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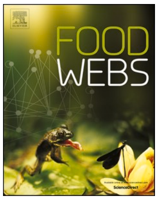

Invasive Brook Stickleback *Culaea inconstans* minimally alters the trophic ecology of four native fishes in Wyoming, USA

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ABSTRACT

Invasive species introductions are a primary threat facing populations of native freshwater fishes. There are multiple mechanisms by which an invader can affect native species, with competition for food resources being one mechanism that can lead to declines in the distribution and abundance of native species. Invaders that are trophic generalists may cause shifts in the trophic ecology of native species and may be better suited for long-term persistence amid environmental stochasticity. Therefore, trophic studies can provide valuable information on the risk an invader poses to native species. Brook Stickleback *Culaea inconstans* is an invasive fish species in Wyoming whose effect on native fish assemblages is poorly understood. Our goal was to understand the potential for competitive interactions between Brook Stickleback and native fishes. We used stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) to evaluate the feeding ecology of Brook Stickleback relative to four native fishes, and to explore whether native fish isotopic niches changed in sympatry with Brook Stickleback. We hypothesized that the isotopic niche of Brook Stickleback would be larger than that of native fishes, suggesting broader resource use. Additionally, we hypothesized that the isotopic niche of native fish populations sympatric with Brook Stickleback would contract. We did not find support for our hypotheses as the isotopic niche of Brook Stickleback was not substantially different from that of native fishes. Further, the isotopic niche of native fishes was not substantially affected by Brook Stickleback presence. As a result, we do not currently see evidence of Brook Stickleback altering the trophic ecology of native fish species. Our results provide insight to the effects of a small-bodied invasive fish species on native fishes in a previously unstudied region, and can help managers prioritize management actions to conserve native fishes.

1. Introduction

Invasive species introductions are a pervasive topic in modern ecology with implications for native fauna and biodiversity worldwide (Wilcove et al., 1998; Sala et al., 2000; Vander Zanden and Olden, 2008). Freshwater ecosystems are particularly sensitive to invasions, as endemic fishes often have limited dispersal capabilities and low population redundancy (i.e., few geographically distinct populations) which heightens the consequences of negative interactions with invaders (Allan and Flecker, 1993; Ricciardi and Rasmussen, 1999; Dudgeon et al., 2006; Vander Zanden and Olden, 2008; Strayer, 2010; Moorhouse and Macdonald, 2015). However, the magnitude and severity of effects from fish invaders can vary widely, spanning from species extirpations or extinctions in worst case scenarios (Rahel, 2002; Cucherousset and Olden, 2011) to positive outcomes in some contexts (Rodriguez, 2006; Sax et al., 2022). As the introduction of invasive fishes becomes increasingly common amid human-mediated transport (Dudgeon et al., 2006; Lodge et al., 2006; Strayer, 2010), and global economic costs of invasions continue to mount (Pimentel et al., 2005), it is crucial to understand which invasive species pose the largest risk to native fish communities to prioritize actions to mitigate their effects.

The success of an invasive species is determined by several factors including its environmental tolerances, growth and fecundity, and feeding ecology (Moyle and Light, 1996; McKinney and Lockwood, 1999; Kolar and Lodge, 2002; Layman and Allgeier, 2012). An invader's feeding ecology can be a key determinant of its long-term success (Moyle

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and Light, 1996; Brandner et al., 2013; Garvey and Whiles, 2017; Schmitt et al., 2019). Many invasive species are trophic generalists with links to multiple trophic levels within the local food web (Olden et al., 2004; Layman and Allgeier, 2012; Schmitt et al., 2019). Trophic generalists may have a competitive advantage over endemic species with a higher degree of trophic specialization, as they are often better suited for occupying diverse systems and dealing with environmental stochasticity and variations in food resource availability (Moyle and Light, 1996; Olsson et al., 2009; Jackson and Britton, 2014; Schmitt et al., 2019). Indeed, increasing diet plasticity often amplifies the effect of an invader on native communities (Shea and Chesson, 2002; Jackson and Britton, 2014). As a result, long-term persistence favors generalist invasive species, particularly with a changing climate and increasingly disturbed aquatic habitats (McKinney and Lockwood, 1999; Rahel, 2002; Rahel and Olden, 2008). In addition to being better suited for long-term persistence, invaders may have direct effects on the trophic ecology of sympatric native fishes.

Predation and competition for food resources are two primary mechanisms through which invasive fishes may affect the trophic ecology of native fishes (Case, 1991; Moyle and Light, 1996; Rahel, 2002; Mills et al., 2004). The effects of predation are well documented (see Witte et al., 1992; Johnson et al., 2008; Zelasko et al., 2016; Hickerson et al., 2019; Booher and Walters, 2021), but the implications of competitive interactions can also be serious and often lead to trophic shifts, declines in native fish body condition and abundance, and extirpation of native species (Blanchet et al., 2007; Walsworth et al., 2013; Britton et al., 2018; Rogosch and Olden, 2020; Wainright et al., 2021). Though interspecific competition is difficult to document, evaluating a species' trophic ecology can allow scientists to make inferences about potential competitive interactions among species (Schoener, 1983; Seegert et al., 2014). Trophic niche overlap between species indicates shared resource use and may suggest the potential for future competitive interactions when resources are limiting (Jackson et al., 2012; Seegert et al., 2014; Coulter et al., 2019). Native fishes may also shift resource use when an invader is present, such that sympatric populations of native fishes with an invader may exhibit a contraction of their trophic niche size relative to allopatric populations due to interspecific competition with a fish invader (Walsworth et al., 2013; Rogosch and Olden, 2020). Conversely, invasive species could take advantage of unused food resources, thus facilitating their establishment while minimizing competitive interactions and producing no change to the realized niche of native species (Shea and Chesson, 2002; Jackson and Britton, 2014). Evaluating the trophic ecology of both allopatric and sympatric populations of native fishes with an invader, as well as species-specific trophic overlap, can further our understanding of the realized effect of an invader on native communities.

