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Nutrient Discharge Limit Assessment for the Rogue River in the Vicinity of the City of Medford Regional Water Reclamation Facility

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Cover photo: 2019 sampling in the Rogue River, Stillwater Sciences

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ACRONYMS AND ABBREVIATIONS

Acronym	Definition
˚C	degrees Celsius
µg/L	micrograms per liter
ac	acre
ac-ft	acre-foot
AFDM	ash free dry mass
APHA	American Public Health Association
BMI	benthic macroinvertebrate
cfs	cubic feet per second
Chl-a	chlorophyll *a*
DO	dissolved oxygen
DL	detection limit
EPT	Ephemeroptera, Plecoptera, Trichoptera
ft	feet / foot
HDPE	high density polyethylene
ID	identification
MGD	million gallons per day
mg/L	milligrams per liter
MT	Montana
N	nitrogen
NH_3-N	ammonia
NIST	National Institute of Standard and Technology
NO_3-N	nitrate
N:P	nitrogen-to-phosphorus ratio
NPDES	National Pollutant Discharge Elimination System
NWEA	Northwest Environmental Advocates
OAR	Oregon Administrative Rules
ODEQ	Oregon Department of Environmental Quality
ORELAP	Oregon Environmental Laboratory Accreditation Program
P	phosphorus
PO_4-P	orthophosphate
QA	quality assurance
QC	quality control
RL	reporting limit
RM	river mile
RMS	root mean square
RMZ	regulatory mixing zone
RPD	relative percent difference
RWRF	Regional Water Reclamation Facility
SAV	submerged aquatic vegetation
SM	Standard Method
TDS	total dissolved solids
TIN	total inorganic nitrogen
TOC	total organic carbon
TP	total phosphorus
USEPA	United States Environmental Protection Agency
USGS	United States Geological Survey

1 INTRODUCTION

1.1 Background

As authorized by NPDES Permit 100985, issued by the Oregon DEQ, the City of Medford (City) Regional Water Reclamation Facility (RWRF) discharges secondary-treated and disinfected effluent to the Rogue River along its south (left) bank in Jackson County, Oregon at river mile (RM) 130.5. The RWRF has a design average dry weather outflow of 31 cubic feet per second (cfs) and hydraulic capacity of 149 cfs during wet weather events. The RWRF discharges into the Rogue River within the Middle Rogue River Sub-basin (HUC 17100308). The middle and upper portions of the Rogue River are located northeast of the Siskiyou Mountains and along the western edge of the Cascade Mountains, with its headwaters near Crater Lake.

In riffle habitat sampling conducted for the Rogue Fly Fishers & Federation of Fly Fishers in 2012, increases in algal density and changes in macroinvertebrate diversity and abundance were attributed to the RWRF discharges to the Rogue River (Hafele 2013). To assess water quality and ecological conditions of the Rogue River in the vicinity of the RWRF wastewater outfall, the City requested that Stillwater Sciences review previous studies (Hafele 2013, Brown and Caldwell 2014, ODEQ 2014) in relation to Oregon's biocriteria (OAR 340-041-0011) and other narrative water quality standards set forth in OAR 340-041-0007(9)-(13). Additionally, Stillwater Sciences conducted follow-up sampling in October 2018 at previous study sites plus some additional sites along the Rogue River upstream and downstream of the RWRF in order to assess the findings of the previous studies and provide estimates of temporal as well as site-to-site variability in the reported indices. Based on analysis and assessment of the previous 2012–2013 studies and additional information collected in 2018, periphyton and BMI indicators data suggest that the resident biological community downstream of the RWRF outfall was likely responding to nutrient enrichment downstream of the RWRF outfall. In addition to increases in apparent algae and macrophyte cover, statistically significant differences in periphyton biomass (cell density, and biovolume) and reductions in BMI indicators (total richness, EPT richness, EPT abundance and total sensitive individuals) were found at sites downstream of the RWRF.

This report summarizes the previous (2012–2013) studies and the 2018 assessment and discusses new information developed in 2019 in accordance with a sampling and analysis plan cooperatively developed with Northwest Environmental Advocates (NWEA) and dated August 16, 2019 to evaluate whether and to what extent nutrient discharge restrictions may be needed to address any RWRF contribution to water quality standards not being met in the river outside the RWRF's regulatory mixing zone (RMZ).

1.2 Purpose

Determine what degree of nutrient discharge reduction may be needed to ensure that the City's municipal wastewater treatment plant does not contribute to exceedances of the biocriteria standard (OAR 340-041-0011) in the Rogue River outside the regulatory mixing zone (RMZ) defined in the City's NPDES Permit.

1.3 Approach to Establishing Nutrient Limits

Although excessive algae biomass accrual is generally associated with reductions in BMI and periphyton diversity measures (Biggs 2000a), a variety of physical and chemical factors affect stream algae (e.g., shade, temperature, substrate, gradient, scour, grazing); nutrient levels alone have been shown to explain only 40–60% of the variations in algal biomass in rivers and streams (Lohman et al. 1992; Dodds et al. 1997). Recognizing uncertainties in linking nutrient levels to stream algae, USEPA (2000a) recommends one or more approaches to establish nutrient discharge limits, such as 1) comparisons of conditions at local or ecoregional reference sites; 2) application of predictive relationships to select nutrient concentrations that will result in appropriate levels of algal biomass, or 3) developing criteria from literature-based thresholds. This report uses a combined approach that relies upon site-specific data in the reach upstream and downstream of the RWRF (reference site approach), comparisons to existing predictive relationships between nutrients and algal biomass, as well as comparisons to existing nutrient thresholds from the literature.

In the sections below we present sampling methods to examine the relationship between Rogue River nutrient levels and periphyton and SAV biomass and community composition, followed by comparisons to predictive relationships and literature-based criteria.

2 METHODS AND RESULTS

2.1 Sampling Design

In order to provide comparability to previous studies as well as to increase the statistical power of conclusions from the analysis of data collected to date, six (6) sites were selected for repeat seasonal surveys, including two locations upstream of the RWRF outfall, the downstream end of the RMZ, and three other locations downstream of the outfall. The study sites are cross-referenced in Table 2-1 to study sites used in previous sampling efforts and shown on Figure 2-1. This was designed to allow sampling of algal biomass and community composition across a gradient of nutrient conditions in the vicinity of the RWRF.

Table 2-1. Summer/Fall 2019 locations for water quality and biological sampling

2019 site	Latitude (WGS84)	Longitude (WGS84)	Previous study site no.				Location relative to RWRF outfall	
			Hafele (2013)	Brown & Caldwell (2014)	ODEQ (2014)	Stillwater Sciences (2019)	Reach	RM
2	42.443384	-122.885543		Riffle 1		2	Upstream	1.1
3	42.438851	-122.897916	US1	Riffle 2	Lower 2	3		0.4
RMZ (N/S)[1,2]	42.438141	-122.905622					Down-stream	0.1
4 (N/S)[1]	42.438716	-122.913395	LS1	Riffle 3	Lower 3	4		0.4
5 (N/S)[1]	42.440351	-122.921109	LS2	Riffle 4	Lower 4	5		0.9
6	42.440134	-122.932468		Riffle 5		6		1.5

1 Due to incomplete mixing downstream of the RWRF outfall, sampling locations were split into north (N) and south (S) transects.
2 Downstream end of NPDES "regulatory mixing zone" (Site RMZ) was sampled for analytical water quality only.

Figure 2-1. 2019 water quality and line transect sampling locations

A brief description of the study sites is included below (See Appendix A for site maps). For detailed periphyton and aquatic vegetation cover estimates for each site, please refer to Section 2.5.1.

- Site 2 is adjacent to the eastern end of the TouVelle State Recreation Site, approximately 1.1 RM upstream of the RWRF outfall and 0.4 RM downstream of Little Butte Creek. Canopy cover was less than 10 percent and confined to the river margin. Average water depth across the stream varied with average water depths less than three feet, however the deepest areas were between three to four feet. Site 2 is characterized by a longitudinal bar with a small side channel on the left (south) bank and the majority of flow directed along the northern, larger channel. Substrate consisted of small sized cobbles and SAV coverage was sparse.
- Site 3 is adjacent to pasture and agricultural fields, approximately 0.4 RM upstream of the RWRF outfall and 1.1 RM downstream of Little Butte Creek. The site is adjacent to pasture and agricultural fields, with canopy cover less than 10 percent and confined to the river margin. Smaller tributaries channels also located upstream of Site 3 include the Modoc Pond drainage channel (0.6 RM), an unnamed drainage ditch which originates from Ken Denmark Wildlife Area and the Jackson County Fire District No. 3 (0.5 RM), and another unnamed drainage channel with multiple origin points (0.3 RM). Average water depth across the stream varied with average water depths less than three feet, however the deepest areas were between three to four feet. Site 3 is a lateral bar with a deeper thalweg along the south bank. Substrate consisted primarily of small sized cobbles and SAV was generally sparse with one large patch originating near the middle of the riffle.
- Site 4 is a lateral bar on the south bank, approximately 0.4 RM downstream of the RWRF outfall, extending from the left bank at Site 4S in a west-northwest direction to form a longitudinal bar along the channel centerline. Site 4N is the extension of the longitudinal bar approximately 0.18 RM downstream of Site 4S. Average water depth across the stream varied with average water depths less than three feet, however the deepest areas were between three to four feet along the right bank adjacent to Site 4N. The site is adjacent to pasture and agricultural fields, with canopy cover less than 10 percent and confined to the river margin. SAV coverage was extensive at Site 4S. SAV was found in a large patch along the northern bank of the gravel bar at Site 4N but was sparse along the majority of the riffle. Substrate at both Sites 4N and 4S consisted of small sized cobbles.
- Site 5 is a large, vegetated, longitudinal gravel bar, approximately 0.9 RM downstream of the RWRF outfall. The majority of flow is directed along the northern bank (Site 5N), with a smaller side channel along the south bank (Site 5S). The site is adjacent to pasture and agricultural fields, with canopy cover less than 10 percent and confined to the river margin. Site 5N lies along the north side of the bar and extends approximately 0.1 miles downstream of Site 5S. Site 5S is a smaller transverse bar feature at the head of the side channel on the south bank. Average water depth across the stream varied with average water depths less than three feet, however the deepest areas within Site 5N were between three to four feet. SAV coverage at Site 5S was greater and more continuous than at Site 5N. Substrate at each site was consistent and consisted primarily of small sized cobbles.
- Site 6 is a point bar with the thalweg located along the northern bank approximately 1.5 RM downstream of the RWRF outfall. The site is adjacent to pasture and agricultural fields, with canopy cover less than 10 percent and confined to the river margin. Average water depth across the stream varied with average water depths less than three feet, however the deepest areas were between three to four feet. Substrate consisted primarily of small sized cobbles and SAV coverage was minimal.

Because previous studies have shown incomplete mixing of effluent across the river cross-section for distances of up to 0.5–1 miles downstream of the RWRF (Brown and Caldwell 2014), several sampling sites were split between the north and south sides of the river channel including the RMZ site, Site 4, and at Site 5 (Appendix A). Sampling activities included monthly water quality grab samples, continuous in situ water quality monitoring events (August and October), cover and abundance of periphyton and submersed aquatic vegetation (SAV), as well as laboratory processing for biomass, species composition, and biomass estimates. The monitoring frequency of these activities is shown in Table 2-2, with methods detailed in Section 2.2.

Table 2-2. 2019 sampling frequency by location for water quality, benthic chlorophyll, biomass and enumeration of periphyton assemblage. (M=Monthly)

2019 site	Water quality		Benthic chlorophyll and biomass [1]	Periphyton enumeration
	In situ and grab samples	Continuous water quality	Cover, Chl-a, biomass	Cell density, biovolume, species ID
2	M (Jun–Nov)		M (Aug-Nov)	2X (Aug, Oct)
3	M (Jun–Nov)	2X (Aug, Oct)	M (Aug-Nov)	2X (Aug, Oct)
RMZ (N/S)[2,3]	M (Jun–Nov)			
4 (N/S)[2]	M (Jun–Nov)	2X (Aug, Oct)	M (Aug-Nov)	2X (Aug, Oct)
5 (N/S)[3,4,5]	M (Jun–Nov)	2X (Aug, Oct)[2]	M (Aug-Nov)	2X (Aug, Oct)
6	M (Jun–Nov)		M (Aug-Nov)	2X (Aug, Oct)

1 Includes analysis of chlorophyll-*a* (Chl-a) and ash free dry mass (AFDM).
2 Due to incomplete mixing downstream of the RWRF outfall, sampling locations were split into north (N) and south (S) transects.
3 Downstream end of NPDES "regulatory mixing zone" (Site RMZ) sampled for analytical water quality only
4 Continuous water quality monitoring occurred only at site 5S.
5 Water quality grab samples at Site 5 were collected as a single composite sample

The City initiated additional water quality grab sampling from the RWRF discharge and at eight (8) of the nine (9) *receiving* water sites in (Table 2-2) beginning in June, with samples collected at Site 5 collected as a single composite rather than the split (N/S) sampling described in the study plan. In addition to samples collected at each location, one equipment blank and one field duplicate were collected during each river sampling event. Including these quality assurance (QA) samples and samples from the RWRF outfall inlet, eleven (11) water quality samples were collected and submitted for laboratory processing during each sampling event. The City intends to continue to collect monthly water quality samples at least at one of the upstream river sites shown in Table 2-2 until DEQ renews the City's NPDES permit.

In addition to surface water grab sampling, continuous water quality monitoring was conducted in August and October at sites 3, 4 (north and south) and at site 5 (south) (Table 2-2). Due to equipment failure discussed further in Section 2.4.2, no data collection occurred at Site 5 during the August 2019 sampling event.

2.2 Sampling and Laboratory Methods

At each sampling location, site name, GPS coordinates, time, date, and crew member names along with observations of site conditions were recorded. Wadeable portions of riffle habitats

(<1m depth) as well as areas of SAV cover were delineated on field maps using a combination of tape, range finder, and GPS. Maps of sampling locations are included in Appendix A.

2.2.1 Surface water grab sampling

Eight (8) sampling sites were sampled for surface water quality by grab sampling, including three locations upstream of the RWRF outfall, as well as four locations downstream of the outfall (see Figure 2-1 and Table 2-2 for site locations). Sites sampled during this study were selected for comparison to previous studies, proximity to the RWRF outfall, and river accessibility. To provide comparability with existing data, sample collection and analysis generally conformed with ODEQ (2009) standard operating procedures for sampling of surface water quality, with minor modifications described below. Constituents to be analyzed for, their analysis methodology, and the method reporting limits are included in Table 2-3. Prior to sampling, equipment blanks were prepared using laboratory supplied deionized water transferred into a pre-cleaned 1 Liter HDPE (Nalgene) bottle used for sampling, with this water also used to examine any potential contamination from the high-density polyethylene (HDPE) bucket used for sample compositing.

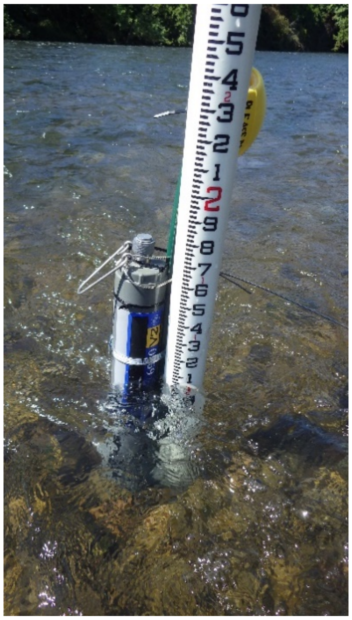
Using a modification of the bucket grab method in ODEQ (2009), surface water grab samples were collected using a 1 Liter HDPE bottle at four (4) equally spaced locations along a transect located at the upstream end of the riffles selected for Chl-a and periphyton sampling. A bottle rinsed with in situ water was filled in the stream at each sampling location. Contents were then transferred to a pre-rinsed HDPE bucket for measurement of in situ water quality and collection of analytical water quality grab samples. In situ water quality, including Temperature, DO, conductivity, and pH were measured using a pre-calibrated multi-probe (YSI Pro Plus), while turbidity was measured using grab samples and a portable turbidimeter (Hach 2100Q). Following recording of in-situ measurements, composited water samples were placed in laboratory supplied sample containers. The samples were preserved as appropriate for the analysis, stored on ice, and delivered to Neilson Research Corporation in Medford for analysis. Sample chain of custody forms are included with analytical water quality results in Appendix B. Samples were analyzed within EPA-specified holding times and were accomplished with appropriate quality control measures. Constituents used for analysis and reporting limits are included in Table 2-3.

Table 2-3. In situ and analytical water quality methods.

Parameter/Constituent	Method	Resolution/Method reporting limit
In-Situ Water Quality (YSI multi-parameter Sonde)		
Temperature	EPA 170.1	+ 0.15 °C
Dissolved oxygen	SM 4500-O	+ 0.2 mg/L or 2% of reading (0-20 mg/L)
pH	SM 4500-H	0.0625 s.u.
Specific conductance	SM 2510A	+ 0.5% of reading (0 -100 mS/cm)
Chlorophyll-a	In Vivo fluorescence	0.1 ug/L Chl; 0.1% RFU
Analytical Chemistry		
Ammonia	EPA 350.1	0.15 mg N/L
Nitrate + Nitrite	EPA 353.2	0.05 mg N/L
Total Kjeldahl Nitrogen	EPA 351.2 Cu	0.0625 mg N/L
Total Phosphorus	SM 4500PE	0.025 mg P/L
Orthophosphate	SM 4500PE	0.025 mg P/L
Total Organic Carbon[1]	SM 5310 C	0.1 mg/L

1 TOC results included in Appendix B only, but not included in analysis.

2.2.2 Continuous water quality monitoring



YSI 6920 deployed at Site 4N

In order to evaluate spatial and temporal patterns in DO and pH that may be affected by aquatic vegetation, multi-parameter water quality Sondes (EQUIPCO YSI 6920) were deployed at four sites upstream and downstream of the RWRF (Table 2-2) and programmed to collect data at 30-minute intervals for a period of not less than 48 continuous hours. Water quality parameters monitored are shown in Table 2-3. Sondes were pre-calibrated using manufacturer's recommended methods with pre-deployment calibration checks performed prior to deployment using methods consistent with USGS methods (Wagner et al 2006). Accuracy of each sonde was verified by instrument calibrations at standard conditions (e.g., oxygen in fully saturated air) and the use of standard solutions (e.g., pH and conductivity). Sondes were deployed at wadeable depths along the upstream end of the riffle at sites shown in Table 2-2. The Sondes were installed using short lengths of fence post or foundation stakes with secondary cable attachments to shore for security.

2.2.3 Sampling for benthic chlorophyll-*a* and ash free dry mass

To inform the development of relationships between ambient nutrient levels and the presence, abundance, and composition of algae and aquatic plant communities, line transect sampling was conducted at wadeable riffle locations shown in Table 2-2 using methods described by the State of Montana Dept. of Environmental Quality (MT DEQ 2011). A single sampling transect was established and photographed at each riffle location most representative of the proportions of riffle depths and SAV cover, with rebar bank pins and flagging to allow for repeat sampling. Sampled transect locations were adjusted in the field as necessary due to the availability of wadeable depth and velocities as well as safety considerations. See Appendix A for sampling maps as well as wadeable portions of the transects that were sampled.

To account for expected sample variance in biomass determinations, MT DEQ (2011) suggests a target total of 16 transect locations to be sampled if the riffle was wadeable in its entirety, with at least 11 samples collected from the wadeable region if the riffle was too swift or deep to wade in its entirety. At each of the sampling locations, representative conditions in an approximately 1 m^2 area were used to determine the selection of the appropriate sampling method (i.e., template or hoop). Quantitative cover estimates in each 1 m^2 station at each transect location were also estimated using methods adapted from Madsen (1999). Site-specific composite samples from each sampling method described below were prepared for laboratory determination of chlorophyll-*a* (Chl-*a*) and ash free dry mass (AFDM).

Scribe or "template" methods involved the collection of cobble sized rocks with large enough flat surfaces to accommodate sampling. Two rocks were collected from each transect location with one sample each collected for Chl-a and the other used for biomass (AFDM). To collect samples, a round 2-inch PVC pipe fitting (template) with an outside diameter of 5.6 cm was placed on each rock, and the algae located outside the scribe removed with a plastic-bristle brush and/or scraped off with a knife and discarded. The circular patch of algae remaining on the rock was then scraped into a basin, and the sample transferred to a 250 ml opaque sample bottle. Figure 2-3 illustrates a rock prepared for sampling of the remaining patch of known area. Periphyton samples for Chl-a analysis were kept in the dark, lab filtered to 0.70 microns, wrapped in aluminum foil, frozen, and shipped on dry ice to the laboratory for analysis. The remaining seven periphyton composite samples were refrigerated and shipped unpreserved on ice for laboratory analysis of AFDM.

Depending upon the accumulation of filamentous algae, macro-algae, or macrophytes, MT-DEQ (2011) provides a secondary "hoop" method of known diameter and area (e.g., approx. 707 cm^2 for 30 cm diameter). For each transect location containing high SAV cover, samples were collected by placing the hoop at the sample location with portions originating upstream or extending downstream of the hoop trimmed away with scissors. Samples were collected by hand by cutting the SAV within the hoop and transferred to a 1-gallon Ziploc bag. Fugitive plant material was captured using a D-frame kick net placed immediately downstream of the hoop with biomass transferred to the Ziploc bag. Once in the lab, hoop samples were rinsed with tap water to separate algae from the SAV with the SAV samples then returned to the Ziploc bag for later determination of AFDM. Rinse water was then lab filtered, wrapped in foil, and frozen to be included in the Chl-a analysis for the transect in accordance with MT DEQ (2011). All samples were wrapped in aluminum foil and refrigerated for shipment to Rhithron Associates Inc. for subsequent Chl-a and AFDM determinations. Field data sheets and sample chain of custody forms are included in Appendices C and D.

Figure 2-2. Example of rocks cleaned of algae, prior to compositing.

Table 2-4. Laboratory methods for biomass and enumeration of periphyton and submersed aquatic vegetation.

Parameter/Constituent	Method	Resolution/Method reporting limit
Aquatic Vegetation Cover	Modified visual/line-transect (Madsen 1999)	<10%
Ash free dry Mass (AFDM)	SM 2540 D, E	0.0001 g
Chlorophyll-*a* in periphyton	EPA 446.0 (Arar 1997)	0.05 ug/L
Diatom and Soft Algae ID and Enumeration	Charles et al (2002)	NA
Aquatic Vegetation ID	Taxonomic Keys (Gilkey and Dennis 2001, Pojar and MacKinnon 2004, Hitchcock and Cronquist 1973)	ID to species, subspecies, or variety, as appropriate

To verify field identification of SAV and macroalgae encountered during transect sampling for benthic Chl-a, separate samples were collected, stored in Ziploc bags, and refrigerated for later identification. Submerged aquatic plants were identified to species, subspecies, or variety, as appropriate, given phenology at the time of sampling. Identification followed taxonomic keys for the Pacific Northwest (Table 2-4) or more specific references to Oregon.

2.2.4 Sampling for periphyton enumeration and community composition

To maintain consistency with previous sampling efforts (Hafele 2013, Brown & Caldwell 2014, Stillwater Sciences 2019) attached algae (periphyton) sampling methods followed those described by the USGS for periphyton (Carpenter, 2003) and used in previous studies. Two rocks were collected from each of ten locations selected randomly from wadeable portions of riffle habitats at each sample site. Selected rocks had a large enough flat surface to accommodate scribe sampling methods described above (Section 2.2.3). Two ten-rock composite samples were prepared for each site using the scribe methods described above for benthic chlorophyll (See Figure 2-3). Composite samples were placed into two 250 ml dark amber sample bottles, labeled, preserved on ice, and refrigerated until shipping to Rhithron Laboratories for identification and enumeration (cell density, and biovolume).

