This manuscript has been peer-reviewed and published as an article in *Freshwater Biology* (DOI: 10.1111/fwb.13478).

Running Head: Consumer response to DOC

Title: Low-level dissolved organic carbon subsidies drive a trophic upsurge in a boreal stream

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Keywords: Allochthony, benthic invertebrate, DOC, productivity, salmonid

Summary

1. Energy pathways in stream food webs are often driven by allochthonous basal resources. However, allochthonous dissolved organic carbon (DOC) is generally viewed as a minor if not insignificant basal resource because much of the DOC pool comprises high molecular weight, recalcitrant compounds and is inefficiently incorporated into biomass. Nevertheless, there is increasing evidence that the relatively small, labile fraction of DOC may indeed fuel microbial activity to a level that stimulates productivity across multiple trophic levels, resulting in a "trophic upsurge." Here, we tested the trophic upsurge hypothesis by subsidizing the labile DOC pool of an Alaskan boreal stream that had relatively high nutrient availability but low levels of naturally occurring DOC.

2. We continuously added ecologically relevant (0.250 mg C/L, ~10% increase above ambient bulk DOC) concentrations of labile DOC (acetate-C) for 62 d to a treatment reach that was statistically indistinguishable in its channel form and chemistry from an upstream reference reach. We measured responses of periphyton production and biomass, whole reach metabolism and nutrient uptake, benthic invertebrate abundances, and juvenile salmonid (Dolly Varden, *Salvelinus malma*) abundance and growth.

3. Measurements of basal ecosystem responses collectively indicated increased energy mobilization at the base of the food web in response to labile DOC addition. Periphyton bacterial production in the treatment reach was generally >1.5x reference reach values, and periphyton ash-free dry mass (AFDM), chl-*a*, and chl-*a*:AFDM were all greater in the treatment reach by the end of the study. Throughout dosing, ecosystem respiration was 1.3x greater in the treatment

reach and dissolved inorganic nitrogen uptake was greater in the treatment reach on eight out of nine measurements.

4. Benthic invertebrate counts, dominated by *Baetis* spp. and Chironomidae, were ~4x greater after 28 dosing days and ~8x greater after 56 days in the upstream portion of the treatment reach. Abundance generally declined with increasing distance from the dosing station. Dolly Varden fry and parr age classes were nearly 2x more abundant in the upstream portion of the treatment reach than in any section of the reference reach and also declined with increasing distance from the dosing station. Further, Dolly Varden tagged with passive integrated transponders prior to the experiment had significantly higher instantaneous growth rates in the treatment reach than those recaptured in the reference reach.

5. The strong consumer responses to small quantities of labile DOC mirrored significant treatment reach increases in basal ecosystem function and therefore demonstrated a response consistent with a trophic upsurge. Terrestrial DOC has historically been viewed as contributing little to metazoan consumers, instead modulating the influence of nutrients and being respired out of a disconnected microbial loop. Because we dosed the treatment reach with a relevant concentration of labile DOC, based on measurements in nearby peatland-draining streams, we suggest that terrestrial DOC deserves more attention as a basal resource for whole food webs, akin to nutrients fueling green (autochthonous) pathways.

Introduction

Ecologists have long recognized that stream ecosystems use and transport significant quantities of terrestrial organic matter, the dominant form typically being dissolved organic carbon (DOC; Fisher and Likens 1973, Dahm 1981, Stanley *et al.* 2012). In comparison to lakes, however, relatively little work has been conducted at relevant temporal and spatial scales to determine the role that terrestrial and wetland (i.e., allochthonous) DOC plays in stream food webs, especially productivity. Much focus has instead been placed on stream ecosystem dependencies on inputs of the particulate fraction of this organic matter, such as leaf litter, which can indeed be a particularly significant resource (Wallace *et al.*, 1999). Instead of being a key resource fueling ecosystem function, DOC is often relegated to the role of a "modulator" of aquatic ecosystem properties because it alters conditions such as light and pH for processes including primary production (Prairie, 2008; Stanley *et al.*, 2012). In contrast, nutrients (e.g., nitrogen and phosphorus) are viewed as the "volume knob" directly controlling the magnitude of ecosystem processes and dominating how biotic ecosystem processes operate (Prairie, 2008). Part of this view stems from a large percentage of the terrestrial DOC pool being resistant to biological degradation on short timescales (Wiegner *et al.*, 2005; Koehler *et al.*, 2012). However, a portion of the DOC pool is in fact labile and readily used by heterotrophic microbial osmotrophs, especially bacteria (Dahm, 1981; Wiegner *et al.*, 2005; Koehler *et al.*, 2012).

Stream consumer energy and biomass are supported to some degree by allochthonous DOC (Hall & Meyer, 1998; Collins *et al.*, 2016a b; Neres-Lima *et al.*, 2017), but limited evidence, especially experimental, exists to suggest that DOC is an important subsidy (i.e., increases consumer production) to stream invertebrate, fish, or other upper-level consumers (Polis, Anderson & Holt, 1997; Brett *et al.*, 2017). Although bacteria and fungi upgrade DOC into consumable biomass, bacteria and fungi contribute weakly to metazoan growth because they lack essential lipids (Guo *et al.*, 2016). In contrast, autochthonous resources (e.g., diatoms) contain essential lipids, so some autochthonous resources are required for consumers relying on poor quality allochthonous resources such as DOC or leaf litter (Tanentzap *et al.*, 2014; Guo *et al.*, 2016; Crenier *et al.*, 2017). Additionally, DOC incorporation into food webs is subject to heavy respiratory losses, such as low bacterial growth efficiency and bacterivorous grazing, so a large proportion of labile DOC assimilated by bacteria is likely never available for metazoan consumers (del Giorgio & Cole, 1998; Hall, Wallace & Eggert, 2000; Berglund *et al.*, 2007; Lischke *et al.*, 2017). Thus, allochthonous DOC may contribute little to stream consumer production.

On the other hand, bacterivorous protists, which do produce lipids essential to metazoan growth, can upgrade the quality of terrestrial DOC-consuming bacteria, better supporting growth of higher trophic levels (Wiegner *et al.*, 2015; Hiltunen *et al.*, 2017); however, this increase in quality incurs respiratory losses of DOC (Findlay, 2010; Anderson, Pond & Mayor, 2017). Additionally, benthic primary production in all but the most closed canopy streams may be adequate to satisfy macroinvertebrate demands for essential lipids (Neres-Lima *et al.*, 2017), allowing allochthonous inputs to supplement algal portions of diets. Whether DOC subsidizes metazoan consumers may depend on the quantity and quality of DOC available to microbes (Faithfull *et al.*, 2011; Hitchcock *et al.*, 2016; Hiltunen *et al.*, 2017), but spatially and temporally appropriate experiments are needed to suggest to what degree environmentally relevant concentrations of DOC can subsidize stream metazoans.

Whole-stream labile DOC additions have been used to examine the role of DOC in streams at spatial scales representative of whole ecosystem responses. Past continuous enrichments lasting longer than one day consistently spurred respiration and nitrogen demand (Bernhardt & Likens, 2002; Johnson *et al.*, 2012; Oviedo-Vargas, Royer & Johnson, 2013), and have even increased macroinvertebrate and fish abundances and production (Warren *et al.*, 1964; Wilcox *et al.*, 2005). While informative, these additions have been extreme in terms of dosing concentrations. Labile DOC is generally not more than 10% of the total stream DOC pool (Kaplan & Newbold, 2003; Berggren *et al.*, 2010; McLaughlin & Kaplan, 2013). Yet, past additions have raised DOC concentrations between 50% and 2000%, at dosing concentrations up to 20 mg C/L. These concentrations more reflect labile DOC inputs from wastewater spills rather than concentrations typically observed across gradients of, e.g., natural or anthropogenic land cover (Stanley *et al.*, 2012), as evidenced by frequently observed blooms of the "sewage bacterium" *Sphaerotilus* spp. during labile DOC enrichments (Warren *et al.*, 1964; Bernhardt & Likens, 2002; Johnson *et al.*, 2012). Similarly, Fuller et al. (2004) reported that their labile DOC enrichment of ~2 mg C/L (~50% increase above ambient) may have been detrimental to some macroinvertebrate taxa due to gill fouling by bacteria.

Extreme labile DOC enrichments also unrealistically overpower energetic inefficiencies in trophic transfer through microbial pathways. They may load ecosystems toward complete bacterial dominance, detrimentally altering conditions or drowning out possible microbial interactions that may occur under more realistic dosing concentrations. For example, algal responses to increased labile DOC availability are often negative, likely because bacteria are excellent competitors for nutrients when they are not dependent on algal-derived DOC (Blomqvist *et al.*, 2001; Bechtold *et al.*, 2012). Responses to extreme enrichments may therefore misrepresent how streams respond to increases in DOC (except in extreme cases) or depend on allochthonous labile DOC. Whole-stream experiments using environmentally relevant concentrations are necessary to answer questions about the role of labile DOC in stream ecosystems and how streams might respond to environmental changes such as browning or land use change (Carpenter, 1996, 1998; Monteith *et al.*, 2007; Stanley *et al.*, 2012; Solomon *et al.*, 2015; Weyhenmeyer *et al.*, 2016).

