Density-weighted $\delta^{13}C$ analysis of detritivory and algivory in littoral macroinvertebrate communities of boreal headwater lakes

Robert L. France

France, R. L., Harvard University, Graduate School of Design, 48 Quincy St., Cambridge, MA 02138, USA

Investigations of the incorporation of terrestrial detritus into aquatic macroinvertebrates through $\delta^{13}C$ analysis are becoming frequent for streams and wetlands, but comparatively little information exists for forest-fringed oligotrophic lakes. Although the most accurate assessment of community patterns in carbon dependency will be made through an organism density-weighted analysis of $\delta^{13}C$, this has never previoulsy been undertaken for any freshwater system. Littoral macroinvertebrates (predominantly amphipods, ephemeropterans and dipterans, as well as odonates and trichopterans) from boreal lakes in northwestern Ontario, Canada displayed ranges of 6‰ to 9‰ in $\delta^{13}C$, all centred about –26‰. The closer agreement between the density-weighted $\delta^{13}C$ distribution for these macroinvertebrates to tree rather than epilithon values, suggests that these organisms may be relying more substantially upon allochthonous detritivory than upon autochthonous algivory for energy sustenance. This finding therefore challenges the precept in some timber management guidelines that dismisses riparian trees as an important energy source for lake foodwebs.

1. Introduction

Coastal researchers have used stable carbon isotope ratios ($^{13}C/^{12}C$ expressed as $\delta^{13}C$) to investigate the possibility of incorporation of vascular plant detritus (either seagrasses or emergent macrophytes) into benthic organisms (reviewed in France 1996a). Similarly, freshwater scientists have also used this technique to measure the assimilation of riparian detritus by aquatic animals (reviewed in France 1996b). A difficulty with all such studies, however, is their attempt to assess detritivory solely from the perspective of the species diversity of $\delta^{13}C$ values rather than, or in addition to, an organism abundance-weighted analysis which may more accurately gauge system-wide patterns of carbon flow. For example, although many more benthic species appear to rely upon attached algae than upon vascular plants for energy sustenance, it is necessary to qualify such a conclusion: "Percentage frequency distributions of species $\delta^{13}C$ values fail to provide any indication about the relative differences which might exist in the contributions of attached algae and

macrophytes to the total carbon pool. It is entirely possible that there are situations wherein the proportionally greater productivity of macrophytes will provide an enormous food base to certain herbivores which are capable of exploiting this abundant food supply. In such cases, the overall consumer productivity would be predominantly macrophyte-based even though on a species-by-species basis, attached algae still support the greater share of the diversity of all animals present" (France 1996a). Indeed, a subsequent $\delta^{13}C$ investigation of food source provenance for benthic animals in Boston Harbour (R. L. France and M. Chandler, unpubl.) supported this contention: although only one of 35 sampled species demonstrated exclusive use of seagrass carbon, due to the overwhelming dominance of this single species, 80% of the carbon food base for the entire macroinvertebrate community was derived from vascular, rather than epiphytic algal, material.

Through the same reasoning, Doucett *et al.* (1996) recently advocated that the best means for gauging the importance of carbon flow from terrestrial to freshwater environments should also be based on such an abundance-weighted $\delta^{13}C$ analysis. The present study takes advantage of the simultaneous estimation of macroinvertebrate density and determination of $\delta^{13}C$ values in order to generate, for the first time, a density-weighted comparative assessment of detritivory and algivory for a freshwater macroinvertebrate community.

2. Description of study lakes and previous stable isotope analyses

Macroinvertebrates were sampled from the littoral zones of four proximally situated, oligotrophic boreal lakes in north-

Table 1. Lake-specific proportional densities (%) obtained from France (1997a).

