THE INFLUENCE OF FLUCTUATING RAMPING RATES ON THE FOOD WEB OF BOREAL RIVERS

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ABSTRACT

A BACI (before-after-control-impact) sampling design was applied to determine the possible effects of ramping rate (RR) regulation on food webs structure and function in a regulated boreal river. We used carbon and nitrogen stable isotope signatures of primary producers, macroinvertebrates and fish to determine variations in the source of carbon fuelling the food web as well as changes in the food web structure under variable RR flow regime. We hypothesized that unrestricted RR would (1) increase the connectivity between terrestrial and aquatic environments allowing for a higher contribution of terrestrial carbon to support the food web and (2) decrease food web length because of frequent disturbances. Unrestricted RR had little influence on δ^{13}C values for the overall food web with most of the differences found between impacted sites compared and control sites, indicating that the proportion of various carbon sources entering the diet of consumers remained unchanged under unrestricted RR. In contrast, significantly higher δ^{15}N values were measured in impacted sites (invertebrates and fish) and as well as under unrestricted ramping flow regime (invertebrates). Further, unrestricted RR was associated to a significant decrease in the difference between macroinvertebrates and fish δ^{15}N signatures, equivalent to a reduction of the length of the food web by at least one trophic level. Results from this study indicate that RR should be taken into consideration in the regulation of operating regimes on rivers. Copyright © 2008 John Wiley & Sons, Ltd.

KEY WORDS: food web; ramping rate fluctuation; regulated rivers; stable isotopes

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INTRODUCTION

Over the last decade, predicting the response of aquatic organisms to flow regulation has become a key issue for the development of sustainable management practices in regulated rivers aiming to maintain biological integrity. In lotic ecosystems, the loss of the natural flow regime has been identified as a major threat to riverine biota (Poff *et al.*, 1997). Identifying the effects of flow alteration is particularly important for hydropower utilities because of the reliance on pulse power generation applied to meet peak energy demands and adapt to variable energy prices (Morrison and Smokorowski, 2000). During hydropeaking operations, which involves generating power to correspond with peak electricity demand, water stored in a reservoir is released over a short time period, resulting in the alteration of several hydrological characteristics of downstream flow, including magnitude, duration, timing, rate of change (ramping rate (RR)) and frequency of changes in flow (Magilligan and Nislow, 2005; Arthington *et al.*, 2006). The complexity surrounding flow has limited our ability to predict the biological effects of its alteration. As a consequence, the functional response of the food web to changes in flow remains unclear (Power *et al.*, 1996), with most studies relying on untested hypotheses when describing the functioning of rivers under different flow regimes (Bunn and Arthington, 2002). Consequently, regulation practices for hydropower utilities are not well defined, although the requirement to restore a natural flow regime based on 'run-of-river' operation is becoming more common (Jager and Bevelhimer, 2007). Nevertheless in most cases, dams are still operated to optimize energy revenue (Jager and Smith, 2007) and there remains a growing need for the development of

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ecological tools to help water resource managers predict and quantify the biotic responses to altered flow regimes (Bunn and Arthington, 2002).

A few studies have reported on the effects of hydropeaking on fish behaviour and habitat, noting increased movement activity and higher stranding susceptibility under rapid flow decreases (Bradford, 1997; Scruton *et al.*, 2003; Scruton *et al.*, 2005) and no measureable impacts on growth as a result of short-term experimental exposures to fluctuating water levels (Flodmark *et al.*, 2004, 2006). Fish, however, are mobile organisms and may be better able to adapt to a fluctuating environment when compared to more sessile lower trophic biota. For example, fluctuating discharge in the regulated Colorado River was responsible for a drastic decrease in algal biomass (Blinn *et al.*, 1995) and similar trends were reported for macroinvertebrate abundance under flow reductions in New Zealand streams as a result of associated habitat loss and decline in food resources (James *et al.*, 2008). Little other detailed documentation of biotic effects of altered flows exists, with virtually no studies having addressed the more subtle issues of changes in food web structure or function under high ramping flow regimes.

In this study, we compare characteristics of the entire food web obtained under natural, restricted and unrestricted RR flow regimes, using carbon and nitrogen stable isotopes (δ^{13}C and δ^{15}N). Stable isotope analysis (SIA) represents a powerful tool to study the transfer of mass (δ^{13}C) and energy (δ^{15}N) through the food web (Fry and Sherr, 1984). In flowing waters, carbon stable isotopes are particularly relevant for tracking the effect of physical variables because the baseline δ^{13}C signature of the food web (algae) is influenced by water velocity via boundary layer effects (Finlay *et al.*, 1999; Trudeau and Rasmussen, 2003). The transfer of carbon to higher trophic levels of the food web involves little fractionation which allows δ^{13}C to be used as a tracer for diet composition (Fry and Sherr, 1984; France, 1996). In flowing waters, δ^{13}C has been applied to determine the relative contribution of algal versus terrestrial carbon sources to the diet of consumers (McCutchan and Lewis, 2002; McNeely *et al.*, 2007). Coupled with δ^{13}C, δ^{15}N provides further insight on the trophic structure of the food web because the δ^{15}N values increase predictably from a food source to a consumer (Post, 2002). Thus we hypothesized that the stable isotope signatures of organisms would be related to RR variations. Specifically, we first hypothesized that a high RR flow regime would be associated with an alteration of food web function through modification of the relative contribution of terrestrial versus algal carbon sources to the food web. Resulting from regular water level fluctuations, the increase in connectivity between aquatic and terrestrial biomes could favour a stronger reliance of the aquatic food web on terrestrial subsidy. Secondly, we hypothesized an alteration of food web structure most notable in a reduction of food web length under high RR flow regime. Disturbances are generally related to an alteration of river structure and function (Sabater, 2008), with flow regulation reducing diversity and further affecting predator-prey interactions in food webs (Power *et al.*, 1996; Bunn and Arthington, 2002).

