Filamentous Algae Monitoring Program:
Cacapon River Microcosm Study 2015

Report to the West Virginia Department of Environmental Protection,

Division of Water and Waste Management

by

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Commission on the Potomac River Basin

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Executive Summary

The Interstate Commission on the Potomac River Basin (ICPRB) augmented its 2015 filamentous algae survey for West Virginia Department of Environmental Quality (WVDEP) with an investigation of the biological impacts of excessive primary production in the Cacapon River (Cacapon Microcosm Study). Continuous monitoring data and stream macroinvertebrate samples were collected in addition to filamentous algae abundance measurements, water chemistry samples, and site observations. The summer of 2015 was unusual in that the extensive blooms of nuisance filamentous green algae typically found at one Cacapon River site did not materialize, and beds of underwater macrophytes became particularly abundant. The results suggest that over-abundances of any primary producer, not specifically nuisance filamentous green algae, can impair recreational and aquatic life uses of West Virginia streams and rivers. The connection to nutrient pollution, and especially phosphorus, may not be evident in summer due to rapid assimilation by abundant macrophytes.

HOBO dissolved oxygen loggers (U26-001) and HOBO temperature and light data Pendant loggers (UA-002-xx) were deployed to measure dissolved oxygen, temperature, and light irradiance for a period of five months at four sites, producing roughly 2000 measurements per logger. Loggers were located upstream, in, and downstream of the highly productive Cacapon River site. From the continuous data, ICPRB was able to accurately record diel variations and maximum/minimum levels for all logger parameters. ICPRB investigated macroinvertebrate community composition inside and outside of the high primary production site, and examined changes in community structure and composition between sites and over time inside the highly productive site. Using an R-package developed by ICPRB, 72 macroinvertebrate metrics were calculated and analyzed to investigate pollution tolerance, taxonomic composition, and functional feeding and behavior groups.

The Cacapon Microcosm Study had four major findings:

1. Nutrient signals, specifically phosphorus, can be lost during the summer season due to the rapid assimilation of the nutrients in dense macrophyte communities.
2. Dense macrophyte communities significantly affect dissolved oxygen concentrations. Sites where excessive macrophyte density exist can regularly experience dissolved oxygen concentration below WVDEP's instantaneous criterion of 5 mg/liter for aquatic life use.
3. Diel variability of dissolved oxygen may be acting as a chronic stressor at sites with dense macrophyte communities.
4. Compared to Cacapon River sites with few or no macrophytes, macroinvertebrate assemblages in dense macrophyte regions had higher percentages of pollution tolerant taxa, lower percentages of pollution sensitive taxa, and more ephiphitic taxa.

The methods used in this study, continuous monitoring of chemical and physical parameters plus macroinvertebrate sampling inside and outside regions with high primary production, show that excessive macrophyte production in West Virginia streams and rivers due to excessive nutrient loading does have measureable, adverse, effects on stream macroinvertebrates. Furthermore, technology based methods used in this study provide economical alternatives to field crew based efforts.

Introduction

Nutrient pollution is a well-known problem in freshwater streams and rivers that often manifests as over production of photosynthetic organisms (USEPA 2007) and nuisance filamentous green algae (NFGA) and their possible impacts on recreational and aquatic life designated uses has been gaining attention in the Mid-Atlantic region of the United States. Despite having defined environmental criteria in place, many states in this region have found themselves without sufficient ability to link NFGA quantitatively to excessive nutrient loading or impairment of aquatic life and recreational designated uses.

Correlations between excess nutrients and reduced biological integrity in waterways are reported in a wide range of research (Tesoriero et al. 2009). Field work, model building exercises, and lab-based studies support the claim that an abundance of nutrients will promote excess primary production (Biggs 2000, Rier and Stevenson 2006), which ultimately may lead to detrimental secondary effects within a system. In the Illinois River Watershed, poultry house density increased in-stream phosphorus (P) concentrations, which promoted significant NFGA cover (Stevenson et al. 2012). The increased productivity ultimately led to diel dissolved oxygen (DO) swings that reached levels believed to be harmful to stream biodiversity. The Lost River of West Virginia saw similar trends in NFGA abundance due to high P concentrations in poultry house runoff water. Phosphorus concentrations in the poultry house runoff water were nearly 10 times higher than runoff concentrations from forested land. Downstream of the poultry operations, low instream P concentrations and a high abundance of aquatic algae suggested that excess P was easily assimilated by algae and other aquatic plants in the Lost River and was responsible for the excessive algal growth (Elrashidi et al. 2008).

Phosphorous pollution can stem from a variety of non-agricultural sources, including residential and commercial (non-agricultural) fertilizer use and sewage outfalls. Summers (2008) reported that golf courses and sewage outfalls were significant sources of the nutrients which stimulated NFGA blooms in the Greenbrier River of West Virginia. The sewage outfall in particular contributed 80 - 90% of the phosphorous loading in a tributary of the Greenbrier River, while the golf courses contributed about 3%. Interestingly, NFGA blooms did not occur in many areas in the Greenbrier basin where relatively high P concentrations should have favored excessive algae growth. The West Virginia Department of Environmental Protection (WVDEP) determined that multiple non-nutrient related water chemistry variables (e.g. hardness, alkalinity, and pH) were actually controlling to a large extent the bioavailability of P. The importance of these confounding factors cannot be overstated when investigating freshwater algae and its relation to stream nutrient concentrations.

Despite the influence of confounding factors, observations made in the Illinois River watershed, Lost River, and Greenbrier River point to a connection between excessive nutrient loading and over-production of primary producers, especially NFGA. It is more difficult to relate nutrient pollution to the secondary biological effects of over-production. Abundant primary producers can cause large diel swings in pH and dissolved oxygen (DO) during periods of rapid photosynthesis, as well as alter habitat composition, all of which stress ecosystem health. During daylight hours, primary production can increase DO levels and decrease CO_2 concentrations in the water column. Decreased CO_2 concentrations in turn raise the pH of the system. At night, respiration by primary producers and animals consumes DO in the water column and produces CO_2, which lowers pH levels. These swings in DO and pH can cause acute stress and in extreme cases can result in large-scale, high profile fish kills (Stevenson et al. 2012). Less extreme diel swings in pH and DO can cause sub-lethal chronic stress that may differentially lower the fitness of various organisms in a community over longer periods and ultimately change community structure (Winter et al. 1996).

Nuisance filamentous green algae (NFGA) blooms can affect streams and rivers by altering physical habitat. Macrophytes and invertebrates that require holding space on rocky substrates experience less available habitat in stream reaches where benthic structure becomes dominated by a dense NFGA bloom (Dudley et al. 1986). By excluding other macrophytes or periphytic algae, NFGA can decrease food availability to some macroinvertebrate grazers (Dudley et al. 1986). Conversely, some primary consumers (grazers) benefit from the abundant food provided by NFGA blooms, as can epiphytes, which further increases production in the system (Dudley et al. 1986). Alterations in primary production and primary consumption cascade through trophic levels non-linearly, and tend to alter vertebrate community composition. In some cases, abundant food sources promote increased growth rates and allow macroinvertebrate prey taxa to attain sizes too large for their macroinvertebrate predators to handle. Species interactions must also be taken into account since algae is a key food source for many grazers. For example, water column nutrients may correspond more to grazing invertebrates than macrophyte biomass if grazers exert enough pressure to limit macrophyte biomass (Bourassa and Cattaneo 1998).

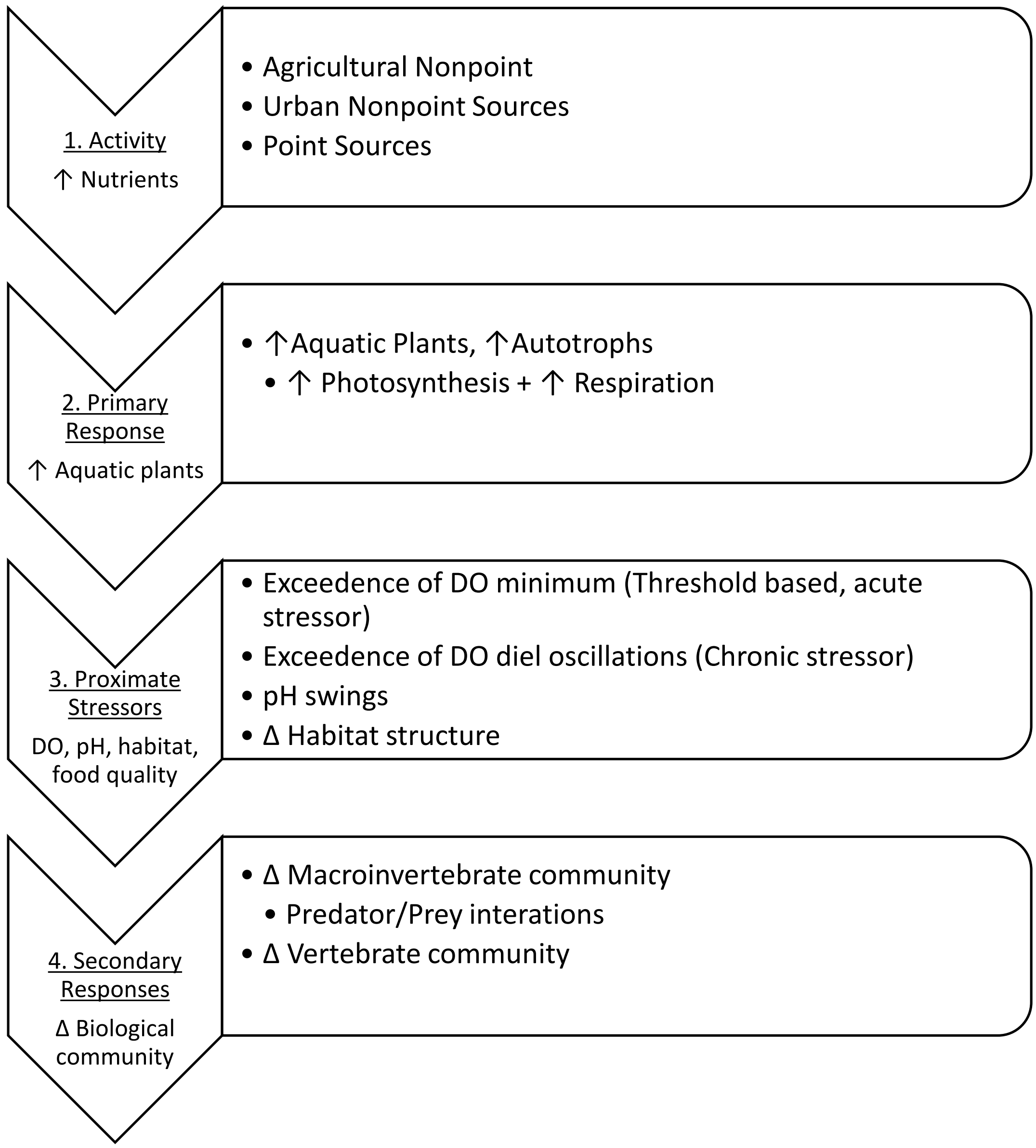


Figure 1. Conceptual model outlining the levels of detectable response variables from an influx of nutrients. Level 1 represents detection of excess nutrients. Level 2 represents detection of excessive primary production due to nutrient loading, the primary response. Level 3 represents detection of changes in environmental structure due to the effects of excessive primary production. Level 4 represents the secondary response of an observed shift in aquatic life (fauna) structure.

State Assessment

In the Mid-Atlantic region, West Virginia and Pennsylvania have taken important but different steps to identify the causes of, and adverse ecosystem responses to, excessive primary production in streams and rivers. After excessive NFGA was reported in the Greenbrier and Tygart Rivers, WVDEP identified phosphorus loading as the most important factor promoting algal blooms in many West Virginia rivers. From these findings, West Virginia developed criteria for the amount of filamentous green algae cover that impairs recreational use of a waterway. Although excessive concentrations of nutrients are not detected very often inside regions of NFGA blooms, NFGA blooms themselves are acting as a signal response for nutrient pollution from upstream and possibly groundwater sources. West Virginia focused their efforts on the primary response variable (increased aquatic plant abundance) rather than instream nutrient concentrations to identify waters needing nutrient reductions. Pennsylvania DEP takes a different approach and, after an initial nutrient screening, deploys continuous monitoring loggers for dissolved oxygen and implements benthic macroinvertebrate sampling. PADEP focuses on secondary responses to excessive nutrients (diel DO swings, aquatic life impacts) rather than the presence of abundant NFGA or aquatic plants in a particular reach (Figure 2).