	L039	L026	L020	L042	Mean
Amphipoda	28	55	46	34	41
Ephemeroptera	19	27	44	23	28
Diptera	47	3	2	40	23
Trichoptera	2	4	2	2	3
Odonata	3	3	2	1	2
Other*	3	8	6	2	5

* Gastropoda, Turbellaria, Coleoptera, Hydracarinida, Pelecypoda

western Ontario, Canada. Precise lake location and study area description are contained in France *et al.* (1998). At the time of this study, these lakes were surrounded by dense coniferous forests that supplied 34 g (m shoreline)$^{-1}$ yr^{-1} of litterfall to the littoral zones. Upon abiotic leaching and microbial conditioning, this litter was actively colonized by a variety of macroinvertebrates for both habitat and food-related purposes due to the rarity of vascular macrophytes and filamentous algae in these lakes. Further limnological details are contained in Steedman *et al.* (1998a, 1998b).

Amphipods (*Hyallela azteca*) dominate these littoral macroinvertebrate communities, with an average proportional representation of 41% (Table 1; France 1997a), as is generally characteristic of circumneutral Canadian Shield lakes. Likewise, the proportional representation of other sampled taxa — 28% for ephemeropterans, 23% for dipterans, 2% for odonates, 3% for trichopterans and 5% for "others" (gastropods, oligocheates, turbellarians, coleopterans, hydracarinids, pelecypods) — is similar to other Shield lakes sampled with identical methods (*see* papers cited in France 1997a).

Due to the influence of water motion on boundary layer diffusion resistance (Hecky & Hesslein 1995), benthic algae become enriched in ^{13}C under situations of low turbulence, such that they exhibit higher $\delta^{13}C$ values than planktonic algae, thereby enabling differentiation between pelagic and benthic foodwebs. For the present study lakes, this resulted in, with the notable exception of lake trout which move between the open-water and inshore regions (France and Steedman 1996), a near complete separation in the $\delta^{13}C$ values for pelagic and benthic animals due to an uncoupling of carbon flows (France 1995).

Closer examination of the isotopic ecology of tadpoles, littoral fishes (France 1997b), and crayfish (France 1996c) in the study lakes revealed a mixed dependence on both epilithic algae and terrestrial detritus. This was made possible because, unlike the situation in many aquatic systems, the potential autochthonous and allochthonous food sources were distinct enough in their $\delta^{13}C$ values (France 1995) so as to successfully enable such a comparative assessment of the relative importance of detritivory and algivory. The present study contains $\delta^{13}C$ data for macroinvertebrates from these study lakes. Of these data, 46% (89 of 189 measurements) are new; the remaining 53% have been previously published, though in only a non-identified form combined anonymously with fish. crayfish and tadpoles (France 1995, 1996d). This paper therefore represents a completely new interpretation of the relative importance of detritus or algae food provenance for these particular types of organisms.

3. Methods

Macroinvertebrates were sampled during the ice-free months of 1992–1995 with use of a hand-held corer, a metal dip-screen and terrestrial litter colonization bags. After allowing time for gut clearance, samples were acid-washed, rinsed and hand-cleaned to remove inorganic and organic contami-

nants. Whole organisms were ground to a fine powder and samples stored frozen in tin combustion capsules.

Samples from 1992–1994 (n = 100) were analyzed for carbon isotopes on a Europa tracermass mass spectrometer interfaced with a Roboprep-CN analyzer which, following weight related bias-correction (G. Cabana and J. Rasmussen, unpubl.) produced an average SD of $\pm$ 0.3‰ for standards. Samples were run in 12 sequences of 5 per tray, each sequence being separated by paired standards (i.e. a blank to clear the combustion tube followed by an identical reference) with duplicates or triplicates never occurring in the same run sequence. Analyses for new samples collected in 1995 (n = 89), were run on a VG Micromass 903E triple-collector mass spectrometer (Univ. of Waterloo) with corresponding weight-related bias correction. Importantly, quality checks showed that duplicate $\delta^{13}C$ determinations of the same material on either machine did not differ. Stable carbon isotope values were not adjusted for variable trophic positions among different macroinvertebrates because ^{13}C-enrichment is very low in freshwater foodwebs in general (France and Peters 1997), and may be absent in these benthic foodwebs in particular (France 1996d).

