

**Controls on the Structure and Function of Tailwater Macroinvertebrate Assemblages –
USU Synthesis of Activities Conducted to Date – Argonne Agreement No. 5F-31981**

Scott Miller

30 November 2016

**OBJECTIVE 1: FACTORS LIMITING THE RECOVERY OF TAILWATER BENTHIC
MACROINVERTEBRATE ASSEMBLAGES**

Task 1: Quantify macroinvertebrate oviposition habitat selectivity in the Green River and the extent to which dam operations alter habitat type and availability

Example Research Questions

- Are macroinvertebrate egg masses randomly distributed or are they differentially located among habitat units (e.g., pools versus riffles), within a habitat unit (e.g., varial zone versus mid-channel), among substrata types (e.g., emergent versus submerged rocks) or other distinct macro- and micro-habitats?
- Is macroinvertebrate recruitment success limited by hydrologic fluctuations and the subsequent type or intermittency of oviposition and egg rearing habitat?
- What is the degree of intra- and inter-specific plasticity in macroinvertebrate oviposition strategies?

Work conducted to date

- Spring 2015 – Protocol testing and preliminary data collection (*Baetis* spp. and Chironomidae midges)
- Summer 2015 – Surveys of oviposition habitat availability and utilization by *Brachycentrus occidentalis* and *Hydropsyche occidentalis* on the Green River below Flaming Gorge Dam (FGD)
- Fall 2015 – Surveys of oviposition habitat availability and utilization. Several unsuccessful attempts, as eggs were not found in sufficient densities.
- Spring 2016 – Surveys of oviposition habitat availability and utilization by *Baetis* spp. and Chironomidae on the Green River below Flaming Gorge Dam (FGD)
- Spring 2016 – Presented research results at the Society of Freshwater Science Annual meeting in Sacramento, CA. Presentations were given on both the oviposition and desiccation work.
- Spring/summer 2016 – experimental manipulations of substrate depth and distance from bank to assess differential oviposition use
- Fall 2016 – data analysis and manuscript preparation
- October 2016 – presentation at Green River Outfitters and Guides Association meeting

Methods Summary

We characterized the oviposition habitat use of four taxa that occur, or originally occurred, in both the Glen Canyon Dam (GCD) and Flaming Gorge Dam (FGD) tailwaters: *Baetis* spp. (Ephemeroptera), *Hydropsyche occidentalis* (Trichoptera), *Brachycentrus occidentalis* (Trichoptera) and *Eukiefferiella* spp. (Diptera). These taxa were further selected because they are thought to exhibit differential oviposition strategies (e.g., edge versus deep

water specialists) and they strike a balance between high densities and traceable oviposition behaviors (i.e., we were able to find the eggs).

To quantitatively characterize oviposition habitat selectivity on the Green River at multiple spatial scales, we focused on three areas below FGD: Tailrace (1 km below dam [kbd]), Little Hole (12 kbd) and Indian Crossing (26 kbd). These areas were selected to represent both geomorphic and hydrologic alteration gradients below FGD, particularly the interaction of width:depth ratios and the magnitude of intra-daily stage height fluctuations. A single 1.5 km river segment was chosen to be representative of the geomorphic diversity within each of the three areas. Within each 1.5 km segment two fast-water (i.e., riffles) and two slow-water (i.e., runs) reaches (maximum length = 75 m) were randomly selected for sampling for a total of four reaches per segment. The three river segments (12 reaches total) were sampled on multiple occasions during the spring and summer of 2015 and 2016, with the goal of quantifying habitat selectivity during peak emergence.

Results from past studies, as well as a pilot study below FGD, indicate that egg masses are disproportionately found near the stream margins, on emergent mineral or wood substrates and in moderate water velocities (Peckarsky et al. 2000, Hoffmann and Resh 2003, Lancaster et al. 2010a, 2010b). As these microhabitats are not present in equal proportions from reach to reach or segment to segment, a simple random sample would result in the under sampling of some microhabitats (e.g., emergent rocks or vegetation) and the oversampling of others (submerged mineral substrate). Therefore, to characterize both the available and selected habitat for oviposition, we followed Reich and Downes (2003) and utilized a two stage sampling procedure. First, each of the 12 reaches were randomly sampled to characterize habitat availability, as well as the habitat utilized by any encountered egg masses. Specifically, 25 particles were randomly selected from the nearshore environment and 25 particles from the mid-channel habitat per reach.

The second stage of sampling involved the stratification of sample reaches by substrate type for a total of five possible strata: 1) submerged mineral substrate, 2) emergent mineral substrate, 3) submerged wood, 4) emergent wood and 5) emergent vegetation. To achieve approximately equal sample sizes among strata ($n = 15$), up to 15 additional points were sampled per strata per reach (note that the 50 random points used to quantify habitat availability counted towards the respective strata in which they were located). In total, we sampled between 75 – 90 points per reach for a total of 300 – 450 points per 1.5 km segment, depending on the number of strata present per reach. At each of the 75 – 90 points per reach, the following parameters were measured: 1) reach type (fast water versus slow water), 2) strata type (submerged mineral substrate, emergent mineral substrate, submerged wood, emergent wood, emergent vegetation), 3) distance to rivers edge, 4) particle depth, size, emergent area and embeddedness, 5) water velocity and 6) the type and number of egg masses.

To further characterize macroinvertebrate oviposition habitat preferences, we experimentally manipulated substrate depth and distance from bank. We focused on these two environmental factors because they form the basis for hypotheses related to hydropeaking and egg viability, they were among the top predictors in habitat utilization models and they were easily manipulated. Specifically, we used 20 x 20 x 20 cm concrete blocks as experimental substrates for oviposition. Substrates were arranged in five transects, with four replicates per transect. The five transects were oriented parallel to the river bank and located approximately 3, 4.5, 7.5, 10.5 and 13.5 m from the wetted edge. Experiments were run with all substrate particles emerging from the water surface and with all particles submerged. For the submerged treatment, we also included an emergent control to ensure that oviposition was actively occurring.

