Limnol. Oceanogr., 38(4), 1993, 872–878

The trophic significance of epilithic algal production in a fertilized tundra river ecosystem

Bruce Peterson, Brian Fry, and Linda Deegan
The Ecosystems Center, Marine Biological Laboratory, Woods Hole, Massachusetts 02543

Anne Hershey
Department of Biology, University of Minnesota, Duluth 55812

Abstract

Fertilization of a pristine tundra river with phosphorus and nitrogen changed the nitrogen and carbon stable isotope ratios in epilithic algae. Determination of $\delta^{15}N$ and $\delta^{13}C$ values in consumers allowed this algal organic matter to be traced in the food web for several kilometers downstream of the fertilizer addition sites. Changes in the isotopic composition of consumers documented the tight coupling between algal production in response to fertilization and measured increases in the growth of insects and fish.

Understanding the controls of biological productivity in flowing waters is of applied and fundamental interest because humans impact and use rivers heavily. However, the analysis of river ecosystem dynamics is made difficult by the complexity of material transfers in systems where water, dissolved nutrients, and suspended particles are continuously moving, insects are drifting, and fish are free to migrate. In our experimental river fertilization study, nutrient addition caused shifts in the carbon and nitrogen stable isotope ratios in the biota which provided a unique opportunity to trace organic matter flows and to help determine the effects of fertilization on a tundra river ecosystem. Phosphorus and nitrogen addition stimulated epilithic algal production which in turn promoted growth of insects and fish.

Experimental design, the Kuparuk River, and isotope methods

This experiment is part of a long-term fertilization study of a meandering 4th-order reach of the Kuparuk River on the North Slope of Alaska. We have reported previously that phosphorus addition stimulates algal growth on the rocky river bottom (Peterson et al. 1983, 1985). For four consecutive summers from 1983 to 1986 we continuously added phosphate from 1 July to 15 August at an average final concentration in the river water of ~10 μg P liter^{-1} above ambient. In 1986 we added nitrogen as ammonium sulfate (average concentration 100 μg N liter^{-1}) at a second location 1.7 km downstream of the P addition site. Here we present results of stable isotope samplings performed during the 1986 experiment when both phosphate and ammonium were added to the river reach that had been fertilized with P every summer since 1983.

The river has a rocky cobble bottom colonized by filamentous algae, diatoms, and bacteria. Large amounts of organic matter enter the river as peat eroding from the river banks and as dissolved organic matter leaching from the tundra landscape. Allochthonous organic matter inputs far outweigh autochthonous production of epilithic algae (Peterson et al. 1986). Four insect species dominate invertebrate biomass and production. Blackfly larvae (*Prosimulium martini*) are filter feeders, a mayfly (*Baetis lapponicus*) is a grazer of the epilithon (microbial slime and diatom community coating rocks), a chironomid (*Orthocladius rivulorum*) grazes on diatoms that colonize the silken tube produced by the insect, and a caddisfly (*Brachycentrus americanus*) is predominantly a filter feeder but also grazes epilithon (Gallepp 1974, 1977; Gallepp and Hasler 1975). The only fish species is the Arctic grayling (*Thymallus arcticus*) represented in the study reach by both young-of-the-year (YOY) and adults.

Samples for stable isotope analysis of filamentous algae, epilithon, insects, and YOY fish were collected after 3–6 weeks of continuous fertilizer addition at stations along 5 km of river: upstream control reach (2 km), phosphorus addition (1 km), and phosphorus plus nitrogen addition (2 km). Adult grayling were held in 1-km subsections of the control reach and of the P+N fertilized reach by means of

weirs from 1 July until sampling after 5 weeks of continuous fertilizer addition. All insect samples were composites of 10–100 individuals, depending on size of the insect. Grayling samples of both young and adults were from individual fish.

Three–six weeks is sufficient time for algae, most insect species, and YOY grayling to achieve isotopic equilibration with their food because of rapid growth and tissue turnover. The two possible exceptions are *B. americanus,* the large caddisfly, and adult grayling. *Brachycentrus* appears to be very nearly equilibrated as this filter-feeding species was isotopically similar to the smaller blackfly in both control and fertilized reaches after 3 weeks of fertilizer addition. Adult grayling are 7–10 yr old and equilibrate slowly except for metabolically active tissue such as gonads and liver.

