Supplemental material

Methods: Analyses of body condition

We first used Fulton's condition factor (Anderson & Neumann 1996) because it requires no species-specific constants, allowing use on hybrids as well as parental species. The equation we used is $K = (W/L^3) \times 100,000$. To avoid distortion due to ontogenetic body shape changes, we excluded individuals <200 mm total length. Although body condition can be affected by differences in body shape (e.g., degree of lateral compression), we assumed that the *Catostomus* species in this study are similar enough in shape to compare body condition across species within the genus using Fulton's condition factor. The only major difference in body shape among these species is that white suckers have a wider caudal peduncle, and bluehead and flannelmouth suckers have a more slender caudal peduncle (Baxter *et al.* 1995). We also used relative weight (W_r) to compare body condition for flannelmouth (Didenko *et al.* 2004) and white suckers (Bister *et al.* 2000), the two species with standard weight equations available (98 individual fish). These equations were developed based on typical length and weight for reference individuals in each species. W_r is thought to be better than Fulton's condition factor for comparing across species with different body shapes, but is also limited because not all species in this study could be included. There are not yet published equations for W_r for bluehead suckers, and more problematically, it is unclear which equation should be used for hybrid individuals or indeed whether any of the species-specific equations are appropriate for hybrid individuals. Hybrids were not included in W_r analyses, both because there is no developed equation, and because hybrids are likely more phenotypically heterogeneous than individuals from parental species, so applying an equation from a parental species would be inappropriate.

Results: Analyses of body condition

A simpler approach to calculating body condition, Fulton's condition factor (K), fails to account for species-level differences in body shape. The *Catostomus* species in this study had similar body shapes, but white suckers have a wider caudal peduncle, while flannelmouth and bluehead have more slender caudal peduncles (Baxter *et al.* 1995). The other approach we used, calculating relative weight (W_r) from preexisting standard weight equations, does account for differences in body shape, but relies on equations developed from reference individuals, and could not be applied to all species, and especially not for hybrids. One particularly troubling aspect of the W_r approach for flannelmouth suckers was that the standard weight equation was developed using reference individuals from the Upper Colorado River basin (Didenko *et al.* 2004), where flannelmouth suckers are endemic, and where white suckers and hybrids have long been present. If there are negative effects of competition, they were incorporated into our conception of what a "standard" flannelmouth sucker should weigh due to the limitations of body condition indexes based on reference populations.

From calculations using Fulton's condition factor, *Catostomus* hybrids were in similar or slightly better body condition than parental species (Fig. S2). Notably, however, the relationship between flannelmouth suckers and white suckers was reversed using W_r, with

flannelmouth suckers in slightly better body condition overall (Fig. S3; Welch Two Sample t-test, $p < 0.05$), which leaves some uncertainty about the accuracy and meaning of these comparisons. However, hybrids must have been at least moderately ecologically successful, because body condition in hybrids was similar to parental species using both metrics. One caveat is that we only sampled adults, which by definition must have been ecologically successful enough to survive to maturity. It is possible that hybrid individuals with less ecologically successful phenotypes were produced, but did not survive early life stages. In some known examples, a broad range of interspecific hybrids can be viable under favorable conditions, but only a subset of hybrid individuals survive in a given environment (as in *Populus* trees; Lindtke *et al.* 2014). It is possible that a similar filtering of a subset of hybrid genotypes occurred with *Catostomus* hybrids, and that the hybrids sampled as adults were those that survived strong selective pressures on ecological traits as juveniles.

Relationship between continuous measures of ancestry (q) and isotopic signature for individual fish

To better understand the relationship between ancestry and isotopic signature, we plotted δ^{13}C and δ^{15}N against q (proportion of ancestry) for each individual fish (Fig. S1, S2). Although many of the individuals in this study were first generation (F1) hybrids and therefore have about 50% of genetic ancestry from each parent, proportion of ancestry (q) is a continuous measure and ancestry proportions did vary within hybrid class. However, isotopic signature is extremely variable even within groups of individuals with similar ancestry.

