RESEARCH ARTICLE

An Experimental Field Evaluation of Winter Carryover Effects in Semi-Anadromous Brown Trout (*Salmo trutta*)

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ABSTRACT

For semi-anadromous brown trout, the decision whether or not to smoltify and migrate to the sea is believed to be made at the end of the preceding summer in response to both local environmental conditions and individual physiological status. Stressors experienced during the fall may therefore influence their propensity to migrate as well as carry over into the winter resulting in mortality when fish face challenging environmental conditions. To evaluate this possibility, we artificially elevated cortisol levels in juvenile trout (via intracoelomic injection of cortisol in the fall) and used passive integrated transponder tags to compare their overwinter and spring survival, growth, and migration success relative to a control group. Results suggest that overwinter mortality is high for individuals in this population regardless of treatment. However, survival rates were 2.5 times lower for cortisol-treated fish and they experienced significantly greater loss in mass. In addition, less than half as many cortisol-treated individuals made it downstream to a stationary antenna over the winter and also during the spring migration compared to the control treatment. These results suggest that a fall stressor can reduce overwinter survival of juvenile brown trout, negatively impact growth of individuals that survive, and ultimately result in a reduction in the number of migratory trout. Carryover effects such as those documented here reveal the cryptic manner in which natural and anthropogenic stressors can influence fish populations. *J. Exp. Zool. 323A:645–654, 2015.* © 2015 Wiley Periodicals, Inc.

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Underlying life-history decisions and trade-offs in wild animals are complex physiological processes (Zera and Harshman, 2001), largely mediated by the endocrine system (Ricklefs and Wikelski, 2002; Crespi et al., 2013). As such, various challenges experienced by an animal (e.g., reductions in food availability, storms, predation events, and interactions with humans) that trigger a glucocorticoid (GC) stress response have the potential to alter life-history decisions and trade-offs through changes in energy allocation (Landys et al., 2006), potentially influencing reproduction and survival (Crespi et al., 2013). Migration associated with seasonal or life-history transitions represent a complex interaction between behavior and

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physiology, which is particularly sensitive to various stressors (Dingle and Drake, 2007). Stress has the potential to have dramatic consequences on the decision of animals to migrate as well as their ultimate fate and fitness. Over the last decade, there has been growing recognition that carryover effects, a concept originally conceived in the medical realm, also occur in an ecological context (Harrison et al., 2011). Ecological carryover effects occur in any situation in which an individual's previous history and experience explains their current performance in a given situation (O'Connor et al., 2014). This concept is often used in the context of animal migration (Harrison et al., 2011; Norris and Taylor, 2006) but is also relevant more broadly across life-history stages (Warne et al., 2012; Warne and Crespi, 2015), time (such as seasonal transitions; O'Connor et al., 2010), or experimental settings (O'Connor et al., 2014). These highly complicated interactions between organismal physiology, behavior, and life-history present experimental biologists with great opportunity to elucidate ecological and evolutionary processes, particularly in the face of changing environments (Wingfield, 2008).

Winter is an extremely challenging period for many animals in temperate regions because it is typically characterized by harsh environmental conditions and reductions in food availability. There are diverse strategies for dealing with winter challenges including migration (e.g., many birds) and hibernation (e.g., some mammals, reptiles, or amphibians). However, some animals (e.g., many fishes) remain active, although often somewhat quiescent compared to their behavior in other seasons, and attempt to forage and survive in winter conditions (Shuter et al., 2012). Many juvenile fish species are particularly prone to overwinter mortality, with fish size and energetic condition influencing survival (Cunjak, '96; Hurst, 2007; Shuter et al., 2012). Fish in fluvial systems also have to deal with dynamic ice conditions (Brown et al., 2011). An added complication is the potential for external perturbations (stressors such as those induced by human activities) to further reduce the likelihood of survival during the winter. O'Connor et al. (2010) demonstrated increased overwinter mortality in largemouth bass (*Micropterus salmoides*) following exposure to an early season stressor (i.e., a carryover effect). For individuals that are able to survive winter conditions, these carryover effects can potentially resonate beyond a single winter, influencing other parts of their life-history, and ultimately organismal fitness and population-level processes (Calow and Forbes, '98).

