Wood mitigates the effect of hydropeaking scour on periphyton biomass and nutritional quality in semi-natural flume simulations

Matthew J. Cashman*[1,2,3], Gemma L. Harvey[1], Geraldene Wharton[1], Bruno Maria Cristina[2],

[1]School of Geography, Queen Mary University of London, Mile End Road
London E1 4NS, United Kingdom
[2]Sustainable Agro-ecosystems and Bioresources Department, Fondazione E. Mach, IASMA Research and Innovation Centre, Via E. Mach 1. San Michele all'Adige (TN), I-38010, Italy
[3]Leibniz Institute of Freshwater Ecology and Inland Fisheries (IGB), Berlin, Germany

*Email address: matthewjcashman@gmail.com

Running Header: Hydropeaking and periphyton nutritional quality
Keywords: Fatty acids, hydropeaking, hydropower, wood, alpine stream, artificial flumes

ABSTRACT

The daily fluctuating discharge from hydroelectric power plants, known as hydropeaking, has been shown to cause catastrophic drift in aquatic insect communities and limit secondary production, but relatively little attention has been given to its effects on periphyton, an important food resource for consumers. We simulated daily 5-hour hydropeaking events over the course of 5 days in spring and summer in an open air, experimental flume system fed by a pristine 2nd order stream in the Italian Alps. We hypothesized that hydropeaking would suppress periphyton biomass and especially nutritional quality (*i.e.* fatty acid content). Hydropeaking resulted in decreased periphyton Chl-*a* and AFDM on tiles, but there was no corresponding loss on wood. Hydropeaking did not alter periphyton elemental nutrient stoichiometry but led to a disproportionate loss of periphyton fatty acid content on both substrates. Ordination of overall fatty acid profiles indicated different periphyton fatty acid profiles by substrate and a shift from physiologically important highly-unsaturated fatty acids to non-essential saturated fatty acids after hydropeaking. These results suggest that hydropeaking may have the potential to depress primary biomass and nutritional quality in downstream ecosystems, and that availability of wood substrate may mitigate part, but not all, of this effect. Since food nutritional quality, especially fatty acid content, has been suggested to be a limiting resource on production in aquatic systems, this may generate an indirect and potentially overlooked limiting effect on aquatic consumers in hydropeaking-impacted alpine rivers.

INTRODUCTION

Hydroelectric power plants fundamentally alter a river's natural flow regime: the seasonality, variability, duration, magnitude, and frequency of flow events which underlie a river's physical processes, biological communities, and ecological function (Poff and Ward 1989; Poff et al. 1997; Bunn and Arthington 2002). The release of water associated with the operations of storage hydropower plants causes a sudden and intermittent, high-frequency (daily – sub-daily), high-intensity (2 – 10-fold or greater increases in discharge) disturbance known as "hydropeaking" (Zolezzi et al. 2011). Hydropeaking results in increased shear stress, rapid changes in water level which can undermine the riverbanks (Grelsson 1985), and wet/dry cycling of the riparian margins that results in vegetation loss and failed establishment of riparian organisms (Northcott et al. 2007). Hydropeaking also degrades fish communities by hampering seasonal migrations and movement to spawning grounds, dewaters and scours redds (Young et al. 2011), increases density-independent fish mortality due to stranding along channel margins (Saltveit et al. 2001; Harnish et al. 2014; Schmutz et al. 2014), and increases daily metabolic expenditures as fish endure high-flow conditions (Finch et al., 2013).

Hydropeaking has also been associated with a longitudinal gradient of decreased benthic invertebrate density and biomass (Céréghino and Lavandier 1998; Céréghino et al. 2002; Céréghino et al. 2004; Bruno et al. 2009; Ellis and Jones 2013). The rapid change in discharge may reduce the availability of benthic habitat (Blaschke et al. 2003; Anselmetti et al. 2007; Bruno et al. 2009) and leave benthic communities vulnerable to increased shear stress, resulting in catastrophic drift (Gore et al. 1989; Gore et al. 1994; Blinn et al. 1995; Céréghino and Lavandier 1998; Bruno et al. 2013; Miller and Judson 2014; Bruno et al. 2015). However, several studies have indicated that the rate of catastrophic drift may be insufficient to account for the reported depletion in the overall benthic community assemblage (Miller et al. 2014). As a result, zoobenthos communities may be limited in hydropeaking-impacted reaches due to other factors besides direct catastrophic removal, and alternative mechanisms need to be explored to account for the longitudinal suppression of these communities.

Understanding how hydropeaking may alter basal food resources for downstream consumers may provide additional insights into the impacts of hydropeaking. In regulated river reaches where impoundments trap detrital inputs from upstream (Blinn et al. 1998), periphyton are likely to assume even greater importance in the trophic base of the food web (Cross et al. 2013).

Periphyton growth and composition are affected by a wide variety of environmental factors, and flow velocity and flood disturbance are considered some of the most important factors regulating primary productivity in river systems (Biggs and Thomsen 1995; Biggs 1996; Biggs et al. 2005; Suren and Riis 2010; Law 2011). While several studies have examined the effect of regulated steady flows on increasing algal biomass (Smolar-Žvanut and Klemenčič 2013; Smolar-Žvanut and Mikoš 2013) and the effect of wet-dry cycling on reducing primary productivity in lateral areas (Blinn et al. 1998), there has been relatively little investigation into the effects of hydropeaking on periphyton (Smolar-Žvanut and Klemenčič 2013; Smolar-Žvanut and Mikoš 2013). Furthermore, little is known about how hydropeaking disturbance may affect periphyton nutritional quality, especially physiologically-important fatty acids.

Algal-derived fatty acids are an integral component of stream food webs (Guo et al. 2016a) and can regulate the efficiency of energy exchange across the plant-animal interface (Müller-Navarra 2008). Polyunsaturated and highly-unsaturated fatty acids (PUFA and HUFA respectively) are hypothesized to be limiting resources in many aquatic systems (Brett and Müller-Navarra 1997; Muller-Navarra et al. 2004; Taipale et al. 2014), and HUFA content of food resources has been linked with growth rates and other fitness measures (*e.g.* survival and fecundity) in various aquatic consumers (Müller-Navarra et al. 2000; Torres-Ruiz et al. 2010; Taipale et al. 2014; Guo et al. 2016b). Importantly, periphyton fatty acid content has been shown to respond to light and nutrients (Cashman et al. 2013; Guo et al. 2015; Guo et al. 2016b), and while fatty acid content is also hypothesized to change with other physical habitat conditions, such as flow, this has not yet been extensively explored in the literature.

Large wood and structural complexity in rivers have been shown to be effective at diversifying local flow patterns (Montgomery et al. 2003), dissipating energy from river flows (Gippel 1995; Curran and Wohl 2003), and slowing flood peaks (Gregory et al. 1985). Large wood has been shown to mitigate flood disturbance for macroinvertebrates (Palmer et al. 1996; Hax and Golladay 1998; Robinson et al. 2004), as wood provides a mosaic of nearby low-flow refugia for less mobile macroinvertebrates (Lancaster and Hildrew 1993), and the structure of large wood itself may passively "catch" drifting invertebrates dislodged from upstream (Palmer et al. 1996). Wood has also previously been shown to increase algal diversity, particularly of shear-stress sensitive taxa, due to the complex texture of the wood surface (Sabater et al. 1998). Therefore,

wood may have the potential to function not only as a refugium for animal species but also to protect periphyton biofilms during scouring high flows.

This study therefore examines the sequences of hydropeaking events on periphyton biofilms and their fatty acid content and explores whether wood mitigates the effect of hydropeaking on periphyton. We tested the following hypotheses:

1) Hydropeaking has a large effect on periphyton resources, as scour from increased discharge is expected to result in reduced periphyton biomass (Chl-*a* and AFDM), elemental nutrient content (C, N, P), and fatty acid content of that biomass.

2) The magnitude of this effect is greater with increasing hydropeaking intensity.

3) Wood substrate mitigates the effects of hydropeaking on both periphyton quantity and quality.

MATERIALS AND METHODS

Experimental setting and design

The experiment was conducted in an experimental, open-air, metal flume system located in the riparian zone of the River Fersina at 577 m a.s.l. (46° 04' 32" N, 11° 16' 24" E) that has been used in an ongoing set of experiments on the impacts of hydropeaking (Bruno et al. 2013; 2015). The Fersina is a 14 km long, snowmelt-fed, 2nd order gravel-bed river with its headwaters at 2005 m a.s.l., drains a 171 km^2 catchment, and joins the Adige River at 181 m a.s.l. in Trento, Trentino Province, Northeast Italy. The open-air, stream-side flume system diverts water directly from the river via a weir into a collecting tank, and the tank feeds five 30 cm wide x 20 m long U-frame metal flumes that contain a sluice gate at the upstream end to control discharge (Figure 1). The flumes are filled to the same depth with two layers of cobbles of approximately 10 cm diameter and a deposited fine layer of silt/sand/gravel has naturally collected around the stones. A baseflow of 0.05 m^3 s^{-1} (velocity: 0.4 m s^{-1}) was established in each flume on the 10th of March 2013, and flume treatments were set up based on a 2x3 factorial design of substrate type (tiles and wood) and hydropeaking intensity.

As in numerous other studies (see Lane et al. 2003 and references therein), unglazed terracotta tiles were used as an artificial hard substrate (Figure 1b; tile surface area: 240 cm^2). Tiles were sampled instead of cobbles since they generate less habitat disruption, reduce sampling effort, and improve sampling precision due to a highly-accurate quantification of the surface area, which

can be difficult to achieve with natural substrates (Lane et al., 2003). We also used wood substrates (wood surface area: 284 cm^2) to be broadly representative of downed pieces of large wood in the channel. In our design, wood pieces were oriented 45 degrees offset from the main line of flow (alternating directions for each subsequent sampling unit), as single-pieces of wood in both natural and restored conditions are frequently oriented with, or slightly offset from, channel flow (Gurnell et al. 2002). Our wood substrate was also fixed to a tile base to prevent movement since downed wood can be stabilized by being buried, braced (Abbe et al. 2003), or fixing to the bed (Roni et al. 2014). The base of both substrates were inserted into the top layer of the channel bed so that the top surface of the tile substrate would not protrude beyond the typical bed level in each flume. The wood substrate, since it was attached to the same tile base, was therefore slightly more prominent in the water column, in accordance with natural systems where wood rests at least in part above the underlying sediment. As a result, wood substrates were explicitly expected to have a different hydraulic effect than tiles and to diversify local flow patterns (Montgomery et al. 2003).

