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STURGEON PAPER

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Natural stranding of Atlantic sturgeon (*Acipenser oxyrinchus* Mitchill, 1815) in Scot's Bay, Bay of Fundy, Nova Scotia, from populations of concern in the United States and Canada

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Summary

Natural mortality of Atlantic sturgeon (*Acipenser oxyrinchus*) has been determined to be low (*M* = 0.07). Reported herein is the mortality by beach stranding of 11 Atlantic sturgeon in Scot's Bay, part of the inner Bay of Fundy in Nova Scotia, Canada on 22 June 2014. Genetic analyses, histological analysis and age determination were performed to determine origin, maturity stage and age of the stranded Atlantic sturgeon. Microsatellite and mitochondrial DNA analyses indicated that four of the Atlantic sturgeon (2 males and 2 females) were from the Saint John River, NB population, which was designated as threatened by the Committee on the Status of Endangered Wildlife in Canada. Seven Atlantic sturgeon (1 male, 5 females, 1 unknown) were from the Kennebec River, Maine population, that was listed as threatened under the Endangered Species Act in the U. S. Ageing of *A. oxyrinchus* Atlantic sturgeon by pectoral fin spine analysis determined that the mean age of the individuals from the Saint John River ($\bar{x}$ = 24.25 years, *SD* = 5.0) and the Kennebec River ($\bar{x}$ = 22.7 years, *SD* = 3.5) were not significantly different. This is the first report of a stranding event of Atlantic sturgeon, and describes a source of natural mortality affecting populations of concern in both Canada and the U. S.

1 | INTRODUCTION

Stranding is a component of natural mortality for fishes and has been reported for several species of sturgeon including Gulf sturgeon (*Acipenser oxyrinchus desotoi*; Parauka, Duncan, & Lang, 2011) and green sturgeon (*Acipenser medirostris*; Thomas et al., 2013). Stranding has not been documented for Atlantic sturgeon (*A. oxyrinchus*). Atlantic sturgeon that suffer mortality from targeted commercial and recreational fisheries (ASSRT, 2007) and as bycatch (Beardsall et al., 2013) are thought to have low rates of natural mortality (*M* = 0.07; Boreman, 1997) as they are large, long-lived with few known predators (Scott & Crossman, 1973).

Atlantic sturgeon is considered threatened and endangered throughout their range primarily due to river damming, compromised water quality, chemical pollution, vessel strikes, and periods of overharvest (COSEWIC, 2011; NOAA, 2012). In Canada, Atlantic sturgeon are designated as threatened by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC [Committee on the Status of Endangered Wildlife in Canada], 2011), and an evaluation for listing under the Species at Risk Act (SARA) is currently underway. In Canada, small-scale fisheries for Atlantic sturgeon remain active in the Saint John River, New Brunswick, and the St. Lawrence River, Quebec (Dadswell, 2006). Both of these fisheries appear to be operating at sustainable levels due to size restrictions and harvest quotas (DFO, 2009; Verreault & Trencia, 2011). In the U. S., four of the five distinct population segments (DPSs) for Atlantic sturgeon are listed as endangered by the Endangered Species Act (ESA), and one (Gulf of Maine DPS) is listed as threatened (NOAA [National Oceanic and Atmospheric Administration], 2012).

Spawning populations of Atlantic sturgeon range from the St. Lawrence River in Quebec to the Satilla River in Georgia. Today 15–20 rivers host successfully reproducing populations of Atlantic sturgeon (Wirgin, Maceda, Grunwald, & King, 2015) compared to

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25–30 rivers historically (ASSRT [Atlantic Sturgeon Status Review Team], 2007). Reproductive isolation among populations is strong, resulting in significant genetic discontinuities among all population pairs (Grunwald, Maceda, Waldman, Stabile, & Wirgin, 2008; King, Lubinski, & Spidle, 2001). Juvenile Atlantic sturgeon are resident within their natal estuaries for 2–10 years (Dadswell, 2006) after which they migrate as subadults into coastal waters (Wirgin et al., 2015) and non-natal estuaries (Waldman et al., 2013) for a decade or longer until they return to spawn as adults in their natal rivers at advanced ages. Their migratory life history makes Atlantic sturgeon susceptible to threats in both marine and fresh estuarine environments.

