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Nutrient levels associated with ecological thresholds of impairment: An approach to estimate numeric nutrient criteria for reservoirs of Puerto Rico[1,2]

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ABSTRACT

Six reservoirs of Puerto Rico were monitored over a 32-month period to establish relationships between their nutrient concentration status and different thresholds of ecological impairment. The selected reservoirs embody the productivity spectrum of reservoirs on the island. Median concentrations of total phosphorus (TP) for epilimnion waters (1 m) were as follows: Cerrillos, 10 µg/L; Cidra, 33 µg/L; Guajataca, 10 µg/L; La Plata, 49 µg/L; Patillas, 6 µg/L; and Toa Vaca, 22 µg/L. Likewise, median concentrations of total nitrogen (TN) were:

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Cerrillos, 0.22 mg/L; Cidra, 0.74 mg/L; Guajataca, 0.28 mg/L; La Plata, 0.55 mg/L; Patillas, 0.23 mg/L; and Toa Vaca, 0.34 mg/L. Strong positive correlations were observed between nutrients and chlorophyll *a* (Chl-*a*). Correlation coefficients were 0.74 for the relationship between TP and Chl-*a*, and 0.66 for TN vs. Chl-*a*. Increases in algal biomass (as measured by Chl-*a*) diminished the depth of light penetration into the water column (i.e., Secchi depth) (r = -0.70). A biological threshold indicative of designated use impairment was established at a Chl-*a* concentration of 24 µg/L based on evidence demonstrating that the extent of reduced light penetration at that Chl-*a* level (due to excessive phytoplankton biomass productivity) hindered the reservoirs' capacity to comply with the aquatic life criteria. Total phosphorus and TN concentrations associated with the Chl-*a* impairment threshold (i.e., 24 µg/L) were established based on a change-point analysis of the data. The resulting values, 0.035 mg/L for TP and 0.43 mg/L for TN, can be considered nutrient thresholds associated with impairment. To protect against impairment, a margin of safety was added based on concentrations defining the lower 5% confidence interval of the bootstrap distribution of values associated with the Chl-*a* impairment threshold (i.e., $p<0.05$-one tail probability). The resulting criteria, 0.026 mg/L for TP and 0.41mg/L for TN, are proposed as basis for establishing the nutrient standards for reservoirs of Puerto Rico. Significant differences in the maximum depth of compliance with the USEPA aquatic life criteria (i.e., DO = 5.0 mg/L) were observed between data points below and above the proposed numeric criteria. These differences, which can be critical to the sustainability of fish populations in reservoirs on the island, attest to the importance of maintaining the nutritional status of waters in our reservoirs at levels below the specified thresholds. The zooplankton community was characterized by a mix of species already known under similar environmental conditions in the Neotropical region. Sixty-five zooplanktonic taxa were identified. Rotifera was the richest group with 37 taxa. Cladocerans were represented by 20 taxa; Copepoda by eight taxa. Absolute abundances of rotifers showed an increase with the increase of several eutrophication indexes. A segmented curved analysis was used to describe the relationship between *Brachionus* rotifers (#ind/L) and phosphorus (lnTP). The relationship yielded a change-point value of 26.58 µg/L for total phosphorus, which coincides with the proposed numeric criteria value. In addition, a change-point of 1.16 meters was obtained for the relationship between *Brachionus* rotifers (#ind/L) and Secchi depth (m), which can be considered as another impairment index for reservoirs on the island.

Key words: numeric nutrient criteria, tropical reservoirs, ecological thresholds of impairment, eutrophication

RESUMEN

Niveles nutricionales asociados con umbrales de impacto ecológico: Alternativa para estimar los criterios numéricos de nutrientes para los embalses en Puerto Rico

Seis embalses de Puerto Rico se monitorearon durante un periodo de 32 meses con el propósito de establecer relaciones entre el estado nutricional de sus aguas y diferentes umbrales de impacto ecológico. Los embalses seleccionados representan el espectro de productividad prevaleciente en los embalses en la isla. Las concentraciones medianas de fósforo total (TP) en el epilimnio (1 m) fueron las siguientes: Cerrillos, 10 µg/L; Cidra, 33 µg/L; Guajataca, 10 µg/L; La Plata, 49 µg/L; Patillas, 6 µg/L; y Toa Vaca, 22 µg/L. Igualmente, las concentraciones medianas de nitrógeno total (TN) fueron: Cerrillos, 0.22 mg/L; Cidra, 0.74 mg/L; Guajataca, 0.28 mg/L; La Plata, 0.55

mg/L; Patillas, 0.23 mg/L; y Toa Vaca, 0.34 mg/L. Se observaron fuertes correlaciones positivas entre nutrientes y clorofila *a* (Chl-*a*). Se obtuvo un coeficiente de correlación de 0.74 para la relación entre TP y Chl-*a*, y de 0.66 para la relación entre TN y Chl-*a*. Aumentos en la biomasa algal (determinada por Chl-*a*) disminuyeron la penetración de luz en la columna de agua (r= -0.70). Se estableció como umbral biológico indicativo de impacto a los usos designados, una concentración de clorofila *a* de 24 μg/L, basado en la reducción en penetración de luz a ese nivel de Chl-*a* y su efecto en la capacidad de los embalses de cumplir con el criterio de vida acuática. Las concentraciones de TP y TN asociadas al umbral de impacto de Chl-*a* (i.e., 24 μg/L) se establecieron basado en un análisis de punto de cambio de los datos. Los valores resultantes, 0.035 mg/L para TP y 0.43 para TN, se pueden considerar como umbrales nutricionales asociados con impacto. Se incorporó un margen de seguridad utilizando la 5ta percentila menor de la distribución de valores "bootstrap" asociados umbral de impacto de Chl-*a* (i.e., 24 μg/L) (i.e., p<0.05- prueba unilateral). Los criterios resultantes, 0.026 mg/L para TP y 0.41 mg/L para TN, representan la base propuesta para el establecimiento de estándares nutricionales en embalses de Puerto Rico. Diferencias significativas en la profundidad máxima de cumplimiento con el criterio de vida acuática de USEPA (i.e., 5.0 mg/L) se observaron entre los datos ubicados por encima y por debajo de los criterios numéricos propuestos. Esta diferencia, que puede ser crítica para la sustentabilidad de la población de peces en la isla, evidencia la importancia de mantener el estado nutricional de las aguas en nuestros embalses a niveles bajo los umbrales propuestos. La comunidad zooplanctónica se caracterizó por una mezcla de especies previamente identificadas bajo condiciones ambientales similares en la región Neotropical. Se identificaron sesenta y cinco taxones de zooplancton. Los rotíferos fueron el grupo más diverso con 37 taxones, seguido de los cladóceros con 20 taxones y los copépodos con ocho taxones. La abundancia absoluta de los rotíferos evidenció un incremento con aumentos en diferentes índices de eutroficación. Se utilizó un análisis de curva por segmentos para describir la relación entre los rotíferos del género *Brachionus* (#ind/L) y el fósforo (lnTP). La relación produjo un cambio de punto a una concentración de 26.58 μg/L de fósforo total, lo cual coincide con el criterio numérico propuesto. Además, se obtuvo un cambio de punto de 1.16 m para la relación entre los rotíferos del género *Brachionus* (#ind/L) y la profundidad Secchi (m), lo cual se puede considerar como un indicador de impacto adicional en los embalses de la isla.

