PROXIMATE COMPOSITION OF PERIPHYTON COMMUNITIES GROWING IN EXPERIMENTAL STREAMS

by

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ABSTRACT

The proximate composition of periphyton communities grown under diverse conditions was analyzed to determine the variability in the potential nutritional value of the community to higher trophic levels. Ash-free protein, lipid and polysaccharide and ash weight were measured.

The analytical methodology employed involved two new modifications. Firstly, the Hach method of nitrogen analysis was modified for periphyton communities and used to estimate protein. The method consistently gave greater nitrogen recoveries than traditional Kjeldahl analyses when used on rather indigestible samples. It is recommended that the applicability of traditional Kjeldahl methods to periphyton analysis be carefully examined. Secondly, numerous methods of ash determination were examined and found to give highly variable results. To improve consistency it is recommended that ashing be carried out for 4h at 450^{o}C with 250mg samples.

Seasonal variation in periphyton proximate composition was noted in all components analysed. Ash-free protein and polysaccharide were highest in summer and autumn, as was production. Lipid was highest in winter and spring, when

the relative dominance of diatoms was at a maximum. Ash weight was also highest in winter as a result of the dominance of diatoms and increased levels of inorganic detritus in the water due to higher run-off.

The effects of current velocity on periphyton proximate composition were examined during autumn; protein was significantly lower in streams with a current velocity of $7cm.s^{-1}$ than at velocities of $14cm.s^{-1}$ and $28cm.s^{-1}$. Lipid levels were significantly higher at a current velocity of $28cm.s^{-1}$. It is postulated that the increased supply of nutrients that results from increases in current velocity stimulates production levels and these in turn lead to increased protein levels. No significant current velocity effect was noted on proximate composition values during winter. Periphyton communities tend to be light and temperature limited at this time of year so the increased supply of nutrients at higher currents is of little consequence.

Reduction in light levels imposed during summer resulted in increased biomass and decreased temporal heterogeneity in communities. Addition of nitrogen, phosphorus, or nitrogen and phosphorus also led to increases in biomass. Proximate composition was unchanged in streams with added nitrogen; streams with added

phosphorus showed a transient increase in polysaccharide levels. Streams with added nitrogen and phosphorus had higher protein levels. Ash-free protein levels were positively correlated ($r=0.9416$, $0.01>p>0.005$) with biomass accrual rates.

Chironomid grazer densities substantially altered periphyton proximate composition values at all times of the year apart from winter. However, they did not alter observed relationships between biomass accrual and ash-free protein, or the range of seasonal variation. It is concluded from this that the measured variability in periphyton proximate composition is due to changes within the primary producer component of the community.

It is suggested that the observed changes in proximate composition have a significant effect on the growth and survival of higher trophic level organisms.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

List of Illustrations

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INTRODUCTION

Definition

The term periphyton refers to the community of all organisms (plant, animal, fungal and bacterial) except the rooted macrophytes, living upon the surfaces of submerged objects in water (Cooke, 1956 modified). This community, of which algal species are the major primary producers, may include protozoans, rotifers, cladocerans and various insect larvae depending on circumstance. The terms, microhaptobenthos, biofilm, biotecton and aufwuchs are alternatives used to describe all or part of this community, but though awkward, the term "periphyton" has become the accepted expression in the English language literature (Weitzel, 1979a; Wetzel 1983a) and is adopted here.

Periphyton research

Research on periphyton communities has traditionally lagged behind that on plankton communities (Brown 1971), and is urgently needed both in lotic (Stockner and Shortreed, 1976), and lentic (Brown, 1971) systems. To facilitate this research, new methodologies need to be developed, and standard methods adopted (Wetzel, 1983b).

The factors affecting periphyton community development, productivity and complexity have been studied to some extent. In lotic systems the response of periphyton to changes in various environmental variables has been greatly clarified and the effects of current (e.g. McIntire, 1966; Antoine and Benson-Evans, 1982), light (e.g. Steinman and McIntire, 1986), inorganic nutrient concentration (e.g. Stockner and Shortreed, 1978; Peterson et al., 1983; Bothwell 1988), and seasonality (e.g. Aizaki, 1978) are relatively well documented. However, the trophic consequences of such natural and artificial perturbations have received very little attention (Wetzel, 1983b; Steinman et al., 1987b), and have therefore been examined in the present work.

Previous work on periphyton suggests a high nutritional value to lotic invertebrates (Anderson and Cummins, 1979; Fuller and Mackay, 1981). Periphyton-invertebrate interactions have been studied both with regard to their effect on the periphyton community (e.g. Kehde and Wilhm, 1972; Steinman et al., 1987a) and invertebrate production (Earnest, 1967; Fuller and Mackay, 1981). Whilst some attention has been paid to the food value of periphyton to lotic invertebrate grazers (e.g. Mason and Bryant, 1975) it has been noted that given these claims of a high

nutritional value surprisingly few detailed proximate analyses have been carried out (Steinman et al., 1987b). Proximate analyses, often misleadingly termed biochemical or chemical analyses, measure the proximate composition (protein, lipid carbohydrate and ash content) of a material. Although the analysis of the proximate composition of periphytic communities does not primarily address the nutritional availability of these compounds, it gives a useful measure of their potential food value. As food quality is recognized to be more important than total consumption rates to grazers (Claude and Goodyear, 1971), its assessment should be a priority in any study of the nutritional potential of periphyton communities. Most estimates of periphyton food quality have relied on measurements of the total energy of the community (e.g. Gray and Ward, 1979). This energy may be more or less available to grazers depending on the species composition of the community. However, the results of Hambrook and Sheath (1987) suggest that other factors such as protein levels and digestibility may play a more important role than total energy in determining food preferences. Thus, the analysis of proximate composition would be the most useful technique. They demonstrated, at least for periphytic Rhodophyta, that preferential grazing does occur

and that in several different invertebrate taxa preference was shown for the alga with the highest protein content, which was actually the alga with the lowest total energy content and simplest thallus structure. However, the measurement of total energy does allow later comparisons to be made with corresponding proximate composition values; for this reason some samples were also subjected to bomb calorimetry.

It has been shown that single species of microalgae undergo significant changes in proximate composition under different environmental conditions (e.g. Sakshaug and Holm-Hansen, 1977; Harrison et al., 1977), even on a daily basis (Hitchcock, 1980). Periphyton, which primarily consists of microalgal species, is presumably subject to further compositional changes as a result of varying species composition. Therefore, the potential for variation in proximate composition is high in periphyton communities and probably has a significant effect upon its value as a food resource.

In general, the ability of microalgal assemblages to act as a food resource has been the focus of more concerted effort in recent research, especially where domestic or "cultured" organisms may act as the consumer. It is well known that microalgae may exhibit very high productivity

and may also have a high nutritional value. In fact, the Cyanophyte Spirulina maxima contains 61-72% protein with an amino acid profile sufficiently rich in many essential amino acids (Wu and Pond, 1981) to act as a food material. To date, by far the bulk of research on the food value of microalgae has been directed towards planktonic species and assemblages. However, at the same time, it has been noted that the productivity of attached organisms (such as are present in periphyton communities) is generally an order of magnitude greater than the productivity of phytoplankton and tychoplankton (Grzenda and Brehmer, 1960; Wetzel, 1963: Swale, 1964; Rodgers and Harvey, 1976). This potential of attached assemblages to act as a food resource therefore warrants investigation. Periphyton, often the major primary producer in stream systems could reasonably be expected to have potential utility as an input to other trophic systems, including those related to aquaculture.

The present work was undertaken to assess the nature of the variability in the proximate composition of periphyton communities, and to determine the effect that such variability has on their potential as a nutritional resource. Specifically, variation in proximate composition under different current, light and inorganic nutrient conditions was addressed, as well as an examination of variation on a seasonal basis.

McMahon et al., (1974) examined periphyton nutritional value at six freshwater habitats in New York State by measuring variation in periphyton C:N ratios on glass slides. A highly significant correlation was found between C:N and the growth rates of the snails Laevapex and Lymnaea feeding on the periphyton. Lower C:N led to increased growth, regardless of biomass levels. One flaw in this approach to examining nutritional value occurs when high protein levels are found in conjunction with, for example, high carbohydrate levels (as are present during the summer in the Humpback Lake experimental streams). In this case a high C:N results when high levels of protein may be available. More recently, Steinman et al. (1987b) examined the effect of grazing by the snail Juga silicula (Gould) and the caddisfly Dicosmoecus gilvipes (Hagen) in laboratory streams and found little association between C:N and protein concentration in algal assemblages. For the present work, measurements of protein, lipid and carbohydrate on both a dry weight and an ash free dry weight (A.F.D.W.) basis were used, to obtain the maximum amount of information on the potential nutritional value of periphyton communities.

Whilst natural stream systems are obviously the best place in which to carry out such research, they are also impractical for several reasons. Firstly, it is very difficult in a stream or river to select multiple reaches which may act as replicates. Secondly, it is extremely difficult to control environmental parameters adequately under natural conditions and as a result, to attribute any differences between replicates to the experimental protocol. Thirdly, different reaches of a stream are likely to have slightly varying immigration patterns which would also mask experimentally induced changes.

The use of laboratory streams has simplified several areas of lotic research (McIntire et al., 1964). Hypotheses and mathematical models generated in laboratory streams have been successfully applied to natural environments (McIntire 1973, 1978; Rodriguez, 1987). However, the applicability of results generated in laboratory streams is seriously hampered as a result of the inability of these systems to mirror the fluctuating environmental conditions that characterize natural streams. These conditions are important in determining community responses (Bott, 1983) and may for example, cause the initiation of sloughing. This has led to the development of experimental streams (man-made streams built

on site and diverting water flow directly from a natural lake or stream in a flow-through system) which are an appropriate compromise between natural and laboratory streams in many situations (Bothwell, 1983). Experimental streams facilitate replication within experiments, as each is identical. In addition they allow control over many environmental variables, whilst providing a habitat subject to the usual temporal variations in precipitation, daylength, light intensity and temperature. It was therefore judged most suitable to carry out such work in experimental streams. Such streams have the further advantages of identical surfaces for colonization, adjustable light, flow rate and nutrient levels, and ease of collection of periphyton biomass with minimal loss, including the collection of adnate diatoms. Also, as a result of their physical proximity they share the same immigration patterns and fluctuations in environmental variables, which have been noted as important in determining community responses (Bott, 1983).

The role of periphyton in determining the productivity of freshwater habitats has received little attention until recently (Perrin et al., 1987). Parkyn and Austin (in prep.) have demonstrated that enhancement of periphyton biomass under natural stream conditions leads to an

increase in the holding capacity of streams for juvenile salmonids. They have further demonstrated direct benthic feeding by these juvenile salmonids on periphyton communities. Food items obtained included larval chironomids, simuliids, adult mites, nematodes, diatoms (e,g, Synedra ulna Kutz, Cymbella cymbiformis Agardh) and chlorophytes (e.g. Ulothrix sp., Spirogyra sp.). The authors suggest that such benthic productivity may be a major component of the diet of juvenile salmonids. Salmonids have been noted to feed heavily on aquatic invertebrates showing digestibility as high as 89.3% in the case of chironomid larvae (De La Noue and Choubert, 1985). As a result of the high numbers of invertebrate grazers within the periphyton during summer and autumn, the community would have a high nutritional value to salmonids. This explains the greater holding capacity of pools with artificially increased periphyton levels. Changes in the proximate composition of periphyton would lead to changes in sustainable grazing levels. This would result in changes in the carrying capacity of streams for salmonids. Data on the variability in periphyton proximate composition would therefore be of interest. They would help to explain what relationships may exist between the vastly increased periphyton levels and the increased

numbers of returning salmon as a result of the whole lake fertilization experiments carried out in British Columbia during the last decade (Stockner, 1981; Shortreed et al., 1984; Stockner and Shortreed, 1985). They would also be of help in predicting the effects of watershed logging on salmonid populations both on a short-term and a long term basis, as logging has been shown to lead to short-term increases in inorganic nutrient levels (and hence in periphyton production) in streams not protected by a 50m buffer zone of trees (Perrin et al., 1984; Gregory, 1980; Stockner and Shortreed, 1976).

MATERIALS AND METHODS

FIELD SITE

Humpback Lake

Humpback Lake is a small surge reservoir in the Greater Victoria Water District (G.V.W.D.), Vancouver Island, British Columbia. It is a major supply of pristine water to the Victoria, Oak Bay, Esquimalt, Saanich and western communities of southern Vancouver Island.

A field site was constructed below the Humpback Lake dam to take advantage of both the highly oligotrophic water and the protection afforded the site within the G.V.W.D. (Illustration I).

Experimental stream system

An experimental stream system was constructed to supply water to 12 experimental streams and 2 large tanks (Illustration I). In addition the system supplied water to a sink and a garden hose which were used for cleaning apparatus before experiments. P.V.C. (poly-vinyl chloride) was used throughout the system as it is light, readily available, easily handled, non-toxic and virtually maintenance free. The major components of the system were, siphon, header (distribution) box and experimental streams.

Siphon

A siphon system modified from Lucey et al., (1986) was used to draw water from a depth of 1m over the dam and down to the header box. No stratification of the lake occurred, as, consequent to its use as the city water supply, it has a very high flushing rate . Three separate siphons were used so that the water supply would be unaffected if any one siphon became blocked.

Header tank

A header tank (capacity - 864L) was designed to receive water from the siphons and distribute it in a regulated manner via a series of gate valves. A constant head was maintained so that current velocity in the experimental streams also remained constant.

Experimental streams

Experimental streams were designed and constructed from 1/8" P.V.C. This afforded adequate strength whilst light weight made for easy maneuverability in the field. The streams consisted of 2.4m long troughs, rhomboidal in cross section with a bottom width of 18.7cm and a top width of 26.3cm. The height was 10.3cm (Illustration II). The troughs were placed on a slight gradient (1.2%) to

facilitate flow, which led to the water depth varying from 7.2cm to 10.0cm from the front to the back of the trough. Variable insertion of substrata was afforded by the use of removable substrata holders held in place by silicone cement. The water distribution system split the experimental streams into 6 pairs. Each pair of streams received its water from a common gate valve. Therefore, the two streams in each pair had identical current velocities. Velocity could be adjusted between 0 and 30cm/s.

Substrata

Precleaned microscope slides (75 x 50mm) were placed in holders in the experimental streams, for the purpose of biomass (dry weight and ash free dry weight), and species composition measurements. The slides were placed parallel to the direction of flow, standing on their longer sides (Illustration II). Samples for the analysis of proximate composition were gathered from the walls of the streams.

Current velocity in experimental streams

Current was adjusted at the Humpback Lake experimental stream system by means of gate valves which regulated flow from the header box to each pair of experimental streams.

The quantitative measurement of current velocities within streams can be very complex. This is primarily a result of turbulence, which has significant effects on current velocity at the substratum level (Round, 1965; Jones, 1978). The majority of current measurements are therefore either qualitative or semi-quantitative.

In the Humpback system, estimates of surface velocity were made following dye tests in the streams. As surface velocities often do not accurately represent total velocity in streams, further velocity estimates were made by measuring the volume of water passing through each pair of experimental streams per unit time. Differences between the velocity estimates were generated primarily by the presence of low current along the sides and back of the experimental streams. When current velocity was subsequently calculated, both measurements were taken into account. Estimates of current velocity should therefore be regarded as semi-quantitative at best.

System check of experimental streams

Experimental streams were run for a period of 1 month (June 21-July 21, 1987) before the first experiment. Glass slides placed in the streams during this period were examined to determine whether any significant differences

in species composition existed between the experimental streams. This involved the examination of randomly selected streams to check for within-stream differences (such as between the front and back ends) and between-stream differences. Samples of periphyton were also removed from the sides of the experimental streams to examine any differences in species composition between the periphyton growing on the glass slides and that on the P.V.C. Results indicated that differences exist between periphyton communities growing on glass slides and on P.V.C. (Austin unpub.). As a result, species composition estimates were always made from samples growing on P.V.C.

Sample collection

Species composition samples were scraped from the sidewalls and bottom of the streams and placed in jars prefilled with trough water containing 5% formalin for later examination. Water-flow to the streams was then turned off and proximate composition samples were collected by manually stripping the walls using paint scrapers.

Water chemistry

Water chemistry analysis was carried out by the Environmental Laboratory, British Columbia Ministry of

Environment. The analytical techniques are summarized in Table I., and follow the methods described in Clark and Shera (1985). Samples were collected in 500mL P.V.C. sample bottles and either sent on ice for immediate analysis or frozen, if appropriate, until the time of analysis.

Selected water chemistry values were monitored during experiments. A summary of the mean and range of these values during the winter months is given in Table II.

ANALYSIS OF PROXIMATE COMPOSITION

Proximate composition consists of the levels of the major cellular components:- protein, lipid, carbohydrate and ash. In the case of periphyton the ash may also contain a significant amount of extracellular debris. A flow chart of the methods of analysis is shown in figure I. A detailed protocol of the analytical methods is presented in Appendix I. Justification for the methodology employed is presented in Appendix II.

Preparation of samples

Potentially large sub-sampling errors may be made when sampling periphyton, because of the clumped nature of the community (Moore, 1952). This is a result of bacterial mucilage, intertwining filaments and basal attachment

structures. Further inaccuracies result from the deposition of inorganic material and organic detritus amongst this "tangle". Methods are therefore necessary which reduce as far as possible this heterogeneity, and allow careful examination of differences between the proximate composition of periphyton communities grown in different conditions.

The following method of sample preparation was used to aid such examination.

a. Periphyton collected in the field was dried in a forced air oven at 50^{o}C until it reached a constant weight.

b. The periphyton was subsequently ground using a coffee grinder (Braun KSM2). This produced a light dry powder, which was sealed in plastic bags and stored at room temperature until analysis. No changes in the weight of the bags or in the proximate composition of the periphyton occurred even after 6 weeks storage.

Initial Step

Dry powdered samples of periphyton were homogenized in a Waring Blender following a modified Bligh & Dyer (1959) method for lipid analysis (Dosanjh, Pers. Comm.) employing a chloroform:methanol:distilled water extraction medium with a ratio of 4:4:3. The homogenization is designed to break up clumps of cells and make the maximum surface area

available for the action of chloroform and methanol. Blending has been shown to significantly reduce sub-sampling error in the quantitative analysis of river periphyton (Biggs, 1987). The samples were then filtered using GF/A glassfiber filters and the filtrate transferred to separating funnels. Paper filters lead to a reduced lipid value with at least some sample types (Limsuwan and Lovell, 1984). It has been suggested that this is due to adsorption of lipid to the paper (Bligh and Dyer, 1959). The residue was carefully removed from the filter paper, dried and weighed. The filter papers were then dried and reweighed, to quantify the amount of residue still remaining on the paper. The loss of this material could then be corrected for in the results.

Lipid analysis

Method

A biphasic system was created in the separating funnel by adding approximately 5mL of distilled water. After 2 hours separation, the (lower) chloroform layer was removed and an aliquot was evaporated to dryness. Several aliquots were taken from initial samples and no differences were found between the amounts of lipid recovered. Crude lipid was estimated from the weight of the dry residue

(Bligh and Dyer, 1959). Spike samples containing 5mg of tripalmitin were used to check recovery (Whyte et al., 1987), mean recovery of tripalmitin was 97.6% with a standard deviation of 7.6% (n=8).

Non-lipid impurities were measured after dissolving the lipid residue in chloroform, to remove it, then weighing any remaining residue (Bligh and Dyer, 1959).

Use of this technique to analyze Whitecrest SDII fish feed showed close correlation between predicted and realized lipid recoveries.

Carbohydrate analysis

Method

Polysaccharide was estimated after a 6h sample digestion in 2N sulphuric acid at 100^{o}C (Whyte, pers.comm.), by a phenol-sulphuric acid assay employing 5% phenol and 18M sulphuric acid to produce the colour reaction. Samples were read against glucose standards following the method of Kochert, (1978). Rice starch (B.D.H.) samples were employed routinely to check the digestion step. Digestion was found to be optimum between 6 and 7 hours. Shorter (3 hour) and longer (16 hour) digestion times led to a 10% decrease in recovery of rice starch.

Monosaccharide was estimated by removing a 5mL aliquot from the methanol layer during lipid extraction (Whyte et al., 1987), from which sub-samples could be taken and analyzed for monosaccharide using a phenol-sulphuric acid assay with glucose standards.

Protein analysis.

Apparatus

Digestion was carried out on 2 Hach Digesdahl units. Each consisted of 100mL volumetric flasks, a thermostatically controlled 25-250W disk element heater, a glass digestion manifold in the form of a Vigreux fractionating column, and a capillary tube/funnel for controlling reagent addition at $3mL.min^{-1}$ (Hach et al., 1987).

Colorimetric assay was carried out using a Pye Unicam U.V./Vis spectrophotometer.

Method

Non-filtrable residue from the initial solvent extraction was scraped off the filter and dried. Subsequently a 0.250g aliquot was placed in a flask for digestion. Solvent extraction was designed to minimize inaccuracies in protein estimation caused by low molecular weight nitrogen compounds. These were removed during filtration and

subsequently lie within the low molecular weight fraction in the methanol layer (Whyte, 1987) after creation of a biphasic system during lipid analysis.

Aliquots of digest were treated by the method described by the Hach "Food and Feed Analysis Instruction Manual" using Hach chemicals and automatic pipettes. This involved a simple Nessler estimation for the determination of Kjeldahl nitrogen. Results were read against an ammonium p-toluenesulphonate calibration graph. Protein was then calculated as N x 6.25. The conversion factor is based on the assumption that all Kjeldahl nitrogen is protein nitrogen and that protein contains 16% nitrogen; this estimation is used in virtually all proximate analysis reports (Hulse et al.,1977).

System Check: Efficiency of Sample Digestion

To check that the digestion apparatus was functioning correctly, samples were run with nicotinic acid, which is an extremely difficult to digest refractory compound. Although more resistant compounds exist, this is the most digestion-resistant compound normally encountered (Hach et al., 1987). Sample digestions using 30% solutions of hydrogen peroxide were compared with digestions using 50% hydrogen peroxide.

Samples consisting of different amounts of nicotinic acid, up to a maximum of 0.250g, were digested under conditions stated to attain full digestion (Hach et al., 1987) using both 30% peroxide and 50% peroxide. Whilst complete recovery was attained using 50% peroxide the best recoveries achieved with 30% peroxide were approximately 90% digestion of nicotinic acid (Table III). With shorter digestion times recovery dropped as low as 50% whilst complete recovery was still obtained with the 50% peroxide.

Results gained using the two different concentrations of peroxide, strongly suggest that only 50% peroxide should be used for determinations in contrast to the statement of Hach et al., (1987).

System check: Comparison between Hach and traditional Kjeldahl Methods

Comparisons were run between the Hach method and the traditional Kjeldahl method to assess differences between them in the estimation of protein from periphyton communities. Initially, replicate samples of freshwater periphyton from the Humpback stream system were sent to the Hach Co. laboratory in Ames, Iowa and the West Vancouver Marine Laboratory for analysis. The Hach method gave a somewhat higher value of 7.5% protein on a dry weight basis (21% A.F.D.W.) than the traditional Kjeldahl method which

measured 5.3% protein (15% A.F.D.W.). The results suggested that further tests were necessary to discover the source of the difference. The possibility that a combination of sample heterogeneity and inter-laboratory variation played a role in the difference could not be discounted. Further tests were therefore run, both with an easily digestible material (fishmeal) and Humpback periphyton which is likely to be rather hard to digest. Results from these tests indicated that little difference exists between the two methods when an easily digestible material is analyzed, but when a more refractive material such as periphyton is analyzed the Hach method gives substantially higher nitrogen recoveries (Table IV). Presumably the difference in nitrogen recovery between the two methods varies depending on the exact species composition of the periphyton. When the community is dominated by relatively easily digestible species, such as the filamentous chlorophytes which were present in the first comparison, the Kjeldahl method gives a value only 30% lower than the Hach method. When relatively indigestible species are present, such as the diatomaceous community analyzed in the second comparison, the difference increases to 45%.

Determination of ash weight

Method

Samples (0.250g) were held in porcelain crucibles (Coors, U.S.A., Type 769-3), and ashed at 450^{o}C for 4 hours in a non forced-air, muffle furnace (Model M-15A-1A; Blue M Elect. Co., Blue Island, Illinois, U.S.A.).

System check: choosing a suitable method

A small sample size of 0.250g was determined as optimal, allowing a suitable number of replicates for ashing. Temperature had to be high enough to remove all organics whilst low enough to remove as few inorganics as possible. Temperatures of 550 ^{o}C may lead to removal of carbonaceous material and cause dehydroxylation of the clay mineral fraction (Appendix III.). A temperature of 450^{o}C was therefore used to avoid these potential problems. Ashing time was set at 4 hours, which is long enough to obtain removal of organics from 250mg samples whilst avoiding the variability caused by temperature gradients in the furnace during longer ashing procedures.

Estimations of ash weight when insufficient sample is available for traditional ashing techniques

During the analysis of small samples insufficient material may be available for the determination of ash weight by traditional means. To address this problem other methods of determining ash weight were investigated.

After acid/peroxide digestion of samples for protein estimation, a proportion of the sample is still undigested and is removed during filtration of the digest. This residue was dried and weighed to examine how accurate an estimate of ash weight it would render. As lipid had already been removed from the sample before digestion, this was taken into account when ash was calculated. Results indicated that this method generally gave a higher estimate of ash weight than traditional ashing techniques. The standard deviation was also higher. However, in 75% of the cases the mean for normal ashing techniques lay within one standard deviation of the mean for this technique (Table V.). A paired sample t-test determined the means to be significantly different ($P<0.0005$). Although this method of estimating ash is obviously inferior to traditional techniques it may be useful in giving a rough estimation of ash weight when insufficient amounts of sample are available to use other methods.

