Effects of Nutrient Replacement on Benthic Macroinvertebrates in an Ultraoligotrophic Reach of the Kootenai River, 2003–2010

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Effects of nutrient replacement on benthic macroinvertebrates in an ultraoligotrophic reach of the Kootenai River, 2003–2010

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Abstract: Large impoundments remove substantial amounts of sediment and nutrients from rivers and often limit production by downstream primary producers and secondary consumers. Nutrient levels and macroinvertebrate and fish abundance in the lower Kootenai River (7^{th} order, mean annual discharge = 454 m^3/s) in Idaho and Montana declined dramatically after Libby Dam was built in 1972. A subsequent study implicated ultraoligotrophic conditions (total dissolved P [TDP] $\leq$ 2 μg/L TDP) as a principal causative agent and prompted an on-going experimental nutrient-addition program for the Kootenai River downstream from Libby Dam, with dosing at the Idaho–Montana border. Pre-treatment monitoring began in 2003 and liquid ammonium polyphosphate fertilizer (10-34-0) was added each year during the growing season from 2006 through 2010 with a target TDP concentration of 3 μg/L and TN : TP near 20 : 1. We studied benthic macroinvertebrate responses to the experimental addition and hypothesized moderate increases in invertebrate richness, abundance, and biomass with little change in assemblage structure. We used a before–after control–impact BACI design with macroinvertebrate samples collected pre- and post-treatment from July to early November 2003–2010 from fertilized and unfertilized reaches. After treatment, mean modified (Oligochaeta and Chironomidae subtaxa excluded) total abundance increased 72%, mean total abundance increased 69%, and mean biomass increased 48%. Abundance of Ephemeroptera, the principal insect order in the study area increased 66%. Filter-feeder abundance also increased, indicating increased suspended organic matter in addition to the attached forms consumed by other benthic macroinvertebrates. The first 5 y of experimental treatment resulted in increased food resources for resident native fishes with no major alteration of macroinvertebrate community structure or trophic pathways.

Key words: benthic macroinvertebrates, large rivers, nutrient addition, water quality, food resources, trophic ecology, NMDS, BACI

Benthic macroinvertebrates have many important ecological functions in rivers and streams. They regulate the flow of materials and energy in lotic ecosystems through food-web linkages involving fish, terrestrial invertebrate, avian, and even mammalian assemblages that generally occupy higher trophic positions (Wallace and Webster 1996, Huryn and Wallace 2000, Baxter et al. 2005, Woodcock and Huryn 2007, Cross et al. 2011). Macroinvertebrates simultaneously support higher trophic level production and consume lower-trophic level organisms (Huryn and Wallace 2000). The intermediate positions of this group of organisms in freshwater food webs has enabled researchers and managers to characterize lotic ecosystems and evaluate responses to large-scale alteration, habitat restoration, and nutrient enhancement by monitoring benthic macroinvertebrates (Quamme and Slaney 2003, Allan and Castillo 2007, Kohler and Taki 2010, Kohler et al. 2012, Bellmore et al. 2013, Cross et al. 2013).

Most published studies of responses of benthic macroinvertebrate communities to nutrient addition have occurred in headwater to mid-order streams. Relatively few studies have dealt with larger rivers, and even fewer have

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involved large rivers so nutrient-limited that they qualify as oligotrophic (Dodds 2006). Thus, our study is a unique contribution to the knowledge needed to understand the ecology of restoring the functions of large rivers via nutrient addition.

A principal rationale for lake and stream fertilization is to mitigate cultural oligotrophication (Stockner et al. 2000) and the associated losses of organismal abundance, biomass, diversity, and biological productivity (Stockner 2003 and references therein, Kohler and Taki 2010, Kohler et al. 2012). Authors of many empirical nutrient-addition studies in streams have demonstrated post-treatment increases in periphyton standing crop, primary production, and invertebrate and fish abundance, biomass, and taxonomic richness (Hyatt and Stockner 1985, Johnston et al. 1990, Perrin and Richardson 1997, Oliver 1998, Ashley et al. 1997, Stockner 2003 and references therein, Quamme and Slaney 2003, Kohler et al. 2008, Kohler and Taki 2010). In unaltered rivers, nutrient levels typically increase downstream, a pattern that is consistent with the predictions of the River Continuum Concept (Vannote et al. 1980). Exceptions to this pattern usually involve impounded rivers, where dams disrupt the natural downstream increase in nutrient concentrations (e.g., serial discontinuity, Ward and Stanford 1983, 1995; the river discontinuum, Cross et al. 2013), or large clear-water Arctic rivers, in which deviations from predicted longitudinal patterns can result from the natural infertility of their watersheds (Peterson et al. 1993a, b, Hershey et al. 1988).

The Kootenai[1] River is a large, 7th-order, floodplain river (mean annual discharge = 454 m^3/s that flows >780 km from its headwaters in southeastern British Columbia, Canada, south into the USA, and north again to Kootenay Lake and, ultimately, the Columbia River (Fig. 1). Nutrient concentrations, nutrient loading, and fish population abundance in the lower river have plummeted during the past 50 y, principally because of impoundment, but also because of pre-dam loss of river connectivity with large areas of historic floodplain and off-channel habitats after extensive levee construction (Northcote 1973, Woods 1982, Anders et al. 2002).

Libby Dam altered downstream hydrologic and thermal regimes (KTOI and MFWP 2004, Burke et al. 2009), and the upstream impoundment (Lake Koocanusa) is a nutrient and sediment sink. Lake Koocanusa retains an estimated 63% of total P and 25% of N and has estimated sediment trapping efficiency approaching 95% (Woods 1982). As a consequence, the river had become ultraoligotrophic by the 1980s (Ashley et al. 1997, Schindler et al. 2011). Daily metabolism in the Kootenai River was reported to be positive (P/R > 1) during only 1 of the 3 growing seasons from 1993 to 1995, results indicating that autotrophic production was rarely sufficient to support energy demands of higher trophic levels (Snyder and Minshall 2005).

