Ecological outcomes of hybridization vary extensively in *Catostomus* fishes

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1 Abstract

Hybridization has been studied extensively to learn about speciation and mechanisms of reproductive isolation, but increasingly we recognize that hybridization outcomes vary geographically and can depend on the environment. At the same time, hybridization can reshape biotic interactions in an ecosystem, leading to ecological shifts where hybridization occurs. Identifying how hybrid individuals function ecologically would link evolutionary outcomes of hybridization to ecological consequences, but relatively few studies have focused on ecological traits of hybrids. We described the feeding ecology of several *Catostomus* fish species and their hybrids by using stable isotopes (δ^{13}C and δ^{15}N) as a proxy for diet and habitat use, and compared two native species, an introduced species, and three interspecific hybrid crosses. We replicated this comparison spatially, including hybrids and parental species from seven rivers in the Upper Colorado River basin where parental species co-occur and the opportunity for hybridization exists. Although relationships between native species in isotopic space varied, individual native species did not fully overlap in isotopic space in any river sampled, suggesting little overlap of resource use between these historically sympatric species. The introduced species overlapped with one or both native species in every river, suggesting similar resource use and potential for competition. Hybrids occupied intermediate, matching, or more extreme (transgressive) isotopic space relative to parental species, and were isotopically variable within and among rivers. We suggest that ecological outcomes of hybridization, like genomic outcomes of hybridization, are likely to vary across locations where a pair of species hybridizes. This variation implies that hybridization might have large unpredictable, idiosyncratic ecological effects on fish assemblages where hybrids occur. Although we found little evidence that hybrids are at a disadvantage ecologically—there were no significant declines in body condition relative to parental species—it is nevertheless possible that abiotic or biotic attributes of a river might constrain the range of interspecific hybrids that are successful, thus contributing to variation in hybridization outcomes across rivers.

Introduction

Hybridization is a critical feature of many speciation processes and serves as a test of repro-
ductive isolation between diverged lineages in secondary contact (Barton and Hewitt 1989,
Harrison 1990). Studies of hybridization are motivated in part by a desire for generality
in our understanding of how biodiversity arises and is maintained. While the increasing
accessibility of large genomic datasets for wild populations has led to a newfound ability to
study replicate outcomes of hybridization in the wild (Narum et al. 2013, McFarlane and
Pemberton 2019), better information in some cases leads to a more nuanced and compli-
cated understanding of evolution, and generates additional questions (Payseur and Rieseberg
2016). Studies of hybridization in multiple locations have uncovered substantial variation
in genomic outcomes of hybridization when a pair of species comes into contact repeatedly
(Nolte et al. 2009, Lepais and Gerber 2011, Haselhorst and Buerkle 2013, Lagache et al. 2013,
Mandeville et al. 2017), and it seems increasingly likely that ecological context, historical
contingency, and stochasticity influence to what extent species remain reproductively iso-
lated from their close relatives. Variable outcomes across replicate instances of hybridization
between the same pair of species suggest not only variation within a species in mechanisms
of reproductive isolation (Cutter 2012, Mandeville et al. 2017), but also variable porosity
of boundaries to gene flow between species. Identifying the degree of ecological dependency
and contingency in hybridization outcomes is therefore essential to understanding speciation
and the persistence of biodiversity.

Mechanisms generating variation in hybridization outcomes remain unidentified in most
hybridizing species pairs (Gompert et al. 2017). Ecological interactions and environmental
conditions likely contribute significantly, and indeed, empirical examples of environmental
dependence are known (e.g. Taylor and Donald McPhail 2000, Young et al. 2016, Muhlfeld
et al. 2017, Mandeville et al. 2019). However, our understanding of the ecological context
and consequences of hybridization is still largely incomplete, which is unfortunate, because

54 ecological context can influence outcomes of hybridization both by affecting relative fitness

55 of hybrid and parental individuals (e.g. Arnold et al. 2012) and by limiting or increasing

56 the opportunities for hybridization (e.g. Lepais and Gerber 2011). Fitness and ecological

57 success of hybrids might also vary across independent instances of hybridization, with some

58 phenotypes and genomic compositions of hybrids being favored in a subset of locations, and

59 alternative traits and genotypes being favored in others. Differential ecological success of

60 hybrids across replicate hybrid zones could selectively filter which genotypes persist (Lindtke

61 et al. 2014), and might contribute to variation in hybridization outcomes across locations.

62 However, few empirical studies have described both variation in hybridization outcomes and

63 the ecological conditions that have either resulted from or shaped these outcomes.

64 In fish, ecological success of interspecific hybrids can depend on environmental conditions

65 (e.g. in sticklebacks; Hatfield and Schluter 1999). The presence of hybrids can alter species

66 interactions so growth, survival rate, or reproductive success of parental species is changed

67 where hybridization occurs (as in trout; Rosenfield et al. 2004, Seiler and Keeley 2009). Phe-

68 notypes related to foraging ability can be especially important in determining outcomes of

69 interactions between traits of hybrids and the environment. Experimental work in fish has

70 shown that hybrids are sometimes less successful than parental species when they have inter-

71 mediate mouth morphology, because of an ecological mismatch between feeding morphology

72 and available resources, leading to worse feeding performance (e.g. sunfish; McGee et al.

73 2015) or reduced growth (e.g. sticklebacks; Arnegard et al. 2014). However, phenotypes

74 of hybrids are hard to predict, and are not always intermediate between parental species

75 (Rieseberg et al. 1999*b*, Stelkens et al. 2009). Instead, phenotypes of hybrid individuals

76 might be similar to one of the parental species, or might differ from both parental species

77 (e.g., Stelkens et al. 2009, Thompson et al. 2019).

78 Our goal for this study was to compare ecological interactions between multiple *Catosto-*

79 *mus* fish species and their hybrid progeny across several rivers in Colorado and Wyoming.

80 The focal *Catostomus* species are part of a clade with extensive and highly variable interspe-

81 cific hybridization between multiple species pairs (Hubbs et al. 1943, McDonald et al. 2008,

82 Mandeville et al. 2015, 2017). We used stable isotopes of carbon and nitrogen as a proxy

83 for diet and spatial resource use, and assessed patterns of ecological overlap first between

84 parental species, and then between parental species and hybrid individuals. We compared

85 patterns of ecological overlap across multiple geographic locations locations where fish were

86 sampled, and analyzed the relationship between these ecological overlaps and genomic out-

87 comes of hybridization in these rivers.

88 Genomic outcomes of hybridization in *Catostomus* can vary substantially over the ge-

89 ographic range in which a pair of species co-occurs (Mandeville et al. 2015, 2017). While

90 some hybridization between *Catostomus* species is precipitated by human-caused species

91 introductions, most notably of *C. commersonii* (white sucker), this group of fishes likely

92 has a long and convoluted history of hybridization (Hubbs et al. 1943, Hubbs 1955, Bangs

93 et al. 2018), including a hypothesized allopolyploid origin (Ferris 1984). Contemporary hy-

94 bridization between six geographically overlapping *Catostomus* species is extensive; several

95 species have hybridized with more than one congener, resulting in at least 12 different hy-

96 brid crosses (Mandeville et al. 2017). In some locations, few crosses were present despite

97 sympatry of multiple parental species; in other locations, many different crosses involving

98 the sympatric parental species occurred. Backcrossed hybrids and later-generation recom-

99 binant hybrids were common in some locations, while other locations contained only first

100 generation hybrids (Mandeville et al. 2015, 2017). Mechanisms driving this variation in hy-

101 bridization outcomes remain unidentified. Environmental variation that affects opportunity

102 for hybridization or intrinsic genetic variation in reproductive isolation could explain the

103 variable hybridization outcomes that we observed in natural populations (Mandeville et al.

104 2017). Another possibility, which we explore in this study, is that geographic variation in the

105 ecological success and survival of hybrids might cause variation in hybridization outcomes.