Presentation of proximate composition data

Proximate composition data are expressed on an A.F.D.W. basis for the purposes of this work, due to the large ash content of the samples. Changes in the levels of protein, lipid and carbohydrate within periphyton communities are often masked by changes in ash weight. Ash weight changes result from increased length of experiments (and hence increased deposition of silt etc.) and the different 3-dimensional forms which communities may develop, which may entrap varying amounts of inorganic particles. This is suitably exemplified by the changes in periphyton proximate composition which were measured on an A.F.D.W. and a dry weight (D.W.) basis, over a period of 3 weeks, during the summer of 1988. Due to the increase in ash levels with time, results based on D.W. showed a large decrease in protein with time, as well as a decrease in carbohydrate and a small increase in lipid. When the results are expressed on an A.F.D.W. basis, they show that there was only a very small decrease in protein, and carbohydrate actually increased; a large increase in lipid was also noted (Figure III.).

Results based on dry weight are useful where the nutritional content of the whole community is of interest, such as when periphyton is incorporated into feed.

However, where selectivity in feeding occurs and when changes in the proximate composition of the species present are being examined, it is important to express results on an A.F.D.W. basis.

BOMB CALORIMETRY TEST

The gross energy of periphyton communities has been relatively frequently calculated as a measure of their potential to act as a source of nutrition (e.g. Gray and Ward, 1979; Hambrook and Sheath, 1987). The energy can be calculated either by bomb calorimetry or by using conversion factors based on the average energy values of the major cellular components, protein, lipid and carbohydrate (Paine, 1971). Richman (1958) found excellent correlation between the use of correction factors and bomb calorimetry results for the green alga Chlamydomonas reinhardi. Similar good correlations were found in comparisons between the chemical data of Carefoot (1967) and the bomb calorimetry data of Paine and Vadas (1969) for 7 marine benthic algae (Paine, 1971).

Bomb calorimetry has also been used to estimate the energy content of periphyton in milkfish ponds (Fong and Ju, 1987), and to demonstrate the effects of eutrophication on periphyton energy content (Eloranta and Kunnas, 1976).

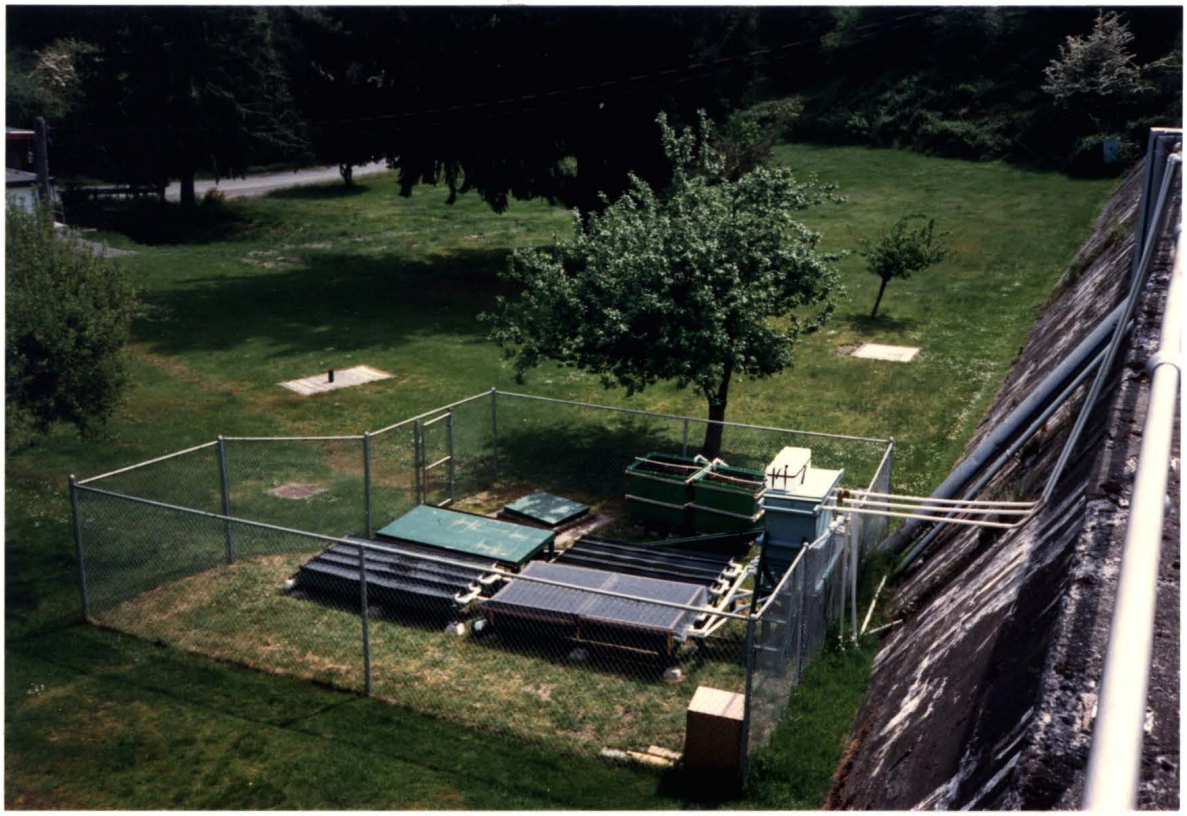
Apparatus.

A plain jacket oxygen bomb calorimeter (Parr Instrument Co.) was used for all calorimetry reported here.

Method

Samples of periphyton were dried, ground and passed through a 40 mesh screen. A Parr pellet press was then used to make approximately 1g pellets of samples (weighed to 3 decimal places), which were then placed in the calorimeter in contact with a weighed piece of fusewire for ignition. The calorimeter was then pressurized with oxygen to approximately 25 atmospheres and ignited. Temperature readings were taken every 30 seconds for 3 minutes prior to ignition and until temperature had risen to a plateau and begun to decline after ignition. Samples of benzoic acid were used both as standards and to calibrate the calorimeter.

Illustration I Humpback Lake experimental site, showing: a). siphon fed water supply from Humpback dam. b). header box. c). experimental streams. d) drainage system.



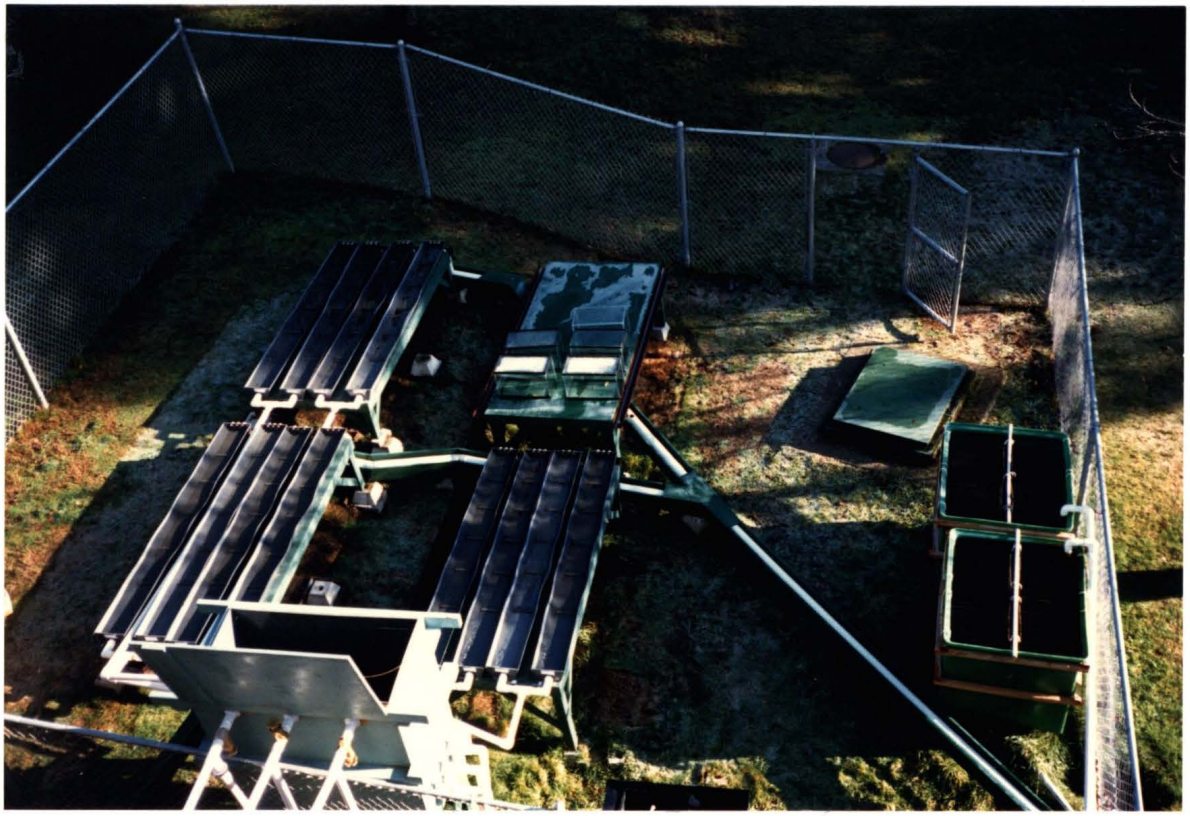


Illustration II Close-up of 2 experimental streams to show orientation of slide-holders.

Table I. Methods used to analyze water quality, from McQuaker (1976) and Clark and Morrison (1985). Abbreviations of analytical techniques follow Table, as do units.

PARAMETER	ANALYTICAL TECHNIQUE	RANGE	UNIT
Alkalinity: Total: pH 4.5	Potentiometric	0.5-500.0	1
Nitrogen Amm.Diss	Froz.Aut.Berth.[a]	0.005-0.500	2
Nitrogen Amm.Diss	Direct Nessler.	0.0-2.0	2
Nit: Nitrate & Nitrite	Froz.Aut.Berth.[a]	0.02-2.00	2
Nitrogen: Organic [N:O]	Calcul. Result[b]	0.01-10.0	2
Nitrogen: Tot. Kjeldahl	Frz.Dig.Aut.Col[c]	0.01-10.00	2
Nitrogen: Total [N:T]	Calcul. Result[d]	0.02-10.0	2
pH	electrometric[e]	0.0-14.0	3
Phos. Diss. o-phosphate	Froz.Aut.Ascorb[f]	0.003-0.500	4
Phosphorus: Tot. Diss.	Frz.Dig.Aut.Asc[g]	0.003-0.500	4
Phosphorus: Total	Frz.Dig.Aut.Asc[g]	0.003-0.500	4
Silica: Reactive	Auto.Moly-Blue[h]	0.5-30.0	5
Specific Conductance	Cond. meter	1-60,000	6

[a] Froz.Aut.Berth. = Automated* Berthelot, frozen method.
[b] [N:O] Calculated Result = difference of Kjeldahl Nitrogen minus Ammonia Nitrogen (TKN - AMM.N = Organic N).
[c] Froz.Dig.Aut.Color = Digestion Automated* Indophenol.
[d] [N:T] Calculated Result = sum of Kjeldahl Nitrogen and Nitrite and Nitrate Nitrogen concentrations (TKN + (NO_2 + NO_3) N = Total N).
[e] Electrometric = pH meter, glass electrode reference electrode OR automated microprocessor-controlled titrator.
[f] Froz.Aut.Ascorbic = Automated* ascorbic acid reduction.
[g] Froz.Dig.Aut.Asco = Persulfate digestion, automated* ascorbic acid reduction.
Froz.Aut.Ascorbic
[h] Auto.Moly-Blue Col. = Automated* ascorbic acid reduction, colorometric.

* Automated - Technicon Automated II.

1 mg/L $CaCO_3$
2 mg/L N
3 Relative Units (negative log of hydrogen ion concentration)
4 mg/L P
5 mg/L SiO_2
6 µs/cm

Table II. Selected water chemistry values for the Humpback Lake experimental stream site during the winter months of 1987-1988.

		December	January	February
Alkalinity mg/L $CaCO_3$	range	--------	-------	13.4
	mean	--------	-------	13.4
	n	--------	-------	2
pH	range	--------	-------	6.6 - 6.8
	mean	--------	-------	6.7
	n	--------	-------	2
Sp. Cond. µs/cm	range	--------	-------	54 - 57
	mean	--------	-------	56
	n	--------	-------	2
Si: react. mg/L SiO_2	range	1.5-2.0	1.8-3.3	2.3-5.8
	mean	1.7	2.6	4.4
	n	3	2	3
N: Org.-Tot. mg/L N	range	0.06-0.11	0.04-0.09	0.03-0.10
	mean	0.09	0.07	0.07
	n	3	2	3
N: Kjel. Tot. mg/L N	range	0.08-0.13	0.05-0.10	0.04-0.10
	mean	0.11	0.08	0.08
	n	3	2	3
N: Tot. mg/L N	range	0.24-0.29	0.20-0.22	0.14-0.24
	mean	0.27	0.21	0.20
	n	3	2	3
N: Amm. mg/L N	range	0.010-0.019	0.010-0.013	<0.005-0.012
	mean	0.015	0.012	-------
	n	3	2	3
N: NO_3+NO_2 mg/L N	range	0.11-0.21	0.12-0.15	0.10-0.14
	mean	0.16	0.14	0.12
	n	3	2	3
P: Ort. dis.P mg/L P	range	<0.003	<0.003	<0.003
	mean	-------	-------	-------
	n	3	2	3
P: Tot. mg/L P	range	0.005-0.008	0.004-0.005	0.003-0.005
	mean	0.006	0.005	0.004
	n	3	2	3
P. Tot dis. mg/L P	range	0.003-0.004	0.003	<0.003-0.003
	mean	0.004	-------	-------
	n	3	2	3

Figure I. Protocol for proximate analysis.

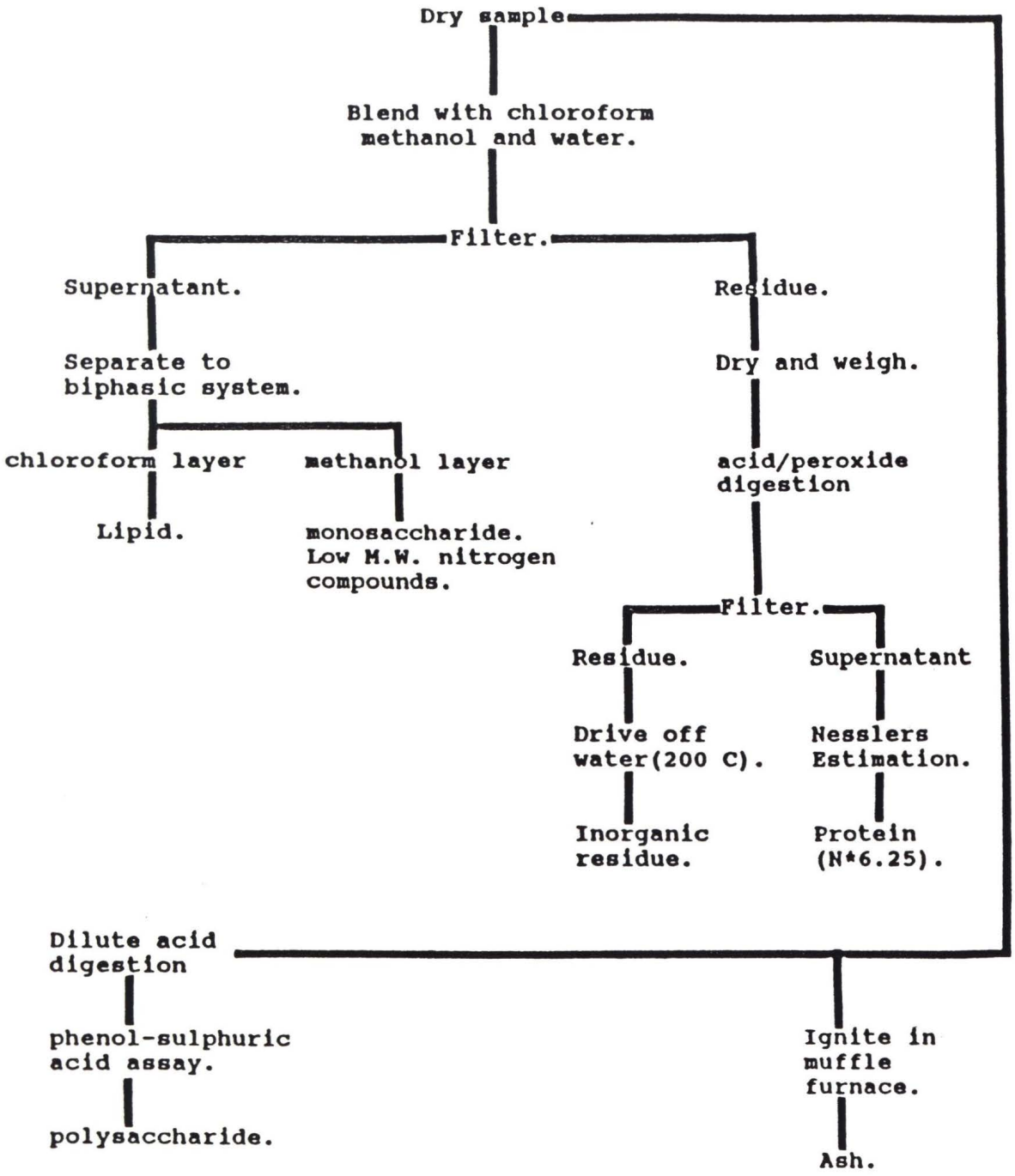

Dry sample
Blend with chloroform methanol and water.
Filter.
Supernatant.
Residue.
Separate to biphasic system.
Dry and weigh.
chloroform layer
methanol layer
acid/peroxide digestion
Lipid.
monosaccharide. Low M.W. nitrogen compounds.
Filter.
Residue.
Supernatant
Drive off water(200 C).
Nesslers Estimation.
Inorganic residue.
Protein (N*6.25).
Dilute acid digestion
phenol-sulphuric acid assay.
Ignite in muffle furnace.
polysaccharide.
Ash.

Table III. The effect of peroxide concentration on recovery of nitrogen from nicotinic acid, using the Hach rapid peroxymonosulphuric acid method of sample digestion.

sample	Hydrogen peroxide	nitrogen theoretical (mg)	nitrogen recovered (mg)	% recovery
1a	50%	11.9	12.0	100.8%[1]
1b	30%	11.9	10.7	89.9%
2a	50%	9.9	9.9	100.0%
2b	30%	9.9	8.8	88.9%
3a	50%	9.7	9.8	101.0%
3b	30%	9.7	8.6	88.5%

[1]n=3 sub-samples for each determination.

Table IV. Comparison between Hach and Kjeldahl methods of protein analysis. Figures are mean percent protein for the fishmeal and periphyton (1) analyses (n=3 for each figure). Figures for periphyton (2) are for each replicate.

	KJELDAHL	HACH
FISHMEAL PROTEIN	74.87	73.06
		73.62
		72.84
PERIPHYTON (1)	5.3	7.50
		7.60
PERIPHYTON (2)	3.8	6.10
	2.7	5.76
		6.10
		5.66
		5.78
		6.12
		(5.92 ± 0.21)[1]

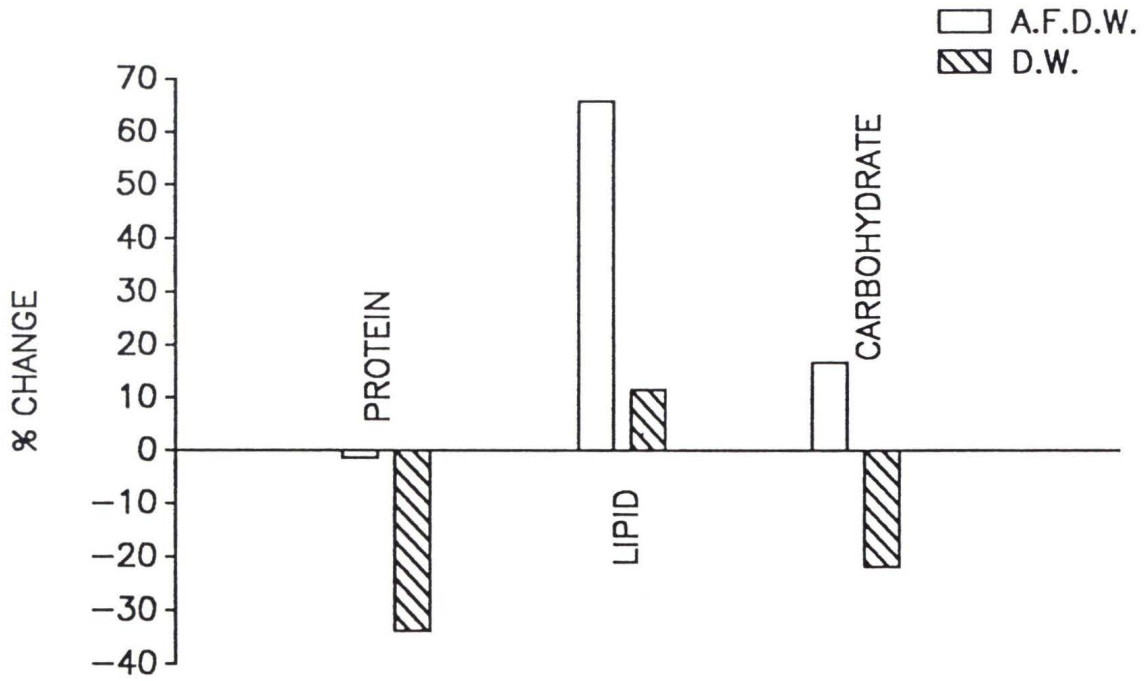
[1]mean ± 95% confidence interval

Table V. A comparison of traditional ashing methods (method 1) with estimations of ash weight based on the proportion of undigested material remaining after acid/peroxide digestion (method 2).

STREAM	METHOD 1[1]	METHOD 2[2]
1	61.3 ± 0.5[3]	62.5 ± 7.3
2	62.8 ± 0.5	64.2 ± 5.0
3	60.7 ± 0.4	68.3 ± 9.8
4	59.8 ± 0.5	62.2 ± 5.1
5	63.8 ± 1.0	68.5 ± 11.3
6	63.9 ± 0.8	65.4 ± 7.6
7	57.8 ± 0.6	50.6 ± 2.5[4]
8	61.4 ± 0.6	75.3 ± 4.5[4]
9	60.6 ± 14	70.5 ± 11.6
10	60.2 ± 1.3	60.4 ± 8.5
11	60.7 ± 1.1	69.4 ± 6.4[4]
12	59.6 ± 0.5	65.6 ± 1.6[4]

[1]n = 9
[2]n = 3
[3]Standard deviation.
[4]Mean of method 1 does not fall within one standard deviation of method 2.

Figure II. Percent changes in proximate composition of periphyton over a 3 week period in June 1988, as a function of the method of measurement, with results based on an A.F.D.W. and a D.W. basis. For lipid and carbohydrate determinations n=3. For protein determinations n=6.


A.F.D.W.
D.W.
% CHANGE
70
60
50
40
30
20
10
0
−10
−20
−30
−40
PROTEIN
LIPID
CARBOHYDRATE

THE EFFECTS OF CURRENT ON PERIPHYTON COMMUNITY PROXIMATE COMPOSITION

Introduction

The effects of current on periphyton community structure have been classified into three general areas (Jan Stevenson, 1984). Firstly increase in nutrient supply with current has been noted (Whitford, 1960). Swift currents sweep away material near the cell surface and produce a steep diffusion gradient, thereby increasing exchange of materials between organism and environment. Whitford (1960) also offered evidence that a minimum current of 15 $cm.s^{-1}$ was required to produce this effect. Secondly increases in current lead to increased shear force, the effects of which are primarily seen in colonization processes, where current is an important factor in determining both the species capable of colonization (Steinman & McIntire, 1986; Jan Stevenson, 1983) and biomass accumulation (McIntire, 1966; Reisen & Spenser, 1970). Thirdly, variations in grazing levels as a result of current may be significant. These have yet to receive detailed study but would presumably exhibit a high degree of temporal and spatial variability within natural streams (Jan Stevenson, 1984).

The aim of the present experiment was to investigate the effect of current on periphyton proximate composition and suggest the likely factors mediating any changes. As the effects of current are manifest through several processes, it is possible that at different times of the year the processes may become more or less important in determining periphyton species composition and proximate composition.

The experiment was carried out once during autumn, when nutrients are traditionally limiting and when the increased supply of nutrients at higher currents, demonstrated by Whitford (1960) would be most beneficial. It was then repeated during winter when communities are light and temperature limited and the effects of shear forces would be expected to be most important.

Method

Four experimental streams were run at each of three velocities, 7cm.s^{-1}, 14cm.s^{-1}, and 28cm.s^{-1} from the 19th August 1987 to the 21st October 1987 (63 days), and from the 8th December 1987 to the 10th February 1988 (64 days). Pairs of streams were randomly assigned to each velocity. At the end of each experiment, biomass was removed for analysis of proximate composition. Data for

changes in the latter were analysed statistically with the SAS computer package (SAS Inst., 1985) using a randomized complete block design (Ostle, 1963). Species composition was determined by light microscopy, employing Utermohl chambers to render qualitative counts of organisms growing in the streams (Austin unpub.). Authorships for species identified can be found in Appendix IV. The density of grazers was estimated at the end of each experiment by sub-sampling the periphyton and counting the number of grazers. Samples were then dried so that an estimate could be obtained of the numbers of grazers per gram dry weight of periphyton.

During the autumn experiment a degree of intra-velocity variation was evident. To further investigate this variation, ash-free protein was collected from 3 separate sections of each stream (the upstream, midstream and downstream), during the winter experiment (8/12/87 - 10/2/88). Comparisons were then made between streams at the same velocity and also between different sections of each stream. The results were then analyzed statistically using a single factor analysis of variance.

Results

Autumn experiment

(19/8/87 - 21/10/87)

Proximate composition

Mean ash-free protein levels varied between 34.36% at a current velocity of 28cm.s^{-1}, and 25.76% at 7cm.s^{-1} (Figure III.). Streams at 28cm.s^{-1} and 14cm.s^{-1} were similar, with significantly higher protein levels than streams at 7cm.s^{-1} (Table VI., Table VII.).

Ash-free lipid values were highest in streams at 28cm.s^{-1} (Figure III.) and were significantly higher than values for 7cm.s^{-1} and 14cm.s^{-1} streams (Table VI., Table VII.).

No significant differences were found between experimental velocities in ash-free polysaccharide values (Table VI., Table VII.), means ranged between 16.6% and 17.4% (Figure III.).