Brook Stickleback *Culaea inconstans* is native to the central-northern latitudes of the United States and Canada, but its distribution has expanded westward and southward (Scholz et al., 2003; McAllister et al., 2010). In Wyoming, Brook Stickleback was first detected in 1993 (Wyoming Game and Fish Department, Fish Division, 1994) and is classified as an aquatic invasive species due to concerns about competition for resources with native fishes (Wyoming Game and Fish Department, 2014). Brook Stickleback is known for its aggressive nature (Reisman and Cade, 1967), which may lead to interference competition with sympatric native fishes. Similarly, the species' generalist diet and morphometric traits, such as a large gape, may facilitate exploitative competition (Tompkins and Gee, 1983; Stewart et al., 2007a; Wieker et al., 2016). These competitive advantages may increase its likelihood of long-term persistence relative to native species. However, studies evaluating the feeding ecology of Brook Stickleback relative to native fishes, and potential effects of Brook Stickleback on native fish resource use, have not been conducted. Brook Stickleback provides a good opportunity to evaluate the effect of a small-bodied generalist invasive species on native fish food webs.

The aim of this study was to evaluate the potential for resource competition between four native fishes and Brook Stickleback. Specifically, the objectives were to 1) characterize the feeding ecology of Brook Stickleback relative to native fishes, 2) evaluate the effects of Brook Stickleback presence on the trophic ecology of sympatric native species, and 3) assess species-specific trophic overlap for native fishes with Brook Stickleback in sympatric populations. We hypothesized that Brook Stickleback would exhibit broader resource use than native fishes, as demonstrated by a larger isotopic niche area. We also hypothesized that the isotopic niche area of native fishes sympatric with Brook Stickleback would contract relative to allopatric populations (Rogosch and Olden, 2020). Our study provides insight into the effects of a small-bodied invasive fish species on native fish trophic ecology, which has implications for invasive species management and restoration of imperiled fishes.

2. Methods

2.1. Study area

Our study took place in the Bighorn and North Platte river drainages of Wyoming, USA (Fig. 1). Located in north-central Wyoming, the Bighorn River drainage has its headwaters in the nearby Absaroka, Wind River, and Bighorn mountain ranges, with tributaries eventually flowing onto the plains where they are influenced by flow modification and municipal use (Bear, 2009). The North Platte River drainage is in the southeast corner of the state; its high elevation tributaries originate in the Never Summer and Medicine Bow mountains of Colorado, and the Sierra Madre and Snowy Range mountains of Wyoming. Tributary streams flow across a landscape used primarily for agriculture before draining into the mainstem of the North Platte on the plains (Bear and Barrineau, 2007).

Both drainages are home to native fish assemblages, with previous studies documenting >15 species in each drainage (Brunger Lipsey et al., 2005; Bear and Barrineau, 2007; Bear, 2009). Our study focused on four native species: Longnose Dace *Rhinichthys cataractae*, Fathead Minnow *Pimephales promelas*, Flathead Chub *Platygobio gracilis*, and Iowa Darter *Etheostoma exile*. These four species have been documented in sympatry with Brook Stickleback in previous sampling and thus provide an opportunity to evaluate their feeding ecology and trophic responses to Brook Stickleback presence. The potential for competitive interactions exists as Brook Stickleback and all four native species are omnivorous. Fathead Minnow is thought to have a greater reliance on vegetative material relative to the other focal species, which rely more heavily on invertebrates as food items (Tompkins and Gee, 1983; Baxter and Stone, 1995; Stewart et al., 2007a). The species vary somewhat in their habitat preferences with Fathead Minnow, Iowa Darter, and Brook Stickleback preferring slower moving waters, while Longnose Dace prefers fast-moving riffles and Flathead Chub prefers rivers and streams with swift currents and high turbidity (Baxter and Stone, 1995; Stewart et al., 2007b). Longnose Dace and Fathead Minnow are abundant across both drainages. The focal distributions of Flathead Chub and Iowa Darter, two species of greatest conservation need in Wyoming, exist in the Bighorn and North Platte river drainages, respectively. Populations of Brook Stickleback are established in both drainages, though populations in the North Platte drainage are more widespread relative to the Bighorn drainage.

