Are migration timing estimates based on tagged Atlantic salmon smolts (*Salmo salar*) biased?

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by

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Cover photo: Atlantic salmon smolts in a keep-net in the river Dale, by Tore Wiers.

ABSTRACT

Telemetry tags (e.g. PIT- or acoustic tags) are increasingly used in management to monitor the migration timing of Atlantic salmon smolts (*Salmo salar*). Recent findings, however, suggest that tagged fish consistently migrate earlier than untagged counterparts based on monitoring with other methods (e.g. trap-nets and video surveillance). It has been postulated that (1) effects from tagging and handling may alter migration behaviour, and (2) that the selection of fish during sampling is not representative of all migrating smolts, yielding bias in migration timing estimates. In the river Dale (Norway), we tagged five groups of wild Atlantic salmon smolts (n=385) at different time points in April-May, recaptured them in a wolf trap, and compared the migration timing to the untagged population from respective tagging dates. Migration timing was not significantly different between tagged (12 mm HDX PIT-tag) and untagged fish. Smolts tagged shortly before initiation of migration showed the most similar migration timing to the untagged population. Migration timing was size-dependent, such that larger individuals migrated earlier than smaller ones. The probability of migration was positively correlated with length and decreased the later in the season smolts were tagged. The migration timing of fish caught in the wolf trap was positively affected by water temperature and discharge. This pattern was not revealed by applying the same model to the tagged fish in the same system, exemplifying the limitations of using telemetry tags to study the mechanism of migration. This study revealed that tagging studies need to be careful when designing, as well as interpreting, their results, particularly related to size-dependent migration behavior.

1.0 INTRODUCTION

Populations of wild Atlantic salmon, *Salmo salar* L., are facing major threats and have experienced pronounced declines throughout its distribution over the past decades (Forseth et al., 2017a; Parrish et al., 1998). Besides its ecological and cultural importance, it has also served as the canvas for artificial selection within the farming industry (Forseth et al., 2017a). Atlantic salmon is an anadromous fish, meaning, it has life stages in both fresh- and saltwater. The adult salmon gather in freshwater rivers to spawn in late autumn (October-November), where the fertilized eggs are placed in the gravel of the riverbed. They hatch during early summer and stay in their natal river as juveniles (*parr*) for 1-5 years before they migrate to the sea (Forseth et al., 2017a). In advance, they transform to smolts (smoltification), a process induced by increased photoperiod (Hoar, 1988; McCormick et al., 2007; Saunders & Henderson, 1970) and involves extensive physiological, morphological and behavioural changes prerequisites for survival at sea (Aas et al., 2011; McCormick, 2012). For example, levels of gill Na^+K^+-ATPase increase to ensure osmoregulatory functioning upon sea entry (high salinity) (McCormick, 1993; Ugedal et al., 2014).

Despite significant reductions in exploitation (WGNAS, 2018), the marine survival of Atlantic salmon is low and has declined since the 1980s (Chaput, 2012; Forseth et al., 2017b). The exact processes that govern marine survival are relatively poorly understood (Jonsson & Jonsson, 2009), but are believed to reflect large-scale changes in ocean ecosystems and climatic factors (WGNAS, 2018). In some regions, particularly Western Norway, salmon lice from fish farms have had an additional impact on the marine survival of salmon populations, reducing the spawning numbers below what is perceived the carrying capacities of the rivers (Forseth et al., 2017b).

Knowledge regarding the timing of salmon smolt migration is extremely important for the understanding of the ecology and recent declines of Atlantic salmon, as it determines how and when a smolt encounters natural and man-made threats during its migration to feeding grounds (Myksvoll et al., 2020). Increased water temperature (Jonsson & Ruud-Hansen, 1985; Jutila et al., 2005; Whalen et al., 1999) and discharge (Hesthagen & Garnås, 1986; Hvidsten & Johnsen, 1993) are regarded as the proximate cues for migration (Jonsson et al., 2009). However, populations may respond differently to these environmental cues (Thorstad et al., 2011). Along the Norwegian coast, smolts enter the sea at different times of the year (Rikardsen et al., 2004), and even within the same watershed, it has been found that smolts in the upper tributary migrate earlier than those from the lower tributary (Stewart et al., 2006). This local adaptation resulted in a simultaneous sea entry from the entire watershed (River Tay, Scotland)

(Stewart et al., 2006). Hence, a possible explanation (ultimate cause) behind the variations in migration timing between populations may be to reach the ocean at a specific time, when conditions are favourable for growth and survival (Hvidsten et al., 2009; Rikardsen & Dempson, 2010).

One important aspect of the timing of migration is related to the likelihood of being infested with parasites during migration. The ectoparasitic salmon lice (*Lepeophtheirus salmonis* and *Caligus* spp) is a natural zooplankton component in the marine environment, but their numbers have increased drastically in line with host availability in fish farms (Jansen et al., 2012). As a consequence, infestation pressure on wild smolts has risen and is currently regarded as one of the most important man-made impacts on wild Atlantic salmon (Forseth et al., 2017b). Although fish farms delouse in spring, lice-induced mortalities in wild smolts are estimated to > 30 % in farm-intensive areas (Production zone 4, 2019) on the west coast of Norway (Vollset et al., 2019a). To mitigate, a newly ratified management system for regulating biomass in fish farms have been implemented – the so-called "Traffic light system" (Nilsen et al., 2017). The system uses models with a variety of factors (e.g. salmon lice concentrations and hydrodynamics) to determine infestation risks and to estimate lice-induced mortalities in wild smolts (Nilsen et al., 2017). In this model system, the timing of outwards migration of wild Atlantic salmon smolts has been repeatedly pointed out as one of the most sensitive parameters (Myksvoll et al., 2020; Nilsen et al., 2017). In many regions, data on outmigration of salmon is scarce, and numerous studies around Norway have therefore been initiated to fill these knowledge gaps (Anon, 2019).

Several methods have been used for monitoring the timing of smolt migration (e.g. video surveillance, traps, telemetry). Results from these studies indicate that the majority of smolts migrate out in May in southern Norway, while some start already from mid-April. For some of the rivers in southern Norway, historical data (5+ years) show considerable among-year variation (up to a month) in migration timing (50 % of smolts) from the same river (Ugedal et al., 2014). These variations are likely caused by climatic differences, where a cold winter and spring result in a later migration.

During the last decade different types of telemetry tags, such as PIT-tags (Box 1.1), have been used to monitor seaward migration in populations of wild Atlantic salmon (Barlaup et al., 2018). Normal practice has been to capture smolts once during spring, before smolt migration, and tag a random sample of the population. The tagged smolts are registered on antennas further downstream. Hence, it is assumed that the tagged group is representative of the entire population in terms of outwards migration. However, it has been a topic of debate whether the selection of fish, handling, and tag effects can alter migration and survival of smolts, in turn producing unwanted biases in the dataset estimating the time of migration. This is supported by recent findings suggesting that tagged fish consistently migrate earlier than other monitoring methods, such as video surveillance and traps (Vollset et al., 2019b).

One explanation for this difference in method estimates might be that the tagged fish is not representative of the population of salmon smolts in the rivers. For example, individuals lacking morphological signs of smoltification could be excluded from tagging, even though they might just be on a later developmental trajectory and thus change coloration and migrate later in the season. Individuals with morphological smolt signs will likely migrate earlier than those not yet fully transformed, and not necessarily be representative of all out-migrating smolts that season. Similarly, tagging-size restrictions can also affect the migration timing estimates if large fish migrate earlier.

These migration timing estimates have large implications for our understanding of Atlantic salmon ecology and for successful management and regulation of farmed fish. Therefore, it is pivotal to resolve these potential biases and understand how the different

Box 1.1. *What is a PIT-tag?*

A PIT-tag (Passively Integrated Transponder) is an internal tag with a unique mark (Fig. 1), providing individual information about survival, behaviour, and spatiotemporal movement (Vollset et al., 2018). Antennas deployed in the river produce magnetic fields which wirelessly charge the tag, allowing it to transmit its identification number back to a reader for registration (Armstrong et al., 1996). Detection range (distance from which a tag can be read) can be limited due to antenna power, tag orientation (parallel or perpendicular) and type (full- or half duplex), operation frequency or interference from other devices. In addition, detection range varies with tag size, the larger the tag the better the range (Biomark, 2019; Burnett et al., 2013).

FIGURE 1. *12 mm HDX PIT-tag (Biomark)*

methods impact the predicted migration timing.

This study aims to test if migration timing estimates from tagging studies of Atlantic salmon smolts are biased.

In this study, we tested the null-hypothesis that migration of wild Atlantic salmon smolts is independent of tagging time, size, handling, and release time during the season.

We investigated this by capturing, tagging, and releasing groups of smolts with 12 mm HDX PIT-tags at five different time points throughout April and May and recapturing them in a wolf trap. Length measurements were taken at tagging and recapture in the wolf trap. Migration timing was compared between each tagged group and the untagged population from the respective tagging dates.

My a priori hypotheses were that:

1. Tagged smolts are not representative of all out-migrating smolts due to either (a) unrepresentative sampling of the population, or (b) effects of tagging and handling on migration.
2. Migration timing is size-dependent. Large smolts likely migrate before small ones because they are physiologically ready earlier.

Also, to assess whether juvenile salmon recaptured in the wolf trap were indeed smolt with the capacity to migrate to sea, I measured gill Na^+K^+-ATPase activity in samples of wild smolts at four time points throughout May. Finally, I investigated the effect of water discharge and temperature on the daily smolt counts in the wolf trap, and if tagged fish to represent outmigration would render a similar response to these environmental factors.

2.0 MATERIALS AND METHODS

2.1 Study site

The present study took place during spring 2019 and was conducted in the river Dale (60º35'N, 5º49'E) on the west coast of Norway, in proximity to the city of Bergen. It is regulated by four hydropower plants that are supplied by water from two reservoirs and several impoundments (BKK; Sauterleute et al., 2016). The river Dale inhabits populations of both *Salmo salar* and anadromous *Salmo trutta* within 4.7 km from the river mouth up to a waterfall, Storefossen, acting as a natural barrier (Sauterleute et al., 2016). The river has a catchment area of 249 km^2 and a mean, yearly discharge of 21 m^3 s^{-1} (BKK; Vollset et al., 2016).

Electrofishing of wild Atlantic salmon smolts was carried out in a ~ 380 m river stretch 500 m upstream of the wolf trap and lowermost power plant (Fig. 2). The river stretch is a residual flow area that includes sandbanks, gravel, and boulders known to be suitable and frequently used spawning grounds for salmonids. A standardized procedure for capture and PIT-tagging of wild smolts was conducted at five different time points in April and May.

