

ARTICLE

# Effects of Multiple Nonnative Fish on an Imperiled Cyprinid, Hornyhead Chub

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**Abstract**

Nonnative fish can have substantial negative effects on the abundance and distribution of native fishes through predation and competition. Nonnative predators are of particular interest because they represent novel threats to native prey species that are not adapted to their presence. Prey species with limited distributions or population sizes may be particularly vulnerable to the effects of nonnative predators. In the Laramie River, four nonnative predators—Brown Trout *Salmo trutta*, Rainbow Trout *Oncorhynchus mykiss*, Brook Trout *Salvelinus fontinalis*, and Smallmouth Bass *Micropterus dolomieu* are present along with a state-imperiled population of Hornyhead Chub *Nocomis biguttatus*. The abundance of Hornyhead Chub has declined with increasing abundance of nonnative predators, with the probability of occurrence of Hornyhead Chub dropping drastically when Smallmouth Bass were present. All four nonnative species preyed on native cyprinids, but Smallmouth Bass relied most heavily on fish as a prey item. Isotopic niche overlap occurred between Hornyhead Chub and all of the nonnative predator species. Our results demonstrate that nonnative predators have the potential to negatively affect the abundance and distribution of Hornyhead Chub through the mechanisms of predation and competition, and predator identity is important in determining the extent of effects. Smallmouth Bass are a greater concern than nonnative salmonids because of their more piscivorous behavior, and their recent upstream expansion may be limiting the downstream distribution of Hornyhead Chub in the Laramie River.

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Interactions between predator and prey species play an important role in structuring aquatic communities (Hoeinghaus and Pelicice 2010; Cucherousset and Olden 2011). Piscivorous fishes can cause declines in the abundance and distribution of prey species through direct predation (Garman and Nielsen 1982; Kershner and Lodge 1995; Jackson et al. 2001) or through competitive

interactions for space or resources (Mills et al. 2004). Assessing the relative importance of competitive versus predatory interactions in driving changes in the abundance of prey species is difficult (Crowder 1980; Ross 2013), but understanding the importance of these drivers is critical to the effective management of fisheries resources, particularly in contemporary ecosystems with nonnative species.

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Nonnative predators represent a novel threat to native prey populations that are not adapted to their presence (Hughes and Herlihy 2012). The number of predator species that are present in a local community and their respective identities are likely important factors that determine the extent of predator effects on prey species. Further, the identity of nonnative predators will be particularly important when they vary in their feeding strategy or habitat use (MacRae and Jackson 2001; Hughes and Herlihy 2012).

Understanding the competitive and predatory effects of nonnative predators is especially important for native species that are of conservation concern. These species are inherently less resilient to new threats because they typically have limited distributions and small population sizes (Labbe and Fausch 2000). For example, nonnative Brook Trout *Salvelinus fontinalis* displace native Cutthroat Trout *Oncorhynchus clarkii* populations from important habitats through competitive exclusion (Shepard et al. 2002; McGrath and Lewis 2007). Elsewhere, the introduction of nonnative Northern Pike *Esox lucius* has led to the decline of geographically isolated and regionally imperiled cyprinids in Montana and darters in Colorado due to predation (Labbe and Fausch 2000; Stringer 2018). Similarly, studies have shown that nonnative Brown Trout *Salmo trutta* and Rainbow Trout *Oncorhynchus mykiss* negatively affect populations of endangered Humpback Chub *Gila cypha*, primarily through predation (Yard et al. 2011). Therefore, species with geographically restricted distributions may be at an increased extirpation risk from nonnative predators due to a lack of population redundancy across the landscape.

Hornyhead Chub *Nocomis biguttatus*, with its highly restricted range, is a species that is greatly in need of conservation efforts in Wyoming (Bestgen 2013). Western populations of Hornyhead Chub have historically occurred in the Platte River tributaries in Wyoming, Colorado, western Nebraska, and western Kansas. Western populations are currently restricted to the Laramie and North Laramie rivers in Wyoming (Propst and Carlson 1986; Baxter and Stone 1995; Bestgen 2013). The Laramie River also has naturally reproducing populations of four nonnative predatory species: Brown Trout, Rainbow Trout, Brook Trout, and Smallmouth Bass *Micropterus dolomieu*. These four nonnatives can all negatively affect the distribution and abundance of the native fish populations (Garman and Nielsen 1982; Dunham et al. 2002; Fritts and Pearsons 2006; Johnson et al. 2008; Yard et al. 2011). The sympatric occurrence of these four species provides a rare opportunity to study the effects of multiple nonnative predators and predator identity on a native fish species that is of significant conservation concern.

Nonnative salmonids have inhabited in the lower Laramie River for decades, with the first stockings of Rainbow

Trout, Brown Trout, and Brook Trout occurring in the late 1930s and early 1940s (S. Gale, Wyoming Game and Fish Department, personal communication). Smallmouth Bass were first introduced into Grayrocks Reservoir, which impounds the Laramie River, in 1981, and they have rapidly expanded their upstream distribution during the last decade (Quist et al. 2005; Hickerson 2018). Smallmouth Bass were not detected upstream of Grayrocks Reservoir during sampling in 2004 or 2005 (Bear and Barrineau 2007), but in 2008 and 2009 Smallmouth Bass were detected 19 and 26 km upstream, respectively (Moan et al. 2010; Bestgen 2013). By 2015, Smallmouth Bass had moved approximately 47 km upstream (Peterson 2017). Currently, the recruitment of Smallmouth Bass occurs throughout the lower Laramie River and individuals have been captured more than 71 river km upstream of Grayrocks Reservoir (Hickerson 2018). The increased upstream movement coincided with a rapidly expanding Smallmouth Bass population in Grayrocks Reservoir after 2009, which is attributed to filling Grayrocks Reservoir after a number of drought years and higher flows—which also likely facilitated the upstream dispersal of Smallmouth Bass (Wyoming Game and Fish Department 2011).

Our objectives were to (1) quantify the relationships between the abundance and distributions of Hornyhead Chub and nonnative predatory species and (2) evaluate the potential for competition, predation, or both between the nonnative predator species and Hornyhead Chub. We examined the four nonnative predator species individually and combined to determine the importance of predator identity.