Preliminary Oviposition Results

Random forest (hereafter, RF) models were fit in R (package ‘randomForest’, version 3.2.2, R Development Core Team 2015) to quantify the relationship between egg density and habitat variables at the segment, channel unit- and point scales. RF is a tree-based approach that fits thousands of classification or regression trees to a dataset where individual trees are developed for a bootstrapped sample of the data by using a random subset of predictors at each split to find the most homogenous groups (Breiman 2001, Liaw and Wiener 2002). Model performance was assessed using the percent variance explained, which is an internal cross validated metric defined as $1 - (\text{mean squared error})/(\text{variance (response)})$, and is analogous to r -squared (Pang et al. 2006). Variable importance was assessed using the percent increase in mean square error (MSE) following removal of each predictor variable, with higher MSE values indicating a greater decrease in model accuracy (Pang et al. 2006) (Fig. 1). Partial dependence plots were constructed to assess the relationship of individual predictors to the response variable by holding the effects of all other predictors in the model constant (Cutler et al. 2007) (Fig. 2).

Model precision was 52% for *B. occidentalis*, 20.8% for *H. occidentalis*, 68% for *Eukiefferiella* spp. and 43% for *Baetis* spp. The relative importance of the ten predictor variables was similar among models, with measures of substrate size, type or emergent area, distance from the bank and water depth being among the top predictors (Fig. 1). Notable differences among models included the strong influence of water velocity for *B.occidentalis* and *Baetis* spp. and to a lesser extent segment for *H. occidentalis*, *Eukiefferiella* spp. and *Baetis* spp. (Fig. 1). In general, all four taxa preferred large, emergent substrates and *B.occidentalis*, *Eukiefferiella* spp. and *Baetis* spp. exhibited a preference for these substrate types being located close to the bank and *H. occidentalis* far from the bank (Figs. 2 & 3). *B.occidentalis*, *Eukiefferiella* spp. and *Baetis* spp. appear to be edge specialists, while *H. occidentalis* can oviposit in deeper, open water.

Preliminary analyses, suggest that downstream gradients in benthic densities are related to oviposition strategy. For example, benthic densities of *H. occidentalis* rapidly recover with distance downstream from Flaming Gorge Dam, while densities of the edge specialist, *B.occidentalis* persist at low levels (Fig. 4).

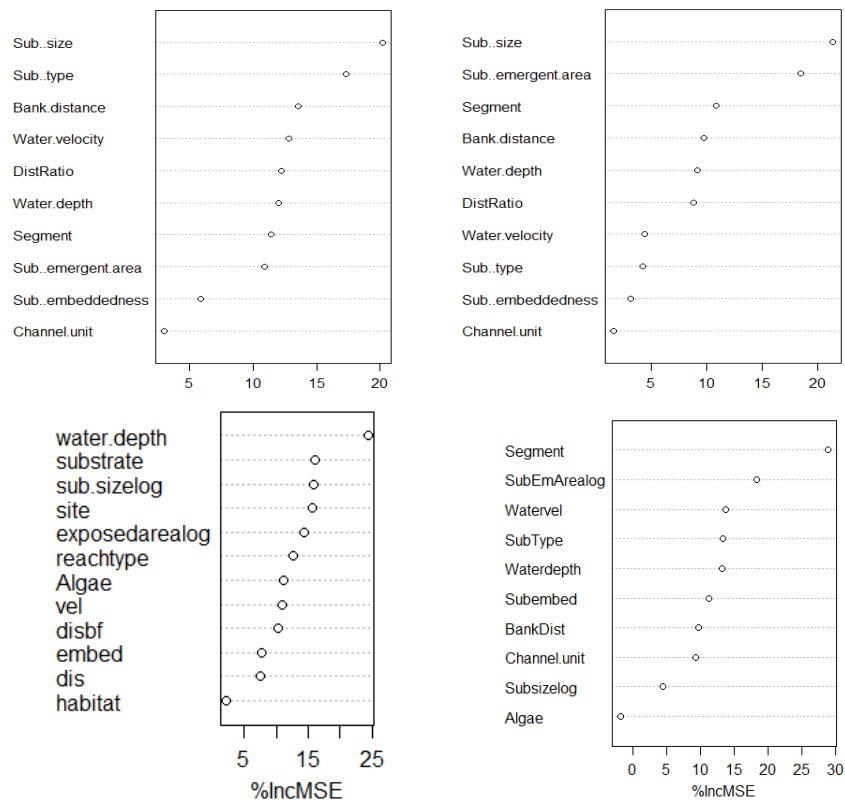


Figure 1. Variable importance plots for the random forest models developed to understand relationships between oviposition egg density and environmental conditions for *B. occidentalis* (top left), *H. occidentalis* (top right), *Eukiefferiella* spp. (bottom left) and *Baetis* spp. (bottom right). The sensitivity of egg density to individual predictors was assessed by quantifying the percent increase in mean square error (MSE) when the validation data for an individual predictor was permuted.

