

Photoperiod control of downstream movements of Atlantic salmon *Salmo salar* smolts

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This study provides the first direct observations that photoperiod controls the initiation of downstream movement in Atlantic salmon *Salmo salar* smolts. Under simulated natural day length (LDN) conditions and seasonal increases in temperature, smolts increased their downstream movements five-fold for a period of 1 month in late spring. Under the same conditions, parr did not show changes in downstream movement behaviour. When given a shortened day length (10L:14D) beginning in late winter, smolts did not increase the number of downstream movements. An early increase in day length (16L:8D) in late winter resulted in earlier initiation and termination of downstream movements compared to the LDN group. Physiological status and behaviour were related but not completely coincident: gill Na⁺/K⁺-ATPase activity increased in all treatments and thyroid hormone was elevated prior to movement in 16L:8D treatment. The most parsimonious model describing downstream movement of smolts included synergistic effects of photoperiod treatment and temperature, indicating that peak movements occurred at colder temperatures in the 16L:8D treatment than in LDN, and temperature did not influence movement of smolts in the 10L:14D treatment. The complicated interactions of photoperiod and temperature are not surprising since many organisms have evolved to rely on correlations among environmental cues and windows of opportunity to time behaviours associated with life-history transitions. These complicated interactions, however, have serious implications for phenological adjustments and persistence of *S. salar* populations in response to climate change.

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Key words: behaviour; degree days; phenology; temperature; thyroid hormone.

INTRODUCTION

Photoperiod is a reliable circannual cue for timing critical life-history events such as migration, reproduction, sexual maturation and metamorphoses (Ebbesson *et al.*, 2011; O'Brien *et al.*, 2012). The importance of photoperiod for timing these life events is conserved across the plant and animal kingdoms. Among animals, photoperiodicity is important in driving phenology of life-history transitions in many invertebrates and at least some species in all major vertebrate taxa (Bradshaw & Holzapfel, 2007; Ebbesson *et al.*, 2011; Singh *et al.*, 2011; Hazra *et al.*, 2012; O'Brien *et al.*, 2012; Richardson *et al.*, 2013). Linkages between photoperiod, physiological and behavioural responses are controlled by the neuroendocrine system. For example,

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sexual maturation and physiological changes associated with reproduction are initiated by the thyroid hormone pathway in three-spined stickleback *Gasterosteus aculeatus* L. 1758 and are similar to changes found in birds and mammals (O'Brien *et al.*, 2012). In salmonids, increased day length has been linked to increased plasma thyroxine (T_4) in Atlantic salmon *Salmo salar* L. 1758 smolts (McCormick *et al.*, 1995) and brain structural re-organization occurs prior to surges in thyroid hormone, affecting responses to environmental stimuli (Ebbesson *et al.*, 2003, 2011).

Smolting is a critical life-history event for anadromous salmonids, involving complex neuroendocrine, physiological and behavioural changes that act together to promote juvenile survival during downstream migration and the transition from fresh water to seawater (Hoar, 1988; McCormick, 2013). Increasing day length has been implicated as the most important environmental factor affecting the physiological aspects of smolting (McCormick *et al.*, 1998, 2002). There is also some evidence for an endogenous rhythm for smolting (Eriksson & Lundqvist, 1982; Duston & Saunders, 1995; McCormick *et al.*, 1995).

The smolt migration is characterized by four main phases (McCormick, 2013): initiation, downstream, estuarine and ocean migrations. Initiation is the initial departure from the freshwater rearing area. Both laboratory studies (Zydlewski *et al.*, 2005; Sykes & Shrimpton, 2010) and field studies (Zaugg & Wagner, 1973; Solomon, 1978; Muir *et al.*, 1994; Sykes *et al.*, 2009) have been used to explore factors that affect the initiation of migration. Although photoperiod has been implicated as the seasonal zeitgeber for physiological events that prepare salmonid smolts for migration, there is limited evidence for the role of photoperiod in regulating behavioural changes associated with smolting (Lundqvist & Eriksson, 1985; Martin *et al.*, 2012). Exposure of steelhead trout *Oncorhynchus mykiss* (Walbaum 1792) to artificial photoperiod regimes followed by release into the wild indicated that experiencing an increased day length was critical to development of downstream migratory behaviour (Wagner, 1974). These results were somewhat confounded by the fact that fishes were released into the wild where they experienced a different photoperiod from what they had been exposed to in laboratory treatments.

Increased day length may act to increase activity (migratory restlessness or zugunruhe) and make fishes sensitive to other factors that initiate migration (*i.e.* releasing factors). It has been suggested that photoperiod may determine the range of dates during which migration can occur but other factors such as water temperature, flow and turbidity may act as releasing factors (Baggerman, 1960). Temperature has been shown to be important in the timing of the behavioural release of smolts (Sykes & Shrimpton, 2010). An increase in temperature is not necessary for initiation of migratory behaviour, but it appears to act in concert with photoperiod to advance smolting, and it is possible that temperature could be a directive factor for migratory release in the absence of photoperiod cues. McCormick *et al.* (1998) suggested that temperature might act as a priming and releasing factor, along with flow and turbidity, to cue downstream movement of smolts. These authors also suggested that in the face of climate change there could be a mismatch of migratory timing and optimum conditions during ocean entry that would result in reduced smolt survival and adult return rates.

Evidence is mounting that a combination of cues is important for the initiation of downstream movement in salmonid smolts, and that photoperiod and temperature may be important predictors of the timing of initiation and the maintenance of migratory behaviours. Zydlewski *et al.* (2005) showed that, while temperature was important for

initiation of downstream movement in *S. salar* smolts, the number of degree days experienced was a better predictor of the timing of initiation of downstream movement, with temperature probably modifying the rate of response to photoperiod. Similarly, Sykes *et al.* (2009) showed that degree days (or accumulated temperature units) were more important than temperature alone for initiating downstream migration of Chinook salmon *Oncorhynchus tshawytscha* (Walbaum 1792) smolts in the field. Sykes & Shrimpton (2010) indirectly demonstrated the importance of photoperiod in controlling the release of downstream movement in *O. tshawytscha* under laboratory conditions when current velocity and temperature conditions were manipulated.

The relationships between environmental variables, behaviour and the neuroendocrine system are difficult to elucidate experimentally due to the difficulty of examining migration under controlled laboratory conditions. Juvenile salmonids may grow well, but in the absence of a seasonal photoperiod cue, the fishes do not exhibit developmental characteristics typical of smolts, *e.g.* neural, endocrine or physiological changes (Ebbesson *et al.*, 2007; Stefansson *et al.*, 2007). Although there has been a long association of smolt migration with elevated thyroid hormones (Hoar, 1988), recent research indicates that changes in thyroid hormones are a consequence of, not causal to, migration (Ojima & Iwata, 2009). Ojima & Iwata (2009) suggested that growth hormone-releasing hormone (GHRH) triggers the onset of downstream migration, whereas increases in plasma T₄ in migrating fishes occur because of the stimuli of active migration itself. Ebbesson *et al.* (2008) highlighted the importance of thyroid to circadian rhythms associated with the light–brain–pituitary axis. Structural changes in the brain, followed by changes in neurotransmitters and receptors commence prior to major surges in circulating thyroid hormone (Ebbesson *et al.*, 2003). Furthermore, thyroid hormones regulate the development of corticotropin-releasing factor (CRF) neurogenesis at the end of physiological smolting (Ebbesson *et al.*, 2011). It is suggested that once the development of the CRF system is complete a negative feedback occurs that downregulates the thyroid system concomitant with increased plasma cortisol levels (Ebbesson *et al.*, 2011). The timing of physiological changes and behavioural expression (*i.e.* downstream migration), however, may or may not be coincident.

Understanding the role of photoperiod in regulating migration, and how the photoperiod cue relates to other environmental influences such as flow and temperature will be critical to predicting the effect of climate change on salmonids. It is expected that in the future, seasonal increases in temperature will occur earlier in the year than currently is the case, whereas seasonal photoperiodicity will not change. If physiological limitations to survival imposed by temperature occur earlier each year, and this shift cannot be anticipated by juvenile salmonids that use seasonal changes in photoperiod, survival of smolts could be compromised by the temporal mismatch between the environmental cue they receive in fresh water and optimal conditions in salt water (McCormick *et al.*, 1998; Visser & Both, 2005; Kennedy & Crozier, 2010). If temperature modifies the phenological response of smolts to the photoperiod cue, or if temperature cues can be interpreted in the absence of a photoperiod cue, then smolts should be able to shift the timing of annual migrations to maintain current survival rates, all other factors being equal. Studies on birds show that species that respond phenologically to temperature as well as to photoperiod can still time migration to match appropriate arrival conditions in reproductive areas [*e.g.* geese *Anser brachyrhynchus* (Bauer *et al.*, 2008), pied flycatcher *Ficedula hypoleuca* (Both, 2010) and blackheaded bunting *Emberiza*

melanocephala (Singh *et al.*, 2011)], while others, those that respond to photoperiod alone, are not likely to match arrival timing as effectively (Møller *et al.*, 2008; Jones & Cresswell, 2010; McNamara *et al.*, 2011).

