Comparison of Environmental DNA Metabarcoding & Conventional Methods for Measuring Species Richness of Fish

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Abstract: Environmental DNA (eDNA) metabarcoding is a biomonitoring tool that can rapidly assess species richness in a variety of environments. It is important that this is calibrated with current, conventional standards of measuring species richness, as currently there is a lack of general understanding of how these methods compare. A dataset containing 121 sites in freshwater and marine systems measuring species richness of bony fish was analyzed using Lin's Concordance Coefficient (CCC) and Bland-Altman analyses to determine agreement in methods. This analysis found that there was significant agreement between these methods up to a species richness of around 75-100 species in freshwater systems, but not in marine systems. This suggests eDNA metabarcoding methods and conventional methods serve best as complements when measuring species richness of an area.

Introduction

Species richness, an indicator of ecosystem health, is measured using a variety of methods. High-throughput sequencing of environmental DNA (eDNA), or eDNA metabarcoding, is a method of rapidly detecting and measuring biodiversity of ecological communities and subsequently its health (Deiner 2017; Olds et. al. 2016). eDNA methods can be applied in multiple environments, including terrestrial, freshwater, and marine environments (Deiner 2017).

eDNA metabarcoding is advantageous because it can detect trace amounts of species in a variety of different environments and may detect rare or elusive fish species that are difficult to detect with conventional surveys (Olds et. al. 2016) and can have less sampling effort and be more cost-effective (Evans et. al. 2017). However, there is a general lack of understanding to how well eDNA metabarcoding methods calibrate to conventional methods of measuring species richness. Previous studies have indicated they calibrate well (Olds et. al. 2016), but others have shown they do not measure the same species richness (Cilleros et. al. 2019). It is important that measurement tools are calibrated to ensure the best methods are used and the measurements are in agreement. A significant difference can indicate one tool being advantageous over the other and that the tool that performs more poorly may have significant effects on biomonitoring and studies measuring ecosystem indicators.

In this analysis, a dataset of peer-reviewed studies measuring species richness using conventional methods and eDNA metabarcoding is analyzed to determine how well eDNA methods calibrate with conventional ones in measuring species richness of bony fish and whether the type of water system – freshwater or marine – are significantly different. The null hypothesis in this analysis is that there is no significant relationship or concordance with the methods, and the alternate hypothesis is that there is a significant relationship or concordance. The prediction is that there is significant agreement with the methods in both water systems.

Methods

This analysis is adapted from McElroy et. al. 2020. This dataset collected peer-reviewed studies that used eDNA metabarcoding and conventional methods of surveying bony fish species richness. The dataset contains 37 studies that covered 121 independent sites where species richness was measured using conventional methods and eDNA metabarcoding.

The dataset was collected by searching for peer-reviewed articles published between January 1, 2008 and April 1, 2020 containing the key terms "environmental DNA", "metabarcoding", and "fish". They selected studies that collected eDNA and measured species richness with eDNA that compared it to species richness as measured by conventional tools. Overall, out of the 121 sites, 17 were in marine systems and the rest were in freshwater (McElroy et. al. 2020). Information that was collected included the first author, the year of publication, the location of the study site, the methods used to measure species richness, and the species richness using the same hydrological units across all studies. For this analysis, the main columns that were used were the type of system, the measured species richness using eDNA metabarcoding methods, and the species richness as measured by conventional methods.

This analysis will use Lin's concordance correlation coefficient (CCC) and Bland-Altman analyses to measure concordance of the methods for both systems, only freshwater systems, and only marine systems. All statistical analyses were conducted in R.

Lin's concordance correlation coefficient (CCC)

This method measures both correlation and agreement between variables. The data is evaluated on how well two variables on a scatter plot fall on a 45° line through the origin (Lin

1989). It compares two measurements of the same variable and is used to compare those methods. It is often used to measure if a method can act as well as a "gold standard". It is like Pearson's correlation coefficient where the values lie between -1 and 1, and relationships are considered strong the closer the value is to +1 or -1. Altman (1991) interprets Lin's CCC as anything above ±0.8 being "excellent" and anything below ±0.2 being "poor". This method is used over Pearson's correlation coefficient in this analysis because a strong correlation does not indicate measurements are the same.