2.2.5 Laboratory methods for determination of biomass and community composition

Samples sent to Rhithron Associates, Inc. were unpacked, examined, and checked against the chain of custody form that accompanied each sample. Gross biomass as ash free dry mass (AFDM) was determined by gravimetric analysis using APHA standard methods 2540 D and E. For Chl-a, pigments retained on filter samples were processed according to EPA method 446.0 (Arar 1997), using a HACH D5000 spectrophotometer. Data for AFDM and Chl-a were normalized to the total template or hoop areas sampled along the transect, and transect averages estimated using methods in MT DEQ (2011).

Samples to be analyzed by microscopy for biovolume and enumeration by species, were preserved upon receipt at the laboratory, with initial sample volumes measured and recorded before samples being thoroughly mixed and split into 2 aliquots for diatom and soft-bodied algae analysis. Permanent diatom slides were then prepared, and subsamples taken and treated with 70% Nitric acid and digested following methods developed by the National Academy of Sciences, Philadelphia (ANSP 2002). Samples were then neutralized with rinses of distilled water and subsample volumes adjusted to achieve adequate densities for slide mounts. Replicate samples were made of each sample and a replicate was selected from each sample batch for identification.

A transect line was made on the cover slip using a diamond scribe mark and a minimum of 600 diatom valves were identified using a compound microscope along each transect. Diatoms were identified to the lowest possible taxonomic level, generally species, following standard taxonomic references. Biovolume measurements were performed consistent with NAWQA protocols and data requirements (ANSP 2002).

Soft-bodied algae samples were identified to genus using a compound microscope following standard taxonomic references. 300 natural units of algae were counted and identified in addition to total cells. Living diatom cells were also included in these counts. Biovolume measurements were performed consistent with NAWQA protocols and data requirements (ANSP 2002).

Quality control procedures involved checking accuracy, precision and enumeration of samples. One sample was randomly selected, and all organisms re-identified and counted by an independent taxonomist. Representatives of each identified taxon were also photographed and verified by Rhithron. Laboratory reports are included in Appendix D.

2.3 Quality Assurance

The objective of data collection for this sampling plan was to produce data that represent, as closely as possible, in situ conditions of the Rogue River in the vicinity of the Medford RWRF with respect to water chemistry affecting algae density and assemblage. Sampling and laboratory quality assurance/quality control (QA/QC) and data reporting were performed in accordance with ODEQ requirements for minimum data acceptance. Quality assurance guidelines included adherence to standard sampling and handling methods, and sampling control through standard chain of custody forms maintained at each laboratory. All collected samples were described by field notes, labeled with the Project name, site identification, sample type, date and time sampled, preservatives used, constituent analyses required, and the sampler's name.

As described further below, data quality parameters used to assess the acceptability of the data were precision, accuracy, representativeness, comparability, and completeness.

2.3.1 Precision

Precision measures the reproducibility of measurements under a given set of conditions. For laboratory analyses of collected water samples, precision is measured through duplicate samples analyzed at a minimum frequency of once per laboratory analysis group or 1 in 10 samples, whichever is more frequent, per matrix analyzed. Laboratory precision was evaluated against quantitative relative percent difference (RPD) performance criteria. Field precision was evaluated by the collection of duplicate samples. One field duplicate per field effort was collected. Field duplicate precision was screened against an RPD criterion of 25% for water samples.

Recognizing that typical coefficients of variation in periphyton and SAV sampling may approach

100%, information about the variability among measurements is inherent to the collection design including at least 11 transect sampling locations as well as conducting multiple sampling events to achieve temporal replication. Nevertheless, duplicate sampling across multiple river assessments conducted by MT DEQ (2011) indicates RPD for algal biomass to be on the order of 30%.

2.3.2 Accuracy

Accuracy is an expression of the degree to which a measured or computed value represents the true value. Accuracy is controlled by adherence to sample collection procedures (i.e., approved sampling methodology) as well as instrument calibrations at standard conditions (e.g., DO in saturated air) or with standard solutions (e.g., pH, conductivity).

For in-situ water quality sampling, the field multiparameter instrument was subjected to pre-deployment calibrations as well as post-sampling calibration checks conducted at the end of each sampling day. Laboratory accuracy was assessed by analyzing "spiked" samples with known standards (surrogates, laboratory control samples, and/or matrix spike) and measuring the percent recovery. Accuracy measurements on matrix spike samples are performed at a minimum frequency of one in 20 samples per matrix analyzed, with results rejected if they were outside of the 80–120% recovery range.

Field accuracy was assessed by preparation of duplicate samples as well as "blanks" prepared using laboratory-supplied de-ionized water labeled as a field sample (i.e., blind). For sample batches with blank results exceeding 10% above the laboratory MRL, all reported results falling below the blank were qualified as provisional data only.

For periphyton and SAV, because of their patchy distribution there is no way to know whether algal biomass or species composition are representative of the true values of these metrics. Based upon studies across multiple rivers used in the development of the MT DEQ (2011) SOP, average benthic Chl-a measured during a sampling event was estimated to be within ± 30% of the true population average at an 80% confidence level.

2.3.3 Representativeness

Representativeness expresses the degree to which data represent the true environmental condition. For this study, the parameters listed in Table 2-2 were selected based on parameters measured in previous studies and typical parameters of concern at other publicly owned treatment works. In addition to comparisons of study findings to a well-founded body of scientific knowledge from reputable sources in the final report, the report was subjected to internal as well as external peer review.

2.3.4 Comparability

Comparability expresses the confidence with which one data set can be evaluated in relation to another data set. Comparability is established through the sampling in comparable habitat types (e.g., wadeable riffle habitats), use of standard sampling and analytical methodologies and reporting formats as in previous studies, the use of standard methods, as well as common National Institute of Standard and Technology (NIST) or other traceable calibration and reference materials.

2.3.5 Completeness

Completeness is a measure of the amount of data that is determined to be valid in proportion to the amount of data collected. Data that have been qualified as estimated because the quality control criteria were not met were considered valid for the purpose of assessing completeness. Data that have been qualified as rejected were not considered valid for the purpose of assessing completeness.

2.4 Water Quality Results

Water chemistry testing was conducted by Neilson Research Corporation in Medford, Oregon (ORELAP ID: OR100016). Laboratory reports for analytical water chemistry are included in Appendix B. QC review of laboratory testing data centered on the accuracy and precision of the reported results. Upon receipt of analytical water quality results from the laboratory, QA/QC reviews included review of laboratory method detection limits (DLs) and reporting limits (RLs), results of method blanks, equipment blanks, and comparisons to relative percent difference (RPD) criteria. In QC reviews of the data, low level exceedances of equipment blanks in some events (Table 2-5) exceedances of RPD criteria were apparent in some samples collected at Site 2. It should be noted that because this location had both low nutrient levels and may be influenced by incomplete mixing of water arriving from Little Butte Creek, composite samples may have captured greater and lesser amounts of water arriving from upstream reaches. Despite these limitations, because multiple sampling events were conducted and the majority met the RPD criterion, the water quality sampling results were accepted for subsequent analyses.

Table 2-5. Analytical water quality results.

Site ID	Sample Date	Ammonia (mg-N/L)	Nitrate/ Nitrite (mg-N/L)	Total Kjeldahl Nitrogen (mg-N/L)	Total Phosphorous (mg-P/L)	Orthophosphate (mg-P/L)	Specific Conductance (umhos/cm)
June 2019							
Site 2	26-Jun	0.074^{J}	<DL	0.275^{J}	0.046	0.0179^{J}	58.2
Site 2 Duplicate	26-Jun	0.080^{J}	<DL	0.220^{J}	0.046	0.0162^{J}	56.1
Site 3	26-Jun	0.082^{J}	<DL	0.210^{J}	0.039	0.0128^{J}	56.8
RMZ North	26-Jun	0.070^{J}	<DL	0.378^{J}	0.049	0.0179^{J}	55.6
RMZ South	26-Jun	0.261^{J}	0.344	0.515^{J}	0.130	0.1060.	72.2
Site 4N	26-Jun	0.435^{J}	<DL	0.182^{J}	0.046	0.0111^{J}	54.9
Site 4S	26-Jun	0.126^{J}	0.098	0.285^{J}	0.066	0.0435	60.4
Site 5	26-Jun	0.147^{J}	0.063	0.322^{J}	0.049	0.0265	58.6
Site 6	26-Jun	0.168^{J}	0.110	0.268^{J}	0.062	0.0384	60.7
RWRF Outfall	26-Jun	7.130	10.300	10.400	3.280	2.7500	519.0

Site ID	Sample Date	Ammonia (mg-N/L)	Nitrate/ Nitrite (mg-N/L)	Total Kjeldahl Nitrogen (mg-N/L)	Total Phosphorous (mg-P/L)	Orthophosphate (mg-P/L)	Specific Conductance (umhos/cm)
July 2019							
Site 2	17-Jul	<DL	<DL	0.195[J, 1]	0.072	0.0362	62.6
Site 2 Duplicate	17-Jul	<DL	<DL	0.083[J, 1]	0.061	0.0347	59.3
Site 3	17-Jul	<DL	<DL	0.135[J]	0.0533	0.0393	60.6
RMZ North	17-Jul	<DL	<DL	0.093[J]	0.046	0.0517	59.4
RMZ South	17-Jul	0.202	0.664	0.540[J]	0.204	0.1650	86.7
Site 4N	17-Jul	<DL	<DL	0.090[J]	0.0624	0.022[J]	60.2
Site 4S	17-Jul	0.125	0.315	0.200[J]	0.138	0.0937	74.6
Site 5	17-Jul	<DL	0.106	0.043[J]	0.074	0.0455	64.1
Site 6	17-Jul	<DL	0.110	0.242[J]	0.125	0.0471	64.9
RWRF Outfall	17-Jul	5.600	12.000	7.030	3.130	2.5800	500.0
August 2019							
Site 2	21-Aug	<DL	<DL	0.580[J]	0.044	0.0238[J]	64.3
Site 2 Duplicate	21-Aug	<DL	<DL	0.470[J]	0.048	0.0269	63.1
Site 3	21-Aug	<DL	<DL	0.122[J]	0.046	0.0300	63.6
RMZ North	21-Aug	<DL	<DL	0.125[J]	0.034	0.0207[J]	62.2
RMZ South	21-Aug	0.285	0.114	0.600[J]	0.138	0.0781	74.3
Site 4N	21-Aug	<DL	<DL	<DL	0.039	0.0238[J]	69.7
Site 4S	21-Aug	0.169	0.065	0.185[J]	0.100	0.0704	75.5
Site 5	21-Aug	<DL	0.025[J]	0.152[J]	0.081	0.0595	67.4
Site 6	21-Aug	<DL	0.029[J]	0.070[J]	0.076	0.0440	71.0
RWRF Outfall	21-Aug	7.310	2.670	10.200	2.260	2.1500	491.0
September 2019							
Site 2	18-Sep	<DL	<DL	0.378[J]	0.059	0.0378[1]	76.9
Site 2 Duplicate	18-Sep	<DL	<DL	0.432[J]	0.072	0.0502[1]	78.0
Site 3	18-Sep	<DL	<DL	0.348[J]	0.059	0.0300	74.8
RMZ North	18-Sep	<DL	<DL	0.262[J]	0.081	0.0300	73.2
RMZ South	18-Sep	0.523	0.291	1.080	0.201	0.1510	99.4
Site 4N	18-Sep	<DL	<DL	0.375[J]	0.064	0.0222[J]	73.4
Site 4S	18-Sep	0.449	0.238	0.695	0.186	0.1360	97.3
Site 5	18-Sep	<DL	0.026[J]	0.618[J]	0.077	0.0362	76.2
Site 6	18-Sep	0.151	0.087	0.265[J]	0.100	0.0611	81.1
RWRF Outfall	18-Sep	10.300	5.220	12.700	3.050	2.7800	563.0

Site ID	Sample Date	Ammonia (mg-N/L)	Nitrate/ Nitrite (mg-N/L)	Total Kjeldahl Nitrogen (mg-N/L)	Total Phosphorous (mg-P/L)	Orthophosphate (mg-P/L)	Specific Conductance (umhos/cm)
RWRF Outfall 00:12	18-Sep	13.500	5.510	15.200	3.280	2.9600	528.0
RWRF Outfall 02:53	18-Sep	13.100	4.720	15.300	3.100	2.8200	551.0
RWRF Outfall 05:57	18-Sep	12.800	4.370	14.300	3.100	2.7400	549.0
RWRF Outfall 08:54	18-Sep	9.770	6.070	12.400	3.030	2.7200	530.0
RWRF Outfall 12:07	18-Sep	7.680	8.060	9.360	3.030	2.6100	553.0
RWRF Outfall 14:51	18-Sep	8.380	6.670	8.950	2.950	2.6100	585.0
RWRF Outfall 17:47	18-Sep	11.600	6.340	14.600	3.260	2.8500	602.0
RWRF Outfall 20:49	18-Sep	16.900	5.000	16.600	3.760	3.4100	616.0
October 2019							
Site 2	16-Oct	<DL	<DL	0.160[J, 1]	0.072	0.0533	74.5
Site 2 Duplicate	16-Oct	<DL	<DL	0.100[J, 1]	0.061	0.0533	75.0
Site 3	16-Oct	<DL	<DL	0.128[J]	0.040	0.0310	73.3
RMZ North	16-Oct	<DL	<DL	0.105[J]	0.041	0.0295	72.5
RMZ South	16-Oct	0.418	0.464	0.633	0.198	0.1560	97.8
Site 4N	16-Oct	<DL	<DL	0.135[J]	0.053	0.0340	72.4
Site 4S	16-Oct	0.191	0.164	0.340[J]	0.093	0.0801	82.5
Site 5	16-Oct	0.123	0.094	0.188[J]	0.067	0.0578	77.5
Site 6	16-Oct	0.123	0.109	0.150[J]	0.072	0.0563	80.1
RWRF Outfall	16-Oct	9.440	1.010	11.700	3.360	2.8400	596.0
November 2019							
Site 2	20-Nov	<DL	<DL	0.208[J]	0.058	0.0340[1]	70.2
Site 2 Duplicate	20-Nov	<DL	<DL	0.178[J]	0.054	0.0459[1]	70.1
Site 3	20-Nov	<DL	<DL	0.192[J]	0.048	0.0340	71.2
RMZ North	20-Nov	<DL	<DL	0.095[J]	0.048	0.0355	69.7
RMZ South	20-Nov	0.242	0.218	0.342[J]	0.130	0.1050	85.8
Site 4N	20-Nov	<DL	<DL	0.065[J]	0.056	0.0370	71.1
Site 4S	20-Nov	0.122	0.111	0.200[J]	0.087	0.0697	80.2
Site 5	20-Nov	0.076[J]	0.072	0.145[J]	0.075	0.0578	74.9
Site 6	20-Nov	0.133	0.136	0.262[J]	0.076	0.0757	79.7
RWRF Outfall	20-Nov	9.760	10.500	12.400	3.730	3.0900	626.0

Site ID	Sample Date	Ammonia (mg-N/L)	Nitrate/ Nitrite (mg-N/L)	Total Kjeldahl Nitrogen (mg-N/L)	Total Phosphorous (mg-P/L)	Orthophosphate (mg-P/L)	Specific Conductance (umhos/cm)
Quality Assurance and Quality Control							
Field Duplicates Relative Percent Difference	*26-Jun*	*8%*	*0%*	*22%*	*0%*	*10%*	*4%*
	17-Jul	*0%*	*0%*	*81%*	*17%*	*4%*	*5%*
	21-Aug	*0%*	*0%*	*21%*	*7%*	*12%*	*2%*
	18-Sep	*0%*	*0%*	*13%*	*20%*	*28%*	*1%*
	16-Oct	*0%*	*0%*	*46%*	*17%*	*0%*	*1%*
	20-Nov	*0%*	*0%*	*16%*	*6%*	*30%*	*0%*
Equipment Blank	*26-Jun*	*<DL*	*<DL*	*0.148*[J]	*<DL*	*<DL*	*1.0*
	17-Jul	*<DL*	*<DL*	*<DL*	*<DL*	*<DL*	*1.1*
	21-Aug	*<DL*	*<DL*	*<DL*	*<DL*	*0.0083*[J]	*<DL*
	18-Sep	*<DL*	*<DL*	*<DL*	*0.008*[J]	*<DL*	*<DL*
	16-Oct	*<DL*	*<DL*	*<DL*	*<DL*	*0.0057*[J]	*1.4*
	20-Nov	*<DL*	*<DL*	*<DL*	*<DL*	*0.0087*[J]	*<DL*
DL		0.064	*0.022*	*0.028*	*0.00277*	*0.00301*	***1***
QL		0.5	*0.05*	*0.625*	*0.025*	*0.025*	***1***

J Result below laboratory method reporting limit (RL), but above method detection limit (DL) and is reported here as a J-flag; therefore, result is an estimated concentration
1 Values exceed the field duplicate relative percent difference threshold of 25%.

2.4.1 Spatial variation in analytical water quality

Analytical water quality results for dissolved nutrients are presented in Figure 2-3 through Figure 2-5 and Table 2-5 above. For presentation purposes, analytical water quality results, are arranged in upstream to downstream order with Site 2 at right. Low nutrient concentrations near or below analytical detection limits (DLs) were observed at sites upstream of the RWRF (Table 2-5). Laboratory DLs are concentrations at which the lab can report with 99 percent confidence that the analytical result is not actually zero. This is usually three times the standard deviation based upon analysis of replicated spike additions to pure water at the expected DL (Oblinger Childress et al. 1999). The RL is more subjective and is set by each lab differently due to matrix interferences and changes in their internal QC results, but typically the RL is set at five times the standard deviation, as determined above, added to the DL. All results falling below laboratory RLs but above the DLs were noted by the laboratory as "J-flagged" to represent lower confidence levels (Appendix B). J-flagged data should be considered semi-quantitative data in that they are not "zero" but replicate samples and analysis would likely show a high degree of variability. Overall, the analytical water quality results indicate that nutrients are highest downstream of the RWRF outfall discharge, with lower concentrations observed at all upstream sites as well as Site 4N, located along the north bank outside of the RMZ Inorganic nitrogen sources (Ammonia, Nitrate plus Nitrate) were near or below detection limits at Site 4N and all sites upstream of the RWRF. Both due to turbulent mixing processes as well as biological uptake, inorganic nitrogen as well as orthophosphate concentrations decrease with distance at downstream sites. A brief summary of the reported ranges is included below by parameter.

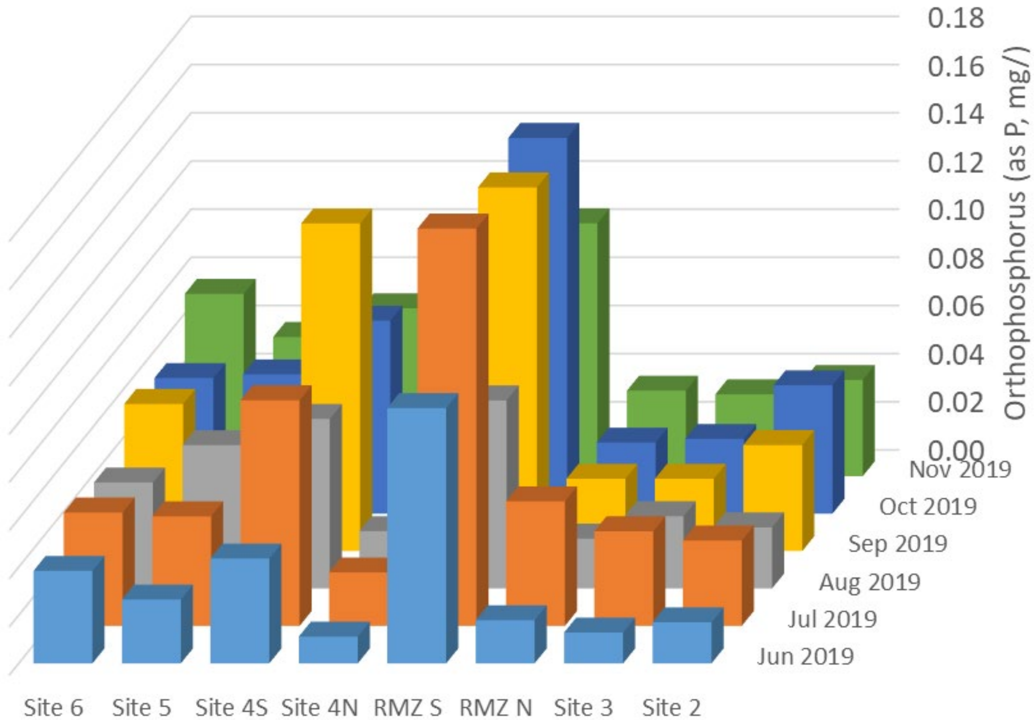


Figure 2-3. Rogue River sites near Medford WWTP analytical results for Orthophosphate.

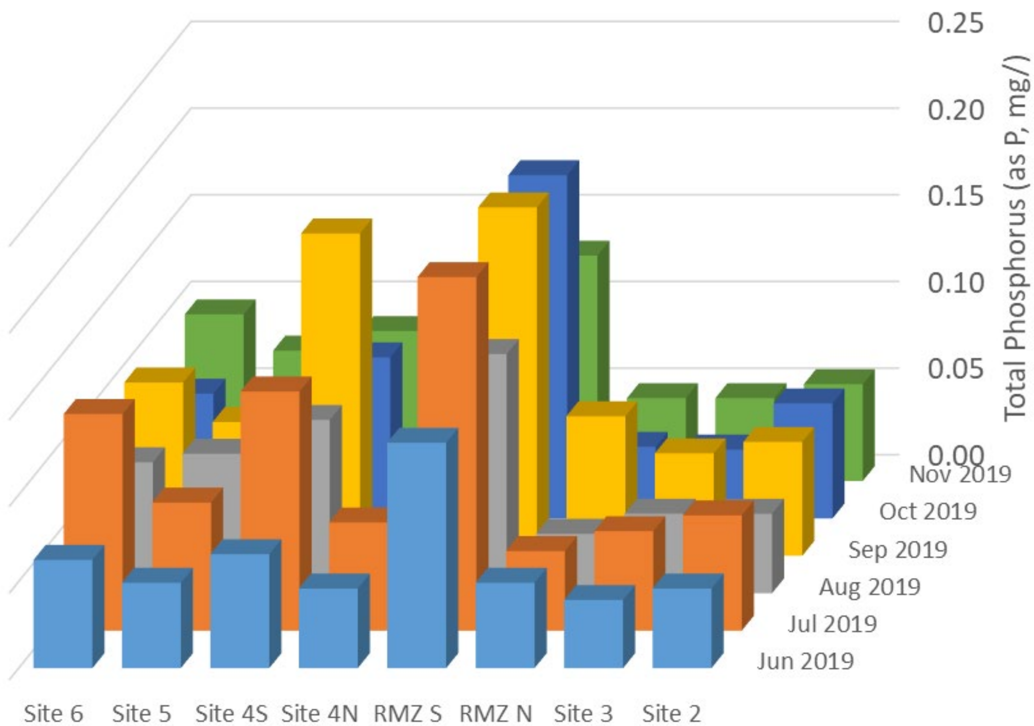


Figure 2-4. Rogue River sites near Medford WWTP analytical results for Total Phosphorous.

