Trophic Structure and the Effects of Agriculture in a Headwater Stream in Southern Ontario using Stable Isotopes and Secondary Production

by

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Abstract

In this thesis, the effect of agricultural activity on lotic macroinvertebrate communities was evaluated at the field scale. Abundance, biomass, secondary production, species richness and diet were compared between habitats upstream and downstream of an agricultural site. Estimates of diet, obtained using stable isotopes of carbon and nitrogen, and secondary production were combined to determine the dependence of individual taxa and each habitat on allochthonous and autochthonous inputs.

Distinctive shifts in the composition and production of benthic invertebrate communities within the 400 metre study reach were consistent with the expected effects of agricultural landuse. Larger, more sensitive forms, especially mayflies, caddisflies and stoneflies were replaced by smaller, more tolerant chironomids, resulting in a 30% reduction in secondary production at the downstream site. A finer and more homogenous stream bed downstream, likely due to increased sedimentation, eliminated habitat and led to poor recruitment of the dominant hydropsychid caddisfly *Hydropsyche slossonae*. Lower density, biomass and production by the shredders was likely a result of the reduced ability of the site to retain coarse particulate matter and lower inputs of allochthonous matter from the cleared farmland. Greater retention of fine detrital sediments also supported increased abundance, biomass and secondary production of Chironomidae.

Periphyton was grown on glass plates suspended in the water column under two conditions of light and water velocity, over two seasons, to test the hypothesis that boundary layer thickness is an important variable affecting stable isotope fractionation in benthic algae. Isotopic signatures for both carbon and nitrogen in samples of periphyton varied with light intensity and season, but not current velocity. These results suggested that isotopic fractionation in periphyton was more strongly influenced by the intensity of metabolic activity than by variations in the thickness of the benthic boundary layer.

Diatom and chlorophyte carbon signatures followed a seasonal pattern in which reduced metabolic fractionation under high growth conditions in summer led to enriched signatures. Carbon signatures of the macroinvertebrates reflected the seasonal variation in algae.

The relative dependence of different functional feeding groups on allochthonous resources followed the expected trend (i.e. shredders were most, and scrapers least, dependent on terrestrial inputs). However, many taxa exploited a wider food base than their feeding group classification implied (e.g. shredders and filterers). The dietary analysis indicated that *D. nivoriunda*, *B. brunneicolor*, *Parakiefferiella* sp. and *Pycnopsyche* sp., should be reassigned different trophic guilds.

Invertebrate diets were similar at sites 1 and 5, suggesting that inputs of autochthonous and allochthonous organic material did not limit secondary production at the downstream site. The analyses support the conclusion that altered

benthic community composition and lower secondary production downstream were primarily due to a shift in stream bed morphology, a likely result of agricultural activity on the adjacent fields. Estimates of autochthonous primary production and allochthonous inputs necessary to support the macroinvertebrate community were within expected ranges for a moderately enriched headwater stream with a partial canopy.

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CHAPTER 1

General Introduction

Agricultural activities can be major contributors to environmental stress. Those activities which lead to the pollution of watercourses include clearing of the land, sub-surface tile drainage, channelisation, tilling, planting, grazing and the application of fertilisers, pesticides and herbicides (Bilby and Bisson 1992; Cooper 1993). Ground water and overland flow convey organic and inorganic sediments and applied chemicals from the disturbed soil into the watercourses draining agricultural catchments.

In this thesis, the effect of agricultural activity on lotic macroinvertebrate communities is evaluated at the field scale. Abundance, biomass, secondary production, species richness and diet are compared between habitats upstream and downstream of an agricultural site. Estimates of diet, obtained using stable isotopes of carbon and nitrogen, and secondary production are combined to determine the dependence of individual taxa and each habitat on allochthonous and autochthonous inputs. The following paragraphs outline the utility of benthic macroinvertebrates in biological assessments, the effects of specific agricultural inputs on invertebrate communities and the objectives of this thesis.

Benthic macroinvertebrates are frequently used to assess the biological aspects of water quality in streams and rivers. Rosenberg and Resh (1996) summarised the characteristics which lend macroinvertebrates, and aquatic insects in particular, to the biomonitoring of aquatic ecosystems: (1) they are ubiquitous; (2) the large number of species exhibit a range of responses; (3) they are relatively sedentary; (4) they can have

long life cycles. Agricultural inputs known to impact benthic communities include suspended sediments, nutrients, organic wastes, pesticides, herbicides and solar irradiance (Dance and Hynes 1980). These are briefly discussed below in order to emphasise the complexity of landuse responses in aquatic ecosystems.

Sediments

An increased rate of erosion and the subsequent sediment loading of lotic environments is one of the physical impacts imposed on aquatic ecosystems. Alterations to the land through sub-surface tile drainage, channelisation, construction of ditches and land clearing can create high sediment loading during initial construction and increased delivery rates in the long term. Tilling, planting and the trampling of riverbanks by grazing animals loosen the soil and may also increase soil erosion. Suspended solids may interfere with invertebrate respiration, abrade the exoskeleton or hinder food collection of filter feeders. Reduced light penetration may limit algal and macrophyte growth, resulting in secondary effects such as the depletion of habitat and high quality autochthonous food sources.

There have been many attempts to determine the effects of sedimentation on lotic benthic populations (Minshall and Minshall 1977; Resh 1977; Williams 1980; Lenat et al. 1981; Erman and Erman 1984). The analysis is complicated by the effects of mean particle size, size heterogeneity and particle surface roughness. The general trend of these studies is that as mean particle size increases, a corresponding increase is observed in overall numbers of invertebrates, number of taxa, number of Ephemeroptera and

secondary production of Trichoptera. Rabeni and Minshall (1977) noted that densities of Chironomidae increased, while densities of Ephemeroptera, Trichoptera and Plecoptera decreased following the application of a layer of silt to the substrate. A similar dominance of Chironomidae and reduction of *Baetis*, *Simulium*, *Cheumatopsyche* and *Stenelmis* was observed by Berkman et al. (1986) in areas of high sedimentation. Sedimentation can result in the loss or creation of habitat for specific organisms by reducing the area of exposed rock surface and smothering periphyton populations. In cases where depth is reduced, air temperatures may exert a greater influence on water temperature fluctuations. Some organisms benefit from sedimentation while many others do not.

Pesticides and Herbicides

Ground and surface water from land treated with pesticides can be highly toxic to non-target aquatic organisms. Dosdall and Lehmkuhl (1989) observed catastrophic drift up to 107 km downstream from the point of methoxychlor treatment in the North Saskatchewan River. The methoxychlor drift response was dependent on species, distance from the injection site, and the time after injection. Initial catastrophic drift and high mortality are typically followed by a reversion phase and recolonization through drift from upstream reaches, migration from the hyporheos, and egg hatching (Wallace and Hynes 1981). Wallace et al. (1991) studied the effect of the insecticide methoxychlor on streams over a 5-year period. The depleted macroinvertebrate population coincided with a two-order-of-magnitude decrease of FPOM exported downstream over the 3-year treatment and 1-year recovery period. The secondary impact of the reduced organic matter available

downstream was not determined. The toxicities of pesticides are probably highly variable and a function of invertebrate morphology, lifestage, size, habitat and sex.

Herbicides have lower acute toxicity to animals than pesticides and the effects on aquatic communities at low levels can be difficult to detect (Cooper 1993). Some herbicides are toxic to algae and, by reducing primary production, they may limit habitat, food availability and dissolved oxygen concentrations.

Nutrients

Inorganic and organic forms of nitrogen and phosphorous may appear in rivers and streams from the application of commercial fertilisers and animal wastes. These important nutrients may enhance algal and macrophytic growth, alter the balance between autochthonous and allochthonous production, and accelerate eutrophication. Excessive algal growth can alter benthic species composition because of physical changes in habitat or altered daily fluctuations in dissolved oxygen concentrations. In extreme cases, the decay of plant biomass or large amounts of organic matter from animal wastes may lead to the proliferation of aerobic decomposers and reduced oxygen levels. Stream characteristics such as flow rate, canopy, depth and substrate type can also influence primary production levels.

Temperature

Agricultural activities may also influence water temperatures. Sedimentation may reduce water depths and increase the diurnal, seasonal and annual water temperature

fluctuations. Clearing vegetation from the riparian zone will increase irradiance and primary production in light-limited cases, lead to elevated temperatures and reduce the capacity to dissolve oxygen (Brazier and Brown 1973; Murphy et al. 1981; Rosenfeld and Roff 1991; Bilby and Bisson 1992). Even small changes in temperatures can affect growth, metabolism, reproduction, emergence and the distribution of aquatic insects. Vannote and Sweeney (1980) concluded that each of the species they studied had an optimal temperature, above or below which larval tissue growth was suppressed more than adult tissue development, resulting in smaller adults and reduced fecundity. The importance of water temperature is emphasised in the work of Benke and Parsons (1990) and Benke and Jacobi (1994). They developed relationships predicting daily growth rates for Simulidae and Ephemeroptera in blackwater streams on the Georgia Coastal Plain, in which daily water temperature was the only independent variable.

Increased temperatures, however, are not necessarily detrimental to aquatic ecosystems. Bilby and Bisson (1992) compared allochthonous and autochthonous contributions to the trophic support of fish populations in clear-cut and old-growth forested streams. They concluded that the higher fish production in the clear-cut site, despite a two-fold larger combined allochthonous and autochthonous input in the old-growth site, reflected the greater production of autochthonous algae, which are more nutritious than allochthonous material.

Assessments of water pollution have focused on alterations in macroinvertebrate community diversity, loss of sensitive species, or increases in the number of tolerant species as a means to evaluate biotic effects. However, the ability of an animal to fix and

retain energy is probably the best measure of success in an environment (Benke 1993). In Chapter 2, the effects of agriculture are assessed using abundance, biomass, species richness and estimates of secondary production at the species level both upstream and downstream of an agricultural site. The effect of increased agricultural activity between 1995 and 1996 is also evaluated.

The remainder of the thesis uses stable isotope methods to identify trophic relationships in the stream and, in concert with estimates of secondary production, to quantify the flow of organic matter among taxa. The utility of stable carbon and nitrogen isotopes as natural tracers of energy flow in marine, estuarine and freshwater ecosystems (Hobson and Welch 1992; Rosenfeld and Roff 1992; Bunn and Boon 1993; Junger and Planas 1994; Hecky and Hesslein 1995; Bootsma et al. 1996) is dependent upon the presence of distinct isotopic signatures among potential primary producers. It is not uncommon, however, for the isotopic ratios of aquatic macrophytes or algae at a site to vary by 10‰ for carbon and 3‰ for nitrogen (Wada and Hattori 1976, 1978; Minagawa and Wada 1984; Kline et al. 1990; Fry and Wainright 1991; Fogel et al. 1992; Bunn and Boon 1993; MacLeod and Barton 1998a). Unfortunately, few researchers have conducted a thorough analysis of the variation in autotrophic stable isotope signatures. This casts doubt on the conclusions drawn from some studies and has limited the resolving power of stable isotope analysis to trace trophic interactions in others (France 1995).

In Chapter 3, the hypotheses that periphyton $\delta^{13}C$ and $\delta^{15}N$ are influenced by boundary layer thickness, growth rate and differences among algal taxa are tested by conducting field experiments in which periphyton is grown on glass plates suspended in

the water column under two conditions of light and water velocity, over two seasons. The experiments also determine the seasonal isotopic signatures of diatoms in the stream.

In Chapter 4, the trophic relationships between primary producers, herbivores and predators are investigated and the relative dependence of the invertebrates on allochthonous and autochthonous material is quantified. The objectives are 1) to determine whether the carbon signatures of chlorophyte and bryophyte primary producers vary seasonally, 2) to assess whether temporal algal signatures are reflected in the macroinvertebrate herbivores, 3) to examine the effect of liquid swine manure, applied to the adjacent farmland, on the nitrogen signatures of the downstream flora and fauna, 4) to evaluate macroinvertebrate dietary shifts between seasons (May to September versus September to May) and between sites (upstream and downstream of agricultural land), and lastly, 5) to determine the overall dependence on allochthonous and autochthonous inputs at sites upstream and downstream of agricultural landuse in a headwater stream.

CHAPTER 2

Secondary Production of Benthic Macroinvertebrates in a Headwater Stream and the Effects of Agriculture at the Field Scale

Abstract

Distinctive shifts in the composition and production of benthic invertebrate communities within a 400 metre study reach were consistent with the expected effects of agricultural landuse. Larger, more sensitive forms, especially mayflies, caddisflies and stoneflies were replaced by smaller, more tolerant chironomids at the downstream site. A finer and more homogenous stream bed downstream, likely due to increased sedimentation, eliminated habitat and led to poor recruitment of the dominant hydropsychid caddisfly *Hydropsyche slossonae*. Lower density, biomass and production by the shredders was likely a result of the reduced ability of the site to retain coarse particulate matter and lower inputs of allochthonous matter from the cleared farmland. Greater retention of fine detrital sediments also supported increased abundance, biomass and secondary production of Chironomidae.

While total numerical abundances were similar at the top and bottom of the study reach, most individual animals were smaller leading to lower secondary production downstream. Increased mortality and/or drift in the late instars was likely due to greater top-down pressure by the additional benthic insectivorous fish species at this site.

General faunal patterns were consistent over both years but increased agricultural activity in 1996 was not reflected in the benthic invertebrates, excepting a major increase in Chironomidae abundance, possibly due to differences in rainfall and stream discharge.

Introduction

Agricultural activities have been identified as major contributors to environmental stress. Those activities which lead to the degradation of watercourses include clearing of the land, sub-surface tile drainage, channelisation, tilling, planting, grazing and the application of fertilisers, pesticides and herbicides (Bilby and Bisson 1992; Cooper 1993). Ground water and overland flow convey organic and inorganic sediments and applied chemicals from the disturbed soil into the watercourses draining agricultural catchments. Agricultural inputs known to impact lotic macroinvertebrate communities include suspended sediments, nutrients, organic wastes, pesticides, herbicides and solar irradiance (Dance and Hynes 1980; Williams 1980; Lenat et al. 1981; Murphy et al. 1981; Wallace and Hynes 1981; Erman and Erman 1984; Dosdall and Lehmkuhl 1989).

Relative to forested streams, macroinvertebrate communities in streams draining agricultural land support fewer taxa and fewer individuals within the most sensitive orders (Ephemeroptera, Plecoptera, Trichoptera) but larger numbers of individuals within tolerant groups (especially Chironomidae), and so have high overall densities (Lenat 1984; Quinn and Hickey 1990; Lenat and Crawford 1994). There may be functional changes as well: Dance and Hynes (1980) observed that the abundance of shredders decreased while that of collectors increased. Most of the work to date has evaluated impacts on a basin scale, whereas Barton (1996) suggested that effects may be more immediate, at least at certain times of the year, and detectable at a finer level. The first objective of this study was to determine whether or not effects can, in fact, be seen at the field scale.

Assessments of water pollution have focused on alterations in macroinvertebrate community diversity, loss of sensitive species, or increases in the number of tolerant species as a means to evaluate biotic effects. The ability of an animal to fix and retain energy is probably the best measure of success in an environment. For this reason, the second objective was to evaluate the effects of agriculture using estimates of secondary production at the species level both upstream and downstream of an agricultural site.

Finally, the third objective was to determine whether a change in landuse between 1995 and 1996 was reflected in the macroinvertebrate communities over this period.

Study Site

The study was conducted in a small headwater stream, Logan Drain, located at Lot 19, Concession 12 in East Nissouri Township, Oxford County near Kintore, Ontario (81°10'W, 43°01'N). The stream originates from springs and flows for approximately 2 km through a mixed hardwood and cedar woodlot before emerging between cultivated fields. From this point until entering the second woodlot 400 metres downstream, the stream has been channelised and was most recently cleaned in 1990. The wetted channel is 1 to 1.5m wide, has year-round baseflow and a mean depth of 12 cm. The stream bed consists mainly of gravel and cobbles (1 to 30 cm diam.) and the riparian buffer strip, consisting of grasses and shrubs, ranges from 3 to 10 metres in width. The surrounding fields have been systematically tile-drained. Alfalfa was grown on the 5.5 ha field to the north of the stream and corn (maize) on the 14.2 ha field to the south, in 1995; both were planted to corn in 1996.

Site 1 extended upstream into the woodlot which borders the headwaters (site 1; Fig. 2.1) and site 5 was situated within the wooded area just below the field (site 5; Fig. 2.1). Sites 2, 3 and 4 were located just below major inflows from a secondary ditch draining 20 cultivated hectares and several tile openings. Sites 1, 2 and 5 had treed canopies while the remaining two were open and subject to elevated irradiance. Data collected from 1994 showed that water entering the stream from the secondary ditch and the tile drains had higher nitrogen concentrations than the stream itself. Intensive sampling to estimate secondary production and assess the overall impact of agricultural activities on the fauna was conducted in riffles at sites 1 and 5. Abiotic data were collected at the intermediate sites to determine the rate of change of the physical and chemical characteristics along the study reach.

Methods

Water temperature at sites 1 and 5 was recorded weekly from 22 June 95 through 09 November 95, monthly from 07 December 95 through 26 April 96, and then biweekly until 10 October 96, using a mercury thermometer. At the remaining sites, temperature readings began on 23 February 1996 and continued on the same schedule as for sites 1 and 5. Other abiotic data gathered by the Earth Sciences Department at the University of Waterloo included concentrations of soluble reactive phosphorus, nitrate-nitrogen, ammonium-nitrogen, total soluble nitrogen, dissolved oxygen, dissolved organic carbon, stream discharge, pH and electrical conductivity. Continuous water temperature recordings were also taken at sites 1 and 4.

Stream bed morphology was quantified by estimating the percentage of each sediment size class (fines <4 mm, small gravel 4-25 mm, medium gravel 25-50 mm, large gravel 50-75 mm, small cobble 75-150 mm, medium cobble 150-225 mm, large cobble 225-300 mm, small boulder 300-600 mm, large boulder >600 mm) in the surface layer of the stream bed within a one metre square quadrat. The process was repeated three times at sites 1 through 5 on 18 July 1996.

In order to reduce the physical impact of sampling, all work was conducted from wooden planks spanning the small stream. A mini-Surber (0.01 m^2) sampling device with a mesh size of 0.200 mm was used on each sampling date to collect 5 samples from sites 1 and 5. The substrate was disturbed to an approximate depth of 5 cm. All samples were preserved immediately using 10% formalin. Samples were collected biweekly from 25 May 95 through November 95, then monthly until the end of September 96. Three floating emergence traps (Fig. 1) were emptied weekly from 19 April 95 until 23 October 95 to reinforce the cohort lifecycle data obtained from the benthic samples, and aid in identification of the insect species.

In the laboratory, each Surber sample was rinsed with water and agitated to suspend the preserved animals. The supernatant was then poured through a 0.100-mm sieve to separate the animals from the debris and the process repeated until no further animals were observed in the supernatant. The animals from each of the 240 samples were sorted from associated detritus under 12x magnification and stored in 70% ethanol. All arthropods collected between 25 May 1995 and 09 May 1996 were then enumerated at

the lowest practical taxonomic level, measured (maximum sclerotized head dimension ± 0.02 mm), and stored in 70% ethanol.

The maximum sclerotized head dimension was length for Ceratopogonidae, and width for all others. Larvae of Tipulidae and Tabanidae do not have external sclerotized body parts; because their numbers were relatively small, every animal was weighed individually.

Linear regressions relating dry mass to head dimension for each of the common taxa were developed using animals from the stored samples. Head dimensions were measured from at least 30 specimens for each species; these individuals were then oven-dried for 48 hours at 60°C and weighed (± 1μm) using a Cahn C-31 microbalance. The animals used covered the complete range of head dimensions found for each taxon. Chironomidae in the first two instars were weighed in groups of five to minimise measurement errors. Equations were then developed using linear regressions of the natural logarithm of dry mass on the natural logarithm of head dimension. These equations were of the form:

$$\text{Ln (dry mass)} = a + b^{*}\text{Ln (head dimension)}$$

where: a and b are regression coefficients for each taxon.

Dry mass was not adjusted for storage in both formalin and ethanol (Mason et al. 1983; Leuven et al. 1985).

While cohort methods could have been used to estimate secondary production for univoltine taxa, they are not applicable to the more complex lifestyles of the multivoltine Chironomidae and Ceratopogonidae. To ensure uniform results, the size-frequency

method (Hamilton 1969; Benke 1984) was used for all taxa. Instrumental to this method is an accurate estimate of the mean cohort production interval (CPI) for each taxon. For most of the invertebrates found in the stream, the CPI could be estimated from the field data. It was necessary to supplement the data with information from other studies of streams in southern Ontario (LeSage 1979; Singh 1981; Singh 1986) to determine the CPI's of the Chironomidae and Ceratopogonidae. Three taxa had asynchronous lifecycles and for each of these the CPI was estimated using a growth equation for Diptera (Morin and Dumont 1994) in which the independent variables were individual dry weight and stream temperature, and the method outlined in Marchant and Yule (1996).

Macroinvertebrates collected between 23 May 1996 and 27 September 1996 were also enumerated at the lowest practical taxonomic level (except Chironomidae, Ceratopogonidae, Simulidae and Oligochaeta) and then stored in 70% ethanol.

Fish were collected by electroshocking at sites 1 and 5 on 08 June 1995 and identified to species in the field.

Results

Dissolved oxygen, pH, electrical conductivity and SRP were similar at all sites along the study reach and had mean values of 10.04 mg/l, 8.20, 545 µS/cm and 14.1 µg/l, respectively (G. Parkin, pers. comm.).

Total discharge, temperature, dissolved organic carbon (DOC) and nitrate concentrations changed along the course of the stream through the study area (Table 2.1). Total discharge was about twice as great in 1996 as in 1995 (Fig. 2.2), primarily because

of much greater precipitation during the months of April through July. The largest differences between years were in May and June when total discharge was 6 to 7-fold greater in 1996. Despite this annual variation, discharge at site 5 was always about twice as great as at site 1 during both years. The addition of water through the study reach came mainly from groundwater (44% in 1995, 60% in 1996) and tile drains (41% and 31%); the secondary ditch accounted for only 15% and 9% of the increase in discharge at site 5 in both years (G. Parkin, pers. comm.). Mean weekly water temperatures ranged from near 0°C from December through February to nearly 18°C in July 1996. Mean temperatures increased downstream from site 1 through site 4, then decreased in the wooded area of site 5. While temperatures at the two wooded sites (1 and 5) were not significantly different ($t = -1.000$, $df = 39$, $p = 0.323$), stream temperature increased at a rate of 3.3 °C/km from site 1 to site 4. This is more than twice that predicted by Barton et al. (1985) for a stream with complete insolation, mainly because of the warm inflow from the secondary ditch. Nitrate concentrations increased steadily downstream from site 1. Despite differences in total discharge, the concentrations of nitrate were remarkably similar at each station in both years. DOC was highest at site 1 and uniformly lower at the sites downstream from the secondary ditch.

The stream bed changed progressively from an even distribution of boulders, cobble, gravel and fines at site 1 to predominantly gravel and fines at site 4 (Fig. 2.3). Some recovery was evident at site 5 with a shift back to cobble and some exposed boulders.

From May 1995 to May 1996, a total of 116 taxa were identified from the two sites. Species richness was greater downstream with 108 taxa identified from site 5 compared to 104 at site 1 (Table 2.2). The increase downstream was due to a greater diversity of Chironomidae and Ceratopogonidae, despite fewer species of mayflies, caddisflies and naidid worms.

Throughout the study period, site 5 supported fewer Ephemeroptera, Plecoptera, Trichoptera and Naididae, but more Coleoptera, Diptera and Tubificidae, relative to site 1 (Table 2.3). Mean annual biomass mirrored the densities with the exception of the Chironomidae and Tipulidae in which biomass was slightly lower downstream. Mean individual biomass was lower in each of the major groups, except Coleoptera and Simulidae, at the downstream site.

The numerical density of Diptera was roughly equal to that of all other groups combined at both sites. The Chironomidae, the largest family in this group, were represented by 49 taxa. While 9 of the 13 most common chironomid taxa (those with densities >40 m^{-2}) were more abundant downstream, the mean biomass per animal was greater or the same upstream for every taxon, so that mean annual biomass at site 5 was 12% less than at site 1, despite the 15% larger mean annual density. The densities of the other Diptera (except *Tipula* spp.) also increased downstream, while mean biomass per animal decreased (except *Simulium* spp. and *Tipula* spp.).

Nine species of Coleoptera were found at the two sites, of which *Optioservus fastiditus* accounted for >99% of the individuals. Mean annual density and biomass were greater downstream by 5% and 7%, respectively.

Of the 4 common Ephemeroptera, *Baetis brunneicolor* was the most abundant and its decline in abundance by 49% accounted for the reduced mayfly presence downstream. The other 3 species were more abundant at site 5 than at site 1. The overall reduction in mayfly density of 10% was accompanied by a decrease in mean biomass per individual from 0.106 mg upstream to 0.08 mg downstream, resulting in a 25% reduction in biomass.

Of the two species of Plecoptera found in this section of Logan Drain, *Allocapnia vivipara* dominated in abundance and biomass at both sites. Density and biomass were 7% and 49% lower, respectively, at the downstream site. The more pronounced change in biomass was due to the mean biomass per animal dropping from 0.053 mg to 0.029 mg.

Mean annual biomass was greater upstream for each of the seven caddisfly (Trichoptera) taxa which were common to both sites. Densities were also greater upstream for each of these, with the exception of the filter-feeder *Cheumatopsyche oxa* (Hydropsychidae) and the shredder *Lepidostoma costalis* (Table 2.3). As with the orders Ephemeroptera, Plecoptera and most Diptera, the mean biomass per animal decreased from 0.478 mg upstream to 0.362 mg downstream.

Mean individual biomass was less in 20 of the 33 most abundant taxa (>40 m^{-2}) at site 5. Biomass was determined using identical weight to head width regressions at both sites, indicating that either the animals in each size-class were smaller (slower growth rates) or that fewer individuals reached the late size-classes (increased mortality or drift) downstream.

Mean individual biomass of the final larval size-class was greater at site 5 in 20 of the 33 taxa studied and in 10 of the 13 chironomid species; however, the percentage of

individuals in the final size-class was smaller in 22 of the 33 common taxa and in 11 of the 13 chironomids downstream. A lower proportion of individuals in the final size-class was indicative of increased mortality and/or drift at that site.

Although biomass was not determined for the Oligochaeta, numerical densities were 44% lower at the downstream site. An 82% decline in the Naididae (*Nais communis, N. bretscheri, N. simplex, Pristina aequiseta, P.* sp., *Pristinella osborni, P.* sp) between sites was partially offset by a 42% increase in the Tubificidae (predominantly *Limnodrilus hoffmeisteri*) downstream.

