

## Parasitic association of *Nanocladius* (Diptera:Chironomidae) and *Pteronarcys biloba* (Plecoptera:Pteronarcyidae): insights from stable-isotope analysis

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**Abstract.** Nymphs of *Pteronarcys biloba* Newman and attached chironomid larvae (*Nanocladius* [*Plecopteracoluthus*] undescribed sp., nr. *branchicolus*) were collected from Catamaran Brook, New Brunswick, in May and November 1997, for stable-carbon- and stable-nitrogen-isotope analysis. *Nanocladius* (*P.*) sp. had mean ( $\pm 1$  SD)  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of  $-27.7 \pm 1.0\text{‰}$  and  $4.9 \pm 0.6\text{‰}$ , respectively, whereas those of *P. biloba* were  $-28.4 \pm 1.0\text{‰}$  and  $1.3 \pm 0.7\text{‰}$ , respectively. *Nanocladius* (*P.*) sp. always had more positive  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values than *P. biloba*, and average fractionation factors (isotope differences between symbiont and host) were  $+1.2\text{‰}$  and  $+3.5\text{‰}$  for carbon and nitrogen, respectively. These results confirm a parasitic relationship between *Nanocladius* (*P.*) sp. and *P. biloba*. No statistical differences were found among the  $\delta^{13}\text{C}$  values of the plecopteran shredder in 4 stream reaches from headwaters to mouth, and  $\delta^{13}\text{C}$  values were similar to those of their expected leaf litter diet. Pteronarcids from the headwater site (Upper Reach) were not parasitized and had distinct  $\delta^{15}\text{N}$  values from those at downstream sites. However, stable-isotope ratios of parasitized stoneflies were not significantly different from those of non-parasitized individuals at the 3 other study locations. *Nanocladius* (*P.*) sp. were more enriched in  $^{15}\text{N}$  than other chironomid genera in Catamaran Brook, including *Ablabesmyia*, a chironomid with predatory feeding habits, and fell within the range of other known invertebrate predators. These findings highlight the usefulness of stable-isotope technology to distinguish between phoresy and parasitism in ectosymbiotic relationships among aquatic organisms.

**Key words:** *Nanocladius*, *Pteronarcys*, parasite, host, ectosymbiosis, chironomid, stonefly, stable-isotope ratios,  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , stream ecology, phoresy, commensalism.

Many chironomids live ectosymbiotically with other benthic invertebrates (Steffan 1967a). Larval associations have been documented for most aquatic insect orders including Plecoptera, Ephemeroptera, Trichoptera, Megaloptera, and Odonata (Steffan 1967b, Gotceitas and Mackay 1980, White et al. 1980, Jacobsen 1995, Dosdall and Parker 1998). Most relationships are phoretic, where the chironomid does not feed on the

host's tissues, but uses the host for dispersal, protection from disturbance and desiccation, and food entrapment (Steffan 1967b, Dosdall and Mason 1981, Dosdall et al. 1986). However, parasitic feeding can occur (e.g., Jacobsen 1995), and the chironomid may obtain its nutrition directly from its host. In most cases, phoresy and parasitism are distinguished by the presence or absence of feeding scars on the host organism, the presence or absence of detrital particles in the chironomid gut, or direct observations of feeding. Alternatively, it is simply assumed that the relationship is phoretic.

Stable-isotope analysis (SIA) may help to distinguish between phoretic and parasitic rela-

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tions among chironomids and their hosts. SIA is a chemical analysis of food-source origins and energy pathways, and is unencumbered by ambiguous visual interpretation of stomach contents and detrital pools (Peterson and Fry 1987). The method is based on the fact that naturally occurring stable-isotope ratios of common elements such as carbon ( $^{13}\text{C}/^{12}\text{C}$ ), nitrogen ( $^{15}\text{N}/^{14}\text{N}$ ), and sulfur ( $^{34}\text{S}/^{32}\text{S}$ ) are assimilated into primary producers with signatures characteristic of various biogeochemical processes, and are passed along food chains with relatively predictable change (reviewed in Fry and Sherr 1984, Peterson and Fry 1987, Rundel et al. 1989, Lajtha and Michener 1994). In addition, SIA provides a composite picture of feeding over a period of time, rather than the single point-in-time sample obtained through gut-content analysis. Typically, at each trophic transfer of food energy, stable-carbon-isotope ratios (expressed as  $\delta^{13}\text{C}$ ) enrich by 0–1‰, whereas stable-nitrogen-isotope ratios ( $\delta^{15}\text{N}$ ) increase by 3–5‰ (DeNiro and Epstein 1978, 1981, Tieszen et al. 1983, Minagawa and Wada 1984, but see Focken and Becker 1998). In aquatic ecology,  $\delta^{13}\text{C}$  values have been used most often to discern between important primary food sources (e.g., aquatic algae versus terrestrial leaf litter; Rounick and Winterbourn 1986), whereas  $\delta^{15}\text{N}$  values have allowed for the accurate determination of trophic position (Vander Zanden et al. 1996). Stable isotopes have also been used to study biotic relationships, e.g., symbiotic relationships among terrestrial plants (Elheringer et al. 1985), marine bivalves (Conway et al. 1989), and ascidians (Kline and Lewin 1999); and trophic relations between rabbit hosts and their endoparasites (Boag et al. 1998).

