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Stress and food deprivation: linking physiological state to migration success in a teleost fish

J.D. Midwood[1], M.H. Larsen[2], K. Aarestrup[2], and S.J. Cooke[1]

[1] Fish Ecology and Conservation Physiology Laboratory, Department of Biology and Institute of Environmental Science, Carleton University, 1125 Colonel By Drive, Ottawa, Ontario K1S 5B6, Canada

[2] National Institute of Aquatic Resources, Freshwater Fisheries, Technical University of Denmark, Vejlsøvej 39, 8600 Silkeborg, Denmark

*Corresponding Author:
Address: 453A Dundurn St. S., Hamilton, ON, L8P 4M1
Email: midwoodj@gmail.com

Summary Statement

Individual energetic state appears to dictate future life-history strategy whereas an artificial stressor impaired growth and reduced survival regardless of life history strategy.

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Abstract

Food deprivation (FD) is a naturally occurring stressor that is thought to influence the ultimate life-history strategy of individuals. Little is known about how FD interacts with other stressors to influence migration success. European populations of brown trout (*Salmo trutta*) exhibit partial migration, whereby a portion of the population smoltifies and migrates to the ocean, and the rest remain in their natal stream. This distinct, natural dichotomy of life-history strategies provides an excellent opportunity to explore the roles of energetic state (as affected by FD) and activation of the glucocorticoid stress response in determining life-history strategy and survival of a migratory species. Using an experimental approach, the relative influences of short-term FD and experimental cortisol elevation (i.e., intra-coelomic injection of cortisol suspended in cocoa butter) on migratory status, survival, and growth of juvenile brown trout relative to a control were evaluated. Fewer fish migrated in both the FD and cortisol treatments; however, migration of cortisol and control treatments occurred at the same time while the FD treatment was delayed for approximately one week. A significantly greater proportion of trout in the FD treatment remained in their natal stream, but unlike the cortisol treatment, there were no long-term negative effects of FD on growth, relative to the control. Overall survival rates were comparable between the FD and control treatments, but significantly lower for the cortisol treatment. Food availability and individual energetic state appear to dictate the future life-history strategy (migrate or remain resident) of juvenile salmonids while experimental elevation of the stress hormone cortisol caused impaired growth and reduced survival of both resident and migratory individuals.

Key Words: Glucocorticoid, stress, starvation, passive integrated transponder tags, freshwater, brown trout

Introduction

For decades, researchers have conducted laboratory studies on the effects of different stressors on the physiology, condition, behaviour and survival of various animals. Such research has formed the basis for major research areas such as comparative physiology (Mangum and Hochachka, 1998) and environmental physiology (Willmer et al., 2009) and helped to shape paradigms related to how organisms respond to different stressors. Although this foundational work is critical, animals in the wild may perceive and respond to stressors very differently than they do in captivity. This has led to the genesis of ecological physiology (Feder and Block, 1991) and broad calls for reinvigorating comparative physiology through field experimentation (e.g., Mangum and Hochachka, 1998; Somero, 2000). Fundamental to ecological physiology is the need to include ecologically-relevant endpoints such as survival and reproduction (Pough 1989; Gilmour et al., 2005) in an attempt to understand the ecological implications of physiological variation (Spicer and Gaston, 1999) and responses to stressors (Pankhurst 2011; Boonstra 2013a). Research in the field is both complex and challenging (Costa and Sinervo, 2004) yet at the same time, provides the ecological relevance needed to understand how physiological state (e.g., stress) may cascade from individuals to explain population-level and evolutionary processes (Calow and Forbes, 1998; Ricklefs and Wikelski, 2002).

The neuroendocrine glucocorticoid (GC) stress response in vertebrates (see Sapolsky et al., 2000) represents an example of a system and response that was long-studied in the laboratory and has only recently been explored in field settings. Through analysis of tissue samples intended to characterize baseline or stress-induced GC levels (see Dantzer et al., 2014) to various GC manipulation studies (reviewed in Sopinka et al., 2015; Crossin et al., 2016), researchers have started to elucidate what is now termed "the ecology of stress" (Boonstra, 2013a). Yet, many challenges remain as studies often use GC manipulations to simulate semi-chronic or chronic stressors that may not be ecologically relevant (Crossin et al., 2016; Sopinka et al., 2015). Natural experiments, where natural processes are re-created directly in the field, avoid this type of issue (i.e., predation, Sheriff et al., 2011; thermal stress, Quigley and Hinch, 2006; flow reduction, Krimmer et al., 2011), but can be challenging to implement on a large-scale in the field while standardizing stress exposure for each individual. While artificial manipulations are often intended to mimic natural phenomena such as storms or other extreme weather events (Romero et al., 2000; Pankhurst, 2011; Wingfield, 2013), studies that characterize the stress of predation, competition, or

Journal of Experimental Biology • Advance article

starvation in the wild are relatively uncommon (Boonstra, 2013b). Relatedly, there is a need to move beyond being simply focused on physiological endpoints to include those that incorporate behaviour and more closely approximate fitness-related factors. This will result in a more complete understanding of the ecology of stress (Boonstra, 2013a).