Ash values were also very similar (Table VI., Table VII.) and means ranged between 60.3% and 61.7% (Figure III.).

Species composition

Communities from experimental streams at 28cm.s^{-1} were dominated by diatoms (Table VIII.). *Achnanthes microcephala*, *Tabellaria fenestrata* and *Synedra* spp. were ubiquitous. A strongly developed canopy of filamentous

chlorophytes was also evident. This was dominated by several Mougeotia spp., with Ulothrix sp. also common. Blue-greens were seen in only a few fields; Oscillatoria sp. and colonies of Merismopedia sp. were most common.

In 14 and 7cm.s^{-1} experimental streams, diatoms showed an even greater dominance than in 28cm.s^{-1} streams, especially A. microcephala; but the chain-forming Tabellaria species were much less prominent. The numbers of filamentous chlorophytes were also lower and were dominated by Geminella sp. in 14cm.s^{-1} streams, whilst Ulothrix sp. dominated in 7cm.s^{-1} streams. Zygnema sp. and Stigeoclonium sp. were also evident in 14 and 7cm.s^{-1} streams. A list of routinely observed species is given in Table IX (Austin, unpub.).

Biomass

Biomass accrual rates showed a definite pattern in which initial growth was inversely related to current, then after approximately three weeks, accrual became positively correlated with current in a linear fashion (Austin unpub.). Accrual rates were plotted against ash-free protein levels (Figure IV.) and the results suggest some type of positive relationship between accrual rate and ash-free protein levels. This was further investigated during a later experiment (Figure VIII).

Biomass estimates were originally calculated from material growing on glass slides within the streams. However, it subsequently became evident that such measurements did not accurately represent the total biomass within experimental streams. As a result, biomass was subsequently estimated using quantitative collections directly from the stream bottom. A later comparison between biomass growing on slides and on PVC rendered a correction factor of x5.0 to convert from glass slides to P.V.C. This estimate may be rather conservative in some cases. The correction factor was subsequently used in the estimation of grazer density per unit area during the autumn experiment.

Grazer density

Large numbers of invertebrate larvae were present within the periphyton (Table X.). The most prevalent genera were Psectrocladius, Chironomus and Cricotopus/Orthocladius (these 2 genera are indistinguishable without detailed examination, including the comparison of adult forms, which were not collected). The invertebrate predator Abalabesmyia was also common. Mean numbers of grazers per square metre were similar in $7cm.s^{-1}$ and $14cm.s^{-1}$ streams being 9750 and 11250 respectively. In $28cm.s^{-1}$

streams the figure was higher by a factor of 6, with 67000 grazers per square meter. The percentage of grazers greater than 5mm in size was fairly constant around 83%. This suggests that consumption rates per grazer were at a maximum at this time of year (Table XXIV.) as longer length suggests a higher instar number (feeding rates being higher in later instars). Intra-velocity variation in grazer density per gram dry weight was very high and no significant differences were noted between different current velocities.

Winter experiment

(8/12/87 - 10/2/88)

Proximate composition

No significant variation was noted in either ash-free protein, ash-free lipid, or ash-free polysaccharide as a result of manipulating current between these dates (Figure V., Table VII., Table XI.). Ash values also showed little variation.

Species composition

In this winter period little difference was observed between periphyton species compositions at different current velocities. Diatoms accounted for more than 99% of the count at all velocities (Table VIII.). T. fenestrata

was the dominant species; Synedra spp. and A. microcephala were sub-dominants. These 3 species made up 96% of the count. Very low numbers of the chrysophyte Dinobryon bavaricum were present in 7 and 24cm.s^{-1} streams. Canopy development was more limited during this experiment and chlorophytes (Mougeotia sp.) were seen in only one field. A list of routinely encountered species is given in Table XII (Austin, unpub.). It should be noted that Tabellaria spp. were primarily present as single cells rather than as chains during the winter experiment.

Biomass

Biomass accrual during the winter experiment was initially greatest in experimental streams with a current velocity of 7cm.s^{-1}. However, after 7 weeks, accrual was positively correlated with current velocity in a linear fashion (Austin unpub.).

Grazer density

Mean grazer density was lower than in the autumn experiment (Table X.) and consisted mainly (88%) of small (<5mm) chironomid larvae. Intra-velocity variation was again very high and no substantial differences were found between streams.

Intra-velocity variation

Ash-free protein levels were not significantly different in streams with a current of 7cm.s^{-1} or 28cm.s^{-1}. However, streams with a current of 14cm.s^{-1} showed a statistical difference. Furthermore, statistically significant differences between different sections of streams were noted in 4 of the 6 streams analyzed (Table XIII.).

Discussion.

The response of periphyton community proximate composition to changes in current velocity was markedly different in the autumn and winter experiments.

During autumn, the higher protein and lipid levels that developed in faster current communities indicate that the capacity of periphyton to provide a source of nutrition is increased at the higher current levels. This is further reinforced as biomass was also greatest at high current velocities. Algal growth during autumn is typically limited by the nutrient supply, principally phosphorus in the case of the experimental streams at the Humpback lake site. Increases in current velocity would therefore have had a significant effect in increasing the supply of nutrients (most importantly phosphorus) and dissolved gases. It is postulated that this current effect is primarily responsible for the differences generated between

experimental streams by the end of the experiment. In addition, rapid growth rates during autumn lead to a greater importance of growth-rate-induced changes in periphyton composition at this time. This is in contrast to the situation in winter, when diatom immigration rates play a more important role. Experimental streams with a current velocity of $7cm.s^{-1}$ during the autumn experiment were probably at a velocity below that necessary to produce the steep diffusion gradient which enables rapid exchange of materials between organism and environment (Whitford, 1960). Those streams at $28cm.s^{-1}$ would have had sufficient velocities. Experimental streams at $14cm.s^{-1}$ showed a species composition similar to $7cm.s^{-1}$ streams yet had higher protein levels suggesting that these also benefitted from the increased supply of nutrients with current.

Biomass accrual during the autumn experiment followed a predictable pattern (McIntire, 1966; Reisen and Spenser, 1970). At lower currents, the decreased shear forces enabled more rapid colonization by filamentous chlorophytes during the initial stages of the experiment. However, after 3 weeks of canopy development by primarily diatomaceous forms, accrual became positively correlated with current. This is a result of both the increased nutrient supply to the community and the development of

small back-eddies and sheltered spots in the streams allowing more rapid colonization (Hamilton and Duthie, 1984), especially by filamentous chlorophytes.

The present results show an increase in the relative dominance of filamentous chlorophytes with current during the autumn experiment. Antoine & Benson-Evans (1982) found that the standing crops of most species of Chlorophyta were inversely related to current. McIntire (1966), working in laboratory streams, also noted that communities dominated by Stigeoclonium, Oedogonium and Tribonema at a low velocity (9cm.s^{-1}) were replaced by a felt-like dark-green or brown growth at a higher current (38cm.s^{-1}). Mougeotia spp. which were the dominant filamentous chlorophytes at 28cm.s^{-1} are generally associated with slowly-flowing waters, lakes and ponds (Smith, 1950); however, faster-current species do exist, and Whitford & Schumacher (1964) noted an increase in metabolic rate directly proportional to current up to a velocity of 40cm.s^{-1} in some species of Chlorophyta. Also, colonization would have been aided by the development of low current sections in the experimental streams, both as a result of eddies on the downstream side of slide-holders and due to the development of fairly adnate felts which lowered current along the surfaces of streams.

This is analagous to the situation in natural streams where fast currents generate many back-eddies and low current areas on the downstream side of rocks and boulders allowing increased settlement (Jan Stevenson, 1984). However, Chlorophyta were less dominant in low flow ($7cm.s^{-1}$) streams; this suggests that although a low current may be needed for initial colonization, the higher currents subsequently allow more prolific growth, as they increase the availability of nutrients to the growing plants. Although the plants are attached in low current areas, they grow so that their filaments extend into high current areas with their increased supply of nutrients.

During autumn, increases in periphyton accrual rates were accompanied by increases in ash-free protein levels. Species composition was similar in $7cm.s^{-1}$ and $14cm.s^{-1}$ streams, yet ash-free protein levels were significantly different. It is therefore very likely that increases in protein were a result of higher production rates, leading to a greater inclusion of cellular carbon into protein.

During winter, nutrient supply is usually at its greatest, nutrient limitation of growth does not tend to occur and algal growth is light and temperature limited. Current velocity was therefore of much less significance during the winter experiment as it had negligible effects

on the latter factors. The difference in total accrual between velocities during these months may have resulted from passive settlement. Bothwell and Jasper (1983), noted that passive settlement may be one of the major factors determining accrual during winter. Jan Stevenson (1984), also noted that immigration was probably the major factor responsible for differences in periphyton abundance during winter. However, the aforenamed author also noted that immigration rate was negatively correlated with current. As the present results show a positive correlation with current it is likely that significant growth was also taking place. The rapid development of mucilage within experimental streams, which reached 95% cover after 5 days (Austin unpub.), may have helped to minimize the effects of shear forces allowing more rapid colonization in higher velocity experimental streams.

The low recruitment potential of incoming waters during the winter (in terms of number of species) was probably at least partially responsible for the lack of significant differences in species composition or periphyton proximate composition between the currents examined. In keeping with the present results, no changes in species composition were noted by either Steinman and McIntire (1986) or Jan Stevenson (1984) when they manipulated current during winter.

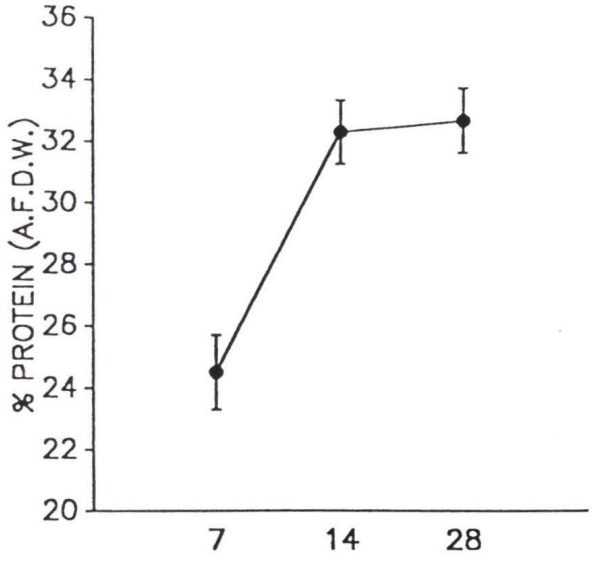
Intra-velocity variation in proximate composition was substantial in some cases (Table XIII.). This may be a result of emigration, isolated sloughing events occurring either as a result of the death of basal cells, or grazing activity (which showed very large intra-velocity variation). These activities appeared to occur more or less randomly within the streams and provided open areas for recolonization, leading to increased heterogeneity. The within-stream variation between upstream, midstream and downstream sections of streams clearly demonstrates the heterogeneity which exists within natural periphyton communities. Such variation should be taken into account when planning sub-sampling techniques. The magnitude of the variation within streams was similar to between-stream variation, suggesting that the scale of the streams used in the present experiments was of the appropriate order to encompass the inherent heterogeneity which exists in periphyton communities. As the experiment was run for 7 weeks the likelihood of emigration events (and hence of increased heterogeneity within streams) was greater than in previous studies which were generally of about 4-5 weeks duration. Even after 4 weeks, Steinman and McIntire (1986) noted a decrease in biomass as a result of sloughing.

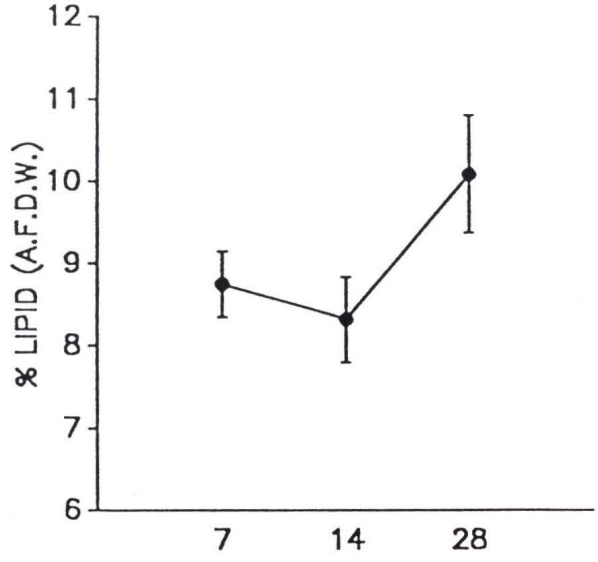
Summary

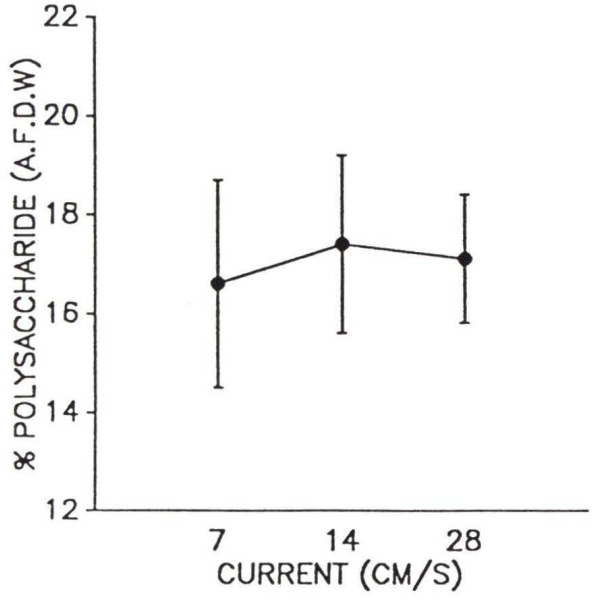
With regard to the effect of current on the proximate composition of periphyton communities, response seemed to be strongly modified by season. Alteration in the factors limiting periphyton growth led to changes in the response of periphyton communities to current velocity.

During autumn, the increased protein values in high velocity streams most likely reflected the higher periphyton production rate, which led to a greater inclusion of cellular carbon into protein (Steinman et al., 1987b). During winter there was little observed effect of current at the velocities examined.

Figure III. Proximate composition of periphyton communities grown at 3 different current velocities during autumn. Each point represents the mean for 4 replicate experimental streams. The error bars denote the 95% confidence limits around the mean. For each replicate stream, n=9 for protein, n=3 for lipid, n=6 for polysaccharide and n=10 for ash.


% PROTEIN (A.F.D.W.)
36
34
32
30
28
26
24
22
20
7
14
28


% LIPID (A.F.D.W.)
12
11
10
9
8
7
6
7
14
28


% POLYSACCHARIDE (A.F.D.W)
22
20
18
16
14
12
7
14
28
CURRENT (CM/S)

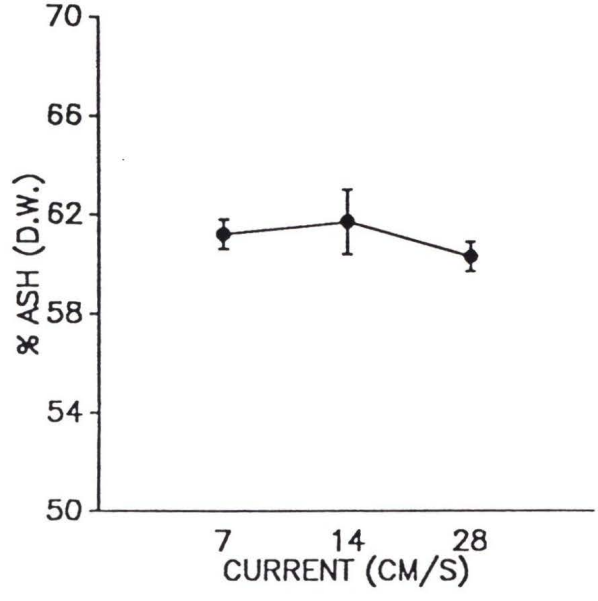

% ASH (D.W.)
70
66
62
58
54
50
7
14
28
CURRENT (CM/S)

Table VI. Summary of statistical analysis of variation in ash-free protein, ash-free lipid, ash-free carbohydrate, and ash as a function of velocity during the winter and autumn experiments. The P (=probability) row shows the probability that the data conform to the null hypothesis that no significant variation exists as a consequence of different current velocities.

AUTUMN

	PROTEIN	LIPID	POLYSACC	ASH
$28cm.s^{-1}$ VS $14cm.s^{-1}$[1]				
F value	2.23	16.76	0.08	0.24
P	0.1859	0.0064	0.7930	0.6382
$28cm.s^{-1}$ VS $7cm.s^{-1}$[1]				
F value	36.62	9.35	NON-EST[2]	0.57
P	0.0009	0.0223		0.4786
$14cm.s^{-1}$ VS $7cm.s^{-1}$[1]				
F value	19.47	1.07	NON-EST[2]	1.56
P	0.0045	0.3401		0.2577

WINTER

	PROTEIN	LIPID	POLYSACC	ASH
$28cm.s^{-1}$ VS $14cm.s^{-1}$[1]				
F value	4.14	0.99	11.83	3.07
P	0.1790	0.4244	0.0751	0.2220
$28cm.s^{-1}$ VS $7cm.s^{-1}$[1]				
F value	7.58	0.13	1.80	2.22
P	0.1105	0.7570	0.3114	0.2749
$14cm.s^{-1}$ VS $7cm.s^{-1}$[1]				
F value	0.56	1.82	4.62	10.30
P	0.5337	0.3095	0.1647	0.0849

[1]Degrees of freedom = 1.
[2]Inadequate number of samples was available for statistical analysis.
Where $P<0.05$ the data may be regarded as not conforming to the null hypothesis.

Table VII. Details of statistical analysis of variation in ash-free protein, ash-free lipid, ash-free carbohydrate, and ash as a function of velocity during the autumn experiment. The P (=probability) column shows the probability that the data conform to the null hypothesis that no significant variation exists as a consequence of different current velocities.

	DEGREES OF FREEDOM	TYPE III SUM OF SQUARES	F VALUE	P[1]
PROTEIN				
ERROR	92	471		
MODEL	11	1794		
vel[2]	2	1447	19.57	0.002
str[3]	3	126	1.30	>0.25
vel*str	6	219		
LIPID				
ERROR	24	17		
MODEL	11	31		
vel	2	21	9.06	0.02
str	3	2.7	0.79	>0.25
vel*str	6	7.0		
POLYSACCHARIDE				
ERROR	18	73		
MODEL	9	66		
vel	2	1.0	0.12	>0.25
str	3	23	0.89	>0.25
vel*str	4	38		
ASH				
ERROR	48	43		
MODEL	11	172		
vel	2	21	0.79	>0.25
str	3	71	1.78	0.25
vel*str	6	80		

[1] Where $P<0.05$ the data may be regarded as not conforming to the null hypothesis.
[2] The variability which is explained by differing current velocities in the streams. Means of sums of squares for vel*str was used as an error term for vel.
[3] The variability explained by between stream differences.

Table VIII. Summary of community composition data (Austin unpub.) for autumn and winter current velocity experiments. Figures are percentages of total cells present at a given velocity.

	AUTUMN 28cm.s^{-1}	14cm.s^{-1}	7cm.s^{-1}
BACILLARIOPHYTA			
I) SINGLE CELLS	41.3	71.7	78.7
II) CHAINS	28.2	7.2	8.1
CHLOROPHYTA			
I) SINGLE CELLS	*[1]	1.6	*
II) FILAMENTS	24.7	17.4	11.1
III) DESMIDIACEAE	*	*	*
CYANOPHYTA	5.0	1.3	1.5
CHRYSOPHYTA	*	*	*
PYRRHOPHYTA	*	*	0.0

	WINTER 28cm.s^{-1}	14cm.s^{-1}	7cm.s^{-1}
BACILLARIOPHYTA			
I) SINGLE CELLS	99.9	99.7	99.1
CHLOROPHYTA			
II) FILAMENTS	*	0.0	0.0
III) DESMIDIACEAE	0.0	*	0.0
CHRYSOPHYTA	0.0	*	*

[1]indicates rare occurrence (1 or 2 fields).

Table IX. Species identified during the autumn current experiment.

BACILLARIOPHYTA
(chain formers)

Tabellaria fenestrata	T. flocculosa
Diatoma vulgare	Fragilaria sp.A
Fragilaria sp.B	F.crotonensis
F.virescens	

(non chain formers)

Synedra sp.A	Synedra sp. B
Synedra sp.C	Achnanthes microcephala
Melosira sp.	Navicula sp.
Pinnularia streptoraphe	Asterionella formosa
Cyclotella sp.	Cymbella sp.
Stauroneis phoenocentron	Gomphonema acuminatum
G.olivaceum	Diploneis elliptica
Cocconeis diminuta	Rhopalodia gibba
Eunotia robusta	Rhopalodia sp.A
Stauroneis sp.	Surirella sp.
Frustulia rhomboides	

CYANOPHYTA

Oscillatoria sp.	Merismopaedia sp.
Anabaena sp.	Gomphosphaeria lacustris

PYRRHOPHYTA
Peridinium sp.

CHLOROPHYTA
(non-filamentous forms)

Oocystis sp.	Dictyoshaerium pulchellum
Pediastrum sp.	Scenedesmus quadricata
Crucigenia rectangularis	Eudorina sp.
Stichococcus sp.	Ankistrodesmus spiralis
Unidentified coccoid A.	

(filamentous forms)

Mougeotia sp.A	Mougeotia sp.B
Mougeotia sp.C	Mougeotia sp.D
Stigeoclonium sp.	Spirogyra sp.
Zygnema sp.	Oedogonium sp.
Microspora sp.	Bulbochaetae sp.
Ulothrix sp.	Geminella sp.

(Desmidiaceae)

Cosmarium blyttii	C.bioculatum
C.punctulatum	C.binum
Xanthideum antilopeum	Staurodesmus cuspidictus
Gonatozygan monotaenium	Arthrodesmus convergens
Staurastrum avicula	Spondylosium planum
Closterium sp.	

CHRYSOPHYTA

Dinobryon bavaricum	D.divergens

Figure IV. Biomass accrual V^S ash-free protein for experimental streams in the autumn current velocity experiment. Accrual data are for a 10 day period between week 3 and week 5. Proximate composition data are for week 7. Each point represents the mean protein value for 4 replicate experimental streams. For biomass data, n=4.

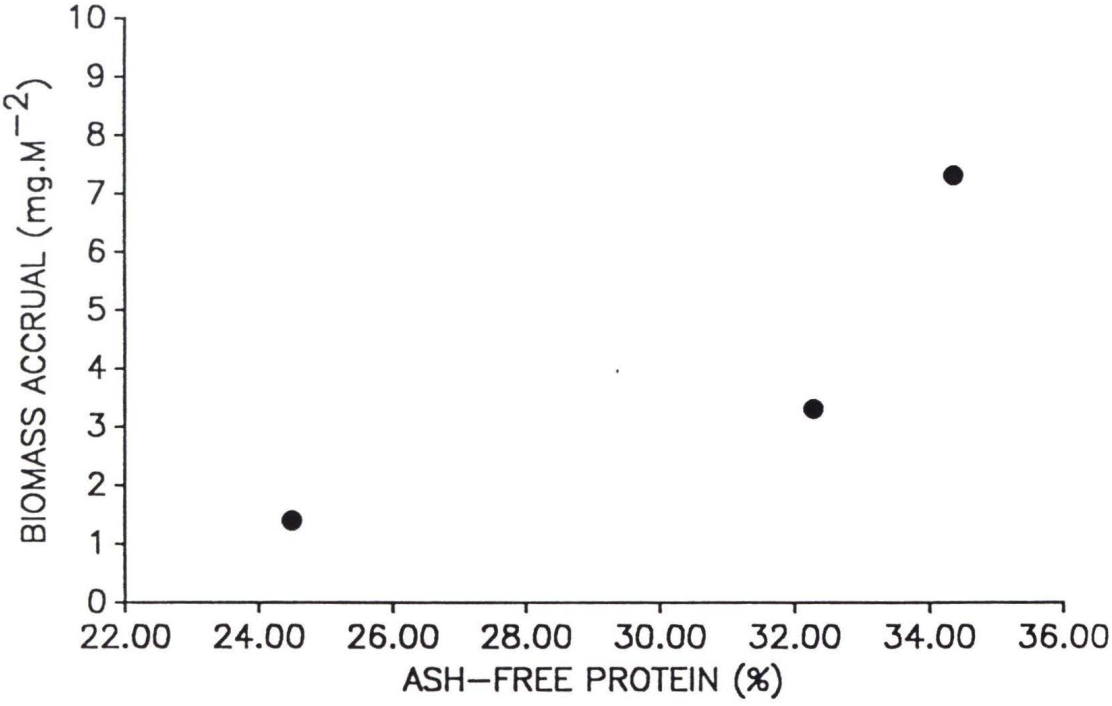

BIOMASS ACCRUAL (mg.M^{-2})
10
9
8
7
6
5
4
3
2
1
0
22.00
24.00
26.00
28.00
30.00
32.00
34.00
36.00
ASH-FREE PROTEIN (%)

Table X. Mean grazer density in experimental streams during the autumn and winter current velocity experiments.

	CURRENT	MEAN NO. GRAZERS (per g dry wt.)	MEAN NO. GRAZERS (per m^2)
AUTUMN			
	7cm.s^{-1}	625 ± 489	9750
	14cm.s^{-1}	375 ± 419	11250
	28cm.s^{-1}	1016 ± 749	67000
WINTER			
	7cm.s^{-1}	43 ± 97	370
	14cm.s^{-1}	29 ± 37	539
	28cm.s^{-1}	50 ± 36	1715

Figure V. Proximate composition of periphyton communities grown at 3 different current velocities during the winter. Each point represents the mean for 2 replicate experimental streams. The error bars denote the 95% confidence limits around the mean. For each replicate stream, n=6 for lipid, n=18 for protein, n=12 for polysaccharide and n=10 for ash.