## METHODS

*Study area.*—The Laramie River flows northeast from the Medicine Bow Mountains in Colorado through southeast Wyoming, and it is impounded by two major reservoirs before reaching its confluence with the North Platte River near Fort Laramie, Wyoming (Figure 1A). The lower Laramie River, downstream of the Wheatland Tunnel Diversion, has one of the most intact and diverse native fish communities in the North Platte River watershed of Wyoming, where sensitive species like Common Shiner *Luxilus cornutus* and Stonecat *Noturus flavus* are relatively abundant (Quist et al. 2005). The flows in this reach are regulated year-round by a series of reservoirs and diversion dams. Historically, Hornyhead Chub were found in the Laramie River from the Wheatland Tunnel Diversion downstream approximately 126 km to the confluence with the North Platte River near Fort Laramie, Wyoming (Baxter and Simon 1970; Baxter and Stone 1995; Quist et al. 2005; Figure 1A). We sampled the current occupied range, a 42-km reach of the Laramie River from the Wheatland Tunnel Diversion to just downstream

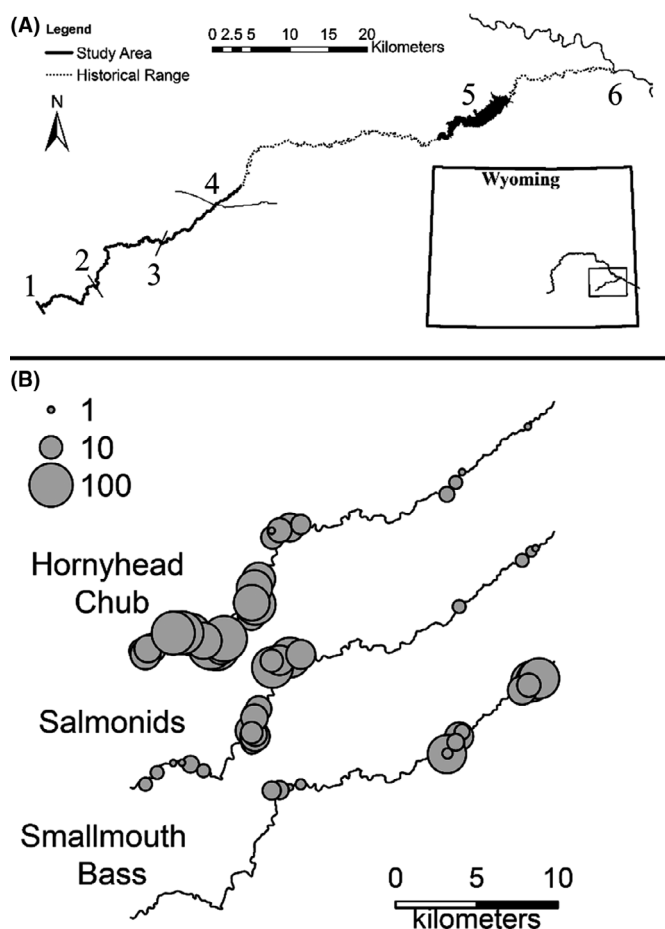


FIGURE 1. Map showing (A) the historical range of Hornyhead Chub within the Laramie River, which went from the Wheatland Tunnel Diversion (1) downstream to the confluence with the North Platte River near Fort Laramie, Wyoming (6; dotted line). The current distribution is the 42-km study reach (wide black line), starting at the Wheatland Tunnel Diversion (1) downstream to near Palmer Canyon Road (4), with reach breaks at Bradbury Gulch (2), and the Cramer Diversion (3). Also included is the location of Grayrocks Reservoir (5). The inset (B) shows the details of the Laramie River study area. The gray points indicate the sites that were sampled during 2016 and 2017, with the size of the points indicating the estimated abundance (fish per hour) of Hornyhead Chub (top), all salmonids (Brook Trout, Brown Trout, and Rainbow Trout; middle), and Smallmouth Bass (bottom) at each site. Hornyhead Chub were most abundant upstream and decreased in abundance downstream. Smallmouth Bass were absent from the upper half of the study area but increased in abundance downstream. Salmonids were most abundant in the middle reach.

of Palmer Canyon Road (Figure 1). Hornyhead Chub have never been collected upstream of the Wheatland Tunnel Diversion, which is a barrier to upstream movement, and they have rarely been found downstream of Palmer Canyon Road since the completion of Grayrocks Reservoir in 1980 (Quist et al. 2005; Bestgen 2013). The 42-km study reach flows through the Laramie River Canyon starting at an elevation of 1,969 meters and dropping

to 1,417 meters (Bestgen 2013). The mean stream temperature in August within the study reach ranges from 17.6°C near the Wheatland Tunnel diversion to 19.8°C downstream near Palmer Canyon Road, which is well within the reported thermal range for Hornyhead Chub (spawning: 16–26°C; Vives 1990; thermal maximum: 35.6°C; Smale and Rabeni 1995) from other parts of their range.

The Laramie River sampling reach was stratified into three subreaches to account for variations in stream habitat and fish community: (1) upper reach (11 km), (2) middle reach (16 km), and (3) lower reach (15 km; Figure 1A). The upper reach is characterized by a moderate gradient; it has few nonnative predatory fish, and Smallmouth Bass are excluded from this reach by at least one potential natural barrier. The middle reach has a relatively high gradient, is dominated by large boulder substrates, is bound by a deep canyon, and supports all four nonnative predatory species. The lower reach has a lower gradient, and Smallmouth Bass are the dominant nonnative predator species. Our study system is unusual because environmental features that are usually associated with the headwaters of a stream (e.g., higher gradients, larger substrates, and canyon bound) are found in the middle of our study area.

We randomly chose seven sampling sites per subreach in 2016 and 2017 for a total of 42 sites. If a randomly chosen site fell on a parcel of private land where access had been denied, fell on sites that were already sampled, or were more than 5 miles from vehicle access, we randomly chose another site until all of the sites fell on accessible land. Additionally, if a randomly chosen site fell on sections of stream that were determined to be too difficult to sample in the field (i.e., not wadeable), we continued upstream to the nearest accessible site. No sites were repeated between 2016 and 2017. The lower and middle reaches have limited amounts of publicly accessible land, so access depended strongly upon the cooperation of private landowners.

The habitat measures that were collected at each sampling site included stream width, stream depth, water velocity, discharge, substrate size, cover type, and macrohabitat length. Additionally, one temperature logger was installed near the Wheatland Tunnel Diversion and another near Palmer Canyon Road. The predicted mean August temperatures for all of the sites were modeled by using the temperature logger data and a multiple regression model following the procedure outlined in Isaak et al. (2009). A full description of the procedure for collecting habitat data can be found in Hickerson and Walters (2019).

*Fish sampling.*—The sampling sites were 100 m in stream length. Three passes of depletion electrofishing with two backpack electrofishers (Smith Root; Model LR-24) and block nets were used to sample the fish. After each pass, all of the fish were identified to species,

counted, and placed in a live-car downstream of the sampling reach. The Hornyhead Chub and the nonnative predators were measured to the nearest millimeter total length (mm TL). We excluded a single site from the upper reach in 2017 from the subsequent analyses because we failed to achieve depletion due to an equipment error.

