Rebuttal of the Report, "Nutrient Discharge Limit Assessment for the Rogue River in the Vicinity of the City of Medford Regional Water Reclamation Facility," by Stillwater Sciences (March 2020)

Report for Plaintiff Northwest Environmental Advocates, Revised May 30, 2020

by

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Executive Summary

The wild and scenic Rogue River near the City of Medford provides critical spawning and general habitat for sensitive salmonid fish, including the threatened southern Oregon/northern California Coho. Since the late 1960s at river mile (RM) 130.5, it has received partially treated effluent from the City of Medford Regional Water Reclamation Facility (RWRF), pursuant to NPDES Permit #100985, Dec. 2011 from the Oregon Department of Environmental Quality (ODEQ). Several studies in 2012-2019 indicated violations of Oregon's biocriteria standard and narrative water quality criteria related to excessive nutrients (nitrogen, N, and phosphorus, P) from the Medford RWRF effluent, and the river segment was listed as impaired (Opalski 2016). In 2018, Northwest Environmental Advocates (NWEA) filed a lawsuit against the Medford RWRF under the citizen suit provision of the Clean Water Act in the U.S. District Court for the District of Oregon alleging that the City of Medford was/is impairing the affected segment for its designated uses for fish and aquatic life. Pursuant to a partial settlement agreement between NWEA and the City of Medford (May 28, 2019), the City agreed to support Stillwater Sciences to conduct further sampling in 2019 to assess water quality, benthic algae, and SAV upstream and downstream from the Medford RWRF. The data were to be used to assess whether lower effluent total N (TN) and total P (TP) are needed to protect the designated uses of the river, and to meet the applicable criteria. If so, revised effluent limits were to be suggested.

Stillwater Sciences and Medford's engineering consultants, West Yost Associates, concurrently released companion reports in March 2020, describing the Stillwater Sciences 2019 sampling effort and some alternatives for improved sewage treatment. The stated goal of the Stillwater Sciences report (p.1) was "to evaluate whether and to what extent nutrient discharge restrictions may be needed to address any Medford RWRF contribution to water quality standards not being met in the river outside the Medford RWRF's RMZ." The report asserted (p.2) that, following U.S. EPA (2000a) guidance, a combined approach was used to establish [suggest] nutrient discharge limits for the Medford RWRF, including site-specific data in the reach upstream and downstream of the outfall (reference site approach), comparisons to existing predictive relationships between nutrients and algal biomass, and comparisons to existing nutrient thresholds from the literature. That writing was inaccurate. Stillwater Sciences did include a robust comparison of data from the Rogue River to many other rivers, but then ignored the finding that the Rogue River is more sensitive to nutrient pollution than the other rivers. Stillwater Sciences did include comparisons to existing nutrient thresholds from the published science literature, but then picked much higher nutrient thresholds than minimally impacted areas of the middle Rogue River as "appropriate" for use in deriving suggested effluent limits for this nutrient-sensitive river.

Thus, Stillwater Sciences failed in its goal to provide a science-based evaluation of the extent to which nutrient discharge restrictions for the Medford RWRF are needed. This rebuttal report addresses four main topics as follows.

Biocriteria, the Medford RWRF, and Additional Required Protection

- The Medford RWRF has failed to comply with the biocriteria standard. Several previous studies have confirmed biological impairment of beneficial macroinvertebrate communities downstream from the Medford RWRF regulated mixing zone (RMZ). Noxious benthic algal and SAV overgrowth fueled by excessive nutrient contamination from the Medford RWRF repeatedly was identified as the main cause.

- ***The Stillwater Sciences study supported those findings***; data from its 2019 study showed excessive N and P contamination downstream from the Medford RWRF, as well as high benthic algal and SAV biomass in the affected river segment. The study additionally demonstrated that the Medford RWRF effluent has created adverse conditions for beneficial aquatic life by promoting unhealthy diel dissolved oxygen variation; and that the excessive inorganic N and P, together with the high conductivity from the effluent, have supported major growth and recent dominance of a notorious noxious responder to sewage, the macroalga *Cladophora*.
- Other parameters of concern are identified as temperature, dissolved oxygen, and pH conditions for salmonid spawning, and for the eggs and young life history stages. At present, returns to the Rogue River of naturally produced Chinook salmon in spring are only about two-thirds of the desired status set by the Oregon Department of Fisheries and Wildlife.
- The Rogue River is already impaired for its designated use for fish and wildlife. An identified cause is violations of Oregon's biocriteria standard due to noxious benthic algal/SAV overgrowth fueled by excessive N and P contamination.

Evaluation of the Stillwater Sciences Study

The Stillwater Sciences study is not science-based. Numerous serious errors and poor approaches characterize its sampling design, field work, laboratory analyses, and data analyses. As examples:

- The timing of the 2019 study *likely missed* the benthic algal biomass maximum, which was the basis for Stillwater Sciences' suggested Medford RWRF effluent TN and TP limits.
- Inadequate reporting limits in Stillwater Sciences' methods for measuring nutrient concentrations resulted in its *fundamental inability to quantify nutrients in control or upstream waters*. As a result, highly uncertain estimates were used for critically important parameters that must be reliably quantified in order to set protective effluent limits.
- Such major mistakes were made in Stillwater Sciences' methods for estimating benthic algal biomass that some important data are *beyond salvage*, and the study yielded *only rough qualitative information* for a key group of responders to nutrient pollution, SAV—and even some of that cannot be trusted.
- The continuous data for dissolved oxygen were inadequate (sparse). The data also indicated questionable calibrations and failures to maintain the datasondes for appropriate data quality control/assurance.
- Stillwater Sciences repeatedly deviated from published field and laboratory protocols that it had agreed to follow, resulting in
  - Unreliable field estimates of primary producer relative abundance as percent cover, due not only to failure to follow protocols but also, even more fundamentally, to failure to fill out field datasheets;
  - Questionable laboratory data for benthic algal and SAV biomass as chlorophyll *a* due to failure to correct the data for inclusion of dead algal/plant remains, inadequate homogenization prior to subsampling, inadequate quantity of subsamples, and steps that promoted pigment degradation prior to analysis;

    - Ash-free dry mass estimates that were beyond salvage due to inclusion of copious non-algal and non-plant debris;
    - Stillwater Sciences' focus on dead+live algae rather than viable algae in abundance assessments using light microscopy, and use of "units" that cannot yield reliable quantitative estimates of algal cell number or biovolume; and
    - Such poor sampling, data recording, and analytical techniques for SAV that even Stillwater Sciences did not use the data in suggesting its effluent limits—although SAV are a known major group of responders to nutrient contamination downstream from the Medford RWRF outfall—in its further efforts to suggest effluent TN and TP limits.

- Stillwater Sciences emphasized the data from wet year 2019 in its efforts to suggest what are supposed to be protective effluent TN and TP limits—despite the fact that data which could have been used from a recent dry year revealed clearer "worst case" conditions since the benthic algae had more time to respond to the nutrient pollution during the lower flows of the dry year.

- Stillwater Sciences considered its 2019 data for maximum benthic algal chlorophyll versus TN and TP concentrations in comparison to data for rivers worldwide. The findings indicate that the middle Rogue River is moderately to highly sensitive to nutrient contamination compared to the many other rivers included in the analysis. Based on the analysis, effluent N and P limits need to be much lower than would be necessary in other, less sensitive rivers, because a very small amount of N or P results in more algal growth in the Rogue River than in the other systems. Stillwater Sciences ignored that important finding in choosing its suggested effluent nutrient limits for the Medford RWRF, by using excessive TN and TP concentrations as "background" levels so that higher effluent limits could be sanctioned.

- Stillwater Sciences included only a vague, brief description of the critical calculations and data used in "choosing" its suggested effluent TN and TP limits. Based on the available information, Stillwater Sciences did not follow the appropriate derivation steps it claimed to have followed. Instead, it apparently selected its targeted effluent limits first, then picked a low dilution factor and excessive background threshold concentrations to fit calculations for those effluent limits. By picking a low dilution factor, Stillwater Sciences conveyed the tacit, false message that downstream effects from the effluent can be remedied by less nutrient reduction than actually will be required.

- Stillwater Sciences' study was confounded by many assumptions that are not supported by science, such as
    - Unsupported assurance that Stillwater Sciences' suggested excessive TN and TP targets will not cause adverse impacts;
    - The false claim that its suggested excessive effluent limits will decrease the noxious alga *Cladophora*, a renowned responder to sewage that recently has become dominant in the affected river segment;
    - The mistaken assertion that phosphorus controls are not needed because nitrogen is limiting in the affected river segment when, clearly, algae and SAV in the affected river segment are so nutrient-saturated that neither N nor P is limiting.

- The recommendation that its excessive, although lower than present, suggested effluent limits should only be applicable to the period from May through October in each future year, which would result in continued degradation of the impaired river segment.

Protective Numeric Effluent Limits for the Medford RWRF

- The extremely high supplies *and* the proportion or supply ratio of N to P together have pushed the aquatic communities in the affected Rogue River segment out of balance to an unhealthy state. As a result, the aquatic communities are less stable, species diversity is depressed, and the food quality at the base of the food web is poorer—the community shifts to dominance of algae and plants, such as *Cladophora*, that often are not very nutritional or unpalatable for other reasons.

- Rivers impacted by partially treated sewage are much more vulnerable to adverse impacts of nutrient pollution in comparison to waters affected by other sources because most of the N and P is highly bioavailable, so that it rapidly stimulates noxious algal and plant growth.

- The following major issues should be considered in order to select protective effluent N and P limits for this Rogue River segment:

  - The effluent limits should be set as daily maxima and weekly averages, applicable year-round. They must be derived using 7Q10 low flow conditions, as well as accurate background concentration data under a low-precipitation year.

  - The U.S. EPA's reference (minimally impacted) approach should be followed in order to derive protective effluent limits. Dramatic reductions in bioavailable forms of *both N and P* are needed to control the noxious algae that have recently become dominant, as well as a TN:TP ratio that is similar to the ratio in minimally impacted waters of this nutrient sub-ecoregion.

  - As noted above, this Rogue River segment is already impaired for its designated use for fish and wildlife, with violations of the biocriteria standard related to excessive N and P contamination. Consistent with federal and state mixing zone guidance and regulations, a mixing zone is not appropriate for this impaired river segment. The permit must also consider contamination of downstream waters by highly soluble nitrate, which can easily be transported down to sensitive marine waters along the Oregon coast.

- Once applied, a lag period (years) should be expected to accomplish visible improvement in decreased biomass of noxious benthic algae/SAV and increased abundance of pollution-sensitive macroinvertebrates as the river shifts toward lower nutrient supplies.

Other Recommended Changes Going Forward

- An adequate monitoring-and-assessment plan should be designed and described in detail in the modified permit for the Medford RWRF, emphasizing macroinvertebrates, benthic algae, SAV, and water quality (temperature, pH, dissolved oxygen, and conductivity; TP and inorganic phosphate; and the nitrogen suite as ammonia-N, nitrate-N, and total Kjeldahl N). Recommendations are included for monitoring and assessment of each of these key components, as well as sampling frequency and methods.

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Section I. Background

The Rogue River headwaters begin in Crater Lake National Park, and the river drains mostly forested areas including waters from both the Klamath and Cascade Mountains (Myer 2013 and references therein). Historically this river was among the first in the nation that was placed under protection of the federal Wild and Scenic Rivers Act. The affected segment lies within the Middle Rogue River watershed, and is depended upon by various salmonid fish species for spawning and general habitat, including the threatened Southern Oregon/Northern California Coho. Since the late 1960s at river mile (RM) 130.5, it has been the receiving waterbody for the partially treated effluent from the City of Medford Regional Water Reclamation Facility (RWRF), discharged pursuant to NPDES Permit #100985, Dec. 2011 from the Oregon Department of Environmental Quality (ODEQ).

During the period from 2012 to 2019, several reports from various entities (a scientist supported by a concerned citizens group, an engineering firm, and the ODEQ), documented adverse impacts from the Medford RWRF on downstream macroinvertebrates and habitat for beneficial aquatic life. These impacts were related to excessive nutrient (nitrogen and phosphorus) discharges in the Medford RWRF effluent and resulting noxious benthic algal and submersed aquatic vegetation (SAV) overgrowth. Thus, the Rogue River was already known to be impaired for its designated use for fish and aquatic life. In December 2016, the U.S. EPA proposed to add, and in December 2018 the U.S. EPA did add, the middle Rogue River (RMs 110.7 to 132.2, including the reach immediately downstream from the Medford RWRF) to Oregon's Clean Water Act section 303(d) list for biocriteria impairment, and described the "Rogue River downstream of Medford STP [sewage treatment plant]" as being "most disturbed" (Opalski 2016). The U.S. EPA's listing decision was based entirely upon data presented in Hafele (2013) and Brown and Caldwell (2014) (see Section II below). Both reports, and other data described herein, make clear that the biocriteria exceedances are due to noxious benthic algal/SAV overgrowth fueled by excessive N and P contamination.

In 2018 Northwest Environmental Advocates (NWEA) filed a lawsuit against the Medford RWRF under the citizen suit provision of the Clean Water Act in the U.S. District Court for the District of Oregon, alleging that "Medford has violated, and continues to violate, the terms of its…permit by discharging polluted effluent…that contributes to myriad detrimental changes to the downstream waters of the Rogue River…." The resulting detrimental changes were identified as excessive growths of nuisance algae and unnatural shifts in the macroinvertebrate community, among others. NWEA described violations, at least since 2012, of two Oregon narrative water quality standards: its "biocriteria" standard and its narrative criteria that prohibit the "development of fungi or other growths having deleterious effect on stream bottoms, fish or other aquatic life" (see Section II below).

Pursuant to a partial settlement agreement between NWEA and the City of Medford dated May 28, 2019, Medford agreed to support Stillwater Sciences to conduct further sampling to assess the water quality, benthic algae (attached on the stream bottom or other substrata) and SAV in a control area immediately upstream from the Medford RWRF in comparison to sites downstream from it. The data were to be used to assess whether lower effluent total nitrogen (TN) and total phosphorus (TP) criteria are needed to protect the designated uses of the affected Rogue River segment for fish and aquatic life and to meet the applicable criteria, and if so, to suggest revised effluent TN and TP limits.

Stillwater Sciences released its final report in March of 2020.

This rebuttal report consists of four main parts: Section II summarizes previous studies about the affected Rogue River segment. Section III provides a thorough critique of the methods, assumptions, calculations, and effluent limits proposed in the Stillwater Sciences (2020) report. Section IV describes how to determine science-based effluent limits that will protect the designated uses of the affected Rogue River segment for fish and aquatic life. Section V includes recommendations for additional protective steps that should be taken to assess compliance in meeting the revised effluent TN and TP limits.

Section II. The Biocriteria Standard and the Medford RWRF

The Medford RWRF has failed to comply with the biocriteria standard, demonstrated by documentation from several recent scientific studies.

II.A. *History of Biocriteria Exceedances*

The first study conducted to document the potential biological impacts of the City of Medford's treated sewage effluent on the Rogue River was undertaken as a result of concerns by local anglers of the Rogue Flyfishers who observed several disturbing conditions at and below the treatment plant's outfall. The unsettling conditions included an extensive amount of foam on the water's surface starting at the outfall and extending hundreds of feet downstream, a strong smell of effluent below the outfall, and a clear change in plant growth on the stream bottom compared to above the outfall that was noticeable for up to a mile downstream. After their concerns raised to the regional office of the Oregon Department of Environmental Quality (ODEQ) were met with no follow-up action (John MacDiarmid, Rogue Flyfishers, pers. comm.), they decided to hire their own consultant to complete a biological assessment of conditions above and below the City's outfall. That study was completed in fall 2012, and a report describing the results was finished in early 2013 (Hafele 2013). The findings from that study showed that the effluent was causing significant exceedance of the state's biocriteria standard based on both macroinvertebrate and periphyton data. In addition to the biocriteria violations, the study also documented nuisance levels of scum, foam, and noxious odors – beginning at the outfall and extending well below the allowed 300 foot mixing zone set forth in the Medford RWRF NPDES permit – which are also violations of mixing zone rules as defined in Oregon Administrative Rules (OAR) 340-41-0053 (2)(a)(C).

Following the Hafele (2013) report, two other independent studies were completed: (1) ODEQ (2014) completed a field study the last week of September 2013 to assess biological conditions along a 31-RM mile reach of the Rogue River that included above and below the City of Medford's water treatment outfall, and (2) the City of Medford hired Brown and Caldwell consulting firm to complete a mixing zone study and biological assessment above and below the City's treatment plant (Brown and Caldwell 2014). Both studies confirmed biological impairment at sites well below the City's allowed mixing zone. For example, the ODEQ report stated: "The observations of this study and Hafele (2013) showed detrimental changes in the resident biological communities for up to a mile below the Medford WWTP. The responses of the algal and macroinvertebrate assemblages were consistent with responses typically associated with nutrient enrichment."

Two additional reports – Hafele (2019; data collected in 2017 and 2018) and Hume (2019) – were completed as part of the litigation between NWEA and the City of Medford. Both reports concluded that biological impairment of macroinvertebrates had occurred below the City's outfall, and identified excessive nutrients from the Medford RWRF effluent as the primary cause of the biocriteria violations. Table 1 and Figure 1 show locations of sample sites sampled in those studies.

Table 1. Sample sites where water quality, flow (discharge), and/or biological samples (macroinvertebrates and/or algae/plants) were collected in studies near the Medford RWRF prior to the Stillwater Sciences (2020) study.*

	Hafele 2013 Report	Hafele 2019 Report		Hume 2019 Report	Brown & Caldwell 2014 Report
Sample Site	**Hafele 2012 samples**	**Hafele 2017 samples**	**Hafele 2018 samples**	**Hume 2018 samples**	**Brown & Caldwell 2013 samples**
1.8 mi u/s				x	
1.1 mi u/s				x	x
0.4 mi u/s (US)	x	x	x	x	x
0.4 mi d/s (LS1)	x	x	x	x	x
0.4 mi d/s (LS1-N)			x		
0.4 mi d/s (LS1-S)			x		
0.9 mi d/s (LS2)	x	x	x	x	x
4.1 mi d/s				x	

* Shaded cells designate sites sampled in all studies. Note that in Hafele (2019), LS1 was sampled on both the north half and south half of the river channel (sites LS1-N and LS1-S, respectively).

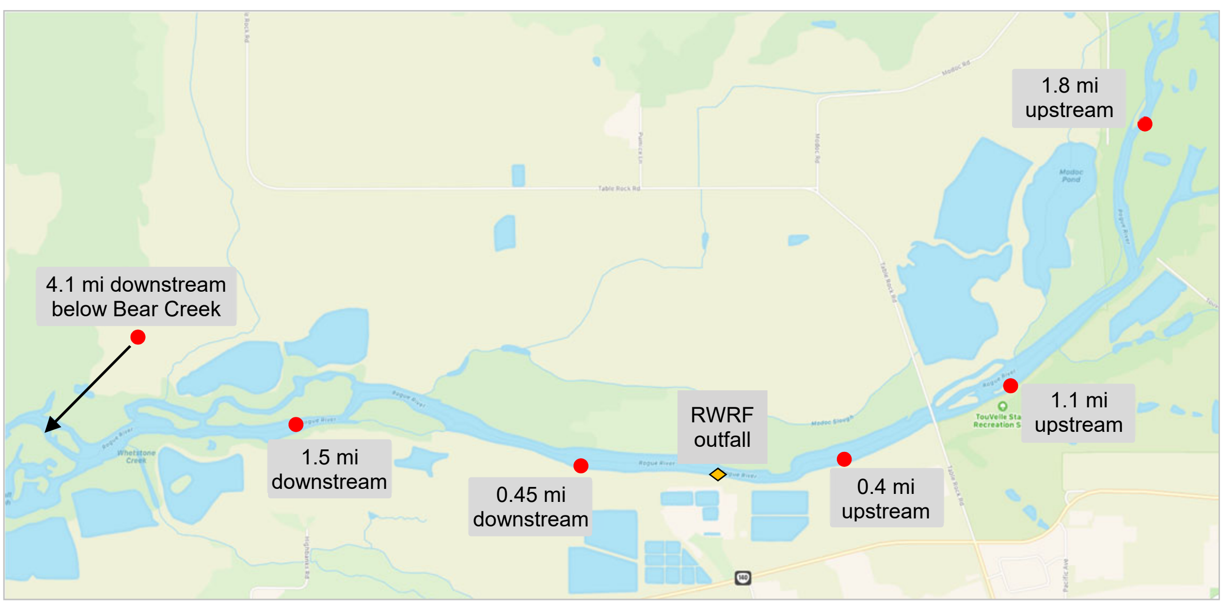

Figure 1. Sample site locations collectively from studies prior to Stillwater Sciences (2020) near the Medford RWRF.

II.B. *Defining Biocriteria Compliance*

Biocriteria is a narrative standard defined in OAR 340-41-0011 as: "Waters of the State must be of sufficient quality to support aquatic species without detrimental changes in the resident biological communities." Four definitions are included in the OARs that help to further explain the meaning of this narrative standard. These four definitions from OAR 340-41-0002 are listed below:

> (6) "Aquatic Species" means plants or animals that live at least part of their life cycle in waters of the state.
> (19) "Ecological Integrity" means the summation of chemical, physical, and biological integrity capable of supporting and maintaining a balanced, integrated, adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of the natural habitat of the region.
>
> (50) "Resident Biological Community" means aquatic life expected to exist in a particular habitat when water quality standards for a specific ecoregion, basin or water body are met. This must be established by accepted biomonitoring techniques.
>
> (75) "Without Detrimental Changes in the Resident Biological Community" means no loss of ecological integrity when compared to natural conditions at an appropriate reference site or region.

The above definitions make clear that the biocriteria standard applies to all aquatic life (e.g., plants, invertebrates, fish) that is expected to occur in a particular habitat. In practice ODEQ primarily relies on macroinvertebrate community data to assess biocriteria compliance (ODEQ Integrated Report 2018), although there is nothing in the standard that restricts assessments only to macroinvertebrates. In addition a "detrimental change" is defined as a "loss of ecological integrity," which is further defined as "the summation of chemical, physical, and biological integrity capable of supporting and maintaining a balanced, integrated, adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of the natural habitat of the region."

Therefore, in order to support ecological integrity that complies with the biocriteria standard, the aquatic environment must support and maintain the (a) chemical integrity, i.e. water quality conditions for temperature, dissolved oxygen, pH and nutrients, etc.; (b) physical integrity, i.e. the physical environment needed for aquatic life to live, feed, and reproduce; and (c) biological integrity, which is a balanced, integrated, and adaptive community with a species composition, diversity, and functional organization (e.g. appropriate mix of predators, grazers, parasites, etc.) when compared to natural habitat (i.e. the habitat unimpaired by human disturbance or reference sites). This language is consistent with that of the Clean Water Act (CWA Section 101(a)).

II.C. *Biocriteria Compliance Assessment*

To apply this narrative standard, ODEQ (2020) has developed specific assessment methodology for biocriteria assessment. For wadeable streams, ODEQ uses a multivariate statistical model called the **PRED**ictive **A**ssessment **T**ool for **OR**egon, or PREDATOR model. As described by ODEQ, "PREDATOR analyzes data from reference sites grouped into three regions in Oregon and models the expected macroinvertebrate taxa." The Rogue River above and below the Medford treatment plant is too large to be considered a wadeable stream, so the network of reference sites used by the PREDATOR model to assess biological impairment are not applicable. ODEQ (2020, p.37), however, describe the following assessment methodology for large rivers and point sources:

> While the PREDATOR O/E model is DEQ's preferred approach and provides the most robust and contemporary method for assessing biological integrity in smaller, wadeable streams and rivers, other approaches may be appropriate for specific cases and datasets. *For example, in studies examining the effects in non-wadeable rivers and/or of point-sources, study designs may look at upstream-downstream changes in macroinvertebrate community composition and function and provide valid information using multi-metric indices (MMIs) or simple metrics such as total richness, dominance, non- insect taxa, tolerance, etc.* [emphasis added].

Based on experience (e.g., Hafele 2019), PREDATOR scores can be relevant for non-wadeable streams and rivers, but should not be used alone.

The ODEQ assessment methodology also states that other aquatic communities can be used to determine biological conditions: *While macroinvertebrates are the most commonly studied community, other aquatic communities such as fish and algae are equally valid for assessing the biological integrity of freshwater systems.*

II.D. *Biocriteria Assessment near the Medford RWRF*

From 2012 to the present, five separate field studies were completed that assessed the macroinvertebrate community above and below the Medford RWRF (Hafele 2013, 2019; Brown and Caldwell 2014, ODEQ 2014, Hume 2019). All five studies found impairment of the macroinvertebrate community that indicated a violation of the biocriteria standard. A summary of some key community metrics is provided in Table 2. These data were all collected from the same three sites in all five studies (Table 1, Figure 1). Brown and Caldwell (2014) sampled one additional site upstream (1.0 RM above the outfall) and one additional site downstream (1.5 RMs below the outfall). Hume (2019) sampled two additional sites farther upstream (approximately 1.0 and 1.8 miles above the Medford RWRF outfall), and two additional sites farther downstream (1.5 RMs and 4.1 RMs below the Medford RWRF outfall; Table 1, Figure 1). The results from the two sites farther upstream showed close agreement with the results at the upstream site closest to the outfall (0.4 RM above the outfall). The site farther downstream (1.5 RMs below the outfall) showed some improvement relative to the other sites below but nearer the outfall, but also some impairment compared to the site 0.4 RM above the outfall. Note that different acronyms for these sites were used in the Stillwater Sciences (2020) report, which is the main focus of this report, but fortunately the locations are similar or, in some cases, identical (see Section III below).

The ODEQ (2020) criteria for biocriteria compliance in wadeable streams are based on the percent of taxa lost at study sites compared to a set of comparable reference sites using the

Table 2. Comparison of macroinvertebrate metrics (percent change from upstream to downstream sites) above and below the Medford RWRF outfall for data collected in five field studies.*

Author(s), Sampling Year	% Total Taxa Loss US - LS1	% EPT Taxa Loss US - LS1	% Sens. EPT Taxa Loss US - LS1	% Non-Insect Taxa Increase US - LS1	% Total Taxa Loss US - LS2	% EPT Taxa Loss US - LS2	% Sens. EPT Taxa Loss US - LS2	% Non-Insect Taxa Increase US - LS2
Hafele 2012	28	64	83	76	15	36	62	51
Hafele 2017	0	40	55	54	0	30	62	43
Hafele 2018	15	50	84	76	9	40	84	79
Brown & Caldwell 2013	20	40	NA	84	0	7	NA	66
Stillwater Sciences 2018	11	40	98	32% decline	37	72	100	63

* US – upstream sampling site (0.37mile above the RWRF outfall);
LS1 – first (lower) downstream site (0.45 mile below the RWRF outfall);
LS2 – second downstream site (0.87 mile below the RWRF outfall).

PREDATOR model. This model is often called a "taxa loss" model because it compares the taxa observed (O) at a study site to the taxa expected (E) to occur at comparable reference sites (O/E). The ratio of O/E can range from 0 to 1, with 1 indicating that all expected taxa were found at the study site, and 0 indicating that none of the expected taxa occurred at the study site. For the region that includes the Rogue River watershed (Western Cordillera and Columbia Plateau), a PREDATOR score (or O/E ratio) less than or equal to (<u><</u>) 0.78 (equivalent to a 22% taxa loss) indicates a biocriteria exceedance if multiple samples were collected (ODEQ 2020).

Because the Rogue River at Medford's RWRF outfall is not a wadeable stream, the PREDATOR model (Hubler 2008) is not directly applicable to assess biocriteria compliance. Also, it is important to note that the O/E output of the PREDATOR model is not the same as simply comparing the difference in total taxa richness between sites. The PREDATOR model generates an expected frequency of occurrence for each taxon found at reference sites and compares the presence of only those "expected" taxa to taxa found at study sites. Total taxa richness, on the other hand, may actually increase at impaired sites due to the addition of more tolerant taxa relative to the decline in sensitive taxa. Even though the percent of non-insect taxa (tolerant taxa) increased at sites below the outfall, Table 1 still shows a decline in total taxa richness below the outfall of from 0-28% and LS1 and 0-37% at LS2. However, because of the increase in tolerant non-insect taxa of 54-84% at LS1 and 43-79% at LS2, total taxa richness underestimates the impairment below the outfall. *The percent loss of EPT taxa and percent sensitive taxa are more appropriate metrics* in assessing the impairment to the macroinvertebrate community. These metrics show large declines at both sites below the outfall compared to the site just upstream (Table 1): Loss of EPT taxa at LS1 ranged from 40-64%; decline of % sensitive EPT tax at LS1 ranged from 55-98%.

II.E. *Other Aquatic Communities*

Besides macroinvertebrates, ODEQ (2012, 2020) recognizes other biological communities such as algae and fish as valid measures for biocriteria compliance. Several studies completed to date have included assessments of aquatic primary producer (photosynthetic) communities, that is, benthic algae and submersed aquatic plants (SAV, macrophytes with vascular tissue) above and below the Medford RWRF (Hafele 2013; Brown and Caldwell 2014, Hume 2019). Like macroinvertebrates, significant changes in the aquatic primary producer communities were found below the outfall compared to upstream sites. Increased nutrients in the effluent from the Medford RWRF were identified as the principle cause of these changes (Hafele 2013, Brown and Caldwell 2014, Hume 2019).

Benthic algae and SAV integrate environmental conditions over time, so they can be valuable indicators of changing ecological conditions from nutrient pollution (Carrick et al. 1988, Lowe and Pan 1996, Carpenter 2003, O'Hare et al. 2018). Both primary producer communities are well known to be sensitive to changes in nutrient conditions (Dodds 1991, 2002; Chambers and Prepas 1994; Dodds et al. 1997; Hilton et al. 2006 and references therein). Nutrient pollution can affect them both directly via increased nutrient availability and uptake, and/or indirectly through overgrowth of certain noxious species resulting in light limitation for photosynthesis (Hilton et al. 2006 and references therein). Benthic algae (relative to SAV) respond more quickly to changes in nutrients than macroinvertebrates (Rosen 1995, Soininen and Könönen 2004). Shifts in benthic algae and SAV taxa and abundance strongly affect higher levels in the food web such as macroinvertebrates and fish because these primary producers largely control food quality and availability, as well as substrata habitat characteristics for macroinvertebrates and fish (Ward 1992, Burkholder and Glibert 2013, Stancheva and Sheath 2016). Benthic algae and SAV also heavily influence water quality parameters such as dissolved oxygen (DO) and pH (Wetzel 2001, Caraco and Cole 2002, Caraco et al. 2006). When present in excessive amounts relative to the natural balance of a river, SAV and filamentous algae especially have caused major diel variation in these parameters (Caraco and Cole 2002, Stevenson et al. 2012) which, in turn, can harm both macroinvertebrates and fish (below). As a result, improvement in the macroinvertebrate and fish communities will depend on improvement in the benthic algal and SAV communities.

