
No Evidence for Long-Term Carryover Effects in a Wild Salmonid Fish

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ABSTRACT

Early-life experiences can shape life histories and population dynamics of wild animals. To examine whether stressful stimuli experienced in early life resulted in carryover effects in later life stages, we conducted several experimental manipulations and then monitored wild fish with passive integrated transponder tags during juvenile out-migration and adult return migration. In total, 3,217 juvenile brown trout (*Salmo trutta*) were subjected to one of six manipulations: chase to exhaustion, thermal challenge, food deprivation, low-concentration cortisol injection, high-concentration cortisol injection, and sham injection, plus a control group. Cortisol and food deprivation treatments were previously shown to have short-term effects on juveniles, such as lower survival to out-migration and changes in migration timing. However, it remained unknown whether any of the six manipulations had effects that carried over into the adult phase. We therefore investigated whether these extrinsic manipulations, as well as intrinsic factors (size and condition), affected probability of return as adults and time spent at sea. Of the 1,273 fish that out-migrated, 146 returned as adults. We failed to detect any effect of treatments on

return rates, while high-concentration cortisol weakly affected time spent at sea in one tagging event. We also found that juvenile condition was positively correlated to likelihood of adult return in only one tagging event. Overall, our findings did not identify either intrinsic factors or extrinsic stressful early-life experiences that have strong effects on fish that survive to adulthood. This suggests that some species may be more resilient than others to stressful stimuli encountered early in life.

Keywords: brown trout, early-life experiences, experimental biology, migration, *Salmo trutta*, stressors.

Introduction

To effectively manage populations of wild organisms, we require a general understanding of how both intrinsic and extrinsic factors affect behaviors (Clutton-Brock and Sheldon 2010). Furthermore, understanding how early-life experiences shape an individual's future decisions would enable us to predict how populations are likely to change under dynamic conditions (e.g., climate change). Early-life experiences can influence fitness traits (e.g., growth, reproduction), and as such, any stimuli that affect these experiences may impact life histories and population dynamics (Lindström 1999) through carryover effects (when an event occurring during one part of an animal's life affects performance during a subsequent part; Harrison et al. 2011; O'Connor et al. 2014; Moore and Martin 2019). One ubiquitous life experience is stress, as individuals often experience challenging stimuli at a young age, but how this type of early-life experience affects later life stages is still widely unknown (Norris 2005; Harrison et al. 2011; O'Connor et al. 2014). Both natural (e.g., low food availability, thermal variability) and anthropogenic (e.g., pollution, disturbance) stimuli can be perceived as stressors by animals, leading to the activation of the hypothalamic-pituitary-adrenal/interrenal axis (Mommsen et al. 1999; Wingfield and Romero 2001; Barton 2002). This elevates levels of glucocorticoid hormones, increasing the mobilization of energy reserves and elevating metabolic rates, thus diverting available resources away from processes such as growth and reproduction (Mommsen et al. 1999; Fullerton et al. 2000). This response is adaptive in the short-term as energy is diverted to bolster self-maintenance and survival but can reduce fitness in the long-term because of the trade-off with reproductive output, immunity, and survival (Wingfield and Romero 2001). Experimental manipulations that mimic aspects of the stress response (e.g., glucocorticoids) can be useful for examining the mechanisms that drive variation in fitness traits in wild animals (Crossin et al. 2016), including in migratory species (Birnie-Gauvin et al. 2020).

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In fish, extrinsic stimuli such as food deprivation (Lendvai et al. 2014), exhaustive exercise (Madaro et al. 2016), and temperature (Folkedal et al. 2012) can increase plasma cortisol levels, the main glucocorticoid hormone in fish. These stressful stimuli can have short-term carryover effects. For example, physiological exertion (i.e., anaerobiosis) in adult sockeye salmon (*Oncorhynchus nerka*) en route to spawning grounds has been shown to carry over and negatively influence reproductive success (Burnett et al. 2014). Exogenously elevated plasma cortisol applied during the freshwater juvenile stage of partially anadromous brown trout (*Salmo trutta*) lowered smolt survival in rivers (Midwood et al. 2014). The same treatment applied to adult female pink salmon (*Oncorhynchus gorbuscha*) also reduced survival as well as reproductive output (McConnachie et al. 2012). However, to our knowledge, there have been no attempts to manipulate stressors experienced by fish during early life phases to determine whether they carry over to influence adult performance. Such studies have however been carried out in mammals (e.g., Mirescu et al. 2004), birds (e.g., Haywood and Perrins 1992; Merilä and Svensson 1997), and reptiles (e.g., O'Steen 1998) and have all demonstrated the (generally) negative effects of early-life stressors on later life stages. Additionally, most studies that have demonstrated carryover effects from early life stages into the adult life stage applied stressors shortly after birth or close to a life-stage transition (reviewed in Harrison et al. 2011). We used an anadromous fish species where individuals were required to undergo a physiological transition between freshwater and saltwater. No studies that we know of have manipulated migratory animals at the juvenile stage that must also overcome some form of physiological transition.