2.2. Site selection

In the Bighorn drainage, study sites ($n = 7$) were limited to lotic habitats known to have both allopatric and sympatric populations of Flathead Chub with Brook Stickleback from previous sampling data collected by the Wyoming Game and Fish Department (WGFD). For sites with multiple historical sampling events, the most recent species assemblage data were used. In the North Platte River drainage we sampled targeted and random sites. Targeted sites were chosen from

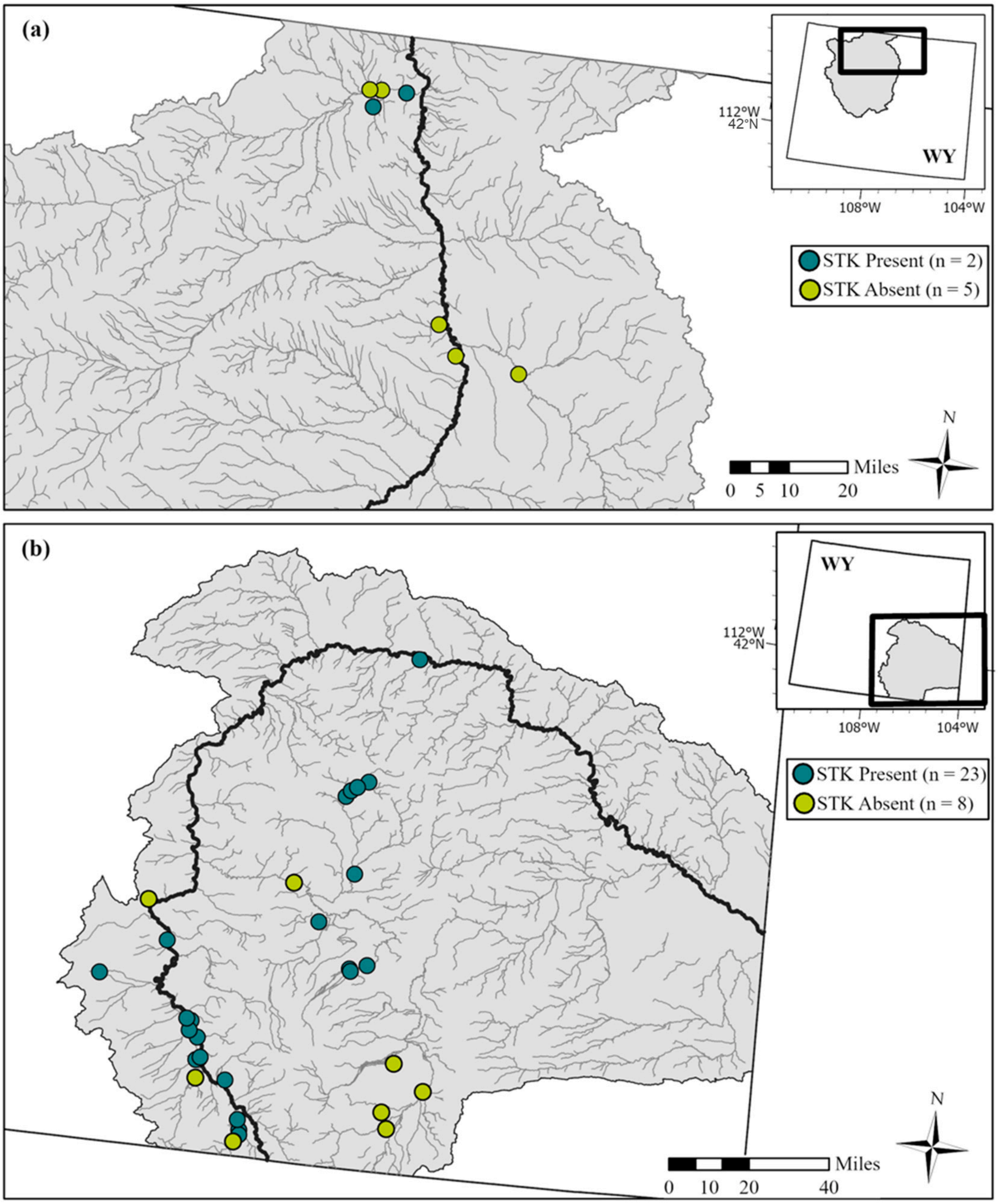

Fig. 1. Sampling locations from the (a) Bighorn and (b) North Platte river drainages of Wyoming, USA. The mainstem Bighorn and North Platte rivers are bolded. The Sweetwater sub-basin was excluded from our sampling in the North Platte drainage due to a lack of Brook Stickleback occurrences in historical sampling data.

historical sampling data collected by the WGFD and datasets collated by researchers at the University of Wyoming. These included lotic ($n = 22$) and lentic ($n = 7$) habitats known to have both allopatric and sympatric populations of Iowa Darter, Longnose Dace, and (or) Fathead Minnow with Brook Stickleback. Random lotic sites were drawn from the National Hydrography Dataset (NHDPlusV2, accessed February 25, 2022, at https://www.epa.gov/waterdata/get-nhdplus-national-hydrography-dataset-plus-data) using Balanced Acceptance Sampling (Robertson et al., 2013) in Program R (version 1.4.1103, R Core Team, 2020). However, drought conditions in 2020 and 2021 led to exclusion of 41 sites due to a lack of water and an additional four sites did not include target species. Thus, only two random sites are included in the study.

2.3. Fish sampling

We used active and passive gears to sample lotic and lentic habitats from July to November 2020, and from June to October 2021. In lotic systems we used two backpack electrofishing units (Smith-Root LR-24) and pulsed DC current to complete a single pass in the upstream direction. We netted all species and did not use block nets. Sampling reaches were 150-m in length for streams with a wetted width <5-m, otherwise a 200-m reach was sampled (Patton et al., 2000). In lentic systems we used un-baited miniature fyke nets and cloverleaf traps set overnight in littoral habitats to capture fish. If target species were not caught in the nets we conducted seine hauls (bag seine) parallel to shore in similar habitats. We identified and counted captured fish and, where possible, kept 15 individuals of each target species for stable isotope analysis (Eckrich et al., 2020). However, if this sample size was unattainable at a given site, we collected all captured individuals and later set a minimum sample size threshold of five individuals of each target species for analysis. We only kept individuals assumed to be adult life stages and of similar size to ensure a consistent amount of muscle tissue for stable isotope analysis. We euthanized individuals with an overdose of Aqui-S20E® (100 mg/L). We froze samples in sample jars filled with water from the sampling location for transport to the University of Wyoming, Wyoming Cooperative Fish and Wildlife Lab for processing.

