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Consequences of captive breeding: Fitness implications for wild-origin, hatchery-spawned Atlantic salmon kelts upon their return to the wild

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ABSTRACT

Broodstock collection and enhancement programs are a widely-used management practice within the Atlantic salmon's (*Salmo salar*) native range. Wild-origin adult salmon captured as part of these programs experience multiple stressors during their time in hatcheries. However, no studies have assessed the potential consequences of hatchery practices on the physiology (stress and immune states), migratory behaviour, and long-term survival of hatchery-spawned kelts that are subsequently released back to their natal river. To address these knowledge gaps, we obtained blood samples from, and acoustically tagged 30 hatchery-spawned kelts and 31 wild-spawned kelts, originating from endangered populations native to a UNESCO Biosphere Reserve in Canada during the autumns of 2014 and 2015. We then tracked individuals for up to two years through their downstream river migration, estuarine residence, ocean entry, and subsequent return as repeat-spawners. Our results indicated that hatchery-spawned kelts showed significantly higher stress levels (elevated plasma cortisol and glucose), as well as potentially altered immune states (increased circulating prostaglandin E_2) in comparison to wild-spawned individuals. Behaviourally, hatchery-spawned kelts exited freshwater prematurely (~66 days earlier on average) compared to wild-spawned counterparts, which was associated with a marked increase in estuarine mortality. Furthermore, survival to repeat-spawning was 0% (0/30) for hatchery-spawned kelts and 6.5% (2/31) for wild-spawned. Given that female repeat-spawners are generally larger and have increased fecundity, our findings suggest that a reduction in the fitness of post-spawners and likelihood of repeat-spawning as a result of hatchery stressors could have population-level consequences. Such impacts should be considered in conservation and management planning.

1. Introduction

In the face of global declines of wild animal populations (Bar-On et al., 2018), recovery efforts have increasingly turned to captive breeding programs as a major restoration tool for reintroducing captive-reared animals into the wild (Snyder et al., 1996). The artificial rearing conditions and stress associated with captivity have been shown to negatively affect the physiology, health and/or behaviour of captive-reared reptiles, birds, fishes and mammals, which has the potential to reduce their survival when freed (e.g., Araki et al., 2008; Arena et al., 2014; Carrete and Tella, 2015; Dickens et al., 2009; Fleming and Petersson, 2001; Fraser, 2008; Kanghae et al., 2016; Kostow, 2009; McPhee and Carlstead, 2010). However, in iteroparous species, little is known about the fitness implications of such practices for the future reproduction of captive-breed adults used for restoration purposes.

For the Atlantic salmon (*Salmo salar*) and other salmonid fishes, the capture of wild broodstock and subsequent progeny release to nature is a widely-used management practice in both anadromous and land-locked populations, and one of the most important investments made in species recovery efforts and conservation planning in North America and Europe (Araki et al., 2008; Fraser, 2008; Kostow, 2009). While protocols vary among jurisdictions, broodstock collection usually

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involves the capture of wild adult salmonids each year in freshwater, when fish are migrating upriver to spawning grounds. They are then transferred to hatcheries where they are held until sexual maturity and are then stripped of eggs and sperm (Araki et al., 2008; Kincaid and Stanley, 1989). Fertilized eggs are incubated and hatched, and alevin are grown to the fry, parr, or smolt stage in hatchery tanks or ponds, until their eventual release into their river of origin. For iteroparous species, the stripped, post-spawned adults are often returned to their natal river as well, where they may resume a wild existence, undergo ocean migrations and, if they survive, spawn again in the future. The duration of adult captivity in hatcheries can range from ~1 to > 6 months, depending on the enhancement program (Kincaid and Stanley, 1989). While significant effort has been directed at improving the rearing conditions and survival prospects of juvenile salmonids in hatcheries (Kincaid and Stanley, 1989), the impacts of captive spawning on wild broodstock has received minimal attention. Despite the stress that captivity and handling can have on spawning salmonids (Barton and Iwama, 1991; Fast et al., 2008), no studies have assessed the potential impacts of such programs on the physiology, seaward migratory behaviour, and long-term survival of post-spawning individuals upon their return to the wild.

In North America and Europe, Atlantic salmon populations (*Salmo salar*) have been decreasing throughout their native range, with many populations at the southern margin of their distribution now considered endangered (COSEWIC, 2010; Hindar et al., 2011). As an iteroparous species, wild Atlantic salmon returning from the ocean can survive a first reproductive event to spawn again a year or two later (and in rare cases up to as many as seven times, Ducharme, 1969). After spawning in autumn, post-spawned individuals (i.e., kelts) spend varying durations in the river before migrating back to sea. Within populations, the seaward migration timing of individual kelts can vary from November (i.e. shortly after spawning) to May (Halttunen et al., 2013; Jonsson et al., 1990; Niemelä et al., 2006). Once at sea, kelts embark on extensive feeding migrations, which can last from a couple of months up to about a year and a half, before returning to spawn as “consecutive” or “alternate” repeat spawners, respectively (Thorstad et al., 2011). In most populations, repeat spawners generally constitute < 11% of a given spawning run, although repeat spawning incidences as high as 42.5% have been documented (Fleming, 1998). Repeat spawners are experienced spawners of large body size, and females have generally much higher fecundities (i.e., number of eggs) than smaller first-time spawners (Reid and Chaput, 2012). These characteristics mean that experienced repeat spawners can make significant contributions to population size, viability, stability, and genetic diversity (Buelow and Moffitt, 2015; Halttunen et al., 2009; Reid and Chaput, 2012; Thorstad et al., 2011). Virtually nothing is known, however, about the behaviour and survival of hatchery-spawned kelts returned to rivers compared to wild-spawned counterparts, and whether repeat spawning probability might be compromised by hatchery operations.

For the adult salmon captured as part of broodstock collection programs, stressors are frequent and numerous. These include the initial capture event and transportation to a hatchery, occasional handling and air exposure, confinement, and the eventual stripping of gametes (procedures described in Kincaid and Stanley, 1989; stressors reviewed in Patterson et al., 2017). In vertebrates, the physiological stress response involves the activation of the hypothalamic-pituitary-interrenal axis (HPI), leading to the secretion of glucocorticoid hormones. Cortisol is the primary glucocorticoid in fish, and is commonly used as an indicator of physiological stress (Barton, 2002). At stress-induced levels, cortisol is an important mediator of energetic trade-offs, which can influence behaviour and survival (Crespi et al., 2013; Crossin et al., 2016b; Midwood et al., 2015). As such, cortisol secretion leads to increases in circulating glucose levels during stress, to power behavioural responses. In the context of captive spawning in hatcheries, stressors can be sustained over an extended period, which can result in the suppression of immune responses, resulting in deleterious health effects and diseases (Wendelaar Bonga, 1997). Due to its role in the inflammatory response, circulating prostaglandin E_2 (PGE_2) levels have been shown to increase in salmonids following fungal infection (*Saprolegnia* spp.; Espelid et al., 1996), thereby inhibiting the expression of several immune-related genes (Belmonte et al., 2014; Fast et al., 2005, 2006). The disruption of physiological mechanisms via stress might thus lead to lethal or sub-lethal behavioural effects (e.g., altered migration timing, reduced feeding and growth, and increased overwinter lipid depletion, Midwood et al., 2014, 2015; Peiman et al., 2017). However, to our knowledge, the effects of hatchery practices on physiological condition, and more generally on the seaward migrations of wild broodstock after their return to natal rivers, are unknown.

