Title: Getting the science right to protect and restore our environment. A critique of Lapointe et al (2019) Nitrogen enrichment, altered stoichiometry, and coral reef decline at Looe Key, Florida Keys, USA: a 3-decade study.

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Data Availability Statement: Data and the R scripts to analyze the data are available at https://github.com/SwampThingPaul/Comment_NEnrichment

Abstract

Eutrophication of coastal ecosystems and decline of essential benthic ecosystems are growing concerns globally. Understanding the drivers associated with a response of an indicator species at an ecosystem or even regional scale hinges on a robust evaluation of data that spans both space and time. Consistent data, suitable and methodical data-handling procedures, appropriate statistical evaluation and logical assessment of hypotheses are essential. Lapointe et al. (2019) suggest that decline of stony coral at Looe Key reef is attributed to local and regional (i.e. discharge >200 km away) nutrient discharges causing eutrophication of coastal waters and the degradation of essential habitat. Furthermore, Lapointe et al. (2019) posit that the restored freshwater flows to the Everglades ecosystem is a causative agent for the observed coral decline observed at Looe Key Reef. While the decline in coral coverage is alarming, rigorous statistical analysis is needed to attribute a true cause-and-effect relationship. This commentary discusses data handling techniques, the application of statistical methods, interoperability of data and evaluation of hypotheses presented by Lapointe et al. (2019) specific to Looe Key reef within the Florida Key Marine Sanctuary. This commentary is not merely a critique of the statistics used by the authors but rather the inappropriate statistical methods utilized and how it affects the acceptance or rejection of the authors' hypotheses and the overall conclusions drawn.

Keywords: frequentist statistics, coastal water quality, outlier analysis, data handling.

"The combination of some data and an aching
desire for an answer does not ensure that a reasonable
answer can be extracted from a given body of data."

- John W. Tukey

In the introduction Lapointe et al. (2019) provides a brief literature review of nitrogen driven eutrophication and its effect on coral reef ecosystems, insofar to raise awareness of impacts to coastal ecosystems. However, the rest of the paper demonstrated a skewed reading of the literature, contradicts established understandings of ecosystem dynamics, and misrepresents the data. While a commentary can focus on each of these topics in detail providing a through and complete thesis of each topic, this commentary will focus on the heart of any quantitative study from which hypotheses are supported or rejected, data handling and statistical analysis. Without an even-handed and robust treatment of the data prior to analysis and appropriate application of statistical analysis, acceptance or rejection of any hypothesis is impossible. The primary objective of this commentary is to discuss the data handling and analyses presented by Lapointe et al (2019) within the context of providing the readers with the tools to understand how the data are presented and analyzed which later the authors use to draw conclusions to support their hypothesis.

A manuscript is only as strong as its methods section. In their paper Lapointe et al. (2019) provides information specific to how they handled the data used to test their hypothesis that "*chronic increase in N availability has increased algal blooms and altered N:P stoichiometry, which have promoted metabolic stress and decline of stony corals at Looe Key*" As noted by the authors, due to the long-term nature of the dataset, a variety of statistical methods was used to analyze the data at different intervals. While the authors should be applauded for a rigorous evaluation of the data, it should be noted that these analyses demonstrate inappropriate data handling and misapplication of statistical methods, which ultimately influences the interpretability of this long-term dataset and the conclusions drawn from it.

Data Handling

As stated by the authors, data determined to be below the minimum detection limit (MDL) were excluded from the calculation of means or ratios. It is assumed that these values were also excluded from any analysis given the reported MDLs by the authors and the data displayed in Fig. 3 of the original manuscript. Proper statistical treatment of data below the MDL include simple substitutions or determining the maximum likelihood method based on data distributions to estimate values but rarely, if ever these data are simply removed from the analyses as the data are valuable in-of-themselves. In the simple substitution method, values reported below the MDL are typically set to the MDL or one- half of the MDL. Removal of data below the MDL ultimately biases any calculated summary statistics, derived values or statistical analyses, thereby not accurately representing the true data distribution or relationships observed in the system (Helsel and Gilliom 1986; Helsel and Hirsch 1992). This bias is especially relevant for evaluating trends within long-term time-series and biological or ecological thresholds. As a demonstration of data handling procedures associated with data below the MDL and its impact on annual trend evaluation (i.e., Kendall's correlation) data for a monitoring location within Everglades National Park (P36; Fig 1) are evaluated using various methods. When values below the laboratory determined MDL are removed from computing the annual mean Nitrate-Nitrite (NOx) concentrations the trend is borderline significant at a level of significance of 0.05 (τ = -0.23, ρ = 0.05). When values reported below the MDL are omitted most of the annual mean concentrations are biased high relative to both the one-half MDL substitution method and the non-parametric Kaplan-Meier method (She 1997)(Fig 1). Replacing values reported less than the MDL with values one-half the MDL is a common data handling technique allowing inclusion of very low values as these are actual data. In this instance, if data reported below the MDL are substituted with values one-half of the MDL, the trend is clearly significant even at a level of significance less than 0.01 (τ = -0.41, ρ < 0.001). Finally, replacing values reported less than the MDL using the non-parametric method results in values between the two other methods and results in a significant declining trend (τ = -0.35, ρ < 0.01). Generally, removing data reported less than the MDL can have significant impacts on data analyses, statistical evaluation and interpretation of results.