*Diet sampling.*—Short-term diet was characterized by using the stomach contents that were collected from all of the nonnative predators that measured over 200 mm TL. The stomach contents were collected using pulsed gastric irrigation (Light et al. 1983), preserved in individual containers with 95% ethanol, and transported to the laboratory for identification. The diet items from each sample were identified to order, counted, and weighed. The fish that were in the diet samples were identified to species when possible. We did not collect Hornyhead Chub stomach contents because of concerns that the survival of the small fish after handling might be low, and we did not want to negatively affect the population.

*Stable isotope sample collection.*—The longer-term patterns in the diets and trophic positions of the sampled fish were assessed by using stable isotope analysis (Post 2002; Boecklen et al. 2011). We collected muscle plugs from all of the nonnative predators that were over 200 mm TL by using a biopsy punch (Premier Medical Products; Uni-Punch; Vinson and Budy 2011). We also collected muscle plugs from Hornyhead Chub from the upper and middle reaches (2016:  $n = 12$ ,  $105.6 \pm 9$  mm TL [mean  $\pm$  SE]; 2017:  $n = 8$ ,  $105.5 \pm 15.9$  mm TL). Trophic baselines (seston and epilithic biofilms) were collected at one site in each reach in July, August, and September of each year. Using a modified portable drill pump, the seston samples were collected in the field by filtering up to 10 L of river water onto precombusted (at 500°C for 4 h) filters (Whatman GF/F; Kelso and Baker 2016). The biofilm samples were collected from rocks (at least five per reach) that were scrubbed with a brush to obtain slurries. The slurries were passed through a 500- $\mu$ m sieve to remove coarse detritus and larger invertebrates, collected in a plastic bottle, and placed on ice in a dark cooler until they were frozen (Jardine et al. 2014). In the laboratory, the samples were oven dried (at 60°C for 48 h), homogenized with a mortar and pestle, weighed to the nearest 0.001 mg, and loaded into tin capsules. The samples were analyzed at the University of Wyoming Stable Isotope Facility by using a Carlo Erba 1110 Elemental Analyzer coupled to a continuous-flow stable isotope ratio mass spectrometer (Thermo Finnigan; THERMO XP). The values for the stable isotopes ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) are reported with respect to the international standards for Vienna PeeDee Belemnite carbonate (V-PDB) and atmospheric nitrogen (AIR), respectively, in parts per thousand (per mille; ‰). The measurements of in-house standards (Liver [UWSIF01] and Alfalfa

[UWSUF05]) across all of the runs were both accurate and precise, with mean  $\pm$  SD of  $-17.8 \pm 0.05$ ‰ for  $\delta^{13}\text{C}$  and  $-6.8 \pm 0.04$ ‰ for  $\delta^{15}\text{N}$  for Liver ( $n = 66$ ) and mean  $\pm$  SD of  $-27.5 \pm 0.04$ ‰ for  $\delta^{13}\text{C}$  and  $-0.2 \pm 0.09$ ‰ for  $\delta^{15}\text{N}$  for Alfalfa ( $n = 6$ ).

*Statistical analysis.*—Three-pass depletion (the Carle–Strub method) was used to estimate abundance for all of the fish species that were encountered at each site by using the “removal” function in the FishR package (Ogle 2017). We evaluated whether the abundance of Hornyhead Chub and nonnative predators differed between years, using a Wilcoxon rank-sum test to determine whether the data from both years could be combined for the analyses.

The relationships between the estimated abundance for Hornyhead Chub and nonnative predators were evaluated by using generalized linear models with a negative binomial error distribution to account for overdispersion (Venables and Ripley 2002). The models were tested for zero-inflation using Vuong's nonnested test, and in the case of zero-inflation a zero-inflated negative binomial model was used (Vuong 1989). To evaluate the effects of nonnative predators on the distribution of Hornyhead Chub, we used logistic regression to model the presence or absence of Hornyhead Chub against the presence of each predator both individually and combined.

Random forest models were run using the randomForest package to model the abundance of Hornyhead Chub as it related to the habitat variables and fish abundances (see the list of variables in Table A.1.1; Breiman 2001; Liaw and Wiener 2002). The models were run with standardized variables, using a regression approach with 1,001 trees. The selection of variables was performed as described in Murphy et al. (2010); the variable selection process calculates a model improvement ratio for each variable and sets a threshold for the number of variables to be retained. The variable set that minimized the mean squared error of the model and maximized the percentage of variation explained was retained as the top model. Relative importance plots were used to quantify and rank the relative ability of the retained variables to predict the abundance of Hornyhead Chub. For the selected variables, partial probability plots were generated, which plot the variable range against the abundance of Hornyhead Chub.

To assess the relative importance of the diet items in the stomachs of nonnative predators, we used an index of relative importance (%IRI). The index of relative importance takes into account the proportional number of diet items, the proportional weight of the items, and the frequency of occurrence of the items in diets (Cortés 1997). The items were grouped into one of seven categories: algae, amphibians, aquatic invertebrates, terrestrial invertebrates, fish, crayfish, and debris.

The frequency of fish present in the diets was calculated for each nonnative predator species by dividing the number of individuals with fish in their stomach by the total number of individuals, excluding those having empty stomachs. The potential relationships between the frequency of fish that was present in diets and total length were assessed by using logistic regression. The sample sizes were not sufficient for a more rigorous quantitative assessment of diet selectivity.

For the stable isotope sampling, we normalized  $\delta^{13}\text{C}$  ratios for the effects of lipid content (Post et al. 2007). The isotope signatures for carbon were further corrected for baseline variation by calculating the difference between the carbon signature for each fish at a given reach and the mean baseline carbon signature at the same reach and dividing by the carbon signature range for all of the baseline samples from that reach (Walsworth et al. 2013). The isotope signatures for nitrogen were corrected for baseline variation by subtracting the difference between the absolute value of the mean nitrogen signature for the reach and the absolute value of the minimum mean nitrogen signature for all of the reaches from the nitrogen signature of each fish from that reach (Walsworth et al. 2013). We then used kernel utilization density methods to estimate the isotopic niches for Hornyhead Chub and of the all nonnative predator species for each year (KUD; Eckrich et al. 2018, in press). The KUD method draws on the existing spatial metrics to estimate isotope niches (e.g., the minimum convex polygon [Layman et al. 2007] and standard ellipses [Jackson et al. 2011]), but importantly it allows users to describe the isotopic niche with a nonparametric estimator that precisely captures the distribution of the data and yields an accurate delineation of niche space and overlap. We extracted the 95% contours (i.e., ellipses) with the rKIN package (Albeke 2017). The percentage of niche overlap between the species was evaluated to assess the potential for competition between nonnative predators and Hornyhead Chub (Eckrich et al. 2018, in press). Trophic position was calculated for all of the nonnative predators that were greater than 200 mm TL and all of the Hornyhead Chub by using the equation presented in Walsworth et al. (2013), assuming a baseline trophic position of 1 and trophic fractionation of 3.4‰ for  $\delta^{15}\text{N}$  (Post 2002). We tested for significant differences in the mean trophic position of the fish between years by using a Wilcoxon rank-sum test, and we tested for differences among the nonnative predator species within each year by using a Kruskal–Wallis rank-sum test with a pairwise Wilcoxon rank-sum test to determine whether the species pairs differed. Finally, we evaluated whether trophic position was related to total length for both nonnative predators and Hornyhead Chub by using generalized linear modeling. All of the statistical analyses were carried out in Program R (R Core Team 2016, version 3.4.2).