We also explored this relationship using simple linear models, where δ^{13}C and δ^{15}N were modeled as a function of several predictor variables, including ancestry from each parental species and a random effects term for each river.

Modeling ecological outcomes of hybridization

To explore potential predictors of the ecological outcomes of hybridization featured in this paper, we modeled hybridization outcomes across rivers. Specifically, we constructed linear models (`lm()` in `R`) to model transgressive phenotypes in hybrids (mean isotopic ratios of hybrids relative to mean isotopic ratios of both parental species jointly) as a function of the ecological differentiation between parental species (distance between mean isotopic ratios of parental species 1 and mean of parental species 2), F_{ST} between parental species (Table S1; calculated using vcftools, Danecek *et al.* 2011), and overlap between parental species standard ellipses. Due to the small number of rivers (4 replicates) with the required data, we were only able to explore the relationship with a single predictor at once. None of these predictors were significantly associated with transgressive phenotypes in flannelmouth× white hybrids. F_{ST} between parental species did not vary substantially; for flannelmouth and white suckers, F_{ST} estimates within a river ranged from 0.25–0.29. This variation is likely either stochastic (and associated with the specific genetic identity and variation in the individuals sampled), or potentially associated with different introduction sources for white suckers.

More generally, modeling ecological outcomes of hybridization in a more robust statistical framework, while highly desirable, would require a greater number of replicates than we currently have. A challenging issue is that hybrid ancestry proportion (q) is a property of individuals, while ecological overlap and genetic differentiation (F_{ST}) are properties of groups. Replicates for understanding patterns of overlap therefore need to be at the river/location level, and many hybrid and parental individuals would need to be sampled from each location. Incorporating ecological and physical characteristics across locations as predictor variables would also be helpful, but this sort of large-scale ecological characterization of multiple rivers is time consuming and costly. We view these sorts of analyses as a potentially fruitful avenue for future research into ecological outcomes of hybridization, both in these study species and other instances of interspecific hybridization.

References

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Site	Species 1	Species 2	F_{ST}
Big Sandy	bluehead	flannelmouth	0.278
Big Sandy	bluehead	white	0.281
Big Sandy	flannelmouth	white	0.289
Little Sandy	bluehead	flannelmouth	0.273
Little Sandy	bluehead	white	0.283
Little Sandy	flannelmouth	white	0.262
Escalante Creek	bluehead	flannelmouth	0.244
Escalante Creek	bluehead	white	0.279
Escalante Creek	flannelmouth	white	0.254
Yampa	bluehead	flannelmouth	0.276
Yampa	bluehead	white	0.305
Yampa	flannelmouth	white	0.288

