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Ecological outcomes of hybridization vary extensively in *Catostomus* fishes

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Abstract

Hybridization outcomes vary geographically and can depend on the environment. Hybridization can also reshape biotic interactions, leading to ecological shifts. If hybrids function differently ecologically in ways that enhance or reduce fitness, and those ecological roles vary geographically, ecological factors might explain variation in hybridization outcomes. However, relatively few studies have focused on ecological traits of hybrids. We compared the feeding ecology of *Catostomus* fish species and hybrids by using stable isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) as a proxy for diet and habitat use, and compared two native species, an introduced species, and three interspecific hybrid crosses. We included hybrids and parental species from seven rivers where hybridization outcomes vary. Relative isotopic niches of native species varied geographically, but native species did not fully overlap in isotopic space in any river sampled, suggesting little overlap of resource use between historically sympatric species. The introduced species overlapped with one or both native species in every river, suggesting similar resource use and potential competition. Hybrids occupied intermediate, matching, or more transgressive isotopic niches, and varied within and among rivers. Ecological outcomes of hybridization varied across locations, implying that hybridization might have unpredictable, idiosyncratic ecological effects.

Keywords

Hybridization; fitness; stable isotopes; ecological interactions

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Introduction

Hybridization tests reproductive isolation between diverged lineages in secondary contact (Barton and Hewitt 1989, Harrison 1990). Studies of hybridization are motivated partly by a desire for generality in our understanding of how biodiversity arises and is maintained, and increasing accessibility of large genomic datasets has led to a newfound ability to study replicate outcomes of hybridization in the wild (Narum et al. 2013, McFarlane and Pemberton 2019). However, mechanisms controlling hybridization outcomes remain unidentified in most species pairs (Gompert et al. 2017), despite evidence that hybridization outcomes often vary substantially across replicate instances of hybridization (e.g. in *Ipomopsis*, sculpins, mice, oaks, spruce, butterflies, toads, barn swallows, trout, and many others; Aldridge and Campbell 2009, Nolte et al. 2009, Teeter et al. 2010, Lepais and Gerber 2011, Haselhorst and Buerkle 2013, Lagache et al. 2013, Gompert et al. 2014, Arntzen et al. 2017, Scordato et al. 2017, Mandeville et al. 2019).

Hybridization outcomes are likely to be shaped by interactions between divergent parental genomes and selection on phenotypes in hybrids across different environments. Both intergenomic and intragenomic incompatibilities can influence hybridization outcomes extensively (Lindtke 2015, Moran et al. 2021). Genomic incompatibilities in hybrids typically scale with evolutionary divergence between parental species (Presgraves 2002, Bolnick and Near 2005), and in some systems hybrids between more divergent species also produce more phenotypic, functional, and ecological novelty in hybrids (Rieseberg et al. 1999, 2007, Stelkens and Seehausen 2009, Stelkens et al. 2009, Holzman and Hulsey 2017, Selz and Seehausen 2019). If genomic incompatibilities alone drive hybridization outcomes without a clear connection to ecological context, or if selection pressures act similarly in all environments, then we might expect relatively consistent outcomes of hybridization across locations, either in overall ancestry or locus-specific ancestry (e.g. Schumer et al. 2018). However, hybridization outcomes do vary in many systems, and it is likely that environment shapes hybridization in some cases. Some clear connections between environmental context and hybridization outcomes have been described (e.g. Taylor and McPhail 2000, Vines et al. 2003, Muhlfeld et al. 2017, Mandeville et al. 2019), but our understanding of the ecological context and consequences of hybridization is still largely incomplete. Not knowing how genomic outcomes link to ecological outcomes is a limitation, because ecological context can influence outcomes of hybridization both by affecting relative fitness of hybrid and parental individuals (e.g. Arnold et al. 2012) and by limiting or increasing the opportunities for hybridization (e.g. Lepais and Gerber 2011). However, few empirical studies have described variation in hybridization outcomes with precise estimates of ancestry from genomic data, and also quantified the ecological interactions that have either resulted from or shaped hybridization outcomes.

Hybridization between fish species provides examples of both environment-dependent fitness of hybrids and broader ecosystem effects of hybridization, although most of these examples come from either laboratory or mesocosm experiments rather than natural populations. For example, stickleback hybrids grow at a reduced rate and have reduced fitness in enclosures under natural conditions relative to in a laboratory setting (Hatfield and Schluter 1999). In both trout and pupfish, sympatry with hybrids has been shown

to modify the fitness landscape for parental species by reducing growth, survival rate, or reproductive success of parental species through competition for resources, space, or mates (Rosenfield et al. 2004, Seiler and Keeley 2009). Phenotypes related to foraging ability can be especially important in determining outcomes of interactions between traits of hybrids and the environment. Experimental work in fish has shown that hybrids can be less successful than parental species when they have intermediate mouth morphology, because of an ecological mismatch between feeding morphology and available resources (e.g. sunfish; McGee et al. 2015) or reduced growth (e.g. sticklebacks; Arnegard et al. 2014). However, phenotypes of hybrids are hard to predict, and are not necessarily intermediate between parental species (Rieseberg et al. 1999, Stelkens et al. 2009). Instead, phenotypes of hybrid individuals might be similar to one of the parental species, or might differ from both parental species (e.g., Stelkens et al. 2009, Holzman and Hulsey 2017, Thompson et al. 2021). Hybrids can even sometimes feed more successfully on novel resources than parental species (Selz and Seehausen 2019), suggesting that under some circumstances hybrids could be better suited to their environment than parental species.

Our goal was to compare ecological interactions between multiple *Catostomus* fish species and their hybrid progeny across rivers in Colorado and Wyoming, incorporating sampling from multiple locations with differing hybridization outcomes, and using precise genomic ancestry estimates. The focal *Catostomus* species are part of a clade with extensive and highly variable interspecific hybridization between multiple species pairs (Hubbs et al. 1943, McDonald et al. 2008, Mandeville et al. 2015, 2017). Genomic outcomes of hybridization in *Catostomus* can vary substantially over the geographic range in which a pair of species co-occurs (Mandeville et al. 2015, 2017). Mechanisms driving this variation in hybridization outcomes remain unidentified. One hypothesis, which we explored in this study, is that geographic variation in the ecological success and survival of hybrids might cause variation in hybridization outcomes. If *Catostomus* hybrids were able to occupy novel ecological niches and thus gain a fitness advantage in some locations but not others, ecological variation and environment-dependent fitness of hybrids might contribute to the extreme variation we have observed in genomic outcomes of hybridization.

While some hybridization between *Catostomus* species is precipitated by human-caused species introductions, most notably of *C. commersonii* (white sucker) from east of the Continental Divide to the Upper Colorado River basin west of the Continental Divide in North America, this group of fishes likely has a long and convoluted history of hybridization (Hubbs et al. 1943, Hubbs 1955, Bangs et al. 2018), including a hypothesized allopolyploid origin (Ferris 1984). Of the three focal species in this study, *C. latipinnis* (flannelmouth sucker) and *C. discobolus* (bluehead sucker) are native to the study region and historically sympatric, while the common introduced species, white sucker, has no previous history of sympatry with the other focal species, but has been introduced to the study region within the last 150 years. Two additional species, *C. catostomus* (longnose sucker, introduced), and *C. platyrhynchus* (mountain sucker, native), were sampled at one location each, and were included in ecological analyses. Contemporary hybridization between *Catostomus* species is extensive; several species have hybridized with more than one congener, resulting in at least 12 different hybrid crosses between six geographically overlapping *Catostomus* species in the US mountain west (Mandeville et al. 2017). Previous genetic work demonstrated

that species with intermediate morphology (Hubbs et al. 1943) are interspecific hybrids (McDonald et al. 2008, Mandeville et al. 2015, Bangs et al. 2018) and that hybridization varied spatially (Mandeville et al. 2017). In some locations, few crosses were sampled despite sympatry of multiple parental species; in other locations, many different crosses involving the sympatric parental species occurred. Backcrossed hybrids and later-generation recombinant hybrids were common in some locations, while other locations contained only first generation hybrids (Mandeville et al. 2015, 2017).

