

Egg incubation temperature influences the population-specific outmigration rate of juvenile brown trout *Salmo trutta*

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Abstract

The present experiment tested if temperature during embryogenesis and parental heritage affected the migratory behaviour of young brown trout *Salmo trutta*. Two parental forms were used, a freshwater resident form and an anadromous form, both from the same river system but geographically isolated since 1993–95. Four groups of young *S. trutta* were produced and reared from (a) freshwater resident parents spawning in a tributary to the River Imsa, Norway, (b) anadromous parents spawning in the main stem of the same river system, (c) resident male × anadromous female parents and (d) resident female × anadromous male parents. The eggs were incubated until first exogenous feeding in River Imsa water, either unheated or heated c. 2.7°C above ambient temperature. Thereafter, all fish experienced the same ambient river temperature until release. Groups were released below an impassable waterfall 900 m upstream of the mouth of the River Imsa, either as age-0 in October 2019 or as age-1 in May 2020. About 7.5% of the released fish moved downstream and were captured in a trap at the outlet. For any given body size, the proportion of warm incubated trout that moved downstream was greater than the proportion of cold incubated trout. It was also found that most emigrants of the October-released *S. trutta* were caught within a month of release. Also, most May-released *S. trutta* emigrated in October. The offspring of the freshwater resident parents emigrated to a larger extent than offspring of anadromous parents. Thus, the difference in emigration with regard to embryonic temperature was phenotypically plastic and may be associated with an epigenetic effect of the thermal conditions during early development. The effect of parental origin suggests there may be genetic divergence between the geographically isolated populations.

KEYWORDS

behaviour, egg temperature, genetic expression, outmigration, parental effect, phenotypic plasticity

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1 | INTRODUCCION

Migration is a widespread phenomenon in the animal kingdom and it occurs across a broad spectrum of taxa, from insects to mammals (Wilcove & Wikelski, 2008). It is believed that migration is under direct genetic control (Zink, 2011), under indirect genetic control influenced by traits such as growth rate, body size and sexual maturation (Forseth *et al.*, 1999; Kelso *et al.*, 2020), or even epigenetically affected by present or previous environmental conditions (Baerwald *et al.*, 2016; Nevoux *et al.*, 2019; Van Leeuwen *et al.*, 2019). Species are not always solely migratory or resident, but may include both migratory and resident phenotypes, sometimes in the same population, where early environmental conditions may play a key role in determining their life history (Jonsson & Jonsson, 1993; Olsson *et al.*, 2006). Although there is a large number of publications discussing causes of migration (*e.g.* Ferguson *et al.*, 2019; Fudickar & Partecke, 2012; Kerr & Secor, 2010, 2011; Menz *et al.*, 2019; Nevoux *et al.*, 2019), our understanding of the underlying mechanisms of this complexity is still limited.

Temperature during embryogenesis is one factor that may affect ecological traits such as growth, reproductive allocations and behavioural decisions of salmonids (Jonsson & Jonsson, 2014, 2019). For instance, Jonsson *et al.* (2005) found that a higher proportion of Atlantic salmon *Salmo salar* L. migrated to sea already at age-1 if they came from year-classes that underwent embryogenesis during winters when temperature was relatively warm. Furthermore, Burgerhout *et al.* (2017) reported that *S. salar* that developed from embryos incubated at 8°C smolted and were ready for migration at a younger age than those incubated at 4°C, possibly because they grew faster, which in turn may be related to differences in metabolic rates (Durtsche *et al.*, 2021; Finstad & Jonsson, 2012). It is unknown whether warmer incubation temperature had an additional effect, contributing to the probability of migrating at a younger age (*cf.* Kelso *et al.*, 2020). Furthermore, year-classes of adult *S. salar* returned from the ocean 2 weeks later in the season if they as embryos were incubated in 3°C warmer water and from first feeding onwards reared under similar conditions until release at smolting (Jonsson & Jonsson, 2018). The same difference was found for three populations, two smolt year-classes and in 2 different years of release. Thus, temperature during embryogenesis may influence behavioural decisions much later in life. In the case of *S. salar*, the timing of the return migration from the sea is probably linked to the seasonal timing of sexual maturation (Jonsson & Jonsson, 2011).

