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Estimating Ecological Thresholds for Phosphorus in the Everglades

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The Florida Everglades, a wetland of international importance, has been undergoing a significant shift in its native flora and fauna due to excessive total phosphorus (TP) loadings (an average of 147 t per annum from 1995 to 2004) and an elevated mean TP concentration (69 μg L^{-1} of TP in 2004) from agricultural runoff and Lake Okeechobee outflow despite the use of 16000 ha of stormwater treatment areas. Here, we present a Bayesian changepoint analysis of long-term experimental research and show that exceeding a surface water geometric mean TP threshold concentration of 15 μg L^{-1} causes an ecological imbalance in algal, macrophyte, and macroinvertebrate assemblages as well as slough community structure. A phosphorus threshold for all trophic levels may be more realistic and protective when presented as a threshold zone (12–15 μg L^{-1}) because estimates of uncertainty must be utilized to accurately define TP thresholds, which change with seasons and water depths. Most interior areas of the Everglades are currently at or below this threshold zone, but the exterior areas near inflow structures (except for the Everglades National Park) are presently receiving double or triple the proposed threshold. Our Bayesian approach, used here to address ecological imbalance along nutrient gradients, is applicable to determining thresholds and stable states in other aquatic ecosystems.

Introduction

Only 51% of its original size, the Everglades remains the largest and most ecologically important subtropical wetland in the United States. This 618000 ha fen, mostly classified as an alkaline peatland, is highly impacted by drainage canals, nutrient additions, and altered hydrology (*1–5*). Its resilient flora and fauna have evolved to survive in an ecosystem disturbed by hurricanes, flooding, seasonal droughts, and fire (*1, 6–8*). Primary productivity is restricted by severe P limitations (*9*), a condition found in similar limestone-based marshes covering extensive areas on the Yucatan Peninsula, northern Belize, and many Caribbean islands (*10*). One prominent hypothesis proposes that the Everglades is unique with respect to the multiple factors causing a high degree of P limitation and organism sensitivity to small additions of P (*11*).

The problem of P-induced eutrophication in the last 30 years has occurred mainly in the northern Everglades due to increased runoff from agricultural lands and eutrophic Lake Okeechobee (*12–14*). A series of outflow structures have created a P-enrichment gradient that often extends 5–8 km into the interior of the wetlands as a result of total phosphorus (TP) loadings averaging 229 t from 1978 to 1988 and 147 t from 1995 to 2004 from agricultural runoff and Lake Okeechobee outflow despite the use agricultural Best Management Practices (BMPs) since 1996 and the construction of 16000 ha of stormwater treatment areas (*14, 15*). In 2004 BMPs had resulted in a 55% cumulative P load reduction to the northern Everglades and a reduction in the three-year flow weighted mean P concentration to 71 μg L^{-1} P.

Although 2004 TP loadings were reduced to 83 t, loading rates per unit area have often exceeded 4 g m^{-2} yr^{-1} at the edge of the fens (*14, 16*). We had earlier shown that these excessive TP loadings would only reach nonimpact levels in the downstream water column when P loading inputs decreased below the ecosystem's P assimilative capacity of 1 g m^{-2} yr^{-1} (*16*). Surface water total phosphorus (TP) input concentrations along these gradients during the 1980s ranged from 173 μg L^{-1} near agricultural outflow gates flowing into the fen to a 2004 stormwater treatment area (STA) mean output concentration of 41 μg L^{-1} of TP, a 76% reduction. However, these values still remain in excess of the 10 μg L^{-1} marsh TP criterion passed by the State of Florida in 2003 and approved by EPA in 2005 (*15*). Specifically the criterion for the Everglades indicates that all measured sites must meet a five-year geometric mean criterion of less than or equal to 10 μg L^{-1} TP in three of five years, have annual concentrations less than or equal to 11 μg L^{-1} P across all stations, and have concentrations less than or equal to 15 μg L^{-1} P annually at all individual stations.

Importantly, 2004 inner concentrations range from 5 μg L^{-1} in the Everglades National Park (ENP) to near 17 μg L^{-1} in the core background areas of Water Conservation Area 2A (WCA-2A) in the northern Everglades (*15*). This suggests that many areas of the Everglades fen are already meeting standards. However, the interior of the most impacted areas of the northern Everglades WCA-2A and most wetland borders adjacent to input canals have TP values two to three times the criterion (*15*). The past two decades of increased P nutrient loadings have resulted in elevated water and soil P concentrations in large portions of the Everglades, typically extending nutrient gradients into the interior of the fen communities (*9, 15–18*). This elevated nutrient gradient has resulted in extensive shifts in algal, macroinvertebrate, and macrophyte species. It has also altered community structure, especially in the highly and moderately P-enriched areas (*19–24*).

These observations of biotic change immediately downstream of the inputs do not elucidate the threshold surface water TP concentration that causes an imbalance of flora and fauna in the Everglades nor do they provide insights into the level of TP that causes changes in both structure and function of aquatic ecosystems resulting in a shift between alternate stable states (*11, 25, 26*). Most importantly, the northern Everglades is now undergoing a reduction in external inputs, and therefore, the Everglades restoration efforts provide us with a unique opportunity to establish and confirm the environmental thresholds that will trigger a reversal from impaired states.

