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Hypo-osmoregulatory capacity during smolting of endangered inner Bay of Fundy Atlantic salmon and other eastern Canadian stocks

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ABSTRACT

Inner Bay of Fundy (iBoF) *Salmo salar* are at risk of extinction due to high losses during the marine phase; cause unknown. As a first step towards testing the hypothesis that compromised smoltification is implicated, we investigated whether genetic differences in hypo-osmoregulatory ability exist between two iBoF stocks (Stewiacke and Gaspereau) and two other Nova Scotia stocks (Sackville and Medway), all reared under the same conditions from the egg stage. In addition, LaHave and Saint John stocks were included, though their rearing history differed from the other four stocks. Plasma osmolality and muscle water content following biweekly 96 h 35 ppt salinity challenge tests (constant 6 °C), was significantly affected by the interaction between genotype and challenge test date. iBoF Stewiacke stock exhibited relatively poor hypo-osmoregulatory ability during April and early May compared to the other five stocks, indicating a later completion of smolting. By comparison, the iBoF Gaspereau stock exhibited similar temporal changes in hypo-osmoregulatory ability to the other stocks. From late May to early July, all stocks exhibited similarly good hypo-osmoregulatory ability. This unusually long smolt window was associated with rearing at 6 °C in well water. Establishing the osmoregulatory capacity of iBoF smolts under 'ideal' lab conditions is useful baseline knowledge that may assist future studies distinguish normal from abnormal among wild or restocked fish.

1. Introduction

The inner Bay of Fundy (iBoF) Atlantic salmon are a distinct evolutionary group on the verge of extinction (COSEWIC, 2006; Vandersteen Tymchuk et al., 2010). The number of returning adults has declined by over 90% in the past 20 years, and survival of the stock is reliant on a live gene bank program (Gibson et al., 2008). Mortality is mostly at sea, but the cause is unknown. Connecting the decline of iBoF salmon with potential marine threats such as predators, aquaculture and climate change remains speculative (Amiro et al., 2008). The freshwater habitat, by contrast, is believed to be suitable for Atlantic salmon since the iBoF watersheds are relatively well-buffered against acid rain, and can support healthy populations of parr when stocked with unfed fry from the gene bank program (Ashfield et al., 1993; Gibson et al., 2004, 2008). Nevertheless, certain environmental factors and a range of contaminants, while not overtly affecting early life stages, can impair completion of smolting and hypo-osmoregulatory ability, resulting in mortality following migration to sea (McCormick et al., 2009). In addition, domestication through supportive breeding programs may have unpredictable and even deleterious effects on fitness (Garcia deLeaniz et al., 2007). Assessing the 'quality' of iBoF smolts is hindered by a lack of published information on their physiology. To begin addressing this deficiency, our objective was to determine whether this genetically discrete group differed in the completion of smolting to other eastern Canadian stocks.

Genetic differences in Atlantic salmon smolt characteristics have been identified among some stocks, providing evidence of local adaptation (Nielsen et al., 2001; Orciari and Leonard, 1996; Stewart et al., 2006). The stocks in the present study have known genetic differences, and originate from rivers with contrasting estuary and coastal environments (Vandersteen Tymchuk et al., 2010; Fig. 1). The iBoF Stewiacke and Gaspereau Rivers drain into the macro-tidal Minas Basin, which can warm rapidly in spring (Dalrymple et al., 1990). By contrast, the Sackville, Medway and LaHave Rivers flow eastward into the Atlantic Ocean with small tides (<2 m) and low temperatures in spring due to the Labrador current. Saint John River fish were included to see whether the hypo-osmoregulatory aspects of smolting of this domesticated stock originating from the outer Bay of Fundy differed from the iBoF stocks from 300 km to the east.

An increase in hypo-osmoregulatory ability is one of a suite of morphological, behavioral and physiological changes that constitute the parr–smolt transformation (Hoar, 1988). Among both Atlantic and Pacific salmon smolts, useful insight of their preparedness for seawater have been gained through either 24 h salinity challenge

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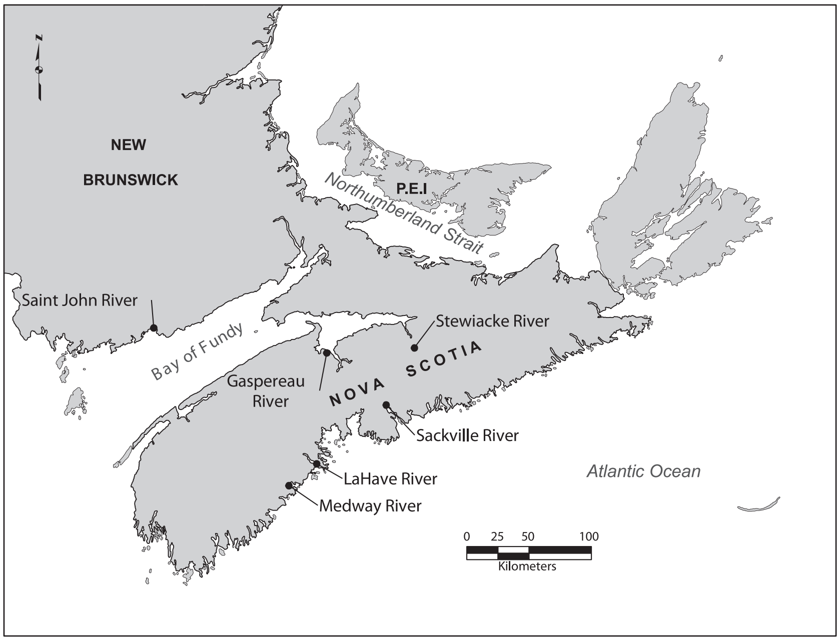

