



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

Journal:	<i>Canadian Journal of Fisheries and Aquatic Sciences</i>
Manuscript ID	cjfas-2016-0255.R1
Manuscript Type:	Article
Date Submitted by the Author:	12-Sep-2016
Complete List of Authors:	Hitt, Nathaniel; U.S. Geological Survey Snook, Erin; U.S. Geological Survey Massie, Danielle; U.S. Geological Survey
Keyword:	brook trout, brown trout, interference competition, thermal refugia, GROUNDWATER < General



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

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14 **Abstract**

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

28

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

31 Introduction

32 Interspecific competition is often influenced by abiotic factors (i.e., condition-specific
33 competition, Dunson and Travis 1991). An understanding of condition-specific competition
34 therefore is necessary to anticipate how changing environmental conditions will affect
35 population dynamics and community composition (Tylianakis et al. 2008; Van der Putten et al.
36 2010). Anticipated increases in stream temperature (Snyder et al. 2015; Isaak et al. 2016)
37 highlight a specific need to understand the relative importance and potential interactions of biotic
38 and abiotic mechanisms of freshwater community assembly (Rahel et al. 2008; Wenger et al.
39 2011). Such knowledge can improve conservation strategies by focusing management actions on
40 the biotic or abiotic conditions that regulate community assembly (Economo 2011).

41 Freshwater fishes provide an important model in this regard because (1) condition-
42 specific competition has been demonstrated across many taxonomic groups (cyprinidae, cottidae,
43 Baltz et al. 1982; salmonidae, Taniguchi and Nakano 2000; fundulidae, cyprinodontidae,
44 Carmona-Catot et al. 2013); (2) the longitudinal addition and replacement of stream fish species
45 is influenced by interactive biotic and abiotic effects (Taniguchi and Nakano 2000; Hitt and
46 Roberts 2012); and (3) introduced fishes have displaced native fish in many cases (Kreuger and
47 May 1991; Cucherousset and Olden 2011). However, mechanistic predictions in this regard are
48 limited in part due to confounded biotic and abiotic effects in observational field studies (see
49 Hearn 1987) and experimental design limitations (see Fausch 1988, 1998; Weber and Fausch
50 2003). Here, we evaluate the relative importance of temperature and brown trout (*Salmo trutta*)
51 on the distribution and behavior of brook trout (*Salvelinus fontinalis*) in an experimental stream
52 system using a fully-crossed and replicated design.

53 The distribution of native brook trout extends from the southern Appalachian mountains
54 (Georgia, USA) to the eastern Canadian Shield (Quebec, Canada) (MacCrimmon and Campbell
55 1969). In the southern portion of their range, brook trout are largely restricted to high-elevation
56 streams in forested catchments (Flebbe 1994; Hudy et al. 2008) that support cooler temperatures
57 (e.g., 7-day maximum of daily-mean temperatures < 23.3 °C, Wehrly et al. 2007). Physiological
58 responses to heat stress have been observed where stream temperatures exceed 21 °C, and 23.4
59 °C has been shown to be the upper limit for brook trout growth (Chadwick et al. 2015). Brook
60 trout utilize groundwater upwelling zones for spawning (Curry and Noakes 1995) as well as for
61 overwintering habitat (Cunjak and Power 1986) and thermal refugia during summer months
62 (Biro 1998; Baird and Krueger 2003; Petty et al. 2012). Observed patterns of brook trout
63 metapopulation structure and population isolation in stream networks (Aunins et al. 2015) may
64 be explained in part by landscape-scale movements to access thermal refugia and spawning
65 habitat (Petty et al. 2012; Kanno et al. 2014a). Thermal refugia from groundwater upwelling
66 zones can buffer stream fish populations from effects of land-use (Steffy et al. 2004; Stranko et
67 al. 2008). However, groundwater upwelling zones are spatially patchy within streams (Kanno et
68 al. 2014b; Dugdale et al. 2015; Snyder et al. 2015), and stream thermal responses to climate
69 change will depend in part on the spatial configuration and depth of groundwater upwelling
70 zones (Meisner et al. 1998; Kurylyk et al. 2014a; Snyder et al. 2015).

71 The downstream distribution limits for native brook trout are often associated with the
72 presence of introduced rainbow trout (*Oncorhynchus mykiss*) or brown trout (*Salmo trutta*)
73 (Vincent and Miller 1969; Gard and Flittner 1974; Kozel and Hubert 1989; Rahel and Hubert
74 1991; Petty et al. 2012). Introduced rainbow trout and brown trout have been linked to declines
75 in native brook trout abundance and occurrence based on time-series analysis (Moore et al. 1983;

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

82 Various mechanisms may account for the observed displacement of native brook trout by
83 introduced brown trout. Brown trout have higher thermal tolerances than brook trout (Eaton et al.
84 1995, *cf.* Lee and Rinne 1980), and therefore warming temperatures may restrict brook trout
85 before brown trout through physiological mechanisms (Flebbe 1994; Fausch 1989; see also
86 Chadwick et al. 2015). Introduced brown trout displace native brook trout for access to flow
87 refugia (i.e., resting positions) (Fausch and White 1981), and this may decrease brook trout
88 growth by increasing respiration relative to consumption (see Hartman and Cox 2008). This
89 hypothesis is supported by the observed decrease of brook trout growth in the presence of brown
90 trout (Carlson et al. 2007; Hoxmeier and Dieterman 2013, *cf.* Öhlund et al. 2008). In addition,
91 redd superimposition or hybridization by brown trout may reduce brook trout abundance and
92 population growth (Sorensen et al. 1995; Essington et al. 1998; Cucherousset et al. 2008), and
93 predation of juvenile brook trout by adult brown trout may account for brook trout population
94 declines (Alexander 1977). Alternatively, observed declines may be because brook trout are
95 more susceptible to harvest by anglers than brown trout when occurring in sympatry (Cooper
96 1952; Marshall and MacCrimmon 1970).

