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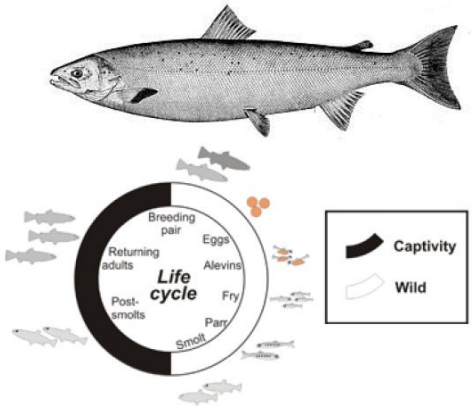
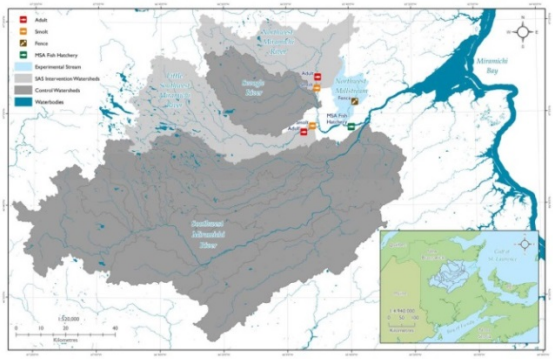
**National Capital Region**

**Canadian Science Advisory Secretariat**
**Science Advisory Report 2018/014**

# REVIEW OF RISKS AND BENEFITS OF COLLABORATION FOR ATLANTIC SALMON TOMORROW'S (CAST) SMOLT-TO-ADULT SUPPLEMENTATION (SAS) EXPERIMENT PROPOSAL (PHASE 1: 2018-2022)




*Smolt-to-adult supplementation (SAS) in the life cycle of Atlantic salmon*



*The Miramichi River in New Brunswick, Canada, highlighting the two main sub-watersheds associated with the SAS experiments. Figure courtesy of the CAST SAS Experiment Proposal: Phase 1 (2018-2022) (Linnansaari et al. 2017).*

## Context:

*Recent broad-scale declines in marine survival suggest that the most substantial threat(s) to wild Atlantic salmon in eastern Canada are found in the marine environment, and that the resulting high marine mortality is the primary constraint to the recovery of Atlantic salmon populations (COSEWIC 2010). Smolt-to-adult supplementation (SAS) has been proposed by Collaboration for Atlantic Salmon Tomorrow (CAST), a multi-stakeholder group, as an activity to circumvent low marine survival and to increase the number of spawners and freshwater juvenile production in the Northwest Miramichi River system (New Brunswick). SAS would be a precedent-setting activity for supplementation of Atlantic salmon populations in DFO's Gulf region, where populations are showing decline but are not yet at immediate risk of extinction. CAST has developed a SAS Experiment Proposal with the goals of determining if SAS is a functional conservation strategy that can be used to supplement Atlantic salmon populations in situations where conservation objectives are not being met due to high at-sea mortality, and to answer identified scientific knowledge gaps regarding the SAS conservation strategy.*

*This Science Advisory Report is from the January 22-23, 2018 national science advisory peer review of the Collaboration for Atlantic Salmon Tomorrow's (CAST) Smolt-to-Adult Supplementation (SAS) Experiment Proposal: Phase 1 (2018-2022). The purpose of the peer review meeting was to assess the risks and benefits of Phase 1 of the proposed SAS program to the long-term integrity, survival and recovery of the wild population of Atlantic salmon in the Miramichi River, and propose mitigation measures to minimize any risks and enhance any benefits. The advice will inform DFO Fisheries and Aquaculture Management, the sector responsible for the issuance of permits for supplementation activities. Participants at the review included invited national and international experts, aboriginal organizations, DFO Ecosystems and Oceans Science, DFO Fisheries and Aquaculture Management,* and participants from the proponent group. Additional publications from this meeting will be posted on the *Fisheries and Oceans Canada (DFO) Science Advisory Schedule as they become available.*

## SUMMARY


- This peer review meeting reviewed the Collaboration for Atlantic Salmon Tomorrow (CAST) Smolt-to-Adult Supplementation (SAS) Experiment Proposal: Phase 1 (2018-2022) (version 4 December 2017), to assess the risks and benefits of the proposed SAS experiment to the long-term integrity, survival and recovery of the wild population of Atlantic salmon in the Miramichi River, and to propose mitigation measures to minimize any risks and enhance any benefits.
- Program 1: Sub-basin Genetic Structure Analysis of the CAST SAS Experiment Proposal poses a negligible risk to the long-term integrity, survival and recovery of the wild population of Atlantic salmon in the Miramichi River.
- Despite indications of a low level of genetic differentiation among the sub-basins in the Miramichi River, collecting smolts across the entire experimental area and keeping individuals from the sub-basins separately in all aspects of the CAST SAS experiment, including the hatchery environment, and releasing adults in the same sub-basins in which they were collected as smolts, would reduce potential genetic risks.
- Program 2: Laboratory Experiments of the CAST SAS Experiment Proposal pose a negligible risk to the long-term integrity, survival and recovery of the wild population of Atlantic salmon in the Miramichi River, as the number of smolts and broodstock taken from the wild for use in the laboratory experiments is minimal compared to the overall population of the Miramichi River.
- Hatchery capacity may be a scientific risk affecting program success as it may limit the flexibility to adjust tank-based environmental conditions such as density, light, temperature, and water quality (water replacement and re-circulation system), and the resulting impacts on hatchery fish involving phenotype, genetic selection and disease.
- Program 3: Experimental River of the CAST SAS Experiment Proposal poses a low risk to the long term integrity, survival or recovery of the wild population of Atlantic salmon in the Miramichi River, as the number of SAS fish proposed for release in the Northwest Millstream is minimal compared to the overall population of the Miramichi River.
- The use of wild non-local fish (from other tributaries in the Miramichi) in Program 3: Experimental River is required since native fish may not be consistently present in the experimental stream, and this will add uncertainty to interpretation of the results.
- Program 4: SAS Impacts on a Natural River poses several risks to the long-term integrity, survival and recovery of the wild Atlantic salmon population in the Miramichi River. An extensive list of risks was presented following an earlier review (DFO 2016). Risks to the wild population of Atlantic salmon in the Miramichi River identified at this peer review meeting are:
    - Risk of transferring disease or pathogens, both from wild to captivity and captivity to wild;
    - Risks to the wild population increases as the proportion of SAS to wild fish (ratio) increases;
    - Risks to the wild population increases as the geographic footprint of the experiment increases;

will only be justified if the experiment has a reasonable probability of attaining its objectives;

- Risk of obtaining a smaller genetic component of the population to support SAS releases if smolts are collected from a localized area (not broadly distributing sampling in time and space); the proposal addresses this risk by planning for multiple collection areas distributed spatially and temporally; and,
- Risk of the Ryman-Laikre effect: an increase in inbreeding and a reduction in total effective population size in a combined captive-wild system, which arises when a few captive parents produce a large proportion of offspring that spawn in the wild; this risk is also addressed in the smolt collection protocols in the proposal.

- The benefits of Program 4: SAS Impacts on a Natural River to the long-term integrity, survival and recovery of the wild population of Atlantic salmon in the Miramichi River include:
  - Opportunity to obtain information about an alternative approach to population supplementation, specifically to address uncertainties identified in an earlier (DFO 2016) CSAS review of SAS;
  - Opportunity to validate and improve population models with empirical estimates for life history parameters and fitness components of SAS and wild spawners;
  - Opportunity to explore a proactive approach to population restoration, where the targeted population has declined from historical levels but is not yet at immediate risk of extinction; and,
  - Opportunity to obtain information about costs and benefits of current wild broodstock and juvenile stocking programs relative to SAS.
- Mitigation measures identified at this peer review meeting which could act to reduce risks to the wild population of Atlantic salmon in the Miramichi River include:
  - Institute a Board to monitor results of the experiment and make adaptive recommendations on program activities on an annual basis, based on pre-agreed triggers and decision points;
  - Conduct detailed scans for diseases and pathogens to reduce the risk of transferring these from the wild to captivity and vice versa, based on representative samples of incoming smolts and SAS adults for release;
  - Examine the genomics of healthy and diseased or dead fish, particularly the genomics of fish from captive cohorts that experienced high mortality before release, to identify any genetic correlates of disease susceptibility;
  - Adjust the ratio of SAS releases to wild spawners annually such that SAS inputs do not exceed returns of wild fish;
  - Conduct the experiment on a smaller scale, for example, only in one sub-basin;
  - Alternate the years of SAS releases if conducting the experiment in two sub-basins;
  - Continue modelling to explore risks and optimize parameters of supplementation; and,
  - Increase sampling efforts to improve statistical power of experiments.

- The proposed program sequencing (Programs 1 through 4) poses a low risk to the long-term integrity, survival and recovery of the wild population of Atlantic salmon in the Miramichi River, as activities in Program 4 are not dependent on activities in Programs 1 to 3.

# BACKGROUND

Recent broad-scale declines in marine survival suggest that the most substantial threat(s) to wild Atlantic salmon in eastern Canada are found in the marine environment, and that the resulting high marine mortality is the primary constraint to the recovery of Atlantic salmon populations (COSEWIC 2010). As a way to circumvent the marine phase of the Atlantic salmon lifecycle and maintain adult numbers, smolt-to-adult supplementation (SAS) has been proposed as an alternative intervention to prevent extirpation, minimize loss of genetic diversity, and maintain Atlantic salmon populations until marine survival becomes favourable (DFO 2008; DFO 2016). SAS consists of the capture of wild salmon at juvenile life stages, rearing them in captivity to the adult stage, and subsequently releasing the adult captive-reared fish back into the river of origin to complete the life cycle (DFO 2016). A science peer review of the risks and benefits of the SAS approach to the fitness of wild Atlantic salmon was conducted in December 2015 (DFO 2016).

Collaboration for Atlantic Salmon Tomorrow (CAST), a multi-stakeholder group, has developed a SAS Experiment Proposal (Linnansaari et al. 2017) with the goals of determining if SAS is a functional conservation strategy that can be used to supplement Atlantic salmon populations in situations where conservation objectives are not being met due to high at-sea mortality, specifically for the Northwest Miramichi River system (New Brunswick), and of answering identified scientific knowledge gaps regarding the SAS conservation strategy. The intent is to circumvent low marine survival, thereby increasing the number of spawners and freshwater juvenile production.

SAS has been used for endangered Atlantic salmon populations of the Bay of Fundy, Canada, and Maine, USA, and for populations of Pacific salmon in precipitous declines, but there has only been a single case of its use in supplementing wild population sizes where declines have not yet reached precipitous levels (see summary in Fraser 2016). SAS would be a precedent-setting activity for supplementation of Atlantic salmon populations in DFO's Gulf region, where populations are showing declines but are not yet at immediate risk of extinction. DFO (2016) indicated that SAS reduces some of the known risks associated with traditional supplementation of juvenile stages but introduces risks at other points in the anadromous life cycle that are not well understood.

The CAST SAS proposal adopts a two-phased approach; Phase 1 is the collection of wild smolts and release of the resulting captive-reared adult salmon, while Phase 2 is a subsequent monitoring period with no SAS releases. This peer review meeting reviewed the CAST SAS Experiment Proposal: Phase 1 (2018-2022) (Linnansaari et al. 2017), to assess the risks and benefits of the proposed SAS experiment to the long-term integrity, survival and recovery of the wild population of Atlantic salmon in the Miramichi River, and propose mitigation measures to minimize any risks and enhance any benefits (Chaput et al. 2016; DFO 2016; DFO 2018; Fraser 2016; Pavey 2016 were used as key references).

# ASSESSMENT

## Program 1: Sub-basin Genetic Structure of Atlantic Salmon on the Miramichi River

The purpose of this first level of study in the CAST SAS Experiment Proposal is to assess the sub-basin genetic structure of Atlantic salmon on the Miramichi River and the requirement of sustaining unique rearing lines for SAS fish production from different sub-basins. Understanding the sub-basin genetic structure of the Miramichi River system is fundamental to initiating a SAS experiment as it establishes the foundation to ensure that smolt rearing lines are appropriately created to protect the natural populations from some of the potential genetic risks, and establishes the baseline level of genetic differentiation between the tributaries. It also lays the basis for detection of genetic changes during and after a SAS experiment. Until recently there has been little published knowledge of the genetics and extent of local adaptation among the sub-basins of the Miramichi River system (Chaput et al. 2016).

The work outlined in the proposal has essentially been completed and results are in review for publication. Results were presented by a study author (L. Bernatchez) and reviewed at the meeting. Seven hundred and seventy-four (774) 1+ age parr were collected in 2016 from electrofishing surveys (see Figure 1). Sites were grouped into major drainages (sub-basins) and samples were genotyped with a 50K SNP array. The results indicate very low average (across loci) levels of genetic differentiation among the sub-basins in the Miramichi River; the most highly differentiated sub-basins were Rocky Brook, Taxis River, and Northwest Millstream. With the exception of these three sub-basins, results indicate that genetic differentiation is sufficiently low that individuals generally cannot be reassigned to sub-basins in the system.

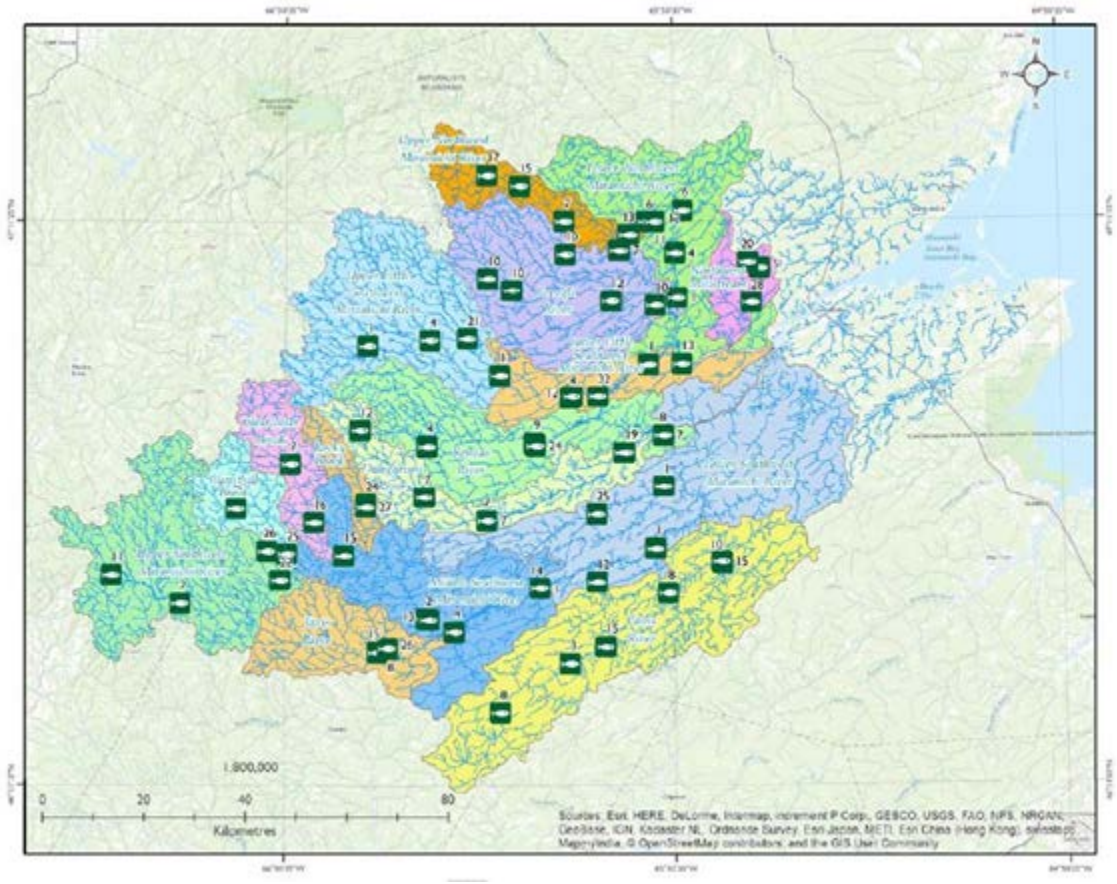



*Figure 1: Sub-basins of the Miramichi River where sampling for genetic baseline material was undertaken in autumn of 2016 with respective sample sizes at each location. Figure courtesy of the CAST SAS Experiment Proposal: Phase 1 (2018-2022) (Linnansaari et al. 2017).*

The level of genetic differentiation between sub-basins was considered unusually low for a system of this size. Factors which could have contributed to lack of differentiation in genetic structure include the long history of supplementation in this system and its high effective population size; the three sites with the most highly differentiated sub-basins are also those with the lowest effective population sizes.

Program 1: Sub-basin Genetic Analysis of the CAST SAS Experiment Proposal poses a negligible risk to the long-term integrity, survival and recovery of the wild Atlantic salmon population in the Miramichi River. Further work to refine and confirm the results of the analysis was recommended.