Brown trout (*Salmo trutta*) are native to almost all of Europe, but have been introduced widely across the entire globe (MacCrimmon et al., '70; Jonsson and Jonsson, 2011). Populations in Denmark exhibit partial migration with a portion of the population in a system becoming resident and the remaining individuals migrating to the ocean (Alerstam et al., 2003). The decision to become a migrant or resident is driven by a variety of environmental and physiological variables including the availability of food, metabolic activity of the individual, and their condition (Thorpe et al., '92; Metcalfe, '98; Cucherousset et al., 2005; Boel et al., 2014), such that individuals with higher metabolic requirements and lower condition scores are more likely to undertake the migration to the ocean (Forseth et al., '99; Morinville and Rasmussen, 2003; Boel et al., 2014). The process of smoltification is inherently stressful, and therefore, the choice of life-history strategy represents a trade-off between increased predation during migration and increased growth and reproductive potential for trout that forage in the marine environment (Hutchings and Myers, '85; Klemetsen et al., 2003). For anadromous brown trout populations, smoltification is thought to commence in the late summer and early fall of the preceding year when local conditions drive a portion of the population to begin a physiological transformation in preparation for the marine environment (Metcalfe, '98).

Given the importance of the fall season for smoltification and the fact that it precedes winter, juvenile trout at this time are likely sensitive to external perturbations that may result in sub-lethal stressors. Such stressors can be caused by natural (e.g., predation attempts, food scarcity) or human (e.g., point source pollution, habitat alterations) induced disruptions to the natural balance (Underwood, '89; Wingfield, 2003; Helmuth, 2009; Baker et al., 2013). Previous work on the influence of stress on wild anadromous trout suggests that, while an artificial stressor applied immediately prior to outmigration does not appear to influence the timing of migration, migration success is depressed relative to a control (Midwood et al., 2014). Similarly, growth of stressed individuals that remained in the stream (residents) was depressed. Over a longer timescale, there is therefore potential for a stressor applied in one season to influence the subsequent smoltification process and also result in carryover effects during the winter. Consequently, our goal was to evaluate the influence of a fall stressor on the overwinter survival, growth, and success of migration of juvenile brown trout. The potential confounding interaction between initial state (e.g., condition) and a fall stressor as well as the impact of initial state on migration status were also explored. Although carryover effects have been well-studied in migratory birds in terms of how conditions of overwintering grounds influence subsequent post-migration reproduction (e.g., Marra et al., '98; Norris, 2005), there is comparatively less known about how a stressor may influence life-history trade-off decisions for species that must weigh the risks and benefits associated with migrating or becoming a resident. Indeed, there is a rich theoretical literature on how physiology (especially endocrinology and oxidative stress) mediates life-history trade-offs (see Zera and Harshman, 2001; Monaghan et al., 2009), yet experimental evaluations of such trade-offs in wild animals are lacking.

We use an experimental approach, comparing the survival, growth, and migration success between a control group and trout that received an artificial stressor (intracoelomic injection of cortisol). This experimental manipulation of GC levels via implantation of a GC-bearing vector is an increasingly common approach for the study of carryover effects in wild animals across a range of taxa (e.g., fish, Gamperl et al., '94; O'Connor et al., 2010;

Midwood et al., 2014; birds, Spée et al., 2011; Davies et al., 2013; reptiles, Meylan et al., 2002; Cote et al., 2006). There are also numerous laboratory-based studies that manipulate GCs in fish (reviewed in Gamperl et al., '94) and provide a strong foundation for conducting field-based studies. Based on previous work with brown trout and other fishes, we predict that growth, migration success, and survival will be lower for fish with manipulated GCs relative to the control. We also predict that the initial state or condition of individual trout will dictate their predisposition to migrate or become a resident such that GC-manipulated individuals (with potentially impaired condition) will be more likely to migrate.

METHODS

Study Site Description

Gudsø Stream is located in central-eastern Jutland, Denmark (Fig. 1). Including a series of sub-tributaries, the stream flows ~16 km before entering into the northwestern Baltic Sea at