2.4. Stable isotope processing

We removed a full-length fillet from each individual to collect muscle tissue. Tissue samples were then oven dried at 60 °C for 48 h, homogenized with a mortar and pestle, and weighed to the nearest 0.001 mg before being packed into 3.5-mm x 5-mm tin capsules. We analyzed samples for stable isotopes of carbon and nitrogen at the University of Wyoming Stable Isotope Facility using an elemental analyzer (Carlo Erba 1110) connected to a continuous flow isotope ratio mass spectrometer (Finnigan Delta V). Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic composition is reported with respect to standards of Vienna PeeDee Belemnite and atmospheric nitrogen, respectively, and is expressed in parts per thousand (per mille, ‰). The acceptable long-term standard uncertainty of carbon and nitrogen isotopic ratio measurements relative to in-house standards at the University of Wyoming is 0.15‰ for $\delta^{13}C$ and 0.2‰ for $\delta^{15}N$.

2.5. Statistical analysis

We used values of $\delta^{13}C$ and $\delta^{15}N$ to describe relevant trophic metrics including niche area, $\delta^{13}C$ range (resource range), $\delta^{15}N$ range (trophic range), and isotopic niche overlap between native species and Brook Stickleback (Layman et al., 2007a). We normalized values of $\delta^{13}C$ for lipid content prior to analysis (Post et al., 2007) and excluded sites that did not meet our minimum sample size threshold of five individuals of a species. We did not correct for baseline variation in carbon and nitrogen values across sites as issues with equipment in the stable isotope facility led to the loss of many macroinvertebrate samples, and high variability in aquatic macrophytes rendered them unreliable as baseline measurements. We estimated the isotopic niche of our four focal native species and Brook Stickleback at each sampling location by calculating the standard ellipse area and extracting the associated 75% contours using package 'rKIN' in Program R (Jackson et al., 2011; Albeke, 2017; R Core Team, 2020; Eckrich et al., 2020). To characterize the feeding ecology of Brook Stickleback relative to native fishes we tested for differences in the niche area, $\delta^{13}C$ range, and $\delta^{15}N$ range of Brook Stickleback and each of our four focal native species, across all sites, using a Wilcoxon rank-sum test. We then evaluated the effect of Brook Stickleback presence on the trophic ecology of native species by using a Wilcoxon rank-sum test to test for differences in the niche area, $\delta^{13}C$ range, and $\delta^{15}N$ range of allopatric and sympatric populations of native species with Brook Stickleback. We calculated the effect size (r) as the Z statistic divided by the square root of the sample size. The common interpretation is $r < 0.3$ indicates small effect, 0.3 - < 0.5 indicates moderate effect and ≥ 0.5 indicates large effect. Finally, we evaluated the percentage of niche overlap between each native species and Brook Stickleback, across all sites where they co-occurred, to assess the species-specific potential for competitive interactions.

Estimates of isotopic niche area and overlap are sensitive to methodology used to characterize a species' niche (Eckrich et al., 2020). We tested three different methods for estimating a species' niche area: minimum convex polygon (MCP), kernel utilization density (KUD), and standard ellipse area (SEA). We did not notice substantial differences in our results between methods, though KUD yielded generally larger niche areas (Eckrich et al., 2020). We chose to use SEA as it is less sensitive to sample size, is more robust to uneven samples sizes than MCP, and offers a niche area estimate that is not inflated in the presence of outliers (Eckrich et al., 2020). We also tested niche area at three different contour levels: 50%, 75%, and 95%. We felt the 75% confidence level provided the most accurate representation of a species' niche, as it accounts for individual variation but does not attempt to encompass extreme outliers. We were also concerned about potential effects of sample size; to ensure sample size was not strongly affecting niche area calculations we randomly re-sampled sites with complete samples (15 individuals). We did not find a relationship between calculated niche area and sample size (Fig. S1).

3. Results

Brook Stickleback feeding ecology generally resembled that of native fishes as the niche area was similar among species (Fig. 2). The niche area of Brook Stickleback was most similar to Fathead Minnow and was not substantially different from the niche areas of Flathead Chub, Iowa Darter, or Longnose Dace (Table 1). The $\delta^{13}C$ range and $\delta^{15}N$ range of Brook Stickleback was also not substantially different than the four native focal species (Table 1, Fig. S2). Native species trophic ecology generally appeared to be unaffected by Brook Stickleback presence (Fig. 3). The niche area of each native species was not substantially different in allopatry than in sympatry with Brook Stickleback (Table 2). Sympatric populations of Fathead Minnow generally exhibited a contraction of niche area with the exception of one sympatric site with a very large niche area; however, results were still not significant after removal of that site (Fig. 3; $p = 0.11$, effect size $r = 0.47$). The $\delta^{13}C$ range and $\delta^{15}N$ range of native fishes was also relatively unaffected by Brook Stickleback presence (Table 2, Fig. S3).