Ecological traits of these species differ. Bluehead suckers eat predominantly algae that grow in fast-moving, shallow portions of rivers (called riffles), while flannelmouth and white suckers eat more aquatic invertebrates and more often occur in deep, slow pools (Tronstad and Estes-Zumpf 2011, Cross et al. 2013, Walsworth et al. 2013). *Catostomus* species vary substantially in mouth morphology. Bluehead and mountain suckers have a distinctive “scraping ridge” that facilitates scraping algal biofilms from surfaces (Baxter et al. 1995, Fig. 1). In contrast, the other species have no scraping ridge, and instead have fleshy lips in varying shapes and textures (Fig. 1). Variation in dietary, behavioral, and morphological traits sets up the expectation that isotopic niche might vary by species. Nitrogen ($\delta^{15}\text{N}$) serves as a proxy for trophic position, and we therefore expected higher values of $\delta^{15}\text{N}$ for fish consuming more invertebrates (Post 2002), namely flannelmouth and white suckers, than for bluehead suckers consuming more algae. Variation in carbon isotopes ($\delta^{13}\text{C}$) can result from intrinsic characteristics of dietary items (e.g. C3 versus C4 plants), but in rivers might also depend on patterns of CO_2 exchange between water and air, source of CO_2 , and flow velocity over biofilms limiting mass transfer and therefore isotopic discrimination (Finlay 2001, Newsome et al. 2007). Carbon stable isotopic ratios might therefore also differ depending on how much individuals feed in riffles or pools in a river, leading to the expectation that $\delta^{13}\text{C}$ might be lower in bluehead suckers, which favor riffles. It is also possible that more separation in $\delta^{13}\text{C}$ could be found in rivers with more heterogeneous habitat, where there is more opportunity for divergent feeding behaviors.

We used stable isotope data to identify ecological overlaps between genetically-defined parental species and hybrids (Layman et al. 2007, Newsome et al. 2007). In hybrid crosses where parental species were expected to differ in isotopic niche (e.g. bluehead×flannelmouth and bluehead×white), we expected intermediacy in diets of hybrids, based on morphology (Fig. 1) and literature on related species (Clarkson and Minckley 1988). In hybrid crosses where parental species shared a similar isotopic niche (e.g. flannelmouth×white), we expected hybrids to have similar diets to parental species (Fig. 1). Based on previous studies of *Catostomus* sucker ecology, we expected that species introductions and hybridization might lead to greater overlap in ecological niche (Walsworth et al. 2013). We compared body condition of parental species and hybrids as a rough estimate of foraging success and ecological suitability to available food resources. Because fertility of hybrids can vary, we consider body condition a partial fitness proxy. Ecological success of hybrids is one component of fitness, but may be independent of fertility and reproductive success in some hybrid crosses. If hybrids or introduced species were at an ecological disadvantage, we would expect to see lower body condition; however, if hybrids or introduced species were at an ecological advantage in some environments, they might be in better body condition.

Methods

We combined stable isotope and genetic analyses of 506 individual fish to identify ecological niches of *Catostomus* suckers with different parental species and hybrid ancestry. Sampling spanned seven different locations in the Upper Colorado River basin in Wyoming and Colorado (Table 1, Fig. 2). Fisheries biologists from state agencies collected fin tissue from each individual fish; tissue was stored in ethanol prior to use for both genetic analyses (Mandeville et al. 2015, 2017) and stable isotope analyses (this study). Ethanol preservation can distort isotopic signatures in fish tissue (Arrington and Winemiller 2002, Kelly et al. 2006), but because all fin samples were treated the same in this study, we did not correct any of the isotope ratios for preservation fractionation. Locations for isotope analysis are a subset of those used for genetic analyses, and were chosen to include rivers with a range of hybridization outcomes, based on the results of Mandeville et al. (2017).

Genomic ancestry of hybrid and parental individuals

We used results of genomic analyses from Mandeville et al. (2017) to identify ancestry of individual fish (Fig. 2). Genomic analyses of hybridization for the individuals in this study used genotyping-by-sequencing data (Parchman et al. 2012). Specifically, we relied on results from a genetic clustering algorithm, entropy (Gompert et al. 2014, Shastry et al. 2021), based on 11,221 single nucleotide polymorphisms (SNPs) to identify parental species and hybrids genetically. This analysis produced precise estimates of each individual fish's ancestry, including proportion of ancestry from each parental species in hybrids. Estimates of interspecific ancestry from entropy also revealed extent of hybridization and backcrossing, so we know that a substantial proportion of hybrids in this study were F_1 (first generation) hybrids or BC_1 (backcrosses resulting from mating between an F_1 hybrid and a parental species). We used both categorical designations of ancestry (species or hybrid cross) and a continuously valued measure of ancestry (proportion of ancestry) as covariates for stable isotopic ratios and body condition of hybrids in this study. We also quantified extent of backcrossing in flannelmouth \times white hybrids (the most geographically widespread cross) in each river using the 95% quantiles of the distribution of proportion of ancestry (q) across hybrid More extensive discussion of genomic outcomes of hybridization in *Catostomus* fishes can be found in Mandeville et al. (2017).

Stable isotope analyses of ecological niche

To obtain stable isotope data, we prepared samples of fin tissue for analysis of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ by first desiccating them in a drying oven (60 °C) for 2–3 days. We chose to use fin tissue because it can be sampled non-lethally and has a relatively slow turnover time, on the order of several months (comparable to muscle, a more frequently used tissue; Jardine Thomas and Crowther 2015). Recent work confirms that $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are highly correlated in muscle and fin in *C. commersonii* and *C. catostomus* (Maitland and Rahel 2021), confirming that use of fin is appropriate for these study species. Dried fin samples were analyzed for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios the University of Wyoming Stable Isotope Facility (UW-SIF) via high temperature oxidation in a Costech 4010 elemental analyzer interfaced to a Finnigan DeltaPlus XP mass spectrometer.

For each species or hybrid cross in each river, as defined by estimates of genetic ancestry (Mandeville et al. 2017), we estimated an ellipse in dual isotopic space to quantify the relative position of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values and variation in those values within a group (Layman et al. 2007). We used the maximum likelihood standard.ellipse function in the siar package in R to fit the standard ellipses (Parnell et al. 2010, Jackson et al. 2011). This model assumes that bivariate isotopic data (isotopic ratios of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) for a group of individuals is best fit by a multivariate normal distribution, and uses sample means and a covariance matrix to estimate parameters of a standard ellipse containing 40% of the data (Jackson et al. 2011), which is analogous to a standard deviation for a univariate normal distribution. Notably, an ellipse of this size is generally consistent in area across variable sample sizes, while approaches enclosing a larger of the proportion are more sensitive to sample size variation and individual outliers. To account for potential underestimation of ellipse size at low sample sizes, an additional correction of $(n-1)/(n-2)$ is applied to produce SEAc, a standard ellipse that is unbiased with respect to sample size. We used the SEAc version of the standard ellipse for all analyses, because simulations suggest that it is less sensitive to sample size variation and small sample size (Jackson et al. 2011), and our sample sizes varied across rivers and species sampled (Table 1). We quantified isotopic similarity of parental species and hybrids within each river using the overlap function in siar to calculate the overlap of standard ellipses (Jackson et al. 2011). We then compared area of isotopic overlap in each pair of species or hybrids across rivers to understand how consistent relative positions of species are in isotopic space. We also examined the relationship between isotopic ratios and a continuous metric of genetic ancestry, using estimates of q (proportion of ancestry) from Mandeville et al. 2017 rather than categorical binning of individuals into species or hybrid crosses.

