Arnold Schwarzenegger
Governor

EVALUATING THE IMPACTS OF MANUFACTURED RECREATION STREAMFLOWS ON THE MACROINVERTEBRATE COMMUNITY OF A REGULATED RIVER

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Prepared By:
Garcia and Associates

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Prepared By:
Ian Chan and Robert Aramayo
Garcia and Associates (GANDA)
1 Saunders Avenue
San Anselmo, California 94960
Commission Contract No. 500-01-044

Prepared For:
Public Interest Energy Research (PIER) Program
California Energy Commission

Joe O'Hagan
Contract Manager

Kelly Birkinshaw
Program Area Lead
Energy-Related Environmental Research

Laurie ten Hope
Office Manager
Energy Systems Research

Martha Krebs
Deputy Director
ENERGY RESEARCH & DEVELOPMENT DIVISION

B.B. Blevins
Executive Director

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Preface

The Public Interest Energy Research (PIER) Program supports public interest energy research and development that will help improve the quality of life in California by bringing environmentally safe, affordable, and reliable energy services and products to the marketplace.

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Evaluating the Impacts of Manufactured Recreation Streamflows on the Macroinvertebrate Community of a Regulated River is the final report for the Management of Ecological Evaluation of Hydropower Pulsed and Manufactured Flow Releases on California's Stream Systems project (contract number 500-01-044) conducted by Garcia and Associates (GANDA). The information from this project contributes to PIER's Energy-Related Environmental Research Program.

For more information about the PIER Program, please visit the Energy Commission's website at www.energy.ca.gov/pier or contact the Energy Commission at 916-654-5164.

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Abstract

Benthic macroinvertebrate data from reaches of the North Fork Feather River, affected and unaffected by monthly pulsed recreation streamflow releases during 2004, were compared using a before-after control-impact (BACI) design. Macroinvertebrate populations were sampled using representative artificial substrates (rock basket samplers) and standard kick-sampling techniques. Macroinvertebrate data were described using the recently developed hydropower multi-metric index (Hydro-MMI), which was designed to be sensitive to the effects of hydropower operations. Analysis of variance (ANOVA) was used to test for a pulsed-flow effect. The control-to-treated difference in Hydro-MMI was significantly different from pre-flow to post-flow ($p < 0.10$). Among basket-sample data, seasonal trends between these two reaches were generally similar; however, kick-sample data suggest a seasonal pattern of increasing richness and abundance in the control reach that was not observed in the treated reach.

Comparisons of the basket- and kick-sampling techniques demonstrated that basket samplers selected for a subset of the benthic community dominated by filter-feeding organisms such as net-spinning caddisflies, which appeared able to quickly colonize and capitalize on the free interstitial spaces in basket samplers. Kick samples provided a better representation of the overall benthic community. Differences in the composition of the two sample types suggest that seasonal trends among basket-sample data primarily followed natural seasonal patterns for the filter-feeder-dominated community that developed in basket samplers, which may be less sensitive to flow-related changes than the larger benthic community.

Keywords: Stream benthos, benthic macroinvertebrates, flow manipulation, pulsed streamflow releases, representative artificial substrate sampling, kick sampling, multi-metric index

Executive Summary

Introduction

Hydroelectric licensing agreements for regulated rivers often include provisions for sudden, short-term significant increases in streamflow (i.e., "pulsed flows") for operational, experimental, or recreational purposes. Understanding the consequences of pulsed flows for resident stream biota is critical for regulatory decision-making; yet the effects this type of flow manipulation are poorly understood, particularly in terms of differences in the magnitude, frequency, duration, and timing (or seasonality) of pulsed-flow events.

Of particular interest are the effects of pulsed flows upon benthic macroinvertebrate communities. Benthic macroinvertebrates (streambed-dwelling invertebrates 0.5 mm or larger) are fundamental components of riverine food webs that provide critical linkages between primary food production and consumption by higher organisms (e.g., fish, birds, mammals). Previous studies by the authors have demonstrated that pulsed streamflows cause temporary increases in invertebrate drift, which may kill, injure, or render benthic organisms "ecologically dead" by displacing them into inhospitable habitats.

Purpose

This project investigated the effects of the pulsed flows, provided for recreational whitewater boating, on benthic macroinvertebrate communities of the North Fork Feather River (NFFR), Plumas County, California. The study compared macroinvertebrate communities from a "treated" reach and an unaffected "control" reach using a limited before-after control-impact (BACI) experimental design. In the treated reach, one-day pulsed flows that were between four and seven times the magnitude of the baseflow were provided once per month from late June through late October 2004, during the typical low-flow season in the NFFR.

Project Objectives

The primary objectives of this study were to:

1. Quantify and compare any short-term differences between the benthic community of the treated and control reaches immediately before and after pulsed recreation streamflow events.
2. Determine if longer-term seasonal trends in the benthic community of the treated reach differed from trends in the control reach following repeated pulsed-flow events.
3. Compare the efficacy of representative artificial substrate sampling vs. standard kick-sampling methods for detecting pulsed-flow-related changes.

Project Approach

The treated and control reaches were sampled concurrently, before and after each pulsed-flow event. Both reaches were sampled using the same representative artificial substrate sampling method that was employed in a previous study of the treated reach (rock basket samplers), as well as a standard kick-sampling technique.

Macroinvertebrate data from pre- and post-flow sampling events in each reach were described using the recently developed hydropower multi-metric index (Hydro-MMI), which was designed to be sensitive to the cumulative effects of hydropower operations. Analysis of variance (ANOVA)—a standard statistical analysis approach—was employed to test for a pulsed-flow effect using this multi-metric index as the response variable. Similar ANOVAs were also used to test for differences between seasonal trends in the treated and control reaches, and to test for differences between the basket- and kick-sampling methods.

Project Outcomes

The control-to-treated difference in Hydro-MMI was significantly different from pre-flow to post-flow ($p < 0.10$). Seasonal trends between these reaches were generally similar among basket-sample data, although the control reach generally scored higher than the treated reach (indicating better biological conditions in the control reach). Seasonal trends in Hydro-MMI were not significantly different ($p > 0.10$); however, some divergence in Hydro-MMI scores as well as in individual metrics was apparent between the treated and control reaches at the end of the sampling season (which may be biologically significant). Moreover, kick-sample data suggest a seasonal pattern of increasing richness and abundance in the control reach that was not observed in the treated reach.

Kick samples provided a better representation of the overall benthic community than basket samples in this study. Basket samplers tended to select for a subset of the benthic community heavily dominated by filter-feeding organisms such as net-spinning caddisflies (primarily of the family Hydropsychidae). These organisms were able to quickly colonize basket samplers and capitalize on free interstitial spaces throughout the volume of these samplers (i.e., including spaces between rocks near the bottom of the sampler, not just the exposed rock surfaces at the top). On average, basket samples overrepresented the abundance of net-spinning caddisflies by approximately 60% relative to kick samples. Several other key species were also either overestimated or underestimated to some degree in basket samples. Statistically significant differences between kick- and basket-sampling methods were detectable (ANOVA, $p < 0.10$), implying that the two sampling methods yield different results.

Conclusions and Recommendations

Overall, short-term differences between the control reach and treated reach were not consistent or large enough to necessarily be considered biologically significant, even though statistical significance was detected in the context of BACI comparisons. Conversely, longer-term seasonal differences, especially among kick-sample data,

suggest biologically significant control-to-treated differences, despite the fact that no statistical significance was detected. Differences in the composition of basket vs. kick samples suggest that seasonal trends among basket-sample data primarily follow the natural seasonal patterns for the filter-feeder-dominated community that developed in the basket samplers; and furthermore, that these filter-feeding organisms may be more robust to flow-related changes by virtue of their specific microhabitat preferences and adaptations.

Therefore, it is recommended that future evaluations of pulsed recreation streamflows in the NFFR be based on direct bottom-sampling methods such as kick sampling instead of artificial substrate sampling. Kick samples appeared to detect pulsed-flow-related disturbances better than basket samples, particularly potential cumulative effects across longer (e.g., seasonal) timescales.

The Hydro-MMI was more useful than individual metrics for both discriminating baseline control-to-treated differences and detecting pulsed-flow-related disturbances. The authors thus recommend that future studies use the Hydro-MMI to compile and evaluate benthic macroinvertebrate data.

Benefits to California

Research regarding the biological effects of hydropower on California's aquatic resources is limited, especially relative to the pulsed-type flow manipulations studied here. An adequate understanding of such effects on benthic macroinvertebrates is notably lacking given the vital importance of these organisms to the ecology of river environments. Indeed, the flow of energy derived from both allochthonous sources (i.e., organic matter produced outside the system) and autochthonous sources (i.e., organic matter produced from within the system) is primarily regulated through macroinvertebrate pathways—for example, as in the breakdown of coarse particulate organic matter by shredder insects or the processing of fine particulate organic matter by filterer and collector species.

Thus, losses in productivity resulting from disturbances to benthic communities can have bioenergetic consequences at multiple trophic levels. To the extent that pulsed flows exacerbate such losses, their bioenergetic consequences may reach beyond the benthic community to include, for example, possible negative impacts on desirable native or valuable sport fisheries, as well as other wildlife dependent on river-borne productivity (e.g., mayflies lost via drift into terminal reservoirs can no longer provide prey for fishes or birds).

Pulsed flows are often provided in a manner that is atypical for both the natural and regulated hydrographs for most hydropower-managed rivers (in terms of the magnitude, duration, frequency, and especially the timing of such flows). Because benthic macroinvertebrates are known to have life cycles cued to periods of high food availability and low flow variability, such atypical pulsed-flow releases may create artificial flood conditions when benthic communities are least adapted to experiencing floods.

This study contributes to a greater understanding of the effects of pulsed-flow manipulations on the biota of California's regulated rivers. In addition, the study demonstrates that the manner in which benthic data are collected is critical to their interpretation, especially in terms of avoiding misinterpretations in a regulatory context. By understanding the effects of pulsed-flow manipulations, resource managers will be better able to balance the various economic, recreational, and environmental demands on riverine resources and support their decisions to the public.

1.0 Introduction

Regulated rivers may experience sudden short-term increases in streamflow (i.e., "pulsed flows") for operational, experimental, or recreational purposes. Understanding the consequences of such flow manipulations for stream biota, including the effects of relative differences in the magnitude, frequency, duration, and timing (or seasonality) of pulsed flows, is critical for regulatory decision-making. The variety of beneficial uses provided for under licensing agreements often poses challenges for resource managers. Recreational mandates, for example, often directly or indirectly conflict with ecological objectives for managing aquatic resources.

In the North Fork Feather River (NFFR), Plumas County, California, pulsed flows are provided on a monthly basis for recreational whitewater boating during the typical low-flow (i.e., baseflow) season of summer and early fall. These recreation streamflow releases are provided as a condition of relicensing for Pacific Gas and Electric Company's (PG&E's) Rock Creek-Cresta Hydroelectric Project (FERC No. 1962). The magnitude of these one-day pulsed flows is typically four to seven times that of baseflow levels, and the timing of these flow events (from late June through late October) is generally contrary to both the natural hydrograph for the region and the regulated hydrograph maintained under normal hydroelectric operations. High flow periods in the Feather River system generally occur in conjunction with winter and spring storm events and spring snowmelt runoff, whereas summer and early fall are typically dry.

Based on temporal (i.e., before-after) comparisons, previous studies in the NFFR have demonstrated that recreation streamflows cause increases in invertebrate drift during and immediately following flow events, which in turn produce short-term differences in post-flow benthic macroinvertebrate communities (GANDA 2005a, GANDA 2005b). However, the extent to which longer-term temporal differences may be attributable to pulsed-flow treatments remains unclear. For example, seasonal declines in benthic community metrics such as richness, diversity, and abundance observed in the NFFR may result from cumulative impacts associated with repeated flow events, or simply from natural life history patterns of various invertebrate taxa. Spatial (i.e., control-impact) comparisons with an unaffected reference reach are required to understand such baseline seasonal changes, and to isolate the effects of natural vs. manufactured sources of variation in benthic community structure and composition.

This study examined the benthic macroinvertebrate community in an upstream reference reach of the NFFR that did not experience pulsed recreation streamflows in 2004. This "control" reach was sampled concurrently with the downstream "treated" reach before and after each recreation streamflow event in 2004. Macroinvertebrate sampling followed the same representative artificial substrate sampling methods employed in previous studies of the treated reach, as well as standard kick-sampling techniques in both reaches. The primary objectives of this study were (1) to quantify and compare any short-term differences between the benthic community of the treated and control reaches immediately before and after pulsed recreation streamflow events; (2) to

determine if longer-term seasonal trends in the benthic community of the treated reach differed from that of the control reach following repeated pulsed-flow events; and (3) to compare the efficacy of representative artificial substrate sampling vs. standard kick-sampling methods for detecting pulsed-flow-related changes.

2.0 Project Approach

2.1. Study Design

The study employed a limited before-after control-impact (BACI) design. The BACI design was limited in that the true BACI design requires multiple sampling events both before and after the treatment at each of the control and impacted sites (Green 1979; Stewart-Oaten et al. 1986). This study was limited to only one pre-treatment and one post-treatment sampling event at each site.

The basic concept was to sample the stream benthos in representative areas of the treated and control reaches immediately before and after each recreation flow event in 2004. Representative artificial substrate sampling (sensu Rosenberg and Resh 1982) was used because substrate and habitat conditions in the NFFR precluded repeated bottom sampling using conventional methods (e.g., standard kick net-type sampling or Surber- or Hess-type bottom sampling). Due to a general lack of wadeable riffle habitats and the smaller substrate material targeted by these standard bottom sampling techniques, overlap of sample sites (i.e., sample superpositioning and general trampling of the area) would have been unavoidable, especially considering the large number of replicates required for before and after sampling of multiple flow events.

Representative artificial substrate samplers are distinguished from standardized artificial substrate samplers by their use of native bottom materials as opposed to artificial surfaces (e.g., rock baskets vs. tile or plate samplers). The use of native materials more closely mimics the existing stream bottom conditions. Given adequate colonization time, these samplers equilibrate with the surrounding stream bottom, and remain transparent to biota and sediment alike. Adequate replication and randomization for this study was achieved by placing a large number of these samplers in each study reach and collecting from them at random.

Representative substrate sampling using rock basket samplers (referred to hereafter as "basket samplers") was preferable to conventional bottom sampling for several other reasons. First, basket samplers were not confounded by the large, boulder-dominated substrates and deeper water typically found in the study area. Sampling with standard nets or fixed-frame samplers in habitats with large, irregular substrates, or swift deep water, is generally inefficient. Large substrates often interfere with the placement of a net frame, creating opportunities to lose specimens. Variation in the size of samples, especially with regard to the surface area of contained substrate particles, is a major limitation in many benthic studies (Rosenberg and Resh 1982). Needham and Usinger (1956), for example, found that 73 standard Surber samples were required to accurately estimate the abundance of invertebrates within a single, relatively homogeneous riffle. Second, basket samplers create a more uniform microhabitat than the natural stream bottom, thereby reducing the variability inherent in benthic samples. As a consequence of increased uniformity, differences found in the biota can be more readily attributed to

treatment effects as opposed to underlying microhabitat differences (Patrick 1982). Some of these types of samplers have been shown to reduce the coefficient of variation of benthic samples by 10–20% when compared to direct sampling approaches (Rosenberg and Resh 1982). Increased similarity generally translates into fewer replicate samples required to achieve a given level of sampling precision.

Potential disadvantages of artificial substrate samplers include uncertainty regarding colonization dynamics, primarily the selectivity of artificial substrates vs. natural substrates (Rosenberg and Resh 1982). However, in this study, the use of native substrate in basket samplers helped more closely replicate conditions in the surrounding stream bottom.

The sampling design for this study built upon that of a larger, concurrent study (funded by PG&E) focusing only on before-after comparisons in the treated reach. Due to time and funding constraints, sampling of the control reach (as part of this study) was completed with only a fraction of the effort of the larger before-after study. Therefore, sampling effort in the two reaches of concern was not equivalent. The control reach had one quarter of the number of basket samples collected as were collected in the treated reach. The kick-sampling effort, however, was the same in both reaches.