Atlantic sturgeon from both Canadian and U. S. spawning stocks as distant as the Hudson River, New York (Wirgin et al., 2012) migrate to and mix in Minas Basin, part of the inner Bay of Fundy, to feed (McLean, Dadswell, & Stokesbury, 2013) from May through October (Dadswell, 2006). Atlantic sturgeon have also been reported from regions adjacent to Minas Basin including Scot's Bay (Wehrell, Dadswell, & Redden, 2008; Figure 1) and Chignecto Bay (Pearson, Gingras, Armitage, & Pemberton, 2007). Earlier DNA analyses showed that the mixed aggregation of Atlantic sturgeon migrating annually into Minas Basin is comprised primarily of individuals from the Saint John River, (>60% of aggregation) and the Kennebec River, Maine (34%–36%), with small contributions from the Hudson River (1%–2%; Wirgin et al., 2012). Therefore, sources of mortality occurring in the inner Bay of Fundy will likely impact individuals in both Canadian and U. S. populations.

In this study we investigated a large stranding event of Atlantic sturgeon in Scot's Bay, located west of Minas Basin. The mortality event provided an opportunity to collect new information on stock composition and to determine, for the first time, the gonad development stages of Atlantic sturgeon while still in their marine life history phase. To evaluate the impact of the mortality event on specific stocks, individuals were genetically assigned to their population of origin, based on their microsatellite DNA genotypes and mitochondrial DNA haplotypes. We assessed the sex, maturity, and age for each Atlantic sturgeon and documented stranding as a component of their natural mortality.

2 | MATERIALS AND METHODS

2.1 | Study site

The Bay of Fundy is a macro-tidal environment of uneven semi-diurnal tides with a tidal amplitude that often exceeds 15 m. Scot's Bay is part of the inner Bay of Fundy where 1–2 km of intertidal sand flats become exposed at low tide. When the tide recedes over this shallow, sloping intertidal zone, the flow rate is high and non-turbulent.

2.2 | Sample-collection and processing

On 22 June 2014 researchers at Acadia University were contacted by a local naturalist who had found 11 dead Atlantic sturgeon on a beach in Scot's Bay, Nova Scotia. All Atlantic sturgeon were stranded within a 30 m stretch of shoreline. The fish were examined for signs of external damage, including those indicative of being caught in fishing gear, which are commonly found on fish taken as bycatch in trawl fisheries (Beardsall et al., 2013).

Microsatellite DNA and mitochondrial DNA control-region sequence analyses were used to determine the population of origin of each fish. Eleven informative microsatellite loci were screened and 205 base pairs of the mitochondrial DNA (mtDNA) control region were sequenced in these samples. DNA samples were obtained from fin clips taken from the deceased specimens that were preserved in 95% ethanol prior to shipping to the New York University laboratory. DNA isolations, mtDNA sequencing and microsatellite DNA sequencing were conducted exactly as previously described (Wirgin et al., 2015).

Genotypes and haplotype frequencies in the samples of these deceased *A. oxyrinchus* Atlantic sturgeon were compared to those from a reference collection set of 1,295 juveniles (<50 cm TL) and adults (>130 cm TL) collected from 11 spawning rivers coast-wide ranging from the St. Lawrence River, Quebec to the Altamaha River, Georgia (Table 1). Leave-one-out tests, as implemented in ONCOR (Kalinowski, Manlove, & Taper, 2008; ONCOR: Software for genetic stock identification. Available at: http://www.montana.edu/kalinowski/Software.htm), were used to determine how well reference specimens could be assigned to the population from which they were collected. Sequentially removing one fish at a time from a reference population and then using the remainder of the reference specimens in that population to estimate its population origin were used to accomplish this. The specimens that had been misassigned to their collection population were then assigned with the highest probability to an alternative reference collection. The Individual Based Assignment testing in ONCOR was then used to assign the 11 deceased specimens of unknown origin to the reference population that would have the highest probability of producing their given genotypes/haplotypes.