To quantify the potential consequences of hatchery confinement and artificial spawning for wild adult Atlantic salmon captured as part of a broodstock enhancement program, we compared the physiology, migration behaviour, and survival of hatchery- and wild-spawned kelts from two populations of endangered salmon native to the Bras d'Or Lake UNESCO Biosphere Reserve in Nova Scotia, Canada. We used a combination of acoustic telemetry and bio-sampling techniques to test the overarching null hypothesis that the physiology, behaviour, and survivorship of hatchery and wild-spawned kelts would not differ. We did so through several comparisons: 1. Physiological stress states (indicated by plasma cortisol and glucose levels); 2. Index of immune states (indicated by plasma PGE_2 levels); 3. Freshwater survival; 4. Freshwater exit timing; 5. Estuarine survival; 6. Estuarine residency period; 7. Estuarine exit timing (i.e., Atlantic Ocean entry timing); and 8. Survival to repeat-spawning. The conservation and management implications of our results are discussed.

2. Methods

2.1. Study system and acoustic receiver array

We conducted our study on two populations of Atlantic salmon native to rivers within the Bras d'Or Lake watershed in Cape Breton, Nova Scotia, Canada. The complex, brackish Bras d'Or Lake forms an inland sea ecosystem, constituting a vast estuary of ~1100 km^2 in area centered at 45°51′37″N, 60°46′44″W (Fig. 1). The Middle and Baddeck rivers are the two largest rivers draining into the Bras d'Or Lake, and support the largest salmon runs in the watershed. Similar to Atlantic salmon populations in other parts of the species' range, spawning escapements have been low during the last few decades, and recent spawner numbers in both of these rivers are < 350 on average (Gibson and Levy, 2014). This prompted the recommendation to list the Eastern Cape Breton populations as endangered by the Committee on the Status of Endangered Wildlife In Canada (COSEWIC, 2010).

For seaward migrating Atlantic salmon leaving these two rivers and passing through the Bras d'Or Lake, the closest access to the Atlantic Ocean is located ~64 km away, either through the Great or Little Bras d'Or Channels (Fig. 1). In order to document the seaward migrations of acoustically tagged Atlantic salmon kelts from spawning areas in the upper Middle and Baddeck rivers, a total of 30 VR2W acoustic receivers (Vemco Ltd., NS, Canada) were deployed throughout the Bras d'Or Lake, forming acoustic detection “gates” that fish must cross en route to the Atlantic Ocean (Fig. 1; see Crossin et al., 2016a for additional details on the array). To evaluate the detection efficiency of the key gates within the acoustic array, we examined the 4 receiver gates fish have to cross on their way to the Atlantic Ocean (i.e., river mouth, Nyanza Bay, and two gates in either the Great or Little Bras d'Or channels, Fig. 1), and determined that all individuals that were detected on a gate were also detected on the previous more inland/upstream gate. Thus, performance of the array was highly reliable to detect tagged fish.

2.2. Fish sampling, tagging and holding conditions

Fish were handled in conformity with guidelines established by the

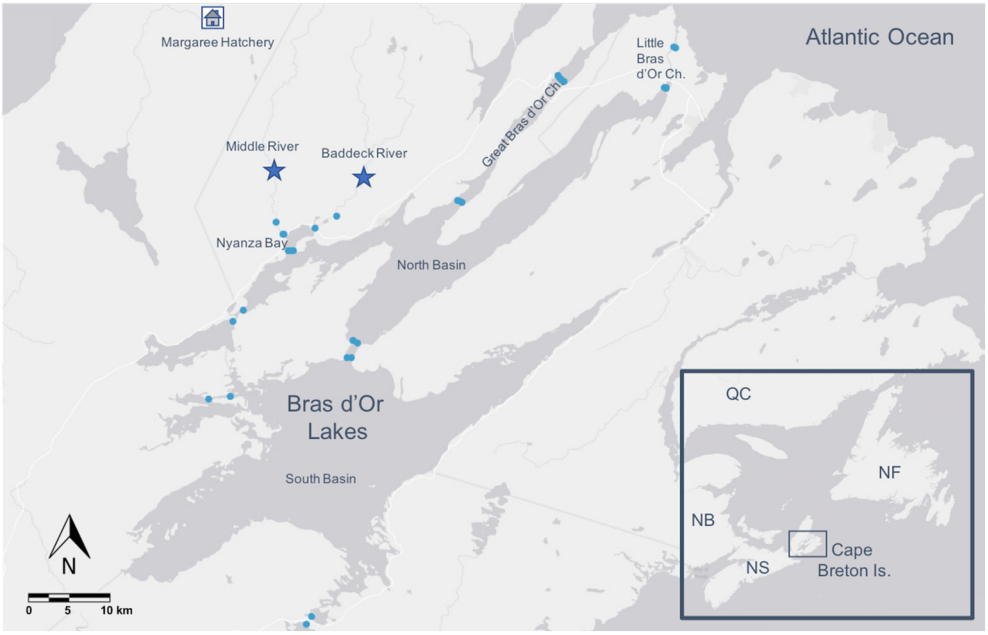

Fig. 1. Map of the acoustic array positioned in the Bras d'Or Lakes, Nova Scotia. Acoustic receivers (n = 30) are indicated by blue circles, and star symbols indicate the release location of wild-origin, hatchery-spawned kelts in both rivers. Wild-spawned kelts of Middle River were captured, tagged, and released between the release site of hatchery-spawned kelts and the river mouth (the map is powered by Esri, HERE, Garmin, NGA, USGS). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Canadian Committee on Animal Care, approved by Dalhousie University and Cape Breton University Animal Care Committees (protocol numbers: 14-105 and 1213-16, respectively), and Scientific Fishing License granted by Fisheries and Ocean Canada (number: 340450).