We added ecologically relevant concentrations of a labile form of DOC to an Alaskan boreal headwater stream that supports juvenile Dolly Varden, an anadromous fish in the family Salmonidae, to estimate how stream consumers depend on and respond to allochthonous DOC. We demonstrated in a companion paper that labile DOC addition subsidized stream microbes, increasing respiration, gross primary production, biofilm production, and whole-stream N demand, also presented here in the context of this study's goals (Robbins *et al*., 2017). We expected this increase in basal ecosystem function to drive a "trophic upsurge" (*sensu* Tanentzap *et al*., 2014) of energy to invertebrates and fish. A trophic upsurge is a bottom-up food web effect, where additional inputs of allochthonous carbon increase heterotrophic carbon mobilization and production at the base of the food web (e.g., bacteria), resulting in greater biomass or production across all higher trophic levels (Tanentzap *et al.*, 2014). A trophic upsurge is therefore subsidization of a food chain (Polis *et al.*, 1997). We hypothesized that added labile DOC would increase densities of fast-growing, multivoltine benthic invertebrate taxa (e.g., chironomids). We also hypothesized that, if multivoltine taxa responded quickly, Dolly Varden fry (age 0+ fish that hatched following snowmelt in late spring) may survive at a higher rate in the treatment reach, translating into higher abundances. Further, we hypothesized that parr (age class 1+; individuals that overwintered for at least one year) would grow faster through increased abundance of invertebrates and conspecific fry, both of which contribute to their diets.

Methods

Site information

We conducted our experiment in 2013 on the western Kenai Peninsula of Alaska in a first-order tributary of the South Fork Anchor River, previously identified as SANC 1203 (King *et al*., 2012, Shaftel *et al*., 2012, Walker *et al*., 2012; 59.77974° N, 151.55518° W; Fig. 1 A&B). We selected SANC 1203 for this experiment for several reasons: (1) well-characterized catchment and water chemistry from previous studies, including cover estimates of wetlands (32 %, largely discharge slope and riparian wetlands that export limited quantities of DOC; Shaftel *et al*., 2012, Walker *et al*., 2012, Whigham *et al*., 2012) and N_2-fixing alder (12.6%; Shaftel et al. 2011, 2012); (2) relatively high nutrient availability, specifically PO_4-P (~20-50 µg/L) related to volcanic deposition in the region and NO_3-N (~200-500 µg/L) directly related to the alder (Shaftel *et al.*, 2012; Callahan *et al.*, 2017); (3) relatively low levels of ambient DOC (1.8 – 3.3 mg/L at baseflow) when compared to peat-rich catchments nearby (10 – 20 mg/L, Walker *et al*., 2012); (4) similar channel width (1.4 m), depth (0.12 m), substrate (gravel-cobble, woody debris), riparian vegetation (bluejoint grass: *Calamagrostis canadensis*) and gradient (5%) over a distance of sufficient length to delineate reaches that would be comparable prior to any experimental manipulation; (5) representative of headwater streams throughout the Kenai lowlands (Whigham *et al.*, 2012) and other boreal regions; and (6) moderate to high densities of juvenile Dolly Varden (*Salvelinus malma*, Fig. 1C), the most widespread and abundant salmonid in headwater streams in this region (King *et al.*, 2012).

Experimental design

We used upstream reference and downstream treatment reaches to assess the effect of labile DOC on stream metazoan consumers. We chose a reach length of 75 m to include sufficient length to represent reach heterogeneity (e.g., multiple riffle-pool sequences), but which also approximated the length over which the labile DOC (acetate) addition might be removed based on median acetate uptake velocities from a whole-stream DOC uptake synthesis (Mineau *et al.*, 2016). The paired experimental reaches were identified based on similarity in width, depth, slope, sinuosity, dominant substrate, riparian topography and vegetation, and water chemistry, resulting in an 80 m intermediate reach that was not part of the study. Gravel and small cobble dominated each reach, and discharge was never measured as more than 4% (0.6 L/s) different between reaches. Wetted width (reference: 1.49 ± 0.26 m; treatment: 1.30 ± 0.30 m (mean ± SD)), depth (reference: 0.12 ± 0.07 m; treatment: 0.12 ± 0.07 m), channel slope (reference: 5.3%; treatment: 4.9%) and sinuosity (reference: 1.07; treatment: 1.06) were statistically indistinguishable between reaches. Dissolved inorganic nitrogen (DIN, almost entirely NO_3-N) at the tops of each reach were typically within 10 µg/L of each other. Similarly, PO_4-P was never more than 3 µg/L different between the tops of the reaches, and background DOC was highly comparable between reaches (usually <<0.2 mg/L different; see Robbins *et al.*, 2017 for detailed nutrient and DOC data). Sampling locations within each reach were designated by meters from the top of the reference (R) and treatment (T) reaches, and weekly water chemistry measures for DOC, DIN, and PO_4-P were taken at the 0, 37.5, and 75 m points in each reach, with an additional 10 m sampling point in the treatment reach (i.e., T10)

DOC was dosed as a solution of sodium acetate ($C_2H_3NaO_2$) from 25 June 2013 (dosing day 1) through 25 August 2013 (day 62). Acetic acid (dissociated acetate in H_2O) is a product of anaerobic wetland and terrestrial processes and so represents a common DOC substrate for microbes in boreal streams (Berggren *et al.*, 2010). We used a model QBG pump (Fluid Metering Inc., Syosset, New York, USA) to deliver dosing stock (75 g/L as C) from a 100-L covered stock tank to the top of the treatment reach (Fig. 1B). Acetate was dosed at a rate of 3.6 mL/min, resulting in a 62-d mean concentration of 250 µg/L acetate-C, about 10% of background DOC in the study stream (mean 62-d DOC, reference reach = 2.52 mg/L). We chose this acetate dosing concentration to mimic labile DOC concentrations found in peatland streams of the western Kenai (~1-1.5 mg/L labile DOC out of 8-13 mg/L total DOC, RDD *unpublished data*), noting that acetate is part of the most bioavailable fraction of the labile DOC pool and lacks the light attenuation potential of natural DOM mixtures (see Discussion). However, 250 µg/L is representative of acetic acid-C concentrations measured in other boreal streams (Berggren *et al.*, 2010).

Weekly estimates of dosed acetate-C, based on discharge fluctuation and confirmed dosing rates, were 142 to 324 µg/L (median= 264 µg/L, mean=250 µg/L). Concentrations fluctuated proportionately with discharge, which ranged from 13.9 L/s to 31.7 L/s during the dosing period. We did not attempt to maintain a constant dosing concentration of acetate-C because DOC naturally fluctuates with discharge, typically being diluted by surface-water runoff in these headwater streams (RSK, unpublished data). We estimated the flux of dosed C into the treatment reach using the change in total DOC concentration from the top of the reach (based on known discharge, dosing rate, and background DOC concentrations [T0]) to the bottom of the reach (T75; data in Robbins *et al.,* 2017). DOC was measured using a Shimadzu TOC-VCSH (Tokyo, Japan). Sample loss between collection and analysis precluded direct measurement of acetate concentrations.

Basal ecosystem response

Basal ecosystem responses and detailed methods presented in this study, except periphyton biomass and its methods, were documented in Robbins *et al*. (2017). In addition to periphyton biomass, we present these basal responses (and limited methods) here to demonstrate an experimental ecosystem response to labile DOC from the bottom-up (i.e., increased ecosystem energy mobilization). However, our goal is not to trace the fate of DOC in terms of a detailed ecosystem budget or determine the relative contributions of different pathways by which energy was transferred to higher trophic levels, which go beyond our data.

Ecosystem metabolism was measured using the one-station diel oxygen change method with propane evasion to correct for reaeration (Bott, 2006). Dissolved O_2 concentrations were logged with YSI EXO1 sondes (YSI, Inc., Yellow Springs, Ohio, USA) placed at the downstream end (75 m) of each reach on dosing days -4 - 0 (pre-dose), 9 - 13, 23 - 27, and 37 - 41. Daily gross primary production (GPP) was calculated as the area under the curve of corrected oxygen change above the mean nightly respiration rate, measured using the rate of decline in oxygen at night. Daily ecosystem respiration (ER) was calculated by scaling the nightly respiration rate to 24 hours. Night periods were determined by nearby NOAA station (Homer, AK) 0 PAR measurements.