2.2. Study Sites

This study focused on two adjacent reaches of the mainstem NFFR: a downstream treatment reach and an upstream control reach (Figure 1). The regulated Rock Creek Reach (the treated reach) below Rock Creek Dam experienced monthly recreation streamflow releases from June through October 2004, whereas the partially regulated Belden Reach (the control reach) between the East Branch NFFR/Upper NFFR confluence and Rock Creek Reservoir did not experience any such flow events. The Belden reach is considered partially regulated because it receives both unregulated flows from the East Branch NFFR and regulated flows from the Upper NFFR.

These flow characteristics made the Belden Reach the most appropriate reference for the regulated Rock Creek Reach. Natural summer baseflow levels in the unregulated East Branch NFFR, for example, are lower than baseflow levels maintained in the regulated or partially regulated reaches downstream. Other regulated reaches of the NFFR adjacent to the Rock Creek Reach either also experienced recreation streamflow events in 2004, or were otherwise unsuitable or less comparable overall than the Belden Reach (e.g., due to differences in elevation and ecotype). Therefore, the partially regulated Belden Reach represented the best available reference condition for this study (i.e., comparable baseflow and temperature regimes without pulsed recreation streamflow releases). Note that for this study, the best reference condition is considered to be one that is as similar as possible to the treated reach with the exception of the pulsed-flow treatments themselves (i.e., one that controls for as many other sources of variation as possible). Therefore, this study does not use the terms “control” or “reference” to refer to

pristine, unregulated conditions, but rather to those conditions that most closely approximate those of the treated reach, minus only the pulsed-flow treatments.

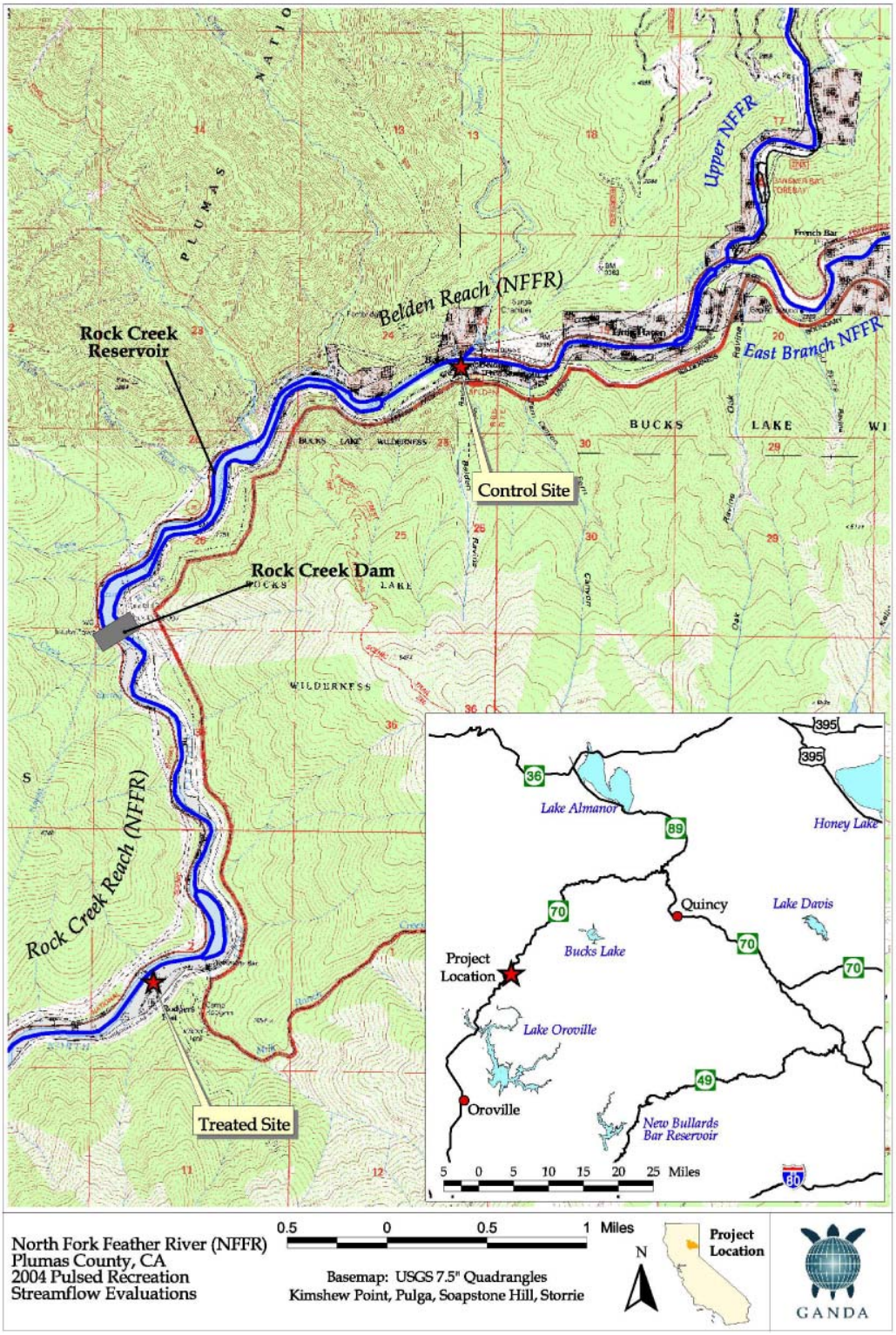

Figure 1. Study area

2.3. Benthic Macroinvertebrate Sampling Using Rock Basket Samplers

Stream benthos samples were primarily collected using modified Coleman-Hynes rock basket samplers (after Coleman and Hynes 1970, with design modifications by Wayne Fields of Hydrozoology). These basket samplers were fabricated from stainless steel and consisted of concentric cylindrical wire baskets with 1½-inch (3.8-cm) mesh and open tops and bottoms. An open-top cylindrical Nitex net (500-micron mesh), fitted with a frame ring and handle assembly at the top, completed the sampling device (Figure 2). Samplers were eight inches (20.3 cm) deep with a six-inch (15.2-cm) inner basket diameter and an eight-inch outer basket diameter. Unlike the original Coleman-Hynes samplers, which tended to accumulate fine sediment during their deployment, these basket samplers allowed sediment to pass through as freely as the surrounding bed substrate.

A total of 120 basket samplers were deployed in 2004: 90 samplers at the treated Rock Creek site, and 30 samplers at the Belden control site. All samplers were deployed in "wadeable" areas along transects within accessible ranges of water depth (approximately 0.25–0.75 m) and velocity (approximately 0.15–1.00 m/s). For each sampler, the substrate was excavated (boulders moved, etc.) to allow deep enough placement of the outer basket such that the top was approximately level with mean height of the surrounding substrate. This process required the use of a prybar and snorkeling equipment. Once the outer basket of each sampler was placed deep enough, a few larger rocks were used to help anchor the sampler. These rocks were placed around the sampler so as not to impede the flow of water and material over or through the sampler (i.e., so that anchor boulders did not create large eddies at the device). After the outer basket was securely positioned, the sample retention net and inner basket assembly was placed inside, and the inner basket was filled with native substrate material (cobble and gravel particles approximately 5 to 10 cm in diameter) collected from the adjacent streambed. This substrate material was cleaned by hand (i.e., scrubbed free of clinging invertebrates), thereby leaving epilithic algal layers essentially intact. Once the inner basket was filled, the sampler was set for collection by depressing the net ring handles so the sample retention net was set securely at the bottom of the space between the baskets (see Figure 2). In order to facilitate easy relocation, samplers were deployed along transects perpendicular to the flow in groups of three to five. For each sampler, the distance along each transect was measured and recorded from a monumented point on the stream bank. All samplers were tagged and numbered using aluminum tree tags and plastic zip ties. The location of individual samplers was pinpointed on aerial photographs.

All samplers were left in situ for a minimum of six to eight weeks prior to sampling to allow equilibration with the surrounding stream bottom. This equilibration time was longer than the minimum times published for recolonization of stream benthos in North America (see Rosenberg and Resh 1982, Klemm et al. 1990). Increased equilibration

times resulted in no significant differences in the macroinvertebrate communities that developed in these basket samplers (GANDA 2005b). In this study, the use of native substrates with the epilithic algal layer left intact presumably reduced the time for the invertebrate community to reach equilibrium in the samplers.

Figure 2. Modified Coleman-Hynes rock basket samplers used for 2004 benthic macro-invertebrate sampling in the NFFR. The sampler on the right shows the net in the retracted or "set" position. Note that the inner basket is not filled with substrate in this photo.

Two days prior to each monthly recreation streamflow event, 25 randomly selected samplers were collected to represent pre-flow conditions for that event (20 from the treated site and 5 from the control site). The day after each flow event, another 25 randomly selected samplers were chosen to represent immediately-post-flow conditions for that event, for a total of 50 samples per month. All selected samplers were chosen randomly from the pool of samplers that had adequately equilibrated with the surrounding substrate (i.e., that had been undisturbed in situ for at least 6–8 weeks). For each sampling event (pre- or post-flow), all 25 samples from both sites were collected in the same day. Sampling always began at the downstream-most transect and proceeded in the upstream direction so as not to disturb any downstream substrate or samplers before collections were made.

Once a randomly selected sampler was located, the sampling process consisted of swiftly raising the net by the ring handles in order to completely surround the inner basket and all of its contents. The sample was then transferred to a processing area on the stream bank where the substrate and other material contained within the sample was separated from collected invertebrates. Individual rocks, and the inner basket itself, were scrubbed by hand in a bucket of clean water to remove clinging invertebrates. The percentage of interstitial space that was occupied by sediment and organic debris in the inner basket of each sampler was visually estimated during the collection of each sample. Such retained material (sand, gravel, organic debris, etc.) was cleaned and separated from the rest of the sample material using a smaller bucket and a No. 35 standard brass soil sieve (500-micron mesh). The sample material was cleaned free of sand, organic debris, etc. (or "elutriated") to the greatest extent possible in the field, fixed and preserved in 10% formalin, labeled inside and out, and sealed in a leak-proof sample container. Samples were later transferred to 70% ethanol after fixing in formalin for several days.

After sampling, all samplers were reset by replacing the cleaned net and inner basket back into the in situ outer basket. The inner basket was then refilled with the cleaned substrate material and the net was reset at the bottom of the space between the baskets. Collection and reset dates for each sampler were carefully recorded to ensure that all samplers at each site were given adequate equilibration time before being re-sampled.

2.4. Benthic Macroinvertebrate Sampling Using Standard Kick Sampling

Stream benthos samples were also collected using standard kick sampling. These samples were collected concurrently with basket samples in both the control and treated reaches during the June and September 2004 sampling events (only). Kick sampling followed a modified version of the California Stream Bioassessment Procedure (CSBP), a standardized field method for collecting benthic samples developed by the California Department of Fish and Game (CDFG 2003a). Per the CSBP protocol, three riffle areas were randomly selected for sampling at each site, and one replicate sample was collected per riffle. Each replicate sample was a composite of three kick-sample collections that covered the widest variety of depths, velocities, and substrate sizes possible in the given riffle area.

Kick samples were collected using a Wildco 9-by-18-inch (23-by-46-cm) rectangular kick net with an oversized 0.5-mm mesh bag. Individual kick-sample collections covered two square feet of the stream bottom. The three kick-sample collections made within each riffle were combined to form a composite sample that represented a total of six square feet (55.7 cm^2) of stream-bottom area. This procedure was repeated at each of the three riffle areas at a given site, such that a total of three replicate samples were collected from each site. All samples were elutriated and cleaned in the field, placed in jars, labeled, and preserved in 10% formalin. CSBP protocols are detailed further in CDFG (2003a).

During CSBP sampling, caution was taken at each site to avoid sampling the exact same area each time. Therefore, all sampled locations per composite sample were marked and described in detail during each CSBP sampling event. Markers consisted of colored nylon mesh bags filled with large cobbles to anchor them in place. These areas were then easy to avoid during subsequent sampling events, and no superpositioning of bottom samples occurred.

2.5. Physical Habitat/Water Quality Sampling

Physical and chemical water quality parameters were sampled prior to each macroinvertebrate sampling event in 2004. The measured parameters included water temperature, pH, specific conductance, salinity, and dissolved oxygen concentration and saturation. Temperature, specific conductance, and dissolved oxygen were measured using a YSI model 85 hand-held meter. Acidity (pH) was measured using a hand-held Oakton pHTester3. Mean depth and stream velocity at base flow (approximately 250 cfs,) were measured for all samplers using a U.S. Geological Survey (USGS) pygmy meter and Rickly Hydrology® AquaCount calculator. Velocity measurements at each basket sampler location were taken at six-tenths total water depth, the USGS standard for mean water column velocity. Velocity measurements were also taken at the sampling devices themselves, or at a point as close to the mean sampler location as possible. These velocity and depth measurements were taken during each benthic macroinvertebrate sampling event at the control site (Belden Reach) because discharge fluctuated throughout the sampling season. For each kick-sampling event, mean water column velocity (at six-tenths total water depth) was measured at the point of each kick-sample collection.

All streamflow data for the Rock Creek Reach were obtained from gage NF57 located immediately below the Rock Creek Dam. Streamflow data for the Belden Reach were obtained from gage NF51 located on the East Branch NFFR upstream of the confluence with the Upper NFFR, and from gage NF70 located on the Upper NFFR just downstream of the Belden Dam.

Water temperature at each site was monitored continuously throughout the study using submersible data-logging temperature probes (Onset Optic Stowaway temperature loggers). Probes were encased in a protective housing and tethered to the bank with stainless steel cable. All probes were deployed so as to remain submerged during fluctuating flow conditions, and were left in situ for the entire sampling season. Two of these probes were deployed at each site for redundancy. Water temperature was recorded once every hour, and data were downloaded at regular intervals during the study.

2.6. Laboratory Procedure

Whenever possible, samples were identified in their entirety; however, most samples were large enough to require subsampling in order to expedite processing. The

subsampling process involved dividing the sample material into coarse and fine portions, and then subdividing only the fine portion (sensu Chan 1998). Our subsampling procedure differed from commonly employed "fixed-count" subsampling methods (e.g., CSBP laboratory procedure [see CDFG 2003a]) in that subsamples were taken from the fine portion of a sample only (i.e., the coarse portion of the sample was always completely identified and enumerated), and subsamples consisted of measured whole fractions of the fine portion as opposed to a limited number of organisms (e.g., a fixed count of 300 or 500 individuals). Jon Lee Consulting performed all laboratory processing and taxonomic identification, with some consultation provided by Wayne Fields of Hydrozoology.

In the laboratory, samples were first drained of preservative and placed in a sorting tray with water for picking. Large and conspicuous invertebrates were picked by eye and retained as part of the coarse portion. After this preliminary picking effort, the remaining sample material was carefully washed through a stacked series of ¼-inch (6.35 mm) and 500-micron (0.5-mm) standard soil sieves to separate the coarse and fine portions of the sample. This "double-sieving" process provides a systematic and repeatable means of separating the coarse and fine portions of benthic samples. The material was agitated through the stacked sieves under a gentle stream of water to ensure adequate separation (with careful attention taken not to damage specimens). Agitation of the stacked sieves during immersion in a water bath helped expedite the cleaning of the coarse portion and promoted the gentle transfer of fine material through the coarse sieve. All the material held on the ¼-inch sieve was picked through completely and all invertebrates were combined with organisms from the preliminary picking effort to form the complete coarse portion.

The fine portion of the sample was defined as that which passed through the ¼-inch sieve and was held on the 500-micron sieve. This material was placed in a standard subsampling tray with numbered grid sections and thoroughly homogenized. The fine portion was then subsampled by sorting one randomly chosen grid section at a time until at least 300 individuals were obtained. The last grid section chosen was always picked through and identified in entirety to permit more accurate estimation of the total fine portion. This subsampling method resulted in more than 300 individuals being identified for each subsample (typically closer to between 350 and 400 organisms). In cases where fewer than 300 individuals were present in the entire fine portion, all grid sections were completely picked and identified (i.e., the fine material was not subsampled).