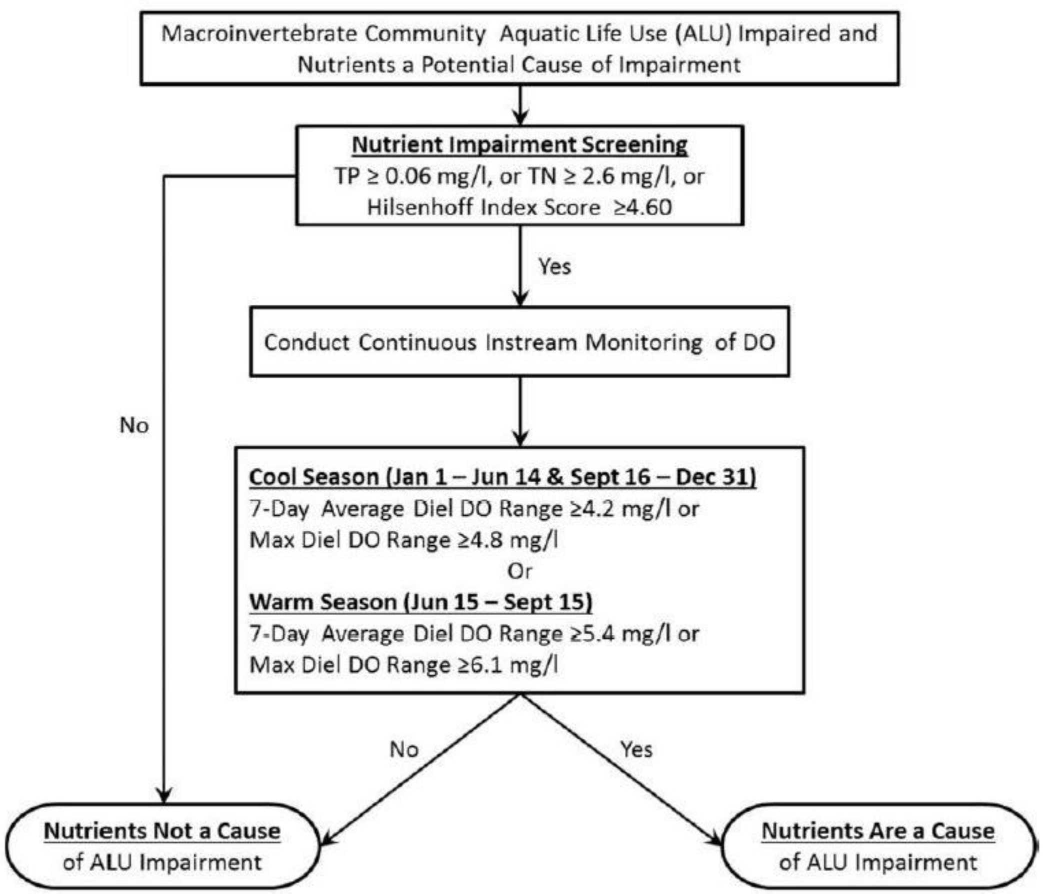


Figure 2 Flow chart of Pennsylvania's nutrient impact assessment protocol. (PADEP Development of Nutrient Impact Assessment Protocol for Identifying Nutrients as a Cause of Aquatic Life Use Impairment in Pennsylvania Wadeable Streams, 2015)

ICPRB Study

The Interstate Commission on the Potomac River Basin (ICPRB) has assisted WVDEP in identifying recreationally impaired reaches of the Cacapon and South Branch Potomac rivers since 2012. The Commission collects water samples, measures NFGA cover, and evaluates ecological condition at 14 sites in these rivers on a biweekly basis during the summer growing season. Information on the WVDEP filamentous algae monitoring program, including the Standard Operating Procedures for algae sampling and water chemistry, can be found on-line at:

http://www.dep.wv.gov/WWE/Programs/wqs/Pages/FilamentousAlgaeinWestVirginia.aspx

The Cacapon microcosm study supplements the filamentous green algae surveys with a more detailed investigation of water quality and biological conditions in an area of dense primary production. The specific objectives of this study are: (1) investigate diel changes in dissolved oxygen concentrations as they relate to the level of primary production, (2) investigate macroinvertebrate benthic community structure at highly vegetated and sparsely vegetated sites and (3) apply PADEP's nutrient impact assessment protocols (Figure 2) to the Cacapon River to determine if an area that routinely fails the West Virginia recreational use criteria due to excess NFGA growth also fails dissolved oxygen criteria and exhibits measureable changes in stream macroinvertebrate communities. We hypothesized that sites with excessive primary production would experience less stable dissolved oxygen levels, with wider diel swings in concentration, and would be more likely to fail aquatic life use criteria. Furthermore, we hypothesized that if DO swings were noticeably large at a site due to abundant primary production, we would also see a shift towards more stress-tolerant microbenthic community composition.

Methods

The Cacapon Microcosm Study consisted of (1) a continuous remote logger component to track diel changes in key environmental conditions for a duration of five months, and (2) a multi-habitat macroinvertebrate benthic sampling component to compare benthic community structure in high production habitats to more stable low production settings. Two monitoring sites on the Cacapon River, CA_WRDS and CA_D_CPBRG, were used in this study as control sites because of their historical absence of dense algal and submerged aquatic vegetation (SAV) blooms. A monitoring site between these two, CA_RMRCK, was selected as the test site because it is located in a river reach with extensive, recurring, well-documented NFGA blooms. CA_YLWSPR was selected as a nearby upstream-of-macrophyte-production site to compare benthic communities between it and CA_RMRCK (Figure 1). Increased primary production, typical of the CA_RMRCK site, often starts within 500m downstream of the CA_YLWSPR site. ICPRB personnel included Gordon Selckmann (GMS, Aquatic Ecologist), Charles A. Dean (CAD, Natural Resources Intern), and Zachary Smith (ZS, Invertebrate Data Analyst).

Region Details

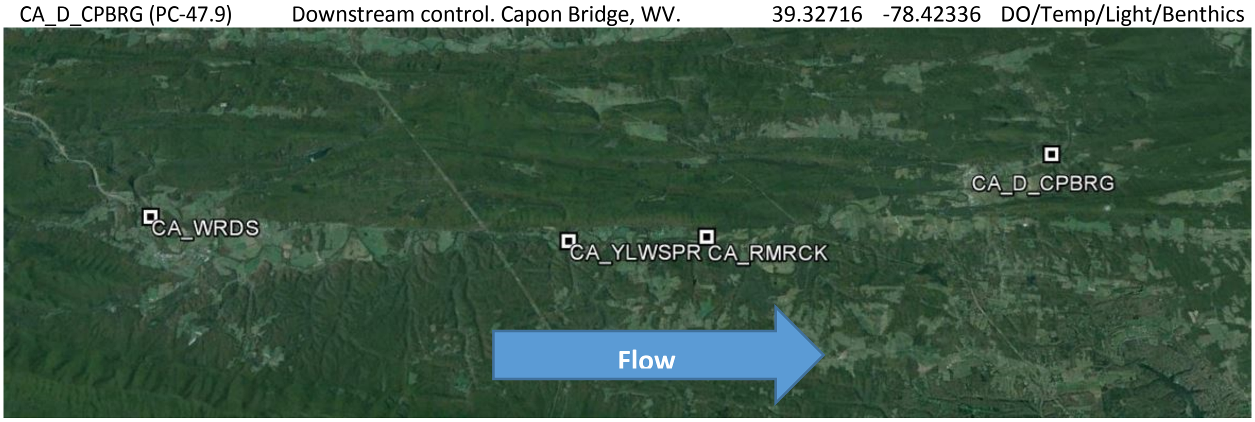
The Cacapon River Watershed (HUC #02070003) is the fourth largest tributary to the Potomac River, draining nearly 1,760 km^2 of the eastern panhandle of West Virginia. The river is contained entirely within the Ridge and Valley Ecoregion (Level III) of the Appalachian Highlands. The three major river segments that make up the Cacapon drainage are the Lost River, the Cacapon River mainstem, and the North River. The Lost River and the Cacapon mainstem are the same lotic system, broken up by the Lost River sinking into a karst seep and reemerging as the Cacapon River in Sandy Ridge, WV. Our study reach was confined to between Sandy Ridge, WV and Capon Bridge, WV, a reach that falls entirely within the Northern Shale Valley Ecoregion (Level IV). The geology of this reach is comprised of resistant shale and sandstone to form the ridges while limestone, dolomite, and calcareous shale underlie the valleys.

The Cacapon River watershed is largely forested, with a small but increasing agricultural and residential land use densities (WVDEP 305b report, 2002). The watershed is 84% forested and dominated by coniferous and deciduous canopy cover. Agricultural development is focused primarily in the head waters of the Lost River where some of the highest densities of poultry farms in the United States are located. The amounts of reported forest area and developed land have changed in favor of greater development in recent years due to the region's proximity to major metropolitan hubs such as Washington D.C. and Baltimore, Maryland.

The four sites selected for this study (Figure 1) were targeted by ICPRB based on recent information gathered at the sites over four years of observation (WVDEP Filamentous Algae Report 2012-2015). The 30.8 km reach of the upper-Cacapon River, from the head waters of the Cacapon to below Capon Springs township, was selected as an ideal microcosm study location due to the relatively short distance between sampling locations, predictability of primary production within reaches, and few significant stream inputs to influence sites water chemistry properties. There is a general trend of decreasing hardness, alkalinity, calcium, magnesium, and nitrogen concentrations with increasing river km away from the genesis of the Cacapon River in Sandy Springs, WV.

Figure 3. Map describing the location and data collected at each of the four sites in the upper Cacapon River.

Site Name	Site Description	Latitude	Longitude	Data Collected
CA_WRDS (PC-78.7)	Negative Control. Farm in Wardensville, WV.	39.07861	-78.61134	DO/Temp/Light/Benthics
CA_YLWSPR (PC-64.1)	Upstream close benthic control @ Rt. 59 bridge	39.18281	-78.50597	Temp/Light/Benthics
CA_RMRCK (PC-60.3)	Positive NFGA bloom site @ Capon River Rd.	39.21969	-78.47605	DO/Temp/Light/Benthics
CA_D_CPBRG (PC-47.9)	Downstream control. Capon Bridge, WV.	39.32716	-78.42336	DO/Temp/Light/Benthics



Continuous Remote Loggers

HOBO dissolved oxygen loggers (U26-001) and Pendants Temp/Light loggers (UA-002-xx) were assembled and calibrated on June 1, 2015. New dissolved oxygen sensor caps (HOBO, U26-RDOB-1) were installed for the 2015 season. A single DO cap has a life span of 6 months from the moment of attachment, which exceeded the duration of the in-water sampling period of any DO sonde in this study. Dissolved oxygen sensors were calibrated for barometric pressure and underwent a 2 step DO saturation calibration process, a 100% saturation and a 0% saturation, before deployment at a site. Temperature and light loggers were activated and monitored for a 3 day period prior to deployment in river to ensure proper function and accuracy.

On June 13th, 2015, three (3) HOBO dissolved oxygen loggers and eight (8) HOBO Pendant Temp/Light loggers were deployed at four sites (Figure 2a-d). The Cacapon at Yellow Springs site (CA_YLWSPR) did not receive a DO logger due to the site's purpose as a benthic community comparison site and budgetary limitations; however, two (2) HOBO Pendants were deployed to compare temperature and light data between sites. The HOBO dissolved oxygen logger recorded dissolved oxygen and water temperature in 10 minute intervals. HOBO Pendant temp and light loggers were used to record water temperature and light irradiance, also recording in 10 minute intervals. For every HOBO dissolved oxygen logger deployed, there were two Pendant loggers deployed above and below the DO sonde within the same thalweg. The multiple temperature loggers within the same thalweg were a means to verify the accuracy of the instruments against each other. A change in voltage or local disturbance would presumably manifest as greater drift in sensor readout when compared to the other sensors within the same thalweg. All loggers were deployed into the main thalweg at a depth of 0.75 m or greater to ensure they would not be dewatered during late summer low flows. All loggers stopped recording November 1st 12:01 AM.

Figure 4a. CA_WRDS site with the locations of the U26-002 (Temp/Light) and U26-001 (DO) loggers. The yellow box represents the sampling area where benthic macroinvertebrates were collected.

Figure 4b. CA_YLWSPR site with the locations of the U26-002 (Temp/Light) loggers. The yellow box represents the sampling area where benthic macroinvertebrates were collected.

Figure 4c. CA_RMRCK site with the locations of the U26-002 (Temp/Light) and U26-001 (DO) loggers. The yellow box the sampling area where benthic macroinvertebrates were collected.

