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Distribution and Impacts of Benthic and Hyporheic Anoxia on the Colorado River Ecosystem Downstream From Glen Canyon Dam, Arizona, USA

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ABSTRACT

The highly regulated Colorado River has undergone many human-caused ecosystem changes, including elimination of extreme flows and decreased fine inorganic sediment transport. We investigated another, previously unrecognized consequence of flow regulation on the Glen Canyon Dam (GCD) tail waters reach – benthic and hyporheic anoxia (BHA). We studied BHA distribution and impacts on sediment and water quality in lower Lake Powell and the GCD tail waters, and we tested mortality effects on macroinvertebrates and the BHA development process.BHA is ubiquitous throughout lower Lake Powell and the tail waters reach in fine sediment deposits. It is more prevalent following periods of high stage (e.g., summer) and is present year-round below the low-stage elevation. Except for [O_2], geochemistry differs little between BHA and non-BHA water and sediments; however, experimental bioassays reveal increased mortality of Heptagenia mayfly larvae subjected to BHA conditions. A combination of microcosm and mesocosm experiments indicate that increased temperature, lack of aeration, and presence of Chara algae, or combinations of those factors enhance BHA development. Our results indicate that impoundment in the reservoir and highly regulated flows in the Colorado River ecosystem downstream from GCD contribute to the development and persistence of BHA, and may affect native macroinvertebrate taxa and fisheries, with impacts amplified by Chara growth, low sediment loads, high water clarity, and restricted flow variability.

Keywords: *anoxia, benthos, Colorado River, Glen Canyon Dam, hyporheic, macroinvertebrates, river regulation*

INTRODUCTION

Large dams severely alter river ecosystems worldwide, leading to degradation of a wide array of fluvial and riparian physical, chemical, and biological characteristics and processes (Dynesius and Nilsson 1994; Poff et al. 1997; Ward and Stanford 1983).The Colorado River in the southwestern U.S. is a prime example of an intensively developed and highly altered system (Stevens et al. 2020b). Fifteen large dams exist along its 2,330 km-length, making it one of the most heavily regulated rivers in the world. The second-largest dam in the coterminous United States, Glen Canyon Dam (GCD) is located in north-central Arizona and regulates flow through lower Glen Canyon and Grand Canyon to Lake Mead. The Colorado River ecosystem (CRE) in this region has been highly altered with respect to flow, water chemistry, sediment dynamics, and biota (Blinn and Cole 1991; Cross et al. 2013; Kennedy et al. 2016; Minckley 1991; Stevens et al. 1997; Stevens 2013).

Largely missing from the river and of particular concern are mayflies (Ephemeroptera), stoneflies (Plecoptera), and caddisflies (Trichoptera) the "EPT" taxa that often are important elements of riverine fisheries foodbases, and which are used as biological indicators of stream ecosystem health (Barbour et al. 1999; Cross et al. 2013; Merritt et al. 2008). These insect orders are relatively abundant in many unaltered tributary streams in the CRE and in other segments. For example, EPT density is relatively high in most unregulated cool watert ributary streams in Grand Canyon,as well as in the dam-regulated Flaming Gorge and other Colorado River reaches upstream in Utah and Colorado (Haden et al. 2003; Oberlin et al. 1999; Vinson 2001), but not in the regulated mainstream in

Grand Canyon (Blinn and Ruiter 2009; Kennedy et al. 2016; Stevens et al. 2020a). Many physical and biological factors potentially are responsible for the absence of EPT in the GCD tailwaters, including altered seasonal water temperature variation, daily and seasonal flow variation, and legacy effects of pre-dam sediment loads on colonization (Cross et al. 2013; Stevens et al. 1997). Kennedy et al. (2016) posit that hydropower flow fluctuations reduce aquatic macro-invertebrate egg survival, restricting the assemblage to those species that oviposit on the water's surface. Other limiting factors, some of which are dam related may, affect aquatic macroinvertebrate survivorship, including water quality, habitat availability (i.e., embeddedness), suspended sediment concentration, and the previously unrecognized development since the mid-1990s of extensive benthic and hyporheic anoxia (BHA) in the reservoir and tailwaters.

Anoxia in benthic and hyporheic sediments negatively affects aquatic benthic and hyporheic geochemistry, habitat quality, biota, and ecosystem interactions (Baker et al. 1999; Boulton et al. 1998; Clements et al. 2000; Hrycik et al. 2017; Winner et al. 1980), but its influence in large regulated rivers, such as the Colorado River downstream from GCD, has received little attention. BHA often develops through excessive production and decomposition of aquatic macrophytic vegetation in habitats with high water clarity and productivity.

Insufficient stream energy exists in lentic and low-velocity settings to export decomposing organic matter, causing BHA to develop and expand. BHA development often is conspicuous, appearing as blackened sediment, and is readily detected (e.g., Marmonier etal. 2004). However, BHA development and feed-back influences on aquatic ecosystem geochemistry, macrophyte assemblages , and higher trophic level structure and function can be complex, counterintuitive, and indirect (Baker et al. 1999; Campbell et al. 2003; Dahm et al. 1987; Fleeger et al. 2003; Hrycik et al. 2017).

For example, experimental chemical reduction of benthic sediments in a highly contaminated Australian estuary generated expected releases of Mn and Fe, but only minor releases of As, Cd, Cu, and Zn. Similarly, minor and slow release of U has been reported at the anoxic sediment-water column interface in Lake Powell (Wildman et al. 2010). Release of anoxia-liberated compounds into the overlying water column can induce bioaccumulation and directly and indirectly affect aquatic assemblages, food webs, and trophic structure and function (Besser et al. 2001; Cain et al. 1992; Campbell et al. 2003; Clements et al. 2000; Hare 1992; Hogsden and Harding 2012; Hrycik et al. 2017; Poulton et al. 1995), with impacts extending into the riparian zone (Moore et al. 1991; Walters et al. 2008). Thus, BHA can influence fluvial ecosystem function, but its influences on the highly regulated CRE downstream from GCD have not been investigated prior to this study.

Minor development of BHA was noted in the GCD tailwaters (Stevens et al. 1997) and has been studied in relation to heavy metal release in Lake Powell reservoir (Wildman et al. 2010). Prior to the onset of highly constrained hydropeaking flows from GCD in the early 1990s, BHA was not recognized as an important phenomenon in the regulated CRE (U.S. Bureau of Reclamation 1996, 2016).

However, since the late 1990s, BHA has become conspicuous in sand-dominated shorelines of Lake Powell and on the channel floor from GCD to the unregulated Paria River confluence (approximately 27 km downstream from the dam), but not to any great extent downstream in Grand Canyon. Development of BHA in the dam tailwaters corresponds with high levels of water clarity and benthic primary production in the tailwaters reach, coupled with reduced daily flow variation after 1990 (Stevens et al. 1997).

An abrupt reduction in mainstream water clarity and benthic production occurs at the Paria River confluence due to fine sediment input, creating a conspicuous, 30-fold stair-step reduction inmacrophyte standing stock downstream in Grand Canyon (Cross et al. 2013; Graf et al. 1991; Stevens et al. 1997). Paria River fine sediment contributions reduce photosynthetically active radiation (PAR) in the water column downstream (Yard et al. 2005), reducing macrophyte production and the deposition of decomposing organic matter. The impacts of BHA on water and benthic sediment quality and benthic macroinvertebrate survivorship in Lake Powell and Glen Canyon tailwaters have not received detailed examination prior to this study. However, previous research has shown that anoxia-related diffusion on Mn and U from pore water into the water column occurs in Lake Powell (Wildman et al. 2010), and elevated concentrations of Hg and Se exist in highertrophic levels, including fish, downstream from the dam (Walters et al. 2015). Sources and consequences of trophic contamination on the river ecosystem have not yet been determined, but the effects of metal mobilization on aquatic organisms can include oxidative stress in algae and transport of those contaminants to higher trophic levels (Pinto et al. 2003); changes in benthic macroinvertebrate composition, behavior, competition, and predation (Fleeger et al. 2003); and overall negative impacts on aquatic invertebrate abundance and diversity (Clements et al. 2000).