The goal of this work was to determine the importance of photoperiod in the initiation of downstream migration associated with smolting. This was accomplished by manipulating photoperiod and examining downstream movement behaviour under laboratory conditions. A statistical approach was used to determine the interaction of photoperiod and temperature in controlling smolt behaviour. Finally, known physiological changes associated with smolting, gill Na^+/K^+ -ATPase (NKA) and circulating thyroid hormone levels were examined to determine their connection to photoperiod-induced changes in downstream migration.

MATERIALS AND METHODS

FISH MAINTENANCE

Salmo salar parr from the White River National Fish Hatchery in Vermont, U.S.A., were transported in winter 1998 to the USGS Conte Anadromous Fish Research Center on the Connecticut River in Turners Falls, Massachusetts. Fish were kept in flow-through ambient river water in 1.5 m diameter tanks at 4–6° C until tagging and experimentation. Fish were fed pelleted Zeigler Brothers feed (www.zeiglerfeed.com) daily *ad libitum* before and during experimentation. On 12 February 1998, juvenile *S. salar* were anaesthetized with 100 mg MS-222 $^{-1}$ buffered with sodium bicarbonate (pH = 7.0), fork length (L_F) and mass (M) were recorded and non-lethal gill biopsies were taken for analysis of gill NKA activity. *Salmo salar* from 8.5 to 11.9 cm L_F were classified as putative parr. Fish >12.0 cm L_F were classified as putative smolts. These lengths and classifications were chosen based on previous work indicating that 12 cm is an appropriate size threshold in February to predict smolting for this strain of *S. salar* (McCormick *et al.*, 2007). All fish were tagged internally with a 23 mm long, 3.4 mm diameter, 0.6 g passive integrated transponder (PIT) tag with a unique eight-digit code (Texas Instruments; www.ti.com). A small incision (c. 4.0 mm) was made ventrally between the pectoral-fins and the tag was placed intraperitoneally. For fish <10.0 cm L_F , one suture was made in the incision to ensure closure.

BEHAVIOURAL EXPERIMENTATION

PIT tags were implanted on 12 February 1998 in putative smolts ($n = 20$ –21 per tank) and parr ($n = 8$ –11 per tank). Upon recovery, fish were immediately placed in six 1.5 m diameter circular tanks (two for each treatment) designed to record upstream and downstream movements of individually marked fish (Fig. 1). Each circular tank had a polyvinyl chloride (PVC) sheet barrier in the middle and an external pump circulated water around the barrier. Water supply to the tanks was flow-through. Water velocity through the narrow portion of the tank was maintained at 50 cm s $^{-1}$ and ranged from 10 to 40 cm s $^{-1}$ around the wide portion of the tank depending on distance from the outside wall. There was gravel and cobble substratum in the widened portion of the tank and black plastic was attached to one-half of the top of the tank to provide overhead cover. As in Zydlewski *et al.* (2005), two antennas were fitted to the narrow portion of each tank. The antennas were 0.3 m apart. Each antenna was wired to a separate TIRIS S2000 reader (Texas Instruments). When in the field of an antenna, a tag would be energized and the reader number, date, time and tag number were recorded on a computer. The readers were programmed to sequentially switch between pairs of antennas in each of four tanks every 5 min (two computers were used to run each set of four tanks). Tag detections at each antenna were recorded for 2.5 min (12–13 reads s $^{-1}$) during every 20 min period of the day. Behavioural recordings began on 19 February 1998 and continued until 23 June 1998.

Each photoperiod regime was simulated in two tanks, both of 1.5 m diameter. For the control treatment (LDN), photoperiod was maintained at simulated natural day length for the latitude of the laboratory (42° 30' N). On 2 March 1998, two additional photoperiod conditions were

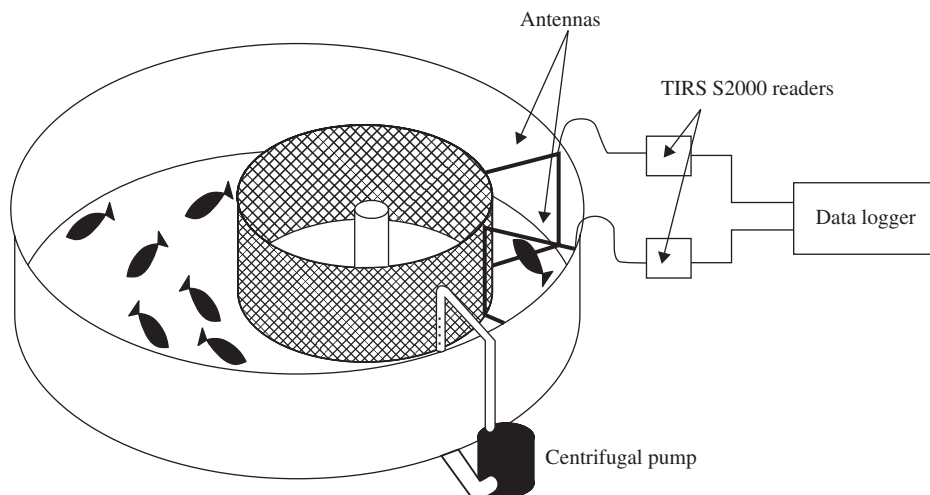


FIG. 1. Schematic representation of the circular stream tank used to monitor movements of individual *Salmo salar*. A barrier to water flow is shown as a cross-hatched structure in the centre of the tank. The barrier helped produce unidirectional water motion throughout the tank. Gravel and cobble substratum was on the bottom of the tank in the widened portion. Two antennas, encircling the water column in the narrowed region of the tank, were wired to readers and a data-logging computer. This figure is reprinted from Zydlewski, G. B., Haro, A. & McCormick, S. D. (2005). Evidence for cumulative temperature as an initiating and terminating factor in downstream migratory behavior of Atlantic salmon (*Salmo salar*) smolts. *Canadian Journal of Fisheries and Aquatic Sciences* **62**, 68–78. (C) Canadian Science Publishing or its licensors.

initiated (Fig. 2): a shortened photoperiod treatment or short day of 10L:14D, and a long day of 16L:8D. All treatments were exposed to ambient river water, which, after 4 April approximated a 1° C increase every fourth day (similar to the increase in water temperature in the Connecticut River over the previous 5 years), until 29 May when the temperature reached 20° C and remained fairly constant until the end of the experiment on 23 June (Fig. 2).

On 10 April and 21 May L_F , M , PIT tag number, non-lethal gill biopsies and non-lethal blood samples were taken from five to six smolts in each tank (for the two LDN tanks this was 12 in April and 10 in May; for both 10L:14D and 16L:8D, a total of 11 smolts were sampled on each date). Gill biopsies were used to determine gill NKA activity and plasma samples were used to determine plasma T_4 concentrations. All fish were returned to their original tanks to analyse behaviour subsequent to physiological sampling. Behavioural experiments were terminated on 23 June 1998. L_F , M , PIT tag number, gill biopsies and blood samples were taken from all fish. Preliminary data analysis indicated that fish of intermediate sizes (11–13 cm L_F) contained both parr and smolt (based on behaviour and gill NKA activity), so they were more conservatively classified as fish between 9.0 and 10.8 cm L_F in February (10.0 ± 0.1 cm, 9.9 ± 0.6 g, mean \pm s.e.) and fish >14.0 cm L_F in February (15.9 ± 0.1 cm, 42.8 ± 1.0 g), respectively (Table I). Only fish meeting these size criteria were used in data analyses. Final sample sizes for behavioural analyses were 12 parr and 34 smolts in the LDN treatment, 13 parr and 24 smolts in the 16L:8D treatment and 12 parr and 21 smolts in the 10L:14D treatment.