Bland-Altman Analysis

Otherwise known as Tukey's mean difference plot, the Bland-Atman Analysis plot, like Lin's CCC, compares the differences between two different methods of measurement (Giavarina 2015). Correlation studies using Pearson's R do not measure agreement, and it is not recommended to compare two different methods of the same measurement (Giavarina 2015). The result is a plot of the difference between two paired measurements against the mean of two measurements. It is recommended 95% of data points lie within 2 standard deviations of the mean difference to indicate a significant agreement (Giavarina 2015). Bland-Altman plots can also show directionality in performance. Using eDNA-measured species richness as the independent x variable and traditional methods as the responsive y variable, the Bland-Altman plot can be interpreted as anything above the mean indicating greater performance, or high species richness measured, from eDNA metabarcoding methods, while anything below the mean indicates better performance by conventional methods.

Results

Bar graphs were constructed using the ggplot2 package in R to illustrate the most commonly-used loci (in the case of eDNA metabarcoding) and methods to measure species richness. The most popular loci used were 12s and a combination of 12s and cytochrome *b* (**Figure 1**). While both marine and freshwater systems had the 12s loci used, there is disparity between the loci used in marine and freshwater systems. This may contribute to a difference of species richness.

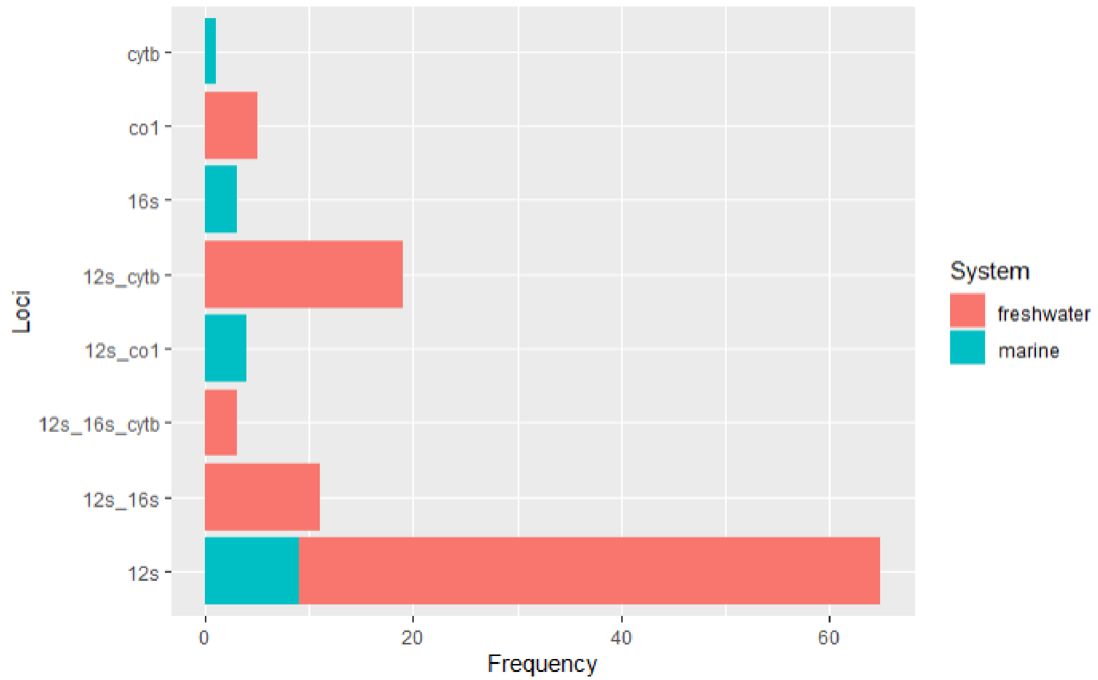


Figure 1. Bar graph of loci used to measure species richness using eDNA, separated by system: freshwater and marine.

