Technical Review of Information Related to Development of Revised

Nutrient Criteria for Colorado Lakes

Prepared for:

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Summary

The Water Quality Control Division (WQCD) has proposed revised nutrient criteria for Colorado lakes, for consideration at the April 2023 Rulemaking Hearing of the Water Quality Control Commission (WQCC). The proposed revisions are described in the WQCD Proponent's Prehearing Statement (PPHS), associated exhibits including the N-STEPS Colorado Lakes Final Technical Report (Ex. O of the PPHS; prepared by Tetra Tech), and the WQCD Supplemental Prehearing Statement (sPHS). The purpose of this report is to 1) summarize important background information about nutrients and algal growth in lakes, 2) provide a technical review of the PPHS, associated exhibits, the data set, and the sPHS, and 3) make recommendations for development and implementation of nutrient criteria for Colorado lakes.

Suspended algae (phytoplankton) are an important source of nutrition for higher trophic levels, but high biomass of phytoplankton can cause water-quality problems that interfere with classified uses. Nutrients, temperature, water-residence time, light, and several other factors affect the growth of algae in lakes. Measures to control algal growth often have been focused on control of total phosphorus (TP), total nitrogen (TN), or both TP and TN. Generally, control of TP is the most effective and economical means to control algal biomass in lakes.

The proposed criteria for TP and TN do not appropriately balance protection of Aquatic Life and Recreation uses for Colorado lakes and should not be adopted by the WQCC without substantial revisions. Specific comments and recommendations are as follows:

1 Seasonal means cannot be estimated reliably from results for one or two sampling events, and larger sample sizes are required for nutrient-rich lakes than for nutrient-

poor lakes. The WQCD should withdraw comments regarding its intent to reduce the minimum sample size for lake assessments, and evaluation of standards compliance for individual lakes should be based on adequate assessment methodologies. If the WQCD desires methodological consistency between development and assessment of standards for nutrients, the minimum sample size for both development and assessment of standards should be at least three for oligotrophic lakes and at least five for mesotrophic and eutrophic lakes.

- 2 The proposed numeric values are over-protective for lakes with low yield of chlorophyll *a* per unit of TP or TN and would provide no real benefits with regard to protection of Aquatic Life and Recreation uses, beyond the benefits that already would be provided by statewide implementation of the 2012 chlorophyll criteria. Implementation of the proposed TP and TN criteria would be costly and could negatively affect fisheries across Colorado. The proposed TN criteria are particularly concerning because of the technological difficulties associated with N removal. The newly proposed numeric values for TP and TN should not be adopted by the WQCC. Instead, the WQCC could retain the interim numeric values for TP and TN that were approved by the US Environmental Protection Agency (EPA) in 2012, and consider adoption of those values for all Colorado lake, except where other, site-specific standards are appropriate. An alternate proposal also could be acceptable if various concerns were addressed and if sufficient time were allowed for technical review of the proposal and its implications.
- 3 In 2012, the WQCD recommended, and the WQCC agreed, that the default 5 μg/L chlorophyll *a* target for lakes with direct-use water supply (DUWS) would be applied on a discretionary basis, and application of any specific value would appropriately balance

protection of all classified uses. The WQCC should continue to consider application of the 5 μ g/L chlorophyll *a* standard on a discretionary basis, as appropriate, but should not approve adoption of the 5 μ g/L standard for all Colorado lakes with DUWS. Application of the 5 μ g/L chlorophyll *a* standard on a statewide basis would not appropriately balance protection of Aquatic Life and Recreation uses.

- 4 If an alternate proposal for TP and TN criteria is developed prior to the April 2023 Rulemaking Hearing, the new proposal should address several other important matters described here. These matters include the proposed framework for site-specific nutrient standards, lake classification, non-linear relationships between nutrients and chlorophyll *a*, and documentation of the methods and assumptions leading to the proposed criteria.
- 5 The proposed framework for site-specific nutrient standards considers only non-algal light attenuation and does not reflect the full range of factors that can alter nutrientchlorophyll relationships for lakes. Site-specific standards for nutrients should be developed from site-specific studies to define expected relationships between nutrients and chlorophyll for individual lakes.
- 6 The lake-classification (partitioning) analyses were completed for a preliminary data set, and the data set has been revised repeatedly and extensively since the partitioning analyses were completed. In addition to the variables that were considered by Tetra Tech, other variables should also be considered for classification of relationships between nutrients and chlorophyll *a*. Furthermore, nonlinear modeling approaches should be considered for lake-classification analyses.

- 7 Only paired data (i.e., chlorophyll *a*, TP, TN results for the same station and date) should be used in calculations of seasonal means. The use of unpaired data weakens the nutrient-chlorophyll relationships that are the basis for the proposed criteria.
- 8 Tetra Tech and the WQCD should provide thorough documentation of the methods associated with any revised proposal, including assumptions and decisions about data processing and data analyses. Results of statistical analyses should be reported unambiguously, and any non-standard statistical terms should be defined explicitly.
- 9 If the WQCD were to develop an alternate proposal for TP and TN criteria, it would be important to understand the expected benefits, in terms of chlorophyll reduction, for a given combination of treatment levels for TP and TN. Phytoplankton require both P and N for growth, and different treatment strategies (i.e., different combinations of investment for control of TP and TN) could achieve similar levels of algal control. Because of the technological barriers associated with N removal, the most cost effective means of attainment of a particular target for chlorophyll *a* may not result from equal investment in removal of TP and TN.

Introduction

Following the March 2012 Rulemaking Hearing, the Water Quality Control Commission (WQCC) adopted interim numeric standards for chlorophyll *a*, total phosphorus (TP), and total nitrogen (TN). The interim values were adopted for Colorado lakes (including reservoirs) larger than 25 acres, with the expectation that standards for lakes smaller than 25 acres would be developed later. The US Environmental Protection Agency (EPA) approved the interim nutrient standards that were adopted in 2012. However, in its July 2016 action letter to the WQCC, the EPA indicated that, for lakes with high yield of chlorophyll *a* per unit of TP or TN, alternative or site-specific values may be more protective than the approved, interim values. Additionally, the EPA recommended in the second enclosure to the 2016 letter that Colorado should "evaluate options for developing more protective alternative values or site-specific standards that can be applied to individual segments." The EPA noted the wide range of temperature and other physical conditions across Colorado lakes, but the EPA did not recommend that more protective, alternative values should be applied to all lakes in Colorado.

The WQCD established a technical advisory committee (TAC) in 2019 to support revision of the nutrient criteria for Colorado lakes, and Tetra Tech was contracted by the EPA to provide technical support for the WQCD and the TAC. In the Proponent's Prehearing Statement (PPHS) for the November 2022 Rulemaking Hearing, the WQCD proposed revised nutrient criteria that were developed with support from Tetra Tech and the TAC. After concerns were raised about technical aspects of the WQCD proposal and potential effects of the proposed criteria on execution of water rights, the WQCC rescheduled the November 2022 Rulemaking Hearing for April 2023. In October 2022, the WQCD submitted

a Supplemental Prehearing Statement (sPHS) that included further revisions to the proposed nutrient criteria. The chlorophyll-*a* values that are being used as targets for development of criteria for TP and TN have remained unchanged and are the same values that were adopted in 2012 (i.e., 5 µg/L for direct-use water supplies, 8 µg/L for other Aquatic Life Cold lakes, and 20 µg/L for other Aquatic Life Warm lakes), except that the WQCD has recommended adoption of the 5 µg/L standard for all lakes with direct-use water supply (DUWS). The PPHS and exhibits, including the Tetra Tech *N-STEPS Colorado Lakes Final Technical Report* dated July 20, 2022 (WQCD Ex. O of the PPHS), describe the data set, assumptions, and decisions associated with development of the proposed nutrient criteria. The sPHS describes recent revisions to the data set, changes to the procedures for screening data, and changes to the proposed standards.

The purpose of this report is to provide 1) background information about nutrientchlorophyll relationships for lakes, 2) a technical review of the WQCD proposal, including the N-STEPS report, the sPHS, and the associated data set, and 3) recommendations for development and implementation of nutrient criteria for Colorado lakes.

Background information about nutrients and algal growth in lakes

Suspended algae (phytoplankton) are an important source of nutrition for higher trophic levels, and fish production in lakes depends on production of phytoplankton biomass (Oglesby 1977, Downing and Plante 1993, Bachman et al. 1996). However, high biomass of phytoplankton (often measured as chlorophyll *a*) can cause a wide range of water-quality problems that interfere with classified uses, and in particular Aquatic Life use and Recreation use (e.g., Smith and Schindler 2009, Wurtsbaugh et al. 2019). High pH and loss

of oxygen from bottom water are common in highly productive lakes, and some groups of phytoplankton produce harmful toxins (e.g., Carmichael 1992, O'Neil et al. 2012). Also, phytoplankton growth can cause taste and odor problems and can contribute to the formation of harmful disinfection byproducts in municipal water supplies (e.g., Watson et al. 2008, Khan et al. 2021).

