TABLE OF CONTENTS

The following Research Findings include a **summary** of highlighted peer-reviewed research and its relevance to protecting Texas' remaining Pristine Stream segments.

Research Finding #1 p. 2 Research Finding #7 p. 16 Small increases in phosphorus in streams, Data from Central Texas streams confirms especially when ambient phosphorus that a phosphorus level at the level of concentrations are low, result in large detection by the TCEQ phosphorus assay increases in algal growth. will cause stream degradation. Research Finding #2 p. 4 Research Finding #8 p. 17 The non-linear relationship between Texas Hill country streams are even more phosphorus and algal growth changes sensitive - only phosphorus levels at or precipitously. below 9 mcg/L were associated with no degradation. Research Finding #3 p. 6 Rampant growth of filamentous algae will Research Finding #9 p. 19 occur in streams whose phosphorus concentration is greater than 30 mcg/L. To protect our low phosphorus streams, all efforts should be made to avoid allowing Research Finding #4 p. 8 any exogenous phosphorus inputs. Degradation occurs any time the total phosphorus concentration is higher than 10 Recommended Steps p. 21 mcg/L. Based on Texas Administrative Code Subchapter A § 309.1, TCEQ already has the Research Finding #5 p. 10 ability to prevent the degradation of Texas' remaining Pristine Streams. Three steps are "Fudging" the restriction cut-point of suggested. phosphorus to a more 'palatable 'level such as 20 mcg/L - does not work.

Research Finding #6 p. 12

Thoughtful discourse, collaboration, and consideration of science is how others have

solved ongoing pollution problems.

#1

Small increases in phosphorus in streams, especially when ambient phosphorus concentrations are low, result in large increases in algal growth.

Reference:

Dodds WK, Smith VH, Lohman K. Nitrogen and phosphorus relationships to benthic algal biomass in temperate streams. *Canadian Journal of Fish and Aquatic Science*, 2002; **59**: 865-874.

Summary:

Based on prior work demonstrating a positive correlation between nutrient concentration and eutrophication in lakes and reservoirs, this paper was one of the first to evaluate the relationship between the nutrient content of streams to their algal biomass. A popular theory explaining eutrophication speculated that eco-region was a significant factor, Published data from 300 distinct sites in 200 rivers in North America and New Zealand were compared to data from 620 USGS monitoring stations. Only a weak ecoregion effect was seen; approximately 40% of the biomass was explained by

concentrations of nitrogen and phosphorus. This relationship was not linear - a 'breakpoint" or 'saturation point' at 30 mcg/L of phosphorus where benthic chlorophyll levels rapidly rise - was clearly demonstrated. When the data correlating total phosphorus to benthic chlorophyll was plotted according to the total nitrogen: total phosphorus ratio, algal biomass still appeared to be driven by the total phosphorus content - and this was observed in both the literature data review and the USGS dataset.

66 Even if it is only possible to explain a limited amount of variance in benthic algal biomass using water column nutrients, most other factors (e.g., light, flooding, grazing) cannot be controlled, leaving management of nutrient loading as the most likely method to control excessive algal biomass in streams."

This research study found that phosphorus, not stream location, was the primary driving factor for algal biomass. This seminal paper concluded that management of nutrient loading (i.e., wastewater effluent discharge) is a logical and important means to control excessive algal blooms. In order to protect Pristine Streams, permits should be required to match the existing phosphorous levels in the watersheds of these classified stream segments.

Supporting resource data:



What does normal, algal biomass look like, compared to an increase in algal biomass? Photographs of typical periphyton biofilm (figure 12 from Reference # 5) showing color and thickness from sites below the TP threshold of <15-20 ug/L (panel A) next to photographs from rocks exposed to higher phosphorus levels (panel B). In panel A, the periphyton is mostly diatoms, calcareous cyanobacteria, and other microbes (bacteria and fungi), with little obvious filamentous green algae. In panel B, the periphyton is comprised of a thinner veneer of mostly diatoms and bacteria, but the overall biomass does not change markedly because of the increase in colonial and filamentous green algae.

#2

The non-linear relationship between phosphorus and algal growth changes precipitously. At a concentration of 16.9 mcg/L, there was a 95% probability that algal growth would rapidly rise. This concentration is below the limits of detection by the current phosphorus assay used at TCEQ (i.e. 20 mcg/L).