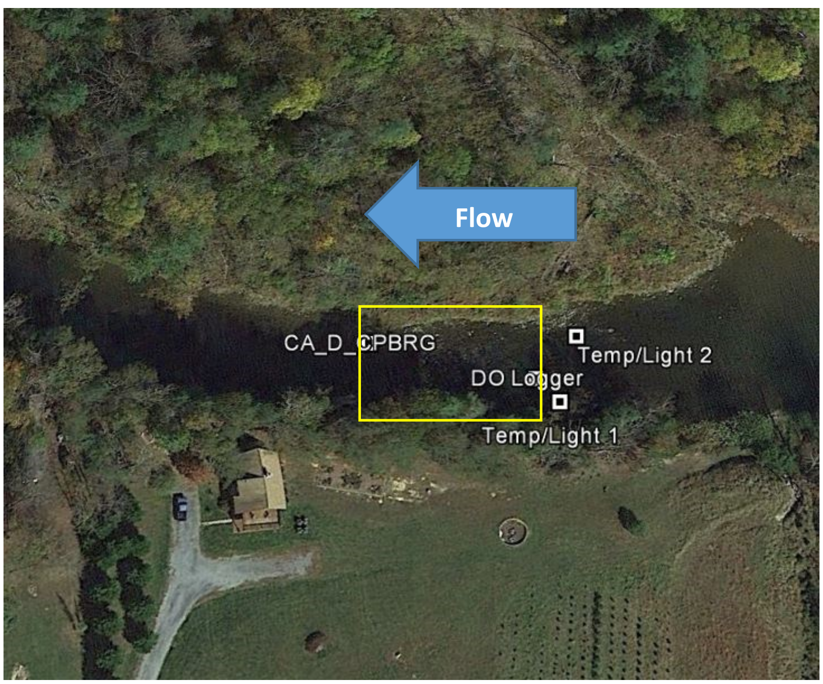


Figure 4d. CA_D_CPBRG site with the locations of the U26-002 (Temp/Light) and U26-001(DO) loggers. The yellow box represents the sampling area where benthic macroinvertebrates were collected.

Continuous monitoring data were downloaded in the field onto a HOBO waterproof shuttle (U-DTW-1) and uploaded to a Windows 7 platform PC running HOBOware software (ver. 3-7-5). Data were collected from each of the dissolved oxygen loggers three times between June and November. Data collected from the loggers at each site were stitched together to form a 5 month final continuous dataset. Within the 5 month period nearly 21,000 events containing both temperature and dissolved oxygen data points were produced from each of the three DO loggers.

HOBO Pendant (temperature and light) loggers set around the DO loggers were left to run continuously for the full duration of the sampling season. Analysis of data after the sensors were recovered revealed reliable temperature readings but compromised light readings. Siltation and benthic macroflora caused unreliable light readouts and therefore were not considered for analysis in this study.

The HOBO DO loggers logged temperature and dissolved oxygen from June 13, 2015 to November 1, 2015. It was decided, post plotting, to limit this analysis to the PADEP defined warm season (June 15 – August 15). The warm season showed the most regular diel patterns and was not effected by rapidly changing water temperatures experienced in the spring and fall. ICPRB used the dissolved oxygen (DO) criteria defined by WVDEP (5 mg/l minimum threshold) and by PADEP (weekly average range and single day range) when analyzing the continuous monitoring data from the upper Cacapon River (Table 1). DO exceedances for each site were reported.

Table 1. Table outlining the dissolved oxygen thresholds investigated for the Cacapon River continuous monitoring data.

Agency	**Dissolved Oxygen Criteria**	**Description**
WVDEP	<5 mg/L	Instantaneous threshold. If dissolved oxygen drops below threshold at any point in time, the river is defined as an impaired waterway.
PADEP	7-Day Average DO Range ≥ 5.4 mg/L	Multi day threshold. If the average diel variation of dissolved oxygen exceeds 5.4 mg/l over the period of seven days, there is sufficient evidence that nutrients are the probable cause of the loss of aquatic life usage (ALU)
PADEP	Single-day DO Range ≥ 6.1 mg/L	Single day threshold. If in a single day a site experiences a diel dissolved oxygen swing that exceeds 6.1 mg/L there is sufficient evidence that nutrients are the probable cause of the loss of the aquatic life usage (ALU).

Macroinvertebrate Collection Protocols

The WVDEP D-Net, multi-habitat approach for low gradient streams was used to collect benthic macroinvertebrates at the four sites in the Cacapon River. Selection of the multi-habitat/ low gradient approach was based on two key factors:

1) Sites with historically dense blooms of filamentous green algae (NFGA) often are located where current velocity is slow due to seasonal low flow, the physical slowing of currents due to dense algal beds (positive feedback loop), broadening of thalweg, etc., and
2) Changes in microhabitat benthic community relative to a localized disturbance from increased primary production and habitat alteration.

Reduced flows and physical barriers make dense algae and SAV bloom locations similar to wetlands and, therefore, require similar protocols. This study should be considered a "special project" and the data used with the understanding that this type of sampling is considered by WVDEP to be non-comparable to the majority of other samples taken by the WV Watershed Assessment Branch (WVWAB). Specifically, the data collected in this study should not be used with the West Virginia Stream Condition Index (WVSCI).

A field site assessment form (Appendix 2) at each sampling event was completed to identify microhabitat composition within a 100m reach. Reaches that were defined in the spring were revisited throughout the 2015 season, with care to not resample previously disturbed habitat while still reflecting the microhabitat composition within the 100m grid. Sampled microhabitats were defined as cobble, snags, vegetated banks, sand, SAV, and NFGA. NFGA and SAV were separated into their own microhabitats classes in order to represent the role of each habitat. A 20-jab sampling effort was proportionally divided among microhabitats based on the preliminary field site assessment. If a microhabitat was rare (< 5%), it was not considered for the 20-jab effort. A "jab" was an aggressive thrust and sweep of the net through an area vigorously kicked (0.5 -1m) to dislodge organisms from the substrate.

The 20 jabs of a single site were combined into a two gallon Nalgene container. Care was taken to remove invertebrates from the interior of the purse as well as from captured organic matter before being placed into 95% EtOH. Since all organic matter was impossible to remove in the field, it was assumed that 95% EtOH would dilute to the correct preservation concentration of roughly 70% EtOH. A final sort of the organic material and invertebrates was completed in a lab setting and specimen containers topped off with fresh alcohol to ensure proper preservation until the invertebrates could be identified by WVDEP. For a more detailed description of WVDEP multihabitat sampling protocols and proper preservation of collected organisms, see WVDEP (2011, V0.1 SOP. Chapter II. Benthic Macroinvertebrate Collection Protocols).

Collected macroinvertebrates were delivered to WVDEP for sorting, genus level identification, and enumeration. Due to time constraints, WVDEP used Watershed Assessment Associates (NY) to enumerate the ICPRB collections. Gross samples were gridded and sorted to a 200 organism count. Identified and enumerated macroinvertebrate data was delivered to ICPRB on March 1, 2016.

The "BIBI" package (R-Statistical Software), recently developed by ICPRB, was used to calculate 72 benthic macroinverterbate metrics. Data were pooled by site so that seasonal changes in community composition and instar development could examined. A nonparmetric Kruskal-Wallis test and post hoc Dunn's test were used to compare the metric values between sampling locations. The results were summarized into a table using the *kd_table* function from the BIBI package. A subsample of metrics were selected for further analysis if the nonparametric tests showed significant differences between sampling locations (alpha = 0.05).

Results

Algae Summer Abundance 2015

The frequency and intensity of nuisance filamentous green algae was far less in the summer of 2015 than in previous years. Instead, excessive primary production in the form of SAV occurred where NFGA blooms historically occurred. Detailed algal abundance measurements, qualitative site observations, water quality, and narrative descriptions of NFGA and SAV growth can be found in the ICPRB West Virginia Filamentous Algae 2015 report (under review). The CA_WRDS site, as expected from previous year observations, did not manifest any NFGA in 2015 and instead produced abundant periphyton. CA_YLWSPR also did not manifest NFGA; nor did it manifest SAV, cyanobacteria, or periphyton. The test site, CA_RMRCK, did not produce NFGA coverage greater than 22% in 2015. The NFGA began to manifest on a shallow gravel bar in early summer, but appeared to be washed away by pulses of early summer high flow. NFGA coverage did not appear at densities greater than 10% for the remainder of 2015. In June, submerged aquatic vegetation (*Hydrilla sp.* and *Valensinaria sp.*) became established in the main thalweg of the CA_RMRCK site and expanded outward towards both banks. SAV coverage estimates for transects at the CA_RMRCK site were around 90% and filled a longitudinal reach roughly 5km long. The CA_D_CPBRG site did not manifest significant NFGA or SAV in 2015. A longitudinal survey conducted from canoes reported dense SAV beds roughly 1.5KM upstream of the CA_D_CPBRG site, however, the loggers and macroinvertebrates at CA_D_CPBRG showed no evidence of excess primary production impacts.

Nutrient Screening 2015

ICPRB evaluated the Cacapon River 2015 nutrient data using PADEP Nutrient Impact Assessment Protocol thresholds, which screen for excessive water column nitrogen and phosphorus (i.e., TP > 0.6 mg/l, TN > 2.6 mg/l). There were four total phosphorus (TP) exceedances and no total nitrogen (TN) exceedances at the four Cacapon sites (Table 3). The phosphorus exceedances occurred on June 3 at all four sites, during the spring and prior to the presence of abundant macroflora. Phosphorus and nitrogen concentrations increased with distance downstream from CA_WRDS. Phosphorus concentrations had dropped by June 17, and stabilized around 0.04 mg/L for the remainder of 2015. Nitrogen levels fluctuated during the summer, possibly reflecting the influence of storm events, but were consistently low in August and September. The stabilization of both phosphorus and nitrogen in the water column suggest that both nutrients are being taken up by primary producers in this river system.

Table 2. Table listing all nutrient exceedances (TN and TP) observed at CA_WRDS, CA_YLWSPR, CA_RMRCK, and CA_D_CPBRG as outlined by PADEP Nutrient Impact Assessment Protocol.

Site	Date	Time	Water Temperature	pH	DO	TN	TP
CA_WRDS	6/3/2015	16:25	16.54	7.66	9.6	1.36	**0.114**
CA_YLWSPR	6/3/2015	15:30	17.03	7.61	8.8	1.278	**0.156**
CA_RMRCK	6/3/2015	15:00	17.38	7.69	8.57	1.402	**0.166**
CA_D_CPBRG	6/3/2015	13:00	18.38	7.89	8.56	1.518	**0.217**

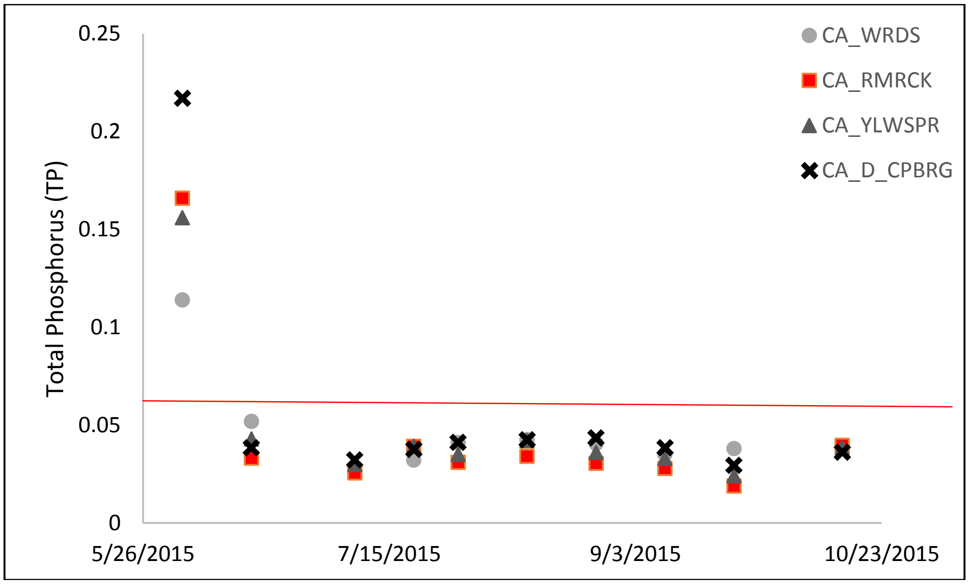


Figure 5. Total water column phosphorus (TP) by date at CA_WRDS, CA_YLWSPR, CA_RMRCK, CA_D_CPBRG. PADEP suggested threshold for total phosphorus (TP ≥ 0.06 mg/L) is depicted by a red line.