2.2.1. Hatchery-spawned kelts

Starting in mid-October 2014 and 2015, 30 pre-spawning adult Atlantic salmon (50.0–91.5 cm in fork length) were captured on the Middle and Baddeck rivers via beach seine as part of the Margaree Hatchery broodstock enhancement program (see Table 1 for sample sizes). Immediately after capture, fish were transferred by hatchery staff to oxygenated transport tanks and transported to the Margaree Fish Hatchery, 48 km away (Margaree Valley, NS; operated by the Nova Scotia Department of Fisheries and Aquaculture; Fig. 1). Each population was held separately in two large tanks (~5500 L) devoid of any kind environmental enrichment (e.g. rocks), which were supplied with a constant flow of ambient water from the adjacent Margaree River. Fish were exposed to natural photoperiods and remained unfed during their time at the hatchery. Salmon reached full sexual maturity about a month later, in mid-November. At that point, hatchery staff collected gametes from salmon over a period of ~2 weeks. Stripped, or post-spawned salmon were then allowed to recover for a minimum of 24 h (average of 4 days, range: 1–17 days). Following recovery, individual salmon were removed via dip net and placed in a padded V-shaped trough prior to anesthesia to collect ~2 mL blood samples via caudal venipuncture (as described in Huston, 1990). Ambient water was pumped into the mouth and over the gills during the procedure. Blood sampling occurred at random times between 9:35–15:40 as hatchery operations allowed and the average time from capture to blood sampling was of 2:43 min (± 1:07 min, range: 1:20–4:50 min). Blood samples were placed in an ice-water slurry before centrifugation (10 min at 1163*g*). The resultant plasma was isolated and frozen at −80 °C in 0.6 mL cryovials. Immediately following blood sampling, kelts were anesthetized using tricaine methanesulphonate (MS222) at 60 mg L^{-1} and V16-4H (69 kHz) acoustic transmitter were surgically implanted into the abdominal cavity (16-mm diameter × 68 mm, 24 g in air; Vemco Ltd., NS, Canada), according to standard surgical procedures (Cooke et al., 2011). All transmitters were programed to ping at random 20–70 s intervals and had an estimated battery life of ~1257 days. Transmitter mass relative to body mass varied between 0.3 and 2.1%; well within the tolerance limits for tag burden (Lacroix et al., 2004). At the end of the tagging procedure, fork length and mass were recorded, scales were collected for ageing, and sex was determined morphologically. Fish were then transferred to a recovery tank filled with ambient water until regaining equilibrium. Hatchery-spawned kelts were then allowed to recover for at least 24 h before being transported back to their natal river and released at their initial capture site (average of 6 days, range: 1–12 days).

2.2.2. Wild-spawned kelts

Starting at the end of November 2014 and 2015, 31 post-spawned Atlantic salmon (51.1–95.0 cm in fork length) were captured on the Middle River by angling (Table 1). Immediately after capture, individual salmon were placed in a padded cradle supplied with ambient river water and blood was sampled as described above. Blood sampling

Table 1
Number of Atlantic salmon tagged (number of females are indicated and the rest are males), average freshwater-exit timing (± SD, in days), as well as freshwater survival and estuarine survival of the three groups, for both years of tagging.

Group	Sample size		Freshwater survival		Freshwater-exit timing		Estuarine survival	
	2014/15	2015/16	2014/15	2015/16	2014/15	2015/16	2014/15	2015/16
Wild - Middle	6 (3 F)	25 (19 F)	33% (2/6)	68% (17/25)	May-1 (± 33 d.)	May-11 (± 10 d.)	100% (2/2)	100% (17/17)
Hatchery - Middle	7 (2 F)	8 (4 F)	57% (4/7)	63% (5/8)	Feb-10 (± 74 d.)	Jan-16 (± 73 d.)	50% (2/4)	60% (3/5)
Hatchery - Baddeck	8 (6 F)	7 (4F)	63% (5/8)	86% (6/7)	Mar-21 (± 85 d.)	Apr-11 (± 60 d.)	60% (3/5)	67% (4/6)

occurred at random times between 9:30–15:30 as catch rates allowed and the average time from hooking to blood collection was of 8:08 min (± 6:28 min, range: 2:02–30:49 min) for wild-spawned kelts (where > 50% of individuals were sampled in < 5:50 min). Following blood sampling, fish underwent surgical procedures for tag implantation at the site of capture, in the same manner as described above for hatchery-spawned kelts. Wild-spawned kelts were then released at the capture site after regaining full equilibrium and swimming capacities about 1 h later. Wild-spawned kelts were tagged and released on average 12 days later than hatchery-spawned kelts (which was later taken into account in statistical analyses).

2.3. Physiological assays

Plasma samples were analyzed for cortisol, glucose and PGE_2. Plasma cortisol was assayed in duplicate using commercially available ELISA kit (#402710, Neogen Corporation, USA), according to the manufacturer's standard procedure and read at 450 nm after the addition of 50 µL of 1 N HCl using a Molecular Devices Spectramax 240pc plate reader (Labconco Corporation, USA). The mean coefficient of variation between cortisol duplicate was 3.2%. Plasma glucose was measured in duplicates using a YSI 2300 STAT Plus Glucose/Lactate Analyzer following methods described in Farrell et al., 2001. Plasma PGE_2 was assayed in duplicate using commercially available forward sequential competitive enzyme immunoassay kit (#KGE004B, Parameter ™, R&D systems Inc., USA), and read at 450 nm with a BioTek Synergy HTX microplate reader (BioTek Instruments, Inc., USA), according to the manufacturer's standard procedure. Due to the complexity of this assay and large plasma volume required, 27 samples could not be measured with confidence (CV's > 35% between duplicates) and were excluded from subsequent analyses. For the reminding 34 samples, the mean coefficient of variation between PGE_2 duplicate was 16.1%.

2.4. Data analysis

2.4.1. Physiology and morphology

To test the first two null hypotheses that stress (plasma cortisol and glucose) and immune state (PGE_2) indicators would not differ between hatchery- and wild-spawned kelts, non-parametric Mann–Whitney *U* tests were computed using the "wilcox.test" function of the "stats" package specifying the argument "paired = FALSE" in R v.3.2.3 (R Development Core Team, 2015). Difference in body condition between hatchery- and wild-spawned kelts was assessed using an analysis of covariance (ANCOVA; "aov" function in R), where body mass (log_{10} transformed) was regressed against body length (log_{10}) for the comparison of slopes and intercepts (Cone, 1989). As the mass-length relationship did not differ significantly between hatchery and wild-spawned kelts ($p = 0.94$) and year ($p = 0.083$), mass residuals were used as an index of individual body condition for subsequent analyses (Kaufman et al., 2007). To better visualize potential morphological differences in mass and body condition between hatchery- and wild-spawned kelts in both years of tagging, parametric Welch Two Sample *t*-tests were computed using the "t.test" function of the "stats" package in R and data were presented in boxplots. Also, potential statistical differences in mean sea age between spawning conditions (hatchery- or wild-spawned) were assessed using non-parametric Mann–Whitney *U* tests. Sea age, in previous number of years spent in the ocean, was determined from scale readings. Mann–Whitney *U* tests were preferred over Welch Two Sample t-tests when the non-normal distribution of the response variable within each group precluded the use of parametric statistics.

