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Evaluation of different averaging methods for calculation of ratios in nutrient data

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With 4 figures and 1 table

Abstract: Nutrient ratios are commonly used in ecology to investigate nutrient limitation in an aquatic environment. Ratios have distinct mathematical properties which should be considered when choosing an averaging method to avoid biased results. Herein, we estimated annual dissolved inorganic nitrogen to dissolved inorganic phosphorus ratios using long-term ecological (LTER) data from three sites in the German Bight by applying various measures of central tendency. Our aim was to examine the sensitivity of nutrient ratios to different modes of averaging and to define the most appropriate method for the calculation of nutrient relationships for stoichiometry. Our results demonstrate that the arithmetic mean of the raw data is upwardly biased, exaggerating the importance of phosphorus-deficient conditions. The geometric mean of the raw data and the arithmetic mean of log-transformed ratios are reliable calculations; however, the median is the most robust method to average nutrient ratios for skewed data. We recommend that ratio data should first be tested for normality, and that the methods used to determine nutrient ratios should be clearly stated, including which averaging method was used.

Keywords: nutrient ratios; nutrient limitation; averaging; Helgoland Roads; Sylt Roads; Norderney data

Introduction

Nitrogen (N) and phosphorus (P) are two major limiting elements for aquatic photosynthesis and hence for primary and secondary productivity. The identification of whether N or P limits productivity in a water body is vital for understanding the ecology of aquatic environments and for water quality management (Beardall et al. 2001; Kolzau et al. 2014; Liu et al. 2020). Redfield (1963) observed a consistent pattern in molar ratios N:P of approximately 16:1 in marine ecosystems, which is often used as a guide for differentiating nutrient limiting conditions (Carlsson et al. 2012; Ganguly et al. 2013; Burson et al. 2016; Miao et al. 2020). More recently Sterner et al. (2008) extended the observations by Redfield to include freshwater systems, interestingly finding slightly different mean ratios in different habitats, but still fairly close to the Redfield ratio. Knowing the nutrient ratios is not of academic interest only, but rather of essential importance for our understanding of the functioning of an ecosystem (Welti et al. 2017). Interestingly, Winfried Lampert started his career working with the then very common single currency approach, mainly focusing on carbon (C) (e.g. Lampert 1977; Lampert 1978; Lampert & Schober 1980), but later included multiple currencies of food quality as well, mainly through his students and colleagues (e.g. Müller-Navarra & Lampert 1996; Sterner et al. 1998; Haupt et al. 2010). The C:N:P ratios are key components in ecological stoichiometry studies used for examining processes including their impact on growth rates and trophic interactions (Boersma 2000; Boersma & Elser 2006; Frost et al. 2006), in nutrient recycling and systems biology (Hessen et

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al. 2013), and in global biochemical models (Galbraith & Martiny 2015). Therefore, it is of utmost importance that we understand what we do when we deal with nutrient ratios.

The problem with ratios is that they have characteristic mathematical properties, including non-normal distributions and non-symmetrical responses to changes in the numerator or denominator (Keene 1995; Hillebrand et al. 2013). This can lead to misleading results when ignored during data processing or analyses. Isles (2020) showed that among 100 randomly selected studies, 70 contained N:P ratios that were averaged out inappropriately. To minimize these issues he suggested, log-transformation of nutrient ratios. However, the log-transformation of original data can be controversial. O'Hara & Kotze (2010) showed that log-transformation performed poorly for count data, which is of course not an issue with nutrient concentrations, but much more importantly Menge et al. (2018) observed that 44 % of 988 ecologists misinterpreted graphs on log-log axes, and especially in ecological stoichiometry, the interpretation of a C:P ratio of 300 is, in the meantime, quite intuitive, whereas a value of 2.47 (10log) or 5.70 (ln) is much more difficult to interpret for many researchers.

In simple experiments, where we manipulate the food and obtain a set of different nutrient ratios, the problem of properly describing the ratios is not so large, although even their multiple measurements already pose somewhat of a problem. The much more challenging exercise is the description of the field, and properly to define and compute descriptors for patterns of changing ratios, especially when dealing with long-term patterns. Hence, in this paper we aim to examine the sensitivity of nutrient ratios to different averaging methods to determine the most appropriate method for the investigation of long-term change in nutrient stoichiometry. To do this, we used as an example several real-world long-term datasets of nutrient levels from the German Bight to calculate annual dissolved inorganic N:P molar ratios using different measures of central tendency, and then compared these with each other and with the Redfield Ratio.