The objectives of this study were to determine distribution and impacts of BHA on the CRE in lower Lake Powell Reservoir and on the CRE in the GCD tailwaters. We report on four components: (1) mapping the distribution and extent of BHA seasonally in lower Lake Powell and the tailwaters of GCD, (2) characterizing the water and sediment chemistry of BHA-affected regions, (3) determining the effects of BHA on macroinvertebrate survival, and (4) determining the conditions (physical, biological, and chemical) under which BHA develops. Failure to recognize BHA since the mid-1990's limits understanding of factors contributing to its onset, impacts, and variation in development rate. Nonetheless, our study illuminates the factors presently facilitating and maintaining BHA in this highly regulated river ecosystem.

METHODS

Study Area

The focal area for the field investigations of BHA included lower Lake Powell (the forebay of the dam) and the GCD tailwaters from the base of the dam to approximately two km downstream from Lees Ferry, including the mouth of the Paria River (Fig. 1). Twelve transects were established for field measurements, including three sites in lower Lake Powell, six sites in the Glen Canyon reach from GCD downstream to Lees Ferry, and three sites downstream from Lees Ferry to river kilometer 2 (Rkm 2), two of which were located downstream from the Paria River confluence.

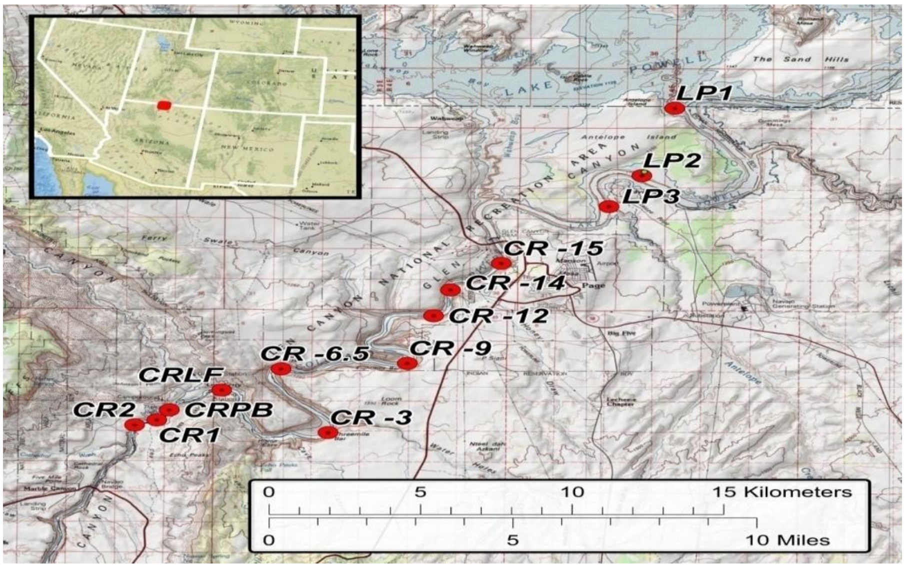

Figure1. *Location map of study site on the Colorado River in Arizona. Inset map shows regional placement of the study site in Glen Canyon National Recreation Area and in relation to the dam. Sampling site abbreviations are as follows: LP (Lake Powell sites), CR (Colorado River below Glen Canyon Dam site; numbers indicate river mile), CRLF (Colorado River at Lees Ferry site), CRPB (Colorado River at Paria Beach, upstream of Paria River confluence), and CR1 and CR2 (Colorado River sites below Paria River confluence). All sampling was conducted on the right side of the river, looking downstream.*

The study area encompassed approximately 30 km from lower Lake Powell to Rkm 2. Riparian vegetation in this reach consisted primarily of saltcedar (*Tamarix spp.*), willow (*Salix gooddingii*), cottonwood (*Populus fremontii*), and seep willow (*Baccharis* spp.*;* Palmquist et al. 2017; Ralston 2005). Shallow-water (<4 m depth) benthic aquatic vegetation consisted of seasonally-varying patches of *Chara* nr. *vulgaris*, horned pondweed (*Zannichellia palustris*), limited cover of filamentous green algae (formerly dominated by *Cladophora glomerata*), and other aquatic macrophytes, all of which respond strongly to increased summertime sunlight in this steep canyon-bound reach. A dense stand of *Fontinalis hypnoides* aquatic moss occupies deeper water benthos throughout the reach. Benthic invertebrates of the reach are dominated by *Potamopyrgus antipodarum* (New Zealand mud snails), *Dressina rostriformis bugensis* (quagga mussel; still in early stages of colonization), *Physa snails*, *Gammarus lacustris scuds*, turbellaria flatworms, Clitellata worms, Chironomidae midges, and rare *Pisidium* finger clams, aquatic earthworms, and micro crustaceans, mayflies, and aquatic beetles in stranding pools (Kennedy et al. 2016; Stevens et al. 1997, 1998; Sublette et al. 1998). Lake Powell stage changes and GCD releases during the study period were seasonally normal in relation to reservoir inflows and operational requirements of the 1996 Adaptive Management Plan (U.S. Bureau of Reclamation 1996) and the 2016 Long-Term Experimental and Management Plan (U.S. Bureau of Reclamation 2016). Lake Powell reservoir pool stage peaked in June-July and gradually decreased throughout the subsequent summer, fall, and winter months, reaching its lowest stage during our March transect sampling period (Fig. 2a). Downstream, river flows varied during the low-release springtime and autumn months from 142 – 396 m^3/s, with daily stage changes < 227 m^3/s, with bimodal (summer and winter) maximum flows of 510 m^3/s (Fig.2b). Daily flow fluctuations created approximately 0.5 m of stage change at transects downstream from the dam.

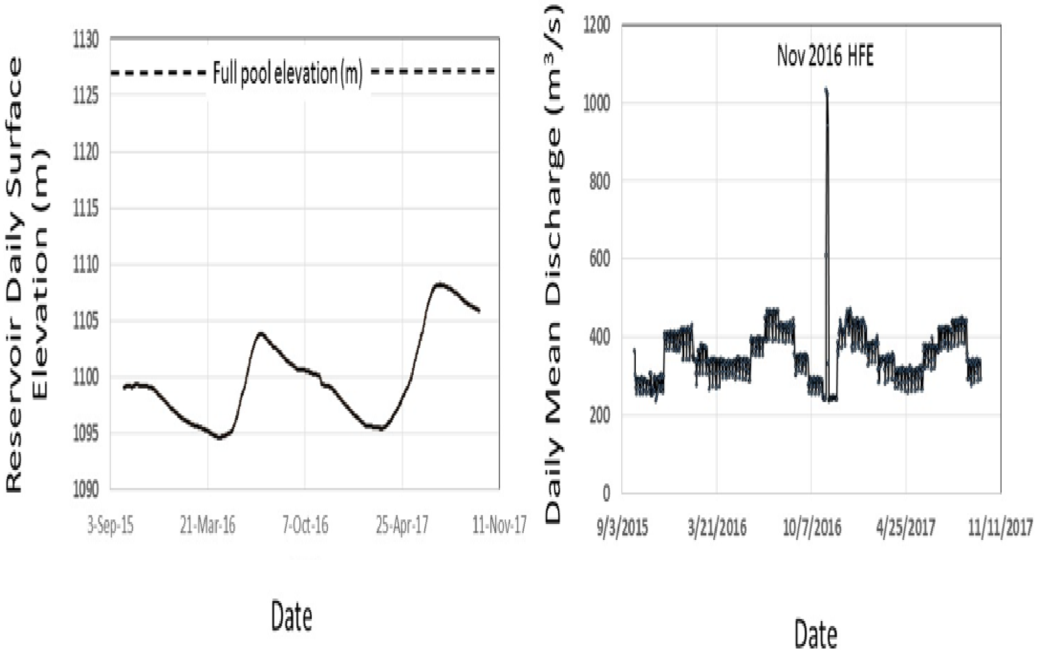

Figure2. *Lake Powell Reservoir stage elevations (left) and Colorado River flow at Lees Ferry during study period (right). HFE = high flow experiment. Data from the U.S. Bureau of Reclamation and the U.S. Geological Survey*

Distribution of BHA in Lower Lake Powell and the Glen Canyon Dam Tailwaters

Each of the 12 transects was visited quarterly (summer, fall, winter, and spring from 2016 to 2017). A Sokkia C330 level, elevation rod, and survey tape were used to measure elevation profiles. Aspect and photography were used to ensure future repeatability, and back-sight elevation and distance were checked before and after each survey to ensure accuracy.