STUDY SITES AND METHODS

Study sites

Three study sites were selected on each of the unregulated Batchawana River (BR) and on the regulated Magpie River (MR) (Figure 1). Both rivers were situated on the northeastern shore of Lake Superior and shared similar annual discharge (Figure 1) and morphology, with river beds made of granitic cobble with areas of gravel and sand as the main substrata. The rivers are typical of the oligotrophic ecosystems that dominate the Canadian Boreal Shield, with low total phosphorus (6.7/8.1 μg L^{-1}, for BR and MR, respectively) and algal biomass (measured as Chlorophyll *a*, 1.1/1.9 μg L^{-1}), and coloured waters (dissolved organic carbon, 7.4/7.5 mg L^{-1}).

Experimental design

On the BR, sample sites were distributed over a 30 km distance. On the regulated MR, the upper site (site 1) was not subject to flow regime changes due to its location 25 km upstream of the Steephill Falls Generating station. The upstream site on the MR was combined with the BR sites and served as controls in the experimental design. On the MR, sample sites 2 and 3 were situated downstream of the dam and were treated as impacted sites subject to flow regime changes. Variations in RRs (m^3 s^{-1} h^{-1}) were applied at the impact sites over a 4-year period. In 2003 and 2004 (before perturbation), the flow regime was subject to restrictions on RRs, which were limited to maximum
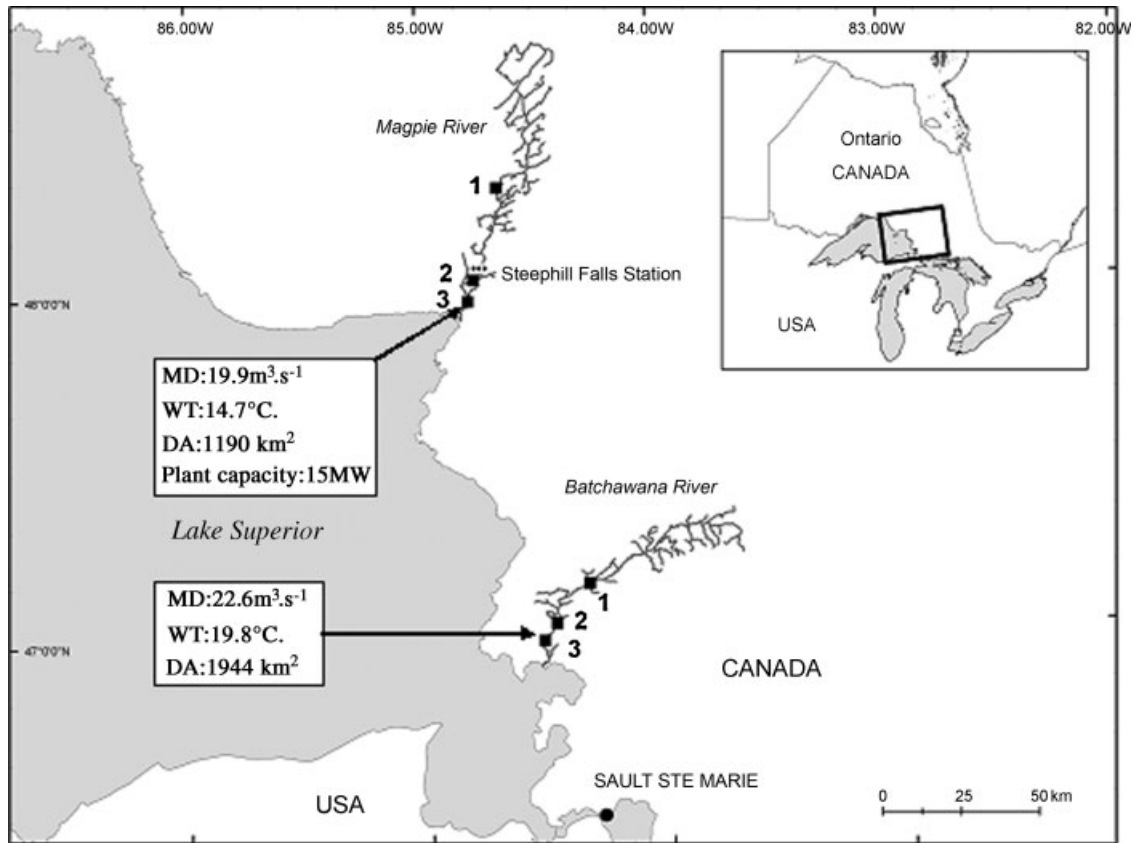
Figure 1. Location of sampling sites on the Magpie River and the Batchawana River marked as solid squares and placed in relation to Ontario on the inset map. MD: mean annual discharge ($m^3 s^{-1}$), WT: mean annual water temperature (°C.) and DA: drainage area (km^2)

changes of $1 m^3 s^{-1} h^{-1}$ from October to November, increasing to $2 m^3 s^{-1} h^{-1}$ from November to the spring freshet, and a maximum of 25% of the preceding hour's discharge from the spring freshet to October. The restriction period was followed by a 2 year (after perturbation, 2005 and 2006) period of unrestricted RR regimes where the operator was allowed to vary flows to meet peak power demands, provided a minimum base flow requirement was met. A summary of the experimental design applied in this study is presented in Figure 2.

