REGULAR PAPER

Asymmetric competition over space use and territory between native brown trout (*Salmo trutta*) and invasive brook trout (*Salvelinus fontinalis*)

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Abstract

Interference competition over food and territory can shape population structure and habitat use within and between species. The introduction of invasive species often leads to novel competitive interactions over shared resources and invaders can eventually exclude the native species from preferred habitats. Invasive brook trout (*Salvelinus fontinalis*) introduced to northern Europe have excluded native brown trout (*Salmo trutta*) from numerous headwater streams. The fact that invasive brook trout can displace the more aggressive brown trout is puzzling. However, the earlier spawning and hatching of brook trout, compared to brown trout, may lead to unequal competition due to size advantage and prior resident status of brook trout at the fry stage. In this study, we examine the effect of competition between brown trout and brook trout using the natural size distribution of the two species. In two consecutive experiments, we first measured space use and feeding of a fry (age 0+) in the presence of a juvenile (age 1+). In experiment 2, we assessed territorial interactions between the species at the fry stage (age 0+) and if smaller brown trout could compensate the disadvantage by manipulating residence duration. Fry of brook trout feed sooner and spend more time close to the larger individual than brown trout fry. We also found that brook trout fry won most territorial contests against brown trout, and that increased residence duration led to longer and more aggressive interactions. The results suggest that smaller brown trout are displaced to suboptimal habitats in the presence of a larger brook trout. Therefore, the later emergence from gravel beds resulting in the naturally occurring size disadvantage of brown trout at the fry stage may lead to unequal territorial interactions that could explain why brown trout are displaced from preferred habitats in sympatry with brook trout.

KEYWORDS
agonistic interactions, competition, intercohort, invasive species, Salmonidae, young-of-the-year

1 | INTRODUCTION

In the late 1800s, the brook trout (*Salvelinus fontinalis*) was introduced from its native range in north-east North America and established a self-sustaining population in many parts of the world (MacCrimmon *et al*., 1971; MacCrimmon & Campbell, 1969). Following the introduction and establishment across the Scandinavian peninsula in 1877 (Aas *et al*., 2018), brook trout have managed to displace native brown trout (*Salmo trutta*) in high-altitude lakes (Spens *et al*., 2007) and rivers (Korsu *et al*., 2010). In sympatric streams, brown trout have been shown to occupy areas downstream whereas brook trout generally displace brown trout from slow-flowing pool sections in cold headwater streams (Korsu *et al*., 2007, 2010, 2012; Závorka *et al*., 2017). In the absence of the other species both brown trout and brook trout are able to occupy the whole longitudinal gradient (Korsu *et al*., 2007). While differences in niche specialization are likely to play a role in this distributional pattern, it is not well understood what mechanism drives this competitive displacement.

Short-term antagonistic behavioural interactions are recognized as important mechanisms facilitating competitive displacement (Hasegawa *et al*., 2004; Shea & Chesson, 2002; Usio *et al*., 2001). However, there is a stark contrast between the aggressive dominance of brown trout over brook trout in competition for habitat (Blanchet *et al*., 2007; DeWald & Wilzbach, 1992; Fausch & White, 1981) and optimal feeding positions (Blanchet *et al*., 2007; Hitt *et al*., 2017), and the fact that the invasive brook trout is able to displace competitively dominant brown trout from headwater streams (Korsu *et al*., 2012). As both growth and fecundity is reduced in brown trout populations in sympatry with brook trout, it is possible that the earlier maturation and faster growth of brook trout increase their competitive advantage in sympatric sites (Öhlund *et al*., 2008). In addition, a study by Cucherousset *et al*. (2008) showed that brook trout start spawning 2–3 weeks earlier than brown trout, followed by a month of overlapping spawning period for the species. The number of days required from fertilization until hatching under a natural temperature regime is also slightly shorter in brook trout than in brown trout (*i.e*., 176 days for brown trout and 168 days for brook trout) (Grande & Andersen, 1990). Therefore, the earlier spawning period and shorter incubation time of brook trout may result in earlier emergence for brook trout compared to brown trout (M. Lovén Wallerius, personal observation; Korsu *et al*. (2009).

After having depleted their yolk sacs, salmonids become dependent on exogenous food intake and will partake in intra-cohort competition for food and territories, causing strong selection pressure on the fry (Elliott, 1990; McNicol & Noakes, 1984). In addition, the fry will also compete with older individuals, *i.e*., intercohort competition, and previous studies have shown that the presence of older individuals causes salmonid fry to select less suitable microhabitats with lower food availability (Höjesjö *et al*., 2016; Kaspersson *et al*., 2012). Thus, the introduction of non-native brook trout may cause increased selection pressure on brown trout fry by additional interspecific competition between the two species. This pressure on brown trout is likely intensified by the body size advantage and territorial prior

residency of invasive brook trout (Johnsson *et al*., 1999; Johnsson & Forser, 2002; Metcalfe *et al*., 2003) caused by their earlier emergence and faster growth rate (Korsu *et al*., 2009; Öhlund *et al*., 2008). This could explain the pattern of reduced densities and recruitment of brown trout following the introduction of non-native brook trout (Korsu *et al*., 2007; Öhlund *et al*., 2008), yet this hypothesis has not been tested experimentally between these species.

In this study we investigate this hypothesis by testing the interspecific interactions between native brown trout and invasive brook trout in two consecutive experiments. First (experiment 1), we examined the effects of hetero or conspecific juvenile fish (age 1+) on space-use and feeding rate in fry (age 0+) and then (experiment 2) we examined if brown trout could compensate for the size disadvantage at the fry stage during territorial conflicts with brook trout when manipulating residence duration. In experiment 1, we predicted that fry of both species would change space use and feeding behaviour in the presence of a juvenile fish (age 1+), but to a larger extent when the potentially more aggressive brown trout was present. In experiment 2, we predicted that brown trout with longer residence duration would participate in more escalated conflicts and that the longer residence duration would increase the chance of winning for brown trout, despite being smaller than the brook trout.