Data were collected across the profile at abrupt changes in elevation or substrate. At each point, elevation and distance were measured and a pit was excavated to record substrate stratification and the presence and depth of BHA, as detected by conspicuous blackening of the sediment. Colorimetric evidence of BHA was confirmed using a YSI™ ORP probe (below). Additional data recorded included vegetation type, water depth, and ecosystem characteristics (e.g., location within the riparian zone). Surveys were made during low water periods, typically on weekends, and extended into the mainstream as far as safety would allow, typically to 1.5 m depth below the low water stage. Beyond the furthest survey point accessible on foot, a Petite Ponar dredge was deployed from the research boat to examine substrate conditions on the channel bed using a calibrated rope and rangefinder to determine depth and distance from the transect benchmark, respectively. Contents from the dredge were examined on site. A five-day experimental high flow was conducted to manage fine sediment mass balance in November 2016, during and after which we measured changes in water geochemistry and BHA distribution on the tailwaters channel floor.

Data were analyzed to determine whether BHA fluctuated seasonally and in response to stage variation. We used linear regression on reservoir data to determine seasonal changes in BHA. Seasonal fluctuation in BHA distribution also were measured against surveyed benchmarksin the tailwaters, and analyzed using a mixed effects model. Season was considered a fixed effect and site a random effect. Data from sites below Lees Ferry were not included due to substantial changes in sediment deposition that limited measurement (BHA was absent in three of the four seasons at one or more of those transects during the study period). A likelihood ratio test was used to detect differences between the null (site only) and experimental (with season included) models using AIC and X^2 as the test statistic. Post-hoc Tukey tests were used to test for seasonal changes if a model indicated significant difference.

Water and Sediment Chemistry

Field water chemistry was measured at each of the 12 transects on each sampling date using a YSI Professional Plus multiparameter instrument, calibrated daily for all parameters. We sampled water quality in the water column and in benthic sediments. BHA water samples were created using a 2:1 river water: BHA sediment slurry, which was settled for 10 min and then decanted. We measured the following field water quality variables: temperature (°C), pH, dissolved oxygen (DO, mg/L), and oxidation-reduction potential (ORP, mV). We used paired t-tests ($\alpha = 0.05$) to test for differences within parameters between the benthos and the water column.

In addition to field measurements, we collected water and benthic sediment samples for laboratory analysis at selected sites for the following components: Hg (water and sediment); nitrate (water and sediments) and nitrite (water); total P (water and sediments); sulfate (water); total U (water); total C and organic matter (sediments); and particle size (sediments). Laboratory water quality samples for the above variables except U were collected in summer and winter at sites CR-6.5R, CRLF, CR2, and LP1 (Fig. 1). Sampling dates were: 19 and 20 August 2016; 6-9, 11-12 November 2016 [high flow experiment (HFE) samples]; and 7-8 March 2017. Water samples for U analysis were collected in BHA sediments in winter at sites CR-12, LP1, and CR2. Water and sediment samples were kept on ice at 4°C until analysis. Laboratory water quality analyses were conducted by Nortest Analytical Lab (Flagstaff, AZ) and by Pennsylvania State University Agricultural Analytical Services Lab (Hg, nitrate, nitrite, total P, sulfate, total C, organic matter, particle size; State College, PA). We used paired t-tests ($\alpha = 0.05$) to test for significant differences in all parameters between BHA samples and non-BHA samples from each site.

Impacts of BHA on Macroinvertebrate Survival

We conducted a bioassay to determine effects of BHA on a common flatheaded mayfly genus in northern Arizona (Ephemeroptera: Heptageniidae: *Heptagenia*). As mentioned above, mayflies are absent in the mainstem Colorado River but are abundant in cool water Grand Canyon tributaries (Cross et al. 2013; Kennedy et al. 2016; Oberlin et al. 1999; Stevens et al. in review). We collected *Heptagenia* larvae from Oak Creek (Coconino County, AZ) in March 2017 at a locality on the Colorado Plateau. The bioassay design consisted of adding 50 mL of BHA sediment into 100 mL of Colorado River water, creating a slurry, and decanting the slurry into experimental chambers. This experimental treatment was compared against controls containing 100% Colorado River water. One larval *Heptagenia* was placed in each chamber (n = 31for each treatment). Survivorship was monitored after the first hour, and then every 12 hours for 8 days. Water quality (temperature, pH, conductivity, and DO) were measured daily throughout the experiment. We performed a regression analysis on the survivorship data and analyzed the regression slopes in the experimental and control groups using a t-test to test for significant differences in mortality rates between BHA water and the control ($\alpha =$ 0.05).We also calculated the point at which 50% of the population perished (LD^{50}) in each treatment.

BHA Development

We investigated the time and factors influencing BHA development through field and laboratory experiments. In the field experiment, twelve 20 L plastic chambers were filled with clean (non-BHA) Colorado River sand and water at site CR-6.5. Six chambers were placed in a floating platform and were unaffected by wave action or river flow fluctuations. Six other chambers were set into the shoreline at the middle stage elevation, with eight cm of lip exposed. One L of living *Chara* algae was added to three chambers in each treatment.

The stationary shoreline chambers containing *Chara* were covered with 1-mm mesh to prevent the algae from dispersing during inundation. For this and the following BHA development experiment, we used *Chara* due to its ubiquitous presence throughout the study area. The experiment began on 17 August 2016 and was completed on 23 September 2016.Water quality (temperature, pH, DO, and ORP) and the depth of BHA development (determined visually by blackening of the sediment) were measured initially and at the end of the experiment.

We used a linear model and two-way ANOVA to test for significant differences in each of the four water quality parameters based on location (floating vs. submerged), presence of *Chara* (present or absent), and the interaction between these two main factors. Subsequent Tukey tests were used in the event of significant differences. Presence and depth of BHA in each chamber were recorded at the conclusion of the experiment.

In order to determine how temperature, aeration, and presence of *Chara* interactively affect the development rate of BHA, we conducted a laboratory microcosm experiment using three 204-L tanks into which smaller chambers containing clean Colorado River sand were placed (Figs. 10, 11). The three large 204L tanks were each maintained at constant temperatures of 5°C, 12°C, and 20°C, spanning the range of water temperatures in the Glen Canyon reach. Each temperature bath contained thirty-two 1 L chambers (a total of 96 individual chambers), each of which were filled with 450 mL of dry non-BHA Colorado River sand and 450 mL of Colorado River water collected from Lees Ferry. Eight treatments in each thermal bath chamber contained *Chara*, *Chara* + aeration, aeration, or neither. Aeration was provided using aquarium pumps and hoses. Each temperature bath was placed under a 120-watt grow light simulating eight hours of direct sunlight. Laboratory temperature mained constant during the 10-day experiment. Water quality parameters (temperature, pH, DO, and ORP) were measured initially (day 0) and on days three, six, and 10. On day 10, each 1 L chamber was removed from the temperature bath and examined for BHA development. Depth of BHA development in the sediment was measured.