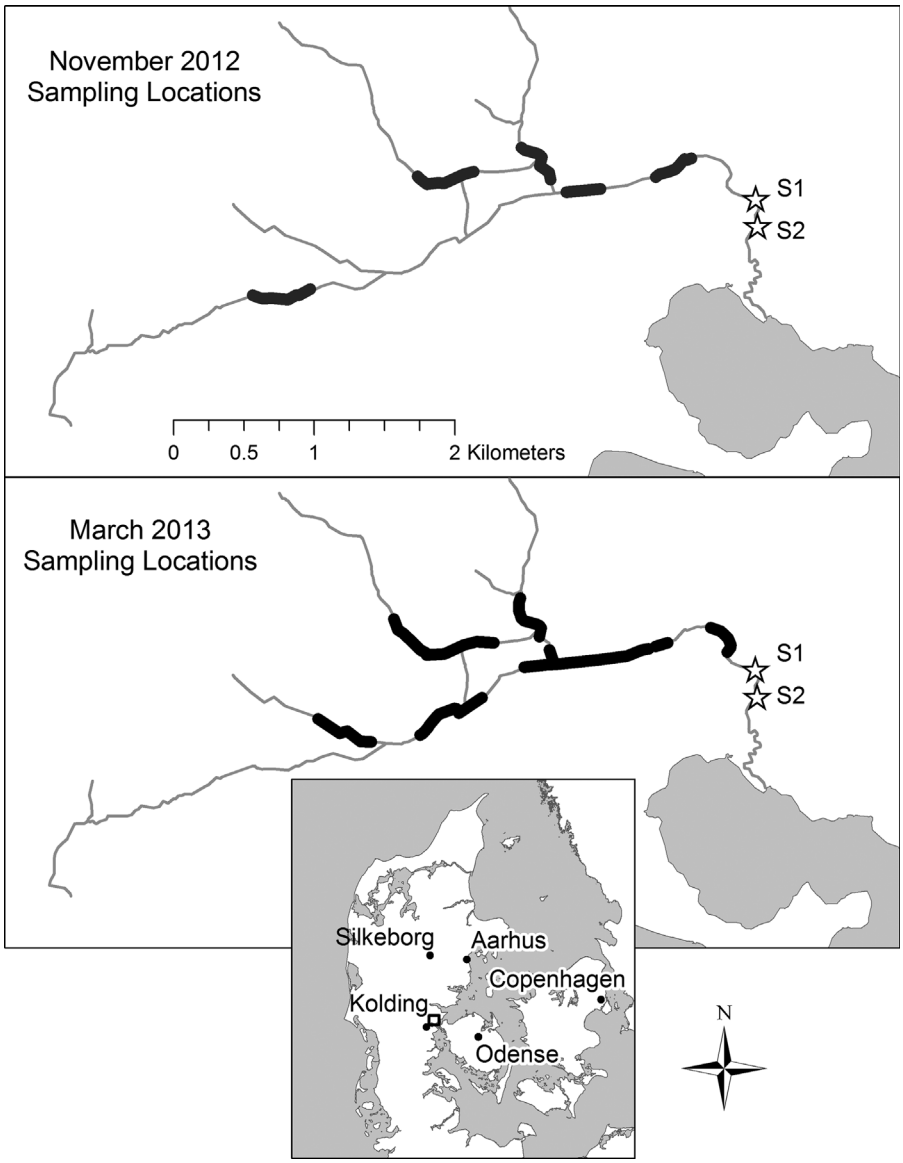

Figure 1. Location of Gudsø Stream and position of the two PIT antennas, S1 and S2. Sampling locations for initial capture (November 2012) as well as areas re-sampled in the spring of 2013 are shown as darker lines. Light gray areas represent water and the outflow location of Gudsø Stream into Kolding Fjord. Bottom inset shows locations of Gudsø Stream north-east of Kolding.

Kolding Fjord. The stream supports a population of semi-anadromous brown trout comprised of both resident and migratory individuals; however, in a previous study approximately two-thirds of the tagged fish migrated to the ocean, with more than half making this migration over a 4-day period in 2013 (Midwood et al., 2014). Two passive integrated transponder (PIT) reading stations established approximately 1 km from the outflow of the stream into the fjord continuously log the passage of PIT tagged fish. Station 1 (S1) is located approximately 150 m upstream of Station 2 (S2). Detection efficiency was estimated for S1 as 93.9% after Zydlewski et al. (2006); it could not be estimated for S2 due to the absence of downstream detections. A small millpond located between S1 and S2 may potentially serve as a sink if individuals cannot find the outflow; therefore, lower detection numbers are to be expected at S2. The millpond was not surveyed during the present study as water depth precluded access.