In general, benthic algal biomass in excess of 100 milligrams of corrected chlorophyll *a* per square meter (100 mg chl$a$/m^2) is undesirable (Horner et al. 1983, Welch et al. 1988; also see Stillwater Sciences 2020). Among the most common noxious benthic algal responders to nutrient pollution are filamentous forms. Such algae begin growth microscopically but rapidly extend beyond benthic microalgal biofilms and are considered as macroalgae (Lapointe et al. 2018 and references therein). Filamentous algae include some of the most well-known, classic responders to nutrient pollution. Peer-reviewed science literature is replete with descriptions of filamentous algal overgrowth in response to nutrient-rich, partially treated sewage effluent (Perrin et al. 1988, Spink et al. 1993, Wilby et al. 1998, Benke and Cushing 2005, Mackay 2006). Many studies have documented that rivers prior to discharge of sewage effluents contained no noticeable growth of filamentous algae, whereas after the discharge began, massive biomass of filamentous algae developed, fueled by the partially treated sewage (e.g., secondary treatment, characteristic of the

Medford RWRF). Other work has documented that improved sewage treatment significantly reduced N and P contamination and eliminated the filamentous green algal slimes, but they took over again if a nutrient-rich regime was re-established by treatment plant malfunction.

Among the most noxious of these filamentous algae is *Cladophora*, the most widely distributed macroalga throughout the world's nutrient-enriched freshwaters (Dodds and Gudder 1992, Higgins et al. 2008). This alga is so highly influential, particularly in nutrient-polluted waters, that it is regarded as an ecosystem engineer (Zulkifly et al. 2013). Maximum *Cladophora* biomass can exceed 900 grams of dry weight per m^2, and its filaments can be two meters (~six feet) long or more (Sandgren et al. 2005, Burkholder 2009, Zulkifly et al. 2013). It thrives especially in phosphorus- and ammonia-enriched alkaline waters with dependable substrata such as rocks and boulders (Pitcairn and Hawkes 1973, Dodds 1991, Ensminger et al. 2000, Burkholder 2009 and references therein). *Cladophora* generally is poorly grazed and is considered a non-preferred food source (Zulkifly et al. 2013). Stillwater Sciences (2020 – below) reported that the benthic algal community downstream from the Medford RWRF has become overwhelmingly dominated by *Cladophora*. Other field studies by Hafele (2013), Brown and Caldwell (2014), ODEQ (2014), and Hume (2019) all documented negative shifts in the benthic algal community below the Medford RWRF that are consistent with nutrient over-enrichment.

Some SAV species are well-known responders to sewage pollution in rivers (Chambers and Prepas 1994). In their resulting rapid overgrowth, they can become even more noxious than benthic algae such as *Cladophora* (Cook et al. 1993). Submersed macrophytes have two potential sources for nutrient supplies, the sediments and the water column (Wetzel 2001). A portion of the nutrient supplies added to the water by the Medford RWRF sewage effluent settles out to the river bottom where it can be available to the SAV and benthic algae; the rest is taken up by suspended algae, adsorbed to suspended soil and detritus particles (dead plant/animal remains), and moved downstream by the river flow (e.g., Withers and Jarvie 2008).

SAV cover at 40% has been considered a eutrophic benchmark of undesirable SAV conditions in nutrient-polluted areas (Maret et al. 2010, Chambers et al. 1999, Suplee et al. 2009). Macrophytes in rivers affected by high nutrient supplies tend to be adept at rapid growth under relatively low light (Hilton et al. 2006). The species reported in Stillwater Sciences (2020, p.33) mostly have those characteristics; their thin, finely dissected leaves optimize light acquisition (Sculthorpe 1967). These plants generally become heavily covered seasonally, and between storm/scouring events, by attached and floating algae which block the light that is essential for SAV photosynthesis (Hilton et al. 2006 and references therein). An example of such heavy cover of SAV by benthic algae is evident at Site LS1 downstream from the Medford RWRF in the Hafele (2013) study (Figure 2). SAV are major responders to nutrient pollution in many rivers worldwide, including the affected Rogue River segment where macrophytes are especially abundant at sites most affected by the Medford RWRF sewage effluent (Stillwater Sciences 2020).

As Munn et al. (2018) wrote, "If only benthic algal biomass is used for assessing the status of aquatic vegetation, streams dominated by extensive macrophyte growth would be misclassified as to their...biological condition." Because of the relationships between nutrient levels and the benthic algal and SAV communities, and between these primary producers and macroinvertebrates, *both* *benthic algae and SAV must be considered when setting nutrient limits for the Medford RWRF treated effluent, and when evaluating future compliance with biocriteria.*

Figure 2. Macrophytes and benthic algae/debris at a site from a previous study (LS2) that was/is strongly influenced by the Medford RWRF sewage effluent. Note that the macrophytes (bright green) are barely visible beneath thick cover of benthic algae and debris (brown material). From Hafele (2013).

II.F. *Other Parameters of Concern*

While biocriteria have been the main focus in assessing impacts on the "fish and aquatic life" beneficial use downstream from the Medford RWRF, other environmental parameters are also of concern, especially temperature, dissolved oxygen (DO), and pH. In particular, salmonid spawning and incubation are protected with more stringent requirements for temperature and DO in the water quality standards. The standards for the Rogue Basin list the spawning use period for salmon and steelhead upstream from the mouth of Bear Creek as September 15 – June 15 (OAR 340-41-0271, Rogue Basin Spawning Use Designations, Figure 271B). During this designated period, the temperature criterion is 13˚C (55.4˚F) and the instantaneous minimum DO concentration is 11 mg/L. The segment of the Rogue River both above and below the Medford RWRF is actively used for spawning by Chinook salmon and other salmonids, with Chinooks observed spawning near all three sample sites (closest upstream site and downstream sites) beginning in September (Oregon Department of Fish and Wildlife [OR DFW] 2019; John MacDairmid, pers. comm.). Returns to the Rogue River of naturally produced Chinook salmon in spring are only about two-thirds of the desired status (set as a 10-year-average of at least 15,000); and hatchery returns have not met expectations in recent years (OR DFW 2019).

An important effect of excessive algal and plant growth from nutrient pollution is large diel (24-hour period) variations in oxygen levels due to photosynthesis during the day and respiration at night (see Section III). This can lead to depressed oxygen levels at night and early morning that fall below the water quality standard to protect salmonid spawning. Continuous DO measurements (30-minute intervals) were collected by Hume (2020) at four locations: 1) the riffle site just upstream of Medford's outfall; 2) the riffle just downstream on the south bank of the river; 3) just downstream on the north bank; and 4) the second site downstream along the south (Figure 1). Note that these four sites are referred to as Sites 3, LS1-S, LS1-N, and LS2, respectively, in Stillwater Sciences (2020; see Section III). Mid-October sampling results showed that DO levels fell below 10.0 mg/L at sites 2 and 4 (downstream sites along the south bank of the river) during the night, while they stayed above 11.0 mg/L at the upstream site and the downstream north bank site (Hume 2020). The north half of the river channel at that downstream site is minimally influenced by the effluent plume, whereas the south half of the river channel is in the plume (Brown and Caldwell 2014). The other downstream site along the southern bank (designated above as 4) is also affected by the effluent plume. These data show that excessive algal and plant growth fueled by high nutrients in the effluent plume resulted in DO concentrations that fell below those required to protect salmonid spawning. The need to protect these important salmonid species further underscores why nutrient limits for the Medford effluent must be set low enough to eliminate excessive algal and plant growth.

II.G. *Additional Protection Required for this 303(d) Listed River*

The Rogue River is already impaired for its designated use for fish and wildlife. An identified cause is violations of Oregon's biocriteria standard due to noxious benthic algal/SAV overgrowth fueled by excessive nitrogen and phosphorus contamination. Consistent with federal and state mixing zone guidance and regulations, a mixing zone is not appropriate for this impaired river segment. The permit must also consider contamination of downstream waters by highly soluble nitrate which can easily be transported down to sensitive marine waters along the Oregon coast.

The state's recent 303(d) list of streams in violation of one or more water quality standards (DEQ Online Integrated Report 2018/2020) includes the Rogue River segment affected by the Medford RWRF (also see Opalski 2016). This segment is impaired for the following designated uses: fish and aquatic life; fishing; private domestic water supply; and public water supply. Causes of impairment were given as temperature year-round, temperature for spawning, biocriteria, and methylmercury.

Of these, violation of the biocriteria standard is especially germane regarding the demonstrated degradation of the Rogue River ecosystem by the Medford RWRF. The parameters known to be contributing to violations of the biocriteria standard by this point source are excessive concentrations of nitrogen and phosphorus in the effluent (previous reports described above; and see Sections III-V below). Both the impaired status of the waterbody into which Medford discharges and the state of the downstream waters must be taken into account in determining the effluent limitations that are required to control nitrogen and phosphorus in the Medford RWRF discharge. National Pollutant Discharge Elimination System (NPDES) permits for point sources such as the Medford RWRF cannot be issued if they allow for the source to "cause or contribute" to violations of water quality standards (40 CFR 122.44(d)(1)(i)).

Notably, ODEQ guidance prohibits the allocation or use of a regulatory mixing zone (RMZ) in situations where a point source discharges to a waterbody that is violating one or more water quality standards (e.g., is "impaired") for such pollutant(s) or parameter(s). As ODEQ states,

> If there is no available dilution due to lack of flow or because the stream is water quality-limited for the parameter in question, water quality criteria should be applied at the end-of-pipe or other alternatives considered (e.g., development of site-specific criteria, use of a variance, change in beneficial uses of the receiving stream (ODEQ 2012).
>
> In general, when a waterbody is listed as impaired for a pollutant and assimilative capacity is not available, the Department does not allow a Regulated Mixing Zone (RMZ) and the factoring of a dilution into the water quality analysis for that pollutant during NPDES permit renewal. The result is that most permitted discharges will not be permitted a mixing zone and will need to meet the water

> quality standard at the point of discharge (ODEQ 2018).

Furthermore, ODEQ regulations (OAR 340-041-0053: Mixing Zones) state:

> A point source for which the mixing zone is established may not cause or significantly contribute to…floating debris, oil, scum, or other *materials that cause nuisance conditions* [emphasis added];

and the mixing zone must be defined so as to:

> minimize adverse effects on the indigenous biological community, especially when species are present that warrant special protection.

Federal regulations (40 CFR §§ 131.2, 131.10(b)) also require water quality standards to protect downstream waters, such that an upstream source cannot cause or contribute to a downstream violation. The Rogue River from its confluence with Little Butte Creek all the way to its confluence with Evans Creek, about 15 RMs downstream from the Medford RWRF, is impaired for the same causes as the area near the outfall (Oregon's 2018/2020 Integrated Report: https://www.oregon.gov/deq/wq/pages/2018-integrated-report.aspz). Nitrate, a major, highly bioavailable form of nitrogen in the treated Medford RWRF effluent and in the modified effluent limits suggested by Stillwater Sciences' (2020), is highly soluble and can travel distances of more than 200 miles downstream (Mallin et al. 1993, Houser and Richardson 2010, Houser et al. 2010). The nitrate in this effluent, if not taken up by primary producers en route, could easily reach nitrogen-sensitive coastal marine waters downstream.

Section III. Evaluation of the Stillwater Sciences Study

III.A. *Introduction*

A stated major goal in Stillwater Sciences (2020, p.1) was "to evaluate whether and to what extent nutrient discharge restrictions may be needed to address any Medford RWRF contribution to water quality standards not being met in the river outside the Medford RWRF's RMZ." The stations sampled during Stillwater Sciences' June through November 2019 study are shown in Figure 3. The report asserted (p.2) that, following U.S. EPA (2000a) guidance, a combined approach was used to establish [suggest] nutrient discharge limits for the Medford RWRF, including site-specific data in the reach upstream and downstream of the outfall (reference [minimally impacted] approach), comparisons to existing predictive relationships between nutrients and algal biomass, and comparisons to existing nutrient thresholds from the literature. That writing, however, did not accurately describe Stillwater Sciences' actions:

- Stillwater Sciences (2020) *did i*nclude a robust comparison of data from the Rogue River to existing predictive relationships between nutrients and algal biomass for various rivers. Remarkably, however, the authors ignored the clear finding from that comparison: the Rogue River is highly sensitive to nutrient pollution in comparison to other rivers.

- Stillwater Sciences (2020) *did* include comparisons to existing nutrient thresholds from the literature, such as the U.S. EPA's (2000b) recommended numeric phosphorus (P) criterion for

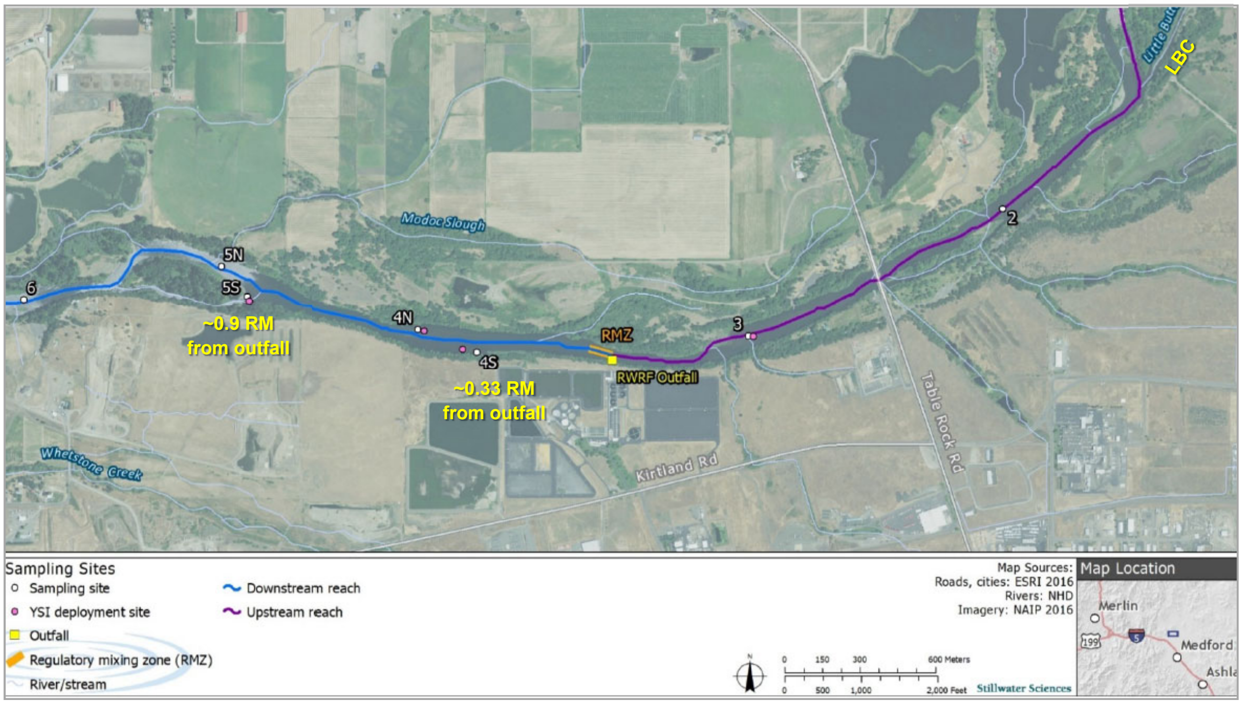

Figure 3. Map from Stillwater Sciences (2020, p.3), modified to show distances (river miles, RMs) from the Medford RWRF to most impacted Sites 4S and 5S (0.33 and 0.9 RM from the outfall, respectively). The upstream confluence of tributary Little Butte Creek (LBC) with the Rogue River is also shown (upper right corner).

the nutrient sub-ecoregion that includes the Rogue River segment affected by the Medford RWRF. Illogically, however, Stillwater Sciences then chose much higher nutrient thresholds than minimally impacted areas of the middle Rogue River as "appropriate" for use in deriving suggested effluent limits in this nutrient-sensitive river.

Thus, Stillwater Sciences (2020) fell far short of its goal to provide a science-based evaluation of the extent to which nutrient discharge restrictions are needed to address the water quality and habitat degradation of the Rogue River by the Medford RWRF. In this section we identify numerous serious errors in Stillwater Sciences' study as described in its 2020 report and appendices, including its sampling design, field work, laboratory analyses, and data analyses.

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III.B. *Sampling Design, Methods, and Data Interpretation*

III.B.1 – Timing

The timing of the 2019 study likely missed the benthic algal biomass maximum upon which Stillwater Sciences' suggested Medford RWRF effluent limits for TN and TP are based.

Studies of benthic algal biomass in aquatic ecosystems affected by human-related sources of nutrient pollution generally focus on measuring biomass across the active growing season to ensure that the maximum is detected (Asarian et al. 2014, 2015; Konrad et al. 2016). This strategy is especially important since some benthic algae can have bimodal growth maxima, usually a higher maximum in spring and a second, lower peak in late summer or fall (Wetzel 2001 and references therein), including *Cladophora* (Madsen and Adams 1988, Whitton 1970). Alternatively, various benthic algae such as diatoms and some macroalgae attain one growth maximum in late winter/early spring (Biggs 1996, Wetzel 2001). Therefore, it is important to conduct preliminary reconnaissance to assess, across an annual cycle, the period(s) of maximum growth for the dominant algae in the specific river of interest (Biggs 2000a, Biggs and Kilroy 2000). As standard procedure across peer-reviewed published studies, sampling for nutrient concentrations to characterize water quality either coincides with the sampling for benthic algal abundance (Welch et al. 1989, Asarian et al. 2015, Konrad et al. 2016), or is conducted 1-2 weeks before the benthic algal samples are taken to capture potential lag effects of nutrient pulses (Hillebrand 2002).

In Stillwater Sciences' 2019 effort, monthly water quality sampling was initiated in June; monthly benthic algal chlorophyll sampling began in mid-July and extended through November; and samples for benthic algal biovolume and cell number were taken only twice, the first in August and the last in October. We are aware that sampling initiation was largely driven by the timing of settlement discussions and development of the sampling plan which was not completed until late July. Our concern with the lack of data from earlier in the year (spring through mid-summer) was raised in follow-up discussions with the City. Although NWEA explicitly requested that "the parties extend the litigation abatement period by up to one year, to April 2021, so that additional field work and laboratory analysis can be conducted" (*see* Letter from James Saul, Earthrise Law Center [counsel for NWEA], to Michael Campbell, Stoel Rives LLP [counsel for City of Medford], February 7, 2020), the City of Medford declined to collect additional data. And although NWEA also noted that "additional field work in the spring and summer months will provide a more complete data set upon which we may be able to determine the appropriate nutrient effluent limitations" *see id*., the important period from April through early July 2019 was not included in Stillwater Sciences' biological monitoring efforts. Yet, previous published studies have shown that various taxa such as *Cladophora* and other benthic algae in other rivers can reach biomass maxima in that period (e.g., Dodds 1991, Ruzycki and Axler 2019). Instead, sampling of the "growing season" was extended through November—yet benthic algal (as well as SAV) growth had subsided by that time based on Stillwater Sciences' data, and based on its descriptions of when benthic algal biomass is highest in the middle Rogue River:

> *Benthic Chl-*a *levels decreased over time indicating that Chl-a biomass likely peaked before or during the August sampling event (p.25)….* and
>
> *We recommend applying the nutrient discharge limits* [we suggested] *for the* [Medford] *RWRF to the period of May 1st through October 31st of each year when benthic algal biomass accrual is usually highest…"* – Stillwater Sciences (2020, p.56).

Thus, the timing of Stillwater Sciences' 2019 study did not capture the benthic algal growing season as described by Stillwater Sciences itself, and likely missed maximum benthic algal biomass entirely. If so, then its suggested effluent limits for TN and TP, set to control maximum benthic algal biomass, will not protect the designated uses of the affected Rogue River segment from further degradation. After all, as an appropriate analogy, no one would trust a doctor who attempted to set a target for how much weight a patient needed to lose, if the doctor did not know how much the person actually weighed.

III.B.2 – Sampling Strategy, Methods, and Analyses

The sampling design, methods, and analyses used by Stillwater Sciences (2020) led to uncertain estimates for critically important water quality parameters in background (upstream or control) conditions; major mistakes in estimating benthic algal biomass, to such an extent that some of the data are beyond salvage; and such poor quality data for SAV that the data can only provide rough qualitative information.

Based on climatic data (National Oceanic and Atmospheric Administration [NOAA] 2020), the sampling period in 2019 described in Stillwater Sciences (2020) was a high-precipitation year relative to conditions in previous studies (e.g., 2018 – see below). Although the sampling period included the lowest flow for that year (1,322 cfs), that flow was still well above the 7Q10 for this segment of the Rogue River (882 cfs). Furthermore, because benthic algal sampling did not begin until August, the impacts of more severe scouring/flushing earlier in this high-flow year on benthic algal growth later in the season cannot be evaluated. Each dataset—for water quality, benthic algal biomass and community structure, and SAV—is considered separately as follows.

III.B.2.a. *Water Quality*

The high practical quantitation limits used in Stillwater Sciences' WQ analyses, adequate for sewage effluent but not for rivers, and the poor QA/QC among replicates, yielded only highly uncertain values for actual nutrient concentrations that likely are in error by as much as an order of magnitude, yet these uncertain values were used to develop the suggested effluent nutrient limits.

Water quality (WQ) was sampled monthly at stations, counting the Medford RWRF outfall, in the 2019 study. Medford obtained additional WQ samples from June through November from the outfall and at 8 of the 9 sampling sites, although it should be noted that samples were collected at Site 5 as a "composite" of samples from sites 5N and 5S—an approach that would have diluted the effluent effect. In its Settlement Agreement with NWEA (May 28, 2019), the City agreed to collect monthly samples from at least one upstream site (but no downstream sites) only until its NPDES permit is renewed.

The U.S. EPA (https://www3.epa.gov/ttn/emc/meetnw/2015/moreado.pdf) provides the following relevant definitions:

MDL (or DL) = minimum detection level (what we really want to know);

PQL (LOQ) = 3x MDL, the practical quantitation level (where we can reliably measure); and

RL = reporting limit – what some labs. consider to be their lowest reportable value, often much higher than the MDL (not desirable). (Note: in some references the RL is considered roughly equivalent to the PQL—e.g., van Buuren 2017.)

The reporting limits used by Stillwater Sciences (Table 3) are much higher than river background concentrations (e.g., U.S. EPA 2000a) for most parameters. For example, the RL for total phosphorus (TP) is so high that any measurements would already (artificially) place the Rogue River into a "mesotrophic" (moderately enriched) category as "best case" (e.g., Dodds et al. 1998). These problems occurred because the methods selected for use cannot capture the low-level nutrient conditions typical of the Rogue River accurately (see U.S. EPA 2000a).

Table 3. A portion of Stillwater Sciences' (2020, p.6) Table 2-3, showing in situ and analytical water quality methods, excluding most parameters collected by datasonde; and modified to correct Stillwater Sciences' error for total Kjeldahl nitrogen reporting limit, and to include attainable PQLs for water quality that more accurately describe nutrient supplies present for potential use by algae and plants in surface waters, including minimally disturbed conditions (Touchette et al. 2007, as an example of the QA/QC requirements of Dr. Burkholder's state-certified laboratory for low-level nutrient analyses in rivers, reservoirs, and estuaries; and U.S. EPA 2000b).

Parameter/Constituent	Method	Resolution/Method reporting limit	Acceptable PQL - Rivers
In-Situ Water Quality (YSI multi-parameter Sonde)			
Chlorophyll-a	In Vivo fluorescence	0.1 ug/L Chl; 0.1% RFU[1]	Note: total or corrected?[2]
Analytical Chemistry			
Ammonia	EPA 350.1	0.15 mg N/L	0.018 mg N/L
Nitrate + Nitrite = NOx	EPA 353.2	0.05 mg N/L	0.011 mg N/L
Total Kjeldahl Nitrogen[3]	EPA 351.2 Cu	~~0.0625 mg N/L~~ 0.625 mg N/L	0.280 mg N/L
Total Phosphorus	SM 4500PE	0.025 mg P/L	0.010 mg P/L
Orthophosphate	SM 4500PE	0.025 mg P/L	0.012 mg P/L
Total Organic Carbon[4]	SM 5310 C	0.1 mg/L	

[1] RFU, relative fluorescence units; cannot be reliably related to actual chlorophyll concentrations, which cannot be measured with the datasonde (Reed et al. 2010). Therefore, the "0.1 µg/L Chl" writing is in error.

[2] The report provides no clarification of whether total or corrected chlorophyll was measured. Rivers are well known for high pheophytin (from dead algae; can artificially elevate the actual chlorophyll concentration from living algae – Wetzel and Likens 2000). Corrected chlorophyll concentrations, that is, chlorophyll concentrations from which pheophytin was subtracted, should have been used.

[3] The value "0.0625", reported by Stillwater Sciences, is an error and is 10-fold too low; elsewhere (p.15), the correct value was given as 0.625 mg N/L (also given in Appendix B, e.g. p.23). It should also be noted that elsewhere Stillwater Sciences (2020, p.19) mentions an analytical detection limit for ammonia as 0.064 mg N/L, still 3.5-fold higher than desirable.

[4] Total organic carbon (TOC) data were included in Appendix B but were not included in Stillwater Sciences' (2020) analysis.

Consider, for example, Stillwater Sciences (2020, p.19): TKN was described as having ranged from 0.2-0.58 mg/L at the upstream sites, but the detection limit (DL) for Stillwater Sciences' method was given as 0.7 mg/L. Given such poor resolution of lower-end nutrient concentrations with the methods used, most of the WQ analyses and the data interpretations are not credible. As another example, consider the following statement:

> "Ammonia-N concentrations at site 4S ranged from 0.12–0.45 mg/L with an average of 0.20 mg/L."

The low end of the range given was below the RL and, therefore, questionable, which also calls into question the average value given.

Stillwater Sciences (2020, p.15) included an explanation of how low concentrations could only be estimated, with high uncertainty, due to lack of improved methods with lower PQLs. The DL was estimated at three times the standard deviation based on published literature values for general water quality (Oblinger Childress et al. 1999)—although the Rogue River is not an "average" river but, rather, is naturally nutrient-poor (oligotrophic, inferred from U.S. EPA 2000b). The RL, described as even "more subjective and set by each laboratory differently…" (*Id.*), is often set at five times the standard deviation added to the DL. In its approach, Stillwater Sciences (2020) ***"J-flagged"*** all results below laboratory RLs but above DLs to indicate lower confidence levels (i.e., ***high uncertainty in the data*****). The data for ammonia, TKN, and ortho-P were frequently "J-flagged,"** most commonly at upstream sites and site 4N, but occasionally at critical Site 4S as well (see Stillwater Sciences' Table 2.5).

Field duplicates indicated poor replication, compounding the above uncertainties in the WQ data. Only one field duplicate per sampling date was collected, and precision was screened against a relative percent difference (RPD) threshold (maximum acceptable) criterion of 25% for water samples. Two of six dates had poor replication for TKN (RPD 46-81%) and ortho-P (RPD 28-30%). Additional replicates to address the poor replicates problem were not collected for these key WQ parameters.

Laboratory accuracy measurements were assessed by analyzing samples that were amended (spiked) with standards (known amounts of nutrient), performed at a frequency of 1 in 20 samples per matrix analyzed. Results were only rejected if they were outside the 80% to 120% recovery range. This wide range of "acceptability for accuracy" is another source of uncertainty.

Overall, the non-science-based "leaps" that were made in extrapolating from the above approach resulted in effluent nutrient limits that will not protect the designated uses of the affected Rogue River segment for fish and aquatic life. Consider, for example, Stillwater Sciences (2020), p.53:

> *Assuming* [emphasis added] that undetected ammonia-N and NOx results are at least ½ of the laboratory DLs, approximately 78% and 38% of the TN and TP in the river upstream from the RWRF is organic N and particulate phosphate. Using 2019 downstream concentration averages, 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. Because inorganic N, particularly ammonium as well as PO_4-P are the nutrient forms most readily absorbed by periphyton (Dodds 2002),…reducing TIN and PO_4-P contributions from the RWRF is the primary control strategy [for periphyton] in the Rogue River. Using the observed proportions of TIN to TN and ortho-P to TP estimated from the 2019 sampling results, corresponding TIN and ortho-P targets were estimated at Site RMZ-S…and the outfall location….

As corrective information, Stillwater Sciences did not use methods that enabled reliable measurement of low nutrient concentrations, so there is no way to know that ammonia (especially) and NOx (which were "undetected" because of the high RLs in the methods used) were or were not as high as half of the amount of the laboratory DLs. Thus, the percentages of TN and TP in the upstream sites are highly uncertain. Moreover, nitrate, not ammonium, is the N form most readily taken up by benthic algal diatoms (Admiraal 1977, Domingues et al. 2011); in fact, high total ammonia, characteristic of the affected river downstream from the mixing zone, is *toxic* to many beneficial algae (Turpin 1991, Glibert et al. 2016); and diatoms are generally the dominant component of natural river benthic algal flora, especially in low-nutrient-background waters (Weilhoefer and Pan 2006, Pan et al. 2012). As for the percentage changes in organic N at site RMZ-S and even at the outfall, many of those TKN values were "J-flagged" as being highly uncertain estimates.

Accordingly, what Stillwater Sciences (2020) describes as "the observed proportions of TIN to TN and ortho-P to TP" were in fact not observed, but rather are unreliable estimates, which in turn were used to "estimate TIN and ortho-P targets" and thereby layer uncertainty upon uncertainty.

Overall, Stillwater Sciences' approach was confounded by lack of information due to an inability to measure low-level river nutrient concentrations. Important parameters such as total ammonia, NOx, and ortho-P ($PO_4^{-3}P$ or SRP)—even (typically much higher) TKN—were "J-flagged" indicating high uncertainty in measurement. Total inorganic N and ortho-P effluent targets were suggested based on these series of uncertain values, regardless.

III.B.2.b. *Continuous 48-Hour Studies (in situ datasondes)*

Stillwater Sciences' continuous DO data show that the Medford RWRF effluent has created adverse conditions for beneficial aquatic life. The data also suggest questionable calibrations and failures to maintain the datasondes for appropriate data quality control/assurance.

Diel DO Flux – As introduced in Section II, the diel DO flux (also referred to as diel DO variation, or the diel DO range or swing) is the total DO fluctuation over a 24-hour period. In river systems that are not receiving nutrient pollution, growth of natural plant species is balanced rather than excessive and does not cause large diel DO swings, whereas unbalanced plant growth (e.g. high production by invasive/exotic plant species) causes such swings (Caraco and Cole 2002, Goodwin et al. 2008). Larger diel DO swings are common under nutrient-polluted conditions, due to over-stimulation of algal/plant growth (Walling and Webb 1992, Morgan et al. 2006). Diel DO swings of 2.3 to 6.6 mg/L have caused behavioral aberrations in fish, reduced spawning, and reduced swimming speed, which would in turn adversely affect the ability of fish both to capture food and avoid predation (e.g., Carlson and Herman 1978, Brady et al. 2009). The maximum diel DO swing that is appropriately protective of aquatic life varies depending on the size of the stream, its flushing rate, and its temperature and light regimes.

Experiments to study diel DO swing effects on the biota of river biota are generally lacking because it is difficult to mimic the simultaneously changing conditions of unidirectional flow, light, temperature, and substrata (bottom rocks, mud, etc.; Izagirre et al. 2007). A more realistic approach has been to develop a strong database that includes nutrient concentrations, algal and plant biomass, and metrics for fish and macroinvertebrates. Few such robust databases are available, due to the need to deploy expensive sensors that measure DO 24/7 for extended periods (weeks) during the growing season for primary producers. The state of Minnesota is using the diel DO range (flux) as a diagnostic measure of P enrichment linked to impairment of beneficial aquatic life, based on a suitable database (Heiskary and Markus 2003, Heiskary et al. 2013). Different diel DO eutrophication standards were developed depending on the region of Minnesota, reflecting the fact that biota in oligotrophic northern, forested, sparsely populated areas would be more sensitive to nutrient pollution and diel DO swings than the rest of the state. Thus, Minnesota is using a eutrophication water quality standard of a maximum DO flux of 1.5 mg DO/L for its sensitive, naturally nutrient-poor streams in the north (Heiskary et al. 2013). In the central, more populated, mixed land-use area, the state has set a eutrophication water quality standard of maximum DO flux of 3.5 mg/L. That number was based on the fact that as the diel DO flux increased to 4 mg/L or more, desirable fish species dramatically declined or were locally extirpated from the central region (Heiskary and Markus 2003, Heiskary et al. 2013).