Isotopic overlap between native species and Brook Stickleback varied widely across species and sites (Fig. 4). At both sites where Iowa Darter occurred with Brook Stickleback, 83% or more of the species' isotopic niche overlapped with that of Brook Stickleback (mean = 91.8%). Flathead Chub and Brook Stickleback isotopic overlap was 100% at the one site where they co-occurred. Isotopic overlap for Fathead Minnow ranged from 0 to 74% (mean = 33.7%, Fig. S4) and from 0 to 100% (mean = 50.1%, Fig. S5) for Longnose Dace.

4. Discussion

Evaluating an invasive species' feeding ecology and its effects on the trophic ecology of native species can provide information crucial to assessing the potential for competitive interactions, which can have implications for the long-term persistence of native species. In our study we found that the isotopic niche area of invasive Brook Stickleback was similar to that of our focal native species. Further, the trophic ecology of native fishes was relatively unaffected by Brook Stickleback presence. Finally, Flathead Chub and Iowa Darter had the highest isotopic overlap with Brook Stickleback. Surprisingly, our results suggest that Brook Stickleback presence currently has limited effects on the trophic ecology of native fish species. However, given high trophic overlap at many sites, there may be potential for competitive interactions when resources are limiting.

Assessing a species' trophic niche provides insight to its feeding ecology (Layman et al., 2007b; Jackson et al., 2012). A species' feeding ecology can inform its habitat preferences, interactions with predators, and its ability to endure environmental stochasticity, all of which inform population dynamics (Moyle and Light, 1996; Olsson et al., 2009; Braga et al., 2012; Jackson and Britton, 2014; Schmitt et al., 2019). For managers interested in conserving imperiled fishes, an understanding of a species' feeding ecology can therefore provide valuable information related to its population dynamics. Evaluation of the niche area for Brook Stickleback and our four focal native species showed that Brook Stickleback resource use was not substantially broader, though the difference between Iowa Darter and Brook Stickleback was marginally significant ($p = 0.06$) and had a moderate effect size ($r = 0.35$). This may suggest that in scenarios where resources are limited, Brook Stickleback has a competitive advantage relative to Iowa Darter. In experimental studies, Threespine Stickleback *Gasterosteus aculeatus* diet variation increased with reduced prey availability demonstrating the competitive advantage of broad resource use (Svanbäck and Bolnick, 2007).

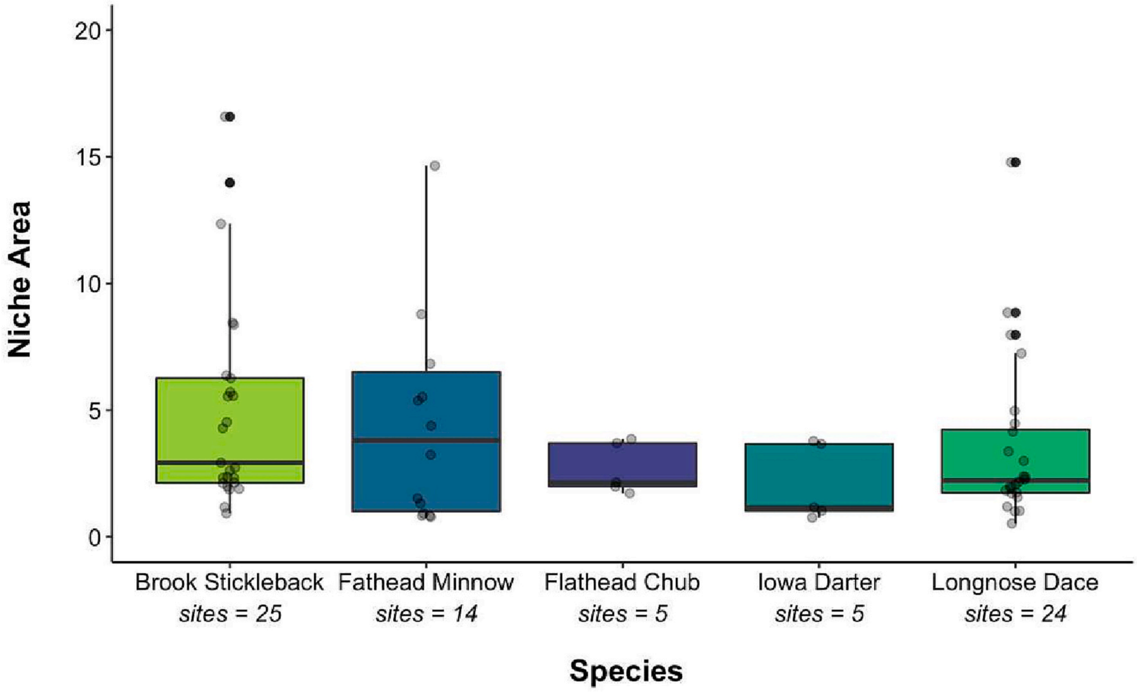

Fig. 2. Niche area of native species is not substantially different from Brook Stickleback. Niche area was calculated using a standard bivariate ellipse at the 75% confidence level. The number of distinct sites analyzed for each species is included below respective labels. Each data point represents the niche area of the corresponding species at a distinct sampling site.

Table 1

Results from Wilcoxon rank-sum tests examining for differences in the niche area, δ^{13}C range, and δ^{15}N range of Brook Stickleback and each of our four focal native species.