Capture, Tagging, and Treatment

From November 4th to 7th, 2012, brown trout were collected from five sections in Gudsø Stream using single-pass backpack electroshocking (Scubla ELT 60 II G, running at 300 V). Fish were immediately netted and placed into a container with regularly exchanged stream water. First, the total length (±1 mm) and wet mass (±0.1 g) of each fish were measured. A relative condition factor (K_R) was developed based on the relationship between log-transformed length and mass of the trout sampled in the current study (after Le Cren, '51). For each individual, K_R was then calculated using the following equation: $K_R = \log(\text{Mass})/(-2.03 + 3.03(\log(\text{Length}))$. Next, a 23 mm PIT tag (Texas Instruments, RI-TRP-RRHP, 134 kHz, 0.6 g mass in air, Plano, TX, USA) was inserted into the body cavity of each fish as an individual marker. A recent evaluation of this technique with Atlantic salmon (*Salmo salar*) suggested that retention of the tags is very high (97%) with no mortality and no impacts on growth relative to a control group (Larsen et al., 2013). Tagged fish were then assigned into either control or cortisol treatments using a stratified random approach to ensure roughly equal sample sizes per treatment. Immediately following PIT tag insertion, cortisol fish received an intracoelomic injection of a suspension of cocoa butter (100% pure cocoa butter, Now Foods, Bloomingdale, IL, USA) and hydrocortisone 21-hemisuccinate (Sigma–Aldrich, St. Louis, MO, USA, Product #H2882-1G) at a dosage of 100 mg kg^{-1} to raise circulating plasma cortisol levels to between 20 ng ml^{-1} and 40 ng ml^{-1} for 2–4 weeks (Pickering, '89). When brown trout are exposed to acute stressors (e.g., variation in water flow or handling stressor; Pickering et al., '82; Flodmark et al., 2002), plasma cortisol typically rises to between 100 ng ml^{-1} and 150 ng ml^{-1} but only stays elevated for 2–4 hr (Pickering et al., '82). Conversely, a chronic stressor (e.g., confinement or crowding) has prolonged elevation (~4 weeks) but lower magnitude (10–20 ng ml^{-1}; reviewed in Pickering, '89). Therefore, the magnitude of cortisol elevation targeted in this study was on the upper end of ecologically relevant values and the extended duration was consistent with the notion of a semi-chronic stress (Nagrodski et al., 2013). Unfortunately, sham treatments (receive injection of only cocoa butter) were not included in the study due to low numbers of captured brown trout in the desired size range (>120 mm total length). However, while there may be some minor negative impacts on growth, survival is not different than a control (Midwood et al., 2014). Fish from both treatments were allowed to recover for approximately 30 min in separate tanks of fresh stream water before being released back into the same section of the stream where they were captured. Animal care approval for this study falls under the Danish Animal Experiment Inspectorate (License Number: 2013-15-2934-00808).

To evaluate growth and survival of trout that remained in the stream over the winter (defined here as November 7th until February 28th), six areas were sampled between February 28th and March 19th, 2013 (Fig. 1). This time window was selected as it is before the peak migration of trout in Gudsø Stream that typically occurs in mid-April (Midwood et al., 2014). All captured trout were scanned to determine if they had been PIT tagged. Total length and mass of recaptured individuals were measured; the mass of the PIT tag (0.6 g) was subtracted from the mass of each recaptured individual. These numbers were used to calculate the percent change in length, mass, and K_R relative to initial capture. Recaptured individuals were released back into the stream. Data were downloaded from the PIT stations in June 2013, after the aforementioned peak spring smolt run. It should be acknowledged that absolute survival was not evaluated; rather detection, both at the stationary antenna and during the spring electrofishing, was used as a surrogate for survival.

Statistical Analysis

A Student's *t*-test was used to determine whether the initial mean length, mass, and K_R differed between the control and cortisol treatments. Based on their known activity, trout within each treatment were divided into three groups: down-stream migrants (detected passing S1 and/or S2; herein referred to as "migrants"), residents (recaptured instream during spring surveys, but not detected passing S1 or S2), and individuals with unknown fates (neither detected nor recaptured, possibly deceased, herein referred to as "unknown"). To determine whether initial state differed among trout that became migrant, resident, or unknown, an analysis of variance (ANOVA) was used to compare initial K_R among these groups. A post-hoc Tukey HSD was used to evaluate significant differences among groups.

To evaluate migration success, Fisher's Exact tests were conducted to compare the total proportion of trout in each treatment reaching S1 and S2 as well as the combined proportion moving past both stations in winter and spring. Similarly, a product-limit log-rank survival analysis was conducted to determine whether the number of detections at S1 differed between the control and cortisol treatments. A Fisher's Exact test was also

used to determine whether more individuals from the control or cortisol treatments were recaptured during spring surveys. By combining individuals from the migrant and resident groups, we were able to estimate overall survival for each treatment, which was compared using a Fisher's Exact test. In terms of shifts in migration strategy, the proportion of trout migrating in the cortisol and control treatments was calculated as the number of trout passing the stations divided by the total number of individuals that were assumed to survive (includes both residents and migrants).

To evaluate the potential interaction between initial state (K_R) and treatment on survival, a logistic regression was performed. The survival response was split into known to survive (includes both migrants and residents, assigned a value of 1) and unknown (assigned value of 0). Finally, a Student's *t*-test was used to compare growth for length, mass, and K_R, measured as the percent change from their initial size to recapture. Recapture data for both migrant and resident trout were pooled for these analyses due to low sample sizes when analyzed separately. Similarly, we did not include initial capture location in the analysis due both to low samples sizes and previous work suggesting there were no differences in migration timing or success (Midwood unpublished data). All analyses were completed in JMP 9.0 (SAS). Trout that were detected at S2 were included in the analysis due to the potential for novel fish detections at this station; however, survival between the two stations was not evaluated since we were unable to evaluate the effectiveness of S2 and it was possible that it had lower detection efficiency than S1. Since diminished detection efficiency at S2 would have equally affected both control and cortisol tagged individuals, a comparison between treatments of trout detected at S2 should still be valid.