BEHAVIOURAL QUANTIFICATION AND ANALYSIS

Movements of parr and smolts were quantified to examine daily behaviour. The frequency distribution of tag detections revealed a cluster of alternating reads (between antennas) one below 0.3 s and one above 0.3 s. Those between 0.3 and 3 s were considered actual movements between antennas. Those below 0.3 s were considered crosstalk between the antennas and were eliminated from the data set. Also, those movements >3 s were probably around the widened portion

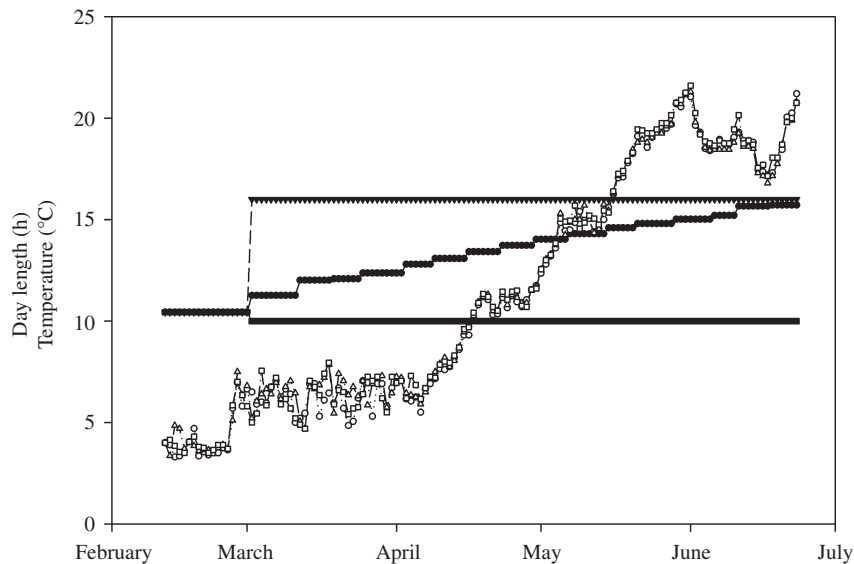


FIG. 2. Temperature (open symbols) and day length (closed symbols) exposure of experimental conditions for *Salmo salar* parr and smolts tested in behavioural evaluation tanks: simulated natural day length (LDN) (●), early increased day length (16L:8D) (▼) and shortened day length (10L:14D) (■). Manipulated photoperiod regimes are indicated (solid symbols).

of the tank and not direct movement between the antennas in the narrow portion of the tank and were also eliminated, because, in order to swim around the inner perimeter of the 1.5 m tanks in 3 s, a 20 cm fish would have to swim at 3.5 body lengths s^{-1} . This was considered not likely based on Fångstam *et al.* (1993) who reported movements of 2.3 body lengths s^{-1} during peak migration for 15–19 cm Baltic *S. salar* using a similar tank design. As such, individual fish total daily number of movements (for the 3 h of the day that recordings were made, 7.5 min h^{-1} , not extrapolated to a full day) was used to examine seasonal patterns of behaviour. Upstream movements were those consecutive detections (within >0.3 s and <3.0 s) that occurred between the downstream and upstream antennas (as dictated by the flow direction in each tank). Similarly, downstream movements were those consecutive detections that occurred between the upstream and downstream antenna. Individual variation is reported as mean \pm s.e.

The total number of downstream movements for each fish was calculated and used as the response variable to model factors affecting initiation and maintenance of downstream

TABLE I. Mean \pm s.e. initial fork length (L_{Fi}) and mass (M_i) and final fork length (L_{Ff}) and mass (M_f) of *Salmo salar* parr and smolts experiencing three different photoperiod treatments (natural photoperiod, LDN, increased day length 16L:18D and decreased day length 10L:14D) in simulated stream conditions in 1998

Mean size	LDN		16L:18D		10L:14D	
	Parr	Smolt	Parr	Smolt	Parr	Smolt
L_{Fi} (mm)	10.6 \pm 0.3	15.8 \pm 0.2	10.1 \pm 0.3	16.0 \pm 0.2	9.7 \pm 0.3	15.9 \pm 0.2
L_{Ff} (mm)	15.4 \pm 0.4	20.9 \pm 0.2	14.3 \pm 0.4	21.2 \pm 0.3	13.5 \pm 0.4	20.5 \pm 0.3
M_i (g)	11.8 \pm 0.2	42.4 \pm 1.4	9.5 \pm 2.3	44.6 \pm 1.7	8.5 \pm 2.4	42.6 \pm 1.8
M_f (g)	43.8 \pm 6.0	92.7 \pm 3.7	36.6 \pm 5.8	95.5 \pm 4.2	30.3 \pm 6.0	92.7 \pm 4.5

movements. Daily counts of downstream movements by smolts were zero-inflated due to (1) imperfect detection of fish and (2) lack of movement by individuals at the beginning and at the end of the smolt window, *i.e.* prior and subsequent to a substantial increase in downstream movements. In addition to being zero-inflated, daily count data were overdispersed due to variation in the positive (*i.e.* non-zero) counts, and as such the modelled distribution of count data was assumed to be negative binomial. Detection probability at PIT antennas was not quantified, and the zeros represented some combination of observation (detection) and process (lack of movement) error. To accommodate zero inflation and overdispersion in positive counts, zero-inflated negative binomial (ZINB) mixture models were used to describe variation in the count of daily downstream movements of individual smolts (Zuur *et al.*, 2009). Because repeated measurements were taken from each fish throughout the study, and there was the potential for heterogeneity in the response due to differences between individuals, a random effect of individual on the intercept was included in all models to explain this random error. All models were constructed and analysed using the 'glmm.ADMB' package (Skaug *et al.*, 2013) in programme R 3.0.2. (R Core Team; www.r-project.org).

A candidate model set was constructed to determine the relative plausibility of hypothesized relationships between photoperiod, temperature, degree days and fish behaviour. Photoperiod was included as a fixed effect with levels that were determined by the experimental treatment to which each fish belonged (LDN, 16L:8D and 10L:14D). Mean daily temperature (T , °C) was included as a continuous fixed effect, and it was hypothesized that downstream movement by smolts would decrease after a threshold temperature, representing the end of the smolt window for each treatment; therefore, a quadratic term was included for temperature in some models to determine if a threshold effect was present. Similarly, cumulative degree day (°D) experienced was included as a continuous covariate and the quadratic effect was included in some models because it was hypothesized that movement would begin to decrease after some threshold. Degree days were calculated as the sum of mean daily temperatures experienced after 1 January 1998.

Previous studies have suggested problems with multicollinearity associated with relations between temperature and cumulative degree day variables (Sykes *et al.*, 2009) so this problem was evaluated to determine the severity of multicollinearity in this study. The evaluation suggested a strong linear relation between mean daily temperature and cumulative degree days (Pearson correlation, $r = 0.87$, d.f. = 5693, $P < 0.001$), indicating that collinearity was a potential problem in this study. To confirm this, variance inflation factors (V_{IF} ; Montgomery *et al.*, 2006) were estimated for each covariate in each candidate model as: $V_{IFj} = \frac{1}{1 - R_j^2}$ where R_j^2 is the coefficient of determination when the j^{th} covariate is regressed on all remaining covariates in each model. When V_{IF} is greater than five, model parameters are poorly estimated and the confidence intervals associated with parameter estimates are inflated by a factor of $\sqrt{C_{jj}}$ (Montgomery *et al.*, 2006). In this study, the V_{IF} for mean daily temperature and cumulative degree days were 12.5 and 4, when the full additive model was considered. Therefore, no model was tested that included both cumulative degree days and mean daily temperature as covariates.

A total of 13 models that represented competing hypotheses were developed using various *a priori* combinations of covariates (Table II), and an information-theoretic approach using Akaike information criterion (AIC) was applied for model selection (Burnham & Anderson, 2002). The effect of each covariate was tested individually and in combination with the others. All models that included the second-order term for cumulative degree days or mean daily temperature also included the first-order term. Two models were developed containing interaction terms: one that hypothesized an interaction between treatment and cumulative degree days, and one that hypothesized an interaction between treatment and temperature. Both of these models included interactions between treatment and both the linear and quadratic terms. Models that included only the linear term for temperature or degree day were developed separately from those including both linear and quadratic terms for the variable of interest to test whether fish movement decreased after some threshold temperature or number of degree days.