Species	Niche Area		δ^{13}C Range		δ^{15}N Range	
	p-value	effect size	p-value	effect size	p-value	effect size
Fathead Minnow	0.51	0.11	0.81	0.04	0.28	0.18
Flathead Chub	0.21	0.24	0.11	0.30	0.18	0.25
Iowa Darter	0.06	0.35	0.14	0.27	0.21	0.23
Longnose Dace	0.08	0.25	0.13	0.22	0.76	0.04

Brook Stickleback presence did not substantially alter the isotopic niche area of native fishes, though sympatric populations of Fathead Minnow showed isotopic niche contraction with the removal of an outlier population. The contraction of Fathead Minnow niche area for most populations in Brook Stickleback presence provides some limited support for our hypothesis and corresponds with previous research showing that native fishes exhibited niche displacement in the presence of an invader (Rogosch and Olden, 2020). However, a past field enclosure experiment between Brook Stickleback and Fathead Minnow found that Brook Stickleback had no effect on the diet of Fathead Minnow when the two species were in sympatry (Abrahams, 1996). It could be that resource availability mediates the strength of competitive interactions, facilitating Brook Stickleback coexistence with native fauna when resources are not limiting (Shea and Chesson, 2002; Jackson and Britton, 2014). We were surprised to not see a stronger effect of Brook Stickleback on native fish niche area, as most fish species studied were of similar size and competitive interactions often become stronger as body size between species becomes more similar (Dick et al., 2017a, 2017b; Britton et al., 2018).

Isotopic overlap was highly variable within and among species. Fathead Minnow showed the lowest level of overlap, which aligns with our observation of reduced niche area for most sympatric populations with Brook Stickleback, but could also reflect Fathead Minnow's more vegetative diet (Baxter and Stone, 1995). Iowa Darter and Flathead Chub exhibited high isotopic overlap with Brook Stickleback, though low sample sizes limit our confidence in the generality of these results. We expected high isotopic overlap with Brook Stickleback for Iowa Darter given the shared diet and habitat preferences of both species (Winn, 1960; Tompkins and Gee, 1983; Baxter and Stone, 1995). This dietary and habitat overlap suggests Iowa Darter may experience competitive interactions with Brook Stickleback if resources were to become limited. In this study, Iowa Darter and Brook Stickleback co-occurrence was limited to lentic waterbodies where we believe productivity was relatively high. Flathead Chub and Longnose Dace exhibited high isotopic overlap with Brook Stickleback at some sites despite both species preferring microhabitats with faster water velocity, in contrast to Brook Stickleback's preference for low-velocity microhabitats (Baxter and Stone, 1995).

The high variability in isotopic overlap among sites (Fig. 4, Fig. S4, Fig. S5) could be related to multiple synergistic factors including population abundances, community composition, and differences in resource availability (Magnan et al., 1994). For example, predator presence may mediate interactions. Brook Stickleback possesses an array of dorsal, pelvic, and anal spines that allow them to exploit habitats that are more vulnerable to predation, therefore allowing for spatial segregation from native species that may have shifted habitat use to limit predation risk (Abrahams, 1996; Brown et al., 1999). Additionally, abiotic factors such as stream width, turbidity, and nutrient inputs can all affect the productivity of a waterbody, potentially limiting (or increasing) food resource availability. We examined whether drainage (North Platte vs. Bighorn) or water type (lentic vs. lotic) contributed to differences in trophic overlap across sites and did not find substantial differences. Finally, variable trophic overlap may reflect temporal variability in resource use due to seasonal movement. Several studies have noted the ability of Brook Stickleback to carry out long distance migrations during the spring spawning period (e.g., Stewart et al., 2007b), which could result in seasonally variable resource use (Hinch

