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

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Early-life stressors have short- but not long-term carryover effects in a wild salmonid fish

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Abstract:	<p>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 tracked wild fish with passive integrated transponder tags from the juvenile to adult stage. In total, 3217 juvenile brown trout (<i>Salmo trutta</i>) 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 outmigration 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. We found that no treatments affected return rates, while high 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 only in one tagging event. Overall, our findings suggest that neither intrinsic factors nor extrinsic stressful early-life experiences have a strong effect on fish that survive to adulthood. This suggests that some species may be more resilient than others to stressful stimuli encountered early in life.</p>

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In prep for Physiological and Biochemical Zoology

Running title: Carryover effects & migration in brown trout

What is known: Early life experiences can influence fitness traits (e.g., growth, reproduction), and as such, any stimuli that affects these experiences may impact life histories and population dynamics. Carryover effects have been observed in a multitude of species, but there have been no attempts to manipulate stressors experienced by fish during early life phases to determine if they carry over to influence adult performance.

What the study adds: We exposed wild juvenile brown trout to a range of stressors (cortisol injections, food deprivation, increased temperature and chase to exhaustion) and tracked fish for up to 4 years to determine whether these early life stressors had long-term effects. We found that despite having some short-term effects, stressors applied in early life had no long-term carryover effects in anadromous brown trout.

22 **Abstract**

23 Early-life experiences can shape life histories and population dynamics of wild animals. To
24 examine whether stressful stimuli experienced in early life resulted in carryover effects in later
25 life stages, we conducted several experimental manipulations and then tracked wild fish with
26 passive integrated transponder tags from the juvenile to adult stage. In total, 3217 juvenile brown
27 trout (*Salmo trutta*) were subjected to one of six manipulations: chase to exhaustion, thermal
28 challenge, food deprivation, low concentration cortisol injection, high concentration cortisol
29 injection, and sham injection, plus a control group. Cortisol and food deprivation treatments were
30 previously shown to have short-term effects on juveniles such as lower survival to outmigration
31 and changes in migration timing. However, it remained unknown whether any of the six
32 manipulations had effects that carried over into the adult phase. We therefore investigated
33 whether these extrinsic manipulations, as well as intrinsic factors (size and condition), affected
34 probability of return as adults and time spent at sea. We found that no treatments affected return
35 rates, while high cortisol weakly affected time spent at sea in one tagging event. We also found
36 that juvenile condition was positively correlated to likelihood of adult return only in one tagging
37 event. Overall, our findings suggest that neither intrinsic factors nor extrinsic stressful early-life
38 experiences have a strong effect on fish that survive to adulthood. This suggests that some
39 species may be more resilient than others to stressful stimuli encountered early in life.

40

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

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53 **Introduction**

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

77 In fish, extrinsic stimuli such as food deprivation (Lendvai et al., 2014), exhaustive
78 exercise (Madaro et al., 2016), and temperature (Folkedal et al., 2012) can increase plasma
79 cortisol levels, the main glucocorticoid hormone in fish. These stressful stimuli can have short-
80 term carryover effects. For example, physiological exertion (i.e., anaerobiosis) in adult sockeye
81 salmon (*Oncorhynchus nerka*) en route to spawning grounds has been shown to carry over and
82 negatively influence reproductive success (Burnett et al., 2014). Exogenously elevated plasma
83 cortisol applied during the freshwater juvenile stage of partially anadromous brown trout (*Salmo*

84 *trutta*) lowered smolt survival in rivers (Midwood et al., 2014). The same treatment applied to
85 adult female pink salmon (*O. gorbuscha*) also reduced survival as well as reproductive output
86 (McConnachie et al., 2012). However, to our knowledge there have been no attempts to
87 manipulate stressors experienced by fish during early life phases to determine if they carry over
88 to influence adult performance. Such studies have however been carried out in mammals (e.g.,
89 Mirescu et al., 2004), birds (e.g., Haywood and Perrins, 1992; Merilä and Svensson, 1997) and
90 reptiles (e.g., O’Steen, 1998), and have all demonstrated the (generally) negative effects of early
91 life stressors on later life stages.

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

104 Here we investigate how both intrinsic factors and mimicked stressors experienced at the
105 juvenile stage in freshwater affect the time spent at sea and successful return of anadromous
106 brown trout upon their return to freshwater as adults. We exposed juvenile brown trout to one of
107 five challenges, mimicking both external and physiological perturbations, and tracked
108 individuals during their outmigration as juveniles and return migration as adults. We simulated
109 three external events: predation avoidance by chasing fish to exhaustion; low food availability by
110 reducing access to food for two weeks; and high temperature by maintaining fish in water 4.2°C
111 higher than stream temperature. We also mimicked one of the main components of the
112 physiological stress response by injecting fish with exogenous cortisol at either a low or a high
113 concentration. This initiated the downstream effects of the stress response (e.g., increased energy
114 mobilization), a method that has been commonly used to examine the effects of stressors on wild