Growth of phytoplankton requires carbon, nitrogen, phosphorus, and other elements that are the building blocks of algal biomass. Alfred Redfield recognized that marine plankton communities have relatively constant N:P ratios (Redfield 1934), although N:P ratios of phytoplankton cells vary somewhat from the nominal Redfield ratio of 16:1 (molar ratio, 7.2:1 as a mass ratio; Smith 1982, Falkowski 2000). Growing algal cells incorporate dissolved, bio-available forms of P and N into biomass, and some groups of cyanobacteria obtain supplementary nitrogen for growth through biological N fixation (e.g., Bradburn et al. 2012). Populations of algal cells can continue to grow as long as bio-available forms of P and N are present and other factors do not limit growth. Ultimately, the maximum biomass of algae in a lake is limited by the total amount of P or N (including N from biological N fixation) that is available for assimilation, depending on which of the two is more scarce relative to growth requirements. However, growth may be co-limited if the N:P ratio of available nutrients matches the requirements for growth, and biomass may not reach the potential maximum set by nutrient requirements if factors other than P or N limit growth.

Nutrient limitation can be demonstrated empirically by an increase in algal growth following addition of one or more limiting nutrients (e.g., Morris and Lewis 1988, Lewis et al. 2008). In lakes where availability of P or N limits growth, phytoplankton biomass as chlorophyll *a* can be strongly correlated with the concentration of the limiting nutrient

(e.g., Dillon and Rigler 1974). However, many factors in addition to P and N can limit algal growth in lakes. For example, growth can be limited by the supply of any required element (e.g., diatoms require silica for growth; Thamatrakoln and Hildebrand 2008). Light availability also limits algal growth, and the upper bounds on algal biomass in nutrient-rich lakes often are determined by light availability (e.g., Krause-Jensen and Sand-Jensen 1998). In addition to self-shading by algal cells, non-algal particles and dissolved humic substances (colored organic matter derived primarily from the decomposition of plants) reduce light available to support phytoplankton growth. Temperature controls rates of biological processes, and even where nutrients are abundant, growth rates of algae are suppressed at low temperatures. Additionally, water temperature affects vertical mixing and thereby indirectly affects the light environment of phytoplankton cells, nutrient availability, and the seasonal succession of phytoplankton communities (e.g., shifts in dominance from diatoms to cyanobacteria). Response of algal growth to nutrients can be suppressed in lakes with short water-residence time (e.g., Dillon 1975, Romo et al. 2013), and residence time is an important factor controlling phytoplankton growth in many Colorado lakes. Finally, depth and other morphometric features of lakes can affect phytoplankton growth (Sakamoto 1966, Fee 1979; Figure 1).



Figure 1. Relationship between maximum phytoplankton biomass, as chlorophyll *a*, and lake depth. Data are from the World Lake Database of the International Lake Environment Committee Foundation (ILEC) and J. McCutchan (unpublished data).

Because factors other than nutrients can limit phytoplankton growth in lakes, and because such factors vary spatially and temporally, prediction of phytoplankton biomass or chlorophyll *a* from TP or TN usually is subject to large uncertainties. Even for individual lakes or small groups of lakes with common physical characteristics, nutrient-chlorophyll relationships can be weak. However, nutrient-chlorophyll relationships are strongly affected by data aggregation, and relationships between chlorophyll and nutrient concentrations can be much stronger for seasonal-mean values than for individual observations (e.g., Jones et al. 1998; see also Ex. P of the PPHS). Limitation by non-nutrient factors is more likely at high concentrations of TP and TN than at low concentrations, and limitation by N is more likely at high concentrations of P than if P availability is low. Thus, for log-log plots, non-linear (e.g., sigmoidal) relationships between TP or TN and chlorophyll are common for large data sets that span a wide range of nutrient concentrations (McCauley et al. 1989, Phillips et al. 2008, Dolman et al. 2012). For lakes generally, temperature, water-residence time, and light availability play important roles in the control of algal growth. For individual lakes, it may not be feasible to control such factors in order to control algal growth. Therefore, measures to control phytoplankton growth often have been focused on control of TP, TN, or both TP and TN. Diversion of sewage from Lake Washington demonstrated the potential for phosphorus control as a means to control phytoplankton growth in lakes (Edmondson 1970). For most lakes, substantial reduction of P loading is accompanied by reduction of algal biomass (e.g., Smith and Shapiro 1981), although modest reductions of P may not correspond to reductions of chlorophyll *a* because many different factors can limit algal growth. Generally, however, control of P has been the most effective and economical means to control phytoplankton biomass in lakes (Fee 1979, Smith 1982, Smith and Schindler 2009).

Control of phosphorus has been highly effective in controlling algae, but some attempts to control algal biomass have involved dual control of nutrients (i.e., control of both P and N). Phytoplankton growth responds positively to additions of P + N in many lakes, and algal growth in downstream ecosystems may be limited by N (e.g., coastal-marine systems; Paerl et al. 2016, Wurtsbaugh et al. 2019). For many anthropogenic sources of nutrients, the molar N:P ratio is less than the 16:1 Redfield ratio. Also, microbial denitrification and annamox can remove large amounts of fixed N from N-rich systems (Pribyl et al. 2005, Piña-Ochoa and Álvarez-Cobelas 2006, McCutchan and Lewis 2008). Thus, the N:P ratio tends to be lower in eutrophic (nutrient-rich) lakes than in oligotrophic (nutrient-poor) lakes (Figure 2). Where the N:P ratio is low, including many nutrient-rich lakes in Colorado, addition of N may stimulate algal growth. Modest reduction of P in lakes with low N:P ratios may not be accompanied by reductions in algal biomass, especially if P is high prior



Figure 2. Relationship between seasonal-mean total nitrogen (TN) and total phosphorus (TP) for Colorado lakes. The bold line indicates the nominal Redfield ratio of 16:1 (molar ratio; mass ratio = 7.2:1), which is typical of phytoplankton biomass.

to initiation of phosphorus control (e.g., Cherry Creek Reservoir; Lewis et al. 2008). However, emphasis on N reduction in efforts to control phytoplankton growth may be unwarranted or even ill advised in some nutrient-rich lakes with low N:P ratios. Some groups of cyanobacteria can compensate for N reduction through biological N fixation, and cyanobacterial dominance is common in lakes where algal biomass is high and N:P ratios are low (Smith 1983, Downing et al. 2001, Schindler et al. 2008). For lakes with high concentrations of total P, N reduction does not necessarily lead to reduction or chlorophyll *a* (Filstrup and Downing 2017). Thus, N control is important as a supplement to P control, but P control is generally the most effective and economical means to control algal biomass in lakes. Where N is controlled specifically to prevent the harmful growth of algae, targets for N control should be considered carefully to avoid unwanted consequences and inefficient use of public resources.

Description of the proposed revisions to nutrient criteria for Colorado lakes

The WQCD PPHS proposes revised criteria for TP and TN for lakes larger than 25 acres, to be considered by the WQCC. The PPHS and the Tetra Tech *N-STEPS Colorado Lakes Final Technical Report* (Ex. O of the PPHS) describe the data that were assembled by CDPHE for development of the newly-proposed criteria, the process of preparing the data for analysis, decisions about classification of lakes, and the methods of data analysis leading to the proposed criteria. The sPHS describes further revisions to the data set, procedures for screening data, and the proposed standards.

Assembly and processing of the data set – CDPHE provided Tetra Tech with waterquality data for almost 200 Colorado lakes. The data set, which reflects sampling and field measurements by local, state, tribal, and federal organizations, includes information about chlorophyll *a*, phosphorus fractions, nitrogen fractions, and other water-quality variables. Because the data were collected by many different organizations, some data processing was necessary before nutrient-chlorophyll relationships could be analyzed. The data set was first made available to the public in November 2021, and a revised data set was made available on July 28, 2022. After further revisions, a third version of the data set was made available with the October 5, 2022 sPHS. This third version of the data set was used in the development of the standards proposed in the sPHS and represents 1384 lake-years (data for chlorophyll a plus TP and/or TN) for 159 Colorado lakes. The sPHS data set includes 23 files (csv format; comma-separated values) that contain results for individual samples and field measurements. Also, the sPHS data set includes a file with means of replicate values for a given station and depth (SiteDate.csv), a file with seasonal-mean values for the primary station on each lake (LakeYear.csv), and other supporting documents.

Data submitted by individual organizations were standardized to achieve consistency of units, variable names, and lake names. Results for lakes less than 20 acres and results for laboratory blanks and other quality-analysis samples were excluded from analyses. Results of replicate analyses for the same station and depth on a given date were averaged. Concentrations reported as zero or below detection were set to half the detection limit. Analyses were restricted to data collected since 1990 and to results for a single station on each lake, typically near the dam or the middle of the lake. Only results for July – September were used in development of the proposed criteria. The minimum sample size for calculation of seasonal-mean values was a single (n = 1) sampling date from the July – September season.