Our objective was to identify the relationship between *Nanocladius* (*Plecoptera*: *coluthus*) undescribed sp., nr. *branchicolus*, and *Pteronarcys biloba* Newman in Catamaran Brook, New Brunswick, using stable-isotope ratios of carbon and nitrogen. *Nanocladius* (*P.*) sp. is a single species in the brook and currently is being described by R. E. Jacobsen, National Park Service, Homestead, Florida. The midge has a 1-y life cycle in Catamaran Brook, and spends most of the year attached to its stonefly host (Giberson et al. 1996). Chironomids were noted first as 2<sup>nd</sup>-instar larvae on the host in mid June, reached 4<sup>th</sup>-instar by the end of August, and overwintered as 4<sup>th</sup>-instar larvae on *Pteronarcys*. Giberson et al.

(1996) reported a parasitic association between the chironomid and the stonefly. *Nanocladius* (*P.*) sp. is also believed to be parasitic on other species of *Pteronarcys* in the eastern USA (R. E. Jacobsen, personal communication). However, some authors have reported that *Nanocladius*, including some on *Pteronarcys*, are phoretic (Gotceitas and Mackay 1980, Dosdall and Mason 1981, Dosdall et al. 1986, Dosdall and Parker 1998). To our knowledge, our study is the 1<sup>st</sup> attempt to verify ectoparasitism among freshwater benthic invertebrates using stable-isotope ratios.

## Methods

### Study site

Catamaran Brook (lat 46°52.7'N, long 66°06.0'W) is a tributary of the Little Southwest Miramichi River, located in a pristine forested area of north-central New Brunswick (Fig. 1). The brook is a well-buffered, circumneutral, 3<sup>rd</sup>-order stream, ~20.5 km long, with a drainage area of 52 km<sup>2</sup> (Cunjak et al. 1993). Riparian vegetation in the basin consists of 60% deciduous trees, including white birch (*Betula papyrifera*), yellow birch (*Betula lutea*), sugar maple (*Acer saccharum*), American beech (*Fagus grandifolia*), and speckled alder (*Alnus rugosa*). The remaining 40% of riparian forest cover consists of conifers, including balsam fir (*Abies balsamea*), red spruce (*Picea rubens*), and eastern white cedar (*Thuja occidentalis*) (Cunjak et al. 1990). Litter fall generally occurs in late September and leaves show rapid conditioning and fungal colonization, especially in autumn (Garnett et al. 2000).

Catamaran Brook is currently the focus of several multidisciplinary studies evaluating logging impacts on Atlantic Salmon (*Salmo salar*) habitat and productivity. Four study reaches (Upper, Middle, Gorge, and Lower) have been surveyed and characterized in the brook for long-term study. The Upper Reach consists of a 180-m stretch in the headwater region, and is characterized by a narrow (1–2 m), highly shaded stream channel, generally coarse substrates, steep slopes, and rapid flow. The Middle Reach, located approximately half way down the stream channel, has a stream width of 6–8 m, riffle gradients of 2–2.3%, and riffle substrates of cobble and gravel. The Gorge Reach, located

