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ORIGINAL ARTICLE

WILEY Ecology of FRESHWATER FISH

Stocked rainbow trout (*Oncorhynchus mykiss*) affect space use by Warpaint Shiners (*Luxilus coccogenis*)

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Abstract

Due to widespread stocking, Rainbow Trout (*Oncorhynchus mykiss* Walbaum) are perhaps the most widely distributed invasive species in the world. Nonetheless, little is known about the effects of stocked Rainbow Trout on native non-game species. We conducted experiments in an artificial stream to assess the effects of hatchery Rainbow Trout on home range and behaviour of Warpaint Shiners (*Luxilus coccogenis* Cope), a common minnow frequently found in stocked Southern Appalachian streams. We used the LoCoH algorithm to generate polygons describing the home ranges used by Warpaint Shiners. When a stocked trout was present Warpaint Shiners: (a) increased home range size by 57%, (b) were displaced into higher velocity mesohabitats, and (c) reduced mean overlap between the home ranges of individual warpaint shiners. Rainbow Trout did not significantly affect the edge/area ratio of Warpaint Shiner home ranges. Warpaint Shiner density (two and five fish treatments) did not significantly affect any response variable. Displacement from preferred microhabitats and increases in home range size likely result in increased energy expenditure and exposure to potential predators (i.e., decreased individual fitness) of Warpaint Shiners when stocked trout are present.

KEYWORDS

biodiversity, cyprinidae, interspecific competition, LoCoH, minnow, social behaviour, spatial relations

1 | INTRODUCTION

Invasive species and habitat alteration are the two main causes of imperilment for freshwater organisms in North America (Jelks et al., 2008). Invasive predators may have strong negative impacts on native species in both freshwater and marine environments (Albins, 2013; Jackson, 2002), nonetheless little is known about the specific mechanisms by which invasives affect native communities other than direct predation. Multiple investigators have shown invasive predators may exert a greater predatory effect on fishes than native predators primarily via an inability of native prey to recognise and employ appropriate antipredator behaviours when invasive predators are present (Kuehne & Olden, 2012; Polo-Cavia, Gonzalo, Lopez, & Martin, 2010; Salo, Korpimaki, Banks, Nordstrom, & Dickman, 2007). Consequently, predation by invasive predators could potentially have catastrophic effects on the structure of native communities. The lack of appropriate behavioural responses and their ecological consequences have been explored theoretically by Sih et al. (2010) and clearly may be linked to the varied impacts that invasive species have in aquatic systems. Nonetheless, the behavioural changes in native species produced by the presence of invasives are not well studied (Elkins & Grossman, 2014).

Rainbow Trout (*Oncorhynchus mykiss* Walbaum) have been stocked on six continents for the creation of sport fisheries and are one of the most widely distributed invasives worldwide (MacCrimmon, 1971; Stankovic, Crivelli, & Snoj, 2015). In the United States alone, Halverson (2008) calculates State and Federal agencies released approximately 9.96×10^6 kg of Rainbow

Trout in 2004. Despite the wide distribution and invasive nature of Rainbow Trout, little is known about its effects on the native fauna and flora, especially in North America. Existing research suggests that Rainbow Trout may have detrimental effects on: (a) native fishes (Hasegawa, Mori, & Yamazaki, 2017; Kaeding, Boltz, & Carty, 1996; McIntosh et al., 2010; Turek, Pegg, Pope, & Schainost, 2016), (b) aquatic invertebrates (Pope, Piovia-Scott, & Lawler, 2009; Wissenger, McIntosh, & Grieg, 2006), (c) amphibians (Hirner & Cox, 2007; Mathews, Pope, Preisler, & Knapp, 2001) and ecosystem interactions (Simon & Townsend, 2003). Extant studies on trout—native fish interactions have focused primarily on the galaxiids in New Zealand (Bonnett & McIntosh, 2004; McDowall, 2006; McIntosh et al., 2010) or native fishes in Patagonian Chile (Penaluna, Arismendi, & Soto, 2009), although several recent studies have occurred in North America (Turek et al., 2016; Weaver & Kwak, 2013). Most of these studies deal with invasive Brown Trout (*Salmo trutta* L.) or a combination of Brown Trout and Rainbow Trout.