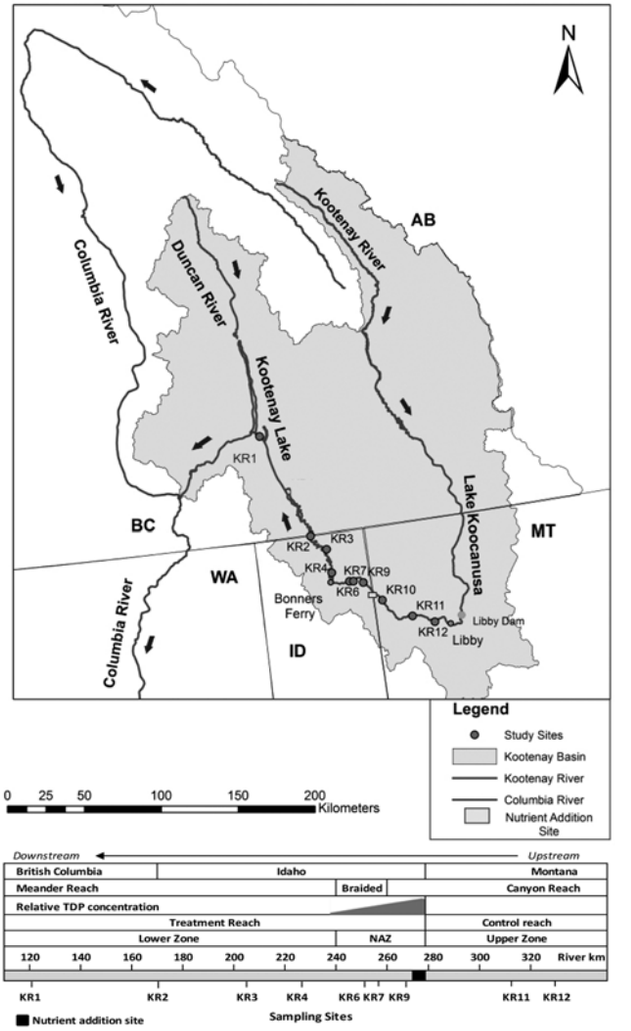

Figure 1. Map of the Kootenai River Basin (shaded) and study area including the location of Libby Dam, sampling sites, the nutrient addition site, geomorphic reaches and data collection zones. Arrows indicate direction of river flow.

Alterations of the Kootenay River system resulting from upstream dam construction and floodplain isolation caused by levee construction in the lower river continue to limit nutrient availability, channel processes, physical-habitat formation, biotic diversity, and ecosystem metabolism in the lower Kootenai River. Ecological effects of these changes include reduced periphyton biomass and accrual rates and reduced abundance, biomass, and diversity of benthic macroinvertebrate and fish assemblages (Snyder and Minshall 2005, Shafii et al. 2010) relative to comparably large unimpounded rivers. Mean pre-treatment benthic chlorophyll *a* values in the Kootenai River ranged from 1 to 4 mg/m^2

1. Spelled Kootenay in Canada.

compared to the range of post-treatment values from >10 to nearly 60 mg/m^2 (Holderman et al. 2009a). Dodds et al. (1998) and Wetzel (2001) suggested a stream benthic chlorophyll threshold of ≤20 mg/m^2 for oligotrophic status. Thus, pre-treatment chlorophyll *a* values indicated ultraoligotrophic status in the Kootenai River.

A consequence of these ecosystem alterations is that numerous native fish populations in the Kootenai River have become imperiled. Abundances of Bull Trout (*Salvelinus confluentus*), Kokanee Salmon (*Oncorhynchus nerka*), Westslope Cutthroat Trout (*Oncorhynchus clarkii lewisi*), Inland Redband Trout (*Oncorhynchus mykiss gairdneri)*, White Sturgeon (*Acipenser transmontanus*), and Burbot (*Lota lota*) now range from near 0 to 60% of previous estimates (KTOI and MFWP 2004), and Inland Redband Trout, Bull Trout, and White Sturgeon are currently listed as threatened or endangered under the US Endangered Species Act (USFWS 1994, KTOI and MFWP 2004). In this study, we focused exclusively on benthic macroinvertebrate community responses to experimental nutrient addition, but many native fishes, which historically provided valuable recreational and subsistence fishery benefits, rely heavily on benthic macroinvertebrates as a food source (KTOI and MFWP 2004, Holderman et al. 2009b).

Our objectives were to determine: 1) whether macroinvertebrate assemblage structure differed among sequential longitudinal river zones (control and treatment), and 2) whether and how assemblage structure in 2 geomorphically distinct river reaches downstream from the nutrient injection site changed after nutrient addition. We hypothesized that a moderate infusion of limiting nutrients to an oligotrophied river would increase benthic macroinvertebrate biomass, abundance, and richness, with relatively minor changes in overall relative abundance.

Based on geomorphic and hydraulic conditions (gradient, substratum, depth, turbulence), we expected that the river reach furthest downstream from the injection site (Lower River Zone [LRZ]) would differ in macroinvertebrate assemblage structure from a reach immediately downstream from the injection point (Nutrient Addition Zone [NAZ]) and the untreated reach immediately upstream from the injection site (Upper River Zone [URZ]). We also expected to see pre-treatment similarities in assemblage structure between the URZ and the NAZ caused, in part, by their shared physical habitat characteristics (both are in the canyon reach; Fig. 1). Despite some shared habitat characteristics between the NAZ and the URZ, we also expected to see some differences in assemblage structure between these 2 zones caused by nutrient addition.

METHODS

Study location

From its headwaters in Kootenay National Park in southeastern British Columbia, the Kootenai River flows south into northwestern Montana where has been impounded since 1972 by Libby Dam, forming Lake Koocanusa (Fig. 1). The Kootenai River is the 2nd largest Columbia River tributary in runoff volume, with historical peak discharges >2832 m^3/s, and the 3rd largest in watershed area (nearly 50,000 km^2) (KTOI and MFWP 2004). The watershed is mostly mountainous and forested and has a continental–maritime climate that produces 500 to 3000 mm of annual precipitation, primarily as snow (Bonde and Bush 1975) (Fig. 1). It is underlain by folded, faulted, metamorphosed Precambrian rock (Ferreira et al. 1992), and supports vegetation communities typical of the Northern Rocky Mountain Forest-Steppe-Coniferous Forest-Alpine Meadow Province (KTOI and MFWP 2004).

Geomorphic reaches From Libby Dam downstream to Kootenay Lake, the river has 3 geomorphically distinct reaches (canyon: 101 km long, braided: 12 km long, meander: 126 km long; Fig. 1). Each reach has distinct channel morphology, gradient, and substrate composition that contribute to reach-specific differences in ecosystem structure and function (Snyder and Minshall 2005). The canyon reach is characterized by an alternately open and constricted gorge incised 50 to 300 m into the local stratigraphy and has little off-channel habitat. The river bed has a moderate gradient (slope: 4×10^{-4} m/m) and flows over predominantly cobble and gravel substrates with several small areas of boulders and exposed bedrock.

The braided reach is immediately downstream from the canyon reach and extends from the mouth of the Moyie River to Bonners Ferry, Idaho, and contains a series of anastomosing channels with reduced bed slope (2×10^{-5} m/m) and stream power. Substrates in the braided reach are predominantly gravels in the larger channels and sand or fine sediments in secondary channels and backwater habitats. Further downstream, the meander reach extends from Bonners Ferry to the delta at the head of Kootenay Lake. This reach lies entirely within the historic floodplain in the Purcell Trench, a glacial valley with very low gradient (slope: 4×10^{-5} m/m) and little hydraulic energy. This 120-km reach has been totally levied, channelized, and isolated from its historic floodplain since the 1950s (KTOI and MFWP 2004). Substrates in the meander reach are mainly sand and silt with areas of shifting sand waves and of exposed lacustrine clay in constricted thalweg locations and outer-bend habitats (Barton 2004, KTOI 2009).

