NRC Research Press

ARTICLE

Surface water with more natural temperatures promotes physiological and endocrine changes in landlocked Atlantic salmon smolts[1]

Amy M. Regish, William R. Ardren, Nicholas R. Staats, Henry Bouchard, Jonah L. Withers, Theodore Castro-Santos, and Stephen D. McCormick

Abstract: Hatchery salmonid smolts are often reared using groundwater with elevated temperatures to maximize growth. Previous work has shown that rearing hatchery smolts in surface water with a more natural thermal regime resulted in increased return rates of adult landlocked Atlantic salmon (*Salmo salar*). We evaluated whether landlocked Atlantic salmon reared in surface water with a natural temperature regime have altered physiological smolt characteristics compared with fish reared in groundwater with elevated winter temperatures. Hatchery fish were sampled three consecutive years from January to May. Additional fish were released as smolts, recaptured, and compared with fry-stocked smolts. Surface water smolts had earlier peaks of plasma T_4, lower T_3 levels, later peak cortisol, and lower gill Na^+/K^+-ATPase activity as compared with groundwater smolts. After release and recapture, surface water fish had elevated plasma T_4 and gill Na^+/K^+-ATPase activity compared with groundwater fish, but less than stream-reared fish. Elevated plasma T_4 in surface water fish in the hatchery and after release may have promoted imprinting and other aspects of smolt development, contributing to the higher adult return rates of a cohort reared in surface water.

Résumé : De l'eau souterraine à des températures élevées est souvent utilisée pour l'élevage de saumoneaux en écloserie afin de maximiser leur croissance. Des travaux antérieurs ont démontré que l'élevage de saumoneaux d'écloserie dans de l'eau de surface dans un régime thermique plus naturel produit de plus hauts taux de retours de ouananiches adultes (*Salmo salar*). Nous avons tenté de déterminer si les saumoneaux de ouananiches élevées dans de l'eau de surface dans un régime thermique naturel présentent des caractéristiques physiologiques modifiées par rapport à celles de poissons élevés dans de l'eau d'origine souterraine à des températures hivernales élevées. Des poissons d'écloserie ont été échantillonnés durant trois années consécutives de janvier à mai. D'autres poissons ont été relâchés en tant que saumoneaux, recapturés et comparés aux saumoneaux ensemencés en tant qu'alevins. Les saumoneaux d'eau de surface présentent des pics plus précoces des teneurs en T_4 plasmatique, des teneurs en T_3 plus faibles, des pics plus tardifs des teneurs en cortisol et une plus faible activité de la Na^+/K^+-ATPase des branchies que les saumoneaux d'eau souterraine. Après le lâcher et la recapture, les poissons d'eau de surface ont des teneurs en T_4 plasmatique et une activité de la Na^+/K^+-ATPase des branchies plus élevées que les poissons d'eau souterraine, mais plus faibles que celles de poissons élevés en cours d'eau. Les teneurs en T_4 plasmatique élevées dans les poissons d'eau de surface en écloserie et après le lâcher pourraient avoir favorisé l'imprégnation et d'autres aspects du développement des saumoneaux, contribuant à expliquer les plus hauts taux de retour d'adultes d'une cohorte élevée dans de l'eau de surface. [Traduit par la Rédaction]

Introduction

In preparation for downstream migration and ocean entry, anadromous Atlantic salmon (*Salmo salar*) smolts undergo a series of morphological, behavioral, and physiological changes that increase their survival in these new environments (McCormick et al. 1998). The vast literature on physiology of anadromous salmon informs us on the presumed behavior and physiology of much less well-studied landlocked salmon. Landlocked salmon, unlike anadromous salmon, do not access seawater as part of their life cycle. Although there is great variability in life history strategies of landlocked salmon (reviewed in Hutchings et al. 2019), here we refer to landlocked Atlantic salmon as salmon that spend their early life in streams, migrate to lakes where

Received 31 July 2020. Accepted 5 January 2021.

A.M. Regish and T. Castro-Santos. USGS, Leetown Science Center, S.O. Conte Anadromous Fish Research Laboratory, Turners Falls, MA 01376, USA.
W.R. Ardren,* N.R. Staats,† and J.L. Withers. US Fish and Wildlife Service, Lake Champlain Fish and Wildlife Conservation Office, Essex Junction, VT 05452, USA.
H. Bouchard.† US Fish and Wildlife Service, Dwight D. Eisenhower National Fish Hatchery, North Chittenden, VT 05763, USA.
S.D. McCormick. USGS, Leetown Science Center, S.O. Conte Anadromous Fish Research Laboratory, Turners Falls, MA 01376, USA; Department of Environmental Conservation, University of Massachusetts, Amherst, MA 01003, USA.

Corresponding author: Amy M. Regish (email: aregish@usgs.gov).

*William Arden served as a Guest Editor at the time of manuscript review and acceptance; peer review and editorial decisions regarding this manuscript were handled by Larry Greenberg.

†Retired.

[1]This article is being published as part of the special issue "Conservation, Ecology, and Evolution of Nonanadromous Atlantic Salmon", arising from an international symposium on landlocked Atlantic salmon held 17–20 June 2018, at the Ecology and Evolutionary Ethology of Fishes Conference in Montréal, Quebec.

subsequent growth and maturation occurs, and return to their natal streams to spawn. Thus, during smolting, landlocked salmon migrate downstream and must adapt to lacustrine conditions, which are quite different from streams. Important differences include loss of rheotactic behavior, development of schooling behavior, and a foraging shift from primarily insectivory to piscivory (Coghlan et al. 2007; Pientka and Parrish 2002).

Landlocked populations undergo downstream migration at a similar size and time of year as anadromous salmon (Schmitz 1995) and maintain traits associated with smolting such as changes in morphology and the capacity to migrate (Nemeth et al. 2003; Hutchings et al. 2019), but in most cases only partially develop the high level of salinity tolerance characteristic of anadromous strains (Nilsen et al. 2003, 2008; McCormick et al. 2019). This could be predicted in landlocked populations where the energetic cost of maintaining elevated salinity tolerance may be considered maladaptive. The observed maintenance of some level of salinity tolerance may be due to the pleiotrophy of hormonal action; the continued selection of beneficial traits of smolting may allow some associated salinity tolerance if they are controlled by the same endocrine system (Lemmetyinen et al. 2013; Piironen et al. 2013; McCormick et al. 2019).

Landlocked salmon populations have been extirpated from large portions of the southern part of the species range in North America, and large-scale hatchery programs have been established to restore landlocked salmon fisheries and natural populations in Lake Ontario and Lake Champlain (Hutchings et al. 2019). Lake Champlain landlocked salmon were extirpated by 1842 due to damming of tributaries, overfishing, deforestation, and subsequent habitat loss caused by the logging industry (Marsden and Langdon 2012). Efforts to restore these populations have relied on stocking hatchery-reared fry and smolts into Lake Champlain tributaries since 1972 (Marsden and Langdon 2012; Brunsdon et al. 2017). Currently, multiple federal and state hatcheries supply hatchery fish for restoration; however, adult returns to rivers and natural spawning goals have not yet been achieved (Hutchings et al. 2019). Recent Lake Champlain landlocked salmon restoration research has focused on modifying stocking treatments (Brunsdon et al. 2017, 2020), recolonization of spawning habitats after dam removal (Hill et al. 2019; Prévost et al. 2020), genetic response to thiamine deficiency (Harder et al. 2020), improving passage in tributaries (Nyqvist et al. 2017; Harbicht et al. 2018), control of sea lamprey (*Petromyzon marinus*) (Marsden et al. 2003), and modification of hatchery rearing conditions and release times (Harbicht et al. 2020).