Tile and wood substrates were installed in the flumes 23 days before the experiment, on the 20th of March 2013. Twelve unglazed terracotta tiles were each placed in flumes A and D, and 12 wood substrates were each placed in flumes B and E. Both substrates (6 of each) were placed in flume C. Substrates were not placed in the first 5 m, as well as the last 1 m, of each flume due to turbulences associated with the inlet sluice gate and the flume outlet. All substrates were equally spaced throughout the remaining 14 m of uniform flow in all flumes. Periphyton were allowed to colonize the substrates naturally for the remaining 23 days until the start of the experiment. Starting on the 12th of April 2013, daily hydropeaking events were simulated in the flumes over the course of 5 consecutive days. In flumes A and B, discharge was increased 3x (to 0.15 m^3 s^{-1}), which resulted in a 2.25x increase in baseflow velocity (to 0.9 m s^{-1}). In flumes D and E, discharge was increased 2x (to 0.10 m^3 s^{-1}), which resulted in a 1.75x increase in baseflow velocity (to 0.7 m s^{-1}). Flume C functioned as the control, as it was not subject to hydropeaking and contained both tile and wood substrates. Hydropeaking was conducted by rapidly raising the sluice gate (< 10 s) at the top of each flume until 2x or 3x discharge was reached. Flumes were kept at this increased discharge for five hours (0900 – 1400) and then quickly (< 10 s) returned to baseflow conditions for 19 hours over five days. duration of hydropeaking was chosen to fall within the range of hydropeaking operations that occur on regulated rivers within the study region of Trentino (Zolezzi et al. 2011). Flow velocity was measured before, during, and after each daily simulation in three

different cross sections per flume using a Global Water Flow Probe hand-held current meter (Global Water Instrumentation, College Station, Texas, USA).

The hydropeaking experiment was repeated to account for seasonal differences in periphyton colonization. The first experiment took place in the spring from 12 to 18 April 2013, during peak spring snowmelt, with daily water temperature ranging from 5.7 – 11.1°C. The second experiment was re-started on August 1st, left to colonize over the same timeframe as the first repetition of the experiment, and hydropeaking simulations occurred from 2 to 6 September 2013, with daily water temperature ranging from 14.7 – 18.8 °C.

Periphyton processing and laboratory analysis

Before the experiment on day 1 and at the conclusion of day 5, 3 periphyton samples were collected from each flume for its respective substrate type (3 wood in B/E; 3 tiles in A/D; 3 wood and 3 tiles in C) according to Stevenson and Bahls (1999). The collected suspensions were mixed, kept on ice, and brought back to the lab where they were frozen at -20°C for less than one week and until all samples could be analyzed simultaneously. After thawing, all periphyton samples were homogenized with a hand blender for 5 s and processed within 24 h for biomass (chlorophyll-*a* [Chl-*a*], ash-free dry mass [AFDM]), elemental nutrient content (carbon, nitrogen, phosphorus content, and C:N:P ratios) and fatty acids. Samples for chlorophyll-*a* were filtered onto a Whatman® 47mm GF/F filter (Sigma Aldritch S.r.l., Milan, Italy), extracted in 90% acetone and stored at -20°C in the dark until analysis on a Schimadzu spectrophotometer for phaeophytin-corrected chlorophyll concentrations (Lorenzen 1967). Periphyton carbon and nitrogen were determined from subsamples dried in 9 x 10 mm tin cups using a Thermo Scientific 2000 CHN analyzer. Periphyton phosphorus was determined following a digestion (Solórzano and Sharp 1980) and analyzed as SRP (Kopp and McKee 1979). All stoichiometric ratios were determined on a molar basis. Aliquots for AFDM and fatty-acid analysis were dispensed onto pre-weighed, pre-ashed Whatman® 47mm GF/F filters (Sigma Aldritch S.r.l., Milan, Italy). AFDM samples were dried overnight at 80°C, weighed for dry weight, and ashed at 450°C for 2 hours and re-weighed for final ash-weight. Fatty acids samples were stored at -20°C under N_2 until extraction.

Fatty acid analysis

Fatty acids were extracted following a method adapted from Torres-Ruiz et al. (2007) and originally modified from Parrish (1999). Samples were extracted in 2 washes of chloroform:methanol (2:1 v/v), sonicated on ice, and the chloroform phase was separated for methylation into fatty acid methyl esters with BF_3 (10–14%) at 80°C. Fatty acid methyl esters were suspended in hexane and measured on an Agilent 6890 gas chromatograph with Agilent 5973-N mass selective detector that was fitted with a CP Sil 88 for FAME fused-silica capillary column (100m x 250 µm x 39 µm) set in splitless mode. Carrier gas (He) flow rate was constant at 0.2 mL min^{-1}. Inlet temperature was 300°C, with initial temperature 70°C with an increase of 720°C min^{-1}. The temperature program was started and held at 80°C for 1 min, increased at a rate of 4°C min^{-1} until a temperature of 220°C, maintained for 4 min, then heated at 4°C min^{-1} until 240°C, where it was maintained for a final 15 min. Detector temperature was set at 280°C. Fatty acid methyl esters were identified by retention times and mass spectra in full scan mode previously calibrated with standards: 37-Component FAME Mix (47885-4), PUFA No1; Marine Source (47033) and PUFA No3; Menhaden Oil (47085-4; all Supelco, Germany). Fatty acid data were examined as the standing stock of fatty acids (per substrate unit area), fatty acid concentration as a relevant measure for consumers (standardized per periphyton ash-free dry mass), and as percentage of all quantified fatty acids for determining the total fatty acid profile.

September samples contained low extract volumes due to both smaller seasonal periphyton accrual and an additional subsampling step added to the extraction procedure, which resulted in many fatty acids entirely below the detection-limits of the GC-MS. Samples were lost before being able to be re-run at a higher concentration. As a result, fatty acid data are only presented for April.

Statistical analysis

A two-way repeated measures factorial design examined the effect of hydropeaking intensity and substrate type over time on periphyton biomass, nutrient content, and fatty acid content. Data were analyzed in R statistical software (R Core Group, Vienna, Austria), using a factorial linear model with "season" (April/September), "time" (Before/After), "hydropeaking intensity" (Control, 2x-discharge, 3x-discharge) and "substrate" (Wood/Tiles) as factor variables, and which was examined with the function ANOVA in the R package car (Fox et al. 2011). Post-hoc comparisons were completed using an F test with holm-adjusted P values using the testInteractions function in the R package phia (Rosario-Martinez 2013) and assessed differences in the response between

control and hydropeaking intensities, and before-after the experiment for each substrate. Fatty acid profiles were then ordinated using principal coordinates ordination (PCO), also known as metric dimensional scaling (Gower 1966), based on a Bray-Curtis dissimilarity index. Changes in the fatty acid profile with experimental conditions were examined using a PERmutational Multivariate ANalysis Of VAriance (PERMANOVA) (Anderson 2001), both in R package vegan (Oksanen et al. 2013). Data were log-transformed to best fit statistical assumptions and α was set at 0.05 for all tests.

RESULTS

Periphyton biomass

Periphyton chlorophyll accumulation at the beginning of the experiment was different between both seasons, with nearly double the initial chlorophyll accumulation in April (2.79 ± 0.56 µg cm^{-2}, mean $\pm$ SE) in comparison to September (1.82 ± 0.13 µg cm^{-2}; Figure 2). Across both experiments, the hydropeaking treatments significantly decreased periphyton chlorophyll-*a* (Table 1; Time*Intensity: $F = 4.36$, df = 2, $P = 0.018$), although there was only a significantly difference in the response between the 3x-discharge intensity and control (Time*Control-3x: $F = 8.13$, df = 1, $P = 0.019$). Response during the experiment also varied by substrate type (Time*Substrate: $F = 7.71$, df = 1, $P = 0.008$), as there was a significant loss on tiles (Time*Tiles: $F = 14.5$, df =1, $P < 0.001$), but not on wood (Time*Wood: $F = 0.016$, df =1, $P = 0.901$). On April tiles, chlorophyll concentrations slightly decreased in the control (-25%), while both hydropeaking intensities decreased ~75%, with the 3x-discharge intensity resulting in the lowest absolute Chl-*a* (Figure 2). On September tiles, there was a strong accumulation in the control (117%), while there was a 31% decrease in the 2x-discharge and 51% decrease in the 3x-discharge intensities. No significant interaction between time and month was detected.

The change in periphyton AFDM with hydropeaking were broadly similar to the trends in Chl-*a* (Figure 3). There was a significant loss in AFDM with hydropeaking (Time*Intensity: $F = 5.91$, df = 2, $P = 0.005$), with a significant loss in the 3x-hydropeaking intensity compared to the control ($F = 11.6$, df = 1, $P = 0.004$). These responses also varied depending on substrate (Time*Substrate: $F = 11.6$, df = 1, $P = 0.001$), as there was a significant loss in AFDM on tile substrates (Time*Tiles: $F = 4.26$, df = 1, $P = 0.045$) but an increase in AFDM on wood

(Time*Wood: F = 7.12, df = 1, P = 0.021). No significant interaction between time and month was detected.

Nutrient content and stoichiometry

Periphyton nutrient stoichiometric ratios averaged 190:20:1 in April and 186:14:1 in September. Periphyton C:N did not significantly change with hydropeaking intensity (Time*Intensity: F = 0.556, df =2, P = 0.578), but there was an overall change on the different substrates solely attributable to time (Time*Substrate: F = 12.9, df = 1, P = 0.0008; Figure 4). Pairwise post-hoc analyses indicated a significant decrease in the C:N ratio on tiles (Time*Tiles: F = 5.158, df = 1, P = 0.028) and a significant increase on wood (Time*Wood: F = 7.735, df = 1, P = 0.016). There was no significant difference in the response of C:N by month (Table 1).