Brown trout *Salmo trutta* L. 1759 displays large variations in its life history phenotypes, ranging from different degrees of freshwater migratory to anadromous individuals within single rivers (Elliott, 1994). It is likely that both genetics and environment influence the decision to migrate (Ferguson *et al.*, 2019; Jonsson & Jonsson, 2011). Typically, *S. trutta* spawn in streams in the autumn, where the young hatch and emerge in the spring. Some leave the nursery stream already in the first summer or autumn, others may stay longer and some may become stream resident. Anadromous *S. trutta* typically smolt before they migrate to sea, that is they go through a

physiological and morphological transformation process, preadapting the fish for sea life (Hoar, 1976; Quigley *et al.*, 2006)

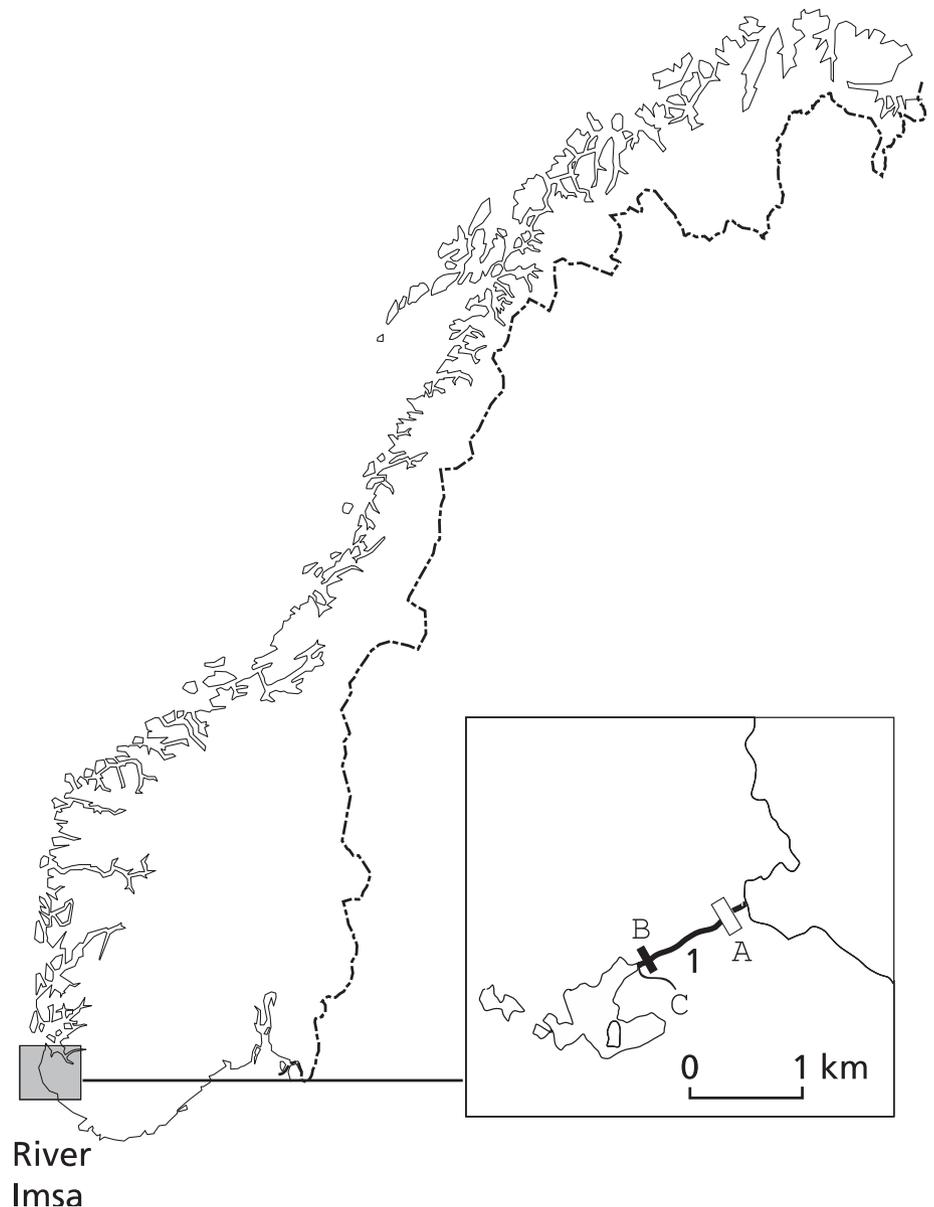
Here, it was tested if parental types and egg incubation temperatures influenced emigration of juvenile *S. trutta* offspring of anadromous and freshwater resident and their associated crosses released into a Norwegian river, the River Imsa. Both anadromous and freshwater resident phenotypes occur in the River Imsa system, but have been geographically isolated from each other since 1993–95, with the resident parents originating from an upstream tributary and the anadromous parents from the main stem of the river. It was hypothesized that juvenile *S. trutta* would emigrate from their stream habitat younger and smaller if temperature during embryogenesis was higher because of a phenotypically plastic, possibly epigenetic, effect. Furthermore, it was hypothesized that offspring of anadromous *S. trutta*, because of their fast growth as juveniles (Jonsson & Jonsson, 2021), would have a higher tendency to emigrate at a younger age than offspring of resident *S. trutta*. Hybrid offspring between anadromous and resident *S. trutta* were expected to have an intermediate tendency to emigrate because of additive genetic/epigenetic effects. As river temperatures are increasing globally, and winters in particular are predicted to be substantially warmer in northern latitudes (IPCC, 2013), understanding how warming winter temperatures impact fish behaviour and ecology has important implications for effective conservation and management.