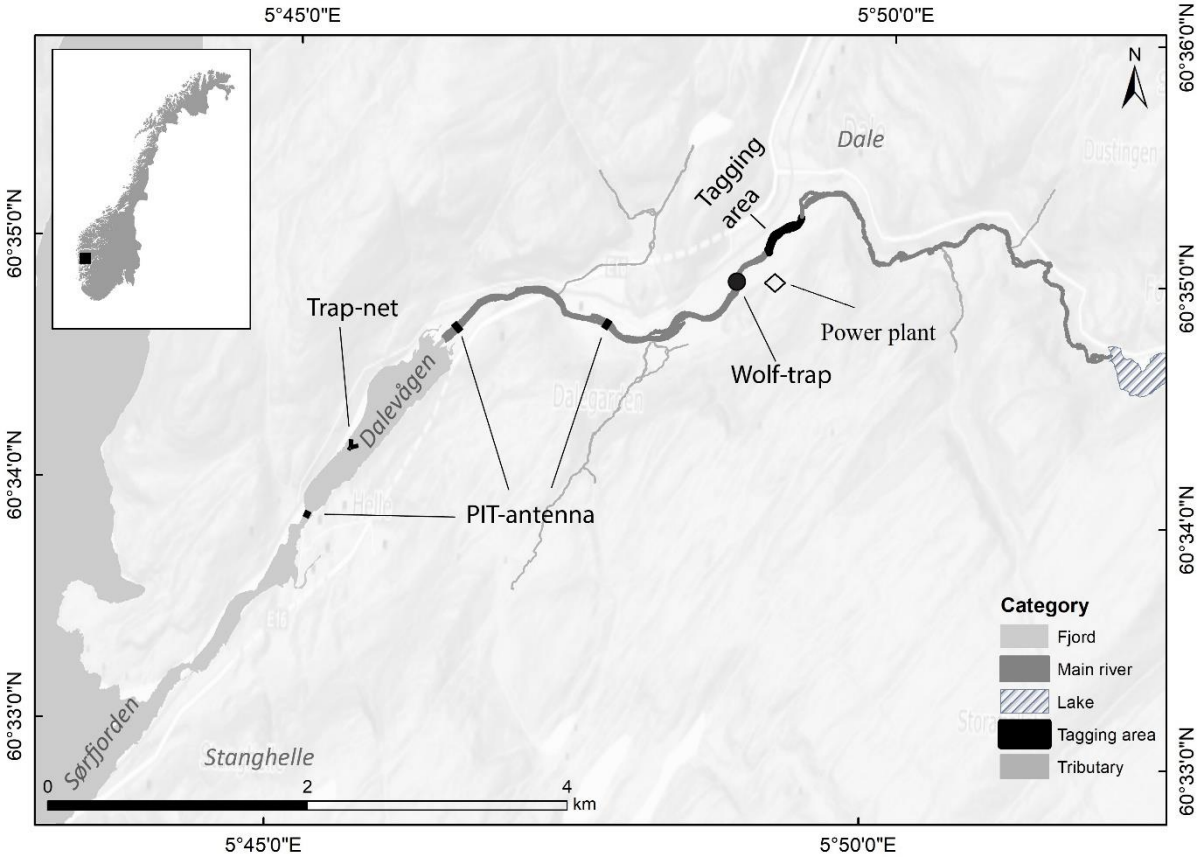

FIGURE 2. *Map showing the river Dale (Main river) in relation to tributaries and Sørfjorden. The locations of capture/tagging area, wolf trap, power plant, PIT-antennas, and trap-net are highlighted.*

2.2 Electrofishing of wild smolts

Electrofishing of wild Atlantic salmon smolts was conducted in accordance with the method described by Bohlin et al (1989).

Fish were electroshocked and retrieved from the water using a hand-net and visually identified as either salmon smolts or trout (*Salmo trutta*) based on morphological characteristics (Box 1.2). Due to size restrictions related to PIT-tagging, only smolts of total length (TL) ≥ 100 mm were captured. The fish were then transferred to a bucket with freshwater, which was frequently replaced to ensure adequate temperature and dissolved oxygen saturation.

Box 1.2. *How to distinguish smolts of Atlantic salmon from anadromous brown trout.*

The morphological characteristics used to distinguish salmon smolts and trout included size and shape of pectoral fins, distance the upper jaw reached posteriorly compared to the back of the eye, colour of adipose fin, in addition to the amounts of spots below the lateral line. Wild Atlantic salmon smolts have bigger, more wing-like pectoral fins compared to trout, and if a vertical line was drawn directly behind the eye the upper jaw would not usually extend behind it (Fig. 3). In terms of spots the salmon have few or none below the lateral line compared to trout. The adipose fin of wild smolts is less pronouncedly red relative to the adipose fin of trout (Fig. 4).

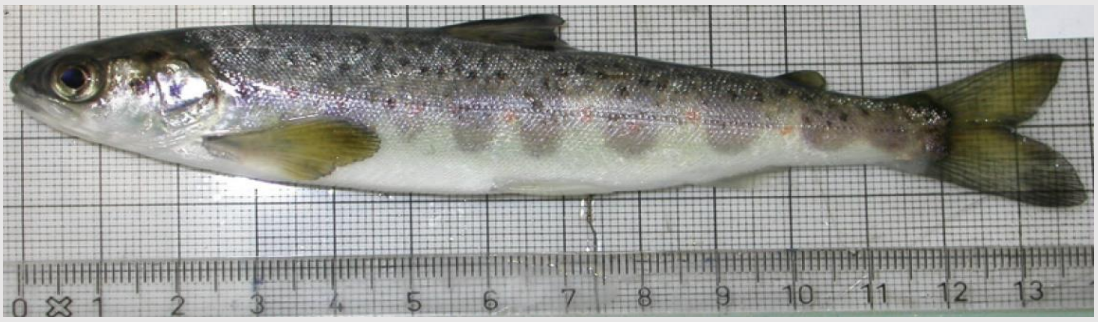

FIGURE 3. *Wild Atlantic salmon smolt (Salmo salar). Photo: NORCE LFI*

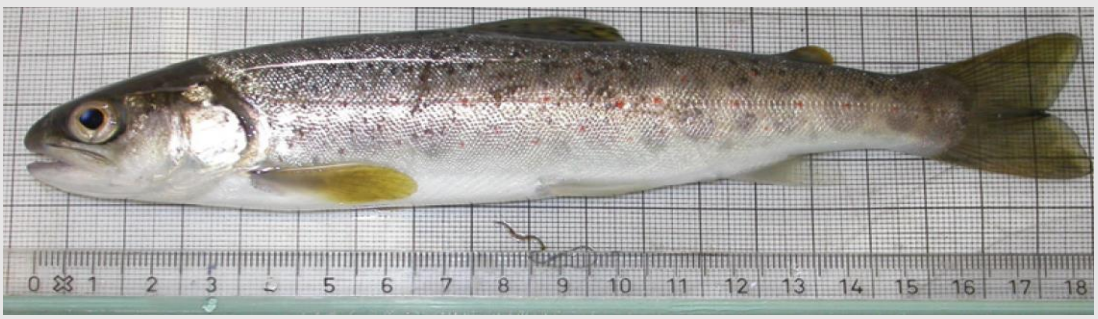

FIGURE 4. *Sea trout smolt (Salmo trutta). Photo: NORCE LFI*

Captured fish were regularly transferred to a keep-net to avoid crowding in the bucket (Fig. 5). The keep-net was positioned in an area with intermediate flow and if possible, shading. They were kept there until electrofishing of the river stretch was finished, approximately two hours for the smolts caught first, and 15 minutes for the ones most recently caught. Due to limited experience with the identification of smolts and early life-stage trout, every fish was double-checked and verified as a salmon presmolt/smolt by an experienced scientist before transferring to the keep-net.

2.3 PIT-tagging procedure and length measurements

Before PIT-tag implantation, fish were transferred in small batches (~ten fish per batch) from the keep-net to anaesthetic solution containing Tricaine mesylate (MS-222) and sodium bicarbonate ($NaHCO_3$) as a buffer, both with a final concentration of 100 mg L^{-1}. After approximately two minutes in anaesthetic solution the fish reached light anaesthesia with partial loss of equilibrium. This was evident when the fish struggled to maintain upright and did not react to stimuli. Thus, sufficient analgesia/pain relief had been induced and fish could be handled safely (Pharmaq, 2019).

Each individual was surgically implanted with a 100 mg, 12 mm long, 2.12 mm wide HDX PIT-tag (www.biomark.com). Beforehand, both the scalpel and the PIT-tag were sterilized in 100 % ethanol. A small (approximately 5 mm) incision was made between the posterior end of pectoral fins on either side of the midventral line (see Prentice et al., 1990). Next, the PIT-tag was inserted with the tip first in a vertical position. Once the tip was inside the abdomen the tag was tilted horizontally and pushed posteriorly (see Gries & Letcher, 2002). This way the tag should be positioned horizontally in the ventral parts of the abdomen. It was then visually inspected to ensure that the tag did not protrude from the wound, which subsequently could lead to tag loss. Afterwards, the fish was registered on a PIT-tag scanner (Biomark) and total length (TL) measured on an attached electronic length measuring board (BigFin scientific), both sterilized and covered in freshwater to protect the epidermal mucus of the fish. The assemblage was connected to an android tablet with DCS Linkstream application (Big Fin scientific). Hence, the tagged smolts could be registered and specific information such as date and length gathered. Next, the smolt was put in a recovery bucket containing freshwater. The tagging procedure took ~ 10-20 seconds per fish.

Once all the fish in a batch had been tagged, they were transferred from the recovery bucket to a second keep-net positioned in calm waters for further recovery (Fig. 5). This procedure was repeated until all smolts had been tagged and registered. Normal swimming

behaviour of fish was visually inspected before release back into the river to reduce the risk of predation-induced mortalities. All tagged smolts were released during daytime. An overview of the tagging dates and number of fish in each of the different PIT-tagged groups of wild Atlantic salmon smolts is presented in Table 1.

TABLE 1. *Tagging date/day and number of fish in each of the different groups of wild Atlantic salmon smolts tagged with 12 mm HDX PIT-tag in April and May.*

Date/day of tagging	Tagging group	Fish in tagging group (N)
15.04.2019/105	1	100
25.04.2019/115	2	98
03.05.2019/123	3	73
16.05.2019/136	4	71
24.05.2019/144	5	43
Sum		**385**

FIGURE 5. *Capture/tagging area of wild Atlantic salmon smolts in the river Dale including the set-up for PIT-tagging with keep-nets.*

2.4 Capture of wild Atlantic salmon smolts in the wolf trap

As an effort to obtain an overview of the temporal distribution for outwards migrating smolts in Dale, a wolf trap was positioned ~ 325 m downstream (60º34’54.2’’N, 5º48’46.0’’E) of the tagging area (Fig. 2). The trap covered the entire river, assuming it would capture all passing fish including the recapture of the different PIT-tagged smolt groups. It was deployed prior to and operative throughout the entire migration period. This allowed for comparisons in temporal migration patterns between tagged smolts and the untagged population.

The trap has a horizontal plate with grooves, onto which the fish swim. Steady water flow pushes the fish into a pipe, transferring them to a tank compartment (Fig. 7). The tank was filled with river water being continuously renewed. A net was attached to a frame surrounding the plate to prevent fish from jumping over or otherwise escaping from the trap (Fig. 6). Every morning, the tank compartment was emptied for fish. The fish caught were scanned for PIT-tags, length measured (TL), and registered on that date as either recaptures or untagged smolts, as outlined in section 2.3.

FIGURE 6. *Wolf trap set-up in the river Dale*

FIGURE 7. *Tank compartment containing fish caught in wolf trap.*

2.4.1 Temperature measurements and discharge data

To gather data and investigate how smolt migration relates to water temperature and water discharge, measurements were taken throughout the year. Temperature measurements were taken hourly in the wolf trap using an OTT Orpheus Mini Logger. Water discharge data were obtained from the power plant (BKK) and collected using a SonTek FlowTracker2.

2.5 Procedure for gill sampling

Gill samples for measurements of Na^+K^+-ATPase activity were taken from outwards migrating wild smolts at four different occasions throughout May, with approximately one-week intervals (Table 2). The objective was to identify if the fish caught in the wolf trap were smolts physiologically prepared for sea entry and to compare ATPase activity between time points. Approximately ten PIT-tagged wild smolts, captured in the wolf trap, were randomly chosen for each gill sampling. If the trap captures contained zero or few tagged individuals, the sample was supplied with untagged smolts. Every smolt was given an ID coupled with information about the origin, date, total length (TL), gill tube ID, and PIT-number (if tagged). Gill sampling was standardized and involved heart puncture before removal of the entire second gill arch. Each gill arch was placed in a labelled tube containing SEI-buffer (250 mM sucrose, 10 mM Na_2EDTA, 50 mM imidazole, pH 7.3) for conservation. Tubes were kept cold before and after insertion of the gill arch, then put in the freezer as soon as possible.

