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Brook trout use of thermal refugia and foraging habitat influenced by brown trout

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Abstract

The distribution of native brook trout (*Salvelinus fontinalis*) in eastern North America is often limited by temperature and introduced brown trout (*Salmo trutta*), the relative importance of which is poorly understood but critical for conservation and restoration planning. We evaluated effects of brown trout on brook trout behavior and habitat use in experimental streams across increasing temperatures (14-23 °C) with simulated groundwater upwelling zones providing thermal refugia (6-9 °C below ambient temperatures). Allopatric and sympatric trout populations increased their use of upwelling zones as ambient temperatures increased, demonstrating the importance of groundwater as thermal refugia in warming streams. Allopatric brook trout showed greater movement rates and more even spatial distributions within streams than sympatric brook trout, suggesting interference competition by brown trout for access to forage habitats located outside thermal refugia. Our results indicate that removal of introduced brown trout may facilitate native brook trout expansion and population viability in downstream reaches depending in part on the spatial configuration of groundwater upwelling zones.

Keywords: interference competition, thermal refugia, groundwater, experimental streams, brook trout, brown trout

Introduction

Interspecific competition is often influenced by abiotic factors (i.e., condition-specific competition, Dunson and Travis 1991). An understanding of condition-specific competition therefore is necessary to anticipate how changing environmental conditions will affect population dynamics and community composition (Tylianakis et al. 2008; Van der Putten et al. 2010). Anticipated increases in stream temperature (Snyder et al. 2015; Isaak et al. 2016) highlight a specific need to understand the relative importance and potential interactions of biotic and abiotic mechanisms of freshwater community assembly (Rahel et al. 2008; Wenger et al. 2011). Such knowledge can improve conservation strategies by focusing management actions on the biotic or abiotic conditions that regulate community assembly (Economo 2011).

Freshwater fishes provide an important model in this regard because (1) condition-specific competition has been demonstrated across many taxonomic groups (cyprinidae, cottidae, Baltz et al. 1982; salmonidae, Taniguchi and Nakano 2000; fundulidae, cyprinodontidae, Carmona-Catot et al. 2013); (2) the longitudinal addition and replacement of stream fish species is influenced by interactive biotic and abiotic effects (Taniguchi and Nakano 2000; Hitt and Roberts 2012); and (3) introduced fishes have displaced native fish in many cases (Kreuger and May 1991; Cucherousset and Olden 2011). However, mechanistic predictions in this regard are limited in part due to confounded biotic and abiotic effects in observational field studies (see Hearn 1987) and experimental design limitations (see Fausch 1988, 1998; Weber and Fausch 2003). Here, we evaluate the relative importance of temperature and brown trout (*Salmo trutta*) on the distribution and behavior of brook trout (*Salvelinus fontinalis*) in an experimental stream system using a fully-crossed and replicated design.

The distribution of native brook trout extends from the southern Appalachian mountains (Georgia, USA) to the eastern Canadian Shield (Quebec, Canada) (MacCrimmon and Campbell 1969). In the southern portion of their range, brook trout are largely restricted to high-elevation streams in forested catchments (Flebbe 1994; Hudy et al. 2008) that support cooler temperatures (e.g., 7-day maximum of daily-mean temperatures $< 23.3^{\circ}\text{C}$, Wehrly et al. 2007). Physiological responses to heat stress have been observed where stream temperatures exceed 21°C , and 23.4°C has been shown to be the upper limit for brook trout growth (Chadwick et al. 2015). Brook trout utilize groundwater upwelling zones for spawning (Curry and Noakes 1995) as well as for overwintering habitat (Cunjak and Power 1986) and thermal refugia during summer months (Biro 1998; Baird and Krueger 2003; Petty et al. 2012). Observed patterns of brook trout metapopulation structure and population isolation in stream networks (Aunins et al. 2015) may be explained in part by landscape-scale movements to access thermal refugia and spawning habitat (Petty et al. 2012; Kanno et al. 2014a). Thermal refugia from groundwater upwelling zones can buffer stream fish populations from effects of land-use (Steffy et al. 2004; Stranko et al. 2008). However, groundwater upwelling zones are spatially patchy within streams (Kanno et al. 2014b; Dugdale et al. 2015; Snyder et al. 2015), and stream thermal responses to climate change will depend in part on the spatial configuration and depth of groundwater upwelling zones (Meisner et al. 1998; Kurylyk et al. 2014a; Snyder et al. 2015).

The downstream distribution limits for native brook trout are often associated with the presence of introduced rainbow trout (*Oncorhynchus mykiss*) or brown trout (*Salmo trutta*) (Vincent and Miller 1969; Gard and Flittner 1974; Kozel and Hubert 1989; Rahel and Hubert 1991; Petty et al. 2012). Introduced rainbow trout and brown trout have been linked to declines in native brook trout abundance and occurrence based on time-series analysis (Moore et al. 1983;

Larson and Moore 1985; Waters 1983, 1999), *in-situ* experiments (Fausch and White 1981; Magoulick and Wilzbach 1998; Hoxmeier and Dieterman 2016), and statistical modeling inferences (McKenna et al. 2013; Wagner et al. 2013). The persistence of sympatric brook trout and introduced trout populations in some locations may be explained by abiotic factors related to stream size (Sheldon 1968; Fausch 1989) or seasonal hydrological variation (Larson et al. 1995; Strange and Habera 1998), suggesting condition-specific competitive effects.

