



Oxidative stress and partial migration in brown trout (*Salmo trutta*)

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44 **Abstract**

45 During migration, animals are typically limited by their endogenous energetic resources which
46 must be allocated to the physiological costs associated with locomotion, as well as avoiding
47 and/or compensating for oxidative stress. To date, there have been few attempts to understand
48 the role of oxidative status in migration biology, particularly in fish. Semi-anadromous brown
49 trout (*Salmo trutta*, Linnaeus 1758) exhibit partial migration, where some individuals smoltify
50 and migrate to sea, and others become stream residents, providing us with an excellent model to
51 investigate the link between oxidative stress and migration. Using the brown trout, we obtained
52 blood samples from juveniles from a coastal stream in Denmark in the fall prior to peak seaward
53 migration which occurs in the spring, and assayed for antioxidant capacity (oxygen radical
54 absorbance capacity) and oxidative stress levels (ratio of oxidized to reduced glutathione). We
55 found that individuals that migrated had higher antioxidant capacity than residents and that
56 future migration date was negatively correlated with both antioxidant capacity and body length
57 in the fall. This study provides the first evidence that oxidative status is associated with
58 migration strategy and timing, months in advance of the actual migration, and provides insight
59 into the role of oxidative status in animal migration.

60

61 **Key-words:** brown trout (*Salmo trutta*, Linnaeus 1758), antioxidant, partial migration, resident,
62 migrant, oxidative stress

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68 **Introduction**

69 Migrations represent some of the most fascinating and energy demanding phenomena in
70 the animal world and are often typified by prolonged elevation in metabolic rate associated with
71 high levels of locomotor activity (Leffler 1993; Jonsson et al. 1997). The idea that elevated
72 metabolism leads to increased production of reactive oxygen species (ROS) via an increased flux
73 of electrons at the level of the electron transport chain is widespread in the literature (Wingfield
74 et al. 1998), though not well supported empirically (Salin et al. 2015 and references therein). An
75 increase in ROS can result in an imbalance between ROS and antioxidants (molecules that delay
76 or inhibit oxidation; Halliwell and Gutteridge 2007) and can lead to oxidative stress and the
77 damage of macromolecules (Asada and Takahashi 1987). When organisms allocate their limited
78 resources to enhance their antioxidant capacity and resist oxidative stress, energy is diverted
79 from other activities such as growth, locomotion, immunity and avoiding predators (Ball and
80 Balthazart 2008; Denver et al. 2009).

81 The relationship between oxidative stress and life history traits is still poorly understood
82 (Speakman et al. 2015). The majority of studies have focused on immediate and short-term
83 effects of ROS but there is increasing realization that oxidative stress will impact life histories
84 over longer timescales (Monaghan et al. 2009; Birnie-Gauvin et al. in press). For example,
85 Costantini et al. (2008) provided evidence that homing pigeons (*Columba livia*, Gmelin 1789)
86 which undergo longer flights experience greater oxidative stress than individuals which undergo
87 shorter flights as well as control individuals (no flight). The few studies published to date that
88 investigated the link between oxidative stress parameters and life-history strategies are often
89 limited to birds (Costantini 2014 and references therein), therefore restricting the generality of

90 those conclusions to other taxa (but see Birnie-Gauvin et al. in press for review). As a result of
91 elevated metabolism and high energetic demands during migration, we would expect migratory
92 species to be better equipped to deal with oxidative stress than non-migratory species (Costantini
93 2008). The same pattern may exist between migratory and resident individuals within a species
94 (i.e., when a species exhibits partial migration, Chapman et al. 2011). No studies that we know of
95 have investigated the potential role of oxidative status as a determinant of partial migration
96 strategy.

