Alton Natural Gas Storage
Estuarial Environmental Monitoring
&
Toxicity Testing
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Alton Natural Gas Storage

Scientific Study Plan for the Shubenacadie Estuary

As required for the understanding of the estuarial food web function and striped bass population dynamics leading to better protection and management of the fish stocks.

This monitoring plan can be adjusted by agreement between Department of Fisheries and Oceans, Dalhousie University researchers and Alton Natural Gas Storage based on new information or questions that may require further investigation. The intent is to continue with the previous eight years of scientific study of the estuary to better protect and manage the fish stocks. Some items overlap with the Alton operation's monitoring but will not be duplicated.

Plankton and fish monitoring

1. River wide monitoring

Alton river site monitoring – determination of Striped bass egg and larvae density (number per cubic metre of water filtered) and developmental stage, plankton sampling (for copepods and other zooplankton), and enumeration of all fish species

30-second standard conical plankton net (250 μm mesh, 0.5m diameter opening; approximately 5 m^3 of water filtered per tow) tows in the main river channel will be conducted every 10 minutes on the 90 minute flood-tide; all tow samples are preserved in 10% formalin and transported to the lab for identification and enumeration; Sampling frequency: from May 1st to July, 15th, will be three days a week

a. Purposes:
   - to quantify temporal striped bass egg and larvae abundance and developmental stage and natural mortality relative to salinity and tidal cycle
   - to analyze gut contents of striped bass from first-feeding larvae to juveniles
   - quantify temporal distribution of invertebrate plankton at the Alton site and seasonal variation in population size relative to temperature and salinity;

- determine food availability to first-feeding larval bass, one of the critical factors affecting year class strength and first-winter survival

b. Sampling impacts:
   - Plankton net sampling is estimated to remove a small fraction of the total number of individuals present in the river on a given flood tide (estimated to be <13% of the eggs spawned by a single female of average fecundity; J. Duston 2015)
   - Approximately 5 m^3 of river water is filtered per 30-second net tow

2. Beach seine net (1mm mesh) sampling on the west sand bank at the Alton site; three 50-m sweeps for enumeration and gut content analysis of larval and juvenile Striped bass; all samples to be retained are euthanized and preserved in formalin for further analysis (age, body size, gut contents); sampling frequency: from July through August, once a week;

a. Purposes: underyearling bass are generally not caught in plankton net tows once they reach a certain size and swimming ability; the seine netting allows the year class to continue to be monitored as they grow, and analyses of gut contents to be tracked through their first growing season.
b. Sampling impacts: fish species other than striped bass, as well as invertebrates (sand shrimp, large mysids) will be released with the least possible harm. Up to 35 young of the year bass per week will be kept for detailed analysis including those captured in plankton net tows, to a maximum of 500 individuals per year.

3. Up-estuary monitoring – Plankton sampling, determination of striped bass egg and larvae spatial distribution, density and developmental stage and enumeration of all fish species
   a. Procedure and purpose: Duplicate 30-second plankton net tows starting at the salt front at high day time tide and sampling approximately every kilometer back downstream to the Alton site to map out the nursery habitat and match with copepod prey density; May - July sampling frequency weekly
   b. Sampling impacts: Similar to flood tide sampling; approximately 5 m^3 of river water are filtered per 30-second plankton net tow

4. Striped bass annual recruitment survey (previously conducted by DFO prior to 2015)
   a) Highway 102 Beach Seine net (1mm mesh) sampling: on the Shubenacadie River at the sand bank adjacent to the Highway 102 Bridge at high slack tide to monitor underyearling Striped bass; two 50-m net sweeps will be conducted; all fish will be counted; a maximum of 30 individuals per species will be retained, with all others returned to the river with the least

b) possible harm. This sampling will take place at two-week intervals three times during late August – end of September

c) Parrsboro Shore Beach Seine net sampling: 1mm mesh net; two 50-m net sweeps at each of six sites along Parrsboro Shore:

i. Ottawa House
ii. Five Islands (Broderick Lane)
iii. Lower Economy (Jacob's Lane)
iv. Economy Point (Sea Breeze Lane)
v. Saints Rest (Wharf Rd.)
vi. Little Dyke Rd

A maximum of 30 individuals per species will be retained for age and body size measures, with all others being returned to the water with the least possible harm. This sampling will take place at two-week intervals three times during late August – end of September.