Various mechanisms may account for the observed displacement of native brook trout by introduced brown trout. Brown trout have higher thermal tolerances than brook trout (Eaton et al. 1995, *cf.* Lee and Rinne 1980), and therefore warming temperatures may restrict brook trout before brown trout through physiological mechanisms (Flebbe 1994; Fausch 1989; see also Chadwick et al. 2015). Introduced brown trout displace native brook trout for access to flow refugia (i.e., resting positions) (Fausch and White 1981), and this may decrease brook trout growth by increasing respiration relative to consumption (see Hartman and Cox 2008). This hypothesis is supported by the observed decrease of brook trout growth in the presence of brown trout (Carlson et al. 2007; Hoxmeier and Dieterman 2013, *cf.* Öhlund et al. 2008). In addition, redd superimposition or hybridization by brown trout may reduce brook trout abundance and population growth (Sorensen et al. 1995; Essington et al. 1998; Cucherousset et al. 2008), and predation of juvenile brook trout by adult brown trout may account for brook trout population declines (Alexander 1977). Alternatively, observed declines may be because brook trout are more susceptible to harvest by anglers than brown trout when occurring in sympatry (Cooper 1952; Marshall and MacCrimmon 1970).

Prior research yields inconsistent conclusions regarding the importance of interspecific competition between brook trout and brown trout as well as the potentially mediating role of

temperature. Experimental stream studies have concluded that brook trout competitively dominate brown trout of equal size (Fausch and White 1986) as well as the converse (DeWald and Wilzbach 1992). Differences between these studies cannot be attributed to stream temperatures (constant 14-15 °C in both cases) but may be due to effects of habitat cover (present in DeWald and Wilzbach (1992) versus absent in Fausch and White (1986)). Nonetheless, observed effects of brown trout on brook trout microhabitat use from DeWald and Wilzbach's (1992) laboratory study are consistent with results of *in-situ* research based on experimental removals of brown trout (Fausch and White 1981). Taniguchi et al. (1998) concluded that brook trout and brown trout were competitively equivalent at temperatures ≤ 20 °C, above which both species became subordinate to creek chub (*Semotilus atromaculatus*). In contrast, brown trout have been shown to increase their competitive advantage over other salmonid species as temperatures increase from 10 to 20 °C (Colorado River cutthroat trout *Oncorhynchus clarki pleuriticus*, DeStaso and Rahel 1994) and from 14 to 17 °C (Arctic char *Salvelinus alpinus*, Jonsson 2009).

Prior experimental research on this topic is limited by spatially homogenous thermal treatments (i.e., uniform heating of tanks), and thus cannot account for thermal habitat patchiness due to groundwater upwelling (Brunke and Gonser 1997; Torgerson et al. 1999; Ebersole et al. 2003; Kanno et al. 2014b; Snyder et al. 2015). Moreover, heterogeneity in resource availability is an important design consideration in testing for interspecific competition (Connell 1983, Weber and Fausch 2003). In this study we compared brook trout in the presence and absence of brown trout with spatially- structured resources defined by thermal refugia and foraging locations. We created thermal refugia by simulating groundwater upwelling through substrates across increasing ambient temperatures (14, 17, 20, 23 °C), and we introduced food from belt feeders

located outside thermal refugia to create spatial structure in foraging habitat quality. This temperature range is useful because it includes approximate temperatures for optimal brook trout growth (13 °C, Baldwin 1957) as well as upper limits to positive growth (23 °C, Chadwick et al. 2015). We hypothesized that both species would increase their use of upwelling zones as ambient temperatures increased, and that competitive advantage would shift from brook trout to brown trout with increasing temperatures.

Methods

We applied experimental treatments to a set of indoor artificial streams located at the Leetown Science Center (US Geological Survey) in Kearneysville, WV (Fig. 1). Each stream consists of connected fiberglass tanks providing pool and riffle habitat as described by Matthews et al. (2006). Streams extend for 7.6 m, each encompassing a surface area of 6.9 m² and volume of 3.8 m³. A 1-horsepower pump supplied a recirculating flow rate of 0.11 m³/minute. Target temperatures were set through a combination of heating (Aqua Logic 12 kW in-line heater) and cooling (Aqua Logic 17.4 kW chiller barrel) (Fig. 1). The bottom of each tank was covered with coarse gravel (~ 25 mm diameter), and natural lighting was supplemented with overhead lighting during daytime hours. Streams were separated to prohibit fish movement between sympatric and allopatric treatments but shared water through a combined sump and filtration system (Fig. 1).