Periphyton C:P was variable throughout the experiments (data not shown), but these changes were not predictable for either hydropeaking treatment (Table 1).

Periphyton fatty acids

Total fatty acids per cm^{-2} of substrate broadly followed the trend of change in periphyton biomass: fatty acid standing stock changed with hydropeaking (Table 1; Time*Intensity: F = 5.05, df = 2, P = 0.015), with a significant loss in both 2x (Time*Control-2x: F = 5.94, df = 1, P = 0.046) and 3x-discharge intensities compared to the control (Time*Control-3x: F = 9.29, df = 1, P = 0.017). However, this response varied by substrate (Time*Substrate: F = 7.01, df = 1, P = 0.014), with tiles under hydropeaking exhibiting a loss of 72% of fatty acid standing stock during the experiment (Time*Tiles: F = 6.98, df =1, P = 0.026), but there was no significant change on wood (Time*Wood: F = 1.35, df = 1, P = 0.257).

When fatty acid content was standardized for changes in periphyton dry mass (*i.e.* mg of fatty acids per gram of periphyton), there was a further loss of periphyton fatty acid content by hydropeaking intensity (Table 1; Time*Intensity: F = 6.781, df = 2, P = 0.005) that was not significantly different by substrate (Time*Substrate: F = 1.851, df = 1, P = 0.176). Overall, total fatty acid content per gram of periphyton AFDM increased 58% in the control but decreased by 83% and 53% on 2x- and 3x-discharge intensities respectively, resulting in significant different responses from the control for both the 2x- (Time*Control-2x: F = 11.6, df = 1, P = 0.007) and 3x-intensity (Time*Control-3x: F = 8.45, df = 1, P = 0.016).

Saturated fatty acids (SAFAs) were the most abundant fatty acid category in the initial sampling of periphyton from all flumes (44–50% of all fatty acids), followed by monounsaturated fatty acids (MUFAs; 25-29%), 20-C highly-unsaturated fatty acids (HUFAs; 13-20%), and 18-C polyunsaturated fatty acids (PUFAs; 7-9%). All fatty acid classes were scoured during hydropeaking, and this loss was not different based on substrate (Table 1). However, the loss was not equivalent across classes: the greatest decrease occurred in the physiologically important HUFAs, where HUFA content decreased 90% in the 2x-discharge treatment, 55% in the 3x-discharge treatment, and increased approximately 15% in the control (Figure 5). This change in HUFA content was significantly different by hydropeaking intensity (Time*Intensity: $F = 5.92$, df $= 2$, $P = 0.001$), with a significant decrease in the 2x treatment (Time*Control-2x: $F = 12.8$, df $= 1$, $P = 0.005$) but not in the 3x treatment ($F = 3.34$, df $= 1$, $P = 0.161$). The physiologically-important eicosapentaenoic acid (EPA: 20:5ω3), representing the largest single component of HUFA (82%) and 15% of all fatty acids, also was scoured in hydropeaking compared to the control for both the 2x (-90%; $F = 9.743$, df $= 1$, $P = 0.014$) and 3x intensity treatment (-61%; $F = 5.165$, df $= 1$, $P = 0.047$).

There was also a scour in the sum of all ω3-fatty acids with hydropeaking (Time*Intensity: $F = 6.13$, df $= 2$, $P = 0.007$), with the response in both 2x ($F = 11.1$, df $= 1$, $P = 0.009$) and 3x-intensities ($F = 5.74$, df $= 1$, $P = 0.049$) significantly lower than the control and no difference by substrate ($F = 1.88$, df $= 1$, $P = 0.183$). In addition, there was also a loss of total ω6-fatty acids with hydropeaking ($F = 6.84$, df $= 2$, $P = 0.005$), with significant losses in both 2x ($F = 11.8$, df $= 1$, $P = 0.007$) and 3x intensities ($F = 7.52$, df $= 1$, $P = 0.02$). However, while the periphyton ω3:ω6 ratio decreased during the course of the experiment (Mean: 2.30 ± 0.1 to 1.89 ± 0.09l; Time: $F = 11.1$, df $= 1$, $P = 0.002$), this was not related to either hydropeaking intensity ($F = 2.375$, df $= 2$, $P = 0.115$) or substrate type ($F = 1.691$, df $= 1$, $P = 0.206$).

The total fatty acid profile of substrates undergoing hydropeaking, when ordinated in a principal coordinates analysis, showed distinct fatty acid profiles according to both substrate and before/after the hydropeaking disturbance (Figure 6). The primary axis, which explained 55.8% of the variation, was negatively correlated with essential HUFA (*e.g.* EPA and DHA) and positively with many SAFA (*e.g.* 16:0, 18:0) and can generally be considered a gradient in nutritional quality. The 2nd axis, which explained 13.4% of the variation, was negatively correlated with 14:0 and both trans (18:2ω6t) and cis (18:2ω6c) forms of the PUFA linoleic acid (LIN) and positively correlated

with 16:1 and the trans and cis forms of 18:1ω9. These fatty acid profiles were significantly different before and after the hydropeaking experiment, (PERMANOVA: Time*Intensity: Pseudo-F = 2.93, P = 0.007), with hydropeaking profiles shifting to lower nutritional quality. Fatty acid profiles were also significantly different by substrate (PERMANOVA: Substrate: Pseudo-F = 4.22, P = 0.006), with tile and wood substrates largely separating along axis 2, and with tile and wood substrates having different changes in profiles under hydropeaking disturbance (PERMANOVA: Time*Substrate*Intensity: Pseudo-F = 2.71, P = 0.001).

DISCUSSION

The objective of this experimental study was to examine the potential for repeated hydropeaking to scour periphyton biomass and affect periphyton nutritional quality for aquatic consumers in hydropeaking-regulated rivers.

Periphyton biomass at the start of the experiment was low compared to other studies of naturally occurring periphyton in alpine areas (Biggs and Close 1989), and the increases in control biomass, especially in AFDM, suggest that periphyton accrual had not yet plateaued on all substrates at the start of the experiment. However, even though low biomass biofilms are typically more flow-resistant than larger biomass accumulations (Biggs and Close 1989; Biggs 1996), our simulated hydropeaking of a 3x-increase in discharge still resulted in the scour of periphyton Chl-a on tile substrates after only 5 days. As a result, periphyton that had the opportunity to plateau may even be expected to result in greater scour than the loss exhibited in this study. In contrast, periphyton on wood did not exhibit any significant change in chlorophyll-a content, suggesting wood might mitigate the effect of hydropeaking scour. This might be explained by the increased roughness of the wood surface, increasing the strength of algal attachment (Sabater et al. 1998), as well as the effect of wood on local flows, effectively creating sheltered areas of lower shear stress.

Hydropeaking created the greatest scour effect in the spring replication of the experiment likely due to the patchy colonization by *Hydrurus foetidus* (Villars.) and an observed higher load of suspended sediments in the water column associated with spring snowmelt (Füreder et al. 2001; Lenzi et al. 2003). Spring snowmelt is also typically associated with dislodged *H. foetidus* in Alpine rivers (Robinson et al. 2002), and increased suspended sediments can increase scour and abrasion of other components of the benthic biofilms, particularly of diatoms, whose silica shells are vulnerable to damage by suspended sediments (Delgado et al. 1991; Francoeur and Biggs

2006). As a result, seasonal differences in the sediment load of water released during hydropeaking may further influence the impacts on downstream periphyton communities.

Overall, the ability for hydropeaking to scour periphyton biomass as seen in this study is in accordance with other studies that have demonstrated the link between high flows and periphyton biomass (Francoeur and Biggs 2006; Davie et al. 2012), including the effect of experimental floods released from reservoirs in alpine rivers (Uehlinger et al. 2003). Although experimental floods are generally single-event disturbances, and not a repeated disturbance such as hydropeaking, these floods could depress periphyton biomass by up to 2 km downstream (Jakob et al. 2003). In contrast, hydropeaking events in alpine rivers are daily occurrences, and in our study we simulated daily repeating hydropeaking pulses, totaling over 25 hours of hydropeaking in 5 days. The effect of repeated hydropeaking may possibly cause greater cumulative effects on the benthos than single flood-events, particularly since the daily recurrence of hydropeaking does not provide sufficient time for periphyton to recover, which may take multiple weeks (Jakob et al. 2003). Moreover, the daily variations in discharge due to hydropeaking can be highly irregular due to the request of the energy market and the resulting patterns in hydropower operations. For instance, in the Adige watershed, Zolezzi at al. (2011) report a bimodal distribution of duration of hydropeaking events, with one peak at about 6–8 h and another one around 18 h for single events, corresponding to the of two typical hydropower generation schemes: half-day production occurring in the morning/ afternoon and whole day production, continuously occurring from morning to evening. Furthermore, the hydropeaking intensity in this study (3 fold increased discharge) was low compared to experimental floods (7 − 30 fold) and the range of intensities (2 − 10 fold) typically exhibited with daily hydropeaking in rivers in the Italian Alps (Zolezzi et al. 2011). The actual effect of repeated scour in hydropeaking-impacted rivers may therefore be expected to be greater than those effects seen in this experimental study, and changes to the periphyton community would be expected to continue over longer time-scales. As a result, effects of hydropeaking may not only result in an initial scour, but in a persistently suppressed baseline of periphyton biomass downstream of water released from storage hydropower plants.

While nutrient stoichiometry did not show significant responses to hydropeaking, it did provide some information about the periphyton community: average stoichiometric ratios were greater than the Redfield Ratio of 7 and 119 for C:N and C:P values respectively, suggesting possible growth limitation by one or both nutrients (Hillebrand and Sommer 1999). However, these

ratios do not account for other physical habitat limitations such as flow, which is likely the limiting factor for growth in hydropeaking-impacted rivers. In addition, periphyton C:N decreased on tiles throughout the experiment, regardless of hydropeaking treatment, suggesting further maturation and concentration of nitrogen in the biofilm on tiles. Similarly, periphyton C:N increased on wood substrates during the experiment and may represent wood breakdown and uptake of wood-C by periphyton or the trapping of C-rich fine particulate organic matter.