2.4.2. Freshwater survival and exit timing

For kelts acoustically tagged in Middle and Baddeck rivers in late fall/early winter of 2014 and 2015, an individual was deemed to have survived in fresh water if it was recorded on a receiver at the river mouth, with subsequent detection on the Nyanza Bay line (confirming that the fish left the river, Fig. 1). For freshwater survivors, the date of freshwater-exit was assigned to the last detection date at a river mouth receiver. To test the third null hypothesis that the probability of freshwater survival would not differ between hatchery- and wild-spawned kelts, binomial logistic regression models were computed using the "glm" function with a "logit" link in R. Spawning condition (i.e., hatchery- or wild-spawned), population of origin, year, sex, and first-degree interactions were considered as potential explanatory variables. The best fitting model was selected using a forward stepwise model building approach, based on AIC scores (using the "step" function in R).

To test the fourth null hypothesis that the freshwater-exit timing would not differ between spawning conditions, general linear models were computed using the "lm" function in R. In addition to spawning condition, population, year, sex, and first-degree interactions were included as predictor variables, and release date was also included as a covariate in the model to control for its potential influence on freshwater-exit timing. The best fitting model was also selected using a forward stepwise model building approach. Furthermore, to visualize differences in the freshwater-exit timing of hatchery- and wild-spawned kelts, Kaplan-Meier product-limit survival curves were computed using the "survfit" function of the "survival" package in R.

To assess how individual level of plasma cortisol, glucose, and PGE_2 might directly influence the freshwater survival and freshwater-exit timing of hatchery-spawned kelts, we compared the concentration of these three parameters between: i. survivors versus mortalities; and ii. for those that survived, between early (fall/winter) versus late (spring) migrants. These comparisons were computed using non-parametric Mann–Whitney *U* tests.

2.4.3. Estuarine survival and residency

For kelts that survived fresh water and entered the Bras d'Or Lake, fish were deemed to have survived in this extended estuary if they were detected subsequently on both receiver gates crossing the Little and Great Bras d'Or channels, which lead to the North Atlantic Ocean (thus indicating seaward movement; Fig. 1). Because estuarine survival was 100% for wild-spawned kelts in both years of tagging, potential statistical differences between spawning conditions could not be assessed using a binomial logistic regression, as the outcome survival probability is equal to 1 for wild-spawned kelts. Alternatively, to test the fifth null hypothesis, statistical differences in estuarine survival of hatchery- versus wild-spawned kelts was assessed by computing a Welch Two Sample *t*-test, which compared the average survival of the tagging groups (2 hatchery- and 1 wild-spawned groups, in both 2014 and 2015; Table 1). For consistency, this method was also used to compare the average group freshwater survival of hatchery- versus wild-spawned kelts to make sure the results were comparable to those of a binomial logistic regression (Table 1).

Further, to assess how individual levels of plasma cortisol, glucose, and PGE_2 might directly influence the estuarine survival of hatchery-spawned kelts, we compared the concentration of these three parameters between survivors and fish that died in the estuary, using non-parametric Mann–Whitney *U* tests.

For the estuarine survivors, Bras d'Or or estuarine residency period was calculated from the moment at which an individual entered the estuary (as given by the last detection at a river mouth receiver) until the moment it left the Bras d'Or Lake to enter the Atlantic Ocean (as given by the last detection at an outermost receiver gate of either one of the Bras d'Or channels, Fig. 1). Accordingly, the estuarine exit timing of individuals or Ocean entry was given by the date of last detection at an outer most Bras d'Or gate. Non-parametric Mann–Whitney *U* tests were used to test the remaining sixth and seventh null hypotheses that the estuarine residency period (i.e., number of days spent) and Ocean entry timing (i.e., date) would not differ between the two spawning

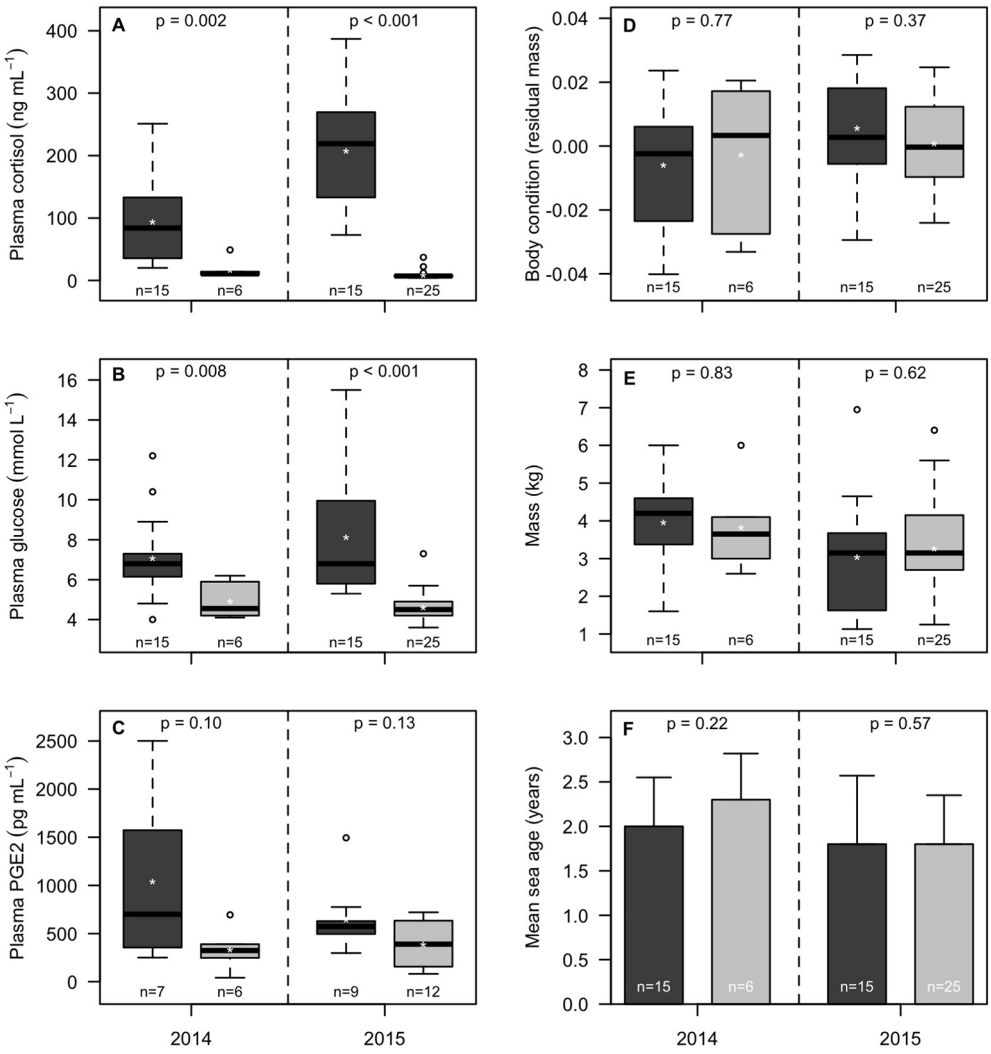

Fig. 2. Physiological, morphological and sea age comparisons between hatchery- (dark grey) and wild-spawned Atlantic salmon kelts (pale grey) from both years of tagging. The boxplots (panels A–E) show median (black lines) and mean values (white dots), as well as the interquartile ranges (boxes) and the 5th and 95th percentiles (whiskers). The barplot (panel F) show mean values with standard deviations. Details on the statistical tests can be found in the Method section.

conditions.