Brook trout were sourced from Ridge Hatchery in Berkeley Springs, WV, and brown trout were sourced from Reeds Creek Hatchery in Franklin, WV in 2015. Both hatcheries are operated by the West Virginia Department of Natural Resources. All fish were adults (mean length = 345 mm TL, mean weight = 622 g), and mean weights and lengths were not significantly different between species ($p > 0.2$ and $p > 0.9$, respectively; Appendix A). Although

brown trout are often larger than brook trout where sympatric in streams (e.g., Fausch and White 1986), the equivalence of body size between species was appropriate in our study because body size usually predicts dominance in salmonids (Fausch and White 1986; Fausch 1988). Fish were acclimated to 14 °C for 7 days prior to experimental trials. Fish were fed 5 mm floating pellets at a rate of ~ 1% total biomass/day (Finfish G pellets, Zeigler Brothers, Inc.) from 12-hour belt feeders in fixed locations (Fig. 1).

We evaluated effects of brown trout on brook trout behavior and habitat selection using a substitutive design by attempting to hold total fish density constant between sympatric and allopatric treatments. This approach is appropriate for assessing the effects of species known to compete in natural systems (Fausch 1998), and prior studies have used this method to assess effects of competition between native and non-native fishes (Taniguchi and Nakano 2000; McMahon et al. 2007). For each stream we assembled experimental populations from 10 individuals for a total linear density of 1.3 fish/m (10 brook trout or 5 brook trout and 5 brown trout), a level within the range of observed densities for stream-dwelling brook trout (Kanno et al. 2015). *Saprolegnia* infection caused 2 brook trout mortalities and reduced allopatric treatment densities to 8 fish (replicates 2-6), but sympatric treatments were held at a uniform density across all replicates. Individual brook trout were randomly reassigned to allopatric and sympatric populations among 6 replicated trials, and allopatric and sympatric treatments were randomly assigned to streams across replicates.

Within each trial, thermal treatments regulated ambient water temperatures at 14, 17, 20, and 23 °C for allopatric and sympatric populations. This temperature range was selected to include upper levels stressful for brook trout physiological function (20-21 °C, Hartman and Cox 2008; Chadwick et al. 2015) without reaching upper incipient lethal levels for brook trout (25-30

°C, Fry et al. 1946; Lee and Rinne 1980). This temperature range also encompasses observed summer temperatures of native brook trout habitat in Virginia (Snyder et al. 2015). Ambient temperatures were held constant during daylight hours and were increased overnight by ~ 1 °C/hour, and similar rates have been observed in natural streams (Constantz et al. 1994).

To create spatial structure in thermal habitat, we simulated groundwater upwelling by introducing spring water (~ 14 °C) into substrates in downstream pools (Fig. 1; Appendix B). Groundwater was run through flexible tubing (1.3 cm diameter) and into a 30 cm square PVC manifold (1.3 cm diameter) that was perforated to allow water flow. Groundwater flow rate was set to 3 L/minute with flow-regulation disks. Temperature loggers (Onset ProV2) were deployed in an upstream pool to measure ambient temperatures and in a downstream pool near the substrate surface to measure incoming groundwater temperatures at hourly increments. Conductivity, dissolved oxygen, and pH were sampled repeatedly at 14 °C and 23 °C to encompass the range of experimental temperatures (Table 1).

We conducted daily visual observations to index brook trout spatial distribution within streams and evenness across connected tanks (i.e., pool and riffle mesohabitat units). We recorded number of fish and their species identity within pool and riffle tanks at least 3-times per day, including morning and afternoon samples. Of the 24 experimental days, 22 (92%) supported 5 or more visual samples per day (mean = 6.8 samples/day). To minimize disturbances to fish, observations were made through plexiglass windows located on the side of pool tanks (see Matthews et al. 2006) that were covered with black plastic when not in use. We quantified distributional evenness within streams using a variant of Simpson's index (E) (Equation 1, Simpson 1949):

191 (1)
$$E = \frac{1/\sum p_i^2}{s}$$

192

193 where p_i is the proportion of brook trout or brown trout in tank i across $s = 5$ tanks per stream (3
194 pools and 2 riffles). Payne et al. (2005) recommended this index for quantifying spatial evenness
195 in organismal distributions, and it has been previously applied for this purpose in fish ecology
196 research (e.g., Didrikas and Hansson 2008).

197 To assess brook trout movement rates, aggressive behaviors, and opercular ventilation
198 rates, we collected underwater video data using a GoPro HERO3 Black Edition (1440 pixels, 48
199 frames/second). We mounted the waterproof camera case to a plastic board (5 cm x 50 cm) to
200 stabilize the camera and allow repeated observations from the same perspective. Each day of the
201 experiment we collected 11 minutes of video from each stream and discarded the first minute to
202 avoid disturbance associated with the placement of the camera (see Biro 1998). Video samples
203 were collected from pools with the highest density of fish during the sampling period. Upstream
204 and downstream pools (see Fig. 1) were sampled equivalently at temperature levels between 14
205 and 20 °C, but downstream pools were sampled in 10 out of 12 cases (83%) when ambient
206 temperatures reached 23 °C and fish preferentially utilized thermal refugia (see Results). Video
207 samples were collected between noon and 3:30 pm daily, and the majority of samples (81%)
208 were collected between noon and 2 pm. We quantified movement rate by species as the number
209 of times a fish moved in or out of the observation tank during the 10-minute video sample. We
210 did not attempt to record individual-level movement patterns.