2 | MATERIALS AND METHODS

2.1 | Fish sampling, housing and open field tests

The study was conducted between autumn 2019 and early winter 2020 in two consecutive experiments. All fish were collected in the Ringsbäcken stream (WGS84 decimal, latitude 57.670827°N, longitude 12.988458°E) in early autumn 2019 using electrofishing (Smith-Root LR-20B, Vancouver, Washington, USA). The downstream section is only populated by brown trout (allopatric section) whereas brown trout and brook trout coexist in the upstream section (sympatric section) (Závorka *et al*., 2017). Due to differences in fry emergence time (M. Lovén Wallerius, personal observation) and low numbers of brown trout fry (0+) in the sympatric section of the stream, all brown trout [both juvenile (1+) and fry (0+)] were collected in the allopatric section of the stream and all brook trout in the upstream sympatric section. All fish were brought back to the laboratory and housed in 10 holding tanks (65 × 35 × 35 cm) according to species and age-class (19 0+ individuals × four tanks and eight 1+ individuals × six tanks). Each holding tank was supplied with flow-through water (2 l/min, 12 ± 1°C), air stone and environmental enrichment in the form of gravel, stones, PVC plates and plastic plants. Two days after fish sampling, all fish were anaesthetized using benzocaine (1.5 ml/l), weighed (g), measured for fork length (FL) and individually tagged using passive integrated transponders (1+ individuals) or tagged with visible implant elastomere (0+ individuals). In total, 125 individuals were collected: 38 0+ brown trout (mean ± s.d. mass = 2.32 ± 0.53 g, FL = 6.19 ± 0.46 cm), 38 0+ brook trout (mean ± s.d. mass = 3.55 ± 0.83 g, FL = 7.24 ± 0.58 cm), 24 brown trout 1+ (mean ± s.d. mass = 11.9

± 4.99 g, FL = 10.8 ± 1.40 cm) and 25 brook trout 1+ (mean ± S.D. mass = 14.5 ± 5.32 g, FL = 11.4 ± 1.26 cm). No difference in length was found between the 1+ brown trout and brook trout (P = 0.18), but 0+ brook trout were significantly longer than 0+ brown trout (P < 0.001). During the length of experiment 1, three rounds of open-field testing were conducted before, during and after the experiment to assess the activity of each individual (David & Dall, 2016). Prior to the open-field tests, individuals were fasted for 24 h before being netted from holding tanks and transferred to white rectangular plastic tanks (71.5 × 54.5 × 30.0 cm for 1+ individuals and 35.0 × 26.5 × 25.0 cm for 0+ individuals) filled with 5 cm of water. All open-field tests were recorded (Sony, IKEA, Sweden, HDR-CX240E handycam) for 25 min, where the first 15 min was used as acclimation and the final 10 min were used as activity scoring. After one batch of fish had been recorded, the water was changed and new fish were introduced into the tanks. All video recordings were analysed with LoliTrack 4.0 (Loligo Systems ApS, Viborg, Denmark).

2.2 | Experiment 1: Intercohort association

The experiment was conducted in eight aquaria (65 × 35 × 35 cm) enriched with gravel, air stone and constant water flow (1.5 l/min) at a temperature of 12 ± 1°C. To avoid any behavioural aggression towards the 0+ individual, the aquariums were divided into two equally sized compartments (32.5 × 35 × 35 cm) using a perforated transparent PVC sheet (intermittently covered by an opaque PVC divider, see Figure 1) to allow visual and chemical cues between the individuals. In addition, the compartment holding the 0+ individual was provided with a Petri dish (placed in the zone close to the mid-section, see below) filled with green gravel to enhance the contrast of food (Johnsson & Kjällman-Eriksson, 2008) and two 8 cm long PVC tubes (3 cm in diameter) placed in the back corners of the compartment to increase heterogeneity (Figure 1). The compartment holding the 1+ individual was only provided gravel to ensure that the 0+ individual had the visual stimuli of the 1+ individual. The experiment was conducted over nine consecutive rounds with four different treatments [treatment 1: 0+ brown trout and 1+ brook trout (n = 17); treatment 2: 0+ brown trout and 1+ brown trout (n = 17); treatment 3: 0+ brook trout and 1+ brook trout (n = 16); treatment 4: 0+ brook trout and 1+ brown trout (n = 16)]. Each round started with the introduction of one haphazardly netted 0+ individual, then 22 h later we recorded space use and feeding for 40 min divided into two 20 min sessions. During the first 20 min we only recorded the space use of the 0+ individual and during the last 20 min the opaque PVC divider was lifted to allow visual contact with the other compartment. Five minutes after the divider was lifted, we introduced food (10 thawed red chironomid larvae) via a tube over the Petri dish and time to first feeding was noted. After the first recording, the PVC divider was lowered and one 1+ individual was introduced in the other compartment. Twenty-two hours later we repeated the same recording procedure as described above, but this time the 0+ individual experienced chemical and visual cues from the 1+ individual in the other
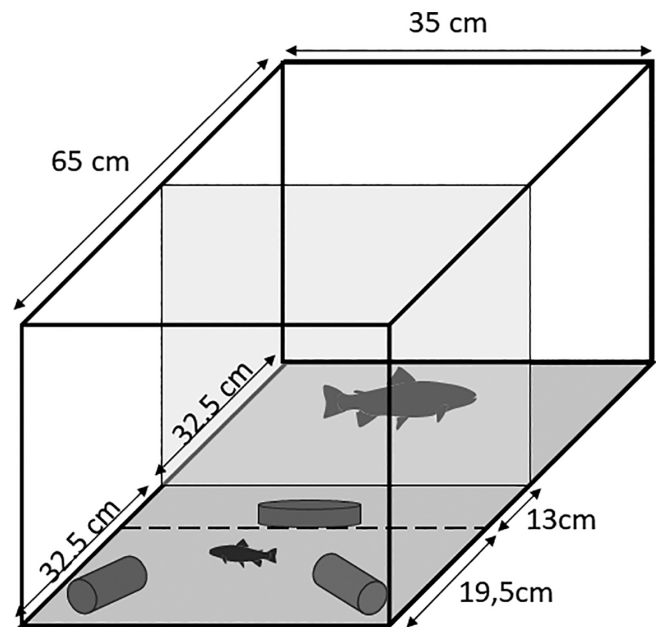
FIGURE 1 Schematic figure over the experimental setup during the interchort association test. The division between the compartments represents the perforated divider that was intermittently covered with an opaque divider to deny visual cues between the 0+ fish and the 1+ fish. The dashed line represents the two zones used to measure space use. The smaller zone close to the other compartment was used to quantify the time spent close to the mid-section and the compartment housing the 1+ individual