97 Semi-anadromous brown trout, *Salmo trutta* (Linnaeus 1758), undergo partial migration,
98 where migratory and resident individuals coexist within the same population (Jonsson and
99 Jonsson 1993). The decision to smoltify and migrate to sea or to assume residency in their native
100 stream is a complex interaction between environment and physiology that is particularly
101 sensitive to stressful conditions (Thorpe et al. 1992; Metcalfe 1998; Cucherousset et al. 2005;
102 Dingle and Drake 2007; Boel et al. 2014). The decision to migrate or not is thought to be made at
103 the end of the summer before spring migration (Metcalfe 1998). We used blood samples
104 collected in the fall to determine whether oxidative stress was associated with migration strategy
105 and migration timing in the spring. Consequently, our goal was to evaluate the link between
106 oxidative stress and partial migration in wild juvenile brown trout in relation to within-year
107 variation in timing of migration and migration strategy (migratory versus resident), and not
108 related to whether migratory individuals undertake migration in one year versus the next year
109 (between-year variation). Larger individuals are more constrained by low food availability in
110 stream environments than smaller individuals and are thus more likely to migrate to marine
111 environments, where food availability is greater (Økland et al. 1993; Thorpe et al. 1998).
112 Additionally, larger individuals often have faster growth rates and higher metabolic rates

113 (Økland et al. 1993; Thorpe et al. 1998), and thus may have higher levels of oxidative stress than
114 smaller individuals. Consequently, we predicted that: (1) larger individuals will migrate, (2)
115 individuals with higher levels of oxidative stress and lower levels of antioxidants will migrate,
116 (3) within migratory individuals, larger individuals will migrate sooner, and (4) individuals with
117 higher levels of oxidative stress and lower levels of antioxidants will migrate sooner.

118

119 **Materials and Methods**

120

121 *Study location*

122 The Gudsø Stream (mean width ~ 2 m) is located in east-central Jutland, Denmark (Figure 1).

123 The stream runs over approximately 16 km and is surrounded by agricultural land. Several
124 tributaries flow into the main stem, before reaching the northwest Baltic Sea at Kolding Fjord.

125 The stream supports natural populations of semi-anadromous brown trout (*Salmo trutta*), eel
126 (*Anguilla anguilla*) and lamprey (*Lampetra planeri*). Two passive integrated transponder (PIT)
127 reading stations (PIT stations 1 and 2) located approximately 1 km from the outflow of the
128 stream into fjord record the passage of PIT tagged fish (Figure 1). The distance between the two
129 PIT reading stations is 150 m. Each reading station consists of two loop-shaped antennas spaced
130 5 m apart, each covering the entire cross-section of the stream. This allowed us to determine the
131 swimming direction of migrating trout. We evaluated the tag detection efficiency of PIT station 1
132 using the formula described in Zydlewski et al. (2006), and found it to be 88%.

133

134 *Fish sampling and tagging*

135 Fish were captured in the main stem of the Gudsø Stream, approximately 2 km upstream of the

136 entrance to the fjord (Figure 1) from October 20 to October 25, 2015. Additional fish were
137 captured from a tributary on November 2 and November 3, 2015. All trout greater than 120 mm
138 in length were captured using single-pass electrofishing gear (Stampes Elektro A/S, Ringkøbing,
139 Denmark) and placed in a 60L container of fresh stream water. The water was changed
140 continuously to provide freshly oxygenated water. Fish were placed in a 0.03g L⁻¹ benzocaine
141 solution until their opercular rate had slowed and fish were unresponsive to external stimuli
142 (usually less than 4 minutes). Total length (± 1 mm) and wet mass (± 0.1 g) were measured for
143 individual fish. A relative condition factor (K) was calculated using equation 1 (Bolger and
144 Connolly 1989). Fish were then tagged with a 23mm PIT tag (Texas Instruments, RI-TRP-
145 RRHP, 134 kHz, 0.6g mass in air, Plano, TX, USA) inserted into their body cavity. Larsen et al.
146 (2013) demonstrated that the retention of these tags in Atlantic salmon (*Salmo salar*, Linnaeus
147 1758) was 97% with no effects on mortality or growth.