115 fish (Sopinka et al., 2015; Crossin et al., 2016). These treatments were applied in different
116 combinations during four tagging events (two in the spring and two in the fall) and their short-
117 term effects on juvenile outmigration are known. In summary, supraphysiological exogenous
118 manipulation of cortisol (high concentration treatment) decreased in-stream survival to
119 outmigration in fall (Midwood et al., 2015) and spring (Midwood et al., 2014, 2016), and caused
120 fish to migrate earlier when applied in the fall (Midwood et al., 2015; Peiman et al., 2017). The
121 food deprivation treatment was the only other stressor to show an effect, most evident when
122 applied in the spring where migration propensity decreased and the timing of migration was
123 delayed (Midwood et al., 2016). In addition to effects on survival and migration, cortisol
124 manipulation had negative effects on growth and condition (Midwood et al., 2014, 2015, 2016;
125 Peiman et al., 2017). Generally, fish of smaller size, lower condition, or lower growth tended to
126 migrate more and earlier (Peiman et al., 2017).

127 Given the effects that the cortisol treatment had on growth, condition, and migration as
128 well as the effects that the food deprivation treatment had on migration in juvenile fish, we
129 predicted that these treatments would extend the time spent at sea as sub-adult fish, perhaps as a
130 means of compensating for growth impairments during the juvenile freshwater phase (Nicieza
131 and Metcalfe, 1997). Compensatory growth has been shown to reduce survival (Johnsson and
132 Bohlin, 2006), and so we also predicted that cortisol and food deprivation treatments would
133 result in lower adult return rates. We made no prediction with regards to intrinsic factors as the
134 association between length/condition at the juvenile stage and adult return can be positive,
135 negative, or non-significant (e.g. Armstrong et al., 2018). This study is novel in that previous
136 work has focused solely on the juvenile stage whereas we are investigating whether intrinsic
137 (size and condition) and extrinsic (stressors) factors experienced in early life carry over into the
138 adult stage.

139

140 **Materials and Methods**

141 *Study locations*

142 The study was performed in two rivers (**Fig. 1**). The River Villestrup is located in north-central
143 Jutland, Denmark, and runs into the Mariager Fjord. The Gudsø stream is located in southeast
144 Jutland, Denmark, and runs into the Kolding Fjord. Both streams are home to natural populations
145 of partially migrant brown trout, with juveniles typically migrating to sea as one-year olds, and

146 migrants generally outnumbering residents 10:1 (Birnie-Gauvin et al., 2017). Both streams are
147 equipped with fixed PIT stations spanning the entire width of the river bed. These stations detect
148 fish tagged with uniquely coded PIT tags year-round. Villestrup has a single PIT station with two
149 antennas (10m apart) located 200m upstream of the river mouth (detection efficiency of the first
150 antenna was estimated at 98.4% across the study period; efficiency at the second antenna cannot
151 be calculated, see Zydlewski et al., 2006). Gudsø is equipped with two PIT stations, each with
152 two antennas (5m apart) located approximately 600m from the river mouth (detection efficiency
153 of the first PIT station was estimated at 98.9% averaged across the two antennas during the study
154 period). Regardless of minor detection inefficiencies, there is no expectation that treatment
155 would affect detection probability.

156

157 *Tagging and treatments*

158 We combined data from field studies carried out during four different tagging events over the
159 course of three years, where a total of 3217 individual fish were captured, tagged and treated.
160 Tagging and treatment occurred during the following dates: (1) March 6 to 12, 2012 (Spring
161 2012, $n = 761$; Gudso stream); (2) November 4 to 7, 2012 (Fall 2012, $n = 475$; Gudso stream);
162 (3) February 28 to March 11, 2013 (Spring 2013, $n = 622$; Gudso stream); and (4) October 21 to
163 29, 2014 (Fall 2014, $n = 1359$; River Villestrup) (**Table 1**). Length and mass measurements were
164 used to calculate a condition factor ($Fulton's K = (mass \times length^{-3}) \times 100$).

165 Detailed descriptions of capture, tagging and treatment procedures are provided in
166 Midwood et al. (2014, 2015 and 2016), as well as Peiman et al. (2017). Briefly, fish were
167 captured by electrofishing and kept in fresh water until processed. Fish were anesthetized with
168 benzocaine ($0.03g l^{-1}$ in water), weighed ($\pm 0.01g$), measured ($\pm 0.1cm$) and tagged with 23mm
169 PIT tags (Texas Instruments, RI-TRP-RRHP, 134 Hz, 0.6 g mass in air, Plano, Texas, USA).
170 Fish were then randomly assigned to a treatment group. Control fish were immediately left to
171 recover in freshly oxygenated water. Sham fish tagged in spring 2012 were given intracoelomic
172 injections of 0.01ml of cocoa butter (NOW Foods, Bloomingdale, Illinois, USA) per gram of
173 fish. Cortisol-treated fish in 2012 and 2013 were injected with either a low (25mg cortisol per kg
174 of fish) or a high (100 mg cortisol per kg of fish) dose of cortisol (hydrocortisone 21-
175 hemisuccinate, Sigma-Aldrich, St. Louis, MO, USA) dissolved in cocoa butter. Sham and
176 cortisol treatments applied in 2014 were performed the same way except vegetable shortening

