



## Testing the Effect of Fish Farms on Salmon Survival, TEFFS

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## Overview

TEFFS is a research initiative to directly measure whether open water fish farming reduces survival of wild sockeye salmon in British Columbia. The goal of TEFFS is to provide clear data so that policy makers can determine whether fish farms should be regulated to protect wild stocks, and to satisfy stakeholders on both sides of the debate that the resulting policy decisions are based on sound science.

Whether fish farming caused the widespread decline of southern British Columbia salmon stocks is hotly debated, and it is unlikely that evidence reported at the Cohen Judicial Inquiry can resolve the controversy. In part, this is because all previous studies used indices, such as sea lice burdens on smolts collected near or far from fish farms, rather than directly measuring smolt survival. This choice was a result of earlier technical limitations preventing direct measurement of marine survival. However, several other issues also are important: (1) indices do not provide a direct causal link to survival and the degree of harm fish farms may actually impose; (2) even if one factor (such as lice or parvovirus) can be unequivocally ruled out, other untested or undescribed diseases may still play a role, leading to a long cycle of studies; and (3) smolts move. For instance, our past studies demonstrate that wild smolts migrate at 8~13 km/day. This makes any association between disease burden and smolt location at the time of capture (near or far from fish farms) problematic.

We are proposing four distinct parts to an overall research program that should resolve the effect of fish farms on wild salmon stocks. These components will do the following:

- (A) Measure the degree to which salmon farm exposure reduces survival of wild smolts over the first ~8 weeks of ocean life after initial exposure;
- (B) Establish whether animals transported and held in holding pens for experimental use have the same migratory behavior and survival as smolts naturally migrating from their natal lakes;
- (C) Develop disease, genomic, and histopathological profiles on smolts that are or are not exposed to fish farms;
- (D) Measure survival to adult return of smolts fed/not fed an initial prophylactic dose of SLICE™ to provide immunity against sea lice before lake release.

This proposal implicitly assumes that POST's existing marine sub-arrays in Juan de Fuca Strait, Strait of Georgia, and Queen Charlotte Strait will be funded by DFO in 2012 and operated by POST to the same high standard as in the past. If the funding requested in this proposal is secured, we will supplement POST's core infrastructure with the additional infrastructure needed to achieve the specific goals of TEFFS; this proposal is neither a substitute nor a replacement for POST.

## A. Experimental Design

The core component of this proposal is a direct test of the effect of fish farm exposure on the survival of wild Fraser River sockeye. This component will contrast the survival of groups of free-ranging smolts first held near fish farm sites or in pristine areas (controls) far from fish farm operations (Figure 1). If fish farms reduce survival by disease transfer, parasite load, or some unknown agent, then there should be a measurable decline in survival of the exposed smolts relative to controls. Using Kintama's protocols for handling and tagging smolts in the Columbia and Fraser Rivers, smolts will be captured as they exit from Cultus and Chilko Lakes and then tagged with acoustic transmitters. Smolts will then be transported to control sites lacking fish farms (such as Bute or Toba Inlets) or treatment sites (near active fish farm operations; Figure 2).

At both control and treatment sites, smolts will be transferred into small-scale open-water holding pens and held for one week to match the approximate time period that migrating wild smolts are exposed to fish farm operations (Figure 2).