In addition to removal of data below the MDL and inappropriate statistical methods, the data were further screened by removing "extreme outliers" using boxplots prior to the application of regression analyses with no detail as to specifics of the boxplot analysis, nor validation that these outliers were truly outliers. An outlier can be defined as a value that has a low probability of originating from the same statistical distribution as the rest of the observed data. In the original development of the boxplot by Tukey (1977), it was never the intention to use boxplots as a method of identifying outliers, but rather to call attention to such data for further investigation. Boxplots are extremely useful for quickly visualization of the central tendency, spread and skewness of the data and to some degree for highlighting extreme data points of interest (Tukey 1977; Zani et al. 1998). However, despite the value of the boxplot to visualize data it equally has several downfalls including providing no information on the underlying data distribution and somewhat arbitrary outlier detection especially for non-normal data distributions. In identifying putative outliers, the thresholds identified by Tukey (1977) were never associated with any theoretical calculations (Hoaglin 2003). Outliers identified using a univariate boxplot simply identifies values that fall outside of 1.5 time the inter-quartile range (IQR) of the first or third quartile (Tukey 1977). The outlier threshold is sample size dependent and the number of outliers detected will increase with sample size making individual outliers undetectable (Kampstra 2008) (Fig 2). As shown by Fig 2, even for normally distributed data the IQR is extremely sensitive to sample size with extreme variability in datasets with small sample sizes eventually narrowing to a much-reduced variability. Given this dependence of sample size of the IQR it is no wonder that the number of outliers detected would also vary with sample size (Fig 2). This synthetic dataset was used for demonstration purposes and was simulated based on a normal distribution with a mean of zero and a standard deviation of one. This synthetic data range, in datasets with a sample size of 1 to 5000, represents a full and extreme range of potential influences of sample size on IQR estimation and potential outlier identification utilizing the 1.5 x IQR methodology. Furthermore, it is possible that the data evaluated by Lapointe et al. (2019) are non-normally distributed complicating the use of boxplots to identify potential outliers as indicated by the simulation study presented above (Fig 2). This deviation of the data from a normal distribution also complicates other analyses that require normally distributed data such as linear modeling (discussed below). Given these issues with the application of boxplots as an outlier analysis it is highly likely that the analysis by Lapointe et al. (2019) has a high type 1 error rate (i.e. false positive error), thereby further affecting any analysis and interpretation of results presented.

In addition to data mis-handling associated with MDL values and other data censoring the authors also state that "*…if either NH_4^+ or NO_3^- were undetectable, the value for other inorganic N species was considered to be the total measurable DIN for the sample.*" They neglected, however, to include analytical methods associated with "*other inorganic N species*" and therefore the accuracy of the stoichiometric ratios reported by the authors is unclear. Rigorous statistical analysis relies on consistent, well-documented and appropriate handling of the data laying the foundation for robust interpretation of data and testing of hypotheses. The analyses conducted by the authors have systemic issues.

Statistical Analyses

In addition to the mis-handling of the data reported above, the authors' analyses rely on the mis-application of statistical methods in an attempt to test their hypothesis. First, the authors use polynomial regression analysis (fifth order) to model temporal relationships of raw data over the study period. It is assumed that these "raw" data are a mix of surface and near-bottom water samples collected on the shallow fore reef spur-and-groove zone and the reef crest in the middle of the Looe Key Sanctuary Preservation Area (LKSPA) as documented by the authors. Given that these data are a time-series and data are "regressed" over time, the analysis violates a major assumption of linear regression/modeling, on which polynomial regression analysis in based. Trend analysis by a linear model typically violates the assumption of independence of model residuals, where the residuals of the model should lack autocorrelation (Helsel and Hirsch 2002). As an example of this autocorrelation, Fig 3 shows dissolved inorganic nitrogen (DIN) observed at Looe Key reef as part of the Florida Keys National Marine Sanctuary (FKNMS) water quality monitoring program between 1995 and 2019 regressed over time using a simple linear model (Fig 3A), the autocorrelation function of the time model (Fig 3B) and scatterplots of the data at various lag intervals (Fig 3C). The model residuals are significantly correlated up to three lag distances indicating that the model residuals are autocorrelated thereby violating a basic assumption of linear modeling. Putting statistical appropriateness of applying linear modeling to time-series data aside for a moment, Lapointe et al. (2019) presents fifth order polynomial regressions as evidence of trends without reporting the actual slopes of the models and relying on visual inspections of the "trend" direction overlaid with what appears to be individual data points (Fig 3 A-F in the original manuscript). While these "trend" models were reported as statistically significant by the authors, the variability inherent in these models, as indicated by the reported R^2 (replicated in Table 1), is so large (i.e. low R^2 values) that it is impossible to attribute an actual trend direction through the data using an inappropriate statistical model that at this point is no better than a data smoothing function. The models reported by the authors only account for 5% to 26% of the data variability across variables. How reasonable is it then to attribute a trend direction or that the model explains the underlying data through time given the lack of model fit? Moreover, the representativeness of the data (i.e. individual years) across the analysis period is limited with significant gaps between years and some years being represented by what appears to be a single value. Putting the application of linear models to time-series data aside, using year as the independent variable with some years represented by one value, other years with multiple values or some years omitted/not sampled confounds the seasonal affects with long-term changes making the analysis dubious. If an actual trend analysis such as Kendall or autoregressive integrated moving average (ARIMA) modeling was applied, with proper data handling to the authors' data, no significant trend would presumably be detected. Based on trend analysis of an independent dataset collected at Looe Key Reef as part of the FKNMS water quality monitoring network, both individual samples and annual mean concentrations indicate no significant trends for total nitrogen (TN), total phosphorus (TP), dissolved inorganic nitrogen (DIN), soluble reactive phosphorus (SRP), DIN:SRP and chlorophyll-*a* concentrations for surface water samples between May 1995 and April 2018 (Fig 4 and Table 2).