Fin tissue was originally sampled for genetic analysis, and because of this focus no food web baseline samples for isotope analysis were collected at the time of sampling. We decided that a post-hoc sampling of the food web would not accurately represent conditions when fish were originally sampled, as food webs vary seasonally and year-to-year (Cross et al. 2013). Rivers likely varied in baseline $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios, as well as the food resources available, making comparisons of absolute values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ across rivers inappropriate. We addressed these constraints by calculating overlaps in isotopic space only within a river, not across rivers, and considering relative positions of species and hybrids in isotopic space within a river rather than absolute values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$.

Clustering of individuals in isotopic space without respect to ancestry

Species and hybrid crosses are commonly considered as “types” in that conspecific individuals are assumed to be more similar to one another than to heterospecific individuals. However, individual variation may lead to differences among individuals of the same species, and it is unclear to what extent we should expect conspecific homogeneity in ecological niche. To assess empirical evidence for isotopic niche clustering in *Catostomus* species and hybrid crosses, we also assessed the distribution of individuals in isotopic space using a hierarchical Bayesian model, isoclust. The model is written in JAGS and implemented through R (code included in Supplement). This model identifies the best-supported number of distinct clusters in isotopic space for each river with no prior

information about ancestry of individuals, thus quantifying how clustered individuals are in resource use without assuming that individuals of a species are similar to one another. We sequentially fit models for 1 to 6 clusters in isotopic space, where each individual is assigned categorically to a cluster, and identified the best fit of model to data using a penalized measure of model deviance (pD). This approach complements our more conventional analyses where we quantified resource use by binning individuals a priori by species. By identifying statistically how many clusters are represented in isotope space, we can better quantify distinctiveness and overlap across species and hybrid categories, rather than simply describing clusters qualitatively based on the assumption that individuals with similar ancestry will be ecologically similar (i.e., grouping by ancestry as in Fig. 3).

Body condition as a measure of ecological success

We calculated body condition using length and weight data for 255 individual fish that had these data available (Bolger and Connolly 1989, Jakob et al. 1996). Body condition is sometimes used as a fitness proxy, but because hybrids can vary in fertility independent of their body condition, we interpret body condition as ecological success but do not fully equate ecological success with overall fitness. We used two methods, Fulton's condition factor (K ; Bolger and Connolly 1989) and relative weight (W_t ; Wege and Anderson 1978). We also modeled body condition as a function of several predictor variables, including isotopic ratios (both carbon and nitrogen), as well as proportional ancestry from white, flannelmouth, and bluehead suckers.

Results

Isotopic niche differentiation of native species and niche overlap with introduced species

Sympatric native species (bluehead and flannelmouth) did not overlap in isotopic space, suggesting little ecological overlap in any of the five rivers where both species were sampled (Fig. 3). In two rivers (Big Sandy River and Escalante Creek), these two species were substantially separated in dual isotope plots, whereas in the other three rivers, standard ellipses for these species were directly adjacent but not overlapping (Fig. 3). Flannelmouth suckers were more enriched in nitrogen than bluehead suckers in four of five rivers where both species were sampled, mostly consistent with our expectation that flannelmouth suckers typically feed on a higher trophic level, but with some variation. In one river (Little Sandy Creek), flannelmouth suckers were differentiated from blueheads only in $\delta^{15}\text{N}$, not in $\delta^{13}\text{C}$. In another location (White River), flannelmouth and bluehead suckers were differentiated only in $\delta^{13}\text{C}$, not in $\delta^{15}\text{N}$.

In contrast to the ecological niche differentiation between native flannelmouth and blue-head suckers, white suckers, a widespread introduced species, overlapped in isotopic niche with at least one of the native species in each river (Fig. 3). In four locations (Big Sandy River, Escalante Creek, Little Snake River, and Muddy Creek), white suckers overlapped with flannelmouth suckers in isotopic space, and Little Sandy Creek, white suckers overlapped with bluehead suckers. In Yampa River, white suckers overlapped with both parental species. The variation in overlap between native and introduced species suggests different ecological effects of the white sucker introduction in different locations.

Matching, intermediate, and transgressive isotopic ratios in hybrids

Relative isotopic niches of hybrids and parental species varied, reflecting likely variation in ecological niches as well. In Escalante Creek, bluehead \times white and bluehead \times flannelmouth hybrids had intermediate isotopic ratios between parental species for both carbon and nitrogen (Fig. 3, Fig. 4), and also slightly overlapped with both parental species. In another river (White River), bluehead \times flannelmouth hybrids overlapped with parental species in carbon isotopic ratio, but had extremely variable nitrogen ratios that were sometimes much lower than either parental species. Flannelmouth \times white hybrids overlapped substantially in dual isotope plots with both white and flannelmouth suckers, suggesting that some hybrid individuals might use similar food resources to parental species. However, in each river sampled, a subset of flannelmouth \times white hybrid individuals also had transgressive isotopic ratios (Fig. 4), or isotopic ratios that substantially exceeded the range of either parental species and were not intermediate between parental species. Location of transgressive hybrids relative to parental species varied substantially, suggesting a variety of ecological niches. In Escalante Creek, these transgressive hybrids were more enriched in carbon than parental species, suggesting that they might use a different carbon source, consistent with foraging in a different location. In the Yampa and Little Snake River, transgressive hybrids were more enriched in nitrogen, suggesting that they might have foraged on a higher trophic level. In Big Sandy River, transgressive hybrids had lower carbon isotopic ratios, suggesting foraging on a lower trophic level.

In addition to considering hybrid individuals of each cross as a group with similar attributes, we also treated ancestry as a continuous correlate of isotopic ratios (Fig. S1, Fig. S2). Hybrids between bluehead and white sucker were mostly intermediate genetically and were first-generation hybrids; as seen using estimated standard ellipses (Fig. 4), these hybrids were usually intermediate in isotopic ratio as well. Hybrids between flannelmouth and white sucker represented more of a continuum in ancestry between the two parental species (specifically, more backcrossed hybrids; see also Fig. 2), but no clear patterns of clinal variation in isotopic ratios were evident (Fig. S1, Fig. S2). We explored modeling isotopic ratios as a function of a suite of predictor variables, including differentiation (F_{ST}) between parental species in each river (Table S1), proportional ancestry from each species, ecological differentiation between parental species, and overlap of parental species. However, only four replicate localities had complete data, which was not a sufficient level of replication to build linear models with multiple predictor variables. We therefore tested for relationships between individual predictors and isotopic signature, but no significant pairwise correlations between isotopic signatures and predictor variables were identified. Additional description of these analyses may be found in the Supplement.