In addition to seasonal-mean values for measured variables, two sets of derived values were calculated. For each sampling event, the TN:TP ratio was calculated as an indicator of nutrient limitation. Expected Secchi transparency (Z_{Secchi}) was predicted from chlorophyll a, as follows (Equation 1; Carlson 1977):

 $ln(Z_{Secchi}, m) = 2.04 - 0.68 ln(chlorophyll a, \mu g/L)$ Equation 1 The ratio of observed to expected Secchi transparency (Secchi O/E ratio) was used as an indicator of non-algal light attenuation.

Classification of lakes – The EPA encouraged the WQCD to consider the effects of temperature on the relationships between nutrient concentration and chlorophyll *a* (i.e., differences in nutrient-chlorophyll relationships between warm and cold lakes). Nutrientchlorophyll relationships were analyzed to identify significant partitions. Aquatic Life Use (Warm, Cold), ecoregion (Plains, Rockies, Xeric), and lake type (natural lake, reservoir)

were considered as categorical variables, and lake area, elevation, Secchi O/E ratio, and TN:TP ratio were considered as continuous variables.

Development of the proposed criteria – A four-step process was used to derive the proposed criteria for TP and TN. The interim chlorophyll values have an allowable exceedance frequency of one in five years and were adjusted from 80th percentiles to median values, using relationships developed from results for a small set of well-sampled lakes; separate relationships were used for Aquatic Life Cold and Aquatic Life Warm lakes. Quantile regression then was used to derive targets for seasonal-mean values of TP and TN. The 0.75 quantile was chosen to represent relationships between nutrients and chlorophyll *a*, for lake-years with high yield of chlorophyll *a* per unit of TP or TN. The resulting seasonal-mean concentrations of TP and TN then were converted to 80th percentile values from the relationships between mean and 80th percentile for well-sampled lakes. The proposed numeric values are shown in Table 1, along with the corresponding values that were adopted in 2012.

Table 1. 2012 criteria for chlorophyll *a* and newly-proposed criteria for total P and total N. Current (interim) criteria for total P and total N (strike-through) are shown for comparison.

	0	Criteria values, μg	/L
Aquatic life use	Chl. <i>a*</i>	Total P**	Total N**
Cold lakes	8	25 20	4 26 380
Warm lakes	20	83 40	910 610

* adopted 2012; proposed application of 5 μ g/L standard to all lakes with DUWS ** proposed; site-specific adjustment for Secchi O/E ratio

Comments on the proposed revisions to nutrient criteria for Colorado lakes

The sPHS data set that was used for development of the proposed criteria was audited to identify errors and assumptions that could invalidate the proposed criteria. Important decisions about partitioning the data set and the development of the proposed criteria were also considered. The purpose of this section is to describe the consequences of the audit findings and various concerns related to development of the proposed criteria for TP and TN.

Detection levels– The PPHS states that each non-detect value was set to half the value of the reported detection limit. Some values below detection originally had been processed incorrectly, but the sPHS explains the revisions that were made to provide consistency for values below detection. Detection limits were defined variously, and the type of detection limit was not specified for approximately 40% of the values in the data set (Table 2). Detection limits were most commonly defined as method detection levels (MDL) or lower reporting levels (LRL), but historical lower reporting limits and practical quantitation levels (PQL) also were used.

	Number of values								
Detection-limit type	Chlorophyll <i>a</i>	Inorganic N (nitrate, nitrite)	Nitrate-N	Nitrite-N	Kjeldahl N	Total N	Soluble reactive P	Total dissolved P	Total P
Historical lower reporting limit	2	22	0	0	2	0	0	0	18
Method detection level (MDL)*	1715	1074	236	63	1114	189	128	88	1474
Lower reporting level (LRL)**	875	424	308	314	406	1182	76	0	1719
Practical quantitation limit (PQL)	0	0	0	0	81	14	0	9	99
Not specified	2160	95	722	355	211	964	521	756	1646

Table 2. Types of detection limits reported in the 23 csv files of the sPHS data set.

* same as method detection limit

** same as laboratory reporting limit

Detection limits varied widely within and among variables (Figure 3). Reported detection limits typically are less than 1 µg/L for chlorophyll *a* and less than 0.1 mg/L for nitrate-N and nitrite-N. For most analyses, detection limits for Kjeldahl N and TN were less than about 0.5 mg/L, and detection limits for P fractions typically were less than 0.01 mg/L. However, some analyses for Kjeldahl N and TN had detection limits above the proposed numeric values for TN. Many of the results used in the Tetra Tech analyses are based on methods that satisfied the analytical requirements dictated by Regulation 85. Because the numeric limits associated with Regulation 85 are much higher than the values that are being proposed here, some monitoring results that were collected to evaluate compliance with Regulation 85 may not be suitable for development of nutrient criteria associated with Regulation 31.



Figure 3. Range of detection limits (DetectionLimit1_Value) for selected variables. Boxes show medians, 25th percentiles, and 75th percentiles; whiskers show ranges, except values more than 1.5 times the interquartile range are shown as outliers (orange symbols).

USGS data – Data provided to Tetra Tech by the WQCD include data collected by the US Geological Survey (USGS). The USGS data file (USGS.csv) that was made available with the PPHS includes data for 44 Colorado lakes. The revised version of the USGS data file that was released with the sPHS includes data for only 27 Colorado lakes. An audit of the earlier (PPHS) file identified notable problems associated with TN results and sampling depths. The sPHS describes measures that were taken to resolve these problems.

TN data in the original (PPHS) USGS data file were analyzed by the AKP01 method (Nutrients, unfiltered water, acidified, alkaline-persulfate digestion, continuous flow colorimetry) or the ALGOR method (Computation by NWIS algorithm). ALGOR TN values were reported by USGS with a less-than symbol (<) preceding each numeric value; these results were assumed to be below detection and were divided by two. The ALGOR values are not measured values, and ALGOR values divided by 2 do not represent TN. Instead, measurements by the AKP01 method or calculated values (i.e., the sum of TKN, nitrate-N,

and nitrite-N) should have been used for TN. Retention of the several hundred ALGOR values that were divided by 2 would bias the numeric values for the TN criteria.

Water-quality data provided by CDPHE to Tetra Tech include results of sampling and field measurements across multiple depths. According to the Final Technical Report, proposed criteria were developed from results for surface samples only (e.g., top, surface, upper 1 m of the water column, photic zone), and data for other depths were excluded from analyses. However, data for many deep-water samples, including samples from near the bottom of the water column, were carried forward in calculations of seasonal-mean values that were presented with the PPHS. Comparison of results in the PPHS csv file with results accessed directly from the National Water Information System (NWIS) confirmed that many of the rows in the csv file for USGS data represented samples from deep water (e.g., > 50 m). Chlorophyll *a*, temperature, and dissolved oxygen can differ greatly between surface water and bottom water, and concentrations of nutrients can differ between surface water and bottom water for various reasons (e.g., nutrient release from sediments during periods of stratification). Because the earlier csv file for USGS data (PPHS data set) reflects sampling from more than 40 lakes, failure to exclude results of bottom samples and other deep-water samples from analyses could cause biases in the proposed criteria for TP and TN.

The sPHS describes steps taken by the WQCD to resolve the problems identified with the USGS data associated with the PPHS. The revised (sPHS) data from the USGS includes values for only a limited set of parameter codes (PCodes; Table 3), and all results for samples from depths greater than 5 feet or 1 m were excluded from analyses. In addition to the

PCode	Description
00003	Depth in feet
00098	Depth in meters
00078	Secchi depth in feet
79701	Secchi depth in meters
00665	Phosphorus, mixed forms, total
62855	Nitrogen, mixed forms, total
70953	Chlorophyll <i>a</i>
00625	Kjeldahl nitrogen
00630	Inorganic nitrogen

Table 3. List of parameter codes (PCode) included with the revised (sPHS) version of the USGS data set (USGS.csv).

parameter codes listed in Table 3, many other parameter codes for USGS analyses are relevant to chlorophyll *a*, TP, TN, and algal growth in lakes. Also, the mixed layer of a lake can extend well below a depth of 5 feet or 1 m. As a result of the steps taken by the WQCD to resolve the problems identified with the PPHS data set, valuable data collected by the USGS were excluded from the analyses for development of the proposed criteria. Table 4 compares the USGS data set for the PPHS with the data set for the sPHS. All values for temperature and dissolved oxygen were removed. Nearly all values for TP and TN, including all values for the AKP01 TN method, were removed.

Number of values October revision **Final Characteristic** August revision Reduction, % (PPHS data set) (sPHS data set) Chlorophyll a 618 347 43.9 Depth, Secchi disk depth 236 181 23.3 Inorganic nitrogen (nitrate and nitrite) 2021 99.2 17 Kjeldahl nitrogen 47 97.2 1685 Total phosphorus, mixed forms 3143 78 97.5 Temperature, water 2340 0 100 Dissolved oxygen (DO) 2094 0 100

Table 4. Comparison of sample size between the August and October revisions of the USGS data set (USGS.csv).