Food deprivation is a ubiquitous natural phenomenon that occurs when a postabsorptive animal, otherwise willing or able to eat, is unable to do so as a result of some extrinsic limitation on food resources (McCue, 2010). Periods of limited food intake (for various time periods ranging from hours to months) due to spatial and temporal heterogeneity of food resources are common in wild animals, limiting population size and biological productivity (McNamara and Houston, 1987). When animals are exposed to periods of reduced food intake, simple bioenergetics principles related to the balance between consumption and expenditure would suggest that energetic conditions would decline with limited energy that could be devoted to growth or reproduction (Kleiber, 1961; McCue, 2010). Indeed, a continuing supply of energy is necessary for an animal to live given that even the most basic physiological processes have an energy cost (Porter and Gates, 1969).

When exposed to food deprivation, particularly lengthy bouts, declines in nutritional condition (which can extend beyond macro-nutrients to include vitamins and minerals; Halver and Hardy, 2002) may lead to impairments in immune function (e.g., Carusso et al., 2011), induce oxidative stress (Pascual et al., 2003), and alter general health (Wang et al., 2006) and even behaviour (e.g., malaise). In extreme cases, food deprivation can lead to mortality – either directly or indirectly, close to when the food deprivation period occurs (i.e., when there is insufficient energy to maintain homeostasis; McCue, 2010) or at a future time (i.e., a carry-over effect; Harrison et al., 2011; O'Connor et al., 2014). However, given that food limitations are common in the wild, it is not surprising that fish have a variety of adaptive biochemical, physiological and behavioural responses to maximize survival (Wang et al., 2006; McCue, 2010).

In teleost fishes, activation of the hypothalamic-pituitary-interrenal (HPI) axis and production of cortisol mobilizes energy that may allow the individual to survive a stressor (reviewed in Mommsen et al. 1999). This activation typically occurs for a short duration and therefore activation of the HPI axis is more often acute than chronic. Continuous activation of the HPI axis results in continued mobilization of energy, reducing growth, disrupting immune function, and preventing the creation of lipid reserves (Espelid et al. 1996; Gregory and Wood 1999; Mommsen et al. 1999; Crespi et al., 2013). These negative consequences are

similar to issues associated with extended food deprivation; however, through activation of the HPI axis these will occur simultaneously rather than as a result of food deprivation.

Using wild juvenile brown trout (*Salmo trutta*; Linnaeus, 1758) as a vertebrate teleost model, we sought to study the ecology of stress in a natural stream system. Specifically, we tested the hypothesis that a natural stressor influences growth rate, survival and life history strategy of juvenile brown trout. To mimic a natural stressor, we exposed fish to a 14-day food deprivation protocol while holding fish in food-limited enclosures in the stream. Food deprivation is particularly relevant to juvenile salmonids, such as brown trout, since low energy stores are associated with poor growth, survival, and their ultimate life-history strategy (Forseth et al., 1999). Further, we tested the hypothesis that exogenous cortisol manipulation (using corticosterone embedded in a cocoa butter carrier – see Gamperl et al., 1994) has a similar influence as the more "natural" food deprivation stressor.

Brown trout were chosen as a model for several reasons. From a logistical perspective, the juveniles reside in small streams, which enables fish to be collected via electrofishing and makes it easier to track individual fish (for survival and behaviour – and if recaptured – for growth and condition) using small passive integrated transponder (PIT) tags (Gibbons and Andrews, 2004). Moreover, the brown trout population in the present study exhibit an interesting life-history strategy in that they exhibit partial migration (Jonsson and Jonsson, 1993; Alerstam et al., 2003), wherein some juveniles smoltify and move downstream to the ocean to feed while others remain in the stream forming resident populations of typically smaller fish.

Materials and Methods

Study Site

Gudsø Stream, located in central-eastern Jutland, Denmark, supports a wild population of partially anadromous brown trout (Fig. 1). This stream, and its connecting tributaries, runs for over 16 km before entering the western Baltic Sea at Kolding Fjord. In general, the stream is shallow (<1.0 m) and less than 2.0 m in width. Approximately 1 km upstream of this connection, two PIT reading stations continuously log the passage of tagged fish. Station 1 (S1) consists of two antenna spaced 10 m apart and is situated approximately 150 m upstream of station 2 (S2), which also consists of two antenna. This paired configuration allows for the determination of the direction of movement of tagged trout. Detection efficiency at S1 was estimated as 98.5%; calculated as the percentage of trout that were known to have passed S1 (i.e., detected at S2) that were actually detected at S1 (after Zydlewski et al., 2006). In the short distance between S1 and S2 the stream flows through a millpond, with a small fish ladder at its outflow just upstream of S2. This millpond has previously been identified as a bottleneck that limits downstream migration for brown trout in the system (Midwood et al., 2014; Midwood et al., 2015); passage at S2 is therefore expected to be naturally lower than S1.