Reference:

King RS, Richardson CJ. Integrating bioassessment and ecological risk assessment: an approach to developing numerical water-quality criteria. *Environmental Management* 2003: **31**(6), 795-809

Summary:

This study was the first of its kind to link experimental phosphorus (P) dosing and field observations to develop numeric nutrient criteria. It was conducted in the Florida Everglades, and while not in Texas, the aquatic character is chemically analogous to Hill Country streams: very high alkalinity, limestone substrate, very low P, and very sensitive to P enrichment. In their study, they used threshold analysis to show that **Total Phosphorus** (TP) between 0.012 and

0.015 mg/L (12-15 mcg/L) caused significant changes in many metrics of biological integrity and thus represented the upper limits of what should be established as a criterion for total phosphorus for the northern Everglades.

Rather than asking the question, "is there a statistically significant relationship between predictor x and response y?" as implied with most hypothesis-testing statistics, this risk-based analysis more explicitly asks "what level of predictor x results in a threshold response of y, and how uncertain is this threshold?"

When treated domestic wastewater effluent is discharged into Pristine Streams, the natural algaelimited, low-phosphorus ecosystem is degraded. **There are consequences to this disturbance. Rather than defining a 'threshold phosphorus level,' TCEQ could stipulate an effluent phosphorous concentration that matches the existing level in these Pristine Streams. This should achieve the needed protection and avoid a degradational shift from oligotrophic to meso- or eutrophic.**

Supporting resource data:

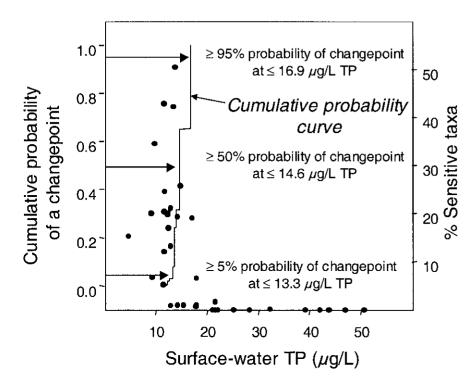


Figure 2. Illustration of the cumulative probability of a changepoint estimated for an individual metric in response to surface-water TP. The cumulative probability curve describes the cumulative risk of a change in a response variable (% sensitive taxa, yaxis [right side]; depicted by filled circles) associated with a range of stressor values. Cumulative probabilities are calculated using 1,000 bootstrap simulations. Any given location along the curve corresponds to a specific cumulative probability of a changepoint (y-axis [left side]) at a specific level of TP (xaxis). In this example, there was at least a 5% cumulative probability, or risk, that a detectable change in the mean and/or variance of the % sensitive taxa metric occurred at or below 13.3 mcg/L TP. In other words, ≥5% of the bootstrap simulations resulted in a changepoint that was ≤ 13.3 mcg/L TP. Similarly, there was ≥50% risk of a change- point ≤ 14.6 mcg/L TP, while there was $\geq 95\%$ probability that a changepoint occurred ≤16.9 mcg/L TP. Data are from the observed P-gradient study.

#3

Rampant growth of filamentous algae will occur in streams whose phosphorus concentration is greater than 30 mcg/L. This threshold is also seen for growth of cyanobacteria (blue-green algae) whose toxins kill small animals. Levels of phosphorus less than 10 mcg/L on a constant basis would be required to protect low phosphorous streams from nuisance filamentous green algae (NFGA) growth.

Reference:

Stevenson, RJ, Rier ST, Riseng CM, Schultz RE, Wiley MJ. Comparing effects of nutrients on algal biomass in streams in two regions with different disturbance regimes and with applications for developing nutrient criteria. *Hydrobiologia* 2006; **561**: 149-165

Summary:

In this paper, data collected in streams throughout Michigan were compared to streams along the Indiana-Kentucky border. The 58 Michigan streams had higher number of inveterate grazers - small insects that eat benthic algae, and their stream flow was fed from groundwater springs. The 46 Kentucky streams had fewer inveterate grazers, and flow was primarily from rapid run off due to steep topography and limestone bedrock. The study looked at Cladophora (a nuisance filamentous green algae), and

evaluated how often a stream had Cladophora, and how much cover this algae had in the stream (higher cover, greater eutrophication). It was speculated that the frequent flood disturbances in Kentucky accounted for the lower frequency of filamentous algae. Cladophora cover was rare if total phosphorus was less than 30 mcg/L.

