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MEASURING LOTIC ECOSYSTEM RESPONSES TO NUTRIENTS:

A Mismatch that Limits the Synthesis and Application of Experimental Studies to Management

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INTRODUCTION

Our team at the U.S. Environmental Protection Agency (EPA) is working on addressing a specific need: identifying and synthesizing scientific information to aid the development and review of numeric criteria by states, tribes, and territories (hereafter, states) to protect aquatic resources from nutrient pollution. Nitrogen and phosphorus pollution is a major stressor of freshwater ecosystems, both across the United States and globally, with nutrients and related stressors (e.g., oxygen depletion) degrading ecosystem services estimated to be worth more than \$2.2 billion annually in the U.S. alone (Dodds et al. 2009). Nutrient pollution remains a high priority of environmental managers and policymakers (Stoner 2011; Beauvais 2016). In the U.S., the Clean Water Act is administered by EPA and provides the regulatory framework to restore and maintain the chemical, physical, and biological integrity of the nation's aquatic resources. Under the Clean Water Act and its implementing regulations, states are required to adopt water quality criteria that protect the designated use they have set for their water resources; EPA reviews and approves the pro-posed criteria before they become water quality standards under the Clean Water Act. Many states have adopted narrative legislative statements that set nutrient criteria in qualitative terms (e.g., "levels that do not cause changes in biotic communities"). States are developing more precise, quantitative numeric values to replace or translate these narrative statements; supporting these numeric nutrient criteria with documented nutrient-stressor response relationships would increase confidence in the resulting targets (U.S. Environmental Protection Agency 2000, 2010).

When using stressor-response approaches for nutrient criteria development, states often use state-level monitoring datasets to statistically relate nutrient stressors to biotic responses (e.g., chlorophyll-a concentration, benthic macroinvertebrate indices) to define nutrient concentrations within which acceptable conditions or minimal changes occur and, based on this information, develop numeric nutrient criteria (e.g., U.S. Environmental Protection Agency 2000, 2010; Heiskary et al. 2013; Heiskary and Bouchard 2015). Other approaches add to our understanding of nutrient stressor-response relationships, including observational field studies; controlled experimental studies in artificial or natural streams; or syntheses of

these different studies (e.g., meta-analyses and systematic reviews). All of these approaches have merit, but it is often difficult to control for confounding factors in observational field studies and to apply the results from experimental studies to natural streams. Generalizations derived from a body of scientific evidence can provide decision makers with greater confidence in proposed numeric limits. To evaluate criteria derived from such complex stressor–response relationships, decision makers may require more generalizable scientific evidence than individual, site-specific studies can provide.

Synthetic approaches such as systematic review and meta-analysis can provide additional scientific information needed for decision-making because these approaches consider all available evidence in a rigorous framework that accounts for study design and identifies potential bias (Dicks et al. 2014; Lortie 2014; Bennett et al. 2018). Such approaches can help in evaluation of criteria derived from primary data and can improve criteria development methods such as using published thresholds or literature-based models (U.S. Environmental Protection Agency 2000). Systematic review integrates evidence across individual studies and weights the strength of individual pieces of evidence (i.e., results of an individual study) based on study quality (Haddaway et al. 2015). This method of targeted synthesis is being increasingly used to investigate questions about human impacts on the environment and can inform environmental regulations and policies that require strong evidence bases (Pullin and Knight 2003; Nichols et al. 2016; Cooke et al. 2017; Collaboration for Environmental Evidence 2018).

In most frameworks that assess study quality in the context of systematic review and synthesis, well-designed, controlled studies such as replicated mesocosm studies or experiments in natural systems (e.g., Before-After-Control-Impact studies) are ranked as highest quality because of their ability to minimize the effects of confounding variables (e.g., variations in canopy cover, flow velocity, other physicochemical parameters) and the potential to uncover causal mechanisms underlying patterns and correlations (Bilotta et al. 2014; Mupepele et al. 2015; Morgan et al. 2016). Such controlled studies could serve as key pieces of evidence of nutrient pollution effects on lotic ecosystems that improve the quality of the evidence base for any synthesis-based investigation of nutrient criteria.