All specimens were identified to species using necessary taxonomic keys. When exact species epithets could not be determined, morphotype designations (e.g., species "A," "B," or "C") were used so that true diversity and richness could be calculated. Some specimens were not identified to the species level (e.g., unidentifiable life stages such as pupae or very early instars, or damaged specimens); however, most taxa were identified to the equivalent of the species level as described above. A reference collection

consisting of all taxa encountered has been established and will be curated indefinitely by Jon Lee Consulting.

2.7. Data Analysis

Macroinvertebrate data were analyzed in terms of three general comparisons: (1) control vs. treated reach differences before and after recreation streamflow events, testing for short-term pulsed-flow effects; (2) seasonal differences between treated and control reaches, testing for longer-term or cumulative effects of repeated pulsed flows; and (3) comparisons of the basket vs. kick-sampling methods.

All taxonomic data were entered into a Microsoft Access database for manipulation and calculation of summary metrics. These metrics included standard CSBP metrics for richness, composition, tolerance/intolerance, and functional feeding group distribution, plus several others as defined in Table 1. Tolerance values and functional feeding group (FFG) classifications for benthic macroinvertebrate metrics were assigned using the most current designations (CDFG 2003b). Metrics were calculated for each replicate sample and then averaged to obtain mean values for each sampling event at each site.

All data analyses and statistical calculations were performed using whole-sample taxa lists for each sample (i.e., actual whole-sample lists for samples that were small enough to be picked through in their entirety, or estimated lists for samples that required subsampling). Estimated whole-sample taxa lists were calculated as the coarse portion total plus the extrapolated fine portion total (i.e., some multiple of the subsample taxa list). For example, if one-third of the fine portion was used as the subsample (e.g., 8 out of 24 grid sections), then the extrapolated fine portion was estimated as three times the subsample taxa list. In this example, the tripled fine portion total, plus the coarse portion total, would equal the estimated whole-sample total.

Sample data were standardized in this manner so that quantitative inventories for each sample equivalently represented the total amount of substrate sampled by each device. Whole-sample data are preferable over standard fixed-count bioassessment data (e.g., 300-bug taxa lists) in that analyses are not fundamentally limited by qualitative characterizations of some fraction of the actual information collected. This problem occurs especially with large rare taxa that are frequently missed during compilation of a random fixed-count subsample. Ensuring the inclusion of large rare specimens in the sample data (by using the "double-sieving" subsampling technique) retains important richness information, and represents a significant improvement in overall data quality. Furthermore, the double-sieving process also creates a more homogeneous fine portion in which the invertebrates are more evenly distributed and easier to subdivide equally, thereby improving the overall quality of the subsample.

In order to reduce the complexity of the information contained in the numerous metrics that describe each sample, data were compiled into a single multi-metric index. A multi-metric index can effectively reduce the uncertainty associated with assessing biological

conditions by combining responsive metrics that provide multiple lines of evidence for diagnosing biological impairment (Karr and Chu 1999). The variability associated with individual metric values, for example, is typically higher than that of the index in which the metrics are aggregated.

The multi-metric index calculated in this study was the "hydropower multi-metric index" (or Hydro-MMI), which was developed by the CDFG Aquatic Bioassessment Laboratory to be sensitive to the cumulative effects of hydropower operations on stream benthic communities (Rehn et al. 2005). The five component metrics of the Hydro-MMI

Table 1. Biological metrics used to describe benthic samples collected from the NFFR using rock basket- and kick-sampling techniques. Listed metric responses are for generalized ecological impairment (specific disturbance events and/or different types of disturbances may cause other responses).

METRIC	DESCRIPTION OF METRIC	RESPONSE TO IMPAIRMENT
RICHNESS-TYPE MEASURES		
# Total Taxa	Total taxa richness	Decrease
# EPT Taxa[1]	Number of mayfly (Ephemeroptera), stonefly (Plecoptera), and caddisfly (Trichoptera) taxa	Decrease
# Ephemeroptera Taxa	Number of mayfly taxa	Decrease
# Plecoptera Taxa	Number of stonefly taxa	Decrease
# Trichoptera Taxa	Number of caddisfly taxa	Decrease
# Coleoptera Taxa[1]	Number of beetle taxa	Decrease
# Diptera Taxa	Number of true fly taxa	Variable
# Chironomid Taxa	Number of taxa in the dipteran family of midges (Chironomidae)	Increase
# Clinger Taxa	Number of taxa with behavioral or morphological adaptations for clinging to substrates	Decrease
# Long-Lived Taxa	Number of taxa with life spans greater than 1 year	Decrease
# Tolerant Taxa	Number of taxa highly *tolerant* of impairment as indicated by tolerance values of 8, 9, or 10	Increase
# Intolerant Taxa	Number of taxa highly *intolerant* of impairment as indicated by tolerance values of 0, 1, or 2	Decrease
Shannon Diversity Index	Index of general sample diversity that incorporates richness and evenness (ln-based)	Decrease
Shannon Evenness	Measure of how evenly taxa abundances are distributed	Decrease
Est. Total # Indiv.	Estimated total number of individuals collected per sample	Variable
COMPOSITION-TYPE MEASURES		
% EPT	Percent of organisms in EPT taxa	Decrease
% Sensitive EPT	Percent of organisms in EPT taxa with tolerance values of 0, 1, 2, or 3	Decrease
% Baetidae	Percent of organisms in the mayfly family Baetidae	Increase
% Hydropsychidae	Percent of organisms in the caddisfly family Hydropsychidae	Increase
% Dominant Taxon	Percent of organisms in the most abundant taxon	Increase
% Dominance (Top 3 Taxa)	Percent of organisms in the three most abundant taxa	Increase
% Tolerant Organisms	Percent of organisms highly *tolerant* of impairment as indicated by tolerance values of 8, 9, or 10	Increase
% Intolerant Organisms	Percent of organisms highly *intolerant* of impairment as indicated by tolerance values of 0, 1, or 2	Decrease
% Tolerant Taxa[1]	Percent of taxa highly *tolerant* of impairment as indicated by tolerance values of 8, 9, or 10	Increase
% Intolerant Taxa	Percent of taxa highly *intolerant* of impairment as indicated by tolerance values of 0, 1, or 2	Decrease
% Collector-Filterers (CF)	Percent of organisms that filters fine particulate matter	Increase
% Collector-Gatherers (CG)	Percent of organisms that collects or gathers fine particulate matter	Increase
% CG+CF[1]	Percent of organisms that filters plus gathers fine particulate matter (combined)	Increase
% Scrapers	Percent of organisms that grazes upon periphyton	Variable
% Non-Gastropod Scrapers[1]	Percent of organisms exlcuding snails and limpets (Gastropda) that grazes upon periphyton	Decrease
% Shredders	Percent of organisms that shreds coarse particulate matter	Decrease
% Predators	Percent of organisms that feeds on other organisms	Variable
MULTI-METRIC INDEX		
Hydropower Mulit-Metric Index (Hydro-MMI)	Composite index of 5 key metrics[1] describing cumulative effects of hydropower operations on benthos	Decrease

1. One of the five key metrics used in the Hydro-MMI

were chosen from over 75 candidate metrics calculated using a combined dataset from nine separate studies of regulated rivers in California managed for hydropower. These five metrics were screened from the pool of candidate metrics based on their biological and/or statistical redundancy, the suitability of their scoring ranges, and their ability to discriminate below-dam sites from above-reservoir reference sites. The Hydro-MMI was selected because it was specifically designed to discriminate hydropower-related effects, of which flow regulation and manipulation, including pulsed flows, are defining features. Standardized scores were assigned to each of the five Hydro-MMI metrics (following Rehn et al. 2005) for both basket and kick samples. Scoring thresholds for Hydro-MMI metrics did not require adjustment or re-scoring for the different sample types collected as part of this project (A. Rehn, Aquatic Biologist, CDFG Aquatic Bioassessment Laboratory, personal communication). These standardized scores (defined in Table 2) were summed to achieve a composite Hydro-MMI score for each sample (adjusted to a 100-point scoring scale as recommended by Rehn et al. [2005]).

Table 2. Hydro-MMI metrics and scoring thresholds used for NFFR basket-sample and kick-sample data collected in 2004

Standardized Score	#EPT Taxa	#Coleoptera Taxa	%CF+CG Individuals	%Non-Gastropoda Scrapers	%Tolerant Taxa
0	≤8	0	≥90	0	≥19
1	9-10		84-89	1-4	18
2	11-12	1	78-83	5-8	16-17
3	13-14		72-77	9-12	14-15
4	15-16	2	66-71	12-16	13
5	17-18		60-65	17-19	11-12
6	19-20	3	54-59	20-22	9-10
7	21-22		48-53	23-26	8
8	23-24	4	42-47	27-30	6-7
9	25-26	5	36-41	31-34	4-5
10	≥27	≥6	≤35	≥35	≤3

Although the standard Hydro-MMI scoring thresholds were applied to the project data, it remains unclear how differences in field sampling methods (e.g., basket vs. kick sampling) and sample processing techniques (e.g., double-sieving vs. fixed-count subsampling) employed in this study affected the final outcome of Hydro-MMI scores. Therefore, no attempt was made to categorize biological conditions described by the sample data as “good,” “fair,” or “poor” relative to other studies per the standard Hydro-MMI criteria. Instead, this study focused on evaluating pulsed-flow effects within the context of the dataset and the confines of the project’s limited BACI design.

In this study, pre-flow and post-flow sampling in the treated and control reaches was performed on the same days. This concurrent sampling was used to eliminate the effects of large-scale environmental variables that were assumed to affect these two reaches equally (e.g., climate). The difference between the control and treated reaches prior to the recreation streamflow release (as estimated by pre-flow data) was considered the baseline difference between the two locations. Post-flow data were used to estimate the control-to-treated difference following the recreation streamflow release. Consequently, the magnitude of the difference between the pre-flow control-to-treated difference and the post-flow control-to-treated difference was considered attributable to the pulsed-flow treatment. One might argue that the magnitude of the difference between the pre-flow and post-flow control-to-treated differences may have alternative explanations (other than the pulsed-flow treatment). The authors acknowledge this limitation, including the implications for interpreting study results in terms of establishing correlations, as opposed to determining definitive causality.

In the context of the study's BACI design, analysis of variance (ANOVA) was used to test for a significant pulsed-flow-related effect with Hydro-MMI as the response variable. ANOVAs were performed using the general linear model function in the Minitab statistical package (Release 13.32). The main hypotheses of interest for testing for this treatment effect using ANOVAs were:

> H_O: The pre-flow control-to-treated difference is the same as the post-flow control-to-treated difference; *and*
>
> H_A: The pre-flow control-to-treated difference is <u>not</u> the same as the post-flow control-to-treated difference

Similar hypotheses were used to test for differences in longer-term seasonal trends between the treated and control reaches, and to test for differences between the basket- and kick-sampling methods.

A common problem among many large-scale ecological studies is the lack of true replication of the treatments (Hurlbert 1984), and this study in the NFFR was no different. In assessing the effects of the pulsed flows, the research team could not randomly assign the recreation streamflow treatments to multiple river reaches. Therefore, this study focused on a single experimental unit receiving the treatment (the treated reach) and a single experimental unit receiving no treatment (the control reach). Even though multiple measurements were taken from each reach during each sampling period, these measurements did not constitute true replicates of the treatments, but rather multiple measurements from each experimental unit. Multiple measurements from an experimental unit are useful, because they increase the sensitivity of the experiment by increasing the precision with which the effects of the treatment are estimated.

The appropriate analysis of an unreplicated or pseudoreplicated experimental design requires a modification of the standard ANOVA model because no degrees of freedom are available for estimation of the error variance (Hurlbert 2004). However, data from an unreplicated or pseudoreplicated experiment can be analyzed with an ANOVA that uses the interaction mean square as an estimate of the true error mean square. A Tukey test of additivity was used to show that the three-factor interaction effect was negligible in this study (i.e., the Month-by-Event-by-Treatment effect); this step was necessary to justify the use of the interaction mean square as an estimate of the true error mean square in the ANOVAs. The preceding hypotheses were tested by examining the Event-by-Treatment interaction in the ANOVAs (using the three-factor interaction mean square as an estimate of the true error mean square) with an alpha (i.e., significance level) of 0.10 (10%). This analysis was performed using basket-sample and kick-sample data.

Abundances of the major taxonomic groups (major invertebrate classes and insect orders) were compared as part of descriptive characterizations of pre- and post-flow benthic community structure in the treated and control reaches. Previous studies have shown that certain taxa (or groups of taxa) are more affected by recreation streamflow releases in the NFFR than others. For example, invertebrate drift monitoring in the Rock Creek Reach during 2002 documented significant increases in typically non-drifting taxa both during and after recreation streamflow events (GANDA 2005a). Indeed, typically non-drifting taxa were frequently among the most common taxa in drift samples during and after 2002 recreation streamflow releases. Pre- and post-flow abundances of fifteen of these "drift taxa" were also tracked in 2004 benthic data.

Seasonal trends among summary macroinvertebrate metrics, and the abundances of the major taxa groups, were compared using pre-flow data collected from the treated and control reaches throughout the sampling season. Statistical differences among seasonal trends in these reaches were evaluated using an ANOVA with Hydro-MMI as the response variable. In this analysis, the dates of the pre-flow sampling events were expressed in terms of Julian date (a time covariate that serves as a continuous variable) in order to allow a "seasonal trend" regression line to be fit separately for the control and treated reaches. Julian dates express standard day-month-year information as a single number (e.g., June 25, 2004, has a Julian date of 13181 and July 23, 2004, has a Julian date of 13209, exactly 28 days later).

The basket- and kick-sampling methods were compared in terms of general qualitative and quantitative differences between sample types (e.g., typical metric values for richness and composition measures, as well as abundance comparisons of the major taxa groups). Statistical differences between the two sampling methods were evaluated using an ANOVA with Hydro-MMI as the response variable.

Error bars on all graphs were calculated as plus and minus two standard errors. Standard error is defined as the standard deviation of the observations divided by the square root of the number of observations. An error range of plus and minus two

standard errors was used because this range corresponds roughly to the 95% confidence interval. Note that for line graphs displaying seasonal trends throughout the study period, lines connecting data points from different monthly sampling events (e.g., between June post-flow and July pre-flow means) are used help illustrate general seasonal trends; however, this does not imply that trends between these data points are necessarily linear during the periods when there was no sampling (between pulsed-flow events).

3.0 Project Outcomes

With the exception of peaks in discharge during recreation streamflow releases in the treated reach, the flow regime in the treated and control reaches was generally similar during the study period. Recreation flows in the Rock Creek Reach peaked between 1,200 and 1,900 cfs, and returned to baseflow levels of approximately 250 cfs typically within the same day. Discharge in the partially regulated Belden Reach was higher immediately prior to the study period (in May) due to snowmelt runoff from the unregulated East Branch NFFR. Baseflow in the Belden Reach was near 200 cfs for most of the study period, although a reduction to near 125 cfs occurred in September when flow in the regulated Upper NFFR was reduced. During the summer of 2004, flows in the Upper NFFR were kept artificially high until just after Labor Day Weekend to enhance recreation. The hydrographs for the treated and control reaches during the study period are plotted in Figure 3.

Temperature regimes were also generally comparable between the treated and control reaches during the study. Stream temperature in the partially regulated Belden Reach was slightly higher in the spring, and slightly lower in the fall, than in the regulated Rock Creek Reach (presumably due to the tempering effect of Rock Creek Reservoir). Stream temperature varied by approximately 3°C per day in the Belden Reach, compared to only 2°C per day in the Rock Creek Reach; however, mean daily temperature was very similar between these reaches (less than a one-degree average difference over the course of the study). Mean daily stream temperature for these reaches during the study period is plotted in Figure 4.

Other measures of physical habitat and water quality were also similar between the treated and control reaches. Average depth, velocity, and substrate composition were comparable for both reaches (Table 3), as were general water quality measures (Table 4).