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The recently promulgated EPA TP criterion concentration of 10 μg L^{-1} was selected because it was considered necessary to maintain the ecological balance and integrity of the ecological community (*14, 15, 27, 28*). If true, then the Everglades has no capacity to assimilate phosphorus much above background concentrations without significant changes occurring in the flora and fauna. Here we present a Bayesian changepoint analysis of long-term experimental research to support the hypothesis that the Everglades has the capacity to assimilate phosphorus above background TP concentrations without causing imbalances in flora and fauna or a shift in stable states. To test this hypothesis, we applied five different concentrations of phosphate (as soluble reactive phosphorus (SRP)) for 6 years at 2 P-limited slough field sites in the northern Everglades (*29*).

Materials and Methods

Field Methods and P Dosing Treatments. We used a mesocosm-scale experiment at two sites to assess the biotic responses of the algal–plant–macroinvertebrate complex in a six-year P dosing in a slough community located in an undisturbed area of the northern Everglades. Two P dosing facilities (hereafter called "flumes") were constructed in the unimpacted interior of WCA-2A (26° 15′ N, 80° 23′ W) in fall of 1990 and then calibrated for over one year. The two sites were almost identical in macrophyte species composition and structure in the calibration year prior to treatment (*29*). Flumes were constructed in open-water sloughs, a habitat that has been shown to be sensitive to P-enrichment (*30, 31*) and ecologically important (*32*). Each site had five walled flumes, 2 m wide × 8 m long with walls approximately 120 cm in height above the slough substrate. An additional unwalled control area of identical size to that of flumes was established on the west side of both sites to concurrently monitor the potential effects of placing walls around slough habitat. Dye and bromide tracers were used to confirm that flumes were isolated and that dilution could be accounted for. Wall effects proved minimal and were also further reduced by only measuring responses in the center meter of the channels. Flumes were oriented N-S and separated by 2 m, where permanent boardwalks were built to allow investigators access for sampling. Each flume was randomly named and assigned one of five soluble reactive phosphate (SRP as Na_2HPO_4) treatments: ~5 μg L^{-1} (mean background concentration [walled and unwalled controls]; 0.25 g m^{-2} y^{-1}), 30 channels, ~22 μg L^{-1} (1.5 g m^{-2} y^{-1}), 50 channels, ~39 μg L^{-1} (2.75 g m^{-2} y^{-1}), 75 channels, ~57 μg L^{-1} (3.5 g m^{-2} y^{-1}), and 150 channels, ~126 μg L^{-1} (8.2 g m^{-2} y^{-1}). SRP was dosed from the northern end of flumes via large mixing tanks. Flumes were dosed on a continuous schedule except during low- or high-water shutdowns or periodic maintenance to specific flumes. Dosing was applied from 30 November 1992 to 21 September 1998. Greater detail on the design and operation of the P dosing study is described by Richardson et al. (*33*). The dosing system created a total phosphorus (TP) and SRP gradient down each flume. Water was sampled every two weeks for SRP, and TP analyses at each meter (1–8) down the length of each channel. Between March 1993 and September 1998, once each month, additional water samples were sampled for a suite of chemical analyses. This includes not only SRP, but TP, dissolved organic P, particulate P, ammonia, nitrate+nitrite, dissolved organic N, particulate N, calcium, potassium, and pH at 2, 4, and 6 m distances down the channel length. Sample collection methods, storage, analysis, and QA/QC procedures were in accordance with standard methods (*34*). A detailed presentation of methods as well as procedures for laboratory and field protocols using blanks, spikes and standards are given in detail in our FDEP QA/QC approved plan (*29*). Biotic responses and water chemistry were measured at each meter location, then selected metrics were compared to the geometric mean water column TP concentrations for either the previous 3 month (algal responses) or 6 month period (macrophytes, macroinvertebrates, and community metrics). Other time periods (1, 2, 8, and 12 month means) were also tested, but the 3 and 6 month periods were chosen because they most closely represent the biological life of each trophic level and because values among other tested time periods proved to be more variable. Daily variations in water depth and temperature were measured at both sites using an Omni Data automated data logger water level system coupled with a Metri-Tape unit and a thermocouple probe placed 10 cm below the water surface. Standard methods and EPA approved methods were used for all analyses.

Ecological Indicators. We initially selected over fifty potential ecological and biological attributes of imbalance for the Everglades study (*35*). Final attributes were selected based on ecological importance and statistical analyses (*35*) at key trophic levels in the Everglades. The attributes tested are categorized as follows: (units shown in parentheses)

- Fast response time: algae–diatom relative abundance (%), diatom density (cell/m^2), diatom biovolume (cm^3/cell); and blue green algae biovolume (cm^3/cell),
- Intermediate response time: Macrophytes- *Utricularia spp* (stem densities), *Utricularia purpurea* (stem densities); Macroinvertebrates–biomass (mg), abundance (number) Oligochaeta (number), microcrustacea (number), sensitive species (%), predators (%), Gastropoda (%);
- Slow response time: Community-macroinvertebrate taxa (number), Bray–Curtis dissimilarity (scaled index), calcareous mat cover (%).