Minimum sample size – The WQCD and Tetra Tech determined that a single sampling event within the July – September season would be adequate for calculation of seasonalmean values used for development of the proposed criteria. Additionally, the WQCD has indicated its intent to change the 303(d) listing methodology for lakes, such that the sample-size requirement for seasonal means (chlorophyll *a*, TP, TN) would be reduced from at least three samples ($n \ge 3$) to a single sample ($n \ge 1$). The WQCD explained in the PPHS that a requirement of $n \ge 3$ would limit its ability to assess lakes on a statewide basis. A single sample (n = 1) per lake-year was the most common for the sPHS data set, followed by monthly sampling (n = 3) and two samples per month (n = 6; Figure 4).



Figure 4. Variation in sample number (growing-seasons means) across lake-years for TP and chlorophyll *a* (left panel) and TN and chlorophyll *a* (right panel).

The WQCD would prefer that the assessment methodology be consistent with the methodology used for development of standards for chlorophyll and nutrients. However, the chlorophyll criteria adopted in 2012 already were derived from analyses of seasonal-means with $n \ge 3$. Thus, assessments based on n = 1 sample would be inconsistent with the methodology that was used for development of the chlorophyll standards. The *2002*

Colorado Nutrient Criteria Development Plan states that "a monitoring plan for determining appropriate nutrient criteria should be designed to detect ... nutrient and algal conditions with statistical rigor", and Ex. P of the PPHS (2012 WQCD rebuttal Ex. 24) gives a detailed explanation supporting the requirement of $n \ge 3$ for seasonal means. Also, other states that have adopted criteria for chlorophyll *a* and nutrients recognized the need for repeated sampling across the growing season and have required a minimum of three or more samples for assessments (e.g., Florida, Missouri, Oregon, West Virginia).

If seasonal variation is very low, the result for a single sampling event could provide a reasonable estimate of the seasonal-mean value. If seasonal variation is high, however, the result from a single sampling event would not provide a reliable estimate of the seasonal mean. Figures 5 – 7 show ranges (July – September for each year) of chlorophyll *a*, TP, and TN for Dillon Reservoir, Cherry Creek Reservoir, and Barr Lake, respectively. For each of the three lakes, concentrations are highly variable for some years, and selection of single values at random for a given year would not provide reliable estimates of the seasonal-mean values.



Figure 5. Seasonal (July – September) ranges of chlorophyll *a*, TP, and TN for Dillon Reservoir. Boxes show medians, 25th percentiles, and 75th percentiles; whiskers show ranges, except values more than 1.5 times the interquartile range are shown as outliers (orange symbols).



Figure 6. Seasonal (July – September) ranges of chlorophyll *a*, TP, and TN for Cherry Creek Reservoir. Boxes show medians, 25th percentiles, and 75th percentiles; whiskers show ranges, except values more than 1.5 times the interquartile range are shown as outliers (orange symbols).



Figure 7. Seasonal (July – September) ranges of chlorophyll *a*, TP, and TN for Barr Lake. Boxes show medians, 25th percentiles, and 75th percentiles; whiskers show ranges, except values more than 1.5 times the interquartile range are shown as outliers (orange symbols).

Analysis of the sPHS data set further illustrates the effect of sample size on variation in seasonal-mean values (Figure 8). For lakes that were sampled eight or more times during the July – September season, seasonal-mean values of chlorophyll *a*, TP, and TN fall within a range of about 1.5 – 2 orders of magnitude. For lakes that were sampled only once during the July – September season, the seasonal means vary over a range of about 3 orders of magnitude.



Figure 8. Effect of sample number on range of seasonal-mean values for chlorophyll *a*, total phosphorus, and total nitrogen for Colorado lakes.

For measurements of chlorophyll *a*, TP, and TN over the July – September season, the standard deviation (SD) increases with the seasonal mean (Equations 2 – 4; Figure 9). These relationships between standard deviation and seasonal mean are similar to the relationship shown by the WQCD in its 2012 Prehearing Statement (Ex. H of the WQCD PPHS). Furthermore, these relationships provide a basis for predicting the range of variation for individual measurements and seasonal-mean values over the July – September season. Figure 10 shows predictions of chlorophyll *a* for lakes with seasonal-mean chlorophyll *a* equal to 8 and 20 μ g/L, as single samples and means (n = 3, n = 7). The predicted range of values for individual measurements is very broad even for lakes with seasonal-mean chlorophyll *a* of 8 or 20 μ g/L, and variation would be higher still for lakes with higher seasonal-mean chlorophyll concentrations. Even for a lake with seasonal-mean chlorophyll *a* equal to 8 μ g/L, individual values are expected to vary from less than 2 μ g/L to over 20 μ g/L.

SD, Chl. a = $Exp(-1.07 + 1.14*ln(Seasonal-mean, chl. a)); r^2 = 0.85$	Equation 2
SD, TP = Exp(-1.14 + 0.98*Ln(Seasonal-mean, TP)); r ² = 0.77	Equation 3
SD, TN = EXP(-1.50 + 1.28*Ln(Seasonal-mean, TN)); r ² = 0.60	Equation 4



Figure 9. Relationships between standard deviation and seasonal mean for chlorophyll *a*, TN, and TP, for Colorado lakes that were sampled 3 or more times within the July – September season.

Mean = $8 \mu g/L$; SD = $3.67 \mu g/L$

Mean = 20 μ g/L; SD = 10.4 μ g/L



Figure 10. Predicted range of single samples (n = 1) and seasonal means (n = 3, n = 7) for chlorophyll *a*, at seasonal-mean concentrations of 8 (left panels) and 20 μ g/L (right panels). Concentrations were selected randomly from log-normal distributions with standard deviations predicted by Equation 2 (SD = 3.67 μ g/L for a mean of 8 μ g/L, SD = 10.4 μ g/L for a mean of 20 μ g/L).

For practically any lake, including unproductive lakes such as Dillon Reservoir, reliable estimates of seasonal-mean values would require results from multiple sampling events. Figure 10 shows the effect of sample size (n = 1, n = 3, n = 7) on expected variation in seasonal-mean chlorophyll *a*. Although the range of variation decreases markedly with increasing sample size, seasonal-mean values for n = 7 extend from about 50% of the true mean to about 50% above the true mean. Even with 25 samples over the July – September season (i.e., about twice per week) for a lake with a true seasonal mean of 8 μ g/L chlorophyll *a*, the probability of a seasonal mean greater than 9 μ g/L is almost 10%. If uncertainty in seasonal means and the relationship between sample size and uncertainty are not considered in assessments, many lakes could be incorrectly listed for impairment, or incorrectly listed as unimpaired.

The relationships shown in Figures 8 – 10 indicate that three sampling dates would be inadequate for reliable determination of seasonal-mean values for some lakes, particularly for mesotrophic and eutrophic lakes. Figure 11 shows the effect of sample size on the relationships between chlorophyll *a* and nutrient concentrations for Colorado lakes. The slopes of relationships are affected by sample size for this data set, and the relationships are much stronger for means with large sample size ($n \ge 5$; $r^2 = 0.67$ for TP and $r^2 = 0.79$ for TN) than for means with small sample size ($n \le 2$; $r^2 = 0.40$ for TP and $r^2 = 0.38$ for TN). The WQCD also found that nutrient-chlorophyll relationships are stronger for seasonal means ($n \ge 3$) than for grab samples (n = 1; Ex. P, 2012 WQCD rebuttal Exhibit 24), and many examples from the peer-reviewed literature (e.g., Jones et al. 1998) support the conclusion that a small sample size is inappropriate for both development and assessment of nutrient criteria.



Figure 11. Effect of sample size for seasonal means on relationships between chlorophyll *a* and nutrient concentrations for Colorado lakes. The solid dark lines show the lines of fit for the regressions of chlorophyll *a* against TP (left panel; $r^2 = 0.67$) and TN (right panel; $r^2 = 0.79$), with $n \ge 5$; gray shading indicates the 95% confidence limits for the regressions with $n \ge 5$. The solid dark lines show the lines of fit for the regressions of chlorophyll *a* against TP (left panel; $r^2 = 0.38$), with $n \le 2$; gray shading indicates the 95% confidence limits for the regressions with $n \le 5.56$ confidence limits for the regressions with $n \le 5.56$ confidence limits for the regressions with $n \le 2$; gray shading indicates the 95% confidence limits for the regressions with $n \le 2$.