Clustering of individuals in isotopic space and ecological niche overlap

Statistically supported clusters of ecologically similar individuals did not correspond to species or hybrid crosses, nor did all individuals of a species fit into the same cluster. Models with 1–3 clusters best fit the isotopic ratio data for individual fish in each river (Fig. S3). The number of statistically supported clusters (1–3) weakly correlated with the number of species (2–5) sampled in each river ($p = 0.046$; Pearson correlation 0.76), but in each location, fewer clusters were identified in isotopic space than there were species present

(Fig. 5A), suggesting that species do not form distinct clusters in isotope space but rather occupy continuous, overlapping isotopic niches. There was no relationship between number of genetic categories (species plus hybrid crosses) and the number of statistically defined clusters in isotope space (Fig. S4), implying that hybrids do not occupy wholly distinct isotopic niche space from parental species. Additionally, with the exception of bluehead suckers in Big Sandy River and Muddy Creek, clusters defined by our model did not correspond to species (Fig. S3). The extent of backcrossing in flannelmouth×white sucker hybridization correlated negatively with the overlap in isotopic niche area in a river; there was less backcrossing of hybrids to parental species in rivers with more ecological overlap between parental species, and more backcrossing in rivers with less ecological overlap between flannelmouth and white suckers (Fig. 5B; $p = 0.026$; Pearson correlation -0.92).

Body condition suggests little variation in ecological success

Body condition of suckers varied among rivers but not in any particularly predictive pattern (Fig. S5, Fig. S6). Using Fulton's condition factor, in some locations white suckers and hybrids had higher body condition than native bluehead and flannelmouth suckers (Fig. S6). Relative body condition of species differed across locations; in some locations (Escalante Creek, White River) body condition for bluehead and flannelmouth suckers were not significantly different, while in one location (Yampa River) flannelmouth suckers had better body condition than bluehead suckers (Welch Two Sample t-test, $p < 0.05$). These results have some caveats, notably that it is difficult to compare Fulton's condition factor across species with slightly different body shapes. We also used relative weight, W_r , to compare body condition in flannelmouth suckers and white suckers, the two species with existing W_r equations, in three locations where adequate numbers of both species were sampled. Estimates of W_r (Fig. S6) contradicted findings using Fulton's condition factor, and showed that flannelmouth suckers had better condition than white suckers in one location (Escalante Creek; Welch Two Sample t-test, $p < 0.05$), but in similar condition in the other two rivers where this both species were present. W_r was similar for flannelmouth suckers and white suckers in the Little Snake River and Yampa River. Taken together, the two metrics of body condition suggest similar ecological success across ancestry classes, with no clear patterns of differential ecological success and no clear implications for the relative fitness of hybrids and parental species. Across all species, W_r and Fulton's condition factor were also positively correlated with $\delta^{13}\text{C}$ (Fig. S7; Pearson correlation = 0.49 and 0.26 respectively, $p < 0.05$ for both). This finding suggests a potential relationship between resource availability and body condition (and by extension fitness), but it would require more detailed environmental data to gain a mechanistic understanding.

Discussion

Relative ecological niche of parental species and hybrid suckers varied across locations, suggesting that ecological outcomes of hybridization varied geographically. Within each genetic category, there was also substantial variation among individuals, especially in hybrids. Importantly, hybrids did not conform neatly to our hypotheses about intermediate versus matching phenotypes relative to parental species (Fig. 1). Transgressive isotopic ratios were common in flannelmouth × white hybrids (Fig. 4). The parental species had

similar isotopic niches in most rivers, but flannelmouthxwhite hybrids were transgressive in different directions in different locations. In contrast, hybrids between parental species with divergent phenotypes did tend to be more intermediate, but relative isotopic niche and degree of variation among hybrids varied geographically (Fig. 4). It is likely that hybridization leads to ecological displacement, and that availability of ecological niches could constrain hybridization under some circumstances. We suggest that there is unlikely to be a consistent ecological outcome of hybridization for the same species pair or suite of species hybridizing in multiple locations. To truly understand ecological effects and environmental dependence of hybridization, we will need to prioritize studies that address both ecological and genomic outcomes of hybridization across locations, including quantifying relative fitness of parental species and hybrids.

Native species did not overlap in isotopic niche, but introduced species and hybrids overlapped substantially

Overlap among species in isotopic space varied across rivers, as did relative positions in isotopic space. However, one consistent trend was that bluehead and flannelmouth suckers, the two native parental species, did not overlap substantially in isotopic space (Fig. 3). In three of five rivers with >1 statistically supported cluster, individuals from these two species were also largely but not entirely assigned to different clusters in our hierarchical Bayesian clustering algorithm (Fig. S3). These results suggest that bluehead and flannelmouth sucker diets generally did not substantially overlap where the species co-occurred. Both species are historically native to the study rivers, and it is possible that non-overlapping isotopic niches (and diets) have evolved to avoid competition, consistent with ecological character displacement in response to competition (Connell 1980, Robinson and Wilson 1994), which can contribute to evolutionary origins and maintenance of species diversity (Schluter 2000, Seehausen 2007).

In contrast, introduced white suckers overlapped in isotopic niche space with flannelmouth, bluehead, or with both species, suggesting some degree of shared resource use between white sucker and one or both native species in each river. Overlap of white suckers with bluehead suckers was unexpected because we anticipated white suckers would be more similar ecologically to flannelmouth than to bluehead suckers, due to mouth morphology (Fig. 1) and previous work showing that white suckers preferentially eat invertebrates rather than algae (Baxter et al. 1995, Cross et al. 2013, Walsworth et al. 2013). These results suggest that different native species might face competition from introduced white suckers in different rivers. Variable position of white suckers relative to the other species in this study also suggests that the resource use of white suckers might be flexible or opportunistic (as in Corse et al. 2009). If this pattern were true, then ecological flexibility and generalist traits of white suckers might have facilitated successful invasion of the Upper Colorado River basin, and might explain to some extent the success of hybrid individuals with white sucker ancestry.

Intermediate, matching, and transgressive phenotypes in hybrids

Hybrids also occupied different isotopic niches relative to parental species in different locations (Fig. 3). Hybridization of bluehead suckers with both flannelmouth and white

suckers produced hybrids that were on average intermediate in isotopic ratios relative to parental species, as anticipated based on predictions of intermediate mouth morphology, although there was substantial variation across individuals (Fig. 1, Fig. 4). Some individual bluehead \times white and bluehead \times flannelmouth sucker hybrids also matched or were more similar to parental phenotypes in isotopic space, suggesting ecological similarity to parental species, consistent with the idea that in some hybrid crosses, traits of one parental species are dominant even in F1 hybrids (Thompson et al. 2021). Bluehead \times white and bluehead \times flannelmouth hybrids typically have intermediate mouth morphology, including a reduced scraping ridge (Hubbs et al. 1943, Hubbs 1955, Quist et al. 2009), and these traits likely drive intermediacy in diet, similar other fish species. Both stickleback and centrarchid fish hybrids use intermediate food sources when they have intermediate feeding morphology (Arnegard et al. 2014, McGee et al. 2015), with variable ecological success depending on the quantity and quality of food resources available. A parental-like feeding phenotype might be advantageous to hybrid individuals if parental phenotypes are optimal for feeding on available resources (as in Arnegard et al. 2014), but also might lead to competition between the parental species and their hybrid offspring if preferred resources are limited (as in Seiler and Keeley 2009). If overlap in isotopic space does correspond to consumption of similar food resources in these rivers, competition between hybrids and parental species might occur. Competition might therefore be a serious concern for conservation of native *Catostomus* species in locations where hybridization is ongoing and resources are limited. We currently do not know to what extent fish production in these rivers is food limited (sensu Cross et al. 2013), so we cannot infer how strong selective pressures associated with competition are, but matching ecological phenotypes suggests that competition is possible.