106 One focal species, bluehead sucker (*Catostomus discobolus*), eats predominantly algae

107 that grow in fast-moving, shallow portions of rivers (i.e., riffles), while the other two widespread

parental species, flannelmouth and white suckers (*C. latipinnis* and *C. commersonii*), 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. *C. discobolus* and *C. platyrhynchus* have a distinctive "scraping ridge" that facilitates scraping algal biofilms from surfaces (Baxter et al. 1995). In contrast, the other species have no scraping ridge, but have fleshy lips in varying shapes and with different textures. Differences in diet and foraging location among species and hybrid crosses can be captured using stable isotopes. Stable isotopes of nitrogen (δ^{15}N) serve as a proxy for trophic position, and would therefore capture the difference between consuming algae and invertebrates; we expect higher values of δ^{15}N for individuals consuming more invertebrates, because they are feeding on a higher trophic level (Post 2002). Stable isotopes of carbon (δ^{13}C) capture differences in carbon composition in diet, which can derive 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 (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. Separation of isotopic niches, as detected by stable isotope analysis, might be a key variable in persistence of closely related species, as in other fish species (e.g. sauger and walleye; Butt et al. 2017). We used stable isotope data to identify ecological overlaps between genetically-defined groups (Layman et al. 2007, Newsome et al. 2007). We compared ecological success of hybrids and parental species using body condition as a proxy for fitness and described the success of parental and hybrid fish in the context of their isotopic niche and overlap with other species or hybrids.

Methods

We combined stable isotope and genetic analyses of 506 individual fish to identify how ancestry of *Catostomus* suckers is related to ecological niche. Sampling spanned seven different locations in the Upper Colorado River basin in Wyoming and Colorado (Table 1). Fisheries biologists from state agencies collected fin tissue from each individual fish. Fin tissue was used for both genetic analyses (Mandeville et al. 2015, 2017) and stable isotope analyses (this study). Locations for isotope analysis were chosen to include rivers with a range of hybridization outcomes, based on the results of Mandeville et al. (2017).

We included individuals from five *Catostomus* fish species, *C. discobolus* (bluehead sucker), *C. latipinnis* (flannelmouth sucker), *C. commersoni* (white sucker), *C. catostomus* (longnose sucker), and *C. platyrhynchus* (mountain sucker). Three focal species—bluehead, flannelmouth, and white suckers—are geographically widespread in the Upper Colorado River basin and were present at most sampling sites, whereas longnose and mountain suckers were sampled in only one location each, but were included because they are part of food web interactions where present. Of the five species included in this study, bluehead, flannelmouth, and mountain suckers are native to the Upper Colorado River basin, and white and longnose suckers were introduced in the Upper Colorado River basin (Baxter et al. 1995). It is unknown exactly when and how white and longnose suckers were introduced, but the introduction probably occurred in the past 100–150 years (Gelwicks et al. 2009, Senecal et al. 2010). Recent work has focused on hybridization between the introduced white sucker and native bluehead and flannelmouth suckers (McDonald et al. 2008, Mandeville et al. 2015), but hybridization also sometimes occurs between the common native *Catostomus* species, bluehead and flannelmouth sucker (Hubbs et al. 1943, Mandeville et al. 2017). Additionally, in some locations mountain and longnose suckers hybridize with the more common species. Hybridization produces spatially heterogeneous outcomes, and different hybrids were sampled in different rivers, with variable amounts of backcrossing to parental species (Mandeville

157 et al. 2015, 2017, Fig. 1).

158 We used results of genomic analyses from Mandeville et al. (2017) to identify ancestry of
159 individuals (Fig. 1). To briefly summarize, genomic analyses of hybridization for the individ-
160 uals in this study used genotyping-by-sequencing data (Parchman et al. 2012). Specifically,
161 we relied on results from a genetic clustering algorithm, `entropy` (Gompert et al. 2014, Shas-
162 try et al. 2021), based on 11,221 single nucleotide polymorphisms (SNPs) to identify parental
163 species and hybrids genetically. This analysis produced precise estimates of each individ-
164 ual fish's ancestry, including proportion of ancestry from each parental species in hybrids.
165 Estimates of interspecific ancestry from `entropy` also revealed extent of hybridization and
166 backcrossing, so we know that a substantial proportion of hybrids in this study were F_1 (first
167 generation) hybrids or BC_1 (backcrosses resulting from mating between an F_1 hybrid and a
168 parental individual). We used both categorical designations of ancestry (species or hybrid
169 cross) and a continuously valued measure of ancestry (proportion of ancestry) as covariates
170 for stable isotopic ratios and body condition of hybrids in this study. More extensive discus-
171 sion of genomic outcomes of hybridization in *Catostomus* fishes can be found in Mandeville
172 et al. (2017).

173 To obtain stable isotope data, we prepared samples of fin tissue for analysis of $\delta^{13}C$ and
174 $\delta^{15}N$ by first desiccating them in a drying oven (60 °C) for 2–3 days. Dried fin samples were
175 analyzed the University of Wyoming Stable Isotope Facility (UW-SIF). UW-SIF ground
176 dried fin into a powder, and then transferred an appropriate mass of sample into a tin.
177 We then measured $\delta^{13}C$ and $\delta^{15}N$ ratios using a Finnigan DeltaPlus XP mass spectrometer
178 connected to a Costech 4010 elemental analyzer. UW-SIF normalized values using known
179 laboratory standards (keratin), and performed quality control on all data.

180 For each species or hybrid cross in each river, as defined by estimates of genetic ancestry
181 (Mandeville et al. 2017), we estimated an ellipse in dual isotopic space to quantify the relative
182 position of $\delta^{13}C$ and $\delta^{15}N$ values and variation in those values within a group (Layman et al.
183 2007). We used the maximum likelihood `standard.ellipse` function in the `siar` package in

184 R to fit the standard ellipses (Parnell et al. 2010, Jackson et al. 2011). This model assumes

185 that bivariate isotopic data (isotopic ratios of δ^{13}C and δ^{15}N) for a group of individuals

186 is best fit by a multivariate normal distribution, and uses sample means and a covariance

187 matrix to estimate parameters of a standard ellipse containing approximately 40% of the

188 data (Jackson et al. 2011). To account for potential underestimation of ellipse size at low

189 sample sizes, a correction of (n-1)/(n-2) is applied to produce SEAc, a standard ellipse that

190 is unbiased with respect to sample size. We used the SEAc version of the standard ellipse

191 for all analyses, because simulations suggest that it is less sensitive to sample size variation

192 and small sample size (Jackson et al. 2011), and our sample sizes varied across rivers and

193 species sampled (Table 1). We quantified isotopic similarity of parental species and hybrids

194 within each river using the `overlap` function in `siar` to calculate the overlap of standard

195 ellipses (Jackson et al. 2011). Because rivers likely varied in baseline δ^{13}C and δ^{15}N ratios,

196 as well as the food resources available, we calculated overlaps only within a river, not across

197 rivers. We then compared area of isotopic overlap in each pair of species or hybrids across

198 rivers to understand how consistent relative positions of species are in isotopic space.

199   We also assessed the distribution of individuals in isotopic space using a hierarchical

200 Bayesian model, `isoclust`, written in `JAGS` and implemented through `R` (Code included in

201 Supplement). This model identifies the best-supported number of distinct clusters in iso-

202 topic space for each river, thus quantifying how clustered individuals are in resource use. We

203 sequentially fit models for 1 to 6 clusters in isotopic space, where each individual is assigned

204 categorically to a cluster, and identified the best fit of model to data using a penalized mea-

205 sure of model deviance (pD). This approach complements our more conventional analyses

206 where we quantified resource use by binning individuals a priori by species. By identifying

207 statistically how many clusters are represented in isotope space, we can better quantify dis-

208 tinctiveness and overlap across species and hybrid categories, rather than simply describing

209 clusters qualitatively based on the assumption that individuals with similar ancestry will be

210 ecologically similar (i.e., grouping by ancestry as in Fig. 2).