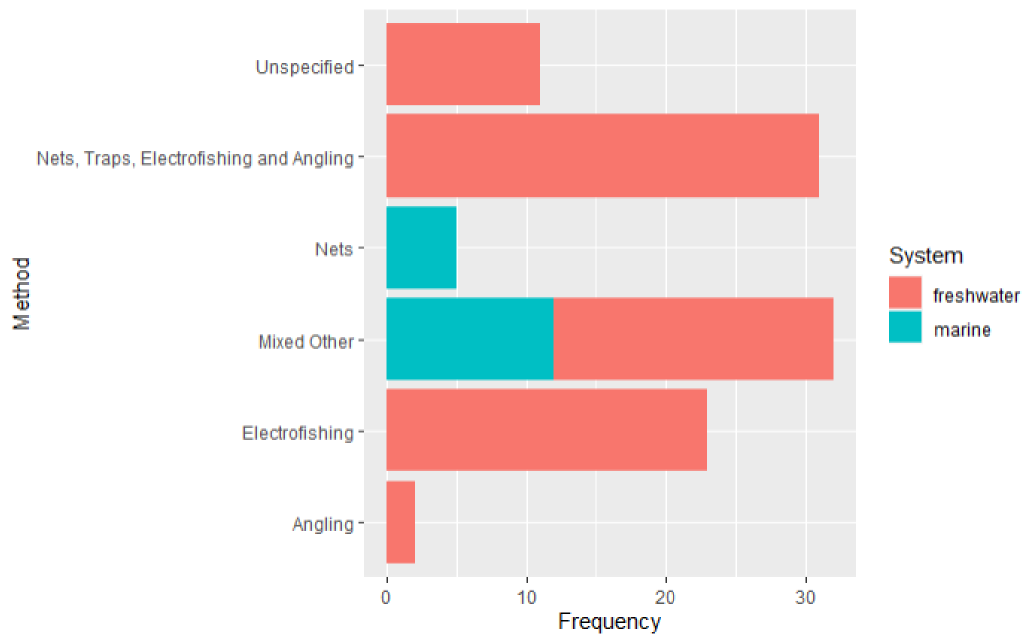


Figure 2. Bar graph of conventional methods used to measure species richness, separated by system: freshwater and marine.

A difference in methods used between marine and freshwater systems was also present in the conventional methods (**Figure 2**). Marine systems were more likely to used mixed methods or nets, whereas freshwater systems used a greater variety. However, any disparities between freshwater and marine systems may be attributed to the smaller amount of studies collected for marine systems (17) compared to freshwater (104). There is a possibility that there is not enough information collected to calibrate these methods in marine systems.

Table 1. Mean and standard deviations of species richness of fish as measured by eDNA metabarcoding.

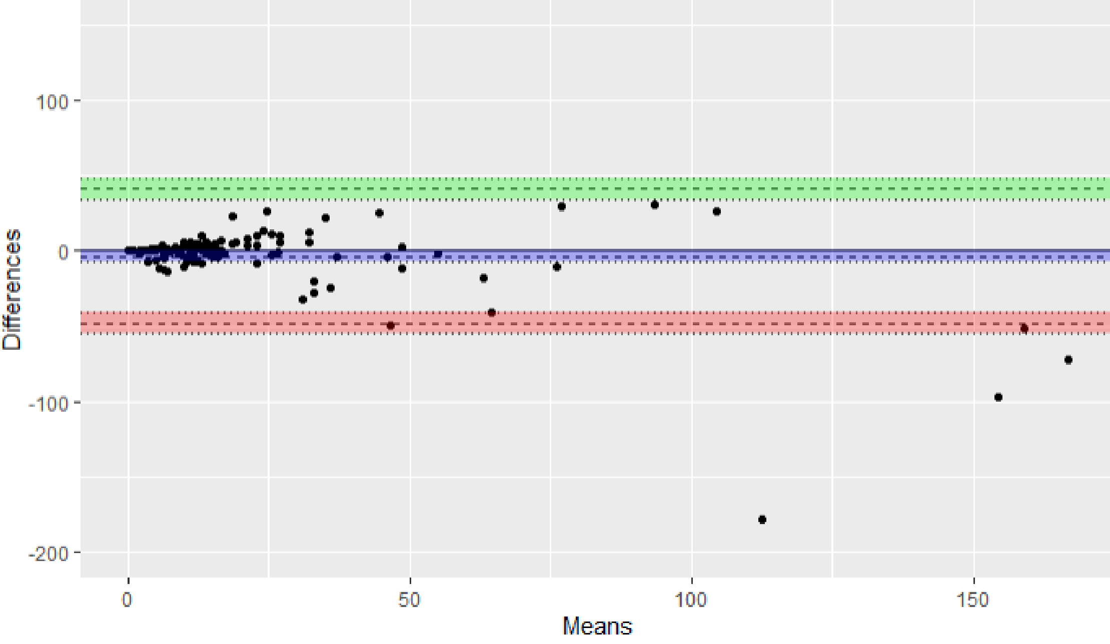
System	**Mean**	**Standard Deviation**
Both	21.03	25.57
Freshwater	16.93	21.91
Marine	46.12	32.15

Table 2. Lin's CCC comparing eDNA metabarcoding methods and conventional methods measuring species richness of fish calculated in both systems, freshwater systems, and marine systems.