2 | MATERIALS AND METHODS

The experiment was conducted in the River Imsa, south-western Norway (59N, 6E; Figure 1). In this river, there is a permanently-installed box trap on top of a three-step fish ladder that catches all fish entering from the sea (Jonsson & Jonsson, 2011). Alongside the box trap is an inclined plane trap (apertures 10 mm; Wolf, 1951) used to capture downstream-migrating *S. trutta*. It catches *S. trutta* longer than c. 7 cm and should take all *S. trutta* longer than c. 10 cm. Hence, the plane trap has the potential to catch all trout in this study as the smallest fish released was 7.1 cm and the trap was fully functional throughout the study period. Nevertheless, proportionally more of the fish smaller than 10 cm may pass the trap unnoticed. This ‘bias’ may be strongest for fish released in October 2019, when many were shorter than 10 cm.

S. trutta used in the experiment were the offspring from crosses of anadromous fish, collected in the box trap as they returned from the sea, and from lacustrine-adfluvial migrants, collected by electrofishing in the Fossbekk, a tributary (brook) to the River Imsa, entering at the outlet of Lake Liavatn, 1 km upstream from the sea (Figure 1). The anadromous parents were wild fish, individually Carlin-tagged as they left the river as smolts when caught in the plane trap and recaptured as adults on their return to the river. *S. trutta* in the tributary Fossbekk are wild. An artificial waterfall, built in 1993–95, has prevented anadromous trout from reaching this brook for nearly 30 years (Jonsson & Jonsson, 2017). Eggs were collected from 12 anadromous females (mean length \pm s.d. 38.0 \pm 3.5 cm, range

FIGURE 1 The River Imsa (1) in south-western Norway, where *Salmo trutta* spawn. (a) Location of the fish trap, c. 150 m upstream of the sea where the anadromous fish were collected. (b) Location of the upstream impassable waterfall, built between 1993 and 1995. (c) The tributary, Fossbekk, which enters the River Imsa at the outlet of Lake Liavatn, where the freshwater resident *S. trutta* spawn. The water flows from C towards A



31.4–44.2 cm, mean mass 577.3 ± 185.9 g, range 315–993 g, mean condition factor 1.05) and 12 freshwater resident females (length 27.9 ± 3.8 cm, range 20.5–34.4 cm, mass 220.5 ± 93.0 g, range 83–423 g, mean condition factor 1.02), crossed with sperm from 12 anadromous males (length 40.4 ± 8.0 cm, range 31.1–53.4 cm, mass 771.3 ± 451.0 g, range 309–1539 g, mean condition factor 1.17) and 12 freshwater resident males (length 26.0 ± 4.1 cm, range 19.5–32.1 cm, mass 167.0 ± 64.5 g, range 76–271 g, mean condition factor 0.95) on November 9, 2018. Four different offspring crosses were created: anadromous male \times anadromous female (AA), anadromous male \times resident female (AR), resident male \times resident female (RR), resident male \times anadromous female (RA). Each adult was used in two different crosses, one by partnering it with a freshwater resident and the other with an anadromous *S. trutta*. Each family group was split in two, one half incubated in ambient River Imsa water and one half in 2.7°C warmer river water (mean \pm s.d. 4.4 ± 1.5 and 7.1 ± 0.6 °C

(Figure 2). The two temperatures were maintained through use of a heat exchanger. There were daily variations in temperature, but heated water was always warmest. The rearing tanks had a water level of 30 cm, a water flow of 2 l min^{-1} and a surface light intensity of approximately 70 lx during daytime (12 h light:12 h dark cycle).

Approximately 100 eggs were acquired for each cross, and cross cohorts were raised separately in incubation trays with constant water flow until the start of exogenous feeding. Mortality was negligible. On 22 February and 9 April 2019, the «warm» and «cold» *S. trutta* were moved into 16 60 l holding tanks, respectively (two tanks for each cross \times incubation temperature). The following day, exogenous feeding was initiated using commercial *S. trutta* pellets (Ewos, Cargill, Bergen, Norway). From 10 April 2019 onwards, all trout were maintained at the same temperature using River Imsa water. During 22 days, from 27 August to 17 September, the growth rates of trout from the eight treatments were monitored. The growth rate of the juveniles was not

significantly influenced by incubation temperature, but offspring of anadromous parents had a higher growth rate than the other three crosses, which had similar growth rates (Jonsson & Jonsson, 2021).