211   Finally, to characterize relative ecological success of parental species and hybrids, we

212 calculated body condition using length and weight data for 255 individual fish that had these

213 data available (Bolger and Connolly 1989, Jakob et al. 1996). Body condition is a fitness

214 proxy, but because hybrids are likely to vary in fertility independent of their body condition,

215 we interpret body condition as a correlate of ecological success but not necessarily of fitness.

216 We used two methods, Fulton's condition factor (K; Bolger and Connolly 1989) and relative

217 weight (W_r; Wege and Anderson 1978). We first used Fulton's condition factor (Anderson

218 and Neumann 1996) because it is simple and requires no species-specific constants, allowing

219 use on hybrids as well as parental species. The equation we used is $K = (W/L^3) \times 100,000$.

220 To avoid distortion due to ontogenetic body shape changes, we excluded individuals <200

221 mm in total length. Although body condition can be affected by differences in body shape

222 (e.g., degree of lateral compression), we assumed that the *Catostomus* species in this study

223 are similar enough in shape to compare body condition across species within the genus using

224 Fulton's condition factor. The only major difference in body shape among these species is

225 that white suckers have a wider caudal peduncle, and bluehead and flannelmouth suckers

226 have a more slender caudal peduncle (Baxter et al. 1995). We also used relative weight (W_r)

227 to compare body condition for flannelmouth (Didenko et al. 2004) and white suckers (Bister

228 et al. 2000), the two species with standard weight equations available (98 individual fish).

229 These equations were developed based on typical length and weight for reference individuals

230 in each species. W_r is thought to be better than Fulton's condition factor for comparing

231 across species with different body shapes, but is also limited because not all species in this

232 study could be included. There are not yet published equations for W_r for bluehead suckers,

233 and more problematically, it is unclear which equation should be used for hybrid individuals

234 or indeed whether any of the species-specific equations are appropriate for hybrid individuals.

235 Hybrids were not included in W_r analyses, both because there is no developed equation, and

236 because hybrids are likely more phenotypically heterogeneous than individuals from parental

237 species, so applying an equation from a parental species would be inappropriate.

Results

Flannelmouth suckers and bluehead suckers, which are both native to the rivers we studied, did not overlap in bivariate isotopic space (carbon and nitrogen isotopes) in any of the five sampling locations where both species were sampled (Fig. 2), suggesting minimal overlap of resource use. In two locations (Big Sandy River and Escalante Creek), these two species were substantially separated in dual isotope plots, whereas in the other three locations, standard ellipses for these species were directly adjacent but not overlapping (Fig. 2). Flannelmouth suckers were more enriched in nitrogen than bluehead suckers in four of five rivers where both species were sampled, suggesting that flannelmouth suckers feed on a higher trophic level. However, in one river (Little Sandy Creek), flannelmouth suckers were differentiated from blueheads only in δ^{15}N, not in δ^{13}C. In another location (White River), flannelmouth and bluehead suckers were differentiated only in δ^{13}C, not in δ^{15}N. In each river in this study, white sucker, a widespread introduced species, overlapped in dual isotope plots with at least one of the native species (Fig. 2). In four locations (Big Sandy River, Escalante Creek, Little Snake River, and Muddy Creek), white suckers overlapped with flannelmouth suckers in isotopic space, and in one location, white suckers overlapped with bluehead suckers (Little Sandy Creek). In one location, white suckers overlapped with both parental species (Yampa River). The variation in overlap between native and introduced species suggests different ecological effects of the white sucker introduction in different locations.

The extent of backcrossing in flannelmouth×white sucker hybridization correlated negatively with the overlap in isotopic niche in a river; there was less backcrossing of hybrids to parental species in rivers with more ecological overlap between species, and more backcrossing in rivers with less ecological overlap between flannelmouth and white suckers (Fig. 4B). Relative resource use of hybrids and parental species varied. In one river (Escalante Creek), bluehead×white and bluehead×flannelmouth hybrids had intermediate isotope values between parental species for both carbon and nitrogen (Fig. 2, Fig. 5),

264 and also slightly overlapped with both parental species. In another river (White River),
265 bluehead×flannelmouth hybrids overlapped with parental species in carbon isotopic ratio,
266 but had extremely variable nitrogen ratios that were sometimes much lower than either
267 parental species. Flannelmouth×white hybrids overlapped substantially in dual isotope
268 plots with both white and flannelmouth suckers in three out of four rivers in this study
269 where flannelmouth×white suckers were sampled, suggesting that some hybrid individuals
270 might use similar food resources to parental species. In two locations (Escalante Creek and
271 the Yampa River), a subset of flannelmouth×white hybrid individuals also had transgressive
272 isotopic ratios (Fig. 6), or isotopic ratios that substantially exceeded the range of either
273 parental species and were not intermediate between parental species. In Escalante Creek,
274 these transgressive hybrids were more enriched in carbon than parental species, suggesting
275 that they might use a different carbon source, consistent with foraging in a different location.
276 In the Yampa River, transgressive hybrids were more enriched in nitrogen, suggesting that
277 they might have foraged on a higher trophic level.

278 For the hierarchical Bayesian clustering analysis, models with 1–3 clusters best fit the
279 isotopic ratio data for individual fish in each river (Fig. 3). However, these clusters of
280 ecologically similar individuals did not correspond to species or hybrid crosses. The number
281 of statistically supported clusters (1–3) was correlated with the number of species (2–5)
282 sampled in each river ($p = 0.0457$; Pearson correlation 0.764), but in each location, fewer
283 clusters were identified in isotopic space than there were species present (Fig. 4A). There
284 was no relationship between number of genetic categories (species plus hybrid crosses) and
285 the number of statistically defined clusters in isotope space. Additionally, with the exception
286 of bluehead suckers in the Big Sandy River and Muddy Creek, clusters defined by our model
287 did not correspond to species (Fig. 3). Within a river, each cluster contained multiple
288 species; similarly, individuals of the same species were typically spread across more than one
289 model-defined cluster.

290 Body condition of suckers varied among rivers but not in any particularly predictive way

291 (Fig. 7, Fig. 8). Using Fulton's condition factor, we compared all species and hybrids. In

292 some locations, white suckers and hybrids had higher body condition than native bluehead

293 and flannelmouth suckers. Relative body condition of species was different in different loca-

294 tions; in some locations (Escalante Creek, White River) bluehead and flannelmouth suckers

295 had similar body condition, while in one location (Yampa River) flannelmouth suckers had

296 significantly better body condition than bluehead suckers. Similarly, in one location all

297 hybrid crosses had similar condition factors to native species (Escalante Creek), while in

298 other locations (Yampa River, White River) flannelmouth×white sucker hybrids had higher

299 body condition than at least one native species. These results have some caveats, notably

300 that it is difficult to compare Fulton's condition factor across species with different body

301 shapes. For this reason, we also used relative weight, W_r, to compare body condition in

302 flannelmouth suckers and white suckers, the two species with existing W_r equations, in three

303 locations where adequate numbers of both species were sampled. Estimates of W_r (Fig. 8)

304 contradicted findings using Fulton's condition factor, and showed that flannelmouth suckers

305 were in significantly better condition than white suckers in one location (Escalante Creek),

306 but in similar condition in the other two rivers where this both species were present. W_r

307 was similar for flannelmouth suckers and white suckers in the Little Snake River and Yampa

308 River. Taken together, the two metrics of body condition suggest similar ecological success

309 across ancestry classes.

Discussion

311 Ecological relationships between parental species and hybrid crosses varied extensively across

312 the seven rivers in this study. Hybrids interacted differently with the parental species and

313 potentially filled different ecological niches in different locations. The degree of variation

314 across these streams–physical, ecological, and variation in the set of fish taxa–and the rela-

315 tively low number of locations (seven) made it difficult to identify specific factors associated

316 with variation across streams. However, inclusion of seven locations is a central feature

317 of this study–had we sampled a single location, we would have identified simpler, but not

318 generally applicable, ecological outcomes of hybridization. We did identify an association

319 between ecological crowding and more extensive hybridization between species. It is un-

320 clear whether this intensified ecological overlap is a consequence of extensive hybridization,

321 or whether hybridization occurs more extensively in areas with more crowded niche space.