Warpaint Shiners (*Luxilus coccogenis* Cope) are a medium sized (maximum 120 mm total length), abundant minnow, found in southern Appalachian streams (Etnier & Starnes, 1993) frequently stocked with Rainbow Trout. These streams typically display substantial environmental variability (Grossman, Hill, & Petty, 1995; Grossman, Ratajczak, Crawford, & Freeman, 1998) that potentially subjects Warpaint Shiners and other native species to a greater probability of extinction when invasives are present (Jelks et al., 2008). Warpaint Shiners represent good study subject for tests of the behavioural impacts of stocked Rainbow Trout for multiple reasons. First, field studies indicate that these species overlap in microhabitat use (Elkins & Grossman, 2014; Grossman et al., 1998) and consume similar prey (Outten, 1957). Second, evidence of similarity in microhabitat and diet is provided by the fact that trout anglers often catch Warpaint Shiners while fishing for trout (Etnier & Starnes, 1993; pers. obs.). Third, Warpaint Shiners may serve as ecological surrogates for multiple minnow species of regional concern, such as the Spotfin Chub (*Erimonax monacha* Cope), Blue Shiner (*Cyprinella caerulea* Jordan) or Bluestripe Shiner (*Cyprinella callitaenia* Bailey & Gibbs) that also may co-occur with stocked Rainbow Trout.

Stocked Rainbow Trout potentially affect Warpaint Shiners via both predation and interspecific competition (Garman & Nielsen, 1982) and even the threat of predation may be sufficient to produce shifts in behaviour and microhabitat that reduces a minnow's fitness (Fraser & Gilliam, 1992). Our previous field research on the effects of naturalised rainbow trout on microhabitat use by native minnows, including Warpaint Shiners, suggests a lack of trout effects (Grossman & Freeman, 1987; Grossman et al., 1998; Rincón & Grossman, 1998); however, these studies involved Rainbow Trout that were much smaller, and likely less aggressive than hatchery trout (Berejikian, Mathews, & Quinn, 1996; Marchetti & Nevitt, 2003). Conversely, in a previous laboratory study, we found the presence of a stocked rainbow trout produced multiple changes in the use of spatial resources and foraging success by Warpaint Shiners (Elkins & Grossman, 2014). In this study, we examined the effects of Rainbow Trout on home range size, shape and overlap, as well as mesohabitat use by Warpaint Shiners.

2 | METHODS

2.1 | Experimental design and artificial stream

Our basic experimental design is presented in Elkins and Grossman (2014) which included a diagram of the artificial stream; consequently, our methodological descriptions are somewhat shortened.

We performed trials in an experimental stream located at the Whitehall Fisheries Laboratory at the University of Georgia. The experimental stream was constructed of 1.9 cm thick acrylic sheeting and had dimensions of 3.05 m (L) × 1.52 m (W) × 0.76 m (H). The stream had a false bottom approximately 30 cm above the base of the tank that allowed for flow to be generated by two electric trolling motors (24.95 kg thrust). Water reemerged at the opposite end of the platform and flowed across the top of this platform in a semi-laminar fashion. We constructed an experimental arena using a pair of block nets across the width of the tank, and this area was subdivided to create three discrete depth zones of equal area: a "riffle" with a depth of 15 cm, a "run" with a depth of 25 cm, and a "pool" with a depth of 35 cm (Elkins & Grossman, 2014). Riffles were covered with a 5 cm layer of small cobbles (maximum diameter 15 cm) and gravel; whereas the run was covered with a 4 cm layer of gravel (max. diameter 2 cm). Substratum for the pool consisted of a 2 cm layer of gravel and sand, and each zone was bordered with a small transition zone consisting of sloping piles of substratum. The tank was shrouded in black plastic to insure visual isolation of observers, and all observations (see below) were made from a blind that minimised disturbance of experimental subjects. Water temperature and photoperiod approximated both spring/fall (water temperature 12°C ± 1°C) and summer conditions (water temperature 17°C ± 1.5°C, photoperiod 12 hr, including dawn and dusk) conditions (Elkins & Grossman, 2014). We measured velocities in the tank with an electronic velocity metre (±0.01 cm/s) before and after each trial to ensure consistency within and among trials. We maintained pH at 7.3 and added aquarium salt (NaCl) at the rate of 7.5 g per 10 gallons of dechlorinated tap water to ease osmotic stress. Water velocity, temperature, and pH ranges were consistent with those recorded at long-term study sites where collection of Warpaint Shiners occurred (Grossman & Ratajczak, 1998).

2.2 | Velocity mapping

To ensure flows were relatively laminar and consistent, before each trial we measured velocities at four locations (the centres of the run and riffle sections and two points directly behind these positions at the midpoint of the pool) at 10 cm above the substrate and adjusted the trolling motors to maintain 22, 16, and 11 cm/s in the riffle, run and pool sections of the tank, respectively. At the conclusion of each trial, we mapped velocities in the tank at a finer scale to model the

TABLE 1 Summary of parameters for experimental trials. Acronyms are as follows: HD is high density (five warpaint) and LD is low density (two warpaint)