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

99 temperature. Experimental stream studies have concluded that brook trout competitively
100 dominate brown trout of equal size (Fausch and White 1986) as well as the converse (DeWald
101 and Wilzbach 1992). Differences between these studies cannot be attributed to stream
102 temperatures (constant 14-15 °C in both cases) but may be due to effects of habitat cover (present
103 in DeWald and Wilzbach (1992) versus absent in Fausch and White (1986)). Nonetheless,
104 observed effects of brown trout on brook trout microhabitat use from DeWald and Wilzbach's
105 (1992) laboratory study are consistent with results of *in-situ* research based on experimental
106 removals of brown trout (Fausch and White 1981). Taniguchi et al. (1998) concluded that brook
107 trout and brown trout were competitively equivalent at temperatures ≤ 20 °C, above which both
108 species became subordinate to creek chub (*Semotilus atromaculatus*). In contrast, brown trout
109 have been shown to increase their competitive advantage over other salmonid species as
110 temperatures increase from 10 to 20 °C (Colorado River cutthroat trout *Oncorhynchus clarki*
111 *pleuriticus*, DeStaso and Rahel 1994) and from 14 to 17 °C (Arctic char *Salvelinus alpinus*,
112 Jonsson 2009).

113 Prior experimental research on this topic is limited by spatially homogenous thermal
114 treatments (i.e., uniform heating of tanks), and thus cannot account for thermal habitat patchiness
115 due to groundwater upwelling (Brunke and Gonser 1997; Torgerson et al. 1999; Ebersole et al.
116 2003; Kanno et al. 2014b; Snyder et al. 2015). Moreover, heterogeneity in resource availability is
117 an important design consideration in testing for interspecific competition (Connell 1983, Weber
118 and Fausch 2003). In this study we compared brook trout in the presence and absence of brown
119 trout with spatially- structured resources defined by thermal refugia and foraging locations. We
120 created thermal refugia by simulating groundwater upwelling through substrates across
121 increasing ambient temperatures (14, 17, 20, 23 °C), and we introduced food from belt feeders

122 located outside thermal refugia to create spatial structure in foraging habitat quality. This
123 temperature range is useful because it includes approximate temperatures for optimal brook trout
124 growth (13 °C, Baldwin 1957) as well as upper limits to positive growth (23 °C, Chadwick et al.
125 2015). We hypothesized that both species would increase their use of upwelling zones as
126 ambient temperatures increased, and that competitive advantage would shift from brook trout to
127 brown trout with increasing temperatures.

128

129 **Methods**

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

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

145 brown trout are often larger than brook trout where sympatric in streams (e.g., Fausch and White
146 1986), the equivalence of body size between species was appropriate in our study because body
147 size usually predicts dominance in salmonids (Fausch and White 1986; Fausch 1988). Fish were
148 acclimated to 14 °C for 7 days prior to experimental trials. Fish were fed 5 mm floating pellets at
149 a rate of ~ 1% total biomass/day (Finfish G pellets, Zeigler Brothers, Inc.) from 12-hour belt
150 feeders in fixed locations (Fig. 1).

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

164 Within each trial, thermal treatments regulated ambient water temperatures at 14, 17, 20,
165 and 23 °C for allopatric and sympatric populations. This temperature range was selected to
166 include upper levels stressful for brook trout physiological function (20-21 °C, Hartman and Cox
167 2008; Chadwick et al. 2015) without reaching upper incipient lethal levels for brook trout (25-30

168 °C, Fry et al. 1946; Lee and Rinne 1980). This temperature range also encompasses observed
169 summer temperatures of native brook trout habitat in Virginia (Snyder et al. 2015). Ambient
170 temperatures were held constant during daylight hours and were increased overnight by ~ 1
171 °C/hour, and similar rates have been observed in natural streams (Constantz et al. 1994).

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

181 We conducted daily visual observations to index brook trout spatial distribution within
182 streams and evenness across connected tanks (i.e., pool and riffle mesohabitat units). We
183 recorded number of fish and their species identity within pool and riffle tanks at least 3-times per
184 day, including morning and afternoon samples. Of the 24 experimental days, 22 (92%) supported
185 5 or more visual samples per day (mean = 6.8 samples/day). To minimize disturbances to fish,
186 observations were made through plexiglass windows located on the side of pool tanks (see
187 Matthews et al. 2006) that were covered with black plastic when not in use. We quantified
188 distributional evenness within streams using a variant of Simpson's index (E) (Equation 1,
189 Simpson 1949):

190

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

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

220 Temperature and brown trout treatments were applied to a fully-crossed design across 6
221 replicated trials. We fit linear mixed models to evaluate the effect of brown trout presence (fixed
222 effect) on brook trout spatial distribution and evenness, movement, aggression, and ventilation
223 while accounting for temperature (14, 17, 20, 23 °C) as random effects. The inclusion of random
224 effects in this context minimizes potential effect of pseudoreplication on statistical inference
225 (Bolker et al. 2009) in our thermal treatment design. We used restricted maximum likelihood
226 procedures to fit mixed-models using library lme4 in R (Bates et al. 2015). We applied z -score
227 transformations to standardize response variables and calculated marginal R^2 (fixed-effects only)
228 and conditional R^2 (fixed and random effects) to summarize model performance (Vonesh et al.
229 1996; Nakagawa and Schielzeth 2013). We assessed the significance of standardized coefficients
230 from 95% confidence intervals calculated from 1000 bootstrapped models with random sampling
231 of the data. Stream identity (1-2) and replicate number (1-6) had insignificant effects on brook
232 trout responses (i.e., standardized model coefficient 95% CIs included 0) and therefore were
233 excluded from further consideration.