*Recommendations for future work*

- Despite indications of weak genetic differences in this study, collecting smolts across the entire experimental area and keeping individuals from the sub-basins separated in all aspects of the CAST SAS experiment, including the hatchery environment, would reduce potential genetic risks. Adults should be released in the same sub-basins in which they were collected as smolts.
- Repeat the sub-basin genetic analysis in fall 2018 to assess temporal variation.

- If possible, analyze historical samples of biological materials (e.g. scales) from the Miramichi River to determine historic level of genetic differentiation prior to supplementation.
- Repeat the sub-basin genetic analysis in the same locations five years after the SAS experiment is initiated in the natural river environment, as outlined in the proposal.

## Program 2: Laboratory Experiments

The purpose of Program 2 of the CAST SAS Experiment Proposal is to assess, in a fully controlled environment, phenotypic and genotypic characteristics of SAS and wild fish, both adults and progeny. Metrics such as fecundity, fertilization success, egg size, and juvenile survival would be used to assess relative fitness of SAS and wild fish. These data would inform any modifications and/or development of rearing practices to continually improve the phenotypic quality of SAS individuals.

Program 2: Laboratory Experiments of the CAST SAS Experiment Proposal poses a negligible risk to the long-term integrity, survival and recovery of the wild Atlantic salmon population in the Miramichi River, as the number of smolts and broodstock taken annually from the wild for use in the laboratory experiments is minimal compared to the overall population of the Miramichi River.

*Environmental factors*

Abiotic factors (e.g., water temperature, salinity), rearing at high densities, rearing in a confined environment, and feeding regimes are examples of the risks associated with the hatchery environment that may lead to deviations from wild characteristics through phenotypic plasticity and domestication selection (an extensive list is provided in Table A2 in DFO 2016). In the current proposal, captive fish would be reared in freshwater, losing exposure to the marine environment as in the wild population. Although a range of factors could influence the fitness of captive-reared fish, the simple fact of being in captivity rather than in the wild will be the overriding factor affecting fitness.

Modifications of environmental factors in captivity such as limiting human interaction, rearing fish in low density conditions, feeding fish from a distance, controlling light and water temperatures, can reduce the risks of phenotypic change (via domestication selection or phenotypic plasticity) of the hatchery fish (individuals which may be used in subsequent programs of the CAST SAS experiment).

*Hatchery capacity*

Hatchery capacity may be a scientific risk affecting program success, as it may limit the flexibility to adjust tank-based environmental conditions such as density, light, temperature, and water quality (water replacement and re-circulation system), and the resulting impacts on hatchery fish. The smolt rearing facility at the Miramichi Salmon Conservation Centre is currently at capacity; adding more tanks to the hatchery would allow for more experimentation to examine the impacts of environmental influences on fitness of hatchery fish.

*Transfer of diseases and pathogens*

In addition to the current efforts being undertaken to monitor and limit diseases and pathogens in the hatchery environment, a sample of representative individuals from incoming smolts and from SAS individuals (adults) upon release should be tested for diseases and pathogens. Disease monitoring should also take place while in captivity and during movement of fish from tank to tank. A Transfer Certificate is required to return animals from the hatchery to the wild,

typically requiring necropsies of a set number of sacrificed individuals. A disease/pathogen scan broader than that required for the Transfer Certificate should be considered.

The following recommendations for improvements to the proposal were made.

*Recommendations*

- Test a sample of representative individuals from incoming groups of smolts and from SAS adults prior to release for health assessment, diseases and specific pathogens. In addition, testing and health monitoring should take place while in captivity.
- Record all dead and euthanized fish with minimal demographic information and history (date, tank, external condition, etc.). A simple gross necropsy, minimally reporting gross external and internal observations, of most fish that are found dead or are euthanized, is recommended. Tissue collection procedures should be performed strategically on selected necropsies, involving representative fish.
- Establish a protocol for staff so that necropsies are performed and reported consistently, and tissues are appropriately collected for possible molecular testing, culture and/or histopathology.
- Examine the genomics of healthy and diseased or dead fish, particularly the genomics of fish from captive cohorts that experienced high mortality before release, to identify any genetic correlates of disease susceptibility.
- Locomotive capacity (swimming) and timing of emergence of progeny should be considered for addition to the list of fitness metrics outlined in the proposal. Timing of emergence of progeny can strongly affect survival in natural environments. Locomotive capacity can be measured by varying water flows in the hatchery tank to increase speed and swimming capabilities of SAS fish.
- While the act of constantly improving rearing conditions based on experience risks complicating interpretation of final results, the current experimental design, which allows for ongoing improvement of rearing conditions, is appropriate to meet the objectives.
- Although the proposal aims to release fish from laboratory experiments into the wild with no monitoring, it would be preferable either to dispose of them or to use these valuable specimens in further experiments. They could be used, for example, in common garden experiment(s) to explore and monitor relative performance of different crosses. The upper part of the Northwest Millstream was identified as a possible location for these types of experiment(s) in the wild.
- Fish from laboratory experiments might also be used for epigenetic and gene expression experiments to help understand the mechanisms behind any intergenerational phenotypic changes observed, particularly those associated with hatchery rearing from the smolt to adult stages. This would quantify the extent of epigenetic reprogramming caused by maternal effects and the rearing environment as well as the subsequent consequences on patterns of gene expression. Assessing these same parameters in the progeny of SAS progeny fish would also be desirable to determine the extent of multigenerational effects.

  This recommendation goes beyond the scope of the proposal but implementation would be beneficial to assessing the long-term impacts of SAS on the population.

## Program 3: Experimental River

The purpose of Program 3: Experimental River is to undertake a small-scale release of SAS adults in a small, natural stream to study how SAS fish will respond to a natural environment and interact with wild salmon. The controlled studies are proposed to take place in a headwater section of the Northwest Millstream (NWMS) (see Figure 2). The proposed experiment would examine SAS and wild adult behaviour, activity levels, and survival, and SAS and wild progeny survival, growth and behaviour. The wild fish for the experiment will likely have to be collected and transported to the experimental river site from another tributary in the Miramichi River, as wild anadromous adults may not be consistently present in the NWMS.

Under the proposed protocol, 20 pairs (20 female, 20 male) each of SAS and wild fish would be released annually for three years in the experimental river. All released fish would be genotyped, radiotagged and PIT tagged, and behaviour, survival and distribution would be followed. Progeny would be sampled by electrofishing in subsequent years, parentage assigned by genotyping, and distribution, survival and behaviour monitored.

Program 3: Experimental River poses a low risk to the long term integrity, survival or recovery of the wild population of Atlantic salmon in the Miramichi River, as the number of SAS fish proposed for release in the NWMS is minimal compared to the overall population (thousands of spawners in recent years, DFO 2018) of the Miramichi River.

In addition to the uncertainties typical of running experiments in a natural river system, the use of wild non-local fish (from other tributaries in the Miramichi) to compare SAS and wild adult behaviour, activity levels and survival, adds uncertainty to the interpretation of the results as the wild non-local fish are not native to the experimental river. Alternative approaches to the experimental river component were considered to enhance its scientific integrity, and the following recommendation was made.

*Recommendation*

- Using progeny from the laboratory experiments, planting known crosses and a controlled number of progeny into the NWMS in a common garden experiment to explore and monitor relative performance of different crosses, rather than depending on spawning of SAS fish in the wild, could reduce experimental uncertainties.

  However, this approach (planting known crosses) would not allow for assessment of spawning interactions between wild and SAS fish in the wild, which is one objective of the proposed CAST SAS experiment.

## Program 4: SAS Impacts on a Natural River

The purpose of Program 4: SAS Impacts on a Natural River is to conduct an experiment to monitor the behaviour and spawning success of SAS adults relative to wild adults in natural rivers, and to assess the subsequent density, survival, growth and genotypic and phenotypic differences in resulting progeny. This part of the study is intended to contribute to the understanding of the generational contribution of SAS adults relative to wild adults, specifically if the SAS parents produce progeny that migrate to the ocean and return to rivers to spawn successfully and contribute to the production of fit juveniles.

The sub-basins proposed for the fourth level of study are Little Southwest Miramichi River (LSW) sub-basin upstream from Sillikers, and the Northwest Miramichi River (NWM) sub-basin upstream of the mouth of Trout Brook (see Figure 2). Sevogle River is to be left untreated and act as a control population within the system. Similarly, the whole Southwest Miramichi River

system will act as a control. Adults are proposed to be released into both sub-basins every year from 2018 to 2022.

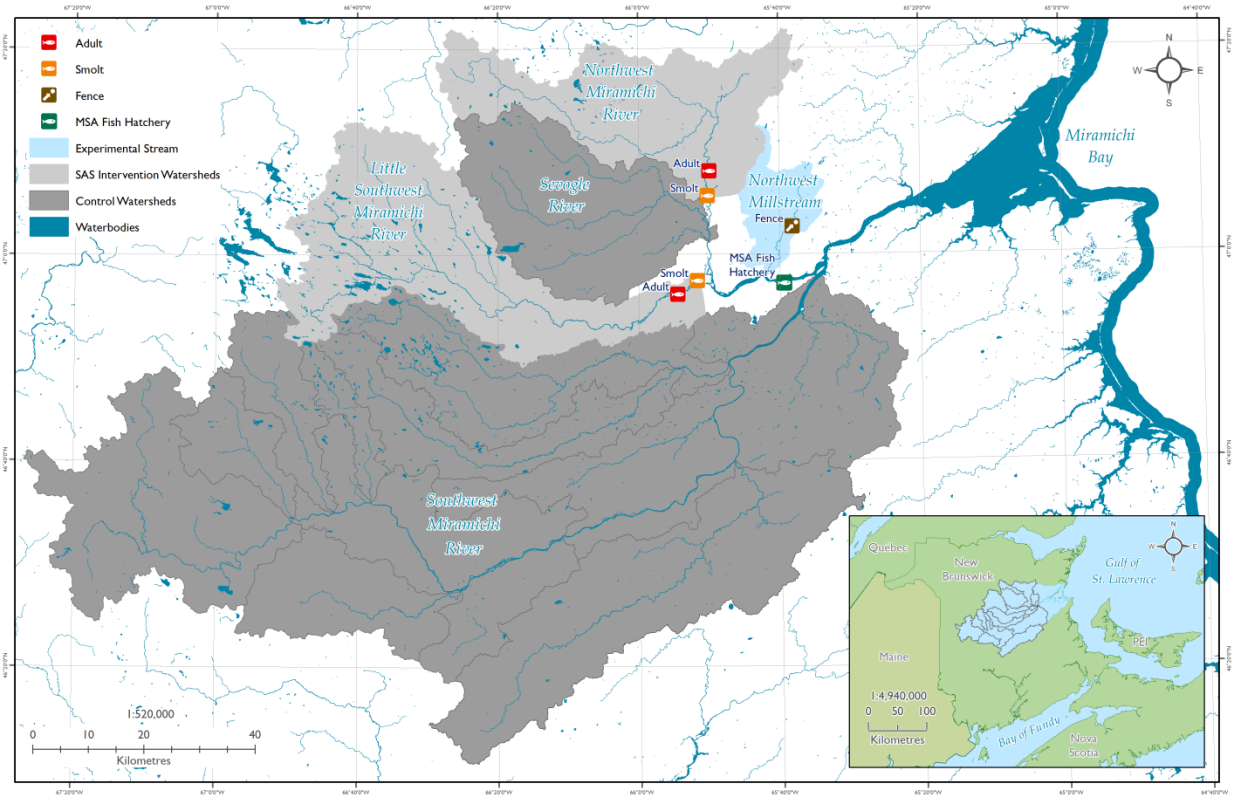



*Figure 2: The Miramichi River in New Brunswick, Canada, highlighting the two main sub-watersheds associated with the SAS experiments: the Experimental Stream (Northwest Millstream), and the two sub-basins with proposed SAS intervention (Little Southwest and Northwest). The orange fish symbols highlight the areas where wild smolts are collected and the red fish symbols indicate the release locations of the mature adults. The Miramichi Salmon Conservation Centre is where salmon are being raised to maturity. Figure courtesy of the CAST SAS Experiment Proposal: Phase 1 (2018-2022) (Linnansaari et al. 2017).*

## Risks of Program 4: SAS Impacts on a Natural River

Program 4: SAS Impacts on a Natural River poses several risks to the long-term integrity, survival and recovery of the wild Atlantic salmon population in the Miramichi River.

An extensive list of risks was presented in DFO 2016 (see Tables A2 and A3), including loss of marine adaptation of SAS adults and resulting introduction of unselected genotypes into the system, early-stage SAS progeny competing with wild juveniles, competition for mates and disruption of wild spawning, and reduction in mean fitness of adult SAS progeny relative to wild returning fish. These remain relevant to assessing the CAST SAS Experiment Proposal.

Specific risks to the wild population of Atlantic salmon in the Miramichi River identified at this peer review meeting are outlined below (some of these had previously been identified in DFO 2016).

- **Risk of transferring disease or pathogens, both from wild to captivity and captivity to wild**

Captive-reared fish are commonly susceptible to increased presence of pathogens or parasites as a result of being reared at higher densities than in the wild, and may experience genetic changes associated with mortality due to differing pathogen/parasite regimes or loading via domestication selection. As a result, release of captive-reared fish could potentially act as a vector of disease to wild fish and may thus contribute to the depletion of wild populations. In addition to risk of disease or pathogens developed in captivity, pathogens, parasites or disease could gain entry from wild fish entering the hatchery environment.

Release of captive fish into the wild requires a Transfer Certificate certifying that the fish are disease-free, but a broader scan for diseases, pathogens and parasites should be considered (see Program 2 recommendations and below under “Mitigation Measures”).

- **Risk to the wild population increases as the proportion of SAS to wild fish (ratio) increases**

  As the proportion of released SAS adults to wild returning salmon increases, so too does the overall risk to the wild population (DFO 2016). The number of SAS adult releases should never exceed the number of wild adult returns, but should be high enough to allow for sufficient statistical power to address knowledge gaps.

  Currently, CAST proposes to release approximately 1850 and 1550 SAS fish into the NWM and the LSW, respectively, in Year 1. In Years 2 through 5, annual releases are proposed to include the surviving mature adults from the 2500 smolt collections from each sub-basin, less the adults required for Programs 2 and 3, with the objective of releasing approximately 2000 fish into each sub-basin each year. The maximum ratio of SAS releases to wild adult returns is proposed to be 1 to 1 (Linnansaari et al. 2017).

  Under the CAST SAS Experiment Proposal, ARIS (Adaptive Resolution Imaging Sonar, an experimental sonar system) will be used to enumerate wild adult returns to the NWM and the LSW, which will allow for further refinement of the ratio of wild to SAS fish in the system. However, there is uncertainty with ARIS counts as, currently, it can only report the number of fish and their respective sizes and must also rely on information such as migration timing and fish behaviour to decipher species composition. As in any assessment of fish abundance, there are uncertainties in this method which should be considered in making abundance determinations.

  Based on recent abundance estimates published by DFO and the proposed number of releases, potential SAS spawners as a proportion of total wild spawners would be in the range of 25% to 50% in the LSW and 40% to 80% in the NWM.

- **Risks to the wild population increases as the geographic footprint of the experiment increases**

  The greater the geographic scale of the SAS experiments, the greater the risk to the wild population of Atlantic salmon in the Miramichi River. The LSW represents 47% of the total Atlantic salmon fluvial habitat in the Northwest Miramichi River system, and the NWM above Sevogle 23%. As currently proposed by CAST, the SAS experiment would thus encompass 70% of the total rearing area in the Northwest Miramichi River system. Although a geographic scale that minimizes the risk to the wild population of Atlantic salmon in the Miramichi River was not identified, there was consensus that concentrating SAS interventions at a smaller scale would reduce the overall risk to the wild population of Atlantic salmon.

- **Risk of not achieving scientifically relevant results or of not addressing knowledge gaps**

  There are risks of not obtaining scientifically relevant results to address knowledge gaps on SAS if the experimental design is insufficiently robust. While these are not direct risks to the integrity, survival and recovery of the wild population as such, the risks to the population incurred by undertaking the proposed experiment should be justified by minimizing scientific risks.

  For example, if too small a percentage of the returning adults are captured, sampled, and genotyped, precise estimates of mean adult-to-adult reproductive success (fitness) of SAS progeny and wild adults will not be possible; this information is needed to assess the genetic risks of SAS activities to wild salmon of the Miramichi. The proposed numbers (and expected proportions) of returning salmon expected to be genotyped are probably currently too low to assess the genetic effects of SAS.

  Other risks to achieving scientific objectives are also outlined in this report.

- **Risk of obtaining a smaller genetic component of the population to support SAS releases**

  If smolts are collected in a localized area and sampling is not distributed in time and space, there is a risk of obtaining a smaller genetic representation of the population to support SAS releases. This risk is addressed in the CAST SAS Experiment Proposal which spreads smolt collection in time and space.

- **Risk of the Ryman-Laikre effect**

  There is a risk of the Ryman-Laikre effect which occurs when a few captive parents produce large numbers of offspring that spawn in the wild, as this will result in an increase in inbreeding and a reduction in total effective population size in the combined captive-wild system (Waples et al. 2016).

  The proposed experimental design should be adequate to avoid this risk but mortality and selection in the captive rearing stage could increase this risk.

