OERA, NRCan and the Nova Scotia Department of Energy – Technology Research and Innovation
to Support the Canadian In-Stream Tidal Energy Sector

Quantifying fish-turbine interactions using VEMCO's new high residency acoustic electronic tagging technology

Project start date and reporting period covered by the report:

November 2017 to December, 2019

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FINAL REPORT 2019

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Executive Summary:

The lack of credible scientific data on the potential effects of instream tidal power is delaying the decision-making process on a technology that shows promise for reducing carbon emissions, and for which Canada could become a global leader in the production of infrastructure. It remains unclear if fishes that occupy Canada's leading tidal energy test site will be negatively affected by turbine installations. Detection of effects on individuals are critical for listed endangered species (i.e. inner Bay of Fundy [iBoF] Atlantic Salmon) where the loss of one individual may have a negative impact on the population. Other more abundant fishes such as Alewife, Striped Bass and Atlantic Sturgeon need impacts evaluated at the population level, as losses of a few individuals are not likely to have a negative effect at the population level.

We used new, innovative Nova Scotian produced High Residency fish tracking technology from VEMCO to determine the spatial and temporal overlap, and interactions of Atlantic Salmon (two life-stages, smolts and kelts, Species At Risk Act [SARA] listed "endangered" iBoF population), Alewife, Striped Bass (COSEWIC designated "Endangered"; COSEWIC 2010) and Atlantic Sturgeon (COSEWIC designated "Threatened"; COSEWIC 2011), with the FORCE test site in Minas Passage.

The main objectives of this study, in stepwise order, were to:

1. Compare the detection efficiency of traditional acoustic tagging technology (coded 69 kHz) versus new HR technology (180 kHz) in Minas Passage where flows and acoustic noise are high;
2. Test and refine a surgical tag implantation procedure for Alewife, as we did not have prior knowledge of post-tagging survival;
3. Determine temporal movements of tagged fishes within the Minas Passage and FORCE test site area using new high residency acoustic tagging technology developed by a Nova Scotia company, VEMCO (Bedford, NS);
4. Assess potential risk of fish-turbine overlap at the FORCE test site

Compared to other work that has been done using acoustic tagging technology in Minas Passage, we were able to collect considerably more information because of the use of the new HR tags and receivers. The benefit of using this technology is that a single ping at 170 kHz from the tag is required to be heard by the receiver to decode a tags unique ID, and the signal is emitted every 1.5 seconds. To decode fish IDs from traditional coded tags a string of 8 or 10 pings needed to be heard. This provides much more opportunity to detect fish moving at high current speeds. Through drifter surveys conducted within Minas Passage (See Appendix B and D), the 170 kHz signal has been shown to be less impacted by background noise when current speeds exceed 2m/s (Stokesbury et al. 2016). This is one of the first studies utilizing this technology to monitor fish movement in high flow environments. The information obtained from HR receivers at the FORCE site has enabled us to predict the likelihood of fish facing temporal and spatial overlap with tidal turbine sites.

Alewife surgical methods were developed in this study. Using a combination of small tags, a lateral tag insertion, and the use of flow-through anesthetic, we demonstrated a survival rate of at least 97% of the tagged individuals to the following day. Furthermore, internal examination revealed few instances of physical damage, with the primary observed injury being the puncture of gonads in ripe individuals. We recommend the use of this tagging method in future tracking studies of Alewife.

During 2018-2019, acoustic transmitters were deployed to detect animal movements and behaviour in Minas Passage. Detection data from a total of 399 transmitters were used in the analysis for this report. VEMCO hydro-acoustic receivers provided autonomous, passive, single-channel, omnidirectional detection of the acoustic transmitters when they were within range. The Ocean Tracking Network (OTN) centred at Dalhousie University placed receivers in a line across Minas Passage (5 km wide) at approximately 400 m intervals. FORCE placed receivers in and close to the FORCE test site to detect the presence of acoustically tagged fishes as they moved within the region.

Fish were surgically implanted with V9, V13 or V16 tags, depending on their size. Some individuals from larger bodied species (e.g., Atlantic Salmon kelts, Striped Bass and, Atlantic

Sturgeon) were double-tagged with traditional coded 69 kHz tags and the new HR 170 kHz and 180 kHz tags so that comparisons of detection efficiency at the FORCE test site could be made. Some of the larger coded tags included pressure sensors to measure fish swimming depth. Atlantic Salmon and Alewife were tagged in the Gaspereau River. Striped Bass were tagged in the Stewiacke River and Atlantic Sturgeon were tagged in Minas Basin.

Results show that the FORCE test site and Minas Passage are used for migration and other movements by tagged fish that were part of this project. Also, tagged fishes from other tagging projects logged on to receivers at both locations (e.g., Atlantic Sturgeon from endangered and threatened populations in the US, White Sharks, etc.).

During 2018 and 2019, 77% (n = 41) and 31% (n = 23) of Atlantic Sturgeon tagged in Minas Basin were detected at the FORCE test site, respectively. Tagged Atlantic Sturgeon travelled pelagically through the FORCE test site and Minas Passage at similar depths to those proposed for tidal turbine operation. There was a decline in the presence of Atlantic Sturgeon at the FORCE test site during flood tide, and a decline in their presence at the OTN line in Minas Passage with increases in current speed. The distance between the FORCE test site and Minas Passage is approximately 3km. Given the estimated abundance of the summer feeding aggregation of Atlantic Sturgeon that visit Minas Basin each summer (Dadswell et al. 2016), our study indicated that at current population estimates at least ~ 2790 unique individuals may overlap with the FORCE test site annually.

During 2018 and 2019, 53% (n = 15) and 6% (n = 49) of Striped Bass tagged in the Stewiacke River, NS were detected at receivers in and close to the FORCE test site. Striped Bass were detected during a total of 77 and 55 days at the FORCE test site throughout 2018 and 2019, respectively. In 2019 the OTN line of receivers suffered malfunctions in their acoustic releases and were not in place for most of the year. Due to the low number of Striped Bass detections during 2019, only 2018 data were analyzed. During 2018, tagged Striped Bass were detected during all months of receiver deployment, with most frequent Striped Bass detections occurring during July (n = 18, 23%) and December (n = 15, 19%).This result provides further evidence that Striped Bass overwinter within Minas Passage as was described in Keyser et al. (2016).

Additionally, consistent with analyses of the behaviour of other fish species at FORCE, the highest proportion of Striped Bass days occurred at the S2 site (2018: n = 47, 61%).

Striped Bass, especially large bass (>60 cm), spent more time in the Minas Passage and near the FORCE test area than any of the other fish species examined. Residency spanned summer, fall and winter. Of the 165 tagged Striped Bass, 52 swam through the FORCE tidal turbine test site in Minas Passage, and many at depths of proposed turbine hub height. Striped bass were detected mostly in the top 40 m of the water column and were located closer to the surface during the night. Maximum travel rate (tide assisted) across Minas Passage was 4.0 m/s. Many tagged Striped Bass moved within Minas Passage throughout the winter months when water temperatures was in the range of 0-3°C. At these temperatures, Striped Bass are expected to have reduced metabolic rates (i.e. sluggish) and may have limited abilities to detect and avoid turbine infrastructure. This species makes near year-round use of the passage, including the FORCE test site during winter, and may be at considerable risk of interaction with turbines.

During 2018 and 2019, 7% (n = 4) and 48% (n = 42) of Atlantic Salmon smolts tagged in the Gaspereau River, NS, were detected at receivers deployed in and close to the FORCE test site. 307 detections occurred during May 15th to June 4th, 2018, with 70% (n = 217) occurring during the month of May. In 2019, 5794 detections of Salmon smolts occurred between May 11th to June 9th, with most detections occurring in the month of May. Consistent with analyses of the behaviour of other fish species at the FORCE site, the highest proportion of Salmon smolts detections occurred at the S2 site for both years (n = 3, 50%; n = 21, 91%).

During 2019, 76% (n = 19) of Atlantic Salmon kelts tagged in the Gaspereau River, NS, were detected at receivers deployed in and close to the FORCE test site. Kelts were detected for a maximum of 53 days at the test site, although detection frequency varied. The site with the highest number of detections and individual kelts was S2, which is closest to the centre of the passage. The average number of days a kelt was detected in the region was 6 days. Mostly Kelts were detected in May, but detections were also made in June, July and August. Sixteen of the 19 kelts detected at the FORCE test site in 2019 also had recorded detections of swim depth (depth from surface). Kelts spent most of their time (94%) in the upper portion (0 to 20 m) of the water column with swim depths averaging (± SEM) 4.33 ± 0.45 m across all three sites. Three Kelts made deep dives greater than 20 m (max swim depth = 48.51 m; average ± SEM = 32.31 ± 1.97 m) at the S2 site. For two of these kelts, the dives were a single occurrence and lasted < 5 min. For the other kelt, dives to depths >20 m occurred on three separate occasions in May and again in August of 2019. These data provide evidence that Atlantic Salmon overlap with the area of the FORCE test site during their sea-bound migration and spend considerable time in the region.

During 2019, 41% (n = 31) of Alewife tagged in the Gaspereau River, NS, were detected at receivers deployed in and close to the FORCE test site. Alewife were detected during a total of 27 days between July 7 to August 12, with most unique detections occurring on July 12 (n = 9). On average, most individuals were detected at the FORCE test site on a single day, with some being detected on 2-3 consecutive days, and one Alewife being detected on 6 separate days. Additionally, consistent with analyses of the behaviour of other fish species at the FORCE site, the highest proportion of Alewife detections occurred at the S2 site (n = 15, 56%). These data provide evidence that Alewife overlap with the area of the FORCE test site during their annual sea-bound migration.

Currently there are no operating turbines within Minas Passage, so the hypothesis that fish avoid operating turbines remains untested. Although the FORCE site represents a relatively small area within Minas Passage (<20% of the passage width), we still don’t know how well migratory fish can control their movements and avoid structures, especially when travelling at times of peak current speed. Our drifter surveys in the Passage detected Atlantic Sturgeon and Atlantic Salmon kelts that were moving with the current. The likelihood of fish-turbine encounters varies among species and may increase with subsequent increases in the number of turbines within the passage (greater cross-sectional area to avoid). The tested HR technology proved to be effective in Minas Passage and we suggest future work includes the combined use of fine-scale arrays and video surveillance around active turbines so that fish-turbine interactions and fish behaviour (e.g., avoidance) can be identified.

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Section 1: Project Introduction

Mortalities have been reported for Atlantic Sturgeon (*Acipenser oxyrinchus oxyrinchus*), Striped Bass (*Morone saxatilis*), and Alewife (*Alosa pseudoharengus*) passing through barrage style hydroelectric facilities (Dadswell et al. 2018). However, it is unknown whether the same is true for fishes migrating through regions with free standing Hydro Kinetic (HK) devices. The FORCE test site serves as a prime location to conduct baseline monitoring to better understand whether HK's pose a risk to local fauna. Currently only a single HK device is being tested at FORCE, taking up 0.02% of the cross-sectional area of the passage (Redden et al. 2014) however this device in non-operational. In this project, we investigated fish overlap with the FORCE test site to provide information critical to informing the proper calculation of effects at the individual (Atlantic Salmon, *Salmo salar*) and population (Atlantic Sturgeon, Striped Bass and Alewife) level. The primary objective of this project was to test new innovative Nova Scotia produced High Residency tracking technology to fill knowledge gaps regarding in-stream turbine encounter (spatial and temporal overlap) and if possible avoidance of operating turbines by commercial and iconic species of fishes that move through Minas Passage. This information is critical for regulators, such as Fisheries and Oceans Canada (DFO) and the Department of the Environment (DOE), who must weigh the environmental costs and benefits of tidal power development.

Bay of Fundy Migratory Fishes

Seventy-one species of fishes have been reported to move through Minas Passage into Minas Basin on a seasonal basis. In a recent study documenting the catch at an intertidal brush weir in 2017 researchers reported that 45 species of fishes were captured (Dadswell et al. 2018). The researchers reported four assemblages of fishes present: 1) Mostly small bodied fishes that are present in Minas Basin year round (i.e. Tomcod, *Microgadus tomcod*, Rainbow Smelt, *Osmerus mordax*, Atlantic Silverside, *Menidia menidia*, Smooth Flounder, *Pleuronectes putnami*, Windowpane Flounder, *Scophthalmus aquosus*, Mummichog, *Fundulus heteroclitus*, Skate, *Raja spp.*, Striped Bass, Sea Raven, *Hemitripterus americanus*, and Three Spined Stickleback, *Gasterosteus aculeatus*); 2) summer migrants into Minas Basin (Sea Lamprey, *Petromyzon marinus*, Spiny Dogfish Shark, *Squalus acathias*, Atlantic Sturgeon, American Eel, *Anguilla* *rostrata*, American Shad, *Alosa sapidissima*, Alewife, Atlantic Menhaden, *Brevoortia tyrannus*, Atlantic Salmon, Monkfish, *Lophius americanus*, Butterfish, *Pampus spp.*, Silver Hake, *Merluccius bilinearis*, Atlantic Mackerel, *Scomber scombrus*, and Winter Flounder, *Pseudopleuronectes americanus*); 3) Cold water fishes that enter the basin in early spring and leave when the water temperature approaches 10 ℃ (i.e. Atlantic Cod, *Gadus morhua*, Haddock, *Melanogrammus aeglefinus*, Pollock, *Pollachius spp*., Halibut, *Hippoglossus hippoglossus*, Ocean Pout, *Zoarces americanus*, Spotted Hake, *Urophycis regia*, and Longfin Hake, *Phycis chesteri*); and 4) rare warm water migrants (i.e. Summer Flounder, *Paralichthyes dentatus*, and Striped Searobin, *Prionotus evolans*) (Dadswell et al. 2018).

Prior Tracking Studies in Minas Passage

There have been several tracking studies aimed at understanding fish movement through Minas Passage and at the FORCE test site. A pilot study conducted in 2011-2012 focused on developing fish tracking techniques for collecting baseline data on the summer and fall movements of Striped Bass, Atlantic Sturgeon, and American Eel (Stokesbury et al. 2012). The primary recommendations from the pilot study included: an extension of the baseline study to include multi-year comparisons of use and movement through Minas Passage, to include tagging and tracking of endangered Atlantic Salmon, and to examine the movement patterns of fish after the installation of an active tidal turbine (then slated for deployment in 2012).

From 2011 – 2013, a large-scale study built on the recommendations made by Stokesbury et al. (2012), by tagging and tracking Atlantic Sturgeon, Striped Bass, American Eel, and Atlantic Salmon Smolts through Minas Passage and at the FORCE test site over multiple tracking years (Redden et al. 2014).

The main recommendations from the multi-year tracking study were to:

1. Test the hypothesis that fish avoid swimming in very fast currents (since detection efficiency was too low to test this hypothesis at the time);
2. Use a range of acoustic technologies and applications to examine fish-turbine interaction;
3. Include species of commercial and conservation importance, and periods of high fish traffic in Minas Passage, in future environmental effects monitoring programs

Project Aims

To address the spatial and temporal overlap of migratory fishes with the FORCE test site, we focused on three species from populations of which there are known population estimates: Gaspereau River Alewife, the Minas Basin summer feeding aggregation of Atlantic Sturgeon, and Stewiacke River Striped Bass. In addition, we included endangered inner Bay of Fundy Atlantic Salmon from two life-stages: smolts and kelts, where any individual losses are considered to have an impact at the population level.

Our project objectives were to:

The main objectives of this study, in stepwise order, were to:

1. Compare the detection efficiency of traditional acoustic tagging technology (coded 69 kHz) versus new HR technology (180 kHz) in Minas Passage where flows and acoustic noise are high;
2. Test and refine a surgical tag implantation procedure for Alewife, as we did not have prior knowledge of post-tagging survival;
3. Determine temporal movements of tagged fishes within the Minas Passage and FORCE test site area using new high residency acoustic tagging technology developed by a Nova Scotia company, VEMCO (Bedford, NS);
4. Assess potential risk of fish-turbine overlap at the FORCE test site

At the time of the project commencement, it was anticipated that at least one active turbine would be installed in the passage. This was not the case, so we were unable to address one of our original objectives, which was to:

5. Model fish interaction and avoidance behaviour with an active turbine

Section 2: General Methodology and Results

2.1 Minas Passage Receiver Deployments

FORCE test site

On May 4th, 2018 four co-located VEMCO VR2W- 69 kHz and four HR2-180 kHz HR receivers attached to streamlined subsurface buoys (SUB) connected to moorings with acoustic releases were deployed at the W1, W2, D1 and S2 test sites (Figure 2.1.1; Figure 2.1.1). The bottom depth of the W1, W2, D1 and S2 sites were 57, 58, 44 and 81 m respectively. Receivers were attached to box-section aluminum mounts and mounted onto SUB buoy's (Figure 2.1.2; Sanderson 2018, Appendix A). The HR2 receiver batteries last approximately 6 months and can record background noise (dB), tilt angle, temperature and interpret signals from traditional coded PPM 180 kHz acoustic tags as well as new High Residency (HR) 180 kHz PPM and 170 kHz HR tags. C-PODs were mounted in the center of the SUB buoy as part of FORCE's monitoring program of Harbour Porpoise, *Phocoena phocoena* (Linnaeus, 1758). The last download of data from the receivers occurred on August 14th, 2019. Receivers were retrieved intermittently throughout deployment for battery changes (Figure 2.1.3). Receivers at the FORCE test site were deployed for 309 days in 2018 and 109 days in 2019. Deployment periods occurred during May 4th to August 14th, 2019.

Minas Passage Line, Ocean Tracking Network

The Ocean Tracking Network (OTN) deployed 12 co-located VEMCO VR2W-180 kHz and 69 kHz omnidirectional hydrophone receivers across Minas Passage (MP) seasonally from 2012 to 2018 (Figure 2.1.1). Data used in this study extended from May 4th 2018 to the latest download on November 26th, 2018. VR2W-180 kHz receivers have a battery life of 15 months and a maximum detection range within MP of 300 m during slack tide, which drops to 220 m when current speeds exceed 3 m/s during flood tide (Sanderson et al. 2017). VR2W- 69 kHz receivers have a larger range than 180 kHz receivers (app 500m for VR2 69 kHz compared to 200 m for VR2 180 kHz); during slack tide the range can extend up to 600m however ranges for coded PPM signals are greatly reduced in current speeds > 2 m/s (Broome et al. 2015). The 8th receiver at the MP line was lost upon deployment. Data was unavailable from the MP line during 2019 due to failure of acoustic releases upon deployment.

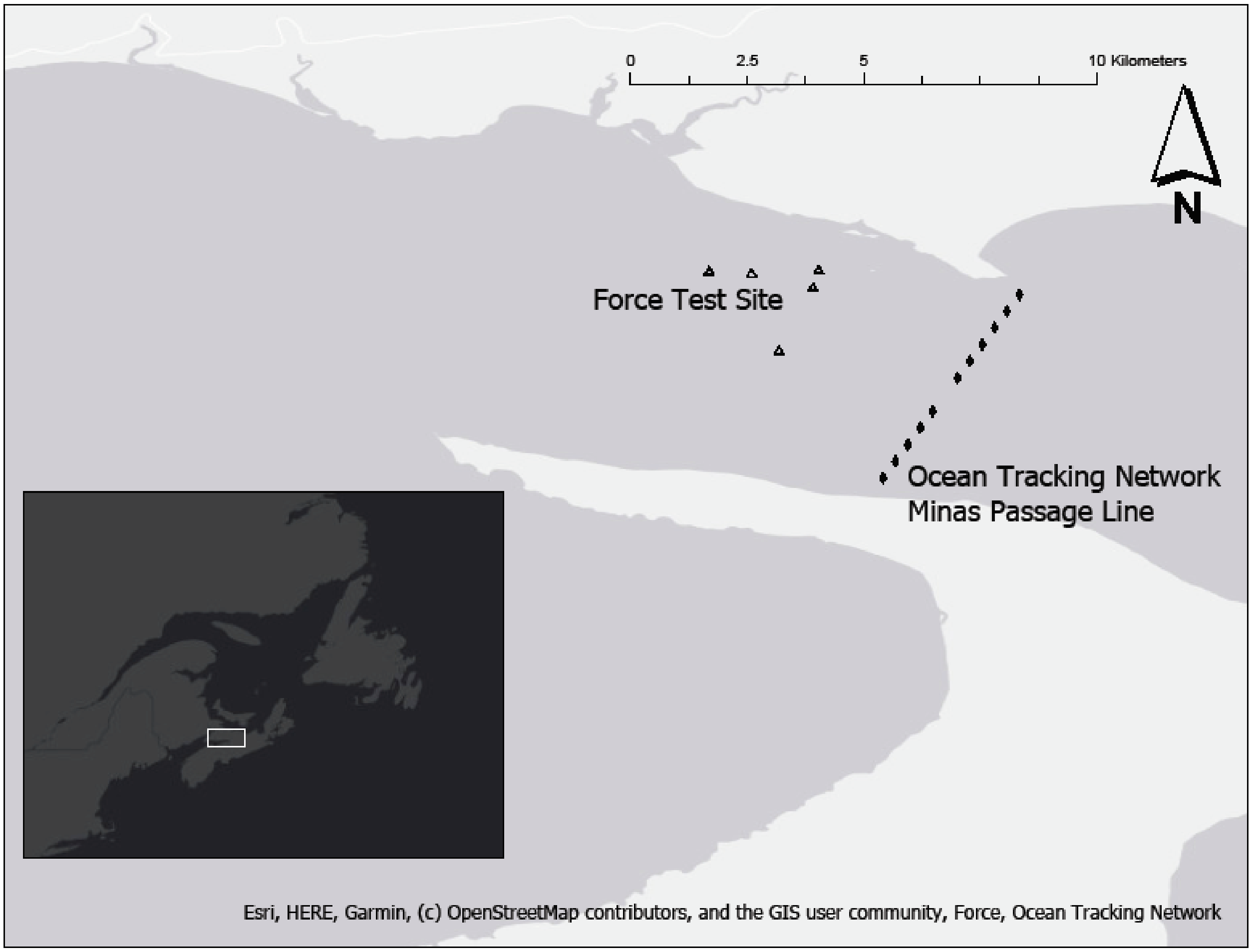

Figure 2.1.1. Location of moored receivers deployed by the Ocean Tracking Network (OTN) and the Fundy Ocean Research Center for Energy (FORCE), respectively, across Minas Passage (2018) and the FORCE test site (2018, 2019).

Figure 2.1.2. Prior to deployment at the Fundy Ocean Research Center for Energy (FORCE) test site VR2W-69kHz and HR2-180kHz receivers were mounted to Streamlined Subsurface Buoys (SUB) buoys by Dr. Brian Sanderson at Acadia University.

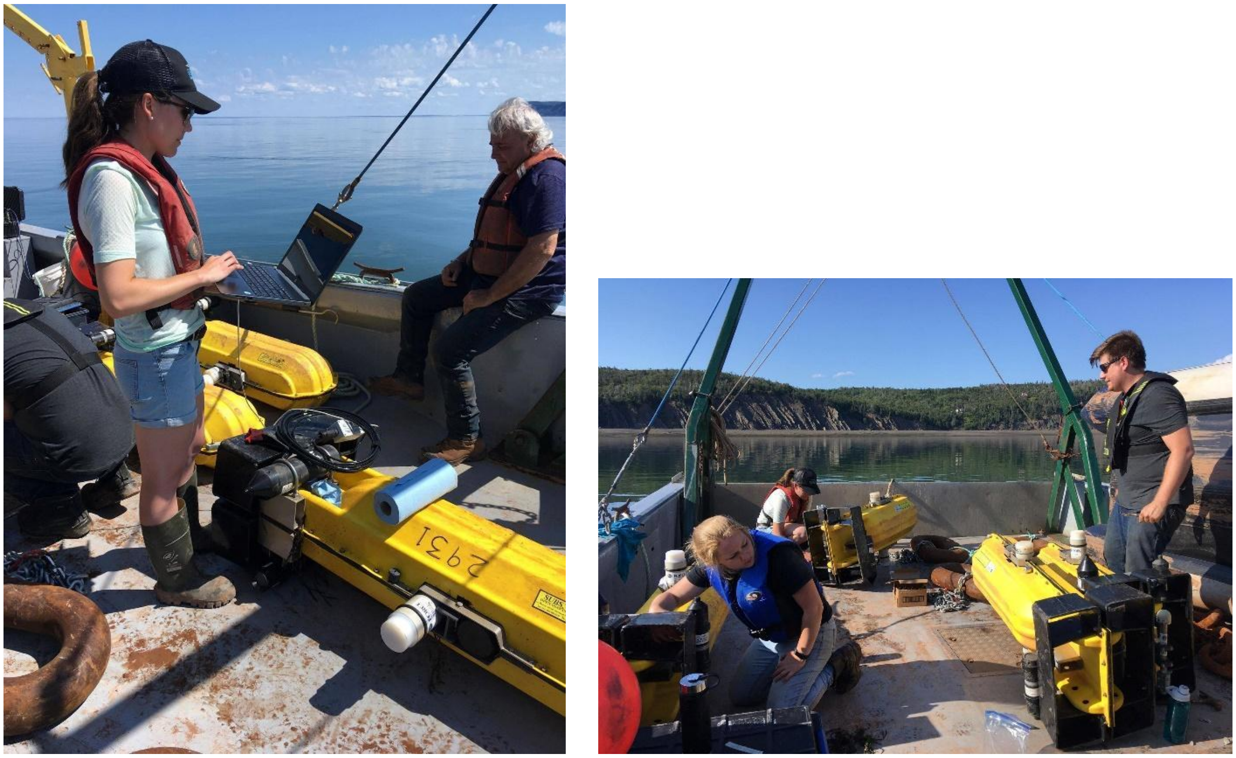

Figure 2.1.3. Jessie Lilly, MSc candidate from Acadia University joined the FORCE crew to download detection data from the four test sites in August 2019.

2.2 Range Tests

The research highlighted in this section is a compilation of a tremendous amount of work led by Dr. Brian Sanderson and colleagues. Detailed reports are found in Appendices B-E and only briefly summarized below. Please refer to the Appendices for additional information and discussion for each section.

Background

There has been, and continues to be, a substantial effort to implant acoustic tags in fish that either migrate through Minas Passage or otherwise inhabit Minas Passage and neighbouring waters. Acoustic receivers continue to be deployed at FORCE and at the Ocean Tracking Network line in Minas Passage as well as many sites in and around Minas Basin. Such receivers detect acoustic tags which establishes the presence of individual tagged fish if it is within range. The range is dependent on several factors, but in Minas Passage, current speeds greatly influence the probability of tag detection (Sanderson and Redden, 2016). Building on previous work, we assessed and tested the range of several tag types within Minas Passage and the Gaspereau River, Minas Basin. This was the first study to test the range of VEMCO's new high residency 170 kHz signals – a tagging technology with promise for better detection efficiency in high current regions.