Water chemistry and temperature monitoring

a) River wide monitoring

Shubenacadie River

- CTD data loggers located up-estuary from Alton site:
  - Opposite the golf course (The Links at Penn Hills)
  - At Highway 102 bridge
  - At Shubenacadie Village
- CTD data loggers located down-estuary from Alton site:
  - Immediately downstream (< 500m) from brine outfall
  - At Gosse Bridge
  - At Maitland

Upriver Stewiacke River

- CTD data logger at the CN rail bridge
- **Frequency: On-going, real-time loggers downloaded bi-weekly during the ice free months, and when it is safe to access them during the winter**

Impacts of water chemistry and temperature monitoring are expected to be minimal as loggers are left to passively record data and are deployed inside PVC piping anchored to the river bottom

Reporting

Reports will be sent to the Department of Fisheries and Oceans as required by the scientific permit.

Procedure for Acute Toxicity Testing of Alton Gas Brine on Striped Bass

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Overview
The objective is to develop a protocol that is satisfactory to all concerned parties for determining the median toxicity threshold (LC_{50}) of the brine water and its constituents on egg, larvae and juvenile stages of striped bass (*Morone saxatilis*). The work will be conducted at Dal-AC. This revised version is based on feedback received on February, 12, 2015 of a first draft submitted to Marc McLean, DFO on January, 26, 2015.

Biological material: Tests will be conducted on wild-domesticated striped bass (WDSB), with hatchery striped bass (HSB) serving as a back-up. WDSB will be derived from eggs caught by plankton net tows in the Stewiacke River. The eggs will be trucked to Dal-AC Aquaculture-Centre and on-reared at 3 to 5 ppt salinity and 18-20 ^{o}C to provide larvae and juveniles for testing. If there is opportunity to catch wild larvae (5-7 mm total length, TL), they will be included in tests. True wild larvae may have experienced salinity ranging from 0.5 to >20 ppt, due to the ebb and flow of the tides, which may alter their salinity tolerance compared to lab reared fish at constant 3-5 ppt.

Advantages of WDSB: Since wild striped bass will be exposed to brine effluent in the Shubenacadie River, it is important to replicate factors as best we can. WDSB will have a greater genetic diversity than HSB, the latter likely will be half-siblings. Also, the nutritional condition of WDSB and HSB yolk sac larvae will likely differ due to potential differences in nutrients derived from the egg yolk. Egg quality from captive broodstock at DAL-AC is inferior to wild eggs: they are smaller, the chorion is weaker, and hatch rates are lower.

Disadvantages of WDSB: a) A batch of wild eggs from a plankton net tow may contain a mixture of developmental stages, which will hatch at different times, generating a confounding factor in the response to the toxicity test. b) Parasites are an unknown confounding factor. c) A sufficient supply of wild eggs is not assured. Wild eggs are only available for about three weeks starting mid-May and spawning is episodic.

Advantages of including HSB as a back-up: a) Response of each developmental stage to brine may prove to be similar to WDSB, increasing our confidence level in the

results. b) Relying 100% on catching sufficient wild eggs to produce enough fish for tests at all developmental stages is risky.

Brine collection and storage: Brine will be collected from the brine pond at the Alton Gas site with a pump (2 inch Honda water pump, loaned from Alton Gas) into a 700L water tank on a ¾ ton HD truck (Dal-AC farm or rent). At Dal-AC, the brine will be stored in 200 L plastic drums in a walk-in cold room (4 ºC) in darkness and aerated.
Dilution water: Tidal water from the Alton Gas settling pond will be pumped (2 inch Honda water pump, loaned from Alton Gas) through a 500 µm screen into a 700L water tank on a ¾ ton HD truck (Dal-AC farm or rent). The water, at Dal-AC, will sit in the tank for about 2 hours to allow the silt to settle, then be siphoned through a 50 µm screen into 200 L plastic drums. Water will be stored in a walk-in cold room (4 ^{o}C) in darkness and aerated. It will be used to both dilute the brine to the test concentrations and dilute away the test salinity after the 1 hour test period.

Shubenacadie River water contains some heavy metals (Al, Fe, Zn, Ni, Cu, Pb, and Cr) that exceed the values recommended by the EPA (2002), and some parameters and heavy metals (ammonia, chlorine, Al, Fe, Pb and Zn) exceed Environment Canada recommendations for toxicity testing specifically for rainbow trout in full freshwater (EC 1998). In addition, the alkalinity, 8-10 mg/L, is lower than recommended (EC 1998). Despite these concerns, using Shubenacadie River water for the tests is the most realistic approach to assessing the toxicity of the diluted brine discharge. The fish are adapted to these conditions. High metal content is common to rivers in Nova Scotia (Dennis and Clair 2012).

Concentrations analyzed: An estimated total of 48 tests will be conducted between May and September, 2016 (Table 1). Each test will include five concentrations will be analyzed at each life stage, plus a control (2 ppt) and brine (100 ppt). Each test salinity will have three replicates. Initially, the test concentrations will be 15, 25, 35, 45 and 55 ppt. However, the range of salinities needed to determine the median toxicity threshold may need adjusting to match the ontogenetic changes in euryhalinity (Cook et al. 2010). The control salinity is 2 ppt and not 0 ppt (freshwater) because Shubenacadie River striped bass eggs and larvae are not found in tidal freshwater in the wild and in culture exhibit poor survival in pure freshwater. Underyearling juveniles (up to 15 g) also exhibit better survival at 2 ppt than in pure freshwater.