Relative to site 1, both the density and biomass of shredders and filter-feeders were lower at site 5; densities of scrapers and gathering collectors were similar but mean annual biomasses were lower. The predators were the only functional feeding group to have a greater biomass at the downstream site.

Of the 20 taxa common to both sites in 1995 and 1996, intersite differences in abundance were similar for 16 taxa in both years (Table 2.4). Chironomids, tubificids and each of the predators (*Dicranota* sp., *Chrysops* sp. and ceratopogonids) were more abundant at the downstream site in both years. Ephemeroptera, naidids and the predominant shredder (*Tipula* spp.) were less abundant downstream in both years. The Chironomidae (1996) were the only taxon to show a significant change between sites 1 and 5 (paired t-test, $t = 2.973$, $df = 4$, $p = 0.041$) in either year. Comparison between years at each site revealed that Ceratopogonidae (paired t-test, $t = 3.031$, $df = 4$, $p = 0.039$), *O. fastiditus* (paired t-test, $t = 3.649$, $df = 4$, $p = 0.022$) and *A. vivipara* (paired t-test, $t = 4.084$, $df = 4$, $p = 0.015$) increased and Naididae (paired t-test, $t = 2.839$, $df = 4$,

$p = 0.047$) decreased significantly in abundance at site 5 in 1996. There were no significant changes in abundance between years at the upstream site.

Of the four taxa which did not exhibit the same trend in both years, *Hydropsyche slossonae* is of greatest interest due to its large biomass and the magnitude of its shift in abundance between years. *H. slossonae* was univoltine at Kintore and recruitment occurred in mid-summer with first instar animals initially observed in July in 1995 and in August in 1996 (Fig. 2.4). The 1995 recruitment at site 1 was sevenfold greater than at site 5 or either site in 1996.

Overall secondary production was 50.71 $g \cdot m^{-2}$ at site 1 and 35.36 $g \cdot m^{-2}$ at site 5 (Table 2.5). The difference is attributable to the lower production of Ephemeroptera, Plecoptera, Trichoptera and *Tipula* (Diptera); production of Chironomidae, Ceratopogonidae, Simulidae, Tabanidae and Coleoptera were the same, or higher, downstream. The lower production downstream reflected a difference in the functional organisation of the benthic community because of a decline in the importance of shredders and filtering collectors.

While not numerically abundant, the large shredders *Tipula* spp. contributed more to total secondary production than any other taxon, and accounted for 31% of the decline downstream. The much lower production by filter feeders, predominantly the hydropsychid caddisflies *H. slossonae* and *C. oxa*, accounted for the remaining decline downstream. Increased production by blackflies and filter feeding midges did not compensate, so filter feeding was less important below the cultivated fields.

Total production by gathering collectors was similar at both sites. Lower production at site 5 by the dominant mayfly, *B. brunneicolor*, was offset somewhat by increased production of *Parametriocnemus lundbeckii*, the *Orthocladius* Group, *Tvetenia paucunca* and *Diamesa nivoriunda*.

Biomass produced by scrapers was also similar at both sites. Lower production by the caddisfly *Neophylax concinnus* and the mayfly *Stenonema vicarium* was offset by an increase from the beetle *O. fastiditus*. The predators were the only functional group to have greater production downstream, but these accounted for less than 5% of the total.

In addition to the two species of fish present at site 1, *Semotilus atromaculatus* (creek chub) and *Rhinichthys atratulus* (blacknose dace), four other species were present at site 5: *Notropis cornutus* (common shiner), *Culaea inconstans* (brook stickleback), *Pimephales promelas* (fathead minnow) and *Etheostoma nigrum* (johnny darter).

Discussion

In small headwater streams with narrow, shallow channels and a more or less complete canopy, allochthonous inputs of detritus are expected to exceed autochthonous production in the stream itself. As stream order increases and shading by riparian vegetation is reduced, primary production by algae and macrophytes begins to dominate the supply of organic material to higher trophic levels (Minshall et al. 1985). Agricultural activities alter the rate of change of longitudinal stream zonation. At Kintore, elevated temperatures, discharge and nitrogen concentrations, in addition to finer bed sediments were observed at the downstream site.

Even small changes in temperature can affect growth, metabolism, reproduction, emergence and the distribution of aquatic insects. Vannote and Sweeney (1980) suggested that each of the species they studied had an optimal temperature, above or below which larval tissue growth was suppressed more than adult tissue development, resulting in smaller adults and reduced fecundity. Lifecycles were established for all of the common taxa found at sites 1 and 5 in order to estimate secondary production. Most of the Diptera had many generations per year, the exact number difficult to determine; the remaining taxa were clearly either univoltine or bivoltine. Recruitment and emergence periods and the number of generations per year appeared identical, so that there was no evidence that the lifecycles of any of these animals differed from one site to another. It is possible, however, that the warmer water temperature downstream contributed to heavier individual biomass in the final size-class of the fauna at site 5, if stream temperature was below optimal for these taxa.

Although the mean biomass of individuals in the final instar was greater at site 5 than site 1 for most taxa, proportionately fewer animals reached the final instar at the downstream site. It is not immediately obvious why this should be so, but increased pressure from the benthic insectivorous fish could have contributed to greater mortality or drift at the downstream site. At site 1, creek chub and blacknose dace were the only species present. Creek chub are known primarily as sight feeders of planktonic or drifting invertebrates, whereas blacknose dace and the additional four species observed at site 5 are benthic insectivores. A trend towards decreased benthic invertebrate size in the presence of insectivorous fish and the absence of piscivorous fish has been well

documented (Flecker and Allan 1984; Bowlby and Roff 1986; Macchiusi and Baker 1992). Greater top-down pressure on the benthic invertebrates at the downstream site is consistent with proportionately fewer final instar animals in the majority of the taxa.

Stream nitrogen and phosphorus concentrations have been associated with the percent of watershed under cultivation, in row crops and tile drained as well as the application of commercial fertilisers and manures (Miller et al. 1982; Neilsen et al. 1982; Wall et al. 1982). These important nutrients may enhance algal and macrophytic growth, alter the balance between autochthonous and allochthonous production, and accelerate eutrophication. Excessive algal growth can alter benthic species composition because of increased food for scrapers, physical changes in habitat or altered daily fluctuations in dissolved oxygen concentrations.

In agricultural streams, however, nutrient enrichment experiments have demonstrated that periphyton communities are seldom limited by nitrogen or phosphorus (Bushong and Bachmann 1989; Munn et al. 1989; Richards et al. 1993) but rather by temperature and light. Although water temperatures were slightly greater downstream at Kintore, both the upstream and downstream sites were partially shaded. The elevated nitrogen concentrations did not lead to any visible increase in standing crop of algal mats or macrophytes downstream with the exception of a brief bloom of filamentous algae at the unshaded mouth of the secondary ditch in the early summer of 1995. In addition, density, biomass and secondary production by scrapers, which rely on attached diatom production, remained constant between sites. These factors suggest little change in food supply and no noticeable impact from the higher nitrogen concentrations and water

temperatures at site 5. Rosenfeld and Roff (1991) estimated primary production on fine substrates to be 20% of that on rocky, and, as a result, the change in the stream bed morphology may have led to reduced primary production in spite of the elevated nitrate levels at the downstream site.

It has been suggested that the deterioration of lowland streams is more a product of physical disruption than of nutrient inputs (Petersen 1992). Channelisation, riparian clearing and percent of watershed in row crops have been associated with increased suspended solids, sedimentation and substrate changes in agricultural streams (Wall et al. 1982; Richards and Host 1994; Rier and King 1996). Sedimentation can result in the loss or creation of habitat for specific organisms by reducing exposed rock surfaces and smothering periphyton populations.

The density of Chironomidae has frequently been positively associated with substrates which retain fine detrital sediments (Minshall and Minshall 1977; Rabeni and Minshall 1977; Berkman et al. 1986). It has been shown that large detrital particles (sticks and pieces of wood > 3.95 mm) are trapped amongst large substrate sizes (cobbles and boulders), and small detrital particles (leaf fragments < 3.95 mm) are more common among finer inorganic particles (Rabeni and Minshall 1977). The small detrital particles are available as food to gathering chironomids and contribute to the shift in community structure from Ephemeroptera, Plecoptera and Trichoptera to Chironomidae (Lenat 1984; Lenat and Crawford 1994) at agricultural sites. In addition, many Chironomidae are adapted for burrowing and can tolerate accumulations of fine sediments. The increased

density, species richness and production by chironomids at site 5 in both 1995 and 1996 supports this view.

Many Chironomidae are generalist feeders and have frequently been shown to benefit from increased organic loading (Rutt et al. 1993). Approximately 90% of the chironomid taxa in Logan Drain belonged to the rhithral tribes (Orthocladinae and Diamesinae), implying that the stream was cool, well-oxygenated and had a predominantly coarse substratum at both the upstream and downstream sites. Annual chironomid production between 3 and 5 $g \cdot m^{-2}$ of dry weight suggests mesotrophic conditions at both sites (Pinder 1995). Slightly polluted streams, as in this study, may provide an ideal balance between abundant food sources and reduced numbers of invertebrate competitors such as mayflies and caddisflies (Lenat 1983).

Although chironomid density and secondary production were greater at site 5, adjusted mean individual biomass was less due to a lower percentage of fourth instar animals in the population, possibly a result of increased drift or predation. Secondary production by predators was 19% greater at site 5, primarily due to the increased abundance of the two largest invertebrate predators, *Dicranota* and *Chrysops*. It seems likely that the greater chironomid densities have created a more favourable habitat for these two predators, while they in turn have contributed to increased chironomid mortality.

Naidid worms can be numerically important in streams with stony substrates. They are generally surface crawlers which graze on bacteria, protists, algae and organic detritus with food selection based primarily on particle size. Organic enrichments of rivers which

have stony beds can result in tenfold increases in naidid abundance. Reduced populations have been observed in habitats with increased fine sediments (Learner et al. 1978) and predaceous chironomids (Loden 1974). Lower naidid densities downstream in this study are likely a result of the greater proportion of fine sediment particles, increased competition or predation.

Tubificid worms are typically burrowers and tolerant of fine sediments, organic enrichment and low oxygen concentrations (Patrick and Palavage 1994; Finogenova 1996; Lafont, Camus and Rosso 1996; Lang and Reymond 1996; Verdonschot 1996). It is probable that the increased abundance of Tubificidae downstream resulted from a combination of increased fines and organic enrichment.

The reduced production by shredders accounted for 29% of the difference between upstream and downstream sites. Shredders convert large organic particles, such as deciduous leaves, to fine particles and faeces which are, in turn, consumed by filter feeders or gathering collectors. Fewer allochthonous inputs from the cleared farmland and the tendency of homogenous substrates to accumulate less coarse particulate organic matter likely contributed to the reduced abundance and production by the predominant shredder, *Tipula* spp., at the downstream site in 1995 and 1996.

The remaining decline in secondary production was a result of a 50% reduction by filter-feeding collectors. Four of the five filter feeding caddisflies, in particular *H. slossonae*, were much less abundant downstream. Comparison of the numbers in each instar at both sites indicate that recruitment of first instar individuals was 44% lower downstream and was largely responsible for the diminished production by *H. slossonae*

(Table 2.6). While the reduced shredder activity, resulting in less fine particulate matter in suspension, must have contributed to the 10.05 g/m^2 reduction in filter feeder production at this site, it may not have been the primary cause.

It has been suggested that each taxon of net-spinning caddisflies partitions a specific fraction of the drifting stream seston (Wallace et al. 1977; Fuller and Mackay 1980), the niche occupied being dictated by the net mesh size, structure and current requirements. Fine meshed nets are constructed in slow currents found on the sides or undersides of rocks while coarse mesh sizes are built in faster water capable of transporting larger particles. Mesh sizes and optimal current requirements vary according to the species and instar of the caddisfly, with late instar animals manufacturing coarser nets than early instars of the same species. In order for these insects to occupy the same riffle, a wide range of current velocities is required. In addition to providing a solid surface to which eggs and nets can be anchored, stream bed morphology and heterogeneity dictate the availability and frequency of the feeding microhabitats important to this group of animals. In general, densities and production of caddisflies increase with substrate particle size (Barber and Kevern 1973; Resh 1977; Rabeni and Minshall 1977).

It is likely that the finer and more homogenous sediments at site 5 may account for much of the lower production of Trichoptera relative to site 1. The fact that other filter-feeding taxa did not replace the caddisflies indicates that all taxa suffered from a lack of suitable substrate, the quantity and/or quality of suspended organic nutrients deteriorated downstream or that nutrient cycling was less efficient at this site. In 1996, it appears that other factors limited the recruitment of *H. slossonae* larvae at site 1 to levels well below

those observed in 1995 and similar to levels found at site 5 in both years. Adult *H. slossonae* were observed between 08 June and 20 July and first instar larvae were observed beginning on 22 June at both sites in 1995. Oviposition must have occurred throughout June and July, a period in which discharge was six times greater in 1996. The much higher discharge in 1996 covered many of the exposed boulders at site 1 and likely led to reduced oviposition.

Secondary production by gathering collectors and scrapers was similar at both sites. Reduced production from intolerant taxa was offset by increased production from tolerant taxa in these two groups. The 19% increase in production by predators downstream was discussed earlier and probably results from the increased density of suitable prey (chironomids) at this site.

Between years, the proportion of the drainage basin in row crops increased and it was expected that this would lead to increased nutrient loading, sedimentation and impact on the benthic fauna. Nitrate and ammonium concentrations downstream were no greater in the second year than the first, although increased precipitation led to greater discharge at both sites in 1996.

In order to evaluate the relative impact of farming intensity between years on the benthic community, taxa which had shown consistent changes in abundance from site 1 to site 5 in both years were studied. An increased impact should be evident by a greater percent change in abundance in 1996. The response of five taxa (Chironomidae, *Tipula* spp., *L. costalis, Chrysops* sp., *C. oxa*) was greater in 1996 than in 1995. While

chironomid abundance was greater at site 5 in both years, the difference was significantly larger in 1996.

The much higher chironomid abundances at site 5 in 1996 were capable of supporting a greater density of predators, including *Chrysops* sp. *C. oxa* was the only filter-feeding caddisfly to have greater densities at site 5 in both years. Discharge was greater downstream in both years and was much larger at both sites in 1996. The hydraulic regime and the quantity and/or quality of suspended particles may have offset the effects of sedimentation on *Cheumatopsyche*.

Significant differences in abundance were detected in the Naididae, Ceratopogonidae, *O. fastiditus*, and *A. vivipara* between years at site 5, whereas no taxa were significantly different at site 1. The larger population of chironomids likely contributed to the increased abundance of predacious ceratopogonids downstream in 1996. *O. fastiditus* and *A. vivipara* are predominantly scrapers and shredder-detritivores, respectively. There was no evidence of increased autochthonous growth or reduced competition which could explain the greater success of *O. fastiditus* in 1996. The increased abundance of *A. vivipara* in 1996, at site 5 in particular, could be a result of the higher discharge.

There was no evidence that the greater farming intensity in the second year resulted in larger changes in the benthic fauna or water chemistry, except perhaps the greater numbers of chironomids. Higher discharge probably diluted the impact from the increase in land under row crops.

When this stream is considered in terms of the river continuum concept (RCC), it is found to conform reasonably well. Shredders and gathering collectors are expected to be the dominant producers in headwater streams in which allochthonous sources are likely to provide the majority of the organic inputs (Minshall et al. 1985). At Kintore, the elevated nutrient concentrations, incomplete canopies and the cleared land served to reduce the roll of allochthonous inputs and production by shredders at the downstream site. Gathering collectors were superseded by filtering collectors at both sites, in contrast to the idealised RCC stream, possibly a result of the sampling procedure in which the rockier habitat of riffles rather than pools were studied.

Benke (1993) analysed the results of 159 studies on stream invertebrate production and concluded that community secondary production is not correlated to discharge or temperature but rather to anthropogenic, shading and impoundment effects. Community production in this study was well within the range observed for other streams with similar discharge (4,100 to 135,000 $mg \cdot m^{-2} \cdot yr^{-1}$). Benke also stated that although trends in production by functional feeding groups generally supported the RCC hypothesis, variation between streams was great. The study in Logan Drain suggests that the effects of agricultural activity on macroinvertebrate communities in headwater streams can be detected immediately, at the field scale. The predominant effect was due to a change in stream bed morphology to a finer substrate, likely the result of sedimentation of eroded soil.

Summary

Distinctive shifts in the composition of benthic invertebrate communities toward assemblages more characteristic of degraded streams were observed within the 400 metre study reach. Larger, more sensitive forms, especially mayflies, caddisflies and stoneflies were replaced by smaller, more tolerant chironomids. The alterations to the macroinvertebrate community between sites appear to be consistent with the effects of sedimentation of eroded soil, and, perhaps, alterations in the hydraulic regime, both of which are likely results of agriculture.

Production by filter-feeding collectors was 50% lower at the downstream site and appeared to be a result of a shift in substrate from an heterogeneous mix of fines, gravel, cobble and boulders at the upstream site to predominantly fines and gravel downstream. Lower density, biomass and production by the shredders downstream was likely a result of the reduced ability of the site to retain coarse particulate matter in the finer and more homogenous stream bed, and possibly lower inputs of terrestrial matter from the cleared farmland. Greater retention of fine detrital sediments also supported increased abundance, biomass and secondary production of the Chironomidae. The higher proportion of fine substrate may also have led to reduced primary production and subsequently, lower secondary production within the scrapers and gathering collectors.

While total numerical abundances of invertebrates were similar at the top and bottom of the study reach, most individual animals were smaller downstream. The additional benthic insectivorous fish species at this site likely increased the top down pressure on the herbivores, led to greater mortality and/or drift of the larger animals, and,

in spite of similar invertebrate densities at both sites, contributed to reduced secondary production.

A reduced sampling effort between May and September 1996 revealed a similar trend towards increased densities of Chironomidae, Ceratopogonidae and Tabanidae and reduced numbers of Ephemeroptera and shredders at the downstream site. The increased agricultural activity between 1995 and 1996 was not detected in the benthic community.

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Table 2.1. Mean values of chemical and physical characteristics of water at the five sample sites in Logan Drain, Kintore. Values in brackets are the number of measurements included in the mean. Mean temperature data is from 23 February 1996 until 10 October 1996 (the dates when all sites were measured). na - data is not available at a particular site.

Site	Dissolved Organic Carbon (mg/l)	Total Soluble Nitrogen (mg/l)	NO_3^--N (mg/l)		NH_4^+-N (mg/l)		Temp. (°C)		Discharge (m^3/day)	
			1995	1996	1995	1996	1995	1996	1995	1996
1	3.30 (21)	2.22 (10)	1.44 (48)	1.85 (25)	0.01 (50)	0.11 (25)	12.8 (22)	12.2 (12)	873	1910
2	1.74 (19)	5.12 (10)	2.86 (48)	2.88 (23)	0.00 (49)	0.09 (23)	12.9 (22)	12.6 (12)	na	na
3	na	na	na	na	na	na	na	13.3 (12)	na	na
4	2.24 (20)	3.51 (10)	3.11 (47)	3.15 (21)	0.00 (50)	0.11 (21)	na	13.3 (12)	na	na
5	2.15 (21)	3.51 (10)	3.22 (49)	3.23 (25)	0.00 (48)	0.09 (25)	12.8 (22)	12.8 (12)	1604	3514

Table 2.2. Total number of taxa identified (Surber samples, emergence traps) in each major group from May 1995 to May 1996 at Sites 1 and 5.

		Site	
Group	Family	1	5
Diptera		64	71
	Chironomidae	45	49
	Ceratopogonidae	3	6
	Tipulidae	6	6
	Tabanidae	1	1
	Empididae	6	6
	Simulidae	3	3
Ephemeroptera		5	4
Trichoptera		11	10
Plecoptera		2	3
Coleoptera		8	7
Megaloptera		1	1
Oligochaeta		10	8
	Naididae	7	5
	Tubificidae	1	1
	Enchytreidae	1	1
	Lumbriculidae	1	1
Nematoda		1	1
Hydracarina		1	1
Decapoda		1	2
Total		**104**	**108**

Table 2.3. Mean annual density, mean annual biomass and mean individual biomass for the common taxa in Logan Drain, Kintore. Some taxa may have more than one feeding group (a = shredder; b = collector (filtering); c = collector (gathering); d = scraper; e = predator; f = detritivore). *Tanytarsus* Group includes *Micropsectra* sp and *Tanytarsus* sp. *Orthocladius* Group includes *Orthocladius* spp. and *Cricotopus* spp. Data collected from May 1995 to May 1996.

Major Group	Taxon	Functional Feeding Group	Mean Annual Density (N/m^2)		Mean Annual Biomass (mg/m^2)		Mean Individual Biomass (mg/individual)	
			Site 1	Site 5	Site 1	Site 5	Site 1	Site 5
Diptera			**2101**	**2496**	**311**	**320**	**0.14**	**0.12**
Chironomidae			1937	2218	38	33	0.02	0.01
	Tanytarsus Group Kieffer	b, c	130	168	13.	14.	0.01	0.00
	Rheotanytarsus sp Thienemann & Bause	b	105	258	14.	25.	0.01	0.01
	Polypedilum spp Kieffer	a, c	326	318	138.	92.	0.04	0.02
	Parametriocnemus lundbecki (Johannsen)	c	415	431	49.	46.	0.01	0.01
	Orthocladius Group van der Wulp	c	135	181	39.	52.	0.02	0.02
	Eukiefferiella claripennis (Lundbeck)	c	50	72	7.	7.	0.01	0.01
	Tvetenia paucunca (Saether)	c	467	534	41.	39.	0.00	0.00
	Parakiefferiella sp Thienemann	c	66	69	4.	4.	0.00	0.00
	Thienemanniella sp Kieffer	c	59	32	4.	2.	0.00	0.00
	Corynoneura sp Winnertz	c	25	33	0.	1.	0.00	0.00
	Paracricotopus sp Thienemann & Harnisch	c	9	7	1.	0.	0.01	0.00
	Diamesa nivorlunda (Fitch)	c	10	17	35.	31.	0.33	0.17
	Trissopelopia ogemawi Roback	e	135	92	30.	17.	0.02	0.01
Tipulidae			14	19	256	252	17.92	13.31
	Tipula spp Linnaeus	a, c	9	6	2542.	2411.	26.21	35.46
	Dicranota sp Zetterstedt	e	4	12	20.	116.	0.44	0.95
Tabanidae	*Chrysops* sp Meigen	e	4	8	56.	82.	1.42	1.01
Ceratopogonidae		e	37	49	11.	11.	0.02	0.02
Simulidae			108	201	10	25	0.09	0.12
	Simulium spp Latrielle	b	51	76	61.	163.	0.11	0.21
	Prosimulium esselbaughi Sommerman	b	56	124	44.	86.	0.07	0.07
Coleoptera	*Optioservus fastiditus* (Leconte)	d, c	**946**	**995**	**53**	**57**	**0.05**	**0.05**
Ephemeroptera			**422**	**378**	**44**	**33**	**0.10**	**0.08**
	Baetis brunneicolor McDunnough	c	313	161	214.	171.	0.06	0.10
	Acerpenna macdunnoughi (Ide)	c	86	184	32.	54.	0.03	0.02
	Paraleptophlebia debilis (Walker)	c	4	8	2.	5.	0.05	0.06
	Stenonema vicarium (Walker)	d, c	18	23	196.	102.	1.08	0.43
Plecoptera			**91**	**85**	**4**	**2**	**0.05**	**0.02**
	Allocapnia vivipara (Claasen)	a, f	87	79	45.	19.	0.05	0.02
	Amphinemura delosa (Ricker)	a, f	3	5	2.	5.	0.06	0.08
Trichoptera			**888**	**511**	**424**	**185**	**0.47**	**0.36**
	Lepidostoma costalis (Banks)	a	4	4	11.	10.	0.24	0.21
	Hydropsyche slossonae Banks	b, e	519	214	2929.	981.	0.56	0.45
	Cheumatopsyche oxa Ross	b, e	161	184	519.	378.	0.32	0.20
	Diplectrona modesta Banks	b, e	36	7	215.	55.	0.58	0.79
	Chimarra obscura (Walker)	b	59	7	137.	40.	0.23	0.54
	Dolophilodes distinctus (Walker)	b	17	11	51.	45.	0.29	0.41
	Neophylax concinnus McLachlan	d	90	81	382.	339.	0.42	0.41
Oligochaeta			**648**	**350**				
Naididae		d, c	458	80				
Tubificidae		c	190	270				
Shredders		a	432	415	274	253	0.63	0.61
Collectors (filtering)		b	1138	1053	398	179	0.35	0.17
Collectors (gathering)		c	1644	1734	43	41	0.02	0.02
Scrapers		d	1054	1100	111	101	0.10	0.09
Predators		e	181	162	11	22	0.06	0.14
Total			**5099**	**4817**	**839**	**599**	**0.16**	**0.12**

Table 2.4. Mean densities (N/m^2) of the major groups of macroinvertebrates found at sites 1 and 5 in Logan Drain, during May through September 1995 and 1996.

Major Group	Taxon	1995		1996	
		1	5	1	5
Diptera		**42393**	**60284**	**47441**	**102078**
Chironomidae		39904	53746	44839	100213
Tipulidae		172	196	255	399
	Tipula spp	152	72	64	16
	Dicranota sp	20	124	80	383
	Pseudolimnephila sp	0	0	111	0
Tabanidae	*Chrysops* sp	0	52	0	140
Ceratopogonidae		112	164	447	591
Simulidae		2205	6126	1900	735
Coleoptera	*Optioservus fastiditus*	**8154**	**8100**	**9916**	**13693**
Ephemeroptera		**10364**	**9029**	**6945**	**5809**
	Baetis brunneicolor	8756	5620	6673	5158
	Acerpenna macdunnoughi	1242	3017	113	270
	Stenonema vicarium	296	220	159	381
	Paraleptophlebia debilis	70	172	0	0
Plecoptera		**220**	**484**	**1634**	**2946**
	Allocapnia vivipara	204	440	1634	2930
	Amphinemura delosa	16	44	0	16
Trichoptera		**15510**	**6397**	**3982**	**5035**
	Lepidostoma costalis	100	63	143	48
	Hydropsyche slossonae	10511	3081	1778	2178
	Cheumatopsyche oxa	1644	2751	1362	2283
	Diplectrona modesta	1014	132	461	318
	Chimarra obscura	1585	52	0	0
	Dolophilodes distinctus	544	222	0	0
	Neophylax concinnus	112	96	238	208
Oligochaeta		**14317**	**5256**	**15729**	**8470**
Naididae		12027	1092	10584	2617
Tubificidae		2289	4164	5145	5853
Total		**90958**	**89550**	**85647**	**138031**

Table 2.5. CPI, voltinism, annual production and P/B for the common taxa in Logan Drain, Kintore. * CPI for these taxa were determined using estimates of growth (Morin and Dumont, 1994) and the method described by Marchant and Yule (1996). The mean value from both sites was used to estimate annual production by the Size-Frequency Method.