## RESULTS

### Abundance and Distribution

The results from the Wilcoxon rank-sum tests for average abundance estimates for both Hornyhead Chub and total nonnative predators did not differ between years ( $W=242.5$ ,  $P=0.398$ ;  $W=152.5$ ,  $P=0.136$  for chub and nonnative predators, respectively), so we combined the data from both years for the analyses. The estimated abundance for Hornyhead Chub was highest at the sites in the upper reach and declined downstream through the middle and lower reaches (see Figure A.2.1). Rainbow Trout were the most abundant nonnative predator in the upper reach but still occurred at relatively low numbers (Table 1). Brook Trout were the most abundant nonnative predator species in the middle reach, occurring at all of the sites that were sampled (Table 1). Smallmouth Bass were by far the most abundant nonnative predator species in the lower reach, occurring at every site that was sampled (Table 1).

There was a strong, negative relationship between the estimated abundance for Hornyhead Chub at a site and the estimated abundance for all nonnative predators at a site ( $P=0.010$ ; Figure 2). This was driven by a strong, negative relationship between the estimated abundance for Hornyhead Chub and the estimated abundance for Smallmouth Bass ( $P<0.001$ ; Figure 2D). The estimated abundance for Hornyhead Chub was not significantly related to the estimated abundance for the salmonid predator species (Brook Trout [ $P=0.538$ ], Brown Trout [ $P=0.437$ ], or Rainbow Trout [ $P=0.089$ ]), but Hornyhead Chub were most abundant at the sites with no salmonid predators.

The presence of Hornyhead Chub was negatively related to the presence of Smallmouth Bass ( $P=0.003$ ). The results indicated that the probability of presence for Hornyhead Chub when Smallmouth Bass were absent ( $P=0.913$ ) was significantly higher than that for when bass were present ( $P=0.444$ ). Hornyhead Chub were absent from 10 of the 18 sites where Smallmouth Bass were captured, and the estimated abundance when bass were present was low ( $6 \pm 6.48$  [mean  $\pm$  SD]). The presence of Hornyhead Chub was not significantly related to the presence of Rainbow Trout ( $P=0.087$ ) or Brown Trout ( $P=0.644$ ), and there was a significant positive relationship between the presence of Hornyhead Chub and Brook Trout ( $P=0.036$ ).

The top random forest model retained 12 variables and had an  $R$ -squared of 0.53. Modeled temperature (1.00) and Smallmouth Bass abundance (0.63) had the highest relative importance values by far, with all of the other variables having importance values less than 0.4 (see Table A.1.1). The abundance estimates for Hornyhead Chub increased with decreasing temperature and decreasing abundance of Smallmouth Bass (see Figure A.2.2).

TABLE 1. Comparison of mean estimated abundance (per 100 meters) of Hornyhead Chub (HHC), Brown Trout (BNT), Brook Trout (BKT), Rainbow Trout (RBT), and Smallmouth Bass (SMB) between the upper, middle, and lower reaches, with minimum and maximum estimates for each species and reach.

	Upper reach ( $n = 13$ )			Middle reach ( $n = 14$ )			Lower reach ( $n = 14$ )		
	Mean (SD)	Min	Max	Mean (SD)	Min	Max	Mean (SD)	Min	Max
HHC	60.85 (48.49)	0	127	23.5 (21.53)	0	73	0.64 (1.28)	0	4
BNT	0.07 (0.27)	0	1	2.21 (3.12)	0	11	0.57 (1.02)	0	3
BKT	0.07 (0.27)	0	1	9.07 (13.34)	1	53			
RBT	1.08 (1.66)	0	5	11.07 (10.63)	0	43	0.07 (0.27)	0	1
SMB	0	0	0	1.07 (2.16)	0	6	27.86 (25.24)	1	72

### Diet Analysis

Stomach samples were collected from 120 Rainbow Trout, 74 Brook Trout, 30 Brown Trout, and 53 Smallmouth Bass, yielding a total of 277 diet samples. Only four of the 277 stomachs that were sampled were empty. Brown Trout, Brook Trout, and Rainbow Trout relied mostly on aquatic invertebrates as a prey source (%IRI = 96.9, 93.2, and 70.9, respectively) with fish being of little importance in the diet (%IRI = 0.45, 0.06, and 0.19 respectively; Figure 3). Smallmouth Bass also had a heavy reliance on aquatic invertebrates (%IRI = 56.18), but crayfish (%IRI = 26.42) and fish (%IRI = 14.59) made up a substantial component of the overall diet (Figure 3).

The frequency for presence of fish in the diet was low for Brown Trout (3.33%), Brook Trout (4.05%), and Rainbow Trout (4.17%), but it was strikingly consistent among the three trout species. Smallmouth Bass were much more piscivorous (25.49%) with over a quarter of all individuals sampled having some kind of fish remains in their stomach. The total length of the predator species was not significantly related to the presence of fish their diets (Rainbow Trout [ $P = 0.144$ ], Brown Trout [ $P = 0.426$ ], Brook Trout [ $P = 0.320$ ], or Smallmouth Bass [ $P = 0.065$ ]; Figure 4).