Statistical Modeling. To first evaluate which abiotic (e.g., water depth, year) and ecological (e.g., algal, macrophyte, macroinvertebrate) variables were the best indicators of TP concentration, we used classification and regression tree (CART) models, an analysis that recursively partitions observations into groups using the best indicators of TP (*36*). The analysis used data collected over more than 6 years, spanning all seasons and a wide range of water depths (15–120 cm).

We then used a Bayesian hierarchical changepoint model, which was specifically designed for detecting changes along a gradient (*17, 37*). Specifically, we assume that the response variable values, $y_1, \ldots, y_n$, collected from the n sites along the P gradient of interest, are random samples from the sequence of random variables $Y_1, \ldots, Y_n$. The corresponding P concentration values are $x_1, \ldots, x_n$, where $x_1 < x_2 < \ldots < x_n$. Assuming that variables $Y_1, \ldots, Y_n$ belong to the same family of distributions with parameter θ. The random variables $Y_1, \ldots, Y_n$ have a changepoint r ($1 \le r \le n$) if the parameter value changes at r:

$$Y_1, \ldots, Y_r \sim \pi(Y_i | \theta_1)$$
$$Y_{r+1}, \ldots, Y_n \sim \pi(Y_i | \theta_2)$$

The corresponding P concentration x_r is the P threshold or changepoint. A uniform prior is used for r and noninformative or vague priors were used for θ_1 and θ_2. The results of the model is presented in the form of a probability distribution of the n P concentration values $x_1, \ldots, x_n$ being the changepoint, or $p(x_i)$, where $\sum_{i=1}^{n} p(x_i) = 1$.

Results and Discussion

Phosphorus Treatments. Six experimental treatment flumes at each site created a surface water TP concentration gradient that ranged from 10 to 75 μg L^{-1} TP along which to test biotic responses (ecological attributes) across several trophic levels (Figure 1). In a P-limited aquatic system such as the Florida Everglades, the biotic assimilation of SRP is rapid, and water column concentrations are reduced to uniformly low concentrations throughout most of the flumes, which negates the formation of a measurable SRP gradient