Table 1. Summary of the number of brown trout tagged and the mean initial length, mass, and condition factor for each treatment with standard deviations.

	Treatment	
Metric	Control	Cortisol
Sample size	232	241
Length (cm)	14.1 ± 2.1	14.1 ± 1.7
Mass (g)	30.8 ± 20.5	29.9 ± 14.0
Relative condition factor (K_R)	1.00 ± 0.03	1.00 ± 0.03
% Reaching S1	20.3_A (47)	8.3_B (20)
% Reaching S2	14.2_A (33)	6.6_B (16)
% Migrants	20.3 (47)	8.3 (20)
% Residents	15.5 (36)	5.8 (14)
% Unknown	64.2 (149)	85.9 (207)
Spring recaps	44_A	16_B
% Overall survival	36.0_A (83)	14.1_B (34)

Migration success to S1 and S2 as well as the proportion of movements that occurred at these stations in the winter (November–February) and spring (March–May) are presented as a percentage and absolute number (in brackets). The percent and absolute number (in brackets) of trout from the treatments that were grouped as migrants, residents, or unknown are also shown. Finally, the number of individuals recaptured in the spring and the overall survival (including data from individuals that moved past S1 and S2 as well as individuals that were recaptured in spring 2013) are presented. Subscript letters indicate significant differences between treatments.

RESULTS

In total, 473 trout were captured, tagged, and treated, 232 control fish and 241 cortisol-manipulated fish (Table 1). There were no significant differences in terms of initial length (*t*-test, $t_{(1)} = -0.083$, $P = 0.934$), mass (*t*-test, $t_{(1)} = -0.549$, $P = 0.583$), or K_R (*t*-test, $t_{(1)} = 0.0002$, $P = 0.999$) between the two treatment groups. There were significant differences in initial K_R among the three trout groups (ANOVA; $F_{(2)} = 5.94$, $P = 0.003$), with K_R significantly lower in migrants relative to those with an unknown fate (mean $\pm$ SD $= 0.99 \pm 0.02$ and 1.00 ± 0.03, respectively, Tukey HSD; $P = 0.007$); residents had an intermediate K_R that was not distinct from the other groups (mean $= 0.99 \pm 0.02$). Given the small absolute difference among groups, the ecological relevance of this finding is explored in the discussion.

There appears to have been substantial overwinter mortality for both the control and cortisol-treated fish, with 64% and 86% (respectively) of trout unaccounted for either by detection at S1 or S2 or during surveys in the spring (Table 1). Despite overall high mortality, significantly fewer trout in the cortisol treatment were detected or recaptured relative to the control (Fisher's Exact Test, $\chi^2_{(1)} = 39.13$, $P < 0.0001$). Trout were observed passing S1 and S2 both during the winter (November 7th until February 28th) and in the spring (March 1st until May 30th); however, 80% of all cortisol trout that passed S1 did so in the winter, significantly more than the control (49%; Fisher's Exact Test, $\chi^2_{(1)} = 4.88$, $P = 0.027$). Similarly, based on the survival analysis, there was significantly lower survival of cortisol-treated trout at S1 than control trout ($\chi^2_{(1)} = 13.56$, $P = 0.0002$; Fig. 2). Regardless of timing, only a small percentage of the control treatment migrated successfully past S1 and S2 ($\leq$20% at both stations); however, this still represented significantly greater passage than the cortisol treatment (<10% at both stations; Fisher's Exact Test, S1, $\chi^2_{(1)} = 13.91$, $P = 0.0002$; S2, $\chi^2_{(1)} = 7.32$, $P = 0.0068$; Table 1). Similarly, based solely on resident trout, trout from the cortisol treatment were also significantly less likely to be recaptured during the spring surveys (Fisher's Exact Test, $\chi^2_{(1)} = 16.22$, $P < 0.0001$; Table 1) than control trout, suggesting again that overall there was lower survival among cortisol-treated fish. There was no clear shift in migration strategy (e.g., resident or migrant) for trout that were known to survive with relatively equal proportions of control (0.52) and cortisol-treated (0.55) trout passing the stations.

