Energetic state and the continuum of migratory tactics in brown trout (*Salmo trutta*)

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Abstract

Alternative migratory tactics, like partial migration, are common in many taxa. The proximate and ultimate drivers underpinning these strategies are unclear, though factors like condition and energetic status have been posited as important predictors. We sampled and PIT tagged 1882 wild brown trout prior to the first so-called decision window, and explored the links between migratory tactics (residency, autumn or spring migration) and body metrics (length and condition), lipids (triglycerides and cholesterol), and sex, in 150 randomly selected individuals. We found that more females adopted the autumn and spring migration tactic than males, while more males adopted the residency tactic than females, likely reflecting sex-biased benefits in anadromy. We also found that autumn migrants were in poorer condition prior to the presumed first decision window than spring migrants and residents. Lastly, we found that both condition and cholesterol were positively correlated to the timing of migration, such that individuals in poorer condition and/or with lower cholesterol migrated earlier. Collectively, these results suggest that energy depletion is an important factor in determining migratory strategy, including timing.

Keywords: cholesterol, partial migration, phenotypic plasticity, residency, triglycerides

Introduction

Life history strategies are ultimately the means by which an individual can maximize its fitness (i.e., number of descendants and genes passed on; McNamara and Houston, 1996; Thorpe et al., 1998). Migratory organisms can thus exploit heterogeneities between spatially separated environments to maximize their fitness. For example, organisms may move between environments that provide optimal feeding opportunities and optimal reproductive success, though migrating between both environments is energetically costly and can be risky (Gross et al., 1988; Alerstam et al., 2003). In fishes, anadromy (i.e., freshwater spawning individuals that migrate to sea to feed) is most prevalent in temperate regions, where marine habitats are more productive than freshwater habitats, whereas catadromy (i.e., marine spawning individuals that migrate to freshwater to feed) is most prevalent in tropical regions, where freshwater habitats are more productive than marine habitats (Gross et al., 1988). The occurrence of partial migration, whereby both migratory and resident phenotypes coexist within the same population, is also relatively common in fishes and other animal taxa (Chapman et al., 2011). Despite its widespread occurrence across taxa, fundamental gaps remain in our understanding of what proximately and ultimately drives partial migration.

Partial migration is well recognized in brown trout (*Salmo trutta*, Jonsson and Jonsson, 1993; Cucherousset et al., 2005), brook trout (*Salvelinus fontinalis*, Morinville and Rasmussen, 2003) and rainbow trout (*Oncorhynchus mykiss*, McPhee et al., 2007), and is thought to be driven by an interaction between environmental and genetic factors. The threshold model suggests that migration is dependent on whether a critical environmentally-sensitive status trait (e.g., physiological condition, size, energetic state) exceeds a predetermined genetic threshold at a certain developmental switch-point, where failure to exceed the threshold would result in migration (Hutchings and Myers, 1994; Thorpe and Metcalfe, 1998; Brodersen et al., 2008; Chapman et al., 2011; Ferguson et al., 2019). While the timing of this developmental switch-point (or decision window) is unknown in brown trout, it is hypothesized to be in late summer in Atlantic salmon (*Salmo salar*; Metcalfe et al., 1990), and is concurrent with the start of the smoltification process in both Atlantic salmon and brown trout migrants. As such, it is likely that the first decision window occurs some time in later summer for brown trout as well. A second decision window, thought to be in early spring (just before migration), determines whether the migration trajectory is continued or discontinued (Rikardsen et al., 2004; Ferguson et al., 2019). The threshold, given its genetic determination, is also likely to be influenced by sex given the presence of a sex bias in the benefits

of migration and residency (Hendry et al., 2004). Despite considerable acceptance of the threshold model however, the factors that may predispose individuals to become migrants or residents remain largely unknown.