The brown trout (*S. trutta* Linnaeus 1758) provides an interesting model for investigating carryover effects because individuals face multiple “decision points” throughout their life, some of which are affected by early-life experiences. For example, after spending the first 1–8 yr of their life in freshwater, brown trout must “decide” whether to migrate to sea (known as anadromous) or to remain in freshwater (known as resident; Klemetsen et al. 2003; Cucherousset et al. 2005). Several studies have indicated that natural factors, such as individual growth and condition (e.g., Forseth et al. 1999; Acolas et al. 2012; Russel et al. 2012), and mimicked stressors, such as low food availability (e.g., Peiman et al. 2017), can influence whether trout migrate and/or the timing of out-migration. Thus, early-life experiences can carry over from the juvenile freshwater stages to affect these decision points. After their marine phase, anadromous individuals return to freshwater to spawn and, as such, provide an opportunity to monitor migrant individuals over long periods using passive integrated transponder (PIT) tags.

Here, we investigate how both intrinsic factors and mimicked stressors experienced at the juvenile stage in freshwater affect the time spent at sea and successful return of anadromous brown trout to freshwater as adults. We exposed juvenile brown trout to one of five challenges, mimicking both external and physiological perturbations, and monitored individuals during their out-migration as juveniles and return migration as adults. We simulated three external events: predation avoidance by chasing fish to exhaustion, low food availability by reducing access to food

for 2 wk, and high temperature by maintaining fish in water 4.2°C higher than stream temperature. We also mimicked one of the main components of the physiological stress response by injecting fish with exogenous cortisol at either a low or a high concentration. This initiated the subsequent effects of the stress response (e.g., increased energy mobilization), a method that has been commonly used to examine the effects of stressors on wild fish (Sopinka et al. 2015; Crossin et al. 2016). The treatments were applied in different combinations during four tagging events (two in the spring and two in the fall), and their short-term effects on juvenile out-migration are known. In summary, supraphysiological exogenous manipulation of cortisol (high-concentration treatment) decreased in-stream survival to out-migration in the fall (Midwood et al. 2015) and spring (Midwood et al. 2014, 2016) and caused fish to migrate earlier when applied in the fall (Midwood et al. 2015; Peiman et al. 2017). The food deprivation treatment was the only other stressor to show an effect, where migration propensity decreased and the timing of migration was delayed, which was most evident when applied in the spring (Midwood et al. 2016). In addition to effects on survival and migration, cortisol manipulation had negative effects on growth and condition (Midwood et al. 2014, 2015, 2016; Peiman et al. 2017). Generally, fish of smaller size, lower condition, or lower growth tended to migrate more and earlier (Peiman et al. 2017).

Given the effects that the cortisol treatment had on growth, condition, and migration as well as the effects that the food deprivation treatment had on migration in juvenile fish, we predicted that these treatments would extend the time spent at sea as sub-adult fish, perhaps as a means of compensating for growth impairments during the juvenile freshwater phase (Nicieza and Metcalfe 1997). Compensatory growth has been shown to reduce survival (Johnsson and Bohlin 2006), and so we also predicted that cortisol and food deprivation treatments would result in lower adult return rates. We made no prediction with regard to intrinsic factors, as the association between length/condition at the juvenile stage and adult return can be positive, negative, or nonsignificant (e.g., Armstrong et al. 2018).

Material and Methods

Study Locations

The study was performed in two rivers (fig. 1). River Villestrup is located in north central Jutland, Denmark, and runs into the Mariager Fjord. The Gudsø stream is located in southeast Jutland, Denmark, and runs into the Kolding Fjord. Both streams are home to natural populations of partially migrant brown trout, with juveniles typically migrating to sea as 1-yr-olds, and migrants generally outnumbering residents 10:1 (Birnie-Gauvin et al. 2017). Both streams are equipped with fixed PIT stations spanning the entire width of the riverbed. These stations detect fish tagged with uniquely coded PIT tags year-round. Villestrup has a single PIT station with two antennas (10 m apart) located 200 m upstream of the river mouth (detection efficiency of the first antenna was estimated at 98.4% across the study period; efficiency of the second antenna could not be calculated; see