Major Group	Taxa	CPI	Voltinism	Annual Production ($mg\ m^{-2}yr^{-1}$)		Annual P/B	
				Site 1	Site 5	Site 1	Site 5
Diptera				**23481**	**21579**	**7.5**	**6.7**
Chironomidae				**3928**	**4293**	**10.3**	**12.8**
	Tanytarsus Group	97	Tri	207	256	15.2	17.5
	Rheotanytarsus sp	157.5	Biv	75	183	5.2	7.1
	Polypedilum spp	157.5	Biv	1239	1367	8.9	14.9
	Parametriocnemus lundbecki	57.5 *	Asyn	786	830	15.9	18.0
	Orthocladius Group	157.5	Biv	200	289	5.0	5.5
	Eukiefferiella claripennis	157.5	Biv	31	43	4.3	5.4
	Tvetenia paucunca	45 *	Asyn	675	729	16.4	18.4
	Parakiefferiella sp	157.5	Biv	22	32	4.8	7.0
	Thienemanniella sp	157.5	Biv	21	18	4.9	7.8
	Corynoneura sp	37.5 *	Asyn	18	13	25.7	13
	Paracricotopus sp	157.5	Biv	7	3	7.0	5
	Diamesa nivoriunda	107.5	Biv	247	294	6.9	9.2
	Trissopelopia ogemawi	157.5	Biv	400	236	13.0	13.3
Tipulidae				**17914**	**13439**	**7.0**	**5.3**
	Tipula spp	340	Uni	17726	12955	7.0	5.4
	Dicranota sp	340	Uni	188	484	9.1	4.1
Tabanidae	*Chrysops* sp	340	Uni	**398**	**817**	**7.0**	**10.0**
Ceratopogonidae		157.5	Biv	**81**	**83**	**7.4**	**7.3**
Simulidae				**1160**	**2947**	**10.9**	**11.8**
	Simulium spp	157.5	Biv	965	2474	15.8	9.9
	Prosimulium esselbaughi	340	Uni	195	473	4.4	5.4
Coleoptera	*Optioservus fastiditus*	705	Semi	**1072**	**1206**	**2.0**	**2.1**
Ephemeroptera				**4649**	**3450**	**10.4**	**10.4**
	Baetis brunneicolor	170	Biv	3485	2100	16.2	12.3
	Acerpenna macdunnoughi	170	Biv	398	723	12.2	13.4
	Paraleptophlebia debilis	120	Uni	36	71	15	13.4
	Stenonema vicarium	335	Uni	730	556	3.7	5.5
Plecoptera				**388**	**247**	**8.1**	**9.9**
	Allocapnia vivipara	245	Uni	379	221	8.3	11.3
	Amphinemura delosa	340	Uni	9	26	3.9	5.2
Trichoptera				**21122**	**8877**	**5.0**	**4.8**
	Lepidostoma costalis	340	Uni	41	41	3.6	4.0
	Hydropsyche slossonae	340	Uni	15024	4647	5.1	4.7
	Cheumatopsyche oxa	340	Uni	2527	1613	4.9	4.3
	Diplectrona modesta	340	Uni	673	134	3.1	2.4
	Chimarra obscura	340	Uni	423	211	3.1	5.3
	Dolophilodes distinctus	157.5	Biv	343	294	6.6	6.4
	Neophylax concinnus	265	Uni	2091	1937	5.5	5.7
Shredders				**19394**	**14610**	**7.1**	**5.8**
Collectors (filtering)				**20432**	**10285**	**5.1**	**5.7**
Collectors (gathering)				**5926**	**5145**	**13.7**	**12.3**
Scrapers				**3893**	**3699**	**3.5**	**3.6**
Predators				**1067**	**1620**	**9.0**	**7.1**
Total				**50712**	**35359**	**6.0**	**5.9**

Table 2.6. Mean annual density and mean individual biomass of *Hydropsyche slossonae* upstream and downstream of the control site in Logan Drain, Kintore, Ontario.

Hydropsyche slossonae	Mean Annual Density (N/m^2)			Mean Individual Biomass (mg/individual)		
Instar	Site 1	Site 5	% Change	Site 1	Site 5	% Change
1	958	537	-44	0.009	0.009	0
2	1277	605	-53	0.035	0.038	9
3	1287	474	-63	0.148	0.157	6
4	1019	297	-71	0.668	0.718	7
5	653	235	-64	3.072	2.829	-8

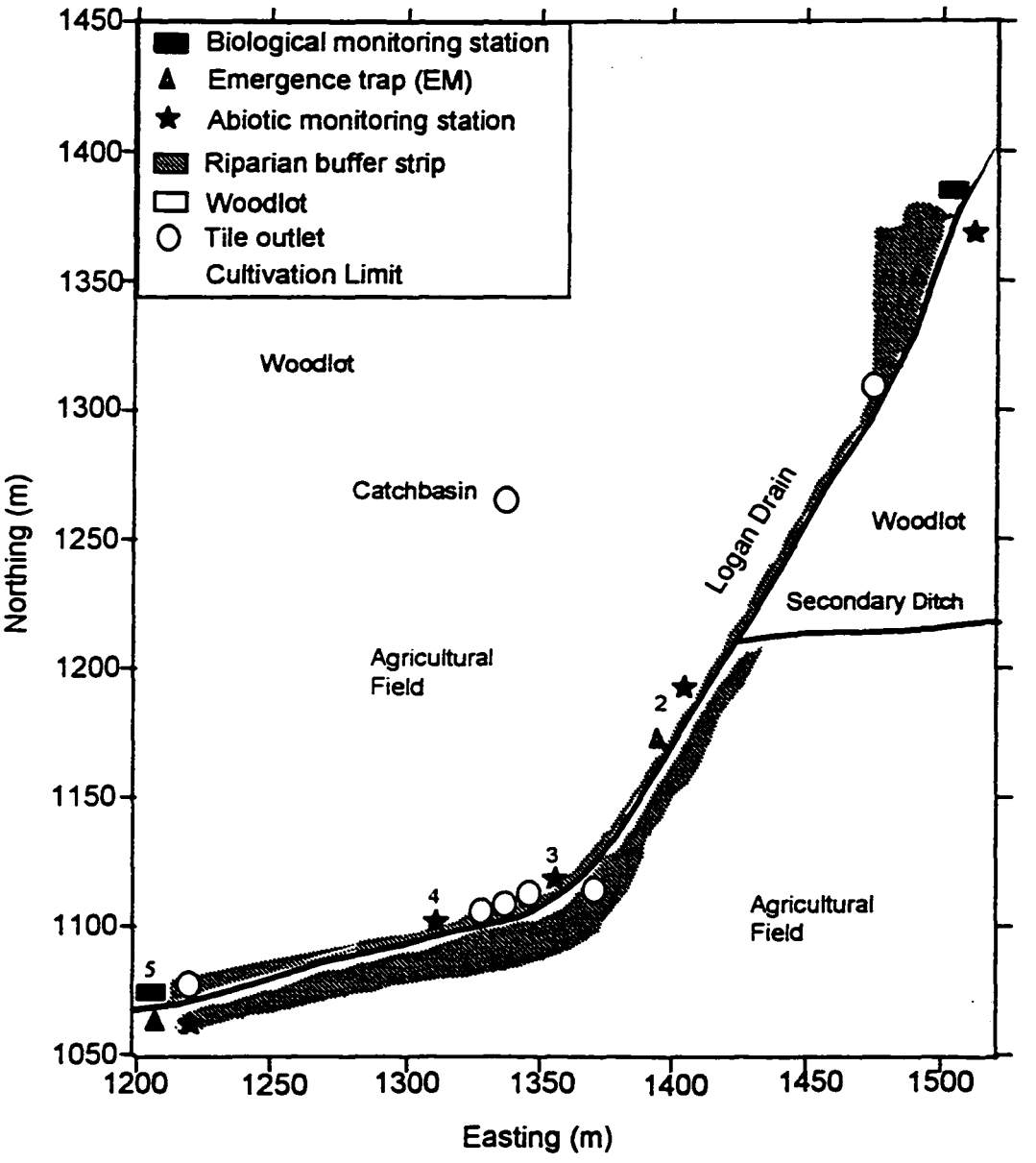

Figure 2.1. Biological monitoring stations in Logan Drain, Kintore, Ontario. Benthic and emergence sampling stations shown. Flow is from upper right to lower left.

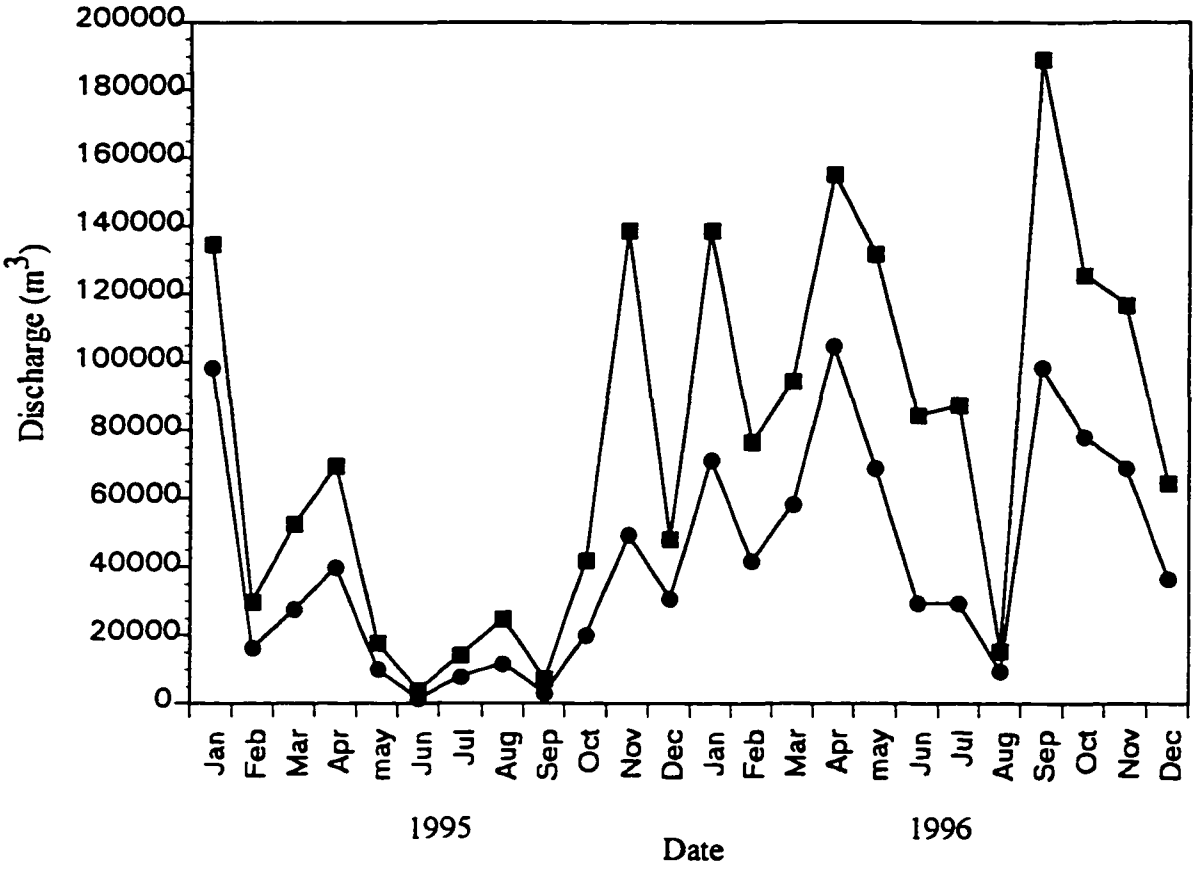

Figure 2.2. Monthly stream discharge (m^3) at sites 1 (solid circles) and 5 (solid squares) from January 1995 to December 1996.

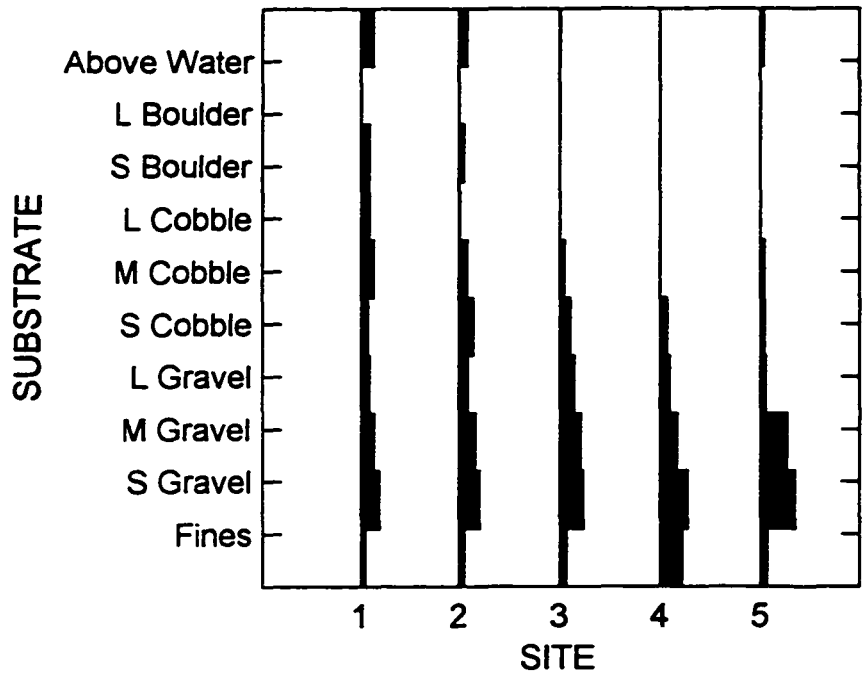

Figure 2.3. Percent surface area of each particle size on the stream bed at the five study sites.

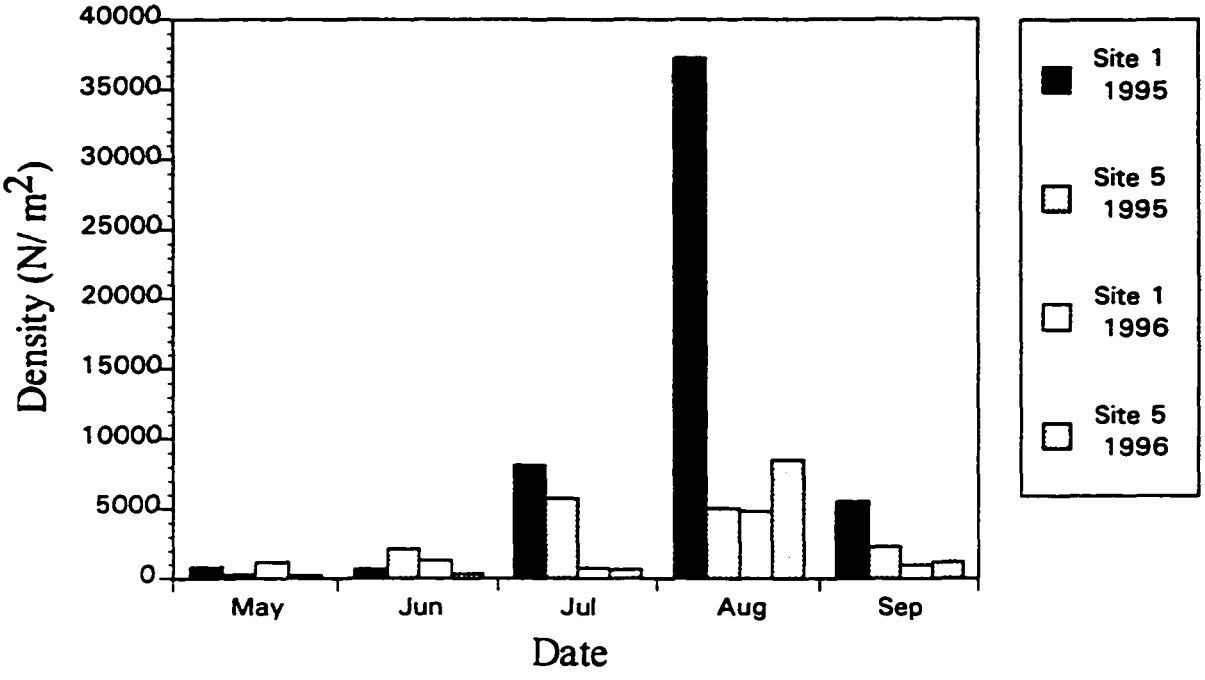

Figure 2.4. Mean density (N/m^2) of *Hydropsyche slossonae* for each month from May to September in 1995 and 1996.

CHAPTER 3

Effects of light intensity, water velocity and species composition on carbon and nitrogen stable isotope ratios in periphyton.

Abstract

Periphyton was allowed to grow on glass plates suspended in the water column of a small stream under two conditions of light and water velocity, over two seasons, to assess the influence of the thickness of benthic boundary layers on stable isotope fractionation. Isotopic signatures for both carbon and nitrogen in samples of periphyton varied with light intensity and season, but not current velocity. In summer, periphyton grown under low light conditions had depleted $\delta^{13}C$ and $\delta^{15}N$ values relative to periphyton grown under high light. In autumn, isotopic signatures were generally more depleted than in summer, but did not vary systematically with light intensity or water velocity. These results suggest that isotopic fractionation in periphyton was more strongly influenced by the intensity of metabolic activity than by variations in the thickness of the benthic boundary layer.

Introduction

The isotopic signatures of aquatic plants and algae are primarily dependent on the concentration and isotopic signature of the dissolved inorganic material being assimilated (Owens 1987; Raven et al. 1993). In addition, the degree of ^{13}C depletion in phytoplankton, benthic algae, and seagrasses is dependent on factors affecting growth rate, such as water temperature, irradiance and nutrients (Wefer and Killingley 1986; Cooper and DeNiro 1989; Muscatine et al. 1989; Fry and Wainright 1991; Laws et al. 1995; Raven et al. 1995; Rau et al. 1997). Less information is available for nitrogen, but studies by Wada and Hattori (1978) and Mariotti et al. (1984) indicate that nitrogen signatures of marine phytoplankton are also dependent on growth rate.

Carbon variability is also dependent on the rate of CO_2 and HCO_3^- diffusion into the cell (Raven et al. 1993). Elevated flow rates can aid in the diffusion of dissolved inorganic species into the chloroplast by reducing the thickness of the boundary layer adjacent to the cell membrane and have been associated with depleted $\delta^{13}C$ values in macrophytes (Osmond et al. 1981; Raven et al. 1982; France and Holmquist 1997). Cooper and McRoy (1988) found no evidence of this effect in their study of the surfgrass *Phyllospadix* spp. France (1995a, 1995b) and Hecky and Hesslein (1995) suggested that more turbulent flow conditions lead to thinner boundary layers around planktonic algae than those adjacent to benthic algae, and this is responsible for the more depleted phytoplankton carbon signatures observed in worldwide compilations of data.

The hypothesis that periphyton $\delta^{13}C$ and $\delta^{15}N$ are influenced by boundary layer thickness, growth rate and differences among algal taxa was tested by conducting field

experiments in which periphyton was allowed to grow on glass plates suspended in the water column of a small stream under two conditions of light and water velocity, over two seasons. The effect of temperature was evaluated by repeating the experiment in late summer (July to mid-September) and in autumn (mid-September to early December).

Methods

Study Site

The study was conducted in a small headwater stream, Logan Drain, located at Lot 19, Concession 12 in East Nissouri Township, Oxford County near Kintore, Ontario (81°10'W, 43°01'N). The stream originates from springs and flows for approximately 2 km through a mixed hardwood and cedar woodlot before emerging between cultivated fields about 200 m upstream of the study site. The channel is 1 to 1.5 m wide, has year-round baseflow and a mean depth of 12 cm. The stream bed at the study site consists mainly of gravel and cobbles (1 to 25 cm diam.) and the banks are lined with grasses and shrubs. The surrounding fields have been systematically tile-drained and were planted to corn in 1996. Although the experiment was conducted between 04 July and 04 December 1996, some stream physical and chemical characteristics were summarised over a longer period to establish trends (Table 3.1).

Water level was measured every 10 minutes with a float-potentiometer-datalogger system. A stage-discharge relationship was developed from magnetic-particle velocity meter readings at six-tenths the water depth and 10 cm increments across the stream taken

on a weekly basis and during major storm events. Water levels were converted to discharge using the stage-discharge relationships.

Stream DIC was sampled in July, September and December of 1996. Samples were collected in dark, airtight 500 ml glass bottles, preserved using mercuric chloride ($HgCl_2$), and refrigerated for no more than 1 week before isotope analysis was performed. CO_2 was extracted from the samples by cryogenic separation in an evacuated, closed-line system, following mixture with 2 ml phosphoric acid (H_3PO_4). Stream DIN was sampled in October 1995, November 1996 and May 1997 and groundwater DIN was sampled in May and November 1996. Water samples were collected in airtight 500 ml plexiglas bottles, filtered through ashed 0.7 μm glass fibre filters, and frozen until analysed.

Isotopic analyses of both DIC and DIN were performed at the Environmental Isotope Laboratory, Department of Earth Sciences, University of Waterloo, Ontario using a VG Isogas (Prism Series II) stable-isotope-ratio mass spectrometer with an analytic precision of ±0.2‰ for both carbon and nitrogen. Isotope ratios are expressed as parts per thousand differences (‰) from a standard reference material:

$$\delta X_{sample} = \left[\frac{R_{sample} - R_{standard}}{R_{standard}} \right] \times 1000$$

where X is ^{13}C or ^{15}N, R is the ratio of either $^{13}C/^{12}C$ or $^{15}N/^{14}N$, and δ refers to the difference between the sample and the reference materials. Standard reference materials,

arbitrarily set at 0‰, are VPDB (Vienna Peedee belemnite) for carbon and nitrogen gas in the atmosphere (Mariotti 1983).

Dissolved CO_2 concentrations were calculated, assuming equilibrium conditions, using pH and alkalinity measurements from 1996 (American Public Health Association 1992). Dissolved $\delta^{13}CO_2$ signatures were calculated from the δ^{13}DIC values, assuming equilibrium conditions, using estimates of CO_2 and HCO_3^- concentrations from pH readings (Pankow 1991) and hydration isotope effects from water temperature measurements (Mook et al. 1974).

Chambers

Six chambers, each with two channels, were constructed of clear 4 mm thick plexiglas (Fig. 3.1). The three "low light" chambers were covered with 90% neutral density filter paper on all outer plexiglas surfaces.

One channel in each chamber was open at both ends so that the rate of flow was similar to the mean stream velocity. The exit of the second was restricted, reducing the velocity to approximately 25% of the high flow channel. The water velocity and irradiance level within both channels of each chamber were measured using a Swoffer Model 2100-stdx current meter and a Li-Cor Model LI1000 light meter on the dates shown in Table 3.2.

The chambers were secured to the stream bed using steel stakes 5 m apart, alternating between high and low light treatments. Periphyton developed on glass plates (10 x 20 cm) mounted horizontally 5 cm below the baseflow water surface in each

channel. Two periods of incubation were used: 04 July to 13 September 1996 (summer) and 13 September to 04 December 1996 (autumn). The chambers were inspected at weekly intervals and cleaned of coarse debris as needed.

The growing plates were divided into three separate scrapes to obtain an alternate test for the effect of boundary layers. Flow over a flat plate is characterised by a viscous layer which begins to grow outward from the leading edge and continues expanding downstream. The boundary layer thickness is defined as the point where the velocity parallel to the plate reaches 99% of the external velocity. Flow approximations over flat-plates are modelled as either laminar or turbulent based on the Reynolds number:

$$Re = \frac{UL}{\nu}$$

where U is the free channel velocity ($m \cdot s^{-1}$), L is the length of the plate (m) and ν is the kinematic viscosity of water ($m^2 \cdot s^{-1}$). Laminar flow occurs at low Reynolds numbers ($Re < 3x10^6$), as in our channels, and boundary layer thickness is given by:

$$T = 5.0(\frac{\nu x}{U})^{1/2}$$

where x is the distance downstream from the leading edge (White 1986).

Carbon and Nitrogen Isotope Signatures

At the end of each incubation period, the glass plates were removed, rinsed in stream water to remove newly sedimented material, bagged, placed on ice in the field, and frozen upon return to the laboratory. Each plate was divided into 8 equal units, numbered 1 to 8 from the upstream edge. Units 2, 5, and 8 were scraped clean, excepting the 25

mm border along each lateral edge which may have been subjected to boundary layer effects from the plexiglas channel sides, and placed into separate petri dishes. The contents of each dish were sorted under a dissecting microscope at 25x magnification in order to remove macroinvertebrates and occasional tufts of filamentous algae. Some of the low light plates yielded too little material for analysis, especially after the autumn incubation.

Most periphyton scrapes remained intact with a layer of microalgae visible beneath an amorphous assemblage of minerals and organic material. It was impossible to separate these without shattering the microalgal mat into individual cells. Therefore, each sample consisted primarily of algae but included other protists, bacteria, fungi and detritus.

After sorting, samples were acidified using 1 N HCL to reduce contamination by inorganic carbon. The samples were centrifuged at low speed for 5 minutes, the supernatant poured off and the process repeated until no further carbon dioxide evolution was observed (France and Holmquist 1997). The samples were diluted with deionized water by the same process until the pH returned to neutral, oven dried at 60°C, and lightly ground to a powder.

Isotopic analyses of the periphyton samples were performed at the Environmental Isotope Laboratory, Department of Earth Sciences, University of Waterloo, Ontario using a Fisons Instruments VG Isochrom-EA continuous flow mass spectrometer with an analytic precision of ±0.20‰ for carbon and ±0.30‰ for nitrogen. All results were adjusted for linearity. The isotope data from the four different conditions in the two study periods were compared using a split plot analysis of variance. The effect of varying

unidentifiable material content in the samples on the carbon and nitrogen signatures was evaluated using linear regressions.

Composition of the Periphyton

Subsamples of the periphyton scrapes were examined microscopically for gross differences in composition. Proportions of algal taxa and unidentifiable material were estimated by viewing 3 fields from the central scrape of each plate. Only one of the three summer low light/low velocity plates had enough material for both isotopic and taxonomic analyses.

Approximately 1mg of each dried sample was suspended in deionized water and allowed to settle into a counting chamber. Algal taxa were identified to genus and enumerated using a Zeiss Axiovert 35 microscope at 1000x magnification. Cell dimensions were determined for each specimen encountered and biomass estimated. The biomass of each taxon observed was expressed as a percentage of the total found under each growing condition by season.

Results

Velocity and Light levels

Mean water velocities in the low flow channels were approximately 25% of the velocities measured in the high flow channels (Table 3.2). The estimated boundary layer thickness ranged from 1.0 mm over unit 2 to 2.1 mm over unit 8 at the highest velocity,

and from 3.1 to 7.0 mm at the lowest velocity. Mean irradiance levels in the low light chambers were <15% of those measured in the high light chambers.