234

235 **Results**

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

245 Allopatric brook trout exhibited different patterns of upwelling habitat use, spatial
246 evenness, and movement than brook trout in the presence of brown trout. The percent of fish
247 occupying upwelling habitat zones (see Fig. 1) increased with temperature for allopatric brook
248 trout as well as sympatric brook trout and brown trout (Fig. 3a), indicating the importance of
249 groundwater upwelling as thermal refugia for both species. However, sympatric brook trout
250 exhibited greater use of the upwelling habitat than allopatric brook trout at all temperatures. For
251 instance, over half of the sympatric brook trout were located in the upwelling zone at all
252 temperatures, whereas allopatric brook trout reached this level of upwelling use at 23 °C (Fig.
253 3a). Mixed-model results indicated monotonic increases in brook trout use of upwelling habitat
254 with increasing temperatures (Fig. 3b). The standardized effect of brown trout presence on brook
255 trout upwelling use was approximately twice that of the highest temperature level (Fig. 3b).
256 Marginal and conditional R^2 values indicated that the relative importance of brown trout was
257 greater than the relative importance of temperature on brook trout upwelling habitat use (0.643
258 and 0.357, respectively; Table 2).

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

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

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

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

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

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

304

305 Discussion

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

317 Both species increased their use of groundwater upwelling zones with increasing
318 temperatures (Fig. 3a) as expected. However, we did not find evidence for our prediction that
319 competitive advantage would shift from brook trout to brown trout with increasing temperatures.
320 Instead, brown trout restricted brook trout distribution and spatial evenness within streams at all
321 temperature levels evaluated (Fig. 4b) suggesting interference competition for access to belt
322 feeders located outside thermal refugia (see Fig. 1). Consistent with our results, field studies
323 have shown that brown trout exhibit faster growth rates than sympatric brook trout (Carlson et al.
324 2007; Hoxmeier and Dieterman 2013), and experimental removals of brown trout have yielded
325 increased brook trout growth rates and biomass (Hoxmeier and Dieterman 2016). Although our
326 results indicate that brown trout displace brook trout from foraging resources across a large range
327 of temperatures (14 – 23 °C), we would expect the energetic effects of brown trout to increase

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

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

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

353 Alternatively, differences among studies may be due to effects of hatchery propagation.
354 Experimental trials have shown hatchery-sourced brook trout to be more aggressive than brook
355 trout collected from wild populations (Moyle 1969) as known from other salmonid species (e.g.,
356 cutthroat trout *Oncorhynchus clarki*, Mesa 1991). Hatchery-sourced brook trout also have
357 exhibited less use of cover and greater movement rates than wild brook trout (Vincent 1960), and
358 brown trout show similar patterns (Bachman 1984). In the present study we used hatchery-
359 sourced fish and found stronger effects of interspecific competition than shown from laboratory-
360 based studies of wild brook trout and brown trout (Fausch and White 1986; Taniguchi et al.
361 1998). It therefore is possible that genetic or behavioral effects associated with hatchery
362 propagation influenced our results. However, our observations were consistent with DeWald and
363 Wilzbach's (1992) study of wild brook trout and hatchery-sourced brown trout, indicating that
364 our results are unlikely to be solely due to source population effects. We recommend *in-situ*
365 experimental removals of brown trout from sympatric brook trout populations following Fausch
366 and White (1981) across a gradient of stream temperatures and thermal refugia configurations to
367 evaluate our results within natural settings.

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

374 within stream networks (Petty et al. 2012) and lake ecosystems (Curry and Noakes 1995; Biro
375 1998) as known for other salmonid species (Chinook salmon *Oncorhynchus tshawytscha*,
376 Torgerson et al. 1999; rainbow trout, Ebersole et al. 2001; lake trout *Salvelinus namaycush*,
377 Snucins and Gunn 1995). Moreover, the absence of groundwater inputs and thermal refugia has
378 been attributed to delays in brook trout spawning during years with above-average air
379 temperatures (Warren et al. 2012) whereas such phenological effects would not be expected in
380 groundwater-dominated locations (e.g., Biro 1998). Brook trout aggregation into upwelling areas
381 was associated with lower movement rates for allopatric and sympatric populations (Table 3) as
382 expected. In contrast, use of upwelling zones was associated with decreased spatial evenness
383 only for sympatric brook trout (Table 3), indicating the capacity for brook trout use of forage
384 habitats in high temperatures ($> 20\text{ }^{\circ}\text{C}$) if brown trout are absent.

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

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

398 Brown trout restricted brook trout movements and spatial distributions within streams,
399 but we did not observe effects of brown trout on brook trout aggression (Fig. 6*b*). Instead, brown
400 trout were generally more aggressive than brook trout at all temperatures evaluated (Fig. 6*a*). We
401 observed multiple instances of brown trout charging and chasing brook trout near belt-feeder
402 locations (see Fig. 1), particularly when ambient temperatures exceeded 20 °C, but these
403 observations were not collected systematically for analysis. The relatively short observation
404 window in our study (10 minutes/day) may explain the absence of an effect, as suggested by the
405 relatively high among-sample variance observed for aggressive behaviors (Fig. 6*a*). By
406 comparison, Hutchinson and Iwata (1997) collected aggression data by sampling at 5-minute
407 increments hourly for multiple days and Dickson and MacCrimmon (1982) observed aggressive
408 interactions for 15 minutes/day. We would expect intraspecific competition to be more intense
409 than interspecific competition because conspecifics would exhibit greater niche-overlap (Connell
410 1983), particularly for brook trout which are known to exhibit greater intraspecific aggression
411 than other salmonid species (Hutchinson and Iwata 1997). Despite the expected importance of
412 intraspecific competition, we observed more aggression in sympatric populations than among
413 allopatric brook trout (Fig. 6*a*), suggesting interference competition by brown trout.