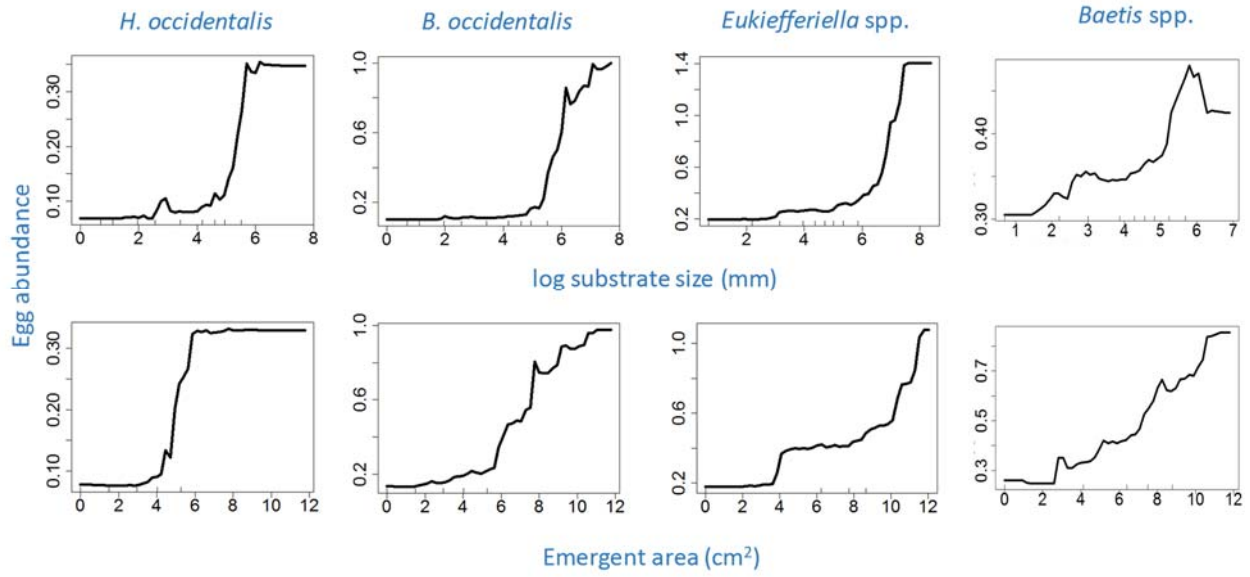


Figure 2. Partial dependency plots of *B. occidentalis*, *H. occidentalis*, *Eukiefferiella* spp. and *Baetis* egg densities exhibiting similar relationships with substrate size and emergent area from each of the four independent Random Forest models. All four taxa show preferences for large, emergent substrates.

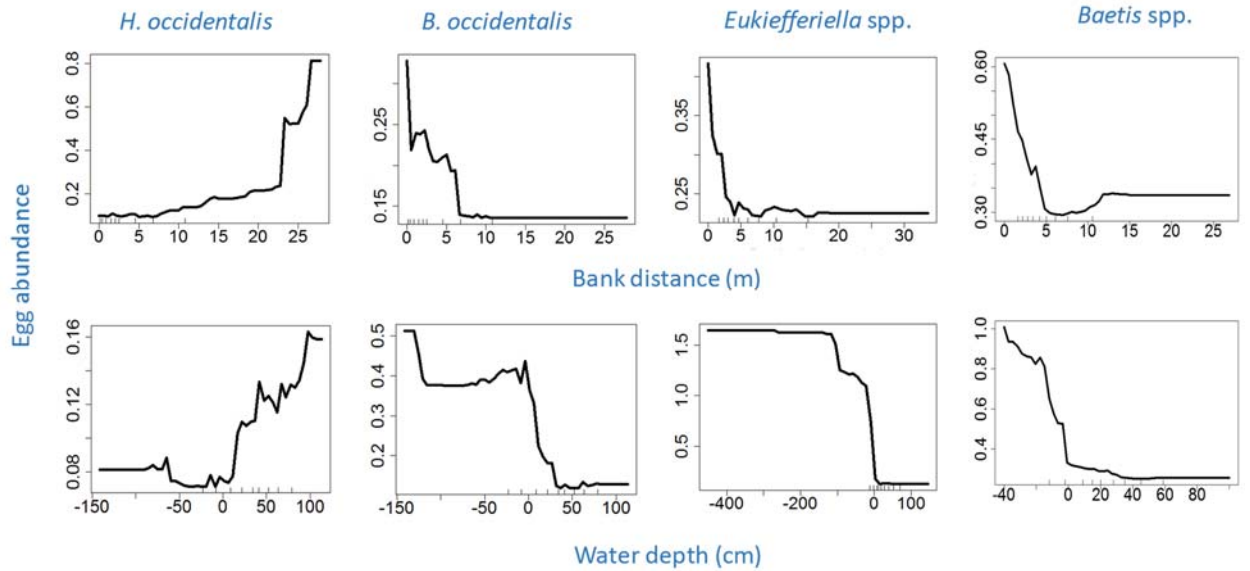


Figure 3. Partial dependency plots of *B. occidentalis*, *H. occidentalis*, *Eukiefferiella* spp. and *Baetis* egg densities exhibiting differential relationships with bank distance and water depth from each of the four independent Random Forest models. *B. occidentalis*, Orthocladiinae and *Baetis* exhibited similar relationships, while *H. occidentalis* differed.

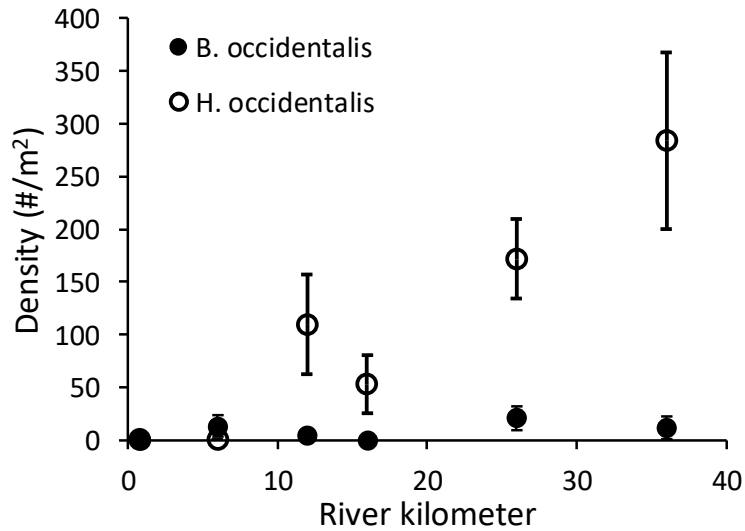


Figure 4. Longitudinal gradients in benthic densities compared between an oviposition edge specialist, *B. occidentalis*, and a taxon capable of ovipositing in deeper water, *H. occidentalis*, below Flaming Gorge Dam.