On 3 October 2019, 1178 *S. trutta* from all crosses were individually tip length measured (Ricker, 1975) and tagged with Passive Integrated Transponder (PIT) tags after being anaesthetized with chlorobutanol (Table 1). They were released on 15 October 2019 downstream of the waterfall, 900 m from the river outlet (the sea) (Figure 1). A second group of 483 fish was measured and tagged on 14 November 2019, 42 days after the first group, and released at the same site on 3 May 2020 (Table 1). Downstream moving *S. trutta* emigrating from the release site were sampled in the plane trap at the river outlet until the end of June 2021. Only two individuals were caught after December 2020.

Differences in length at recapture of the various groups were tested with three-way univariate ANOVA using the General Linear Model statistics of SPSS 27 with cross, incubation temperature and year at release as factors. The same model was used to test for differences in size between released and recaptured *S. trutta* at the river mouth. Tests for significant differences in proportion that outmigrated between the four offspring crosses, RR, AA, RA and AR, were based

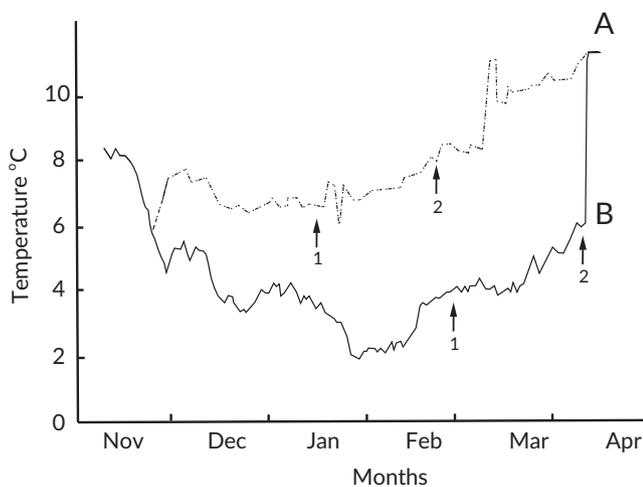


FIGURE 2 Temperature from fertilization on 9 November 2018 to egg hatching (1) and start of external feeding (2) of *Salmo trutta* incubated as embryos in either ambient (solid line) or heated (broken line) River Imsa water. A gives the warm and B the cold incubation temperature

TABLE 1 Numbers of recaptured/released *Salmo trutta* offspring of the crosses resident × resident (RR), resident × anadromous (RA), anadromous × resident (AR) and anadromous × anadromous (AA) incubated as embryos at either ambient temperature or 2.7°C warmer (heated) River Imsa water

Year	Heated				Ambient				Total
	RR	RA	AR	AA	RR	RA	AR	AA	
2019	21/131	24/149	20/155	11/149	5/149	4/148	6/147	3/150	94/1178
2020	11/60	5/62	3/58	1/60	5/60	3/61	2/60	0/60	30/483
Total	32/191	29/211	23/213	12/211	10/209	7/209	8/207	3/210	124/1661

on the normal approximation to the binomial distribution, with number of fish as the data input, shown below (Siegel, 1956). The two-sample test is expressed by:

$$Z^* = \frac{\sum_{i=1}^4 t_i^2}{\sum_{i=1}^4 t_i^2}$$

$$i = 1$$

Z^* is chi-square distributed with 4 degrees of freedom.

$$t_i = \sqrt{((n_i m_i)/(n_i + m_i))(p_i - q_i)/(\sqrt{r_i}(1 - r_i))}$$

t_i is t-distributed with $n_i + m_i - 2$ degrees of freedom.

$$r_i = (x_i + y_i)/(n_i + m_i), p_i = x_i/n_i, q_i = y_i/m_i$$

where x_i and y_i are the numbers of *S. trutta* migrants caught while migrating out of the system grouped according to the incubation treatment in each of the four parent groups, and n_i and m_i are the respective total numbers of *S. trutta* in the same groups. Due to size-based differences in trap detection, we evaluated the effect of incubation temperature on the proportion of out-migrants by making comparisons within comparable length groups at tagging (< 9, 9–10, 10–11, and >11 cm).

2.1 | Ethics statement

The care and use of the experimental fish complied with Norwegian animal welfare laws and policies as approved by the Norwegian Animal Research Experimentation Agency with the Animal Care and Use ID 15450.

3 | RESULTS

Approximately 7.5% of *S. trutta* released were recaptured in the trap at the mouth of the River Imsa (Table 1). The rest were small and emigrated unnoticed, died or became resident in the river. Mean lengths at tagging of the fish that outmigrated from the various groups varied from 8.8 to 13.6 cm. The ANOVA showed a significant effect of incubation temperature ($F_{1,108} = 51.8, P < 0.001$) and time of tagging ($F_{1,108} = 27.4, P < 0.001$). There was no significant effect of parent cross on size at tagging of the outmigrating *S. trutta* ($F_{3,108} = 0.3, P = 0.31$) nor were the interaction terms significant (all $P > 0.12$). The

2020 released fish were measured in autumn, 1 month later than the 2019 released ones, when the fish were still growing.