177 (100% vegetable shortening, Crisco, OH, USA) was used as a vehicle. Both vehicles have been
178 validated for use with cortisol to elevate levels of plasma cortisol in fish (Birnie-Gauvin et al.
179 2018). Food-deprived fish were kept in a pen placed in the stream for 14 days to reduce foraging
180 opportunities, which resulted in a loss of mass (Midwood et al., 2016; Peiman et al., 2017).
181 Thermally-challenged fish were maintained at $4.2 \pm 0.1^\circ\text{C}$ above stream water temperature for 30
182 minutes (Peiman et al., 2017). Fish chased to exhaustion were placed in an annulus arena and
183 chased for 3 minutes, after which time most fish no longer responded to external stimuli (Peiman
184 et al., 2017). Following all treatments, fish were left to recover in freshly oxygenated stream
185 water until equilibrium was regained. All fish were released the same day near their site of
186 capture, except food-deprived fish that were released after 14 days in the pens. Note that not all
187 treatments were performed at each tagging event.

188 All procedures were performed in accordance to and with permission from the Danish
189 Animal Experiment Inspectorate (under license numbers 2012-DY-2934-00007 and 2013-15-
190 2934-00808, administered by the Technical University of Denmark).

191

192 *Migration and return*

193 All PIT data were downloaded March 1, 2018. Note that for all smolt outmigration data we
194 cannot differentiate between stream residency and death for individuals not detected at the PIT
195 antennas. Adult return date was defined as the first detection at the lowermost antenna, and a fish
196 was considered to have returned if it was detected at the downstream and upstream PIT antennas
197 in that order after having been detected migrating as a smolt. All return migrations occurred after
198 at least 2.5 months at sea. Time spent at sea was calculated as the duration (number of days)
199 between smolt outmigration and adult return date.

200

201 *Statistical analyses*

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

206 Mass and length were highly correlated, so only length was used for subsequent analyses
207 to avoid collinearity. No fish tagged in fall 2012 returned, so data from this tagging event could

208 not be included in any analyses. Very few fish (1 to 6) tagged in fall 2014 returned, so data from
209 this tagging event were excluded from analyses due to low statistical power, though results from
210 this tagging event are still presented in the figures.

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

$$214 \text{Adult return}_i \sim \text{Bernoulli}(\pi_i, 1)$$

$$215 E(\text{Adult return}_i) = \pi_i$$

$$216 \text{var}(\text{Adult return}_i) = \pi_i \times (1 - \pi_i)$$

$$217 \text{Logit}(\pi_i) = \alpha + \beta_1(\text{treatment}_i) + \beta_2(\text{length}_i) + \beta_3(\text{condition}_i)$$

218 Significance was tested using likelihood ratio tests (LRT). We also explored differences in adult
219 return probability using a chi-square analysis (a one-sided test) but opted for the GLM approach
220 as this allowed us to simultaneously test for length and condition effects and because the results
221 were the same (see Supplemental Materials).

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

$$224 \text{Time at sea}_i \sim \text{Gamma}(\mu_i, \tau)$$

$$225 E(\text{Time at sea}_i) = \mu_i$$

$$226 \text{var}(\text{Time at sea}_i) = \frac{\mu_i^2}{\tau}$$

$$227 \text{Log}(\mu_i) = \alpha + \beta_1(\text{treatment}_i) + \beta_2(\text{length}_i) + \beta_3(\text{condition}_i)$$

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

232 Data from each tagging event were analysed separately as the type and number of levels
233 of the independent variable (treatment) varied among the events. All statistical analyses were
234 performed in R version 3.1.2 (R Development Core Team 2014). Model validation was
235 performed by visual inspection of the residuals and no violations were encountered. Collinearity
236 between independent variables was assessed using variance inflation factors (VIFs; see Zuur et
237 al., 2009). Significance was evaluated as $p < 0.05$.

238

239 **Results**

240 *Likelihood of smolt migration*

241 Of the 3217 fish tagged, a total of 1273 smolts migrated out of their natal stream. As noted
242 previously, these data have been analysed in Midwood et al. (2014, 2015 and 2016) for spring
243 2012, fall 2012 and spring 2013 tagging events respectively, and in Peiman et al. (2017) for the
244 fall 2014 tagging event. As such, these data were not reanalysed statistically. Briefly, we note
245 that high-cortisol fish had lower outmigration rates than controls in all tagging events. Food-
246 deprived fish were less likely to migrate than controls in spring 2013 (**Fig. 2**).