Material and methods

To examine the sensitivity of N:P data in averaging methods, we used dissolved inorganic N:P ratios from measurements at three sites in the German Bight: Helgoland Roads, Sylt Roads and Norderney. Dissolved inorganic nitrates (NO_3^-), nitrites (NO_2^-), ammonium (NH_4^+) and phosphates ($[PO_4]^{3-}$) expressed in µmol/l, have been continuously recorded on a work-daily basis in Helgoland Roads since 1962 (Raabe & Wiltshire 2009), twice weekly in Sylt Roads since 1984, and weekly or biweekly in Norderney station since 1987. Here, we used data from 1987 to 2019. Helgoland and Sylt Roads LTER Data can be retrieved from the databank PANGAEA (www.pangaea.de); Norderney data can be requested from the Lower Saxony Water Management, Coastal Defence and Nature Conservation Agency (NLWKN).

The N:P ratios were calculated as the sum of NO_3^-, NO_2^- and NH_4^+, divided by $[PO_4]^{3-}$. We estimated the annual nutrient ratios by applying four averaging methods: the simple arithmetic mean, the median, the geometric mean and the harmonic mean (Table 1). The annual N:P ratios were compared with the annual arithmetic mean N divided by annual arithmetic mean P ($[N_{(\text{annual mean})}]$: $[P_{(\text{annual mean})}]$) to examine the sensitivity in the specific method of averaging (Isles 2020). Annual ratios were also calculated as arithmetic means from natural log-transformed ratios. We used the Redfield Ratio to examine whether annual N:P, calculated by different averaging methods, gave similar indications of nutrient limitations.

Spearman's rank correlation was applied to measure the strength of the monotonic relationship between the annual ratios as estimated by different averaging methods. This is a non-parametric correlation estimator robust to outliers and appropriate for data which do not follow a normal distribution (Croux & Dehon 2010). The Spearman's rank correlations were evaluated for significance at a 5 % level (alpha = 0.05). A correlation test with a *p*-value less than alpha is considered statistically significant. The variance, the distribution and the symmetry of the data were examined using boxplots, histograms, and the Kernel density distribution.

Results

The central trend values of the annual N:P molar ratios calculated with the different averaging methods are conspicuously different from each other in all datasets (Fig. 1). The annual arithmetic means were significantly higher than the Redfield Ratio from the mid-80s onwards in Helgoland Roads (Fig. 1A), from the early 00's onwards in Sylt Roads (Fig. 1C) and around 2002 in Norderney (Fig. 1E), indicating P-limited conditions. The annual harmonic means, by contrast, were below or close to the Redfield threshold, particularly