Fig. 1. Atlantic salmon natal rivers. Stewiacke and Gaspereau Rivers: Inner Bay of Fundy. Saint John River: New Brunswick, outer Bay of Fundy. Sackville, LaHave and Medway Rivers: Atlantic coast.

tests followed by a measurement of blood salt levels, or 96 h tolerance tests quantifying percent survival (Blackburn and Clarke, 1987; Saunders et al., 1985; Staurnes et al., 2001). We adopted this approach to characterize the hypo-osmoregulatory aspects of smolting of two hatchery reared iBoF salmon stocks and to identify genetic differences from other stocks in a 'common-garden' experiment.

2. Materials and methods

All fish were 1+ year-old pre-smolts. The federal Department of Fisheries and Oceans (DFO) supplied the two iBoF stocks (Stewiacke, Gaspereau) from its live gene bank (LGB; DFO, 2008), and the three other mainland Nova Scotia stocks (Sackville, Medway, LaHave). The sixth stock, Saint John River (New Brunswick), was from a commercial producer (Merlin Fish Farms Ltd., Wentworth NS). The parents of the Stewiacke fish were LGB F1's reared from parr to adult at DFO Coldbrook Biodiversity Facility (Nova Scotia). The parents of the other Nova Scotia stocks were also reared at Coldbrook for between 3 months and 5 years prior to spawning in fall 2006. Gaspereau parents included returning wild and LGB F1 adults caught at the fish ladder at White Rock between 2001 and 2006, and smolts collected at a downstream by-pass at White Rock (2003–2005). Medway parents were collected as parr (2003–2004). Sackville parents were mostly wild returning adults collected in summer 2006, and some fish captured as juveniles. LaHave parents were returning adults of hatchery origin collected at Morgan Falls in summer 2006. The Coldbrook facility has a flow-through water supply of a surface and ground water mix (pH 7, annual temperature range from 0.7 to 15.5 °C). The eggs of Stewiacke, Gaspereau, Medway and Sackville stocks were fertilized and water-hardened at Coldbrook, then transferred to DFO Mersey Biodiversity Facility (Nova Scotia), and reared separately in tanks supplied with a flow-through surface water (pH 5.8, hardness 4.0 mg/L, alkalinity not detectable, 0.4 to 21 °C annual range). On March 31, 2008, a random sample of n = 120 pre-smolts (>12 cm fork length) from each of these four stocks were trucked from Mersey to Nova Scotia Agricultural College (NSAC). LaHave stock were reared at Coldbrook until close to first feeding, then about 500 sac-fry were transferred in May 2007 to NSAC and reared in well-buffered ground water (10 °C). In March 2008, n = 120 LaHave fish >12 cm fork length were selected for the experiment. Saint John River pre-smolts (n = 160) were overwintered at about 1 °C in well-buffered water then trucked on March 20, 2008 from Merlin Fish Farms Ltd. to NSAC.

By March 31, n = 60–80 of each stock was randomly distributed to each of two black plastic rearing tanks (70 cm diameter, 140 L vol.) supplied with a flow-through well water at 6 to 7 °C (pH 7.9, hardness 123 mg/L eqv.$CaCO_3$; alkalinity 133 mg/L). We chose this temperature as it was likely high enough not to limit the completion of smolting, and low enough to avoid desmoltification. Photoperiod was a simulated annual cycle (lat. 45°N, abrupt dawn/dusk). Fish were hand-fed to apparent satiation four times daily with a commercial salmon diet (Ewos Vextra). All fish acclimated quickly to the rearing tanks and were feeding normally within 3 days. Food was withheld 2 days prior to salinity challenge tests to minimize fecal waste in the test tanks.

Ninety-six hour, 35 ppt salinity challenge tests were repeated biweekly between April 8 and July 4. The twelve salinity challenge test tanks (clear acrylic, 75 cm*30 cm*36 cm deep, vol. 70 L) shared a common recirculated water supply maintained at 6 °C by a chiller. Silica diffusers in each tank maintained oxygen at >90% saturation. At least 24 h before each test, the system was filled with clean seawater (31 ppt, filtered to 30 μM. Source: Institute for Marine Biosciences, Sandy Cove, near Halifax). Salinity was increased to 35 ppt by addition of Kent synthetic sea-salt (J&L Aquatics, BC). Each test started between 09.00 and 10.00 h. Eight fish were netted from each rearing tank and directly transferred to a randomly assigned salinity challenge test tank, giving two replications of test tanks per stock. During each test, food was withheld, oxygen was checked daily, mortalities were removed twice daily and body size recorded (body weight BW to 0.1 g, fork length FL to 0.1 cm). At 96 h, each survivor was euthanized (MS222 overdose), BW, FL, sex and maturity recorded. Condition factor was calculated as $100(BW/FL^3)$. Blood was taken from severed caudal vessels with a heparinized glass tube (Caraway, Fisher Scientific), and a piece of dorsal musculature was dissected out. Blood samples were centrifuged (6000 rpm, 6 min) and plasma stored at −20 °C until osmolality was determined in duplicate using a freezing point osmometer (MicroOsmette, Model#5004, Precision Systems Inc., Natrick, MA). Muscle water content, a useful measure of hypo-osmoregulatory ability in juvenile Atlantic salmon