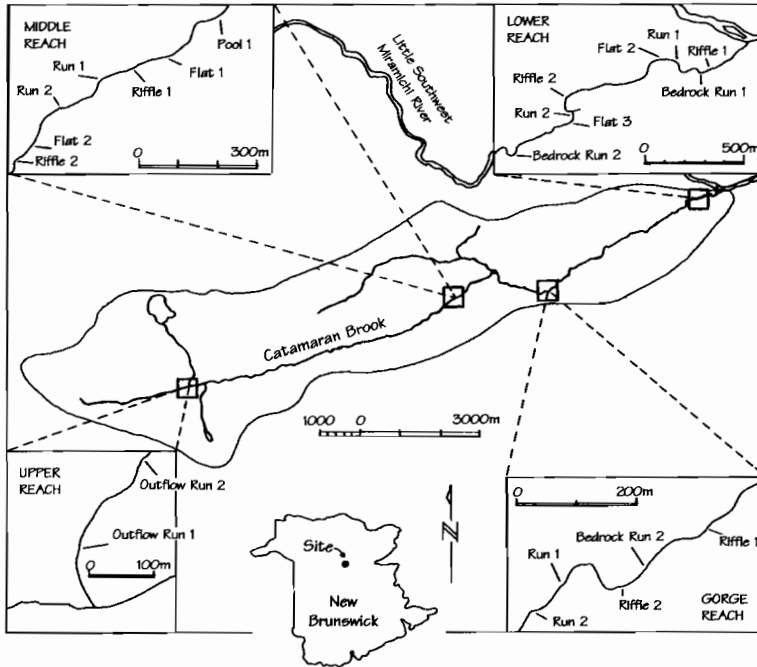


FIG. 1. Catamaran Brook, New Brunswick, eastern Canada, showing the location of the 4 main study reaches in the drainage basin.

~2 km downstream of the Middle Reach, runs through a bedrock outcrop area and is 6–8 m wide with riffle gradients of ~2% and a primarily bedrock substrate with some gravel and cobble. The Lower Reach consists of the lower 2 km of the stream and is 8–12 m in width, has riffle gradients of 1.6–1.75%, and riffle substrates of gravel, cobble, and boulder. Other details of geochemistry, hydrology, and biology can be found in Cunjak et al. (1993) and Giberon and Caissie (1998).

#### Invertebrate sampling

Invertebrates were collected on 28–30 May 1997 from run-riffle habitats, using a D-frame kick net with 250- $\mu$ m nylon mesh from the 4 main study reaches in Catamaran Brook. Collections of *P. biloba* and *Nanocladius* (*P.*) sp. were supplemented on 2–4 November 1997, but no isotopic differences were found between those sampled on the 2 dates (*t*-test for independent means,  $p > 0.05$ ). On both of these dates, *Nanocladius* (*P.*) sp. were attached to *Pteronarcys* nymphs as 4<sup>th</sup>-instar larvae. Kick samples were preserved with 85% ethanol (v/v) and stored in

plastic bags. Invertebrates were sorted from debris and identified to the lowest possible taxon using the keys of Merritt and Cummins (1996). *Nanocladius* (*P.*) sp. were removed from their silken tubes, which were attached to the bodies of *P. biloba*. Free-living chironomids (defined here as those not found attached to a host organism) were removed from debris and identified to genus by the inspection of head capsules mounted on glass slides using the keys of Wiederholm (1983).

#### Stable-isotope analysis

Stable-carbon- and stable-nitrogen-isotope analysis required only 0.6 mg tissue (dry mass) per sample. Invertebrates were oven-dried at constant temperature (60°C) for 24 to 48 h and ground to a fine powder using either a mortar and pestle or a ball-mill grinder. Up to 10 individuals of smaller taxa (e.g., other chironomids, simuliids, elmids, etc.) were required for each replicate sample. For larger organisms (e.g., pteronarcids, perlids, hydropterygids, tipulids, etc.), an aliquot of powdered tissue was used for each individual. All *Nanocladius* (*P.*) sp. found

on a single stonefly were combined, and the pooled sample was used in the analysis. Under no circumstances were *Nanocladius* (*P.*) sp. pooled among hosts.

Stable-isotope ratios are expressed as delta values ( $\delta$ ) and are measures of a parts-per-thousand (or *per mil*) difference (‰) between the isotope ratio of a sample and that of an international standard according to the formula:

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} = [(R_{\text{sample}} - R_{\text{standard}})/R_{\text{standard}}] \times 1000$$

where  $R = {}^{13}\text{C}/{}^{12}\text{C}$  or  ${}^{15}\text{N}/{}^{14}\text{N}$ . Samples that are *more negative* are *depleted* and contain less  ${}^{13}\text{C}$  or  ${}^{15}\text{N}$ ; samples that are *less negative* are *enriched* and contain more of the heavier isotopes. International standards are carbonate rock from the Peedee Belemnite formation (Craig 1957) and nitrogen gas in the atmosphere (Mariotti 1983). These standards are, by definition, set at a value of 0‰.