211 Aggression was quantified from daily video samples as the sum of nips, charges, and
212 chases following Keenleyside and Yamamoto (1962) and Hartman (1963). Ventilation rate was
213 quantified as the beating rate of opercula for brook trout from daily video samples to provide an

index of oxygen consumption, resting metabolic rate, and stress responses (Cairns et al. 1982; Millidine et al. 2008). For each video sample, we measured the time for 30 operulum beats in seconds (b) and calculated ventilation rate per minute as $3600/2b$. We sampled ventilation rates for 5 randomly-selected brook trout and averaged values across individuals within each video sample. Similar methods have been used to assess ventilation rates in salmonid fishes (Walker et al. 1988).

Temperature and brown trout treatments were applied to a fully-crossed design across 6 replicated trials. We fit linear mixed models to evaluate the effect of brown trout presence (fixed effect) on brook trout spatial distribution and evenness, movement, aggression, and ventilation while accounting for temperature (14, 17, 20, 23 °C) as random effects. The inclusion of random effects in this context minimizes potential effect of pseudoreplication on statistical inference (Bolker et al. 2009) in our thermal treatment design. We used restricted maximum likelihood procedures to fit mixed-models using library lme4 in R (Bates et al. 2015). We applied z -score transformations to standardize response variables and calculated marginal R^2 (fixed-effects only) and conditional R^2 (fixed and random effects) to summarize model performance (Vonesh et al. 1996; Nakagawa and Schielzeth 2013). We assessed the significance of standardized coefficients from 95% confidence intervals calculated from 1000 bootstrapped models with random sampling of the data. Stream identity (1-2) and replicate number (1-6) had insignificant effects on brook trout responses (i.e., standardized model coefficient 95% CIs included 0) and therefore were excluded from further consideration.

Results

Observed ambient temperatures closely approximated target levels: 95% of all hourly temperature measurements were within 0.7 °C of the target temperatures (Fig. 2). Temperatures near upwelling zones ranged from ~ 14-19 °C and created thermal refugia 6-9 °C below ambient temperatures (Fig. 2). Upwelling temperatures were several degrees warmer in the last three replicates than the first three but were below ambient temperatures of 17-23 °C in all replicates (Fig. 2). Water quality was typical of calcium-rich springs that service the Leetown Science Center (Kozar et al. 2007) as characterized by alkaline pH and high conductivity. Specific conductance and pH increased with temperature (Table 1), likely due to increased calcium dissolution and associated ionic loading.

Allopatric brook trout exhibited different patterns of upwelling habitat use, spatial evenness, and movement than brook trout in the presence of brown trout. The percent of fish occupying upwelling habitat zones (see Fig. 1) increased with temperature for allopatric brook trout as well as sympatric brook trout and brown trout (Fig. 3a), indicating the importance of groundwater upwelling as thermal refugia for both species. However, sympatric brook trout exhibited greater use of the upwelling habitat than allopatric brook trout at all temperatures. For instance, over half of the sympatric brook trout were located in the upwelling zone at all temperatures, whereas allopatric brook trout reached this level of upwelling use at 23 °C (Fig. 3a). Mixed-model results indicated monotonic increases in brook trout use of upwelling habitat with increasing temperatures (Fig. 3b). The standardized effect of brown trout presence on brook trout upwelling use was approximately twice that of the highest temperature level (Fig. 3b). Marginal and conditional R^2 values indicated that the relative importance of brown trout was greater than the relative importance of temperature on brook trout upwelling habitat use (0.643 and 0.357, respectively; Table 2).

Spatial evenness of brook trout within streams decreased in the presence of brown trout, whereas temperature had insignificant effects. Allopatric brook trout were more evenly distributed than sympatric brook trout at all temperature levels, and allopatric brook trout were more similar to sympatric brown trout than sympatric brook trout in this regard (Fig. 4a). Brown trout reduced brook trout spatial evenness within streams (i.e., 95% CI < 0), but temperature levels had no effect in this regard (i.e., 95% CIs include 0) (Fig. 4b). Likewise, the effect of brown trout accounted for 99% of the conditional R^2 for the model of brook trout spatial evenness (Table 2).

Inter-tank movement rates of brook trout decreased in the presence of brown trout. Allopatric brook trout showed greater movement rates than sympatric brook trout or sympatric brown trout at all temperature levels (Fig. 5a). This result could not be attributed to differences in brook trout density (i.e., twice the number of brook trout in allopatric vs. sympatric treatments) because the difference in observed movement rates was more than twice as high in the allopatric vs. sympatric populations (Fig. 5a). Moreover, the movement rates for allopatric brook trout were generally greater than for the total movement observed for all fish in the sympatric treatments (i.e., brown trout and brook trout combined) (Fig. 5a). Although fish moved into thermal refugia as ambient temperatures increased (Fig. 3), temperature was unrelated to brook trout movement from video samples (i.e., 95% CIs include 0) whereas brown trout showed a significant negative effect in this regard (Fig. 5b). The conditional R^2 of the brook trout movement model was 0.377, of which 98% was attributable to the effect of brown trout (Table 2).

Observed brook trout aggression was not influenced by temperature or brown trout. The linear mixed-model for brook trout aggression supported a low conditional R^2 (0.09), and

therefore much of the observed variation was not accounted for by thermal or biotic treatments (Table 2). Brown trout aggression generally increased with temperature (Fig. 6a), but the effect of brown trout on brook trout aggression rates was insignificant (Fig. 6b). Moreover, the relative importance of temperature exceeded the importance of brown trout in partitioning the conditional R^2 for the model of brook trout aggression (Table 2).