Depending on the body mass of macroinvertebrate taxa, different individuals (same time, same location) were combined to provide 2–4 mg samples for replicated analyses. Because neither lake nor season differences in $\delta^{13}C$ were observed (ANOVA, Duncan's Multiple Range tests, p-values > 0.05), samples were pooled for a single composite average analysis as in previous studies in these proximally located, limnologically nearly indistinguishable basins (i.e. these particular study lakes were selected after a rigorous screening process so as to be as similar as possible, several basins serving as references for others which later had their watersheds experimentally clearcut; Steedman *et al.* 1998a). Between 18 and 40 individual $\delta^{13}C$ determinations were made per inter-lake composite taxon grouping. With the exception of the agglomerate "other" category, macroinvertebrate species (usually a 2 to 4 species subsample from the total of 5 to 6 species per Order found to exist in these lakes) were grouped together only if: (a) identified individuals were of the same functional feeding-guild as determined from gut content data and stable nitrogen isotope analysis of relative trophic position, and (b) no statistical differences existed in their separate $\delta^{13}C$ values (ANOVA, Duncan's Multiple Range tests, p-values > 0.05) before pooling.

The density-weighted frequency distributions of $\delta^{13}C$ for macroinvertebrates were produced by multiplying the proportional distributions of $\delta^{13}C$ values determined for each taxon by the average proportional density of each of the various taxa within the total sampled zoobenthos communities (Table 1) from France (1997a).

4. Results and discussion

As observed previously in these study lakes for crayfish due to systematic ontogenetic diet shifts (France 1996c), and for tadpoles and littoral fishes due to simple idiosyncratic diet plasticities (France 1997b), the macroinvertebrate taxa examined here were found to display wide ranges (6‰ to 9‰) in $\delta^{13}C$, either grouped together as an inter-lake composite (Fig. 1) or when examined lake-specifically (Fig. 2). This $\delta^{13}C$ variability occurred irregardless of whether the taxa groupings were composed of individuals from multiple Orders (as for "other" = gastropods, oligochaetes, turbellarians, coleopterans, hydracarinids and peleypods), several species within a single Order having a similar feeding-guild and trophic position (as for trichopterans, emphemeropterans, odonates or dipterans), or even for only a single species (as for *Hyalella azteca* amphipods). Because most studies are based on only a single, or at best, a few $\delta^{13}C$ measurements per species or taxa grouping, these findings seriously question the concept common in the literature of ascribing precise isotopic fidelities, often referred to with the phrase "signature" in an attempt to give an impression of limited diet plasticity, thereby justifying the small sampling program used.

The density-weighted assessment of community $\delta^{13}C$ for macroinvertebrates in the present boreal lakes was found not to differ substantially from what the frequency distribution of the combined taxa $\delta^{13}C$ diversities would indicate (Fig. 3). This occurred because all 6 of the taxa groupings examined in this study displayed uniform modal $\delta^{13}C$ values of –26‰ (Fig. 1). In other systems such as Boston Harbour (R. L. France and M. Chandler, unpubl.), density-weighted distributions of $\delta^{13}C$ may be very different from those based on the simple species diversity ascription of values.

Fifteen percent of all sampled macroinvertebrates had $\delta^{13}C$ values synonymous with terrestrial detritus, whereas one percent of the organisms had $\delta^{13}C$ values indicative of epiphyton (Fig. 1). Clearly, as also found for crayfish, tadpoles and fishes in these same lakes (France 1996c, 1997b), the majority of macroinvertebrates displayed neither exclusive detritivory nor algivory, but instead some mixture of the two. The overall closer agreement between the community density-weighted $\delta^{13}C$ distribution to that of terrestrial rather than epilithon values, implies, however, that detritivory is probably the more important avenue of food provenance in this particular system.