Palabras clave: criterios numéricos de nutrientes, embalses tropicales, niveles umbrales de impacto ecológico, eutroficación

INTRODUCTION

In the United States of America, cultural eutrophication has been a predominant water quality concern for over 50 years. Nutrient over-enrichment of surface waters stimulates immoderate growth of aquatic macrophyte vegetation and undesirable abundance of phytoplankton. The eventual decay of macrophyte and planktonic biomass can lead to short- and long-term cycles of anoxia (USEPA, 1999; Campbell and Edwards, 2001). In turn, low dissolved oxygen concentrations reduce fish growth rates, increase fish mortality, and alter the distribution and behavior of aquatic organisms (Breitburg, 2002). Anoxia also promotes biogeochemical processes such as sulfate reduction to hydrogen sulfide, the recycling of ammonia and phosphates, as well as the mobilization of heavy metals (e.g., Fe, Mn) from bottom sediments (USEPA, 2003). These conditions not only affect the water quality of the lake ecosystem, but that of downstream waters.

Concerns with eutrophication have increased in recent decades with the identification of several planktonic strains capable of producing toxins that are hazardous to human and animal health. Some of these compounds have been reported to affect the liver (hepatoxins), neurological (neurotoxins), and/or gastrointestinal systems, while others have been reported to cause kidney disease (Pan et al., 2002). Recurring formation of toxic cyanobacteria blooms has rapidly become an issue of increasing concern for health and water quality officials worldwide. Altogether excessive nutrient concentrations are detrimental to the designated uses of water bodies (raw source of potable water, aquatic life, navigation, fishing, or swimming) and make water treatment for drinking purposes more difficult and expensive (USEPA, 2000).

Elevated nutrient concentrations constitute the second most important impairment cause in the USA, affecting close to 20% of the total lake/reservoir population (USEPA, 2009). Lakes with excess nutrients were 2.5 times more likely to exhibit poor biological health relative to those with adequate nutrient levels. Interestingly, nutrients have yet to be identified as a significant impairment cause in reservoirs of Puerto Rico (PREQB *303d list,* 2012). The fact that nutrients are not officially considered a major pollution cause of surface waters in Puerto Rico is probably due to the lack of adequate standards that enable identification of nutrient impaired waters. The current water quality standard for reservoirs of Puerto Rico, 1,000 µg TP/L, corresponds to the NPDES permit limit applicable to point sources as assigned by USEPA at their effluent discharge point. Said level was never intended to regulate phosphorus concentrations at an ecosystem level. Using it as such clearly precludes the identification of nutrient impaired waters and the protection of a resource that is critical for the well-being of our society. Over 70% of the water used for human consumption in Puerto Rico comes from its reservoirs (Ortiz-Zayas et al., 2004). The high dependence of the population of Puerto Rico on reservoir water supply supports the implementation of sound protective measures to ensure sustainability of these ecosystems. Regulating the nutrient status of our reservoirs water should be at the forefront of these efforts.

The United States Environmental Protection Agency (USEPA) recommends two main approaches for the estimation of numeric nutrient criteria, namely: a) establishing reference conditions (reference criteria), or b) identifying ecological thresholds of integrity for protecting the designated uses of the waters (USEPA, 2010). Reference criteria (a) may be established from frequent distribution analyses by selecting the nutrient concentrations corresponding to the 75th percentile of the distribution of median values from a reference population of reservoirs (i.e., reservoirs in watersheds with no historic human presence nor intervention). Alternatively, one could select concentrations corresponding to the 25th percentile of the distribution of median values from the complete reservoir population (e.g., complete reservoir network) in a particular ecoregion. When based on a "reference population" criteria numbers represent background or pristine concentrations, whereas if the complete population is used the obtained values represent "best currently attainable" conditions. Reference conditions could also be estimated from paleolimnological analyses of sediment samples dated prior to the intensification of human activities in a particular region (not applicable to reservoirs).

An alternative to the frequency distribution approach is to establish *numeric nutrient criteria* based on predictive relationships between a stressor (i.e., nutrients) and a biological or ecological response variable at thresholds indicating significant impact to the ecological integrity of the systems (USEPA, 2010). The latter may allow a certain degree of nutrient enrichment (i.e., criteria may not be set at "pristine" or "best attainable conditions") by choosing to set the protective limits at concentrations that protect against designated use impairment.

Both of these approaches have been followed in Puerto Rico. Reference conditions were determined in a study conducted from 2003 to 2005 using samples from the complete reservoir network (19 major reservoirs) of Puerto Rico (Martínez et al., 2005). This manuscript presents results of a recently concluded study aimed at identifying nutrient levels associated with ecological thresholds of impairment in order to define the numeric criteria (Martínez et al., 2014).

Determination of numeric nutrient criteria

When calculated based on a reference population, reference criteria usually fall at, or below, the transition between "nutrient limiting" and "nutrient enriched" conditions. In contrast, numeric criteria could be established at the transition between nutrient enrichment and impairment, with impairment being defined by either an indicator of ecological integrity or by evidence indicating detriment to a designated use of the waters. Numeric criteria should ultimately be tied to the protection of one or more of the designated uses of the waters and would ideally involve the use of stressor-response relationships that relate a response variable (e.g., Chl-*a*, algal community index, depth of light penetration, etc.) to a causal variable (i.e., nutrients) (USEPA, 2010).

In Puerto Rico all reservoirs are evaluated based on the following designated uses: primary and secondary contact, aquatic life, and sources of potable water. In the case of reservoirs, one of the most conservative uses for protection purposes and nutrient criteria development is aquatic life. An ecological limit that prevents exuberant growth of aquatic macrophytic vegetation, recurring algae blooms and/or a significant deterioration of the aquatic community structure could successfully be used to define limiting nutrient conditions. Based on this conceptual framework we proposed using Chl-*a* to define a quantitative ecological threshold that prevents impairment to the designated uses of our reservoirs.

MATERIALS AND METHODS

Observational approach

Field measurements and nutrient analyses

The observational strategy involved the recurrent sampling of six of Puerto Rico's most important reservoirs, namely: La Plata, Guajataca, Cerrillos, Patillas, Cidra, and Toa Vaca (Figure 1). The selected res-

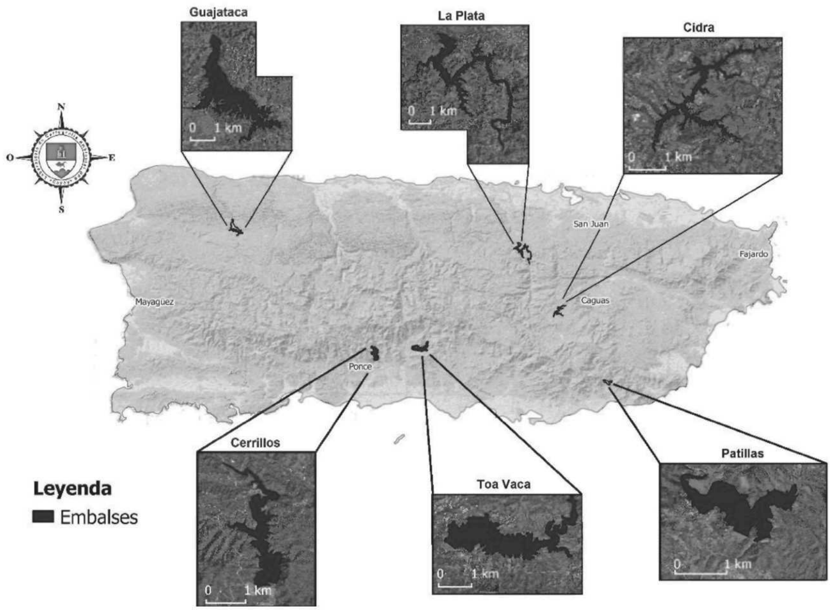


FIGURE 1. Map of selected reservoirs in Puerto Rico (Map by "Laboratorio de Cartografía Ambiental", UPR-Cayey).