Stable isotope analysis

To characterize the carbon and nitrogen stable isotope signatures of the base of the food web ($\delta^{13}C_{BASE}$ and $\delta^{15}N_{BASE}$), aquatic vegetation (epilithic algae, filamentous algae and occasionally macrophytes (*Potamogeton richardsonii*, *Ceratophyllum demersuman* and *Vallisneria americana*)) and primary consumers (snails and mussels) were collected in the spring (mid-June) and late summer (mid-August) of each year (2003–2006) of the experiment. Epilithic algae, sampled at each site (3 replicates) in summer 2006, were brushed from cobble and filtered (GF/C-Whatman). Due to low abundance, no separation was performed on these samples to extract algae from other detritus. Mats of filamentous algae and macrophytes were collected every year at each site when present, and placed in plastic bags. Aquatic invertebrates were collected using a surber sampler (mesh size 363 μm) and areas of cobble and gravel were vigorously disturbed by kicking with the net held downstream. Samples from riffles and pools were collected and pooled together to avoid potential effects of water velocity on carbon signatures. At shore, organisms were sorted according to main taxonomic groups or family (Table I). Fish were captured by electrofishing in the same habitats as for invertebrates, measured (fork-length) and sorted according to species (Table I). All samples were kept frozen until processing for SIA.
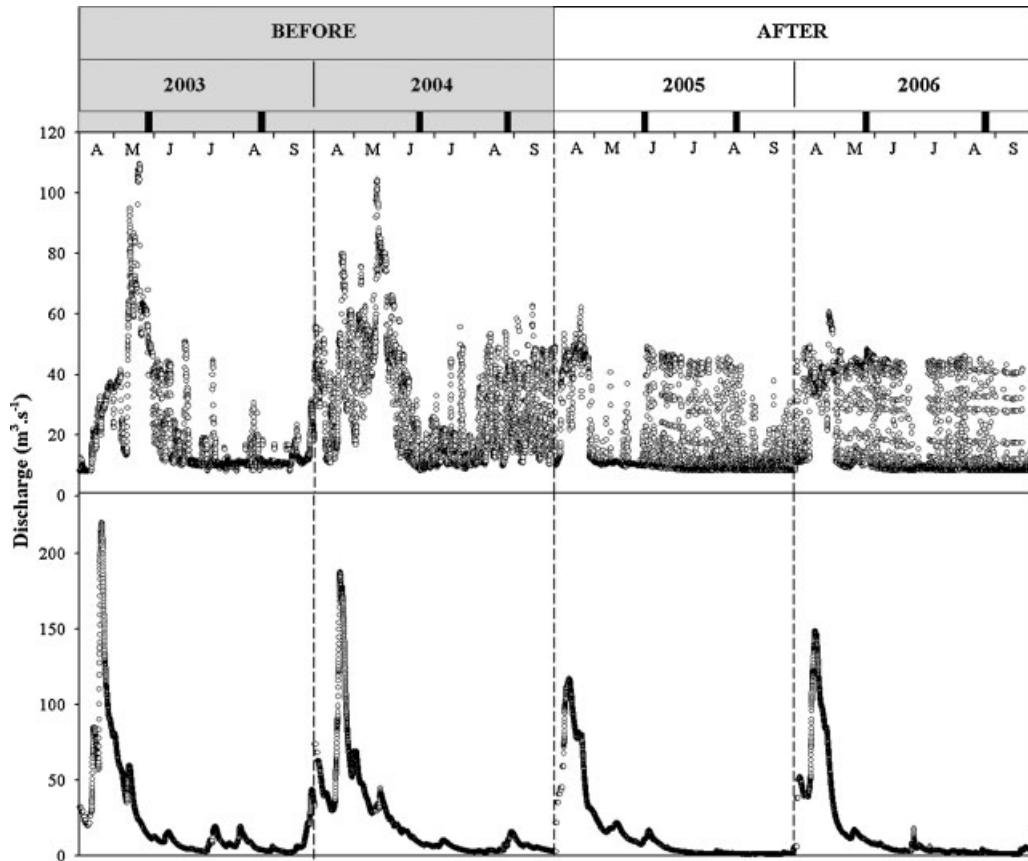
Figure 2. Impacted (top) and control (bottom) sites hydrographs in relation to BACI experimental design. Black bars indicate stable isotope sampling dates and letters refer to month of year

In the laboratory, samples from each taxon were dried at 50°C and ground into fine powder using a mixer mill (Retsch® MM301) or a mortar and pestle in the case of small sized invertebrate samples. The muscle tissue from up to 10 individuals covering the size range of each species found at a given site were analysed, whereas plant and invertebrate samples were analysed in triplicate. Lipids were not extracted in any of the samples because of consistent C/N ratios measured between taxa for invertebrates (mean ± SE: 4.7 ± 0.03) or over the size range for fish (mean ± SE: 3.8 ± 0.01). When lipid or C/N ratios for all samples are uniformly low, lipid extraction or normalization has little influence on measured $\delta^{13}C$ values (Post et al., 2007). In addition, none of the samples were acidified prior to combustion because of the relatively low concentration of inorganic carbonates in circumneutral Canadian Shield waters. All SIA were performed at the University of Waterloo-Environmental Isotope Laboratory on a Thermo Finnigan Mat Delta Plus Mass Spectrometer, coupled to a Carlo Erba Elemental Analyzer (NA1500). Results are given using in standard δ notation with $\delta = [(R_{sample}/R_{reference}) - 1] \times 1000$, expressed in parts per thousand (‰) and $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$ (Verardo et al., 1990). A secondary standard (cellulose) of known relation to the international Pee Dee Belemnite standard and atmospheric nitrogen were used as the reference material, respectively, for carbon and nitrogen. Precision on SI measurement was calculated as the standard deviation of signatures obtained from repeat analysis of a given sample and on average was 0.11 and 0.08‰, respectively, for $\delta^{13}C$ and $\delta^{15}N$.

Hydrology

Several sources of data for discharge were used to cover the 4-year time period of the experiment. For the upper site on the MR (site 1), we used data from an upstream station monitored by the Ontario Ministry of Natural

Table I. Main family or taxa analysed for SIA, with corresponding carbon and nitrogen signatures (%), according to BACI sampling design