Use of paired data – For each lake, seasonal-mean values were calculated separately for chlorophyll *a*, TP, and TN. Paired measurements (i.e., chlorophyll *a*, TP, TN all collected on the same date) were not required, as was the case for development of the approved (2012) interim criteria. Failure to use only paired measurements could mask relationships between chlorophyll *a* and nutrients, and this problem is compounded for lakes that were sampled infrequently. For example, a single TP measurement from late July (i.e., near the warmest time of the year) might not have any meaningful relationship to a single chlorophyll *a* measurement from late September.

Lake classification – The EPA recognized the importance of temperature as a factor controlling algal growth in lakes. In response to the recommendation for development of separate empirical relationships for warm and cold lakes, Tetra Tech evaluated nutrient-chlorophyll relationships for different classes of lakes. Aquatic Life Use (Warm, Cold),

ecoregion (Plains, Rockies, Xeric), and lake type (natural lake, reservoir) were considered as categorical variables, and lake area, elevation, Secchi O/E ratio, and TN:TP ratio were considered as continuous variables. Tetra Tech showed results of statistical analyses for the split between Aquatic Life Use Warm and Aquatic Life Use Cold lakes, but similar information about results of statistical tests was not shown for partitioning of the data set based on other classifications. Furthermore, the decision to classify lakes on the basis of Aquatic Life Use was made before the data set was finalized, and no temperature data are included in the sPHS version of the USGS data set.

Seasonal-mean temperature differs significantly between Aquatic Life Cold and Aquatic Life Warm lakes in Colorado, but there is substantial overlap in temperature between Cold and Warm lakes (Figure 12). Algal growth is more directly related to temperature than to Aquatic Life Use, but temperature was not considered as a variable for partitioning the data set. Also, algal growth in lakes varies with lake depth and water-residence time, but neither depth nor residence time was considered by Tetra Tech as a partitioning variable. Furthermore, the Tetra Tech Report does not provide enough information to evaluate whether Aquatic Life Use is the most appropriate variable for partitioning the relationships between nutrient concentrations and chlorophyll *a*. Decisions about lake classification (and covariates for regression analyses, see below) are important for development of nutrient criteria because statewide relationships between chlorophyll *a* and any single variable (TP, TN, temperature, depth, water-residence time, etc.) can have poor predictive power, particularly for analyses of grab-sample (n = 1) data.



Figure 12. Water temperatures (July – September) for Cold and Warm lakes. Boxes show medians, 25th percentiles, and 75th percentiles; whiskers show ranges, except values more than 1.5 times the interquartile range are shown as outliers (orange symbols).

In the sPHS data set, the ranges of TP and TN are larger for Aquatic Life Warm lakes than for Cold lakes. Because non-linear relationships between nutrients and chlorophyll *a* are common for data sets that span a wide range of concentrations, the significant split (partition) between Aquatic Life Cold and Warm lakes may reflect the relatively narrow range of values for Cold lakes and the choice of linear regression models, rather than a fundamental difference in the nutrient-chlorophyll relationships between Cold and Warmlakes in Colorado. If the relationships were developed for data sets spanning similar ranges of concentration, or if non-linear regression models were used, it might be appropriate to derive a single set of relationships for Cold and Warm lakes. At least for Warm lakes, there is significant non-linearity for the relationship between chlorophyll *a* and TP. There is no indication of a non-linear relationship between chlorophyll and TP for Cold lakes, but the range of TP values is narrow for Cold lakes, and the relationship between chlorophyll and TP for Cold lakes is not strong ($r^2 = 0.11$; Figure 13).



.1.2.3.4.5.6.7.8.9 Quantile Density Contours

Figure 13. Quantile-density contours for relationships between chlorophyll *a* and nutrient concentrations (Cold lakes, upper panels; Warm lakes, lower panel). Contours show the probability densities for lake-years in the LakeYear file of the sPHS data set.

Development of the proposed criteria – Interim table-value standards for chlorophyll *a* were adopted in 2012 for protection of Aquatic Life use and Recreation uses. Revised criteria for TP and TN are being considered to prevent harmful growth of algae that could jeopardize these uses. The proposed criteria for phosphorus were developed from empirical relationships between TP and chlorophyll *a*, and Secchi O/E ratio (the ratio of observed to expected Secchi transparency) was considered as a covariate for Aquatic-Life Cold lakes. Separately, the proposed nitrogen criteria were developed from empirical

relationships between TN and chlorophyll *a*. Thus, the proposed criteria for TP do not depend on information about TN, and vice versa.

Information about projected attainment of the proposed standards is shown in Figure 14. These data indicate that about 30% of Cold lakes and over 40% of Warm lakes in the sPHS data are impaired for chlorophyll *a*. However, the seasonal-mean values for TP and TN would exceed the proposed table values in four of five years for 55 – 65% of lakes in the data set. Lakes that fall in the lower right quadrant of the graphs in Figure 14 indicate over-protection with regard to TP or TN (i.e., attainment of the chlorophyll *a* standard without attainment of the TP standard or TN standard).

Figure 14. Relationships between chlorophyll *a* and TP (left panels) and TN (right panels) for lakes in the sPHS data set. Values are 80th percentiles across lake-years. Upper panels show relationships for Cold lakes, and lower panels show relationships for Warm lakes. Horizontal dashed lines indicate the numeric chlorophyll *a* values of 8 μ g/L and 20 μ g/L for Cold and Warm lakes, respectively. Vertical dashed lines indicate the proposed numeric values for TP and TN.



The purpose of nutrient criteria is fundamentally different from the purpose of criteria for most other regulated constituents. For TP and TN, implementation of criteria is not to protect classified uses directly, but rather to guard against harmful growth of algae (chlorophyll *a*), which does pose a direct risk to specific classified uses. If attainment of criteria for TP is sufficient for attainment of the chlorophyll *a* standards, and therefore sufficient for protection of the relevant classified uses for a lake, concurrent attainment of the proposed criteria for TN would be redundant. Similarly, if attainment of criteria for TN is sufficient for attainment of the chlorophyll *a* standards, attainment of criteria for TN criteria would be unnecessary. This type of redundancy often is unnecessary and may be costly or even harmful.

Although dual control of nutrients is appropriate, algal growth requires both phosphorus and nitrogen. Therefore, the status of P control and other site-specific factors should be considered in any decisions about N control. Dominance by cyanobacteria, including groups that produce toxins, is common in lakes with high nutrient concentrations and low TN:TP ratios. Where P is abundant, enrichment with N can stimulate production of toxins by some taxa (e.g., Dolman 2012). However, as long as P is abundant, reduction of N without P reduction can maintain conditions that favor dominance by N-fixing cyanobacteria. Under certain conditions, N reduction may even stimulate algal growth (Filstrup and Downing 2017). Ultimately, the primary concern related to prevention of harmful algal growth is high concentrations of P. Where concentrations of P are low, phytoplankton generally do not cause water-quality problems in lakes.

TP and TN differ with regard to treatment technologies. Even the most efficient biological-treatment processes do not reduce TN concentrations to the range of

background concentrations for many natural systems, and biological N removal is energy intensive (McCarty 2018). Treatment by reverse osmosis (RO) can reduce nitrogen in wastewater to low levels but is costly, and disposal of RO brine is problematic. In contrast, phosphorus concentrations can be reduced to low levels with existing treatment processes (e.g., facilities upstream of Dillon Reservoir), although the cost of phosphorous removal is not trivial.

Section 31.17 (i) of *The Basic Standards and Methodologies for Surface Water* (Regulation 31) allows for site-specific flexibility though adoption of alternatives to the table-value standards for chlorophyll *a*, TP, or TN. Thus, if it is determined that the table values for specific segments are not sufficiently stringent, or are overly stringent, the WQCC can adopt alternate standards for those specific segments. Thus, the WQCC already has adopted regulations to ensure the balanced protection of Aquatic Life use and Recreation use. Statewide adoption of nutrient criteria that are overly protective for many lakes in Colorado was not recommended by the EPA in its 2016 action letter, nor is it necessary.

Recommendations for revisions to the proposed nutrient criteria

The WQCD PPHS for the April 2023 Rulemaking Hearing proposes revised criteria for TP and TN for Colorado lakes. The proposed criteria are based on analyses of a new data set for Colorado lakes, as described in the PPHS, Ex. O of the PPHS (N-STEPS Colorado Lakes Final Technical Report), and the sPHS. The WQCD and its partners have addressed many of the data problems that were identified before the hearing was rescheduled by the WQCC. However, other critical matters have not been addressed by the WQCD. Decisions about

minimum sample size weaken the strength of relationships between chlorophyll *a* and nutrient concentrations, and more importantly, seasonal means cannot be estimated reliably from results of a single sampling event. Contrary to the assertions of the WQCD, the use of quantile regression in development of criteria for TP and TN and independent development of criteria would not provide further protection for Aquatic Life use and Recreation use, and the proposed criteria are over-protective for lakes with low yield of chlorophyll *a* per unit of TP or TN. Implementation of the proposed criteria would lead to unnecessary public spending by diverting funding away from problems that directly affect Aquatic Life and Recreation uses. Furthermore, the WQCD should withdraw its proposal to apply the 5 µg/L chlorophyll *a* standard to all lakes with DUWS. If these and other matters cannot be addressed prior to the April 2023 Rulemaking Hearing, the WQCC should not adopt the proposed criteria but should instead retain the 2012 interim numeric standards for TP and TN, which already have been approved by the EPA.