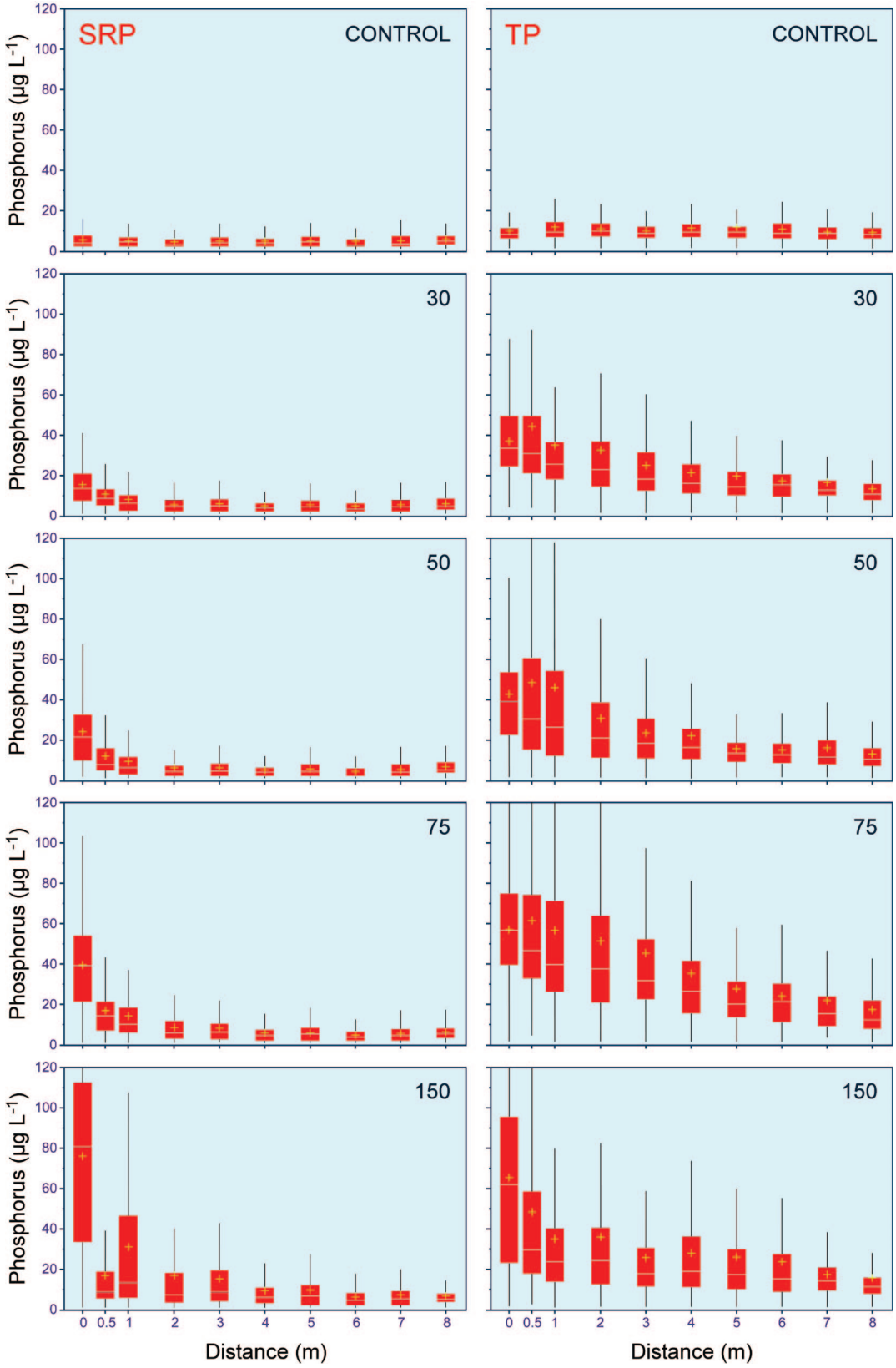

FIGURE 1. The mean water column soluble reactive phosphorus (SRP) and total phosphorus (TP) concentrations in the dosing flume channels at one-meter intervals downstream of the influent head boxes. The flume channels were labeled unwalled controls (UC), walled controls (WC), and 30, 50, 75, and 150 to represent P treatment status (see channel label numbers in upper right corner). Actual SRP dosing concentrations are given in the methods section. The top, midline, and bottom of each box represents the 75th, 50th (median), and 25th percentile of the data, respectively. The means (+) represent the TP found at dosing sites 1 and 2 during the dosing period (1992–1998). The vertical lines represent the 10th and 90th percentiles, respectively. Water samples were replicated at each date and measurements were taken at midcolumn water depths. Water depths ranged from >120 cm during the wet season to <10 cm during the dry season. Error bars represent the standard error of the mean. ($n = 1117$).

(Figure 1). Therefore, TP serves as a more reliable measure of P-enrichment and is typically used by agencies to set water quality standards. The long-term data set of TP concentrations in the experiment allowed us to test responses over each season and at different water depths, which seasonally ranged from <15 cm to over 120 cm. This experimental TP gradient closely matched the concentration range of existing edge-to-interior TP gradients found in the Everglades (*15, 16, 38*). However, ecological attributes often show little change along a gradient until a critical environmental value (threshold) is reached (*39*), and quantitative description of such exposure-response relationships (*40, 41*) are very difficult to obtain. To address this problem, we utilized a Bayesian changepoint analysis (*17*) to estimate TP thresholds for selected biological response variables across multiple trophic levels. Here we define ecological imbalance as a significant alteration in distribution parameters (e.g., mean and/or variance) of the biological response variable (attribute) found above versus below a gradient changepoint. The significance is determined by a small (<0.05) probability of no changepoint.

Ecological Responses. The density (stems m^{-2}) of *Utricularia purpurea* (bladderwort), a floating aquatic macrophyte, was selected as the best indicator of TP using CART (Figure 2A). Almost no stems per m^{-2} of *U. purpurea* were found at TP concentrations averaging ≥21 $\mu g\ L^{-1}$ (Figure 2B). By contrast we found up to 63 stems m^{-2} in channel locations with TP concentrations near 12 $\mu g\ L^{-1}$. The second best indicator was the combined population of *Utricularia (U. purpurea+ U. fibrosa + U. foliosa)* closely followed by water depth (data not shown). All three *Utricularia* species—a key component of the ecologically significant floating periphyton mat community (*21, 32, 38*)—demonstrated a consistent-pronounced decline with increasing P concentrations (Figure 2C). This may be due to their inability to photosynthesize in waters devoid of CO_2, a condition found at higher pH levels (*42*) in P-enriched areas of the Everglades. In fresh waters the total amount of free CO_2 available for photosynthesis is variable and highly pH dependent. The p*K* dissociation relationships of CO_2, HCO_3^- and CO_3 indicate that free CO_2 is the dominant form in the water column at a pH of 5 and below, whereas HCO_3^- is dominant from pH 7–9. Above a pH of 9.5, CO_3 is the main form of inorganic carbon in the water column (*43*). Supporting the "CO_2 limitation" hypothesis is the rise in pH in the Everglades. It averages 7.5 and ranges from a low of 7.2 at night to a high of >10 during the day in the P treatment channels (*29*). Daytime alkalinity values were often found to exceed 300 $\mu g\ L^{-1}$ as $CaCO_3$. Another interaction takes place within the floating periphyton mat, where a significant reduction in *Utricularia* is concurrent with the disappearance of the periphyton community, which primarily occurs at higher P levels (*21*). We have found in periphyton removal experiments that a loss of plant buoyancy occurs when the highly oxygenated periphyton mat disappears and *Utricularia* sinks deeper into the water column (*15, 31*).