322 Both explanations are plausible and we discuss them in greater detail below.

Patterns of ecological overlap among species and hybrids

324 Ecological overlap as measured by isotopic niches (Layman et al. 2007, Newsome et al.

325 2007) showed high variability among rivers and this variability correlated with the extent

326 of hybridization (Fig. 4). Relationships among species in isotopic space varied across rivers.

327 However, one consistent trend was that bluehead and flannelmouth suckers, the two native

328 parental species, did not overlap substantially in isotopic space (Fig. 2). In three out of the

329 five rivers with greater than one statistically supported cluster, individuals from these two

330 species were largely but not entirely assigned to different clusters in our hierarchical Bayesian

331 clustering algorithm (Fig. 3). We suggest that bluehead and flannelmouth sucker diets

332 generally did not substantially overlap where the species co-occurred. Because both species

333 are historically native to the study locations, it is possible that non-overlapping isotopic

334 niches have evolved to avoid competition. The idea of ecological character displacement in

335 response to competition has a long history in ecology (Connell 1980, Robinson and Wilson

336 1994), and has previously been invoked to explain evolutionary origins and maintenance of

337 species diversity (Schluter 2000, Seehausen 2007). Our results are consistent with evolution

338 of divergent ecological niches in flannelmouth and bluehead suckers.

339 In contrast, introduced white suckers overlapped in isotopic niche space with flannel-

340 mouth, bluehead, or with both species, suggesting some degree of shared resource use be-

341 tween introduced white sucker and one or both native species at every location sampled.

Overlap of white suckers with bluehead suckers in some locations was unexpected because we anticipated white suckers would be more similar ecologically to flannelmouth than to bluehead suckers, due to similar mouth morphology and because white suckers preferentially eat invertebrates (Baxter et al. 1995, Cross et al. 2013, Walsworth et al. 2013). This overlap in isotopic space suggests 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.

Hybrid individuals also occupied different isotopic space relative to parental species in different locations (Fig. 2). Hybridization of bluehead suckers with both flannelmouth and white suckers produced some hybrids that were intermediate in isotopic ratios relative to parental species (Fig. 5). However, some individual bluehead×white and bluehead×flannelmouth sucker hybrids also matched parental phenotypes in isotopic space, suggesting similarity of resource use to parental species. Intermediacy in diet might be linked to intermediacy in mouth morphology. Hybrid individuals with bluehead×white and bluehead×flannelmouth ancestry have intermediate mouth morphology, including a reduced scraping ridge (Hubbs et al. 1943, Hubbs 1955, Quist et al. 2009). Intermediate morphology in hybrids corresponds to intermediate diets in 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 native parental species and their hybrid offspring if preferred resources are limited (as in Seiler and Keeley

369 2009). If overlap in isotopic space does correspond to consumption of similar food resources

370 in these rivers, competition between hybrids and parental species might occur. Competi-

371 tion might therefore be a serious concern for conservation of native *Catostomus* species in

372 locations where hybridization is ongoing and resources are limited. We currently do not

373 know to what extent fish production in these rivers is resource limited, so we cannot infer

374 whether selective pressures associated with competition are strong, but matching ecological

375 phenotypes suggests that competition is possible.

376 Hybridization can also produce individuals with phenotypes that differ from either parental

377 species and are not intermediate; indeed, novel phenotypes produced by hybridization have

378 promoted evolutionary diversification in diverse groups of organisms (e.g. Rieseberg et al.

379 2003a, Marques et al. 2019, Meier et al. 2019). We observed transgressive isotopic ratios

380 in a subset of flannelmouth×white hybrid individuals (Fig. 6). These individuals exceeded

381 values for any other *Catostomus* species in these rivers, including parental species of the

382 hybrids. In Escalante Creek, δ^{13}C values for a subset of flannelmouth×white hybrids were

383 transgressive. In the Yampa River, δ^{15}N ratios for a subset of individuals were transgres-

384 sive. Transgressive values for δ^{13}C likely correspond to use of different carbon resources,

385 potentially in a different part of the river (Finlay 2001). Transgressive values for δ^{15}N could

386 also correspond to use of a different portion of the river (e.g., feeding in a deep pool where

387 denitrification is occurring), but nitrogen isotope values are more commonly interpreted as

388 a rough indicator of trophic position (Post 2002).

389 Individual fish with transgressive δ^{15}N might be feeding on a higher trophic level by eating

390 more invertebrates than flannelmouth or white suckers, or consuming some other resource

391 like larval fish. It is not possible to identify causes of elevated δ^{15}N without additional data

392 like direct observation of stomach contents and a detailed survey of the base of the food

393 web, which are goals for future study. Examples of transgressive phenotypes in hybrids are

394 well-documented in other organisms (Rieseberg et al. 1999a, Stelkens and Seehausen 2009),

395 and hybrids can use different resources and habitats than parents (Rieseberg et al. 2003b,

396 Gompert et al. 2006, Rieseberg et al. 2007). In at least one other group of organisms, ci-

397 chlid fishes, likelihood of transgressive phenotypes is correlated with evolutionary distance

398 (Stelkens et al. 2009), but in *Catostomus* fishes the transgressive hybrids appear to result

399 from hybridization between closely related and ecologically similar taxa (flannelmouth and

400 white suckers). Transgressive phenotypes are unlikely to be fit when they initially arise,

401 as they do not reflect a long history of adaptation, but some fraction of transgressive phe-

402 notypes might confer a novel ability to use certain resources or habitats, and have been

403 implicated as a potential source of evolutionary novelty in diversification (Rieseberg et al.

404 2003*b*, Stelkens and Seehausen 2009). Regardless of specific drivers of elevated δ^{13}C and

405 δ^{15}N, transgressive isotopic ratios suggest that some individual hybrids might use different

406 resources than parental species. In both of these rivers, isotopic ratios of hybrids had more

407 among-individual variation than isotopic ratios of parental species, as shown by the slightly

408 larger standard ellipse areas. Consumption of alternative resources could be advantageous in

409 more crowded ecological niche space, such as locations containing many species and hybrids

410 that overlap in dietary requirements.

Food web effects of hybridization

412 In rivers with extensive hybridization, niche space occupied by *Catostomus* species and

413 their hybrids was more compressed (Fig. 2), and occupied by more species and hybrids that

414 potentially overlap in resource use, suggesting that one potential effect of hybridization is

415 ecological crowding, with potential for competition. In locations where two parental species,

416 flannelmouth sucker and white sucker, overlapped more in isotopic space, there was less

417 backcrossing and hybridization was mostly constrained to first generation hybrids (Fig. 4).

418 These lines of evidence suggest that although there might be some filtering of hybridization

419 outcomes by environmental conditions and available niche space, it is perhaps more likely

420 that hybridization in *Catostomus* suckers could reshape food webs. Non-native species alter

421 food web dynamics in freshwater fishe communities, including isotopic niche (Sagouis et al.