Capture & Treatment

On 28 February and 1 March 2013, four sections of Gudsø Stream were sampled using single-pass backpack electroshocking (Scubla ELT 60 II G, running at 300 volts). In each section, between 46-53 brown trout greater than 12 cm in total length were collected for a total of 202 individuals. The total length (cm) and wet mass (g) of each individual was recorded and a uniquely coded 23 mm PIT tag (Texas Instruments, RI-TRP-RRHP, 134kHz, 0.6 g mass in air, Plano, Texas, USA) was inserted into their body cavity following methods outlined in Midwood et al., (2014). A similar approach in the con-generic Atlantic salmon (*Salmo salar*) was found to have high rates of tag retention and survival (Larsen et al. 2013). Trout were then placed into one of four 100-L barrels with approximately 50 individuals in each, which were subsequently secured in the stream near to their location of capture (Fig. 1). These barrels had 1.0 cm holes drilled across their surface to allow stream water to flow through, but prevent the trout from escaping. Limited shelter was available within the barrels in the form of larges stones used to weigh the barrel down in the stream. Trout were kept in these barrels for 14 days to simulate a two-week food deprivation period. Prior to their release on 14 and 15 of March, 2013, the individual total length and wet mass (minus 0.6 g to

account for the PIT tag) of trout in this "food deprivation" treatment (herein FD) were again assessed to determine whether there were changes as a result of the holding period.

Also on 14 and 15 March, 2013, an additional 421 trout were collected from 5 sections of Gudsø Stream, which overlapped the areas where trout in the FD treatment were captured (Fig. 1). The total length and wet mass were measured for all of these additional trout and they were PIT tagged in the same manner as the FD treatment. After tagging, these trout were assigned to one of two groups using a stratified random approach to ensure approximately equal sample sizes and size distributions. The first group (210 in total) was assigned to the control treatment (herein control) and was released following their recovery. The remaining 211 trout were assigned to a cortisol treatment (herein cortisol), where each individual received an intra-coelomic injection of a suspension of cocoa butter (100% pure cocoa butter, Now Foods, Bloomingdale, IL) and hydrocortisone 21-hemisuccinate (Sigma-Aldrich, Product #H2882-1G) at a dosage of 100 mg kg^{-1}. A recent validation study carried out under natural conditions found that this treatment raised circulating plasma cortisol levels in brown trout over 200 ng ml^{-1} (Birnie-Gauvin et al. In Submission), which is above documented levels for an acute handling stressor (130 ng ml^{-1}; Pickering et al. 1982) and considerably more than what has been previously reported in a similar laboratory-based validation study (20-40 ng ml^{-1}; Pickering 1989). These elevated levels persisted for at least three days, but had returned to baseline conditions (equal to a control and sham treatment) after six days. Consequently, this treatment is consistent with a semi-chronic stressor (i.e., longer than acute but not particularly prolonged such that it would be chronic). Sham treatments were not included in the present study, but previous work on brown trout has suggested that relative to a control, treatment with cocoa butter alone reduced growth rates (length), but did not affect survival (Midwood et al. 2014). Circulating plasma cortisol levels in sham treated brown trout were also not found to differ significantly from a control (Birnie-Gauvin et al. In Submission). Animal care approval for this study falls under the Danish Animal Experiment Inspectorate (License Number: 2013-15-2934-00808).

On 18 and 19 June 2013 five sections of the stream were re-sampled using single-pass backpack electrofishing to capture trout that did not migrate and instead had become resident in the stream (Fig. 1). Previous estimates of brown trout capture efficiency with this technique range between 52-90% (Buttiker 1992) and are typically higher in narrow shallow systems like Gudsø Stream since brown trout are actively drawn to the anode. All trout were scanned (Agrident, APR350) to determine their PIT tag number and their length and wet mass were measured. For the recaptured trout, the instantaneous growth rate for both length

(G_L) and mass (G_M) were calculated according to equation 1 (after Schreck and Moyle, 1990).

$$G = (\log_e Y_2 - \log_e Y_1)/(t_2 - t_1) \text{ eq. 1}$$

Where Y_1 is the length or mass at the time of tagging (t_1) and Y_2 is their length or mass at the time of recapture (t_2). The length and mass for trout at the time of their release was used for Y_1. Therefore, the length and mass of FD trout after the two-week holding period were used for Y_1. A relative condition factor (K_R; after Le Cren, 1951) was developed for the sample population based on the relationship between the log-transformed length and mass. The K_R for each individual was then calculated based on equation 2.

$$K_R = \log(\text{Wet Mass})/(-1.84 + 2.81(\log(\text{Length})) \text{ eq. 2}$$

Statistical Analysis

A paired t-test was used to determine the extent of the changes in mass and K_R for trout in the FD treatment following the two-week holding period. Product-limit log-rank survival analyses were conducted to determine whether the number of detections at S1 and S2 differed among the three treatments. Logistic regression was used to compare the relative proportions of trout in each treatment that were recaptured in the stream or were known to survive (either recaptured or detected passing S1 or S2).

Analysis of variance (ANOVA) was used to compare G_L and G_M among treatment groups. Similarly, ANOVA was also used to compare the mean number of days it took trout in each treatment group to pass S1. When significant, a post-hoc Tukey HSD was conducted to determine which treatments differed. All analyses were completed in JMP 9.0 (SAS Institute, Cary, NC, USA) with alpha evaluated at $p=0.05$.