To further analyse the relationship between degree days and downstream movement, the transition period from low to elevated downstream activity was defined as the absolute date when an individual smolt's downstream movements exceeded the 95th percentile of parr movements (20 downstream movements per day). For each smolt, the dates when activity exceeded this level and returned to this level were determined along with the associated environmental conditions on

TABLE II. Results of zero-inflated negative binomial (ZINB) model selection for models of *Salmo salar* smolt movement (count of daily downstream movements) in relation to photoperiod treatment (P), mean daily temperature (T , T^2), and cumulative degree days ($^{\circ}D$, $^{\circ}D^2$) and interactions. The number of parameters estimated in each ZINB: k , AIC_i is the Akaike information criteria for each i^{th} model and Δ_i is the difference between the AIC of each i^{th} model and the best model in the candidate set, and w_i is the relative probability that each i^{th} model is the best in the candidate set

Model	k	AIC_i	Δ_i	w_i
Count $\sim PT + PT^2 + (1 \text{Tag})$	13	35358.2	0.0	1.000
Count $\sim P^{\circ}D + P^{\circ}D^2 + (1 \text{Tag})$	13	35789.8	431.6	0.000
Count $\sim P^{\circ}D + (1 \text{Tag})$	10	36248.2	890.0	0.000
Count $\sim P + T + T^2 + (1 \text{Tag})$	9	36506.8	1148.6	0.000
Count $\sim T + T^2 + (1 \text{Tag})$	7	36576.4	1218.2	0.000
Count $\sim P + ^{\circ}D + ^{\circ}D^2 + (1 \text{Tag})$	9	36624.0	1265.8	0.000
Count $\sim ^{\circ}D + ^{\circ}D^2 + (1 \text{Tag})$	7	36702.4	1344.2	0.000
Count $\sim PT + (1 \text{Tag})$	10	36843.2	1485.0	0.000
Count $\sim P + ^{\circ}D + (1 \text{Tag})$	8	36843.6	1485.4	0.000
Count $\sim ^{\circ}D + (1 \text{Tag})$	6	36916.4	1558.2	0.000
Count $\sim P + T + (1 \text{Tag})$	8	37250.6	1892.4	0.000
Count $\sim T + (1 \text{Tag})$	6	37316.4	1958.2	0.000
Count $\sim P + (1 \text{Tag})$	7	37859.4	2501.2	0.000

those dates. These values and their differences were compared across treatments using analysis of variance (ANOVA).

PHYSIOLOGICAL SAMPLING AND ANALYSIS

Approximately four primary gill filaments taken from fish in February, April, May and June were placed in individual tubes with 100 μl of ice-cold SEI buffer (250 mmol of sucrose l^{-1} , 10 mmol of Na_2EDTA l^{-1} and 50 mmol of imidazole l^{-1} , $\text{pH} = 7.3$) and frozen within 1 h at -80°C . Gill NKA activity was measured using the microassay method of McCormick (1993). Plasma samples collected in April, May and June were assayed for T_4 . Concentrations were assayed by a direct radioimmunoassay (Dickhoff *et al.*, 1978) as modified by McCormick & Naiman (1984). A two-way repeated measures ANOVA on ranks (as the data were not normal and did not have homogeneous variances) was used to test for treatment and date effects on gill NKA activity and *post hoc* tests were used for multiple comparisons of treatment and date means. The thyroid hormone data set did not complete a full-repeated measures design; therefore, a one-way ANOVA comparing treatments within each month was used instead.

RESULTS

Downstream movements of smolts were influenced by the photoperiod treatments experienced. Upstream and downstream movements were documented for parr and smolts from February to June. Net movement was negligible in both parr and smolt from February to early April (Fig. 3). In mid to late April, downstream movements of smolts started to exceed those of parr for individuals in the LDN [Fig. 3(d)] and 16L:8D [Fig. 3(e)] treatments, but not in the 10L:14D treatment [Fig. 3(f)]. The movement patterns of parr did not change substantially over time in any of the photoperiod treatment groups [Fig. 3(a)–(c)].

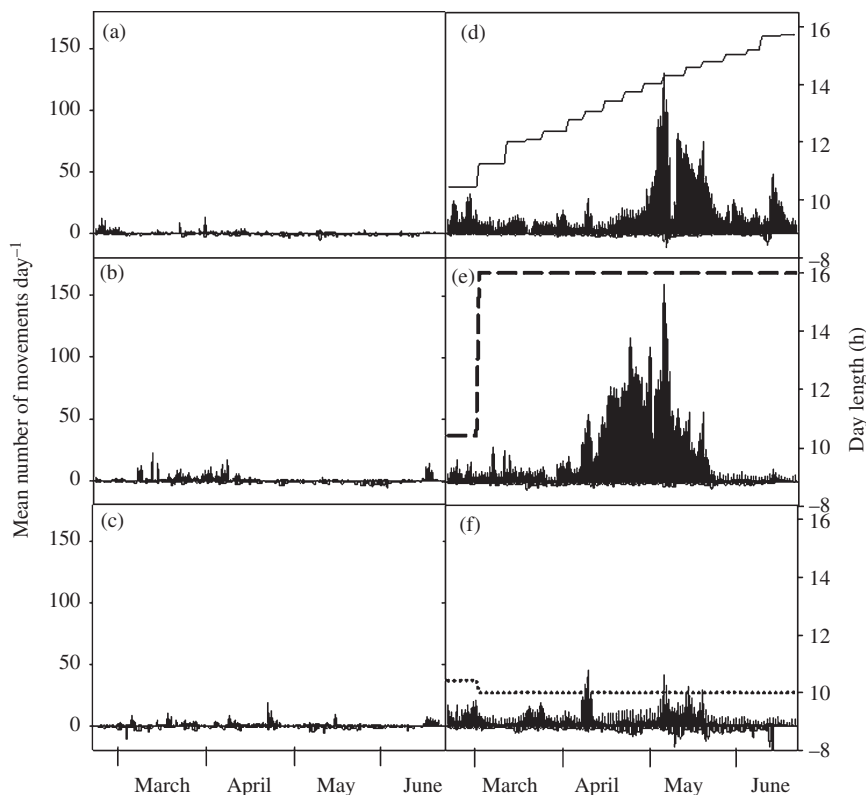


FIG. 3. Seasonal daily movements (negative, upstream and positive, downstream) of *Salmo salar* (a–c) parr and (d–f) smolts maintained under three photoperiod regimes: (a, d) (—), simulated natural day length (LDN), (b, e) ----, 16L:8D and (c, f) 10L:14D. The x-axis of each panel indicates the day of the year. The y-axis of each panel is the mean \pm S.E. number of movements per individual parr or smolt. The y2-axis is number of hours of light. All treatments had the same temperature regime shown in Fig. 2.

Smolts held in the LDN and 16L:8D treatments demonstrated significantly different patterns of downstream movements. Smolts in the LDN treatment demonstrated an increased frequency of downstream movements around 30 April [Fig. 3(d) and Table III] with a peak occurring during the week of 8 May. The average number of downstream movements declined to parr levels by 26 May (Table III). While the pattern and duration of downstream movement were similar in the early photoperiod treatment [16L:8D; Fig. 3(e) and Table III], the increase and decrease in downstream movements occurred significantly earlier for the 16L:8D treatment, on 17 April and 13 May (Table III), than in the LDN treatment. Yet, the number of days of increased activity did not differ between the LDN and 16L:8D treatments, lasting for an average of 26 days in both treatments.

With a shortened photoperiod (10L:14D), smolt behaviour was very different from behaviours in both the LDN and 16L:8D treatments. Overall, the number of downstream movements was greatly reduced under 10L:14D [Fig. 3(f)]. The increase in downstream movement was significantly later than in the other treatments, around 9 May (v. 17 or 30 April) with the decline to parr levels occurring only 8 days later

TABLE III. Mean \pm S.E. initial and final dates for when *Salmo salar* smolt downstream movements exceeded and decreased to (respectively) the 95th percentile of the downstream movement activity of parr when maintained under three different photoperiod treatments (natural photoperiod, LDN, increased day length 16L:18D and decreased day length 10L:14D) in simulated stream conditions in 1998. Different superscript lowercase letters after values in a row indicate statistically significant ($P < 0.05$) differences of the values within that row

Variable	Treatment		
	LDN	16L:18D	10L:14D
Date of increased activity	30 April \pm 0.9 ^a	17 April \pm 1.1 ^b	9 May \pm 1.6 ^c
Temperature (° C)*	12.4 \pm 0.2 Median = 12.4 ^a	12.1 \pm 1.3 Median = 9.8 ^b	15.3 \pm 0.2 Median = 15.0 ^c
Day length (h)	14.3	16	10
Degree days	695 \pm 11 ^a	577 \pm 13 ^b	835 \pm 24 ^c
Date of decreased activity	26 May \pm 2.2 ^a	13 May \pm 1.4 ^b	17 May \pm 2.3 ^{a,b}
Temperature (° C)	18.1 \pm 0.3 ^a	16.2 \pm 0.4 ^b	17.4 \pm 0.6 ^{a,b}
Day length (h)	14.8	16	10
Degree days	1114 \pm 42 ^a	920 \pm 23 ^b	965 \pm 39 ^{a,b}
Number of active days	26.15 \pm 2.56 ^a	25.96 \pm 2.04 ^a	4.00 \pm 1.72 ^b

*Data failed normality test (Shapiro–Wilk), Kruskal–Wallis run on ranks; therefore, median and range are more appropriate descriptors and were significantly different.

on 17 May. The average duration of downstream activity was significantly shorter (4 days) under the short day (10L:14D) treatment than the other treatments (26 days; ANOVA, $P < 0.001$; Table III).