TABLE 2. *Date of gill sampling, sample size, and proportion PIT-tagged of wild Atlantic salmon smolts captured in the wolf trap.*

Date of gill sampling	Sample size (N)	Proportion PIT-tagged
03.05.2019	10	0
16.05.2019	5	1
24.05.2019	10	9
31.05.2019	10	9

2.6 Measurement of Na^+K^+-ATPase activity in gill samples

Gill Na^+K^+-ATPase activity was analyzed according to the method described in McCormick (1993). The gill filaments obtained from the wolf trap were thawed before assemblage of the kinetic assay. The production of adenosine diphosphate (ADP) in the presence of Na^+K^+-ATPase is ouabain-sensitive (ouabain inhibits Na^+K^+-ATPase). The reaction is enzymatically coupled to the oxidation of nicotinamide adenine dinucleotide (NADH) by pyruvate kinase and lactic dehydrogenase, which could be directly measured on a Spark multicode microplate reader at 340 nm (25ºC, 60 cycles, 10 min). Protein in the homogenate was determined by bicinchoninic acid method according to Smith et al (1985). The Na^+K^+-ATPase activity was measured as the difference in activity, with and without ouabain present as an inhibitor, expressed as µmol ADP mg protein^{-1} h^{-1}. For detailed information see Appendix 1.

2.7 Statistical analysis

All data analyses were conducted in R, version 3.6.0 (R Core Team, https://www.r-project.org/). The following additional packages were used: Tidyverse package set (Wickham, 2017), patchwork (Pedersen, 2019), mgcv (Wood, 2017), and AICcmodavg (Mazerolle, 2019). Corrected Akaike information criterion (AICc) was used to select the best linear models (lm) and generalized linear model (glm) by stepwise selection (backwards elimination). The best generalized additive models (GAM) were found by comparing all combinations and selecting the model with the lowest AIC.

2.7.1 Na^+K^+-ATPase activity in out-migrating wild Atlantic salmon smolts

A linear model was used to compare gill Na^+K^+-ATPase activity (measured as µmol ADP mg protein^{-1} h^{-1}) between migrating smolts captured in the wolf trap at four different sampling dates in May, and to investigate if length affected Na^+K^+-ATPase activity.

2.7.2 Capture efficiency of the wolf trap

Although the wolf trap was assumed to capture all fish passing it, a proportion of the tagged smolts were detected on PIT-antennas or in the trap-net downstream without being captured in the wolf trap first. Assuming the likelihood of being observed downstream of the wolf trap is the same for all individuals, it is possible to estimate the total number of tagged smolts that were able to cross the wolf trap without being captured (X1), using equation 1.

$$\frac{X1}{X2} = \frac{Y1}{Y2} \qquad \text{[eq.1]}$$

Where X1 = total number of tagged smolts able to cross wolf trap, X2 = smolts crossed wolf trap and detected downstream (n=10), Y1 = smolts released from wolf trap (n=212), Y2 = smolts released from wolf trap and detected downstream (n=47).

2.7.3 Does tagging length and tagging date affect probability of migration?

To investigate if the time of tagging during the season and the length of a smolt at tagging affected the probability that a smolt would migrate, I ran a logistic regression using a generalized linear model (GLM) with binomial distribution (Appendix 3). In addition, the mean length at tagging was compared between (1) the smolts that had, and (2) the smolts that had not migrated for each PIT-tagged group of wild Atlantic salmon smolts. That being (1) those either recaptured in wolf trap, detected on PIT-antennas or trap-net downstream, and (2) those not.

2.7.4 Is the migration timing different between PIT-tagged smolts and the untagged population?

To test if there was a temporal difference in out-migration between a PIT-tagged group and the untagged population, a two-sided Kolmogorov-Smirnov test (KS-test) was performed on cumulative relative proportions. The test statistic, D, represents the maximum absolute difference between the two cumulative relative proportions (Kirkman, 1996). If the two samples were drawn from the same distribution (H_0), the D-statistic should be close to zero. The p-value is the probability of finding a D-statistic which is at least as large as what we found, if the null-hypothesis is true (Kirkman, 1996). To reduce the chance of type 1 error, the H_0 was tested using Bonferroni adjusted alpha levels of 0.01 per test (0.05/5). Because untagged fish started out-migrating before some of the groups were tagged, temporal patterns in out-migration of each group was compared to the untagged population from each respective tagging date. For example, migration in group 2 (tagged 25.04.2019) was compared to the migration of the untagged population from that tagging date.

2.7.5 Is length at migration for PIT-tagged smolts and untagged smolts the same?

A potential difference in mean length at out-migration between tagged and untagged smolts was tested using a two-sample t-test.

2.7.6 Is timing of migration a function of tagging length and tagging date?

A linear model was used to test if migration timing (day of the year) depended on the length of smolts when tagged and the date of tagging (day of the year) (Appendix 3).

2.7.7 Does water discharge and temperature affect migration timing in Atlantic salmon smolts?

A potential relationship between daily counts of wild Atlantic salmon smolts in the wolf trap and date (day of the year), mean daily water discharge and temperature was investigated using a generalized additive model (GAM) with a negative binomial distribution, due to the potential non-linearity between response and predictors (Appendix 3).

Low count numbers late in the season are not necessarily a small response to predictors, but rather due to the emptying of fish from the river throughout the season. Therefore, each observation was weighted based on the number of fish remaining in the river at the time of the observation. Because the wolf trap was emptied for fish each morning, I also investigated if discharge or temperature lag by one day would be better explanatory variables rather than the measurements the day of capture, with the rationale that the captures in the wolf trap were potentially caused by discharge or water temperature from the previous night. Potential autocorrelation was investigated using acf and pacf on model residuals (van RiJ, 2016).

To investigate if tagged smolts would render the same final model as data from the whole population, I used the same modelling procedure as explained for the data in the wolf trap for PIT-group 1 (n=64). The model was fitted using a Poisson distribution because residual variance indicated a better fit. The response of the other tagged groups to date, water discharge, and temperature could not be explored because there were not enough recaptures in the wolf trap from these groups to model a response.

3.0 RESULTS

3.1 Na+K+-ATPase activity in out-migrating wild Atlantic salmon smolts

No explanatory variables were included in the best linear model (lowest AICc), indicating that date of gill sampling and length of smolts did not affect Na^+K^+-ATPase activity in wild Atlantic salmon smolts captured in the wolf trap. Na^+K^+-ATPase activity ranged from 5.0 to 16.4, with the grand mean being 11.2 (± 3.1 SD) µmol ADP mg protein^{-1} h^{-1} (Fig. 8).

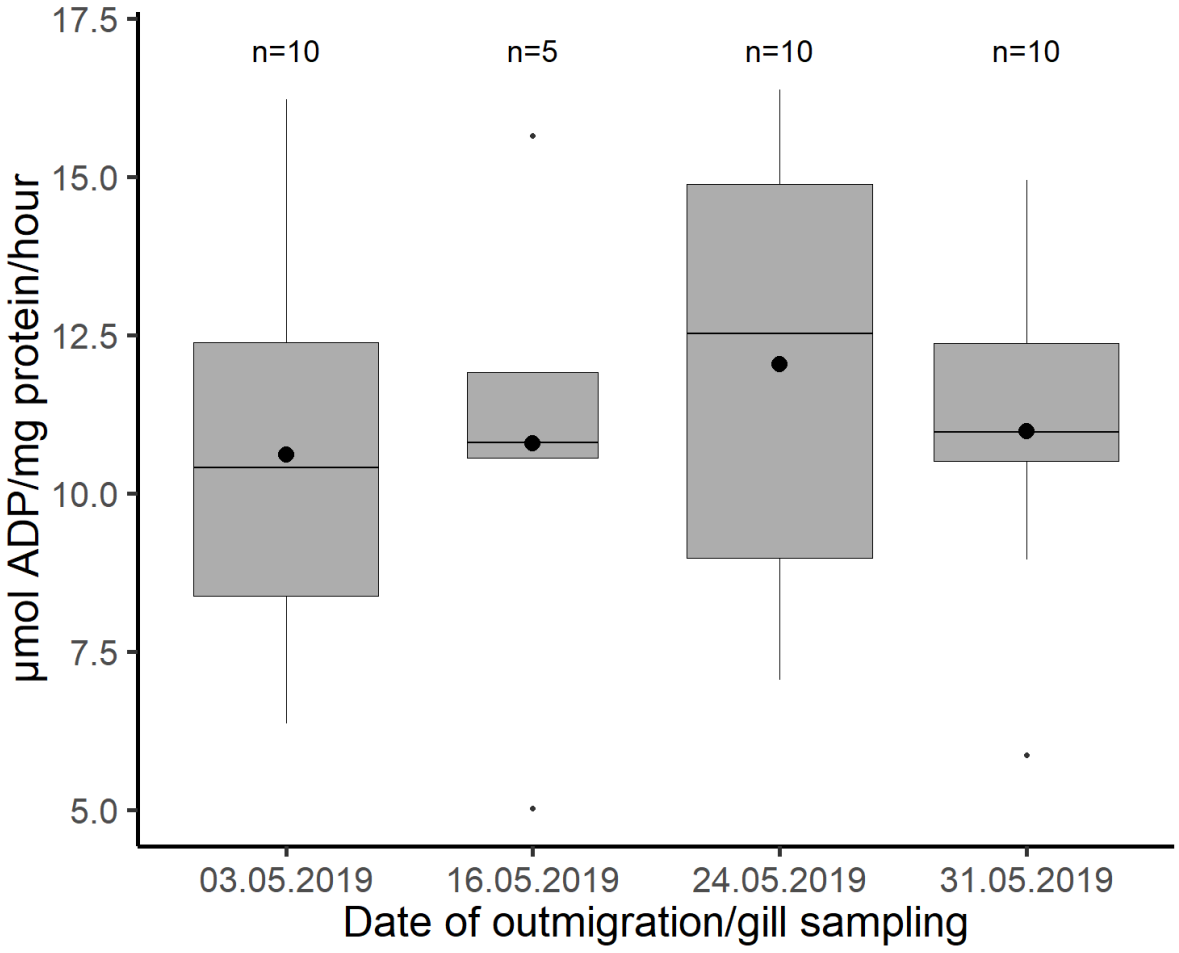

FIGURE 8. *Na^+K^+-ATPase activity in wild Atlantic salmon smolts at the date of outmigration (wolf trap capture) during May. Line in the box represents the median, the dot represents the mean.*

3.2 Capture efficiency of the wolf trap

Out of the 385 PIT-tagged smolts in the river Dale, a total of 231 (60%) were recaptured in the wolf trap. Subtracting the tagged smolts euthanized for gill sampling (n=19) results in 212 smolts released from the wolf trap, out of which 47 (22 %) were observed on PIT-antennas (70%) or in the trap-net (30%) downstream of the wolf trap. The wolf trap was assumed to capture all fish passing it, but still, 10 of the remaining 154 smolts were detected downstream. Seven of the 10 were detected on PIT-antennas, the rest were caught in the trap-net. No pattern was found in either group origin or detection timing.

From the 154 tagged smolts that were not recaptured in the wolf trap, an estimated 45 individuals (29%) had been able to cross the wolf trap. That results in an estimated 109 tagged smolts left upstream of the wolf trap (28 %), whereas an estimated 276 (231+45) tagged smolts migrated (72 %).