The authors employed correlation analysis *en masse*. The adage "*correlation does not imply causation"* should be applied here as the authors compare water quality metrics (i.e. nutrient concentrations) with population size of Monroe county, rainfall, Shark River flow (SRS) rate, maximum water temperature and coral cover. In interpreting data analyses such as these to avoid spurious and erroneous correlations, understanding the used data is critical. However, the authors provide very little information for data sources such as Monroe county population data, why they are relevant to this analysis, why rainfall across an approximate 400 km distance or why discharge from a selected set of discharge locations 150 km away from the area of interest (i.e. Looe Key) are relevant to compare water quality conditions specific to Looe Key reef. It should be noted that intra- and inter-annual rainfall patterns can differ across the 400 km straight line distance from

Orlando to Flamingo (Fig 5), driven by a multitude of factors including atmospheric interactions between land and sea breezes and land cover (Burpee and Lahiffi 1984; Pielke et al. 1999). Additionally, the inclusion of discharges to SRS via the S12A, S12B, S12C and S12D neglects a significant component to the discharge to eastern SRS (S333) and conveyance components such as those that control flow from the south Dade, Taylor slough and coastal basin portions of the system (Rose et al. 1981; Rudnick et al. 1999; Sutula et al. 2001; Brown 2014; Sandoval et al. 2016). It appears that a large portion of the discussion relies on this analysis and after a close inspection of the authors' Fig 4, spurious and erroneous correlations become apparent. For instance, some potential erroneous correlations include a negative correlation of Monroe county population size and maximum water temperature and a positive correlation of SRS discharge with population size. Other spurious correlations include the correlation of ammonium (NH_4), nitrate (NO_3), DIN, f-ratio and DIN:SRP where NH_4 and NO_3 were used to calculate DIN, *f*-ratio and DIN:SRP and therefore are not independent. As suggested by Pearson (1897) and later Kronmal (1993) the use of a ratio, especially when comparing this ratio to a component of the ratio in any statistical analysis can lead to incorrect or misleading inferences. The only correlations of note would be that of SRS discharge compared to chlorophyll-a, NO_3 and DIN; however in light of the discussion above (i.e., unrealistic distance from Looe Key to cause an effect) and the general reduction of N-species concentration within the freshwater Everglades and SRS specifically (Julian et al. 2018), it is highly probable that these results are also erroneous in nature.

In addition to Spearman correlation analysis of all variables, the authors conducted a stepwise regression analysis to model the variation in annual mean living coral coverage and chlorophyll-a concentrations by "*other environmental variables*", which the authors conclude are maximum values of all variables across the entire study without any rationale. The authors are distilling their environmental variable time-series datasets with presumably hundreds of samples to one value per variable. Moreover, this data reduction and comparison is conducted using an unbalanced comparison with data with a perceived distribution (i.e., mean values from a normal distribution) versus a distribution free variable (maximum values), essentially comparing apples (mean biological response) to bananas (maximum environmental variables). The authors also mention that "*Highly correlated variables, such as* NH_4^+ *and* NO_3^- *with DIN, were not included together in the model building to avoid consideration of redundant information*" but did include variables such as DIN, DIN:SRP and *f*-ratio, which by their definition have the same degree of redundancy as DIN:SRP and *f*-ratio are both calculated using DIN. Inclusion of DIN:SRP and *f*-ratio in addition to DIN again injects some degree of spurious correlation (Pearson 1897; Jackson and Somers 1991; Kronmal 1993). Furthermore, the authors states "*Stepwise multiple regressions on coral cover against temperature, Shark River Slough flow, population, chlorophyll a, DIN, SRP, DIN:SRP, and f ratio using P values as selection criteria showed that annual coral cover was best predicted by DIN concentrations*". However, this is not a valid model selection approach when using a stepwise multiple regression framework. The significance of the model has nothing to do with selection of the model variables but rather the probability that the model is a better fit than the null model using the F-test. Typically, stepwise models are selected based on the Akaike Information Criteria (AIC) where the number of model variables and the maximum value of the likelihood function for the model is used to assess a candidate model for goodness of fit. Additionally, the AIC method penalizes candidate models for overfitting. The preferred model is one with the minimum AIC value (Akaike 1974; Burnham and Anderson 2002), indicating the best model given the available information. Furthermore, it is considered good practice to run the step-wise regression both forward and backward as the approach can produce different results. Ultimately, the reader does not know whether the best model was selected as nothing related to the stepwise multiple regression analysis was presented.