ervoirs represent the trophic status range of reservoirs on the island while contributing close to 60% of the total reservoir storage volume. Twenty-five sampling events were performed at each reservoir (24 at Toa Vaca). All project activities (e.g., sampling and analysis) were performed by AES personnel in conformity with procedures described in an EPA-approved Quality Assurance Project Plan (QAPP). Samples were obtained in triplicate, one at each of three points located along a spatially defined transect at the center (mid) portion of each reservoir. In all, a total of 447 surface samples were analyzed in this study. All samplings were performed between 9:00 and 11:00 am. Samples were obtained with a horizontal-type 2-L Van Dorn sampler at a depth of 1 m. Samples were also collected at 5 m, 10 m, and 15 m exclusively for nutrient analyses (i.e., not Chl-*a*). Water samples for nutrient analyses were transferred to pre-labeled polypropylene bottles and preserved on-site by acidification. Separate aliquots were used for Chl-*a* analyses. Samples for Chl-*a* analyses were filtered on site through glass fiber filter (Whatman GF/F). In situ measurements of pH, temperature (°C), ORP, and dissolved oxygen (mg/L) were performed with a YSI 6600 multiparameter sonde (Yellow Springs Instruments, Yellow Springs, OH)[6]. Water transparency was determined with a 20-cm Secchi disk (SD). Vertical (depth) profiles (1 m resolution) of pH, electrical conductivity, dissolved oxygen, water temperature, turbidity, oxidation-reduction potential were obtained at the geographical mid sampling station (Station B) of each reservoir. Samples (including Chl-*a* filters) were transported at 4° C to the Soil and Water Chemistry Laboratory at the University of Puerto Rico within six hours of collection. Algal biomass was estimated by means of the Chl-*a* acetone extraction method and quantification of Chl-*a* using a model 10-AU fluorometer (Turner Designs Inc., Sunnyvale, CA). Nutrient analyses included dissolved and total reactive P (EPA method 365.2), TKN (EPA method 351.2), and nitrate-N (EPA method 353.1). Samples for nitrate-N, and dissolved P were filtered through a 0.45 µm Gelman acrodisc filter before analysis. The relationship between indicators of phytoplankton community structure (i.e., taxonomic richness, diversity) and trophic status was evaluated (detailed results on phytoplankton community composition will be presented in a forthcoming paper).

[6]Company or trade names in this publication are used only to provide specific information. Mention of a company or trade name does not constitute an endorsement by the Agricultural Experiment Station of the University of Puerto Rico, nor is this mention a statement of preference over other equipment or materials.

Zooplankton analyses

Nine sampling events were performed on each reservoir for zooplankton analyses. At each sampling, length and depth-integrated samples of the entire mixed layer (epilimnion) were obtained by longitudinal and oblique tows within the first meter using a bongo net system fitted with two metered 64 µm mesh nets. The samples were transferred to 500-mL bottles and preserved with 4% formalin. Number of taxa and individuals per volume of sample were estimated based upon the volume dispensed in Sedgewick-Rafter chambers. Number of taxa per reservoir corresponds to the cumulative richness after scanning these chambers. Species were identified using published keys and Santos-Flores (2001).

Statistical analyses

The statistical analysis focused on the relationship of nutrient parameters (TP and TN) and Chl-*a*. Initially, simple and multiple regression models were fitted for log-transformed values. Using these relationships, conditional probability analysis (i.e., change-point identification), and hierarchical modeling (Qian et al., 2003; Paul and McDonald, 2005) were used to establish specific nutrient thresholds in relation to biological indicators.

Use of change-point models for the determination of nutrient thresholds

In order to determine a threshold (x_c) for a given nutrient x, given that we know the corresponding threshold (y_c) for an environmental response y, we considered $P(y>y_c \mid x>x_i)$ for all possible observed values of x_i. For a given value x_i of the nutrient, this expression indicates the probability that the environmental response exceeds the threshold since the nutrient exceeds the given value x_i. This is computed for all possible observed values of x_i, and plotted versus x_i. In this graph, the value of the nutrient which "separates" best the response into two groups, x_c, is called the change-point value for the relationship $P(y>y_c \mid x>x_i)$ vs. x_i. This x_c value is the nutrient concentration which minimizes the residual sum of squares for a simple model for the response (this model has two means for the environmental response, one associated with values smaller than x_c, and the other one associated with values larger than x_c). Here we applied a least squares method to determine TN and TP thresholds (i.e., x_c for TN and TP) based on an estimated Chl-*a* impairment criteria (y_c) (Paul and McDonald, 2005). In each case, 1,000 bootstrap samples were obtained, and the threshold was estimated as the average of the samples. The 95% confidence intervals were estimated from the bootstrap samples using the percentile method (Martínez-Suárez, 2010).

The relationship between the maximum depth with DO concentrations greater than 5 mg/L (DO≥5 mg/L) vs. Secchi depth (SD) or Chl *a*, was evaluated using a segmented curve model. This non-linear model considers an exponential curve up to a value of Chl *a*=g. For values of Chl *a* larger than g, the relationship is constant (horizontal line). The model was fitted using the least squares methodology.

RESULTS AND DISCUSSION

The reservoirs chosen exemplify the nutrient status/productivity spectrum of reservoirs on the island (Table 1). Cidra and La Plata embody reservoirs with a high degree of enrichment, while Toa Vaca and Guajataca represent a transition phase in the productivity spectrum. Conversely, Cerrillos and Patillas represent near reference conditions (Table 1). As expected, reservoirs with higher nutrient and productivity status (i.e., Cidra, La Plata) have lower depth of light penetration (i.e., Secchi depth) than reservoirs with relatively lower nutrient and Chl-*a* values (i.e., Patillas, Cerrillos) (Table 1).

Pearson correlation coefficients for the complete data set showed positive correlations between TKN and Chl-*a* ($r = 0.65$), TN and Chl-*a* ($r = 0.66$), and TP and Chl-*a* ($r = 0.74$). The relationship between TP and Chl-*a* (Figure 2) is similar to that reported in numerous studies worldwide (Table 2). Differences in slopes have been attributed to the effect of grazing (presence of large grazers lower the slope), stratification (stratification reduces the slope), and algal community composition, among other factors (Mazumder, 1994; Felip and Catalan, 2000; Fernandes-Cunha et al. 2013). Coefficients of determination (r^2) seem to indicate that total phosphorus has less explanatory power of chlorophyll *a* variability in tropical lakes/reservoirs than in temperate lakes (Table 2). Among the hypotheses suggested to explain this pattern are:

TABLE 1.—*Means separation among reservoirs of causative (nutrients) and response [Chl-a and Secchi depth] variables in selected reservoirs.*

	TKN-N	TN	TP	Chl-*a*	Secchi-depth
		mg/L		µg/L	meters
Cidra	0.68 A[1]	0.72 A	0.038 A	37.02 A	1.00 F
La Plata	0.50 B	0.52 B	0.043 A	21.01 B	1.36 E
Toa Vaca	0.30 C	0.31 C	0.022 B	9.04 C	1.76 D
Guajataca	0.25 D	0.25 D	0.009 C	6.18 D	1.99 C
Cerrillos	0.18 E	0.20 E	0.009 C	4.76 E	2.69 A
Patillas	0.16 E	0.18 E	0.007 D	4.47 E	2.49 B

[1]Within a given column, means with the same letter are not significantly different as determined by Fisher's Least Significant Difference (LSD) test ($P<0.05$).