In brown trout, multiple migration tactics are common within the same population, exhibiting a sort of migration continuum that includes stream residents, fluvial and adfluvial potamodromous individuals (i.e., river and lake migrants), short-distance marine migrants and long-distance marine migrants (Klemetsen et al., 2003; Cucherousset et al., 2005; Boel et al., 2014; Bordeleau et al., 2018). More recently, acceptance for the presence of a class of autumn migrants has grown (Winter et al., 2016; Aarestrup et al., 2018; Birnie-Gauvin and Aarestrup, 2018), though observations of autumn migrants dates back to the 1980s (e.g., Youngson et al., 1983). It has been suggested that the adopted migratory tactic is related to intrinsic metabolic needs of individual trout (Cucherousset et al., 2005). In contrast to dichotomous migratory tactics, a migration continuum suggests the presence of behavioural responses to resource availability, which may change through space and time along the migratory route. Migratory tendency is negatively correlated with the costs (Bohlin et al., 2001) and distance of migration (Kristoffersen et al., 1994). For example, Boel et al. (2014) found that energetically depleted brown trout were more likely to migrate, and of migrants, those with lower triglycerides were more likely to abort seaward migration and become short-distance lake migrants. As such, the distance and duration of migration undertaken by an individual may be partly controlled by energetic status.

Vertebrates that undergo changes and adaptations relating to life history strategy can be characterized by a lipid storage phase, followed by a phase of depletion (Sheridan and Kao, 1998). In salmonids in particular, lipid depletion and alterations in lipid metabolism are a key aspect of the smoltification process (i.e., the process by which migrants prepare for their entry in seawater; Sheridan et al., 1983, 1985; Sheridan 1989). Prior to migration, available resources tend to be allocated toward protein anabolism, and not toward lipid storage as is the case for residents (Morgan et al., 2002). In salmonids, low energetic (nutritional) status can be characterized as low plasma concentrations of cholesterol, triglycerides, total protein, phosphorus and calcium (Congleton and Wagner 2006). Within this context, lipid status, as determined by plasma levels of cholesterol and triglycerides, is a likely indicator of migratory tactic in salmonids.

We currently still lack studies attempting to characterize the mechanisms that animals use to achieve the optimal life history pattern. To explore relationships between body metrics, energetic status,

sex and migratory tactics, we sampled juvenile brown trout prior to the proposed first developmental switch-point to characterize their lipid resources and monitored their life-history strategy using telemetry. Samples were obtained before the migratory decision itself was made, thus allowing us to: 1) characterize differences in status traits (length, condition, lipids) among three migratory phenotypes prior to the first migratory decision window in both females and males; 2) explore whether these status traits and sex influence migratory tactics; and 3) determine whether these status traits influence the timing of migration among female and male migrants.

Methods

Study site

The Gudsø stream is located in the southeastern region of Kolding, Jutland, Denmark. The stream runs for 6 km before entering the Kolding fjord. It is home to a wild population of brown trout, with both migratory and resident phenotypes. The stream is equipped with two Passive Integrated Transponder (PIT) stations (S1 and S2), each equipped with two antennas, located approximately 150m from one each other, with the downstream-most station situated 500m from the fjord outlet (*Figure 1*). Note that while fish could reside in the 500m stretch between the downstream PIT station and the fjord, previous electrofishing surveys indicated that tagged fish did not reside there. All fish were tagged and released more than 1 km from the PIT stations.

Capture, sampling, and tagging

Between July 3 and 19, 2017, a total of 1882 fish were captured using electrofishing, netted, and immediately placed in a 60L container of fresh, oxygenated, stream water. Shortly thereafter, fish were transferred into a 400L tank with continuously flow of freshly oxygenated stream water. Subsequently, fish were anesthetized in 0.03g L⁻¹ benzocaine in fresh water until their ventilation rate had slowed significantly. Fish were then measured (total length, ± 0.1 cm) and weighed (± 0.1 g) before being tagged with a 23mm PIT tag (Texas Instruments, RI-TRP-RRHP, 134 Hz, 0.6 g mass in air, Plano, Texas, USA). All fish had their adipose fin clipped to enable differentiation from non-tagged fish in the stream and for sexing (see below). Blood samples (0.1mL) from the caudal vasculature were obtained using a

150 1-inch 25-gauge heparinized needle, and centrifuged for 2 minutes immediately. Fish were then left to
151 recover in a 500L container of fresh stream water, and released near their site of capture.