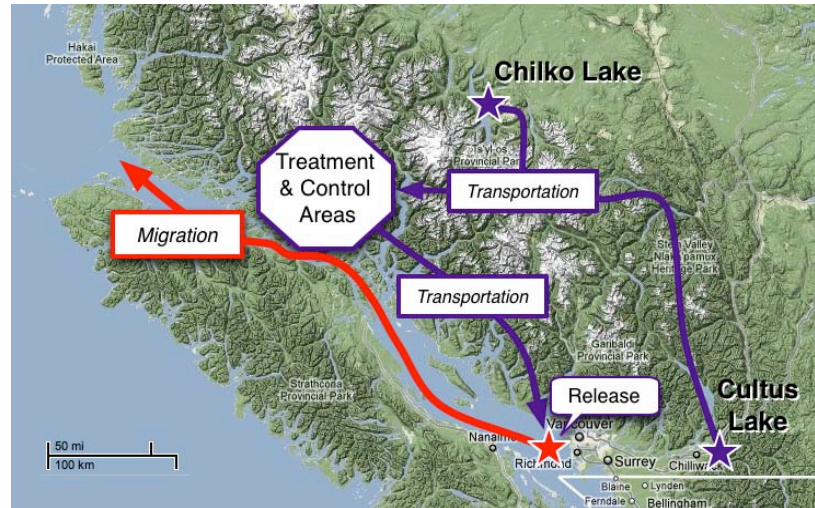


Figure 1. Overview of the TEFFS manipulative experiment. Arrows show the movement of fish from the source populations.

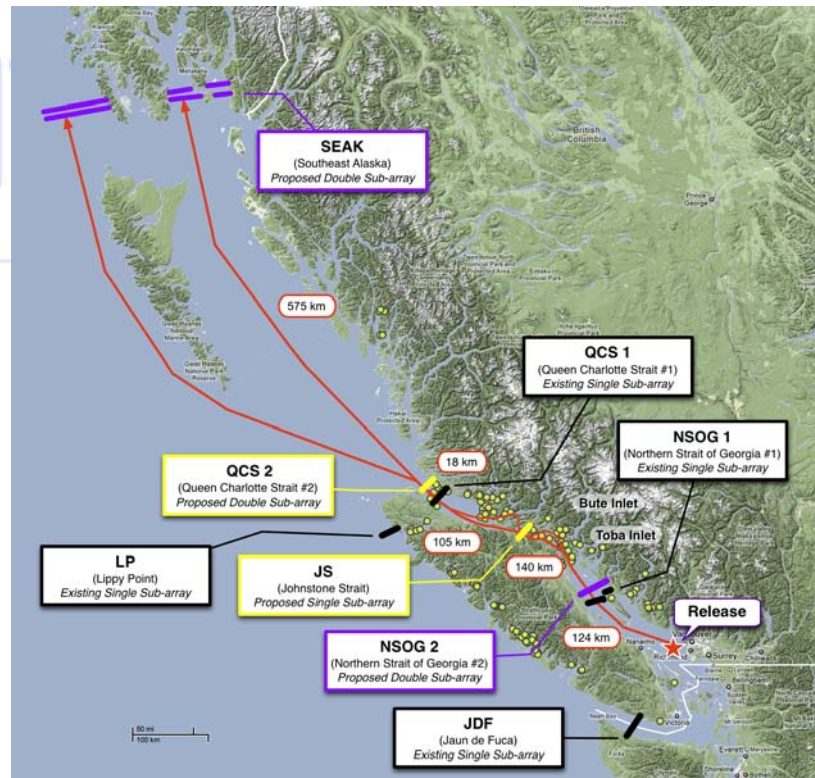


Fig. 2. Overview of possible acoustic sub-array configurations discussed in the Appendix on array design. Black lines indicate existing POST sub-arrays, the two yellow lines indicate the additional sub-arrays yielding maximum statistical power amongst the designs considered; purple lines indicate other sub-array locations that were considered and rejected. Yellow dots indicate existing fish farm tenures.



as they migrate through Discovery Passage. After holding, both control and treatment groups will be transported to a point in the Strait of Georgia near the mouth of the Fraser River and released. Marine survival will then be determined using the existing POST acoustic array and two seasonal acoustic sub-arrays deployed over the migration period to boost the statistical power of the study and provide additional calibration data as the smolts migrate out of the area<sup>1</sup>. Figure 2 shows the location considered for additional lines, while Appendix II reports the results from the power analysis used to determine the preferred array configuration.

A flowchart (Figure 3) outlines the approach; the exposed and control smolts whose marine survival will be measured and compared after release are shown in column A.