3. Results

3.1. Physiology and morphology

In contrast to the first null hypothesis, both stress indicators, plasma cortisol and glucose, were significantly higher in hatchery- versus wild-spawned kelts, for both years of tagging (Fig. 2). The difference was especially strong for plasma cortisol, the primary stress hormone, which was ~15-fold higher in hatchery-spawned kelts (averaging of 149 ng mL^{-1} vs 10 ng mL^{-1}). This is despite the fact that it took 5:25 min longer on average to capture and draw blood from wild-spawned kelts and that, as a result, we might be overestimating the baseline stress levels of these fish. However, blood-sampling time was not positively correlated with plasma cortisol or glucose (p-values > 0.31, Pearson's r). While both stress indicators were significantly elevated in the hatchery-spawned kelts of both sexes (p-values < 0.003), differences with wild-spawned kelts were stronger in females (200 vs 8 ng mL^{-1} and 8.2 vs 4.6 mmol L^{-1}) than in males (92 vs 16 ng mL^{-1} and 7.0 vs 4.9 mmol L^{-1}) - for plasma cortisol and glucose, respectively. Although PGE_2 could only be measured with confidence for 34 out of 61 individuals (Fig. 2), a significant difference was nevertheless evident between groups when pooling years (p = 0.015, Mann–Whitney *U* test), with hatchery-spawned kelts expressing a 2.2-fold increase in circulating levels (819 pg mL^{-1}) versus wild-spawned kelts (375 pg mL^{-1}). Furthermore, males (779 vs 361 pg mL^{-1}) and females (870 vs 384 pg mL^{-1}) from both spawning conditions expressed similar average plasma PGE_2. This result counters the second null hypothesis that this index of immunological state or health would not differ between the two spawning conditions. In all of these physiological tests, population of origin for the hatchery fish was not a significant factor in either year (p-values > 0.064, Mann–Whitney *U* tests), which allowed us to pool Middle and Baddeck river fish in our hatchery group for these analyses. Furthermore, no differences in sea-age (i.e., prior number of years spent at sea) or morphology (i.e., body condition, mass and length) were found between hatchery- and wild-spawned kelts, for both years of tagging (p-values > 0.22, Welch Two Sample *t*-tests; Fig. 2).

3.2. Freshwater survival and exit timing

From the 61 kelts (30 hatchery- and 31 wild-spawned) that were tagged in 2014 and 2015, 39 (20 hatchery- and 19 wild-spawned) were detected leaving fresh water and entering the Bras d'Or Lake, for a combined freshwater survival of 67% and 61% for hatchery- and wild spawned kelts, respectively. Freshwater survival of the six different

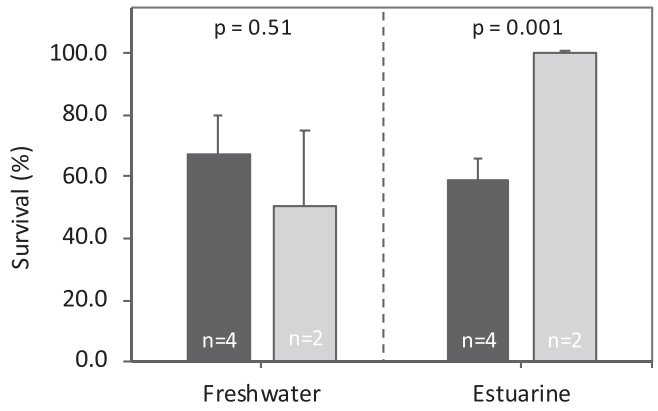

Fig. 3. Comparisons of the mean freshwater and estuarine survival of hatchery- (dark grey) and wild-spawned kelts (pale grey), with bars representing standard deviations. Statistical differences were computed using Welch Two Sample *t*-tests comparing the average survival of the six tagging groups: 4 hatchery-spawned groups (Middle 2014, Middle 2015, Baddeck 2014, Baddeck 2015) versus 2 wild-spawned groups (Middle 2014, Middle 2015; Table 1).

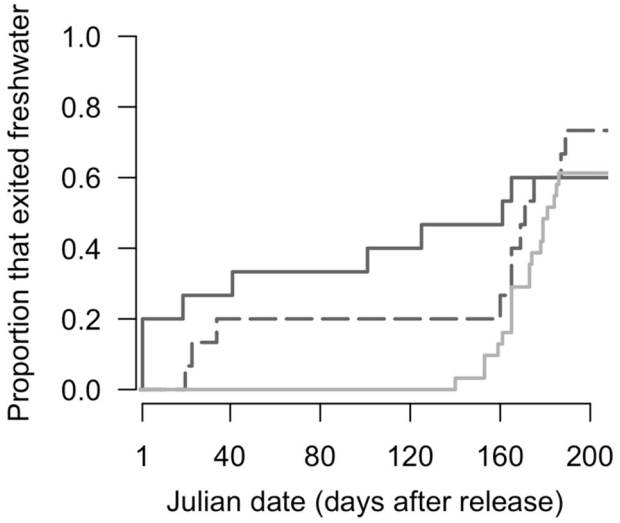

Fig. 4. Kaplan-Meier product-limit survival fit of freshwater-exit timing for the different tagging groups: wild-spawned kelts from Middle River (solid pale grey line); hatchery-spawned kelts from Middle River (solid dark grey line); and hatchery-spawned kelts from Baddeck River (dashed dark grey line). Day 1 represents Nov-21 in 2014–15, and Nov-24 in 2015–16. Mar-21, which marks the limit between early (fall/winter) and late (spring) migrating kelts, occurred on day 121 after release in 2014–15, and on day 119 in 2015–16.

tagging groups varied between 33% and 86%, with the greatest variation in wild-spawned kelts (33% in 2014 versus 68% in 2015, Table 1). No statistically significant differences were found between hatchery- and wild-spawned kelts in relation to freshwater survival probability ($p = 0.66$, binomial logistic regression). In fact, following a stepwise model building approach, the null model had a lower AIC value than alternative models. This is also consistent with the absence of a statistically significant difference between the average group freshwater survival of hatchery- (67%) and wild-spawned (51%) kelts ($p = 0.51$, Welch Two Sample *t*-test, Fig. 3). These findings support the third null hypothesis that there is no difference in the freshwater survival of hatchery- and wild-spawned kelts.