Photoperiod treatment resulted in differences in overall activity level, expressed as the total daily number of downstream movements (Fig. 3). The number of downstream movements was significantly higher in the LDN and 16L:8D treatments than in the 10L:14D treatment. The most parsimonious model used to describe downstream movement of smolts under the conditions tested was the model that included synergistic effects of photoperiod treatment and temperature (Table II). Interactions between treatment (photoperiod) and temperature indicate that peak movement occurred at colder temperatures in the 16L:8D treatment [Fig. 4(a)] than in LDN, and temperature did not influence a peak in downstream movement of smolts in the 10L:14D treatment. Similarly, smolts in the 10L:14D treatment moved less in response to increasing degree days than those in LDN and 16L:8D [Fig. 4(b)] with those in 16L:8D increasing their activity at a significantly lower number of degree days (*i.e.* earlier) than those in LDN.

The effect of cumulative degree days since 1 January on initiation of smolt movement differed significantly among the different photoperiod treatments (ANOVA, $F_{2,61} = 67.167$, $P < 0.001$; Table III). For the LDN and 16L:8D treatments, increased activity occurred at 695 and 577 degree days, respectively. For smolts in the 10L:14D treatment, their downstream activity increased (exceeded parr levels) at 835 °D. Absolute temperature at which downstream activity increased was a better predictor of downstream movement behaviour than degree days. Downstream movements increased between 12 and 15° C for all treatments and there were no significant differences (Table III) (Kruskal–Wallis, $H = 21.192$, d.f. = 2, $P < 0.001$). Loss of downstream activity (decreased below parr levels) for fish under LDN and 16L:8D differed and occurred in late and mid May when temperatures reached 18 and 16° C,

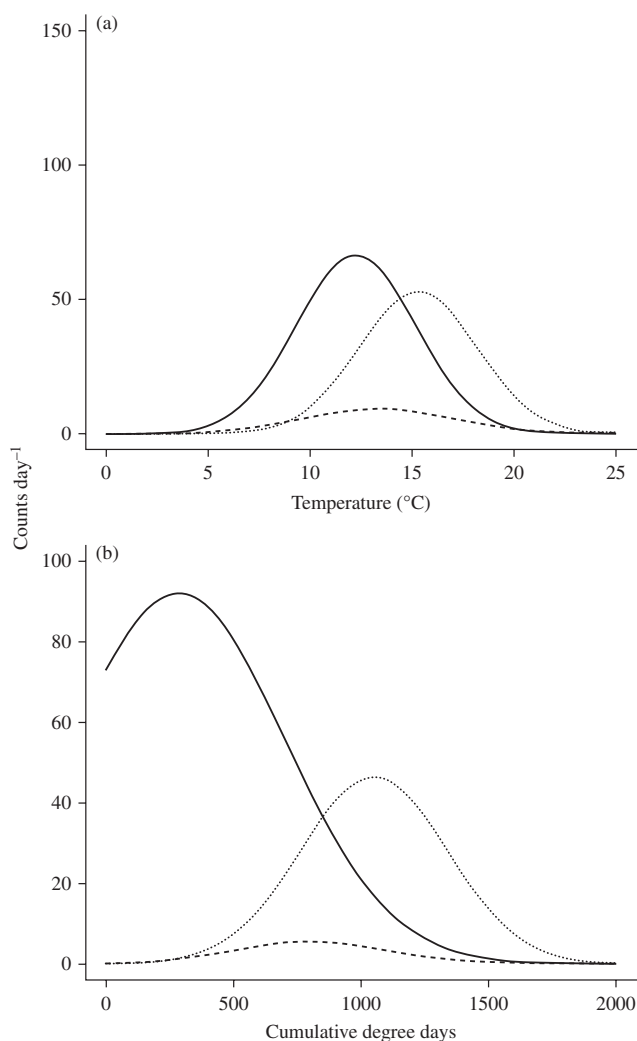


FIG. 4. Modelled downstream movements of *Salmo salar* smolts (counts day⁻¹), demonstrating the interactions of photoperiod treatment [—, 16L:8D; ---, 10L:14D; , simulated natural day length (LDN)] with (a) mean daily temperature and (b) cumulative degree days.

respectively. The decline in activity exhibited by fish in the 10L:14D treatment occurred at a similar temperature, 17° C.

Based on morphological appearance in June (silvering, loss of parr marks and dark-fin margins), all fish >14 cm in April underwent the parr–smolt transformation. Final L_F and M differed significantly between parr (final L_F = 14.4 cm) and smolts (final L_F = 20.9 cm) within treatments (Table I).

Photoperiod treatment had a significant effect on gill NKA activity (Fig. 5) and plasma T_4 levels (Fig. 6). Significant differences (two-way repeated measures ANOVA on ranks, treatment effect: $P < 0.001$, d.f. = 2; date effect: $P < 0.001$, d.f. = 3; interaction: $P < 0.001$) were due to significantly higher gill NKA activity for

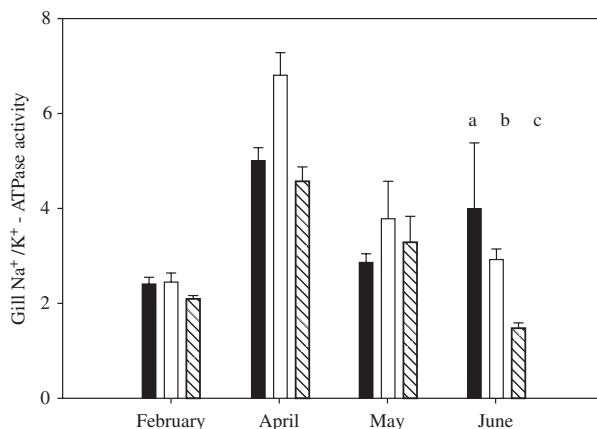


FIG. 5. Mean \pm S.E. from repeated measures analysis of variance (ANOVA) on gill Na⁺/K⁺-ATPase (NKA) activity non-lethally sampled from *Salmo salar* smolts maintained under three different photoperiod treatments [simulated natural day length (LDN) (■), 16L:8D (□) and 10L:14D (▨)] in simulated stream conditions in 1998. Within each time, statistical differences are indicated by different lowercase letters above the bars. For comparison, parr NKA activities were 1.46 ± 0.115 (LDN), 1.29 ± 0.053 (16L:8D) and 2.06 ± 0.78 (10L:14D).

smolts in all treatments in the month of April (v. February, May and June). In addition, there were significant differences among the treatments in June when gill NKA activity was highest for smolts in the LDN treatment and lowest for smolts in the 10L:14D treatment. Final gill NKA activity of parr was different between treatments (Figure 5; Kruskal–Wallis, $H=9.682$, d.f. = 2, $P<0.01$), the values were significantly lower than for smolts in all treatments except 10L:14D (two-way ANOVA on ranks, treatment effect: $P<0.001$; parr v. smolt effect: $P<0.001$; interaction: $P<0.001$). Photoperiod treatment effects were observed for plasma T₄ levels in April (Fig. 6; ANOVA, treatment effect: $F_{2,32}=16.686$, $P<0.001$). Smolts experiencing 16L:8D demonstrated the highest levels of plasma T₄ in April. In May, T₄ levels were significantly lower than in both April and June and there were no treatment differences in May or June.

DISCUSSION

This study provides the first direct observations that an increase in day length must be experienced for the initiation of downstream behaviour in *S. salar* smolts. In fact, it is shown that smolts exposed to an early increase in day length advanced the initiation of their downstream movement. With a shortened day length (10L:14D), however, smolts did not initiate downstream movement and only showed a slight increase in activity between 9 and 17 May. They never increased downstream activity to the level expressed by those smolts under simulated natural photoperiod conditions (LDN) which demonstrated increased downstream movement in late April, a peak in early May and a decline to parr levels by late May, as expected.