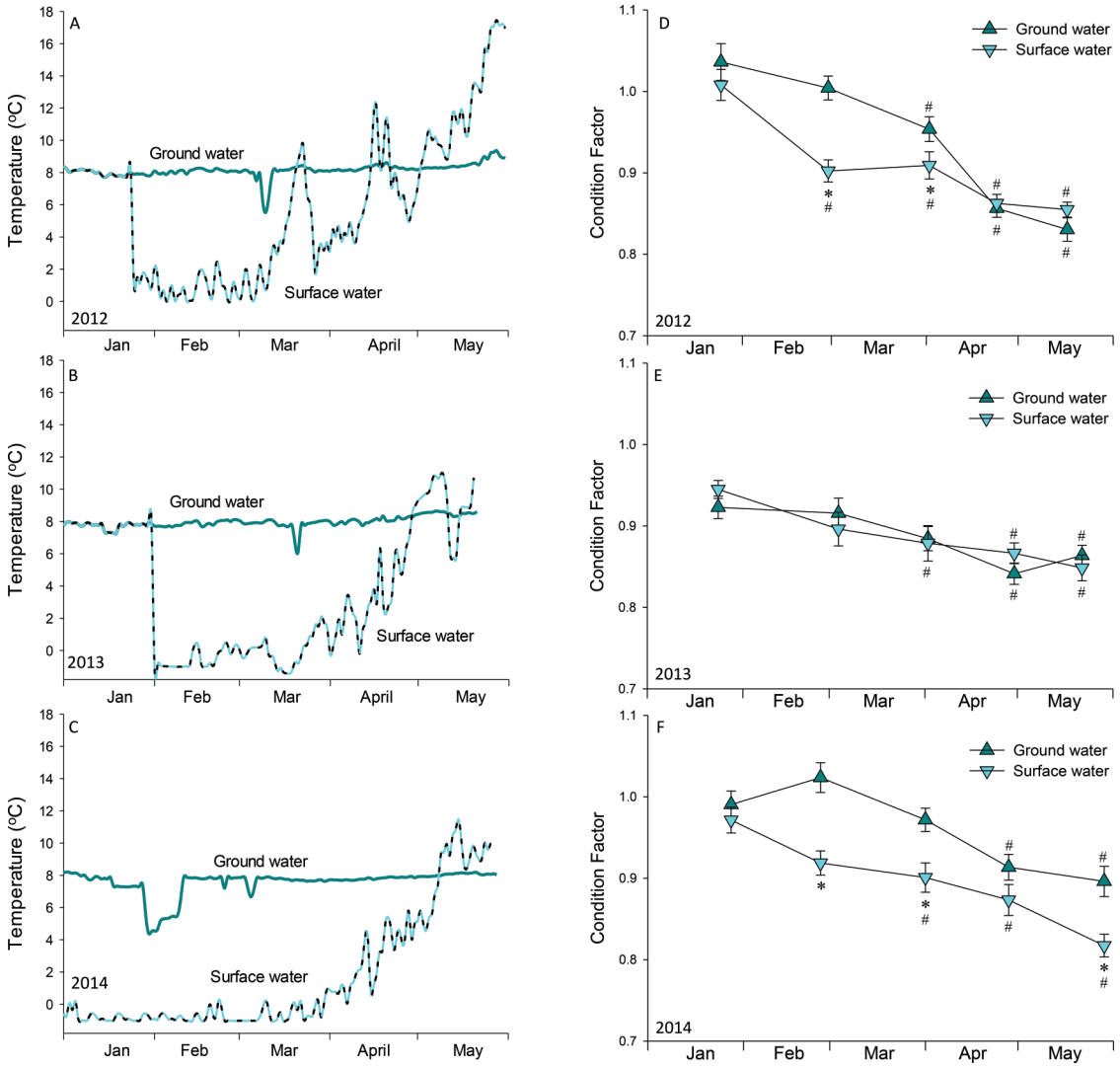
Both anadromous and landlocked salmon juveniles reared for population restoration and released as smolts are often subject to an increased temperature regime. Hatchery practices have sought to produce yearling smolts of larger size, with the aim of reducing rearing costs and improving the smolt-to-adult survival rate. Although large size hatchery stock may reduce their risk of predation, artificial hatchery selection of phenotypes such as large body size may not translate into success in a more natural setting (Saikkonen et al. 2011). In contrast, by exposing juvenile salmonids to more natural conditions, hatcheries may reduce growth rates but improve smolt development. This has been demonstrated in anadromous hatchery-reared Chinook salmon (*Oncorhynchus tshawytscha*) where the best smolt-to-adult return rates came from hatchery-reared groups that experienced a strong seasonal change in growth rate (Beckman et al. 2017). The use of surface water for rearing landlocked smolts, which more closely resembles natural thermal conditions than standard groundwater conditions, has been explored by Harbicht et al. (2020). They demonstrated that return rates of spawning adults in Lake Champlain increased 4.8-fold (3-year average estimate based on returns to the Winooski River, Winooski, Vermont, 16 km from the lake) compared with the standard hatchery production method of cohorts reared in warmer groundwater in winter months. They conclude that their results do not support the idea that larger size-at-release among hatchery-reared landlocked salmonids always improves survival to maturity and migratory returns and that more natural growth trajectories in the hatchery can improve survival to adulthood relative to optimized production methods while increasing migratory returns. To our knowledge, the physiological patterns brought on by more seasonal changes in hatchery-reared juvenile landlocked smolts have not been examined.

Smolt development is triggered by environmental cues including photoperiod, temperature, and flow (Jonsson and Ruud-Hansen 1985; Zydlewski et al. 2005; Jensen et al. 2012) and is driven by hormonal changes (McCormick et al. 2007; Nilsen et al. 2008). Increases in thyroid hormones (THs) are thought to be important in morphological changes (McCormick 2013) and the process of imprinting (Dittman et al. 1996; Lema and Nevitt 2004), whereas increased growth hormone (GH) and cortisol levels are both strongly linked to increased salinity tolerance and contribute to the metabolic and osmoregulatory changes that occur during smolting. Cortisol is involved in several smolt-related processes and is acknowledged as one of the main regulators of hypo-osmoregulatory mechanisms (Bisbal and Specker 1991; Nilsen et al. 2008). Of particular importance to anadromous smolts is the development of salinity tolerance and increased gill Na^+/K^+-ATPase (hereinafter NKA) enzyme activity, which have been found to be strongly correlated. This enzyme is also elevated in landlocked salmon smolt gill and shows the same temporal pattern of increasing protein abundance and activity level, although to a lesser extent (McCormick et al. 2019). In anadromous salmon these transitions result in the development of traits that support the survival of smolts in the ocean (Hoar 1988), while in landlocked salmon these smolt development patterns are thought to be important in landlocked salmon survival and fitness in freshwater systems. In particular, traits associated with imprinting and migration from streams to lakes allow for exploitation of freshwater habitats that promote growth and maturation and homing. (Nemeth et al. 2003; Piironen et al. 2013; Keefer and Caudill 2014).

In anadromous Atlantic salmon, photoperiod has been shown to set the overall timing of smolt development, with temperature having important secondary effects (McCormick et al. 1998). Constant high winter temperature (10 °C) has been shown to accelerate increases in gill NKA activity and salinity tolerance compared with ambient seasonal temperatures (McCormick et al. 2000). Constant elevations in temperature (e.g., 5 and 15 °C) can alter the timing of the peak of smolt development by several weeks (Handeland et al. 2004). In addition to what happens within the hatchery, substantial endocrine and physiological changes of smolts can occur after release (McCormick et al. 2003). These changes may be impacted by differences in temperature between the rearing hatchery and the receiving stream and can have impacts on subsequent survival and imprinting (McCormick et al. 1998). The effects of temperature on the process of imprinting have not, to our knowledge, been examined in any salmonid species.

The aim of this study was to evaluate the physiological impacts of rearing landlocked salmon on surface water with a more natural winter and spring thermal regime, which has been shown to increase return rates (Harbicht et al. 2020) compared with fish reared on groundwater at elevated winter temperatures. We hypothesize that these changes in hatchery conditions would optimize juvenile rearing and would be reflected in measured physiological smolt indices. To compare the two treatments, we measured common smolt indices over time, including thyroid hormones (T_4 and T_3) that are associated with downstream migration and imprinting, cortisol, which controls metabolism and osmoregulation, and gill NKA activity, which has been widely used as a marker of smolt development in anadromous salmon. We also examined the timing of downstream migration and physiological

Fig. 1. Water temperatures of groundwater (solid lines) and Furnace Brook surface water (dashed lines) in 2012 (A), 2013 (B), and 2014 (C). Condition factor at each sampling time point for 2012–2014 (D–F) for groundwater fish are represented as dark upward pointing triangles, and experimental surface water fish are represented as lighter downward facing triangles. Symbols represent means, and error bars show standard error. Significant difference ($P < 0.05$) within treatment in condition factor from initial time point by one-way ANOVA followed by Dunnett post hoc comparison is indicated by a number symbol (#), and an asterisk (*) indicates significant difference between treatments.



changes of the two groups after release and compared them with fish that had been fry-stocked and stream-reared.

Materials and methods

Hatchery conditions and release–recapture methods

In the fall of 2010, 2011, and 2012, Sebago Lake strain captive brood stock salmon embryos (Havey and Warner 1985) raised at the Vermont Department of Fish and Wildlife Bald Hill Fish Culture Station (Newark, Vermont, USA) were transferred immediately after fertilization to US Fish and Wildlife Service (USFWS) D.D. Eisenhower National Fish Hatchery (Pittsford, Vermont, USA). Embryos were maintained in vertical tray incubators with groundwater (~8–10 °C, from two onsite wells, below 50 ft. depth (1 ft. = 30.48 cm), not influenced by surface water) through hatching and yolk sac absorption. At this stage fish were transferred to 2.4 m diameter tanks supplied with heated groundwater (~15 °C) and fed ad libitum four times daily. They remained on groundwater through their first winter and spring. When temperatures of Furnace Brook (hereinafter termed surface water) exceeded groundwater temperatures (late June), juveniles were moved to raceways containing surface water for maximal growth. In the fall, fish were moved to groundwater until January. On 24 January 2012 and 29 January 2013, the experimental treatment fish were moved to raceways containing surface water until May (Figs. 1A–1C). The 2014 cohort was kept on surface water from 28 June 2013 through smolting in 2014. The chemical composition of groundwater and surface water was, respectively, as follows: Na: 4.0, 0.8 mg·L^{-1}; Cl: 7.6, 0.8 mg·L^{-1}; Ca: 27, 13 mg·L^{-1}; Mg: 13.0, 5.8 mg·L^{-1}; SO_4: 5.1, 4.7 mg·L^{-1}; hardness: 121, 56 mg·L^{-1}; conductivity: 250, 110 μmho·cm^{-1} (1 mho = 1 seimens); pH: 7.9, 7.4. Aside from water source and temperature differences, photoperiod, tank densities, and feeding schedules were the same for both groundwater and surface water fish.

Fish from groundwater and surface water were sampled from raceways at five time points from January through the peak of smolting in May (n = 12 per water source, per time point, 2012, 2013, 2014). Hatchery smolts from each treatment group were released into the Huntington River (tributary to the Winooski River 56 river kilometres (rkm) upstream from Lake Champlain;

see Nyqvist et al. 2017). These fish were released to determine movement behavior and conduct physiological sampling following recapture in a downstream smolt trap. Releases were as follows: 4 April 2012 (n = 568 each groundwater and surface water), 1 May 2013 (n = 500 each groundwater and surface water), and 29 April 2014 (n = 500 each groundwater and surface water). A rotary screw trap (hereinafter, smolt trap) (E.G. Solutions, Corvallis, Oregon) was deployed in the Huntington River at rkm 0.5 on 1 April 2012. The trap consisted of two 8 m floating pontoons between which a revolving mesh-covered cone was suspended. The large end of the cone (2.4 m diameter) faced upstream, and an internal screw built into the cone's center axle rotated the cone as the water current exerted pressure on it. Downstream-migrating fish that entered the cone were passed to the end of the cone and collected in a live box. The trap was tied to the shore and positioned in the upstream end of a pool at the end of a shallow riffle that funneled much of the flow into the cone. Released fish were recaptured in the smolt trap and sampled in 2012 on 5 and 12 April, 23 May, and 27 May. Migrating stream-reared smolts were also captured and sampled from the smolt trap.

To examine migration behavior of stocked hatchery-reared smolts, we radio-tagged an equal number of groundwater (n = 10) and surface water (n = 10) smolts and released them just downstream of the Winooski One Dam (rkm 16.51) on 17 April 2013. We used the methods of Nyqvist et al. (2017) for tagging and monitoring movement of tagged fish. Four stationary automatic receiver stations were positioned throughout the river from just below the dam to the river mouth (Stations 2–5 were located at rkm 0.17, 3.29, 9.97, and 15.57, respectively) to continuously monitor smolt movements for 23 days from 17 April to 9 May 2013. In addition, two mobile tracking events that covered the entire study reach from the river mouth to Winooski One Dam occurred on 18 April and 8 May 2013. Initiation of migration was determined by detection at Station 5. Migration speed was calculated as time of first detection at Station 5 to time of first detection at Station 2 divided by the length of river between these stations.