Dissolved inorganic nitrogen (DIN) and PO_4-P net uptake were calculated each week in each reach following Webster & Valett (2006). We measured ambient nutrient concentrations in triplicate at each sampling location once weekly during the study (dosing days -21, -14, -6, 1, 8, 14, 22, 28, 36, 42, 50, and 56). We regressed the log-transformed nutrient concentrations against distance downstream from the top of a reach (0, 37.5, 75, plus a 10 m point for the treatment reach), where the inverse slope of the regression is the uptake length (S_w). To standardize for differences in reach characteristics, we used the depth (z) and stream velocity (u) to calculate uptake velocity (V_f) for each nutrient as $V_f = uz/S_w$. V_f is therefore a measure of nutrient demand that is comparable across stream reaches.

Bacterial biomass production (BBP) and photosynthesis (PS) of periphyton were measured following methods in Scott *et al*. (2008). Seven medium-sized gravel rocks were collected at R10 and T10 on dosing days 14, 28 and 56. Gravels were incubated with site water from each reach in 60 mL jars, with one formalin killed control, three foil-wrapped 'dark' incubations and three unwrapped 'light' incubations. Jars were placed under grow lights (300-350 µE m^{-2} s^{-1}) in a water bath representative of ambient stream temperatures (10 - 12°C) in the lab in Homer, AK. Periphyton microbial activity was measured by injecting labelled substrates into each jar. PS was measured with ^{14}C-bicarbonate and BBP with ^{3}H-leucine. After two hour incubations, each jar was killed with formalin. Radioactivity of periphyton in each sample was measured by scintillation counting and then related to incorporation of the labelled substrate as a measurement of PS or BBP. Rates of substrate incorporation were standardized to rock surface area.

Periphyton biomass was measured as both ash-free dry mass (AFDM) and algal pigment (chl-*a*) following Biggs & Kilroy (2000). We collected 3-5 large gravel rocks from each sampling location (excluding T0) in each reach on days -9, 28, and 56. Periphyton was scraped from each rock with a toothbrush into a slurry of known volume. For AFDM, an aliquot of periphyton slurry was filtered onto a pre-ashed, pre-weighed glass fiber filter (0.7 µm) and dried for 48 hours at 60 °C. Dried filters were weighed and then ashed at 500 °C for four hours and weighed again. AFDM was determined by subtracting the ashed mass from the dry mass. For chl-*a*, an aliquot of slurry was filtered onto another glass fiber filter, placed in a vial with EtOH, heated in a water bath at 85 °C until boiling, and left at room temperature in the dark overnight. We measured absorbance of the chlorophyll extract on a Lambda 35 UV/Vis spectrophotometer (Perkin Elmer, Inc., Waltham, Massachusetts, USA). Absorbance was converted to chl-*a* concentration using known equations (Biggs & Kilroy 2000). We standardized AFDM and chl-*a* to rock surface area.

Benthic invertebrate sampling

We sampled benthic invertebrate assemblages by placing five Hester-Dendy (HD) artificial substrate samplers (0.16 m^2 sampling area; Wildco, Buffalo, New York, USA) at sampling locations 5-10 m, 35-40 m, and 70-75 m downstream from the top of each reach (15 samplers per reach). We placed HDs at 5-10 m, rather than 0-5 m, to ensure adequate solute mixing had occurred before water reached the HDs. HD samplers allowed us to estimate invertebrate response to DOC additions on a standardized substrate similar to the woody debris and submerged riparian roots found throughout both stream reaches without disturbing the benthos. We considered alternative quantitative methods (e.g., Surber, Hess samplers), but we deemed them excessively disruptive to other key benthic measurements given the relatively small size of the stream and the fact that we already had disrupted the substrate on day (-)17 during backpack electrofishing (see next).

We secured HDs to cobbles with zip-ties and distributed them longitudinally (~1 m apart) along the thalweg at each sampling location. We deployed HDs on dosing days 1 - 28, and days 29 - 56. We did not attempt to deploy samplers pre-dosing because 1) we had disturbed substrate by wading in the stream during electrofishing and 2) there was not sufficient time between spring runoff and the first day of dosing for colonization of HDs, as 28 d is the standard deployment time for HDs (King & Richardson, 2008). HD samples were collected by gently lifting the HD off the stream bottom and simultaneously placing a 250 μm mesh sieve under the submerged HD prior to removal from the water. HDs were then put into a large storage bag and immediately stored on ice. Upon return to the laboratory, invertebrates were removed from disassembled HD samplers with a toothbrush while rinsing with tap water into a 250 μm sieve and stored in 5% buffered formalin (v/v) for later identification. Due to loss of sample during transport, some within-sampling location replication was lost on day 28, with a minimum of two HDs per sampling location (all but one location had N=3 or more; Table S1). Individual invertebrates were counted and identified to the lowest practical taxonomic unit, typically genus. Chironomidae (non-biting midges) and Naididae (small Oligochaeta) were identified at the family level, whereas Amphipoda (scuds), Hydrachnidia (water mites), Nemata (nematodes), Ostracoda (seed shrimp) and Turbellaria (flatworms) were identified at these coarser levels of taxonomic classification.

Salmonid sampling

We sampled juvenile Dolly Varden (Salmonidae: *Salvelinus malma*) by three-pass electrofishing with a Smith-Root LR-24 (Smith-Root, Inc., Vancouver, WA, USA) in three subsections of each reach (0 - 25 m, 25 - 50 m, 50 - 75 m) on dosing days (-) 17 and 62. Each subsection was separated at the top and bottom with a block net (4 mm mesh) that was secured tightly to the benthos and stream bank with stakes, and weighted down with large cobbles to eliminate any gaps. We checked block nets for trapped fish at the end of each of the 3 passes. Captured fish were placed in an aerated bucket, anesthetized with 70 mg/L tricaine methane sulfonate (MS-222), measured for fork length and weight (g), and released. We expressed fish abundance as total observed counts and not as densities (no./m^2) because reaches were identical in length and not statistically different in mean width (reference: 1.49 ± 0.26 m; treatment: 1.30 ± 0.30 m). Further, we did not use depletion models to extrapolate observed counts per pass to total fish counts because these models are biased and unreliable (Rosenberger & Dunham, 2005).

Parr (year 1+ or older) captured on day (-)17 were tagged with 8.4 mm passive integrated transponder (PIT) tags (Biomark MiniHPT8, Boise, ID, USA; Bailey et al. 1998, Chittenden et al. 2008). We tagged 39 parr from each reach (78 total), and PIT tags were used for mark-recapture estimation of growth rate.

Data analysis

We report response ratios for each basal ecosystem response as the treatment reach mean divided by the reference reach mean, and tested for reach differences between those means. Error in DIN and PO_4-P V_f estimates was determined by propagating slope error from the regression models to V_f and calculating 95% confidence intervals (CIs). We concluded that V_f was significantly different between reaches on a given date when the 95% CIs for the slope differences did not overlap zero. Between-reach differences in GPP and ER were determined by treating daily measurements as replicates because our methodology produced no error estimate for daily metabolism rates. Thus, we compared GPP and ER rates between reaches within measurement periods (e.g., days 9 – 14) using generalized least squares (gls) modelling that included a variance weighting function (varIdent) to allow variance heterogeneity by date range (Zuur *et al.*, 2009). We tested for differences in AFDM, chl-*a*, and chl-*a*:AFDM using random effects models that also included variance weighting functions (varIdent) by sampling date. We treated sampling location as a random effect after observing that sampling location was not meaningfully related to within-reach AFDM, chl-*a*, and chl-*a*:AFDM means or variances (i.e., no clear pattern relating periphyton biomass to within-reach location). Inclusion of random effects and variance weighting was based on model comparison using AIC and analysis of model residuals. We performed generalized least squares (gls) and mixed modelling (lme) in R package nlme (Pinheiro *et al.*, 2019).

We tested for treatment and sampling location (distance) effects (e.g., gradient response) on counts of total invertebrates and the taxa representing 95% of the total abundance (N = 5 taxa) by fitting generalized linear models (GLMs) with counts on each HD as the response variable, with date nested within a reach*distance interaction. We hypothesized that there would be no effect of distance in the reference reach, whereas, if added DOC had any effect on abundance, we would also observe the highest values at 5-10 m and a decline in abundance down the length of the treatment reach. Such a gradient response is considered strong evidence for attributing effects of experimental manipulations in unreplicated experiments (Barley & Meeuwig, 2017). Based on the distribution of taxon counts, overdispersion, residual deviance and AIC for each model (compared to simpler models), we specified GLMs with negative binomial error families and log link functions (Zuur *et al.*, 2009). We chose not to employ multivariate analyses (e.g., mvabund; Wang *et al*., 2012) because we were most interested in detecting increases in abundance of dominant taxa that could fuel growth and abundance of Dolly Varden rather than changes in species composition, which we did not expect. Hester-Dendy samples were replicates by distance (5 m, 37 m and 75 m), reach (reference, treatment), and date (dosing days 28 and 56). Invertebrate GLMs were fit using the glm.nb function in the MASS package (Venables & Ripley, 2013) in R (version 3.4.3, R Core Team, Vienna, Austria). Post-hoc multiple comparisons were performed using pairwise contrasts with Tukey correction (emmeans package;

Lenth 2018). No mean counts for any modelled taxa were significantly different between reference reach locations (all $P>0.05$), so we presented treatment/reference post-hoc contrasts only between corresponding reference and treatment locations (e.g., T5 and R5) for simplicity. That is, reference reach counts were statistically homogeneous for modelled taxa, so it is roughly equivalent to compare the counts at a treatment reach location to counts at any reference reach location.