3.3 Does tagging length and tagging date affect probability of migration?

The mean length of smolts that did (n=241) and did not (n=144) migrate was 133 mm (± 8 SD) and 131 mm (± 10 SD), respectively. The probability of migration increased with length at tagging (glm, $\beta = 0.02$, $z = 2.11$, $p = .04$). For example, when tagged on day 115, a smolt of length 160 mm (78.9 %) had a 15 % higher probability of migrating than a smolt of length 130 mm (63.9 %) (Table 3). Smolts tagged later in the season had a lower probability of migration (glm, $\beta = -0.02$, $z = -2.10$, $p = .04$; Table 3). For instance, a smolt of length 130 mm tagged on day 144 (51.8 %) had a 16 % lower probability of migrating than a smolt of similar size tagged on day 105 (67.8 %).

TABLE 3. *The difference in mean length (TL) at tagging between (1) the proportion that did, and (2) did not migrate out for the five PIT-tagged groups of wild Atlantic salmon smolts. Out-migrated=yes defined as smolts either recaptured in wolf trap, detected on PIT-antennas, or caught in trap-net downstream. Out-migrated=no represents those not recaptured nor detected. Tagging group ID and date are presented.*

Group of PIT-tagged smolts	**Date/day tagged**	**Number of smolts (n)**		**Mean total length (mm) at tagging (SD)**		**Difference in mean total length (mm) at tagging between (1) smolts that out-migrated and (2) those that did not**
		Out-migrated		**Out-migrated**		
		No	Yes	No	Yes	
1	15.04.2019/105	32	68	130.8 (9.6)	131.9 (8.4)	+ 1.1
2	25.04.2019/115	35	63	129.1 (11.5)	130.4 (8.5)	+ 1.3
3	03.05.2019/123	31	42	130.3 (8.7)	132.1 (8.6)	+ 1.8
4	16.05.2019/136	22	49	136.9 (10.9)	136.6 (7.0)	- 0.3
5	24.05.2019/144	24	19	131.3 (9.8)	138.0 (7.1)	+ 6.7

The proportion of tagged smolts migrating (recaptured in the wolf trap) was similar for group 1-4, ranging from 56 to 66 %. In group 5, tagged the latest (24.05.2019), only 18 individuals out of the 43 tagged (42%) ended up migrating (Table 4).

TABLE 4. *Overview of tagging time, sample size, and proportion out-migrated (recaptured in the wolf trap) for the five PIT-tagged groups of wild Atlantic salmon smolts.*

Group of PIT-tagged smolts	**Date/day tagged**	**Smolts in group (N)**	**Proportion recaptured in wolf trap (n)**	**Proportion recaptured in wolf trap (%)**
1	15.04.2019/105	100	64	64
2	25.04.2019/115	98	61	62
3	03.05.2019/123	73	41	56
4	16.05.2019/136	71	47	66
5	24.05.2019/144	43	18	42
Sum		**385**	**231**	

3.4 Is the migration timing different between PIT-tagged smolts and the untagged population?

Although on average all five tagging groups migrated later than the comparative non-tagged smolts, none of them were significantly different after Bonferroni correction (Group 1: KS-test: $D = 0.202$, $p = .39$; Fig. 9A, n=64; group 2: KS-test: $D = 0.267$, $p = .17$; Fig. 9B, n=61; group 3: KS-test: $D = 0.211$, $p = .52$; Fig. 9C, n=41; group 4: KS-test: $D = 0.415$, $p = .05$; Fig. 9D, n=47; and group 5: KS-test: $D = 0.515$, $p = .08$; Fig. 9E, n=18). The cumulative out-migration of group 1, tagged before migration had started, was most similar to the untagged population out of the different tagged groups (KS-test: $D = 0.202$, $p = .39$; Fig. 9A, n=64).

The difference in median out-migration timing (50 % of the group) for tagged smolts was 3-9 days (Fig. 9; Table 5). The largest difference occurred in groups 2 and 3 (tagged late April-early May), with nine and eight days later than the untagged population, respectively (Table 5). The smallest difference in median out-migration was in group 1 (n=64), the earliest tagging date, and group 5 (n=18), the latest tagging date. Note that, at the time of tagging for group 5 (24.05.2019), 74 % of the untagged population had already migrated out.

Table 5. *Median out-migration day (50% of the group) for each PIT-tagged group compared to that of the untagged population (from date of tagging for respective groups). The difference (number of days) in median out-migration timing is presented.*

Group of PIT-tagged smolts	Median outmigration date/day (50% of PIT-group)	Median outmigration date/day (50% of group) for untagged population	Difference (number of days) in median out-migration timing for PIT-group compared to untagged population
1	20.05.2019/140	16.05.2019/136	+ 4
2	25.05.2019/145	16.05.2019/136	+ 9
3	30.05.2019/150	22.05.2019/142	+ 8
4	30.05.2019/150	25.05.2019/145	+ 5
5	03.06.2019/154	31.05.2019/151	+ 3

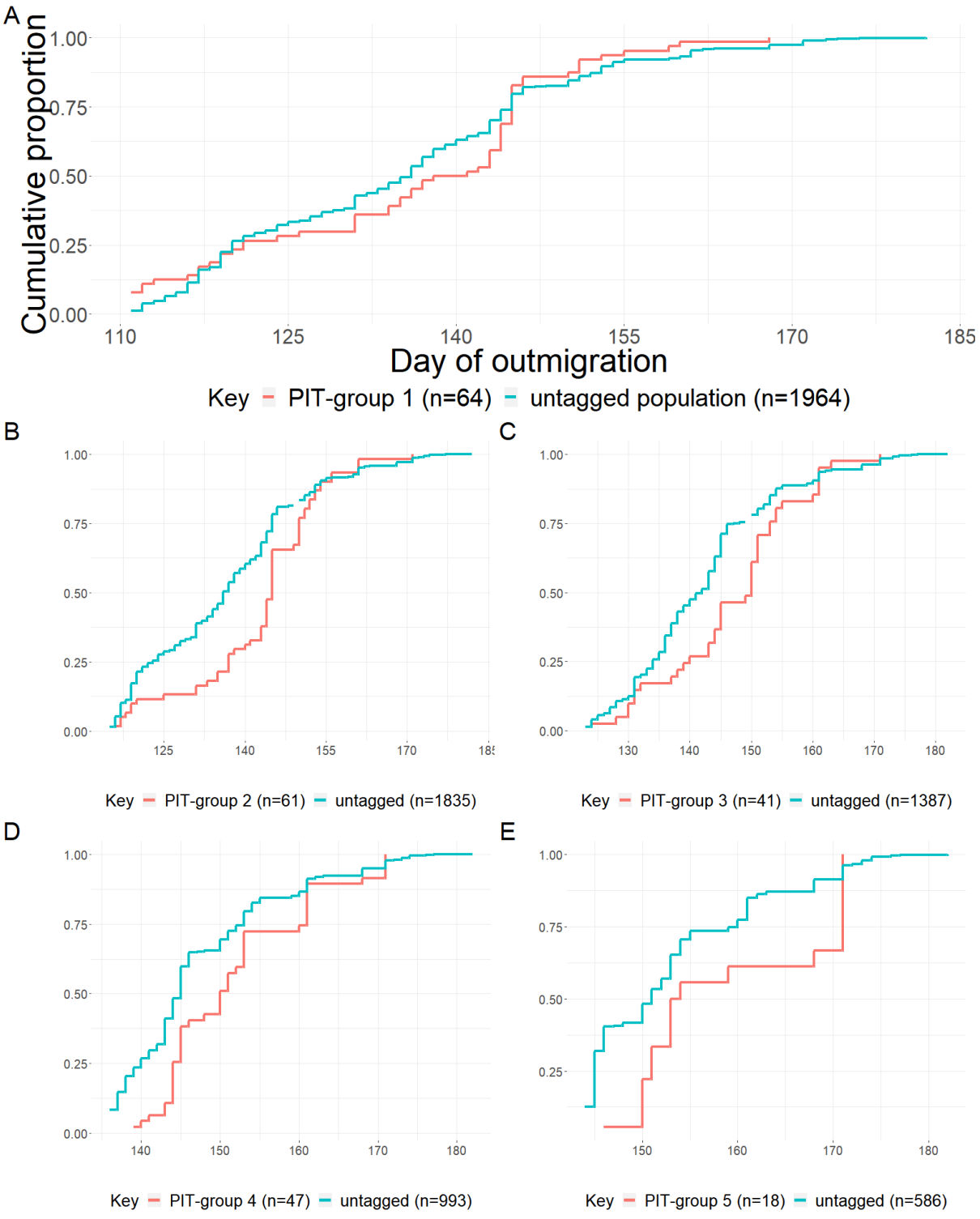

FIGURE 9. *Cumulative relative proportion of out-migration day for PIT-tagged smolts and the untagged population for A) PIT-group 1 (tagged day 105/15.04.2019, n=64), B) PIT-group 2 (tagged day 115/25.04.2019, n=61), C) PIT-group 3 (tagged day 123/03.05.2019, n=41), D) PIT-group 4 (tagged day 136/16.05.2019, n=47), and E) PIT-group 5 (tagged day 144/24.05.2019, n=18).*

3.5 Is length at migration for tagged and untagged smolts the same?

The total length of PIT-tagged smolts (n=231) at the time of out-migration (wolf trap capture) ranged from 115-164 mm, while that of the untagged population (n=1964) ranged from 106-199 mm. The tagged smolts covered 97.8 % of the length distribution of untagged smolts, missing 0.92 % (smaller than 115 mm) at the lower tail and 1.27 % (larger than 164 mm) at the upper tail (Fig. 10). The difference in mean length between tagged (139 mm ± 8 SD) and untagged smolts (141 mm ± 10 SD) was not significant (two-sample t-test (df=2193) = 1.8, p = .07).