Trial	Warpaint shiner dens.	Season	Warpaint SL (mm)	Trout SL (mm)	Trout mass (g)	Chub SL (mm)	Chub mass (g)
1	HD	SUM	69–75	189	78.3	145	58.7
2	HD	SUM	55–91	216	144.6	142	50.0
3	HD	SUM	80–118	232	180	148	51.7
4	HD	SUM	67–82	192	130.3	180	82.5
5	LD	SUM	68–74	189	128.3	180	83.0
6	LD	SPR	105–112	133	35.0	116	25.0
7	LD	SPR	87–88	201	109.5	152	61.5
8	HD	SPR	81–108	173	77.3	152	61.5
9	LD	SPR	50–51	127	28.5	107	22.5
10	LD	SUM	83–90	174	69.4	173	77.9
11	HD	SPR	60–63	212	128.0	140	38.1
12	HD	SUM	62–66	250	232.5	144	36.9
13	HD	SPR	71–79	210	127.1	141	35.8
14	LD	SPR	62–64	145	54.4	100	16.2
15	LD	SPR	77–78	171	57.3	110	19.2
16	HD	SPR	68–73	255	229.7	110	18.2
17	LD	SUM	69–69	223	92.8	130	33.0
18	LD	SUM	76–77	217	184.8	116	27.2

current regime experienced by each fish. The tank was gridded off in 10 × 10 cm squares and velocity measured at 2 cm from the substrate in every square. We completed similar measurements using a 20 × 20 cm grid at 10 cm from the substrate and a 30 × 30 cm grid at the surface. Substratum-associated measurements were made using a smaller grid to capture turbulence caused by cobbles and the sloping tank bottom between habitat sections and because the majority of the fish activity we observed was near the bottom. We used a 2-dimensional spline procedure in Arc/Info (ESRI 2006) to interpolate between points with measured velocities and create a continuous current map for the experimental area with a cell size of 1 × 1 cm. This raster layer then served as a reference and contained the predicted velocity for every location within the tank.

We installed 8 prey release outlets spaced evenly across the front of the tank (see Figure 1 of Elkins & Grossman, 2014), even with the substratum. *Chironomidae* larvae (bloodworms) were dispensed at the head of the tank (riffle and run sections), and carried the length of the tank, simulating the natural drift of prey items. For each trial, we added 10.8–11.0 g of bloodworms to two water reservoirs above the tank, which were aerated to keep bloodworm prey in suspension. The reservoirs sat above computer-controlled release valves programmed on staggered 90-s cycles such that one of the four valves was open for 1-s out every 22.5 s (Wagner, 2004).

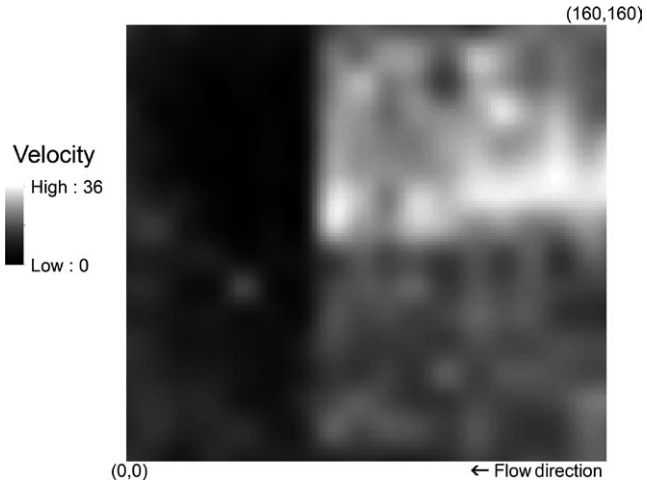

FIGURE 1 Current Map at z = 2 cm interpolated from point measurements. The figure displays current moving from upstream right side to downstream. The riffle and run are on the right side of the map and the pool on the left

2.3 | Collection of experimental subjects

We obtained Rainbow Trout from the Georgia Department of Natural Resources (DNR) hatchery at Lake Burton, Habersham County. Rainbow trout were collected from the batch of trout to be stocked that week. Trout were held in a chilled (approximately 14°C) closed 480 L tank and fed commercial trout chow (2.4 mm pellets) to simulate hatchery conditions. We collected healthy Warpaint Shiners and River Chub from Coweeta Creek, Macon County, North Carolina, using seine nets as described by Wagner and Grossman (2014). After acclimation for at least 12 hr in monospecific holding tanks, all Warpaint Shiners were anesthetised using a buffered

solution of MS222 (Tricaine Methane Sulphonate) at a concentration of 1.07 g to two L of holding tank water, weighed (±0.01 g), and measured for length (standard length, SL, ±1.0 mm). While anesthetised, each fish was marked with a coloured acetate tag (approximately 2 mm^2) just below the dorsal fin which has been shown to not affect behaviour in other minnows (Wagner & Grossman, 2014). We then returned Warpaint Shiners to the holding tanks to recover for 2 days prior to experiments, during which we fed them bloodworms and administered a prophylactic dose (3 mg/L) of the antibiotic Kanamycin Sulphate. The typical period between collection and initiation of an experiment was 4 days (collect Thursday, mark Friday, recover Saturday and Sunday) although this period was occasionally extended to 6 days due to weather or other scheduling constraints. The size ranges of Warpaint Shiners used in trials typically was less than 10 mm (SL) and Table 1 lists size ranges of all species. All fish were euthanised after trials in a supersaturated solution of CO_2.