422 2015, Rogosch and Olden 2020), but the role of hybrids is less well documented. One

423 study of *Catostomus* ecology that used stable isotopes, Walsworth et al. (2013), showed

424 that introduction of white suckers and other non-native species into tributaries of the Upper

425 Colorado River basin could increase food chain length and increase niche crowding, consistent

426 with our findings here. However, Walsworth et al. 2013 excluded hybrids, which make up

427 a large fraction of the *Catostomus* fish present in many parts of the Upper Colorado River

428 basin (Gill et al. 2007). In our study, it is notable that although the number of identified

429 distinct clusters in isotopic space was correlated with number of parental species (Fig. 4),

430 the same relationship did not hold for number of clusters and number of ancestry classes,

431 i.e., parental species plus hybrid crosses. This finding suggests that hybrids were not likely

432 to be accessing different resources or occupying available niches, except perhaps in the case

433 of individuals with transgressive phenotypes, but rather were overlapping with ecological

434 resource use of parental species, with the potential for competition. It is plausible that the

435 degree of ecological crowding in a river might correspond to increased selection pressures for

436 foraging success, as hybrids, non-native parental species, and native parental species compete

437 for resources and space. Competition and

438 It is unclear what fitness consequences these interactions between parental species and

439 hybrids might have. Attempts to use body condition as a measure of ecological success and

440 proxy for fitness produced equivocal results, because different measures of body condition

441 produced opposing patterns (Fig. 7, Fig. 8). These contradictory results illustrate some of

442 the methodological challenges associated with estimating body condition (Peig and Green

443 2010), and suggest caution is warranted in attaching too much weight to body condition as a

444 fitness proxy. A simpler approach, Fulton's condition factor (K), fails to account for species-

445 level differences in body shape. The *Catostomus* species in this study had similar body

446 shapes, but white suckers have a wider caudal peduncle, while flannelmouth and bluehead

447 have more slender caudal peduncles (Baxter et al. 1995). The other approach we used,

448 calculating relative weight (W_r) from preexisting standard weight equations, does account

449 for differences in body shape, but relies on equations developed from reference individuals,

450 and could not be applied to all species, and especially not for hybrids. One particularly

451 troubling aspect of the W_r approach for flannelmouth suckers was that the standard weight

452 equation was developed using reference individuals from the Upper Colorado River basin

453 (Didenko et al. 2004), where flannelmouth suckers are endemic, and where white suckers

454 and hybrids have long been present. If there are negative effects of competition, they were

455 incorporated into our conception of what a "standard" flannelmouth sucker should weigh

456 due to the limitations of body condition indexes based on reference populations.

457 From calculations using Fulton's condition factor, *Catostomus* hybrids were in similar

458 or slightly better body condition than parental species (Fig. 7). Notably, however, the

459 relationship between flannelmouth suckers and white suckers was reversed using W_r, with

460 flannelmouth suckers in slightly better body condition (Fig. 8), which leaves some uncertainty

461 about the accuracy and meaning of these comparisons. However, hybrids must have been

462 at least moderately ecologically successful, because body condition in hybrids was similar to

463 parental species using both metrics. One caveat is that we only sampled adults, which by

464 definition must have been ecologically successful enough to survive to maturity. It is possible

465 that hybrid individuals with less ecologically successful phenotypes were produced, but did

466 not survive early life stages. In some known examples, a broad range of interspecific hybrids

467 can be viable under favorable conditions, but only a subset of hybrid individuals survive in

468 a given environment (as in *Populus* trees; Lindtke et al. 2014). It is possible that a similar

469 filtering of a subset of hybrid genotypes occurred with *Catostomus* hybrids, and that the

470 hybrids sampled as adults were those that survived strong selective pressures on ecological

471 traits as juveniles.

472 More empirical studies of fitness in hybrid individuals, including *Catostomus* hybrids,

473 will be needed to develop a more mechanistic understanding of hybridization. Clarifying this

474 point is essential because relative fitness of parental species and hybrids has a profound effect

475 on how hybridization affects the evolution of species (reviewed in Arnold M and Hodges 1995,

476 Arnold et al. 2012). In some instances of interspecific hybridization, as in tiger salamanders,

477 hybrids survived at higher rates than either parental species (Fitzpatrick and Shaffer 2007).

478 However, it is important to distinguish between the well-being of individuals—i.e., survival,

479 growth, and ecological success—and reproductive success. Survival and growth cannot be

480 equated with fitness if hybrids cannot reproduce, and there are many examples of hybrid

481 sterility (e.g. Sweigart et al. 2006, Good et al. 2008). There might also be fitness differences

482 between first-generation hybrids and later generation hybrids, particularly if there is hetero-

483 sis in F_1 hybrids. For example, in native westslope cutthroat trout (*Oncorhynchus clarkii*

484 *lewisi*) and introduced rainbow trout (*Oncorhynchus mykiss*) hybridization, F_1 hybrids had

485 relatively high fitness, including high reproductive success, but later-generation hybrids had

486 markedly lower fitness (Muhlfeld et al. 2009). Studies of fitness in wild, free-living popu-

487 lations are logistically challenging, but would add to our understanding of the causes and

488 consequences of interspecific hybridization.

Conservation implications

490 The common native parental species in this study, bluehead and flannelmouth suckers, are

491 currently the target of extensive management and conservation efforts by state agencies (Gel-

492 wicks et al. 2009, Senecal et al. 2010). Previous work on genomic outcomes of hybridization

493 suggested that hybridization might act as a demographic sink due to reduced conspecific

494 reproduction and apparent absence of hybrids beyond the F_1 generation in some crosses

495 (Mandeville et al. 2015, 2017). We can also now infer some potential ecological effects of

496 hybridization. Hybrids and non-native parental species overlapped substantially in isotopic

497 space with native parental species, which suggests that hybrids and introduced species oc-

498 cupied similar ecological niches to native species, and therefore might compete for resources.

499 Additionally, in some rivers hybrids and non-native species had better body condition than

500 native species by one metric, suggesting that hybrids and introduced species might sometimes

501 exploit resources better than parental species. When combined, these lines of evidence sug-

gest 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 in some rivers. 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.

Conclusions

In this study, we showed that ecological niches occupied by hybrid individuals varied within and across rivers. Across replicate locations where hybridization occurs, 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. 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.