Isotopes were analyzed on a Micromass VG Isochrom continuous-flow isotope-ratio mass spectrometer connected to a Carlo Erba elemental analyzer at the Environmental Isotope Laboratory (EIL) (University of Waterloo, Waterloo, Ontario, Canada). Repeat analyses of common laboratory standards yielded results that were both accurate and precise (International Atomic Energy Agency [IAEA] standard CH6:  $\delta^{13}\text{C} = -10.5 \pm 0.2\text{‰}$  [mean  $\pm 1$  SD,  $n = 32$ ]; IAEA-N1:  $\delta^{15}\text{N} = 0.6 \pm 0.3\text{‰}$  [ $n = 25$ ]). Replicate samples of a lipid-extracted fish standard (EIL-70) also gave reliable  $\delta$  values for carbon and nitrogen ( $\delta^{13}\text{C} = -20.7 \pm 0.2\text{‰}$  [ $n = 38$ ], and  $\delta^{15}\text{N} = 16.4 \pm 0.3\text{‰}$  [ $n = 38$ ]).

#### Statistical analysis

Statistical analyses were performed using SYSTAT (version 7.0, SPSS Inc., Chicago). Maximum Type-I error rates were set at  $\alpha = 0.05$ . Normality and homogeneity of variance assumptions were checked using plots of the residuals. Significant ANOVA results were followed by multiple comparisons using the conservative Tukey's HSD post-hoc test (Sokal and Rohlf 1995). Model-2 linear regressions (Sokal and Rohlf 1995) were used to determine the significance of relationships between *Nanocladius* (*P.*) sp. and *P. biloba*  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values.

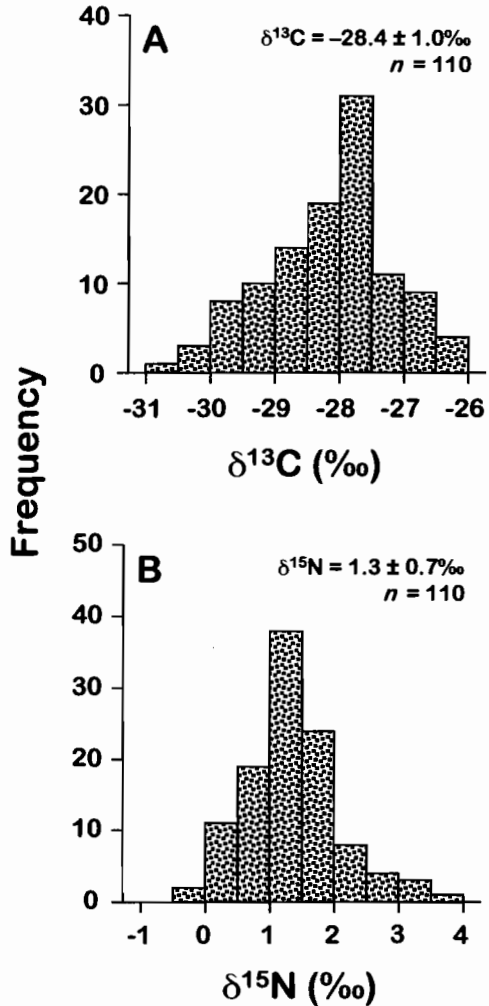


FIG. 2. Stable-isotope-ratio histograms (A:  $\delta^{13}\text{C}$  and B:  $\delta^{15}\text{N}$ ) of *Pteronarcys biloba* collected in late May and early November 1997 from Catamaran Brook, New Brunswick (samples pooled across date and location). Average values ( $\pm 1$  SD) and total sample size ( $n$ ) are included.

#### Results

One-hundred and ten *P. biloba* nymphs were collected, 84 of which were hosts to *Nanocladius* (*P.*) sp. Chironomid densities ranged from 1 to 12 individuals per host (average  $\pm 1$  SD =  $4.4 \pm 2.6$ ). No pteronarcids in the Upper Reach were parasitized, and non-parasitized nymphs in other locations were usually small (<15 mm in length). Most *Nanocladius* (*P.*) sp. were at-

TABLE 1. Stable-isotope ratios ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) of submerged deciduous leaves from common riparian trees in the Catamaran Brook drainage basin. Collections were made in late May 1997 from the Lower Reach. Values are means  $\pm 1$  SD, with sample sizes in parentheses.