2.4 | Treatments and fish observations

An experiment began with a two-day control period with a focal group of either two (low density, LD) or five Warpaint Shiners (high density, HD treatment) observed three times daily. We made the first observation generally between 10 a.m. and noon and the last observation ended before 6 p.m. To avoid satiation effects, we suspended feeding for a minimum of 30 and a maximum of 90 min between observation periods (see below). High and low-density treatments were 0.7 and 2.1 fish/m^2 respectively, which were similar to densities observed in the field (pers. obs.). We then began a series of randomly chosen (via a random number generator) two-day long treatments which included addition of: (a) one Rainbow Trout (trout treatment, TR), (b) one River Chub, *Nocomis micropogon* (Cope), (a large minnow that served as a "large fish" control, CH), or (c) an additional Warpaint Shiner (density control, DC). The chub was used because it is the largest non-game drift-feeder sympatric with Warpaint Shiners in our study system. We added treatment fish during late afternoon, after the completion of the day's third observation session and removed them after completion of the two-day treatment period. We minimised acclimation stress by matching temperature treatments to the seasonal conditions under which fish were collected. We completed nine trials at each density: five LD trials at 12°C and four at 17°C; and four HD at 12°C and five at 17°C.

We based observational methods on those of Rincón and Grossman (1998). Warpaint Shiners were introduced into the experimental stream in late afternoon and acclimated overnight. Observations commenced 30 min after the initiation of the feeding system to allow fishes to acclimate to prey releases. We observed individual fish at 300 s intervals for periods of 2 min each, during which we recorded: (a) fish identity, (b) X, Y, and Z coordinates and (c) distance of the nearest con- and heterospecifics. After 2 min of observations, we randomly chose a new focal fish until all individuals had been observed. In all, we made three circuits resulting in 30 min of observations (5 fish × 2 min × 3 rounds) in each high-density observation session or 12 min of observations (2 fish × 2 min × 3 rounds) per session of the low-density trials. All observations were recorded on microcassette and transcribed after the completion of the trial.

2.5 | Statistical analyses

2.5.1 | Warpaint space use

We estimated Warpaint Shiner home ranges for each treatment in two dimensions from the 30-s positional (X, Y) data using the LoCoH tool in R (R Development Core Team 2008). LoCoH is a non-parametric, kernel-based method of home range estimation (Getz & Wilmers, 2004). In contrast to other algorithms, LoCoH is capable of generating home ranges containing non-contiguous areas and is particularly well suited for describing habitat use by organisms residing in patchy environments like streams (Getz & Wilmers, 2004). We used the "adaptive sphere-of-influence" LoCoH method to calculate home range, as recommended by Getz et al. (2007). This method produces minimum convex polygons (local hulls) based on prespecified areas/number of points, e.g., a 25th percentile isopleth, hull encloses 25% of points. We chose a value for α by following the "minimum spurious hole-covering" rule (Getz et al., 2007). We allowed the algorithm to selectively add up to 2 cm of random "jitter" to observations, to ensure overlapping points are not discarded and 2 cm is probably within the range of observation error.

We employed the adaptive LoCoH method to construct a local hull (Getz et al., 2007). In brief, although the number of nearest-neighbour points used to construct a local hull is variable; local hulls are constructed by including all points within a variable sphere around a root point so the sum of the inter-point distances is less than or equal to α (Getz et al., 2007). Although it is possible to ensure that the 100% isopleth contains all the recorded points by selecting a large value for α (e.g., for our tank, approximately 440 cm), the isopleths generated using values of α greater than ~150 cm consistently resulted in unrealistically large home ranges and covered areas rarely occupied by Warpaint Shiners (the equivalent of spurious hole-covering in our habitat). Getz et al. (2007) showed the adaptive method was fairly insensitive to small changes in alpha. Consequently, we used an α value of 100 for our data, which produced home ranges consistent in shape and scale with our observations, and did not exhaustively tune the value for each individual trial. We also specified that each hull must contain at least two points to ensure 100% isopleths always would be generated.

We generated a home range for each focal Warpaint Shiner for each treatment (WP, DC, CH, and TR). We based individual home ranges (Wray, Creswell, White, & Harris, 1992) on the 50% use isopleths, because the fish were interacting and feeding within this spatial range and because this isopleth excluded infrequent movements outside of the core area.