Brook trout ventilation rates were influenced by temperature but not the presence of brown trout. Ventilation rates increased monotonically across temperature levels from ~55 beats/minute at 14 °C to ~80 beats/minute at 23 °C (Fig. 7a). The effect of brown trout on brook trout ventilation was insignificant as indicated by 95% CIs (Fig. 7b). In contrast, temperature levels showed monotonically increasing effects on brook trout ventilation rate (Fig. 7b), and temperature was the only significant predictor in the model, given that <0.01% of the conditional R^2 (0.763) was accounted for by the effect of brown trout (i.e., marginal R^2) (Table 2).

Effects of brown trout were also evident in the correlation structure of brook trout responses. Upwelling habitat use was negatively associated with movement rates for both allopatric and sympatric brook trout (Spearman $r > |0.43|$, $p < 0.01$, respectively), but only sympatric brook trout exhibited a significant correlation between spatial evenness and upwelling habitat use (Table 3). Upwelling habitat use was positively correlated with brook trout ventilation rates in the absence but not presence of brown trout (Table 3). Brook trout ventilation rates also were positively correlated with aggressive behaviors for allopatric and sympatric brook trout, but only in the presence of brown trout was brook trout aggression significantly correlated with movement rates (Table 3). Only 4 of the 10 significant ($p < 0.05$) correlations in brook trout response variables were shared between allopatric and sympatric treatments (Table 3).

Discussion

Our results indicate that brown trout affect brook trout behavioral thermoregulation and access to foraging habitats. This study expanded on prior research by regulating the spatial structure of thermal refugia and foraging resources across increasing temperatures. We used experimental streams with a fully-crossed experimental design to partition the relative importance of biotic and abiotic conditions for brook trout while accounting for variation across replicates and spatial positions of streams within the laboratory. Although observed interspecific interactions were not strictly temperature-dependent, brown trout restricted brook trout distributions and limited their access to foraging habitats outside thermal refugia. Our study underscores the importance of resource spatial configuration in streams (Dunning et al. 1992; Schlosser 1991), and our results have implications for native brook trout conservation in the context of climate change and non-native species management.

Both species increased their use of groundwater upwelling zones with increasing temperatures (Fig. 3a) as expected. However, we did not find evidence for our prediction that competitive advantage would shift from brook trout to brown trout with increasing temperatures. Instead, brown trout restricted brook trout distribution and spatial evenness within streams at all temperature levels evaluated (Fig. 4b) suggesting interference competition for access to belt feeders located outside thermal refugia (see Fig. 1). Consistent with our results, field studies have shown that brown trout exhibit faster growth rates than sympatric brook trout (Carlson et al. 2007; Hoxmeier and Dieterman 2013), and experimental removals of brown trout have yielded increased brook trout growth rates and biomass (Hoxmeier and Dieterman 2016). Although our results indicate that brown trout displace brook trout from foraging resources across a large range of temperatures (14 – 23 °C), we would expect the energetic effects of brown trout to increase

with water temperature (see Carlson et al. 2007) because both species exhibit density-dependent growth during summer months (Jenkins et al. 1999; Utz and Hartman 2009) and brook trout metabolic efficiency decreases rapidly as temperatures exceed 20 °C (Hartman and Cox 2008). Given the similarity of ambient and groundwater temperatures at 14 °C (Fig. 2), we interpret the prevalence of sympatric brook trout in the upwelling habitat at this temperature level as displacement from upstream foraging habitat rather than selection for groundwater habitat. Additional research is needed to test this interpretation by manipulating the spatial location of foraging resources relative to groundwater upwelling locations.

Our results differ from prior research showing competitive equivalency of these species across temperature levels (Taniguchi et al. 1998) and showing brook trout out-competing brown trout at relatively low temperatures (14 °C, Fausch and White 1986). However, our results are consistent with prior research showing brown trout dominance over brook trout in experimental settings (DeWald and Wilzbach 1992) and field studies (Öhlund et al. 2008). Differences with prior studies may be explained by effects of fish size, source stock, or spatial structure of limiting resources. We evaluated large adult fish (310-380 mm total length, Appendix A), whereas Fausch and White (1986) evaluated juvenile trout and Taniguchi et al. (1998) evaluated smaller trout (107-165 mm fork length) than our study. Field studies have revealed stronger interspecific competition between adult brook trout and brown trout than juveniles (Fausch and White 1981; Hoxmeier and Dieterman 2013; *cf.* Hutchinson and Iwata 1997), and this may explain why we observed competitive effects that other studies did not. However, this is probably not a complete explanation because at least one study showing brown trout dominance over brook trout evaluated fish of similar size as in Taniguchi et al. (1998) (mean TL = 107 mm, DeWald and Wilzbach 1992). Our results probably represent conservative estimates of brown trout effects in

natural ecosystems because brown trout tend to be larger than brook trout when occurring in sympatry (Fausch and White 1981; Carlson et al. 2007).