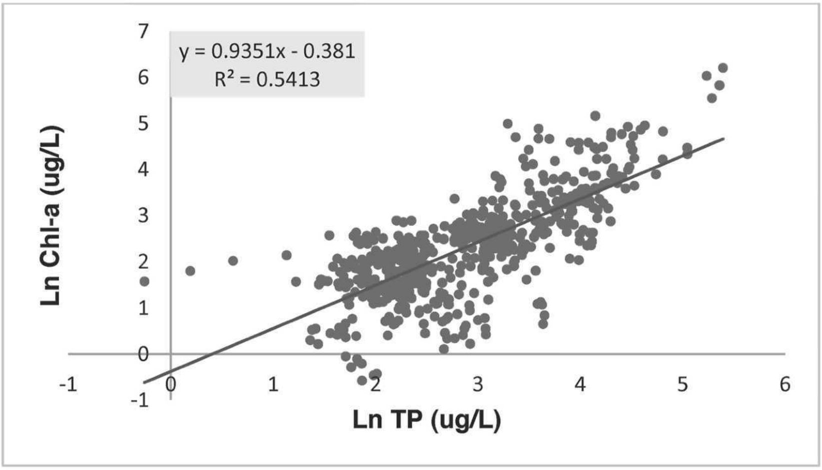


FIGURE 2. Relationship between total phosphorus (TP) (µg/L) and chlorophyll *a* (Chl-*a*) (µg/L).

a greater relevance of nitrogen as a potential limiting nutrient in tropical systems, greater presence of non-algal particulates in tropical systems, and differences in morphometry and hydrologic regimes (White, 1989; Huszar et al., 2006). Despite all this, phosphorus still represents a major controlling factor of primary productivity in tropical reservoirs.

As expected, increases in algal biomass density (as measured by Chl-*a*) diminish the light penetration depth of the lake profile (i.e., Secchi depth). This is evidenced by the negative correlation coefficient obtained between these two parameters (r = -0.70) (Figure 3). In fact, Chl-*a* and nutrients (i.e., TP, TKN, TN) were negatively correlated with Secchi depth (SD). SD is a widely used tool in lake (reservoir) limnology. It offers a practical and reliable approach to estimating the extent of light penetration in the water column. The extent of light penetration, particularly that corresponding to the photosynthetic active radiation [PAR (400-700 nm)] fraction, greatly influences the spatial and temporal distribution of phytoplankton which, in turn, significantly impacts the dynamics of dissolved oxygen (DO), a critical parameter to aquatic life. Inorganic turbidity might significantly interfere with the relationship between algal biomass and light penetration. To reduce this effect our protocol prohibited sampling less than five days after a 2 yr/24 h rainfall event. Most of our samplings were performed under dry conditions as demonstrated by our turbidity values: median = 1.10 NTU; Q_{75}= 4.60 NTU for surface (1 m depth) measures.

TABLE 2.—*Selected regression equations for the relationship between total phosphorus and chlorophyll a.*

Linear regression equation	r^2	Description	Reference
Ln Chl-*a*= 0.94Ln TP – 0.381	0.54	Puerto Rico's reservoirs	This study
Ln Chl-*a* = 0.94Ln TP – 0.806	0.71	North American and European Lakes	Mazumder (1994)
Ln Chl-*a* = 0.87Ln TP – 0.622	0.89	Eastern North American Lakes	Nürnberg (1996)
Ln Chl-*a* = 0.80Ln TP – 0.576	0.64	Worldwide Lakes	Nürnberg (1996)
Ln Chl-*a* = 0.87Ln TP – 0.898	0.69	North American and European Lakes	Praire et al. (1989)
Ln Chl-*a* = 0.70Ln TP – 0.35	0.42	Tropical and Subtropical Lakes	Huzar et al. (2006)
Ln Chl-*a* = 1.10Ln TP – 1.94	0.53	Tropical and Subtropical Reservoirs	Fernandes-Cunha et al. (2013)
Ln Chl-*a* = 0.82LnTP – 0.19	0.84	Tropical Reservoirs	González and Quirós (2011)
Ln Chl-*a* = 0.96Ln TP – 1.27	0.94	OECD study	White (1989)
Ln Chl-*a* = 1.44Ln TP – 2.44	0.72	Worldwide Lakes	Carlson (1977)

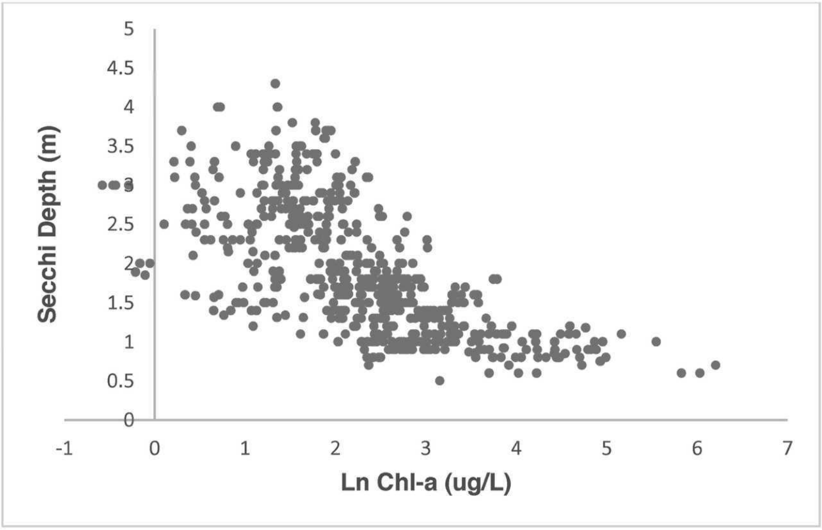


FIGURE 3. Relationship between chlorophyll *a* (Chl-*a*) and Secchi depth.

The inverse relationship between Chl-*a* (an index of algal biomass productivity) and the extent of light penetration as measured by SD has led to worldwide adoption of SD as a trophic state-defining criterion. Secchi depth values at or below 1 m are considered indicative of impairment in lakes (Forsberg and Ryding, 1980; Nürnberg, 1996; Carlson and Simpson, 1996). We used our vertical profile data (DO, pH, temp., ORP) collected at Station B (i.e., station at the middle of the sampling transect) to explore in more detail the relationships among Chl-*a*, SD and DO in order to define a Chl-*a* threshold that could be used to establish the numeric criteria. The inverse relationship between SD and Chl-*a* (Figure 4) describes that there is a non-linear decrease in SD with Chl-*a*, followed by a well-defined threshold (change-point) below which SD does not change significantly with further Chl-*a* increase (within the Chl-*a* range observed in this study). A segmented curve approach was used to identify the Chl-*a* value that best defines this transition (Table 3, Figure 4). The resulting value (23.6 µg/L Chl-*a*) represents a potential ecological threshold for establishing the nutrient criteria. At Chl-*a* values below 23.6 µg/L there is an exponential increase of light penetration with reductions in Chl-*a*, whereas at values greater than 23.6 µg/L Chl-*a*, SD values are less sensitive to change and have reached values that are considered indicative of impairment (i.e., 1 m).

