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Invaders induce coordinated isotopic niche shifts in native fish species

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1 Invaders induce coordinated trophic niche shifts in native fish species

2 Abstract

3 Food-web investigations inform management strategies by exposing potential interactions
4 between native and nonnative species and anticipating likely outcomes associated with species
5 removal efforts. We leveraged a natural gradient of compositional turnover from native-only to
6 nonnative-only fish assemblages, combined with an intensive removal effort, to investigate
7 underlying food-web changes in response to invasive species expansion in a Lower Colorado
8 River tributary. Nonnative fishes caused coordinated isotopic niche displacement in native fishes
9 by inducing resource shifts toward lower trophic positions and enriched carbon sources. By
10 contrast, nonnative fishes didn't experience reciprocal shifts when native fishes were present.
11 Asymmetrical outcomes between native and nonnative fishes indicated species displacement
12 may result from competitive or consumptive interactions. Native species' isotopic niches
13 returned to higher trophic levels after nonnative green sunfish removal, indicating removal
14 efforts can support trophic recovery of native fishes like desert suckers and roundtail chub. Using
15 stable isotope analysis in pre-removal assessments provides opportunities to identify asymmetric
16 interactions whereas post-removal assessments could identify unintended consequences, like
17 mesopredator release, as part of adaptive decision making to recover native fishes.

18 Keywords: food-web, invasive species, non-native, mechanical removal, control, community,
19 stable isotopes, trophic niche, niche overlap

20 Introduction

21 Freshwater ecosystems are highly susceptible to species invasions (Gallardo et al. 2016),
22 where challenges persist to identify and predict potential ecological impacts (Jackson et al.
23 2017). Trophic interactions between native and nonnative species can propagate through entire
24 food-webs (Lodge et al. 2006, McCue et al. 2019), often resulting in management decisions that
25 target invasive species for removal. However, nonnative removal efforts may lead to
26 unintentional outcomes if nonnative species have become integrated into communities through
27 new or modified interactions among resident native and nonnative species (David et al. 2017).
28 This has led to calls to account for food-web structure and species interactions in nonnative
29 species control strategies (Zavaleta et al. 2001; Ballari et al. 2016). Knowledge of food-web
30 interactions can help prevent unexpected consequences and lead to strategies such as prioritizing
31 scheduled removal of interacting species (Bode et al. 2015), identifying situations that require
32 simultaneous eradication of multiple invaders (Ballari et al. 2016), and determining whether
33 eradication actions are beneficial (Kopf et al. 2017).

34 Nonnative invasive fishes have demonstrated impacts on riverine food-webs (Eby et al.
35 2006; Cucherousset and Olden 2011; Gallardo et al. 2016). The most commonly reported direct
36 impacts come from introduced top predators, who are often successful invaders in aquatic
37 ecosystems (e.g., Eby et al. 2006). Introduced predators lengthen food chains by occupying
38 higher trophic levels than resident species, either by direct consumption, displacing native
39 predators toward lower quality resources, or both (reviewed in Sagouis et al. 2015). Direct
40 predation is the predominant mechanism by which local native species populations at lower
41 trophic levels decline (Mollot et al. 2017). Other negative species interactions such as
42 competition or intra-guild predation may develop between nonnative and native species who

43 share the same trophic level, resulting in resource partitioning and a restricted resource base for
44 native species (David et al. 2017). As a result, native fish that modify their behavior in response
45 to these interactions may suffer decreased growth, reproduction, and/or recruitment rates
46 (Blanchet et al. 2007, Britton et al. 2018). Predicting nonnative species impacts becomes more
47 complicated with multiple species introductions (Jackson 2015), as does predicting the
48 consequences of their removal.

49 Numerous native species recovery efforts involve nonnative fish control or eradication
50 through mechanical removal programs (Rytwinski et al. 2019). Previous efforts to remove target
51 species, however, have infrequently alleviated negative impacts or promoted native species
52 conservation (Prior et al. 2018). Successful eradication efforts tend to be in areas with few
53 nonnative species where barriers prevent subsequent recolonization (e.g., Marks et al. 2010;
54 Buktenica 2013), but not always (e.g., Meyer et al. 2006). Most removal efforts that suppress,
55 but do not eradicate target species, result in compensatory nonnative population responses (e.g.,
56 Zipkin et al. 2008; Zelasko et al. 2016) or an equivocal community response across native and
57 nonnative fishes (e.g., Franssen et al. 2014; Propst et al. 2015). A recurring explanation for
58 unsuccessful outcomes (i.e. failure to recover native fish populations) is that unforeseen species
59 interactions resulted in detrimental impacts through hyperpredation, meso-predator release, or
60 trophic cascades (Zavaleta et al. 2001; Ballari et al. 2016). Awareness of unsuccessful removal
61 programs has led to growing recognition that understanding food-web interactions among native
62 and nonnative species may help anticipate management outcomes (Prior et al. 2018).

63 Nonnative fishes are a long-standing threat to the conservation of native fishes in dryland
64 rivers of the United States (Minckley and Deacon 1968). Species introductions and subsequent
65 establishment and proliferation have resulted in mixed origin assemblages where nonnative

66 species equal or outnumber native species richness (Schade and Bonar 2005; Pool et al. 2010).
67 The primary concern is that nonnative species, both large- and small-bodied, consume larval
68 native fishes and prevent them from recruiting to adult life stages (Carpenter and Mueller 2008).
69 Many of the larger nonnative species in the American Southwest are somewhat piscivorous and
70 have larger gapes than their native species counterparts (Arena et al. 2012). Thus, the greatest
71 focus of nonnative species management is centered around the impacts of widespread aggressive
72 species like green sunfish (*Lepomis cyanellus*), which are suspected of being responsible for
73 eliminating or suppressing native fish populations due to both predatory and competitive
74 interactions (Lemly 1985; Dudley and Matter 2000).

75 We investigated food-web interactions among vulnerable native and nonnative fishes
76 using a field study conducted across a spatial gradient in community composition turnover,
77 coupled with a targeted management effort to eradicate nonnative fishes from a stream system.
78 Our objectives were to determine (1) changes in native and nonnative species resource use in the
79 presence of each other; (2) degree of isotopic niche overlap and evidence of trophic dispersion in
80 mixed native-nonnative assemblages; and (3) potential isotopic niche recovery of native species
81 upon mechanical removal of nonnative green sunfish. We employed carbon and nitrogen stable
82 isotope analyses to make inferences about mechanisms underlying food-web changes in response
83 to species invasion and form subsequent predictions about how nonnative species removal efforts
84 would affect native species recovery (e.g., Vander Zanden et al. 1999; Marks et al. 2010). To
85 fulfill the first two objectives, we compared species' isotopic niche space within native-only,
86 mixed-origin, and nonnative-only fish assemblages by leveraging an existing gradient of
87 nonnative species dominance along an invasion front in a dryland river. For the third objective,

88 we compared native species isotopic niche space before and after removing nonnative green
89 sunfish to test if removal efforts can ecologically benefit native fishes.

90 Based on previous research, we had the following expectations. First, predation of native
91 fishes would cause piscivorous nonnative species to shift to significantly higher trophic position.
92 This would manifest as nonnative species demonstrating more enriched N values in mixed
93 assemblages than nonnative-only assemblages. We also expected nonnative fishes to have higher
94 trophic position than native fishes in mixed assemblages. Consequently, mixed assemblages
95 would have a higher trophic range compared to either native or nonnative-only assemblages.
96 Second, intraguild competition would cause native species to shift basal resource use in the
97 presence of nonnative species. Therefore, native species would occupy more enriched or
98 depleted C values in mixed assemblages relative to their position in native-only assemblages.
99 Consequently, mixed assemblages would display a larger carbon range compared to native or
100 nonnative-only assemblages. Third, nonnative green sunfish removal efforts would result in
101 “restored” native species isotopic niche space, or in other words, be reflected in a shift opposite
102 in direction to the changes observed from native-only to mixed assemblages. Using the scenario
103 described above in response to competitive interactions, if native species carbon values had
104 become more enriched in the presence of nonnative species, we would expect native species
105 carbon values to become more depleted following green sunfish removal efforts.