To protect naturally low levels of productivity and algal biomass in streams that are hydrogeomorphically similar to our study streams, nutrient concentrations should probably be constrained to ≤ 10 mcg/L for total phosphorus and 400 mcg for total nitrogen.

To prevent nuisance growths of Cladophora, higher criteria in the range of 30 mcg/L TP and 1,000 mcg Total Nitrogen (TN) may be satisfactory."

The data suggest a limit of <10 mcg/L for total phosphorus in a stream is required to maintain its clarity. The addition of even the most stringently treated domestic wastewater to our low phosphorus Pristine Streams would exceed this threshold. Further, the expected 'hiccups' of effluent discharge (when effluent temporarily exceeds the permit restriction) would certainly assure stream degradation, and nuisance algae growth. To protect from NFGA in Pristine Streams, no discharge of effluent adding additional phosphorus should be permitted.

Supporting resource data:

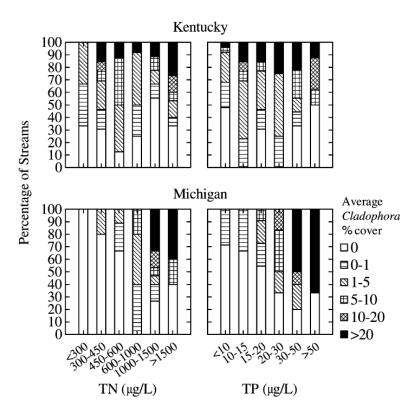


Figure. Histograms representing the percentage of streams in different nutrient categories and in different regions with increasing cover of Cladophora. Width of the bands in each bar represents the percentage of streams in a nutrient category with specific ranges of average Cladophora cover. Codes for those ranges are in the legend.

#4

Degradation occurs any time the total phosphorus concentration is higher than 10 mcg/L.

Reference:

Stevenson RJ, Hill BH, Herlihy AT, Yuan LL, Norton SB. Algae-P relationships, thresholds, and frequency distributions guide nutrient criterion development. Journal of the North American Benthologic Society, 2008, 27(3): 783-799.

Summary:

This paper analyzed periphyton samples collected from 149 streams throughout the Mid-Atlantic Highlands region of the US (Pennsylvania, West Virginia, Maryland, Virginia, and parts of North Carolina and New York states).

The authors found that as the phosphorus level increased, distinct changes in 10 of 11 algal attributes were observed - all markers of water degradation.

... low stressor criteria, such as 10 mcg/L, could prevent degradation of high-quality sites... A 10 mcg TP/L criterion would constrain productivity to natural levels, maintain phosphorus-resource constraints on species membership, and prevent proliferation of invasive algae that require high nutrient concentrations."

> "Increases in TP from 10 to 30 mcg/L were associated with responses of benthic diatom assemblages that indicated release from nutrient limitation... some biological variables different little among streams when TP was less than 10 mcg/L but differed greatly as TP increased from 10 to 30 mcg/L"

Pristine Streams in Texas have undetectable phosphorus (<0.06 mg/L or <60 mcg/L) in ≥90% of the samples obtained by TCEQ from January 1, 2010- January 1, 2020. The actual phosphorus level is likely in the 3-6 mcg/L range. This paper supports the Pristine Streams Team assertion that these streams will be degraded if additional phosphorus is discharged into them.

Supporting resource data:



What does NFGA look like? **Figure 35 from reference 5** Comparison of periphyton growing on ceramic tiles among the 3 P treatments on Day 14 (left) and Day 28 (right). Note the obvious difference in color between Control and Low/High P treatments, even on Day 14. By Day 28, Low/High P tiles were covered in green algae.

#5

"Fudging" the restriction cut-point of phosphorus to a more 'palatable 'level - such as 20 mcg/L - does not work. Algal blooms do not respond to cut-points. Algae blooms and water degradation are driven by phosphorus levels.

Reference:

King, RS, Brooks BW, Back JA, Taylor JM, Fulton BA. Linking Observational and Experimental Approaches for the Development of Regional Nutrient Criteria for Wadeable Streams. Section 104(b)(3) Water Quality Cooperative Agreement #CP-966137-01 U. S. EPA Region 6, Dallas, TX, 2009.