compartment. The space use of the 0+ individual was measured as time spent in two zones, one zone close to the mid-section and the compartment with the 1+ individual (13 cm away from the PVC divider), and the other zone containing rest of the compartment (Figure 1). After the two recording sessions were done, both individuals were removed and new 0+ individuals were introduced. To reduce the number of fish used, all 1+ individuals were used twice during the experiment.

2.3 | Experiment 2: Territorial interactions and impact of prior residency

Two months after finalizing the first experiment, a subset of the 0+ individuals were reused in a second experiment. The experiment was conducted in two parts: in one the brook trout and brown trout had equal residence duration (1 day each), conducted between 9 and 12 December 2019, and in one we manipulated the prior residence of the brown trout (either 2 or 4 days) conducted between 10 and 31 January 2020. In the equal residence experiment, we assessed territorial interactions between 32 pairs of brook trout (mean ± S.D. mass = 3.46 ± 0.73 g, FL = 7.37 ± 0.43 cm) and brown trout (mean ± S.D. mass = 2.69 ± 0.75 g, FL = 6.47 ± 0.52 cm). Each experimental tank had a size of 24 × 18 × 13 cm (L × W × H) and was provided with 12 ± 1°C flow-through water (0.5 l/min). The tank walls had a

nontransparent grey interior and a movable opaque PVC wall placed in the middle. The wall created two equally sized compartments 12 × 9 × 13 cm (L × W × H) in each tank, both enriched with gravel. At the start of the experiment, one brook trout and one brown trout were gently netted and put separately in the two compartments. One day after introducing the two fish, the PVC plate was removed so the two opponents could interact. All contests were recorded for 15 min or terminated by the observer when a clear winner could be determined to avoid injury due to aggressive behaviour of the winner. From the video recordings, winner, contest duration and number of agonistic interactions (*i.e.*, displace, circle, attack, chase and nip) during the recorded time (15 min or less) were noted (Johnsson & Åkerman, 1998). Similarly, for the prior residence experiment all fish were measured for mass and fork length to create 25 pairs of interspecific dyads (12 in the 4 day residency and 13 in the 2 day residency). As the experiment was conducted over the course of a month, fish were measured after the completion of a residence dyad to ensure correct mass and length measurements in the analysis (mean ± S.D. brook trout mass = 3.26 ± 0.55 g, FL = 7.48 ± 0.42 cm; brown trout mass = 2.58 ± 0.69 g, FL = 6.65 ± 0.48 cm). The same tanks were used as in the equal residence experiment, with the exception that the two compartments were manipulated. One was enriched with a gravel covered bottom and a cobble placed in the middle to provide a reference point for the fish and to further enrich the territorial environment. The other compartment was barren and did not have any added enrichment, thus creating one enriched territory and one barren territory. Each prior residence treatment started with the introduction of one brook trout in the enriched territory. After an initial 3 days of brook trout holding the enriched territory, it was gently moved to the barren territory of the experiment tank and a brown trout was introduced to the enriched territory originally occupied by the brook trout. The brown trout were then left in the enriched territory for either 2 or 4 days depending on treatment. Fish were fed *ad libitum* in both compartments throughout the residence advantage experiment but not during contests. Following the method in Johnsson & Forser (2002), 2 or 4 days after replacing the brook trout, the PVC divider was gently lifted and the brook trout was gently moved with a PVC plate back into the enriched territory. At the same time, the brown trout was gently moved to the opposite wall in the enriched territory to assure similar disturbance levels for the brook trout and the brown trout. Following the moving of both fish, the PVC divider was put back in place and the PVC plates were removed so that the two opponents could contest the enriched territory. The observation of contests and data collection in the prior residence experiment followed the same procedure as in the equal residence experiment.

2.4 | Ethical statement

2.5 | Data analysis

Four binomial generalized linear models were used to analyse the proportion of time spent close to the mid-compartment in experiment 1. Relative length difference between the 0+ individual and the 1+ individual was calculated as $(FL_{1+} - FL_{0+}) \div FL_{0+}$, and the mean activity of the three rounds of open-field testing and treatment (four groups) were used as predictors in all four models. Akaike information criterion (AIC) was used to estimate whether relative mass or relative length should be used as a predictor for all models. A Dunnet's *post hoc* test was performed to compare differences between the baseline treatment (brown 0+ and brook 1+) and the other treatments. A Cox proportional hazard regression was estimated to analyse if the predictors activity and treatment affected the time to first feeding of the 0+ individuals. The model accounted for only one event per individual, *i.e.*, the response variable was time to feeding for the first time. In the equal residence duration experiment, we used a logistic regression to analyse if relative mass difference between the individuals affected the probability of winning a contest. Relative mass difference was calculated as $(Mass_{brook} - Mass_{brown}) \div Mass_{brown}$. Due to high overdispersion when modelling agonistic interactions, a negative binomial generalized linear model was used to assess if the number of agonistic interactions was affected by relative mass between species. Contest duration in the equal residence experiment was transformed to logarithmic values to ensure normally distributed residuals. The transformed data were analysed using a linear model and relative mass difference was used as predictor. In the prior residence duration experiment, a binomial generalized linear model was used to assess if the predictors treatment (*i.e.*, 2 or 4 days of prior residence) and relative mass difference affected the chance of winning a contest. To account for overdispersion, a negative binomial generalized linear model was used to analyse if agonistic interactions were affected by the predictors treatment and relative mass difference. Contest duration in the prior residence experiment was transformed to logarithmic values to ensure normally distributed residuals. The transformed data were analysed using a linear model and treatment and relative mass difference were used as predictors. All statistical analyses were conducted using R, version 4.0.3.