We tested for differences in Dolly Varden fry (age 0+) and parr (age 1+) abundances between reaches using chi-squared tests, where the 'expected' abundance was an even distribution of the observed total abundance across both reaches (i.e., a 50-50 split). We also tested for differences in size-frequency distributions by reach using Kolmogorov-Smirnov tests and visualisation of empirical cumulative distribution functions.

Instantaneous growth rates (IGRs) were used to quantify the rate of change of mass for each Dolly Varden parr that was recaptured in the same reach it was initially tagged prior to dosing. Only one recaptured individual migrated between reaches (moved from reference to treatment). IGRs were calculated as $[\ln(W_f)-\ln(W_i)]/t$, where W_f is weight (g) at recapture, W_i is weight (g) at initial capture and t is growth period (79 days for all recaptured individuals; Hopkins 1992). Because we expected Dolly Varden growth rates to decrease with increasing size, we used IGRs to provide a comparable measure of growth across differently sized individuals (Elliott, 1975; Hopkins, 1992). We suspected that individual growth responses could also be size-dependent (e.g., gape can influence prey size), so we used $\ln(W_i)$ as a covariate in ANCOVA regressions (IGR predicted by Reach*$\ln(W_i)$; lm function in R) to control and test for differences in initial weight by reach. We interpreted a significant Reach factor and/or

Reach*ln(W_i) interaction as evidence for differences in salmonid growth between reference and treatment reaches.

For all statistical hypothesis tests, we set α=0.05.

Results

DOC Flux

Our nominal dosing concentration, chosen based on observed concentrations of labile DOC in nearby peatland-draining streams, resulted in the addition of 20 kg acetate-C to the treatment reach over the course of the experiment. Changes in DOC concentration through the treatment reach indicated total removal of added DOC on most dates, and substantially more than the dose was taken up on some dates suggesting a 'priming' of the ambient DOC pool (see Robbins *et al.*, 2017). Thus, we estimate that 20-25 additional kg DOC were taken up in the treatment reach compared to the reference reach.

Basal ecosystem

Each basal ecosystem response (BBP and PS were not measured pre-dosing) was largely similar across reaches pre-dosing, conforming to the similarity in reach physicochemical characteristics, and most responded to DOC addition at some point during the study (Table 1). Throughout DOC addition, ER was ~1.3x higher in the treatment reach relative to the reference reach, whereas treatment GPP did not respond significantly through any measurement period (this does not exclude positive or negative responses on particular days within each measurement period). BBP was ~2.6x greater in periphyton in the treatment reach on days 16 and 56, but not significantly different on day 30. PS was never significantly different between reaches.

Periphyton AFDM and chl-*a* were 2.5 and 18.5x greater, respectively, in the treatment reach on day 56, but similar on dosing days -9 and 28. Similarly, the ratio of chl-*a*:AFDM in periphyton was similar between reaches on dosing days -9 and 28, but 6.7x greater in the treatment reach on day 56. DIN V_f was generally 1.5 to 3.5x greater in the treatment reach during dosing, with eight out of nine sampling dates having significantly greater DIN V_f with DOC addition. PO_4-P V_f was never significantly different between reaches, even after dosing, and was rarely ever measurable in either reach.

Invertebrate response

Early instar or young larvae of just a few taxa dominated the invertebrate communities on the HD samplers. By far, *Baetis* spp. (Ephemeroptera: Baetidae; a small, multivoltine mayfly nymph) and Chironomidae larvae (Diptera; non-biting midges) were the most abundant taxa, comprising 46% and 39% of total benthic invertebrates, respectively (Table S1). Naididae (Oligochaeta, freshwater worm), *Zapada* nymphs (Plecoptera: Nemouridae; a uni- or semi-voltine stonefly), and *Cinygmula* spp. (Ephemeroptera: Heptageniidae; a univoltine mayfly) nymphs comprised 5, 4%, and 1% of the total benthic invertebrates, respectively. Chironomidae was 80% of the total abundance on day 28, *Zapada* comprised 7% of the total abundance on day 28, with no other taxa comprising more than 2% of the day 28 total (Table S1). Small size-class individuals comprised a slightly larger proportion of the counts in the treatment reach on day 28 (Reference: 84%, treatment: 88%) and day 56 (reference: 93%, treatment: 97%).

Modelling of the taxa comprising 95% of benthic invertebrate abundance suggested generally positive responses to the DOC addition. Both *Baetis* and Chironomidae strongly responded to the DOC addition. *Baetis* nymphs exhibited significant gradient effects on both days 28 and 56, where the treatment response to DOC addition was greatest near the dosing station (T5) and weaker downstream (Fig. 2). *Baetis* were estimated as ~75x more abundant at T5 than R5 (P=0.016), which had only one individual, on day 28. On day 56, *Baetis* nymphs were 16.5x and 8.5x more abundant at T5 and T37.5 than the corresponding reference reach locations, respectively (both P<0.0001), with no significant response at T75 (Fig. S2). Chironomidae larvae generally had higher abundances at T5 compared to T37.5 and T75 on both sampling dates, suggesting a slight gradient effect (Fig. 2). On day 28, Chironomidae were 5x and 3.5x more abundant at T5 and T75 compared reference reach locations (both P<0.01). On day, 56, Chironomidae were 5.6x and 4.7x more abundant at T5 and T37.5 than the corresponding reference reach locations (both P<0.0001). Throughout the study, Naididae were 2.5x greater in the reference reach compared to the treatment reach (P<0.0001), with no effect of sampling location (Table S2). *Zapada* spp. responded positively to DOC addition only on day 28 at T37.5 (P=0.029, Table S2). *Cinygmula* spp., however, did not respond significantly to the DOC addition (Table S2). Other identified taxa were generally more abundant in the treatment reach, and no identified taxon was considerably less abundant in the treatment reach compared to the reference reach (Table S1).

The response of the total benthic invertebrate community on the HDs corresponded strongly to the response of the dominant taxa (*Baetis* and Chironomidae), and overall suggested a strong gradient response to the DOC addition, as well (Table S2). On day 28, total invertebrates were ~4x more abundant at T5 than R5 (P<0.001) and 3x greater at T75 than R75 (P=0.013). On day 56, total invertebrates were 7.7x greater at T5 (P<0.0001), 4.8x greater at T37.5 (P<0.0001), and 2.7x greater at T75 (P=0.0092) than corresponding reference reach locations.

Fish response

Dolly Varden abundances for both parr and fry age classes were highly similar between reaches pre-dosing (fry χ^2=0.129, P=0.719; parr abundances identical between reach), with low numbers of very small fry in both reaches because they had recently hatched and were either just emerging or were too small to capture by electrofishing (Fig. 3). There were 39 parr in each reach during pre-dosing, and 25 and 21 fry in the reference and treatment reaches, respectively (Table 2). On day 62, both reaches had considerably higher parr and fry abundances due to immigration and fry emergence through the growing season; however, both fry (χ^2=36.23, P<0.0001) and parr (χ^2=11.63, P=0.0007) abundances were ~2x greater in the treatment reach compared to the reference reach (Fig. 3). Both fry and parr qualitatively followed a gradient response in the treatment reach, with the total abundance declining from 174 fish in the top subreach (0 – 25 m) to 104 fish in the bottom subreach (50 – 75 m). Total Dolly Varden in the reference subreaches ranged from 81 – 86 (Table 2). Length-frequency cumulative distribution functions for each age class and date were statistically similar between reaches (Fig. 3, KS-test, all P>0.2), indicating that we did not detect a difference in overall parr or fry lengths between reaches.

We recaptured 39 of the 78 Dolly Varden parr that were captured and PIT tagged pre-dosing. Twenty and 19 parr were recaptured in the treatment and reference reach, respectively. Except one individual that migrated from the reference reach to the treatment reach (excluded from growth analysis), each individual recaptured at the end of the study was found in the reach where it was initially captured. Young Dolly Varden tend to have high reach fidelity (Bryant *et al.*, 2009). We therefore calculated IGRs for 19 individuals in each reach.