106 Methods

107 Study site

108 The study was conducted in the Bill Williams River Basin, Arizona, United States (Fig.
109 1). The upper part of the watershed flows through the transition zone between Sonora and

110 Mohave desert scrub and interior chaparral on a landscape that is primarily used for recreation
111 and grazing. The native fishes in the upper part of the Bill Williams watershed are common to
112 the encompassing lower Colorado River Basin and include desert sucker (*Catostomus clarki*),
113 Sonora sucker (*Catostomus insignis*), roundtail chub (*Gila robusta*), speckled dace (*Rhynchithys*
114 *osculus*) and occasional longfin dace (*Agosia chrysogaster*). Desert sucker, Sonora sucker, and
115 roundtail chub are considered vulnerable species of concern due to habitat loss and impacts from
116 nonnative species. Roundtail chub has been a candidate for listing under the endangered species
117 act several times, and currently is managed by the state of Arizona under a conservation
118 agreement with US Fish and Wildlife Service. Nonnative fish distributions form an invasion
119 gradient along the watercourse. In downstream reaches of the watershed nonnative fishes occur
120 in greater species richness and higher abundances, becoming less common toward the
121 headwaters. The gradient has manifested over the last half century as nonnative fishes spread
122 upstream from initial locations of stocking, predominantly Alamo Reservoir constructed in 1968
123 (Pool and Olden 2015). Nonnative fishes in the upper basin include green sunfish (*Lepomis*
124 *cyanellus*), black bullhead (*Ameiurus melas*), yellow bullhead (*Ameiurus natalis*), red shiner
125 (*Cyprinella lutrensis*), and occasional fathead minnow (*Pimephales promelas*) and common carp
126 (*Cyprinus carpio*). Native and nonnative species composition of these assemblages have
127 remained relatively unchanged in recent decades (Pool and Olden 2015).

128 Changes in species isotopic niches across an assemblage invasion gradient

129 We examined potential interactions between native and nonnative species using a spatial
130 comparison of food-web structure over a gradient of invasion. Previous experience in the basin
131 and an understanding of species distributions in the upper watershed allowed us to identify river
132 reaches that support nonnative-only assemblages, mixed assemblages, and native-only

133 assemblages. While designing the study, we identified 4 sites to ostensibly represent each
134 assemblage type, for a total of 12 sampling sites in the upper watershed. We used these 12 sites
135 to compare how species isotopic niches changed when occupying single origin (native- or
136 nonnative-only) assemblages to mixed assemblages. First, we examined individual species shifts
137 in isotopic niche space as a measure of species' responses to novel community members. Then,
138 we examined the degree of niche overlap between native and nonnative species in mixed
139 assemblages as a measure of potential competition where the two groups co-occurred. Lastly, we
140 examined the overall assemblage structure as a measure of potential food-web dispersion due to
141 changes in trophic position and resource use among species in each assemblage type.

142 Fish assemblages were surveyed at each site using standard backpack electrofishing
143 (Bonar et al. 2009) along with opportunistic seining and angling to collect fish in 50-m stretches
144 of the river, incorporating both riffle and pool habitats. After surveys were completed, we
145 identified that there were in fact 4 native-only, 2 nonnative-only, and 6 mixed sites. Sites were
146 separated by at least 500 m to reduce the likelihood of sampling the same individuals at
147 neighboring sites. We typically sampled two neighboring sites per day between April 5, 2016
148 and April 14, 2016.

149 At each site, we obtained tissue samples from fishes, macroinvertebrates, and
150 allochthonous and autochthonous primary producers to conduct a food-web investigation
151 according to natural-abundance stable isotopes. Using this method, $^{13}\text{C}:^{12}\text{C}$ is a proxy for basal
152 carbon resources and $^{15}\text{N}:^{14}\text{N}$ is a proxy for trophic position, which in combination represent
153 resource use. Fish were identified to species, enumerated, and measured for total length (mm)
154 and mass (g). A subset of fish, targeted at 10 – 12 individuals, were temporarily held prior to
155 release, in order to remove the distal margin of anal or caudal fins for stable isotope analysis. We

156 limited the subset of fish to minimize the number of individuals that were handled for extended
157 periods of time in consideration of species of conservation concern. Fin clips were taken from
158 the distal margin to promote fin regeneration, minimize infection (Jardine et al. 2011), and target
159 soft tissue membranes over harder fin rays. Because fin membrane tissue and fin rays have
160 different turnover rates, we used fin margins as a standardized tissue sample for comparison
161 across individuals (Hayden et al. 2015). Collecting fish fins is a less harmful and suitable
162 alternative to collecting muscle tissue (Hanisch et al. 2010; Jardine et al. 2011; Tronquart et al.
163 2012). Additionally, in diet-switch experiments comparing fin and muscle tissue demonstrate
164 similar half-lives between the two tissue types (e.g., Suzuki 2005; Thornton 2015). Fin-clipped
165 individuals were representative of captured body lengths in order to incorporate variation caused
166 by ontogenetic, sex, or other individual differences.

167 Dominant macroinvertebrates were collected using a 500- μm D-frame kick-net deployed
168 on the substrate and in-stream vegetation. We collected 3 samples selecting different habitats
169 (e.g. riffles with rocky substrate, vegetated margins of pools) within the same 50-m sampling
170 reach where fish sampling occurred. Macroinvertebrates were initially brushed with our hands
171 from stones and vegetation in front of the net before kicking for 60 s (Hauer and Resh 1996).
172 Invertebrates were rinsed into a sorting tray where they were separated from large debris and
173 identified to order or family. If after sorting invertebrates into vials, the biomass of dominant
174 collector, filterer or gatherer taxa appeared too low for stable isotope analysis, one or two
175 additional kick samples were performed to increase biomass in the sample. Primary producers
176 were collected from multiple habitats to represent basal food-web resources. We collected
177 epilithic algae and detritus (fine particulate organic matter [FPOM]) from rocks in pools,

178 filamentous algae (FILALG) from flowing water habitats, and macrophytes and leaf litter (coarse
179 particulate organic matter [CPOM]) from channel margins.

180 Animal tissues were preserved in salt for later processing. Salt is an effective preservation
181 method for biological samples collected in remote field settings (Arrington and Winemiller
182 2002) and has been used successfully for stable isotope analysis investigations (e.g. Spurgeon et
183 al. 2015). Following Spurgeon et al. (2015), FPOM, FILALG, and CPOM samples were dried in
184 sunlight and stored before processing.