On 5 May 2014, we sampled gill tissue from groundwater-reared salmon for measurement of gill NKA activity from 10 fish per 10 mm divisions ranging from total length of 90 to 200 mm to validate our size threshold for smolt development (see Sampling section below).

Sampling

Fish of total length 150 mm and greater from each treatment were selected to exclude fish that would be too small to smolt in the spring (McCormick et al. 2007). Fish were anesthetized with MS-222 (100 mg MS-222·L^{-1}, pH 7.0), weighed to the nearest 0.1 g, and total length was recorded to the nearest 1 mm. The range of total lengths for each sampling time point in each year is represented in Table 1. Blood was collected in heparinized 1 mL syringes from the caudal blood vessels and centrifuged at 2500g for 5 min. Plasma was removed and stored at −80 °C for analysis. Gill biopsies for NKA activity consisting of four to six primary gill filaments were taken and placed into 100 μL SEI buffer (250 mmol·L^{-1} sucrose, 10 mmol·L^{-1} Na_2EDTA, and 50 mmol·L^{-1} imidazole, pH 7.3) and stored at −80 °C. All fish rearing occurred under USFWS guidelines, in accordance with accepted guidelines for the use of fish in research (American Fisheries Society, American Institute of Fishery Research Biologists, and American Society of Ichthyologists and Herpetologists) (Jenkins et al. 2014), and all sampling was carried out in accordance with US Geological Survey institutional guidelines and an approved Institutional Animal Care and Use Committee protocol (SP-9065).

Plasma thyroid hormones

Plasma triiodothyronine (T_3) and thyroxine (T_4) were measured by a direct radioimmunoassay (Dickhoff et al. 1978). Briefly, 10 μL of plasma was incubated in 12 mm × 75 mm glass tubes with antibodies directed to either T_3 or T_4 (Fitzgerald Antibodies, Acton, Massachusetts, USA) and ^{125}I-labeled radioligand triiodothyronine or thyroxine (Perkin Elmer, Waltham, Massachusetts, USA) at a concentration previously determined to achieve 50% binding at 5000 counts per minute. Samples were incubated at 37 °C for 30 min, precipitated with polyethylene glycol at 4 °C, and centrifuged for 20 min at 2000g at 4 °C. The resulting supernatant was aspirated, and tubes containing the precipitate were counted for 2 min using a Beckman 5500 Gamma counter. Plasma hormone concentration was calculated using a four-parameter curve of standard samples (1–100 ng·mL^{-1}) run in triplicate in each assay.

Table 1. Total length (mm) of Atlantic salmon juveniles sampled in 2012–2014.

Year	Water source	Jan.	Feb.	Mar.	Apr.	May
2012	Groundwater	155–188	153–212	175–241	171–229	197–214
	Surface water	150–204	157–190	152–180	166–204	150–217
2013	Groundwater	164–197	170–226	180–237	204–251	218–254
	Surface water	160–196	150–202	165–202	162–206	160–207
2014	Groundwater	158–200	177–224	177–225	184–230	195–242
	Surface water	160–194	164–215	163–195	154–215	162–222

Note: Sampling dates in 2012: 25 January, 29 February, 2 April, 24 April, and 17 May. 2013 sampling dates: 24 January, 4 March, 2 April, 30 April, 22 May. 2014 sampling dates: 28 January, 26 February, 1 April, 28 April, 29 May. Values are ranges of size for each sampling time point for each year.

Plasma cortisol

Plasma cortisol levels were measured by a validated direct competitive enzyme immunoassay as outlined in Carey and McCormick (1998). Sensitivity, as defined by the dose–response curve, was 1 to 400 ng·mL^{-1}. The lower detection limit was 0.2 ng·mL^{-1}, and samples below this concentration were appointed the detection limit value. Using a pooled plasma sample, the mean intra-assay variation was 7.8%, and the mean interassay variation was 9.5%. Hatchery fish were bled within 5 min to ensure that cortisol levels were not elevated due to handling stress. Cortisol was not measured in fish captured by smolt trap.

Gill NKA activity

NKA activity of gill homogenates was determined using a temperature-regulated microplate method (McCormick 1993). Ouabain-sensitive NKA activity was measured by coupling the production of ADP to NADH using lactic dehydrogenase and pyruvate kinase in the presence and absence of 0.5 mmol L^{-1} ouabain. Samples (10 μL) were run in duplicate in 96-well microplates at 25 °C and read at a wavelength of 340 nm for 10 min on a BioTek Synergy2 microplate reader using Gen5 software (BioTek, Winooski, Vermont, USA). Protein concentration of the homogenates was determined using a BCA protein assay (Pierce, ThermoFisher Scientific, Waltham, Massachusetts, USA).

Statistics

All data graphed are represented as mean ± one standard error of the mean. For all analyses, the probability of establishing statistical significance was $P < 0.05$. Fish condition factor (CF) was estimated from Fulton's condition factor, $K = W/TL^3 \times 100$, where W is total mass (g) and TL is total body length (mm). Differences within water source treatment over time were compared with initial sampling date by one-way analysis of variance (ANOVA) followed by Dunnett post hoc comparison and denoted by number symbols (#) on graphs. Differences between groundwater and surface water groups over time at the hatchery were compared by two-way ANOVA followed by Student–Newman–Keuls (SNK) post hoc comparisons using Statistica Software (Tibco Software, Palo Alto, California, USA) and denoted by asterisks (*). Comparison of hatchery fish with postrelease captured hatchery fish were compared by one-way ANOVA followed by SNK post hoc comparisons. Stream-

Table 2. Summary of two-way ANOVA results for physiological parameters for all hatchery sampling.

	2012	2013	2014
CF			
Treatment	0.0041	0.8946	<0.0001
Date	<0.0001	<0.0001	<0.0001
Treatment × Date	0.0014	0.4836	0.0930
T_3			
Treatment	0.9561	0.0337	<0.0001
Date	<0.0001	<0.0001	0.0984
Treatment × Date	0.0882	0.0546	0.0012
T_4			
Treatment	0.0907	<0.0001	0.8336
Date	<0.0001	<0.0001	<0.0001
Treatment × Date	<0.0001	<0.0001	<0.0001
Cortisol			
Treatment	0.8154	0.5127	0.9696
Date	<0.0001	<0.0001	0.0001
Treatment × Date	<0.0001	0.0140	0.0250
NKA			
Treatment	0.0004	0.0365	<0.0001
Date	<0.0001	<0.0001	<0.0001
Treatment × Date	0.0064	0.0617	<0.0001

Note: Here, *P* values are reported for treatment (groundwater versus surface water), date sampled, and the interaction of treatment and date. Degrees of freedom for all comparisons: treatment df = 1, date df = 4, and interaction df = 110–118. CF = condition factor; T_3 and T_4 = thyroid hormones; NKA = Na^+/K^+-ATPase.

reared fish were binned to the nearest 4-day period for physiological comparisons with hatchery-reared smolt recaptures.

Results

Hatchery sampling

Condition factor decreased in groundwater and surface water fish groups 2012, 2013, and 2014 (Figs. 1D–1F; Table 2). In 2012 and 2014, condition factor decreased sooner in the surface water fish relative to the groundwater fish, which is reflected by the significant interaction (Table 2).

For all three study years, plasma T3 hormone levels showed a similar pattern in terms of changes in hormone concentration over time (Figs. 2A–2C; Table 2). In both groups there was a general decrease in plasma T3 over time, with surface water fish showing earlier decreases in 2013 and 2014 (Figs. 2B and 2C). Plasma T_4 levels had the opposite pattern, with levels increasing in both groups over time (Figs. 2D–2F). Plasma T4 levels increased earlier in the surface water group in each year, with significantly higher levels in the surface water fish in late February and (or) early April compared with groundwater fish (Table 2). By May the surface water fish had reduced plasma T_4 levels, both compared with earlier in the year and compared with groundwater fish (Figs. 2D–2F; Table 2).

Plasma cortisol levels in each year were low in winter in both groups and then became elevated in late spring (Figs. 3A–3C). The increase in plasma cortisol occurred earlier in the groundwater fish (April) relative to surface water fish (May) in each year and is reflected by significant effect of sampling date and the interaction of treatment and date (Table 2).

Gill NKA activity increased over time in both groups in each year (Figs. 3D–3F). The timing of increases in gill NKA activity were similar in groundwater and surface water fish in 2012 and 2013 with peaks occurring in late April. In both of these years, gill NKA activity levels were significantly higher in the groundwater fish (Table 2). In 2014, gill NKA activity of groundwater fish increased steadily throughout the spring with highest levels in late May, whereas stream water fish had highest levels in late April, which then decreased substantially in late May. As with earlier years, the peak levels of gill NKA activity were higher in groundwater than in stream water fish in 2014 (Table 2).

Physiology of recaptured hatchery smolts and captured stream-reared smolts

Hatchery fish were released into the Huntington River on 4 April 2012. Many of the released fish were caught one day later in the smolt trap (4–6 km downstream), with surface water fish comprising most of the fish captured on 5 April (surface water n = 34, groundwater n = 5; Fig. 4A). A total of 55 surface water fish were recaptured and 21 were sampled (n = 16 on 5 April, n = 2 on 12 April, and n = 3 on 23 May). A total of 24 groundwater fish were recaptured and nine were sampled (n = 5 on 5 April, n = 3 on 12 April, and n = 1 on 27 May). The migration pattern of stream-reared fish for the 3 years represented in Table 3 shows that the median outmigration date of both hatchery treatments precedes that of stream-reared smolts by more than 2 weeks.

Postrelease changes in plasma T_3 levels of hatchery fish showed a downward trend immediately after release with both treatments significantly lower than cohorts sampled in the hatchery 3 days earlier (Fig. 4B; P = 0.001). No difference between hatchery treatment groups were detected, and no difference in circulating T_3 levels were detected from the date first captured (5 April 2012) compared with subsequent recapture dates (12 April, 23 and 27 May) for either surface water or groundwater treatment groups (P = 0.62). Although recapture numbers were too few to make statistical comparisons with stream-reared fish in late May, both surface and groundwater plasma T_3 levels were lower than that in stream-reared fish on those dates.

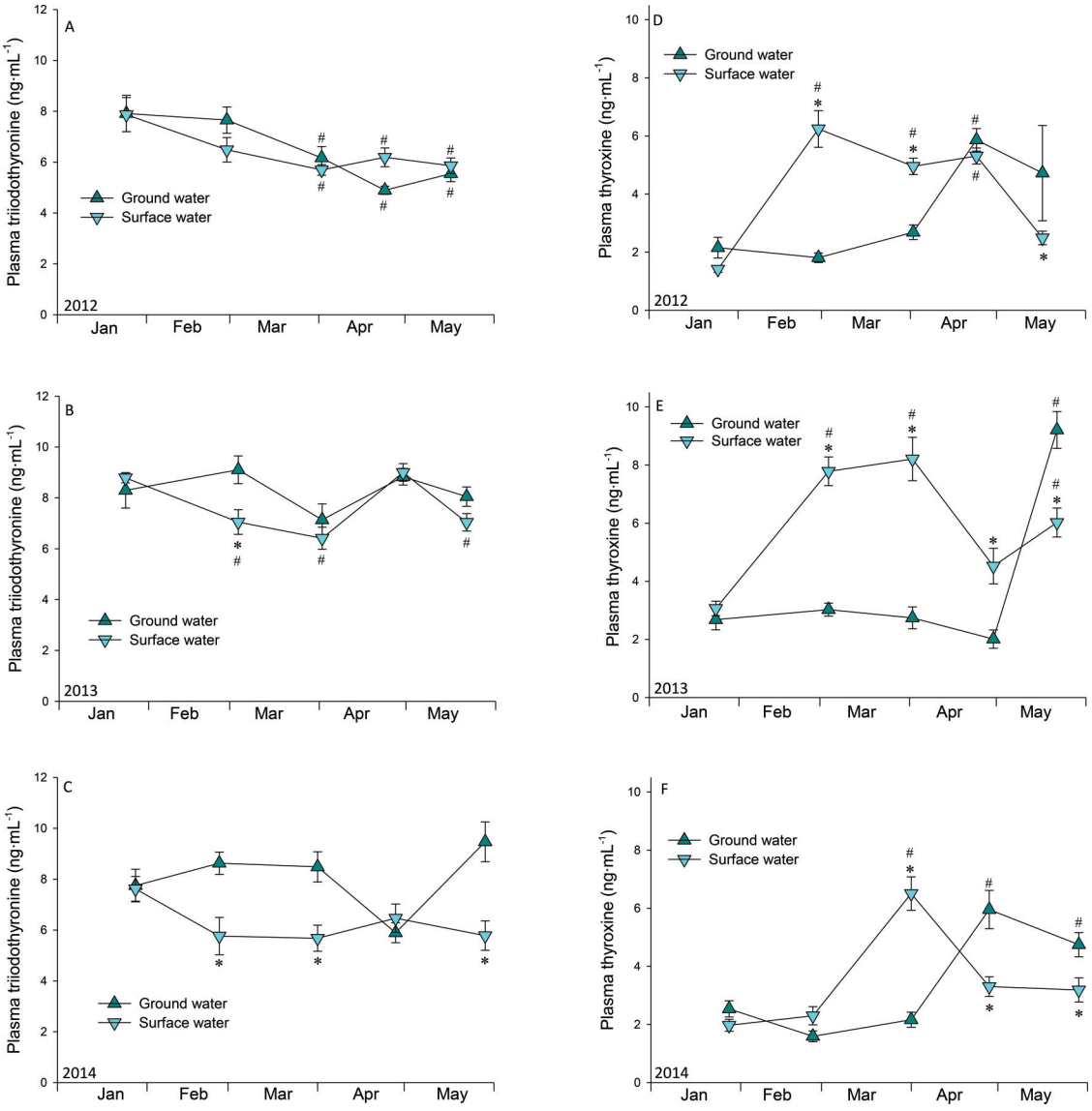
Following release on 3 April, plasma T_4 was elevated in both treatment groups ($P < 0.0001$) when recaptured on 5 April as compared with hatchery levels, with the surface water fish levels significantly lower than levels in groundwater fish on this date (Fig. 4C). Subsequent recapture dates showed significant decrease of plasma T_4 in groundwater fish captured on 12 April sample ($P < 0.0001$) and a significant elevation in the surface water group on 23 May as compared with the 5 April sampling date ($P < 0.0001$). In contrast, a further a decrease in plasma T_4 was detected in the groundwater fish, although sample size on this date was inadequate to make statistical comparisons.

Release of hatchery smolts from both water sources into the Winooski River had a significant effect on gill NKA activity ($P < 0.0001$), although groundwater fish NKA activity was not significantly different compared with the 2 April cohort of hatchery fish when recaptured on 5 April (Fig. 4D). Gill NKA activity of surface water fish was significantly lower than levels at the hatchery on 2 April and levels in groundwater recaptured group on 5 April. On 23 May, NKA activity significantly increased in surface water fish compared with both the 2 April hatchery date (P = 0.002), the 5 April recaptured surface water fish, and the 27 May groundwater recaptured fish (P = 0.026). Gill NKA activity was similar in late May compared with stream-reared smolts, although numbers were inadequate to make meaningful comparisons.

Downstream movement of radio-tagged hatchery smolts

Of 20 radio-tagged fish stocked below Winooski One Dam, 15 initiated migration (seven groundwater and eight surface water smolts). We did not observe a difference in the duration of time from release to initiation of migration between groundwater (mean of 2.0 days) and surface water smolts (mean of 3.1 days; note that fish 144 was not included in this analysis because it was not detected at station 5; t = 0.66, df = 10.16, P = 0.52). Of the fish

Fig. 2. Plasma thyroid hormones measured in Atlantic salmon sampled fish at five time points beginning in late January through late May. Groundwater fish are represented as dark upward pointing triangles, and experimental surface water fish are represented as lighter downward facing triangles. Symbols represent means, and error bars show standard error. Plasma T_3 and T_4 graphs are shown for 2012 (A and D), 2013 (B and E), and 2014 (E and F). Significant difference within treatment from initial sampling time point by one-way ANOVA followed by Dunnett post hoc comparison is indicated by a number symbol (#), and an asterisk (*) indicates significant difference in two-way ANOVA followed by Student–Newman–Keuls (SNK) post hoc analysis ($P < 0.05$).



that initiated migration, two surface water and four groundwater smolts successfully made it to the lake in an average of 10.2 days with a range of 1.6 to 20.3 days. One groundwater fish returned upstream to the dam after 7 days in the lake. We did not test for differences in migration speed between treatment groups due to small sample size and grouped data from both treatments to get mean migration speed of 12.5 km·day^{-1} with a range of 0.9 to 47.6 km·day^{-1}. Detailed movement patterns for all 15 smolts that initiated migration are presented in Supplemental Fig. 1[2].

Size threshold for landlocked Atlantic salmon smolts

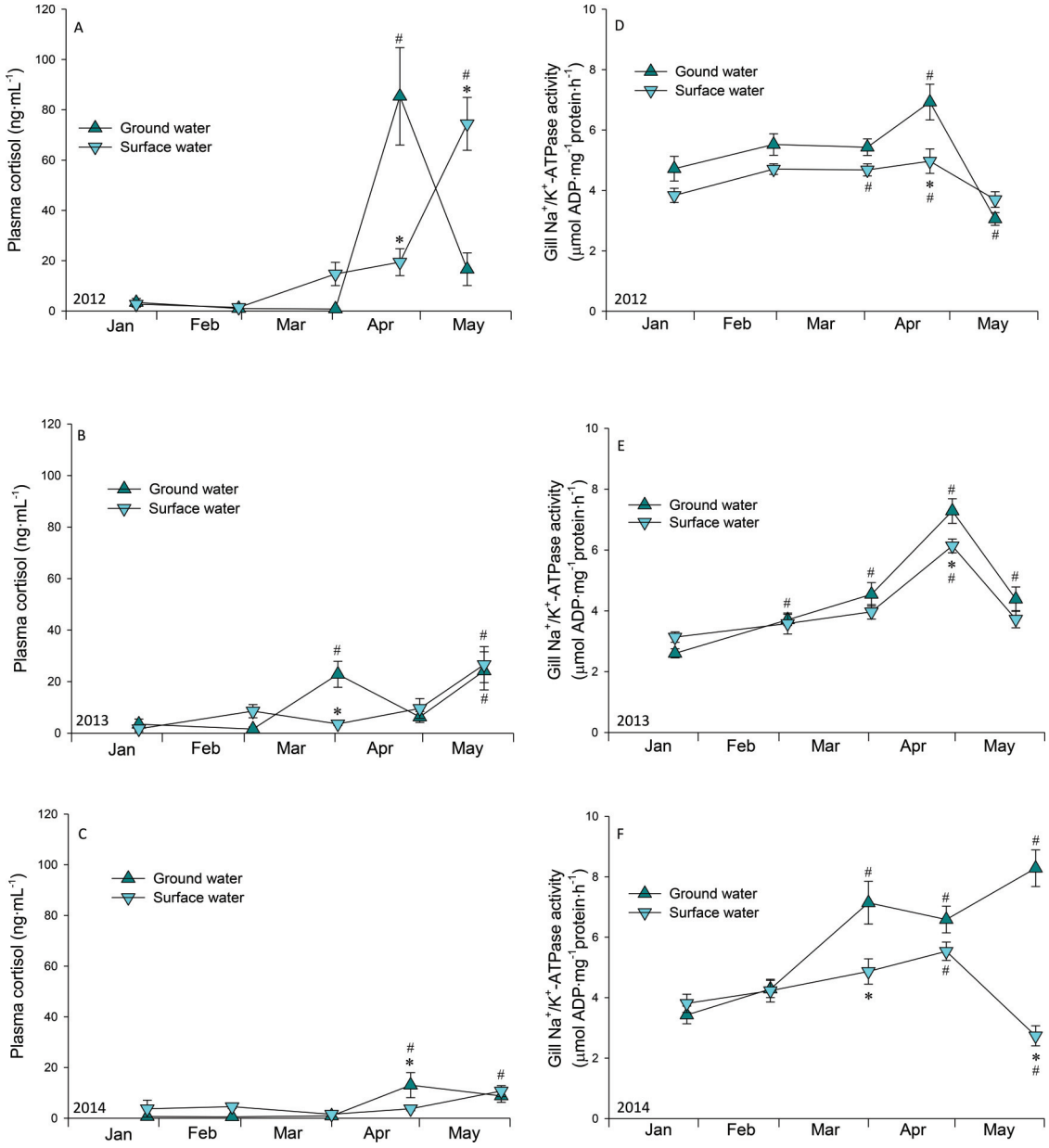
Hatchery-reared juvenile landlocked salmon measured and sampled in early May showed that increased size is associated with increased gill KNA activity (r^2 = 0.49). Sizes ranging from 90 to 200 mm total length were binned into 10 mm groups of 10 fish and compared by one-way ANOVA ($P < 0.0001$), followed by Tukey HSD post hoc comparisons. Statistical analyses indicate that fish with total length greater than 140 mm had significantly higher gill NKA activity than fish in the smallest size class (90–100 mm; Fig. 5).

Discussion

We compared physiological responses of juvenile hatchery-reared landlocked Atlantic salmon exposed to surface water conditions with lower winter temperatures and seasonal increases in temperature (January–May) with fish reared in groundwater with elevated winter temperatures and minimal seasonal variability.

[2]Supplementary data are available with the article at https://doi.org/10.1139/cjfas-2020-0295.

Fig. 3. Plasma cortisol measured in Atlantic salmon sampled fish at five time points beginning in late January through late May for 2012–2014 (A–C). Gill NKA activity for 2012–2014 are also represented (D–F). Groundwater fish are represented as dark upward pointing triangles, and experimental surface water fish are represented as lighter downward facing triangles. Symbols represent means and error bars show standard error. Significant difference within treatment from initial sampling time point by one-way ANOVA followed by Dunnett post hoc comparison is indicated by a number symbol (#), and an asterisk (*) indicates significant difference in two-way ANOVA followed by SNK post hoc analysis (Text should read $P < 0.05$).



While fish from both treatments in all years did develop smolt-like physical characteristics (silvering, reduced condition factor, and movement behavior) and physiological indices (increased thyroid hormones, cortisol, and gill NKA activity), the timing of when these changes occurred differed between the two water sources. Of the physiological parameters we measured, plasma T_4 differed the most between the two treatment groups. Plasma T_4 in surface water fish was elevated earlier in all years compared with groundwater fish. Plasma T_4 was further elevated in both groups after river release, especially late in the migratory period in surface water fish. Plasma cortisol was lower in surface water fish until May when levels matched or exceeded those seen in groundwater fish. Gill NKA was lower in surface water than groundwater fish until 6 weeks after release and upon recapture when levels were elevated in surface water fish but not in groundwater fish. These physiological differences during hatchery-rearing and after release may have contributed to increased smolt-to-adult return rates for a cohort of surface water fish observed by Harbicht et al. (2020).

In our size threshold analysis, fish larger than 140 mm total length in early May had significantly higher gill NKA activity than the smallest fish, indicating the larger fish had undergone smolt development. Intermediate levels in fish between 110 and 130 mm may indicate that some fish were undergoing smolting and others were not. These findings demonstrate that all the fish that were sampled and tagged in this study (140 total) were

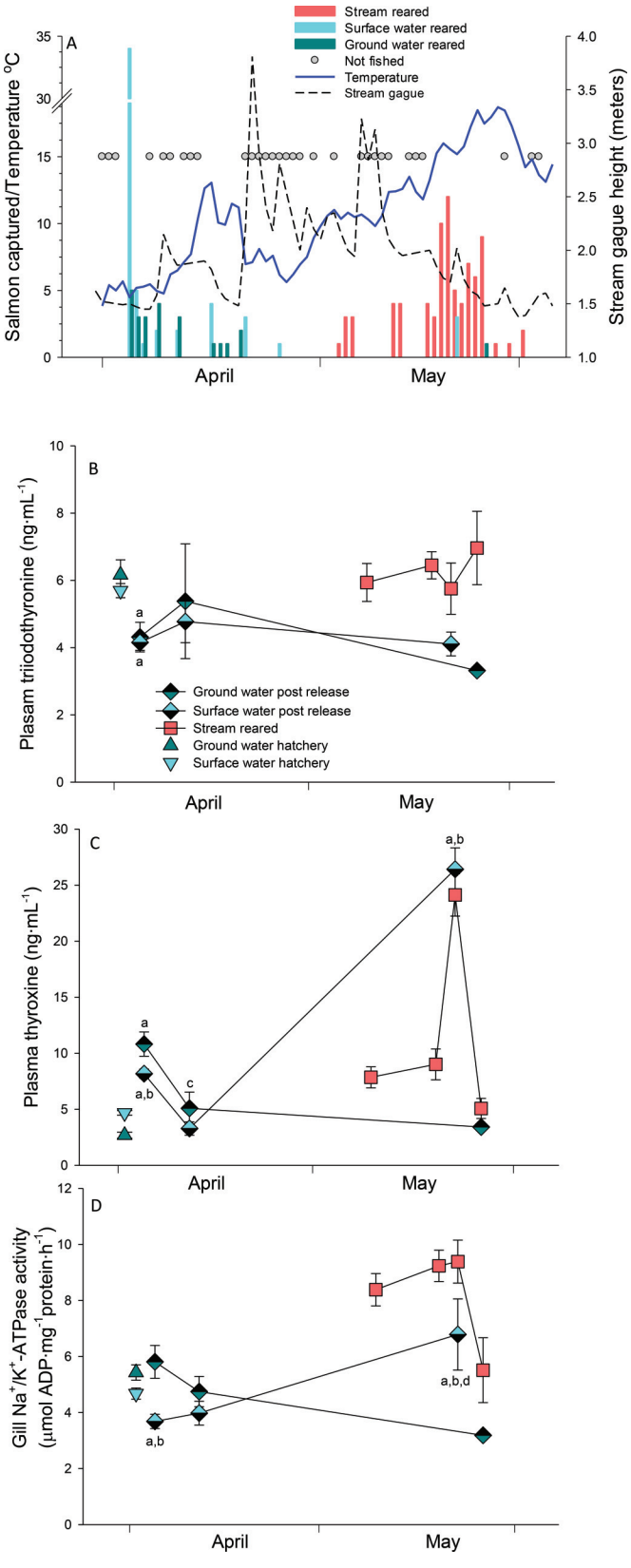


Fig. 4. (A) Time course of smolt trap captures of stream-reared fish (dark rose bars), groundwater hatchery-released fish (dark cyan), and surface water hatchery-released fish (light cyan) for the 2012 smolt season. Stream temperature is represented by the solid line, and stream gauge height (m) is shown by the dashed line. Grey circles represent days where the smolt trap was not operating. (B–D) Plasma T_3, T_4, and gill NKA activity are shown for 2012 hatchery smolts (surface water shown by downward facing light cyan triangles, groundwater shown by upward facing dark cyan triangles) 2 days before stream release (2 April 2012), stream-captured hatchery-released smolts (light cyan–black diamonds show surface water, dark cyan–black diamonds showngroundwater), and captured stream-reared smolts (dark rose squares). Significance ($P < 0.05$) is noted by lowercase letters, where "a" = postrelease sample versus hatchery sample, "b" = difference between groundwater and surface water treated fish, and "c" = difference over time from first recapture date (5 April). [Colour online.]

sufficiently large (>150 mm) to have smolted. From a hatchery management perspective, these results suggest that most landlocked Atlantic salmon of the Sebago strain that are greater than 140 mm in late winter will undergo smolt development.

We found lower levels of plasma T_3 in surface water fish while plasma T_4 was consistently elevated in comparison with groundwater fish in hatchery sampling February through March. Thyroid hormones have long been associated with developmental changes in salmonids, and a "surge" in plasma T_4 levels is well established in the parr–smolt transformation (Specker and Schreck 1982; Hoar 1988). The parr–smolt transformation has been identified as a critical period for olfactory imprinting, and it is suggested that thyroid hormones play a role in olfactory learning and imprinting (McCormick et al. 1998; Morin et al. 1989), likely via thyroid hormone-stimulated olfactory sensory neurons (Lema and Nevitt 2004). Thyroid hormones have also been associated with the initiation of silvering (Leatherland 1982) and downstream migratory behavior (Iwata 1995) and are thought to have preparatory and perhaps synergistic, but not direct, effects on seawater tolerance (McCormick et al. 1987).

Circulating levels of plasma thyroid hormones in smolting salmon can be affected by a host of environmental cues, such as photoperiod (McCormick et al. 2000), turbidity (Specker et al. 2000), water velocity (Youngson and Webb 1993), and a change in water source (Hoffnagle and Fivizzani 1990). Temperature effects on plasma T_4 have been observed in striped bass (*Morone saxatilis*) (Parker and Specker 1990), where premetamorphic larval striped bass T_4 was elevated when temperatures were decreased. Similarly, Levin and Bolotovskiy (2015) suggests that in northern latitudes, elevated T_3 is seemingly associated with adaptation to colder thermal environments in common roach (*Rutilus rutilus*) (Levin and Bolotovskiy 2015). Larsen et al. (2001) showed that in coho salmon (*Oncorhynchus kisutch*) exposed to low temperature for the months of January and February then returned to constant temperature at 10 °C through smolting, plasma T_4 levels were not different than levels seen in fish that had been held at warm temperatures (Larsen et al. 2001). These results differ from the present study where cooler winter temperature were associated with higher levels of plasma T_4. In agreement with the present study are the findings of McCormick et al. (2002), where a marked decrease in plasma T_4 concentration occurred following exposure to increased temperature (McCormick et al. 2002). We hypothesize that the longer period of elevation in plasma T_4 in surface water fish may have improved overall smolt development, including the capacity for imprinting after release.

We found that plasma cortisol was elevated earlier in groundwater fish than in surface water fish, with peak cortisol levels in April. Plasma cortisol of surface water fish showed a more gradual increase, and peak levels were observed in May (Figs. 3A–3C). This steady rise is generally consistent with observations in literature where cortisol peaks with peak salinity tolerance in smolting salmon (Specker and Schreck 1982; Young 1986; McCormick et al.

Table 3. Dates of start and median and end dates for stream-reared, surface water, and groundwater smolts captured in a smolt trap on the Huntington River in 2012–2014.

Year	Water source	*n*	Start	Median	End
2012	Stream-reared	79	6 May	21 May	5 June
	Surface water	55	5 April	5 May	23 May
	Groundwater	24	5 April	9 May	27 May
2013	Stream-reared	79	26 April	17 May	6 June
	Surface water	180	2 May	3 May	20 May
	Groundwater	107	2 May	3 May	21 May
2014	Stream-reared	24	8 May	20 May	5 June
	Surface water	58	30 April	4 May	10 June
	Groundwater	89	30 April	30 April	22 May

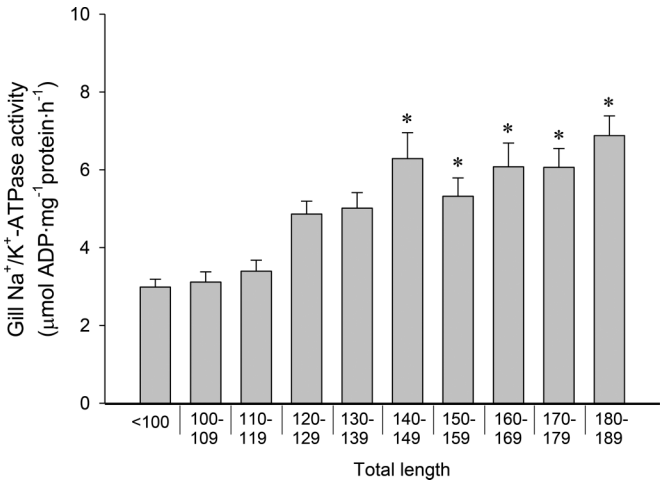
Note: Stocking dates for surface and groundwater fish are 4 April 2012, 1 May 2013, and 29 April 2014.

2007). Plasma cortisol increases in spring during smolt development of anadromous Atlantic salmon (Shrimpton and McCormick 1998), but spring increases are less pronounced in landlocked strains (Nilsen et al. 2008; McCormick et al. 2019). The rearing environment can have a pronounced effect on the spring rise in plasma cortisol in smolts, and in some cases plasma cortisol has been found to be substantially lower in hatchery-reared relative to wild fish (Shrimpton et al. 1994). Smolts display increased sensitivity to stress compared with parr (Carey and McCormick 1998), and a heightened stress response may be important for survival during downstream migration. In this study, groundwater fish showed a peak in plasma cortisol more than a month earlier than surface water fish, and this timing was synchronous with elevated gill NKA activity in only 1 of 3 years of the study (2012). Although the physiological ramifications are currently unclear, it is possible that the early elevation of plasma cortisol in groundwater fish may have altered the timing of key developmental changes and diminished their capacity for survival after release.

In the present study, we observed elevations of gill NKA activity in both groups during the spring, with peak levels being slightly higher in groundwater fish. These results are consistent with other studies that have shown that elevated rearing temperature results in higher levels of gill NKA activity. In a study on anadromous Atlantic salmon with two temperature treatments that were similar to those in the present study, McCormick et al. (2000) observed that elevations in gill NKA activity occurred earlier in the constant elevated winter temperature group, but that the timing and magnitude of peak levels were similar to the natural temperature group. These results are not unlike the findings of the present study where the timing of changes in gill NKA activity during smolt development were comparable, but levels were generally higher in the groundwater group. Other studies using constant (seasonally unchanging) levels of temperature throughout smolt development have found advances in the timing of peak gill NKA activity (Handeland et al. 2004), which might be detrimental for fish being released into natural temperature water, since the peak of smolt development may have already passed by the time fish are released (McCormick et al. 1998). Elevated temperature shortens the physiological smolt window, the time during which smolts can maintain maximum performance during downstream migration and ocean entry (Björnsson et al. 2011). Although landlocked fish do not have to deal with the physiological challenge of osmoregulation in seawater, the timing of gill NKA is likely to be tied to other aspects of smolt development and may thus still be indicative of greater fitness after release.

Although we have interpreted most of the differences between the surface and groundwater groups in terms of their temperature difference, we cannot rule out the possibility that other differences in these two water sources may have played a role in the physiological and endocrine difference we observed. The surface water at Eisenhower hatchery had slightly lower conductivity, hardness, pH, and ion levels relative to groundwater (see Materials and methods). The levels of pH were only slightly different (7.9 versus 7.4) and not within a range that would normally affect physiological or endocrine changes during smolting (Monette et al. 2008). Sockeye salmon (*Oncorhynchus nerka*) reared in hard water (234–244 mg·L^{-1}) and released into very soft water (11–12 mg·L^{-1}) experienced high mortality compared with those reared on soft water (Trushenski et al. 2019). Although these differences in hardness were more than those observed between groundwater and surface water at the hatchery (121 versus 56 mg·L^{-1}), the increased hardness observed in the groundwater may have some effect on postrelease mortality, though such a phenomenon has not been shown for Atlantic salmon. It is also possible that difference in chemical composition at the hatchery played a role in the olfactory imprinting process after release. It is currently thought that amino acids produced by microbial activity are used to provide stream-specific cues for juvenile salmon (Shoji et al. 2003; Ueda 2012) and act as attractants in homing salmon (Shoji 2000). Perhaps prior experience with natural surface water, which likely contains more amino acids than groundwater, contributes to a greater capacity for imprinting of smolt after release.

Fig. 5. Total length and gill NKA activity of smolt. Values are mean + standard error of 10 fish per group. Significant difference ($P < 0.05$) from the <100 mm group is represented with an asterisk (*).



Postrelease changes and comparison with stream-reared fish

Behavior of radio-tagged smolts were consistent with downstream migration patterns observed in other studies on Atlantic salmon smolts. For example, 70% of the radio-tagged smolts stocked in this study initiated downstream migration compared with 62% of tagged landlocked smolts observed by Nyqvist et al. (2017) in the Winooski River. The mean migration speed of 12.5 km·day^{-1} was similar to migration speeds observed for landlocked (Nyqvist et al. 2017) and anadromous Atlantic salmon (Aarestrup et al. 2002). The average number of days from stocking to entry into the lake was slightly over 10 days, providing a window for imprinting to the Winooski River.

Hatchery fish were captured at the smolt trap soon after release into the Huntington River (Fig. 4A). Time from stocking to capture at the smolt trap did not differ between the surface and groundwater groups. However, wild smolts were captured later than hatchery smolts, and the outmigration timing of wild smolts was similar to timing observed for anadromous Atlantic salmon smolts (Fig. 4A; McCormick et al. 1998). Time to initiation

of migration observed for radio-tagged smolts in this study averaged between 2 and 3 days, which is consistent with the rapid movement observed by smolts stocked into the Huntington River and recaptured in the smolt trap.

Compared with levels in the hatchery, an increase in plasma T_4 and a decrease in plasma T_3 was observed in both groundwater and surface water groups within the first few days after release into the Huntington River. Although the sample sizes were small, the levels of plasma T_4 were even more strongly upregulated a month later. At this time stream-reared smolts were also migrating and had similar, high levels of plasma T_4. Previous work on anadromous fish has also shown that there are strong increases in plasma T_4 after release of hatchery fish into natural waters (McCormick et al. 2003). Although imprinting can occur throughout the life of fish, changes experienced during the final stage of smolt development appear to be most important in guiding the upstream migration of salmon as adults (Dittman et al. 1996). Hasler (1978) demonstrated that coho salmon exposed to odors prior to the parr–smolt transformation stage did not demonstrate long-term imprinting memories unless their T_4 levels were also experimentally elevated (Hasler et al. 1978). It has been suggested that the experience of "novel water", such as when wild fish move downstream or into a new tributary or when hatchery fish are released, is an important cue for increased plasma T_4 levels (Hoffnagle and Fivizzani 1990), which could in turn promote imprinting. It would therefore be of interest to examine olfactory tissue and the brain itself to see whether T_3 levels or thyroid hormone receptors are altered after smolt experience novel water. Development of molecular or physiological markers for understanding when olfactory imprinting has occurred would greatly facilitate studies of wild and hatchery-released fish.

Gill NKA activity of fish recaptured within 1 week of release (mid-April) did not differ substantially from those sampled at the hatchery immediately prior to release. However, surface water fish captured later in the migratory period (late May) had 80% higher levels compared with fish at the hatchery and were similar in magnitude to stream-reared fish. Similar increases after release and differences in gill NKA activity between hatchery and stream-reared smolts from the Huntington River were also observed by Nyqvist et al. (2017). Postrelease increases in gill NKA activity have also been observed in several studies on anadromous Atlantic salmon (McCormick et al. 2013, 2014). The fact that smolts reared in the wild have higher levels than fish in the hatchery indicates that cues to complete smolt development may be absent in the hatchery. Nonetheless, results of studies on postrelease changes indicate that smolt development can become more complete after hatchery fish are released.

Summary

Harbicht et al. (2020) examined the same surface water and groundwater smolt cohorts that we examined in this study, allowing us to directly compare our physiological and endocrine results with smolt-to-adult survival rates observed in their study. In the Winooski River stocked groups, they observed a 2.9-, 3.3-, and 8.3-fold increase in smolt-to-adult survival rate (smolt release years 2012–2014, respectively) in surface water compared with groundwater fish. In the present study we observed increased plasma T_4 levels both in surface water fish in the hatchery as well as after release and hypothesize the more natural water source and temperature cycle in surface water resulted in more complete and well-timed smolt development, including olfactory imprinting. This study emphasizes that exposing landlocked juveniles to a natural surface water source with seasonal temperature variations reduced winter growth rates but promoted natural hormonal cycles prior to release that may be causal to increased return rates. We suggest that these rearing methods may be applicable to other hatchery rearing programs for landlocked Atlantic salmon and perhaps to other anadromous salmonids as well.

Acknowledgements

The authors thank Andrew Weinstock and Michael F. O'Dea for assistance in sampling and NKA assays. William Olmstead, Steve Smith, B.J. Allaire, and David Gibson also provided help collecting samples at the hatchery and in the field. The findings and conclusions in the article are those of the authors and do not necessarily represent the views of USFWS. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the US Government. There is no conflict of interest declared in this article. Funding for this report was provided by the USFWS Award No. FRFR48370550002M2.

References

Aarestrup, K., Nielsen, C., and Koed, A. 2002. Net ground speed of downstream migrating radio-tagged Atlantic salmon (*Salmo salar* L.) and brown trout (*Salmo trutta* L.) smolts in relation to environmental factors. Hydrobiologia, **483**(1–3): 95–102. doi:10.1023/A:1021306907338.

Beckman, B.R., Harstad, D.L., Spangenberg, D.K., Gerstenberger, R.S., Brun, C.V., and Larsen, D.A. 2017. The impact of different hatchery rearing environments on smolt-to-adult survival of spring Chinook salmon. Trans. Am. Fish. Soc. **146**(3): 539–555. doi:10.1080/00028487.2017.1281168.

Bisbal, G.A., and Specker, J.L. 1991. Cortisol stimulates hypo-osmoregulatory ability in Atlantic salmon, *Salmo salar* L. J. Fish Biol. **39**(3): 421–432. doi:10.1111/j.1095-8649.1991.tb04373.x.

Björnsson, B.T., Stefansson, S.O., and McCormick, S.D. 2011. Environmental endocrinology of salmon smoltification. Gen. Comp. Endocrinol. **170**(2): 290–298. doi:10.1016/j.ygcen.2010.07.003. PMID:20627104.

Brunsdon, E.B., Fraser, D.J., Ardren, W.R., and Grant, J.W.A. 2017. Dispersal and density-dependent growth of Atlantic salmon (*Salmo salar*) juveniles: clumped versus dispersed stocking. Can. J. Fish. Aquat. Sci. **74**(9): 1337–1347. doi:10.1139/cjfas-2015-0488.

Brunsdon, E.B., Prévost, A., John Fraser, D., Rundle Ardren, W., and Grant, J.W.A. 2020. Microhabitat use of landlocked juvenile Atlantic salmon (*Salmo salar*). J. Gt. Lakes Res. **46**(2): 347–355. doi:10.1016/j.jglr.2020.01.007.

Carey, J.B., and McCormick, S.D. 1998. Atlantic salmon smolts are more responsive to an acute handling and confinement stress than parr. Aquaculture, **168**: 237–253. doi:10.1016/S0044-8486(98)00352-4.

Coghlan, S.M., Jr., Cain, G.R., and Ringler, N.H. 2007. Prey selection of subyearling Atlantic salmon and rainbow trout coexisting in a natural stream. J. Freshw. Ecol. **22**(4): 591–607. doi:10.1080/02705060.2007.9664820.

Dickhoff, W.W., Folmar, L.C., and Gorbman, A. 1978. Changes in plasma thyroxine during smoltification of coho salmon, *Oncorhynchus kisutch*. Gen. Comp. Endocrinol. **36**: 229–232. doi:10.1016/0016-6480(78)90027-8. PMID:738596.

Dittman, A.H., Quinn, T.P., and Nevitt, G.A. 1996. Timing of imprinting to natural and artificial odors by coho salmon (*Oncorhynchus kisutch*). Can. J. Fish. Aquat. Sci. **53**: 434–442. doi:10.1139/f95-185.

Handeland, S.O., Wilkinson, E., Sveinsbø, B., McCormick, S.D., and Stefansson, S.O. 2004. Temperature influence on the development and loss of seawater tolerance in two fast-growing strains of Atlantic salmon. Aquaculture, **233**(1–4): 513–529. doi:10.1016/j.aquaculture.2003.08.028.

Harbicht, A.B., Castro-Santos, T., Gorsky, D., Hand, D.M., Fraser, D.J., and Ardren, W.R. 2018. Environmental, anthropogenic, and dietary influences on fine-scale movement patterns of Atlantic salmon through challenging waters. Can. J. Fish. Aquat. Sci. **75**(12): 2198–2210. doi:10.1139/cjfas-2017-0476.

Harbicht, A.B., Fraser, D.J., and Ardren, W.R. 2020. Minor shifts towards more natural conditions in captivity improve long-term survival among reintroduced Atlantic salmon. Can. J. Fish. Aquat. Sci. **77**(5): 931–942. doi:10.1139/cjfas-2019-0201.

Harder, A.M., Willoughby, J.R., Ardren, W.R., and Christie, M.R. 2020. Among-family variation in survival and gene expression uncovers adaptive genetic variation in a threatened fish. Mol. Ecol. **29**(6): 1035–1049. doi:10.1111/mec.15334. PMID:31837181.

Hasler, A.D., Scholz, A.T., and Horrall, R.M. 1978. Olfactory imprinting and homing in salmon. Am. Sci. **66**(May–June): 347–355. PMID:677550.

Havey, K.A., and Warner, K. 1985. The landlocked Salmon (*Salmo salar*): its life history and management in Maine. Maine Dept. Inland Fish. Wildl. Sport Fishing Institute, Washington, D.C.

Hill, N.L., Trueman, J.R., Prévost, A.D., Fraser, D.J., Ardren, W.R., and Grant, J.W.A. 2019. Effect of dam removal on habitat use by spawning Atlantic salmon. J. Gt. Lakes Res. **45**(2): 394–399. doi:10.1016/j.jglr.2019.01.002.

Hoar, W.S. 1988. The physiology of smolting salmonids. *In* Fish physiology. Vol. XIB. *Edited by* W.S. Hoar and D. Randall. Academic Press, New York. pp. 275–343. doi:10.1016/S1546-5098(08)60216-2.

Hoffnagle, T.L., and Fivizzani, A.J. 1990. Stimulation of plasma thyroxine levels by novel water chemistry during smoltification in Chinook salmon (*Oncorhynchus tshawytscha*). Can. J. Fish. Aquat. Sci. **47**(8): 1513–1517. doi:10.1139/f90-169.

Hutchings, J.A., Ardren, W.R., Barlaup, B.T., Bergman, E., Clarke, K.D., Greenberg, L.A., et al. 2019. Life-history variability and conservation status of landlocked Atlantic salmon: an overview. Can. J. Fish. Aquat. Sci. **76**(10): 1697–1708. doi:10.1139/cjfas-2018-0413.

Iwata, M. 1995. Downstream migratory behavior of salmonids and its relationship with cortisol and thyroid hormones: A review. Aquaculture, **135**(1–3): 131–139. doi:10.1016/0044-8486(95)01000-9.

Jenkins, J.A., Bart, H.L., Bowker, J.D., Bowser, P.R., MacMillan, J.R., Nickum, J.G., et al. 2014. Guidelines for use of fishes in research—revised and expanded, 2014. Fisheries, **39**: 415–416. doi:10.1080/03632415.2014.924408.

Jensen, A.J., Finstad, B., Fiske, P., Hvidsten, N.A., Rikardsen, A.H., and Saksgård, L. 2012. Timing of smolt migration in sympatric populations of Atlantic salmon (*Salmo salar*), brown trout (*Salmo trutta*), and Arctic char (*Salvelinus alpinus*). Can. J. Fish. Aquat. Sci. **69**(4): 711–723. doi:10.1139/f2012-005.

Jonsson, B., and Ruud-Hansen, J. 1985. Water temperature as the primary influence on timing of seaward migrations of Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. **42**(3): 593–595. doi:10.1139/f85-076.

Keefer, M.L., and Caudill, C.C. 2014. Homing and straying by anadromous salmonids: A review of mechanisms and rates. Rev. Fish Biol. Fish. **24**(1): 333–368. doi:10.1007/s11160-013-9334-6.

Larsen, D.A., Beckman, B.R., and Dickhoff, W.W. 2001. The effect of low temperature and fasting during the winter on metabolic stores and endocrine physiology (insulin, insulin-like growth factor-I, and thyroxine) of coho salmon, *Oncorhynchus kisutch*. Gen. Comp. Endocrinol. **123**(3): 308–323. doi:10.1006/gcen.2001.7677. PMID:11589631.

Leatherland, J.F. 1982. Environmental physiology of the teleostean thyroid gland: a review. Environ. Biol. Fishes, **7**: 83–110. doi:10.1007/BF00011829.

Lema, S.C., and Nevitt, G.A. 2004. Evidence that thyroid hormone induces olfactory cellular proliferation in salmon during a sensitive period for imprinting. J. Exp. Biol. **207**(19): 3317–3327. doi:10.1242/jeb.01143. PMID:15326208.

Lemmetyinen, J., Piironen, J., Kiiskinen, P., Hassinen, M., and Vornanen, M. 2013. Comparison of gene expression in the gill of salmon (*Salmo salar*) smolts from anadromous and landlocked populations. Ann. Zool. Fenn. **50**(1–2): 16–35. doi:10.5735/086.050.0102.

Levin, B.A., and Bolotovskiy, A.A. 2015. Discovery of latitudinal gradient of triidothyronine concentrations in ectotherms as revealed from a cyprinid fish, the common roach *Rutilus rutilus*. Biochem. Syst. Ecol. **62**: 128–136. doi:10.1016/j.bse.2015.08.007.

Marsden, J.E., and Langdon, R.W. 2012. The history and future of Lake Champlain's fishes and fisheries. J. Gt. Lakes Res. **38**(Suppl. 1): 19–34. doi:10.1016/j.jglr.2011.09.007.

Marsden, J.E., Chipman, B.D., Nashett, L.J., Anderson, J.K., Bouffard, W., Durfey, L., et al. 2003. Sea lamprey control in Lake Champlain. J. Gt. Lakes Res. **29**(Suppl. 1): 655–676. doi:10.1016/S0380-1330(03)70522-X.

McCormick, S.D. 1993. Methods for non-lethal gill biopsy and measurement of Na^+,K^+-ATPase activity. Can. J. Fish. Aquat. Sci. **50**(3): 656–658. doi:10.1139/f93-075.

McCormick, S.D. 2013. Smolt physiology and endocrinology. *In* Euryhaline fishes. *Edited by* S.D. McCormick, A.P. Farrell, and C.J. Brauner. Academic Press, Amsterdam. pp. 199–251.

McCormick, S.D., Saunders, R.L., Henderson, E.B., and Harmon, P.R. 1987. Photoperiod control of parr–smolt transformation in Atlantic salmon (*Salmo salar*): changes in salinity tolerance, gill Na^+,K^+-ATPase activity and plasma thryoid hormones. Can. J. Fish. Aquat. Sci. **45**: 1462–1468. doi:10.1139/f87-175.

McCormick, S.D., Hansen, L.P., Quinn, T.P., and Saunders, R.L. 1998. Movement, migration, and smolting of Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. **55**(S1): 77–92. doi:10.1139/d98-011.

McCormick, S.D., Moriyama, S., and Björnsson, B.T. 2000. Low temperature limits photoperiod control of smolting in Atlantic salmon through endocrine mechanisms. Am. J. Physiol. Regul. Integr. Comp. Physiol. **278**(5): R1352–R1361. doi:10.1152/ajpregu.2000.278.5.r1352.

McCormick, S.D., Shrimpton, J.M., Moriyama, S., and Björnsson, B.T. 2002. Effects of an advanced temperature cycle on smolt development and endocrinology indicate that temperature is not a zeitgeber for smolting in Atlantic salmon. J. Exp. Biol. **205**(22): 3553–3560. PMID:12364408.

McCormick, S.D., O'Dea, M.F., Moeckel, A.M., and Björnsson, B.T. 2003. Endocrine and physiological changes in Atlantic salmon smolts following hatchery release. Aquaculture, **222**(1–4): 45–57. doi:10.1016/S0044-8486(03)00101-7.

McCormick, S.D., Shrimpton, J.M., Moriyama, S., and Björnsson, B.T. 2007. Differential hormonal responses of Atlantic salmon parr and smolt to increased daylength: A possible developmental basis for smolting. Aquaculture, **273**(2–3): 337–344. doi:10.1016/j.aquaculture.2007.10.015.

McCormick, S.D., Sheehan, T.F., Björnsson, B.T., Lipsky, C., Kocik, J.F., Regish, A.M., and O'Dea, M.F. 2013. Physiological and endocrine changes in Atlantic salmon smolts during hatchery rearing, downstream migration, and ocean entry. Can. J. Fish. Aquat. Sci. **70**(1): 105–118. doi:10.1139/cjfas-2012-0151.

McCormick, S.D., Haro, A., Lerner, D.T., O'Dea, M.F., and Regish, A.M. 2014. Migratory patterns of hatchery and stream-reared Atlantic salmon *Salmo salar* smolts in the Connecticut River, U.S.A. J. Fish Biol. **85**(4): 1005–1022. doi:10.1111/jfb.12532. PMID:25263185.

McCormick, S.D., Regish, A.M., Ardren, W.R., Björnsson, B.T., and Bernier, N.J. 2019. The evolutionary consequences for seawater performance and its hormonal control when anadromous Atlantic salmon become landlocked. Sci. Rep. **9**(1): 968. doi:10.1038/s41598-018-37608-1. PMID:30700821.

Monette, M.Y., Björnsson, B.T., and McCormick, S.D. 2008. Effects of short-term acid and aluminum exposure on the parr-smolt transformation in Atlantic salmon (*Salmo salar*): Disruption of seawater tolerance and endocrine status. Gen. Comp. Endocrinol. **158**(1): 122–130. doi:10.1016/j.ygcen.2008.05.014. PMID:18606407.

Morin, P.-P., Dodson, J.J., and Dore, F.Y. 1989. Thyroid activity concomitant with olfactory learning and heart rate changes in Atlantic salmon, *Salmo salar*, during smoltification. Can. J. Fish. Aquat. Sci. **46**(1): 131–136. doi:10.1139/f89-017.

Nemeth, M.J., Krueger, C.C., and Josephson, D.C. 2003. Rheotactic response of two strains of juvenile landlocked Atlantic salmon: implications for population restoration. Trans. Am. Fish. Soc. **132**(5): 904–912. doi:10.1577/T02-090.

Nilsen, T.O., Ebbesson, L.O.E., and Stefansson, S.O. 2003. Smolting in anadromous and landlocked strains of Atlantic salmon (*Salmo salar*). Aquaculture, **222**(1–4): 71–82. doi:10.1016/S0044-8486(03)00103-0.

Nilsen, T.O., Ebbesson, L.O.E., Kiilerich, P., Björnsson, B.T., Madsen, S.S., McCormick, S.D., and Stefansson, S.O. 2008. Endocrine systems in juvenile anadromous and landlocked Atlantic salmon (*Salmo salar*): Seasonal development and seawater acclimation. Gen. Comp. Endocrinol. **155**(3): 762–772. doi:10.1016/j.ygcen.2007.08.006. PMID:17904138.

Nyqvist, D., McCormick, S.D., Greenberg, L., Ardren, W.R., Bergman, E., Calles, O., and Castro-Santos, T. 2017. Downstream migration and multiple dam passage by Atlantic salmon smolts. North Am. J. Fish. Manag. **37**(4): 816–828. doi:10.1080/02755947.2017.1327900.

Parker, S.J., and Specker, J.L. 1990. Salinity and temperature effects on whole-animal thyroid hormone levels in larval and juvenile striped bass, *Morone saxatilis*. Fish Physiol. Biochem. **8**: 507–514. doi:10.1007/BF00003408. PMID:24221038.

Pientka, B., and Parrish, D.L. 2002. Habitat selection of predator and prey: Atlantic salmon and rainbow smelt overlap, based on temperature and dissolved oxygen. Trans. Am. Fish. Soc. **131**(6): 1180–1193. doi:10.1577/1548-8659(2002)131<1180:HSOPAP>2.0.CO;2.

Piironen, J., Kiiskinen, P., Huuskonen, H., Heikura-Ovaskainen, M., and Vornanen, M. 2013. Comparison of smoltification in Atlantic salmon (*Salmo salar*) from anadromous and landlocked populations under common garden conditions. Ann. Zool. Fenn. **50**(1–2): 1–15. doi:10.5735/085.050.0101.

Prévost, A.D., Hill, N.L., Grant, J.W.A., Ardren, W.R., and Fraser, D.J. 2020. Patterns of reproductive success among reintroduced Atlantic salmon in two Lake Champlain tributaries. Conserv. Genet. **21**(1): 149–159. doi:10.1007/s10592-019-01243-8.

Saikkonen, A., Kekäläinen, J., and Piironen, J. 2011. Rapid growth of Atlantic salmon juveniles in captivity may indicate poor performance in nature. Biol. Conserv. **144**(9): 2320–2327. doi:10.1016/j.biocon.2011.06.010.

Schmitz, M. 1995. Seasonal changes in hypoosmoregulatory ability in landlocked and anadromous populations of Arctic charr, *Salvelinus alpinus*, and Atlantic salmon, *Salmo salar*. Environ. Biol. Fishes, **42**(4): 401–412. doi:10.1007/BF00001471.

Shoji, T., Ueda, H., Ohgami, T., Sakamoto, T., Katsuragi, Y., Yamauchi, K., and Kurihara, K. 2000. Amino acids dissolved in stream water as possible home stream odorants for masu salmon. Chem. Senses. **25**(5): 533–540. doi:10.1093/chemse/25.5.533. PMID:11015325.

Shoji, T., Yamamoto, Y., Nishikawa, D., Kurihara, K., and Ueda, H. 2003. Amino acids in stream water are essential for salmon homing migration. Fish Physiol. Biochem. **28**(1–4): 249–251. doi:10.1023/B:FISH.0000030544.64774.f6.

Shrimpton, J.M., and McCormick, S.D. 1998. Seasonal differences in plasma cortisol and gill corticosteroid receptors in upper and lower mode juvenile Atlantic salmon. Aquaculture, **168**: 205–219. doi:10.1016/S0044-8486(98)00350-0.

Shrimpton, J.M., Bernier, N.J., and Randall, D.J. 1994. Changes in cortisol dynamics in wild and hatchery-reared juvenile coho salmon (*Oncorhynchus kisutch*) during smoltification. Can. J. Fish. Aquat. Sci. **51**(10): 2179–2187. doi:10.1139/f94-219.

Specker, J.L., and Schreck, C.B. 1982. Changes in plasma corticosteroids during smoltification of coho salmon, *Oncorhynchus kisutch*. Gen. Comp. Endocrinol. **46**(1): 53–58. doi:10.1016/0016-6480(82)90162-9. PMID:7060935.

Specker, J.L., Eales, J.G., Tagawa, M., and Tyler, W.A. 2000. Parr-smolt transformation in Atlantic salmon: Thyroid hormone deiodination in liver and brain and endocrine correlates of change in rheotactic behavior. Can. J. Zool. **78**(5): 696–705. doi:10.1139/z99-258.

Trushenski, J.T., Larsen, D.A., Middleton, M.A., Jakaitis, M., Johnson, E.L., Kozfkay, C.C., and Kline, P.A. 2019. Search for the smoking gun: identifying and addressing the causes of postrelease morbidity and mortality of hatchery-reared Snake River sockeye salmon smolts. Trans. Am. Fish. Soc. **148**(5): 875–895. doi:10.1002/tafs.10193.

Ueda, H. 2012. Physiological mechanisms of imprinting and homing migration in Pacific salmon *Oncorhynchus* spp. J. Fish Biol. **81**(2): 543–558. doi:10.1111/j.1095-8649.2012.03354.x. PMID:22803723.

Young, G. 1986. Cortisol secretion in vitro by the interrenal of coho salmon (*Oncorhynchus kisutch*) during smoltification: relationship with plasma thyroxine and plasma cortisol. Gen. Comp. Endocrinol. **63**: 191–200. doi:10.1016/0016-6480(86)90156-5. PMID:3023179.

Youngson, A.F., and Webb, J.H. 1993. Thyroid hormone levels in Atlantic salmon (*Salmo salar*) during the return migration from the ocean to spawn. J. Fish Biol. **42**: 293–300. doi:10.1111/j.1095-8649.1993.tb00329.x.

Zydlewski, G.B., Haro, A., and McCormick, S.D. 2005. Evidence for cumulative temperature as an initiating and terminating factor in downstream migratory behavior of Atlantic salmon (*Salmo salar*) smolts. Can. J. Fish. Aquat. Sci. **62**(1): 68–78. doi:10.1139/f04-179.