Results

In total, 627 trout were captured, tagged, and treated with similar initial length, wet mass, and K_R among treatments (Table 1). There was a significant decline in mass for trout in the FD treatment following the two-week holding period ($t_{(201)}= -11.54$, $p<0.0001$) with an average of 1.02±1.26 g of mass lost (3.8±2.8% of their initial biomass, ranging from a maximum loss of 4.2 g [-13.8%] to a net gain of 1.2 g [+4.9%]). There was also a significant concomitant decline in K_R following the holding period ($t_{(201)}= -237.23$, $p<0.0001$, mean diff = -0.433±0.026).

For all treatments, less than half of all tagged fish were detected passing S1 and S2. The proportion passing the stations was lower for both the FD and cortisol treatments relative

to the control (Table 1). This was confirmed with the survival analysis, with significantly lower passage of both FD and cortisol treated trout relative to the control at both S1 and S2 (Table 2). There were no differences, however, in survival to either station between the FD and cortisol treatments. While it is clear that there was reduced passage at both stations for the FD and cortisol treatments (Fig. 2a and 2b), passage of the FD treatment at S1 also took significantly longer (ANOVA, $F_{(2)}=3.88$, $p=0.022$; Table 1) than the cortisol treatment (Tukey HSD, $p=0.028$). Although marginally non-significant (Tukey HSD, $p=0.057$), there was a trend towards fish in the FD treatment taking longer than the control treatment. Indeed, passage by the FD treatment took between 6 and 7 days longer, than the control and cortisol treatments, respectively (Table 1).

A total of 80 trout were recaptured during the June surveys. There was a significantly greater proportion of trout from the FD treatment (0.20) recaptured during these surveys relative to the control (0.10) and cortisol treatments (0.08; $\chi^2_{(2)}=13.989$, $p=0.0009$; Table 1; Fig. 3). In contrast, there was a significantly smaller proportion of trout from the cortisol treatment that were known to survive (i.e., recaptured or detected at S1 or S2) and no differences between the control and FD treatments ($\chi^2_{(2)}=6.675$, $p=0.0355$; Fig. 3). There were also significant differences among treatments for both G_L and G_M (ANOVA, $F_{(2)}=1.6094$, $p<0.0001$ and $F_{(2)}=16.1997$, $p<0.0001$, respectively). For both instantaneous growth rates the cortisol treatment was significantly lower relative to both the control and FD treatments (Tukey HSD, $p<0.001$). There were no differences, however, between the control and FD treatments for either G_L or G_M (Tukey HSD, $p>0.8$).

Discussion

For fish, and indeed many invertebrates, food deprivation represents a common natural stressor associated with spatio-temporal variation in the abundance of appropriate food items. In the present study we exposed fish to a 14 day period of food restriction, which given the cool water temperatures, would presumably result in modest food deprivation and declines in energetic condition (e.g, Byström et al., 2006). Fish were held in the river in barrels with holes such that some natural forage would occasionally pass through the barrels, although much less than would be available to them if they were at liberty and had access to smaller-bodied fish, terrestrial invertebrates and benthic invertebrates. On average fish lost 3.8% of their initial mass although a few individuals gained a small amount of mass (up to 4.9%) or maintained their initial mass, while several fish exhibited extreme loss of mass (up

to 13.8%). Overall, there was a general decline in body condition and negative growth. The fact that we had variable responses to food limitation would not be unexpected given individual differences in behaviour, physiology and genotype (Koolhass et al., 1999; Adriaenssesn and Johnsson, 2011; Adriaenssens and Johnsson, 2013). The food limitation we imposed on the fish occurred during a life-history period where food is exceedingly important. Juvenile brown trout feed extensively while in streams to prepare for migration, therefore, even small reductions in food intake during early life stages of salmonids can be deleterious (Jonsson and Jonsson, 1998). Conversely, increases in food availability (e.g., through supplemental feeding) can improve survival and stream carrying capacity (Mason, 1976). When exposed to transient periods of food deprivation, compensatory growth is possible (Nicieza and Metcalfe, 1997), but negatively influences survival in the future (Johnson and Bohlin, 2006).

Unfortunately, we cannot completely discount that trout held as part of the FD treatment were not also stressed due to their holding in tanks in somewhat crowded conditions. Pickering and Stewart (1984) explored the effects of crowding on cortisol and growth in brown trout and found elevated cortisol levels in brown trout kept in crowded (~1 fish per L) but not in non-crowded (~0.1 fish per L) tanks. Growth was also suppressed in the more crowded tank, but this was attributed more to competition for food than the activation of the HPI axis since the growth impairments continued for the duration of the study, despite cortisol levels returning to baseline conditions within 39 days (study lasted for 110 days; Pickering and Stewart, 1984). In the present study, densities were intermediate between these two treatments (~0.5 fish per L), therefore while the FD trout likely experienced an increase in circulating cortisol, it was well below what the cortisol treatment experienced. Consequently, as in Pickering and Stewart (1984), we are confident that the FD treatment rather than a moderate crowding stressor caused the declines in mass.