152 Fish captured in the study ranged between 11.0 and 18.9cm, but no scales were taken to age the
153 fish. While it is possible that this size range reflects two year classes (1+ and 2+ years), the length
154 distribution of all captured individuals would seem to indicate otherwise (**Figure 2**). Given that fish
155 were sampled in the summer, precocious maturity (especially common in males) could not be
156 identified.

157 Between June 1 and June 8, 2018, the entire stream was resampled by electrofishing to
158 determine which individuals had assumed residency. All protocols were approved by the Danish
159 Experimental Animal Inspectorate (2017-15-0201-01164).

161 *Determination of phenotypes and migration timing*

162 Data from the PIT stations were downloaded 1 June 2018, following the peak smolt migration period in
163 Denmark. To be considered as migrants, fish had to be detected at one of the upstream *and* one of the
164 downstream PIT antenna, in that order (to confirm directionality), and subsequently not detected for >
165 1 month to ensure the fish migrated. No fish returned between migration and the download of the PIT
166 data on 1 June 2018. Migration date was considered as the date the fish was last detected on the
167 downstream PIT station (after having been detected at the upstream station). Migration timing was
168 subsequently calculated as the number of days between migration date and date of tagging (i.e., days
169 post-tagging).

170 Fish detected to have left the stream between September and November were considered
171 ‘autumn migrants’, while those detected to have left the stream between February and April were
172 considered ‘spring migrants’. Between 1 June and 8 June 2018, the Gudsø stream was resampled via
173 electrofishing in its entirety to determine which individuals had become ‘residents’ (i.e., fish recaptured
174 alive that did not leave the stream). Fish that were not detected to leave the stream nor recaptured
175 during resampling nor predated (see below) were considered to have an ‘unknown fate’. These fish are
176 not considered in any analyses, but data on body metrics is presented in **Table 1**.

177 We were also able to identify individuals that were predated by cormorants (*Phalacrocorax*
178 *carbo*) by scanning nearby colonies with portable backpack PIT scanners. These were not considered
179 separate phenotypes, but data on body metrics is also presented for ‘predated’ individuals in **Table 1**.

180

181 *PIT station and electrofishing efficiency*

182 PIT station efficiency for S1 (upstream station) was found to be 89%, and calculated as follows
183 (Zydlewski et al. 2006):

184
$$Efficiency_{S1} = \frac{detections_{common}}{(detections_{S2} + detections_{common})} \times 100$$

185 Where $detections_{common}$ are the number of detections common to S1 and S2 and $detections_{S2}$ are
186 detections unique to S2. Note that the efficiency for S2 cannot be calculated. The PIT stations were
187 operational throughout the entire period of the study as flow conditions did not change to a degree that
188 would preclude functionality.

189 Electrofishing efficiency was estimated using the depletion method during the stream
190 resampling in June 2018, using 3 stretches of 100m in varied locations across Gudsø stream
191 (representing varied habitats). Briefly, each stretch was electrofished once, where all collected fish
192 were kept in a bin. The stretch was immediately electrofished a second time. Electrofishing efficiency
193 was estimated as 95.2%, and calculated as follows:

194
$$Electrofishing\ efficiency = \frac{N_{pass\ 1} - N_{pass\ 2}}{N_{pass\ 1}} \times 100$$

195 Where $N_{pass\ 1}$ is the number of fish captured on the first pass and $N_{pass\ 2}$ is the number of fish captured
196 on the second pass.

197

198 *Plasma energetic correlates and condition*

199 A random subsample of individuals were selected to assess plasma lipids and for sexing: 50 residents,
200 and 100 migrants, spanning a migration date between August 2017 to May 2018. Thus, the subsample
201 of 100 migrants included autumn migrants, spring migrants, as well as migrants that may have left the
202 stream outside of the September-November and February-April periods. Plasma triglyceride levels
203 were measured using a triglyceride colorimetric assay kit (Cayman Chemical Item No. 10010303). All
204 samples were assayed in duplicates, and the average absorbance was used to extrapolate triglyceride
205 concentration. Intra-assay variation was between 0 and 7.41%, while inter-assay variation was 2.57%.
206 Plasma cholesterol levels were measured using a cholesterol fluorometric assay kit (Cayman Chemical
207 Item No. 10007640). Samples were diluted 1:200, and assayed in duplicates. The average fluorescence

208 of the duplicates was used to extrapolate cholesterol levels. Intra-assay variation was between 0 and
209 2.5%, while inter-assay variation was 4.7%. Both indices have previously been used as indicators of
210 nutritional status in salmonids (Congleton and Wagner, 2006).