Brine (100 ppt) is included to determine the effect of an accidental discharge of brine. The suggestion by the reviewers to use 240 ppt salinity to match the highest possible concentration seems unreasonable. No teleosts can survive 240 ppt, similarly 100 ppt is likely highly toxic.

Temperature and salinity interact to affect survival of striped bass early life stages (Cook et al. 2010). Spawning usually occurs at 14-18 ^{o}C, after which a cooling trend to 11-12 ^{o}C is quite common during the Nova Scotia spring. Hence toxicity tests will be run at both 12 and 18-20 ^{o}C for the egg and larvae stages only (Table 1).

For quality control purposes, an additional series of trials will be conducted with "Instant Ocean" sea-salt as a reference toxicant.

Life stages: Six life stages will be analyzed:

1) Eggs; 2) Yolk-sac larvae; 3) 5-10 days post hatch (dph) larvae; 4) 10-20 dph larvae; 5) Early juveniles (30 mm total length, TL); 6) Large juveniles (120 mm TL).

The appendix lists the specific experimental conditions for each stage:

Table 1: Estimated Number of Salinity Toxicity Tests on Shubenacadie Striped Bass

Material to analyze	Biological material	Temperature	Eggs	Yolk-sac	4-10 dph	10-20 dph	30 mm	120 mm
Brine	Wild	12 ºC	2	2	2	2	0	0
		18-20^{o}C	2	2	2	2	1	1
	Captive	12 ºC	2	2	2	2	0	0
		18-20^{o}C	2	2	2	2	1	1
Instant Ocean	Wild	18-20^{o}C	1	1	1	1	1	1
	Captive	18-20^{o}C	1	1	1	1	1	1
Sub-total			10	10	10	10	4	4
Total								**48**

References

Cook, AM., Duston, J., and Bradford, R.G. (2010). Temperature and salinity effects on survival and growth of early life stages of Shubenacadie River striped bass. Transactions of the American Fisheries Society, 139:749-757.

Dennis IF and TA Clair 2012. The distribution of dissolved aluminum in Atlantic salmon (*Salmo salar*) rivers of Atlantic Canada and its potential effect on aquatic populations. Canadian Journal of Fisheries and Aquatic Science, 69:1174-1183.

EC 1998. Biological test method: toxicity tests using early life stages of salmonid fish (Rainbow trout). Second ed. Report: EPS 1/RM/28-1E. 122 pp.

EPA 2002. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. 266 pp.

Table 2: Egg Stage Test Conditions

Test type	Static non-renewal
Exposure time	1 h, then dilute to 2 ppt in one hour
Test duration	To hatch (range: 12 to 96h)
Temperature	12 ºC and ambient (18-20 ºC)
Light quality	Dim ambient laboratory illumination
Light intensity	30 lux
Photoperiod	24 h
Test chamber size	4 L (McDonald jars)
Test solution volume	1 L for 1 h, then fill jar to 4 L during dilution
Renewal of test solutions	No renewal
Age of test organisms	Eggs
Nº organisms per test chamber	100
Nº replicate chambers per concentration	3
Nº organism per concentration	300
Feeding required	No
Test chamber cleaning	Cleaning not required
Test solution aeration	Gentle aeration needed to maintain eggs in suspension. O_2 will checked 2 times per day
Dilution water	Shubenacadie River
Test concentrations	Control (2 ppt), five concentrations (15, 25, 35, 45, 55 ppt) and brine (100 ppt)
Endpoint	Hatch and mortality
Volume of brine (100 ppt) required	Minimum 2 L per test
Test acceptability criterion	≥70% survival in wild egg controls ≥50% survival in eggs from captive broodstock

Table 3: Yolk-Sac Larvae Test Conditions

Test type	Static non-renewal
Exposure time	1 h, then dilute to 2 ppt
Test duration	24 h
Temperature	12 ºC and ambient (18-20 ºC)
Light quality	Dim ambient laboratory illumination
Light intensity	30 lux
Photoperiod	24 h
Test chamber size	4 L (McDonald jars)
Test solution volume	1 L for 1 h, then fill jar to 4 L during dilution
Renewal of test solutions	No renewal
Age of test organisms	1-3 days post hatch
Nº organisms per test chamber	50
Nº replicate chambers per concentration	3
Nº organism per concentration	150
Feeding required	No
Test chamber cleaning	Cleaning not required
Test solution aeration	No aeration, O_2 will checked 3x per day
Dilution water	Shubenacadie River
Test concentrations	Control (2 ppt), five concentrations (15, 25, 35, 45, 55 ppt) and brine (100 ppt)
Endpoint	Mortality
Volume of brine (100 ppt) required	Minimum 2 L per test
Test acceptability criterion	≥70% survival in controls