247

248 *Adult survival*

249 Of the 1273 smolts that migrated, 145 (11.4%) survived to return as adults at least once. No fish
250 tagged in fall 2012 returned, and very few tagged in fall 2014 returned, so the adult analysis is
251 based on returns from the spring 2012 and spring 2013 tagging events. Though few fish returned
252 from fall 2014 tagging (the only event in River Villestrup), these all returned within one year,
253 whereas 34.4% and 41.2% of fish originating from Gudsø stream in 2012 and 2013, respectively,
254 spent more than one year at sea.

255

256 *Likelihood of adult return*

257 Likelihood of return was higher for juveniles that had higher condition in spring 2012 (LRT =
258 5.268, $df = 1$, $p = 0.022$) but was not affected by length (LRT ≤ 1.239 , $df = 1$, $p \geq 0.061$). In
259 spring 2013, neither condition nor length were associated with likelihood of return (LRT \leq
260 1.239, $df = 1$, $p \geq 0.266$). Treatment had no effect on likelihood of return in either study year (all
261 $p \geq 0.260$, **Fig. 3**).

262

263 *Time spent at sea*

264 Time spent at sea was not affected by length ($F \leq 0.245$, $p \geq 0.625$) or by condition as juveniles
265 ($F \leq 0.518$, $p \geq 0.474$) for either tagging event. Treatment had a weak effect on time spent at sea
266 in the spring of 2012 ($F = 2.594$, $df = 3$, $p = 0.058$) such that the high cortisol group spent longer
267 time at sea than controls, but not in spring 2013 ($F = 1.128$, $df = 2$, $p = 0.338$, **Fig. 4**).

268

269 **Discussion**

270 Given the delay in juvenile outmigration and loss of mass in food-deprived fish, as well as the
271 decreased survival to juvenile outmigration and negative effects on juvenile growth and
272 condition in cortisol-treated groups, we expected that individuals from these groups would have
273 lower adult return rates and/or spend a longer time at sea to compensate for a time of reduced
274 growth in early development. Despite the presence of short-term carryover effects, however, we
275 found only a single, weak long-term carryover effects for juveniles that outmigrated. This
276 suggests that the effects of stressors experienced early in life do not have a strong effect on adult
277 brown trout.

278 We found that juveniles in better condition in the spring 2012 but not spring 2013 tagging
279 event were more likely to return, and that length had no effect on return rates. Many studies have
280 investigated the link between length/condition and aspects of migration, but the relationship
281 between these parameters is complicated by the timing of measurements relative to age and
282 outmigration time (Ferguson et al., 2017). Because body length and condition were measured at
283 the time of tagging and not at the time of migration, our data does not reflect that some
284 individuals have accelerated growth shortly before migrating (Ferguson et al., 2017).
285 Furthermore, the relationship between juvenile size/condition and migration success has been
286 shown to vary considerably across studies, both in sign and magnitude (see Gregory et al., 2018
287 and references therein). While a wide range of studies support a ‘bigger-is-better’ paradigm
288 where marine survival of juveniles to adulthood is positively correlated to size and condition at
289 migration (e.g. Saloniemi et al., 2004; Armstrong et al., 2018), other studies question the
290 generality of this paradigm (Birnie-Gauvin et al., 2019; del Villar-Guerra et al., 2019). For
291 example, optimal length at juvenile outmigration was ‘medium-size’ in river Imsa, Norway
292 (Jonsson et al., 2017). Thus, the influence of length and condition on marine survival (and thus
293 adult return rates) may vary among years and rivers, as is supported by our results.

294 The negative effects on juvenile growth and/or condition following exogenous cortisol
295 injections (Midwood et al., 2014, 2015; also reviewed in Sopinka et al., 2015) and food
296 deprivation (Byström et al., 2006) have already been documented. An increase in time at sea
297 would be consistent with the literature suggesting that longer marine phases represent a means of
298 compensating for a period of low growth during early life (Jonsson and Bohlin, 2006). Our
299 findings suggest this is not the case as cortisol treatment had only a weak effect on time spent at

300 sea in one tagging event. We also found no indication that juvenile length or condition affected
301 time spent at sea. Sea trout are known to vary extensively in their behaviour, with some
302 individuals being more active and even adjusting their behaviour when affected by external
303 stressors such as sea lice by making short trips to the estuaries to shed their lice (Halttunen et al.,
304 2018). Thus smaller or low-condition brown trout may adjust their behaviour by more active
305 foraging rather than extend their time spent at sea.