Minimum sample size – Historically, the WQCD has required at least three sampling events per season for lake assessments, and both the WQCD (Water Quality Control Division 2013) and the EPA (United States Environmental Protection Agency 2000) recommend that monitoring for development of nutrient criteria should be conducted in a statistically rigorous manner. Additionally, the EPA specified that "sampling should occur repeatedly during the growing season to be able to precisely characterize individual lakes" and that "statistical power analysis can be used to determine the appropriate sample size based on the purpose of the sampling and the acceptable error" (United States Environmental Protection Agency 2000). The WQCD has arbitrarily set the minimum sample size for standards development to n = 1 and is proposing to set the minimum

sample size for assessments to n = 1, in order to maintain methodological consistency between development and assessment of standards. The logic in these decisions is flawed. The minimum sample size for assessments should be determined according to the requirements related to the desired statistical precision, as recommended by the EPA (see also Figures 9 – 10 and associated text above), and then the sample size for standards development should be set equal to the sample size chosen for assessments.

Nearly half of the seasonal-mean values used to derive the proposed criteria are based on only one or two measurements within the July – September season. Even if analytical precision for individual measurements is excellent, seasonal means cannot be reliably estimated from only one or two values. For mesotrophic and eutrophic lakes in Colorado, three sampling dates would be inadequate for reliable calculation of seasonal-mean values (Figures 5 – 10). For development of criteria for TP and TN, seasonal-mean values for chlorophyll *a*, TP, and TN should be calculated from at least three values, and preferably more than three values, from the July – September period. For assessments of mesotrophic and eutrophic lakes, the sample size for calculation of seasonal means should be at least five, and preferably more than five.

In the PPHS, the WQCD indicates its intention to change the sample-size requirement for lake assessments from a minimum of three samples per season to a single sample per season. Whether the purpose is standards development or assessment of standards compliance, seasonal means cannot be reliably estimated from results for a single sample. If lake assessments are based on a single sample per year, even oligotrophic lakes with low seasonal-mean chlorophyll *a* concentrations may be incorrectly listed for impairment, and the probability of inaccurate assessments would be higher still for mesotrophic and

eutrophic lakes. Sample-size requirements for lake assessments should be based on expectations about seasonal variation in regulated constituents (e.g., relationships for chlorophyll *a*, TP, and TN shown in Figures 9 – 10; see also EPA 2000, WQCD 2013) and the required level of uncertainty for assessments. Decisions about listings for impairments are made for individual lakes, and evaluation of standards compliance for individual lakes should be based on adequate assessment methodologies. The minimum sample size for assessment of the discretionary 5 μ g/L chlorophyll *a* standard for lakes with DUWS (i.e., five sampling events from the March – November season) may be inadequate and should also be reconsidered. Furthermore, currently available information about seasonal variation in chlorophyll *a*, TP, and TN for individual lakes in Colorado (e.g., Figures 5 – 10) suggests that seasonal-mean values probably should be calculated as geometric means rather than arithmetic means.

Development of criteria – In its July 2016 action letter, the EPA indicated its approval of the interim standards for chlorophyll *a* that were adopted by the WQCC in 2012. The EPA did not act on the TP and TN values for running waters but approved, with recommendations, the TP and TN values for lakes and reservoirs. The EPA was concerned specifically about lakes with high chlorophyll *a* per unit of TP or TN (i.e., "high-yield" lakes) and recommended that, in the application of numeric standards to individual segments, "refinements to the interim values may be necessary in order to be protective" of Aquatic Life and Recreation uses. However, the EPA did not recommend that more stringent standards for TP and TN be adopted for all lakes in Colorado.

In response to the recommendations of the EPA, the WQCD and Tetra Tech used quantile regression to develop newly-proposed numeric standards for TP and TN. The

result of the quantile-regression approach is a new set of numeric standards for TP and TN that would be applied as default standards unless a use-attainability analysis (UAA) or other site-specific analysis supports a different set of standards. However, the newly proposed standards for TP and TN are inappropriate with regard to balanced protection of Aquatic Life use and Recreation use in Colorado lakes, particularly for lakes with low yield of chlorophyll *a* per unit of TP or TN.

It is not high yield of chlorophyll *a* per se that threatens Aquatic Life and Recreation uses, but rather harmful growth of algae as indicated by high chlorophyll a. A lake with very low nutrient concentrations (e.g., TP ~ 1 μ g/L) can have high yield of chlorophyll *a* per unit of TP or TN, and a lake with low yield of chlorophyll *a* per unit of TP or TN can have high chlorophyll *a* if nutrient concentrations are sufficiently high. Interim standards for chlorophyll *a* were adopted in 2012 for protection of Aquatic Life and Recreation uses (i.e., $8 \mu g/L$ for Cold lakes, 20 $\mu g/L$ for Warm lakes). These numeric values already can be adopted by the WQCC for lakes on a statewide basis and are expected to be consistent with protection of Aquatic Life and Recreation uses for Colorado lakes. Failure to attain the relevant standard for chlorophyll *a* would result in 303(d) listing for impairment, and regardless of chlorophyll *a* yield per unit of TP or TN, control of TP and/or TN would be considered as a means to control phytoplankton biomass in any lake listed for impairment for chlorophyll a. Also, Section 31.17 (i) of Regulation 31 allows for site-specific flexibility though adoption of alternative standards (chlorophyll *a*, TP, TN) for specific segments, as necessary to provide balanced protection of classified uses.

The currently proposed (sPHS) standards for TP and TN go beyond what would be necessary to protect Aquatic Life and Recreation uses for Colorado lakes. Moon et al.

(2021) explained that it is important to consider that phytoplankton biomass in lakes is affected by various non-nutrient factors, so that "nutrient criteria are not under-protective or over-protective." By using the 0.75 quantiles in derivation of statewide criteria for TP and TN, the WQCD has developed a set of proposed numeric standards that would be protective of Aquatic Life and Recreation use in lakes with high yield of chlorophyll *a* per unit of TP or TN, even without adoption of a numeric standard for chlorophyll *a*. This approach is flawed for two reasons. First, the interim chlorophyll *a* standards of 8 and 20 μ g/L already have received approval by the EPA and can be adopted on a statewide basis by the WQCC, and Section 31.17 (i) allows for additional site-specific flexibility. Secondly, statewide adoption of the currently proposed criteria for TP and TN would be overprotective for lakes with low yield of chlorophyll *a* per unit of TP or TN (Figure 14). Even the approach that was used in development of the 2012 interim criteria resulted in numeric values for TP and TN that would be over-protective in about half of the cases (i.e., numeric values for TP and TN would be consistent with chlorophyll a values below the adopted standard for about half of the lakes). The sort of redundancy that is being proposed by the WQCD would provide no obvious benefit with regard to protection of Aquatic Life and Recreation uses, but the cost of implementation would be high, and overprotection (i.e., reduction of chlorophyll *a* in lakes that do not exceed the approved interim values for chlorophyll *a*) could negatively affect fisheries throughout Colorado.

Because the quantile-regression approach was used for development of the currently proposed criteria, but not for the interim (2012) criteria, concerns about over-protection are particularly important for the nitrogen criteria. Algal cells require both phosphorus and nitrogen, and control of algal growth in lakes can be achieved through control of either TP

or TN, or by control of both TP and TN. The proposed criteria for TP and TN were derived independently, as was the case for development of the interim (2012) criteria. However, both TP and TN criteria proposed in the sPHS are based on the 0.75 quantile, and high yield of chlorophyll *a* per unit of TP or TN is not likely to occur simultaneously for both TP and TN. In a lake where algal biomass is adequately controlled through control of TP, the need for N control would be lessened. Similarly, the need for P control would be reduced where algal growth is controlled adequately through control of N. Phosphorus control is a reliable means of control for harmful algal growth, in Colorado (e.g., in Dillon Reservoir) and generally. Furthermore, it is often technologically infeasible to treat TN in municipal wastewater to the levels necessary to achieve the desired level of control for algal biomass in lakes (e.g., consistent with the adopted numeric values for chlorophyll *a*). Therefore, particularly for TN, the proposed numeric values are not economically justifiable and would provide little or no benefit with regard to protection of Aquatic Life and Recreation uses, in comparison with the existing, interim values for TP and TN.