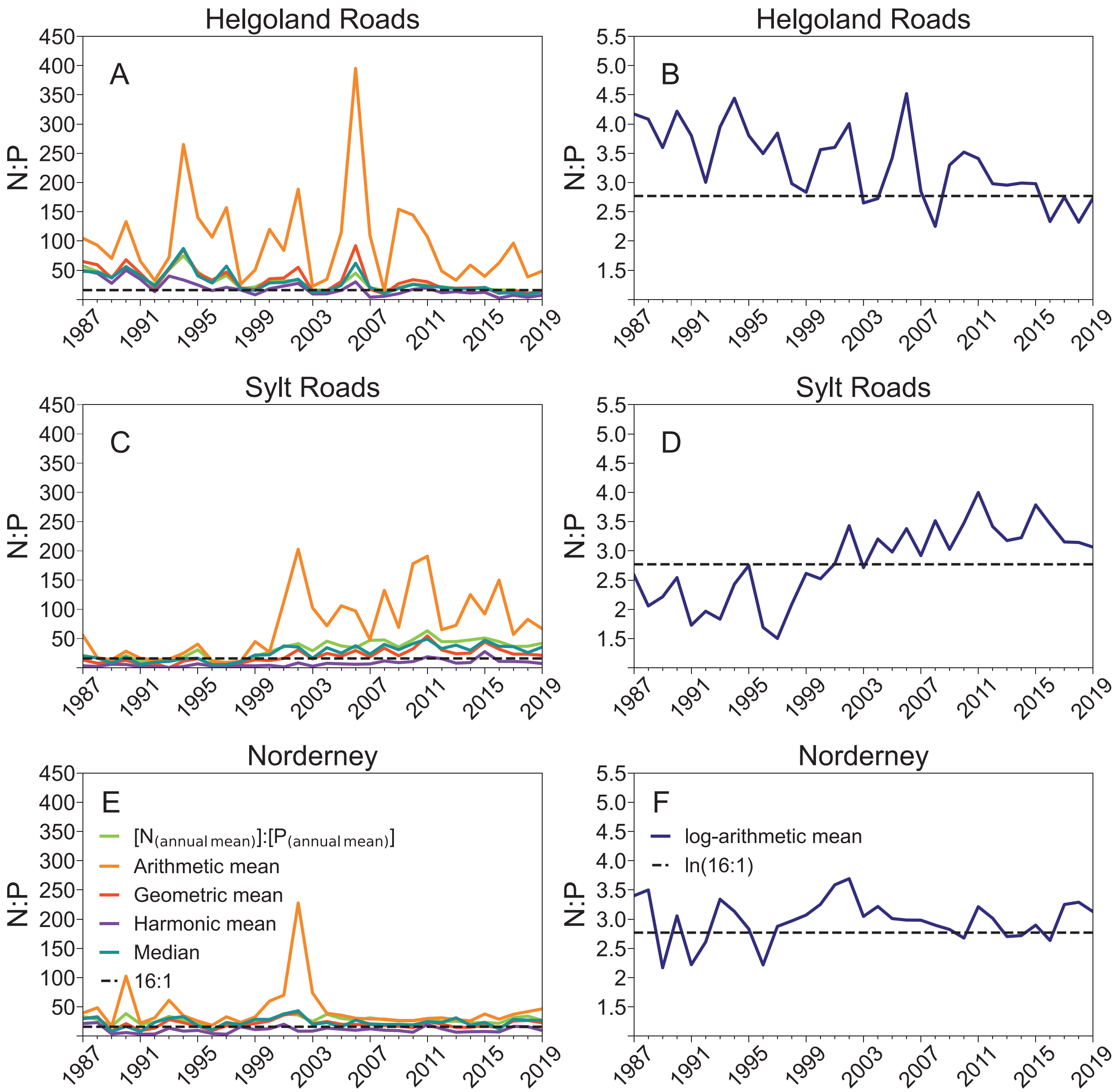

Fig. 1. Annual N:P molar ratios in Helgoland Roads **(A, B)**, Sylt Roads **(C, D)** and Norderney **(E, F)** from 1987 to 2019; estimated as annual arithmetic mean N divided by annual arithmetic mean P ([$N_{(annual\ mean)}$]: [$P_{(annual\ mean)}$]) (green line), arithmetic mean (orange line), geometric mean (red line), harmonic mean (purple line), median (cyan line) and arithmetic mean of the log-transformed ratios (blue line). The black dashed horizontal line is the Redfield Ratio.

in Sylt Roads and Norderney (Fig. 1C, E), implying N-limitation. The annual geometric means, medians, annual ratios of the annual arithmetic means ([$N_{(annual\ mean)}$]: [$P_{(annual\ mean)}$]) (Fig. 1A, C, E) and log-arithmetic means (Fig. 1B, D, F) fluctuated around the Redfield Ratio, suggesting P-limitation to a lesser extent and more frequent N-limited conditions than the arithmetic means. This implies that depending on the averaging method, we would conclude different things on the nature of limitations, which is of course less than satisfying.

Box plots of the raw nutrient ratios displayed the variance of samples within each year and the respective outliers for all datasets (Fig. 2). Outliers with a N:P ratio that exceeded 4000 were observed for the Helgoland Roads dataset (Fig. 2A), the same period that the highest arithmetic means was found (Fig. 1A). Similarly, years with the highest outliers (Fig. 2B, C)