Methods

Several studies were undertaken from 2018-2019 and summarized in detailed reports in Appendices B-E.

Summaries

Detection Range Testing in Minas Passage June 2018 – Appendix B

Using various transmitters and receivers, we were able to investigate tag detection of 69 kHz PPM, 180 kHz PPM, and 170 kHz HR tags along paths that went from drifter to mooring as well as between drifters within Minas Passage, in order to define the effective area of detection at some time when a tagged fish is detected by a receiver. A drifting 69 kHz PPM tag was well detected when current speed was low but poorly detected at current speeds above 3 m/s.

Calculations show that fast currents might disrupt period encoding by changing the received interval between PPM pulses from that transmitted, and reflections from the sea surface and high ambient sound levels can degrade detection of both 69 kHz PPM signals and 180 kHz PPM signals. In contrast, 170 kHz HR technology shows promise for use in high current speeds. A moored HR2 receiver seems to detect drifting 170 kHz HR tags a little better than drifting 180 kHz PPM tags. A drifting HR2 receiver detects drifting 180 kHz PPM tags with close to the same efficiency as drifting 170 kHz HR tags.

Testing HR Tags at Whiterock – Appendix C

On 19 March 2018 our field team1 tested the reception of an acoustic tag by the HR receiver located in the Gaspereau River, immediately downstream of a hydroelectric dam. Our scientific objective was to measure paths taken by tagged fish after they had passed through the dam or the two bypass pipes and cleared the tail race. Only a few tagged fish were detected by receivers within the array. Unfortunately, there were no instances when the same tag signal was detected by 3 or more receivers. Thus, VPS position finding was not possible and using trajectories to study fish behaviour was not possible.

Drifter with a VR2W-180kHz PPM Receiver, A Pilot Study, Sep-Oct 2019 – Appendix D

Using drifters as an instrument platform platform for a VEMCO VR2W receiver, we conducted a pilot study to detect fish that carry a 180 kHz PPM acoustic tag in Minas Passage. Three sets of quasi-stable trajectories are evident in the present measurements and a fourth is known from previous work. Our drifting VR2W receiver detected a tag 180 kHz PPM tag (50039) that had been implanted into a salmon kelt by ACADIA University in Spring 2019. Four sequences of detection times were obtained. An Atlantic sturgeon was also detected over a 277 s period on 19 Sep 2019 during the ebb tide. A drifter that approximately follows a fast-moving water mass seems to be a better platform than a mooring for detecting an acoustically tagged fish in some circumstances.

Windsor Causeway, Equipment Test, 1-20 Nov 2019 – Appendix E

Vemco 170/180 kHz acoustic tags have been implanted within the body cavities of Tomcod on the ocean side of the Windsor Causeway. These, as well as a range test tag, were used to test the detection efficiency of HR2 receivers placed on ledges of the gates of the Windsor impoundment. Detection efficiency was about 6-7% for 170 kHz HR and about 33-40% for 180 kHz PPM signals. HR signals are messed up by reflections from very nearby surfaces. Thus, to work well on a tidal turbine installation one must take care to position the receiver so that it will not suffer from reflected signals and so that it is also optimally oriented relative to the current. In contrast, PPM signals are not much influenced by reflections from very nearby surfaces. If PPM signals have shorter pulses (say < 1 ms instead of > 6 ms) then they may serve very well for localization under some circumstances. Additionally, the shorter pulses would enable larger pulse amplitude (and more pulses) to be achieved with the same energy (battery).

HR versus PPM Detections from Tagged Atlantic Sturgeon and Striped Bass

We compared the detections of two signals transmitted from tagged Atlantic Sturgeon and Striped Bass at different current speeds in Minas Passage. The V9-2x signal emits an HR-170kHz signal every 5 seconds and a PPM-180kHz signal every 30 seconds. If detections from both signals are equally received than the ratio of detections should equal 6 (30/5). There was not a significant difference between the frequencies of detections versus current speed between the HR-170 and PPM-180kHz signal for both Striped Bass and Sturgeon, respectively (χ^2= 10.30, p = 0.17, d.f. = 7; χ^2= 3.86, p = 0.28, d.f. =3). A large proportion of possible PPM signals were missed at all current speeds; for Striped Bass ratios ranged between 9-27, and for Atlantic Sturgeon ratios ranged between 8-51 (Table 2.1). Ratios appeared to be high regardless of current speed or tidal stage (ebb, flood, slack).

Table 2.2.1. Comparison of the detections of the HR-170 kHz and PPM-180 kHz signal at varying current speeds at the FORCE test site during May 4th to March 29th 2019. This table includes detections of Striped Bass (SB) and Atlantic Sturgeon (AS). The V9-2x tags emit an HR and PPM signal every 5 and 30 seconds respectively. If both signals are detected equally than the ratio between HR to PPM would be 6.

	No. Detections					
Current Speed (m/s)	HR		PPM		Ratio (HR/PPM)	
	SB	AS	SB	AS	SB	AS
-3	53	N/A	3	N/A	18	N/A
-2	161	51	6	1	27	51
-1	463	57	29	7	16	8
0	603	64	40	4	15	16
1	413	N/A	42	N/A	10	N/A
2	105	N/A	12	N/A	9	N/A
3	169	40	13	3	13	13
4	22	N/A	2	N/A	11	N/A

2.3 Validating Methods for Surgical Tag Implantation

Background

The Bay of Fundy is an important site for tidal power development in Atlantic Canada due to its large tidal amplitudes and fast currents. In 2009, the Fundy Ocean Research Centre for Energy (FORCE) created a site to test large-scale tidal turbines in Minas Passage – a narrow channel that flows into the Minas Basin macrotidal estuary at the head of the Bay of Fundy (FORCE 2011). This area is particularly important to migratory species such as the Alewife (*Alosa pseudoharengus*), an anadromous forage fish in the family *Clupeidae*, commonly found in rivers across Maritime Canada. A large population of this species is found in the Black River-Gaspereau River watershed in the Annapolis Valley, Nova Scotia. Alewives typically migrate upstream during May and June, where they are targeted by commercial and recreational fisheries of local economic importance (Gibson and Myers 2001). The escaping fish must then pass two fish ladders, located at generating stations along the river, before reaching Gaspereau Lake where spawning occurs. It is believed that the species undergoes a single spawning event before returning to the marine environment (Collette and Klein-MacPhee 2002).

Overall, biological information about the Gaspereau River stock of Alewife is limited, due in part to a lack of data on passage efficiency at fish ladders, as well as at-sea survival (Gibson and Daborn, 1998). The development of tidal energy in the Minas Passage could pose a new threat to the species, given the potential of fish sustaining injuries or mortality caused by interactions with turbine blades (Dadswell and Rulifson 1994). The likelihood of fish-turbine encounters occurring may vary with fish size, swimming depth, residency, and water temperature, and must therefore be measured *in-situ* for each species of interest. Acoustic telemetry is a promising method that could address this question; this technology allows researchers to track fish with the use of internally implanted acoustic transmitter tags, which can be picked up by receivers in the study area. However, Clupeid fishes are extremely sensitive to handling (Rounsefell and Dahlgren 1933), making the invasive tagging protocol difficult to execute. Therefore, there is a need for developing surgical techniques that will minimize handling stress and ensure a good survival rate for small pelagic fishes. Once an effective tagging method is established, acoustic tracking can be used to investigate the post-spawning migration of Alewife through Minas Basin, in order to estimate residency at the FORCE test site and inform management efforts.

Methods

Ninety-six adult Alewife (52 ripe, 44 spent) were captured between the months of May and June, 2019, from the White Rock fish ladder on the Gaspereau River. Each fish was sexed, weighed and measured before being anesthetized with a buffered solution of methanesulfonate (MS-222) at a concentration of 2 g/10 L for surgery. Dummy (non-transmitting models) of VEMCO V5 acoustic tags were implanted into the intracelomic cavity, 1-2 mm off the mid-ventral line by making an 8-10mm long vertical incision between the ribs (Figure 2.3.1). This method differs from the traditional approach to tagging fish, with the tag being inserted into the ventral side. Incisions were closed with two simple interrupted sutures (Ethicon monofilament nylon sutures, reverse cutting 4–0, 1.5 metric, 45 cm, PS-2 18 mm, 3/8 circle needle), and the fish allowed to recover for 5 minutes in a closed, 100L water tank.

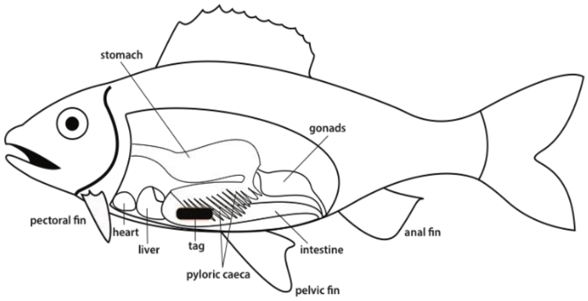

Figure 2.3.1. Position of the intracelomic tag implantation on the right lateral side of the fish, 1-2 mm off the mid-ventral line, with internal anatomy.

After this time, the lid of the tank was removed, and the behavioural condition of the fish was assessed based on reflex impairment. Reflexes assessed included ventilation, orientation, swimming vigour, light response and tactile response, each of which was assigned a score of 0 if non-impaired, and 1 if impaired. Scores were added up to form an individual Reflex Action Mortality Predictor (RAMP) score, which assesses the overall viability of each animal.

Fish were then allowed to recover for 24 hours, after which they were euthanized by anesthetic overdose, and a necropsy health assessment was conducted. Fish were assessed for internal signs of bleeding or hemorrhage, condition of the incision, organ damage or discoloration, and tag position within the body cavity. Each condition was again assigned a score of 0 or 1, and the scores were summed for an average physical impairment score.

Results

Overall, the developed tagging method proved effective in minimizing the effects on fish. Average RAMP and physical impairment scores were both 0.2, with only 4/96 fish experiencing an impairment of all reflexes, and no fish receiving a full impairment score in the necropsies. The most commonly impaired reflex was response to light stimulus (Figure 2.3.2). Fish sex and size had no observable effect on the outcomes of the tagging, however water temperature was found to increase the amount of reflexes impaired, with more impairments seen above 12˚C (Figure 2.3.2).

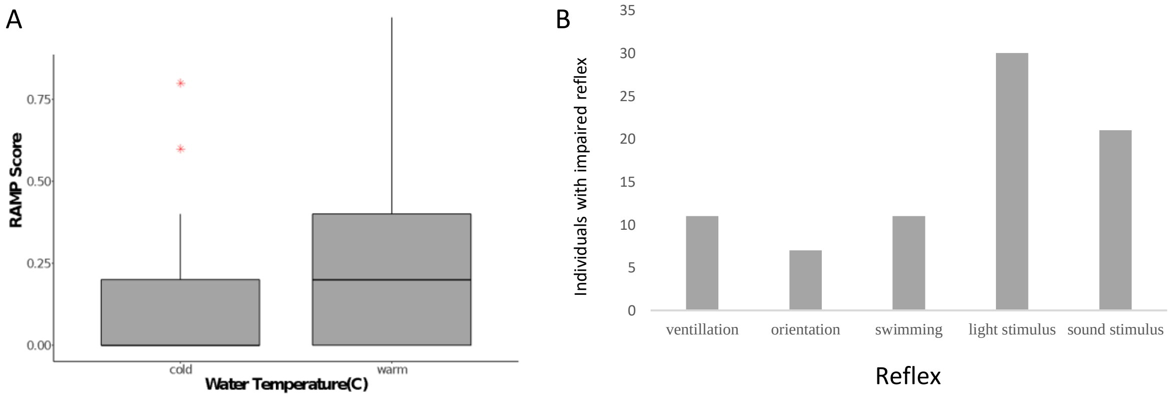

Figure 2.3.2. A) Boxplot showing median, quartile and maximum observed RAMP scores based on water temperature, where water temperature is described as cold between 9˚C to 12˚C, and warm between 14˚C to 18˚C. B) Histogram of the frequency of impairment for the five observed reflexes: ventilation, orientation, swimming vigour, light response and tactile response.

The primary internal injury observed was a puncture of the gonads by the tag, however, this was only observed in pre-spawned fish (Figure 2.3.3). As this study aims to study postspawning migration, the gonads of the tagged fish will be reduced, and this type of injury will be avoided. Some individuals also showed evidence of bleeding around the incision site, however, the damage was not thought to have been significant enough to result in mortality.

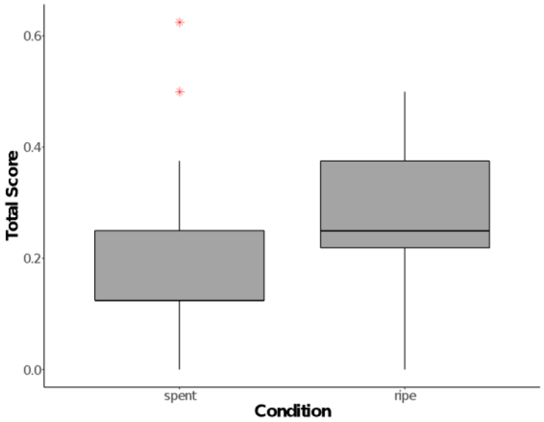

Figure 2.3.3. Boxplot showing median, quartile and maximum observed physical impairment scores in ripe and spent Alewife.

Conclusions

The tagging method developed for this study, which uses a combination of small tags, a lateral tag insertion, and the use of flow-through anesthetic, is an effective way for tagging sensitive Clupeid fishes that minimizes tagging effect and ensures the viability of tagged individuals. Overall, most fish were able to recover full functionality of their reflexes within 5 minutes following tagging, and 97% of the tagged individuals survived into the following day. Furthermore, internal examination revealed few instances of physical damage, with the primary observed injury being the puncture of gonads in ripe individuals. Therefore, we recommend the use of this tagging method in future tracking studies of Alewife.

Primary factors to consider when tagging fish include the physical condition of individuals (ripe vs. spent), as well as water temperature. We found it best to tag post-spawned fish as their gonads are reduced, which allows more space in the body cavity for tag insertion. In addition, it is advisable to conduct tagging earlier in the season, when water temperatures are closer to 10˚C, as this reduces stress.

2.4 Animal Use of Minas Passage/FORCE Test Site

All four species monitored were assessed for their presence at, and use of, Minas Passage, and specifically, the FORCE test site. The following is a brief outline of the methods used to analyze the telemetry data collected during 2018 and 2019.

Summary of Data Analysis

"Individual days" were calculated for each species at each site at FORCE (W1, W2, S2, and D1) during 2018 and 2019 and on the MP line (MPS001-MPS012) during 2018. Individual days were referred to as days when a single specimen was detected (Broome 2014; Keyser et al. 2016). The proportion of individual days at all receivers within the FORCE site was compared to the total possible number of days to determine how frequently tagged animals utilized the FORCE test site during 2018 and 2019. The total number of possible detection days was equal to the duration between the first and last detection of an individual at the FORCE test site and MP line.

Atlantic Sturgeon *Acipenser oxyrinchus oxyrinchus*

Background

From May-October approximately 9,000 sub-adult Atlantic Sturgeon (L_F >150cm F_L, age >26 years) inhabit MB annually to feed on the large abundance of benthic organisms in the intertidal zone (McLean et al. 2013; Dadswell et al. 2016). In 2012, five distinct population segments (DPS) of U.S Atlantic Sturgeon were listed as endangered and one population as threatened by the U.S Fish and Wildlife Service (NOAA 2012a; NOAA 2012b). In Canada two DPS, the SJR and SLR population was designated as threatened by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC 2011). Through genetic analysis of Atlantic Sturgeon captured in Minas Basin, Wirgin et al. (2012) determined that 61% of Atlantic Sturgeon within Minas Basin originated from the SJR, 34% from the Kennebec River (KR), 2% from the Hudson River and 1% from the James River. Atlantic Sturgeon from the SJR and KR are designated as threatened, and sturgeon from the Hudson River and James River are listed as endangered.

Atlantic Sturgeon remain within the basin until October before migrating to overwintering habitats in the Bay of Fundy and along the USA coast (Beardsall et al. 2016; Taylor et al. 2016). They have been shown to migrate through Minas Passage at a depth of approximately 31 m, a similar depth as the proposed HK devices (Stokesbury et al. 2016). The behavior of Atlantic Sturgeon around HK's is unknown. Since Atlantic Sturgeon from both threatened and endangered populations migrate into Minas Basin, it is important to determine whether Atlantic Sturgeon will face temporal and spatial overlap with operating HK's. The objective of this study was to determine spatial and temporal overlap of Atlantic Sturgeon with the FORCE test site.

Methods

Prior to 2019 all fishing was conducted under the Department of Fisheries and Oceans Scientific License to Fish #322595. Fishing conducted during 2019 was registered under license # 330657. Atlantic Sturgeon surgical procedures were performed under Acadia Animal Care Committee protocol #07-11. Detailed methods for the capture and sampling of Atlantic Sturgeon can be found in Appendix F. Capture and release of Atlantic Sturgeon occurred in Minas Basin, NS (Figure 2.4.1).

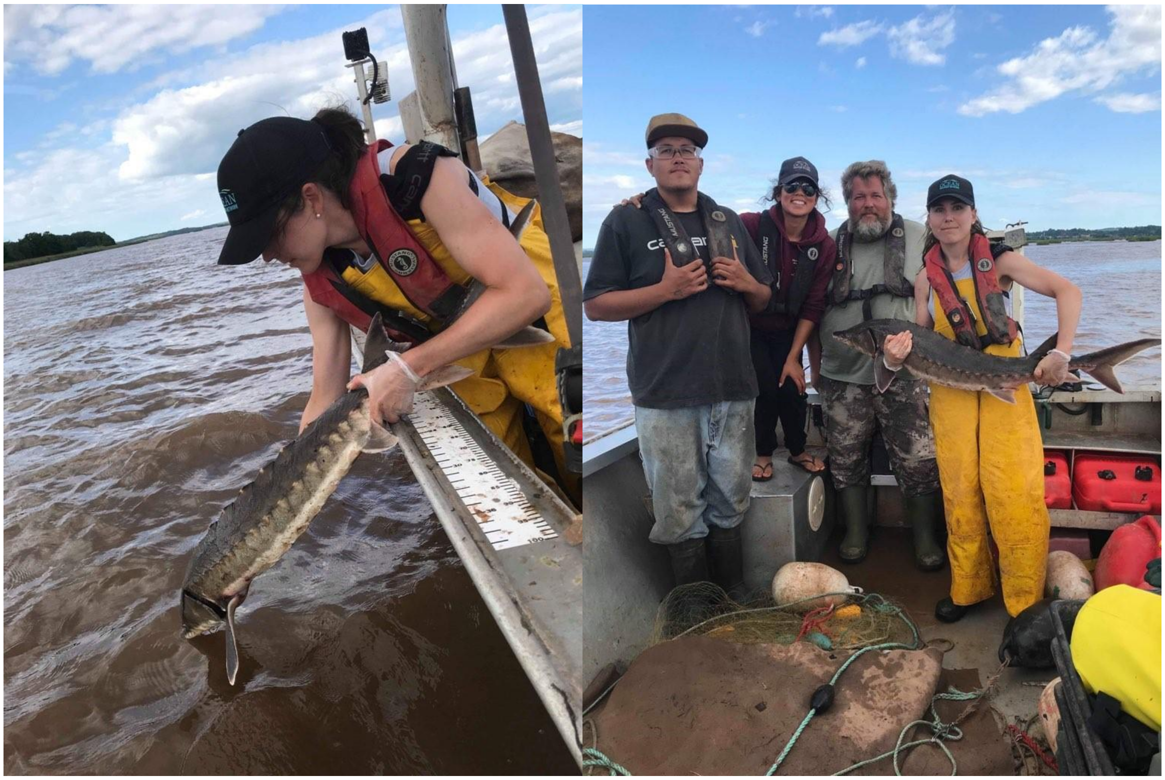

Figure 2.4.1. Atlantic Sturgeon were captured in Minas Basin, NS, by brush weir, directed otter-trawl, or set gill nets.

Results

From 2010-2019, 194 Atlantic Sturgeon were acoustically implanted with long-lifespan acoustic transmitters (10 year lifespan). During 2018 and 2019, approximately 40% of individuals reported to have had active tags were detected at the FORCE test site (Table 2.4.1). In comparison to the FORCE test site, a higher proportion of specimens were detected at the MP line (Table 2.4.1). The mean L_F of Atlantic Sturgeon tagged in 2018 and 2019 and detected at the FORCE test site and MP line (n = 19) was 133.24 ± 26.04 cm, indicating that they were sub adults. Individuals from the Saint John (FORCE, n=2; MP, n = 3) and Kennebec Rivers (FORCE: n=4; MP, n = 6) were detected at the FORCE test site and MP line in 2018 and 2019. Atlantic Sturgeon were detected on <9% and <30% of possible detection days at the FORCE test site in 2018 and 2019, and <20% of possible detection days at the MP line in 2018 (Table 2.4.1).

Table 2.4.1. Detection summary for Atlantic Sturgeon detected at the FORCE test site and Minas Passage (MP) line in 2018 and 2019. The number of possible detection days for each Atlantic Sturgeon was dependent on the first and last detection at the FORCE test site and MP line, as well as the date the individual was tagged and released. In 2018, data from V9-2x and VR2W-69 kHz receivers was obtained from the FORCE test site and MP from, May 5th to November 16th and April 21st to November 18th, respectively. In 2019, Atlantic Sturgeon were detected by HR2 and VR2W-69 kHz receivers at the FORCE test site from May 9th to August 8th.

	Date Tagged	No. active tags	No. possible detection days		No. detected (%)		Detection days (%)	
Year			FORCE	MP	FORCE	MP	FORCE	MP
2018	<2018	26	175	198	8	23	15	40
	June 13th	4	136	158	3	3	3	6
	July 8th	2	111	132	0	1	0	3

	July 17th	7	102	123	1	3	2	5
	July 19th	14	100	121	7	7	7	13
	Total	53	-	-	19	37	24	57
2019	<2019	53	91	-	21	-	28	-
	July 13th	5	26	-	1	-	1	-
	July 18th	1	1	-	-	-	0	-
	July 19th	4	4	-	1	-	1	-
	July 23rd	5	5	-	-	-	0	-
	August 2nd	6	6	-	-	-	0	-
	Total	74	-	-	23	-	30	-

Atlantic Sturgeon detection days were not evenly distributed throughout all months of the study (Appendix Figure 2.3). Typically, Atlantic Sturgeon were initially detected at FORCE and MP in late May-early June and were last detected in September-October, with peaks in detections occurring during mid-summer and early fall (Appendix Figure 2.3). The highest proportion of Atlantic Sturgeon days at FORCE and the MP line occurred at the S2 site (2018: n =15, 50%; 2019: n = 9, 43%; Figure 2.4.2), and MPS001 site (2018: n = 24, 42%; Figure 2.4.2), respectively. Atlantic Sturgeon did not appear to exhibit a preference for what region of Minas Passage they utilized each month (Appendix Figure 2.5).

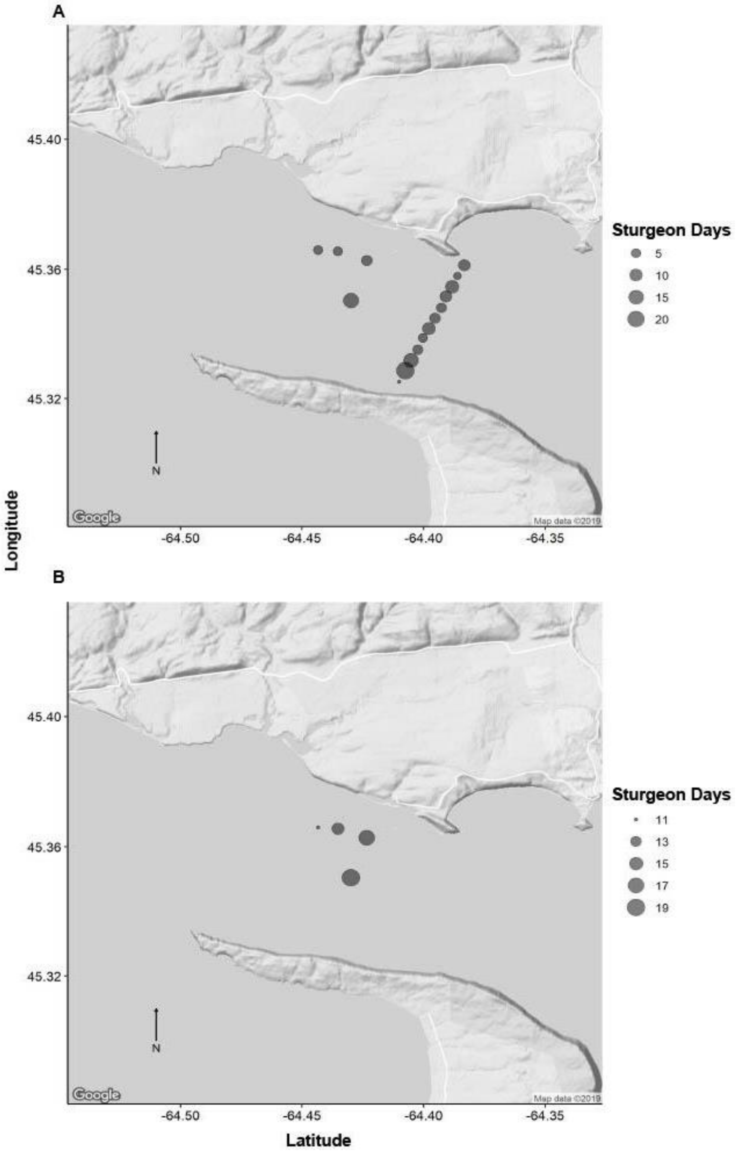

Figure 2.4.2. Map of the number of days Atlantic Sturgeon (sturgeon days) were detected at the Fundy Ocean Research Center for Energy (FORCE) test site and Minas Passage line (A) during 2018 and at the FORCE test site during 2019 (B). In 2018, data from V9-2x and VR2W-69 kHz receivers were obtained from the FORCE test site and MP from May 5th to November 16th and April 21st to November 18th, respectively. In 2019, Atlantic Sturgeon were detected by HR2 and VR2W-69 kHz receivers at the FORCE test site from May 9th to August 8th. Data was not available for receivers at the MP line during 2019.

