. validus</i>	6.5

Results from Channel 9 & 10 are not dealt with in this thesis.









Appendix 5 - Percentage removal rates vs time for selected water quality parameters

Each graph (1 - 10) refers to a different horizontal-flow wetland. The final two graphs (11 & 12) are for the up-flow wetlands. Each pair of wetlands received a different hydraulic loading of dairy shed wastewater. These graphs show changes in concentration of major parameters during the period of the study. Bayesian curves have been fitted to the data points (x). The solid line represents the mean, and the fine dashed line the 85% confidence intervals of the mean. Where the confidence intervals of two superimposed curves do not overlap, they are significantly different at the 95% level.

The treatments which correspond to each channel are:

Channel	Type	Planted	Nominal Retention time (d)
1	Horizontal flow	-	3
2	Horizontal flow	<i>S. validus</i>	3
3	Horizontal flow	-	2
4	Horizontal flow	<i>S. validus</i>	2
5	Horizontal flow	-	5.5
6	Horizontal flow	<i>S. validus</i>	5.5
7	Horizontal flow	-	7
8	Horizontal flow	<i>S. validus</i>	7
9	Horizontal flow	Mixed species	3
10	Horizontal flow	<i>S. validus</i> harvested	3
11	Up-flow	<i>S. validus</i>	2.2
12	Up-flow	<i>S. validus</i>	6.5

Results from Channel 9 & 10 are not dealt with in this thesis.