3.1. Short-Term Pulsed-Flow Effects

Tukey tests of additivity demonstrated nonsignificant three-factor interactions between the month (June through October), treatment (treated vs. control), and event (pre- vs. post-flow) factors for both basket-sample data and kick-sample data (p = 0.299 and 0.742, respectively). Therefore, it was valid to perform ANOVAs (using the three-factor interaction mean square as an estimate of the true error mean square) to test for a pulsed-flow-related effect using both basket- and kick-sample data in the context of the study's BACI design.

3.1.1. Basket-Sample Data

The Event-by-Treatment interaction was statistically significant (p = 0.011) in the ANOVA for basket sample data, using Hydro-MMI as the response variable—, meaning the difference in average Hydro-MMI score of basket samples between the treated and control reaches was not the same before and after pulsed-flow treatments. The Minitab

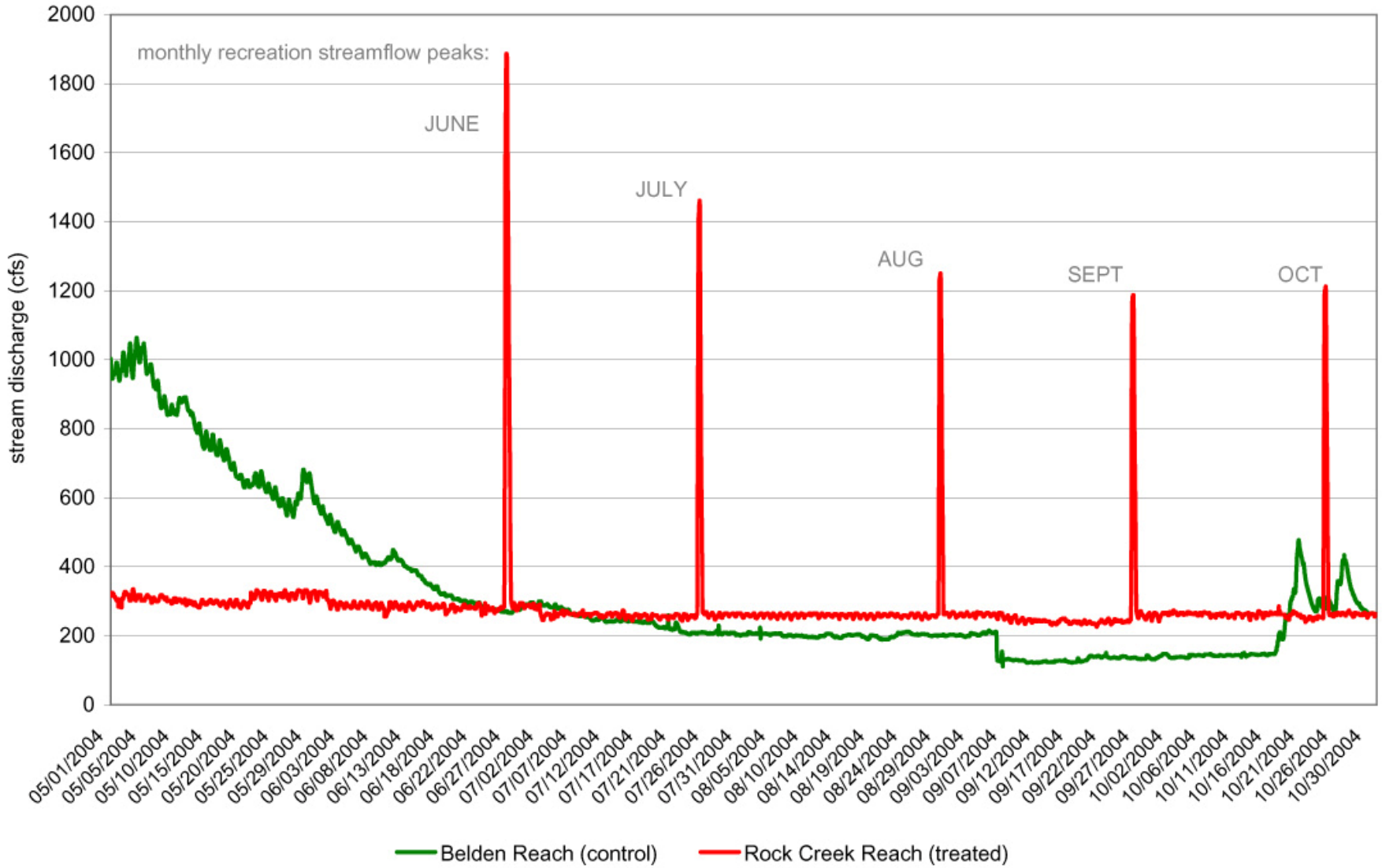

Figure 3. Hydrograph for treated and control reaches (NFFR 2004)

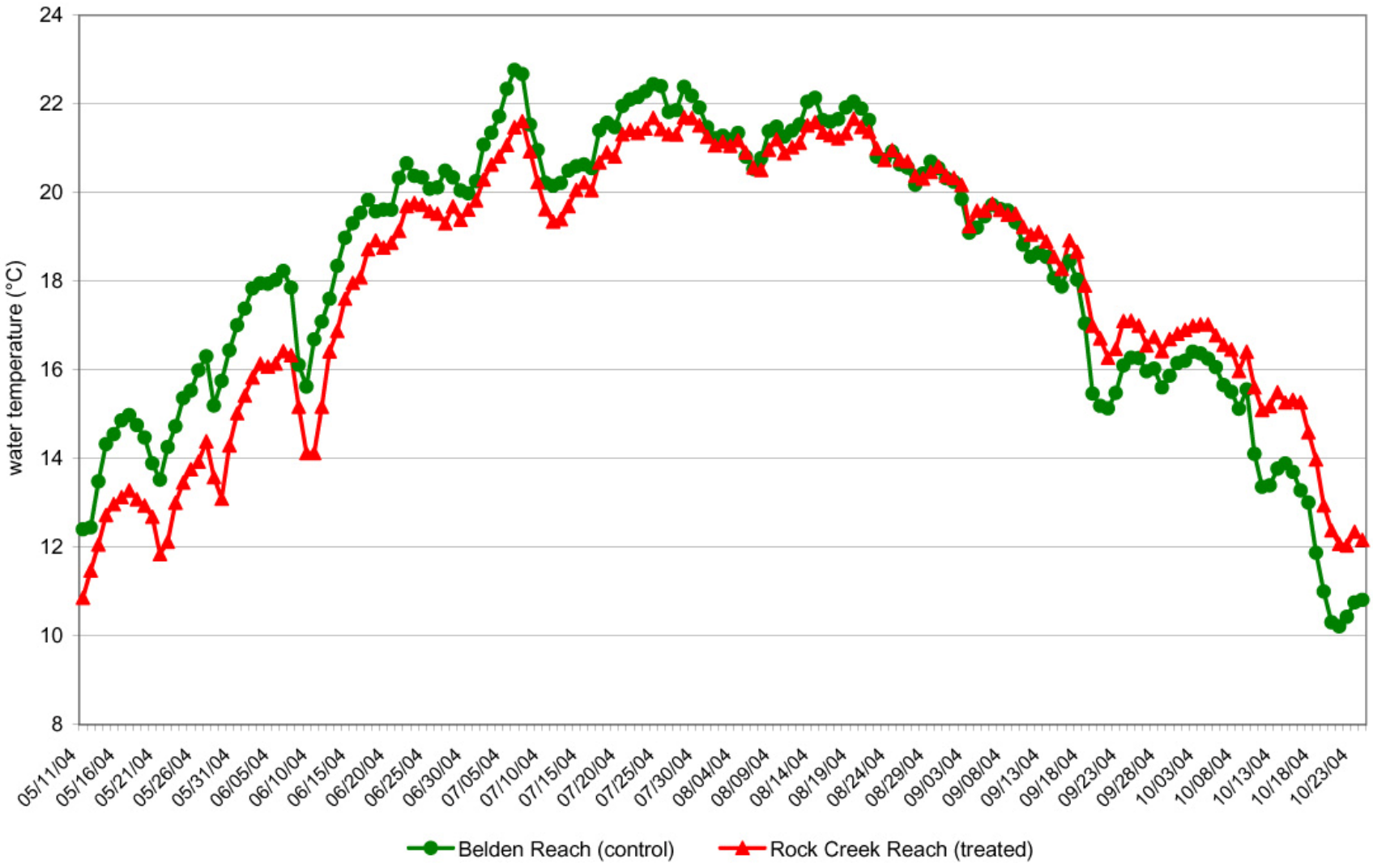

Figure 4. Mean daily stream temperature for treated and control reaches (NFFR 2004)

Table 3. Summary of physical habitat data from CSBP Bio-assessment Worksheet and Physical Habitat Quality Form

2004 Kick Samples	**Rock Creek (treated)**	**Belden (control)**
REACH-WIDE PHYSICAL HABITAT SCORES		
epifaunal substrate (0-20)	17	18
embeddedness (0-20)	16	15
velocity/depth regimes (0-20)	16	16
sediment deposition (0-20)	17	16
channel flow (0-20)	20	15
channel alteration (0-20)	20	15
riffle frequency (0-20)	N/A	N/A
bank stability (0-20)	20	18
vegetative protection (0-20)	20	18
riparian zone width (0-20)	18	12
total physical habitat score (0-180)	164	143
SITE PHYSICAL HABITAT		
riffle width (m)	32.3	47.0
avg. depth (ft)	1.8	1.6
avg. velocity (m/s)	0.6	0.6
%canopy cover	3	1
substrate complexity (0-20 scale)	18	18
riffle embeddedness (%)	16	15
substrate consolidation	med	med
%fines (<0.1")	10	12
%gravel (0.1-2")	7	10
%cobble (2-10")	38	40
%boulder (>10")	45	38
%bedrock (solid)	0	0
riffle gradient (%)	1.2	1.5

Table 4. Water quality data from CSBP Bioassessment Worksheet. Shaded values are for the Rock Creek Reach (treated) and unshaded values are for the Belden Reach (control).

2004 Kick Samples	**JUNE**				**SEPT**			
	PRE		**POST**		**PRE**		**POST**	
date	6/26/04	6/26/04	6/29/04	6/29/04	9/24/04	9/24/04	9/27/04	9/27/04
time	9:40	16:00	9:11	12:02	9:30	14:06	9:27	14:30
temp (°C)	18.4	22.5	18.3	19.6	15.2	17.2	15.5	17.1
conductance	N/A	164.9	115.2	162.4	116.8	193.1	119.7	188.9
pH	8.1	8.5	8.1	8.4	8.2	8.7	8.1	8.7
DO (mg/L)	7.0	6.5	6.8	7.2	8.5	9.1	8.1	9.1
DO saturation	74%	75%	72%	78%	86%	94%	82%	94%

output for this ANOVA is provided in Table 5. The control-to-treated difference in average Hydro-MMI scores for each sampling event is illustrated in Figure 5. This difference was generally smaller following recreation streamflow releases than it was before. However, note that a difference of only a few Hydro-MMI points (as is typically the case here) may not be biologically significant (regardless of the finding of statistical significance in the ANOVA).

Table 5. Minitab output for the ANOVA to test for a pulsed-flow treatment effect among basket-sample data using a general linear model with Hydro-MMI as the response variable (DF= degrees of freedom; SS= sum of squares; MS= mean square; F= F test statistic; P= probability).

General Linear Model: Average MMI vs. Month, Event, Treatment

Source	DF	SS	MS	F	P
Month	4	63.2845	15.8211	22.52	0.005
Treatment	1	23.7620	23.7620	33.82	0.004
Error	4	2.8105	0.7026		

*These tests use error term = Month*Treatment

Source	DF	SS	MS	F	P
Event	1	1.7405	1.7405	10.68	0.031
Event*Month	4	1.2620	0.3155	1.94	0.269
Event*Treatment	1	3.2805	3.2805	20.13	**0.011**
Error	4	0.6520	0.1630		

*These tests use error term = Month*Event*Treatment

Average values for individual metrics are summarized per sampling event for basket-sample data in Table 6. Mean values for the treated reach are shaded, and mean values for the control reach are not shaded.

Pre- and post-flow abundances of the major taxonomic groups (major invertebrate classes and insect orders) are presented in Table 7 for basket samples. No distinct control-to-treated differences in abundance were evident for the major taxa groups from pre- to post-recreation streamflows. Pre- and post-flow abundances for these groups were typically variable in both reaches. In general, the treated reach had relatively more clams (Bivalvia), and fewer mites (Arachnida), worms (Oligochaeta), beetles (Colepotera), and caddisflies (Trichoptera) than the control reach.

Similarly, no distinct control-to-treated differences in abundance were evident from pre- to post-flow for taxa that were the most abundant in the drift during and after 2002 recreation streamflows. Of these "drift taxa," most were also abundant in 2004 benthic samples; however, several were not well represented in benthic samples and thus were not listed in Table 7 (chironomids of the *Dicrotendipes* spp. and *Cardiocladius* spp., the worm *Ophidonais serpentina*, freshwater mites of the *Lebertia* spp.; the hemipteran *Sigara mckinstryi*, and the net-spinning caddisfly *Psychomyia flavida*).

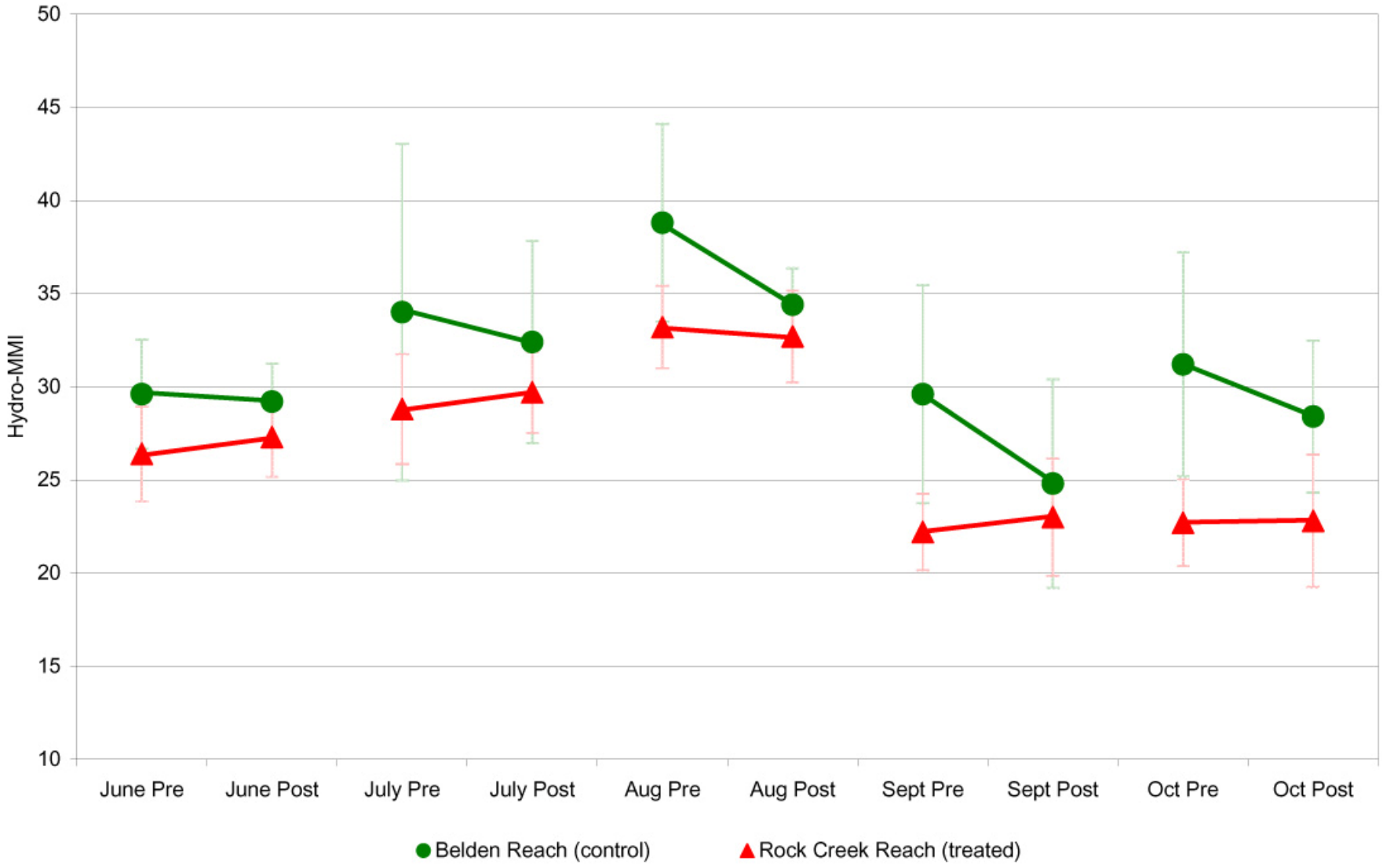

Figure 5. Average Hydro-MMI for treated and control reaches (2004 NFFR basket sampling)

Table 6. Macroinvertebrate metric summary (2004 basket sampling). Listed values are mean metric values per sample for each sampling event and location (shaded= Rock Creek Reach (treated), unshaded= Belden Reach (control).