3 | RESULTS

3.1 | Experiment 1: Intercohort association

During the first day, when no 1+ individual was present in the other compartment, no significant difference was found between the treatments in proportion time spent in the zone close to the mid-section of the aquarium before (Table 1a and Figure 2a) and after (Table 1b and Figure 2b) lifting the opaque divider. During the second day before lifting the cover, there was a trend for 0+ brook trout in presence of 1+ brown trout to spend more time in the zone close to the mid-section than 0+ brown trout in the presence of 1+ brook trout (Dunnet *post hoc* test, $P = 0.086$) (Table 1c and

Figure 2c). After lifting the divider, 0+ brook trout in the presence of 1+ brown trout spent significantly more time in the zone close to the mid-section than 0+ brown trout in the presence of 1+ brook trout (Dunnet *post hoc* test, *P* = 0.041) (Table 1d and Figure 2d). No difference in space use was found when comparing difference between days within a treatment (*post hoc* test, *P* > 0.05 for all treatments). Activity measured during the open-field test did not influence time spent in the zone close to the mid-section during any of the days. When measuring time to first feeding, no significant difference was found between the treatments during the first day (Table 2a and Figure 3a). However, during the second day, 0+ brook trout in the presence of 1+ brown trout were significantly faster to feed than 0+ brown trout in presence of 1+ brook trout (Table 2b and Figure 3b).

3.2 | Experiment 2: Territorial interactions and impact of prior residency

In the equal residence experiment, 12 out of 32 contests ended up without interactions between the species. In the remaining 20 contests there was no difference between species winning the territorial contest (binomial test, *P* > 0.05) where brown trout won seven and brook trout 13. Brook trout were significantly larger than brown trout (*t*-test, *P* < 0.001), with an average of 0.471 ± 0.731 g difference. Relative mass did not affect the outcome of the contest, although there was a trend towards larger brook trout winning more contests (logistic regression, *z* = 1.718, *P* = 0.087) (Figure 4). Although relative mass difference did not affect the contest duration (linear model, *z* = −0.475, *P* > 0.05) (Figure 5a), a larger relative mass difference

TABLE 1 Generalized linear model analysing the effect of treatment, activity and relative length on time spent close to the mid-section for the 0+ individual

a) Coefficients	Estimate	Std. error	z value	P value
Intercept	1.017	1.571	0.648	0.517
Relative length	0.925	1.248	0.742	0.458
Activity	−0.001	0.001	−1.409	0.159
Brown 0+ and brown 1+	−0.041	0.762	−0.054	0.957
Brook 0+ and brook 1+	1.437	0.917	1.567	0.117
Brook 0+ and brown 1+	0.453	0.883	0.513	0.608
b) Coefficients	Estimate	Std. error	z value	P value
Intercept	1.396	1.587	0.880	0.379
Relative length	0.0645	1.222	0.536	0.592
Activity	−0.001	0.001	−1.934	0.053
Brown 0+ and brown 1+	−0.179	0.759	−0.237	0.812
Brook 0+ and brook 1+	0.832	0.857	0.971	0.331
Brook 0+ and brown 1+	0.858	0.895	0.959	0.337
c) Coefficients	Estimate	Std. error	z value	P value
Intercept	−2.385	1.612	−1.479	0.139
Relative length	0.749	1.219	0.615	0.538
Activity	0.001	0.001	0.828	0.407
Brown 0+ and brown 1+	0.973	0.784	1.241	0.214
Brook 0+ and brook 1+	1.633	0.872	1.871	0.061
Brook 0+ and brown 1+	1.971	0.934	2.109	0.034 *
d) Coefficients	Estimate	Std. error	z value	P value
Intercept	−1.085	1.682	−0.645	0.519
Relative length	−0.755	1.302	−0.580	0.562
Activity	0.001	0.001	0.688	0.491
Brown 0+ and brown 1+	0.553	0.802	0.690	0.490
Brook 0+ and brook 1+	1.043	0.861	1.210	0.226
Brook 0+ and brown 1+	2.512	1.036	2.424	0.015 *