Species	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
American beech ( <i>Fagus grandifolia</i> )	$-31.0 \pm 1.0$ (4)	$1.3 \pm 2.0$ (4)
Speckled alder ( <i>Alnus rugosa</i> )	$-29.5 \pm 0.8$ (5)	$-1.1 \pm 0.6$ (5)
Sugar maple ( <i>Acer saccharum</i> )	$-30.5 \pm 0.5$ (4)	$-0.6 \pm 0.7$ (4)
White birch ( <i>Betula papyrifera</i> )	$-30.9 \pm 0.3$ (3)	$0.4 \pm 0.6$ (3)
Yellow birch ( <i>Betula lutea</i> )	$-30.4 \pm 0.3$ (3)	$0.2 \pm 0.8$ (3)

tached to the femora or were located on the thoracic pleura beneath the wingpads.

The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of *P. biloba* ranged from  $-31\text{‰}$  to  $-26\text{‰}$ , and  $-0.5\text{‰}$  to  $+4\text{‰}$ , respectively (Fig. 2), and were slightly more enriched than those of their presumed leaf litter diet (Table 1). Although no differences were noted in the  $\delta^{13}\text{C}$  values of *P. biloba* among the 4 study reaches (Tukey's HSD,  $p > 0.05$ ),  $\delta^{15}\text{N}$  varied and *P. biloba* from the Upper Reach were more enriched in  $^{15}\text{N}$  than those from all other sites (Fig. 3). However, stable-isotope ratios of non-parasitized individuals at 3 other locations did not differ from parasitized ones (Tukey's HSD,  $p > 0.05$ ) (Fig. 3).

Model-2 linear regression showed a significant relationship between the stable-isotope ratios of *Nanocladius* (*P.*) sp. and those of *P. biloba* ( $p < 0.001$ ; Fig. 4). This relationship indicated that isotopically more enriched *Nanocladius* (*P.*) sp. were found on isotopically more enriched hosts. Average isotope differences, or fractionation factors, between *Nanocladius* (*P.*) sp. and *P. biloba* were  $+1.2 \pm 0.7\text{‰}$  and  $+3.5 \pm 0.5\text{‰}$ , for carbon and nitrogen, respectively.

*Nanocladius* (*P.*) sp. were not isotopically distinct from most other chironomids with respect to  $\delta^{13}\text{C}$ , but *Eukiefferiella* and *Heterotrissocladius* were more  $^{13}\text{C}$ -depleted than other genera (Table 2). *Nanocladius* (*P.*) sp. were, however, much more  $^{15}\text{N}$ -enriched than all other midges sampled including *Ablabesmyia* (Table 2). Stable-nitrogen-isotope ratios showed a strong relationship with feeding type in Catamaran Brook, and *Nanocladius* (*P.*) sp. was similar to invertebrates with predatory habits (Fig. 5).

## Discussion

### Relationship between *Nanocladius* and Pteronarcys

Laboratory feeding experiments have shown that animals are isotopically more enriched than

their diet (DeNiro and Epstein 1978, 1981, Tieszen et al. 1983). For carbon ( $\delta^{13}\text{C}$ ), animals are usually only slightly more enriched than their food (i.e., 0 to 1‰) but, for nitrogen ( $\delta^{15}\text{N}$ ), trophic fractionation is much higher (i.e., 3 to 5‰; Peterson and Fry 1987). Isotopic enrichment occurs along food chains because metabolic processes, such as respiration and excretion, preferentially use lighter isotopes ( $^{12}\text{C}$  and  $^{14}\text{N}$ ), leaving more of the heavier isotopes ( $^{13}\text{C}$  and  $^{15}\text{N}$ ) to accumulate in animal tissues (Steele and Daniel 1978, Hobson and Clark 1992). With respect to our study, if *Nanocladius* (*P.*) sp. were feeding parasitically on *P. biloba*, then: 1) the chironomid should be isotopically more enriched than its host, 2) isotopic differences between the 2 animals should fall within the range of expected values for diet-tissue fractionation measured in previous laboratory studies, and 3) individual variation in stable-isotope ratios between host and parasite should be correlated. Our results conform to these 3 expectations. *Nanocladius* (*P.*) sp. had  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values that were, on average, 1.2‰ and 3.5‰ more enriched than those of *P. biloba*, similar to values reported in the literature. In addition, stable-isotope ratios correlated well between *Nanocladius* (*P.*) sp. and *P. biloba*, with isotopically enriched chironomids found on isotopically enriched hosts. Thus, stable-isotope ratios confirm that *Nanocladius* (*P.*) sp. is parasitic on *P. biloba* in Catamaran Brook.