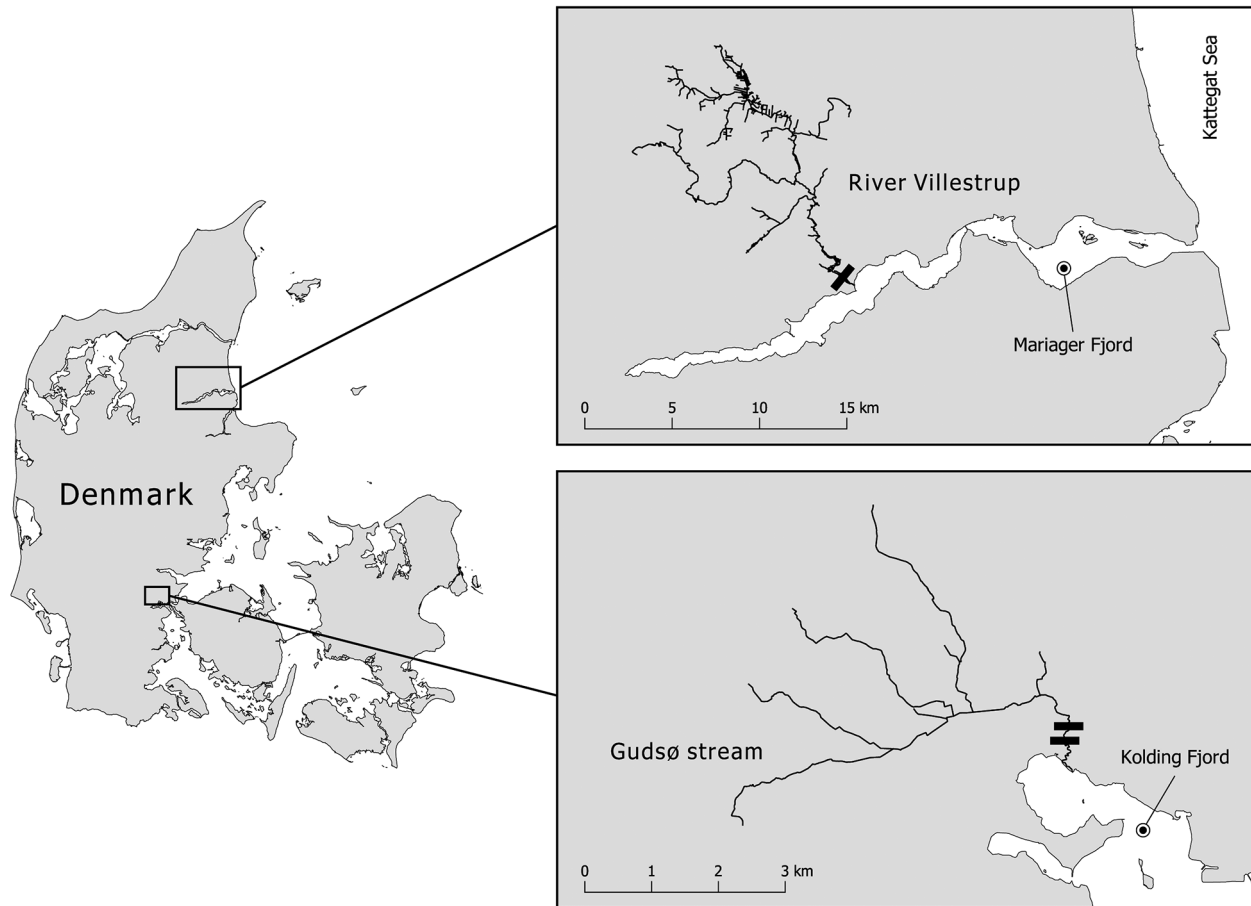


Figure 1. River Villestrup, northern Jutland, and Gudsø stream, southern Jutland, Denmark. Passive integrated transponder stations are represented as black bars.

Zydlowski et al. 2006). Gudsø is equipped with two PIT stations, each with two antennas (5 m apart) located approximately 600 m from the river mouth (detection efficiency of the first PIT station was estimated at 98.9% averaged across the two antennas during the study period). Regardless of minor detection inefficiencies, there is no expectation that treatment would affect detection probability.

Tagging and Treatments

We combined data from field studies carried out during four different tagging events over the course of 3 yr, where a total of 3,217 individual fish were captured, tagged, and treated. Tagging and treatment occurred during the following dates: (1) March 6–12, 2012 (spring 2012; $n = 761$; Gudsø stream), (2) November 4–7, 2012 (fall 2012; $n = 475$; Gudsø stream), (3) February 28–March 11, 2013 (spring 2013; $n = 622$; Gudsø stream), and (4) October 21–29, 2014 (fall 2014; $n = 1,359$; River Villestrup; table 1). Length and mass measurements were used to calculate a condition factor (Fulton's $K = (\text{mass} \times \text{length}^{-3}) \times 100$).

Detailed descriptions of capture, tagging, and treatment procedures are provided in Midwood et al. (2014, 2015, 2016), as well as in Peiman et al. (2017). Briefly, fish were captured by elec-

trofishing and kept in freshwater until processed. Fish were anesthetized with benzocaine (0.03 g L^{-1} in water), weighed ($\pm 0.01 \text{ g}$), measured ($\pm 0.1 \text{ cm}$), and tagged with 23-mm PIT tags (RI-TRP-RRHP, Texas Instruments, Plano, TX; 134 Hz, 0.6 g mass in air). Fish were then randomly assigned to a treatment group. Control fish were immediately left to recover in freshly oxygenated water. Sham fish tagged in spring 2012 were given intracoelomic injections of 0.01 mL of cocoa butter (NOW Foods, Bloomingdale, IL) per gram of fish. Cortisol-treated fish in 2012 and 2013 were injected with either a low (25 mg of cortisol per 1 kg of fish) or a high (100 mg of cortisol per 1 kg of fish) dose of cortisol (hydrocortisone 21-hemisuccinate, Sigma-Aldrich, St. Louis, MO) dissolved in cocoa butter. Sham and cortisol treatments applied in 2014 were performed the same way except that vegetable shortening (100% vegetable shortening, Crisco, Orville, OH) was used as a vehicle. A validation study showed that exogenous manipulation of high doses of cortisol in vegetable shortening increased plasma cortisol to supraphysiological levels for 6 d after treatment and plasma cortisol was still elevated but at normal stress-induced physiological levels at day 9 after treatment, but high doses of cortisol in cocoa butter increased plasma cortisol only to normal stress-induced physiological levels at day 3 and had no effect by day 6 (Birnie-Gauvin et al. 2018). Food-deprived fish were kept in

Table 1: Number, mean total length (\pm SD), and condition factor (\pm SD) of juvenile brown trout at initial capture assigned to each treatment group during each tagging event