2004 Basket Samples	JUNE				JULY				AUGUST				SEPTEMBER				OCTOBER			
	PRE		POST		PRE		POST		PRE		POST		PRE		POST		PRE		POST	
RICHNESS-TYPE MEASURES																				
# Total Taxa	39.2	35.6	45.8	34.4	42.5	44.8	44.4	45.8	41.1	41.4	43.2	41.4	37.9	39.2	37.3	38.0	34.2	37.8	35.0	36.0
# EPT Taxa	12.8	14.2	15.3	15.6	15.3	15.6	14.6	15.8	15.8	17.2	16.5	17.6	13.6	13.8	13.1	13.6	13.5	15.2	13.4	13.8
# Ephemeroptera Taxa	5.7	5.0	6.7	4.4	5.4	7.2	4.8	5.6	6.3	6.8	6.8	7.2	5.3	5.4	4.7	4.8	5.1	5.2	5.2	4.8
# Plecoptera Taxa	1.4	1.8	2.0	3.2	2.2	2.4	2.2	2.2	1.5	2.6	1.7	2.4	1.5	1.6	1.4	2.2	1.4	2.6	1.3	2.2
# Trichoptera Taxa	5.7	7.4	6.6	8.0	7.7	6.0	7.6	8.0	8.0	7.8	8.0	8.0	6.9	6.8	7.0	6.6	7.1	7.4	7.0	6.8
# Coleoptera Taxa	0.2	0.6	0.3	0.4	0.5	0.8	1.0	1.4	1.1	1.4	1.1	0.4	0.9	1.2	1.0	0.6	1.0	2.0	0.9	1.6
# Diptera Taxa	21.7	14.6	23.3	14.8	20.6	21.6	20.6	21.4	15.9	16.0	17.5	17.2	14.9	15.2	14.5	13.6	12.0	10.8	11.9	8.2
# Chironomid Taxa	17.0	11.6	17.9	10.8	15.5	17.2	15.8	17.8	11.2	12.2	12.4	12.6	11.0	12.0	9.9	10.6	8.4	7.4	7.8	5.4
# Clinger Taxa	20.4	18.6	22.0	20.0	21.1	20.8	20.0	22.0	20.1	23.2	20.3	23.0	17.3	19.6	17.4	17.4	17.0	19.2	16.9	17.8
# Long-Lived Taxa	0.7	0.4	1.1	0.8	1.3	0.4	2.1	0.2	1.9	1.0	2.2	0.2	2.0	1.2	2.0	1.8	1.9	1.2	2.0	1.6
# Tolerant Taxa	2.1	1.4	2.8	1.4	3.1	3.2	3.7	4.4	3.6	2.4	4.3	2.6	4.8	3.6	4.5	4.0	5.0	5.2	4.9	5.2
# Intolerant Taxa	6.4	6.0	7.3	7.0	7.4	7.4	7.2	8.2	6.4	7.6	6.4	8.6	5.6	5.2	4.6	5.4	5.6	6.0	5.5	5.2
Shannon Diversity	2.59	2.38	2.65	2.31	2.52	2.80	2.57	2.84	2.59	2.71	2.80	2.71	2.63	2.60	2.63	2.52	2.46	2.47	2.57	2.42
Shannon Evenness	0.71	0.67	0.69	0.65	0.67	0.74	0.68	0.74	0.70	0.73	0.75	0.73	0.73	0.71	0.73	0.70	0.70	0.68	0.72	0.68
Est. Total # Indiv.	3844	3955	3137	5800	1725	1539	1847	2830	1190	1739	1470	1262	1671	1803	1690	1510	941	1382	1085	1410
COMPOSITION-TYPE MEASURES																				
% EPT	65.8	72.9	68.5	76.1	65.1	55.2	64.7	57.6	66.1	66.3	60.6	67.5	62.7	62.1	64.9	70.5	72.9	78.0	76.0	74.6
% Sensitive EPT	3.3	2.3	3.1	2.5	3.5	4.6	3.9	5.3	5.7	6.3	6.2	7.1	3.1	2.4	3.1	2.9	4.1	5.1	5.6	4.5
% Baetidae	16.7	14.5	17.1	12.4	6.0	6.2	4.8	3.7	6.1	5.2	10.2	6.9	10.7	5.6	13.6	6.8	14.2	13.0	19.3	7.6
% Hydropsychidae	45.4	53.2	47.7	57.4	53.9	41.6	53.8	45.4	45.1	45.3	37.7	48.1	42.0	47.0	40.5	52.1	44.4	51.0	40.7	49.0
% Dominant Taxon	27.6	31.7	27.5	30.0	33.2	25.2	33.5	25.8	33.4	28.1	24.6	32.4	28.5	30.6	28.0	29.6	31.6	30.9	25.8	31.3
% Dominance (Top 3 Taxa)	54.4	59.8	55.4	62.1	58.0	50.3	57.8	47.8	53.8	51.4	46.0	51.6	49.9	55.1	51.6	56.5	55.6	59.3	53.2	58.4
% Tolerant Organisms	0.9	0.5	1.2	0.6	1.3	1.9	2.0	2.2	4.0	2.4	4.0	3.3	6.4	6.1	7.0	6.4	11.3	8.1	10.2	10.3
% Intolerant Organisms	4.0	2.6	3.5	2.3	5.5	8.1	5.6	9.6	7.0	9.0	7.1	9.0	5.4	5.5	4.5	4.6	5.5	6.3	6.8	5.1
% Tolerant Taxa	5.1	3.4	6.0	4.0	7.1	6.7	8.3	9.5	8.5	5.9	9.7	6.3	12.5	9.2	12.0	10.6	14.6	13.5	14.0	13.9
% Intolerant Taxa	16.2	17.2	16.1	20.3	17.6	16.8	16.3	18.1	15.8	18.4	14.8	20.7	15.0	13.3	12.3	14.4	16.3	16.3	15.5	14.8
% Collector-Filterers (CF)	62.7	60.4	61.9	65.4	71.0	58.5	69.9	57.6	64.3	62.2	58.8	61.3	60.8	58.4	62.4	63.2	59.9	58.3	55.7	59.8
% Collector-Gatherers (CG)	31.4	35.0	31.9	28.7	18.5	24.6	17.7	25.6	18.4	20.7	24.4	20.5	28.8	26.2	25.7	20.9	23.0	22.4	28.1	17.1
% CF+CG	94.1	95.5	93.8	94.1	89.5	83.1	87.6	83.3	82.8	82.8	83.2	81.8	89.6	84.7	88.1	84.0	83.0	80.7	83.7	76.9
% Scrapers	2.4	1.0	1.7	0.4	2.3	6.0	2.6	2.7	5.5	3.4	5.3	3.6	1.7	2.2	1.9	1.6	2.4	3.9	3.2	2.7
% Non-Gastropod Scrapers	2.4	1.0	1.6	0.4	2.3	6.0	2.5	2.6	5.4	3.4	5.1	3.6	1.5	1.5	1.6	1.3	2.2	3.9	3.1	2.6
% Shredders	0.4	0.1	0.5	0.4	0.3	0.4	0.4	0.1	0.2	0.1	0.1	0.1	0.0	0.0	0.1	0.0	0.1	0.1	0.1	0.2
% Predators	1.5	1.6	2.2	1.8	3.6	6.4	5.1	7.1	7.2	8.0	6.9	10.1	6.2	11.4	7.3	13.0	8.9	11.6	8.8	16.2
MULTI-METRIC INDEX																				
Hydro-MMI	26.4	29.6	27.3	29.2	28.8	34.0	29.7	32.4	33.2	38.8	32.7	34.4	22.2	29.6	23.0	24.8	22.7	31.2	22.8	28.4

Table 7. Pre- and post-flow abundance of major taxa groups (2004 basket sampling). Listed values are mean number of individuals per sample for each sampling event (shaded= Rock Creek Reach [treated], unshaded= Belden Reach [control]).

2004 Basket Samples	JUNE				JULY				AUG				SEPT				OCT			
	PRE		POST		PRE		POST		PRE		POST		PRE		POST		PRE		POST	
MAJOR TAXA GROUPS																				
Arachnida	4	6	7	15	9	11	10	30	4	14	9	14	15	36	12	20	15	40	15	34
Bivlavia	1	0	4	0	1	1	3	0	12	0	16	0	32	4	36	6	46	5	41	12
Crustacea	1	0	4	0	1	1	2	0	1	2	3	0	1	0	1	1	1	2	1	4
Enopla	1	1	0	0	4	1	8	2	14	16	16	28	27	58	30	43	28	47	36	57
Gastropoda	1	0	2	0	1	0	1	1	1	0	3	0	2	12	3	4	2	0	1	0
Nematoda	2	3	1	0	11	6	10	7	4	5	5	2	3	0	3	2	2	1	3	1
Oligochaeta	155	531	114	269	21	45	38	148	27	66	42	64	61	150	56	69	12	52	34	49
Turbellaria	1	0	2	0	1	1	1	5	3	0	0	0	6	1	1	1	0	0	2	2
Coleoptera	1	10	2	7	2	4	7	19	6	14	5	2	5	8	5	5	4	13	3	13
Diptera	1139	560	870	1103	548	559	551	857	330	414	490	294	432	359	427	216	134	124	111	106
Ephemeroptera	643	618	528	746	129	153	126	179	112	164	198	137	221	138	255	132	163	224	244	150
Plectoptera	17	32	23	77	25	24	32	97	34	54	50	39	28	24	32	27	19	23	22	34
Trichoptera	1880	2193	1578	3583	972	733	1057	1485	639	987	632	678	838	1007	827	983	515	851	571	942
DRIFT TAXA*																				
Baetis tricaudatus	502	518	418	646	74	35	68	29	46	39	108	18	167	62	207	73	122	158	183	94
Simulium spp.	373	57	169	99	75	39	84	21	70	51	132	19	96	25	184	26	37	13	28	5
Nais communis/variabilis	55	225	32	117	4	6	10	21	2	2	3	3	7	12	5	5	5	5	12	7
Hydroptila sp. B	4	3	3	3	13	10	16	22	24	61	21	31	12	13	11	10	36	36	30	37
Leucotrichia pictipes	3	1	9	3	9	0	5	0	26	5	34	2	5	1	4	0	0	0	0	0
Corbicula fluminea	0	0	3	0	0	0	2	0	11	0	16	0	31	4	35	5	46	5	40	11
Acentrella turbida	1	0	2	0	2	1	2	0	2	0	3	1	6	1	7	1	16	10	20	7
Cheumatopsyche campyla	911	888	824	1421	530	314	568	588	330	465	311	408	434	463	362	372	237	335	243	317
Antocha monticola	26	2	28	11	29	6	29	9	14	11	19	5	10	6	6	2	5	13	6	12
Hydropsyche californica	383	180	345	270	241	77	267	164	107	80	101	51	126	86	178	120	89	79	118	119
Nais pardalis	3	50	4	8	1	1	1	11	1	5	2	4	3	13	3	4	0	3	2	9
Potthastia gaedii group	8	2	4	0	13	12	10	11	3	4	3	7	11	17	9	4	3	5	3	2
Prostoma graecense	1	1	0	0	4	1	8	2	14	16	16	28	27	58	30	43	28	47	36	57
Cricotopus bicinctus	41	7	14	3	7	22	5	50	3	15	2	11	2	7	0	8	1	2	1	3
Nais bicuspidalis	53	0	16	0	3	3	5	1	3	1	1	1	2	9	5	4	1	11	3	11

*taxa from 2004 benthic samples that were also among the most abundant in drift samples collected during and after 2002 recreation streamflow releases in the NFFR (see GANDA 2005a)

3.1.2. Kick-Sample Data

The Event-by-Treatment interaction was not significant (p = 0.410) in the ANOVA for kick sample data using Hydro-MMI as the response variable, meaning the difference in average Hydro-MMI score of kick samples between the treated and control reaches was similar before and after pulsed-flow treatments. The Minitab output for this ANOVA is provided in Table 8. The control-to-treated difference in average Hydro-MMI scores for each sampling event is illustrated in Figure 6.

Table 8. Minitab output for ANOVA (using a general linear model) to test for a pulsed-flow treatment effect among kick-sample data with Hydro-MMI as the response variable (DF= degrees of freedom; SS= sum of squares; MS= mean square; F= F test statistic; P= probability).

General Linear Model: MMI Mean vs. Month, Event, Treatment

Source	DF	SS	MS	F	P
Month	1	6.6978	6.6978	1.49	0.437
Treatment	1	37.5845	37.5845	8.35	0.212
Error	1	4.5000	4.5000		

*These tests use error term = Month*Treatment

Source	DF	SS	MS	F	P
Event	1	3.5645	3.5645	7.13	0.228
Event*Month	1	12.5000	12.5000	25.00	0.126
Event*Treatment	1	0.8845	0.8845	1.77	**0.410**
Error	1	0.5000	0.5000		

*These tests use error term = Month*Event*Treatment

Average values for individual metrics for kick-sample data are summarized for the June and September sampling events in Table 9. Mean values for the treated reach are shaded, and mean values for the control reach are not shaded.

Pre- and post-flow abundances of the major taxonomic groups (major invertebrate classes and insect orders) are presented in Table 10 for kick samples. No distinct control-to-treated differences in abundance were evident for the major taxa groups from pre- to post-recreation streamflows. However, particularly in June, several of the most abundant drift taxa increased substantially from pre- to post-flow in the treated reach, but not in the control reach (e.g., *Baetis tricaudatus*, *Simulium* spp., *Hydroptila* sp. B., *Cheumatopsyche campyla*, *Hydropsyche californica*).