We observed transgressive isotopic ratios in a subset of flannelmouth \times white hybrid individuals (Fig. 4). These individuals exceeded values for any other *Catostomus* species in these rivers, including parental species of the hybrids, potentially pointing to novel food or habitat use. Hybrids were transgressive in different directions in different rivers (Fig. 4). Without additional data on stomach contents or isotopic baselines, we cannot pinpoint specific causes for these transgressive isotopic values, but we highlight some potential explanations. Hybrids with transgressive $\delta^{15}\text{N}$ probably feed on a higher trophic level, potentially eating more invertebrates than flannelmouth or white suckers, or consuming some other resource like larval fish. Transgressive values for $\delta^{15}\text{N}$ could also correspond to use of a different portion of the river (e.g., a deep pool where denitrification is occurring), but nitrogen isotope values are more commonly interpreted as a rough indicator of trophic position (Post 2002). Transgressive values for $\delta^{13}\text{C}$ likely correspond to use of different carbon resources, potentially in a different part of the river (Finlay 2001), consistent with possible divergence in habitat use. One caveat associated with interpretation of $\delta^{13}\text{C}$ separation in isotope space more generally, however, is that habitat heterogeneity is required for divergence in $\delta^{13}\text{C}$ in association with different foraging behavior to emerge. More geomorphic and hydraulic data would be required to better assess how habitat heterogeneity would shape isotopic ratios, and to understand trends in $\delta^{13}\text{C}$ across rivers.

Novel phenotypes and resource use in hybrids are well-documented in other organisms (e.g. sunflowers, butterflies, and cichlids; Rieseberg et al. 1999, Gompert et al. 2006, Stelkens and Seehausen 2009), and have promoted evolutionary diversification in diverse groups

of organisms (e.g. Rieseberg et al. 2003, Marques et al. 2019, Meier et al. 2019). Some transgressive hybrids might be better able to use novel resources than parental species, and might therefore be better poised to take advantage of ecological opportunity (Selz and Seehausen 2019). In cichlid fishes, likelihood of transgressive phenotypes is correlated with evolutionary distance (Stelkens et al. 2009) in some cases, but in contrast, trophic morphology of hybrids can be more transgressive in closely related hybridizing species pairs (Holzman and Hulsey 2017). In *Catostomus* fishes the transgressive hybrids resulted from hybridization between closely related and ecologically similar taxa (flannelmouth and white suckers), while more evolutionarily distant crosses (bluehead \times white and bluehead \times flannelmouth) produced intermediate or matching hybrids. Transgressive phenotypes in hybrids between closely related and ecologically similar species (flannelmouth \times white) suggest that ecological competition and character displacement could help drive this pattern (Connell 1980, Robinson and Wilson 1994).

Food web effects of hybridization

Ecological outcomes of hybridization varied geographically, but some trends were apparent from our comparison of ecological and genomic outcomes of hybridization. In locations where flannelmouth and white sucker (the two most widespread parental species), overlapped more in isotopic space, there were fewer backcrossed hybrids and hybridization was mostly constrained to first generation hybrids (Fig. 5B). This pattern might reflect filtering of hybridization outcomes by environmental conditions and available niche space, if backcrossed hybrids are unable to compete in locations where parental species have greater overlap and therefore those hybrids do not survive to be sampled as adults. However, the causality could run the other direction: hybridization might entirely reshape food webs, promoting greater niche overlap. Non-native species commonly alter food web dynamics in freshwater fish communities, including isotopic niche (Sagouis et al. 2015, Rogosch and Olden 2020), but the role of hybrids is less well documented. One study of *Catostomus* ecology that used stable isotopes, Walsworth et al. (2013), showed that introduction of white suckers and other non-native species into tributaries of the Upper Colorado River basin could increase food chain length and increase niche crowding, consistent with our findings here. However, Walsworth et al. 2013 excluded hybrids, which make up a large fraction of the *Catostomus* fish present in many parts of the Upper Colorado River basin (Gill et al. 2007). In our study, although the number of identified distinct clusters in isotopic space was correlated with number of parental species (Fig. 5A), the same relationship did not hold for number of clusters and number of ancestry classes, i.e., parental species plus hybrid crosses. This finding suggests that hybrids were not likely to be accessing different resources or occupying distinct available niches, except perhaps in the case of individuals with transgressive phenotypes, but rather were overlapping with ecological resource use of parental species, with the potential for competition.

It is unclear what fitness consequences these interactions between parental species and hybrids might have. Attempts to use body condition as a measure of ecological success and proxy for fitness produced equivocal results, because different measures of body condition produced opposing patterns (Fig. S5, Fig. S6). These contradictory results illustrate some of the difficulties associated with estimating body condition (Peig and Green 2010), and

suggest caution is warranted in attaching too much weight to body condition as a component of fitness. However, based on the lack of clear signal in our two metrics of body condition, it is likely that hybrids and parental species are in similar body condition, indicating that no particular species or hybrid cross is limited by fit to ecological resources.

More empirical studies of fitness in hybrid individuals, including *Catostomus* hybrids, will be needed to develop a mechanistic understanding of hybridization. Clarifying the fitness of hybrids is essential because relative fitness of parental species and hybrids has a profound effect on how hybridization influences the evolution of species (reviewed in Arnold and Hodges 1995, Arnold et al. 2012). In some instances of interspecific hybridization, hybrids survive at higher rates than either parental species (as in tiger salamanders, Fitzpatrick and Shaffer 2007). However, it is important to distinguish between the well-being of individuals—i.e., survival, growth, and foraging success—and reproductive success. Survival and growth cannot be equated with fitness if hybrids cannot reproduce, and there are many examples of hybrid sterility (e.g. Sweigart et al. 2006, Good et al. 2008), but also of viable, fertile offspring between quite divergent lineages. Generally, viability and fertility of hybrids is negatively correlated with genetic divergence (Bolnick and Near 2005, Stelkens et al. 2010), with additional genomic incompatibilities more likely with increased evolutionary distance (Moran et al. 2021). If genomic incompatibilities make some hybrid crosses inviable, fitness related to ecological success might be less important for these species pairs. There are frequently fitness differences between first-generation hybrids and later generation hybrids (i.e. heterosis in F_1 hybrids). For example, in native westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) and introduced rainbow trout (*Oncorhynchus mykiss*) hybrids, F_1 hybrids had relatively high fitness, including high reproductive success, but later-generation hybrids had markedly lower fitness (Muhlfeld et al. 2009). Studies of fitness in wild populations are logistically challenging, and have not yet been completed in *Catostomus* suckers, but would substantially improve our understanding of the causes and consequences of interspecific hybridization.