In addition to using stepwise regression, the authors also used linear regressions comparing biological responses (i.e., coral coverage and chlorophyll-a concentration) to significant environmental variables from the stepwise regression. It is unclear what new information could be gained from an additional regression analysis that could not be drawn from the stepwise regression. The Spearman rank correlation combined with stepwise and linear regression analyses presented by the authors are all generally testing the same data thereby conducting multiple statistical tests independently, which is inadvisable (Peres-Neto 1999), increases type I error, is generally poor-analytical form and is generally perceived as hunting for results or data fishing. Specific to linear models (which includes stepwise regression), the most parsimonious model that fits the assumptions of linear models is the simplest model with the greatest explanatory predictive power (Akaike 1974; Burnham and Anderson 2002). Rather than reporting the results of the stepwise regression, the authors focus on only the results of the linear regression comparing the individual metrics in isolation and leaving the readers wondering why the stepwise regression was performed in the first place ultimately providing no explanatory value to the study.

Much like in the debate regarding seagrass die-off in Florida Bay (Zieman et al. 1999, 2004; Lapointe and Barile 2004), the discussion of water quality driven algae blooms and coral reef interaction (Lapointe 1997, 1999; Hughes et al. 1999) and a more recent discussion of water quality related to reef impacts on the Great Barrier Reef (Bell et al. 2014a, b; Furnas et al. 2014), Lapointe et al. (2019) rely on selective literature citations and a skewed and improper evaluation of limited data to overstate the support for their hypothesis regarding the cause of changes to coral at Looe Key. At the same time, they continue to ignore the published interpretations of the data that they selectively used to create their narrative that Everglades restoration has the potential to impact coral reef ecosystems. Moreover, as outlined above, it is not certain that the treatment of data allows for a subjective and rigorous analysis to support (or reject) the hypothesis presented by the authors. Finally, their analyses lack serious spatial reasoning as evident by including rainfall data across an approximately 400 km distance and discharge volumes from over 150 km away from the study area. While N enrichment of coastal waters is problematic resulting in negative ecosystem impacts, actions are needed to address these impacts and help restore critical coastal and estuarine ecosystems. The study by Lapointe et al. (2019) attempt to draw the conclusion that restoration of the largest continuous wetland ecosystem in North America is causing adverse ecosystem impacts to coral reefs hundreds of kilometers away. This flies in the face of countless studies that call for Everglades restoration for the benefit of the ecosystem in totality and does the opposite of “Getting the science correct”.

Conflict of interest

The author declare that they have no conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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Competing Interest

The authors declare that they have no conflict of interest.