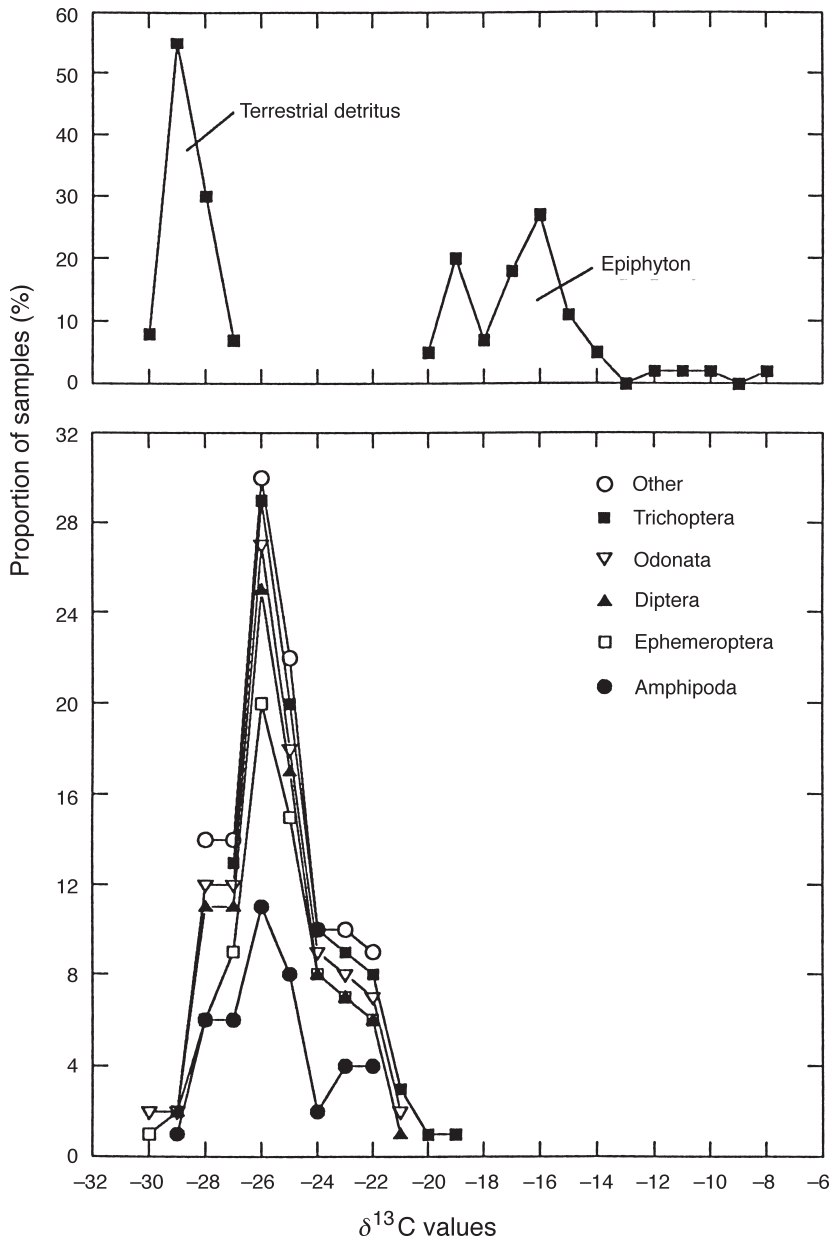Rau (1980) found that the total carbon derived from terrestrial sources in adult insects emerging

Fig. 1. Proportional distributions of $\delta^{13}C$ for terrestrial detritus, epiphyton and littoral macroinvertebrates composited from four oligotrophic boreal lakes in northwestern Ontario, Canada. Autotroph values have been published previously as means ± SDs in France (1997b) and as box and whisker plots in France (1995). About half of the $\delta^{13}C$ values for macroinvertebrates have appeared anonomously combined with fishes, crayfish and tadpoles in France (1995) and France (1996d); the other half of the data are new to this analysis, as are the present taxa ascriptions of $\delta^{13}C$ ranges.

from a subalpine lake was 47% for chironomids, 51%–87% for two types of limnephilids, and 0% for an emphemeropteran which relied exclusively on periphyton. Clearly, as Rau (1980) and the present study suggest, allochthonous detritivory can be a major source of energy sustenance for certain insects and other littoral macroinvertebrates inhabiting clearwater oligotrophic lakes surrounded by coniferous forests.