## Benefits of Program 4: SAS Impacts on a Natural River

Benefits to the long-term integrity, survival and recovery of the wild population of Atlantic salmon in the Miramichi River include:

- Opportunity to obtain information about an alternative approach to population supplementation, specifically to address uncertainties identified in DFO (2016) by obtaining on-the-ground information;
- Opportunity to validate and improve demographic models by obtaining empirical estimates for important life history parameters such as survival rates, and relative fitness components of SAS and wild spawners and progeny;
- Opportunity to explore a proactive approach to population restoration where the targeted population, although at low abundance relative to historical levels, is not yet at immediate risk of extinction; and
- Opportunity to obtain information about costs and benefits of current wild broodstock and juvenile stocking programs relative to SAS, since fish from ongoing juvenile supplementation programs would also be sampled in the proposed experiment.

## Mitigation Measures to Reduce Risks for Program 4: SAS Impacts on a Natural River

The meeting considered several mitigation measures which could act to reduce risk of the proposed activities.

- **Institute a Board to monitor results of the experiment and make adaptive recommendations on program activities on an annual basis**.

  An adaptive management plan is included in the CAST Experiment Proposal, an important risk management feature of an experimental program of this type. A Board should be instituted to monitor results and make adaptive recommendations on program activities on an annual basis. Triggers and decision points should be agreed upon in advance of the experiment, while allowing for adjustments on all elements, including program sequencing, upon annual review.

  Examples of actions which could be taken should the Board identify a risk of irreversible genetic harm to the population include cryopreservation of wild Atlantic salmon sperm and releasing all female SAS fish to reduce SAS-wild interactions.

- **Conduct detailed scans for diseases and pathogens to reduce the risk of transferring disease or pathogens, both from wild to captivity and captivity to wild.**

  Test representative samples of incoming smolts and SAS individuals upon release for diseases or pathogens, both while in captivity and during transfer. Returning fish to the wild will require a Certificate of Transfer certifying that the animals are free from specific conditions, but a broader scan of diseases, pathogens and parasites could be considered, in order to increase certainty.

  Further details of recommended fish health control measures which would mitigate risk are found under Program 2 “Recommendations”.

- **Adjust the ratio of SAS to wild fish annually such that SAS inputs do not exceed returns of wild fish**.

  The number of SAS salmon released into a given tributary should not exceed the number of wild adult returns to the tributary in any given year; a 1:1 ratio of SAS to wild fish was discussed at the meeting as representing a reasonable balance between mitigating possible SAS effects on the wild population and maximizing statistical power for detecting differences in the fitness of SAS progeny (SAS F1 salmon) compared to wild salmon. Adjusting SAS releases annually based on a desired ratio would be challenging as it is currently not possible to forecast returns to sub-basins. Using recent estimates of overall returns, it may be possible to get an approximate value of the returns against which to base numbers of annual releases of SAS females.

- **Conduct the experiment on a smaller scale, for example, in only one sub-basin**.

  Conducting the experiment on a smaller geographic scale would reduce the scope and potential risk of the experiment, which, as proposed, would involve 70% of fluvial habitat of the Northwest Miramichi River system. Three potential sub-basins were discussed:

  - The LSW sub-basin, 40% of fluvial habitat in the total Northwest Miramichi system, drains into tidal waters, reducing the risk of SAS fish straying into other sub-basins.
  - The NWM sub-basin, 23% of the fluvial habitat of the Northwest Miramichi system, is upstream of the Sevogle River entrance, potentially allowing for strays (including those affected by SAS) into the Sevogle.

- The NWMS is only 3% of the fluvial habitat in the system. As native fish may not be consistently present in the system, the use of wild non-local fish (from other tributaries in the Miramichi) would be required. In addition there is persistent blockage of this system by beaver dams.

Reducing the scale of the experiment could also allow for the capture and genotyping of a larger proportion of adult returns (including potential SAS F1 and F2 progeny), thereby increasing statistical power to detect fitness differences between SAS and wild salmon.

- **Alternate the years of SAS releases if conducting the experiment in two sub-basins.**

  Alternating the years of SAS releases, for example releasing SAS adults in the LSW sub-basin in Year 1, in the NWM sub-basin in Year 2, and so on subsequently, would reduce the temporal scope of the experiment in each sub-basin while allowing for comparison of results in replicate sub-basins.

- **Continue modelling to explore risks and optimize parameters of supplementation**.

  The use of modelling to explore risks and optimize parameters of supplementation should be continued. Models could then be validated with empirical data from SAS experiments.

- **Increase sampling efforts to improve statistical power of experiments**

  The proposal addresses sampling of returning adults (i.e., tissue samples, size, and sex) in addition to counts. Increasing the proportion of returning salmon that are captured, genotyped, and pedigreed each year would increase the overall robustness of the experimental design, and improve the statistical power to assess the long-term fitness consequences of SAS interventions on the wild population. Sampling of 50% to 70% of returning adults was suggested.

  In the context of fitness estimation, increased sampling intensity on a smaller number of returns into a smaller environment would increase statistical power. However, in the context of demographic responses, a smaller experiment would reduce the capability to detect demographic level response.

  Increasing sampling efforts would improve explanatory power of the experiment but would not mitigate risk to the wild population.

In addition to the above mitigation measures, recommendations were made for additional studies which would contribute to better understanding of the long-term impacts of SAS.

- **Design sampling to strengthen multigenerational analysis and to trace pedigree of returning fish**.

  To fully assess the impact of SAS on population integrity, survival and recovery, contributions of generations beyond the F1 (to F2, F3) should be examined. Sampling a high proportion of returning adults to experimental tributaries would be important in order to trace the pedigree of returning subsequent generations. All catchable returning adults would have to be sampled to the highest number possible to ensure adequate sample size to examine F2+ contributions. The number of samples analyzed should be based on a power analysis of the magnitude of differences required in order to identify fitness level differences (e.g., 10%).

  The current proposal does not address generations subsequent to the F1, and numbers sampled in the current proposal would be inadequate to accurately detect genotypic contributions of SAS and wild fish to generations beyond F1.

- **Examine epigenetic and gene expression of individuals in the natural environment**.

  Recent research on a range of taxa including fishes suggests that environmentally induced epigenetic changes (such as those which might occur in captive rearing) may be passed from parents (maternal and paternal) to offspring, although there is limited knowledge of these potential effects to date and this is an area of active research.

  To address this knowledge gap, individuals in the natural environment representing both wild and SAS progeny (matched for developmental stage) could be collected to perform epigenetic and gene expression experiments similar to those undertaken on fish derived from the laboratory experiments (see Recommendations in Program 2: Laboratory Experiments).

### Program sequencing

The proposed program sequencing (Programs 1 through 4) poses a low risk to the long-term integrity, survival and recovery of the wild population of Atlantic salmon in the Miramichi River, as activities in Program 4 are not dependent on activities in Programs 1 to 3.

### Sources of Uncertainty

Smolt-to-adult supplementation (SAS) has only recently been initiated as an approach to supplement populations of Atlantic salmon that are considered at high risk of extinction. To date there is little empirical information to quantify risks and benefits of SAS to a wild population and to propose mitigation measures.

The proposed activities in the CAST SAS Experiment Proposal are intended to provide information to address some uncertainties identified in an earlier review (DFO 2016).

## CONCLUSION

This peer review sought to bring the appropriate expertise, including technical and traditional ecological knowledge, to assess the risks and benefits of the proposed SAS experiment to the long-term integrity, survival and recovery of the wild population of Atlantic salmon in the Miramichi River, and to propose mitigation measures to minimize risks and enhance benefits.

The goal of the CAST SAS Experiment Proposal: Phase 1 (2018-2022) is to address scientific knowledge gaps and determine if SAS is a functional conservation strategy that can be used to circumvent the marine phase of the Atlantic salmon lifecycle and maintain spawning escapements in situations where populations are experiencing high at-sea mortality. CAST is proposing to initiate SAS in the Northwest Miramichi River system (New Brunswick), which would be a precedent-setting activity for supplementation of Atlantic salmon populations in DFO's Gulf Region where populations are showing decline but are not yet at immediate risk of extinction. Although SAS has been used as a recovery action for endangered or highly depleted salmon populations, there is very limited experience to date with use of this approach in populations which are at relatively low abundance but not yet at immediate risk of extinction.

Programs 1 and 2 of the CAST SAS Experiment Proposal pose a negligible risk to the long-term integrity, survival and recovery of the wild population of Atlantic salmon in the Miramichi River. Program 3: Experimental River poses a low risk as the number of SAS fish proposed for release in the NWMS is minimal compared to the overall population of the Miramichi River.

Program 4: SAS Impacts on a Natural River poses several risks to the long-term integrity, survival and recovery of the wild Atlantic salmon population in the Miramichi River, including those identified in DFO (2016). Specific risks addressed in this review include: risk of

transferring disease or pathogens, both from wild to captivity and captivity to wild; increase in risk to the wild population as the proportion of SAS to wild fish (ratio) increases; increase in risk to the wild population with increase in the geographic footprint; risks associated with disproportionate contribution to spawners in the wild of adults from a limited gene pool of hatchery-reared fish.

Benefits include an opportunity to obtain experimental and field information on the efficacy of the SAS approach to assess its validity as a functional conservation tool and to validate population models; an opportunity to assess a proactive approach to conservation for Atlantic salmon populations which are declining but not yet at risk; and an opportunity to address uncertainties identified in an earlier CSAS review (DFO 2016).

Mitigation measures identified to reduce risks to the wild population of Atlantic salmon in the Miramichi River include: carrying out measures to reduce the risk of transferring diseases and pathogens; adjusting the ratio of wild to SAS salmon annually; conducting the experiment on a smaller geographic scale, e.g. in one of the sub-basins identified in the proposal; alternating the years of SAS releases in different sub-basins; and conducting modelling to assess risks. Setting up a Board to monitor results of the experiment and recommend adaptive measures to address risks during implementation would be an important mitigation measure. Increasing sampling efforts to increase the power of multigenerational analysis and to trace the pedigree of returning subsequent generations would help to assess the long-term fitness consequences of SAS interventions on the wild population but this would be an addition to the CAST proposal as reviewed. The proposed approach of running the experiment for a limited time (five years) mitigates the risk of long-term SAS impacts on a wild system.

There remain a number of uncertainties about the impacts of SAS and of the proposed experiment, but the activities proposed would help to address some of these. Uncertainties and knowledge gaps might also be addressed by reviewing the results of existing SAS programs in other river systems, which may be sufficiently advanced that they could provide useful information.

## SOURCES OF INFORMATION

This Science Advisory Report is from the January 22-23, 2018 national science advisory peer Review of risks and benefits of Collaboration for Atlantic Salmon Tomorrow's (CAST) Smolt-to-Adult Supplementation (SAS) Experiment Proposal (Phase 1: 2018-2022). Additional publications from this meeting will be posted on the Fisheries and Oceans Canada (DFO) Science Advisory Schedule as they become available.

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# APPENDIX OR APPENDICES

# COLLABORATION FOR ATLANTIC SALMON TOMORROW (CAST) SMOLT-TO-ADULT-SUPPLEMENTATION (SAS) EXPERIMENT PROPOSAL: PHASE I (2018-2022)

## SEEKING ANSWERS TO IDENTIFIED SCIENTIFIC KNOWLEDGE GAPS REGARDING THE SMOLT-TO-ADULT SUPPLEMENTATION CONSERVATION STRATEGY

**4 December, 2017**

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# 1. Background

Canada's Policy for Conservation of Wild Atlantic Salmon (DFO 2009) dictates that management intervention should increase when Atlantic salmon (*Salmo salar*) populations decline below selected benchmark values under which a population is no longer considered to be healthy. Declines in Atlantic salmon populations below conservation targets have been observed in many index rivers in Atlantic Canada (e.g., ICES 2017). Supplementation, or "stocking", is often seen as a rational and reasonable response to a situation where a population of salmon is depleted, or perceived to be depleted (IBIS 2013).

Scientific consensus on salmon stocking suggests that assumptions of net benefits of traditional stocking programs are frequently not valid (IBIS 2013). Indeed, controlled studies have found that progeny of hatchery fish have decreased fitness in the wild compared to progeny of wild fish (e.g. Reisenbichler and Rubin 1999).

Management intervention is important because the risks to population perseverance increase as a population declines below its established conservation target (CAFSAC 1991). These risks include, but are not limited to accentuation of annual fluctuations in run size, increased susceptibility to extinction from genetic, demographic, or environmental catastrophes, decreases in productivity, and permanent changes in demographic characteristics of the spawning population (CAFSAC 1991).

Smolt-to-adult supplementation (SAS; a term coined by Fraser (2016)) has emerged as a potential conservation strategy for Atlantic salmon (and other salmonid) rivers where populations have declined below targeted conservation levels (CSAS 2016). The SAS strategy, in its simplest form, captures outward migrating, wild Atlantic salmon smolts and rears them to adults in either fresh- or saltwater (CSAS 2016). Upon maturity, the adults are returned to the river of their origin to spawn in the wild. Such a strategy is hypothesized to be beneficial in populations where the population decline is attributable to high at-sea mortality, i.e., SAS effectively circumvents this at-sea bottleneck for the population. The SAS strategy has benefits in comparison to traditional juvenile stocking methods based on broodstock collections; however, the SAS strategy may similarly have risks that are not thoroughly understood (Fraser 2016).

The SAS strategy is not a novel idea. Smolt-to-adult supplementation first emerged in Thomas (1996) as a conservation strategy for rainbow (steelhead) trout (*Oncorhynchus mykiss*). Thomas (1996) outlined the scientific and monitoring needs, potential risks, and potential promise as a conservation tool. Fraser (2016) provided a detailed outline of the potential risks and benefits related to the SAS strategy, and recently, a 20-year report of experiences of adult supplementation in Idaho was compiled and has added significantly to the scientific knowledge-base regarding the strategy (Kozfkay et al. 2017).

The SAS strategy has already been implemented where wild salmonid populations have declined below conservation targets (Dempson and Furey 1997; Dempson et al. 1999; Berejikian et al. 2008; Jones et al. 2014; Venditti et al. 2013; Kozfkay et al. 2017), and ongoing

SAS programs for Atlantic salmon exist in New Brunswick in the Saint John River by Fisheries and Oceans Canada (Outer Bay of Fundy populations; releases ranging from 339 to 1348 adult SAS spawners between 2003 to 2015 in sympatry with wild salmon; Jones et al. 2015) and in the Upper Salmon River by Parks Canada (Inner Bay of Fundy populations; releases ranging from 429 to 965 adult SAS spawners between 2015 to 2017 in allopatry; Corey Clarke, Parks Canada, unpubl. data). In New Brunswick, the preliminary observations and anecdotal data suggest successful spawning and population maintenance (Jones et al. 2015; C. Clarke, Parks Canada, unpubl. data). Still, there are a number of key knowledge gaps and generally, a paucity of data exists that assess SAS individuals and their progeny against their truly wild counterparts, particularly in the natural environment (CSAS 2016).

Collaboration for Atlantic Salmon Tomorrow (CAST) was created to address key knowledge gaps in our understanding of the Atlantic salmon populations of eastern Canada and specifically those factors hypothesized to be limiting factors for population success. One major component of CAST is the rigorous assessment of the SAS strategy as a supplementation tool. The identified knowledge gaps associated with the use of SAS as a management tool are outlined in CSAS (2016). These questions form the science premise for the SAS studies proposed in the CAST program. Herein, we identify how the proposed studies address the current knowledge gaps. To that end, the CAST premise is that the proposed SAS project in the Miramichi River, New Brunswick, is a science experiment seeking to compare the quantifiable aspects of the SAS fish to their wild counterparts across a variety of experimental settings and thus truly understand the merit of this conservation strategy which includes assessing the possible risks and benefits for a salmon population's recovery.

This document outlines the proposed CAST SAS studies. This is a novel experiment and thus it will always be adaptive in structure, i.e., as new knowledge is gained, the experimental components may require adjustments. In addition, the core science team of CAST has been the Canadian Rivers Institute (CRI) at the University of New Brunswick (UNB), Universite Laval, Cooke Aquaculture, and the Miramichi Salmon Association (MSA), but also includes Fisheries and Oceans Canada (DFO – Gulf Region) and Mi'gmawe'l Tplu'taqnn Incorporated (MTI). Once all parties are satisfied with the review of the current document, then the proposal will move from a "draft" to "final" version.

# 2. General Description of the SAS Experiment

## 2.1. Objectives of the SAS Program

The main objective of the CAST SAS program is to determine if Smolt-to-Adult Supplementation is a functional conservation strategy that can be used to supplement Atlantic salmon populations in situations where conservation targets (as defined by authorities managing respective populations) are not being met due to high at-sea mortality but before populations decline into state necessitating at-risk classification. A large number of knowledge gaps pertaining to SAS strategy have been identified (CSAS 2016; Fraser 2016); the objective of CAST SAS program is to answer the most critical of the identified knowledge gaps, including the most important question to be addressed (as per CSAS 2016): *quantify and compare the*

*lifetime fitness of SAS progeny versus wild progeny in the natural environment to examine the extent to which SAS may reduce marine adaptation*.