Conclusions

Atlantic Sturgeon were detected on a higher proportion of days at the MP line than at the FORCE test site, with the highest probability of being detected at southern receivers within Minas Passage. Most Atlantic Sturgeon detected at the FORCE test site during 2018 were also detected prior to, or after, a detection within central Minas Passage. During flood tide water flowing out of MB is jetted northwards by Cape Split and past the FORCE test site, creating the highest current speeds within central MP (Karsten et al. 2008; Broome and Redden 2012). Based on the distance between locations, and time difference between detections, the estimated swimming speed was similar to the modelled mean current speeds between consecutive detections at the FORCE test site and MP line. Atlantic Sturgeon could be using tidal stream transport within this region, a behavior that has been reported for Juvenile European Sturgeon (*Acipenser Sturio* Linnaeus,1758) occupying high flow environments within the Gironde Estuary, France (Taverny et al. 2002).

Tide type (ebb/flood) and current speed were the only predictors of Atlantic Sturgeon presence at both sites. Atlantic sturgeon was more likely to be present at the FORCE test site on ebb tides and at the MP line when current speeds were lower. During a flood tide water is more evenly distributed within MP due to friction caused by the bottom substrate than during an ebb tide (Karsten et al. 2008). There is also a more uniformly directed flow of water from the north to south coastlines of Minas Passage on an ebb tide. If Atlantic Sturgeon are travelling with the current, then it is more likely that they would overlap with the FORCE test site during an ebb tide. However, background noise within Minas Passage is higher during flood tide than during an ebb tide (Martin and Vallarta 2012). Due to the low number of Atlantic Sturgeon detected in this study, data from V16 and V9-2x tags had to be combined for analysis. Lower frequency sounds are more likely to be impeded by background noise within MP than high frequency sounds (Taverny et al. 2002; Sanderson et al. 2017). Most of the Atlantic Sturgeon detected at the FORCE test site consisted of individuals tagged with V16-69kHz tags. The lower number of detections of Atlantic Sturgeon during flood tide could be attributed to the decline in signal receptions of the V16-69kHz tags.

The decline in detecting Atlantic Sturgeon with an increase in current speed at the MP line could be attributed to their inability to remain stationary within this region. Sturgeon are poor swimmers and are unable to maintain their critical swim speed for prolonged periods of time (Peake et al. 1995). The maximum swim speed of Atlantic Sturgeon has not been reported. However, the ability of adult Lake Sturgeon (106- 132 cm TL) to swim against a current rapidly declines when current speeds exceed 1.5 m/s (Peake et al. 1995). The depth averaged current speeds within MP are much higher than 1.5 m/s. During ebb and flood tide the speeds are reported to each 4.1m/s and 4.3m/s, respectively (Cornett and Bourban 2008). We suggest, however, that the decline in Atlantic Sturgeon detections with increasing current speed is more likely attributed to the performance of the tagging technology.

The bottom of Minas Passage consists mainly of bedrock, gravel and boulders up to 5m in diameter and the FORCE test site is located on a volcanic plateau surrounded by bedrock ridges (Fader 2009, 2011). On average Atlantic Sturgeon at the FORCE test site and MP line were found to occupy the mid-water column. Due to their potential inability to visualize and sense their environment it would make sense for Atlantic Sturgeon to remain in the upper water column within this region. Stokesbury et al. (2016) noted that Atlantic Sturgeon travelled pelagically through Minas Passage. In this study the only significant predictor of Atlantic Sturgeon presence in the upper water column at the FORCE test site was tide type, with a higher likelihood of detecting an Atlantic Sturgeon in the upper water column during ebb than flood tide. The entry point of Minas Passage into Minas Basin is characterized by deep channels consisting of bedrock (Fader 2009). When water is jetted through Minas Basin, into Minas Passage and towards the FORCE test site on ebb tide Atlantic Sturgeon may be lifted with the current due to the bathymetry. Sturgeon had a higher likelihood of being detected closer to the bottom substrate at the MP line with an increase in temperature. Sediment suspension within MP decreases with an increase in temperature (Dadswell et al., 1983; Envirosphere 2009). Atlantic Sturgeon may occupy deeper depths with an increase in temperature due to the increased visibility in the region.

Compared to teleosts, sturgeon have poor vision (Miller 2004) and they spend much of their time associated with the benthic substrate (Bramblett and White 2001; Taverny et al. 2002;

Bennett et al. 2005; Dadswell 2006; Beardsall et al. 2016). Poor vision, especially in turbid environments like MP, would make it difficult to discriminate obstacles including tidal turbines (Hammar et al. 2013). While there are currently no operational turbines within FORCE, as the currently deployed HK device has ceased to operate, plans are in place to test two MW turbines over the next few years (FORCE 2018). Most proposed tidal turbines function best within mid-water column where current speeds are often the highest (Stokesbury et al. 2016; Zhou et al. 2017). Larger species, such as Atlantic Sturgeon appear to be at a greater risk of contacting turbine blades due to their increased surface area. Atlantic Sturgeons morphology make them inadept at conducting rapid maneuvers (Webb 1986; Kieffer et al. 2001). It is highly unlikely that Atlantic Sturgeon could control their behavior within MP.

Striped Bass *Morone saxatilis*

Background

Starting in May, Bay of Fundy Striped Bass populations, designated by COSEWIC as endangered, migrate into river systems located within Minas Basin, NS to spawn. Striped Bass then exit their natal rivers during summer to feed on the rich abundance of fishes that inhabit Minas Basin (Broome et al. 2015; COSEWIC 2012). Striped Bass have also been reported to use Minas Passage as an overwintering site (Keyser et al. 2016). During the winter months the metabolism of Striped Bass is drastically reduced making it unlikely that they could control their behaviour within Minas Passage (Keyser et al. 2016). Due to the strong presence of Striped Bass within Minas Passage it is important to determine when a proportion of the Bay of Fundy Striped Bass population may overlap with FORCE.

Methods

On August 20th, 2018, 34 Striped Bass were captured through angling and tagged in the Shubenacadie/Stewiacke Rivers, NB. Fifteen Striped Bass were double tagged with V9-2x 180kHz and V16-69 kHz acoustic tags, 19 Striped Bass were tagged with just V16-69 kHz acoustic tags (Table 2.4.2). On June 14th, 2019 15 Striped Bass were captured through angling and double tagged with V9-2x 180kHz and V16-69 kHz acoustic tags (Figure 2.4.3). Striped Bass were double tagged so that specimens could be detected at HR acoustic receivers at the FORCE test site, as well as V16-69kHz receivers located throughout their migratory range.

Table 2.4.2. Summary of V16-69kHz and V9-2x tag types implanted into Striped Bass during 2018 and 2019 (n = 49). During 2018 15 Striped Bass were double tagged with V16-4x and V9-2x tags and during 2019 all Striped Bass were double tagged.

Year	VEMCO tag type	Weight (g)	Length (mm)	Power	Number Deployed	Battery life (days)	Approx. tag death (yyyy-mm)
2018	V9-2x	26.10	3.70	143	15	392	2020-06
	V16-6x	95	34	158	34	1633	2026-02
2019	V9-2x	26.10	3.70	143	15	392	2020-06
	V16-4x	95	34	158	15	2435	2026-02

Figure 2.4.3. Striped Bass were captured by angling in 2018 and 2019 in the Shubenacadie/Stewiacke Rivers, NS.

Results

During 2018 and 2019, Striped Bass were detected on 88 and 7% of possible detection days, respectively (Table 2.4.3). During 2018 and 2019, the average number of days Striped Bass were detected was 5.0 ± 5.0 days and 1.0 ± 1.0 day (Table 2.4.3). Striped Bass tagged and detected at FORCE test site mainly consisted of adults (2018: 87.10 ± 5.45 cm FL; 2019: 75 ± 0.10 cm FL).

Table 2.4.3. Detection summary for Striped Bass detected at the FORCE test site in 2018 and 2019. The number of possible detection days for each Striped Bass was dependent on the first and last detection at the FORCE test site, as well as the date the individual was tagged and released. In 2018, data from V9-2x and VR2W-69 kHz receivers was obtained from the FORCE test site and from May 5th to November 16th. In 2019, Striped Bass were detected by HR2 and VR2W-69 kHz receivers at the FORCE test site from May 9th to August 8th. During 2019 only Striped Bass tagged during 2019 were detected at the FORCE test site.

Year	Date Tagged	No. active tags	No. possible detection days	No. detected (%)	Detection days (%)
2018	August 20th 2019	15	77	53	88
2019	June 14th, 2019	49	55	6	7

In 2018, Striped Bass were present at the FORCE test site during June to December, in 2019 detections of Striped Bass at FORCE only occurred during July to August (Figure 2.4.4). Due to the low number of days Striped Bass were detected at the FORCE test site during 2019 only a graph depicting the detections during 2018 was created. The highest proportion of Striped Bass days at the FORCE test site during 2018 occurred during July (n = 18, 23%) and December (n = 15, 19%), when temperatures from HR receivers were 14 and 4 °C, respectively (Figure 2.4.4). The highest proportion of Striped Bass days at the FORCE test site occurred at the S2 site in 2018 (n

= 47, 61%; Figure 2.4.5). During 2019 the highest proportion of Striped Bass days at FORCE occurred at the D1 (n = 2, 50%) and W2 (n = 2, 50%) site in 2019 (Figure 2.4.5).

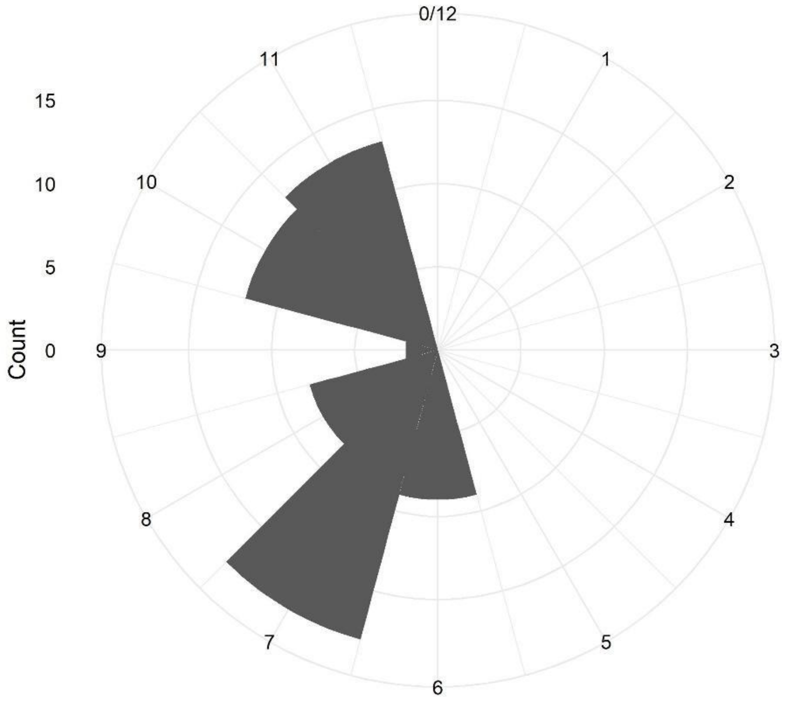

Figure 2.4.4. Month versus days Striped Bass (Striped Bass days) were detected at the Fundy Ocean Research Center for Energy (FORCE) test site 2018. In 2018, data from V9-2x and VR2W-69 kHz receivers was obtained from the FORCE test site from May 5th to November 16th. There were only four days when Striped Bass were detected during 2019, therefore the data was not plotted. The low number of Striped Bass days was likely related to the lack of data available from the FORCE test site during 2019.

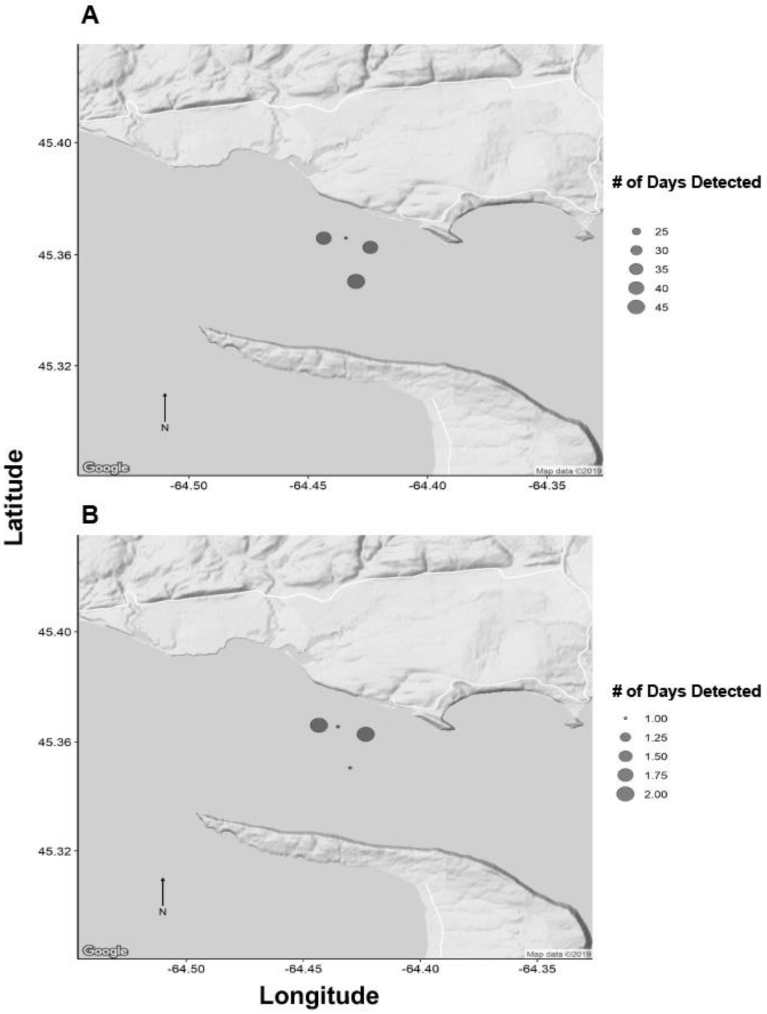

Figure 2.4.5. Map of the number of days Striped Bass (striped bass days) were detected at the Fundy Ocean Research Center for Energy (FORCE) test site and Minas Passage line (A) during 2018 and at the FORCE test site during 2019 (B). In 2018, data from V9-2x and VR2W-69 kHz receivers were obtained from the FORCE test site from May 5th to November 16th. In 2019, Atlantic Sturgeon were detected by HR2 and VR2W-69 kHz receivers at the FORCE test site from May 9th to August 8th.

Conclusions

Only adults were tagged and subsequently detected at the FORCE test site. The potential adverse effects of the presence of HK devices to other life-stages of striped bass is currently unknown. Adults are required to increase recruitment to the population, and any disruptions to the adult cohort could prolong the endangered designation of Striped Bass under COSEWIC. During 2018 most tagged Striped Bass were detected at the FORCE test site, and specimens utilized Minas Passage throughout all seasons. The largest proportion of detections during 2018 occurred during the winter. This is consistent with the findings of Keyser et al. (2016) who also reported that Striped Bass are frequently detected during December to March within Minas Passage. Water temperatures within Minas Passage can reach a minimum < 1 °C (Keyser et al. 2016) and Striped Bass have been reported to become lethargic and decrease feeding when water temperatures fall below 10°C (Keyser et al. 2016). This suggests that during winter it would be unlikely that Striped Bass could engage in evasion behaviours around an HK device deployed at FORCE.

Striped Bass were also found to mainly utilize the S2 test site. This site is located directly within the center of Minas Passage where the current is the strongest (Karsten et al. 2008; Broome and Redden 2012). Keyser et al. (2016) noted that it is unlikely that Striped Bass would be capable of swimming against the strong current within this region, and they may be engaging in tidal stream transport to and from Minas Basin. This is concerning as Striped Bass have been reported to occupy the top 40m of the water column, the depth at which HK's optimally function (Keyser et al. 2016). During 2019, there were a low proportion of striped bass at the FORCE test site which could be attributed to the sparseness of receiver data available during this period.

In this study Striped Bass were double tagged with both V16-4x and V9-2x acoustic tags. This was the first study attempting to compare the traditional and new tagging technologies developed by VEMCO in a fish. The old coded tagging technology (V16-4x) emits a signal at 69kHz which is impeded by background noise when current speeds exceed 2m/s within Minas Passage (Broome et al. 2015). The new HR technology (V9-2x) emits an acoustic signal at 170kHz which can be detected at high current speeds (Sanderson et al. 2017). Unfortunately, there were too few doubles tagged Striped Bass detections during 2018 and 2019 to compare the detection efficiencies of the two tagging technologies. Data offloaded during the next download at the FORCE test site will hopefully contain enough detections to undertake statistical analysis. This information is necessary to inform other researchers regarding the prime technology to use for detecting fishes in high flow environments.

Atlantic Salmon *Salmo salar*

Background

Atlantic Salmon are anadromous, spawning in freshwater and migrating to estuarian and marine environments to feed and grow (Baum et al. 1997). Salmon are physiologically and energetically prepared for the migration from freshwater to saltwater when they undergo the parrsmolt transformation into smolts approximately 1-3 years after hatching. Atlantic Salmon at sea are referred to as post-smolts until they spawn and are then termed kelts. Kelts are iteroparous (multiple spawn events); iteroparity is a life-history strategy that can help maintain populations when survival to first spawning (recruitment) is low. Spawning either occurs in consecutive years and is referred to as repeat spawning, or in alternate years (Jonsson et al. 1991). Although the return rate for spawners can range from 0-90% across native river systems, research continues to demonstrate the importance of repeat spawners in stock maintenance (Bardonnet and Baglinere 2000; Bordeleux et al. 2019).

Atlantic Salmon populations have suffered severe declines in abundance since the 1990’s (Jonsson et al. 1999). Reduced marine survival of immature (e.g., smolts) and previously spawned adults (kelts) has been determined to be the most prominent factor in the declines in abundance for Atlantic Salmon from North American (DFO 2018) and European (ICES 2017) populations. Quantifying marine survival, and attributing mortality events to specific biological (e.g., predators or prey distributions) or physical variables (e.g., temperature shifts, anthropogenic disturbances) is complex (Lacroix 2013, Strøm et al. 2019). There is a lack of detailed information on the marine migration and distribution of Atlantic Salmon at sea, from different regions and populations. Much of our understanding of Atlantic Salmon marine distribution comes from mark and recapture of conventionally tagged fish, with recapture being fisheries dependent (Reddin and Shearer 1987, Dadswell et al. 2010). Traditionally, assumptions have been made that individuals from different populations migrate along similar routes, at similar times to the same destinations (Dadswell et al. 2010, Lacroix 2013). Assumed shared migration routes and ocean distribution for salmon from different populations may hamper the identification of threats to survival that may be area specific (Lacroix et al. 2008, Lacroix 2013).

Atlantic Salmon abundance in the Maritimes has been in decline for over two decades (CSAS 2017). Many populations are extirpated and the inner Bay of Fundy (iBoF) salmon are listed as endangered under the Species at Risk Act. Historically, the iBoF populations had some of the highest return rates for kelts, with the majority spawning after 1 year at sea and spawning multiple times in consecutive years (Ritter 1989). The last major decline in recruitment occurred in the 1980s and since that time the iBoF kelt returns have effectively disappeared (COSEWIC 2010). Understanding all potential sources of marine mortality for endangered iBoF Atlantic Salmon is critical since any high at-sea mortality would be catastrophic to populations that have relied on the consecutive return of repeat spawners of higher fecundity (Jessop 1986; Lacroix 2013). The objective of this study was to determine spatial and temporal overlap of two life-stages of Atlantic Salmon (smolts and kelts) with the FORCE test site. Due to the Endangered conservation status of iBoF Atlantic Salmon, it is important to determine whether Atlantic Salmon will face temporal and spatial overlap with operating HK's.

Methods

Prior to 2019 all smolt fishing was conducted under the Department of Fisheries and Oceans Scientific License to Fish #322595. Fishing conducted during 2019 was registered under license # 330657. Smolts and kelts were obtained from the Gaspereau River (GR) watershed. The GR is one of the inner BoF rivers with an endangered salmon population (COSEWIC 2006). Each year, smolts and kelts are captured in the summer and fall in a trap 18 km from the river mouth at a small hydropower dam. Kelts determined to be of GR origin are held and spawned at the Live Gene Bank Coldbrook Biodiversity Facility, Nova Scotia. In the Fall, tens of thousands of unfed fry are released back into the GR watershed (wild-exposure) and then recaptured as smolts to be raised in captivity and spawned before being released as kelts again. The Biodiversity Facility retained 30 4-yr old female GR-origin wild-exposed kelts to tag for our project. Salmon surgical procedures were performed under Acadia Animal Care Committee protocol #07-18. Detailed methods for the capture and sampling of Atlantic Salmon can be found in Appendix F.

Results - smolts

2018

307 detections of 4 Salmon Smolts occurred during May 15th to June 4th from data obtained from the HR2 receivers at the FORCE test site in 2018. 70% (n = 217) occurred during the month of May and smolts were detected on a total of 8 separate days. Salmon smolts were detected at the W2 site on the highest number of days (n=6) (Figure 2.4.6). One Salmon Smolt was detected at the FORCE test site on 6 separate days, most (n=3) were detected on a single day. Salmon Smolts were not detected at more than one site on multiple days therefore a Kruskall-Wallis test could not be applied to test for a difference between the mean number of days individuals were detected at each site.

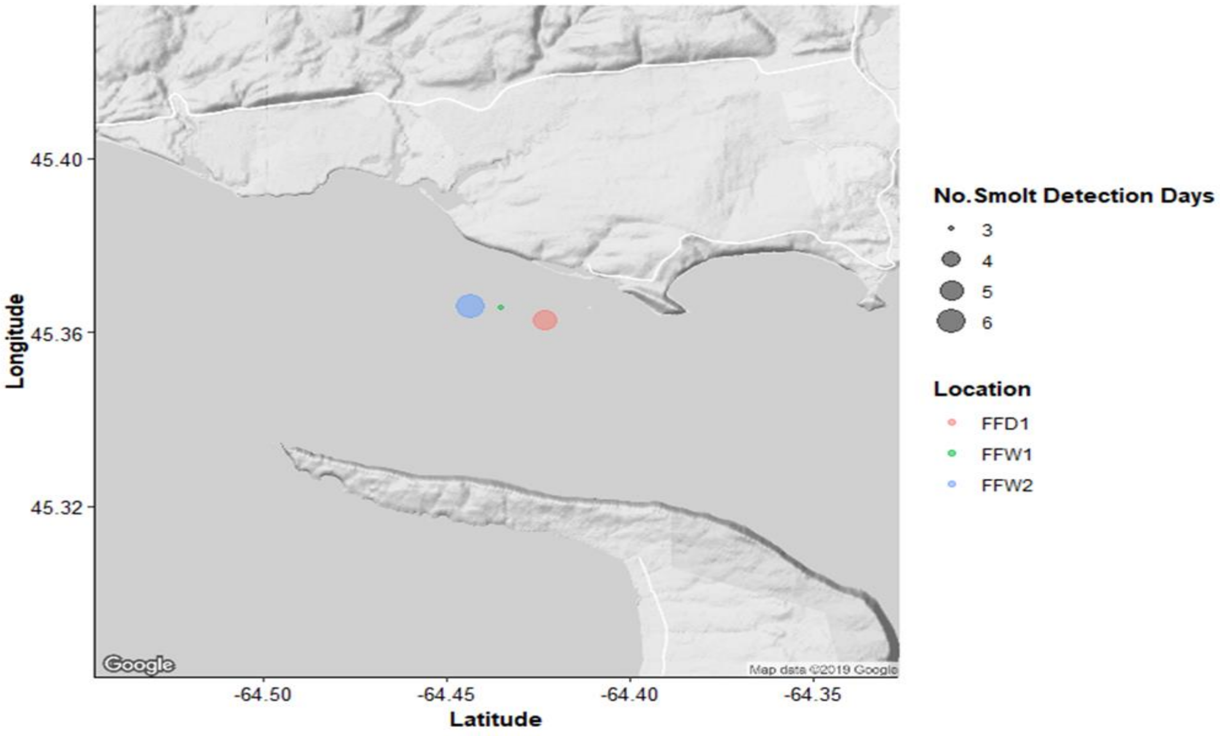

Figure 2.4.6. The total number of days Salmon Smolts were detected at each of the four test sites during May 5th 2018 to March 29th 2019. Striped Bass were detected during May 5th to November 16th 2018.

2019

Salmon Smolts were detected on 23 days during May 11th to June 9th from data obtained from the HR2 receivers FORCE test site. Salmon Smolts were detected at the S2 site on the highest number of days (n=21) (Figure 2.4.6). On average, smolts spent 2.1 ± 1.1 days at the FORCE site, with one individual spending 5 days, and many spending between 2-4.

Results - kelts

In April 2019, 25 wild-exposed hatchery female Atlantic Salmon kelts (Mean ± SEM, L_F= 0.63 ± 0.011 m, Age = 4 years) were acoustically implanted at the Department of Fisheries and

Oceans Live Gene Bank hatchery in Coldbrook, Nova Scotia. Two transmitter types were used, a HR V9-2x (tag life = 320) and a V13P-1x 69kHz (tag life = 437) equipped with a pressure sensor for recording fish swim depth. Kelts were released into the Gaspereau River on May 9th (Figure). Nineteen of 25 released kelts were detected at the FORCE test site between May 11th and August 2019.

Figure 2.4.7. Atlantic Salmon Kelts were tagged at the Department of Fisheries and Oceans Live Gene Bank hatchery in Coldbrook, NS, and released into the Gaspereau River, NS, in May 2019.

Kelts were detected for a maximum of 53 days at the test site, although detection frequency varied and the site with the highest number of detections and individual kelts was S2, which is closest to the centre of the passage (Figure 2.4.8). The average number of days a kelt was detected in the region was 6 days. Mostly kelts were detected in May, but detections were also made in June, July and August.