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Figures

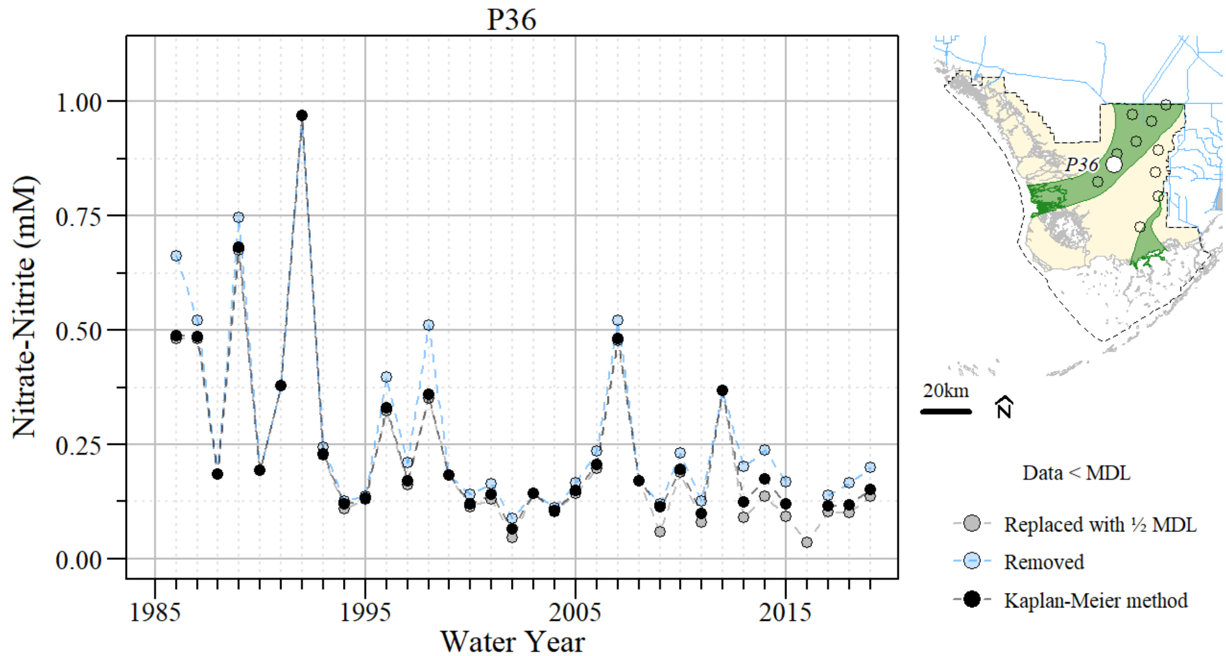

Figure 1. An example of how data treatment can influence annual mean concentrations for data at or near the minimum detection limit (MDL). Data reported at or below the minimum detection limit (MDL) were either replaced with ½ the MDL, removed or estimated using the Kaplan-Meier non-parametric approximation method. Data retrieved from South Florida Water Management District (SFWMD) online environmental database for the period of record between May 1985 to May 2019 (entire sampling period) for a monitoring location within Everglades National Park (25.528, -80.796 WGS84). Only useable data were used in this analysis consistent with methods presented by Julian et al. (2018).

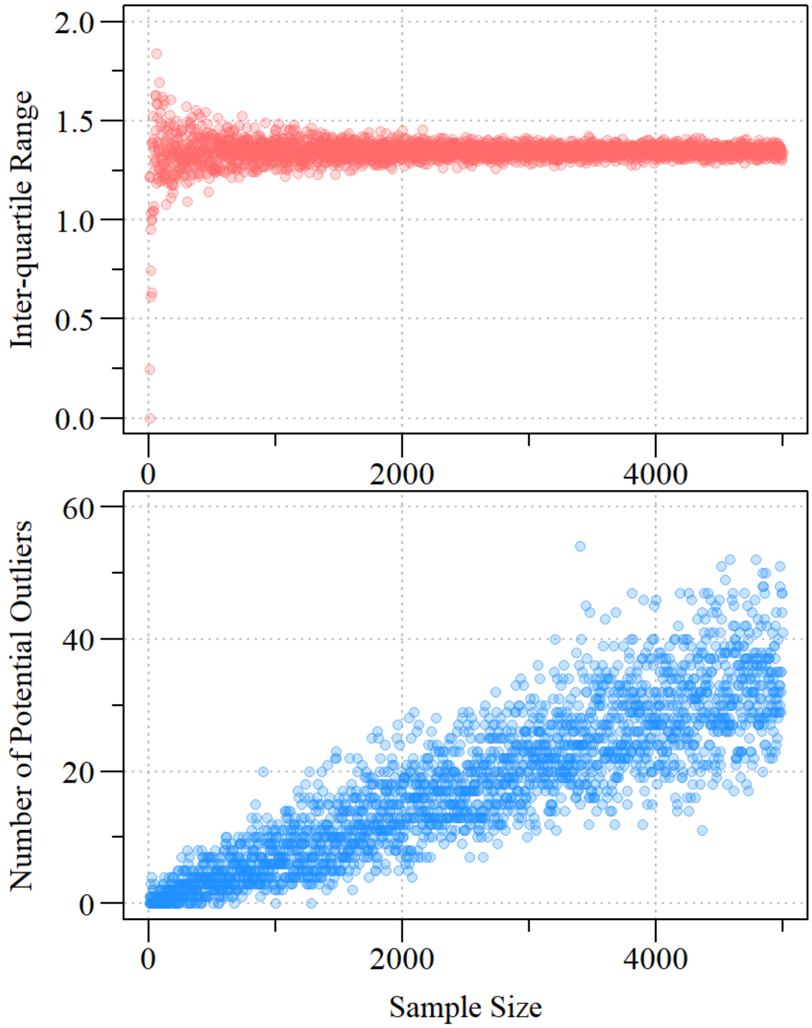

Figure 2. Inter-quartile range as a function of sample size (top) and number of potential outliers detected using a univariate boxplot (bottom) from a normally distributed simulated dataset with a mean of zero and a standard deviation of one (μ = 0; σ = 1).