Tagging event	Spring 2012 Gudsø	Fall 2012 Gudsø	Spring 2013 Gudsø	Fall 2014 Villestrup
Control:				
<i>n</i>	190	228	206	223
Mean total length (cm)	14.7 \pm 2.0	14.1 \pm 1.9	14.3 \pm 1.4	14.4 \pm 2.1
Condition factor	.92 \pm .06	1.01 \pm .09	.88 \pm .05	.96 \pm .06
Sham:				
<i>n</i>	192	—	—	224
Mean total length (cm)	14.6 \pm 1.7			14.3 \pm 1.9
Condition factor	.92 \pm .06			.96 \pm .06
Low cortisol:				
<i>n</i>	191	—	—	—
Mean total length (cm)	14.6 \pm 1.8			
Condition factor	.93 \pm .07			
High cortisol:				
<i>n</i>	188	247	213	230
Mean total length (cm)	14.7 \pm 2.0	14.1 \pm 1.8	14.4 \pm 1.6	14.0 \pm 2.1
Condition factor	.92 \pm .06	1.01 \pm .09	.88 \pm .05	.96 \pm .06
Food deprived:				
<i>n</i>	—	—	203	235
Mean total length (cm)			14.4 \pm 1.5	14.1 \pm 1.8
Condition factor			.90 \pm .05	.97 \pm .06
Thermal:				
<i>n</i>	—	—	—	222
Mean total length (cm)				14.1 \pm 1.8
Condition factor				.96 \pm .06
Chase:				
<i>n</i>	—	—	—	225
Mean total length (cm)				14.3 \pm 1.9
Condition factor				.96 \pm .06

Note. Dashes indicate that the treatment was not used in that tagging event.

a pen placed in the stream for 14 d to reduce foraging opportunities, which resulted in a loss of mass (Midwood et al. 2016; Peiman et al. 2017). Thermally challenged fish were maintained at $4.2^{\circ} \pm 0.1^{\circ}\text{C}$ above stream water temperature for 30 min (Peiman et al. 2017). Fish chased to exhaustion were placed in an annulus arena and chased for 3 min, after which time most fish no longer responded to external stimuli (Peiman et al. 2017). Following all treatments, fish were left to recover in freshly oxygenated stream water until equilibrium was regained. All fish were released the same day near their site of capture, except food-deprived fish that were released after 14 d in the pens. Note that not all treatments were performed at each tagging event. All procedures were performed in accordance with and with permission from the Danish Animal Experiment Inspectorate (under licenses 2012-DY-2934-00007 and 2013-15-2934-00808, administered by the Technical University of Denmark).

Migration and Return

All PIT data were downloaded March 1, 2018. Note that for all smolt out-migration data, we cannot differentiate between stream

residency and death for individuals not detected at the PIT antennas. Adult return date was defined as the first detection at the lowermost antenna, and a fish was considered to have returned if it was detected at the downstream and upstream PIT antennas in that order after having been detected migrating as a smolt. All return migrations occurred after at least 2.5 mo at sea. Time spent at sea was calculated as the duration (number of days) between smolt out-migration and adult return date.

Statistical Analyses

Because the short-term effects on juvenile out-migration have already been published (Midwood et al. 2014, 2015, 2016; Peiman et al. 2017), our analysis focused on long-term effects, specifically return rates as adults and time spent at sea. We nonetheless present results on the proportion of smolt out-migration across tagging events for comparative purposes only.

Mass and length were highly correlated, so only length was used for subsequent analyses to avoid collinearity. No fish tagged in fall 2012 returned, so data from this tagging event could not be included in any analyses. Very few fish (one to six per treatment)

tagged in fall 2014 returned, so data from this tagging event were excluded from analyses because of low statistical power, although results from this tagging event are still presented in the figures.

Generalized linear models (GLMs) with Bernoulli distribution and logit link function (i.e., logistic regression) were used to investigate whether treatment, length, and condition influenced the likelihood of adult return (yes or no) from the sea:

$$\begin{aligned}\text{adult return}_i &\sim \text{Bernoulli}(\pi_i, 1), \\ E(\text{adult return}_i) &= \pi_i, \\ \text{var}(\text{adult return}_i) &= \pi_i \times (1 - \pi_i), \\ \text{logit}(\pi_i) &= \alpha + \beta_1(\text{treatment}_i) + \beta_2(\text{length}_i) \\ &\quad + \beta_3(\text{condition}_i).\end{aligned}$$

Significance was tested using likelihood ratio tests (LRTs). We also explored differences in adult return probability using a χ^2 analysis (a one-sided test) but opted for the GLM approach, as this allowed us to simultaneously test for length and condition effects and because the results were the same (see the appendix).

For adults that returned, a GLM with gamma distribution and log link function was used to test whether treatment, length, and condition influenced time spent at sea (number of days):

$$\begin{aligned}\text{time at sea}_i &\sim \text{gamma}(\mu_i, \tau), \\ E(\text{time at sea}_i) &= \mu_i, \\ \text{var}(\text{time at sea}_i) &= \frac{\mu_i^2}{\tau}, \\ \log(\mu_i) &= \alpha + \beta_1(\text{treatment}_i) + \beta_2(\text{length}_i) \\ &\quad + \beta_3(\text{condition}_i).\end{aligned}$$

One fish treated with the high dose of cortisol in spring 2012 exerted extreme influence on the model fit and was thus excluded from this analysis (for analysis with the outlier included, see the appendix). This individual spent 946 d at sea (average time at sea for the remaining high-cortisol fish tagged in spring 2012 was 361.1 d and ranged from 176 to 625 d).

Data from each tagging event were analyzed separately, as the type and number of levels of the independent variable (treatment) varied among the events. In addition, each tagging event occurred under different environmental conditions, which may have exerted some influence on the outcomes, providing further support for separate analyses. All statistical analyses were performed in R version 3.1.2 (R Development Core Team 2014). Model validation was performed by visual inspection of the residuals, and no violations were encountered. Collinearity between independent variables was assessed using variance inflation factors (see Zuur et al. 2009). Significance was evaluated as $P < 0.05$.