185 Changes in species isotopic niches in response to nonnative green sunfish removal

186 In 2017, We collaborated with Arizona Game and Fish Department to obtain fish and
187 macroinvertebrate tissue samples before and after one year of intensive mechanical removal of
188 nonnative green sunfish at McGee Wash (Fig. 1). McGee Wash is a predominantly intermittent
189 tributary, with a 2 km perennial reach that serves as reliable fish habitat throughout the year,
190 separated by a 3.2 km downstream stretch of intermittent channel before its confluence with the
191 perennial mainstem. Removal efforts occurred along the entire perennial reach commencing in
192 August 2017 and continuing on at least a monthly interval. The effort was part of a conservation
193 and mitigation program to secure existing populations of native roundtail chub (USFWS 2011).
194 Green sunfish were targeted with a variety of gear to perform the mechanical removal effort
195 including backpack electrofishing, minnow traps, seining, and hook and line angling. Native fish
196 caught in sampling gear were returned back to the stream. On two occasions, personnel from
197 AZGFD collected fish fin clips of 12-15 individuals per species along with larval mayfly
198 (Ephemeroptera – Baetidae) primary consumers to represent the baseline for stable isotope
199 analysis. Tissue samples were collected on August 10, 2017, when removal efforts were
200 initiated, and again on October 3, 2018, to represent one year of removal efforts, for the before

201 and after removal comparison. Between the two tissue collection dates there were 17 total
202 removal events. Fin tissue turnover time is estimated to be approximately 30 days (Suzuki et al.
203 2005; Thornton et al. 2015), so collecting the tissues at least 30 days after the year-long removal
204 efforts increased the probability that native fish tissues reflected a time period of suppressed
205 green sunfish abundance. As is the case for small tributaries in dryland river basins, the McGee
206 Wash fish assemblage was less speciose, including two native species (roundtail chub and desert
207 sucker) and one nonnative species (green sunfish).

208 Stable isotope processing

209 All tissue samples were dried for 24 - 48 h at 60 °C, homogenized with mortar and pestle,
210 and encapsulated in 5 x 9 mm tin capsules (1 mg animal tissue; 6 mg plant tissue). Tissues were
211 sent to University of California-Davis Stable Isotope Facility and analyzed for ratios of stable
212 isotopes ($^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$) using an elemental analyzer (PDZ Europa ANCA-GSL)
213 interfaced to an isotope ratio mass spectrometer (PDZ Europa 20-20; Sercon Ltd., Cheshire,
214 UK). Data are reported as permil (‰) relative differences from standards of Vienna PeeDee
215 Belemnite for carbon and from atmospheric nitrogen, expressed as delta “ δ ” units. Long-term
216 standard deviations for estimates of natural abundance stable isotope values based on reference
217 material at UC Davis are 0.2‰ for $\delta^{13}\text{C}$ and 0.3‰ for $\delta^{15}\text{N}$.

218 Fin clips of each fish species were processed for stable isotope analysis (SIA) to estimate
219 mean and variation among species at each site within an assemblage type. After initial lab
220 preparation, some fin tissue samples were too small to run SIA. Due to lab processing and
221 variability in catch during sampling, actual sample sizes for the analyses of stable isotope values
222 vary from the target of 10 individuals per species per site (Table 1).

223 We selected Diptera-Simuliidae (collector/filterer) and/or Ephemeroptera-Baetidae
224 (collector/gatherer) to represent primary consumers as baseline isotope values for subsequent
225 fish isotopic shift analyses. We used primary consumers as our baseline to account for spatial
226 and temporal variability across a watershed (Jardine et al. 2014), thus representing more robust
227 indicators of resource use (Bunn et al. 2013). We chose these organisms because isotope values
228 (mean [SD]) of these two macroinvertebrate families overlapped with each other and with
229 FILALG and CPOM values, but were less variable than plant tissues (see online supplementary
230 material¹, Fig. S1).

231 Statistical analysis of stable isotope niches

232 Stable isotopes help indicate nonnative species impacts on food-webs by integrating
233 outcomes of trophic relationships. We examined native and nonnative species shifts in isotopic
234 niche space with directional statistics for both the invasion gradient and green sunfish removal
235 following Schmidt et al. (2007). Directional statistics calculate magnitude and direction of
236 change in isotopic niche space with estimates of mean and variability comparable to other
237 multivariate analyses. Because C and N isotope values are analyzed simultaneously, this method
238 can reveal insights that may be unclear when focusing on a single element. Factors affecting an
239 individual's isotope values (e.g. trophic discrimination) are not essential to elucidate structural
240 food-web patterns using directional statistics (Layman et al. 2012).

241 We used a hierarchical structure to determine species' isotopic niche shifts between
242 native- or nonnative-only assemblages sites compared with mixed assemblages. Mean pairwise
243 site differences were used as replicates to calculate overall mean and dispersion in the directional

¹Supplementary data are available with the article through the journal Web site at

244 change (θ) and vector magnitude (r) of isotopic niche shifts between assemblage types. We
245 accounted for variability among individuals at a site by bootstrap sampling individuals within
246 each species prior to calculating site-to-site differences. The number of individuals in each
247 bootstrap sample depended on the minimum number of fin clips of a given species available
248 among sites for each assemblage comparison. For example, among 5 mixed sites and 4 native-
249 only sites where desert suckers were caught, sample size ranged from 3 – 10 (mean = 8) fin clips
250 (Table 1). Therefore, isotope values from 3 desert sucker individuals were sampled from each
251 native-only and each mixed assemblage site for each bootstrap resampling. Ontogenetic and
252 body-size differences were accounted for by comparing individuals exhibiting the most similar
253 body lengths between sites from each assemblage type. We performed 1,000 bootstrap samples
254 of individuals from each species to account for uneven sample sizes among sites and for multiple
255 potential body length matches. By comparing pairwise site differences rather than directly
256 comparing pairwise individual differences, we accounted for site-to-variability in a hierarchical
257 manner, creating a similar analysis structure via bootstrapping, as nested ANOVAs would in
258 traditional multivariate statistics. For the temporal comparison before and after nonnative fish
259 removal effort, which took place at one location, we calculated directional statistics for pairwise
260 differences among individuals of each species between pre- and post-removal sampling events.
261 For both assemblage and nonnative removal comparisons, we performed a Rayleigh's test of
262 circular uniformity to assess the significance of mean directionality and a Wilcoxon signed rank
263 test to assess significance ($\alpha = 0.05$) of vector magnitude from zero among replicate samples
264 within each species. Rao's homogeneity test was performed to assess differences in mean
265 directionality among species (Batschelet 1981). Directional (i.e. circular) statistics were
266 performed with package 'CircStats' in software program R (Lund and Agostinelli 2012).

267 Spatial and temporal variation in baseline carbon and nitrogen isotope values must be controlled
268 for in riverine food-web studies (Hadwen et al. 2010; Jardine et al. 2014). We accounted for site
269 differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of primary consumer baselines with a normalization
270 procedure: $\delta X_{cor_{ij}} = \delta X_{fish_{ij}} - \delta X_{base_{j, min}}$, where X_{cor} represents corrected C or N isotope
271 values, X_{fish} is the isotope value of fish i at site j , and X_{base} is the minimum isotope value of
272 assuming macroinvertebrate primary consumers at site j (following Hobson et al. 2012). This
273 correction assumes primary consumers have consistent feeding and tissue assimilation of stable
274 isotopes across sites, and thus changes in fish isotope values reflect changes in fish feeding
275 behavior. Although baseline isotope values varied between individual sites, no longitudinal
276 trends were observed (Fig. S2).

277 We also examined niche overlap and trophic dispersion of native and nonnative species at
278 mixed assemblage sites, where the two groups co-occur. We used a Bayesian approach to
279 calculate standard ellipse areas, corrected for sample size (SEAc), as an indicator of potential
280 resource competition, and isotopic diversity indices, as an indicator of trophic dispersion.
281 Isotopic niche overlap between native and nonnative species pairs at mixed assemblage sites was
282 evaluated using the package 'SIBER' in software program R (Jackson et al. 2011). To examine
283 trophic dispersion, we tested the probability that pairwise comparisons of native-only, nonnative-
284 only, and mixed assemblages had equal basal resource range ($\delta^{13}\text{C}$ range) trophic range ($\delta^{15}\text{N}$
285 range), total trophic area (TA), centroid distance (CD), nearest neighbor distance (NND), and
286 standard deviation in nearest neighbor distance (SDNND) (Jackson et al. 2011). All analyses
287 were performed in software program R v.3.4.0 (R Core Team 2017).