| Common name | Family or taxa | $\delta^{13}C$ | | | | $\delta^{15}N$ | | | |
| | | C | | I | | C | | I | |
		B	A	B	A	B	A	B	A
Invertebrates									
Stonefly*	Perlidae, Pteronarcyidae, Capniidae	−28.9	−28.4	−34.3	−33.5	4.8	4.9	10.1	9.2
Caddisflies*	Limnephilidae, Hydropsychidae, Philopotamidae, Leptoceridae	−29.0	−28.2	−31.9	−33.3	3.4	3.8	5.5	8.0
Crayfish	*Cambarus bartonii*	−25.6	−25.4	−28.1	−28.4	5.6	5.4	8.9	8.9
Clams	*Elliptio complanata*	−30.7	30.8		−33.4	3.5	3.9		6.3
Damselflies*	Calopterygidae	−30.8	−28.0			6.5	5.1		
Dragonflies*	Macromiidae, Gomphidae, Aeshnidae, Petaluridae	−28.5	−27.9	−31.3	−30.6	5.2	5.2	7.9	7.9
Mayflies*	Heptageniidae, Baetidae, Isonychiidae, Baetiscidae	−29.4	−29.6	−33.6	−34.4	3.7	5.0	7.4	10.1
Snails*	*Lymnaea stagnalis, Promenteus exacuous*	−28.1	−27.1	−29.1	−29.6	4.1	3.5	6.1	6.2
Water bugs	Athericidae	−27.0		−27.9		4.0		6.9	
Water striders*	Gerridae	−26.7	−28.3	−28.9	−29.2	4.9	4.9	7.1	5.7
Water boatman	Corixidae	−32.9	−29.2	−30.9	−34.9	5.0	3.2	6.8	10.6
Crane flies	Tipulidae	−28.6	−28.4	−27.8	−33.0	3.6	4.8	4.4	9.1
Fish									
Brook trout*	*Salvelinus fontinalis*	−26.5	−26.2	−30.9	−30.4	7.7	7.0	10.7	11.0
Common white sucker*	*Catostomus commersonii*	−27.9	−24.0	−28.0	−28.6	7.1	7.0	9.5	9.5
Blacknose dace*	*Rhinichthys atratulus*	−25.2	−26.1			7.5	7.1		
Longnose dace*	*Rhinichthys cataractae*	−27.6	−27.5	−30.5	−30.3	7.0	7.1	9.8	10.0
Northern Redbelly Dace	*Phoxinus eos*	−29.5	−27.2		−30.2	7.8	7.9		7.3
Sculpin*	*Cottus ricei*	−27.3	−27.8	−32.2	−32.4	7.4	7.6	11.7	11.6
Trout perch*	*Percopsis omiscomaycus*	−23.8	−24.6	−30.0	−29.6	8.1	8.2	11.6	10.1
Logperch	*Percina caprodes*	−25.9	−28.4		−28.1	7.6	8.4		10.4
American brook lamprey	*Lampetra appendix*	−24.0	−25.5			3.0	2.9		
Burbot	*Lota lota*	−30.4	−29.4	−30.0	−28.8	8.5	9.1	10.8	11.5
Creek chub	*Semotilus atromaculatus*	−25.8	−25.5			7.5	7.3		
Lake chub	*Couesius plumbeus*	−25.4	−25.9	−29.2	−28.9	6.9	7.5	10.0	9.5
Iowa darter	*Etheostoma exile*	−30.5	−28.5	−26.8	−28.2	10.5	10.6	7.7	8.5
Five spine stickleback	*Culaea inconstans*	−30.0	−28.8	−30.0	−28.7	8.0	8.0	10.2	9.6

*Indicates occurrence at all sampling sites.

Resources (OMNR). For the lower sites of the MR (sites 2 and 3), hourly discharge data at the Steephill Power Plant were provided by Brookfield Power Corporation (2003–2004) and from a downstream station monitored by the Ontario Ministry of Natural Resources (2005–2006). Data from the OMNR consisted of continuous flow measurements (Solinst levelloggers), recorded every 15 min and we used hourly mean values for consistency of measurement frequency at all sites. On the BR, flow measurements from a long-term monitoring station of the Water Survey of Canada (station 02BF001) were used for the 4-year time period. The geographic coordinates of discharge and stable isotope sampling sites were entered into a hydrometric geographic information system program (OFAT v.2) to determine site-specific drainage area and associated discharge values. RR was calculated as the difference in discharge between hourly observations. Combined up-flow and down-flow changes in RR followed a normal distribution centred on zero. Therefore, absolute values were used to investigate the effect of RR amplitude on the stable isotope signatures of taxa at a given site. For comparison with the stable isotope data collected in spring and summer, mean seasonal RR values were calculated.

Statistical analysis

The detection of anthropogenic disturbances on natural ecosystems is a critical step in the management of natural resources and in the evaluation of associated impacts. In this study, a before-after-control-impact (BACI) experimental design was applied to detect the effect of RRs on the stable isotope signatures of resident organisms. The BACI design requires data from multiple control and impacted sites sampled before and after a perturbation. The statistical methods resulting from this approach are based on a two-way analysis of variance that is used to identify significant differences before and after the disturbance (B), as well as significant differences between control and impacted sites (C) and their interaction (B $\times$ C). The interaction term is of main interest when assessing the overall significance of the perturbation in a BACI design, and will be significant when a change occurs at the impact site but not at the control site. Simple correlation analysis was used as a measure of the strength of the relationship between RR and stable isotope signatures.

Based on obtained isotope signatures, we were able to calculate food web length, and analyse the data based on a BACI approach, to provide additional insights on the functioning and response of the food web to RR flow regimes. The length of the food web, from invertebrates to fish, was calculated as:

$$\Delta^{15}N = Max[\delta^{15}N_{FISH}] - Min[\delta^{15}N_{INV}]$$

where Max and Min values refer to the highest and the lowest signatures obtained within all taxa of a given community (fish and invertebrates), at a given site and sampling date. A variation in $\Delta^{15}N$ is related to variation in the length of the food web for consumers as baseline effects are removed.

JMP 6.0.3 (SAS Institute) was used to perform all reported statistical analyses, including miscellaneous variance and correlation analyses reported below, with significance judged at the $\alpha = 0.05$ level of significance in all analyses.

RESULTS

Hydrology

Main hydrological characteristics according to BACI are presented in Figure 2. Mean discharge was higher at the impacted sites (25.6 m^3 s^{-1}) compared to the control sites (19.5 m^3 s^{-1}), with the pattern being consistent before and after RR manipulation. In addition, a decrease in discharge was observed before and after RR manipulation in the control (−66%) and impacted sites (−63%) (Figure 2). Seasonal variations were related to the spring freshet and consisted in higher discharge in both rivers in spring (31.4 m^3 s^{-1}) compared to summer (9.6 m^3 s^{-1}).