211 Condition was calculated as Fulton's (K) using the following equation:

212
$$K = \frac{\text{mass (g)}}{\text{length (cm)}^3} \times 100$$

213

214 *Sexing*

215 DNA from adipose fins was extracted using the Chelex method (Estoup et al., 1996) with Qiagen
216 Multiplex PCR kits. All PCR amplifications were performed in a final volume of 6 µl with 0.5 µl
217 template DNA and a concentration of 0.2µM for each primer. PCR reactions were conducted following
218 the kit manufacturer's recommendation, using an annealing temperature of 57°C and 25 cycles. The
219 PCR product was run on a Seq Studio Automated Sequencer, with Genemapper (version 4) for
220 genotyping. Sexing was done using the sdY primer (Quéméré et al., 2014) with a peak presence at 180-
221 200 base pairs corresponding to males and the absence of a peak corresponding to females. Two other
222 primers (ssa85 and SsOsl408) were used for positive control. Of the 150 selected samples, 147 were
223 successfully sexed (3 samples could not be sexed due to poor DNA extraction results).

224

225 *Data analysis*

226 Statistical analyses were conducted on the 147 randomly selected individuals for which plasma
227 triglyceride and cholesterol concentrations, and sex were available. One spring migrant had an
228 abnormally high condition factor ($K = 1.40$), and was removed from the analysis, leaving 146
229 individuals for further analyses [98 migrants (including 41 autumn migrants, 44 spring migrants and 13
230 migrants that migrated outside the autumn and spring peaks) and 48 residents]. Data were inspected for
231 homogeneity of variance and collinearity, where correlation coefficients <0.70 were considered
232 acceptable. Length and mass were highly correlated (correlation coefficient = 0.96), thus only length
233 was used in the analyses.

234 To test for differences in length, condition, triglycerides and cholesterol among phenotypes
235 (categorical, 3 levels: resident, autumn migrant and spring migrant) and sex (categorical, 2 levels:
236 female and male), individual two-way analyses of variance (ANOVA) with Type III sums of squares

were conducted for each response variable, including both main effects and interactions. Significant differences were evaluated by *post hoc* comparisons (Tukey-Kramer test). Differences in sex ratio among the three phenotypes was evaluated by a Chi-square analysis (goodness-of-fit test). Note that the 13 migrants that migrated outside the autumn and spring peaks (i.e., August, December, January and May migrants) are not included in these analyses to avoid overlap between the autumn and spring seasons.

To explore relationships between plasma lipid concentrations and migration timing (numerical, continuous variable), we used generalized linear models (GLM). All 98 migrants (i.e., autumn migrants, spring migrants *and* fish that migrated outside the autumn and spring) were included in this analysis, but residents (i.e., non-migrants) were not. We followed a step-by-step approach of model simplification from a full model (including length, condition, triglycerides, cholesterol and sex) based on the Akaike information criterion (AIC, **Table 2**). The GLM was fitted with a Gamma distribution and log link function. The best model fit suggested that length, condition and cholesterol should be retained as explanatory variables. The final model was as follows:

$$\begin{aligned}
 \text{Day of migration}_i &\sim \text{Gamma}(\mu_i, k) \\
 E(\text{Day of migration}_i) &= \mu_i \\
 \text{var}(\text{Day of migration}_i) &= \frac{\mu_i^2}{k} \\
 \log(\mu_i) &= \rho_1 \\
 \rho_1 &= \alpha + \beta_1 \cdot \text{Condition}_i + \beta_2 \cdot \text{Length}_i + \beta_3 \cdot \text{sqrt}(\text{Cholesterol})_i
 \end{aligned}$$

Where α is the intercept, and β is the slope (**Table 3**). Note that square root transformations were applied to triglycerides and cholesterol data to satisfy underlying assumptions of normality and homogeneity of variance in residuals. All statistical analyses were performed using R version 3.6.3 (R Development Core Team, 2020), using lattice (Deepayan, 2008), ggplot2 (Wickham, 2009) and MASS (Venables and Ripley, 2002) R packages.