288

289 Results

290 Changes in species isotopic niches across an assemblage invasion gradient

291 Species occupied different isotopic niche space depending on whether they occurred in
292 native-only, nonnative-only, or mixed assemblages (Fig. 2). In native-only assemblages, suckers,
293 roundtail chub, and dace occupied relatively high and similar trophic positions ($\delta^{15}\text{N}$ value) but
294 displayed some niche partitioning among basal carbon sources (Fig. 2a). Native species
295 demonstrated a marked niche shift away from this isotopic position in the presence of nonnative
296 species within mixed assemblages (Fig. 3a). All four native species showed more enriched basal
297 carbon sources (increased $\delta^{13}\text{C}$ value) and lower trophic position (decreased $\delta^{15}\text{N}$ value) in
298 mixed assemblages relative to native-only assemblages. This isotopic niche shift was
299 directionally significant among species (Rayleigh's $Z = 0.81 - 0.97$, $0.0001 < P < 0.001$), and all
300 four native fishes shifted in a similar manner. Neither directional mean (Rao's test statistic = 2.
301 5, $P = 0.47$) nor variance (Rao's test statistic = 6.7, $P = 0.08$) differed among species. The
302 magnitude of these isotopic shifts was also significant for all native fishes. Desert sucker isotope
303 values shifted most at 3.9 ‰ ($V = 210$, $P < 0.0001$), followed by 3.1‰ for Sonora sucker ($V =$
304 21, $P < 0.05$), and 2.4‰ for speckled dace ($V = 21$, $P < 0.05$) and roundtail chub ($V = 136$, $P <$
305 0.0001). Despite our attempt to account for differences in body size, desert and Sonora suckers
306 and roundtail chub had a smaller average total length in native than mixed assemblages (Table
307 S1). However, larger body size is typically correlated with higher $\delta^{15}\text{N}$ values (Fig S3 and S4);
308 the opposite expectation from what was observed between native and mixed assemblages.

309 In nonnative-only assemblages, bullhead catfish and green sunfish, appeared to use a
310 narrow range of carbon sources but had distinct trophic positions, whereas red shiner appeared to
311 be more of a generalist with relatively large SEAc (Fig. 2c). Nonnative species exhibited more

312 variable responses in isotopic niche shifts in the presence of native species within mixed
313 assemblages (Fig. 3b). Green sunfish were the only species to have significant changes between
314 nonnative-only and mixed assemblages. Their isotopic niche shifted by a magnitude of 2.2‰ on
315 average ($V = 78$, $P < 0.001$), but directional changes were indistinguishable from random ($Z =$
316 0.18 , $P = 0.69$). Although not significant, larger-bodied nonnative species like bullhead catfish
317 and green sunfish tended to display a higher trophic position (increased $\delta^{15}\text{N}$ value) but showed
318 little change in basal carbon source. The small-bodied red shiner tended to have a more depleted
319 basal resource signature and trophic position (decreased $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values) when comparing
320 nonnative-only to mixed assemblages (Fig. 3b).

321 Even though all of the native species shifted in isotopic niche space in the presence of
322 nonnative species relative to native-only assemblages, they still had overlapping niches where
323 they co-occurred in mixed assemblages (Fig. 2b). The magnitude of niche overlap, measured by
324 standard ellipse area between native and nonnative species in mixed assemblages, depended on
325 the species pair (Table 2). Among native species, roundtail chub overlapped most with nonnative
326 fishes, followed by speckled dace, desert sucker and Sonora sucker. The highest niche overlap
327 occurred between native roundtail chub and nonnative bullhead catfish, followed by overlap
328 between native speckled dace and bullhead catfish. However, both roundtail chub and speckled
329 dace had greater than 20% overlap with nonnative green sunfish as well (Table 2).

330 Isotopic diversity indices revealed trophic dispersion when native and nonnative species
331 co-occurred (Fig. 4). Isotopic richness (i.e. isotopic niche space occupied by a group of
332 organisms) was higher in mixed assemblages than native- or nonnative-only assemblages. Mixed
333 assemblages had larger trophic range ($\delta^{15}\text{N}$ range; Fig. 4a) than either native (mean difference =
334 1.77 , 95% CI = $[1.62, 1.92]$) or nonnative assemblages (mean diff. = 1.82 , CI = $[1.72, 1.92]$) but

335 a smaller resource range ($\delta^{13}\text{C}$ range; Fig. 4b) than native assemblages (mean diff. = 0.27, CI =
336 [0.08, 0.46]) resulting in larger overall trophic area (mean diff. native = 1.43, CI = [1.18, 1.68];
337 mean diff. nonnative = 2.03, CI = [1.84, 2.22]; Fig. 4c). This increasing trophic area was
338 accompanied by a higher centroid distance (mean diff. native = 0.18, CI = [0.14, 0.23]; mean
339 diff. nonnative = 0.25, CI = [0.20, 0.30]; Fig 4d). . Estimates of isotopic redundancy or evenness
340 indicated no differences among assemblage types using metrics of nearest neighbor distance
341 (NND) and standard deviation in NND.

342 Changes in species isotopic niches in response to nonnative green sunfish removal

343 Native species' isotopic niches showed marked responses to intensive removal of green
344 sunfish in McGee Wash, shifting toward pre-invasion (sunfish-free, native-only assemblage)
345 values. Monthly efforts conducted over one year resulted in a removal of 11,579 green sunfish,
346 representing a 97% decline in adult captures between the first and last removal dates (Fig. 5).
347 Following one year of green sunfish removal efforts, native species displayed a shift toward
348 higher trophic positions along $\delta^{15}\text{N}$ axis, and little change in $\delta^{13}\text{C}$ values, relative to fish captured
349 prior to removal (Fig. 6). For roundtail chub, but not desert sucker, their isotopic niche shifted
350 significantly in directionality ($Z = 0.92$, $P < 0.0001$) and magnitude (3.0%, $V = 120$, $P < 0.01$).

351 Discussion

352 Food-web investigations inform management strategies by exposing potential interactions
353 among target nonnative species and other community members that may compromise achieving
354 desired conservation outcomes. This knowledge can be used to help plan nonnative removal
355 (Kopf et al. 2017) or other restoration efforts (e.g., Bellmore et al. 2017; Spurgeon et al. 2015)
356 aimed at recovering native species and restoring ecosystems. Nonnative species are often the

357 target of restoration because they alter trophic interactions leading to changes in food-web
358 structure, energy flow and ecosystem function (David et al. 2017; Jackson et al. 2017). Here,
359 native species, in the presence of nonnative species, shifted to isotope values representing lower
360 trophic levels and more enriched basal resources. Resource shifts resulting from competition or
361 predation may lead to reduced reproduction, growth rates, and survival (Chase et al. 2002). We
362 speculate that, over time, asymmetric competitive interactions are at least a partial mechanism
363 for species replacement occurring in watersheds invaded by introduced fish species, ultimately
364 leading to nonnative-dominated assemblages (Bøhn et al. 2008). In our study, roundtail chub
365 appeared to be most impacted by negative interactions with nonnative species. Roundtail chub
366 had considerable isotopic niche shifts from native to mixed assemblages, but even after a
367 significant shift in isotope space, had the largest isotopic niche overlap with introduced species
368 in mixed assemblages. However, roundtail chub also benefitted more from nonnative removal
369 efforts, as indicated by a greater shift in magnitude and concentrated directionality, relative to
370 desert sucker, after green sunfish removal suppressed their populations in McGee Wash. These
371 results are promising for the recovery of native fishes via nonnative species management because
372 it indicates removal efforts may not only be beneficial from a demographic standpoint, but from
373 an ecological one as well.