The Rogue River is a naturally low-nutrient, oligotrophic system (Naiman and Bilby 1998, U.S. EPA 2000b), as are northern Minnesota streams, suggesting that it would be similarly sensitive to diel swings. In 2019, Stillwater Sciences collected continuous 24-hour DO data only twice (48 hours) in August and in October, at only four sites. Equipment malfunction resulted in no data at one site (5S) during August. Despite the extremely sparse data collected, the study documented adverse conditions for aquatic life (Stillwater Sciences 2020, p.24, Figure 2-6) with a DO flux of 4 mg/L at critical site 4S in October. That finding indicates that the Rogue River has been degraded by the Medford RWRF effluent to such an extent that its diel DO variation rivals that of streams in the Minnesota "corn belt."

<u>General Sonde Data Quality</u> –Scrutiny of the Stillwater Sciences (2020) report and appendices revealed sensor malfunctions and high potential for serious sensor drift during the two (very) short-term (48-hour) studies in August and October 2019:

- Some sensor malfunctions during the August 48-hour study were not detected during post-deployment checks (p.19), such as sporadic malfunction of the DO optical sensor wiper at Site 4N. The DO optical sensor at Site 5 failed immediately after deployment during the August study, although it was described as having functioned well during both pre- and post-calibration checks.
- Calibration checks pre- and post-deployment did not detect malfunction related to pH readings at Site 4S (p.20) in the October study, but subsequent time series plots showed high variability between readings that were attributed to possible sensor malfunction.
- August study – DO at Site 3 differed by ~0.3 mg/L pre- versus post-calibration, indicating potential for substantial probe drift in the field. Similarly, pH differed by ~0.6 unit pre- versus post-calibration (appendices, p.173). Post-deployment field check information was not included.
- October study – At Site 5, the pH differed by ~0.4 unit pre- versus post-calibration, suggesting the potential for substantial probe drift in the field (appendices, p.187). At Site 3, pH was 0.2 to 0.4 unit off, and DO was 0.5 mg/L off pre- versus post-calibration (appendices, p.181). Post-deployment field check information was not included.

III.B.2.c. *Benthic Algae*

Although most of the data are questionable due to use of poor methods and interpretations that were opposite of what the data actually showed, one point is clear: The excessive phosphorus and high *conductivity from the Medford RWRF effluent have fueled massive growth of a notorious algal ecosystem engineer, the macroalga* Cladophora*.*

To determine relationships between noxious primary producers and nutrients in the Rogue River, Stillwater Sciences was tasked with accurately quantifying both cover and biomass of SAV and benthic algae (Stillwater Sciences Rogue River Sampling Plan, Section 1.2). Our overall assessment, detailed below, is that neither cover nor biomass of primary producers was quantified in a way that produced reliable and reproducible results. This failure stems from improper implementation of the agreed-upon (with NWEA) field methods, improper laboratory methods, and issues with biomass calculations. In addition, we call into question the treatment of data in statistical analyses used to develop relationships between nutrients and primary producer biomass.

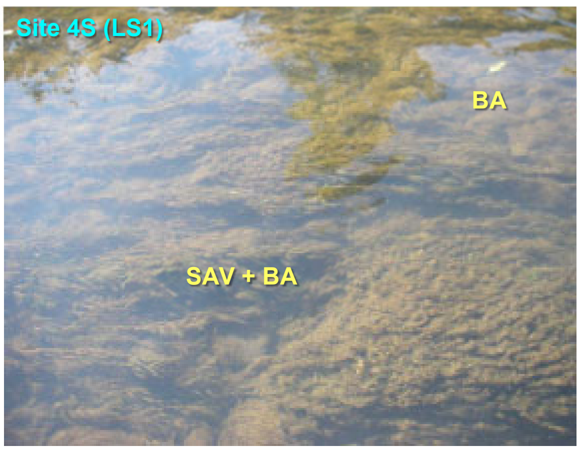
The benthic algal community (growing on or attached to substrata such as rocks, sediments, SAV etc.) was referred to by Stillwater Sciences as periphyton. In clarification, periphyton technically includes the total microbial consortium (bacteria, protozoans, fungi etc.) in benthic biofilms (Wetzel 2001). The affected segment of the Rogue River actually has much more than microalgal periphyton; the excessive inorganic phosphorus and nitrogen, together with high conductivity from the Medford RWRF effluent, have also fueled growth of an "algal ecosystem engineer"—the notorious algal responder to sewage, *Cladophora* (Carpenter 2003, Lapointe et al. 2018 and references therein – see below).

Sampling – Methods developed by the Montana Department of Environmental Quality (MT DEQ 2011) were agreed upon for the 2019 study. The methods that were supposed to have been followed included three protocols for collecting benthic algae, depending on the substratum and the conditions at a transect location (MT DEQ 2011): At each sampling location, the sample should "represent conditions prevalent in a ~1 m^2 area around the transect." The sample is collected using (i) a template for substrata dominated by small boulders, cobble and gravel without heavy filamentous algal growth; (ii) a hoop for transects dominated by filamentous algae, regardless of the substrata; or (iii) a core for transects dominated by silt-clay substrata without heavy filamentous algal growth.

Based on the report and discussions with Stillwater Sciences personnel, some steps in the agreed-upon methods for benthic algal chlorophyll and biomass sampling were not followed, leading to inaccurate estimates of benthic algal biomass. In addition, we call into question the numbers provided in the report (Tables 2-8 and 2-9) regarding the calculation of chlorophyll and ash-free dry mass (AFDM) on an areal basis. These numbers differ from our calculations using the data provided in the Appendices to the report, and the calculation methodology outlined in MT DEQ (2011). Inaccurate field collection of biomass samples coupled with inaccurate calculations renders the relationships between benthic algal biomass and nutrients that were developed in the Stillwater Sciences (2020) report invalid.

Some examples are given below of the numerous important steps involved in reliable sampling that were not followed by Stillwater Sciences (2020):

- *The hoop sampling method was not used correctly, resulting in substantial underestimates of benthic algal biomass* – Stillwater Sciences used it only to collect SAV, deviating from protocol which required that *all* algal/plant growth within the hoop area must be collected including SAV, filamentous algae, and other benthic algae scraped off rocks (MT DEQ, section 2.2.2 vs. Stillwater Sciences 2020, p.8). Instead, in the study, SAV samples were not collected unless SAV covered at least 20% of the hoop area (see Stillwater Sciences 2020, Table 2-7, footnote 1). Moreover, there is no indication that benthic algae were collected using any other method when 20% SAV cover was present and the hoop method was used. Yet, benthic algae were usually abundant in sites where SAV covered 20% or more of the area (Figure 4). Stillwater Sciences' inadequate biomass collection, using the hoop method to collect only SAV, led to major underestimates of benthic algal chl*a* and AFDM at all sampling locations that included a mix of SAV and benthic algae.



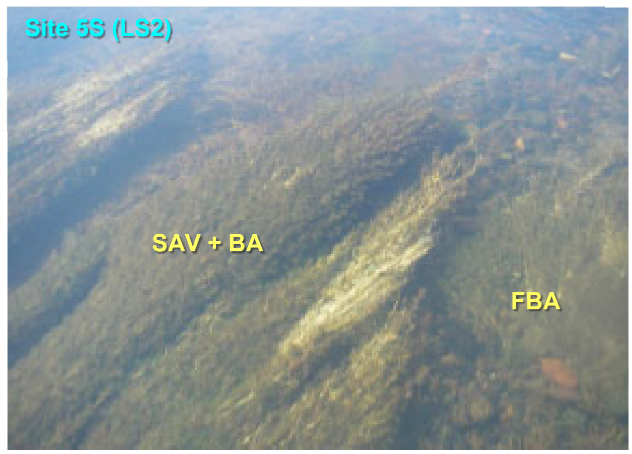


Figure 4. Examples of the common occurrence of abundant benthic algae in (BA), including filamentous forms (FBA), in locations where submersed aquatic vegetation (SAV) covered 20% or more of the area. Photos of Sites LS1 and LS2 (Hafele 2013) correspond to Stillwater Sciences (2020) Sites 4S and 5S.

- *Calculations of reach-wide benthic chlorophyll a and AFDM were incorrect* – MT DEQ (2011, Section 5.2.4) protocol uses a weighted average approach to calculate reach-wide benthic chlorophyll and AFDM along transects characterized by a mix of SAV and benthic algal cover. Weighted averages account for collection of unequal numbers of hoop and template samples, depending on sampling logistics. Stillwater Sciences (2020, p.32) stated that values were scaled based on the proportion of SAV samples taken to the total number of transect points sampled, but provided no specifics as to how this was done. We checked reach-wide estimates following MT DEQ (2011) calculations protocol and using data from the appendices to the Stillwater Sciences report. Our analysis indicates that the Stillwater Sciences calculations do not follow the agreed-upon calculation method outlined by MT DEQ (2011), and that the Stillwater Sciences calculations underestimated AFDM by as much as 88% when SAV coverage was 20% or higher along transect sites. Overall, the relationships between nutrients and primary producer biomass depicted in Stillwater Sciences' Figures 3-1 to 3-4 were based on methods and calculations that repeatedly deviate from the MT DEQ (2011) protocols, so that the data for biomass estimates as chlorophyll a content and AFDM, and the interpretations from those data are unreliable.

- *There was no apparent accounting for algae growing epiphytically on SAV when estimating percent cover* – As Table 2-7 (Stillwater Sciences 2020, p.24) shows, all percent cover information per site per sampling date sums to 100%. This is extremely unrealistic because benthic algae, especially filamentous algae, commonly colonize SAV and form a thick, abundant overstory (Wharfe et al. 1984, Spink et al. 1993, Thiébaut and Muller 1999) (e.g., see Figures 2 and 4).

- *Samples for benthic algal chl*a *were not filtered immediately in the field but, rather, after hours of holding time apparently without temperature control, which would have led to underestimates due to substantial chlorophyll degradation* – Protocol (MT DEQ 2011, Section 2.1.3) requires that chlorophyll samples are filtered immediately in the field, in recognition of the fact that chlorophyll samples can degrade rapidly in light and at temperatures above ambient (Wetzel and Likens 2000). According to the report, however, Stillwater Sciences (p.7) instead "kept [samples] in the dark", and once the samples were transported to the laboratory—that is, when all other sites planned for sampling that day were finished, sometimes after ~7-8 hours—finally "lab-filtered" the samples. There is no mention of having ensured appropriate temperature control throughout that hours-long delay. Thus, there is high potential that a portion of the chlorophyll that initially was present degraded, resulting in substantial underestimates of benthic algal biomass.

- *Samples for benthic algal chl*a *analysis apparently were not composited under low light* – MT DEQ (2011, p.13) noted that "Because Chl*a* readily breaks down in light [that is, in all except green light], samples must be composited in subdued light at the laboratory prior to processing." The above-mentioned filtering process could not have been done in darkness, and there was no mention of having used low-light (or, more preferably, low-green-light) conditions when samples were filtered in the laboratory. That important step stipulated in MT DEQ (2011) does not appear to have been followed.

<u>Chlorophyll *a*</u> (algal biomass indicator) – The report indicated a lack of understanding about what the chlorophyll measurement means and how it should be interpreted. Chlorophyll *a* is degraded to pheophytin in dead algae (Hendry et al. 1987), and inclusion of pheophytin in a chl*a* measurement over-estimates the algal biomass indicated by chl*a* in living algal cells (Biggs and Kilroy 2000, Wetzel and Likens 2000). This is especially a problem when attempting to assess benthic algal biomass because the benthic habitat accumulates the dead remains of plants and algae (Biggs and Kilroy 2000)—so that pheophytin can contribute up to 60% of the measured chl*a* content (Marker et al. 1980).

Yet, *directly countering the requirement given in MT DEQ (2011), Stillwater Sciences did not include a simple step to correct for pheophytin in their chl*a *measurements* (see, e.g., Axler and Owen 1994, Welschmeyer 1994, Steinman et al. 2017) which is important for both data quality and data interpretation (Biggs and Kilroy 2000). In other words, the benthic algal chl*a* data were confounded on some dates by high pheophytin (related to seasonal changes in algal growth, washout from precipitation, etc.), because a simple corrective step was not included in the procedure followed by Stillwater Sciences (confirmed in the Table 2-8 legend, "total benthic Chl-*a*.")

In chl*a* analysis, variation among subsamples can be as high as 25% for benthic algal communities with high abundance of filamentous algae such as *Cladophora* (Biggs 1987, Biggs and Kilroy 2000), characteristic of all the sites downstream from the Medford RWRF. To obtain a realistic estimate of the benthic algal biomass as chl*a*, *it is strongly recommended to analyze a subsample that consists of at least 3- to 5-mL aliquots of homogenized material, which considerably reduces subsampling variability* (Biggs and Kilroy 2000). The extra homogenization step is needed to ensure uniform subsampling of samples with many filaments. Benthic algal samples instead were simply filtered at Stillwater Sciences (although they should have been filtered immediately in the field, as explained above—MT DEQ 2011) and then frozen and shipped (on dry ice) to a commercial laboratory (Rhithron Associates, Inc. – Appendix C) for chlorophyll analysis. Scrutiny of Appendix C revealed that only 0.2 mL of the filtered but un-homogenized material was usually analyzed, at most 1 mL.

Stillwater Sciences (2020, p.19) noted that all the datasondes recorded anomalous chl*a* readings (as relative fluorescence units, RFU) in both the continuous 48-hour studies (August, October). Low RFU readings were interspersed with "improbably high" readings that were off-scale in many instances, probably due to sensor fouling. The RFU data would have provided only qualitative (relative) information about suspended microalgal concentrations in the water column (Reed et al. 2010).

<u>AFDM</u> – *Our overall assessment is that Stillwater Sciences' (2020) AFDM data cannot be reliably interpreted and should not have been used in efforts to assess relationships between benthic algal biomass and nutrients in the Rogue River.* This evaluation is based on the following points.

AFDM is a measure of the total amount of organic materials in a sample, <u>not</u> the total amount of living algae. It includes living phototrophic and heterotrophic organisms, detritus, and usually some terrestrial leaf-fall debris (Biggs and Kilroy 2000, Steinman et al. 2017). Although AFDM measurement is straightforward, extreme methodological problems were indicated from Stillwater Sciences' writing and data:

- Benthic algal communities typically accumulate abiotic and biotic debris such as sediments and dead animal/plant remains (Burkholder 1996, Biggs 2000a). Unless this debris is identified and mostly removed from the sample, it causes significant error in estimating the amount of benthic algae present (Biggs 2000a). There is no indication in the Stillwater Sciences report/appendices that any effort was made to remove non-algal debris. The extremely high "apparent" AFDM values at upstream Sites 3 and 2 in August, reported in Stillwater Sciences' Table 2-8, very likely reflected the inclusion of such debris (analogous to use of total chlorophyll rather than corrected chlorophyll, above), especially since algal biovolume data for August indicated very low biomass.
- Major errors can easily occur in drying, ashing, and weighing only a small amount of material (here, for example, such as would have been expected from sampling upstream Sites 3 and 2). Thus, AFDM is undependable in estimating low benthic algal biomass unless measurements

are very carefully made (Biggs 2000a). We assessed Stillwater Sciences' data for this potential problem by calculating autotrophic indices (AIs). The autotrophic index (Weber 1973: AI = AFDM (milligrams/m^2) / corrected chl*a* (milligrams/m^2), if based on sound benthic chl*a* and AFDM measurements, can indicate the proportions of the aquatic community comprised of heterotrophic versus phototrophic organisms. AI values are sometimes used to infer non-polluted conditions with little organic debris, versus communities affected by organic pollution (Biggs 2000a). Here, however, values for corrected chl*a* are not available for calculation of an AI as explained above; and it is already well known that the river segment of concern is downstream from a major sewage effluent discharge.

Nevertheless, we found it instructive to calculate AIs from Stillwater Sciences' Table 2.6 (p.19) as a check on data quality (Table 4 below). ***First***, we checked Tables 2.8 and Table 2.9. The titles of these tables read:

> Table 2-8: Chlorophyll-a (Chl-a) and Ash Free Dry Mass (ADFM [corrected, AFDM]) results from point transect sampling at 2019 Rogue River study sites; and
>
> Table 2-9: Chlorophyll-a (Chl-a) and Ash Free Dry Mass (ADFM [corrected, AFDM]) results from SAV samples only during point transect sampling at 2019 Rogue River study sites.

We therefore inferred that Table 2-8 pertained to benthic algal chlorophyll and AFDM, whereas Table 2-9 pertained to SAV. A footnote to Table 2-8 mentioned information about sampling both "SAV and periphyton," which we assumed to have been applicable to both tables. ***Second***, we considered published literature values for AIs. Healthy benthic algal communities in unpolluted stream segments typically have AIs of 100-200 (Biggs and Kilroy 2000), which we expected at upstream Sites 3 and 2 a short distance upstream from the Medford RWRF outfall. Benthic algae in these sites were dominated by low biomass of diatoms in August, and by diatoms (Site 2) or very low biomass of green algae with cyanobacteria and diatoms (Site 3) in October (Stillwater Sciences 2010, Figure 2-10 – see Figure 5).

Biggs and Kilroy (2000) cautioned that for healthy low-biomass benthic algal communities, errors associated with weighing usually result in spurious AI values, up to 2000. Our calculations of AI values from Stillwater Sciences' data yielded extremes in AI values well above 2000 (Table 4). The apparent failure to homogenize samples adequately before subsampling, and the inappropriate use of extremely small subsamples, likely contributed to the poor-quality data indicated by these AI values.

MT DEQ (2011) instructed that if samples were properly collected, AFDM of benthic algae could be combined with AFDM of SAV for reach-wide estimates, per site, of total benthic primary producer AFDM. This potential use of the data was not possible, however, due to improper sampling (above).

Biovolume (algal biomass indicator) – The chl*a* data *should* have generally paralleled the biovolume data, as both are estimates of benthic algal biomass. This expectation is especially true for filamentous green algae such as *Cladophora* and *Oedogonium*, which (unlike diatoms, whose cells are mostly occupied by a large vacuole) have chlorophyll ~evenly distributed throughout their cells. Note the writing of Stillwater Sciences (2020, p.25):

> Chl-a results were highest during the August sampling events….Despite being upstream of the Medford RWRF, Site 2 had higher Chl-a levels than some downstream sites….

The benthic algal chl*a* data, if corrected for pheopigments as explained above, should have yielded the same general findings about overall benthic algal abundance as biovolume data. Stillwater Sciences did not mention the fact that biovolume data showed about ten-fold higher benthic algal abundance in October rather than August (Figure 5). It should be noted that benthic

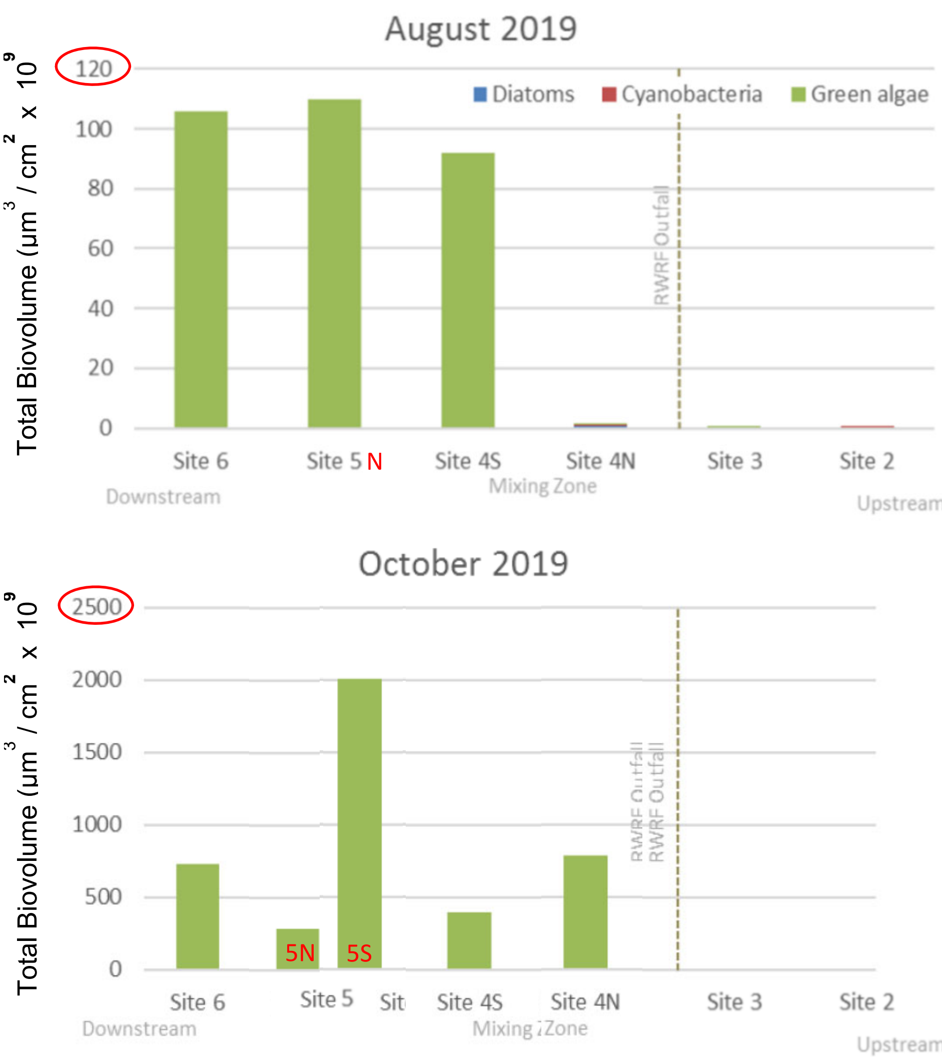


Figure 5. Benthic algal biovolume (estimate of biomass; Stillwater Sciences 2020, Figure 2-10), modified to emphasize differences in the vertical scale for the two months, and to provide clarification that data were not available for Site 5S which is known to be influenced by the Medford RWRF. Note that (i) sites downstream from the sewage outfall are dominated by the notorious "phosphorus-loving" noxious green macroalga, *Cladophora,* along with another known pollution-tolerant green macroalga, *Oedogonium* (Francke and Den Oude 1983, Burkholder 2009, Stevenson et al. 2012, and references therein); (ii) these noxious algae were absent at the upstream sites, and (iii) biomass was about 10-fold higher in October than in August.

algae at upstream Site 3 in October consisted mostly of green algae (Stillwater Sciences' Figure 2-10, lower right panel). The genus was not clarified in the report; in the appendices (p.268), the entries for the October sampling at Site 3 stated "unidentified chlorophyte" and *Oedogonium*. Regardless, their biomass at Site 3 was extremely low relative to that of green algae at the downstream stations (Figure 5).

Table 4. Autotrophic index (AI) values calculated from Stillwater Sciences' data (Table 2-8) for benthic algal biomass as total chlorophyll *a* (uncorrected for pheopigments) and AFDM (from samples that were not cleaned to remove debris).

Site (2019)	August	September	October	November
Upstream Control Site 3	**2,330**	**2,598**	**4,482**	**2,154**
Upstream Control Site 2	1,866	383	552	420
Site 4N*	**2,431**	999	554	536
Site 4S	199	546	2,394	11,170
Site 5N	-----	**2,663**	1,835	**2,553**
Site 5S	510	4,442	3,499	8,738
Site 6	83	163	932	1,065

* Values in red/bold, at upstream control and downstream north shore sites considered to have been minimally affected by the RWRF effluent, are clearly spurious, reflecting poor-quality data. Values in red are questionable. In south shore sites 4S and 5S, known to have been strongly impacted by the RWRF effluent, extremely high values in brown are considered questionable as well.

** The benthic algal (total) corrected chlorophyll value at 4N in August (241 µg/L) is regarded as questionable, as it exceeded the value from effluent-impacted Site 4S (183 µg/L).

<u>Cell Number (Density)</u> – The same fundamental error was repeated in Stillwater Sciences' interpretation of its data for *benthic algal cell numbers* (density) as for biovolume—misstating repeatedly that benthic algal cell number was higher in August than in October, when actually the reverse was true. For example, Stillwater Sciences (2020, p.56) wrote: "we found peak biomass occurring in August with lower levels during subsequent sampling events (Figure 2-11 and Figure 2-12)." Both figures referenced had did not pertain to biomass; they instead showed cell number/ species composition of diatoms and cyanobacteria, respectively. Both clearly revealed much higher cell numbers of these taxa in October than in August. Curiously, total benthic algal cell number data were not presented.

<u>Light Microscopy Methods for Biovolume and Cell Number</u> – The information given in Appendix C by Rhithron Associates, Inc. was detailed for total dead+live ("digested") diatoms. No information was provided, however, on what should have been the focus of these analyses—live benthic diatom cells. Analogous to use of total chlorophyll rather than corrected chlorophyll, focus on total diatoms makes it impossible to retrospectively estimate actual live abundance because many diatoms in benthic algal samples—commonly the majority of the cells in warmer periods—are dead when collected except during optimal (cold) seasons for diatom growth (e.g., Burkholder

and Wetzel 1989, Burkholder et al. 1990, Burkholder 1996). Stillwater Sciences (2020, p.10) stated that living [that is, viable when collected] diatom counts were included with the "soft-bodied algae" counts (below), but no information was provided in the Appendices about those counts or how they compared to the "total dead+live counts" that unfortunately were the sole focus of the diatom-related components of the report. Rhithron Associates, Inc. (Appendix C) stated that inclusion of these [unavailable] viable diatom cell counts would allow for calculation of diatom species abundance, yet there was no mention of any actual use of live-diatoms information throughout Stillwater Sciences' analysis.

In contrast to the detailed methods given for dead+live diatoms, the description of the methodology used for "soft-bodied algae" (algae unlike diatoms or other taxa with hard coverings)—such as filamentous macroalgae, which overwhelmingly dominated the Rogue River segment most adversely affected by the Medford RWRF sewage effluent—was so poorly described that it would not be possible to replicate:

> …the sample was manually homogenized and emptied into a porcelain evaporating dish. A small, random sub-sample of algal material was pipetted into a standard Palmer-Maloney counting chamber using a disposable Pasteur pipette. Visible (macroscopic) algae were also sub-sampled, in proportion to their estimated abundance relative to the total volume of algal material in the sample, and added to the liquid fraction on the slide.

This was poor technique that led to questionable estimates of benthic algal abundance (biovolume, cell number) for the following reasons:

- "Manually homogenized" – Biggs and Kilroy (2000) cautioned that great care must be used when attempting to homogenize "certain green filamentous algae such as *Cladophora* [because] they do not break apart easily." Many published procedures recommend inclusion of detailed information about this step, which is fundamental to accurate quantification of algal filaments. Information is missing about *how* the samples for "soft-bodied algae" were homogenized. There was no mention of any checks for variation among cell counts post-homogenization (e.g., see Burkholder and Wetzel 1989). These steps are fundamentally important for obtaining accurate estimates of filamentous benthic algal abundance.
- Rhithron Associates, Inc. also included the above vague description of their methods: ("Visible (macroscopic) algae were also sub-sampled…"). *How* was their abundance estimated relative to the total algal material? How was it "added" to the Palmer-Maloney chamber? Was the entire chamber counted? If not, was a rigorous check conducted for homogenization of settling by the algal cells and the filaments across the chamber? These important steps are not mentioned and likely were not taken. Rhithron Associates, Inc. stated that "internal quality control procedures for soft-bodied [filamentous] algae involved review and verification of digital photographs." What does that *mean*, quantitatively? What was actually done to check the quality of the analyses?
- Stillwater Sciences (2020, p.10) stated that "300 natural units" of soft-bodied [mostly filamentous] algae were counted and identified. There is no further mention of that

information in the report or its appendices. This statement, in combination with the writing from Rhithron Associates, Inc. (Appendix C), leaves readers with no idea as to what was actually done to assess filamentous benthic algal abundance (cell numbers, biovolume) in light microscopy.

What can be said about use of "units" is that this is, at best, a qualitative rather than quantitative approach. One unit is defined as one filament, regardless of its length. Therefore, a unit can include 3 cells or 80 cells; 300 units could include 900 cells or 24,000 cells—leading to an old adage among benthic algal ecologists that "units are useless" in quantifying benthic filamentous algae. What should have been done was to have quantified filament lengths and related those data (through linear regression analysis) to cell numbers per filament in order to obtain the total cell number and, from there, a quantitative biovolume estimate.

Significance – We are not alone in our evaluation of the benthic algal assessment in Stillwater Sciences (2020) as highly questionable. Stillwater Sciences (p.11) itself wrote, "For periphyton [and SAV, below], 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* [emphasis added]." That startling admission is accurate, but not for the reason given. The patchy distribution of periphyton (and SAV) *can* make quantitative assessments more difficult, but there are published methods for overcoming such problems of uneven colonization (Biggs and Kilroy 2000). Stillwater Sciences maintained that, based on information from MT DEQ (2011), average benthic chl*a* measured per sampling event was probably within ± 30% of the true average, with an 80% confidence level. Those numbers assume sound techniques, however, such as correction for pheopigments. The estimates obtained by Stillwater Sciences could easily have been ± 40-50% or more of the true average, with even lower confidence levels, because of critical protocol steps that were not followed as explained above. Nevertheless, these data were the foundation used to develop Stillwater Sciences' suggested Medford RWRF effluent nutrient limits. Consider the statements on pp. 51-52:

> Based upon commonly recommended periphyton biomass thresholds of maximum Chl-a less than 100 mg/m^2…., site-specific relationships developed from data collected during 2019 were used to estimate mean seasonal TP concentrations….

Thus, the uncorrected benthic algal chlorophyll estimates from Stillwater Sciences' assessment were directly used to estimate mean seasonal nutrient concentrations and then extrapolated yet further to develop the suggested Medford RWRF effluent nutrient limits. Stillwater Sciences' unreliable benthic algal biomass estimates, based on major deviations from accepted protocols, render the relationships between benthic algal biomass and nutrients developed in the report, and the Medford RWRF effluent nutrient limits derived from them, invalid.

III.B.2.d. *Submersed Aquatic Vegetation*

Submersed aquatic vascular plants are major responders to the high nutrient supplies from the Medford RWRF sewage effluent, yet they were poorly assessed and then ignored in Stillwater Sciences' attempt to develop suggested effluent nutrient limits.

Submersed aquatic vegetation was aptly described by Stillwater Sciences (2020) and earlier reports (see Section II) as a group of major responders to the high nutrient supplies in the Medford RWRF sewage effluent. SAV was also found to be highly abundant especially in affected downstream sites 4S and 5S. As Stillwater Sciences (2020) noted, critical Site 4S, most influenced by the Medford RWRF sewage effluent downstream from the RMZ, had the highest SAV cover (30 to 59%) (excluding erroneous data for Site 4N – see pp.29-31 below). Such findings are consistent with the published science literature (e.g., Smith and Barko 1990, Hood et al. 2014, O'Hare et al. 2018). Thus, SAV merits major consideration in developing protective nutrient effluent limits for the affected Rogue River segment. Yet, remarkably, SAV was not considered by Stillwater Sciences in developing its suggested effluent nutrient limits.