Conservation implications

Bluehead and flannelmouth suckers remain the target of extensive management and conservation efforts (Gelwicks et al. 2009, Senecal et al. 2010). Previous work on genomic outcomes of hybridization suggested that hybridization might act as a demographic sink due to reduced conspecific reproduction and apparent absence of hybrids beyond the F_1 generation in some crosses (Mandeville et al. 2015, 2017). We can also now infer some potential ecological effects of hybridization. Hybrids and non-native parental species overlapped substantially in isotopic space with native parental species, which suggests that hybrids and introduced species occupied similar ecological niches to native species, and therefore might compete for resources. Additionally, in some rivers hybrids and non-native species had higher body condition than native species by one metric, suggesting that hybrids and introduced species might sometimes exploit resources better than parental species. When combined, these lines of evidence suggest additional potential obstacles to conservation of native species: hybrids and introduced species are likely to compete with native species, but hybrids do not contribute substantially to the gene pool. Thus, hybridization might sometimes be both a demographic sink and a resource sink. However,

the variability uncovered by both this study and genetic studies of hybridization suggest that these dynamics might vary substantially by river, making outcomes of hybridization more complicated to predict. If we had studied these ecological dynamics in a single river, our conclusions would have been incorrect; it is critical to recognize that ecological outcomes of hybridization, like genomic outcomes, may vary substantially and might require different conservation strategies in different locations.

Conclusion

In this study, we showed that ecological niches occupied by hybrid individuals varied within and across rivers. Across multiple locations, hybrids of similar ancestry had variable feeding ecology, either matching parental species isotopic ratios, intermediate between parental species, or displaying a transgressive isotopic ratio beyond the range of either parental species. This variation might have arisen due to greater plasticity in hybrid feeding phenotypes, or might suggest that postzygotic selection constrained hybrids to the range of phenotypes that would be ecologically successful in a given location. Additionally, differing genetic composition of hybrids, either due to selection on recombinant hybrid genotypes or associated with locus-specific genotypes at functionally important loci, could contribute to variation in ecological outcomes of hybridization. We also suggest that overall food web structure might either influence hybridization, or be altered by the presence of hybrids and non-native species. Together, these results make a compelling case for more study of how ecological interactions can constrain or facilitate hybridization, and how hybridization might alter ecological function of communities and modify fitness landscapes for hybridizing species.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data accessibility

Isotope data and genetic summary statistics presented in this paper are available on Dryad at <https://doi.org/10.5061/dryad.0cfxpnw1f>. R scripts used in analysis are available on

Zenodo at <https://doi.org/10.5281/zenodo.6958091>. Genetic data associated with this paper are archived in connection with the publication of Mandeville et al. 2017.

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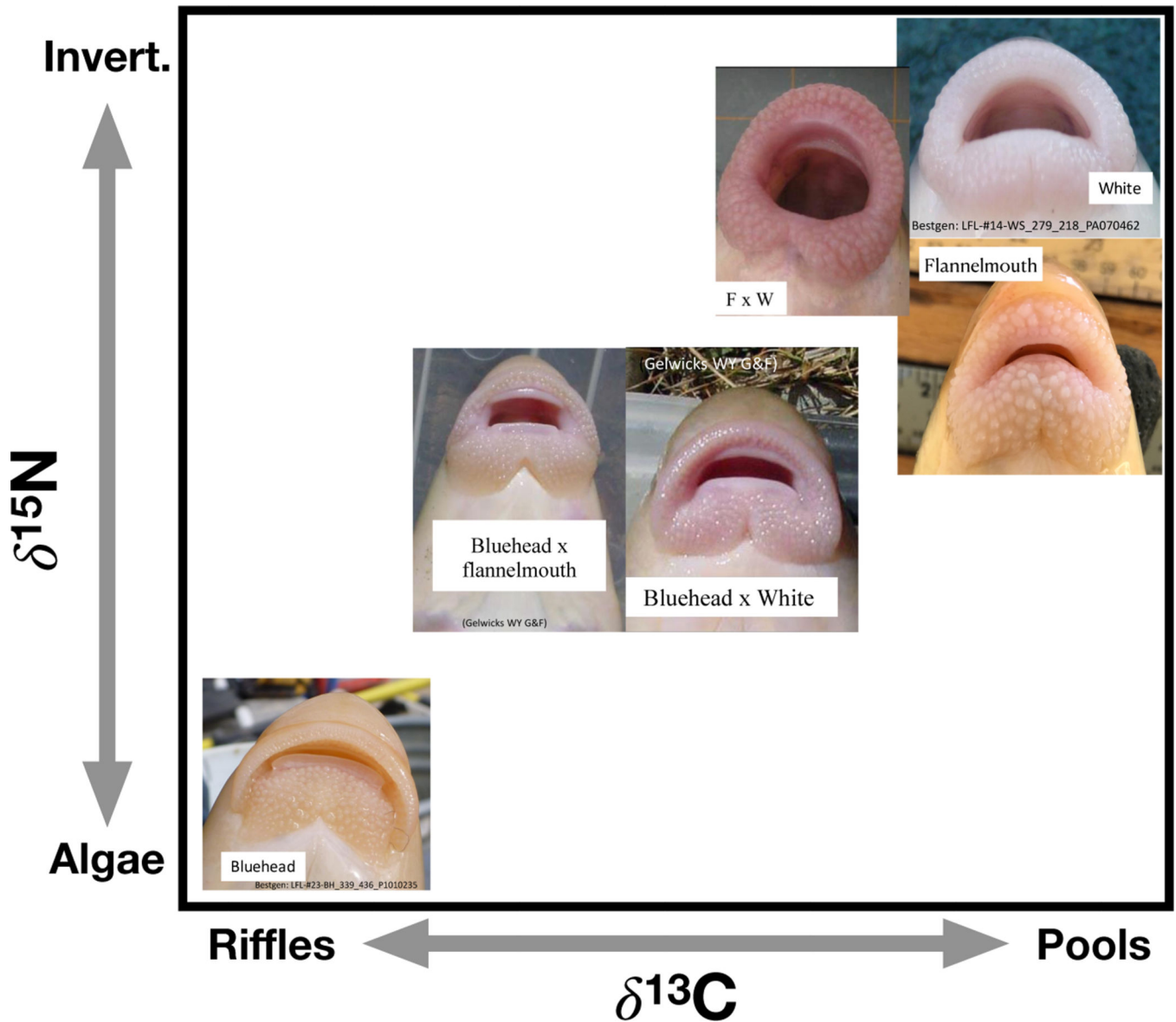


Figure 1:

Hypothesized distribution of the three most widely sampled *Catostomus* fish species and their hybrids in isotopic space. Previous studies and mouth morphology suggested that bluehead suckers eat more algae, and feed primarily in riffles, leading to lower $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Flannelmouth and white suckers are expected to eat more invertebrates and in pools, leading to higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Due to morphological intermediacy, bluehead×flannelmouth and bluehead × white hybrids were expected to be dietarily intermediate between parental species. Flannelmouth × white hybrids were expected to match the dietary phenotypes of their parental species. Mountain and longnose sucker were excluded from this figure because each species (and a small number of hybrids) were found in only one river each. Based on mouth morphology, mountain suckers are expected to be similar to bluehead suckers, while longnose would likely be more similar to flannelmouth and white suckers.