Results

Likelihood of Smolt Migration

Of the 3,217 fish tagged, a total of 1,273 smolts migrated out of their natal stream. As noted previously, these data have been analyzed in Midwood et al. (2014, 2015, 2016) for spring 2012, fall 2012, and spring 2013 tagging events, respectively, and in Peiman et al. (2017) for the fall 2014 tagging event. As such, these data were not reanalyzed statistically. Briefly, we note that high-

cortisol fish had lower out-migration rates than controls in all tagging events. Food-deprived fish were less likely to migrate than controls in spring 2013 (fig. 2).

Adult Survival

Of the 1,273 smolts that migrated, 146 (11.5%) survived to return as adults at least once (table 2). No fish tagged in fall 2012 returned, and very few tagged in fall 2014 returned, so the adult analysis is based on returns from the spring 2012 and spring 2013 tagging events. Although few fish returned from fall 2014 tagging (the only event in River Lillestrup), these all returned within 1 yr, whereas 34.4% and 41.2% of fish originating from Gudsø stream in spring 2012 and 2013, respectively, spent more than 1 yr at sea.

Likelihood of Adult Return

Likelihood of return was higher for juveniles that had higher condition in spring 2012 (LRT = 5.268, $df = 1$, $P = 0.022$) but was not affected by length (LRT ≤ 1.239 , $df = 1$, $P \geq 0.061$). In spring 2013, neither condition nor length was associated with likelihood of return (LRT ≤ 1.239 , $df = 1$, $P \geq 0.266$). Treatment had no effect on likelihood of return in either study year (all $P \geq 0.260$; fig. 2).

Time Spent at Sea

Time spent at sea was not affected by length ($F \leq 0.245$, $P \geq 0.625$) or by condition as juveniles ($F \leq 0.518$, $P \geq 0.474$) for either tagging event. Treatment had a weak effect on time spent at sea in spring 2012 ($F = 2.594$, $df = 3$, $P = 0.058$) such that the high-cortisol group spent longer time at sea than controls ($P = 0.0279$), but not in spring 2013 ($F = 1.128$, $df = 2$, $P = 0.338$; fig. 3).

Discussion

Given the delay in juvenile out-migration and loss of mass in food-deprived fish, as well as the negative effects on juvenile growth and condition in cortisol-treated groups, we expected that individuals from these groups would have lower adult return rates and/or spend a longer time at sea to compensate for a time of reduced growth in early development. Despite the presence of short-term carryover effects, however, we failed to detect strong effects of treatment on adult return or time spent at sea. This suggests that the effects of stressors experienced early in life did not have a strong effect on adult brown trout.

We found that juveniles in better condition in the spring 2012 tagging event but not those in the spring 2013 tagging event were more likely to return and that length had no effect on return rates. Many studies have investigated the link between length/condition and aspects of migration, but the relationship between these parameters is complicated by the timing of measurements relative to age and out-migration time (Ferguson et al. 2017). Because body length and condition were measured at the time of tagging and not at the time of migration, our data do not reflect that some