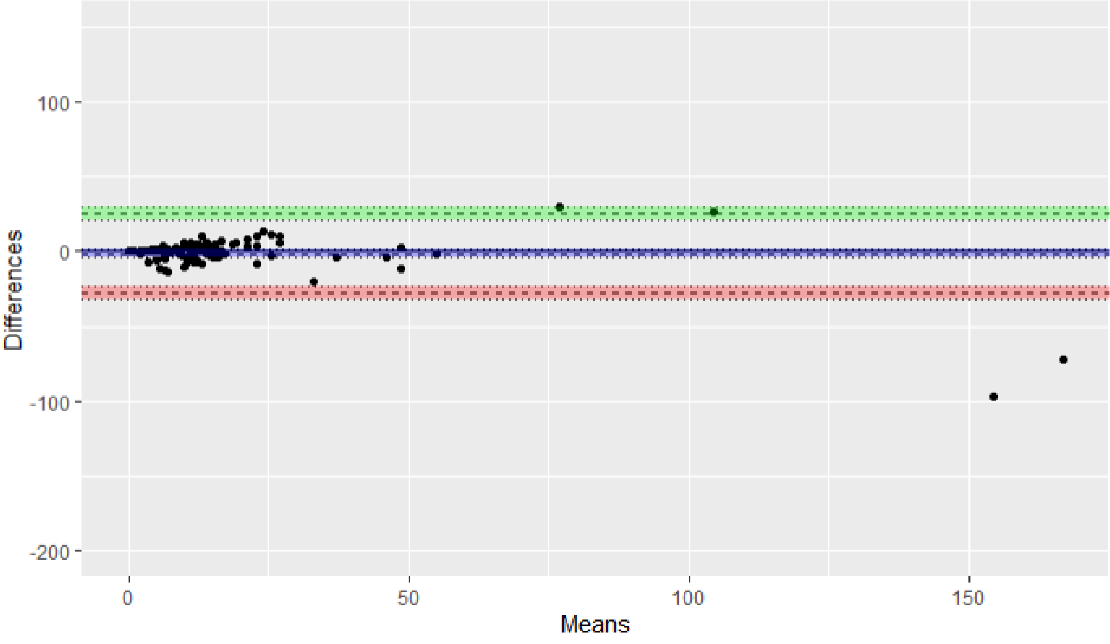
System	**CCC**	**Lower $CI_{95\%}$**	**Upper $CI_{95\%}$**
Both	0.74	0.66	0.80
Freshwater	0.86	0.82	0.90
Marine	0.35	-0.001	0.65

Lin's CCC was calculated using the agree.cc function in the R package agree with the method "mvn.jeffreys" and the default confidence level of 0.95 (**Table 2**). There appears to be agreement in both systems and in freshwater systems, but not in marine systems. The 95% confidence interval in marine systems overlaps 0, meaning there is no significant concordance.

(a)



(b)