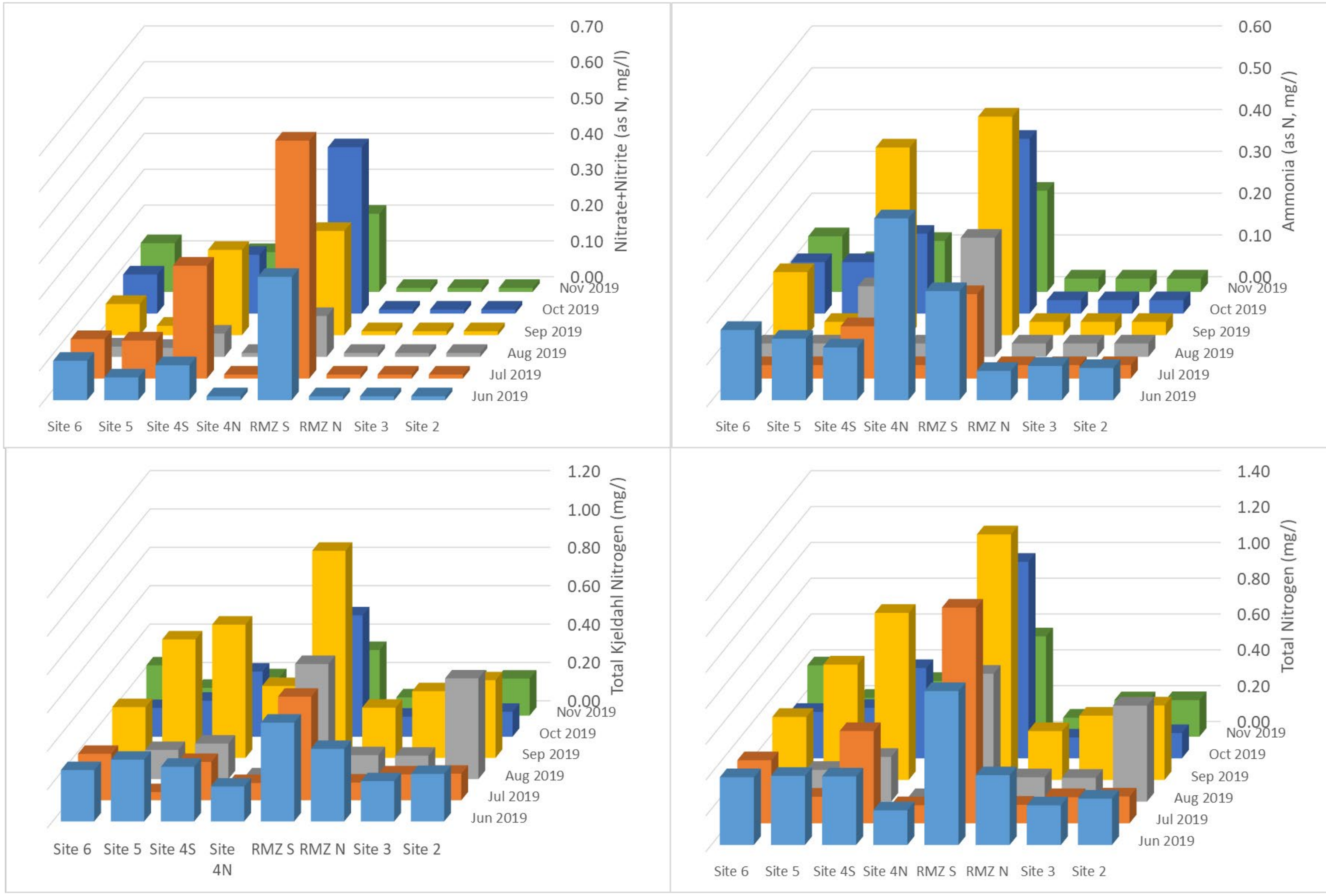


Figure 2-5. Analytical water quality results for Nitrate-Nitrite, Ammonia, Total Kjeldahl Nitrogen, and Total Nitrogen.

Orthophosphate (PO_4-P) ranged from 0.03–0.05 mg/L with an average of 0.04 mg/L at Sites 2 and 3 upstream of the RWRF and 0.01–0.14 mg/L with an average of 0.05 mg/L at all sites downstream of the RMZ (Figure 2-3). Orthophosphate ranged from 0.08–0.13 mg/L with an average of 0.13 mg/L at RMZ-S. Total phosphorus (TP) ranged from 0.04–0.07 mg/L with an average of 0.05 mg/L in the reach upstream of the RWRF and 0.04–0.19 mg/L with an average of 0.08 mg/L at all sites downstream of the RMZ (Figure 2-4). Within the RMZ, Total P ranged from 0.13–0.20 mg/L with an average of 0.17 mg/L at RMZ-S (Table 2-5).

Total Kjeldahl nitrogen ranged from 0.12–0.58 mg/L with an average of 0.19 mg/L at Sites 2 and 3 upstream of the RWRF, and <DL–0.70 mg/L with an average of 0.24 mg/L at all sites downstream of the RMZ (Figure 2-5). Total Kjeldahl nitrogen ranged from 0.34-1.08 mg/L with an average of 0.62 mg/L at RMZ-S (Table 2-5).

Ammonia-N concentrations at Sites 2 and 3 upstream of the RWRF were 0.07 mg/L and 0.08 mg/L respectively during the June sampling event and less than the analytical detection limit (DL) of 0.064 mg/L during all other sampling events. (Figure 2-5). Ammonia-N concentrations downstream of the RMZ varied at each site as concentrations at site 4N were below DL during each sampling event and site 5 and 6 were below DL during the July, August, and September sampling events. Ammonia-N concentrations at site 4S ranged from 0.12–0.45 mg/L with an average of 0.20 mg/L (Table 2-5). Ammonia-N concentrations ranged from and 0.20–0.52 mg/L with an average of 0.32 mg/L at RMZ-S.

Nitrate concentrations at Sites 2 and 3 upstream of the RWRF were less than the DL (0.02 mg/L) (Figure 2-5). Nitrate-N concentrations were also less than DL at Site 4N, but ranged from and 0.03–0.32 mg/L with an average of 0.11 mg/L at all other sites downstream of the RMZ (Table 2-5). Nitrite-N concentrations ranged from and 0.11–0.66 mg/L with an average of 0.35 mg/L at RMZ S.

2.4.2 Continuous water quality sampling

Four continuously recording multi-parameter water quality sondes (YSI 6920) were deployed at four locations within the Project Area during the weeks of August 20 and October 14 , 2019 to characterize in-situ water quality parameters (temperature, pH, Chl-a, DO, and specific conductivity) at approximately 30-minute intervals for up to 48 hours. Water quality sondes were deployed at upstream and downstream locations to characterize conditions both upstream and downstream of the RWRF (Figure 2-1). After the 48-hour monitoring period, post-deployment calibration checks were performed and recorded in a calibration log (Appendix B) and data were downloaded into MS Excel.

Data quality reviews included identification of periods when the sondes were deployed in the Rogue River as well as identification of results that were unexpectedly higher or lower than to be expected, as well as identification of other anomalies related to sensor fouling or other issues. The primary data exclusion was applied to time periods when the sondes were not physically deployed in the Rogue River. In other words, records were excluded for dates and times prior to when the sondes were deployed at the sampling sites as well as records from periods after the sonde was retrieved from the site. These records can be roughly determined by comparing the deployment and retrieval times that were recorded (noting that sonde records are standardized at 30-minute recording intervals) and by examining the pressure variable and depth variable which provides an indication when the sonde is positioned in the water column. Both methods were used to determine which records to exclude.

Sensor malfunctions during the August sampling event that were not detected during post deployment checks resulted in the exclusion of data from the data record for the following reasons:

1. Subsequent to the sonde retrieval and data download on 8/22/2019, it was noted that the DO optical sensor wiper malfunctioned at the Site 4N Sonde on 8/19/2015 at 15:00. No other data concerns were detected, and the wiper was functioning during both pre and post calibration checks.
2. Subsequent to the sonde retrieval and data download on 8/22/2019, it was noted that the DO optical sensor at the Site 5 Sonde failed immediately following deployment. No other data concerns were detected, and the DO sensor was functioning during both pre and post calibration checks.

Anomalous chlorophyll-a readings were apparent across all sondes deployed during both the August and October sampling events. Because the nearest source of suspended algae was upstream in Lost Creek Lake upstream of William Jess Dam at RM 158, Chl-a concentrations were expected to be near zero in the lotic Rogue River habitats upstream of the RWRF. Inspection of the data from the August and October events shows low Chl-a readings interspersed with improbably high readings with results that were off scale in most instances. While this may potentially be the result of particulate fouling of the optical sensor, a decision was made to not report the accumulated Chl-a data.

Following QA/QC review, continuous water quality sampling data collected during both August (8/20/2019-8/22/2019) and October (10/14/2019–10/17/2019) sampling events was summarized by quantiles (Table 2-6). Diel patterns over a 48-hour period during the August and October sampling events are shown in Figure 2-6 and Figure 2-7. Available electronic data records collected during August and October 2019 are available upon request.

Table 2-6. Continuous water quality monitoring data summary.

Parameter	Units[1]	Quartile	Site 3		Site 4N		Site 4S		Site 5	
			August	October	August	October	August	October	August	October
Temperature	°C	Median	15.1	8.0	13.4	6.5	15.6	9.4	15.6	8.8
		Q 1	14.4	7.3	12.7	5.9	14.8	8.6	14.9	8.1
		Q 3	16.5	8.6	14.9	6.8	17.4	9.6	17.4	9.0
pH	s.u.	Median	8.2	7.3	8.3	7.7	7.5	6.4	7.3	7.3
		Q 1	7.7	7.1	8.0	7.4	7.1	6.3	6.9	7.1
		Q 3	8.7	7.7	8.9	8.1	8.0	6.6	7.7	7.5
pH	mV	Median	-49.7	-10.2	-71.2	-45.4	-23.2	47.9	-50.9	-20.8
		Q 1	-70.0	-27.1	-103.8	-71.2	-46.4	38.3	-71.5	-32.9
		Q 3	-25.0	-1.6	-54.6	-31.2	-7.0	53.5	-27.8	-14.5
DO	mg/L	Median	9.7	11.3	10.2	11.9	10.1	10.3	NA[1]	10.5
		Q 1	9.1	11.0	9.3	11.4	9.6	9.7	NA[1]	10.0
		Q 3	10.4	12.0	11.5	12.8	11.2	12.1	NA[1]	11.7
DO saturation	%	Median	96	95	98	97	105	90	NA[1]	90
		Q 1	89	93	93	93	95	84	NA[1]	85
		Q 3	104	101	111	104	114	105	NA[1]	100
Specific conductivity	uS/cm	Median	62.0	72.0	72.0	73.0	122.0	119.0	90.0	100.0
		Q 1	62.0	69.0	72.0	73.0	111.0	114.5	85.0	96.8
		Q 3	63.0	81.0	73.0	74.0	125.0	124.5	91.5	103.0

1 DO sensor malfunction

Water temperature

Continuous records of water temperature collected during the August sampling event show median temperatures between 13.4°C to 15.6°C (Table 2-6). Continuous records of water temperature collected during the October sampling event show median temperatures between 6.5°C and 9.4°C (Table 2-6).

pH

Continuous records of pH collected during the August sampling event show median pH ranging between 7.3 and 8.3 across all sites, with site 4N exhibiting the highest pH (Table 2-6). Because photosynthesis by periphyton raises the pH through a shift in the inorganic carbon equilibrium that results from algal uptake of carbon dioxide, these results show continued effects of algal accumulations shown in Brown and Caldwell (2014) as well as these conditions being influenced by potential nutrient sources upstream of the RWRF. Note that Brown and Caldwell (2014) found pH > 7.5 at Riffle 3 (Site 4) during October 2013. pH exceeded ODEQ criteria (pH 6.5–8.5) for short periods at sites upstream of the RWRF (Sites 3 and 4N) during August (Figure 2-6), with minor exceedances with pH>8.5 at Site 4N as well as pH < 6.5 at Site 4S during October (Figure 2-7). Although calibration checks conducted before and after deployment suggested that pH readings at Site 4S were accurate, the time series plot shows high variability between readings that suggests the observed low pH readings at Site 4S may be due to a sensor malfunction during the October sampling event. pH readings at Site 4S were within ODEQ criteria and similar to those found at Site 5 during August (Figure 2-6).

Dissolved oxygen

Continuous records of DO collected during the August sampling event upstream of the RWRF showed averages ranging from 9.1 mg/L to 9.7 mg/L with corresponding percent saturation ranging from 89 to 96 percent (Table 2-6). Downstream of the RWRF, DO showed averages ranging from 9.3 mg/L to 11.5 mg/L with corresponding percent saturation ranging from 93 to 114 percent. The DO sensor at Site 5 malfunctioned and as a result, no DO data is available during the August sampling event. Continuous records of DO collected during the October sampling event upstream of the RWRF showed averages ranging from 11.0 mg/L to 12.0 mg/L with corresponding percent saturation ranging from 93 to 101 percent. Downstream of the RWRF, DO showed averages ranging from 9.7 mg/L to 12.8 mg/L with corresponding percent saturation ranging from 84 to 104 percent. Site 4N exhibited the highest DO levels across all sites during the October sampling event. As discussed for pH above, these results are consistent with photosynthetic DO production by periphyton during daylight hours at locations both upstream and downstream of the RWRF.

Specific conductivity

Continuous records of specific conductivity collected during the August and October sampling events show moderate levels of conductivity with the influence of dissolved solids from the RWRF effluent discharge indicated at downstream sites. Median specific conductivity in August ranged from 62 uS/cm to 122 uS/cm with a similar range (72 uS/cm to 119 uS/cm) found during the October monitoring event (Table 2-6). Across both sampling events, the sonde deployed above the RWRF at Site 3 had the lowest specific conductivity while Site 4S downstream of the RWRF had the highest.

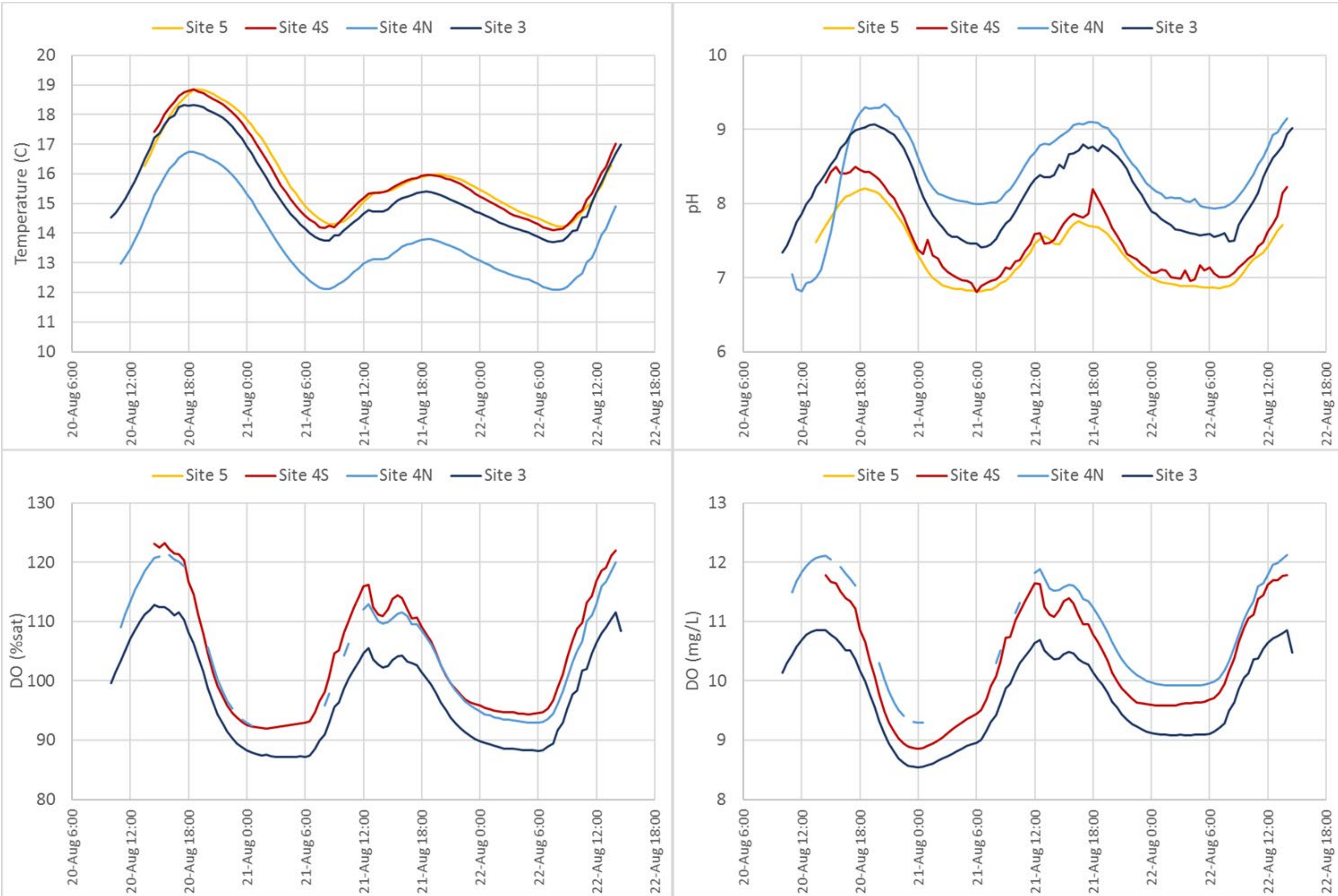


Figure 2-6. Diel patterns of selected in situ water quality parameters in the vicinity of the Medford RWRF during August 2019.

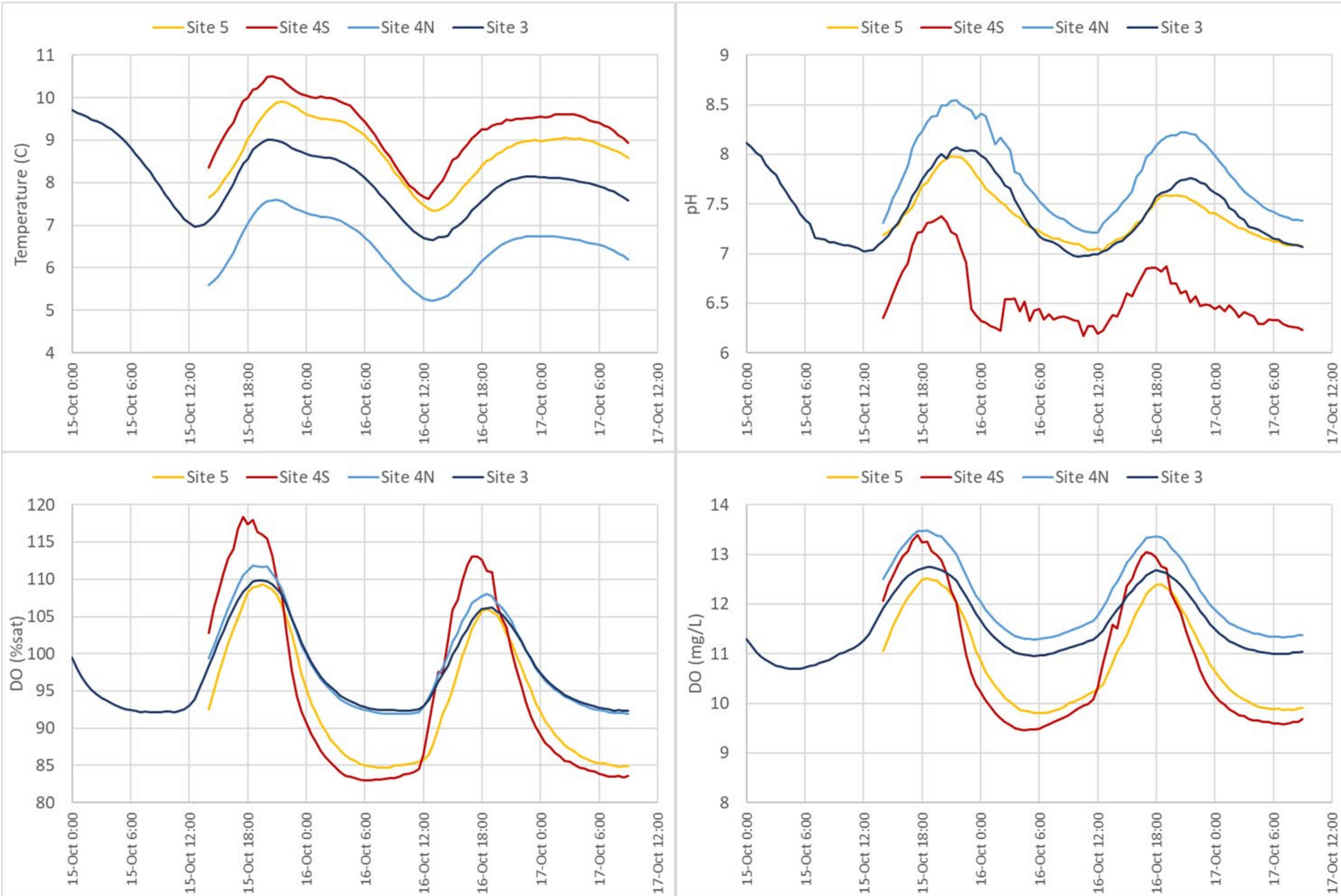


Figure 2-7. Diel patterns of selected in situ water quality parameters in the vicinity of the RWRF during October 2019.

2.5 Periphyton and Submersed Aquatic Vegetation Results

2.5.1 Cover estimate

Quantitative cover estimates and sampling frequency from point transect data are shown in Table 2-7 below. Samples collected along the south side of the channel downstream of the RWRF at Sites 4S and 5S had the highest SAV cover estimates overall for each sampling event. Site 2 had the lowest SAV cover ranging from 2–7% across all sampling events with Site 4S exhibiting the highest cover (30–59%). Note that Site 5 was sampled at the south side of the channel in the August sampling event with subsequent sampling events split into north and south transect samples during September through November; no data for Site 5N are available for the August sampling event.

Table 2-7. Quantitative cover estimates from transect data at 2019 Rogue River study sites.

Site	Point transect data					Cover estimates		
	Visual cover	Stations	SAV present	SAV hoop samples [1]	Periphyton template samples	Algae	SAV	Open
August 2019								
2	90%	11	1	0	11	88%	2%	10%
3	90%	11	4	0	11	83%	7%	10%
4N	90%	11	5	0	11	82%	8%	10%
4S	90%	16	16	7	9	41%	50%	10%
5S	90%	11	10	3	8	54%	36%	10%
6	90%	11	11	0	11	72%	18%	10%
September 2019								
2	90%	11	1	0	11	88%	2%	10%
3	90%	12[2]	3	2	10	74%	17%	10%
4N	90%	11	9	0	11	75%	15%	10%
4S	90%	16	16	5	11	50%	41%	10%
5N	90%	11	11	2	9	59%	31%	10%
5S	90%	11	11	3	8	52%	38%	10%
6	90%	11	11	1	10	65%	25%	10%
October 2019								
2	90%	11	4	0	11	83%	7%	10%
3	90%	22[3]	9	4	18	70%	20%	10%
4N	90%	11	9	0	11	75%	15%	10%
4S	90%	16	16	9	7	32%	59%	10%
5N	90%	11	11	0	11	11	72%	18%
5S	90%	16	16	3	13	59%	32%	10%
6	90%	11	10	0	11	74%	16%	10%

Site	Point transect data					Cover estimates		
	Visual cover	Stations	SAV present	SAV hoop samples [1]	Periphyton template samples	Algae	SAV	Open
November 2019								
2	90%	11	4	0	11	83%	7%	10%
3	90%	11	7	1	10	72%	18%	10%
4N	90%	11	7	0	11	79%	11%	10%
4S	90%	16	11	4	12	60%	30%	10%
5N	90%	11	9	0	11	75%	15%	10%
5S	90%	11	11	3	8	52%	38%	10%
6	90%	11	8	0	11	77%	13%	10%

1 SAV samples not collected unless cover exceeded approximately 0.2 m^2 out of 1 m^2 observed
2 Site conditions prohibited the sampling of all 16 stations
3 Chl-a samples were collected the day after AFDM samples were collected

2.5.2 Benthic chlorophyll and ash free dry mass

Using transect sampling methods adapted from MT DEQ (2011) and described in Section 2.2.3, Chl-a and AFDM samples were taken during each sampling event and sent to Rhithron Associates, Inc. for analysis of biomass as Chl-a and AFDM using methods shown in Table 2-4. For presentation purposes, benthic chlorophyll-a (Figure 2-8) and AFDM (Figure 2-9) results are arranged in upstream to downstream order with Site 2 at right. Chl-a results were highest during the August sampling events and ranged from 241 mg/m^2 at Site 4N to 107 mg/m^2 at Site 3 (Table 2-8). Despite being upstream of the RWRF, Site 2 had higher Chl-a levels than at some downstream sites which may be due to nutrients supplied from Little Butte Creek, a tributary approximately 0.5 river miles upstream of Site 2. Chl-a levels at Site 3 are lower than at Site 2 which was consistent with lower total nitrogen at Site 3 and may indicate dilution of nutrients as mixing continues downstream of Little Butte Creek. Benthic Chl-a levels decreased over time indicating Chl-a biomass likely peaked before or during the August sampling event. AFDM results were also highest during the August sampling event with a range of 587 g/m^2 at Site 4N to 11.9 g/m^2 at Site 6. Except for Site 4N, Sites 2 and 3 had higher AFDM levels than sites downstream of the RWRF. This pattern was only observed during the August sampling event and may have preceded a die-off at these upstream sites. Subsequent months indicate a shift to sites downstream of the RWRF having higher levels of biomass as AFDM than sites upstream of the RWRF. Laboratory reports are available in Appendix D.