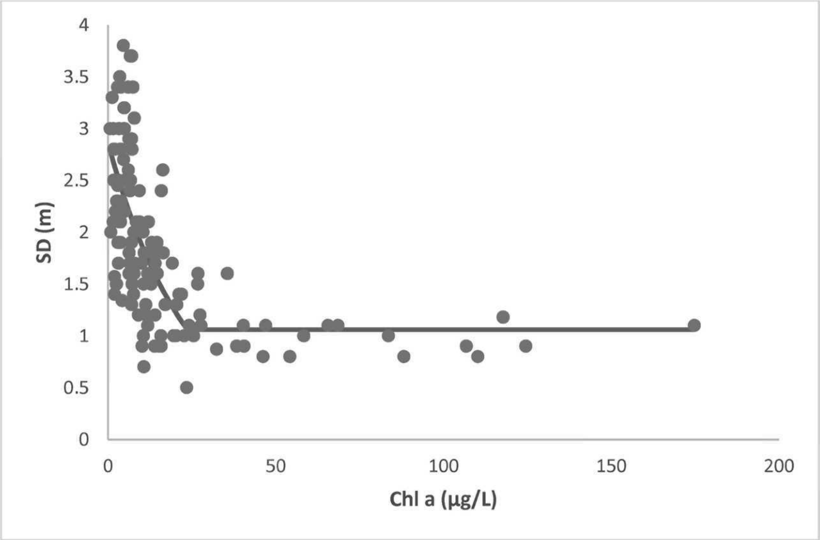


FIGURE 4. Relationship between chlorophyll *a* (Chl-*a*) and Secchi depth (SD) at the water column profile station (Station B). Line represents the best fit of a segmented curved approach. The first segment was a negative exponential curve, and the second segment a horizontal line. The change-point value for Chl-*a* (parameter g) was estimated at 23.6 µg/L.

The significance of the 23.6 µg/L Chl-*a* threshold as defining criterion is sustained by the relationship between Chl-*a* and an ecological DO threshold obtained from the same data set. A plot of Chl-*a* values vs. the maximum depth denoting DO concentrations equal or greater than 5 mg/L results in a similar relationship as that of Chl-*a* and SD (Figure 5). We expressed DO concentrations in terms of the maximum depth in compliance with the USEPA aquatic life criteria (DO ≥ 5mg/L) due to the importance of this parameter for reservoirs of Puerto Rico. At present, all of our reservoirs are listed as impaired (PREQB 303(d) 2012) for violation of the USEPA DO criteria. In our

TABLE 3.—*Results of a segmented curve fitting exercise on the relationships between Chl-a and SD, and Chl-a vs maximum depth with DO values ≥ 5 mg/L (station B).*

	Parameter Estimates				
Relation	Parameter (g) Estimate	Standard error	Pr > |t|	Lower interval	Upper interval
Chl *a* - SD	23.6	3.69	<0.0001	16.316	30.95
Chl *a* - Max. Depth DO ≥ 5 mg/L	28.3	5.14	<0.0001	18.1	38.5

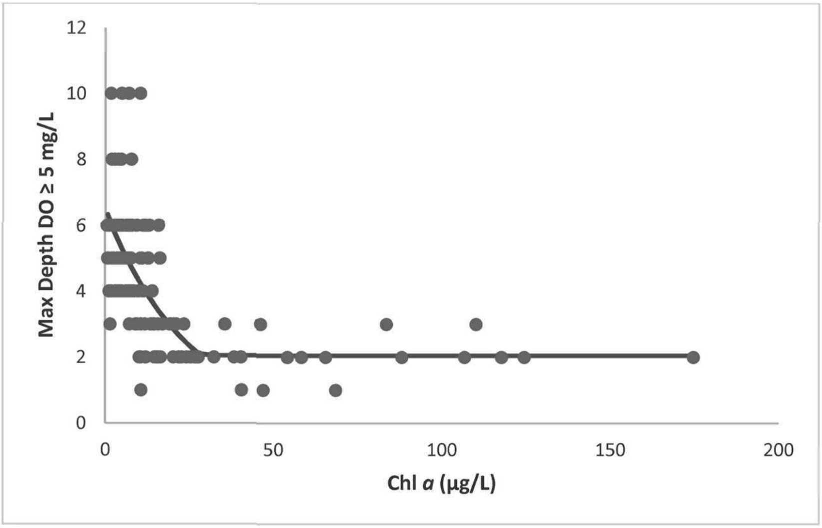


FIGURE 5. Relationship between chlorophyll *a* and the maximum depth with dissolved oxygen (DO) values ≥ 5 mg/L. Data at the water column profile station (Station B). Line represents the best fit of a segmented curved approach. The first segment was a negative exponential curve, and the second segment a horizontal line. The change-point value for Chl-*a* (parameter g) was estimated as 28.3 µg/L.

view, said determination is a result of an inadequate assessment protocol rather than a true DO impairment as a result of eutrophic conditions. The current assessment process consists of a comparison of average DO values from discrete measurements gathered at three depths (i.e., 1 m, 5 m, and 15 m) with the 5 mg/L limit established by USEPA. Since most tropical reservoirs experience hypolimnion anoxia (< 0.2 mg/L DO) during most part of the year (regardless of trophic status) it is extremely unlikely that our reservoirs meet the 5 mg/L regulatory threshold with the current assessment protocol.

Tropical lakes and reservoirs are more susceptible to hypolimnion oxygen depletion than their temperate counterparts (MacKinnon and Herbert, 1996; Lewis, 2000). This is attributed to the reduced solubility of oxygen in warm waters, the long periods of thermal stratification exhibited by lakes in the tropics, coupled with the higher rates of microbial metabolism experienced at higher temperatures (Townsend, 1996, 1999). For instance, both Patillas and La Plata reservoirs exhibit hypolimnion anoxia during most of the year despite having contrasting trophic status (Figure 6). Said condition precludes that hypolimnion anoxia be used as an indicator of