We used a three-factor ANOVA followed by a Tukey post-hoc test to determine differences within and among the three factors (temperature, *Chara* presence, and aeration) with respect to the depth of BHA development ($\alpha = 0.05$).

RESULTS

Distribution of BHA in Lower Lake Powell and Glen Canyon Dam Tailwaters

Quarterly surveys on each transect revealed that BHA was commonplace throughout the GCD forebay and in the tail waters between the dam and Lees Ferry.

BHA was ubiquitous in sand and silt sediment deposits below the annual low water stage of Lake Powell reservoir at all sites dominated by aquatic macrophytic vegetation or containing buried shoreline organic matter. Mainstream tail water flows varied daily and monthly during the study duration, varying from a low of near 142 m^3/s to nearly 510 m^3/s during July and August of both 2016 and 2017, except for the three day-long experimental high flow (Fig. 2b).

BHA was present at all Glen Canyon reach transects from the dam downstream to Lees Ferry and occurred erratically at the transects downstream from Lees Ferry.

In general, we noted development of BHA in the reservoir in areas with fine sediment below the seasonal low water stage, and in the river upstream from the Paria River confluence, particularly in settings dominated by dense benthic macrophyte cover (*Chara*, *Zanichellia*, and other macrophytes). Little BHA was detected in the thalweg of the mainstream channel at depths >10 m; however, BHA was detected beneath gravels and cobbles in shallow cobble bar habitats (e.g., site CR-9).BHA distribution varied seasonally in the reservoir, decreasing in elevation over time from August 2016 to March 2017 ($R^2 = 0.91$, $m = -0.03$, $P = 0.009$; Fig. 3). BHA was not detected during the springtime (May, high stage) sampling in the reservoir, but was common from mid-summer through the winter, with declining stages ($X^2_3 = 9.85$, $P = 0.02$). Changes between summer/fall (an increase in extent of BHA) and fall/winter (a decrease in extent of BHA) were significantly different (P = 0.05; Fig. 3). BHA was continually present below the annual minimum stage in Lake Powell and developed gradually on the rising stage of the reservoir, retreating in autumn as stage elevation decreased.

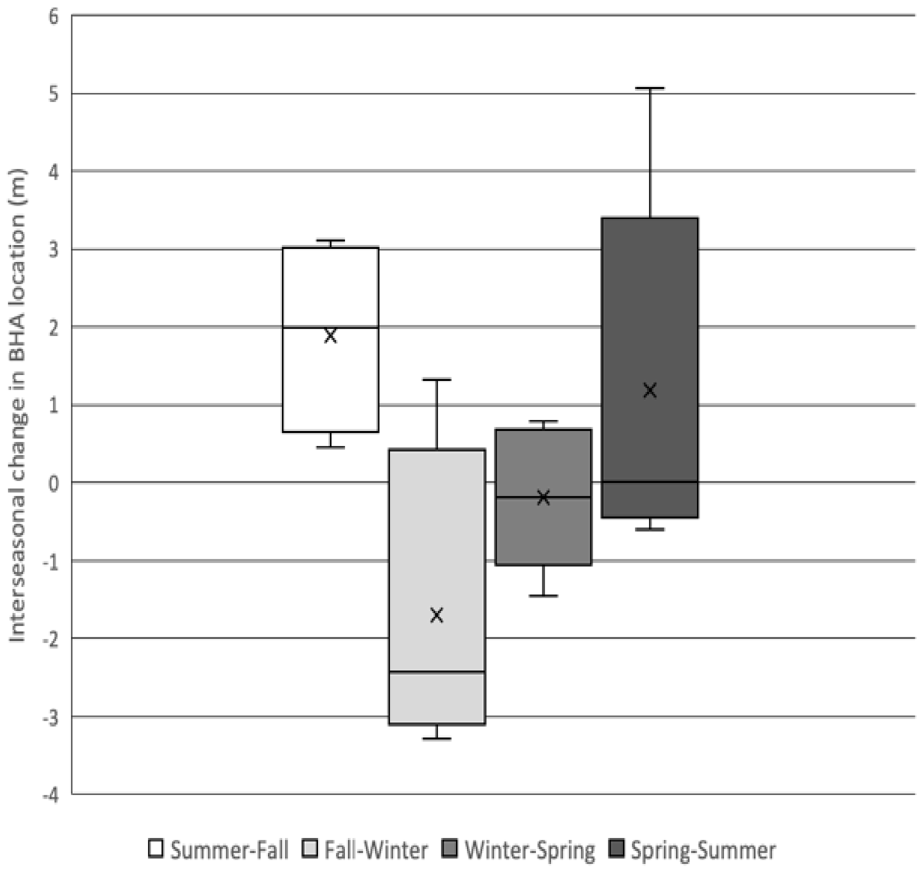

Figure3. *Boxplot of interseasonal change in BHA location below Glen Canyon Dam. Lake Powell sites are not included. "Summer-Fall" indicates change in BHA elevation on sampling transects from summer to fall seasonal sampling. "Fall-Winter" indicates change from fall to winter; "Winter-Spring" indicates changes from winter to spring; and "Spring-Summer indicates change from spring to summer. Positive change values indicate expansion of BHA coverage on transect over the noted time period. Negative change values indicate retreat of BHA coverage.*

Downstream from the dam, BHA was ubiquitous below the annual low stage elevation CR 0.5, and developed slowly following prolonged summertime inundation. BHA development retreated through following lower water levels in autumn springtime. Site CR-3 was an outlier in relation to BHA seasonal change due to transect-specific hydrological and biological variables (i.e., dense living wetland vegetation and nearly constant inundation in a shallow return current channel depression), and that site was eliminated from our analyses. Additionally, BHA was absent from CR-9 during fall sampling and, therefore, was not included in seasonal change calculations from summer to fall and fall to winter. We observed that the November 2016 high flow experiment flow velocity scoured some of the organic floc from the floor of the tailwaters channel, and resulted in widespread development of a thin (five mm-thick) surficial, buff-colored (oxygenated) sand layer on the floor of the channel, overlying BHA deposits. While we did not have sufficient data to rigorously investigate change in BHA distribution downstream from Lees Ferry, we noted its occasional presence at the three downstream sites. Traces of BHA were found among the cobbles at CRPB (upstream of the Paria River confluence) during the summer and fall, but BHA was absent during the winter and springtime. Traces of BHA also were present in shoreline sand deposits at CR1 (downstream of the Paria River confluence), particularly following Paria River flooding and deposition of extensive quantities of organic matter in late summer. The furthest downstream site, CR2, developed considerable amounts of BHA to >30 cm depth following Paria River flooding; however, that sandbar subsequently eroded away, leaving a steep shoreline channel margin that did not display BHA. Thus, transects downstream from the Paria River confluence exhibited BHA development only following Paria River deposition of fine sediments and organic matter. Observations during several river trips through Grand Canyon during the study period revealed that BHA was restricted only to highly localized shallow backwaters, and was virtually non- existent along the mainstream and its many sandbars, where higher levels of turbidity reduce aquatic PAR and aquatic macrophyte growth (Yard et al. 2005).

Water and Sediment Chemistry

Field water chemistry measurements revealed no significant differences ($P > 0.05$) between any of the parameters on the benthic surface versus the water column. Our laboratory water quality analyses revealed no detectable release of nitrate, nitrite, or sulfate from BHA sediments into the water column (Table 1). However, during the summer 2016 sampling period, we detected an increase in total P concentration from water extracted from BHA sediments (non-BHA water mean P = 0.02 mg/L, BHA water mean P = 2.45 mg/L, $P = 0.022$) from three sites (2 riverine, 1 reservoir). The remainder of the samples (sites and dates) contained total P below the detectable limit.