In this study we also manipulated cortisol titers of fish experimentally using exogenous cortisol implants. We did so to test whether the semi-chronic cortisol manipulation had a similar influence as the more "natural" food deprivation stressor. In terms of growth, brown trout in the cortisol treatment had significantly lower instantaneous growth rates for both length and mass relative to the control and FD treatments, which is consistent with previous exogenous manipulations of cortisol in this species (Midwood et al., 2014, 2015) and others teleost fishes (e.g., rainbow trout, *Oncorhynchus mykiss*, Gregory and Wood 1999). In a similar manner as the FD treatment, reduced food intake likely contributed to a reduction in growth in the cortisol treatment; however, instead of having restricted access to

food, increased plasma cortisol levels can suppress a fish's appetite (Anderson et al., 1991; Gregory and Wood 1999). Furthermore, even if the cortisol treated fish consumed food, the additional consequences of activation of the HPI axis, including increased metabolic rates and a reduction in the efficiency of food absorption, would further impair growth and development as well as survival (Barton et al. 1987; Metcalfe et al. 1995; Gregory and Wood 1999). In contrast, when the FD treated fish were released back into the stream, they were free to consume *ad libitum* allowing for compensatory growth. An important caveat, however, to this conclusion is that unlike previous work (e.g., Gregory and Wood 1999), a sham treatment was not included in the present study. We therefore cannot conclusively state that the observed reduction in growth rate in the cortisol treatment was solely caused by increased circulating cortisol. Indeed, the vector itself (cocoa butter) may have partially affected growth, as has been documented in previous studies (Hoogenboom et al. 2011; Midwood et al. 2014). Hoogenbooom et al. (2011) suggest that a vector may trigger an immune response that increases the basal metabolic rate and consequently reduces growth. While we cannot completely discount this possibility, the exogenous cortisol manipulation used in the present study has been shown to elevate plasma cortisol levels above that of a sham treatment (Birnie-Gauvin et al. In Submission). As a result, while cortisol treated fish were likely affected by the well-documented growth impairments associated with activation of the HPI axis, their growth may have been further impaired due to an elevated immune response. Further research, however, is warranted to assess the independent affects of the vector and exogenous cortisol manipulation on growth.

Food deprivation generally leads to elevations in GC levels in salmonid fishes (Barton et al., 1988; Barton and Iwama, 1991; Barton, 2002). Moreover, baseline cortisol levels tend to increase close to smoltification with a concomitant increase in stress responsiveness during that period (Barton et al., 1985). Relative to control fish, individuals that were food deprived or exposed to cortisol exhibited altered migratory behaviour. The survival analysis revealed significantly lower passage of both FD and cortisol treated trout relative to the control at both downstream antennas. Moreover, for the FD treatment there was a clear delay (6-7 days) in the timing of their downstream passage. Since brown trout tend to migrate with high spring flows (Bohlin et al., 1993; Aarestrup et al., 2002) this delay for FD fish may mean they miss these flood events. Indeed, around day 40 of the study there was an increase in the number of control and cortisol fish that migrated, but no similar "spike" in the number of FD treatment fish that did so (Fig. 2). It is possible that the fish that were starved were still attempting to compensate for the period of food deprivation such that they would have the nutritional

resources necessary to smoltify, migrate and make the transition to life in marine waters. Smoltification is energetically costly (Folmar and Dickhoff, 1980), with lipid metabolism playing a number of critical roles (Sheridan, 1989).

Brown trout exhibit partial migration such that a component of the population becomes resident. In this study we observed that significantly more fish in the FD treatment became resident. Interestingly, an equal number of FD and control trout were known to survive (based on recapture and PIT antennas). In contrast, there were fewer cortisol treated trout in both the migrant and resident groups. These findings suggest that the FD group did not have reduced mortality, but rather their propensity to migrate was reduced. The idea that food deprivation could influence life-history strategy is not surprising given that life-history is closely linked to energetics and the endocrine system (Ricklefs and Wikelski, 2002). There is a reasonably large body of literature on the physiological and energetic correlates of partial migration. For example, fast-growing individuals will be constrained by food limitations in a habitat more rapidly than slow-growing individuals and therefore exhibit increased propensity to migrate in search of additional foraging opportunities (Jonsson and Jonsson, 1993; Chapman et al., 2011; Boel et al., 2014). Furthermore, since migration is associated with increased energy expenditure, individual energetic state may balance the migratory decision (Chapman et al., 2011).

Overall, we found that a natural stressor (FD) and a more experimental stressor (exogenous cortisol manipulation) resulted in alterations in migration behaviour and/or survival relative to control fish. However, there were notable differences in growth rates for cortisol treated fish as well as in the life-history decision to either migrate or become resident for FD fish relative to controls and cortisol-treated fish. The lack of concordance in rates of partial migration between FD and cortisol-treated fish is itself interesting since both treatments reduce energy and nutritional states and therefore should similarly influence the propensity of an individual to migrate (Chapman et al., 2011). Despite this apparent discrepancy, our previous work has found no difference in migration timing (Midwood et al. 2014) nor in migration propensity (Midwood et al. 2015) for cortisol treated fish relative to a control. It is evident, therefore, that the additional effects of higher plasma cortisol levels that have been previously noted (e.g., increased metabolic rate, reduced gut absorption, impaired immune function, etc.) increased mortality regardless of an individual's migration strategy, but failed to reduce their propensity to migrate. In contrast, food deprivation has been found to impede swimming performance in some fish species (i.e., grass carp, *Ctenopharynodon idellus*; Cai et al. 2014), while the stress response does not (Gregory and Wood 1999). This

may be a possible explanation for the apparent differences in rates of partial migration, therefore a focused evaluation of the swimming performance and energetics of individuals from these treatments is warranted.