2.5.2 | Space use analysis

To test whether Rainbow Trout affected the area or shape of the Warpaint Shiners' home ranges, we used ArcMap (ESRI 2006) to calculate the area, perimeter, and edge/area ratio for each set of 50% isopleths. To test the hypothesis that Warpaint Shiners displayed increased overlap in home ranges when Rainbow Trout were present, we overlaid their isopleths for each trial and treatment and calculated the amount of overlap in home ranges among Warpaint Shiners both as an absolute area and as a percentage of each fish's home range. We used mixed linear models to test for significant differences in mean home range size, percentage overlap, or edge/area ratio of the Warpaint Shiners, using density, season, and treatment as fixed effects (including an interaction between density and season) and a random effect of individual fish (alpha = 0.05). All models estimate the additive effects of the other parameters relative to a baseline of low-density (LD) and spring (SPR) conditions.

Measured velocities ranged from zero in the pool to 32 cm/s at the head of the riffle. Although our intention was to analyse velocity in three dimensions, the mean depth-to-substrate we observed was 3.25 cm (*SD* 3.03 cm), 95% of the observations were below 7 cm, and the mean depth-to-substrate did not vary by treatment ($p = 0.099$) so we used the interpolated velocity grid at $z = 2$ cm for all velocities. We calculated mean velocity (±*SD*) within each core area polygon using the Iterative Zonal Statistics ArcScript (Albeke, 2009), which used the core area layer as an analysis mask for the velocity map to extract the pixels describing the current profile within each Warpaint Shiner home range. We then calculated 95% confidence intervals for Warpaint Shiners in each treatment and tested the null hypothesis that velocity did not differ among treatments.

3 | RESULTS

Velocities ranged from slightly negative (−2 cm/s) in the pool mesohabitat to approximately 35 cm/s at the top of the riffle, with intermediate velocities in the run and some turbulence at the boundaries between the mesohabitats (Figure 1). Individual fish frequently displayed multiple home range polygons, which was consistent with observations. These polygons were fairly stable for the WP, DC, and CH treatments (Figures 2 and 3). Regardless of Warpaint Shiner density or seasonal temperature, mean velocities in core areas occupied by Warpaint Shiners in the control (WP) was either the lowest of the treatments or statistically indistinguishable from the lowest. In three of four cases, mean velocities displayed by fish in the control (WP) were indistinguishable from the DC treatment. In all cases, the mean velocity experienced by a focal Warpaint Shiner during TR was significantly higher than under WP (i.e., control; Figure 4).

Fish addition treatments elicited different responses from Warpaint Shiners. Edge/area ratios of Warpaint Shiner's home ranges were unaffected by Rainbow Trout. Under baseline conditions (LD, SPR, WP) the mean edge/area ratio was 0.37, and no other treatment was significant (Tables 1 and 2). Rainbow Trout significantly increased the average home range of a Warpaint Shiner, from 641 cm^2 (baseline) to 1,009 cm^2 (*df* 566, $t = 5.18$, $p < 0.01$), an increase of 57%. The only other significant effect was the CH control, which reduced mean home range to 440 cm^2 (*df* 566, $t = 4.09$, $p < 0.01$). All fish addition treatments decreased overlap in mean home range significantly from the baseline (18% overlap at LD, SPR, WP). When an additional Warpaint Shiner was present (DC) overlap dropped to 9% (*df* 566, $t = -4.73$, $p < 0.01$) and when a River Chub was present (CH), mean home range overlap dropped to 5% (*df* 566, $t = -6.95$,