Summary:

This study was the first in **Texas to combine** experimental phosphorus dosing in streams with field observations to show that phosphorus, especially when phosphorus is added to streams with low levels of ambient phosphorus, cause algal communities to change rapidly. In an experimental mesocosm, three phosphorus conditions were compared: 1) control channels = ambient phosphorus of 6 mcg/L; 2) Low phosphorus channels = 20 mcg/L, and 3) high phosphorus channels = 100 mcg/L. After 28 days, both the low and high phosphorus channels were covered with nuisance filamentous algae. The

consequence of this nuisance algae is significant - dissolved oxygen concentrations fell precipitously to 3 mg/L when TP exceeded 200 mcg/L. Other significant changes indicative of degradation occurred at far lower levels than 20 mcg/L, supporting the assertion that 20 mcg/L is the level where these Texas streams clearly become impaired. Management levels would need to achieve less than 20 mcg/L of phosphorus on a year-round basis downstream from any treated domestic wastewater discharge to maintain natural conditions of limestone-based, low phosphorus Hill Country streams.

66 The weight of evidence from both the field stream study and experimental stream study demonstrates that streams of the study area are very sensitive to phosphorus enrichment. There is a very high probability that streams exposed to surface-water TP levels exceeding 20 ug/L, and possibly 15 ug/L, will experience a sharp decline in biological integrity, including loss of characteristic structure (periphyton and macrophytes), loss of numerous species >

This study documents that low phosphorus streams cannot tolerate increases in phosphorus load from wastewater discharge. Pristine Stream protection is a small ask: <1% of Texas streams that have low phosphorus, and the addition of phosphorus from treated domestic wastewater effluent discharge should be prevented to maintain the water quality and clarity of these few streams.

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(algae and macroinvertebrates), minimum dissolved oxygen levels unsuitable for supporting native fauna during low flows, and increase likelihood of nuisance algal growth that limits recreational use of streams."

Supporting resource data:

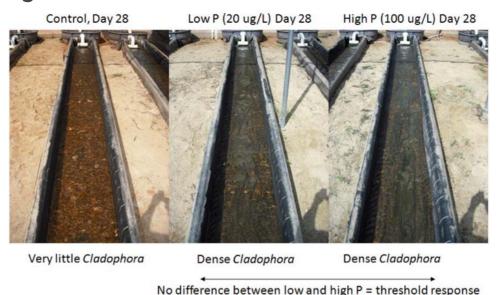


Figure 38. A longitudinal view of Control, Low-P, and High-P streams on Day 28.

Cladophora (filamentous green algae) biomass Bare rocks (non-transplant)

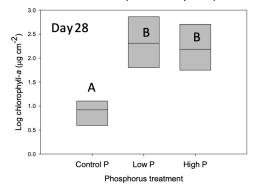


Figure 39. Results of ANOVA comparing mean Cladophora chlorophyll-a (ug/cm²) among P treatments on Day 28. Means with the same letter are not different (p>0.05).

#6

Thoughtful discourse, collaboration, and consideration of science is how others have solved ongoing pollution problems.

References:

Stevenson RJ, Bennett BJ, Jordan DN, French RD. Phosphorus regulates stream injury by filamentous green algae, DO, and pH with thresholds in responses. *Hydrobiologia*, 2012, **695**: 25-42.

Cook SC, Housley L, Back JA, King RS, Freshwater eutrophication drives sharp reductions in temporal beta diversity. *Ecology* 2018: **99**(1),47-56.

Cook SC, Back JA, King RS. Compensatory dynamics of lotic algae break down nonlinearly with increasing nutrient enrichment. *Ecology*, 2022. **103**(4), e3613.

King RS, Matlock M, Benefield R, Chard S, Philips S. Oklahoma-Arkansas Scenic Rivers Joint Phosphorus Study. 2016: IR-2016.11.8.

Haggard, B., Smithee, D, Benefield, R, Chard, S, Matlock, M, Phillips, S, King, R., 2017. Final Report to Governors from the Joint Study Committee and Scientific Professionals. Arkansas Water Resources Center, Fayetteville, AR. MSC384, 72.

Summary:

The consequences of excessive Phosphorus pollution in streams is not confined to regulatory boundaries. The excessive filamentous algae growth cross state regulatory agencies boundaries. The above 5 articles - 3 research papers published in peer-reviewed journals + 2 official reports

summarize the research examining algae growth in streams flowing from Arkansas to Oklahoma. Practices in Arkansas watersheds resulted in significant runoff from poultry waste applied as fertilizer to fields (PHD = poultry house density), leading to degraded water quality in Oklahoma.