Samples for isotope analyses were preserved either frozen or in alcohol and analyzed in Woods Hole, Massachusetts. Samples were dried at 60°C, pulverized with CuO and Cu, sealed in evacuated Vycor tubes, combusted at 900°C for 1 h, and cooled during 4 h to room temperature. Pure CO_2 and N_2 were separated cryogenically and measured for isotopic composition with a precision of ±0.1‰ on a Finnigan 251 isotope ratio mass spectrometer. $\delta^{13}C$ and $\delta^{15}N$ values are reported relative to PDB and air N_2 standards respectively, where $\delta X = [(R_{sample}/R_{std}) - 1] \times 10^3$; $X = {}^{13}C$ or ^{15}N and $R = {}^{13}C:{}^{12}C$ or $^{15}N:{}^{14}N$.

Stable isotope distributions before fertilization

The prospects for using stable isotopes to trace organic matter flow appeared unpromising prior to the fertilization experiment because differences in $\delta^{13}C$ and $\delta^{15}N$ values between important organic matter sources were small. For example, the carbon isotope ratios in peat ($\delta^{13}C = -27.1$‰), in terrestrial plants (−26.0 to −27.3‰), and in epilithon (−27.2‰) collected in 1979 were not sufficiently distinctive to provide a useful tracer of carbon flow (Peterson et al. 1986). In addition, the $\delta^{15}N$ values of the added fertilizer ammonium (0‰), peat (+0.3‰), and epilithon (+1.9‰) differed only slightly.

Before ammonium addition, $\delta^{15}N$ values of epilithon and insects were similar in both reaches. For example, in June 1986 immediately before fertilization, samples of epilithon from the control and fertilized reaches had similar mean $\delta^{15}N$ values of 3.4±0.4‰ and 3.1+0.2‰. Samples of *Baetis* from control and fertilized reaches collected the previous summer (July 1985) had $\delta^{15}N$ values of 2.7±0.4‰ and 3.1±0.1‰.

Isotopic shifts following fertilization

Natural isotopic fractionations which occurred in the P- and N-fertilized reaches during algal photosynthesis for ^{13}C and during uptake of ammonium for ^{15}N produced surprisingly large shifts in algal $\delta^{13}C$ and $\delta^{15}N$ values. We used these isotopic shifts induced by fertilization to trace the flow of organic matter produced by epilithic algae (autochthonous production) in the riverine food web. After 3–5 weeks of fertilization, $\delta^{15}N$ values of algae and insects in the ammonium-fertilized reach were strikingly different from the control reach. Samples of filamentous green algae had a mean $\delta^{15}N$ value of +3.5‰ in the control reach but averaged −6.3‰ at the station 1.3 km downstream (3-k station) of the ammonium addition site (Table 1). The low $\delta^{15}N$ values of filamentous algae in the fertilized reach indicate that the added ammonium, which had a measured $\delta^{15}N$ value of 0.0‰, was strongly fractionated by ~9‰ during uptake by algae (Macko et al. 1987). These low values of $\delta^{15}N$ provided a tracer for algal organic matter produced in the nutrient-enriched stream reach as it moved through the food web. There was no measurable shift in the $\delta^{15}N$ value of detrital peat held in litterbags (Table 1). *Orthocladius* and *Baetis,* the two grazing insects, had large negative $\delta^{15}N$ shifts, reflecting a strong dependence on organic matter produced in the reach fertilized with both ammonium and phosphorus (Fig. 1, top). The filter-feeding blackfly (*Prosimulium*) and the caddisfly (*Brachycentrus*) exhibited marked but smaller nitrogen isotope shifts in the N+P-fertilized reach. Samples of insects collected farther downstream exhibited increasing $\delta^{15}N$ values reflecting either or both the progressive ^{15}N enrichment of the ammonium and algal nitrogen pools as the added fertilizer was taken up in transit or a lesser isotopic discrimination as the ammonium concentration was reduced by uptake and nitrification.