Next, we used the Bayesian changepoint method to detect variations in response of the population of *U. purpurea* to TP at different seasons each year (Figure 3A–D). Here each existing data point was assigned a probability of being the changepoint and the TP concentration associated with the highest probability was selected as the TP threshold. For example, *U. purpurea* displayed a variation in TP thresholds over different seasons with geometric mean values of 17.2, (August 1995), 22.3 (March 1996), 12.4 (April 1998), and 16.6 $\mu g\ L^{-1}$ TP (August 1998, near the end of the 6-y experiment) (Figure 3D). In all cases the probability of each selected threshold exceeded 0.97. Importantly, the temporal variation among TP thresholds suggested that seasonal and yearly differences must be integrated into the development of a TP

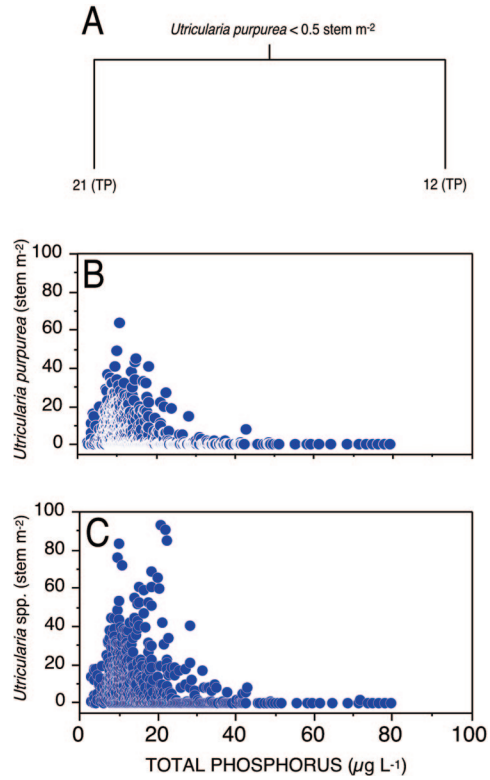

FIGURE 2. (A). A CART (classification and regression tree) model was used as an inverse ANOVA (*41*) to establish the association between total phosphorus (TP) and candidate biological and abiotic variables (e.g., macrophytes species, water depth, algal species). *Utricularia purpurea* stem counts were selected as the variable that was most closely associated with P concentrations in the water column of the Everglades. (B) The number of all *Utricularia purpurea* stems/m² in response to mean TP concentrations found in the water column in August. The TP values are the geometric mean ($\mu g\ L^{-1}$ TP) of the water column phosphorus in found during the six months growth period for the plant species. Data for all seasons and years are combined to give a general response pattern for *U. purpurea* during the entire experiment (1992–1998). (C) The number of *Utricularia* spp. stems/m² in response to mean TP concentration in the water column in August of 1992–1998.

threshold for each attribute. To accomplish this we calculated an overall mean threshold and credible interval (CI) for *U. purpurea* for the entire experimental period by averaging the geometric mean P thresholds across each time period. The 95% Credible Interval (CI) is the interval of P concentrations that includes 95% of the probability mass of the changepoint distribution. In Bayesian analyses each data point has a probability that it is the threshold but the threshold is assigned to the highest maximum probability data point distribution (*17*). The lower bound (x_L) is largest P concentration such that $\sum_{i=1}^{L} p(x_i) \leq 0.025$ and the upper bound (x_U) is the smallest P concentration such that $\sum_{i=U}^{n} p(x_i) \leq 0.025$. Because the distribution is discrete, the selected 95% credible interval (x_L, x_U) may include more than 95% probability mass. In other words, the CI would give a conservative estimate of the range of upper and lower bounds to the TP threshold. The mean P threshold for *U. purpurea* was 14.8 $\mu g\ L^{-1}$ TP with a 95% CI that ranged from 13.6 to 15.7 $\mu g\ L^{-1}$ TP (Table 1).

To examine the ecological imbalance of the community across trophic levels, we completed a similar hierarchical changepoint analysis over each season and year to identify biological attributes that were consistent metrics of ecological imbalance (*44*). After an initial screening of our database with CART to identify candidate species and other ecological

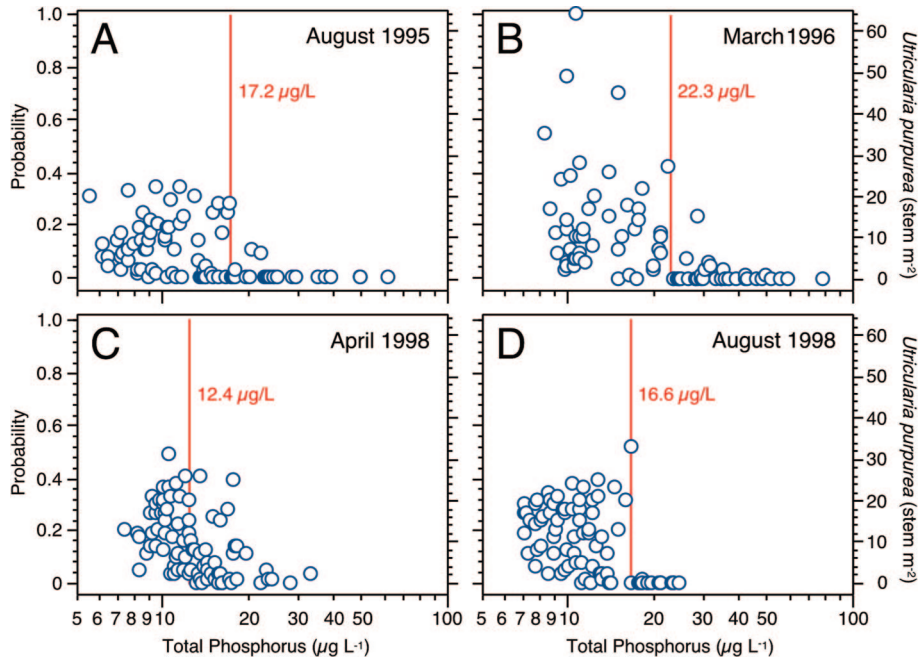

FIGURE 3. (A to D) A decrease in the stem counts of *Utricularia purpurea* per m^2 to increasing water column total phosphorus (TP) concentrations in the dosing channels during the summer and spring of 1995–1998. The selected TP threshold was based on Bayesian analyses and is shown as a "red" vertical line. Values range from a low of 12.4 μg L^{-1} TP in April of 1998 to a high of 22.3 μg L^{-1} TP in March of 1996. The mean and variance of the populations above and below the thresholds have been shown to be significantly different as estimated by a nonparametric deviance analyses (*17*). The probability for all the thresholds exceeds 0.97.