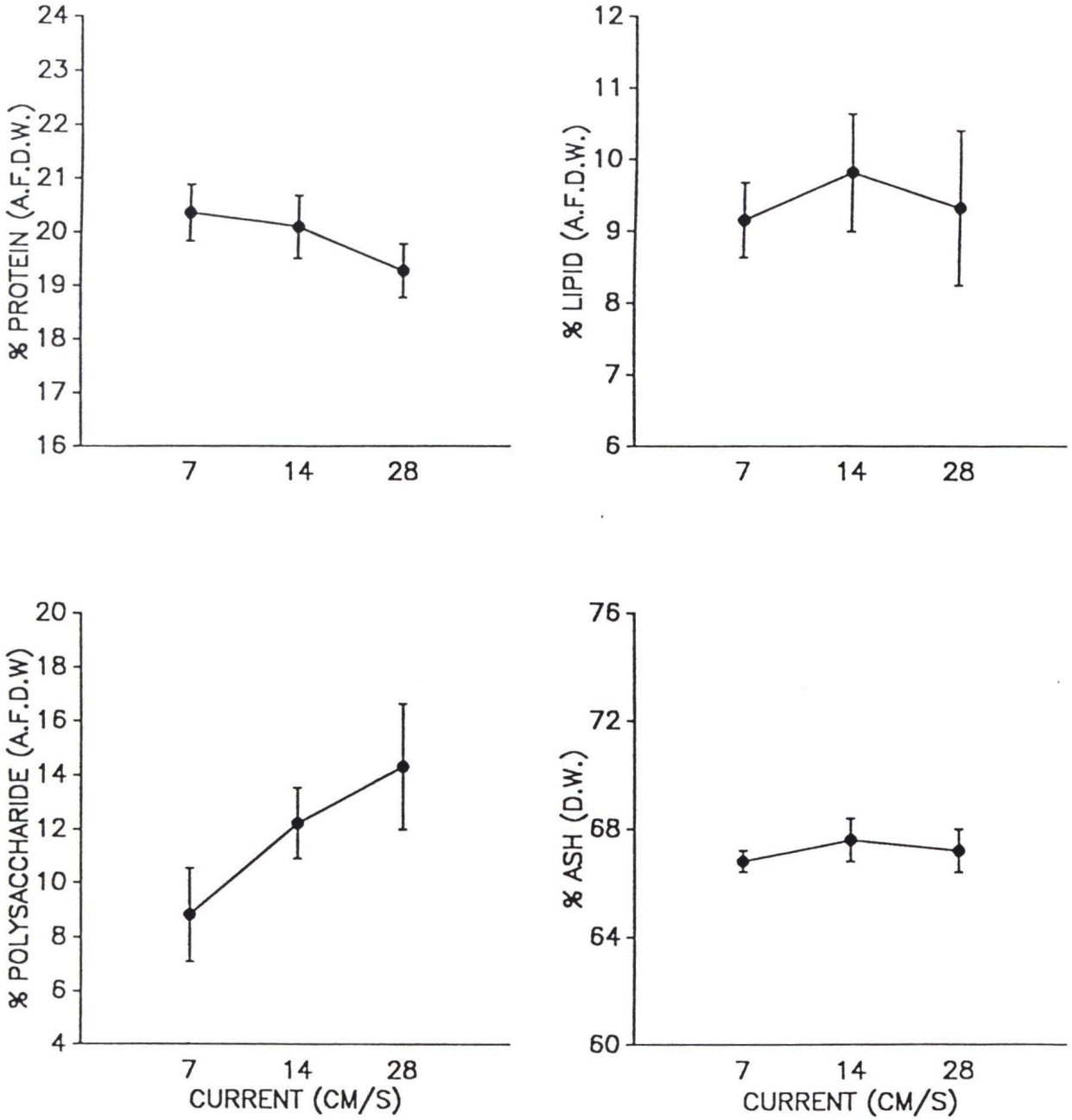

% PROTEIN (A.F.D.W.)
24
23
22
21
20
19
18
17
16
7
14
28
% LIPID (A.F.D.W.)
12
11
10
9
8
7
6
7
14
28
% POLYSACCHARIDE (A.F.D.W)
20
18
16
14
12
10
8
6
4
7
14
28
CURRENT (CM/S)
% ASH (D.W.)
76
72
68
64
60
7
14
28
CURRENT (CM/S)

Table XI. Details of statistical analysis of variation in ash-free protein, ash-free lipid, ash-free carbohydrate, and ash as a function of velocity during the winter experiment. The P (=probability) column shows the probability that the data conform to the null hypothesis that no significant variation exists as a consequence of different current velocities.

	DEGREES OF FREEDOM	TYPE III SUM OF SQUARES	F VALUE	P[1]
PROTEIN				
ERROR	98	222		
MODEL	5	43		
vel[2]	2	22	4.09	0.20
str[3]	1	16	5.81	0.18
vel*str	2	5		
LIPID				
ERROR	30	30		
MODEL	5	30		
vel	2	2.9	0.98	0.51
str	1	24	16.42	0.09
vel*str	2	2.9		
POLYSACCHARIDE				
ERROR	63	1123		
MODEL	5	480		
vel	2	348	5.96	0.14
str	1	70	2.58	>0.25
vel*str	2	58		
ASH				
ERROR	67	215		
MODEL	5	12		
vel	2	9	1.38	0.16
str	1	2.3	0.75	0.11
vel*str	2	1.7		

[1] Where $P<0.05$ the data may be regarded as not conforming to the null hypothesis.
[2] The variability which is explained by differing current velocities in the streams. Means of sums of squares for vel*str was used as an error term for vel.
[3] The variability explained by between stream differences.

Table XII. Species identified during the winter current experiment.

BACILLARIOPHYTA

Tabellaria fenestrata

T. flocculosa

Gomphonema acuminatum

Synedra sp.A

Synedra sp.B

Synedra sp.C

Achnanthes microcephala

Diatoma vulgare

Fragilaria sp.

F. crotonensis

Melosira sp.

Asterionella formosa

Cyclotella sp.

Cymbella sp.

Stauroneis phoenocentron

CHLOROPHYTA

Mougeotia sp.

(Desmidiaceae)

Cosmarium blyttii

CHRYSOPHYTA

Dinobryon bavaricum

Table XIII. Summary of statistical analysis of intra-velocity variation in ash-free protein during the winter current experiment.

$7cm.s^{-1}$[1]	str1 v^s str3	0.1141[2]
$14cm.s^{-1}$	str6 v^s str7	0.0199
$28cm.s^{-1}$	str10 v^s str11	0.6558

[1] n=18
[2] P (=probability) for the null hypothesis that there is no significant difference in ash-free protein values between streams. Where $P<0.05$, the data may be regarded as not conforming to the null hypothesis.

	UPSTREAM v^s MIDSTREAM	UPSTREAM v^s DOWNSTREAM	MIDSTREAM v^s DOWNSTREAM
STR1[3]	0.0001[4]	0.0013	0.0001
STR3	0.1668	0.0858	0.6543
STR6	0.1266	0.0023	0.0575
STR7	0.0108	0.2104	0.1585
STR10	0.5958	0.9459	0.6431
STR11	0.0003	0.0046	0.2199

[3] n=6 for each portion of a stream.
[4] P for the null hypothesis that no significant difference exists in ash-free protein between the 2 sections of the stream under examination.

THE EFFECT OF LIGHT AND NUTRIENTS ON PERIPHYTON COMMUNITIES

Introduction

Inorganic nitrogen and orthophosphate are the two most common limiting nutrients in lotic ecosystems. In general, nitrogen limited streams most often originate in volcanic formations (as is the case in 50% of the waters in Washington state), whilst phosphorus limited streams most often drain glacial or granitic areas, common in British Columbia (Gregory, 1980).

Ratios of inorganic nitrogen to orthophosphate have commonly been used to determine which of the two is limiting in a given situation. Cellular N:P ratios are approximately 15 (Ryther and Dunstan, 1971). This led Redfield (1958) to suggest that at higher ratios phosphorus would be limiting whilst at lower ratios nitrogen would be limiting. Later studies with Scenedesmus sp. found a higher optimal cellular ratio of 30 (Rhee, 1978) and it is now becoming clear that optimal N:P ratios are species specific. A minimum value of 40 for the N:P ratio in Humpback Lake (measured by weight) was derived from historical water chemistry data (Austin unpub.), and

strongly suggests phosphate is the limiting of the two nutrients at this site.

It has been demonstrated for the phosphate limited rivers and streams of southern British Columbia that increases in phosphorus concentration can lead to large increases in periphyton biomass (Stockner and Shortreed, 1978; Bothwell, 1985) and that the resulting increased food availability can potentially enhance wild stocks of anadromous salmonids (Perrin et al., 1987). The influence that such water chemistry changes have on the nutritional value of periphyton communities is also of importance, as it may be reflected in production at higher trophic levels. Such an effect should be of interest with regard to the impact on lotic systems, of both forest and lake fertilization programs, such as those carried out in British Columbia for the last decade (Stockner and Shortreed, 1985; Perrin et al., 1984). Whilst the increase in quantity of available food following such manipulations can be clearly demonstrated (Perrin et al, 1984), the effect on food quality of the periphyton remains largely unknown. The importance of such studies is heightened by the results of recent research which has demonstrated that saturation of growth rates can occur at very low phosphorus concentrations (0.3-0.6µg P L^{-1}) (Bothwell, 1988).

Therefore very small additions of nutrients may cause large scale changes in lotic periphyton and hence in the whole lotic system.

While the effect of both nitrate and phosphate enrichment on the proximate composition of a few freshwater algal species has been examined (e.g. Giddings, 1975; Rhee, 1978), it is unlikely that these responses can be extrapolated to the community level. The major problem in this respect are the fact that nutrient limitation influences algal community competition and succession (Rhee, 1974). Subsequent changes in species composition may result and probably affect the proximate composition of the community. Hence, proximate composition changes can occur in communities independently of the cellular level responses to changing water chemistry noted by Rhee (1978).

Light energy also plays an important role in determining the species composition of periphyton communities (e.g. Steinman and McIntire, 1986). It is therefore likely that changes in light energy would result in changes in the proximate composition of these communities. While light is not usually considered a limiting factor during summer, excessive light may lead to inactivation of chloroplasts (Cock, 1982), bleaching of chlorophylls may be evident,and reduced growth may result

(Lorenzen, 1979). Bothwell and Stockner (1980) demonstrated U.V. inhibition of microbial accumulation, under ambient light conditions, in troughs similar to the present experimental streams. Necrosis of basal cells in filaments may also occur as a result of reduced light intensity on the stream bottom due to heavy canopy development. The purpose of this section of the experiment was to determine whether or not light levels play a significant role in determining biomass accrual during the summer months and whether changes in the proximate composition of the periphyton community result from changes in light levels.

Method

Manipulation of Light levels

Reduction of ambient light levels was achieved using P.V.C. mesh screening attached to a wooden frame above the experimental streams (Illustration III). Light levels were measured using a QSL 100 lightmeter (Biospherical Instruments Inc.) calibrated for both wet and dry readings. Light levels under the mesh screens were approximately 33% of incident light. Light readings were also taken from the bottom of the streams (underneath the canopy) throughout the experiment.

Manipulation of nutrient levels

Carboys (20L) containing stock solutions of nitrogen as 2.35M sodium nitrate ($NaNO_3$) and phosphorus as 0.11M disodium hydrogen orthophosphate (Na_2HPO_4) were used in conjunction with a drip feed system (Flexiflo gravity gauge set,Ross Co., Ohio) to accomplish desired concentrations of N and P (Table XIV.). The nutrients were added at a rate of $1.5mL.min^{-1}$ via a venturi system into the pipes leading to the experimental streams. This ensured that thorough mixing was achieved before the nutrients entered the streams. The carboys were replenished and drip rates checked every 3 days. Distilled water rather than Humpback Lake water was used to dilute the nutrients to the required concentrations, as this reduced any growth inside the carboys or the drip feed lines.

Water chemistry

Water temperature profiles were similar for all experimental streams. Initial temperatures were approximately 12.0°C, final temperatures were approximately 15.5°C. Temperature increases along the length of the streams varied between 1.0 and 1.5°C. The rapid increase in temperature towards the end of the experiment is a reflection of improved weather conditions. In the period between 03/6/88 and 15/6/88 mean air

temperatures increased by 32% from 11.0 to 14.5^{o}C and mean daily hours of sunshine increased by 86% from 6.7h to 12.5h.

pH varied between 7.0 and 7.9 during the experiment. All experimental streams registered similar values (Table XV.).

Dissolved oxygen varied between 9.5 and 10.4mg O_2L^{-1} during the experiment. There were no changes in the percent saturation of the water (approximately 96%). The changing dissolved oxygen values reflect the change in water temperature. Oxygen bubbles were evident throughout the experiment, especially attached to filamentous chlorophytes in the streams.

Biomass

Biomass was measured by inserting a rigid cylinder into the streams, which effectively sealed off a 77cm^2 area of stream bottom. All organisms were then removed from the area by a combination of suction and scraping. The water from within the cylinder was also removed and material recovered by filtration. All material was then dried at 50^{o}C and weighed. Results are expressed as grams (dry weight) of biomass per square meter.

Biomass accrual (i.e. change in biomass with time) was calculated and the relationship between this and protein levels was examined. Accrual between week 2 and week 4 was chosen as protein levels were available for week 4. Use of final accrual and protein levels would have been misleading as sloughing and grazing tends to increase beyond week 4 (Steinman and McIntire, 1986). As a result, accrual and production become less similar.

Grazer data

The total number of grazers was counted in samples taken for analysis of species composition and related to the dry weight of each sample.

Analysis of results

The 95% confidence limits are plotted around each mean for all results. More detailed statistical analysis of the data would need larger numbers of replicates than were practically available. To aid the reader, where means were different at the 95% confidence level, the term "significantly different" has been used.

Results

Proximate composition

Ash-free protein values were within the range of previous values (see figure X., summer value), however protein

values on a dry weight basis were the highest recorded. Protein values were fairly similar on days 28 and 46 of the experiment, except for streams with one third of the normal light level (33%hv), which showed higher ash-free protein on day 46 (figure VI.). On day 46 streams with both nitrogen and phosphorus added (NP and NP33%hv) had significantly higher mean protein than control streams. NP33%hv streams in particular also had higher ash-free protein than both 33%hv and N streams (figure VI.).

Ash-free lipid values showed little variability by day 46, the highest values were in 33%hv and NP33%hv streams and these were significantly higher than the values for P streams. No replicates were obtainable on day 28, however, 33%hv, N and control streams had substantially lower values than on day 46 (figure VI.).

On day 28 ash-free polysaccharide values were similar in all streams apart from phosphorus enriched streams (P) which had substantially higher polysaccharide values. By day 46 ash-free polysaccharide values in P streams had declined and were similar to N, NP and control streams. NP33%hv and 33%hv streams had significantly lower values (figure VII.).

Ash values on day 46 were similar in N, P and control streams, NP streams had significantly higher ash values, 33%hv and NP33%hv streams had significantly lower values, NP33%hv streams having the lowest. Whilst no replicates were available for day 28 the values suggest that between day 28 and day 46 there was a general increase in ash content of all streams apart from NP33%hv streams which stayed the same (figure VII.).

Biomass

Variation in biomass accrual was demonstrated under different environmental regimens (Figure VIII.).

In control streams, biomass increased steadily for approximately three weeks before reaching a relatively steady state of approximately 12 $g.m^{-2}$ dry weight; this was maintained for the remainder of the experiment. The untreated streams under low light conditions (33%hv) showed steadily increasing biomass throughout the experiment, with final values of 22$g.m^{-2}$.

Biomass in phosphate enriched streams (P) increased very rapidly during the first three weeks, then dropped sharply to levels similar to control streams for the remainder of the experiment. Biomass initially increased at a much slower rate in nitrate enriched streams (N) before peaking after approximately five weeks, followed by

a rapid reduction in biomass towards the end of the experiment.

Biomass increases in streams enriched with both nitrate and phosphate (NP and NP33%hv) were similar during the first three weeks of the experiment. Subsequently biomass declined rapidly in NP streams while it continued to increase in NP33%hv streams with values of 60g.m^{-2} by the end of the experiment.

Species composition

Communities were examined both on day 28 and day 46. All communities were dominated by diatoms (Table XVI.). On day 28 the dominant species were Tabellaria fenestrata and Synedra spp. in control, 33%hv, and NP streams, with Achnanthes microcephala being sub-dominant. In N and P streams a similar pattern was evident except A. microcephala was the dominant species. NP33%hv streams had very high numbers of Diatoma vulgare and T. fenestrata, with Synedra spp., A. microcephala and Fragilaria virescens as sub-dominants.

Filamentous chlorophytes were present in all streams on day 28, with relatively high levels in P, NP, and control streams, and low levels in 33%hv, NP33%hv and N streams. These were mostly Mougeotia spp., significant amounts of Zygnema sp. and Ulothrix sp. were also present.

By day 46, A. microcephala was the dominant species in all but 1 treatment, having increased from 18% to 52% of the count. In NP33%hv streams, it made up only 6% of the count and F. virescens was the dominant species, with T. fenestrata and Synedra spp. as sub-dominants.

Numbers of filamentous chlorophytes were much lower than on day 28 in control, NP and P streams, which were fairly similar (Table XVI.). In N, 33%hv and NP33%hv streams, levels of filamentous chlorophytes had increased since day 28.

A list of routinely observed species can be found in Table XVII. (Austin, unpub.).

Grazer density

Levels of invertebrate larvae varied between 294 and 542 per gram dry weight of biomass (Table XVIII.) and showed a high degree of spatial variability. Numbers of grazers per unit area were highest in NP33%hv and 33%hv streams and substantially lower in all other streams. Most of the larvae (77%) were greater than 5mm in length, suggesting a greater potential for consumption than during the winter and spring, when only 12% and 32% of the larvae respectively were larger than 5mm.

Light levels

Incident light varied between 1.0 - 3.9 x $10^{17}\mu E.cm^{-2}s^{-1}$ for midday readings during the course of the experiment (Table XIX.). In low light (screened) streams, light reaching the bottom was as little as 10-33% by the end of the experiment. Canopy development in streams under normal light conditions never reduced light levels to less than 67% of incident light.

Protein levels and biomass accrual

Protein levels were plotted against biomass accrual for day 28. A significant ($0.005<p<0.01$) linear relationship was demonstrated, increased protein levels occurring in conjunction with increased accrual rates (Figure IX.).

Discussion

Control streams

Ash-free protein and polysaccharide values were similar on days 28 and 46; lipid and ash levels increased with time. Increased lipid values have been associated with nutrient depletion and senescence (Shifrin and Chisholm, 1981) and increases in the relative number of diatoms (Opute, 1974). As biomass was steadily increasing in these streams, even under relatively heavy grazing pressure, production rates must also have been high; therefore senescence is unlikely

and the increased lipid levels must have been due to the increased numbers of diatoms. Ash values are generally higher in diatoms than chlorophytes (Moore, 1977). As levels of chlorophytes declined between day 28 and day 46, this would explain the corresponding increase in ash levels.

Addition of nitrogen

Addition of nitrogen as sodium nitrate had little observable effect on total protein, lipid or polysaccharide levels, final ash levels were also similar. Research with single species of microalgae has demonstrated that organisms growing under conditions of excess nitrogen may accumulate the excess in the protein fraction, leading to increases in measured protein levels (e.g. Rhee, 1978, for Scenedesmus sp.). As no increase in protein level was measured it must be assumed that changes in species composition and production rate had a more important effect on community proximate composition than the types of changes noted by Rhee (1978).

Ash levels increased between day 28 and day 46, even though there was a reduction in the relative number of diatoms. However, the high ash value on day 46 may be a result of increased numbers of dead cells within the material collected, as the communities in these streams were undergoing heavy sloughing at the time of collection.

Stimulation of biomass production by nitrogen, a non-limiting nutrient was demonstrated. Previous authors (e.g. Peterson et al., 1983; Pringle and Bowers, 1984; Fairchild and Lowe, 1984) have observed that nitrogen addition has no effect on biomass in phosphorus limited streams. However, in keeping with the present results, De Vries et al. (1985) also noted biomass stimulation by both nitrogen or phosphorus when both nutrients were at low concentrations. They suggested that this demonstrates the inadequacy of the single limiting nutrient concept under sub-optimal concentrations of both nitrogen and phosphorus.

Addition of phosphorus

Addition of disodium hydrogen orthophosphate to experimental streams resulted in a significant increase in polysaccharide on day 28 of the experiment. However, by day 46 there were no differences in proximate composition between these streams and control streams. The very high polysaccharide levels on day 28 were not associated with strong dominance by any particular species and may be a result of increased photophosphorylation and sugar production, which due to nitrogen limitation, may have been incorporated into carbohydrate rather than protein. Previous authors have also noted an increase in C:N under phosphorus limitation (Perry, 1976; Sakshaug and Holm-Hansen, 1977; Goldman, 1979).

Addition of phosphorus and nitrogen

The N:P ratio of the added nutrients was 35:1, maintaining phosphorus at a level slightly closer to the optimal cellular ratio than normally found in Humpback Lake water. This addition resulted by day 46 in higher protein and ash levels than control streams. The higher protein levels may be a reflection of increased production rates, leading to a greater incorporation of cellular carbon into protein (Steinman et al., 1987b). Biomass in NP streams was higher than in all other streams under normal light conditions (Illustration IV.) again as a result of increased production rates. However, it is of note that there was little difference in biomass between control and NP streams by the end of the experiment, even though grazing levels per gram biomass were not substantially different.

The increased levels of diatoms in NP streams on day 46 would account for the increase in ash levels. Increased deposition of inorganic detritus as a result of time and canopy development is unlikely, as NP33%hv streams showed no increase in ash weight between day 28 and day 46.

Reduction of light levels

Periphyton growing in streams with 1/3 of incident light had similar protein, lipid and carbohydrate levels to control streams. Ash weights however were significantly

lower by day 46, as a result most likely of the lower levels of diatoms in these streams. Species composition differences were evident between these streams and control streams; levels of chlorophytes were higher and diatoms were less dominant. As these changes in species composition did not lead to changes in proximate composition it is suggested that changes in proximate composition are generated primarily by production rate differences. As there was little difference in net production between 33%hv streams and control streams, little difference in proximate composition would therefore be expected.

Addition of nitrogen and phosphorus and reduction of light levels

Addition of nutrients to periphyton growing in low light streams resulted in higher lipid, protein and ash levels on day 28. Lipid and ash levels were presumably high due to the larger proportion of diatoms in these streams compared to control streams. The high protein levels (which were similar to those in NP streams) seem to be a result of increased incorporation of carbon into protein due to high production rates. By day 46, carbohydrate and ash levels were lowest in NP33%hv streams. It is of considerable interest that these communities exhibited very little

change in proximate composition between day 28 and day 46. Also, they did not undergo any "crash" in biomass in contrast to other streams even though grazing levels were relatively high. There were large changes in species composition between days 28 and 46, which again suggests the greater importance of growth rate than species composition in determining proximate composition.

Algal production in temperate streams is generally believed to be restricted by light availability (e.g. Murphy and Hall, 1981; Sheath et al., 1986). Indeed, a negative correlation between light levels and periphyton biomass was demonstrated in the experimental streams when additional phosphorus was present. However, in contrast to this belief, no correlation was seen between light levels and biomass when natural phosphorus limited water was used. This confirms the results of Bothwell (1988) who noted that changes in light levels were not correlated with biomass accrual under strong nutrient limitation.

Biomass patterns, sloughing and grazing

All streams with either added nitrogen, phosphorus, or nitrogen and phosphorus exhibited greater biomass than control streams. Of particular interest is the fact that these streams (with the exception of NP33%hv streams), whilst exhibiting increased biomass at some stage during

the experiment, had rather similar values to control streams by the end of the experiment.

Previous workers have observed sloughing events within periphyton communities, and these generally occur between week 3 and 5 after the initiation of growth (e.g. Eloranta and Kunnas, 1976; Steinman and McIntire, 1986). In addition, the development of the grazer component of the periphyton took a similar amount of time during this experiment. Therefore, both sloughing events (McIntire et al., 1964) and grazing pressure (Gregory, 1980) should be regarded as component forces responsible for the observed "crash" in periphyton biomass between week 3 and 5.

Although high numbers of grazers were present in streams under low light conditions, the periphyton in these streams did not exhibit a crash, and it is possible that sloughing events were greatly reduced. Gregory (1980) also noted that the amount of drift in streams exposed to direct sunlight, as a result of logging, was much greater than in shaded (unlogged) streams. In the present experiment pronounced bleaching of chlorophylls occurred in streams under normal light conditions. This stress may have been a causal factor in the initiation of sloughing.

Sloughing due to necrosis of basal cells as a result of reduced light levels under the canopy was unlikely. Measurements taken under the canopy at the time showed sufficient light for growth. The lowest recorded light penetration for any treatment carried out in incident light was 0.225 x $10^{17}\mu E.cm^{-2}s^{-1}$, whilst the average light penetration in NP33%hv streams was only 0.246 x $10^{17}\mu E.cm^{-2}s^{-1}$.

Summary

The influence of light and nutrients on periphyton communities

Changes in periphyton proximate composition as a result of nutrient addition appear to be related to the ensuing changes in production rate. Ash-free protein has been shown to vary with the rate of biomass accrual (Figure IX.) and this would be expected. Previous authors have demonstrated a similar relationship between C:N and growth rate in marine phytoplankton (Sakshaug and Holm-Hansen, 1977; Goldman, 1979). Bothwell (1985), investigating the effect of increasing phosphorus limitation (i.e. decreasing growth rate) on lotic periphyton communities found C:N to be a useful, if somewhat conservative estimate of differences between sites. Lipid levels are also known to

vary as a consequence of growth rate (Shifrin and Chisholm, 1981).

Species composition changes did not appear to play a major role in determining proximate composition during the present experiment; several rather large changes in species composition resulted in no change in proximate composition.

Predictions of proximate composition changes made from work with single species did not appear to be valid for periphyton communities. Species composition changes result both as a function of environmental conditions and community maturation. The complexity of these changes and their subsequent effect on production rates and hence upon community proximate composition are unlikely to be mirrored by a single species.

Although changes in the proximate composition of single species may occur within the periphyton they are not identifiable, as production rate and species composition generated changes are of a greater magnitude.