In contrast, periphyton fatty acids exhibited significant changes with hydropeaking, both as total fatty acids per substrate surface area and as fatty acid content within the remaining periphyton (standardized by dry mass). While the loss of total fatty acids could be expected with the general scour of periphyton biomass, the loss in fatty acid content after standardizing for the scour in periphyton biomass indicates a disproportionate loss of fatty acid content, and thus nutritional quality, in the periphyton. This loss was not limited to common saturated fatty acids, but these losses were seen in physiologically-important ω3 and ω6 fatty acids. Overall, ordination of the entire fatty acid profile resulted in a clear separation from before to after hydropeaking, accounting for a change in the fatty acid profile from HUFAs to saturated fatty acids, and indicate a clear shift in the periphyton community and a loss of nutritional quality for consumers.

The different response between nutrient stoichiometry and fatty acid content is important. The fact that fatty acid content, but not nutrient stoichiometry, responds to physical habitat change is a phenomenon that has been previously noted (Cashman et al. 2013) and suggests that fatty acid content is a more sensitive measurement of disturbance than nutrient stoichiometry. In addition, it has been suggested that fatty acid content is also a more relevant measure of nutritional quality as growth relationships associated with elemental nutrient content have been shown to co-vary with an underlying direct relationship with fatty acid content (Müller-Navarra et al. 2000). As consumers cannot synthesize all physiologically-required biochemical compounds *de novo* and must obtain these essential compounds in their food (Müller-Navarra 2008), the loss of fatty acid content from food resources is extremely important for the function of the overall food web (Guo et al. 2016a). The loss of fatty acid content in downstream periphyton might result in decreased growth rates for macrozoobenthic taxa (Torres-Ruiz et al. 2010; Guo et al. 2016b), and may have effects on other fitness measures such as fecundity and survival, as noted in other aquatic consumers (Müller-Navarra et al. 2000; Taipale et al. 2014). These cascading effects may therefore contribute to the observed longitudinal loss of macroinvertebrates below hydropeaking plants

(Céréghino and Lavandier 1998; Céréghino et al. 2002; Céréghino et al. 2004; Bruno et al. 2009; Ellis and Jones 2013)

Our study examined fatty acid content primarily because it influences nutritional quality, yet fatty acid profiles can also reflect benthic algal species composition (Honeyfield and Maloney 2015), particularly since specific fatty acids are related to specific algal taxonomic groupings (Taipale et al. 2013). As a result, the distinct fatty acid profiles before and after hydropeaking and on tile and wood reflect a shift in overall periphyton composition. Although fatty acids have been used to quantify taxonomic shifts in phytoplankton (Strandberg et al. 2015), this method has yet to be adapted for periphyton communities. However, field studies in the eastern European Alps have demonstrated a decrease of periphyton biodiversity in hydropeaking-impacted rivers (Smolar-Žvanut & Mikoš, 2013). Due to the ability for fatty acids to reflect disturbance and taxonomic change in the total periphyton community, as well as providing information relevant to nutritional quality for consumers and ecosystem function, we recommend the incorporation of fatty acid methods into routine periphyton monitoring.

CONCLUSION

Our experimental study suggests that repeated hydropeaking may decrease algal biomass and reduce the nutritional quality of periphyton for aquatic consumers in hydropeaking regulated rivers. The examination of wood substrates in this study also suggests that hydropeaking-impacted rivers that lack habitat heterogeneity may be particularly vulnerable to flow disturbance, and that wood has the potential to mitigate some of these impacts by retaining higher periphyton biomass, even if hydropeaking can still scour its fatty acid content. Since high-quality periphyton are one of the most important food resources for benthic consumers in river ecosystems (Torres-Ruiz et al. 2007, Guo et al. 2016a), this nutritional limitation may affect ecosystem processes and consumer communities in hydropeaking-impacted rivers.

As this study was a preliminary field experiment, more work is required to confirm if these dynamics translate in hydropeaking rivers. However, these results may have important implications for river management, as a potentially-overlooked, "bottom-up" nutritional effect can be fundamental in shaping the distribution of macroinvertebrate communities below hydropeaking plants. As hydropeaking diminishes the quantity and quality of periphyton, alternative basal food sources, such as seston released from the reservoir during hydropeaking, are likely to increase in proportional importance in the trophic base and may drive a shift in community structure, such as

to collector-filterer taxa (Voelz and Ward 1996). As a result, those taxa unable to feed on transported seston or obtain dietary HUFA requirements through other means, such as increased carnivory (Torres-Ruiz et al. 2010), may preferentially abandon the system due to the effects of scour limiting benthic food resources. These nutritional changes may partly explain the failure of some hydropeaking-impacted rivers to reclaim good ecological status even after restorations. Since many ecohydraulic models are calibrated on steady flow conditions (Person et al. 2014), models may be failing to account for the effect of lost nutritional quality on trophic efficiency and ecosystem production.

This study also provides support for restorations using wood in order to mitigate the negative impacts of hydraulic extremes on instream communities. Although periphyton on wood still proportionally lost fatty acid content, its increased resistance to biomass scour during hydropeaking still results in an increased availability of total fatty acids for downstream consumers. Mitigating the environmental impacts of hydropeaking in Alpine areas, while maintaining the economic and energy generation needs of hydroelectric power, is of vital importance. Operational mitigation measures, such as lowering peak discharge or drawdown range, have high costs and low cost-effectiveness; in contrast, structural mitigation measures are relatively inexpensive and have strong potential to diminish the effects of hydropeaking while having minimal effect on the economics of hydroelectric power (Person et al 2013). Although there are clear concerns around the dislocation of wood during high flows and the potential hazard to downstream infrastructure (see Wohl et al. 2016 and references therein), fixing wood to the channel bed may prevent displacement in downstream regions and provide a feasible and cost-effective structural mitigation measure. Even if the specific hazard of large wood in a hydropeaking river would preclude the use of wood, this study has provided an additional piece of evidence that the homogenization and simplification of river channels has the potential to affect ecosystem function. Improving the availability of wood, and likely other aspects of structural complexity, may therefore potentially mitigate discontinuities in primary production and nutritional quality in hydropeaking-impacted rivers.

ACKNOWLEDGEMENTS

REFERENCES

Abbe TB, Brooks AP, Montgomery DR (2003) Wood in river rehabilitation and management. In: Gregory SV, Boyer KL, AM Gurnell (eds) The Ecology and Management of Wood in World Rivers. American Fishery Society Symposium 37, Bethesda, Maryland, USA, pp 367-389.

Anderson MJ (2001) Permutation tests for univariate or multivariate analysis of variance and regression. Can J Fish Aquat Sci 58:626-639. doi: 10.1139/f01-004

Anselmetti FS, Bühler R, Finger D, Girardclos S, Lancini A, Rellstab C, Sturm M (2007) Effects of Alpine hydropower dams on particle transport and lacustrine sedimentation. Aquat Sci 69:179-198. doi: 10.1139/cjfas-58-3-626

Biggs BJ, Close ME (1989) Periphyton biomass dynamics in gravel bed rivers: the relative effects of flows and nutrients. Freshwater Biol 22:209-231. doi: 10.1111/j.1365-2427.1989.tb01096.x

Biggs BJ, Thomsen HA (1995) Disturbance of stream periphyton by perturbations in shear stress: time to structural failure and differences in community resistance. J Phycol 31:233-241. doi: 10.1111/j.0022-3646.1995.00233.x

Biggs BJF (1996) Patterns in benthic algae of streams. In: Stevenson RJ, Bothwell ML, Lowe RL (eds) Algal Ecology: Freshwater Benthic Ecosystems. Academic Press, San Diego, USA, pp 31-56

Biggs BJF, Nikora VI, Snelder TH (2005) Linking scales of flow variability to lotic ecosystem structure and function. River Res Appl 21:283-298. doi: 10.1002/rra.847

Blaschke AP, Steiner KH, Schmalfuss R, Gutknecht D, Sengschmitt D (2003) Clogging processes in hyporheic interstices of an impounded river, the Danube at Vienna, Austria. Int Rev Hydrobiol 88:397-413. doi: 10.1002/iroh.200390034

Blinn DW, Shannon JP, Benenati PL, Wilson KP (1998) Algal ecology in tailwater stream communities: the Colorado River below Glen Canyon Dam, Arizona. J Phycol 34:734-740. doi: 10.1046/j.1529-8817.1998.340734.x

Blinn W, Shannon JP, Stevens LE, Carder JP (1995) Consequences of fluctuating discharge for lotic communities. J N Am Benthol Soc 14:233-248. doi: 10.2307/1467776

Brett M, Müller-Navarra D (1997) The role of highly unsaturated fatty acids in aquatic foodweb processes. Freshwater Biol 38:483-499. doi: 10.1046/j.1365-2427.1997.00220.x

Bruno MC, Cashman MJ, Maiolini B, Biffi S, Zolezzi G (2015) Responses of benthic invertebrates to repeated hydropeaking in semi-natural flume simulations. Ecohydrol 9:68-82. doi: 10.1002/eco.1611

Bruno MC, Maiolini B, Carolli M, Silveri L (2009) Impact of hydropeaking on hyporheic invertebrates in an Alpine stream (Trentino, Italy). Ann Limnol-Int J Lim 45:157-170. doi: 10.1051/limn/2009018

Bruno MC, Siviglia A, Carolli M, Maiolini B (2013) Multiple drift responses of benthic invertebrates to interacting hydropeaking and thermopeaking waves. Ecohydrol 6:511-522. doi: 10.1002/eco.1275

Bunn SE, Arthington AH (2002) Basic principles and ecological consequences of altered flow regimes for aquatic biodiversity. Environ Manage 30:492-507. doi: 10.1007/s00267-002-2737-0

Cashman MJ, Wehr JD, Truhn K (2013) Elevated light and nutrients alter the nutritional quality of stream periphyton. Freshwater Biol 58:1447-1457. doi: 10.1111/fwb.12142.