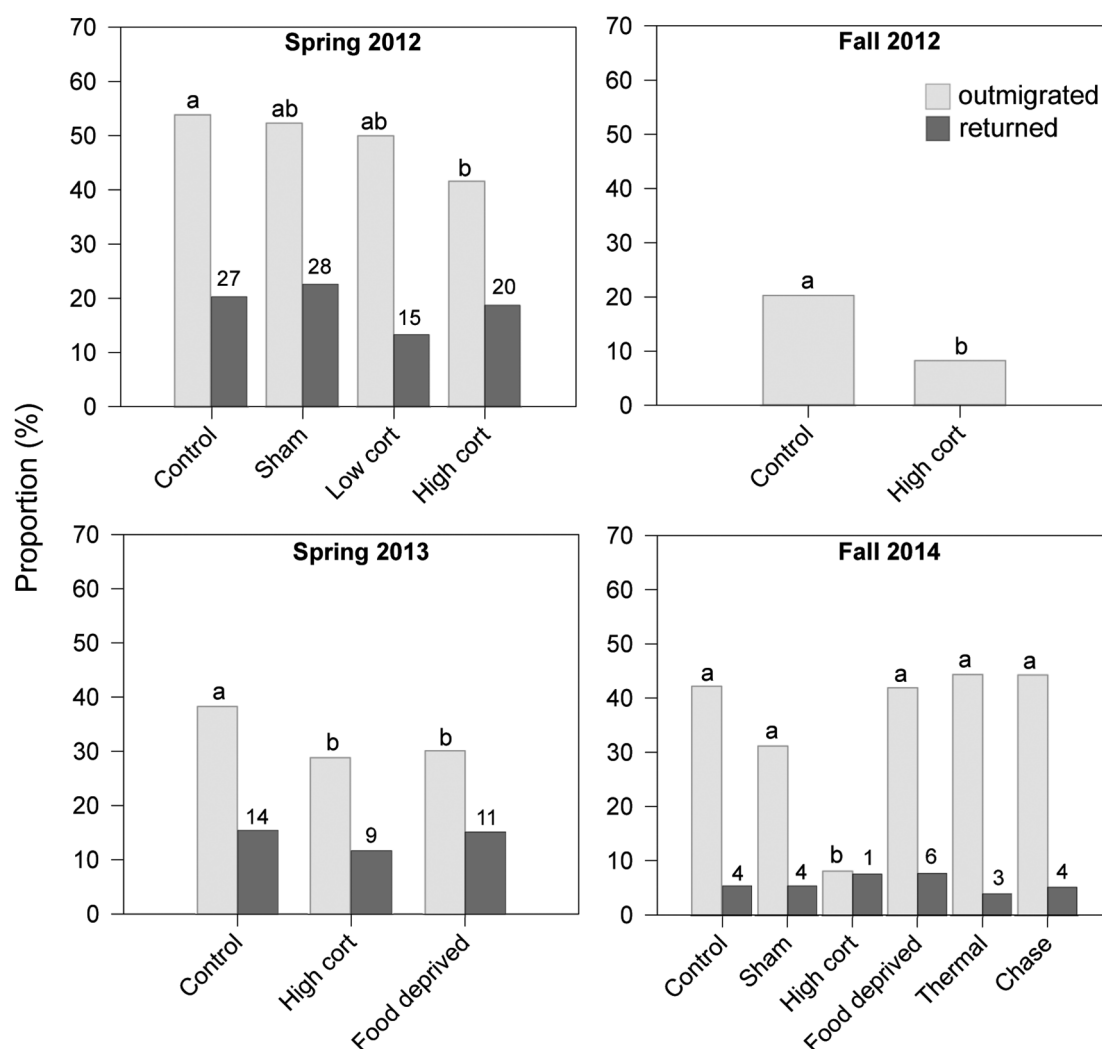


Figure 2. Juvenile out-migration and adult return migration. Proportion of tagged juvenile brown trout (*Salmo trutta*) that out-migrated within each tagging event for each treatment is shown in light gray. Different lowercase letters indicate significant differences ($P < 0.05$), as analyzed in Midwood et al. (2014, 2015, 2016) and Peiman et al. (2017) for spring 2012, fall 2012, spring 2013, and fall 2014, respectively. Proportion of out-migrated juvenile brown trout within each tagging event and treatment that returned to freshwater as an adult is shown in dark gray. The number of fish is presented above the bars. No fish returned in fall 2012, and fall 2014 was not analyzed because of low sample size. No treatment effects were found. Cort = cortisol.

individuals have accelerated growth shortly before migrating (Ferguson et al. 2017). Furthermore, the relationship between juvenile size/condition and migration success has been shown to vary considerably across studies, both in sign and in magnitude (see Gregory et al. 2018 and references therein). While a wide range of studies support a “bigger is better” paradigm where marine survival of juveniles to adulthood is positively correlated to size and condition at migration (e.g., Saloniemi et al. 2004; Armstrong et al. 2018), other studies question the generality of this paradigm (Birnie-Gauvin et al. 2019; del Villar-Guerra et al. 2019). For example, optimal length at juvenile out-migration was medium size in River Imsa, Norway (Jonsson et al. 2017). Thus, the influence of length and condition on marine survival (and thus adult return

rates) may vary among years and rivers, as is supported by our results.

The negative effects on juvenile growth and/or condition following exogenous cortisol injections (Midwood et al. 2014, 2015; also reviewed in Sopinka et al. 2015) and food deprivation (Byström et al. 2006) have already been documented. An increase in time at sea would be consistent with the literature suggesting that longer marine phases represent a means of compensating for a period of low growth during early life (Jonsson and Bohlin 2006). Our results failed to reveal strong treatment effects, as cortisol treatment had only a weak effect on time spent at sea in one tagging event. We also found no indication that juvenile length or condition affected time spent at sea. Sea trout are known to vary

Table 2: Number of tagged fish, number of out-migrated (and percentage of tagged) fish, number of returned (and percentage of out-migrated) fish, and time at sea for each tagging event

Tagging event	Location, tagging dates	Initially tagged fish	Out-migrated fish (%)	Returned fish (%)	Time at sea (d)
Spring 2012	Gudsø, Mar. 6–12	761	477 (62.7)	90 (18.9)	118–625 ^a
Fall 2012	Gudsø, Nov. 4–7	475	75 (15.8)	0	NA
Spring 2013	Gudsø, Feb. 28–Mar. 11	622	273 (43.9)	34 (12.5)	83–648
Fall 2014	Villestrup, Oct. 21–29	1,359	448 (33.0)	22 (4.9)	190–325
Total		3,217	1,273 (39.6)	146 (11.5)	

Note. All treatment groups have been combined. NA = not applicable.

^aIncludes one outlier that spent 946 d at sea that was excluded from analysis.

extensively in their behavior, with some individuals being more active and even adjusting their behavior when affected by external stressors, such as by making short trips to the estuaries to shed their sea lice (Halttunen et al. 2018). Thus, smaller or low-condition brown trout may adjust their behavior by more active foraging rather than extend their time spent at sea.