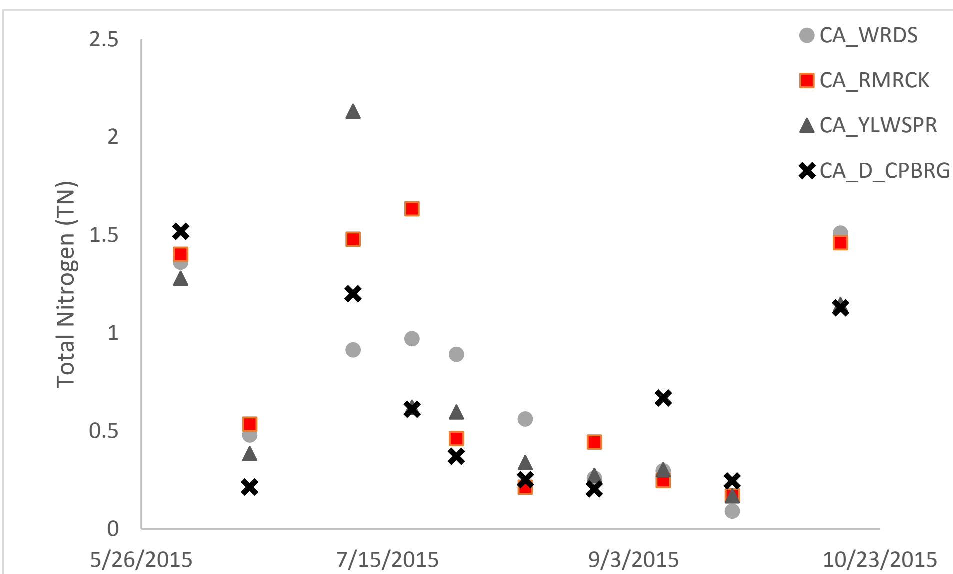


Figure 6. Total water column nitrogen (TN) by date at CA_WRDS, CA_YLWSPR, CA_RMRCK, CA_D_CPBRG. All total nitrogen measurements fell below PADEP defined threshold of TN ≥ 2.6 mg/L

Dissolved Oxygen

The dissolved oxygen loggers at CA_WRDS (Figure 3a), CA_RMRCK (Figure 3b), CA_D_CPBRG (Figure 3c) allowed ICPRB to observe in high resolution the diel oscillations that occur at each site. ICPRB used three different thresholds to define dissolved oxygen impairment in the Cacapon River (Table 2). At no time did the upstream control site, CA_WRDS, violate the defined PADEP thresholds or WVDEP criteria for dissolved oxygen. Following WVDEP's DO threshold requirement which states that a single exceedance (DO concentration below 5 mg/L) impairs the waterway, both CA_RMRCK and CA_D_CPBRG are impaired sites. CA_RMRCK was impaired 54 of 74 analyzed days (72.9% of warm season) while CA_D_CPBRG was impaired 16 of 90 days (17.7% of warm season). Differences in total analyzed days is due to anomalies from disturbance in the continuous data.

Pennsylvania DEQ uses two dissolved oxygen calculations to define impairment: a diel change greater than 6.1 mg/l on a single day and a 7-day average diel change greater than 5.4 mg/l. During the summer, CA_WRDS did not experience a change in DO greater than 6.1 mg/L on any single day, nor did it exceed an average change greater than 5.4 mg/L, therefore CA_WRDS by PADEP standards would not be considered an impaired waterway. CA_RMRCK was impaired for 62 single days out of 70 days (88.8% of the warm season) and CA_D_CPBRG was impaired 1 day out of 88 (1.1% of the warm season). With respect to PADEP's 7-day average threshold, CA_RMRCK was impaired for 100% of the warm season and CA_D_CPBRG was not impaired (0% of the warm season).

Table 2. Percent of days deemed impaired by WVDEP and PADEP's dissolved oxygen thresholds. Partial days and days that experienced disturbance were removed from analysis.

	# of Days with DO Exceedances (Warm Season) in 2015		
Threshold	**WVDEP DO <5 mg/L**	**PADEP Diel DO Range Δ 6.1 mg/L**	**PADEP 7-day Average Diel DO Range Δ 5.4 mg/L**
CA_WRDS	0/87	0/87	0/73
CA_RMRCK	54/74	62/70	52/52
CA_D_CPBRG	16/90	1/88	0/76

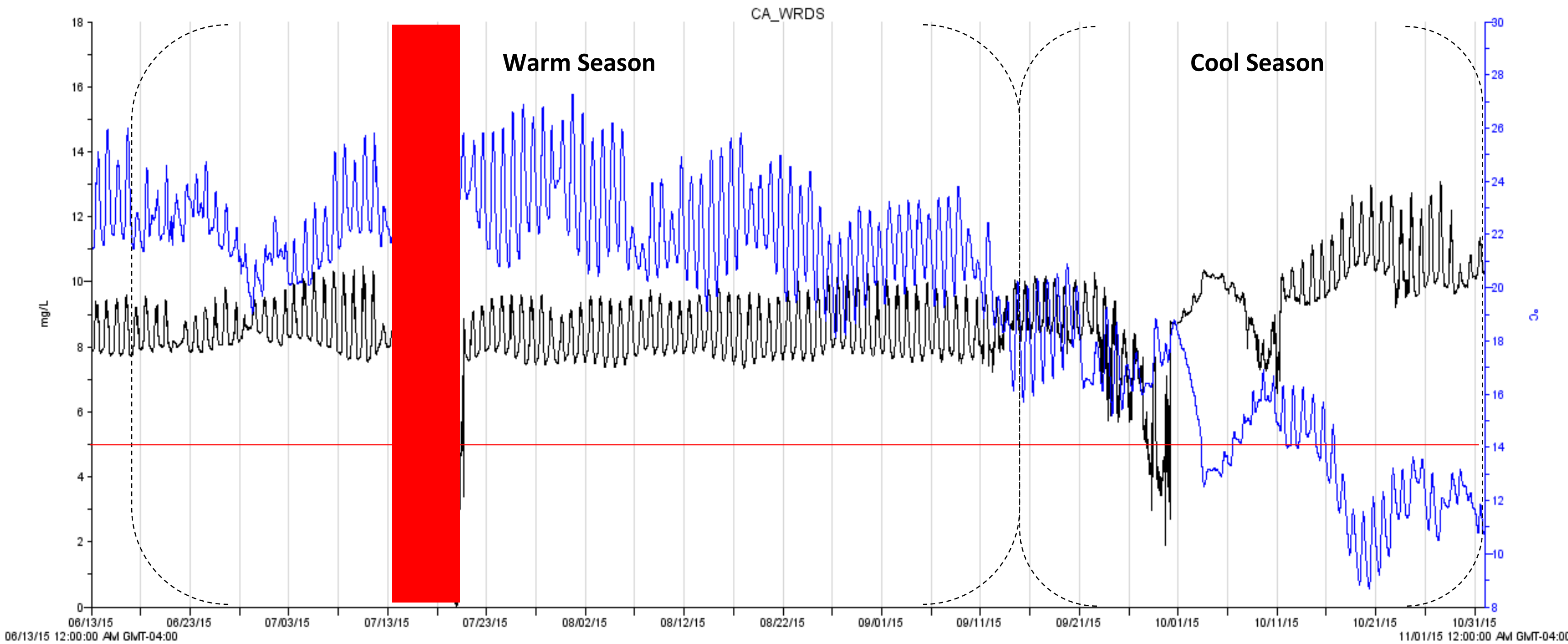


Figure 7a. Temperature (Blue) and dissolved oxygen (DO, Black) profiles for the site; Cacapon River at Wardensville (PC-78.7). The red horizontal line marks the West Virginia biological criteria threshold for dissolved oxygen (5 mg/l) for warm-water streams. Red shaded area depict disturbance events that reported questionable results and were therefore removed.

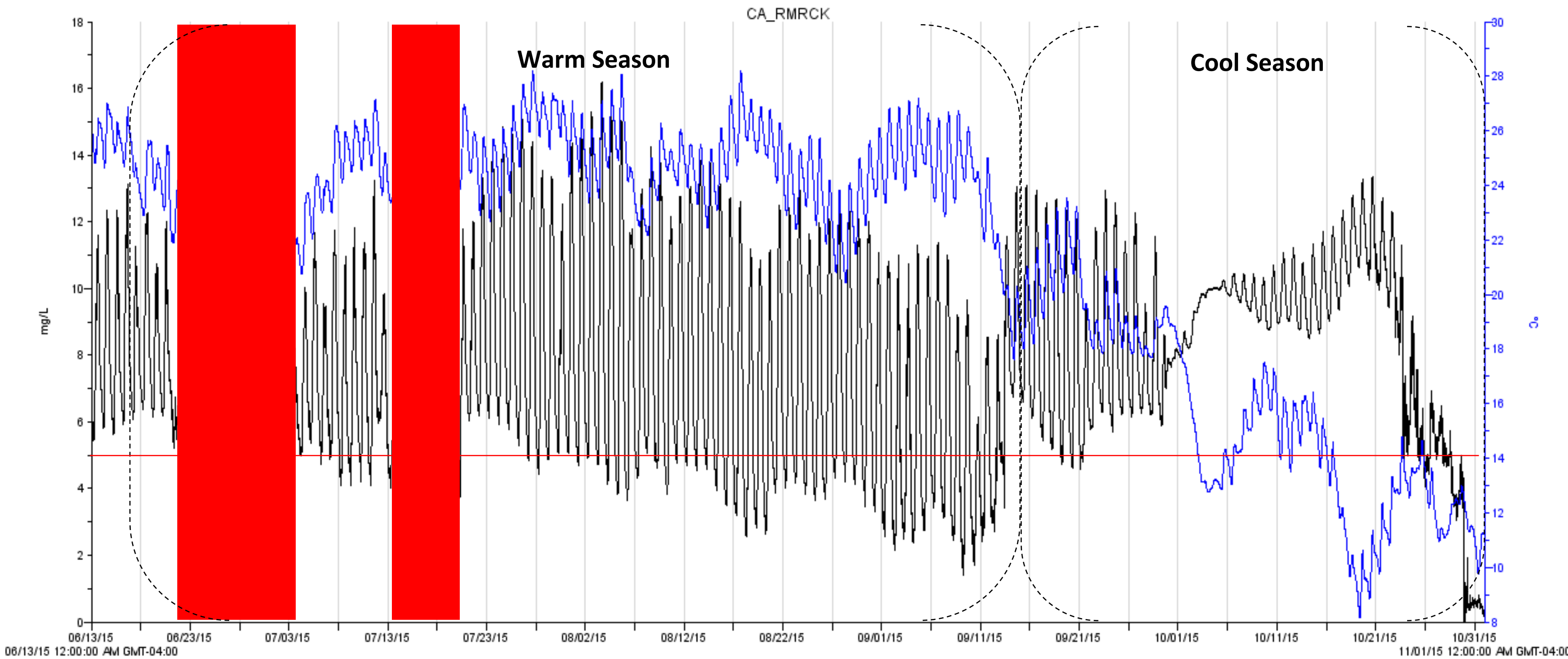


Figure 7b. Temperature (Blue) and dissolved oxygen (DO, Black) profiles for the site; Cacapon River at Camp Rim Rock (PC-60.3). The red horizontal line marks the West Virginia biological criteria threshold for dissolved oxygen (5 mg/l) for warm-water streams. Red shaded area depict disturbance events that reported questionable results and were therefore removed