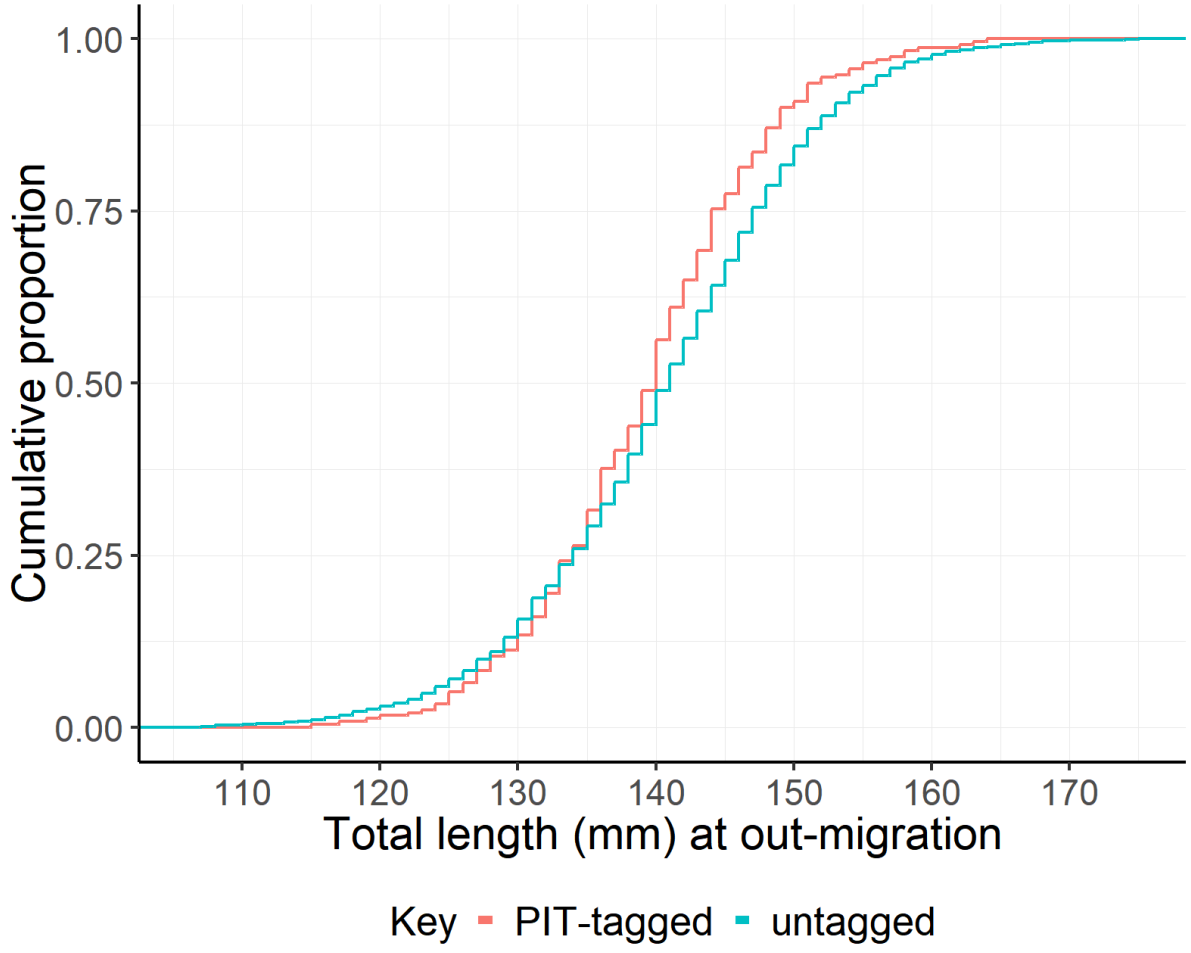

FIGURE 10. *Cumulative relative proportion of total length (mm) for PIT-tagged (n=231) and untagged (n=1964) wild Atlantic salmon smolts at time of out-migration (wolf trap capture). Two outliers of 188 and 199 mm were removed from the length distribution of untagged smolts for graphical purposes.*

3.6 Is timing of migration a function of tagging length and tagging date?

The best linear model (lowest AICc) to explain the timing of out-migration included length at tagging and the tagging date, without interaction (adjusted R^2= .313). Day of out-migration decreased with tagging length, indicating that larger fish would migrate at an earlier date than smaller fish (lm, β = - 0.39, *t* = - 4.2, *p* <.05; Fig. 11). All tagging groups were significantly different from each other where the group tagged first (PIT-group 1) migrated first and the group tagged last (PIT-group 5) migrated last (*p* < .05).

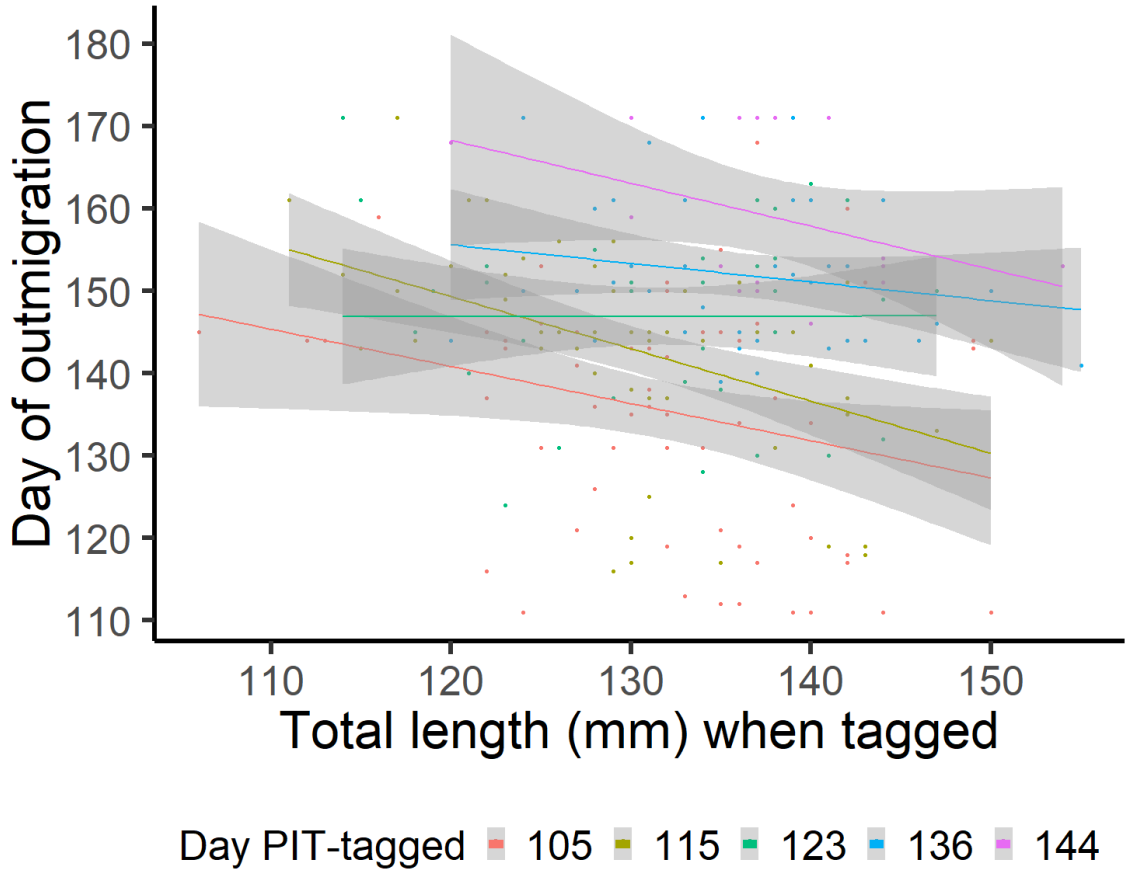

FIGURE 11. *Relationship between out-migration timing (wolf-capture) and the tagging length (TL) of smolts for the different PIT-tagged groups of wild Atlantic salmon smolts. Linear regression lines for each group presented. Day 105=group 1 (tagged 15.04.2019, n=64), 115=group 2 (tagged 25.04.2019, n=61), 123=group 3 (tagged 03.05.2019, n=41), 136=group 4 (tagged 16.05.2019, n=47), and 144=group 5 (tagged 24.05.2019, n=18).*

3.7 Does water discharge and temperature affect migration timing in Atlantic salmon smolts?

The generalized additive model (GAM) with the best fit (lowest AIC) to predict out-migration of wild Atlantic salmon smolts (daily counts in the wolf trap including tagged smolts) included mean water discharge the day before capture and mean water temperature the day of capture, both as linear smooth terms (Table 6). The model indicates that when discharge was high the number of fish being captured the next day was higher (Fig. 12A). Smolt counts were also positively correlated with temperature on the day of capture (Fig. 12B). In contrast, the final model using the number of tagged fish from PIT-group 1 per day as a response variable did not include any explanatory variables (Table 6), and the correlation with both water discharge and temperature was clearly not significant (Fig. 12A and B), exemplifying that a tagging study would not have revealed a response to discharge or temperature in this system.

Table 6. *Overview of the predictors included and coherent AIC value in each GAM model investigated to explain daily counts of a) all wild Atlantic salmon smolts captured in the wolf trap (n=2195), and b) tagged smolts from group 1 (n=64). Delta AIC between models presented. Predictor explanations: Date= the date of smolt count, water discharge=mean daily discharge the day of smolt count (m^3s^{-1}), lag water discharge = mean daily discharge the day before smolt count (m^3s^{-1}), water temperature= mean daily temperature the day of smolt count (ºC), lag water temperature=mean daily temperature the day before smolt count (ºC).*

Response	GAM model	Predictors included	AIC	Delta AIC
Daily counts of wild smolts in the wolf trap (n=2195)	1	Lag water discharge, water temperature	231.4	0.0
	2	Date, lag water discharge, water temperature	233.6	2.2
	3	Date, lag water discharge, lag temperature	234.0	2.6
	4	Date, water discharge, water temperature	235.2	3.8
Daily counts of tagged smolts from group 1 (n=64)	1	Date	54.2	0.0
	2	Date, water discharge, temperature	56.0	1.8
	3	Date, lag water discharge, temperature	57.0	2.8
	4	Date, water discharge, lag temperature	60.2	6.0

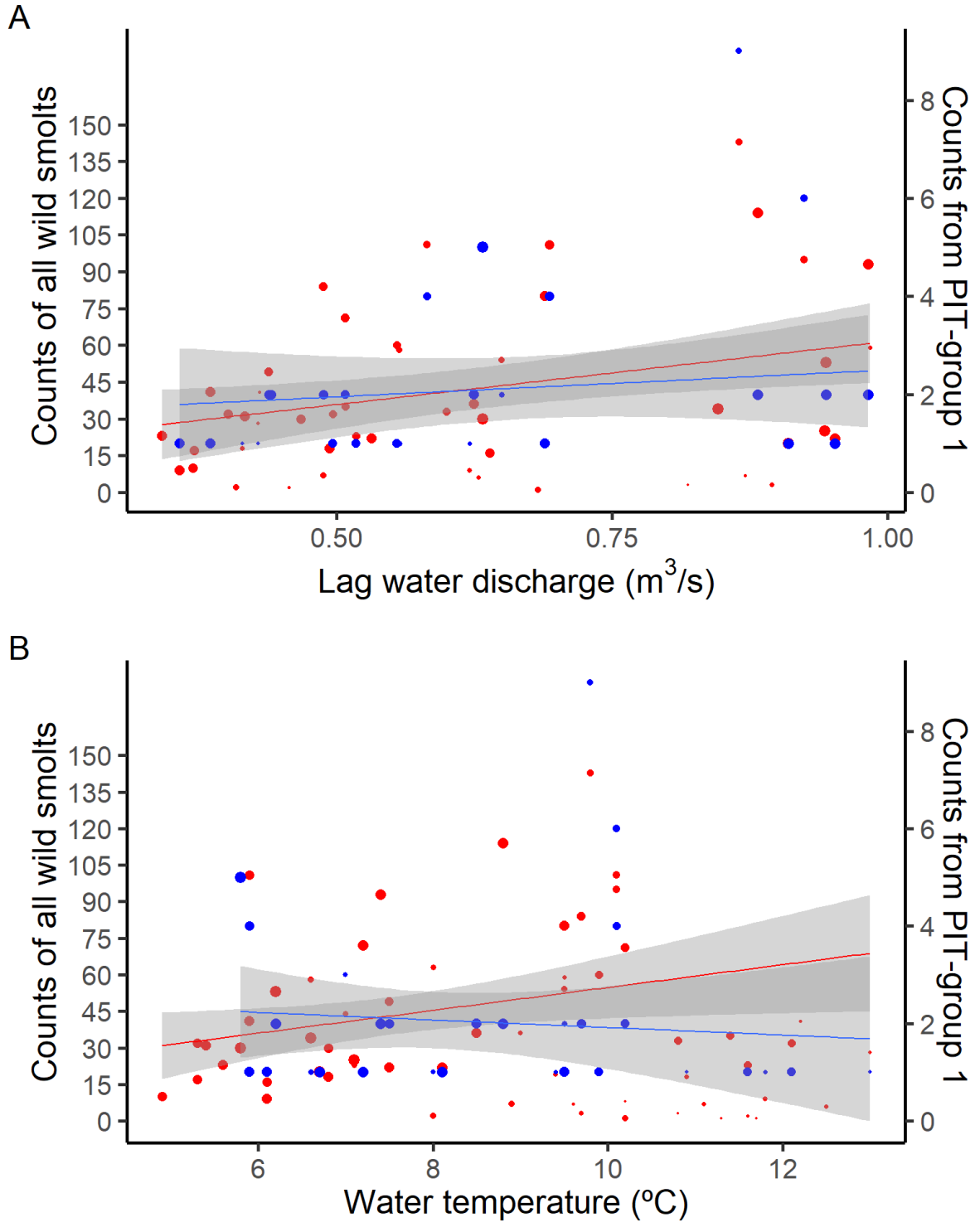

FIGURE 12. *Daily counts of wild Atlantic salmon smolts in the wolf trap as response to A) mean daily water discharge the day before, and B) mean daily water temperature the day of capture. The red points and related red regression line correspond to counts of all wild smolts (n=2195) and are read on the left y-axis. The blue points and related blue regression line correspond to counts of tagged smolts from group 1 (n=64) and are read on the right y-axis. The size of the dots represents the weight of the observation.*