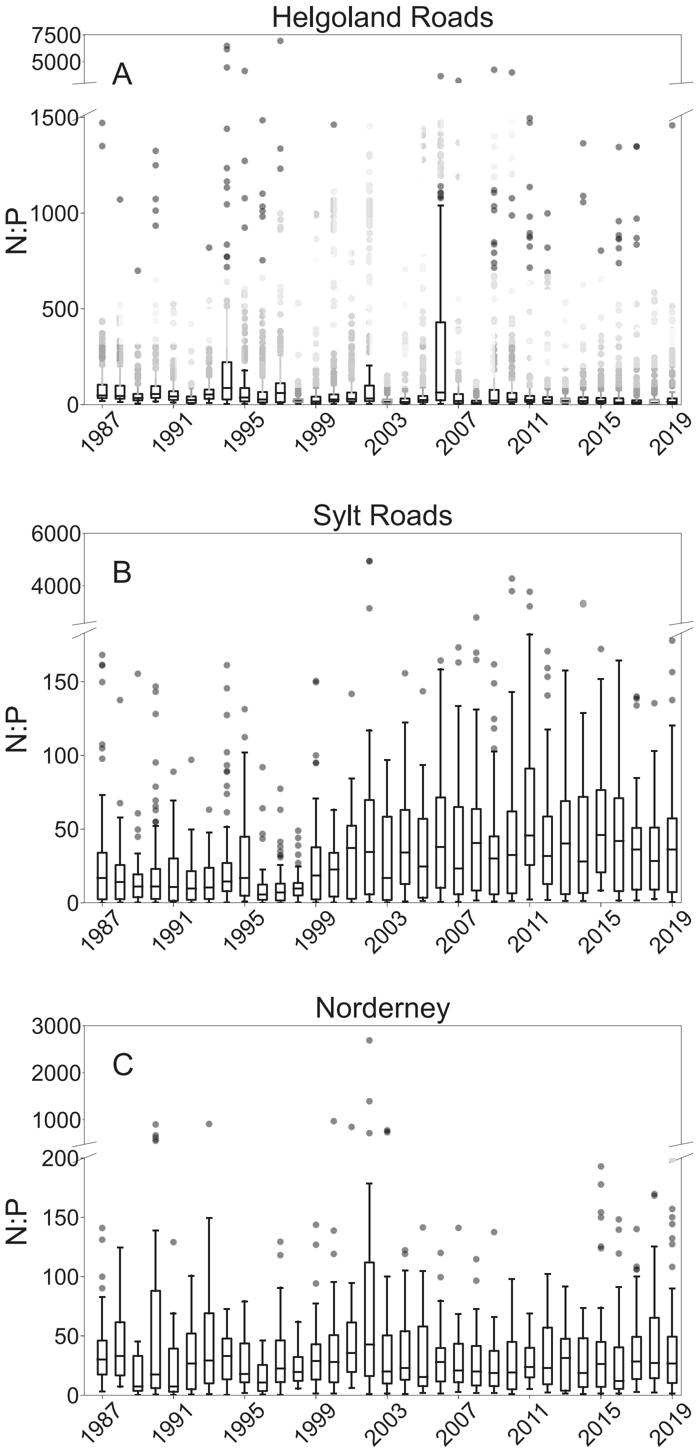

Fig. 2. Boxplots of Helgoland Roads **(A)**, Sylt Roads **(B)**, and Norderney **(C)** N:P raw data. The black circles are the outliers.

coincided with years with the highest arithmetic means in Sylt Roads and Norderney datasets (Fig 1C, E).

Relationships between the annual ratios estimated by different averaging methods were assessed with Spearman's correlation coefficient (ρ) (Fig. 3). The arithmetic means presented the weakest monotonic relationships and were always higher than annual ratios calculated with the other averaging methods for all datasets (Fig. 3A–D). Harmonic means showed moderate or strong positive correlation with medians , geometric means, and the annual ratio of the annual arithmetic means ([$N_{(annual\ mean)}$]: [$P_{(annual\ mean)}$]) for all