For all *S. trutta* released, mean length at tagging was longer for those recaptured than for those released ($F_{1,1753} = 11.0$, $P = 0.001$; Table 2). There were significant effects on length at tagging of year of release ($F_{1,1753} = 151$, $P < 0.001$) and of egg incubation temperature ($F_{1,1753} = 209$, $P < 0.001$). There was no significant effect of the crosses ($F_{3,1753} = 1.48$, $P = 0.2$) or interactions between the variables tested. It is important to note that the 2020 release was measured 42 days after the 2019 group was measured, explaining size differences at release between these groups.

Most migrants released in October 2019 were captured in the plane trap within the first 10 days after release (69%), although 16% emigrated 11–12 months later, from the middle of September to the middle of October 2020, i.e., the year after release. The remaining fish were captured sporadically over the year. The 2020-released *S. trutta* emigrated to some extent in May 2020 (35%), but even more entered the plane trap from the middle of September to the middle of October 2020 (46%). A few were also sampled in summer and late autumn. The difference in timing of the outmigration relative to time of release between the two releases, tested as the proportion outmigrating during the first month after release versus later, showed a highly significant difference in timing of the outmigration between the two releases ($\chi_1^2 = 10.4$, $P < 0.001$; Figure 3). Thus, whether released in spring or autumn, large proportions outmigrated during autumn.

It was not investigated whether any of the downstream migrants had smolted physiologically, but among those that were recaptured in the trap during spring and summer, 15 (of 124 recaptures) were silvery in coloration and therefore registered as smolts. Among these, 13 were from the warm incubated groups and two were incubated at ambient river temperature. Nine were offspring of RR parents and

only one was an AA offspring, suggesting that offspring of RR parents could smoltify after at least five generations of geographical isolation from the anadromous trout occurring below the waterfall. Also, a larger proportion of the warm incubated fish emigrated downstream from all four length groups, <9 cm to ≥ 11 cm ($Z^* = 27.0$, 4 df, $P < 0.001$; Figure 4). Thus, egg incubation in warmer water induced outmigration of juvenile *S. trutta* and possibly also younger age at smolting.

There were also differences between the crosses as offspring of resident parents emigrated more than offspring of anadromous parents (Figure 5). The proportion of hybrids that emigrated was

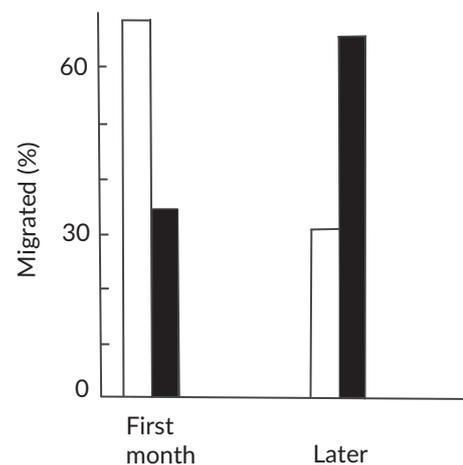


FIGURE 3 Percentage outmigration during the first month or later after releases in October 2019 (white bars) or May 2020 (black bars) of *Salmo trutta*

TABLE 2 Mean total length (L_T cm) and standard error (s.e.) at tagging of *Salmo trutta* offspring of river resident and anadromous parents incubated as embryos at either ambient temperature or 2.7 °C warmer River Imsa water (heated), released downstream of the waterfall in the river, approximately 900 m above the river outlet, either in October 2019 or in May 2020. The ANOVA showed a significant effect of incubation temperature ($F_{1,108} = 51.8$, $P < 0.001$) and time of tagging ($F_{1,108} = 27.4$, $P < 0.001$). There was no significant effect of parent cross on size at tagging of the outmigrating *S. trutta* ($F_{3,108} = 0.3$, $P = 0.31$) nor were the interaction terms significant (all $P > 0.12$)

Year		Incubation temperature	L_T	s.e.
2019	Released	Ambient	8.95	0.05
	Captured	Ambient	9.23	0.21
	Released	Heated	10.61	0.05
	Captured	Heated	11.13	0.13
2020	Released	Ambient	10.37	0.07
	Captured	Ambient	10.49	0.38
	Released	Heated	12.24	0.07
	Captured	Heated	13.30	0.28