306 Individuals from every treatment and the control groups spent highly variable times at
307 sea, similar to other sea trout populations (Klemetsen et al., 2003). However all Villestrup adults
308 spent <1 year at sea, whereas 34-41% of returning adults from Gudsø spent >1 year at sea,
309 perhaps as a result of differences in adjacent sea conditions in northeast (Villestrup: Mariager
310 Fjord, Kattegat Sea-like conditions) and southeast Jutland (Gudsø: Kolding Fjord, Western
311 Baltic-like conditions; refer to **Fig. 1**) during those years. There is some evidence suggesting that
312 Mariager Fjord is the most productive fjord (in terms of phytoplankton, invertebrates and fish) in
313 Denmark (Kaas et al., 1996), and thus conditions in Mariager Fjord may be more favourable than
314 those in Kolding Fjord. As a result, one potential explanation for the observed differences in
315 return timing may be that brown trout in Mariager Fjord reach sufficient reproductive potential to
316 make the return migration in <1 year worthwhile. However, we only had one tagging event in
317 Villestrup and so cannot generalize across years, and other studies in the same system have
318 observed a relatively high instance of 1+ sea-winter fish (e.g., del Villar-Guerra et al., 2019),
319 suggesting that the mechanisms underlying the decision to spend more (or less) time at sea is
320 more complex than a simple function of food availability. Alternatively, the time of tagging and
321 manipulation may have an effect on juvenile survival or outmigration propensity, as our two fall
322 tagging events results in either zero or very few adult fish return, in contrast to our two spring
323 tagging events. Future research should consider recapturing individuals during the spawning
324 season to assess carryover effects on reproductive potential. Initial sample sizes for such studies
325 should be guided by our findings that <15% of tagged fish will return as adults (0% in some
326 treatments), and should thus be closer to 1000 fish per treatment. This would offer fundamental
327 knowledge on the extent to which carryover effects may affect population dynamics in the wild.
328 Our study is further limited in that only fish that survived the applied stressors and migrated to
329 the sea in the short term could be used for the long-term portion of the analysis.

330 While many studies have found carryover effects in adults across seasons in diverse taxa
331 (e.g., Robb et al., 2008; Crossin et al., 2010; Harrison et al., 2011 and references therein;
332 Rockwell et al., 2012; Betini et al., 2013; Latta et al., 2016), in general studies that have
333 demonstrated carryover effects from early life stages into the adult life stage applied stressors or
334 used naturally-occurring conditions shortly after birth (e.g., hatching, weaning, or the larval
335 stage, Blount et al., 2003; Chelgren et al., 2006; Descamps et al., 2009; Saino et al., 2018). Thus,
336 stressors occurred at what is perhaps a particularly sensitive life stage, and consequences may be
337 more severe (i.e., death), and for the survivors long-lasting (i.e., carry over into later life stages),
338 as a result. Though most studies did not or could not measure mortality, it could be that species
339 differ in whether stressors have stronger short-term effects on mortality vs longer-term carryover
340 effects on the survivors (individuals that were robust enough to survive the stressor). The present
341 study summarized the short-term effects of stressors (which were mortality/residency and
342 changes in outmigration timing) applied at the late juvenile stage, but found only weak effects on
343 adults in the long-term, perhaps because stressors were applied when fish were 10 months or
344 more past the larval stage (i.e., tagged in the spring), or because of high mortality at both life
345 stages leaving only robust individuals returning (on average, only 36% of tagged fish
346 outmigrated, and only 11.4% of outmigrating smolts returned as adults). To our knowledge, the
347 longest recorded impact of an applied stressor in wild fish was a cross-seasonal effect in adult
348 largemouth bass (*Micropterus salmoides*) (O'Connor et al., 2010). Adults were subjected to
349 increased circulating levels of cortisol for 5 days in the fall and were then followed over the next
350 7 months, with cortisol-treated adults dying sooner than control fish during a winterkill event.
351 The studies that found longer lasting effects in fish used natural variation in larval traits such as
352 growth rate, condition, or early life environment (e.g. Shima and Swearer, 2010; Saboret and
353 Ingram, 2019), again highlighting that the life stage at which a stressor occurs may be an
354 important factor in determining whether effects carryover to adulthood.

355 How close to a life stage transition an organism is may also be a critical factor in studies
356 of carryover effects (e.g., Chelgren et al., 2006; Touchon et al., 2013; Dingeldein and Wilson
357 White, 2016). For example, Chelgren et al. (2006) manipulated food availability in Northern red-
358 legged frog (*Rana aurora*) tadpoles that were near metamorphosis to the adult stage, and found
359 that food influenced the timing of metamorphosis and the performance of adults as they moved
360 from aquatic to terrestrial habitats. If physiological and molecular processes that enable the

361 transition from one life stage to the next have already begun when the stressor is applied, long-
362 term effects on the survivors may be more prevalent. It also may be that in species where
363 maturity is reached early in life, ‘long-term’ effects are easier to detect as there may be less
364 environmental variation and fewer other new stressors also acting on these individuals over their
365 short life spans.