Results

Of the 1882 tagged and sampled juvenile brown trout, 493 migrated, 96 assumed residency and 17 were detected at nearby cormorant colonies (**Table 1**). Fish migrated between 10 August 2017 to 23

May 2018 (**Figure 3**), with the majority of fish migrating in spring (February-April: 302 spring migrants) and autumn (September-November: 124 autumn migrants), representing the two ‘peaks’ in migration typically observed in this river (Birnie-Gauvin and Aarestrup 2019). The remaining 1276 individuals had an unknown fate, and may have: 1) died in the stream, 2) been predated by herons and cormorants (colonies for both species are found nearby), 3) assumed residency but were not captured upon re-electrofishing of the stream, or 4) migrated but were not detected at the PIT antennas. The two latter options are less likely given our electrofishing efficiency of 95.2% and PIT detection efficiency of 89% at the S1 (Zydlewski et al., 2006; note that efficiency at S2 cannot be measured). Overwinter mortality can be high for brown trout, and is likely to account for many of the fish with unknown fate (Elliott, 1993; Midwood et al., 2015).

Within the 146 individuals selected for sexing as well as triglyceride and cholesterol analysis, 26 females and 15 males migrated in autumn, 33 females and 11 males migrated in spring, and 4 females and 44 males resided. Five (5) females and eight (8) males migrated outside of the autumn and spring peaks; these are not included in the ANOVA analyses, but are included in the analyses testing the effect of plasma lipids, body metrics and sex on migration timing (see GLM results below).

The sex ratio differed significantly from an even distribution (goodness-of-fit test, $\chi^2 = 47.046$, $df = 2$, $p < 0.001$) such that significantly more males assumed residency than females, and more females migrated in the autumn and spring than males (**Figure 4**).

Length differed between sexes ($F = 8.909$, $df = 1$, $p = 0.00341$), with females being longer than males, but not among phenotypes ($F = 2.886$, $df = 2$, $p > 0.05$, **Figure 5**). Condition differed among phenotypes ($F = 6.009$, $df = 2$, $p = 0.00321$), with autumn migrants having significantly lower condition factors than both spring migrants (Tukey: $p = 0.0151$) and residents (Tukey: $p = 0.0160$, **Figure 5**). Plasma triglyceride ($F \leq 0.5194$, $df \leq 2$, $p \geq 0.5961$) and cholesterol ($F \leq 2.187$, $df \leq 2$, $p \geq 0.1167$) concentrations did not differ among phenotypes or between sexes (**Figure 5**).

Condition ($p = 0.00358$) and cholesterol ($p = 0.0389$) were significantly positively correlated to migration day (**Table 3**, **Figure 6**). Length was not significantly correlated to migration day ($p = 0.119$).

Discussion

Understanding the proximate and ultimate drivers of divergent phenotypes is a fundamental goal of evolutionary ecology (Naish and Hard, 2008). In the field of salmonid research, the drivers of facultative migration in brown trout have piqued the curiosity of researchers for decades. The threshold model has been useful in understanding (at least in part) the drivers of migratory decision in salmonids (Hutchings and Myers, 1994; Thériault et al., 2007). According to this framework, if an individual's status exceeds a genetically-determined threshold at a specific decision window (late summer), this individual should assume residency and mature in freshwater (Dodson et al., 2013; Ferguson et al., 2017, 2019). The model also suggests that a second decision window (late winter, early spring) exists, where size and/or energetic status must exceed another threshold for an individual to continue migration. Failure to exceed the threshold would lead to migration being deferred to the next season or later (Ferguson et al., 2019).