## B. Comparison with Natural Migration

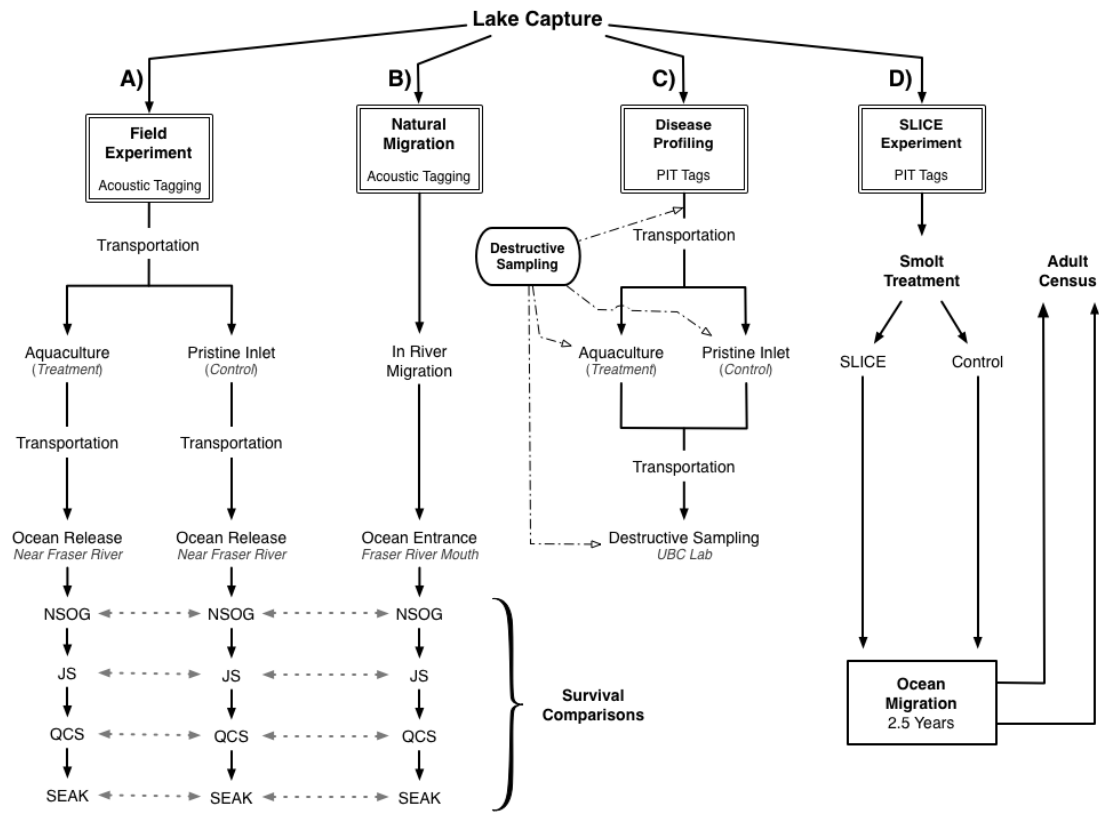
Studies that do not directly manipulate exposure by holding animals close to fish farms rely on the capture of naturally migrating individuals in the ocean whose prior history of exposure to fish farms is uncertain. Although manipulative experiments provide a clear way to vary exposure, questions still arise. For example, is the exposure level used in the study representative of the exposure actually experienced by wild smolts or will the transported and held smolts behave as normally migrating smolts after release?

To address these questions, we will also conduct an observational experiment similar to our six years of prior work tracking salmon smolts, and which will ground-truth the results of the manipulative experiment (Figure 3, Column B). The natural experiment will consist of releasing acoustically tagged Chilko and Cultus Lake sockeye smolts at the lake outlets and allow normal migration down-river and into the ocean, as in past years (2004-07, 2010-11). The data will provide a survival baseline comparable to prior years, and will provide guidance on how similarly the smolts held in holding pens behave to smolts migrating naturally.