### Pteronarcys as a primary consumer

The stable-isotope ratios of *P. biloba* confirmed that this stonefly was feeding primarily on coarse particulate organic matter in Catamaran Brook. Deciduous leaf litter collected from the brook ranged from  $-31\text{‰}$  to  $-29\text{‰}$  for  $\delta^{13}\text{C}$ . The  $\delta^{13}\text{C}$  values for *P. biloba* were similar, but

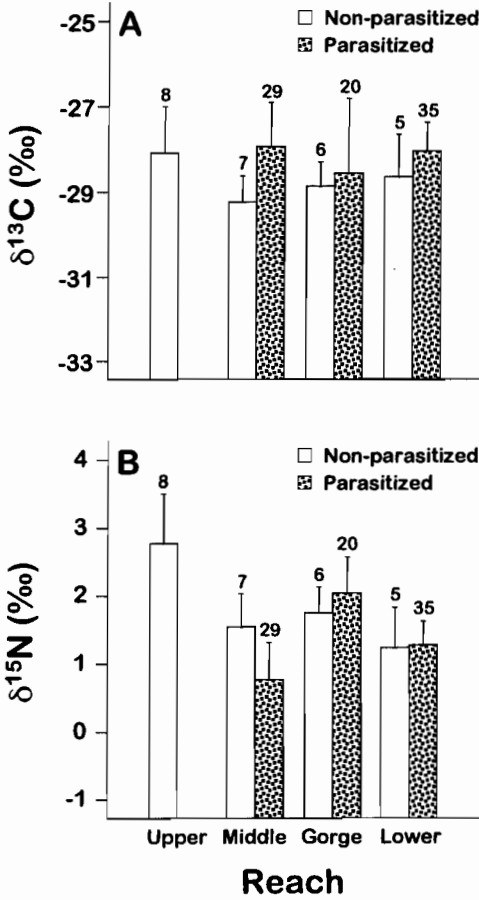


FIG. 3. Stable-isotope ratios (A:  $\delta^{13}\text{C}$  and B:  $\delta^{15}\text{N}$ ) of parasitized and non-parasitized nymphs of *Pteronarcys biloba*, collected in late May and early November 1997 from Catamaran Brook, New Brunswick. Values are averages (+1 SD) for each reach (pooled across dates). Sample sizes are shown above the error bars.

slightly more enriched at  $-31\text{‰}$  to  $-26\text{‰}$ . Because  $\delta^{13}\text{C}$  values are used to elucidate primary energy sources, values for *P. biloba* indicate that it obtained most of its energy from terrestrial leaf litter as opposed to aquatic plants, which in Catamaran Brook are usually more depleted ( $-35\text{‰}$  to  $-31\text{‰}$ ) than allochthonous inputs (R. R. Doucett, unpublished data). Furthermore, trophic enrichment occurs in such a way that animals at the base of food chains possess lower  $\delta^{15}\text{N}$  values than animals that feed at higher levels (Minagawa and Wada 1984). *Pteronarcys biloba* in our study had  $\delta^{15}\text{N}$  values between  $-0.5\text{‰}$  and  $+4.0\text{‰}$ . These  $\delta^{15}\text{N}$  values were

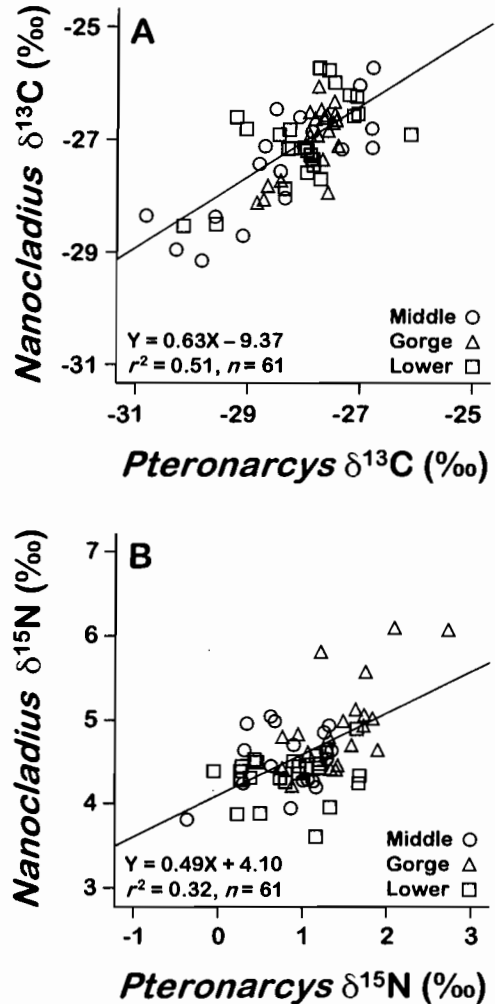


FIG. 4. Linear relationship between  $\delta^{13}\text{C}$  (A) and  $\delta^{15}\text{N}$  (B) of *Nanocladius* (*Plecopteracoluthus*) sp. and its host, *Pteronarcys biloba*, collected in late May and early November 1997 from Catamaran Brook, New Brunswick (samples pooled across date and location). Model-2 regression equations and coefficients of determination ( $r^2$ ) are also given for each relationship. Sample size ( $n$ ) is 61 because 23 of the 84 *P. biloba* nymphs collected did not host enough chironomids to reach the mass required for stable-isotope analyses. Sampling locations as in Fig. 1.