Table S1: F_{ST} between species in each of four focal rivers, calculated using `vcftools`.
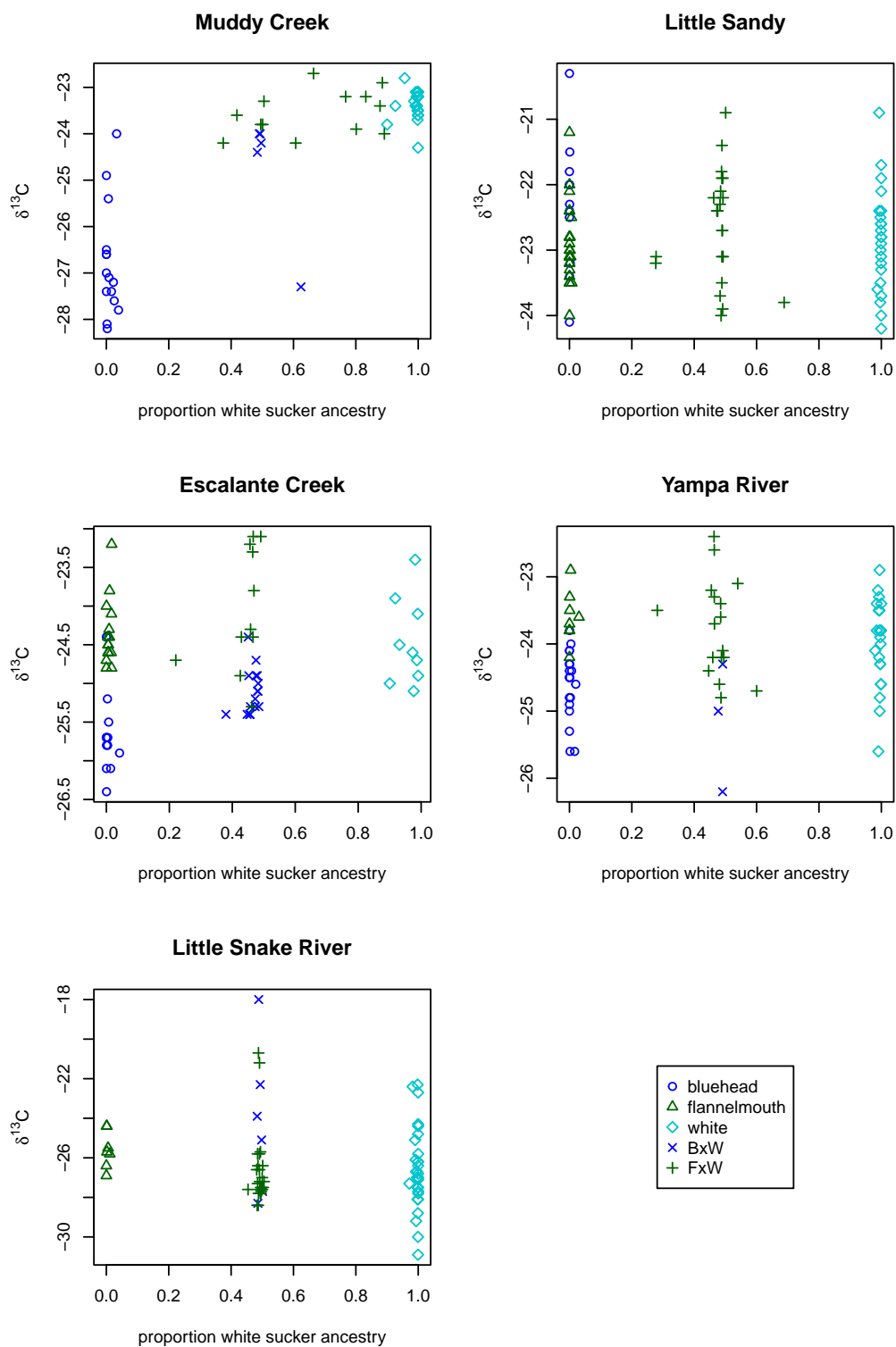
Figure S1: δ^{13}C plotted against proportional white sucker ancestry