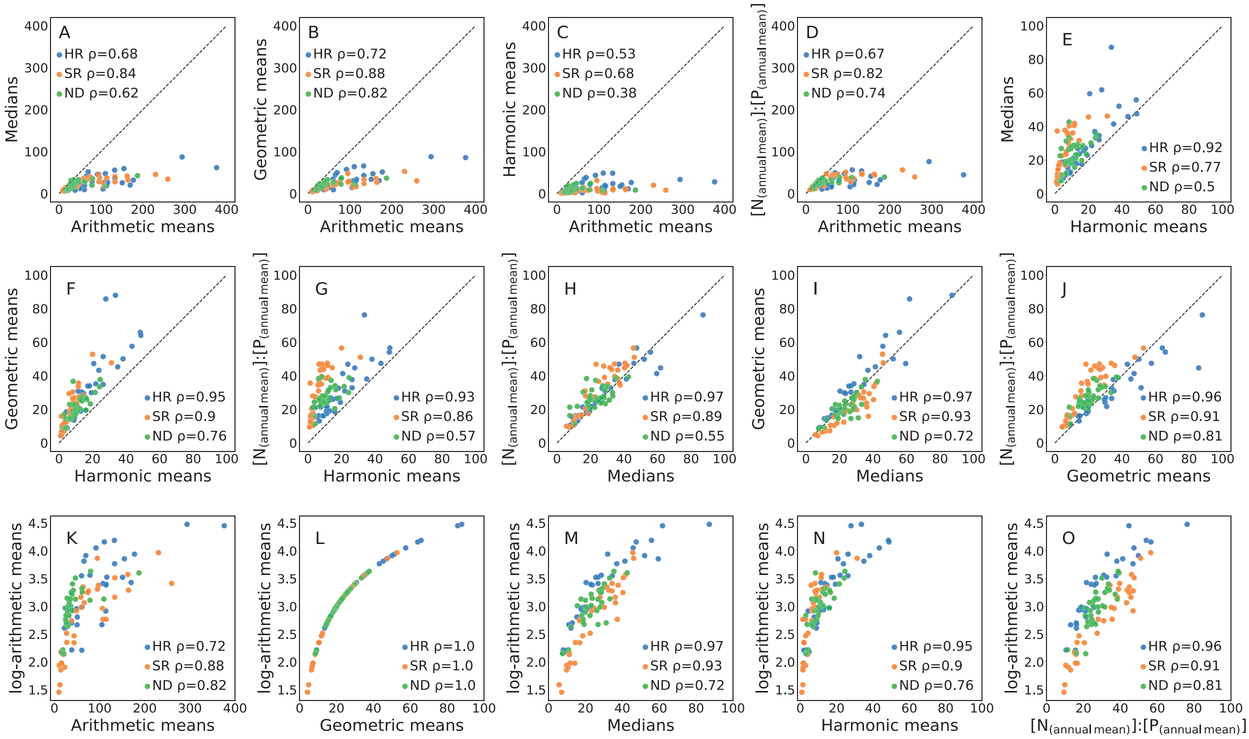

Fig. 3. Comparisons of different methods of calculating annual N:P molar ratios of raw **(A–J)** and log-transformed **(K–O)** data of Helgoland Roads (HR), blue dots; Sylt Roads (SR), orange dots; and Norderney (ND), green dots. The annual ratios of the annual mean N divided by the annual mean P are represented as $[N_{(annual\ mean)}]:[P_{(annual\ mean)}]$. Rho (ρ) is the Spearman's correlation coefficient, and the black dashed diagonal line is the 1:1 line. All correlations were statistically significant (*p*-values < 0.01). Note the higher y-axis maximum with the arithmetic mean.

datasets, but they always deviated from 1:1 line importantly, presenting the lowest values (Fig. 3, E–G). Medians compared to the annual ratios of the annual means ($[N_{(annual\ mean)}]$: $[P_{(annual\ mean)}]$) presented a very strong correlation for Helgoland (ρ > 0.97) and Sylt Roads (ρ > 0.89) but a moderate correlation (ρ = 0.55) for Norderney data (Fig. 3H). Strong positive correlations were observed for all datasets between medians and geometric means (ρ > 0.72) (Fig. 3I), as well as between geometric means and the annual ratios of the annual means ($[N_{(annual\ mean)}]$: $[P_{(annual\ mean)}]$) (ρ > 0.81) (Fig. 3J). In these cases, the ratios' deviation from a 1:1 line increased with higher ratios. The annual ratios of the annual arithmetic means ($[N_{(annual\ mean)}]$: $[P_{(annual\ mean)}]$) were slightly higher compared to the medians (Fig. 3H) and the geometric means (Fig. 3J), especially for the Sylt Roads and Norderney datasets. As the geometric mean is the antilogarithm of the arithmetic mean of the logarithms of the data (Table 1), there was a perfect correlation between the geometric means and the log-arithmetic means (ρ = 1) (Fig. 3L); and all correlations between other averaging methods and the two were identical (Fig. 3I, M; J, O; F, N; B, K). All the aforementioned correlations were statistically significant with p-values much lower than alpha (*p*-values < 0.01).

To examine the symmetry of log-transformed ratios (not averaged), we chose three years as examples: 2002 in Helgoland Roads with positive-skewed raw data (median close to the lower quartile Fig. 2A), 2001 in Sylt Roads with negative-skewed raw data (median close to the upper quartile Fig. 2B) and 1987 in Norderney with almost symmetrical distributed data (median in the middle of the boxplot Fig. 2C). Histograms and Kernel density distributions of log-transformed N:P (Fig. 4) showed that skewness remained in 2002 and 2001, with arithmetic mean higher than median (Fig. 4A) and arithmetic mean lower than median (Fig. 4B) respectively. In the symmetrical distribution, the arithmetic mean and the median were equal (Fig. 4C).