DIC and DIN Concentrations and Isotopic Signatures

CO_2 concentrations fluctuated between 1.8 and 3.1 mg·litre^{-1} indicating that Logan Drain is likely supersaturated at all times. δ^{13}DIC was stable at -12.06‰ over the duration of the experiment (sd = 0.20, n = 3). Dissolved $\delta^{13}CO_2$ signatures were approximately 1.2‰ more depleted in December (-23.29‰) than in July (-22.27‰) or August (-21.88‰) 1996, primarily because of increased fractionation during equilibrium dehydration of HCO_3^- at lower water temperatures (Mook et al. 1974).

The low NH_4^+ concentrations (Fig. 3.2) prevented the estimation of $\delta^{15}NH_4^+$ signatures and also indicate that ammonium was not likely to be the primary nitrogen source in the stream, although concentrations did increase into the autumn experimental period. NO_3^- concentrations were high throughout both of the experimental periods (Fig. 3.2). Elevated nitrate concentrations were a result of annual spring and fall applications of liquid swine manure and commercial fertilisers to the surrounding fields.

The stream $\delta^{15}NO_3^-$ signature varied from 8.1‰ to 10.1‰. These readings were similar to the 6‰ to 11‰ range found in samples of shallow groundwater from the adjacent tiled field collected in May by Cey (1996), and in November 1996.

Periphyton $\delta^{13}C$ and $\delta^{15}N$ Signatures

Periphyton $\delta^{13}C$ ranged from -33.7‰ to -26.9‰ in the summer and from -36.3‰ to -30.8‰ in autumn, with means of -29.6‰ and -33.3‰, respectively (Fig. 3.3). $\delta^{15}N$ ranged from 5.3‰ to 7.3‰ (mean of 6.2‰) in the summer and from 0.8‰ to 4.2‰ (mean of 2.7‰) in autumn. In the summer period, comparisons between high and low light conditions showed that the periphyton carbon signatures were significantly more enriched ($p = 0.045$, $df = 1,4$) when grown under high light. A significant interaction was observed ($p = 0.043$, $df = 4$) between light and velocity in which the effect of light was greater under high than low velocity conditions. $\delta^{15}N$ periphyton signatures were also enriched under the high light summer conditions ($p = 0.069$, $df = 1,4$). No effect of light was observed during autumn. Neither velocity or scrape location appeared to have any influence on $\delta^{13}C$ or $\delta^{15}N$ in either time period. Periphyton signatures for both carbon ($p < 0.001$, $df = 1,4$) and nitrogen ($p < 0.001$, $df = 1,4$) were significantly more depleted in the autumn than in the summer.

Composition of Algal Communities

In the summer, species of *Acnanthes* dominated the biomass found in each of the experimental conditions (Table 3.3). *Amphora sp.* dominated the fall scrapes under all conditions and approached 75% of the mass in the low light samples. Unidentifiable material appeared to be composed of clumps of diatoms held together by extracellular material (Lock et al. 1984). The lack of correlation between the proportion of this

material in each sample and either $\delta^{13}C$ or $\delta^{15}N$ signatures, suggests that it was primarily of autochthonous origin.

Discussion

Isotopic signatures for both carbon and nitrogen in the periphyton samples were influenced by light intensity and season, but not by current velocity or species composition. Periphyton grown under high light conditions in summer were generally more enriched in both ^{13}C and ^{15}N than periphyton grown under low light or at lower temperatures in autumn. In summer under high light, periphyton from the high velocity channels was, on average, slightly enriched in ^{13}C relative to samples from low velocity channels. This is opposite to what would be expected from a boundary layer effect, suggesting that isotopic fractionation primarily reflects the intensity of metabolic activity.

Increasing either light intensity or water temperature should increase metabolic activity in periphytic algae. When the rate of photosynthetic activity is high, the concentration of CO_2 within the cell decreases and enzymatic discrimination between $^{12}C/^{13}C$ is reduced because a larger percentage of the available CO_2 is assimilated (Sharkey and Berry 1985). The same applies to the stable isotopes of nitrogen (Wada and Hattori 1978; Mariotti et al. 1984). The samples from high light intensity during the summer experiment had more enriched ^{13}C and ^{15}N signatures than those from low light or autumn. This result is consistent with the more depleted ^{13}C signatures reported under lower light intensities or slower growth rates in a variety of marine and freshwater plants, both in

culture and in the field (Wefer and Killingley 1986; Cooper and DeNiro 1989; Muscatine et al. 1989; Laws et al. 1995; Raven et al. 1995; Hemminga and Mateo 1996).

The effects of boundary layer thickness on periphyton carbon and nitrogen isotope signatures were evaluated both by manipulating water velocity and by analysing different portions of the glass plates. Elevated flow rates can aid in the diffusion of dissolved inorganic species into the chloroplast by reducing the thickness of the boundary layer adjacent to the cell membrane. Depleted $\delta^{13}C$ values in macrophytes have been associated with fast flowing water (Osmond et al. 1981; Raven et al. 1982; Hecky and Hesslein 1995; France and Holmquist 1997) and if this effect were important to periphyton, more depleted signatures would have been observed in the high than in the low velocity channels. This was not the case. The resistance to diffusion is a function of the length and nature of the path that must be crossed. The resistance to CO_2 and NO_3^- diffusion through the complex spatial structure of the epilithon and the algal cell membranes are likely more important than the thickness of the outer boundary layer. In addition, the supersaturated concentrations of dissolved CO_2, high NO_3^- concentrations and internal recycling of both CO_2 and NO_3^- within the periphytic film may also have reduced the importance of boundary layer diffusion. Regardless, flow rate did not influence the $\delta^{13}C$ or $\delta^{15}N$ signatures of the algal scrapes.

Estimates of boundary layer thickness also increased downstream over each plate. This did not yield increased discrimination on the leading, in comparison to the trailing, edges of the plates. Again, boundary layer thickness did not affect algal carbon or nitrogen isotope signatures.

The lack of isotopic differences between the high and low light conditions in autumn indicates that growth rates were probably already limited by low water temperatures under both light levels. The low water temperatures restricted algal metabolism, growth rates, demand for both carbon and nitrogen and allowed greater discrimination against the heavier isotopes. As a result, all autumn isotope signatures were more depleted than in summer.

Water temperature affected the carbon signatures in autumn through two mechanisms. Mean carbon signatures changed from -28.3‰ in summer high light to -30.9‰ in summer low light and to -33.3‰ in autumn. Of the 2.4‰ differential between summer low light and autumn signatures, 1.2‰ stems from enhanced equilibrium hydration effects and the remaining 1.2‰ from a further reduction in algal metabolic rates at the lower autumn water temperatures.

Mean nitrogen signatures became progressively depleted from summer high light (6.7‰) to summer low light (5.8‰) and finally to autumn (2.7‰). In addition to water temperature effects on algal metabolic activity, depleted autumn nitrogen signatures could have been influenced by changes in stream nitrogen concentrations, signatures or the preferential assimilation of an alternate nitrogen source. Stream NO_3^- concentrations were consistent throughout the two experimental periods and unlikely to have contributed to the depleted signatures observed in autumn. Stream NH_4^+ concentrations were elevated in autumn and may have been preferentially assimilated or contributed to increased nitrogen influx into the algal cells at that time. Stream nitrate signatures ranged between 8.1‰ and 10.1‰ and fluctuations throughout our experimental period may also have contributed to

the lighter autumn signatures. The enriched stream nitrate signatures appear to be a result of the manure applications, a sample of which measured 9.1‰ in the fall of 1995, although denitrification of commercial fertilisers and soil organic nitrogen could also have led to similar values (Smith et al. 1991).

Water velocities in both the slow and fast channels were uniformly higher in autumn than in summer and, according to the boundary layer hypothesis, also could have contributed to the depleted carbon and nitrogen signatures observed then. It seems doubtful, however, that increased water velocities led to this effect. Larger differences in water velocity between the slow and fast channels failed to significantly influence the periphyton signatures in either summer or autumn. In addition, linear regressions of carbon and nitrogen signatures against water temperature, channel velocity, light level and unidentifiable material content failed to find a significant contribution from channel velocity in either season or over both seasons combined.

The homogenous species composition in the summer epilithic community implies that algal community composition was not responsible for the variation found in either the carbon or nitrogen signatures. It has been shown that some aquatic primary producers possess a CO_2 concentrating mechanism that allows algae to actively transport DIC across the cell membrane and create higher cell concentrations of CO_2 than would otherwise exist (Raven et al. 1993). Sharkey and Berry (1985) demonstrated that this mechanism was inducible in *Chlamydomonas reinhardtii* at low dissolved CO_2 concentrations. They assumed that HCO_3^- was the carbonate species pumped into the algal cell, converted to CO_2 and subsequently fixed by ribulose bisphosphate carboxylase. In their model, CO_2

fixed by the cell must have the same isotopic composition as the transported HCO_3^-, resulting in heavier algal signatures as well as the higher cell concentrations of CO_2. Similarly, it has been suggested that different taxa may preferentially assimilate alternate species of dissolved inorganic nitrogen, with separate isotopic signatures (Falkowski 1990). Although a shift in diatom community composition occurred from summer to autumn, there was no evidence of a relationship between carbon or nitrogen signatures and composition of the periphytic assemblage.

The possibility existed that varying unidentifiable biomass, of unknown origin, within each scrape could affect isotopic ratios. However, from the linear regressions, no relationship between carbon or nitrogen signatures and the proportion of unidentifiable material in the samples was observed, perhaps because most of this material appeared to be derived from diatoms.

The mean range of carbon signatures within each experimental condition was 3.5‰ in both summer and fall, whereas that for nitrogen was 1.1‰ in summer and 1.9‰ in fall. Such variability is inherent in field experiments and probably reflects factors such as different degrees of shading of individual chambers by grasses and shrubs on the stream bank, the periodic interference with flow and light by branch and leaf snags or the natural variability in colonisation of the chamber surfaces. Over the two experimental periods, the complete range of carbon and nitrogen signatures encompassed 9.4‰ and 6.5‰, respectively. The implication of these results is that the use of stable carbon and nitrogen isotopes as natural tracers of energy flow in lotic habitats may require an intensive sampling effort to understand the complete microhabitat and seasonal variation in

autochthonous signatures. Such effort may, however, yield more detailed information about where and when consumers actually feed.

Acknowledgements

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Table 3.1. Physical and chemical measurements in Logan Drain from 1994 to 1996. Numbers in brackets are sample sizes.

Characteristic	Mean
Discharge - summer 1996 ($m^3 \cdot s^{-1}$)	0.02594 (1703)[a]
- autumn 1996 ($m^3 \cdot s^{-1}$)	0.05889 (1970)[a]
Temperature - summer 1996(°C)	15.4 (1699)[a]
- autumn 1996(°C)	8.5 (1970)[a]
Dissolved Oxygen ($mg \cdot l^{-1}$)	10.04 (2)[a]
Alkalinity ($mg \cdot l^{-1}$ $CaCO_3$)	249.9 (9)[b]
pH	8.20 (42)[c]
Electrical Conductivity ($\mu S \cdot cm^{-1}$)	545 (11)[a]
DOC ($mg \cdot l^{-1}$)	2.2 (20)[a]
NO_3^--N ($mg \cdot l^{-1}$)	3.1 (81)[a]
NH_4^+-N ($mg \cdot l^{-1}$)	0.04 (82)[a]
TSN ($mg \cdot l^{-1}$)	3.6 (11)[a]
SRP ($\mu g \cdot l^{-1}$)	14.1 (9)[b]
TP ($\mu g \cdot l^{-1}$)	29.6 (10)[b]

Note: Sources of data: a = G. Parkins, b = J. Winter, c = Upper Thames Conservation Authority, personal communications.

Table 3.2. Mean water velocity and irradiance levels measured in each experimental channel over summer (04 July to 13 September 1996) and autumn (13 September to 04 December 1996) periods. Values are averages of 3 replicates from each experimental condition. Numbers in brackets are standard deviations.

	High Light		Low Light	
	High Velocity	Low Velocity	High Velocity	Low Velocity
Velocity ($m \cdot s^{-1}$)				
04 Jul 96	0.14 (0.03)	0.03 (0.02)	0.20 (0.05)	0.02 (0.01)
18 Jul 96	0.28 (0.03)	0.09 (0.06)	0.26 (0.02)	0.06 (0.04)
01 Aug 96	0.17 (0.05)	0.07 (0.03)	0.23 (0.10)	0.08 (0.01)
15 Aug 96	0.16 (0.05)	0.07 (0.02)	0.20 (0.08)	0.03 (0.02)
13 Sep 96	0.28 (0.05)	0.06 (0.07)	0.39 (0.04)	0.04 (0.03)
04 Dec 96	0.57 (0.08)	0.12 (0.10)	0.44 (0.14)	0.14 (0.07)
Irradiance (μE)				
04 Jul 96	133 (86)	144 (73)	11 (6)	12 (6)
18 Jul 96	456 (102)	425 (17)	43 (2)	49 (1)
01 Aug 96	692 (746)	409 (218)	116 (77)	65 (44)
15 Aug 96	547 (78)	563 (136)	48 (17)	46 (14)
04 Dec 96	452 (91)	442 (81)	66 (26)	73 (21)

Table 3.3. The algal community structures by % biomass in each of the experimental conditions from the summer and autumn study periods. Values for each condition are means of 3 fields of view from each of 3 replicate plates. The summer low light/low velocity condition is the mean of 3 fields from 1 plate.

	Summer 1996				Autumn 1996			
	High Light		Low Light		High Light		Low Light	
Algal Taxa	High Velocity	Low Velocity	High Velocity	Low Velocity	High Velocity	Low Velocity	High Velocity	Low Velocity
Amphora sp	3	11	11	22	28	31	72	79
Acnanthes sp	39	36	28	23	8	3	1	1
Navicula sp	3	10	5	15	10	3	1	1
Cocconeis sp		1	6	20	<1	31	6	6
Cymbella sp	2	1	1	2	<1	1	<1	
Gomphonema sp	1	2	1	1	5	2	<1	
Nitzschia sp					1			
Surilella sp	<1			1				
Synedra sp		<1						
Diploneis sp	<1							<1
Unidentifiable	51	39	48	15	49	30	9	14

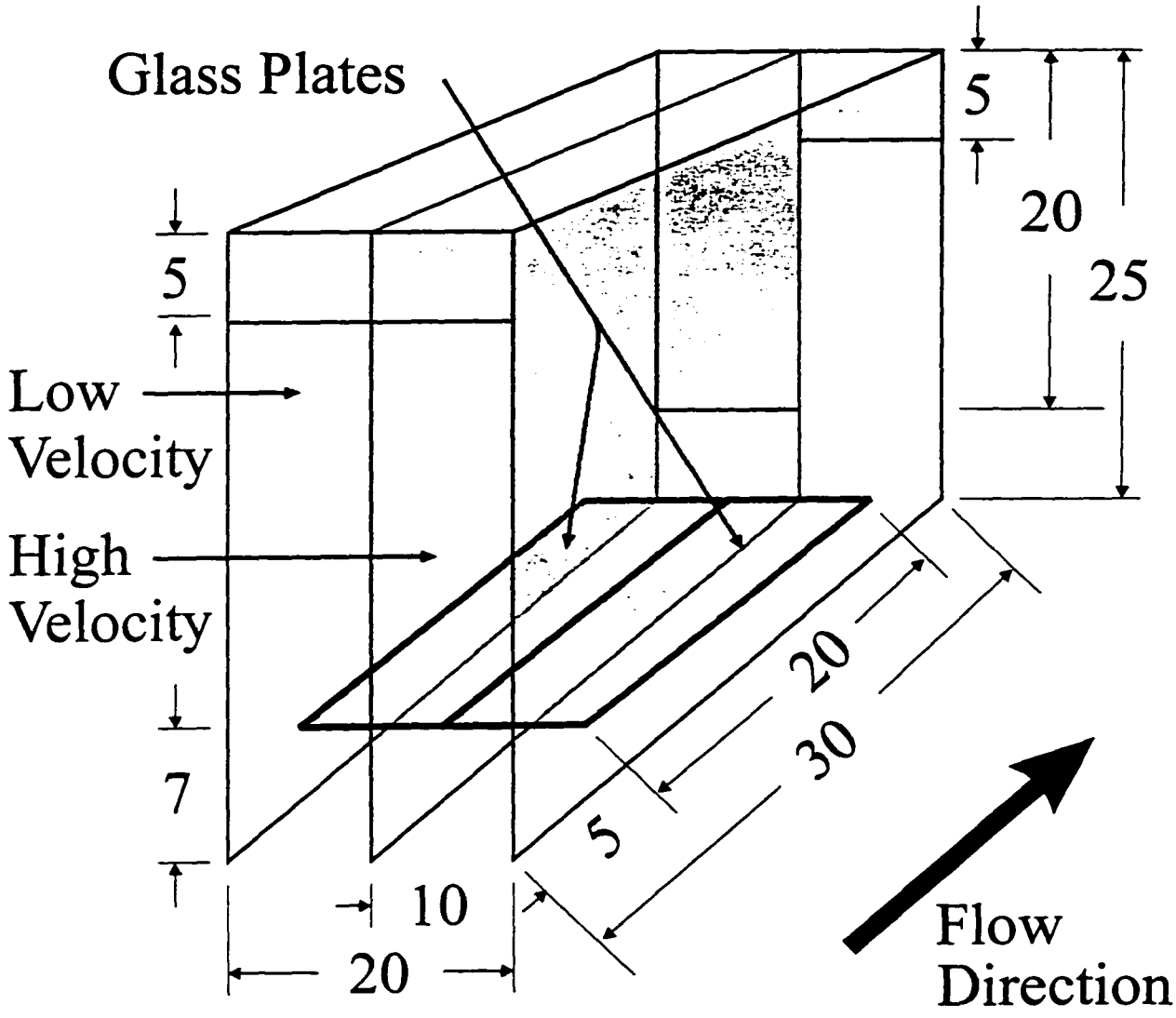

Figure 3.1. High light chamber with high and low velocity channels. Glass plates are 10 x 20 cm. Mean water level and depth over glass plates are 12 cm and 5 cm, respectively. All dimensions are in cm.

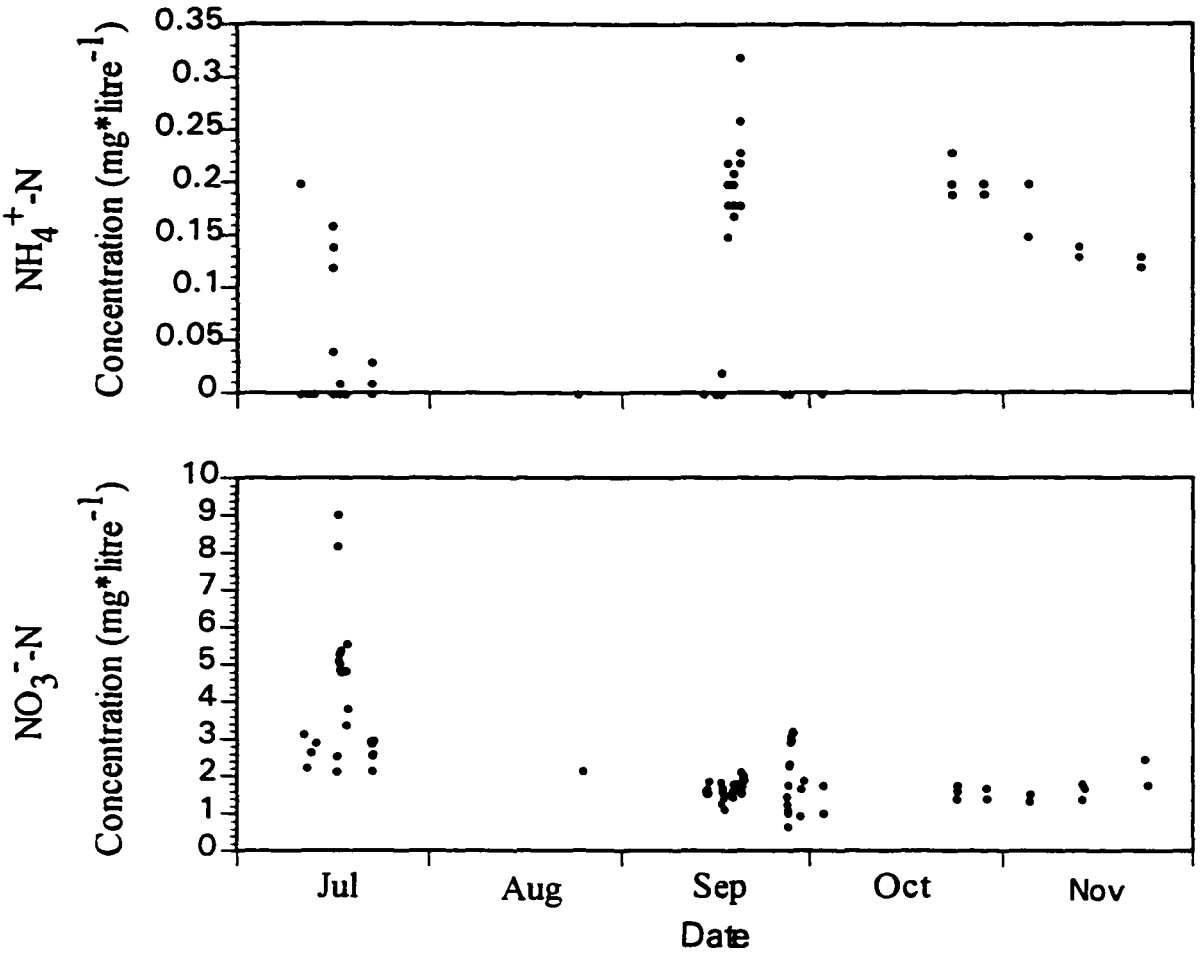

Figure 3.2. Nitrate and ammonium concentrations (mg·litre^{-1}) from July 1996 to November 1996.

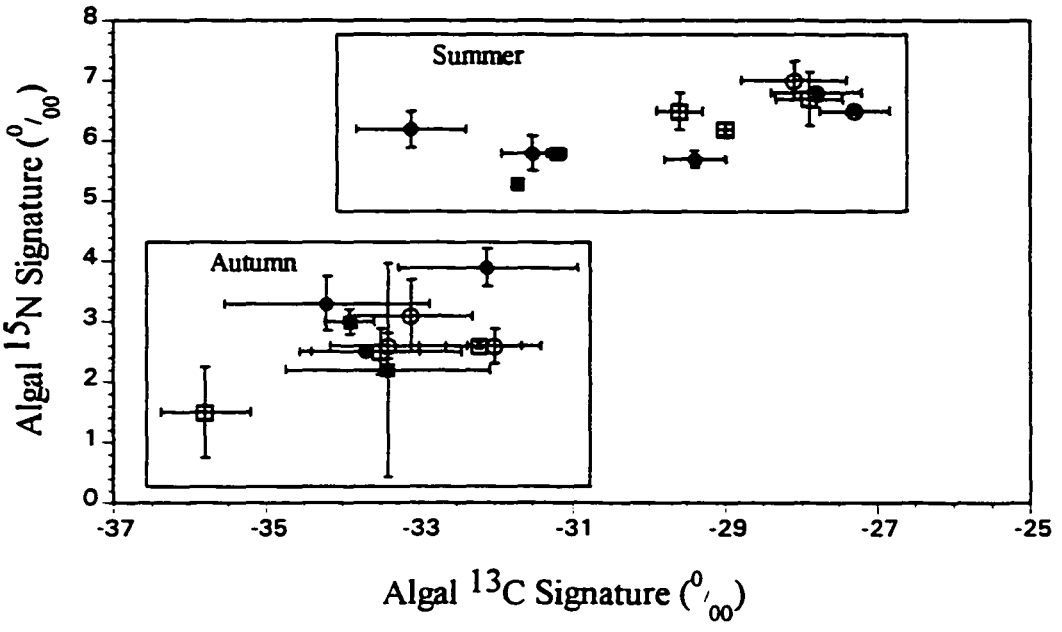

Figure 3.3. Mean stable carbon and nitrogen isotope signatures of periphyton scrapes from summer and autumn. Means are averages of the three positions analysed on each glass plate. High light (non-filled), low light (filled), high velocity (circles) and low velocity (squares). Error bars are standard deviations.

Chapter 4

The trophic structure of a headwater stream in southern Ontario using stable isotopes and secondary production

Abstract

Carbon and nitrogen stable isotope signatures of diatoms, chlorophytes, bryophytes, macroinvertebrates and fish were examined over two years in a headwater stream draining wooded and agricultural land in southern Ontario. Chlorophyte and diatom carbon signatures followed a seasonal pattern in which reduced metabolic fractionation under high growth conditions in summer led to enriched signatures. Signatures were most depleted in winter, while those in spring and autumn were intermediate. Carbon signatures of the macroinvertebrates reflected seasonal variation in the algae. The application of commercial and organic fertilisers did not result in heavier nitrogen signatures in either the autochthonous producers or macroinvertebrates at the downstream site.

Functional feeding group designations were useful predictors of diet at the group level. The relative dependence of the different groups on allochthonous resources followed the expected trend (i.e. shredders were most and scrapers the least dependent on terrestrial inputs). However, many taxa exploited a wider food base than their designation

implied (e.g. shredders and filterers). The dietary analysis suggests that four taxa should be assigned to different trophic guilds. *Parakiefferiella* sp., *D. nivoriunda* and *B. brunneicolor* were almost completely dependent on benthic algae so should be considered scrapers. Likewise, the diet of *Pycnopsyche* sp. suggests it is more of a gatherer than a shredder. Food availability rather than morphological adaptions appeared to be most important in determining the diet of individual taxa.

The similarity of invertebrate diets at sites upstream and downstream of the agricultural site suggest that inputs of autochthonous and allochthonous organic material did not limit secondary production at the downstream site. The altered benthic community composition and lower secondary production downstream were primarily due to a shift in stream bed morphology, likely a direct result of agricultural activity on the adjacent fields. Autochthonous primary production and allochthonous inputs necessary to support the macroinvertebrate community were within expected ranges for a moderately enriched headwater stream with a partial canopy.

Introduction

The river continuum concept (Vannote et al. 1980) describes changes in trophic structure along the course of a river and emphasises the importance of terrestrial plant debris to undisturbed headwater streams. Contributions by allochthonous and autochthonous carbon sources to lotic communities have usually been determined by gut content analysis of the most abundant invertebrates. Although the method provides a high resolution of certain foods, the relative abundances of foods in the gut do not necessarily reflect their importance to an animal. More recently, stable isotope analysis (SIA) has been used successfully to determine the importance of different carbon sources to individual taxa. The advantage of SIA is that the isotope ratios reflect materials that are assimilated by the animal rather than materials present in the gut. The study presented here is the first in which SIA will be combined with estimates of secondary production to quantify the dependence of individual taxa and a stream ecosystem on terrestrial and aquatic primary production.