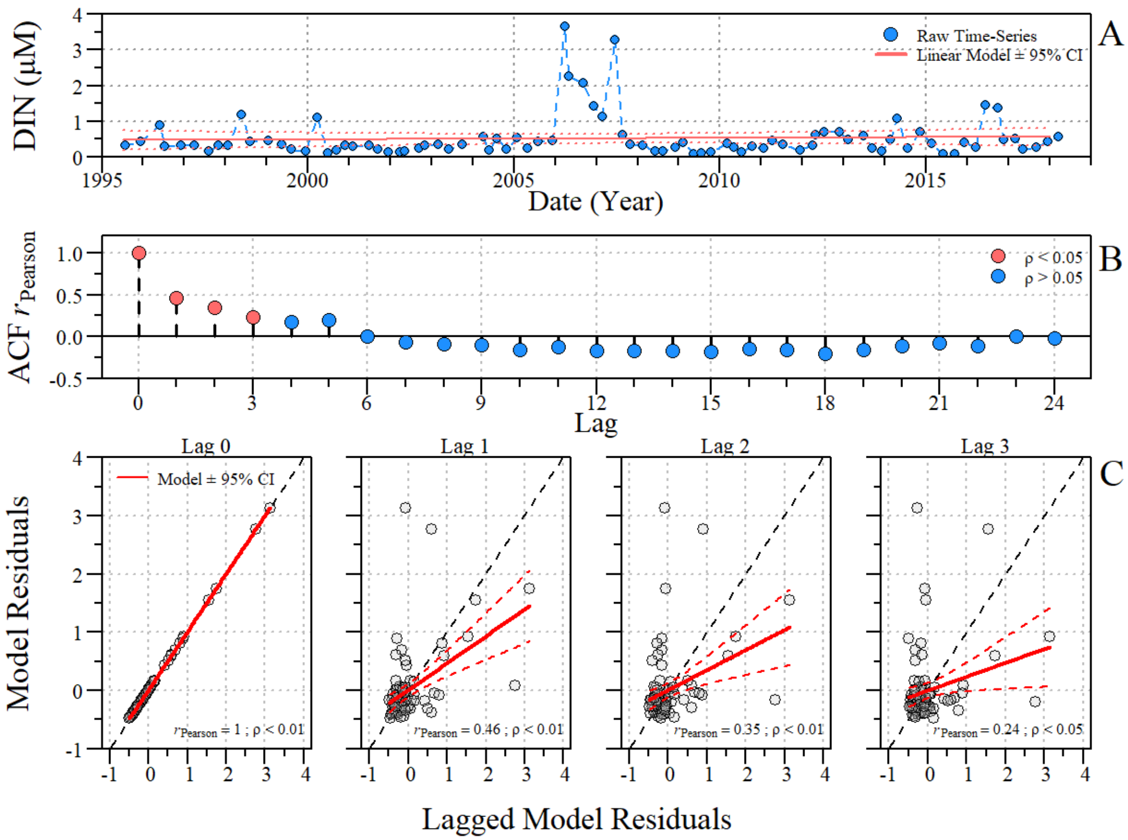

Figure 3. A) Surface water dissolved inorganic nitrogen (DIN; Ammonium + Nitrate + Nitrite) concentrations observed between May 1995 to May 2018 collected at Looe Key Reef (25.548, -81.397 WGS84) (Briceño and Boyer 2019). B) Autocorrelation function (ACF) of model residuals from the time-series linear model presented in part A. C) Lagged correlations of time-series linear model presented in part A corresponding in the ACF$_{r_{\text{Pearson}}}$ presented in part B. Only lag zero to three are presented here. All analyses conducted using the base R-library.