The CAST SAS program is designed as a science experiment aimed at providing answers not only to the objective above, but also to provide a better understanding of potential phenotypic and genotypic deviations between SAS and wild Atlantic salmon and consequences of potential deviations. Specific hypotheses to be addressed by the different SAS studies are detailed in Section 5. Ultimately, the studies aim to determine if juvenile fish (smolts) collected from the wild and grown into adulthood in a captive environment (F0 generation) produce progeny in wild? If yes, then are their progeny (F1 generation) viable and therefore, does the supplementation strategy fulfill the objectives of producing added, wild-like progeny into the system? There are several aspects of life history that may be different in SAS vs. wild fish and to this end, the CAST SAS project will monitor the performance of the SAS fish compared to wild in four levels of studies.

The conservation targets of the Atlantic salmon population are consistently not being achieved in the Miramichi River system (see Section 2.3; CSAS 2017a). Based on DFO's Atlantic salmon management policy, the SAS strategy is a candidate as a conservation tool in the near future if the population trajectory remains unaltered in the Miramichi River (CAFSAC 1991; DFO 2009). Consequently, it is critical to first assess the effectiveness and risks of a SAS conservation strategy before a full implementation at a scale required to achieve adequate conservation status in this system (see Size of the experiment in section 5.4). Importantly, the CAST SAS project is an experiment and not a stocking program.

## 2.2. Experimental Components – General Description

The CAST SAS program is proposed as a two-phased approach at four scales of study. In Phase 1, wild smolt collections are proposed to occur for five years (five smolt "cohorts") during the period 2016-2020. The first release of maturing salmon is proposed in the autumn of 2018 and continue to 2023 when the last salmon from the 2020 smolt cohort are predicted to be fully mature (as Multi-Captivity Winter salmon, or MCW,) and be ready for release. While the Phase 1 is characterized by the smolt collections and adult salmon releases, the monitoring begins already in Phase 1. Phase 2 is monitoring period with no experimental releases, minimally continuing until 2029 when the first Multi-Sea Winter (MSW) salmon are expected to return from the last release cohorts (of 2023).

**The first level of study** will establish the genetic structure among tributaries of the Miramichi River. It is a fundamental baseline and building block for the other experiments, i.e., it establishes the foundation for rearing smolts (relevance based on tributary "strains"), establishes the baseline level of genetic uniqueness between the tributaries and lays the groundwork for future management planning using supplementation strategies. Data from these studies will also be used relevant genetic (parentage) tool for monitoring the success of SAS releases. **The second level of study** is the necessary laboratory experiments that provide a fully controlled environment where basic SAS fish characteristics can be assessed, e.g., maturation rate and size at maturation, compared to wild fish performance. Fundamental

information on fecundity, fertilization success, egg size and fitness relative to wild salmon, defined as survival to eyed stage, survival to hatch (yolk-sac fry) and survival to swim-up (start of external feeding) will be assessed. These parameters are a key to development of the rearing practices to maximize survival and health of SAS fish in captivity.

**The third level of study** will be a controlled experiment in a small, natural stream to understand how SAS fish will respond to natural environment and interact with wild salmon. The proposed stream, Northwest Millstream (NWMS), flows directly to the Miramichi River estuary (Figure 1). The NWMS is annually blocked with numerous impassable or near impassable beaver dams and salmon migration to upstream sections where the experiment is proposed is naturally highly impeded. A barrier fence, with a trap to account for any wild fish that may migrate up to the fence will be erected in the upstream section of the NWMS and will be maintained during the autumn of each year of the experiment.

**The fourth level of study** is an experiment in natural rivers, with controls, where the population scale assessments of the SAS strategy occur. Monitoring the behaviour and spawning success of the SAS adults relative to wild counterparts and the subsequent density, survival, growth and genotypic and phenotypic differences in resulting juveniles will involve a variety of telemetry, genetic and other field-based methods.

## 2.3. Area of Experiment – The Miramichi River

The Miramichi River consists of two major branches: the Southwest Miramichi (approx. 7 700 $km^2$ drainage area) and the Northwest Miramichi (approx. 3 900 $km^2$ drainage area; Figure 1) (Chaput et al. 2016). The Miramichi River has historically had the largest run of Atlantic salmon in North America (Chaput et al. 2016). However, its Atlantic salmon population has experienced a multi-decadal decline and adult returns to the river in 2014 were the lowest in recorded history meeting only 22 % of the approx. 7300 MSW female salmon conservation requirement (Randall 1985; Douglas et al. 2015), in the Northwest Miramichi River (CSAS 2015).

The Atlantic salmon population in the Miramichi River is managed as a composite, but DFO has generated branch-specific, stock monitoring for the Southwest (SW) and Northwest (NW) Miramichi River since 1992 (CSAS 2015). DFO (2006) prescribes management planning guidelines, including triggers for intervention when a stock's status declines from "Healthy" to "Cautious" to "Critical" status. The critical "Cautious" and "Critical" change occurs at 2.4 eggs / $m^2$ of fluvial, rearing habitat (Elson 1957; Gibson and Claytor 2012) and represents a level below which it is hypothesized that serious harm occurs to the stock (CAFSAC 1991). Based on these management thresholds, the overall Miramichi River has achieved "Cautious" status only three times over the past 20 years, while being in the "Critical" level for the remaining years (CSAS 2017a).

There is a disparity in the performance of the two main branches regarding conservation targets (CSAS 2017a). The Northwest Miramichi has met the conservation requirements only three times in the last two decades and underperforms in relation to the Southwest (CSAS 2017a). The Northwest Miramichi still meets general standards for a minimum viable population, or a median estimate of 4 169 individuals based on a meta-analysis of 30 years of published

estimates for vertebrates (Traill et al. 2007). The consequence of the depressed Atlantic salmon population status is the increased vulnerability to inverse density dependence or the Allee effect (Allee et al. 1949). While effects of inverse density-dependent mechanisms are not widely studied, and are generally poorly understood for salmon populations, possible mechanism of the Allee effect for the Atlantic salmon in the Miramichi, is relatively lower survival because antipredator strategies become inefficient in small groups of prey (Courchamp et al. 1999). Such mechanism may manifest especially if predator-prey dynamics are unbalanced, e.g., the very abundant Striped Bass population in river's estuary which is estimated to be experiencing a >10-fold exceedance of its conservation target (CSAS 2017b).

Supplementation activities in the Miramichi River have a long history. The Miramichi Salmon Conservation Centre, where proposed SAS fish husbandry will take place, is the oldest fish hatchery in Canada (est. 1873). Supplementation methods and quantities have varied over the years including supplementation of first-feeding fry, fall fingerlings, 1+ parr, and smolts (Chaput et al. 2016). Supplementation activities occur annually in both the Northwest and Southwest systems. Stocking numbers have ranged from 13 000 to 133 000 fish per year in the Northwest (excluding Little Southwest, where additional 800 to 106 400 have been stocked annually) and 9 000 to 469 400 in the Southwest Miramichi in the period of 1978 to 2008 (Chaput et al. 2016); other stocking programs and quantities precede those compiled in Chaput et al. (2016). Since 2010, the supplementation activity has been first-feeding fry stocking (occurring in early summer) and generally targeting areas where natural production in previous years have been determined to be low (MSA 2016). In 2016, approximately 91 171 first-feeding Atlantic salmon fry were stocked into 45 sites; 15 483 and 75 688 fry were stocked in the Northwest and Southwest Miramichi, respectively (MSA 2016). Unfortunately, there has been very little assessment of the efficiency of the stocking strategies in the Miramichi system. In a recent study, Wallace and Curry (2017) determined the effectiveness of juvenile stocking was undetectable. CAST SAS plan includes monitoring the success of the current broodstock program in rivers where SAS activity will take place using genetic tools.

The "Experimental Stream" for the controlled studies is a small stream where the history of past anthropogenic activity and logistics of maintaining a barrier fence during autumn spawning period make. Northwest Millstream (NWMS) as the ideal candidate (Figure 1). The NWMS was obstructed by a dam without a fish pass in 1947 (Moore & Chaput 2007). The center spillway of the dam was opened in 1979 to allow spawner access above the dam and NWMS was stocked (stock source unknown) in 1970's and 1980's. The dam was removed in 2005. Juvenile surveys (1994 to 2004) upstream of the dam indicate that the areas upstream of the dam were inaccessible in most years (Moore & Chaput 2007).

A conduit fence that acts as a barrier to adult salmon emigration from the experimental area can feasibly be maintained 13 km from the stream mouth (M. Hambrook, personal observations; T. Linnansaari, unpubl. field data from 2017). The experimental area upstream is approximately 19 km in linear stream length consisting of 3rd (10km) and 4th (9km) order streams and associated 1st and 2nd order streams.

The population-scale SAS experiments in natural rivers is proposed for the Northwest Miramichi system. The sub-basins selected for the SAS studies are proposed as:

1) Little Southwest Miramichi River (LSW) upstream from Sillikers (Upper Oxbow; Figure 1); and
2) The main Northwest Miramichi River (NWM) upstream of mouth of Trout Brook (Figure 1).

These are the locations for smolt collections, and adult releases are planned upstream in the first, suitably-sized holding pools with accessibility using fish transport truck (Figure 1). These are appropriately sized tributaries (and sub-populations) for assessing a population-scale effect in the wild. In addition, these sub-basins provide feasible logistics for monitoring in the SAS experiment, e.g., smolt collections, automating salmon counts using sonar, electrofishing, and PIT-tag reader deployment (details in Section 5.4).

The natural structure of the overall Miramichi River watershed provides for several control systems for the SAS experiments where the juvenile abundance, relative to SAS rivers, can be monitored. Spatially, the Sevogle River is a control population that exists within the Northwest system and the Main Southwest provides an untreated subwatershed (Figure 1). Juvenile assessment data exists for the experiment and control rivers since the 1970's (e.g. Moore & Chaput 2007), and monitoring is ongoing by the DFO and will be supplemented by CAST in coordination with DFO. Adult return assessments exist for the Main Southwest and Northwest composite. Both juvenile and adult data sets are long-term, providing a temporal assessment in the control set. In addition, there are 10+ rivers where similar data sets exist thus providing more control sets for the SAS experiment in terms of potential population recovery trends. Although high natural variability in juvenile density and size data exists, it is hypothesized that the average density in the SAS intervention rivers will increase above background levels in proportion to the SAS fish releases while the densities are predicted to remain at background levels in control rivers as dictated by the returns of only wild salmon (assuming population trajectories remain similar or decline further). The growth of juvenile salmon is predicted to remain relatively unaltered relative to control rivers as the effects on juvenile growth are typically observed via exploitation competition already at low population densities (Grant & Imre 2005; Imre et al. 2005).

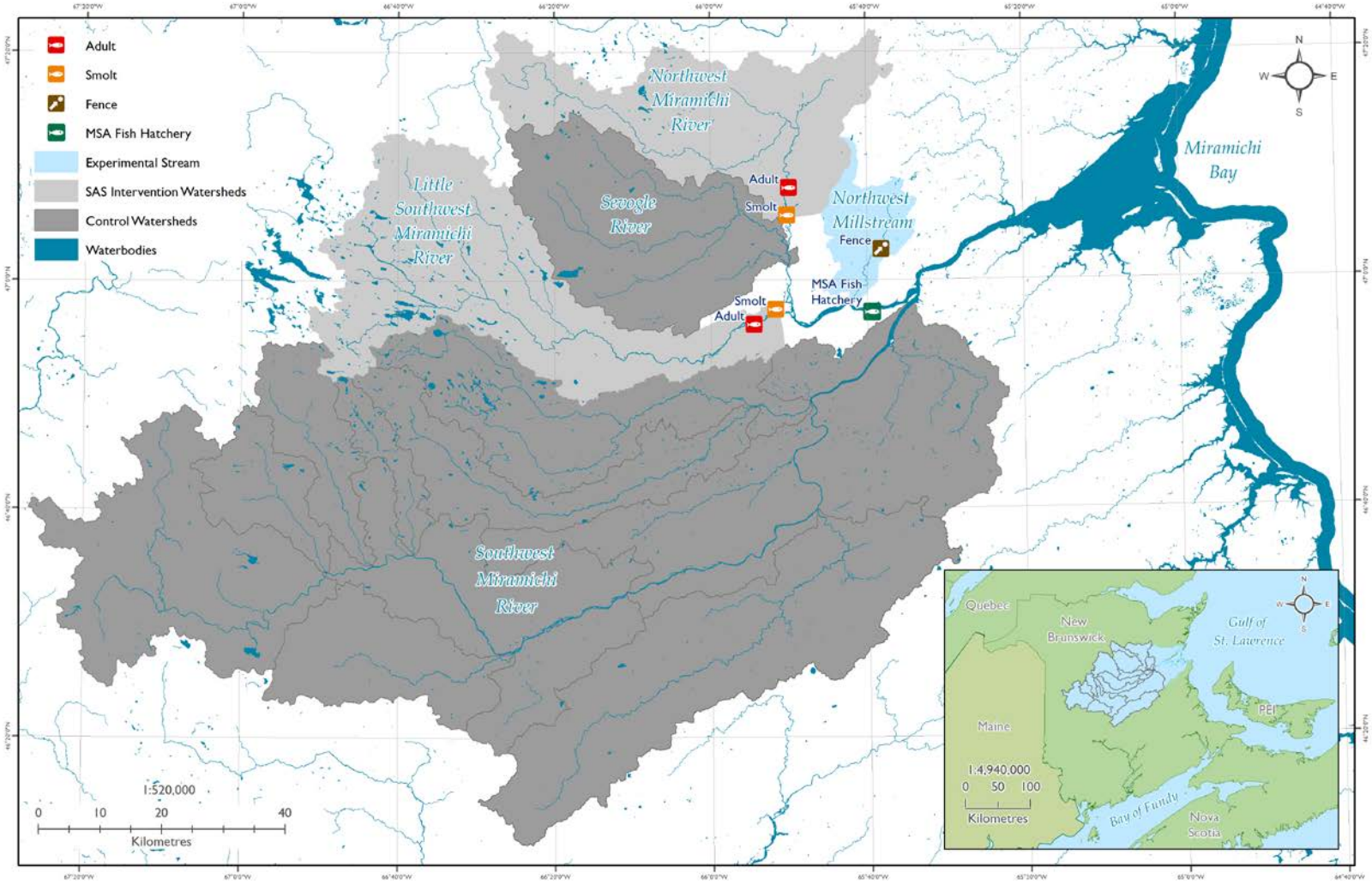


*Figure 1. The Miramichi River in New Brunswick, Canada, highlighting the main sub-watersheds associated with the SAS experiments: the Experimental Stream (Northwest Millstream), and the two tributaries with proposed SAS intervention (Little Southwest and Northwest). The orange fish symbols highlight the areas where wild smolts are collected and the red fish symbols indicate the release locations of the mature adults. The Miramichi Salmon Conservation Centre ("Hatchery") is where salmon are being raised to maturity.*

# 3. Smolt Capture, Operational Protocols in Captivity, and *Status Quo* of the Collections to Date

## 3.1. Smolt collections

Wild Atlantic salmon smolts are annually collected by the Miramichi Salmon Association staff during the month of May using standard rotary screw traps (RST) on the LSW Miramichi (Sillikers) and NW Miramichi upstream of Trout Brook (Figure 1). The RSTs are installed as soon as the water level recedes to operable conditions, typically in the early part of May prior to start of the smolt migration. Two or three RSTs are operated in each tributary, depending on availability.

The traps are fished each morning and 25 smolts are hand counted into pails that have a perforated cover and holes in the upper half of the pail to allow for water movement through the pail when in the transportation tank, yet can be carried while still retaining ½ pail of water. The transportation tank can accommodate nine pails, or 225 smolts / lot. The protocol of smolt transportation by individual pails ensures only one handling of the smolts (from RST to pail) and the smolts are simply poured into their initial holding tanks at the Miramichi Salmon Conservation Centre (without additional stress of netting, etc.).

Smolts for the SAS experiments are collected randomly and throughout the full smolt migration period to avoid unintentional selection as per Fraser (2016). At the start and end of the migration, smaller quantities of smolts are collected (less than a lot of 225 / day), however, multiple lots of 225 smolts will be collected during the peak migration days. Based on the experience from smolt migration in previous years, it is anticipated that approximately 15 % of the total catch will be collected in the first and last 10 days of migration, and 70 % of the total will be collected during a week of most intense migration typically in mid-May.

## 3.2. Operational Protocols in Captivity

**Facilities**

The Miramichi Salmon Conservation Centre (MSCC) is a Miramichi Watershed Management Committee facility and operated long term by the Miramichi Salmon Association. It is located at South Esk, New Brunswick (Figure 1; “MSA Fish Hatchery”). The MSCC has been used primarily for juvenile supplementation programs, but many other husbandry operations over the years, and it is currently being modified for the SAS program-specific needs. Construction of new facilities is near complete at this time (December 2017), and herein we describe the facilities as they are planned (predicted completion January 2018). SAS fish are cared for under the supervision of both the experienced MSCC and Cooke Aquaculture experts.

Each new smolt cohort will first enter a Quarantine Building (QB) for five months, then move to Big Greenhouse (BG) where each stock-specific smolt cohort will be held in their own tanks until maturity without the need to transfer fish between tanks thus minimizing transport and handling. The buildings and their tank systems are equipped with secure, back-up systems ensuring fish welfare in case of emergencies, i.e., to avoid non-random die-offs that could lead to unintentional selection in the hatchery environment (Fraser 2016).

Quarantine Building – The QB has 10 – 2.5m (diameter) tanks where the new smolts are held from May until late autumn of each year. The water to QB is from deep wells that is UV-sterilized, degassed and oxygenated. The well water is heated indirectly by a plate heat exchanger that extracts heat from the brook water to provide fluctuation to the water temperature; temperature range is predicted to be 7 – 14 °C (heat exchanger is currently being installed). Natural light is used in the tank area, however, overhead fluorescent lights are turned on when staff are working in the building (coinciding with daytime hours). Each tank is continuously monitored for oxygen level and each tank is equipped with plate diffusers with back-up oxygen bottles in case of emergencies. This building and the adjacent well pump has a backed-up, emergency diesel generator and a second emergency generator. Oxygen is generated by electricity. An alarm system monitors water levels and pressure connected to a telephone answering service. Access to the building is controlled including personnel wearing room-specific footwear. Entry to the tank room is via a disinfection station including a footbath and hand sanitizing station.

Big Greenhouse – This building is undergoing significant changes to accommodate the SAS program. The tank-space of the building is enclosed by a large (white) industrial tarp where the fish receive diffused, natural light. The renovations involve dividing the building into two separate units with a dividing wall and disinfection station between the two sections such that each section will house a separate cohort of SAS fish where they will live throughout their captive growth period. Six new glass-coated steel circular tanks 6.8m (diametre) x 2.1m (depth) have been installed in each end inside existing tanks. Two extra tanks in each side are used for water treatment equipment. Each tank has a centre drain for waste and a side pod where clearer water is removed to be treated and recycled. The water from both drains go through separate drum filters to remove solids and subsequently, a portion is pumped through a UV-sterilizer to an aeration facility where CO2 is removed and oxygen is added before returning to the tanks. The new well water is UV-sterilized, heated by using a plate heat exchanger to capture heat from brook water and then degassed and oxygenated providing fluctuating temperature. Water temperature range is predicted to fluctuate between 7 and 12 °C in summer, while the temperature will be maintained at 7 °C in winter to maintain fish growth. Each tank has continuous oxygen monitoring equipment and if oxygen levels drop, back-up oxygen through plate diffusers automatically turns on in each tank.

Each section of the BG has its own alarm system that monitors oxygen and water levels to numerous areas. The alarms are reported through telephone lines or a cellular network to an answering service. Each tank is equipped with a side window that can be removed to allow access through the tank wall to remove fish. A new tarp on the building has ports to permit a pipe to protrude through the wall to a holding tank to facilitate moving the large salmon to the transport (stocking) truck upon maturity. All the equipment in this facility and two adjacent production wells are backed-up by two large diesel generators, with one generator backing up the other in case it fails. Disinfection stations, as described above, are also in use for anyone working this building.

During the first two years of collections (2016 and 2017), smolts have been held in existing tanks at the MSCC while planning and construction has been occurring. A smolt cohort

collected in 2015 that has been used to start preliminary laboratory analysis and tracking studies, has been in new BG tanks since March 2017 and the 2016 cohort will go into new tanks in December 2017. The 2017 cohort entered into the modified new tanks in the refurbished QB and will be moved into the new large grow-out tanks in BG upon completion in winter 2018.

**Fish Husbandry Procedures (dietary and health practices)**

Dietary practices are of critical importance to transition the wild smolts onto consume of commercial fish feed. Upon arrival to the facility, the smolts are initially fed chopped krill for a week after which a semi-moist food mixture (Cooke Aquaculture) is introduced during a "transitional period". The krill has proven to be critical as a transitional diet to ensure smolts shift from natural food items to commercial feed (M. Hambrook, personal observations). The semi-moist food is mixed with krill in order to entice the smolts to feed. Chopped krill is continued to be supplied during the "transitional period" after the fish have been given abundant, semi-moist food to ensure the transition has the greatest potential for success for each fish. After two weeks of feeding semi-moist/krill mixture, plain semi-moist food is introduced and fed to satiation and a semi-moist/krill mixture is fed in addition so that even the fish that are slower in transitioning will be fed. After two weeks, the smolts are introduced to dry food (Skretting). Dry pellets are fed first until feeding rate is reduced, and then the dry pellets mixed with krill is fed until feeding rate is reduced further followed by the semi-moist food to ensure every fish is feeding. The fish are fed slowly and observed carefully to judge how much food is being consumed thus minimizing food waste and its accumulation in the tank.

To date, it has been observed that introduction to dry food is the longest transition period. Dry food is also placed on belt feeders to feed into the evening. By late July, the fish consume dry food only. Once the fish have been habituated to dry food with automatic feeders, hand feeding still occurs four times daily to observe fish behaviour. This will continue until salmon are near ready to be released back into the wild and have stopped feeding.

Fish healthcare is proactive. As a preventative treatment, smolts in the Quarantine Building are given a salt bath or formaldehyde treatment every week. After moving into the BG, fish are given a salt bath every two weeks. The bath is a 2% salt mixture that is premixed in a separate tub and pumped into the tanks. Formaldehyde is mixed at a 1:4,000 ratio and is sprinkled into the tank. Both treatments are one hour exposures with the water flow turned off with the exception for a trickle of high oxygenated water going into the tanks.

Daily food allocations are recorded as well as treatments, mortalities, and visitors to the buildings. Mortalities, when they occur, are removed as soon as noticed and may be kept for veterinarian/lab analysis or disposed. A dedicated veterinarian from Cooke Aquaculture is on stand-by on a priority basis and is available should any indication of a disease outbreak be evident.

A separate handling event for each smolt cohort is planned to inventory the fish numbers, measure their size, to individually tag each fish with a Passive Integrated Transponder (PIT-tag; 23mm glass encapsulated half-duplex "silver bullet" manufactured by OregonRFID), and to collect tissue material for genetic analysis (fin clip). PIT-tags will be injected into the flesh under the dorsal fin; vertical injection is being currently experimented with to optimize detection

distance during the in-stream monitoring phase which uses a flat-bed PIT antennas (see Section 5.4). The genetic material is required for establishing the parentage analysis for monitoring (see Section 4). The fish are externally tagged using tributary-specific, colored T-anchor tags and the adipose fin of the fish is removed for additional external identification purposes.

## 3.3. *Status quo* (31 October 2017) of the CAST SAS program

Wild Atlantic salmon smolt collections for the CAST SAS program started in 2015, and have continued in spring of 2016 and 2017. The initial intention was that the 2015 cohort would be released in the autumn of 2017, however, the release did not take place due to pending agency approvals. Certain laboratory studies (see Section 5.2) and radiotracking of 40 SAS individuals in the Experimental stream (Section 5.3) were authorized.

In 2015, an authorized collection of 1,100 smolts from the LSW Miramichi River (May 17 to 27), 191 smolts from the Sevogle River (May 20 to 24), and 200 smolts from the NW Miramichi River upstream of Trout Brook (May 16 to May 27) were secured for a total of 1,491 fish introduced into the MSCC facilities. In 2016, 2500 (May 13 to 27) and 2132 (May 11 to June 3) smolts were collected from LSWM and NWM, respectively. In 2017, 2500 smolts were collected from both systems (May 16 to 29)).

The practice of collecting wild smolts and raising them to maturity is new to the MSCC. The first years of a new husbandry initiative are necessarily challenging as both fish handling and facilities must be continuously adapted to maximize successful rearing. Unfortunately, in 2015 significant mortalities occurred due to a “failed smolt syndrome” where the new smolts didn’t accept the food that was offered. Smolts that did start feeding performed very well with minimal mortalities thereafter. The diet was modified in 2016, *i.e.*, the introduction of the krill and semi-moist diets with appropriate transition periods, as described above, and the smolts readily accepted the food. However, late in the summer 2016, a large mortality event occurred as a result of the protozoa Costia (*Ichtyobodo* spp) on the gills that came into the facility with the fish. Again, improving from 2016 by developing appropriate treatment and filtering protocols with on-demand priority veterinarian service, the 2017 smolt cohort collection is feeding well with a regular preventative treatment regime in place. The current collections and survival (or % remaining) of SAS smolts is shown in Table 1.

*Table 1. Numbers of wild Atlantic salmon smolts collected from tributaries of the Miramichi River by stock and surviving numbers in 31 October (2017) at the Miramichi Salmon Conservation Centre. *denotes numbers where the number of fish is also affected by the use of 2015 mature fish for the laboratory and tracking purposes, and survival for this cohort of fish is better described as the number of fish remaining in the facility.*

| Smolt Cohort | Stock | Initial Collection | Survivors (31 October 2017) | Survival (%) |
|---|---|---|---|---|
| 2015 | Northwest | 200 | 148 | 74.0 |
| 2015 | Sevogle | 191 | 59* | n/a |
| 2015 | Little Southwest | 1100 | 362* | n/a |
| 2016 | Northwest | 2132 | 1710 | 81.8 |

| Smolt Cohort | Stock | Initial Collection | Survivors (31 October 2017) | Survival (%) |
|---|---|---|---|---|
| 2016 | Little Southwest | 2500 | 1205 | 48.3 |
| 2017 | Northwest | 2500 | 2255 | 90.3 |
| 2017 | Little Southwest | 2500 | 2092 | 83.5 |

It should also be noted that the collection of 5 000 smolts annually represents a very small risk to the wild Atlantic Salmon population in the Miramichi River given the assumed very high mortality rate at sea. Assuming 80:20 grilse:MSW ratio (Chaput et al. 2016) and 1% survival for grilse and 0.2 % survival for MSW salmon (Chaput et al. 2016), 5 000 smolt translates to ~40 grilse and ~2 MSW salmon lost to the smolt collections.

The 2015 smolt cohort was ready for release back into their tributaries of origin (LSW and NW) in the autumn 2017, however, the release was delayed due to approvals for authorization. Some mature fish from 2015 cohort were used in the laboratory experiments (N=20) and radiotracking project (N=40; additional fish were used as the group originally radiotagged became overripe while waiting for authorization approvals, and re-tagging of a new group of fish had to take place twice). The rest of the mature fish, as indicated in Table 1, were handled to remove eggs and milt, and are being held at MSCC and are being reconditioned for a release in 2018.

Due to the developing program, construction and systems upgrades at the Miramichi Salmon Conservation Centre, all mature Atlantic salmon consist of MCW component at this time; the 2016 smolt cohort is not expected to mature as grilse due to developing program. A grilse release component is expected to be produced from the 2017 smolt cohort.

# 4. Genetic Monitoring Tools of the Program; General Approach

The main objective of the CAST SAS program is to examine the generational contribution of SAS adults to wild adults. Simultaneously, the currently used broodstock collections – juvenile stocking program can be assessed using same methods. This assessment is best achieved using parentage analysis approaches based on genetic markers wherein sampled progeny can be assigned to specific parents and therefore, parent type (CSAS 2016). Also, monitoring of genetic markers allows for detection of alterations in allele frequencies over time in both the SAS and control river populations.

Genetic parentage analyses are based on the principles of Mendelian inheritance where individuals of a sexually reproducing diploid species receive one gene copy (allele) from each of their parents. By screening offspring and their potential parents for variation in molecular genetic loci, inference can be made regarding the parents of a given individual. Various analytical approaches (e.g. exclusion, categorical allocation, sibling-reconstruction) and statistical frameworks (e.g. Bayesian, maximum likelihood) exist for conducting genetic parentage analyses (for a review see: Jones et al. 2010). Each of these approaches are suited to different types of parentage questions, the genetic markers used, available information about the breeding systems, sampling of the individuals, as well as computing power and time (Jones et al. 2010). Parentage-based tagging (Anderson and Garza 2006), unlike genetic stock

identification, does not rely on genetic differences among populations to assign individuals. Instead, it relies on being able to unambiguously assign parentage to adults of known identity.

The use of parentage analysis for monitoring the proposed SAS strategy in the Northwest Miramichi River has been reviewed and simulation work suggests that a parentage-based tagging approach would be effective (Pavey 2016). Pavey (2016) recommended the use of single nucleotide polymorphism (SNP) genetic markers, provided that a panel of sufficiently variable markers is available, due to their flexibility and scalability. Preliminary analysis of ~50K SNP markers characterized in juvenile Atlantic salmon from 16 sub-basin populations in the Miramichi River indicate that there are more than 14K SNPs with a minor allele frequency greater than 0.3 (the level of variation used by Pavey (2016) in simulations) suggesting there are no limitations to using SNPs for parentage in this system. SNP data come from the 2016 surveys of juveniles (see Section 5.1.). Our intention is to select a panel of approximately 500 of these highly variable SNPs to design a low density SNP array that will provide discriminatory power to assign parentage unambiguously to SAS fish. A 500 SNPs with the shown level of variation will theoretically provide 100% power to assign individuals (Pavey 2016) as we intend to use more SNPs with greater variation than Pavey's (2016) simulation study that had 100% power. The chances of not assigning an individual if the true parent is in the dataset (e.g., not assigning a SAS offspring; false negative) are negligible to zero (Pavey 2016).

Existing parentage-based tagging programs for monitoring supplemented populations (e.g., Steele et al. 2013; Beacham et al. 2017) have demonstrated good success in parentage assignment using panels of 100-300 SNP markers (many fewer than we have proposed). For example, Beacham et al. (2017) assigned 92% of 1599 known origin Coho salmon from 15 hatchery populations to the appropriate year-class within broodstock with SNPPIT (Anderson 2010) with 100% accuracy. The lowest proportion assigned for a hatchery population was 72.4%; however, only ~90% of the broodstock for this population was genotyped and SNPPIT is not capable of making single parent assignments. This highlights the importance of genotyping every SAS fish to obtain the highest probability of correctly assigning SAS offspring. Furthermore, when software that can make single parent assignments (COLONY; Jones and Wang 2010) was used, the overall success rate of assigning known-origin offspring to the correct year-class within broodstock was 99.9% (1597/1599). Similar results were observed by Steele et al. (2013) with assignment rates of known origin steelhead generally >95% with no false positives. It is important to note both of these studies included large numbers of potential parents in their assignment procedures that could not biologically have be the parents of the tested offspring. Despite their inclusion, no false positive assignments were made. This is consistent with modeling simulations that suggest low to no false assignments with the proposed number of SNP markers (e.g., Pavey 2016).

To monitor fish with SAS parental origin, all SAS fish will be tissue sampled while they are in captivity, DNA will be extracted from the tissue samples, and their genotypes characterized using the low-density SNP array (similar sampling will be done to all adult salmon used for the ongoing broodstock program). These samples provide reference parents for comparing the genotypes of juvenile collections and eventual adult returns to the river. Beginning in the year following release of the first SAS adults, and continuing for six years after the last releases of

SAS adults, life stages that could be biological descendants of SAS fish will be collected and non-lethal tissue samples taken using large scale electrofishing and smolt collection surveys (See section 5.4 for sample sizes). DNA will be extracted from these samples using non-lethal fin clips (Dietrick and Cunjak 2006) and all individuals will be assayed for their genotypes with the low-density SNP array.

A hierarchical approach to assigning parentage, such as that presented by Beacham et al. (2017), that would first use SNPPIT to rapidly identify parent pairs for individuals whose parents are both in the database and then a computationally more intensive approach (COLONY) to match unassigned individuals to single SAS parents. This type of approach will facilitate identification of SAS x SAS offspring as well as SAS x wild offspring in a given set of samples while potentially reducing processing time. Other new software that uses a similarly efficient hierarchical approach to assignment (Huisman 2017) may be used to verify parentage and provide confidence in assignments as recommended by Pavey (2016). The intention of this parentage analysis is to identify the number of SAS offspring relative to wild offspring and track the ratio of SAS:wild through each life stage to assess the relative survival of SAS offspring until they return as adults. We expect the sampling at the smolt life stage to be the least biased toward sampling of related or otherwise non-randomly mixed individuals and may provide the most accurate assessment of the survival proportion of SAS offspring during the juvenile freshwater phase.

In addition to the parentage-based tracking of performance this genotype data will provide, it will allow us to assess any allele frequency changes from the basin-wide baseline samples to assess any potential impacts of releasing SAS fish (e.g., domestication effects). While we expect these effects to be negligible, any detected changes can be followed up by genotyping samples with a higher density SNP array (50K) to assess the magnitude and risks posed by these differences. Genetic samples will be collected also in the control rivers (Figure 1), however, the control river samples will be submitted to genetic analysis only if differences in allele frequencies are observed in SAS intervention rivers between the SAS progeny and wild progeny. As the allele frequency changes in the intervention rivers may be due to temporal variability, the control river samples can then be analysed (using the 50K SNP array) to assess amount of temporal variability in control baseline in the absence of SAS intervention, and the amount of temporal variability between control and SAS intervention rivers can be compared.

Parentage-based methods will also be used in the Experimental Stream (Northwest Millstream, NWMS). In NWMS, all wild adult salmon are also genetically sampled prior to release to the experimental arena. The parentage-based analysis in the experimental stream is therefore predicted to result in high degree of analytical power to answer the knowledge gaps regarding genetic and phenotypic differences between the SAS and wild fish up to the smolt migration endpoint. However, NWMS experiment is not large enough to be able to assess SAS strategy in its main objective of producing returning adults from the ocean (See Section 5.3).