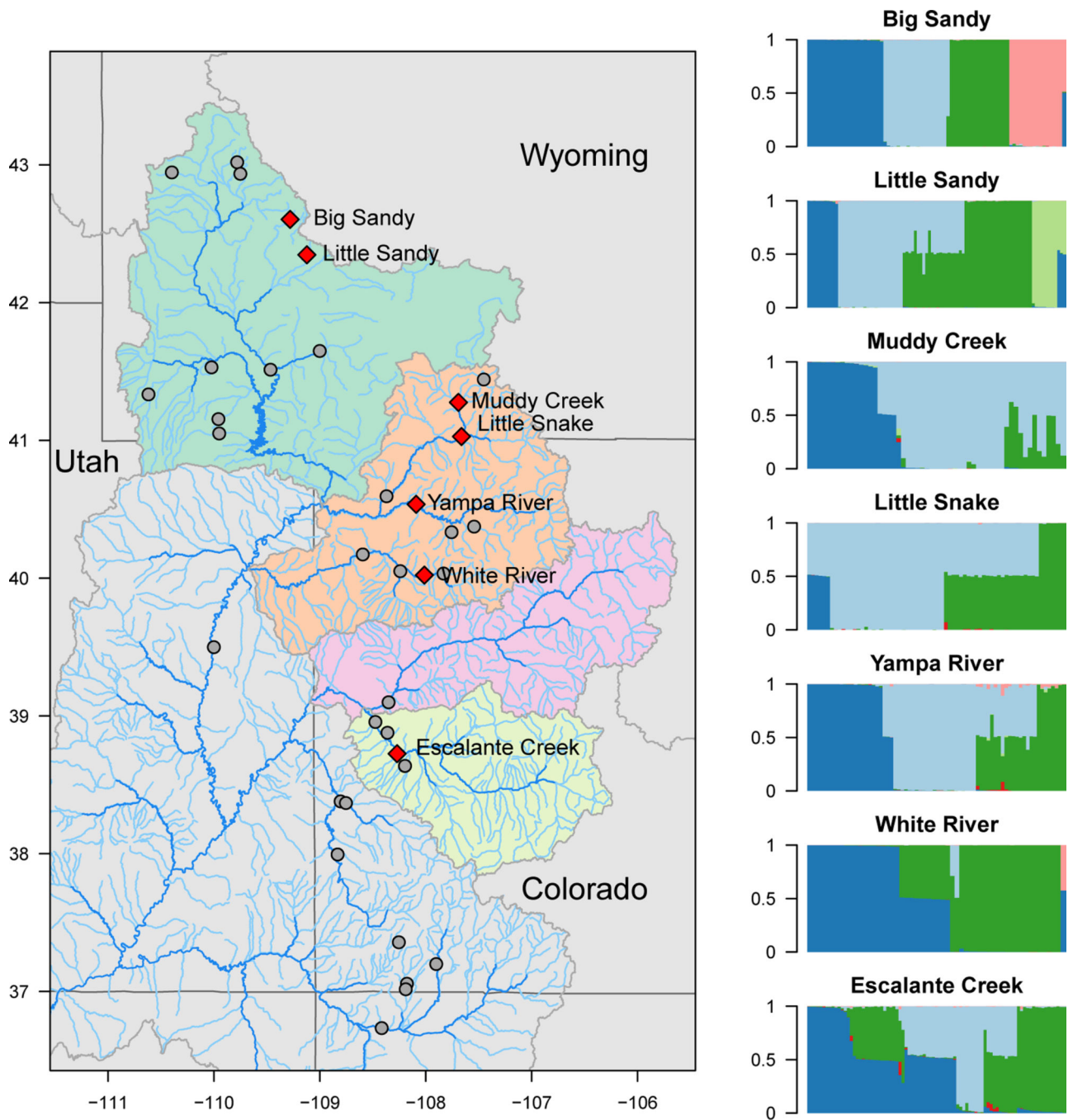


Figure 2:

Seven rivers in Wyoming and Colorado and corresponding genetics outcomes of hybridization. Gray dots on the map at left represent all sampling points from Mandeville et al. (2017); the focal rivers for this study are shown with red diamonds. Barplots at right show proportional genetic ancestry of individual fish used for stable isotope analysis (dark blue = bluehead sucker; light blue = white sucker; dark green = flannelmouth sucker; pink = longnose sucker; light green = mountain sucker). A range of hybrids between *Catostomus* species were present (Mandeville et al. 2017). The most common crosses were

flannelmouth×white (closely related, F1 and backcrossed hybrids), and bluehead×white and bluehead×flannelmouth (more distantly related; mostly F1 hybrids).

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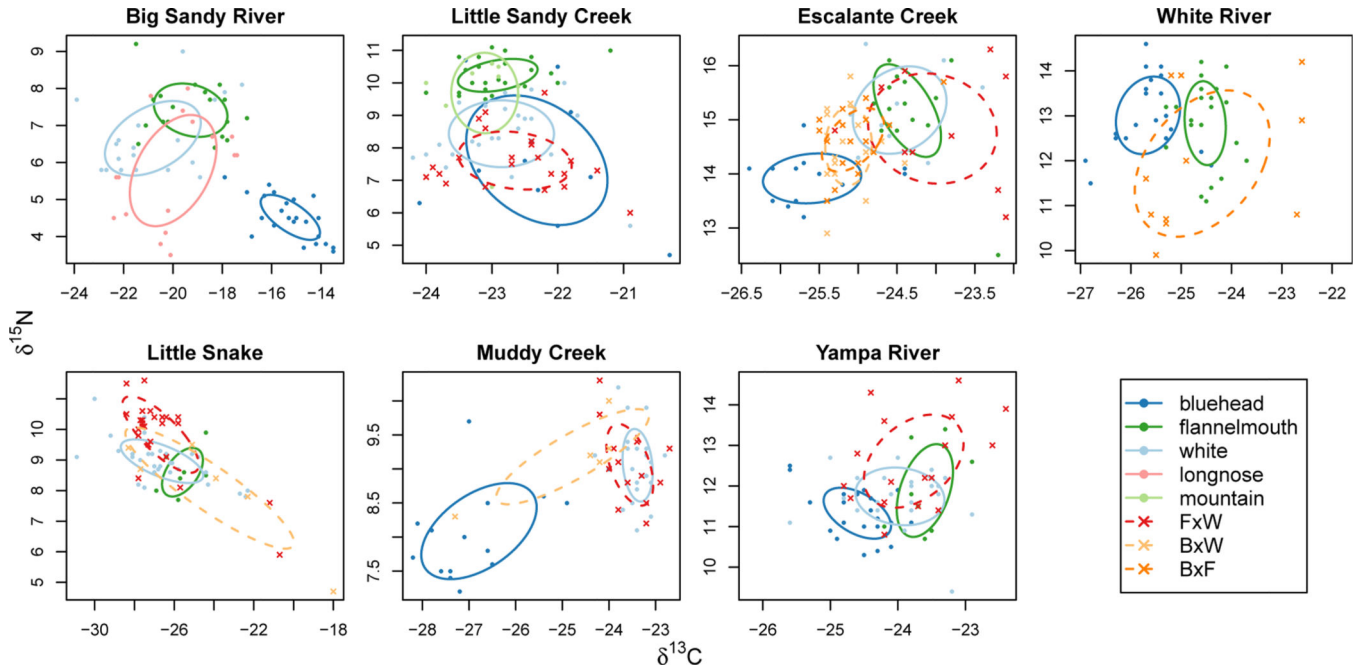


Figure 3:

Standard ellipses summarize stable isotopic ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$; plots oriented the same as Fig. 1) by encompassing approximately 40% of individual fish within each of five species and three hybrid crosses. Ellipses were estimated using a maximum likelihood and a bivariate normal distribution, implemented in SIAR (Jackson et al. 2011). Species ellipses are solid lines; hybrid ellipses are dashed lines. Ecological relationships between parental species varied across rivers, but the two native parental species (bluehead and flannelmouth) did not overlap in isotopic space in any sampled locations. Hybrids among these three parental species had matching, intermediate, or transgressive isotopic ratios relative to parental species, and are shown more clearly in Fig. 4.