Alternatively, differences among studies may be due to effects of hatchery propagation. Experimental trials have shown hatchery-sourced brook trout to be more aggressive than brook trout collected from wild populations (Moyle 1969) as known from other salmonid species (e.g., cutthroat trout *Oncorhynchus clarki*, Mesa 1991). Hatchery-sourced brook trout also have exhibited less use of cover and greater movement rates than wild brook trout (Vincent 1960), and brown trout show similar patterns (Bachman 1984). In the present study we used hatchery-sourced fish and found stronger effects of interspecific competition than shown from laboratory-based studies of wild brook trout and brown trout (Fausch and White 1986; Taniguchi et al. 1998). It therefore is possible that genetic or behavioral effects associated with hatchery propagation influenced our results. However, our observations were consistent with DeWald and Wilzbach's (1992) study of wild brook trout and hatchery-sourced brown trout, indicating that our results are unlikely to be solely due to source population effects. We recommend *in-situ* experimental removals of brown trout from sympatric brook trout populations following Fausch and White (1981) across a gradient of stream temperatures and thermal refugia configurations to evaluate our results within natural settings.

Our study demonstrated the importance of thermal refugia for trout. We observed that trout aggregated into thermal refugia when ambient temperatures exceed 20 °C (i.e., >50% abundance; Fig. 3a). Baird and Krueger (2003) also identified a behavioral response of brook trout where stream temperatures exceeded 20 °C such that observed body temperatures were on average 4 °C cooler than ambient temperatures, indicating fish movements to access thermal refugia. Field studies have also demonstrated brook trout movements to access thermal refugia

within stream networks (Petty et al. 2012) and lake ecosystems (Curry and Noakes 1995; Biro 1998) as known for other salmonid species (Chinook salmon *Oncorhynchus tshawytscha*, Torgerson et al. 1999; rainbow trout, Ebersole et al. 2001; lake trout *Salvelinus namaycush*, Snucins and Gunn 1995). Moreover, the absence of groundwater inputs and thermal refugia has been attributed to delays in brook trout spawning during years with above-average air temperatures (Warren et al. 2012) whereas such phenological effects would not be expected in groundwater-dominated locations (e.g., Biro 1998). Brook trout aggregation into upwelling areas was associated with lower movement rates for allopatric and sympatric populations (Table 3) as expected. In contrast, use of upwelling zones was associated with decreased spatial evenness only for sympatric brook trout (Table 3), indicating the capacity for brook trout use of forage habitats in high temperatures ($> 20^{\circ}\text{C}$) if brown trout are absent.

The spatial structure of groundwater exchange processes in streams is expected to regulate the utility of thermal refugia for fishes (Poole et al. 2006). Hyporheic exchange at the microhabitat scale (i.e., 10^{-1} - 10^0 m scale, Harvey and Bencala 1993) is nested within groundwater discharge dynamics at the reach-scale (i.e., 10^1 - 10^2 m scale, Dugdale et al. 2015; Snyder et al. 2015), and both scales may structure thermal habitat for fishes (Frissell et al. 1986; Fausch et al. 2002; Poole et al. 2006). Our study evaluated thermal refugia 6-9 $^{\circ}\text{C}$ below ambient temperatures within a relatively small distance (7.6 m-long streams), and this temperature difference is larger than expected effects of hyporheic exchange at the meter-scale (Harvey and Bencala 1993) but resembles potential thermal heterogeneity observed at the stream reach scale (Dugdale et al. 2015; Snyder et al. 2015) and stream network scale (i.e., inter-stream effects, Kaya et al. 1977; Benda et al. 2004; Dugdale et al. 2013). Our study therefore provides a

perspective most applicable for stream reaches near downstream distribution limits of brook trout.

Brown trout restricted brook trout movements and spatial distributions within streams, but we did not observe effects of brown trout on brook trout aggression (Fig. 6*b*). Instead, brown trout were generally more aggressive than brook trout at all temperatures evaluated (Fig. 6*a*). We observed multiple instances of brown trout charging and chasing brook trout near belt-feeder locations (see Fig. 1), particularly when ambient temperatures exceeded 20 °C, but these observations were not collected systematically for analysis. The relatively short observation window in our study (10 minutes/day) may explain the absence of an effect, as suggested by the relatively high among-sample variance observed for aggressive behaviors (Fig. 6*a*). By comparison, Hutchinson and Iwata (1997) collected aggression data by sampling at 5-minute increments hourly for multiple days and Dickson and MacCrimmon (1982) observed aggressive interactions for 15 minutes/day. We would expect intraspecific competition to be more intense than interspecific competition because conspecifics would exhibit greater niche-overlap (Connell 1983), particularly for brook trout which are known to exhibit greater intraspecific aggression than other salmonid species (Hutchinson and Iwata 1997). Despite the expected importance of intraspecific competition, we observed more aggression in sympatric populations than among allopatric brook trout (Fig. 6*a*), suggesting interference competition by brown trout.