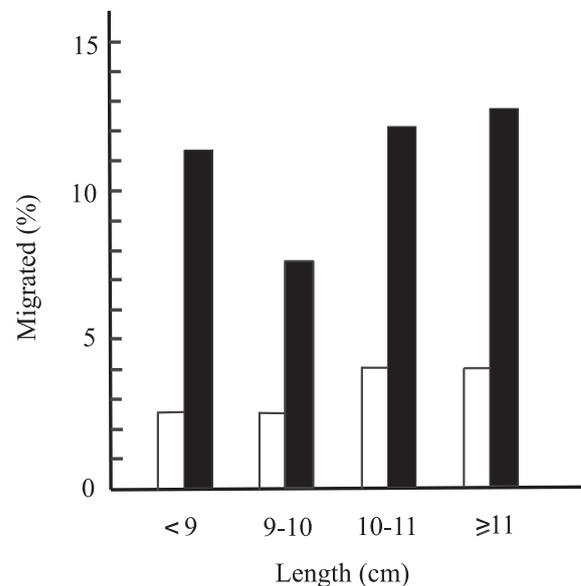


FIGURE 4 Percentage outmigration of pooled crosses of freshwater resident and anadromous *Salmo trutta* in length groups from <9 cm to ≥ 11 cm at tagging, incubated in either unheated (white bars) or heated (black bars) River Imsa water

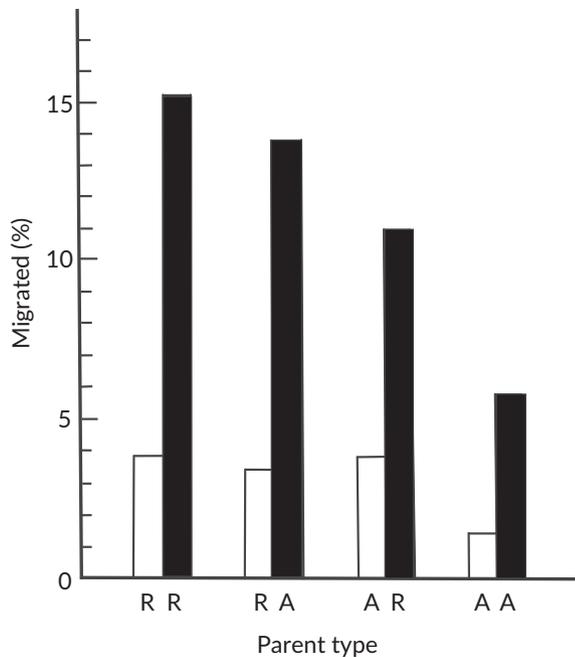


FIGURE 5 Percentage outmigration of *Salmo trutta* offspring of male × female freshwater resident (RR), anadromous (AA) and hybrids between freshwater resident and anadromous (RA, AR) incubated in either unheated (white bars) or heated (black bars) River Imsa water

intermediate between the proportions of pure resident and pure anadromous offspring. The difference between the proportions of the crosses in tendency to emigrate was highly significant ($Z^* = 57.3$, 4 df, $P < 0.001$). There was no significant difference in the proportion that emigrated of the various crosses between the 2 years of release.

4 | DISCUSSION

The research lent support to the contention that when incubated in heated water, juvenile *S. trutta* showed a stronger tendency to emigrate at a young age from their release site in the river than if incubated in colder water. This difference could not be explained by differences in body size at tagging (cf. Figure 4). Thus, incubation temperature likely led to an environmentally-induced difference in migratory tendency as no genetic differences between heated vs. unheated groups within cross types were expected, given that the fish were randomly allocated to treatments (e.g., RR ambient versus RR heated, etc.). There was no indication of differential selective mortality between ambient versus heated incubation groups before release, as mortality was quite low for all eight groups (two incubation temperatures × four crosses).

The low rates of detection of released *S. trutta* are likely caused by the trap's low capture efficiency for fish smaller than 10 cm (Table 1). However, the size variation between the emigrating groups (parental crosses or length groups) was small and should be little influenced by the selectivity of the trap. Furthermore, by comparing effects of incubation temperature within size groups, size-based

differences in detection efficiency are accounted for. The fact that there was no significant difference in the emigration rates of the offspring groups between the 2 years of release, even though the 2020 fish were larger at tagging as a consequence of 42 days later tagging, lends further support to the notion that the effect of the trap on the results was small.