4.0 DISCUSSION

Tagging studies of Atlantic salmon smolts to reveal migration patterns have been conducted for several decades (Bourgeois & O'Connell, 1988; Halfyard et al., 2012; Thorstad et al., 2012). The results from such studies have direct implications for management, and it is therefore of utmost importance to understand if they have any inherent biases. In this study, it was found that the migration timing was not significantly different between tagged and untagged fish. Smolts tagged shortly before migration started showed the most similar migration timing to the untagged population. Furthermore, migration timing was size-dependent, whereas within a group of tagged smolts larger individuals migrated earlier than smaller ones. Thus, it is essential to sample the entire size-distribution of smolts to minimize biases in migration timing estimates. The probability of migration increased with length, whereas it decreased the later in the season that the tagging was conducted. Finally, it was found that the migration timing was positively affected by water temperature and water discharge (the day before smolt count), but a similar response was not observed when modelling based on tagged smolts from group 1, exemplifying that a tagging study would not reveal a response to discharge or temperature in this system. This study reveals that tagging studies need to be careful when designing, as well as interpreting their results, particularly related to size-dependent migration behaviour.

4.1 Comparison of migration timing between tagged and untagged smolts

The capture, handling, and tagging procedures include many stressors that may alter survival or migration behaviour in smolts; however, the timing of outmigration was not significantly different between tagged and untagged fish. One of the a priori hypotheses was that tagged smolts are not representative of all out-migrating smolts due to effects from tagging and handling, with the rationale that this may affect survival, fitness, or behaviour of smolts. After all, smolts are exposed to multiple stressors including electroshock, time out of water, anaesthesia, handling, confinement, and internal implantation of the tag. Although mortality rates after tagging can be affected by the tag-to-size ratio (Lacroix et al., 2004; Larsen et al., 2013; Sigourney et al., 2005) they are generally considered negligible (Gries & Letcher, 2002; Larsen et al., 2013; Prentice et al., 1990). Tag loss rates are also minor (Foldvik & Kvingedal, 2018; Gries & Letcher, 2002; Larsen et al., 2013), and repetitive electroshocking does not affect growth or survival in smolts (Sigourney et al., 2005). However, these results are based on hatchery-smolts not subject to natural stressors (e.g. foraging, predator-avoidance); meaning, the validity of these results to the wild may be limited. In addition, knowledge about potential adverse, indirect effects from tagging is scarce although some studies suggest that swimming capacity (Lacroix et al., 2004; Larsen et al., 2013), buoyancy regulation (Macaulay et al., 2020), and growth rate (Lacroix et al., 2004; Prentice et al., 1990; Sigourney et al., 2005) can all be depressed after tagging, especially short-term. Consequently, such tagging effects may alter survival rates (e.g. predation-induced mortality) or migration behaviour. Hypothetically, after tagging a smolt may migrate out immediately to seek better growth opportunities at sea, or it could prolong its stay in the river to recuperate from tagging before migration. In this study, although each group of tagged smolts on average migrated later than the untagged population, none of them were significantly different. Because between-year and between-river variation in migration can be considerable (Ugedal et al., 2014), similar research in other river systems could add additional weight to these findings. Here, migration was not significantly different between tagged and untagged fish, thus, the null-hypothesis that migration is independent of tagging time, size, handling, and release time during the season could not be rejected.

Only the first group was tagged before fish had started migrating in the river Dale, potentially explaining why temporal patterns in migration were not as similar for the remaining four groups. After tagging, individuals may need time to recuperate and adapt to the extra burden exerted by the tag. If so, such an effect from tagging may have manifested itself more in group 2-5 than 1. Whereas tagged smolts in group 1 could recuperate from the tagging

procedure before the smolt run, the other groups could not (untagged smolts had already started migrating). In turn, this potentially produced the "lag-periods" in migration compared to the untagged population, which were especially noticeable in groups 2 and 3 (although the overall migration was not significantly different). Median migration in these groups was nine and eight days delayed, respectively. These results indicate that an early tagging date (before the migration has started), potentially counteracting a tagging effect, produces the most representative sample in terms of migration timing. Subsequently, if outmigration timing in a river is unknown, smolts should be tagged early to reduce migration timing bias towards later in the season. On the other hand, because of growth, the number of fish eligible for tagging (tagging-size restriction) is probably lower the earlier tagging is conducted. As a result, the sample would have an overrepresentation of large individuals. Thus, tagging fish earlier in the season could cause an early migration estimate, because (1) larger individuals migrate earlier than smaller ones, and (2) the sample would not include migrating fish who are just on a later developmental trajectory (lacking morphological smolt signs). Perhaps, an even earlier tagging date in this study (~ one to two months before migration is initiated), could have presented even more valuable insight in terms of the effect of tagging date on migration timing estimates. Nevertheless, this study suggests that tagging shortly before migration is initiated produces similar temporal patterns in migration between tagged and untagged fish.

4.2 Size-dependency on migration timing

In concordance with the a priori hypothesis, the timing of migration was size-dependent, whereas within a group of tagged smolts larger individuals migrated earlier than smaller ones. Previously, a size-dependency on migration phenology has been documented in the river Imsa (Norway), where especially small (< 13 cm) and large (> 20 cm) fish migrated outside the regular smolt migration period (April-June), in October-March and July-September, respectively (Jonsson et al., 2017). Diel migration patterns also seem size-dependent (Haraldstad et al., 2017; Ibbotson et al., 2011). Consequently, a size-dependency on migration appears evident on both a diel and seasonal scale. A possible explanation for the observed pattern could be that size-selective predation is reduced by synchronizing the migration with conspecifics of equal size. Although small individuals have more potential piscivorous fish predators (Parker, 1971; Poe et al., 1991), it has been proposed that large individuals may also be targeted to maximize the cost/benefit ratio for predators (Mather, 1998). Thus, an anti-predator strategy where migrating smolts are neither unusually small nor large may be

advantageous. In terms of monitoring, this result suggests that the migration timing estimate is affected by the size distribution of the sample, which potentially can be skewed due to either tagging-size restrictions or how and when animals are sampled. Subsequently, an overrepresentation of small individuals will lead to a delayed migration timing estimate, whereas an overrepresentation of large individuals will lead to an earlier migration timing estimate. Thus, a tagged sample covering the size-distribution of all migrating smolts is important for representative migration estimates.

4.3 Tagging date and length of smolts affects probability of migration

The probability of migration increased with length of smolts at tagging, whereas it decreased the later in the season tagging was conducted. There are several possible explanations for why migration probability was size-dependent. First, smaller individuals can be subject to higher natural mortality (e.g. size-selective predation) (Parker, 1971; Poe et al., 1991), and second, larger individuals may be further progressed in the smoltification process, whereas smaller individuals must prolong their stay in the river until all preparatory changes are complete. These smoltification changes (physiological, biochemical, morphological, and behavioural) are prerequisites for the high-salinity marine phase (Hoar, 1988; Thorpe et al., 1998). For instance, to conceal themselves in the pelagic environment they transform morphologically to a dark back, white belly, and silvery sides (Aas et al., 2011). Once these smoltification changes are complete, the smolt can start migrating downstream (Aas et al., 2011). However, if a smolt is not exposed to seawater within a certain 'smolt-window' it can de-smoltify, again taking on a darker appearance and reverting physiological functions (Stefansson et al., 2008). This de-smoltification process is accelerated by warm water temperature (Soivio et al., 1988), possibly explaining why the probability of migration decreased for smolts tagged later in the season. Another explanation could be that the likelihood of capturing individuals with other life histories, for instance, precocious males that do not migrate (Aas et al., 2011), increases during the season. For example, only 42 % of the smolts tagged late May migrated whereas 56-66 % in the other groups did. In terms of monitoring, this is indicative that telemetry studies investigating the spatiotemporal movement of smolts should tag them either before or early during the migration period to increase the probability of obtaining migrating individuals.

4.4 Water discharge and temperature as proximate cues for migration

Water discharge and temperature affected migration timing of smolts in the river Dale, however, it would not have been revealed in a tagging study. The data including all wild Atlantic salmon smolts captured in the wolf trap (n=2195) show that water temperature and water discharge (the day before) positively affected migration timing. This coincides with other findings stating that downstream migration is initiated by water discharge (Hesthagen & Garnås, 1986; Hvidsten & Johnsen, 1993), water temperature (Jonsson & Ruud-Hansen, 1985; Jutila et al., 2005; Whalen et al., 1999), or a combination of these environmental cues (Hvidsten et al., 1995; Ugedal et al., 2014). Although a few rivers, such as Dale, can deploy wolf traps and gather extensive migration data, most rivers cannot (e.g. too high discharge) (Ugedal et al., 2014). Therefore, it is interesting to see if models based on a tagging study would render the same migration response to proximate cues such as water discharge and temperature. If so, that could be used to better understand Atlantic salmon ecology and temporal patterns in migration in different populations. In this study, the model based on tagged smolts (n=64, tagged before the migration started) did not render a similar migration response to water discharge and temperature. The final model did not include any explanatory variables, and the correlation with discharge and water temperature was clearly not significant. The absence of a response in tagged smolts may be due to a different reaction to environmental factors. For instance, it could be that tagging alters migration behaviour and that alternative cues for migration (e.g. social cues) (Hansen & Jonsson, 1985; Hvidsten et al., 1995), become more important than the water temperature and discharge. However, findings suggest that tagged and untagged smolts exhibit a similar response to water discharge and temperature (Aarestrup et al., 2002), indicating that other factors play a role. A second possible explanation for the absence of migration response in tagged smolts is small sample size. For a variety of reasons, such as upholding the reduction principle in animal welfare, telemetry studies often have smaller (Økland et al., 2006; Urke et al., 2013) or approximately equal sample sizes as this study (Urke et al., 2019). However, as shown here, it can be problematic to use tagging studies to make inferences about the effects of environmental factors (e.g. water discharge and temperature) on migration as tagged smolts did not reveal the same response as that obtained when using all smolts captured in the wolf trap.

