*Quantification of turnover along gradients (QTAG)*

We developed QTAG to describe microbial turnover across salinity in each of our datasets. Prior to analysis, QTAG filters OTU tables by removing (a) low abundance observations from each sample (<5 reads on a per sample basis), (b) low occurrence OTUs (present in <3 samples), and (c) all OTUs with fewer than 10 total reads from the dataset. QTAG first fits a model that best describes the OTU abundance data across a salinity gradient (Step I). Then, it uses this model to classify each OTU based on characteristics of its abundance distribution (Step II). Finally, it finds each OTU’s tolerance range by finding the narrowest interval within which 95% of its cumulative abundance occurs (Step III). Since the process of choosing the best-fit model does not make assumptions about the specialist type of the OTU, or vice versa, the output for both goals are independent from each other. See Figure 2 for a visual representation of QTAG steps.

*Step I: Model fitting*. QTAG fits a piecewise model that assumes that the relative abundance of an OTU across a gradient can be described by three horizontal segments representing *groups* *A,* *B*, and *C* (Figure 2). The segments are divided by boundaries X and Y, which are whole integer salinity values. The program iterates through all combinations of X and Y (where X is less than Y), and calculates the mean relative abundance for each group (*,,*), which form the piece-wise model. The fit of the model is calculated by summing the error squared between the data and the piece-wise model. The model with the lowest error squared is used for part two.

*Step II: Classification of specialist type*. In step two, QTAG takes the best-fit model from step one and classifies it as one of 13 specialist sub-types by using three sets tests to describe the shape of the data. First, QTAG tests whether the segment means are significantly different from each other (Mann-Whitney U test) to assign each OTU to one of the three main specialist types. If is significantly higher than *,* the OTU is classified as freshwater, if   is significantly higher than or   the OTU is classified as brackish, and if is significantly higher than it is classified as marine. Second, QTAG differentiates OTUs that are exclusively found at in a salinity category (e.g. fresh-restricted) from those that peak in the salinity category but also occur at lower abundances in other salinities (e.g. fresh-peaking). This is accomplished by comparing the means of each group ( ,, or   ) to the low-abundance threshold (LAT; default value is 10% of maximum relative abundance of that OTU) to test for overall ubiquity. Third, QTAG tests unclassified OTUs for patchy but salinity-restricted distribution patterns. Since zero-inflation of OTU occurrences can be common in large datasets and may reduce the power of Mann-Whitney U tests, we use a comparison of variance (Levene’s test) to test whether an OTU is found inconsistently, but exclusively, in a certain area of the salinity gradient. This classification is labelled ‘bloom’-type, because it describes a situation where an OTU might periodically ‘bloom’ in the environment, resulting in zero-inflated observations within a certain salinity range.

In total, there are 13 classification types that an OTU could be sorted into based on the overall distribution of the OTU’s relative abundance across salinity (fresh/brackish/marine/other) and the specificity to that particular salinity range (restricted/ peaking/ blooming).

*Step III: Tolerance Range:* Finally, after classifying each OTU as one of 13 sub-types, QTAG estimates the tolerance range of each OTU by finding the narrowest continuous salinity interval that most closely includes 95% of each OTU ‘s cumulative relative abundance across the salinity gradient. OTU occurrences outside this interval are marked as “outliers” and plotted as individual points in tolerance plots in downstream plots. Within each OTU’s tolerance range, QTAG also estimates average relative abundance for all samples with non-zero sequence counts for that OTU using a moving 5ppt window.