Discussion

The above results demonstrate that the arithmetic means exaggerated P-limited conditions in all sites of the German Bight, while the other averaging methods

Table 1. Measures of central tendency (Zar 1996).

Arithmetic Mean	$\frac{\sum_{i=1}^{n} X_i}{n}$
Median	$X_{(n+1)/2}$
Geometric Mean	$\text{antilog}\left(\frac{\log X_1 + \log X_2 + \ldots + \log X_n}{n}\right) = \frac{\sum_{i=1}^{n} \log X_i}{n}$
Harmonic Mean	$\frac{n}{\sum \frac{1}{X_i}}$

estimated significantly lower N:P ratios than arithmetic means, suggesting P-limitation to a much lesser extent (Fig. 1). The period that arithmetic means were very high, extremely high values of N:P and increasing variance existed in the original datasets (Fig. 2). Specifically, an apparent increase in extreme ratios existed in the recent years in the Helgoland and Sylt Roads datasets, implying that arithmetic means (as predictors) were worsening.

The extreme ratios are not methodological outliers but are mainly driven by the dynamics of nutrient uptake in spring. Phosphorus is depleted first, leading to strongly increasing N:P values caused by algal growth. Hence, simply removing these from the dataset is not a valid solution. It is important to note, however, that elevated N:P, suggesting P-limited conditions, can be also the outcome of an increase in N without the corresponding depletion in P (non-limiting concentration of both nutrients). Therefore, it is important to always consider changes in actual nutrient concentrations as well. In general, the comparison of ambient N:P against the Redfield ratio to determine whether plankton growth in a given water mass will become N or P limited is a coarse indicator, and supplementation with other methods is suggested (Beardall et al. 2001). However, nutrient ratios are indicative of which nutrient is or will become limiting (Burson et al. 2016) and are useful in long-term monitoring programs when other estimates are not available (Isles 2020). Herein, nutrient ratios and comparison with the Redfield ratio are used to investigate the sensitivity of nutrient ratios to different modes of averaging, rather than to infer the potential for nutrient limitation of phytoplankton assemblages.

The arithmetic mean is biased upwards because it is affected by extremely high values (Stevens 1955; Zar 1996) and the increasing variance (Isles 2020). Two possible ways to deal with the bias they produce are: 1) the use of a measure which is not affected by extremely high or low measurements, and 2) the transformation of the original data. Medians are resistant to extreme values and, despite being less informative than arithmetic means (Stevens 1955; Zar 1996), they are preferred when dealing with skewed data (Tian 2020; Hollister et al. 2021). Geometric and harmonic means correct the skewness through transformation of the original data, and they are generally recommended for averaging ratio-scaled positive data (Stevens 1955; Zar 1996). Geometric means give equal weight to each ratio (Stevens 1955; Zar 1996), and therefore they are more appropriate for averaging nutrient ratios (Daly et al. 1999, Isles 2020). Arithmetic means of log-transformed ratios (Isles 2020; Kim et al. 2021) and the annual ratio of the annual arithmetic means (Isles 2020) are also utilized for averaging nutrient ratios.

Here, we found that the arithmetic means of individual ratios produced different results compared to the annual ratios of the annual arithmetic means ($[N_{(\text{annual mean})}]$:$[P_{(\text{annual mean})}]$) (Fig. 3D). The annual ratios of the annual means were strongly correlated to medians and geometric means but tended to show higher values, particularly in the Sylt Roads and Norderney datasets (Fig. 3H, J). This approach is inappropriate, despite the strong correlations with the medians and geometric means, as the annual arithmetic mean of a nutrient can be biased due to data that follow a non-normal distribution (Ricker 1973; Daly et al. 1999). We also found strong correlation between the medians and 1) the geometric means; and 2) the log-arithmetic means for all datasets (Fig. 3I, M), which indicate the quantitative similarities between them. However, medians may be preferred to express central tendency in