After controlling for initial size, there was a highly significant reach effect on Dolly Varden growth (Fig. 4, Reach*log Initial Weight interaction P=0.006). Individual fish that were larger than average prior to dosing were more likely to have higher growth rates in the treatment reach than in the reference reach. The smallest individuals prior to dosing had similar IGRs by reach (Fig. 4). IGR for individuals in the reference reach significantly decreased with initial size.

Discussion

Our study demonstrates that environmentally relevant concentrations of labile DOC can subsidize stream consumers, a result consistent with the trophic upsurge hypothesis (Tanentzap *et al.*, 2014). Metabolism, nutrient spiraling, and periphyton biomass (basal responses) showed increased energy mobilization and acquisition of nutrients into the base of the food web in response to labile DOC addition (Robbins *et al.*, 2017), which elicited strong increases in invertebrate abundance, fish abundance, and some fish growth. This result is particularly compelling because DOC is generally thought to be inefficiently transferred to metazoan consumers, with much of it being respired rather than supporting biomass production of basal energy sources needed to fuel a trophic upsurge (Faithfull *et al.*, 2011; Hitchcock *et al.*, 2016; Hiltunen *et al.*, 2017). Although a detailed C mass balance and stable isotope tracing would yield useful mechanistic information regarding our experimental responses, we did not measure stable isotope compositions and are unable to estimate invertebrate masses or inputs of C in the form of drift and migration of invertebrate and fish biomass, and thus cannot calculate *in situ* production rates. However, the fact that we observed substantial increases in macroinvertebrate and fish consumers, as well as fish growth rates, implies that even small concentrations of labile DOC could supply an appreciable quantity of energy (C) to stream consumers.

Benthic invertebrates were ~8x more abundant near the dosing station in comparison to any reference reach sampling locations at the end of the study (August). Bacterial C, derived from labile DOC, can be a dominant C source for stream macroinvertebrate consumers (Hall & Meyer, 1998; Collins *et al.*, 2016b), and the few labile DOC addition studies including macroinvertebrate responses have shown that labile DOC can subsidize macroinvertebrate populations, albeit at very high dosing concentrations (Warren *et al.*, 1964; Fuller *et al.*, 2004; Wilcox *et al.*, 2005). The relatively moderate treatment responses in July (day 28) compared to August (day 56) suggest a time lag for the system to translate increased microbial production into increased invertebrate abundances, or for invertebrates to respond reproductively. Large increases in *Baetis* spp. and Chironomidae densities in the treatment reach supported our hypothesis that small-bodied, multivoltine taxa would display the strongest responses to the C addition. More numerous populations of multivoltine taxa in the treatment reach might have translated increased survivorship or growth from a bolstered resource base to increased reproductive output, because the common uni- or semi-voltine taxa (*Zapada* spp. and *Cinygmula* spp.) did not strongly increase in response to labile DOC addition. The increase in *Baetis* spp. and Chronomidae was dominated by small size-class individuals, implying increased survivorship of recently hatched nymphs/larvae, rather than strong immigration from upstream drift. Further, the Chironomidae were dominated by Orthocladiinae and tube-making Tanytarsini (RSK, personal observation), which do not drift much.

Substantial increases in macroinvertebrate abundances led to a near doubling of Dolly Varden abundance nearest the dosing station. This increased carrying capacity for Dolly Varden, particularly fry, was likely sustained by the considerable numbers of *Baetis* spp. and Chironomidae. Dolly Varden feed on both drifting and benthic individuals of these taxa (Nakano

& Furukawa-Tanaka, 1994; Nakano, Miyasaka & Kuhara, 1999). Juvenile salmonid production was nearly seven times greater in labile DOC-enriched reaches of an experimental stream in one other study (Warren *et al.*, 1964). That study employed screens to avoid movement of yearling trout stocked at low densities (compared to the densities found here), whereas our study reaches were unconstrained and fish were free to move into, throughout or between reaches. Dolly Varden in our study also congregated closer to the DOC source, where invertebrate counts were highest, suggesting that the increased macroinvertebrate abundances sustained greater Dolly Varden abundance. Increased Dolly Varden abundance may have been due to both increased migration to food-rich habitat and increased survivorship from a bolstered resource base.

In that light, parr growth improved in the treatment reach, specifically in initially larger individuals. Increased salmonid growth suggests a strong subsidy effect of labile DOC at the top trophic level for this system. Larger individuals may better exploit the enhanced invertebrate resource base, possibly outcompeting smaller individuals for space and food (Abbott, Dunbrack & Orr, 1985). Wipfli et al. (2003) also suggested growth rates of coho (*Oncorhynchus kisutch*) fry in experimental streams enriched with salmon carcasses were size dependent due to a dominance hierarchy where larger fry could acquire food faster than smaller fry. Similarly, larger parr might have had larger mouth gape to cannibalize the abundant conspecific fry in the treatment reach as an additional, highly nutritious food source. Other juvenile salmonids prey on salmonid fry, and this predation can increase as the predators increase in size relative to the fry, suggesting that larger individual fish can exploit the availability of larger food items (Ruggerone & Rogers, 1992; Pearsons & Fritts, 1999; Nowak *et al.*, 2004).

Our study was designed to experimentally simulate one role of a specific landscape element (peatlands) in the context of another specific landscape element (alder). We chose a study stream with a catchment comprising a high proportion of upland alder stands (N_2-fixing terrestrial plant) and low catchment peatlands, leading to high inorganic N and low DOC. Additionally, we chose an acetate dosing concentration to approximate the labile DOC found in nearby peatland-dominated streams. High N availability probably facilitated some of the observed responses, because nutrients increase microbial growth efficiency on DOC and can also boost the production of high essential nutrient-containing algae, perhaps in synergy with increased DOC (Robbins *et al.*, 2017). In fact, N rich boreal streams likely contain numerous C limited compartments, while use of labile C in wetland-dominated systems may be limited by low nutrient availability (Burrows *et al.*, 2017). Thus, our study implies the confluence of catchments with attributes that complementarily alleviate biogeochemical limitations (i.e., high peatland but low alder with high alder but low peatland) may form a permanent ecosystem control point that form stream segments with enhanced biogeochemical processing that benefits consumer production (Bernhardt *et al.*, 2017; Robbins *et al.*, 2017). Holistically understanding stream ecosystems, from microbes to top consumers, requires consideration of any specific landscape elements that provide significant basal resources, especially if their flowpaths converge (Laudon & Sponseller, 2018).

Our study strongly demonstrates that environmentally relevant concentrations of labile DOC can play an important role in aquatic food webs, particularly streams. Relatively low concentrations of labile DOC can fuel microbial respiration and growth, in turn subsidizing multiple trophic levels despite known inefficiencies in heterotrophic pathways (Robbins *et al.*, 2017, this study). Though we tout the small dosing concentration in the context of other DOC addition studies, our treatment reach took up all added DOC, an additional 20-25 kg of C (at least 70% was likely respired; del Giorgio and Cole 1998). This emphasizes that small concentrations can constitute large fluxes over time. Further, the dosing concentration is representative of the highly labile fraction of DOC potentially stemming from peatland-dominated streams. So while the observed responses may be an upper bound for natural labile DOC subsidies, some natural systems export this large quantity of DOC over a relatively short period of time, representing a large energetic potential.

Except in oligotrophic lakes where associated organic nutrients increase primary production, DOC is often discounted as a subsidy because the light-attenuating fractions of the DOM pool subtract from high quality algal production (Jones, Solomon & Weidel, 2012; Finstad *et al.*, 2014; Benoît, Beisner & Solomon, 2016; Kissman *et al.*, 2017). Similar limitations likely exist in streams, but consumer production may be more resilient to light-attenuating DOM because of their shallow profiles, especially in riffles, and already high dependencies on allochthonous C due to riparian canopies. Frost *et al*. (2007) added natural (i.e., colored) DOM to shallow stream mesocosms and observed only very minor reductions in benthic photosynthetically active radiation, while also observing increases in chlorophyll-a for much of the study. Further, headwater stream systems are already generally light-limited and higher trophic levels may be better at using allochthonous organic matter than commonly studied lake pelagic consumers. When compared to pelagic lake systems there may be a much higher potential for DOC, particularly its labile fraction as we have shown here, to subsidize rather than subtract from stream food webs. However, this needs testing within the context of a naturally light-attenuating DOM source, because high quantities of labile DOM are usually accompanied by high quantities of colored and recalcitrant DOM.