Our results show nutrient enrichment strongly influences the structural aspect of seasonal biodiversity, which likely has widespread impact on the driven functioning of stream ecosystems."

— From Cook et al.

Some streams received additional phosphorus from wastewater effluent (Urban).

Stephenson et al describes Nuisance Filamentous Green Algae (NFGA) biomass assessed by percent cover of stream bottom. Benthic algal biomass was assessed by Chlorophyll A. Both phosphorus from poultry waste and human waste was associated with benthic and NFGA biomass. These relationships are depicted in Figure 3 on page 15. Both phosphorus sources were significantly associated with algal growth in the spring; urban sources of phosphorus were significantly associated with benthic algal biomass in the summer. The lack of an association between NFGA cover and phosphorus concentrations in the summer was felt to be due to water temperature impairment of NFGA growth.

Cook et al. 2 publications, published in one of the top journals in all of biologic science - reported one study focused on macroinvertebrate and another focused on algae. Both studies found that exceeding 0.025 mg/L (25 mcg/L) of total phosphorus in the Ozark Highland rivers resulted in sharp, nonlinearly degradation of macroinvertebrate and algal communities, respectively.

King et al. is arguably the most in-depth study conducted on the effects of phosphorus on nuisance filamentous algae growth and other biological endpoints in the USA. The location was the Ozark Highlands on the border between Oklahoma and Arkansas. The study was solicited by both states in response to a 1992 Supreme Court decision in favor of Oklahoma dictating that an upstream entity may not violate the water quality standards of a downstream entity (includes landowners), a landmark case in environmental law. The three-year study included 35 rivers spanning a range of phosphorus from 0.005 to 0.25 mg/L TP. Every river was sampled 6x per year, resulting in an enormous database and. ultimately, an analysis that showed without any doubt that 0.035 mg/L TP was associated, in every stream above this level, with nuisance filamentous algal blooms. Blooms started to occur at levels near 0.025 mg/L but the joint study committee, comprised of members from both states, compromised and decided to recommend 0.035 mg/L as the threshold. For management, the authors recommended a criterion that was lower than 0.035 mg/L, closer to 0.02, and this was only >



66 Phosphorus pollution stimulated an increase in algal biomass in IRW (Illinois River Watershed) streams to what has been characterized to be a nuisance level and could be defined as an injury to aesthetics. NFGA cover in minimally disturbed, low phosphorus streams usually had <10% NFGA cover. When total phosphorus was above 27 mcg/L, NFGA cover averaged 36% cover."

- From Stevenson et al.

to protect streams from nuisance filamentous algal blooms. Diatom communities significantly changed at 0.019 mg/L TP, and thus a criterion to protect streams from biological impairment based on diatoms would need to be 0.01-0.015 mg/L TP. Note that the study streams were primarily limestone based and had similar water chemistry to those of the Texas Hill Country.

Relevance to Pristine Streams:

It's important to point out the stakeholders involved in this research were working to remedy existing pollution problems. **Instead of mitigating pollution, TCEQ has an opportunity to prevent** pollution. The research is clear on this matter: Any increase in phosphorous concentration in low phosphorous streams will cause degradation.

The JOINT STUDY
COMMITTEE and its
scientific professionals
(Dr. Ryan King)
employed to complete
the JOINT STUDY
specifically and
unanimously
recommend:

A six-month average total phosphorus level of not to exceed 0.035 mg/L based on water samples taken during the CRITICAL CONDITION*, as previously defined, was necessary to protect the aesthetics beneficial use and scenic river

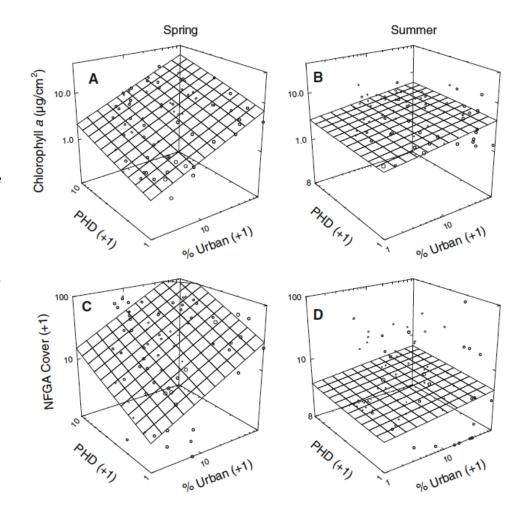
(Outstanding Resource Water) designations assigned to the designated Scenic Rivers."