In contrast to our fourth null hypothesis, hatchery-spawned kelts exited freshwater significantly earlier than wild-spawned counterparts. Using general linear models, the model with the lowest AIC score included spawning condition (either hatchery- or wild-spawned), sex, and population of origin as statistically significant explanatory variables in addition to controlling for release date (final model in Table 2). An interaction between spawning condition and sex was also retained in the best fitting model, indicating that the difference in timing between hatchery- and wild-spawned kelts from the Middle River was greater in females than in males (Table 2), although the difference was statistically significant for both sexes. In Middle River, female hatchery-spawned kelts exited fresh water on average ~132 days earlier than wild-spawned counterparts ($p < 0.001$, Table 2), while that difference was reduced to ~70 days in males ($p = 0.040$, post-hoc test). Furthermore, in both years some hatchery-spawned kelts left the river on the very first day of release (Nov 21st and Nov 24th, in 2014 and 2015 respectively), while no wild-spawned kelts left the river before Apr 9th (Fig. 4). No significant inter-year differences in exit timing were found for hatchery- and wild-spawned kelts from Middle River, nor for hatchery-spawned kelts from Baddeck River ($p > 0.46$, Mann–Whitney *U* tests; Table 1), which allowed us to pool data from both years of study. Unlike in Middle River, no hatchery-spawned kelts from Baddeck River left the river before December 13th (Fig. 4), and hatchery-spawned kelts from Baddeck River left significantly later than hatchery-spawned kelts from Middle River, a difference of ~86 days on average ($p = 0.008$, Table 2). Pooling hatchery-spawned kelts from both populations and sexes, hatchery-spawned kelts exited freshwater on average ~66 days earlier than wild-spawned kelts, a difference that remained statistically significant ($p = 0.009$, Mann–Whitney *U* test). Moreover, no wild-spawned kelt (0/19) exited the Middle River before spring, while a combined 45% (9/20) of hatchery-spawned kelts did (67% in Middle River and 27% in Baddeck River; Fig. 4).

In assessing how individual levels of plasma cortisol, glucose, and PGE_2 might directly influence the freshwater survival and exit timing of hatchery-spawned kelts, unexpectedly the only significant difference found was an elevated cortisol level in freshwater survivors (182 pg mL^{-1}) versus hatchery-spawned kelts that presumably died in rivers (83 pg mL^{-1}, $p = 0.009$, Mann–Whitney *U* test). However, this pattern seemed to be driven by hatchery-spawned females with significantly higher cortisol levels than males (200 pg $mL^{-}1$ versus 92 pg mL^{-1}, $p = 0.002$), and who also showed higher freshwater survival probability (81%, 13/16) than males (50%, 7/14), although the difference was not statistically significant ($p = 0.079$, binomial logistic regression). Nonetheless, these results indicated that, at the individual level, high cortisol concentrations did not directly impair freshwater survival probability. In addition, lower recovery times (between artificial spawning and blood sampling) were not correlated increased plasma cortisol, glucose or PGE_2 in either year (p-values > 0.39, Pearson's r). Furthermore, individual level of plasma cortisol did not differ between early and late migrating hatchery-spawned kelts ($p = 0.71$), nor did glucose ($p = 0.058$) and PGE_2 levels ($p = 0.93$, Mann–Whitney *U* tests).

Table 2
Output of the best fitting linear regression model of freshwater-exit timing (in days after release) as a function of spawning condition and population of origin, while controlling for individual differences in release date. The baseline outcome (or intercept), represents female hatchery-spawned kelts from the Middle River.

Explanatory variable	Coefficient	SE	*t*-Statistic	p-Value
Intercept	35.0	21.0	1.7	0.10
Spawning condition (wild)	131.6	26.3	5.0	< 0.001
Sex (male)	59.4	24.4	2.4	0.021
Spawn. cond. (wild):sex (male)	−62.0	35.2	−1.8	< 0.001
Release date	0.288	1.0	0.3	0.77

Multiple $R^2 = 0.50$.

3.3. Estuarine survival and residency

Of the 39 kelts (20 hatchery-spawned and 19 wild-spawned) that

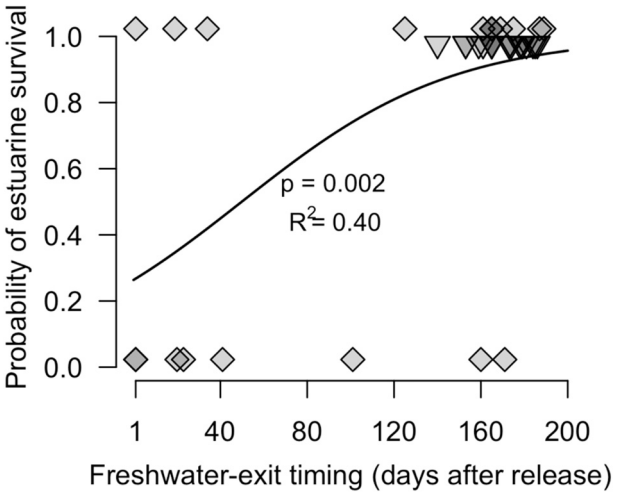

Fig. 5. Binomial logistic regression of the probability of estuarine survival as a function of freshwater-exit timing for hatchery- (diamonds, n = 20) and wild-spawned (triangles, n = 19) kelts combined. Day 1 represents Nov-21 in 2014–15, and Nov-24 in 2015–16. Mar-21, which marks the limit between early (fall/winter) and late (spring) migrating kelts, occurred on day 121 after release in 2014–15, and on day 119 in 2015–16. The coefficient of determination (R^2) of the logistic regressions was computed using the "lrm" function of the "rms" package in R (Nagelkerke, 1991).

were detected leaving the rivers and moving into the Bras d'Or Lake system, a total of 31 kelts (12 hatchery- and 19 wild-spawned) survived to leave the system and reach the Atlantic Ocean (Table 1). Bras d'Or or estuarine survival of the six different tagging groups varied between 50% and 100% (Table 1), with an average group survival of 59% versus 100% for hatchery- and wild-spawned kelts, respectively (54% vs 100% for females alone). In contrast to the fifth null hypothesis, hatchery-spawned kelts showed significantly lower estuarine survival in comparison to wild-spawned kelts (p = 0.001; Fig. 3).