Direct-use water supplies – In 2012, the WQCC adopted chlorophyll *a* criteria for lakes with DUWS. However, the prehearing statement and associated exhibits for the 2012 Rulemaking Hearing explain why specific numeric values should not be applied to all lakes. Instead, specific numeric values would be applied only on a discretionary basis, to supplement or obviate additional treatment where there are specific concerns about disinfection byproducts, and after consideration of the following factors: 1) whether a public water system experiences problems related to algal growth, 2) whether use restrictions are caused by problems in the DUWS, 3) whether application of the standard would appropriately balance protection of all classified uses, and 4) whether a more

protective standard would be required because of other, site-specific considerations. In Ex. 10 of the PPHS for the 2012 Rulemaking Hearing, which is a part of Ex. H of the 2022 PPHS, the WQCD stated that:

The criterion is proposed as a preventive measure that may offer a viable alternative, or supplement, to additional treatment in situations where there is concern about the level of DBPs in a distribution system. However, it is not intended as a means of guaranteeing that the public water system will remain in compliance with DBP MCLs.

Since adoption of the chlorophyll *a* criteria in 2012, the WQCC has been able to consider any proposal to implement a site-specific chlorophyll *a* value for a lake with DUWS, application of specific numeric values has been limited to Pueblo Reservoir and Standley Lake. Thus, neither water providers nor the WQCD have recognized the need for widespread application of the 5 μ g/L standard for lakes with DUWS. In its proposal to apply the 5 µg/L standard to all lakes with DUWS, the WQCD has failed to consider whether application of the 5 µg/L standard on a statewide basis would be necessary or would appropriately balance protection of classified uses in lakes with DUWS. The WQCC did not intend that discretionary application of the chlorophyll *a* standard for lakes with DUWS would expire as part of the phased-implementation plan for nutrient criteria. Furthermore, the WQCD recommended in 2012 that any specific chlorophyll-*a* values for DUWS should be applied without a translation to TP or TN, because such translations have been developed for summer-average means rather than March – November means. No such translator has been developed for lakes with DUWS, and any translator for such a purpose should be developed on a site-specific basis from data collected over the March -November season. Therefore, the WQCD should withdraw its proposal to apply a specific numeric value for chlorophyll *a* to all lakes with DUWS.

Other matters of concern – Several other matters of concern should be resolved before revised nutrient criteria are considered by the WQCC. These matters include site-specific adjustment of numeric values for TP and TN and remaining concerns about processing of the data set.

In the PPHS, the WQCD proposes a framework for site-specific adjustment of the criteria for TP and TN. The equations proposed by the WQCD are functions of the Secchi O/E ratio, which is an indicator of non-algal light attenuation. Non-algal light attenuation is not the only factor other than nutrients that can limit phytoplankton growth in lakes. For many Colorado lakes, phytoplankton growth is strongly influenced by temperature or water-residence time. Thus, the proposed framework for development of site-specific standards for TP and TN does not reflect the wide range of factors that can alter nutrient-chlorophyll relationships across Colorado lakes. Site-specific standards for nutrients should be developed from site-specific relationships between nutrients, chlorophyll *a*, and other variables, rather than relationships derived from a statewide data set for a single variable.

The use of linear relationships to predict log(chlorophyll *a*) from log(TP) or log(TN) can lead to biases in predictions of chlorophyll *a* (McCauley et al. 1989; see also 2016 letter from the EPA to the WQCC), and for Warm lakes in Colorado, the yield of chlorophyll *a* per unit of TP tends to be lower for lakes with TP > 0.1 mg/L than for lakes with lower concentrations of TP (Figure 13). Tetra Tech should have tested for significant non-linear relationships between log(chlorophyll *a*) and log(TP) and log(TN), and non-linear regression models should be used if significant non-linearity is found.

The lake-classification analyses were conducted prior to release of the sPHS data set, and a large body of relevant information was stripped from the data set in the effort to address problems that were identified in auditing the USGS data (Table 4). The classification analyses should have been repeated after revisions to the data set. Also, other factors that affect algal growth in lakes (e.g., lake depth, water-residence time) and nonlinear modeling approaches should have been considered in the classification analyses. Finally, the results of the classification analyses, associated statistical tests, and the rationale for decisions about classification of lakes were not well documented by Tetra Tech. Such documentation should be provided, as necessary for review of the WQCD proposal or any revised proposal.

The 2012 Rulemaking Hearing was delayed for completion of a cost-benefit analysis. If the WQCD proceeds with development of revised criteria for TP and TN, it will be important to consider the costs and benefits of implementation for the proposed criteria, with particular emphasis on criteria for TN and potential effects on fisheries. Beyond the normal considerations for a cost-benefit analysis, it will be important to understand the expected benefits, in terms of chlorophyll reduction, for a given combination of treatment levels for TP and TN. Phytoplankton require both P and N for growth, and different treatment strategies (i.e., different combinations of investment for control of TP and TN) could achieve similar levels of algal control. Because of the technological barriers associated with N removal, the most cost effective means of attainment of a particular target for chlorophyll *a* may not result from equal investment in removal of TP and TN.

Conclusions

The WQCD has proposed revised criteria for TP and TN for Colorado lakes, to be considered by the WQCC at the April 2023 Rulemaking Hearing. The proposed criteria are based on work described by Tetra Tech in WQCD Ex. O of the PPHS (*N-STEPS Colorado Lakes Final Technical Report*), the PPHS, and the sPHS. The newly proposed criteria for TP and TN are inappropriate with regard to balanced protection of Aquatic Life and Recreation uses for Colorado lakes, and the proposed criteria should not be adopted by the WQCC.

Minimum sample size – There is no reasonable scientific basis for estimation of seasonal means from results for a single sampling date, particularly for assessments, and guidance documents published by the WQCD and the EPA recommend multiple sampling dates. Evaluation of standards compliance for individual lakes should be based on adequate assessment methodologies. For many lakes, and particularly for nutrient-rich lakes, even three dates for July – September may be inappropriate for calculation of seasonal means.

Development of criteria –It is not high yield of chlorophyll *a* per se that threatens Aquatic Life and Recreation uses but rather harmful growth of algae as indicated by high chlorophyll *a*. Aquatic Life and Recreation uses would be adequately protected as long as the interim chlorophyll *a* standards for protection of those uses (i.e., 8 µg/L for Cold lakes and 20 µg/L for Warm lakes) are implemented on a statewide basis and those specific numeric values are adequately protective. Section 31.17 (i) of Regulation 31 provides further protection though site-specific flexibility for adoption of alternatives of the tablevalue standards for chlorophyll *a*, TP, or TN. Use of a high quantile for development of criteria for TP and TN, as for the currently proposed (sPHS) values, is not only unnecessary but also potentially harmful. The proposed criteria would be over-protective for lakes with

low yield of chlorophyll *a* per unit of TP or TN, which is a particular concern because of the technological difficulties associated with N removal and because of the potential for harm to fisheries throughout Colorado.

Direct-use water supplies – In 2012, the WQCD recommended, and the WQCC agreed, that the default 5 μ g/L chlorophyll *a* target for lakes with DUWS would be applied only on a discretionary basis, and application of any specific value would appropriately balance protection of all classified uses.

Other matters of concern – The proposed framework for site-specific nutrient standards considers only non-algal light attenuation and does not reflect the full range of factors that can alter nutrient-chlorophyll relationships for lakes. Site-specific standards for nutrients should be developed from site-specific studies to define expected relationships between nutrients and chlorophyll *a* for individual lakes.

The lake-classification analyses were completed for a preliminary data set. In addition to the variables that were considered by Tetra Tech, other variables also should have been considered for classification of relationships between nutrients and chlorophyll *a*, and nonlinear modeling approaches should have been considered.

Additionally, Tetra Tech and the WQCD should provide thorough documentation of the methods for development of proposed criteria, including assumptions and decisions about data processing and data analyses. Results of statistical analyses should be reported unambiguously, and any non-standard statistical terms should be defined explicitly.

The 2012 Rulemaking Hearing was delayed for completion of a cost-benefit analysis. If the WQCD proceeds with development of revised criteria for TP and TN, it will be important to consider the costs and benefits of implementation for the proposed criteria,

with particular emphasis on criteria for TN. If the WQCD were to develop an alternate proposal for TP and TN criteria, it would be important to understand the expected benefits, in terms of chlorophyll reduction, for a given combination of treatment levels for TP and TN. Phytoplankton require both P and N for growth, and different treatment strategies (i.e., different combinations of investment for control of TP and TN) could achieve similar levels of algal control. Because of the technological barriers associated with N removal, the most cost effective means of attainment of a particular target for chlorophyll *a* may not result from equal investment in removal of TP and TN.

Final recommendations – The WQCC should not approve the revised criteria as proposed in the PPHS, associated exhibits, and the sPHS. If the numeric chlorophyll-*a* values for protection of Aquatic Life and Recreation uses are implemented for all Colorado lakes, except those for which site-specific values are appropriate, the WQCC could retain the existing (2012) interim criteria and provide strong protection for Aquatic Life and Recreation uses for all Colorado lakes. An alternate proposal also could be acceptable if sufficient time were allowed for technical review of the proposal and its implications. Finally, the WQCC should not adopt the 5 μg/L chlorophyll *a* standard for all lakes with DUWS and instead should continue to adopt the 5 μg/L chlorophyll *a* standard on a discretionary basis, as appropriate.