Céréghino R, Cugny P, Lavandier P (2002) Influence of intermittent hydropeaking on the longitudinal zonation patterns of benthic invertebrates in a mountain stream. Int Rev Hydrobiol 87:47-60. doi: 10.1002/1522-2632(200201)87:1<47::AID-IROH47>3.0.CO;2-9

Céréghino R, Lavandier P (1998) Influence of hypolimnetic hydropeaking on the distribution and population dynamics of Ephemeroptera in a mountain stream. Freshwater Biol 40:385-399. doi: 10.1046/j.1365-2427.1998.00353.x

Céréghino R, Legalle M, Lavandier P (2004) Drift and benthic population structure of the mayfly *Rhithrogena semicolorata* (Heptageniidae) under natural and hydropeaking conditions. Hydrobiologia 519:127-133. doi: 10.1023/B:HYDR.0000026499.53979.69

Cross WF et al. (2013) Food-web dynamics in a large river discontinuum. Ecol Monogr 83:311-337. doi: 10.1890/12-1727.1

Curran JH, Wohl EE (2003) Large woody debris and flow resistance in step-pool channels, Cascade Range, Washington. Geomorphology 51:141-157. doi: 10.1016/S0169-555X(02)00333-1

Davie AW, Mitrovic SM, Lim R (2012) Succession and accrual of benthic algae on cobbles of an upland river following scouring. Inland Waters 2:89-100. doi: 10.5268/IW-2.2.441

Delgado M, De Jonge V, Peletier H (1991) Effect of sand movement on the growth of benthic diatoms. J Exp Mar Biol Ecol 145:221-231. doi: 10.1016/0022-0981(91)90177-X

Ellis LE, Jones NE (2013) Longitudinal trends in regulated rivers: a review and synthesis within the context of the serial discontinuity concept. Environ Rev 21:136-148. doi: 10.1139/er-2012-0064

Finch C, Pine WE, Limburg KE (2015) Do hydropeaking flows alter juvenile fish growth rates? A test with juvenile humpback chub in the Colorado River. RRA 31:156-164. doi: 10.1002/rra.2725

Fox J, Weisberg S (2011) An {R} Companion to Applied Regression, Second Edition. Sage, Thousand Oaks CA, USA. http://socserv.socsci.mcmaster.ca/jfox/Books/Companion

Francoeur SN, Biggs BJ (2006) Short-term effects of elevated velocity and sediment abrasion on benthic algal communities. In: Advances in Algal Biology: A Commemoration of the Work of Rex Lowe. Springer Netherlands, pp 59-69

Füreder L, Schütz C, Wallinger M, Burger R (2001) Physico-chemistry and aquatic insects of a glacier-fed and a spring-fed alpine stream. Freshwater Biol 46:1673-1690. doi: 10.1046/j.1365-2427.2001.00862.x

Gippel CJ (1995) Environmental hydraulics of large woody debris in streams and rivers. J Environ Eng-ASCE 121:388-395. doi: 10.1061/(ASCE)0733-9372(1995)121:5(388)

Gore JA, Nestler JM, Layzer J (1989) Instream flow predictions and management options for biota affected by peaking-power hydroelectric operations. Regul Rivers: Res Mgmt 3: 35-48. doi: 10.1002/rrr.3450030106

Gore JA, Niemela S, Resh VH, Statzner B (1994) Near-substrate hydraulic conditions under artificial floods from peaking hydropower operation: A preliminary analysis of disturbance intensity and duration. Regul Rivers: Res Mgmt 9:15-34. doi: 10.1002/rrr.3450090103

Gower JC (1966) Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika 53:325-338. doi: 10.1093/biomet/53.3-4.325

Gregory KJ, Gurnell AM, Hill CT (1985) The permanence of debris dams related to river channel processes Hydrolog Sci J 30:371-381. doi: 10.1080/02626668509491000

Grelsson G (1985) Vegetational changes on two eroding banks of a short-term regulated river reservoir in northern Sweden. Nord J Bot 5:581-614. doi: 10.1111/j.1756-1051.1985.tb01695.x

Growns IO, Growns JE (2001) Ecological effects of flow regulation on macroinvertebrate and periphytic diatom assemblages in the Hawkesbury–Nepean River, Australia. Regul Rivers: Res Mgmt 17:275-293. doi: 10.1002/rrr.622

Gurnell AM, Piégay H, Swanson FJ, Gregory SV (2002) Large wood and fluvial processes. Freshwater Biol. 47: 601-619. doi: 10.1046/j.1365-2427.2002.00916.x

Guo F, Kainz MJ, Sheldon F, Bunn SE (2015) Spatial variation in periphyton fatty acid composition in subtropical streams. Freshwater Biol. 60: 1411-1422. doi: 10.1111/fwb.12578

Guo F, Kainz MJ, Shaldon F, Bunn SE (2016a) The importance of high-quality algal food sources in stream food webs – current status and future perspectives. Freshwater Biol. doi:10.1111/fwb.12755

Guo F, Kainz MJ, Sheldon F, Bunn SE (2016b) Effects of light and nutrients on periphyton and the fatty acid composition and somatic growth of invertebrate grazers in subtropical streams. Oecologia 1-14. doi:10.1007/s00442-016-3573-x

Harnish RA, Sharma R, McMichael GA, Langshaw RB, Pearsons TN, Hilborn R (2014) Effect of hydroelectric dam operations on the freshwater productivity of a Columbia River fall Chinook salmon population. Can J Fish Aquat Sci 71:602-615. doi: 10.1139/cjfas-2013-0276

Hax CL, Golladay SW (1998) Flow disturbance of macroinvertebrates inhabiting sediments and woody debris in a prairie stream. Am Midl Nat 139:210-233.

Hillebrand H, Sommer U (1999) The nutrient stoichiometry of benthic microalgal growth: Redfield proportions are optimal. Limnol Oceanogr 44:440-446. doi: 10.4319/lo.1999.44.2.0440

Honeyfield D, Maloney K (2015) Seasonal patterns in stream periphyton fatty acids and community benthic algal composition in six high-quality headwater streams. Hydrobiologia 744:35-47. doi: 10.1007/s10750-014-2054-7

Jakob C, Robinson C, Uehlinger U (2003) Longitudinal effects of experimental floods on stream benthos downstream from a large dam. Aquat Sci 65:223-231. doi: 10.1007/s00027-003-0662-9

Kopp JF, McKee GD (1979) Methods for chemical analysis of water and wastes National Technical Information Service Springfield, Va, Report No PB 297686,(32319):490

Lancaster J, Hildrew AG (1993) Flow refugia and the microdistribution of lotic macroinvertebrates. J N Am Benthol Soc 12:385-393. doi: 10.2307/1467619

Lane CM, Taffs KH, Corfield JL (2003) A comparison of diatom community structure on natural and artificial substrata Hydrobiologia. 493:65-79. doi: 10.1023/A:1025498732371

Law RJ (2011) A review of the function and uses of, and factors affecting, stream phytobenthos. Freshwater Reviews 4:135-166. doi: 10.1608/FRJ-4.1.448

Lenzi MA, Mao L, Comiti F (2003) Interannual variation of suspended sediment load and sediment yield in an alpine catchment. Hydrolog Sci J 48:899-915. doi: 10.1623/hysj.48.6.899.51425

Lorenzen C (1967) Determination of chlorophyll and pheopigments: spectrophotometric equations. Limnol Oceanogr 12:343-346. doi: 10.4319/lo.1967.12.2.0343

Miller SW, Judson S (2014) Responses of macroinvertebrate drift, benthic assemblages, and trout foraging to hydropeaking. Can J Fish Aquat Sci 71:675-687. doi: 10.1139/cjfas-2013-0562

Montgomery DR, Collins BD, Buffington JM, Abbe TB (2003) Geomorphic effects of wood in rivers. In: Gregory SV, Boyer, KL, Gurnell AM (eds) The Ecology and Management of Wood in World Rivers. American Fishery Society Symposium 37, Bethesda, Maryland, USA, pp 21-47.

Müller-Navarra DC, Brett MT, Liston AM, Goldman CR (2000) A highly unsaturated fatty acid predicts carbon transfer between primary producers and consumers. Nature 403:74-77. doi: 10.1038/47469

Muller-Navarra DC, Brett MT, Park S, Chandra S, Ballantyne AP, Zorita E, Goldman CR (2004) Unsaturated fatty acid content in seston and tropho-dynamic coupling in lakes. Nature 427:69-72.

Müller-Navarra DC (2008) Food web paradigms: the biochemical view on trophic interactions. Int Rev Hydrobiol 93:489-505. doi: 10.1002/iroh.200711046

Northcott K, Andersen DC, Cooper DJ (2007) The influence of river regulation and land use on floodplain forest regeneration in the semi-arid upper Colorado River Basin, USA. River Res Appl 23:565-577. doi: 10.1002/rra.1007

Oksanen J et al. (2013) Vegan: Community Ecology Package. R package version 2.0-10. http://CRAN.R-project.org/package=vegan

Palmer MA, Arensburger P, Martin AP, Denman DW (1996) Disturbance and patch-specific responses: the interactive effects of woody debris and floods on lotic invertebrates. Oecologia 105:247-257. doi: 10.1007/bf00328554

Parrish CC (1999) Determination of total lipid, lipid classes, and fatty acids in aquatic samples. In: Arts MT, Wainman BC (eds) Lipids in Freshwater Ecosystems. Springer New York, pp 4-20. doi: 10.1007/978-1-4612-0547-0_2

Person E, Bieri M, Peter A, SChleiss AJ (2004) Mitigation measures for fish habitat improvement in Alpine rivers affected by hydropower operations. Ecohydrology 7:580-599. doi: 10.1002/eco.1380

Poff NL et al. (1997) The natural flow regime. BioScience 47:769-784. doi: 10.2307/1313099

Poff NL, Ward JV (1989) Implications of streamflow variability and predictability for lotic community structure: a regional analysis of streamflow patterns. Can J Fish Aquat Sci 46:1805-1818. doi: 10.1139/f89-228