Assessing the factors influencing estuarine survival, freshwater-exit timing was the only significant predictor of estuarine survival (p = 0.002, binomial logistic regression; Fig. 5), with an increase of 0.02 in the log odds of survival for every additional day spent in river before migrating out to the estuary. While all wild-spawned kelts (n = 19) exited the river to enter the Bras d'Or in the spring and subsequently survived to reach the Atlantic Ocean, estuarine survival was much lower for hatchery-spawned kelts (Fig. 3). Looking specifically at the physiological, morphological, and phenological factors, as well as sex that influenced the estuarine survival probability of hatchery-spawned kelts, the model with the lowest AIC score only included freshwater-exit timing (following forward stepwise model building). In fact, spring-migrating hatchery-spawned kelts showed significantly higher estuarine survival (82%, n = 11) than earlier migrating counterparts (33%, n = 9; p = 0.037, binomial logistic regression). Furthermore, individual levels of plasma cortisol, glucose, and PGE_2 did not differ significantly between estuarine survivors and hatchery-spawned kelts that died (p > 0.2, Mann–Whitney *U* tests).

The residency period of kelts in the Bras d'Or estuary varied between 5 and 191 days. Although more variable in hatchery-spawned kelts (54 ± 59 days), their average estuarine residency period did not differ significantly from wild-spawned counterparts (31 ± 16 days; p = 0.64, Mann–Whitney *U* test), consistent with the sixth null hypothesis. Interestingly, the three early-migrating, hatchery-spawned kelts that survived to the Ocean showed highly contrasting estuarine residency patterns. Two of them spent the entire winter in the Bras d'Or system with residency periods of 159 and 191 days, while the third one left the system on December 21st (9 days estuarine residency period) and is the only individual that was detected entering the North Atlantic Ocean before spring. Furthermore, consistent with the seventh and last null hypothesis, the Bras d'Or exit timing (or Ocean entry) did not differ significantly between the two spawning conditions (p = 0.70, Mann–Whitney *U* test). Apart from the only individual that left the system in December, fish exited in a narrow period between May 30th and June 21st.

3.4. Survival to repeat-spawning

Of the 61 Atlantic salmon kelts that were initially tagged over the two years of study, 0% (0/30) of hatchery-spawned kelts survived to come back to the river as repeat-spawner, while 6.5% (2/31) of wild-spawned kelts did. Both kelts that survived were wild-spawned, 2SW females that were tagged in late November 2015, which then came back to the Middle River on Sep-7 and Sep-10, 2017, as alternate repeat spawners. Strictly looking at females, 0% (0/16) of hatchery-spawned kelts survived to repeat spawning, while 9.1% (2/22) of wild-spawned counterparts did. These two kelts spent 489 and 504 days in the marine environment between spawning events.

4. Discussion

Overall, our findings indicate that hatchery-spawned Atlantic salmon kelts differed from their wild-spawned counterparts physiologically, behaviourally, and in relation to survival. Physiologically, hatchery-spawned kelts showed significantly elevated stress levels (high plasma cortisol and glucose) as well as potentially altered immune state (through significantly elevated plasma PGE_2 levels) compared to wild-spawned kelts (Fig. 2). While no differences in freshwater survival were found between the two spawning conditions, hatchery-spawned kelts showed significantly earlier freshwater-exit timing, a difference of ~66 days. This likely resulted in significantly lower estuarine survival probability for hatchery-spawned kelts, with estuarine survival being the lowest in early-migrating, hatchery-spawned kelts. Furthermore, none (0/30) of the hatchery-spawned kelts survived to spawn again, while 6.5% (2/31) of wild-spawned counterparts did, coming back as alternate repeat-spawners (0% vs 9.1% for females alone). While these are low numbers, it supports the idea that the difference in estuarine survival might be carried through the rest of the marine migration. Collectively, these findings suggest an effect of hatchery operations on individual fitness with potential influence on the likelihood of repeatspawning. This in turn could have consequences for population level processes, which we discuss below.

Physiological condition differed markedly between hatchery- and wild-spawned kelts, suggesting that the stress associated with confinement and handling in the hatchery was at least an indirect driver of their premature freshwater-exit timing and reduced survivorship. Although elevated plasma cortisol, glucose, and PGE_2 levels were not associated with premature freshwater-exit timing nor decreased survival, this might have been due to the time lag between initial blood sampling dates, return to the rivers, and subsequent migration dates. For the purpose of this study (i.e., documenting the physiological stress resulting from hatchery operations), hatchery-spawned kelts were sampled at the hatchery, prior to their transportation back to their river of origin. Therefore, the stress state and immune health indicator measured did not necessarily correspond to the final levels, upon hatchery-spawned kelts return into the wild. We can speculate, however, that the elevated stress response in hatchery fish had some disruptive effect on some other physiological system (or systems) related to the perception of seasonal migratory cues, resulting in altered migratory behaviour. But whatever the mechanism, we show that hatchery exposure resulted in a mismatch between migration timing and survival, which had implications for individual fitness. Despite the absence of direct links between physiology and subsequent migratory decision in our study, stress and corticosteroids can have a broad range of impacts on the immune system by acting directly on immune cells or thorough secondary agents (Espelid et al., 1996). It is also possible that

all hatchery-spawned kelts had an increased susceptibility to infections through a threshold cortisol effect. For example, in adult brown trout (*Salmo trutta*), chronic elevation of plasma cortisol above 30 ng mL^{-1} was sufficient to increase fish susceptibility to infectious diseases (Pickering and Duston, 1983). Furthermore, plasma cortisol has been shown to reduce the number of lymphocytes to abnormally low level in brown trout (Pickering, 1984). In Atlantic salmon, cortisol injections (which raised circulating concentration to levels similar to those observed in our study) had an immunosuppressive effect through the alteration of lymphocyte functioning (Espelid et al., 1996). Similarly, Atlantic salmon subjected to a prolonged stressor (i.e., infection of the parasitic copepod *L. salmonis*) showed significantly elevated plasma cortisol levels along with increased PGE_2 levels relative to uninfected fish. This led to inhibition of immune-related genes interleukin-1ß (IL-1ß) and major histocompatibility (MH) class II (Fast et al., 2006). In addition, *Saprolegnia parasitica*, a parasitic mycelium commonly observed on salmonids in aquaculture and in nature, can produce PGE_2 to suppress the expression of genes related to cellular immunity (Belmonte et al., 2014). Although we did not quantify the external fungal infection (e.g., *Saprolegnia* spp.) in our fish, we noted qualitatively that the hatchery-spawned kelts had higher incidences of fungus on their fins, flanks, and nose relative to the wild-spawned fish. This is likely due to fish handling and abrasion occurring in hatchery tanks, which might have contributed to the increased PGE_2 levels in those fish. In juvenile anadromous brown trout in the wild, fish with artificially elevated cortisol levels showed amplified weight loss, increased overwinter freshwater mortality, and earlier freshwater-exit timing with a majority of fish entering the estuary during winter (Midwood et al., 2015; Peiman et al., 2017). In a similar study, juvenile anadromous brown trout with cortisol injections expressed reduced growth and lower survival during the spring downstream migration (Midwood et al., 2014). Although these studies were conducted on a different species at a different life-history stage, their findings complement our current findings that hatchery-spawned kelts, with elevated stress levels and potentially altered immunity, showed premature freshwater-exit timing. While PGE_2 is a common biomarker of inflammation in mammals (de Grauw et al., 2009; Morris et al., 2013; Solcà et al., 2016; van de Water et al., 2016), and increases in circulating levels of PGE_2 were previously associated with altered immune-competence in salmonids (Espelid et al., 1996; Fast et al., 2005, 2006; Gómez-Abellán and Sepulcre, 2016; Knight and Rowley, 1995), we recognize that plasma PGE_2 needs further characterization and validation as its role as a biomarker for inflammatory/immunological status in teleosts. In general, there is a paucity of biomarkers for non-destructively assessing salmonid/teleost health, and PGE_2 among other protein-based assessments could provide a much clearer picture in the future.