We contend that further understanding the role of DOC in stream ecosystems, particularly as a subsidy, will require a greater understanding of microbial interactions with light availability and catabolic losses within the microbial loop. Microbial (primarily algal-bacterial) interactions may be the 'gate-keeper' for how and whether labile DOC influences nutritionally important autotrophic biofilms (Scott *et al.*, 2008; Kamjunke, Herzsprung & Neu, 2015), and the microbial loop should control the degree to which inputs of labile DOC are lost as respired CO_2 prior to any trophic upgrading (e.g., incorporation by protozoan bacterivores) that could supply essential lipids. It is interesting to note that we observed a strong preservation of chl-*a* in the treatment reach at the end of the study when light availability was strongly inhibited by riparian vegetation cover. The explanations for this response go beyond the scope of this paper, but it does suggest that labile DOC may indeed interact with autotrophic structure and function in more complicated ways than have been observed in other studies. The interaction of green, autotrophic and brown, heterotrophic pathways needs much more attention to fully understand allochthonous C fate in freshwater ecosystems. Additionally, there is a strong need for future studies to build understanding of how regional sources of carbon (e.g., peatlands) may influence streams through differing, potentially very large, contributions of labile DOC. We suggest allochthonous DOC controls the 'volume' of stream ecosystems more positively than has been appreciated in lakes (Prairie, 2008).

Acknowledgements

Laura T. Johnson made numerous helpful suggestions during the planning stages of the study. Becky Shaftel digitized alder cover using aerial photographs as part of her previous research in this catchment. Terry Thompson, Steve Baird, and many others at the Kachemak Bay Research Reserve provided logistical support that allowed us to complete this project. The Center for Reservoir and Aquatic Systems Research provided analytical support and cost sharing. Project funding came from Alaska Sustainable Salmon Fund 44709 awarded to CM Walker, RS King, and DF Whigham. CJR was partially supported by NSF DEB-1442595. The authors declare that they have no conflict of interest.

Data availability statement: Upon acceptance of this manuscript, data will be archived in a repository such as Dryad.

Conflict of interest statement: The authors declare no conflicts of interest.

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Table 1. Basal ecosystem responses to the acetate addition in this study. Response ratios (treatment reach rate or stock divided by reference reach rate or stock) are presented for whole-stream gross primary production (GPP), ecosystem respiration (ER), dissolved inorganic N (DIN) and PO_4-P uptake velocity (V_f), bacterial biomass production (BBP), and photosynthesis (PS), aforementioned quantified in Robbins *et al.* (2017), and epilithic periphyton biomass (ash-free dry mass, AFDM) and algal pigment concentration (chl-*a*), and their ratio. Ratios >1 indicate response was greater in Treatment reach, while <1 indicates response was greater in Reference reach. PS/BBP and AFDM/Chl-*a* were measured on separate and slightly differently sized rocks. Negative values were unique to PO_4-P, and indicate that one reach's V_f was trending toward net production of PO4, but this never occurred at a statistically significantly level. Asterisks denote significant ($P<0.05$) between-reach differences for periphyton and metabolism responses or non-zero overlapping 95% confidence intervals for differences between reach V_fs. The response ratio for DIN V_f on day 28 is likely inflated because the reference reach value was very low and not different from zero.

Response	Day	Ratio	SE	Difference	Response	Day	Ratio	SE	Difference
ER	-4 - 0	1.08	0.06		**DIN V_f**	-21	1.19	2.54	
	9 - 14	1.33	0.05	*		-14	1.67	0.73	
	23 - 28	1.33	0.09	*		-6	0.58	0.38	
	37 - 42	1.35	0.07	*		1	1.59	0.21	*
GPP	-4 - 0	1.09	0.10			8	2.58	0.88	*
	9 - 14	1.09	0.08			14	1.66	0.36	
	23 - 28	0.85	0.06			22	2.79	0.73	*
	37 - 42	0.87	0.15			28	9.02	7.22	*
BBP	16	2.54	0.76	*		36	2.02	0.63	*
	30	1.60	0.72			42	2.31	1.00	*
	56	2.64	0.79	*		50	3.78	2.21	*
PS	16	1.25	0.88			56	2.20	0.81	*
	30	1.45	0.30		**PO4 V_f**	-21	-3.91	10.6	
	56	0.22	1.40			-14	2.57	3.91	
AFDM	-9	1.04	0.16			-6	-1.73	2.56	
	28	0.97	0.12			1	2.32	6.28	
	56	2.54	0.47	*		8	0.15	0.55	
Chl-*a*	-9	0.88	0.18			14	-1.54	1.97	
	28	1.53	0.46			22	-0.33	2.87	
	56	18.51	5.65	*		28	0.14	1.99	
Chl-*a*:AFDM	-9	0.83	0.11			36	-10.63	34.9	
	28	1.67	0.61			42	-0.61	1.37	
	56	6.67	2.33	*		50	-0.51	0.50	
						56	-0.11	0.38	

Table 2. Dolly Varden (*Salvelinus malma*) parr and fry counts and total masses (g) of individuals collected from each subreach (0-25 m, 25-50 m and 50-75 m) in the reference reach (R) and treatment (T) reach on days (-)17 (pre-dosing) and 62.

		Fry		Parr	
	Subreach	Count	Mass (g)	Count	Mass (g)
Pre-Dosing	R 0-25 m	8	5.9	15	106
(Day (-)17)	R 25-50 m	9	3.9	12	91
	R 50-75 m	8	3.5	12	86
	T 0-25 m	4	1.5	15	138
	T 25-50 m	15	6.5	12	92
	T 50-75 m	2	2.3	12	79
Day 62	R 0-25 m	51	46	30	443
	R 25-50 m	72	66	14	304
	R 50-75 m	61	51	24	373
	T 0-25 m	128	106	46	736
	T 25-50 m	111	103	44	603
	T 50-75 m	80	73	24	383

Figure Captions

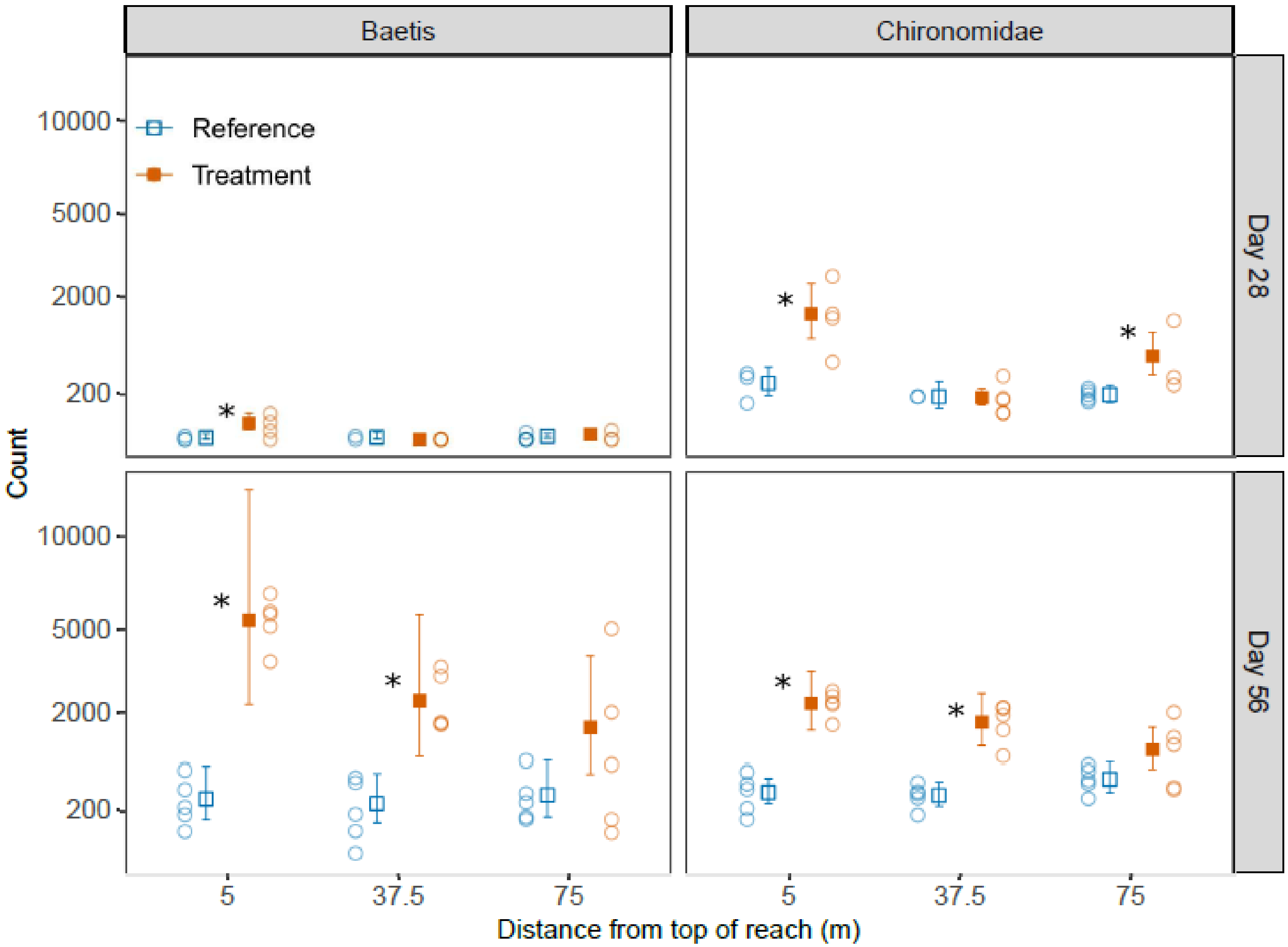
Fig. 1. Photos of the study stream, SANC 1203. A) The upper portion of the treatment reach pre-labile DOC dosing in early June; B) Labile DOC (acetate) dosing station, with protective tarp removed from stock container, at the top of the treatment reach in early August, showing extensive vegetation growth; C) large Dolly Varden (Salvelinus malma) parr (age 1+) captured while electrofishing.