The utility of stable carbon and nitrogen isotopes as natural tracers of energy flow in marine, estuarine and freshwater ecosystems (Hobson and Welch 1992; Rosenfeld and Roff 1992; Bunn and Boon 1993; Junger and Planas 1994; Hecky and Hesslein 1995; Bootsma et al. 1996) is dependent upon the presence of distinct isotopic signatures among potential primary producers. It is not uncommon, however, for the isotopic ratios of aquatic macrophytes or algae at a site to vary by 10‰ for carbon and 3‰ for nitrogen (Wada and Hattori 1976, 1978; Minagawa and Wada 1984; Kline et al. 1990; Fry and Wainright 1991; Fogel et al. 1992; Bunn and Boon 1993; MacLeod and Barton 1998).

Unfortunately, few researchers have conducted a thorough analysis of the variation in autotrophic stable isotope signatures and this casts doubt on the conclusions of some studies and has limited the resolving power of SIA to trace trophic interactions in others (France 1995c).

Carbon and nitrogen isotope ratios are primarily dependent on the concentrations and signatures of source inorganic materials, in addition to factors affecting growth rate such as water temperature, irradiance and the concentrations of nitrogen and phosphorous. These environmental factors vary both spatially and temporally. It has been shown that fractionation between inorganic carbon and nitrogen and the tissues of primary producers is greatest under low, and least under high, growth rates (Wada and Hattori 1976, 1978; Owens 1987; Rau 1997; MacLeod and Barton 1998). An earlier study of this stream (MacLeod and Barton 1998) described seasonal variation in periphyton carbon and nitrogen signatures. The first objective of the present work was to determine whether the signatures of other aquatic primary producers (chlorophytes and bryophytes) also varied seasonally.

As a result of the variation in the isotopic signatures of lotic primary producers, it is clear that temporal and spatial variation is also likely to occur at higher trophic levels. Few studies utilising stable isotopes to determine the primary sources of organic carbon in stream fauna have sampled extensively enough to determine temporal and spatial variation in the signatures of the potential carbon sources or in the animal taxa of interest. The second objective was to evaluate whether seasonal variation in autotrophic primary

producers was reflected in the signatures of consumers, in particular the scrapers and collector gatherers which typically assimilate a high proportion of algae.

Inputs to streams draining agricultural land include nutrients, organic wastes, suspended solids, pesticides, herbicides and solar irradiance (Dance and Hynes 1980; Lenat et al. 1981; Erman and Erman 1984; Dosdall and Lehmkuhl 1989). Different sources of nitrate, which can sometimes be distinguished by their $^{15}N/^{14}N$ ratios, include nitrified soil organic nitrogen, nitrogenous fertilisers, and animal or sewage wastes. Animal wastes, typically the most enriched of these sources, generally have signatures in excess of 10‰ (Wilson et al. 1994). The third objective was to determine whether the application of liquid swine manure to the study site resulted in enriched nitrogen signatures in the downstream flora and fauna.

A mixing model was used to determine the relative dependence on allochthonous versus autochthonous inputs by each taxon, upstream and downstream from the agricultural site. The fourth objective was to evaluate shifts in macroinvertebrate diets between seasons. The results were combined with estimates of secondary production for the 33 most common taxa in order to quantify the utilisation of allochthonous and autochthonous production upstream and downstream of the agricultural activities in this headwater stream. The final objective was to determine whether a reduced dependence on allochthonous sources could be detected through a shift in consumer carbon signatures away from allochthonous and towards autochthonous producers at the downstream site.

Methods

Study Site

The study was conducted in a small headwater stream, Logan Drain, located at Lot 19, Concession 12 in East Nissouri Township, Oxford County near Kintore, Ontario (81°10'W, 43°01'N). The stream originates from springs and flows for approximately 2 km through a mixed hardwood and cedar woodlot before emerging between cultivated fields. From this point until entering the second woodlot 400 metres downstream, the stream has been channelised and was most recently cleaned in 1990. The wetted channel is 1 to 1.5m wide, has year-round baseflow and a mean depth of 12 cm. The stream bed consists mainly of gravel and cobbles (1 to 25 cm diam.) and the stream is bordered by a strip of grasses and shrubs, ranging from 3 to 10 metres in width. The surrounding fields have been systematically tile-drained. Alfalfa was grown on the north side of the stream and corn (maize) to the south in 1995; both sides were planted to corn in 1996. Fertiliser inputs consisted primarily of liquid swine manure and were applied in April and September 1995 and May 1996.

Site 1 extended upstream 20 metres into the woodlot which borders the headwaters (site 1; Fig. 4.1) and site 5 encompassed 10 metres of the stream within the wooded area just below the field (site 5; Fig. 4.1). Both of these sites were partially shaded during summer and autumn, but sites 2, 3 and 4 (Fig. 4.1) received direct insolation through much of the day. Secondary production was measured at sites 1 and 5 in 1995/1996 (chapter 2).

Carbon and nitrogen signatures of dissolved inorganic matter (DIM), particulate organic matter (POM) and autochthonous primary producers were determined at all sites. Signatures of common stream fauna were determined at sites 1 and 5. The chemical and physical characteristics of the stream are summarised in Table 4.1 and the sampling dates for all field collections are in Table 4.2.

Carbon and Nitrogen Isotope Signatures

Stream DIC and DIN signatures were determined as described by MacLeod and Barton (1998) on the dates shown in Table 4.2. Dissolved $\delta^{13}CO_2$ signatures were calculated from the δ^{13}DIC values, assuming equilibrium conditions, using estimates of CO_2 and HCO_3^- concentrations from pH readings (Pankow 1991) and hydration isotope effects from water temperature measurements (Mook et al. 1974).

Samples for POM in the water column consisted of 4 litres of stream water strained through a 40µm screen and collected in plastic jugs at each site, kept on ice in the field, pumped across GF/C (1.2µm) pre-combusted glass fibre filters in the laboratory and then frozen. The filters were acidified with 1 N HCL and dried at 60°C for 48 hours prior to isotopic analysis. Sample dates are shown in Table 4.2.

Fresh foliage from fourteen common terrestrial plants was collected on 08 June 1995, placed on ice in the field and frozen upon return to the laboratory. Prior to isotopic analysis, the samples were rinsed with deionized water, oven dried at 60°C, and ground using a Retsch MM2000 ball mill.

Potential sources of autochthonous organic matter included diatoms, chlorophytes, and aquatic bryophytes. Diatom carbon and nitrogen signatures were determined in summer (04 July to 13 September) and autumn (13 September to 04 December) in 1996 (MacLeod and Barton 1998) and in winter (04 December 1996 to 13 March 1997). Bryophytes and chlorophytes were scraped from rocks at all sites on the dates shown in Table 4.2, rinsed in stream water to remove newly sedimented material, bagged, placed on ice in the field, and frozen upon return to the laboratory. Prior to isotopic analysis, the samples were acidified for 24 hours, dried at 60°C for 48 hours and ground to a powder with a mortar and pestle.

Aquatic macroinvertebrates were collected in spring (May and June) and in autumn (September; *Sialis* sp., *Calopteryx* sp. and *Aeshna* sp. were collected in February) using a kick-sweep technique across the width of the stream at sites 1 and 5 in an attempt to sample all habitats within each site. Samples from different years at each site were kept separate and served as repeats when taxa were collected more than once. The animals were bagged and placed on ice in the field, sorted to the family level in the laboratory and subsequently frozen. Prior to isotopic analysis, all animals were sorted to the lowest possible taxonomic level, head capsules measured to determine age of the common taxa, and the largest taxa (e.g. *Hydropsyche slossonae*, *Cheumatopsyche oxa*, *Diplectrona modesta* and *Tipula* spp.) were gutted. Only late instar animals were selected to ensure that biomass assimilated throughout the complete lifecycle of each taxon was analysed. The small size of many of the Diptera (ceratopogonids, empidids and most chironomids) necessitated the use of all instars in each sample to ensure sufficient biomass for analysis.

Samples were dried at 60°C for 48 hours, ground to a powder with a mortar and pestle, and stored in airtight glass vials. Between 1 and 50 animals, depending on size and abundance, were used in each sample.

Fish were collected by electroshocking at sites 1 and 5 on 08 June 1995. They were sorted to species, bagged and placed on ice in the field, and frozen upon return to the laboratory. Sections of muscle tissue dorsal to the lateral line were excised, dried at 60°C for 48 hours, ground to powder and stored in airtight glass vials. Up to three individuals of each species were sampled from each site.

Isotopic analyses of DIC and DIN were performed at the Environmental Isotope Laboratory, Department of Earth Sciences, University of Waterloo, Ontario using a VG Isogas (Prism Series II) stable-isotope-ratio mass spectrometer with an analytic precision of ±0.2‰. Isotopic analyses of the remaining samples were performed at the Environmental Isotope Laboratory, Department of Earth Sciences, University of Waterloo, Ontario using a Fisons Instruments VG Isochrom-EA continuous flow mass spectrometer with an analytic precision of ±0.2‰ for carbon and ±0.3‰ for nitrogen. All results were adjusted for source linearity problems. Isotope ratios are expressed as parts per thousand differences (‰) from a standard reference material:

$$\delta X_{sample} = \left[\frac{R_{sample} - R_{standard}}{R_{standard}} \right] \times 1000$$

where X is ^{13}C or ^{15}N, R is the ratio of either $^{13}C/^{12}C$ or $^{15}N/^{14}N$, and δ refers to the difference between the sample and the reference materials. Standard reference materials, arbitrarily set at 0‰, are VPDB (Vienna Peedee belemnite) for carbon, and nitrogen gas in the atmosphere (Mariotti 1983). Statistical comparison of the isotope signatures between sites, seasons and functional feeding groups was conducted using paired t-tests with individual taxa serving as replicates.

An isotopic mixing model was used to estimate the relative proportion of secondary production which was dependent on allochthonous versus autochthonous organic matter. The following general equation (modified from Doucett et al. 1996) was used:

$$\%\text{Allochthonous} = \left(\frac{\delta^{13}C_{animal} - \delta^{13}C_{autochthonous} - fx}{\delta^{13}C_{allochthonous} - \delta^{13}C_{autochthonous}}\right) \times 100$$

where f is the average enrichment of ^{13}C between an animal and its food, x is the trophic position of an animal above the primary producers, and $\delta^{13}C_{allochthonous}$ and $\delta^{13}C_{autochthonous}$ values are the mean signatures of allochthonous and autochthonous inputs, respectively.

Because the average trophic enrichment for carbon has not been determined for most animals over any food sources, we ran the model using both 0‰ (France 1996) and 1‰ (DeNiro and Epstein 1978) enrichments in order to obtain estimates which would encompass the most widely quoted values and provide a likely range of allochthonous dependence for each animal. Trophic position was determined using the $\delta^{15}N$ data from this study. Shredders, scrapers, gatherers and filterers were assumed to have a trophic position of 1 and those animals known to rely on predation were assigned trophic

positions based on the level of ^{15}N enrichment above the mean nitrogen signature of the four predominantly herbivorous groups; an enrichment of 3.4‰ corresponded to one trophic level increment (Minagawa and Wada 1984). This method is a modification of that proposed by Cabana and Rasmussen (1996) and it allowed us to minimise the effects of varying nitrogen signatures among the primary producers.

Results and Discussion

Autochthonous Primary Producers

Seasonal variation was evident in the carbon signatures of diatoms and chlorophytes, both of which exhibited enriched values under summer conditions and more depleted values in winter (Table 4.3). MacLeod and Barton (1998) observed that diatom signatures were significantly lighter when grown under low light than under high light conditions in summer and were significantly lighter in autumn than summer. Nitrogen signatures were also most enriched in summer relative to both autumn and winter (Table 4.3). The most abundant taxa in these samples were species of the diatoms *Acnanthes*, *Amphora*, *Navicula* and *Cocconeis*.

Carbon signatures of chlorophytes were depleted relative to diatoms (Fig. 4.2). Diatoms were embedded in the epilithon whereas Chlorophytes were not. Increased resistance to diffusion through the complex spatial structure of the epilithon likely led to lower cellular concentrations of CO_2 in diatoms than in chlorophytes. Lower cellular concentrations are associated with reduced enzymatic discrimination between ^{12}C and ^{13}C because a larger percentage of the available CO_2 is assimilated (Sharkey and Berry 1985).

Chlorophyte nitrogen signatures were most depleted in winter and became more enriched throughout the year to a maximum in autumn. *Cladophora*, *Spirogyra*, *Ulothrix* and an unidentified unicellular green algae were the most abundant taxa in the these samples.

The isotopic signatures of aquatic plants and algae are primarily dependent on the concentration and isotopic signature of the dissolved inorganic material being assimilated (Owens 1987; Raven et al. 1993), but the degree of fractionation is also dependent on factors affecting growth rate, such as water temperature, irradiance and nutrients (Wefer and Killingley 1986; Cooper and DeNiro 1989; Muscatine et al. 1989; Fry and Wainright 1991; Laws et al. 1995; Raven et al. 1995; Rau et al. 1997; MacLeod and Barton 1998). Less information is available for nitrogen, but Wada and Hattori (1978) and Mariotti et al. (1984) indicate that nitrogen signatures of marine phytoplankton are also dependent on growth rate.

Estimates of dissolved $\delta^{13}CO_2$ varied by less than 3.4‰ seasonally, so do not account for the observed fluctuations in chlorophyte signatures. MacLeod and Barton (1998) concluded that seasonal variation of the diatom signatures in this stream was due to the effect of temperature on growth rates. The temporal response of chlorophytes suggests that high water temperatures in summer led to increased metabolic rates and reduced enzymatic fractionation within these taxa as well. Enriched nitrogen signatures of diatoms in summer and of chlorophytes in summer and autumn may also result from increased metabolic rates, although inputs from fertilisers in spring and autumn (Fig. 4.2) also likely contributed.

Isotopic signatures of bryophytes were much less variable (Fig 4.2). The annual mean carbon and nitrogen signatures were -40.0‰ and 7.6‰, respectively, and the nitrogen signatures were more enriched than any other abundant primary producer except grass. Bryophytes appeared to grow continuously throughout the year. The samples consisted of complete shoots so signatures were similar on all sampling dates.

Allochthonous Primary Producers

Carbon signatures from allochthonous plants, excluding maize, fell within a narrow range from -29.5‰ to -24.3‰ (Table 4.4), with a mean signature of -27.4‰. Maize was more enriched, -12.7‰, due to its C4 metabolism. Conversely, nitrogen signatures from these plants varied widely from -0.3‰ to 9.2‰ and had a mean signature of 3.7‰. The range of terrestrial nitrogen signatures encompassed the range from autochthonous sources and precluded the use of nitrogen signatures to trace organic inputs to the invertebrate fauna.

There was no evidence of a seasonal trend in either the mean carbon or nitrogen signatures of particulate organic matter (Fig. 4.2). Mean carbon signatures fell within a narrow range from -27.0‰ to -25.6‰ with an annual mean of -26.5‰ and therefore were not influenced by the seasonal variation in the autochthonous sources. This implied that the POC signature was predominately terrestrial, as was reported by Rounick and Hicks (1985) from a second order New Zealand stream. POC was 0.9‰ more enriched than the mean signature of the most abundant terrestrial plants. This could be the result of a greater percentage of the most enriched plants entering the stream, trophic enrichment by

bacteria and other microbes colonising the POC (POC samples are likely composed of the most refractory terrestrial material which may be heavier than whole tissues), or that prefiltering removed isotopically light materials. Although mean nitrogen signatures ranged from 0.2‰ to 6.7‰, variation within dates was greater, -3.4‰ to 18.3‰, and also implied a terrestrial source. Autochthonous nitrogen signatures were stable within seasons and, even over the course of a year, did not exhibit the range found in PON, whereas PON and allochthonous signatures had a similar mean and range of signatures. The variation in PON signatures suggests that, in some cases, individual terrestrial plant species dominated the biomass collected on a filter and strongly influenced its isotopic signature.

Primary Consumers - comparison of diets between seasons

Carbon and nitrogen signatures were determined for 57 taxa. Carbon signatures were significantly more depleted in spring samples than in autumn samples at both site 1 (paired t-test, $df=19$, $p<0.01$) and site 5 (paired t-test, $df=20$, $p=0.02$). Nitrogen signatures were also more depleted in spring at both sites; however, the changes were not significant. There were no significant differences in overall carbon or nitrogen signatures between the upstream and downstream sites in either season. As a result, the faunal isotope data from sites 1 and 5 were consolidated by season in order to compare diets from September to May (spring) with diets from May to September (autumn) (Table 4.5). Both carbon (paired t-test, $df=29$, $p<0.01$) and nitrogen (paired t-test, $df=21$, $p=0.05$) animal signatures were significantly lighter in spring than in autumn.

Isotope Mixing Model

An isotopic mixing model was used to determine whether the more enriched invertebrate carbon signatures in autumn than in spring were due to the temporal fluctuations in algal signatures or to seasonal changes in invertebrate diets.

Nitrogen signatures of the bryophytes were more enriched than those of the herbivores so could not have formed a substantial proportion of any macroinvertebrate's diet. Other researchers have also suggested that mosses and macrophytes are infrequent sources of food for stream consumers (Allan 1995). The diatom and chlorophyte nitrogen signatures indicated that either could have been assimilated by aquatic invertebrates and therefore both were incorporated into the autochthonous endpoint of the mixing model.

The relative areal coverage of the stream bed by algae was estimated to be 77.9% diatoms and 22.1% chlorophytes (J. Winters, pers. comm.). Autochthonous endpoints were calculated by weighting the signatures of diatoms and chlorophytes accordingly (Table 4.6). Recruitment occurred in summer for most of the macroinvertebrates in this study and therefore the mean summer benthic algal (diatom and chlorophyte) signature was used as the autochthonous endpoint in the autumn (September invertebrate samples and *Sialis* sp., *Calopteryx* sp. and *Aeshna* sp. from the February sample) mixing model. *Neophylax concinnus* and *N. oligius* were in diapause over this period so the same spring endpoint was also used in the autumn. In the spring (May and June invertebrate samples) mixing model, the mean of the spring and autumn algal signatures was used because growth between the autumn and spring sample dates was concentrated in these two

periods for most taxa. In this model, it was assumed that diatom signatures were similar in spring and autumn. The four exceptions included *N. concinnus*, *N. oligius*, *D. nivoriunda* and *P. esselbaughi* which all demonstrated active growth through the winter. The mean algal signature over autumn, winter and spring was used as the autochthonous endpoint for these taxa. The mean of the spring, summer and autumn algal signatures was used for taxa with lifecycles of two or more years in both the spring and autumn mixing models. These included Elmidae (*Optioservus fastiditus*, *Dubiraphia quadrinotata* and *Stenelmis crenata*) and fish.

The allochthonous endpoint (-27.4‰) was the mean signature of the most abundant leaf litter, which included trees and grass. Maize was not included because it was removed from the adjacent fields at autumn harvest.

The macroinvertebrates were summarised according to functional feeding groups (Merritt and Cummins 1996) because diets were expected to be more similar within feeding rather than taxonomic groups (Table 4.5). In spring, allochthonous inputs contributed approximately 40% to the biomass assimilated by herbivores, with shredders most and scrapers least dependent (not weighted by secondary production). Terrestrial material was less important in autumn, 25%, primarily due to a change in diet by the shredders and to a lesser extent by the filterers. The groups which typically depend the most on autochthonous production exhibited the strongest seasonal trends. Carbon signatures of the scrapers, gatherers and filterers were 1.7‰ - 1.8‰ heavier in autumn than in spring, whereas the shredders were unchanged. The heavier autumn signatures

reflected the temporal trend observed in diatoms and chlorophytes rather than a shift in diet towards allochthonous sources over that period.

The extreme variation in the nitrogen signatures of the allochthonous and autochthonous inputs makes it difficult to evaluate the level of trophic enrichment of the herbivores over their food sources. An enrichment of approximately 3.4‰ was expected and, with the exception of scrapers in autumn, the difference between the signatures of each feeding group and their potential foods approached this value.

The unique lifecycles of some scrapers minimised the impact of summer benthic algae on their isotopic signatures in autumn. The three elmids, *O. fastiditus* larvae and adults and *S. crenata* adults, have slow growth rates over two or more years. The two species of *Neophylax* were in a state of diapause and therefore were not expected to reflect summer algal signatures. *N. concinnus* was heavier in autumn, probably due to the continual excretion of lighter nitrogen throughout diapause (Gannes et al. 1997). The heavier nitrogen signatures of *Stenonema vicarium* in the spring samples was likely due to some predation by the final instars of this taxa in late winter and early spring.

Shredders

The larvae of caddisflies (Trichoptera), crane flies (Diptera) and stoneflies (Plecoptera) are the major consumers of coarse particulate matter (CPOM) in forested streams. As expected, in spring, the shredders were the most dependent on allochthonous carbon of the herbivorous feeding groups (Fig. 4.3).

The dependence on allochthonous material was much lower in autumn than in spring. Five of the six common shredders were almost completely dependent on autochthonous material over the summer. Recruitment occurred in early summer for *Tipula* spp., *Allocapnia vivipara*, *Amphinemura delosa* and *Lepidostoma costalis* but little growth was evident until October for any of these taxa. The data suggest that early instars of these animals are herbivores and their dependence on allochthonous organic matter is important only after the autumn leaf fall.

The lower than expected overall dependence on allochthonous material, even in spring, suggests that bacteria, fungi and diatoms colonising allochthonous detritus were assimilated more readily than terrestrial leaf litter. Althoug fungal signatures are likely of allochthonous origin, bacteria may incorporate both terrestrial and aquatic organic material. Several species of "shredding" caddisflies and the cranefly, *Tipula abdominalis*, have limited capacity to digest the major structural polysaccharides of plant tissue but contain enzymes active against polysaccharides widely distributed in fungi and algae (Bjarnov 1972; Martin et al. 1980). *Pycnopsyche* larvae prefer leaf packs with well developed fungal and bacteria colonies (Mackay and Kalff 1972). Naiman (1983) estimated that algae on leaf litter contributed up to 35% of the primary production in a first order stream in Canada, suggesting that algal material could provide a large proportion of the nutrients assimilated by shredders. These studies imply that the contribution of autochthonous material to the biomass produced by shredders may be underestimated in gut content studies and can exceed that from allochthonous sources in streams where periphyton is abundant.

The caddisfly, *Pycnopsyche* sp., had the lowest allochthonous dependence in this group. Gut content studies by Cummins (1964) and Williams and Williams (1982) highlighted the flexible feeding habits of members of this genus, which included a shift from detrital to periphytal feeding in fifth instars of both *P. lepida* and *P. guttifer*. As a result, it may be misleading to include this taxon in the shredder feeding guild.

Scrapers

The scrapers include animals which utilise scraping, brushing or grazing feeding modes to ingest periphyton. As expected, scrapers had the most depleted carbon and nitrogen signatures, demonstrated the least dependence on allochthonous food sources (0% to 31%), and had carbon signatures which did not overlap those from terrestrial sources (Fig. 4.4). There was no evidence of a dietary shift between the spring and autumn samples as heavier carbon signatures in autumn reflected the more enriched autochthonous signatures over the summer.

The very depleted spring *N. concinnus* signature was likely due to a diatom diet, supplemented by filamentous and unicellular algae. Recruitment and growth to fifth instar occurred in autumn and winter when algal signatures were most depleted. The accumulation of lipids, which tend to be isotopically lighter than other biological components (Gannes et al. 1997), prior to a period of diapause through the summer may also have contributed to the depleted signatures. The autumn signatures were from individuals which had not fed since entering diapause in spring and the slightly heavier

signatures at this time were likely due to the utilisation of stored lipids for respiration over the summer.

N. oligius signatures were heavier in spring than those for *N. concinnus*. Beam and Wiggins (1986) noted that recruitment and development were temporally advanced in *N. oligius* relative to *N. concinnus* at another site in southern Ontario. Recruitment occurred in October rather than in November and all larvae had entered diapause by April rather than July. The advanced cycle probably resulted in the accumulation of a greater percentage of biomass earlier in the year when algal signatures were heavier.

Other studies have also emphasised the importance of diatoms to species of *Neophylax*. Mayer and Likens (1987) estimated that algae contributed up to 50% of the gut contents and supported up to 75% of the growth of *N. aniqua* in Bear Brook, New Hampshire. Wiggins (1996) stated that *Neophylax* feeds primarily on diatoms.

Optioservus fastiditus larvae had a semi-voltine lifecycle in this stream (chapter 2) and the adults are also thought to be long-lived. As a result, growth rates were slow, little organic matter was assimilated over the summer, and carbon signatures were similar in spring and autumn. The slightly heavier signatures of adults, relative to larvae, suggests the assimilation of some allochthonous material. Alternatively, MacLeod and Barton (1998) found periphyton in this stream to be more enriched when grown under high than low light levels in summer and the heavier signatures could be due to feeding in more exposed locations. White (1989) concluded that some adult Elmidae have a chemical defence against predation which is concentrated in their elytra. This may allow adult *O. fastiditus* to feed in more exposed habitats and avoid direct competition for resources with

their larvae. Detritus and diatoms dominate the gut contents of *Optioservus* and *Stenelmis* larvae (Tavares and Williams 1990).

Of the scrapers examined, *S. vicarium* had the greatest dependence on terrestrial material, 22% to 31%. Webb and Merritt (1987) observed higher growth rates in this species when grown on periphyton than on leaf detritus, whereas Richardson and Tarter (1976) analysed foreguts and observed that allochthonous detritus mixed with mineral particles was most abundant, followed by diatoms and then filamentous algae. These studies suggest that *S. vicarium* will preferentially ingest periphyton when it is abundant but will also take FPOM when necessary. The elevated nitrogen signatures in spring suggest that late instars may supplement their algal diets with other animals.

Gatherers

Collector-gatherers are considered deposit feeders of FPOM; however, the wide range of signatures in this group suggests that many of these taxa occupy specific niches within the lotic environment (Fig. 4.5). As is the case with CPOM, it is thought that the associated microbes rather than the FPOM substrate may be the primary source of carbon. In spite of the more enriched carbon signatures in autumn, dependence on terrestrial matter was similar in spring (39%) and autumn (40%). The heavier signatures in autumn were due to the enriched algal signatures in summer.

Three of the taxa in this group, *Parakiefferiella* sp., *Diamesa nivoriunda* and *Baetis brunneicolor*, were algivorous with little contribution from terrestrial sources in either season, suggesting that they would be more appropriately classified as scrapers.