more enriched than those of leaf litter ( $-1\text{‰}$  to  $+1\text{‰}$ ), but were more depleted than most other benthic invertebrates and fish (Doucett et al. 1996) sampled at the same sites.

Although intraspecific differences in stable-isotope ratios have been previously correlated

TABLE 2. Stable-isotope ratios ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) of late-instar chironomids collected in late May 1997 from the Lower Reach of Catamaran Brook, New Brunswick. Values are means  $\pm$  1 SD, with sample sizes in parentheses. Different superscripts represent means that are significantly different from one another (Tukey's HSD,  $p < 0.05$ ).

Genus	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
<i>Ablabesmyia</i>	$-27.6 \pm 0.7$ (8) <sup>a</sup>	$3.4 \pm 0.3$ (8) <sup>c</sup>
<i>Eukefferiella</i>	$-30.3 \pm 1.0$ (5) <sup>b</sup>	$1.5 \pm 0.7$ (5) <sup>a</sup>
<i>Heterotrissocladius</i>	$-28.7 \pm 2.7$ (6) <sup>ab</sup>	$2.0 \pm 0.5$ (6) <sup>ab</sup>
<i>Micropsectra</i>	$-27.2 \pm 0.7$ (4) <sup>a</sup>	$3.1 \pm 0.2$ (4) <sup>bc</sup>
<i>Microtendipes</i>	$-27.2 \pm 0.5$ (14) <sup>a</sup>	$2.5 \pm 1.0$ (14) <sup>b</sup>
<i>Nanocladius</i> ( <i>Plecopteracoluthus</i> ) sp.	$-27.1 \pm 0.8$ (29) <sup>a</sup>	$4.6 \pm 0.5$ (29) <sup>d</sup>

with environmental stress (Ambrose and DeNiro 1987, Hobson et al. 1993), differences in *P. biloba*  $\delta^{15}\text{N}$  values do not appear to be related to parasitism by *Nanocladius* (*P.*) sp. The most  $^{15}\text{N}$ -enriched *P. biloba* in our study were non-parasitized individuals from the Upper Reach. Parasitized stoneflies were not isotopically distinct from non-parasitized ones. Differences in *P. biloba*  $\delta^{15}\text{N}$  (and  $\delta^{13}\text{C}$ ) may be a result of: 1) dif-

ferences in the stable-isotope ratios of leaf litter at the 4 stream reaches, 2) variable assimilation of attached algal material, or 3) varying degrees of facultative omnivory. Pteronarcids are thought to be large particle detritivores and important consumers of leaf material in headwater streams (Cummins 1974). Recent evidence suggests that pteronarcids may ingest some algae (Freilich 1991), and that the relative importance