374 Comparisons across the invasion gradient of native-only, nonnative-only and mixed
375 assemblages indicated that native and nonnative species responded to each other's presence
376 asymmetrically. Native species displayed coordinated shifts toward lower trophic levels and
377 more enriched C values, whereas nonnative bullheads and green sunfish tended to increase
378 trophic level, though inconsistently. More enriched C values in native fishes were similar to
379 isotope values from samples of FPOM collected from in-stream rocks from slow-flowing

380 habitats. The largest shifts were observed in the two sucker species, which suggests suckers in
381 particular may have shifted to a higher reliance on consuming trophically lower resources such
382 as algal periphyton and/or invertebrate grazers when nonnative species were present.

383 Comparable results have been reported following restoration efforts in Fossil Creek, a stream
384 with a similar fish assemblage. In the Fossil Creek study, nonnative smallmouth bass
385 (*Micropterus dolomieu*) and green sunfish replaced native speckled dace, roundtail chub, and
386 suckers in the highest trophic positions of the food-web when they co-occurred (Marks et al.
387 2010). Furthermore, roundtail chub and desert sucker diets shifted away from a diet including
388 predatory invertebrates, while nonnative fish diets indicated reliance on predatory invertebrates
389 and fish (Marks et al. 2010). Because larger bodied nonnative fishes like bullhead catfish and
390 green sunfish in our study likely consumed fish and predatory invertebrates in both nonnative-
391 only and mixed assemblages, this may help explain why their trophic position did not increase
392 significantly when they were in the presence of native species. For example, based on body size
393 and gape width alone, bullhead catfish could opportunistically consume smaller green sunfish or
394 red shiner individuals. In reflecting on potential diet items of both native and nonnative species,
395 however, it is important to note that isotope values are quantitative indicators of changes in niche
396 that may not be equivalent to trophic niche. Shifting isotope values may not only reflect changes
397 in diet, but changes in habitat use or growth rates, which affect diet assimilation into fish tissues.

398 For this reason, using diet data to corroborate stable isotope values is often recommended as a
399 best practice when making trophic inferences (Fry 2013). Although we did not collect diet data,
400 to minimize handling time and stress associated with extracting stomach contents of native
401 species of conservation concern, niche partitioning is a likely outcome of co-occurrence
402 dynamics between native and nonnative species. In streams, consumers are typically dependent

403 on few common resources, namely algal derived sources of carbon and nitrogen (e.g., Bunn et al.
404 2013), and low resource diversity leads to more competition, diet partitioning, and consumption
405 of non-optimal energetic food sources among species (e.g., Latli et al. 2019).

406 After experiencing significant isotopic niche shifts, some native species continued to
407 show considerable overlap with nonnative species. When nonnative and native species have
408 similar ecology, changes in native species behavior may not be enough to offset competitive
409 and/or consumptive interactions with nonnative species (e.g. Ayala et al. 2007). We found that
410 roundtail chub and speckled dace had more overlap with nonnative fishes than either sucker
411 species. Considering diet and morphology, roundtail chub and speckled dace are functionally
412 more similar to nonnative species like green sunfish and red shiner when compared to suckers,
413 and thus may have been unable to partition high quality resources, activity times, or habitat use,
414 resulting in smaller niche shifts, higher niche overlap, and higher potential for facing detrimental
415 impacts from nonnative fishes. Because suckers have different resource dependencies (i.e., more
416 reliance on grazing algae) than nonnative species, they may have been able to partition resources
417 more successfully, resulting in greater niche shifts and less niche overlap with nonnative species
418 in mixed assemblages. Again, similar results were observed in Fossil Creek. Although roundtail
419 chub shifted to a diet dominated by small filter feeder invertebrates in the presence of nonnative
420 species, desert sucker diets remained more balanced among feeding groups but included more
421 grazing invertebrates (Marks et al. 2010). High resource overlap between pelagic native and
422 nonnative species has been reported elsewhere in the Colorado River Basin (CRB; Walsworth et
423 al. 2013; Spurgeon et al. 2015). Studies combining stable isotope analysis with diet evaluation
424 also demonstrate native and nonnative species share overlapping diets of invertebrate taxa (e.g.,

425 Pilger et al. 2010; Whiting et al. 2014). Thus, niche overlap metrics are probably a faithful
426 representation of competition for shared resources and changing diets.

427 Nonnative species introductions commonly result in trophic dispersion (Cucherousset et
428 al. 2012). We found that larger isotopic niche areas in mixed assemblages, compared to other
429 assemblage types, was driven mainly by an increase in trophic ($\delta^{15}\text{N}$) range caused by native
430 species displacement to lower trophic levels. Native species shifts in C and N isotope values,
431 unaccompanied by increased values in redundancy (NND and SDNND), signified that resource
432 partitioning was the most likely response of native species to the presence of nonnative species.
433 Although isotopic diversity indices are correlated and sensitive to the number of samples and
434 biased by small sample sizes, our total sample size per assemblage type met the recommended 10
435 – 30 individuals (Jackson et al. 2011). Even centroid distance, which is less sensitive to species
436 number, considered the “core” niche (Brind’Amour and Dubois 2013), was larger in mixed
437 assemblages relative to native- or nonnative-only assemblages. Our findings are typical of
438 riverine ecosystems, where top predator additions increase total area of isotopic niches, primarily
439 by increasing trophic range ($\delta^{15}\text{N}$; Sagouis et al. 2015). Contrary to previous studies, however,
440 we found this increased trophic area was also associated with native species niche shifts.

441 We found considerable niche overlap, and trophic dispersion in mixed assemblages
442 relative to single origin assemblages, which provides evidence to support our hypothesis that
443 native and nonnative species are potential competitors. Despite this evidence, definitively
444 establishing interspecific competition without knowing corresponding changes in demographic
445 rates presents a challenge in observational studies like ours. Documenting changes in growth,
446 survival, or other vital rates through time is difficult due to the arduousness of obtaining repeated
447 measurements of the same individuals and controlling for other variables that affect vital rates.

448 However, the degree of trophic dispersion and niche shifts we observed have been accompanied
449 by reduced native species growth rates and provided evidence for interspecific competition in
450 experimental settings (e.g., Britton et al. 2018). High competition potential has also been inferred
451 from functional convergence (Arena et al. 2012). Arena and colleagues (2012) showed that
452 common native and nonnative fishes of the CRB with similar trophic niches also exhibited
453 similar prey capture behavior, but nonnative fishes did exhibit larger gape width that allowed for
454 potential asymmetric competitive interactions (i.e., intraguild predation). Intraguild predation
455 may be facilitated by ontogenetic shifts in species interactions. In the CRB, nonnative species
456 prey upon small-bodied and young-of-year native fishes (Dudley and Matter 2000; Pilger et al.
457 2010), and trophically similar adults have been observed to interfere and compete with one
458 another (e.g., Karp and Tyus 1990; Spurgeon et al. 2015), providing additional evidence for this
459 hypothesis. Throughout the CRB, nonnative fishes have been observed to occupy higher trophic
460 levels than native fishes (Pilger et al. 2010; Walsworth et al. 2013). Together the available
461 information about mixed assemblages in the CRB, combined with our observation that native but
462 not nonnative species shift isotopic niche space in each other's presence, suggests asymmetric
463 competition is occurring and may therefore contribute to reducing local persistence of native
464 species.