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

381 Biological systems naturally work in sequence, and thus every ‘decision’ or ‘event’ in an
382 animal’s life is likely to have downstream effects (Harrison et al., 2011). In this study, we found
383 that the consequences of delayed growth during early life induced by cortisol and food
384 deprivation treatments do not appear to have strong effects that carryover through adult life in
385 brown trout. Species vary in how they cope with early-life stressors (Clavel et al., 2011; Harrison
386 et al., 2011), and in some species these effects may carry over into adulthood. This may be one
387 reason why species vary in their ability to cope in a changing world (e.g., inherently variable
388 levels of food availability and extreme weather conditions mediated by human activity; Angelier
389 and Wingfield, 2013).

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637 **Table 1.** Number, mean total length in cm (\pm SD), and condition factor (\pm SD) of juvenile brown
 638 trout at initial capture assigned to each treatment group during each tagging event. Dashes
 639 indicate the treatment was not used in that tagging event.

640

Tagging event	Spring 2012	Fall 2012	Spring 2013	Fall 2014
<i>System</i>	<i>Gudsø</i>	<i>Gudsø</i>	<i>Gudsø</i>	<i>Villestrup</i>
Control	190	228	206	223
	14.7 \pm 2.0	14.1 \pm 1.9	14.3 \pm 1.4	14.4 \pm 2.1
	0.92 \pm 0.06	1.01 \pm 0.09	0.88 \pm 0.05	0.96 \pm 0.06
Sham	192	-	-	224
	14.6 \pm 1.7	-	-	14.3 \pm 1.9
	0.92 \pm 0.06	-	-	0.96 \pm 0.06
Low cortisol	191	-	-	-
	14.6 \pm 1.8	-	-	-
	0.93 \pm 0.07	-	-	-
High cortisol	188	247	213	230
	14.7 \pm 2.0	14.1 \pm 1.8	14.4 \pm 1.6	14.0 \pm 2.1
	0.92 \pm 0.06	1.01 \pm 0.09	0.88 \pm 0.05	0.96 \pm 0.06
Food deprived	-	-	203	235
	-	-	14.4 \pm 1.5	14.1 \pm 1.8
	-	-	0.90 \pm 0.05	0.97 \pm 0.06
Thermal	-	-	-	222
	-	-	-	14.1 \pm 1.8
	-	-	-	0.96 \pm 0.06
Chase	-	-	-	225
	-	-	-	14.3 \pm 1.9
	-	-	-	0.96 \pm 0.06

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652 **Figure Captions**

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654 **Fig. 1.** River Villestrup, northern Jutland, and Gudsø stream, southern Jutland, Denmark. PIT
655 stations are represented as dark bars.

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657 **Fig. 2.** Juvenile outmigration. Proportion of tagged juvenile brown trout (*Salmo trutta*) that
658 migrated within each tagging event for each treatment. Dissimilar letters indicate significant
659 differences ($p < 0.05$), as analysed in Midwood et al. (2014, 2015, 2016) and Peiman et al.
660 (2017) for spring 2012, fall 2012, spring 2013, and fall 2014, respectively.

661

662 **Fig. 3.** Adult return migration. Proportion of outmigrated juvenile brown trout (*Salmo trutta*)
663 within each tagging event and treatment that returned as an adult to freshwater. Number of fish
664 presented above the bars. Fall 2014 was not analysed. No treatment effects were found.

665

666 **Fig. 4.** Time spent at sea. Average time (in days) spent at sea for brown trout (*Salmo trutta*)
667 within each tagging event for each treatment. One outlier from the high cortisol treatment in
668 Spring 2012 was excluded. Error bars represent one standard deviation of the average value.
669 Only a weak high cortisol effect was found in spring 2012.