Ventilation rates increased monotonically with ambient temperature levels but were invariant to the presence or absence of brown trout (Fig. 7*b*). This pattern indicates increased oxygen consumption at higher temperatures, and such a temperature effect has been observed in brook trout and brown trout (Job 1955; Beamish 1964). Observed brook trout ventilation rates ranged from approximately 50-90 beats/minute (Fig. 7*a*) which was somewhat lower than

observed for smaller brook trout at 11 °C (approximately 95 beats/minute, Walker et al. 1988). Although the magnitude of observed ventilation rate increases across temperatures is generally consistent with previously reported increases over time in response to acid stress (pH = 4.8, Walker et al. 1988), caution is warranted in interpreting ventilation rate as a stress response because increased ventilation responses may not necessarily indicate the magnitude of the stressor (Barreto and Volpato 2004). Nonetheless, observed ventilation rates sampled primarily from the groundwater upwelling zone at 23 °C (see Fig. 1) did not provide a complete refuge from physiological effects associated with ambient temperature exposure. This may be due to the mixing of ambient and upwelling water in the groundwater upwelling zone. Dye tests indicated that groundwater upwelling from substrates provided a discrete spatial zone of cold water (Appendix B), but fish movement would increase mixing of water temperatures. This may also explain the observed differences in upwelling-zone temperatures across experimental replicates (Fig. 2).

An important limitation of our study is the lack of individual-level inferences. For instance, we could not resolve the individual identities of allopatric brook trout that periodically utilized high temperatures to access foraging habitat. Future research using passive integrated transponder tags and fixed antennas is recommended to address this question. Moreover, our design randomly re-assigned brook trout to allopatric and sympatric treatments across replicates, and therefore we could not discern effects of brown trout on individual growth rates as an index of foraging efficiency as previously demonstrated (Fausch and White 1986; Taniguchi et al. 1998; DeWald and Wilzbach 1992). We also cannot resolve potential social interactions that structure dominance hierarchies within and between trout species (Newman 1956; White and Gowan 2013). However, because we randomly assigned allopatric and sympatric treatments to

streams across replicates, handling-effects were equally distributed among all individuals and thus are not expected to introduce directional biases.

Our results provide direct evidence for interspecific competition between brook trout and brown trout by use of a substitutive design whereby we attempted to hold total fish densities constant. This approach is appropriate for investigating interspecific competition between species known to compete in natural systems, and is more appropriate for this purpose than additive designs which conflate total fish density with species composition (Fausch 1988, 1998; Weber and Fausch 2003). Total fish densities in our study ranged from 1.3-1.5 fish/m, within the observed range of brook trout densities in Appalachian streams (Kanno et al. 2015). However, mortality of 2 brook trout reduced densities within the sympatric treatment after the first replicate due to infection of *Saprolegnia* sp., an oomycete mold causing lethality through haemodilution (Woo and Bruno 2011). DeWald and Wilzbach (1992) observed a similar pattern whereby brook trout in the presence of brown trout succumbed to *Saprolegnia* infection whereas neither allopatric brook trout nor brown trout were affected. They interpreted this effect as a stress indicator in brook trout exposed to brown trout (DeWald and Wilzbach 1992), and our observations are consistent with their hypothesis.

Conservation strategies for native fishes often include goals for expansion of occupied habitat, and our results suggest that removal of brown trout may facilitate this goal for brook trout at their downstream limits. We showed that both species preferentially selected thermal refugia when ambient temperatures exceeded 20 °C, and that brown trout restricted brook trout access to forage resources at such temperature levels. Anticipated increases in stream temperatures (Snyder et al. 2015; Isaak et al. 2016) therefore will be important for brook trout not only through direct physiological mechanisms (Chadwick et al. 2015) but also through

indirect pathways associated with brown trout competition. Management for thermal refugia in streams may be feasible in some cases (Kurylyk 2014b), but management for non-native trout abundance has been shown to be feasible albeit energy intensive (Larson and Moore 1985). Furthermore, downstream expansion of native brook trout could enhance fisheries management by increasing brook trout growth in downstream locations. Limitations of this approach will depend in part on the spatial configuration of thermal refugia at the stream reach scale and the willingness of the angling community to conserve native fishes.

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Figure captions

Fig. 1. Experimental stream design. Two replicate streams are shown with pools as circles and riffles as troughs between pools. Arrows indicate recirculating flow direction through the overflow sump (S), system pump and filters (PF), heating unit (HE), chilling unit (C). The locations of groundwater upwelling (GW) and belt feeders (B) created spatial structure for thermal and forage habitats.

Fig. 2. Observed hourly temperatures during experimental trials. Solid lines show ambient stream temperatures and dashed lines show upwelling water temperatures measured near substrates over 6 experimental trials (replicates). Horizontal dashed lines show target temperature levels (14, 17, 20, 23 °C), and vertical dashed lines indicate midnight. Auxiliary measures of upwelling zone temperature are indicated by “x”.

Fig. 3. (a) Upwelling habitat use for allopatric brook trout (A, filled), sympatric brook trout (S, grey) with sympatric brown trout (B, open), and total sympatric populations (hatch-marks) across ambient temperature levels. Bars show mean values, and whiskers show 1 standard deviation from the mean. (b) Effects of brown trout and temperature on brook trout upwelling habitat use. Points indicate fitted model coefficients, and line segments indicate bootstrapped 95% confidence intervals. Line segment colors distinguish fixed and random effects. Upwelling zones are mapped as GW in Fig. 1.