Other studies have also emphasised the importance of algae to these taxa. Singh (1986) found that diatoms were most abundant in the guts of *D. nivoriunda* except in winter when detritus dominated. Fuller et al. (1986) and Wallace and Gurtz (1986) attributed higher densities, secondary production and larger adults in *Baetis* spp. to increased consumption of diatoms. Between 61% and 74% of the production by *Baetis* spp. in a southern Appalachian hardwood catchment was attributed to the assimilation of diatoms by Wallace and Gurtz (1986). In contrast, Shepard and Minshall (1984) did not detect a preference by *B. tricaudatus* for either detritus or diatoms in a laboratory study evaluating the relative densities of nymphs in areas of either detritus or diatoms.

Three taxa had increased dependence on terrestrial matter over the summer. The chironomids, *Parametriocnemus lundbeckii* and *Tvetenia paucunca*, increased from 54% to 72% and from 57% to 86%, respectively, between spring and autumn. Singh (1986) recorded 74% detritus and 20% diatoms in the guts of fourth instar larvae of *P. lundbeckii* in another headwater stream in southern Ontario. Singh did not estimate the terrestrial contribution to detritus in his study but if it is assumed to be completely allochthonous and that the assimilation of algae is three times greater than detritus (Benke and Jacobi, 1994), then allochthonous material contributed approximately 55% to the biomass of these taxa, similar to this study. The *Orthocladius* group (*Orthocladius* spp. and *Cricotopus* spp.) shifted from 10% to 64% allochthonous dependence from spring to autumn. Hershey et al. (1988) noted that diatoms were the primary food source for *Orthocladius rivulorum* in the Kuparuk River, Alaska, whereas Mackey (1978) found mostly detritus in the guts of both *Cricotopus bicinctus* and *C. sylvestris* in the River Thames, U. K.

The remaining taxa were either less dependent on terrestrial organic matter in autumn than spring or samples were only available from one of the two seasons. The chironomid, *Eukiefferiella claripennis*, had a greater dependence on allochthonous material in spring (50%) than in autumn (0%). Singh (1986) also observed a shift from a predominantly detrital dependence in autumn and winter towards diatoms in spring and summer. He attributed the change in feeding habits to increased primary production in spring and summer and less allochthonous material available in summer. Mattingly (1987) determined that the mouthparts of *Paraleptophlebia* spp. were adapted to both shredding and gathering and that these mayflies had short gut content passage times and low assimilation efficiencies, a combination of characteristics which permit consumption of large amounts of detrital material from a wide range of sources. She also suggested that this genus assimilates attached microbes in addition to the substrate. My results also suggest that a large proportion of allochthonous detrital material is assimilated.

Filterers

Carbon signatures of the filter feeders implied that terrestrial inputs accounted for 45% and 31% of the assimilated biomass in spring and autumn, respectively. FPOM in the water column had a terrestrial signal and although some filter-feeders were strongly dependent on allochthonous matter, it was surprising that, in general, autochthonous inputs were the dominant source of food for this group (Fig. 4.6).

The two philopotamid caddisflies, *Chimarra obscura* and *Dolophilodes distinctus*, were strongly dependent on allochthonous matter, 69% and 77% in spring, respectively,

likely from FPOM in the water column. Both species spin sac-like nets with fine mesh sizes in slow currents on the under side of rocks and other objects. The small contribution from algae was probably due to grazing of diatoms growing on and in the vicinity of the nets. Parker and Voshell (1981) determined that detritus contributed from 85% to 100% of gut biomass and between 65% and 89% to the annual biomass produced by *C. obscura* in the North and South Anna rivers in Virginia. Benke and Wallace (1980) recorded that detritus (87%) and diatoms (10%) were the most abundant sources of food in the guts of *D. distinctus* in a headwater stream of the Tallulah River in Georgia. Wallace and Malas (1976) also analysed the foreguts of *D. distinctus* and recorded predominantly FPOM and some diatoms.

The three hydropsychid caddisfly filter-feeders (*Hydropsyche slossonae*, *Cheumatopsyche oxa* and *Diplectrona modesta*) depended on terrestrial material for between 27% and 37% of their assimilated biomass in spring. Fuller and MacKay (1981) evaluated the growth responses of third, fourth and fifth instar *H. slossonae* to diets of leaf or fecal detritus, diatoms and animal material. Weight increases were 7.0 and 4.6 times higher on animal and diatom diets, respectively, than on either leaf or fecal material. They concluded that diatoms contributed the most, and detritus the least, to larval growth in each instar, and that autotrophy may be a significant factor affecting the diversity and abundance of filter-feeders in streams. Mecom (1972) found detritus and filamentous algae to be the most abundant food in late spring and summer while diatoms were most abundant in the guts of *Hydropsyche* spp. in mid-winter and early spring. Mecom attributed the shift in dependence to the temporal availability of organic material in the

larval microhabitats. Benke and Wallace (1980) observed a much greater contribution from animals (60%) for *D. modesta* than in our study. The nitrogen signatures of these three were not strongly enriched in comparison to the other herbivores, even though each of these animals are known to supplement their diet with other animals and all three were found with insect larvae in their mouths and/or guts throughout this study. The higher nitrogen signatures in autumn were consistent with observations by Fuller and MacKay (1980) that higher percentages of animal material were present in the guts of late instar *H. slossonae* over the summer, although the contribution was always less than 15%. In Logan Drain, the diets of these taxa consisted primarily of autochthonous production, supplemented by POM (terrestrial origin) and some predation.

The blackflies, *Simulium* spp. and *Prosimulium esselbaughi*, assimilated little terrestrial matter and likely grazed on periphyton to meet their nutritional needs. Chapman and Demory (1963) determined that the guts of *Simulium* spp. frequently contained minute crustacea, diatoms, green algae, and chironomid larvae and that in one study this taxon fed solely on diatoms. The two chironomid filter-feeders, *Tanytarsus* Group and *Rheotanytarsus* spp., depended on allochthonous matter for up to 73% of their assimilated biomass.

Invertebrate Predators

Carbon and nitrogen signatures of predators were more enriched than any of the other feeding groups. The mean nitrogen signatures of the shredders, scrapers, gatherers and filterers were 6.6‰ in spring and 7.0‰ in autumn, whereas the invertebrate predators

were more enriched by 2.0‰ and 2.8‰, respectively. This is slightly less than the expected enrichment of 3.4‰, suggesting some selectivity in the types of prey consumed (Fig. 4.7). Terrestrial inputs contributed 59% in spring and 20% in autumn to the invertebrate predators. In spring, the dependence on allochthonous material was greater than that of their prey. Many of the taxa in this group are not obligate predators and supplement their animal diets with both terrestrial and aquatic plants and algae. The reduced dependence on allochthonous matter in autumn was primarily due to the reduced allochthonous material in their prey over their summer, in addition to increased ingestion of autochthonous material.

The three Empididae, *Hemerodromia* sp., *Chelifera* sp. and *Clinocera* sp. were less than 1.3‰ heavier than the mean herbivore nitrogen signature. They are likely preying on the isotopically lightest taxa in addition to ingesting plants and algae.

Trissopelopia sp., *Chrysops* sp. and adult Dytiscidae shared a mixed diet of herbivores and allochthonous material. Carbon signatures suggest a strongly terrestrial diet whereas nitrogen signatures indicate that predation was also important. Pechuman et al. (1961) noted a strong dependence on non-animal material in *Chrysops* sp. and many adult dytiscids are actually scavengers (White and Brigham 1996). The classification of these taxa as predators contributed to the surprisingly high overall dependence on terrestrial material by this guild in spring.

Depleted carbon and enriched nitrogen signatures indicate that Ceratopogonidae were predacious between autumn and spring, yet mostly algivorous over the summer. Nitrogen signatures of *Cambarus robustus*, *Sialis* sp., *Calopteryx* sp., *Aeshna* sp.,

Ephydridae and the Acarina suggest that these taxa were primarily predacious, although the carbon signature of *C. robustus* indicates that it supplemented its diet with terrestrial material and was likely omnivorous.

The mean carbon signatures of the invertebrate predators were 2.2‰ and 2.1‰ more enriched than the herbivores in spring and autumn, respectively. This trophic enrichment was greater than is commonly accepted and is probably due to a combination of factors. First, it is likely that many predators feed on herbivores occupying the most exposed habitats in which periphyton growth rates and isotopic signatures are highest. As a result, carbon signatures of the predators are dependent on the most enriched herbivores. Second, some predators may have supplemented their diets with terrestrial plant or arthropod material (e.g. *Chrysops* sp. and adult Dytiscidae). Finally, the ^{13}C enrichment per trophic level by predators over their prey may actually be greater than the 0‰ to 1‰ range assumed here.

There is some evidence that higher trophic groups (i.e. insectivorous fish) may exhibit a greater trophic enrichment of carbon than is commonly accepted. While most authors assume a range of trophic enrichment in carbon between 0‰ and 1‰, this range may not be applicable in all circumstances. A relatively high carbon trophic enrichment of between 1.5‰ and 1.7‰ was observed in fish over their foods by DeNiro and Epstein (1978), Rounick and Hicks (1985), Fry (1988) and at one site by Doucett et al. (1996). It is possible that the trophic enrichment of carbon by predators over their prey is greater than that existing between herbivores over plants and algae.

Fish

Fish had the most enriched carbon and nitrogen signatures of the taxa studied (Fig. 4.8). Of the six species, *Semotilus atromaculatus* (creek chub) had the heaviest carbon signature, -25.8‰, which implied a 97% terrestrial diet that was likely due to predation on terrestrial arthropods. Other researchers have also suggested that terrestrial arthropods may contribute up to 100% of the diet of this species in wooded, low order streams (Lotrich 1973; Angermeier 1985). Inputs of terrestrial arthropods to headwater streams can range from 1,050 to 38,430 $mg \cdot m^{-2} \cdot yr^{-1}$, equal the biomass produced by lotic invertebrates, and may dominate the diet of some fish (Mason and MacDonald 1982; Bowman 1988; Cloe and Garman 1996). Garman (1991) observed that the majority of terrestrial insects in the drift of a third order stream in Virginia occurred in mid-stream within the upper 2cm of the water column, rather than adjacent to the substrate or at the stream margin. He stated that morphological and behavioural constraints possessed by individual fish taxa regulate the utilisation of this resource. As a result, active, non-benthic fish such as *S. atromaculatus* and *Notropis cornutus* (common shiner), may be best suited to exploit terrestrial arthropod inputs. Mendelson (1975) also reported more terrestrial prey in the guts of mid-water species than bottom dwelling fish. Terrestrial inputs contributed 61% to the diet of *N. cornutus*, much of this likely in the form of terrestrial arthropods.

Benthic invertebrates were the primary source of carbon in the diet of *Pimephales promelas* (fathead minnow), *Rhinichthys atratulus* (blacknose dace) and *Etheostoma nigrum* (johnny darter). Their dependence on allochthonous material, 39% to 43%, was

similar to the herbivores in spring and autumn. In addition, nitrogen signatures were approximately one trophic level greater, from 3.4‰ to 4.2‰ heavier, than the mean invertebrate signatures in spring and autumn.

Culea inconstans (brook stickleback) had the most enriched nitrogen signature, 13.0‰, which suggested a complete dependence on invertebrate predators, and eggs and young of the year of fish. Scott and Crossman (1973) described the brook stickleback as mainly carnivorous, with a diet which included eggs and larvae of fish, while Vander Zanden et al. (1997) placed it at a higher trophic level than suckers, trout, burbot, white bass and cyprinids.

Quantification of Allochthonous and Autochthonous Carbon Inputs

Annual secondary production and isotope data were combined to quantify the contributions by terrestrial and lotic sources of organic matter to the most abundant taxa and to the stream ecosystem at sites 1 and 5. In order to compare the relative contributions upstream and downstream of the agricultural land, separate mixing models were run at each site. Mean signatures from both sites were used only when insufficient data were available from a particular site. The lifecycle of each taxon dictated whether the spring or autumn mixing model was used. Growth through the final instars by all of the univoltine and some of the multivoltine taxa occurred primarily between September and May, and for these animals, the spring mixing model was used. For the remaining multivoltine taxa, in which there were both winter and summer generations, the mean from the spring and autumn mixing models was used. For taxa in which isotopic signatures

were not available from the desired period the percent contribution from the alternate period was used. For taxa in which isotopic data were not available, the mean percent allochthonous dependence of the functional feeding group to which it belongs was used. For example, the chironomid, *Corynoneura* sp. is a gatherer for which isotopic data were not obtained. The mean percent allochthonous dependence of the other gatherers was used for this taxon.

The river continuum concept (Vannote et al. 1980) provides a template of aquatic ecosystems along the course of a river and emphasises the importance of terrestrial plant debris to undisturbed headwater streams. My data clearly show that while terrestrial matter may be important to certain groups (i.e. shredders after the autumn leaf fall and young *S. atromaculatus* which are sight feeders on planktonic organisms), autochthonous sources can be of greater importance under certain conditions. Logan Drain has moderate nutrient enrichment and a partial canopy, and benthic algae were the source of over 64% of the biomass assimilated by the herbivorous macroinvertebrates (Table 4.7).

The invertebrate taxa in each of the functional feeding groups from both sites 1 and 5 obtained the majority of their organic material from autochthonous sources. This suggests that autochthonous production was abundant at both sites and that the animals were able to utilise the more nutritious benthic algae rather than terrestrial organic matter. It is curious that shredder production remained high (>39% of herbivore production), in an environment where algae provided the majority of the organic matter so that scrapers and gatherers would be expected to be most successful. It seems probable that the shredders actually assimilate periphyton growing on leaf litter, whereas the scrapers and

gatherers grazed predominantly on epilithic algae. The high production by filter-feeders and their strong dependence on autochthonous carbon sources was also surprising. The terrestrial origin of suspended POM likely requires many of the filter-feeding taxa to graze on diatoms and chlorophytes rather than to rely solely on their filtering capabilities. The results strongly suggest that in spite of morphological adaptations for particular types of feeding, food availability is most important in determining the diet of individual taxa.

Secondary production declined the most within the shredder and filter-feeding groups at the downstream site. As suggested in chapter 2, the lower production by shredders was likely a result of reduced terrestrial inputs to the stream from the cultivated land between sites and a more homogenous stream bed of finer particle sizes which retained less coarse particulate matter. The similarity of carbon signatures between sites suggests that the shredders did not increase the proportion of autochthonous foods in their diets to offset the reduced supply of leaf litter downstream.

Production by filter-feeders was 50% lower at the downstream site due to the reduced success of *H. slossonae*. The dependence of this guild on allochthonous inputs was similar at both sites, with the exception of *H. slossonae* which increased from 18% upstream to 37% downstream. In chapter 2, I concluded that a change in the stream bed towards finer sediments at the downstream site eliminated the hard surfaces necessary for the five large Trichoptera filter-feeders to successfully attach their nets. Increased competition for habitat likely led to crowding, limited grazing on the epilithon and resulted in a greater reliance on filter-feeding of terrestrial POM.

Rosenfeld and Roff (1991) found primary production on fine sediments to be 20% of that on rock surfaces. Lower periphyton production contributed to reduced secondary production in each of the herbivorous groups downstream.

Two fish species were present at the upstream site, creek chub and blacknose dace, of which only one is a benthic insectivore. At the downstream site, four additional species, common shiner, brook stickleback, fathead minnow and johnny darter, all of which are benthic insectivores, increased top-down pressure on the invertebrate community. The downstream fish population contributed to lower abundances, biomass, secondary production and mean individual biomass at that site.

Minimum inputs of autochthonous and allochthonous materials necessary to support secondary production in the stream were estimated based on ecological efficiencies described by Benke and Jacobi (1994). These included net production efficiency (annual production/assimilation) of 40%, assimilation efficiencies (assimilation/ingestion) of 30%, 10% and 70% and therefore gross efficiencies (annual production/ingestion) of 12%, 4% and 28% for diatoms, vascular plant and amorphous detritus, and animals, respectively. Secondary production was greatest at site 1 so minimum terrestrial and stream inputs were estimated based on this site. Autochthonous primary production necessary to support 34,750 $mg \cdot m^{-2} \cdot yr^{-1}$ secondary production by the common herbivores must have approached 289,583 $mg \cdot m^{-2} \cdot yr^{-1}$. Rosenfeld and Roff (1991) estimated daily net production to average 72,000 $mg \cdot m^{-2} \cdot yr^{-1}$ and 940,000 $mg \cdot m^{-2} \cdot yr^{-1}$ (using 2 mg dry mass ~ 1 mg carbon) at forest and meadow sites in southern Ontario

headwater streams, respectively; a range which encompassed that necessary to support the biomass produced by the macroinvertebrates in our study.

Allochthonous inputs necessary to support 14,895 $mg \cdot m^2 \cdot yr^{-1}$ secondary production by the herbivores must have approached 372,375 $mg \cdot m^2 \cdot yr^{-1}$. Estimates of allochthonous litter inputs commonly exceed this in headwater streams (Allan 1995). Inputs to the downstream site may have been less due to the reduced riparian cover and may have contributed to lower shredder production at site 5. Production by *S. atromaculatus*, the most abundant fish at these sites (pers. obs.), has been estimated at 2,200 $mg \cdot m^2 \cdot yr^{-1}$ in a first order stream in eastern Kentucky (Lotrich 1973) and would require an input of 7,857 $mg \cdot m^2 \cdot yr^{-1}$ of terrestrial arthropods. As discussed earlier, this is also well within the range expected in headwater streams.

Production by invertebrate predators was greatest at the downstream site, 1620 $mg \cdot m^2 \cdot yr^{-1}$, and would require ~5,786 $mg \cdot m^2 \cdot yr^{-1}$ production by herbivorous invertebrates. Herbivore production exceeded 33,000 $mg \cdot m^2 \cdot yr^{-1}$ and easily supported the predators. The estimates of secondary production by the invertebrate predators is likely low as this study did not estimate crayfish or fish production.

The number of trophic levels at each site were estimated using the mean nitrogen signature of the herbivores from spring and autumn, 6.8‰, as the baseline for the second trophic level and an enrichment of 3.4‰ per trophic level. At site 1, *R. atratulus* had the heaviest nitrogen signature, 10.9%, and a trophic position of 3.2. At site 5, *C. inconstans* was the most enriched taxa, 13.0‰, and had a trophic position of 3.8. These results

approximate the trophic position of 3.7, for *C. inconstans*, estimated by Vander Zanden et al. (1997) from the literature.

In conclusion, through the use of stable isotope methods, this study has provided a comprehensive analysis of carbon flow from terrestrial and lotic sources through individual taxa and a stream ecosystem. The success of the stable isotope method is primarily dependant on whether potential dietary components have isotopically distinct signatures. The fact that carbon signatures of many lotic primary producers are dependant on growth rate demands a sampling procedure which will detect both seasonal and microhabitat variations within this food source. In addition, it is critical that animal lifecycles, including periods of maximal growth and diapause, are known.

Secondly, it is necessary to estimate the trophic enrichment of animals over their foods. The 0‰ to 1‰ range used here approximates the trophic enrichment found in most studies. However, there is evidence that trophic shifts are both consumer and food specific (Focken and Becker 1998) and further work is needed to clarify metabolic fractionation of stable carbon isotopes in consumers (Gannes et al. 1998).

Summary

Chlorophyte and diatom carbon signatures followed a seasonal pattern in which reduced metabolic fractionation under high growth conditions in summer led to enriched signatures. Winter signatures were most depleted while spring and autumn were intermediate. Temporal carbon signatures of the macroinvertebrates reflected the seasonal variation in algae. The application of commercial and organic fertilisers to the adjacent

land did not result in heavier nitrogen signatures in either the autochthonous primary producers or macroinvertebrates at the downstream site.

Functional feeding group designations were useful predictors of diet at the group level. The relative dependence by the different groups on allochthonous resources followed the expected trend (i.e. shredders were most, and scrapers least, dependent on terrestrial inputs). However, many taxa exploited a wider food base than their feeding group classification implied (e.g. shredders and filterers). The dependence of shredders on terrestrial inputs increased from 11% over summer to 54% after the autumn leaf fall. Four taxa had diets which suggest that they would be more suited to alternate trophic guilds. *Parakiefferiella* sp., *D. nivoriunda* and *B. brunneicolor* are routinely classified as gatherers, whereas in this study they were clearly grazing on benthic algae. *Pycnopsyche* sp. is classified as a shredder but as this and other studies have shown, the fifth instars are more likely to be gatherers. Few of the predators were completely dependent on animal diets. *Chrysops* sp., *T. ogemawi*, Empididae and adult Dytiscidae had either lower nitrogen signatures or a greater dependence on allochthonous inputs than expected from obligate predators. Food availability rather than morphological adaptions appeared to be most important in determining the diet of individual taxa.

The similarity of the invertebrate diets at sites 1 and 5 suggest that inputs of autochthonous and allochthonous organic material did not limit secondary production at the downstream site. The analyses support the conclusions from chapter 2 that changes to the benthic community composition and lower secondary production downstream were due primarily to a shift in stream bed morphology towards a finer and more homogenous

substrate at site 5. This effect was a direct result of agricultural activity on the adjacent fields.

The number of trophic levels increased from 3.2 upstream to 3.8 downstream due to an increased diversity of fish species, which included the brook stickleback, at site 5. Using previously developed ecological efficiencies, autochthonous and allochthonous inputs necessary to support the macroinvertebrate community were within expected ranges for a moderately enriched headwater stream with a partial canopy.

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Table 4.1. Mean values of chemical and physical characteristics of water at the five sample sites in Logan Drain, Kintore. Values in brackets are the number of measurements included in the mean. Mean temperature data is from 23 February 1996 until 10 October 1996 (the dates when all sites were measured). na - data is not available at a particular site.

Site	Dissolved Organic Carbon (mg/l)	Total Soluble Nitrogen (mg/l)	NO_3^--N (mg/l)		NH_4^+-N (mg/l)		Temp. (°C)		Discharge (m^3/day)	
			1995	1996	1995	1996	1995	1996	1995	1996
1	3.30 (21)	2.22 (10)	1.44 (48)	1.85 (25)	0.01 (50)	0.11 (25)	12.8 (22)	12.2 (12)	873	1910
2	1.74 (19)	5.12 (10)	2.86 (48)	2.88 (23)	0.00 (49)	0.09 (23)	12.9 (22)	12.6 (12)	na	na
3	na	na	na	na	na	na	na	13.3 (12)	na	na
4	2.24 (20)	3.51 (10)	3.11 (47)	3.15 (21)	0.00 (50)	0.11 (21)	na	13.3 (12)	na	na
5	2.15 (21)	3.51 (10)	3.22 (49)	3.23 (25)	0.00 (48)	0.09 (25)	12.8 (22)	12.8 (12)	1604	3514

Table 4.2. Sample dates for the carbon and nitrogen isotope measurements in Logan Drain, Kintore

Date	Stream DIC	Stream DIN	Groundwater DIN	Stream POC/PON	Allochthonous	Chlorophytes and Bryophytes	Macroinvertebrates	Fish
8 Jun 95	y			y	y	y	y	y
12 Oct 95		y						
2 Nov 95	y			y		y		
7 Dec 95	y			y		y		
29 Mar 96	y			y		y		
23 May 96	y		y	y		y	y	
18 Jul 96	y			y		y		
27 Sep 96	y			y		y	y	
Nov 96		y	y					
6 Dec 96	y			y		y		
9 May 97		y				y	y	
11 Feb 98						y	y	

Table 4.3. Seasonal autochthonous primary producer and POM carbon and nitrogen signatures. Winter (January to March), Spring (April to June), Summer (July to September), Autumn (October to December). ns - not sufficient data.

	Winter				Spring				Summer				Autumn			
	M	min	max	sd	M	min	max	sd	M	min	max	sd	M	min	max	sd
$\delta^{13}C$																
Autochthonous																
diatoms	-33.8	-35.0	-32.8	1.1					-29.6	-33.7	-26.9	2.0	-33.3	-36.3	-30.8	1.3
chlorophytes	-44.2	-44.2	-44.2	ns	-38.2	-40.7	-36.1	1.5	-35.6	-39.2	-34.1	2.1	-44.7	-45.2	-44.3	0.6
aquatic bryophytes	-41.7	-42.5	-41.0	0.7	-38.5	-41.7	-34.7	3.4	-40.8	-42.1	-38.4	1.6	-39.9	-39.9	-39.9	ns
POC	-25.6	-25.9	-25.0	0.4	-27.0	-31.3	-24.9	2.4	-26.5	-27.3	-24.9	0.7	-26.3	-27.0	-25.4	0.5
$\delta^{15}N$																
Autochthonous																
diatoms	3.7	2.6	4.6	0.9					6.3	5.3	7.3	0.5	2.7	1.5	3.9	0.6
chlorophytes	2.7	2.7	2.7	ns	4.2	2.7	5.9	1.0	5.7	4.8	6.4	0.6	6.3	5.8	6.9	0.8
aquatic bryophytes	7.1	6.5	7.6	0.6	7.0	5.2	8.8	1.5	8.7	8.0	9.2	0.5	7.9	7.9	7.9	ns
PON	6.2	5.8	6.6	0.6	6.1	0.0	18.3	5.1	6.3	-0.7	15.1	4.2	3.6	-3.4	8.0	3.3

Table 4.4. Carbon and nitrogen signatures of the most abundant terrestrial plant foliage.

Terrestrial Plants	$\delta^{13}C$	$\delta^{15}N$
Cornus spp. (Dogwood)	-27.8	-0.3
Crataegus sp. (Hawthorn)	-25.5	0.9
Ulmus americana (White Elm)	-28.9	0.5
Salix nigra (Black Willow)	-26.6	5.7
Grass	-29.5	9.2
Populus tremuloides (Trembling Aspen)	-28.9	0.5
Tilia americana (Basswood)	-24.5	3.2
Mean (most abundant plants)	**-27.4**	**2.8**
Spiraea latifolia (Meadowsweet)	-27.9	4.3
Impatiens capensis (Jewelweed)	-28.8	2.9
Caltha palustris (Marsh-marigold)	-27.0	8.4
Sambucus canadensis (Elderberry)	-24.3	2.4
Rubus odoratus (Raspberry)	-28.1	2.3
Equisetum sp. (Horsetail)	-28.1	7.6
Mean (all plants except maize)	**-27.4**	**3.7**
Maize	-12.7	7.1

Table 4.5. Mean carbon and nitrogen signatures and % (range) allochthonous contribution in spring and autumn. Ranges calculated from 0‰ to 1‰ potential range in trophic enrichment of carbon. na - insufficient material for analysis. * - Fish were sampled in summer. l - larvae. a - adult.