$$K = \left(\frac{\text{mass}}{\text{length}^3} \right) (100) \quad (1)$$

149 *Blood sampling*

150 Blood samples of 0.1mL were obtained from the caudal vasculature of individual fish using a
151 heparinized 1.5 inch 25-gauge needle. Within 10 minutes of sampling, blood was centrifuged at
152 6,000 rpm for 2 minutes, after which plasma was separated from red blood cells (RBCs). RBCs
153 were flash-frozen in liquid nitrogen and then stored at -80°C. Fish were allowed to recover in a
154 60L container of fresh stream water and were released near their site of capture. These
155 standardized techniques were approved by the Danish Animal Experiments Inspectorate (License
156 Number: 2013-15-2934-00808).

157

158 *Antioxidant capacity*

159 Samples of RBCs were homogenized on ice in 1:5 lysis buffer (20mM Tris-HCl, 137mM NaCl,
160 1% NP-40, 10% glycerol, 2mM EDTA) using a handheld Tissue Master 125 (Omni
161 International, Kennesaw, GA). Lysates were centrifuged at 13,000 rpm for 5 minutes at 4°C in a
162 Hermle Labnet Z216MK (Mandel, Guelph, ON). Supernatants were separated into two: one half
163 for the antioxidant capacity assay, and the other half for the oxidative stress level assay (see
164 below). Samples were stored at -80°C until the Oxygen Radical Absorbance Capacity (ORAC)
165 assay was performed (as described in Wilson et al. 2012). ORAC assays were performed using a
166 Cytation 5 microplate reader (BioTek Instruments Inc., Winooski, VT, USA) and black 96-Well
167 Costar microplates. Fluorescence was measured with an excitation wavelength of 485 nm and
168 emission of 520 nm. Gen5 data analysis software (2.07.17 BioTek Instruments Inc., Winooski,
169 VT, USA) was used to analyze the fluorescence data.

170 Each reaction well contained 20 μ L of either sample, blank (75 mM potassium phosphate
171 (pH 7.4)), or standard (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox); 0-400
172 μ M), and 3.82 μ M fluorescein in 75 mM potassium phosphate (pH 7.4). The plate was incubated
173 at 37°C for 20 min before rapidly adding the free radical generator 2,2"-azobis (2-
174 amidinopropane) dihydrochloride to a final concentration of 79.83 mM. The plate was placed
175 immediately in the microplate reader and the fluorescence was read every 80 seconds for 90
176 minutes. The area under the fluorescence decay curve was determined for the samples and
177 Trolox standards to determine the Trolox equivalency, commonly used as a benchmark for
178 antioxidant capacity. Total protein of samples was determined using the BioRad assay and final
179 values are reported in Trolox equivalents/ μ g total protein. All samples were run in duplicates,
180 and the mean of both values was used as the final parameter value.

181 The ORAC assay provides a measure of low molecular weight antioxidants, and is one of
182 the few techniques which takes the reaction of ROS to completion (Cao and Prior 1999). Like
183 most antioxidants, low molecular weight antioxidants are typically derived from diet, and are
184 therefore expected to remain relatively consistent through time, provided that an individual
185 continues to feed on the same food sources.

186

187 *Oxidative stress levels*

188 Following the homogenization of the RBC samples in lysis buffer described above, supernatants
189 (half of the total amount) were further homogenized in 1:5 5% sulfosalicylic acid solution
190 (bubbled with N₂ gas). Sample lysates were centrifuged at 13,500 rpm for 10 minutes at 4°C.
191 Supernatants were used to assess total glutathione (TGSH), reduced glutathione (GSH) and
192 oxidized glutathione (GSSG) [TGSH = GSH + 2GSSG]. Glutathione assays were performed
193 using an Epoch microplate reader with Gen5 data analysis software (Biotek Instruments,
194 Winooski, VT, USA) and clear 96-Well Costar microplates. Glutathione assays were performed
195 by following the rate of reduction of 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) by GSH at
196 412nm compared to a standard curve of GSH.