Table 2-8. Chlorophyll-*a* (Chl-*a*) and Ash Free Dry Mass (ADFM) results from point transect sampling at 2019 Rogue River study sites.

Chlorophyll-*a* (mg/m²)				
Site	**August**	**September**	**October**	**November**
2	160.20	45.35	54.24	47.77
3	107.04	24.93	18.75	23.60
4N	241.38	43.82	68.97	45.62
4S	183.07	50.65	25.18	7.81
5N	NA[1]	89.21	67.89	40.59
5S	158.03	34.04	14.85	11.46
6	143.68	68.78	53.66	45.26
AFDM (g/m²)				
Site	**August**	**September**	**October**	**November**
2	298.90	17.38	29.96	20.08
3	249.41	64.76	84.03	57.29
4N	586.80	43.78	38.19	24.46
4S	36.34	27.65	60.28	87.24
5N	NA[1]	237.56	124.55	103.63
5S	80.60	151.19	51.96	100.14
6	11.89	11.22	50.05	48.21

1 Transect samples for collections of SAV and periphyton were limited to Site 5S for the August sampling event, but was split into north (Site 5N) and south (Site 5S) transects for collections during September through November

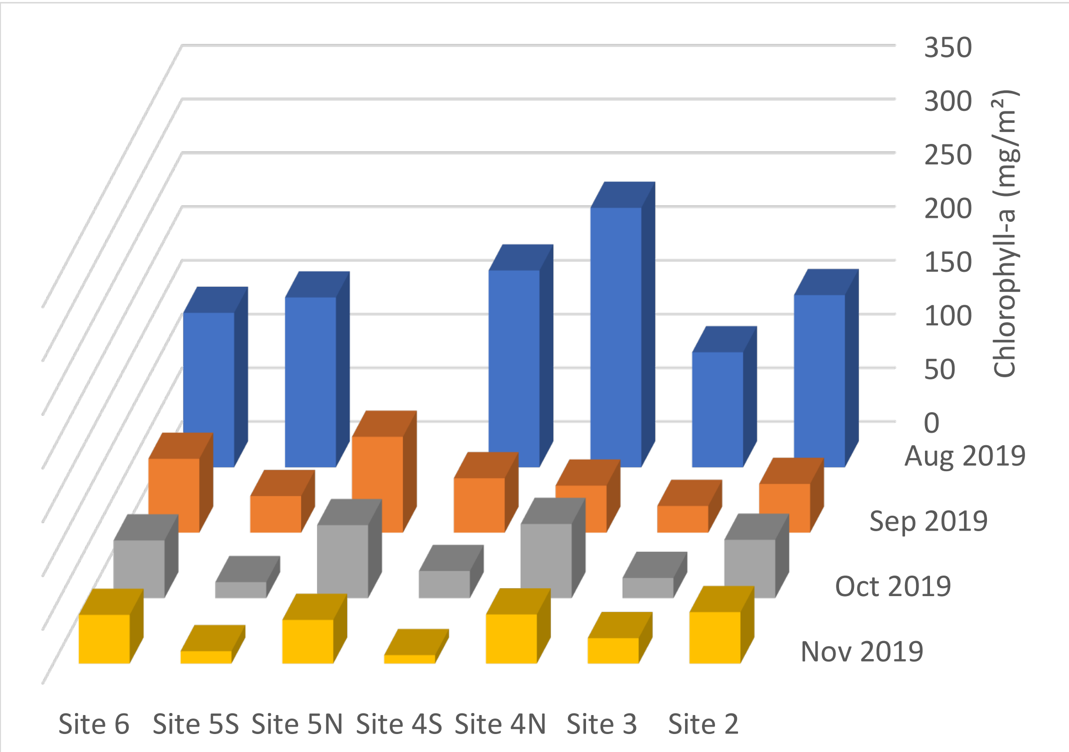


Figure 2-8. Rogue River sites near the Medford WWTP results for total benthic Chl-*a* from line transect sampling.

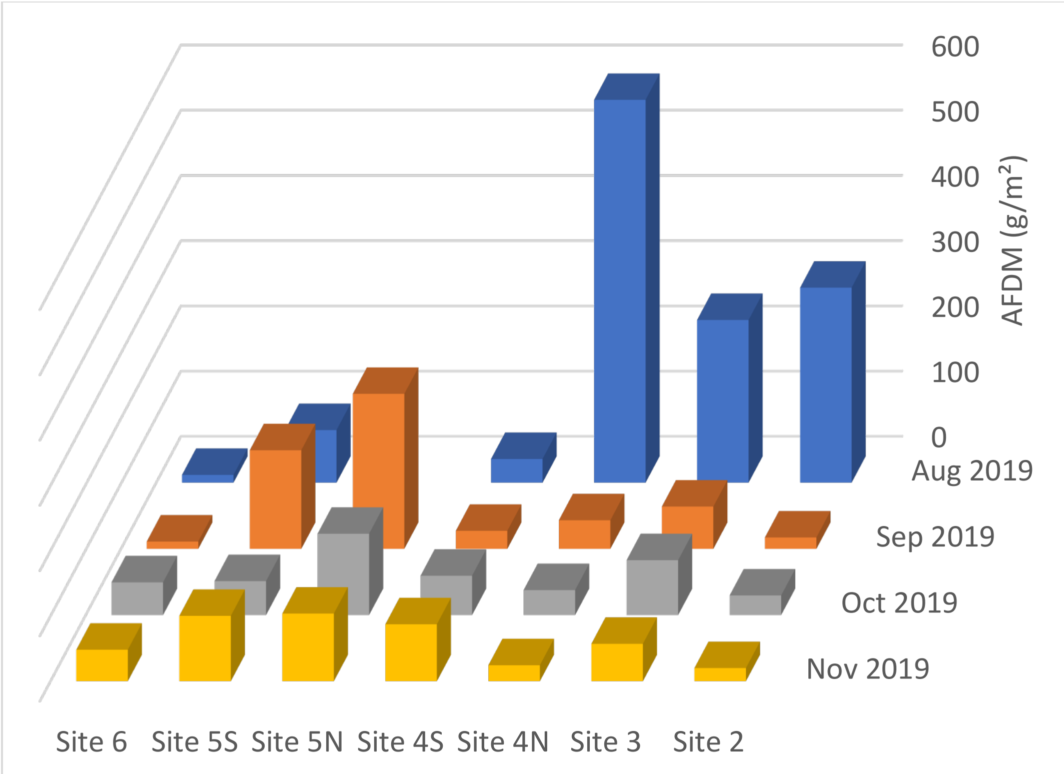


Figure 2-9. Rogue River sites near the Medford WWTP results for Ash Free Dry Mass from line transect sampling.

2.5.3 Periphyton ID, biovolume and cell density

Periphyton samples were taken during the August and October sampling events and were sent to Rhithron Associates, Inc. for microscopic analysis of biovolume, cell densities, and community composition[1]. For samples collected in August, taxonomic precision for identification and enumeration measured by the Bray-Curtis index was 90.84% and PTD was 9.16% for the randomly selected taxonomic QC sample. For samples collected in October, the corresponding measures were 88.83% (Bray Curtis)and 11.82% (PTD). Data entry efficiency was 100% for the project. Laboratory reports and worksheets for total biovolume, cell density, and community composition are included in Appendix D.

In addition to benthic Chl-a and AFDM estimates of biomass (Section 2.5.2), algae total biovolume (Figure 2-10) and cell density (Figure 2-11 and Figure 2-12) metrics selected for analysis include those used by Hafele (2013) and Brown and Caldwell (2014). Algae total biovolume estimates the total volume occupied by each type of algae. Cell density assesses the number of cells per unit area. For presentation purposes, biovolume and cell density estimates are arranged in upstream to downstream order with Site 2 at right.

[1] Diatoms were generally identified to species and soft bodied algae (e.g., cyanobacteria and green algae) were identified to genus.

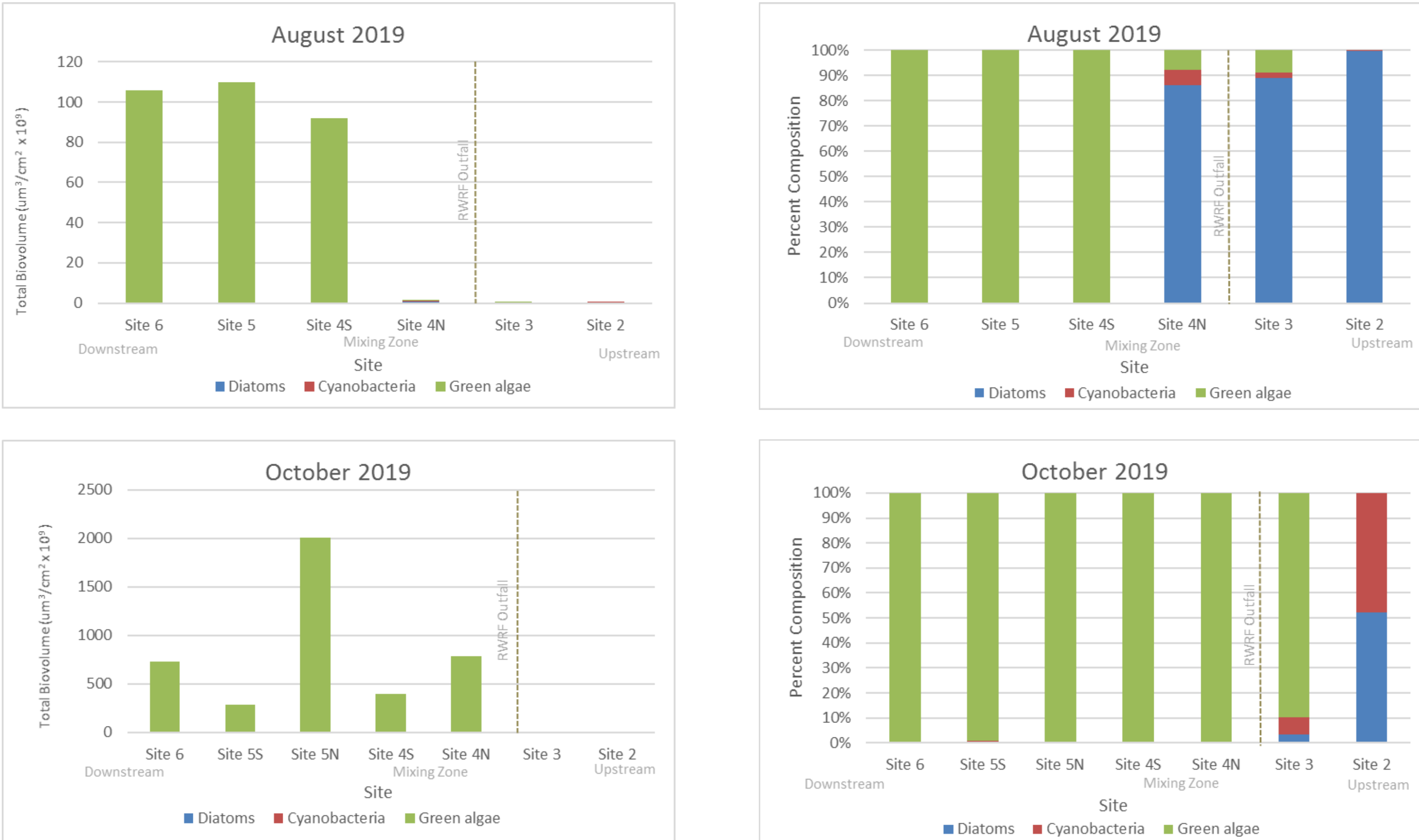
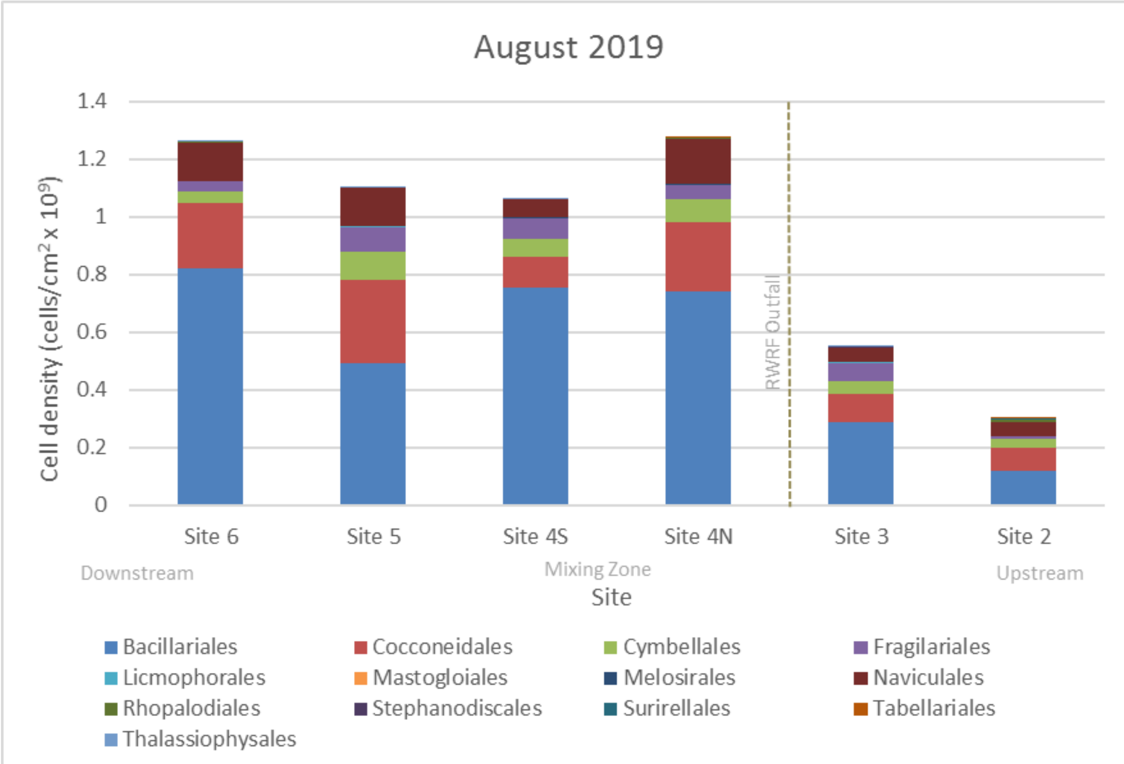


Figure 2-10. Algae total biovolume and percent community composition during August and October 2019.



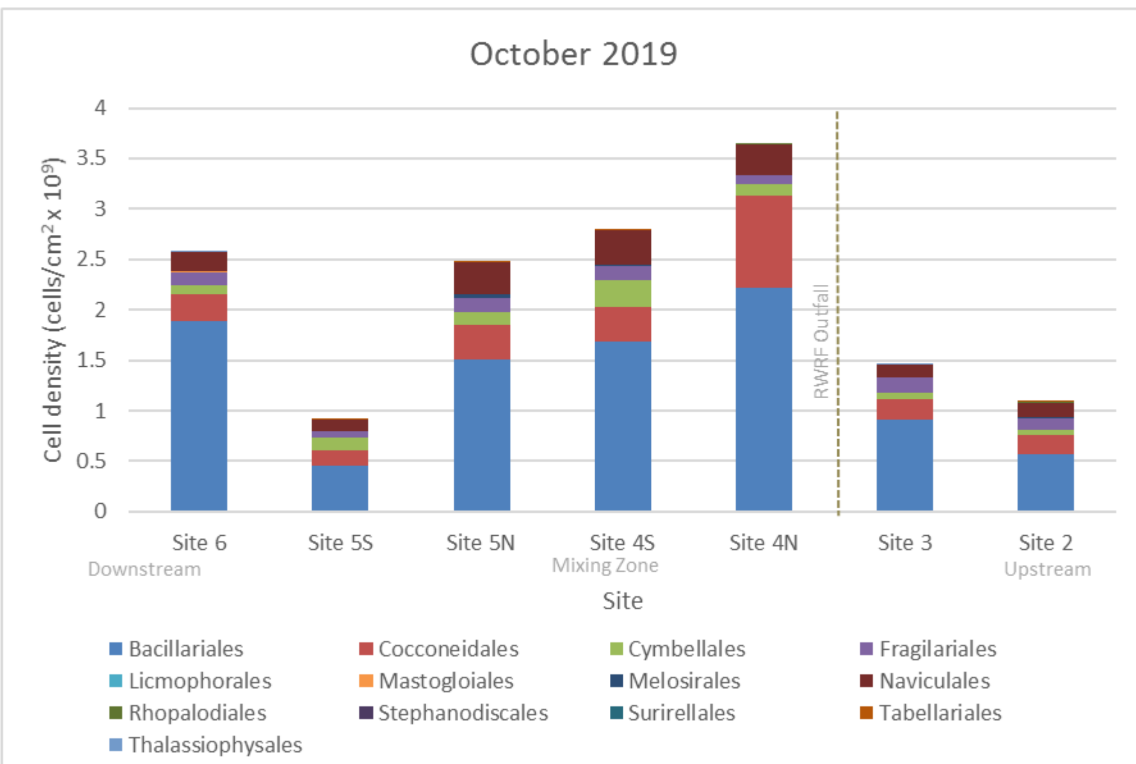
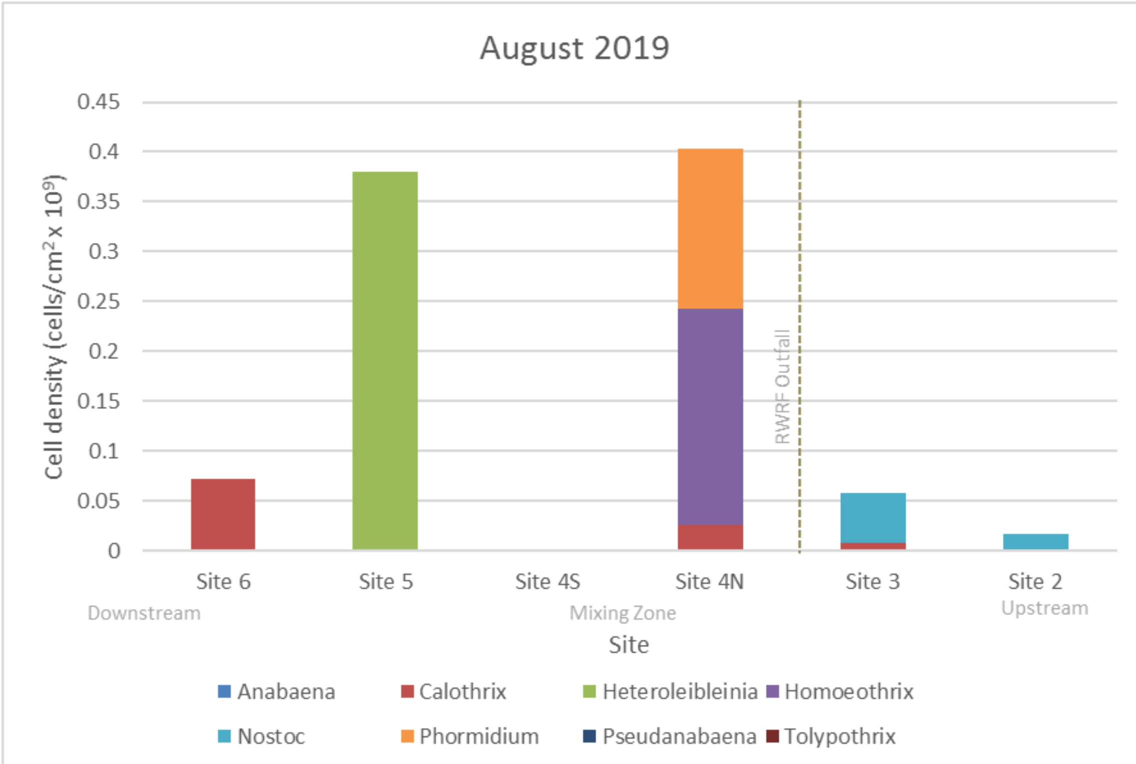


Figure 2-11. Diatom cell density and community composition by order during August (top) and October (bottom), 2019.



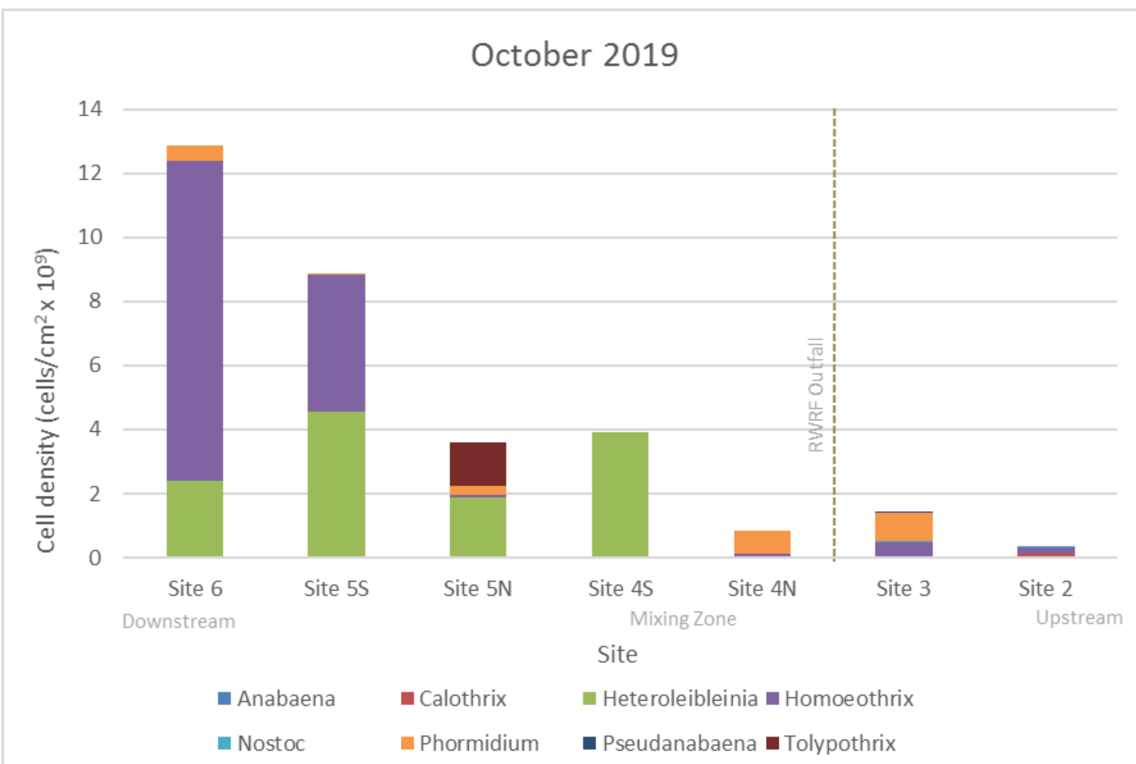


Figure 2-12. Cyanobacteria cell density and community composition by genus during August (top) and October (bottom), 2019.