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

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

432 An important limitation of our study is the lack of individual-level inferences. For
433 instance, we could not resolve the individual identities of allopatric brook trout that periodically
434 utilized high temperatures to access foraging habitat. Future research using passive integrated
435 transponder tags and fixed antennas is recommended to address this question. Moreover, our
436 design randomly re-assigned brook trout to allopatric and sympatric treatments across replicates,
437 and therefore we could not discern effects of brown trout on individual growth rates as an index
438 of foraging efficiency as previously demonstrated (Fausch and White 1986; Taniguchi et al.
439 1998; DeWald and Wilzbach 1992). We also cannot resolve potential social interactions that
440 structure dominance hierarchies within and between trout species (Newman 1956; White and
441 Gowan 2013). However, because we randomly assigned allopatric and sympatric treatments to

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

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

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

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

472

473 **Acknowledgements**

474 The authors thank C. Snyder, Z. Johnson, D. Smith, S. Faulkner, D. Weller, J. Mullican,
475 M. Morgan, J. Roach, S. Strecky, M. Hudy, S. Phillips, D. Spooner, and 2 anonymous reviewers
476 for assistance with this manuscript. Fish handling protocols were approved by USGS IACUC
477 review. Funding was provided by the US Geological Survey Chesapeake Bay Program. E. Snook
478 was supported by a USGS contract with IMSG. Any use of trade, product, or firm names does
479 not imply endorsement by the US government.

480

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Draft

830 **Figure captions**

831

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

837

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

843

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

851

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

859

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

866

867 **Fig. 6.** (a) Aggressive behavior rates for allopatric brook trout (A, filled), sympatric brook trout
868 (S, grey) with sympatric brown trout (B, open), and total sympatric populations (hatch-marks)
869 across ambient temperature levels. Bars show mean values, and whiskers show 1 standard
870 deviation from the mean. (b) Effects of brown trout and temperature on brook trout aggressive
871 behavior rates. Points indicate fitted model coefficients, and line segments indicate bootstrapped
872 95% confidence intervals. Line segment colors distinguish fixed and random effects.

873

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.

Draft

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

883

Draft

884 **Table 2.** Coefficients of determination for linear mixed models. The marginal R^2 indicates
 885 variance explained by brown trout (fixed effect), and the conditional R^2 indicates variance
 886 explained by brown trout and temperature (fixed and random effects) (see Nakagawa and
 887 Schielzeth 2013). The relative importance of temperature ranges from 0-1 and is calculated as
 888 $(\text{conditional } R^2 - \text{marginal } R^2)/\text{conditional } R^2$, and the relative importance of brown trout is
 889 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

890

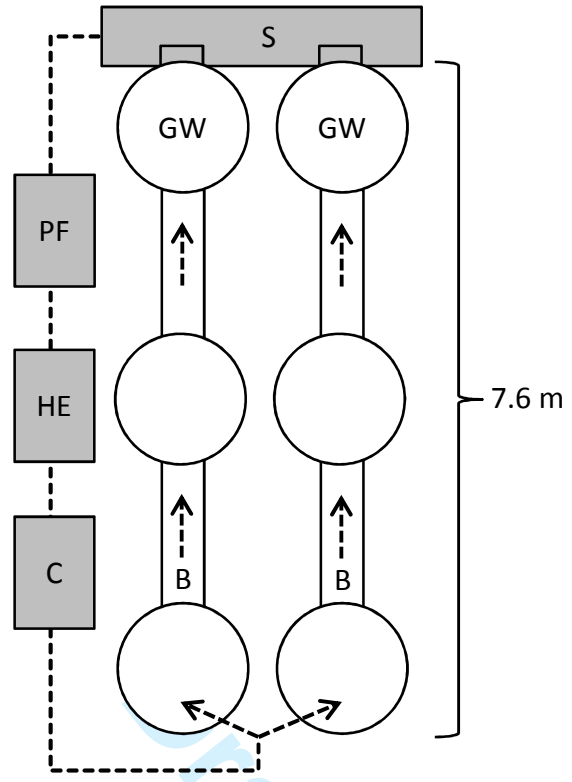
891 **Table 3.** Correlation matrices for brook trout upwelling habitat use (UHU), brook trout spatial
 892 evenness (SPE), brook trout movement rate (MOV), brook trout aggressive behavior rate (AGG),
 893 and brook trout ventilation rate (VEN) in the presence and absence of brown trout across all
 894 temperature levels. Upper diagonals contain Spearman correlation coefficients and lower
 895 diagonals contain associated p -values. Coefficients with $p < 0.05$ are indicated by *. Sample size
 896 for allopatric and sympatric treatments included 24 observations (4 temperature levels x 6
 897 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	–