# 5. Detailed description of proposed studies, hypothesis framework, and knowledge gaps to be addressed

The following sections describe the four levels of research discussing the science premise, hypothesis to be tested, general methods (including sample sizes and duration, as applicable), knowledge gaps to be answered (as defined in CSAS 2016), and metrics to be measured (as per Fraser 2016). Triggers and decision rules regarding when the experiment will be altered, continued or halted is discussed with respect to the studies in natural rivers, where the risk to wild Atlantic salmon population must be considered. There is currently no perceived risk mechanism to affect wild Atlantic salmon in the sub-basin genetics study, laboratory experiments or the Experimental Stream study and therefore, triggers and decision rules to halt these studies are not considered.

## 5.1. Program 1: Sub-basin genetic structure of Atlantic salmon on the Miramichi River

*Science Premise:*

Foremost in any recovery strategy assessment should be an understanding of the genetic population structure for the species of concern (Jonsson et al. 1999). Knowledge regarding the genetic structure, i.e., the uniqueness of each sub-basin in the Miramichi, is necessary as baseline information to ensure SAS production lines, i.e., the smolt cohort management, are appropriately created so as to protect the natural populations from potential genetic risks of the SAS experiments (and as would be required when using SAS as an intervention strategy). Additionally, understanding the genetic structure prior to initiating SAS experiment will allow the identification of any potential genetic changes both during and after the cessation of the SAS experiment.

*Hypothesis*: Genetic structuring of the Atlantic salmon population occurs and is defined by the sub-basins in the Miramichi River system

*Conducted studies and ongoing activities:*

- In 2016, 16 sub-basins (Figure 2) were sampled by electrofishing in collaboration with DFO and MSA (target sample size of N=50 age 1+, Atlantic salmon parr in each sub-basin); A total N=774 was collected (Table 2)
- Samples have been genotyped using a 50k SNP chip in winter 2017 (Wellband et al., unpublished data); genotyping was achieved for 743 individuals
- Based on preliminary analysis, traditional population genetic statistics identified statistically significant genetic divergence among most pairwise comparisons of sub-basins. However, the overall magnitudes of pairwise divergence among rivers were very low given what was expected to a river system this size (FST = 0.0006 – 0.0147) (K. Wellband et al., Laval/UNB, unpublished data). Naïve genetic clustering routines (i.e. those with no prior knowledge of sample grouping) failed to discriminate meaningful clusters of individuals within the Miramichi River; however, this result is not unexpected due to the reduced power of these techniques to detect population structure when the magnitudes of divergence are weak (FST < 0.05).

- Development of a lower density (LD; approx. 500) SNP chip for conducting parentage analysis (see Section 4) in subsequent SAS studies is ongoing (UNB/Laval with Cooke Aquaculture)
- Sub-basin genetic study provides necessary baseline information to assess SAS impacts on natural rivers (Section 5.4)
- A second, complete assessment of genetic structuring in five years will be used to evaluate temporal genotypic stability, which also provides an additional check on genetic effects of potential straying and reproduction by SAS–origin fish.

*Knowledge gap (CSAS 2016) to be addressed:*

- Genetic structure among the sub-basins of the Miramichi River system is currently unknown. This study will assess genetic structure and verify the requirement of sustaining unique rearing lines for SAS fish production from different sub-basins separate (ongoing working hypothesis).
- Provide a genetic baseline to assess potential genetic changes as a result of SAS experiments in natural rivers.

*Table 2. The number of genetic tissue samples collected in 16 sub-basins in the Miramichi River in the autumn 2016 (See also Figure 2)*

| SubBasin | N | Notes | |
|---|---|---|---|
| Upper NW Miramichi | 52 | | |
| Lower NW Miramichi | 63 | 13 samples from a tributary (Sutherland Brook) flowing into tidal parts of Northwest | |
| Sevogle | 49 | | - |
| Upper LSW Miramichi | 40 | | - |
| Lower LSW Miramichi | 49 | | - |
| Lower SW Miramichi | 54 | | - |
| Middle SW Miramichi | 49 | | - |
| Upper SW Miramichi | 52 | | - |
| Renous | 52 | | - |
| Dungarvon | 52 | | - |
| Cains | 59 | | - |
| Taxis | 49 | | - |
| Burnthill | 27 | | - |
| Clearwater | 23 | | - |
| Rocky Bk | 51 | | - |
| Northwest Millstream | 53 | | - |
| Total | 774 | | - |

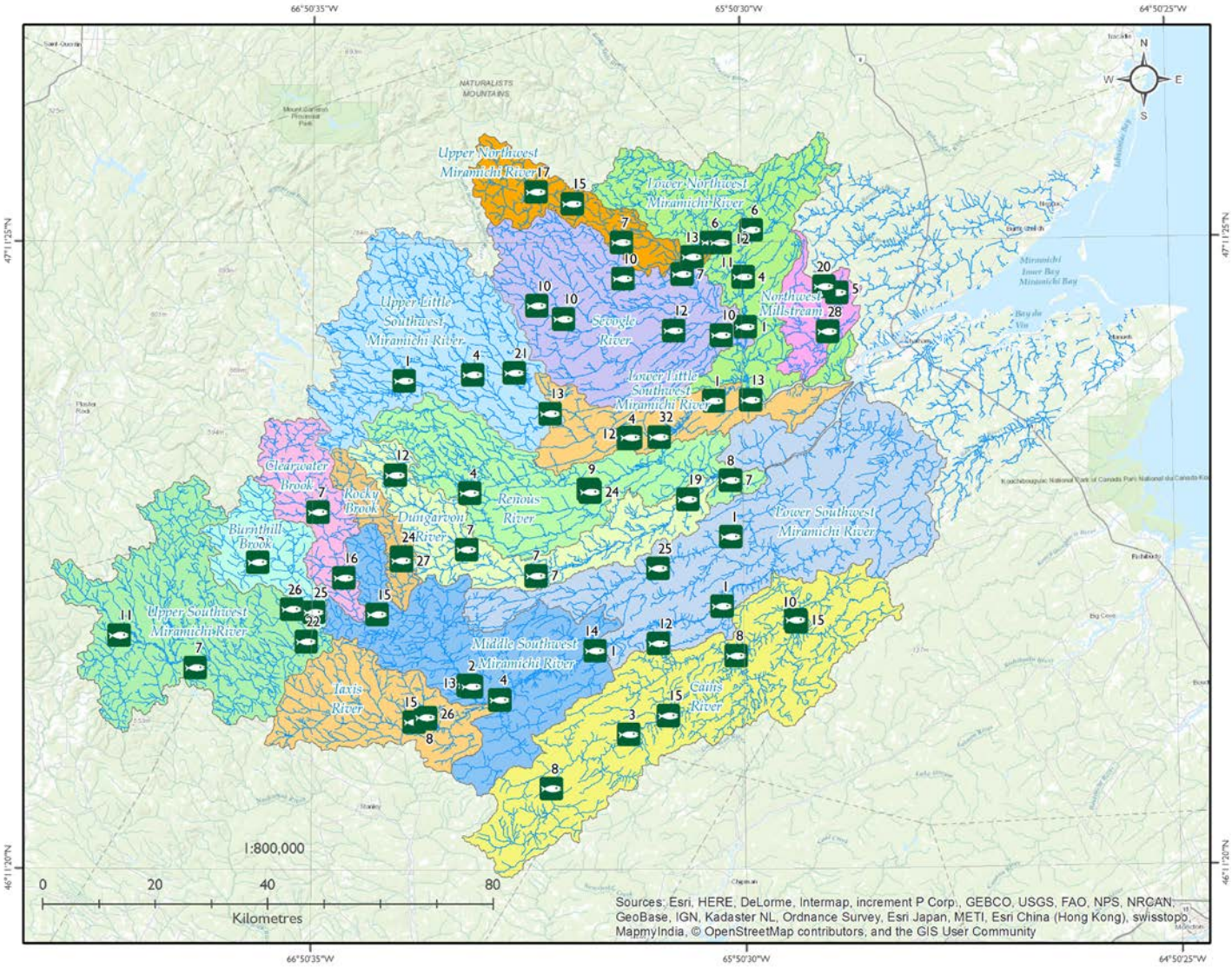


*Figure 2. Sub-basins (N=16) of the Miramichi River where sampling for genetic baseline material was undertaken in autumn of 2016 with respective sample sizes at each location (Total = 774 fish samples).*

## 5.2. Program 2: Laboratory experiments

*Science Premise:*

The laboratory experiments provide a fully controlled environment where fundamental data on SAS fish characteristics, both phenotypic and genotypic, (e.g., maturation rate, size at maturation, maturation timing) can be assessed for progeny and adults, including the relative differences between SAS and wild fish. Fundamental information is collected on fecundity, egg size, and fitness which is defined here as survival to eyed stage, survival to hatch (yolk-sac fry), and survival to swim-up (start of external feeding). In addition, findings are expected to lead to modifications and development of the rearing practices to continually improve the phenotypic quality of SAS salmon. Importantly, these are controlled situations where comparisons between groups, i.e., SAS versus wild, is a relative assessment. These results help us to understand the mechanisms of differences, if they exist, between groups that will (1) help define the model parameters for full production SAS programs in the future, i.e., the cumulative progeny production for a SAS adult and its progeny's survival to its spawning event (1 SAS adult = X future egg deposition), and (2) will help us to model the predicted SAS contribution to the total population (numbers), i.e., spawners, juveniles, and smolts in a subbasin.

*Hypothesis*: A SAS fish will differ in basic biological (phenotypic) traits from wild at the adult stage. Survival to described end-points is expected to be lower in SAS than wild, however, no genotypic or phenotypic differences are predicted a priori for the surviving progeny.

*Proposed studies; general methods:*

[Adults]

- Maturation rate of the adult SAS fish (by sex) is measured for five years of the experiment
- Size-at-maturation by sex is measured
- Maturation timing by sex is assessed
- Fecundity and egg sizes are compared annually; N = 20 (targeted) adult females (each of wild and SAS; only 10 wild females and males were available in 2017; 10 + 10 SAS salmon were similarly used in 2017)
- Fertilization success by males; N=10 males of both wild and SAS (N=8 used in 2017 due to wild availability)
    - Repeated for 2 years;
    - Fertilization of sub-samples of eggs (N=100) from selected wild females (N=3) by both wild (N=10; N=8 in 2017) and SAS males (N=10; N=8 in 2017); eggs from each female randomly selected to two batches of equal size (N=100) and fertilized by milt from SAS and wild male
    - Total 1000 eggs / female / treatment
- Survival studies
    - 10 females and 10 males of each wild and SAS to generate wild x wild, SAS x SAS, wild x SAS and SAS x wild crosses for a common garden experiment resulting in 40 crosses; 3 replicates / each cross

  - (for 2017; crosses were fertilized 20- 27 October)
  - Egg to eyed stage survival
  - Egg to hatch survival
  - Egg to swim-up survival
  - Swim-up to first feeding fry survival and growth
  - Surviving SASxSAS, SASxwild, wildxSAS crossed first-feeding fry to be distributed to the lower sections of Northwest Millstream below the barrier fence (13 km section) unless otherwise determined; all genotypes are known
  - Surviving Wildxwild crosses distributed to tributaries of origin as part of MSA's normal supplementation program (genetic identity of parents known, and success of the surviving fish will be determined as a part of the SAS monitoring program)
- Potentially, examine gene expression and epigenetic differences between the experimental groups at the swim-up fry stage (contingent on additional, future funding).

*Knowledge gap (CSAS 2016) to be addressed:*

- Characteristics of SAS adults relative to wild
- Characteristics of SAS eggs relative to wild
- Survival (fitness) of SAS progeny relative to wild in controlled hatchery environment up to first-feeding fry stage (Note that spawning of adults and thus mate selection, is not free unlike in SAS strategy in wild. Similarly, hatching will be in hatchery environment where selection may occur. Therefore, the experiment is not a true test of SAS strategy as it would occur in wild)

*Metrics (Fraser 2016; Table 2)*

- [Adult]: Growth and body size, body shape (a separate undergraduate thesis project undertaken in autumn 2017 at UNB), maturation rate, reproductive timing, egg size and fecundity
- [Juvenile]: Survival to described end-points, and size at the final endpoint relative to wild

Deviations in mean and variance in above metrics between SAS and wild fish will be assessed and statistically compared (Fraser 2016).

*Adaptive Planning - Risk assessment*: Lowered survival of wild (female) x SAS (male) crosses to described endpoints could indicate a risk to wild Atlantic salmon if similar result was applicable in natural environment (i.e. wild eggs would have lowered survival if fertilized by SAS male). Lowered survival of SAS x SAS crosses and SAS (female) x wild (male) crosses is not a critical risk, as wild egg production is not affected by lowered survival of SAS progeny (wild sperm is not a limiting factor; SAS intervention occurs in areas well under the carrying capacity of juvenile production; see DFO (2017a)). Caveat in the studies in laboratory is that unlike actual SAS program in the wild, the mate selection is not natural, and differences in laboratory may not be reflective of SAS program in wild.

## 5.3. Program 3: Experimental River

*Science Premise:* To better understand how SAS salmon will respond in the natural environment relative to wild Atlantic salmon, a controlled experiment is designed to occur in a small, natural stream. The proposed stream, Northwest Millstream (NWMS), flows directly to Miramichi estuary (Figure 1) and has a history of stocking of various Atlantic salmon strains into the system. The NWMS is annually blocked by numerous beaver dams and salmon migration to upstream section is naturally impeded. The lack of natural connectivity in stream and repeated, mixed stock introductions are good guarantees that potential effects of SAS introductions on wild stocks are minimized in the NWMS. A barrier (conduit) fence will be erected in the upstream section of the NWMS in the autumn of each year and will be maintained through the spawning period for each year of the experiment (proposed 3 years of SAS introductions). The section upstream of the barrier will act as the experimental section for SAS vs. wild comparisons. The experiment will examine 1) SAS and wild adult behaviour, activity levels, and survival and 2) SAS and wild progeny survival, growth, and behaviour. The wild fish required for the experiment are proposed to be collected from DFO index trap, supplemented by seining and angling to collect additional wild fish as required. First release of 20 SAS females and 20 SAS males took place in late October 2017; no wild fish could be captured in 2017 due to lateness of getting authorization to collect wild fish for the purpose (permit received October 26).

*Hypotheses:*

The experiments will test deviations of the SAS phenotype and genotype from the wild in a controlled natural setting.

At adult stage, the following hypothesis is examined:

1. SAS adult behaviour is similar to the behaviour of wild fish.

   Predictions are that:

   - SAS fish will spawn in similar areas to the wild;
   - SAS adult spawning timing is similar to the wild adults;
   - SAS adults find mates and spawn successfully;
   - SAS post-spawner survival is similar to wild adults

At juvenile life stage, the following hypothesis is set forward:

2. SAS adults produce progeny that are phenotypically and genotypically no different from progeny produced by wild fish.

Predictions are that:

- Eggs from SAS adults emerge successfully in ratio compared to wild as would be predicted from relative survival studies from the lab studies (i.e., fecundity and the egg viability may be less (or more) than that of wild).
- SAS juveniles survive successfully in the wild at similar rate as the wild juveniles
- SAS juvenile growth in the wild is similar to the wild juveniles
- SAS juvenile behaviour in the wild is similar to the wild juveniles

- Allele frequencies in the examined loci are no different between SAS and wild juveniles

*Proposed studies:* Monitoring of movements and spawning behaviour of SAS and wild adult fish will use radiotelemetry; monitoring will be repeated for 3 years. All salmon, SAS and wild, are genetically sampled (for progeny parentage assignments). The juveniles will be assessed using electrofishing surveys; the annual electrofishing surveys to collect fin-clips (genetic material) of progeny for parentage analysis will be carried out for five years. A sub-group of the captured juveniles will be tracked with Passive Integrated Transponder (PIT) tags to monitor the behaviour, survival, and growth of known individual juveniles from the two different groups.