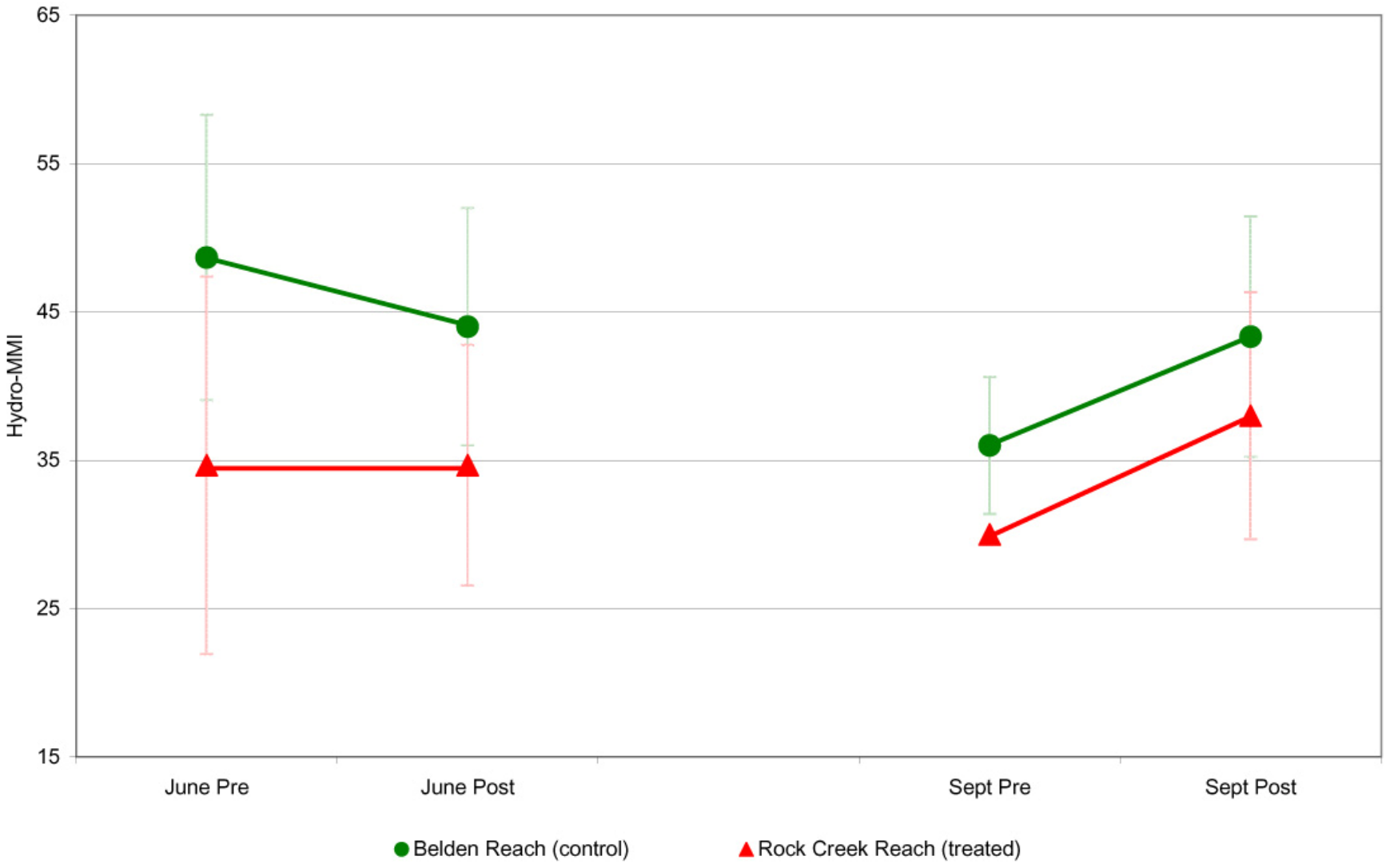

Figure 6. Average Hydro-MMI for treated and control reaches (2004 NFFR kick sampling)

Table 9. Macroinvertebrate metric summary (2004 kick sampling). Listed values are mean metric values per sampling event (shaded= Rock Creek Reach [treated], unshaded= Belden Reach [control]).

2004 Kick Samples	JUNE				SEPTEMBER			
	PRE		POST		PRE		POST	
RICHNESS-TYPE MEASURES								
# Total Taxa	62.7	45.7	58.0	46.3	46.0	45.7	44.0	48.3
# EPT Taxa	24.7	23.3	22.0	22.3	19.0	18.3	19.7	20.3
# Ephemeroptera Taxa	10.0	9.0	8.7	8.7	5.3	6.7	6.7	7.7
# Plecoptera Taxa	3.0	1.7	2.3	1.0	2.7	2.7	3.0	2.7
# Trichoptera Taxa	11.7	12.7	11.0	12.7	11.0	9.0	10.0	10.0
# Coleoptera Taxa	2.0	2.0	1.7	1.7	2.0	2.3	2.3	3.0
# Diptera Taxa	21.3	12.3	23.7	14.7	13.3	16.0	12.7	14.0
# Chironomid Taxa	18.0	8.7	18.7	10.7	9.3	10.0	9.3	10.0
# Clinger Taxa	32.0	26.7	28.7	26.7	23.3	25.0	24.3	27.0
# Long-Lived Taxa	3.7	1.3	2.7	1.3	3.0	1.3	2.7	1.7
# Tolerant Taxa	11.3	4.3	9.0	6.0	9.0	7.3	7.0	8.0
# Intolerant Taxa	17.7	15.3	15.0	13.0	10.0	10.3	10.3	11.0
Shannon Diversity	3.03	2.96	2.99	2.97	2.93	2.96	2.94	3.01
Shannon Evenness	0.73	0.78	0.74	0.78	0.77	0.78	0.78	0.78
Est. Total # Indiv.	3169	3351	4659	2802	3275	4282	3861	5723
COMPOSITION-TYPE MEASURES								
% EPT	71.3	79.2	72.2	76.8	71.2	66.8	66.3	65.1
% Sensitive EPT	7.7	11.9	7.4	11.1	5.5	5.0	6.7	6.6
% Baetidae	34.2	36.9	32.1	35.7	19.9	22.4	18.3	17.4
% Hydropsychidae	27.2	25.7	27.3	23.8	34.8	35.0	32.3	34.2
% Dominant Taxon	19.9	22.6	24.9	19.4	21.7	16.6	21.7	15.7
% Dominance (Top 3 Taxa)	46.4	44.7	46.4	42.1	41.7	41.8	43.3	38.8
% Tolerant Organisms	5.0	4.0	4.8	5.8	12.2	10.2	15.5	12.1
% Intolerant Organisms	7.7	12.3	7.8	10.2	6.2	6.6	7.5	7.7
% Tolerant Taxa	18.4	9.5	15.5	12.9	19.4	16.1	16.0	16.2
% Intolerant Taxa	28.1	33.6	25.9	28.2	21.4	22.6	23.4	22.9
% Collector-Filterers (CF)	34.6	27.9	35.2	25.8	49.7	45.4	44.0	44.2
% Collector-Gatherers (CG)	48.5	47.5	45.8	45.8	25.5	31.3	27.3	27.8
% CF+CG	83.1	75.3	81.0	71.6	75.2	76.7	71.3	72.0
% Scrapers	6.6	11.1	6.9	12.5	6.1	9.0	7.6	9.9
% Non-Gastropod Scrapers	6.5	11.0	6.9	12.5	5.9	8.9	7.5	9.9
% Shredders	0.4	0.1	0.2	0.1	0.5	0.0	0.0	0.2
% Predators	7.8	10.2	7.1	12.0	15.3	13.1	18.8	16.0
MULTI-METRIC INDEX								
Hydro-MMI	34.7	48.7	34.7	44.0	30.0	36.0	38.0	43.3

Table 10. Pre- and post-flow abundance of major taxa groups (2004 kick sampling). Listed values are mean number of individuals per sample for each sampling event (shaded= Rock Creek Reach [treated], unshaded= Belden Reach [control]).

2004 Kick Samples	JUNE				SEPT			
	PRE		POST		PRE		POST	
MAJOR TAXA GROUPS								
Arachnida	99	108	92	145	217	176	273	406
Bivlavia	29	2	45	0	120	27	107	24
Crustacea	2	2	9	4	2	11	1	8
Enopla	3	0	0	0	20	100	60	165
Gastropoda	6	2	0	0	7	5	7	0
Nematoda	19	0	22	2	0	0	8	0
Oligochaeta	126	137	133	129	72	72	59	208
Turbellaria	11	9	27	0	9	5	7	11
Coleoptera	41	101	56	80	56	187	122	381
Diptera	566	307	875	272	422	712	508	721
Ephemeroptera	1238	1421	1757	1146	699	1095	816	1148
Plectoptera	24	49	32	27	110	64	140	176
Trichoptera	1003	1211	1610	997	1525	1819	1747	2448
DRIFT TAXA*								
Baetis tricaudatus	523	796	1032	551	389	524	421	559
Simulium spp.	67	29	217	27	117	354	100	257
Nais communis/variabilis	13	9	14	13	0	5	0	17
Hydroptila sp. B	39	61	104	85	55	9	32	29
Leucotrichia pictipes	0	36	2	23	2	3	2	18
Corbicula fluminea	26	2	42	0	118	27	107	11
Acentrella turbida	141	53	83	32	91	67	104	39
Cheumatopsyche campyla	545	270	708	216	647	541	920	768
Antocha monticola	152	76	228	97	31	35	42	56
Hydropsyche californica	189	108	249	46	217	218	251	220
Nais pardalis	0	29	0	7	0	7	0	58
Potthastia gaedii group	3	23	15	2	14	20	25	44
Prostoma graecense	3	0	0	0	20	100	60	165
Cricotopus bicinctus	9	0	7	2	0	2	2	7
Nais bicuspidalis	7	0	3	0	0	9	0	6

*taxa from 2004 benthic samples that were also among the most abundant in drift samples collected during and after 2002 recreation streamflow releases in the NFFR (see GANDA 2005a)

3.2. Seasonal Trend Comparisons

Longer-term seasonal trends in the treated and control reaches were evaluated using an ANOVA with pre-flow data from basket samples only. In this test, the Treatment-by-Julian Date interaction was not significant (p = 0.400 and 0.229, respectively), meaning that the seasonal trends in Hydro-MMI were similar in the treated and control reaches (among basket samples). The Minitab output for this test is provided in Table 11. Average pre-flow Hydro-MMI values are plotted for the season in Figure 7. Note that, although differences among basket-sample data were not statistically significant in these tests, there is some divergence in Hydro-MMI scores between the treated and control reach later in the season (which may be biologically significant).

Table 11. Minitab output for ANOVA (using a general linear model) to compare seasonal trends in the control and treated reaches with the Hydro-MMI score as the response variable (DF= degrees of freedom; Seq SS= sequential sum of squares; Adj SS= adjusted sum of squares; Adj MS= adjusted mean square; F= F test statistic; P= probability).

General Linear Model: MMI vs. Treatment, Julian Date

```
Factor      Type  Levels  Values
Treatment   Fixed    2   Control Treated

Analysis of Variance for MMI, using Adjusted SS for Tests

Source                 DF     Seq SS     Adj SS     Adj MS       F      P
Treatment               1     178.80      15.85      15.85    1.43  0.234
Julian Date             1      74.32      20.09      20.09    1.81  0.181
Treatment*Julian Date   1      16.19      16.19      16.19    1.46  0.229
Error                 121    1341.04    1341.04      11.08
Total                 124    1610.35
```

Among basket samples, seasonal trends in richness and abundance were similar in both reaches. Taxa richness was generally low in June, highest in July, and declined thereafter in both the control and treated reaches, although taxa richness was slightly higher in the control reach (Figure 8); note that some divergence between these reaches is also evident, particularly later in the season. Total macroinvertebrate abundance declined sharply from June to July, and then remained relatively unchanged for the rest of the months in both reaches, although abundance was slightly higher in the control reach (Figure 9). In general, one would expect richness and abundance to trend upward into the fall (Hynes 1970; Leland et al. 1986; McElravy et al. 1989); however, this trend was not observed among basket-sample data.

Among kick samples, seasonal richness and abundance were not similar between the treated and control reaches (considering only June vs. September data). In the control reach, the mean taxa richness of kick samples was similar in June and September; but in

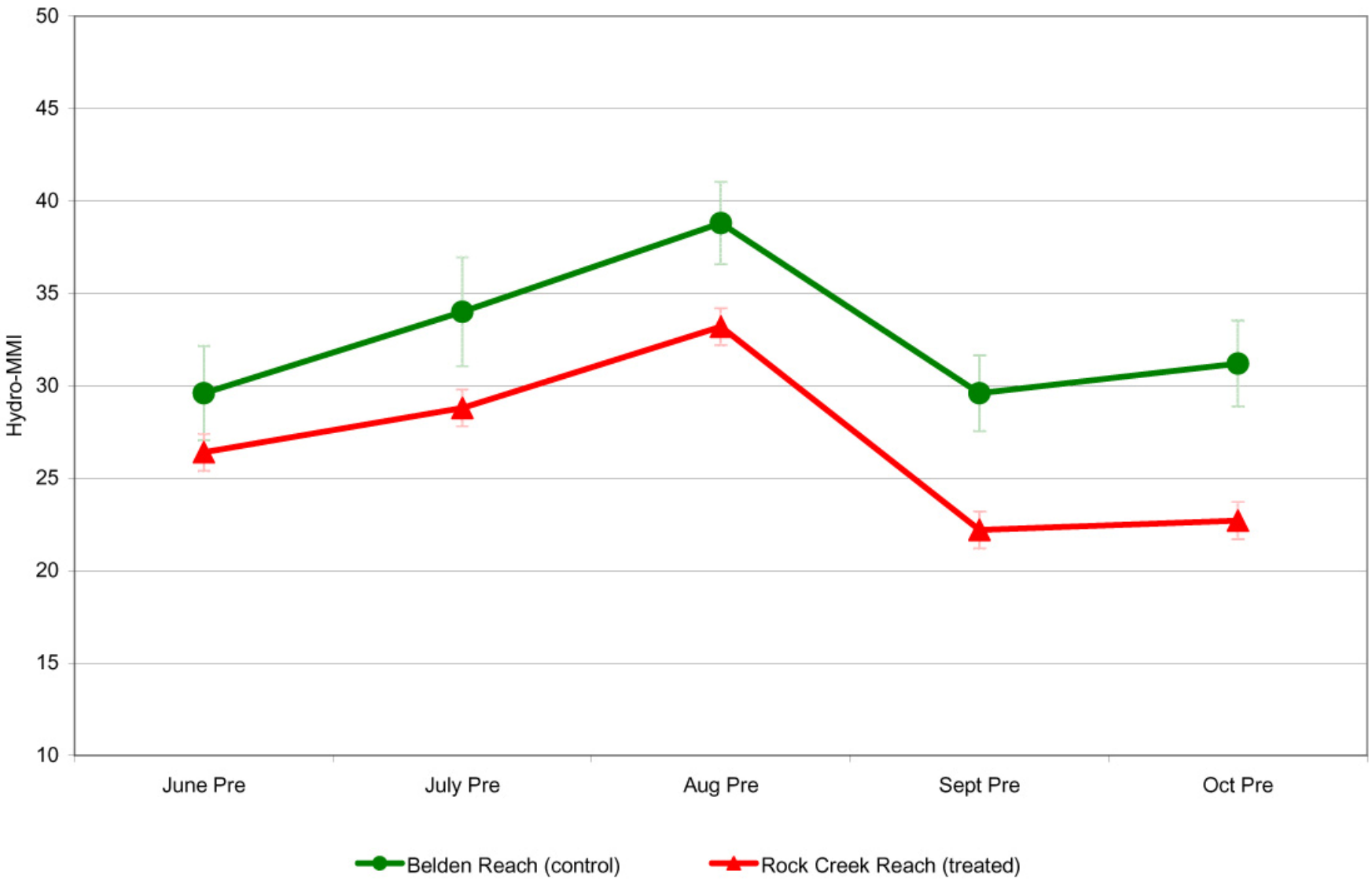

Figure 7. Seasonal Hydro-MMI for treated and control reaches (2004 NFFR basket sampling)

Figure 8. Seasonal trends in taxa richness (2004 NFFR basket sampling)