<u>Inadequacies and Errors in Stillwater Sciences' Approach to SAV Assessment</u> – Stillwater Sciences was tasked with quantifying both percent cover and biomass of SAV (Stillwater Sciences Rogue River Sampling Plan, Section 1.2). Cover of SAV was to be estimated in two ways: (1) visual cover estimate for the entire site, by delineating areas of SAV on field maps; and (2) noting presence/absence of species at 11 locations along a transect following methods of Madsen (1999). Stillwater Sciences (2020) also assessed SAV biomass following methods of MT DEQ (2011). SAV biomass was assessed as AFDM, generally cleaned of epiphytes (Stillwater Sciences 2020, p.8); and as chl*a* content, which is not generally considered a useful measurement of SAV abundance since their chlorophyll content is highly dependent on the available light (Dennis and Isom 1984). Stillwater Sciences attempted to use both SAV chl*a* content and AFDM measurements to obtain an overall estimate of the biomass of primary producers (i.e., benthic algae + SAV) per unit river bottom area (but see below).

As there were for approaches and analyses regarding water quality and benthic algae, there were major errors and inadequacies in Stillwater Sciences' (2020) assessment of macrophytes in the study:

- *Lacking in the report and appendices was (i) specific information about how quantitative cover estimates of algae, SAV, and open area (Table 2-7) were calculated, (ii) maps delineating SAV areas, and even (iii) any* ***actual data*** *for SAV percent cover*.
- *Field data sheets for SAV point-transect sampling did not include a list of SAV species present at each location*; only the general presence of SAV was noted.
- *Sampling for SAV in general was grossly inadequate*, despite the fact that these macrophytes are known major responders to the Medford RWRF sewage effluent. SAV samples for benthic AFDM and chlorophyll quantification were not collected unless SAV cover was ~20% or more within the 1 m^2 areas observed along transects (see Table 2-7, footnote 1). It should also be noted that the 20% lower limit conflicted with information given in Table 2-4 (Stillwater Sciences, p.9) which stated that the "Resolution/Reporting Limit" for SAV percent cover was less than 10%. Moreover, the MT DEQ (2011, p.8) protocol stipulates that (i) [even] if macrophyte cover is *less than 5%* of the river bottom area, *macrophytes should be sampled* and separated from any filamentous algae in the sample immediately; (ii) if more than 5% macrophyte cover is present, the entire sample should be collected and filamentous algae

should be separated from the macrophytes when the sampling personnel are back on the river bank; and (iii) both the filamentous algae and the macrophyte samples should be saved for inclusion in AFDM estimates. Stillwater Sciences provided no explanation about why SAV was not collected unless there was at least 20% cover, but clearly MT DEQ protocol was not followed.

- In many cases, abundant SAV was noted on field datasheet copies included in the report appendices, yet biomass samples were not collected. For example, Table 2-9 showed results from SAV chl*a* and AFDM at the study sites, but the report's authors cautioned that the results were "provided for comparative purposes only, since the low number of hoop samples collected for analysis of SAV chl*a* and AFDM is inconsistent with the minimum sample size assumptions in the MT DEQ (2011) methodology." However, MT DEQ (2011) assumes no minimal number of hoop samples for biomass estimates; rather, the protocol requires that a minimum of 11 samples of SAV or benthic algae be collected within the wadeable portion of the transect. According to Stillwater Sciences' Table 2-7, this minimum sample size was met at all sampling sites on all dates. On p.204 of the appendices was the statement "SAV present but not enough to hoop; lots of drift SAV." Unfortunately, the drift SAV was not sampled, either. Thus, the contribution of SAV to benthic biomass was routinely underestimated.

- *There was no mention of any effort to separate live from dead SAV material for biomass estimates*, even by a simple method such as color (removal of brown leaves and shoots; Dennis and Isom 1984, Thomas 2013). This problem is analogous to use of AFDM and total chlorophyll for benthic algae described above; SAV material that was dead when collected should not have been included in biomass estimates.

- *The SAV chlorophyll data for August are invalid because the August data are not comparable to those for other sampling dates because sampling methods were changed* – In the August sampling effort, chl*a* estimates were confounded by mistakenly combining SAV+algae, including filamentous forms (see Stillwater Sciences 2020, Table 2-9, footnote 2). MT DEQ (2011) protocol requires physical separation of such algae before determining chlorophyll content. The error was corrected for other sampling efforts, but the change in methodology means that the chlorophyll data for August and other sampling dates are not comparable. Therefore, statistical analyses and interpretations combining the August "SAV" chlorophyll data with the other data are invalid.

- *August 2019 samples for SAV AFDM were not analyzed*, as another inconsistency that occurred in SAV sampling (see Stillwater Sciences 2020, Table 2-9, footnote 3). Because the August AFDM estimates for the biomass of benthic primary producers are missing SAV, statistical analyses and interpretations about the biomass of benthic primary producers in August are invalid.

- *Reach-wide estimates of total benthic primary producer biomass were not attempted* – MT DEQ (2011) instructed that a weighted-average approach could be used to combine benthic algal + SAV biomass data for an overall, reach-wide estimate of total benthic primary producer biomass per site. Stillwater Sciences (2020, section 3.2.1) did not attempt reach-wide estimates, as the report explained, 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." There was no apparent recognition of the fact that the weighted average approach *accounts* for such differences, and that the approach is not limited by the number of hoop samples.

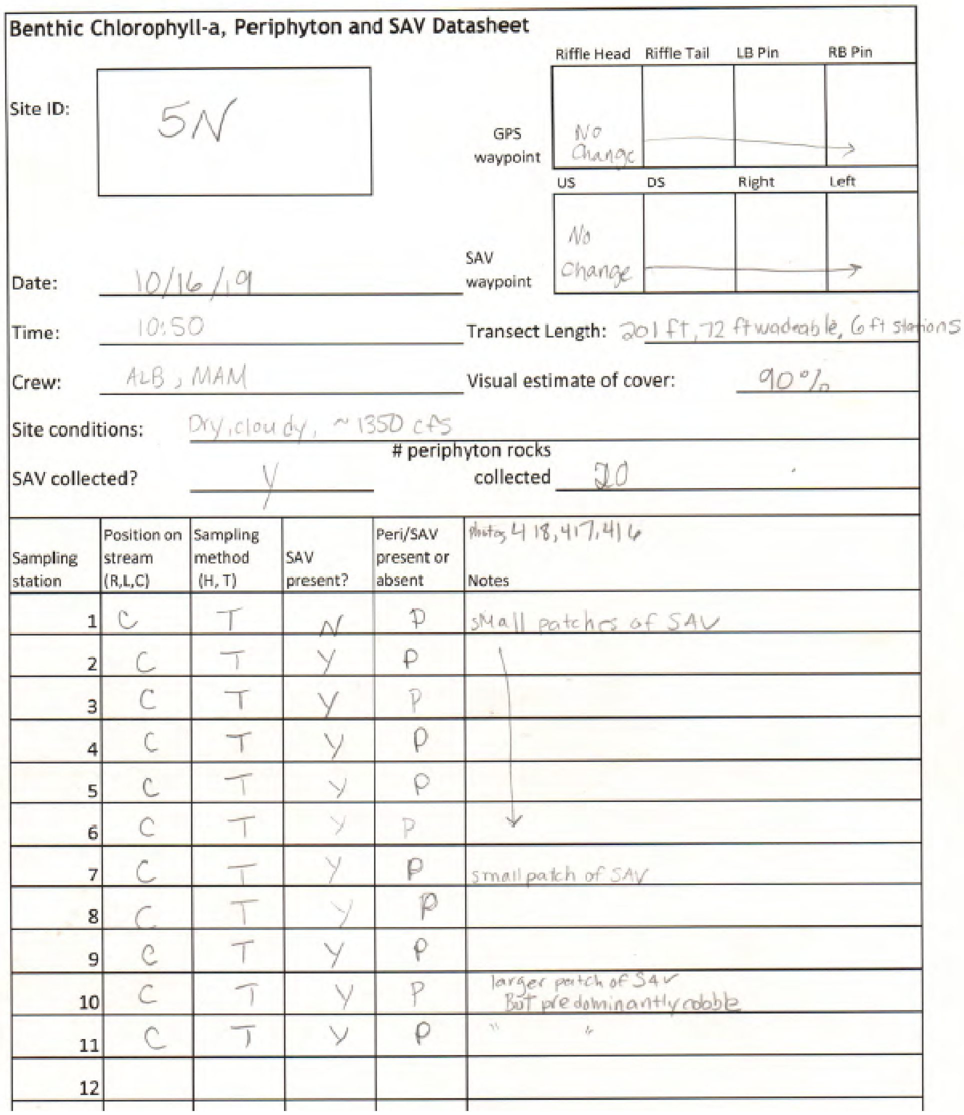
- *Data documentation for SAV in general was grossly inadequate*, making the analysis and interpretation of SAV responses to nutrients impossible—despite the fact that these macrophytes are major responders to the Medford RWRF sewage: For example, Table 2-9 showed results from SAV chl*a* and AFDM at the study sites, but the report's authors cautioned that the results were "provided for comparative purposes only, since the low number of hoop samples collected for analysis of SAV chl*a* and AFDM is inconsistent with the minimum sample size assumptions in the MT DEQ (2011) methodology." (Again, note that there is no minimal number of hoop samples required by MT DEQ 2011 protocol.) In field notes (appendices) were entries such as "lots of SAV present, but not enough to sample" (Site 4S, October 15, appendices). On p.204 of the appendices was the statement "SAV present but not enough to hoop; lots of drift SAV."

  As another example of this serious problem, consider the field datasheet for Site 5N in October 2019 (Figure 6). Table 2.7 listed the percent SAV cover for this site (72%) as the highest for any site downstream from the Medford RWRF outfall in the October sampling effort. The field datasheet provides no such information. The datasheet reflects a conceptual problem as well: Note the column, "SAV present" next to the column "Peri [periphyton, i.e., benthic algae]/SAV present or absent." The first column is filled with "Y" ["yes" responses] except for "sampling station" (point location) #1, "N" ["no" response]. The entries for the second column are all "P" [present]. For "sampling station" (point) #1, the only logical interpretation is that periphyton were present at that location, but not SAV. Yet, the comment written at the side of that entry states that "small patches of SAV" were present.

  In addition, there is no indication from the field datasheet that more than "small patches" of SAV were present except perhaps at locations #10 and #11 ("larger patches of SAV but predominantly rubble"). So, how was the overall evaluation of SAV at Site 5N in October calculated to be 72% (Table 2-7)? This extremely high number clearly is not supported by the datasheet, leading readers to regard even the (usually easily assessed) percent SAV cover data contained in the report as highly questionable.

Stillwater Sciences' failure to include SAV in reach-wide biomass calculations obviously resulted in substantial underestimates of total primary producer biomass at sites where hoop samples were collected, and even at sites where SAV samples were not collected since SAV was ignored unless coverage along transect points was at least 20%. Correlation analyses between nutrients and AFDM may have yielded negative correlations because of omission of SAV in the analyses.

Stillwater Sciences (2020, p.11) wrote, *"For SAV [and periphyton], because of their patchy distribution there is no way to know whether…biomass or species composition are representative of* *the true values of these metrics."* We also consider the SAV data highly uncertain, as for the benthic algal analyses—not because of patchiness in distribution but, rather, because of the lack of rigorous sampling for SAV and the inconsistent data collection/analysis that characterize Stillwater Sciences' report.



Benthic Chlorophyll-a, Periphyton and SAV Datasheet

Site ID: 5N

GPS waypoint	Riffle Head	Riffle Tail	LB Pin	RB Pin
	No Change			

SAV waypoint	US	DS	Right	Left
	No Change			

Date: 10/16/19

Time: 10:50

Crew: ALB, MAM

Site conditions: Dry, cloudy, ~1350 cfs

SAV collected? Y

Transect Length: 201 ft, 72 ft wadeable, 6 ft stations

Visual estimate of cover: 90%

periphyton rocks collected 20

Sampling station	Position on stream (R,L,C)	Sampling method (H, T)	SAV present?	Peri/SAV present or absent	photos 418, 417, 416 Notes
1	C	T	N	P	small patches of SAV
2	C	T	Y	P	
3	C	T	Y	P	
4	C	T	Y	P	
5	C	T	Y	P	
6	C	T	Y	P	
7	C	T	Y	P	small patch of SAV
8	C	T	Y	P	
9	C	T	Y	P	
10	C	T	Y	P	larger patch of SAV But predominantly cobble
11	C	T	Y	P	" "
12					

Figure 6. The field data sheet for Site 5N, October sampling effort, 2019 (appendices pdf, p.214). Note that percentage cover data are not included except for a "visual estimate of [total benthic algal + SAV] cover as 90%." Descriptions for the 11 locations (transect points) that were evaluated mention nothing about SAV except that it was present (locations 8 and 9), or mention SAV as having been present in "small patches" (locations 1-7). Remaining sites 10 and 11 were described as having had "larger patches of SAV," but the two points were covered by "predominantly rubble." Yet, the overall SAV cover at Site 5N in October was listed by Stillwater Sciences (2020, Table 7-2) as 72%, higher than at any other site throughout the 2019 study. This entry is highly questionable. More generally, the inadequate entries on the field datasheets call into question the other SAV percent cover data at all sites on all sampling dates.

Significance of Omitting SAV from Consideration in Setting Effluent Nutrient Limits – Rooted plants in aquatic habitats are similar to those on land in having vascular tissue that helps to form their roots, stems, flowers, and leaves (Sculthorpe 1967). Some noxious species can become separated from the bottom substrata to form floating masses in the water as well, as did the SAV mentioned above (top of this page). As Stillwater Sciences (2020) described, SAV is abundant at several key sites downstream from the Medford RWRF discharged sewage effluent, but not upstream. Despite having been omitted from Stillwater Sciences' effort to develop effluent nutrient limits, *SAV is an **essential** response variable that must be included in efforts to develop relationships between nutrient supplies and primary producer biomass, and to set protective effluent nutrient limits, for this Rogue River segment.*

III.C. *Stillwater Sciences' Excessive Background (Control or Threshold) Nutrient Conditions*

Stillwater Sciences (2020, p.34) selected a maximum appropriate level of benthic algal biomass at 100 mg [corrected] chlorophyll *a* per square meter of river area, based on various peer-reviewed studies published for other rivers (Horner et al. 1983; Nordin 1985; Welch et al. 1988; Dodds et al. 1997; Biggs 2000a,b). Stillwater Sciences' analysis, described with the goal of suggesting effluent nutrient levels for the Medford RWRF that would maintain benthic algal biomass at or below that level, was based on three lines of evidence:

First, Rogue River data from wet year 2019 and dry year 2018 (data not shown) ostensibly were used to evaluate apparent responses to nutrient levels upstream versus downstream from the Medford RWRF outfall. Unfortunately, the much more instructive low-flow ("worst case" for pollution effects assessment) year 2018 was only cursorily considered, and mostly ignored. Second, the Rogue River was compared to other rivers based on predictive relationships from those other systems, but the main finding from that comparison was ignored, namely, that the Rogue River is more sensitive to nutrient pollution than most rivers.

Third, various nutrient thresholds were considered from published literature, including rivers ranging from pristine to highly nutrient-polluted. From that literature compilation, Stillwater Sciences used in its analysis, without supporting rationale, the 25th percentile threshold for "background" TN that was about twice as high as the TN in control sites upstream from the Medford RWRF. The 25th percentile threshold for TP from the compilation *was rejected* as too low to be "feasible"—although it was identical to the U.S. EPA recommendation for 25th percentile (minimally impacted) streams in the sub-ecoregion that contains the middle Rogue River, and similar to the site-specific background TP concentration estimated from regression analysis and control site concentrations. Instead, Stillwater Sciences, without supporting rationale, used in its analysis a background TP concentration that was more than three-fold higher than the U.S. EPA recommendation for minimally impacted rivers in the middle Rogue River sub-ecoregion, and twice as high as the control site concentrations a moderate distance (1.1 mile or less) upstream from the Medford RWRF.

III.C.1 Consideration of Inappropriate Sites as Controls

In evaluating the impacts of sewage effluent discharges, accepted assessment protocol is to compare water quality in upstream waters near the outfall versus in affected downstream waters near the outfall (e.g., Marriott 1997, Peschke et al. 2019). There typically are various sources of pollution draining into a river within a middle watershed region. That is why it is important for the upstream "control" sampling sites to be located within a short (minimal) distance from the sewage outfall area—so that it is possible to assess the influence of *that* sewage outfall on the river.

The upstream sampling sites closest to the Medford RWRF and, thus, germane to this evaluation (Figures 1 and 3, above) are at distances of 0.4 RM (Site 3, the key minimally impacted site, nearest the outfall) and 1.1 RMs from the outfall (Site 2). The downstream sites are within a segment 1.5 RMs downstream from the Medford RWRF (Site 6, described by Stillwater Sciences as outside the detectable influence of the effluent), as described in Section III.B.1. Without explanation, however, Stillwater Sciences (p.36) suddenly expanded its analysis beyond the 2019 sampling design to considerable distance in both upstream and downstream directions (Table 5). The expanded consideration included Gold Hill, ~10 RMs downstream from the outfall. Additional

Table 5. Stillwater Sciences' (2020) Table 3-1, modified for correction and clarification. Note that:

i) The lack of appropriate low-level nutrient analyses led to weak "guesstimates" of nutrient conditions at critically important True Control Sites 3 and 2, and no estimate at all for ammonia-N.

ii) According to Stillwater Sciences' analysis, Site 6 was downstream from the influence of the RWRF effluent and, therefore, should not have been included as an affected site "in the vicinity" of the RWRF. Its inclusion artificially reduced the apparent impact of the RWRF effluent on nutrient concentrations in nearby waters.

	Location	Nitrate only ~~TIN~~ (mg/L)	TN (mg/L)	PO_4-P (mg/L)	TP (mg/L)
U.S. EPA recommendation	minimally impacted **Background ~~"reference"~~ conditions based upon 25th percentile of ~~historical~~ all streams data (nutrient-poor to nutrient-rich) samples reported between 1990–1998 (USEPA 2000b)**				
	Level III ecoregion 78 (Klamath Mountains)	0.04	0.18	~~NA~~ ≤ 0.03	0.03
	Long-term (1990–2019) summer/fall (June-October) average nutrient concentrations too far **upstream of Medford RWRF**				
~10 RMs upstream	Rogue River at Dodge Park (DEQ Site 10423)	0.02	0.13	0.03	0.04
P-impaired, 3 RMs upstream	Little Butte Creek at Agate Rd (DEQ Site 10462)	0.03	0.64	0.06	0.10
"Control" or "Threshold" Sites	**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
Affected sites within 1.5 miles downstream from the RWRF outfall, except that Site 6 should not have been included	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
Irrelevant –	**Long-term (1990–2019) summer/fall (June-October) average nutrient concentrations** too far **downstream of Medford RWRF**				
~10 RMs downstream	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. Uncertain; inadequate methods for low-level nutrient analyses

1 Site assumed to be unaffected by RWRF discharge as determined by dye-tracer study of outfall plume (Brown and Caldwell 2014). 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 $NO_2^- + NO_3$.

Uncertain; inadequate methods for low-level nutrient analyses upstream sites considered for "background" (control or threshold) conditions included the Rogue River at upstream Site 1 and Dodge Park (Dodge Bridge County Park), 1.8 RMs and ~10 RMs upstream from the Medford RWRF.

Another upstream site considered, Lower Little Butte Creek (Stillwater Sciences, p.36) empties into the Rogue River ~1.5 RMs upstream from the Medford RWRF outfall. Available water quality data for that tributary are from a site about 3 RMs upstream from the Medford RWRF outfall. At that location, the stream drains a mostly agricultural area (cattle operations, cropland). The U.S. EPA added Little Butte Creek to Oregon's most recent 303(d)-list (2012, approved in 2018), as impaired due to high phosphorus pollution (see https://www.oregon.gov/deq/wq/Pages/2012-Integrated-Report.aspx - waterbodies added to the list by EPA). Stillwater Sciences (p.35) misinformed readers by asserting that its approach "recognized the presence of *local nutrients in the **immediate vicinity** of the Medford RWRF* [emphasis added] such as sources arriving from Little Butte Creek."

Stillwater Sciences' data presented earlier in the report demonstrated otherwise: As related above, upstream Sites 3 and 2 had such low-nutrient (including phosphorus) conditions that Stillwater Sciences' inadequate reporting levels could not detect them. Stillwater Sciences selected upstream Control Sites 3 and 2, averaged with downstream sites 4N and RMZ-N, as the sites potentially representative of background conditions, but then rejected the TN and TP data from all of those sites in its calculations for effluent nutrient limits (below).

III.C.2. Relationships Between Benthic Algae and Nutrients

*Linear regression analyses indicated that benthic algal [total] chlorophyll (but not other abundance parameters) was positively related to concentrations of both TN and TP in wet year 2019. While benthic chl*a *data were not available for dry year 2018, the relationships were much stronger for available abundance parameters biovolume and cell density in the dry year. In addition, use of the drier year enables assessment of "worst case" conditions, which should be used to develop protective effluent nutrient limits.*

As brief background for this subsection, Stillwater Sciences attempted to assess its 2019 data, along with limited consideration of 2018 data, for inferences about relationships between benthic algae and water-column nutrient concentrations. Linear regression analysis was also conducted to check whether the data from two years of the summer-fall "seasons" (2019 and the previous year, 2018) indicated that nutrients in the Rogue River study area stimulate benthic algal growth.

III.C.2.a. *AFDM Data Beyond Salvage*

Stillwater Sciences conducted linear regression analysis of *maximum AFDM* versus overall average TN and TP at the study sites during August – November. The findings, apparent inverse relationships (Figure 3-1), were opposite of what commonly has been reported for other rivers and studies (Biggs and Kilroy 2000). Curiously, Stillwater Sciences attributed the aberrant findings to "a predominance of green algae during the August survey" without supporting rationale. While green algae were dominant among other algal groups, their biomass (biovolume) in August actually was very low (see Figure 5 above).

The AFDM data are not credible and should not be used (see pp.17-18 above). Furthermore, inclusion of "Site 5" data for August confounded the entire analysis because only Site 5S data were available for August, versus both Sites 5N and 5S for the rest of the study (see Stillwater Sciences' Table 2-8). Inconsistent data such as these cannot be statistically analyzed.

III.C.2.b. *Wet Year 2019 versus Dry Year 2018*

Linear regression analysis was conducted on "seasonal" (defined by Stillwater Sciences as the period from August through November) average and maximal data for benthic algal abundance over the study period, comparing two years—wetter year 2019 and the much drier year 2018 (Figures 7 and 8). Although low-flow conditions in both years were comparable, the typical high discharge associated with winter storms occurred in 2019, but not in 2018 (Figure 7).

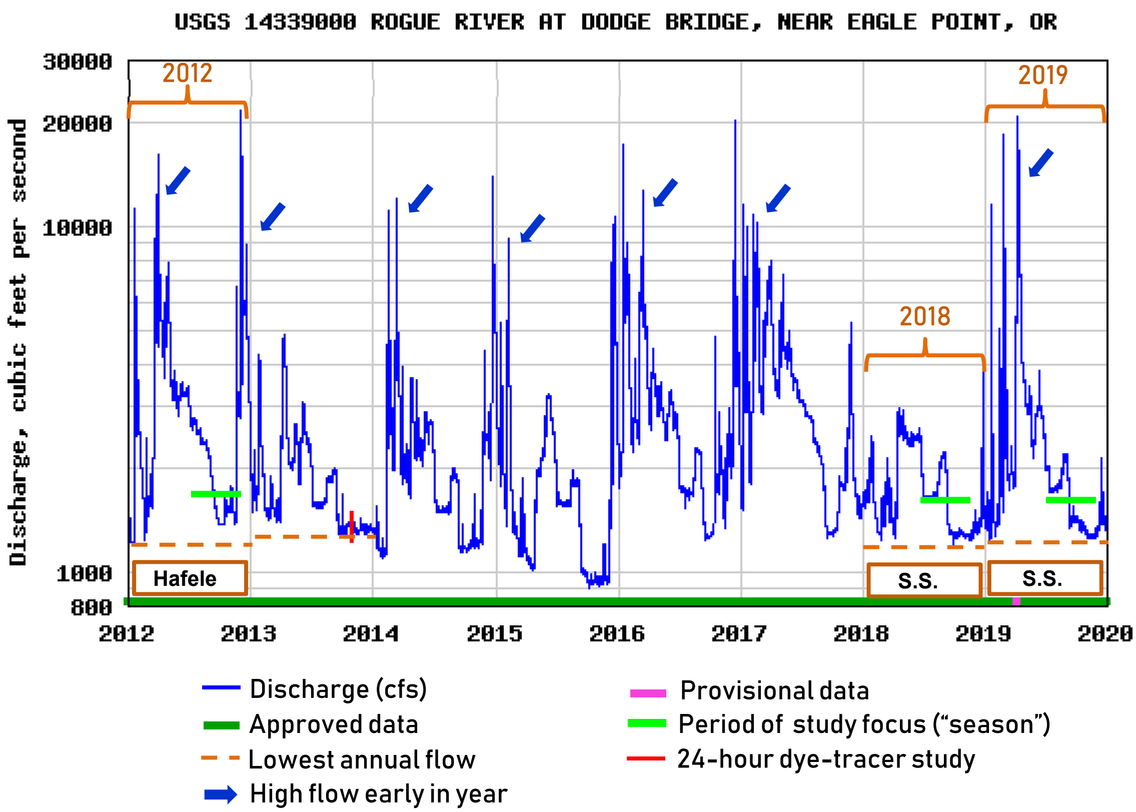


Figure 7. Discharge data over annual periods from 2012 through 2019 at the upstream reference USGS gaging station used by Brown and Caldwell (2014). Developed from data plotted by the USGS Water Information System: Web Interface (https://nwis.waterdata.usgs.gov/or/nwis).

November is not included in the Figure 8 precipitation data because, as explained above, it is typically characterized by lower, subsiding algal growth, as was shown by the 2019 data in this study. In previous studies—as examples, data collected in 2012 and 2018 (Hafele 2013, Hume 2019)—benthic algal abundance consistently has been described as stimulated by nutrient supplies from the Medford RWRF. Clearly the driest year of the three was 2018, whether considered from

August through October on a monthly or annual basis (Figure 8, left and middle panels, respectively) or over the total "growing season" (here, April through October). The three years differed markedly in precipitation during August through October (Figure 8). In contrast, total precipitation in the extended growing season (April through October) was similar in 2012 and 2019, but much lower in 2018.

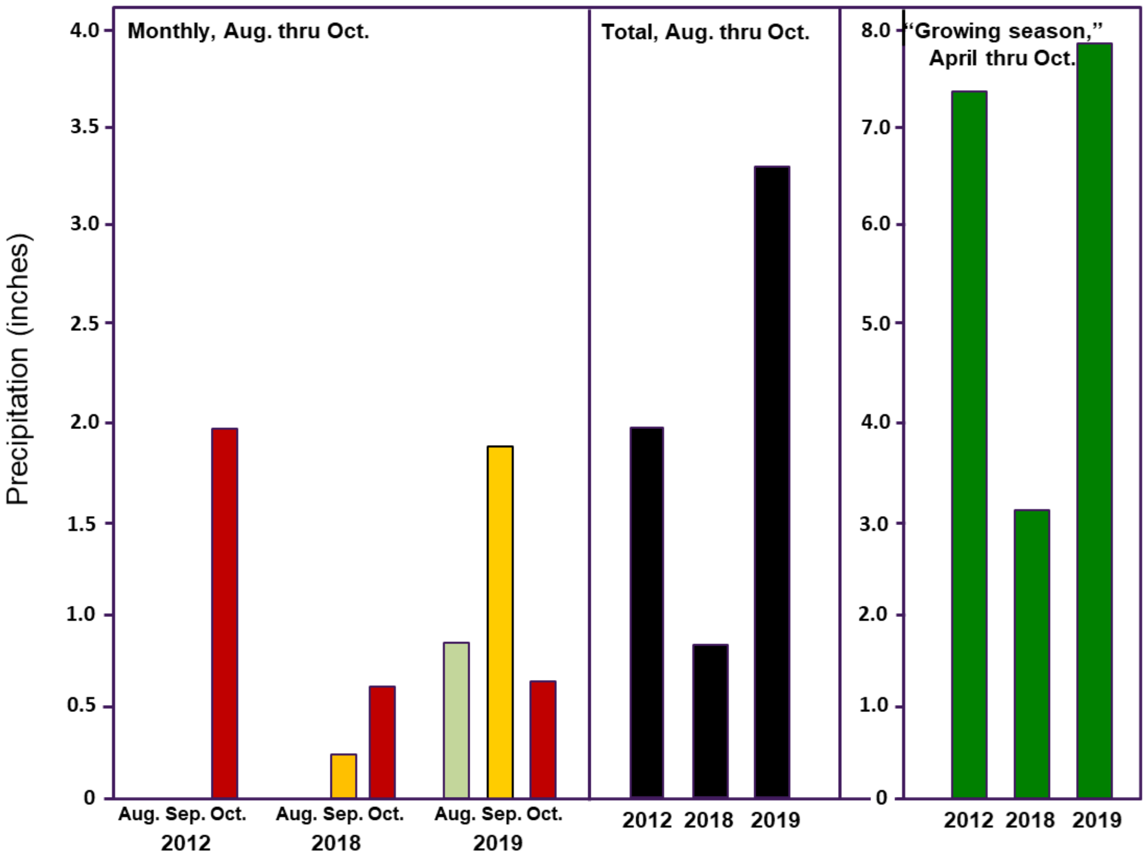


Figure 8. Precipitation during three years of data collection for algal abundance – 2012 (Hafele 2013), 2018, and 2019 (both Stillwater Sciences efforts): Left) monthly precipitation from August through October; Middle) total precipitation from August through October; and Right) total precipitation from April through October. Raw data were obtained from NOAA (2020).

Benthic algal abundance is influenced by short-term weather conditions, which is why sampling for representative average biomass (typically on a monthly basis; Biggs and Kilroy 2000) should not be conducted during a recovery period (1-2 weeks or more) after a moderate or more major rain event, as the high water can scour algal biomass from the system (Biggs and Close 1989). Abundance is also influenced by longer-term dry periods because, if not too severe, they allow for maximal biomass accrual with minimal washout/scouring by storms (Biggs 2000a,b; Lake 2000). *Worst-case conditions for noxious benthic algal [and SAV] growth in response to nutrient pollution occur during low-precipitation years*, when nutrients are more slowly carried downstream, discharged sewage effluent is a higher proportion of the flow and less diluted over longer periods, and substantial benthic algal biomass is able to accumulate. Such conditions also show the strongest relationships between benthic algal biomass and nutrient concentrations. Thus, these conditions offer the best opportunity to develop protective numeric criteria for minimizing noxious algal biomass because they enable consideration of river health (even) under the most ideal conditions for algal growth and strong response to nutrient supplies.

III.C.2.c. *Stillwater Sciences' Site-Specific TN and TP Targets for River Water Quality*

As expected based on the above explanation, in wetter year 2019, Stillwater Sciences' "seasonal" (August through November) analysis yielded positive but weak linear relationships between overall *average* benthic algal biomass as (total) chl*a* and average TN and TP concentrations (report, Figures 3-2 and 3-3). There were also very weak or no relationships between overall average cell number or biovolume versus overall average TN or TP (August and October: cell number, R^2 = 0.0413 to 0.2989; biovolume, R^2 = 0.0007 to 0.0821). (But note that the latter analyses "mixed apples and oranges," since cell number and biovolume were assessed only twice (August, October), whereas the TN and TP concentrations used were the overall averages per site from August through November.)