Note: A binomial proportion distribution of the proportion of time spent close to the mid-section was used as the response variable. Treatment brown 0+ and brook 1+ was used as the baseline level of the corresponding variables. (a) Day 1 before removing divider, (b) day 2 after removing divider, (c) day 2 before removing divider and (d) day 2 after removing divider. Asterisks indicate significant differences (*P* < 0.05*, *P* < 0.01**, *P* < 0.001***).
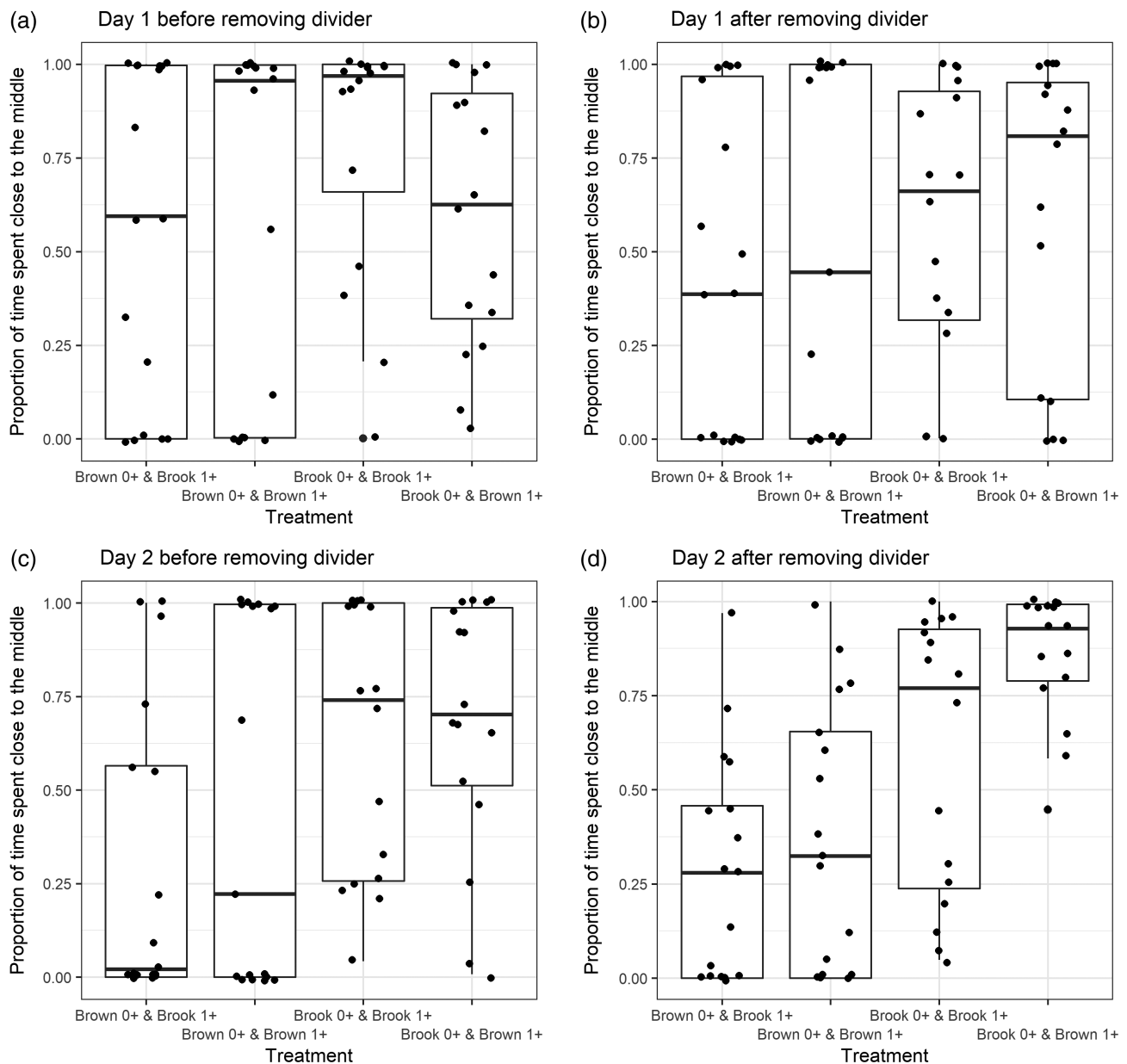
FIGURE 2 Box plot showing the intercohort association as the difference in the proportion of time spent close to the midsection between the treatments during (a) day 1 before removing the divider, (b) day 1 after removing the divider, (c) day 2 before removing the divider and (d) day 2 after removing the divider

significantly lowered the number of agonistic interactions between the species (GLM negative binomial, $z = -2.18$, $P < 0.05$) (Figure 5b). In the prior residence experiment, brook trout were significantly larger than brown trout (t-test, $P < 0.001$), an average of 0.624 ± 0.384 g larger. Four contests in the 2-day treatment and one in the 4-day treatment had no clear interactions or winner, and in the remaining 20 contests brook trout won 19. No difference in relative mass was found between the 2-day and 4-day treatment groups (t-test, $t = -0.690$, $P > 0.05$). However, a 4-day residence advantage increased the duration of the contest by an average of 28.31 s compared with a 2-day residence (linear model, $t = 2.442$, $P < 0.05$) (Figure 6a), whereas larger relative mass difference between the two contesting individuals decreased the contest duration (linear model,

$t = -3.713$, $P < 0.01$). Similarly, a 4-day residence advantage increased the number of agonistic interactions by an average of 6.95 interactions compared to the 2-day treatment (GLM negative binomial, $z = 2.167$, $P < 0.05$) (Figure 6b), and larger relative mass difference between the individuals decreased the number of agonistic interactions (GLM negative binomial, $z = 3.936$, $P < 0.001$).

4 | DISCUSSION

The results of the first experiment showed that juveniles of brook trout and brown trout behave differently in the presence of a 1+ hetero- or conspecific, whereas brown trout fry (0+) generally

TABLE 2 Cox proportional hazard regression estimating the effect of treatment and activity on the time individuals took to feed for the first time when the divider was lifted during (a) day 1 and (b) day 2

a)	Coef	Exp(coef)	Se(coef)	z value	P value
Activity	0.000	1.000	0.000	0.681	0.496
Brown 0+ and brown 1+	−0.820	0.440	0.649	−1.263	0.207
Brook 0+ and brook 1+	0.184	1.202	0.488	0.378	0.706
Brook 0+ and brown 1+	−0.248	0.779	0.547	−0.454	0.650
b)	Coef	Exp(coef)	Se(coef)	z value	P value
Activity	0.001	1.000	0.000	0.648	0.517
Brown 0+ and brown 1+	0.126	1.135	0.457	0.277	0.782
Brook 0+ and brook 1+	0.607	1.836	0.405	1.498	0.134
Brook 0+ and brown 1+	0.861	2.367	0.410	2.101	0.035*