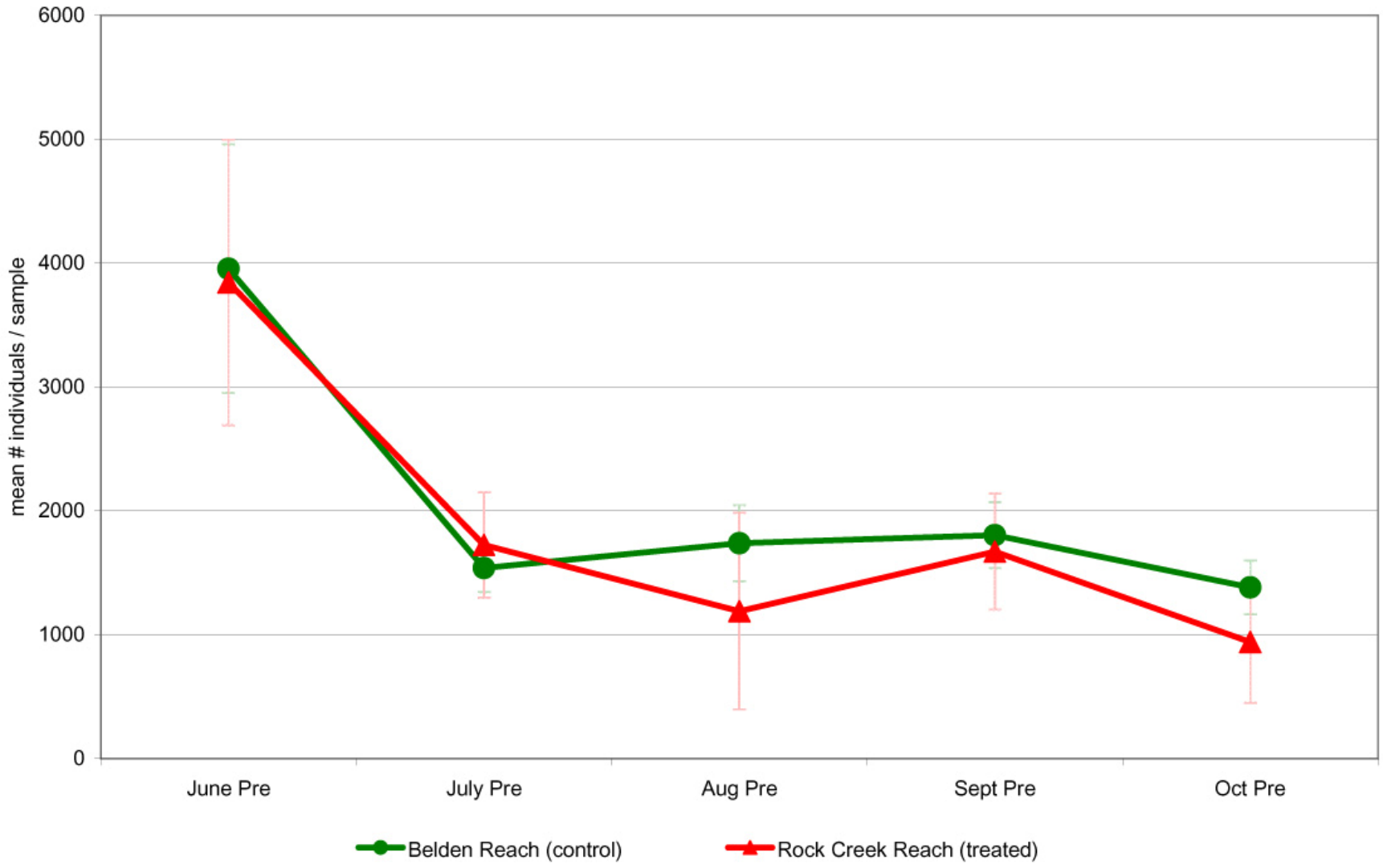

Figure 9. Seasonal trends in macroinvertebrate abundance (2004 NFFR basket sampling)

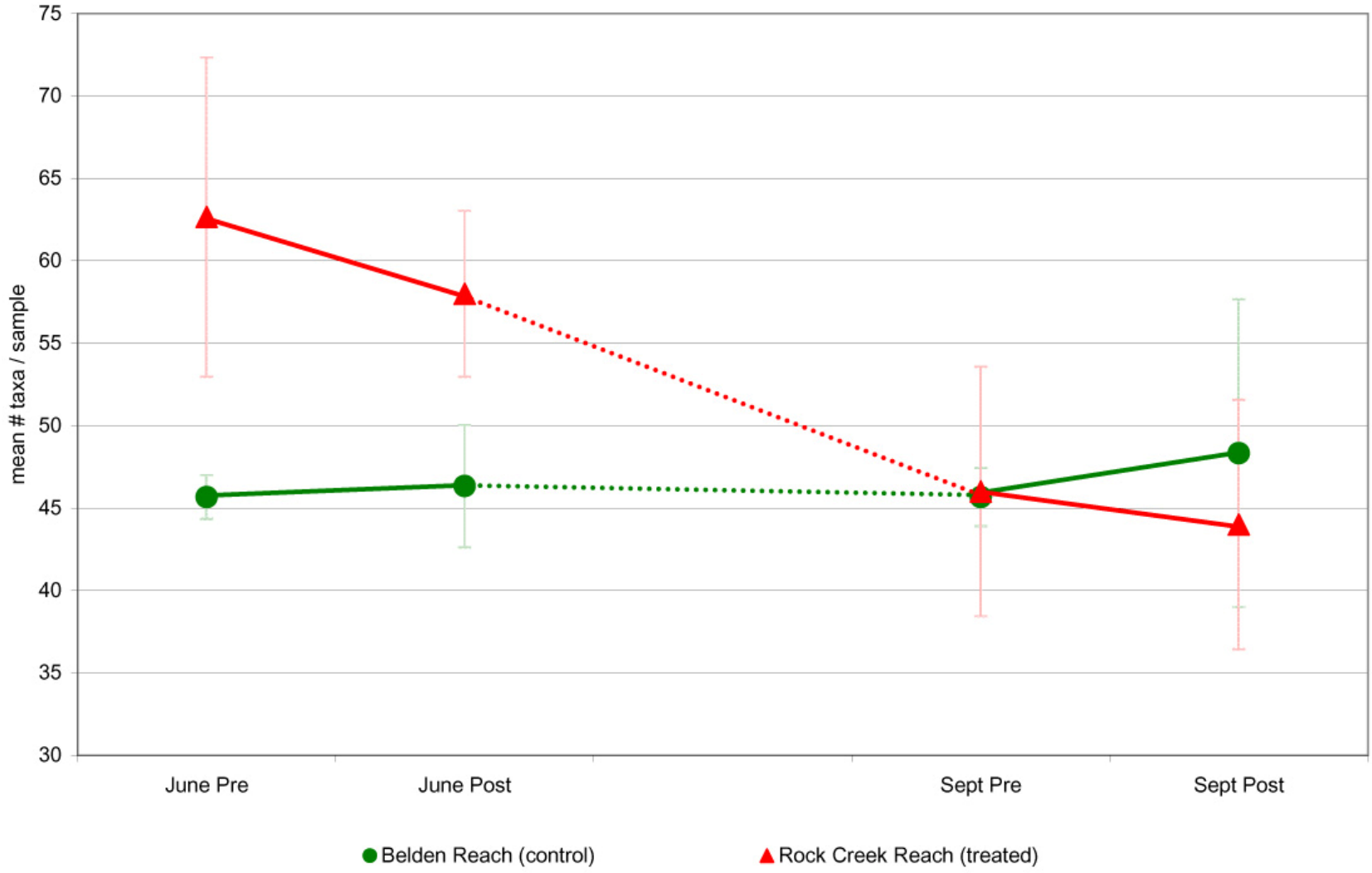

Figure 10. June vs. September taxa richness (2004 NFFR kick sampling)

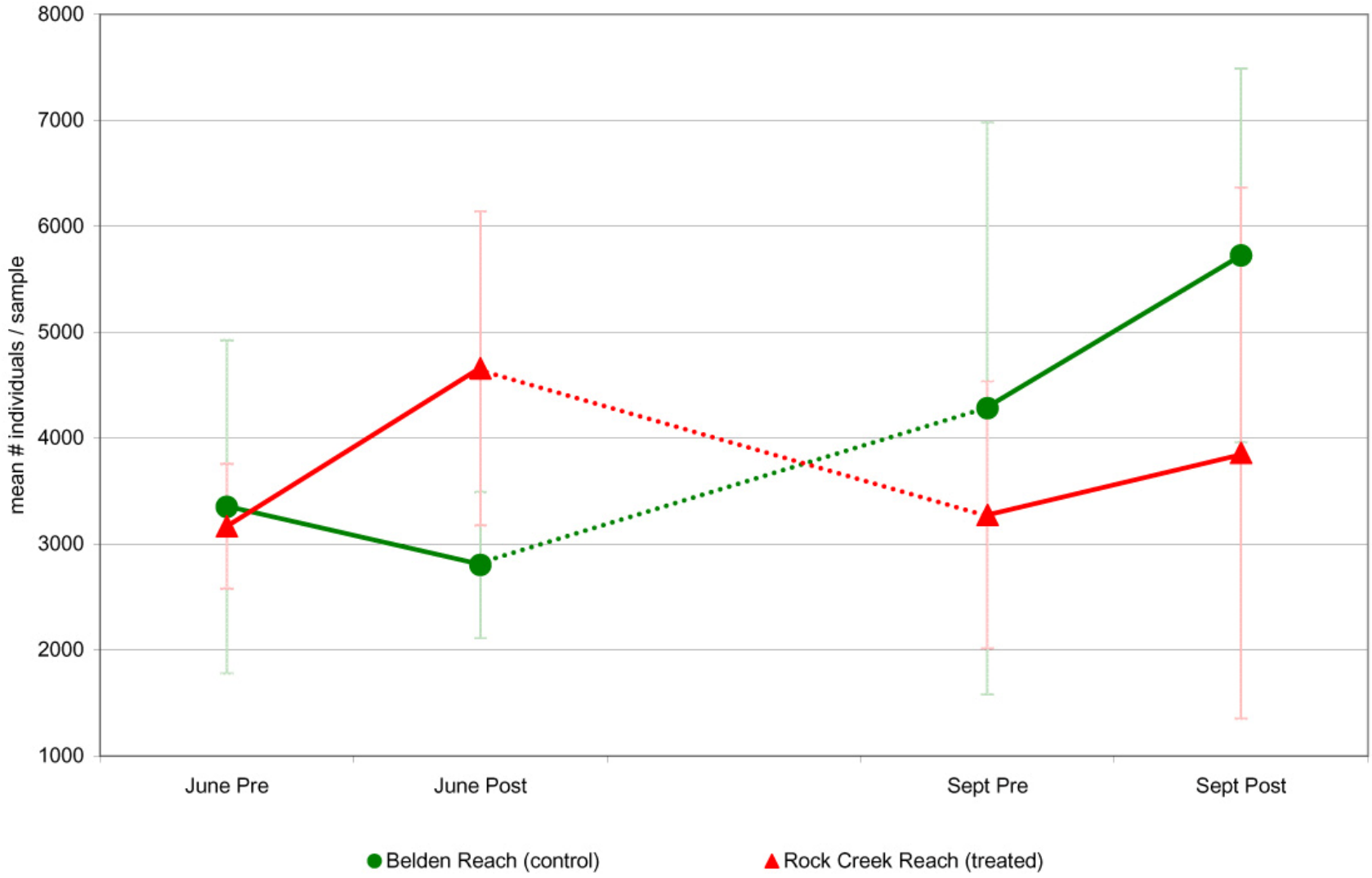

Figure 11. June vs. September macroinvertebrate abundance (2004 NFFR kick sampling)

the treated reach, richness was much lower in September than in June (Figure 10). Mean abundance in kick samples from the control reach was also higher in September than in June; but abundance remained relatively unchanged in the treated reach during these months. Overall mean abundance in kick samples was much higher for the control reach by late September (Figure 11). Although kick-sample data were limited in this study relative to basket-sample data, richness and abundance in kick samples appeared to trend upward (as would be expected) in the control reach more so than in the treated reach.

Natural seasonal trends in the abundance of the major taxa groups were also apparent. Among basket samples, freshwater mites (Arachnida), clams (Bivalvia), and proboscis worms (Enopla) were more prevalent later in the season; while worms (Oligochaeta), true flies (Diptera), mayflies (Ephemeroptera), and caddisflies (Trichoptera) were generally most abundant in the early season and decreased throughout the season in both reaches (see Table 7).

3.3. Basket- vs. Kick-Sampling Methods

Samples collected directly from the bed substrate generally provide better estimations of the existing benthic community than samples collected using devices left in situ with artificial substrates, even representative artificial substrates (Rosenberg and Resh 1982). Representative artificial substrate samplers, after all, only represent the native substrate material; they do not duplicate the microhabitat variability of the natural stream bottom. Thus, the kick samples collected during our study were assumed to provide a better representation of the overall benthic community of the NFFR than our basket samples. Kick sampling was not practical as the primary collection method due to the lack of smaller substrates necessary for bottom sampling, and due to the subsequent limitations regarding superpositioning and general disturbance of the stream bottom. These problems would have been unavoidable during repeated kick sampling before and after multiple pulsed-flow events.

Kick samples were larger overall than basket samples. In terms of volume, kick samples were the equivalent of approximately ten times the amount of substrate sampled in a basket sampler. Each individual kick sample was a composite of three 1-by-2-ft (30.5-by-61-cm) kick-sample collections made across the widest variety of productive microhabitats in a given riffle area. The amount of substrate sampled by an individual basket sampler was restricted to the volume of the inner cylinder containing the representative rock substrate. By virtue of this size discrepancy, taxa richness, abundance, and diversity were generally higher for kick samples than basket samples: a mean of 50 vs. 40 total taxa, and 3,890 vs. 1,953 total individuals for kick and basket samples, respectively.

In general, basket samples overestimated the abundance of caddisflies (Trichoptera), especially net-spinning caddisflies, true flies (Diptera), and aquatic earthworms (Oligochaeta), and underestimated the abundance of mayflies (Ephemeroptera), beetles

(Coleoptera), and freshwater mites (Arachnida) relative to kick samples (Table 12). Basket samples had 59% more filter-feeding organisms on average than kick samples (mean filterer composition was 62% in basket samples and 39% in kick samples). Among the filter feeders, the mean composition of net-spinning caddisflies was 65% higher in basket samples than in kick samples (56% vs. 34% in baskets and kick samples, respectively).

Table 12. Average percent composition of the major taxa groups in kick vs. basket samples

TAXA GROUP	BASKET	KICK
Arachnida	1%	5%
Oligochaeta	6%	3%
Coleoptera	0%	3%
Diptera	20%	14%
Ephemeroptera	11%	30%
Trichoptera	58%	40%
Net-Spinning Caddisflies	56%	34%
Filter Feeders	62%	39%

In particular, basket samples overrepresented the abundances of the net-spinning caddisflies *Hydropsyche occidentalis*, *Cheumatopsyche campyla*, and *Chimarra utahensis*; chironomids of the *Tvetenia discoloripes* group and genus *Rheotanytarsus*; and oligochaete worms of the genus *Nais*. Basket samples underrepresented the abundances of the baetid mayflies *Baetis tricaudatus*, *Acentrella insignificans*, and *Acentrella turbida*; freshwater mites of the genus *Sperchon*; the beetle *Zaitzavia parvula*; the cranefly *Antocha monticola*; chironomids of the genus *Cardiocladius*; and blackflies of the genus *Similium* (Table 13).

Table 13. Average percent composition of select taxa in kick vs. basket samples

TAXON	BASKET	KICK
Hydropsyche occidentalis	21%	9%
Cheumatopsyche campyla	24%	15%
Chimarra utahensis	5%	3%
Tvetenia discoloripes group	5%	1%
Rheotanytarsus spp.	4%	1%
Nais spp.	6%	1%
Baetis tricaudatus	7%	15%
Acentrella insignificans	1%	9%
Acentrella turbida	0%	2%
Sperchon spp.	1%	5%
Zaitzevia parvula	0%	3%
Antocha monticola	0%	2%
Cardiocladius spp.	0%	1%
Simulium spp.	2%	4%
Glossosoma oregonense	0%	1%
Leucotrichia pictipes	0%	2%

Although caddisflies were generally abundant (and overrepresented) in basket samples, some caddisflies were poorly represented in basket samples relative to kick samples (e.g., *Glossosoma oregonense* and *Leucotrichia pictipes*). Caddisflies generally increased through the sampling season in kick samples, but decreased over this period in basket samples (see Table 7 and Table 10). Among basket samples, this seasonal decrease likely reflects the natural cycle for the dominant net-spinning caddisflies, while the overall seasonal increase seen in kick samples is more indicative of the natural pattern for caddisflies in general (i.e., not just the subset selected for in basket samplers).