197 For the measurement of TGSH, the reaction media contained 20µL of sample, 5 IU/mL
198 glutathione reductase, 0.5M potassium phosphate buffer (pH 7.0), 0.3 mM nicotinamide adenine
199 dinucleotide 2'-phosphate (NADPH), and 60 mM DTNB and reduction was read for 30 minutes
200 and compared to a GSH standard curve (0-8 µM). To quantify only GSSG, 50 µL of the initial
201 supernatant and the GSSG standards (0-0.5263 µM) were treated with 44.7 mM 2-vinylpyridine
202 and 227.27 mM KPi in a total volume of 110 µL and allowed to incubate at room temperature for
203 90 minutes to derivatize the GSH. Once complete, the GSSG was measured in the same manner

204 as TGS_H using the methods described above. GS_H values were calculated using the equation
205 described above. Final values are reported in mM concentrations. All samples were run in
206 duplicates, and the mean of both values was used as the final parameter value.

207 We opted to measure glutathione as it is the most abundant antioxidant in eukaryotic cells
208 and critical for the protection against oxidative damage (Owen and Butterfield 2010). Moreover,
209 glutathione is costly to generate, and is therefore seldom broken down, making it useful to
210 investigate effects over longer timescale (i.e., tends to remain fairly constant over time within an
211 individual).

212 *Evaluation of predation and migration*

214 Two cormorant (*Phalacrocoracidae* sp., Linnaeus 1758) colonies are located approximately 2
215 and 5 km away from the Gudsø stream. Each colony was scanned on March 14-15, 2016 by two
216 people, each sweeping the entire area of the colonies once to detect excreted PIT tags. Scanned
217 PIT tags allowed us to determine which fish had died from cormorant predation.

218 The main stem and one tributary of the Gudsø stream were resampled entirely between
219 February 29 and March 2, 2016 to evaluate residency. Recaptured fish were assumed to be
220 residents unless the fish was later detected at the PIT reading stations. PIT data were downloaded
221 on October 27th, 2016, past the peak migration period for smolts which occurred in March-April
222 2016. All fish detected at the PIT antennas were considered to be migrants. Fish that were neither
223 detected at the PIT antennas, recaptured in the spring or found in the cormorant colonies were
224 defined as unknown.

225 *Statistical analysis*

227 Statistical analyses were conducted using R version 3.2.3 (R Core Team 2015; nlme and
 228 AICcmodavg packages by Pinheiro et al. 2016 and Mazerolle 2016, respectively). To explore
 229 potential relationships between individual life history strategy (migratory vs. resident) and
 230 oxidative stress levels (OSL) or antioxidant capacity (AOX) obtained for each fish, we fit the
 231 following generalized linear model, with length and condition (K) as covariates:

$$232 \quad \text{strategy}_i \sim \text{Bin}(\pi_i, 1)$$

$$233 \quad E(\text{strategy}_i) = \pi_i$$

$$234 \quad \text{var}(\text{strategy}_i) = \pi_i * (1 - \pi_i)$$

$$235 \quad \text{logit}(\pi_i) = \eta_i$$

$$236 \quad \eta_i = \beta_1 + \beta_2 * \text{length}_i + \beta_3 * K_i + \beta_4 * \text{OSL}_i + \beta_5 * \log(\text{AOX}_i)$$

237 The model states that the life history strategy of fish i follows a binomial distribution with
 238 probability parameter π_i and $n = 1$, i.e. a Bernoulli distribution. π_i is specified through a logit-link
 239 by the predictor function η_i to be a linear function of the included covariates (length_i , K_i , OSL_i
 240 and AOX_i).

241 For the migratory fish, we modelled day of migration (DOM, unit: day of year, where
 242 Day 300 = October 28, 2015) as a function of individual oxidative stress metrics and day of
 243 tagging (DOT, unit: day of year) by the following model fitted using generalized least squares:

$$244 \quad \text{DOM}_i \sim N(\mu_i, \sigma^2 * \text{length}_i^{2 * \delta})$$

$$245 \quad E(\text{DOM}_i) = \mu_i$$

$$246 \quad \text{var}(\text{DOM}_i) = \sigma^2 * \text{length}_i^{2 * \delta}$$

$$247 \quad \mu_i = \beta_1 + \beta_2 * \text{length}_i + \beta_3 * K_i + \beta_4 * \text{OSL}_i + \beta_5 * \log(\text{AOX}_i) + \beta_6 * \text{DOT}_i$$

248 The model assumes DOM of fish i follows a Gaussian distribution with mean μ_i specified as an
 249 identity linked predictor function of the included covariates (length_i , K_i , OSL_i , AOX_i and DOT_i).

250 A covariate variance structure incorporating length_i was used to accommodate variance
251 heterogeneity.

252 For both models, OSL was transformed to a categorical variable representing whether or
253 not individual OSL values were zero (i.e., true zeros) or not. Additionally, AOX was log-
254 transformed as preliminary analysis indicated the models would otherwise violate underlying
255 assumptions.

256 We tested whether length was correlated with OSL or AOX using a linear regression
257 independently within migratory and resident individuals.

258

259 **Results**

260 A total of 414 juvenile brown trout were initially captured, tagged and sampled in the fall. Of
261 those fish, 24 were recaptured in the spring, of which only 13 (3.1%) were not detected at the
262 PIT antennas, and are therefore assumed to be residents. We found that 147 (35.5%) of all tagged
263 individuals were known migrants, 11 (2.7%) were predated by cormorants and 241 (58.2%) had
264 an unknown fate (presumably mortalities). Of the 147 migratory individuals from the study, a
265 subsample of 48 was randomly chosen to perform oxidative stress assays (with some individuals
266 randomly chosen within specific time intervals to ensure coverage across the migratory season),
267 while all 13 residents were assessed. Two of the migratory individuals were considered as
268 outliers and therefore removed from subsequent analyses ($n = 46$).

269 In the fall, migratory individuals had a higher antioxidant capacity than resident
270 individuals (GLM, $Z = -2.05$, $P = 0.042$; Figure 2). No significant relationships were detected
271 between life-history strategy and oxidative stress levels ($Z = -0.688$, $P = 0.49$), length ($Z = -1.63$,
272 $P = 0.10$) or condition ($Z = 0.25$, $P = 0.80$). See Table 1 for all parameter values.

273 Within migratory individuals, day of migration was negatively correlated with
274 antioxidant capacity ($t = -2.20$, $P = 0.0393$, Figure 3A) and body length ($t = -3.81$, $P = 0.0005$,
275 Figure 3B), but was not associated with oxidative stress levels ($t = -1.35$, $P = 0.18$) or condition
276 ($t = 0.64$, $P = 0.52$).

277 Within migratory individuals, body length was positively correlated with oxidative stress
278 levels ($t = 2.36$, $P = 0.023$), but not antioxidant capacity ($t = -1.89$, $P = 0.065$). Within resident
279 individuals, body length was not correlated with oxidative stress levels ($t = -0.35$, $P = 0.73$) or
280 antioxidant capacity ($t = -0.68$, $P = 0.51$).

281

282 Discussion

283 Migration is an energetically demanding activity, which involves physiological costs
284 such as a consistently elevated metabolic rate, thus depleting finite resources more rapidly and
285 potentially increasing the production of reactive oxygen species (ROS) compared to non-
286 migratory individuals (Leffler 1993; Jonsson et al. 1997; Costantini et al. 2007). Migratory
287 individuals must therefore have the ability to cope with the increased ROS production that
288 migration induces, which implies having sufficient repair mechanisms and antioxidants
289 (Costantini 2008). Here we tested whether this overall greater ability to deal with high levels of
290 ROS production is apparent long before migration actually occurs.