In the process of developing a targeted systematic review on biotic responses to nutrients in rivers and streams, we set out to find studies examining causal linkages, especially experimental studies that would provide high-quality evidence to evaluate biotic responses to total nitrogen (TN) and total phosphorus (TP), which are the primary nutrient components for which numeric nutrient criteria are being derived in the United States (Bennett et al. 2017). However, we found that many experimental studies we examined from our broad literature search did not report TN or TP. To determine if this lack of TN and TP data was consistent across studies, we searched Web of Science™ for experimental studies that examined lotic ecosystem responses (broadly defined to include multiple levels of biological organization) (see the full dataset and methods at www.epa.gov/sciencehub/...). We screened the titles and abstracts of returned papers for relevance (experimental studies in streams/stream mesocosms that manipulated nutrients). We supplemented this search by examining the bibliographies of relevant articles (alphabetically by author) until we had obtained a total of 100 articles. To be clear, this process itself was not a systematic review. Rather, we aimed

to gain a rapid, representative overview of the experimental nutrient literature. From the included studies, we extracted or summarized the following information: study type, study duration, nutrient treatments, nutrients measured, inclusion of TN and/or TP response to nutrient additions, and a description of what nutrient forms were reported. We compared the proportion of these studies that measure TN or TP with the proportion of numeric nutrient criteria based on TN or TP (based on a U.S. EPA database available online at <https://www.epa.gov/nutrient-policy-data/state-progress-toward-developing-numeric-nutrient-water-quality-criteria>).

We found that, of the 17 states with river and stream numeric nutrient criteria, 15 (88%) have criteria based on TP and 9 (53%) have criteria based on TN (Table 1, Fig. 1). Ten states have numeric criteria based on some dissolved nutrient, but any single dissolved form was represented by only a few states. Also, at more local scales of nutrient pollution management, most total maximum daily loads (TMDL)—the pollution “budgets” developed by states for specific water bodies or sections not meeting water quality standards—across lotic and lentic systems in the U.S. for nutrients are based on TP or TN (U.S. Environmental Protection Agency 2017). By contrast, of the 100 studies of ecological responses to nutrients in stream mesocosm (46 studies) or field nutrient addition (54 studies) experiments published from 1987–2016 that we collected (see the full dataset and methods at [www.epa.gov/sciencehub/...](http://www.epa.gov/sciencehub/)), only 8% reported TN or TP values (including some only reporting TN:TP ratios) for nutrient addition treatments, whereas more than 85% reported values for some dissolved nutrient form (Fig. 2). Thus, there was a mismatch in the nutrient constituents most often measured in experimental studies and those constituents used by states to develop numeric nutrient criteria under the Clean Water Act.

EXPERIMENTAL STUDIES TO BETTER INFORM NUMERIC NUTRIENT CRITERIA DEVELOPMENT

Management decisions concerning complex environmental responses to human impacts require strong evidence bases that are robust to uncertainty associated with natural variability and limitations of study designs in natural systems. Systematic review is one method to build strong evidence bases in a comprehensive and transparent way. Experimental studies that control for natural variability and confounding factors could be used as high-quality evidence in a systematic review, but only if they meet inclusion criteria of the target question of the systematic review. Targeted questions that focus on the effects of TN and TP on lotic systems are most relevant to development of numeric nutrient criteria because these nutrient forms are currently being used by states in the regulatory process and will continue to be used based on state monitoring programs and federal recommendations. Thus, the lack of TN or TP measurements reported in experimental studies is not a flaw in achieving the objectives of these studies but does represent a potential barrier to using results from these experiments to inform management decisions.

Experimental results using nutrient measures other than TN or TP provide a great deal of insight into understanding nutrient dynamics in stream and river ecosystems and certainly support nutrient criteria development in general. For instance, strong responses of biota to

dissolved nutrients in controlled experimental studies at relevant field concentrations provide good evidence for causal relationships. However, a synthesis of these results in combination with observational stressor-response and other studies to develop a strong evidence base informing numeric criteria for TN or TP is possible only if responses of TN or TP to the nutrient addition treatments are measured and reported. Otherwise, experimental studies measuring only dissolved forms would either be excluded from the scope of the synthesis (e.g., Bennett et al. 2017), or would have to be synthesized independently of TN/TP studies when evaluating effect sizes and conducting the meta-analysis. The inability to include a majority of experimental stream studies in our targeted systematic review represents a missed opportunity to obtain high-quality evidence to inform a high-priority management need to better protect freshwater resources. This extends beyond development of numeric nutrient criteria to TMDL development and evaluation of restoration targets, since these also are frequently based on total nutrient forms.