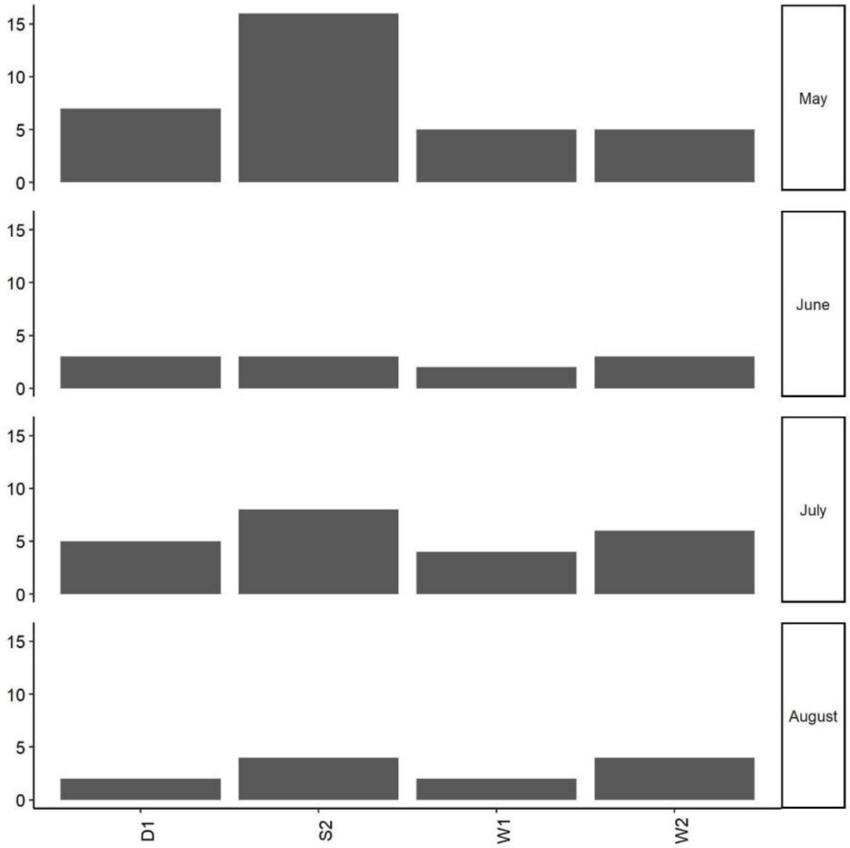

Figure 2.4.8. Detection frequency of 19 acoustically tagged kelts at the FORCE test site from May – August 2019.

Swim Depth

Sixteen of the 19 kelts detected at the FORCE test site in 2019 also had recorded detections of swim depth (depth from surface). Bottom depths for each receiver at the FORCE test site were estimated when water levels were at 0 m. Bottom depths were estimated by adding/subtracting the bottom depth obtained upon receiver deployment at the FORCE test site/MP line to the water level estimated through the hydrodynamic model during the time of deployment. For the W1, W2, D1 and S2 site the zero tide levels were 44.4m, 39.61m, 28.63m and 68.21m and respectively. The average (± SEM) depth from bottom reading when water levels were 0 m, was 61.91 m (± 0.76), 41.93 m (± 0.66) and 37.83 m (± 0.32), at the S2, W1 and W2 sites, respectively. Kelts spent most of their time (94%) in the upper portion (0 to 20 m) of the water column with swim depths averaging (± SEM) 4.33 ± 0.45 m across all three sites (Figure 2.4.9). Swim depths averaged 6.29 m (± 0.76), 2.47 m (± 0.66), and 1.78 m (± 0.32) at the S2, W1, and W2 sites, respectively. Three kelts made deep dives greater than 20 m (max swim depth = 48.51 m; average ± SEM = 32.31 ± 1.97 m) at the S2 site (Figure 2.4.9). For two of these kelts, the dives were a single occurrence and lasted < 5 min. For the other kelt, dives to depths >20 m occurred on three separate occasions in May and again in August of 2019.

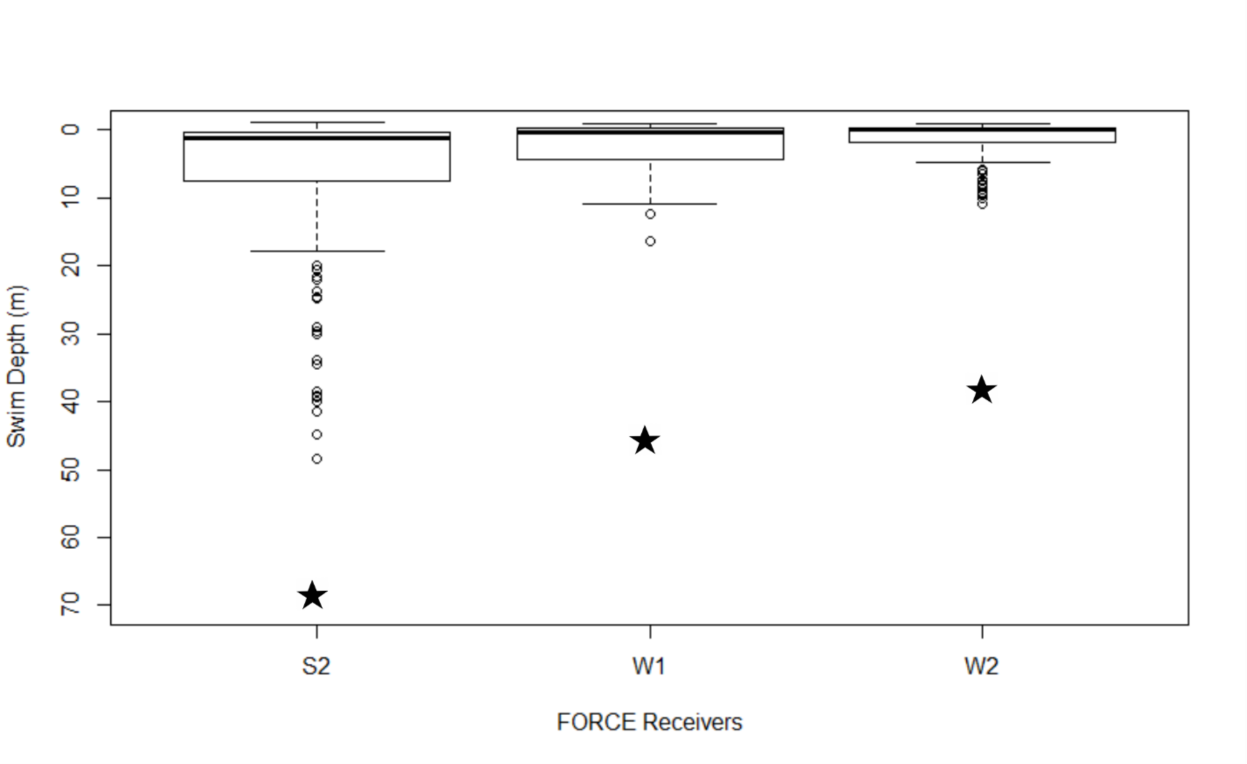

Figure 2.4.9. Boxplots represent swim depths (m) for 16 Atlantic Salmon kelts recorded at the FORCE test site between May and August 2019. Stars represent the estimated bottom depths for each receiver at the FORCE test site when water levels were at 0 m. Bottom depths were estimated by adding/subtracting the bottom depth obtained upon receiver deployment at the FORCE test site/MP line to the water level estimated through the hydrodynamic model during

the time of deployment. For the W1, W2, D1 and S2 site the zero tide levels were 44.4m, 39.61m, 28.63m and 68.21m and respectively. Only receivers with depth detections are shown.

Conclusions

This was the first study to examine the spatial and temporal use of the FORCE test site for two life stages (Smolt and Kelt) of endangered iBoF Atlantic Salmon. Like Atlantic Sturgeon, Striped Bass, and kelts, the smolts were primarily detected on receivers closest to the centre of the passage but were typically only detected on a single day as they migrated from Minas Basin out to sea. In comparison to 2018, more Salmon smolts were detected at the FORCE test site in 2019.

Kelts spent a considerable amount of time in the region, particularly near the S2 site. This behaviour is comparable to what was observed in Striped Bass which were also found to primarily utilize the S2 site where the current is strongest. The drifter survey outlined at the beginning of this report and in Appendix D also detected one of our tagged Salmon kelts in the region and concluded that the kelt was likely engaging in tidal stream transport to and from Minas Basin; as with striped bass, it is unlikely Atlantic Salmon kelts are capable of swimming against the strong current in this region. Detections from kelts made on receivers within the Minas Basin estuary and the outer Bay (Scot's Bay) provided us with a snapshot of Kelt behaviour as they move in and out of Minas Basin, through Minas Passage. Although some individuals only traversed the Passage a single time on their way out to sea, others moved considerably throughout the region, foraying between systems and being detected up to 53 days at the FORCE test site in the span of only 4 months of monitoring. Kelts were found to occupy the upper water column most frequently (< 20 m), however occasional suspected foraging dives were made to 48 m at the S2 site. This is concerning as HK's optimal functionality is the upper portion of the water column where kelts were often found.

Alewife *Alosa psedoharengus*

Background

Alewife (locally known as Gaspereau) are a dominant species of forage fish in the Black River – Gaspereau River system in Nova Scotia, supporting a lucrative commercial fishery (McIntyre et al. 2007). Fisheries target adult Alewife as they migrate upstream through the estuary and river (Gibson and Myers 2001) with the use of gillnets in tidal waters, and with weir and dip-net apparatus (defined in regulations as square-nets) in non-tidal waters (Jessop and Parker, 1988). Due to their local importance, fishing mortality counts have been recorded since 1964, and population assessments using escapement counts have been conducted intermittently since 2000 (Gibson 2000; Gibson and Myers, 2001; Gibson 2003; McIntyre et al. 2007).

The 2001 assessment found that the Gaspereau River alewife population exhibited the characteristics of a heavily exploited stock, with a nearly 80% exploitation rate, and the majority of fish in the spawning run belonged to only 2 age classes (McIntyre et al. 2007). A five-year fishery management plan came into effect for the start of the 2002 season, with the goal of meeting the spawning escapement target of 400,000 adults through a reduction in fishing mortality and improved fish passage. Exploitation rates have been lower in every year since the implementation of this management plan, with escapement surpassing the target for the past 3 years (Gibson, unpublished data).

However, there has been no observed evidence that alewife longevity or incidence of repeat spawning has increased in response to the management efforts (McIntyre et al. 2007). Furthermore, recruitment of age-four spawners was still low during the last assessment, indicating a potential source of concern over post-escapement and/or post-spawning survival. Concerns with the status of Gaspereau populations, and the fisheries they support, have been expressed by clients and stakeholders at Gaspereau advisory meetings held throughout Maritimes Region since 2004 (McIntyre et al. 2007). These concerns may indicate a general decline in Alewife status across their range. The potential role of in-river versus regional influences (beyond fishing effects) on population size and post-spawned survival have not previously been evaluated, therefore a lot of uncertainty remains about the optimal management targets for this species.

Understanding potential sources of mortality occurring during the post-spawning return to the ocean is important to more accurate assessments of the Gaspereau River population. The objective of this study was to examine the post-spawning movement of Alewife in the coastal habitats of the Minas Basin, and determine spatial and temporal overlap with the FORCE test site.

Methods

Seventy-Five spent (post-spawned) Alewife were captured on June 17-22, 2019, from the White Rock fish ladder on Gaspereau River (GR) watershed, NS, using a dip net. Fish were tagged with VEMCO/Innovasea V5, HR model tags with an estimated 63-day battery life. Each fish was sexed, weighed and measured before being anesthetized with a buffered solution of methanesulfonate (MS-222) at a concentration of 2 g/10 L for surgery. Tags were implanted into the intracelomic cavity, 1-2 mm off the mid-ventral line by making an 8-10mm long dorso-ventral incision between the ribs, and incisions closed with two simple interrupted sutures (Ethicon monofilament nylon sutures, reverse cutting 4–0, 1.5 metric, 45 cm, PS-2 18 mm, 3/8 circle needle). Fish allowed to recover until vertical equilibrium and swimming ability were regained, before being released directly at the base of the fish ladder.

Results

1796 detections of 31 individuals occurred between July 7th to August 12th from data obtained from the HR2 receivers FORCE test site. 73% (n = 1307) occurred during the month of June, over the course of 27 separate days (Figure 2.4.10). Alewife were detected at the S2 site on the highest number of days (n=15) (Figure 2.4.11). One Alewife was detected at the FORCE test site on 6 separate days, with an average of 1.6 ± 1.1 detection days per individual. Males and females were detected in near equal proportion (52% female, 48% male).

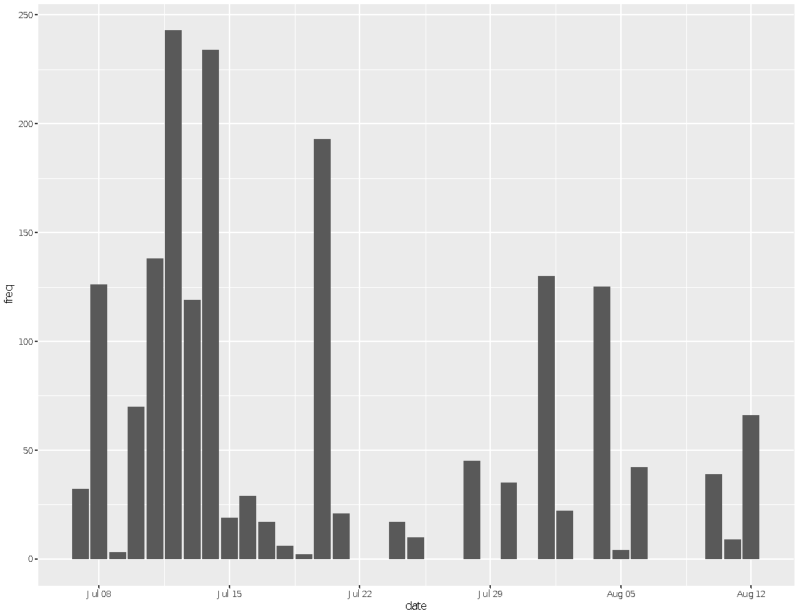

Figure 2.4.10. Detection period of Alewife at the FORCE test site between July 7th to August 12th 2019.

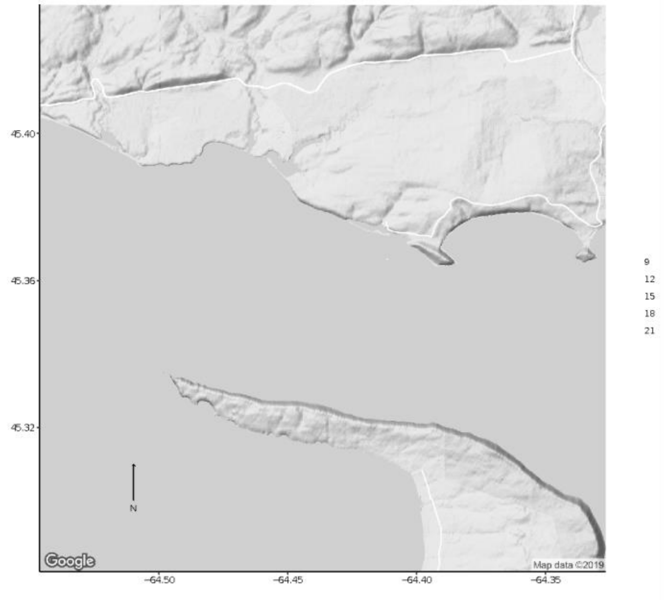

Figure 2.4.11. The total number of days Alewife were detected at each of the four stations at the FORCE test site during July 7th to August 12th, 2019.

Conclusions

This is the first study to examine the spatial and temporal use of the FORCE test site by Alewife. Like many of the species described above, Alewife were primarily detected on receivers at the S2 site, closest to the centre of the passage. Most fish were only detected on a single day, presumably as they migrated from Minas Basin out to sea, however several Alewife were present for 2-4 days, with a maximum of 6 days for one individual. Given their size, it is unlikely that Alewife could swim against the strong current within this region, and they may be engaging in tidal stream transport to and from Minas Basin, similar to Striped Bass and Atlantic Salmon. Further analysis of detections from receivers within the Minas Basin estuary and the Gaspereau River might provide us some insight into the behaviour of Alewife, as they move out of the river and into the Minas Basin, then through Minas Passage towards the sea.

Section 3: General Conclusions and Recommendations

General Conclusions

In this project we have vastly improved our ability to track fishes migration and behaviour in a macro-tidal estuary. Moving in sequences, we have developed surgical procedures that enable researchers to tag commercially valuable river herring (Alewife and the closely related blueback herring). Before this project, there had been very limited tagging of these very important species due to their perceived inability to survive surgery and return to normal behaviour.

Much work was done during this project to determine the effect of current speed on our ability to decode acoustic tags transmitting at 69, 170 and 180 kHz. Traditions coded acoustic tag technology has used 69 kHz tags. Theses tags are generally larger, but their advantage is that they have a longer battery life. Because we want to get to the point where we can track fishes movements in 3 dimensions we testing new innovative tagging technology at 170 and 180 kHz that enabled researchers to triangulate positions of tagged fishes to within a metre. The ability of these signals to be detected at different current speeds, both on moored equipment and by drifting receivers, was tested by Dr. Sanderson in, what I believe to be, the most thorough range test of acoustic tags in a high current environment in the world. Appendices B-E contain the results of these experiments. Dr. Sanderson also quantified the ability of moored broad band hydrophone *ic*Listen technology to decode acoustic tags. Enabling researchers to look at historical data from these hydrophones deployed on the non-operable turbine currently in Minas Passage. Although this ability had not been foreseen at the beginning of the study, and was therefore not detailed in the proposal, it contributes greatly to the tool box fish trackers can use to accurately detect movement and behaviour of fishes, when operating turbines are deployed in areas of high energy globally, and in Minas Passage in particular.

Having done due diligence in surgical techniques and quantifying the range of acoustic signals in areas of high current, we also determined overlap of several species with the FORCE test site, and areas adjacent where turbines are proposed to be deployed. This was done for commercially valuable Alewife, and species of concern, Atlantic salmon (smolts and kelts), Striped Bass and Atlantic Sturgeon. We mow have estimates of interaction of these species with the area targeted for turbine deployment. These estimates will continue to improve in precision as more data is collected on fish movement through Minas Passage. For Alewife, a commercial species, and Atlantic Sturgeon and Striped Bass and estimates of interaction can be matched with estimates of abundance to predict percentage of the population that are likely to overlap with areas of turbine operation. These estimates are central to developing predictive models of impacts of turbines on species of fishes in Minas Passage. Also, for larger bodied species this information can be matched with depth preferences such as for Atlantic Sturgeon.

Predictive models will also need species specific information on fishes' ability to detect and avoid operating turbines. In this study we have begun the foundation of tracking studies that could enable researchers to investigate fishes' detection and avoidance behaviour, by improving surgical techniques, quantifying receiver range, and defining temporal overlap between fishes and areas of interest. Also, we have identified a new combination of Nova Scotia manufactured technology, VEMCO tags and Ocean Sonic's *ic*Listen hydrophones, that can be used to track tagged fishes in 3 dimensions. Working with these companies to test and to increase the ability of their equipment to function in this high current area is central to developing accurate predictive models of fish/turbine interaction. Also central to this modeling exercise is to have a functioning turbine in Minas Passage where developers work with scientists to quantify any impacts of turbine deployment.

Recommendations

1. Reflective surfaces nearby an HR2 receiver greatly degrade detection of 170kHz-HR signals but have a smaller impact on detection of 180kHz-PPM signals. Reflective surfaces should be avoided, when possible, when deploying HR receivers.

2. Probability of detection of 170kHz-HR signals is reduced in fast currents but the effect of current is not so great on 170kHz-HR signals as it is on 69kHz-PPM signals. Tag programming can be adjusted to emphasize the 170 kHz-HR signals.

3. The primary disadvantage of 180kHz-PPM tags is not that power requirements limit the number of transmitted signals. *Ic*ListenHF hydrophone measurements reveal that at all but very close range it is only the first fraction of a PPM pulse that is relatively free of confounding reverberation. Making pulses of shorter duration would enable more transmissions with larger amplitude and ensure better reception.

4. Providing care is taken to mount the HR2 receiver on the PLAT-I so it is streamlined with the flow and sufficiently removed from reflective surfaces, both 180kHz-PPM and 170kHzHR tags were very well received at short range (less than 50m) in the tidal currents of Petit Passage, NS and range tests indicate that 170kHz-HR tags/receivers stand a good chance of being able to measure tracks of tagged animals in the immediate vicinity (less than 100m) of a tidal turbine in Minas Passage.

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5. Mounting a Vemco VR2W receiver on a drifter has been demonstrated to be an effective way to measure currents and to monitor the movement of acoustically-tagged fish.

6. On multiple occasions, all three of the synchronized *ic*ListenHF hydrophones on the OpenHydro platform were able to detect the same signal from 69kHz-PPM acoustic tag that had been implanted within a fish. Thus, with more well-placed hydrophones it should be possible to measure trajectories of tagged fish near a in instream turbine.

7. The OpenHydro platform has Acoustic Doppler Current Profilers (ADCPs) that operate at 500kHz. These ADCPs also transmit unwanted noise at frequencies that interfere with detections of 180kHz-PPM signals, 170kHz-HR signals and porpoise vocalizations. Close to the turbine, this unwanted noise can be detected by marine mammals and alosine herring --- which may influence their behaviour.

8. Recommendations made by Redden et al. (2014) still apply and include, coupling various monitoring approaches, including the FORCE FAST platform, sensors in autonomous landers and SUBS buoys, and possibly fish weir surveys, to monitor the movements of commercial species (schooling herring and other susceptible species) and species of “conservation concern”, as designated by COSEWIC and/or listed under SARA. Endangered inner Bay of Fundy Atlantic Salmon require further monitoring and consideration given the number of individuals and extent of time spent in Minas Passage was more extensive than previously recognized.

9. Use available fish detection datasets and associated environmental data (e.g. current speed and water temperature) to model collision probabilities for fish species of interest. Although successfully done for Atlantic Sturgeon in this report, the information gathered over time for other species (Striped Bass, American Eel, Alewife, Atlantic Tomcod) will allow for this method to be applied.

10. Include periods of high fish traffic through Minas Passage when designing an environmental effects monitoring program (EEMP).

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Appendix F

Additional Information on Capture/Sampling

Detailed Capture & Sampling Methods

Atlantic Sturgeon

From 2010 to 2018, 174 Atlantic Sturgeon were captured and surgically implanted with V16-69 kHz acoustic tags (Table 1). In 2018, 23 Atlantic Sturgeon were implanted with V9-HR 2x 180 kHz and four with V16-6x 69 kHz acoustic tags in Minas Basin, Nova Scotia (Table 1). During 2019, 10 Atlantic Sturgeon were double-tagged both with V9-2x and V16 acoustic tags, and 10 were tagged with V9-2x tags (Table 1). Atlantic Sturgeon tagged prior to 2019 were captured through otter trawl and brush weir. Captain Glanville Travis of the vessel "Terri and Sandi" was hired to conduct Otter Trawls lasting between 30-60 minutes. The vessel consisted of a 24m box trawl net, with 14cm stretched mesh and modified rock hopper equipment (Dadswell et al. 2016). Weir fishing was conducted at locations in the intertidal zone of the southern and northern shores of MB. All weirs were composed of 2.5cm stretched mesh attached to 2.2m wooden posts shaped in a V. The tip of the V was directed towards the ocean and the length of the wings extended 1-2km. The walls of the weir are covered in brush, making it difficult for fish to view the walls when submerged during high tide (Porter et al. 2018). On ebbing tides Atlantic Sturgeon were funneled to a trap containing a pool of water located at the tip of the V where they were sampled (Figure 1). During 2019, Atlantic Sturgeon (n = 20) were captured by drifting two attached monofilament gill nets within Minas Basin. The net was 190m long by 5m deep and the stretch mesh panels were 10 and 13cm, respectively. Nets were drifted during for periods ranging from 15 – 30 minutes.

Figure 1. Atlantic Sturgeon were captured and sampled in a fish weir

Atlantic Sturgeon were measured for fork (FL) and total length (TL). External identification Floy dart tags (Floy Tag & Manufacturing Inc., Seattle, Washington) were inserted into the dorsal fin and anchored around the pteriogyte bone (Dadswell et al. 2016). For surgeries that took place at the weir, Atlantic Sturgeon were placed onto their dorsal side in a shallow pool (Logan-Chesney 2016). On the trawl and after gill net capture Atlantic Sturgeon were placed onto a tarp on a flat bin located on the vessel. Prior to the surgeries, all instruments and tags were disinfected with 10% Betadine ™ solution and rinsed with 0.9 % sodium chloride solution. A no.22 scalpel was used to make a 30mm incision 40-60mm below the pelvic fin. V16 tags were activated by removing an activation magnet, and V9-2x tags were activated by using a VEMCO tag activator. Tags were inserted into the peritoneal cavity through the incision. Absorbable # 1 and #0 monofilament polydioxanone sutures (PDS) with reverse cutting edge were used to make two double square does not suture to close the incision (Johnson and Johnson, Markham, Ontario; Moser et al. 2000). Size 0 sutures were used after 2017, as the larger needle size has been reported to make it easier to puncture through sturgeons' tough skin and reduce surgery time (M.F. McLean, Acadia University, 23 Westwood Avenue, Wolfville, NS, personal communication, 2018).

Striped Bass

On August 20th, 2018, 34 Striped Bass were captured through angling using barbless circle hooks baited with Alewife *Alosa pseudoharengus* (Wilson, 1811) in the Shubenacadie River, NB. Upon capture Striped Bass were places in an anaesthetic bath composed of river water and 40mg/L of a 10% Eugenol (clove oil) solution. Once fish lost equilibrium (approx.5 minutes) and displayed signs of reflex impairment they were removed for surgery. Striped Bass were measured for fork (FL) and total (TL) length (mm) and weighed using a spring scale. Fish were placed into a V-shaped cradle with their gills immersed in water. A 2cm incision was made below the pelvic fin adjacent to the ventral midline. Acoustic tags were inserted into the peritoneal cavity and the incision site was closed with two double square knot sutures (Ethicon® FS 5-0). Post surgery Striped Bass were placed into a holding tank to fully recover. 15 Striped Bass were double tagged with V9-2x 180kHz and V16-69 kHz acoustic tags, 19 Striped Bass were tagged with just V16-69 kHz acoustic tags. On June 14th, 2019 15 Striped Bass were double tagged with V9-2x 180kHz and V16-69 kHz acoustic tags. Striped Bass were double tagged so that specimens could be detected at HR acoustic receivers at the FORCE test site, as well as V16-69kHz receivers located throughout their migratory range.