Fry *et al.* (1986) reviewed the $\delta^{13}C$ literature on seagrass communities and posited that the relative importance of vascular plant detritus may increase in low productivity systems where algal productivity is low. Similarly, because the productivity of epilithic algae in oligotrophic boreal lakes in northwestern Ontario is very low (Schindler *et al.* 1973), benthic animals there may be more likely to rely upon terrestrial detritus for part of their sustenance than is the case for animals residing in tropical locations (Araujo-Lima *et al.* 1986, Forsberg *et al.* 1993, Hamilton *et al.* 1992, France 1998) where algal productivity is higher
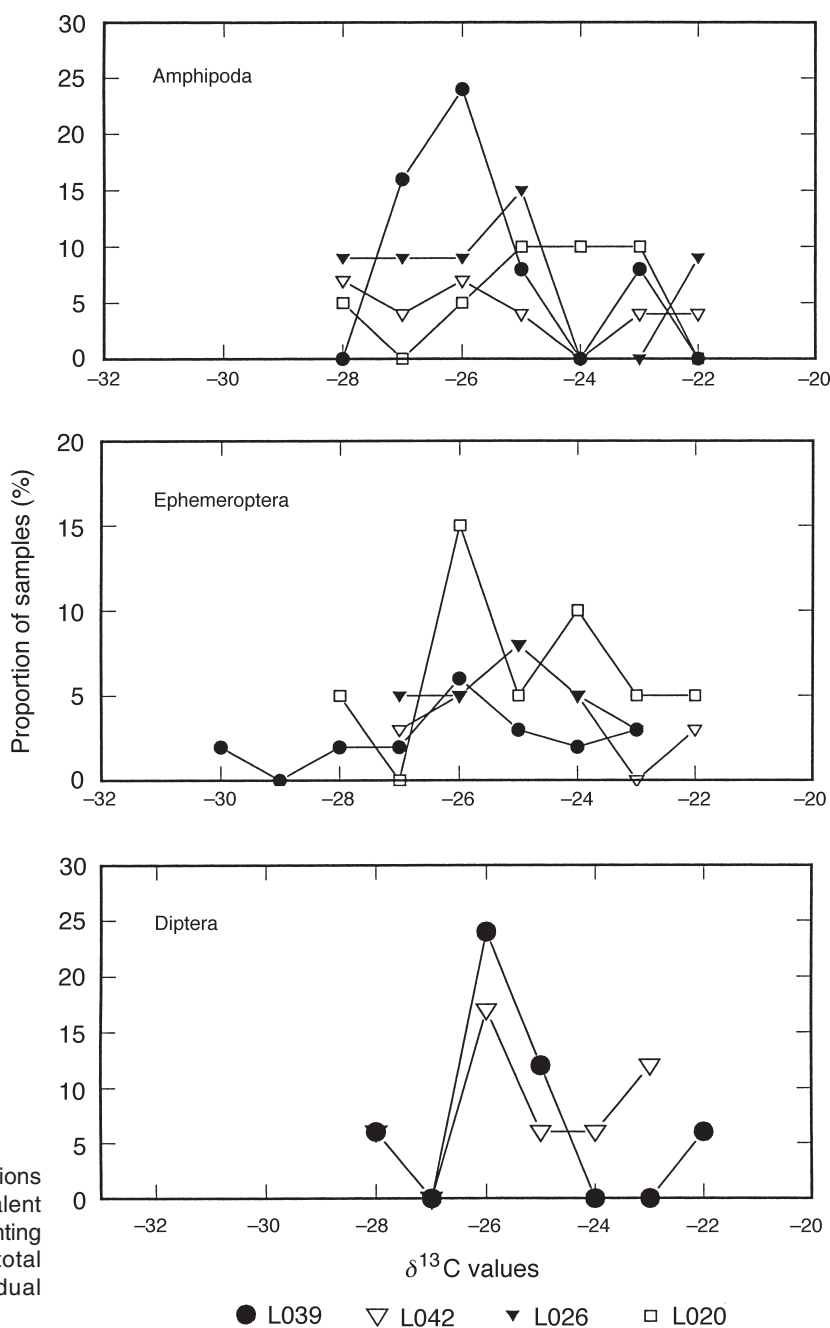
Fig. 2. Proportional distributions of $\delta^{13}C$ for the most prevalent macroinvertebrates representing greater than 25% of the total fauna (Table 1) in individual study lakes.