TABLE 1. Results from Bayesian Changepoint Analysis on All Trophic Levels from the Phosphorus Dosing Experiment in 1993–1998

metric	observed response (*n*)[a]	mean changepoint[b]	maximum probabilities	95% CI[b]	lower and upper probabilities
community					
mat cover	decrease (216)	19.2	0.88	18.4–20.0	0.003–0.000
Bray–Curtis-dissimilarity (macroinvertebrates)	increase (180)	13.0	0.54	9.4–15.8	0.010–0.011
macroinvertebrates					
% tolerant species	increase (180)	13.0	0.77	9.8–19.4	0.004–0.004
% sensitive species	decrease (72)	12.4	0.67	8.4–26.2	0.016–0.012
% predators	decrease (72)	8.2	0.96	7.9–8.7	0.003–0.664
% microcrustacea	increase (180)	19.9	0.83	16.5–20.7	0.000–0.000
% oligochaeta	increase (108)	18.3	0.99	17.1–18.3	0.000–0.000
macrophytes (stem counts)					
total *Utricularia* species	decrease (468)	15.6	0.94	15.0–15.9	0.002–0.002
Utricularia purpurea	decrease (432)	14.8	0.84	13.6–15.7	0.000–0.005
algae					
% diatom on *Eleocharis cellulosa*	decrease (108)	14.5	0.91	12.1–22.2	0.003–0.003
% diatom on plexiglas	decrease (72)	23.5	0.68	14.0–40.3	0.001–0.006
% diatom on floating mats	decrease (144)	15.3	0.75	12.0–22.3	0.004–0.007

[a] (n = number of sampling periods times 36 sample locations that displayed a significant TP changepoint) [b] Changepoints and 95% credible intervals are based on the geometric means of total phosphorus (μg L^{-1} TP) in water.

metrics (over 50 metrics were tested), we selected biological indicator groups that covered multiple trophic levels including "fast" (periphyton), "intermediate" (macroinvertebrate composition, plant density), and "slow" (percent mat cover) process variables from both the natural state and the altered states. Imbalance was calculated as a change in the mean and/or variance of the attribute response variable found above versus below the biological changepoint with the highest maximum probability. The maximum probability is the mode of the changepoint probability distribution: $p(x_r) = \max(p(x_1),...,p(x))$. It is the P concentration that is most likely to be the threshold, and it gives an indication of how well defined the threshold is within a particular metric (*17*).

At all levels the composition, diversity or population structure of the attribute were significantly altered above the TP threshold as compared to below the changepoint as shown, for example, in Figure 3 A–D. Importantly, the Bayesian hierarchical model takes into account the natural variation in the attributes and thus provides a robust probability estimate of the TP threshold. We then selected metrics at several trophic levels with the highest ecological value and probability threshold to calculate a mean annual TP threshold concentration for each metric (Table 1). This multimetric approach is based on the premise that while no one particular attribute of a biotic assemblage will always be a reliable indicator of imbalance, a suite of attributes used in combination will greatly increase our ability to detect impairment (*44, 45*). Collectively, our selected attributes represent taxonomic composition, species richness and diversity, tolerance/intolerance, and trophic and community structure (Table 1).