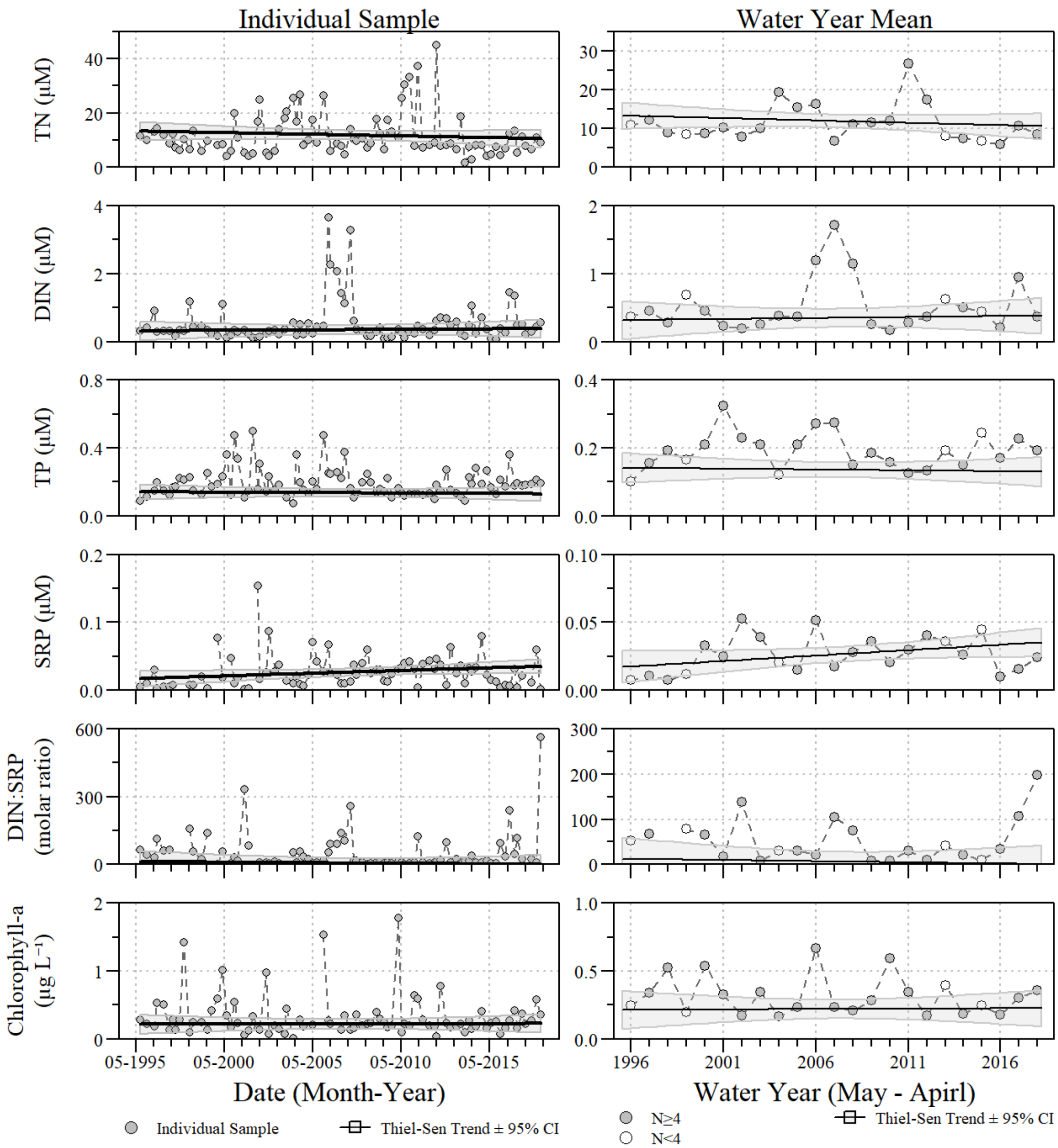

Figure 4: Surface total nitrogen (TN), dissolved organic nitrogen (DIN; Ammonium + Nitrate + Nitrite), total phosphorus (TP), soluble reactive phosphorus (SRP), molar ratio of DIN and SRP and chlorophyll-a concentrations observed between May 1995 to May 2018 collected at Looe Key Reef (25.548, -81.397 WGS84) (Briceño and Boyer 2019). Left: Data presented are individual samples with Theil-Sen Trend (± 95% Confidence Interval). Right: Annual arithmetic mean values based on Florida Water Year with Theil-Sen Trend. All data below the minimum detection limit (MDL) were replaced with one-half the MDL and Theil-Sen Trend was estimated using the mblm function in the mblm R-library (Komsta 2013). Trends from individual samples included all data while trends for annual mean values were estimated with only years with greater than four samples per year.