(Kalff 1991).

The present results support previous isotope findings for other organisms in demonstrating that littoral foodwebs in boreal headwater lakes in northwestern Ontario, Canada may be tightly coupled to their surrounding forests in terms of carbon flow. This has important managerial impli-

cations for regulating the methods of clearcutting in this region. For example, the present day timber management guidelines for Ontario state: "Because of their larger area lakes are not as dependent on adjacent vegetation as a food source as are streams. Much of the energy available to the food chain of lakes is fixed by phytoplankton and mac-
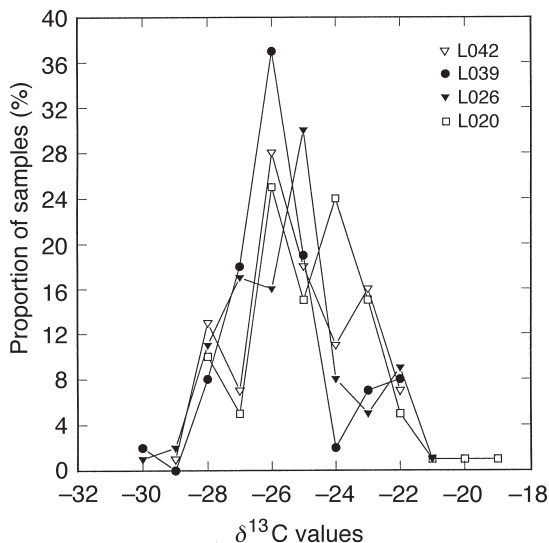
Fig. 3. Proportional distributions of δ^{13}C for the total macroinvertebrate communities in each of the four study lakes.

rophytes through photosynthesis. Hence the value of nearby timber for this purpose is not great ..." (Ont. Min. Natur. Resour. 1988). Therefore, because of the assumption that the input of detritus from riparian forests is relatively unimportant as a food resource to lake consumers, clearcutting is often permitted right to the lakeshore edge in Ontario (France *et al.* 1996, Steedman *et al.* 1998b). The present findings suggest that the justification for these current riparian logging guidelines may be in need of review.

References

Araujo-Lima, C., Forsberg, B. R., Victoria, R. & Mortinelli, L. 1986. Energy sources for detritivorous fishes in the Amazon. — Science 234: 1256–1258.

Doucett, R. R., Barton, D. R., Guiguer, K. R. A., Power, G. & Drimmie, R. J. 1996. Comment: critical examination of stable isotope analysis as a means for tracing carbon pathways in stream ecosystems. — Can. J. Fish. Aquat. Sci. 53: 1913–1915.

Forsberg, B. R., Araujo-Lima, L., Martinelli, L. A., Victoria R. L. & Bonassi, J. A. 1993. Autotrophic carbon sources for fish of the central Amazon. — Ecology 74: 643–652.

France, R. 1995. Differentiation between littoral and pelagic foodwebs in lakes using stable carbon isotopes. — Limnol. Oceanog. 40: 1310–1313.