If industry support and additional funding can be obtained, tamper-proof acoustic receivers will also be deployed at each BC fish farm site in Discovery Passage. These extra receivers will provide an estimate of natural exposure experienced by tagged sockeye smolts during migration (which will be operationally defined as the time duration smolts remain within acoustic range of the farms) as well as information on the relative behaviour of the three groups (treatment & control groups; natural lake-migrants) in the Discovery Passage waterways containing fish farms. Because fish farm sites are distributed widely throughout the Discovery Passage/Broughton Archipelago region (Figure 2), the receivers will provide additional information on the extent which migrating sockeye smolts use these areas. Past experience with Cultus Lake sockeye showed that smolts occasionally penetrated deep into Howe Sound, an inlet further south, before continuing their migration northward.

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<sup>1</sup> We assume that the baseline POST array will be operational, but our analysis has found that to achieve the statistical precision necessary to measure small differences in survival additional supplementary sub-arrays will be required. These sub-arrays are an enhancement to POST.



**Figure 3.** Handling and treatment of smolts from the overall experiment. Columns A & B represent acoustic tag-based experiments while C represents smolts held for disease profiling. Column D represents a direct experiment to measure whether treating smolts with SLICE™, a sea lice prophylactic, improves their survival to adult return 2.5 yrs later.

## C. Disease Profiling

Additional groups of smolts (without acoustic tags) will also be transferred to the control and farm sites from the lake sites and held in separate holding pens. Smolts will be periodically collected and sacrificed to see whether genomic and biochemical indicators of disease or physiological stress develop over time and whether their prevalence and intensity is greater for farm-exposed animals (Figure 3, Column C). The genomic profiles generated for each individual smolt will be compared with the results from traditional histopathological screening, providing a better understanding of how the new genomic methods match established protocols.

Handling will be the same as for the acoustic tagged individuals transported to the treatment and control sites, except that only small (and inexpensive) PIT tags will be used to identify individuals. Detailed protocols are outlined in Appendix I, as well as a summary of the diseases and physiological conditions that will be surveyed.

The growth and survival of PIT-tagged smolts over time will also be followed within the holding pens, providing a baseline survival rate in predator-free conditions to compare with the freely-migrating smolts. This will also allow us to assess whether it will be possible in subsequent years to hold and feed acoustic tagged smolts for longer periods of time near fish farms prior to release, increasing exposure levels beyond those likely encountered when freely migrating; the current one week holding period reflects a balance between maximizing farm exposure and minimizing the risk from holding wild fish in pens for long periods of time.

## D. Effect of Sea Lice

A limitation of studies A-C is that they can only measure the degree to which mortality or physiological & genomic changes are expressed for 1-3 months after exposure (the study period)<sup>2</sup>. If disease transfer occurs but takes longer to develop, these studies will not detect them. Sea lice have been frequently identified as a potential disease vector or direct source of mortality for wild smolts. To address this, we will implement as part of a full scale-project in 2013 and beyond, a simple experimental design where two large groups of Cultus Lake hatchery smolts are implanted with PIT tags and one group is fed SLICE™-supplemented feed for one week prior to release (SLICE™ provides immunity to sea lice for several months, long enough for the smolts to migrate beyond the fish farms). The protocol is detailed in (Jackson *et al.* 2011) and the response variable is the proportion of released smolts that survive to return as adults to the spawning grounds 2.5 years later<sup>3</sup>.

## Pilot Study

While the general concept of TEFFS is straightforward, and all major elements of this study have previously been successfully carried out by the proponents, some components involve novel procedures and the work has not been conducted as a single package focused on testing sockeye smolt survival after exposure to fish farms. The main uncertainties concern (1) logistics for long-distance transfer of sockeye smolts to & from seawater holding pens in the Discovery Passage area and (2) maintenance of smolts in pens for 1-4 weeks.