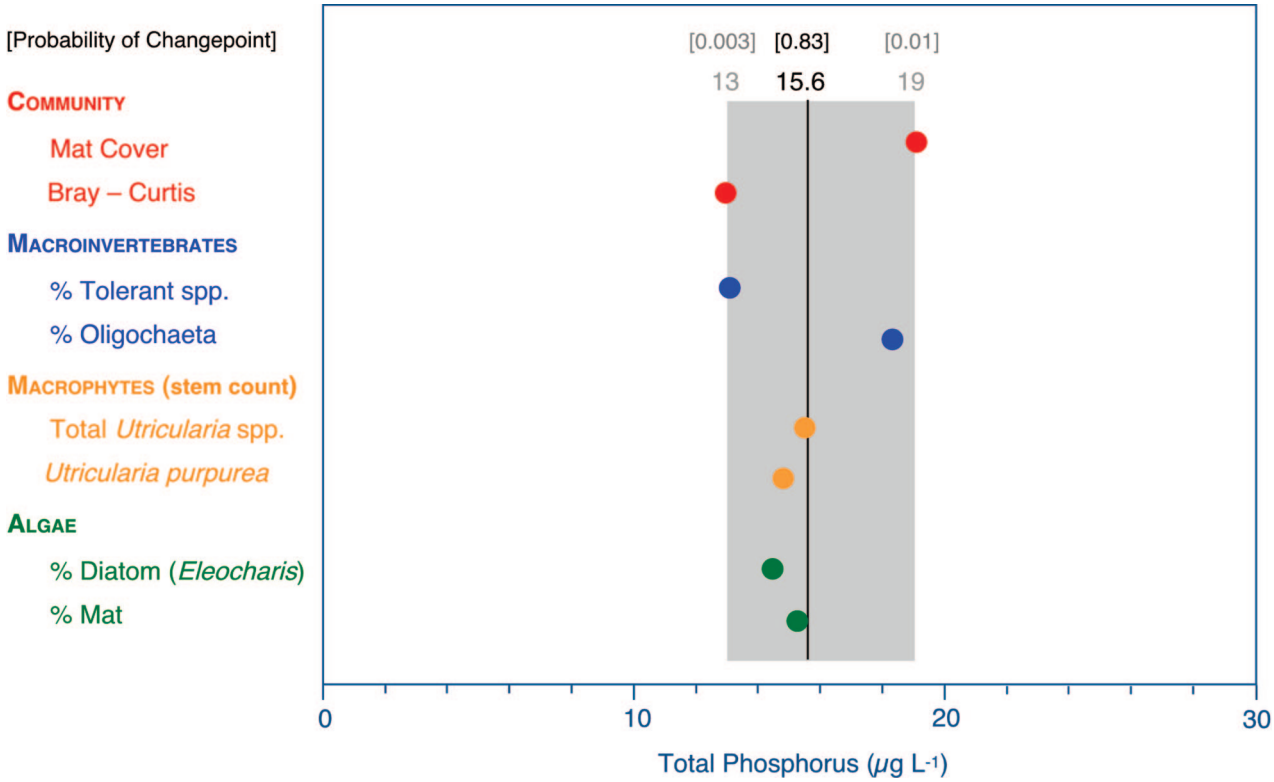

FIGURE 4. A hierarchical graph of the mean total phosphorus (TP) thresholds along with the corresponding 95% credible interval limits (CI) for each trophic level attribute. The mean TP threshold for all trophic levels combined is 15.6 μg L^{-1} with a CI of from 13 to 19 μg L^{-1} TP. The approved EPA TP criterion of 10 μg L^{-1} TP is below the range of all quantified trophic level thresholds (*27, 28*).

The observed responses among trophic levels demonstrated a remarkably similar pattern of response (Table 1). Thresholds were almost all above 10 μg L^{-1} TP and below 20 μg L^{-1} TP, with macroinvertebrate predators displaying the lowest TP changepoints, whereas diatoms growing on artificial Plexiglas substrates had the highest TP threshold. The most consistent TP threshold with the highest probability was for *Utricularia* species. This metric showed very little variation as noted by the narrow CI (Table 1). The 95% CI intervals were the tightest for the metrics with the highest number of sampling points and varied greatly for the % diatoms on Plexiglas, which had only two sampling dates with changepoints.

To assess uncertainty for each of the selected thresholds, we evaluated the maximum probability and CIs for each metric. Providing an estimate of uncertainty is important because no clear consensus definition for imbalance of natural populations of flora and fauna exists in the ecological literature (*46*). The weakest maximum probabilities were found for Bray–Curtis dissimilarity (BCD, 0.54), % sensitive macroinvertebrate species (0.67) and % diatoms on Plexiglas (0.68) (Table 1). Again, this was probably related to the small number of sampling dates that showed a significant TP threshold for these metrics. Probabilities in excess of 0.90 were found for % Oligochaeta, % predators as well as total *Utricularia* species, and % diatoms on *Eleocharis* stems. Maximum probabilities averaged 0.89, 0.84, 0.78, and 0.71 for the plant macrophytes, macroinvertebrates, algae, and community metrics, respectively. The metrics with both high probabilities and narrow CIs indicate thresholds that accurately reflect a major imbalance in the attributes above the changepoint thresholds for each group as compared to the attribute characteristics below the changepoints (Table 1). The probabilities that the threshold existed at the lower or the higher extreme of the 95% CI were also tested by developing individual probability ratios of the selected changepoint to the lower and higher CIs. In all cases the probability that the changepoint is less than or equal to the lower CI or greater than or equal to the upper 95% CI was <0.01, with most metrics being <0.005 (Table 1). Thus, the probability that the threshold is at the lower or upper 95% CI is close to 0.

To develop a uniformly weighted, integrated threshold, we selected two metrics at each trophic level based on data for at least 100 sampling points, the narrowest CI, and highest maximum probability (Table 1, Figure 4). The overall mean changepoint was 15.6 μg L^{-1} TP, a value nearly identical to the overall mean of all changepoints shown in Table 1 (Figure 4). The BCD index had the lowest TP threshold (13 μg L^{-1} TP), lowest maximum probability (0.54), and a wide CI, while mat cover had the highest TP threshold (19.2 μg L^{-1} TP) with a maximum probability of 0.88 and a narrow CI. The most robust ecological attribute over the entire 6 year period of the experiment was *Utricularia* spp., with a mean changepoint of 15.6 μg L^{-1}, TP a very narrow CI of 15.0–15.9, and a maximum probability of 0.94 (Table 1, Figure 4). The ecological importance of *Utricularia spp.* as a key matrix component of the Everglades periphyton mat community (*1, 21, 31*) coupled with their highly predictable response to P additions ((*25*), Table 1) suggest they may be highly sensitive indicators of the P threshold for the Everglades, especially *Utricularia purpurea*. These findings also suggest that *Utricularia purpurea* may be both an important keystone and P indicator species for the Everglades.