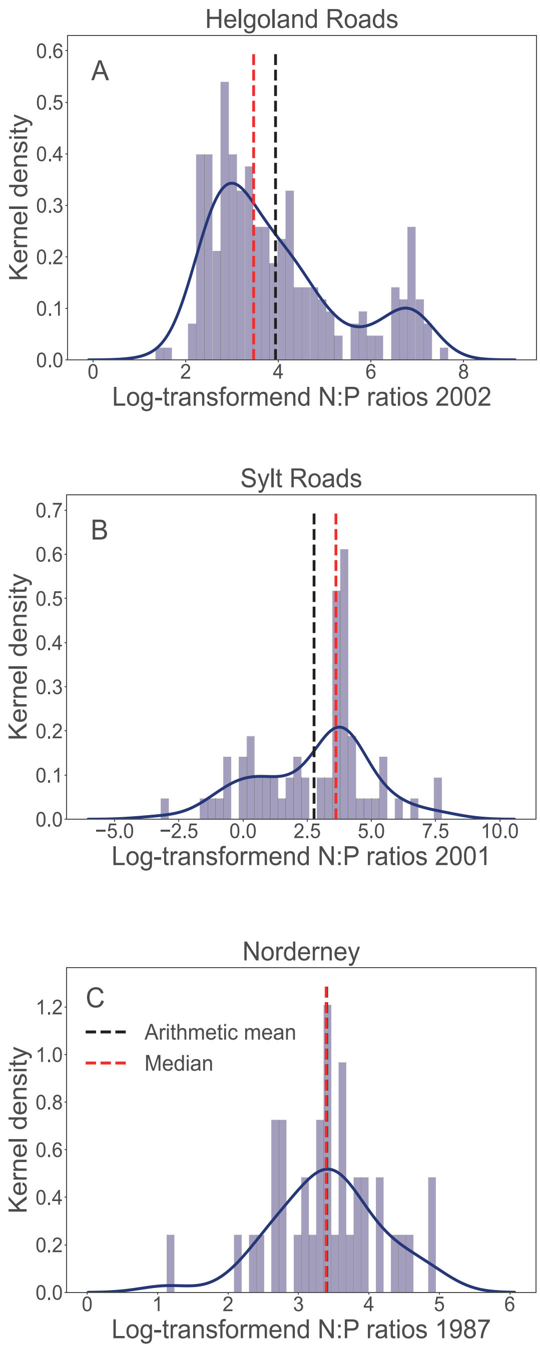

Fig. 4. Example histograms and Kernel density distributions of log-transformed N:P ratios in 2002 in Helgoland Roads **(A)**, in 2001 in Sylt Roads **(B)** and in 1987 in Norderney **(C)**. The black dashed line is the annual arithmetic mean and the red dashed line is the annual median of the log-transformed ratios

nutrient ratios, because even log-transformed ratios can retain skewness (Fig. 4A, B). If further analysis is needed, like correlation with other variables, non-parametric methods should be conducted (Tian 2020; Kim et al. 2021; Hollister et al. 2021).

So, what have we learned and why is it important? Many surveys in marine and limnic systems use time-series data to investigate the nature of the limitations of the water bodies. If the original data are plotted, and no averaging takes place, then it is perfectly reasonable to use untransformed data, as these represent biologically-relevant ratios. However, when amalgamating these data into monthly or yearly averages, it is very important to consider that the way this averaging takes place affects the results of the analysis. In general, an arithmetic mean will overestimate the N:P ratios, and as a result will lead to potentially erroneous conclusions that a waterbody is P-limited, where in fact this is completely driven by a small period in spring where P is depleted faster than N, with the resulting very high N:P values. Especially, when we consider management options and mitigation strategies, it is vital that we take this into account.

Our recommendations apply to all ratio data in aquatic sciences, including C:nutrient and other stoichiometric data. Here, we primarily addressed large datasets, where the median and geometric mean should be the same or similar for data with a normal distribution. The geometric mean may be more efficient than the median for smaller datasets.

Conclusions

Our findings highlight that the mathematical type of averaging method of N:P ratios is crucial to the interpretation of conditions in which limitation by N or P dominates. The distribution of the nutrient ratios and the existence of extreme values should be first examined to avoid potential errors in the identification of limiting nutrients. Medians, geometric means and log-arithmetic means are all preferred instead of the arithmetic means. We recommend using medians for averaging nutrient ratios for highly variable and extremely skewed data. Finally, we urge that the type of processing of nutrient ratios used in studies should be clearly stated in scientific reports, including which averaging method was used, as this is neither an obvious nor a trivial procedure.

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