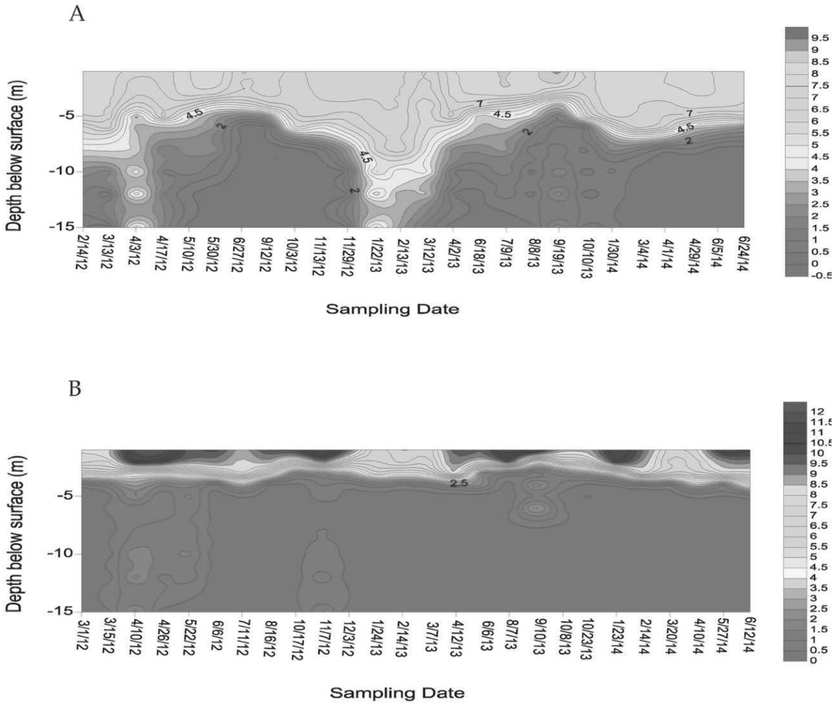
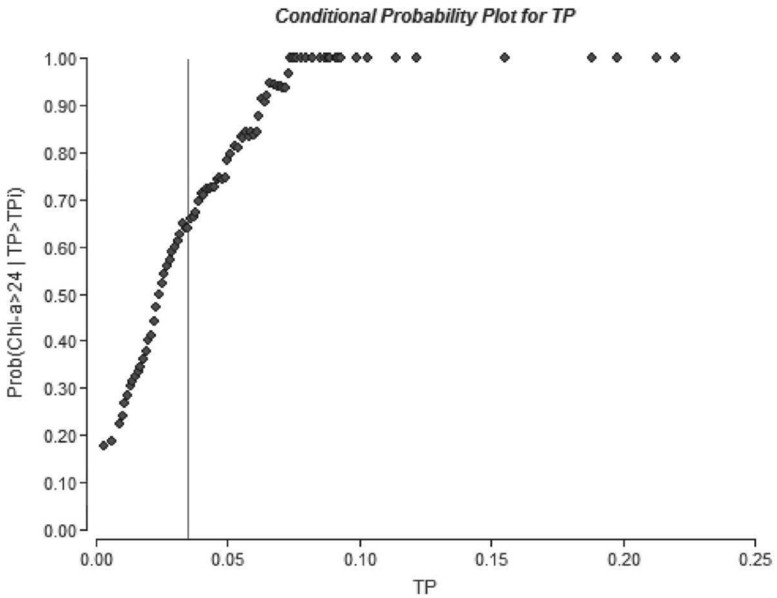


FIGURE 6. Dissolved oxygen (DO) isopleths for two reservoirs of Puerto Rico of contrasting trophic status, namely: Patillas (A) and La Plata (B). Median TN, TP and Chl *a* concentrations for Patillas are 0.19 mg/L, 0.007 mg/L and 4.56 µg/L, respectively. Median TN, TP and Chl *a* concentrations for La Plata: 0.55 mg/L, 0.045 mg/L and 21.83 µg/L, respectively.

trophic status in tropical lakes/reservoirs as is commonly used in the temperate region. The well-documented differences in DO dynamics between tropical lakes/reservoirs and temperate systems demand a thorough characterization prior to adopting guidelines and assessment protocols developed in temperate ecosystems. There is a need to develop an adequate assessment protocol that can discriminate the effects of excessive primary productivity on DO dynamics in tropical reservoirs where hypolimnion anoxia is not exclusively linked to trophic status. Evaluating the maximum depth at which relevant ecological DO thresholds are met for reservoirs across the trophic spectrum may provide a practical diagnostic tool to distinguish between nutrient impaired and non-impaired reservoirs in the tropics (provided abiotic factors that influence DO dynamics do not vary significantly between the target populations as in our case).

The segmented curve approach for the relationship between Chl-*a* and the maximum depth in compliance with the aquatic life criteria yields a Chl-*a* concentration of 28.3 µg/L (Table 3). Significant increases in said parameter (i.e., max depth DO ≥ 5 mg/L) are observed at Chl-*a* concentrations below 28.3 µg/L. This type of data arrangement could be useful in developing a protocol for assessing compliance with aquatic life criteria in reservoirs where hypolimnion anoxia naturally occurs. The value of 28.3 µg/L is similar to that obtained for the relationship between Chl-*a* and SD. The latter (i.e., 23.6 µg/L) carries greater sensitivity because SD values were measured at 0.1 m resolution intervals whereas the DO profiles are based on discrete measurements obtained at 1 m resolution. Altogether, the evidence suggests that at Chl-*a* values close to 24 µg/L the extent of light penetration in our reservoirs is significantly reduced (due to excessive phytoplankton biomass productivity) which hinders the capacity of our reservoirs to comply with the aquatic life criteria. On the other hand, an exponential increase in depth of light penetration is observed at Chl *a*- values < 24 µg/L. As stated, this is reflected in an increase in the maximum depth with dissolved oxygen concentrations in compliance with aquatic life criteria. We, therefore, recommend using 24 µg/L Chl-*a* as an impairment threshold to define the numeric criteria of reservoirs of Puerto Rico.

Total phosphorus and TN concentrations associated with our Chl-*a* impairment threshold (i.e., 24 µg/L) were established based on a change-point analysis of the data (Figure 7, Table 4). The resulting values, 0.035 mg/L for TP and 0.43 mg/L for TN, can be considered nutrient thresholds associated with impairment. To protect against impairment the Numeric Nutrient Criteria should incorporate a margin of safety. We have chosen to use the concentrations defining the lower tail of the 90% confidence interval of the bootstrap distribution of values associated with our Chl-*a* impairment threshold (i.e., $p<0.05$-one tail probability) to prevent impairment. These values are: 0.026 mg/L for TP and 0.41 mg/L for TN. Based on our data set, reservoirs exhibiting said concentrations would also reach the Chl-*a* impairment threshold only 5% of the time. Thus, we propose using 0.026 mg/L for TP and 0.41 mg/L for TN as a basis for establishing the nutrient standards for reservoirs of Puerto Rico. A least square comparison of the means of the maximum depth complying with the aquatic life criteria between populations separated by the criteria thresholds (i.e., 0.026 mg/L for TP and 0.41 mg/L for TN) results in highly significant differences for both nutrients (Fig-



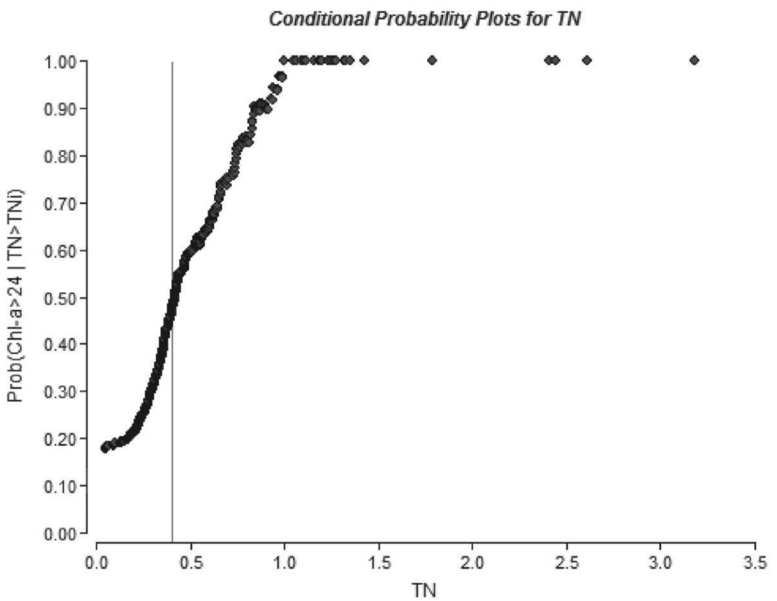


FIGURE 7. Total phosphorus and TN conditional probability plots for our ecological Chl-*a* threshold.