Robinson CT, Aebischer S, Uehlinger U (2004) Immediate and habitat-specific responses of macroinvertebrates to sequential, experimental floods. J N Am Benthol Soc 23:853-867. doi: 10.1899/0887-3593(2004)023<0853:iahrom>2.0.co;2

Robinson CT, Tockner K, Burgherr P (2002) Seasonal patterns in macroinvertebrate drift and seston transport in streams of an alpine glacial flood plain. Freshwater Biol 47:985-993. doi: 10.1046/j.1365-2427.2002.00835.x

Roni P, Beechie TJ, Pess GR, Hanson KM (2014) Wood placement in river restoration: fact, fiction and future direction. Can J Fish Aquat Sci 72:466-478. doi: 10.1139/cjfas-2014-0344

Rosario-Martinez H (2013) phia: post-hoc interaction analysis. R package version 0.1–3. http://CRAN.R-project.org/package=phia

Sabater S, Gregory SV, Sedell JR (1998) Community dynamics and metabolism of benthic algae colonizing wood rock substrata in a forest stream. J Phycol 34:561-567. doi: 10.1046/j.1529-8817.1998.340561.x

Saltveit S, Halleraker J, Arnekleiv J, Harby A (2001) Field experiments on stranding in juvenile Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*) during rapid flow decreases caused by hydropeaking. Regul Rivers: Res Mgmt 17:609-622. doi: 10.1002/rrr.652

Schmutz S et al. (2014) Response of fish communities to hydrological and morphological alterations in hydropeaking rivers of Austria. River Res Applic 31: 919-930. doi: 10.1002/rra.2795.

Smolar-Žvanut N, Mikoš M (2013) The impact of flow regulation caused by hydropower dams on the periphyton community in the Soča River, Slovenia. Hydrolog Sci J 59:1032-1045. doi: 10.1080/02626667.2013.834339

Smolar-Žvanut N, Klemenčič AK (2013) The impact of altered flow regime on periphyton. In: Maddock I, Harby A, Kemp P, Wood P (eds) Ecohydraulics: An Integrated Approach. Wiley & Sons, Ltd, Chichester, UK, pp 229-243. doi: 10.1002/9781118526576.ch13

Solórzano L, Sharp JH (1980) Determination of total dissolved phosphorus and particulate phosphorus in natural waters. Limnol Oceanogr 25:754-758. doi: 10.4319/lo.1980.25.4.0754

Stevenson RJ, Bahls LL (1999) Periphyton protocols Revision to Rapid Bioassessment Protocols for Use in Streams and Rivers: Periphyton, Benthic Macroinvertebrates, and Fish. http://nepis.epa.gov/. Accessed 7 April 2016

Strandberg U, Taipale SJ, Hiltunen M, Galloway AWE, Brett MT, Kankaala P (2015) Inferring phytoplankton community composition with a fatty acid mixing model. Ecosphere 6:16. doi: 10.1890/ES14-00382.1

Suren AM, Riis T (2010) The effects of plant growth on stream invertebrate communities during low flow: a conceptual model. J N Am Benthol Soc 29:711-724. doi: 10.1899/08-127.1

Taipale S, Strandberg U, Peltomaa E, Galloway AWE, Ojala A, Brett MT (2013) Fatty acid composition as biomarkers of freshwater microalgae: analysis of 37 strains of microalgae in 22 genera and in seven classes. Aquat Microb Ecol 71:165-178. doi: 10.3354/ame01671

Taipale SJ et al. (2014) Differing *Daphnia magna* assimilation efficiencies for terrestrial, bacterial, and algal carbon and fatty acids. Ecology 95:563-576. doi: 10.1890/13-0650.

Tang T, Niu S, Dudgeon D (2013) Responses of epibenthic algal assemblages to water abstraction in Hong Kong streams. Hydrobiologia 703:225-237. doi: 10.1007/s10750-012-1362-z

Torres-Ruiz M, Wehr JD, Perrone AA (2007) Trophic relations in a stream food web: importance of fatty acids for macroinvertebrate consumers. J N Am Benthol Soc 26:509-522. doi: 10.1899/06-070.1

Torres-Ruiz M, Wehr JD, Perrone AA (2010) Are net-spinning caddisflies what they eat? An investigation using controlled diets and fatty acids. J N Am Benthol Soc 29:803-813. doi: 10.1899/09-162.1

Uehlinger U, Kawecka B, Robinson CT (2003) Effects of experimental floods on periphyton and stream metabolism below a high dam in the Swiss Alps (River Spöl). Aquat Sci 65:199-209. doi: 10.1007/s00027-003-0664-7

Voelz NJ, Ward JV (1996) Microdistributions of filter-feeding caddisflies (Insecta: Trichoptera) in a regulated Rocky Mountain river. Can J Zool 74:654-666. doi: 10.1139/z96-074

Wohl E, Bledsoe BP, Fausch KD, Kramer N, Bestgen KR, Gooseff MN (2016) Management of large wood in streams: an overview and proposed framework for hazard evaluation. JAWRA 52:315-335. doi: 10.1111/1752-1688.12388

Wu N et al. (2010) Impacts of cascade run-of-river dams on benthic diatoms in the Xiangxi River, China. Aquat Sci 72:117-125. doi: 10.1007/s00027-009-0121-3

Young P, Cech J, Jr., Thompson L (2011) Hydropower-related pulsed-flow impacts on stream fishes: a brief review, conceptual model, knowledge gaps, and research needs. Rev Fish Biol Fisheries 21:713-731. doi:10.1007/s11160-011-9211-0

Zolezzi G, Siviglia A, Toffolon M, Maiolini B (2011) Thermopeaking in Alpine streams: event characterization and time scales. Ecohydrol 4:564-576. doi: 10.1002/eco.132

TABLES

Table 1: Statistical results of the general linear model testing the effects of hydropeaking and substrate type on measured periphyton properties. Total FA cm^{-2} indicates mass of fatty acids available per cm^{-2} of substrate. All other fatty acid values are fatty acid content standardized by periphyton AFDM (dm^{-1}; *i.e.* mg of fatty acids per gram of periphyton). All factors examined are repeated measures for change over the course of the experiment (interactions with time). Post-hoc results indicate differences in the response rate between control and hydropeaking intensities, and before-after for substrates; +/− indicates whether treatment increased (+) or decreased (−) response variable, with significance indicated by * at $P < 0.05$, ** at $P < 0.01$, and *** at $P < 0.001$.

	Hydropeaking Intensity (df = 2)				Substrate (df = 1)				Month*Intensity (df = 2)		Month*Substrate (df = 1)	
	F	P	Post-hoc		F	P	Post-hoc		F	P	F	P
Chl-*a*	**4.36**	**0.018**		**-3****	**7.71**	**0.008**	**-T*****		0.166	0.848	3.453	0.069
AFDM	**5.91**	**0.005**		**-3****	**11.6**	**0.004**	**-T***	**+W***	1.23	0.300	0.0255	0.874
C:N	0.556	0.578			**12.9**	**<0.001**	**-T***	**+W***	1.35	0.271	0.0369	0.848
C:P	1.06	0.357			2.72	0.125			2.36	0.108	0.114	0.738
Total FA cm^{-2}	**5.05**	**0.015**	**-2***	**-3***	**7.01**	**0.014**	**-T***					
Total FA dm^{-1}	**6.78**	**0.005**	**-2****	**-3***	1.85	0.176						
$\sum$SAFA dm^{-1}	**6.47**	**0.006**	**-2***	**-3***	1.96	0.174						
$\sum$MUFA dm^{-1}	**6.64**	**0.005**	**-2****	**-3***	2.41	0.134						
$\sum$PUFA dm^{-1}	**5.57**	**0.011**	**-2***	**-3***	1.62	0.219						
$\sum$HUFA dm^{-1}	**5.92**	**0.001**	**-2****		2.28	0.145						
$\sum$ω3 dm^{-1}	**6.13**	**0.007**	**-2****	**-3***	1.88	0.183						
$\sum$ω6 dm^{-1}	**6.84**	**0.005**	**-2****	**-3***	2.62	0.119						
20:5ω3 dm^{-1}	**5.78**	**0.009**	**-2***	**-3***	2.45	0.131						
20:4ω6 dm^{-1}	**3.842**	**0.036**	**-2***		0.301	0.588						
22:6ω3 dm^{-1}	**6.62**	**0.005**	**-2*****		2.48	0.129						

FIGURE CAPTIONS

Figure 1: Photographs of the experimental flume system alongside the River Fersina a) Water is diverted from the main river channel by the weir (i) into a collecting tank (ii) located just above the flumes. Discharge from the collecting tank into each of the five flumes (iii) is controlled by an adjustable sluice gate. Water exits the bottom of the flumes through a spout (iv) where it re-enters the channel. b) View looking downstream indicating flume name, substrate and hydropeaking treatment in each of the five flumes, along with pictures of both the tile substrate (top) and wood substrate (bottom). White strips at the downflume end of the tiles were used for Simuliidae colonization for a separate experiment.

Figure 2: Changes in chlorophyll-a concentrations on tile and wood substrates for both hydropeaking intensity treatments in both seasons. Values are mean ± 1 standard error. Data are paired, with pre- and post-experiment samples adjacent for comparison.

Figure 3: Changes in ash-free dry mass (AFDM) on tile and wood substrates for both hydropeaking intensity treatments in both seasons. Values are mean ± 1 standard error. Data are paired, with pre- and post-experiment samples adjacent for comparison.

Figure 4: Changes in periphyton C:N ratio on tile and wood substrates for both hydropeaking intensity treatments in both season. Values are mean ± 1 standard error. Data are paired, with before and after samples adjacent for comparison. Redfield C:N ratio for periphyton is 7.

Figure 5: Change in 20+ C highly unsaturated fatty acids (HUFA) per unit dry mass of periphyton. Only April values are shown due to loss of samples and low sample volumes in September. Data are paired, with before and after samples adjacent for comparison.