Smolts in the control (LDN) treatment demonstrated behaviour remarkably comparable to those recorded under similar control conditions before 1 year (Zydlewski *et al.*,

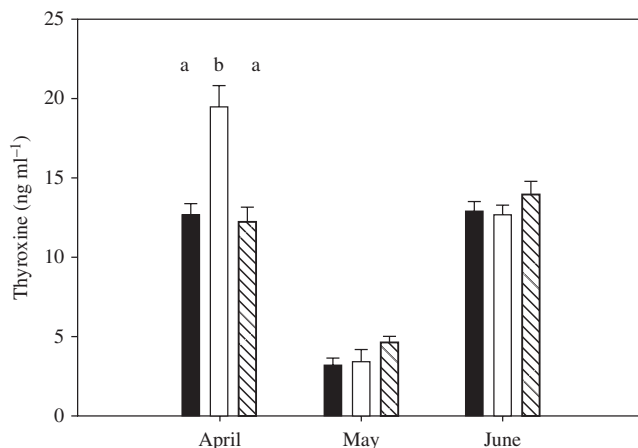


FIG. 6. Mean \pm S.E. levels of plasma thyroxine non-lethally sampled from *Salmo salar* smolts maintained under three different photoperiod treatments [simulated natural day length (LDN) (■), 16L:8D (□) and 10L:14D (▨)] in simulated stream conditions in 1998. Within each time, statistical differences are indicated by different lowercase letters above the bars.

2005). The date of increased activity was identical, 30 April, and the number of degree days did not differ, 695 ± 11 °D in this study and 688 ± 11 °D in the previous work by Zydlewski *et al.* (2005). The temperature of increased activity, however, was different: 12.4 ± 0.2 °C here and 9.1 ± 0.5 °C in Zydlewski *et al.* (2005). Together, this suggests that without increasing day length, temperature alone cannot modulate the initiation of downstream movement in *S. salar* smolts. This is consistent with the idea that photoperiod determines the range of dates during which migration occurs and other environmental conditions act as releasing factors (McCormick *et al.*, 1998).

An early increased day length also resulted in earlier initiation of movement. While movements occurred significantly earlier for fish experiencing an early and sustained photoperiod cue, they expressed similar activity intensity as those in the control treatment. Termination of movement in the sustained photoperiod treatment (16L:8D) was significantly earlier and at a lower temperature and number of degree days than the control (LDN). The number of active days was similar, however, *c.* 26 days total, indicating that behavioural responses to increased day length can occur out of synchrony with increasing temperature but that the duration of the smolt window (dates between which downstream movement activity exceeds parr downstream movement activity) is not affected. Zydlewski *et al.* (2005) found that termination of migration in smolts was related to number of degree days they experienced. These results indicate that initiation of migration is strongly dependent on photoperiod (with some interaction with temperature), whereas termination may be independent of photoperiod and strongly controlled by cumulative temperature. While the results show a clear link between degree days and smolt behaviour, the connection between the hours of light experienced, or light days, has not been explored and warrants further consideration.

The peak of downstream movement documented in the LDN treatment, during the first week of May, corresponded with migratory behaviour reported annually for wild fish at similar latitudes (Whalen *et al.*, 2011). In addition, here and in the field, migration occurred for a few weeks and then stopped. Numerous field studies have been

conducted to understand the phenology of smolt migration; however, many of these studies generated data that are correlative and subject to sampling bias (Zaugg & Wagner, 1973; Solomon, 1978; Muir *et al.*, 1994). Even in a more controlled study with the release of laboratory-reared fish, the link between temperature and photoperiod in the timing and duration of the smolt migration was difficult to elucidate (Wagner, 1974). The smolt migratory period could be extended when the temperature cycle was out of phase and behind the photoperiod cycle, but when temperature was out of phase and ahead of increasing day length, the migratory period was shortened. The complicated interactions of photoperiod and temperature should not be surprising since many organisms have evolved to rely on correlations among environmental cues and windows of opportunity to time behaviours associated with life-history transitions (Visser *et al.*, 2010; Bauer *et al.*, 2011). McNamara *et al.* (2011) suggested that sensitive life-history events that have high fitness consequences, such as smolting, should be regulated using multiple cues since single cues will often only have a low correlation with the best timing of such events. As such, dependence on one cue is risky, whereas combining multiple cues can increase the correlation between the cue and the best possible time; for example, photoperiod and temperature jointly determine the migration timing of geese (from Bauer *et al.*, 2008).

As previously hypothesized, photoperiod and temperature appear to interact to release downstream movement by *S. salar*. In this study, it was possible to separate photoperiod from temperature to show that the peak timing of smolt movement can be affected by temperature once a photoperiod cue has been received. Here and in other laboratory behavioural studies of *S. salar* and *O. tshawytscha* (Zydlewski *et al.*, 2005; Sykes & Shrimpton, 2010), initiation of smolt downstream movement has been jointly linked to temperature and photoperiod. Both studies concluded that a combination of either photoperiod or degree days was most strongly linked to initiation of downstream movement. Degree days were more strongly correlated with the behavioural smolting process than mean daily temperature, whereas the loss of behavioural smolt characteristics appeared to be more influenced by cumulative daily temperature.

Circannual rhythms have been suggested for controlling phenology and seasonal timing of critical life-history events (Visser *et al.*, 2010). For downstream migration of *S. salar* smolts, Lundqvist & Eriksson (1985) suggested that a circannual rhythm was involved in the annual cycle of swimming behaviour in previously mature male *S. salar*. They found an increase in downstream movements during spring with a reversal of swimming behaviour in late summer under constant photoperiod and temperature. Similarly, Martin *et al.* (2012) found that a weak residual cyclic activity was still detected and concomitant with recorded variations in water temperature when smolts were held under continuous darkness. An analogous residual activity in the 10L:14D treatment was found. When photoperiod was shortened, there was a weak (and highly variable) behavioural response, suggesting the possibility of a circannual rhythm since the behaviour was not completely absent. The physiological data also suggest a circannual rhythm of gill NKA activity. This has also been found in previous studies when day length was kept constant (McCormick *et al.*, 1995). Full expression of the behavioural response, however, may be more affected by multiple environmental conditions than the physiological response, *i.e.* requiring the presence of other releasing factors. It should be noted here that temperature increase did not allow the release of migratory behaviour with shortened photoperiod, suggesting the overriding control of photoperiod for the release of this behaviour.

In this study, smolt physiological status and behaviour were related but not completely coincident. Gill NKA activity increased in smolts in all treatments between February and April. Previous work has established that gill NKA activity can be advanced by an early increase in day length (McCormick *et al.*, 1995). In this study, gill NKA activity in April was 25% higher in 16L:8D fish relative to LDN fish, but activities did not differ significantly. Previous studies in this region have established that the peak of gill NKA activity under similar conditions occurs in late April or early May (McCormick *et al.*, 2002), and it is likely that the peak of gill NKA activity was missed early as a necessary consequence of minimizing disturbances to smolt behaviour. Had gill tissue been sampled closer to the peak of smolting, more obvious effects of photoperiod on gill NKA activity may have been observed. An effect of physically sampling fish on their behaviour in April was observed, however, so subsequently going into the tank was avoided until after the peak of behavioural changes.

The role of thyroid hormone in downstream migration of smolts is unclear (McCormick, 2013). Some evidence suggests a permissive nature of the hormone (Ebbesson *et al.*, 2011) and others indicate that plasma thyroid hormones increase in response to the act of migration (Ojima & Iwata, 2009), but all suggest a complicated relationship with multiple hormone systems. Plasma T_4 has been shown to increase in hatchery smolts after release and in wild smolts during migration (Iwata *et al.*, 2003; McCormick *et al.*, 2003; Ojima & Iwata, 2009), providing indirect evidence that thyroid hormones (TH) respond to migration. Indirect evidence also suggests that increases in thyroid hormones during migration are an outcome of other neuroendocrine factors rather than a driving factor for migration (Iwata *et al.*, 2003; McCormick *et al.*, 2003; Ojima & Iwata, 2010). When injected with gonadotropin-releasing hormone (GnRH) and CRF, juvenile *Oncorhynchus kisutch* exhibited downstream movement and had increased plasma T_4 levels (Ojima & Iwata, 2010). Ebbesson *et al.* (2011) also suggests a positive relationship between CRF and thyroid hormone during smolt development and proposed that thyroid hormone is stimulatory during the CRF neurogenesis early in development, ultimately resulting in the release of thyroid-stimulating hormone, with plasma T_4 peaking near the end of development (Ebbesson *et al.*, 2011).