At present, there is little knowledge of phenotypically plastic effects of temperature during embryogenesis on behavioural decisions. However, Burgerhout *et al.* (2017) found that if *S. salar* embryos were incubated in warmer water, they smolted and were ready for emigration to sea at a younger age, possibly because of faster growth. But this may not only result from differences in growth. Greenberg *et al.* (2021) observed that 1-year-old juveniles of *S. salar* incubated as eggs in heated water had a slimmer, more smolt-like body shape than similar-sized *S. salar* offspring that were produced from colder eggs. This may suggest that the warm offspring were ready for migration younger and smaller than those incubated in colder water. This view corresponds to our finding that warm-incubated offspring emigrated at a smaller body size. In the present research, differences in growth or size cannot explain the difference in emigration because we compared similar sized fish (Figure 4). However, this may explain why there were some smolt-like emigrants at age-1, most of which were incubated in heated water, and, moreover, these fish were younger than observed among wild offspring in this river (Jonsson & Jonsson, 2009). Earlier, Jonsson and Jonsson (2018) reported that adult *S. salar* returned later in the season from the ocean for spawning in the River Imsa if they as embryos were incubated in warmer water, regardless of their age or size at smolting. Thus, embryo temperature may influence later behavioural decisions, as found for the present *S. trutta*.

To our knowledge, there are few other reported examples from fish research where egg incubation temperature influences behavioural decisions. From amphibians and reptiles, it is known that egg incubation temperature can influence behavioural performance of tadpoles and hatchlings (e.g., Elphic & Shine, 1998; Seebacher & Grigaltchik, 2014; Siviter *et al.*, 2019). For instance, the swimming behaviour and stroke force of the turtle *Elusor macrurus* are influenced by egg incubation temperature (Micheli-Campbell *et al.*, 2011). A recent experiment with the oviparous lizard, the yellow-bellied three-toed skink *Saiphos equalis*, showed that elevated embryo temperature reduced the exploratory behaviour and foraging efficiency of the hatchlings (Beltrán *et al.*, 2020). Apparently, egg incubation temperature programmed hormone production, which is of major importance for many physiological processes that precede behavioural decisions (Singh *et al.*, 2020; While *et al.*, 2018). Our results demonstrate that incubation temperature influenced migratory behaviour, but given the range of responses across other species, the effects of incubation temperature likely extend beyond the responses evaluated in this study.

The present experiment did not support the second hypothesis, that offspring of anadromous *S. trutta* emigrated the most. Instead, offspring of freshwater resident parents emigrated the most, anadromous the least. This difference in outmigration may result from

selective differences between offspring of lacustrine-adfluvial and anadromous *S. trutta* in the river. The pure lacustrine-adfluvial *S. trutta* originated from parents spawning in the small tributary Fossbekk. This population has been geographically isolated from the anadromous for two and a half decades or possibly more if there has been fine-scale population structure in the watercourse. Although this period seems short, this may be sufficient to create genetic differentiation and/or lead to epigenetic changes (Sandlund & Jonsson, 2016).

Differences in genetic expression do not need to be caused by differences in genetic structure or composition. They can also result from differences in environments such as temperature at the embryonic stage, or be a parental effect initiated by environmental differences (Jonsson & Jonsson, 2014, 2019; Turner, 2009; Van Leeuwen *et al.*, 2019). The anadromous and lacustrine-adfluvial parents have experienced contrasting water qualities, feeding opportunities and risks of predation. The anadromous parents fed to a large extent in sea water with good feeding opportunities, reflected by the larger bodies and higher condition factor of the anadromous fish when compared with freshwater residents. Higher condition factor implies that parents possess more metabolic resources that can influence the fitness-enhancing traits of the offspring, such as more nutrients and/or hormones in the eggs. Such a maternal effect has been exhibited for a high number of species from mammals to insects (Bonduriansky & Crean, 2018; Chavatte-Palmer *et al.*, 2018; Deas *et al.*, 2019). Also, predator cues experienced by parents can influence offspring behaviour and personality traits, as demonstrated for the three-spined stickleback *Gasterosteus aculeatus* L. (Hellmann *et al.*, 2020). In these examples, an epigenetic mechanism, such as DNA methylation, may be involved. In vertebrates, methylation of CpG sequences (*i.e.*, a cytosine nucleotide directly followed by a guanine nucleotide in the linear base sequence) of regulatory DNA regions downregulate genetic expression, and conversely demethylation leads to upregulated genetic expression (Greenberg & Burc'his, 2019). Baerwald *et al.* (2016) investigated differences between anadromous and freshwater resident rainbow trout *Oncorhynchus mykiss* (Walbaum) and found 57 differentially methylated regions between smolts and freshwater residents that may influence migration-related transitions and smolting. Furthermore, Le Luyer *et al.* (2017) demonstrated that rearing environment influenced epigenetic variation in coho salmon *Oncorhynchus kisutch* (Walbaum). They reported that differentially methylated DNA regions exhibited enrichment for biological functions that may affect their capacity to migrate and survive. There is still little information about causes of different DNA methylations, although low body temperature in general is associated with higher DNA methylation levels (Kakutani, 2002; Varriale & Bernardi, 2006).