The identifiable fishes in the diet samples from Smallmouth Bass included Creek Chub *Semotilus atromaculatus* ( $n = 3$ ), Central Stoneroller *Campostoma anomalum* ( $n = 2$ ),

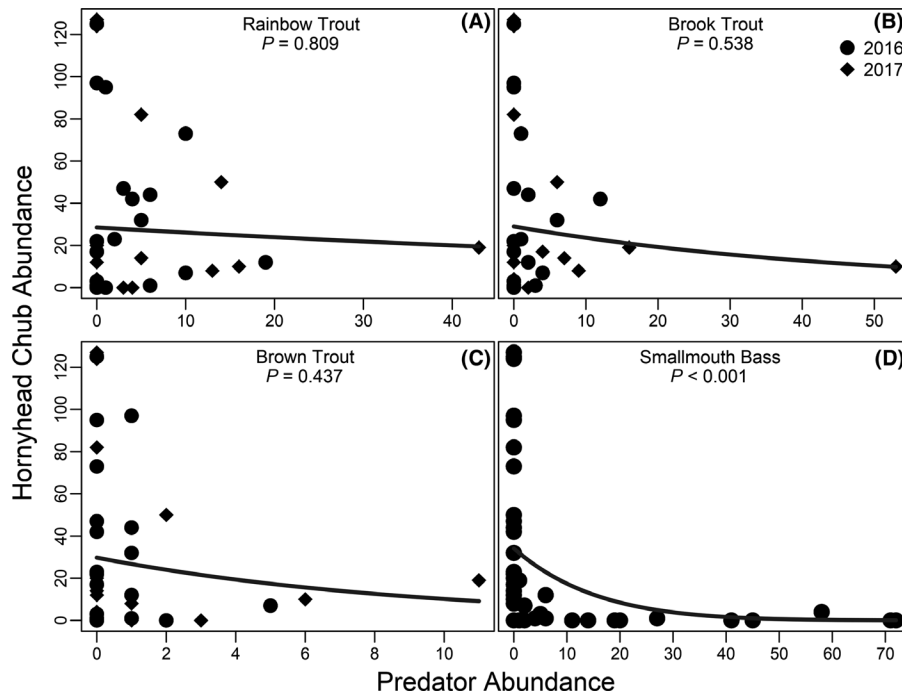


FIGURE 2. Relationship between the estimated abundance for Hornyhead Chub and the estimated abundance for salmonid predator species (Brook Trout [ $P = 0.538$ ], Brown Trout [ $P = 0.437$ ], and Rainbow Trout [ $P = 0.089$ ]). There is a strong negative relationship between the estimated abundance for Hornyhead Chub and the estimated abundance for Smallmouth Bass ( $P < 0.001$ ).

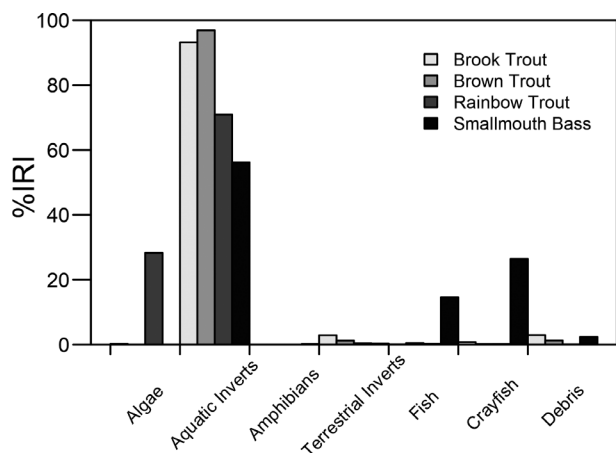


FIGURE 3. Index of relative importance (%IRI) values for seven prey categories for Brook Trout, Brown Trout, Rainbow Trout, and Smallmouth Bass (light to dark bars, respectively). All of the species relied heavily on aquatic invertebrates, but Smallmouth Bass also consumed substantial amounts of fish and crayfish. The consumption of algae by trout was likely incidental while trying to capture invertebrates.

Stonecat ( $n=3$ ), and Smallmouth Bass ( $n=1$ ). The identifiable fishes in the salmonid stomachs included Common Shiner ( $n=3$ ), Creek Chub ( $n=2$ ), and Hornyhead Chub ( $n=3$ , two of which had been consumed by Rainbow Trout and one by a Brook Trout). Generally, the most abundant prey fish were the ones that were consumed, with Hornyhead Chub typically being consumed at sites where they were relatively abundant.

### Stable Isotope Analysis

Predator niche size (determined with KUDs) varied among species and years (Figure 5). From 2016 to 2017, niche contraction was observed in Rainbow Trout and Brown Trout and niche expansion was observed in Brook Trout and Smallmouth Bass. The estimated isotopic niche overlap between Hornyhead Chub and nonnative predators varied between years, and the variation was predominately due to niche shifts of the predators. In 2016, Hornyhead Chub had the greatest niche overlap with Rainbow Trout (56.3%), with Brown Trout (39.1%), Smallmouth Bass (28.8%), and Brook Trout (10.6%) having slightly less overlap (Figure 5). In 2017, the niche overlap was reduced for Rainbow Trout (36.9%) but increased for Smallmouth Bass (75.9%), Brown Trout (46.0%), and Brook Trout (32.6%; Figure 5).

The trophic positions of all of the nonnative predators were significantly different between years ( $W=1819$ ,  $P < 0.001$ ). The mean trophic position was significantly different between predator species in 2016 ( $\chi^2 = 31.259$ ,  $df = 3$ ,  $P < 0.001$ ), but not in 2017 ( $\chi^2 = 3.003$ ,  $df = 3$ ,  $P = 0.391$ ). The trophic position for Smallmouth Bass ( $3.04 \pm 0.21$  [mean  $\pm$  SD]) was significantly higher than that for Rainbow Trout ( $2.77 \pm 0.15$ ,  $P < 0.001$ ) and Brook Trout ( $2.71 \pm 0.12$ ,  $P < 0.001$ ), but not Brown Trout ( $2.88 \pm 0.22$ ,  $P = 0.13$ ) in 2016. In 2017, Smallmouth Bass had the highest mean trophic position ( $3.19 \pm 0.15$ ), but it was not significantly different from the three nonnative salmonid species (Rainbow Trout,  $3.13 \pm 0.13$ ; Brook Trout,  $3.12 \pm 0.15$ ; Brown Trout  $3.12 \pm 0.23$ ). The trophic position of