Plasma T_4 levels appeared to be elevated prior to expression of downstream movement in this study. For example, plasma T_4 in April was significantly greater in the 16L:8D group compared to the LDN group 7 days prior to the date downstream movement was initiated in the 16L:8D treatment. This is inconsistent with Ojima & Iwata (2009) indicating that changes in thyroid hormones are a consequence of, not causal to, migration, but consistent with Ebbesson *et al.*'s (2003, 2011) hypothesis that the response to increased day length allowed the endocrine system to be more responsive than it was prior to movement. It is possible that the circadian rhythm associated with the light–brain–pituitary axis was altered by an early (and prolonged) increase in day length, resulting in structural changes and increased neurotransmitter levels with an ultimate earlier surge in circulating thyroid hormone. It is also important to note that the monthly samplings conducted in this study may have resulted in missing the peak of thyroid hormone and a shift in the development of fish in the 16L:8D treatment may account for these differences as well. Clearly, more research in this area is needed to examine the mechanisms connecting neuroendocrine, physiological and behavioural responses related to smolt migration phenology. Ultimately, interdisciplinary research with links among physiology, evolutionary ecology and molecular

genetics, as suggested by Visser *et al.* (2010), will be required to better explain the mechanisms controlling the initiation of smolt migration.

This study and statistical approach provide another tool for predicting smolt behaviour and timing under varying conditions in the natural environment that could be useful for management. The predictive model used here can be applied to estimate appropriate conditions (photoperiod and temperature) to stock hatchery-reared smolts during the smolt window. More variability in the behavioural response of fish that experienced unchanging day length (16L:8D or 10L:14D) than those experiencing simulated natural photoperiod (LDN) was observed. The variability in the response of smolts held in 16L:8D suggests that there may be individual plasticity in the response of *S. salar* smolts to these conditions of photoperiod and temperature. This is important when considering the cue-phenotypic responses and selection processes associated with climate change. Kennedy & Crozier (2010) provided evidence that smolt emigration timing is shifting based on local climate variation, resulting in a possible mismatch between the freshwater and marine environment. Otero *et al.* (2014) reported that, globally, *S. salar* smolt migration timing has been shifting significantly towards earlier median emigration: 2.8 ± 0.3 days per decade and over a 50 year period, 13.8 ± 1.5 days earlier. It is therefore important to consider smolt phenology in the context of climate change.

This study indicates that without a photoperiod cue a critical life cycle event, smolt downstream movement, will not occur. This is consistent with Anderson *et al.*'s (2013) suggestion that a cue, such as photoperiod, that is insensitive to climate change, may be relied on more by animal's with long migration routes. As temperature appears to modify the phenological response to the photoperiod cue, then smolts should be able to shift the phenology of annual migrations to maintain current survival rates, all other factors being equal. Temperature cues cannot be interpreted in the absence of a photoperiod cue and under warming climate conditions temperature-mediated responses could ultimately occur outside of the appropriate day length window. While an unrealistic shift to a 10 h day length is presented, these results should not be taken lightly since populations with migration timings that are cued by photoperiod are expected to exhibit weaker phenotypic plasticity for adapting to climate-induced mismatches between the environment where the cue for migration is received and optimal conditions in the target environment (Anderson *et al.*, 2013). Experiments with finer-temporal variation in day length (or natural photoperiod with fine-scale temperature changes) would be needed to determine when the day length window would be mismatched with the increase in temperature cues needed to initiate migration. Such experiments could be used to elucidate the effects of this shift on the degree of mismatch between environmental conditions and migration timing. Such an approach would be necessary to build on this and other work (McCormick *et al.*, 2002; Zydlewski *et al.*, 2005; Sykes *et al.*, 2009) to develop a yardstick (Visser & Both, 2005) for interpreting shifts in phenology that result in a match or mismatch with the environmental changes associated with climate change.

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References

- Anderson, J. J., Gurarie, E., Bracis, C., Burke, B. J. & Laidre, K. L. (2013). Modeling climate change impacts on phenology and population dynamics of migratory marine species. *Ecological Modelling* **264**, 83–97.
- Baggerman, B. (1960). Factors in the diadromous migrations of fish. *Zoological Society Symposium, London* **1**, 33–60.
- Bauer, S., Gienapp, P. & Madsen, J. (2008). The relevance of environmental conditions for departure decision changes en route in migrating geese. *Ecology* **89**, 1953–1960.
- Bauer, S., Nolet, B. A., Giske, J., Chapman, J. W., Åkesson, S. & Hedenström, A. (2011). Cues and decision rules in animal migration. In *Animal Migration—A Synthesis* (Milner-Gulland, E. J., Fryxell, J. M. & Sinclair, A. R. E., eds), pp. 68–87. Oxford: Oxford University Press.
- Both, C. (2010). Flexibility of timing of avian migration to climate change masked by environmental constraints en route. *Current Biology* **20**, 243–248.
- Bradshaw, W. E. & Holzapfel, C. M. (2007). Evolution of animal photoperiodism. *Annual Review of Ecology, Evolution, and Systematics* **38**, 1–25.
- Burnham, K. P. & Anderson, D. R. (2002). *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*, 2nd edn. New York, NY: Springer.
- Dickhoff, W. W., Folmar, L. C. & Gorbman, A. (1978). Changes in plasma thyroxine during smoltification of coho salmon, *Oncorhynchus kisutch*. *General and Comparative Endocrinology* **36**, 229–232.
- Duston, J. & Saunders, R. L. (1995). Advancing smolting to autumn in age 0+ Atlantic salmon by photoperiod, and long-term performance in sea water. *Aquaculture* **135**, 295–309.
- Ebbesson, L. O. E., Ekström, P., Ebbesson, S. O. E., Stefansson, S. O. & Holmqvist, B. (2003). Neural circuits and their structural and chemical reorganization in the light-brain-pituitary axis during parr-smolt transformation in salmon. *Aquaculture* **222**, 59–70.
- Ebbesson, L. O. E., Ebbesson, S. O. E., Nilsen, T. O., Stefansson, S. O. & Holmqvist, B. (2007). Exposure to continuous light disrupts retinal innervation of the preoptic nucleus during parr-smolt transformation in Atlantic salmon. *Aquaculture* **273**, 345–349.
- Ebbesson, L. O. E., Björnsson, B. Th., Ekström, P. & Stefansson, S. O. (2008). Daily endocrine profiles in parr and smolt Atlantic salmon. *Comparative Biochemistry and Physiology A* **151**, 698–704.
- Ebbesson, L. O. E., Nilsson, T. O., Helvik, J. V., Tronci, V. & Stefansson, S. O. (2011). Corticotropin-releasing factor neurogenesis during midlife development in salmon: genetic, environmental and thyroid hormone regulation. *Journal of Neuroendocrinology* **23**, 733–741.
- Eriksson, L.-O. & Lundqvist, H. (1982). Circannual rhythms and photoperiod regulation growth and smolting in Baltic salmon (*Salmo salar* L.). *Aquaculture* **28**, 113–121.
- Fängstam, H., Berglund, I., Sjöberg, M. & Lundqvist, H. (1993). Effects of size and early sexual maturity on downstream migration during smolting in Baltic salmon (*Salmo salar*). *Journal of Fish Biology* **43**, 517–529.
- Hazra, P., Sinha, A., Mondal, P. & Khan, T. N. (2012). Calendar-effects and temperature-impacts in migratory waterbirds at three tropical Indian wetlands. *Acta Oecologica* **43**, 60–71.
- Hoar, W. S. (1988). The physiology of smolting salmonids. In *Fish Physiology*, Vol. XIB (Hoar, W. S. & Randall, D. J., eds), pp. 275–343. New York, NY: Academic Press.
- Iwata, M., Tsuboi, H., Yamashita, T., Amemiya, A., Yamada, H. & Chiba, H. (2003). Function and trigger of thyroxine surge in migrating chum salmon *Oncorhynchus keta* fry. *Aquaculture* **222**, 315–329.
- Jones, T. & Cresswell, W. (2010). The phenology mismatch hypothesis: are declines of migrant birds linked to uneven global climate change? *Journal of Animal Ecology* **79**, 98–108.
- Kennedy, R. J. & Crozier, W. W. (2010). Evidence of changing migratory patterns of wild Atlantic salmon *Salmo salar* smolts in the River Bush, Northern Ireland, and possible associations with climate change. *Journal of Fish Biology* **76**, 1786–1805.
- Lundqvist, H. & Eriksson, L.-O. (1985). Annual rhythms of swimming and behaviour and seawater adaptation in young Baltic salmon, *Salmo salar*, associated with smolting. *Environmental Biology of Fishes* **14**, 259–267.