Studies that have explored the relationship between potential status traits (e.g., energetic status, size, condition, growth rate) and the decision to migrate have often been conducted under laboratory settings, where fish that turn silver are considered migrants and those that do not are considered residents (e.g., Sloat and Reeves, 2014; Archer et al., 2019). While this approach has merit, it does not necessarily reflect the natural conditions of the fish's home river, and generally does not reflect ratios of migrant to resident observed in nature. In addition, using visual cues like silver colouration as an indication of migration has been shown to be inaccurate (del Villar-Guerra et al., 2019). Studies have also tended to measure status traits sometime after the migratory decision itself (for example during the parr-to-smolt transformation), and thus may not accurately reflect the trait at the time the decision was made. In this study, we attempted to address some of these limitations by sampling individuals before the presumed first decision window occurs, using telemetry to track the true life-history strategy, and further included autumn migrants as an additional phenotype given its increasing acceptance as part of the salmonid lifecycle. While the exact timing of the first decision window is not known, observations suggest that both brown trout and Atlantic salmon (i.e., closely related species) start to smoltify at the end of the summer (e.g., Metcalfe et al. 1990). By sampling fish in July, we were able to explore energetic status prior to the first decision window.

Unsurprisingly, we found that more females migrated than males, and more males assumed residency than females. Given that female reproductive success is limited by gamete production, and thus by size, there is a sex bias in the balance of benefits of migration and residency (Fleming, 1996;

Hendry et al., 2004; Quinn, 2018). Thus, females gain more by migrating to sea because they can attain larger sizes, and thus greater fecundity and egg size, in addition to attracting more mates and defend spawning sites better (Fleming and Reynolds, 2004). Male reproductive success in contrast tends to be limited by access to mates rather than gamete production (Fleming, 1998). They often mature as residents and can adopt a sneaking strategy where they fertilize eggs at a very small size (Gross, 1985; Jonsson and Jonsson, 1993). Those males may still adopt a migratory strategy later in life (Dellefors and Faremo, 1988; L'Abée-Lund et al., 1990), but little investigation has been conducted on this aspect, thus offering an interesting avenue for future research.

We found that on average, autumn migrants were in poorer condition prior to the first decision window than both residents and spring migrants. Fish in poorer condition may have higher metabolic demands and thus often adopt a migratory strategy to sustain their physiological demands (Sogard et al., 2012; Sloat and Reeves, 2014; Ferguson et al., 2017). In combination with the sex-biased benefits of anadromy, we may expect that females in poor condition have much to gain from migrating to sea earlier, and thus migrate in the autumn. However, we found no significant effect of sex on condition suggesting that low condition may drive both females and males to migrate in autumn. In addition, fish of low condition are less likely to survive harsh winter conditions, as resources – lipids in particular – become increasingly depleted (Cunjak 1988). Migrating to sea earlier, despite making fish more susceptible to predation, provides greater food abundance and thus an opportunity to replenish resources. Food abundance at sea is however greater in spring compared to winter, and thus postponing migration provides the benefit of staying relatively safe from predation in freshwater for as long as possible, while maximizing the feeding opportunities at the time of migration. In this context, spring migrants also have much to gain from migrating to sea. Moreover, because these spring migrants were in better condition than autumn migrants at the time of the first decision window, they were more likely to survive harsh winter conditions in freshwater, and thus deferred migration to the spring. This decision is expected, given that migration is more costly and risky than residency (Gross, 1987; Brönmark et al., 2014; Schwinn et al., 2018). In contrast, residents typically mature earlier and tend to have lower metabolic costs than migrants due to lower metabolic rates, so a higher condition factor at the first decision window is concurrent with existing literature on the subject (Sloat and Reeves, 2014). Together, these findings support the threshold model, and suggest that condition prior to the first decision window may be a good predictor of, or an important factor affecting, migratory strategy

though we suggest discriminating between autumn and spring migrants (**Figure 7**). Specifically, both autumn and spring migrants would fall below the threshold at the decision window, but we would expect autumn migrants to fall below spring migrants (i.e., a lower level of the status trait).