The 95% CI range for all metrics varied from a low of 13 μg L^{-} TP to a high of 19 μg L^{-1} TP. This span of 6 μg.L^{-1} TP represents a measure of the uncertainty of the estimate, which in part may be due to the natural variation found in the TP thresholds across seasons, water depths, and years (Figure 3A–D). Moreover, 92% of all TP thresholds tested are above 10 μg L^{-1} TP and below 20 μg L^{-1} TP. Our dosing study results support the hypothesis that the TP threshold for the Everglades is best represented by a TP zone—not a single number—and that it is above 10 μg L^{-1} TP, but should not

exceed 15 μg L^{-1} TP. Importantly, our results at each trophic level show a similar, well-defined TP threshold with a high probability of maintaining a balanced flora and fauna within the Everglades. The dosing research supports our hypothesis that the Everglades has the capacity to assimilate P slightly above background concentrations and that a P threshold protective for all trophic levels in the northern Everglades would best be defined as a threshold zone between 12 and 15 μg L^{-1} due to seasonal and water level effects.

In the face of these experimental findings, it can be argued that the small spatial scale of our mesocosm study is not representative of the Everglades ecosystem (*15, 30, 38*). To test if our mesocosm results were representative of the TP gradient in the Everglades, we completed TP changepoint analyses (*47*) for macroinvertebrates along a 10 km nutrient (TP, N, Ca, etc.) gradient (*9*) and found that the average for all five metrics (Table 1) in the dosing channels was slightly lower (14.4 μg L^{-1} TP) than found along the gradient (15.5 μg L^{-1} TP). In addition our earlier studies (*16*) along this gradient found that no changes in the community structure, productivity or diversity at loadings of 0.4 g m^2 yr^{-1}, a loading that resulted in <18 μg L^{-1} TP in the water column. These findings suggest that our mesocosm study results are representative and give a conservative estimate of the existing TP thresholds at the landscape scale.

A current major concern is the fact that the exterior portions of all Everglades areas adjacent to input canals, except the ENP, are not meeting either the proposed EPA criterion of 10 μg L^{-1} TP or our TP ecological exceedence threshold of 15 μg L^{-1}. Recent TP gradient analyses (*38, 15*) clearly demonstrate elevated TP water column concentrations (>15 μg L^{-1} TP and sometimes as high as 100 μg L^{-1} TP) within the first 1 km into the wetlands. Unfortunately, even greatly reduced input TP water concentrations will result in a build up of soil P concentrations (*16*) due to the massive amounts of water being added to the Everglades (i.e., low TP concentrations times large volumes of water equal elevated P loadings). Thus, waters and soils near the input structures will continue to have elevated TP concentrations, although not as high as the northern areas of WCA-2A, which currently have more than 1500 mg kg^{-1} in the top 0–10 cm of soil (*9, 12, 25, 38*). This residual P will result in an upflux of P from the soil sediment to the water column (*25*) and in conjunction with elevated TP input water concentrations will result in TP water concentrations far in excess of the Everglades TP threshold for several km down gradient.

The weight-of-evidence produced from the dosing and gradient analyses implies that the P threshold protective for all trophic levels would best be defined as a threshold zone between 12 and 15 μg L^{-1}, and a TP concentration >15 μg L^{-1} is likely to cause degradation of plant and macroinvertebrate assemblage structure and function, a reflection of biological integrity, in the study area. While a TP concentration of 15 μg L^{-1} TP is a reasonable estimate of a TP concentration that will maintain a balance in the flora and fauna at the northern edge of the Everglades peatland, some species, especially in the interior of the southern Everglades and ENP, may require a value closer to the EPA approved criterion of 10 μg L^{-1} TP. Our threshold findings result from the development of a numerical estimate of the associated risk for metrics at each trophic level as well as an integrated estimate of risk from the combined hierarchical analysis compared with the Index of Biological Integrity (IBI) estimate (*47*). These methods give very similar results and provide a robust estimate of the TP threshold for the Everglades.

Importantly, our Bayesian approach represents a reliable and innovative way of quantifying ecological thresholds along gradients based on estimates of changes in both mean and population variance coupled with a probability analysis. The quantification of ecological thresholds for multiple-trophic levels rather than relying on single-species responses or arbitrary estimates of imbalance provides for a robust estimate of the TP threshold (*44*) and is applicable to other aquatic ecosystems. However, a Bayesian hierarchical modeling approach needs to be developed in the future to statistically integrate the changepoint analysis method presented here to form an ecosystem level threshold distribution to better represent the interactions among fast and slow responding species. We have explored the potential of combining multiple response variables into a single model to study the interactions between these responses. Preliminary results suggest that a Bayesian multilevel ANOVA method can be used to integrate multiple factors affecting thresholds and the interaction of thresholds at several trophic levels (*48*). This integrated approach should also provide risk-based criteria to assess ecological resilience and predict the threshold for alternative state changes.

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