Linear relationships were much stronger for overall *maximum* benthic algal chl*a* versus both overall average TN and TP ((R^2 = 0.9793 and 0.7262, respectively, August through November). These relationships were used to estimate "site-specific" targets for the area affected by the Medford RWRF, assuming acceptable benthic algal biomass of maximum chl*a* less than 100 mg/m^2 (as recommended for rivers in general by Welch et al. 1988, Dodds et al. 1997, Suplee et al. 2012, etc.; Figure 9). Stillwater Sciences (2020) erred in applying its regression equations; the correct site-specific target values (mean, late summer-fall: 205 µg TN/L and 48 µg TP/L) are slightly higher than stated in the report (190 µg TN/L, 40 µg TP/L).

The Stillwater Sciences (2020) report did not include the 2018 data (October only – Hume 2019), or the findings from linear regression of maximum benthic algal chl*a* versus overall average TN or TP. The R^2 values likely would indicate even stronger positive relationships between maximum chl*a* and overall average TN and TP concentrations than in 2019, as there were also very strong positive relationships between cell number and biovolume versus both N and TP (Aug., Oct.: cell number, R^2 = 0.9895 for TN and 0.9451 for TP; biovolume, R^2 = 0.9186 for TN and 0.8608 for TP).

III.C.2.d. *The Rogue River 2019 Data Compared to Models of Other Rivers*

Stillwater Sciences' analysis indicated high sensitivity of the middle Rogue River to nutrient inputs in comparison to other rivers: that is, benthic algal growth per unit N or P is much higher in the Rogue River than in other rivers based on published models.

As the next step in its considerations, Stillwater Sciences (2020) attempted to fit the 2019 Rogue River data to several published models relating benthic algal chl*a* and TN or TP in other rivers. The comparison was somewhat confusing because the models focus on annual benthic chl*a*, whereas the Rogue River data were from summer/fall. In many rivers, benthic algal abundance is highest in colder periods (e.g., Sheath and Burkholder 1985, Everitt and Burkholder 1991). Unfortunately, mean annual benthic chl*a* data for the Rogue River in the study area are not available, so it is not possible to know how the summer-fall data compare to an annual mean.

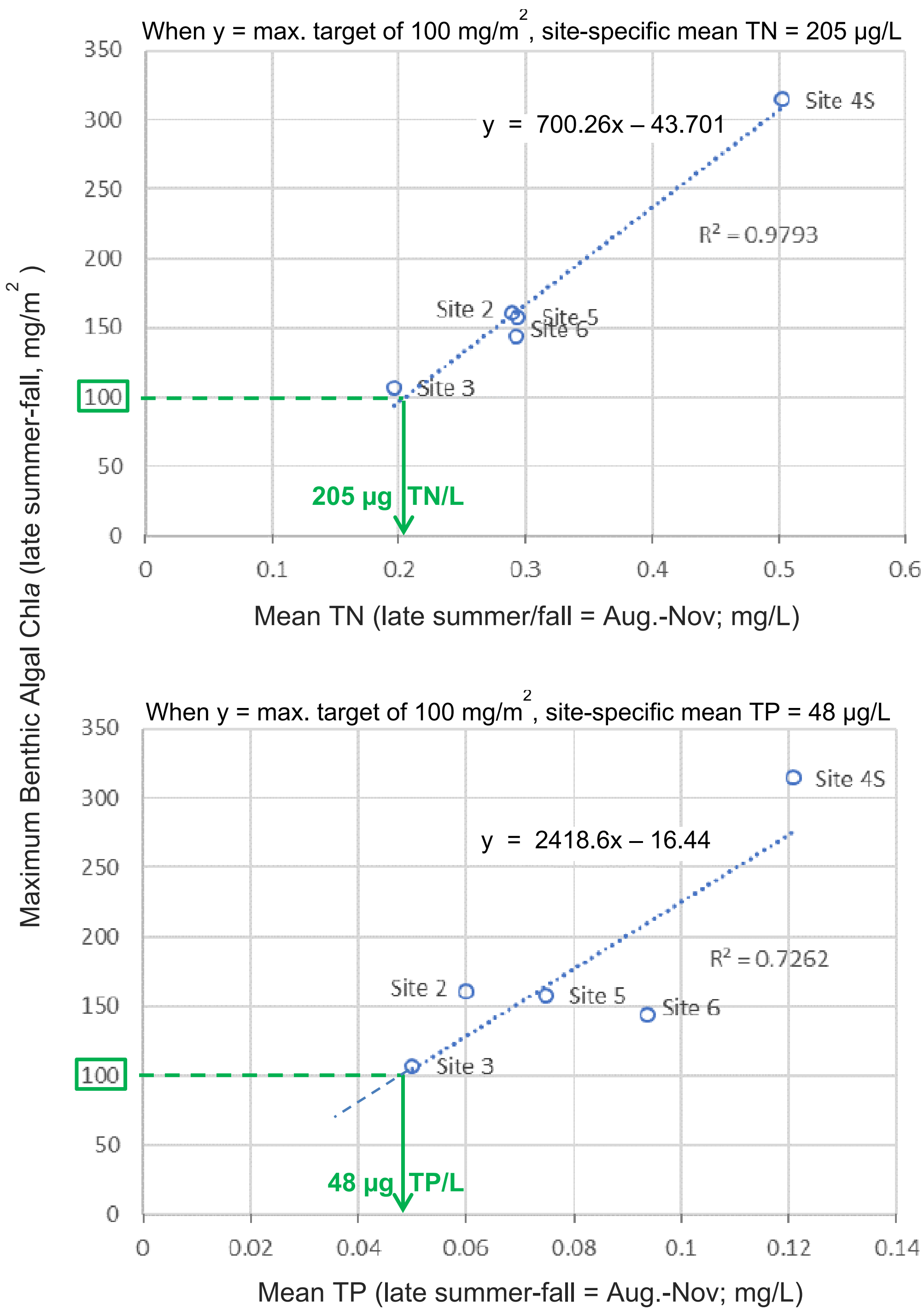


Figure 9. Site-specific targets for TN and TP concentrations near the Medford RWRF, based on linear regression analysis of the 2019 data (Stillwater Sciences (2020: maximum benthic algal chlorophyll *a*, uncorrected for pheopigments, versus mean TP from August through November). Targets are corrected from Stillwater Sciences (Figure 3-4). Note that these values, based on the equations developed by Stillwater Sciences, are slightly higher than those reported (Stillwater Sciences 2020, Figure 3-4: 190 µg TN/L and 40 µg TP/L).

Assuming that the comparison is valid, ***the findings indicate that the Rogue River system is moderately to highly sensitive to nutrient concentrations compared with the many other rivers described in the publications considered by Stillwater Sciences.*** For 4 of 6 Rogue River sampling sites, the predictions fall above all of the curves—that is, a small amount of TN results in much higher predicted benthic algal abundance in the Rogue River than in the other rivers (Figure 10, upper panel). The other two sites fall on or near the highest predicted relationship line (from Chételat et al. 1999), that is, the relationship predicting the highest benthic algal chlorophyll per unit TN (Figure 9). The Rogue River predictions by sampling site are more variable, with overall moderate to high sensitivity in comparison to the other rivers (sites falling to the left half of the graph except for Site 4S, most strongly affected by the Medford RWRF sewage effluent; Figure 10, lower panel). These findings fit the general profile of the Rogue River, historically oligotrophic although draining volcanic P-containing rock areas (Myer 2013 and references therein).

The relatively high sensitivity of the middle Rogue River to TN and TP inputs is a significant and expected finding from the literature comparison: It means that this river is extremely sensitive to nutrient pollution in comparison to a range of other rivers that have been analyzed in the published literature. Therefore, the effluent N and P nutrient limits need to be much lower than would be necessary in other rivers, since a very small amount of N or P results in much more algal growth than in the other, less sensitive rivers commonly studied and discussed in the science literature (e.g., Lohman et al. 1992; Chételat et al. 1999; Biggs 2000b; Dodds et al. 2002, 2006).

III.D. Suggested Effluent TN and TP Limits Much Too High to Protect the Rogue River

Despite an analysis showing that the affected Rogue River segment is highly sensitive to N and P pollution, Stillwater Sciences' suggested effluent limits were excessively high. Those effluent limits were based on a suite of errors and unsupported assumptions, "wet year" rather than low-flow conditions, and data from Corn Belt midwestern streams and other inappropriate waters.

III.D.1. *Use of Excessive "Background" RMZ-S Nutrient Levels for Calculations*

Stillwater Sciences (2020, p.50) acknowledged that nutrient thresholds (that is, its ***uncertain estimates*** for control site TN and TP concentrations) for the Rogue River near the area affected by the Medford RWRF were within the range of U.S. EPA-suggested 25th percentile concentrations from all streams data within the broader sub-ecoregion. The analysis from that point, however, de-emphasized control (upstream/effluent-unaffected) thresholds in deriving effluent nutrient limits:

- Stillwater Sciences (2020, pp.50-51) first asserted that benthic algae accumulated at the control sites despite relatively low nutrient levels, so that setting nutrient criteria based on matching upstream average conditions "may not necessarily reduce periodic periphyton accumulation…."

  As corrective information, total chl*a* data were *apparently* elevated at Sites 2, 3, and 4N in August (RMZ-N data were not available). Comparison of Figures 2-8 and 2-9 indicates, however, that the spurious AFDM data and the failure to correct for pheopigments in the total chl*a* data strongly influenced the questionably elevated chl*a* estimates. The more reliable biovolume and cell density estimates support this evaluation (Figures 2-11 and 2-12); those

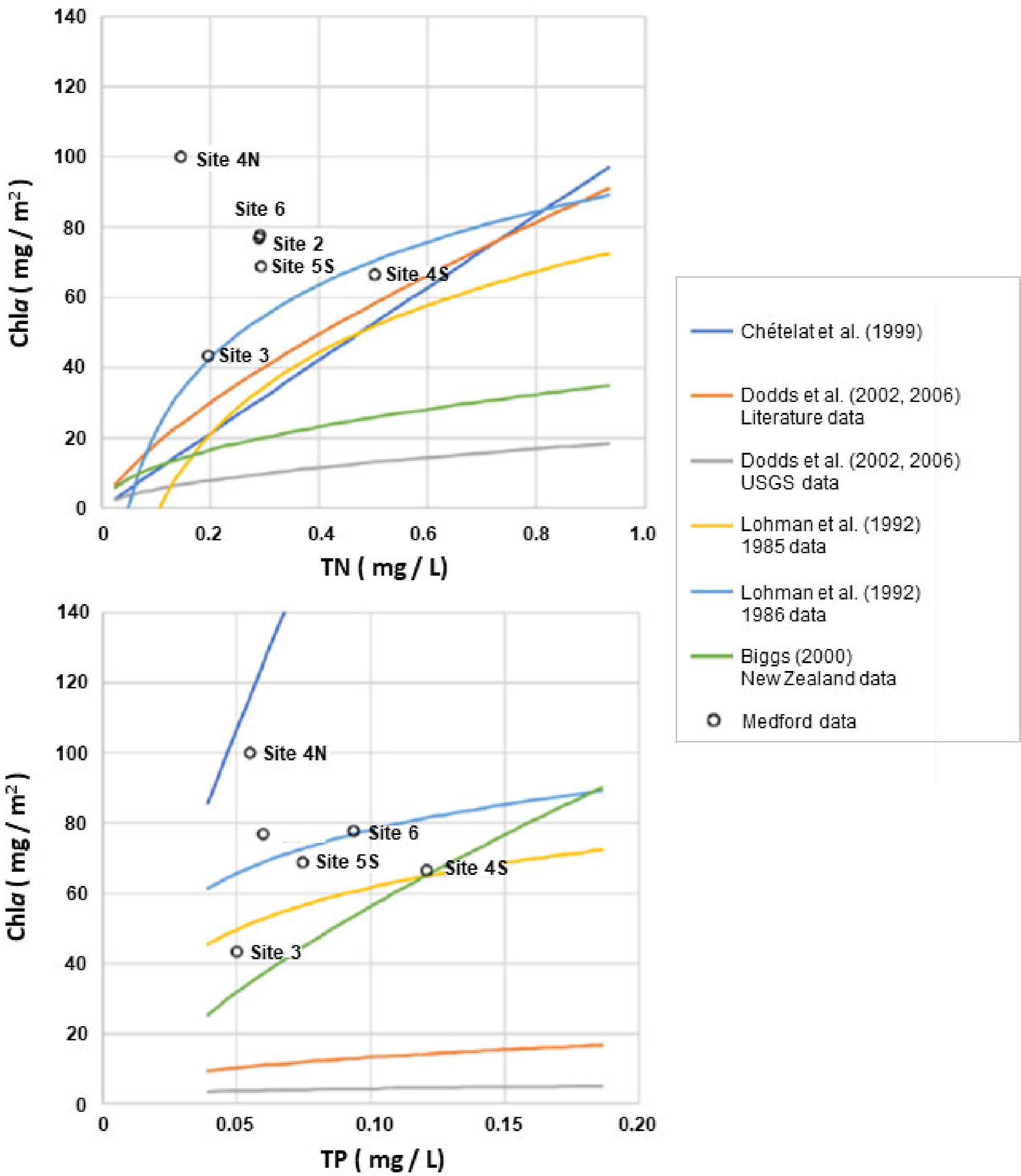


Figure 10. Comparison of "seasonal" data (August through November 2019) for benthic algal biomass as (total, uncorrected) chlorophyll a versus published models (various rivers) for observed and predicted mean annual benthic algal chlorophyll a, based on seasonal average TN or TP concentration (upper and lower panels, respectively). From Stillwater Sciences (2020, Figures 3-9 and 3-10, corrected to include the correct citations for Dodds et al.). Note that the Lohman et al. (1992) model , described by Stillwater Sciences as having best fit to the Rogue River 2019 data, was actually a poor fit for the TN and TP data at about half of the sites.

measures of benthic algal abundance (even including dead as well as live cells) were actually very low at the control sites in both August and October (see Figure 5 above).

- The range of nutrient thresholds then considered by Stillwater Sciences reflected the poor sampling design and errors described above (e.g., study period, lack of accurate data for critical threshold concentrations at all control sites, use of only wet year 2019 data, questionable interpretation of published literature, and errors in calculating site-specific threshold values for mean "seasonal" TN and TP concentrations).

  Compounding these problems was (i) an error in the data source, and (ii) highly inappropriate selection of the data used as the basis for the selected effluent TN level (see Table 6, Figure 11). The proposed effluent limitation for TN was based on a draft report, Miltner (2011, Figure 1), which included data from laboratory experiments, mesocosm studies, and field studies in the Corn Belt of the Midwest, eastern U.S., Europe, Texas, New Zealand, etc. The brief comparison in Table 6 shows estimated actual control or threshold conditions near the Medford RWRF, versus Stillwater Sciences' approach using Miltner (2011) for "background" conditions.

Table 6. Comparison of control / threshold values for the affected Rogue River segment, versus values considered and then selected by Stillwater Sciences (2020) as effluent TN and TP limits for the Medford RWRF.

Source	TN (mg/L)	TP (mg/L)
U.S. EPA 25th percentile of rivers in the ecoregion	**0.18**	**0.03**
Control or threshold - Rogue River segment (average of Sites 3, 2, 4N, and RMZ-N),	less than 0.19[a]	0.05
Miltner et al. (2011) - rivers	up to 3.26	up to 0.63
25th percentile of that compilation	0.40 (algae), 0.60 (invertebrates)	0.02 (algae), 0.03 (invertebrates)
Other reference considered (Dodds et al. 1998)[b] Oligotrophic / mesotrophic boundary (many rivers)	0.70	0.025
Stillwater Sciences' RMZ-S thresholds (background) Origin for TP: 60% reduction of effluent P	**0.40**	**0.100**
Effluent TN and TP (outfall)	**5.65** (87% as DIN)[c]	**1.35** (86% as $PO_4^{-3}P$)[c]

[a] Was poorly measured by Stillwater Sciences (see Section I of this white paper); instead, was estimated as an uncertain ("J-flagged") approximation in the report. Actual measurements for both dissolved inorganic forms NOx and ammonia were not available throughout the June - November study.

NOx – Total Kjeldahl N (TKN = organic N + NOx) values—that is, organic N values—were substituted and used for NOx instead. TKN values in minimally disturbed (control) river waters are usually much higher than NOx (e.g., U.S. EPA 2000a, Stanley and Maxted 2008). Thus, the uncertain values used for NOx likely were much higher than the actual NOx concentrations would have been.

DIN – The seasonal average for DIN (referred to by Stillwater Sciences as TIN, total inorganic N, not used in most science reports) was estimated, with high uncertainty, as the sum of the individual detection limits, that is, as 200 µg/L or 0.2 mg/L (see Section II, Table 5 of this white paper).

[b] Should not be confused with Dodds et al. (1997), the publication erroneously cited by Stillwater Sciences that does not contain the information.

[c] The forms of N and P that are highly bioavailable to alga and SAV are dissolved inorganic N (DIN = nitrate or NOx + ammonia), and phosphate ($PO_4^{-3}P$, also called ortho-P or soluble reactive phosphorus, SRP) (Wetzel 2001, Dodds 2002). The Stillwater Sciences recommendations call for the Medford RWRF effluent to contain 4.91 mg DIN/L and 1.16 mg PO_4^{-3}-P/L.

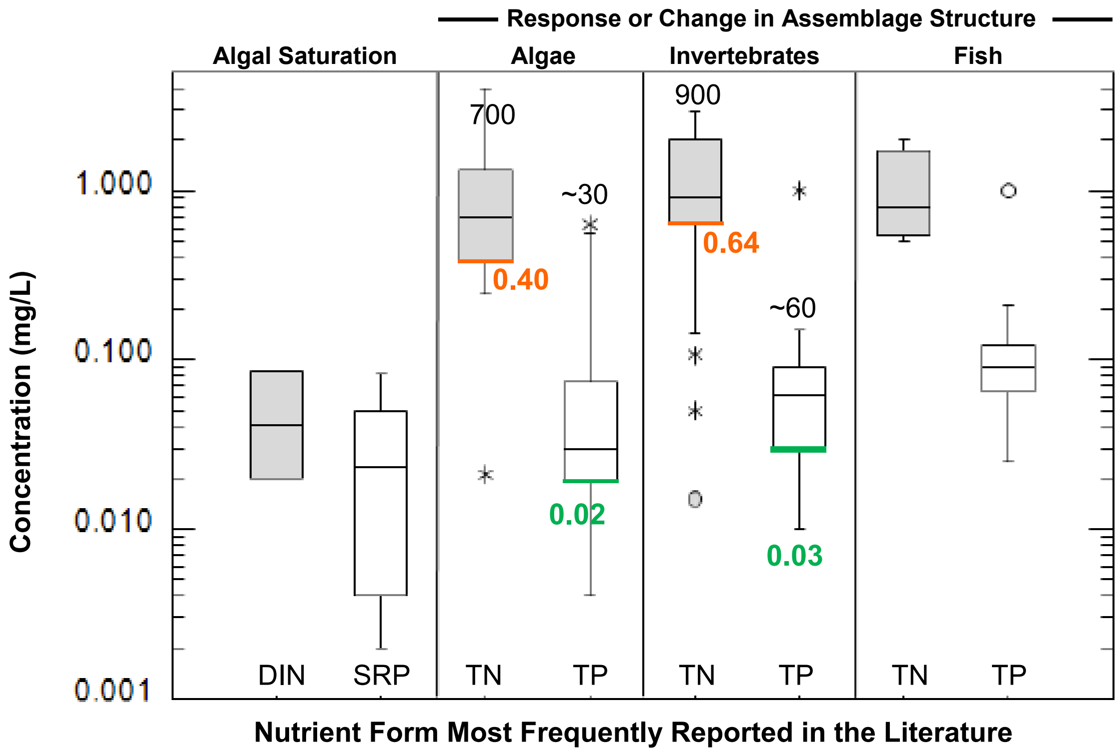


Figure 11. The figure from Miltner (2011), used by Stillwater Sciences to derive its"25th percentile" background values for TN (orange), but not TP (green), at RMZ-S in its Scenarios 5 and 6. These percentiles were based on all compiled data from laboratory, mesocosm, and field experiments (1985-) assessing algal or invertebrate response to N and P across the world, including corn belt, Appalachian, eastern U.S., European, New Zealand, and other streams, Values in black are medians.

Stillwater Sciences (2020, p.49) noted that Miltner (2011) draft report was compiled to support development of numeric nutrient water quality standards for rivers in Ohio, a state with surface waters that are heavily influenced by corn belt agriculture and confined animal feed operations. The TN concentration for Stillwater Sciences' overall recommended effluent TN limit (0.4 mg TN/L at Site RMZ-S), was taken from that compilation. The corresponding TP concentrations in Miltner (2011), 0.02 to 0.03 mg TP/L (Table 6, Figure 11), however, were regarded by Stillwater Sciences as too low to be "feasible" for the RMZ-S "acceptable background" or threshold TP concentration. Concentrations suggested by Dodds et al. (1998) for the boundary between oligotrophic (nutrient-poor) and mesotrophic (moderately nutrient- enriched) rivers were also rejected by Stillwater Sciences as too low for TP. The rationale given was that the resulting RMZ-S estimated values would have been lower than Control Site conditions, although that was incorrect (see Section III.C.2.c).

Instead, more than 3-fold higher TP value than EPA's recommendation for minimally impacted waters in the area, and double the average TP value for control sites, was selected as Stillwater Sciences' recommended background or threshold TP concentration at Site RMZ-S (Table 6). The value selected by Stillwater Sciences as "acceptable" for RMZ-S TP, critical to the calculations for effluent limits (below) was not based on peer-reviewed science. It was not based on any "background" or "control" upstream information. It was, however, identical to the TP concentration associated with Little Butte Creek, an impaired stream added to the state's 303(d) list by the U.S. EPA due to phosphorus pollution. It also "matched" the number calculated by Stillwater Sciences for a 40% reduction in effluent TP content (Stillwater Sciences' Table 3-2, "40% reduction of Avg. at Site RMZ-S"). Such a reduction is much less than needed, and much less than can be achieved by the City of Medford (below). Moreover, further scrutiny of the Stillwater Sciences (2020) report indicated to us that, although this RMZ-S information was presented prior to the discussion about suggested effluent limits, Stillwater Sciences (2020) picked effluent limits and a low dilution factor first, and then picked RMZ-S values to fit in equation 2 (below).

III.D.2. *Erroneous Calculations, A "Major Disconnect," and Poor Assumptions*

Stillwater Sciences' calculation of TN and TP effluent limits used flow conditions much higher than low flow, a "created" dilution number, and a suite of unsupported assumptions, resulting in excessive effluent nutrient limits that will fail to protect the designated uses of the affected waters for fish and aquatic life.

III.D.2.a. *Dilution Factor and Effluent Limits Apparently "Selected" First*

The ODEQ specifies that mixing zone and water quality analyses must reference critical ambient and effluent conditions to ensure that impacts to receiving waters are assessed conservatively (protectively, that is, considering "worst case" pollutant impacts). See OAR 340-041-0053(2)(b)(B) (prohibiting point sources with an allowed mixing zone from causing or significantly contributing to exceedances of water quality standards outside the mixing zone "under normal annual low flow conditions," used as the 7Q10 which is the lowest seven-day average flow that occurs, on average, once every ten years).

The dilution factor should be calculated from actual 7Q10 low-flow conditions, which must be used to identify future effluent TN and TP limits that will protect the designated uses of this river segment. Accurate calculations shown below yielded much higher dilution factors than Stillwater Sciences' dilution factor from the average flow and average conductivity data (July – November 2019) Stillwater Sciences claimed to have used.

Based on the information Stillwater Sciences provided, Stillwater Sciences simply picked a low dilution number to "fit" excessive TN and TP concentrations for the effluent and RMZ-S that Stillwater Sciences had already chosen. By picking a low dilution factor, Stillwater Sciences conveyed the tacit, false message that downstream effects from the effluent can be remedied by less nutrient reduction than is actually required. In reality, a downstream effect that occurs in higher river flow requires greater nutrient reduction.

In effluent discharge, for a given concentration of pollutant that can have known downstream impacts, calculating the dilution factor accurately (that is, not "pretending" that the river can dilute, and thereby safely handle, more pollution than it actually can) is essential in order to protect against continued degradation both near the legally allowed mixing zone and farther downstream.

During *low-flow periods (associated with low dilution factors)*—for example, between rainstorms—a river has lower volume and is less able to dilute incoming pollution. As a result, pollutant concentrations remain relatively high for longer, and impacts are worse. That is why low-flow periods are often referred to as "worst case," that is, worst conditions, when pollutant effects are most severe. Noxious algal blooms typically develop when the water level is relatively low, the flow slows down, scouring effects are less, and the nutrient supplies (N and P concentrations) in the incoming sewage effluent remain high for longer because there is not as much river water to dilute them and wash them downstream. In contrast, during high-flow periods, the river has more volume and its dilution factor is higher; it can dilute the pollution more so that its impacts are less severe.

The only detailed study available on flow (discharge) in the segment of the Rogue River near the Medford RWRF effluent outfall was conducted on a day when flow was almost double the lowest flow often considered in calculating the dilution factor—the 7Q10 (Figures 7 and 12). The river width along the downstream transect averaged 229 feet, but the plume spread across only ~75% of the river width; complete mixing did not occur until the plume had traveled about two miles downstream (Brown and Caldwell 2014; note that Stillwater Sciences 2020, Table 3-3, considered Site 4N as a "control" site reflecting the inference that the plume does not reach the north shore, and cited Brown and Caldwell 2014 in support). The mixing situation would be expected to be worse (slower, with less dilution for longer) during low-flow conditions, which must be used to develop effluent limits that protect the designated uses of this river segment for fish and

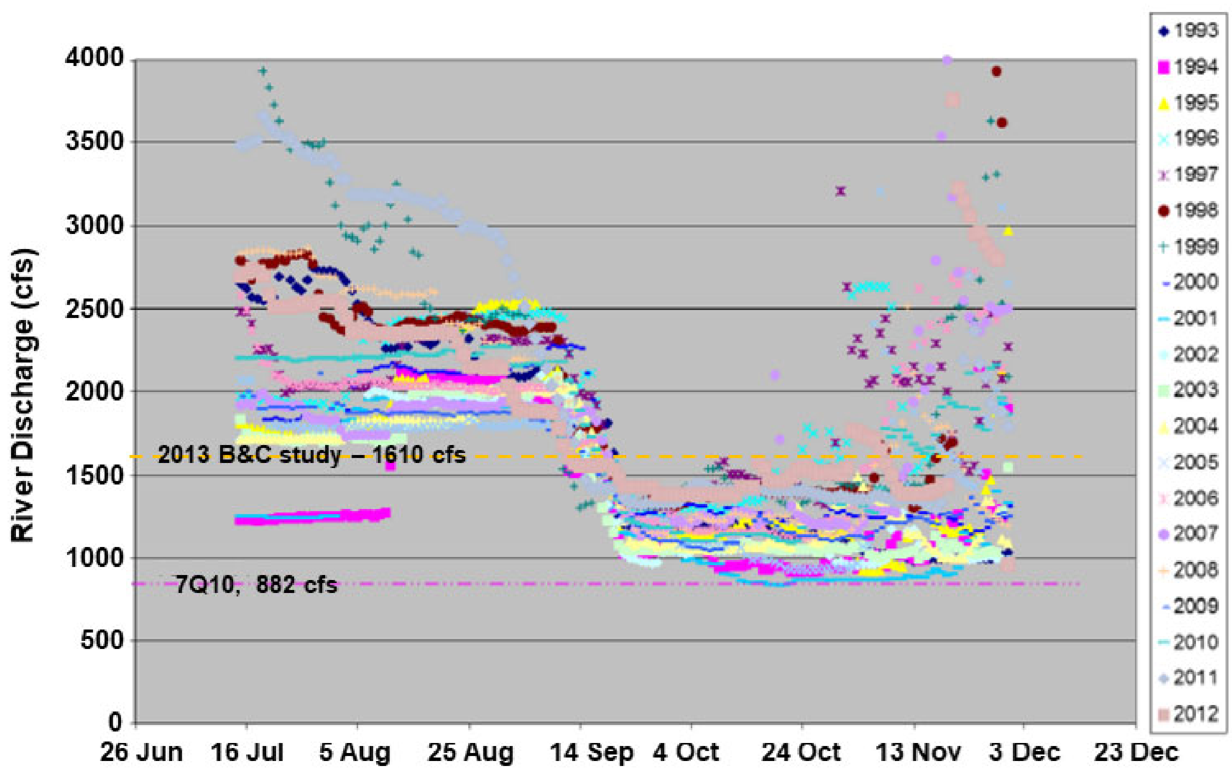


Figure 12. Rogue River discharge downstream from the RWRF outfall from 1993 through 2012 . From Brown and Caldwell (2014).

aquatic life. As only ~75% of the river flow (based on river width and Brown and Caldwell 2014) would have diluted the effluent, 75% of the low flow condition represented by the 7Q10 should have been considered in order to strengthen protective effluent limits for affected areas downstream from the RMZ. Alternatively, if the City of Medford believes that 100% of the river flow near the outflow should be used for dilution calculations, another mixing zone study should be required to support that view.

Stillwater Sciences (2020, p.53) asserted that it used "seasonally averaged" flow (June through November) from wet year 2019, to calculate what should have been an excessive river dilution factor for estimating effluent TN and TP limits. Yet, Stillwater Sciences' dilution factor was suspiciously low (Table 7). **We uncovered a disconnect** between the "average seasonal flows" that Stillwater Sciences claimed to have used in estimating the dilution factor, and the conductivity data that Stillwater Sciences also claimed to have used, versus the purported dilution factor of 25 (claimed to have been approximated from 26.4) that actually was used in Stillwater Sciences' calculations.

Stillwater Sciences (2020, p.53) described the dilution factor as being possible to estimate using two different equations, but only the first of these uses the 7Q10:

$$\text{Dilution} = \frac{Q_{UPSTREAM}}{Q_{OUTFALL}} \quad \text{or} \quad \text{Dilution} = \frac{(C_{RMZ} - C_{OUTFALL})}{(C_{UPSTREAM} - C_{RMZ})}$$

According to USGS data, the average "seasonal" flow from June through November 2019 was 1,655 cfs (Table 7). A range of outfall discharges was considered in checking Stillwater Sciences' calculations, from Brown and Caldwell (2014) which reported that in only one day (October 17, 2013), outflow discharge ranged from 15.8 to 22.3 mgd (24.4 to 34.5 cfs), averaging 18.2 mgd (28.2 cfs). Using these discharge data from wet year 2019 in the first equation above and the average outfall discharge from Brown and Caldwell (2014),

$$\text{dilution factor} = 1{,}655 \text{ cfs} / 28.2 \text{ cfs} = 59$$

Using the conductivity data that Stillwater Sciences claimed to have used,

$$\text{dilution factor} = \frac{(80.8\ \mu\text{mhos/cm} - 555\ \mu\text{mhos/cm})}{(69.2\ \mu\text{mhos/cm} - 80.8\ \mu\text{mhos/cm})} = 41$$

The numbers do not match, which may mean that Stillwater Sciences did not use average flow data for July-November 2019 (its numbers were not provided). What is clear is that both dilution factors are much higher than the dilution factor of 25 that Stillwater Sciences used (Table 7).