Individuals from every treatment including the control groups spent highly variable times at sea, similar to other sea trout populations (Klemetsen et al. 2003). However, all Villestrup adults spent <1 yr at sea, whereas 34%–41% of returning adults from Gudsø spent >1 yr at sea, perhaps as a result of differences in adjacent sea conditions in northeast (Villestrup: Mariager Fjord, Kattegat Sea-like conditions) and southeast (Gudsø: Kolding Fjord, western Baltic-like conditions; refer to fig. 1) Jutland during those years. There is some evidence suggesting that Mariager Fjord is the most productive fjord (in terms of phytoplankton, invertebrates, and fish) in Denmark (Kaas et al. 1996), and thus conditions in Mariager Fjord may be more favorable than those in Kolding Fjord. As a result, one potential explanation for the observed differences in return timing may be that brown trout in Mariager Fjord reach sufficient reproductive potential to make the return migration in <1 yr worthwhile, although our low adult return rates argue against favorable conditions in this fjord. However, we had only one tagging event in Villestrup and so cannot

generalize across years, and other studies in the same system have observed a relatively high instance of 1+ sea-winter fish (e.g., del Villar-Guerra et al. 2019). This suggests that variation in food availability and other environmental factors (such as temperature, salinity, or high predation pressure; e.g., Thorstad et al. 2012) across years likely affected time spent at sea and survival for all treatments, although these conditions were not monitored during our study. However, we have no reason to expect that differences between years or fjord systems would differentially affect our interpretations of treatment effects conducted within years. If anything, we would predict that harsher environmental conditions at sea within any given year would have a larger impact on survival or return timing of individuals still affected by treatments and so make detecting treatment effects easier. Given that we had one tagging event with no returns and one with few returns, this suggests that conditions at sea were harsh regardless of treatment. Additionally, the time of tagging and manipulation may have had differential effect on adult survival, as it was our two fall tagging events that resulted in either zero or very few adult fish return, in contrast to our two spring tagging events. This is another reason why we analyzed all tagging events separately. Nonetheless, investigating the influence of sea conditions on the behavior of fish that have undergone various treatments provides an interesting avenue for future research.

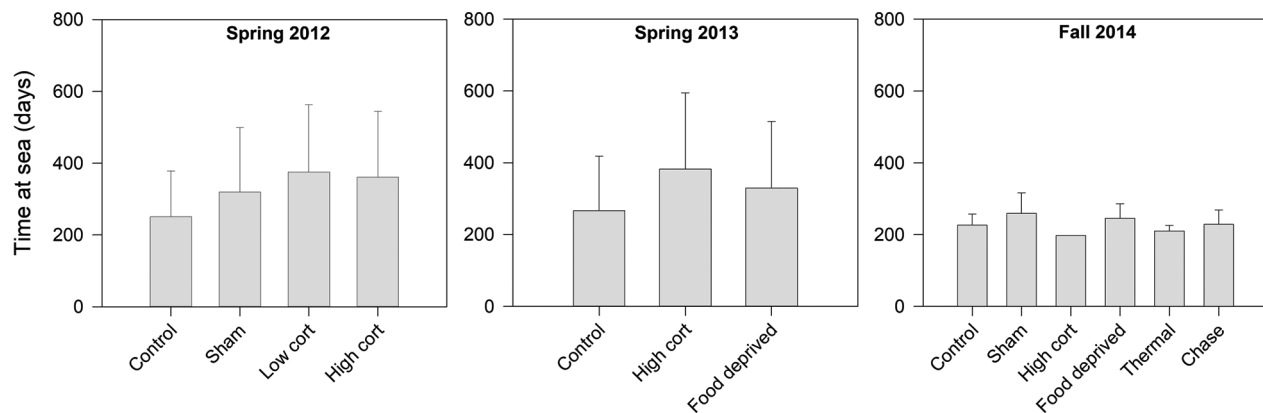


Figure 3. Time spent at sea. Average time (in days) spent at sea for brown trout (*Salmo trutta*) within each tagging event for each treatment. One outlier from the high-cortisol treatment in spring 2012 was excluded. Error bars represent 1 SD of the average value. Fall 2014 was not analyzed. Only a weak high-cortisol effect was found in spring 2012. Cort = cortisol.

Although the marine survival of first-time migrants in the present study (11.5%) was on the lower end of what has previously been observed in brown trout in similar systems (15.7%–20.3%; Birnie-Gauvin and Aarestrup 2019), the first marine migration has been identified as a particularly challenging life stage, with high mortality being common (8%–71% across multiple systems; Thorstad et al. 2012). Even though we failed to detect strong effects of treatments on adult returns, carryover effects may not be manifested until later life stages. Future research should consider recapturing individuals during the spawning season to assess carryover effects on reproductive potential. A simple power analysis (see Peterman 1990) indicates that at a minimum, 16 returned adults per treatment are needed to have a statistical power of 0.80. All treatments except one in spring 2012 had more than 16 individuals return as adults (low-cortisol group had 15), and thus the finding that treatments did not have strong effects that carried over to influence return rate and time at sea is supported with adequate statistical power for that tagging event. Using an average sample size of 11 returned adults per treatment (the average number of returned fish across treatments in spring 2012, spring 2013, and fall 2014), our statistical power is approximately 0.65 (but as low as 0.11 for groups with especially low return rates tagged in fall 2014), so results from those tagging events are less robust. Future studies should be guided by our findings that 1.6%–11.8% of initially tagged fish may return as adults (0% in some treatments), and initial sample sizes should thus be closer to 1,000 fish per treatment. Although we invested significant time and resources in this work, it is clear that even more expansive efforts are needed to fully unravel the mysteries of animal migration (Bowlin et al. 2010). This would offer fundamental knowledge on the extent to which carryover effects may affect population dynamics in the wild. Our study is further limited in that only fish that survived the applied stressors and migrated to the sea in the short-term could be used for the long-term portion of the analysis.