Taxa	Spring			Autumn		
	Carbon	Nitrogen	% Allochthonous	Carbon	Nitrogen	% Allochthonous
Shredders	-30.7	5.9	54 (±6)	-30.8	6.3	11 (±2)
Polypedilum spp	-30.5	5.1	57 (±6)	-30.8	6.0	2 (±2)
Tipula spp	-31.5	5.7	45 (±6)	-32.9	6.0	0 (±0)
Pseudolimnophila sp	-30.9	na	52 (±6)	na	na	na
Allocapnia vivipara	-29.6	6.6	68 (±6)	-31.3	6.0	0 (±0)
Amphinemura delosa	-31.7	4.7	42 (±6)	na	na	na
Lepidostoma costalis	-30.1	6.3	62 (±6)	-30.0	na	13 (±14)
Pycnopsyche sp	-33.2	6.1	25 (±6)	na	na	na
Limnephilus sp	-30.6	5.4	56 (±6)	-28.8	4.5	45 (±15)
Ptilostomis sp	-28.5	7.3	81 (±6)	-30.7	9.0	3 (±3)
Scrapers	-35.3	5.9	5 (±2)	-33.5	5.8	9 (±5)
Optioservus fastiditus (l)	-34.9	5.2	0 (±0)	-34.8	6.0	0 (±0)
Optioservus fastiditus (a)	-33.5	5.6	2 (±2)	-32.8	4.9	7 (±7)
Stenelmis crenata (a)	na	na	na	-33.1	5.6	5 (±5)
Stenonema vicarium	-33.5	7.1	22 (±6)	-29.3	5.4	31 (±14)
Neophylax concinnus	-38.5	5.2	0 (±0)	-37.7	7.0	0 (±0)
Neophylax oligius	-36.0	6.6	0 (±0)	na	na	na
Gatherers	-32.5	7.1	39 (±4)	-30.7	7.5	40 (±8)
Parametriocnemus lundbecki	-30.7	7.3	54 (±6)	-27.9	7.9	72 (±14)
Orthocladius Group	-34.4	7.0	10 (±6)	-28.2	7.8	64 (±14)
Eukiefferiella claripennis	-31.1	6.8	50 (±6)	-34.0	na	0 (±0)
Tvetenia paucunca	-30.5	7.5	57 (±6)	-27.4	7.2	86 (±14)
Parakiefferiella sp	-38.2	7.4	0 (±0)	na	na	na
Diamesa nivorlunda	-37.2	6.2	0 (±0)	-34.8	8.6	0 (±0)
Dubiraphia quadrinotata (l)	na	na	na	-29.2	5.6	64 (±6)
Dubiraphia quadrinotata (a)	na	na	na	-30.9	7.3	36 (±8)
Baetis brunneicolor	-36.4	6.5	0 (±0)	-34.7	7.9	0 (±0)
Acerpenna macdunnoughi	-31.6	7.2	44 (±6)	-29.0	na	41 (±14)
Paraleptophlebia debilis	-30.0	7.4	63 (±6)	na	na	na
Isopoda	-26.4	na	100 (±0)	na	na	na
Oligochaeta	-30.7	7.2	54 (±6)	na	na	na
Filterers	-31.5	7.0	45 (±6)	-29.8	8.1	31 (±8)
Tanytarsus Group	-33.8	8.6	18 (±6)	-27.8	na	73 (±14)
Rheotanytarsus sp	-29.2	6.5	72 (±6)	na	na	na
Simulium spp	na	na	na	-29.6	8.5	22 (±14)
Prosimulium esselbaughi	-32.4	6.4	32 (±6)	na	na	na
Hydropsyche slossonae	-33.0	7.4	27 (±6)	-31.7	8.6	0 (±0)
Cheumatopsyche oxa	-32.8	6.2	30 (±6)	-31.5	7.1	0 (±0)
Diplectrona modesta	-32.2	6.5	37 (±6)	-30.6	8.0	4 (±4)
Chimarra obscura	-29.5	7.2	69 (±6)	-27.3	8.1	87 (±14)
Dolophilodes distinctus	-28.8	7.5	77 (±6)	na	na	na
All Herbivores	-32.2	6.6	40 (±5)	-31.0	7.0	25 (±7)
Predators	-30.0	8.6	59 (±10)	-28.9	9.8	20 (±6)
Trissopelopia ogemawi	-29.0	8.4	72 (±9)	-25.7	na	na
Chrysops sp	-27.7	8.9	86 (±10)	-32.4	8.5	0 (±0)
Hemerodromia sp	-31.6	7.6	43 (±8)	-28.4	8.0	40 (±5)
Chelifera sp	-30.8	7.8	51 (±8)	-26.9	na	na
Clinocera sp	na	na	na	-28.4	7.3	42 (±2)
Ceratopogonidae	-31.3	9.3	43 (±11)	-29.0	7.8	22 (±4)
Ephydridae	na	na	na	-31.7	9.4	0 (±0)
Dytiscidae (l)	-29.6	na	na	na	na	na
Dytiscidae (a)	-28.4	8.0	80 (±9)	na	na	na
Acarina	-32.4	8.9	30 (±10)	-29.3	9.5	9 (±9)
Sialis sp	na	na	na	-28.3	10.4	33 (±15)
Calopteryx sp	na	na	na	-29.1	9.4	13 (±10)
Aeshna sp	na	na	na	-28.7	9.2	25 (±10)
Cambarus robustus	-29.5	9.8	64 (±12)	na	na	na
All Invertebrates	-31.7	7.0	44 (±5)	-30.4	7.5	24 (±7)
Fish	-29.0	11.2	54 (±16)	na	na	na
Semotilus atromaculatus *	-25.8	10.2	97 (±3)	na	na	na
Rhinichthys atratulus *	-30.0	10.9	41 (±18)	na	na	na
Notropis cornutus *	-28.7	11.1	61 (±18)	na	na	na
Culaea inconstans *	-29.7	13.0	42 (±22)	na	na	na
Pimephales promelas *	-30.1	11.0	39 (±18)	na	na	na
Etheostoma nigrum *	-29.9	11.2	43 (±18)	na	na	na

Table 4.6. Autochthonous endpoints used in the spring (May and June invertebrate samples) and autumn (September invertebrate samples) mixing models. Allochthonous endpoint was -27.4‰.

Life-History Type	Spring	Autumn
a. growth predominantly through autumn and spring	-35.8‰	-30.9‰
b. growth predominantly through autumn, winter and spring (*Diamesa*)	-35.5‰	-30.9‰
c. growth strictly through autumn, winter and spring (*Neophylax*)	-35.5‰	-35.5‰
d. growth predominantly through spring, summer and autumn (lifespan greater than one year - Elmidae larvae and adults, Fish)	-33.7‰	-33.7‰

Table 4.7. Contributions by allochthonous and autochthonous sources to secondary production by the common invertebrates in Logan Drain, Kintore. Ranges calculated from 0 to 1 potential range in trophic enrichment of carbon. a - Taxa which grew predominantly through autumn, winter and spring and for which the spring percentages (from Table 4.5) were used to determine carbon sources. b - Taxa which were multivoltine, grew through all seasons and for which the mean of the spring and autumn percentages (from Table 5) were used to determine carbon sources.

Spring	Production (mg dry weight • m^{-2} • yr^{-1})					
Taxa	Site 1			Site 5		
	Allochthonous	Autochthonous	Total	Allochthonous	Autochthonous	Total
Shredders	**8592 (±518)**	**10802 (±518)**	**19394**	**6841 (±482)**	**7769 (±482)**	**14610**
Polypedilum spp (b)	353 (±21)	886 (±21)	1239	800 (±120)	567 (±120)	1367
Tipula spp (a)	7977 (±479)	9749 (±479)	17726	5830 (±350)	7125 (±350)	12955
Allocapnia vivipara (a)	258 (±15)	121 (±15)	379	150 (±9)	71 (±9)	221
Amphinemura delosa (a)	3 (±1)	6 (±1)	9	20 (±1)	6 (±1)	26
Lepidostoma costalis (a)	1 (±2)	40 (±2)	41	41 (±2)	0 (±2)	41
Scrapers	**168 (±10)**	**3725 (±10)**	**3893**	**111 (±7)**	**3588 (±7)**	**3699**
Optioservus fastiditus (a)	0 (±0)	1072 (±0)	1072	0 (±0)	1206 (±0)	1206
Stenonema vicarium (a)	168 (±10)	562 (±10)	730	111 (±7)	445 (±7)	556
Neophylax concinnus (a)	0 (±0)	2091 (±0)	2091	0 (±0)	1937 (±0)	1937
Gatherers	**1446 (±129)**	**4480 (±129)**	**5926**	**1660 (±219)**	**3485 (±219)**	**5145**
Parametriocnemus lundbecki (b)	570 (±57)	216 (±57)	786	427 (±64)	403 (±64)	830
Orthocladius Group (b)	74 (±10)	126 (±10)	200	103 (±14)	186 (±14)	289
Eukiefferiella claripennis (b)	8 (±1)	23 (±1)	31	1 (±0)	42 (±0)	43
Tvetenia paucunca (b)	530 (±32)	145 (±32)	675	525 (±74)	204 (±74)	729
Parakiefferiella sp (b)	0 (±0)	22 (±0)	22	0 (±0)	32 (±0)	32
Diamesa nivoriunda (a)	0 (±0)	247 (±0)	247	0 (±0)	294 (±0)	294
Thienemanniella sp. (b)	9 (±1)	12 (±1)	21	6 (±1)	12 (±1)	18
Corynoneura sp. (b)	8 (±1)	10 (±1)	18	4 (±0)	9 (±0)	13
Paracricotopus sp. (b)	3 (±0)	4 (±0)	7	1 (±0)	2 (±0)	3
Baetis brunneicolor (b)	52 (±2)	3433 (±2)	3485	179 (±11)	1921 (±11)	2100
Acerpenna macdunnoughi (b)	169 (±24)	229 (±24)	398	369 (±52)	354 (±52)	723
Paraleptophlebia debilis (a)	23 (±1)	13 (±1)	36	45 (±3)	26 (±3)	71
Filterers	**4689 (±306)**	**15743 (±306)**	**20432**	**3552 (±266)**	**6733 (±266)**	**10285**
Tanytarsus Group (b)	94 (±13)	113 (±13)	207	116 (±16)	140 (±16)	256
Rheotanytarsus sp (b)	54 (±3)	21 (±3)	75	132 (±8)	51 (±8)	183
Simulium spp (b)	212 (±30)	753 (±30)	965	544 (±76)	1930 (±76)	2474
Prosimulium esselbaughi (a)	62 (±4)	133 (±4)	195	151 (±9)	322 (±9)	473
Hydropsyche slossonae (a)	2704 (±162)	12320 (±162)	15024	1719 (±103)	2928 (±103)	4647
Cheumatopsyche oxa (a)	758 (±45)	1769(±45)	2527	468 (±28)	1145 (±28)	1613
Diplectrona modesta (a)	249 (±15)	424 (±15)	673	50 (±3)	84 (±3)	134
Chimarra obscura (a)	292 (±18)	131 (±18)	423	146 (±9)	65 (±9)	211
Dolophilodes distinctus (b)	264 (±16)	79 (±16)	343	226 (±14)	68 (±14)	294
All Herbivores	**14895 (±963)**	**34750 (±963)**	**49645**	**12164 (±974)**	**21575 (±974)**	**33739**
Predators	**761 (±56)**	**306 (±56)**	**1067**	**1170 (±76)**	**450 (±76)**	**1620**
Trissopelopia ogemawi (b)	288 (±26)	112 (±26)	400	170 (±15)	66 (±15)	236
Chrysops sp (a)	342 (±21)	56 (±21)	398	703 (±42)	114 (±42)	817
Ceratopogonidae (b)	26 (±3)	55 (±3)	81	31 (±3)	52 (±3)	83
Dicranota sp. (a)	105 (±6)	83 (±6)	188	266 (±16)	218 (±16)	484
Total	**15656 (±1019)**	**35056 (±1019)**	**50712**	**13334 (±1050)**	**22025 (±1050)**	**35359**

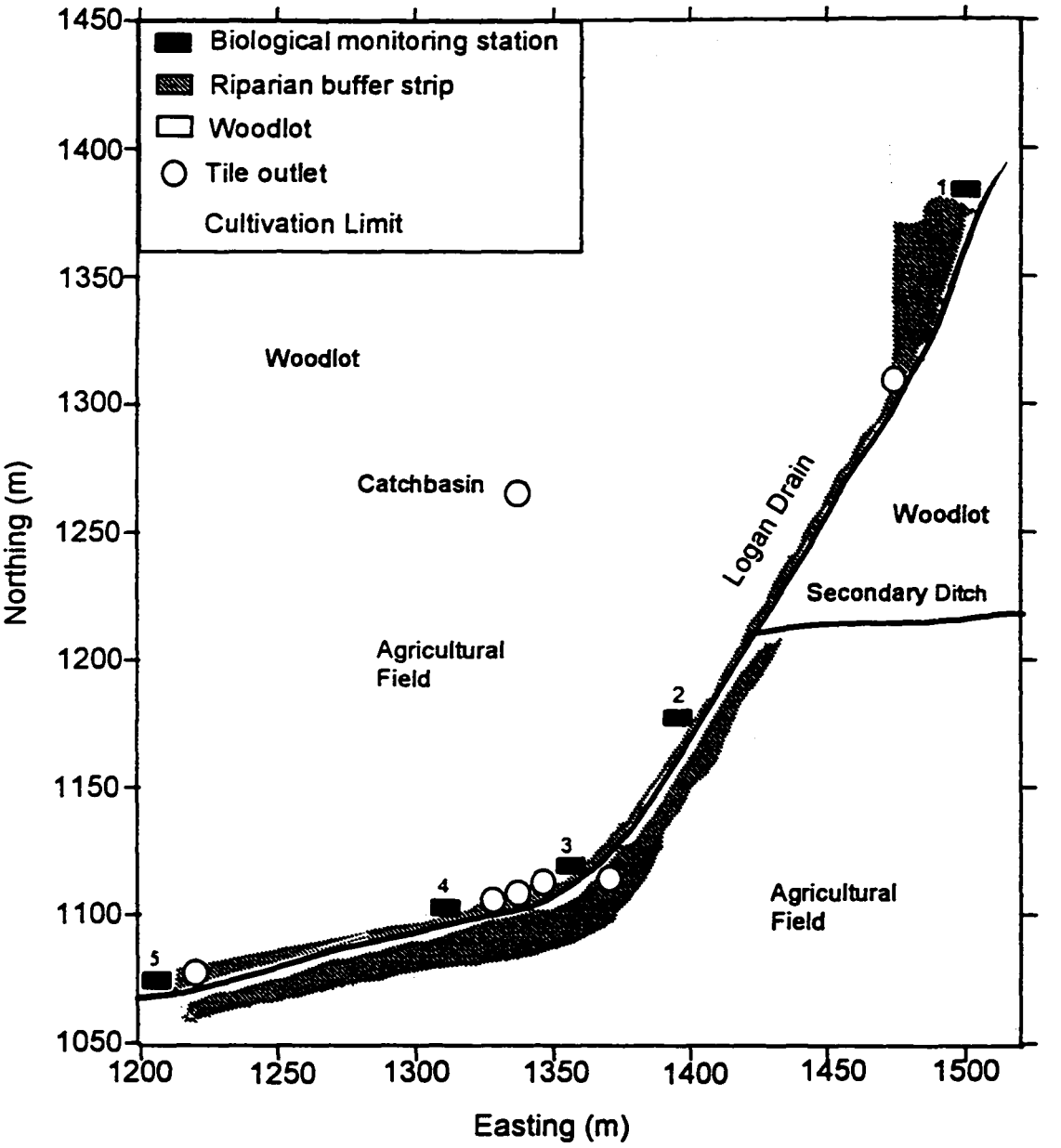

Figure 4.1. Biological monitoring stations in Logan Drain, Kintore, Ontario. Flow is from upper right to lower left.

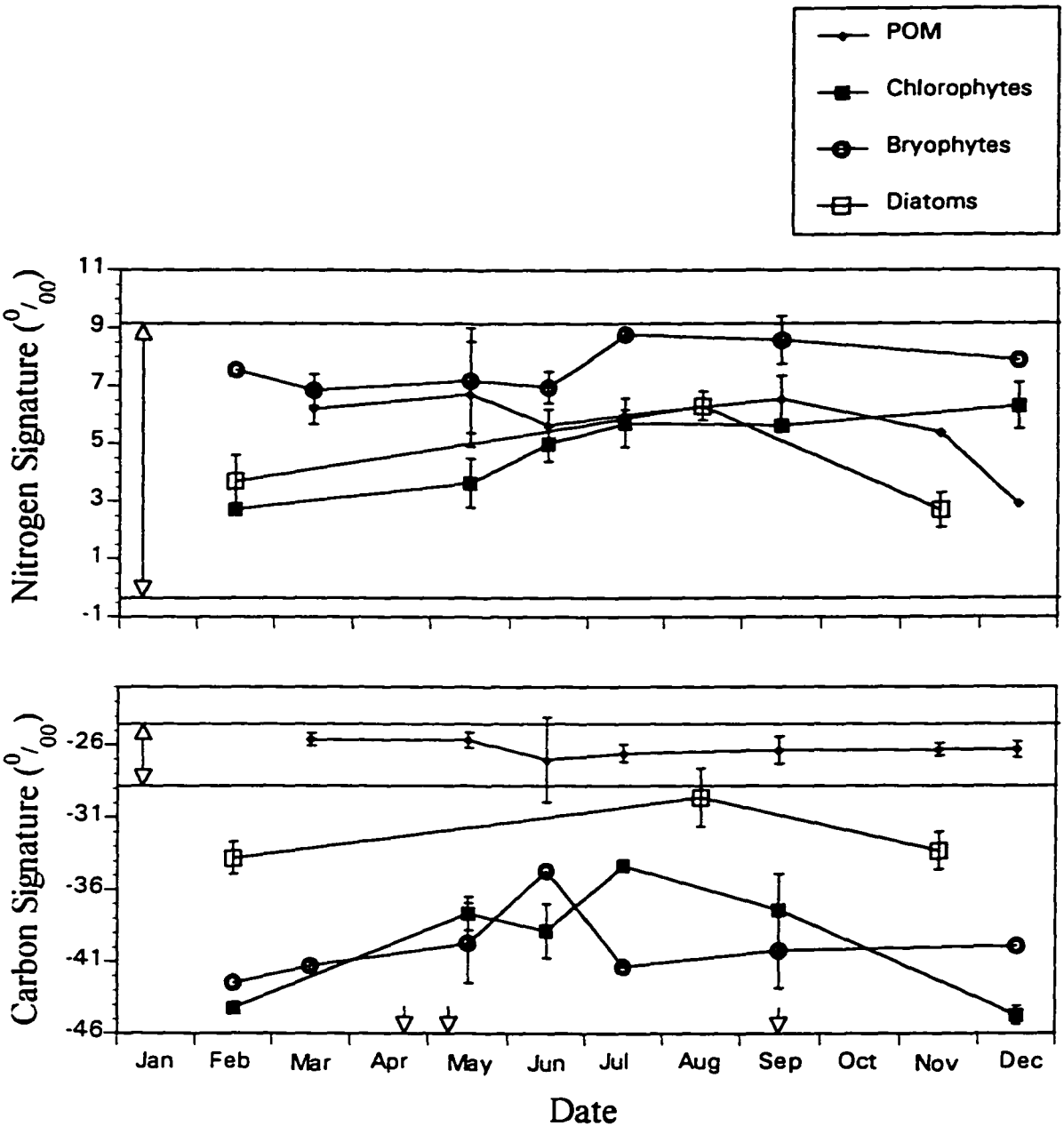

Figure 4.2. Carbon and nitrogen signatures (mean ± std. dev.) of POM, chlorophytes, bryophytes and diatoms (‰). Double ended arrows indicate range of terrestrial foliage signatures. Single ended arrows indicate dates of manure applications (17 April 1995, 18 September 1995, 8 May 1996).

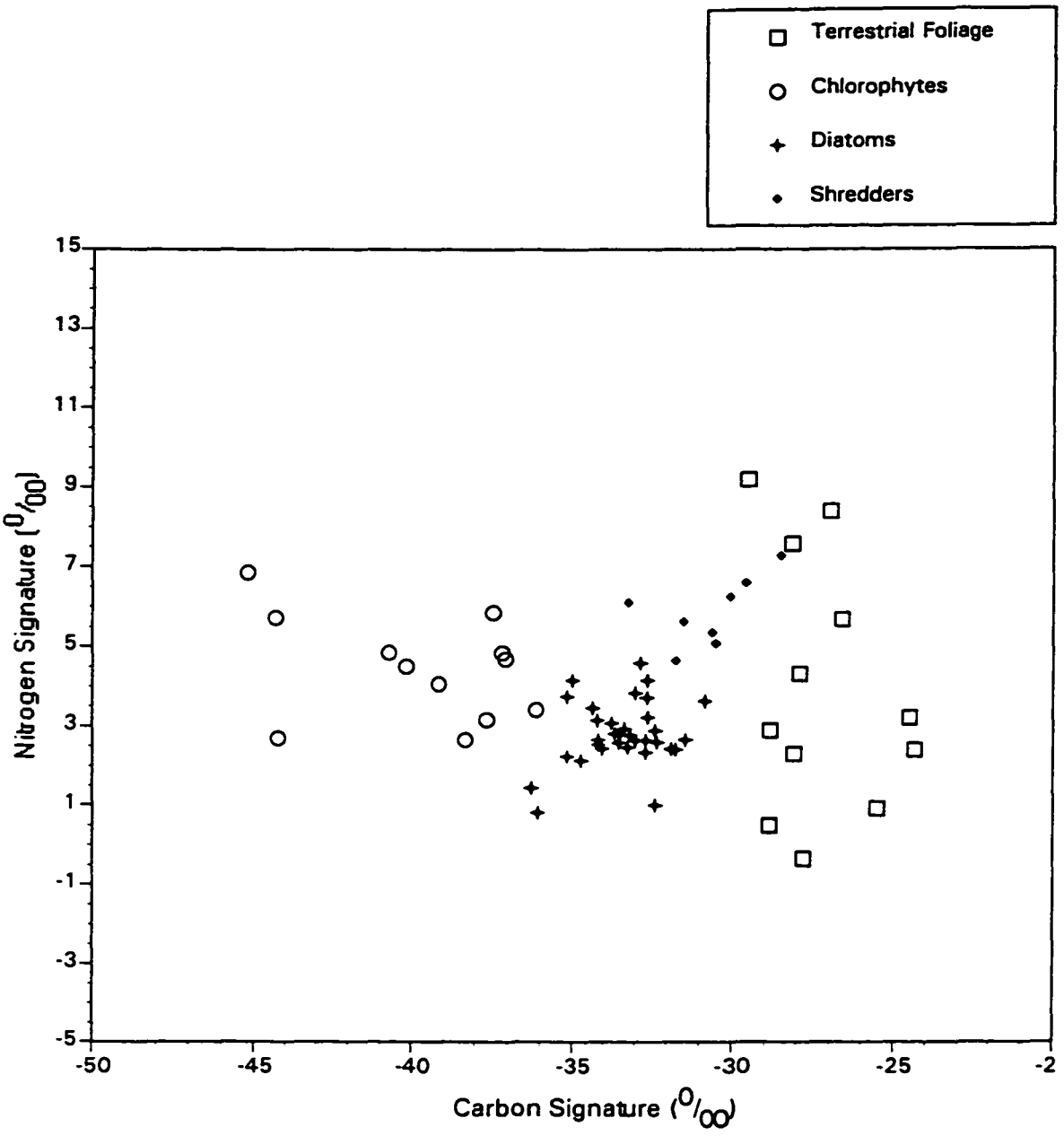

Figure 4.3. Carbon and nitrogen signatures (‰) from terrestrial plants, chlorophytes, diatoms and shredders in spring.

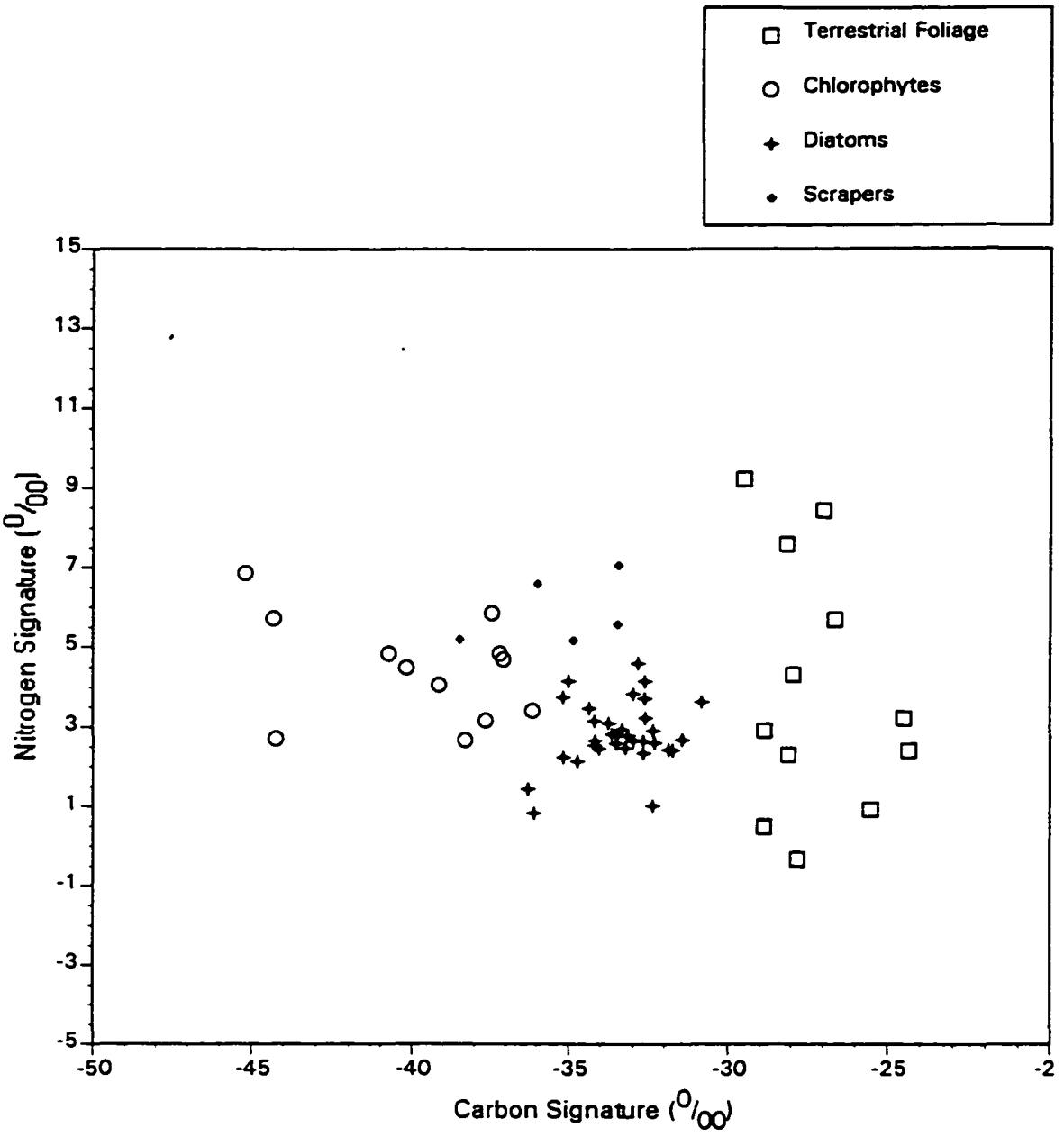

Figure 4.4. Carbon and nitrogen signatures (‰) from terrestrial plants, chlorophytes, diatoms and scrapers in spring.