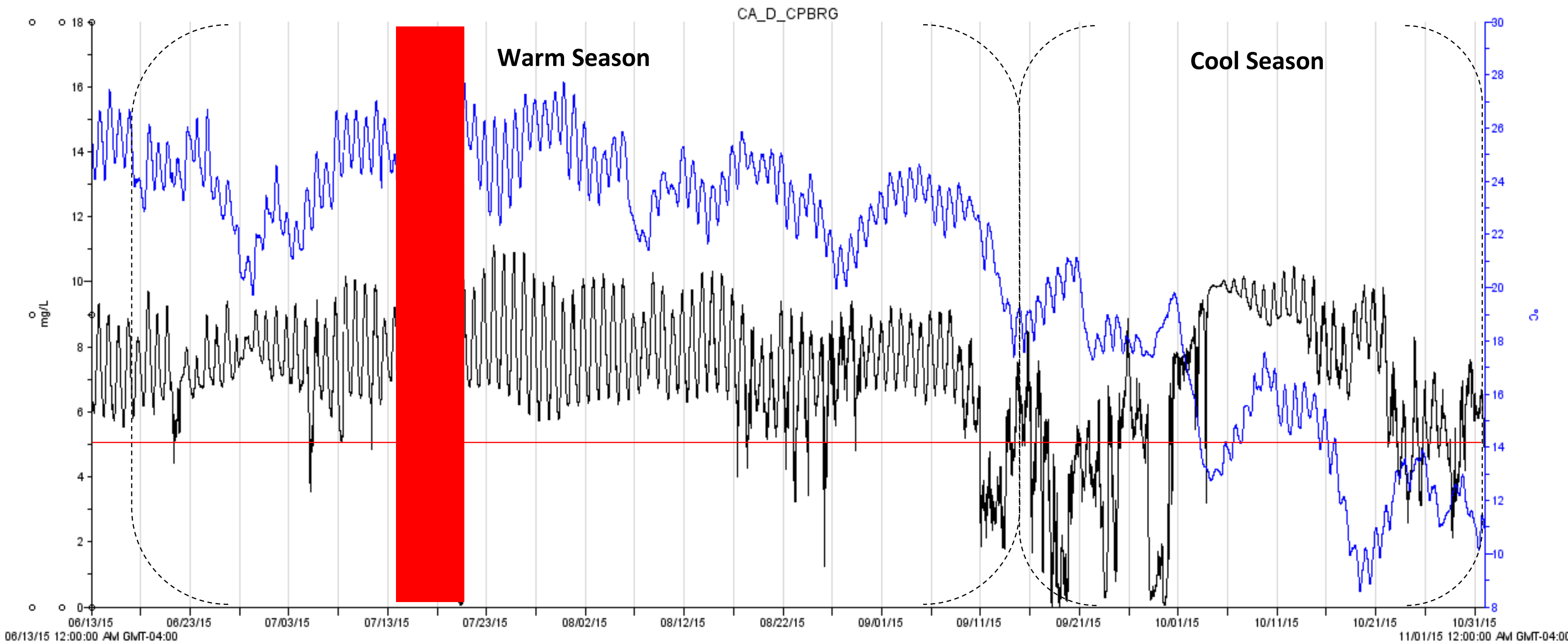


Figure 7c. Temperature (Blue) and dissolved oxygen (DO, Black) profiles for the site; Cacapon River at River's Edge Farm (PC-47.9). The red horizontal line marks the West Virginia biological criteria threshold for dissolved oxygen (5 mg/l) for warm-water streams. Red shaded area depict disturbance events that reported questionable results and were therefore removed

Macroinvertebrate Collections

A total of 20 macroinvertebrate collections were made in 2015. Collections were picked at the 200 count level according to WVDEP standard protocols. Seventy four (74) metrics were calculated from the sample counts. Twenty four (24) metrics detected significant difference between sites. In general, the benthic communities observed at CA_WRDS and CA_D_CPBRG were noticeably different from those CA_YLWSPR and CA_RMRCK. A list of the metrics showing significant differences ($p < 0.05$) is presented in Table 3 and intrasite post-hoc comparisons are presented in Appendix 4.

Tolerance Scores

Of the 72 metric tests run on the invertebrate taxa data, 8 were "tolerance" metrics aimed at defining a community's ability to endure anthropogenic stress. A Kruskal-Wallace non-parametric test revealed four tolerance metrics with significant differences between sites: the family level Hilsenhoff (*FAMILY_HBI*), a Maryland created tolerance metric identifying urban stress intolerant species (*PCT_URBAN_INTOL*), a percent moderately tolerate (tolerance score 4-6/10) metric (*PCT_MOD_TOL*) and a percent tolerant (>6/10) metric (*PCT_TOLERANT*).

Average Hilsenhoff scores observed at the CA_YLWSPR (5.33) and CA_RMRCK (4.86) test sites were higher than the upstream CA_WRDS (4.36) and downstream CA_D_CPBRG (4.25) control sites. The upstream control site CA_WRDS, was significantly different from CA_RMRCK ($p = .002$) and approached significance from CA_YLWSPR ($p = 0.054$). The downstream control site was only significantly different from CA_RMRCK ($p = 0.005$). Percent urban intolerant (PCT_URBAN_INTOL) and percent tolerant (PCT_TOLERANT) are the inverse of the other. Significant site differences ($p < 0.002$) in both metrics suggest a more pollution tolerant community occurs at high production sites CA_RMRCK and CA_YLWSPR compared to CA_WRDS and CA_D_CPBRG. There were no difference observed between the two control sites or the two test sites. Moderately tolerant taxa (4-6/10) were observed significantly more often ($p = 0.011$) at control sites than at CA_RMRCK or CA_YLWSPR. CA_WRDS has significantly more moderately tolerant than both CA_YLWSPR and CA_RMRCK ($p = 0.001$ and 0.014 respectively). CA_D_CPBRG had significantly more moderately tolerant taxa than the CA_RMRCK site ($p= 0.011$) and approached significantly more moderate taxa at the CA_YLWSPR site ($p = 0.067$).

Community Structure

There was a significant shift in the EPT taxa compositions, specifically in Tricoptera, which depended on macrophyte presence and river location. CA_WRDS and CA_D_CPBRG had a significantly larger percentage of *Tricoptera* ($p = 0.001$) compared to the test sites CA_RMRCK and CA_YLWSPR. When the metric was corrected to exclude non-tolerant *Tricoptera,* there was an ever more apparent trend with a significant loss ($p = 0.006$) of sensitive and moderately sensitive T*ricoptera* taxa. There were significantly more mayflies (*Ephemoptera*) observed at CA_WRDS than any other site. Amphipods ($p = 0.002$) and non-insect organisms ($p = 0.002$) made up a significantly higher percentage of the benthic community in the dense macrophyte environment than was observed at any other location. Conversely, beetles (*Coleoptera*) were found to make up a significantly higher percentage of the community at the control sites than the macrophyte abundant sites ($p = 0.003$).

Behavior

Significant differences in macroinvertebrate behavior metrics (habit and functional feeding groups) were observed between test and control sites. Control sites had significantly higher percentages of clingers (p = 0.018) and collectors (p = 0.012) compared to the test sites. Control sites also had a significantly greater percentages of filterers (p = 0.001) compared to test sites. Conversely test sites CA_RMRCK and CA_YLWSPR had significantly higher percentages of sprawlers (p = 0.014), climbers (p = 0.005), and predators (p = 0.027) compared to the test sites.

Table 4. Table depicting significant metrics ($p \leq 0.05$, Kruskal-Wallace nonparametric test) comparing sampled sites. Values reported within this table are averages of the five sample dates. Bolded metrics are community tolerance metrics, non-bolded metrics are metrics that reflect community structure and invertebrate behavior.

	STATION_ID	***CA_WRDS***	***CA_YLWSPR***	***CA_RMRCK***	***CA_D_CPBRG***
Tolerance Scores	**FAMILY_HBI (Hilsenhoff)**	**4.36**	**5.33**	**4.86**	**4.25**
	PCT_URBAN_INTOL	**97.37**	**82.41**	**71.36**	**94.90**
	PCT_TOLERANT*	**7.20**	**28.23**	**36.97**	**10.32**
	PCT_MOD_TOL**	**60.02**	**32.37**	**40.50**	**55.81**
Community Structure	PCT_EPT_RICH_NO_TOL	41.16	31.33	28.63	44.85
	PCT_EPHEMEROPTERA	37.43	58.24	37.32	29.46
	PCT_TRICHOPTERA	15.48	2.73	11.31	30.43
	PCT_RETREAT_CADDISFLY	13.10	2.16	9.16	28.29
	PCT_TRICHOPTERA_NO_TOL	7.85	1.69	2.68	7.29
	PCT_COLEOPTERA	33.52	10.13	9.06	17.67
	PCT_COTE	87.83	76.10	64.71	79.19
	PCT_POTEC	89.00	78.01	66.38	85.70
	PCT_AMPHIPODA	0.00	0.22	9.76	0.72
	PCT_NON_INSECT	1.93	11.79	18.80	2.39
Behavior (Habit/FFG)	PCT_COLLECT	71.81	50.41	55.64	76.26
	PCT_FILTER	21.17	3.93	11.25	39.58
	PCT_PREDATOR	3.75	9.91	10.31	5.11
	PCT_CLIMB	1.98	7.84	13.53	4.78
	PCT_CLING	74.63	54.76	45.60	78.35
	PCT_SPRAWL	2.28	12.91	19.98	9.07

**PCT_TOLERANT – Percent tolerant taxa (tolerance score >6)*

***PCT_MOD_TOL - Percent moderately tolerant taxa (tolerance values 4-6)*

Discussion

The primary goals of this study were to:

1) Investigate diel changes in dissolved oxygen concentrations as they relate to the level of primary production,
2) Investigate macroinvertebrate benthic community structure at high and low primary production sites, and
3) Apply PADEP's nutrient impact assessment protocols to the Cacapon River to determine if an area that routinely fails the West Virginia recreational use criteria for NFGA growth might also be biologically degraded.

Our results suggest that excessive primary production causes significant changes in the diel dissolved oxygen concentration profile as well as alters physical habitat structure in the affected reach. Acute and chronic dissolved oxygen stress in combination with alteration of primary habitat corresponded to changes in the macroinvertebrate community structure. The results suggest that areas of over production have reduced biological integrity and would be impaired if PADEP's nutrient protocols were applied.

In reviewing Cacapon River data collected since 2012 as part of the WVDEP Filamentous Algae Project, ICPRB confirmed that excess levels of total phosphorus, the system's limiting nutrient, are found only in the late spring/early summer samples. In 2015, the spring total phosphorus (TP) concentration was observed to be nearly 4 times greater (Figure 5) than the maximum threshold allowed for West Virginia waters (0.6 mg/liter). The early summer peak in total phosphorus (TP) rapidly diminishes to detection limit levels by mid-June and remains low for the remainder of the growing season. Total nitrogen followed a similar trend. Levels of total nitrogen (TN) were highest early in the year and steadily decreased through the summer season. The reduced rate of nitrogen loss over time compared to total phosphorus loss is likely due to the limiting nutrient phosphorus proportionally limiting nitrogen take-up.

The seasonal TP and TN declines are likely due to the high levels of biological stream activity that occurs in summer. Macrophytes are able to rapidly assimilate available phosphorus and nitrogen in the water column in summer, and concentrations of water column nutrients during peak production are typically not indicative of total nutrients bound within the system. Nutrient sinks such as sediments or plant and animal biomass are not currently reflected in the water chemistry collection results. Accrual of macrophyte biomass is a function of a specific growth rate which is dependent on available limiting nutrients, light availability for photosynthesis, and water temperature. Many attempts to create dissolved nutrient-macrophyte biomass models have met with minimal success (Homer and Welch 1981, Jones et al. 1984, Aizaki and Sakamoto 1988, Biggs and Close 1989, Lohman et al. 1992, Biggs 1995, Dodds et al. 1997, Chetelat et al. 1999). Although the general processes involving nutrient assimilation are well understood, creating nutrient-based models is difficult due to the complexity of biological interactions, multiple nutrient sinks, and environmental/physical factors that can all effect biomass at any point in time (Biggs 2000, Chambers and Prepas 1994).

High concentrations of water column nutrients before the summer growing season (Figures 5 and 6) confirm large nutrient loads are entering the river. The nutrient assimilation in summer appears to be accomplished by multiple primary producers, and the dominant primary producer at a given site seems to depend on stream morphology and flow velocity. The uppermost CA_WRDs site is shallow and faster-flowing, with coarse substrate, and dominated by periphyton. The wider, slower-flowing, CA_RMRCK site with a sand and gravel bottom had extensive macrophyte beds in 2015 and abundant NFGA blooms in the previous three years. The downstream CA_D_CPBRG site is a pool transitioning to a riffle and has not had significant NFGA or macrophytes. However, a longitudinal survey in 2015 found dense macrophyte beds roughly 1.5 km upstream of the site.

The Cacapon River monitoring sites with less primary production in 2015 had safe oxygen levels and modest diel variability while the CA_RMRCK site with excessive primary production had a highly variable, oxygen stressed environment. The periphyton-dominated CA_WRDS site, with no upstream macrophytes or NFGA, experienced stable DO concentrations and diel changes of around 2.5mg/L for the complete warm period of 2015, with the exception of rain events. This stable, well oxygenated environment supported a macroinvertebrate community that was more pollution sensitive and was associated with the physical substrate habitat. The CA_RMRCK site experienced substantially larger diel swings in DO, with frequent drops below 5 mg/L. The site held significantly more pollution tolerant and epiphytic taxa. Exceedances of the WVDEP DO threshold criterion and the PADEP diel daily range and weekly average diel range occurred in over 75% of sampled days in 2015. CA_RMRCK was therefore chronically oxygen stressed for much of the summer season, which explains the longitudinal shift from more sensitive to a more pollution tolerant macroinvertebrate taxa as represented by the HBI and percent tolerance scores. Additionally, the increased macrophyte biomass (change in habitat) appears to have smothered the benthic community as represented by a shift from clinging taxa (benthic obligate) to climbing taxa (epiphyte obligate). The benthic community represents a more epiphyte dominant community than it does a benthic community at high production sites. High HBI scores at CA_RMRCK suggest anthropogenic nutrient inputs are causing the change in primary producer and macroinvertebrate communities. The HBI is a metric used to measure the biological impact of municipal wastewater. Elevated HBI scores at the CA_YLWSPR and CA_RMRCK sites suggest degraded conditions specifically due to municipal waste.