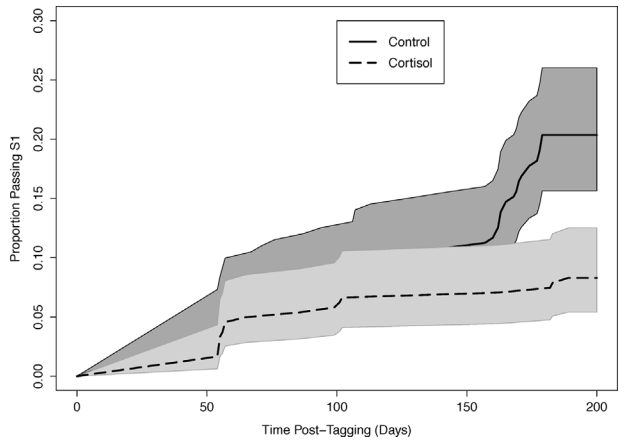

Figure 2. Visualization of the output from the product-limit log-rank survival analysis. Trout from the cortisol treatment had significantly lower survival to S1 than control trout. Shaded areas show the 95% confidence intervals.

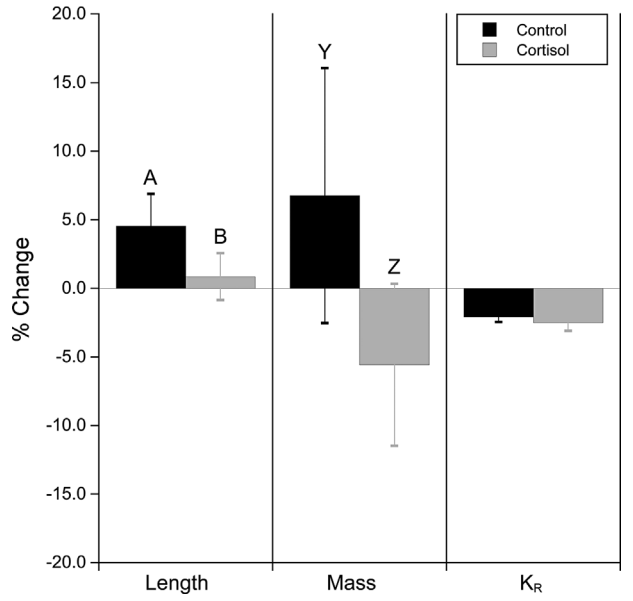

Figure 3. Percent change in length, mass, relative condition (K_R) for trout recaptured in the spring of 2013 prior to the main downstream migration. Letters indicate significant differences and the bars show the mean with standard deviation.

There was no interaction detected between K_R and treatment in determining the ultimate fate of a trout (Wald $\chi^2_{(1)} = 0.1$, $P = 0.793$). However, both individual terms were significant (K_R, Wald $\chi^2_{(1)} = 11.9$, $P = 0.001$; Treatment, Wald $\chi^2_{(1)} = 30.3$, $P < 0.0001$) with greater survival for trout in lower initial condition and trout in the control treatment.

Due to the low number of cortisol-treated trout recaptured that became migrants ($N = 3$), data from both migrant and resident groups were pooled to compare differences in growth between treatments. Over the 112 to 130 days from tagging to recapture, control trout grew significantly more both in terms of length ($t_{58} = -5.779$, $P < 0.0001$; Fig. 3) and mass ($t_{58} = -4.951$, $P < 0.0001$; Fig. 3) than cortisol-treated trout. In fact, rather than exhibiting growth, cortisol-treated trout on average lost 5.6% of their body mass compared to a 6.8% gain for trout in the control group (Fig. 3). There was no difference in the percent change in K_R between the two treatments ($t_{58} = -0.338$, $P = 0.563$; Fig. 3).

DISCUSSION

This study represents a simple experimental test of winter carryover effects in juvenile salmonids, with particular focus on how a fall stressor can have resulting impacts on survival, growth, and downstream migration success. Previous work has documented carryover effects in long-distance migrants (e.g., American redstarts, Norris et al., 2004; Black-tailed godwit, Gunnarsson et al., 2005; Light-bellied Brent goose, Inger et al., 2010; Sockeye salmon, Donaldson et al., 2010), which, in addition to exhibiting carryover effects in the winter (e.g., O'Connor et al., 2010), may be exposed to numerous and varied stressors during their migration. Our study is one of the first to integrate the concepts of carryover effects, winter ecology, and migration and evaluate their influence on the life history decision to migrate or remain resident. For cortisol-treated fish, the majority of the response metrics (e.g., growth, downstream migration success, and survival) were depressed relative to fish in a control group, suggesting that impairments to pre-winter condition in brown trout influence individual overwinter survival and migration success. However, we found no evidence to support the prediction that elevated GCs influenced the determination of a migratory or resident life-history strategy. Despite a strong theoretical basis for physiological status mediating life-history decisions (Zera and Harshman, 2001), our experimental approach failed to document such a relationship, at least in the context of elevated GCs, in a wild fish.