Algae biomass as total biovolume was greater at sites downstream of the RWRF outfall than upstream of the outfall (Figure 2-10) during August and October, and was primarily comprised of the three groups below.

- **Green algae** (Chlorophyta) – the most diverse group of algae, with over 7,000 species, and common in freshwater. Green algae include macroalgae species (e.g. *Cladophora sp.)*
- **Diatoms** (Bacillariophyta) – a major algae group and one of the most common types of phytoplankton. Diatom cells are characteristically encased within a cell wall made of silica.
- **Cyanobacteria** – photosynthesizing bacteria that can often be a nuisance aquatic species.

Based on total biovolume, the algae community during August was dominated by diatoms at sites above the RWRF outfall and at Site 4N and green algae was dominant at sites below RWRF (Figure 2-10) During October, green algae was dominant at all sites, except for the most upstream site (Site 2). Green algae include macroalgae species that are adapted to grow larger than diatoms and cyanobacteria; therefore, when present green algae can be larger in biovolume than the other algae groups. Although the percent community composition of diatoms and cyanobacteria is small compared to green algae downstream of the RWRF outfall, the cell densities of diatoms and cyanobacteria were generally greater at sites downstream of the RWRF outfall than upstream of the outfall during August and October (Figure 2-11 and Figure 2-12). The higher total biovolume and cell densities of algae downstream of the RWRF outfall indicate that nutrients supplied from the outfall may contribute to higher productivity of all phytoplankton groups present.

The differences in biovolume and species composition at locations outside the RMZ (Site 4N) may be attributed to site specific differences in nutrient mixing, hydrology, and species composition. Algae biovolume at Site 4N, which supports a smaller biovolume in August than the other downstream sites (sites 4S, 5, 6), exhibits higher diatom and cyanobacteria cell density than other sites. Because this Site lies outside of the RWRF outfall plume, this may explain the lower concentrations of green algae during August at that location (Figure 2-5). The increase in biovolume at Site 4N and presence of green algae during October may be due to more favorable conditions for green algae growth (e.g., lower flows) in the Rogue River, since the presence of green algae was dominant at most sites in October, including one site (Site 3) upstream of the RWRF outfall (Figure 2-10).

Trends in algae community composition and distribution across sites varied by algae group. The distribution of diatom community by order was similar across all sites; diatoms within the order Bacillariales had the highest cell density (Figure 2-11). The number of diatom species identified were similar between sites (49–97 species). However, the samples with the highest diatom diversity were collected at Site 2, the most upstream site, during August (ninety-two species) and October (ninety-nine species) (Appendix D). In general, more cyanobacteria genera were identified in October than August and similar numbers of genera were identified at sites upstream and downstream of the RWRF outfall (1–7 genera). Of the genera identified upstream of the RWRF outfall, *Nostoc* sp. was the dominant genus during August. Species within this Nostoc genus have be classified as low nutrient indicators (Stancheva and Sheath 2016). Green algae below the RWRF outfall were predominantly *Cladophora* sp. and *Oedogonium* sp. (Appendix D). Of these genera, *Cladophora sp*. were 70–100% of the green algae biovolume at sites located below the RWRF outfall and were not present in samples collected above the RWRF outfall. Green algae species within the *Cladophora* sp. and *Oedogonium* genera have been classified as high nutrient indicator species and have been positively correlated with increased nutrients in other riverine systems (Stancheva and Sheath 2016, Stevenson et al. 2006, Penick et. al. 2012, Marks and Lowe 1989).

2.5.4 Submersed aquatic vegetation, benthic chlorophyll and ash free dry mass

Biomass results and species identification from hoop samples collected at transect locations with SAV present (See Appendix A for locations) are discussed in this section. Table 2-9 includes Chl-*a* and AFDM averages from hoop samples to better display how SAV contributes to site specific totals described in Section 2.6.2. Identification of representative SAV samples collected during each sampling events is included in Table 2-10. Common species included common Water-crowfoot (*Ranunculus aquatilis*) and Water starwort (*Callitriche hermaphroditica*), with less common occurrences of native and non-native pondweeds (*Potamogeton sp*.), Waterweed (*Elodea sp.*) and other species. Methods for determining benthic Chl-a and biomass (AFDM) per unit area were developed using methods described by the State of Montana Department of Environmental Quality (MT DEQ 2011). The MT DEQ sampling design frame is designed to limit variance in wadeable streams by incorporating 16 transect stations with a minimum of 11 for transects that are not entirely wadeable. MT DEQ methodology also consolidates hoop and template samples from a sampling event together. Most sites selected for repeat sampling in 2019 were too hazardous to wade in their entirety and as such, the majority of transects had only 11 stations. Table 2-9 shows results from Chl-a and AFDM from SAV samples at each study site, with values scaled based on the proportion of SAV samples taken to the total number of transect points sampled (Table 2-7). However, it should be noted that these results are provided for comparative purposes only, since the low number of hoop samples collected for analysis of Chl-a and AFDM is inconsistent with the minimum sample size assumptions in the MT DEQ (2011) methodology.

Table 2-9. Chlorophyll-*a* (Chl-*a*) and Ash Free Dry Mass (ADFM) results from SAV samples only during point transect sampling at 2019 Rogue River study sites.

Chlorophyll-a (mg/m²)				
Site	**August**	**September**	**October**	**November**
2				
3		1.51	0.97	3.23
4N				
4S	9.03[2]	3.98	0.16	2.73
5N[1]		0.48		
5S[1]	24.20[2]	0.63	3.02	2.66
6		1.24		
AFDM (g/m²)				
Site	**August**	**September**	**October**	**November**
2				
3		46.68	46.02	24.23
4N				
4S	NA[3]	24.66	34.22	26.54
5N[1]		55.80		
5S[1]	NA[3]	33.20	29.98	9.82
6		4.79		

1 Transect samples for collections of SAV and periphyton as Site 5 were limited to Site 5S for the August sampling event but were split into north (Site 5N) and south (Site 5S) transects for collections during September through November.
2 Chl-a samples at Sites 4S and 5S during August were extracted directly from SAV, whereas results for all other surveys were analyzed from attached periphyton separated from SAV samples.
3 Due to miscommunication with the laboratory, SAV samples collected during August were not analyzed for AFDM.

Table 2-10. Identified SAV species at Rogue River sites near the Medford WWTP.

Site	Species	Status	Month			
			August	**September**	**October**	**November**
2	*Callitriche hermaphroditica*	Native			X	
	Elodea canadensis	Native	X		X	X
	Elodea sp.	Native		X		
	Potamogeton crispus	Non-Native		X		X
	Potamogeton foliosus	Native	X			
	Potamogeton robbinsii	Native			X	
	Ranunculus aquatilis	Native	X	X	X	X
3	*Elodea sp.*	Native		X		
	Potamogeton crispus	Non-Native				X
	Ranunculus aquatilis	Native	X	X	X	X
4N	*Callitriche hermaphroditica*	Native		X	X	X
	Elodea sp.	Native		X		
	Potamogeton robbinsii	Native				X
	Ranunculus aquatilis	Native	X	X	X	X

Site	Species	Status	Month			
			August	September	October	November
4S	*Callitriche hermaphroditica*	Native	X	X	X	
	Elodea canadensis	Native	X			
	Lemna minor	Native				X
	Potamogeton crispus	Non-Native	X	X		
	Potamogeton robbinsii	Native			X	
	Ranunculus aquatilis	Native			X	X
5N	*Callitriche hermaphroditica*	Native			X	
	Elodea nuttallii	Native			X	X
	Elodea sp.	Native		X		
	Potamogeton crispus	Non-Native		X	X	
	Potamogeton robbinsii	Native				X
	Ranunculus aquatilis	Native		X	X	X
5S	*Callitriche hermaphroditica*	Native	X	X	X	
	Elodea nuttallii	Native				X
	Elodea sp.	Native		X		
	Equisetum spp.	Native	X			
	Potamogeton crispus	Non-Native	X	X	X	X
	Potamogeton robbinsii	Native			X	
	Ranunculus aquatilis	Native	X	X	X	X
6	*Callitriche hermaphroditica*	Native	X	X	X	
	Elodea canadensis	Native	X			
	Elodea sp.	Native				X
	Ludwigia palustris	Native	X			
	Potamogeton crispus	Non-Native		X		X
	Potamogeton foliosus	Native	X			
	Ranunculus aquatilis	Native		X	X	X

3 ANALYSIS AND ASSESSMENT

Recognizing uncertainties in linking nutrient levels to stream algae, we analyzed data collected during 2018 and 2019 to evaluate spatial patterns and apparent responses to nutrient levels at locations upstream and downstream of the RWRF outfall. Next, predictive relationships from other river systems were used to provide a basis of comparison to the results in the Rogue River. Lastly, literature-based nutrient thresholds were compared to develop an overall recommendation for target nutrient concentrations that will result in appropriate levels of algal biomass in the Rogue River.

3.1 Regional and Local Reference Conditions

One of the simplest approaches in establishing nutrient limits recommended by USEPA (2000a) is to compare conditions at local or ecoregional reference sites. The primary method recommended is to derive criteria from ambient nutrient concentrations observed at a population of reference sites that represent least impacted or best attainable conditions. Ecoregions are one means of classifying areas based on similarities of natural geographic features (e.g., geology, soils, climate, hydrology, vegetation, and wildlife) and land use patterns. U.S. EPA divided the continental United States into 14 nutrient ecoregions (aggregates of level III ecoregions) with similarities in characteristics expected to affect nutrient concentrations. The middle Rogue River watershed upstream of the RWRF lies within the Rogue/Illinois Valley (level IV ecoregion 78a) and within the Klamath Mountains (level III ecoregion 78) (Klamath Mountains) (USEPA 2012). Lacking more detailed data, Table 3-1 summarizes low (25^{th} percentile) background nutrient concentrations between 1990–1998 developed for river and streams in the Klamath Mountains (USEPA 2000b) in comparisons with 2019 data summaries and longer term data sets (ODEQ 2019) upstream and downstream of the RWRF. At the ecoregion level and at the Dodge Park site upstream of the RWRF, long term (1990–2019) median nutrient concentrations are generally low with TIN and TN ranging from 0.02–0.04, and 0.13–0.18 mg/L respectively. Corresponding PO_4-P and TP concentrations are 0.03–0.04 mg/L. In contrast however, corresponding TN and TP concentrations in Little Butte Creek over this time period were 0.64 and 0.14 mg/L, respectively. During summer and fall 2019, averages of the data presented in Table 2-5 across all sites unaffected by the RWRF outfall (Sites 2, 3, RMZ-N, 4N) showed non-detects for NH_3-N, and NO_2^- + NO_3 -N, which was reported at an upper bound TIN <0.09 mg-N/L, representing the sum of the individual DLs. Seasonal averages of TKN results at these sites were used to estimate a TN of 0.19 mg/L. Seasonal TP concentrations from 2019 shown in Table 3-1 reflect slightly elevated concentrations compared to ecoregional reference conditions found in USEPA (2000b). Increased nutrient concentrations were found at sites downstream of the RWRF (Sites RMZ-S, 4S, 5, 6) during 2019. At Gold River, downstream of the Bear Creek confluence, long-term average TN and TP concentrations were 0.54 and 0.08 mg/L, respectively. Recognizing the presence of local nutrients in the immediate vicinity of the RWRF such as sources arriving from Little Butte Creek, average concentrations at the sites outside of the hydraulic influence of the RWRF outfall (Sites 2, 3, RMZ-N, 4N) are within the range of the low (25^{th} percentile) concentrations for nitrogen, but with TP concentrations higher locally than was found in the surrounding ecoregion (USEPA 2000b).

Table 3-1. Ambient Rogue River water quality in comparison to 2019 sampling results.

Location	TIN (mg/L)	TN (mg/L)	PO_4-P (mg/L)	TP (mg/L)
Background "reference" conditions based upon 25th percentile of historical samples reported between 1990–1998 (USEPA 2000b)				
Level III ecoregion 78 (Klamath Mountains)	0.04	0.18	NA	0.03
Long-term (1990–2019) summer/fall (June-October) average nutrient concentrations upstream of Medford RWRF				
Rogue River at Dodge Park (DEQ Site 10423)	0.02	0.13	0.03	0.04
Little Butte Creek at Agate Rd (DEQ Site 10462)	0.03	0.64	0.06	0.10
2019 Summer/Fall (June–November) average nutrient concentrations in the vicinity of the Medford RWRF				
Avg. of Sites 2 and 3 (upstream of RWRF), Sites RMZ-N[1], and 4N[1]	<0.09[2]	<0.19[J,3]	0.03[J]	0.05
Medford RWRF Outfall	18.23	20.99	2.87	3.34
Regulatory Mixing Zone (RMZ-S)	0.66	0.98	0.09	0.17
First Riffle Downstream of RWRF (4S)	0.36	0.47	0.05	0.12
All Downstream Sites (RMZ-S, 4S, 5, 6)	0.33	0.50	0.08	0.11
Long-term (1990–2019) summer/fall (June-October) average nutrient concentrations downstream of Medford RWRF				
Rogue River at Gold Hill (DEQ Site 10421)	0.21	0.54	0.06	0.08

J One or more 2019 sampling results below laboratory method reporting limit (RL), but above method detection limit (DL) and is reported here as a J-flag; therefore, seasonal average is an approximation.
1 Site assumed to be unaffected by RWRF discharge as determined by dye-tracer study of outfall plume (Brown and Caldwell 2014).
2 Ammonia-N (NH_3-N) and NO_2^-+ NO_3 were not detected between July and November. Seasonal TIN average reported as the sum of the individual DLs.
3 Because NO_2^- + NO_3 were not detected between July and November, value based upon the sum of the TKN results and DLs for NO_2^- + NO_3.

3.2 Exploratory Relationships Between Stream Nutrients and Response Metrics

As noted in Section 2.5, relative abundance measures (AFDM, Chl-a, biovolume, cell density) from transect sampling at sites upstream and downstream of the RWRF were variable with the upstream sites exhibiting the highest biomass in the August survey and downstream sites only slightly higher than upstream sites in the three remaining surveys. In the sections below, we describe exploratory analyses of the response of the relative abundance to measured stream nutrients were conducted to examine spatial patterns as well as for comparisons to reference conditions upstream of the RWRF outfall.

3.2.1 Ash free dry mass of periphyton and submersed aquatic vegetation

Transect samples of periphyton samples were analyzed for AFDM in each of the four monthly surveys from August to November, with SAV results analyzed for the September through November events (Table 2-8 and Table 2-9). Although MT DEQ (2011) includes methods for integrating results from both periphyton template sampling of stream cobbles and hoop-based collections of SAV, because of large differences in AFDM and Chl-a biomass measures between periphyton and SAV samples and the low total numbers of SAV (hoop) samples collected (Table 2-7), we elected to base regressions upon periphyton samples only. In initial plots, high biomass estimates at sites upstream of the RWRF in August resulted in a negative relationship between seasonally averaged nutrients and maximum AFDM (Figure 3-1). These results were unexpected and were attributed to a predominance of green algae during the August survey (Section 2.5.3). Because downstream sites exhibited slightly higher AFDM than upstream sites in the three remaining surveys, relationships between average nutrients and AFDM during September through November were also plotted (Figure 3-2).

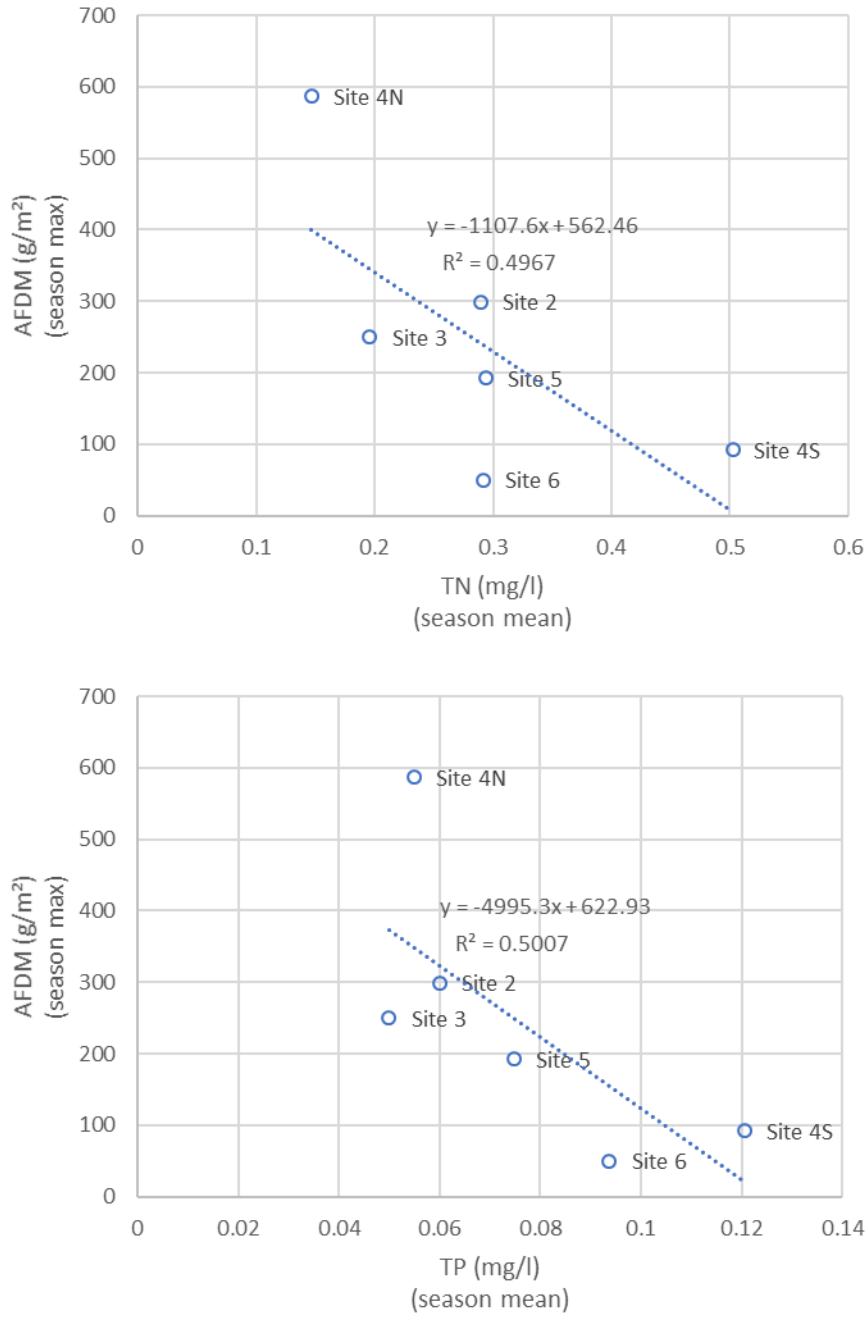


Figure 3-1. Maximum seasonal AFDM in periphyton samples versus seasonally averaged nutrients (TN upper, TP lower) at study sites in the vicinity of the RWRF between August and November 2019.

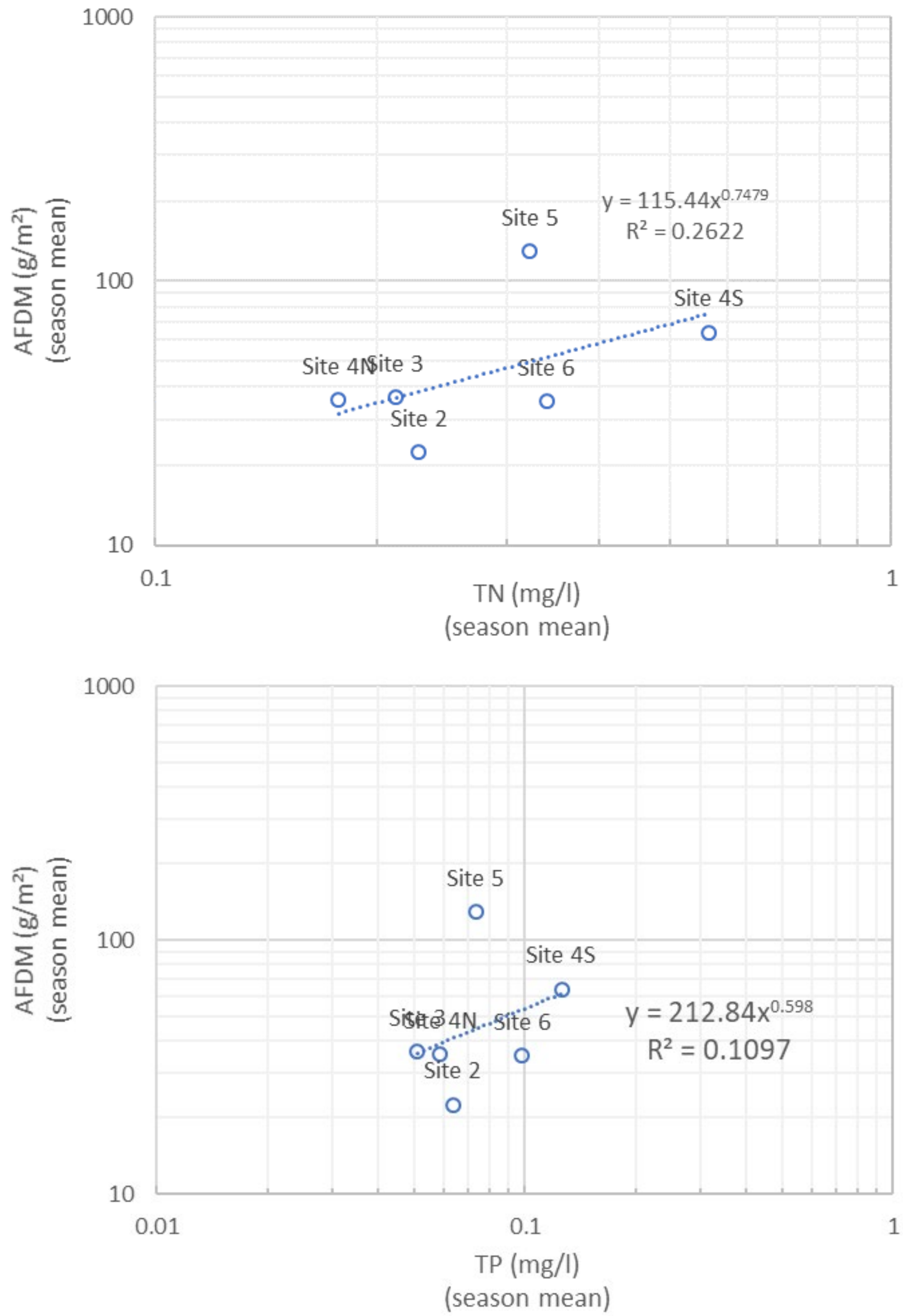


Figure 3-2. Average of AFDM in periphyton samples versus stream nutrients (TN upper, TP lower) at study sites in the vicinity of the RWRF between September and November 2019.

3.2.2 Periphyton chlorophyll-a

Composited transect samples of periphyton and SAV were also analyzed for Chl-a in each of the four monthly surveys from August to November, with results reported separately for template and hoop sampling methods. Positive relationships were identified between seasonal averages of Chl-a and total nutrients (Figure 3-3). Because previous studies have found highly variable Chl-a estimates at study sites (Biggs 2000a; Chételat et al 1999; Dodds et al 2002, 2007), a decision was made to regress maximum Chl-a on seasonally averaged nutrients. Note that because apparent discrepancies between the spatial extent of water quality and periphyton transects were found at Site 4N during the August surveys, exploratory regressions were attempted excluding data from Site 4N (Figure 3-4).