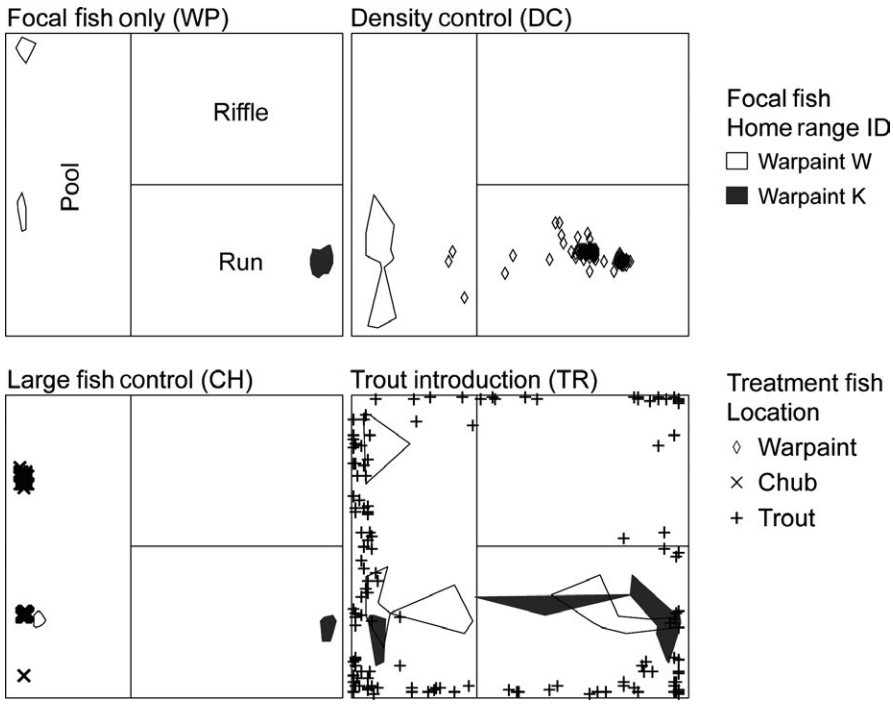

FIGURE 2 Home ranges derived for Trial 20, a two fish trial (letters refer to the individual fish i.e., Warpaint W and K). The colours of the polygons reflect the colours of the tags applied to the focal Warpaint Shiners in this LD trial under SUM conditions. Diamond, x, and cross symbols reflect the measured positions of the Warpaint Shiner, Chub, or Trout during respective treatments

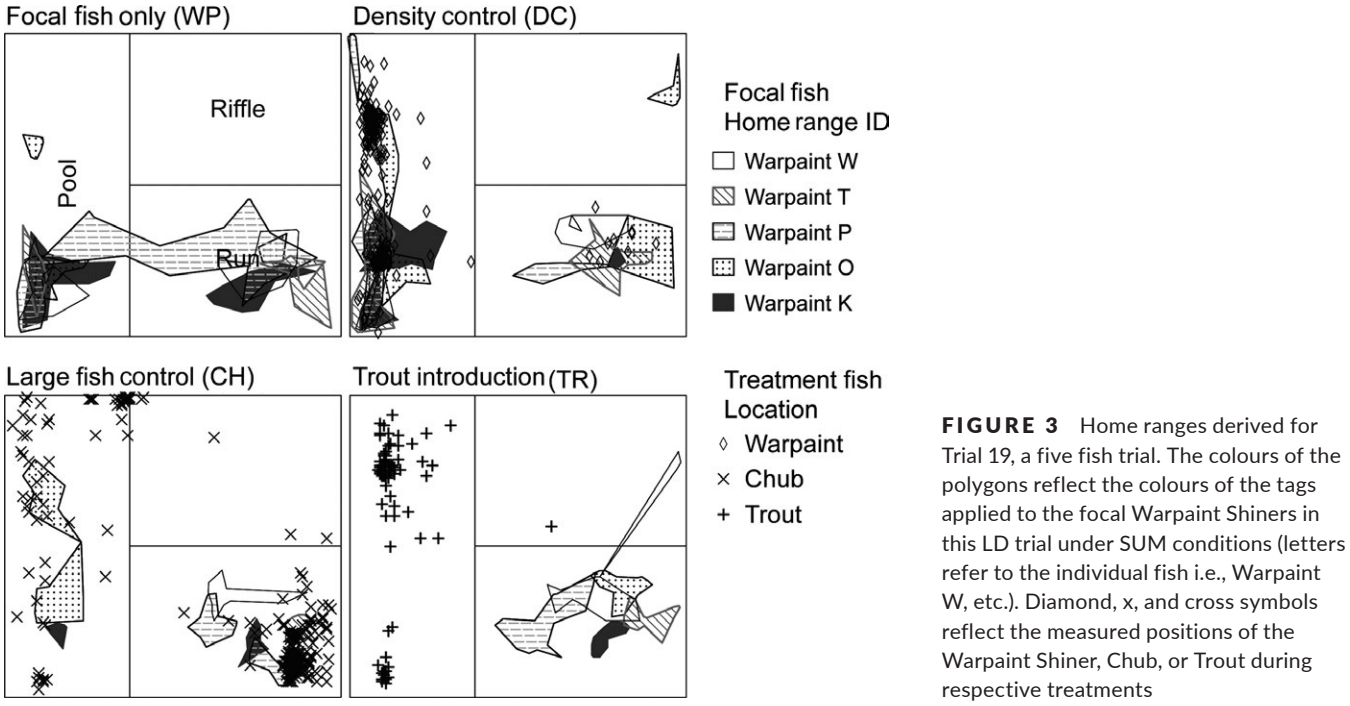

FIGURE 3 Home ranges derived for Trial 19, a five fish trial. The colours of the polygons reflect the colours of the tags applied to the focal Warpaint Shiners in this LD trial under SUM conditions (letters refer to the individual fish i.e., Warpaint W, etc.). Diamond, x, and cross symbols reflect the measured positions of the Warpaint Shiner, Chub, or Trout during respective treatments