The downstream control site, CA_D_CPBRG held very few macrophytes, much like CA_WRDS, and so saw few DO stress exceedances and a stable macrophyte community. One key difference between these two sites however was the presence of an upstream dense macrophyte community. The larger diel DO variation and few DO exceedances at CA_D_CPBRG when compared to CA_WRDS can be explained by a highly productive reach below the town of Capon Bridge. Gray water outflow was observed from the town's water treatment facility which is the probable cause of dense SAV beds lasting several kilometers downstream. The large SAV bloom stopped roughly a kilometer above the site. This observation holds two important implications; (1) it demonstrates that the effects of highly productive reaches are relatively localized and (2) the large SAV beds appear to have removed a significant amount of the nutrients. HBI scores for the CA_D_CPBRG site suggested municipal waste water pollution (from the gray water input) was not a stressor. This finding suggests that the SAV beds largely removed nutrients from the water, so much so that benthic community composition returned to acceptable levels a short distance downstream. The proximity of the SAV bed to the testing site provided a good example of how the river biota are capable of repairing an aquatic system. What is still unknown is whether the nutrients are sequestered and held in the soil by the macrophytes or if there is a seasonal release of these nutrients in Fall. Additional nutrient transport studies should be considered to determine the longer term fate of nutrients sequestered in biomass.

Considerations for Managers

Our study suggests that nutrient pollution resulting in excessive primary production, can be detected at four different tiers: (1) direct detection of nutrient threshold exceedances, (2) a primary response level where macrophyte abundance is measured, (3) a proximate stressor level which detects acute and chronic levels of dissolved oxygen (or pH) stress using continuous monitoring technology, and (4) a secondary response level where benthic community structure is affected by both the change in habitat structure and stressor environment.

Tier 1. Nutrient Detection

Detection of excess nutrients is pivotal in the linking of terrestrial stressors to the aquatic environment. Water column grab samples may be adequate for describing point source nutrient inputs and cool season nutrient availability but may not adequately describe nutrients that are bound in the soil and biological tissues during highly productive seasons. Collection of nutrient data should therefore be implemented as seasonal (Spring or Fall) or targeted (known point source) monitoring in lotic systems with abundant macrophyte communities.

Tier 2. Primary Response

At the primary production response level, an overabundance of macrophytes or NFGA can be easily assessed using visual based reporting. As the WVDEP Filamentous Algae protocol has shown, recreational impairment can be successfully assessed using a visual transect method. Percent macrophyte or NFGA coverage/abundance measures can be correlated to user's perceived loss of recreation, and used to create a defendable recreation-based use criteria. If the goal of a manager is solely to define recreational criteria, this method accomplishes that. What this method fails to detect (because it is not the intended purpose) is what effect over-production has on the ambient biological community. Macrophyte measurements should be collected when investigating the biological implications of a bloom as a means to correlate macrophyte abundance to the related biological impact.

Tier 3. Proximate Stressor

The third tier managers can consider when investigating the effects of over production is the detection of a proximate stressor. As shown in this study and well documented in the literature, an over producing reach is capable of significantly altering diel water chemistry, particularly dissolved oxygen and pH. If either of these water chemistry parameters fall outside the physiologically acceptable bounds for aquatic life, high profile events such as fish die offs and "dead" rivers can be the result. Agencies have developed threshold-based criteria for DO and pH in order to list waterways as impaired and address the relevant stressors. Threshold-based criteria have been useful when grab samples were the best logistical option. Unfortunately, one of the criticisms of grab samples is that they are a single snapshot of a dynamic system usually collected at a time of day when the stress is not evident. Factors such as the time of day, temperature, light intensity, biological activity, and seasonality can give significantly different results. Consider, for example, results from ICPRB's work in the Cacapon and South Branch Rivers. Despite attempts to collect all water samples in the same day, dissolved oxygen levels will reflect the amount of photosynthesis/respiration occurring at the time of day sampled. A site with abundant amount of macrophyte biomass sampled in the morning could have the same DO concentration as a site lacking macrophytes at noon.

Logistically, the effort required to detect diel swings in water chemistry parameters over long periods of time at multiple locations is unreasonable for any field crew collecting grab samples. One answer to this logistical dilemma, is the use of continuous monitoring sensors to passively collect data. Continuous monitoring data can create thousands of data points at no increased effort to field crews, except for time required for initial installation and eventual removal. The initial investment in the technology is well worth the level of resolution gained with continuous monitoring data, especially as costs should continue to decline as the technology further develops.

The ability to detect exceedances is greatly increased with the new technology. Thresholds that were created for lower resolution methods and daytime collections are still based in aquatic life physiology but may no longer be appropriate when analyzing high resolution data. Results from our study show that the number of dissolved oxygen exceedances (Table 1) are based on the threshold definition and ultimately are synthesized down to a binary pass/fail count result. A single value threshold analysis may be an over simplification of continuous high resolution data, especially since continuous monitoring data may be able to define the type of stress as acute or chronic, as well as quantify number of exceedances.

Dissolved oxygen CDF curves are useful tools capable of defining threshold exceedances as well as describe dose durations for large amounts of sonde data. CDF curves are more powerful descriptors of environmental conditions when compared to previous methods such as descriptive statistics or time series analysis. A CDF r-script was provided by Dustin Schull (PADEP) and modified by ICPRB. The R script was prepared to tally the number and the duration of events that exceeded DO thresholds of interest. Events that exceeded cutoff values were then ranked by magnitude of duration. Four cutoff values (thresholds) were selected as 6 mg/L, 5 mg/L, 4 mg/L, 3 mg/L for the purpose of this study (Figure 8). Observation of both the frequency and duration of hydrologic events, above a given level of interest and for a given time period provides the necessary data to characterize site-specific curves for concentration, duration, and frequency of events. An example of a Concentration-Duration-Frequency plot for the site CA_RMRCK is presented in Figure 8. From this analysis managers can identify different types of stressor conditions (acute vs chronic thresholds) for aquatic life use criteria.

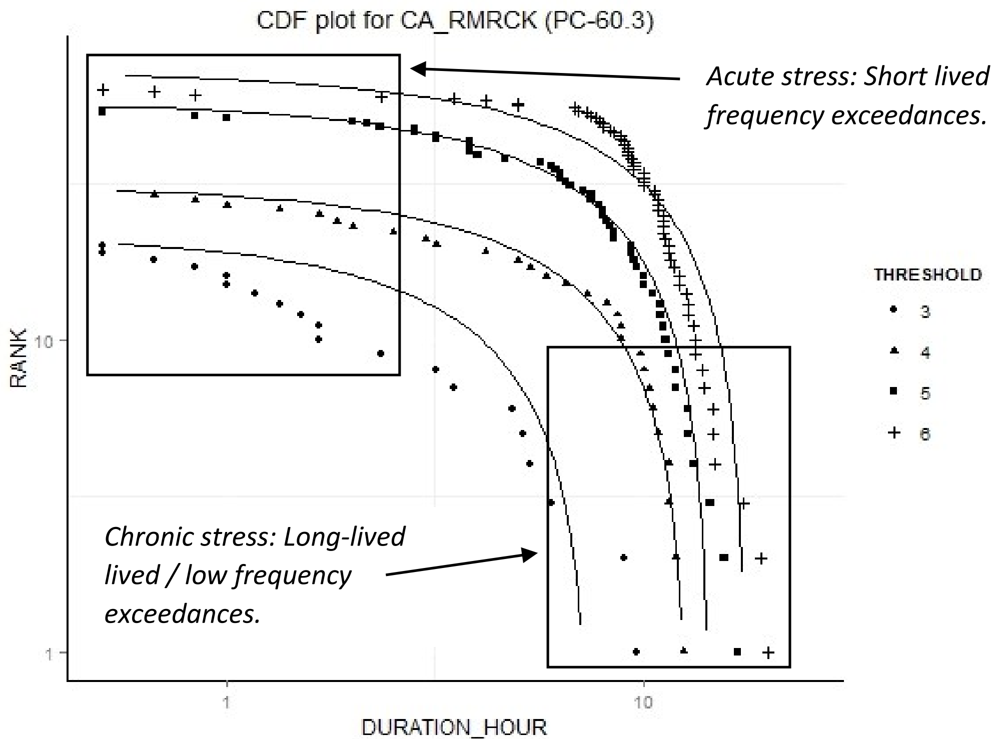


Figure 8. Concentration-Duration-Frequency (CDF) curve created using continuous monitoring data from the 2015 Cacapon River at CA_RMRCK site.

Tier 4. Secondary Response

At the secondary production response level, the impacts of the primary production response and related proximal stressors manifest as degradation in the condition of the macroinvertebrate community. This fourth tier strengthens the case that nutrient pollution is affecting the entire ecosystem, and not just causing nuisance macrophyte blooms. It should not be difficult for managers to implement their macroinvertebrate protocols when needed as many field crews are well versed in these methodologies. ICPRB chose to use a multihabitat approach to look at community structure within macrophyte beds but other stream macroinvertebrate surveys methods could be considered for specimen collection as well (ie. for inclusion in IBI/MMI applications). The key to documenting impacts of excessive NFGA or macrophytes on stream invertebrates is sampling within or near the areas of high production during periods of high growth.

Final thoughts

The four tiered approach presented here connects nutrient pollution to NFGA and macrophyte abundance as well as biotic integrity. Aside from the initial investment of new technology and the time required to analyze macroinvertebrate collections, these methods could easily be implemented into already existing protocols.

Conclusion

The 2015 Cacapon River microcosm study was designed to investigate the biological implications of nuisance level filamentous green algae blooms. Due to an uncharacteristic year which resulted in a dearth of filamentous green algae production, the results reported in this study instead attempt to address a broader question, what are the biological implications of over production in a system? Using continuous logger technology and multi-month benthic sampling, ICPRB is able to report on the diurnal variability of dissolved oxygen at highly productive sites as well as report on the macroinvertebrate community structure. Elevated levels of primary production found in the Cacapon River caused excessive dissolved oxygen (DO) variability relative to sites devoid of significant primary production. During diel DO swings, nocturnal respiration in macrophytes dense locations caused dissolved oxygen levels to diminish below WVDEP aquatic life thresholds regularly during the warm season. Excessive dissolved oxygen daily variability was elevated at the CA_RMRCK site dense with macrophytes. PADEP standards would impair the system due to DO variability exceeding both daily DO range and 7-day average DO range requirements.

Although this study cannot specifically link nutrient pollution to macrophyte abundance and change in benthic community structure in the upper Cacapon River, there is a wealth of literature that suggest the relationship exists between nutrients and biological impact. We observed shifts from benthic communities with sensitive and moderately pollution tolerant taxa at minimal macrophyte abundance sites to pollution tolerant taxa at sites with excessive macrophyte abundance. An average family level Hilsenhoff Biotic Index score (HBI) was calculated for each of the four sites for the duration of 2015 and showed that CA_YLWSPR and CA_RMRCK are affected by stressors that our two control sites, CA_WRDS and CA_D_CPBRG did not experience. Shifts in pollution intolerant indicator order, specifically *Trichoptera*, support there is an environmental pressure exerted on systems with high primary production that is not experienced by more stable sites.

Following PADEP's protocols, continuous monitoring of DO would have been implemented based on the microbenthic community results (specifically HBI scores) collected at our macrophytes positive site. The DO concentrations observed at CA_RMRCK failed all WVDEP and PADEP thresholds for DO stress. According to PADEP's protocol for identifying excessive nutrients loadings, nutrients pollution is a cause to list this site as impaired based on loss of aquatic life use. This report suggests that excessive primary production, but not specifically algae, is a cause for concern in freshwater systems.

This study outlines a structure that combines WVDEP's algae quantification methods for defining recreational impairment and PADEP's methods for relating excessive nutrient loading to aquatic life uses. These two protocols work synergistically to inform the effects of excessive nutrients on freshwater uses. Results from this study suggest the semi-quantitative narrative of WVDEP's recreational impairment protocols fill a gap in the PADEP nutrient impact assessment defining how much of the primary response of excessive nutrients, macrophyte production, is required to reveal a secondary response, a change in community structure. Inversely, WVDEP's qualitative protocols can document NFGA abundance and comment on the loss of recreational usage, however, is missing the secondary biological component that PADEP's protocols emphasis. A hybrid protocol will allow for a powerful statement connecting both recreational and biological uses with nutrient pollution and it may be able to screen against naturally occurring production versus anthropogenically influenced production reaches. Ultimately, this approach could reduce the effort required by agencies.