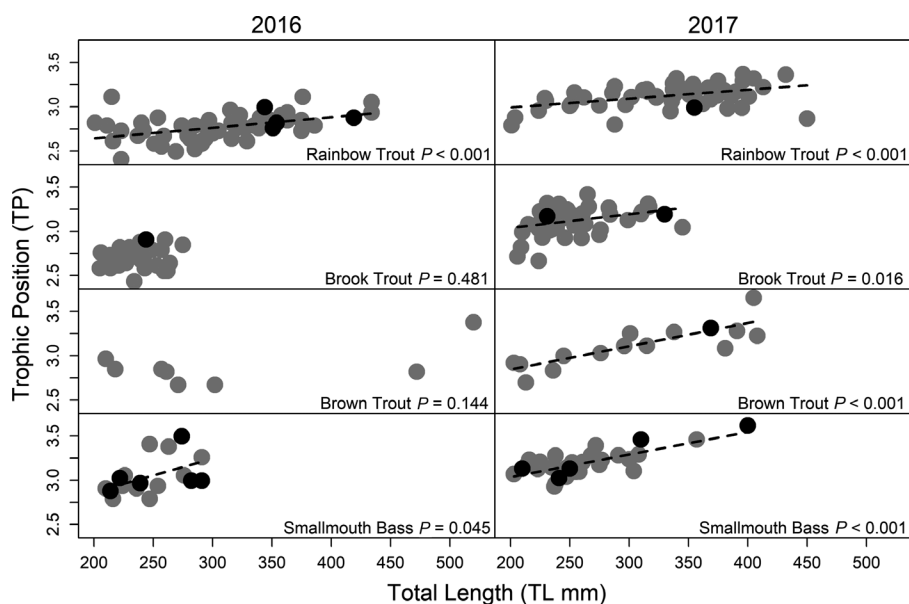


FIGURE 4. Trophic position (y-axis) as a function of total length (x-axis) for all four nonnative predator species and each study year. The panels show the  $P$ -value for each species and year and the fitted regression line for the significant relationships. The black circles indicate individuals that had fish remains in their stomachs at the time of capture.

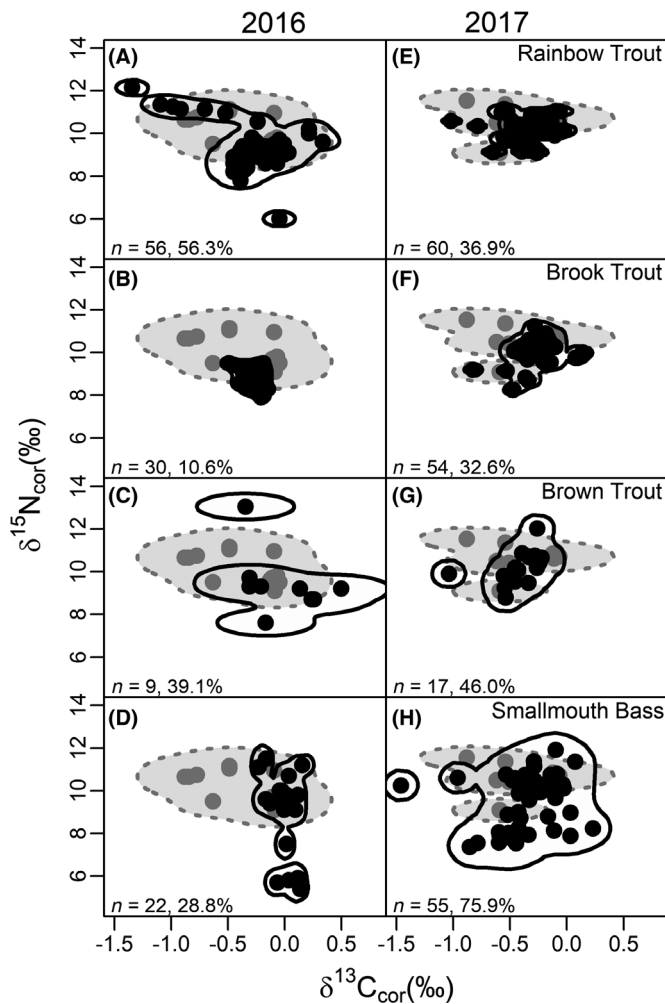


FIGURE 5. Kernel isotopic niche estimates for Hornyhead Chub (gray circles, dashed line) varied in overlap with nonnative predators (black circles, solid black line) between (A–D) 2016 ( $n = 12$ ) and (E–H) 2017 ( $n = 8$ ). The percentages indicate the amount of niche overlap between Hornyhead Chub and a single predator species: (A, E) Rainbow Trout, (B, F) Brook Trout, (C, G) Brown Trout, and (D, H) Smallmouth Bass.

Hornyhead Chub (2016:  $2.83 \pm 0.11$ , 2017:  $3.02 \pm 0.30$ ) was comparable to that of the nonnative predator species. Trophic position was significantly related to total length for all of the nonnative predator species each year, with the exception of Brook Trout ( $P = 0.481$ ) and Brown Trout ( $P = 0.144$ ) in 2016 (Figure 4).

## DISCUSSION

The results of this study indicate that levels of abundance and distributions for Hornyhead Chub are negatively correlated with the presence of nonnative predators in the Laramie River. Furthermore, species identity seems to be important for determining the extent of predator effects. At sites with relatively high abundance of

nonnative salmonids, Hornyhead Chub persist, although at lower abundances. In contrast, there is limited overlap between sites with Smallmouth Bass and Hornyhead Chub and when they overlap abundance is low for Hornyhead Chub. Although all of the sampled nonnative predator species were found to consume native cyprinids, Smallmouth Bass had fish in their diets more frequently, had fish in their stomachs at smaller sizes, and had a higher mean trophic position than did nonnative salmonids. This suggests that in the Laramie River, Smallmouth Bass predation could be a greater threat to native fish species, such as Hornyhead Chub, than that from nonnative salmonids.

Differences in the degree of piscivory and habitat use among predator species could explain why Hornyhead Chub appear to be able to coexist with nonnative salmonids but not with Smallmouth Bass. In small Ontario lakes, it was proposed that use of deeper thermal refugia by salmonids during summer thermal stratification explained why salmonids had less of an effect than did Smallmouth Bass, which occupied the same habitats as the sensitive cyprinids year-round (MacRae and Jackson 2001). Further, predation risk from Smallmouth Bass has previously been found to drive Hornyhead Chub movement from preferred habitats into shallow water refugia (Schlosser 1987) and limited refuge microhabitat availability for small-bodied native fish has been shown to result in high spatial overlap and predation by Smallmouth Bass (Johnson et al. 2008). Smallmouth Bass are sympatric with Hornyhead Chub throughout much of their distribution in the Midwest, where they prey on Hornyhead Chub, but predation does not lead to extirpation (Becker 1983). The Hornyhead Chub that inhabit the Laramie River might be more susceptible to predation because Smallmouth Bass are a novel predator in the system, so they may not be behaviorally adapted to predation pressure or there may be a lack of refuge habitat in the Laramie River (Schlosser 1987; Matter and Mannan 2005).

In the Laramie River, Hornyhead Chub showed a strong longitudinal decrease in abundance moving downstream, which corresponds to increasing temperatures and increased Smallmouth Bass presence and abundance. There was a slight downstream increase in stream temperature ( $\sim 2\text{--}3^\circ\text{C}$ ), with Smallmouth Bass primarily found at sites with predicted mean August temperatures greater than  $19^\circ\text{C}$ . This temperature threshold corresponds with what has been found in other studies on Smallmouth Bass (Lawrence et al. 2012). Disentangling the effect of temperature and that of Smallmouth Bass is not possible with only field data. While Hornyhead Chub appear to prefer cooler temperatures, we do not believe temperatures  $>19^\circ\text{C}$  are a limiting factor for Hornyhead Chub because they are well within the range required for spawning ( $16\text{--}26^\circ\text{C}$ ) and the maximum daily peak water temperatures were far below lethal levels ( $35.6^\circ\text{C}$ ; Vives 1990; Smale



and Rabeni 1995). In addition, Hornyhead Chub was historically present at downstream locations (Quist et al. 2005) and a natural fish barrier appears to be preventing further upstream invasion of Smallmouth Bass (Hickerson 2018).