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<sup>2</sup> We ignore here the use of acoustic tags for smolts programmed to turn off (to conserve battery power) and then turn back on at the time the adults migrate back to freshwater. We have successfully used this technique to measure survival to adult return of both Cultus and Sakinaw Lake sockeye smolts (Welch *et al* 2011; Wood *et al*, In Press), but do not initially advocate their use because large hatchery smolts are required. Failure to find an effect of fish farms using large hatchery smolts could be argued to be the result of small wild smolts being more susceptible to disease, thus not resolving the core issues.

<sup>3</sup> The Jackson *et al.* 2011 study on Atlantic salmon found that SLICE™ had very little effect on ocean survival of Irish salmon.

We are requesting funding for a one-year pilot phase in 2012 to validate the experimental design for components A-C, identify logistical problems, and demonstrate the success of the approach before scaling up to the full experiment. We will use the success of the 2012 pilot to establish feasibility and justify federal and provincial funding for a 3-5 yr program to achieve a definitive result over multiple years.

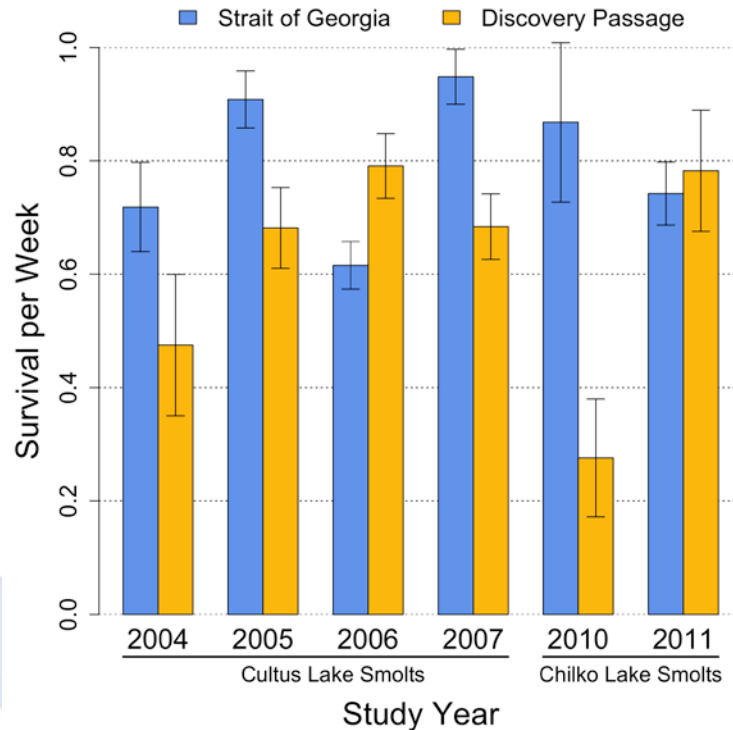
### 2012 Deliverables

The key deliverable will be a successful operation resulting in a target detection of approximately 20 control and 20 treatment smolts at the Queen Charlotte Strait line (assuming equal survival for both groups). Based on previous Cultus and Chilko Lake smolt survival and detection rates for acoustic tags, this will require a total of approximately 500 acoustic tags (Appendix II& III), about 1/3<sup>rd</sup> the acoustic tag numbers annually needed once the experimental process is validated and fully scaled up (1,000-1,500 tags/year, plus additional tags for smolts released at the lake outlets (if used; see Appendix II). The larger tag numbers required in later years will likely require the use of an enhanced array operating at two frequencies (currently the array is single frequency) in order to allow use of smaller acoustic tags that can be implanted into smaller individuals; larger smolts are limited in number and critics might argue that the experimental results may not apply to smaller individuals. Using smaller tags in later years of the study will provide both the numbers needed for the experiment and also expand the sample population to include smolts as small as 95 mm, smaller than previously tagged smolts ( $\geq 125$  mm). A separate project, with funding support by OTN, will tag Chilko smolts with much smaller 180 KHz tags in 2012 and provide baseline information on relative performance which can be used to feed the major 2013-2015 project proposal.