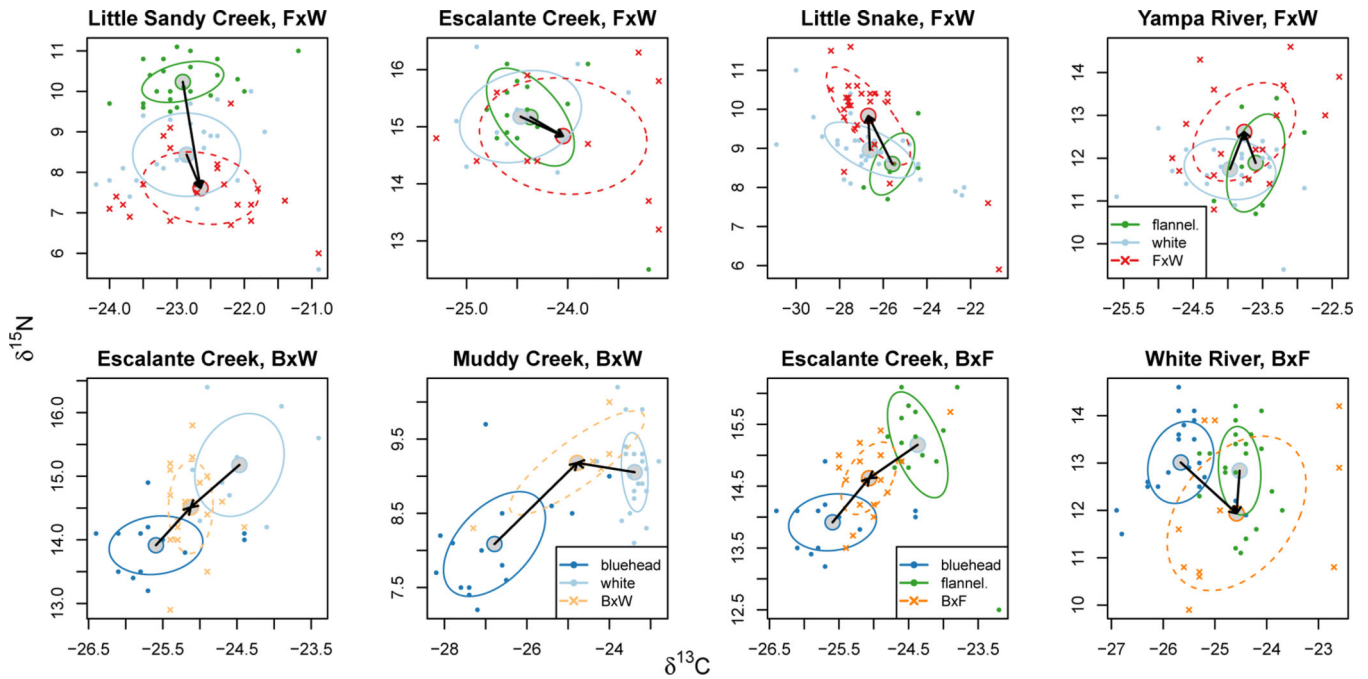


Figure 4:

Subset plots show pairwise relationships of parental species and hybrids. Means for genetically-defined species and hybrid crosses are shown with gray points; individual fish are denoted by points color-coded by species or hybrid cross. Flannelmouth \times white sucker hybrids (top row) displayed matching and transgressive isotopic ratios relative to parental species, as shown by arrows from the mean values for parental species to the mean value for hybrids. Bluehead \times white sucker hybrids (bottom row, left) were intermediate between isotopic values for the two parental species. Bluehead \times flannelmouth sucker hybrids (bottom row, right) were intermediate between values for the two parental species in Escalante Creek, but individuals were a mixture of matching and transgressive relative to parental species in the White River.

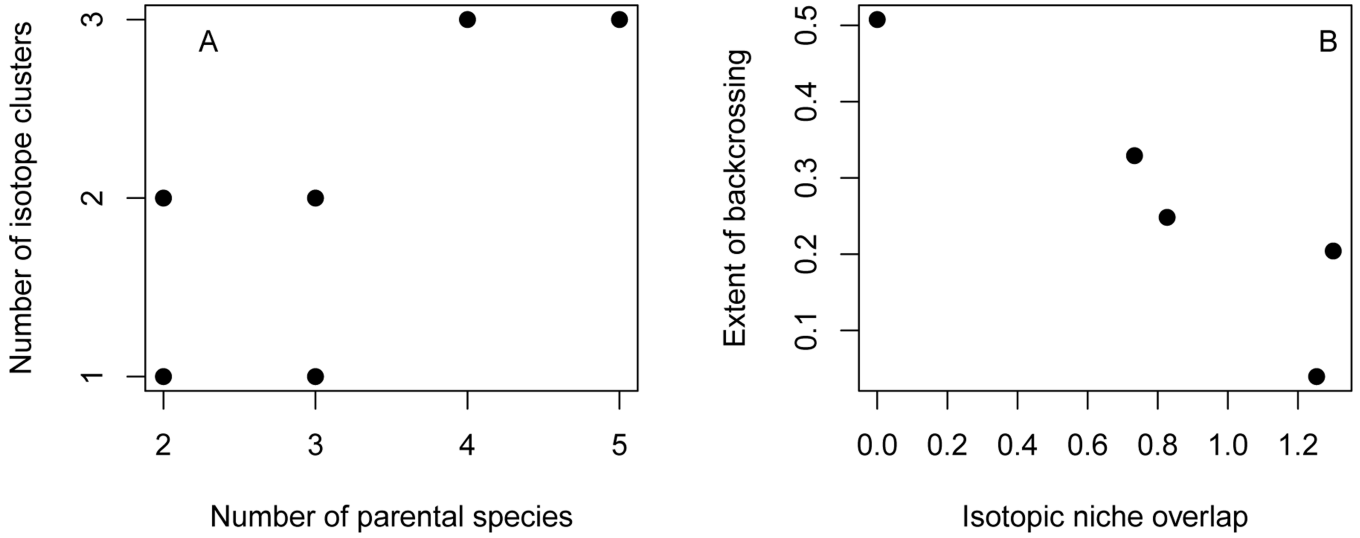


Figure 5:
A) Number of clusters of ecologically similar individuals (identified by a hierarchical Bayesian model) correlated with the number of parental species sampled in a river (Pearson correlation 0.764, $p < 0.05$). B) Extent of backcrossing in flannelmouth×white hybrids negatively correlated with isotopic niche area overlap in 6 river locations (Pearson correlation -0.922 , $p < 0.05$).

Table 1:

Fish were sampled from seven rivers in the Upper Colorado River basin. Individuals sampled included parental species and hybrids, abbreviated in the table below. (B = blue-head, F = flannelmouth, W = white. Hybrids are represented with a × connecting parental species, i.e. flannelmouth×white = F×W).

Location	Drainage	Individuals	Species	Hybrids (> 3 ind)
Big Sandy River (WY)	Green River	61	B, F, W	-
Little Sandy Creek (WY)	Green River	80	B, F, W	F×W
Escalante Creek (CO)	Gunnison	85	B, F, W	B×F, B×W, F×W
White River (CO)	White	55	B, F	B×F
Little Snake River (WY)	Yampa	68	F, W	B×W, F×W
Yampa River (CO)	Yampa	72	B, F, W	B×W, F×W
Muddy Creek (WY)	Yampa	58	B, W	B×W, F×W