Atlantic Salmon

Surgery was required to internally implant each of the 25 kelts with a V9 HR acoustic transmitter (Figure 2, 26 mm in length x 9 cm diameter, 2 g - weight in water, ~500 d battery life). Surgical procedures, including handling, optimal anesthesia concentrations, and post-release recovery care, have been carefully studied to ensure reduced stress and optimal survival. Best practices have been developed in our lab at Acadia University for use on many fish species including Striped bass (*Morone saxatilis*), Atlantic Sturgeon, Atlantic Herring (*Clupea harengus*), and Alewife (*Alosa pseudarengus*). Briefly, 25 kelts were immersed in 100mg/L concentration of MS-222 until vertical equilibrium is lost. A 10mg/L concentration of anesthetic was continuously delivered through the gills throughout the surgical procedure to maintain sedation (Figure 3). This concentration and delivery method was successful for use on Alewife and Atlantic Salmon smolts and resulted in fast recovery times (Tsitrin et al. unpublished data). Once anesthetized, fish were placed right-side down on a foam cradle and a small incision was made to insert the tag into the peritoneum (Figure 4). To reduce the risk of tag loss, incisions were closed with two simple interrupted sutures (nonabsorbable Ethicon monofilament nylon sutures, reverse cutting 4–0, 1.5 metric, 45 cm, PS-2 18 mm, 3/8 circle needle), secured with two throws. A similar procedure was used for surgical implantation of tags on salmon smolts (Figure 4).

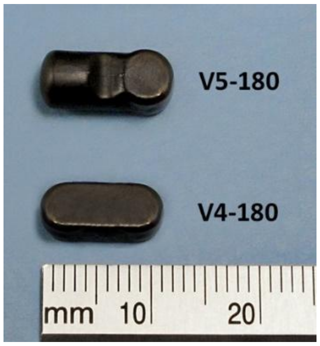

Figure 2. Size chart of VEMCO V5-180 kHz acoustic transmitters used on an Alewife study at Acadia University. The V9-180 tag is slightly larger with a longer battery-life for use on larger fish. Photo from https://VEMCO.com/products/v4-v5-180khz/

Figure 3. Example of the foam holding cradle used for surgery on Atlantic Salmon smolts, with orally-delivered anesthesia.

Figure 4. Atlantic Salmon smolt from the Gaspereau River being tagged with a VEMCO HR acoustic tag, in spring 2018.

Appendix G

Use of new High Residency and traditional coded acoustic tagging technology to predict if Atlantic Sturgeon (*Acipenser oxyrinchus* Mitchill, 1815) spatially and temporally overlap with a tidal turbine test site in Minas Passage, Nova Scotia

By Jessie Lilly, MSc, Acadia University, Wolfville, N.S., Canada

This work also appears as data chapter 2 in Jessie's MSc thesis.

Abstract

In 2009, the Fundy Ocean Research Centre for Energy (FORCE) was established to test tidal turbines in Minas Passage, a tidal strait that connects the inner Bay of Fundy to Minas Basin, Nova Scotia. Minas Basin is a summer feeding aggregation site for Atlantic Sturgeon (*Acipenser oxyrinchus*, Mitchill 1815) from endangered and threatened populations in Canada and the US. In this study, new High Residency (HR; V9-2x) acoustic tagging technology developed by VEMCO coupled with coded pressure measuring acoustic tags (V16) were used to determine if Atlantic Sturgeon passed through the FORCE test site. Also, detection efficiency of the two tag types was compared in response to environmental variation (current). Atlantic Sturgeon HR data was combined with detections from Atlantic Sturgeon tagged during previous years in Minas Basin with V16-69 kHz coded tagging technology. During 2018 and 2019, 77% (n = 41) and 31% (n = 23) of Atlantic Sturgeon tagged in Minas Basin were detected at the FORCE test site, respectively. Tagged Atlantic Sturgeon travelled pelagically through the FORCE test site and Minas Passage at similar depths to those proposed for tidal turbine operation. There was a decline in the presence of Atlantic sturgeon at the FORCE test site during flood tide, and a decline in their presence at the Ocean Tracking Network line in Minas Passage with increase current speed. The distance between the FORCE test site and Minas Passage is approximately 3km. Given the estimated abundance of the summer feeding aggregation of Atlantic Sturgeon that visit Minas Basin each summer, our study indicated that at current population estimates

at least ~ 2790 unique individuals may overlap with the FORCE test site annually. Future studies should tag a higher proportion of Atlantic Sturgeon with HR tagging technology to ensure that detections are recorded during high current speeds. Additionally, VEMCO positioning studies should be conducted to assess fine scale movement of Atlantic Sturgeon near an operating tidal turbine.

Introduction

The dramatic rise in greenhouse gas emissions over the last century has led to an urgent need for nations to develop clean and sustainable forms of energy (Jacobson 2009). Renewable energy production is mostly composed of hydroelectric and wind generation (Huaman and Jun 2014). Hydroelectric facilities extract energy by placing a barrage across an estuary, or river (Zhou et al. 2017). These facilities can influence the hydrology of rivers, and impact local fish populations (McCartney 2009; Dadswell et al. 2018). Fish passages created within hydroelectric facilities are often designed in a way that is suitable to a subset of species, and others are forced to move across spillways or through turbines (Cooke et al. 2002; Čada et al. 2006; Calles et al. 2012). Some studies have shown that there is a strong positive relationship between the size of fishes and their likelihood of injury when encountering turbines (Dubois and Gloss 1993; Amaral et al. 2014; Dadswell et al. 2018). Larger fish are less likely to evade turbine blades due to the close spacing and high revolutions per minute. For larger species such as Atlantic Sturgeon, mortalities observed downstream from the Annapolis Turbine site in Nova Scotia were mainly linked to mechanical strike.

Recently, industry has invested in the extraction of tidal energy through free standing hydrokinetic devices (HK; Jacobson 2009; Kedar and Fodase 2018). Hydrokinetic devices can operate on both flood and ebb tides. The turbine blades are propelled by marine currents and transfer energy to grid connected generators (Rourke et al. 2010; FORCE 2018). In comparison to traditional barrage style turbines, HK's are thought to reduce the potential impact on marine fauna since they do not span entire estuaries or streams (Shen et al. 2016; Bevelhimer et al.

2017). One is targeted for the deployment and operation of HK turbines is the Bay of Fundy, Nova Scotia, Canada.

The Bay of Fundy (BoF) has the highest tides in the world with a tidal range of 16m, making it a prime area for the development of tidal turbine technology. Minas Passage (MP) is a narrow corridor that is connected to the Bay of Fundy to Minas Basin (MB) (Karsten 2011). It has been estimated that approximately 7 GW of power could be extracted from MP, however this would result in an estimated 40% decrease in tides within MB but if only 2.5 GW of energy was extracted, the change in amplitude would only be 5% (Karsten et al. 2008).

With support of the federal and provincial government, the Fundy Ocean Research Center for Energy (FORCE) was established in 2009 to test tidal turbines on the northern shore of MP near Black Rock, Nova Scotia (FORCE 2018). Currently five companies hold test births in the region, but so far only Cape Sharp Tidal has tested their Open Hydro HK design. During 2016 – 2018, Cape Sharp Tidal Venture (CSTV) deployed a 16m diameter, 2 MW Open Hydro HK. Plans are in place to test other HK designs in this region within the next few years. Although HK's are proposed to have a lower impact on the environment, *a priori* environmental monitoring is required before the deployment of turbine arrays. A significant knowledge gap exists on the behavior of marine species around HK's (Roche et al. 2016; Fraser et al. 2018). A technology that holds promise for determining the movement, migration and behavior of fishes around HK deployments is acoustic telemetry.

Acoustic telemetry enables researchers to determine species specific behavior of fishes in the marine environment (Broome et al. 2015; Keyser et al. 2016). Starting in May each year, approximately 24 species of fish migrate into MB through MP to feed, including Atlantic Sturgeon and Striped Bass, *Morone saxatilis* (Walbaum, 1792). During 2014 and 2015 acoustically tagged Atlantic Sturgeon were monitored passing through the OTN line (Stokesbury et al. 2016). In 2015, Broome et al. (2015) used VEMCO (VEMCO/Innovasea Ltd, Bedford, NS) acoustic technology to monitor the movement of Striped Bass through the FORCE test site. The 69 kHz tags operate using the pulse position modulation system (PPM), emitting 8 transmission pulses with a nominal delay of 75 seconds. All 8 transmission pulses must be detected by a VR2W receiver in order to decode a tags unique ID (Guzzo et al. 2018). Broome et al. (2015) reported that Striped Bass detections were drastically reduced when current speeds within MP exceeded 2m/s. The long nominal delay of the signal may have prevented the detection of a majority of tagged Striped Bass. Recently, VEMCO has developed new High Residency (HR) acoustic tagging technology. This technology consists of tags that emit both the traditional PPM signal every 30 seconds at 180 kHz and a new HR signal every 5 seconds at 170 kHz (Guzzo et al. 2018; VEMCO 2018). Sanderson et al. (2017) reported that unlike the 69 kHz signal, the 170 kHz signal is impeded less by ambient noise when current speeds exceed 2 m/s within MP. Additionally, only one HR transmission pulse is required to be logged by a receiver in order to decode a tags unique ID; making this a promising technology to use when assessing the behavior of fish around HK devices in high flow environments.

From May-October approximately 9,000 sub-adult Atlantic Sturgeon (L_F >150cm F_L, age >26 years) inhabit MB annually (Dadswell et al. 2016) to feed on the large abundance of benthic organisms in the intertidal zone (McLean et al. 2013). Through genetic analysis of Atlantic Sturgeon captured in MB, Wirgin et al. (2012) determined that 61% of Atlantic Sturgeon within MB originated from the SJR, 34% from the Kennebec River (KR), 2% from the Hudson River and 1% from the James River. Atlantic Sturgeon from the SJR and KR are designated as threatened, and sturgeon from the Hudson River and James River are listed as endangered.

Atlantic Sturgeon remain within MB until October before migrating to overwintering habitats in the BoF and along the USA coast (Beardsall et al. 2016; Taylor et al. 2016). They migrate through MP at a depth of approximately 31 m, a similar depth as the proposed HK devices (Stokesbury et al. 2016). The behavior of Atlantic Sturgeon around HK's is unknown. Since Atlantic Sturgeon from both threatened and endangered populations migrate into MB, it is important to determine whether Atlantic Sturgeon will face temporal and spatial overlap with operating HK's. The objective of this study was to determine spatial and temporal overlap of Atlantic Sturgeon with the FORCE test site.

Methods

Study site description

MP is a 5km wide, 15km long passage that connects the BoF to MB (Karsten 2011). The current speed within MP is highest in the northern portion during flood tide, exceeding 5 m/s. Depths in this region of MP are the shallowest ranging between 30-55m, and water from the incoming flow is diverted northwards by Cape Split (Figure 1). During ebb tide current speeds remain even across the width of MP. Approximately 14 billion tons of water flows through MP during each flood tide and the high flow through the Passage creates a residual current of 0.8 m/s within MB (Godin 1968). MB is a shallow macrotidal embayment of approximately 115 000 hectares. At low tide, 2 km of mudflats are exposed, providing habitat to benthic invertebrates that feed on the diatoms and bacteria (Yeo and Risk 1979; Percy 2001). The benthic invertebrates within MB provide nutrition to many species of shore birds and bottom feeding fish who spend the summers in MB before migrating to overwintering habitats (Yeo and Risk 1979; McLean et al. 2013; Beardsall et al. 2016).

Receiver deployment and retrieval

FORCE test site

On May 4th, 2018 four VEMCO VR2W- 69 kHz and four HR2-180 kHz HR receivers attached to streamlined subsurface buoys (SUB) connected to moorings with acoustic releases were deployed at the W1, W2, D1 and S2 test sites (Figure 1; Figure 2). The bottom depth of the W1, W2, D1 and S2 sites were 57, 58, 44 and 81 m respectively. Receivers were attached to box-section aluminum mounts and mounted onto SUB buoy's (Figure 2). The HR2 receiver batteries last approximately 6 months and can record background noise (dB), tilt angle, temperature and interpret signals from traditional 180kHz acoustic tags as well as new HR2 180kHz tags. C-PODs were also mounted in the center of the SUB buoy as part of FORCE's monitoring program of Harbour Porpoise, *Phocoena phocoena* (Linnaeus, 1758). The last download occurred on August 14th, 2019; receivers were retrieved intermittently throughout deployment for battery changes. Receivers at the FORCE test site were deployed for 309 days in 2018 and 109 days in 2019. Deployment periods occurred during May 4th to August 14th, 2019.

Minas Passage

The Ocean Tracking Network (OTN) deployed 12 VEMCO VR2W-180 kHz and 69 kHz omnidirectional hydrophone receivers across MP seasonally from 2012 to 2018 (Figure 1). Data used in this study extended from May 4th to the latest download on November 26th, 2018. VR2W-180 kHz receivers have a battery life of 15 months and a maximum detection range within MP of 300 m during slack tide, which drops to 220 m when current speeds exceed 3 m/s during flood tide (Sanderson et al. 2017). VR2W- 69 kHz receivers have a farther range than 180 kHz receivers; during slack tide the range can extend up to 600m and declines to 300m during peak flow. The 8th receiver at the MP line was lost upon deployment. Data was unavailable from the MP line during 2019 due to failure of acoustic releases upon deployment.

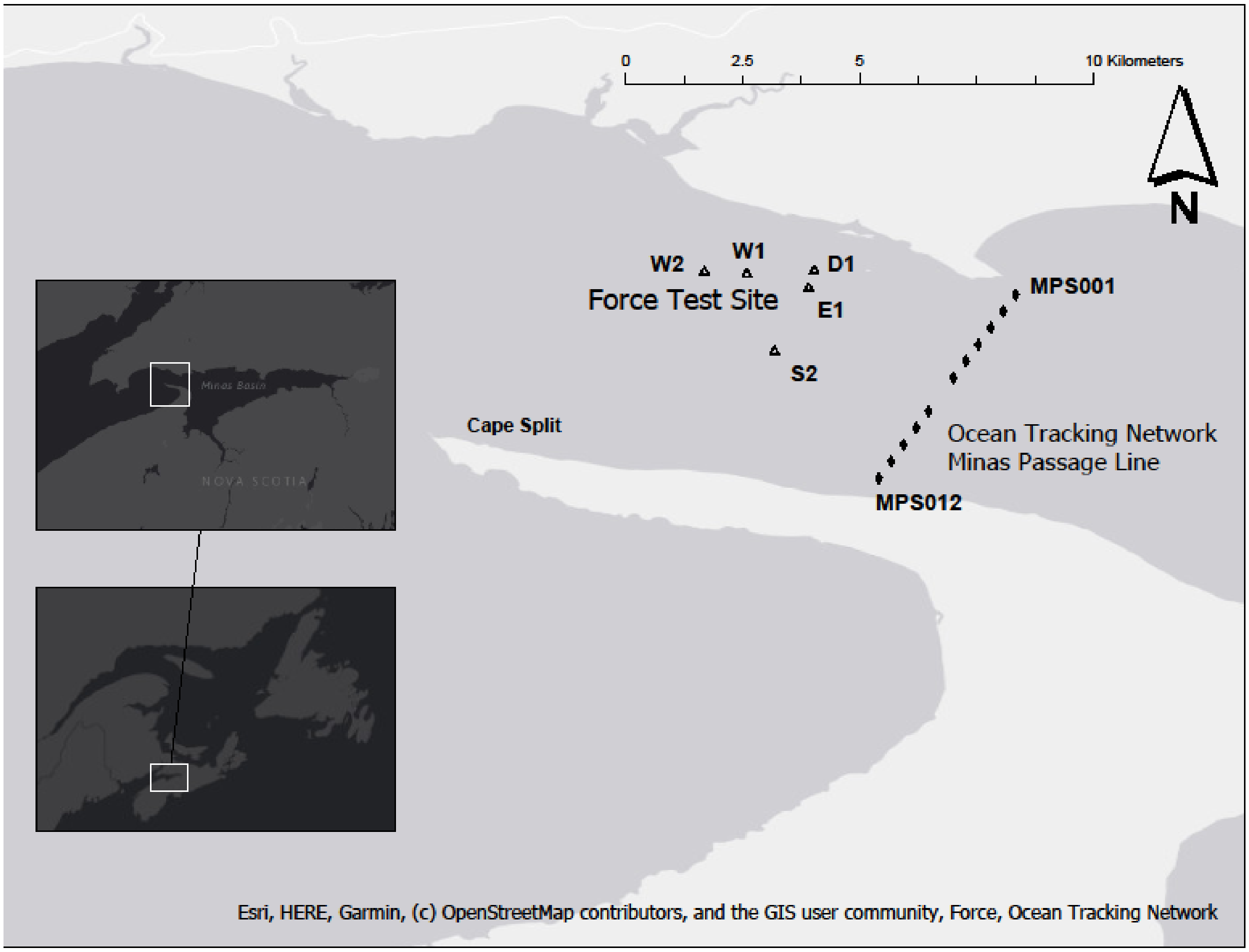

Figure 1. Location of moored receivers deployed by the Ocean Tracking Network (OTN) and the Fundy Ocean Research Center for Energy, respectively, across Minas Passage (2018) and the FORCE test site (2018, 2019).

Figure 2. Prior to deployment at the Fundy Ocean Research Center for Energy test site VR2W-69kHz and HR2-180kHz receivers were mounted to Streamlined Subsurface Buoys buoys by Dr. Brian Sanderson at Acadia University.

Atlantic Sturgeon Capture

From 2010 to 2018, Atlantic Sturgeon were captured and surgically implanted with V16-69 kHz acoustic tags (Table 1). In 2018, Atlantic Sturgeon were implanted with V9-HR 2x 180 kHz and with V16-6x 69 kHz acoustic tags in MB, Nova Scotia (Table 1). During 2019, Atlantic Sturgeon were double-tagged both with V9-2x and V16 acoustic tags, and some were tagged with V9-2x tags. Atlantic Sturgeon tagged prior to 2019 were captured by otter trawl and brush weir. Captain Glanville Travis of the vessel “Terri and Sandi” was hired to conduct Otter Trawls lasting between 30-60 minutes. The vessel consisted of a 24m box trawl net, with 14cm stretched mesh and modified rock hopper equipment (Dadswell et al. 2016). Weir fishing was conducted at locations in the intertidal zone of the southern and northern shores of MB. All weirs were composed of 2.5cm stretched mesh attached to 2.2m wooden posts shaped in a V. The tip of the V was directed towards the ocean and the length of the wings extended 1-2km. The walls of the weir are covered in brush, making it difficult for fish to view the walls when submerged during high tide (Porter et al. 2018). On ebbing tides Atlantic Sturgeon were funneled to a trap containing a pool of water located at the tip of the V where they were sample. During 2019, Atlantic Sturgeon were captured by drifting two attached monofilament gill nets within MB. The net was 190m long by 5m deep and the stretch mesh panels were 10 and 13cm, respectively. Nets were drifted for periods ranging from 15 – 30 minutes.

All fishing was conducted under the Department of Fisheries and Oceans Scientific License to Fish #322595. Fishing conducted during 2019 was registered under license # 330657. Atlantic Sturgeon surgical procedures were performed under Acadia Animal Care Committee protocol #07-11.

Acoustic Tagging

Atlantic Sturgeon were measured for fork (FL) and total length (TL). External identification Floy dart tags (Floy Tag & Manufacturing Inc., Seattle, Washington) were inserted into the dorsal fin and anchored around the pteriogyte bone (Dadswell et al. 2016). For surgeries that took place at the weir, Atlantic Sturgeon were placed onto their dorsal side in a shallow pool (Logan-Chesney 2016). On the trawl and after gill net capture Atlantic Sturgeon were placed onto a tarp on a flat bin located on the vessel. Prior to the surgeries, all instruments and tags were disinfected with 10% Betadine ^{TM} solution and rinsed with 0.9 % sodium chloride solution. A no.22 scalpel was used to make a 30mm incision 40-60mm below the pelvic fin. V16 tags were activated by removing an activation magnet, and V9-2x tags were activated by using a VEMCO tag activator. Tags were inserted into the peritoneal cavity through the incision. Absorbable #1 and #0 monofilament polydioxanone sutures (PDS) with reverse cutting edge were used to make two double square knot suture to close the incision (Johnson and Johnson, Markham, Ontario; Moser et al. 2000). Size 0 sutures were used after 2017, as the larger needle size makes it easier to puncture through sturgeons' tough skin and reduce surgery time.

Table 1. Summary of V16-69 kHz and V9-2x tag types implanted in Atlantic Sturgeon during 2010-2019 (n = 194). Atlantic Sturgeon were not tagged during 2013, and 10 Atlantic Sturgeon were double tagged with V16P-6x and V9-2x tags during 2019.

Year	VEMCO tag type	Weight (g)	Length (mm)	Power	Number Deployed	Battery life (days)	Approx. tag death (yyyy-mm)
2010	V16-6x	95	34	158	15	1633	2015-02
	V16P-6x	98	36	158	10	1287	2014-02
	V16TP-6x	98	36	158	5	1609	2015-01
2011	V16P-6x	98	36	158	53	1287	2015-01
2012	V16-6x	95	34	158	20	1633	2017-02
	V16P-6x	95	36	158	15	1287	2016-04
						1581	2016-12
2014	V16P-6x	95	36	158	10	2751	2021-12
2015	V16-6x	95	34	158	6	1633	2019-11
	V16P-6x	95	36	158	7	2751	2023-01
2016	V16P-6x	95	36	158	2	2751	2024-03
2017	V16P-6x	95	36	158	4	2282	2023-11
2018	V16P-6x	95	36	158	4	2282	2025-09
	V9-2x	26.10	3.70	143	23	320	2020-06
2019	V16P-6x	95	36	158	10	2282	2025-10
	V9-2x	26.10	3.70	143	20	159	2020-01

Data Analysis

Using Vemco User Environment (VUE), OTN offloaded .vrl files from VR2W-69 kHz and 180 kHz receivers at the MP line, and VR2W-69 kHz receivers at the FORCE test site. Vdat files from the HR2-180 kHz receivers were offloaded using Vemco's FATHOM software. All data files were transformed to .csv files and analyzed in R version 3.5.0 (R Development Core Team 2018). Data was filtered for false detections using the *GLATOS* package (Holbrook et al. 2018).

Detections were considered false if the duration between consecutive detections was less than the tags minimal nominal delay (V16-69 kHz: 30 seconds (PPM), V9-2x: 5 seconds (HR2) and 30 seconds (PPM)).

Initial Analyses

Sturgeon Days

"Sturgeon days" were calculated for each site at FORCE during 2018 and 2019 and on the MP line during 2018. Sturgeon days were referred to as days when a single sturgeon was detected (Broome 2014; Keyser et al. 2016). The proportion of sturgeon days at all receivers within the FORCE site was compared to the total possible number of days to determine how frequently Atlantic Sturgeon use the FORCE test site during 2018 and 2019. The total number of possible detection days was equal to the duration between the first and last detection of an Atlantic Sturgeon at the FORCE test site and MP line. A Watsons two sample non-parametric test was used to determine if the distribution of sturgeon days was significantly different between the FORCE test site and MP line.

Comparing HR Signals

A χ^2 test of independence was used to determine if there were significant differences in the frequencies of detections versus current speed between the HR-170 kHz and PPM-180 kHz signal of Atlantic Sturgeon tagged with V9-2x tags. If all signals of the HR- 170 kHz and PPM- 180 kHz are equally received by an HR2 receiver then the ratio between signals should be 6, since the PPM signal is emitted approximately 30 seconds and the HR signal every 5 seconds. This value was used to compare the efficiency of the PPM to HR signal at the FORCE test site during 2018 and 2019.

Environmental Factors

A binomial General Linear Model (GLM; link=logit) was fit in R using maximum likelihood estimation to determine whether current speed, water level, and time of day (day versus night, and tide type (flood versus ebb) were predictors of Atlantic Sturgeons hourly presence at the FORCE test site and MP line (Zuur et al. 2009). Data was available from the FORCE test site during May 5th, 2018 to August 14th, 2019. Current speed and water level data was obtained through a hydrodynamic model of the Bay of Fundy (Karsten et al. 2008). Using the R packages rgeos (Bivand and Rundel 2018) and map tools, (Bivand and Lewin-Koh 2018) sunrise, sunset and dawn and dusk were calculated from the mean time of detection of each interval (Keyser et al. 2016). Sturgeon days were split into one-hour periods, and the detection of a sturgeon during a one-hour period was modelled as a binary response (1: sturgeon presence; 0: sturgeon absence) (Martin et al. 2019). Collinearity was assessed using Spearmen's rank correlation tests; correlations between variables did not exceed 0.5 thus all variables were retained during model selection (Hollensead et al. 2018). A stepwise backwards selection procedure was used to select the model with the lowest Akaike Information Criterion (AIC) using the step function in R (Zuur et al. 2013). This selection procedure started with the full model and variables were removed until the model with the lowest AIC value was fit; statistically significant variables were retained following the last step ($p<0.05$) (Hollensead et al. 2018). To assess the goodness of fit of a model, a likelihood ratio test was used to compare the final model to the null model (intercept only model). McFaddens R^2 was used to assess model fit, this pseudo R^2 is more robust to changes in sample size, number and distribution of parameters than other tests (Hemmert et al. 2018). A good fitting model has an McFaddens R^2 value between 0.2-0.4 (Bass et al. 2018).

Atlantic Sturgeon depth

A binomial General Linear Model (GLM; link=logit) was fit in R using maximum likelihood estimation to determine whether current speed (m/s), water temperature (°C), time of day (day versus night and tide type (flood versus ebb) were predictors of hourly presence in the top half of the water column at both the FORCE test site and MP line. Atlantic Sturgeon presence was modelled as "sturgeon hours" to help control for the non-independence of detections. A sturgeon hour was defined as the presence of individual sturgeon during an hour.

When water levels were at 0 m, bottom depth was estimated by adding/subtracting the bottom depth obtained upon receiver deployment at the FORCE test site/MP line to the water level estimated through the hydrodynamic model during the time of deployment. For the W1, W2, D1 and S2 site the zero tide levels were 44.4m, 39.61m, 28.63m and 68.21m and respectively. For the MPS line zero tide levels ranged between 30.65m at MPS001 to 123.208m at MPS007 (Table 2.2). Water level during each hour was added to the zero tide levels for an accurate representation of bottom depth during time of detection. Atlantic Sturgeon depth reading during a sturgeon hour was relative to their distance below the surface. The average location of Atlantic Sturgeon within the water column during a sturgeon hour was modeled as their proportion off the bottom. Where the proportion off the bottom was calculated by:

$$Proportion = \frac{Bottom\ depth - mean\ hourly\ Atlantic\ Stureon\ depth}{Bottom\ depth}$$

Hourly proportion within the water column was modelled as a binary response, where proportions > 0.50 were assigned a one, indicating that Atlantic Sturgeon were in the upper water column. Proportions < 0.50 were assigned a zero indicating that Atlantic Sturgeon were in the lower water column. Collinearity was assessed using spearmen's rank correlation tests and all variables were retained (<0.5; Hollensead et al. 2018). A stepwise backwards selection procedure was also used to select the model with the lowest AIC.