The pilot will also demonstrate whether transported smolts continue their normal migration route by successfully tracking smolts across the existing acoustic lines in the northern Strait of Georgia, Queen Charlotte Strait and Juan de Fuca Strait. It will also generate better estimates of variability (i.e., how much survival varies between treatment or control sites) that will allow a more precise calculation of the required tag sample sizes prior to deployment of a full-scale study in 2013-2015.

### What can be measured?

In 4 of 6 previous years of study using naturally migrating Fraser sockeye smolts, ocean survival rates of acoustic tagged smolts were lower in the northern (Discovery Passage) region than in the southern (Strait of Georgia) region (Fig. 4). Although the northern area contains fish farms, we emphasize that it also seems to have more abundant marine life (seabirds, marine mammals) and the smolts reach the northern region later; it is also possible that the tag's output signal may weaken over time or that the northern sub-array (QCS) may have poorer performance than the southern (NSOG) sub-array for as-yet unidentified reasons. For these reasons, it is important to not ascribe the observed survival difference to a single specific factor such as fish farms. The experimental comparison of treatment and control groups using the temporary second line at Queen Charlotte Strait will allow disentangling these complex factors and our analysis (Appendix II) indicates that given the difference in survival rates apparent in Fig. 4 in at least some years we should have high statistical power to resolve the effect of fish farms if they are indeed the cause of the observed lower survival rates in the northern area.



*Figure 4. Sockeye survival in the Strait of Georgia (blue) and in Discovery Passage/Queen Charlotte Strait (gold). 2004-2007 survival data are from (Welch et al. 2009; Welch et al. 2011); 2010 & 2011 results are for 2-year old wild Chilko Lake sockeye smolts, which are substantially smaller (unpublished). 2011 Chilko data were analyzed assuming the same detection efficiency for the Queen Charlotte Strait sub-array as observed for the NSOG sub-array in 2011 (48%); in prior years we have assumed  $p_{OCS}=70\%$ , the long-term performance. (If we assume a detection efficiency of 70% for Queen Charlotte Strait the Discovery Passage survival rate is slightly lower than the Strait of Georgia survival rate but not significantly different). In 4 of 6 study years, survival rates were lower in the northern area. Error bars are  $\pm 1$  SE.*



### Timelines

A timeline for 2012 work elements is presented in Appendix IV. To achieve the 2012 objectives will likely require a funding decision by the end of February in order to manufacture necessary equipment.

### Study Limitations

We solicited comments from colleagues as part of the proposal development process and during the request for letters of support (attached). The comments fall into three categories, which we outline here:

- a. **Tagging and Handling Procedures too Stressful.** Several individuals commented that sockeye smolts are “too delicate” to withstand the proposed tagging and transport protocols. Evaluating this concern is an important goal of the 2012 pilot. In Kintama’s previous work we have not encountered greater difficulty using sockeye relative to the other three species we have tagged. Scott Hinch has also tagged, transported, and held Chilko sockeye smolts (with seawater transition) at UBC and his Master’s student is completing her thesis on the tagging results (Kintama’s tagging protocols were largely used). Dr Simon Jones of DFO also informs us that he has transported and held sockeye for months at PBS with no evidence of unusual problems. In sum, we see little evidence for a practical problem using sockeye, and successful execution of the 2012 study is a critical step to securing longer-term funding from government for the scaled-up experiment.
- b. **Control fish become exposed to fish farms.** Smolts not initially exposed to fish farms during the holding period likely become exposed to fish farms during their subsequent migration northwards and thus “convert” from true controls to exposed smolts, compromising the experimental test. Control and exposed smolts will first reach the NSOG sub-array about 2 weeks after release and the farms about 3 weeks after release (3 & 4 weeks if we include the 1 week holding period). Before reaching the farms controls have not been exposed, so any measurable survival difference can be ascribed to farm exposure. Migration from NSOG to QCS sub-arrays will take an additional 3 weeks. Thus, if shortly after farm exposure significant mortality is expressed, we will identify it using the NSOG sub-array. Alternatively, if significant mortality is only expressed after first exposure with a >3 wk latency period, then control smolts that become exposed to farms during their migration will not have time to die before reaching the QCS sub-array; the contrast in mortality will thus still be measurable and ascribable to farm exposure. (We hope, if 2012 work is successful, to hold smolts for longer exposure periods in future years, increasing both the level of farm exposure and the time period we can monitor for survival problems)<sup>4</sup>.

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<sup>4</sup> We also note that all current field studies, which sample smolts near or far from farm sites, have no control over the smolt’s prior movements, and thus neither their exposure level nor the time period they can be studied after exposure; the current proposal controls both factors and also substantially extends the duration survival can be measured over. We note that the siting of additional receivers at each fish farm in 2013 and later years has a good likelihood of allowing us to reconstruct the prior exposure history of each smolt, and allow us to assess whether higher farm exposure during migration reduces survival. In principle, this approach should also be extensible to the control groups’ potential re-exposure.

- c. **Mortality may be chronic & long-term (>7 wks), not acute and short term.** This experimental design is initially focused on measuring potential short term acute disease expression, where significant mortality occurs within the time period that smolts require to reach the final sub-array. Our earlier work (Fig. 4) indicates significantly higher mortality in the Discovery Passage area, suggesting rapid disease onset is likely. If a farm-transmitted pathogen (or suite of pathogens) merely induces a long-term chronic response to the disease then it will be necessary to measure relative survival to adult return to fully capture the potential losses to mortality and resolve this uncertainty. We have previously been successful in doing this by using hatchery-reared smolts and programming acoustic tags to transmit for two periods: (a) a few months on the outward smolt migration and (b) several months during the adult return migration ~2.5 years later (Welch et al 2011). However, our results suggest that there seems to be substantial short-term expression of mortality, so the proposed design will make the study more statistically powerful and shorter (there will be no need to wait 2 years for the adults to return).
- d. **Access to Smolts.** In April 2011, Kintama was denied a permit to transport 200 tagged Cultus Lake hatchery-reared sockeye smolts to validate the handling approach using sockeye. We hope to resolve this problem, but at the current date have not done so. We are currently working to address this issue with DFO; should we fail, we will switch to using Seymour River steelhead, which have experienced an equally profound decline in marine survival over the same time period. We have substantial experience working with this stock and with the Seymour Steelhead Society (see attached letter of support), but would prefer to work with Cultus sockeye because the current controversy concerns the effect of fish farms on sockeye, not steelhead.

## Scientific Standards

This project will be operated to explicitly meet or exceed all elements of the new ARRIVE guidelines for animal-based research studies (Kilkenny *et al.* 2010). All data and analyses will be made freely available with no limitation, a power analysis will be used to guide the study design, the data analysis will be based on a doubly blinded randomized trial with the analyst(s) not knowing the group allocations until after the analysis is complete, and the ethical standards for animal care will be subjected to review by an independent institutional animal care committee.

## Participants

Drs Scott Hinch (UBC), Tony Farrell (UBC), Kristi Miller (DFO), Carl Schwartz (SFU), Gary Marty (Province of BC), and Brian Riddell (PSF) will join Kintama as co-PIs. Hinch will take primary responsibility for the physiological analyses and Miller will take primary responsibility for the genomic assessments. Marty will be responsible for the histopathology screening of the smolts. (All PIs will receive samples from the same animals, allowing Miller's genomic approach to be matched with the traditional histopathology techniques). Farrell will be responsible for the SLICE™ experiment. Kintama will take overall responsibility for design and operation of the supplementary acoustic arrays, management of the collected data, and will

be responsible for survival analyses jointly with Carl Schwartz and our post-doctoral fellow, Dr Wendell Challenger.

POST will contribute data from its existing three sub-arrays. A copy of all telemetry data will be placed on the POST public access database as soon as it is collected, and no restrictions will be placed on its use.