Longitudinal habitat shifts could also be driving fish presence and abundance, but the lower Laramie River does not show clear longitudinal habitat shifts. Turbidity is a habitat characteristic known to be important for Hornyhead Chub (Mammoliti 2002). We did not measure stream turbidity, but we did not observe longitudinal changes in turbidity in the summer. However, silt released from the Wheatland Tunnel Diversion during October drawdowns likely caused increased turbidity for some of the upper sites in the fall. Previous research on the Laramie River suggested that low summer discharge was one factor limiting Hornyhead Chub distribution (Bestgen 2013). However, our research occurred during a period of relatively high and stable flows, primarily regulated by diversions, and discharge was not found to be an important limiting factor (Hickerson and Walters 2019). In general, a comparison between Laramie River sites with and without Hornyhead Chub found that differences in the fish communities were much greater than differences in habitat (Hickerson and Walters 2019). The fish community data suggest that the low abundance of Hornyhead Chub in the lower reach coincides with the upstream invasion of Smallmouth Bass (Quist et al. 2005; Bear and Barrineau 2007; Moan et al. 2010).

The results for both diet and isotope signatures support some degree of fish consumption for Smallmouth Bass and salmonids, but combining these approaches provided a more resolved understanding of piscivory in this system. Stable isotope analysis is better able to detect piscivory than stomach content analysis alone (Vinson and Budy 2011), and our isotope data suggests that a higher proportion of nonnative predators are piscivorous than our diet data indicates—fish that had fish in their diet had a similar trophic position to fish that did not. Stomach contents can rapidly degrade to the point where they become unidentifiable, especially for food items such as larval fish, and may only represent the previous few hours or days of feeding (Schooley et al. 2008). We did not find any Hornyhead Chub in the stomachs of Smallmouth Bass, but Smallmouth Bass preyed on morphologically similar native cyprinids (Creek Chub and Central Stoneroller), which suggests that Smallmouth Bass would likely consume Hornyhead Chub when they encounter them. Trophic position increased with fish size, as has been commonly documented elsewhere (McIntyre et al. 2006; Vinson and Budy 2011). Our data suggest that all of the examined individuals are piscivorous to some extent, but larger individuals are likely either eating a higher proportion of fish relative to other diet items (e.g.,

invertebrates) or larger fish than are smaller individuals. This demonstrates the importance of using multiple diet tracers when investigating predation because had we relied on diet data alone we would have concluded that only a few individuals were piscivorous and that piscivory had no relationship to the total length of predators.

The stable isotope results also provided insight into the potential for competition between species. Niche overlap between Hornyhead Chub and nonnative predators was variable but substantial across species and years (10–75% overlap). While we did not find any evidence of food limitation, the existence of niche overlap is evidence that competition for food resources could also be occurring among Hornyhead Chub and nonnative predators.

Our results are limited because the data were collected during the summer months of July, August, and September. The diet composition of predators is known to shift seasonally, especially when prey fish are vulnerable during spawning (Museth et al. 2003). Therefore, we may not be fully accounting for the annual diet composition of the nonnative predator species and could be missing seasonally important periods of piscivory (i.e., during nest guarding and spawning by Hornyhead Chub). We were also limited to sampling sections of the river where permission to access private property was granted. As a result we were not able to account for some potentially important sections within the lower reach where the Laramie River transitions out of the canyon onto the prairie.

Our results add to the growing concern about nonnative predators and have important implications for fisheries managers tasked with conserving at-risk native fish species. Reservoirs are often stocked with nonnative predatory fish, and they can serve as a source population for the dispersal of nonnatives into lotic habitats that are occupied by native fishes. This pathway has been repeatedly implicated by the loss of native fish species (Labbe and Fausch 2000; Mammoliti 2002; Quist et al. 2005). The recent expansion of Smallmouth Bass out of Grayrocks Reservoir appears to be having important ramifications for the Hornyhead Chub population in the Laramie River, as Hornyhead Chub are generally not found in reaches with Smallmouth Bass and this is not well explained just by habitat characteristics. Hornyhead Chub are now found without Smallmouth Bass in only 18 km of their historical range of about 126 km in the Laramie River, a range reduction of nearly 86%. In contrast, Hornyhead Chub appear to persist in the presence of nonnative salmonids. This could be due to differences in the degree of piscivory and habitat use between the predator species. Understanding the relative effects of different predator species can help to prioritize and target management actions for the conservation of native fishes.

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### Appendix 1: Study Variables and Partial Probability Data

TABLE A.1.1. Variable importance for the 48 variables that were included in the random forest model procedure for Hornyhead Chub abundance. The asterisks denote the variables that were included in the top model.

Variable Name	Importance
Predicted mean August water temperature*	1
Smallmouth Bass*	0.6343
Longnose Dace*	0.3182
Common Shiner*	0.2755
White Sucker*	0.2383
Minimum water velocity*	0.2357
Stonecat*	0.2156
Total nonnative predators*	0.2101
Longnose Sucker*	0.1924
Creek Chub*	0.1884
Central Stoneroller*	0.1402
Mean width*	0.085
Common Carp	0.0822
Johnny Darter	0.0626
Mean boulder cover	0.0558
Total nonnative trout	0.0546
Mean undercut cover	0.0425
Brook Trout	0.0336
Bank stability	0.0316
Mean overhead cover	0.0294
Shorthead Redhorse	0.0216
Mean woody debris cover	0.0153
Percent riffle	0.0141
Percent cascade	0.0107
Percent pool	0.0093
Green Sunfish	$8.00 \times 10^{-4}$
Substrate diversity	$1.00 \times 10^{-4}$
Bigmouth Shiner	0
Iowa Darter	0
Plains Topminnow	0
Sand Shiner	0
Channel Catfish	0
Brook Stickleback	0
Width : depth ratio	$-5.00 \times 10^{-4}$
Mean max depth	-0.0017
Percent run	-0.0033
Fathead Minnow	-0.0033
Mean discharge	-0.0051
Rainbow Trout	-0.0053
Brown Trout	-0.0069
Mean substrate	-0.0073
Walleye	-0.0073
Slope	-0.0113
Riffle : run ratio	-0.0119
Mean depth	-0.0193
Mean velocity	-0.0445
Mean cover	-0.0564
Max velocity	-0.0573