Data-collection zones We grouped data into 3 spatial zones for statistical analysis: Upper River Zone (URZ; the control portion entirely within the canyon reach), Nutrient Addition Zone (NAZ; the treated portion occupying the furthest-downstream 20 km of the canyon and the entire braided reach), and Lower River Zone (LRZ; the entire meander reach) (Fig. 1).

Study-site characteristics We chose 10 sampling sites to quantify the effects of nutrient addition on the abundance, biomass, and taxonomic composition of the macroinvertebrate assemblage. We added nutrients immediately downstream from the Idaho–Montana border at river km 275.8. An s-shaped river bend and tributary 1.3 km downstream from the addition site produced complete vertical and transverse mixing. Seven treatment sites were 4.8 to 152.5 km downstream of the nutrient addition site, whereas 3 control sites were 4.6 to 49.0 km upstream of the nutrient-addition site (Fig. 1). We numbered sites sequentially, starting at the most downstream location, but not all sites were used in this study. The LRZ was represented by sites KR 2 to 4, the NAZ by sites KR 6, 7, and 9, and the URZ by sites KR10 to 12 (Fig. 1).

Nutrient addition

We added nutrients by dosing the river with liquid agricultural-grade ammonium polyphosphate fertilizer ($[NH_4 PO_3]_n$; 10-34-0) at a single site in Idaho (Fig. 1). Nutrient addition was facilitated by a gravity-flow system including fertilizer storage tanks, a mixing-head box, dispensing pumps, and flow-monitoring meters. In 2005, we added nutrients to maintain an in-river total dissolved P (TDP) concentration of 1.5 µg/L at the dosing site. From 2006 through 2010, the target concentration was 3.0 µg/L. This program also was designed to add N fertilizer (liquid ammonium nitrate [$NH_4 NO_3$]; 32-0-0) if needed to maintain a minimum in-river TN : TP ratio of ≥20 : 1 to avoid potential co-limitation by N and to prevent the growth of blue-green algae. We maintained proper nutrient dosing volumes and dilution rates by checking an on-site US Geological Survey gaging station daily and adjusting dosing volumes accordingly.

Sample collection and processing

We sampled benthic macroinvertebrates on multiple occasions each year from March through December 2003–2010. However, to focus on the growing season, we used only samples collected from July through early November. No samples were available from the LRZ for that period in 2005. We collected 6 replicate samples per site in each zone (LRZ, URZ, NAZ). We used a 500-µm mesh size for all samplers and sorting screens following EPA guidelines (Barbour et al. 1999). In the NAZ and URZ (KR6–12), we used a Surber sampler (0.5 × 0.5 m) to collect macroinvertebrates, whereas in the LRZ (KR1–4), we used a boat-mounted petite Ponar dredge (15 × 15 cm). In the URZ and NAZ, we sampled exclusively in near-shore shallow (<1 m) riffle or run habitats. In the LRZ, we sampled primarily in near-shore habitats that ranged in depth from 3 to 10 m. Some sampling occurred in the deeper thalweg areas, but because of the difficulty of taking Ponar samples in deeper water (>12–15 m), most sampling occurred in near-shore, mud-bottom habitat. We sampled monthly in all zones during 2004. However, after 2004, we sampled seasonally (excluding winter). During the monthly and seasonal sampling regimes, we took samples when flows and other logistical constraints, such as dam operations, allowed. We preserved captured specimens in 95% ethanol.

Laboratory procedures

Macroinvertebrate samples were processed by EcoAnalysts, Inc. (Moscow, Idaho) according to standard US Environmental Protection Agency (EPA) Rapid Bioassessment Protocols (Barbour et al. 1999). All samples were processed in their entirety with no subsampling. After sorting, all macroinvertebrates were identified to the lowest practical taxonomic level (usually genus or species). Total dry mass (g) was measured for Ephemeroptera, Plecoptera, Trichoptera, Coleoptera, Diptera, Chironomidae, Oligochaeta, Gastropoda, Bivalvia, Acari, Crustacea, Annelida, and other after drying for ≥8 h at 105°C (±5°C). Gastropods, bivalves, and cased Trichoptera (mainly *Brachycentrus*) were not removed from their shells or cases for weighing. However, all of these taxa were relatively rare. Large bivalves (Unionidae) were excluded from biomass measurements.

Statistical analyses

We initially considered multiple measures of richness and abundance for statistical analysis. Richness metrics included total number of taxa, total taxa excluding subtaxa of Chironomidae and Oligochaeta (NCO); Chironomidae; Oligochaeta; Ephemeroptera, Plecoptera, and Trichoptera (EPT), and Margalef's Index for total and NCO richness. Abundance metrics included total, NCO, Ephemeroptera, Baetidae, EPT, Chironomidae, and filterers. We used NCO richness because it more closely approximated results from published studies, in which Chironomidae usually are identified to family and Oligochaeta to order, than did total richness, which included lower-level taxa from these 2 groups (e.g., genus or species) in our study.

Each year, sampling intensity varied among months and sites from July through early November, so we aggregated data by pre- and post-treatment periods and analyzed by river zone. Mean site values for all assemblage metrics during 2005 were similar to those in 2003 and 2004 and were intermediate between those and 2006 values. For this reason and because nutrient addition was initiated in July 2005 at ½ the concentration of subsequent years, we defined the pre-treatment period as 2003–2005. We defined the post-treatment period as 2006–2010.

We explored the spatial clustering of sampling units based on assemblage response metrics with nonmetric multidimensional scaling (NMDS) (Rabinowitz 1975, Kohler and Taki 2010). We considered 11 response metrics for NMDS analysis and retained 6 for visualizing the structure of the data in 2-dimensional space: NCO richness; NCO, Chironomidae, Ephemeroptera, and filterer abun-

dances; and total biomass of all taxa excluding Bivalvia. We assessed adequacy and completeness of the NMDS analyses with diagnostic scree plots and predicted correlations. We $\sqrt[4]{(x)}$-transformed all metrics before analysis. Initial diagnostics and an associated scree plot indicated that retention of 2 axes was sufficient to describe the assemblage data and to down-weight the importance of abundant taxa.

We used before–after control–impact analysis (BACI; e.g., Smith 2002, Stewart-Oaten et al. 1986) to test for effects of nutrient addition on response metrics. We used repeated measures analysis of variance (rmANOVA) to test for differences in response metrics before and after nutrient addition between treated and untreated river zones. We were particularly interested in the zone × time-period interaction when nutrient-addition effects were discernible from simple temporal effects. We log(x)-transformed abundance and biomass response metrics to meet the normality assumptions of the specified analyses. Statistical analyses were done with SAS (version 9.3; SAS Institute, Cary, North Carolina).