The effect of light on periphyton communities is dependent on the nutrient status of the water. When nutrients are available, a negative correlation exists between the observed light levels and periphyton accrual. When nutrients are limiting, changes in light levels cause little difference in these parameters. Furthermore, it is

suggested that irradiation may be in part responsible for creating conditions under which sloughing increases within periphyton communities. In the present experiment, those communities growing at normal light levels exhibited a "stressed" development, as noted by the bleached appearance of filaments, and subsequently sloughed in contrast to the low light unbleached communities.

The importance of proximate composition changes to lotic systems

It is clear from the results that the combined addition of nitrogen and phosphorus may cause an increase in ash-free protein levels which addition of the limiting nutrient alone does not cause. This effect is magnified when light levels are such that photodestruction of pigments is minimal. Rhee (1978) stated that the seemingly synergistic effects of simultaneous N and P addition stem from competition and coexistence based on optimal cellular N:P, whereas De Vries (1985) questioned the applicability of the single limiting nutrient theory to such situations, when the concentrations of both nutrients are sub-optimal. I believe that the single limiting nutrient theory may be suspect in such cases. However, the effects of competition as noted by Rhee (1978), are likely to be of greater magnitude in community level studies. Certainly in the

present experiment the differences generated in species composition and production rate as a result of competition, were the major phenomena responsible for change within the periphyton.

Increases in the levels of both N and P in streams are likely to cause an increase in food quality as well as food availability to invertebrate larvae and other grazers. Other treatments, in contrast, may only increase the quantity of food available. It is therefore likely that the increased food quality that results from simultaneous N and P addition would lead to greater grazer survival and growth rates. This would eventually be transferred into more rapid growth of higher level consumers such as salmonids and subsequently lead to greater survival and hence production rates of these organisms(Perrin et al., 1984).

Illustration III Setup of Humpback Lake experimental stream system during the investigation of the effects of nutrients and light on periphyton communities. Note the carboys of nutrients on top of the header box and the P.V.C. mesh screening over the experimental streams on the right hand side to reduce light levels.

Table XIV. Environmental parameters manipulated in the Humpback experimental stream system during the period May 17 - July 1, 1988.

stream	Current	Light (% ambient)	$N\text{-}NO_3$ addition ($mg.L^{-1}$)	$P\text{-}PO_4$ addition ($\mu g.L^{-1}$)
1 (33%hv)[1]	$14cm.s^{-1}$	33	-----	-----
2 (33%hv)	$14cm.s^{-1}$	33	-----	-----
3 (NP33%hv)	$14cm.s^{-1}$	33	0.079	2.25
4 (NP33%hv)	$14cm.s^{-1}$	33	0.079	2.25
5 (N)	$14cm.s^{-1}$	100	0.079	-----
6 (N)	$14cm.s^{-1}$	100	0.079	-----
7 (P)	$14cm.s^{-1}$	100	-----	2.14
8 (P)	$14cm.s^{-1}$	100	-----	2.14
9 (NP)	$14cm.s^{-1}$	100	0.076	2.03
10 (NP)	$14cm.s^{-1}$	100	0.076	2.03
11 (CNTRL)	$14cm.s^{-1}$	100	-----	-----
12 (CNTRL)	$14cm.s^{-1}$	100	-----	-----

[1]name of protocol

Table XV. Mean water temperature, pH, and dissolved oxygen readings for the Humpback Lake experimental streams, 17 May-1 July, 1988. (Measurements were made on a weekly basis).

STREAM (no.)	PROTOCOL	MEAN WATER TEMPERATURE WEEK 1-3 (°C)	MEAN WATER TEMPERATURE WEEK 4-7 (°C)	MEAN pH	MEAN DO_2 (mgL^{-1})
1	33%hv	11.7	16.4	7.3	9.9
2	33%hv	11.7	16.4	7.3	10.0
3	NP33%hv	11.7	16.4	7.3	10.0
4	NP33%hv	11.7	16.4	7.3	10.0
5	N	12.0	16.5	7.3	10.1
6	N	12.0	16.5	7.3	10.1
7	P	12.0	16.4	7.3	10.1
8	P	12.2	16.5	7.4	10.1
9	NP	12.1	16.5	7.4	9.9
10	NP	12.1	16.4	7.4	10.0
11	CNTRL	12.1	16.4	7.4	9.9
12	CNTRL	12.1	16.2	7.4	10.0

Figure VI. Protein and lipid values (as a percentage of ash free dry weight) for periphyton samples collected on day 28 and day 46 (the final day) of an experiment to determine the effects of N, P and light on periphyton proximate composition, May 17 - July 1, 1988. Error bars denote 95% confidence limits around the mean. Each point represents the pooled samples of 2 experimental streams. On day 28, n=3 for protein and n=2 for lipid. On day 46, n=6 for protein and n=3 for lipid.

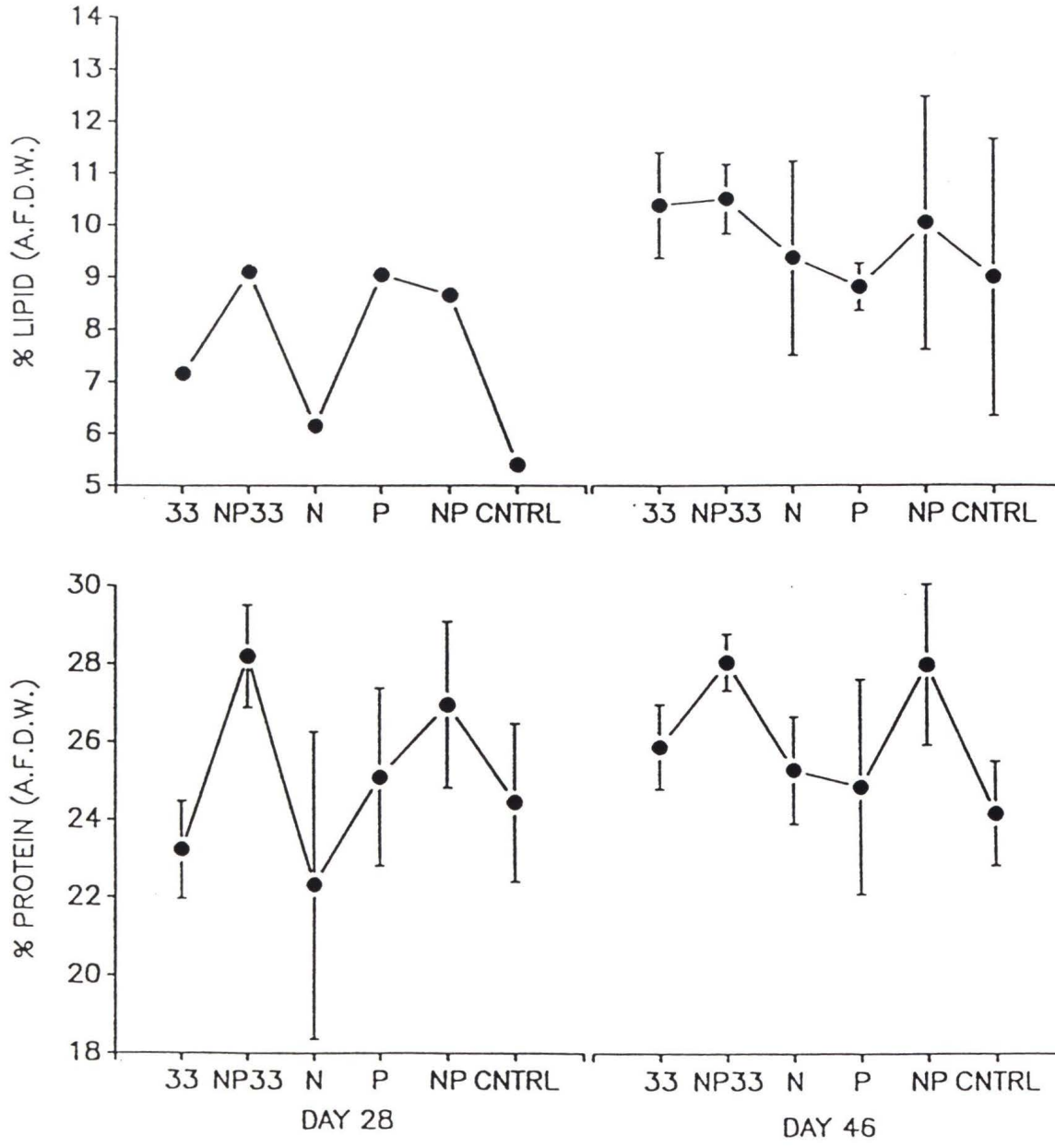

% LIPID (A.F.D.W.)
14
13
12
11
10
9
8
7
6
5
33 NP33 N P NP CNTRL
33 NP33 N P NP CNTRL
% PROTEIN (A.F.D.W.)
30
28
26
24
22
20
18
33 NP33 N P NP CNTRL
33 NP33 N P NP CNTRL
DAY 28
DAY 46

Figure VII. Polysaccharide values (as a percentage of ash free dry weight) and ash values (as a percentage of dry weight) for periphyton samples collected on day 28 and day 46 (the final day) of an experiment to determine the effects of N, P and light on periphyton proximate composition, May 17 - July 1, 1988). Error bars denote 95% confidence limits around the mean. Each point represents the pooled samples of 2 experimental streams. On day 28, n=1 for ash and n=3 for polysaccharide. On day 46, n=5 for ash and n=3 for polysaccharide.

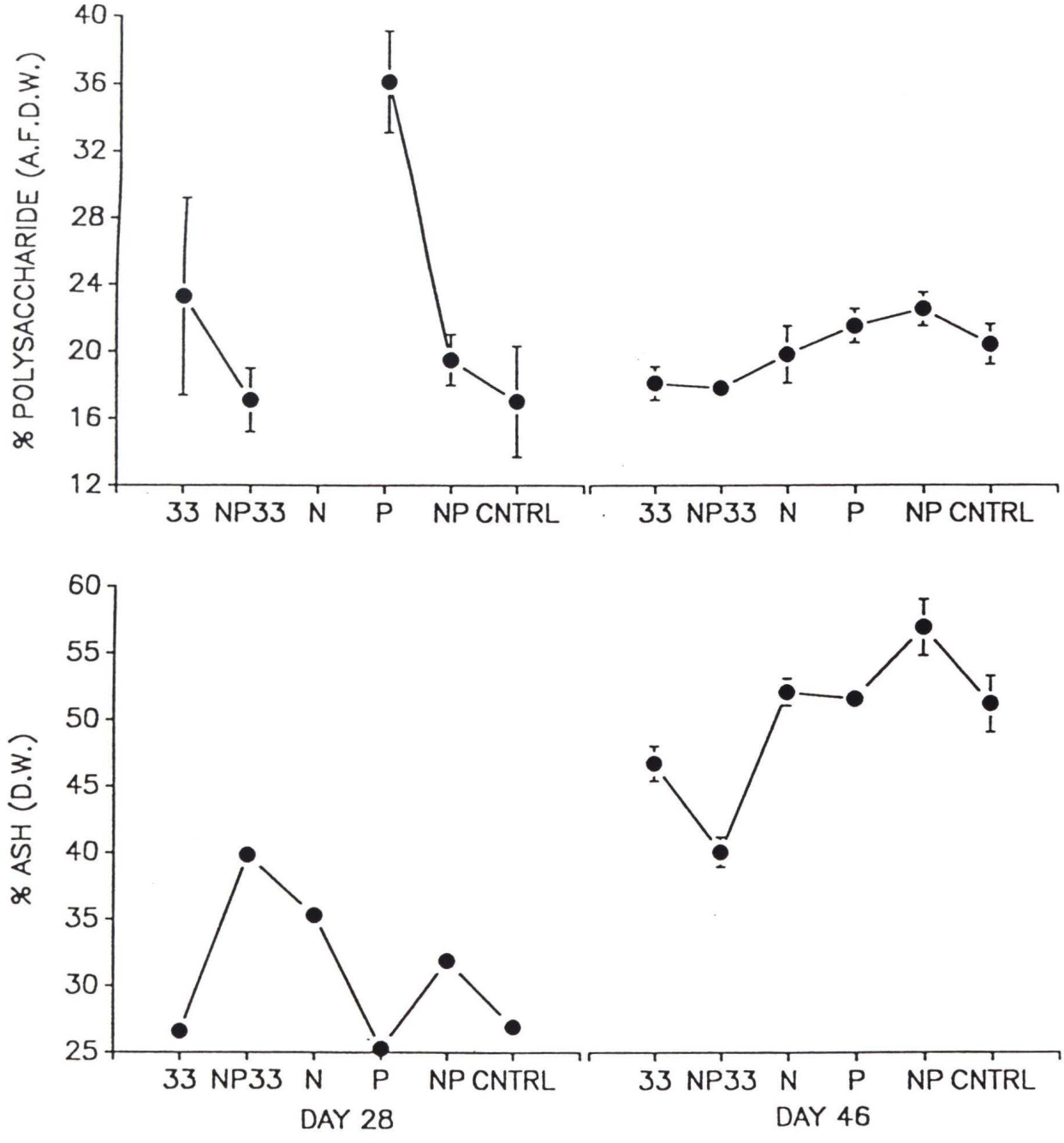

% POLYSACCHARIDE (A.F.D.W.)
40
36
32
28
24
20
16
12
33 NP33 N P NP CNTRL
33 NP33 N P NP CNTRL
% ASH (D.W.)
60
55
50
45
40
35
30
25
33 NP33 N P NP CNTRL
33 NP33 N P NP CNTRL
DAY 28
DAY 46

Figure VIII. Biomass (g dry wt.m^{-2}) variation with time under different environmental regimens in the Humpback experimental stream system, from May 17 - July 1, 1988. For each point, n=1.

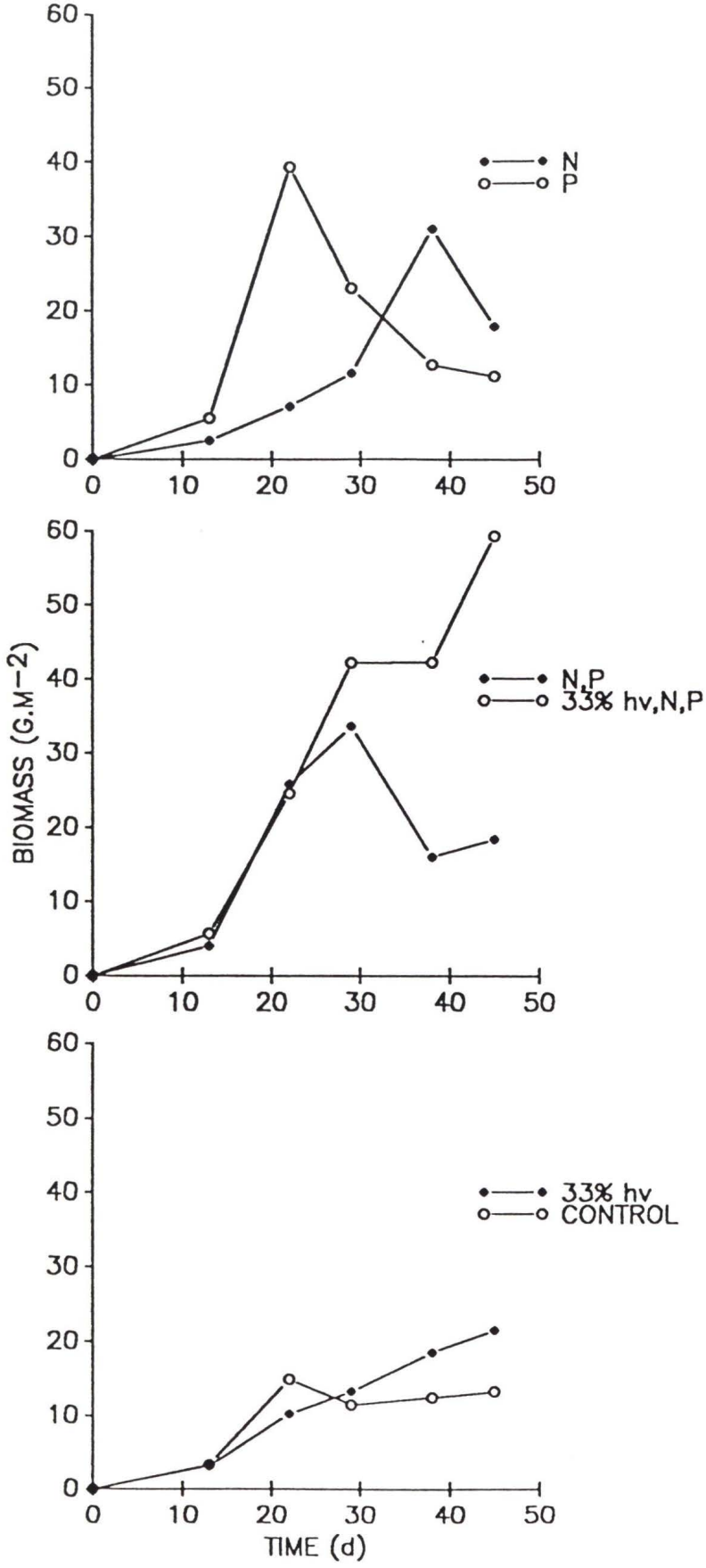

BIOMASS (G.M-2)
TIME (d)
N
P
N,P
33% hv,N,P
33% hv
CONTROL
0
10
20
30
40
50
60

Table XVI. Summary of community composition data (Austin unpub.) for the summer experiment on the effect of nutrients and light on periphyton communities . Figures are percentages of total cells present in a given treatment.

	DAY 28					
	33%HV	NP33%HV	N	P	NP	CNTRL
BACILLARIOPHYTA						
I) SINGLE CELLS	53.3	21.9	60.1	51.2	35.9	45.5
II) CHAINS	30.8	69.7	23.1	31.9	41.8	31.4
CHLOROPHYTA						
I) SINGLE CELLS	0.0	0.0	1.3	0.0	0.0	0.0
II) FILAMENTS	9.3	8.4	5.2	15.4	17.7	19.0
III) DESMIDIACEAE	*[1]	0.0	*	0.0	*	*
CYANOPHYTA	0.0	0.0	2.1	*	*	1.5
CHRYSOPHYTA	6.3	0.0	8.1	1.4	4.2	2.2
PYRRHOPHYTA	*	0.0	0.0	0.0	0.0	0.0

	DAY 46					
	33%HV	NP33%HV	N	P	NP	CNTRL
BACILLARIOPHYTA						
I) SINGLE CELLS	71.5	26.8	77.0	86.6	75.4	85.5
II) CHAINS	6.0	43.4	3.0	6.6	21.2	5.3
CHLOROPHYTA						
I) SINGLE CELLS	0.0	1.0	*	*	*	*
II) FILAMENTS	14.3	26.8	12.6	3.3	1.6	2.8
III) DESMIDIACEAE	*	0.0	*	*	*	*
CYANOPHYTA	*	1.3	2.1	0.0	*	1.6
CHRYSOPHYTA	7.2	2.7	4.4	2.6	1.4	4.2
PYRRHOPHYTA	*	0.0	*	0.0	*	*

[1]indicates rare occurrence, less than 1%

Table XVII. Species identified during the summer experiment to investigate the effects of nutrients and light on periphyton communities.

BACILLARIOPHYTA
(chain formers)

Tabellaria fenestrata	T.flocculosa
Diatoma vulgare	Fragilaria sp.A
Fragilaria sp.B	F.crotonensis
F.virescens	

(non chain formers)

Synedra sp.A	Synedra sp.B
Synedra sp.C	Achnanthes microcephala
Melosira sp.A	Navicula sp.
Melosira sp.B	Asterionella formosa
Cyclotella sp.	C.compta
C.bodanica	Cymbella cymbiformis
Cymbella sp.	Gomphonema constrictum
Stauroneis phoenocentron	Gomphonema Acuminatum
G.olivaceum	Rhopalodia gibba
Rhizosolenia eriensis	Nitzchia sigmoidea
Eunotia curvata	N.vivax
E.robusta	

CYANOPHYTA

Oscillatoria sp.	Merismopaedia sp.
Anabaena sp.	Lyngbya sp.

PYRRHOPHYTA
Peridinium sp.

CHLOROPHYTA
(non-filamentous forms)

Scenedesmus quadricata	Ankistrodesmus fulcatis
S.bijuga	A.spiralis

(filamentous forms)

Mougeotia sp.A	Mougeotia sp.B
Mougeotia sp.C	Mougeotia sp.D
Spirogyra sp.	Geminella sp.
Zygnema sp.	Oedogonium sp.
Microspora sp.	Ulothrix sp.

(Desmidiaceae)

Cosmarium blyttii	C.moniliforme
Cosmarium sp.	C.punctulatum
micrasterias sp.	

CHRYSOPHYTA

Dinobryon bavaricum	D.divergens

Table XVIII. Numbers of grazers (per gram dry weight of periphyton and per square meter) in experimental streams during the summer experiment investigating the effects of light and nutrients on periphyton communities (n=2).

PROTOCOL	MEAN NO. GRAZERS (per g dry wt.)	MEAN NO. GRAZERS (per m^2)
CNTRL	491	6530
33%hv	542	11650
NP33%hv	477	28300
N	325	5800
P	327	3650
NP	294	5400

Table XIX. Summary of incident light data for Humpback Lake experimental streams, May 17-July 1, 1988, (noon readings). Mean penetration of light to the bottom of each stream during the experiment is also presented, based on weekly light measurements.

STREAM (no.)	PROTOCOL	INCIDENT LIGHT MAXIMUM (10^{17} μE.cm^{-2}s^{-1})	INCIDENT LIGHT MINIMUM (10^{17} μE.cm^{-2}s^{-1})	INCIDENT LIGHT MEAN (10^{17} μE.cm^{-2}s^{-1})	MEAN PENETRATION (%)
1	33%hv	1.35	0.32	0.79	85
2	33%hv	1.35	0.32	0.79	82
3	NP33%hv	1.35	0.32	0.79	74
4	NP33%hv	1.32	0.32	0.79	55
5	N	3.75	1.00	2.39	80
6	N	3.60	1.00	2.36	85
7	P	3.75	1.00	2.40	83
8	P	3.75	1.00	2.39	83
9	NP	3.60	1.00	2,33	73
10	NP	3.60	1.00	2.36	69
11	CNTRL	3.75	1.00	2.40	82
12	CNTRL	3.90	1.00	2.44	85

Figure IX. Biomass accrual (n=1) V^S ash-free protein (n=6) for experimental streams in the summer experiment on the effects of nutrients and light. Accrual data are for the 16 day period preceding the removal of material for analysis of proximate composition. The correlation coefficient for the line, $r = 0.9416$. With 4 degrees of freedom the relationship between accrual and protein is linear component significant ($0.005<p<0.01$).

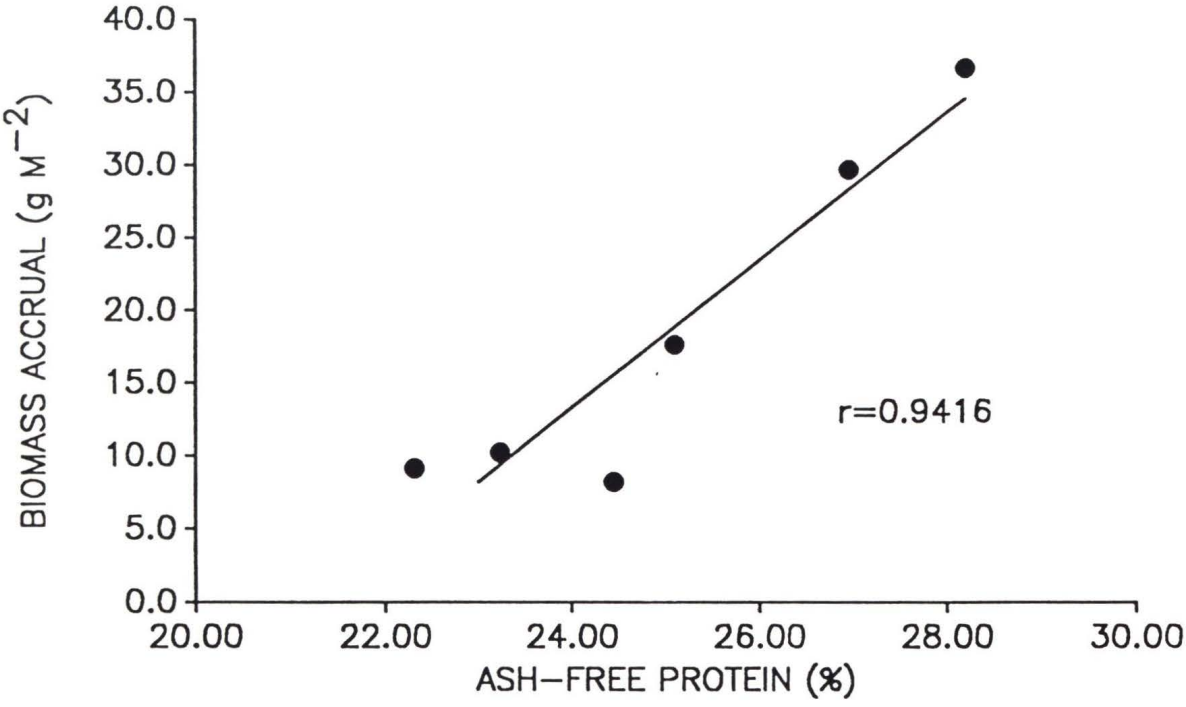
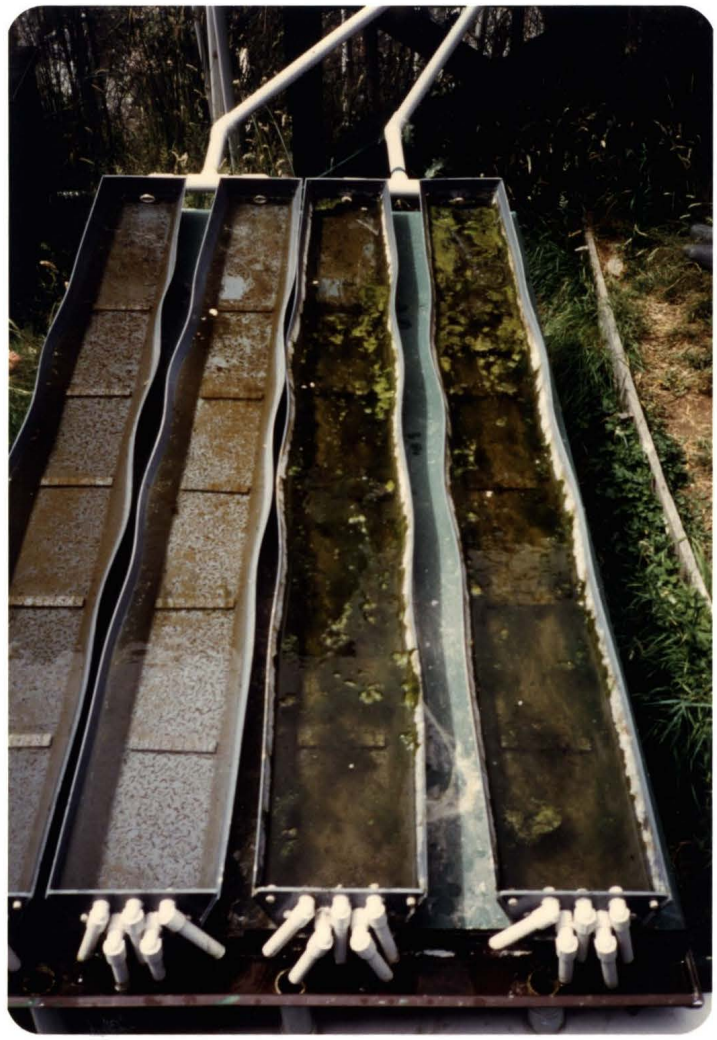

40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
BIOMASS ACCRUAL (g M^{-2})
r=0.9416
20.00
22.00
24.00
26.00
28.00
30.00
ASH-FREE PROTEIN (%)

Illustration IV Demonstration of the effect of N + P addition on periphyton accrual. The experimental streams on the left hand side are controls, those on the right hand side have an extra 0.079mg.L^{-1} of $N\text{-}NO_3$ and 2.25µg.L^{-1} of $P\text{-}PO_4$.