The design of this experiment resulted in a great range in RRs (range: 0.5–3.4 m^3 s^{-1} h^{-1}, C.V.:104%, Figure 3). Overall, mean RR values were lower in control sites than impacted sites (0.7/2.7 m^3 s^{-1} h^{-1}). At the impacted sites, unrestricted RR condition was associated with an increase from 1.9 to 3.5 m^3 s^{-1} h^{-1} (Figure 3). There was no significant difference in RR between spring and summer across both rivers ($p = 0.36$) and, therefore, mean seasonal values were used to explore RR relationships with stable isotope data.

Stable isotope compositions

The stable isotope dataset for aquatic vegetation was unbalanced because of the limited number of species found at some sampling sites. In addition, the number of samples analysed for periphyton was small as a result of the inconsistent distribution of periphyton among sites and the analytical limitations imposed by the low weight of collected material. Accordingly, the mean values of several taxa were used to estimate the baseline signatures required for the comparison of food web signatures among sites at a given sampling time. Included in the baseline calculation were mollusc (*Elliptio complanata*) and gastropod (*Lymnaea stagnalis, Promenteus exacuous*) organisms known to have isotopic signatures related to that of primary producers (Post, 2002). $\delta^{13}C_{BASE}$ and $\delta^{15}N_{BASE}$ values, respectively, ranged from −38.2 to −20.7% (mean: −28.4%) and −1.0 to 7.9% (mean: 4.4%). Based on BACI analysis, the $\delta^{13}C_{BASE}$ remained invariant at all sites, regardless of RR variations (Figure 4, Table II). A significant difference in the $\delta^{15}N_{BASE}$ was found between control and impacted sites (Figure 5, Table II), with higher $\delta^{15}N_{BASE}$ values observed at impacted sites (I: 5.5% and C: 3.4%). No significant differences

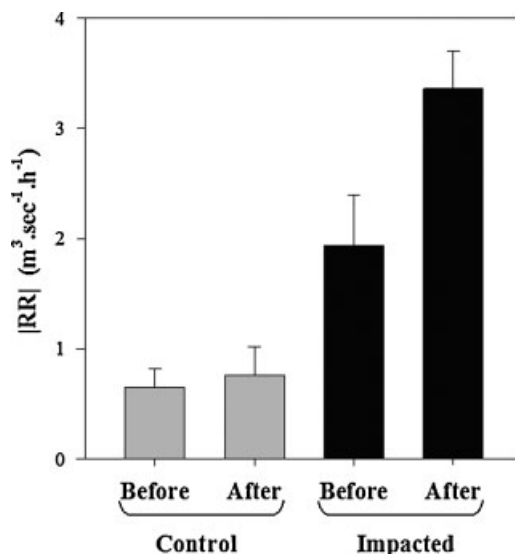Figure 3. Mean ($\pm$S.E.) ramping rate ($m^3 s^{-1} h^{-1}$) values as computed from the BACI experimental design

in $\delta^{15}N_{BASE}$ were found before and after RR manipulations or for the interaction (B $\times$ C), implying that RR changes did not affect the signatures of taxa at the base of the food web (Table II).

The carbon and nitrogen isotopic signatures of up to 12 invertebrate families ($\delta^{13}C_{INV}$ and $\delta^{15}N_{INV}$) were determined at each sampling site (Table I). Invertebrate $\delta^{13}C$ and $\delta^{15}N$ values ranged, respectively, from -41.0 to -20.5% (mean: -29.3%) and 0.0 to 15.4% (mean: 5.6%). A restricted maximum likelihood (REML) analysis of variance accounting for unbalanced design (Fletcher and Underwood, 2002) was applied to evaluate the portion of variance related to taxonomy and seasonality. Taxonomy explained 19 and 5.6% of the total variance of invertebrate carbon and nitrogen signatures, respectively. Signatures among seasons were similar over the course of the experiment as less than 1% of the total variance of both isotopes was associated to this variable. Given these results, mean taxonomic and seasonal values were used for BACI analysis of variance. BACI analysis of variance revealed significantly lower $\delta^{13}C_{INV}$ values at impacted sites compared to control sites (I: -31.3%, C: -27.8%). Further, unrestricted RR was responsible for slightly but significantly lower $\delta^{13}C_{INV}$ values, as shown by the significant B $\times$ C interaction (Figure 4, Table II). The variance of $\delta^{15}N_{INV}$ values was strongly related to BACI variables, with significantly higher values observed at impacted sites than at control sites (I: 7.6% and C: 4.5%) (Figure 5).
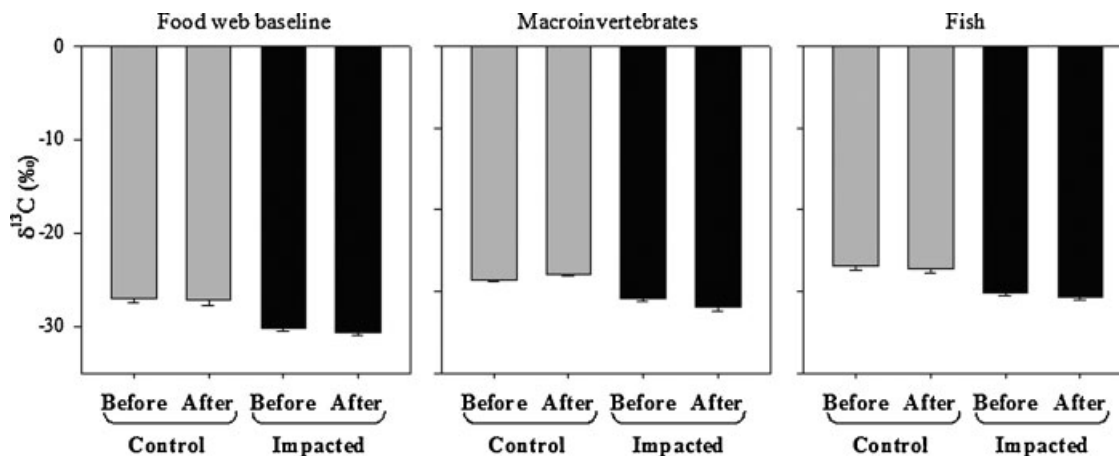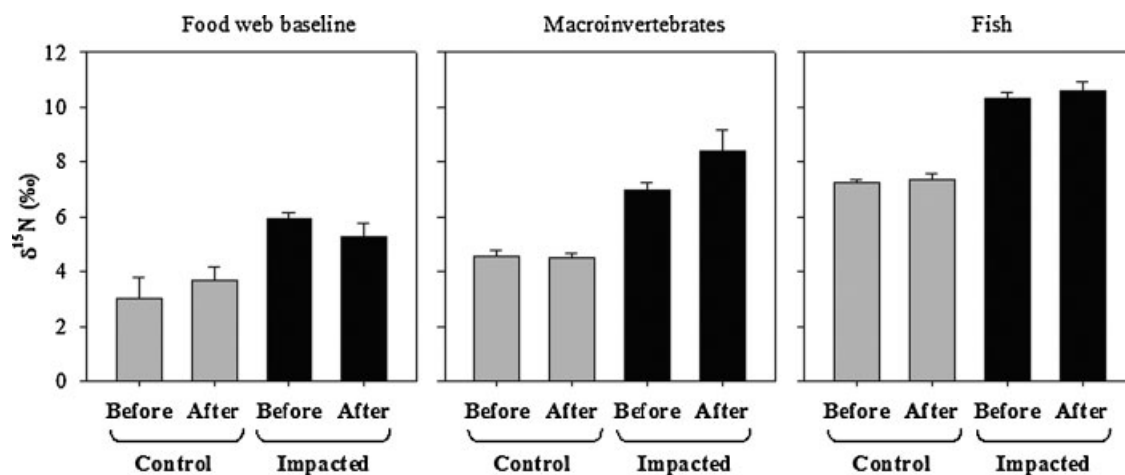