Note: Treatment brown 0+ and brook 1+ was used as the baseline level of the corresponding variables. Asterisks indicate significant differences ($P < 0.05^*$, $P < 0.01^{**}$, $P < 0.001^{***}$).
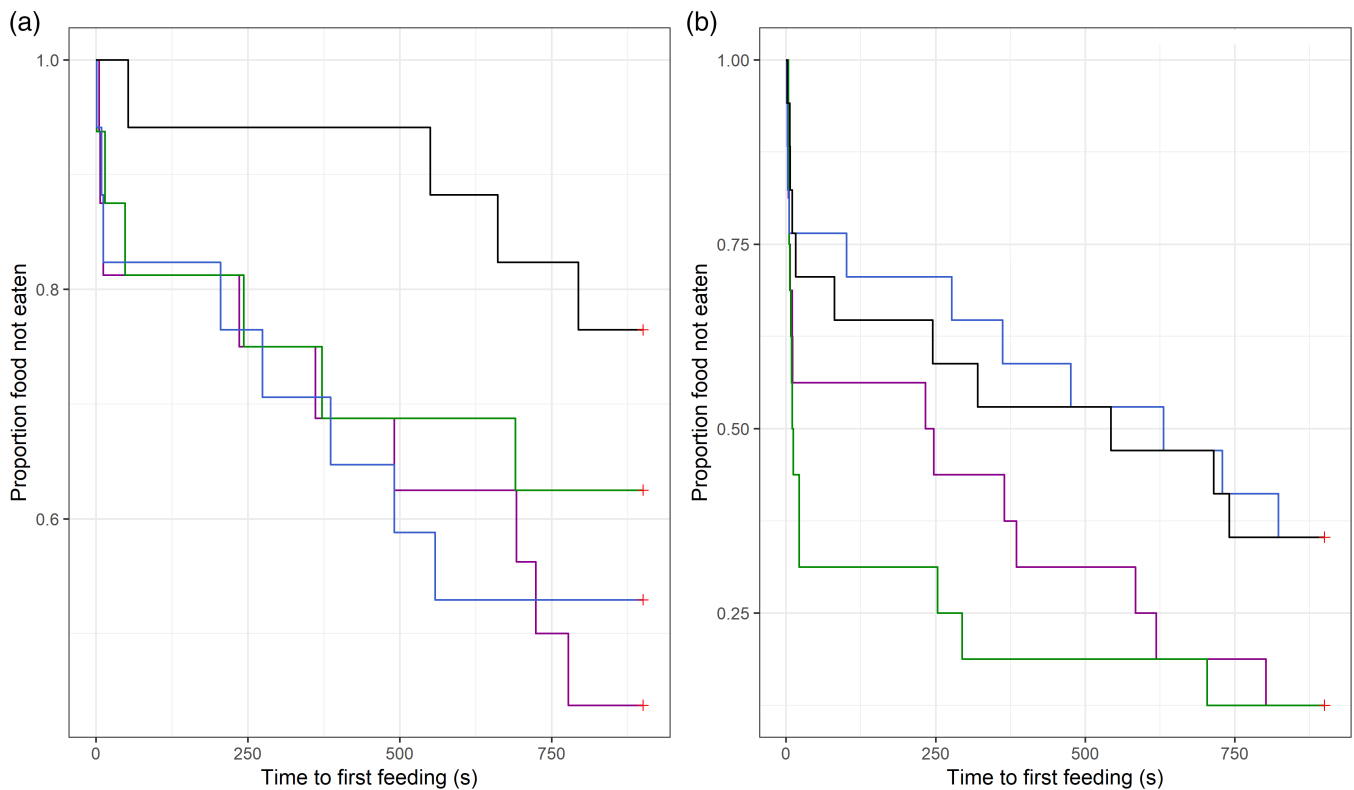

FIGURE 3 Time-to-event curves illustrating time to feeding as the proportion of food not eaten on the y axis and time to first feeding (s) between the treatments during (a) day 1 after lifting the divider and (b) day 2 after removing the divider. —— brook 0+ and brook 1+; —— brook 0+ and brown 1+; —— brown 0+ and brook 1+; —— brown 0+ and brown 1+

positioned themselves further away from larger individuals if the larger fish were a brook trout. Interestingly, brook trout fry were faster to capture food with 1+ brown trout than brown trout fry in the presence of a 1+ brook trout. Moreover, and in agreement with previous studies measuring contest between salmonids (*e.g.*, Hasegawa *et al.*, 2012; Nakano, 1995; Young, 2004), the results from the second experiment showed that size may be a major determinant for outcomes of interspecific interactions between brook and brown trout at the juvenile stage, and that brook trout were competitively dominant over brown trout when contesting a territory due to their

larger body size. Furthermore, a longer prior residency (4 days *vs.* 2 days) also resulted in longer contest duration and higher numbers of aggressive interaction between the species. The earlier emergence may thus give the invasive brook trout a size advantage and a prior residence effect over native brown trout in interference competition for food and territory, which might explain the competitive displacement of brown trout by the invader.

The behavioural differences of the 0+ individuals towards the 1+ individuals may reflect differences in innate sociality between the two species (Bisazza *et al.*, 2000). Previous studies have shown that a

group of 0+ brook trout had a stronger shoaling tendency than 0+ brown trout (Lovén Wallerius *et al.*, 2017), and that brown trout aggregate more with brook trout than conspecifics of the same cohort (Larranaga *et al.*, 2019), maybe indicating that brook trout are more
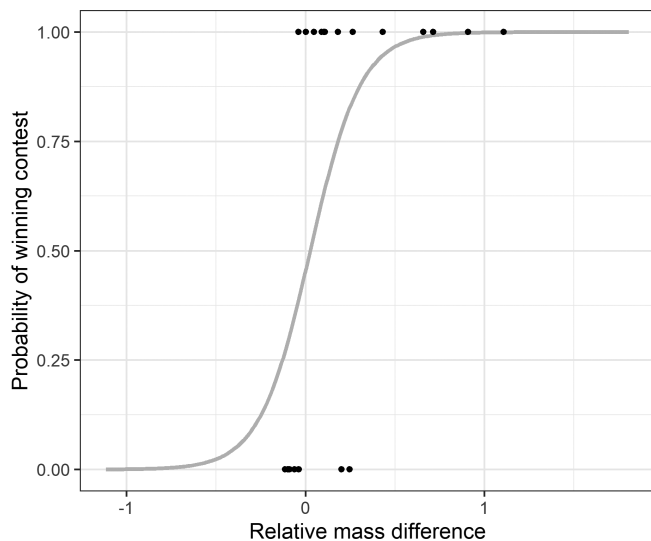