While many studies have found carryover effects in adults across seasons in diverse taxa (e.g., Robb et al. 2008; Crossin et al. 2010; Harrison et al. 2011 and references therein; Rockwell et al. 2012; Betini et al. 2013; Latta et al. 2016), in general, studies that have demonstrated carryover effects from early life stages into the adult life stage applied stressors or used naturally occurring conditions shortly after birth (e.g., hatching, weaning, or the larval stage; Blount et al. 2003; Chelgren et al. 2006; Descamps et al. 2008; Saino et al. 2018). Thus, stressors occurred at what is perhaps a particularly sensitive life stage, and consequences may be more severe (i.e., death) and, for the survivors, long-lasting (i.e., carry over into later life stages) as a result. Although most studies did not or could not measure mortality, it could be that species differ in whether stressors have stronger short-term effects on mortality versus longer-term carryover effects on the survivors (individuals that were robust enough to survive the stressor). Here, we first summarized the numerous short-term effects of stressors (which were mortality/residency and changes in out-migration timing) applied at the late juvenile stage during four separate tagging events (Midwood et al. 2014, 2015, 2016; Peiman et al. 2017), but our long-term analysis found only weak effects on adults, perhaps because stressors were applied when fish were 10 mo or more past

the larval stage (i.e., tagged in the spring) or because of high mortality at both life stages leaving only robust individuals returning (on average, only 36% of tagged fish out-migrated, and only 11.5% of out-migrating smolts returned as adults). To our knowledge, the longest recorded impact of an applied stressor in wild fish was a cross-seasonal effect in adult largemouth bass (*Micropterus salmoides*; O'Connor et al. 2010). Adults were subjected to increased circulating levels of cortisol for 5 d in the fall and were then followed over the next 7 mo, with cortisol-treated adults dying sooner than control fish during a winterkill event. The studies that found longer-lasting effects in fish used natural variation in larval traits, such as growth rate, condition, or early-life environment (e.g., Shima and Swearer 2010; Saboret and Ingram 2019), again highlighting that the life stage at which a stressor occurs may be an important factor in determining whether effects carry over to adulthood.

How close to a life-stage transition an organism is may also be a critical factor in studies of carryover effects (e.g., Chelgren et al. 2006; Touchon et al. 2013; Dingeldein and Wilson White 2016). For example, Chelgren et al. (2006) manipulated food availability in northern red-legged frog (*Rana aurora*) tadpoles that were near metamorphosis to the adult stage and found that food influenced the timing of metamorphosis and the performance of adults as they moved from aquatic to terrestrial habitats. If physiological and molecular processes that enable the transition from one life stage to the next have already begun when the stressor is applied, long-term effects on the survivors may be more prevalent. It also may be that in species where maturity is reached early in life, long-term effects are easier to detect, as there may be less environmental variation and fewer other new stressors also acting on these individuals over their short life spans.

The present study used an anadromous fish species where individuals were required to undergo a physiological transition between freshwater and saltwater. Many studies have considered the effects of stressors on migrating adult animals (Norris and Taylor 2006 and references therein; O'Connor et al. 2014 and references therein), but none that we know of have manipulated migratory animals at the juvenile stage that must also overcome some form of physiological transition. Yet there is evidence that this may be important, as natural variation in salinity experienced during the larval stage in the facultatively diadromous common bully (*Gobiomorphus cotidianus*) affects vulnerability to parasites, trophic position, and diet preference in adulthood (Saboret and Ingram 2019). Species that face a physiological transition while migrating are likely challenged to a greater extent because energy must also be allocated to the physiological processes necessary to deal with this transition, although our current study suggests that this is not the case for brown trout. This offers an interesting avenue for future research in migratory fish, where stressors could be applied at hatching to explore whether very early-life-stage stressors are more likely to have carryover effects in adulthood in diadromous versus nondiadromous fishes.

Biological systems naturally work in sequence, and thus every “decision” or “event” in an animal’s life is likely to have downstream effects (Harrison et al. 2011). In this study, we found that the consequences of delayed growth during early life induced by

cortisol and food deprivation treatments do not appear to have strong effects that carry over through adult life in brown trout despite having reasonable statistical power. Species vary in how they cope with early-life stressors (Clavel et al. 2011; Harrison et al. 2011), and in some species, these effects may carry over into adulthood. This may be one reason why species vary in their ability to cope in a changing world (e.g., inherently variable levels of food availability and extreme weather conditions mediated by human activity; Angelier and Wingfield 2013). However, the evolutionary basis for and consequences of carryover effects are just beginning to be explored (Moore and Martin 2019).

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APPENDIX

Additional Analysis for Adult Return Rate

We further explored treatment effects on adult return rate using a χ^2 analysis (one-sided test). We found no treatment effects in either spring 2012 ($\chi^2 = 3.607$, $df = 3$, $P = 0.307$) or spring 2013 ($\chi^2 = 0.526$, $df = 2$, $P = 0.769$).

Additional Analysis for Time Spent at Sea

To include the outlier fish that spent 946 d at sea in the spring 2012 high-cortisol treatment, we used a nonparametric Kruskal-Wallis test (because including this outlier created nonhomogeneous variance in the data) followed by Wilcoxon pairwise comparisons. A treatment effect was detected in spring 2012 ($\chi^2 = 8.05$, $df = 3$, $P = 0.0447$), but this effect could not be detected in the post hoc analysis. The high-cortisol ($P = 0.063$), low-cortisol ($P = 0.063$), and sham ($P = 0.45$) treatments did not differ from the control group. The use of the same nonparametric test yielded the same nonsignificant result as in the main text for spring 2013 ($\chi^2 = 3.390$, $df = 2$, $P = 0.184$).

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