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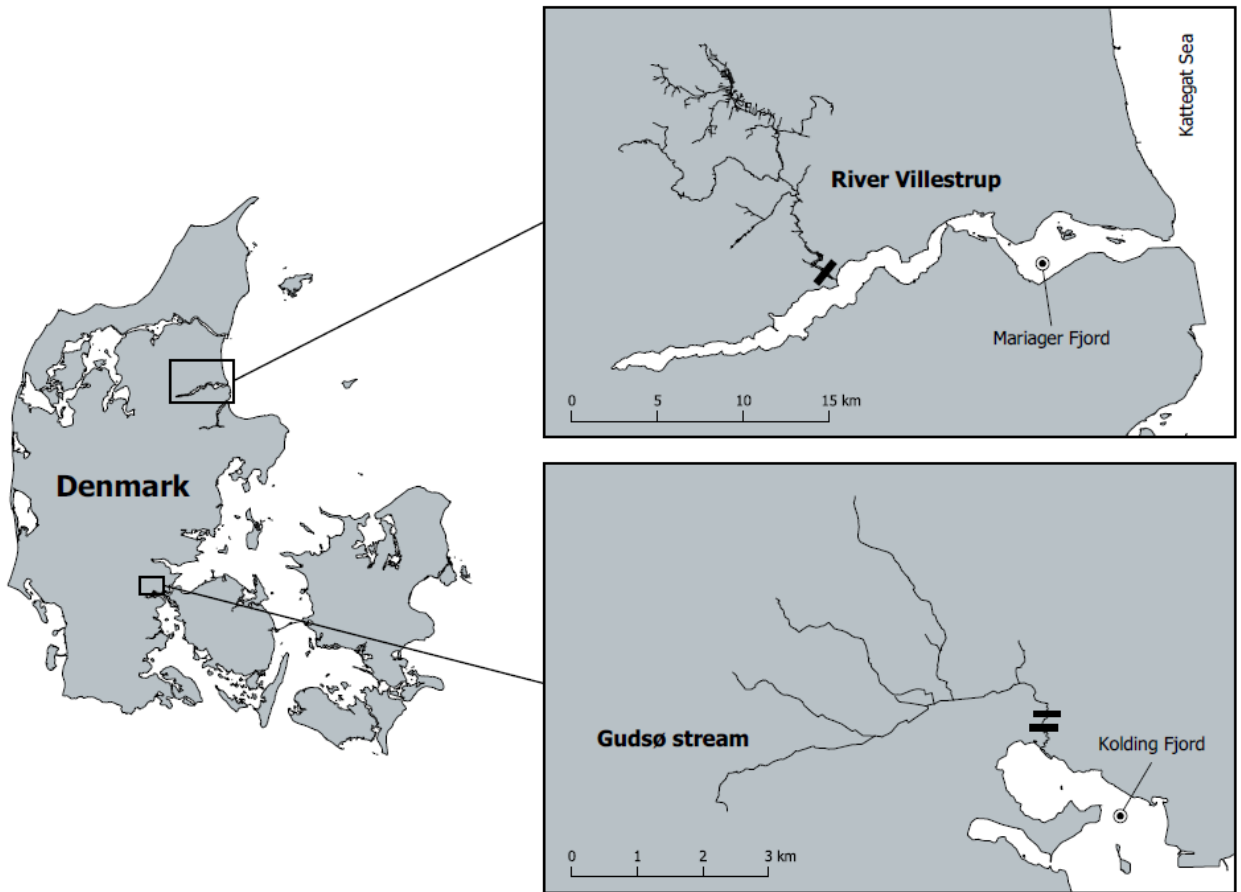
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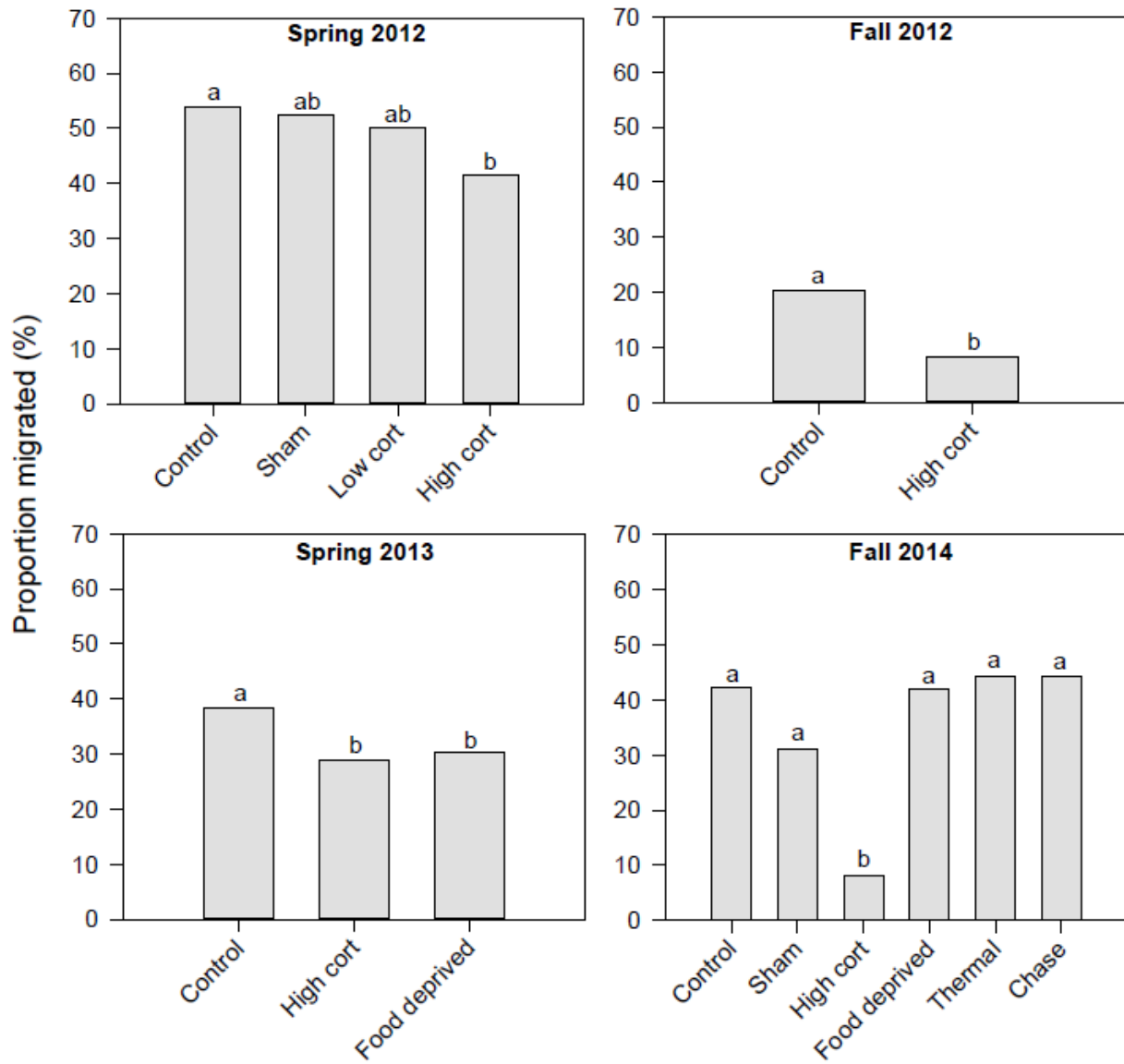
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683 **Figure 1**