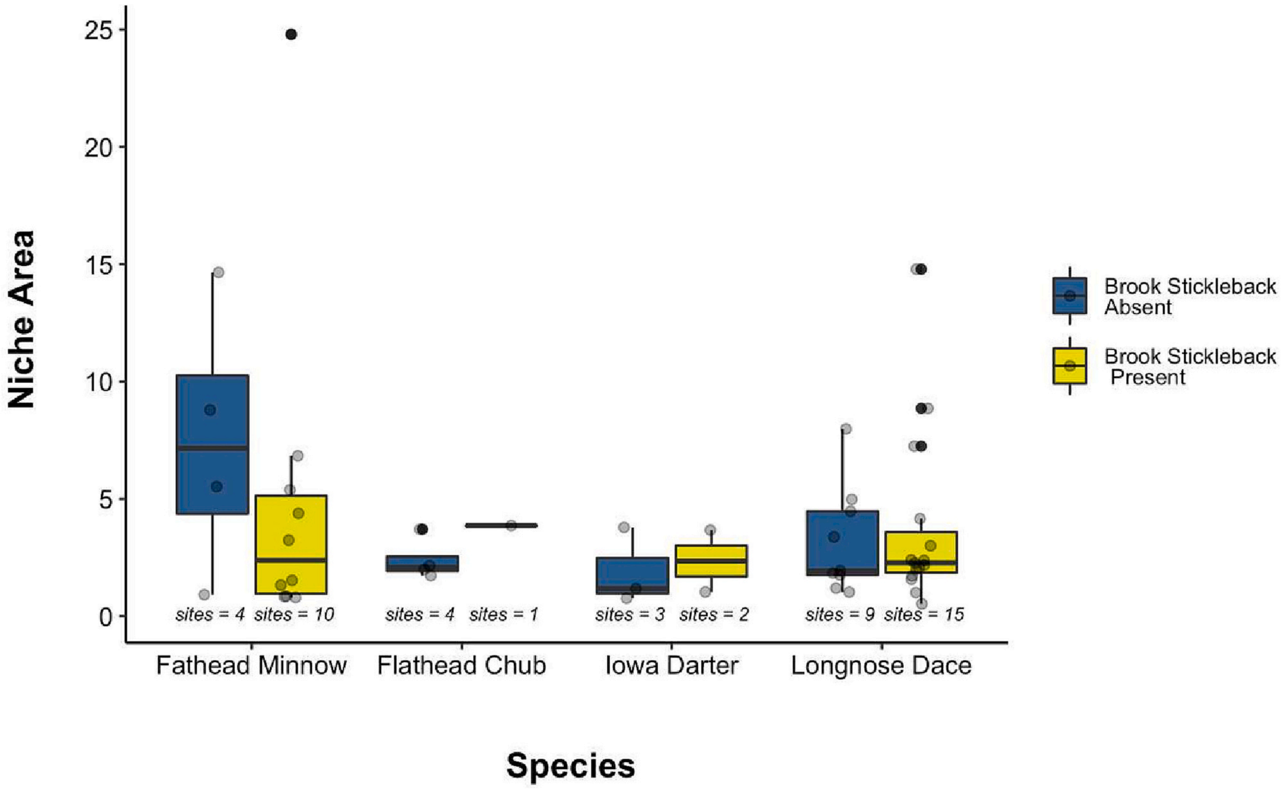

Fig. 3. Niche area for native species was not substantially different in allopatry than in sympatry with Brook Stickleback, though many Fathead Minnow populations showed evidence of isotopic niche contraction. Niche area was calculated using a standard bivariate ellipse at the 75% confidence level. The number of distinct sites analyzed for each species under allopatric and sympatric scenarios is included above respective labels. Each data point represents the niche area of the corresponding species at a distinct sampling site.

Table 2
Results from Wilcoxon rank-sum tests examining for differences in the niche area, $\delta^{13}C$ range, and $\delta^{15}N$ range of the four focal native species in allopatry vs. sympatry with Brook Stickleback.

Species	Niche Area		$\delta^{13}C$ Range		$\delta^{15}N$ Range	
	p-value	effect size	p-value	effect size	p-value	effect size
Fathead Minnow	0.24	0.34	0.62	0.15	0.45	0.23
Flathead Chub	0.40	0.63	0.40	0.63	0.40	0.63
Iowa Darter	1.00	0.00	1.00	0.00	0.40	0.52
Longnose Dace	0.86	0.04	0.61	0.11	0.79	0.06

et al., 2006).

Our interpretation of results is limited by low sample sizes for Flathead Chub and Iowa Darter, both species of greatest conservation need in Wyoming. Flathead Chub overlapped infrequently with Brook Stickleback on the landscape, potentially due to differences in habitat preferences and Brook Stickleback's more limited distribution in the Bighorn drainage (Baxter and Stone, 1995). We detected Iowa Darter at a limited number of sites and often in low relative abundances, despite the suitability of our sampling locations for Iowa Darter and its shared habitat preferences with Brook Stickleback (Baxter and Stone, 1995). Iowa Darter has experienced declines in abundance and distribution across the North Platte drainage in recent years. Habitat degradation and non-native species introductions have been suggested as the primary reasons for Iowa Darter's decline (Wyoming Game and Fish Department, 2017). Though our results showed no substantial effect of Brook Stickleback presence on Iowa Darter's trophic ecology, competition between the species over the last three decades could have contributed to Iowa Darter's decline and current overlapping populations may be trending in a similar direction, highlighting the benefit of continued monitoring. Future work focusing on collecting species of greatest conservation need at a greater number of sites across the state would provide a more comprehensive representation of species interactions.

Another study limitation was the inability to distinguish between environmental variation among sites and the influence of STK occurrence, limiting comparisons to within a site. Correcting for baseline variation in carbon and nitrogen values would allow for comparison of changes in species' isotopic ecology and overlap across sites. Due to equipment issues, our only available baseline across sites were macrophytes that can use atmospheric or aqueous carbon for cell processes, reducing their reliability as a baseline (Osmond et al., 1981; Finlay, 2001). We also did not conduct stomach content analysis to verify whether species shared specific food items, as small body size and morphological traits such as pharyngeal teeth in Fathead Minnow made identifying food items to corroborate stable isotope results challenging. As a result, trophic overlap could indicate similar resource use or feeding on different resources with similar isotopic values. Future work would benefit from ensuring adequate sample material of a temporally stable baseline organism and including diet analysis or fatty acid analysis to corroborate stable isotope results (Post, 2002; Anderson and Cabana, 2007; Kristensen et al., 2016; Rubenson et al., 2020). Lastly, repeated temporal sampling would be beneficial to improve understanding of seasonal shifts in species' diets and provide a more complete picture of species interactions. As fishes complete successive stages of their life history they often exploit different resources based on current needs, resource availability, and presence of predators and (or) competitors.