Figure 4. Mean ($\pm$S.E.) $\delta^{13}C$ (%) values for baseline, macroinvertebrate and fish taxa, as computed from the BACI experimental design

Table II. BACI results for baseline (BASE), invertebrates (INV) and fish (FISH) carbon (δ^{13}C) and nitrogen (δ^{15}N) stable isotope signatures

	Source of variation	δ^{13}C			δ^{15}N		
		S.S.	F	p	S.S.	F	p
	Before/after (B)	14.5	1.9	0.2	0.2	0.1	0.8
BASE	Impact/control (C)	2.2	0.3	0.6	46.8	18.5	0.0002
	B × C	5.7	0.7	0.4	0.5	0.2	0.7
	Before/after (B)	3.0	0.5	0.5	29.1	11.3	<0.01
INV	Impact/control (C)	516.9	81.9	<0.0001	507.8	196.9	<0.0001
	B × C	43.9	7.5	<0.01	21.8	8.4	<0.01
	Before/after (B)	1.6	0.5	0.5	1.6	0.6	0.4
FISH	Impact/control (C)	188.1	55.6	0.0001	218.0	81.5	<0.0001
	B × C	0.4	0.1	0.7	0	0	1.0

Unrestricted RR was responsible for a significant increase in δ^{15}N $_{INV}$ values at impacted sites (from 4.9 to 8.4%) as shown by the significant interaction (B × C) (Table II). The importance of RR on δ^{15}N$_{INV}$ value variation was further confirmed by correlation ($r = 0.66$; $n = 46$; $p < 0.001$) (Figure 6).

Samples of up to 10 fish species per site were analysed for stable isotopes to compute mean δ^{13}C$_{FISH}$ and δ^{15}N$_{FISH}$ at each site, with six species present at most sites (Table I). No significant relationship was found between body size and isotope signatures (ANCOVA, $p < 0.05$), likely as a result of the small range of fish body sizes in the studied systems (mean: 50.2 and 56.9 mm, CV: 37.3 and 54.2% for BR and MR, respectively). Mean δ^{13}C$_{FISH}$ and δ^{15}N$_{FISH}$ signatures ranged from −35.2 to −20.2% and 2.0 to 13.7%, respectively. Similarly to invertebrate signatures, a small portion of the variance of fish δ^{13}C and δ^{15}N was related to taxonomy (18.4 and 3.9% respectively) and seasonality (0.2 and 1.6%, respectively). Thus, following BACI analysis was based on mean seasonal and taxonomic fish signatures. Significant differences in δ^{13}C$_{FISH}$ and δ^{15}N$_{FISH}$ were observed between control and impacted sites (Figure 4, Figure 5, Table II), with lower δ^{13}C values (I: −30.4%, C: −27.0%) and higher δ^{15}N values (I: 7.6%, C: 4.5%) measured below the dam than at reference sites before and after the perturbation. Based on correlation analysis, δ^{15}N$_{FISH}$ was positively related to RR ($r = 0.77$; $n = 46$; $p < 0.0001$) whereas no significant relationship was found with δ^{13}C$_{FISH}$ (Figure 6).

Figure 5. Mean (±S.E.) δ^{15}N (%) values for baseline, macroinvertebrate and fish taxa, as computed from the BACI experimental design