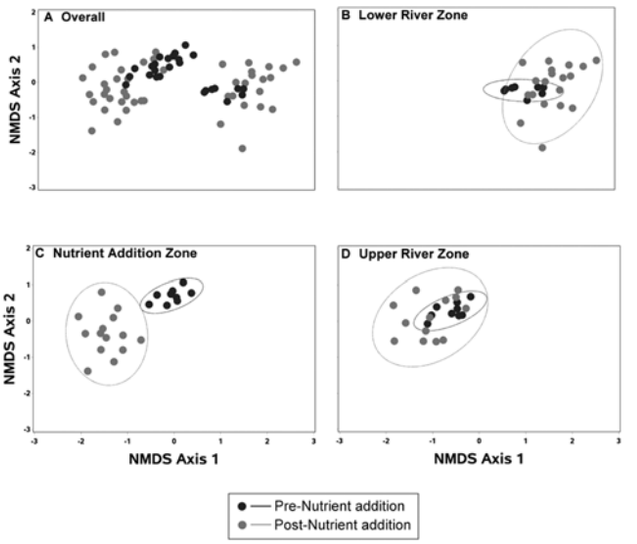

Figure 2. Plot of data points along the first 2 nonmetric multidimensional scaling (NMDS) axes for all river zones (A), and partitioned by the Lower River Zone (B), the Nutrient Addition Zone (C), and the untreated Upper River Zone (D). Ellipses represent a 95% confidence region for each period and zone combination.

RESULTS

Ordination analysis

The structure of the macroinvertebrate assemblage changed during the post-treatment period. The change was strong in the NAZ and weaker in the LRZ, and little to no change occurred in the URZ. The relationship between observed and predicted NMDS ordinal data was close to linear and had a high correlation (r = 0.97) and minimal badness of fit (0.11). Axis 1 was strongly negatively correlated with all abundance, richness, and biomass measures, except Chironomidae abundance, which was the single dominant factor of the 2nd axis (Table 1). The overall NMDS plot showed 2 distinct clusters of data points, but the groupings did not reflect pre- and post-treatment periods (Fig. 2A). However, decomposing the plot into river regions revealed some correspondence to these large clusters (Fig. 2B–D). The LRZ made up 1 cluster that was well separated from the other zones (Fig. 2B). Within the LRZ, pre- and post-nutrient-addition clusters, a positive post-treatment shift along the 1st axis, and a reduction in variability along the 2nd axis were evident. In the remaining data, individual data points for the NAZ and URZ overlapped, but separate plots of the 2 regions indicated differing internal structure (Fig. 2C, D, respectively). The NAZ had the clearest separation between pre- and post-nutrient addition periods and a negative shift along both axes and increased variability on the 2nd axis after treatment (Fig. 2C). However, pre- and post-treatment data points overlapped the most in the URZ (Fig. 2D). The cluster position of this river zone shifted little, and a

Table 1. Pearson correlations and associated p-values for 6 benthic invertebrate responses with the 2 nonmetric multidimensional scaling (NMDS) axes. NCO = total taxa excluding subtaxa of Chironomidae and Oligochaeta.

	NMDS Axis 1		NMDS Axis 2	
Response	Correlation	$p > 0$	Correlation	$p > 0$
NCO abundance	−0.98	<0.001	0.01	0.921
Ephemeroptera	−0.93	<0.001	0.05	0.688
Filterers	−0.91	<0.001	−0.04	0.736
NCO richness	−0.90	<0.001	−0.05	0.675
Total biomass	−0.87	<0.001	−0.05	0.654
Chironomidae	−0.33	0.004	−0.94	<0.001

mild increase in variability along both axes followed treatment.

Spatial and temporal responses of assemblage metrics

The intersite patterns for the various richness measures examined were similar, so we have presented only the results for NCO richness here (Fig. 3A). All abundance responses also were similar, so we have presented only the results for NCO, Ephemeroptera, and filterer abundances (all used in the final NMDS model; Fig. 3B–D).

General comparison Benthic macroinvertebrate response patterns of the LRZ differed from those of the URZ and NAZ (Fig. 3A–D, Table 2) during the pre- and post-treatment periods. These patterns were consistent with the different habitat conditions in the LRZ (lower velocity; unstable, fine substrates) and the upstream reaches (higher velocity; gravel and cobble substrates). NCO richness and abundance values were markedly lower in the LRZ than in the 2 upstream zones in most years. The assemblage was composed of ≥50% fewer taxa in the LRZ than in the other zones, and composition in the LRZ was dominated by chironomids and oligochaetes, which constituted 86 to 96% of total abundance (Table 2). Assemblage attributes in the URZ and NAZ were very similar, but with some differences described below.

Comparison among sites within zones Intersite variability for NCO richness and all abundance metrics in the LRZ was low before and after nutrient addition (Fig. 3A–D). In the NAZ, richness and all abundance metrics were markedly higher in the post- than the pre-treatment period and were higher than values in the LRZ. Some differences in the responses were seen between pre- and post-treatment periods in the URZ, but they were smaller than those observed in the NAZ.

The 6 most abundant Ephemeroptera in each year of the study varied from year to year resulting in a total of 18 taxa (data not shown). Three taxa (*Caenis*, *Callibaetis*,