465 Identifying interactions and assessing recovery efforts in freshwater ecosystems using
466 stable isotope analysis can be a valuable approach to understanding underlying mechanisms of
467 successful efforts. In one application involving nonnative species control, stable isotope and diet
468 analysis suggested trout removal efforts led to increased survival and recruitment of endangered
469 juvenile fishes via reduced predation and resource competition (Coggins et al. 2011; Whiting et
470 al. 2014). In another study, removing a nonnative piscivore, allowed a native piscivore to re-

471 establish trophic links that resulted in quick and substantial food-web recovery of the native fish
472 community (Lepak et al. 2006). Likewise, we found that nonnative removal efforts in McGee
473 Wash resulted in the apparent recovery of native species with respect to returning to isotopic
474 values typically expressed in native-only assemblages. Following nonnative removal, roundtail
475 chub and desert sucker increased trophic position, with significant recovery for roundtail chub, a
476 state-listed threatened species and candidate for federal listing under the U.S. Endangered
477 Species Act. Similarly, after nonnative removal efforts in Fossil Creek, speckled dace and
478 roundtail chub increased the trophic position they occupied as well as increasing population size,
479 with densities up to 150 times higher than densities prior to removal efforts, while desert sucker
480 densities were about 50 times higher (Marks et al. 2010). Removing green sunfish from first
481 order streams in North Carolina also resulted in positive responses of native fish abundance and
482 biomass (Lemly 1985). In all of these cases of successful recoveries resulting from nonnative
483 fish management, greater than 90% of the invasive fish populations was removed. Whether
484 benefits can be identified from less intense control efforts remains unknown. This presents a
485 great opportunity for future research to explore the relationship between nonnative species
486 densities and trophic responses in native species to provide valuable insights on the amount of
487 effort needed to have positive effects on native species.

488 Successful or unsuccessful nonnative removal efforts are influenced by the food-web and
489 ecosystem context, which raises two important considerations of our study. First, the number of
490 species and trophic links in an assemblage affects food-web structure (David et al. 2017). The
491 fish assemblage in the upper Bill Williams River basin is depauperate compared to other
492 temperate rivers, and McGee Wash in particular only had a single nonnative species, green
493 sunfish, which is a predator and competitor of native fishes. In more speciose food-webs with

494 multiple invaders, a higher potential exists for unforeseen species interactions (e.g.,
495 mesopredator release or hyperpredation) and trophic cascades to affect the outcome of nonnative
496 removal efforts (Zavaleta et al. 2001; Ballari et al. 2016). Second, despite not finding
497 longitudinal trends in primary productivity or basal resource isotope values, trophic structure can
498 be affected by changes in productivity alone, without changes in predator composition or
499 introducing new basal resources (McMeans et al. 2015). Thus, we encourage more research on
500 impacts of nonnative species introductions and removal efforts to determine the challenges and
501 opportunities of ecosystem recovery in riverine systems.

502 In conclusion, nonnative species removal efforts are likely to have positive ecological
503 benefits with respect to native species recovery. We suggest food-web interactions before and
504 after nonnative species removal efforts may help inform management and conservation decisions
505 to help protect native species biodiversity and ecosystem function (e.g., Kopf et al. 2017). Pre-
506 removal assessment will help prevent ecological surprises like mesopredator or competitive
507 release that may result in increases of non-target invasive species. Post-removal assessment,
508 and/or assessment prior to native species reintroduction efforts, will help support functioning
509 populations of native species and overall ecosystem integrity as part of an adaptive decision-
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715 harvest: release from competition? *Canadian Journal of Fisheries and Aquatic Sciences*,
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717 Tables

718 Table 1. Sample sizes of fin clips used in stable isotope analysis and subsequent statistical

719 analysis.

Site	Assemblage type	Native species				Nonnative species		
		Desert sucker	Sonora sucker	Roundtail chub	Speckled dace	Bullhead sp.	Red shiner	Green sunfish
<u>Community Comparison</u>								
Conger-01	Native	10	0	10	8	0	0	0
Conger-02	Native	10	0	10	4	0	0	0
Francis-01	Native	3	3	10	0	0	0	0
Francis-02	Native	10	10	10	0	0	0	0
Burro-01	Mixed	10	3	10	8	0	0	9
Burro-02	Mixed	10	0	9	7	0	0	9
Burro-03	Mixed	0	4	0	0	7	9	4
Burro-04	Mixed	10	0	0	0	10	7	10
Francis-03	Mixed	6	0	10	0	10	0	10
Francis-04	Mixed	10	3	10	9	10	0	10
Burro-05	Nonnative	0	0	0	0	10	0	10
Burro-06	Nonnative	0	0	0	0	0	10	9
<u>Nonnative removal</u>								
McGee Wash	Before	15	0	15	0	0	0	NA
McGee Wash	After	15	0	12	0	0	0	NA

720

721

722 Table 2. Isotopic niche overlap between native and nonnative species in mixed assemblage sites
 723 as measured by standard ellipse area corrected for sample size*.

<u>Nonnative</u> <u>species</u>	<u>Native species</u>			
	Desert sucker	Sonora sucker	Roundtail chub	Speckled dace
Bullhead spp.	16.6 (4.7)	7.6 (1.8)	36.0 (1.4)	25.2 (--)
Red shiner	12.6 (--)	9.5 (--)	--	--
Green sunfish	18.5 (3.5)	13.6 (8.0)	23.3 (1.8)	22.3 (2.5)

724 * Overlap is the percent area of 95% prediction ellipses using Bayesian estimation that is shared between two
 725 species. Overlap is shown in the body of the table as mean (SE). Species comparisons were made only for sites
 726 where two species co-occur in a mixed assemblage. Estimates that could not be made based on sample size or lack
 727 of co-occurrence data are indicated by a dash.

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730 Figure captions

731 Figure 1. Study area map of upper Bill Williams River basin. Approximate sample locations of
732 fish tissue collection surveys are denoted by pie charts showing the proportional richness of
733 native (green) and nonnative (orange) species. Removal efforts, targeting nonnative green
734 sunfish, took place at McGee Wash. The inset map shows the location of the Bill Williams River
735 basin and extent indicator of the study area in northwestern Arizona (AZ), USA. Esri (2015,
736 ArcGIS v10.2), U.S. Geological Survey and U.S. Environmental Protection Agency (2012,
737 National Hydrography Dataset Plus v2).

738 Figure 2. Isotopic niche spaces occupied by fish species in native-only (a), mixed (b), and
739 nonnative-only (c) assemblages. Isotopic niche space is delineated using standard ellipse areas
740 corrected for sample size (SEAc). Native and nonnative species are expressed as solid and
741 dashed lines, respectively. Isotopic content is expressed in “ δ ” units as the relative difference in
742 parts per thousand between sample and conventional standards for ratios of carbon ($^{13}\text{C}/^{12}\text{C}$)
743 and nitrogen ($^{15}\text{N}/^{14}\text{N}$).

744 Figure 3. Polar plot of isotopic niche shifts of species compared (a) from native-only to mixed
745 assemblages or (b) from nonnative-only to mixed assemblages. Vectors (solid lines) represent
746 the mean isotopic differences of individuals between a native- or nonnative-only site and a mixed
747 site. A bootstrap sampling procedure was used to randomly select sites within each assemblage
748 type and individuals of similar body size within each site for comparison (see text for details).
749 Directional isotope differences are represented by the angle of change (θ), where each circular
750 sector on the plot is 20° . The length of each vector represents the total magnitude of niche shifts
751 in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotopes. Units of magnitude (per mil) are indicated along the plot's
752 radial grid. Directional mean (dashed radial line) and variance (arc on circumference) across all

753 pairwise site comparisons are displayed for each species. Each species' isotopic niche shifts are
754 represented by vectors of a unique color indicated by species labels adjacent to the nearest
755 dashed radial line (color codes same as in Fig. 2).

756 Figure 4. Isotopic diversity indices of native-only, nonnative-only, and mixed species
757 assemblages. Diversity indices include (a) nitrogen range, (b) carbon range, (c) centroid distance,
758 and (d) total area in isotopic niche values. The black points correspond to the mean value for
759 each assemblage, and the boxed area reflects the 95, 75 and 50% credible intervals. Letters
760 indicate groups with significant differences (i.e. 95% CI of differences between groups do not
761 overlap zero).