for flannelmouth, bluehead, and white suckers and their hybrids (only rivers with sufficient white sucker hybrids shown).
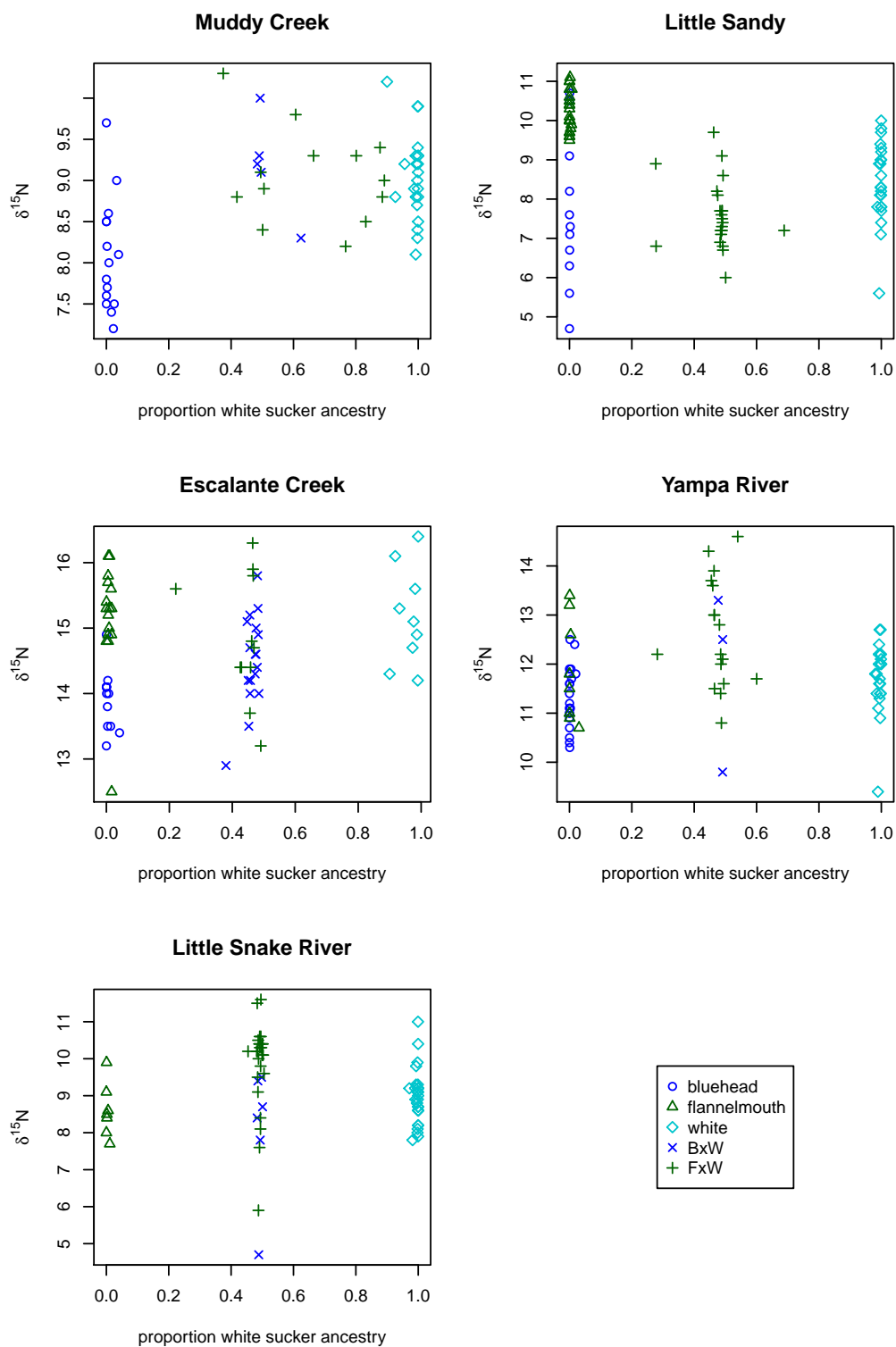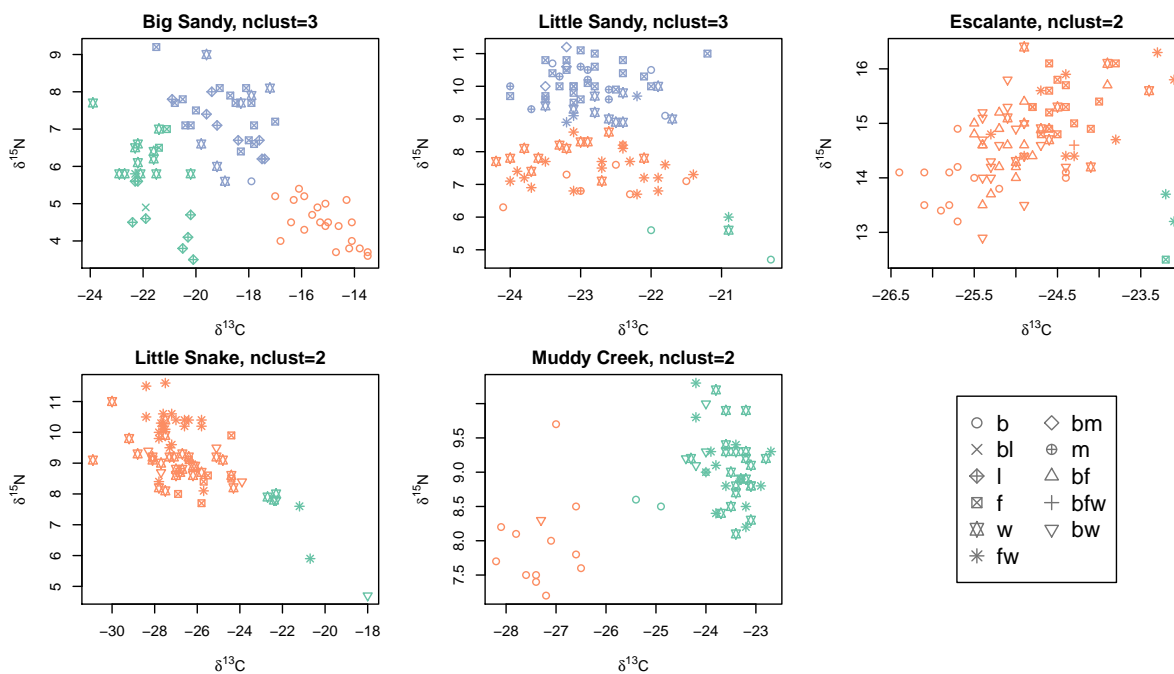
Figure S2: δ^{15}N plotted against proportional white sucker ancestry for flannelmouth, bluehead, and white suckers and their hybrids (only rivers with sufficient white sucker hybrids shown).