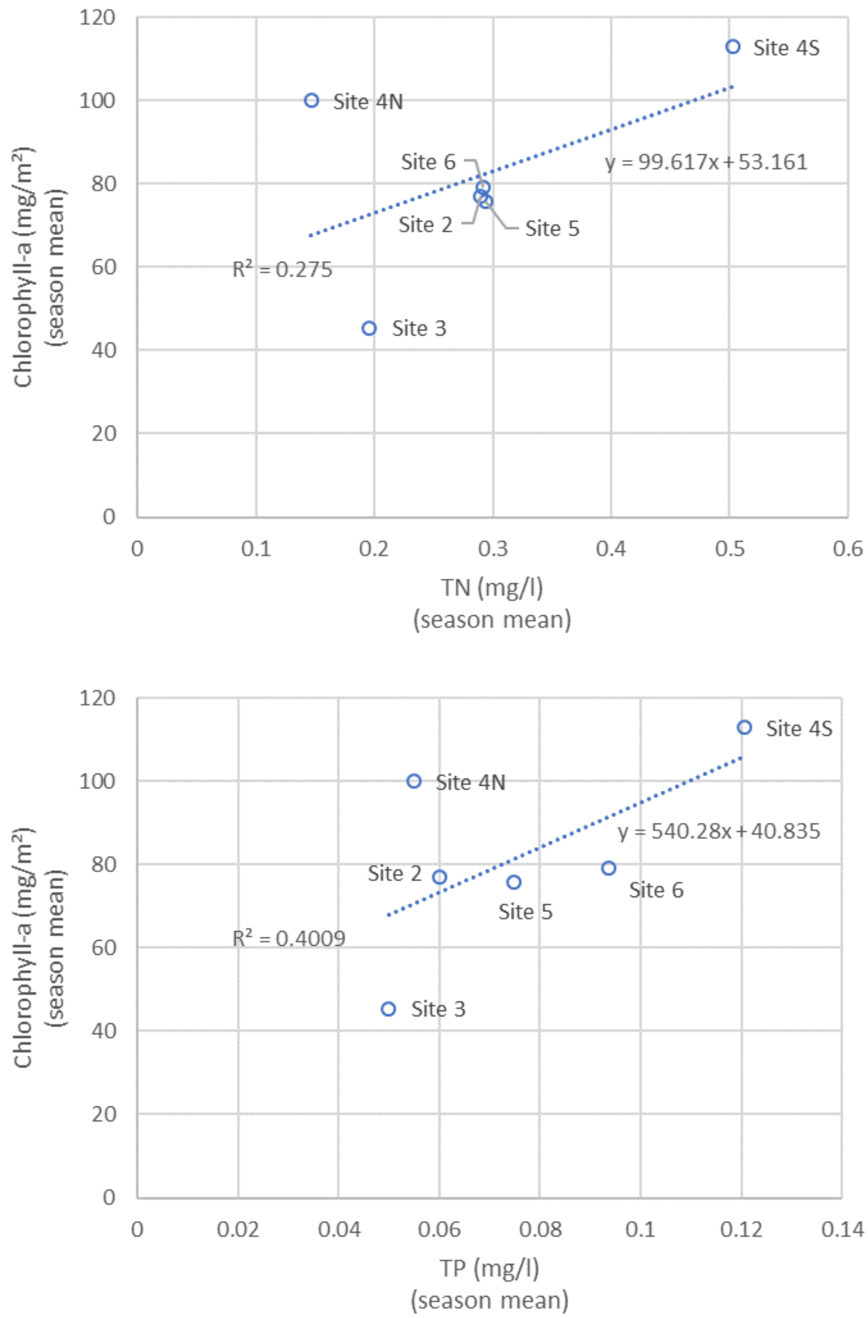


Figure 3-3. Seasonal averages of Chl-a in periphyton samples versus stream nutrients (TN, TP) at study sites in the vicinity of the RWRF between August and November 2019.

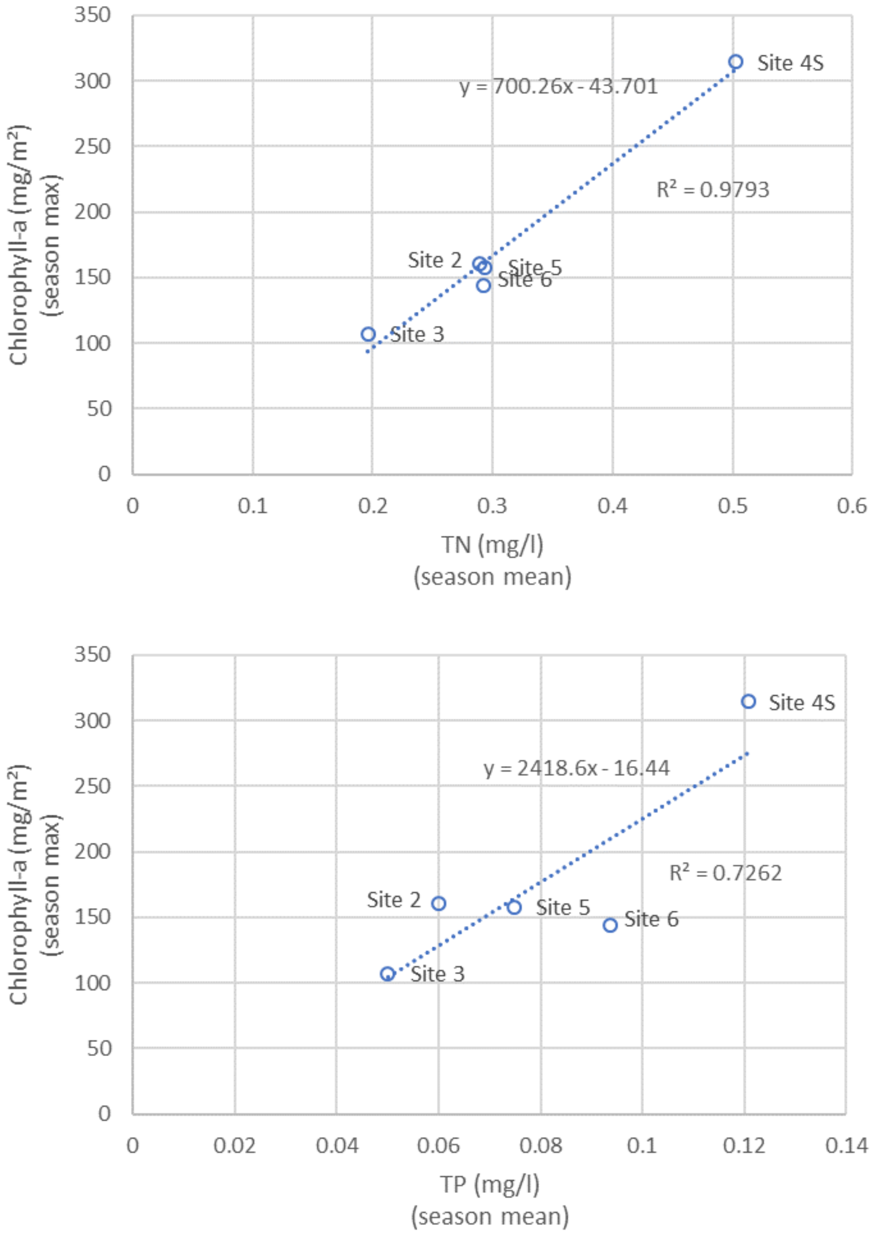
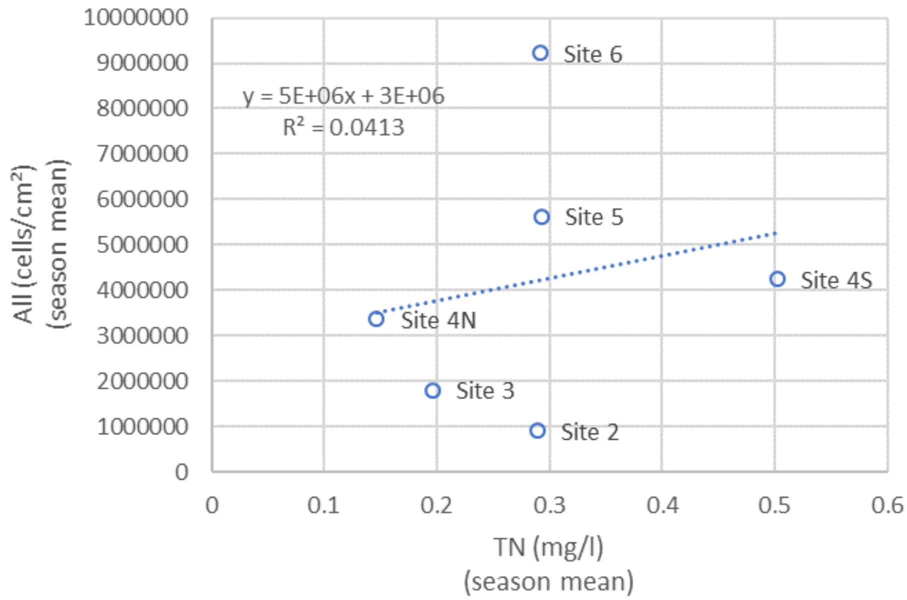


Figure 3-4. Maximum seasonal Chl-a in periphyton samples versus seasonally averaged nutrients (TN, TP) at study sites in the vicinity of the RWRF between August and November 2019.

3.2.3 Cell density of periphyton

Periphyton cell density from composited transect samples collected during the August and October sampling events was determined by cell counts through microscopy with results shown in Figure 2-11 and Figure 2-12. Weak positive relationships of cell counts were identified with total nitrogen, with somewhat stronger relationships with total phosphorus (Figure 3-5). In comparison to 2019 results, much stronger relationships between cell density and both total nitrogen and total phosphorus were identified in samples collected during October 2018 (Figure 3-6). It should be noted, however, that cell density results in 2019 were over an order of magnitude lower than was found in the October 2018 sampling event.



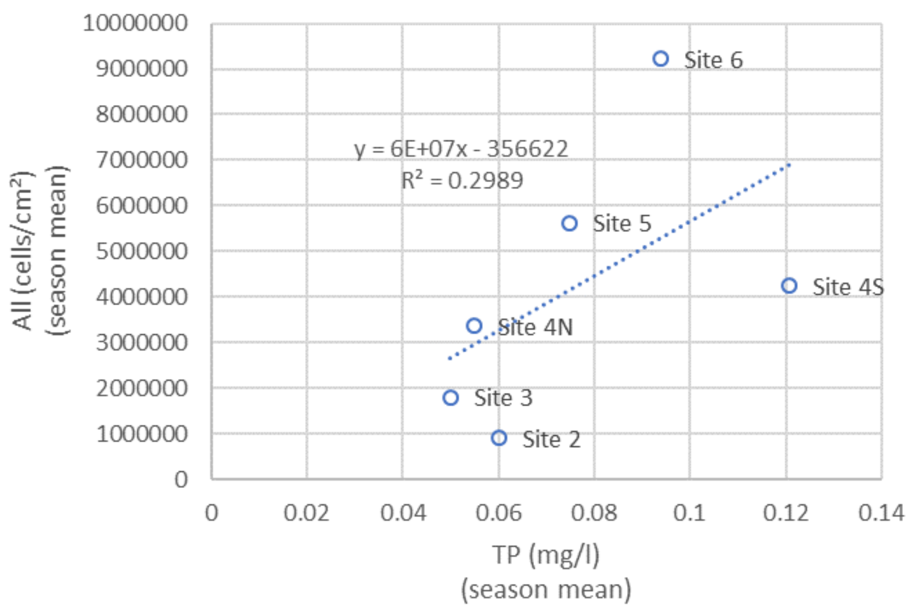


Figure 3-5. Seasonally averaged cell counts from composited periphyton samples versus stream nutrients (TN, TP) at study sites in the vicinity of the RWRF during August and October 2019.

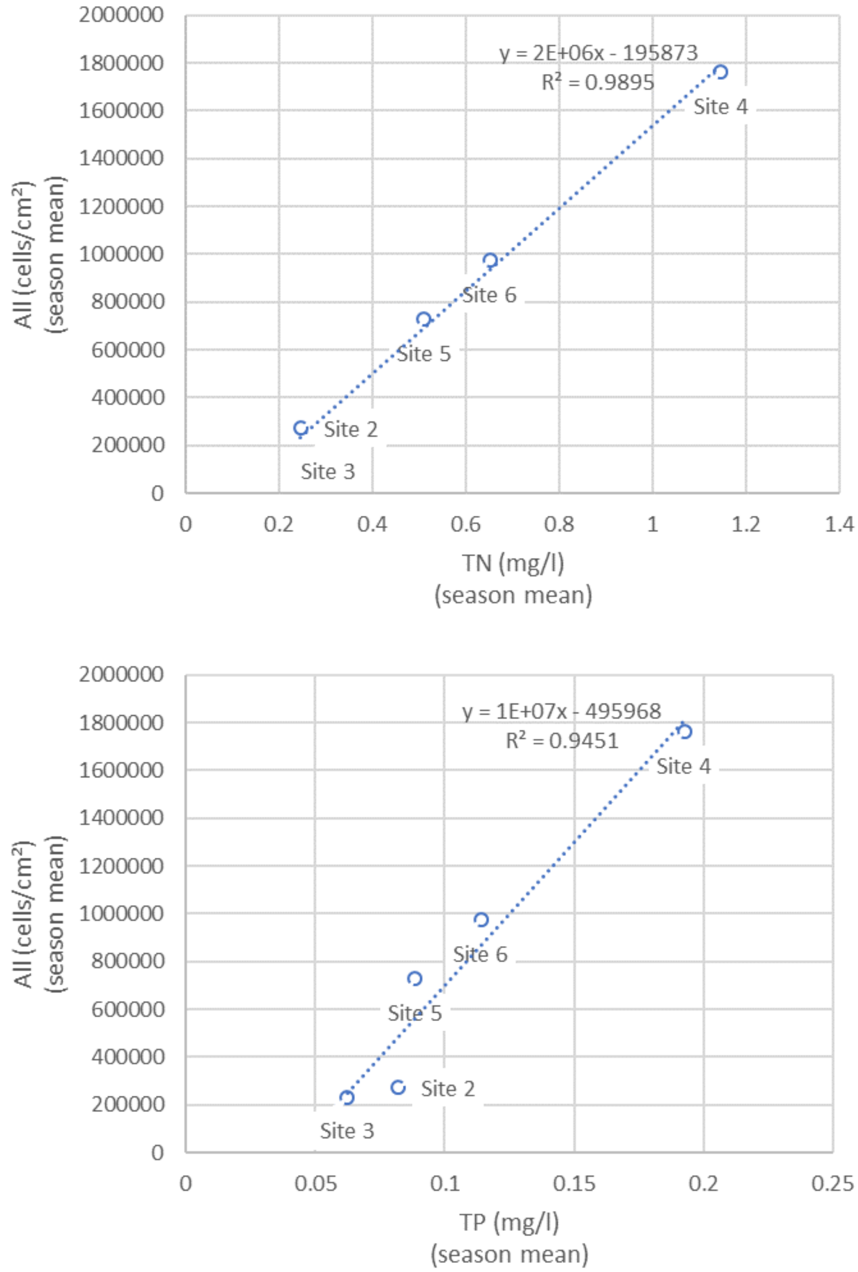


Figure 3-6. Cell Counts from composited periphyton samples versus stream nutrients (TN, TP) at study sites in the vicinity of the RWRF during October 2018.

3.2.4 Total biovolume of periphyton

Total biovolume of composited transect samples collected during the August and October sampling events was calculated from cell dimensions and cell counts (Figure 2-10). Consistent with other studies (Porter at al 2008), only weak relationships were identified between total biovolume and total nutrients in samples collected during August and October 2019 (Figure 3-7). In comparison to 2019 results, however, stronger relationships between total biovolume and both total nitrogen and total phosphorus were identified in samples collected during October 2018 (Figure 3-8). As with cell density, biovolume results in 2019 were more than an order of magnitude lower than was found in the October 2018 sampling event.

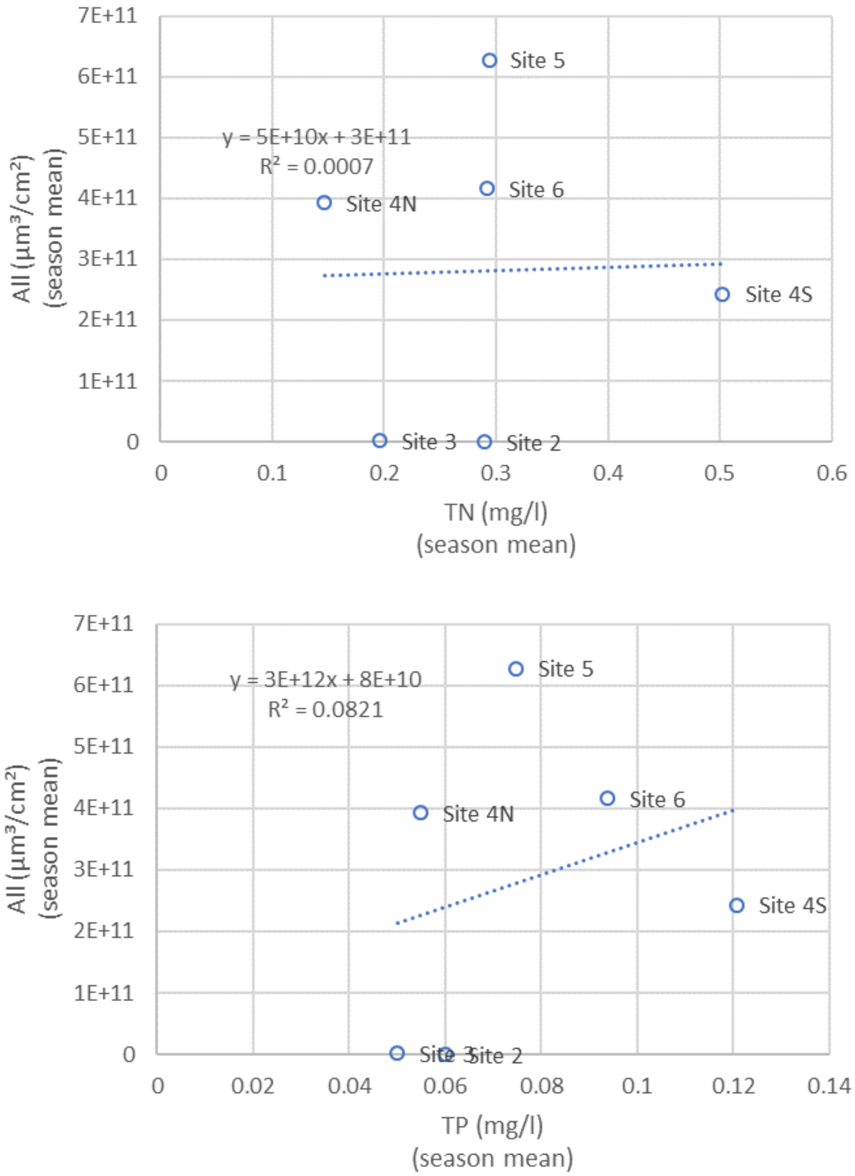
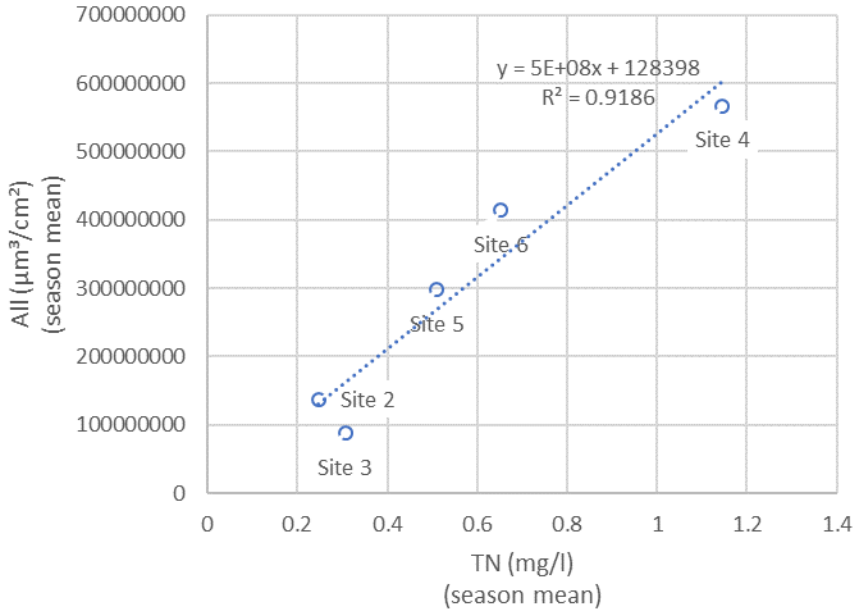


Figure 3-7. Total biovolume of periphyton from composited template samples versus stream nutrients (TN, TP) at study sites in the vicinity of the RWRF during August and October 2019.



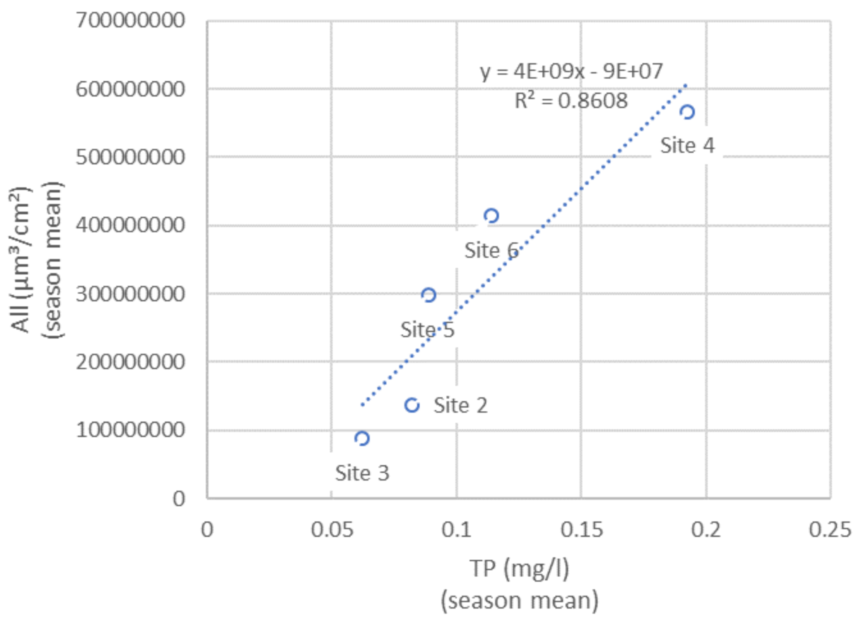


Figure 3-8. Total biovolume of periphyton from composited template samples versus stream nutrients (TN, TP) at study sites in the vicinity of the RWRF during October 2018.

3.3 Predictive Relationships Between Nutrients and Periphyton in other River Systems

Mathematical models from other systems may potentially be used to provide insights into the relationships between different parameters that may not be apparent from the data at hand. Empirical models that correlate TN and/or TP with benthic algal biomass that were evaluated include studies by Lohman et al. (1992), Dodds et al. (1997), Chételat et al. (1999), and Biggs (2000). Model predictions using observed TN and TP (Figure 3-9 and Figure 3-10) are discussed below along with model fit comparisons to observed benthic Chl-a estimates from data collected in the vicinity of the RWRF during summer/fall 2019.