Table 2. Bottom depth at the 0m water level at each receiver site deployed within Minas Passage (MPS). Bottom depth was calculated for Minas Passage by adding/subtracting the bottom depth obtained upon receiver deployment to the water level estimated through the hydrodynamic model at the time of deployment.

MPS Site	001	002	003	004	005	006	007	008	009	010	011	012
Level (m)	30.65	46.68	72.13	82.34	80	83.10	123.21	117.50	102.59	70.67	46.6	41.3

Results

Atlantic Sturgeon Behavior

During 2018 and 2019, approximately 40% of individuals reported to have had active tags were detected at the FORCE test site (Table 3). In comparison to the FORCE test site, a higher proportion of individuals were detected at the MP line (Table 3). The mean L_F of Atlantic Sturgeon tagged in 2018 and 2019 and detected at the FORCE test site and MP line (n = 19) was 133.24 ± 26.04 cm, indicating that they were sub adults. Individuals from the Saint John (FORCE, n=2; MP, n = 3) and Kennebec (FORCE: n=4; MP, n = 6) Rivers were detected at the FORCE test site and MP line in 2018 and 2019. Atlantic Sturgeon were detected on <9% and <30% of possible detection days at the FORCE test site in 2018 and 2019, and <20% of possible detection days at the MP line in 2018 (Table 3).

Table 3. Detection summary for Atlantic Sturgeon detected at the Fundy Ocean Research Center for Energy (FORCE) test site and Minas Passage (MP) line in 2018 and 2019. Sturgeon tagged during 2010-2017 are referred to as <2018 in the year column, sturgeon tagged prior to 2019 are referred to as <2019 in the year column. The number of possible detection days for each Atlantic Sturgeon was dependent on the first and last detection at the FORCE test site and MP line, as well as the date the individual was tagged and released. In 2018, data from V9-2x and VR2W-69 kHz receivers was obtained from the FORCE test site and MP from, May 5th to November 16th and April 21st to November 18th, respectively. In 2019, Atlantic Sturgeon were detected by HR2 and VR2W-69 kHz receivers at the FORCE test site from May 9th to August 8th.

Year Tagged	Date Tagged	No. active tags	No. possible detection days	No. detected (%)	Detection days (%)

Year			FORCE	MP	FORCE	MP	FORCE	MP
<2018		26	175	198	8	23	15	40
2018	June 13th	4	136	158	3	3	3	6
2018	July 8th	2	111	132	0	1	0	3
2018	July 17th	7	102	123	1	3	2	5
2018	July 19th	14	100	121	7	7	7	13
	Total	53	-	-	19	37	24	57
<2019		53	91	-	21	-	28	-
2019	July 13th	5	26	-	1	-	1	-
2019	July 18th	1	1	-	-	-	0	-
2019	July 19th	4	4	-	1	-	1	-
2019	July 23rd	5	5	-	-	-	0	-
2019	August 2nd	6	6	-	-	-	0	-
	Total	74	-	-	23	-	30	-

Atlantic Sturgeon detection days were not evenly distributed throughout all months of the study (Figure 3). Typically, Atlantic Sturgeon were initially detected at FORCE and MP in late May-early June and were last detected in September-October, with peaks in detections occurring during mid-summer and early fall (Figure 3). The highest proportion of Atlantic Sturgeon days at FORCE and the MP line occurred at the S2 site (2018: n =15, 50%; 2019: n = 9, 43%; Figure 4), and MPS001 site (2018: n = 24, 42%; Figure 4), respectively. Atlantic Sturgeon did not appear to exhibit a preference for what region of Minas Passage they utilized each month (Figure 5).

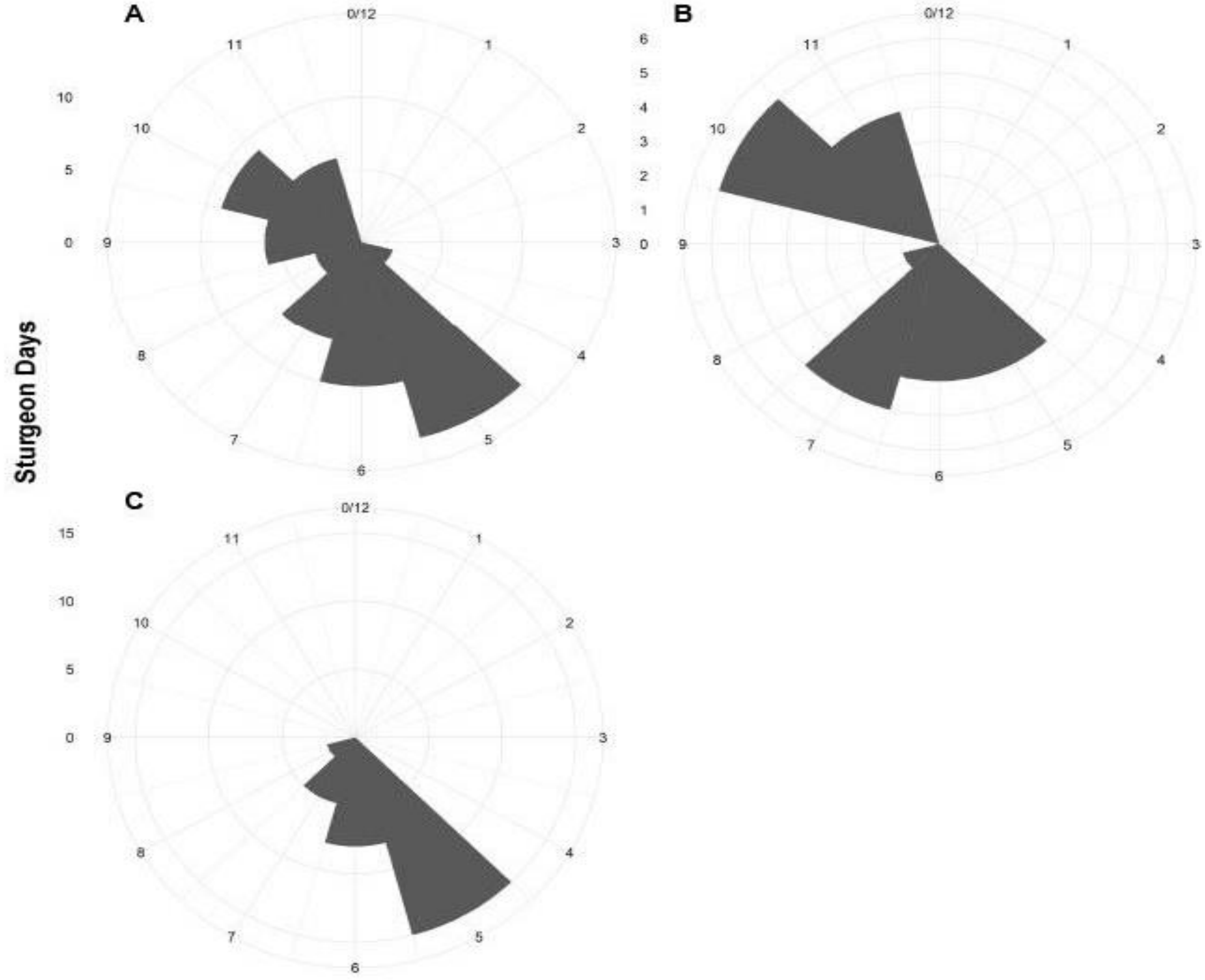

Figure 3. Month versus days Atlantic Sturgeon (sturgeon days) were detected at the Fundy Ocean Research Center for Energy test site (A) and Minas Passage line (B) in 2018 and at the FORCE test site during 2019 (C). In 2018, data from V9-2x and VR2W-69 kHz receivers was obtained from the FORCE test site and MP from May 5th to November 16th and April 21st to November 18th, respectively. In 2019, Atlantic Sturgeon were detected by HR2 and VR2W-69 kHz receivers at the FORCE test site from May 9th to August 8th.

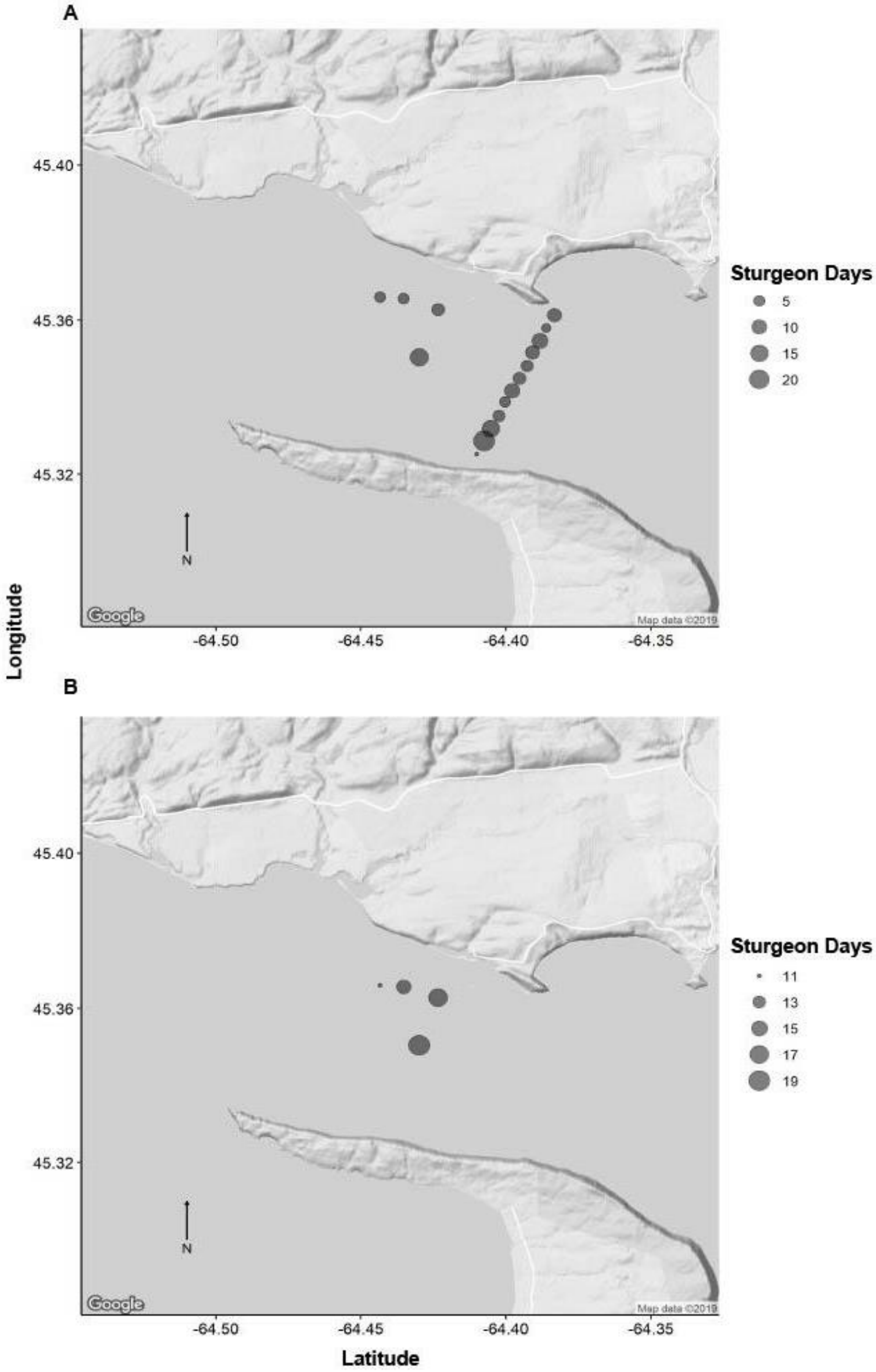

Figure 4. Map of the number of days Atlantic Sturgeon (sturgeon days) were detected at the Fundy Ocean Research Center for Energy (FORCE) test site and Minas Passage line (MP) (A) during 2018 and at the FORCE test site during 2019 (B). In 2018, data from V9-2x and VR2W-69 kHz

receivers were obtained from the FORCE test site and MP from May 5th to November 16th and April 21st to November 18th, respectively. In 2019, Atlantic Sturgeon were detected by HR2 and VR2W-69 kHz receivers at the FORCE test site from May 9th to August 8th. Data was not available for receivers at the MP line during 2019.

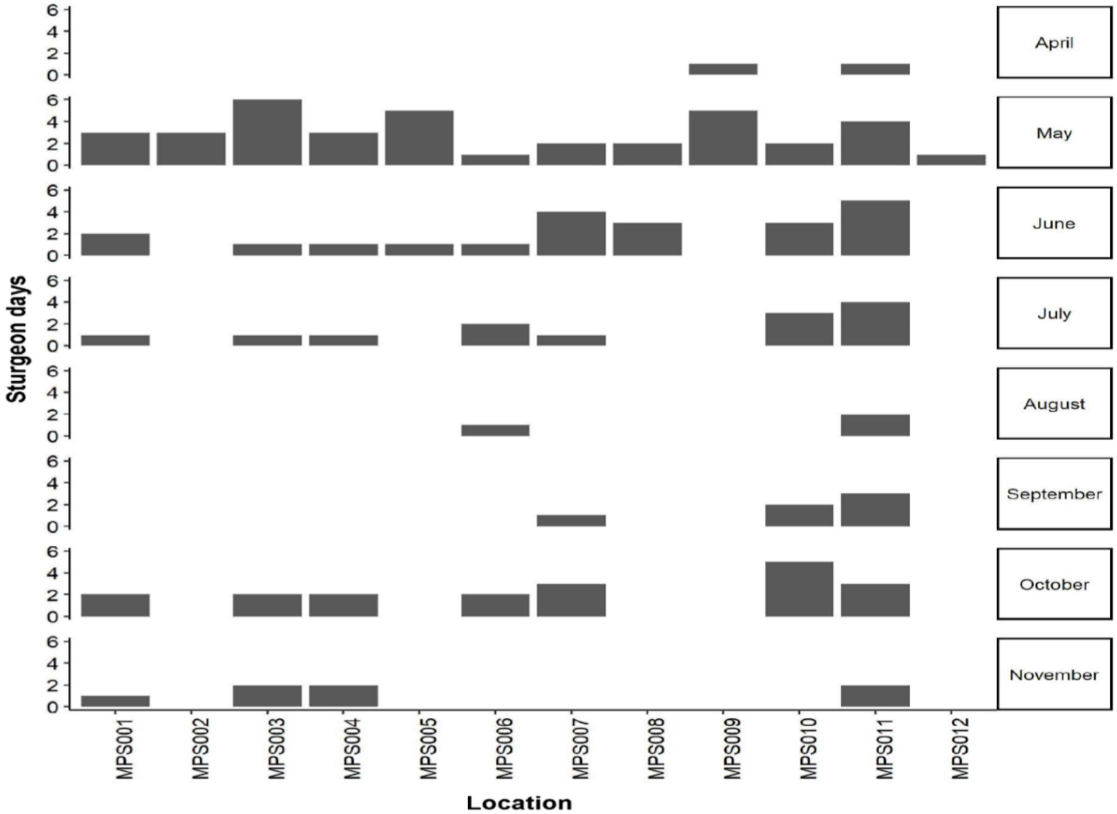

Figure 5. Distribution of Atlantic Sturgeon days versus month and site at the Minas Passage line. Atlantic Sturgeon were detected from April 21st to November 18th, 2018. Data from the Minas Passage line was not available for 2019.

Comparison between sites

In 2018, there was no significant difference between the distribution of days Atlantic Sturgeon were detected at the FORCE test site and MP line ($U^2 = 0.08$, $p > 0.10$). There were 17 days, from May-November 2018, where 14 sturgeon were detected at both sites. There did not appear to be a significant difference between the estimated swim speed of Atlantic Sturgeon and the mean current speed during consecutive detections between FORCE to MP (Table 4; two sample t-test, t = 1.47, df = 4, p = 0.22). However, the estimated swim speed of Atlantic Sturgeon was significantly higher during consecutive detections between MP to FORCE (Table 4; two sample t-test, t = 4.82, df = 6, p = 2 × 10^{-3}).

Table 4. Duration between consecutive detections of Atlantic Sturgeon on the same day between the Fundy Ocean Research Center for Energy test site and Minas Passage line. Consecutive detections occurred during May 11th to November 13th, 2018.

Site	No. Sturgeon	No. cons. detections	Avg. duration cons. Detections (hh:mm ± SD)	Avg.Sturgeon swim speed (m/s)	Avg. current speed (m/s)
FORCE - MP	10	12	00:16 ± 00:08	3.95 ± 2.29	1.49 ± 0.93
MP - FORCE	7	13	00:21 ± 00:26	2.44 ± 0.62	1.06 ± 0.51

HR versus PPM acoustic signals

Atlantic Sturgeon tagged with V9-2x tags were detected at current speeds ranging from 0 to 3 m/s, during both ebb and flood tide at the FORCE test site in 2018 (n = 8) and 2019 (n = 16; Table 5). There was not a significant difference between the frequencies of detections and current speed between the HR-170 and PPM-180kHz (n = 212, χ^2= 3.72, p = 0.44, df = 4; Figure 2.6) during 2018. In 2019, however, there was a significant difference (n = 956, χ^2= 43.20, p = 3.52 × 10^{-7}, df = 7) likely attributed to the increase in signal receptions during this year (Figure 6). A large proportion of possible PPM signals were missed at all current speeds; ratios ranged between 0 - 15.50 (Table 5). Ratios appeared to be high regardless of current speed or tidal stage (ebb versus flood; Table 5).

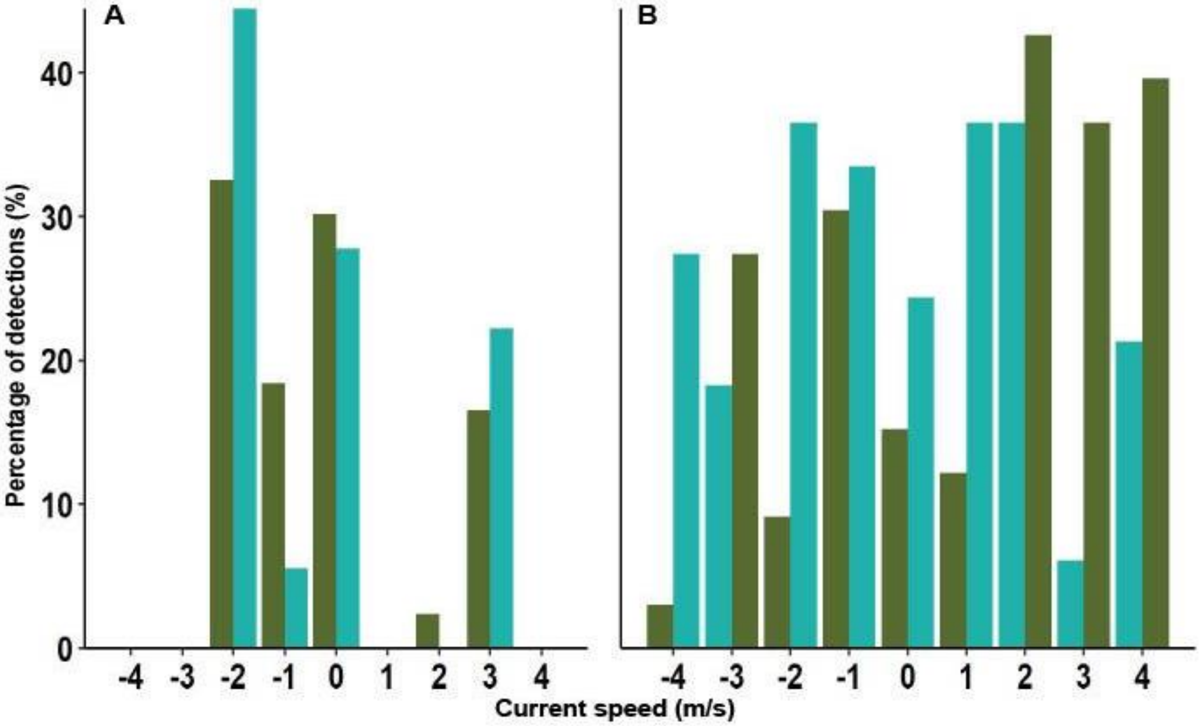

Figure 6. Comparison of the percentage of detections versus current speed of the two acoustic signals (HR-170 kHz, PPM- 180kHz) emitted from V9-2x high residency (HR) acoustic technology during 2018 (A) and 2019 (B). Green and blue bars represent detections from the HR-170 kHz signal and the PPM-180 kHz signal, respectively. The V9-2x tags emit a HR-170kHz, and PPM-180kHz signal with a nominal delay of five and 20 seconds, respectively. During 2018 Atlantic Sturgeon tagged with V9-2x tags were detected at the FORCE test site from July 21st to November 16th, and during 2019 Atlantic Sturgeon were detected from May 14th to August 11th. Data was only available at the FORCE test site to August 14, 2019.

Table 5. Comparison of detections from HR-170 kHz and PPM-180 kHz signals at varying current speeds at the Fundy Ocean Research Center for Energy (FORCE) test site from May 4th to December 2nd, 2018. Atlantic Sturgeon were internally tagged with V9-2x transmitters which emit an HR and PPM signal every 5 and 30 seconds, respectively. If both signals are detected equally

than the ratio between HR to PPM would be 6. Negative current speed values correspond to ebb tide and positive current speed values correspond to flood tide.

	No. of detections					
Current speed (m/s)	HR		PPM		Ratio (HR/PPM)	
	2018	2019	2018	2019	2018	2019
-3	0	35	0	2	NA	17.50
-2	69	80	8	6	8.63	13.33
-1	39	334	1	55	39	8.56
0	64	134	5	48	12.80	10.31
1	0	93	0	6	NA	15.50
2	5	65	0	6	NA	10.83
3	35	38	3	1	11.67	3.12
4	0	48	0	4	N/A	12

V16-6x versus V9-2x signals

During 2018 and 2019 Atlantic Sturgeon tagged with V16-6x (2018: n = 9; 2019: n = 7) and V9-2x tags (2018: n = 8; 2019: n = 7) were detected at current speeds ranging between 0 – 4 m/s (Figure 7). However, in comparison to V16-6x detections a higher proportion of V9-2x signals were detected at current speeds greater than 3m/s (Figure 7). Detections of the V9-2x tag were more likely to occur during flood tide than ebb tide, and detections of the V16-6x tags were more likely to occur during ebb tide than flood tide (Figure 7; Table 6).

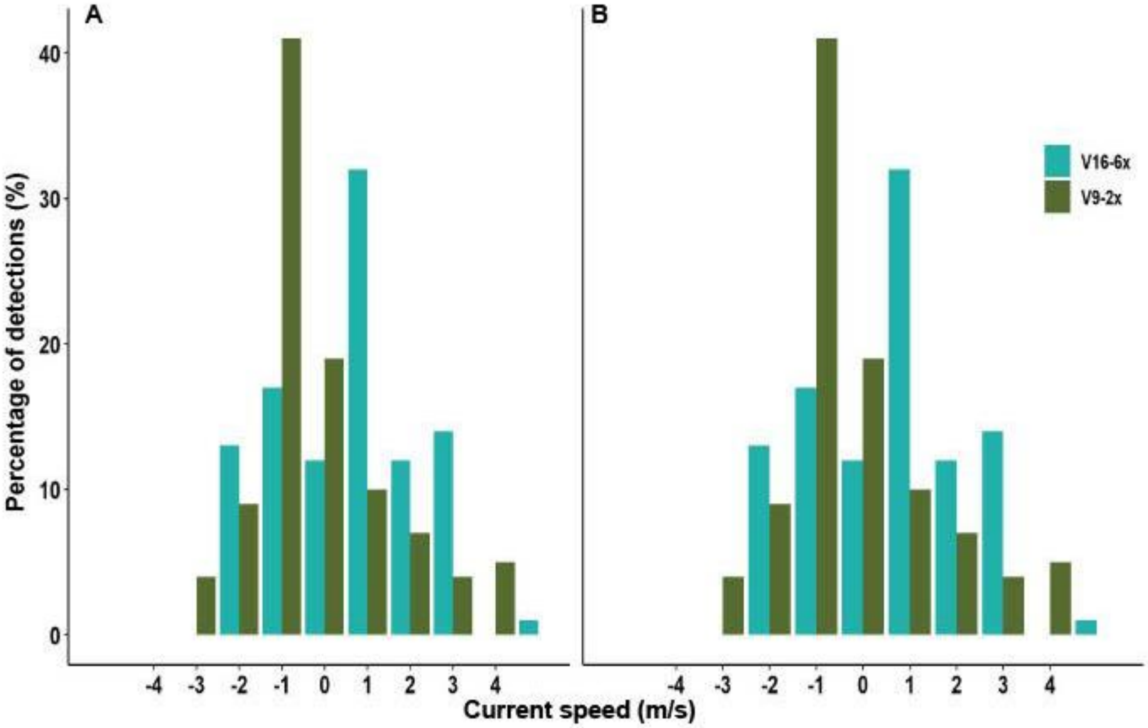

Figure 7. Comparison of the percentage of detections versus current speed of Atlantic Sturgeon tagged with V16-6x 69 kHz and V9-2x 180 kHz tags during 2018 (A) and 2019 (B). Green and blue bars represent detections from the V16-6x signal and the V9-2x signal, respectively. The V16-6x tags emit a 69kHz signal with a nominal delay between 20-45 seconds. The V9-2x tags emit a HR-170kHz, and PPM-180kHz signal with a nominal delay of five and 20 seconds, respectively. During 2018 and 2019 Atlantic Sturgeon tagged with V16-6x tags were detected at the FORCE test site during May 5th to November 6th and May 9th to August 8th, respectively.

Table 6. Comparison of detections from V16-6x and V9-2x tags during different tide types at the Fundy Ocean Research Center for Energy test site during 2018 and 2019. During 2018 and 2019 Atlantic Sturgeon tagged with V16-6x tags were detected at the FORCE test site during May 5th to November 6th and May 9th to August 8th, respectively. During 2018 Atlantic Sturgeon tagged with V9-2x tags were detected at the FORCE test site from July 21st to November 16th, and during 2019 Atlantic Sturgeon were detected from May 14th to August 11th. Data was only available at the FORCE test site to August 14, 2019.