291 Migrant and resident brown trout show differences in morphological (e.g., color and body
292 form) traits, which could play a role in forging an individual's oxidative status. For example,
293 resident individuals display yellow bellies and red spots on their sides, both of which can result
294 from the presence of carotenoids, an important source of antioxidants (Youngson et al. 1997).
295 Smolts in contrast, undergo massive physiological and morphological changes to prepare for

296 migration, such as silvering in colour and increased sodium potassium ATPase activity in the
297 gills (Hoar 1988; Aarestrup et al. 2000; Nielsen et al. 2004), but there is no indication that they
298 divert antioxidant resources to do so. We found that antioxidant capacity, measured as the
299 oxygen radical absorbance capacity (i.e., low molecular weight antioxidants), was higher in
300 migratory individuals than in resident individuals. These antioxidants were elevated days to
301 months in advance of migration. It is possible that residents deflect resources from building
302 antioxidant capacity to invest in coloration (i.e., carotenoids in this case). In contrast, migratory
303 individuals may invest their resources into building antioxidant capacity to deal with the
304 demands of migration. This hypothesis is further supported by our finding that migrants with
305 higher antioxidant capacity migrate earlier, perhaps as a sign that fish are ready to migrate (i.e.,
306 physiologically prepared to deal with oxidative stress during migration). This also predicts that
307 later migrating individuals would increase their levels of antioxidants as they approached their
308 migration date, an intriguing area for future study.

309 We observed that larger individuals migrated sooner, which is a well-supported pattern in
310 the literature (Metcalf et al. 1990; Bohlin et al. 1996). Because fish of larger size may have
311 higher growth rates and higher metabolic rates than their smaller counterparts (Økland et al.
312 1993; Thorpe et al. 1998), we would predict that larger individuals have higher levels of
313 oxidative stress. Our data suggests this is the case only within migrants, where oxidative stress
314 levels were positively correlated with length, possibly emphasizing that larger individuals are
315 more constrained by low food availability in freshwater stream environments.

316 It is possible that our findings reflect other physiological differences such as differences
317 in growth rate or standard metabolic rate (SMR) among individuals, which may make an
318 individual more likely to adopt one strategy over the other. Specifically, individuals with higher

319 growth rates and metabolic rates may have higher antioxidant capacities to compensate for
320 higher metabolic demands, and may be more likely to migrate as these individuals are more
321 constrained by food availability. For example, Sloat and Reeves (2014) showed that rainbow
322 trout (*Oncorhynchus mykiss*, Walbaum 1792) with high SMR were more likely to smoltify and
323 migrate than those with lower SMR. As such, the higher antioxidant capacities we observed in
324 migrant individuals in the present study may reflect a compensatory mechanism rather than
325 individual readiness to migrate. Future studies should consider measures of SMR and oxidative
326 stress indices in the context of partial migration to answer this question, though this may
327 represent a challenge in field studies.

328 We cannot exclude the possibility that sex and age played a role in the patterns in
329 oxidative parameters observed in this study. While it has been reported that females tend to
330 migrate more often than males (reviewed in Jonsson and Jonsson 1993), most studies of partial
331 migration do not sex juvenile fish (e.g., Morinville and Rasmussen 2003) as this process requires
332 lethal sampling or expensive laboratory assays. However, even if the observed resident/migrant
333 differences were due to sex, this itself is an intriguing possibility as no other studies that we
334 know of have documented differences in oxidative parameters due to sex in immature wild fish.
335 Similarly, age was not determined for these fish and may have impacted oxidative status. Though
336 the link between oxidative stress markers and age has been established in humans (Harman
337 1956), we know very little about fish in that aspect (Martinez-Alvarez et al. 2005), which would
338 provide an interesting avenue for future studies. Nonetheless, only fish between 12 and 20 cm
339 were used, which are typically thought to be 16 to 18 months of age (personal observation, K.
340 Aarestrup; Jonsson 1985) and so it is unlikely that the relationships between oxidative
341 parameters and migratory strategy can be attributed to age. Furthermore, because some