Acknowledgments

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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 = bluehead, 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
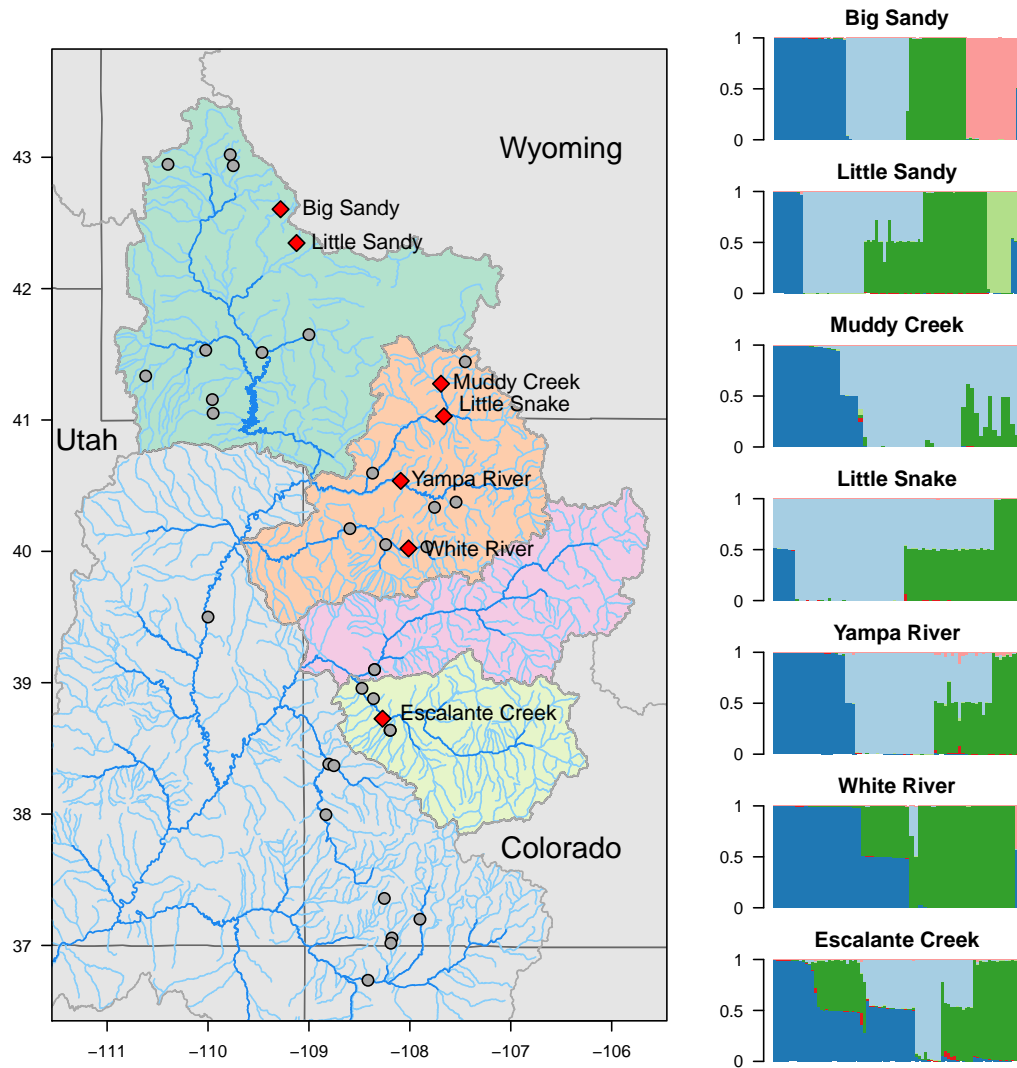
Figure 1: Seven rivers in Wyoming and Colorado and corresponding genetics outcomes of hybridization had indicated that a range of hybrids between *Catostomus* species were present (Mandeville et al. 2017). Gray dots represent all sampling points from Mandeville et al. (2017); the focal rivers for this 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).
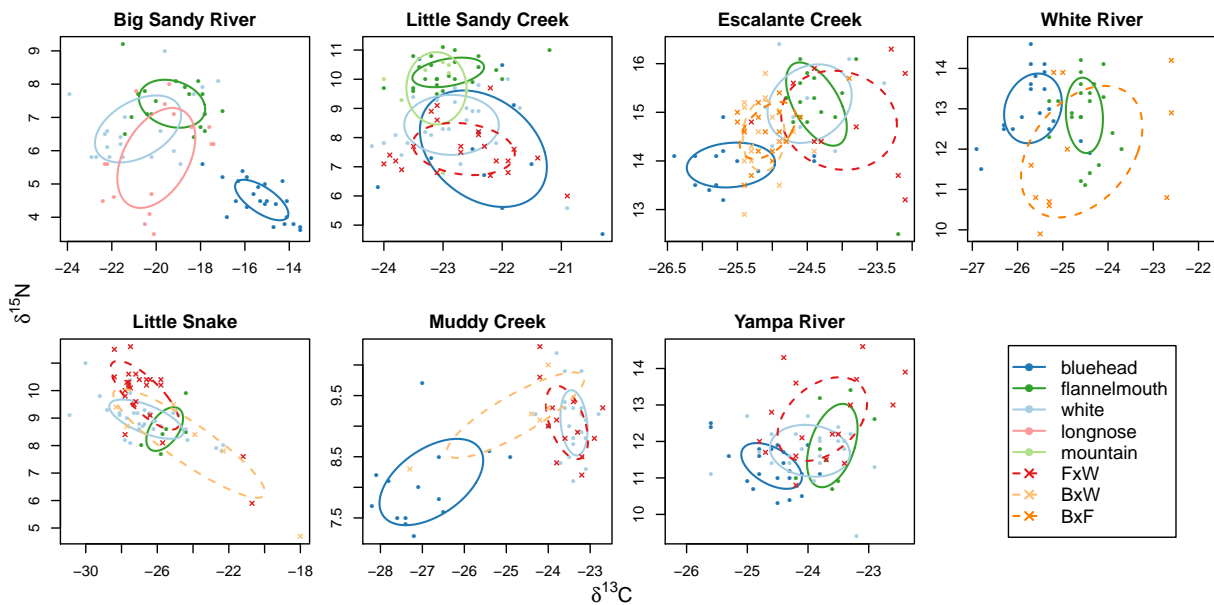
Figure 2: Standard ellipses summarize stable isotopic ratios (δ^{13}C and δ^{15}N) by encompassing approximately 40% of individual fish within each of five species and three hybrid crosses. Ellipses were estimated using a maximum likelihood and a bivariate normal distribution, implemented in SIAR (Jackson 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 signatures relative to parental species.
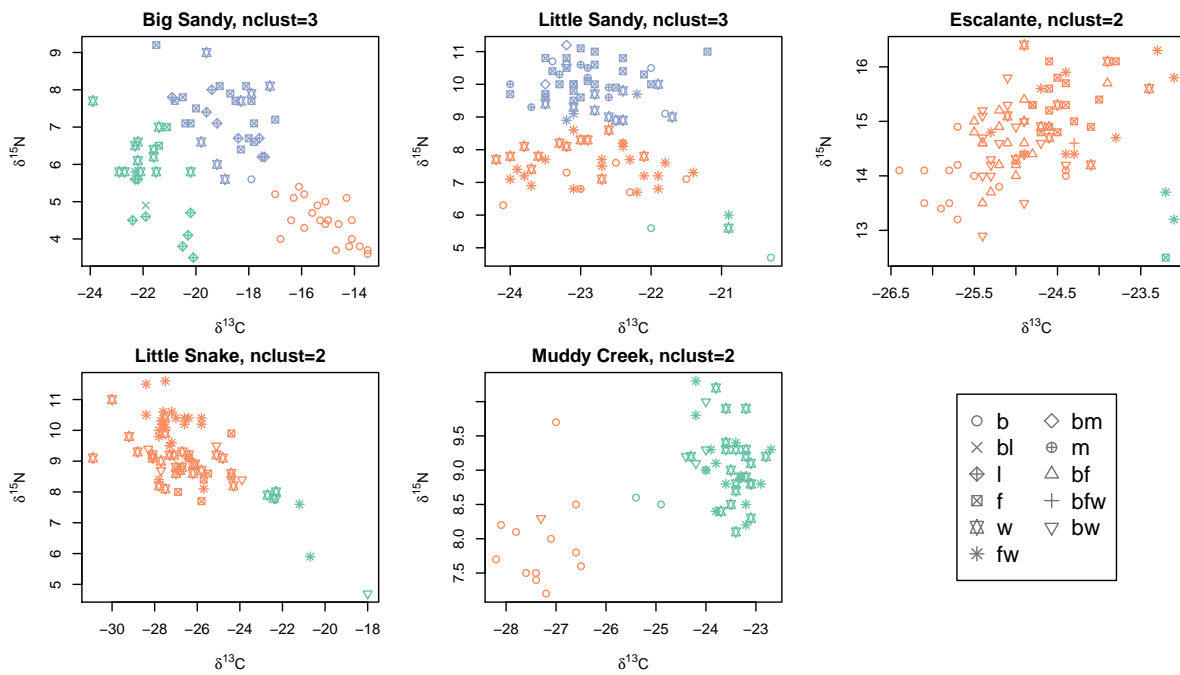
Figure 3: Results of a hierarchical Bayesian clustering algorithm were plotted in dual isotopic space for the five rivers where the optimal number of clusters was greater than 1. Point color corresponds