SEASONALITY IN PERIPHYTON PROXIMATE COMPOSITION

Introduction

Distinct seasonal patterns have been identified in periphyton communities (Hooper-Reid and Robinson, 1978; Aizaki, 1978; Shortreed et al., 1984). Variation in periphyton production, standing crop and species composition have all been investigated to some extent. It would seem logical to expect that such variation would be reflected in the corresponding proximate compositions, and hence, food value of periphyton communities. McMahon et al. (1974) have examined variation in periphyton food value employing C:N ratios. They found ratios varied from 10.7:1 down to 2.1:1 between early spring and winter, clearly indicating that changes in nutritional value of the periphyton do occur. Furthermore, they correlated low C:N ratios with increased growth of the snails Laevapex sp. and Lymnaea sp., which, however, was relatively independent of the fluctuating biomass levels. Whilst these investigations indicate the importance of seasonality in periphyton nutritional value, the use of C:N ratios to estimate nutritional value can be misleading. For example, in cases where high protein levels are found in conjunction

with high carbohydrate levels (as are present during the summer in the Humpback Lake experimental streams), a high C:N would be recorded, suggesting low nutritional value even though high levels of protein are present. Steinman et al. (1987b) also examined variation in periphyton nutritional value and found little association between C:N and protein levels. The experiment reported here was undertaken as it is important to know the spectrum of variability in periphyton proximate composition to be able to assess the potential nutritional value of particular periphyton communities on anything more than a short term basis.

Method

The proximate composition of periphyton communities developed in Humpback Lake experimental streams running at a velocity of 14cm/s in autumn, winter, spring and summer experiments was compared. These streams acted as controls in further experiments. Therefore, they received no unnatural perturbations. A summary of the weather data for each of the 4 experiments is given in Table XX.

The experiments were run on the following dates:-

Autumn	19/08/87 to 21/10/87	63 days
Winter	08/12/87 to 10/02/88	64 days
Spring	21/02/88 to 26/04/88	67 days
Summer	17/05/88 to 01/07/88	45 days*

*The summer experiment had to be cut short due to constraints imposed by the Greater Victoria Water District.

Results

Variation in proximate composition

Ash-free protein values showed highly significant variation on a seasonal basis (Table XXI.). Values were lowest in spring and subsequently increased through summer and autumn before declining again in winter (Figure X.).

Ash free lipid values also showed some significant variation on a seasonal basis (Table XXI.). Mean ash-free lipid levels were highest during winter, and were significantly higher than lipid levels during the autumn (Figure X.).

Ash free polysaccharide values showed some significant variation. Winter polysaccharide levels were significantly lower than spring, summer and autumn levels, which were not significantly different (Table XXI., Figure X.).

Ash values showed highly significant variation on a seasonal basis (Table XXI.). Values were lowest in spring and gradually increased through summer, autumn and winter (Figure X.).

Variation in species composition

Diversity was greatest during summer and autumn when the number of commonly encountered species observed reached a maximum of 67 (Table IX.). By winter, the number of routinely observed species had dropped to 19 (Table XII.).

Diatoms were dominant throughout the year (Table XXII.), ranging from 78.9% of the total count during autumn to 99.9% of the count during winter.

Amongst the diatoms Achnanthes microcephala, Synedra spp. and Tabellaria fenestrata were always the dominant species, totalling between 76.5% and 94.4% of the diatom count in autumn and winter respectively. T. fenestrata was mostly present as single cells during the winter and as chains during the rest of the year.

Chlorophyta were generally present in low numbers (less than 3% of the total count). However, their numbers increased to 19% of the count during autumn. The major species were all filamentous, with Mougeotia spp. and Ulothrix sp. most commonly encountered throughout the year. Geminella sp. was dominant during autumn but was not encountered at other times.

Low numbers of Cyanophytes were noted during summer and autumn; these were primarily Oscillatoria sp. and Merismopedia sp..

Chrysophytes (Dinobryon bavaricum and D. divergens) were present throughout the year only in low numbers (<1%). However, a bloom of D. bavaricum was noted just after the end of the summer experiment.

Species lists for spring and summer can be found in Table XXIII. and Table XVII. respectively.

Presence of grazers

The density of grazers per gram dry weight of biomass and per unit area was highest in summer and autumn and lowest during winter (Table XXIV.). Although instar numbers were not ascertained, an estimate of the proportion of later instars was gained by determining the number of larvae greater than 5mm in length. This increased through the year from 12% in winter to 83% in autumn. Spatial heterogeneity in grazer density was very high throughout the year.

Discussion

Periphyton proximate composition varies substantially over the year. The highest protein and carbohydrate levels were noted in conjunction with high biomass accrual (net production) rates during summer and autumn. The high production rates presumably lead to greater inclusion of cellular carbon into protein (Steinman et al., 1987b). There was little correlation between standing crop and proximate composition.

The increased protein and carbohydrate levels during summer and autumn lead to a much greater capacity for periphyton to act as a significant source of nutrition at these times. McMahon et al., (1974) noted that snail growth was correlated with nutritional value rather than standing crop. Whilst variability in standing crop is very high on a short term basis, little significant effect is discernible on a seasonal basis (McIntire and Phinney, 1965; Tominaga and Ichimuma, 1966; Watanabe et al, 1975; Aizaki, 1978). Higher grazing levels and emmigration are presumed to occur during periods of high production so that increases in standing crop are minimized.

Steinman et al., (1987b) noted herbivore induced changes in the proximate composition of periphyton

communities growing in laboratory streams which included changes in lipid and protein quality. However, the extent to which these changes were controlled by changes in production rates was unclear. Furthermore, the mechanism by which grazing effects and other environmental factors, such as light levels, temperature and species composition interact to yield increased production rates and, hence, protein levels during summer and fall has yet to be clearly asertained. As a result the analysis of community level changes in proximate composition, and especially of species composition related changes in proximate composition, is complex. The aforenamed authors also noted a limitation of community level analyses in "the inability to determine unequivocally whether certain chemicals are associated with specific taxa".

An increase in ash-free lipid values was noted during winter and this coincided with the period of lowest production rates. Two factors have previously been demonstrated to lead to increased lipid levels. Lipid may build up in senescent cells (Shifrin and Chisholm, 1981) which are relatively more numerous at low production rates. Second, diatoms have been noted to have higher lipid levels than other algae (Opute, 1974; Shifrin and Chisholm, 1981); these organisms reached their greatest relative dominance during winter.

Ash values increased during the summer and autumn, presumably as a result of increasing canopy development leading to a more efficient trapping of the suspended inorganic particles being carried through the stream. The adnate growths produced during winter had the highest ash weight which may be an indication of reduced turnover (and hence a larger proportion of dead material) within the periphyton. Furthermore, the increased run-off at this time would likely lead to greater sediment loads in the incoming water, and hence in the periphyton.

Summary

Seasonal patterns in periphyton proximate composition have been identified in the Humpback Lake experimental stream system. These suggest that the nutritional value of periphyton communities determined primarily by protein levels (Hambrook and Sheath, 1987) is highest during summer and autumn, when production and grazing rates also reach a maximum. Standing crop does not show a similar pattern (Figure XI.). This supports the hypothesis that the link between periphyton production rates and grazer growth rates is relatively independent of periphyton standing crop (McMahon et al., 1974).

Table XX. Summary of weather data during experimental timecourses. Figures are from the Victoria weather office at Victoria International Airport.

	SPRING	SUMMER	AUTUMN	WINTER
MEAN MAXIMUM TEMPERATURE (^{o}C)	11.8	18.1	20.8	06.5
MEAN MINIMUM TEMPERATURE (^{o}C)	3.1	7.7	7.7	-0.3
TOTAL PRECIPITATION (mm)	181	047	002	202
MEAN DAILY SUNSHINE (h)	5.4	8.3	9.2	2.2

Table XXI. Summary of statistical analysis of proximate composition.

	DEGREES OF FREEDOM	TYPE I SUM OF SQUARES	F VALUE	P[1]
PROTEIN	3	3557.96	227.32	0.0001
LIPID	3	12.74	3.73	0.0242
POLYSACCHARIDE	3	258.89	8.02	0.0002
ASH	3	2774.20	199.51	0.0001

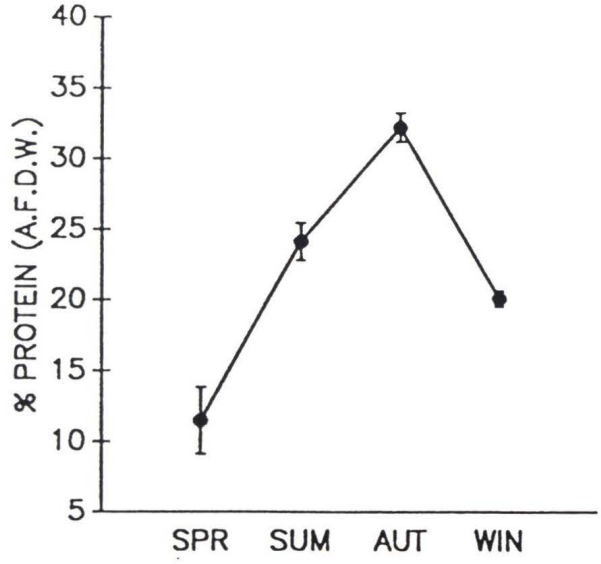
	PROTEIN	LIPID	POLYSACC	ASH
SPRING VS SUMMER[2]				
F value	82.99	0.11	1.69	32.29
P[3]	0.0001	0.7479	0.2000	0.0001
SPRING VS AUTUMN[2]				
F value	359.50	1.33	2.80	272.06
P	0.0001	0.2602	0.1014	0.0001
SPRING VS WINTER[2]				
F value	62.22	0.39	4.59	472.14
P	0.0001	0.5366	0.0377	0.0001
SUMMER VS AUTUMN[2]				
F value	65.17	0.82	0.04	83.88
P	0.0001	0.3749	0.8418	0.0001
SUMMER VS WINTER[2]				
F value	15.96	1.44	7.75	204.84
P	0.0001	0.2410	0.0079	0.0001
AUTUMN VS WINTER[2]				
F value	497.50	11.07	19.86	80.13
P	0.0001	0.0027	0.0001	0.0001

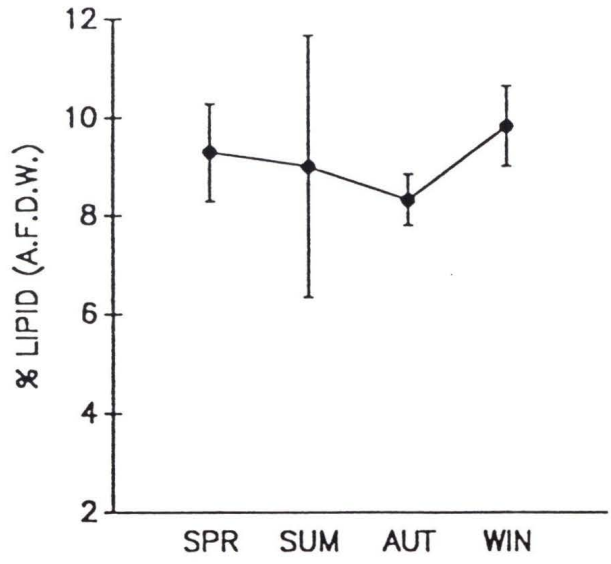
[1] P (=probability) that the proximate composition data conform to the null hypothesis that there was no significant variation in the factor under investigation (protein, lipid, polysaccharide or ash) over the four seasons. Where $P<0.05$, the data may be regarded as not conforming to the null hypothesis.

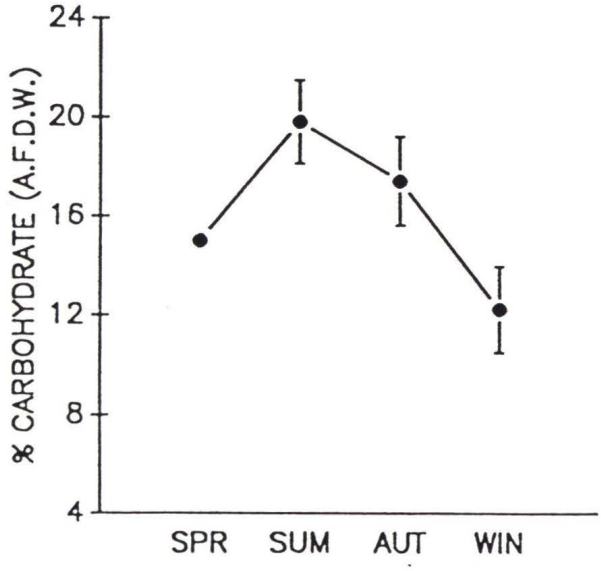
[2] Degrees of freedom = 1.

[3] P (=probability) that the data conform to the null hypothesis that there was no significant difference in the factor under investigation (protein, lipid, polysaccharide or ash) between the two seasons named. Where $P<0.05$, the data may be regarded as not conforming to the null hypothesis.

Figure X. Seasonal variation in proximate composition. SPR = spring, SUM = summer, AUT = autumn, WIN = winter. Error bars denote 95% confidence limits around the mean. Minimum sample sizes were Protein n=6, Lipid n=3, Carbohydrate n=3, ash n=5.


% PROTEIN (A.F.D.W.)
40
35
30
25
20
15
10
5
SPR
SUM
AUT
WIN


% LIPID (A.F.D.W.)
12
10
8
6
4
2
SPR
SUM
AUT
WIN


% CARBOHYDRATE (A.F.D.W.)
24
20
16
12
8
4
SPR
SUM
AUT
WIN

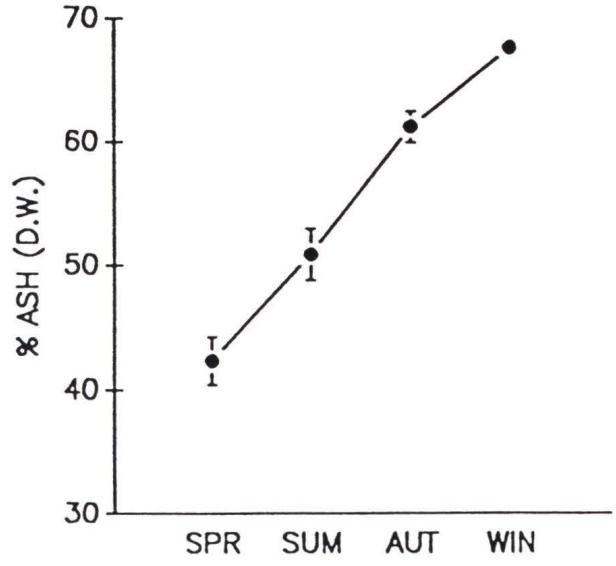

% ASH (D.W.)
70
60
50
40
30
SPR
SUM
AUT
WIN

Table XXII. Summary of periphyton species composition over an annual cycle in the Humpback Lake experimental stream system.

	SPRING	SUMMER	AUTUMN	WINTER
BACILLARIOPHYTA				
I) SINGLE CELLS	70.0[1]	85.5	71.7	99.9
II) CHAINS	26.5	5.3	7.2	0.0
CHLOROPHYTA				
I) SINGLE CELLS	0.0	*[2]	1.6	0.0
II) FILAMENTS	2.1	2.8	17.4	0.0
III) DESMIDIACEAE	0.0	*	*	*
CYANOPHYTA	0.0	1.6	1.3	0.0
CHRYSOPHYTA	*	4.2	*	*
PYRRHOPHYTA	0.0	*	*	0.0

[1]Figures are percentage of total count.
[2]denotes rare occurrence (less than 1%).

Table XXIII. Species list of organisms identified during the spring.

BACILLARIOPHYTA

(chains)

Tabellaria fenestrata

T. flocculosa

Fragilaria crotonensis

Diatoma vulgare

(single cells)

Gomphonema olivaceum

Synedra sp.A

Synedra sp.B

Synedra sp.C

Achnanthes microcephala

Melosira sp.

Asterionella formosa

Cymbella sp.

Nitzchia sigmoidea

Navicula sp.

Rhizosolenia eriensis

CHLOROPHYTA

(filaments)

Mougeotia sp.

Ulothrix sp.

CHRYSOPHYTA

Dinobryon bavaricum

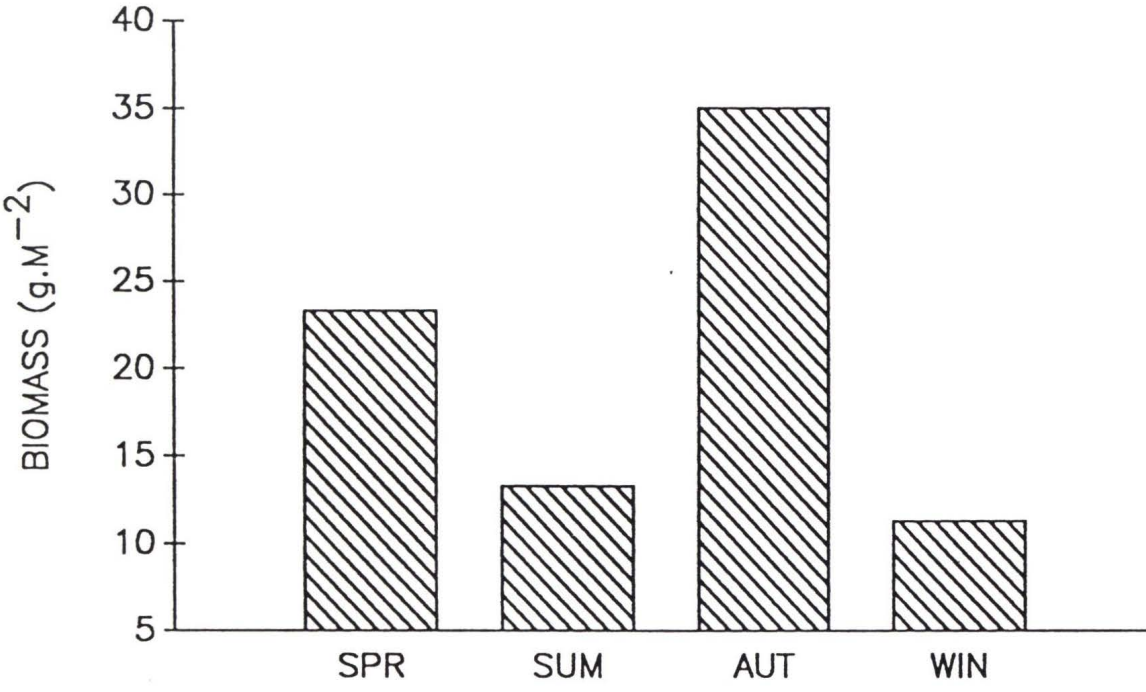
D.divergens

Table XXIV. Grazing levels in the experimental streams. Autumn and winter values also show the 95% confidence limits. Only one sample was available for spring and two for summer.

SEASON	MEAN NO. GRAZERS (per g dry wt periphyton)	MEAN NO. GRAZERS (per m^2)	PERCENT OF LARVAE >5mm
SPRING	151	3520	32%
SUMMER	491	6530	77%
AUTUMN	375 ± 419	11250[1]	83%
WINTER	29 ± 37	539	12%

[1]Estimation of biomass was from glass slides during the autumn. This method was found to significantly underestimate biomass in the experimental streams. The figure presented was derived from glass slide data using a correction factor of x5.0 (developed during a later experiment).

Figure XI. Seasonal variation in periphyton biomass in the Humpback Lake experimental streams. n=4 for spring, autumn and winter; n=2 for summer.


40
35
30
25
20
15
10
5
BIOMASS (g.M^{-2})
SPR
SUM
AUT
WIN

THE USE OF BOMB CALORIMETRY IN ESTIMATING PERIPHYTON NUTRITIONAL VALUE

Introduction

Estimates of periphyton food quality have been made using measurements of the total energy potentially available to grazers (e.g. Gray and Ward, 1979). Data comparing energy values of lentic periphyton, chironomid larvae and aquatic detritus reveal that the periphyton community at times has the lowest total energy content of the three (e.g. Eloranta and Kunnas, 1976). However, the availability of this energy varies between the constituents of the periphyton measured. A working estimate of assimilation efficiency has been derived from the literature by Benke and Wallace (1980) for freshwater invertebrates. This assumes 70% assimilation efficiency for animal food, 30% efficiency for algal food and 10% efficiency for detritus.

As lotic periphyton communities especially may act as a trap for detritus, it is likely that at least part of the energy value for any given periphyton community is derived from detrital material entrapped within the community. Therefore as the amount of entrapped detrital material increases within periphyton, its assimilation efficiency by

aquatic invertebrates will decrease. Hence, a general estimate for the assimilation of periphyton will be of less use. It would therefore seem reasonable to assess the amount of energy within a given periphyton community which is derived from the detrital component, so that the assimilation value of the whole can be estimated.

The use of bomb calorimetry in the present instance enables comparisons to be made between calorimetric data and data generated from proximate analyses using the appropriate conversion factors. This is a useful comparison as detritus often contains large amounts of indigestible compounds such as lignin and very low amounts of protein, lipid and carbohydrate (Calow, 1975). As a result data generated using bomb calorimetry include energy released from the indigestible lignins and associated compounds, whereas these would not effect data derived using conversion factors. Comparison between the two figures would therefore give an estimate of the amount of indigestible detrital material present in the periphyton samples.

The relevance of this approach is that modifications can subsequently be made to predictions of potential nutritional value once the amount of indigestible detrital material within the periphyton is known.

Method

Periphyton from 2 different protocols effective during the summer experiment were subjected to bomb calorimetry. These were from control streams and from streams subjected to 1/3 of incident light with additional phosphorus and nitrogen (NP33%hv). These two samples were chosen as they demonstrated different species compositions, proximate compositions, accrual rates and sloughing patterns, all of which have the potential to alter the relationship between periphyton energy values measured by the two different methods.

Dried samples for bomb calorimetry were carefully mixed with benzoic acid (10% W/W) to aid proper combustion (Eloranta and Kunnas, 1976).

The conversion factors used to estimate periphyton energy content for carbohydrate, lipid and protein were, respectively, 17.18kJ.g^{-1}, 39.60kJ.g^{-1} and 23.67kJ.g^{-1} (Paine, 1971).

Results

Energy values for NP33%hv streams were substantially higher than in control streams (Table XXV.). Measurements of

energy content derived from conversion factors were slightly lower than those derived by bomb calorimetry. The difference between the 2 methods of calculating energy values was greatest in NP33%hv streams, derived energy values being 11.4% lower.

Discussion

If the standard deviations for the bomb calorimetry results are also applied to the derived energy values, then the outside limits of their means lie 3.0% and 5.4% lower than bomb calorimetry results for control and NP33%hv streams respectively. This suggests a rather close parity between the 2 methods of measuring energy for the periphyton communities under investigation. The use of bomb calorimetry data would therefore be warranted in this instance.

In situations where relatively high levels of lignin or similarly indigestible compounds may be encountered, it would be advisable not to use bomb calorimetry to estimate periphyton energy values, as the difference between the methods is likely to become significant. Calow (1975) found lentic detritus which was 32% lignin on a dry weight basis, with very little lipid, protein or carbohydrate and

an energy value of over 7.0KJ.g^{-1}. Such levels among the periphyton would obviously give a rather false image of the potential nutritional value of the communities if bomb calorimetry data alone were relied upon.

Table XXV. Comparison of energy values for periphyton derived from bomb calorimetry and mean proximate composition values.

	CONTROL STREAMS	NP33%hv STREAMS
CALORIMETRY VALUES[1]	6800 ± 187[2]	9804 ± 296
DERIVED VALUES[1]	6218	8686
DIFFERENCE IN VALUES	582	1118
% DIFFERENCE	8.6%	11.4%

[1]units are joules per gram dry weight.
[2]denotes ± 1 standard deviation; n=3.