There is increasing interest in experimentally manipulating GCs in wild animals (Sopinka et al., 2015; Crossin et al., 2016) to simulate different natural and anthropogenic stressors. To our knowledge this is one of the first studies to directly compare and contrast the consequences of a natural challenge (i.e., starvation via food deprivation) with exogenous cortisol manipulation via implants. Although the consequences of those manipulations were not consistent with respect to partial migration, they did result in similar levels of mortality. Given the potential to use GC manipulations to understand how animals respond to natural and anthropogenic stressors (Sopinka et al., 2015), including novel ones, and explore difficult-to-study phenomena like carry-over effects (O'Connor et al., 2014), we submit that more studies of this nature are warranted. We also encourage future studies that simultaneously manipulate food intake and cortisol (*sensu* Small et al., 2006) on fish with different levels of initial energy density and nutritional status to try to understand the relative roles of different mechanisms on the responses of wild fish to stress.

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Competing Interests

No competing interests declared

Author Contributions

JDM, MHL, KA, and SJC all helped to design the study. JDM and MHL completed the necessary fieldwork and compiled the data. JDM completed the statistical analyses and JDM and SJC wrote the initial draft of the manuscript. All authors reviewed and revised the manuscript prior to submission.

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Figures

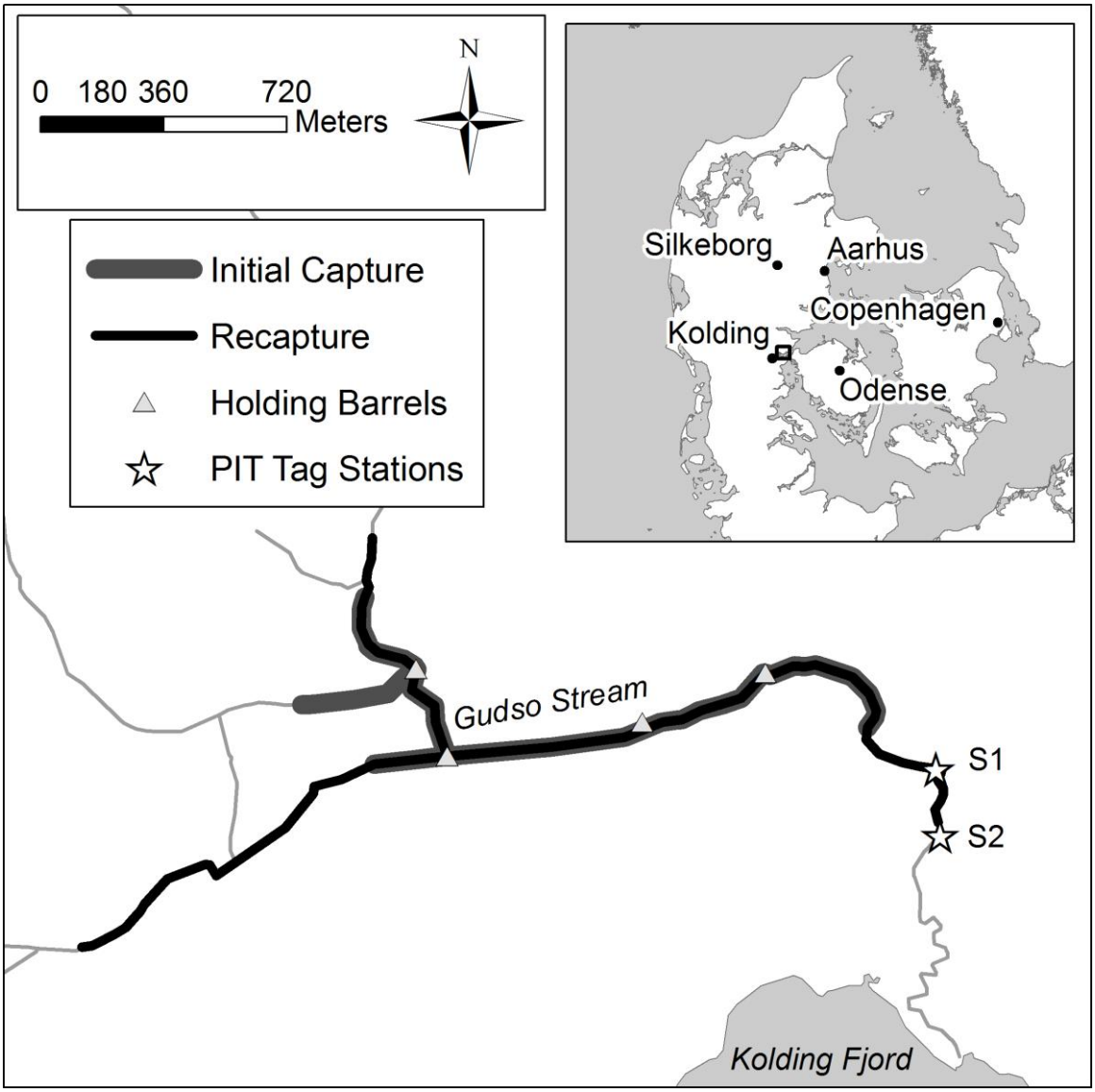

Figure 1 – Location of Gudsø Stream, northeast of Kolding in Jutland, Denmark. The portions of the stream where brown trout were initially captured in Spring 2013 and where they were recaptured in June 2013 are shown. The four holding locations for the food deprivation containers and the location of the PIT reading stations are also shown.