4.5 Limitations of the study

One of the essential questions to answer was whether fish captured in the wolf trap, positioned ~2-3 km upstream of the estuary, was due to within-river movements rather than actual migrating smolts. To investigate this, gill Na^+K^+-ATPase activity (NKA) as a measure of smoltification was investigated in wild smolts (n=35) captured in the wolf trap at four different time points during May (Appendix 2). It was found that NKA activity was not affected by the length of smolts, did not differ between sampling dates, and that the overall average NKA activity was 11.2 µmol ADP mg protein^{-1} h^{-1} (± 3.1 SD). Unfortunately, gill samples from parr were not collected, removing the possibility of within-population comparisons in NKA activity between parr and smolts. Albeit, the overall NKA activity observed represents a threefold increase compared to ~3-4 µmol ADP mg protein^{-1} h^{-1} found in parr in other studies (McCormick, 1993; McCormick et al., 2013). This is in line with the two to fivefold increase in gill NKA activity commonly observed during the parr-smolt transformation (D'Cotta et al., 1996). Note, NKA levels could have been affected by slight deviations in the conservation procedure of gill samples. Even though the gill sample tubes were put in a temporary freezer within 0.5 h, as described in McCormick (1993), they were not frozen (only cold) during transportation (~ 2h) back to a long-term freezer. Also, for four months they were stored at – 20 °C as opposed to – 80 °C for up to three months (McCormick, 1993). This could have caused sample degradation, further reducing observable levels of NKA activity. Nevertheless, except for the two outliers (5.0 and 5.9) potentially representing presmolts or within-river movements, the overall average NKA activity was comparable or higher than observed smolt levels in other studies (Stefansson et al., 2012; Strand et al., 2011). This, and the threefold increase in NKA activity compared to parr, is indicative that the majority of wild smolts captured in the wolf trap during May were smolts physiologically prepared for sea entry.

Another potential source of error in this study is that tagged smolts did not cover the size distribution of all migrating smolts that season. In turn, this could have affected temporal patterns in migration. To evaluate this, the length distributions of tagged and untagged smolts at out-migration/wolf trap capture were compared. Tagged smolts covered 97.8 % of the length distribution of untagged smolts, missing a marginal 0.92 % at the lower tail and 1.27 % at the upper tail. Although the overlap is substantial, it is not indisputable evidence that the length distribution was identical at the time of tagging. Growth rates in tagged smolts can be lower than in untagged smolts for ~1-3 months after tagging (Lacroix et al., 2004; Prentice et al., 1990; Sigourney et al., 2005), which could have shifted the length distribution between the time

points. Also, there was not a significant difference in mean length (TL) between tagged (mean:139 mm ± 8 SD) and untagged smolts (mean:141 mm ±10 SD) at outmigration. Overall, these findings suggest that the sampled smolts, ≥ 100 mm at tagging, covered the size distribution of all migrating smolts from the Dale river.

It is also noteworthy that the wolf trap did not capture all migrating fish. 231 (60 %) of the tagged smolts were captured in the wolf trap, however, an estimated 45 out of the remaining 154 tagged smolts were able to cross it without being captured. It is unknown when exactly this occurred, removing the possibility to compare temporal migration patterns in these individuals to the untagged population. During high discharge, the catchability of wolf traps can decrease (Ugedal et al., 2014), possibly explaining the observed pattern. Nevertheless, the wolf trap captured the majority of migrating smolts enabling comparisons in migration timing to the untagged population.

4.6 Implications of the study

Tagging-size restrictions related to the use of telemetry tags (e.g. PIT- and acoustic tags) can alter the size-distributions of samples, further affecting study parameters such as spatiotemporal movement. All tags, whether externally attached or internally implanted, will likely influence the fish. Therefore, animal welfare committees often use a tag-to-fish weight/length ratio to define the potential impact and set tagging-size restrictions accordingly (Vollset et al., 2018). Leading up to the decision, the committee weighs different aspects up against each other. If the tagging size restriction is set too low it can potentially have negative effects on the survival, fitness, and behaviour of animals. Set unnecessarily high, the restriction can exclude a large proportion of the size distribution from tagging. All the above can affect the tagged sample and determine whether it is representative of the population or not. Recently, the Norwegian animal research authority suggested a tagging-size restriction of ≥ 140 mm when using internal acoustic tags in smolts (Knut Wiik Vollset 2020, personal comment). This would arguably reduce the impact on the animals, but the question is whether a sample restricted to smolts ≥ 140 mm is still representative. To exemplify, in this study only 20.8 % (n=48) of the migrating, tagged smolts (n=231) was ≥ 140 mm at the time of tagging. With that restriction, the median migration of tagged smolts (From group 1, n=11) would be 16 days earlier than the untagged population according to our data. In contrast, the restriction of ≥ 100 mm in the current study did not exclude smaller individuals migrating later in the season. Due to the size-dependency on migration timing, this could partly explain why median migration from the same group was

four days later than the untagged population. This exemplifies that the size-distribution of a tagged sample can have a considerable effect on the migration timing estimate.

A size-dependency on migration timing may explain why telemetry studies have shown earlier migration estimates than other methods. Whereas the smolts in this study were implanted with the smallest PIT-tag (12 mm), other telemetry studies conducted in Norway, such as Urke et al (2019) in the river Eio, have primarily used larger acoustic tags (18-22 mm) with correspondingly larger smolts (140 mm ± 15 SD). Consequently, this could explain why median migration in 2018 was early (17th of May) using acoustic telemetry (Urke et al., 2019), while previous studies in the same system, using video surveillance and trap-nets, showed a later migration (~29th of May) (Skoglund et al., 2012). Having said that, between year variations in migration timing can be up to a month (Ugedal et al., 2014), and thus cannot be neglected as a possible explanation. All monitoring methods have potential biases (Vollset et al., 2019b), and the extent of these may vary throughout the migration period (e.g. avoid traps as day-length increases). Unfortunately, few rivers have more than one monitoring method (Vollset et al., 2019b). Therefore, to further investigate the effect of these biases on migration timing estimates, several methods should be compared within the same river system (Vollset et al., 2019b).

Migration timing estimates that are either too early or too late may have large implications for management and our understanding of post-smolt survival of Atlantic salmon. Seasonal dynamics of salmon louse in fish farms are repetitive (Aldrin et al., 2013), causing an increase in infestation pressure on post-smolts as summer progress (Kristoffersen et al., 2018). Therefore, lice-induced mortalities in post-smolts is dependent on both migration timing and residency through fjords and coastal areas (affected by progression rates and location of the natal river) (Kristoffersen et al., 2018; Nilsen et al., 2017). In fact, Bøhn et al (2020) found that chemically unprotected smolts (against salmon lice) had 50 times higher mortality risk than protected smolts when migration timing was late (June) and infestation pressure high. In contrast, unprotected fish migrating during lower infestation pressures (May) did not have a lower likelihood of survival (return to river) than treated fish (Bøhn et al., 2020). Accordingly, migration timing is crucial and one of the most sensitive parameters when modelling parasite-induced mortalities (Nilsen et al., 2017). The accuracy of these models can be improved by monitoring more rivers and quantifying the uncertainties in methods used to estimate migration timing (Nilsen et al., 2017). Although more research is needed in other rivers, the results in this study help enlighten some of the potential biases produced in telemetry studies, a method that

is increasingly used to monitor populations. In turn, by resolving these biases the management practices and telemetry-based migration estimates can be enhanced.

5.0 CONCLUSION

Migration timing in Dale was not significantly different between tagged and untagged fish. The gill Na^+K^+-ATPase activity observed in fish captured in the wolf trap indicated that they were migrating smolts, and the size distribution of tagged smolts was representative of the untagged population. Furthermore, this study provides desired knowledge regarding some of the uncertainties in telemetry data. First, results suggest that an early tagging date, before migration has started, produces the most representative sample. Second, migration timing is size-dependent, such that larger individuals migrate earlier than smaller ones. This finding advocates that future telemetry studies must consider that the size distribution of the sample (affected by e.g. tagging-size restrictions) may cause a bias in migration timing estimates, which in some cases can be considerable. Further, the probability of migration increased with length, whereas it decreased the later in the season that the tagging was conducted. The likelihood of obtaining migrating individuals in the sample therefore decreases with time. Results in this study also show that migration timing was positively affected by water temperature and discharge, although a tagging study would not reveal a similar response in this system. These findings will contribute to management practices and the use of telemetry, further enhancing the accuracy of migration timing estimates.

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7.0 APPENDICES

Appendix 1:

This protocol derives from McCormick (1993) and is among the predominantly used methods in the scientific community for measuring Na^+K^+-ATPase activity in gills (Richards *et al.*, 2003; Mancera & McCormick, 2000; Hiroi & McCormick, 2007).

The production of ADP (Ouabain-sensitive) in the presence of Na^+K^+-ATPase is enzymatically coupled to the oxidation of nicotinamide adenine dinucleotide (NADH). This equimolar removal of NADH can be directly measured on a microplate reader at 340 nm. The assay consists of the following three reactions; firstly, adenosine triphosphate (ATP) is hydrolysed to adenosine diphosphate (ATP) and phosphate, catalysed by Na^+K^+-ATPase. Secondly, pyruvate kinase aids in the yield of pyruvate and ATP from ADP and phosphoenolpyruvate. Lastly, pyruvate and NADH react to produce lactate and NAD^+ with the help of lactate dehydrogenase (McCormick, 1993).

Reaction 1: ATP $\xrightarrow[NaK-ATPase]{} ADP + P_i$

Reaction 2: ADP + Phospoenolpyruvate $\xrightarrow[Pyruvate\ kinase]{} Pyruvate + ATP$

Reaction 3: Pyruvate + NADH $\xrightarrow[Lactate\ dehydrogenase]{} Lactate + NAD^+$

ATPase activity was determined as the difference in absorbance, due to removal of NADH, in the absence and presence of ouabain (specific inhibitor of Na^+K^+-ATPase) (McCormick, 1993).

Assay mixture

An assay mixture containing 0.22 mM β-Nicotinamide adenine dinucleotide (NADH), 0.7 mM adenosine triphosphate (ATP), 2.8 mM phosphoenolpyruvate (PEP), 50 Mm imidazole buffer (IB), 4 U mL^{-1} lactic dehydrogenase (LDH) and 5 U mL^{-1} pyruvate kinase (PK) was prepared

immediately in advance to the assay. Before mixing chemicals, the calculated volumes of both PK and LDH were centrifuged in a 5424 R centrifuge (Eppendorf, Hamburg, Germany) for 8 minutes (1200 g, 4°C) resulting in a pellet with supernatant on top. To finish the assay mixture, the supernatant was removed, and pellet suspended in 500 µl IB (McCormick, 1993).

ADP standard curve

An ADP standard curve was run to ensure the assay mixture was of adequate quality. This involved the preparation of four different ADP standards (Table 7) containing ADP stocks (4 mM) and imidazole buffer (50 mM) (McCormick, 1993). The different ADP stock solutions were made beforehand by a lab technician, stored at - 80 ºC and thawed just prior to use.

TABLE 7. *Overview of the four ADP standards used to produce the ADP standard curve. Concentration and content of each standard presented.*

ADP standard	Concentration (nmoles 10µl^{-1})	50 mM Imidazole buffer (µl)	4 mM ADP stock
1	0	200	0
2	5	175	25
3	10	150	50
4	20	100	100

Before adding the assay mixture + salt solution (189 mM NaCl, 42 mM KCl, 10.5 mM MgCl, and 50 mM imidazole, pH 7.5) to the ADP standards, it was placed in a water bath (25ºC) for ~ five min, and then shaken thoroughly on a shaker.

Each ADP standard was added in triplicates (10 µl) on a Nunc plate (Nunc plate #269620, VWR 732-2746). Furthermore, 200 µl of assay mixture + salt solution (189 mM NaCl, 42 mM KCl, 10.5 mM MgCl, and 50 mM imidazole (pH 7.5)), was added to each well before measuring the absorbance of the different standards at 340 nm (60 cycles, 10 sec intervals, 10 min running time) in a Spark multicode microplate reader. The slope of the endpoint standard curve should be 17-19 mOD (milli optical density unit) nmole ADP^{-1}. The disappearance of NADH is measured, and thus the standard curve will be negative.