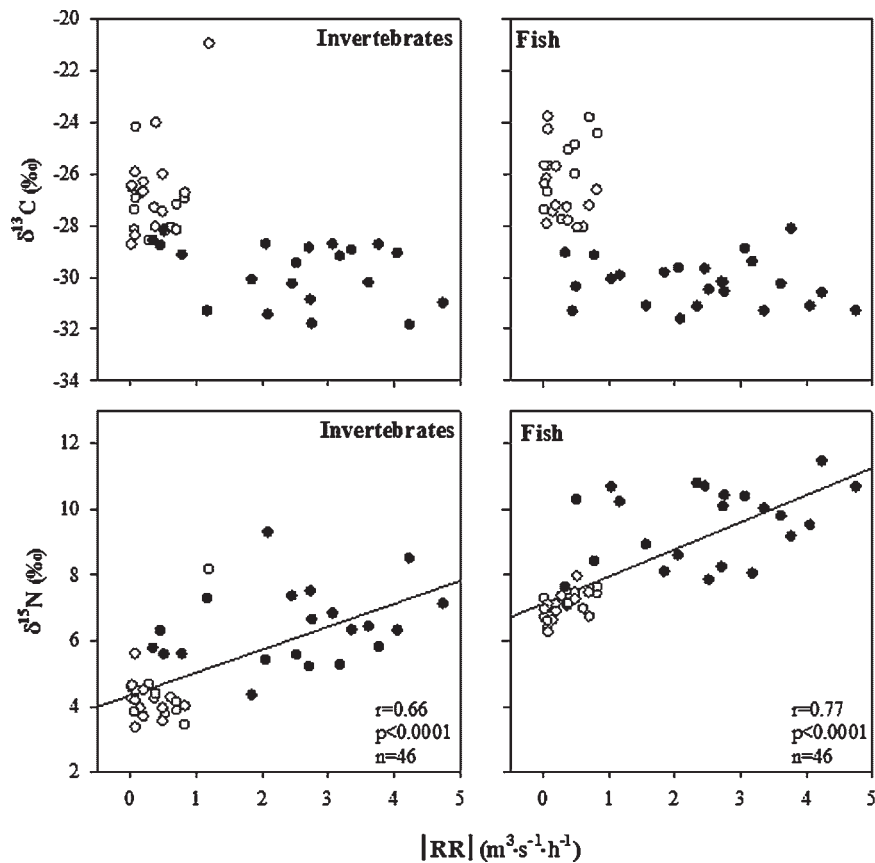Figure 6. Linear relationships between ramping rates (m³ s⁻¹ h⁻¹) and δ^{13}C (%) and δ^{15}N (%) for invertebrates (left) and fish (right), in the Magpie River (black) and the Batchawana River (white)

Testing for variations in food web length

Nitrogen variation computations (Δ^{15}N) confirmed that there was a significant effect of unrestricted RR flow regime on the length of the food web (Figure 7). Although Δ^{15}N remained similar at control sites and impacted sites under restricted RR, unrestricted RR significantly lowered Δ^{15}N values, indicating a reduction of the food web length under high RR flow regime.

DISCUSSION

This study is the first to have experimentally tested the effects of RRs on food web structure and function and has found support for the general hypothesis that the stable isotope values of affected organisms would change in relation to the RR. Although there was no evidence to suggest that high RRs were associated with a shift in the source of carbon supporting the food web, there was a measurable alteration of the food web structure noted in the reduction of food web length. Stable isotope analyses have been successfully applied to decipher some of the complexity of food web structure in lentic systems (Hecky and Hesslein, 1995; Grey and Jones, 1999; Vander Zanden *et al.*, 1999; Schindler and Lubetkin, 2004), but there is much less known about the functioning of lotic waters based on this tool. Furthermore, despite the relevance of SIA for detecting anthropogenic disturbances (Cabana and Rasmussen, 1994; Vander Zanden *et al.*, 1999), stable isotopes have not been applied to the study of flow perturbation effects on lotic food webs. As results from this study demonstrate, SIA is capable of determining the importance of RR as a key structural influence on the food webs of regulated rivers.
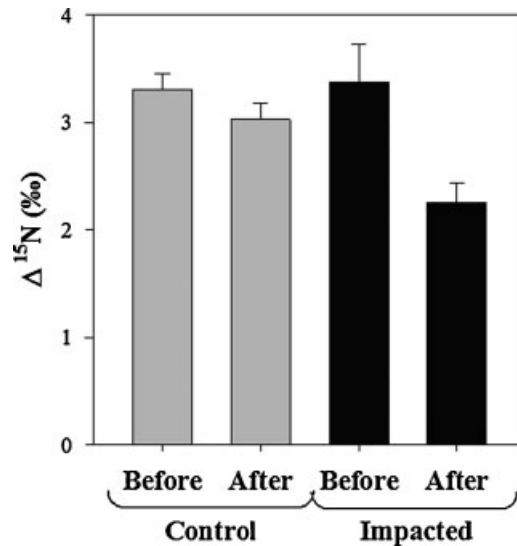
Figure 7. Mean seasonal (±S.E.) $\Delta^{15}N$ (%) as computed from the BACI experimental design

Sources of variation in $\delta^{13}C$

Previous studies have highlighted the control of physical variables related to flow over the $\delta^{13}C$ variations of aquatic organisms. In particular, flow velocity is a key variable controlling baseline carbon signatures by influencing CO_2 supply and isotopic discrimination which in turn affect mass transfer through the diffusive boundary layer (Trudeau and Rasmussen, 2003; Singer *et al.*, 2005). There is known to be a negative relationship between the $\delta^{13}C$ of herbivores, their algal diet and water velocity in productive rivers that suggests water velocity affects algal $\delta^{13}C$ strongly when CO_2 availability is low relative to photosynthetic rates (Finlay *et al.*, 1999; McCutchan and Lewis, 2001). The relationship highlights the importance of removing baseline effects when attempting to describe carbon transfer through lotic food webs (Finlay *et al.*, 1999; McCutchan and Lewis, 2001). However, in this study, $\delta^{13}C$ values obtained for each trophic level of the food web were similar in our BACI design and not related to RR variations. Our results indicate that unlimited RRs either did not modify water velocity sufficiently to affect baseline $\delta^{13}C$ values or that in oligotrophic shield systems the relationship between velocity and baseline $\delta^{13}C$ does not hold. For example, in unproductive California rivers, there was no significant relationship between baseline $\delta^{13}C$ values and current velocity (Finlay *et al.*, 1999) and in oligotrophic headwater streams water velocity has no effect on algal $\delta^{13}C$ (MacLeod and Barton, 1998). The absence of either relationship may serve to limit the impacts of RR on carbon transfer in the Boreal shield riverine ecosystems which contain a large number of hydropeaking operations in Canada.