Figure 6: Principal coordinates ordination (metric multidimensional scaling) of periphyton fatty acid profiles both before (filled symbols) and after (hollow symbols) the hydropeaking experiment on both tiles (square) and wood (triangle) substrates of only hydropeaking treatments (control excluded for clarity). PCO axis 1 accounts for 55.8% of the total variation, while axis 2 accounts for 13.4%. Substrates and before/after experiment are separated into 4 different quadrants (I-IV). Bottom figure shows the relationship between axes and individual fatty acids.
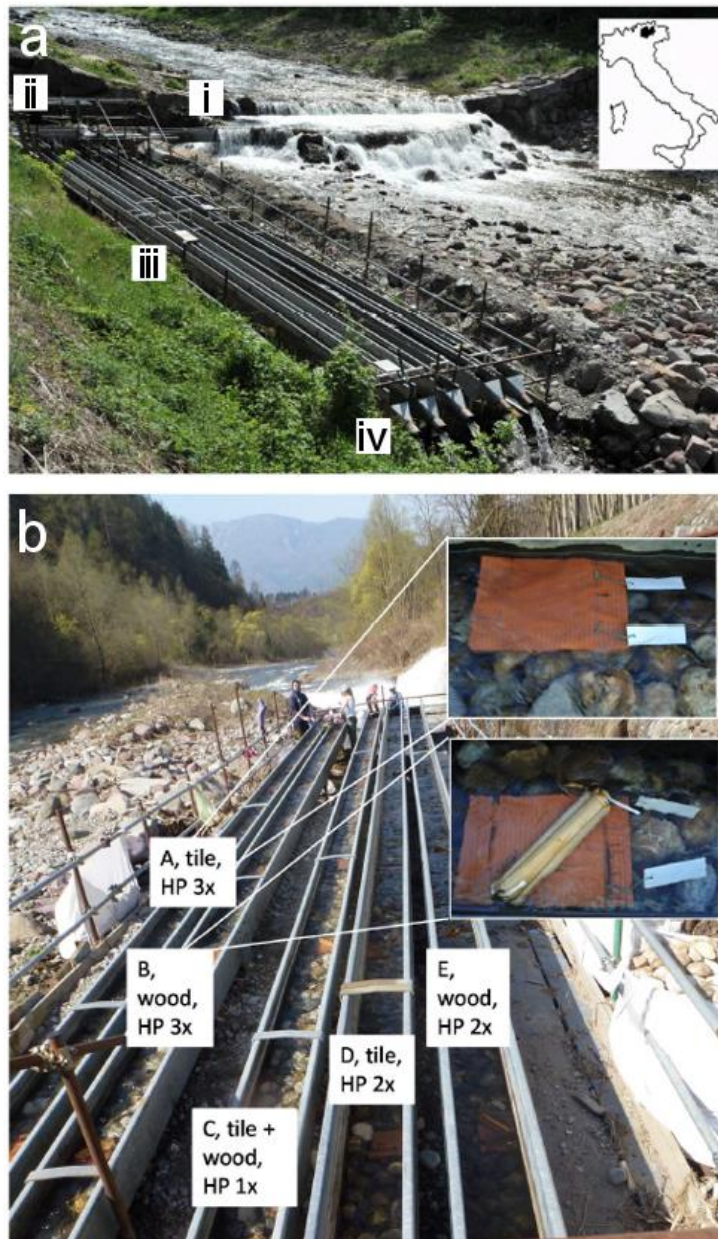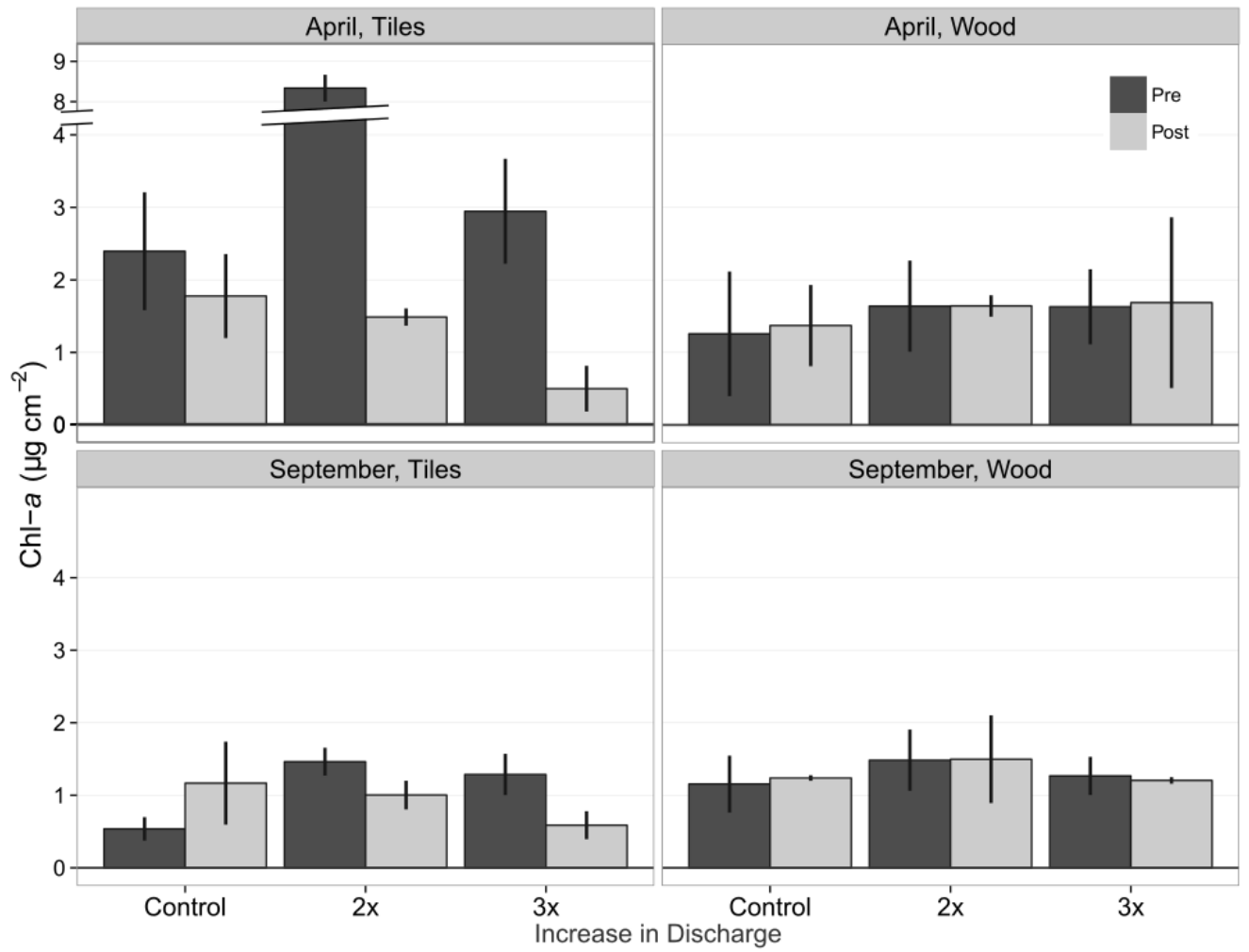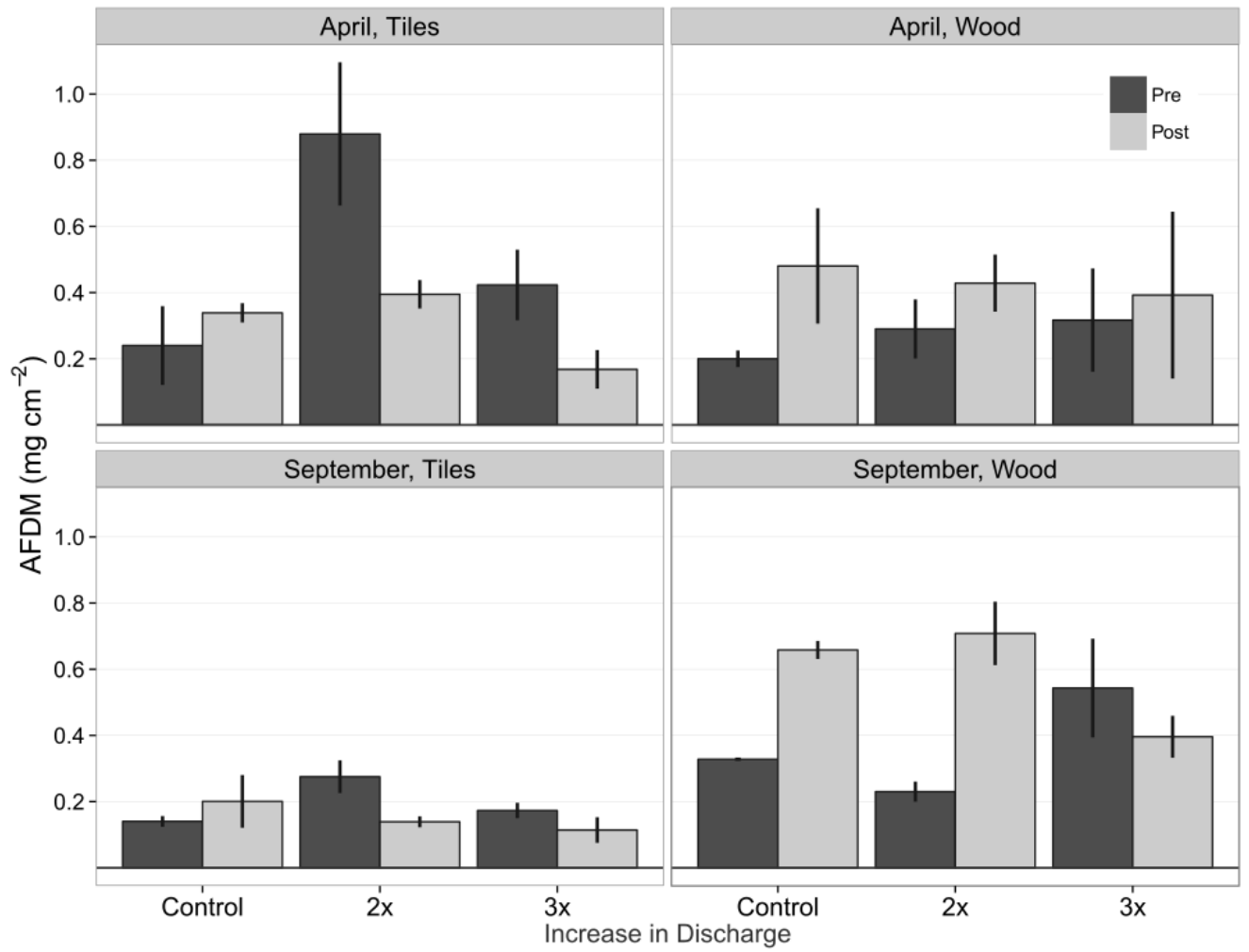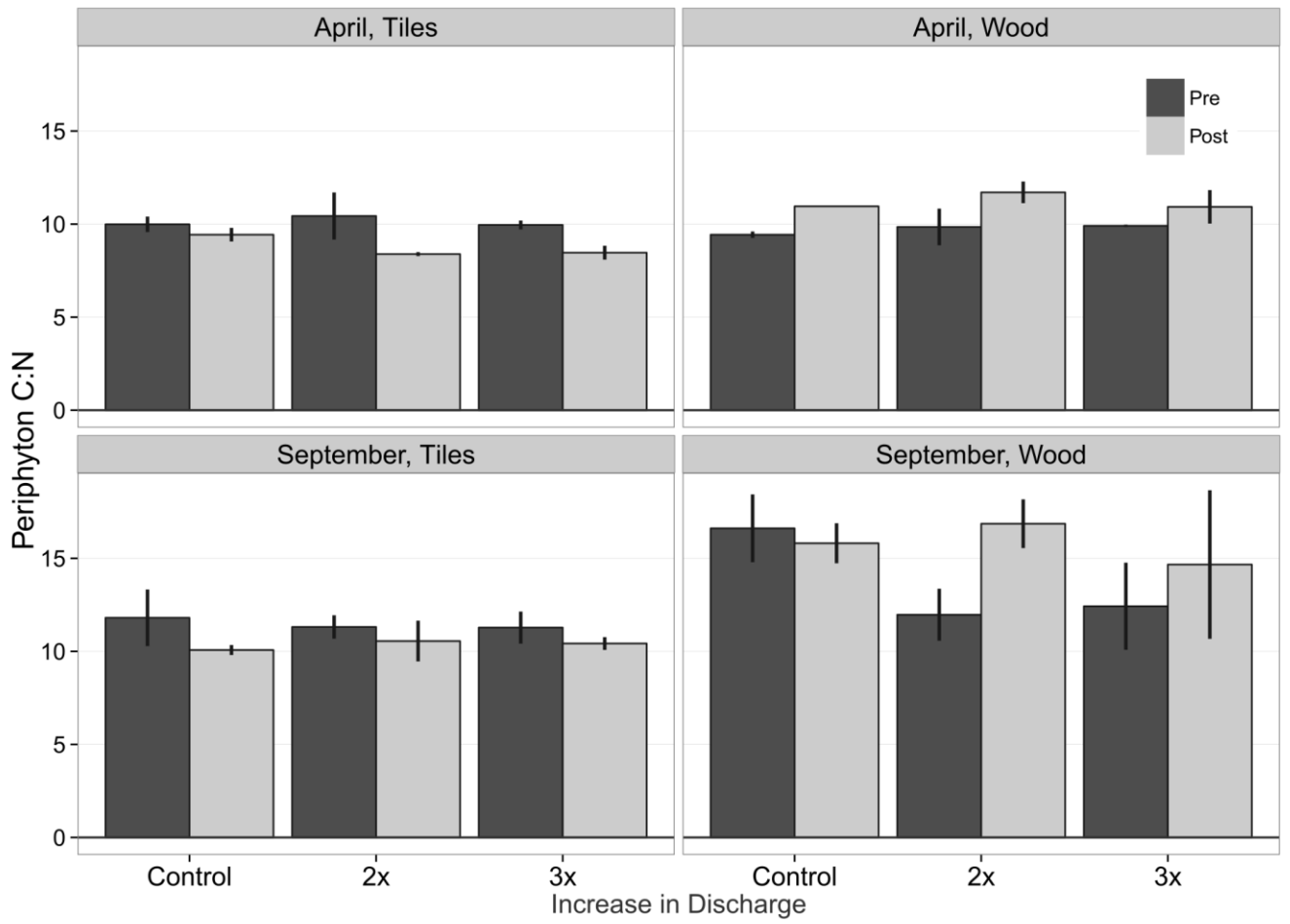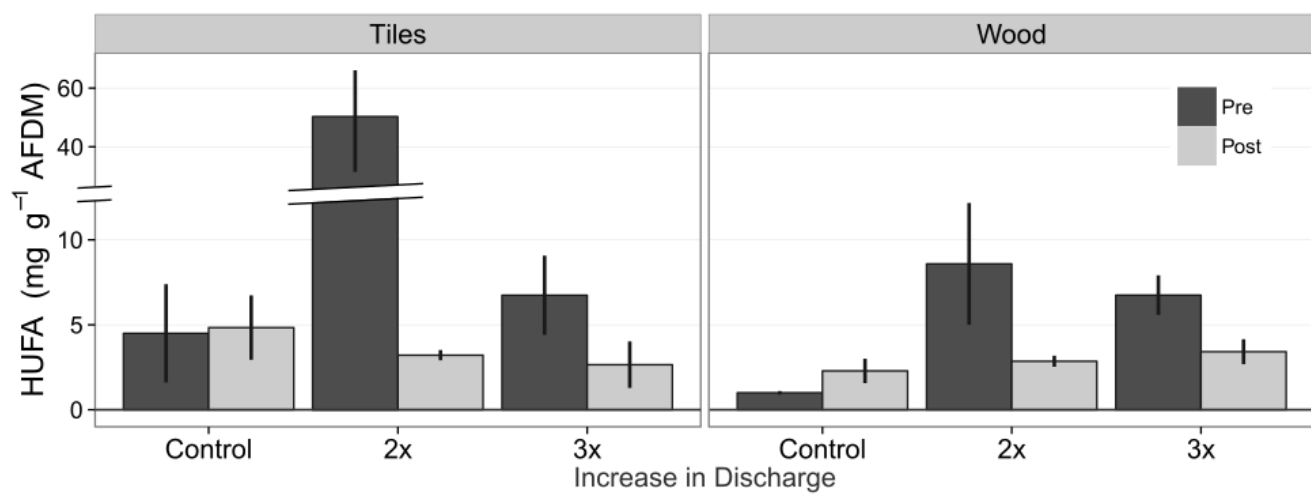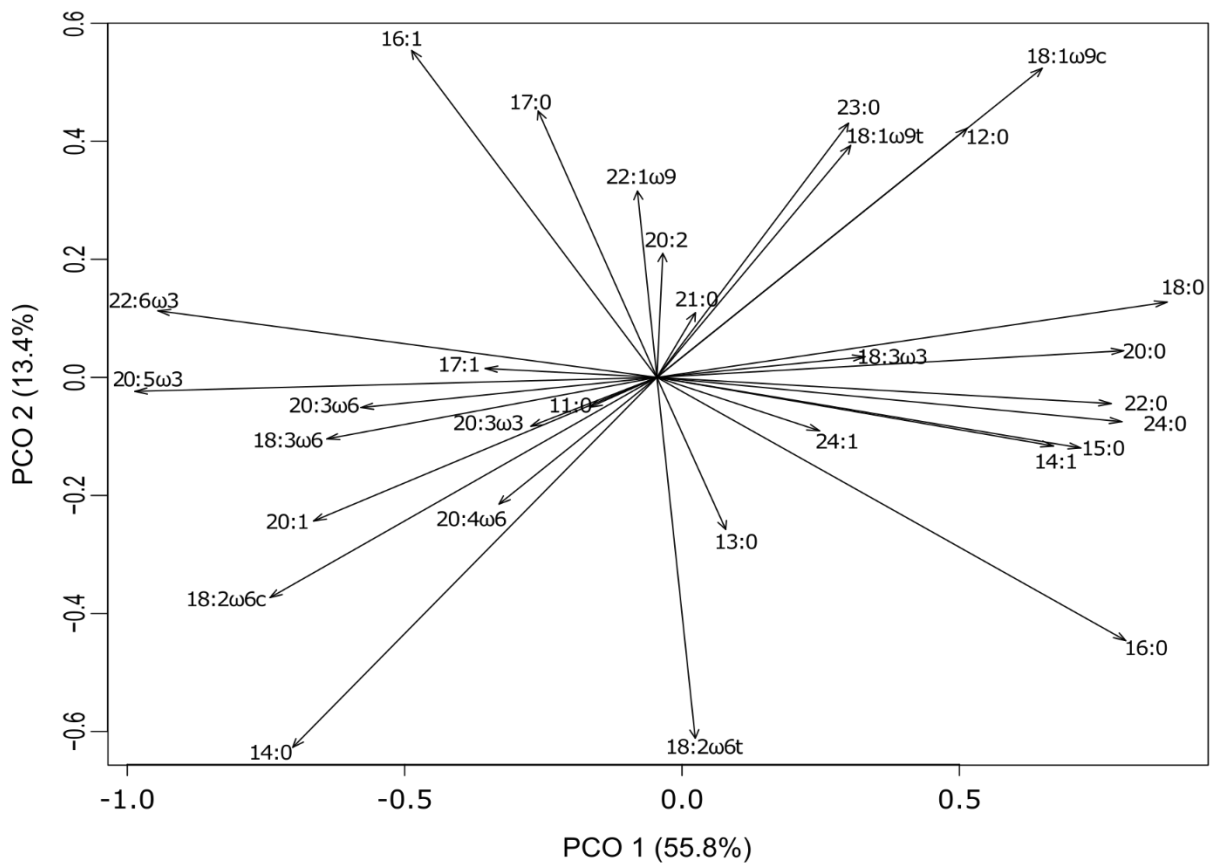
FIGURES

Figure 1:

Figure 2:

Figure 3:

Figure 4:

Figure 5:

Figure 6: