NRC Research Press

ARTICLE

Physiological and endocrine changes in Atlantic salmon smolts during hatchery rearing, downstream migration, and ocean entry

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Abstract: Billions of hatchery salmon smolts are released annually in an attempt to mitigate anthropogenic impacts on freshwater habitats, often with limited success. Mortality of wild and hatchery fish is high during downstream and early ocean migration. To understand changes that occur during migration, we examined physiological and endocrine changes in Atlantic salmon (*Salmo salar*) smolts during hatchery rearing, downstream migration, and early ocean entry in two successive years. Gill Na^+/K^+-ATPase activity increased in the hatchery during spring, increased further after river release, and was slightly lower after recapture in the ocean. Plasma growth hormone levels increased in the hatchery, were higher in the river, and increased further in the ocean. Plasma IGF-I remained relatively constant in the hatchery, increased in the river, then decreased in the ocean. Plasma thyroid hormones were variable in the hatchery, but increased in both river- and ocean-captured smolts. Naturally reared fish had lower condition factor, gill NKA activity, and plasma thyroxine than hatchery fish in the river but were similar in the ocean. This novel data set provides a vital first step in understanding the role and norms of endocrine function in smolts and the metrics of successful marine entry.

Résumé : Si des milliards de saumoneaux issus d'écloseries sont relâchés annuellement afin d'atténuer les impacts de l'activité humaine sur les habitats d'eau douce, l'efficacité de ces efforts est souvent limitée. La mortalité des poissons sauvages et provenant d'écloseries est élevée durant l'avalaison et le début de la migration en mer. Pour mieux comprendre les changements qui s'opèrent durant la migration, nous avons examiné les changements physiologiques et endocriniens chez des saumoneaux de saumon atlantique (*Salmo salar*) durant l'alevinage en écloserie, l'avalaison et le passage en mer pendant deux années consécutives. L'activité de l'ATPase Na^+/K^+ branchiale a augmenté au printemps en écloserie, puis après la libération en rivière et avait légèrement diminué après la recapture en mer. Les teneurs de l'hormone de croissance plasmatique ont augmenté en écloserie, puis davantage en rivière ainsi qu'en mer. L'IGF-I plasmatique est demeuré relativement inchangé en écloserie, a augmenté en rivière, pour ensuite diminuer en mer. Les teneurs en hormones thyroïdiennes plasmatiques étaient variables en écloserie, mais avaient augmenté chez les saumoneaux capturés tant en rivière qu'en mer. Si, en rivière, les poissons issus de l'alevinage naturel présentaient des coefficients d'embonpoint, une activité de la NKA branchiale et des teneurs en thyroxine plasmatique plus faibles que ceux des poissons issus d'écloseries, ces valeurs étaient semblables pour les deux groupes de poissons une fois en mer. Ce nouvel ensemble de données constitue un premier pas critique vers la compréhension du rôle et des normes de la fonction endocrine chez les saumoneaux, ainsi que vers la caractérisation des paramètres associés à un passage en mer réussi. [Traduit par la Rédaction]

Introduction

As part of their normal life history, Atlantic salmon migrate as juveniles from freshwater to seawater. Atlantic salmon undergo a transformation that is adaptive for life in the ocean, from stream-dwelling parr to downstream migrating smolt. The parr–smolt transformation, or smoltification, includes a number of behavioral, morphological, and physiological changes, including the development of a high degree of salinity tolerance (Hoar 1988). In spite of these adaptive changes, downstream migration and entry into seawater is a life-stage transition linked to high natural mortality (Lacroix et al. 2005; Kocik et al. 2009; Dempson et al. 2011). Movement through areas of high predation and variable food resources are thought to be the primary drivers of this mortality (Spence and Hall 2010). The timing of smolt migration in relation to estuarine and ocean conditions that support high survival has been suggested to play an important role in interannual differences in early marine survival (McCormick et al. 1998). In addition to this "ecological" smolt window, the timing of smolt preparedness with seawater entry (a "physiological" smolt window) may also play a role in survival. This may be particularly true for hatchery fish, for which the timing or magnitude of smolt development may be altered from that which occurs in nature (McCormick 2009). This may explain, in part, the lower return rates of hatchery smolts relative to those of wild fish.

Smolt development is driven by a combination of developmental and environmental information. For Atlantic salmon, once a critical size threshold is reached, certain components of the neuroendocrine axis become sensitive to increased day length (Ebbesson et al. 2003; McCormick et al. 2007). The activation of the light–brain–pituitary axis leads to increased plasma levels of growth hormone (GH), leading, in turn, to increased plasma levels of insulin-like growth factor I (IGF-I) (Stefansson et al. 2012). Plasma cortisol levels also increase, and in concert these hormones drive cellular and biochemical changes in the gill, including increased gill Na^+/K^+-ATPase (NKA) activity that results in

Received 31 March 2012. Accepted 26 September 2012.

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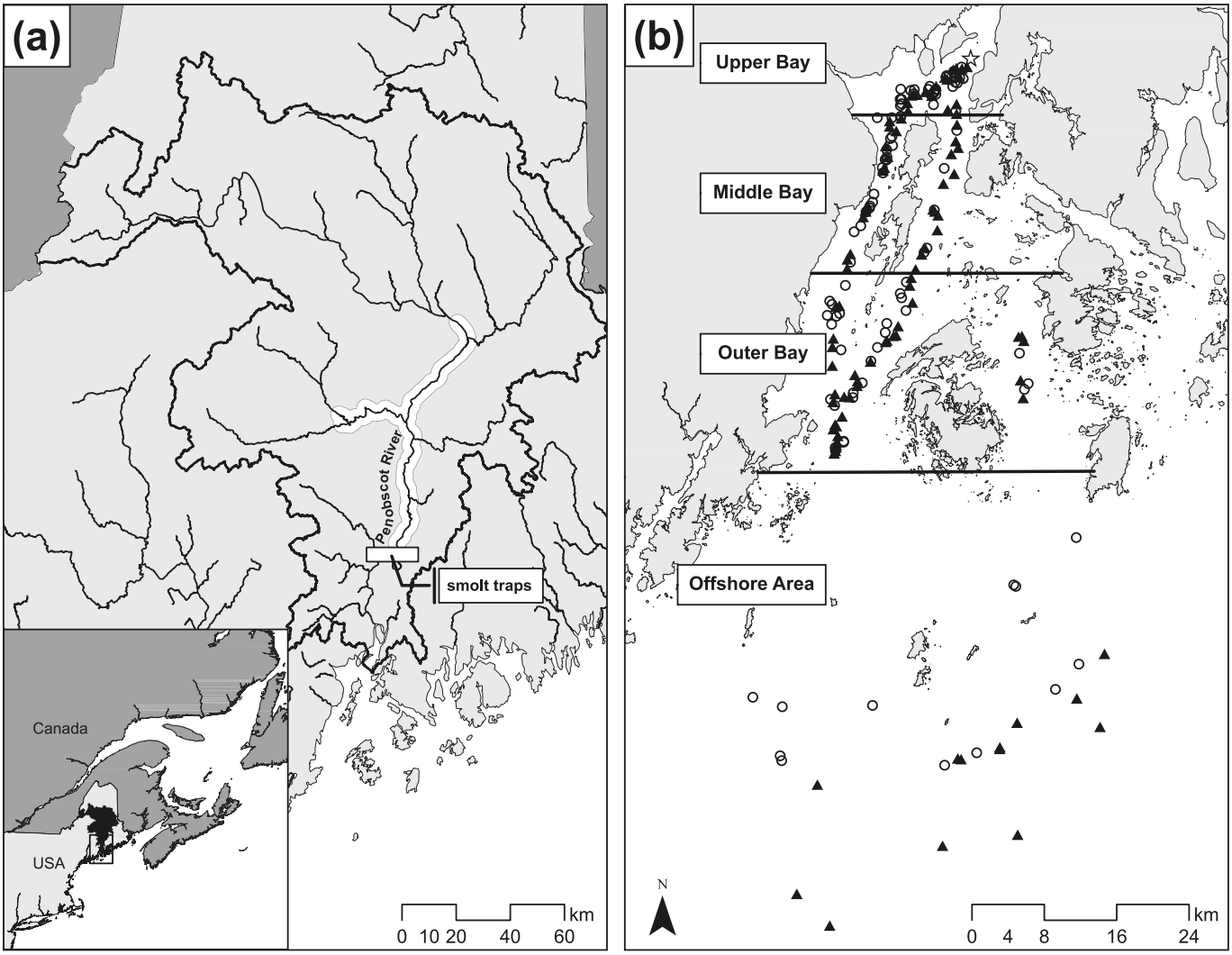
Fig. 1. Map of Penobscot River watershed (left) and Penobscot Bay and offshore areas (right). Atlantic salmon smolts were captured in the river just downstream of Veazie Dam. Location of trawl stations are designated with open circles (2003) and closed triangles (2004).



increased salinity tolerance (McCormick et al. 2007). Thyroid hormones (THs), thyroxine (T_4) and triiodothyronine (T_3), also increase during smolt development and are thought to be involved in altered behavior, morphological changes, and imprinting (Hoar 1988).

Much of the understanding of environmental regulation of endocrine changes during smolt development is derived from laboratory or hatchery studies of hatchery-reared fish, with only a few studies having examined hormonal changes of juvenile salmon smolts in the wild. (Björnsson et al. 2011). Atlantic salmon released from the hatchery and recaptured in freshwater during downstream migration have elevated plasma levels of GH, IGF-I, and T_4, similar to levels seen in naturally reared smolts (McCormick et al. 2003). Plasma GH levels of wild Atlantic salmon smolts migrating through an estuarine fjord in Norway were transiently elevated, but were lower when Atlantic salmon were captured in nearshore marine waters (Stefansson et al. 2003). In contrast, plasma GH and TH levels increased, but IGF-I levels progressively decreased, as wild Atlantic salmon migrated from the river, through an estuarine fjord and into the near-coastal and ocean environments (Stefansson et al. 2012). To date, there have been no studies examining physiological changes in hatchery-reared Atlantic salmon smolts in the marine environment, nor any studies that have followed Atlantic salmon through time in the freshwater, river, and near-coastal ocean environments. Thus, in spite of the emerging importance of estuary and early marine phases as a period critical to salmon survival (Thorstad et al. 2012), there is currently limited information on the physiological and endocrine changes that occur during this period. In the present study, changes in plasma levels of GH, IGF-I, T_4, and T_3 and gill NKA activity of Atlantic salmon smolts were examined during hatchery rearing, downstream migration, estuarine entry, and initial ocean migration.

Materials and methods

Hatchery rearing and release

A multilife stage stocking program is utilized to maintain the Penobscot River (Maine, USA) Atlantic salmon population. The broodstock are Penobscot-origin sea run adults captured at a trapping facility located at Veazie Dam, 48 km upstream from the northern extent of Penobscot Bay (Fig. 1*a*). The majority of adult returns originate from smolt stocking and hence so do the broodstock. Adults retained as broodstock are transported to and maintained at the Craig Brook National Fish Hatchery in Orland, Maine. Following strip spawning in the fall, eggs are incubated in egg trays for 6 months until hatching and partial yolk absorption the following spring. They are then transferred to 2 m diameter

tanks at the Green Lake National Fish Hatchery in Ellsworth, Maine, for first feeding until they reach approximately 4 cm, at which time they are transferred to outdoor 6 or 9 m diameter tanks under ambient light conditions and fed with water from Green Lake. Approximately 1 year after hatching, fish were sampled on the following dates (temperature of fish tanks in parentheses): 13 February (2.0 °C), 12 March (2.3 °C), 4 April (2.7 °C), 15 April (2.7 °C), 1 May (4.8 °C), and 7 May 2003 (6.7 °C); and 19 February (1.5 °C), 16 March (2.1 °C), 11 April (3.3 °C), and 28 April 2004 (5.3 °C). Fish were not fed the morning of sampling, which occurred between 0900 and 1130 h.

One year-old parr, smolts, and approximately 1.1 million unmarked fry salmon are released annually into the Penobscot River drainage. Stocked fry are indistinguishable from individuals originating from natural spawning, and therefore these two origins are collectively referred to as naturally reared. To maximize smolt production at the hatchery, fish smaller than 120 mm are culled and released throughout the drainage in the fall as parr. Most of the released parr are expected to remain in the river for 20 months ("20-month parr") before emigrating, but some portion will emigrate the following spring with their cohort ("8-month parr"; Sheehan et al. 2011). Approximately 375 000, 321 000, and 369 000 parr were stocked in 2002, 2003, and 2004, respectively, throughout the drainage (river kilometres 47–148). In 2003 and 2004, approximately 550 000 smolts were stocked annually into the Penobscot River. Stocking locations occurred throughout the drainage (river kilometres 44–149), although three sites received 65% of the smolts (Milo, Mattawamkeag, and Howland at river kilometres 120, 142, and 99, respectively; Fig. 1*a*).

River capture

Smolts were captured during their downstream migration in the Penobscot River via rotary screw traps (E.G. Solutions, Inc., Corvallis, Oregon, USA) located just downstream of the Veazie Dam (river kilometre 48, Fig. 1*a*). Rotary screw traps passively collect downstream migrating fish when they enter through the large end of a revolving and half-submerged screened cone suspended between two pontoons. As the river current turns the cone, the fish are guided downstream into a holding tank, where they are held in a low-stress environment in ambient river water and low flow (Music et al. 2010) until retrieved for sampling during daylight hours between 0800 and 1100 h. Smolts migrated primarily at night, and traps were tended to daily, so smolts were assumed to be confined for 10 h or less.

Smolt traps were running from 21 April to 20 June 2003 and 20 April to 14 June 2004, during which 418 and 1565 smolts were captured, respectively. Of these captures, 89 smolts in 2003 and 191 smolts in 2004 were sampled for physiological analyses. In both years, the first smolt capture occurred on 24 April. In 2003, 25% of catch occurred before 13 May, 50% of the catch before 19 May, and 75% before 24 May. In 2004, 25%, 50%, and 75% of the catch occurred before 6, 9, and 12 May, respectively. The last smolts captured each year occurred on 17 June 2003 and 10 June 2004, respectively.

Temperature was recorded by Onset data loggers attached to the rotary screw trap. In 2003, the temperature on the date of the first captured fish was 5.5 °C and on the last date captured was 17.8 °C. The total number of degree-days (cumulative mean daily temperature) for May 2003 was 390.3, during which 88% of the fish migrated. In 2004, the temperature on the date of the first capture was 8.2 °C and on the date of the last capture was 18.4 °C. The total number of degree-days for May 2004 was 451.2, during which 92% of the fish migrated.

Discharge patterns were similar between years. Mean discharge was 503 and 352 $m^3 \cdot s^{-1}$ in 2003 and 2004, respectively, for the period 1 April through 30 June. Peak discharge in 2003 occurred on 25 April at 46–100 $m^3 \cdot s^{-1}$, and in 2004 peak discharge occurred on 16 April at 33–700 $m^3 \cdot s^{-1}$. Discharge in both years generally remained below the running 5-year mean (104-year record), except for an approximate 1-week period in late April and early May 2003, when it rose slightly above. Thus, the slightly earlier river migration in 2004 was associated with higher temperatures and with earlier and lower mean discharge.

Ocean capture

Marine surveys for Atlantic salmon post-smolts were conducted in Penobscot Bay and the nearshore water of the Gulf of Maine. Standard pair-trawling techniques were used to capture post-smolts in the ocean, and a detailed description specific to this study can be found in Sheehan et al. (2011). Briefly, a modified pelagic net was towed at the surface to capture emigrating smolts. The cod end of the trawl was fitted with an aluminum aquarium holding tank with an interior volume of 0.74 m^3. Captured post-smolts entered the holding tank and remained in a low-velocity holding area until the aquarium was retrieved on board and the contents were removed for sampling. Tows were 30 min in duration, except for the 2004 Offshore Area tows, which were 60 min. Given haul-back time (15 min), smolts were in the aquarium a maximum of 45 or 75 min prior to sampling.

We divided Penobscot Bay and coastal waters into four ecological zones: the Upper Bay, Middle Bay, Outer Bay, and Offshore Area. These areas (Fig. 1*b*) were delineated on the basis of surface water temperature and salinity, as they are important habitat characteristics for surface-oriented smolts. Bay migration distance was also calculated for each sampling location. A starting location just north of the uppermost Upper Bay station was selected, and from this point a minimum number of migration paths were drawn throughout the bay. Migration path for each station was then determined based on these predetermined paths. The distance to each station was calculated by linking a predetermined migration path to each station surveyed. Migration path distances were determined in ArcMap using the Measure tool (ArcGIS 10.1, ESRI, Redmond, California, USA). Bay migration distance (from the starting location to each station) was calculated using R (R Development Core Team 2011) in conjunction with the argosfilter package.

In 2003, smolt trawls began on 6 May and ended on 24 May. A total of 485 smolts were captured, of which 350 were sampled for physiological analyses. In 2004, smolt trawls began on 6 May and ended on 23 May, with 696 smolts captured and 374 sampled for physiological analyses. Smolts were sampled at random from each haul, with the exception that moribund or dark (stressed) fish were not sampled.

Surface waters in the bay experienced considerable seasonal warming in both years. In the Upper Bay, minimum surface temperatures were 6.3 and 7.6 °C at the start of the survey and warmed to a maximum of 13.4 and 12.4 °C in 2003 and 2004, respectively. The coolest surface temperatures were registered in the Outer Bay and ranged from 5.0 to 7.9 °C and 4.8 to 8.2 °C in 2003 and 2004, respectively. Surface temperatures generally decreased as collections moved offshore and increased throughout the migratory period. Salinity also increased as collections moved through the bay into the waters of the Gulf of Maine. The mean Upper Bay salinities recorded were 19.3 ± 4.5 ppt (mean ± standard deviation; range 12.3–32.6 ppt) and 24.2 ± 1.9 ppt (range 18.2–32.6 ppt) in 2003 and 2004, respectively. Offshore Area mean salinities were 30.4 ± 0.5 ppt and 31.7 ± 0.2 ppt.

We determined the origin of ocean-captured smolts by the presence of marks, clips, or scale pattern analysis (Renkawitz and Sheehan 2011). In 2003, the vast majority of fish captured for physiological sampling were hatchery-reared fish released as smolts (hatchery smolts), with only a small number (n = 3) subsequently identified as either hatchery-reared fish released as parr or naturally reared fish. In 2004, the majority of fish were hatchery smolts (n = 337), while 14 fish were identified as hatchery-

reared fish released as parr and 9 fish were identified as naturally reared fish (n = 9).

Sampling protocol

Smolts were anesthetized with MS-222 (100 mg·L^{-1}, pH 7.0) and weighed to the nearest 0.1 g, and fork length was recorded to the nearest 0.1 cm. Blood was collected from the caudal vasculature in heparinized 1 mL syringes and centrifuged at 3200g for 5 min at 4 °C. Plasma was removed and stored at −80 °C for later analyses. Gill biopsies (4–6 primary filaments) were cut from the first or second gill arch and placed into 100 μL ice-cold SEI (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 for later analysis. Physiological sampling was usually non-lethal and fish were returned to the river or ocean, but there were a small number of fish in the trawl survey that died after sampling (19 in 2003 and 15 in 2004). For these fish, gut content was removed and its energy content estimated as outlined in Renkawitz and Sheehan (2011).

Laboratory analyses

NKA activity was determined with a kinetic assay run in 96-well microplates at 25 °C and read at a wavelength of 340 nm for 10 min as described in McCormick (1993). Gill tissue was homogenized in 150 μL of SEID (SEI buffer and 0.1% deoxycholic acid) and centrifuged at 5000g for 30 s. Two sets of duplicate 10 μL samples were run, one set containing assay mixture and the other assay mixture and 0.5 mmol·L^{-1} ouabain. The resulting ouabain-sensitive ATPase activity is expressed as micromoles of ADP per milligram of protein per hour. Protein concentrations were determined using the BCA (bicinchoninic acid) protein assay (Pierce, Rockford, Illinois, USA). Both assays were run on a THERMOmax microplate reader using SOFTmax software (Molecular Devices, Menlo Park, California, USA).

Plasma GH levels were measured using a double-antibody salmon GH radioimmunoassay developed by Bolton et al. (1986) and modified by Björnsson et al. (1994). Plasma IGF-I levels were measured by a radioimmunoassay validated for salmonids (Moriyama et al. 1994). T_4 and T_3 concentrations were measured by a direct radioimmunoassay described by Dickhoff et al. (1978) and modified by McCormick et al. (1995). Sensitivity as defined by the dose–response curve was 1–64 ng·mL^{-1} for T_4 and 0.5–16 ng·mL^{-1} for T_3. Intra- and inter-assay coefficients of variation for these assays were 4%–11% and 3%–5%, respectively.

Statistics

In 2003, sample sizes for GH, IGF-I, T_4, and T_3 were 116–127 from the hatchery, 58–71 from the river, and 161–175 in the ocean. In 2004, samples sizes in the hatchery, river, and ocean were 95, 200, and 364 for gill NKA activity; 72, 94, and 183 for GH and IGF-I; and 91, 128, and 364 for T_4 and T_3. Physiological differences in hatchery smolts over time were analyzed by one-way analysis of variance (ANOVA) followed by a Tukey honestly significant difference (HSD) test to compare individual dates. Smolts from the hatchery, river, and ocean were compared using one-way ANOVA followed by Tukey HSD, comparing smolts in the hatchery on the last sampling date before release (7 May in 2003 and 28 April in 2004) and all fish sampled in the river and ocean. Changes over time in the river were analyzed using regression analysis. Hatchery smolts, fish released from the hatchery as parr, and naturally reared fish that were captured in the ocean were compared using one-way ANOVA for 2004 only owing to small sample sizes of non-hatchery smolts in 2003. With the exception of plasma T_3, no significant differences among these groups were detected. Thus all groups were combined for subsequent analysis. For physiological parameters of smolts captured in the ocean, the Akaike information criterion (AIC) was used to choose the most parsimonious model that could include length, condition factor, date of capture, distance offshore, surface temperature, and salinity. A multiple regression analysis that included those independent variables identified by AIC was then run to determine the explanatory power (R^2) of the model. All statistical analyses were carried out using Statistica 6.0 (Tulsa, Oklahoma, USA).

Results

In 2003, the length (fork length) of fish in the hatchery increased progressively with time ($p < 0.0001$; Fig. 2a), with a mean value of 18.4 ± 0.3 cm on 7 May. Smolts recaptured in the river had a mean length of 17.4 ± 0.2 cm, but were not significantly different from fish in the hatchery on 7 May (p = 0.30). There was no significant change over time in the length of fish captured in the river (p = 0.68). The size of fish captured in the bay and coastal ocean (overall mean value 18.7 ± 0.10 cm) was greater than that of smolts captured in the river ($p < 0.0001$), but was not different from that of fish in the hatchery on 7 May (p = 0.89). There was no obvious difference in length of ocean-captured smolts with time or offshore distance (Fig. 2b). AIC regression analysis indicated a significant relationship of length of ocean-caught smolts with salinity (R^2 = 0.014; p = 0.027). Correlation analysis indicated a significant relationship of length with date of capture and salinity (Table 1).

In 2004 the length of fish in the hatchery increased progressively with time ($p < 0.017$; Fig. 2c), with a mean fork length of 18.6 ± 0.3 cm on 28 April. Smolts recaptured in the river had a mean length of 17.9 ± 0.2 cm, but were not significantly different from fish in the hatchery or the ocean (p = 0.32). There was a significant decrease over time in the length of fish captured in the river ($p < 0.0001$; R^2 = 0.11). There was no obvious difference in length of ocean-captured smolts with time or offshore distance (Fig. 2d). Length of ocean-caught smolts did not vary significantly with temperature, salinity, date, distance, or condition factor (AIC model p = 0.089; Table 1).

Condition factor (CF) of hatchery smolts in 2003 decreased progressively from 1.12 ± 0.02 in February to 1.00 ± 0.02 on 7 May ($p < 0.0000$; Fig. 3a). Smolts recaptured in the river had a significantly lower mean CF (0.95 ± 0.01) compared with hatchery smolts on 7 May (p = 0.043). There was no significant change over time in CF of smolts captured in the river (p = 0.17). CF of fish captured in the ocean (0.95 ± 0.01) was not different from that of hatchery smolts (p = 0.076) or river-captured smolts (p = 0.66) on 7 May. There was no obvious difference in CF of ocean-captured smolts with time or offshore distance (Fig. 3b). AIC regression analysis indicated a significant relationship of CF of ocean-caught smolts with date and offshore distance (p = 0.0046), but with low explanatory power (R^2 = 0.032). Correlation analysis indicated no significant relationship of CF with date, distance offshore, temperature, or salinity (Table 1).

CF of hatchery smolts in 2004 decreased progressively from 1.18 ± 0.01 in February to 1.03 ± 0.01 on 28 April ($p < 0.0001$; Fig. 3c). Smolts recaptured in the river had a CF of 1.00 ± 0.01, which was not significantly different from that of hatchery smolts on 28 April (p = 0.25). There was a significant decrease over time in the CF of fish captured in the river ($p < 0.0001$; R^2 = 0.10). CF of fish captured in the ocean (0.95 ± 0.01) was significantly lower than that of hatchery smolts on 28 April (p = 0.0005) and that of river-captured smolts ($p < 0.0001$). There was no obvious difference in the CF of ocean-captured smolts with time or offshore distance (Fig. 3d). AIC regression analysis and correlation analysis indicated no significant relationship of the CF of ocean-caught smolts with length, date, distance offshore, temperature, or salinity (p = 0.2; Table 2).

In 2003, a freezer malfunction resulted in a loss of samples for analysis of gill NKA activity. In 2004, gill NKA activity of smolts increased with time in the hatchery ($p < 0.0001$; Fig. 4a). The highest gill NKA activity in the hatchery occurred on 28 April. After release, smolts recaptured in the river had significantly greater gill NKA activity (12.9 ± 0.2 μmol ADP·(mg protein)$^{-1}$·h^{-1}) than smolts in the hatchery on 28 April (10.9 ± 0.6 μmol

Fig. 2. Length of Atlantic salmon smolts during hatchery rearing (Hatchery) and after recapture in the Penobscot River (River) and coastal Gulf of Maine (Ocean) in 2003 and 2004. Values for the hatchery were for a specific date (month and day; $n = 24$), whereas smolts captured in the River ($n \geq 68$) and in the Ocean ($n \geq 150$) were captured over 5–7 days (date shown is the median date of the group). In panles *b* and *d* ocean-caught fish are grouped according to four oceanographic regions with increasing distance from the river. Asterisk in dates for hatchery fish indicates a significant difference from initial sampling date ($p < 0.05$, Tukey HSD). Lines over histograms with different letters indicate a significant difference ($p < 0.05$, Tukey HSD).

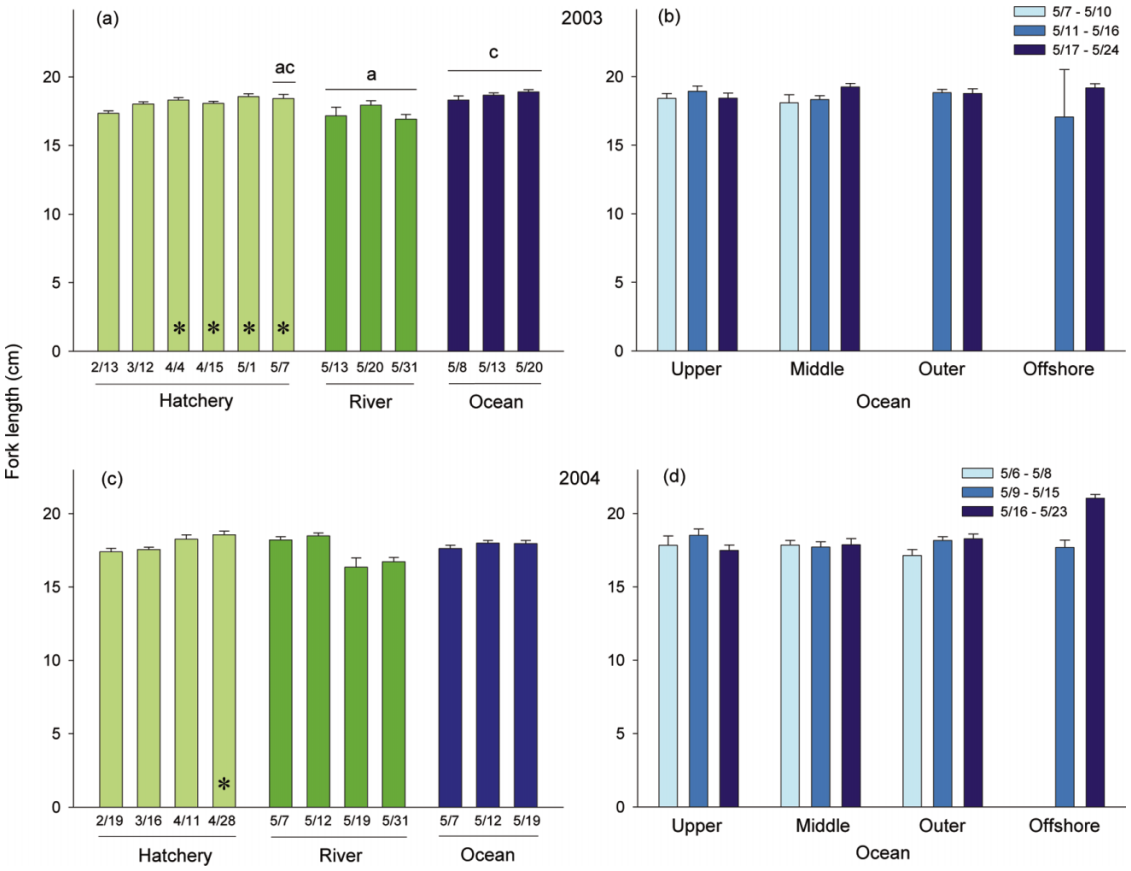


Table 1. Correlation matrix of physiological parameters and abiotic factors for ocean-caught Atlantic salmon smolts in 2003.

	CF	GH	IGF-I	T_4	T_3	Date	Distance	Temperature	Salinity
L	**0.27**	**–0.19**	0.14	–0.07	**0.17**	**0.12**	0.08	0.02	**0.12**
CF		**–0.26**	0.01	0.1	0.12	–0.11	0.11	–0.09	0.01
GH			0.09	0.02	–0.04	**0.25**	**–0.25**	**0.26**	0.03
IGF-I				0.15	–0.03	**–0.19**	**–0.34**	0.1	**–0.26**
T_4					0.05	**–0.2**	**–0.41**	0.03	**–0.46**
T_3						0.06	**0.2**	–0.07	**0.21**
Date							**0.49**	**0.19**	**0.66**
Distance								**–0.31**	**0.8**
Temperature									**–0.29**

Note: L, length; CF, condition factor; GH, plasma growth hormone; IGF-I, insulin-like growth factor I; T_4, thyroxine; T_3, triiodothyronine. Values in bold are significant ($p < 0.05$).

ADP·(mg protein)$^{-1}$·h^{-1}; $p = 0.0005$, Tukey HSD). There was a significant decrease in gill NKA activity over time in the river ($p < 0.0001$; $R^2 = 0.28$). Gill NKA activity was significantly lower in the ocean than the overall mean for fish in the river ($p < 0.0001$). There was a general trend of gill NKA activity decreasing with time in ocean-captured smolts (Figs. 4*a*, 4*b*). AIC regression analysis indicated a significant relationship of gill NKA activity with date, length, and CF ($p < 0.0000$; $R^2 = 0.12$). Simple correlation analysis indicated a significant negative relationship of gill NKA activity with length and date (Table 2).

In 2003, plasma GH increased with time in the hatchery ($p < 0.0001$; Fig. 5*a*). The highest plasma GH levels in the hatchery occurred on 1 May. After release, smolts recaptured in the river had significantly greater plasma GH levels (12.0 ± 0.9 ng·mL^{-1}) than smolts in the hatchery on 7 May (1.2 ± 0.3 ng·mL^{-1}; $p < 0.0001$). There was no significant change in plasma GH over time in the river ($p = 0.79$). Plasma GH levels increased further in the ocean-captured smolts (24.8 ± 1.0 ng·mL^{-1}) and were greater than in both the hatchery smolts and river-captured smolts ($p < 0.0001$). There was a general trend of plasma GH to increase with time and decrease with distance offshore in ocean-captured smolts (Figs. 5*a*, 5*b*). AIC regression analyses of plasma GH levels identified a model including length, CF, date, distance, temperature, and salinity ($p < 0.0000$; $R^2 = 0.27$). Correlation analysis indicated a significant

Fig. 3. Condition Factor of Atlantic salmon smolts during hatchery rearing (Hatchery) and after recapture in the Penobscot River (River) and coastal Gulf of Maine (Ocean) in 2003 and 2004. Values for the hatchery were for a specific date (month and day; $n = 24$), whereas smolts captured in the River ($n \geq 68$) and in the Ocean ($n \geq 150$) were captured over 5–7 days (date shown is the median date of the group). In panels *b* and *d* ocean-caught fish are grouped according to four oceanographic regions with increasing distance from the river. Asterisk in dates for hatchery fish indicates a significant difference from initial sampling date ($p < 0.05$, Tukey HSD). Lines over histograms with different letters indicate a significant difference ($p < 0.05$, Tukey HSD).

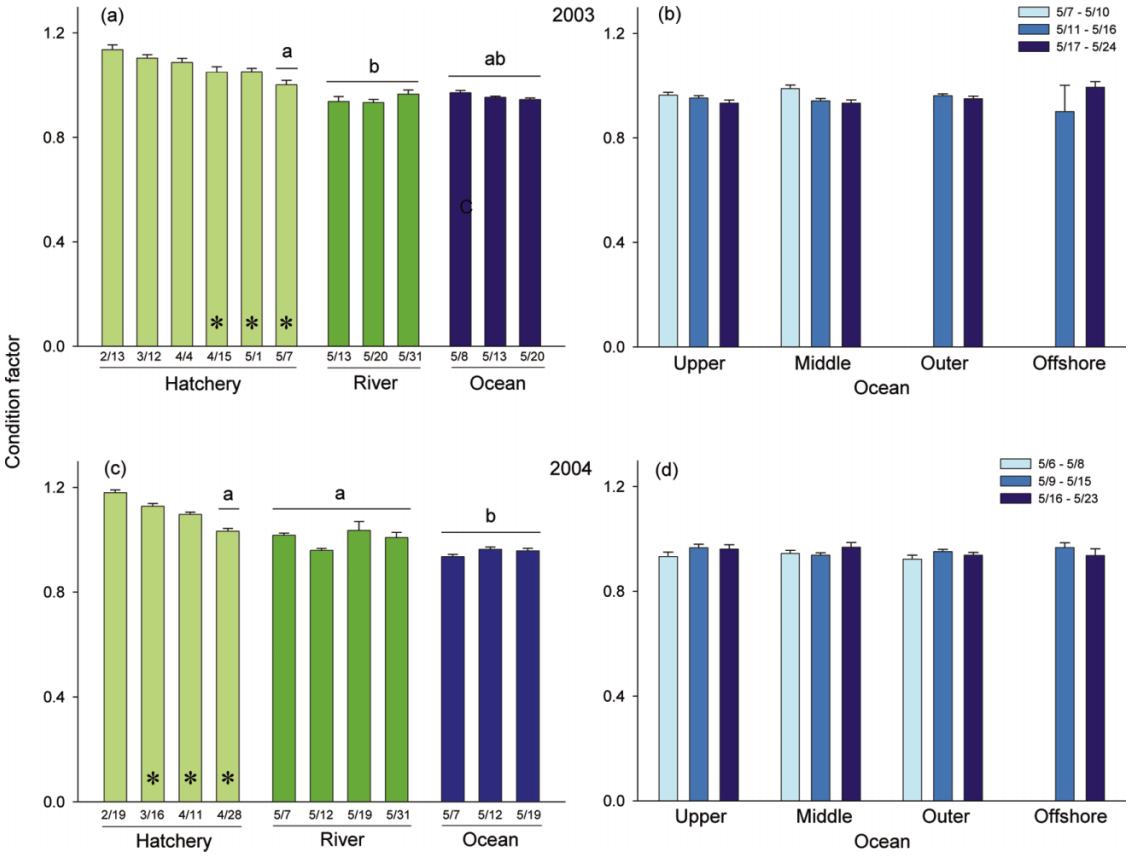


Table 2. Correlation matrix of physiological parameters and abiotic factors for ocean-caught Atlantic salmon smolts in 2004.

	CF	NKA	GH	IGF-I	T_4	T_3	Date	Distance	Temperature	Salinity
L	–0.02	**–0.29**	–0.12	0.05	0.06	**0.3**	0.04	0.06	–0.04	0.09
CF		0.1	**–0.15**	**0.27**	0.03	**0.16**	0.07	0.01	–0.02	–0.01
NKA			–0.04	0.06	0.05	0.02	**–0.18**	–0.02	–0.04	–0.05
GH				–0.04	**0.21**	–0.05	–0.08	**–0.37**	**0.24**	**–0.41**
IGF-I					–0.05	**0.44**	**–0.28**	–0.08	–0.02	–0.01
T_4						0.08	0.09	**–0.34**	**0.23**	**–0.44**
T_3							–0.04	0.05	0	0.06
Date								–0.06	**0.34**	–0.02
Distance									–0.63	**0.81**
Temperature										–0.67

Note: L, length; CF, condition factor; NKA, sodium–potassium ATPase activity; GH, plasma growth hormone; IGF-I, insulin-like growth factor I; T_4, thyroxine; T_3, triiodothyronine. Values in bold are significant ($p < 0.05$).

positive relationship of plasma GH levels with date and temperature and a significant negative relationship with length, CF, and distance offshore (Table 2).

In 2004, plasma GH increased with time in the hatchery primarily owing to an increase on 28 April (5.3 ± 2.2 ng·mL^{-1}, $p < 0.0001$; Fig. 5*c*). After release, smolts had slightly elevated plasma GH levels (8.0 ± 1.1 ng·mL^{-1}) that were not significantly different from those of smolts in the hatchery on 28 April ($p = 0.51$). There was no significant change in plasma GH over time in the river-captured smolts ($p = 0.42$). Plasma GH levels increased further in the ocean-captured smolts (22.4 ± 0.8 ng·mL^{-1}) and were greater than in both the hatchery smolts and river-captured smolts ($p < 0.0001$). There was a general trend of plasma GH decreasing with distance offshore in ocean-captured smolts, but an effect of time was not obvious (Figs. 5*c*, 5*d*). AIC regression indicated that CF, distance, temperature, and salinity were included in the best explanatory model ($p < 0.00001$; $R^2 = 0.19$). Correlation analysis indicated a significant positive relationship of plasma GH levels with temperature and a significant negative relationship with distance offshore and salinity (Table 2).

In 2003, plasma IGF-I levels of hatchery smolts were lower in April and early May compared with that in February, and then increased to 47.8 ± 1.8 ng·mL^{-1} on 7 May (Fig. 6*a*). After release, smolts recaptured in the river had significantly greater plasma

Fig. 4. Gill Na^+/K^+-ATPase activity of Atlantic salmon smolts during hatchery rearing (Hatchery) and after recapture in the Penobscot River (River) and coastal Gulf of Maine (Ocean) in 2004. Values for the hatchery were for a specific date (month and day; $n = 24$), whereas smolts captured in the River ($n \geq 68$) and in the Ocean ($n \geq 150$) were captured over 5–7 days (date shown is the median date of the group). In panels *b* and *d* ocean-caught fish are grouped according to four oceanographic regions with increasing distance from the river. Asterisk in dates for hatchery fish indicates a significant difference from initial sampling date ($p < 0.05$, Tukey HSD). Lines over histograms with different letters indicate a significant difference ($p < 0.05$, Tukey HSD).

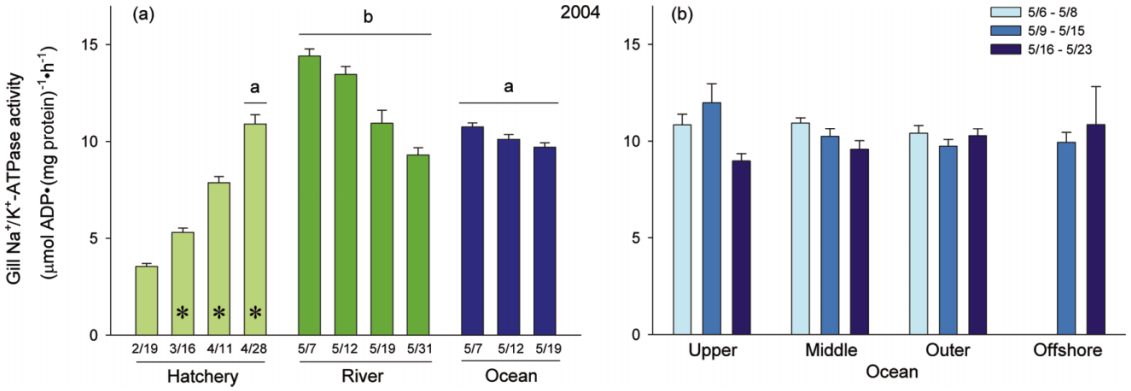
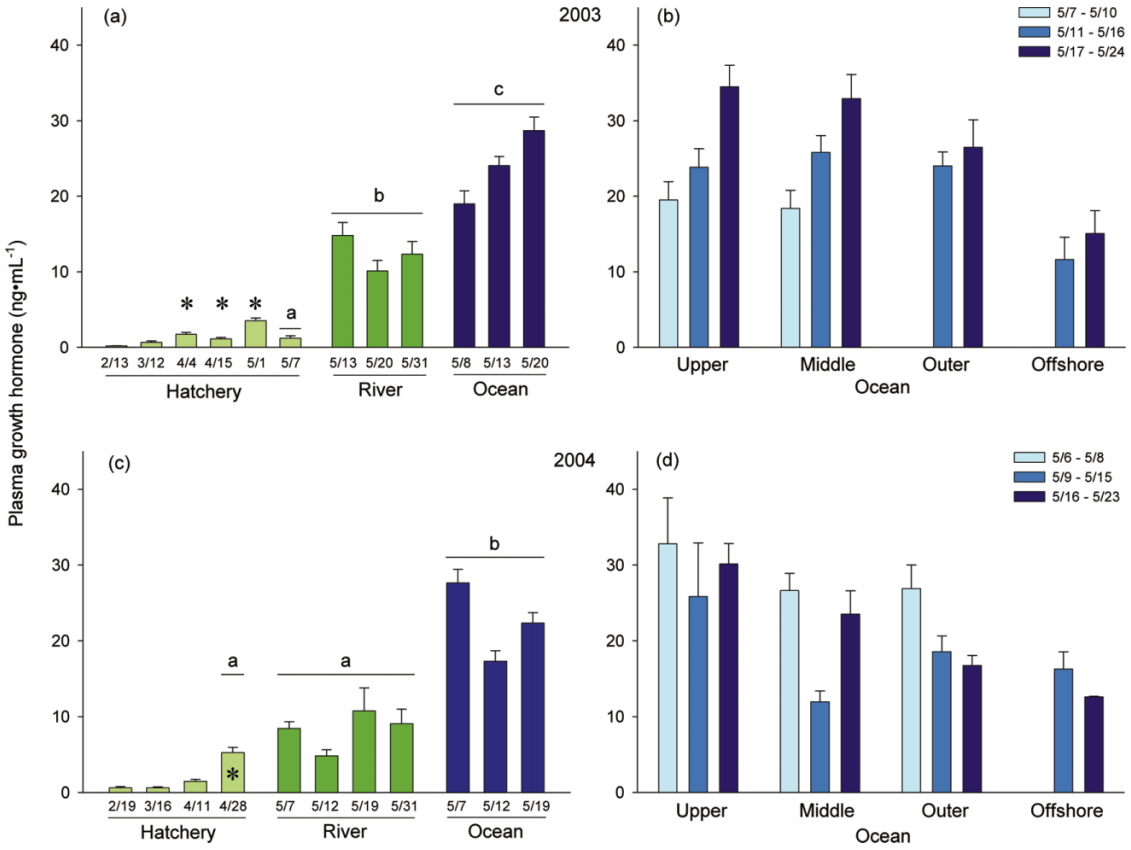
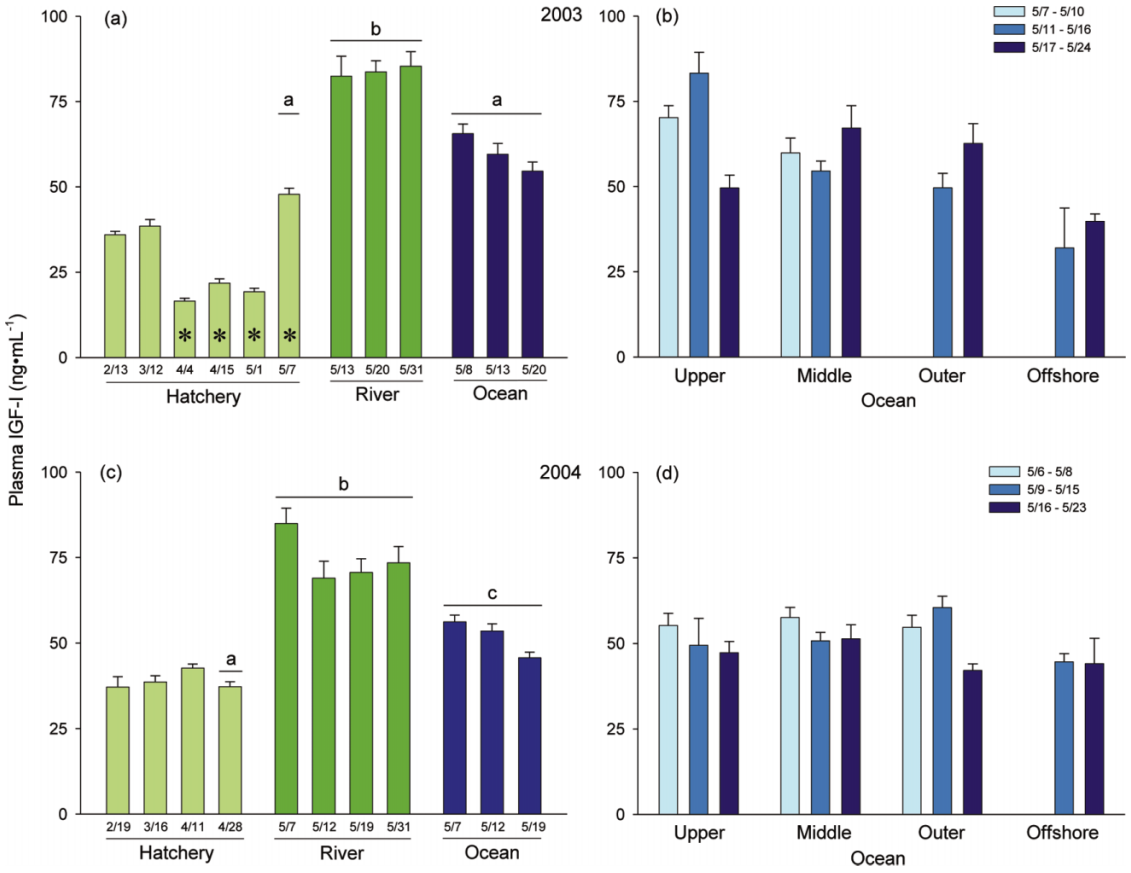


Fig. 5. Plasma growth hormone (GH) of Atlantic salmon smolts during hatchery rearing (Hatchery) and after recapture in the Penobscot River (River) and coastal Gulf of Maine (Ocean) in 2003 and 2004. Values for the hatchery were for a specific date (month and day; $n = 24$), whereas smolts captured in the River ($n \geq 68$) and in the Ocean ($n \geq 150$) were captured over 5–7 days (date shown is the median date of the group). In panels *b* and *d* ocean-caught fish are grouped according to four oceanographic regions with increasing distance from the river. Asterisk in dates for hatchery fish indicates a significant difference from initial sampling date ($p < 0.05$, Tukey HSD). Lines over histograms with different letters indicate a significant difference ($p < 0.05$, Tukey HSD).



IGF-I levels (84.1 ± 2.4 ng·mL⁻¹) than smolts in the hatchery on 7 May ($p < 0.0001$). There was no significant change over time in plasma IGF-I levels of smolts captured in the river ($p = 0.46$). Fish captured in the ocean had lower plasma IGF-I levels compared with smolts captured in the river ($p < 0.0001$), but did not differ significantly from fish on 7 May in the hatchery ($p = 0.16$). In ocean-captured smolts there was a general trend of plasma IGF-I levels decreasing with time and with distance offshore (Figs. 6*a*, 6*b*). AIC regression analysis of plasma IGF-I levels identified a model including length, date, and distance ($p = 0.0000$; $R^2 = 0.15$). Correlation analysis indicated a significant negative relationship of plasma IGF-I levels with date, distance, and salinity (Table 1).

Fig. 6. Plasma insulin-like growth factor I (IGF-I) of Atlantic salmon smolts during hatchery rearing (Hatchery) and after recapture in the Penobscot River (River) and coastal Gulf of Maine (Ocean) in 2003 and 2004. Values for the hatchery were for a specific date (month and day; $n = 24$), whereas smolts captured in the River ($n \geq 68$) and in the Ocean ($n \geq 150$) were captured over 5–7 days (date shown is the median date of the group). In panels *b* and *d* ocean-caught fish are grouped according to four oceanographic regions with increasing distance from the river. Asterisk in dates for hatchery fish indicates a significant difference from initial sampling date ($p < 0.05$, Tukey HSD). Lines over histograms with different letters indicate a significant difference ($p < 0.05$, Tukey HSD).



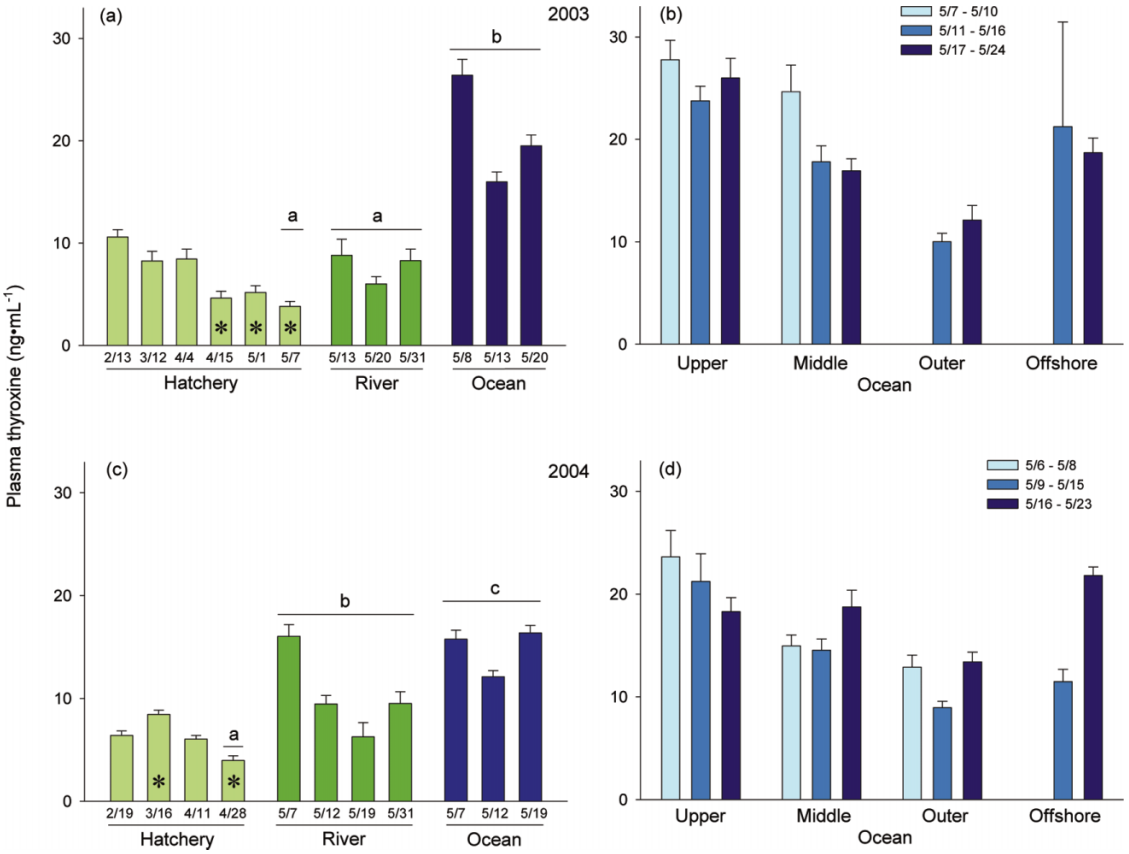
In 2004, plasma IGF-I levels in the hatchery remained relatively constant over time ($p = 0.046$; Fig. 6*c*) and was 37.2 ± 3.7 ng·mL^{-1} on 28 April. After release, smolts recaptured in the river had significantly greater plasma IGF-I levels (75.3 ± 1.8 ng·mL^{-1}) than smolts in the hatchery on 28 April ($p < 0.0001$). There was no significant change over time in plasma IGF-I levels of smolts captured in the river ($p = 0.06$). Fish captured in the ocean had lower plasma IGF-I levels compared with smolts captured in the river ($p < 0.0001$), but higher levels compared with smolts in the hatchery on 28 April ($p = 0.0018$). In ocean-captured smolts there was a general trend of plasma IGF-I levels decreasing with time, but no obvious trend with distance offshore (Figs. 6*c*, 6*d*). AIC regression analysis of plasma IGF-I levels identified a model including length, CF, date, distance, temperature, and salinity ($p < 0.0001$; $R^2 = 0.21$). Correlation analysis indicated a significant negative relationship of plasma IGF-I levels with date and a positive relationship with CF (Table 2).

In 2003, plasma T_4 levels decreased with time in the hatchery ($p < 0.0001$; Fig. 7*a*) and were lowest on 7 May (3.8 + 0.5 ng·mL^{-1}). After release, smolts recaptured in the river had slightly higher plasma T_4 levels (7.5 ± 0.6 ng·mL^{-1}), though not significantly different from those of smolts in the hatchery on 7 May ($p = 0.40$). There was no significant change in plasma T_4 levels over time in the river ($p = 0.98$). Plasma T_4 levels increased to 19.5 ± 0.96 ng·mL^{-1} in ocean-captured fish and were significantly greater than those in both hatchery smolts (fourfold increase) and river-captured smolts (less than twofold increase, $p < 0.0001$). In ocean-captured smolts there was a general trend of plasma T_4 levels decreasing with time (Figs. 7*a*, 7*b*) and decreasing progressively in the Upper Bay, Middle Bay, and Outer Bay, but subsequently increasing offshore (Fig. 7*b*). AIC regression analysis of plasma T_4 levels identified a model including date and distance ($p < 0.001$; $R^2 = 0.17$). Correlation analysis indicated a significant negative relationship of plasma T_4 levels with date, distance, and salinity (Table 1).

In 2004, plasma T_4 levels changed significantly with time in the hatchery ($p < 0.0001$; Fig. 7*c*), increasing in March, and then decreasing at the time of release on 28 April (3.9 + 1.6 ng·mL^{-1}). After release, smolts recaptured in the river had significantly higher plasma T_4 levels (12.0 ± 0.7 ng·mL^{-1}, $p < 0.0001$) compared with smolts in the hatchery on 28 April. There was a significant decrease in plasma T_4 levels over time in the river ($p < 0.0001$). Plasma T_4 levels increased to 14.5 ± 0.4 ng·mL^{-1} in ocean-captured fish and were significantly greater than those in both hatchery smolts (3.5-fold increase) and river-captured smolts (20% increase, $p < 0.0001$, Tukey HSD). In ocean-captured smolts there was a general trend of plasma T_4 levels decreasing with time (Figs. 7*c*, 7*d*) and decreasing progressively in the Upper Bay, Middle Bay, and Outer Bay, but subsequently increasing offshore (Fig. 7*d*). AIC regression analysis of plasma T_4 levels identified a model including length, date, temperature, and salinity ($p < 0.0001$; $R^2 = 0.23$). Correlation analysis indicated a significant positive relationship of plasma T_4 levels with temperature and a significant negative relationship with distance and salinity (Table 2).

In 2003, plasma T_3 levels did not change over the course of sampling in the hatchery ($p < 0.097$; Fig. 8*a*). After release, smolts

Fig. 7. Plasma thyroxine (T_4) of Atlantic salmon smolts during hatchery rearing (Hatchery) and after recapture in the Penobscot River (River) and coastal Gulf of Maine (Ocean) in 2003 and 2004. Values for the hatchery were for a specific date (month and day; $n = 24$), whereas smolts captured in the River ($n \geq 68$) and in the Ocean ($n \geq 150$) were captured over 5–7 days (date shown is the median date of the group). In panels *b* and *d* ocean-caught fish are grouped according to four oceanographic regions with increasing distance from the river. Asterisk in dates for hatchery fish indicates a significant difference from initial sampling date ($p < 0.05$, Tukey HSD). Lines over histograms with different letters indicate a significant difference ($p < 0.05$, Tukey HSD).



recaptured in the river had similar levels to those of fish in the hatchery on 7 May (7.5 ± 0.4 and 7.7 + 0.4 ng·mL^{-1}, respectively; $p = 0.99$). Although there was a trend of increasing plasma T_3 levels over time in the river, this relationship was not statistically significant ($p = 0.057$) and had low explanatory power ($r^2 = 0.064$). Plasma T_3 levels increased to 16.1 ± 0.4 ng·mL^{-1} in ocean-captured fish and were more than twofold greater than those in both hatchery and river-captured smolts ($p < 0.0001$). In ocean-captured smolts there was no obvious trend in T_3 levels in relation to date or distance offshore (Figs. 8*a*, 8*b*). AIC regression analysis of plasma T_3 levels identified a model including length, date, temperature, and salinity ($p = 0.0016$; $R^2 = 0.10$). Correlation analysis indicated a significant positive relationship of plasma T_3 levels with length, distance, and salinity (Table 1).

In 2004, plasma T_3 levels were lower in April compared with those in February and March ($p < 0.0001$; Fig. 8*c*). After release, smolts recaptured in the river had elevated levels compared with smolts in the hatchery on 28 April (10.6 + 0.5 and 5.1 ± 1.2 ng·mL^{-1}, respectively; $p = 0.99$). There was a significant increase in plasma T_3 levels over time in the river ($p < 0.0001$; $R^2 = 0.10$). Plasma T_3 levels increased to 13.3 ± 0.3 ng·mL^{-1} in ocean-captured fish and were more than twofold greater than those in hatchery smolts and 25% greater than those in river-captured smolts ($p < 0.0001$). AIC regression analysis of plasma T_3 levels identified a model including length, CF, and date ($p < 0.0001$; $R^2 = 0.12$). Correlation analysis indicated a significant positive relationship of plasma T_3 levels with length and CF (Table 2).

A summary of the changes in the hatchery, river, and ocean for plasma GH, IGF-I, T_4, and T_3 in 2004 is shown in Fig. 9, allowing for direct comparison of the relative changes in each of the hormones. This graph shows that all four hormones increased during river migration. During initial seawater entry plasma in the Upper Bay, GH, T_4, and T_3 increased, whereas IGF-I decreased. Migration further offshore resulted in lower levels of plasma GH and T_4, but moderately increasing levels of T_3. The greatest magnitude of increase (approximately fivefold) occurred in plasma GH and T_4.

Of the 89 smolts captured and sampled in the Penobscot River in 2003, 72 were hatchery smolts, 13 were naturally reared, and 4 could not be identified. There were no significant differences in any of the physiological parameters except for plasma GH, which was significantly higher in naturally reared smolts. Of the 191 smolts captured and sampled in the Penobscot River in 2004, 153 were hatchery smolts, 15 were released as parr (8 months prior to migration), and 23 were naturally reared. The fish released as parr were significantly smaller and had higher plasma T_3 than hatchery and naturally reared smolts (Table 3). Naturally reared smolts had significantly lower CF and lower plasma T_4 than hatchery smolts and smolts released as parr.

Of the 360 fish captured in the ocean in 2004 for physiological sampling, 337 were hatchery smolts, 14 were released from the hatchery as parr, and 9 were naturally reared smolts. Fish released as parr were significantly shorter (14.8 ± 0.6 cm) than both hatchery smolts and naturally reared fish (18.0 ± 0.1 and

Fig. 8. Plasma triiodothyronine (T_3) of Atlantic salmon smolts during hatchery rearing (Hatchery) and after recapture in the Penobscot River (River) and coastal Gulf of Maine (Ocean) in 2003 and 2004. Values for the hatchery were for a specific date (month and day; $n = 24$), whereas smolts captured in the River ($n \geq 68$) and in the Ocean ($n \geq 150$) were captured over 5–7 days (date shown is the median date of the group). In panels *b* and *d* ocean-caught fish are grouped according to four oceanographic regions with increasing distance from the river. Asterisk in dates for hatchery fish indicates a significant difference from initial sampling date ($p < 0.05$, Tukey HSD). Lines over histograms with different letters indicate a significant difference ($p < 0.05$, Tukey HSD).

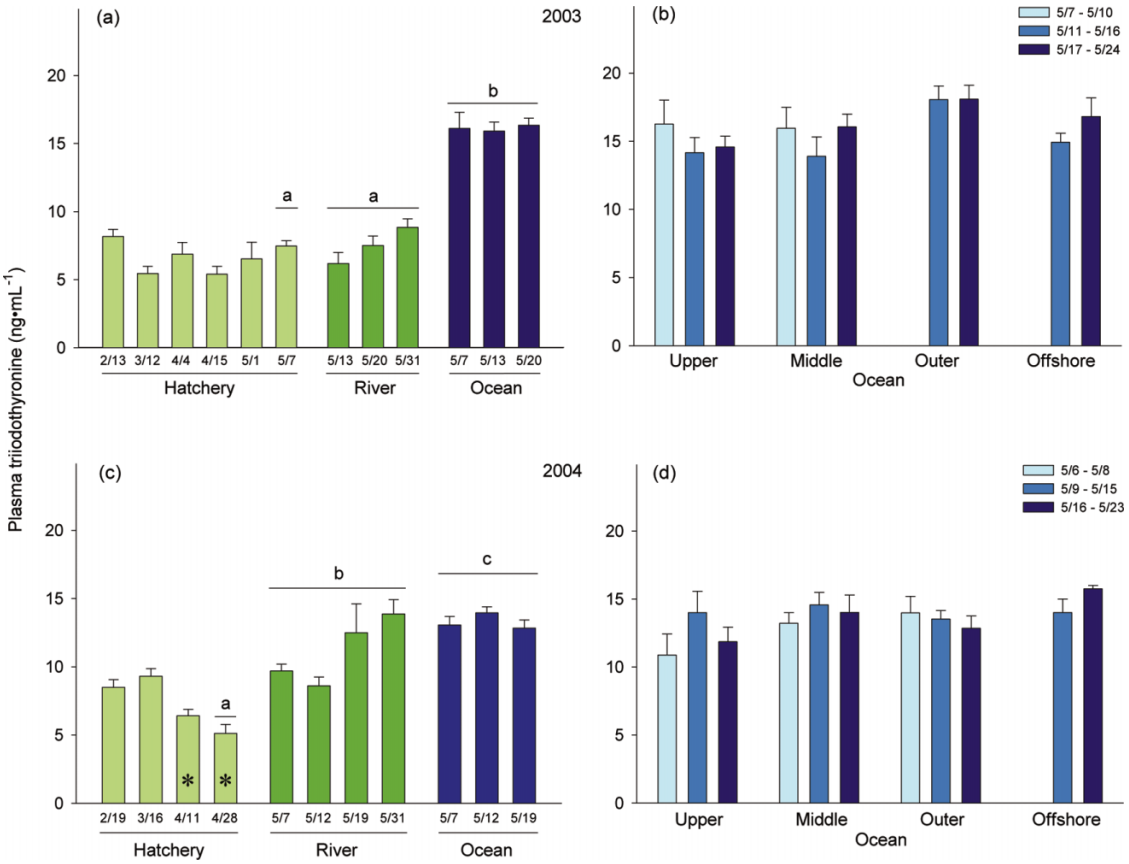
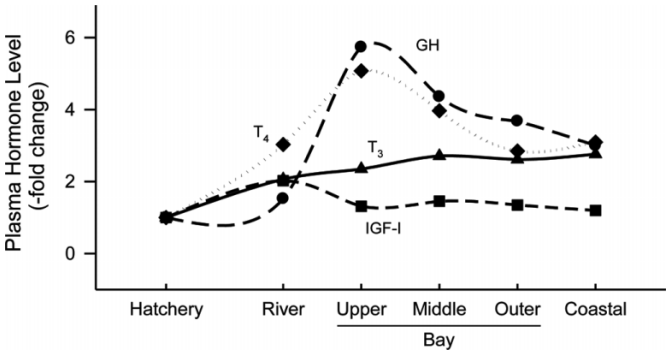


Fig. 9. Summary of changes in plasma GH, IGF-I, T_4, and T_3 levels in Atlantic salmon smolts at the end of hatchery rearing and after river and coastal migration in 2004. Values for each hormone at the last sampling prior to release from the hatchery on 28 April were set to 1.0 and changes expressed relative to this value.



18.1 ± 0.7 cm, respectively; $p < 0.05$). There was no significant difference among these groups in condition factor, gill NKA, plasma GH, IGF-I, or T_4 (Table 4). Plasma T_3 was higher in hatchery smolts (13.6 ± 0.3 ng·mL^{-1}) than in fish released as parr or naturally reared (11.0 ± 1.6 and 9.2 ± 2.0 ng·mL^{-1}, respectively; $p = 0.03$), though no differences were detected among the groups using post hoc tests ($p > 0.3$).

Table 3. Biometric and physiological parameters of Atlantic salmon smolts captured in the Penobscot River in 2004.

	Hatchery smolts	Hatchery parr	Naturally reared fish
Length (cm)	18.1±0.2a	15.4±0.5b	17.8±0.4a
Condition factor	1.01±0.01a	1.03±0.02a	0.94±0.02b
Gill NKA activity (μmol ADP·(mg protein)$^{-1}$·h^{-1})	13.2±0.3a	10.8±0.8b	10.2±0.7b
Plasma GH (ng·mL^{-1})	7.4±0.9	9.8±2.1	10.3±2.6
Plasma IGF-I (ng·mL^{-1})	76.7±2.7	79.5±6.4	57.7±7.7
Plasma T_4 (ng·mL^{-1})	12.6±0.8a	13.3±2.3a	7.7±1.7b
Plasma T_3 (ng·mL^{-1})	10.3±0.5a	15.0±1.4b	10.8±1.1a

Note: Fish were identified as hatchery smolts ($n = 153$), hatchery parr ($n = 15$), or naturally reared fish ($n = 23$). Values are means ± standard error, and those with different letters were significantly different. Condition factor = 100 (mass × length^{-3}).

In 2003, there was a positive correlation between offshore distance and date (Table 1), since more of the offshore sampling occurred later in this year. In 2004, there was no significant correlation between offshore distance and date (Table 2), as the sampling occurred more evenly in this year. In both 2003 and 2004, there was a significant positive correlation of distance offshore with salinity and a negative correlation of station distance with temperature (Tables 1 and 2).

Our physiological sampling was intended to be non-lethal, and thus analysis of gut content was limited to a small number of trawl-captured fish that died after physiological sampling. There

Table 4. Biometric and physiological parameters of Atlantic salmon smolts captured in the ocean in 2004.

	Hatchery smolts	Hatchery parr	Naturally reared fish
Length (cm)	18.0±0.1a	14.8±0.6b	18.1±0.7a
Condition factor	0.95±0.01	0.92±0.02	0.95±0.03
Gill NKA activity (μmol ADP·(mg protein)$^{-1}$·h^{-1})	10.1±0.1	9.6±0.7	10.7±0.9
Plasma GH (ng·mL^{-1})	21.6±0.9	28.7±5.4	29.3±4.1
Plasma IGF-I (ng·mL^{-1})	51.2±1.2	50.1±6.8	39.1±5.1
Plasma T_4 (ng·mL^{-1})	14.3±0.4	14.9±2.2	15.2±2.7
Plasma T_3 (ng·mL^{-1})	13.6±0.3	11.0±1.6	9.2±2.0

Note: Fish were identified as hatchery smolts (n = 337), hatchery parr (n = 14), or naturally reared fish (n = 9). Values are means ± standard error, and those with different letters were significantly different. Condition factor = 100 (mass × length^{-3}).

was a negative correlation between gut energy content and plasma IGF-I in 2003 (r = −0.54) and a positive correlation between gut energy content in plasma T_4 (r = −0.38) in 2004. However, these trends were not consistent between years, and there were no other significant correlations between gut energy content and physiological or endocrine parameters.

Discussion

The present study, carried out over two consecutive spring seasons, with expected natural year-to-year variation in environmental variables such as river and ocean temperatures and water flow, paints a remarkably clear picture of the endocrine and physiological changes that take place in Atlantic salmon smolts from rearing in the hatchery through downstream river migration, ocean entry, and migration. In the following discussion these sequential, important life history steps for the anadromous Atlantic salmon are examined.

Hatchery rearing

During hatchery rearing, branchial NKA activity increases and CF decreases, in line with the well-documented developmental changes in physiology and endocrinology of Atlantic salmon smoltification (Prunet et al. 1989; Björnsson et al. 1989; McCormick et al. 1995) that are preadaptive to seawater entry. The zeitgeber for these changes is the increase in daylength during spring (McCormick et al. 2002), activating the light–brain–pituitary axis and leading to increased GH release (Ágústsson et al. 2001), with temperature as a modulating environmental factor (McCormick et al. 2002). GH, together with cortisol, act as drivers of morphological and functional changes in branchial ionocytes, leading to increased NKA activity and greater salinity tolerance of juvenile salmon (McCormick 2001). GH also stimulates hepatic IGF-I release (Moriyama 1995) and as well as local IGF-I production, stimulating skeletal growth and strength (Wargelius et al. 2005), including a relative lengthening of the caudal peduncle (Winans and Nishioka 1987). This results in a slimmer body shape, at least partly explaining the smoltification-related decrease in CF seen in the present as well as previous studies on salmon smoltification (Hoar 1988).

At the hatchery, plasma GH levels of the smolts in the present study started to increase during spring, reaching peak levels in late April and early May. Although this change is relatively modest compared with subsequent changes in GH levels, the increase is highly significant. The lack of change in plasma T_4 levels during smolt development in the hatchery is somewhat surprising. Increased plasma T_4 in hatchery-reared salmon has been seen in other smolting salmonids, especially Pacific salmon. However, studies under controlled laboratory conditions have also found minimal changes in plasma T_4 at the same time that plasma GH and cortisol are increasing (McCormick et al. 2002). Fish in the hatchery were always sampled in the morning, a fairly typical sampling protocol that allows for detection of seasonal changes in plasma T_4 in smolts (McCormick et al. 1995). Plasma thyroxine increases during smolt development are thought to be caused in part by exposure to novel water and other environmental cues (Hoar 1988). Under natural river conditions, snow melt, rain events, and increased biological production may provide chemical and temperature cues for imprinting and initiation of migration. One possible explanation for the absence of increased plasma T_4 in this and other hatchery-based studies, especially those like Green Lake Hatchery that use deep lake water, is that changes in temperature and chemical composition of the water are muted compared with those experienced by fish in the wild and provide only limited cues for increased plasma T_4.

River release and migration

Following the gradual increase in gill NKA activity observed in the hatchery, gill NKA activity of smolts captured in the river were even further elevated, a phenomenon that has been observed previously in hatchery-reared fish in the Connecticut River (McCormick et al. 2003), another large hatchery-dependent Atlantic salmon population approximately 400 km southwest of the Penobscot basin. The increase in gill NKA activity between hatchery release and recapture in the river is likely due to increased growth hormone and cortisol levels seen during river migration (present study; McCormick et al. (2003), as these hormones are known to be the main stimulators of gill NKA (McCormick 2001). Throughout the freshwater migratory period there was a consistent decline in gill NKA activity with time. This decrease has been observed previously in smolts on the Penosbscot River and is likely driven by the temperature-dependent loss of smolt characteristics (McCormick et al. 1999).

The river-released smolts show several significant changes in endocrine profiles. These are not just due to further ontogenetic smolt developmental changes, as development proceeded at a much more rapid pace than that occurring in the hatchery. Previous studies on Atlantic salmon smolts in the Connecticut River have shown that the physiological and endocrine changes taking place in the river are much more pronounced than those occurring in fish retained in the hatchery (McCormick et al. 2003). Thus, it seems likely that smolts are responding to some of the physicochemical conditions in the river, which differ from those in the hatchery, demonstrating an ongoing environmental modulation of the smoltification process during the freshwater phase. The increased plasma GH levels in the river-released fish, in line with previous studies (Björnsson et al. 2011), indicate that environmental factors other than day length are impacting GH regulation. Concomitantly, there is a large increase in IGF-I levels, possibly GH-driven, at least to some extent.

In the river, the thyroid system also appears to be activated, and this was especially clear during the 2004 season. The experience of "novel water," either within a hatchery or after release, has been suggested as an important cue for increased plasma T_4 levels (Hoffnagle and Fivizzani 1990), which in turn are involved in the process of imprinting. Chum salmon (*Oncorhynchus keta*) fry had greater increases in plasma T_4 during the downstream migratory period when reared in stream water versus hatchery well water (Iwata et al. 2003). The elevated thyroid hormones observed in both the river and after early ocean entry may be an indication of continued imprinting during these periods (Dittman and Quinn 1996).

Ocean entry and migration

Overall, gill NKA activity of fish captured in the ocean was lower than that of river-captured fish, though there was no apparent effect of time or distance. In laboratory studies of Atlantic salmon smolts, gill NKA activity increased in response to salinity (Stefansson et al. 2009), though the degree of increase likely depends on the

initial levels and the absolute salinity levels. Recent evidence shows that there are two major isoforms of NKA in the gills of Atlantic salmon that are differentially regulated by salinity (Mackie et al. 2005; Nilsen et al. 2007; McCormick et al. 2011). The observed decrease in NKA activity following migration into the marine environment may be the result of decreases in the NKAα1a (freshwater) isoform, while the NKAα1b (seawater) isoform likely increases or stays constant. Wild Atlantic salmon smolts in the Vosso River in Norway (Stefansson et al. 2003) have lower gill NKA activity than smolts captured in the Penobscot River (present study), but smolts captured in the North Sea have higher gill NKA activity compared with fish captured in the Gulf of Maine. Such differences may be driven by a variety of factors, including lower river temperatures and higher ocean salinities in Norway, as well as genetic differences.

Movement from the river to the estuarine and ocean environment caused significant increases in plasma GH, T_4, and T_3, but decreases in IGF-I. This pattern was robust and consistent in both years. The differential response of hormones during this early estuarine and ocean period indicates differential endocrine responses to environmental conditions encountered during early marine migration. The driving forces for these responses are not entirely clear. Although we found correlations with several biotic (physiological) and abiotic variables, it is difficult to come to firm conclusions because there is substantial correlation among the distance, salinity, and temperature variables we analyzed (Tables 1 and 2). It should also be noted that fish sampled here are a subset of the total population released from the hatchery and necessarily include surviving fish and those accessible to capture. With these precautions in mind, we can draw some inferences from the statistical relationships among the biotic and abiotic variables and changes in hormone levels of smolts during estuarine and near-coastal migration.

Plasma GH levels were higher in the river smolts than in the ocean smolts for each study year, especially in the Upper Bay, indicating stimulation of GH by early estuarine migration. Upon movement from river to the Upper Bay, there is an increase in salinity and a decrease in temperature. Exposure of Atlantic salmon smolts to seawater causes an increase in pituitary GH mRNA levels (Ágústsson et al. 2003) and plasma GH (Handeland et al. 2000; Arnesen et al. 2003). A further increase in plasma T_4 levels occurs with exposure to seawater compared with levels found during smolt development for Atlantic salmon (McCormick and Saunders 1990; Young et al. 1995) and coho salmon (*Oncorhynchus kisutch*) (McCormick and Saunders 1990; Young et al. 1995). Exposure to lower temperature after seawater transfer of Atlantic salmon smolts has been shown to result in lower levels of plasma GH (Handeland et al. 2000). Plasma T_4 levels in salmon are generally lower under cooler water temperatures in freshwater (McCormick et al. 2000; Larsen et al. 2001), though to our knowledge temperature impacts on salmonids in seawater have not been examined. Since laboratory studies have generally found salinity stimulatory and cool temperatures inhibitory to plasma GH, T_4, and T_3, it seems likely that the higher levels of these hormones seen during initial marine migration are due to elevated salinity. Although GH has a well-established role in increasing the salinity tolerance of smolts during their development in freshwater, the precise role of GH and thyroid hormones during their transition from freshwater to seawater is unclear.

Interestingly, plasma IGF-I levels were consistently lower in fish in the Upper Bay and all ocean areas compared with levels in fish captured in the river, indicating a differential response compared to GH and thyroid hormones. Plasma IGF-I levels have been found to increase following exposure to salinity in rainbow trout (*Oncorhynchus mykiss*) (Shepherd et al. 2005). Plasma IGF-I levels are generally lower with decreasing temperature in Chinook salmon (*Oncorhynchus tshawyscha*) (Beckman et al. 1998; Larsen et al. 2001; McCormick et al. 2002) and Atlantic salmon (Beckman et al. 1998; Larsen et al. 2001; McCormick et al. 2002). Thus, during early marine migration the decrease in IGF-I caused by temperature may be greater than the stimulatory effect of salinity, resulting in lower IGF-I levels.

In ocean-caught smolts, for each study year there was a significant negative relationship between plasma GH levels and distance from the river and CF and a positive relationship with temperature. As noted above, laboratory studies have found increasing plasma GH levels with increasing temperature. Since there was an inverse relationship between temperature and distance from the river, the latter relationship with GH may be in part driven by temperature. There was no significant correlation between condition factor and any of the abiotic variables of date, distance, temperature, or salinity in either year. If CF were an accurate measure of the energetic content of fish (which is not always the case; Trudel et al. 2005), then the negative relationship with plasma GH in the present study may be driven by energetic or food intake differences among individuals captured in the ocean. Laboratory studies have found that higher plasma GH levels in salmonids occur during periods of food withdrawal (Björnsson et al. 2011). In a detailed time-course study of Chinook salmon, significant increases in plasma GH levels occurred within 3 days of food withdrawal (Pierce et al. 2005). There was a positive relationship between plasma T_3 levels and the CF of ocean-caught smolts. Previous work has shown a graded, positive response between plasma T_3 levels and food intake of post-smolt Atlantic salmon (McCormick and Saunders 1990). Further work will be needed to determine whether energetic or feeding differences may in fact be important determinants of endocrine status in ocean migrating salmon.

There was a general trend for naturally reared fish captured in the ocean trawls to have higher plasma GH and lower plasma IGF-I and T_3 levels than was found in hatchery fish, though none of these differences were statistically significant. These endocrine trends observed in naturally reared fish would be characteristic of limited food intake, but this is an unlikely explanation given the greater food intake seen for naturally reared versus hatchery-reared smolts in a companion study (Renkawitz and Sheehan 2011). Comparison of all hatchery fish to the small number of naturally reared fish, irrespective of location or timing, may have masked differences in endocrine status. A more expansive survey that includes more naturally reared fish and an expanded analysis of feeding, growth, and energetic state would be useful for determining possible differences between wild and hatchery-reared fish.

Stefansson et al. (2012) recently examined endocrine changes in wild Atlantic salmon post-smolts migrating off the coast of Norway. Their results for plasma T_4 were similar to our results in that levels of this hormone increased initially in fish in the fjord and stayed high in fish along the coast and offshore. In contrast with our results, they found decreasing plasma GH and increasing IGF-I as fish spent more time in the ocean and moved offshore. It seems likely that these differences are due to the different time and spatial scales examined in the two studies. Our sampling in coastal and offshore areas occurred within a relatively short time and distance from the river, and fish may have been in seawater for only a few days to a week (Renkawitz et al. 2012). In contrast, Stefansson et al. (2012) examined changes that occurred up to 7 weeks after river migration and 1000 km offshore. Thus, the lower plasma GH and higher IGF-I levels likely reflect entrance into a rapid growth phase as seawater acclimation is complete and growth conditions become optimal. This is supported by observed increases in muscle RNA/DNA ratios and total body lipid and energy content of fish captured in the ocean in late June.

The different capture methods used in the hatchery, river and ocean resulted in fish being held for differing periods of time prior to sampling. Fish in the hatchery were sampled immediately, whereas fish in smolt traps were held for up to 10 hours

before sampling, and fish captured by trawling were held for 45–75 minutes before sampling. The appearance of all sampled fish was normal and not highly darkened as occurs in highly stressed salmon, indicating that they were not strongly affected by the sampling and holding procedures (Music et al. 2010). Previous studies on Atlantic salmon smolts indicate that gill NKA activity is not influenced by stresses lasting for a few hours (Carey and McCormick 1998). Plasma GH, IGF-I, and thyroid hormones may be influenced by stress, but the degree to which this occurs, if at all, will be dependent on the length and severity of the stressor (Deane and Woo 2009). We have indirect evidence that the procedures used here had relatively little influence on circulating hormone levels. We previously examined plasma levels of GH, IGF-I, and thyroid hormones in actively migrating hatchery-reared and wild smolts that were captured by angling or at smolt collection facilities and sampled within 3 min of initial disturbance (McCormick and Björnsson 1994; McCormick et al. 2003). Plasma hormone levels of smolts in these studies were nearly identical to those found for smolts captured in the Penobscot River in the present study and were similarly elevated relative to fish in the hatchery, indicating that the use of smolt traps does not have a strong influence on circulating hormone levels. While comparable data are not available for ocean-captured fish, the relatively short time (≤1.25 h) that fish were subjected to stress makes it even less likely that endocrine status of these fish was affected by the capture process.

Endocrine status of individuals has the potential to provide information on performance and overall fitness (Husak et al. 2009; McCormick 2009). One long-term goal of the approach adopted in our study is to provide a physiological baseline for changes that occur during downstream migration and ocean entry. This information can then be used towards developing a predictive indicator of behavior and survival of Atlantic salmon in the marine environment. This could occur either by sampling fish before release, as has been done in several studies on smolt development and subsequent survival (Beckman et al. 1999), or more rarely when sampling smolts after they have been released into the wild (McCormick et al. 2003). Comparison of wild and hatchery-reared fish will also be of value, particularly in light of the lower survival of the latter. Complex behaviors with large variation among individuals have been observed during estuarine migrations of Atlantic salmon (Kocik et al. 2009; Dempson et al. 2011), which may derive in part from the degree or timing of smolt development at the time of seawater entry. An emerging question for managers is how behaviors vary across stocks, what is normal or adaptive, and what behaviors may indicate physiological–environmental mismatches that impact survival. Combining hormone titer measurements with tracking of individual movement and survival is an important next step that should provide novel insight to the links between endocrine status, behavior, and survival. There is a strong link between early marine growth and overall smolt-to-adult survival in Atlantic salmon (Friedland et al. 2000; Thorstad et al. 2012), providing a strong impetus to understand the link between hormones, growth, and survival in smolts soon after seawater entry. Such an approach may be capable of detecting differences in groups of fish, such as different release dates, rearing strategies, or strains of fish. It may also be possible to monitor and predict annual and even individual differences in survival based on physiological metrics. The present study provides an initial examination of the environmental factors that affect hormonal changes in Atlantic salmon smolts after release and is a necessary first step in linking endocrine changes to performance and survival in the marine environment.

Acknowledgements

We thank Fred Trasko and the staff of the Green Lake National Fish Hatchery for their help in providing and sampling fish. We acknowledge Ed Hastings for his contributions to this effort; we miss his enthusiasm and companionship. Justin Stevens, National Marine Fisheries Service, Northeast Fisheries Science Center (NMFS–NEFSC), provided the map in Fig. 1. Alicia Miller (NMFS–NEFSC) provided the distance to each station for the marine surveys. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the US Government.

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