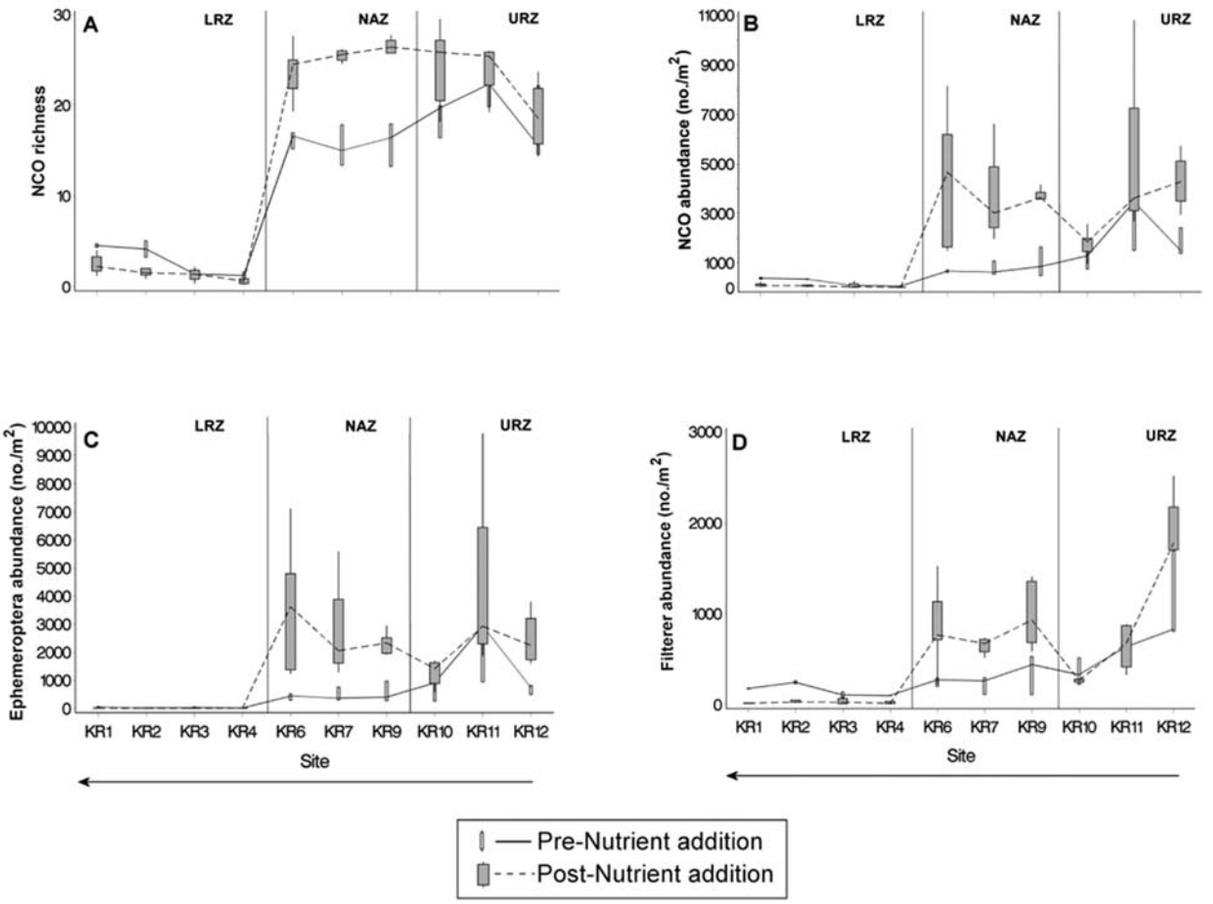

Figure 3. Overall trends across sampling sites for total taxa excluding subtaxa of Chironomidae and Oligochaeta (NCO) richness (A), and NCO (B), Ephemeroptera (C), and filterer (D) abundance. The respective trend lines pass through the corresponding median values at each site. Box ends are quartiles, and whiskers are ranges. The vertical lines designate the 3 river zones: the Lower River Zone (LRZ), the Nutrient Addition Zone (NAZ), and the Upper River Zone (URZ). Arrows indicate the direction of river flow.

Tricorythodes) were found only in the LRZ. Eight taxa (*Acentrella insignificans*, *Acentrella turbida*, *Baetis tricaudatus*, *Ephemerella inermis/infrequens*, *Heptagenia*, *Rhithrogena*, *Paraleptophlebia*, *Seratella tibialis*) occurred regularly among the 6 most abundant taxa in the URZ and NAZ, and *Drunella grandis* occurred in 6 of the 8 y in the URZ. The remaining 6 most abundant taxa appeared only sporadically among sites and years (URZ: *Attenella margarita* in 2010, *Epeorus* in 2008 and 2010; NAZ: *Drunella coloradensis/flavilinea*, *Cinygmula*, and *D. grandis* in 2008, *Nixe* in 2010). Seven Ephemeroptera taxa (*Acentrella*, *Caenis*, *Callibaetis*, *E. inermis/infrequens*, *Nixe*, *Paraleptophlebia*, *Tricorythodes*) occurred in samples from the LRZ, but always were rare. *Callibaetis* and *E. inermis/infrequens* were the most abundant Ephemeroptera pre-treatment, *E. inermis/infrequens* maintained its dominance post-treatment, but *Callibaetis* disappeared.

Examination of filterer abundance using the relative abundance of the top 6 taxa in each year (data not shown) indicated that the most abundant filterers were hydropsychid caddisflies (Trichoptera:*Hydropsyche* in all zones, followed by *Cheumatopsyche* in the NAZ and URZ) and blackflies (Diptera:*Simulium*). Brachycentridae (Trichoptera:*Brachycentrus americanus* or *occidentalis*) were common and 2 other Trichoptera: *Arctopsyche* and *Wormaldia* appeared sporadically in the NAZ and URZ, but not in the LRZ.

Comparison of zones among years The 6 most abundant taxa in any given year (Table 2) made up 92.6 to 99.4% of the total abundance in the LRZ, 65.3 to 88.7% in the NAZ, and 78.8 to 91.7% in the URZ (except during 2010 when they made up only 57.3%). Chironomidae was the only abundant taxon detected in all zones in all sampled years. It constituted from 44.4 to 71.0% of the total abundance in the LRZ, and together with Oligochaeta, accounted for 86.2 to 96.5% of the total abundance in the LRZ. In the other 2 zones, chironomids generally accounted for <50% of the total abundance (except in the NAZ in 2009). The highest chironomid abundance occurred after nutrient addition in the NAZ (13.4–16.4% pre-treatment vs 18.7% in 2008 and 30.7–54.0% in the other post-treatment years). The relative abundance of Chironomidae increased in the URZ during post-treatment years but was more sporadic and partially overlapped the pre-treatment values (17.6–19.9% pre-treatment and 11.8–32.6% post-treatment). A striking exception to post-treatment trends was the decrease in relative abundance of Chironomidae at all sites, especially in the NAZ (7.0%) and URZ (5.6%) during 2006.

The number of taxa constituting the top 6 in any given year over all years was similar among zones (12–14 taxa) but, except for Chironomidae, the top 6 numerically dominant taxa differed widely in composition among zones and years (Table 2). *Ephemerella* were present in all years in the URZ and NAZ. A core group of taxa that appeared periodically throughout the study was similar in composition in the URZ and NAZ but differed substantially in the LRZ. In the URZ and NAZ, *Hydropsyche*, *Rithrogena*, and *Paraleptophlebia* were among the top 6 taxa during most pre- and post-treatment years. In the NAZ and LRZ, only 2 taxa were not collected after nutrient addition began (Oligochaeta and *Zaitzevia*; Ceratopogonidae and *Callibaetis*, respectively). In all 3 zones, 4 more new taxa were collected (sporadically) than disappeared. Across all sites, the number of taxa appearing or disappearing from the list did not differ between sampling dates or between pre- or post-treatment periods. NCO richness generally increased by ~10 taxa during the 8 y of study. However, minimal changes in metric values associated with nutrient addition were observed in the NAZ.

BACI analyses

We did not consider benthic macroinvertebrate assemblage responses in the LRZ for further analyses because of the lack of a pronounced treatment effect and because of the differences in physical habitat and metric responses between the LRZ and the 2 upriver zones. The BACI analysis of data from the NAZ and URZ indicated that the zone main effect was not significant, but the period × zone interaction was significant for all metrics (Table 3).

All interactions showed the same overall response pattern. Some positive changes during the pre- and post-treatment periods were noted in the untreated URZ, but the changes in the NAZ were greater during the same periods. Plots of relative change in metric values between pre- and post-treatment periods at each sampling location showed this pattern consistently (Fig. 4A–D). All NAZ sites showed relatively large positive changes after nutrient addition, whereas much smaller changes and some decreases (e.g., filterers at KR10) were seen in the URZ. At all sites in the NAZ, NCO richness increased by ~≥20% (Fig. 4A), NCO abundance by 57 to 74% (Fig. 4B), Ephemeroptera abundance by 62 to 79% (Fig. 4C), and filterer abundance by ≥47% (Fig. 4D).