Overwinter Survival

While the ultimate fate of each individual cannot be known for certain, since the majority of trout were neither detected at the stations nor recaptured in the stream, we conclude that there is naturally high overwinter mortality in the Gudsø Stream system for juvenile brown trout. Previous work has found a high-degree of variability in natural overwinter mortality of brown trout, ranging from 16% to 65% (Elliot, '93; reviewed in Huusko et al., 2007) and our values for control trout with unknown fates are at the upper end of this range. Similar to O'Connor et al. (2010), we found that a pre-winter stressor (injection of cortisol) significantly impacted

survival with just over one third as many cortisol treated trout surviving relative to control. This increased mortality emphasizes the challenges faced by fish during the winter and shows how pre-winter condition may play a role in dictating their ability to survive the winter. We caution, however, that since we did not assess the timing of mortality in cortisol treated trout, it is possible that they experienced increased mortality shortly after treatment. A more detailed evaluation of post-treatment survival is therefore warranted. Regardless, the scale of winter mortality is of a magnitude that warrants attention of resource managers to ensure it is being appropriately incorporated into population models. For example, survival is much lower during winter than the initial marine mortality of smolts that is normally hypothesized to be a critical life stage (Thorstad et al., 2012; Aarestrup et al., 2014; del Villar-Guerra, 2014).

While there were significant differences in the initial condition of individuals in the migrant and unknown groups, the absolute difference in condition was only 0.01. As a result, it is likely that this difference is not ecologically relevant. Furthermore, this initial state was not found to interact with the stress treatment, suggesting that the treatment had an impact regardless of the initial state of the individual. Storage of lipids prior to the winter is an important part of the overwinter survival strategy of many animals (e.g., salmonids, Berg and Bremset, '98; reptiles, Derickson, '76; birds, Blem, '76). Prolonged activation of the glucocorticoid pathway and the resulting increase in metabolic activity can prevent the creation of these reserves or deplete established stores (Sheridan, '86; reviewed in Mommsen et al., '99). This loss of lipid reserves is evident in the cortisol trout that survived the winter given their lower mass and overall limited growth. Since low lipid reserves prior to winter leads to a decrease in survival (e.g., rainbow trout, Biro et al., 2004), it is probable that a decrease in lipid reserves in the cortisol treatment resulted in lower survival of these individuals. In addition, as discussed in Midwood et al. (2014), it is also possible there are differences in predation rates between the two treatments resulting from differences in behavior or energetic ability. However, to our knowledge, there is as yet no evidence for increased predation of stressed individuals. Finally, it is also conceivable that GC manipulation promoted foraging and thus energy accumulation through time, although that is not something that we were able to find support for in the literature. Because we were unable to sample fish repeatedly, it is not possible to identify the specific manner in which GC manipulations manifested, which limits our ability to elucidate how GCs, overwinter survival and migration success are linked.

Migration Timing and Strategy

For trout that were known to survive, roughly equal proportions of control and cortisol trout passed the stations. This suggests that once an individual made the decision to migrate downstream at the end of the summer or early fall, a subsequent stressor did not appear to alter their migratory or resident life history strategy. However, given low samples sizes in both treatments due to high overwinter mortality, this is not conclusive evidence of an absence of an effect of stress on the propensity to migrate and this hypothesis should be explored further.

Typically, migration to the ocean occurs during the spring; therefore, it was surprising to find trout moving downstream in the winter. Indeed, 80% of all migratory trout in the cortisol treatment and 49% of control trout moved downstream past the stations in the winter. An important caveat to note regarding this observation is that there is still approximately 1 km of stream habitat (albeit poor trout habitat with relatively deep and slow moving water and sandy sediments) downstream of the stations; therefore, it cannot be conclusively stated that the movements observed during the winter were made by ocean-bound migrants or that they actually entered saline waters during winter. These winter migrants may instead reflect a portion of the population that spends the winter in the section of Gudsø downstream of the stations. That none of these individuals was observed moving back upstream, as might be expected if they were using this solely as overwintering habitat, suggests that they (1) did not survive, (2) migrated to the ocean, or (3) became residents downstream. Positioning of detection antenna closer to the estuary, analysis of return data, electro-shocking downstream of the PIT antenna, or alternative tagging methods (e.g., radio or acoustic telemetry) could help resolve the ultimate destination of these types of individuals.