Experimental substrate manipulation results

- Similar to observational studies (i.e., RF models of oviposition habitat preferences), egg densities were significantly greater for emergent versus submerged substrate particles (Fig. 5).
- Stage-height fluctuations associated with hydropeaking have the capacity to reduce the number of emergent substrate particles; the preferred habitats for macroinvertebrate oviposition (Figure 6).

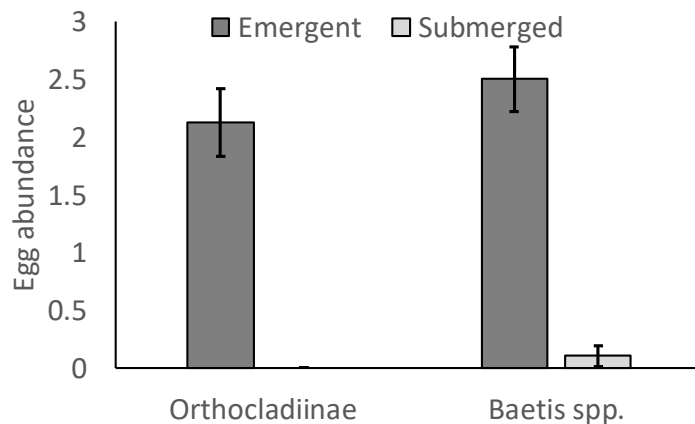


Figure 5. Orthocladiinae and *Baetis* spp. egg densities compared between experimental emergent and submerged particles.

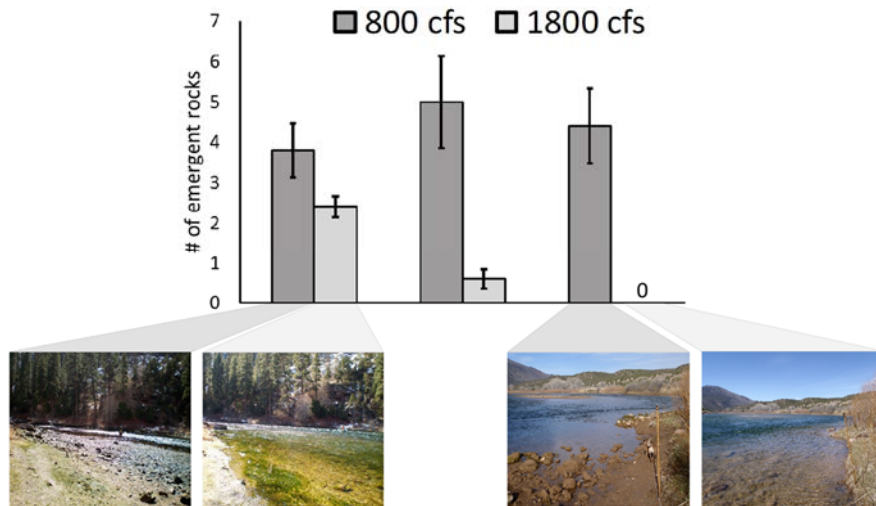


Figure 6. Number of emergent rocks compared between 800 and 1800 cfs at the Tailrace and Indian Crossing study segments.

Next Steps

- Finalize analytical approaches for data analysis (e.g., mixed effects models, randomForest modeling)
- Work with the broader research team to quantify egg densities and oviposition habitat preferences of macroinvertebrates on the Colorado River below Glen Canyon Dam
- Final report and manuscript preparation – carryover into 2017

Task 2: Quantify egg desiccation tolerance for macroinvertebrates common to large western rivers

Example Research Questions

- What is the desiccation tolerance of common tailwater macroinvertebrate egg-masses?
- Does the desiccation tolerance of egg-masses vary as a function of season and/or air temperature? For example, do *Baetis* spp. eggs subject to nighttime wetting and drying experience lower mortality rates than daytime wetting and drying?

Work conducted to date

- July 2015 – Pilot field experiment conducted for *Brachycentrus occidentalis* and *Hydropsyche occidentalis*
- November 2015 – Completed lab *Baetis* spp. desiccation experiment
- April 2016 – Conducted trial run of Chironomidae desiccation experiment
- July 2016 – Completed lab and field *Brachycentrus occidentalis* desiccation experiments
- September 2016 – Completed lab *Hydropsyche occidentalis* desiccation experiment

Methods Summary

We quantified egg desiccation tolerance for four common, large river taxa: *Brachycentrus occidentalis* (Trichoptera), *Hydropsyche occidentalis* (Trichoptera), *Baetis* spp. (Ephemeroptera) and Chironomidae (Diptera). Desiccation tolerance for *B. occidentalis* was quantified in the field and lab, while experiments for the three other taxa were all conducted in the lab. Specifically, the field experiment was a completely randomized, one-way factorial design where egg masses attached to mineral substrates were assigned a ‘desiccation’ treatment with four levels: 0 (control), 0.5, 1, 2, 4 and 8 hours of river-side desiccation (n = 16 per treatment level). The treatment levels greater than one hour represent common durations for varial zone exposure and drying below hydroelectric dams, while treatment durations less than two hours were added to understand general desiccation tolerance. After the completion of each treatment egg masses were rehydrated, scraped from rocks and placed in river water on ice and returned to the lab.

For the lab experiments, eggs were collected by either deploying artificial substrates or collecting rocks with eggs from the Green River below FGD and Fontenelle Dam. Artificial and river substrates were transported to the lab where the eggs were scraped from tiles and placed in petri dishes containing water. Individual egg masses were assigned to one of six treatments in a completely randomized factorial design: 0 (control), 0.5, 1, 2, 4, and 8 hours of desiccation, with 10 – 16 egg masses per treatment.

In the lab egg masses were photographed under a dissecting microscope, individual eggs per mass counted using Image J software and reared at room temperature (23°C) on a 12 h day-night light cycle for 5 weeks or until egg masses were no longer viable. For all experiments, relative egg hatching success was computed as the ratio of hatched eggs to the total number of eggs per egg mass. We tested for differential hatching success among treatments using a generalized linear model fit to a binomial distribution.