Summary of Conclusions

1) There were no significant nuisance filamentous algae (NFGA) blooms observed between Sandy Springs, West Virginia and Capon Bridge, West Virginia in 2015.
2) Phosphorus was observed above PADEP maximum concentration criteria (0.06 mg/L) early in the season before aquatic macrophytes were present.
3) High concentrations of water column nutrients before the summer growing season (Figures 5 and 6) confirm large nutrient loads are entering the river. The nutrient assimilation in summer appears to be accomplished by multiple primary producers, and the dominant primary producer at a given site seems to depend on stream morphology and flow velocity
4) Despite lack of NFGA, the Cacapon River at Camp Rimrock produced an excessive amount of submerged aquatic vegetation (SAV), raising the question is the presence of NFGA the top concern for identifying nutrient inputs, or should the focus of assessment efforts be on regions of over production in general?
5) The diurnal dissolved oxygen stability of a site is dependent on the site's level of primary production.
    a) Sites that were devoid of excessive over production (NFGA and/or SAV) experienced more stable diurnal dissolved oxygen concentrations.
    b) Sites that experienced a heavy macrophytes density saw large daily variation in dissolved oxygen concentrations whereas sites that did not have abundant macrophyte density saw small variation in DO.
6) A single threshold criteria does not adequately describe DO requirements for a healthy biotic community.
    a) Single day DO concentration thresholds should be set at levels of immediate biological loss.
    b) Dissolved oxygen diel range (Single day range and multi-day average range) may be more suited to addressing long term, chronic DO stress.
    c) Concentration-duration-frequency (CDF) curves could be a tool for accessing acute and chronic stressors using continuous monitoring technology.
7) Benthic macroinvertebrate community structure was altered in the presence of excessive primary production.
    a) There was a shift to more pollution tolerant taxa in sites that experienced large amounts of primary production.
    b) Sites with little primary production hold proportionally more pollution sensitive/moderately tolerant taxa.
    c) Control sites had the highest percentage of urban intolerant taxa.
    d) There is a higher percentage of predators proportionally at high primary production sites.
    e) There are proportionally more caddisflies (*Trichoptera)* at sites lacking excessive primary production.
    f) There are significantly more collectors at sites that did not experience excessive primary production.

Future work

Ideally, in-stream monitoring would be spatially, temporally, biologically, and chemically comprehensive. Limited resources, however, always preclude such an exhaustive monitoring effort and researchers must utilize methods capable of collecting the most information with minimum time, effort, and expense. Temporally defined sampling strategies should be considered when addressing the topic of nutrient pollution. Direct detection of excessive water column nutrients is the best means to report on nutrient pollution. Due to biological activity and the diminishing chemical signal in summer, however, water chemistry analysis of suspended nutrients should be considered when investigating a known point source or applied to low production periods (fall –spring). Primary and secondary stressors analysis as surrogates for nutrient detection should be considered during periods of elevated biological activity (summer). This research shows that there is a strong correlation between excessive nutrients and ecological shifts due to macrophyte structure.

Analysis of nutrient sources should include, if resources allow, analysis of nutrient source pathways and the bioavailability of nutrients in sediment to complement current water column concentrations. In the Cacapon region, karst geology is a significant factor to consider due to the calcareous soils and bedrock and propensity for subsurface flow. Ground water source tracking may point nutrient input to new sources. Additionally, soil samples analyzed by MgCl extraction have been reported to be a more accurate predictor of macrophyte production than water column nutrients in some systems (Mebane et al. 2014) and therefore might be considered for future studies.

Continuous monitoring of water quality measures such as pH and DO also are valuable because large macrophyte blooms can cause harmful pH and DO swings overnight when field crew monitoring typically is not implemented. The use of continuous monitoring allows biologists to collect large amounts of data depicting the diel oscillations at sites at relatively low cost beyond the initial investment. One unforeseen outcome of using higher frequency and resolution technology is the increased detection of exceedances that have not been recorded before. Threshold based criteria, for example West Virginia's single exceedance impairment of dissolved oxygen when a minimum threshold of 5 mg/L, may be too strict in impairing waterways. In some cases, exceedances were extremely short lived while others may be due to environmental factors such as rain events. Revision of the threshold based impairment for more tiered based impairment, such as PADEP's methods of range-based exceedances, or concentration-density-frequency (CDF) analysis should be considered to adjust for the heightened resolution of continuous monitoring data.

In-stream light levels and temperature are important considerations in any study of NFGA blooms since light and temperature can often limit distribution and growth. Light and temperature levels can be continuously monitored with inexpensive sondes or light-logging pendants.

Finally, results from this study were focused on the summer (warm) season to limit the effects of multiple variables (water temperature, day length, etc.). Nutrient concentrations or the biological condition of sites that were dominated by macrophytes in the cold season were not looked at. As nutrients that were formerly tied up in macrophyte biomass re-suspend in the water column we would expect to see increased nutrient concentrations. Additional research is required to describe the Spring and Fall turnover and the fate of formerly assimilated nutrients.

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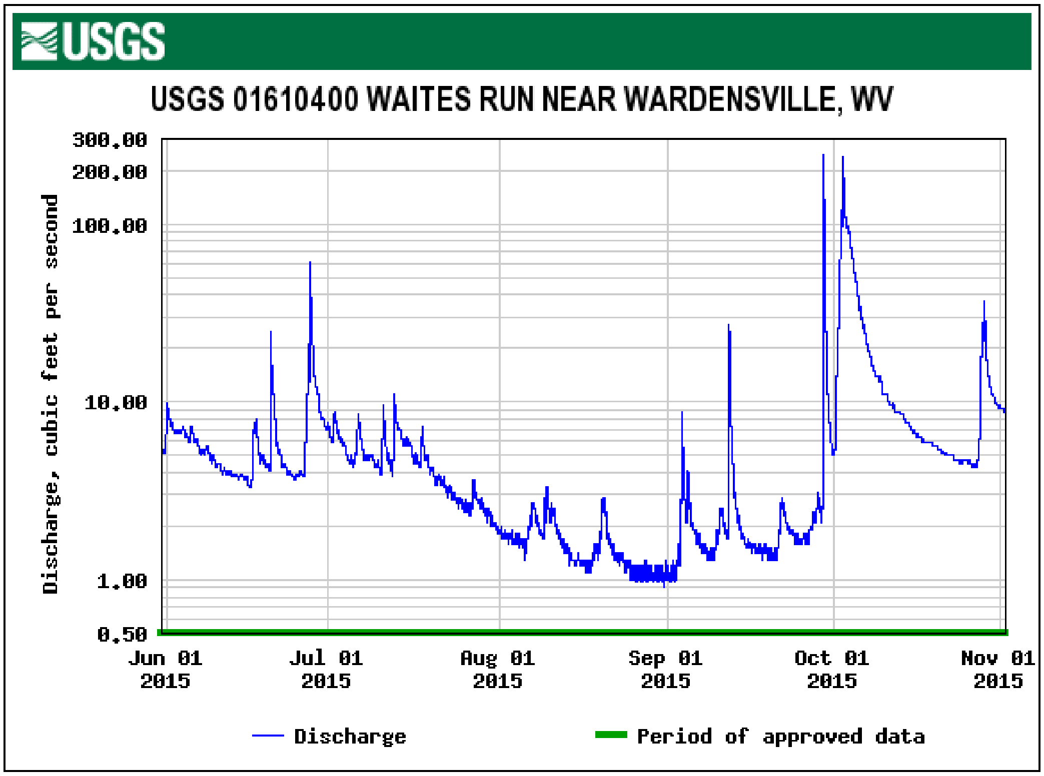
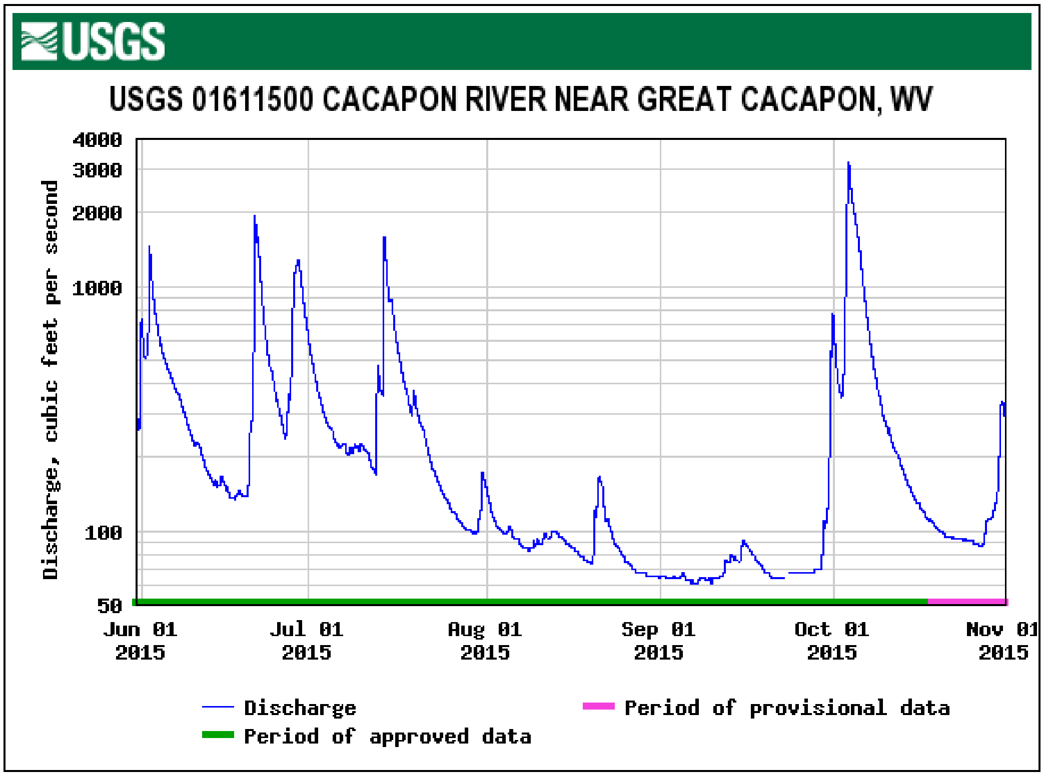
Appendix 1. USGS 2015 river discharge at Waites Run (near CA_WRDS) and Great Cacapon, WV.





Appendix 2. Data sheet for macroinvertebrate collections.

Site Name		**AN Code:**	
Date	/ /	**Time**	D: / L:
Lat		**Long**	
Agency	**ICPRB**	**Primary Inv:**	**GM Selckmann**

Multihabitat Sampling Habitat	Collected by: Wading					
	Cobble %		Snags %		Veg Banks %	
	Sand %		SAV %		Algae %	
Effort/Habitat (# kicks)	Cobble	/20	Snags	/20	Veg Banks	/20
	Sand	/20	SAV	/20	Algae	/20

General Comments:

Qualitative Measure					
Periphyton	0	1	2	3	4
Filamentous Green Algae	0	1	2	3	4
Blue Green Algae	0	1	2	3	4
Moss	0	1	2	3	4
SAV	0	1	2	3	4
EAV	0	1	2	3	4
Floating Veg	0	1	2	3	4
Total Aquatic Plants	0	1	2	3	4

Environmental Conditions (D/L):		Rain in 7 days? Y / N		Past 24 Hours? Y / N	
Storm (Heavy Rain)	○○	Dark (D)	DO	Light (L)	DO
Rain (Steady Rain)	○○		Conduct		Conduct
Showers (Inter)	○○		TDS		TDS
Cloud Cover	○○		Water Temp		Water Temp
Clear	○○		pH		pH

Inorganic Substrate Components			**Organic Substrate Components**		
Type	Diameter	% Comp	Type	Characteristic	% Comp
Bedrock			Detritus	sticks, wood, cPOM	
Boulder	> 256 mm (10")				
Cobble	64-256 mm (2.5"- 10")		Mud/Muck	black, fines, fPOM	
Gravel	2-64 mm (0.1" - 2.5")				
Sand	0.06 - 2 mm (gritty)		Marl	grey, shell fragments	
Silt	0.004-0.06mm				
Clay	<0.004 mm				

*Appendix 3a. The CA_WRDS daily and 7 day dissolved oxygen (mg/L) range for the warm season 2015. ** Dates where 24 hours of data were not available to average.*