We found no differences in average lipid resources among autumn migrants, spring migrants and residents. This may suggest that lipid resources in general, or plasma triglycerides and cholesterol specifically, are not status traits, though this would be in disagreement with previous findings (Jonsson and Jonsson, 2005; Boel et al., 2014). However, a lack of difference in average lipid resources prior to the first decision window may not be surprising. This is because we are comparing average group values (of 41 to 48 individuals), but the threshold varies for each individual. For example, a value of 'X' may be below the threshold of individual A, but may be above the threshold of individual B. In this case, individual A would migrate, but individual B would assume residency, even if both have the same value for a specific status trait. In other words, the fact that we found no differences in triglycerides or cholesterol among the three phenotypes does not mean that these two lipid correlates are not components of a status trait, but instead may reflect that each individual has a different reaction norm/threshold to which it responds. This idea, though difficult to tackle, should be explored further to pinpoint the factors that determine individual thresholds. Experimental work may be particularly useful here, where genetic background, food availability, temperature, and other factors would need to be controlled for and experimentally manipulated (e.g., Archer et al. 2019). Alternatively, assessing an extensive suite of physiological measurements (e.g., metabolic rate, thermal performance, cardiac performance, blood parameters, etc.) across a range of environmental conditions may allow us to develop more accurate thresholds for individuals, and enable more accurate predictions about their life-history strategies by providing us with a better understanding of the factors that affect life-history decisions.

The hypothesis that energetic status plays a role in the migratory decision is supported by our finding that cholesterol and condition are correlated to the timing of migration. Specifically, we found that both plasma cholesterol and condition factor were positively correlated to the timing of migration, suggesting that individuals with lower cholesterol and poorer condition migrate earlier. The timing of migration, which is a strategy in itself, may be driven by low resource availability to maintain metabolic functions, and thus migration may be initiated when individuals reach a specific low threshold. Though timing was not investigated in their study, Boel et al. (2014) found that migrants had

lower condition and lipid stores, and suggested that individuals adopting migratory strategies were energetically depleted before migrating. In another study, Bordeleau et al. (2018) found that sea trout that migrated farther to sea were in poorer condition, and that individuals with lower triglycerides remained at sea longer. Again, this would indicate that energy depletion drives migratory strategies such that more energy-depleted individuals are willing to take greater risks (like migrating instead of assuming residency, or going farther and/or staying longer at sea). Our findings support this hypothesis, as autumn migrants had lower condition than spring migrants and residents, and energy-depleted migrants (low condition and low cholesterol) migrated earlier. This is likely driven by a need to replenish energy stores, and may involve a willingness to undertake more risky strategies.

In conclusion, the present study demonstrates a link between two posited status traits (condition and lipids) and life-history strategies, thereby supporting the threshold model, even with the consideration of a 'new' autumn migrant phenotype. In addition, the study supports the notion that a link exists between metabolic costs and life-history strategies (Morinville and Rasmussen, 2003), that sex differences are likely at play as early as the first decision window, and that migration timing is likely driven (at least in part) by energy depletion. We contribute to the existing body of literature by providing valuable information on the endogenous mechanisms that drive the migration continuum in brown trout. Understanding these mechanisms, and the factors that affect them, can help conserve facultative migratory species currently declining worldwide, by helping to maintain intraspecific variability, and thus increasing resilience against global changes (Schindler et al., 2015)

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Author contributions

KBG, MHL and KA conceived the study. KBG and MHL performed the field work and sampling. KBG performed the laboratory analyses. KBG performed the data analyses and wrote the first draft of the manuscript. KBG, MHL and KA commented and edited the manuscript.

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623 **Tables**

624 **Table 1. Biometrics.** Mean total length (cm), mass (g) and condition (Fulton's K) ($\pm$ SD) for all tagged
 625 migrant and resident brown trout (*Salmo trutta*), as well as trout that were predated by cormorants, and
 626 individuals with an unknown fate. Samples sizes for each phenotype are presented in italics. Range for
 627 biometrics is provided in brackets.