References

- Bachmann, R. W., B. L. Jones, D. D. Fox, M. Hoyer, L. A. Bull, and D. E. Canfield. 1996.
 Relations between trophic state indicators and fish in Florida (USA) lakes. Can. J. Fish.
 Aquat. Sci. 53: 842 855.
- Bradburn, M. J., W. M. Lewis, Jr., and J. H. McCutchan, Jr. 2012. Comparative adaptations of *Aphanizomenon* and *Anabaena* for nitrogen fixation under weak irradiance.
- Carmichael, W. W. 1992. Cyanobacteria secondary metabolites the cyanotoxins. J. Appl. Bacteriology 72: 445 – 449.
- Dillon, P. J. 1975. The phosphorus budget of Cameron Lake, Ontario: the importance of flushing rate to the degree of eutrophy of lakes. Limnol. Oceanogr. 20: 28 39.
- Dillon, P. J., and F. H. Rigler. 1974. The phosphorus-chlorophyll relationship in lakes. Limnol. Oceanogr. 19: 767 – 773.
- Dolman, A. M., J. Rücker, F. R. Pick, J. Fastner, T. Rohrlack, U. Mischke, and C. Wiedner. 2012. Cyanobacteria and cyanotoxins: the influence of nitrogen versus phosphorus. PLoS ONE 7: e38757.
- Downing, J. A., and C. Plante. 1003. Production of fish populations in lakes. Can. J. Fish. Aquat. Sci. 50: 110 – 120.
- Downing, J. A., S. B. Watson, and E. McCauley. 2001. Predicting cyanobacteria dominance in lakes. Can. J. Fish. Aquat. Sci. 58: 1905 1908.
- Edmondson, W. T. 1970. Phosphorus, nitrogen, and algae in Lake Washington after diversion of sewage. Science 169: 690 691.

Falkowski, P. G. 2000. Rationalizing elemental ratios in unicellular algae. J. Phycol. 36: 3 – 6.

- Fee, E. J. 1979. A relation between lake morphometry and primary productivity and its use in interpreting whole-lake eutrophication experiments. Limnol. Oceanogr. 24: 401 416.
- Filstrup, C. T., and J. A. Downing. 2017. Relationship of chlorophyll to phosphorus and nitrogen in nutrient-rich lakes. Inland Waters 7: 385 400.
- Jones, J. R., M. F. Knowlton, and M. S. Kaiser. 1998. Effects of aggregation on chlorophyllphosphorus relations in Missouri reservoirs. J. Lake and Reservoir Management 14: 1 – 9.
- Khan, A. L., E. R. Sokol, D. M. McKnight, J. F. Saunders, A. K. Hohner, and R. S. Summers.
 2021. Phytoplankton drivers of dissolved organic material production in Colorado reservoirs and the formation of disinfection by-products. Frontiers in Environ. Sci. 9:
 Article number 673627.
- Krause-Jensen, D., and K. Sand-Jensen. 1998. Light attenuation and photosynthesis of aquatic plant communities. Limnol. Oceangr. 43: 396 407.
- Lewis, W. M., Jr., J. F. Saunders III, and J. H. McCutchan, Jr. 2008. Application of a nutrientsaturation concept to the control of algal growth in lakes. Lake and Reservoir Management 24: 41 – 46.
- McCarty, P. L. 2018. What is the best biological process for nitrogen removal: when and why? Environ. Sci. Technol. 52: 3835 3841.
- McCauley, E., J. A. Downing, and S. Watson. 1989. Sigmoid relationships between nutrients and chlorophyll among lakes. Can. J. Fish. Aquat. Sci. 46: 1171 1175.

- McCutchan, J. H., Jr., and W. M. Lewis, Jr. 2008. Spatial and temporal patterns of denitrification in an effluent-dominated plains river. Verh. Internat. Verin. Limnol. 30: 323 328.
- Moon, D. L., J. T. Scott, and T. R. Johnson. 2021. Stoichiometric imbalances complicate prediction of phytoplankton biomass in U.S. lakes: implications for nutrient criteria. Limn. Oceanogr. 66: 2967 2978.
- Morris, D. P., and W. M. Lewis, Jr. 1988. Phytoplankton nutrient limitation in Colorado mountain lakes. Freshwater Biol. 20: 315 327.
- Oglesby, R. T. 1977. Relationships of fish yield to lake phytoplankton standing crop, production and morphoedaphic factors. J. Fish. Research Board Canada 34: 2271 2279.
- Paerl, H. W., J. T. Scott, M. J. McCarthy, S. E. Newell, W. S. Gardner, K. E. Havens, D. K. Hoffman, S. W. Wilhelm, and W. A. Wurtsbaugh. 2016. It takes two to Tango: when and where dual nutrient (N & P) reductions are needed to protect lakes and downstream ecosystems. Environ. Sci. Tech. 50: 10805 – 10813.
- Phillips, G., O.-P. Pietiläinen, L. Carvalho, A. Solimini, A. Lych Solheim, and A. C Cardoso.
 2008. Chlorophyll-nutrient relationships of different lake types using a large European dataset. Aquat. Ecol. 42: 213 226.
- Piña-Ochoa, E., and M. Álvarez-Cobelas. 2006. Denitrification in aquatic environments: a cross-system analysis. Biogeochemistry 81: 111 130.
- Pribyl, A. L., J. H. McCutchan, Jr., W. M. Lewis, Jr., and J. F. Saunders III. 2005. Whole-system estimation of denitrification in a plains river: a comparison of two methods.
 Biogeochemistry 73: 439 455.

- Watson, S. B., J. Ridal, and G. L. Boyer. 2008. Taste and odor and cyanobacterial toxins: impairment, prediction, and management in the Great Lakes. Can. J. Fish. Aquat. Sci. 6: 1779 – 1796.
- O'Neil, J. M., T. W. Davis, M. A. Burford, and C. J. Gobler. 2012. The rise of harmful cyanobacteria blooms: the potential roles of eutrophication and climate change. Harmful Algae 14: 313 – 334.
- Redfield, A. C. 1934. On the proportions of organic derivatives in sea water and their relation to the composition of plankton. *In* Daniel, R. J. (Ed.) James Johnstone Memorial Volume. University Press of Liverpool. pp. 176 192.
- Romo, S., J. Soria, F. Fernández, Y. Ouahid, and Á. Barón-Solá. 2013. Water residence time and the dynamics of toxic cyanobacteria. Freshwater Biology 58: 513 – 522.
- Rus, D. L., C. J. Patton, D. K. Mueller, and C. G. Crawford. 2012. Assessing total nitrogen in surface-water samples – Precision and bias of analytical and computational methods:
 U.S. Geological Survey Scientific Investigations Report 2012–5281, 38 p.
- Sakamoto, M. 1966. Primary production by phytoplankton community in some Japanese lakes and its dependence on lake depth. Arch. Hydrobiol. 62: 1 28.
- Schindler, D. W., R. E. Heckey, D. L. Findlay, M. P. Stainton, B. R. Parker, M. J. Paterson, K. G.
 Beaty, M. Lyng, and S. E. M. Kasian. 2008. Eutrophication of lakes cannot be controlled
 by reducing nitrogen input: results of a 37-year whole-ecosystem experiment. Proc. Nat.
 Acad. Sci 32: 11254 11258.
- Smith, V. H. 1982. The nitrogen and phosphorus dependence of algal biomass in lakes: an empirical and theoretical analysis. Limnol. Oceanogr. 27: 1101 1112.

- Smith, V. H. 1983. Low nitrogen to phosphorus ratios favor dominance by blue-green algae in lake phytoplankton. Science 221: 669 671.
- Smith, V. H., and D. W. Schindler. 2009. Eutrophication science: where do we go from here? Trends Ecol. Evol. 24: 201 – 207.
- Smith, V. H., and J. Shapiro. 1981. Chlorophyll phosphorus relations in individual lakes. Their importance to lake restoration strategies. Environ. Sci. Tech. 15: 444 – 451.
- Thamatrakoln, K., and M. Hildebrand. 2008. Silicon uptake in diatoms revisited: a model for saturable and nonsaturable uptake kinetics and the role of silicon transporters. Plant. Physiol. 146: 1397 1407.
- United States Environmental Protection Agency. 2000. Nutrient Criteria Technical Guidance Manual – Lakes and Reservoirs. EPA-822-B00-001.
- Water Quality Control Division. 2013. Nutrient Criteria Development Plan Update. 24 pp, including the 2002 Nutrient Criteria Development Plan for Colorado.
- Wurtsbaugh, W. A., H. W. Paerl, and W. K. Dodds. 2019. Nutrients, eutrophication and harmful algal blooms along the freshwater to marine continuum. WIREs Water 2019: e1373.