	% of Detections			
	V16-6x		V9-2x	
Tide Type	2018	2019	2018	2019
Flood	48	59	19	27
Slack	42	12	30	19
Ebb	27	30	50	54

Depth versus environmental predictors

FORCE test site

During 2018 (n = 8) and 2019 (n = 7) Atlantic Sturgeon with depth tags were detected on 14 and 9 days, respectively at the FORCE test site. When comparing Atlantic Sturgeon depth to the total bottom depth, Atlantic Sturgeon were mostly detected at a proportion of 0.51 ± 0.19 from the bottom (range = 0 to 0.87). Atlantic Sturgeon appeared to remain within 30% of the bottom regardless of current speed, water level, time of day or temperature (Figure 8).

The full binomial GLM (AIC: 50.76) indicated that mean hourly current speed (m/s; z = 1.20, p = 0.23), temperature (°C ; z = 0.72, p = 0.47) , level (m; z = 0.74, p = 0.46), time of day (day/night; LightNight, z = -0.33, p = 0.74), and tide (ebb versus flood; TideFlood, z = -2.42, p = 0.08) were not significant predictors of Atlantic Sturgeon presence in the top half of the water column (Table 7). The final model (AIC: 43.81) included tide (ebb/flood; TideFlood, z = -1.90, p = 0.06) and was significantly different from the null model (LRT, χ^2 = 5.20, df = 1, p = 0.02). McFaden's R^2 was 0.12, suggesting that the variation explained by the model was low. The odds of detecting an Atlantic Sturgeon in the upper water column were slightly higher during flood versus ebb tide (Table 7). The probability of presence in the upper water column during an ebb and flood tide was 9.09% and 5.42%, respectively.

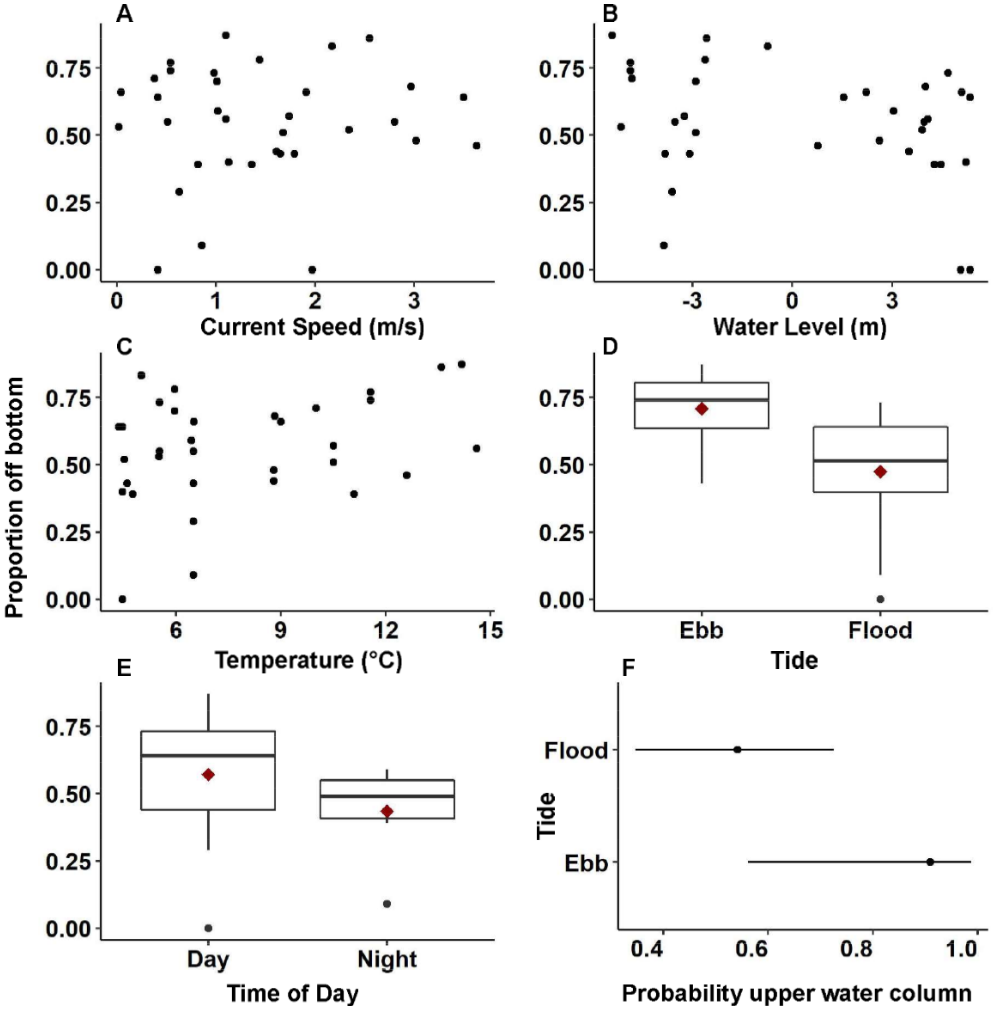

Figure 7. Characteristics (A, current speed; B, water level; C, water temperature; D, tide type; E, time of day) influencing Atlantic Sturgeon (n = 8) mean hourly depth distribution (proportion off the bottom) at the FORCE test site from May 5th to July 11th, 2018 and May 9th to August 9th 2019. Data was available from VR2W-69 kHz receivers until August 14th, 2019. The horizontal line in the boxplots represents the median proportion, and the red dot represents the mean. The only significant predictor of Atlantic Sturgeons hourly presence in the upper water column was tide type (ebb/flood; F). Error bars (F) represent the 95% confidence intervals of the predicted probability of observing Atlantic Sturgeon in the upper water column.

Minas Passage line

Atlantic Sturgeon tagged with V16P-6x tags (n=16) were detected at the MP line on 27 days. When comparing Atlantic Sturgeons depth to the total bottom depth, Atlantic Sturgeon were mostly detected at a proportion of 0.57 ± 0.24 (range: 0.14 to 0.89) from the bottom. Atlantic Sturgeon appeared to be evenly distributed throughout the water column regardless of current speed, water level, time of day and tide (Figure 8).

The full binomial GLM (AIC: 68.91) indicated that mean hourly current speed ($z = 0.004$, $p = 0.99$), water level ($z = 0.52$, $p = 0.60$) time of day (day/night; LightNight, $z = 0.23$, $p = 0.82$) and tide (ebb versus flood; TideFlood, $z = 1.46$, $p = 0.14$) were not significant predictors of Atlantic Sturgeon presence in the top half of the water column (Table 7). The final model (AIC: 63.25) included mean hourly temperature ($z = -2.48$, $p = 5.0 \times 10^{-2}$) and tide (ebb/flood; TideFlood, $z = 1.50$, $p = 0.14$). Temperature was the only significant predictor of Atlantic Sturgeon depth distribution; therefore, tide was dropped from the model (LRT, $\chi^2 = 2.24$, df = 1, $p = 0.13$; AIC: 63.50; Table 7). McFaden's R^2 was 0.05, suggesting that the variation explained by the model was low. The odds of detecting an Atlantic Sturgeon in the upper water column are predicted to decrease by 0.75 for a one unit increase in temperature (Table 7). The probability of presence in the upper water column during the mean (10.4 °C), min (5°C) and max (16.7 °C) temperature was 65%, 85% and 19%, respectively (Figure 8).

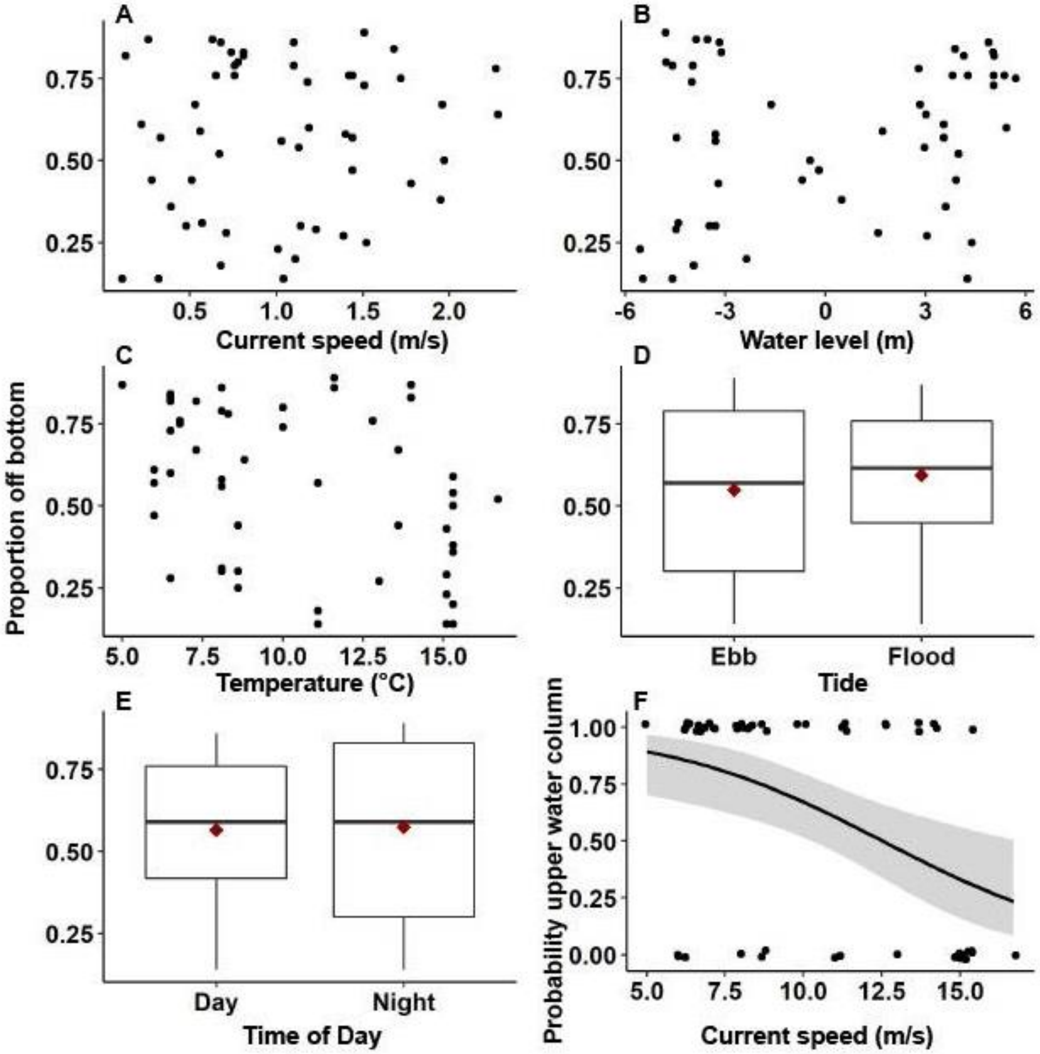

Figure 8. Potential factors influencing Atlantic Sturgeons (n = 16) mean hourly (n = 52) depth distribution (proportion off the bottom) at the Minas Passage line from May 5th to November 4th, 2018. Depth was modelled as the proportion of Atlantic Sturgeon that were located within the water column from the bottom; where one corresponded to the upper water column (> 0.50) and zero corresponded to the lower water column (> 0.50). Current speed (m/s;A), water level (m; B), Tide (ebb/flood; C), light (night/day; D) were not significant predictors of Atlantic Sturgeon use of the upper water column. Temperature (° C) was a significant predictor of Atlantic Sturgeon presence in the upper water column (F). The probability of observing Atlantic Sturgeon within the water column decreased as temperatures rose to 16.7 °C (E). The shaded regions represent the 95% confidence intervals (F).

Table 7. Full binary logistic models (GLM) obtained using in backwards selection to assess factors that influence Atlantic Sturgeon hourly presence in the upper water column at the Fundy Ocean Research Center for Energy test site and Minas Passage line (Full). Data used for the FORCE test site included Atlantic Sturgeon detections that ranged from July 21st to November 16th, 2018 and May 14th to August 11th, 2019. Data used for the MP line consisted of Atlantic Sturgeon detections from May 5th to November 4th, 2018. Backwards selection indicated that the model with the lowest AIC for the FORCE test site included tide type (ebb/flood). The model with the lowest AIC for the MP line included temperature (°C) and tide type (ebb/flood) (M1), however temperature was the only significant variable and was included in the final model (M2).

Location	Model	AIC	Parameters	Estimate ± SE	z-value	P-value	Odds ratio
FORCE	Full	50.761	Intercept	1.93 ± 1.61	1.20	0.23	
			Speed	-0.14 ± 0.41	-0.35	0.73	0.87
			Temperature	0.10 ± 0.15	0.72	0.47	1.11
			Level	0.10 ± 0.13	0.74	0.46	1.10
			Tide-Flood	-2.42 ± 1.39	-1.75	0.08	0.09
			Light-Night	-0.32 ± 0.99	-0.33	0.74	0.73
	Final	43.81	Intercept	-2.30 ± 1.05	2.20	0.03	
			Tide-Flood	-2.14 ± 1.23	-1.90	0.06	0.12
MP	Full	68.91	Intercept	3.03 ± 1.34	2.25	0.02	
			Speed	-0.002 ± 0.66	0.004	0.99	1.0

		Temperature	-0.27 ± 0.66	-2.71	7.0×10^{-3}	0.76
		Level	0.04 ± 0.08	0.52	0.60	1.04
		Tide-Flood	1.00 ± 0.69	1.46	0.14	2.74
		Light-Night	0.18 ± 0.79	0.23	0.82	1.20
Final	63.50	Intercept	3.55 ± 1.08	3.28	1.0×10^{-3}	
		Temperature	-0.28 ± 0.09	-3.03	5.45×10^{-2}	0.75

Environmental predictors of Atlantic Sturgeon presence

FORCE

Atlantic Sturgeon (2018: n = 19; 2019: n = 30) were detected a total of 85 hours (2018: n = 35; 2019: n = 50) at the FORCE test site. The full binomial GLM (AIC: 642.27) indicated that, current speed (m/s; z = - 2.04, p = 0.84), water level (m; z = 1.12, p = 0.26) , tide (flood; p = -5.96, p = 2 × 10^{-9}), and light (night; z = 0.55, p = 0.58) were not significant predictors of Atlantic Sturgeon presence at the FORCE test site (Table 7).

The final model (AIC: 637.81) obtained through backward selection included tide type as a predictor (Flood; z = - 6.08, p = 1.17 × 10^{-9}) of Atlantic Sturgeon presence, although the deviance explained by the model was low (McFadens R^2 = 0.07; Table 8). The probability of detecting an Atlantic Sturgeon during a sturgeon hour on flood and ebb tide was 3% and 12%, respectively (Table 8; Figure 9).

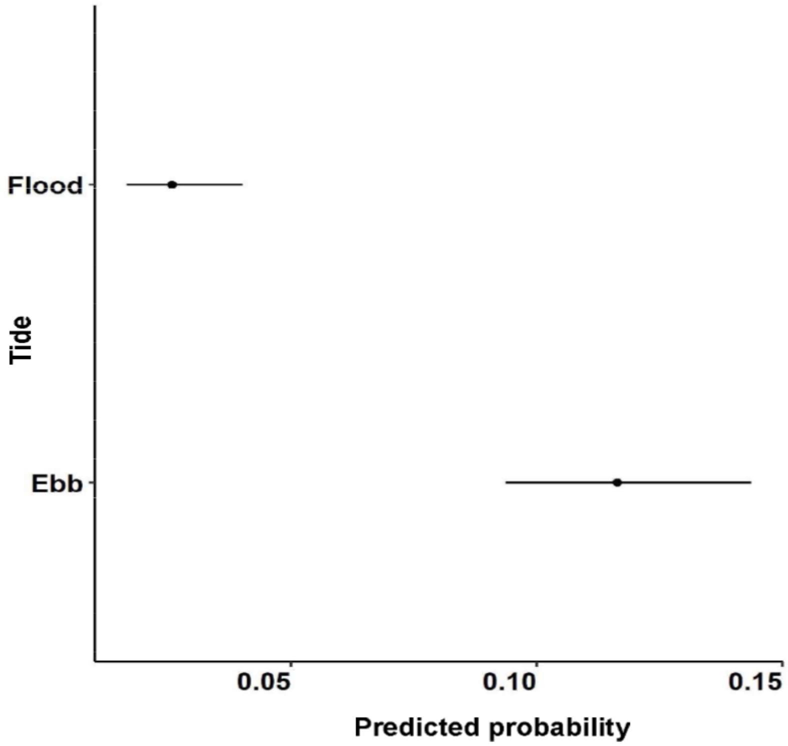

Figure 9. Model predicted probability of Atlantic Sturgeon hourly presence at the Fundy Ocean Research Center for Energy (FORCE) test site on a sturgeon day. Sturgeon days occurred during May 5th to November 16th in 2018 and during May 9th to August 9th in 2019. An Atlantic Sturgeon day was defined as a day when a sturgeon was detected by VR2W-69 kHz or V9-2x HR acoustic receivers (Keyser et al. 2016). The model obtained through backwards selection consisted of tide type (ebb/flood; B); tide type was the only significant predicter of Atlantic Sturgeon presence. Graphs were created using the model predicted probability (Lennox et al. 2017).

MP

Atlantic Sturgeon (n = 37) were detected on a total of 112 hours at the MP line during 2018. The full binomial GLM (AIC: 755.72) indicated that mean hourly water level (m; z = 0.13, p = 0.67), time of day (Night; z = -1.72, p = 0.24) and tide (Flood; z = 0.17, p = 0.87) were not significant predictors of Atlantic Sturgeon hourly presence on a sturgeon day at the MP line. The model with the lowest AIC (751.34) included speed (z = -2.75, $p = 5.91 \times 10^{-3}$) as a predictor (Table 8). The model containing speed was significantly different then the null model (LRT, $\chi^2 = 7.81$, df = 1, p = 5.19×10^{-3}), but the deviance explained by the model was low (McFadens $R^2 = 0.01$). For a one unit increase in current speed, the odds of detecting an Atlantic Sturgeon decreased by 0.74 (Table 8). The probability of detecting an Atlantic sturgeon during the mean (1.52 m/s), minimum (0.001 m/s) and maximum (4.07 m/s) current speeds was 9.23 %, 14.0%, and 4.60%, respectively (Figure 10).

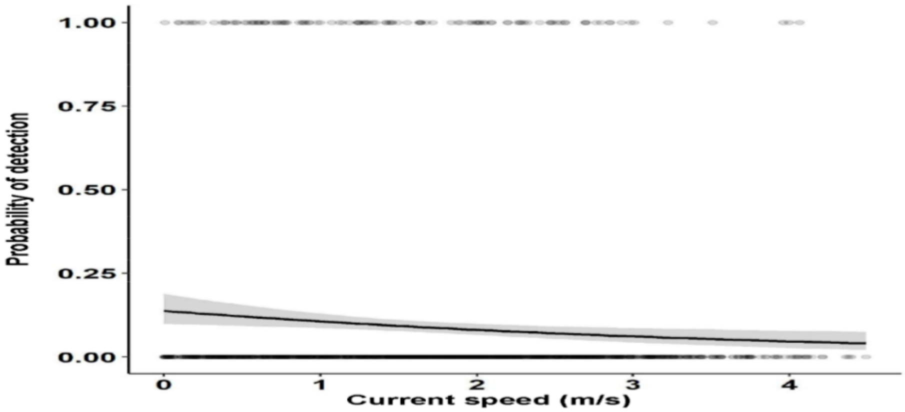

Figure 10. Binomial logistic regression with 95% confidence bands, displaying the relationship between the probability of detecting an Atlantic Sturgeon during a sturgeon hour on a sturgeon day at the Minas Passage (MP) line versus them mean current speed (m/s). A sturgeon hour was defined as an hour when an Atlantic Sturgeon was detected at the MP line, and a sturgeon day was defined as a day when Atlantic Sturgeon were detected (Keyser et al. 2016).

Table 8. Full (Full) binary logistic models (GLM) obtained using backwards selection to assess factors that influence Atlantic Sturgeons hourly presence of an Atlantic Sturgeon at the Fundy Ocean Research Center for Energy (FORCE) test site and Minas Passage line (MP). Data used for the FORCE test site included Atlantic Sturgeon detections that ranged from July 21st to November 16th, 2018 and May 14th to August 11th, 2019. Data used for the MP line consisted of Atlantic Sturgeon detections from May 5th to November 4th, 2018. Backwards selection indicated that the model with the lowest AIC at the FORCE test site included current speed (m/s) and tide type (ebb/flood) (Final), however neither variables were significant (Final). The final model at the MP line included current speed, and current speed was a significant predictor of Atlantic Sturgeons hourly presence (Final).

Location	Model	AIC	Parameters	Estimate ± SE	z-value	P-value	Odds ratio
FORCE	Full	280.54	Intercept	-2.34 ± 0.39	-5.95	2.74×10^{-9}	
			Speed	-0.27 ± 0.19	-1.39	0.17	0.76
			Level	0.04± 0.05	0.73	0.47	1.04
			TideFlood	0.15 ± 0.36	0.42	0.67	1.16
			LightNight	-0.57 ± 0.38	-1.50	0.13	0.56
	Final	277.26	Intercept	-2.33 ± 0.36	-6.42	1.36×10^{-10}	
			Speed	-0.23 ± 0.19	-1.20	0.23	0.79
			TideFlood	-0.56 ± 0.38	-1.47	0.14	0.57
MP	Full	755.72	Intercept	-1.73 ± 0.22	-7.71	1.23×10^{-14}	
			Speed	-0.31 ± 0.11	-2.81	5.01×10^{-3}	0.73
			Level	0.01 ± 0.03	0.43	0.69	1.01
			TideFlood	0.03 ± 0.20	0.17	0.87	1.03
			LightNight	-0.25 ± 0.21	-1.17	0.24	0.78
	Final	277.26	Intercept	-1.84 ± 0.19	-9.52	2.0×10^{-16}	
			Speed	-0.30 ± 0.11	-2.75	5.91×10^{-3}	0.74

Discussion

Mortalities have been reported for Atlantic Sturgeon passing through barrage style hydroelectric facilities (Dadswell et al. 2018). However, it is unknown whether the same is true for fishes migrating through regions with free standing HK devices. The FORCE test site serves as a prime location to conduct baseline monitoring to better understand whether HK's pose a risk to local fauna. Currently only a single HK device is being tested at FORCE, taking up 0.02% of the cross-sectional area of the passage providing space for fish to engage in avoidance behavior around operational HK's (Redden et al. 2014). However, if the percentage of Atlantic Sturgeon detected at the FORCE test site during 2019 provides a correct sub-sample of the population migrating into MB (~9000 sturgeon) then at least approximately 2790 Atlantic Sturgeon from endangered and threatened populations would overlap with the FORCE test site annually (Dadswell et al. 2017). Atlantic Sturgeon appeared to be at the greatest risk of overlapping with HK's upon entry and exit into MB. Consistent with the findings of Stokesbury et al. (2016) most Atlantic Sturgeon detections within MP occurred in early summer (May- July) and late fall (September-November).

Atlantic Sturgeon were detected on a higher proportion of days at the MP line than at the FORCE test site, with the highest probability of being detected at southern receivers within MP. Most Atlantic Sturgeon detected at the FORCE test site during 2018 were also detected prior to, or after, a detection within central MP. During flood tide water flowing out of MB is jetted northwards by Cape Split and past the FORCE test site, creating the highest current speeds within central MP (Karsten et al. 2008; Broome and Redden 2012). Based on the distance between locations, and time difference between detections, the estimated swimming speed was similar to the modelled mean current speeds between consecutive detections at the FORCE test site and MP line. Atlantic Sturgeon could be using tidal stream transport within this region, a behavior that has been reported for Juvenile European Sturgeon (*Acipenser Sturio* Linnaeus,1758) occupying high flow environments within the Gironde Estuary, France (Taverny et al. 2002).

Tide type (ebb/flood) and current speed were the only predictors of Atlantic Sturgeon presence at both sites. Atlantic sturgeon was more likely to be present at the FORCE test site on ebb tides and at the MP line when current speeds were lower. During a flood tide water is more evenly distributed within MP due to friction caused by the bottom substrate than during an ebb tide (Karsten et al. 2008). There is also a more uniformly directed flow of water from the north to south coastlines of MP on an ebb tide. If Atlantic Sturgeon are travelling with the current, then it is more likely that they would overlap with the FORCE test site during an ebb tide. However, background noise within MP is higher during flood tide than during an ebb tide (Martin and Vallarta 2012). Due to the low number of Atlantic Sturgeon detected in this study, data from V16 and V9-2x tags had to be combined for analysis. Lower frequency sounds are more likely to be impeded by background noise within MP than high frequency sounds (Taverny et al. 2002; Sanderson et al. 2017). Most of the Atlantic Sturgeon detected at the FORCE test site consisted of individuals tagged with V16-69kHz tags. The lower number of detections of Atlantic Sturgeon during flood tide could be attributed to the decline in signal receptions of the V16-69kHz tags.

The decline in detecting Atlantic Sturgeon with an increase in current speed at the MP line could be attributed to their inability to remain stationary within this region. Sturgeon are poor swimmers and are unable to maintain their critical swim speed for prolonged periods of time (Peake et al. 1995). The maximum swim speed of Atlantic Sturgeon has not been reported. However, the ability of adult Lake Sturgeon (106- 132 cm TL) to swim against a current rapidly declines when current speeds exceed 1.5 m/s (Peake et al. 1995). The depth averaged current speeds within MP are much higher than 1.5 m/s. During ebb and flood tide the speeds are reported to reach 4.1m/s and 4.3m/s, respectively (Cornett and Bourban 2008). We suggest, however, that the decline in Atlantic Sturgeon detections with increasing current speed is more likely attributed to the performance of the tagging technology.

The VR2W-180 kHz receivers at the MP line are only capable of detecting 180kHz PPM signals emitted from the V9-2x tags and the signal could have been lost if Atlantic Sturgeon were travelling in currents in excess of 2m/s. At the FORCE test site, it was determined that for the V9-2x tag detections, the absence of the HR-170kHz signal would have resulted in a large proportion of missed detections. Additionally, it was noted that a higher proportion of V9-2x tag detections at the FORCE test site occurred when current speeds exceeded 3m/s in comparison to traditional V16-69 kHz tagging technology. Detections of V9-2x tags were also more likely to occur during ebb tide than flood tide, and the opposite was true for the V16-6x tags. This result was unexpected as the background noise within MP is higher during flood than ebb tide, and the V9-2x tag has been shown to be less impeded by background noise at high current speeds (Sanderson et al. 2017). The difference in detections during the two tide types could be related to the lower number of Atlantic Sturgeon tagged with V9-2x tags being detected at the FORCE test site. Since Atlantic Sturgeon were not double tagged with the two tags types statistical comparisons could not be conducted to determine whether there was a significant difference between when the tags were detected. Future studies should increase the proportion of V9-2x tags and deploy HR receivers across the MP line to compare models fitted using data from coded (V16) and HR (V9-2x) tagging technology.