Among iteroparous salmonids, the energetic cost of spawning is reported to be highest in Atlantic salmon, with both sexes investing upwards of 60–70% of their total somatic energy reserves (Jonsson et al., 1997). In combination with sparse post-spawning feeding opportunities in fresh water throughout the winter months, the total degree of somatic depletion through reproduction is a key determinant of individual survival (Belding, 1934). Variation in survival probability is mediated by variation in post-spawning condition, which may influence decisions about the timing of seaward migration. Halttunen et al. (2013) showed that Atlantic salmon kelts with low body condition factors initiated their seaward migrations early in the winter months, as opposed to kelts with better condition factors that migrated later in the spring. However, we did not observe this relationship; although hatchery-spawned kelts exited fresh water significantly earlier than wild spawned kelts (~66 days on average), this difference in phenology was independent of individual body condition (i.e., residual mass). However, whereas Halttunen et al. (2013) examined variation in post-spawning body condition and its implication on the seaward migratory timing of naturally-spawned kelts, we examined differences between artificially-spawned versus naturally-spawned kelts. As such, given the observed increase in plasma cortisol and glucose for hatchery-spawned kelts, we cannot rule out that aspects of their energy reserves perhaps not reflected in body condition index might have been depleted as a result of elevated stress levels and increased basal metabolism.

Ice covered freshwater habitats are believed to offer safe overwinter refuges for salmonids when available (Komadina-Douthwright et al., 1997), raising concerns that early migrating individuals (i.e., those deciding to leave the river before spring) might experience reduced estuarine and marine survival due to increased predation, low food availability, and/or sub-optimal environmental conditions in winter (Halttunen et al., 2013; Peiman et al., 2017). We have shown that early winter migrants, which were entirely composed of hatchery-spawned individuals, had a significantly lower probability of estuarine survival (~2.5-fold lower) relative to later spring migrants. This might potentially reflect an additive effect of entering the estuarine environment at a time when conditions are sub-optimal, plus secondary effects of elevated stress levels and potentially weakened immunity. Interestingly, hatchery-spawned kelts that did migrate later in the spring had similar survival as their wild-spawned counterparts, and had similar overall patterns of behaviour for estuarine residency period and ocean-entry timing. This might indicate that some individuals have the potential to recover from hatchery exposure and avoid the deleterious consequences of early migration. However, none of those hatchery-spawned kelts survived to spawn again.

4.1. Management implications

The physiological, behavioural, and survival consequences for wild adult Atlantic salmon captured as part of broodstock collection program were significant, and might be of particular concern to hatchery operations in other jurisdictions more broadly. In the present context, considering the small numbers of spawners collected each year from the Middle and Baddeck rivers for broodstock collection purposes (7–8 fish collected from populations estimated at 312 and 215 spawners respectively between 2007 and 2011, Levy and Gibson, 2014), as well as the low frequency estimate of repeat spawning in Middle and Baddeck rivers (3.4% and 4.8% respectively, Gibson and Levy, 2014), the potential population-level impact of an increase in kelt mortality is likely minimal. This is important considering that these populations were proposed to be listed as endangered (COSEWIC, 2010). However, the importance of repeat spawners to Atlantic salmon population dynamics is slowly being recognized. For example, female repeat spawners, and especially alternate repeat spawners, have increased reproductive potential due to their larger size and associated higher fecundity. Comparing maiden spawners with alternate repeat spawners that matured at the same sea-age (1SW or 2SW), alternate repeat spawners have fecundities ~2.8-fold (~7200 vs 2539 eggs) and ~1.7-fold greater (~11,000 vs 6320 eggs) for salmon that matured at 1SW and 2SW, respectively (Reid and Chaput, 2012). Previous estimates suggested that repeat spawning frequencies from 3% to 30% can contribute to 8% up to > 40% of the total annual egg deposition in Atlantic salmon populations (Halttunen, 2011; Hubley and Gibson, 2011; Moore et al., 1995; Randall, 1989). Therefore, efforts to increase juvenile freshwater survival (e.g. egg to fry survival) via hatchery rearing practices should consider the potential trade-off in the reduced survival of hatchery-spawn kelts and the lost future recruitment potential from this reduction of iteroparity. While further research is required, the frequency and composition of repeat spawners (e.g., sex ratio, numbers and proportion of consecutive versus alternate females) are likely to be good proxies for assessing the potential importance of kelts to population level processes. And while these components vary greatly across the species' global distribution and among years (Chaput et al., 2006; Fleming, 1998), potential population level effects of a reduction in kelt survival should be considered.

In threatened populations of anadromous rainbow trout or steelhead (*Oncorhynchus mykiss*), another iteroparous salmonid species that

shares similar incidence of repeat-spawning as Atlantic salmon (Fleming, 1998), increasing iteroparity has been identified as a goal to aid in stock recovery through management actions such as the development of appropriate dam downstream passages, kelt transportation and reconditioning (Keefer et al., 2008; summarized in Penney and Moffitt, 2014). However, our current findings suggest that nutritional requirements should not be the only consideration to improve the survival of broodstock kelts returned to the wild, but that the stress imposed through captivity and handling might also have cascading effects on the physiology, behaviour, and survival. Therefore, management practices that mitigate the many stressors that wild salmon experience when captured for broodstock programs should be considered (e.g., minimizing confinement period, reducing air exposure time during striping and handling frequency, allowing moderate flow in hatchery environment to favors exercise, Patterson et al., 2004). Reducing such stressors could be beneficial for adult salmonids, and also for the survival of eggs and juveniles that might also be negatively affected by high stress levels through maternal effects (Patterson et al., 2004; Sopinka et al., 2016). In terrestrial and aquatic species more broadly, mitigating the stressors imposed on reproductive animals through captive breeding programs may be key in improving the survivorship of both post-reproductive adults and juveniles reintroduced into the wild. Research and management efforts should be directed at the evaluation and integration of stress mitigation measures, moving towards improving animal welfare and the effectiveness of captive breeding programs.

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