THE INFLUENCE OF PROTEIN DERIVED FROM LARVAL CHIRONOMIDS ON PERIPHYTON PROTEIN VALUES

Introduction and method

The definition of periphyton used in this work includes insect larvae as part of the community. During summer and autumn, when the density of insect larvae reaches a maximum, the protein derived from these organisms may have a significant impact on total periphyton proximate composition. Chironomid larvae in particular, as the dominant grazers, may influence periphyton proximate composition in the Humpback experimental streams. As the density of grazers within the periphyton varies considerably on a seasonal basis (Table XXIV.), it is probable that their influence on the proximate composition of the community also varies. Removal of this variable from proximate composition data should reveal more clearly the response in proximate composition shown by the primary producers under different environmental protocols.

To investigate this hypothesis, insect larvae were removed from samples of periphyton, dried and weighed. All those larvae identified were found to be chironomids.

Using a literature value for mean protein levels in chironomid larvae (Yurkowski and Tabachek, 1979), observed protein levels for periphyton communities were examined to determine the impact of chironomid protein on total protein measurements on both a seasonal basis and during individual experiments.

Results

During winter the low densities of chironomids resulted in little change in periphyton protein estimates (Figure XII.). Chironomid protein makes up approximately 0.68% of the total protein levels during this period, which, on an ash-free basis is equal to 0.13g of chironomid protein in the 19.26g of total protein recorded per 100g.

In spring, numbers of chironomid larvae increased (as did their average size (Table XXIV.)). In addition, observed protein levels were very low. As a result, the contribution of chironomid protein increased to 10.7% of the total protein (Figure XII.).

By summer, larval density had increased to between 294 and 542 organisms per gram dry weight of periphyton biomass in the different environmental regimens employed, and demonstrated substantial heterogeneity. Insufficient

numbers of samples were available to examine the significance of differences in grazer density between protocols, therefore the recorded levels for each protocol were used to estimate grazer protein effects. The percentage of total protein derived from the larvae varied between 7.9% and 12.8%. When these values were removed from total protein estimates for each protocol there was little alteration in the observed relationship between biomass accrual rates and ash-free protein level, which remained linear component significant at the $0.02>P>0.01$ level (Figure XIII.)

Larvae reached a maximum density during autumn, and were responsible for 19.9% of the total protein (Figure XII.). Due to the enormous heterogeneity in density estimates (Table VII.), little difference could be discerned between grazer densities. The trend between biomass accrual rates and ash-free protein remained identical, except for being shifted to the left in the same manner as Figure XIII. (as protein levels were reduced by 19.9%).

Discussion

A substantial proportion of total periphyton protein is

derived from chironomid larvae at all times of the year apart from winter.

Whilst invertebrate larvae are present in periphyton communities, their impact on the measurement of its energy content and proximate composition is often neglected. The presence of aquatic detritus and other grazers may add further confusion to examinations primarily focussed on the algal component. Literature values quoted by Eloranta and Kunnas (1976) also suggest that at least 15% of the total energy in periphyton communities is not derived from the algal component.

The difficulty of extracting small (<1cm) larvae from tangles of filamentous and chain-forming algae may account for the lack of literature on the topic. However, removal of larvae from filter papers after filtration of dilute samples is fairly easy. If both the larvae and periphyton are subsequently dried, estimates of their relative inputs to the proximate composition of the total community can subsequently be derived.

Grazers have been shown to modify the proximate composition (Steinman et al., 1987b), biomass and species composition (Sumner and McIntire, 1982; Steinman et al., 1987a) of periphyton communities. They may also mediate

algal interactions by further physical means which are only indirectly related to algal consumption rates (Hart, 1985). In order to acquire a more thorough understanding of the interactions between different trophic levels in lotic systems, careful examination of the role of periphyton proximate composition changes in determining invertebrate growth and survival rates is required. To achieve this, the large numbers of grazers which exist within the periphyton will have to be either removed or prevented from establishing themselves in order to obtain base line data on the proximate composition changes within the primary producers.

Figure XII. A comparison between estimates of seasonal variation in ash-free protein for periphyton communities, with (circles and solid line) and without (triangles and dashed line) the inclusion of chironomid larval protein. n=2 for estimates of chironomid larval protein.

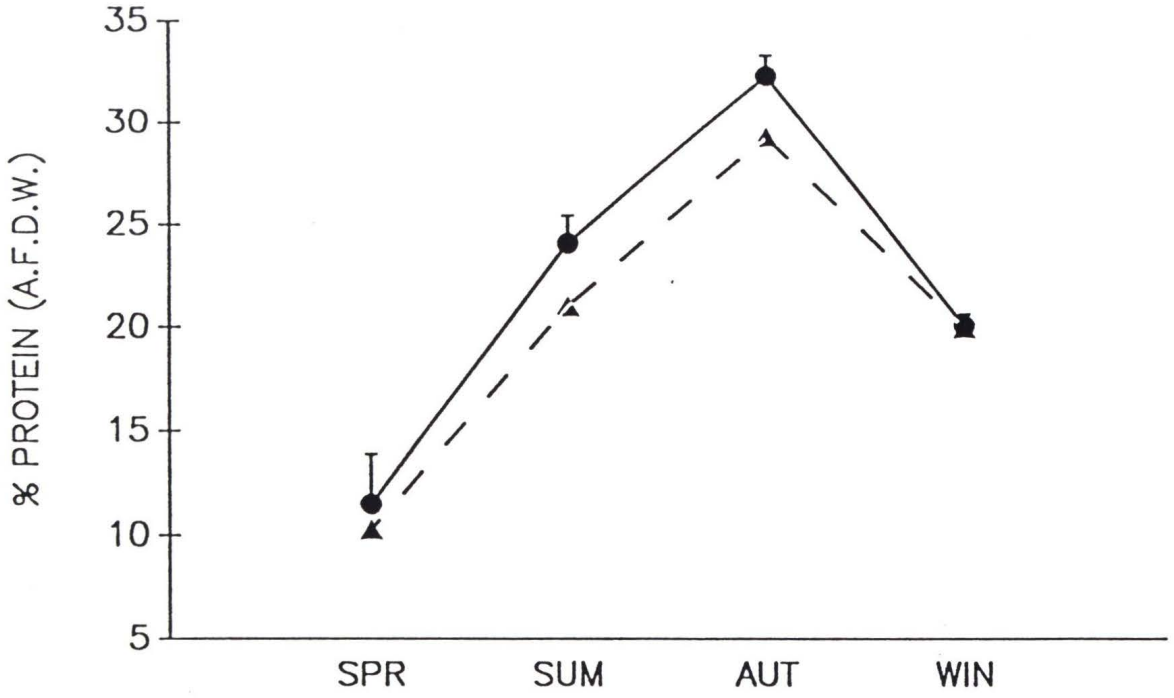
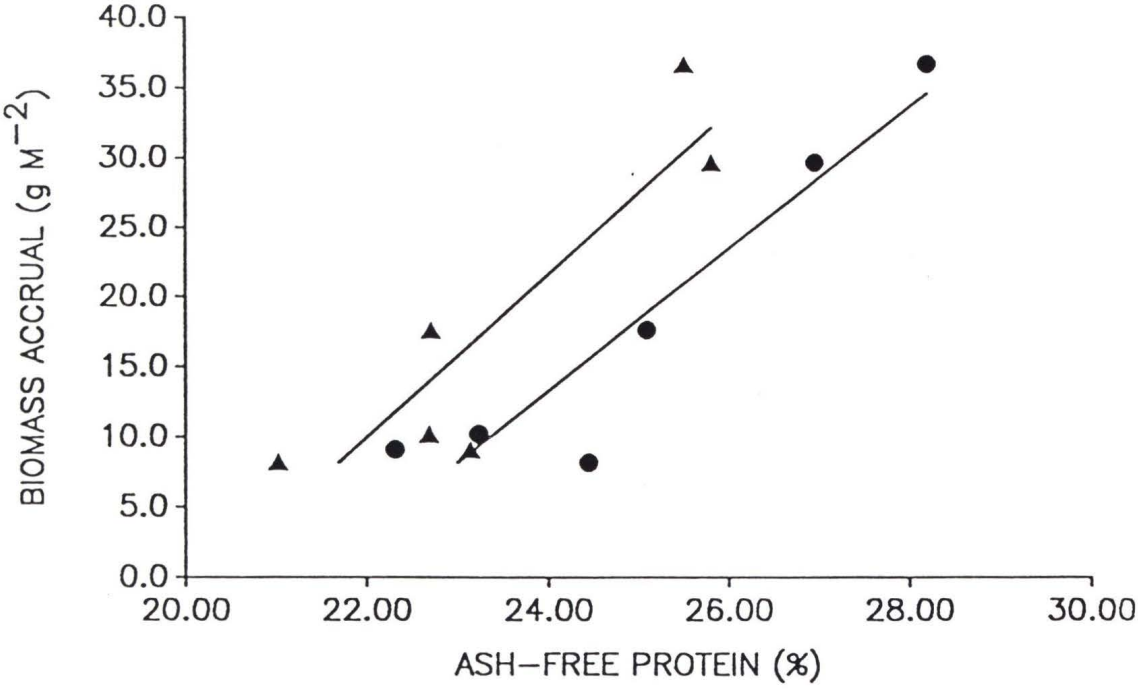

% PROTEIN (A.F.D.W.)
35
30
25
20
15
10
5
SPR
SUM
AUT
WIN

Figure XIII. A comparison of the relationship between ash-free protein levels and biomass accrual rates when chironomid larval protein is included (circles and solid line) and removed (triangles and dashed line) from ash-free protein estimates. r= 0.9416 and 0.8946 for estimates with and without chironomid protein respectively. n=2 for estimates of chironomid larval protein.


40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
BIOMASS ACCRUAL (g M^{-2})
20.00
22.00
24.00
26.00
28.00
30.00
ASH-FREE PROTEIN (%)

SUMMARY

Aims

It has been demonstrated that a need exists to learn more about the proximate composition of periphyton communities. A primary motivation for such work is the need to determine the potential nutritional significance of periphyton communities to organisms at higher trophic levels (both invertebrates and vertebrates). To this end, the aim of the present work was to examine and explain variability in periphyton proximate composition under different environmental conditions and on a seasonal basis.

Methodology

To realize these aims it has been necessary both to modify existing methodology and develop new methods for the analysis of periphyton communities.

With regard to the measurement of ash free dry weight, it has been demonstrated that the lack of a standard method has led to the development of many incompatible methodologies which serve to confuse rather than enhance comparisons between the work of different authors (Ridley-Thomas *et al.*, 1989).

Comparison of the Hach peroxymonosulphuric acid method with traditional Kjeldahl methods of protein determination suggests that further research is necessary to examine the relative suitability of the methods to different tasks. The ability of the Kjeldahl method to adequately digest diatomaceous samples is questioned by the results reported here.

The experimental stream system design first reported in Lucey et al. (1986) has proved to be an inexpensive and useful tool for ecological studies with periphyton communities. The scale of the streams is of an appropriate order to encompass the inherent heterogeneity which exists within periphyton communities. However, the ability of experimental streams on such a scale to adequately incorporate the spatial heterogeneity which exists within grazer (macroinvertebrate) populations needs further investigation.

Within the experimental streams, levels of phosphorus were in the $\mu g.L^{-1}$ range, very small changes in these levels are sufficient to change community composition. To adequately measure these effects, more accurate methods are necessary for the determination of phosphorus than are generally used, and have been used in this study. This suggestion is corroborated by Bothwell (1988) for all lotic research in British Columbia.

Variation in periphyton proximate composition

Seasonal variation

Results indicate that seasonal differences in protein, lipid, carbohydrate and ash occur (Figure X.). Protein and carbohydrate levels were highest in summer and autumn, when production and grazer density reach a maximum. A significant linear relationship was demonstrated between protein levels and periphyton accrual rates during the summer. This supports the statement of Steinman *et al.* (1987b), who suggested that at higher production rates a greater amount of cellular carbon may find inclusion into protein. The increased carbohydrate levels would similarly stem from increased production.

Lipid was highest during the winter, when diatoms, which tend to have relatively high lipid levels (Opute, 1974; Shifrin and Chisholm, 1981) were dominant. Low production rates during winter can also lead to increased lipid levels as a consequence of reduced turnover and senescence (Shifrin and Chisholm, 1981). Changes in ash weight were dependent on several major factors. Community architecture may influence ash levels, more 3-dimensional communities trapping a greater amount of inorganic detritus. The relative dominance of diatoms is also an

important factor, as, due to their silica frustule they yield more ash than chlorophytes. Finally, the amount of inorganic material in the incoming water could influence ash levels; as runoff is highest in winter (Table XX.) the levels of inorganic material in the water may also increase at this time.

Effects of current

Significant differences in proximate composition were generated as a result of increased current during autumn, 1987. Higher currents led to increased protein levels; increases in biomass accrual rate and lipid levels were also noted. It is hypothesized that the higher protein levels result from the greater supply of nutrients which are available at increased current velocities. A similar experiment run in winter generated no permanent differences in proximate composition, although biomass showed a similar linear response to the autumn experiment. During winter, light and temperature tend to be the limiting factors and the increased nutrient supply which results from higher current velocities would be of much less importance. Shear effects during winter did not lead to changes in species composition.

Enhancement with inorganic nutrients

Enhancement of incoming water with phosphorus, nitrogen or a combination of the two, led (during summer, 1988) to large increases in periphyton accrual (net production).

Changes in proximate composition with time were noted in all treatments and appear to be related to the ensuing changes in production. The present results indicate that ash-free protein varies with biomass accrual (net production) before the onset of sloughing (Figure IX.). Further examination of this relationship would require the measurement of gross production.

Carbohydrate levels showed less variability than protein. However, it seems likely that the relationship between production rates and periphyton proximate composition involves some interplay between carbohydrate and protein synthesis depending on the levels of available nitrogen. The present data lays the groundwork for such an investigation, providing a baseline which should be used to determine an appropriate methodology.

Lipid levels which have previously been linked with production are also strongly linked with species composition changes (Shifrin and Chisholm, 1981). Relationships between environmental variables and lipid levels therefore appear more complex. However, species composition changes did not appear to play a major role in

determining proximate composition during individual experiments; several rather large changes in species composition resulted in no change in proximate composition.

Effects of light

Reduction of ambient light levels to 33% of normal, affected periphyton species composition and proximate composition. The usual pattern of sloughing was also effected, and no "crashes" were noted in community biomass.

The effect of grazers

Grazing has been shown to modify periphyton community proximate composition (Steinman et al., 1987) and species composition (Gregory, 1980). It is therefore important to have an estimate of the significance of the grazer component during any experiment conducted on, or with, periphyton.

Examination of grazer effects in the Humpback experimental streams revealed a general seasonal pattern in which grazers were at a maximum during summer and autumn. At any one time, grazer densities were highly spatially variable and no substantial differences were noted in grazer density per unit biomass between different protocols (during individual experiments). As substantial biomass differences were often generated between different

protocols in an experiment, grazer numbers expressed on an areal basis showed substantial differences between protocols.

Indirect effects of grazers on periphyton proximate composition and species composition through adjustment of production rates were not assessed. The direct effect of the inclusion of protein from chironomid larvae in periphyton protein estimates was investigated and found to be substantial. However, the removal of this protein from total protein estimates did not alter the observed relationship between ash-free protein and biomass accrual; nor did it alter the spectrum of variability in periphyton proximate composition. As a result of these findings it can be stated that the observed variability within periphyton community proximate composition in the Humpback Lake experimental streams is a result of changes within the primary producer component of the community.

Conclusions

The interactions between various environmental variables in determining the composition of periphyton communities are complex and have yet to be fully understood. In general, these effects can lead to changes in the proximate composition of periphyton via changes in production rates,

species composition or more subtle intracellular modifications. The present results suggest that the dominant avenue of change is through modification of production rate. However, other changes also play an important role. For example, species composition changes in favor of diatoms lead to increased ash levels (Moore, 1977) and lipid levels (Shifrin and Chisholm, 1981). Also qualitative changes in lipid and protein may occur as a result of grazing (Steinman et al., 1987b).

The nutritional value of periphyton communities

Assuming the assimilation efficiencies derived by Benke and Wallace (1980) for freshwater invertebrates (30% efficiency for algal food), the variation measured in periphyton proximate composition must have a substantial effect on lotic energy pathways. The importance of the changes may at times be overlooked when total energy values are used to calculate potential nutritional value. As lotic periphyton communities especially may act as traps for detritus, energy values may include large amounts of indigestible compounds such as lignins. These compounds would lead to decreased digestible energy and increased total energy values. Hence, general estimates of potential nutritional value based on total energy estimates are prone to give misleading data.

Russell-Hunter (1970) has estimated that the minimum C:N ratio which animals require in their diet is 17:1 or about 16.5% protein by dry weight. McMahon et al. (1974) noted that below this critical ratio periphyton quality, in terms of protein content, appears to be more important than available biomass in supporting herbivore growth and reproduction. Periphyton communities developed in the Humpback experimental streams during both summer and autumn had dry weight values both above and below this critical value depending on the particular protocol. As such, the effect of the protocols in causing changes in periphyton proximate composition at these times is likely to be very important in terms of grazer growth rates, and subsequently in terms of the growth and survival rates of higher trophic level organisms. One finding of particular importance in this respect was that addition of nitrogen and phosphorus together causes an increase in periphyton protein levels, which addition of the limiting nutrient alone does not cause. Water fertilization programs designed to increase the yield of the top trophic level organisms should therefore employ both nitrogen and phosphorus additions regardless of the similar increases in periphyton biomass when the limiting nutrient alone is added. If the present findings are extrapolated to such a situation, increases in

biomass of summer and autumn periphyton communities, which often have protein levels in excess of 16.5% (the critical concentration of Russell-Hunter (1970)) would have limited effects on the growth and survival rates of grazers unless accompanied by increased protein levels. This requires the addition of both nutrients. Furthermore, due to the low periphyton protein levels during winter and spring, the only significant benefits of fertilization at these times would be in terms of total biomass rather than nutritional quality.

Reports of direct feeding on periphyton by salmonids in natural systems (Parkyn et al., in prep.) have recently raised new questions on the role of benthic feeding in these fish. With regard to the nutritional availability of periphyton to salmonids, I estimate that a minimum of 16.6% of periphyton protein would be available to salmonids during the autumn. This estimate is generated from the proportion of chironomid larval protein within the periphyton assuming 83.6% digestibility (De La Noue and Choubert, 1985). Further work would be necessary in order to determine whether any of the remaining periphyton protein is also available to these fish.

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APPENDIX I

A protocol for proximate analysis

Initial step

1 Weigh 0.450 - 0.500g of sample.

2 Homogenize sample with 10mL chloroform, 20mL methanol and 3mL of water for 2 minutes.

Blending has been shown to significantly reduce subsampling variation in the quantitative analysis of river periphyton (Biggs, 1987). The action is designed to break up clumps of cells and make the maximum surface area available for action of chloroform and methanol.

3 Add 10mL chloroform and homogenize for a further 30 seconds.

The chloroform:methanol ratio reaches its final state, added chloroform further leaches lipid from the cells.

4 Pour sample into filter apparatus.

5 Wash out homogenizer cup with methanol/chloroform/ water rinse bottle.

This removes any residue from the homogenizer cup. The methanol/chloroform/water rinse should have the same ratio as the extraction mixture.

6 Filter the sample.

Preweighed Whatman GF/A glassfiber filters are used to achieve filtration. Significant differences in lipid recoveries have been noted when using different types of filter (Limsuwan and Lovell, 1984). Glassfiber filters have been used for this filtration in the recent past (Whyte et al., 1987). A small amount of chloroform:methanol:water (4:4:2) is used to rinse the residue.

7 Scrape residue from filter paper into an evaporating dish and put both paper and residue in incubator at 50°C.

The residue is dried and used later for protein analysis. The filter paper is reweighed to determine how much material was lost through adhesion to the paper.

8 Transfer filtrate to a separating flask.

9 Add a few drops of water at a time until a biphasic system is created.

The filtrate goes a milky colour at the point when enough water has been added, a biphasic system can then be created by agitation of the separating flask. Further addition of water at this point leads to emulsification of the lipid.

10 Allow approximately 2 hours to separate.

Care should be taken that lipid does not adhere to the glass walls in the top part of the flask. This can be avoided by carefully swirling the separator so that the liquid runs round the top of the flask, which effectively removes any residues.

11 Take a 5mL aliquot of the methanol layer and put this in a test-tube, put on cap, seal with parafilm, label and put in fridge.

This aliquot can be used to estimate mono- and oligo-saccharides as well as low molecular weight nitrogen compounds (Whyte et al., 1987).

Lipid analysis

12 Measure chloroform volume (bottom layer).

This is done by marking the level of the chloroform layer in the separating flask then measuring this amount into a calibrated cylinder after analysis is complete. Alternatively the separating flask can be calibrated

13 Pour out at least 10-15mL of the chloroform layer.

14 Weigh aluminium evaporating dish.

Weighing is done to 4 decimal places for all measures concerned with evaporation of the chloroform layer.

15 Add 10mL of the chloroform layer to the dish.

Automatic pipettor used.

16 Evaporate chloroform layer to dryness.

17 Reweigh dish.

18 Check for impurities by rinsing 3 times with chloroform, drying the dish again and reweighing.

Lipid is removed during the chloroform rinses and any residues are impurities which need to be subtracted from the weight of fat.

19 Calculation.

$$\% \text{ fat} = \frac{[\text{wt. fat(g) x chloroform vol. (mL).}]\text{x}10}{\text{sample weight (g).}}$$

Protein analysis

P1 Measure out exactly 0.250g of dry material from the residue collected in step 7. into a digestion flask.

P2 Digest with 5mL of sulphuric acid and 20mL of a 50% solution of hydrogen peroxide at 454^{o}C, until 5 minutes after the peroxide addition is complete.

Sulphuric acid is added to the flask which is then put on the heat. Charring of the sample continues for 4 minutes, 20mL of 50% hydrogen peroxide is then added to the feed funnel on the Vigreux capillary flow manifold system (Hach Co.), this feeds into the flask over the next 7 minutes. A 5 minute clearing time follows during which excess peroxide

decomposes. This is essential as peroxide may interfere with the colorimetric assay.

P3 Remove flask from heat and let cool. Dilute to 100mL with deionised water.

Thorough mixing with water leads to a volume change of the liquid, so it is advisable to check that the volume has stopped changing before proceeding further.

P4 Weigh out Whatman GF/A glassfiber filters.

The same number of filters as sample flasks.

P5 Filter sample.

Whatman GF/A glassfiber filters.

P6 Standards are made using ammonium p-toluenesulphonate digests (steps P2-P4). 0.00mg (control), 36.48mg (6.75% protein), 51.07mg (9.45% protein), 72.96mg (13.50% protein). 102.14mg (18.90% protein) and 145.91mg (27.00% protein).

The more dilute samples (which could not be made up with sufficient accuracy using the balances available), were made up by dilutions of the more concentrated samples.

P7 Blanks and standards may be kept for a short while (1-2 weeks), in a refrigerator.

P8 Take 0.10mL aliquots of samples and standards and place in spectrophotometer tubes.

P9 Add 6.25mL Polyvinyl alcohol ($0.1gL^{-1}$)..

P10 Add 0.25mL Nessler's reagent.

P11 Mix thoroughly, then read transmittance at 460nm.

P12 Calculation. Protein (%) can be read from the calibration graph.

Carbohydrate analysis

Polysaccharide

C1 Weigh out a 25mg sample in an ampoule.

C2 Add 2.5mL of 2N sulphuric acid.

C3 Seal the ampoule.

An oxygen/propane flame generates the required temperature.

C4 Store for 6 hours at 100°C. in muffle furnace.

C5 Reopen ampoule.

C6 Remove contents and pass through glass fiber filter under suction.

C7 Pipette 70µL of sample into a test tube and dilute to 1mL with distilled water.

C8 Make up standards using glucose at the following concentrations. 0µg.mL^{-1} (blank), 20µg.mL^{-1}, 40µg.mL^{-1}, 70µg.mL^{-1}, 120µg.mL^{-1} and 170µg.mL^{-1}.

C9 Mix samples (the filtrate), standards and control solutions (1mL) with 1mL of 0.53M phenol solution.

C10 Add 5mL of concentrated sulphuric acid rapidly and directly to the solution surface without touching the sides of the tube.

An Oxford pipettor (bottle top pipettor) is suitable.

C11 Determine absorbance at 490nm after an hour.

C12 Determine sample concentration from calibration graph.

Remember to take account of all sample dilutions.

Monosaccharide

M1 Take 1mL aliquots of the aqueous methanol layer (from step 11) and dilute to 5, 10, and 15mL with deionised water.

This ensures that 1 sample will fall within the sensitivity limits of the assay.

M2 Follow steps C8-C12.

Ash weight

A1 Preheat muffle furnace to 450^{o}C.

A2 Place clean crucibles in muffle furnace for 1 hour.

This helps to clean any residues off the crucibles.

A3 Remove crucibles from furnace, place in desiccator for 10 minutes to cool, then weigh.

A4 Weigh out 0.250g aliquots of sample into each crucible. The number of aliquots per sample depends on the amount of sample available, the preferred number being 10.

A5 Place crucibles in furnace for 4 hours, then remove to desiccator for 10 minutes to cool, then weigh.

A6 Calculate percent ash.

$$\% \text{ Ash} = \frac{\text{Final sample weight}}{\text{Initial sample weight}} \times 100.$$

APPENDIX II

Justification of methodology

1.Lipid

Chloroform / methanol extraction has been used to determine lipid for a large number of small aquatic organisms including algae (Yurkowski and Tabachek, 1979), as well as for purely phycological analyses (Ben Amotz et al., 1985; Tornabene, 1985; Suen et al., 1987).

The chloroform / methanol extraction procedure is a simple and rapid method of total lipid extraction which was developed from lipid decomposition studies in fish. Initial extraction has been shown to recover approximately 94% of the extractable lipid; the further 6% being recovered by re-blending the residue with chloroform (Bligh and Dyer, 1959), though this is not always carried out. Some authors have replaced this step by further rinsing of the residue with chloroform / methanol / water (Whyte, 1987), used during these studies, or chloroform alone. Treatment of the residue with 6N hydrochloric acid, has also been shown to yield a further 6% in the case of fish muscle, at least part of which may have originated from inositides, whose protein complexes are resistant to

chloroform / methanol extraction. However, the acid treatment may also produce some fat-like material from non-lipid material (Bligh and Dyer, 1959).