FIGURE 4 Observed winners (circles) of contests of the equal residency duration together with predicted win probabilities (blue line). Predicted win probabilities were estimated by a logistic regression model where a probability of 0.00 equals a 100% win probability for brown trout and a probability of 1.00 equals a 100% win probability for brook trout. Negative values on relative mass difference correspond to brown trout being the larger individual of the pair, while positive values correspond to brook trout being the larger individual

social towards conspecifics and heterospecifics than the less social and more aggressive brown trout (Alanärä *et al.*, 2001). Moreover, intercohort competition in salmonids has shown that subordinate salmonids are displaced into suboptimal habitats (Keeley, 2001; Nakano, 1995), eventually leading to reduced growth (Kaspersson *et al.*, 2012; Kaspersson & Höjesjö, 2009; Louhi *et al.*, 2014). Thus, the avoidance response of 0+ brown trout in the presence of 1+ brook trout may indicate that larger heterospecifics also displace juvenile brown trout with a potential long-term negative effect on growth (Öhlund *et al.*, 2008; Závorka *et al.*, 2017; but see Korsu *et al.*, 2009). The results from the intercohort association experiment could also indicate differences in responsiveness towards visual and chemical cues of larger individuals (Hirvonen *et al.*, 2000; Ward & Currie, 2013). This would suggest that the the 0+ brown trout were more responsive to chemical and visual cues of a potential competitor than the 0+ brook trout, as they spent more time further away when the divider was down and later lifted up. Additionally, larger individuals of both species could not only function as potential intercohort competitors, but also as predators of the 0+ individuals (Hirvonen *et al.*, 2000; Svenning & Borgström, 2005; Taniguchi *et al.*, 2002). As such, the stronger avoidance response of the smaller 0+ brown trout could indicate that they were more susceptible to predation than the larger 0+ brook trout due to gape limitations of the 1+ individuals (Keeley & Grant, 2001). With the multitude of cues present in a stream, it is unlikely that there is only one factor that determines the spatial distribution of individuals, rather it may be a combination of visual and chemical cues (Brown, 2003; Höjesjö *et al.*, 2015), presence of predators (Ferrari *et al.*, 2010) and habitat variability (Höjesjö *et al.*, 2004; Watz *et al.*, 2019) that helps form hierarchies and spatial distribution of individuals (Kaspersson & Höjesjö, 2009; Nakano, 1995).
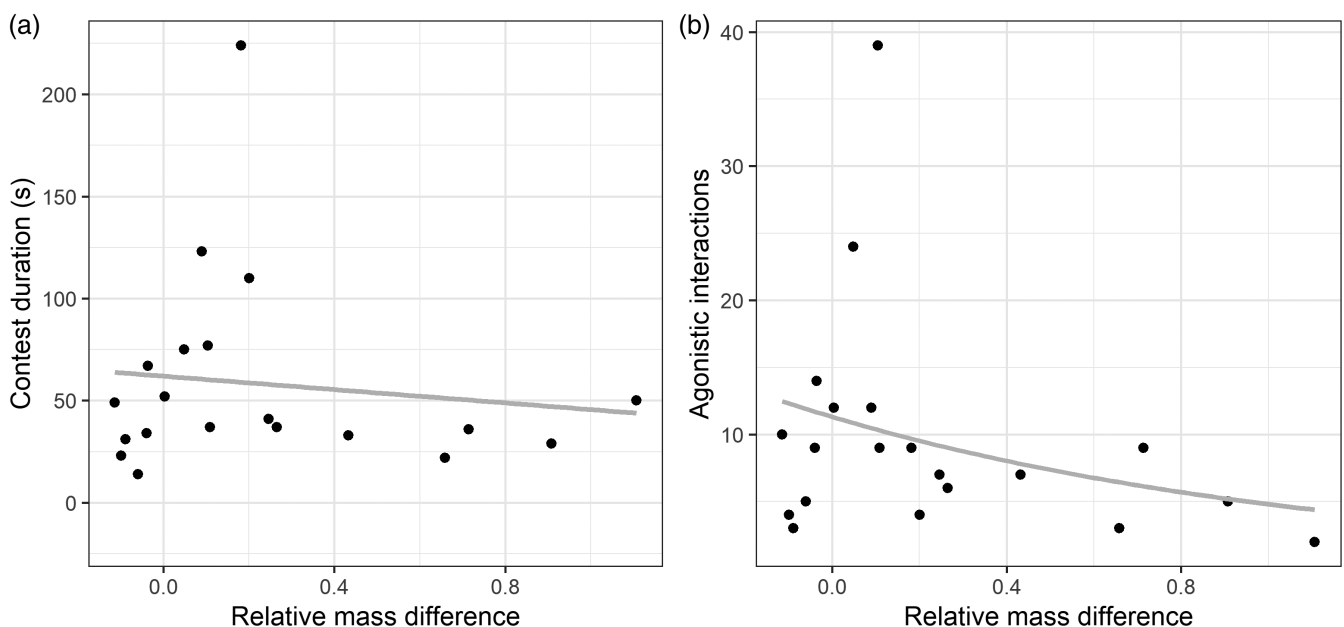
FIGURE 5 The effect of relative mass difference between the individuals on (a) contest duration and (b) agonistic interactions in the equal residence duration experiment
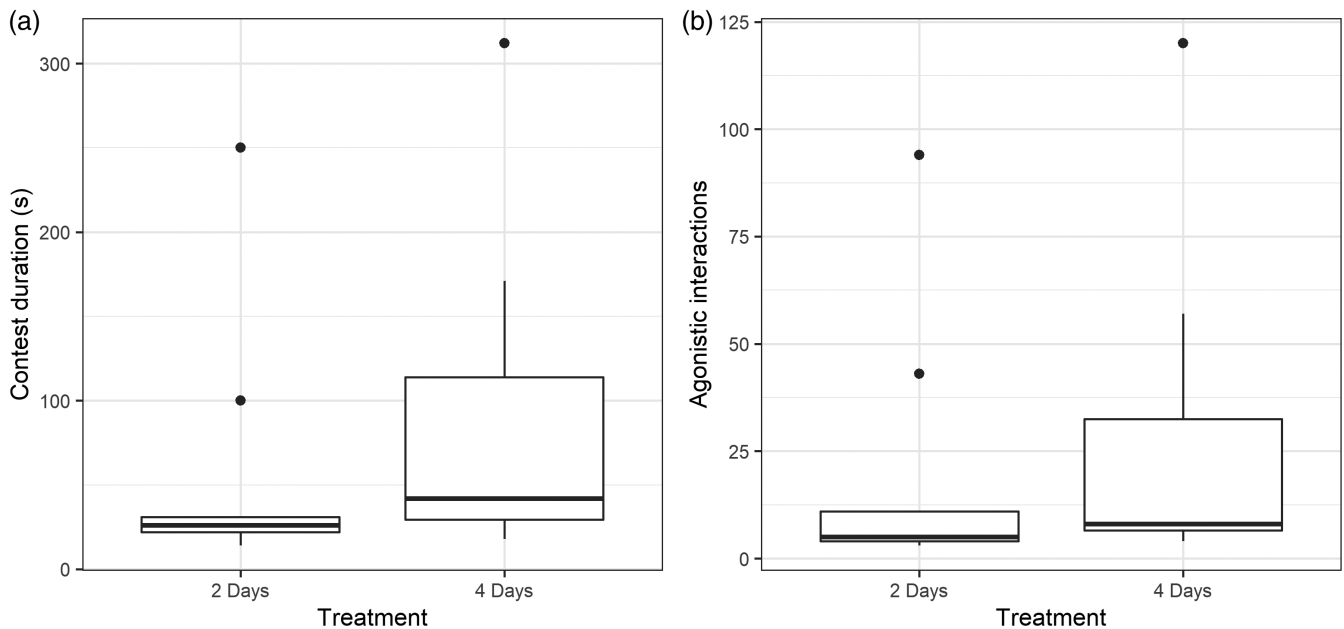
FIGURE 6 Boxplots showing the difference in (a) contest duration and (b) number of agonistic interactions when brown trout had a 2 day or 4 day residence duration