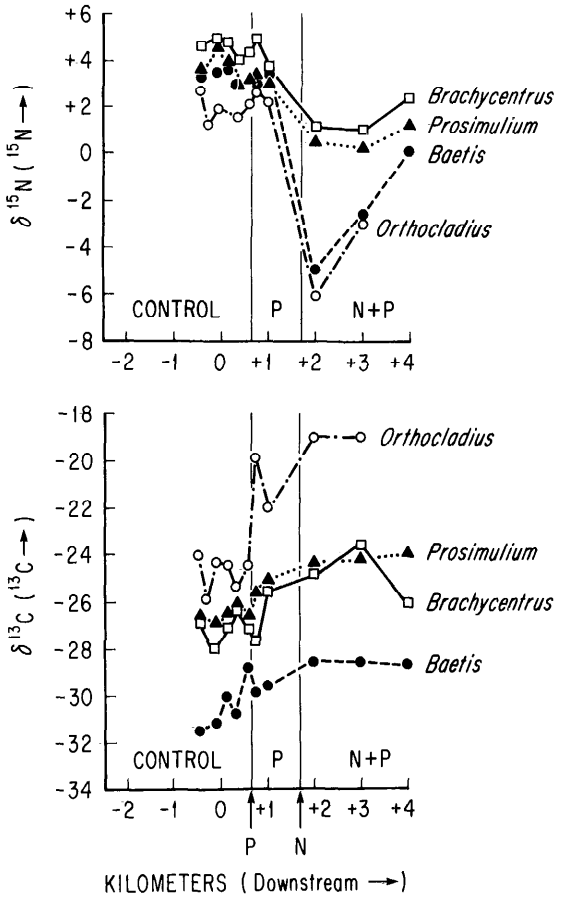

Fig. 1. The $\delta^{15}N$ and $\delta^{13}C$ values of the four dominant insect species collected in late July and early August along a transect of riffle sites in the control, P- and N+P-fertilized reaches of the Kuparuk River. 0 km is the site of initial fertilization in 1983 (Peterson et al. 1985). P addition at 0.59 km and N+P addition at 1.7 km.

The experimental fertilization unexpectedly produced a second tracer of autochthonous primary production by shifting the $\delta^{13}C$ values of some algae, which was measured indirectly via analysis of insect grazers of algae (Fig. 1, bottom). The $\delta^{13}C$ values of insects averaged from 2.2 to 5.8‰ higher (enriched in ^{13}C) in the N+P-fertilized reach than in the control reach. Values from the P-only reach were intermediate (Table 1). The shift in ^{13}C content was most dramatic in *Orthocladius* which feeds on the diatom *Hannaea arcus* on its silken tube (Hershey et al. 1988) rather than on the more mixed diets consumed by *Baetis* and the filter feeders. Filamentous algae were not isotopically heavier in the fertilized reach than in the control reach but averaged −19.7‰ and thus were apparently not an important dietary component for the grazing or filter-feeding insects in the river, which were more depleted in ^{13}C.

We do not attribute the ^{13}C shift in the microalgae to change in $\delta^{13}C$ values of ambient CO_2 as the partial pressure of CO_2 in river water was well above saturation (~700 ppm) and was not measurably affected by the fertilizer additions (Kipphut unpubl. data). Rather, the ^{13}C shift probably reflects more rapid enzymatic fixation of CO_2 relative to rates of CO_2 supply (Osmond et al. 1981; Sharkey and Berry 1985) and in the N+P-fertilized reach an enhanced CO_2 flux through the phosphoenolpyruvate carboxylase (PEPase) pathway with lower isotopic discrimination than the RuBP carboxylase pathway (Guy et al. 1989). The carbon isotopic changes in insects were almost certainly a reflection of the ^{13}C shift in the algal component of their diet since the ^{13}C content of peat decomposing in litter bags did not change even after two consecutive summers in the river and 50% weight loss (Table 1). For example, detrital soil organic matter (peat) held in litterbags for two summer seasons in the control, P-enriched, and P+N-enriched (year 2 only) reaches had $\delta^{13}C$ values of -24.9 ± 0.2, -25.1 ± 0.2, and -24.8 ± 0.1, which are not statistically different from the initial value of −25.2‰. The shift in carbon isotope ratios in the biota is important because it indicates the linkage between photosynthetic algal production and the changes in consumer isotope ratios. If only $\delta^{15}N$ values had changed, one could argue that perhaps heterotrophic bacteria using allochthonous organic matter but taking up the added ammonium were the primary food resources causing shifts in consumer isotope ratios.