- From Cook et al.

Supporting resource data:

Figure 3 from Stephenson et.al.

Relationships between benthic algal biomass (as chl a cm-2 and NFGA cover) and TP during spring (A, C) and summer (B, D). The crosshatched planes in A-C and the line in D indicate the linear relationships between nutrient concentrations and land uses. PHD = poultry house density. Urban = wastewater discharge density



#7

Data from Central Texas streams confirms that a phosphorus level at the level of detection by the TCEQ phosphorus assay will cause stream degradation.

Reference:

Taylor JM, King RS, Pease AA, Winemiller KO. Nonlinear response of stream ecosystem structure to low-level phosphorus enrichment. *Freshwater Biology*, 2014: **59**(5); 969-84.

Summary:

This study was focused on both algae and fish among 64 streams sampled throughout central Texas. This work was funded by TCEQ for the development of indicators of nutrient enrichment in Texas streams. The study showed that both algae and fish communities were degraded (biologically impaired) when TP exceed 0.02 mg/L (20 mcg/L).

Relevance to Pristine Streams:

The consistency of threshold numbers when phosphorus enrichment of a stream occurs, is remarkable. These are numbers not derived by speculation, but from firm and reproducible observational and experimental evidence. There is no reason for TCEQ to continue to permit treated domestic wastewater effluent discharges into Pristine Streams knowing, based on this overwhelming evidence, that degradation will occur.

66

Our results provide new insights into interpretation and analysis of assemblage-level responses to nutrient enrichment. Our findings indicate that a numerical criterion for surface water TP of c. 20 ug/L would be needed to maintain natural algae assemblages and at least two specialist fishes within our study region. Proliferation of weedy alga species and increased abundance of invasive fishes are also likely when surface water concentrations exceed these thresholds. While many streams likely exceed these thresholds, managers should consider potential low-level enrichment effects when developing criteria for ecosystems to protect existing nutrient-limited streams."

#8

Texas Hill country streams are even more sensitive – only phosphorus levels at or below 9 mcg/L were associated with no degradation.

Reference:

Bellinger, B., and Richter, A. 2018. Phosphorus Concentration Ranges and Periphyton Responses. City of Austin, Watershed Protection Department, Environmental Resource Management Division, SR-18-11; March 2018

Summary:

Surface water in streams surrounding Austin were extensively characterized. The total phosphorus concentrations ranged from 3.2 - 184 mcg/L with a mean of 36.6 mcg/L. Several sophisticated measures of algal growth were measured - rather than relying on periphyton chlorophyl A concentrations - the phosphorus, nitrogen and carbon content of the benthic scrapings were measured and normalized to area and mass. Using GAM modeling, which does not assume a predetermined outcome, a clear and consistent reduction in periphyton content was seen with increasing stream phosphorus levels.



In this study we evaluated the potential of diatoms from streams in the Austin region, spanning a wide trophic gradient, for establishing preliminary nutrient criteria thresholds. Based on this initial study, establishment of a preliminary numeric nutrient criteria near 9 µg/L TP would be protective of stream health, above which a stream would be considered in a degraded condition. It was also determined that current TP laboratory minimum detection limits were not sensitive enough to adequately capture ambient phosphorus concentrations near the proposed ecologically relevant threshold."

When does degradation begin? This study supports the assertion that **degradation begins below** the detection limits of utilized laboratories: If Pristine Streams are to be protected, no amount of additional phosphorus should be permitted to be added to these streams.

Supporting resource data:

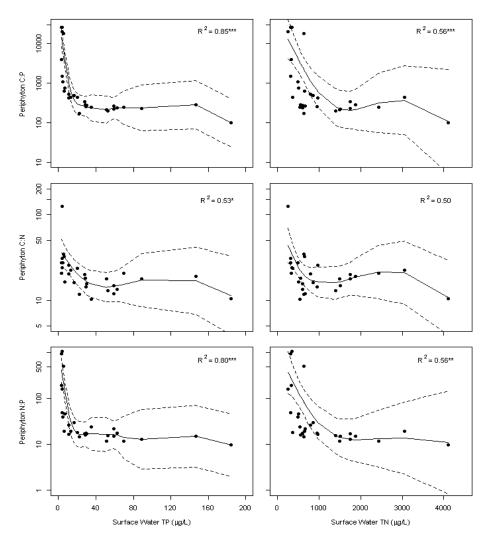


Figure 4: Relationships between periphyton C:P, C:N and N:P (y-axis; top-to-bottom) against surface water TP (x-axis; left column) and TN (x axis; right column) with GAM smoothers. Solid lines are predicted values while dashed lines are 95% confidence intervals. R² =(null deviance - residual deviance)/null deviance; P < 0.001 indicated by ****, p < 0.01 indicated by ** and p < 0.05 indicated by *.

From the Figure above, note the rapid decline in benthic algal mass. The authors conclude that Texas Hill Country streams are extremely sensitive to low level phosphorus, as low as 0.009 mg/L, which is below reporting limits for all labs used by TCEQ and regional water authorities.

#9

More studies of Texas streams replicate prior data. **Dramatic increases in algal growth were** seen when total phosphorus exceeded 20-25 mcg/L. To protect our low phosphorus streams, all efforts should be made to avoid allowing any exogenous phosphorus inputs.

Reference:

Taylor JM, Back JA, Brooks BW, King RS. Spatial, temporal and experimental: three study design cornerstones for establishing defensible numeric criteria in freshwater ecosystems. *Journal of Applied Ecology*, 2018: **55**(5), 2114-23.

Summary:

This paper unequivocally shows that central Texas limestone streams are extremely sensitive to low levels of phosphorus enrichment from treated wastewater and agricultural sources. The relationship between the phosphorus content of the stream and algal degradation is not linear - there is a distinct threshold. The investigation includes not

only observational data, but

data from an experimental

dosing of phosphorus in an

experimental stream facility.

Both sets of data observational and experiential arrive at a clear conclusion: total phosphorus concentrations (TP) accounts for Texas stream impairment. Levels of TP that were associated with dramatic changes in algae were 0.020-0.025 mg/L, (20-25 mcg/L) but note this is the level that caused changes. Management would need to stay well below these levels to avoid algae problems and impairment to Pristine rivers.

66 Stressor-response studies specifically designed to identify levels of nutrients strongly associated with undesirable ecological conditions are needed to inform numeric nutrient criteria that protect inland waters. In this study, algal species composition was nonlinearly correlated with total phosphorus (TP) throughout the 2year field study. This occurred despite temporal shifts in species composition between two hydrologically distinct years and over eight >

Further evidence from our own state that Pristine Streams that contain ambient phosphorus concentrations under the detection limits of the TCEQ assay (< 20 mcg/L) will be degraded if any exogenous phosphorus source is added.

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seasons. Threshold analysis identified synchronous declines in several diatom species that culminated in assemblage thresholds associated with TP concentrations >20 and 25 μ g/L for 2006 and 2007 respectively. We present a framework that includes spatial, temporal and experimental components, and has broad applicability for use in different ecological settings to evaluate ecological endpoints and set limits for a variety of contaminants threatening freshwater ecosystems throughout the world."

RECOMMENDED STEPS

Based on Texas Administrative Code Subchapter A § 309.1, TCEQ already has the ability to prevent the degradation of Texas' remaining Pristine Streams: "In order to achieve compliance with water quality standards within certain segments, more stringent effluent quality limitations other than basic secondary treatment may be required to protect water quality." By requiring the applicant's Texas Pollutant Discharge Elimination System (TPDES) effluent to match the existing phosphorus concentration in the stream, pristine water quality should be preserved.



Step 1: Modify the Model

To preserve the low phosphorous nature of Pristine Streams, the TCEQ model used to define effluent parameters can be modified. Requiring phosphorus effluent levels to match ambient stream phosphorus concentration should enable TCEQ to meet their mandate to protect water quality.



Step 2: Inform the Applicant

During the interview prior to the submission of an application for a proposed TPDES into a Pristine Stream watershed, the applicant will be made aware that the effluent proposed to be discharged must match the phosphorus level of the Pristine Stream.



Step 3: Mandate a Match

During the scientific review, a Receiving Water Assessment (RWA) will be conducted, and ambient phosphorus levels of the classified Pristine Stream segment will be measured on three occasions using sensitive phosphorus assays with detection limits of <1 mcg/L to provide criteria for draft permit phosphorus levels.