762 Figure 5. Timeline of green sunfish captures during mechanical removal effort, performed by
763 Arizona Game and Fish Department at McGee Wash. Removed green sunfish in a seine (a; photo
764 credit J. Olden), one of many gears used during the mechanical removal efforts. Total number of
765 individuals removed from the start of the removal effort through April 2019 (b) with individuals
766 divided by age/size classes, young-of-year (YOY; ≤ 50 mm) and larger (Age-1+; > 50 mm).
767 Droplines indicate dates on which removal efforts took place. Black arrows indicate when fin
768 clip tissues were collected from fishes for stable isotope analysis for the before and after removal
769 comparison.

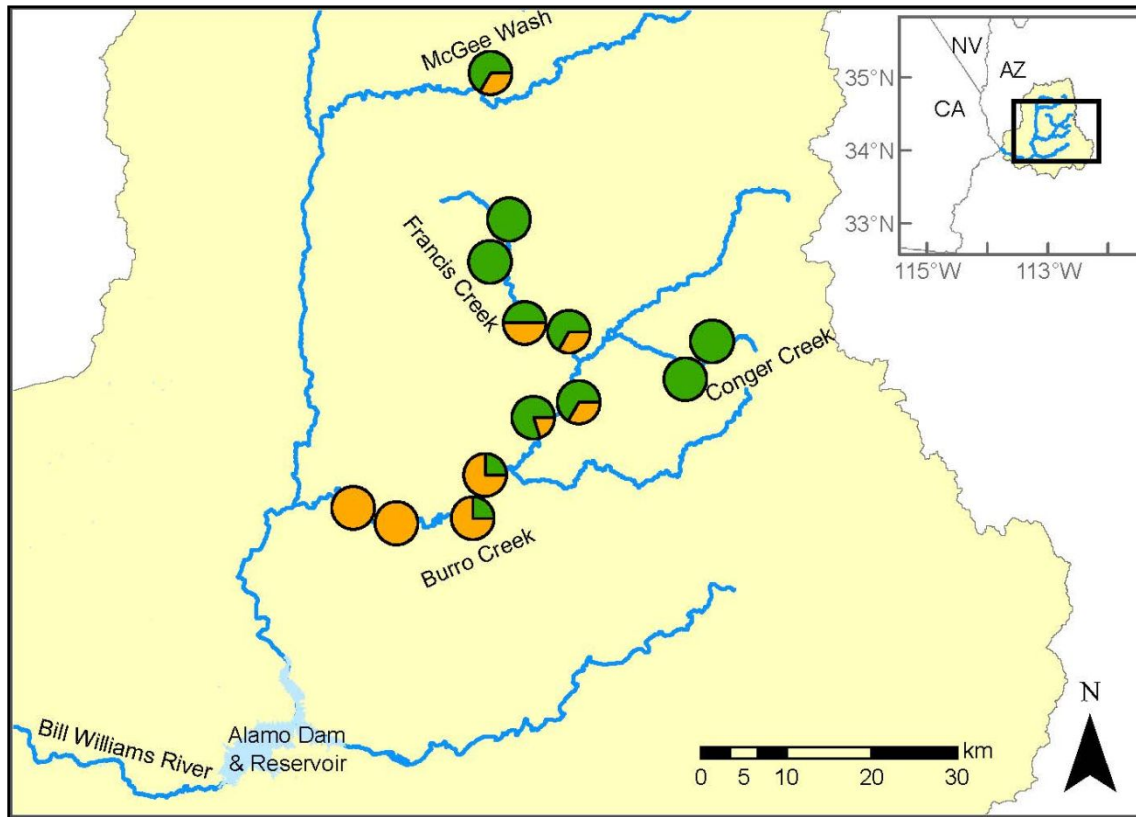
770 Figure 6. Polar plot of isotopic niche shifts of species before and after a year of nonnative green
771 sunfish removal efforts at McGee Wash. Each solid line vector represents the mean pairwise
772 isotopic differences between individuals of a species before and after removal according to
773 bootstrap sampling ($n = 1,000$ per species). Directional isotope differences are represented by the
774 angle of change (θ), where each circular sector is 20° . The length of each vector represents the
775 total magnitude of niche shifts in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotopes. Units of magnitude (per mil) are

776 indicated along the plot grid radius. Directional mean (dashed radial line) and variance (arc on
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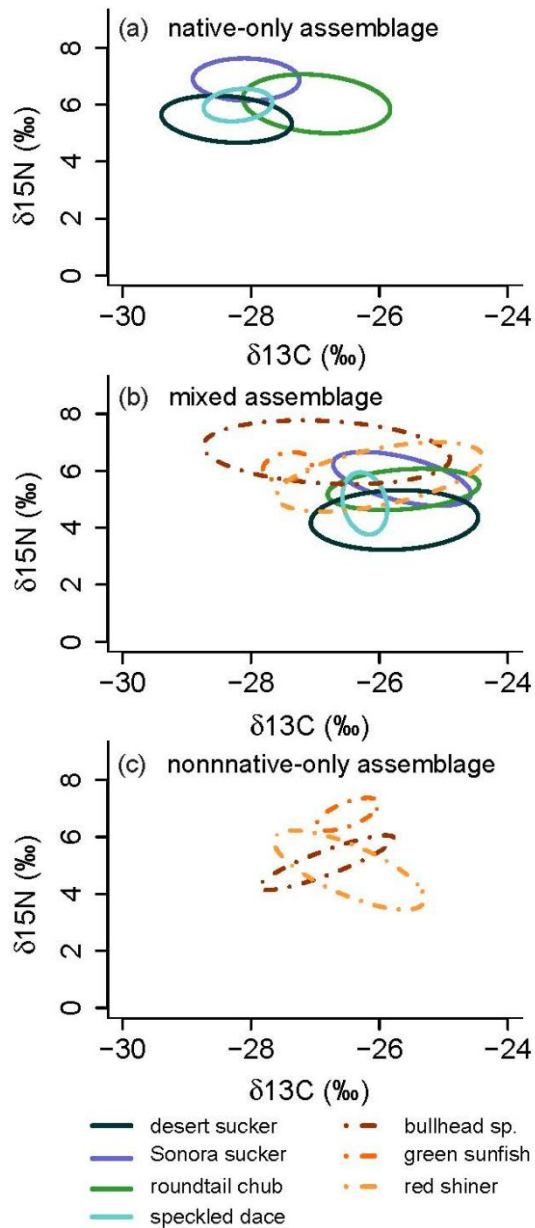
781 Figures



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789 Agency (2012, National Hydrography Dataset Plus v2).