The CR-12 sample contained 0.019 mg/L U, which is slightly greater than the reported detection limit but below the EPA's maximum contamination limit (MCL) for drinking water (MCL = 0.03 mg/L). The remaining two water samples (LP1 and CR2) analyzed for U revealed concentrations at or below the detection limit (0.01 mg/L).

Table1. *Results of water and sediment laboratory analysis of BHA and non-BHA ("clean") water and sediment. Values shown are means across sites (+/- SD). BDL = below detectable limit. T-tests between treatments indicated no significant differences among any water quality variables.*

	Mercury (mg/L)	**Nitrate (mg/L)**	**Nitrite (mg/L)**	**Total P (mg/L)**	**Sulfate (mg/L)**	**Uranium (mg/L)**
BHA Water	BDL	0.15 (+/- 0.17)	BDL	1.20 (+/- 1.40)	197.41 (+/- 22.14)	0.02*
Clean Water	BDL	0.26 (+/- 0.14)	BDL	0.22 (+/- 0.37)	194.64 (+/- 17.79)	n/a
	Mercury (mg/L)	Total P (mg/kg)	Total C (%)	Organic matter (%)	Nitrate (ppm)	
BHA Sediment	BDL	111.37 (+/- 92.66)	0.61 (+/- 0.42)	0.28 (+/- 0.38)	14.12 (+/- 15.05)	
Clean Sediment	n/a	134.46 (+/- 151.06)	1.39 (+/- 2.16)	1.67 (+/- 2.88)	7.92 (+/- 4.00)	
*single data point						

Laboratory sediment analyses indicated no significant differences between the tested parameters in BHA vs. non-BHA sediments (Table 1). However, there were several variables of interest despite the lack of significance. Concentration of Hg was generally below minimum detectable levels in all sediment samples, with the exception of sediments from the Lees Ferry transect. Hg concentration in non-BHA sediments at Lees Ferry was 0.007 mg/L, 3.5-fold higher than the EPA MCL standard for drinking water (0.002 mg/L), and slightly higher than the World Health Organization's drinking water guidelines (0.006 mg/L). The occurrences of that level of contamination in non-BHA sediments may reflect legacy impacts of a marina that formerly existed near that transect location.

Effects of BHA on Macroinvertebrate Survival

The bioassay experiment involving subjection of *Heptagenia* mayfly larvae to 10-day treatments of BHA sediment with Colorado River water and controls without BHA sediments revealed significant negative impacts of BHA on *Heptagenia* survival rate in the BHA treatment ($t_{60} = 2.213$, $P = 0.015$; Fig. 4). By the fifth day, all mayflies in the BHA treatment had died, whereas only 40% of mayflies in the control treatment had perished. Water quality differed as well, with pH and DO being lower in the BHA treatment (pH: $t_{28} = 7.08$, $P < 0.001$; DO: $t_{23} = 6.67$, $P < 0.001$; Table 2). The 50 percent mortality rate (LD_{50}) in the BHA treatment occurred 18.3 hr after the start of the experiment, whereas

LD_{50} in the control group was estimated to occur 562 hr after the start of the experiment.

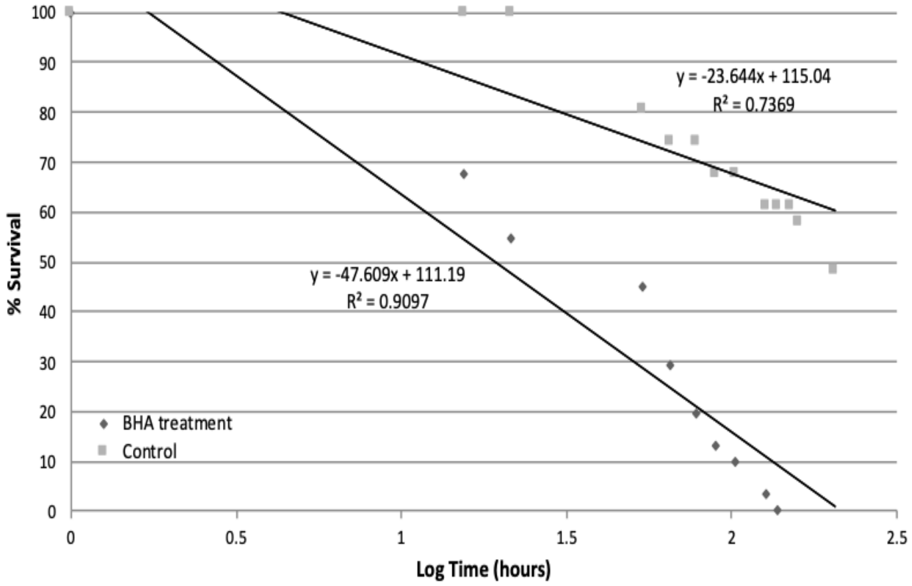

Figure4. *Percent survival of Heptagenia sp. mayflies in the presence of BHA sediments ("HA treatment" and controls (non-BHA sediment) over the 8-d microcosm experiment. The x-axis represents the log_{10} hours since the start of the experiment.*

BHA Development

The field mesocosm experiment revealed significant development of BHA within the 38-day experimental period. BHA developed only in chambers into which *Chara* had been added. Submerged chambers exhibited a lower water temperature ($F_{1,7} = 239.06$, $P < 0.001$), but no other water quality effects were detected between the location treatments (submerged vs. floating; Table 3).The presence of *Chara* led to decreased dissolved oxygen concentration ($F_{1,7} = 46.89$, $P < 0.001$), pH ($F_{1,7} = 42.11$, $P < 0.001$), and ORP ($F_{1,7} = 15.68$, $P = 0.005$) regardless of location, indicating that these factors may be integral to the development of BHA (Table 3). A small pilot experiment also revealed that exposing black BHA sediments to the atmosphere resulted in dissipation of BHA and return to normal buff-colored sand in less than 10 hours.

Table3. *Water quality measurements (mean +/- SE) from the field mesocosm BHA development experiment (location treatment = submerged vs. floating; vegetation treatment = Chara absent vs. Chara present). Only final measurements are shown (taken at the end of the 38-d experimental period). Significance testing was accomplished through ANOVA (see footnotes for details on notation).*

	Temp (°C)	DO (mg/L)	pH	ORP (mV)
Submerged				
***Chara* absent**	20.7 (+/- 0.51)*	7.4 (+/- 0.28)^a	8.3 (+/- 0.13)^a	107.8 (+/- 60.80)^a
***Chara* present**	20.25 (+/- 0.05)	4.14 (+/- 1.94)^b	7.37 (+/- 0.22)^b	-74.1 (+/- 38.60)^b
Floating				
***Chara* absent**	15.27 (+/- 0.18)*	6.55 (+/- 0.48)^a	8.47 (+/- 0.16)^a	154.17 (+/- 50.56)^a
***Chara* present**	16.3 (+/- 0.06)	0.33 (+/- 0.22)^b	7.62 (+/- 0.10)^b	-40.43 (+/- 20.59)^b
*Significant difference between submerged and floating treatments ($p < 0.001$)				
Different superscripts represent significant differences between *Chara* treatments within location ($p < 0.005$).				