Planned Studies in NWMS (others may be added in our adaptive approach based on new knowledge gained)

- Release of 20 pairs (20 females and 20 males) of both SAS and wild Atlantic salmon to NWMS upstream of the barrier fence
  - All released fish genotyped, radiotagged (Lotek MCFT2 tags) and PIT tagged
  - All released fish are externally tagged (T-anchor tags); different colour code for SAS and wild adults to facilitate behavioural observations using direct streamside observations
  - Repeated for 3 years
- Project 1 - Monitoring of movement and behaviour of adults
  - Assessing spawner distribution and survival, spawning behaviour, redd locations
  - Active monitoring (radiotags) in streamside surveys
  - Two passive (radio)monitoring stations installed to monitor movements during spawning; a number of passive PIT tracking stations will also be used to monitor movements
  - One passive monitoring station retained to monitor post-spawner emigration after barrier fence is removed in late autumn.
  - Repeated for 3 years
  - Monitoring of survival of the post-spawners to potential repeat-spawning in both SAS and wild fish (radiotags with multi-year battery life)
- Project 2 - Distribution and survival of SAS progeny
  - Annual electrofishing surveys to collect fin-clips (genetic material) of progeny for parentage (SNP) analysis; annual monitoring for 5 years (assuming majority of smolts migrate at age 2 or 3).
  - Parentage resolved using LD SNP chip developed based on the sub-basin study (see Section 4 and Section 5.1)
  - Inter-stage survival and growth monitored based on repeated electrofishing surveys, and genetic analysis
  - 10 annual electrofishing sites, N= 50 / age group / site (YOY and “parr”); Total annual genetic sample size N = 500 for 5 years

- Project 3 - Movements and behavior of juveniles
    - Monitor behaviour and movements of PIT-tagged individuals
    - Use of genetics to resolve parentage (post-tagging; the parentage unknown at the time of tagging, but representatives of both wild and SAS groups are likely to be tagged by randomizing the tagging efforts and 1:1 ratio of parents released into the system).
    - Install passive PIT antenna arrays in strategic locations to monitor movements and emigration of PIT tagged juveniles throughout their residence in NWMS [will also monitor adult movements in autumn]
    - Inter-stage survival and growth of known individuals of known parentage
    - PIT methods to be used are half-duplex Oregon RFID systems with 12mm and 23 mm "silver bullet" tags with improved detection distance. The reader systems will be novel satellite-synchronized multiplex systems to maximize detection distance (currently not available commercially, but will be available for CAST SAS studies)
- Project 4 - Behavioural studies in small stream enclosures
    - Direct observation of PIT-tagged individuals to assess aggression / boldness
    - Fish will be PIT tagged, and genetically analyzed as 0+ to determine parentage; in a subsequent year (1+ or 2+ parr), recaptures will be obtained and balanced design of SASxSAS, wild x wild and crosses will be selected to stream enclosure studies to study differences in behavioural metrics.

**Size of the experiment**

The Experimental Stream component of the SAS program is designed to allow high level of control in a necessarily, small-scale natural environment. This allows the experimenter to control the ratio of SAS to wild fish in a semi-closed "arena". Having higher level of control means, however, that the experimental section is relatively small stream where aspects relating to generational output of an actual SAS program becomes infeasible, i.e., too few SAS adult returns can be generated to have statistical power to examine generational contribution of SAS adults relative to wild adults in producing returning adults.

*Knowledge gaps (CSAS 2016) to be addressed:*

- Behaviour (migratory rigor, pairing ability, spawning behaviour, post-spawning behaviour) and interactions of the released SAS adults relative to wild counterparts in the natural environment
- Competition for mates and disruption of wild spawning
- Spawn timing relative to wild
- Fitness (individual and group) of SAS adults relative to wild counterparts during juvenile stages
    - Relative *individual fitness* of both SAS and wild Atlantic salmon (relative production of juveniles by known SAS and wild parents; all parents genetically sampled) during juvenile to smolt stages
    - Relative *group fitness* of SAS adults relative to wild (ratio of SAS:Wild progeny through years within cohort) during juvenile to smolt stages

- Behaviour and growth and morphology of SAS juveniles relative to wild juveniles

*Metrics (Fraser 2016; Table 2)*

- Adults - Body shape, Reproductive timing, Migratory rigor, Activity levels
- Juveniles - Inter-stage survival (fitness), growth, body shape, Activity levels, movement behaviour, aggression/boldness

Deviations in mean and variance in above metrics between SAS and wild fish will be assessed and statistically compared (Fraser 2016).

## 5.4. Program 4: SAS impacts on a natural river

*Science Premise:* A successful SAS program, i.e., its value as a potential conservation strategy, requires an understanding of the ***generational contribution of SAS adults relative to wild adults***, i.e., can the SAS parents produce progeny that migrate to ocean ***and return back*** to rivers to spawn successfully and contribute to the production of successful (fit) juveniles in the river. This objective can only be achieved at a population scale and in a system (river) where SAS adult introductions are of sufficient numbers to generate progeny that: 1) can be quantitatively assessed in river (counted in each age class) and 2) are sufficient to complete the cycle of becoming adults returning to the river to spawn and be quantitatively assessed. The SAS origin returning adults have to be of sufficient numbers to invoke a change in the wild juvenile production that can be quantitatively assessed. Importantly, the study must be replicated, i.e., two or more systems are required.

*Hypotheses:*

1) The experiments will test deviations of the SAS phenotype and genotype from the wild in a natural setting.

At adult stage, the following hypothesis is examined:

1. SAS adult behaviour is similar to the behaviour of wild fish.

   Predictions are that:

   - SAS fish will spawn in similar areas to the wild;
   - SAS adult spawning timing is similar to the wild adults;
   - SAS adults find mates and spawn successfully;
   - SAS post-spawner survival is similar to wild adults

For the SAS progeny, the following hypothesis is set forward:

2. SAS adults produce progeny that are phenotypically and genotypically no different from progeny produced by wild fish.

   Predictions are that:

   - Eggs from SAS adults emerge successfully in ratio compared to wild as would be predicted from relative survival studies from the lab studies (i.e., fecundity and the egg viability may be less (or more) than that of wild).

- SAS juveniles survive successfully in the wild to smolt stage at similar rate as the wild juveniles
- SAS juveniles growth in the wild is similar to the wild juveniles
- Allele frequencies in the examined loci are no different between SAS and wild juveniles
- Survival of SAS smolts to adulthood and return back to the river of origin is similar to the wild fish, i.e. there is no SAS-induced loss of marine adaptation

2) The SAS experiments will result in a demographic increase in juvenile density in the SAS intervention rivers relative to the background level in the multi-year electrofishing dataset, whereas similar increase in comparison to background level in control rivers will not be observed.

*Proposed studies:* The current plan is to release mature SAS adults to two proposed intervention rivers (See Section 2.3). A first release is planned in autumn 2018. In general, releases are 15 months post-capture for majority of males (i.e., grilse), and 27 months for the majority of females (i.e., Multi-Captivity Winter, MCW). Maturity can be detected late in the season, thus only a late season release is initially planned. Early diagnostics of maturity using ultrasound will be explored to enable mid-summer releases. It is anticipated that not all SAS will mature in these timeframes and the non-mature fish will be held for an additional year at the MSCC. The females from the smolt cohort collected in 2015 (originally destined for release in autumn 2017) is anticipated to be fully mature in 2018 and will be released in mid-summer 2018 coinciding with the wild run; the fate of the males from the 2015 cohort is to be discussed in the CSAS Expert review in January 2018 as the space limitations may not allow keeping them at MSCC after spring 2018 (potential release during kelt migration in April 2018).

The current proposal is that SAS fish will be released upstream of the smolt collection areas (Figure 1 - Sillikers in the LSW and Wayerton Bridge at NWM). The release plan may evolve as new information is learned regarding their behaviour and sub-population genetics during this experiment. The period of release is 15 September to 15 October corresponding to a natural migration peak in the Northwest Miramichi (Chaput et al. 2016); a mid-summer releases will be used if maturity can be predicted reliably using ultrasound methods. Releases will initially occur daily in lots of ~75-100 / river depending on water conditions, i.e., water temperature and levels appropriate for adult salmon.

Fifty (50) SAS adults and 50 wild adults will be equipped with radiotags to monitor movements and behavior, annually for 3 years in each tributary.

The number of released SAS salmon will be explicitly known each year. All SAS fish will be marked with a PIT-tag, and the rivers where the fish are released will be monitored at selected locations using multiple large flatbed PIT antennas that will detect if any of the SAS fish leave the river systems (double antennas will be installed to stream-bed to ensure detection of movement directionality; Figure 3). The number and sizes of wild Atlantic salmon entering the SAS intervention rivers will be monitored using the Adaptive Resolution Imaging Sonars (Figure 3) in a parallel CAST project (full study described elsewhere). This allows calculation of a ***ratio*** of wild:SAS fish in the system, and this ratio can be used to further monitor the success of their juvenile production (see Metrics section below).

The juvenile production will be monitored in a large-scale electrofishing program starting 2019, and continuing throughout the CAST SAS Phase 1 for at least 5 years (it continues in Phase 2 of the monitoring program). The parentage of the juveniles will be resolved using genetic SNP markers (see Section 4); the premise is that we will know the DNA of all our SAS fish, and therefore, will be able to identify any juveniles produced by SAS parents (both SASxSAS and SASxwild families). The objective of the juvenile monitoring is to document if the ratio of the wild:SAS fish is the same as the ratio of wild:SAS during the adult phase, and whether the ratio remains similar as the fish age (indicating similar intercohort survival between wild and SAS progeny). We will additionally sample the broodstock adults used in the ongoing juvenile stocking program to additionally compare the success of this program relative to wild and SAS juvenile and smolt production.

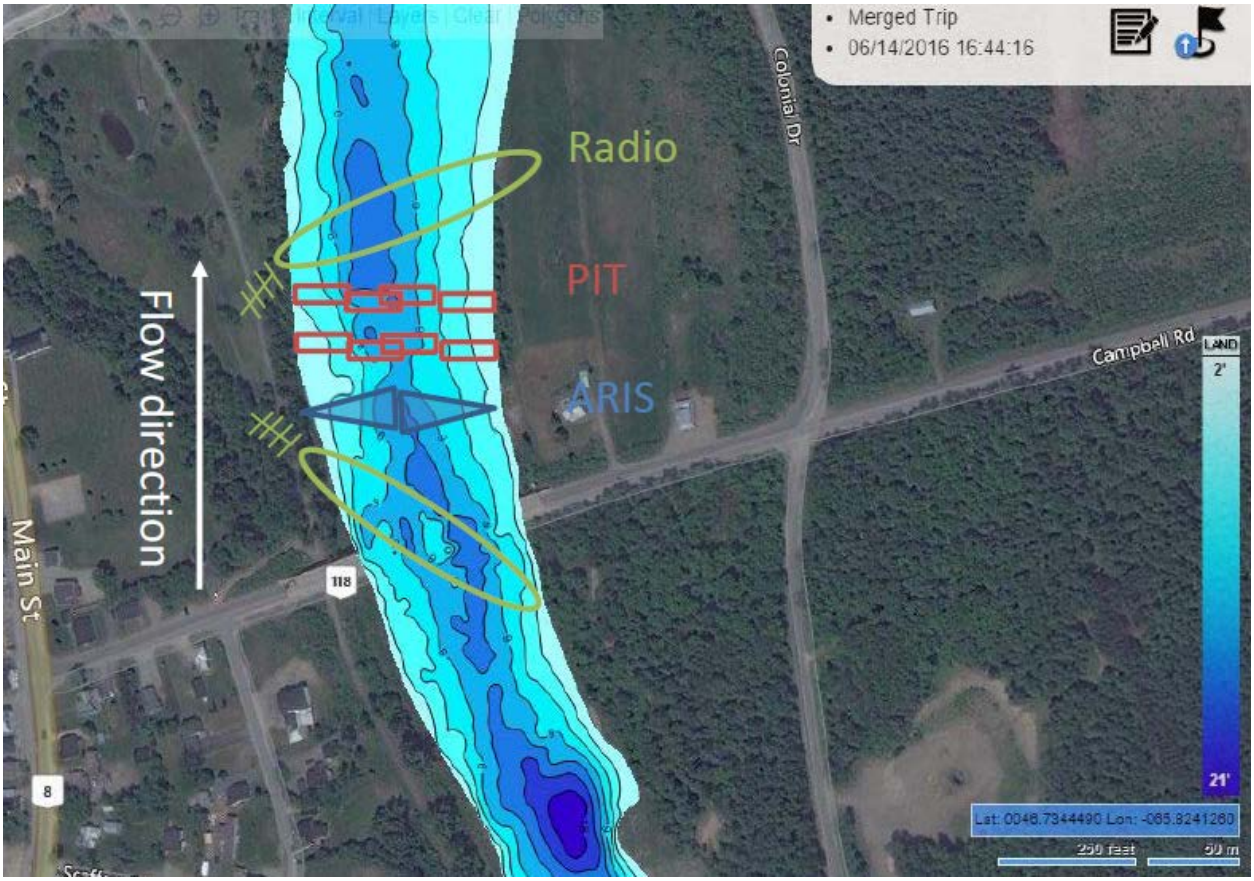


*Figure 3. Schematic representation of methods to establish the number of adult salmon (wild or SAS) upstream of original SAS smolt collection locations. Wild salmon will be enumerated by the ARIS sonar units; SAS salmon will be detected by both ARIS and associated PIT arrays. In addition, a sub-population of both SAS and wild salmon will be additionally detected by the radiotelemetry, allowing for a method to test the efficiency of both ARIS and PIT methods. (The schematic is for illustrative purposes only, and does not accurately represent the proposed study sites).*

Planned Studies (others may be added in our adaptive approach based on new knowledge gained):

- All SAS adults are sampled using genetic tools (LD SNP tool) and are PIT tagged (23mm Half-duplex "silver bullet"). All SAS fish are also externally marked using T-anchor tags so that the local First Nations communities can positively identify SAS fish, if captured (see Section 6).
- Project 1 - Monitoring movements and behavior of post-release adults
    - 50 of each of SAS and wild adults radiotagged / tributary for 3 years
    - Wild salmon sourced by seining / trapping and angling in the intervention tributaries; additional wild fish may be available from a potential concurrent DFO radiotracking study
    - Active tracking and passive monitoring stations at key locations (N = 4 locations in LSWM; areas locally known as Upper Oxbow; Catamaran Brook; Junction of Lower North Branch LSWM; and junction of North Pole Brook: N= 3 locations in NW; Wayerton Bridge area; junction of Little River; third location to be determined)
    - Lotek radiotelemetry systems; MCFT2 tags with multi-year batteries (approx. 2 years)
    - Fish will be tracked continuously through the spawning season, occasionally in winter as ice conditions allow (targeting 2 events), and weekly during the kelt outmigration period to establish the behaviour of SAS adults relative to wild fish. Tracking will be undertaken using a variety of methods based on area (vehicle; 4-wheeler; foot; aircraft). Data collected includes:
        - Spawning – Establishes that (1) SAS fish spawn and (2) serves as a guide where potential SAS progeny may be encountered during progeny monitoring efforts.
        - Winter behaviour, survival, and movement during kelt migration – Establishes if SAS fish may become multi-year spawners.
        - At-sea survival (SAS post-spawned fish) and among tributary straying
        - Repeat-spawning – Information on SAS fish rates of return relative to wild fish will be collected as the proportion of radiotagged fish returning (multi-year tags)
- Project 2 - Monitoring of post-release using PIT telemetry
    - Flat-bed PIT arrays to monitor of potential emigration of SAS adults; SAS adults are introduced to areas upstream of PIT arrays.
    - Half-duplex multiplex systems with satellite synchronization and marker tags to test for functionality
    - Required to establishing how many SAS fish remain in each tributary through the spawning period, i.e., is required to establish a ratio between adult SAS:wild salmon in each tributary during spawning time
- Project 3 – Counting returning wild adults
    - ARIS 1800 units are used to establish the number of wild salmon, and to assess the size distribution of returning adults (ARIS study is described elsewhere in full; units have been operated in 2016 and 2017, and daily fish counts by size category can be observed at www.castsalmon.com)

- PIT arrays are associated with ARIS units to solve whether migrants are wild or SAS (all SAS fish PIT tagged and detection of fish by PIT array while in sonar indicates a SAS fish; synchronization of PIT-arrays to be used are new satellite-based systems; antenna installation methods have been tried in another project (the Upper Salmon River SAS program) in 2016 and 2017, and have proven reliable)

- Project 4 - Assessing contribution of SAS progeny
  - Annual electrofishing surveys of 20 sites / tributary with 25 young-of-the-year and 25 parr collected / site for parentage analysis (i.e., 1000 juveniles / tributary / year for minimum of 5 years).
  - All genetic samples will be small, non-lethal fin-clips, from which juveniles are expected to rapidly recover (Dietrick and Cunjak 2006). If possible, other concurrent electrofishing programs may secure samples (e.g., DFO/MSA e-fish programs)
  - Genetic material collected from control rivers through the DFO and MSA electrofishing programs
    - Samples serve as baseline and analysed if genetic deviations between wild and SAS progeny in intervention tributaries are observed
  - Resolve parentage using SNP genetic tools (see Section 4)
  - Average inter-stage survival and growth monitoring by origin (SAS, wild or crosses, including broodstock stocking)
  - Assessment of changes in allele frequencies
    - Annual comparison of samples of wild juvenile Atlantic salmon (as determined by the parentage analysis, i.e. fish determined to be of non-SAS or non-broodstock origin) in the SAS intervention areas (Lower and Upper sub-basins in the Little Southwest Miramichi, and the Upper Northwest Miramichi) to the wild *baseline* (2016) genetic information from those areas and assessed for deviations. Are the allele frequencies changing in wild juveniles before and after SAS intervention?
    - Genetic samples from SAS progeny will also be compared to the wild baseline (pre-intervention, or 2016) data, as well as to the data from wild progeny collected *during* intervention. Are the allele frequencies different between SAS and wild juveniles either before or during SAS intervention?
    - If differences are detected between SAS fish and the wild baseline, the tissue samples collected from the control rivers (Sevogle River, and selected areas in the Southwest Miramichi River) will be analysed using the SNP tools. These data will be compared to the baseline data at the same control sites prior to the start of the SAS intervention. Are the allele frequencies changing in wild juveniles in control rivers before and after SAS intervention?
    - If differences exist in the control areas and the intervention areas, then the data are indicative of natural temporal variability in allele frequencies within each tributary irrespective of the SAS intervention. If differences in allele frequencies pre- and post-intervention are exhibited only in the SAS intervention rivers but not in control rivers, then halting of further SAS releases into natural rivers is to be considered pending further analysis. Results will be discussed by the Science Team (CAST/DFO/MTI) to determine if genetic evidence is sufficient to trigger the adaptive planning actions, e.g., modification of studies (see below).