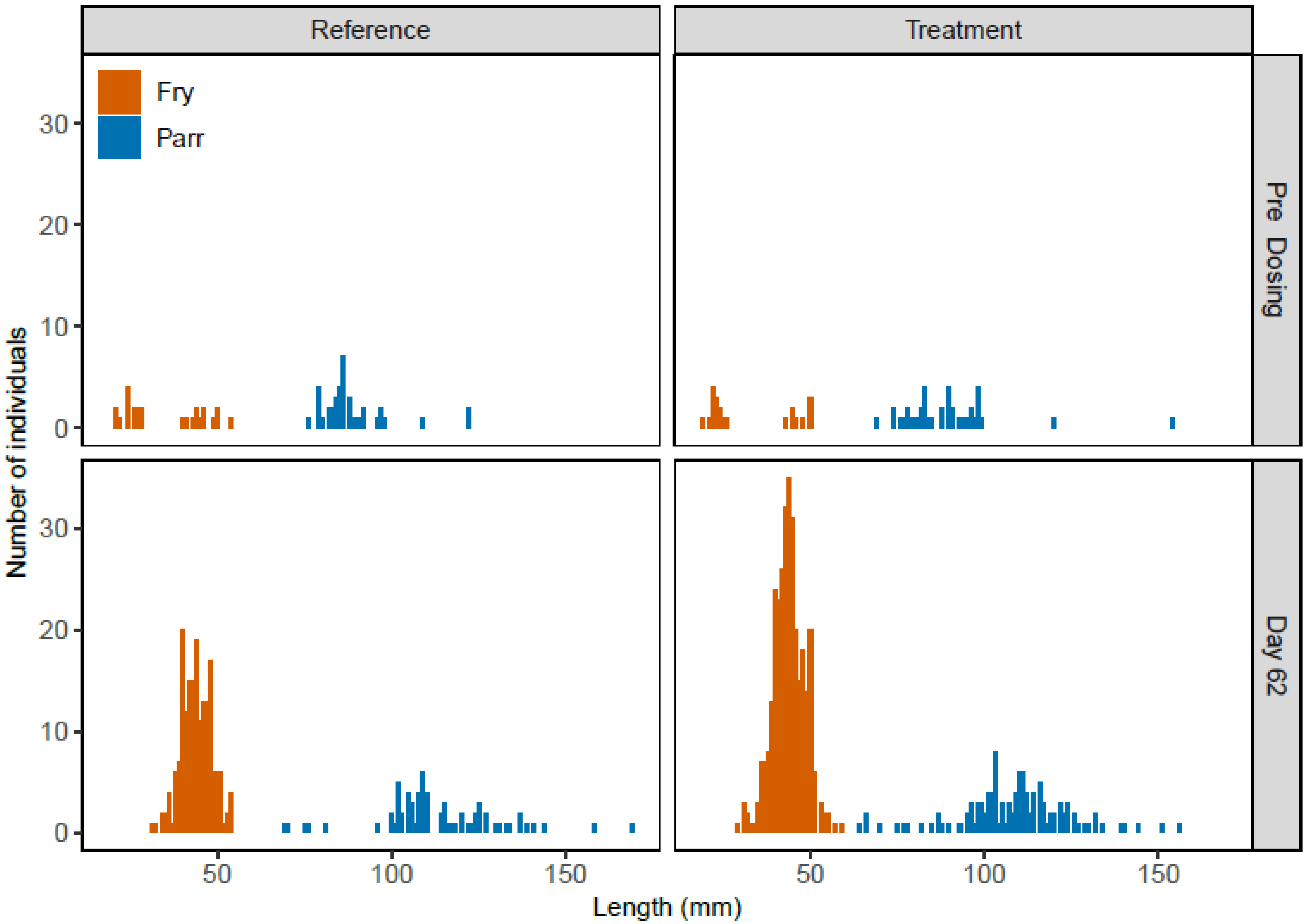
Fig. 2. Counts for Baetis spp. and Chironomidae (two taxa accounting for 85% of total benthic invertebrates counted, Table S1) from Hester-Dendy samplers in the reference (open blue) or treatment (closed orange) reach. Samplers were incubated in the stream reaches from dosing days 1 – 28 and 29 - 56. Samplers were deployed at ~1 m intervals at points 5, 37.5 and 75 m downstream from the top of each reach – each sampler was treated as a replicate from those locations. Circles indicate counts from each individual Hester-Dendy sampler. Squares and error bars are predicted mean and ±1 standard error from negative binomial regressions. Asterisks represent significant ($P<0.05$) post-hoc contrast between treatment mean and reference mean. Because reference reach counts were never different among sampling locations, we compared each treatment reach location’s mean to its corresponding reference reach location for simplicity.

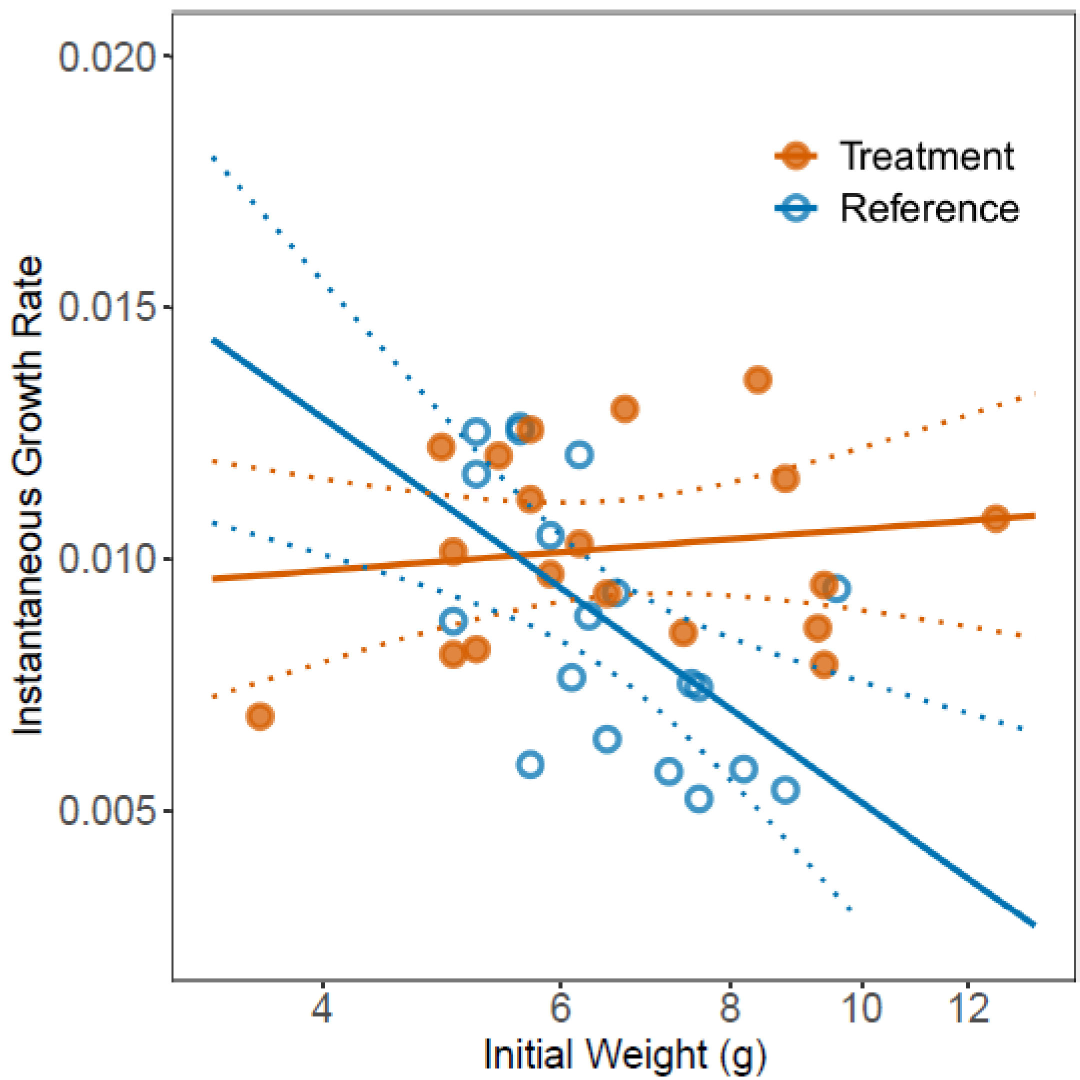
Fig. 3. Dolly Varden (Salvelinus malma) size-frequency histograms for fry (age 0, orange) and parr (age 1+, blue) age classes captured by electroshocking the reference reach and treatment reach on days (-)17 (pre-dosing) and 62 (post-dosing).