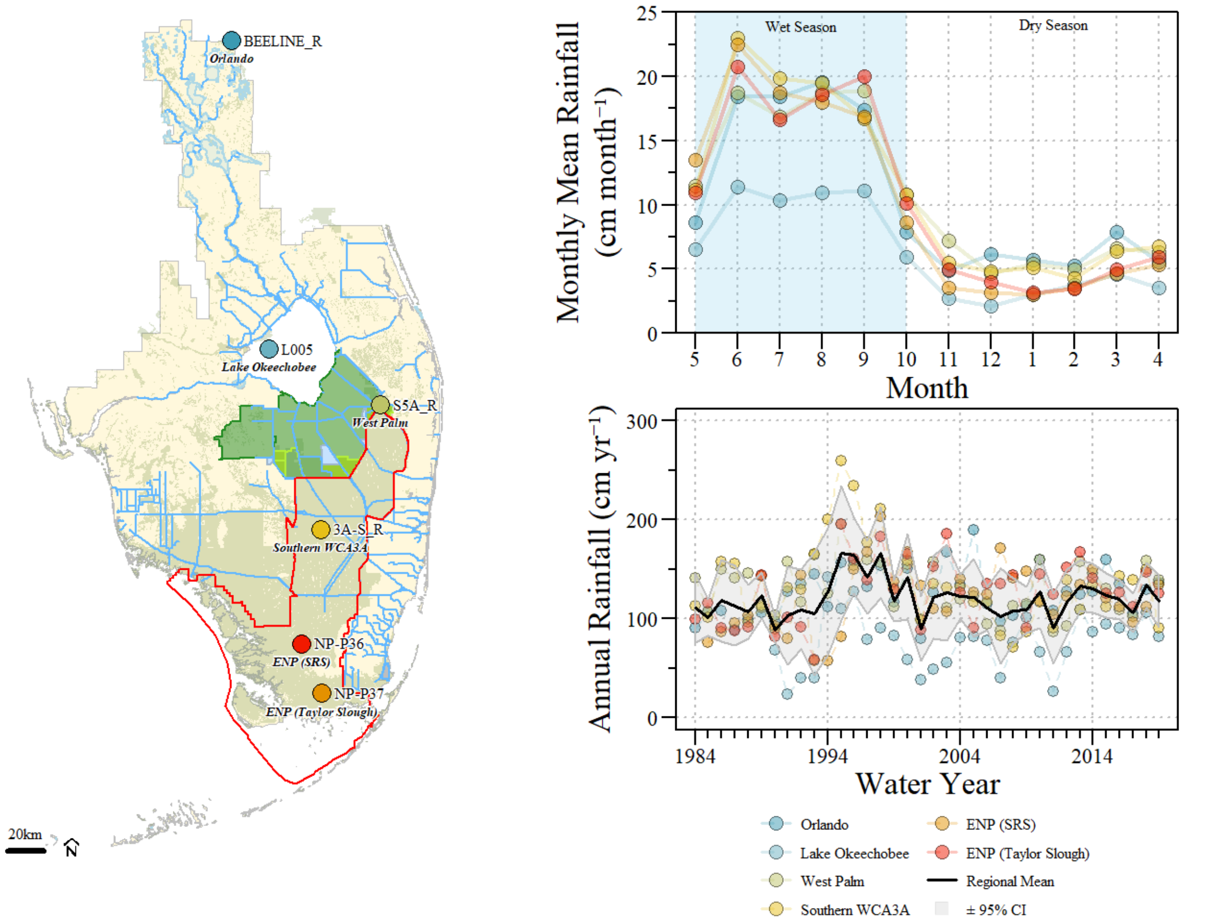

Figure 5. Monthly mean rainfall for each site over the period of record (top right) and annual total rainfall for sites from Orlando to Everglades National park between May 1 1983 and April 30 2019 (Water Year 1984 to 2019; bottom right). Regional mean ± 95% confidence interval calculated from these six sites. Data retrieved from South Florida Water Management District (SFWMD) online environmental database for the period of record between May 1984 to April 2019 for a monitoring location identified by the map (left). Only months and years with a complete record was used for mean and confidence interval calculation. Site “BEELINE_R” was used for Orlando; site “L005” was used for Lake Okeechobee; site “3A-S_R” was used southern Water Conservation Area 3A; site “NP-P37” was used for Everglades National Park (Shark River Slough) and; site “NP-P36” was used for Everglades National Park (Taylor Slough).

Tables

Table 1. Summary of fifth order polynomial regression results presented by Lapointe et al. (2019) for parameters regressed over time.

Parameter	R^2	F-statistic	Degrees of Freedom	ρ-value
Ammonium (NH_4^+)	0.05	356	3, 162	0.016
Nitrate (NO_3^-)	0.15	11.1	3, 165	<0.001
Dissolved Inorganic Nitrogen (DIN)	0.26	20.5	3, 162	<0.001
Soluble Reactive Phosphorus (SRP)	0.06	4.37	3, 150	0.005
DIN:SRP	0.22	15.1	3, 150	<0.001
Chlorophyll-*a*	0.15	8.28	3, 123	<0.001

Table 2: Kendall trend results of individual and annual mean for total nitrogen (TN), dissolved organic nitrogen (DIN; Ammonium + Nitrate + Nitrite), total phosphorus (TP), soluble reactive phosphorus (SRP), molar ratio of DIN and SRP and chlorophyll-a concentrations observed between May 1995 to May 2018 collected at Looe Key Reef by Florida International University Southeast Environmental Research Center (Briceño and Boyer 2019). All data below the minimum detection limit (MDL) were replaced with one-half the MDL. Kendall trend analysis was performed with base R and Theil-Sen Trend was estimated using the mblm function in the mblm R-library (Komsta 2013). Trends from individual samples included all data while trends for annual mean values were estimated with only years with greater than four samples per year.

Data Aggregation	**Parameter**	**N**	**Kendall τ**	**ρ-value**	**Thiel-Sen Slope Estimator[1]**
Individual	TN	88	-0.07	0.63	-0.12
	DIN	87	0.08	0.25	0.002
	TP	88	0.007	0.92	-0.0005
	SRP	81	0.12	0.10	0.0008
	DIN:SRP	79	-0.08	0.29	-0.52
	Chlorophyll-*a*	85	-0.003	0.97	0.0005
Water Year Mean	TN	19	-0.03	0.89	0.14
	DIN	19	0.06	0.73	0.006
	TP	18	-0.20	0.26	-0.004
	SRP	18	-0.02	0.94	-0.0004
	DIN:SRP	17	0.04	0.84	-0.59
	Chlorophyll-*a*	19	-0.01	0.58	-0.006

[1] units of slope for TN, DIN, TP and SRP are μM Yr^{-1}, DIN:SRP are Yr^{-1} and Chlorophyll-*a* are μg L^{-1} Yr^{-1}