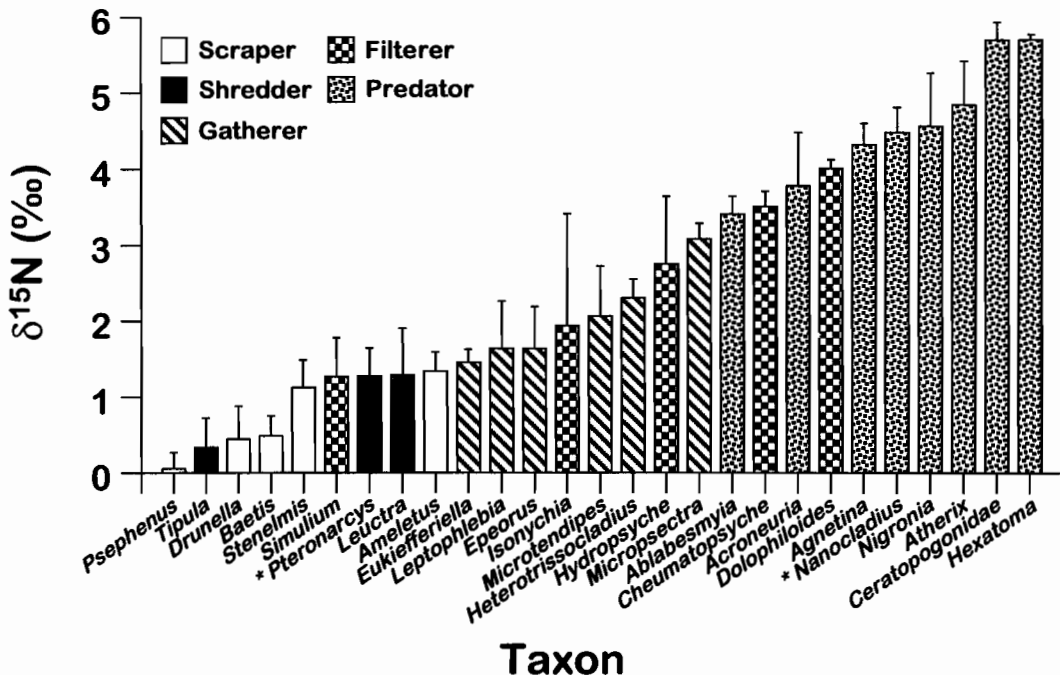


FIG. 5. Stable-nitrogen-isotope ratios ( $\delta^{15}\text{N}$ ) of late-instar benthic invertebrates sampled from run-riffle habitats in the Lower Reach, Catamaran Brook, New Brunswick, in late May 1997. Values are averages  $\pm$  1 SD. Sample sizes ranged from 3 to 20 replicates. Functional-feeding groups were designated according to descriptions in Merritt and Cummins (1996). Trophic patterns were similar at other sites and on other dates (R. R. Doucett, unpublished data). Asterisks denote the 2 main study taxa, *Pteronarcys biloba* and *Nanocladius* (*Plecopteracoluthus*) sp.

of autochthonous sources may increase with stream size (Plague et al. 1998). Gut analyses also have shown that pteronarcids may ingest animal material along with their detrital diet (Freilich 1991). More information on *P. biloba* gut contents and leaf litter composition along Catamaran Brook would help to clarify this issue.

#### *Nanocladius trophic position*

Isotopic comparisons with other benthic invertebrates clearly showed that *Nanocladius* (*P.*) sp. were functioning as parasites and not as detritivores. *Nanocladius* was isotopically more enriched than all other chironomids including the predator *Ablabesmyia* (Merritt and Cummins 1996). The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of other chironomids suggested that they were feeding on detritus, or fine particulate organic matter, which in Catamaran Brook had stable-isotope ratios of  $-28\text{‰}$  to  $-27\text{‰}$  and  $-1\text{‰}$  to  $0\text{‰}$  for carbon and nitrogen, respectively (R. R. Doucett, unpublished data). *Eukiefferiella* was significantly more depleted ( $-30\text{‰}$ ) than all other chironomids, which indicates that it grazed on algae and consumed detrital material. Finally, *Nanocladius* (*P.*) sp. were among some of the most  $^{15}\text{N}$ -enriched invertebrates sampled in this study, with  $\delta^{15}\text{N}$  values that were more similar to those of the predators, *Agnatina* (Plecoptera), *Atherix* (Diptera), and *Nigronia* (Megaloptera) than to those of invertebrates with herbivorous diets.

In conclusion, it was encouraging to observe field data that conformed to the isotope fractionation factors obtained previously by others in the laboratory. Many studies using SIA to determine feeding relations among aquatic organisms presume that trophic enrichment between diet and animal is  $0\text{‰}$  to  $1\text{‰}$  for  $\delta^{13}\text{C}$  and  $3\text{‰}$  to  $5\text{‰}$  for  $\delta^{15}\text{N}$  because it is often too difficult to obtain exact measurements of diet-animal isotope fractionation in the field. The validity of this underlying assumption is crucial to the success of SIA in ecology, but has generally gone untested, prompting a call by some (Gannes et al. 1997) to retreat to the laboratory for further testing of isotope patterns and dietary relations. Parasitic associations are ideal situations in which to test and confirm trophic enrichment of stable isotopes because the diet of the parasite is known and the fractionation factors can be measured accurately. Enrichment of  $+1.2\text{‰}$  for  $\delta^{13}\text{C}$  and  $+3.5\text{‰}$  for  $\delta^{15}\text{N}$  between *P. biloba* and

*Nanocladius* (*P.*) sp. demonstrated that at least 1 of the many tenets of isotopic ecology appears to hold true in field situations.

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