RECOMMENDATIONS

Ideal studies for investigating nutrient effects would pair experimental and field-based approaches and include measurement of nutrient forms relevant to the question at hand and to management (e.g., Taylor et al. 2018). To achieve this goal, we first recommend more complete and transparent data reporting (Reichman et al. 2011; Lortie 2014), as several studies measured total nutrient fractions but either did not report these measures or measured them only prior to the experimental addition of nutrients. Second, to balance the utility of translating experimental results into decision-relevant evidence with costs to researchers, we recommend at minimum analyzing water samples for and reporting TN and/or TP for each nutrient addition treatment during experiments. Three replicate water samples analyzed for TN or TP would allow assessment of variability and provide a mean value so that the experimental results can be characterized and evaluated in terms of these management-relevant nutrient constituents. Measurement of total nutrients also allows for more transparency in reporting the conditions of experimental systems to aid interpretation of results and evaluation of relevance; for instance, whether artificial systems experienced ranges of nutrient conditions similar to natural streams. Finally, development of continuous sensors and refinement of automated analyzers for TN and TP could further aid characterization of nutrient pollution effects in these terms, and efforts at various stages of development are underway in multiple labs (e.g., Tong et al. 2010; Denice Shaw, U.S. EPA, personal communication). With these small changes requiring fairly minimal effort, researchers can increase the utility of their experimental nutrient addition studies and increase the evidence base to better inform the management and protection of stream and river ecosystems.

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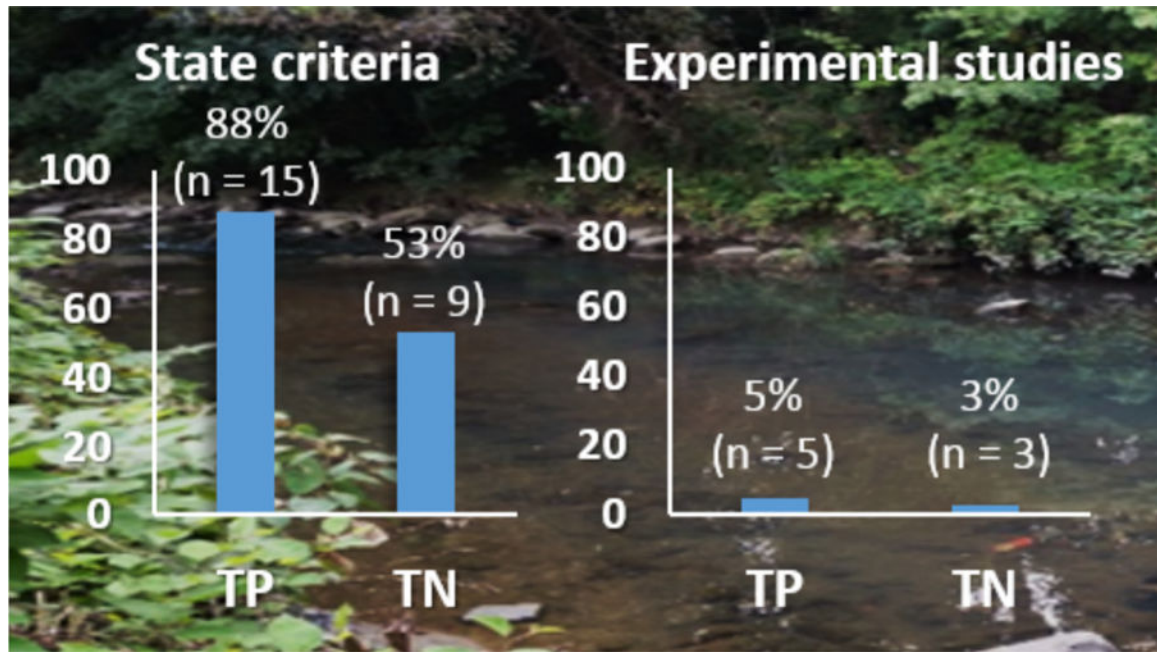
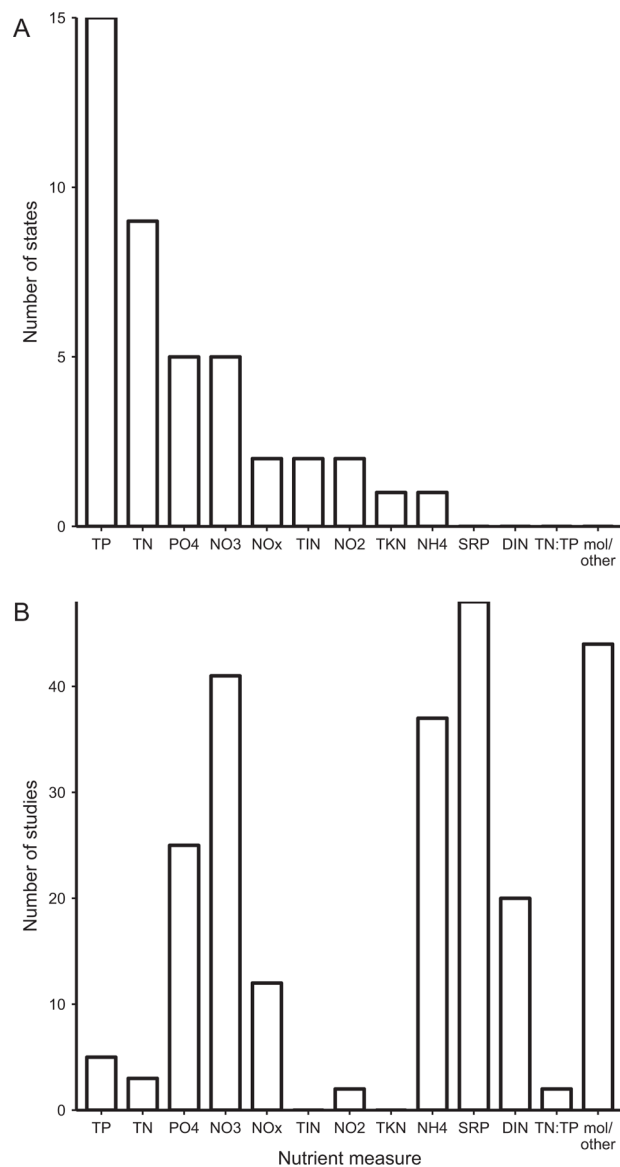