DISCUSSION

Benthic macroinvertebrate assemblage responses to nutrient enrichment

Low levels of ammonium polyphosphate fertilizer added to the Kootenai River to achieve and maintain an in-river target concentration of 3 µg P/L and a TN : TP ratio near 20 : 1 had the intended effects of increasing benthic macroinvertebrate abundance and biomass. Average NCO and Ephemeroptera abundances increased 66 and 72%, respectively, as a result of fertilization. In addition, mean total abundance increased by 69%, and mean biomass increased by 48%. These increases were accompanied by other changes in assemblage metrics (all increases) generally considered to be indicative of improved biological

Table 2. Percent dominant taxa from 2003 to 2010 for the 3 river zones. Numbers in parentheses are the number of years present before and after nutrient addition, respectively. Dashes indicate that the taxon was not present. Total abundance (no./m^2) is given for each river zone. Years 2003–2005 were pre-nutrient addition (pre), 2006–2010 were post-nutrient addition (post).

River zone/period present	Taxon	2003	2004	2005	2006	2007	2008	2009	2010
Upper River Zone									
Present in all years	Chironomidae	19.9	17.9	17.6	5.6	30.0	15.4	32.6	11.8
	Ephemerella sp.	21.7	39.6	33.4	57.9	35.0	32.2	16.6	20.1
Present in fewer years	*Hydropsyche* (3, 4)	23.9	12.6	14.3	10.4	8.4	10.5	6.1	–
	Rithrogena (3, 4)	7.9	4.5	10.4	13.7	4.6	11.8	9.2	–
	Simulium (2, 4)	2.6	3.4	–	–	4.4	9.6	6.9	6.1
	Optioservus (2, 1)	4.0	–	3.4	–	–	–	–	6.1
	Paraleptophlebia (1, 1)	–	4.2	–	2.0	–	–	–	–
	Baetis tricaudatus (1, 2)	–	–	5.5	2.1	5.3	–	–	–
Present only in pre		–	–	–	–	–	–	–	–
Present only in post	Oligochaeta	–	–	–	–	–	4.4	–	–
	Serratella tibialis	–	–	–	–	–	–	9.4	–
	Epeorus	–	–	–	–	–	–	–	6.8
	Acentrella insignificans	–	–	–	–	–	–	–	6.4
Total abundance (no./m^2)		1551.7	3039.0	2717.6	6565.4	3666.2	4215.3	4854.0	1698.0
Nutrient Addition Zone									
Present in all years	Chironomidae	16.4	13.4	14.2	7.0	30.7	18.7	54.0	43.7
	Ephemerella inermis/infrequens	8.9	12.6	16.4	50.8	22.0	20.9	10.3	5.3
Present in fewer years	*Hydropsyche* (2, 5)	14.4	–	10.7	4.0	4.5	8.7	8.3	6.7
	Rithrogena (3, 4)	13.1	17.6	24.3	19.5	13.8	22.6	3.6	–
	Cheumatopsyche (2, 3)	9.6	–	8.6	5.3	3.9	7.5	–	–
	Paraleptophlebia (2, 2)	–	6.0	4.7	3.4	–	9.2	–	–

Present only in pre	Oligochaeta	5.6	14.9	–	–	–	–	–	–
	Zaitzevia	–	6.1	–	–	–	–	–	–
Present only in post	*Glossosoma*	–	–	–	–	1.6	–	–	–
	S. tibialis	–	–	–	–	–	–	4.3	–
	Antocha	–	–	–	–	–	–	3.2	–
	A. insignificans	–	–	–	–	–	–	–	13.3
	Nixe	–	–	–	–	–	–	–	4.7
	Attenella margarita	–	–	–	–	–	–	–	3.7
Total abundance (no./m^2)		898.4	756.6	1334.3	6564.4	5996.0	4834.0	5493.1	4165.2
Lower River Zone									
Present in all years	Chironomidae	68.1	71.0	–	44.4	65.0	44.6	51.5	68.2
	Oligochaeta	18.1	16.9	–	52.1	30.1	48.4	44.0	27.0
Present in fewer years	*Probezzia* (2, 4)	1.2	2.1	–	1.3	–	2.2	1.3	1.5
	Gyraulus (2, 3)	0.9	1.0	–	–	1.3	0.8	–	0.2
	Hyallela (1, 2)	–	1.0	–	0.2	0.8	–	–	–
	Helobdella stagnalis	–	0.6	–	–	–	0.4	–	–
Present only in pre	Ceratopogonidae	3.4	–	–	–	–	–	–	–
	Callibaetis	2.2	–	–	–	–	–	–	–
Present only in post	*Dubiraphia*	–	–		0.4	0.7	1.1	–	1.0
	E. inermis/infrequens	–	–		1.0	–	–	–	–
	Pionidae	–	–		–	0.3	–	–	–
	Lebertia	–	–		–	–	–	0.5	–
	Nematoda	–	–		–	–	–	0.4	0.5
	Hydropsyche	–	–		–	–	–	0.4	–
Total abundance (no./m^2)		1264.6	1213.8	–	1244.0	1725.4	1054.5	412.1	3018.6

Table 3. Results ($p > F$) of the Before–After Control–Impact analysis for 6 benthic invertebrate response metrics. NCO = total taxa excluding subtaxa of Chironomidae and Oligochaeta. n = 45.

Source	df	NCO richness	NCO abundance	Ephemeroptera abundance	Biomass	Chironomidae abundance	Filterer abundance
Period	1	0.002	0.001	0.001	0.010	0.001	0.009
Year(period)	6	0.014	0.005	<0.001	0.135	<0.001	0.168
Zone	1	0.635	0.260	0.445	0.174	0.498	0.440
Period × zone	1	0.009	0.003	0.005	0.007	0.001	0.007

integrity (Karr 1991, Minshall 1996, Barbour et al. 1999). In contrast, no such responses were seen during the first year (2005) of nutrient augmentation when the TDP target was only 1.5 µg/L. This result suggests that the lower dosing rate did not have a widespread or sustained effect on primary or secondary production. Thus, the 1st-y results supported our decision to include 2005 in the prefertilization category.