Several taxa that were underrepresented in basket samples included "drift taxa" that were among the most abundant taxa collected in drift samples during and after pulsed-flow treatments in 2002 (e.g., *Baetis tricaudatus*, *Leucotrichia pictipes*, and *Acentrella turbida*). For example, in June, the pre-to-post-flow increase in the abundance of *Baetis tricaudatus* (by far the most abundant taxon in the 2002 drift study) was much more pronounced in kick-sample data than in basket-sample data (a 97% pre-to-post-flow increase for kick samples compared to a 17% pre-to-post-flow decrease for basket samples).

Statistical differences between the two sampling methods were evaluated using an ANOVA with June and September data only. Using Hydro-MMI as the response variable, the Treatment-by-Method interaction was significant ($p = 0.067$), meaning the difference in average Hydro-MMI score between the sampling methods was not the same in treated and control reaches. The difference in average Hydro-MMI between kick and basket samples was higher in the control reach than the treated reach (a difference of 11.8 vs. 7.6 Hydro-MMI points, respectively). This test implies that the different sampling methods yield different results.

4.0 Conclusions and Recommendations

The direct impacts of elevated streamflows on benthic communities are related primarily to increases in velocity, shear stress, scour, and particularly bedload transport, which cause physical disturbance and removal of benthic organisms into the drift. Invertebrate drift can be broadly categorized into three classes: (1) *constant drift* defined as the continual occurrence of low numbers of most species, (2) *behavioral drift* indicated by characteristic behavioral patterns resulting in a consistent diel periodicity (usually greater drift during nighttime hours), and (3) *catastrophic drift* resulting from physical disturbance of the benthic fauna, e.g., by floods, high temperatures, or pollution (Hynes 1970; Waters 1972; Brittain and Eikeland 1988; Allan 1995; Merritt and Cummins 1996). Invertebrate drift, including catastrophic drift, is composed of animals that enter the drift both actively (reflecting a voluntary choice to do so) and passively (being forced to do so) (Wilzbach et al. 1988; Poff and Ward 1991). In the case of pulsed flows, changes in the physical environment of a stream may occur relatively suddenly, often eliciting both active and passive drift responses. Invertebrate drift monitoring in the NFFR during 2002 demonstrated that catastrophic drift occurred each month as a result of these pulsed recreation streamflow events (GANDA 2005a).

4.1. Conclusions

4.1.1. Short-Term Pulsed-Flow Effects

This research builds upon the results of other studies that have demonstrated that pulsed recreation streamflow releases in the NFFR produce short-term disturbances to the macroinvertebrate community (GANDA 2005a, 2005b). This study detected statistically significant treated-to-control differences (ANOVA, $p < 0.10$) in the context of before-after control-impact comparisons (BACI) using a multi-metric index designed to be sensitive to the effects of hydropower operations (the Hydro-MMI). However, the authors suggest that observed short-term differences are not *biologically* significant because they were based on too few points on the Hydro-MMI scale, were generally smaller following flow events, and largely resulted from changes at the control site (not the treated site).

Therefore, short-term (i.e., pre-to-post-flow) control-to-treated differences observed in this study were not consistent or large enough overall to be considered ecologically important in and of themselves. Variability in individual metrics was generally high between and within sites, and between and within sampling events. While natural variability is inherent in samples of stream benthos, previous studies have demonstrated increased variability immediately following pulsed-flow events (GANDA 2005b). Higher variability was attributable in part to rearrangement and redistribution of benthic taxa during and immediately following pulsed-flow treatments, particularly between different velocity regimes. In the short-term, benthic organisms were often displaced from their preferred velocity regime, with slower water areas being more affected in general than swifter water areas. In the current study, a comparatively

smaller sampling effort in the control reach likely contributed to greater variability of the control sample averages.

4.1.2. Longer-Term Seasonal Trends

In temperate areas, the richness and abundance of benthic communities in streams is typically highest in the fall, prior to disturbances associated with winter floods. These measures generally increase from summer to fall as the result of the predominant spring-summer emergence patterns of most aquatic insects. The growth of eggs and early instars through the summer and into the fall (to minimum sample-able sizes) generally results in higher richness and abundance in benthic samples collected in the fall (Hynes 1970; Minshall 1981; Leland et al. 1986; McElravy et al. 1989). Yet, previous studies of pulsed recreation streamflows in the NFFR showed that benthic community measures generally declined through the sampling season in representative artificial substrate (i.e., basket) samples (GANDA 2005b). Taxa richness and abundance, for example, decreased from June through October in these studies. However, because previous studies in the NFFR focused on before-after comparisons only, the extent to which these seasonal differences were attributable to pulsed-flow treatments as opposed to natural seasonal patterns (e.g., simply a late onset of upward trends in the fall) remained unclear.

The present study incorporated spatial (i.e., control-impact) comparisons with an upstream control reach in order to provide an understanding of baseline seasonal changes, and to help isolate the effects of natural vs. manufactured sources of variation. Results indicate that similar seasonal decreases occurred among basket samples in both the treated and control reaches during the study period. Trends in taxa richness and abundance were generally parallel for these reaches, although these measures were slightly higher for the control reach. Because basket samplers tended to favor a particular subset of the benthic community (see discussion below), seasonal trends among basket-sample data may have primarily followed the natural seasonal patterns for these dominant taxa, which were similar for both reaches.

Although no statistically significant seasonal control-to-treated difference in Hydro-MMI was detectable in the context of BACI comparisons using basket-sample data, some divergence was visible between the treated and control reaches later in the season (e.g., control-to-treated differences were generally greatest in October; see Figure 5 and Figure 7). A slight divergence between control and treated reaches later in the season was evident for taxa richness and abundance as well (see Figures 8 and 9). This divergence, although not statistically significant, may be biologically significant in that the expected upward seasonal trend in benthic richness and abundance may be delayed (or reduced) in the treated reach, possibly as a result of cumulative pulsed-flow disturbances.

On the other hand, kick samples collected during this study suggest seasonal differences between the treated and control reaches that were greater than in basket samples. Seasonal trends in richness and abundance in kick samples were *not* similar between

these reaches. Taxa richness generally declined between June and September in the treated reach, but remained relatively unchanged in the control reach (see Figure 10). At the same time, abundance remained relatively unchanged in the treated reach, but increased substantially in the control reach in September (see Figure 11).

The potential for disturbance of the benthic community may be related to the timing of flow events. Previous studies of recreation streamflow events in the NFFR demonstrated that catastrophic drift effects were orders of magnitude greater during the late summer and fall months than earlier in the season (GANDA 2005a). Seasonal differences in the susceptibility of benthic organisms to disturbance may be associated with the general positioning behavior of insects on substrates. Kohler (1983) found that, overall, individuals tended to occur more frequently on top of substrate surfaces in September than in August, and that drift was likely highly correlated with such positioning behavior.

Seasonal differences in overall susceptibility to disturbance also reflect evolutionary adaptations in the morphology and life history of lotic organisms. Just as different stream types are predicted to exhibit different levels of resilience to flooding (Fisher and Grimm 1988), differential resilience to flood disturbance can be predicted between temporally separated assemblages present during different seasons in the same stream (e.g., based on the timing of disturbances in relation to sensitive or critical life history stages, etc. [Giller et al. 1991]). Benthic macroinvertebrates are known to have life cycles cued to take advantage of periods of high food availability and low flow variability (Canton et al. 1984; Sweeney 1984; McIntosh et al. 2002). In temperate areas, it is reasonable to expect more flood-adapted assemblages or life stages to be present in the winter as opposed to summer months; hence, the implication that summer high-flow events may have greater potential for disturbance. Giller et al. (1991), for example, found that overall recovery of the macroinvertebrate community from catastrophic winter floods was relatively rapid, but recovery from a catastrophic summer flood was poor. In fact, in their study, macroinvertebrate densities reached only 40% of pre-flood levels three years after the event.

4.1.3. Conclusions Regarding Basket- vs. Kick-Sampling Methods

Kick samples provided a better overall representation of the benthic community than basket samples during the study. Basket samplers tended to select for a subset of the benthic community that was heavily dominated by filter-feeding organisms such as net-spinning caddisflies (primarily Hydropsychidae). These organisms were able to quickly colonize and capitalize on free interstitial spaces provided by design in basket samplers. Filter feeders are fundamental components of benthic communities in regulated rivers that are often space-limited (Mackay 1992). Intra- and inter-specific competition for space among filter feeders can result in aggressive interactions between individual larvae, often leading to density-dependent drift.

Drift is the primary mechanism for colonization of new or disturbed substrates (Williams and Hynes 1976, 1977; Townsend and Hildrew 1976; Minshall and Petersen 1985). Mackay (1992) noted that taxa that were prominent in the drift were the earliest colonizers of denuded habitat, including hydropsychid caddisflies on rocks with surface irregularities (such as those used in the basket samplers). Overall, basket samplers overrepresented the abundance of these net-spinning caddisflies (and filter feeders in general) by approximately 60% relative to kick samples. Other investigators have also recorded high hydropsychid dominance (as high as 90% of all insects) in basket-type samplers (Hall 1982).

Statistically significant differences between kick- and basket-sampling methods were detectable (ANOVA $p < 0.10$). These tests imply that the different sampling methods are not comparable in the sense that they may yield different results in the treated and control reaches. As discussed above, seasonal trends among basket-sample data may primarily follow the natural seasonal patterns for the subset of taxa that dominated basket samplers. Indeed, artificial substrates (even representative artificial substrates) may more effectively measure the colonization potential at a site, rather than the natural community structure at that site (Rosenberg and Resh 1982).

Moreover, certain taxa may be less sensitive to flow-related changes by virtue of their specific microhabitat preferences and adaptations. Net-spinning caddisflies, for example, require at least enough flow to bellow out their nets so that they can efficiently filter food particles from the water column (Haddock 1977). The basket samplers in this study effectively created a vertical stratum of interstitial habitat that was unique relative to the structure of the surrounding bed substrate. Early colonizers such as net-spinning caddisflies were able to capitalize on interstitial spaces throughout the volume of the basket samplers (i.e., including spaces between rocks near the bottom of the sampler, not just the exposed rock surfaces at the top), provided those spaces were not filled in with sediment. Consequently, a greater volume of interstitial habitat was available in these samplers that was somewhat protected from higher flows. Therefore, the filter-feeder-dominated community that was selected for in basket samples during this study may have been less sensitive overall to pulsed-flow-related disturbances than that of the larger, natural benthic community present in the surrounding substrate. In this sense, the selectivity of basket samplers likely reduced the potential of basket-sample data to detect pulsed-flow-related disturbances. Thus, kick-sample data, although limited in extent relative to basket-sample data in this study, provided better evidence of pulsed-flow-related effects.

4.2. Recommendations

- Evaluations of pulsed recreation streamflows in the NFFR should be repeated using a similar BACI study design based on kick sampling only. Kick samples appear to detect pulsed-flow-related disturbances better than basket samples at these scales.

- Limitations regarding the amount of sample-able substrate may be overcome by reducing the number of replicates collected per sampling event and staggering sampling locations among sub-sites to allow longer recolonization periods between sampling events.
- The sampling design should focus equivalent effort in the control and treated reaches in order to minimize variability between sites due to artifacts from sampling.
- Post-flow samples should be collected 7–10 days following a given flow event, as opposed to the day after a flow event, when immediately post-flow variability is higher.
- The study design should be improved by having multiple pre-flow and multiple post-flow sampling events per disturbance event, probably in different sub-sites.
- The hydropower multi-metric index (Hydro-MMI) should be calculated in future evaluations of the effects of pulsed flows on benthic communities. The Hydro-MMI was more useful than individual metrics for both discriminating baseline control-to-treated differences and detecting pulsed-flow-related disturbances.
- Future studies of the effects of recreation streamflows should include some variation in the timing of events. Pulsed flows clustered more in the spring, for example, as opposed to spread out during the typical low-flow season of summer and early fall, would more closely mimic the natural hydrograph, possibly resulting in a reduced potential for cumulative disturbances.

4.3. Benefits to California

Research regarding the biological effects of hydropower on California's aquatic resources is limited, especially relative to the pulsed-type flow manipulations studied here. An adequate understanding of such effects on benthic macroinvertebrates in particular is notably lacking given the vital importance of these organisms to the ecology of lotic environments. Benthic macroinvertebrates are fundamental components of riverine food webs that provide critical linkages between primary production and consumption at higher trophic levels (e.g., fish, birds, mammals, etc.). Indeed, the flow of energy derived from both allochthonous (i.e., organic matter produced outside the system) and autochthonous (i.e., organic matter produced from within the system) sources is primarily regulated through macroinvertebrate pathways—for example, as in the breakdown of coarse particulate organic matter by shredder insects or the processing of fine particulate organic matter by filterer and collector species (Vannote et al. 1980; Wallace and Webster 1996).

Thus, losses in productivity resulting from disturbances to benthic communities can have bioenergetic consequences at multiple trophic levels. Flow manipulations are known to cause increases in invertebrate drift (i.e., catastrophic drift), which may kill, injure, or otherwise render benthic organisms "ecologically dead" (e.g., by displacing

them into inhospitable habitats). The displacement of benthic organisms from riffles or other productive erosional habitats into depositional features such large pools or terminal reservoirs common in regulated rivers constitutes a loss of benthic productivity. To the extent that pulsed flows exacerbate such losses, their bioenergetic consequences may reach beyond the benthic community to include, for example, possible negative impacts on desirable native and/or valuable sport fisheries, as well as other wildlife dependent on river-borne productivity (e.g., mayflies lost via drift into terminal reservoirs can no longer provide prey for fishes or birds).

Provisions for pulse-type flow manipulations are increasingly being incorporated into hydropower licensing agreements under mandates for recreational beneficial uses. The current lack of understanding regarding the biological effects of these flow manipulations are particularly problematic given that pulsed flows are often provided in a manner that is atypical for both the natural and regulated hydrographs for most hydropower-managed rivers (in terms of the magnitude, duration, frequency, and especially the timing of such flows). Because benthic macroinvertebrates are known to have life cycles cued to take advantage of periods of high food availability and low flow variability, such atypical pulsed-flow releases may create artificial flood conditions when benthic communities are least adapted to experiencing floods; the detrimental impacts of such disturbance to the benthic community are not well known. The results of this study, therefore, are valuable in that they contribute to a greater understanding of the effects of pulsed-type flow manipulations on the biota of California's regulated rivers.

In addition, this study demonstrates that the manner in which benthic data are collected is critical to their interpretation, especially in terms of avoiding misinterpretations in a regulatory context. As such, this research directly informs the applied aspects of resource monitoring and data acquisition, and ultimately the regulatory decision-making processes that determine how aquatic resources are managed in California rivers. Moreover, the results of this research translate to rivers far beyond California where similar regulatory and ecological challenges already exist or are beginning to emerge.

The results and recommendations of this study can be used to establish appropriate and efficient methods for evaluating natural resources in this setting and managing them in a manner that is economically, socially, and environmentally responsible. By understanding the effects of pulsed-flow manipulations, resource managers will be better able to communicate with project stakeholders regarding such impacts in order to balance the various economic, recreational, and environmental demands on riverine resources. Understanding the ecological outcomes of flow-management decisions is essential for providing for the widest variety of beneficial uses in conjunction with hydropower generation and supporting these decisions to the public.

Improvements to the process for managing regulated rivers will ideally result in perpetuation and enhancement of the quality of California's unique natural resources and heritage, while also improving opportunities for the people of California to enjoy and benefit from them. Information gained through this study will ultimately be submitted to a peer-reviewed journal to better share these findings with biologists, managers, and other resource professionals throughout California and beyond.

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6.0 Glossary

ANOVA	Analysis of variance, a statistical technique for testing for differences in the means of several groups
BACI	Before-after control-impact experimental design (used in modified form by this study)
CBSP	California Stream Bioassessment Procedure, a standardized field method developed by CDFG
CDFG	California Department of Fish and Game
FFG	Functional feeding group (grouping of invertebrate taxa based on feeding adaptations)
Hydro-MMI	Hydropower multi-metric index, an analytical tool designed to be sensitive to the effects of hydropower operations
NFFR	North Fork Feather River (Plumas County, California)
PG&E	Pacific Gas and Electric Company
USGS	United States Geological Survey