The bottom of MP consists mainly of bedrock, gravel and boulders up to 5m in diameter and the FORCE test site is located on a volcanic plateau surrounded by bedrock ridges (Fader 2009, 2011). On average Atlantic Sturgeon at the FORCE test site and MP line were found to occupy the mid-water column. Due to their potential inability to visualize and sense their environment it would make sense for Atlantic Sturgeon to remain in the upper water column within this region. Stokesbury et al. (2016) noted that Atlantic Sturgeon travelled pelagically through MP. In this study the only significant predictor of Atlantic Sturgeon presence in the upper water column at the FORCE test site was tide type, with a higher likelihood of detecting an Atlantic Sturgeon in the upper water column during ebb than flood tide. The entry point of MP into MB is characterized by deep channels consisting of bedrock (Fader 2009). When water is jetted through MB, into MP and towards the FORCE test site on ebb tide Atlantic Sturgeon may be lifted with the current due to the bathymetry. Sturgeon had a higher likelihood of being detected closer to the bottom substrate at the MP line with an increase in temperature. Sediment suspension within MP decreases with an increase in temperature (Dadswell et al. 1983; Envirosphere 2009). Atlantic Sturgeon may occupy deeper depths with an increase in temperature due to the increased visibility in the region.

Compared to most teleost's, sturgeon have poor vision (Miller 2004) and they spend much of their time associated with the benthic substrate (Bramblett and White 2001; Taverny et al. 2002; Bennett et al. 2005; Dadswell 2006; Beardsall et al. 2016). Poor vision, especially in turbid environments like MP, would make it difficult to discriminate obstacles including tidal turbines (Hammar et al. 2013). While there are currently no operational turbines within FORCE, as the currently deployed HK device has ceased to operate, plans are in place to test two MW turbines over the next few years (FORCE 2018). Most proposed tidal turbines function best within mid-water column where current speeds are often the highest (Stokesbury et al. 2016; Zhou et al. 2017). Larger species, such as Atlantic Sturgeon appear to be at a greater risk of contacting turbine blades due to their increased surface area. Atlantic Sturgeons morphology make them poor at conducting rapid maneuvers (Webb 1986; Kieffer et al. 2001). It is highly unlikely that Atlantic Sturgeon could control their behavior within MP at high current speeds. This study is the first to provide baseline information on the behavior of Atlantic Sturgeon at the FORCE test site in the absence of a HK device. Future studies should focus on using VEMCO positioning system (VPS) arrays to map out the exact movements of Atlantic Sturgeon in this region. This would provide evidence as to whether Atlantic Sturgeon are able to engage in avoidance behavior around an operating tidal turbine.

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Appendix H

Improved protocol for acoustic tagging of sensitive Clupeid fishes

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This work also appears in chapter 1 of Liza's MSc thesis.

Background

Understanding how animals move and interact with their environment is fundamental to answering big-picture questions in aquatic ecology, such as migration patterns, habitat use, physiology and energetics, population dynamics, survival, and behaviour, all of which are essential components of management and conservation science (Donaldson et al. 2014). In recent years, acoustic telemetry has emerged as a promising method for studying the spatial and temporal distribution of individuals, particularly in aquatic ecosystems, where direct observation of animals is often challenging. The term simply refers to the use of sound (acoustics) to relay information across space (telemetry), and the use of this technology in wildlife fisheries studies has expanded dramatically during the last few decades (Brownscombe et al. 2019).

Acoustic telemetry relies on two pieces of equipment, working in tandem to record animal movement: transmitters, or electronic tags, which broadcast a series of "pings" (sound pulses) that can be picked up and identified by receivers (Cooke and Wagner 2004). Each signal is uniquely coded, thereby providing individual IDs for the tagged animals that can be matched to their biometrics. As long as a tag is within the detection range of a receiver, each transmission will be recoded and saved with the date and time, as well as any additional information that may be programed into the tags, such as sensors to monitor biological and/or environmental information. Receivers are generally deployed in networks or arrays across the study area in order to provide insight into the movement patters of tagged fish (Cooke and Wagner 2004).

Data derived from telemetry studies has helped to address questions on the ecology of a diverse range of taxa, including invertebrates, fish, amphibians, reptiles, birds, and mammals, across both freshwater and marine environments and from various life stages (Cooke et al. 2004).

While tags can be attached to animals in different ways, the majority of tracking studies use surgical procedures to implant tags directly into the peritoneal cavity of fish, particularly for small-bodies species (Cooke and Wagner 2004). This approach reduces the effects of drag or biomaterial buildup experienced by animals with externally mounted equipment (Jepsen et al. 2015), as well as the instances of tag loss associated with both external attachment and gastric implants (Wagner and Cooke 2005). However, the procedure comes at a cost, as increased handling can have negative effects on fish behaviour and viability, and may even be lethal (Cooke et al. 2011).

Handling can impact fish in many ways: through inducing physical injuries or scale loss (Bauer 2005), the sublethal effects of stress on physiology and behaviour (Barton et al. 1986, Brezeale 2013), increased susceptibility to disease (Caputo et al. 2009), and reduced swimming performance, which may leave them vulnerable to predation (Adams et al. 1998). These effects may be compounded or allayed by the choice of tag design, the handling method used by researchers, the maintenance of asepsis, ambient environmental conditions, and the maturity, physical condition, and size of the fish (Jepsen et al. 2002, 2013). If the proper tagging technique is followed, surgical tag implantation may have very little impact on fish (Koed and Thorstad 2001). However, herring-like species are generally considered to be sensitive to handling (Rounsefell and Dahlgren 1933) and confinement (Hershberger et al. 2006). While some studies have proven successful in tagging Pacific herring (Seitz et al. 2010, Eiler and Bishop 2016), such studies are few, and are often limited in scope and sample size. Even less data exist for other Clupeid species such as Alewife (Eakin 2017, McCartin et al. 2019) or Atlantic herring (Eggers et al. 2015), which puts into question the feasibility of using acoustic tracking to monitor these small-bodied, laterally compressed fishes.

Assessing post-tagging survival and behaviour of fish is particularly challenging, as it is difficult to monitor the fate of individuals via direct observation. Furthermore, latent effects can result in the mortality of individuals previously deemed viable, and which would have been released during a tagging study (Parrish and McPherson 1963). Consequently, there is a need for some method of vitality assessment in tagged fish that could help predict post-tagging mortality. One such method, which provides both a quick and non-invasive method for quantifying fish vitality before release, is the use of or reflex impairment indices or Reflex Action Mortality Predictors (RAMP) (Davis 2007, 2010, Raby et al. 2012). Reflexes are defined as involuntary, stereotyped movement induced by a peripheral stimulus (Landau 1986), and are linked to fitness outcomes such as reduced growth, impaired predator evasion, and delayed mortality (Davis 2007). Responses can be quantified as present or absent, and the total RAMP score can be used as proxy for the likelihood of post-release mortality. In this way, RAMP is an effective tool for rapid real-time assessment of fish stress, with applications in catch-and-release fisheries, bycatch, migration, and tracking studies (Raby et al. 2012, Brownscombe et al. 2015, McLean et al. 2016).

However, this method only assesses visual external manifestations of stress. Furthermore, there are still a number of questions that remain about the potential consequences of tagging procedures on fish, such as how tagging proficiency can best be evaluated (Cooke et al. 2011). Necropsy-based assessments offer another method for quantifying stress, and include both external and internal observations and allow for the measurement of condition indices (Blazer et al. 2018). While these are not possible in telemetry studies, conducting necropsies in laboratory-based tagging trials could offer more insight into the ramifications of this procedure in sensitive fishes.

This study examines the feasibility of implanting acoustic tags in Alewife from the Gaspereau River population in Nova Scotia, Canada, to evaluate the utility of this tool in the field. We use reflex impairment, as well as post-mortem examination to assess the stress response of Alewife to internal tag implantation, as well as the influence of external and internal factors (environmental conditions, handling time, fish size) on these conditions.

Methods

Pilot Trials

Prior to beginning the study, we conducted a series of pilot trials from May to July 2018 that would help establish a baseline tagging protocol. 24 Alewife from the Black River-Gaspereau River stock were captured at the White Rock Fish Ladder through dip netting. The animals were split into 4 groups to test which concentration of the anesthetic tricaine methanesulfonate (MS-222) resulted in the fastest induction of surgical anesthesia (characterized by a total loss of equilibrium, swimming ability or reactivity), followed by a fast recovery time (i.e. the regaining of equilibrium, swimming ability and response to tactile stimuli). The concentrations tested were: 50mg/L, 100mg/L and 200mg/L. From these trials, it was determined that the optimal anesthesia delivery method for Alewife consists of immersion into a 200 mg/L concentration of MS-222 until fish loose vertical equilibrium, followed by anesthesia delivery through the gills to maintain sedation during the surgical procedure, at a water temperature of about 15˚C.

In addition, we conducted trials to test the effect of suturing. The standard surgery protocol calls for the use of sutures to close the incision site, as this facilitates healing and reduces the risk of infection (Wagner et al. 2011). However, sutures can also cause tissue trauma at entry and exit points, and where the sutures come into contact with skin (Wagner et al. 2011, Deters et al. 2012). In studies using PIT tags, which are smaller in comparison to traditional acoustic transmitters, researchers have moved away from the standard surgical protocol, instead using needles to inject tags into the body cavity through a non-sutured incision (Cook et al. 2014). This method is generally accepted to improve survival and a reduce tagging bias, and several studies have shown fish surviving for multiple years following the procedure, such as Nau et al. (2017). The acoustic tags used in this study are a new family of V5, High Residency (HR) tags developed by VEMCO/Innovasea (Bedford, Nova Scotia, Canada), measuring 11 x 3.6 x 5.5mm – this is closer to the size of larger PIT tags, which can measure from 12 to 32mm, compared to the 26mm V9 model used in previous studies on herring (Eiler and Bishop 2016). This new technology opens up the possibility to move away from standard surgical procedures used for acoustic tags, instead adopting techniques employed for implanting PIT tags, which are less impactful on small-bodied fishes.

25 ripe Alewife were captured from the White Rock Fish Ladder for trial surgeries. Captured fish were immediately transferred to a holding tank (270L capacity)) filled with fresh, circulating water from the ladder. Individuals were selected at random to be part of one of three trial groups: tagging with suturing, tagging without suturing, or control. The tagging protocol was adapted from previous studies on Pacific herring (Clupea pallasii; Seitz et al. 2010; Eiler and Bishop 2016), PIT tagging practices (Cook et al. 2014), as well as methods developed by Dr. Hardie of the Department of Fisheries and Oceanrs for tagging Atlantic salmon smolts (Hardie personal communications). Prior to surgery, the fish were anesthetized with a concentration of 2g/10L of MS-222, buffered with sodium bicarbonate ($NaHCO_3$) at a 1:2 ratio. The animal was considered anesthetized when it had lost vertical equilibrium and ceased responding to tactile stimuli (tail grab). Fish were then measured (weight and length), and tagged with non-transmitting (dummy) models of the V5-HR tags.

Anesthetized fish were placed right-side down in a foam cradle designed to hold small laterally compressed fish (Figure 1). A continuous flow-through apparatus was set up to provide a steady supply of oxygenated, anesthetic solution (1g/10L concentration) through the gills, helping to keep the fish moist and sedated during surgeries. Because acoustic tags are wider than PIT tags, we could not use a needle for tag insertion. Instead, an incision just large enough (8-10 mm) to allow passage of the dummy tag was made on the right lateral side of the fish, one to two millimetres off the mid-ventral line, similar to a PIT tagging protocol. The incision was cut vertically between the ribs, and the tag lightly pushed through the incision and forward into the peritoneal cavity. Using this method, the tag becomes positioned in the abdominal cavity, just posterior to the pyloric ceca and above the pelvic girdle (Figure 2). If the scalpel blade slips and cuts too deep through the epithelial tissue, it will likely follow the inside wall of the abdominal cavity without passing through vital internal organs. Furthermore, this position allows easier access and more control for the tagger, due to Clupeid fishes being laterally compressed.

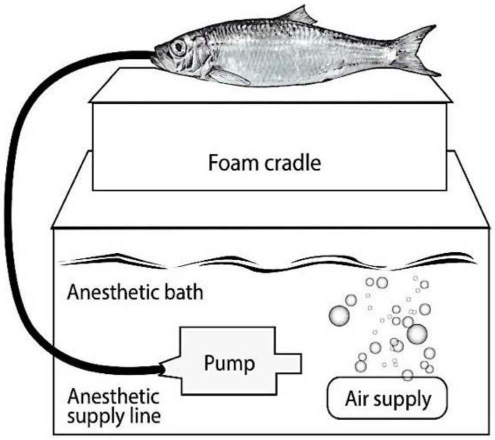

Figure 1. Continuous flow-through anaesthetic apparatus to maintain sedation during surgeries, designed to hold small, laterally compressed fish.

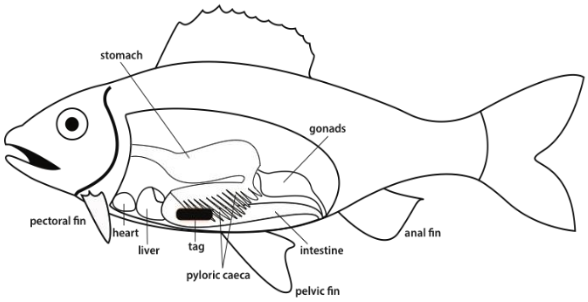

Figure 2. Position of the tag following surgical implantation on the right lateral side of the fish, with internal anatomy shown.

Some of the animals were then recovered immediately, without any further treatment of the incision site. For the remaining fish, incisions were closed with two simple interrupted sutures (nonabsorbable Ethicon monofilament nylon sutures, reverse cutting 4–0, 1.5 metric, 45 cm, PS-2 18 mm, 3/8 circle needle), secured with four throws. The control fish were simply anesthetized then recovered, without any other manipulation. In total, 8 Alewives were tagged using the suturing method, and 17 Alewives were tagged without suturing. We recorded the time it took each animal to recover equilibrium and normal swimming behaviour (indistinguishable from control fish) following the surgery, after what the animals were retained for 72 hours to monitor for any latent mortalities or signs of infection around the sutures/incision site.

An analogous tagging procedure was tested on Atlantic herring, captured aboard a Scotia Garden Seafood purse sein fishing vessel operating out of Yarmouth, NS. Animals were captured on the German Bank's spawning grounds on 29 September and 3 October 2018, and tagged using the described method while still onboard the ship. 25 herring were tagged without suturing, and 20 had the incision closed with sutures before being recovered. Recovery times were recorded for each fish, and the animals were held for 2 hours before being released.

In addition to the change in tagging site, additional precautions were taken to minimize the effect of tagging on fish. All surgical instruments were soaked in a sterilizing solution and rinsed with sterile saline water before and after use. Precautions were taken to minimize handling damage to fish, such as using soft dip nets or plastic containers for fish transfer, keeping working surfaces moist, and covering the animals' eyes during surgeries.

Based on these pilot trials, we saw no differences in the recovery time or survival between sutured and non-sutured fish for both species, with most animals recovering within a minute after being placed back into flowing water (Figure 3), and no observed mortalities. We concluded that the described tagging method was well suited for small pelagic forage fish such as Alewife and Herring, and was applicable to telemetry studies.

Validating the Surgery Protocol

96 adult Alewife (52 ripe, 44 spent) were captured between the months of May and June, 2019, from the White Rock fish ladder on the Gaspereau River. Each fish was sexed, weighed and measured before being anesthetized with MS-222, and tagged with dummy (non-transmitting models) of VEMCO V5 acoustic tags, as per the pilot trials. Because no negative effect was observed from suturing, it was decided to close all incisions with two simple, interrupted sutures,

as this method is widely recognized to be effective at reducing infection in tagged animals (Wagner et al. 2011).

Following the surgeries, fish were allowed to recover for 5 minutes in a closed, 100L water tank. This was based on the maximum recorded recovery time from the pilot trials. After this time, the lid of the tank was removed, and the behavioural condition of the fish was assessed based on reflex impairment. Reflexes assessed included ventilation, orientation, swimming vigour, light response and tactile response, each of which was assigned a score of 0 if non-impaired, and 1 if impaired. Ventilation was considered unimpaired if the fish exhibited regular opercular movement for 30s. An unimpaired orientation response was noted if the fish maintained vertical equilibrium in the water, and unimpaired swimming consisted of sustained, regular movement for at least 30s. A startle response was expected for both the light (lifting tank lid) and tactile (tail grab) stimuli, in which unimpaired fish shows rapid forward motion in response to stimuli. Total RAMP scores for each individual were calculated as a simple proportion of the five measured reflexes that were impaired (0 = no reflexes impaired; 1 = all reflexes impaired).

Fish were then allowed to recover for 24 hours, after which they were euthanized by anesthetic overdose, and a necropsy health assessment was conducted. Fish were assessed for internal signs of bleeding or hemorrhage, condition of the incision, organ damage or discoloration, and tag position within the body cavity. Each condition was again assigned a score of 0 or 1, and A total physical impairment index was calculated as the proportion of physical parameters that had been impaired or damaged. Both sets of scores were modelled individually using a General Linear Model with a Gaussian distribution (significance level of 0.05; R version 3.6.1) based on physical parameters of the fish (weight, length, sex and condition), as well as external factors (water temperature and total handling time), in order to identify parameters that most influence the outcomes of a surgery.

Results

Overall, the developed tagging method proved effective in minimizing the effects on fish. Average RAMP and physical impairment scores were both 0.2, with only 4/96 fish experiencing an impairment of all reflexes, and no fish receiving a full impairment score in the necropsies. The most commonly impaired reflex was response to light stimulus (Figure 4). Fish sex and size had no observable effect on the outcomes of the tagging, however water temperature was found to increase the amount of reflexes impaired, with more impairments seen above 12˚C (Figure 4).

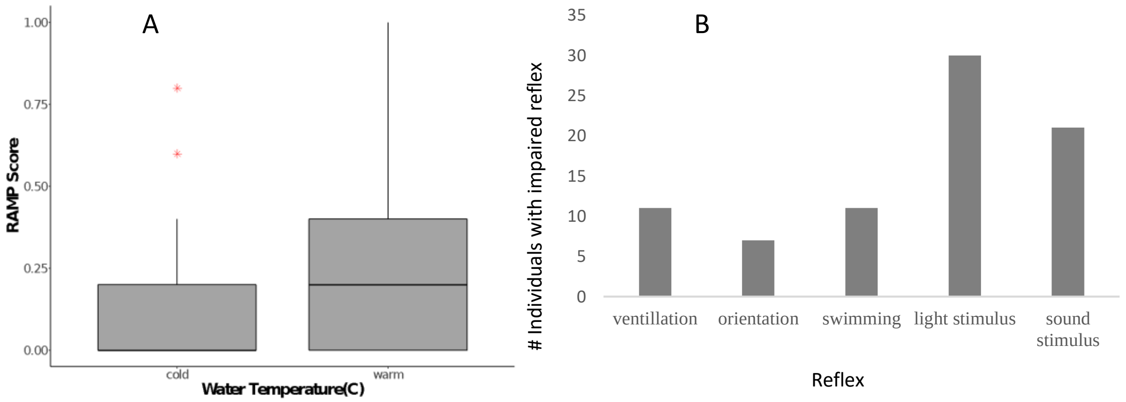

Figure 2. A) Boxplot showing median, quartile and maximum observed RAMP scores based on water temperature, where water temperature is described as cold between 9˚C to 12˚C, and warm between 14˚C to 18˚C. B) Histogram of the frequency of impairment for the five observed reflexes: ventilation, orientation, swimming vigour, light response and tactile response.

The primary internal injury observed was a puncture of the gonads by the tag, however, this was only observed in pre-spawned (ripe) fish, in which the gonads generally take up the entire body cavity (Figure 5). Some individuals also showed evidence of bleeding around the incision site.

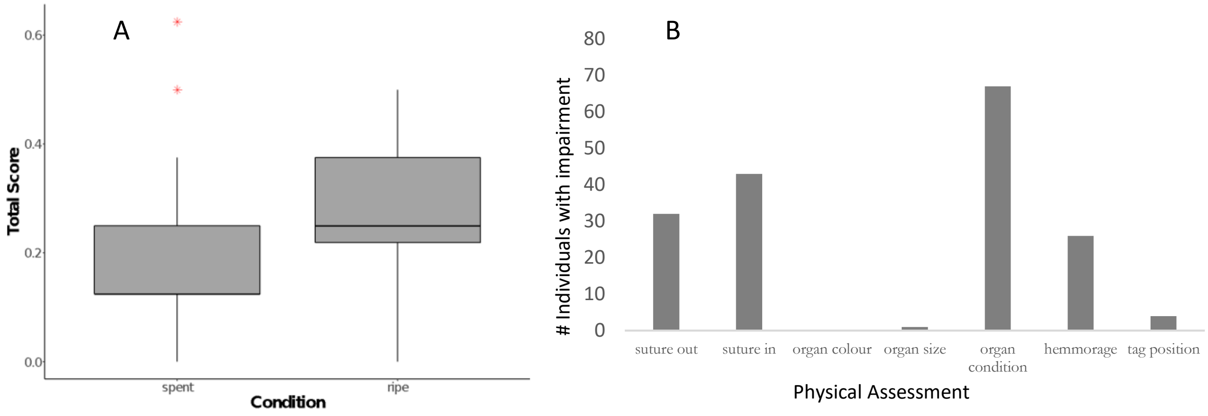

Figure 3. Boxplot showing median, quartile and maximum observed physical impairment scores in ripe and spent Alewife.

Discussion

Overall, the surgical method developed in this study is appropriate for tagging sensitive, small-bodied fishes of the Clupeid family. Average RAMP and physical impairment scores were low, and most individuals appeared healthy 24 hours following the surgery, with the exception of 3 observed mortalities in the last round of tagging.

The underlying assumption of reflex impairment is that it has a basis in the physiological stress response (Davis 2010). Greater reflex impairment scores are associated with physiological exhaustion and reduced vitality in many fish species (Davis 2007, 2009, Raby et al. 2012, Brownscombe et al. 2015, McLean et al. 2016). Therefore, this method can be used as a fast and effective approach to assessing the likelihood of tagged animals surviving after release. Unfortunately, we were not able to identify individual fish once they were released into the larger holding tank for the 24 hour holding period, therefore, we cannot assess whether the observed mortalities occurred in those fish that had scored highest on their reflex impairment. This is a pertinent question in tagging studies, and should be assessed further under laboratory settings. However, based on the wealth of research in reflex impairment in numerous species, it can generally be expected that individuals with low RAMP scores will have a high chance of survival (Davis 2010).

Interestingly, we did not find any allometric differences in RAMP scores, which is contrary to the observation in most species of smaller fish having an improved ability to recover from disturbance compared to larger individuals (Gingerich and Suski 2012). This is likely because all of the alewife captured in this study were of a similar size (28 cm ± 10mm), which likely does not provide enough contrast to observe different recovery times. Instead, the primary factors observed to affect post-tagging recovery were the sexual condition of individuals (ripe vs. spent), as well as water temperature. It is not clear whether the puncture of gonads by a tag can have long-lasting impact on the individuals. However, given that this happens regularly in PIT tagging studies, in which many individuals are still detected several years after tagging, and that the size of the acoustic tags used in this study is comparable to that of large PIT tags, this injury is not expected to be life-threatening (Nau et al. 2017). However, we recommend this be explored in future long-term laboratory studies, with fish being held for a longer period to assess latent changes in growth rate or behaviour.

A bigger influence on tagging outcome appears to be water temperature. In the Gaspereau River, Alewife spawning generally occurs at water temperature of 5˚C to 10˚C (Stone et al. 1992). Over the course of the run (from late April to early July), water temperature in the river can change dramatically, with upwards of 18˚C near the end of summer (E. Tsitrin, personal observation). Numerous studies have implicated the influence of water temperature on the success of tagging, with fish suffering increased physiological stress, decreased immune response, and mortality at higher temperatures (Bunnell and Isely 1999, Walsh et al. 2000, Cooke et al. 2011).Temperature also affects the anesthesia induction and recovery times, with the thermal stress effect being considered more severe in water >14˚C (Jepsen et al. 2002). In this study, temperature of the river water (temperature of the anaesthetic bath was maintained close to the river environment) ranged from 9-11˚C in May (start of the run) to 15-18˚C in June (end of the run). This, coupled with the immunosuppression observed in fish when (Watts et al. 2001) likely resulted in greater stress experienced by fish tagged at the end of the season. These observations corresponds with the increase in RAMP scores observed at higher temperatures, as well as the only observed mortalities happening at the end of the study period, when water temperatures were at their highest.

The necropsy-based health assessment provides additional insight into the unseen effects of tagging. Though overall low, the post-mortem scores revealed some degree of physical damage in almost all individuals. Aside from tags being pushed into the gonads in ripe fish, some animals were observed to have bleeding or signs of inflammation around the incision and sutures. Although this is consistent with previous studies on the effects of suturing (Wagner et al. 2011, Deters et al. 2012), the general consensus in surgical tagging is that the use of sutures increases the overall chance of survival (Mulcahy 2003). In a long-term trial of acoustic tagging with suturing in Pacific herring, which was conducted under controlled laboratory conditions, most animals showed no signs of an incision at the end of the study, which suggests that most incisions are able to heal if given enough time (Seitz et al. 2010). However, the same study had one animal in which the incision site never closed and the sutures had loosened. Further studies are needed to assess the impact of suturing and the rate of wound-healing in free-ranging fish.

Conclusions

The tagging method developed for this study, which uses a combination of small tags, a lateral tag insertion, and the use of flow-through anesthetic, is effective at minimizes tagging effect and ensuring the viability of tagged individuals. Despite their sensitivity to handling, the fish responded well to the tagging procedures, with most recovering full functionality of their reflexes within 5 minutes following tagging, and 97% of the tagged individuals surviving into the following day. Internal examination revealed few instances of physical damage that would be considered life-threatening. Therefore, we recommend the use of this tagging method in future tracking studies of Clupeids.

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