The laboratory microcosm BHA development experiment provided significant insight into the interactive effects of water temperature, DO concentration, and the presence of *Chara* on rates of BHA development on water quality. Water quality (pH, DO, and ORP) varied significantly over the course of this 10-day experiment (Fig. 5). Presence of *Chara* led to decreased pH, DO, and ORP, particularly at higher temperatures and in the absence of aeration. All three treatment variables in the laboratory microcosm experiment (presence of *Chara*, temperature, and aeration) significantly affected the development of BHA (measured as the depth of darkened sediment at the end of the experiment): *Chara*: $F_{1,84} = 1244.75$, $P < 0.001$; temperature: $F_{2,84} = 12.77$, $P < 0.001$; aeration: $F_{1,84} = 21.96$, $P < 0.001$. No BHA development occurred in the absence of *Chara*, and sediment in all chambers with *Chara* present developed conspicuous BHA. In addition to presence of *Chara*, higher temperatures ($T_{20} > T_5$, $P < 0.001$; $T_{20} > T_{12}$, $P = 0.037$; $T_{12} > T_5$, $P = 0.0341$) and lack of aeration (non-aerated > aerated, $P < 0.005$) led to increased BHA development rates. In addition to effects of each variable independently, significant interactions occurred among all variables with respect to BHA development. Aeration in the presence of *Chara* significantly reduced BHA depth as compared to non-aerated treatments with *Chara* ($P < 0.001$). A three-way comparison showed that aeration at elevated temperatures can mitigate temperature effects, resulting in decreased BHA development (i.e., no significant differences were detected between aerated treatments with *Chara* present at 12°C or 20°C versus non-aerated treatments with *Chara* present at 5°C). This provides further evidence that aeration can significantly reduce BHA development rate. However, the differences between the low (5°C) and the middle (12°C) temperature treatment in the absence of aeration did not change extent of BHA development. At higher temperatures (20°C), BHA development significantly increases in the absence of aeration. The highest temperature in our experiment facilitated the most extensive development of BHA.

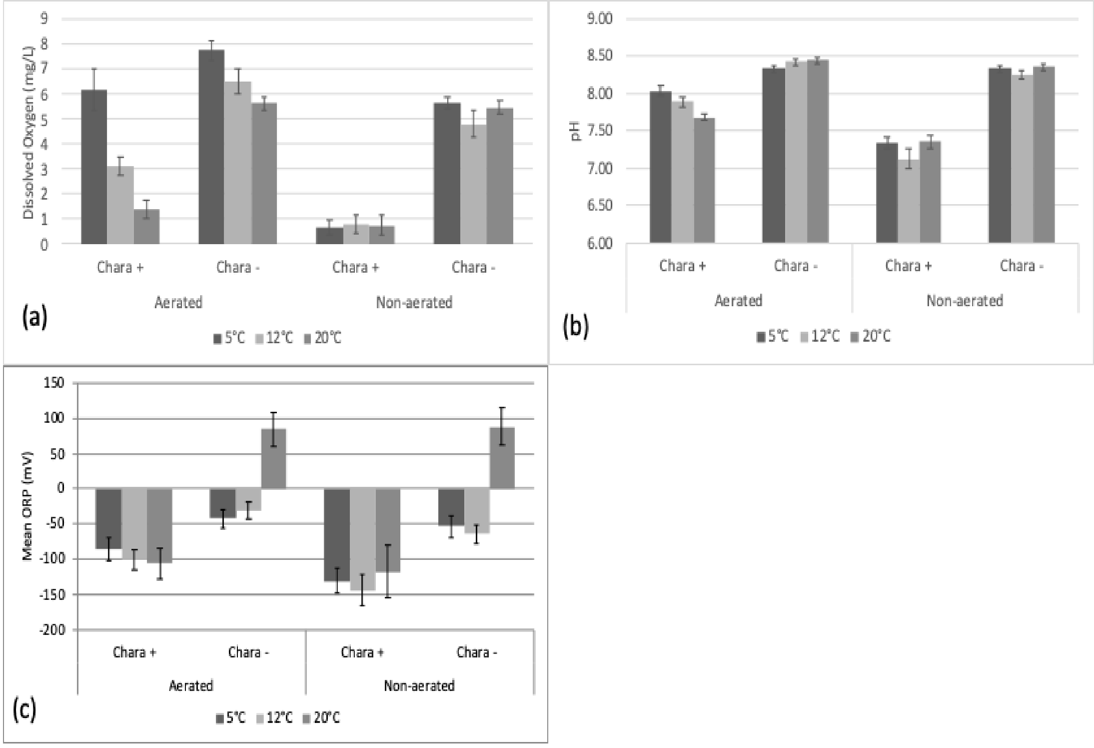

Figure 5. *Mean water quality measurements (+/- SE) from the BHA development microcosm experiment. Measurements were taken three times over the 10-day experimental period. (a) mean dissolved oxygen, (b) mean pH, and (c) mean oxidation-reduction potential.*

DISCUSSION

Distribution of BHA in Lower Lake Powell and the Glen Canyon Dam Tailwaters

Our investigation reveals the previously-unrecognized ubiquitous distribution of BHA in lower Lake Powell and the GCD tailwaters, a phenomenon that likely warrants consideration in CRE adaptive ecosystem management. The vast majority of the BHA-affected channel lies below the seasonal lowest stage elevations of Lake Powell and the Colorado River channel downstream to the Paria River confluence. BHA was particularly prominent during summer in lentic, sand-dominated sections of the reservoir and channel floors, where aquatic macrophytic vegetation cover was most dense. Cobble bars and benthic sediments in higher velocity sections of the channel revealed some, but less extensive, BHA development. Minimal BHA occurrence in the tailwaters thalweg suggests that PAR limitation along with higher velocities and reduced macrophyte cover limits BHA development there. These results are supported by our field and lab BHA development experiments (see Objective 4). Depth of BHA ranged from >30 cm to unknown depths in most of our excavated soil pits.

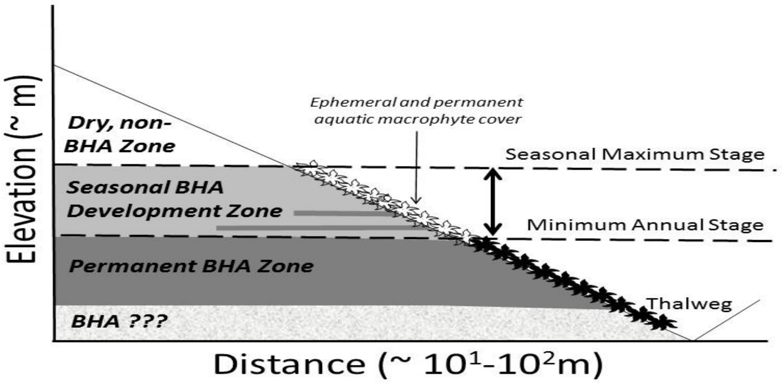

Figure 6. *Elevational cross section showing zones of benthic hyporheic anoxia (BHA) development in the Colorado River tailwaters section below Glen Canyon Dam, Arizona. Ephemeral aquatic macrophyte cover is symbolized by the open symbols in the Seasonal BHA Development Zone; permanent aquatic macrophyte cover is symbolized by the closed symbols in the Permanent BHA Zone (below the minimum annual river stage).*

Seasonally, the extent of BHA development along shorelines was greatest during early fall, dissipating somewhat during winter and springtime. This pattern occurred in both the reservoir and river, suggesting that BHA development tracks the increase or recession in inundation along the shoreline (Fig. 6). At its greatest extent in fall 2016, we estimate that the total area of BHA in the 25 km-long Glen Canyon tailwaters reach was 0.65 km_2. Due to difficulty in assessing the full depth of BHA sediment at each transect site, the volume of BHA-influenced sediment in the GCD tailwaters remains unknown. BHA was detected in the CRE downstream from the Paria River confluence ephemerally in fine sediment deposits containing high concentrations of organic matter. However, the presence of BHA at the two sites downstream from the Paria River confluence did not follow the same pattern as sites upstream. Instead, BHA expanded primarily following a spring time Paria River flood that deposited a substantial volume of fine sediment and organic matter, creating conditions suitable for BHA development. That BHA dissipated as the deposits quickly eroded away. Detection of BHA development at those two transects was complicated by the patchy, ephemeral distribution of sediment deposits and significant variation in tributary flows.