Overwinter Change in Mass

Growth depression following a stressor is a common response that has been observed in birds (e.g., Barn owls, Almasi et al., 2013; Abert's towhees, Davies et al., 2013), fishes (e.g., Largemouth bass, O'Connor et al., 2011), reptiles (e.g., Common lizard, Cote et al., 2006), and mammals (e.g., Belding's ground squirrel, Brooks and Mateo, 2013). This impairment is driven by increased metabolic rate and the resulting loss of energy reserves (Chan and Woo, '78; Morgan and Iwama, '96; O'Connor et al., 2011); indeed the cortisol-treated fish in this study lost over 5% of their mass over the winter while the control treatment gained over 6%. While these types of losses can be compensated for in a comparatively short time period (e.g., 1 month), there is potential for this compensation to have a carryover effect in the future life of these individuals resulting in lower survival in the ocean (Johnsson and Bohlin, 2006). Thus, even if a stressed individual survived the winter, their long-term survival may still be impaired. This type of long-term carryover effect can be challenging to document, but is critical to evaluating the complete impact of short-term stressors.

Previous work has also suggested that brown trout may experience growth depression over the winter in the absence of a stressor due to higher energetic costs associated with maintaining metabolic activity (Cunjak and Power, '87; Berg and Bremset, '98). Our results suggest that brown trout in Danish streams continue to grow throughout the winter in the absence of a stressor (as witnessed

by the increased mass in the control treatment). This discrepancy may be related to the severity of the winter wherein ice may form over streams in some regions. In Gudsø Stream, water temperatures typically remained well above 1°C (mean = 3.8 ± 2.0°C) in the winter (unpublished data not shown).

Caveats

An important caveat of the work presented here is that the ultimate fate of individuals not detected at the stations or recaptured in the stream cannot be known for certain. That being said, a comparison of the proportion of individuals in the two treatments detected passing the stations and recaptured in the streams likely represents a valid comparison since there is no reason to believe that the probability of detection in either of these two instances is dependent on treatment. Detection at PIT stations is more dependent on the orientation of the tag and the strength of the electromagnetic field (Burnett et al., 2013); therefore, the detection (or missed detection) rate would likely be similar for the two treatment groups. Capture of brown trout using electrofishing is dependent on size, but typically is quite efficient since they are actively drawn to the anode (efficiency range 52–90%; Büttiker, '92); this, in combination with a narrow stream (typically less than 2 m) and shallow water depths (<0.5 m), likely resulted in high capture efficiency in sampled reaches. Our recapture efforts in the spring of 2013 covered a larger area than the initial fall surveys (21.6% of Gudsø Stream vs. 10.7%, respectively) but, without sampling all areas of the stream (>16 km), we cannot conclusively determine the proportion of the population that survived the winter. However, efforts were likely sufficient to provide a comparison of cortisol versus control treatments since we sampled throughout the stream in areas that likely provide good habitat (e.g., overhanging cover, instream structure) and also resampled areas that were part of the initial fall surveys.

Finally, we must caution some of the interpretations of the growth results, especially as they relate to the cortisol treatment. Without a sham treatment, we cannot directly differentiate between the impact of exogenous cortisol manipulation and the cocoa butter vector. As previously mentioned, a sham treatment of cocoa butter did influence the growth, but not survival, of wild brown trout (Midwood et al., 2014). There was still, however, a trend toward lower growth rates for the cortisol treated individuals relative to the sham treatment. Therefore, in the current study, we cannot be certain of the portions of the change in growth that can be attributed to the cortisol treatment and the cocoa butter vector. Both the current study and Midwood et al. (2014) had low recapture rates (~5–10%) largely due to the timing of recapture efforts post-migration. By recapturing cortisol treated wild trout during their migration issues associated with low sample sizes could be overcome and this would help to partition the impacts of the cortisol treatment and the cocoa butter vector on growth.

CONCLUSION

Carryover effects have the potential to be the main drivers behind variability in individual behavior, reproduction, and ultimately, survival (Harrison et al., 2011). A more complete understanding of their potential influence on survival, growth, migration success, and selection of a life history strategy (e.g., to migrate or become resident) will help determine the drivers behind population dynamics. This study used a simple experimental approach to document the negative impacts to growth and survival of an overwinter carryover effect following a late fall stressor. Notwithstanding the aforementioned caveats, it is evident from this and other studies that winter is a challenging period for juveniles and that a preceding stressor can have a later impact on the survival of affected individuals. It is therefore important for environmental managers and researchers to recognize that impacts from a stressor applied during one season may not manifest themselves until later in the life history of an individual. We must be aware of the potential influence of experience on the current behavior, condition, and ultimately survival of each individual, which is relevant to conservation and management of wild animals in a changing world (Wingfield, 2008; O'Connor and Cooke, 2015).

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