TABLE 4.—*Change-point estimates for TP and TN based on an impairment chlorophyll a threshold of 24 µg/L.*

Stressor	Chl-*a* level (µg/L)	Change-Point	90% CI	
TP	24	0.0347	0.0260	0.0490
TN	24	0.4322	0.4035	0.4898

ures 8 and 9; Table 5). Median values of the maximum compliance depth are 5 m and 2 m for populations below and above the specified thresholds, respectively. Thus, a significant improvement in the distribution depth of dissolved oxygen is achieved with compliance with the suggested nutrient criteria.

Zooplankton analysis

Despite their considerable potential as effective indicators of environmental change and their fundamental importance in the transfer of energy and nutrient cycling in aquatic ecosystems, the zooplanktonic communities have not been widely used as indicators of ecosystem condition (Stemberger and Lazorchak, 1994). The zooplankton community of the six reservoirs was characterized by a mix of species already known under similar environmental conditions in the Neotropical region, and a group of species with a totally opposed behavior. Sixty-five zooplanktonic taxa were identified. Rotifera was the richest group with

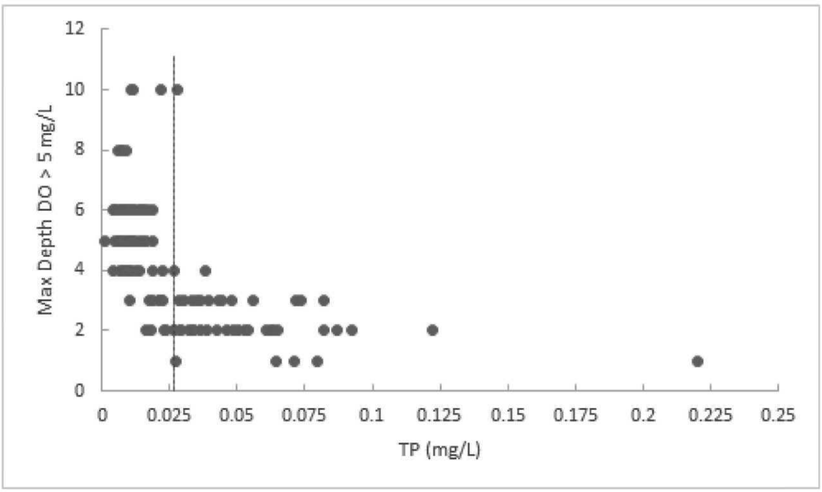


FIGURE 8. Relationship between total phosphorus (TP) concentration and the maximum depth (m) in compliance with the USEPA aquatic criteria. The numeric criteria (TP = 0.026 mg/L) is included as a reference line.

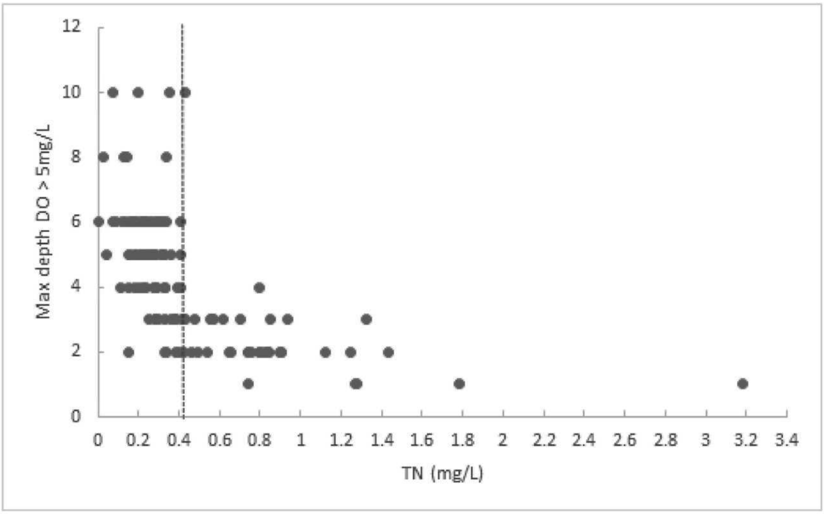


FIGURE 9. Relationship between total nitrogen (TN) concentration and the maximum depth (m) in compliance with the USEPA aquatic criteria. The numeric criteria (TN = 0.41 mg/L) is included as a reference line.

37 taxa. The cladocerans were represented by 20 taxa and Copepoda by eight taxa. This pattern is common in tropical freshwaters (Neves et al., 2003). The zooplankton community showed a clear rarity of large zooplankters like *Daphnia* spp. The low diversity of *Daphnia* seems to be an outstanding feature of cladoceran assemblages in tropical systems, either oligotrophic or eutrophic (Pinto-Coelho et al., 2005).

In this study, small-sized species like rotifers predominated. Among the cladocerans, also small forms such as *Bosmina longirostris* and *Ceriodaphnia cornuta* (*cornuta-rigaudi* complex) occurred frequently at high densities. Copepoda were mainly represented by immature forms of nauplii and copepodites, but nauplii were not

TABLE 5.—*Comparison between groups below and above the TP and TN protective thresholds (i.e., 0.026 mgTP/L and 0.41 mgTN/L).*

Least Squares Means					
Group	Estimate	Standard Error	DF	t Value	Pr > |t|
TP<0.026mg/L	5.1310	0.1815	126	28.28	<0.0001
TP>0.026 mg/L	2.4545	0.2068	126	11.87	<0.0001
TN<0.41 mg/L	5.0698	0.1818	126	27.89	<0.0001
TN>0.41 mg/L	2.4524	0.2188	126	11.21	<0.0001

considered in our analyses as these were below the sampling limit allowed by the 63 µm mesh and they are difficult to assign to particular taxa. However, adult cyclopoids showed a well-documented distribution pattern, with higher numerical densities at poor environmental conditions, decreasing to low values in lakes of better water quality.

The highest absolute densities of zooplankton were mainly due to the presence of the rotifers *Brachionus* spp. and *Asplanchna* spp., whose total numerical densities were comparable to values from tropical South American reservoirs. Values determined for local reservoirs followed a gradient, decreasing when trophic conditions improved, like in Cerrillos or Patillas, where lower numbers for these species (sometimes less than 100 ind/m^3) were detected. High species richness of Rotifera has been considered a common trend in tropical reservoirs (Starling, 2001). The dominance of rotifers in these tropical habitats has been attributed to hydrodynamics, which removes individuals from the deep and littoral zones to the limnetic zone. This is the case of genera such as *Lecane*, *Platyas*, *Lepadella*, *Colurella* and *Cephalodella*. However, the dominance of rotifers in tropical reservoirs cannot be considered a general rule. Although our results may indicate that rotifers are numerically dominant in the reservoirs, it must be pointed out that large numbers of nauplii (immature stages of Copepoda) were also observed in some instances.