NCO abundance at NAZ sites during our study averaged ~700/m^2 before fertilization and ~4500/m^2 after fertilization (800–5400/m^2 when Chironomidae and Oligochaeta abundances were included). These values are comparable to those published by Bonde and Bush (1975), who reported a mean density of 3520/m^2 organisms sampled from shallow riffles (<0.5m) in the Kootenai River before completion of Libby Dam (1969–1971). Snyder (2001) reported mean benthic macroinvertebrate densities of 358 to 2508/m^2 in the Kootenai River during the 1994–1995 summer seasons downstream from Libby Dam, whereas Holderman and Hardy (2004) reported a pre-treatment (2002–2004) mean of 1177 /m^2 in the NAZ sites (n = 220). Mean macroinvertebrate densities of 3944, 62,938, and 38,233/m^2 were reported from the analogous nearby systems of Priest, Coeur d'Alene, and Salmon Rivers, respectively (Royer and Minshall 1996). In addition, R. Wisseman (Aquatic Biology Associates, Inc., Corvallis, Oregon, personal communication) reported that a range of 10,000 to 30,000 insects/m^2 is typical for larger streams and rivers in the Pacific Northwest. These local and regional values suggest that the Kootenai River below Libby Dam has yet to achieve its full production potential. However, a recent study of 7 Pacific Northwest rivers reported means of 39 to 46 benthic macroinvertebrate taxa/site (Hughes et al. 2012), which is comparable to the mean site total richness values of 38 to 41 in the NAZ postfertilization (cf. annual means of 21–25 taxa in the NAZ prefertilization).

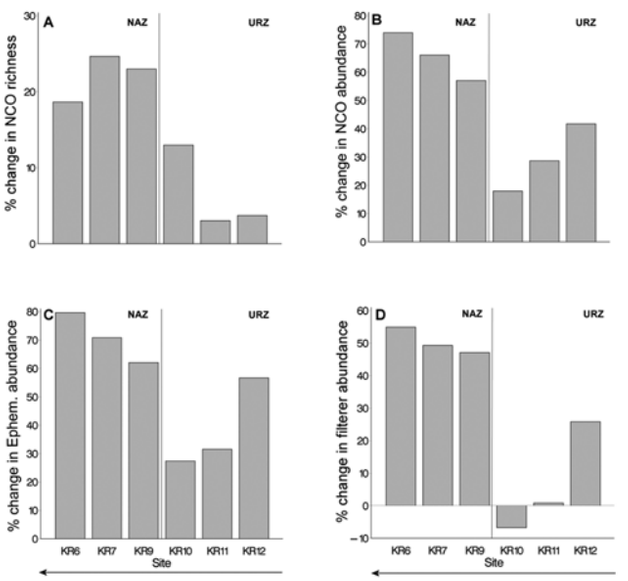

Figure 4. Relative change in total taxa excluding subtaxa of Chironomidae and Oligochaeta (NCO) richness (A), and NCO (B), Ephemeroptera (Ephem.) (C), and filterer (D) abundance from the prenutrient-addition period to the post-nutrient-addition period. The vertical lines designate 2 river zones: the Nutrient Addition Zone (NAZ) and the Upper River Zone (URZ). Arrows indicate the direction of river flow

The Kootenai River is ≥30× larger in terms of discharge than any other free-flowing river sections where nutrients have been added experimentally. Few other investigators have added nutrients throughout the growing season, and most that did lacked pre-treatment data. For these reasons, comparable pre- and post-nutrient-addition results at these scales are limited. The next largest streams in this category are the Kuparuk River in the Arctic tundra (e.g., Peterson et al. 1985, 1993a, b, Deegan et al. 1997) and the Keogh, Salmon, and Adam Rivers, and Big Silver Creek in southern coastal British Columbia, which have mean annual discharges of ~5 to 15 m^3/s (Slaney et al. 2003, Wilson et al. 2003). In all of these rivers, the increases in macroinvertebrate abundance resulting from P or P+N additions were comparable to those in our study.

Post-treatment changes also occurred in the macroinvertebrate assemblage in the URZ. For example, NCO abundance in the URZ increased by an overall average of 31.4% and biomass increased by 13% from the pre- to post-treatment periods. These sampling sites were upriver from the injection site, so the increases were indepen-

dent of our nutrient addition and, therefore, must be attributed to temporal variation and upstream phenomena, including reservoir conditions, Libby Dam operations, and contributions from the Fisher and Yaak Rivers, lesser tributaries, and possibly the Libby and Troy sewage treatment facilities. However, such influences were simultaneously applied to the NAZ and, hence, any additional changes in the NAZ can be attributed to nutrient addition. Hoyle (2012) reported mean NO_3+NO_2 values of 124.6 μg/L for URZ sites and 116.5 μg/L in NAZ sites from 2006 through 2010. Total P (TP) concentration was significantly higher in the NAZ than in the URZ ($p < 0.001$), with median values of 4.7 μg/L in the URZ and 7.8 μg/L in the NAZ (Hoyle 2012). Mean TDP concentrations at the URZ sites ranged between the detection limit (2.0 μg/L) and 5.0 μg/L, whereas analogous values in the NAZ ranged between 2.0 and 15.7 μg/L. TDP concentrations were significantly higher in the NAZ than in the URZ ($p = 0.03$; Hoyle 2012). TDP values were often below the detection limit in the URZ, but concentrations were significantly greater in the NAZ than in the URZ ($p < 0.001$; Hoyle 2012).

Increased macroinvertebrate NCO abundance and total abundance and biomass (and other metric values) in the URZ and the NAZ were associated with increased algal growth on tile substrate from ~2 mg/m^2 chlorophyll *a* prefertilization to 15 to 30 mg/m^2 post-fertilization in the NAZ (Holderman et al. 2009a). Several investigators have found that addition of N and P, but especially P, substantially increased both algal (e.g., Elwood et al. 1981, Peterson et al. 1985, 1993a, b, Hershey et al. 1988, Perrin et al. 1987, Johnston et al. 1990) and microbial growth (Peterson et al. 1985, Hullar and Vestal 1989, Gulis and Suberkropp 2003, Greenwood et al. 2007). Increased macroinvertebrate abundance and biomass also paralleled increased heterotrophic activity on organic matter produced locally or imported from upstream. However, we did not measure this nonalgal component of the epilithon.

The longitudinal fertilization effect on the macroinvertebrates observed in our study appeared to dissipate by the downstream end of the 36-km-long treatment zone. For example, we found little evidence of nutrient addition 45 km downstream from the dosing site at KR4 in terms of composition or standing stocks of benthic macroinvertebrates collected from the channel bed. This trend is consistent with observations from ongoing water-quality/nutrient-monitoring and chlorophyll accrual studies over the same reach of the Kootenai River. Holderman et al. (2009a) and Hoyle (2012) reported a consistently decreasing downstream gradient of chlorophyll *a* and total chlorophyll (*a* + *b*) biomass and accrual rates from 2005 through 2010 over the same river reach. However, these observations may have been consequences of the sharp differences in habitat characteristics between the 2 zones (i.e., upstream canyon vs downstream meander reach) and the absence of suitable substrate for biofilm and benthic invertebrate colonization in the LRZ, rather than only of the absence of added nutrients in the water. Macroinvertebrates in unsampled littoral habitats in the LRZ conceivably could have benefitted from nutrient addition.