Preliminary Results

- We observed differential susceptibility to desiccation among the three taxa analyzed to date (Fig. 7). *Baetis* spp. appeared to be the most sensitive, followed by *H. occidentalis* and finally *B. occidentalis* was the least affected by desiccation.

- Despite differential responses among taxa, all three exhibited significantly reduced hatching success in response to desiccation durations typical of hydropeaking, with *H. occidentalis* and *Baetis* spp. approaching zero hatch success after experiencing two or more hours of desiccation
- The nearly 20% hatching success for *B. occidentalis* after eight hours of desiccation is likely attributable to egg morphology; *B. occidentalis* eggs are encased in a gelatinous mass that is thought to protect the eggs from desiccation and other adverse conditions.
- Chironomidae experiment
 - Trying to salvage results given difficulties determining the number of eggs per egg mass

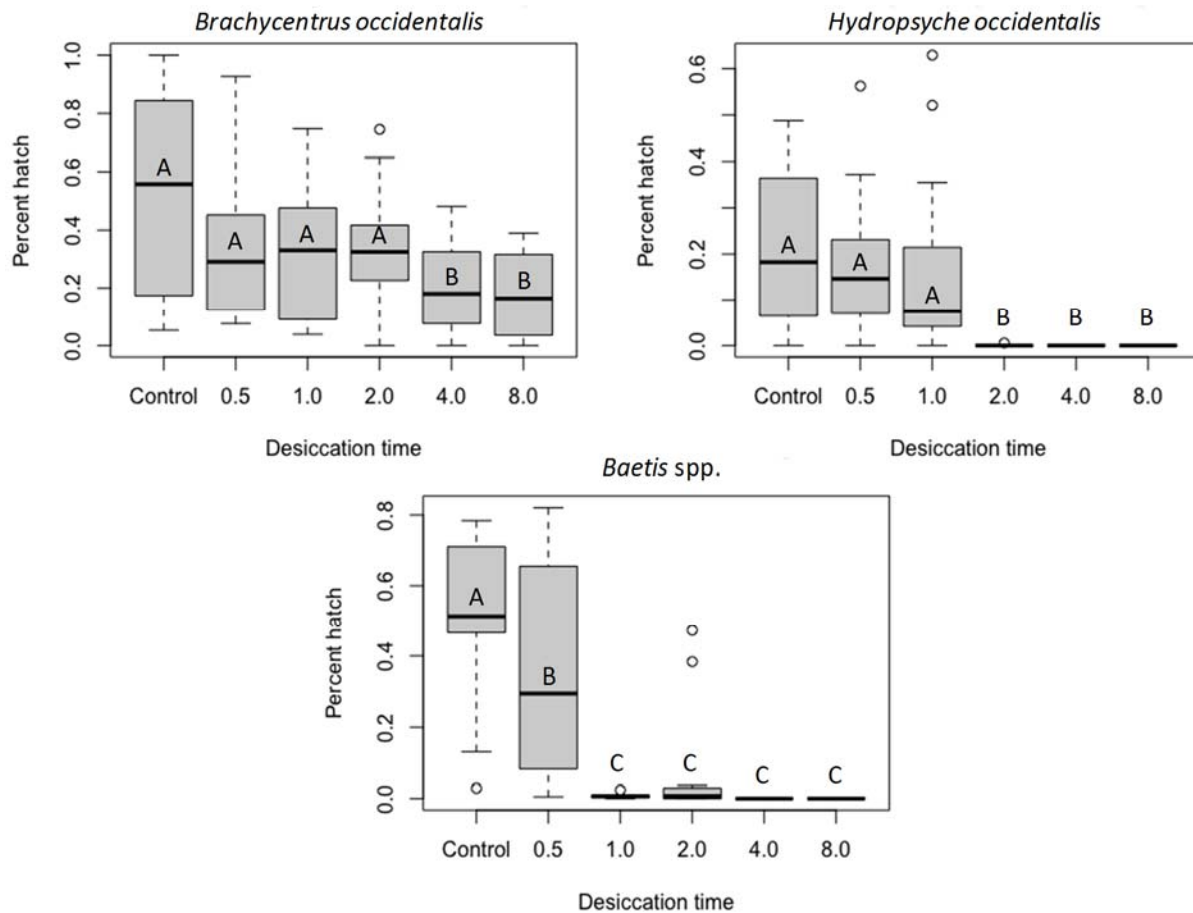


Figure 7. Proportional hatch success for *B. occidentalis* (top left), *H. occidentalis* (top right) and *Baetis* spp. compared among the six treatment levels. Note the different y-axis scales among panels. Different letters indicate statistical significance at the 0.05 alpha level.

Next Steps

- Working to salvage Chironomidae experiment
- Final report and manuscript preparation – carryover into 2017

Task 3: Western tailwater synthesis – Factors associated with differential response and recovery patterns to dam management

Example Research Questions

- Does macroinvertebrate assemblage composition differ among western tailwaters?
- What anthropogenic (e.g., alterations to hydrologic, thermal, and sediment regimes) and natural (e.g., drainage density, watershed area, elevation, ambient air temperature) variables explain variability in assemblage composition?
- Do dams preclude certain macroinvertebrate life history traits (e.g., thermal preference, mode and strength of dispersal, oviposition strategy)? If so, what are the environmental filters?

Work conducted to date

- Co-published a manuscript in Bioscience outlining the conceptual framework by which hydropeaking or load following flows could preclude macroinvertebrate taxa with certain oviposition strategies
- Started compiling a database of macroinvertebrate life history strategies for analyses of a regional macroinvertebrate database compiled by GCMRC and USU
- Analyzing macroinvertebrate recovery patterns below Flaming Gorge Dam and relating these to environmental gradients such as water temperature, substrate size and embeddedness, turbidity and periphyton biomass

Methods Summary

- Compiled data for macroinvertebrate samples collected below western tailwaters and associated hydrologic, thermal, and physiographic conditions.