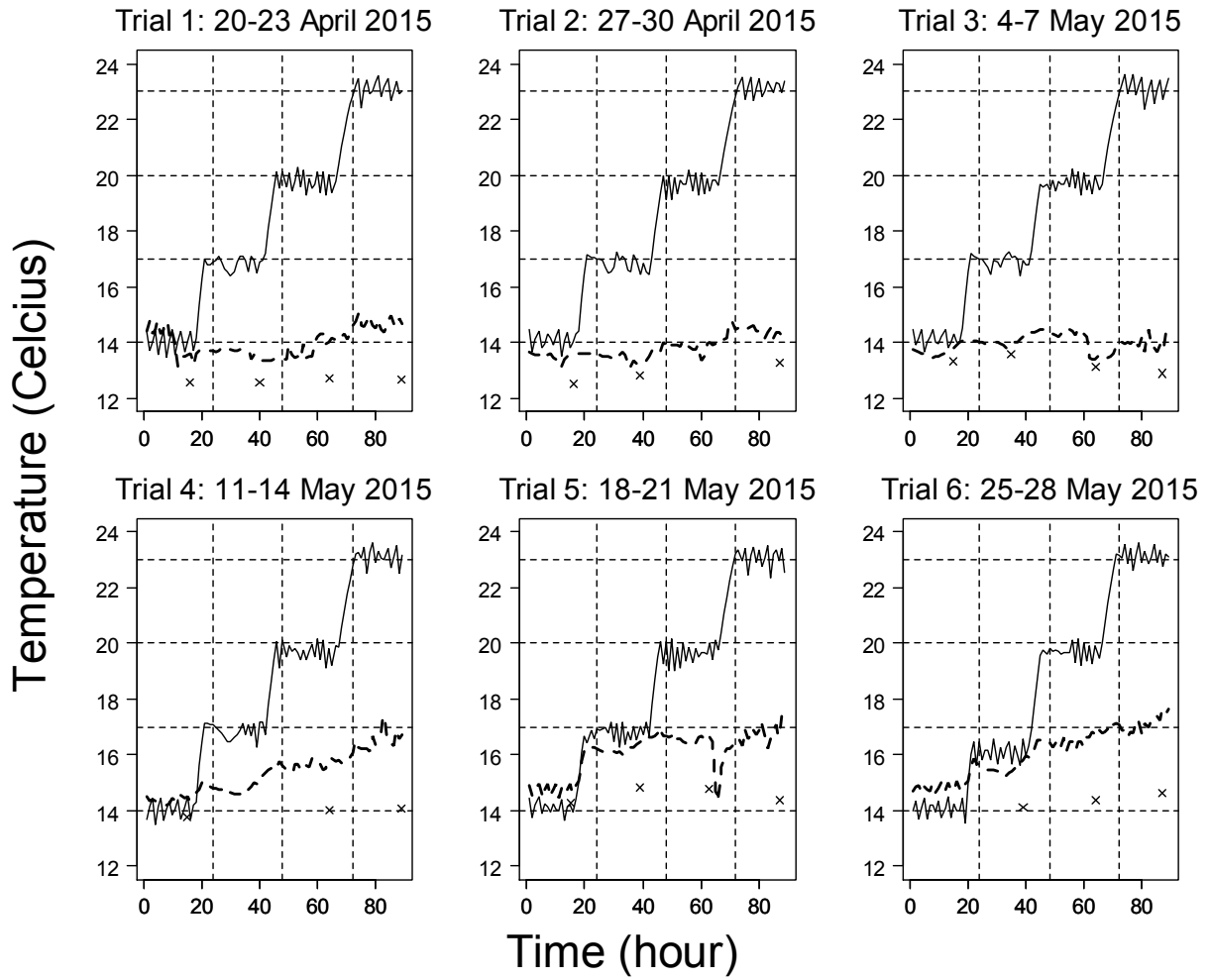
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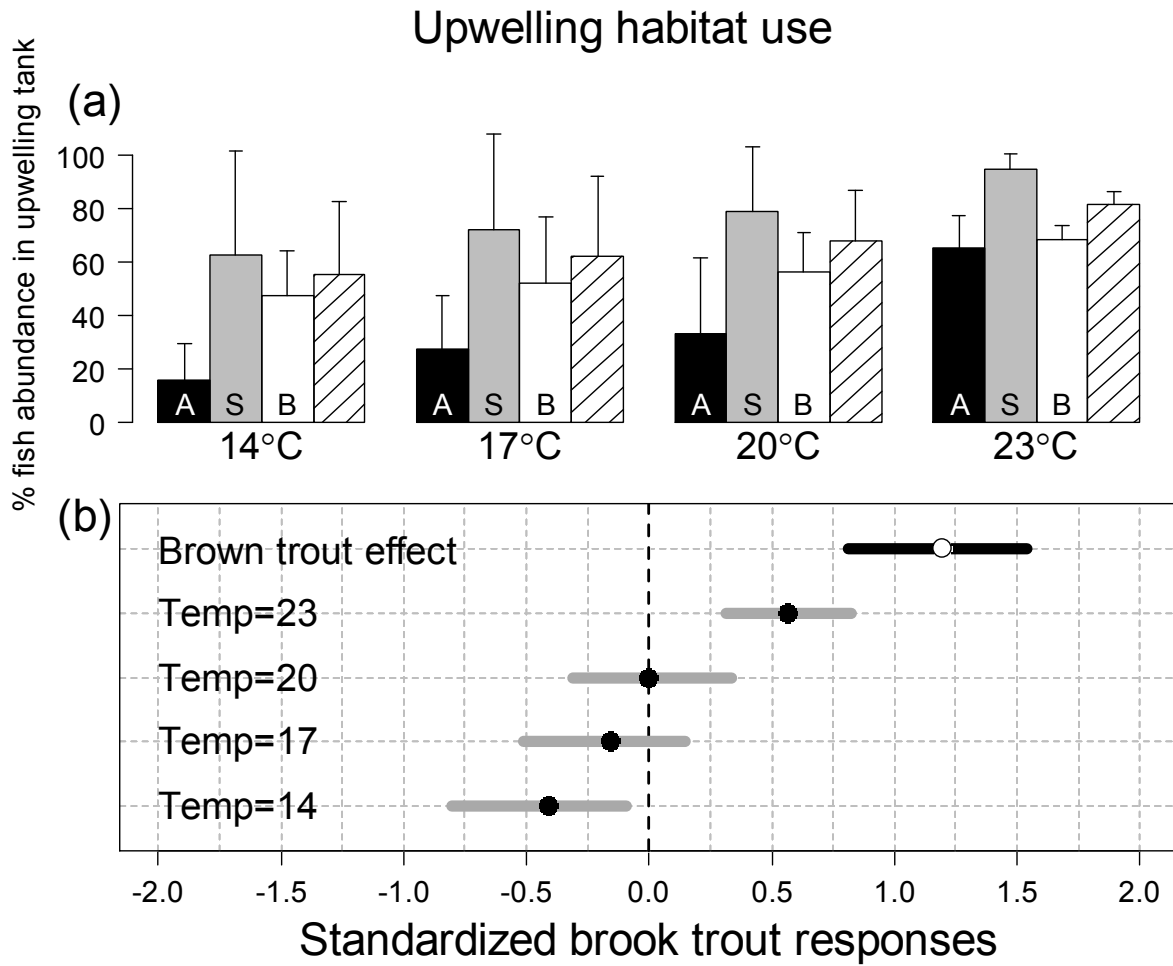
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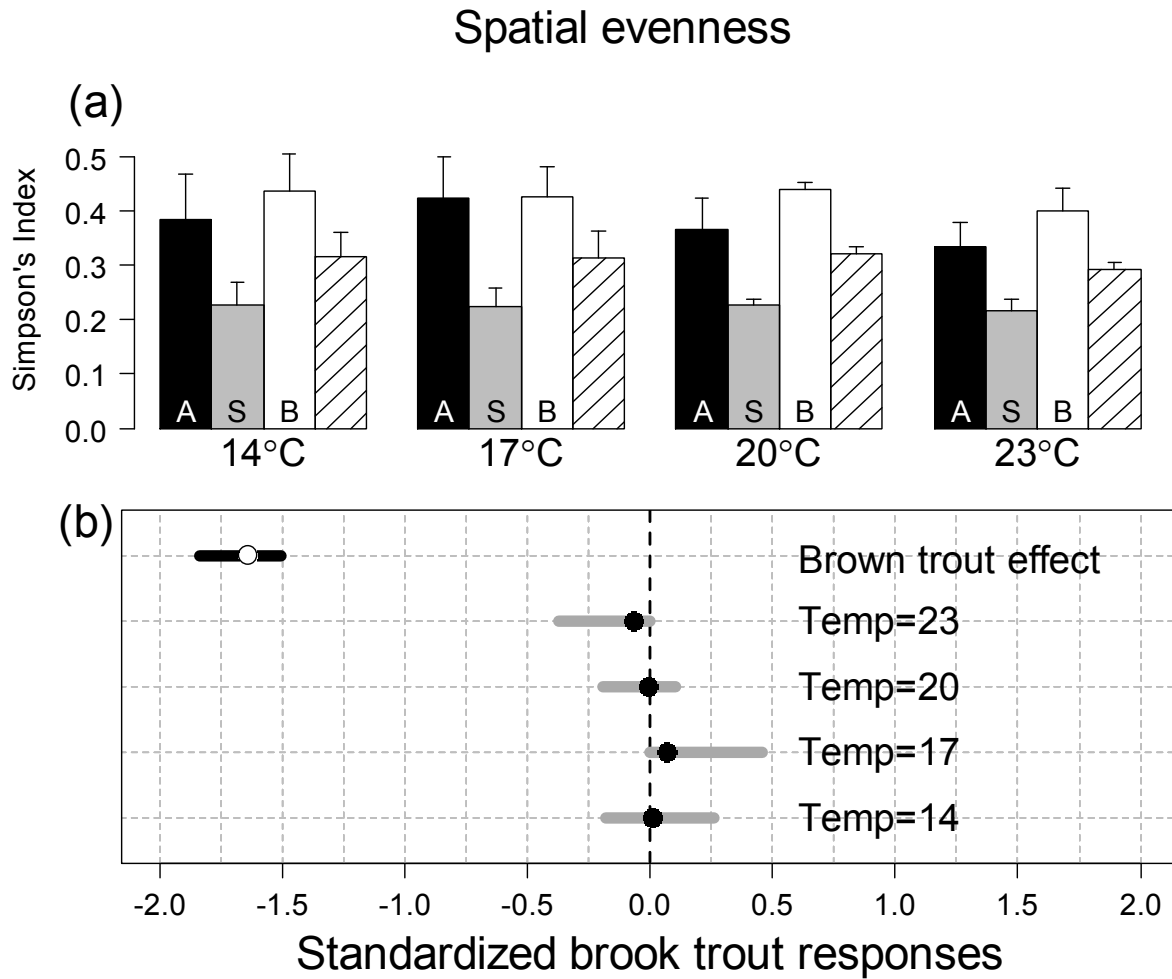