Reliance of consumers on algal production

We can determine the reliance of consumers on algal production in the fertilized stream reaches by focusing on changes in $\delta^{15}N$ values. The ammonium addition altered the nitrogen isotope ratios at all levels in the food web (Fig. 2). Comparisons of $\delta^{15}N$ values of each insect species between the control and N+P-fertilized reach indicate the degree of dependence

Table 1. Comparisions of the $\delta^{13}C$ and $\delta^{15}N$ values (‰) in components of the Kuparuk food web in the control, P-, and N+P-fertilized reaches. Collections were taken 3–5 weeks after the start of fertilization. Values given are means ± SE (*n*). Values for peat (C and N) and for filamentous algae (C only) were not affected by fertilization and are pooled.

	$\delta^{13}C$			$\delta^{15}N$		
	Control	P	N+P	Control	P	N+P
Organic matter sources						
Peat	←	−26.8±0.3(12)	→	←	+1.1±0.3(9)	→
Epilithon	−29.2±0.1(2)	−27.7±0.8(3)	−28.0±0.3(3)	1.9±0.1(2)	+2.5±0.2(3)	0.0±0.4(3)
Filamentous algae	←	−19.7±1.9(6)	→	+3.5±0.1(2)	+2.6(1)	−6.3±0.1(2)
Insects						
Brachycentrus	−27.1±0.3(4)	−26.6±1.0(2)	−24.9±0.7(4)	+4.6±0.2(3)	+4.4±0.6(2)	+1.5±0.4(3)
Prosimulium	−26.6±0.2(4)	−25.3±0.2(2)	−24.2±0.1(4)	+3.8±0.4(3)	+3.2±0.0(2)	+0.6±0.3(3)
Baetis	−30.9±0.3(4)	−29.8±0.2(2)	−28.6±0.0(4)	+3.3±0.1(3)	+3.2±0.2(2)	−2.5±1.5(3)
Orthocladius	−24.8±0.3(6)	−20.9±1.5(2)	−19.0±0.0(2)	+1.9±0.2(3)	+2.4±0.3(2)	−4.6±1.6(2)
Fish						
Thymallus arcticus						
YOY muscle	−27.8±0.1(5)	—	−25.1±0.4(5)	+6.9±0.0(5)	—	+3.6±0.4(5)
YOY gut contents	−30.0±0.7(4)	—	−27.0±0.4(4)	+4.3±0.2(4)	—	+0.5±0.1(4)
adult muscle	−26.2±0.1(5)	—	−25.5±0.3(5)	+8.3±0.2(5)	—	+7.6±0.3(5)
adult liver	−27.5±0.7(3)	—	−25.0±0.1(3)	+7.3±0.2(3)	—	+5.3±0.3(3)
adult gonad	−27.3±0.5(3)	—	−26.0±0.0(3)	+7.1±0.3(3)	—	+4.8±0.4(3)