- Martin, P., Rancon, J., Segura, G., Laffont, J., Boeuf, G. & Dufour, S. (2012). Experimental study of the influence of photoperiod and temperature on the swimming behaviour of hatchery-reared Atlantic salmon (*Salmo salar* L.) smolts. *Aquaculture* **362–363**, 200–208.
- McCormick, S. D. (1993). Methods for non-lethal gill biopsy and measurement of Na^+ , K^+ -ATPase activity. *Canadian Journal of Fisheries and Aquatic Sciences* **50**, 656–658.
- McCormick, S. D. (2013). Smolt physiology and endocrinology. In *Euryhaline Fishes, Fish Physiology*, Vol. 32 (McCormick, S. D., Farrell, A. P. & Brauner, C. J., eds), pp. 199–251. Amsterdam: Academic Press.
- McCormick, S. D. & Naiman, R. J. (1984). Osmoregulation in the brook trout, *Salvelinus fontinalis*. II. Effects of size, age and photoperiod on seawater survival and ionic regulation. *Comparative Biochemistry and Physiology A* **79**, 17–28.
- McCormick, S. D., Björnsson, B. Th., Sheridan, M., Eilertson, C., Carey, J. B. & O'Dea, M. (1995). Increased daylength stimulates plasma growth hormone and gill Na^+ , K^+ -ATPase in Atlantic salmon (*Salmo salar*). *Journal of Comparative Physiology* **165**, 245–254.
- McCormick, S. D., Hansen, L. P., Quinn, T. P. & Saunders, R. L. (1998). Movement, migration, and smolting of Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences* **55**, 77–92.
- McCormick, S. D., Shrimpton, J. M., Moriyama, S. & Björnsson, B. Th. (2002). Effects of an advanced temperature cycle on smolt development and endocrinology indicate that temperature is not a zeitgeber for smolting in Atlantic salmon. *Journal of Experimental Biology* **205**, 3553–3560.
- McCormick, S. D., O'Dea, M. F., Moeckel, A. M. & Björnsson, B. Th. (2003). Endocrine and physiological changes in Atlantic salmon smolts following hatchery release. *Aquaculture* **222**, 45–57.
- McCormick, S. D., Shrimpton, J. M., Moriyama, S. & Björnsson, B. Th. (2007). Differential hormonal responses of Atlantic salmon parr and smolt to increased daylength: a possible developmental basis for smolting. *Aquaculture* **273**, 337–344.
- McNamara, J. M., Barta, Z., Klaassen, M. & Bauer, S. (2011). Cues and the optimal timing of activities under environmental change. *Ecology Letters* **14**, 1183–1190.
- Møller, A. P., Rubolini, D. & Lehikoinen, E. (2008). Populations species that did not show a phenological response to declining. *Proceedings of the National Academy of Sciences of the United States of America* **105**, 16195–16200.
- Montgomery, D. C., Peck, E. A. & Vining, G. G. (2006). *Introduction to Linear Regression Analysis*, 4th edn. Hoboken, NJ: John Wiley and Sons Inc..
- Muir, W. D., Giorgi, A. E. & Coley, T. C. (1994). Behavioral and physiological changes in yearling Chinook salmon during hatchery residence and downstream migration. *Aquaculture* **127**, 69–82.
- O'Brien, C. S., Bourdo, R., Bradshaw, W. E., Holzapfel, C. M. & Cresko, W. A. (2012). Conservation of the photoperiodic neuroendocrine axis among vertebrates: evidence from the teleost fish, *Gasterosteus aculeatus*. *General and Comparative Endocrinology* **178**, 19–27.
- Ojima, D. & Iwata, M. (2009). Central administration of growth hormone-releasing hormone triggers downstream movement and schooling behavior of chum salmon (*Oncorhynchus keta*) fry in an artificial stream. *Comparative Biochemistry and Physiology A* **152**, 293–298.
- Ojima, D. & Iwata, M. (2010). Central administration of growth hormone-releasing hormone and corticotropin-releasing hormone stimulate downstream movement and thyroxine secretion in fall-smolting coho salmon (*Oncorhynchus kisutch*). *General Comparative Endocrinology* **168**, 82–87.
- Otero, J., L-Abée-Lund, J. H., Castro-Santos, T., Leonardsson, K., Storvik, G. O., Jonsson, B., Dempson, J. B., Russell, I. C., Jensen, A. J., Bagliniere, J., Dionne, M., Armstrong, J. D., Romakkaniemi, A., Letcher, B. H., Kocik, J. F., Erkinaro, J., Pooke, R., Rogan, G., Lundqvist, H., Maclean, J. C., Jokikokko, E., Arnekleiv, J. V., Kennedy, R. J., Niemelä, E., Caballero, P., Music, P. A., Antonsson, T., Gudjonsson, S., Veselov, A. E., Lamberg, A., Groom, S., Taylor, B. H., Taberner, M., Dillane, M., Arnason, F., Horton, G., Hvidsten, N. A., Jonsson, I. R., Jonsson, N., McKelvey, S., Næsje, T. F., Øystein, S., Smith, G. S., Sægvog, H., Stenseth, N. C. & Vøllestad, L. A. (2014). Basin-scale phenology and

- effects of climate variability on global timing of initial seaward migration of Atlantic salmon (*Salmo salar*). *Global Change Biology* **20**, 61–75. doi: 10.1111/gcb.12363
- Richardson, A. D., Keenan, T. F., Migliavacca, M., Ryu, Y., Sonnentag, O. & Toomey, M. (2013). Climate change, phenology, and phenological control of vegetation feedbacks to the climate system. *Agricultural and Forest Meteorology* **169**, 156–173.
- Singh, J., Budki, P., Rani, S. & Kumar, V. (2011). Temperature alters the photoperiodically controlled phenologies linked with migration and reproduction in a night-migratory song-bird. *Proceedings of the Royal Society B* **279**, 509–515.
- Solomon, D. J. (1978). Migration of smolts of Atlantic salmon (*Salmo salar* L.) and sea trout (*Salmo trutta* L.) in a chalkstream. *Environmental Biology of Fishes* **3**, 223–229.
- Stefansson, S. O., Nilsen, T. O., Ebbesson, L. O. E., Wargelius, A., Madsen, S. S., Björnsson, B. Th. & McCormick, S. D. (2007). Molecular mechanisms of continuous light inhibition of Atlantic salmon parr-smolt transformation. *Aquaculture* **273**, 235–245.
- Sykes, G. E. & Shrimpton, J. M. (2010). Effect of temperature and current manipulation on smolting in Chinook salmon (*Oncorhynchus tshawytscha*): the relationship between migratory behaviour and physiological development. *Canadian Journal of Fisheries and Aquatic Sciences* **67**, 191–201.
- Sykes, G. E., Johnson, C. J. & Shrimpton, J. M. (2009). Temperature and flow effects on migration timing of Chinook salmon smolts. *Transactions of the American Fisheries Society* **138**, 1252–1265.
- Visser, M. E. & Both, C. (2005). Shifts in phenology due to global climate change: the need for a yardstick. *Proceedings of the Royal Society B* **272**, 2561–2569.
- Visser, M. E., Caro, S. P., Oers, K., Schaper, S. V. & Helm, B. (2010). Phenology, seasonal timing and circannual rhythms: towards a unified framework. *Proceedings of the Royal Society B* **365**, 3113–3127.
- Wagner, H. H. (1974). Photoperiod and temperature regulation of smolting in steelhead trout. *Canadian Journal of Zoology* **52**, 219–234.
- Whalen, K. G., Parrish, D. L. & McCormick, S. D. (2011). Migration timing of Atlantic salmon smolts relative to environmental and physiological factors. *Transactions of the American Fisheries Society* **182**, 289–301.
- Zaugg, W. S. & Wagner, H. H. (1973). Gill ATPase activity related to parr-smolt transformation and migration in steelhead trout (*Salmo gairdneri*): influence of photoperiod and temperature. *Comparative Biochemistry and Physiology B* **45**, 955–965.
- Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A. & Smith, G. M. (2009). *Mixed Effects Models and Extensions in Ecology with R*. New York, NY: Springer Science and Business Media.
- Zydlewski, G. B., Haro, A. & McCormick, S. D. (2005). Evidence for cumulative temperature as an initiating and terminating factor in downstream migratory behavior of Atlantic salmon (*Salmo salar*) smolts. *Canadian Journal of Fisheries and Aquatic Sciences* **62**, 68–78.

Electronic Reference

- Skaug, H., Fournier, D., Nielsen, A., Magnusson, A. & Bolker, B. (2013). *Generalized Linear Mixed Models using AD Model Builder, R Package Version 0.7.7*. Vienna: R Foundation for Statistical Computing. Available at https://r-forge.r-project.org/R/?group_id=847.