After a successful quality check, the assay mixture was used as a basis for two separate

assay solutions. Assay solution A and B were made with imidazole buffer and ouabain, respectively, both with a final concentration of 0.5 mM. Just before the protocol for each sampling microplate, both solutions were then separately mixed with salt solution in a 3:1 ratio and kept on ice.

Protocol for Na^+K^+-ATPase activity measurements

The frozen gill samples for Na^+K^+-ATPase activity measurements were taken out of the freezer and kept on ice throughout the following procedure.

Before analysis, gill filaments (n=4-6, McCormick, 1993) were thawed, kept on ice, and homogenized (10-15 seconds using a motor pestle, VWR 431-0100, VWR, Radnor Pennsylvania, USA) in 125 µl containing 80 % (v/v) SEI buffer (250 mM Sucrose, 10 mM Na_2EDTA , 50 mM Imidiazole, pH 7.3) and 20 % (v/v) SEID buffer (0.5 % (w/v) Na deoxycholate acid in SEI buffer). Every sample was visually inspected to check if properly homogenized. The homogenized samples were then centrifuged (5000 g,1 min, 4ºC) to precipitate cell debris (McCormick, 1993).

From each sample, quadruplicates of supernatant (10 µl) were loaded in a 96-well Nunc microplate (Nunc plate #269620, VWR 732-2746) for the measurements of Na^+K^+-ATPase activity. In addition, three replicates of supernatant (10 µl) were added to a Costar plate (Sigma CLS9017, Sigma-Aldrich, St. Louis, Missouri, USA) for protein analysis. Both microplates were kept on ice-cold gel packs. Once half of the samples had been added to the microplates, assay solution A and B were positioned in water bath at 25ºC (McCormick, 1993).

Furthermore, on the Nunc plate 200 µl of solution A was added to half of the replicates, whilst 200 µl of solution B were added to the remaining half (McCormick, 1993).

The absorbance was measured at 340 nm using a temperature-controlled Spark multicode microplate reader with kinetic assay (25 ºC, 60 cycles, 10s intervals, 10 min running time). Results were expressed as mOD $10\mu l^{-1}$ min^{-1} (mOD=milli optical density unit) (McCormick, 1993).

Protein analysis

Using a bicinchoninic acid method (Smith et al., 1985) the protein concentration in every sample was determined. From the Pierce BCA Protein Assay kit (Thermo fisher Scientific, Massachusetts, USA), 200 µl of working reagent consisting of reagent A and B in a 50:1 ratio was added to every triplicate on the costar plate. Covered in parafilm the plate was shaken for 30 seconds on an IKA VXR basic Vibrax (IKA, Staufen, Germany), before incubation for 30

min at 37 ºC in the INCU-Line digital incubator (IKA, Staufen, Germany). The plate was cooled for two-three minutes, parafilm removed, and placed in the plate reader at a wavelength of 562 nm (endpoint assay). Result outputs were in µg 10 µl^{-1} (McCormick, 1993).

Na^+K^+-ATPase activity calculation

Result outputs from the microplate reader are used to obtain Na^+K^+-ATPase activity in µmol ADP mg protein^{-1} h^{-1} using the following calculations.

Na^+K^+-ATPase activity was measured as the difference in activity, with and without ouabain present as inhibitor, in the unit mOD 10 µl^{-1} min^{-1}. This is divided by the standard curve slope (mOD nmole ADP^{-1}) resulting in the unit nmole ADP 10µl^{-1} min^{-1} (Equation 2). Furthermore, this measurement is divided by the protein reading in µg 10 µl^{-1} and multiplied by 60 resulting in ATPase activity measurements in µmol ADP mg protein^{-1} h^{-1} (Equation 3).

Equation 2:

$$\frac{Na^+K^+\text{-ATPase(mOD 10 µl}^{-1}\text{ min}^{-1})}{\text{standard curve slope (mOD nmole ADP}^{-1})} = \text{nmole ADP 10 µl}^{-1}\text{ min}^{-1}$$

Equation 3: $$\frac{\text{nmoles ADP 10µl}^{-1}\text{min}^{-1}}{\text{µg 10 µl}^{-1}} * 60\text{ min} = \text{µmol ADP mg protein}^{-1}\text{ h}^{-1}$$

Appendix 2:

Appendix 2. *Specific information for each wild Atlantic salmon smolt euthanized for gill sampling in the wolf trap. Sampling date, total length (mm), PIT ID, and all values according to equations 2 and 3 (Appendix 1) required to estimate Na^+K^+-ATPase activity levels presented. mOD= milli optical density unit, output from microplate reader.*

Smolt ID	Gill sampling date	Total length (mm)	PIT ID	ATPase activity difference with and without ouabain (mOD 10 µl^{-1} min^{-1})	ADP standard curve slope (mOD nmol ADP^{-1})	Protein reading (µg protein 10 µl^{-1})	Final measurement (µmol ADP mg protein^{-1} h^{-1})
1	03.05.2019	166	NA	18.14165	17.9	7.493426	8.1
2	03.05.2019	137	NA	10.77095	17.9	3.217729	11.2
3	03.05.2019	125	NA	9.05065	17.9	2.110691	14.4
4	03.05.2019	130	NA	2.8968	17.9	1.38496	7.0
5	03.05.2019	132	NA	9.4883	17.9	3.221315	9.9
6	03.05.2019	136	NA	9.21685	17.9	2.417629	12.8
7	03.05.2019	146	NA	20.3543	17.9	4.205246	16.2
8	03.05.2019	135	NA	10.98405	17.9	4.009761	9.2
9	03.05.2019	136	NA	16.5617	17.9	5.073108	10.9
10	03.05.2019	139	NA	4.5696	17.9	2.406441	6.4
11	16.05.2019	135	NA	13.81465	17.9	2.958013	15.7
12	16.05.2019	137	982126057879385	6.0732	17.9	1.708846	11.9
13	16.05.2019	140	NA	6.81665	17.9	2.163269	10.6
14	16.05.2019	130	NA	1.93551	17.9	1.289679	5.0
15	16.05.2019	139	NA	9.3666	17.9	2.908269	10.8
16	24.05.2019	130	NA	5.59055	17.9	1.571016	11.93
17	24.05.2019	145	982126057877079	9.065	17.9	4.014143	7.57
18	24.05.2019	126	982126057879368	10.8699	17.9	2.643028	13.79
19	24.05.2019	137	982126057651525	13.30185	17.9	2.923307	15.25
20	24.05.2019	158	982126057882072	5.62685	17.9	1.174303	16.06
21	24.05.2019	134	982126057878865	3.06385	17.9	0.936388	10.97
22	24.05.2019	125	982126057879408	5.51865	17.9	1.41089	13.11
23	24.05.2019	126	982126057651555	2.97425	17.9	1.414077	7.05
24	24.05.2019	140	982126057651522	4.5601	17.9	1.836653	8.32
25	24.05.2019	132	982126057879404	10.03895	17.9	2.054582	16.38
26	31.05.2019	139	NA	7.72785	17.9	2.387436	10.8
27	31.05.2019	156	982126057879370	5.67545	17.9	2.122628	9.0
28	31.05.2019	144	982126057878890	4.32625	17.9	1.381218	10.5
29	31.05.2019	149	982126057651558	5.2246	17.9	2.98391	5.9
30	31.05.2019	137	982126057878046	7.5729	17.9	2.005064	12.7
31	31.05.2019	141	982126057878923	4.3215	17.9	1.258974	11.5
32	31.05.2019	138	982126057877133	7.82145	17.9	2.489038	10.5
33	31.05.2019	134	982126057879361	4.3479	17.9	1.314744	11.1
34	31.05.2019	140	982126057879421	8.14385	17.9	2.111026	12.9
35	31.05.2019	130	982126057877069	7.8428	17.9	1.758397	15.0

Appendix 3:

The generalized linear model (GLM) with binomial distribution used to run a logistic regression and determine the probability of migration depending on tagging length and tagging date of smolts.

log_migrated1<-glm(migrated~LENGDE+Dato.y, data=data_logmod, family="binomial")

where migrated is the response and corresponds to if a smolt had migrated (1) or not (0). LENGDE refers to tagging length of smolts (mm) and Dato.y refers to tagging date (day of the year) of smolts. The dataset used consists of all 385 tagged smolts.

Model summary:

```
Call:
glm(formula = migrated ~ LENGDE + Dato.y, family = "binomial",
    data = data_logmod)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-1.7403  -1.3482   0.8643   0.9804   1.2536

Coefficients:
             Estimate Std. Error z value Pr(>|z|)
(Intercept) -0.695568   1.661007  -0.419   0.6754
LENGDE       0.024940   0.011833   2.108   0.0351 *
Dato.y      -0.017184   0.008177  -2.102   0.0356 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for binomial family taken to be 1)

    Null deviance: 509.02  on 384  degrees of freedom
Residual deviance: 501.56  on 382  degrees of freedom
AIC: 507.56

Number of Fisher Scoring iterations: 4
```

The linear model (lm) used to investigate if day of migration (day of the year) was affected by length of smolts at tagging (mm) and the tagging date (day of the year).

fit2<-lm(Dato.x~LENGDE.y+factor(Dato.y),data=n_pit_recap_wolf)

where Dato.x refers to the day of outmigration, LENGDE.y to length at tagging and Dato.y to the tagging date. The dataset used consists of all tagged smolts recaptured in the wolf trap (n=231).

Model summary:

```
Call:
lm(formula = Dato.x ~ LENGDE.y + factor(Dato.y), data = n_pit_recap_wolf)

Residuals:
    Min      1Q  Median      3Q     Max
-27.589  -7.120   0.542   7.883  34.511

Coefficients:
                   Estimate Std. Error t value Pr(>|t|)
(Intercept)       187.23416   12.40153  15.098  < 2e-16 ***
LENGDE.y           -0.39230    0.09336  -4.202 3.81e-05 ***
factor(Dato.y)115   6.65356    2.05235   3.242  0.00137 **
factor(Dato.y)123  11.37122    2.28892   4.968 1.34e-06 ***
factor(Dato.y)136  18.18398    2.23654   8.130 2.86e-14 ***
factor(Dato.y)144  25.86200    3.09804   8.348 7.08e-15 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 11.44 on 225 degrees of freedom
Multiple R-squared:  0.3282,    Adjusted R-squared:  0.3133
F-statistic: 21.98 on 5 and 225 DF,  p-value: < 2.2e-16
```

The final generalized additive model (GAM) with negative binomial distribution used to investigate the relationship between daily counts of all wild smolts captured in the wolf trap and mean daily water discharge the day before smolt count and mean daily water temperature the day of smolt count.

mod1d=gam(count~s(offsetWd)+s(Temperature.C),weights=vekt,data=mod_data, family=nb)

where count refers to daily number of smolts captured in the wolf trap, offsetWd refers to the mean daily water discharge the day before smolt count, and Temperature.C refers to mean daily water temperature the day of smolt count. S refers to smooth term, and vekt refers to weight term.

Model summary:

```
Family: Negative Binomial(2.356)
Link function: log

Formula:
count ~ s(offsetWd) + s(Temperature.C)

Parametric coefficients:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)   3.9789     0.1708    23.3   <2e-16 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Approximate significance of smooth terms:
                edf Ref.df Chi.sq p-value
s(offsetWd)   1.041  1.080  2.742  0.0976 .
s(Temperature.C) 1.001  1.002  2.538  0.1112
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

R-sq.(adj) =  -0.0313   Deviance explained = 17.4%
-REML = 113.85  Scale est. = 1         n = 59
```