Atlantic sturgeon were measured for total length (TL) and pectoral fin spines were removed and dried for a month, and then sectioned (0.4 mm thick) for age determination by counting annuli under transmitted light (Stewart et al., 2015). Sex and maturity stage were determined by cutting three samples of gonad, which were fixed in Bouin's Fluid and embedded in paraffin. Samples were sectioned with

FIGURE 1 Eleven stranded dead Atlantic sturgeon (*Acipenser oxyrinchus*) lined up for sampling in June 2014, at Scot's Bay, Nova Scotia

TABLE 1 Proportion of Atlantic sturgeon (*Acipenser oxyrinchus*) specimens correctly assigned to the reference population in which they were collected and the population to which individuals were most likely misassigned as determined by using Individual Based Assignment testing implemented in ONCOR

Reference Populations	*N*	% Correct Classified	Population w/Largest Misclassification	% Misassigned to the population
St. Lawrence River	49	95.9	Saint John	2.0
Saint John River	223	97.8	Kennebec	1.3
Kennebec River	41	85.4	Saint John	4.9
Hudson River	216	90.3	Delaware	6.5
Delaware River	107	85.0	Hudson	14.0
James River	113	91.2	Altamaha	2.7
Albemarle Sound	82	85.4	Altamaha	4.9
Edisto River	94	91.5	Altamaha	4.3
Savannah River	99	71.7	Altamaha	15.2
Ogeechee River	135	71.1	Edisto	13.3
Altamaha River	136	78.7	Savannah	12.5

a rotary microtome at 10 μm. Each tissue section was placed on a poly-L-lysine coated slide, and floated with reverse osmosis water. Slides were placed on the slide warmer (37°) until dry (approximately 24 hr). Hematoxylin & Eosin (H&E), Gomori Trichrome, Orcein, Periodic Acid Schiff (PAS) and Acridine Orange were used for staining. The slides were analyzed via histological observation in terms of sex and gonadal features (Van Eenennaam et al., 1996).

3 | RESULTS

Eleven dead Atlantic sturgeon were found on the southern end of the Scot's Bay beach (N 45 17.750; W 64 24.051), during low tide on 22 June 2014. There were no signs of entanglement or any other physical damage (Figure 1). The fish appeared to be robust and were free of any signs of disease or high levels of parasites. Therefore, we suggest that the sturgeon died of natural causes, most likely by stranding. Local people have brought us Atlantic sturgeon specimens in other years (one in 2011, two in 2012), suggesting that Atlantic sturgeon stranding events may occur annually in Scot's Bay, but generally only include a few sturgeon.

3.1 | DNA analyses

Mean accuracy of assignments of specimens to the reference river in which they were collected using Individual Based Assignment (IBA) testing was 85.8%, but varied greatly among populations (Table 1). Assignment accuracy to the Saint John River was 97.8% with most misassignments occurring for the proximal Kennebec River, Gulf of Maine DPS (1.3%). In contrast, the greatest misassignment was seen in rivers within the U.S. South Atlantic DPS, particularly the Savannah and Ogeechee rivers where correct assignment was 71.7% and 71.1%, respectively. In total, correct assignment was much higher for populations in the northern portion of the species' range, particularly in the Canadian management unit, than to populations in the U.S. South Atlantic DPS.

Baseline mtDNA and microsatellite DNA data on the 11 reference populations were used in IBA testing to assign the population origin of

TABLE 2 Summary of Atlantic sturgeon (*Acipenser oxyrinchus; n* = 11) found in Scot's Bay in June 2014 describing origin, sex, stage of oogenesis (females) or spermatogenesis (males), total length (cm), and age (years)

Origin	Sex	Stage	Total Length (cm)	Age (years)
Saint John	*F*	Small and Stage 2 oocytes	137	19
Saint John	*F*	Small and Stage 2 oocytes	146	24
Saint John	*M*	Early Stage 1	150	23
Saint John	*M*	Early Stage 1	187	31
Kennebec	*M*	Early Stage 1	134	17
Kennebec	*F*	Small and Stage 2 oocytes	136	20
Kennebec	Unknown	N/A	138	24
Kennebec	*F*	Stage 2	140	24
Kennebec	*F*	Small and Stage 2 oocytes	145	22
Kennebec	*F*	Stage 2	161	24
Kennebec	*F*	Stage 2	181	28

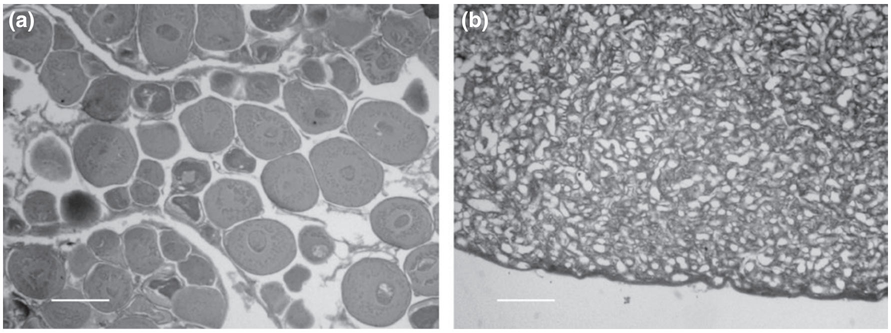

FIGURE 2 Bright field micrographs of gonads of Atlantic sturgeon (*Acipenser oxyrinchus*). a) Section of an ovary showing oocytes at stage 2 oogenesis (Hematoxylin and Eosin). b) Section of a testis showing stage 1 spermatogenesis (PAS); scale bar = 200 μm

the 11 deceased specimens from the inner Bay of Fundy. IBA testing indicated that four of the deceased specimens were from the Saint John River population and seven were from the Kennebec River population (Table 2). Assignment probability for all 11 specimens was 100%.

3.2 | Length and age

Total lengths of Atlantic sturgeon from the Saint John River (n = 4, $\bar{x}$ = 155.0 cm, *SD* = 22.0, minimum = 137 cm, maximum = 187 cm) and from the Kennebec River (n = 7, $\bar{x}$ = 147.9, *SD* = 17.2, minimum = 136 cm, maximum = 181 cm) were not significantly different (*t* test, t_9 = 2.262, p = .281). Atlantic sturgeon display sexually dimorphic growth (Stewart et al., 2015), however, the low number of samples did not allow us to test for significance in growth rates between males and females from the two populations. Ages of Atlantic sturgeon from the Saint John River (n = 4, $\bar{x}$ = 24.3 years, *SD* = 5.0, range = 12) and from the Kennebec River (n = 7, $\bar{x}$ = 22.7 years, *SD* = 3.5, range = 11) were not significantly different (*t* test, t_9 = 2.262, p = .280).

3.3 | Sex and maturity

The two female Atlantic sturgeon from the Saint John River and two from the Kennebec River displayed small and stage 2 oocytes (Table 2; Figure 2). Three females from the Kennebec River displayed stage 2 oocytes (Table 2). All female Atlantic sturgeon from both populations <146 cm TL had small and stage 2 oocytes, except for a 140 cm TL female from the Kennebec that had stage 2 oocytes. Both females >146 cm TL displayed stage 2 oocytes. All male Atlantic sturgeon sampled (2 from the Saint John River and 1 from the Kennebec River) had gonads in early stage 1 spermatogenesis (Table 2).

4 | DISCUSSION

Determining stranding rates is critical because they form a component of annual mortality (Nagrodski, Raby, Hasler, Taylor, & Cooke, 2012). It is particularly important when a species is of conservation concern. Here we report stranding as a component of natural mortality for Atlantic sturgeon from a COSEWIC designated threatened population in Canada and an ESA listed threatened DPS in the United States. Stranding is a source of mortality for a variety of both marine mammals and fishes, although formal reports of marine mammal stranding events are more common. For sturgeons, these stranding events may be natural (Parauka et al., 2011), or anthropogenic (Thomas et al., 2013). Most records of sturgeon stranding events occur in river systems, due to either natural seasonal water level changes, or changes caused by dams and flood diversions (Thomas et al., 2013). In 2004, Hurricane Ivan caused the stranding of Gulf Sturgeon in the Escambia River, Florida and nearby bays (Parauka et al., 2011). This, toether with our observations, suggests that Atlantic sturgeon and their close relatives may be naturally susceptible to stranding in various habitats. Atlantic sturgeon feed in shallow water of the intertidal zone of Minas Basin during high tide, traveling with the tide to maximize time spent on the submerged tide flats (McLean, Simpfendorfer, Heupel, Dadswell, & Stokesbury, 2014). This behavior may put them at risk of stranding as the tide recedes.