to cluster membership as estimated by our model; point shape corresponds to species or hybrid cross. Estimates of the optimal number of clusters in isotopic space and membership of individuals in those clusters suggest that defined clusters did not correspond closely to species or hybrid categories in most cases.
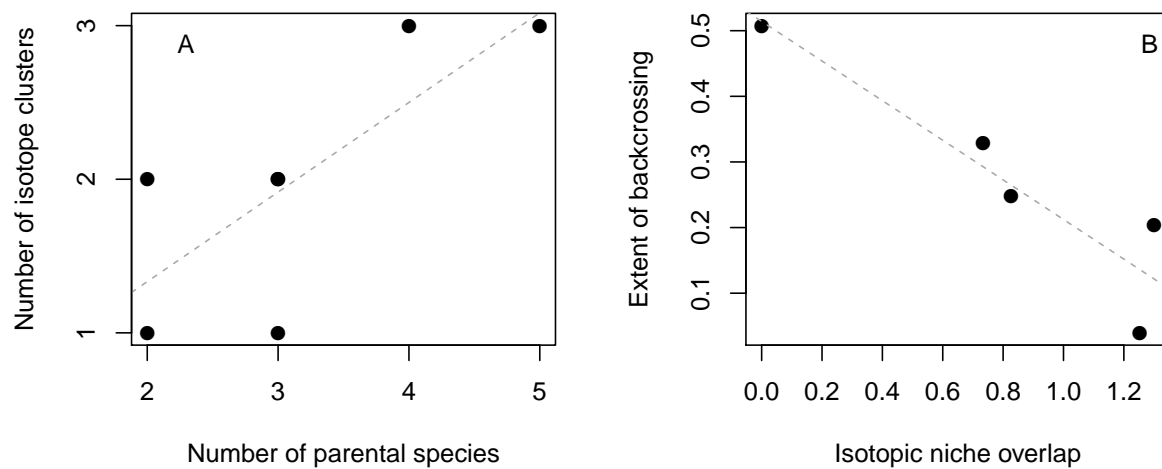
Figure 4: A) Number of clusters of ecologically similar individuals (identified by a hierarchical Bayesian model) correlated with the number of parental species sampled in a river. B) Extent of backcrossing in flannelmouth×white hybrids negatively correlated with the degree of isotopic niche overlap in 6 river locations.
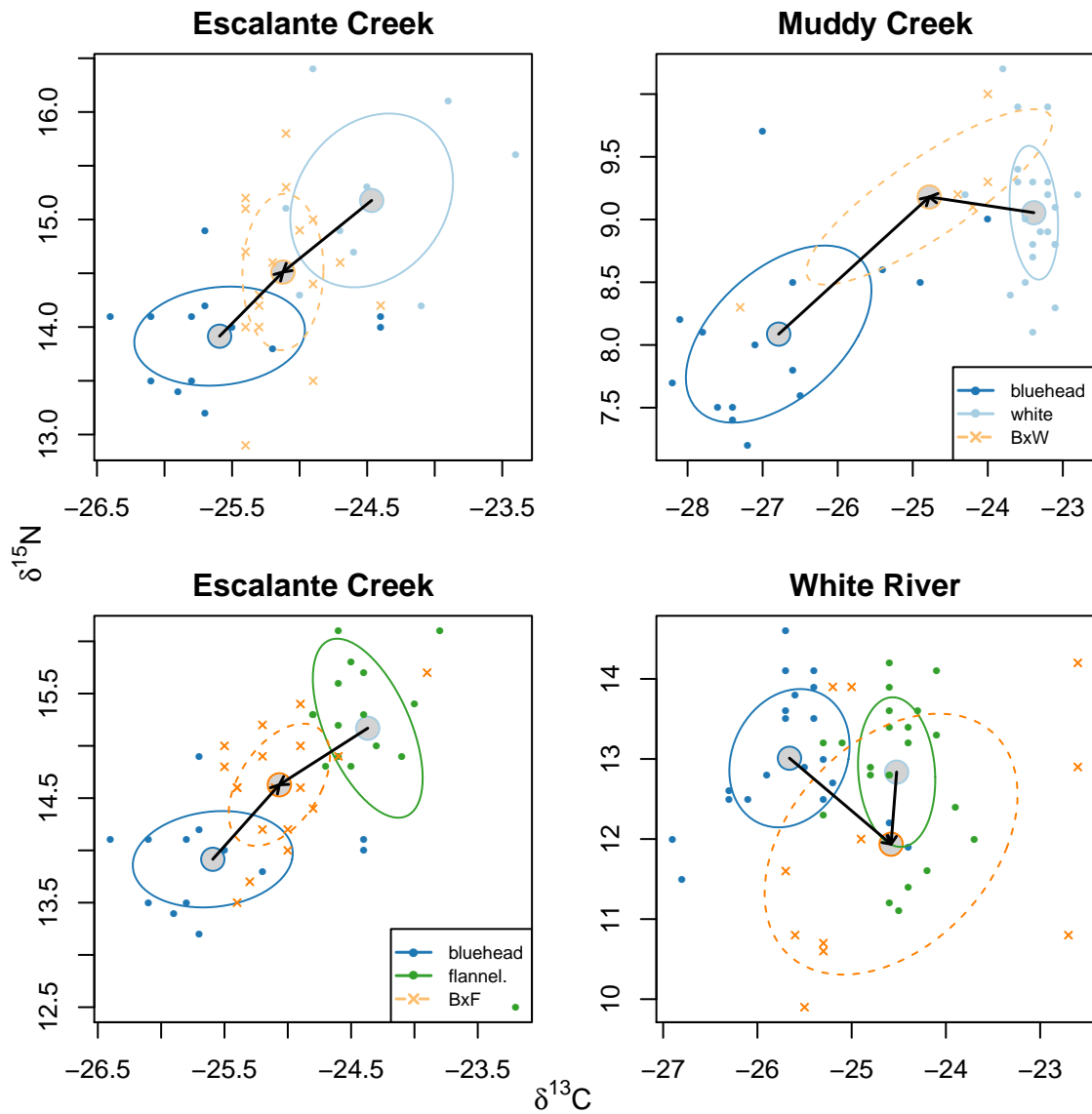
Figure 5: Mean carbon and nitrogen isotopic ratios for bluehead×white sucker hybrids (top panels) were intermediate between values for the two parental species in both carbon and nitrogen, as shown by arrows from the mean for parental species to the mean for hybrid individuals. Mean carbon and nitrogen isotopic ratios for bluehead×flannelmouth sucker hybrids (bottom panels) were intermediate between values for the two parental species Escalante Creek, but transgressive relative to parental species in the White River as shown by arrows from the mean for parental species to the mean for hybrids. Means are shown with gray points; individual fish are denoted by points color-coded by species or hybrid cross.