In conclusion, we do not see evidence for current competitive interactions among Brook Stickleback and native fishes, though the potential for such interactions exists. In systems where food resources are limited, competition seems most likely to occur between Brook

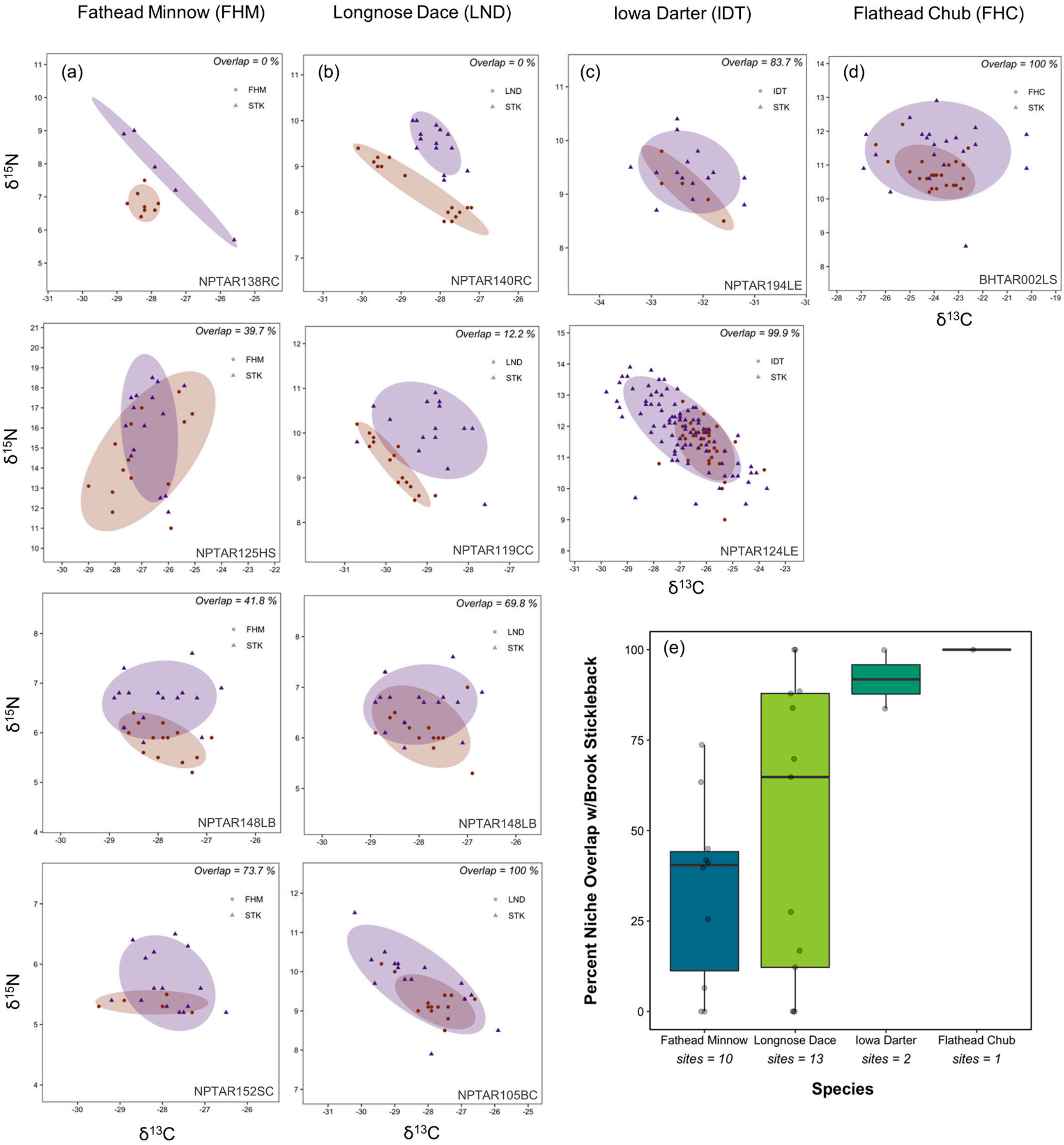

Fig. 4. Isotopic niche overlap between native fishes and Brook Stickleback (STK) varied across species and sites. Overlap is shown at a selection of sites where the species co-occurred with Brook Stickleback for (a) Fathead Minnow and (b) Longnose Dace, and at all sites for (c) Iowa Darter and (d) Flathead Chub. Species are separated by column and each panel represents one site. Within a panel, each point represents a single fish, with species differentiated by color. Site IDs are provided in the bottom right of each panel; IDs that begin with “NP” are in the North Platte drainage and sites that begin with “BH” are in the Bighorn. The variation in native species isotopic niche overlap with Brook Stickleback across all sites, and number of distinct sites analyzed for each species, is also included (e). See Fig. S4 and Fig. S5 for all isotopic niche overlap plots for Fathead Minnow and Longnose Dace with Brook Stickleback.

Stickleback, Fathead Minnow, and Iowa Darter given the species’ shared habitat preferences and isotopic results. Future research focusing on potential effects for Iowa Darter would be valuable given its status as a species of a greatest conservation need. For managers interested in conserving native populations, trophic studies provide insight on complex species interactions and allow for characterization of the risk posed to native communities by invaders, which is crucial for prioritizing management actions. In addition, our study contributes to a growing body of knowledge about the effects of invasive species relative to species traits (Ruesink, 2005; Howeth et al., 2016). In our study, a generalist small-bodied invertivore appears to have a limited effect on native fish resource use. This contrasts with previous work in Wyoming on large bodied invasive piscivores that found strong negative effects of piscivore presence for native fish abundance (Hickerson et al., 2019; Booher and Walters, 2021). As introductions of invasive species become increasingly common amid anthropogenic disturbance, trophic studies of invaders will play a crucial role in guiding the conservation and recovery of native species.

CRediT authorship contribution statement

Jacob S. Ruthven: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization. **Annika W. Walters:** Conceptualization, Methodology, Resources, Writing – review & editing, Supervision, Funding acquisition, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fooweb.2023.e00275.

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