We had hypothesized that high RR flow regimes would facilitate exchange between aquatic and terrestrial biomes and increase the contribution of terrestrial organic matter fuelling the aquatic food web. On the boreal shield, terrestrial carbon signatures are rather uniform (close to −28%), with most of the variation in d13C of consumers related to that of photosynthetic organisms (Marty and Planas, 2008). Based on these two end-members, several studies have reported on the variation in the relative contribution of terrestrial carbon to consumers (Grey *et al.*, 2001; Pace *et al.*, 2007). In this study, consumer $\delta^{13}C$ values were found to be similar in impacted sites before and after RR manipulations, and no relationship was found between organism carbon signatures and RR values. These results indicate that the main source of carbon fuelling the food web remained consistent, regardless of the flow perturbation. Although a detailed analysis of the carbon sources fuelling the food web of our systems was outside the scope of the study, autochthonous carbon has been reported as the primary carbon source for temperate lotic food webs in summer baseflow conditions with watershed areas ranging from 0.2 to 4000 km^2 (Finlay, 2001).

The data here provided no evidence of a change in the utilization of carbon sources under ramping flow regimes. Nevertheless, the data to date do not contradict the hypothesis concerning a potential increase in terrestrial carbon

reaching the water. Peaking operations are often associated with shoreline flooding, which will facilitate the entry of terrestrial organic carbon into lotic systems that may be used as a substrate for primary producers (Finlay, 2001). Availability of terrestrial organic matter is key to supporting algal production and consumers in hydroelectric reservoirs where nutrient limitations occur (Marty *et al.*, 2005). A similar effect is likely under RR regimes in oligotrophic riverine ecosystems, where dams trap terrestrial organic matter from upstream reaches, releasing it in spates to downstream reaches as peaking power demands dictate.

In our experimental results, the carbon isotopic values of organisms were significantly lower in impacted sites relative to reference sites. The differences likely relate to the characteristics of the carbon cycling occurring in the upstream reservoir. In reservoirs, processes such as respiration, methane oxidation and photolysis are responsible for the release of low $\delta^{13}C$ dissolved inorganic carbon compared to that of lakes (Marty and Planas, 2008). The trend toward low $\delta^{13}C$ values is also likely in consumers as DIC is incorporated in the biota through trophic transfer. Similar processes may explain the observed $\delta^{13}C$ values of consumers found in this study, with the lower $\delta^{13}C$ values of DIC measured below the dam compared to reference sites (Marty, unpublished data) supporting the above described reservoir effect.

$\delta^{15}N$ dynamic and food web structure response to RR

Based on $\delta^{15}N$ analysis, we were able to demonstrate the influence of RR variations on the food web structure of lotic ecosystems, with most of the $\delta^{15}N$ variation being explained by differences between impacted and references sites and a significant relationship between RR and both invertebrate and fish $\delta^{15}N$ signatures. Several mechanisms may be involved in explaining the observed results. Variations in $\delta^{15}N$ between impacted and reference sites may reflect the consumption of different food sources with a distinct nitrogen signature. Variations may also reflect differences in the baseline signatures of the two food webs. Multiple mechanisms may be responsible for the production of ^{15}N-enriched inorganic nitrogen, including microbial mineralization and nitrification/denitrification processes (Vander Zanden and Rasmussen, 1999; Leggett *et al.*, 2000). As noted above for carbon, it is likely that these processes occurred in the upper reservoir and have conditioned the downstream $\delta^{15}N_{BASE}$ as a result of connectivity between upstream and downstream reaches imposed via the transport of organic matter and nutrients through the dam (Vannote *et al.*, 1980).

In addition, higher $\delta^{15}N$ values in impacted sites may relate to the influence of abiotic variables on the food web. Laboratory experiments with *Daphnia magna* and *Hyallela* have demonstrated that the isotopic values of organisms are influenced by temperature dependent fractionation, with the difference between predator and prey $\delta^{13}C$ and $\delta^{15}N$, respectively, increasing and declining with increasing temperatures as a result of changes in organism physiological rates (Power *et al.*, 2003). Similar temperature dependent effects have also been observed experimentally with fish (Sweeting *et al.*, 2007). The temperature dependent pattern of fractionation differences leading to higher $\delta^{15}N$ and lower $\delta^{13}C$ organism values may have occurred here, with the impacted sites of the MR receiving the colder water from the upstream reservoir. For example, under simulated hydropeaking flow regimes, colder temperatures have altered prey availability as well as food intake, digestion, absorption and excretion of fish. (Lagarrigue *et al.*, 2002; Flodmark *et al.*, 2004). Thus, the increase in $\delta^{15}N_{INV}$ values observed under unrestricted RR regimes may stem more from an indirect RR effect mediated through organism physiological responses to both flow rate change and temperature, than as a result of a direct effect of flow rate change per se.

Many of the previous studies of flow regulation effects on riverine biota have focussed on fish species, presumably because fish are known to be integrators of ecosystem stressors (e.g. Power, 2002). Although we expected $\delta^{13}C$ or $\delta^{15}N$ of macroinvertebrates and fish to respond similarly to RR changes, macroinvertebrates were more responsive than fish, and overall the unrestricted RR flow regime was responsible for a significant decrease in $\Delta^{15}N$ values. Such results highlight the control of RR variations on trophic positioning and food web length. Considering the variability in fractionation values used to distinguish between 2 trophic levels (2.0 ± 1.0 SD, (McCutchan *et al.*, 2003)), the unrestricted RR regime was responsible for the reduction of the lotic food web length between macroinvertebrates and fish by the equivalent of one trophic level. Theory (Duffy *et al.*, 2005) and empirical research (Pace *et al.*, 1999) have shown that food web length can have significant impacts on the structure and function of ecosystems as a whole, with changes in food web length often occurring either as a result of the removal of the apical predator or a change in its degree of omnivory (Power, 1995; Post and Takimoto, 2007).

Disturbance is commonly thought to affect food and in our experiment it is likely the causative factor. Nevertheless, the subtlety of the observed effects and the differential sensitivities of the studied trophic levels indicate further work is required to completely assess RR effects on the trophic dynamics within boreal river macroinvertebrate and fish communities. Such work should consider the diet preferences of sampled taxa to better assess possible changes in omnivory. Evidence of the effects of RR variations on the food web provided by this study suggests that environmental flow management practices aiming to maintain flow regimes supporting a range of abiotic, biotic and functional ecosystem characteristics should consider unrestricted RRs as a potential source of ecosystem disturbance with significant implications for food web structure.

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