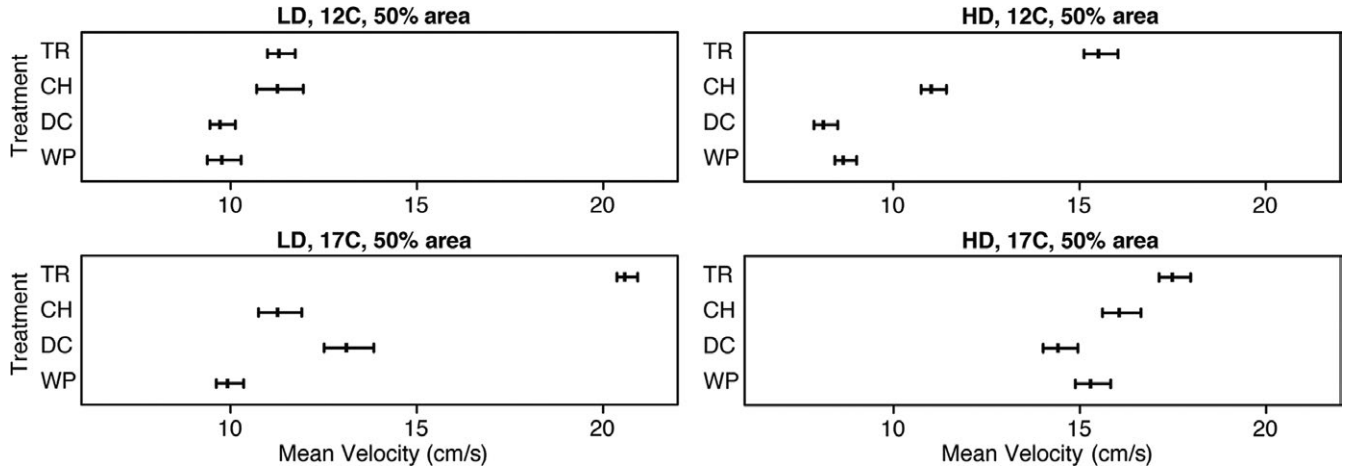

FIGURE 4 Mean (95% CI) velocities for the core areas used by Warpaint Shiners in all trials

$p < 0.01$). The presence of a Rainbow Trout (TR) reduced the home range overlap the least, to 11% (df 566, $t = -3.84$, $p < 0.01$).

4 | DISCUSSION

Our results suggest the presence of a stocked invasive Rainbow Trout altered the behaviour of Warpaint Shiners by forcing them into larger home ranges with higher mean velocities. These behavioural changes likely reduced the fitness of individual Warpaint Shiners by increasing the energetic costs of occupying a microhabitat (increased activity and swimming costs; Grossman, 2014). Velocities at the position of a fish are likely the most important component of habitat selection for stream fishes in systems where predation is not important, such as many southeastern Appalachian cold-water streams (Grossman, 2014; Grossman, Rincon, Farr, & Ratajczak, 2002). For example, Hill and Grossman (1993) and Grossman et al. (2002) showed four minnow species, including Warpaint Shiner, and small naturalised Rainbow Trout were capable of choosing microhabitats that maximised their net energy gain in a Southern Appalachian stream. Although microhabitat shifts by Warpaint Shiners in the presence of stocked trout may seem relatively small, it is important to remember that over the lifetime of a fish, they likely would represent a substantial energetic drain. In addition, our experimental apparatus, although a reasonable simulation of a Southern Appalachian stream, was still smaller than most streams occupied by Warpaint Shiners; this likely limited the magnitude of their responses to a stocked trout.

TABLE 2 Linear model parameters and statistics for edge/area ratios, home range size and home range overlap

	Parameter estimate (*SE*)	*df*
Edge/area ratio		
(Intercept)	0.37 (0.03)***	147
CH	0.00 (0.03)^{ns}	147
DC	0.04 (0.03) ^{ns}	147
TR	−0.04 (0.03)^{ns}	147
SUM	−0.05 (0.04)^{ns}	46
HD	−0.03 (0.03)^{ns}	46
SUM × HD	0.01 (0.05)^{ns}	46
Home range size		
(Intercept)	640.65 (156.45)***	566
CH	−136.45 (69.3)*	566
DC	−73.19 (69.3)^{ns}	566
TR	368.76 (71.2)***	566
SUM	240.57 (225.7)^{ns}	51
HD	−101.59 (173.8)^{ns}	51
SUM × HD	281.84 (261.7)^{ns}	51
	Value	***df***
Home range Overlap		
(Intercept)	0.18 (0.03)***	566
CH	−0.13 (0.02)***	566
DC	−0.09 (0.02)***	566
TR	−0.08 (0.02)***	566
SUM	−0.03 (0.05)^{ns}	51
HD	0.06 (0.03)^{ns}	51
SUM × HD	−0.08 (0.05)^{ns}	51

Notes. *T*-tests evaluate significant treatment and interaction effects. Acronyms are as follows: (a) CH represents the chub treatment, (b) DC represents the density control (six warpaint), (c) TR is the trout treatment, (d) SUM is the effect of summer temperatures, (e) HD is the effect of high density, and (f) SUM × HD is the interaction term for summer and high-density treatments.