Preliminary Results

- See GCMRC AMP presentation and Bioscience paper

Next Steps

- Complete field collection of environmental data below Flaming Gorge Dam during spring 2017
- Relate downstream changes in diversity and community composition to environmental gradients
- Report preparation

OBJECTIVE 2: TESTING FOODBASE MANAGEMENT ASSUMPTIONS: QUANTIFYING MACROINVERTEBRATE DIVERSITY – PRODUCTIVITY AND DIVERSITY – STABILITY RELATIONSHIPS

Task 1: Quantify macroinvertebrate diversity – productivity and diversity – stability relationships for the Green River below FGD

Research Questions

- Does macroinvertebrate secondary production exhibit longitudinal gradients below FGD?
- Do downstream macroinvertebrate diversity gradients explain spatial variability in secondary production?
- Does the magnitude of inter- and intra-annual variability in macroinvertebrate assemblages vary as a function of macroinvertebrate diversity?

Work conducted to date

- Completed the collection of samples to estimate secondary production at seven sites below FGD in November of 2016.
- Processed November – May samples processed including the measurement of a subset of individuals for production estimates
- Started compiling length-weight regressions

Methods Summary

- Monthly sampling of seven sites below FGD representing a diversity gradient (Fig. 8)
- At each site, composite samples (8 samples) were collected from both fast- and slow-water habitats
- The proportional availability of fast- versus slow-water habitats was estimated for scaling biomass estimates
- Identification of 300 randomly selected individuals per sample + big/rare search
- Identification of taxa to genus level except for midges
- 30 random individuals of each unique taxon measured to 0.1 mm
- Production methods to be determined, but likely a combination of the size frequency method and cohort-based techniques

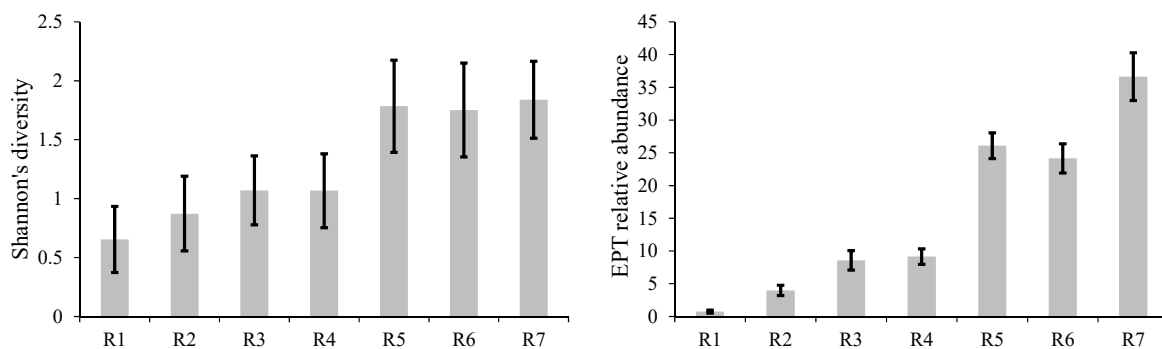


Figure 8. Downstream trends in macroinvertebrate diversity (left) and the relative abundance of Ephemeroptera, Plecoptera and Trichoptera (EPT) taxa (right) for the seven long-term

monitoring stations below Flaming Gorge Dam. Data are 19 year averages (\pm standard deviations).

Preliminary Results

- NA

Next Steps

- Complete processing of June – November samples. Limiting sample processing and production estimates to fastwater habitats at this point
- Estimate secondary production
- Relate production to diversity
- Identify other systems for comparison of macroinvertebrate diversity – productivity relationships
- Report preparation

OBJECTIVE 3: CONTINUATION OF THE GREEN RIVER SENTINEL MONITORING NETWORK OF BENTHIC MACROINVERTEBRATE ASSEMBLAGES AND FISH DIETS

Task 1: Long-term benthic macroinvertebrate and fish diet monitoring below Fontenelle and Flaming Gorge Dams

Example Research and Monitoring Questions

- What are the temporal and spatial trends in periphyton biomass, macroinvertebrates and fish diets?
- How do macroinvertebrate assemblages and periphyton biomass respond to within and among-year hydrologic variability (e.g., spring high flow events, double-peaking)?
- Do fish diets and the condition of the sport fishery track changes in the quantity and type of macroinvertebrates prey resources?

Work conducted to date

- Collected quarterly benthic samples for two sites below Fontenelle Dam and seven below FGD
- Processed $\frac{3}{4}$ of 2016 water year samples
- August 2016 – completed processing of 2015 gut samples
- September 2016 – conducted annual fish gut sampling in collaboration with UDWR
- October 2016 – presentation at Green River Outfitters and Guides Association meeting
- Development of new bi-annual report to be shared with Green River stakeholders

Methods Summary

We will sample benthic macroinvertebrates, periphyton biomass and fish diets following the methods used on the Green River since 1994. Specifically, benthic macroinvertebrates will be sampled seasonally (January, April, July and October) at each of the nine monitoring stations. All samples will be collected from riffle habitats with a Hess net (0.86 ft², 250 μ m mesh), except for the lowest most site, which will be sampled with a kick net because of the predominantly sand substrate. When possible, samples will be collected below 800 cfs to maximize sample representativeness and efficiency. Eight samples will be collected at each monitoring station per sampling event, composited into a single sample and preserved in 95% ethanol. In the laboratory, samples will be processed using a 300 count subsampling procedure paired with a “big/rare” search to remove taxa potentially missed during the initial sorting process (Cuffney et al. 1993, Vinson and Hawkins 1996).

Periphyton biomass will also be sampled seasonally at six stations below FGD; the most downstream site has historically not been sampled because of the absence of periphyton resulting from the highly mobile sand substrate. Periphyton biomass will be estimated by collecting samples from ten D₅₀ sized rocks from riffle habitats. The attached periphyton will be removed from a small area (5.03 cm²) on the upper surface of each rock using a circular template and composited into a single sample. Samples will be frozen and returned to the laboratory where they will be thawed, dried at 60° C, weighed and fired in a muffle furnace at 550° C for two hours to obtain ash-free dry mass (AFDM).