Phenotype	Length (cm)	Mass (g)	Condition
Migrants (<i>493</i>)	13.3 $\pm$ 1.3 (11.0 – 17.1)	24.7 $\pm$ 7.5 (12.7 – 52.2)	1.0 $\pm$ 0.07 (0.77 – 1.4)
Residents (<i>96</i>)	13.1 $\pm$ 1.2 (11.1 – 16.2)	23.8 $\pm$ 7.5 (13.2 – 50.7)	1.0 $\pm$ 0.06 (0.83 – 1.2)
Predated (<i>17</i>)	14.2 $\pm$ 1.8 (11.2 – 17.8)	31.0 $\pm$ 11.8 (15.3 – 56.5)	1.0 $\pm$ 0.10 (0.89 – 1.3)
Unknown fate (<i>1276</i>)	13.6 $\pm$ 1.5 (11.0 – 18.9)	27.0 $\pm$ 10.3 (12.4 – 72.0)	1.0 $\pm$ 0.07 (0.74 – 1.4)

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630 **Table 2. Model selection.** Model selection process and composition of the best three fitting regression
 631 models (i.e., lowest AIC).

Rank	Model structure	AIC	Δ AIC
1	~ Condition + Cholesterol + Length	1065.21	0.00
2	~ Condition + Cholesterol + Length + Triglycerides	1065.98	0.77
3	~ Condition + Cholesterol + Length + Triglycerides + Sex	1067.54	2.33

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635 **Table 3. Parameter estimates.** Parameter of the best fitted model (lowest AIC) predicting migration
636 timing (number of day post-tagging) as a function of length (cm), condition and cholesterol (μM). The
637 model followed a Gamma distribution.

Explanatory variable	Coefficient	SE	t-statistic	p-value
Intercept	0.0119	0.00426	2.798	0.00631
Length	0.000337	0.000214	1.580	0.118
Condition	-0.00960	0.00321	-2.992	0.00358
Cholesterol	-0.000618	0.000295	-2.096	0.0389

Figure captions

Figure 1. Gudsø stream, southeastern Jutland, Denmark. The stream is equipped with two PIT stations (S1 and S2), each with two antennas. A well-known cormorant colony (circle) is found nearby in Kolding Fjord, where PIT tags from predated juvenile brown trout (*Salmo trutta*) can be found.

Figure 2. Size distribution of all tagged brown trout (*Salmo trutta*) captured between 3 and 19 July 2017 ($n = 1882$).

Figure 3. Distribution of migration timing between August 2017 and May 2018, (A) divided by 5-day bins, and (B) divided by month. Fish migrated in all months of the year with two peaks, a larger peak in spring (February-April) and a relatively smaller peak in autumn (September-October).

Figure 4. Sex ratios of autumn-migrating, resident and spring-migrating juvenile brown trout (*Salmo trutta*).

Figure 5. Boxplot of autumn-migrating, resident and spring-migrating juvenile brown trout (*Salmo trutta*) values of (A) length (cm), (B) condition, (C) plasma triglycerides (mg/dl, square-root transformed), and (D) plasma cholesterol (μM , square-root transformed), separated by sex. The boxplot shows median values (black lines), the interquartile ranges (boxes) and the 10th and 90th percentiles (vertical lines). Dissimilar letters denote significant difference in mean values as identified by Tukey-Kramer *post hoc* test. We note that length (A) was affected by sex (but not phenotypes) such that females tended to be larger than males.

Figure 6. Modelled migration day (days post-tagging) as a function of (A) condition and (B) plasma cholesterol levels (μM , square-root transformed) (B) for female (grey) and male (black) migrating brown trout (*Salmo trutta*), according to the most supported model based on the AIC criterion. Models suggest a positive relationship between condition and migration day for both females and males, as well as a positive relationship between plasma cholesterol and migration day for both females and

690 males. Note that for (A) length and cholesterol values were held at mean values; for (B) length and
691 condition were held at mean values. Note also that day 100 = 17 October.

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693 **Figure 7.** Updated threshold model at the first decision window accounting for autumn migrants. At
694 the time of the decision window, the level of the status trait for autumn migrants is expected to be
695 lower than spring migrants. We note that the exact trajectories of the status trait before and after the
696 decision window are not known (i.e. the status trait could increase, decrease or remain the same over
697 time; it is the level of the status trait at the time of the decision window that will dictate whether the
698 fish migrates, and when). Depicted trajectories are for illustrative purposes only and based off of the
699 typical representation of the threshold model (e.g., Thorpe et al., 1998; Ferguson et al., 2019).

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720 **Figures**

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722 **Figure 1.**

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736 **Figure 2.**

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