Why do *S. trutta* to a large extent emigrate in the autumn? It may be that the observed migration pattern represents an active migration where the released *S. trutta* search downstream for a suitable winter or feeding habitat, or a passive displacement where many individuals abandon their position in the river and move downstream with the strong water current. Both options are possible and not mutually exclusive. In spring, there may be active migration, where the few

individuals with smolt-like characteristics moved downstream. This would thus occur at the same time (May) as the wild *S. trutta* smolts moved to sea (Jonsson & Jonsson, 2009). Furthermore, it is probable that offspring of lacustrine-adfluvial *S. trutta*, the group that emigrated the most, had an intrinsic tendency to move downstream. From the tributary, these fish may naturally have emigrated in the autumn to spend the winter in the lake. When released in the River Imsa, their migratory response might have been similar, but given the location of the release site, the fish ended up in the fish trap instead of the lake. This possibly results from generations of genetic adaptation. This type of movement may have occurred even before the waterfall was built in the River Imsa. Autumnal movement from streams into lakes is a typical behavioural adaptation of lacustrine-adfluvial *S. trutta* spawning in small tributaries (Jonsson & Jonsson, 2011), and it is also observed in several other lacustrine-adfluvial trouts and charrs (*Salmo*, *Oncorhynchus* and *Salvelinus*) (Jonsson *et al.*, 2019).

The anadromous *S. trutta* in the River Imsa have no access to a lake, and there is probably selection against moving to the fjord as presmolts. This is because of the salt water barrier, *i.e.*, ~30‰ salt in the fjord water just outside of the river mouth (Jonsson *et al.*, 2016). Also, emigrating too early at a small size is costly, owing to size-selective early marine mortality. In the estuary, risk of predation is probably higher than in the river. Estuarine predators are cod *Gadus morhua* L, saithe *Pollachius virens* (L.), seals and sea birds, and these predators are not present upstream of the trap in the River Imsa.

S. trutta may also disperse downstream because the temperature drops in the autumn, when it becomes relatively more costly for the fish to maintain position in the strong current (Priede, 1985). This may be particularly true for juvenile *S. trutta* incubated as embryos in warm water, as they have been shown to have lower metabolic rate and aerobic scope than those incubated in colder water (Durtsche *et al.*, 2021). In parallel, Archer *et al.* (2021) reported that the standard metabolic rate was lower when juveniles of *S. trutta* were reared at an elevated temperature. Aerobic scope is the difference between the maximum and the standard metabolic rates, and it represents the capacity of organisms to increase their aerobic metabolic rates above the maintenance level to carry out various activities, such as feeding, swimming and defence (Fry, 1971). Experiments with zebrafish *Danio rerio* (Hamilton), a much-used model organism, have shown that temperature during embryogenesis influences energy metabolic pathways and acclimation capacity later on in life (Schnurr *et al.*, 2014). Moreover, temperatures experienced by embryos have knock-on effects on the routine metabolic rates of free-swimming brook trout *Salvelinus fontinalis* (Michill) fry (Cook *et al.*, 2018). Thus, trout and charr (*Salmo*, *Salvelinus*) incubated in warm water have a lower energy capacity available for activity because of their lower aerobic scope. This may be critical when water temperature and aerobic scope decrease in the autumn (Priede, 1985). Warm-incubated fish may thus abandon their position in the river sooner and move downstream to find a more suitable winter habitat, instead of remaining resident in the swiftly flowing river.

The present results suggest that warmer climates may have fitness consequences for *S. trutta*. In particular, winters are expected to

be substantially warmer in predicted global change scenarios, and our results suggest that one can expect behavioural changes in *S. trutta*, with relatively more offspring that leave the nursery area sooner, although high temperatures experienced by parr after embryogenesis might have a different effect (Archer *et al.*, 2020). For example, Archer *et al.* (2020) found lower smolting rates in *S. trutta* exposed to warmer rearing temperatures as parr. The migratory direction of the young offspring in the autumn, whether it be upstream or downstream, is inherited, depending on whether the fish are adapted to spawning upstream or downstream of wintering areas (Jonsson *et al.*, 1994). A warmer temperature may also result in younger age at smolting (Jonsson *et al.*, 2005). How earlier outmigration influences fitness will depend on the predator system, feeding opportunities and water quality of the habitat they move into, but, in general, small *S. trutta* are more vulnerable to predation and other adverse environmental conditions than larger conspecifics (Vainikka *et al.*, 2021). Our results, coupled with further evaluation of incubation temperature effects on fish behaviour and ecology, have important relevance to effective fisheries conservation and management, especially under changing climatic conditions.

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AUTHOR CONTRIBUTIONS

Both authors planned the experiment. B.J. analysed the material and wrote the first draft of the manuscript. L.G. contributed to the writing, discussion and presentation of the results.

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Note. *Salmo trutta* offspring were released in the River Imsa in October 2019 and May 2020 and recaptured until the end of June 2021.

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