Macroinvertebrate drift and fish diets will be sampled bi-annually at two sites below FGD, Tail Race (0.8 km downstream) and Little Hole (12 km downstream). The sampling events will coincide with the Utah Division of Wildlife Resources (UDWR) fish sampling in the spring

and fall. Invertebrate drift will be sampled at dusk (ca. 1900 h) at the downstream end of riffle habitats by deploying seven nets for 30 minutes along a transect perpendicular to the river bank. Samples will be preserved in 95% ethanol and returned to the laboratory for identification at the lowest taxonomic resolution possible and biomass determined at the family level. On the same sampling dates, the diets of approximately 90 fish will be sampled based on a target of sampling two species (brown and rainbow trout), three size classes within each species and 15 replicates of each. All fishes will be collected by UDWR personnel by boat electrofishing between 2000 and 2200 h. Once collected, fish will be anesthetized with tricaine methanesulfonate (MS-222), identified, measured to the nearest millimeter, weighed to the nearest 0.1 kg and their stomach contents sampled by pulsed gastric lavage. The stomach contents will be preserved in 95% ethanol and identified and weighed at the family level.

Results – benthic samples through 2015 water year

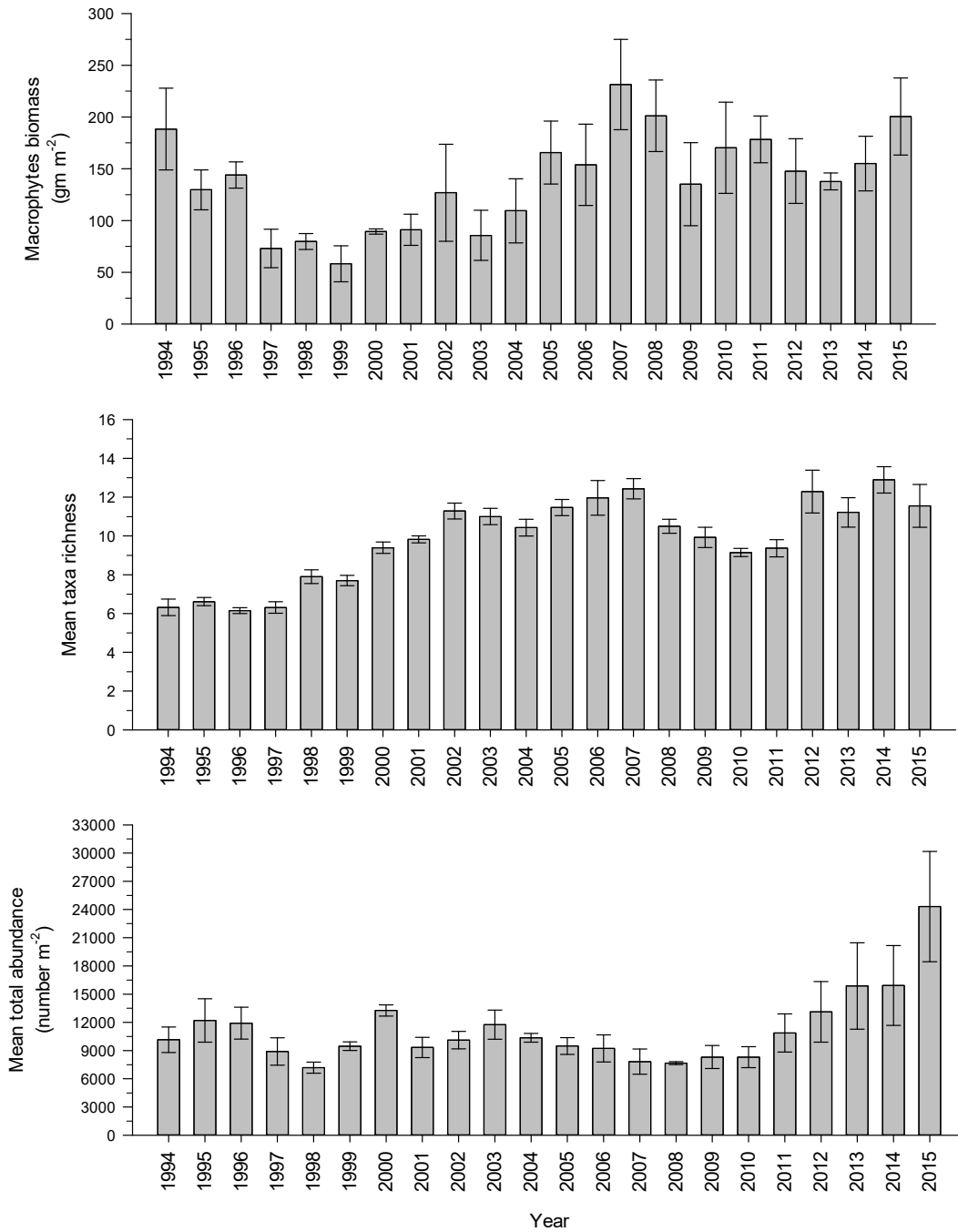


Figure 9. Annual means (\pm SE) for macrophyte biomass (top), macroinvertebrate taxa richness (middle) and macroinvertebrate density (bottom) from 1994 – September, 2015 for 7 sites downstream of Flaming Gorge Dam. Individual bars represent the average of 7 sample stations over four collection periods (January, April, July, September) per year.