Fig. 4. (a) Spatial evenness within streams for allopatric brook trout (A, filled), sympatric brook trout (S, grey) with sympatric brown trout (B, open), and total sympatric populations (hatch-marks) across ambient temperature levels. Bars show mean values, and whiskers show 1 standard deviation from the mean. (b) Effects of brown trout and temperature on brook trout spatial evenness. Points indicate fitted model coefficients, and line segments indicate bootstrapped 95% confidence intervals. Line segment colors distinguish fixed and random effects. Spatial evenness was calculated from a modification of Simpson's Index (equation 1).

Fig. 5. (a) Inter-tank movement rates for allopatric brook trout (A, filled), sympatric brook trout (S, grey) with sympatric brown trout (B, open), and total sympatric populations (hatch-marks) across ambient temperature levels. Bars show mean values, and whiskers show 1 standard deviation from the mean. (b) Effects of brown trout and temperature on brook trout movement rates. Points indicate fitted model coefficients, and line segments indicate bootstrapped 95% confidence intervals. Line segment colors distinguish fixed and random effects.

Fig. 6. (a) Aggressive behavior rates for allopatric brook trout (A, filled), sympatric brook trout (S, grey) with sympatric brown trout (B, open), and total sympatric populations (hatch-marks) across ambient temperature levels. Bars show mean values, and whiskers show 1 standard deviation from the mean. (b) Effects of brown trout and temperature on brook trout aggressive behavior rates. Points indicate fitted model coefficients, and line segments indicate bootstrapped 95% confidence intervals. Line segment colors distinguish fixed and random effects.

874 **Fig. 7.** (a) Ventilation rates for allopatric brook trout (A, filled) and sympatric brook trout (S,
875 grey) with brown trout across ambient temperature levels. Bars show mean values, and whiskers
876 show 1 standard deviation from the mean. (b) Effects of brown trout and temperature on brook
877 trout ventilation rates. Points indicate fitted model coefficients, and line segments indicate
878 bootstrapped 95% confidence intervals. Line segment colors distinguish fixed and random
879 effects.

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880 **Table 1.** Water quality parameters measured across the range of experimental temperatures.
 881 Median values are shown with median absolute deviation in parentheses. Data were collected
 882 with a YSI Professional Plus Multiparameter Instrument ($n=18$ per temperature level).

Variable	Temperature		Wilcoxon rank-sum test	
	14 °C	23 °C	<i>W</i>	<i>p</i>
Conductivity ($\mu\text{S}/\text{cm}$)	473 (11.3)	582 (3.0)	0	< 0.0001
Dissolved oxygen (mg/L)	8.5 (0.3)	8.3 (0.4)	240	< 0.01
pH	8.2 (0.05)	8.3 (0.03)	80	< 0.01

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Table 2. Coefficients of determination for linear mixed models. The marginal R^2 indicates variance explained by brown trout (fixed effect), and the conditional R^2 indicates variance explained by brown trout and temperature (fixed and random effects) (see Nakagawa and Schielzeth 2013). The relative importance of temperature ranges from 0-1 and is calculated as $(\text{conditional } R^2 - \text{marginal } R^2)/\text{conditional } R^2$, and the relative importance of brown trout is calculated as 1 minus the relative importance of temperature.

Response variable	Marginal R^2 (fixed effect)	Conditional R^2 (fixed and random effects)	Relative importance of temperature (random effects)	Relative importance of brown trout (fixed effect)
Upwelling habitat use	0.347	0.540	0.357	0.643
Spatial evenness	0.689	0.698	0.013	0.987
Movement	0.370	0.377	0.019	0.981
Aggression	0.0003	0.093	0.997	0.003
Ventilation	0.0001	0.763	1.000	< 0.001

Table 3. Correlation matrices for brook trout upwelling habitat use (UHU), brook trout spatial evenness (SPE), brook trout movement rate (MOV), brook trout aggressive behavior rate (AGG), and brook trout ventilation rate (VEN) in the presence and absence of brown trout across all temperature levels. Upper diagonals contain Spearman correlation coefficients and lower diagonals contain associated p -values. Coefficients with $p < 0.05$ are indicated by *. Sample size for allopatric and sympatric treatments included 24 observations (4 temperature levels x 6 replicates), respectively.

Treatment	Variable	UHU	SPE	MOV	AGG	VEN
Brown trout						
absent	UHU	–	-0.15	-0.54*	0.20	0.44*
	SPE	0.49	–	0.02	0.07	-0.24
	MOV	<0.01	0.93	–	0.17	-0.22
	AGG	0.36	0.76	0.43	–	0.51*
	VEN	0.03	0.25	0.30	0.01	–
Brown trout						
present	UHU	–	-0.55*	-0.43*	-0.09	0.21
	SPE	<0.01	–	0.25	0.09	0.08
	MOV	0.03	0.25	–	0.55*	0.33
	AGG	0.68	0.68	<0.01	–	0.59*
	VEN	0.34	0.72	0.12	<0.01	–

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Appendix A. Length-weight plots for brook trout and brown trout prior to experimental trials. A random subset of the individuals shown was used for experimental trials.

Appendix B. Photo of dye test showing coldwater upwelling from substrates in an experimental stream channel.