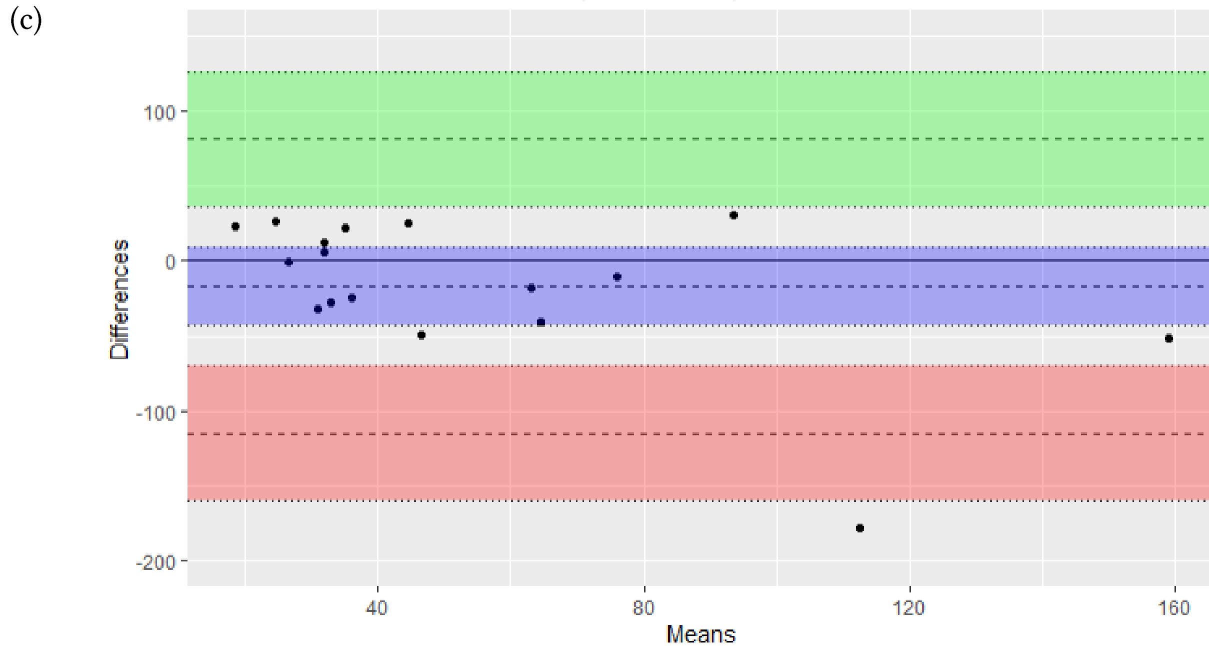


Figure 3. Bland-Altman plots constructed of eDNA metabarcoding methods and conventional methods measuring species richness of fish calculated in both systems (a), freshwater systems (b), and marine systems (c). The blue band is the mean (dashed line) with 95% confidence intervals, and the green and red bands indicate two standard deviations above and below the mean respectively with 95% confidence intervals. Anything above the mean difference would mean eDNA metabarcoding identifying more species, and anything below the mean difference would indicate conventional methods identifying more species.

Bland-Altman plots were constructed using the blandr.draw function in the blandr R package, which uses the ggplot2 library. In both systems (**Figure 3a**), there is agreement that starts to deviate (but still lie within two standard deviations of the mean) at the 50 species mark, and then a lot more after the 100 species mark. After the 100 species mark, conventional methods measure more species richness. In freshwater systems (**Figure 3b**), a similar pattern is observed. After the 75 species mark, there are two sites that measure more species richness using eDNA methods and two sites measuring higher richness in conventional methods. There is not as strong agreement in marine systems (**Figure 3c**), and the large confidence interval bands indicate a greater uncertainty.

Discussion

Lin's CCC showed moderate agreement in both systems (n = 121, CCC = 0.74, $CI_{95\%}$: 0.66, 0.80), which indicates agreement between traditional and eDNA metabarcoding methods. However, evaluating methods by different systems showed only good agreement in freshwater systems (n = 104, CCC = 0.86, $CI_{95\%}$: 0.81, 0.90) but not in marine systems (n = 17, CCC = 0.35, $CI_{95\%}$: -0.04, 0.65). This suggests that these methods are likely calibrated in freshwater systems.

The results of the Bland-Altman plots were similar to Lin's CCC results where eDNA metabarcoding agrees with or calibrates well with traditional methods of measuring species richness, but not when only marine systems are taken into account.

Based on these results, it can be concluded that eDNA methods perform just as well as traditional methods in freshwater systems. The null hypothesis that there is no agreement between methods can be rejected for both systems and freshwater systems, but not in marine systems. It is not clear if there is agreement in marine systems and there were not as many observational sites in marine systems than freshwater systems. Some species were only detected by one or the other method, meaning they would work best as complementary methods to capture all species diversity. Also, looking at the Bland-Altman plots, species richness was measured in near-agreement up to a certain point (around 75-100 species), and then deviated from there. This suggests both methods are similar up to a specific level of species richness. This is similar to the results found in Cilleros et. al. 2019, which suggests eDNA approaches can be an effective rapid assessment tool on a broader scale and conventional methods can be used on a local scale. Thus, using both methods appears to be useful in capturing the species richness of an area.

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