Stillwater Sciences (2020, p.53) wrote,

> 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 [µS]/cm [µS/cm] at sites upstream of the RWRF outfall, at the RMZ-S site, and at the RWRF outfall, respectively. Using Equation 1 above [dilution factor = discharge upstream divided by outfall discharge], the seasonally averaged dilution for the Medford RWRF outfall is 26.4….

Table 7. Dilution factors (D.F.s) calculated for various discharge conditions in the Rogue River. Highlighted in bold is "wet year 2019 average, July through November).

Condition	Discharge (cfs)	Dilution Factor (D.F.) = $Q_{upstream}$ / $Q_{outfall}$ min.	avg.	max.	Effluent conc.from flow data, avg. D.F.*, and Stil.Sci.' excessive RMZ-S concs.**
Discharge - Rogue River + Little Butte Creek					
7Q10	882	36.1	31.3	25.6	6.97 mg TN/L, 1.67 mg TP/L
30Q5	998	40.9	35.4	28.9	7.83 mg TN/L, 1.87 mg TP/L
75% of 7Q10 [reflecting RMZ coverage in the river]	662	27.1	23.5	19.2	5.34 mg TN/L, 1.28 mg TP/L
Brown & Caldwell (2014; 1 day, mid-Oct. 2013: calculated, vs. average transect measurement	1,437 calc.; 1,600 avg. trans. meas.	----	56.7	----	12.31 mg TN/L, 2.94 mg TP/L
wet year 2019 avg. (July - Nov)	**1,655**	48.0	**58.7**	67.8	**12.73 mg TN/L, 3.04 mg TP/L**
as above (asserted use of this avg.)	1,655	Stillwater Sci.'s D.F.: **25** [26.4]			
2019 minimum daily (July - Nov.)	1,322	54.2	46.9	38.3	10.25 mg TN/L, 2.45 mg TP/L
Outfall (15.8 to 22.3 mgd, 1 day in Oct. 2013 - B&C)					
minimum (15.8 mgd)	24.4				
average (18.2 mgd)	28.2				
maximum (22.3 mgd)	34.5				
Rogue River Conductivity Data (2019, Jun - Nov)	**Conductivity (µS/cm)**	**D.F. = ($C_{RMZ\text{-}S}$ - $C_{OUTFALL}$) / ($C_{UPSTREAM}$ - $C_{RMZ\text{-}S}$)**			-----
upstream (measured)	69.2	**40.9**	vs. Stillwater Sci.'s D.F.: **25** [26.4] (yet asserted use of these data)		
RMZ-S (measured)	80.8				
Effluent at outfall (measured)	555				

* Avg. D.F. based on the range of outflow discharge numbers in Brown and Caldwell (B&C, 2014).

* Stillwater Sciences (2020) RMZ-S "threshold or background" concentrations: 0.4 mg TN/L, 0.1 mg TP/L; its suggested effluent limits: 5.65 mg TN/L, 1.35 mg TP/L.

Note that the calculations reflected above used the same values for "control" background as Stillwater Sciences (2020: 0.19 mg TN/L and 0.04 mg TP/L, from linear regression of Aug.-Nov. 2019 maximal benthic algal biomass as total chlorophyll *a* versus average TN or TP concentration) for comparison with Stillwater Sciences' asserted D.F. calculations, although the corrected values for control background should be 0.205 mg TN/L and 0.048 mg TP/L (see Figure 12 of this rebuttal report).

Thus, Stillwater Sciences described, ***as its initial step***, having used (USGS) discharge data for the 2019 study period (July through November) to calculate a "seasonally averaged dilution [factor] for the Medford RWRF outfall." In fact, it could not have (Table 7, Figure 13). Stillwater Sciences also described using specific conductivity measurements, which would have been applied to its equation 2 (second equation on Stillwater Sciences, 2020, p.52, repeated above, for calculating a dilution factor)—but it could not have.

So, how then did Stillwater Sciences derive the low dilution factor of 25, during the wet year of 2019? Stillwater Sciences (2020) provided none of its actual calculations, specific flow data used, etc. Lacking that information, and based on the specific information and numbers that report *did* provide, Stillwater Sciences first set the dilution factor to 25, approximating the dilution factor for this river segment under low flow conditions, which is well-known information (e.g., in Brown and Caldwell 2014; and see Table 7); and then "worked backward" using target effluent TP and TN numbers that had already been chosen (5.65 mg TN/L and 1.35 mg TP/L, approximating treatment level scenario

> 4 from a "companion report" to the Stillwater Sciences (2020) report, by West Yost Associates 2020—5.5 mg TN/L and less than 1 mg TP/L).

Those numbers were used to solve for the RMZ-S concentrations so that the equation for TN was resolved as:

$$\frac{\text{(RMZ-S conc. 0.4 mg TN/L – effluent conc. 5.65 mg TN/L)}}{\text{(upstream conc. 0.19 mg TN/L – RMZ-S conc. 0.4 mg TN/L)}} = 25$$

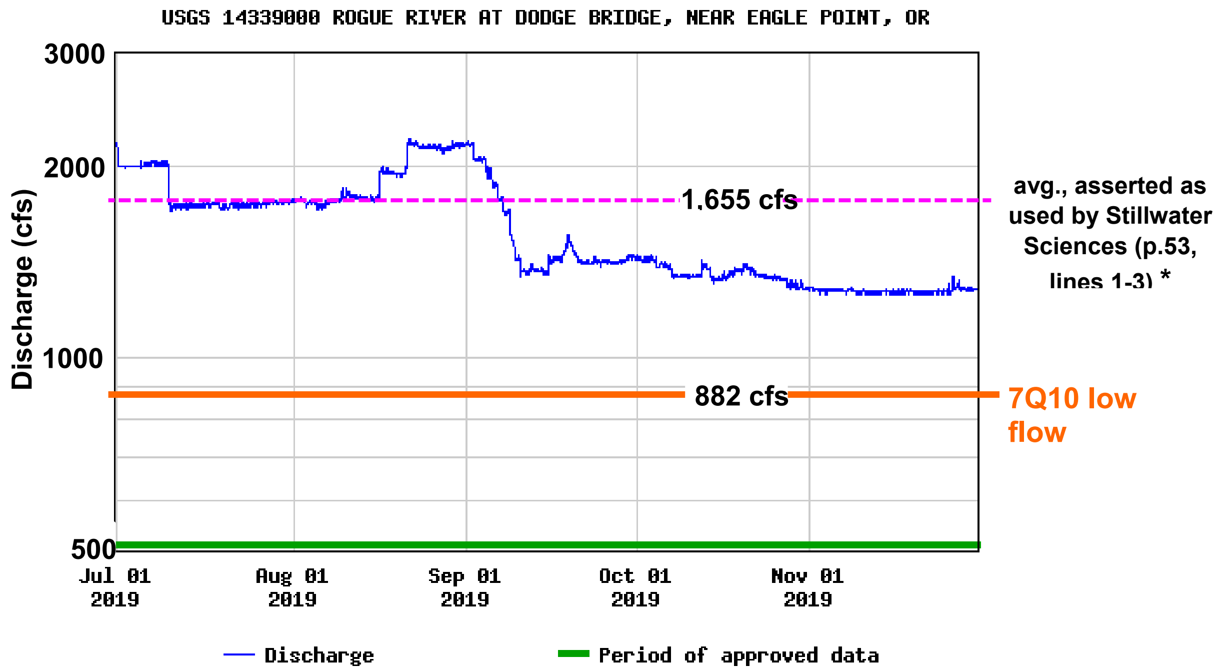


Figure 13. Rogue River discharge upstream from the Medford RWRF during July through November 2019. *Note that, while the graph does not include the relatively small amount of additional flow from Little Butte Creek, the discharge values would only be increased by ~3% by the flow from that tributary (see Brown and Caldwell 2014). Also note that the 7Q10 flow and the July 1 through November 30 2019 average flow values reflect the tributary flow input. USGS data and graphics available at https://nwis.waterdata.usgs.gov/nwis/uv?cb_00060=on&format=gif&site_no=14339000&period=&begin_date=2019-07-01&end_date=2019-11-30 .

This equation demonstrates how Stillwater Sciences "derived" the dilution factor of 25 using excessive background concentrations of TN. It also demonstrates that this "selected" dilution factor—intended for the purpose of demonstrating the actual dilution taking place in the Rogue River—was not an accurate description but, rather, a means to achieve the desired outcome.

Of the two nutrients, the more problematic for matching Stillwater Sciences' desired outcome in equation 2 was phosphorus. Thus, Stillwater Sciences used the high TN level of 0.4 mg/L from Miltner (2011), but rejected the level given for TP (0.02 to 0.03 mg/L – Figure 11) as "too low" — despite the fact that 0.03 mg/L matched the U.S. EPA sub-ecoregion value of 0.03 mg TP/L and was close to Stillwater Sciences' "site-specific" value of 0.04 (actually 0.048) mg TP/L from its own linear regression analysis of data from the Rogue River segment. The derived effluent limit for TN was only a little higher than for treatment scenario 4 in West Yost Associates (2020), but the TP limit selected by Stillwater Sciences still ended up being substantially higher than the "less than 1 mg/L" (West Yost Associates 2020, p.3) for TP in order to "fit" the equation with the already-"selected" effluent TN concentration and dilution factor. The final equation was resolved as:

$$\frac{\text{(RMZ-S conc. 0.1 mg TP/L – effluent conc. 1.35 mg TP)}}{\text{(upstream conc. 0.05 mg TP/L – RMZ-S conc. 0.1 mg TP/L)}} = 25$$

This equation again shows, this time for TP, how Stillwater Sciences "derived" the dilution factor of 25 using excessive background concentrations. Again, this purported dilution factor of 25—intended for the purpose of demonstrating the actual dilution taking place in the Rogue River—was not an accurate description but, rather, a means to achieve the desired outcome.

It is important to note at this point that *all of the treatment scenarios evaluated by West Yost Associates (2020), including scenario 4, were inadequate* to protect the designated uses of the Rogue River from degradation due to excessive Medford RWRF effluent TN and TP concentrations. Improved techniques for biological nutrient removal (BNR) are available that were not considered, cost-effective techniques that can reduce TN and TP to much lower levels than the scenarios evaluated (U.S. EPA 2007a,b).

In summary, what Stillwater Sciences (2020) did—picking a dilution factor that purports to describe what takes place in the river—is not the same as identifying the dilution factor actually calculated from the 7Q10 which must be used to identify future effluent TN and TP limits that will protect the designated uses of this river segment. Stillwater Sciences mistakenly claimed that equation 1 yielded a dilution coefficient of 26.4 ("rounded" to 25) through use of average flow from July-November 2019, and from average conductivity for that period. Accurate calculations (Table 7), however, yielded much higher actual dilution factors from those data. *By picking a low dilution factor, Stillwater Sciences conveyed the tacit but false message that downstream effects from the effluent can be remedied by less nutrient reduction than is actually required. In reality, a downstream effect that occurs in higher river flow requires greater nutrient reduction.*

III.D.2.b. *Unsupported Assumptions*

Stillwater Sciences then made a series of incorrect assumptions:

- Stillwater Sciences falsely assumed that it could not use control (threshold upstream) concentrations as the target water quality conditions for Site RMZ-S because the site-specific relationships developed from linear regression analysis produced estimates of RMZ-S targets (Figure 9: 205 µg TN/L, 48 µg TP/L) that were less than the average upstream control or threshold concentrations (Table 6: ~190 µg TN, 50 µg TP/L). As shown here, *these concentrations are comparable and, therefore, achievable.*

- Because the excessive reporting limits in Stillwater Sciences' water quality analyses prevented *any* accurate measurements for inorganic N concentrations in the critically important Control Sites, those concentrations were *uncertain estimates* as explained in Section III. Control ammonia and NOx were each assumed to be "at least one half" of the inappropriately high detection limits used by Stillwater Sciences, although those forms of inorganic N could have been much less. There was no apparent effort to assess what these values should have been based on actual data from other studies. The phosphate ($PO_4^{-3}P$) data for the Control Sites were also "J-flagged" as *uncertain* (see Table 5 and footnotes). Using those already uncertain numbers and the 2019 WQ data, Stillwater Sciences built upon the uncertainty by *further estimating* that ~78% of the TN and 38% of the TP in the river upstream from the Medford RWRF was organic N and particulate P, respectively.

The proportion estimated for particulate P at control sites was much too low, especially considering that 2019 was a wet year (when a high proportion of particulates would have been carried into the river by stormwater) and based on many peer-reviewed studies, since upstream from the Medford RWRF the major influence on nutrient levels is nonpoint from agriculture and other sources. Most of the phosphorus in the TP measurement (90% or more) from those sources is particulate (Mainstone and Parr 2002, Jarvie et al. 2006, Withers and Jarvie 2008, Neal et al. 2010, Millier and Hooda 2011).

- Stillwater Sciences used the wet year 2019 average concentration data from July through November to assess the contributions of inorganic N and P ($PO_4^{-3}P$) to TN and TP measurements at RMZ-S (67% DIN and 52% TP, respectively). This step seriously compounded the lack of protection from its suggested effluent limits that resulted from its failure to use low-flow conditions. The effluent has very high proportions of inorganic N and P (86-87% of the TN and TP; Table 6). Within a few hundred feet, these proportions had dropped to only two-thirds of the total for inorganic N, and only about half of the total for inorganic P. During low flow conditions, without such high dilution, the proportions of highly bio-available N and P forms that reached RMZ-S would have been expected to be much higher.

In summary, the above steps in Stillwater Sciences' analysis led to effluent TN and TP limits that are much too high to achieve compliance with Oregon's biocriteria standard and protect the designated uses of the affected Rogue River segment for fish and aquatic life:

- ✓ Rejection of the threshold (background) concentrations indicated from the site-specific relationships as "infeasible" wrongly supported Stillwater Sciences' use of much higher TN and TP levels as "acceptable background" for RMZ-S and, in turn, much higher suggested effluent nutrient levels (Table 6).
- ✓ Overestimating "Control" (background) Sites inorganic N (ammonia, NOx) artificially decreased the impact from the Medford RWRF effluent downstream by creating "higher" background bioavailable N.
- ✓ Underestimating the contribution of particulate P to Control Sites TP wrongly inflated the proportion of highly bioavailable phosphate in the Control Sites, artificially decreasing the impact from the Medford RWRF effluent downstream by creating "higher" background bioavailable P.
- ✓ Use of wet year 2019 data resulted in artificially lower proportions of bioavailable forms of N and P at RMZ-S than if the data had been taken under low-flow conditions. This point is ironic considering that Stillwater Sciences' asserted emphasis was to reduce inorganic N and P contributions from the Medford RWRF as the primary control strategy for reducing benthic alga[l] accrual. Stillwater Sciences' actions accomplished the opposite of that professed goal.
- ✓ Use of a dilution factor that was not based on average seasonal flow or low flow (June – Nov 2019), or on average conductivity data but, rather, appears to have been "picked" first along with effluent TN and TP levels.
- ✓ Numbers that "don't add up" (Table 7) – effluent TN and TP concentrations much higher than those suggested by Stillwater Sciences were obtained when the dilution factors was calculated

using the average flow data or conductivity data from July through November 2019—yet those were the data claimed to have been used by Stillwater Sciences (2020, p.53) to calculate its dilution factor.

III.D.3. *Overall Findings Without Scientific Basis*

Numerous other assertions without scientific basis, poor rationale, and non-protective recommendations characterized the overall recommendations and conclusions (Stillwater Sciences 2020, pp. 55-59).

III.D.3.a. *Nonsensical Assertion That Vagaries in Flow Mainly Cause the High Algal/SAV Biomass Downstream*

Stillwater Sciences (2020, p.56) aptly noted that stream velocity is an important factor influencing benthic algal and SAV growth and accumulation, but then made the following illogical leap:

> For this reason, local patterns in velocity distribution as well as the frequency of high flow events may explain the relative amounts of periphyton and SAV biomass at locations upstream and dowsntream [downstream] of the RWRF.

In reality, previous studies described in Section II—conducted by a scientist, an engineering firm, ODEQ, and Stillwater Sciences itself (Hume 2019)—all concluded that the high nutrient inputs from the Medford RWRF are causing or contributing to the high algal/SAV biomass downstream from the outfall. Differences in flow above versus below the outfall are minimal except for the turbulence created by the outfall itself. The "elephant in the room" is not flow but, rather, the extreme nutrient contamination added by the Medford RWRF.

III.D.3.b. *Unsupported Assurance That the Suggested Excessive TN Targets Will Not Cause Adverse Impacts*

Stillwater Sciences (2020, p.55) stated, vaguely and without scientific basis, that although the excessive target TN and TP concentrations selected for Site RMZ-S "still exceeded"—actually, were twice as high as—upstream concentrations, they "will likely not result in detrimental changes in the resident biological community as indicated by metrics used in this and previous studies." The specific meaning of that sentence is unclear, but in reality, the excessive concentrations suggested for both effluent limits and RMZ-S will continue to cause violations of the biocriteria standard (see Section IV).

Stillwater Sciences (2020, p.55) acknowledged that its suggested TN targets exceeded the concentrations needed to maintain benthic algal biomass below its own target (p.34) of less than 100 mg chl*a*/m^2. Its purported rationale was that the excessive TN targets were within (actually, equal to) the 25th percentile of data (low effects range) for shifts in benthic algae as identified by Miltner (2011), the draft compilation study of rivers ranging from oligotrophic to highly nutrient-polluted—and lower than the 25th percentile of data associated with shifts in benthic macroinvertebrates from that compilation. Although these percentiles have nothing to do with the middle Rogue River, and despite the fact that Stillwater Sciences' (2020) analysis of the Rogue River compared to various other modeled rivers showed that the Rogue appears to be much more sensitive to nutrient pollution than most other rivers, Stillwater Sciences (2020, p.53) falsely asserted that its use of 25th percentile numbers for TN from Miltner (2011) would "ensure that the [Medford] RWRF does not contribute to exceedances of the State of Oregon biocriteria standard." That assertion is not based on science.

III.D.3.c. *False Assertion That the Suggested Excessive Effluent Limits Will Decrease* Cladophora

Stillwater Sciences (2020, p.56) stated that its suggested effluent limits "may be expected to reduce the dominance of green algae (e.g., *Cladophora*) relative to diatoms" in areas downstream from the Medford RWRF. Two references were cited in support of this statement. The first, Sosiak (2002), was not in the reference list. The only "Sosiak (2002)" reference we could find is listed in the References Section of our report. It did not indicate that concentrations as high as Stillwater Sciences' suggested effluent nutrient limits would decrease *Cladophora* relative to diatoms. The second reference cited did not support Stillwater Sciences' statement either; instead, it described *Cladophora*, once established, as continuing to thrive in very low concentrations of TP and SRP, five-fold or lower than Stillwater Sciences' suggested TP target at RMZ-S (Suplee et al. 2008, p.39; Suplee et al. 2012).

III.D.3.d. *Nitrogen Limitation Wrongly Invoked to Justify Suggested Excessive P Targets*

The recommended TP target at RMZ-S was also acknowledged (Stillwater Sciences 2020, pp.55-56) to be [much] higher than the 25th percentile of data in Miltner (2011) literature compilation. Stillwater Sciences cited two studies that did not rigorously assess the potential for nutrient limitation (Stillwater Sciences 2019 which is the same as Hume 2019 in Section I of this report, and Brown and Caldwell 2014) to make the mistaken assertion that "the Rogue River is generally nitrogen limited in the vicinity of the RWRF…, *making phosphorus reductions potentially unnecessary."* [emphasis added]

That statement shows a fundamental lack of scientific understanding about both nutrient limitation and the noxious high-P-optima macroalga, *Cladophora*, which has taken over and become the dominant benthic alga downstream from the Medford RWRF (see Figure 5 above, Stillwater Sciences' data). The extreme P contamination in that area, relative to background (minimally impacted or reference) conditions in the river historically, are matched by extremely high N contamination. Nutrient ratios historically were used to infer limitation of N or P of natural algal and SAV assemblages in nutrient-poor waters (Burkholder and Glibert 2013). Attempting to use nutrient ratios to determine whether N or P is limiting algal growth when both N and P supplies are excessive is flawed, as shown by this analogy: A person goes to a restaurant for a steak dinner. The waiter apologetically says that supplies are limited: only 140 steaks and 50 potatoes are available. Which one, steaks or potatoes, will the customer run out of first? The obvious answer is, neither; the supplies of both are much too high, as no one can consume (the smaller number of) 50 potatoes at dinner. Although this statement may seem obvious, nutrient ratios are often incorrectly: ***N:P ratios can only be used to infer limitation when the supply of N or P is actually limiting*** (see Section IVA.2).

The nutrient conditions downstream from the Medford RWRF are **saturating**. Studies repeatedly have shown that at limiting N and P concentrations, algae show a linear response to increasing nutrient inputs up to a threshold point when the algae become nutrient-saturated (Munn et al. 2018 and references therein; Figure 14). Above those N and P threshold concentrations, there is no further response to nutrient inputs. Once saturation is reached, as in the affected Rogue River segment, benthic algal biomass will not noticeably decrease in response to nutrient reductions until the N and P are pushed back to limiting levels for algal growth.

The Medford RWRF has been discharging partially treated sewage into the affected segment of the Rogue River for decades. The benthic algae in the affected segment shifted long ago from the naturally occurring flora to dominance by species that are either high-nutrient-tolerant or high-nutrient-optimal. The recent shift to *Cladophora*, which is now dominant, yet was not mentioned in reports over the past 1-7 years, is a classic example (see Section IV.A.4). This noxious alga thrives under high ammonia and high phosphate relative to background concentrations, characteristic of the Medford RWRF effluent. Without question, major reductions of P, as well as N, well beyond the

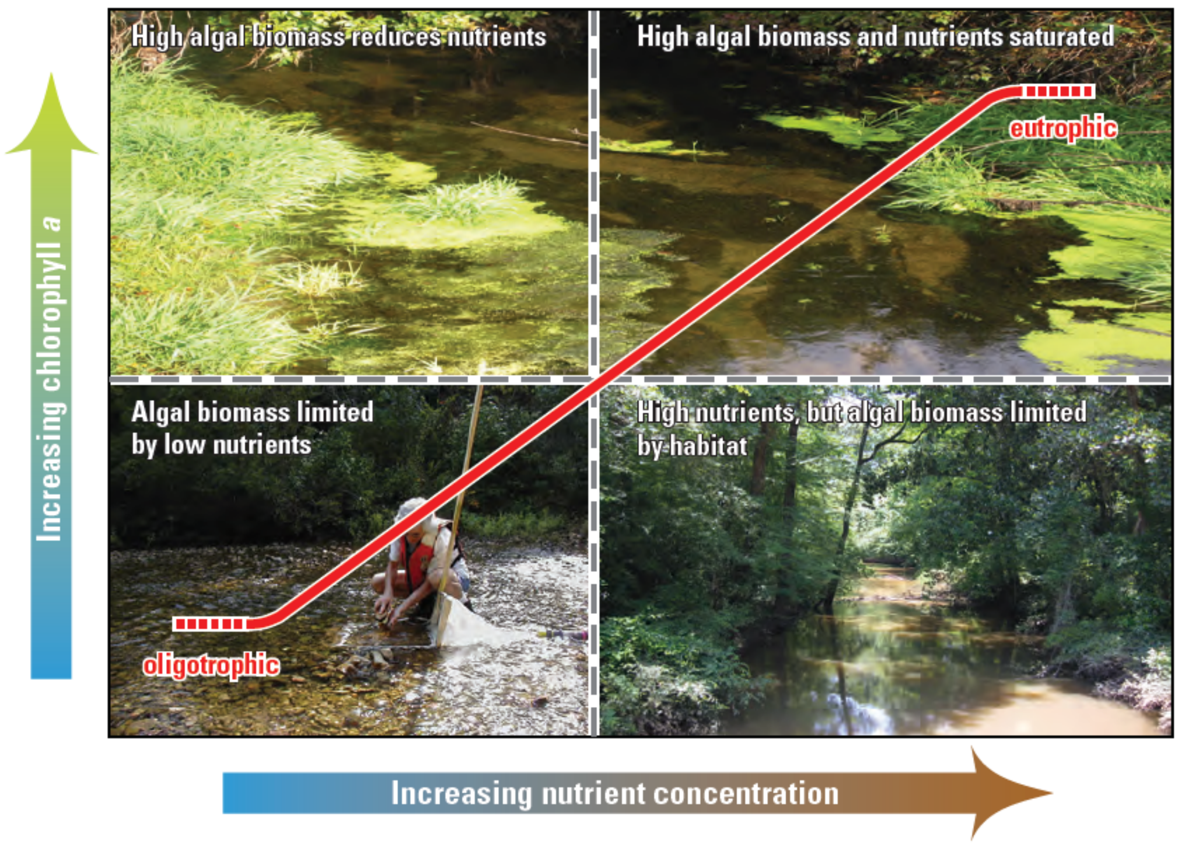


Figure 14. The Nutrient-Algal Biomass Conceptual Model, showing the complex interactions between nutrients and algal biomass (here, as corrected chlorophyll *a*). The red line represents the expected linear response of algal biomass to increasing nutrient (N, P) concentrations. Rivers fall into one of the four quadrants depending on the interaction of nutrients, habitat, and benthic algal biomass across the gradient of increasing nutrient concentrations (supplies) ranging from nutrient-poor (oligotrophic) to nutrient over-enriched. Along the linear portion of the red line, nutrients are still at limiting levels so that there is an increase in algal biomass per unit increase in nutrient supply. Note, however, that saturation occurs (dashed horizontal red line in upper right quadrant) with excessive nutrient supplies, wherein there is no further increase in algal biomass with an increase in nutrients. From Munn et al. (2018).

targets suggested by Stillwater Sciences, will be required to minimize violations of the biocriteria standard in the affected river segment.

III.D.3.e. *Suggested Excessive Effluent Limits for Only Half of Each Year, Then Back to Even Higher Limits*

Stillwater Sciences (2020, p.56) recommended continuance of the present extreme effluent limits for May through October of each future year. However, Stillwater Sciences presented no information about benthic algal biomass in the affected Rogue River segment from mid-autumn through mid-spring, and only began sampling benthic algal biomass in July for the 2019 study. Overlooking those important points, Stillwater Sciences re-emphasized that in its 2019 effort, peak benthic algal biomass was found in August, and asserted that effluent nutrients would have minimal effect on benthic algae except during May through October. There was no apparent recognition of the fact that benthic algal biomass in many north temperate rivers is maximal in spring—such as maximal *Cladophora* biomass in Montana streams during May-June shortly after spring runoff (Dodds 1991, Lohman and Priscu 1992). Importantly, as well, in north temperate waters, maximal *Cladophora* biomass has been strongly linked to high nutrient concentrations *in winter* as well as during the rest of the year: there was a significant linear relationship between annual maximum *Cladophora* biomass and mean *winter* phosphate concentration (Parker and Maberly 2000). ***Such findings underscore the importance of setting effluent TN and TP limits for the Medford RWRF as daily maxima and weekly averages applied year-round.***

In making the above recommendation, Stillwater Sciences additionally did not address downstream impacts. For example, as previously mentioned (Section II.G), nitrate is highly soluble and can travel distances of more than 200 miles downstream. Thus, if nitrate concentrations are not minimized during late fall through mid-spring, the high nitrate concentrations could easily reach nitrogen-sensitive coastal marine waters downstream, as well as segments of the Rogue River downstream that have been identified as violating DO criteria

III.D.3.f. *Suggested Excessive Effluent Limits As Non-Protective Average Monthly Concentrations*

Stillwater Sciences (2020, p.57) additionally recommended that its suggested effluent TN and TP limits should be considered as average monthly concentrations, based on the seriously flawed rationale that “short-term pulses of nutrients” (12 hours to 2 weeks) did not significantly affect biomass accrual in several other studies of benthic algae in rivers.

Here, Stillwater Sciences “compared apples to oranges” and also failed to understand the concept of nutrient saturation. First, the Medford RWRF effluent is not added to the affected Rogue River segment as a short-term pulse. It is added continuously as a major source of nutrient contamination relative to background conditions. Second, as explained above, the nutrient conditions downstream from the Medford RWRF are **saturating**. In nutrient-contaminated rivers where concentrations are saturating, no apparent response of benthic algae to additional nutrient inputs should be expected (Figure 14).

Stillwater Sciences argued that exceedances of the suggested excessive effluent TN and TP limits should be allowed to varying degrees throughout each month, as long as the overall monthly averages are at or below the effluent targets, because the exceedances "should not result in excess periphyton accrual." That is not the issue—excess periphyton accrual does not occur in response to more nutrient addition when nutrient concentrations are already saturating. The goal should be to push nutrient concentrations back toward limiting levels for algal growth. Violations ideally should be infrequent rather than routinely allowed. Therefore, as a more protective approach also mentioned above, we recommend that the effluent limits for TN and TP in the Medford RWRF permit are considered as daily maxima and weekly averages for TN and TP (e.g., as in Washington Department of Ecology 2011, ODEQ 2016). Daily maximal limits can be calculated on a statistical basis to mirror the desired monthly average.

Section IV. Protective Numeric Effluent Limits for the Medford RWRF

IV.A. Brief Primer on Nutrients and Aquatic Primary Producers

IV.A.1. *Key Nutrients Nitrogen and Phosphorus*

Cultural eutrophication (more simply, eutrophication) is the process of extremely high N and P contamination of surface waters relative to natural levels. As a result, the affected aquatic ecosystem is pushed out of balance to an unhealthy state (Burkholder and Glibert 2013). Cultural eutrophication promotes major adverse shifts in the structure of algal, plant, and animal communities, generally affecting dominant components of every trophic level from microbial decomposers to the larger animals at the top of the aquatic food web, and significantly reducing biodiversity.

Nitrogen and phosphorus are the nutrients usually emphasized in sewage impact assessment because, in considering the nutrient supplies needed for algal and plant growth under natural conditions, N and P are the essential nutrients that algae and plants run out of first (Vallentyne 1974, Wetzel 2001). Under natural conditions, low N and P supplies can limit algal and plant growth. Total nutrient levels (both N and P) have been more strongly correlated with suspended microalgal biomass than soluble nutrient forms (e.g., Dodds et al. 1997). ***Both nutrients are important to control*** because in surface waters, N and P together describe algal biomass estimates better than either nutrient alone (Smith 1982, Prairie et al. 1989, Dodds and Smith 2016); "more P means more chlorophyll per unit N, and vice versa" (Dodds and Smith 2016).

When an essential nutrient is needed for an organism to complete its life history, the supply of that nutrient has a direct effect(s) on the organism's physiology and growth. No other nutrient can be substituted for N or P in meeting the physiological needs of aquatic primary producers. The scientific recognition of "primary nutrient limitation" dates back to Liebig's Law of the Minimum in the 1840s, from agricultural literature on crop yield (Liebig 1847): If a nutrient is limiting, then its amount in algal or plant cells will decline to a low level, and the nutrient in lowest concentration relative to the specific requirements for that alga or plant will limit growth. That is, the nutrient in least supply relative to the need of the plant or alga will limit growth, whereas increases in plentiful nutrients will have little effect.