Farther downstream through Grand Canyon, BHA was rarely detected in the mainstream and only at a few low-velocity tributary mud deposits during several river trips in 2016 and 2017. The duration and extent of BHA development in the CRE in Grand Canyon is limited, but BHA is occasionally observed in low-gradient tributaries that deposit fine sediment or precipitate travertine and support benthic macrophytic vegetation during periods of extended base flow (e.g., the Little Colorado River and Kanab and Havasu Creeks; L.E. Stevens, personal observations). These rare occurrences of BHA development are in accord with our upstream observations and experiments, and support the hypothesis that naturally dynamic hydrographs normally limit BHA development.

Water and Sediment Geochemistry

BHA impacts on Colorado River water quality appear to be relatively minor at the scale of individual sampling points based on laboratory analyses of water column and BHA sediment sampling. The concentration of total P was slightly elevated during summer in BHA water samples as compared to non-BHA water samples.

Sediment analyses revealed no significant patterns regarding nitrate, total P, total C, or organic matter. However, taken at the scale of the entire tailwaters reach, below-detection-limit concentrations of these compounds may occur, a process that warrants further study. Hg was detected in sandy sediments at Lees Ferry; however, contrary to expectations, slightly greater concentrations of Hg were found in non-BHA sediments there. The elevated Hg concentrations found in non-BHA sediments at Lees Ferry exceed the EPA's maximum contaminant level (MCL) for drinking water (MCL = 0.002 mg/L), and non-BHA sediments contained greater concentrations than the World Health Organization's drinking water guidelines (WHO guideline = 0.006 mg/L). Higher levels of Hg in Lees Ferry sediments may reflect the legacy of long-term human use at this historic site (i.e., the Spencer mining operation, the historic trading post and settlement, and a marina formerly located there). Overall, these data indicate that benthic anoxia is not mobilizing Hg at readily detectable levels at the micro-site scale. Again, however, minor releases of Hg and U across the entire channel bed may be occurring and warrant monitoring. Our results appear to be consonant with the findings of Wildman et al. (2010) in Lake Powell, who reported minor seasonal releases of U from benthic sediments. However, low levels of geochemical activity within BHA sediments or at the sediment-water interface may result in cumulative, long-term, element-specific release or sequestration. In addition, toxic compounds may be released from mobilized BHA sediments during HFEs, a phenomenon also warranting further study.

Effects of BHA on Benthic Macroinvertebrates

Larval *Heptagenia* mayfly mortality was significantly greater in the presence of BHA sediment than in controls in our laboratory experiment, possibly due to toxicity or physical obstruction of respiration by the fine particulate organic BHA sediments. *Heptagenia* are common in cool water tributaries in Grand Canyon (Oberlin et al. 1999; Stevens et al. 2020b review), but the only water bodies supporting mayflies in the study area are off-channel, groundwater-fed backwater habitats, supporting the hypothesis that BHA sediments may contribute to embeddedness and hinder colonization of mayfly populations in the mainstream. *Heptagenia* larvae are flowing water species and are far more sensitive to poor water quality than are introduced amphipods or New Zealand mudsnails, which are abundant in the Glen Canyon reach below the dam on BHA-dominated sediments. No mayfly species are presently found in the mainstream of the Colorado River in Glen or Grand Canyons, an absence that is the focus of intensive federal concern and study (e.g., Kennedy et al. 2016). The high degree of mortality caused by BHA sediments may be one factor contributing to this absence, particularly in lower velocity waters where the extremely fine particle size of BHA sediment may physically obstruct respiration in mayfly gills, leading to mortality. Thus, the physical impacts of extensive organic floc and the impacts of low DO concentration may limit mayfly presence, rather than chemical properties of the BHA sediment. Additional bioassays can help determine which of several flow, sediment, and geochemical mechanisms affect different potential macroinvertebrate colonizers. A primary concern regarding BHA impacts on macroinvertebrates centers on the quality of the food base available to trout in the tailwaters reach.

The mainstream CRE macroinvertebrate assemblage in Glen and Grand Canyons is depauperate, but the specific mechanism(s) responsible remain under study. Kennedy et al. (2016) suggest that egg mortality due to daily hydropower flow fluctuations eliminate taxa that oviposit at the water's edge. Thus, egg mortality due to desiccation under fluctuating flows may reduce CRE macroinvertebrate diversity and abundance. Our study suggests there also may be negative impacts due to BHA development, at least in the tailwaters reach. With multiple mechanisms potentially hindering macro invertebrate assemblage development, the aquatic food base for trout in the tailwaters remains limited and likely cannot be substantially enhanced without significant changes to river ecosystem management.

Quagga mussels have become established in high densities (>3000 individuals/m^2 detected in our dredge samples in 2016) in the study area, and may greatly affect river ecosystem processes. These non-native bivalves are well-known for their ability to filter water, removing phytoplankton and decreasing organic turbidity. Increased density of closely-related invasive zebra mussel (*Dreissena polymorpha*) in the Great Lakes significantly reduce turbidity due to this biological filtering, affecting major ecosystem processes (Churchill et al. 2016; Lowe and Pillsbury 1995; Skubinna et al. 1995). In addition to increasing PAR availability, dreissenid mussels also excrete additional nutrients into aquatic systems, some of which may affect phosphate concentration and aquatic production.

This process may support additional benthic filamentous algal growth (e.g., *Cladophora*) beyond that expected in the absence of invasive mussels (Francoeur et al. 2017). The CRE tailwaters channel was dominated by *Cladophora* prior to 1995 (Stevens et al. 1997), and recovery of *Cladophora* may somewhat reduce the dominance of *Chara*, thereby reducing the extent of BHA. However, the impacts of quagga mussel invasion on this ecosystem remain highly speculative.

BHA Development and Management

Based on meso- and microcosm experiments, the rate of BHA development can be relatively rapid, occurring within 10 days. Our initial field experiment emphasized the importance of macrophytic vegetation to BHA development and suggested that minor stage fluctuations (0.5 m/day) only slightly limit BHA development. BHA development increases in the presence of productive and decaying aquatic macrophytic vegetation (i.e., *Chara*), warm water temperatures, and low DO concentration. We conceptually modeled this process to visually explain the causes of BHA development in the GCD tailwaters (Fig. 7). This model emphasizes the strongly bottom-up nature of physical conditions and dam operations that generate the template on which benthic macrophytic vegetation develops, and the influences of that vegetation on primary and secondary production, including the recreational trout fishery.