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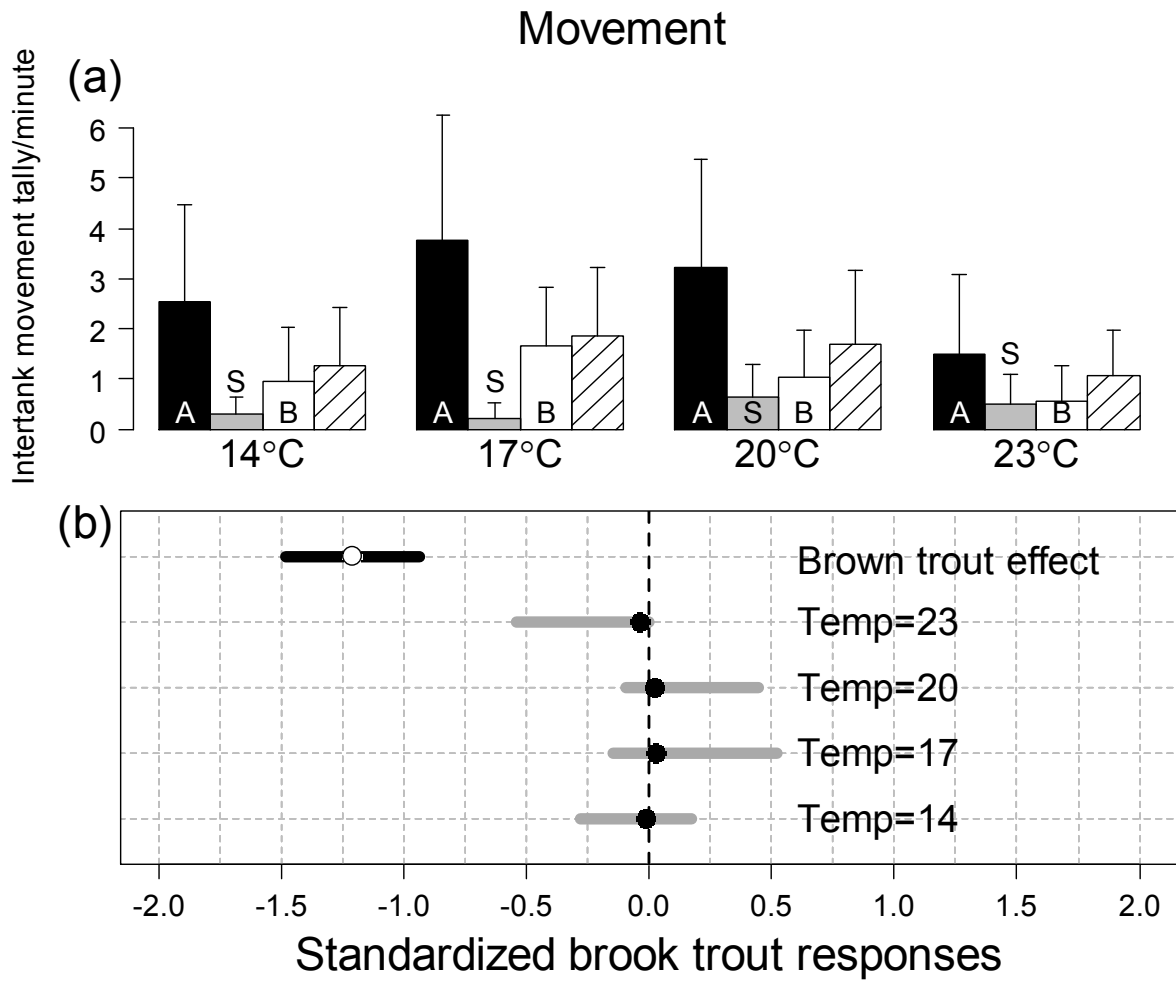
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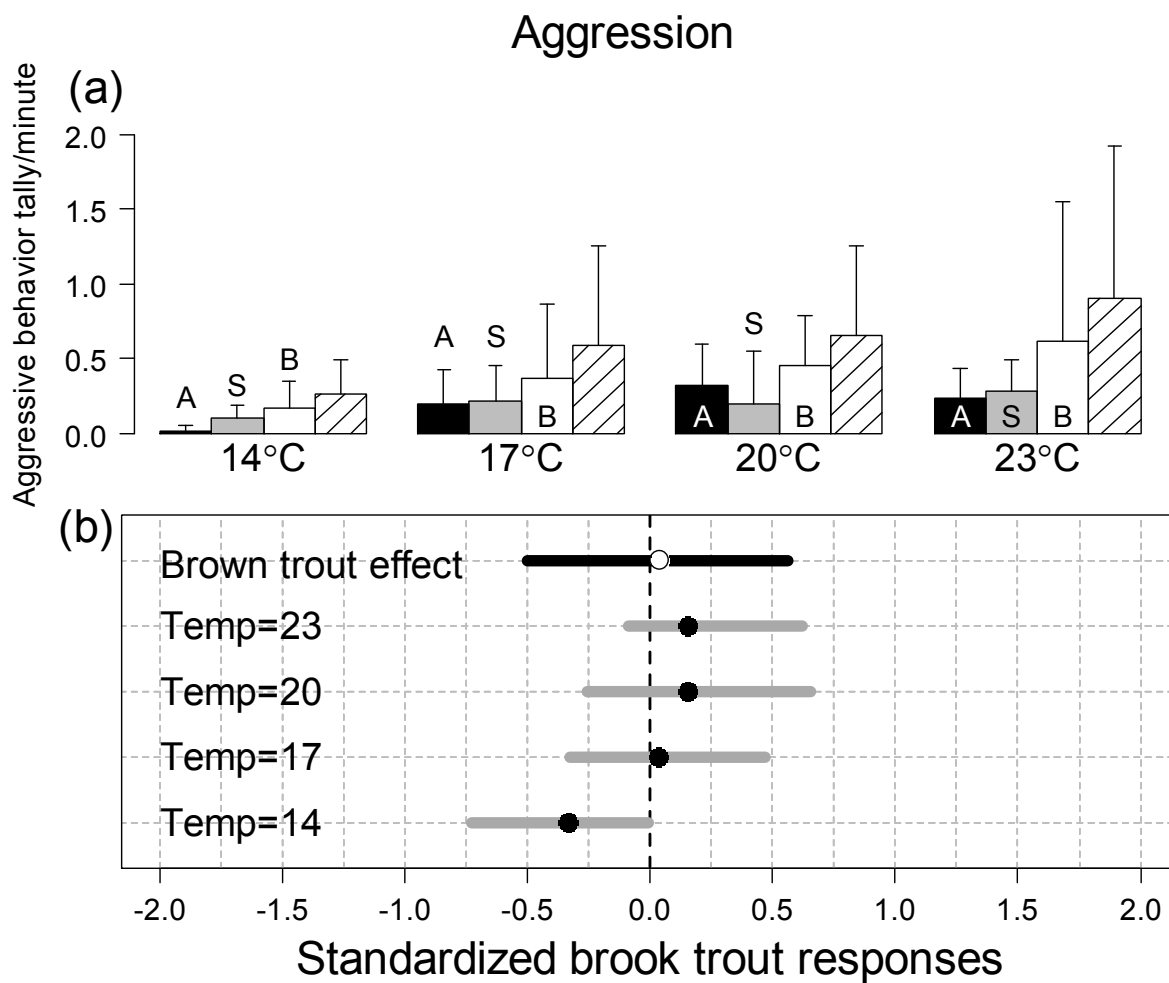