Nitrogen is an essential nutrient, required for organisms to make amino acids, proteins, enzymes and coenzymes, nucleotides, and nucleic acids (genetic material), chlorophyll pigments, and various other essential algal pigments (Taiz 2010). The forms of N that many algae and plants generally use for growth are *ammonia* (ionized form, ammonium) and *nitrate*, two types of *inorganic N* (Wetzel 2001). Algae and plants can also use certain forms of *organic N* for growth, such as urea and some simple amino acids (Hecky and Kilham 1988). In natural (background) conditions, most of the TN consists of organic N; inorganic N forms are usually low, typically < 200 µg NO_3^--N/L and < 20 µg NH_4^+-N/L, respectively (Stanley and Maxted 2008, and references therein).

In assessing aquatic systems for N abundance to primary producers, either TN or inorganic N forms are usually considered. Processes involving N in surface waters are more complicated than those involving P, partly because nitrate, another inorganic N form called nitrite, and ammonia/ammonium can be toxic to algae and plants, depending on the concentration. Thus, low amounts are beneficial, but high amounts are detrimental and these nutrient forms act as chemical poisons (Glibert et al. 2016 and references therein). Moreover, ammonia/ammonium can be an oxygen-demanding substance because it takes up oxygen during the process (called nitrification) when much of it is converted to nitrate (Stumm and Morgan 1996). The highly bioavailable forms, ammonia (ionized form, ammonium) and nitrate, are most important in controlling algal and plant growth, with limited storage capability inside the cells (Glibert et al. 2016 and references therein). Whereas nitrate is highly soluble, ammonium acts much more like phosphate in being highly insoluble and easily adsorbed to particulate materials (Stumm and Morgan 1996).

In addition to serving as plant nutrients, high levels of nitrate and ammonia in surface waters can be toxic to aquatic life (Camargo et al. 2005, Camargo and Alonso 2006, Hickey 2013, Glibert et al. 2016 and references therein). Nitrate exposure in the water column can adversely affect many metabolic and reproductive processes. Nitrate interferes with fish steroid hormone synthesis, and adversely affects fish fecundity and sperm motility/viability (Poulson et al. 2018 and references therein). At concentrations ranging from 0.2 to 5.1 mg nitrate-N/L—which would include the RMZ-S concentration suggested by Stillwater Sciences (2020; see West Yost Associates 2020) in association with its suggested effluent TN limit—nitrate exposure has decreased immune response, reproductive activity, and embryo dry weight; acted as an endocrine disruptor; and induced adverse hematological and biochemical changes in aquatic fauna (Poulson et al. 2018). Certain aquatic/amphibious species known to occur in the Rogue River are especially sensitive to nitrate toxicity. For example, a threshold concentration of 1.1 mg nitrate-N/L was toxic to young stages of salmonid fish and caused significant increases in egg mortality (Kincheloe et al. 1979). Early instar caddisfly larvae sustained adverse effects from chronic exposure at 1.4 to 2.4 mg nitrate-N/L (Camargo and Ward 1995).

Unionized ammonia damages gill epithelia, causing asphyxiation; stimulates suppression of the Krebs cycle in respiration, causing progressive acidosis and reduction in blood oxygen-carrying capacity; inhibits ATP production in the brain; disrupts blood vessels and osmoregulatory activity in the liver and kidneys, and suppresses the immune system (Camargo and Alonso 2006, and references therein). These adverse physiological impacts have led to reduced feeding activity, fecundity, survival, and population size (Environment Canada 2001, Camargo and Alonso 2006).

Concentrations of unionized ammonia ranging from 0.01 to 0.02 mg/L in long-term exposure—an order of magnitude less than the effluent concentrations suggested by Stillwater Sciences 2020 (see West Yost Associates 2020)—have been recommended to protect sensitive aquatic life such as salmonids (U.S. EPA 1999; Environment Canada 2003).

Phosphorus is also an essential nutrient, required for organisms to make the energy currency for cells, ATP, and other nucleotides. In addition, P is a component of nucleic acids such as DNA, certain proteins, several important coenzymes, membrane phospholipids that are required for cell survival; and P is attached to many different substances that are important in photosynthesis and respiration (Taiz 2010). Algae and plants generally use the inorganic phosphate ion (P_i or $PO_4^{-3}P$, also referred to as orthophosphate, or soluble reactive phosphorus SRP) for growth (Reynolds and Davies 2001, Wetzel 2001, Peters and Bergmann 2011), so it is highly bioavailable.

Phosphate is relatively insoluble in alkaline surface waters, such as the middle Rogue River (Stumm and Morgan 1996), and readily adsorbs to sediment particles and detritus (Froelich 1988, Wetzel 2001). Nevertheless, algae and plants can use enzymes called phosphatases to gain access to some of the adsorbed phosphate supply (Burkholder and Wetzel 1990, and references therein). In natural (background) conditions, P_i is usually very low in supply, typically < 5-10 µg/L (Wetzel 2001). If surface waters have phosphate concentrations exceeding 25 µg/L (25 parts per billion), that is an indication of nutrient pollution from sewage (treated effluent P is mostly phosphate) and certain other human-related sources (Carpenter et al. 1998, Correll 1998). *Algae and plants luxury-consume phosphate (that is, they take much more phosphate up than they need at the time, and are able to access some of the stored P for later use)*, so much so that the phosphate ions left in solution are usually much, much lower in supply than the P stored in their cells. For that reason, in assessing aquatic systems for P abundance to primary producers, the TP concentration, which includes the P in the algal and plant cells, is usually used (Wetzel 2001).

IV.A.2. ***Importance of Both Nutrient Supplies (Concentrations) and Supply Proportions (Ratios)***

Nutrient pollution can damage aquatic ecosystems in two basic ways: First, through an increase in the available ***amounts*** (***concentrations or supplies***) of N and P that stimulate outbreaks (blooms) of noxious algae and plants; and second, through a shift in the proportion (***supply ratio***) of N relative to P supplies (Figure 15). Surface waters affected by nutrient pollution usually have both problems: The N and P supplies are extremely high in comparison to background (reference or minimally impacted) conditions, and the N:P ratio is skewed so that the aquatic communities have been pushed out of what is referred to as "stoichiometric balance" (Burkholder and Glibert 2013).

Many surface waters worldwide, including the affected Rogue River segment, are *not only over-enriched with P and N but also in a state of stoichiometric imbalance*—which is worse than simply eutrophic (nutrient-rich). Stoichiometric imbalance is defined as a forced trophic state in a waterbody that develops when the supply of one nutrient (generally P or N), is altered either due to enrichment from human activities or management-related nutrient control (Burkholder and Glibert 2013). While 7 is commonly considered to be a balanced N:P ratio (by mass; Redfield 1958), the natural background (minimally impacted) TN:TP ratio in middle Rogue River water is 6 (basis: U.S.

EPA 2000a). Sewage treatment has targeted P removal without N removal, or more P than N removal (Glibert et al. 2011). For that reason, a common "signature" N:P ratio for raw or partially treated sewage is ~5.5 or less (Metcalf and Eddy 1991, Henze et al. 1997).

Nutrient pollution causes damage in two basic ways

Amount of N and P (SUPPLY or concentration) available to stimulate noxious algal/plant growth.

~100 µg P/L or more can cause noxious growth; the Medford RWRF effluent has more than 3,000 µg P/L.

+

Proportion (supply **RATIO**) **of N-to-P** (N:P, by mass)

Minimally impacted* (natural balance): N:P ratio = 6*

* Derived from the U.S. EPA (2000a) recommendations for the nutrient sub-ecoregion containing the affected Rogue River segment.

Figure 15. Diagram depicting the importance of BOTH nutrient supplies and nutrient supply ratios in cultural eutrophication. These numbers typically refer to conditions in the growing season.

Nutrient ratio science (nutrient stoichiometry) is defined as the study of changes in the relative proportions of critical nutrients (especially N and P) available in the water, in comparison to differences in the allocation of these elements in organisms ranging from benthic algae and SAV to macroinvertebrates, fish, and other higher levels of aquatic food webs. The water-column TN:TP ratio heavily influences the quality of primary producers as food for aquatic animals. The response to sewage usually involves a shift in the benthic algal community from diatoms that are a good food resource to filamentous green algae that are poorly consumed by many grazers (Hilton et al. 2006, Zulkifly et al. 2013; Figure 16). Nutrient stoichiometry compares nutrient ratios in the water versus in algal cells, SAV, and animals (Glibert et al. 2011 and references therein). Nutrient ratio science is rooted in what is called the Redfield ratio (Redfield 1958: 7 N to 1 P, by mass, for balanced or healthy algal growth), wherein scientists noticed that water-column ratios of several essential nutrients for algal growth were similar to the nutrient ratios inside the algal cells. Rapidly growing algae exhibit nutrient uptake ratios similar to the Redfield ratio, reasonable since the cycles of major and minor nutrients are closely related to biological processes in aquatic systems.

Although the Redfield ratio is widely applied to indicate aquatic ecosystem health, nature is more complicated, so that the optimum ratio can vary somewhat depending on the algal or plant species (Hillebrand et al. 2013). In the middle Rogue River, for example, U.S. EPA (2000a) recommendations for TN and TP concentrations in minimally impacted waters indicate a TN:TP ratio of 6 (Table 5). As mentioned, this ratio fits the general profile of the Rogue River, historically oligotrophic although draining volcanic P-containing rock areas (Myer 2013 and references therein). Deviations from the Redfield ratio in N:P ratios have been interpreted to indicate nutrient limitation (N limitation inferred when the TN:TP ratio is below 7; P limitation inferred when the TN:TP ratio is above 7). As explained above (p.53), however, the affected Rogue River segment is not nutrient-limited considering the naturally occurring algal and plant communities; rather, it is nutrient-saturated.

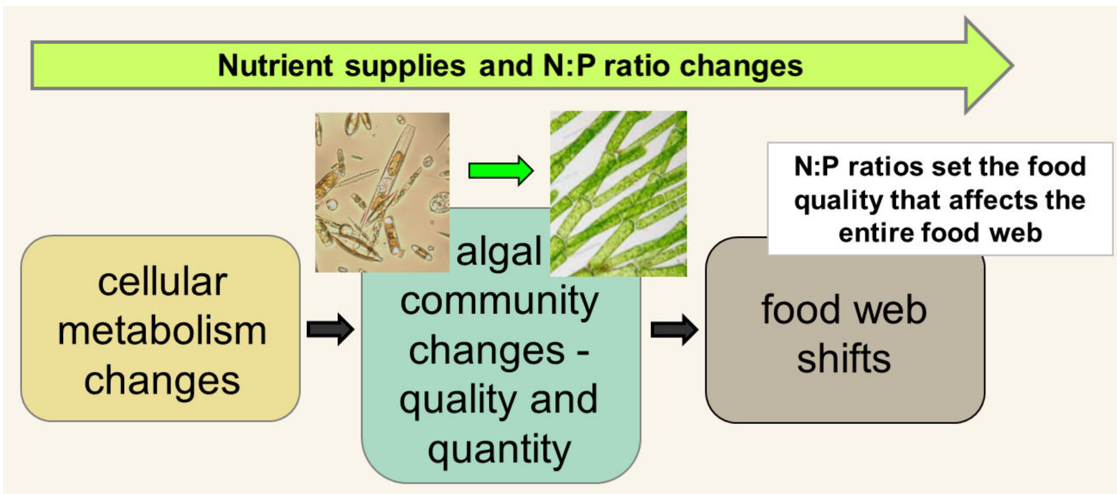


Figure 16. Conceptual diagram of commonly reported changes in the algal base of river food webs from sewage.

Background: Nutrient supplies and N:P ratios control algal dominance which, in turn, controls food quality at the base of the river food web (Glibert et al. 2011 and references therein). Algal species vary by 10,000-fold in size, from relatively small diatoms to large-celled filamentous green forms (Finkel et al. 2010). They have different needs, analogous to different physiological needs by mice versus elephants, and their innate optimum N:P ratios differ. The most favored algal physiology changes across the gradient of N and P availability; N:P ratios affect cell size, pigments, enzyme activities, and growth rate (Finkel et al. 2010, Glibert et al. 2011).

Sewage shifts food quality at the base of the food web from beneficial diatoms to filamentous green algae such as *Cladophora*, causing a "domino effect" that (directly or indirectly) adversely impacts higher trophic levels from microscopic animal-like protozoans to macroinvertebrates and fish (see text for supporting references).

IV.A.3. *Nutrient Behavior and Benthic Algal/SAV Response*

Excessive nutrient contamination from discharge of sewage after secondary treatment causes N and P saturation of the naturally occurring algal and plant communities, selects for certain noxious species that thrive in the polluted conditions, and pushes the entire ecosystem out of balance.

High N and P pollution causes enrichment of both the water column and the bottom sediments. Enrichment of benthic habitats occurs because much of the inorganic N and P are adsorbed to particulates that settle out; in addition, the inorganic N and P are taken up by biota that eventually die and settle out (Froelich 1988, Demars et al. 2005, Venkiteswaran et al. 2019). Much of the incoming water-column N and P can be moved downstream with the volume of flow. If external nutrient sources (here, through improved sewage treatment) are reduced and water-column concentrations decline, the N and P tend to move from the sediment into the water column to re-establish the previous unhealthy equilibrium (Froelich 1988, Wetzel 2001). Overly abundant rooted SAV can assist in that process by taking up nutrients from the bottom sediment, translocating them to their shoots, and then releasing them to the water upon senescence, death, and decomposition (Landers 1982, Smith and Adams 1986). It can require a decade or more for N and P enrichment from the bottom sediments into the overlying water to subside (Meals et al. 2010).

Aquatic ecosystems that receive high nutrient enrichment from sewage are much more vulnerable to adverse impacts of nutrient pollution in comparison to waters affected by other sources, because the majority (often ~80 to 95%) of the N and P in partially treated sewage is inorganic N and P, the forms most directly available for algal/plant uptake (Young et al. 1982, Millier and Hooda 2011, Venkiteswaran et al. 2019). For that reason, wastewater discharges are ranked as the highest nutrient source in "ecological relevance," that is, in terms of composition (solubility and concentration) and patterns of delivery (mode and timing) (Withers and Jarvie 2008). In other words, the N and P supplies in sewage-affected systems are much more potent in causing adverse impacts. Thus, from a study of north temperate rivers, Jarvie et al. (2006) concluded that "point sources [of nutrients] provide a greater risk for river eutrophication than diffuse sources from agricultural land, even for rural areas with high agricultural losses." Sewage sources have major impacts on stream and river ecosystems, not only in the water column but also in the sediments, which become a nutrient-rich repository (House and Denison 1998, Haggard et al. 2001, Venkiteswaran et al. 2019).

Risk of increased impacts from P-laden sewage on segments farther downstream can be greater as well (Withers and Jarvie 2008). Large dissolved N and P loads from treated sewage have been shown to saturate the stream communities, including the primary producers, and depress nutrient retention efficiency, in comparison to streams of similar size with lower dissolved nutrient inputs (Marti et al. 2004). In fact, surface waters dominated by nutrients from point sources, including downstream waters, are considered to need strengthened protection from nutrient pollution *because* of their enhanced vulnerability to high inputs of bioavailable nutrient forms (Mainstone and Parr 2002, Bowes et al. 2010, Neal et al. 2010).

Changes in the abundance of benthic/floating algae, especially filamentous macroalgae, and SAV are often the most visible signs of a changing nutrient regime in rivers (Hilton et al. 2006). Both benthic algae and SAV directly consume the inorganic N and P supplies in sewage (Mainstone and Parr 2002, Withers and Jarvie 2008, Hood et al. 2014). In general, high-nutrient-tolerant and high-nutrient-optimal filamentous benthic algae and SAV become dominant in areas affected by partially treated sewage. Both communities of primary producers have two potential sources of nutrients, the water column and the sediments (Wetzel 2001). "Canopy" or "overstory" species of benthic algae that can grow up into the overlying water as debris accumulates have a competitive advantage over other species because of much greater, continual access to the rich water-column nutrient source (Burkholder 1996). Although rooted SAV in more natural environments obtain most of their nutrient supplies from the bottom sediments (Wetzel 2001), the extremely high water-column concentrations from the sewage effluent promote high leaf uptake (see Carignan 1982, Sosiak 2002) until that source is blocked by thick cover of algae that colonize the bigger plants (Hilton et al. 2006; e.g, see Figure 4). SAV often become heavily covered with microalgae and debris as high nutrient contamination continues, and are eventually eliminated if they are unable to obtain enough light for photosynthesis. Over time, areas affected by high nutrient contamination from partially treated sewage commonly become covered by a few species of noxious benthic filamentous algae (Hilton et al. 2006 and references therein).

The response of algae and plants to nutrient additions typically is non-linear (Withers and Jarvie 2008; Stevenson et al. 2008, 2012), partly because a portion (~10-60%) of the N and P entering a stream or river generally is adsorbed to particulates as well as taken up by the biota (Jarvie et al. 2002, Bowes et al. 2003, Hood et al. 2014, Xia et al. 2018). Rather than traveling directly for long distances downstream, nutrient forms other than nitrate tend to "spiral"—that is, when the biota die or the chemistry changes in the area immediately adjacent to the particles, ammonium or phosphate ions are released back into the water. At the sediment-water interface, deoxygenation of stream waters (for example, through enhanced microbial activity linked to high organic matter inputs in partially treated sewage) generally results in the release of highly bioavailable ammonium or phosphate back to the overlying water (Mainstone and Parr 2002, House 2003, Xia et al. 2018). The process of ammonium, nitrate, and phosphate uptake or adsorption followed by release is repeated as these ions slowly "spiral" downstream (Newbold et al. 1983, Bowes et al. 2003, Withers and Jarvie 2008). A spiral of N or P is defined as the distance through which an inorganic N or P ion completes one cycle from the dissolved ionic form to particulate/organic transformation and then back to the dissolved ionic form. Radiolabeled tracer studies have shown that inorganic N and P uptake, turnover, and regeneration times in rivers can occur rapidly, in seconds, minutes or hours (Mulholland et al. 1985). Much of the particulate N and P that is temporarily unavailable can be released through de-absorption or decomposition of organic remains, to affect the immediate area or contribute to P impairment downstream.

IV.A.4. Special Case: *the Filamentous Green Macroalga,* Cladophora

Among the harmful algae best known to proliferate in response to sewage nutrients in freshwater rivers are *Cladophora* species (Stevenson et al. 2012, Zulkifly et al. 2013, and references therein). *Cladophora* commonly overgrows and displaces beneficial aquatic plants, reduces densities of sensitive macroinvertebrates, and depresses fish spawning; its thick decaying mats can also cause or contribute to low-oxygen stress (Whitton 1970, Ward and Ricciardi 2010). The mats also commonly sustain and nourish growth of fecal bacteria (Whitman et al. 2003, Ishii et al. 2006), which are added to the river in the treated effluent according to the Medford RWRF permit.

Freshwater *Cladophora* especially thrives in N- and P-enriched waters with dependable substrata for attachment such as rocks and boulders (Pitcairn and Hawkes 1973, Dodds and Gudder 1992, Lohman and Priscu 1992). It favors relatively fast-flowing alkaline waters (pH higher than 7 and less than < 10; Bellis and McLarty 1967, Whitton 1970) with relatively high conductivity (Biggs and Price 1987). The seasonal progression of *Cladophora* growth can vary substantially, with maxima occurring in late spring, summer, or fall depending on the environmental conditions and the population or strain (e.g., Bellis and McLarty 1967, Whitton 1970, Biggs and Price 1987, Dodds

1991, Parker and Maberly 2000, Vodacek 2012). *Cladophora* has high nutrient optima; for example, it has been found to achieve optimal growth under a relatively high P regime (0.6 mg TP/L; Leland and Porter 2000). Its relatively large cells can luxury-consume and store substantial P (Young et al. 2010), with smaller amounts of N storage as well (see Lohman and Priscu 1992).

Once established, *Cladophora* can persist for years, overwintering as robust basal growth that can regenerate major biomass when conditions are favorable (e.g., as the water warms) As Zulkifly et al. (2013, p.13) wrote,

> Only if the resistant basal structure dies, or the environment changes so that growth is depressed, will *Cladophora* be likely to disappear.

Under ample light characteristic of the affected Rogue River segment, the most promising strategy available to depress *Cladophora* growth is N and P reductions. Accordingly, various studies have recommended major reductions in nutrient inputs to rivers by interception (N and P stripping) at wastewater treatment facilities (e.g. Canale and Auer 1982, Parker and Maberly 2000). *Cladophora* largely depends on the water column for nutrients, and therefore responds relatively rapidly to nutrient reductions down to limiting levels (Parker and Maberly 2000). Both inorganic N and inorganic P have been shown to control *Cladophora* growth (Wharfe et al. 1984, Freeman 1986, Lohman and Priscu 1992), which underscores the need for major reductions in both nutrients to decrease noxious *Cladophora* and protect the designated uses of the affected Rogue River segment for fish and aquatic life.

IV.B. Major Issues in Selecting Protective Effluent N and P Limits

Acceptable effluent limits for TP and TN should be set as daily maxima and weekly averages, applicable year-round. They must be derived using low-flow conditions, accurate upstream (background) concentration data, dramatic reductions in bioavailable inorganic nutrient forms, and a TN:TP ratio that is similar to the ratio in minimally impacted ("reference") waters of the nutrient sub-ecoregion.

IV.B.1. *Recommended Approach for Deriving Acceptable Effluent TN and TP Limits*

The ***reference approach*** (U.S. EPA 2000a,b), based on TN and TP conditions in rivers minimally impacted by nutrient pollution in the nutrient sub-ecoregion containing the middle Rogue River, is recommended for estimating effluent targets that will protect designated uses for fish and aquatic life near the Medford RWRF. The responses of many beneficial biota to changes in nutrient concentrations are non-linear, so that a small increase in nutrient concentration near a threshold corresponds to a relatively large change in the biota. The threshold concentration where often-abrupt undesirable change in biota occurs, such as loss of sensitive species, is often only slightly higher than the reference concentration (e.g., Robertson et al. 2008). Thus, the reference approach, used protectively, favors anti-degradation.

IV.B.2. *Dilution Factor Based on Low Flow Conditions*

Four critical "ingredients" are required to derive effluent limits that will protect the designated uses of the Rogue River downstream from the Medford RWRF. First, calculations must be based on low-flow conditions to account for adverse impacts during "worst-case" conditions. Thus, the dilution factor is usually calculated from the 7Q10, that is, the lowest discharge averaged over a period of 7 consecutive days that can be statistically expected to occur once every 10 years. For example, following Stillwater Sciences' (2020, p.52) equation 1 (above) and the flow data given in Table 7, the dilution factor for the maximum Medford RWRF effluent volume (i.e., worst case) would be 25.6:

$$\text{dilution factor} = \frac{\text{7Q10 discharge upstream, 882 cfs}}{\text{maximum outflow discharge, 34.5 cfs}} = 25.6$$

While the above dilution factor appears similar to the dilution factor that was "selected" by Stillwater Sciences, *this dilution factor was actually calculated from 7Q10 low-flow conditions*. Stillwater Sciences' dilution factor (Section III.D.2.a), as Stillwater Sciences described how it was calculated, should have been much higher because substantially higher flow conditions purportedly were used than the 7Q10, even substantially higher conditions than low flow during 2019 (Table 7, Figure 13). As explained above, Stillwater Sciences (2020) asserted that it used average flow and average conductivity data (July-Nov.) in wet year 2019 for its calculations. Neither was used; instead, based on the available information, Stillwater Sciences' dilution factor was "selected" along with excessive effluent TN and TP concentrations, and then RMZ-S excessive TN and TP concentrations were selected to match those numbers in equation 2.

As explained in Section III.D.2.a, by using a "selected" low dilution factor to fit excessive TN and TP levels in the effluent and at RMZ-S, Stillwater Sciences conveyed to readers the tacit but false message that downstream effects from the effluent can be remedied by less nutrient reduction than is actually required. In reality, a downstream effect that occurs in higher river flow requires greater nutrient reduction.

IV.B.3. *Accurate Data for Background Conditions and RMZ-S Concentrations Under Low Flow Conditions*

The second required ingredient is accurate data for background conditions. Ideally, the dilution factor derived from low flow conditions is used to solve for acceptable effluent TN and TP concentration targets (Stillwater Sciences' equation 2, above)—but the errors (uncertainty) associated with trying to estimate very low nutrient levels often lead to large overestimates of the actual concentrations. Without accurate data to characterize background conditions, equation 2 cannot be used reliably to estimate acceptable effluent TN and TP concentrations.

Accurate background data must be in hand for TN and TP concentrations immediately upstream from influence of the effluent outfall, <u>and</u> for downstream RMZ-S concentrations measured during low flow conditions. The 2019 wet year data correspond to a much higher dilution factor (Table 7); thus, concentrations downstream from the Medford RWRF outfall were lower (more diluted) than during low flow conditions. Calculations that relied upon these lower downstream concentrations would mistakenly result in higher effluent limits than would be needed to protect the river biota during worst-case periods.

Since the needed data are lacking for both upstream minimally impacted conditions and RMZ-S nutrient concentrations under low-flow conditions, the U.S. EPA's (2000c) recommended TN and TP concentrations for minimally impacted (25^{th} percentile all-streams data) rivers in this nutrient sub-ecoregion could be considered as the upstream/background targeted values (Table 5: 0.18 mg TN/L, 0.30 mg TP/L). Note that these concentrations are fairly close to, but higher than, the estimated control or upstream values from Stillwater Sciences' (2020) corrected linear regression analyses (0.205 mg TN/L, 0.048 mg TP/L). They likely are inflated because the only data for benthic algal biomass data from the Stillwater Sciences (2020) study, needed for the regression analyses, were for total rather than corrected chlorophyll *a* as explained above.

While the U.S. EPA recommendations for minimally impacted conditions can be used for background (upstream) TN and TP concentrations, *there is no appropriate substitution for accurate data for RMZ-S nutrient concentrations under low-flow conditions*. In recognition of that problem, here for illustration only, we roughly approximated the proportion of the effluent concentration that reaches RMZ-S on average based on the only data available—for wet year 2019 (Table 8). Note that RMZ-S contained 5.6-5.7% of the effluent TN and TP concentrations. If the desired TN and TP

Table 8. Parameter concentrations during the 2019 study, compiled from Stillwater Sciences (2020, Table 2-5), and the proportion (percentage) of the effluent concentrations that reached RMZ-S.

Parameter	Background		RMZ-S		Effluent at Outflow		Reaching RMZ-S
Ammonia (mg/L)	------		0.335	(0.285)	8.44	(9.76)	4.0%
NOx (mg/L)	[0.04][b]		0.350	(0.291)	6.39	(5.77)	5.5%
TKN (mg/L)	0.196	(0.136)	0.639	(0.600)	10.90	(11.7)	5.9%
TP (mg/L)	0.053	(0.052)	0.174	(0.198)	3.13	(3.18)	5.6%
SRP (mg/L)	0.034	(0.037)	0.131	(0.151)	2.70	(2.83)	4.9%
TN (mg/L)	0.187	(0.164)	0.989	(1.097)	17.29	(17.47)	5.8%

[a] Means; numbers in parentheses are medians.

[b] TKN = organic N + ammonia; the U.S. EPA's 25th percentile value for NOx is in brackets, needed to calculate TN: TN = TKN + NOx.

concentrations at RMZ-S are set as equal to the upstream control/background concentrations (taken from U.S. EPA recommendations as explained above), and if 5.6-5.7% of the effluent TP and TN concentrations characterize RMZ-S, then

X (the TN effluent target) = 0.18 mg TN/L x 100% divided by 5.8% ⟶ *X = 3.10 mg TN/L*; and

X (the TP effluent target) = 0.03 mg TP/L x 100% divided by 5.6% ⟶ *X = 0.54 mg TP/L* .

However, these estimates of acceptable effluent concentrations are too low because the nutrient concentrations at RMZ-S (taken during a wet year) were more dilute than they would have been under 7Q10 conditions (or even under low flow conditions for 2019; see Figure 13), so the percentages representing the amounts arriving from the outfall area (the denominator of each equation above) are too low. Nevertheless, it is clear that the Stillwater Sciences' (2020) suggested target effluent concentrations (5.65 mg TN/L, 1.35 mg TP/L) are much too high to protect the downstream Rogue River segment from biocriteria violations. These excessive targets were obtained, in part, because accurate upstream concentrations were not available, and because low-flow conditions were not used.

IV.B.4. *Dramatic Reductions in Highly Bioavailable Inorganic N and P*

The excessive effluent TN and TP limits suggested by Stillwater Sciences (2020) will not protect the Rogue River from further violations of the biocriteria standard. At RMZ-S, TN and TP would be 0.4 mg/L and 0.1 mg/L, respectively, most of it highly bioavailable. These high nutrient levels will continue to fuel noxious algal/SAV overgrowth, leading to loss of habitat needed by macroinvertebrates and fish, and to increased incidence of harmful diel DO variation. For example, the now-established dominant benthic alga, *Cladophora* downstream from the Medford RWRF outfall is highly efficient in biomass production per unit N or P (Gerloff and Fitzgerald 1976, Auer and Canale 1982, Lohman and Priscu 1992). Once *Cladophora* is established, ammonia-N and phosphate concentrations must be dramatically decreased (to ten-fold lower concentrations than Stillwater Sciences selected for RMZ-S) to reduce *Cladophora* growth back down below noxious levels (Welch et al. 1989, Parker and Maberly 2000, Dodds 1991, Suplee et al. 2012).

The information presented above about *Cladophora* (also see Section IV.A.4) underscores a critical need to achieve dramatic reductions in bioavailable forms of N and P (total ammonia, NOx, and SRP), in order to shift the benthic algal community downstream from the Medford RWRF away from dominance by this noxious alga. Biological nutrient removal technologies available for more than a decade ***can*** achieve treated sewage effluent concentrations of 0.05 mg ammonia-N/L, 1-2 mg nitrate-N/L, and 0.01 mg SRP/L (U.S. EPA 2007a,b). Such technologies would make it possible to achieve the reductions in effluent bioavailable N and P needed to push *Cladophora* growth below noxious levels (< 100 mg chl*a*/m^2 river area). Unfortunately, the five treatment alternatives presented in the companion document to the Stillwater Sciences (2020) report (West Yost Associates 2020) would allow 5 to 15 mg TN/L (up to ~85% of it, highly bioavailable) and < 1 mg TP/L, mostly as highly bioavailable SRP (phosphate).

IV.B.5. N*utrient Ratios (TN:TP) for Minimally Impacted Conditions*

The fourth critical ingredient that must be considered in order to derive acceptable effluent TN and TP limits is targets that reflect the TN:TP ratio of minimally impacted waters in the nutrient sub-ecoregion. As explained above (Section IV.A.2), this issue—supply ratios—is often overlooked, but it is just as important as the need to set appropriate effluent TN and TN concentrations (supplies). The U.S. EPA (2015) recognized that success in efforts to control noxious algal/plant growth in waters which have been driven out of balance in nutrient stoichiometry as well as nutrient supplies will require reducing both N and P pollution (that is, N and P co-management) toward re-establishing minimally impacted conditions for N:P ratios. Background minimally impacted conditions for the middle Rogue River indicate a TN:TP ratio of 6 (U.S. EPA 2000b). While our rough illustration yielded effluent limits with a TN:TP ratio very close to that (5.9), note that Stillwater Sciences' suggested effluent limits would continue to impose, in downstream water, an unbalanced TN:TP ratio of only 4.2.

IV.C. Summary: Important Considerations in Setting Effluent Limits for the Medford RWRF

The important issues that must be addressed in selecting final nutrient effluent limits for the Medford RWRF are summarized as follows:

- The reference approach, based on both TN and TP *concentrations* and the TN:TP *ratio* in minimally impacted waters within the same nutrient sub-ecoregion (U.S. EPA 2000a), should be used to set nutrient limits for the Medford RWRF effluent that will protect the designated uses of the affected Rogue River segment for fish and aquatic life.