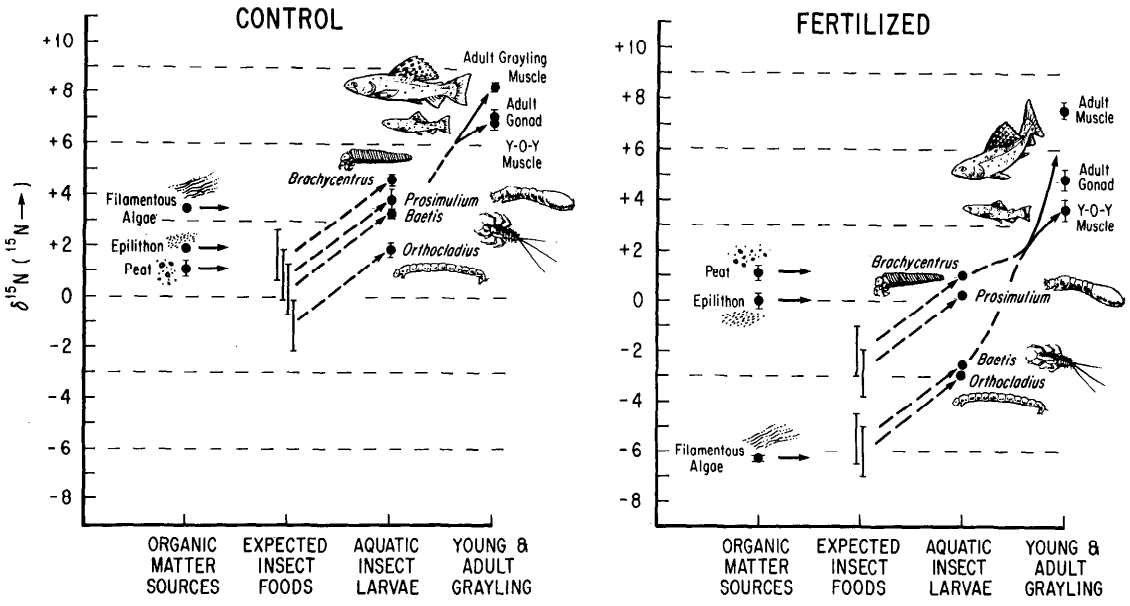

Fig. 2. Comparison of the nitrogen isotope distributions in the food web of the control reach and the 3-km station of the N+P-fertilized reach of the river. The range of values expected for insect foods was calculated by adjusting the insect $\delta^{15}N$ values downward by the usual 2–4‰ trophic transfer shift in $\delta^{15}N$ values (Minagawa and Wada 1984); they are presented here only as visual guide for comparison with measured values for organic matter sources. Actual diets may of course include mixtures of sources.

of these consumers an algal production in the N+P-fertilized reach. The grazers *Orthocladius* and *Baetis* must have relied on local algal production almost exclusively because their $\delta^{15}N$ values were strongly shifted to values consistent with a diet composed entirely of algae from the N+P-fertilized reach (Fig. 2). We know that *Orthocladius* and *Baetis* do not consume the filamentous algae but it is apparent that the microalgae consumed by these grazers had $\delta^{15}N$ values similar to the filamentous algae. The consumers do not have the same $\delta^{15}N$ value as their foods since consumers, once equilibrated with their diet, are expected to have $\delta^{15}N$ values 2–4‰ higher than their foods due to metabolic fractionation of nitrogen (Minagawa and Wada 1984). Filter-feeding caddisflies and blackflies experienced smaller nitrogen isotope shifts, about half as large as the grazers. They must depend partly on algal production within the fertilized stream reach and partly on allochthonous detritus or on organic matter imported from upstream reaches.

For both YOY and adult grayling the magnitudes of the shifts in $\delta^{15}N$ values indicate that the bulk of their diet was composed of insects that relied heavily on algal production in the N+P-fertilized reach. The gut contents and muscle of YOY grayling from the 3-km station in the N+P-fertilized reach were depleted in ^{15}N by 3.8 and 3.2‰ relative to samples from the control reach (Table 1, Fig. 2). Since the ^{15}N shift in the YOY is of the same magnitude as the isotopic shifts in the insects (= −3.1 to −5.8‰), we conclude that YOY at the 3-km station were primarily dependent on insect larvae produced in the N+P-fertilized reach. The YOY grayling experienced a >40-fold gain in weight during the fertilization period (Deegan and Peterson unpubl. data), so they would have been fully equilibrated with the isotopic composition of their food by early August.