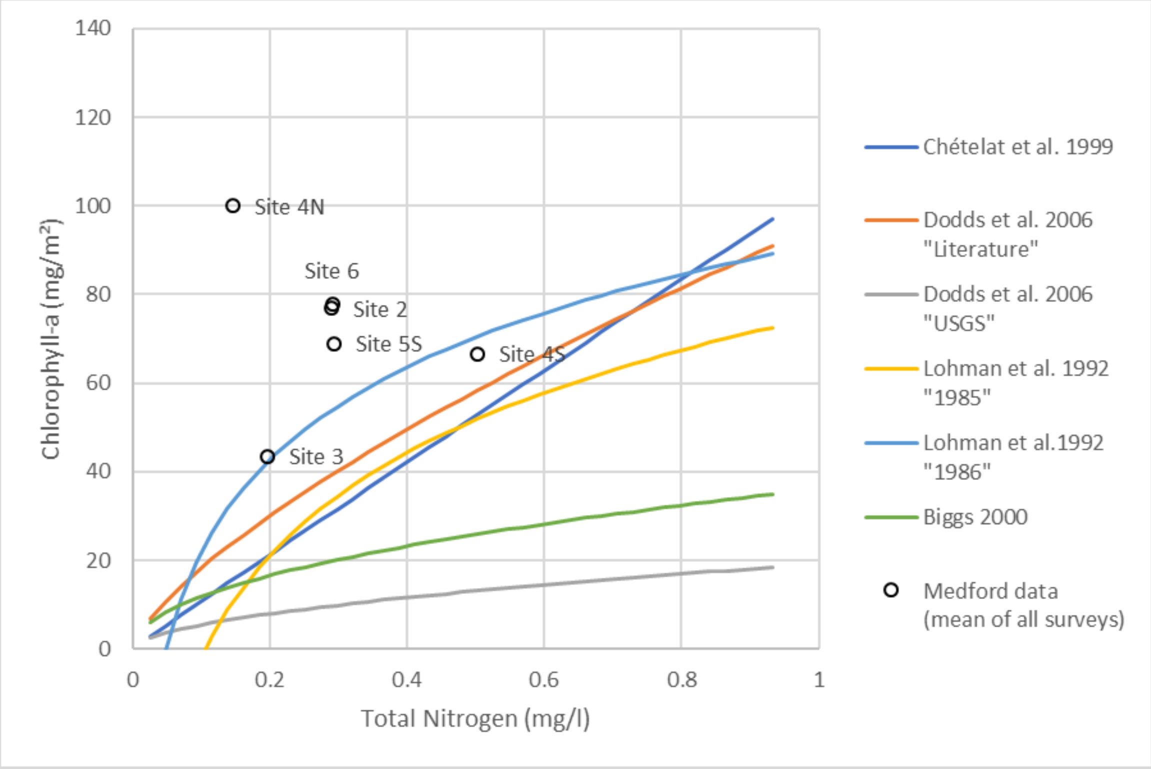


Figure 3-9. Comparison of observed and predicted mean annual Chl-a from selected models based upon seasonal averages of total nitrogen in the vicinity of the Medford RWRF during summer/fall 2019

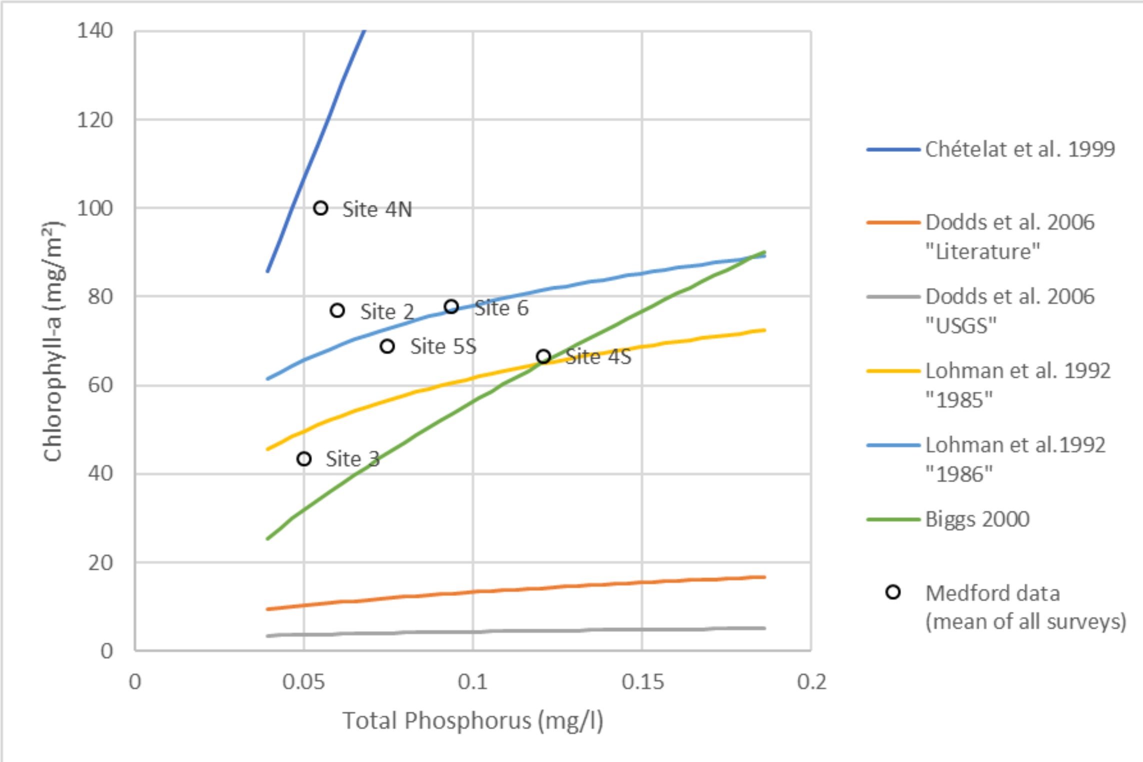


Figure 3-10. Comparison of observed and predicted mean annual Chl-a from selected models based upon seasonal averages of total phosphorus in the vicinity of the Medford RWRF during summer/fall 2019.

3.3.1 Dodds et al. (2002, 2006) nitrogen and phosphorus relationships to benthic algal biomass

Dodds et al. (2002, 2006) used data from published studies representing approximately 200 sites in North America, New Zealand, and Europe to develop mathematical equations to predict benthic chlorophyll-a values. They recommend using the equations only when local relationships have not been developed. Regression equations were developed to estimate mean Chl-a or maximum Chl-a using TN, TP, or both TN and TP concentrations.

In applying earlier parameterizations of these models to conditions in the Clark Fork River, MT, Dodds et al. (1997) predicted that if seasonal mean TN concentrations in the Clark Fork River are reduced to 0.275 mg/L, the maximum chlorophyll-a values would be 100 mg/m2. Additionally, if TN concentrations do not exceed 0.252 mg/L and TP concentrations are kept below 0.035 mg/L, Chl-a values in the Clark Fork River were expected to remain below 100 mg/m^2.

Based on Figure 3-9 and Figure 3-10 it is apparent that Dodds et al. (2006) "USGS" greatly underpredicts mean annual Chl-a values given nutrient concentrations measured in these study sites, with calculated root mean square (RMS) errors of 65 mg/m^2 and 70 mg/m^2 for TN and TP, respectively. Likewise, Dodds et al. 2006 "Literature" underpredicts mean Chl-a with RMS errors of 40 mg/m^2 for TN and 67 mg/m^2 for TP, suggesting that these models are not representative of the relationship between nutrient conditions and benthic algal biomass observed in this system.

3.3.2 Lohman et al. (1992) nutrient relationships with algal biomass in the Ozark mountains, Missouri

Lohman et al. (1992) conducted a two-year study collecting nutrients and periphyton data from 22 sites on 12 streams in the Ozark Mountains, Missouri, and developed relationships to predict benthic Chl-a values from TN and TP concentrations. Chl-a was found to be positively correlated for both study years with respect to log TN ($r^2 = 0.58, 0.60$) and log TP ($r^2 = 0.47, 0.60$). Lohman et al. (1992) credit the strength of their regression analysis on the use of long-term averages (a March – November "annual" average) and the wide range of TN (range of site annual means: 0.148 – 9.188 mg/L) and TP concentrations (range of site annual means: 0.006 – 3.264 mg/L).

Lohman et al. (1992) created independent regression equations with datasets collected in 1985 and 1986. Figure 3-9 and Figure 3-10 illustrate that equations utilizing data from 1986 best predict mean annual Chl-a values under observed nutrient concentrations with RMS errors of 31 mg/m2 and 18 mg/m2 for TN and TP respectively, the lowest RMS errors observed for each nutrient among all models considered in this analysis. Chl-a predictions formulated with data collected in 1985 are slightly weaker, with RMS errors of 48 mg/m^2 for TN and 23 mg/m^2 for TP.

3.3.3 Chételat et al. (1999) nutrient relationships with algal biomass and community composition in southern Ontario and Western Quebec, Canada

Chételat et al. (1999) conducted a three-year study collecting nutrients and periphyton data from riffle habitats at 33 sites on 13 rivers in southern Ontario and western Quebec, Canada, during summer low flow conditions. Chl-a values were correlated with TP ($r^2 = 0.56$), and TN ($r^2 = 0.50$) and also with specific conductivity ($r^2 = 0.71$). Interestingly, TN and TP concentrations were also positively correlated with conductivity ($r^2 > 0.70$, $p < 0.001$). TP, TN, and conductivity were negatively correlated with catchment area, indicating that the smaller rivers in the Chételat et al. (1999) study had higher nutrient concentrations. In comparing slopes of the Chételat et al. (1999) model to studies in New Zealand (Biggs and Close 1989) and the Ozarks (Lohman et al. 1992), stream size and drainage area appeared to affect the comparability of models across watershed.

Figure 3-9 and Figure 3-10 clearly show that the model described in Chételat et al. (1999) underpredicts mean annual Chl-a values under TN concentrations observed in study sites, and overpredicts Chl-a values under observed TP concentrations, with an RMS error of 47 mg/m^2 for TN and 96 mg/m^2 for TP.

3.3.4 Biggs (2000) dissolved nutrient-chlorophyll-a relationships for benthic algae

Biggs (2000) integrated research from previous studies in New Zealand rivers to develop models for predicting mean monthly and maximum Chl-a as a function of PO_4-P, TIN, and days of accrual following stream scour events. Combining flooding disturbance frequency of flow events in excess of 1 m/s velocity and stream enrichment factor as measured by cellular nitrogen he developed models to predict mean periphyton biomass with positive correlations between days of accrual and TIN (r^2=0.437, p<0.179) and PO_4-P (r^2=0.488, p<0.038).

Models developed in Biggs (2000) significantly underpredict mean annual Chl-a values under observed TN concentrations (Figure 3-9), with an RMS error of 56 mg/m^2. The model also underpredicts mean annual Chl-a values given observed TP concentrations (Figure 3-10), albeit to a lesser degree, with an RMS error of 36 mg/m^2

3.3.5 Model selection summary

Although the water quality and algal biomass results presented in this report reflect both annual and monthly variability, empirical models such as those examined here may be useful for predicting relationships between nutrient concentration and benthic algae biomass under some circumstances. However, because algal biomass accrual is influenced by site-specific characteristics such as drainage and catchment area, flow rate, and flood frequency in addition to nutrient concentration, it can be difficult to assess the applicability of preexisting models in novel study areas that may not share watershed or other characteristics of other systems.. Despite these limitations, comparisons of site-specific data collected for this study most closely fits the model detailed in Lohman et al. (1992) for the 1986 dataset (RMS error for TN = 31 mg/m^2, RMS error for TP = 18 mg/m^2. In general, and with the possible exception of the study by Chételat et al (1999), all other relationships tended to under predict observed Chl-a at study sites in the Rogue River.

3.4 Summary of Algal Associations with Nutrients Reported in the Literature

While relationships between suspended algae abundance and water column nutrients have been well established in both laboratory and lake settings (Cooke et al 1993), a variety of physical and chemical factors affect stream algae (e.g., shade, temperature, substrate, gradient, accrual period between high flow scour events, invertebrate grazing); nutrient levels alone have been shown to explain only 40–60% of the variations in algal biomass in rivers and streams (Dodds et al. 1997, Biggs 2000a). We provide a brief overview of literature associations between nutrient levels, algal biomass, and algal community structure. These have been largely reproduced from a literature review and quantitative comparisons of nutrient associations with algae, BMI, and fish assemblage data conducted by Miltner et al (2011) to support the development of nutrient water quality standards for rivers and streams in Ohio (Appendix F).

While laboratory and mesocosm studies have shown that algal growth rates can be saturated at low concentrations of nutrients, many field studies have shown changes in algal abundance or composition over several orders of magnitude in nutrient concentrations (Figure 3-11 and Appendix F). Several states have compiled these and other references in developing nutrient criteria recommendations with similarly broad ranges in recommended nutrient criteria. The broad range in study results across a range of ecoregional settings suggest that periphyton may respond to nutrient concentrations above theoretical saturation concentrations due to diffusive mass transfer limitation from the turbulent water column through the boundary layer into periphyton. A conceptual model tested by Larned et al (2004) describes the interactions between nutrients, water velocity, periphyton canopy structure relative to the diffusive boundary layer. They concluded that nutrient uptake is generally mass-transfer controlled and rarely kinetically controlled, which may explain the lack of sensitivity to water column concentrations in some studies as well as the larger range in periphyton responses to elevated nutrients in both field and mesocosm settings (Figure 3-11).

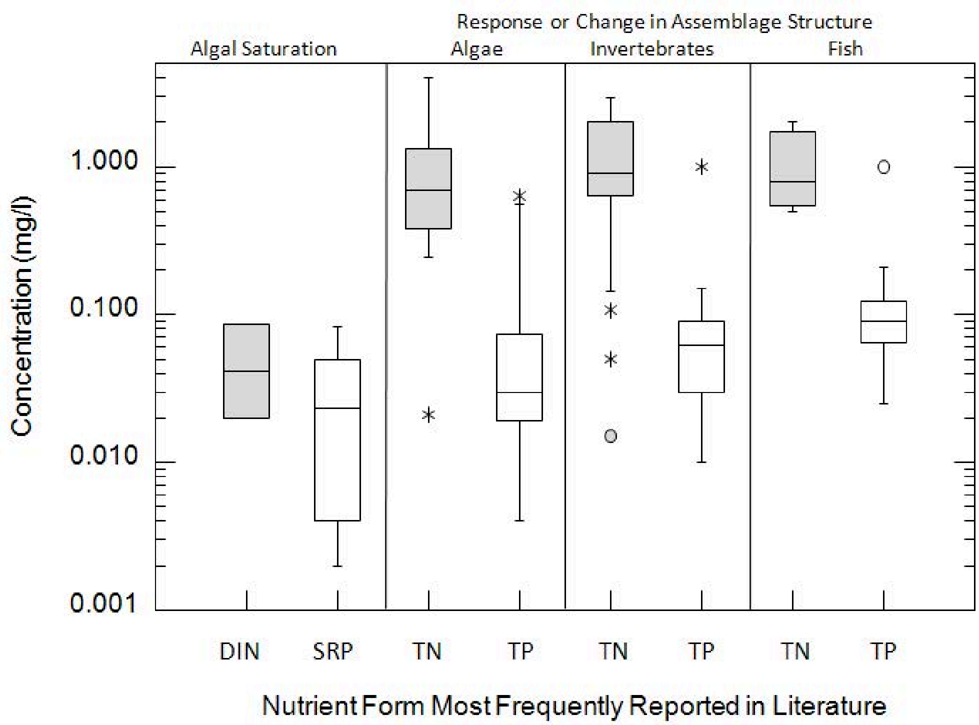


Figure 3-11. Quantiles of reported nutrient ranges associated with algal growth saturation, and changes to community assemblage of algae, macroinvertebrates, and fish (modified from Miltner et al 2011).[2]

3.5 Synthesis and Discussion

In this assessment, we have proceeded with an assumption that excess nutrients leading to accrual of algal biomass identified in previous studies (Hafele 2013, Brown and Caldwell 2014, Stillwater Sciences 2019) are the proximate factors in explaining reduced habitat suitability for some BMI taxa at sites downstream of the RWRF. As described in Section 1.3, we have adopted an approach that combines comparisons of conditions at local or ecoregional reference sites (Section 3.1); application of predictive relationships to select nutrient concentrations that will result in appropriate levels of algal biomass (Section 3.2 and 3.3); as well as literature-based thresholds (Section 3.4).

3.5.1 Range of nutrient thresholds considered

As discussed in Section 3.1 average concentrations at the sites upstream of the RWRF outfall (Sites 2, 3, RMZ-N, 4N) are within the range of the low (25th percentile) concentrations for the larger surrounding ecoregion (Table 3-1). It should be noted however, that algal accumulation was observed at these sites despite the presence of these relatively low nutrient levels (Section 2.5.2), and setting nutrient criteria based upon matching average upstream conditions may not necessarily reduce periodic periphyton accumulation or prevent local shifts in the algal and

[2] Boxes represent 25th, 50th and 75th percentile, with outliers shown outside the "whiskers" defined as 1.5 times the inter-quartile range (IQR = 75th minus 25th percentile values). Dissolved inorganic nitrogen (DIN) and Soluble Reactive Phosphorus assumed to be equivalent to TIN and PO_4-P.

benthic macroinvertebrate community composition. Nevertheless, the summer /fall average nutrient concentrations at sites upstream of the RWRF during 2019 are included as a potential instream target for establishing discharge limits at the RWRF outfall (Table 3-2). In addition to incremental reductions of average nutrient concentrations at the downstream end of the regulatory mixing zone (Site RMZ-S), other potential nutrient thresholds are discussed below.

Table 3-2. Potential nutrient thresholds for the Rogue River in the vicinity of the Medford RWRF.

Description	TIN (mg/L)	TN (mg/L)	PO_4-P (mg/L)	TP (mg/L)
Thresholds based on average nutrient concentrations during summer/fall (July-November) 2019				
Avg. of Sites 2 and 3 (upstream of RWRF), Sites RMZ-N[1], and 4N[1]	<0.09[2]	<0.19[J,3]	0.03[J]	0.05
Avg. at Site RMZ-S	0.66	0.98	0.09	0.17
30% reduction of Avg. at Site RMZ-S	0.46	0.69	0.06	0.12
40% reduction of Avg. at Site RMZ-S	0.40	0.59	0.05	0.10
50% reduction of Avg. at Site RMZ-S	0.33	0.49	0.04	0.08
60% reduction of Avg. at Site RMZ-S	0.26	0.39	0.03	0.07
Site specific relationships of predicted biomass from measured nutrient concentrations during summer/fall 2019				
2019 regression model TN and TP predictions to meet seasonal maximum of 100 mg/m² Chl-a		0.19		0.04[4]
2019 regression model TN and TP predictions to meet seasonal mean of 35 g/m² AFDM		0.21		0.05
Literature relationships of predicted biomass from measured nutrient concentrations during summer/fall 2019				
Lohman et al (1992) regression model TN and TP predictions to meet seasonal mean of 50 mg/m² Chl-a		0.25		0.02[4]
Thresholds based on literature reviews of TN and TP effects upon algae and stream biota				
Miltner et al (2011) 25th percentile TN and TP effects level on algae		0.40		0.02[4]
Miltner et al (2011) 25th percentile TN and TP effects level on benthic macroinvertebrates		0.60		0.03[4]
Dodds et al (1997) Oligotrophic/Mesotrophic Boundary		0.70		0.025[4]

J One or more 2019 sampling results below laboratory method reporting limit (RL), but above method detection limit (DL) and is reported here as a J-flag; therefore, seasonal average is an approximation.
1 Site assumed to be unaffected by RWRF discharge as determined by dye-tracer study of outfall plume (Brown and Caldwell 2014)
2 Ammonia-N (NH_3-N) and NO_2^-+ NO_3 were not detected between July and November. Seasonal TIN average reported as the sum of the individual DLs.
3 Because NO_2^- + NO_3 were not detected between July and November, average value based upon the sum of the TKN results and DLs for NO_2^- + NO_3
4 TP threshold is below seasonally averaged TP concentrations found at sites outside the hydraulic influence of the RWRF Outfall (Sites 2, 3, RMZ-N, 4N).

Based upon commonly recommended periphyton biomass thresholds of maximum Chl-a <100 mg/m² (Dodds et al 1997; Nordin 1985; Quinn 1991), site-specific relationships developed from data collected during 2019 (Figure 3-4) were used to estimate mean seasonal TN and TP concentrations of 0.19 and 0.04 mg/L, respectively. Because a corresponding relationship for maximum seasonal AFDM resulted in a negative relationship with increasing nutrients (Figure 3-1), application of maximum biomass criteria of AFDM <35 g/m^2 (Biggs 2000b, Suplee et al 2008) could not be used. Instead, Table 3-2 includes an estimate of mean seasonal TN and TP necessary to meet this target based upon a regression of seasonal mean AFDM included in Figure 3-2. In addition to the site-specific relationships developed from data collected during 2019, we have included estimates of TN and TP of 0.25 mg/L and 0.02 mg/L predictions to meet a seasonal mean biomass of Chl-a <50 mg/m^2 (Lohman et al 1992; Dodds et al 1997).

In addition to site specific relationships (Section 3.2) and literature relationships between stream biomass and dissolved nutrients, we have included nutrient thresholds from meta-analyses of studies from the wider literature. Using the lower quartile of nutrient ranges found to result in shifts in algal community structure, Miltner et al (2011) identifies thresholds below 0.4 mg/L and 0.02 mg/L as TN and TP, respectively. When considering benthic macro-invertebrate community structure, increased lower quartile thresholds of 0.6 mg/L TN and 0.03 mg/L TP were identified (Figure 3-11). Lastly, higher estimates of 0.7 mg/L TN and TP of 0.025 mg/L TP were included based upon thresholds exceeding the oligotrophic-mesotrophic in studies examined by Dodds et al (1997).

3.5.2 Mixing Model development and scenario evaluation

As discussed in Section 3.1 average concentrations at the sites upstream of the RWRF outfall (Sites 2, 3, RMZ-N, 4N) are within the range of the low (25th percentile) concentrations for nitrogen, but with TP concentrations higher locally than was found in the surrounding ecoregion (Table 3-1). In determining appropriate nutrient discharge limits for the Medford RWRF, we must account for ambient concentrations in the Rogue River upstream of the outfall, concentrations in the outfall, as well as the ratio of the two discharges within the period of interest. Here, we apply a simple material balance within the RMZ which relies upon assumptions of both steady state conditions as well as conservation of mass principles (Nazaroff and Alvarez-Cohen 2001). Recognizing both diffusion and dispersion mixing processes occur in the mixing zone downstream of the RWRF outfall, an idealized material balance for the RMZ may be considered as follows using measurements of specific conductivity to estimate dilution.

Accumulation = Sum of Inflows – Sum outflows ± Generation/Decay

Under steady state conditions and considering a nonreactive tracer such as dissolved solids (i.e., Specific Conductivity) this may be formulated as follows:

$$d\frac{CV}{dt} = 0 = Q_{upstream} \times C_{upstream} + Q_{outfall} \times C_{outfall} - \left(Q_{upstream} + Q_{outfall}\right) \times C_{RMZ}$$

With abbreviations for discharge (Q), river outfall concentration (C), as well as a hypothetical differential control volume (V) where mixing is assessed. Under a steady state assumption (i.e., no accumulation or loss within the control volume), the above differential may be rearranged to solve for either the dilution ratio ($Q_{upstream}/Q_{outfall}$) or to solve for $C_{outfall}$ as follows:

$$Dilution = \frac{Q_{upstream}}{Q_{oufall}} = \frac{C_{RMZ} - C_{outfall}}{C_{upstream} - C_{RMZ}} \quad \text{Equation 1}$$

$$C_{outfall} = C_{RMZ} + Dilution \times \left(C_{RMZ} - C_{upstream}\right) \quad \text{Equation 2}$$

Based upon grab samples collected by the City between July and November (Table 2-5), specific conductivity measurements averaged 69.2, 80.8 and 555 uS/cm at sites upstream of the RWRF outfall, at the RMZ-S site, and at the RWRF outfall, respectively. Using Equation 1 above, the seasonally averaged dilution for the Medford RWRF outfall is 26.4, roughly one half of the ratio of the river and effluent discharges during summer and fall 2013 (Brown and Caldwell 2014). Assuming a dilution ratio of 25:1 and using the range of potential nutrient thresholds shown in Table 3-2, Equation 2 was used to estimate nutrient discharge limits from the RWRF outfall as a series of scenarios shown in Table 3-3. Applicable TN and TP targets were applied at the downstream end of the regulatory mixing zone (Site RMZ-S) based upon the range of TN and TP thresholds from Table 3-2. Asssuming that undectected ammonia-N and NO_3-NO_2 results (Table 2-5) are at least one half of the laboratory DLs, approximately 78% and 38% of the TN and TP in the river upstream of the RWRF is in the form of organic nitrogen and particulate phosphate. Using 2019 averages of the higher concentrations found at downstream sites, these proportions change to 33% of TN and 48% of TP at Site RMZ-S and fall to 13% and 14% within the RWRF at the outfall location (Table 3-1). Because inorganic nitrogen, particularly ammonium (NH_4^+) as well as PO_4-P are the nutrient forms that are most readily absorbed by periphyton (Dodds 2002), we emphasize that reducing TIN and PO_4-P contributions from the RWRF as the primary control strategy for reducing benthic algae accrual at sites in the Rogue River. Using the observed proportions of TIN to TN and PO_4-P to TP estimated from the 2019 sampling results, corresponding TIN and PO_4-P targets at Site RMZ-S were estimated at Site RMZ-S, with Equation 2 used to estimate the TIN and PO_4-P requirements at the outfall location (Table 3-3).