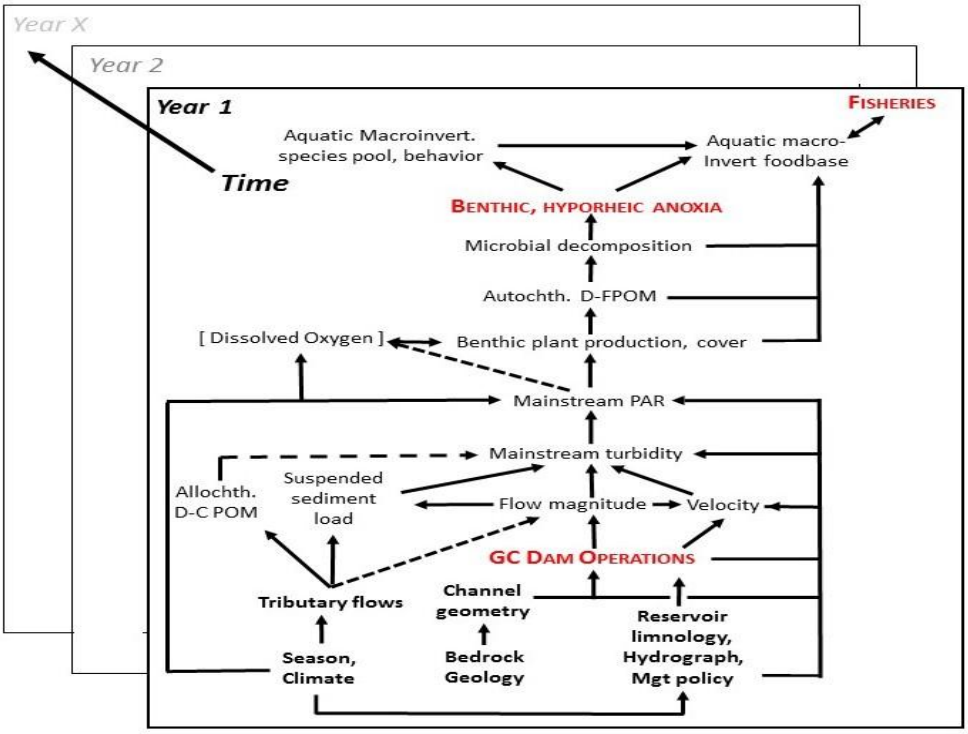

Figure7. *Conceptual model of BHA development through time. Bold text indicates stronger effects than non-bold text. Dashed lines indicate weaker effects than solid lines.Red text indicates key management concerns. Abbreviations: DO - dissolved oxygen; D-C POM - dissolved-to-coarse particulate organic matter; DS - downstream; PAR - photosynthetically active radiation.*

Prior to the mid-1990s, epiphytic algae were the functional foodbase for the Glen Canyon reach of the river ecosystem, with *Cladophora* formerly providing the substrate for ephiphyte growth, and the epiphytes supported benthic grazers (Chironomidae and Gammaridae; Stevens et al. 1997). *Chara* now dominates the reach and is known to influence aquatic ecological processes in lentic habitats in complex ways (Kufel and Kufel 2002; Torn et al. 2010); however, dominance and the impacts of *Chara* on regulated river ecology have not been widely reported. Nearly 40 species of *Chara* have been described around the world, and several species, such as *C. vulgaris*, are globally distributed. While nutrient uptake and mobilization from the benthos is considered to be relatively unimportant in most Characeae, several potential biogeochemical interactions may be of relevance in the CRE. *Chara* precipitates calcite during peak growth periods, a process that binds or sorbs P in calcite or benthic sediments. *Chara* may deliver dissolved O_2 into benthic substrata, enhancing nitrification, and preventing release of Fe-bound P from bed sediments. Physically, dense *Chara* beds can restrict suspension of fine sediments, and temporally-extended growth periods may increase the influence of *Chara* on nutrient dynamics into the non-growing season. In addition, *Chara* may decompose more slowly than vascular aquatic taxa, extending the duration of its nutrient influences in temperate latitudes. While Kufel and Kufel (2002) concluded that dense *Chara* stands function as nutrient traps in shallow lakes, *Chara* also has been widely reported to support N-fixing cyanobacteria. Due to its ability to develop dense stands in lentic settings, it occasionally has been the focus of control efforts (e.g., Guha 1995). P is generally regarded to be a limiting nutrient in the GCD tailwaters. If substantial N-fixation by *Chara* epiphytes is occurring in the Glen Canyon tailwaters, *Chara* may be influencing N:P ratios, the development and persistence of BHA, as well as aquatic macroinvertebrate composition and structure. Research on epiphyte distribution and composition in the *Chara*- and *Zanichellia*-dominated macrophyte beds, and the changing roles of these macrophyte taxa in reservoir-to-river nutrient dynamics are warranted to better understand how the contemporary macrophyte assemblage influences the riverine foodbase.

Our laboratory microcosm experiment clarified the roles of *Chara* (and possibly other aquatic macrophyte species), DO concentration, and water temperature as key drivers in BHA development in Lake Powell and the Glen Canyon tailwaters. Dense macrophyte beds in the reservoir and the Glen Canyon reach produce abundant decaying vegetation, and DO concentration is low due to hypolimnetic releases and glide-dominated structure of the reach, which abruptly changes at the first major riffle just downstream from the Paria River confluence. However, water temperature in the Glen Canyon reach remains low and constant, generally varying from 8-12°C over the course of the year (Wright et al. 2009).

River ecosystem restoration through adaptive management has included much discussion of installation of temperature control devices on the dam to more closely mimic pre-dam temperature conditions. Our findings support those of Walters et al. (2000) and contemporary concerns that increased warming will lead to undesired ecosystem impacts, including increased BHA development and enhanced non-native warm water fish populations. Downscaling the impact of any of these three factors (i.e., decreasing water temperature, removing *Chara* biomass, or increasing aeration), or tandem combinations thereof, likely will retard and reduce BHA development; however, no single treatment, and likely no minor shift in combined treatments of these three factors will greatly reduce BHA from the tailwaters reach. High levels of discharge and scour apparently limited BHA development in the Glen Canyon reach during pre-dam time and from 1963-1991 under maximum hydropeaking flows. Since regulation of dam releases to maximize fine sediment mass balance in 1991, BHA has gradually developed, and now dominates the reach, despite repeated interannual high flow experiments (flows of 1000-1275 m^3/s). These flow experiments are generated by power plant capacity releases coupled with jet tube releases but are only about half the mean annual pre-dam flow (2500 m^3/s; Topping et al. 2003), and are insufficient to scour the bed, move cobbles, and reduce BHA. While significantly higher flows are not politically practicable under the present flow management regime (U.S. Bureau of Reclamation 2016), anomalous unplanned higher flows, such as occurred from 1983-1986 rarely may occur but should be studied carefully in relation to their effects on BHA development and other river ecosystem processes.

Conclusions

The spatial extent of BHA development in the GCD tailwaters exceeded our original expectations, with approximately 0.65 km^2 (approximately 20%) of the benthic CRE between the dam and the Paria River confluence. Currently BHA is limited to fine sediment deposits in Lake Powell, but it occurs widely in the tail waters reach. The phenomenon appears to be yet another unanticipated consequence of river regulation, arising from reduced daily flow fluctuations after curtailment of highly varying hydro peaking flows in 1991. BHA development rate is controlled by constraints in flow variation, high water clarity, low DO concentration, the presence of abundant growth and decay of macrophytic vegetation, and can be accelerated under warmer water temperatures. Anoxia does not greatly alter local water column geochemistry, but may influence it at the reach scale. BHA was experimentally demonstrated to be detrimental to sensitive larval macroinvertebrates, such as a common lotic mayfly species, possibly though physical impacts on respiration. Consequently, BHA may limit potential aquatic macroinvertebrate assemblage and food base development in the tail waters reach. BHA does not presently occur to any great extent downstream in Grand Canyon, but could begin to develop if suspended sediment load decreases, under prolonged warmer water flows, and during prolonged periods of water clarity that foster increased macrophyte (particularly *Chara*) growth in the mainstream channel floor. While mitigation of any of those controlling factors may limit BHA development, only much more intensive scour than presently occurs under experimental high flows is likely to significantly reduce BHA. However, such scour may send a wave of BHA contaminants downstream that could detrimentally influence the native fisheries in Grand Canyon. Therefore, we recommend that river managers consider conducting well-controlled, well-replicated channel floor disturbance experiments in the Glen Canyon reach before undertaking reach-wide experiments to mitigate BHA. Our results reveal yet another complex, unanticipated consequence of flow regulation on the CRE influenced by GCD. Our study is in accord with the premises of Schmidt et al. (1998) that the roles of science are to inform ecosystem stewards of ecological characteristics and processes, potential management consequences, and opportunities to plan and test options in adaptive ecosystem management.

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