The results from the territorial contest after manipulating residence duration was rather surprising as only one brown trout won the territorial contest. This result is contrary to previous studies on Atlantic salmon (*Salmo salar*) and brown trout, where longer residence duration increased the chance of winning a contest (Cutts *et al.*, 1999; Johnsson & Forser, 2002; Metcalfe *et al.*, 2003; O'Connor *et al.*, 2000). Yet, and in agreement with Johnsson and Forser (2002), our results showed that brown trout having a longer residence duration partake in a longer contest with more agonistic interactions between the species. As size is a major predictor of winning intraspecific contests in salmonids (Johnsson *et al.*, 1999), the most likely explanation for brook trout winning all but one of the territorial contests was that the size differences were too big between the species compared to the size difference in the equal residence duration experiment. On the other hand, dominance status in interspecific interactions between salmonids may be determined by behavioural differences between the species in addition to body size (Hasegawa *et al.*, 2004; Thornton *et al.*, 2017). This effect was partially seen when brook trout and brown trout had equal residency, as seven brown trout won the territorial contest against brook trout despite being smaller. Our study highlights how ecologically relevant interactions may be misinterpreted when trying to impose ecologically irrelevant size matching between species and not accounting for actual size differences between species in the wild (Hasegawa *et al.*, 2012; Young, 2004). As a consequence, the later emergence of native brown trout fry could lead to a competitive disadvantage against invasive brook trout and force brown trout fry to habitats where growth might be negatively affected (O'Connor *et al.*, 2000). However, the earlier hatching and size advantage of brook trout in the wild (M. Lovén Wallerius, personal observation; Korsu *et al.*, 2009) may also affect

the territorial contests between the species due to earlier ontogenetic habitat shifts of brook trout (Cantin & Post, 2018; Hasegawa *et al.*, 2012). This possible change of habitat could ultimately lead to reduced interspecific competition at the fry stage, but later on increase competition and negatively affect reproduction of older brown trout cohorts, eventually leading to species replacement (Korsu *et al.*, 2007). However, whether a couple of weeks of earlier brook trout emergence is enough to induce a habitat shift (Rose, 1986) and affect competition with brown trout in the study system is yet to be discovered.

The significance of the current study lies in its experimental nature, where we show strong intercohort responses and differences in competitive ability between the species at an early life stage. However, the question and caution remain whether these laboratory-based results also apply to natural conditions (Korsu *et al.*, 2009, 2010). Under natural conditions, the results presented here could imply that smaller 0+ brown trout may be displaced to suboptimal habitats due to their inferior competitive abilities towards heterospecific 0+ brook trout and their tendency to keep longer distances and approach food slower in presence of an adult heterospecific. This could potentially create a hierarchy between and within year classes and species, where 0+ brown trout acquire less food of lower quality in the presence of the invasive species (Závorka *et al.*, 2021). As the two species have characteristic habitat niches, where brook trout prefer the cold upstream slow flowing pool sections (Korsu *et al.*, 2010), our slow-flow regime could have favoured brook trout in the contests and space use against brown trout, and partly explain the pattern of upstream replacement of brown trout by brook trout (Korsu *et al.*, 2007, 2012). Thus, if the habitat characteristics and environmental conditions of the downstream sections favour brown trout,

these could function as a barrier against further brook trout expansion as the conditions might give brown trout a competitive advantage over brook trout (Hoxmeier & Dieterman, 2019).

This study highlights how mismatching life cycles of native and invasive species can have a profound impact on their competitive interactions (Alexander & Levine, 2019). This mismatching not only covers size differences during interference competition, but could also affect habitat use (Hasegawa *et al.*, 2012) and have a negative impact on reproduction of the native salmonids (Cucherousset *et al.*, 2008; Kitano *et al.*, 2014). As environmental conditions have influenced the world-wide success of non-native salmonids (Fausch, 2008; Hasegawa, 2020; Hoxmeier & Dieterman, 2019; Kitano, 2004), future studies should consider how environmental factors such as temperature influence the reproduction and early development of eggs and fry to realistically assess the extent of competition pressure that invasive salmonids impose on the native species at an early life stage.

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AUTHOR CONTRIBUTIONS

M.L.W, J.H and L.Z developed the overall research question and experimental design. M.L.W and V.M performed the experiments and analysed the data. M.L.W and V.M wrote the paper with contributions from L.Z and J.H.

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