The absolute abundance of rotifers denoted an increase with increased eutrophication (Figure 10). Within the rotifer group, the relationship between the genus *Brachionus* and phosphorus was used to establish the effect of this nutrient as a stressor (Figure 11). A segmented curved approach yielded a change-point value of 26.58 µg TP/L, which coincides with our numeric criteria value. Also, a change-point value of 1.16 meter (SD) was obtained for the relationship between *Brachionus* rotifers (ind/L) and Secchi depth (Figure 12). These change-points can be considered thresholds for an ecological regime shift in reservoirs of Puerto Rico.

The evidence presented herein supports establishing regulatory limits for nutrients at the following concentrations: 26 µg/L for TP and 0.41 mg/L for TN, to ensure compliance of our reservoirs with their designated uses and protect their ecological integrity. The proposed values are in accordance with nutrient standards adopted by other states of the USA (http://cfpub.epa.gov/wqsits/nnc-development). It is recommended that the reservoir's condition should be established based on median values of at least five consecutive samplings obtained from the surface (1 m) at the center or dam sections during the dry season.

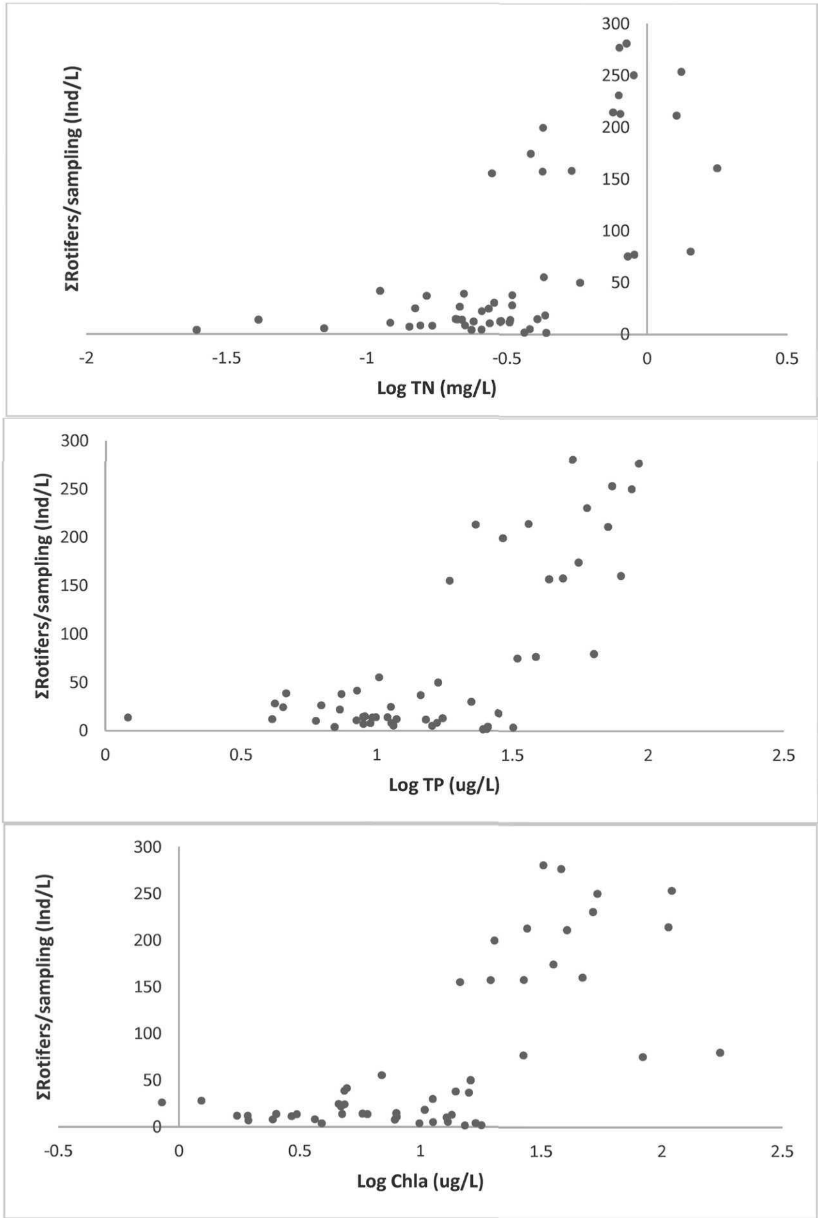


FIGURE 10. Relationship between the density of rotifers (ind/L), nutrients and chlorophyll *a*.

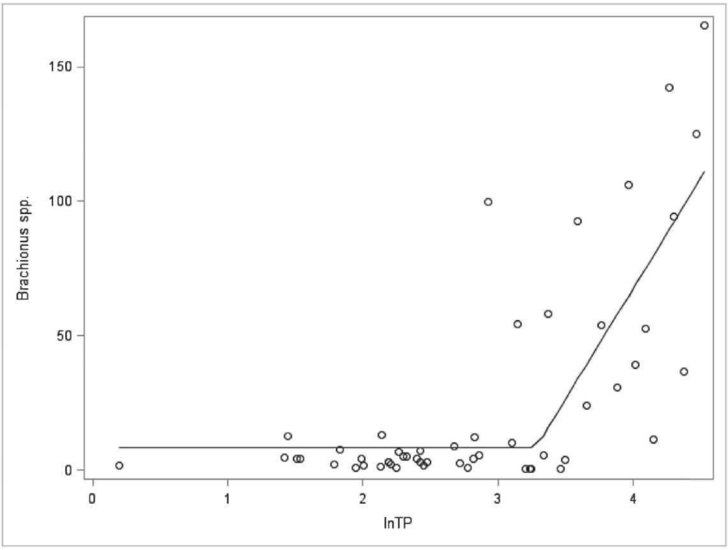


FIGURE 11. Relationship between *Brachionus* rotifers (ind/L) and phosphorus (lnTP). Line represents the best fit of a segmented curved approach. The change-point value for total phosphorus (parameter g) was estimated as Ln of 3.28 (= 26.58 μg/L).

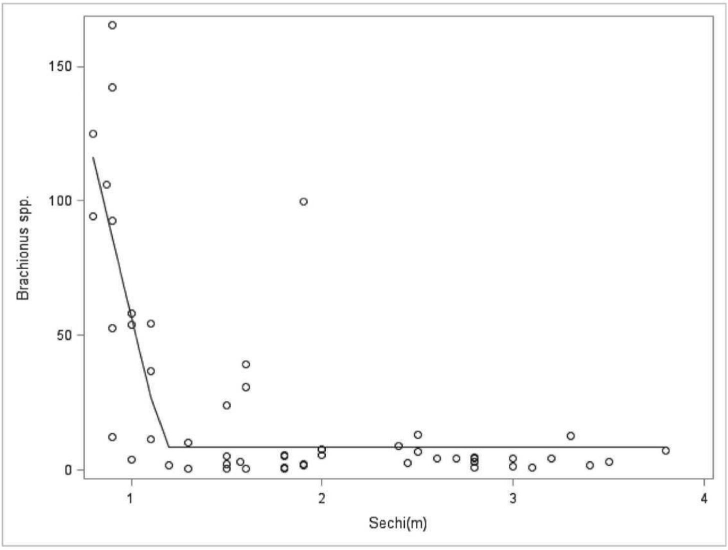


FIGURE 12. Relationship between *Brachionus* rotifers (ind/L) and Secchi depth (m). Line represents the best fit of a segmented curved approach. The change-point value for Secchi depth (parameter g) was estimated as 1.16 meter

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