Appendix 2: Additional Data

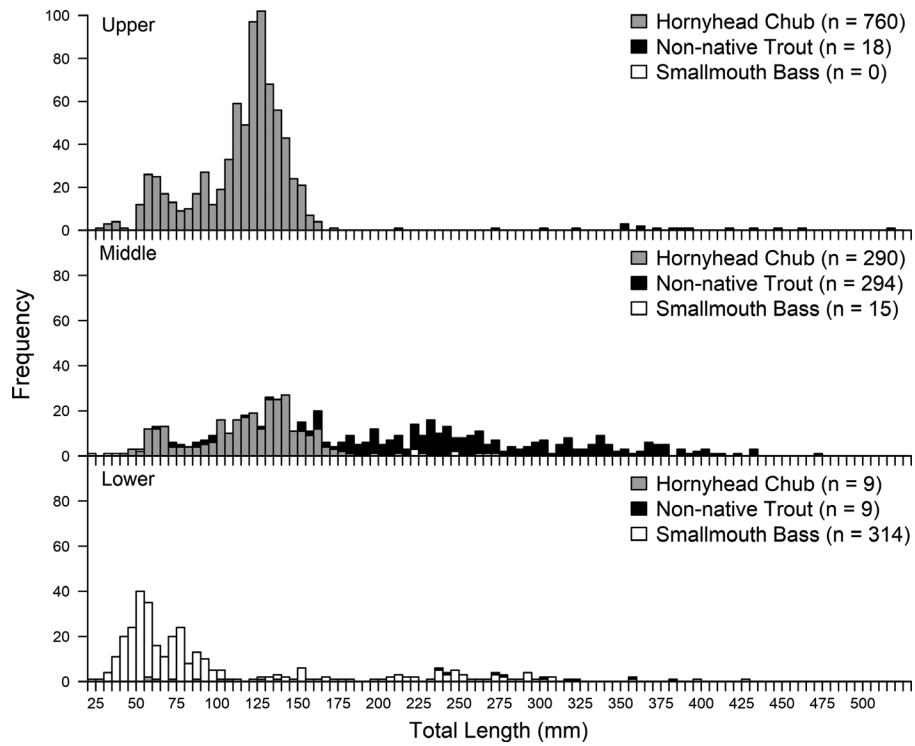


FIGURE A.2.1. Length-frequency histogram of all Hornyhead Chub, nonnative salmonids, and Smallmouth Bass that were captured in the upper, middle, and lower reaches of the Laramie River in 2016 and 2017. Included is the number of fish of each taxa that were captured in each reach.

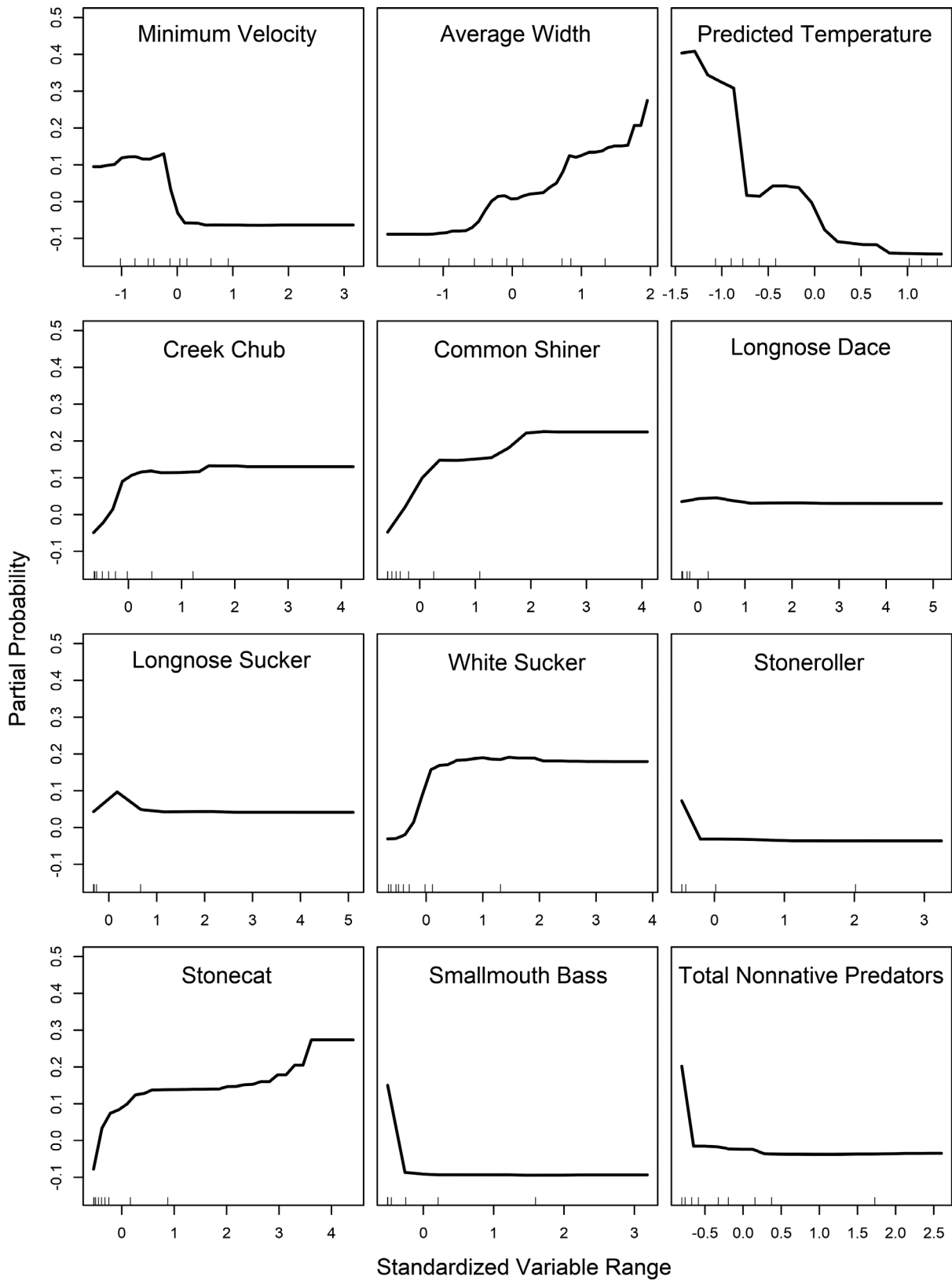


FIGURE A.2.2. Partial probability plots for each of the 12 variables included in the top random forest model for Hornyhead Chub abundance. All of the variables were standardized.