- Low flow conditions (7Q10) and maximum benthic algal/SAV biomass, as determined from appropriate sampling over an annual cycle (Section V), must be used to set the effluent limits for TN and TP concentrations and for the TN:TP ratio in the effluent and RMZ-S water.

- Using the reference approach, the bioavailable inorganic N (as ammonia, nitrate) and P (phosphate or SRP) concentrations at RMZ-S will be low enough to decrease *Cladophora* growth below noxious levels (< 100 mg corrected chl*a*/m^2 river area; see Section IV.B.2). Such reductions can be accomplished with BNR technology, but not with the treatment scenarios considered in the companion document to Stillwater Sciences (2020), West Yost Associates (2020).

- The effluent limits for TN and TP concentrations should be set at a TN:TP ratio approximating that of minimally impacted waters in the same nutrient sub-ecoregion (U.S. EPA 2000a).

- To protect the Rogue River segment from continued violations of the biocriteria standard, these effluent limits should be set as daily maxima and weekly averages.

- The effluent limits should be applied year-round (see Sections III.D.3.e-f) to control the biomass of noxious benthic algae such as *Cladophora*.

- Once applied, a lag period of several years should be expected (see Sections III.D.3.e and IV.A.3) to accomplish visible improvement in decreased biomass of noxious benthic algae/SAV and increased abundance of pollution-sensitive macroinvertebrates as the river shifts toward lower nutrient supplies (Dodds 1991, Meals et al. 2010).

- As explained in Sections I and II.G, consistent with Federal and State mixing zone guidance and regulations (OAR 340-041-0053), and given that the Rogue River segment into which Medford discharges is now listed as "impaired" for (i.e., does not comply with) Oregon's biocriteria water quality standard pursuant to Section 303(d) of the Clean Water Act, a mixing zone is not appropriate for the impairment-related pollutants (including nutrients). Therefore, Medford's discharges should ensure compliance with the biocriteria standard at the point of discharge.

Section V. Other Recommended Changes Going Forward

V.A. Assessment of Compliance

The critical goal of setting protective nutrient limits in the Medford RWRF permit is to reduce nutrient levels in the effluent low enough to ensure that (i) all water quality standards are met downstream, **and** (ii) the biocriteria standard is met (i.e., water quality no longer negatively affects the aquatic communities). Realizing this goal will require an adequate sampling/analysis plan to track future biological conditions and water quality at sites such as those shown in Figure 17. The modified permit should include a description of this plan, with key components for monitoring/assessment described as follows.

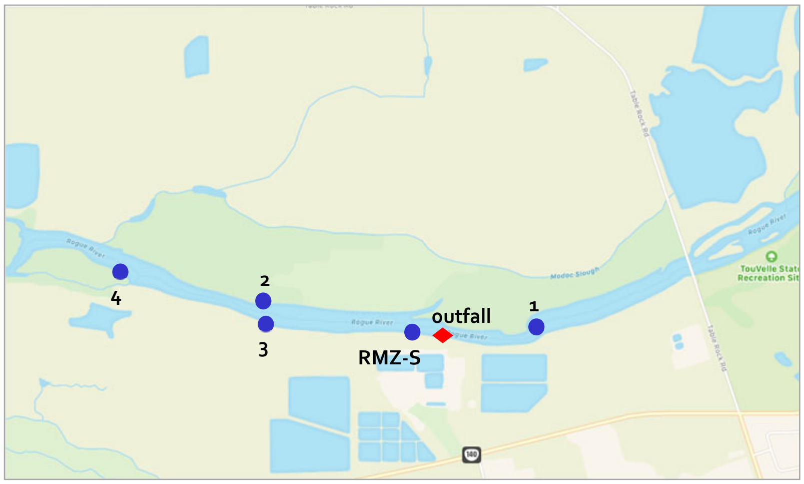


Figure 17. Map showing recommended compliance monitoring sites for benthic macroinvertebrates, benthic algae, SAV, and water quality (grab samples for nutrient concentrations). In situ datasondes for continuous (diel) monitoring should be included at Sites 1, 3, 4, and 5.

Site 1 (formerly Site 3): Upstream from the Medford RWRF outfall.

RMZ-S – At downstream edge of mixing zone within 100 feet of south bank of river.

Site 2 (formerly 4N) – First riffle downstream from outfall (0.4 mile) along north bank.

Site 3 (formerly 4S) – First riffle downstream from outfall along south bank of river.

Site 4 (formerly 5S) – Second riffle downstream from outfall (0.9 mile) along south bank of river on north side of island.

V.A.1 – Biocriteria

Data from three communities—macroinvertebrates, benthic algae (including drift), and SAV—will be needed to evaluate how the new effluent nutrient limits affect aquatic life over time, and whether the biocriteria are being met. Macroinvertebrates reflect the overall integrated environmental conditions (water quality and habitat) over an annual cycle (Ward 1992, Karr and Chu 1999). In addition, the macroinvertebrate community is emphasized by ODEQ (2019) to assess biological condition. Primary producers generally provide the most sensitive and direct responses to changes in water quality and nutrient concentrations (Rosen 1995). The more rapidly growing algae will provide the most rapid, sensitive indication of the efficacy of the new nutrient limits (Paul et al. 2017). SAV are excellent long-term integrators (days to weeks) of nutrient availability or change in the nutrient regime (Gerloff and Krombholtz 1966, Burkholder et al. 2007, Paul et al. 2017). Because the algal and plant communities directly affect food and habitat conditions for the macroinvertebrates (Hynes 1972, Ward 1992, Dodds 2002), without adequate improvement in the primary producers, recovery of the macroinvertebrate community will not occur. Thus, all three communities—benthic algae, SAV, and macroinvertebrates—must be monitored for biocriteria compliance assessment in the Rogue River near the Medford RWRF.

While the fish community is of critical importance in the Rogue River ecosystem, several issues make this group problematic for inclusion in compliance assessment. First, previous data focusing on the fish community in the affected segment have not been collected, so there is no way to determine past conditions for comparison with future conditions. Second, fish have relatively long lives, so that changes in their community – either positive or negative – can take years to occur. Third, resident fish are highly mobile and anadromous fish are not present the entire year; thus, changes in their populations may be unrelated to changes in the RWRF effluent quality. Overall, if the targeted water quality parameters (below) meet in-stream standards below the RWRF mixing zone and the algae, SAV, and macroinvertebrate communities are in compliance with biocriteria – and if temperature and DO meet salmonid spawning criteria (below) – then the fish community will be adequately protected.

V.A.1.a. *Macroinvertebrates*

Field and Laboratory Methods - Field sampling and laboratory analysis methods for macroinvertebrates have been well established by ODEQ (2009). Although the field methods have been developed primarily for wadeable streams, the same methods have been used effectively in wadeable riffle areas of larger rivers such as the Rogue River. The ODEQ field methods were used in all the previous studies that assessed macroinvertebrate communities above and below the Medford RWRF (Hafele 2013, 2019; Brown and Caldwell 2014; Hume 2019) with exception of the sampling devices used: Brown and Caldwell (2014) used a Portable Invertebrate Box Sampler (PIBS); Hume (2019) used a Surber sampler; and Hafele (2013, 2019) used a D-frame kick net as described in ODEQ (2009). All three sampling devices are widely used to collect macroinvertebrate samples (Merritt et al. 2008).

For consistency in methods to evaluate compliance by the Medford RWRF with the biocriteria standard, we suggest that ODEQ should specify within the new NPDES permit the type of sampling device to be used for future macroinvertebrate sampling. Macroinvertebrates should be sampled annually, including for at least five years after improved effluent limits are imposed, to capture data across multiple years with different weather and streamflow conditions. Sample collection should occur under stable flow conditions during the low-flow period in the fall and before fall rains occur that substantially increases stream flows, typically from mid-September through the first week of October. Sampling was also conducted during that period in the previous studies, so that the data can be compared with those earlier datasets in tracking macroinvertebrate community health.

Laboratory sample processing procedures should continue to follow the consistent approach used in the previous studies, wherein at least 500 organisms should be randomly sub-sampled from each field sample and then identified to the lowest practical level. The level of identification recommended for specific taxa groups is described in ODEQ (2009).

Data Analysis – The methods used to evaluate the macroinvertebrate data will be critically important in determining future biocriteria compliance by the Medford RWRF. As previously noted, the ODEQ PREDATOR model is used to assess macroinvertebrate results for wadeable streams. The PREDATOR model, however, is not calibrated for large rivers, such as the Rogue River, so it is not directly applicable in this situation. A common alternative approach is to use a set of invertebrate community attributes (metrics) (Carter et al. 2006). Individual metrics reflect different yet predictable biological responses to human disturbance (Karr and Chu 1999). When multiple metrics are combined into a single index value, they are often referred to as an IBI (Index of Biotic Integrity) or MMI (Multimetric Index).

<u>Metrics and Compliance Assessment</u> – Various metrics have been used in the previous studies to compare the macroinvertebrate communities upstream and downstream from the Medford RWRF outfall and evaluate changes (Table 9). Metrics reflect differences in various aspects of community structure and function, and their sensitivity differs depending on the types of stressors (Karr and Chu 1999). Based on the "Ecological Integrity" definition of the Biocriteria standard, the selected metrics must assess the "species composition, diversity, and functional organization of the community" (OAR 340-41-0002). In addition, the metrics selected for assessment of biocriteria compliance by the Medford RWRF must be responsive to the effects of nutrient enrichment. The following suite of metrics is provided not as a final list but, rather, as the minimal core of metrics needed to develop a sensitive and robust macroinvertebrate community assessment. A final list should be developed in consultation with ODEQ and an advisory group of scientists with appropriate expertise.

- *EPT Taxa Richness* (decreases with increasing disturbance): As a group, the EPT taxa (Ephemeroptera, mayflies; Plecoptera, stoneflies; and Trichoptera, caddisflies) are sensitive to a range of environmental perturbations including nutrient enrichment. Thus, EPT Taxa Richness is a commonly used metric in assessment of macroinvertebrate community health (Davis and Simon 1995). In the previous studies, this metric has been sensitive to changes downstream from the Medford RWRF outfall.

- *% Sensitive Taxa* (decreases with increasing disturbance): Because some EPT taxa are relatively tolerant of poor water quality, this metric excludes the tolerant EPT, and also includes other non-EPT taxa that are sensitive to poor water quality. Several lists of "sensitive" taxa have been established over the past few decades. One of the most recent was contributed from a U.S. EPA project that involved expert macroinvertebrate taxonomists from the Northwest. An attribute score ranging from 1 to 6 was assigned to most Northwest taxa (R. Hafele, pers. comm.), with 1 indicating high intolerance to disturbance and 6 indicating high tolerance. This metric could be refined for biocriteria compliance assessment based on such new information.

- *% Non-insect Taxa* (Increases with increasing disturbance): Non-insect invertebrate taxa mostly consist of species of worms, snails, and molluscs, many of which increase in abundance and diversity with increasing organic enrichment. Nearly all are considered to be tolerant of poor water quality.

- *% Tolerant Individuals* (increases with increasing disturbance): This is another metric that can be useful for assessing nutrient enrichment and poor water quality.

Table 9. Biological metrics used in previous studies to assess macroinvertebrate community health and evaluate biocriteria compliance by the Medford RWRF.

Metric	Predicted response to increasing disturbance	Hafele (2012, 2019)	Brown and Caldwell (2014)	Hume (2019)
Taxa Richness/Composition metrics				
Total Abundance (#/m²)	Decrease	√	√	√
EPT Abundance (#/m²)	Decrease	√	√	√
Total Taxa Richness	Decrease	√	√	√
EPT Taxa Richness	Decrease	√	√	√
Tolerants & Intolerants				
% Sensitive EPT	Decrease	√		
% Total Sensitive Individuals				√
% Intolerant Taxa	Decrease	√	√	√
% Tolerant Individuals	Increase			√
% Oligochaeta	Increase	√	√	
% Non-Insect Taxa	Increase	√	√	
% Total Non-Insect Individuals				√
Feeding and other Habits				
% Clingers	Decrease		√	
% Shredders	Decrease		√	
Population attributes				
% Dominant taxon	Increase		√	√
*Multi-Metric Index (MMI) Score	Decrease	√		
**PREDATOR Score	Decrease	√		

* The MMI score is a composite of six metrics: % EPT Taxa Richness; % Individuals of Top 5 Taxa; scraper Taxa Richness; EPT Taxa Richness; % Clinger Taxa Richness; % Tolerant Taxa Richness. This MMI was developed by the EPA for the National Rivers and Stream Assessment program for the Western Mountain region (U.S. EPA 2016).

** In Hafele (2019), PREDATOR scores were calculated for the samples collected in 2012, 2017, and 2018 after discussion with ODEQ (Shannon Hubler, pers. comm.). While not calibrated for large rivers, areas within the affected Rogue River segment are wadeable; and the PREDATOR scores showed a significant loss of taxa below Medford's outfall (up to 65% taxa loss) compared to above the outfall (12-26% taxa loss).

- *% Long-Lived Taxa Abundance* (decreases with increasing disturbance): Most invertebrate taxa are relatively short-lived (life cycles of one year or less). While this is beneficial for seeing a response to improving or declining stream conditions quickly, it is also helpful to look at longer lived taxa (2-4 year life cycles). Because long-lived taxa require adequate environmental conditions over several years to survive, they help evaluate longer term trends in water quality. Many long-lived taxa are also intolerant of poor water quality (e.g. several species of stoneflies), so their presence at levels similar to unimpaired sites indicates good stream conditions across several seasons.
- *MMI Score* (decreases with increasing disturbance): This multi-metric index was developed by the U.S. EPA (2016) to assess streams across the Western Mountain Region (which includes the Rogue River) for the National River and Streams Assessment program. Thus, it has been vetted and tested for this region, and provides a helpful, broader context for assessing macroinvertebrate community health upstream and downstream from the Medford RWRF outfall.

All of these metrics previously have indicated, to varying degrees, significant impairment to the macroinvertebrate community below the Medford RWRF outfall. Going forward, a standardized assessment approach should be selected and implemented for future determination of biocriteria compliance and added to the new NPDES permit by ODEQ. To assess the macroinvertebrate communities adequately for biocriteria compliance, two components must be addressed: 1) which metrics or model will be used to assess the macroinvertebrate communities, and 2) how the selected metrics will be evaluated to determine whether the biocriteria standard has been met. Both components must be defined for reliable biocriteria compliance assessment. The following suggested conceptual framework can be used to develop the final set of selected methods, as reviewed and accepted by an advisory group of scientists with appropriate expertise.

The goal of the macroinvertebrate assessment is to determine if the ecological integrity below Medford's outfall is degraded compared to the communities measured upstream. In other words, an upstream vs downstream analysis that will be able to detect whether differences exist between upstream and downstream sample sites and, if so, whether those differences resulted from water quality impairment rather than natural variability versus sampling error. The statistical tools for these analyses are readily available and should be selected with counsel from ODEQ. By comparing the data with the multiple years of data previously collected, it will be possible to set appropriate metric limits outside of which represent biological impairment and non-compliance with the biocriteria standard.

While we suggest that biocriteria compliance below the Medford RWRF may be based on a comparison of upstream to downstream sites, it is important to recognize that the upstream sites themselves are not without impacts. For example, previous MMI scores show that upstream sites fall into the moderate, and occasionally into the poor, level of impairment category. Therefore, while they may provide an upstream control for downstream sites, they do not represent attainment of high biological health. As other control measures are put in place in the watershed to better control nonpoint sources of organic enrichment, it is expected that the biological condition of these upstream sites will improve over time. Thus, the nutrient limits for the Medford RWRF should be set to allow for additional downstream improvement as upstream water quality conditions improve.

V.A.1.b. *Benthic Algae*

Attached benthic algal abundance is probably the most established indicator of river water quality with respect to nutrient conditions, along with use of benthic macroinvertebrate indices (Newman et al. 2005). Maximal biomass is considered of most value in assessing stream health over time in response to the nutrient regime (Biggs 2000b, Paul et al. 2017 and references therein). The protocols in MT DEQ (2011), if followed in detail (Table 10), will yield reliable estimates for attached benthic algal abundance (corrected chlorophyll *a* or AFDM) and relative abundance (percent cover) per transect and per reach that can be used to compare sites and track changes in relative abundance over time.

Adequately homogenized subsamples can also be analyzed for community composition and group (e.g., diatoms, green algae, cyanobacteria) or taxa abundance as biovolume under light microscopy using well-accepted, detailed procedures. The mean Shannon-Weaver diversity index requires fewer samples per site than for biomass estimates (Biggs 1988). Many indices have been developed using overall benthic microalgal assemblages, or the diatom assemblage, or the "soft-bodied algae" assemblage to assess nutrient and associated organic pollution in rivers (e.g., Watanabe et al. 1986, Kelly et al. 1995, Kelly and Whitton 1995, Lavoi et al. 2014). We suggest that a combination of indices emphasizing both diatoms and "soft-bodied" algae such as filamentous green taxa would be most advantageous for compliance assessment of the Medford RWRF over time (e.g., Fetscher et al. 2013, 2014; Stancheva and Sheath 2016).

Both quantitative and qualitative estimates of benthic algal abundance can rapidly become complicated because some benthic algae do not stay microscopic, and do not necessarily even stay in place. Benthic algal biofilms covering various substrata (from rocks and wood to plants) are commonly considered to be up to about 2.5 centimeters (1 inch) thick (Burkholder 1996 and references therein). Filamentous algae exceeding that length are considered macroalgae (Stancheva and Sheath 2016, Lapointe et al. 2018). They can range in length from a few centimeters to several meters, and they can be much more challenging to assess; they can break away from the substrata or remain loosely attached, easily dislodged and easily lost from samples; or they can form drift accumulations against rocks or other surfaces. Thus, assessment of only attached microalgae, or excluding benthic algae on SAV, can miss most of the biomass. The following comments are intended to augment the information outlined in Table 10, considering all three components: attached microalgae, attached filamentous macroalgae, and benthic algae forming drift.

<u>Attached Microscopic Algae</u> – The MT DEQ (2011) protocols are most straightforwardly applied to attached microalgae. We emphasize here the importance of measuring the biomass of algae attached to SAV (see Figures 2 and 4), as well as the attached algae on rocks, for compliance assessment of the Medford RWRF. The maximal microalgal biomass target recommended for healthy stream conditions is 100 mg/m^2 (Biggs 2000 and references therein). Another suggested target is the amount of each year when high biomass occurs. For example, Biggs (2000) described highly nutrient-enriched streams as having more than 100 mg chl*a*/m^2 for about 40% of the year, versus moderately enriched streams with that amount of biomass for less than 1% of the year.

Benthic algal biomass on rocks and sediments can rapidly accumulate and then be scoured from the system by disturbance, sometime by even a gentle storm (Biggs 2000b, Biggs and Kilroy

Table 10. Guidance for preliminary reconnaissance to determine period(s) of maximal abundance for benthic algae and SAV, and associated water quality, and steps/procedures needed to select final protective effluent TN and TP limits.

Reconnaissance - Benthic Algae and SAV * Conduct preliminary reconnaissance, sampling at least monthly, through an annual cycle to verify the period(s) of maximum growth for the dominant algae and SAV in the river segment. This information should guide the entire subsequent compliance assessment effort in the period(s) of maximal abundance. * Determine appropriate data management and statistical procedures for all sample types before beginning assessment. Reconnaissance and Compliance Assessment - Water Quality * Characterize effluent weekly and other sites biweekly throughout the same annual cycle as above, using adequate PQLs to measure low-level nutrient concentrations for total ammonia, NOx, TKN, TP, and SRP. Biweekly sampling may capture potential lag effects of nutrient supplies. Include at least 10% duplication of samples on each sampling date (U.S. EPA 2002a). * Routine compliance assessment - sample weekly (effluent) to biweekly (the other sites in Figure 17) during the major growth periods for the primary producers.
Diel Data (salmonid spawning habitat) * Use apropriately calibrated and maintained datasondes to collect diel hourly data for temperature, pH, DO, and conductivity from April through November each year.
Benthic Algae B1and SAV (see report text for sampling frequencies) Follow MT DEQ (2011) protocols in detail, augmented by other information as needed (e.g., from Dennis and Isom 1984, Burkholder and Wetzel 1989, Barbour et al. 1999 [U.S. EPA], Biggs 2000a, Biggs and Kilroy 2000, Wetzel and Likens 2000, Moulton et al. 2002 [USGS], Suplee (2004), Flotemersch et al. 2006 [U.S. EPA]): * Use the hoop method correctly to assess both groups of primary producers and then to calculate reach-wide total benthic chl*a* and AFDM. * Include epiphytes in both percent cover and biomass estimates for benthic algae. This often will result in total percent cover estimates that exceed 100% in sites with abundant primary producers. * Chl*a*: Filter in the field (microalgae), or transport on ice in darkness to the laboratory and homogenize, then filter (filamentous algae, SAV). Follow careful homogenization steps, especially important for samples containing filamentous algae. Conduct all laboratory steps under low light (preferably, low green light), and correct for pheopigments. * AFDM: Clean samples of most debris; homogenize samples adequately before subsampling; and make measurements using a calibrated analytical balance. For SAV, collect/clean both above- and below-ground structures, and separate live from dead material prior to measurement. * Biovolume and Cell Number (benthic algae): - In all analyses, focus on algae that were viable when collected (basis: apparently intact cell contents). - Carefully homogenize samples, with extra steps needed for samples containing filamentous algae. - Check to ensure uniform settling across the counting chamber using statistical analysis of chamber transect data. - Avoid use of units because that measurement is semi-quantitative at best. For each filamentous taxon, assess at least 25 filaments for cell number and biovolume per filament. Then use linear regression analysis to obtain reliable factors for converting filament length to quantitative estimates for cell number and biovolume data. * SAV (additionally): Do not impose an arbitrary resolution or reporting limit; record quantitative percent cover on datasheets; and, for corrected chlorophyll and AFDM data, collect samples consistently on all dates to enable statistical analysis of the data.

2000, and references therein). Monthly sampling is usually recommended for characterizing periods of maximal microalgal abundance over an annual cycle (Table 10, reconnaissance). Once that information is in hand, sampling once a season can be adequate unless there are unusual weather conditions such as extended cold or early warming. Since high biomass generally accrues and persists for one to three months, however, monthly sampling during the season(s) with maximal biomass is a better approach than once per season (Konrad et al. 2016).

Benthic algae colonizing plant surfaces, called epiphytes or epiphytic algae, also need to be assessed as part of the total benthic algal abundance estimates, because they are clearly a substantial component of the primary producers in affected sites below the Medford RWRF outfall (Section II, Figures 2 and 4). The is effort can be intensive because it requires removing the epiphytes from the plant leaves and shoots without scraping the chlorophyll- and AFDM-contributing plant tissues. Care must be taken not to include plant tissues in the algal sample during the cleaning procedure because they can significantly overestimate the epiphytic algal biomass.

Samples collected for SAV biomass estimates (below) must be cleaned of epiphytes (MT DEQ 2011). That cleared epiphytic material can be thoroughly homogenized and then analyzed for biomass and AFDW estimates per unit area of river bottom as a less arduous approach than attempting to report the epiphytic algal abundance per unit plant surface area. Detailed protocols for characterizing the abundance and community structure (cell number, biovolume) of epiphytic algae are available in Burkholder and Wetzel (1989). It is recommended that thoroughly homogenized epiphytic algal samples should be viewed under light microscopy and assessed for taxa dominance by algal group (division or phylum level) and by genus (note that identification of viable cells when collected to species level is usually not possible with light microscopy).

<u>Attached and Drift Filamentous Macroalgae</u> – The general MT DEQ (2011) protocols for sampling algae per unit substratum surface area are applicable to filamentous macroalgae if applied with care. The protocol described in detail by Biggs and Price (1987) should be used, however, for thick mats of long filaments, wherein sections of the growth should be gently lifted from the river bottom until just clear of the water and 10x10 cm^2 samples should be cut from it using surgical scissors. Such samples taken for biomass estimates can be extrapolated from the area sampled to estimate biomass over the total area covered. This method was described as generally necessary for thick *Cladophora* growth. The number of replicate samples per site (usually three) varied depending on patchiness of colonization based on stratified proportional variance sampling). Biggs and Price (1987) also provide detailed protocols that should be followed for sample processing.

Filamentous macroalgae such as *Cladophora* can rapidly increase in size over a few days to 1-2 weeks (Higgins et al. 2008). For communities with abundant filamentous macroalgae, weekly to biweekly sampling during the maximal period(s) for growth is recommended to improve abundance estimates, including drift, between storm events (Newman et al. 2005, Biggs and Kilroy 2000, and references therein). For relative abundance as percent cover, MT DEQ (2011) protocols can be used along with modifications imposed for thick filamentous growth from Biggs and Price (1987). Biomass and relative abundance estimates should be similarly estimated per unit stream reach if drift filamentous algal accumulations occur.

The total maximal biomass estimate for sites with noticeable growth of filamentous algae has been recommended by some specialists as less than the biomass target for benthic microalgae lacking such algae because noxious, high-biomass algal growth can adversely impact beneficial fauna more easily through diel DO swings, smothering of habitat, and food quality shifts. For both Montana rivers and a river in the United Kingdom with abundant *Cladophora*, AFDM estimates exceeding 50 g/m^2 were considered to indicate noxious or nuisance growth (Wharfe et al. 1984, Suplee et al. 2012).

For relative abundance as percent cover, MT DEQ (2011) protocols can be used along with modifications imposed for thick filamentous growth from Biggs and Price (1987). Biomass and relative abundance estimates should be similarly estimated per unit stream reach if drift filamentous algal accumulations occur. Filamentous algal coverage estimates of 20% (Welch et al. 1988) to 30% (Biggs 2000) have been proposed as thresholds for noxious or nuisance conditions. Filamentous algal coverage of the streambed by about 20% (Welch et al. 1988) could also be used in compliance assessment as a threshold for undesirable, noxious growth.

V.A.1.c. *Submersed Aquatic Vegetation*

The protocols in MT DEQ (2011), if followed in detail (Table 10), will yield reliable estimates for SAV abundance (chlorophyll *a*, AFDM) or relative abundance (percent cover) per transect and per reach that can be used to compare sites and track changes in relative abundance over time. Here we augment those protocols with additional instructive information. All or some combination of the parameters described below can be statistically compared for upstream SAV versus SAV downstream from the Medford RWRF to assess whether the downstream community is increasingly comparable to the upstream community over time.

<u>Abundance as Chlorophyll *a* and AFDM per Square Meter</u> – – MT DEQ (2011) advises application of the hoop method even for minor macrophyte cover. Alternatively, in areas where SAV coverage is less than 5%, coverage could be noted as "minor." Entire plants (above- and below-ground biomass) should be collected. Living/senescent material is roughly separated from dead biomass (by color: green/yellow-green versus brown/black, respectively; and whitish versus black roots/rhizomes; Thomas 2013). The dead materials should be discarded. The living/senescent material should be cleaned of debris (for example, by gently wiping/rinsing, or by washing over a coarse filter with mesh size 250 μm^2). Chl*a*/m^2 (corrected for pheopigments) should be determined from the aboveground sample (leaves+shoots) after homogenization. The chl*a* data can be combined with similar data for benthic and drift algae to estimate the total biomass of primary producers/m^2 for the stream reach (MT DEQ 2011).

The remaining homogenized aboveground material should be processed following MT DEQ (2011) protocol to estimate AFDM/m^2 river bottom. Similarly, AFDM should be estimated for the belowground material. It is recommended that above- and belowground AFDM/m^2 are measured separately because valuable insights can thereby be gained about plant allocation of resources to above- and belowground tissues can vary depending on the nutrient regime (Dennis and Isom 1984, U.S. EPA 2002b, Burkholder et al. 2007, and references therein). The two measurements can be combined for a total AFDM estimate.

Relative Abundance As Percent Cover – At each transect location, cover of SAV (as well as benthic algae and filamentous algae, as above) should be estimated for a 1-m^2 area centered on the transect (11 to 16 points in total, as in MT DEQ 2011), using a viewing bucket with a 50-dot grid (Stevenson and Bahls 1999). Field datasheets should include specific percent cover estimates for each transect location, along with notes about taxa dominance if visually obvious. As mentioned previously (Section II), SAV cover at 40% has been considered a eutrophic benchmark of undesirable SAV conditions in nutrient-polluted areas (Maret et al. 2010, Chambers et al. 1999, Suplee et al. 2009). Macrophytes in rivers affected by high nutrient supplies tend to be adept at rapid growth under relatively low light (Hilton et al. 2006).

Other Useful Information: Taxa, Indices, and Tissue Content – SAV often cannot be identified to species because reproductive structures needed for that task are not present (Hamel et al. 2001, Fassett 2006). Specimens should be identified to the lowest taxon possible, usually genus.

Individual SAV taxa, groups of taxa, or communities all have been used to indicate river nutrient regime or enrichment status (e.g., Schneider and Melzer 2003, and references therein). For example, the Mean Trophic Rank (MTR) is an index based on the presence and abundance of SAV, where taxa are assigned a score based on their known tolerance of nutrient pollution (Dawson et al. 1999, Holmes et al. 1999). The Trophic Index for Macrophytes (TIM) has been used to indicate the trophic status of rivers (Fabris et al. 2009), and the Macrophyte Biological Index for Rivers (IBMR) was developed to assess eutrophication status and the degree of organic pollution (Haury et al. 2006). These indices differ in required input parameters and application. Use of one or more of them would be of value in monitoring SAV community health and nutritional status under a declining nutrient regime in the affected Rogue River segment.

Because aquatic plants are excellent integrators of seasonal nutrient conditions, the spatial and temporal variations in SAV C:N:P ratios (above- versus below-ground tissues) have been used with some success to indicate aquatic plant nutrient enrichment status in response to a major nutrient source or a changing nutrient regime (Burkholder et al. 2007, Moe et al. 2019, and references therein). Increased tissue N and P as a result of nutrient enrichment commonly has been reported, and SAV from low-nutrient habitats generally have significantly higher C:N and C:P ratios than plants from high nutrient regimes.

V.A.2 – Water Quality Monitoring Requirements

In addition to compliance assessment for biocriteria, it will also be critical to monitor adequately for water quality (Table 10) at the outflow (weekly) and both upstream and downstream from the Medford RWRF, at the four stations (biweekly) shown in Figure 17. The same methods used by Stillwater Sciences (2020) will be appropriate for compliance assessment (U.S. EPA 1993, 1997; Rice et al. 2017)—except that the laboratory procedures and instrumentation must be modified to achieve suitable PQLs (Section II, last column of Table 3) for more accurate low-level nutrient analyses of minimally impacted sites.

For continuous monitoring of diel DO, datasondes should be installed at the four sites near the outfall (Figure 17) and set to collect data hourly for temperature, DO, pH, and conductivity. These data will be needed to ensure that appropriate conditions are maintained for salmonid spawning, eggs, and young life history stages downstream from the Medford RWRF. Accordingly, datasondes should be installed in early spring after high streamflow has subsided, and should be retrieved in late November or just before the onset of high fall flows. The data will also be needed to assess whether harmful diel DO variation occurs during periods of high benthic algal and/or SAV growth. The datasondes should be calibrated/checked for calibration more frequently than cited in manufacturers' specifications when placed in areas with high abundance of microalgae and other microbes that can quickly coat sensor membranes (Reed et al. 2010).

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