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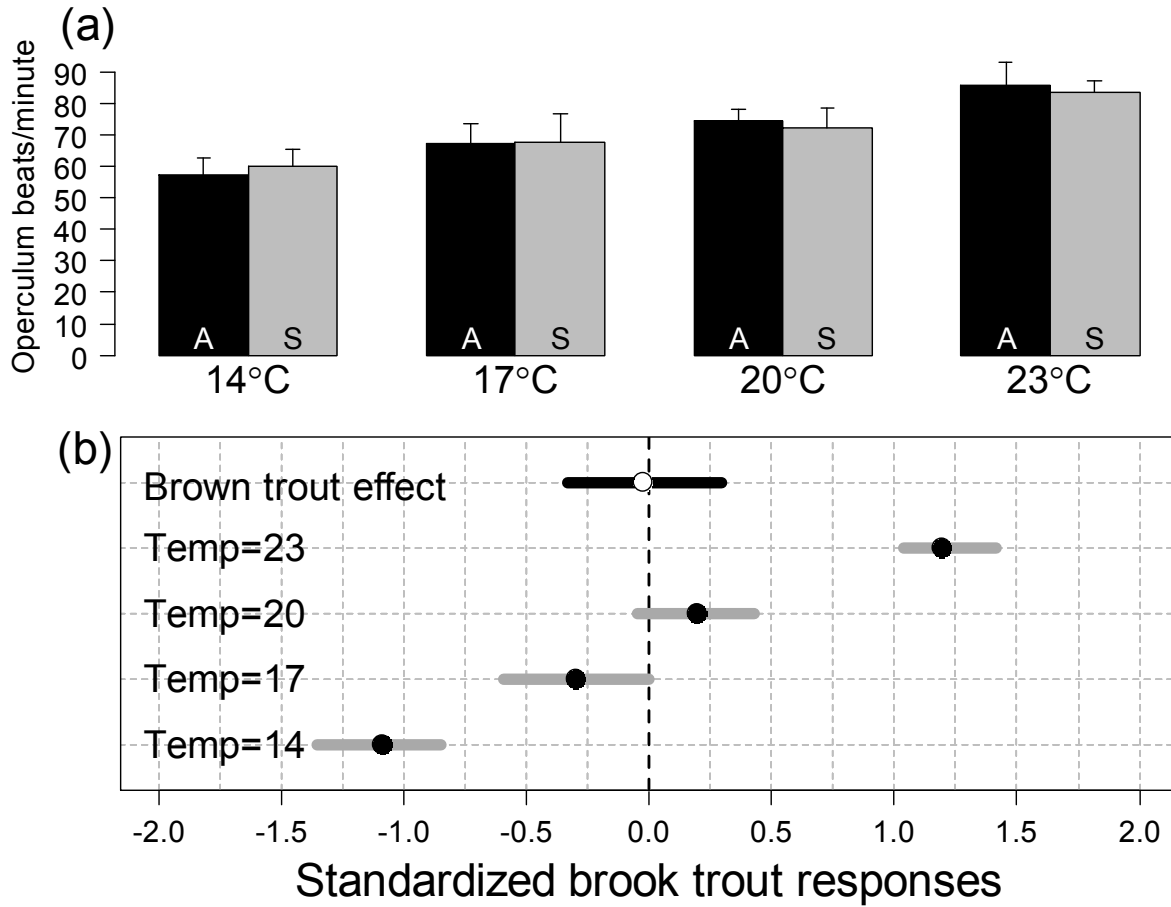