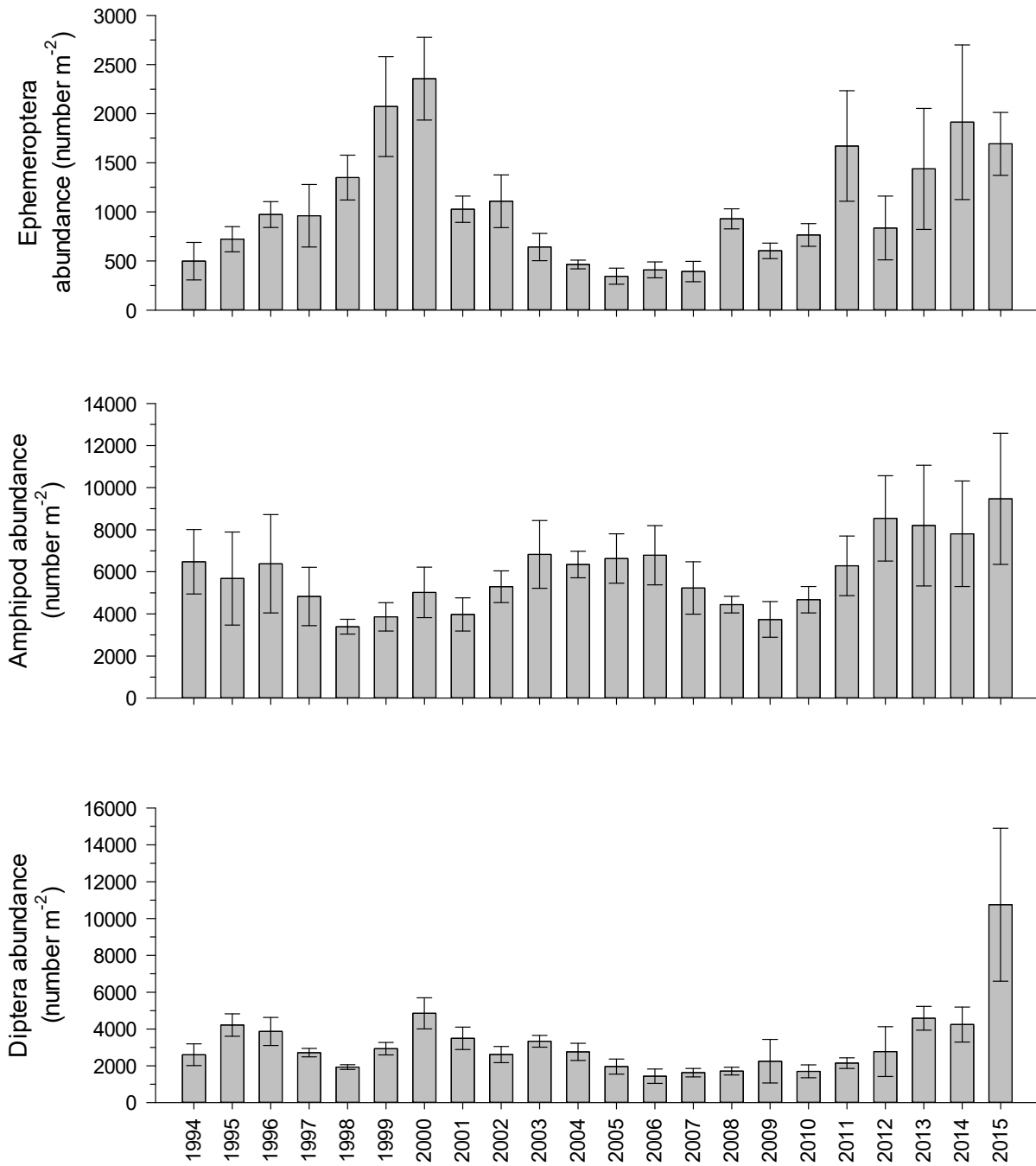


Figure 10. Annual means (\pm SE) for Ephemeroptera, Amphipoda and Diptera densities from 1994 – September, 2015 for 7 sites downstream of Flaming Gorge Dam. Individual bars represent the average of 7 sample stations over four collection periods (January, April, July, September) per year.

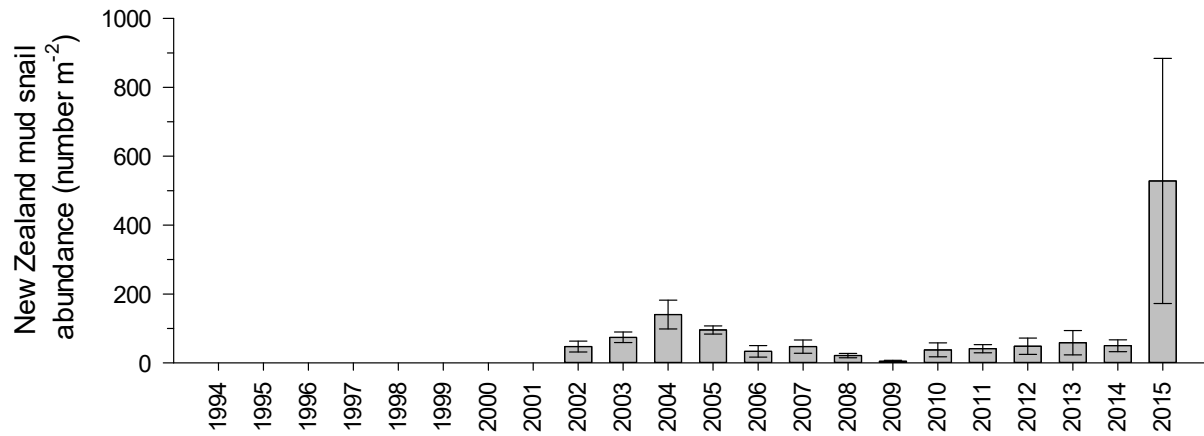


Figure 11. Annual mean (\pm SE) New Zealand mud snail densities from 1994 – September, 2015 for 7 sites downstream of Flaming Gorge Dam.

Next Steps

- Complete 2017 water year quarterly benthic and periphyton sampling at seven sites below FGD and two sites below Fontenelle Dam
- Complete processing of fall 2016 gut samples
- Complete processing of 2017 water year samples
- Bi-annual reporting to Green River stakeholders