Table 4: 5-10 dph Larvae (Swim Bladder Inflated, 'First Feeding' Stage) Test Conditions

Test type	Static non-renewal
Exposure time	1 h, then dilute to 2 ppt
Test duration	48 h
Temperature	12 ºC and ambient (18-20 ºC)
Light quality	Dim ambient laboratory illumination
Light intensity	30 lux
Photoperiod	24 h
Test chamber size	4 L (McDonald jars)
Test solution volume	1.5 L for 1 h, then fill jar to 4 L during dilution
Renewal of test solutions	No renewal
Age of test organisms	5 – 10 day post hatch
Nº organisms per test chamber	50
Nº replicate chambers per concentration	3
Nº organism per concentration	150
Feeding required	No
Test chamber cleaning	Cleaning not required
Test solution aeration	Gentle aeration, O_2 checked 2x per day
Dilution water	Shubenacadie River
Test concentrations	Control (2 ppt), five concentrations (15, 25, 35, 45, 55 ppt) and brine (100 ppt)
Endpoint	Mortality
Volume of brine (100 ppt) required	Minimum 3 L per test
Test acceptability criterion	70% or greater survival in controls

Table 5: 10-20 dph Larvae Test Conditions

Test type	Static non-renewal
Exposure time	1 h, then dilute to 2 ppt
Test duration	72 h
Temperature	12 ºC and ambient (18-20 ºC)
Light quality	Dim ambient laboratory illumination
Light intensity	30 lux
Photoperiod	24 h
Test chamber size	4 L (McDonald jars)
Test solution volume	2 L for 1 h, then fill jar to 4 L during dilution
Renewal of test solutions	No renewal
Age of test organisms	10 - 20 days post hatch
Nº organisms per test chamber	50
Nº replicate chambers per concentration	3
Nº organism per concentration	150
Feeding regime	Feeding of *Artemia* may be required to prevent cannibalisms
Test chamber cleaning	Cleaning not required
Test solution aeration	Gentle aeration, O_2 checked 2x per day
Dilution water	Shubenacadie River
Test concentrations	Control (2 ppt), five concentrations (15, 25, 35, 45, 55 ppt) and brine (100 ppt)
Endpoint	Mortality
Volume of brine (100 ppt) required	Minimum 4 L per test
Test acceptability criterion	70% or greater survival in controls

Table 6: 30 mm Total Length Juveniles Test Conditions

Test type	Static non-renewal
Exposure time	1 h, then dilute to 2 ppt
Test duration	96 h
Temperature	Ambient (18-20ºC)
Light quality	Dim ambient laboratory illumination
Light intensity	300 lux
Photoperiod	24 h
Test chamber size	15 L (green plastic buckets))
Test solution volume	14 L
Renewal of test solutions	No renewal
Age of test organisms	30 – 40 days post hatch
Nº organisms per test chamber	10
Nº replicate chambers per concentration	3
Nº organism per concentration	30
Feeding required	No
Test chamber cleaning	Cleaning not required
Test solution aeration	Aeration provided, O_2 checked 2x daily
Dilution water	Shubenacadie River
Test concentrations	Control (2 ppt), five concentrations (15, 25, 35, 45, 55 ppt) and brine (100 ppt)
Endpoint	Mortality
Volume of brine (100 ppt) required	Minimum 25 L per test
Test acceptability criterion	90% or greater survival in controls

Table 7: Test Conditions and Procedures for Striped Bass 120 mm Juveniles

Test type	Static non-renewal
Exposure time	1 h, then dilute to 2 ppt
Test duration	96 h
Temperature	Ambient (18-20ºC)
Light quality	Normal ambient laboratory illumination
Light intensity	300 lux
Photoperiod	24 h
Test chamber size	15 L (green plastic buckets)
Test solution volume	14 L
Renewal of test solutions	No renewal
Age of test organisms	100 days post hatch
Nº organisms per test chamber	10
Nº replicate chambers per concentration	3
Nº organism per concentration	30
Feeding required	No
Test chamber cleaning	Cleaning not required
Test solution aeration	Aeration provided, O_2 will checked daily
Dilution water	Shubenacadie River
Test concentrations	Control (2 ppt), five concentrations (15, 25, 35, 45, 55 ppt) and brine (100 ppt)
Endpoint	Mortality
Volume of brine (100 ppt) required	Minimum 25 L per test
Test acceptability criterion	90% or greater survival in controls