FIG. 1. Percentage of state numeric criteria for total phosphorus (TP) and total nitrogen (TN) (left) and percentage of experimental studies measuring TP and TN (of 100 examined) (right).

**FIG. 2.**

(A) Number of U.S. states using particular measures of N and P in developing numeric nutrient criteria (out of 17 states with river and stream criteria). $\text{NO}_x = \text{NO}_2^- + \text{NO}_3^-$. TIN = total inorganic nitrogen. TKN = total Kjeldahl nitrogen. See more details in Table 1. (data from <https://www.epa.gov/nutrient-policy-data/state-progress-toward-developing-numeric-nutrient-water-quality-criteria>). (B) Number of experimental studies reporting particular measures of N and P in response to nutrient addition (out of 100 studies examined). Mol/other = molar concentration, molar ratio, and other measures.

TABLE 1.

Use of nutrient measures in state numeric nutrient criteria for rivers and streams

Basis for WQ Criteria												
State/Territory	Status/Coverage	TN	TP	NO ₃ ⁻	NO ₂ ⁻	NO _x	NH ₄ ⁺	PO ₄ ^a	TIN ^b	TKN ^c	SRP	
Am. Samoa	statewide	+	+									
Arizona	partial	+	+					+				
California	partial	+	+	+				+	+		+	
Florida	partial	+	+			+						
Guam	statewide			+				+				
Hawaii ^e	statewide	+	+			+	+					
Minnesota	statewide	+										
Montana	partial	+	+									
N. Marianas Is. ⁺	statewide	+	+					+				
Nevada	partial	+	+	+				+		+		
New Jersey	statewide	+	+	+								
New Mexico	partial	+	+									
New York ^f	statewide									+		
Oklahoma ^g	partial		+									
Puerto Rico	statewide	+	+									
Vermont ^h	partial		+								+	
Wisconsin	statewide		+									

Note: Data from <https://www.epa.gov/nutrient-policy-data/state-progress-toward-developing-numeric-nutrient-water-quality-criteria> "Partial" describes states in which nutrient criteria cover only a subset of river or streams in the state or territory. Both partial and statewide criteria often cover water bodies supporting a variety of "designated uses" including drinking water, aquatic life, fishing, swimming, and other recreation, unless specifically noted.

^a Includes criteria that use "phosphates" and "orthophosphate".

^b Total inorganic nitrogen.

^c Total Kjeldahl nitrogen.

^d Applies to freshwater springs.

- ^e Applies to open coastal waters which includes rivers and streams under some tidal influence.
- ^f Applies to fish propagation and trout waters.
- ^g Applies to scenic rivers statewide.
- ^h Applies to “ecological” waters with designated uses for aquatic life, aesthetics, swimming/contact recreation, boating, fishing and other recreation.