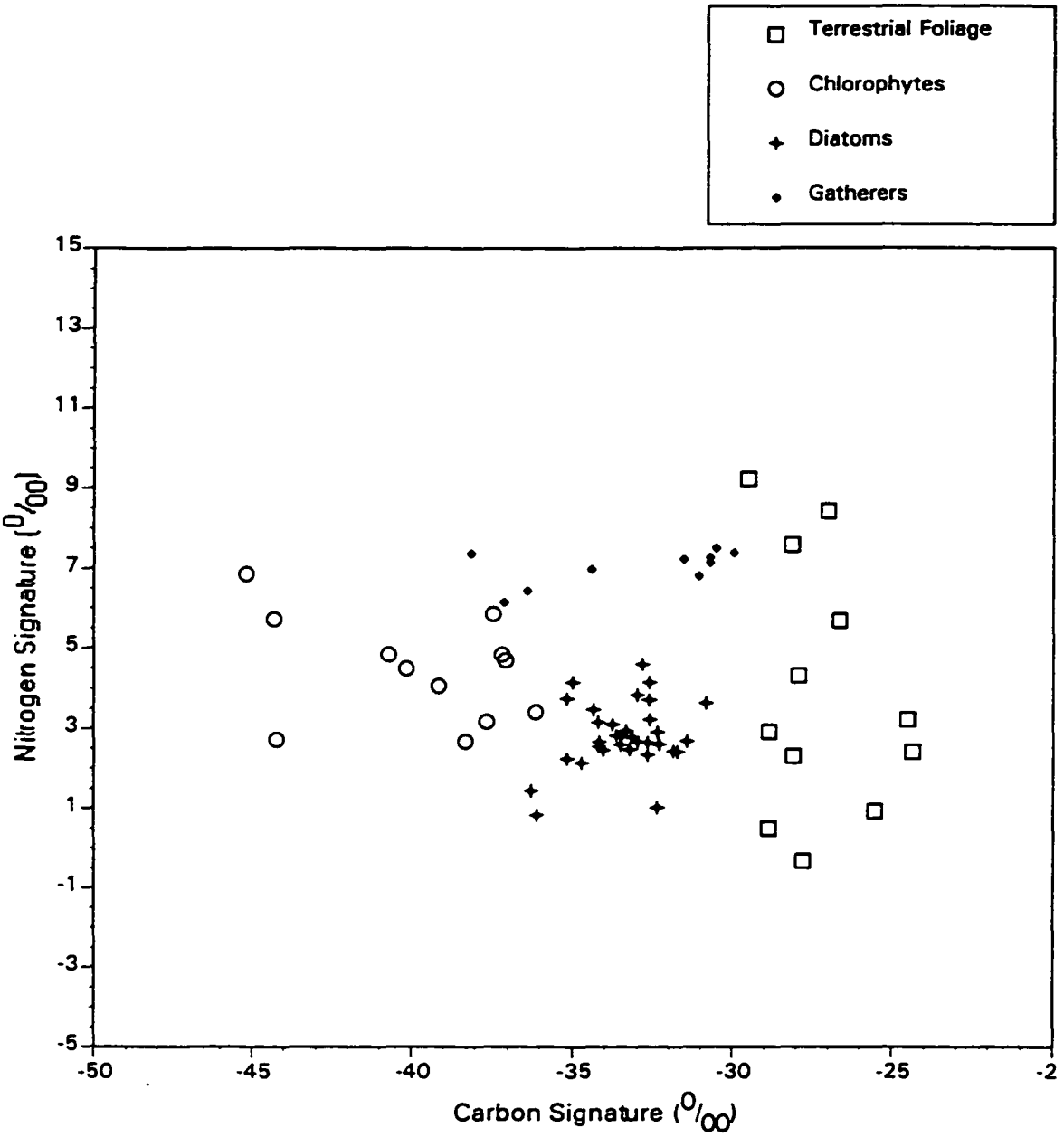

Figure 4.5. Carbon and nitrogen signatures (‰) from terrestrial plants, chlorophytes, diatoms and gatherers in spring.

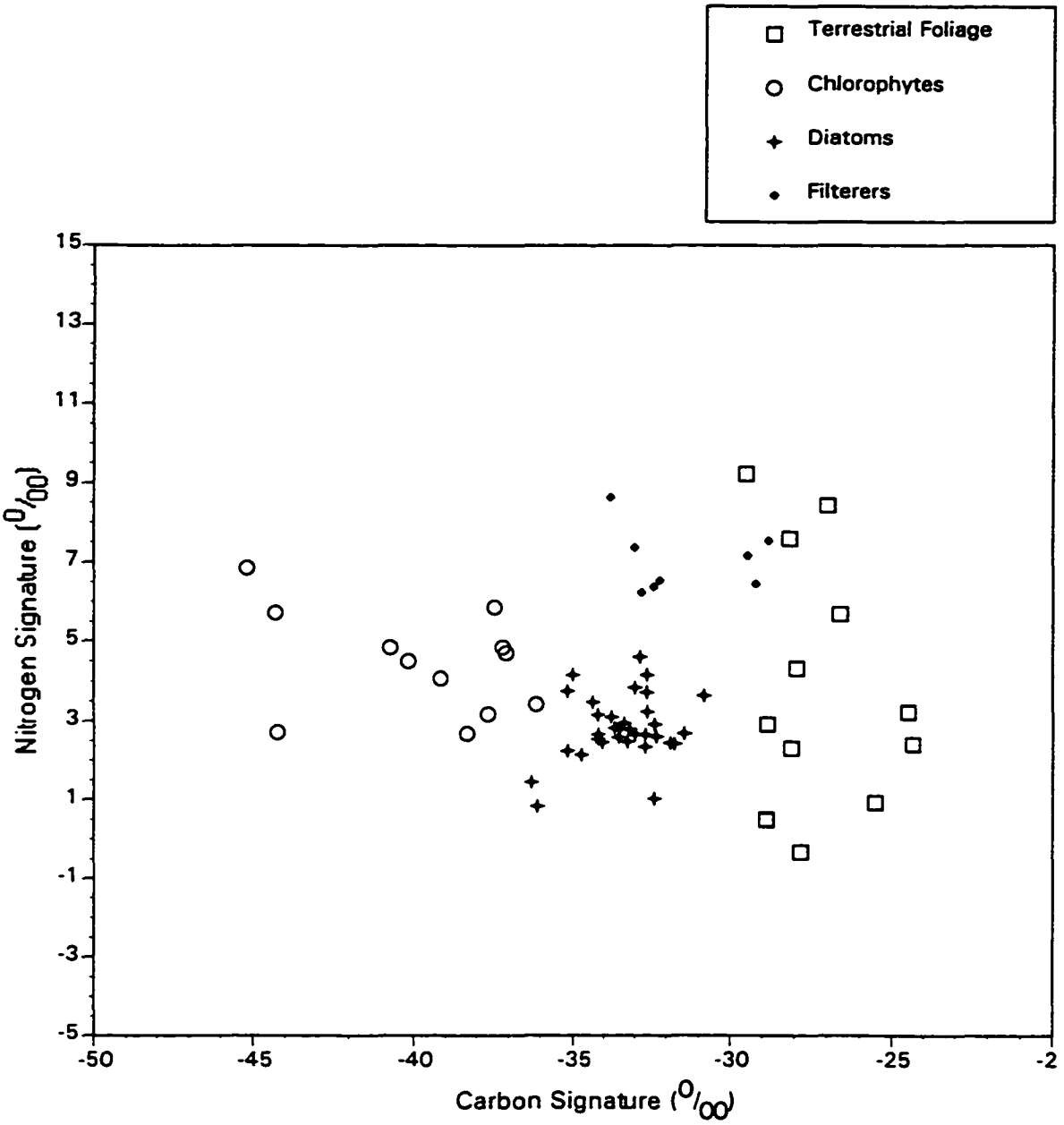

Figure 4.6. Carbon and nitrogen signatures (‰) from terrestrial plants, chlorophytes, diatoms and filterers in spring.

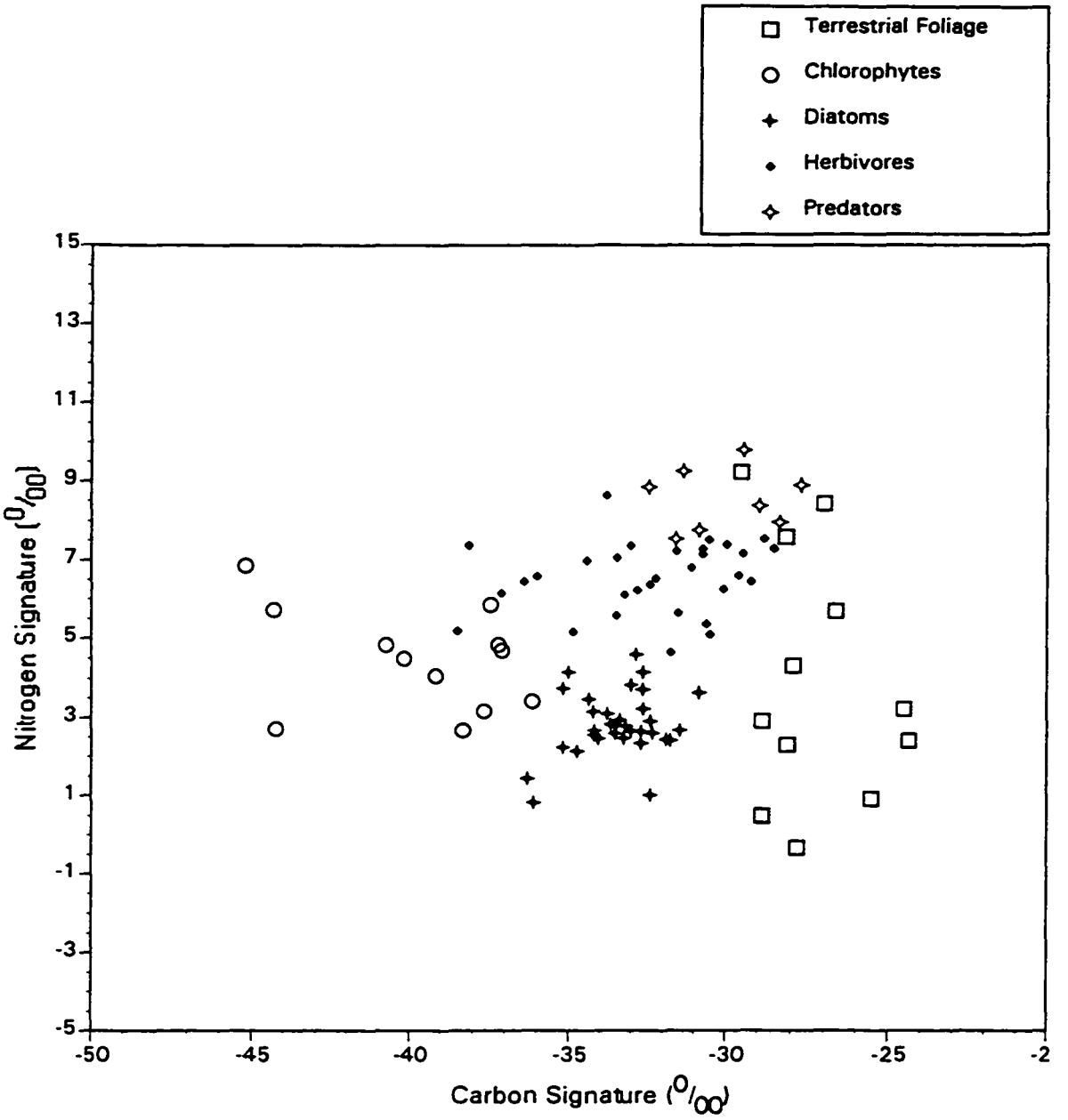

Figure 4.7. Carbon and nitrogen signatures (‰) from terrestrial plants, chlorophytes, diatoms, herbivores and predators in spring.

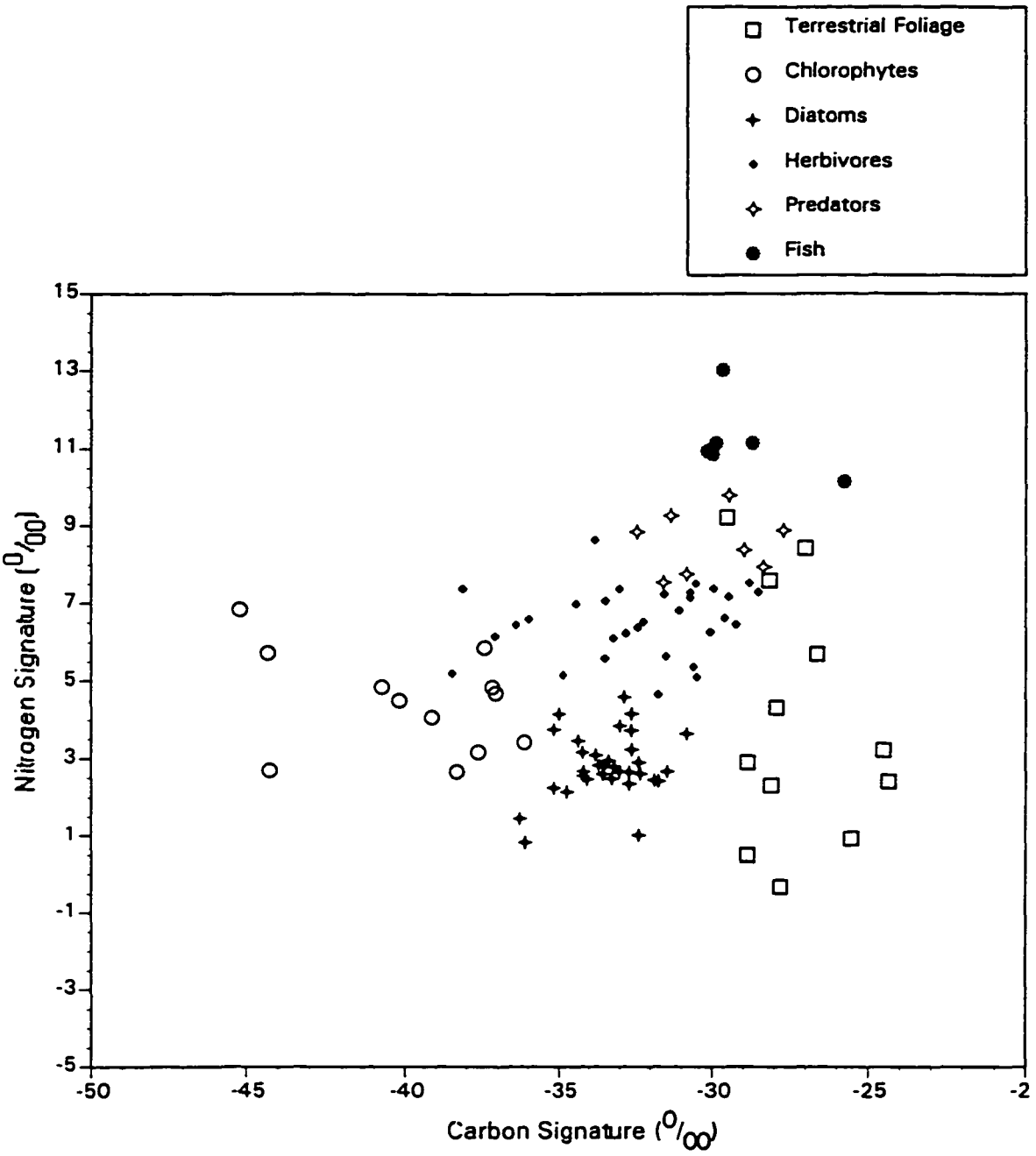

Figure 4.8. Carbon and nitrogen signatures (‰) from terrestrial plants, chlorophytes, diatoms, herbivores, predators and fish in spring.

CHAPTER 5

General Conclusions

In this thesis, the effect of agricultural activity on lotic macroinvertebrate communities was evaluated at the field scale. Abundance, biomass, secondary production, species richness and diet were compared between habitats upstream and downstream of an agricultural site. Estimates of diet, obtained using stable isotopes of carbon and nitrogen, and secondary production were combined to determine the dependence of individual taxa and each habitat on allochthonous and autochthonous inputs.

In general, alterations to the macroinvertebrate community between sites were consistent with the effects of sedimentation of eroded soil, and, perhaps, alterations in the hydraulic regime, both of which are likely results of agriculture (Chapter 2). Distinctive shifts in the composition of benthic invertebrate communities toward assemblages more characteristic of degraded streams were observed within the 400 metre study reach. Larger, more sensitive forms, especially mayflies, caddisflies and stoneflies were replaced by smaller, more tolerant chironomids. The increased agricultural activity in 1996 did not result in a greater impact on the benthos, but this is probably due to the much greater precipitation during that summer.

Production by filter-feeding collectors was 50% lower at the downstream site, primarily due to poor recruitment of the dominant hydropsychid caddisfly *Hydropsyche slossonae*, as a result of a shift in substrate from an heterogeneous mix of fines, gravel, cobble and boulders at the upstream site to predominantly fines and gravel downstream.

Lower density, biomass and production by the shredders downstream was likely a result of the reduced ability of the site to retain coarse particulate matter in the finer and more homogenous stream bed, and possibly lower inputs of terrestrial matter from the cleared farmland. Greater retention of fine detrital sediments also supported increased abundance, biomass and secondary production of the Chironomidae. The higher proportion of fine substrate may also have led to reduced primary production and subsequently, lower secondary production within the scrapers and gathering collectors.

While total numerical abundances of invertebrates were similar at the top and bottom of the study reach, most individual animals were smaller downstream. The additional benthic insectivorous fish species at this site increased the top-down pressure on the herbivores, led to greater mortality and/or drift of the larger animals, and, in spite of similar invertebrate densities at both sites, contributed to reduced secondary production.

Trophic relationships were identified using stable isotopes of carbon and nitrogen. The method provides estimates of diet based on assimilation rather than on ingestion and is therefore advantageous over gut content studies. In the past, the limitation of stable isotope analysis has been through inadequate sampling of benthic algae and a poor ability to accurately determine seasonal algal $\delta^{13}C$ and $\delta^{15}N$. The third chapter assessed the sources of variation of these signatures in periphyton (predominantly diatoms) grown on glass plates suspended in the water column under two conditions of light and water velocity, over two seasons. Isotopic signatures for both carbon and nitrogen in samples of diatoms varied with light intensity and season, but not current velocity. In summer, diatoms grown under low light conditions had depleted $\delta^{13}C$ and $\delta^{15}N$ values relative to

diatoms grown under high light. In autumn, isotopic signatures were generally more depleted than in summer, but did not vary systematically with light intensity or water velocity. These results suggested that isotopic fractionation in periphyton was more strongly influenced by the intensity of metabolic activity than by variations in the thickness of the benthic boundary layer.

In the fourth chapter, temporal samples of chlorophytes, bryophytes and macroinvertebrates were evaluated and trophic dependencies determined. Chlorophyte carbon signatures were more depleted than diatom signatures. Increased resistance to diffusion through the complex spatial structure of the epilithon likely led to lower cellular concentrations of CO_2 in diatoms than in chlorophytes. Lower cellular concentrations are associated with reduced enzymatic discrimination between ^{12}C and ^{13}C because a larger percentage of the available CO_2 is assimilated (Sharkey and Berry 1985). Chlorophyte carbon signatures also followed a seasonal pattern in which reduced metabolic fractionation under high growth conditions in summer led to enriched signatures. Winter signatures were most depleted while spring and autumn were intermediate. Temporal carbon signatures of the macroinvertebrates reflected the seasonal variation in algae. Nitrogen signatures were similar at both upstream and downstream sites within autochthonous primary producers and macroinvertebrates and therefore an effect due to the application of commercial and organic fertilisers to the agricultural land was not apparent.

Functional feeding group designations were useful predictors of diet at the group level. The relative dependence of different groups on allochthonous resources followed

the expected trend (i.e. shredders were most, and scrapers least, dependent on terrestrial inputs). However, many taxa exploited a wider food base than their feeding group classification implied (e.g. shredders and filterers). The dependence of shredders on terrestrial inputs increased from 11% over summer to 54% after the autumn leaf fall. The dietary analysis suggests that four taxa should be assigned different trophic guilds. *D. nivoriunda*, *B. brunneicolor* and *Parakiefferiella* sp. were almost completely dependent on carbon fixed by benthic algae so should be considered scrapers. Likewise, the diet of *Pycnopsyche* sp. suggests it is more of a gatherer than a shredder. Food availability rather than morphological adaptions appeared to be most important in determining the diet of individual taxa.

The similarity of the invertebrate diets at sites 1 and 5 suggests that inputs of autochthonous and allochthonous organic material did not limit secondary production at the downstream site. The analyses support the conclusions from chapter 2 that changes to the benthic community composition and lower secondary production downstream were primarily due to a shift in stream bed morphology, likely a direct result of agricultural activity on the adjacent fields. Autochthonous primary production and allochthonous inputs necessary to support the macroinvertebrate community were within expected ranges for a moderately enriched headwater stream with a partial canopy.

The stable isotope analyses in this thesis emphasise the seasonal variation in carbon and nitrogen signatures which can be expected in both producers and consumers in lotic environments. It is apparent from this study that past efforts to determine lotic algal signatures have been inadequate to detect either habitat or seasonal variations and the

work in this thesis should provide a benchmark for future research in this area. The study also highlights the need for a comprehensive knowledge of animal life-cycles to confidently utilise stable isotopes to define feeding habits and trophic relationships.

The combination of lotic secondary production and stable isotope analysis to quantify trophic relationships is unique in the literature and led to the identification of major pathways of organic matter in the ecosystem. The study strongly suggests that microbial, fungal and diatom colonies on both FPOM and CPOM are important sources of carbon assimilated by stream macroinvertebrates. It is hoped that future researchers will improve on these techniques and develop methods to determine the relative importance of allochthonous and autochthonous inputs to microbial production in streams.

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IMAGE EVALUATION
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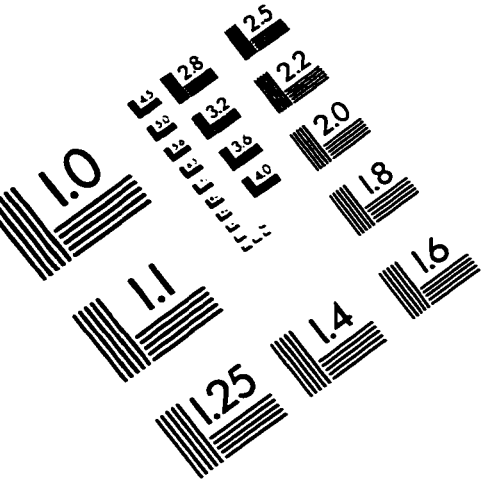

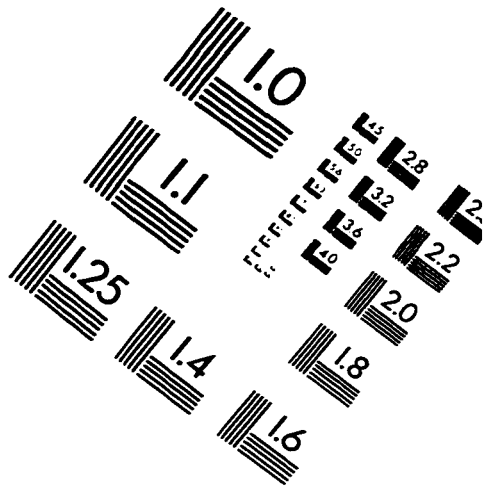

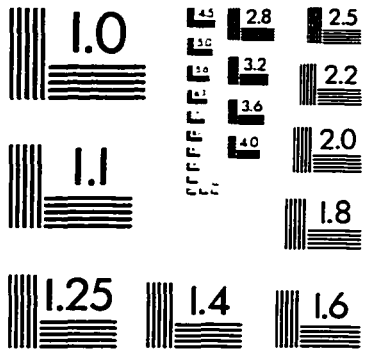

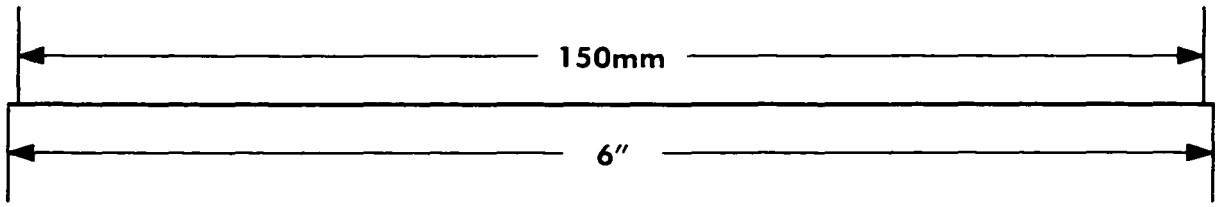

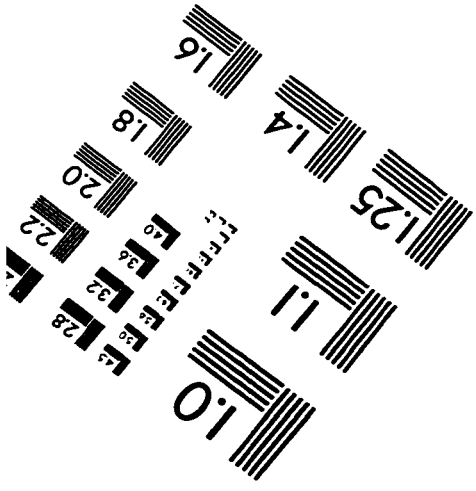

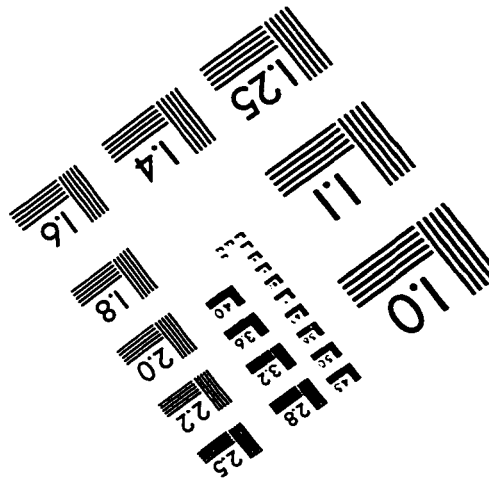

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