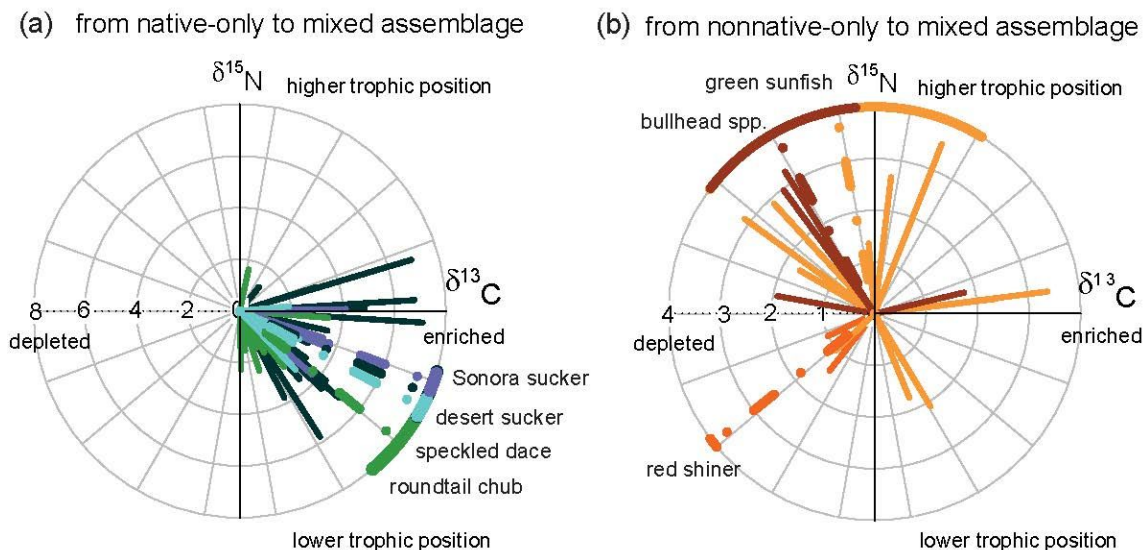
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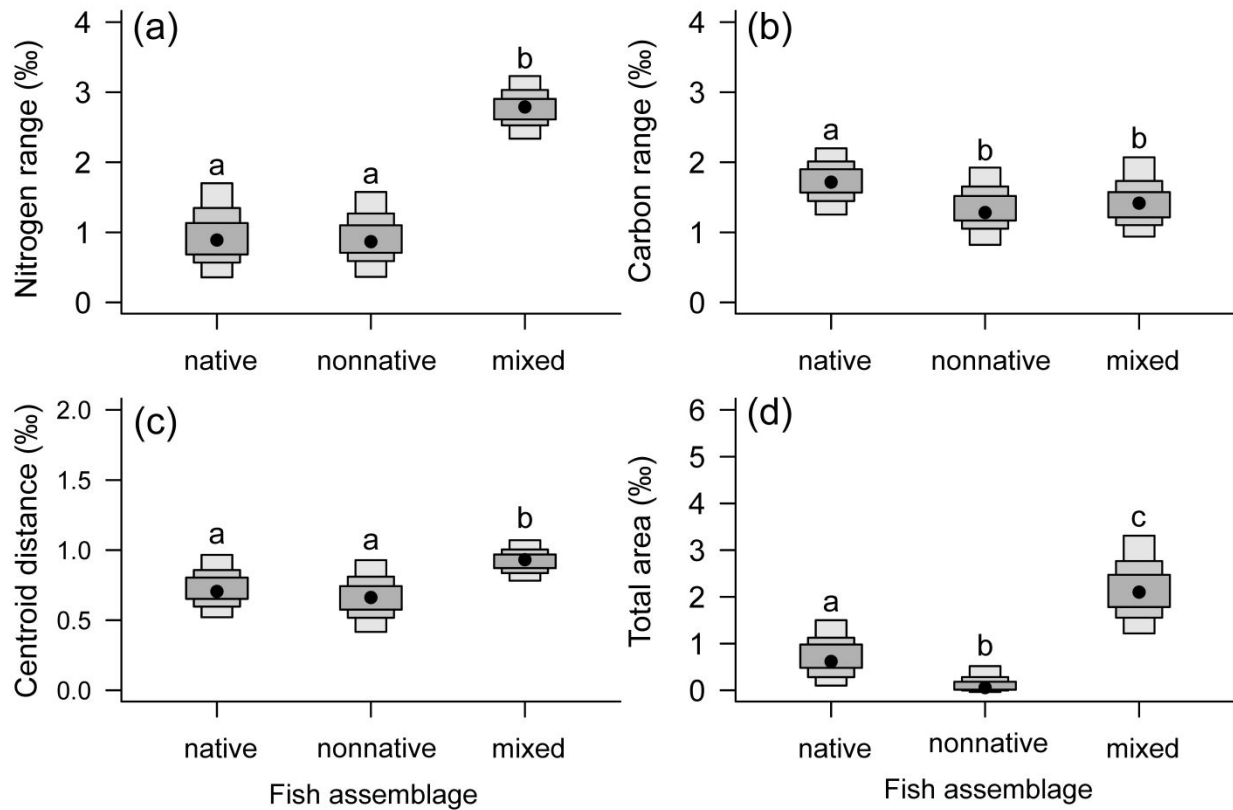
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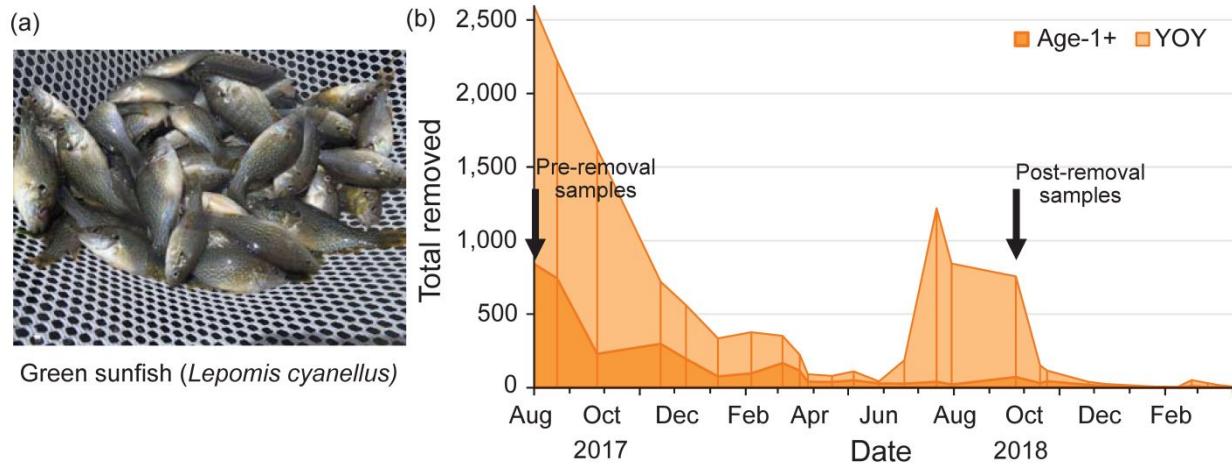
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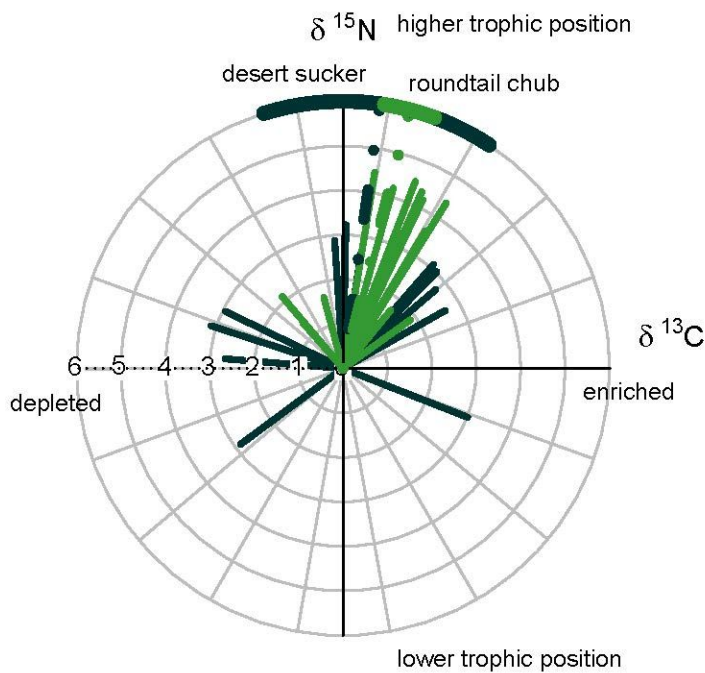
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827

Shift from before to after removal



828

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839