Journal of Experimental Biology • Advance article

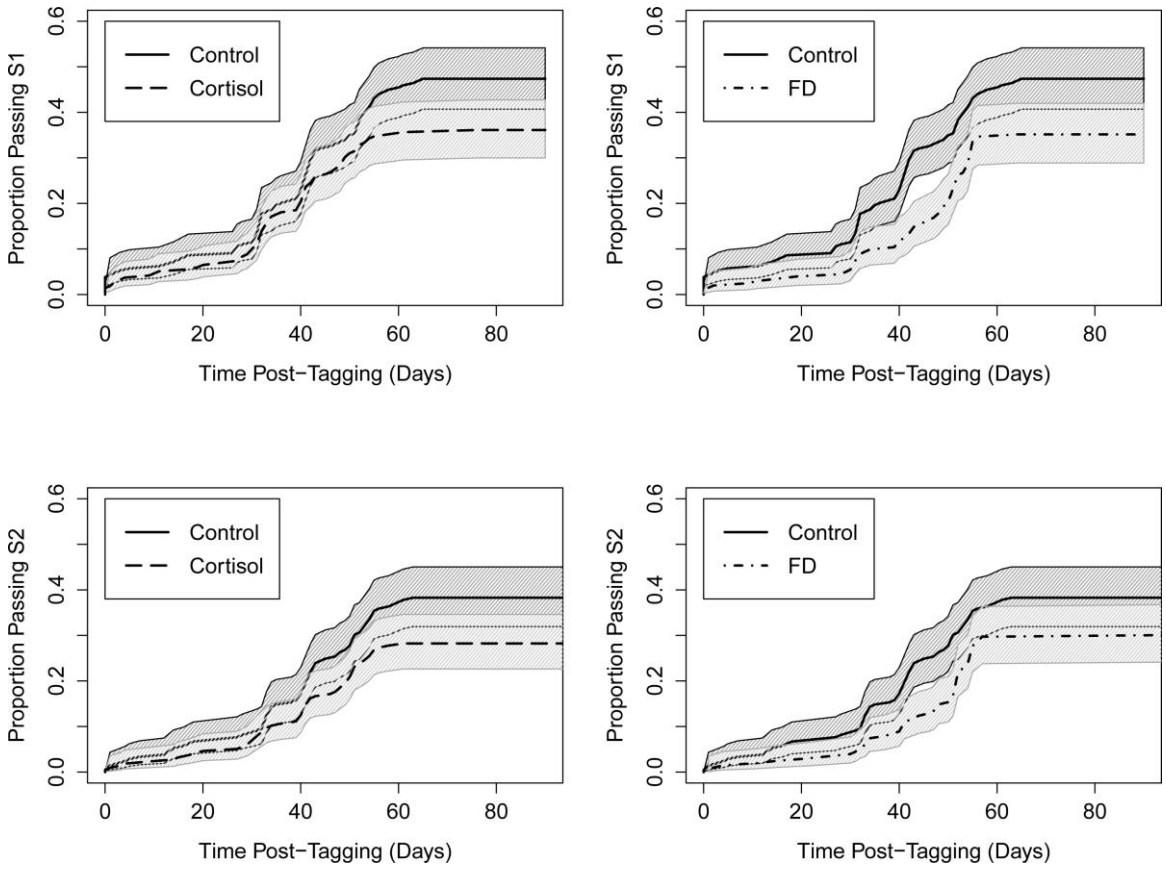

Figure 2 – Visualization of the output from the product-limit log-rank survival analyses. The top two figures are for brown trout passage at S1 (Control N=99, Cortisol N=78, and Food Deprivation N=71) and the bottom two panels are for passage at S2 (Control N=80, Cortisol N=61, and Food Deprivation N=61). The shaded areas show the 95% confidence intervals for each treatment.