Similar findings have been observed by investigators working in different habitats including Fausch (1984) with coho salmon (*Oncorhynchus kisutch* Walbaum) and (Metcalfe, 1986) with Rainbow Trout. Unlike the minnows and Rainbow Trout studied by Hill and Grossman (1993) and Grossman et al. (2002), intraspecific dominance played a strong role in individual energy gain in the studies by Fausch (1984, 1988, 1998) and Metcalfe (1986). Our findings regarding the negative consequences of invasive Rainbow Trout on habitat use by native species are similar to those displayed by two galaxiid species and one trichomycterid in a Chilean stream (Penaluna et al., 2009), as well as the Argentinian Creole Perch (*Percichthys trucha* Valenciennes, Otturi, Battini, & Barriga, 2016). Hanisch, Tonn, Paszkowski, and Scrimgeour (2012) in a study of dace (*Chrosomus* spp.) and fathead minnows (*Pimephales promelas* Rafinesque) in Canadian lakes also found similar responses to the presence of stocked trout. Conversely, Weaver and Kwak (2013) found few shifts in microhabitat use by native fishes in Southern Appalachian streams, when the effects of habitat variation were taken into account, and Grossman and de Sostoa (1994) did not observe microhabitat shifts in an assemblage of native cyprinids in a Spanish river when Rainbow Trout were introduced. In the latter case, it is worth noting that all cyprinid species except one were significantly larger in size than Warpaint Shiners.

It is unlikely that the responses displayed by Warpaint Shiners in the presence of trout were antipredator responses for several reasons. Although Elkins and Grossman (2014) showed intraspecific distances among Warpaint Shiners decreased significantly, from 56 to 46 cm, when Rainbow Trout were present, the current analysis shows mean home range size increased, and overlap also decreased, suggesting Warpaint Shiners were not aggregating to avoid a predator. Perhaps this is a result of "conflicting pressures" as hypothesised by Magurran (1990), which suggests the advantage of schooling as an antipredator defence may be outweighed by other selection pressures, such as the need to obtain suitable rations (increased intraspecific competition for food). We did observe Warpaint Shiners were typically tightly clumped before the initiation of feeding during the morning session, which lends support to this idea.

When a Rainbow Trout was present, the positions of the Warpaint Shiners were less stable (the larger home ranges during this treatment suggesting more movement among observations) and Warpaint Shiners occupied significantly higher velocities than when alone (WP) or with an additional conspecific (DC, 3 cases of 4). The mean velocity experienced by Warpaint Shiners increased during the CH control, as well, although less than when a Rainbow Trout was present (3 cases of 4). This result may be a result of collaborative foraging by Warpaint Shiners behind the River Chub. Unlike the trout, the River Chub in our trials would occasionally forage benthically, both among cobbles in the riffle and in other areas of the tank where prey may have lodged. When this occurred, Warpaint Shiners would trail the chub presumably to feed on potential prey displaced by the chub. Whether this foraging behaviour produced the observed change in microhabitat use is unknown, but our observations show these differences were due to an association rather than avoidance, as observed with Rainbow Trout (Elkins & Grossman, 2014).

Although direct predation is the clearest mechanism by which salmonids might negatively impact native fishes, our results suggest invasive stocked Rainbow Trout produced more subtle behavioural shifts in space use by Warpaint Shiner; a result confirmed by earlier work (Elkins & Grossman, 2014). These behavioural shifts likely result in reduced fitness for individual Warpaint Shiners, because of their increased energetic costs. Although our treatments only lasted 48 hr, if the observed behaviours are manifested by Warpaint Shiners in streams where Rainbow Trout are regularly stocked, it could result in negative consequences at both the individual and population-level. This is especially problematical if similar responses are displayed by species of concern.

Because many streams are stocked repeatedly, Rainbow Trout have become established in many streams outside of their native

range (Crawford & Muir, 2008), especially in cold-water streams of the Southeastern United States. Given that the Southeastern United States is the centre of diversity for freshwater fishes north of Mexico, there is potential for substantial negative impacts on native fish assemblages. Clearly, managers should use caution in making decisions to stock streams that currently support threatened or vulnerable native fish populations with Rainbow Trout.

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