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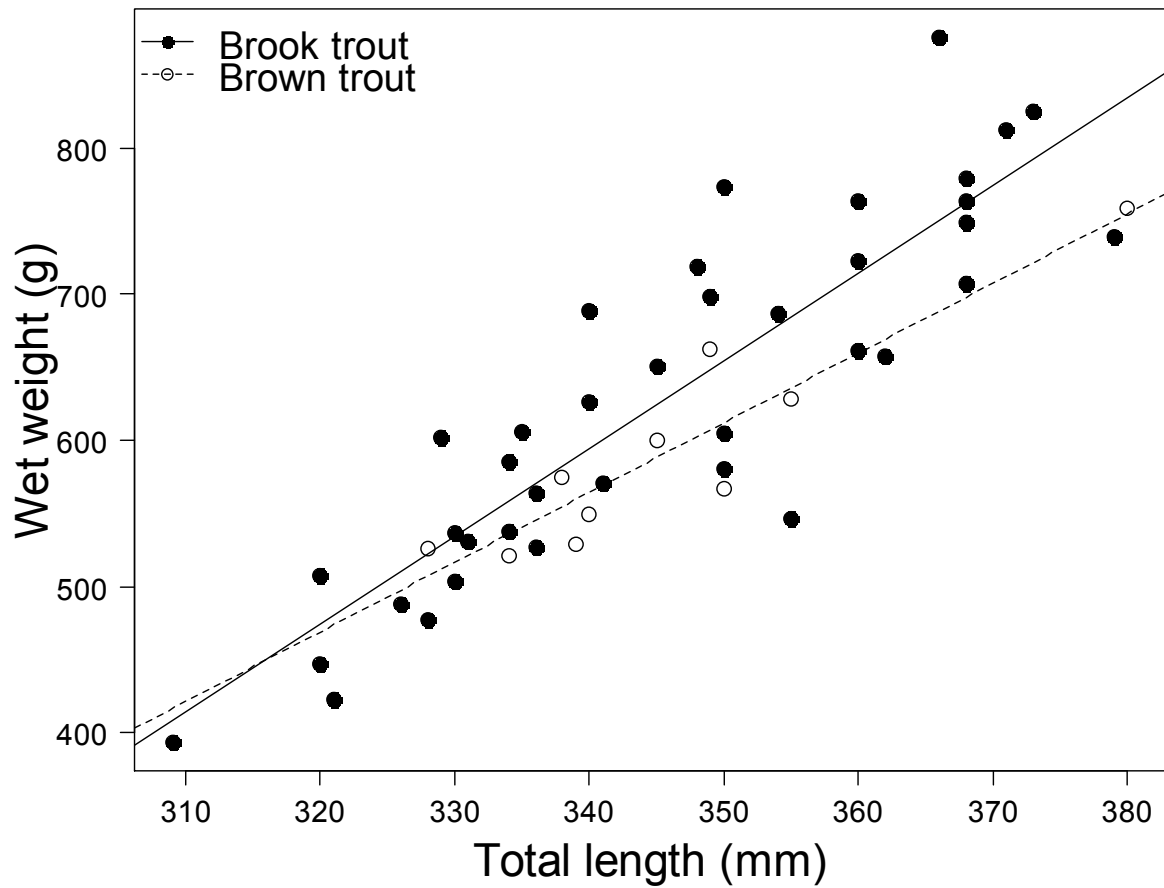
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Ventilation rate



909

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.