Although Crude fat is usually determined for foods and feedstuffs by extraction with an organic solvent, it has been shown that ether extractions may grossly underestimate fat values in some feeds (especially extrusion processed feeds). This is due to bonding between carbohydrates and triglycerides during processing (Limsuwan and Lovell, 1984). Heat-induced linkages may also occur between proteins and lipid (Pomeraz and Melcan, 1978). As a result, chloroform/methanol extraction was deemed a more suitable method for the present work.

2.Carbohydrate

A colorimetric method of determination was found most suitable for the analysis of carbohydrate. With regard to microalgae, the Dubois method or phenol-sulphuric acid assay is a commonly used assay (Myklestad & Haug, 1972; Hitchcock, 1980; Fabregas et al., 1986). It is simple, rapid and very sensitive, and the colour produced is very stable. However, the extinction coefficients of different sugars do show some variation in the assay (Kochert, 1978).

In addition, cysteine, non-carbohydrate reducing agents, heavy metal ions and protein have been shown to interfere with this assay (Chaplin and Kennedy, 1986). However, Kochert (1978) has noted that the test is largely unaffected by the presence of protein.

The method depends on the fact that simple sugars, oligosaccharides, polysaccharides and their derivatives with free or potentially free reducing groups give an orange-yellow colour when treated with phenol and sulphuric acid (Dubois et al., 1956); the sulphuric acid causes hydrolysis of glycosidic linkages. Cysteine and orcinol may also be used with sulphuric acid. However the colour in the cysteine assay is liable to distinctive shifts and the test is more liable to contamination. The orcinol-sulphuric acid assay has a reduced interference from uronic acids and deoxy sugars (Chaplin and Kennedy, 1986). However, this latter procedure seems to be less commonly used by phycologists, possibly because the reagents degrade rapidly and have to be made up each day.

3.Protein

Choice of a Kjeldahl Method

Criticisms of the use of the Kjeldahl method for estimating

protein have been made on the basis that it measures total nitrogen and not protein. Due to the presence of various other nitrogen compounds such as amino sugars and nucleic acids, measuring protein by a total nitrogen technique may lead to serious over-estimations (O'Leary, 1985).

However, the lack of solubility of algal protein in the standard reaction mixtures for dye binding procedures is a major problem in direct protein measurements. Coupled with this, there is a certain amount of differential binding of dye to proteins. This property of the dye is still a concern in cases (such as with periphyton), where the protein standard may bear little relationship to the material being examined. It has been noted that the Lowry method (Lowry et al., 1951) responds strongly to aromatic amino acids and that samples and standards should be similar in composition (Jernejc et al., 1986). A standard with the same protein quality as the periphyton community, with its unique mix of plant, animal, fungal, and bacterial cells is simply not available. In the case of a heterogeneous community such as periphyton, the advantages of a powerful digestion procedure should not be underestimated in avoiding possible selectivity during analysis between organisms whose protein is readily soluble and those whose protein is not. It has been shown that

even textural changes in wheat between the maturation and harvest periods will lead to different responses to the Biuret reaction (Williams and Butler, 1982).

The use of chloroform / methanol / water extraction before sample digestion improves the accuracy of the Kjeldahl method. Low molecular weight nitrogenous compounds are removed during the extraction and subsequently lie in the methanol layer. These might otherwise lead to an incorrectly high estimate of protein. The result is a much more accurate technique for determining protein concentrations using a nitrogen analysis (Whyte, pers. comm.). This has made it a more accurate and hence more popular technique than dye-binding for this field of study. The preference for a Kjeldahl method of protein analysis is echoed in the literature of algal biochemical studies.

The Chemistry

The fact that the literature is extensive regarding various modifications of the Kjeldahl method for determining nitrogen points to the inadequacies which plague this difficult but necessary procedure (Norton *et al.*, 1987). The Hach system is a recently developed modification of the

Kjeldahl method which eliminates several of these inadequacies. This rapid peroxymonosulphuric acid method for the estimation of total nitrogen (Hach et al., 1985; Hach et al., 1987), is a fast and easy to use method of protein analysis. Favorable comparisons have been made with other methods of analysis for a wide sample range (Watkins et al.,1987). The efficiency of the method is achieved by the combination and improvement of two previous modifications; firstly, "precarbonization" of samples with sulphuric acid, also used by Miller and Miller (1948); and secondly, the use of peroxide as a digestion reagent. Peroxide has been quoted as a reagent in the past (Kleemann, 1921; Koch and McMeekin, 1924), and is recognized as a rapid and powerful oxidizer of organic matter (Analytical Methods Committee, 1967). The importance of multiple additions has been reported (Miller and Miller, 1948), as well as the use of dropwise additions (Koch and McMeekin, 1924; Florence and Milner, 1979). The Hach method uses a Vigreux capillary flow manifold system to achieve a constant addition of peroxide. The condenser successfully retains peroxide in the reaction mixture, as the peroxide condenses on the column and is returned to the flask, whilst water vapor is removed. With the further

addition of peroxide from the capillary flow system the concentration of peroxide in the flask increases, speeding up digestion time (Hach et al., 1987).

The combination of both controlled peroxide addition and sample precarbonization leads to a much more rapid digestion, without the need for a catalyst. Digestion time for the refractory compound nicotinic acid is 17.5 minutes, compared to 3 hours using the best of the Kjeldahl catalysts available (Hach et al., 1987).

One further advantage of the lack of catalyst is that the digest is not contaminated by salts or metal catalysts and can be further analysed for other elements (Hach et al., 1985). The additional function of the equipment for water analysis on an "effluent-in, effluent-out" basis, adds considerably to its value for analysis of growth in aquatic environments.

APPENDIX III

VARIABILITY IN THE DETERMINATION OF ASH FREE DRY WEIGHT FOR PERIPHYTON COMMUNITIES: A CALL FOR A STANDARD METHOD.

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In Press, Water Research, London.

ABSTRACT

An evaluation of diverse procedures for determining ash free dry weight (AFDW) has been carried out on a homogeneous periphyton biofilm. Results indicate a considerable degree of variability in AFDW depending on ignition time, ignition temperature and sample size. A standard method is called for; in the interim it is recommended that all three variables be reported to facilitate comparisons. Caution should be exercised when comparing results gained using dissimilar or incompletely quoted procedures.

INTRODUCTION

Calls have been made to standardise methodologies in many aspects of periphyton research (Wetzel 1983; Hammons, 1981; Weitzel, 1979) and one of the first steps in doing this should be the careful assessment of the relative accuracy and reliability of available methods. Chemical (proximate) analysis of periphyton communities has received little attention in the past (Steinman et al., 1987) and thus little "standard" methodology has been developed.

The assessment of periphyton biomass has traditionally been performed in several different ways including dry weight and ash free dry weight (AFDW), as well as estimations of accrual from chlorophyll (Horner and Welch 1981), and ATP levels (Holm-Hansen, 1973). AFDW has probably been one of the most useful estimations because it makes some attempt to separate the biomass of a periphyton community from the biomass of the inorganic particulates which often become entrapped within it.

The estimation of AFDW is achieved through subtracting the weight of material remaining after ignition in a muffle furnace (fixed residue) from the dry weight of the sample (total residue). Depending on the temperature, ignition loss may include organic matter, carbon, sulphur

(sulphites and sulphides), carbon dioxide from carbonates, water of hydration, and occasionally nitrogen oxides or ammonia (ASTM, 1978). The temperature at which the material is ashed will obviously affect the proportions of each category that are removed.

It would therefore be expected that if sample ashing is carried out using a variety of different temperatures, times and sample sizes, differences in fixed residues will likely occur. Consequently, the amount of useful information obtained from ashing is contingent upon each procedure being performed at the same temperature and for the same length of time using samples of equivalent size. There is evidence to suggest that particle size of the sample may also have a significant effect when minimal ignition times are used. Nevertheless, in the case of periphyton there appears to be no specific standard method for ashing of samples; an examination of the periphyton literature reveals a plethora of different ashing methods (Table 1).

Standard ashing procedures have been set for various sample types, however, there is a suprising variability between standards set by different associations. The American Society for Testing and Materials (ASTM, 1978) notes that, for carbonacous samples, temperatures in excess

of 500 ^{o}C are unsuitable. Unfortunately, whilst recommending "the report of analysis should give the ignition temperature and length of ignition time," they record only temperature themselves. The Association of Official Analytical Chemists (AOAC, 1984) recommends two methods for plant material i) 600 ^{o}C for 2 hours with 2g samples, or ii) ignition at 525 ^{o}C to a "white ash". However, these two methods can yield different results since the first method does not produce a white ash. The American Public Health Association (APHA 1985) suggests temperatures of 550 ^{o}C for 1 hour on "Solid or semi-solid" samples such as river and lake sediments, which have an initial wet weight of 25-50g. In periphyton literature, few authors state why they have chosen their particular ashing technique. An examination of several different ashing methods has therefore been carried out to identify what, if any, significant differences exist between published methods.

MATERIALS AND METHODS

SAMPLE MATERIAL

A periphyton biofilm consisting primarily of Cladophora glomerata (L.) Kuetzing 1845, was collected from a small flooded quarry near Shawnigan Lake, Vancouver Island, British Columbia. This biomass was dried (55 ^{o}C to constant weight), ground to a powder (Braun Coffee Grinder, Type KSM 2) and sieved (0.0625 mm mesh) to improve sample homogeneity.

ASHING PROCEDURE

Different sized aliquots of the sample were placed in porcelain crucibles (Coors, U.S.A., Type 769-3), ashed in a non-forced-air muffle furnace (Model M-15A-1A; Blue M Elect Co., Blue Island, Illinois, U.S.A.) at various temperatures, for various lengths of time. Six replicate crucibles were used for all 2g samples, whilst 10 crucibles were used for smaller sample sizes. The percentage weight loss was calculated for each replicate crucible set, and mean and standard deviation calculated and plotted.

RESULTS

1. As ashing duration increases, the amount of material volatilised is seen to increase, as did variability between replicates (Figure 1).

2. As sample size increases, the amount of material volatilised decreases; variability between replicates also decreased with sample size decrease (Figure 2).

3. As temperature increases, the amount of material volatilised is also seen to increase. (Figure 3).

DISCUSSION

The degree of variability exhibited between results indicates that the comparison of results obtained by different methods would be misleading. When protein, lipid and carbohydrate (or levels of other cellular components) are estimated on the basis of AFDW for freshwater periphyton, with its often large inorganic component, a significant compounding of errors can occur.

For example, if biofilm protein concentrations of 10% were determined and expressed on an AFDW basis, then given the variability of Figures 1 to 3, results would range from 65.6% to 42.6% protein, depending on the ashing method chosen (Table 2). Such differences based on different AFDW analyses render comparisons inappropriate. Therefore it is essential that a standard periphyton ashing technique be established to both facilitate comparisions of published

results and to serve as a safeguard when comparing previous work.

Concentration has been centred upon small sample sizes in this study, because it is the belief of the authors that a "standard sample size" should be as small as necessary, without sacrificing accuracy, allowing the maximum number of workers to conform to the standard. A small sample size should also increase the speed of the analysis and allow an optimal number of replicates.

Methods of ashing to "grey" or "white" ash were not chosen since variability between replicates substantially increased when longer ashing times were used. We believe this is due to the effect of small temperature gradients within the muffle furnace which, over a prolonged time, have an increasing effect on sample variance. The gradients were detected by noting the correlation between position of crucibles in the furnace and the resultant AFDW. Similar gradients were also found in a trial conducted with a comparable furnace.

CONCLUSION

Significant differences in ash residues were found as a result of using different ashing methods, indicating the necessity of determining how temperature affects volatilization of specific periphyton organic and inorganic

constituents. Certainly more than just the organic portion is lost when using the majority of recommended techniques. ASTM (1978) cautions against using temperatures in excess of 500 ^{o}C when carbonaceous material is involved, whereas, higher levels are suggested in many other published methods. The most frequent temperature used is probably 550 ^{o}C, though this may cause dehydroxylation of the clay mineral fraction (Biggs 1987). The validity of comparing periphyton AFDW results should be questioned since such results are obtained using non-standard practices. A call is made to establish a "standard" procedure for estimating periphyton "ash free dry weights".

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TABLE 1. Comparision of recommended ignition temperatures and times for conducting Ash Free Dry Weight estimations.

MATERIAL	TEMP ^{o}C	TIME Hours	REFERENCE
Brown algae	400	6.0	Hellebust and Craigie (1978)
Carbonaceous matter	500 (max)	-	ASTM (1978)
Sediment	550	1.0	APHA (1985)
Plant matter	550	2.0	Clark and Shera (1985)
Animal feed	600	2.0	AOAC (1984)
Periphyton	400	4.0	Biggs (1987)
Periphyton	450	-	Gons and Van Keulen (1983)
Periphyton	475	-	Reuter et al., (1983)
Periphyton	500	-	McIntire (1966)
Periphyton	500	1.0	APHA (1985)
Periphyton	500	4.0	Stockner and Shortreed (1978)
Periphyton	525	0.5	Hill and Webster (1982)
Periphyton	550	2.0	Meulemans and Heinis (1983)
Periphyton	550	3.0	Muller (1983)
Periphyton	600	2.0	Eloranta (1982)

Table 2. Variability in estimated protein levels, calculated on an AFDW basis, using results from the ashing trials.

TEMP °C	TIME (Hr)	SAMPLE (mg)	% PROTEIN	REFERENCES (Table 1)
525	0.5	75	65.6	Hill & Webster
550	1.0	200	53.9	APHA
600	1.0	150	47.6	-
600	2.0	200	45.5	-
600	16.0	2000	42.6	AOAC

Figure 1. Loss of organics following ignition at 600°C, as a function of oven residence time; solid circles = 200mg, open circles = 2000mg. Vertical bars = two standard deviations.

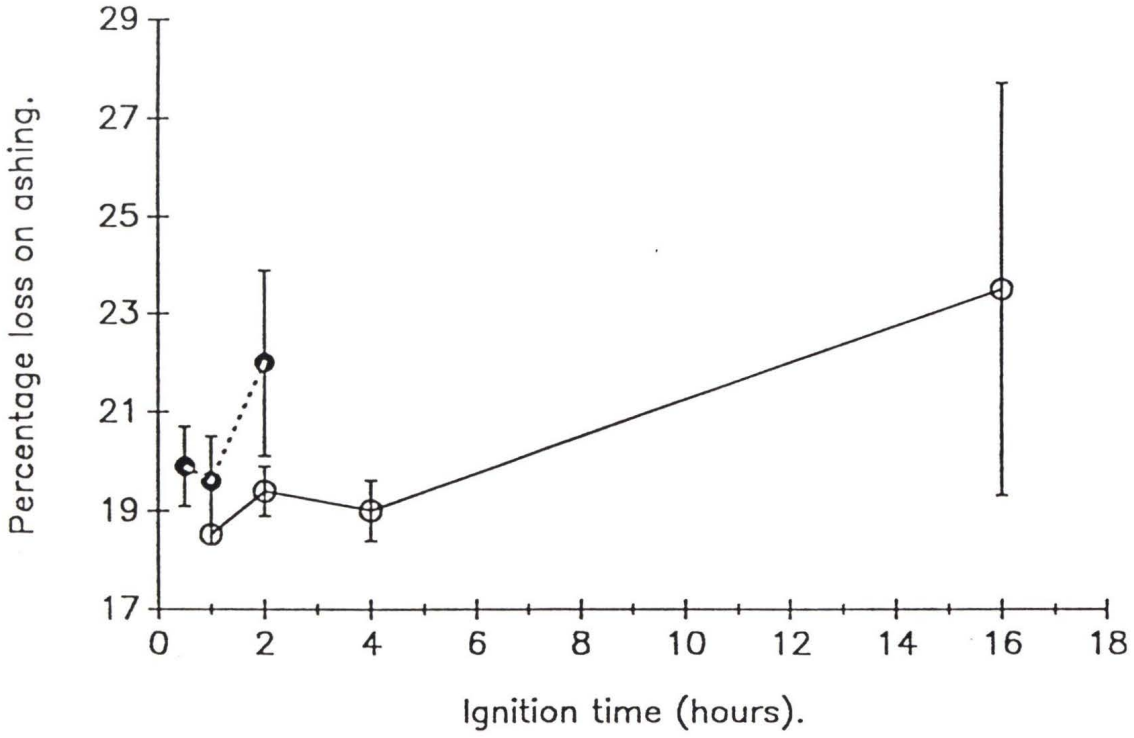

Percentage loss on ashing.
29
27
25
23
21
19
17
0
2
4
6
8
10
12
14
16
18
Ignition time (hours).

Figure 2. Loss of organics following ignition (600 ^{o}C), as a function of sample size; oven residence time = 1.0h. Vertical bars = two standard deviations.

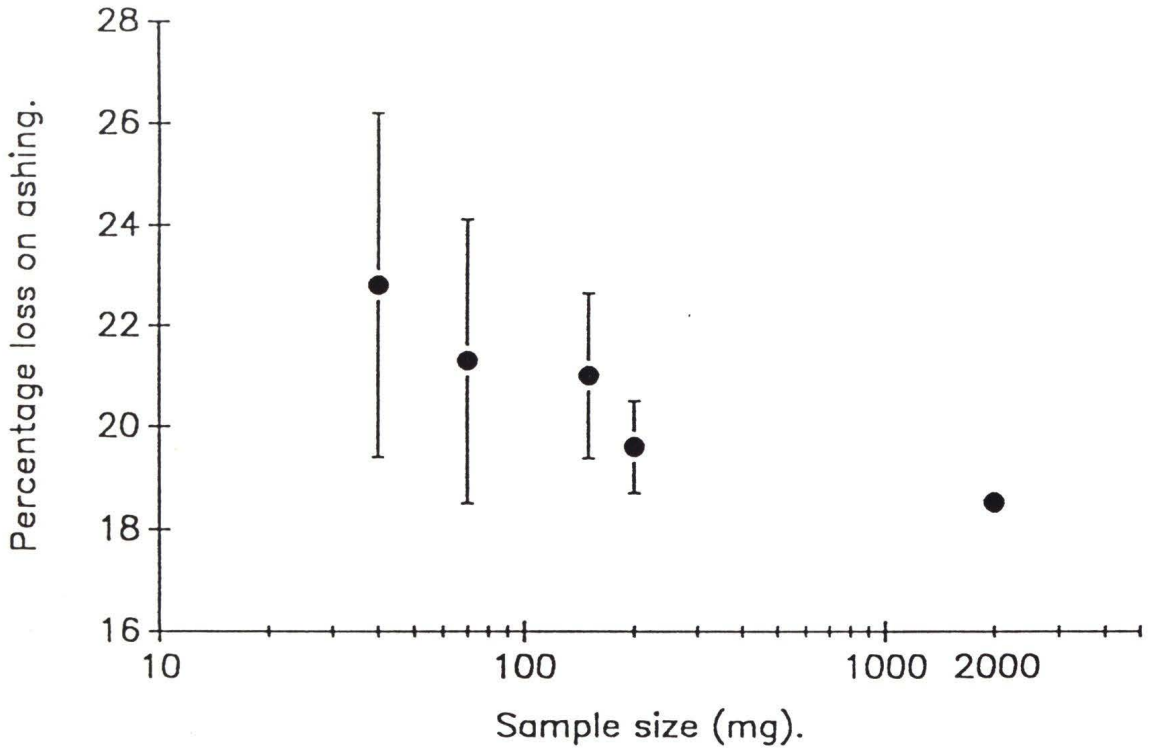
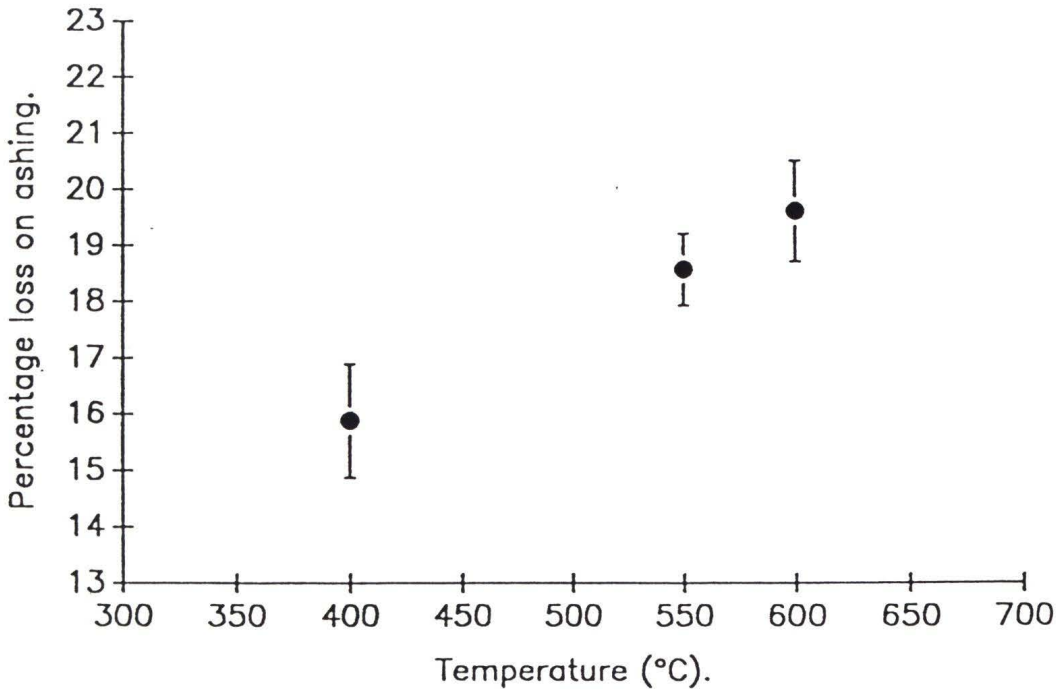

28
26
24
22
20
18
16
Percentage loss on ashing.
10
100
1000
2000
Sample size (mg).

Figure 3. Loss of organics following ignition at selected temperatures; sample size = 200mg, oven residence time = 1.0 h. Vertical bars = two standard deviations.


Percentage loss on ashing.
23
22
21
20
19
18
17
16
15
14
13
300
350
400
450
500
550
600
650
700
Temperature (°C).

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APPENDIX IV.

Full list of species identified, with authorships

BACILLARIOPHYTA
(chain formers)
- Diatoma vulgare Bory***
- Fragilaria sp.A
- Fragilaria sp.B
- Fragilaria crotonensis Kitton***
- Fragilaria virescens Ralfs***
- Tabellaria fenestrata (Lyngb.) Kutz.***
- Tabellaria flocculosa (Roth) Kutz.***

(non chain formers)
- Achnanthes microcephala (Kutz.) Grun. 1880****
- Asterionella formosa Hasall***
- Cocconeis diminuta Pant****
- Cyclotella sp.
- Cyclotella bodanica O. Mull.***
- Cyclotella compta (Ehrbg.) Kutz.***
- Cymbella cymbiformis Font.*****
- Cymbella sp.
- Diploneis elliptica (Kutz) Cleve***
- Eunotia curvata (Kutz.) Lagerst.****
- Eunotia robusta Ralfs***
- Frustulia rhomboides (Ehrbg.) De Toni***
- Gomphonema acuminatum var. acuminatum Ehrbg.***
- Gomphonema constrictum Ehrbg.***
- Gomphonema olivaceum (Lyngb.) Kutz.***
- Melosira sp.A
- Melosira sp.B
- Navicula sp.
- Nitzchia sigmoidea W. Smith +
- Nitzchia vivax
- Pinnularia streptoraphe Cleve****
- Rhizosolenia eriensis H.L. Smith***
- Rhopalodia gibba (Ehrbg.) O. Mull.***
- Stauroneis phoenocentron Ehrbg.***
- Surirella sp.
- Synedra sp.A
- Synedra sp B
- Synedra sp.C

CYANOPHYTA
- Anabaena sp.
- Gomphosphaeria lacustris Chodat 1898*
- Lyngbya sp.
- Merismopaedia sp.
- Oscillatoria sp.

PYRRHOPHYTA
- Peridinium sp.

CHLOROPHYTA
(non-filamentous forms)
- Ankistrodesmus fulcatis (Corda) Ralfs 1848*
- Ankistrodesmus spiralis (Turner) Lemmerman 1908*
- Crucigenia rectangularis (A. Braun) Gay 1891*
- Dictoshaerium pulchellum Wood 1874*
- Eudorina sp.
- Oocystis sp.
- Pediastrum sp.
- Scenedesmus bijuga (Turp.) Lagerheim 1893*
- Scenedesmus quadricata (Turp.) de Brebisson 1835*
- Stichococcus sp.
- Unidentified coccoid A.

(filamentous forms)
- Bulbochaetae sp.
- Geminella sp.
- Microspora sp.
- Mougeotia sp.A
- Mougeotia sp.B
- Mougeotia sp.C
- Mougeotia sp.D
- Oedogonium sp.
- Spirogyra sp.
- Stigeoclonium sp.
- Ulothrix sp.
- Zygnema sp.

(Desmidaceae)
- Arthrodesmus convergens Ehrbg. 1848++
- Cosmarium sp.A
- Cosmarium bioculatum Breb.**
- Cosmarium binum Nordst.**
- Cosmarium blyttii Wille**
- Cosmarium moniliforme (Turp.) Ralfs**
- Cosmarium punctulatum Breb.**
- Gonatozygan monotaenium DeBary**
- Micrasterias sp.
- Spondylosium planum (Wolle) W & G.S. West**
- Staurastrum avicula Breb.**
- Staurodesmus cuspidictus (Breb.) Teiling**
- Xanthideum antilopeum (Breb.) Kutz**

CHRYSOPHYTA

Dinobryon bavaricum Imhof 1890*
Dinobryon divergens Imhof 1887*

SOURCES

* Prescott (1962)

** Lind and Brook (1980)

*** Bourrelly (1968)

**** Patrick and Reimer (1966)

***** Patrick and Reimer (1975)

+ Von Euler (1951)

++ Prescott et al. (1982)

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Proximate composition of periphyton communities growing in experimental streams.

[redacted]

Christopher Ridley-Thomas

4/4/89

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