In adult grayling, metabolically active tissues such as gonads, which are produced in large part in summer because grayling are spring spawners, were depleted in ^{15}N by 2.7‰ (Table 1, Fig. 2). This shift is similar in magnitude to that of the YOY and consistent with a primary dependence on aquatic insects produced in the N+P-fertilized reach, even though adult fish

have been shown to ingest large quantities of allochthonous detritus and some terrestrial insects as well. For example, guts of adult grayling collected in 1985 (Hershey et al. unpubl. data) contained 26% (dry wt) aquatic insects, 5% terrestrial insects, and the remaining 69% detritus, mainly peat. Not all tissues in adult grayling were as close to isotopic equilibration as gonad tissue. For example, the ^{15}N shift in muscle tissue of adult fish was only half as great as in gonad tissue, reflecting the slower nitrogen turnover in muscle (Fig. 2). This rate of change in ^{15}N content of adult grayling muscle tissue is equivalent to a half-life for muscle nitrogen of 40 d according to the method of Tieszen et al. (1983), if we assume that at equilibration the change in adult muscle $\delta^{15}N$ value would be similar to the changes in gonad tissue and YOY.

Carbon isotopic shifts in the consumers followed the same pattern of change seen in the ^{15}N distributions. The young and adult fish tissues also reflected the entire ^{13}C enrichment measured in the insects. *Baetis* and *Brachycentrus,* the two most important invertebrate prey for adult grayling, were enriched in ^{13}C by 2.3 and 2.2‰ respectively in the N+P-fertilized reach (Table 1). The muscle tissue of YOY grayling was enriched by 2.7‰ and the metabolically active liver of adult fish was enriched by 2.5‰. The ^{13}C data indicate, as do the ^{15}N data, that both young and adult fish derived the bulk of the organic matter in their diet from autochthonous primary production in the N+P-fertilized reach.

Conclusions

The fertilization experiment introduced isotopic tracers into the river that were used to analyze ecosystem processes in several ways. The large shift in ^{13}C in organisms implicated photosynthesis as the process most responsible for the change in producer and consumer stable isotope ratios. The simultaneous shifts in algal and consumer $\delta^{13}C$ and $\delta^{15}N$ values provided a means for determining the dominant food resources of consumers and for tracing organic matter transfers in the food web. Upstream-downstream transects of samples provided a yardstick for defining the downstream extent of the fertilization-induced perturbations of the carbon and nitrogen cycles (Fig. 1). We conclude that the riverine food web from nutrients to algae to insects to fish is tightly linked in river reaches of kilometers or less. In spite of large inputs of allochthonous peat and dissolved organic matter from the tundra, which, on the basis of mass, strongly dominate the carbon cycle (Peterson et al. 1986), all trophic levels of the riverine food web are highly responsive to fertilization which primarily stimulates epilithic diatoms and filamentous algae (Peterson et al. 1985, 1993).

The most significant finding from the stable isotope perspective is that naturally occurring stable isotopes may serve as tracers even in ecosystems where no useful isotopic signals are measurable under undisturbed conditions (Peterson et al. 1986). Either experimental or natural disturbances that cause changes in natural isotope fractionating processes such as photosynthesis, nitrogen uptake, nitrification, denitrification, or sulfate reduction may be anticipated to cause changes or shifts in stable isotope distributions which propagate throughout the ecosystem. In this experiment, we did not use the natural signals present in the undisturbed ecosystem as tracers nor did we follow the fate of an added isotopically enriched or depleted substance. Rather, it was the shift in the magnitude of natural stable isotopic fractionations which labeled newly produced algal organic matter and allowed us to follow subsequent carbon and nitrogen transfers in the ecosystem. By measuring these shifts in isotope distribution over time, we established relationships among ecosystem processes and components that were not apparent from other measurements and for which alternative means of measurement were not available. The magnitude and timing of the shift in isotopic composition was the parameter of primary interest rather than the isotopic ratios themselves. In this respect our study represents a new dynamic approach to the use of isotopes as tracers in ecosystems. The dramatic shifts provide a reminder that the isotopic compositions of organisms in nature are variable, frequently reflecting changes in the cycles of elements of which they are a part.

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