Date	Daily Range	7 Day Average	Date	Daily Range	7 Day Average
6/15/2015	1.79		8/5/2015	2.06	1.92
6/16/2015	1.9		8/6/2015	2.09	2.00
6/17/2015	1.5		8/7/2015	1.74	2.01
6/18/2015	1.64		8/8/2015	1.95	2.03
6/19/2015	1.42		8/9/2015	1.89	2.00
6/20/2015	1.49		8/10/2015	1.69	1.93
6/21/2015	0.33	1.44	8/11/2015	1.64	1.87
6/22/2015	0.93	1.32	8/12/2015	1.63	1.80
6/23/2015	1.1	1.20	8/13/2015	1.79	1.76
6/24/2015	1.39	1.19	8/14/2015	2.09	1.81
6/25/2015	1.58	1.18	8/15/2015	2.25	1.85
6/26/2015	1.78	1.23	8/16/2015	2.2	1.90
6/27/2015	1.29	1.20	8/17/2015	2.19	1.97
6/28/2015	0.81	1.27	8/18/2015	2.34	2.07
6/29/2015	1	1.28	8/19/2015	2.17	2.15
6/30/2015	1.33	1.31	8/20/2015	1.89	2.16
7/1/2015	1.37	1.31	8/21/2015	1.91	2.14
7/2/2015	1.7	1.33	8/22/2015	2.09	2.11
7/3/2015	1.62	1.30	8/23/2015	2.42	2.14
7/4/2015	1.83	1.38	8/24/2015	2.2	2.15
7/5/2015	2.16	1.57	8/25/2015	2.22	2.13
7/6/2015	2.15	1.74	8/26/2015	2.4	2.16
7/7/2015	2.52	1.91	8/27/2015	2.16	2.20
7/8/2015	2.65	2.09	8/28/2015	2.37	2.27
7/9/2015	2.75	2.24	8/29/2015	2.35	2.30
7/10/2015	2.94	2.43	8/30/2015	2.47	2.31
7/11/2015	2.76	2.56	8/31/2015	2.31	2.33
7/12/2015	0.81	2.37	9/1/2015	2.33	2.34
7/13/2015**	0.77		9/2/2015	2.26	2.32
7/20/2015**	1.15		9/3/2015	2.21	2.33
7/21/2015	1.53		9/4/2015	2.37	2.33
7/22/2015	1.55		9/5/2015	2.42	2.34
7/23/2015	1.62		9/6/2015	2.2	2.30
7/24/2015	1.78		9/7/2015	2.18	2.28
7/25/2015	1.94		9/8/2015	2	2.23
7/26/2015	2.15		9/9/2015	2.41	2.26
7/27/2015	2.12	1.81	9/10/2015	1.88	2.21
7/28/2015	2.16	1.90	9/11/2015	2.29	2.20
7/29/2015	1.75	1.93	9/12/2015	2.3	2.18
7/30/2015	1.52	1.92	9/13/2015	1.67	2.10
7/31/2015	1.63	1.90	9/14/2015	1.7	2.04
8/1/2015	1.85	1.88	9/15/2015	1.85	2.01
8/2/2015	2.07	1.87			
8/3/2015	2.18	1.88			
8/4/2015	2.1	1.87			

*Appendix 3b. The CA_RMRCK daily and 7 day dissolved oxygen (mg/L) range for the warm season 2015. ** Dates where 24 hours of data were not available to average.*

Date	Daily Range	7 Day Average	Date	Daily Range	7 Day Average
6/15/2015	6.73		8/10/2015	8.33	9.56
6/16/2015	7.21		8/11/2015	8	9.26
6/17/2015	5.2		8/12/2015	8.39	8.78
6/18/2015	5.9		8/13/2015	9.28	8.99
6/19/2015	4.58		8/14/2015	9.2	9.16
6/20/2015	5.83		8/15/2015	9.2	9.14
6/21/2015**	1.66		8/16/2015	9.24	8.81
7/4/2015**	4.95		8/17/2015	9.43	8.96
7/5/2015	6.32		8/18/2015	8.61	9.05
7/6/2015	4.83		8/19/2015	8.05	9.00
7/7/2015	6.89		8/20/2015	8.5	8.89
7/8/2015	6.88		8/21/2015	8.63	8.81
7/9/2015	7.74		8/22/2015	8.39	8.69
7/10/2015	7.17		8/23/2015	8.97	8.65
7/11/2015	9.13	6.99	8/24/2015	7.83	8.43
7/12/2015**	3.88		8/25/2015	8.34	8.39
7/20/2015**	4.64		8/26/2015	8.39	8.44
7/21/2015	5.99		8/27/2015	8.1	8.38
7/22/2015	7.12		8/28/2015	8.43	8.35
7/23/2015	8.22		8/29/2015	7.93	8.28
7/24/2015	8.94		8/30/2015	8.58	8.23
7/25/2015	8.87		8/31/2015	7.74	8.22
7/26/2015	9.81		9/1/2015	8.26	8.20
7/27/2015	9.57	8.36	9/2/2015	8.84	8.27
7/28/2015	9.08	8.80	9/3/2015	8.28	8.29
7/29/2015	8.47	8.99	9/4/2015	8.93	8.37
7/30/2015	7.48	8.89	9/5/2015	8.22	8.41
7/31/2015	9.31	8.94	9/6/2015	7.98	8.32
8/1/2015	9.93	9.09	9/7/2015	7.88	8.34
8/2/2015	10.38	9.17	9/8/2015	6.73	8.12
8/3/2015	11.47	9.45	9/9/2015	8.27	8.04
8/4/2015	11.07	9.73	9/10/2015	4.46	7.50
8/5/2015	11.2	10.12	9/11/2015	6.13	7.10
8/6/2015	8.14	10.21	9/12/2015	5.9	6.76
8/7/2015	8.65	10.12	9/13/2015	8.15	6.79
8/8/2015	9.18	10.01	9/14/2015	6.3	6.56
8/9/2015	9.14	9.84	9/15/2015	6.52	6.53

*Appendix 3c. The CA_D_CPBRG daily and 7 day dissolved oxygen (mg/L) range for the warm season 2015. ** Dates where 24-hour data were not available to average.*

Date	Daily Range	7 Day Average	Date	Daily Range	7 Day Average
6/15/2015	2.92		8/2/2015	3.88	4.00
6/16/2015	3.32		8/3/2015	3.89	3.94
6/17/2015	2.49		8/4/2015	3.53	3.84
6/18/2015	3.51		8/5/2015	3.86	3.76
6/19/2015	3.11		8/6/2015	2.66	3.61
6/20/2015	2.87		8/7/2015	2.5	3.46
6/21/2015	2.49	2.96	8/8/2015	3.27	3.37
6/22/2015	1.07	2.69	8/9/2015	3.15	3.27
6/23/2015	1.28	2.40	8/10/2015	2.77	3.11
6/24/2015	2.26	2.37	8/11/2015	3.18	3.06
6/25/2015	1.82	2.13	8/12/2015	3.46	3.00
6/26/2015	2.67	2.07	8/13/2015	3.85	3.17
6/27/2015	1.37	1.85	8/14/2015	3.69	3.34
6/28/2015	0.8	1.61	8/15/2015	3.63	3.39
6/29/2015	1.28	1.64	8/16/2015	3.42	3.43
6/30/2015	1.63	1.69	8/17/2015	5.28	3.79
7/1/2015	2.26	1.69	8/18/2015	4.68	4.00
7/2/2015	1.88	1.70	8/19/2015	2.25	3.83
7/3/2015	2.45	1.67	8/20/2015	3.21	3.74
7/4/2015	2.74	1.86	8/21/2015	4.17	3.81
7/5/2015	5.9	2.59	8/22/2015	4.94	3.99
7/6/2015	2.49	2.76	8/23/2015	5.84	4.34
7/7/2015	3.68	3.06	8/24/2015	5.08	4.31
7/8/2015	5.09	3.46	8/25/2015	2.45	3.99
7/9/2015	3.56	3.70	8/26/2015	7.57	4.75
7/10/2015	3.53	3.86	8/27/2015	3.47	4.79
7/11/2015	5.04	4.18	8/28/2015	3.07	4.63
7/12/2015	2.62	3.72	8/29/2015	4.44	4.56
7/13/2015	2.27	3.68	8/30/2015	2.23	4.04
7/14/2015	1.63	3.39	8/31/2015	2.13	3.62
7/15/2015	0.62	2.75	9/1/2015	2.59	3.64
7/16/2015**	1.09		9/2/2015	2.54	2.92
7/20/2015**	2.53		9/3/2015	2.28	2.75
7/21/2015	3.07		9/4/2015	2.24	2.64
7/22/2015	3.66		9/5/2015	2.86	2.41
7/23/2015	4.27		9/6/2015	2.55	2.46
7/24/2015	4.15		9/7/2015	2.56	2.52
7/25/2015	4.4		9/8/2015	2.66	2.53
7/26/2015	4.64		9/9/2015	2.96	2.59
7/27/2015	4.3	4.07	9/10/2015	5.5	3.05
7/28/2015	4.25	4.24	9/11/2015	2.94	3.15
7/29/2015	4.4	4.34	9/12/2015	2.08	3.04
7/30/2015	3.74	4.27	9/13/2015	4.61	3.33
7/31/2015	3.55	4.18	9/14/2015	3.49	3.46
8/1/2015	3.9	4.11	9/15/2015	3.53	3.59

Appendix 4. Table depicting Kruskal Wallace nonparametric significant statistical test results and Dunn's post-hoc comparisons (right). Significant (α = 0.05) Dunn's post-hoc results are shaded in grey.

Metric	Kruskal Wallace Statistic	df	p-value	CA_WRDS - CA_YLWSPR	CA_WRDS - CA_RMRCK	CA_D_CPBRG - CA_WRDS	CA_D_CPBRG - CA_YLWSPR	CA_D_CPBRG - CA_RMRCK	CA_RMRCK - CA_YLWSPR
PCT_FILTER	16.5086	3	0.001	**0.008**	0.131	0.067	**0.000**	**0.004**	0.100
PCT_TRICHOPTERA	16.5543	3	0.001	**0.009**	0.244	**0.049**	**0.000**	**0.009**	**0.049**
PCT_RETREAT_CADDISFLY	16.1429	3	0.001	**0.016**	0.395	**0.031**	**0.000**	**0.016**	**0.031**
PCT_URBAN_INTOL	15.7543	3	0.001	**0.003**	**0.000**	0.182	**0.035**	**0.004**	0.211
PCT_TOLERANT	15.2514	3	0.002	**0.004**	**0.000**	0.244	**0.024**	**0.004**	0.244
PCT_NON_INSECT	15.1829	3	0.002	**0.009**	**0.001**	0.395	**0.019**	**0.001**	0.182
PCT_AMPHIPODA	14.3898	3	0.002	0.216	**0.000**	**0.037**	0.157	**0.037**	**0.003**
PCT_COLEOPTERA	14.2457	3	0.003	**0.001**	**0.000**	0.054	0.075	**0.039**	0.374
PCT_POTEC	13.4	3	0.004	**0.014**	**0.000**	0.182	0.100	**0.006**	0.110
PCT_CLIMB	12.8629	3	0.005	**0.016**	**0.000**	0.067	0.261	**0.021**	0.082
PCT_TRICHOPTERA_NO_TOL	12.5837	3	0.006	**0.002**	**0.012**	0.395	**0.004**	**0.023**	0.261
PCT_COTE	11.6857	3	0.009	**0.011**	**0.001**	0.061	0.227	**0.039**	0.155
PCT_EPT	11.6857	3	0.009	**0.035**	0.354	**0.006**	0.244	**0.002**	**0.014**
PCT_MOD_TOL	11.2057	3	0.011	**0.001**	**0.014**	0.244	**0.011**	0.067	0.211
PCT_COLLECT	10.9314	3	0.012	**0.011**	**0.031**	0.335	**0.003**	**0.011**	0.335
PCT_EPHEMEROPTERA	10.9086	3	0.012	**0.008**	0.457	0.244	**0.001**	0.211	**0.011**
PCT_SPRAWL	10.6229	3	0.014	**0.009**	**0.001**	0.067	0.196	0.054	0.227
Family_HBI	10.44	3	0.015	0.054	**0.002**	0.374	0.100	**0.005**	0.100
PCT_CLING	10.12	3	0.018	0.100	**0.009**	0.297	**0.035**	**0.002**	0.143
PCT_PREDATOR	9.16	3	0.027	**0.016**	**0.005**	0.297	0.054	**0.021**	0.335
PCT_EPT_RICH_NO_TOL	8.1637	3	0.043	0.074	**0.033**	0.269	**0.020**	**0.007**	0.344