- Project 5 - Assessing SAS contributions to smolt migrations
    - Smolts will be captured annually in the rotary screw traps (MSA operated)
    - Fin clip samples (1000 / tributary) collected for genetic analysis to solve parentage (SAS and wild population assumed well mixed during the smolt migration; sampling throughout the run)
- Project 6 - Monitoring of returning adults (progeny of potential SAS adults)
    - 250 non-SAS adults (no PIT-tag) handled in each of the DFO Cassilis (NWM; branch with SAS intervention) and Millerton (Southwest Miramichi; control branch; Figure 1) trap nets will be tissue sampled (caudal fin clip) and analyzed to assess: 1) temporal, genetic characteristics of returning, wild salmon; and 2) proportion of SAS-derived adults (estimated to begin being detected at 5 years after first release).
    - The proportion sampled between Millerton and Cassilis index traps may change over time if the results indicate that SAS fish are almost exclusively returning to NW Branch (as hypothesized, although natural straying between the two branches occur), and thus, we favour sampling to detect “effect” (ratio of SAS originated fish returning relative to the wild) rather than document “risk” (monitoring the potential occurrence, i.e. straying, of SAS originated fish into non-natal branch). However, preliminarily, the sampling program will use a balanced design.
    - Additional adults will be sampled at conservation barriers (e.g. headwaters at NWM) and fishing camps along the LSW and NWM. N = 100 per tributary is targeted.

**Scale of the experiment**

The main objectives of the *in-situ* experiment is to 1) address the largest outstanding scientific knowledge gap regarding the SAS strategy, *i.e*., compare the lifetime success of offspring of SAS adults vs. wild adults in the natural environment; and 2) examine whether the SAS program will be able to increase juvenile production in the intervention rivers relative to controls. To be successful, the experiment must be large enough that we achieve appropriate sample sizes of *returning adults* whose parents were SAS fish, and that produced quantities of juveniles are not masked by natural interannual variation in juvenile densities.

The main method of assessing this outcome is to compare the ratio of SAS:wild (and their crosses) in the randomly sampled, out-migrating, smolt cohorts in each of the two SAS intervention rivers, to the ratio of SAS:wild (and their crosses) of returning adults in one-to-three years after each smolt cohort has migrated to ocean. The majority of smolts migrate at freshwater age 2 or 3, while <3% migrate at age 4 (Chaput et al. 2016). Complete assessment of the success of each year class requires assessing the smolt production over the three outmigration seasons and the subsequent returns to MSW stage.

Accurate calculations of required SAS “seeding” quantities can only be estimated based on available information. This is because the success rate of SAS fish to produce progeny is not known at the current time, and the freshwater survival of juveniles or the ocean survival of adults (either wild or SAS) are not explicitly known. It is estimated that 2500 smolts from each SAS tributary can generate a production of 2,000 SAS adults (assuming 80 % survival to release in captive environment), 50% of which are predicted to be female (but will be explicitly know at the time of release).

A release of 1000 SAS females / replicate tributary is considered an absolute minimum to ensure a good likelihood for securing adequate sample size of returning adults whose parents were of SAS origin. While only a very coarse estimate, it is predicted that 1000 females will produce an approximate egg deposition of 6.2 million (Assumptions: 80 % of the females mature as MSW at average weight of 5 kg; 20 % mature as grilse at average weight of 2.5 kg, and an average fecundity of 1385 eggs/ kg; Chaput et al. 2016; Cunjak & Therrien 1998). An egg-to-smolt survival of 2.5 % (a mid-range estimate from available juvenile salmon studies; Cunjak & Therrien 1998) would translate into approx. 156 000 smolts being produced. Estimating an ocean survival of 1 % from smolt to grilse, and 0.2 % for smolt to MSW (lower range estimates from Miramichi in the 1998 to 2006 time series; Chaput et al. 2016) and 80 % return rate of smolt class as grilse (Chaput et al 2016), an estimated 1246 grilse and 62 MSW salmon may be estimated to return (/ 1000 SAS females). Sampling genetic material to confirm whether SAS:wild ratio is similar to the ratio during smolt migration requires a chance to capture of a representative number of the returning SAS individuals. Assuming that 10 % of the returning adult salmon could be sampled by DFO index trap, sample collections from outfitter lodges, barrier traps, angling and seining salmon pools, a sample of 120 -130 SAS fish / tributary could therefore be obtained / returning adult cohort, assuming original “seeding” of 1000 females. It is emphasized that the above calculation is only a coarse estimate, however, provides justification for the size of the SAS experiment in natural streams, in absence of any other meaningful way to predict various stages of survival or capture rates of salmon as they return. It is also noted that the production sizes of females (i.e., 2.5 kg and 5 kg) as well as egg-to-smolt survival of 2.5%, may be “liberate”, meaning that the *de facto* return may be *less* than the above prediction, calling for potentially larger “seeding” of SAS adults. However, the potential risk to wild Atlantic salmon increases as the SAS “seeding” numbers increase, and therefore, until better information is available regarding the risks related to the experiment, the proposal is to compromise with a collection of 2500 smolts / tributary.

Furthermore, in order to test for the potential of SAS intervention to increase juvenile production in a system where the average inter-annual variation in average densities is approx.. 80% with a mode of 35 % (DFO e-fish data), the modeled increase in adult recruitment needs to be at least in the range of 2000 adults / tributary to be able to detect an “effect” (i.e. increase in juvenile density above background “noise” level; R. A. Curry, unpubl.).

Currently, the CAST program is predicting to start with a release consisting of approximately 1850 and 1550 SAS salmon for the Northwest and the Little Southwest Miramichi, respectively (Table 1; 2015 and 2016 cohorts with some minor mortality; assuming 50% females). Current prediction for releases in years 2 to 5 (continuing to year 6 to release all the multi-captivity winter fish) is to annually release the surviving mature adults (less the adults required for laboratory experiments, Experimental stream studies and veterinary testing) from the 2500 smolt collections to NW and LSW (in years 2 to 5, or 2019-2022/23). For context, the conservation requirement for the Northwest Miramichi system is estimated as 7300 large (MSW) females and a corresponding amount of adult males (Randall 1985).

The return rates of wild adult Atlantic salmon to either Little Southwest Miramichi or Northwest Miramichi above Trout Brook are not known (CSAS 2017a). In CAST’s salmon counting

program (ARIS program), there will be an assessment of adults returns in both the NWM and LSW. For context, the average of the median adult returns to the Northwest Miramichi system (i.e. includes both SAS intervention areas and the Sevogle River control, and other small streams) is approximately 5 000 MSW and 12 000 grilse (CSAS 2017a). Based on CAST's ARIS project the return, the return in LSW in 2017 (at the end of October) was 4287 grilse and 1576 MSW salmon.

In a SAS, or any supplementation program, the potential risks may increase when SAS releases represent an increasing proportion of the total number of wild adults in the population at spawning time (CSAS 2016). The releases of CAST SAS program of up to approximately 1000 adult females / tributary (in 2019 and onwards) would comprise a small-to-modest quantity compared to the amount of adult females required to meet the conservation status. While the wild returns a number of years into the future cannot be predicted with any confidence, a maximum number of SAS releases in relation to wild adult returns are proposed to be a maximum of 1:1 until new information is available to assess this ratio and its potential impact on the wild salmon. The actual ratio will be known at the time of the releases based on the CAST ARIS program. Similarly, if the ARIS program indicated that the wild salmon return is so strong that the conservation targets would be exceeded by additional SAS releases, further discussion regarding the SAS release quantities would have to take place.

*Knowledge gap (CSAS 2016) to be addressed:*

- Behaviour (migratory rigor, between tributary straying, pairing ability, spawning behaviour and timing, post-spawning behaviour and survival) and interactions of the released SAS adults relative to wild counterparts in the natural environment,
- At-sea survival information of SAS repeat-spawning adults relative to wild repeat-spawning adults based on redetection of radiotagged and PIT tagged adults in years following spawning.
- Relative fitness at various life-stages including first generation returns of anadromous adult stages (i.e. generational contribution of SAS adults relative to wild adults).
    - Relative group fitness of SAS adults relative to wild (ratio of SAS:Wild progeny through years within cohort) during juvenile to smolt stages.
    - Relative group fitness of first generation (F1) returning adults of SAS fish relative to wild adults (ratio of SAS:Wild F1 adults in each SAS tributary relative to the ratio in the tributary during smolt migration phase).
- Inter-stage survival and growth of juveniles of SAS and SAS-wild hybrid relative to wild salmon in natural environment.
- Characteristics of smolt migration (timing, size, age at smoltification) of SAS and SAS-wild hybrid relative to wild salmon.
- Characteristics of anadromous adult progeny of SAS and SAS-wild hybrid to assess cross-generational differences in genotype or phenotype relative to wild salmon; i.e. assessment of loss of marine adaptation.

*Metrics (Fraser 2016; Table 2)*

- Adult - Morphology, Reproductive timing, Migratory rigor, Activity levels

- Juvenile - Inter-stage survival (fitness), growth, body shape, genetic differences

Deviations in mean and variance in above metrics between SAS and wild fish will be assessed and statistically compared (Fraser 2016).

*Adaptive Planning - Triggers and decision rules for altering, continuing or halting the SAS studies*

- Assessment of the SAS program in natural rivers, relative to wild fish, is based on following the development of SAS:wild ratio. This ratio is established starting from spawner escapement by 1) knowing how many SAS adults are released in each of the intervention rivers, 2) monitoring potential emigration of SAS fish from the rivers based on PIT arrays, and 3) establishing the amount and sizes of wild fish based on ARIS counts.
    - If the ratio of SAS:wild fish declines in favour of wild fish when measured in the young-of-the-year (YOY) life stage, this may indicate that SAS fish are not as effective in propagating as wild fish. Such outcome does not pose a risk to the wild Atlantic salmon, but indicates inefficiency of the SAS program (e.g. lower fecundity, lower egg survival).
    - If the ratio of SAS:wild fish increases in favour of SAS fish when measured in the young-of-the-year life stage, it may indicate that SAS fish are outcompeting wild fish or otherwise negative affecting wild population. Continuing of SAS program would have to be re-assessed.
    - If the ratio of SAS:wild fish continues to significantly change from the ratio observed at YOY stage, it would indicate a possibility that certain traits, either phenotypic or genotypic, have been transferred to the juveniles. The changes in ratios would have to be carefully assessed, and the consistency between the two SAS intervention rivers would have to be compared to rule out potential effects due to sampling bias. Continuing of SAS program would have to be re-assessed after such analysis.
    - If the ratio of SAS:wild fish would change from smolt stage to returning adult stage, and the ratio would change to be biased to higher returns of wild fish, the outcome would indicate loss of marine adaptation. Continuing of SAS program would have to be re-assessed, although, it is also considered that the SAS-originated salmon not returning due to the potential loss of marine adaptation are being eliminated by natural selection, and would therefore not be further propagating in the system, should this scenario be at play. Therefore, the potential problem of loss of marine adaptation may be considered to be “self-correcting”.
    - It should be noted that assessment of SAS:wild ratio is best assessed in smolt migration stage when the population is thoroughly mixed. Monitoring of the ratio during the juvenile stages is a subject to initial spatial distribution that is somewhat dictated by the spawner distribution. Random sampling across 20 electofishing sites may alleviate chances of sampling locations where only SAS fish, or wild fish, have spawned, however, the smolt stage will represent the best assessment time-point in the freshwater (juvenile) life stage.
- If growth metrics (average size-at-age measured in either length or weight) in wild juvenile salmon drops below values documented in long-term baseline DFO dataset in SAS intervention rivers, but not in control rivers, SAS program could be having an effect on growth of wild Atlantic salmon. Continuing of SAS program would have to be re-assessed.
- If significant changes in allele frequencies in SAS juveniles vs wild juveniles would be observed in SAS intervention rivers, but no changes in wild juveniles in control rivers

compared to (wild) baseline would be observed, continuing of SAS program would have to be re-assessed and potentially halted.

- If the Atlantic salmon population stock status develops in the Northwest Miramichi River system to a point where adult returns not only meet the conservation targets set forth by DFO (CSAS 2017a), but increase enough that population status can be considered “healthy” (See section 2.3), then the juvenile densities across the system may increase to a point that density-dependent factors through interference competition may start limiting juvenile production (density-dependent effects on growth occur primarily already at low population densities: Grant & Imre 2005; Imre et al. 2005). Adding juveniles in such conditions may be considered a risk to wild Atlantic salmon in case SAS originated juveniles would outcompete wild fish. If such conditions are observed due to natural increase in adult returns, continuing the SAS program would be re-assessed. For the current time, however, increases in spawning escapement result in concomitant increases in juvenile densities (as expected if the population follow a Beverton-Holt stock-recruitment relationship; Chaput et al. 2016) indicating juvenile production well below carrying capacity (non-plateau part of the Beverton-Holt relationship). A prime example of the expectation for juvenile densities following an increase in adult spawning escapement above conservation target was observed in 2011 in the Miramichi system, when both the Northwest and Southwest Miramichi branches exceeded the minimum conservation requirement and the fry and parr indices showed a corresponding increase in years following the 2011 spawning cohort (Chaput et al. 2016). Such increases would not be evident in a system where juvenile production was limited by excessive density-dependent responses that manifest as changes in survival.

# 6. Considerations related to First Nations perspectives

Atlantic salmon plays vital importance for the livelihoods and culture of the First Nations communities that have lived along the Miramichi River since time immemorial. Three Mi’kmaq First Nations live in immediate proximity of the Miramichi River; The Eel Ground, the Metepenagiag and the Esgenoôpetitj First Nations. All three rightsholder communities have indicated the importance and continued resilience on Atlantic salmon for their community members, and have indicated that it is important that any Atlantic salmon activity in the area is brought into attention to the communities so that the concerns and comments, as they specifically relate to the First Nations perspectives, are heard, understood, and taken into account.

To this end, CAST members have met a number of times with a group representing the interest of the aforementioned rightsholder communities (Mi'gmawe'l Tplu'taqnn Inc; MTI). Through these meetings, it has been collectively identified that exchange of knowledge and direct involvement in the on–the-ground work are important ingredients to ensure the First Nations interests are not overlooked. It is recognized that the exchange of knowledge will be best in multiple formats, and is a two-way dialogue involving both the “Western science” (as described in this proposal with regard to SAS studies) and an Indigenous Knowledge regarding Atlantic salmon in the area of concern. While CAST is currently working directly with MTI to better understand the potential scope of an Indigenous Knowledge Study, it is also recognized that the exchange of knowledge, in two-way manner, is a required component for the proposed CAST SAS work so that the concerns and comments from the rightsholders can be appropriately considered. CAST, MTI and DFO discusses the CAST-scope of work (all CAST projects, but to

include SAS activity when it occurs) on a monthly information sessions via teleconference. While the teleconferencing is able to inform the fisheries management staff of the MTI, it is not sufficient to cover the wider membership of the First Nations. Therefore, community outreach is required, and will take place in the form of community sessions that are to be arranged, as needed, in all aforementioned Mi'kmaq communities in collaboration with the MTI. Such sessions will be arranged prior to releases of fish into rivers in an effort to disseminate the required information regarding the CAST SAS program to the community members. In addition, similar sessions may be required as data from the program becomes available to inform community members regarding the success of the project; such sessions would be arranged, as necessary, with MTI staff.

With regard to technical aspects of the proposed work, certain components are in place directly to facilitate First Nations concerns. It is important that SAS adults are identifiable following a release such that the members of the First Nations communities are able to recognize the background of a potentially captured adult salmon as a SAS fish. All adult SAS fish are externally identifiable via adipose fin clipping, and T-anchor tags. Also, as explained previously, SAS salmon are identifiable by internal PIT-tag, and a hand-held PIT-tag reader can be provided to each community, as required. As the SAS adults will be identifiable, community members can decide whether to release a SAS fish upon potential capture (preferred), or to use it for sustenance. The meat quality of the SAS fish in comparison to the meat quality of wild fish has not been assessed, but can be done if it is deemed necessary by the rightsholders (the SAS program objective is to produce spawners to increase juvenile production, not fish for human consumption).

It is also considered that the removal of the proposed (up to) 5000 wild smolts / year may result only in a marginal reduction in the ability for First Nation membership to capture returning adults in subsequent years; this is due to the estimated high at-sea mortality (estimates for smolt-to-adult survival range 0.3 % to 3.3% for grilse, and 0.2 % to 2.2 % for MSW between 2006 -2010 (Chaput et al. 2016), but with estimated subsequent decline in sea-survival in recent years) and low-to-moderate trapnet efficiency in First Nations food fisheries (4.1% to 17.5 % for grilse; 4.5% to 14.2% for MSW; Chaput et al. 2001).

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