Figure S3: Results of a hierarchical Bayesian clustering algorithm were plotted in dual isotope space for the five rivers where the optimal number of clusters was greater than 1. Point color corresponds to cluster membership as estimated by our model; point shape corresponds to species or hybrid cross. Estimates of the optimal number of clusters in isotope space and membership of individuals in those clusters suggest that defined clusters did not correspond closely to species or hybrid categories in most cases.
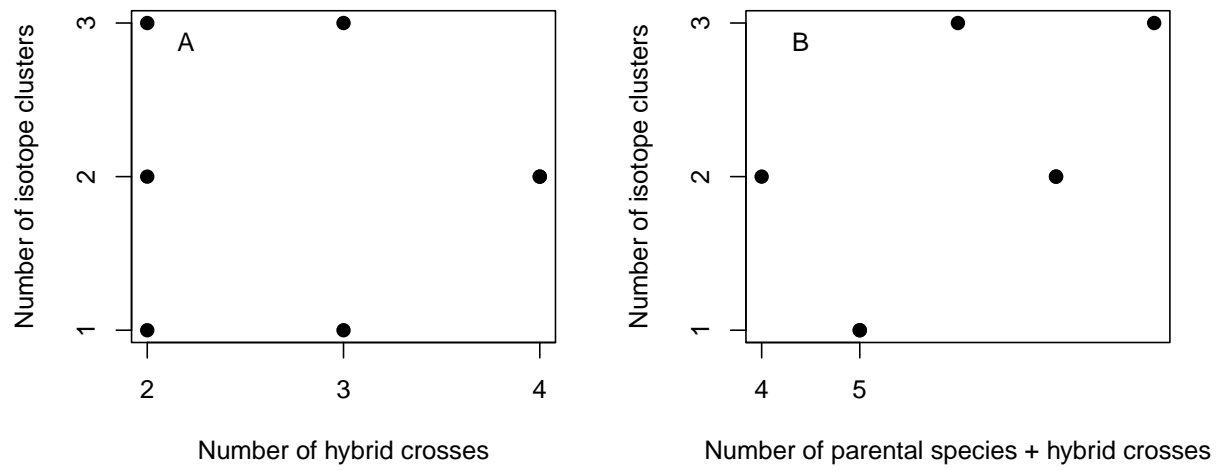
Figure S4: The number of inferred clusters in isotopic space was not significantly correlated with either A) the number of distinct hybrid crosses in a location or B) the total number of distinct genetic categories (parental species + hybrid crosses).