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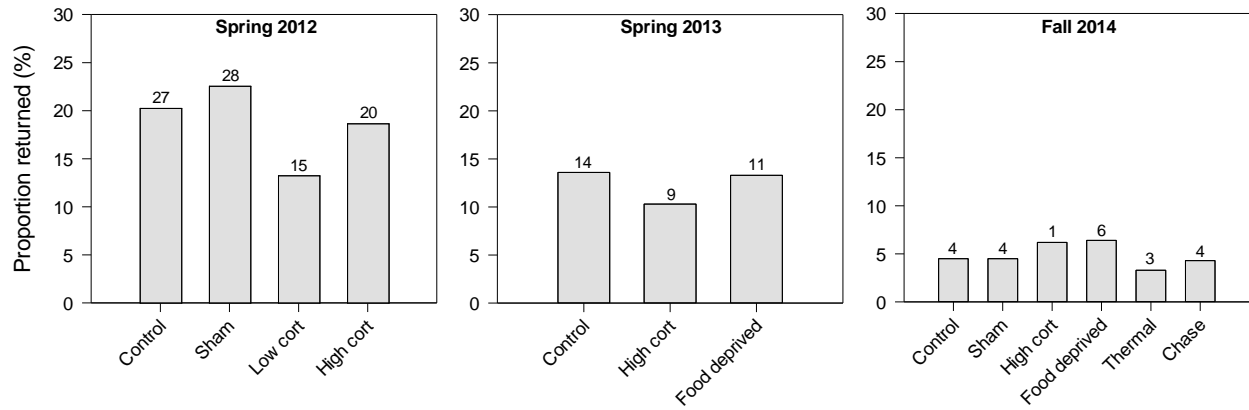
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708 **Figure 3**

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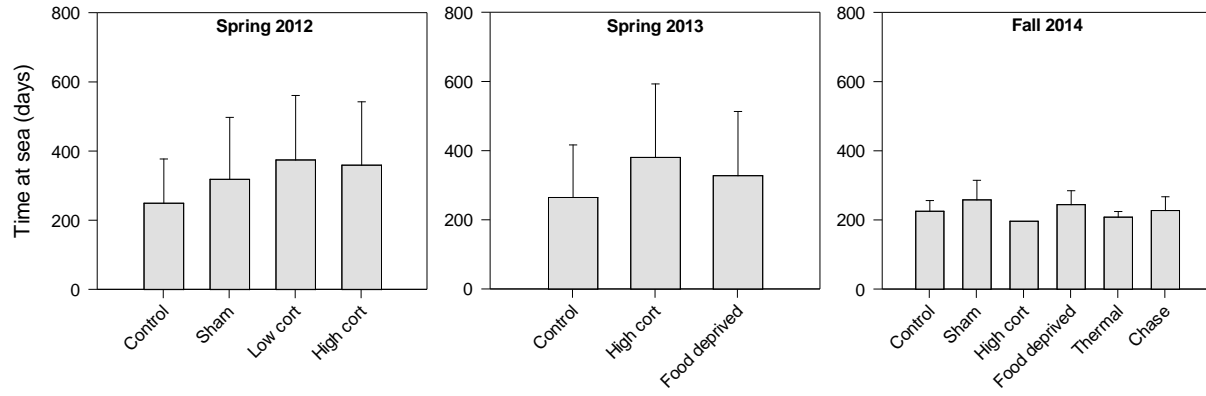
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727 **Figure 4**

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