Table 3-3. Evaluation of outfall concentrations required to meet nutrient threshold scenarios for the Rogue River in the vicinity of the Medford RWRF.

Calculation/ Description	TIN (mg/L)	TN (mg/L)	PO_4-P (mg/L)	TP (mg/L)	Notes
Average nutrient concentrations upstream of the Medford RWRF during Summer/Fall (June–November) for comparison purposes only					
Avg. of Sites 2 and 3 (upstream of RWRF), Sites RMZ-N[1], and 4N[1]	<0.09[2]	<0.19[J,3]	0.03[J]	0.05	
Medford RWRF Outfall	18.23	20.99	2.87	3.34	
Scenario 1 – 40% reduction of Avg. TN and TP measured at Site RMZ-S during summer/fall 2019					
Scenario TN and TP thresholds at Site RMZ-S		0.59		0.10	
Estimated TIN and PO_4-P target at Site RMZ-S[2]	0.44		0.07		
Estimated Outfall Target[3]	9.17	10.56	1.18	1.37	
Percent Reduction at Outfall	50%	50%	59%	59%	
Scenario 2 – 60% reduction of Avg. TN and TP measured at Site RMZ-S during summer/fall 2019					
Scenario TN and TP thresholds at Site RMZ-S		0.39		0.07	
Estimated TIN and PO_4-P target at Site RMZ-S[2]	0.27		0.05		
Estimated Outfall Target[3]	4.74	5.46	0.43	0.50	
Percent Reduction at Outfall	74%	74%	85%	85%	

Calculation/ Description	TIN (mg/L)	TN (mg/L)	PO_4-P (mg/L)	TP (mg/L)	Notes
Scenario 3 – 2019 Site-specific predictions of TN and TP to meet maximum of 100 mg/m^2 Chl-a					
Scenario TN and TP thresholds at Site RMZ-S		0.19		0.04	TP target < Avg. at upstream sites
Estimated TIN and PO_4-P target at Site RMZ-S[2]	0.09		0.02		PO_4-P target < Avg. at upstream sites
Estimated Outfall Target[3]	0.17	0.19	<0.03	<0.05	Infeasible PO_4-P, TP target
Percent Reduction at Outfall	99%	99%	>100%	>100%	
Scenario 4 – Lohman et al (1992) regression model predictions of TN and TP to meet mean of 50 mg/m^2 Chl-a					
Scenario TN and TP thresholds at Site RMZ-S		0.25		0.02	TP target < Avg. at upstream sites
Estimated TIN and PO_4-P target at Site RMZ-S[2]	0.14		0.00		PO_4-P target < Avg. at upstream sites
Estimated Outfall Target[3]	1.52	1.75	<0.03	<0.05	Infeasible PO_4-P, TP target
Percent Reduction at Outfall	92%	92%	>100%	>100%	
Scenario 5 - Apply 25th Percentile TN and TP associated with invertebrate community shifts from Miltner et al (2011)					
Scenario TN and TP thresholds at Site RMZ-S		0.40		0.02	TP target < Avg. at upstream sites
Estimated TIN and PO_4-P target at Site RMZ-S[2]	0.27		0.00		PO_4-P target < Avg. at upstream sites
Estimated Outfall Target[3]	4.91	5.65	<0.03	<0.05	Infeasible PO_4-P, TP target
Percent Reduction at Outfall	73%	73%	>100%	>100%	
Scenario 6 - Apply 25th Percentile TN and TP associated with invertebrate community shifts from Miltner et al (2011)					
Scenario TN and TP thresholds at Site RMZ-S		0.60		0.03	TP target < Avg. at upstream sites
Estimated TIN and PO_4-P target at Site RMZ-S[2]	0.45		0.01		PO_4-P target < Avg. at upstream sites
Estimated Outfall Target[3]	9.42	10.85	<0.03	<0.05	Infeasible PO_4-P, TP target
Percent Reduction at Outfall	48%	48%	>100%	>100%	
Recommended Thresholds					
Final RMZ-S thresholds	0.27	0.40	0.07	0.10	
Estimated Outfall Target[3]	**4.91**	**5.65**	**1.16**	**1.35**	
Percent Reduction at Outfall	73%	73%	60%	60%	

J One or more 2019 sampling results below laboratory method reporting limit (RL), but above method detection limit (DL) and is reported here as a J-flag; therefore, seasonal average is an approximation.
1 Site assumed to be unaffected by RWRF discharge as determined by dye-tracer study of outfall plume (Brown and Caldwell 2014)
2 Ammonia-N (NH_3-N) and NO_2^-+ NO_3 were not detected between July and November. Seasonal TIN average reported as the sum of the individual DLs.
3 Because NO_2^- + NO_3 were not detected between July and November, value based upon TKN results. Including DLs for NO_2^- + NO_3 would increase seasonal average estimate by 0.02 mg-N/L.

Overall, the results presented in Table 3-3 above show that four of the scenarios for PO_4-P targets at Site RMZ-S were below the average concentrations found at sites upstream of the RWRF (Sites 2, 3, RMZ-N, and 4N) during summer/fall 2019, and so would require discharge concentrations below background. The site-specific relationships developed for this study (Scenario 3) produced estimates of required RMZ-S targets that were less than the average upstream concentrations shown in Table 3-1. The relationships by Lohman et al (1992) shown in Scenario 4 produced low TIN targets, and PO_4-P targets that were less than the upstream concentrations. Using the low range (25th percentile) effects levels identified for algae and benthic macroinvertebrates (Figure 3-11), Scenarios 5 and 6 resulted in recommended TIN target levels of 0.27 mg/L and 0.45 mg/L at Site RMZ-S, respectively, but also PO_4-P targets that were less than the upstream concentrations.

Based upon observed dilution levels and the mixing assumptions in the study, we recommend targets at the RMZ-S of 0.40 mg/L and 0.10 mg/L as TN and TP, with corresponding TIN and PO_4-P targets of 0.27 mg/L and 0.07 mg/L, respectively. To achieve these concentrations, the recommended nutrient limits at the RWRF outfall location are 4.91 mg/L TIN and 1.2 mg/L PO_4-P on a seasonally averaged basis. These discharge limits would represent approximately a 73% reduction in outfall TIN concentrations, and 60% of PO_4-P concentrations at the RWRF outfall under current operations. The resulting nutrient concentrations in the Rogue River at Site RMZ-S and further downstream of the RWRF, although still exceeding upstream concentrations, will likely not result in detrimental changes in the resident biological community as indicated by metrics used in this and previous studies, including periphyton biomass, periphyton and benthic macroinvertebrate community composition.

For comparison purposes, analytical water quality results from samples collected during 2018 (See Table 3-4, Stillwater Sciences 2019) show TN concentrations of 0.9 mg/L at Site 4, decreasing to 0.7 mg/L at Site 7, approximately 0.2 mi downstream of the Bear Creek confluence. At the same time algal biomass metrics at Site 7 (See Figure 3-2, Stillwater Sciences 2019) as well as some BMI indicators such as EPT (See Table 3-5, Stillwater Sciences 2018) approached those from sites upstream of the RWRF in the 2018 sampling, while nutrient concentrations were only slightly lower than those at sites in close proximity to the RWRF outfall. While the recommended TN targets in the Rogue River are above those predicted to maintain benthic Chl-a levels below levels assessed in Scenarios 4 (mean seasonal Chl-a <50 mg/m^2) and Scenario 3 (maximum seasonal Chl-a <100 mg/m^2) on the basis of site specific and literature relationships, they are within the low effects range (25th percentile) for algae identified by Miltner et al (2011), and well below the corresponding quantiles for effects levels associated with shifts in benthic macro-invertebrates or fish assemblage structure (Figure 3-11), thereby ensuring that the RWRF does not contribute to exceedances of the State of Oregon biocriteria standard (OAR 340-041-0011).

For phosphorus, the recommended TP targets at Site RMZ-S are above those found in the literature (Table 3-2) and general statewide DEQ guidelines (> 0.08 mg/L total P) (Hicks 2005). Previous studies, however, have concluded that the Rogue River is generally nitrogen limited in the vicinity of the RWRF (Stillwater Sciences 2019, Brown and Caldwell 2014), making phosphorus reductions potentially unnecessary. Nonetheless, because observed nutrient levels may be influenced by uptake along the channel bottom, there is a potential for co-limitation by phosphorus (Francour et al 1999). For this reason, we have recommended PO_4-P reductions roughly proportional to the TIN reductions above. In additon to expected reductions in periphyton biomass with reduced TN and TP concentrations, because algal community compositon has been shown to be affected by nutrient levels in some systems (Sosiak 2002, Suplee et al 2012), the recommended nutrient reductions may be expected to reduce the dominance of green algae (e.g., *Cladophora*) relative to diatoms in the reaches downstream of the RWRF.

Stream velocity is an important determinant of the growth and accumulation of both periphyton (Biggs et al 1999) as well as SAV (Chambers et al 2011). For this reason, local patterns in velocity distribution as well as the frequency of high flow events may also explain the relative amounts of periphyton and SAV biomass at locations upstream and dowsntream of the RWRF. Further, SAV abundnace has been shown to be affected by sediment rather than water column nutrient concentrations (Jones et al 2012). While we would expect that the proposed nutrient reductions discussed above may result in reduced SAV cover and abundance at locations dowsntream of the RWRF, conditions supporting SAV (e.g., low veocity zones, stream sediment accumulation) may continue even with the application of the recommended nutrient limits discussed above.

3.5.3 Seasonal and monthly application of recommended nutrient discharge limits

Recognizing that a range of factors (e.g., nutrients, light, photoperiod, temperature, scour velocity, substrate, grazing) have been associated with seasonal cycles of periphyton biomass accrual and depletion (Biggs 1995), abundance in many river systems is seasonal, with peak abundance and diversity typically occurring in late summer or early fall (Bahls 1993; Francoeur et al., 1999). In this Study we also found peak biomass occurring in August with lower levels during subsequent (September–November) sampling events (Figure 2-11 and Figure 2-12). Previous studies indicate that nutrient availability limits algal biomass accrual in summer more than at other times of year, when other factors such as water temperature and light availability play a more significant role (Rosemond et al 2000). Additionally, flood scour events that have been shown to limit algal biomass (Biggs & Close, 1989; Biggs, 2000) are more likely to occur during winter months. Considering water discharge rates over the past 20 years at Dodge Bridge (USGS Gage No. 14339000), regular rain and snowmelt between late November and mid-April significantly increases the likelihood of high flow scour events during those months, with very few high flow events occurring between July and September (Figure 3-12). Because light intensity, photoperiod, and water temperatures are also lower during winter months, nutrient availability is likely to have a reduced impact on algal biomass between November and April. Accordingly, we recommend applying the nutrient discharge limits developed for the RWRF to the period of May 1st through October 31st of each year when benthic algal biomass accrual is usually highest and when elevated nutrient concentrations are likely to have the highest relative impact at locations downstream of the RWRF. During winter months, we believe that hydraulic disturbance (i.e., detachment, or bed scour), lower water temperatures, and limited light availability are likely to limit the potential effects of nutrients discharged to the Rogue River upon periphyton accrual.

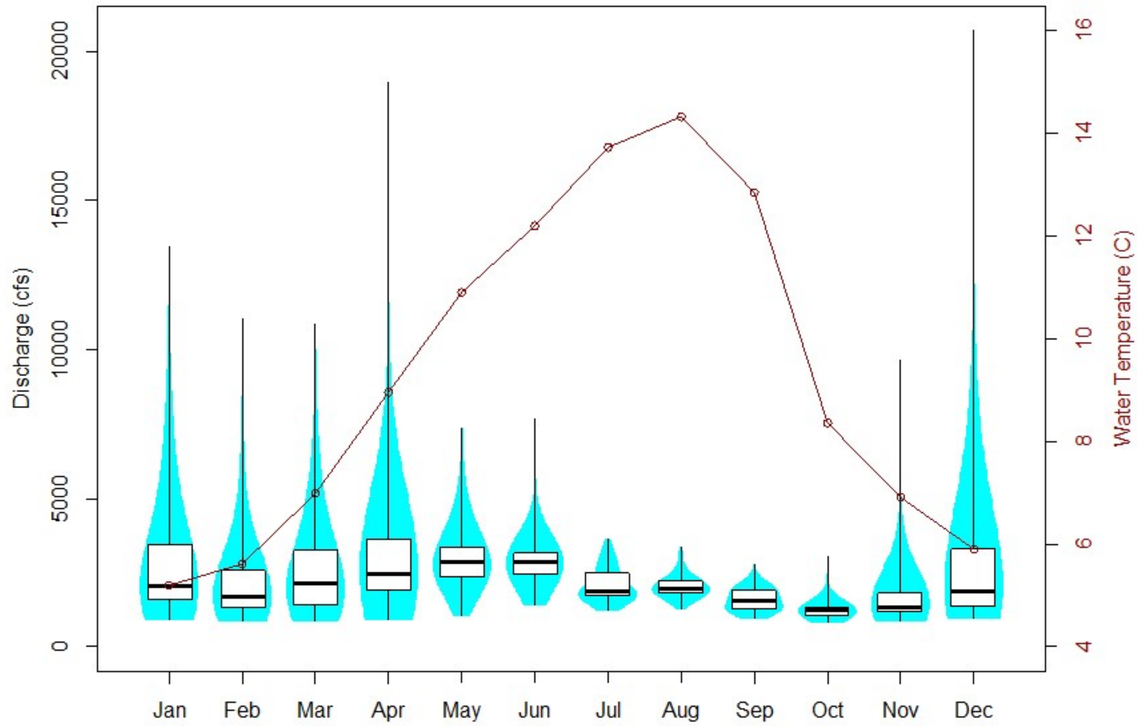


Figure 3-12. Violin and box plots showing variations in daily mean discharge for 10/1/1999 through 10/1/2019 as well as mean daily water temperatures from 10/1/2007 through 10/1/2019 plotted by month in the Rogue River at Dodge Bridge (USGS 14339000)[3]

In addition to the seasonal application of the recommended nutrient discharge limits, we propose that recommended limits at the RWRF should be applied on an average monthly basis. In studies examining periphyton growth in response to short term variations in nutrient concentrations, Elsdon and Limburg (2008) found that short-term pulses of nutrients (<2 weeks) had no significant effect on biomass accrual in rural streams in New Zealand. Other experiments conducted in Kentucky at shorter times scales showed pulses of PO_4-P and NO_3-N lasting up to 12 hours had little effect upon algal biomass (Humphrey and Stevenson 1992). Based upon these studies, we propose that exceedances of the proposed nutrient discharge limits due to variations in RWRF operations on a time scale of days (e.g., < 14 days) should not result in excess periphyton accrual as long as the discharge limits are met on a monthly average basis.

4 CONCLUSIONS AND RECOMMENDATIONS

Based upon three previous studies (Hafele 2013, Brown and Caldwell 2014, ODEQ 2014) examining periphyton and benthic macro-invertebrate community composition in relation to discharges from the City of Medford's (City) municipal wastewater treatment plant, Stillwater Sciences (2019) identified nutrient enrichment associated with RWRF effluent as a contributing

[3] Violin plots after Hintze and Nelson (1998) showing probability density (blue shading) of daily discharge by month, superimposed by box plots showing 25th, 50th, and 75th percentiles as well as extreme daily discharge values.

factor to increased algal biomass and associated changes in macroinvertebrate community structure downstream of the regulatory mixing zone (RMZ) defined in the City's NPDES Permit. To determine appropriate nutrient discharge limits needed to ensure that the City's regional water reclamation facility (RWRF) does not contribute to exceedances of the State of Oregon biocriteria standard (OAR 340-041-0011) in the Rogue River outside the RMZ, the City developed a study plan for additional sampling and analysis during 2019 in cooperation with Northwest Environmental Advocates (NWEA).

Following USEPA (2000a) recommendations for establishing such limits, this study employs a combined approach, incorporating updated site-specific sampling along a gradient of water quality conditions in the vicinity of the RWRF, a review of predictive relationships between algal periphyton biomass and nutrient concentrations in the literature, and a fit of those predictive relationships to site-specific data collected during summer and fall of 2019. Additional literature-based nutrient thresholds were also summarized from studies in the United States, Canada, Australia, and New Zealand. Based on analysis and assessment of these approaches and using comparisons to historical water quality data in the nearby Rogue River and Klamath Mountains ecoregion (USEPA 2000b), this report makes the following findings.

- Analytical testing results of dissolved sources of nitrogen and phosphorus that may be used as nutrients by periphyton and submersed aquatic vegetation were found to be near regional background levels at sites upstream and outside the hydraulic influence of the RWRF outfall (2019 study sites 2, 3, RMZ-N, and 4N), with elevated concentrations found in monthly sampling conducted at all sites under the hydraulic influence of the RWRF outfall (2019 study sites RMZ-S, 4S, 5, and 6).
- Grab sampling results at sites downstream of Little Butte Creek (2019 study Site 2), as well as at sites downstream of the RWRF outfall (2019 study sites RMZ-S, 4S), also indicated lateral variations in nutrient concentrations across the channel, indicating incomplete mixing at distances of up to 0.5 miles or more from the RWRF outfall and tributary locations.
- To examine the influence of the RWRF discharge and stream periphyton upon in situ water quality, continuous multiparameter water quality monitoring instruments (sondes) were deployed at locations upstream and downstream of the RWRF. As found in previous studies, diel variation in dissolved oxygen (DO) and pH are consistent with algal photosynthetic exchanges of dissolved gases (DO and CO2 [aq]) at locations both upstream and downstream of the RWRF. During August and October 2019, DO concentrations and percent saturation met ODEQ criteria (8.0 mg/L) at all locations. pH exceeded ODEQ criteria (pH 6.5–8.5) for short periods at sites upstream of the RWRF (2019 Study Sites 3 and 4N) during August, with minor exceedances with pH>8.5 at Site 4N as well as pH <6.5 at Site 4S during October.
- Based upon sampling of attached periphyton at transects established upstream and downstream of the RWRF, periphyton biomass (cell density, biovolume, chlorophyll-a, ash free dry mass) was generally greater at sites under the hydraulic influence of the RWRF outfall (2019 Study sites 4S, 5S, 5N, and 6) as compared to Site 4N and upstream Sites 2 and 3.
- Changes in periphyton community structure were also observed at locations under the hydraulic influence of the RWRF outfall relative to upstream sites, with a shift from a predominance of diatom species upstream of the RWRF outfall to green algae species at downstream sites.

- Exploratory regression-based relationships between periphyton biomass metrics and stream nutrient concentrations were constructed from sampling data collected in 2019, with positive associations found between the following parameters:
  - Mean seasonal AFDM and mean seasonal nutrients (TN and TP).
  - Maximum seasonal Chl-a and mean seasonal nutrients (TN and TP).
  - Mean seasonal cell density and mean seasonal nutrients (TN and TP).
  - Mean seasonal biovolume and mean seasonal nutrients (TN and TP).
- Mathematical models from other systems that correlate TN and/or TP with benthic algal biomass were also evaluated in comparison to sampling data collected in 2019, including regression relationships by Lohman et al. (1992), Dodds et al. (1997), Chételat et al. (1999), and Biggs (2000). While underpredicting site specific Chl-*a* biomass data collected in the Rogue River in 2019, TN and TP relationships by Lohman et al (1992) provided the best fit to sampling data collected in 2019.
- Recognizing there are a number of uncertainties in linking nutrient levels to stream periphyton, many field studies have shown changes in algal abundance or composition over several orders of magnitude in nutrient concentrations. Preliminary nutrient thresholds to be applied at Site RMZ-S and sites downstream of the RWRF included:
  - Site specific model predictions of TN and TP corresponding to a maximum seasonal biomass of 100 mg/m^2 Chl-*a*
  - Literature-based (Lohman et al 1992) model predictions of TN and TP corresponding to a mean seasonal biomass of 50 mg/m^2 Chl-*a*
  - Literature-based TN and TP thresholds corresponding to the low (25th percentile) effects levels associated with shifts in community structure of algae, benthic macroinvertebrates, and fish assemblages (Miltner et al 2011).
- Using a mixing model approach to estimate RWRF outfall concentrations necessary to meet various nutrient thresholds at Site RMZ-S, a series of scenarios examined the range of TN and TP thresholds identified during this study. Overall, the range of results indicates that, while varying levels of TN reductions may be accomplished, targets approaching observed concentrations at sites upstream of the RWRF were found to be unnecessary. Based upon these results we recommend targets at the RMZ-S of 0.40 mg/L and 0.10 mg/L as TN and TP, with corresponding TIN and PO_4-P targets of 0.27 mg/L and 0.07 mg/L, respectively. Although the recommended TP thresholds are slightly above those found in the literature and general statewide DEQ guidelines (<0.08 mg/L total P) (Hicks 2005), previous studies have concluded that the Rogue River is generally nitrogen limited in the vicinity of the RWRF (Stillwater Sciences 2019, Brown and Caldwell 2014), making phosphorus reductions potentially unnecessary.
- Based upon observed dilution levels and the mixing assumptions in the study, the recommended nutrient discharge limits at the RWRF outfall location are 4.91 mg/L TIN, 5.65 mg/L TN, 1.16 mg/L PO_4-P, and 1.35 mg/L TP on an average monthly basis from May 1st through October 31st of each year.
- Based upon review of studies examining periphyton growth dynamics in response to short term variations in nutrient concentrations (Elsdon and Limburg 2008, Humphrey and Stevenson 1992), exceedances of the proposed nutrient discharge limits due to variations in RWRF operations on a time scale of days (e.g., < 14 days) should not result in excess periphyton accrual as long as the discharge limits are met on a monthly average basis.

This report recognizes the difficulty in establishing the precise relationship between nutrient availability and algal biomass in a complex river ecosystem. Results that are developed from a data set collected over one or two seasons and spanning a narrow range of nutrient conditions may be expected to produce uncertain predictions. For example, the presence of significant periphyton biomass upstream of the RWRF outfall during August 2019 sampling suggests the potential for upstream nutrient sources to continue to contribute to periphyton accumulation in the vicinity of the RWRF. Further, because SAV abundance has been shown to be strongly affected by variations in stream velocity and sediment associated nutrients, localized accumulations of SAV may continue to occur at sites upstream and downstream of the RWRF even with the adoption of the proposed nutrient limits. Despite these limitations, the proposed nutrient discharge limits represent approximately a 73% reduction in TIN concentrations and a 60% reduction in PO_4-P concentrations at the RWRF outfall. Subject to the influences of stream velocity and other factors affecting periphyton and SAV biomass, the resulting nutrient concentrations in the Rogue River at Site RMZ-S and locations further downstream of the RWRF, although still exceeding upstream concentrations, will likely not result in detrimental changes in the resident biological community as indicated by metrics used in previous studies, including periphyton biomass, periphyton and benthic macroinvertebrate community composition. Recommended TN thresholds are within the low effects range (25th percentile) for algae identified by Miltner et al (2011), and TN and TP thresholds are well below the corresponding quantiles for concentrations associated with shifts in benthic macro-invertebrates or fish assemblage structure, thereby ensuring that the RWRF does not contribute to exceedances of the State of Oregon biocriteria standard (OAR 340-041-0011) outside the RMZ.

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