Effects of nutrient addition on water quality as indicated by macroinvertebrate assemblage metrics

Dodds (2006) emphasized the importance of exploring how the effects of stream nutrient enrichment are propagated through the food web to influence biotic integrity and the associated ecosystem benefits provided by the stream ecosystem. Legitimate concerns exist regarding the addition of nutrients to streams in an era when negative impacts of nutrient loading and subsequent eutrophication are widespread (Miltner and Rankin 1998, Wang et al. 2007). However, nutrient addition has successfully enhanced biological productivity in culturally oligotrophic systems in temperate latitudes, especially in ultraoligotrophic systems (Anders and Ashley 2007). Dodds (2006) and others (e.g., Bourassa and Cattaneo 1998) recognized that increases in invertebrate abundance, such as we found, may be accompanied by losses in diversity that are considered undesirable.

Previous nutrient-enrichment experiments in oligotrophic natural streams and stream mesocosms corroborate our findings that nutrient additions typically increase abundance and biomass of primary and secondary consumers with relatively minor changes in assemblage structure (e.g., Mundie and Simpson 1991, Peterson et al. 1993a, b, Perrin and Richardson 1997, Quamme and Slaney 2003, Slaney et al. 2003, Wilson et al. 2003). Studies involving the addition of salmon carcasses and their analogs also support our Kootenai River results (e.g., Wipfli et al. 1998, Chaloner et al. 2004, Claeson et al. 2006, Kohler et al. 2008, Kohler and Taki 2010). The results of carcass studies are confounded by inclusion of proteinaceous organic matter in the mix of primary nutrients involved, but sufficient overlap exists in the results of these experiments with those of experiments using just N or P to justify their inclusion. Most of these investigators showed positive bottom-up effects on abundance, biomass, and primary and secondary production whether they used nutrients directly or in the form of fish tissue. In nutrient-addition studies, benefits accrue to the fish assemblage after nutrient addition (Johnson et al. 1990, Wipfli et al. 2003). Significant increases in abundance, biomass, and growth of mountain whitefish (*Prosopium williamsoni*) followed nutrient addition in the Kootenai River (Shafii et al. 2010).

Consumer biomass in primary production-based systems typically increases in response to nutrient enrichment when the edibility of primary producers remains high (Rosemond et al. 1993). Most experimental additions of nutrients aimed at enhancing biotic productivity while supporting sustainable and functional assemblages and foodweb functions

have involved much lower treatment levels than those associated with either meso- or eutrophic conditions. Most studies of P fertilization have involved concentrations of 10 to 20 μg/L or less, but very few have been as low as the 3-μg/L in situ concentration targeted in our study. P and N generally have been applied at concentrations and ratios intended to enhance the existing diatom and nonfilamentous chlorophyte algal components while avoiding replacement by blue-green bacteria or undesirable filamentous green algae, such as *Cladophora* or *Spirogyra*, which are associated with decreased food availability and quality for macroinvertebrate consumers (Elwood et al. 1981, Perrin et al. 1987). In the Kootenai River, the target N to P ratio near 20 : 1 and the low concentrations of N and P maintained the diatom-dominated native epilithon with no evidence of blue-green bacteria (Holderman et al. 2009a, Hoyle 2012). Eutrophication can lead to dense, undesirable growth of algae and aquatic macrophytes, resulting in habitat degradation, hypoxia, and degraded macroinvertebrate assemblages. Such conditions are not currently found in the Kootenai River, where mean chlorophyll *a* biomass values in the NAZ (15–30 mg/m^2; Holderman et al. 2009a) are near the oligotrophic–mesotrophic boundary suggested by Dodds et al. (1998).

Macroinvertebrate taxa that respond to increased autotrophic production have short generation times and can respond rapidly at the population level (characteristics of r-strategists) (Newbold et al. 1981). These taxa commonly include Diptera and Ephemeroptera. Chironomidae (Diptera) typically is the predominant taxon that responds to enrichment, followed by Simuliidae, mayfly (Ephemeroptera) nymphs (especially Baetidae, but including Heptageniidae e.g., *Cinygmula*), and occasionally by caddisfly (Trichoptera:Brachycentridae) and riffle beetle larvae (Coleoptera:Elmidae) (Peterson et al. 1993a, Hershey et al. 1988, Deegan et al. 1997, Wipfli et al. 1998, Claeson et al. 2006, Kohler et al. 2008, Kohler and Taki 2010). Most of these taxa, except Chironomidae, are regarded as indicators of good water quality. In our study, increases in most indicators of good water quality (notably Ephemeroptera taxa) offset much of the increases in abundance and relative abundance of Chironomidae that otherwise might have been perceived as a negative indicator. For example, we found that 8 diverse taxa of mayflies occurred regularly among the top 6 Ephemeroptera in the URZ and NAZ and 3 taxa (*E. inermis/infrequens*, *Rhithrogena*, *Paraleptophlebia*) consistently accounted for an average of 82% (range 56–97) of the Ephemeroptera present in the NAZ after fertilization. The presence of large numbers of midges is often associated with reduced water quality, but this association is an oversimplification because Chironomidae often are abundant even in pristine streams, and their dominance is enhanced by natural disturbances, such as wildfire (Williams and Feltmate 1992, Minshall et al. 2001). However, the observed increase in Chironomidae after fertilization in the Kootenai River (Table 2) is consistent with findings of other experimental additions of low-to-medium levels of nutrients to streams and mesocosms (e.g., Perrin and Richardson 1997, Quamme and Slaney 2003, Slaney et al. 2003, Wilson et al. 2003). Chironomids are important prey for fishes, especially juvenile stages (Warren et al. 1964, Power 1992). In the Kootenai River, a postfertilization increase in mountain whitefish abundance was associated with their increased consumption of chironomids (Holderman et al. 2009b, Shafii et al. 2010).

Consistent effects during the first 5 y of fertilization

Macroinvertebrate community metric responses were relatively consistent over the 5 y of nutrient addition reported here, but altered longer-term responses are possible. For example, we found a gradual decline in NCO abundance of 4000 individuals between the 1st fertilized year (2006) and 2010 even though NCO abundance remained ~2× greater than in the prefertilization period. Long-term changes in biological responses or response patterns in the lower Kootenai River could result from: 1) stochastic or planned changes in the Kootenai River ecosystem, including hydropower operations at Libby Dam, 2) temporal variation in precipitation, temperature, and runoff patterns associated with climate change, and 3) changes in fisheries management, including the planned release of hatchery-produced fish. Furthermore, many authors have reported nutrient-addition response patterns after ≥5 y that differed from initial post-treatment responses. For example, Slavik et al. (2004) documented large changes in assemblage structure over a 16-y P-addition experiment in the Kuparuk River after initial post-treatment increases in algal biomass and productivity. Their results suggested that these changes contributed to increased growth rates and densities of some insect species and young-of-the-year (age-0) and adult fish (Peterson et al. 1985, 1993a, b, Deegan and Peterson 1992). Other authors have reported changes in biological production and trophic interactions associated with annual variation in water years and changes in predator–prey relationships over time (Deegan et al.1997, Davis et al. 2010). Thus, an array of empirical findings show that responses to nutrient addition are not always predictable and do not always permeate the food web to benefit higher trophic levels as intended. These studies serve as reminders of the possible unanticipated consequences from long-term nutrient addition in streams and rivers, such as the Kootenai.

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