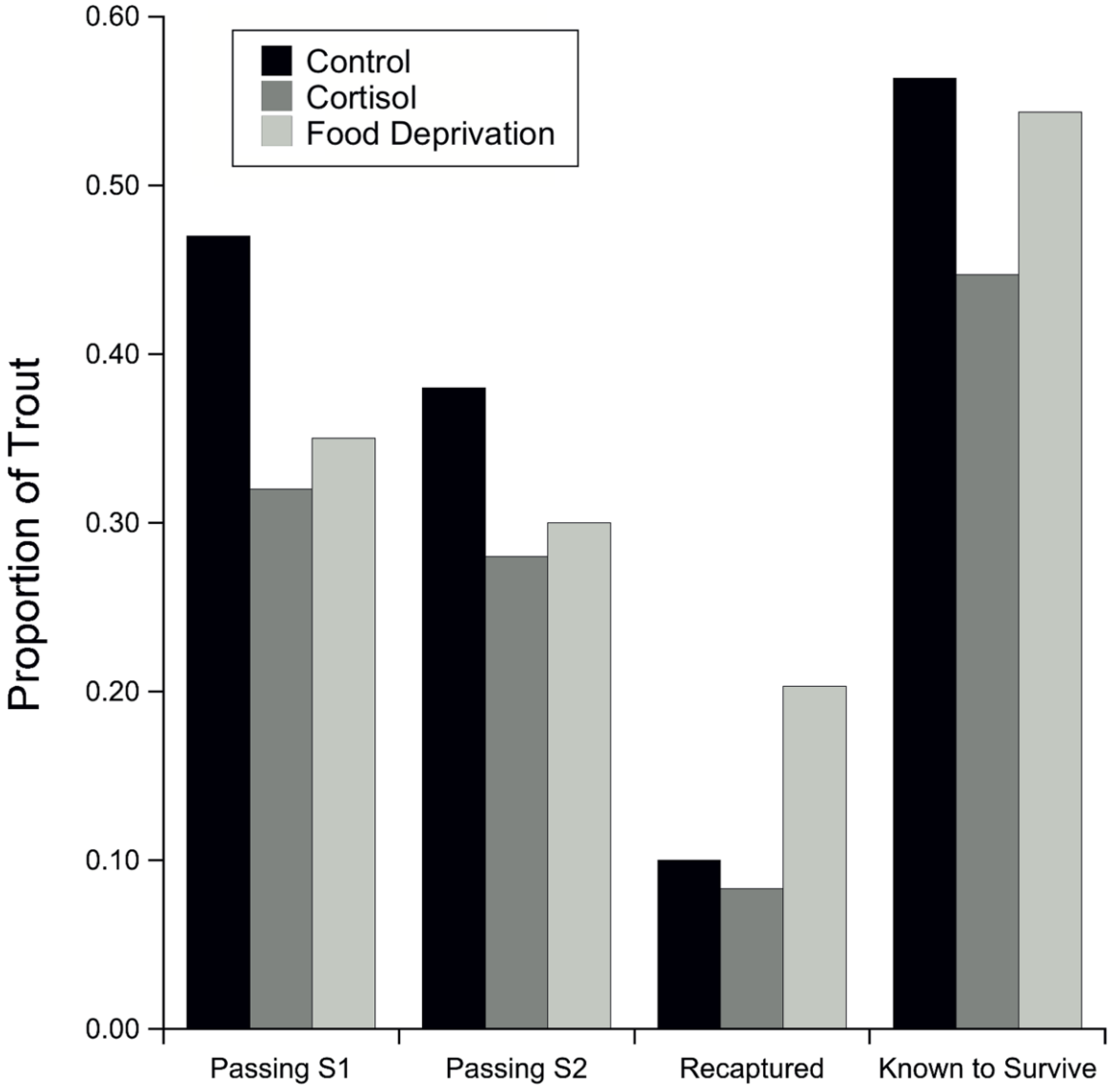

Figure 3 – Proportion of trout in each treatment that passed S1 and S2, were recaptured during the June surveys, or were known to have survived (recaptured or detected at either S1 or S2).

Table 1 – Mean initial length, mass and relative condition (K_R) plus standard deviation (SD) for trout in each of the three treatment groups. The ranges (min-max) for each metric are shown in the brackets. The mean number of days between release and passage to S1 are presented; subscript letters show means that are significantly different (ANOVA and Tukey HSD). The total number of trout that were detected passing S1 and S2 and the number of trout that were recaptured in the stream during June surveys are also shown. Finally, the mean instantaneous growth rates for length and mass for recaptured trout are shown with SD.

	Treatment		
Metric	**Control**	**Cortisol**	**Food Deprivation**
Sample Size	209	216	202
Length (cm)	14.3±1.4	14.3±1.6	14.4±1.5
	(12.0-19.7)	(12.0-22.8)	(12.0-19.8)
Mass (g)	26.6±8.4	26.7±10.2	27.5±9.1
	(14.6-80.5)	(14.9-106.6)	(12.1-65.7)
K_R	1.00±0.02	1.00±0.02	1.00±0.02
	(0.95-1.06)	(0.94-1.10)	(0.90-1.05)
Days to S1 Passage	$36.5±17.7_{AB}$	$35.4±16.0_{A}$	$42.4±15.0_{B}$
Num. Passing S1	99	78	71
Num. Passing S2	80	61	61
Num. Recap.	21	18	41
Recap. G_L	0.002±0.001	0.001±0.001	0.002±0.0005
Recap. G_M	0.008±0.002	0.005±0.002	0.008±0.002

Table 2 – Output from the log-rank survival analysis. For all analysis the Degrees of Freedom was 1 and alpha was set to p=0.05.

Station	Treatments Compared	χ^2	p-value
S1	Control – Cortisol	4.62	0.032
	Control – Food Deprivation	7.98	0.005
	Cortisol – Food Deprivation	0.42	0.515
S2	Control – Cortisol	4.75	0.029
	Control – Food Deprivation	4.02	0.050
	Cortisol – Food Deprivation	0.04	0.849