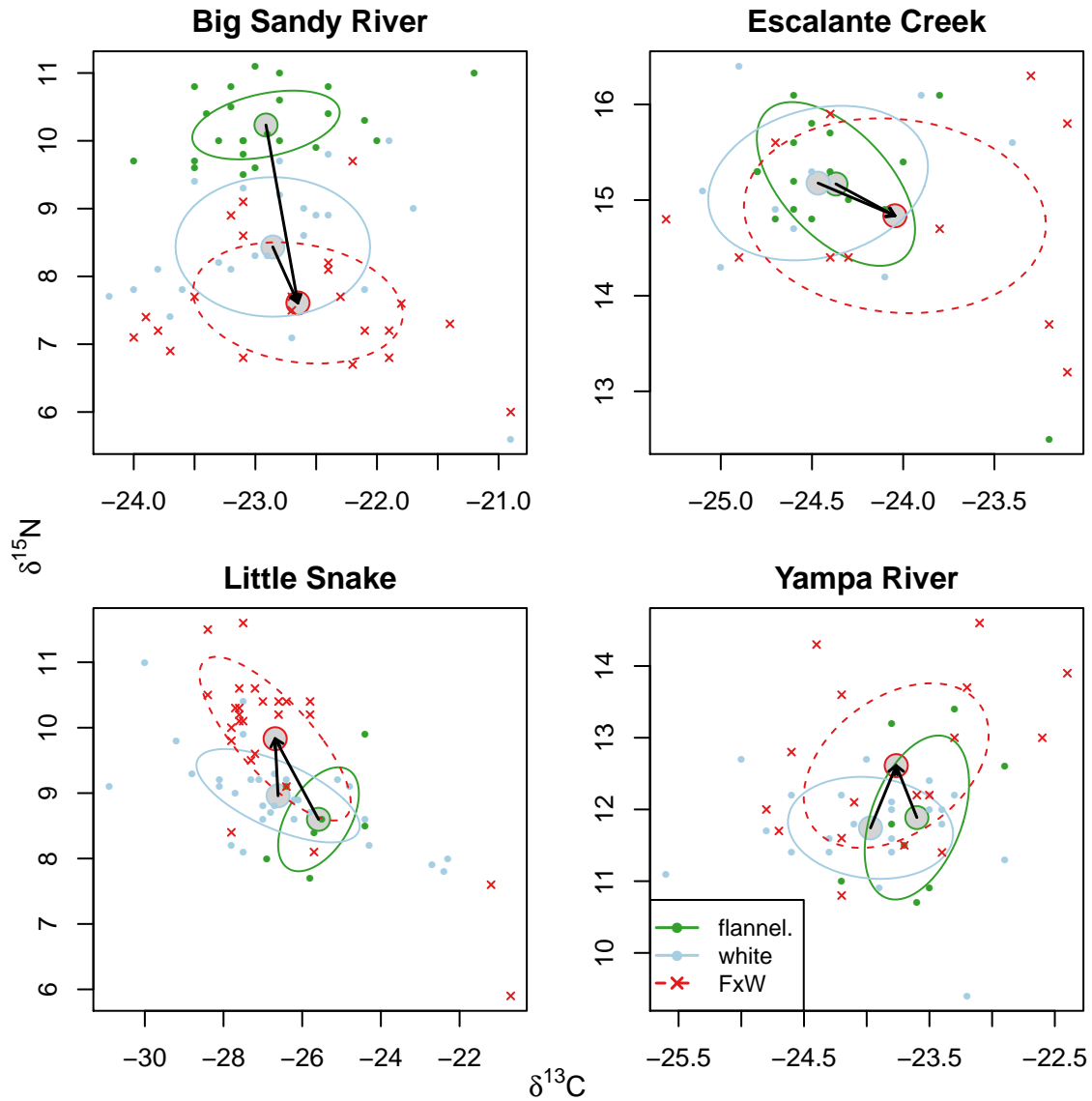
Figure 6: Mean carbon and nitrogen isotopic ratios for flannelmouth and white suckers were similar in most locations. However, as shown by arrows from the mean for parental species to the mean for hybrid individuals, mean values for flannelmouth×white hybrids were transgressive relative to means of parental species. Means are shown with gray points; individual fish are denoted by points color-coded by species or hybrid cross.
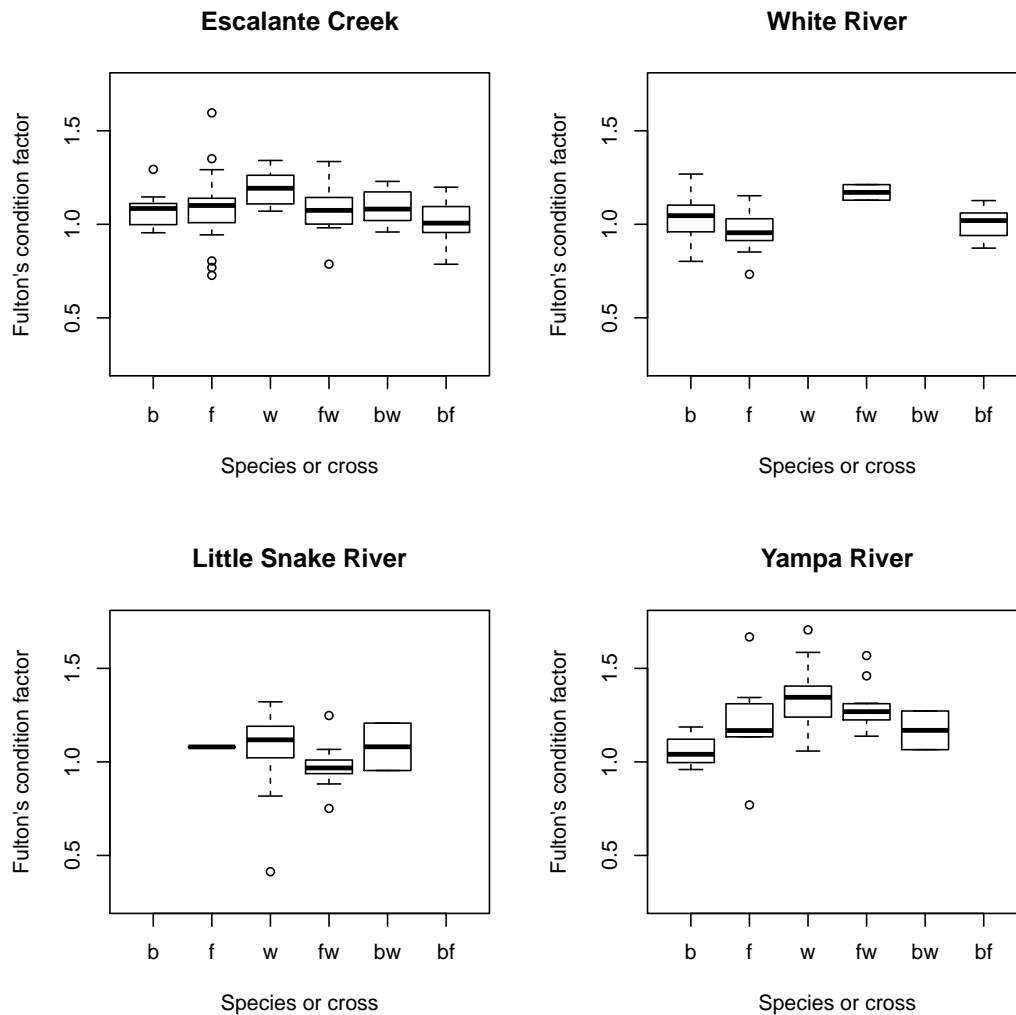
Figure 7: Hybrids and non-native white suckers had similar or higher body condition indices compared to native bluehead and flannelmouth suckers using Fulton's condition factor. Relative condition of species and hybrid crosses varied across rivers.
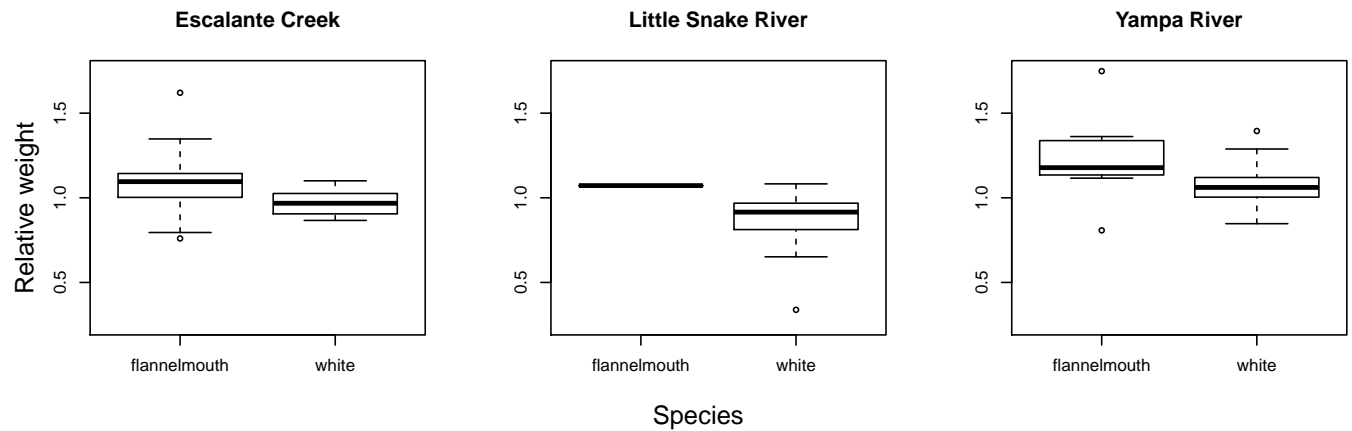
Figure 8: Flannelmouth suckers and white suckers had similar relative weights according to W_r equations available for these two species. In one river, Escalante Creek, flannelmouth suckers were in better condition according to W_r estimates; in the other 3 rivers no differences between species were identified.