342 individuals may migrate after 3 years of stream residency (Økland et al. 1993), we cannot say for
343 certain that individuals identified as residents in this study will always remain residents; they
344 may in fact migrate during the following year. However, the factors affecting migration between
345 years may be different than those affecting migration within any given year and thus, our study
346 focused on the potential physiological aspects that underpin partial migration within one year.

347

348 **Conclusion**

349 To better understand the physiological factors that may promote the evolution of partial
350 migration in fish, we examined oxidative stress markers in both migrant and resident individuals
351 of brown trout. During migration, these fish must distribute their limited resources toward
352 swimming efforts, immunity, and predator avoidance among other physiological demands, in
353 addition to coping with the elevated production of ROS. We show that for migrant fish, these
354 resources are also invested toward building antioxidant capacity days to months before migration
355 occurs. Our study suggests that antioxidant capacity is associated with migratory status and
356 migratory timing in brown trout: migrants have higher antioxidant capacity than residents, and
357 within migrants those with higher antioxidant capacity migrate sooner. This has important
358 ecological implications: (1) increased antioxidant capacity may be a component of smoltification
359 for migratory fish, and (2) fish exposed to stressful conditions may be less able to invest resource
360 in antioxidants, which may delay fish in their migration, and impact population dynamics
361 (Theriault et al. 2008). Our findings support the hypothesis that migrants have mechanisms to
362 cope with the added ROS production induced by a sustained increase in metabolic rate during
363 migration.

364

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372

373

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523 **Figure Captions**

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525 **Figure 1.** Map of the Gudsø stream, Jutland, Denmark. Inserted circle shows location of the
526 stream in Denmark. Sampling locations are highlighted in the thick black trace, A) for the fall
527 sampling, and B) for the spring sampling (adapted with permission from Birnie-Gauvin et al.
528 2017, *J. Exp. Biol.* 220: 1693-1700).

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531 **Figure 2. Antioxidant capacity.** Antioxidant capacity (ORAC; Oxygen Radical Absorbance
532 Capacity) in Trolox equivalents per μg protein for migrants ($n = 46$) and resident individuals ($n =$
533 13). There is a significant association between life-history strategy and antioxidant capacity
534 (GLM, $P < 0.05$).

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536 **Figure 3. Migration timing.** (A) migration day (Day 300 = October 28, 2015) as a function of
537 antioxidant capacity (ORAC; Oxygen Radical Absorbance Capacity) in Trolox equivalents per
538 μg protein ($n = 46$, GLM, $P = 0.0393$). (B) migration day as a function of body length ($n = 46$,
539 GLM, $P = 0.0005$). Lines represent model predictions, dotted lines indicate 95% confidence
540 intervals.

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546 **Table 1. Measured variables for migratory and resident individuals.** All values presented are
 547 mean (\pm SD) for migrants ($n = 46$) and residents ($n = 13$).

	Strategy	
	Migrants	Residents
Mass (g)	28.6 \pm 16.3	25.1 \pm 8.9
Length (cm)	14.3 \pm 1.9	13.6 \pm 1.3
Condition	0.9 \pm 0.08	1.0 \pm 0.08
TGSH (mM)	250.7 \pm 178.0	75.6 \pm 45.8
GSH (mM)	250.7 \pm 177.9	75.6 \pm 45.8
GSSG (mM)	0.006 \pm 0.01	0.002 \pm 0.004
GSSG/GSH	0.00002 \pm 0.00003	0.00002 \pm 0.00003
ORAC (TE/ μ g protein)	13.0 \pm 9.7	8.5 \pm 2.2
Protein (μ g)	3.0 \pm 1.2	5.5 \pm 1.2

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