France, R. 1996a. Stable isotopic survey of the role of macrophytes in the carbon flow of aquatic foodwebs. — Vegetatio 124: 67–72.

France, R. L. 1996b. Carbon-13 conundrums: limitations and cautions for use of stable isotopes in ecotonal stream research. — Can. J. Fish. Aquat. Sci. 53: 1916–1919.

France, R. L. 1996c. Ontogenetic shift in crayfish δ^{13}C as a measure of land-water ecotonal coupling. — Oecologia 107: 239–242.

France, R. L. 1996d. Absence or masking of metabolic fractionations of ^{13}C in a freshwater benthic food web. — Freshw. Biol. 36: 1–6.

France, R. L. 1997a. The importance of beaver lodges in structuring littoral communities in boreal headwater lakes. — Can. J. Zool. 75: 1009–1013.

France, R. L. 1997b. Stable carbon and nitrogen isotopic evidence for ecotonal coupling between boreal forests and fishes. — Ecol. Freshw. Fish. 6: 78–83.

France, R. L. 1998. Estimating the assimilation of mangrove detritus by fiddler crabs in Laguna Joyuda, Puerto Rico, with use of dual stable isotopes. — J. Trop. Ecol. 14: 413–425.

France, R. L. & Peters, R. H. 1997. Ecosystem differences in the trophic enrichment of ^{13}C in aquatic foodwebs. — Can. J. Fish. Aquat. Sci. 54: 1255–1258.

France, R. & Steedman, R. 1996. Energy provenance for juvenile lake trout in small Canadian Shield lakes shown by stable isotopes. — Trans. Amer. Fish. Soc. 125: 512–518.

France, R., Culbert, H. & Peters, R. 1996. Decreased carbon and nutrient input to boreal lakes from particulate organic matter following riparian clearcutting. — Environ. Manag. 20: 579–583.

France, R., Peters, R. & McCabe, L. 1998. Spatial relationships among boreal riparian trees, litterfall and soil erosion potential with reference to buffer strip management and coldwater fisheries. — Ann. Bot. Fennici 35: 1–9.

Fry, B., Macko, S. A. & Zieman, J. C. 1986. Review of stable isotopic investigations of food webs in seagrass meadows. — Florida Mar. Res. Publ. 42: 189–209.

Hamilton, S. K., Lewis, W. M. & Sippel, S. J. 1992. Energy sources for aquatic animals in the Orinoco River floodplain: evidence from stable isotopes. — Oecologia 89: 324–330.

Hecky, R. E. & Hesslein, R. H. 1995. Contributions of benthic algae to lake food webs as revealed by stable isotope analysis. — J. North Amer. Benthol. Soc. 14: 631–653.

Kalff, J. 1991. The utility of latitude and other environmental factors as predictors of nutrients, biomass and production in lakes worldwide: problems and alternatives. — Verh. Verien. Internat. Limnol. 24: 1235–1240.

Ontario Ministry of Natural Resources. 1988. Timber

management guidelines of the protection of fish habitat. — Ontario Ministry of Natural Resources, Toronto, Ontario. 14 pp.

Rau, G. H. 1980. Carbon-13/carbon-12 variation in subalpine lake aquatic insects: food source implications. — Can. J. Fish. Aquat. Sci. 37: 742–746.

Schindler, D. W., Frost, V. E. & Schmidt, R. V. 1973. Production of epilithiphyton in two lakes of the Experimental Lakes Area, northwestern Ontario. — J. Fish. Res. Bd. Can. 30: 1511–1524.

Steedman, R. J., Kushneriuk, R. S. & France, R. L. 1998a. Initial rsponse to catchment deforestation of small boreal forest lakes in northwestern Ontario, Canada. — Verh. Verein. Internat. Limnol. [In press].

Steedman, R., France, R., Peters, R. & Kushneriuk, R. 1998b. Effects of riparian deforestation on littoral water temperatures in small boreal forest lakes. — Boreal Environ. Res. 3: 161–169.