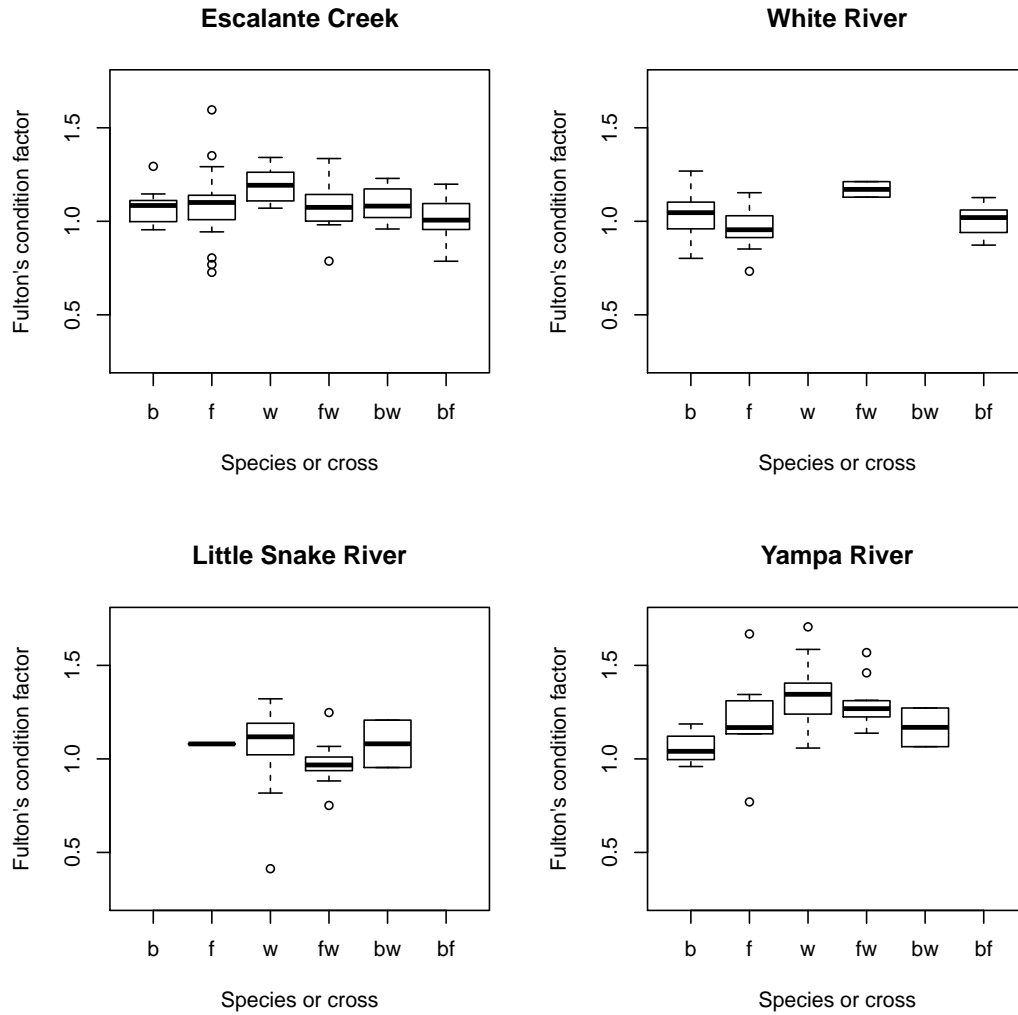
Figure S5: Hybrids and non-native white suckers had similar or higher body condition indices compared to native bluehead and flannelmouth suckers using Fulton's condition factor. Relative condition of species and hybrid crosses varied across rivers.
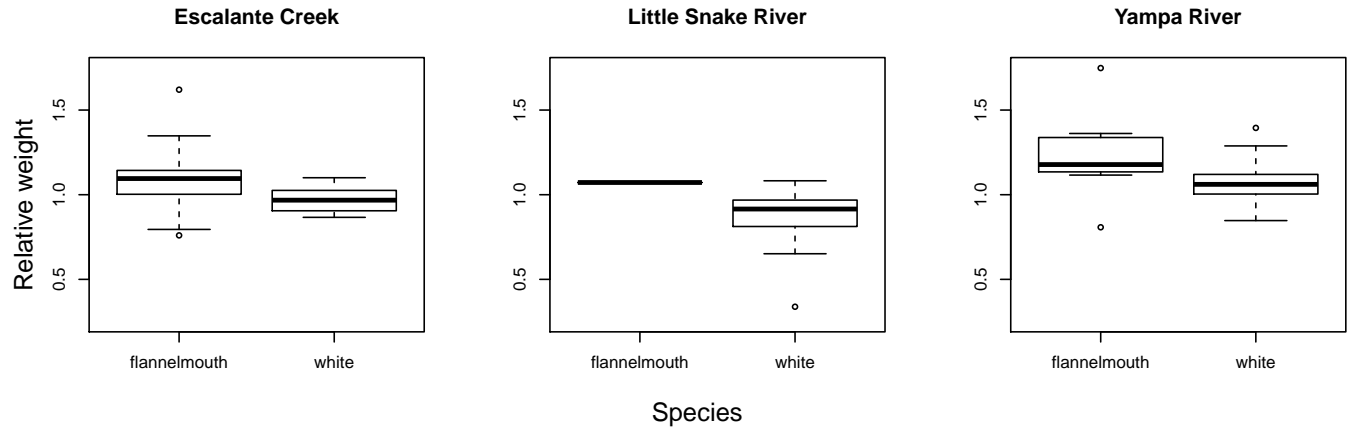
Figure S6: Flannelmouth suckers and white suckers had similar relative weights according to W_r equations available for these two species. In one river, Escalante Creek, flannelmouth suckers were in better condition according to W_r estimates; in the other 3 rivers no differences between species were identified.
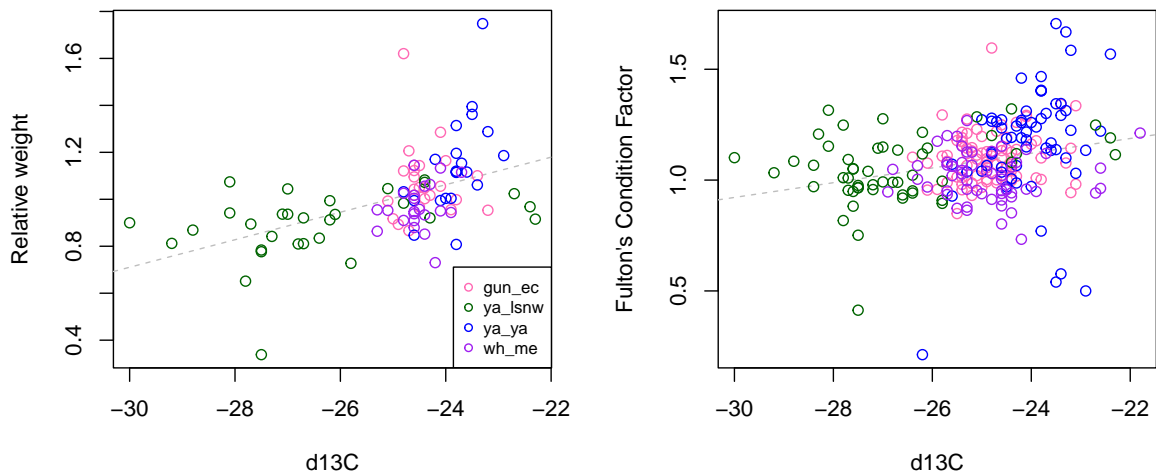

Figure S7: Both measures of body condition were positively correlated with $\delta^{13}C$.