ONLINE SUPPLEMENT 16.4

QUANTITATIVE ANALYSIS OF FUNCTIONAL TRAITS DATA

Efforts to quantify relationships between fish species functional traits and environmental conditions is, by definition, multivariate in nature. Early comparative approaches used the ordination technique *principal components analysis* (PCA) to explore patterns in fish trait characteristics at the assemblage structure (Winemiller and Rose 1992). In this approach, it is important to first remove the statistical effect of body size because body size is itself highly correlated with many other species traits; this is most often done by using the residuals from length × mass regression models. More recently, the PCA method has been replaced with nonmetric multidimensional scaling (MDS; see '*Multidimensional scaling*' in main text); this is an improvement because fish traits data rarely meet key assumptions (e.g., normally distributed data) of PCA. For example, Brown et al. (2009) used nonmetric MDS to relate traits of stream fish assemblages to urbanization in nine metropolitan areas across the United States. Furthermore, Monte Carlo permutation tests (e.g., the 'PERMDISP2' procedure in the R package *vegan*; Anderson 2006) can now be used to conduct formal tests of significance when comparing functional groups within multivariate datasets (e.g., Mims et al. 2010).

Emerging methods to analyze assemblage-level fish data using both species traits *and* environmental data include 'RLQ' analysis (Doledec et al. 1996) and 'fourth-corner' analysis (Legendre et al. 1997, Dray and Legendre 2008).[1] These methods require three input matrices: the **R** (environment × site) matrix, the **L** (species occurrence/abundance × site) matrix, and the **Q** (species × trait) matrix. Both analyses return a **D** matrix that quantifies the correlations between species traits and environmental variables, organized using the site × species matrix. For instance, using fourth-corner analysis, Keck et al. (2014) showed that stream fishes exhibiting opportunistic life history strategies in Tennessee River streams were indeed highly associated with disturbed habitats and minimal flow regulation, while streams with intermediate levels of

[1] Both RLQ and fourth-corner analysis can be run using the *ade4* package in R (Thioulouse and Dray 2007).

disturbance and regulated flows supported fishes with periodic life history strategies. By adopting a species × trait × environment approach, then combining this information with data on species' distributions (i.e., occurrence data), fish ecologists are quickly gaining novel and powerful insight to the factors that underlie observed relationships between stream fishes and their environments.

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