Fig. 4. Instantaneous growth rates (IGR) of PIT-tagged Dolly Varden (Salvelinus malma) parr recaptured in the reference (blue open circles) or treatment (orange closed circles) reach (N=19 per reach). All tagged fish were recaptured in the same reach as captured, with one exception that was excluded from this analysis. IGRs are regressed against Dolly Varden weights at initial capture (log-scaled x axis). Solid lines are predicted regression slopes and dotted lines are 95% confidence intervals for the predicted slopes. ANCOVA slopes were significantly different by reach (Reach*log(Initial Weight) interaction P=0.006).


A
B
C
10 20 30 40 50 60 70 80 90 100 110 120 130 140


Baetis
Chironomidae
Reference
Treatment
Day 28
Day 56
Count
10000
5000
2000
200
*
5
37.5
75
Distance from top of reach (m)


Reference
Treatment
Fry
Parr
Pre Dosing
Day 62
Number of individuals
0
10
20
30
50
100
150
Length (mm)


0.020
0.015
0.010
0.005
Instantaneous Growth Rate
Treatment
Reference
4
6
8
10
12
Initial Weight (g)

Supplementary Table (Appendix S1) Legends

Table S1. Counts for all benthic invertebrate taxa identified at each sampling location in reference (R) and treatment (T) reaches, 5, 37.5 and 75 m downstream from the top of each reach. Counts are totals from Hester-Dendy samplers at each sampling location. Due to sample loss in transport, the number of Hester-Dendy samplers counted is given after the sampling location.

Table S2. Summary of GLMs for total benthic invertebrate counts and the taxa comprising the 95% of the total abundance observed on Hester-Dendy samplers pulled at 28 and 56 days into the acetate addition.

Appendix S1.

“Low-level dissolved organic carbon subsidies drive a trophic upsurge in a boreal stream”

Caleb J. Robbins, Alyse D. Yeager, Stephen C. Cook, Robert D. Doyle, Jasmine Maurer, Coowe M. Walker, Jeffrey A. Back, Dennis F. Whigham, and Ryan S. King

Table S1. Counts for all benthic invertebrate taxa identified at each sampling location in reference (R) and treatment (T) reaches, 5, 37.5 and 75 m downstream from the top of each reach. Counts are totals from Hester-Dendy samplers at each sampling location. Due to sample loss in transport the number of Hester-Dendy samplers counted is given after the sampling location.

	Day 28						**Day 56**						
	Reference			Treatment			Reference			Treatment			
Taxon	5 m (N = 3)	37.5 m (N = 2)	75 m (N = 5)	5 m (N = 4)	37.5 m (N = 5)	75 m (N = 3)	5 m (N = 5)	37.5 m (N = 5)	75 m N = 5)	5 m (N = 5)	37.5 m (N = 5)	75 m (N = 5)	Taxon Sum
***Baetis* spp.**	1	1	5	100	0	8	1636	1382	1860	27092	11725	8046	51856
Chironomidae	930	357	979	6194	848	2054	2022	1839	2939	11301	8698	5476	43637
Naididae	0	0	0	0	0	0	711	688	338	1116	1776	1528	6157
***Zapada* spp.**	155	143	197	238	73	313	548	620	519	1037	754	634	5231
***Cinygmula* spp.**	48	53	62	23	93	46	113	129	99	134	169	103	1072
Oligochaeta	8	1	21	23	32	50	27	21	18	148	416	227	992

Ostracoda	16	3	30	8	42	24	53	114	23	160	416	65	954
Amphipoda	5	16	4	31	10	13	54	67	62	84	180	92	618
Acarina	26	1	30	105	32	26	40	59	23	83	62	69	556
Nemata	20	7	33	50	39	20	9	14	24	64	96	77	453
Turbellaria	8	6	8	6	60	58	33	67	28	67	50	42	433
***Neoplasta* spp.**	0	0	9	18	4	3	9	10	46	110	60	22	291
Simuliidae	2	0	2	3	3	7	60	43	22	76	40	31	289
Tipulidae	40	20	43	35	16	39	5	1	0	0	0	5	204
***Rhyacophila* spp.**	9	2	12	10	13	12	25	25	13	17	18	19	175
***Ecclisomyia* spp.**	0	1	3	1	12	18	6	14	0	8	14	75	152
***Isoperla* spp.**	0	0	0	0	0	0	9	20	8	32	6	13	88
Limnephilidae	0	0	0	0	0	0	16	14	5	6	18	3	62
***Ameletus* spp.**	0	0	1	2	6	0	0	4	0	2	0	5	20
Collembola	0	2	1	0	0	0	1	1	0	6	4	3	18
***Epeorus* spp.**	0	0	0	0	1	3	0	1	0	12	0	0	17
Chloroperlidae	0	0	0	0	0	0	1	0	0	3	8	4	16
Tardigrada	0	0	0	0	0	0	0	0	4	8	0	0	12
Copepoda	0	0	0	0	0	0	0	0	4	0	0	1	5
***Psychoglypha* spp.**	0	0	0	0	0	5	0	0	0	0	0	0	5
Psychodidae	0	0	0	0	0	1	2	0	1	0	0	0	4
Perlodidae	0	0	0	0	0	0	0	0	3	0	0	0	3
Coleoptera	0	0	0	0	1	0	0	0	0	0	0	0	1
Drunella doddsi	0	0	0	0	0	1	0	0	0	0	0	0	1
Ectoprocta	0	0	1	0	0	0	0	0	0	0	0	0	1
***Grensia* spp.**	0	0	0	0	0	1	0	0	0	0	0	0	1
Heteroptera	0	0	1	0	0	0	0	0	0	0	0	0	1
Total	1268	613	1442	6847	1285	2702	5380	5133	6039	41566	24510	16540	113325

Table S2. Summary of GLMs for total benthic invertebrate counts and the taxa comprising the 95% of the total abundance observed on Hester-Dendy samplers pulled at 28 and 56 days into the acetate addition.

	Analysis of Deviance Table					**Estimated Marginal Means (**			
Modelled Taxon	Model Parameter	LR Chisq	df	P	Deviance explained (%)	Day	5m Ratio	5m P	3
All	date	152.7	1	**<0.0001**	85.1	28	4.045	**0.0012**	
	distance	21.2	2	**<0.0001**		56	7.73	**<0.0001**	
	date*reach	94.8	2	**<0.0001**					
	date*distance	10.4	2	**0.0054**					
	date*distance*reach	18.1	4	**0.0012**					
Chironomidae	date	46.3	1	**<0.0001**	78.4	28	4.99	**<0.0001**	
	distance	21.9	2	**<0.0001**		56	5.59	**<0.0001**	
	date*reach	80.4	2	**<0.0001**					
	date*distance	19.5	2	**<0.0001**					
	date*distance*reach	21.2	4	**0.0003**					
Baetis spp.	date	204.9	1	**<0.0001**	83.8	28	75	**0.016**	N
	distance	10.8	2	**0.0046**		56	16.5	**0.0003**	
	date*reach	33.3	2	**<0.0001**					
	date*distance	12.4	2	**0.0021**					
	date*distance*reach	12.6	4	**0.0134**					
Naididae	reach	15.2	1	**<0.0001**	38.0	28	NA – No naidids obse		
	distance	1.77	2	0.412		56	Only main factor reac Treatment/Reference		
	reach*distance	3.23	2	0.199					
Zapada spp.	date	35.393	1	**<0.0001**	51.9	28	1.15	1	
	distance	1.972	2	0.373		56	1.89	0.656	
	date*reach	2.781	2	0.249					
	date*distance	5.204	2	0.074					
	date*distance*reach	18.347	4	**0.0011**					
Cinygmula spp.	date	7.4154	1	**0.0065**	22.7	No significant Treatment/Ref			
	distance	4.472	2	0.1069					
	date*reach	1.719	2	0.4234					
	date*distance	1.864	2	0.3939					
	date*distance*reach	2.892	4	0.576					