Fishers and other residents around Minas Basin and Scot's Bay have commented on finding or have brought us Atlantic sturgeon found dead on the shores of tidal estuaries and beaches; however, it is probable that these events are seldom formally reported. The shoreline of Minas Basin is not densely populated. Stranding events may be more frequent than indicated by the number of formal reports. Based on our results, natural stranding events in the inner Bay of Fundy are contributing to the natural mortality of Atlantic sturgeon populations along the east coast of North America, particularly for individuals from the Saint John River and Kennebec River populations.

The Atlantic sturgeon spawning population in the Saint John River has most recently been estimated at 1,000–3,000 adults per year (Bradford et al., 2016). As the males spawn approx. every 2 to 3 years and females spawn every 3–5 years (Dadswell, 2006), the overall spawning population is likely much larger than 1,000–3,000 adults. The Atlantic sturgeon spawning population in the Kennebec River has not been calculated, however, more than 570 fish have been captured and marked in recent years, indicating that the total population exceeds this number (Stokesbury, Stokesbury, Balazik, & Dadswell, 2014). Implications of sturgeon stranding events on population abundance have been investigated for green sturgeon populations in California, U. S. (Thomas et al., 2013). It was determined that an individual stranding event was not likely to have an effect at the population level, although reoccurring events at times of low abundance might (Thomas et al., 2013). As the Saint John River and Kennebec River populations of Atlantic sturgeon consist of several hundreds to thousands of adults, this single stranding event would likely have little consequence at the population level. Regardless, documenting stranding for Atlantic sturgeon is important for the proper estimation of the natural mortality rate.

Assignments of the 11 deceased inner Bay of Fundy specimens to their correct population of origin were dependent on the assignment accuracies of specimens to our 11 reference collections. Assignment accuracies of individual Atlantic sturgeon to their population of origin varied among populations, but were much higher at the northern compared to the southern limits of their distribution. This was particularly true for the Saint John River and St. Lawrence River populations, within the Canadian management unit, where assignment accuracies exceeded 95%. Specifically, for the Saint John River collection of 223 adult specimens, all of which were adults, only five specimens were misassigned, and of these three were misassigned to the Kennebec River. Our assignment accuracy to the Kennebec River population was substantially lower at 85.4%; the greatest number of misassigned specimens was to the Saint John River (4.9%). While our Saint John River reference collection was robust in size and collected over a greater than 20-year period, our collection from the Kennebec River was less so, and only collected in a single year. Nonetheless, given that seven of the 11 stranded specimens assigned to the Kennebec River and their assignment probabilities were all 100%, we feel confident in stating that the majority of stranded specimens were of U.S origin and most likely were all from the Gulf of Maine DPS.

Total lengths of Atlantic sturgeon from the Saint John River and the Kennebec River were not significantly different. A latitudinal trend in growth rates of Atlantic sturgeon demonstrates that individuals from more southern populations grow more rapidly than northern individuals (Stewart et al., 2015). As such, Atlantic sturgeon from the Saint John River should have a slower growth rate than sturgeon from the Kennebec River. This trend might have emerged if greater numbers of sturgeon had been examined.

We found no population level differences for size or age, however, the histological results do provide us with evidence of the reproductive stage of Atlantic sturgeon while at sea in this region. Based on histological analysis, the Atlantic sturgeon were likely just beginning to prepare for their first spawning event, or they were feeding between spawning events. All female Atlantic sturgeon were at or near stage 2 of oogenesis.

In this study we described for the first time a stranding event for Atlantic sturgeon and noted that this is a component of their natural mortality. In addition, we obtained and analyzed samples that provide information on stock structure, maturity and growth that would normally require sacrificing the fish. We recommend that biologists monitor and report sturgeon strandings and use these unfortunate events to gather critical biological information from sturgeon in their poorly understood marine phase.

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