## TEFFS Advisory Board

Names released pending approval; members have been drawn from both the industry and NGO community as well as practicing scientists to advise the process and represent all viewpoints.

## Budget

Total Budget, 2012 Pilot: \$2.56M.

Total foundation support requested: \$2.21M, including HST and Pacific Salmon Foundation overhead. This will support all costs of tagging, transport, and holding of smolts; full genomic, physiological, and histopathology screening by Co-PIs of smolts; additional acoustic sub-arrays needed to achieve required statistical power; and analysis and write-up of the results.

We assume POST will separately fund cost of maintaining and operating the existing 3 POST lines at \$350K for 2012. Kintama will fund a pilot deployment of 5-10 disinfected receivers which the aquaculture industry can place on their farms (thereby avoiding biosecurity concerns) from outside staff visiting the farms. Kintama's design should allow certification that these receivers remain operational and in-water for the duration of the 2012 pilot.

Likely full Project (2013-2015): \$3M~\$3.5M/yr, depending upon final scope.

## Acknowledgements

Cover photos courtesy of the BC Salmon Farmers Association and John Day, Kintama.

## Appendix I. Disease Profiling

Disease profiling will be assessed by sampling additional smolts at both Cultus and Chilko lakes, and at weekly intervals after smolts are moved into holding pens near or far from fish farms. Physiological and genomic profiling will identify whether a difference in immune or physiological response in control and fish-farm exposed smolts develops over time, using sampling similar to that used for adult sockeye returning to the Fraser River. We will assess stress and ionoregulatory status from plasma (cortisol, glucose, lactate, Na, K, Cl, osmolality) and gill tissue samples (isoforms of Na/K ATPase). Also, both histopathological and viral (e.g., parvovirus, ISA) samples will be collected for later disease assessments using gill, brain, heart, liver, head kidney, trunk kidney, spleen, intestinal caeca, skin and skeletal muscle, plus blood smears to rule out blood pathogens (two types of viruses are common in blood cells of some Pacific salmon populations). For a subset of acoustic tagged smolts, we may take a small sample of gill tissue to look for viral signatures prior to releasing the fish and tracking their fate. (A decision on this will depend upon our assessment of whether the tissue sampling could compromise survival post-release).

By PIT-tagging the smolts at the lake, it will be possible to relate the genomic and physiological profiles that develop over time in the ocean to data collected on individuals at the time of capture (size, condition factor).

## Appendix II. Statistical Power

Statistical power is the probability of rejecting the null hypothesis that fish farms have no effect on survival when farms really do have an effect. It is, in other words, the probability of observing a reduction in survival if it actually exists. Before conducting any large-scale experiments it is important to understand the ability of the proposed design to answer the scientific question. If an experiment only has a 20% or 30% chance of success, then there is likely little reason to proceed. If, however, there is an 80% chance of success there is good reason to proceed, as this is generally considered to be sufficiently high power by the scientific community.

Within the context of the TEFFS experiment, we are looking for differences in survival between control and fish farm exposed groups. In order to make these power comparisons, we assumed baseline marine survival levels to different sub-array locations that were taken from our prior sockeye tagging work using the POST array.



Specifically, we modeled baseline smolt survival as 60% per 100 kilometers of migration travel. That is, survival  $S$  was defined as

$$S = \zeta^{D/100},$$

where  $\zeta = 0.6$  is a survival rate of 60% per 100 kilometers and  $D$  is the distance in kilometers. For example, from the release point to the NSOG sub-array (Fig. 2) is 125 km, so we can expect survival to be  $S = 0.6^{125/100} = 0.528$ , or 52.8%.

This survival rate was used to predict the survival of control smolts to various sub-array locations, that is  $S_{control} = \zeta^{D/100}$ . For fish farm exposed smolts we assumed that exposure would affect the baseline survival rate by some factor  $c$ , such that  $S_{farm} = (c \cdot \zeta)^{D/100}$ , where the value of  $c$  may vary from 0 to  $1/\zeta$ . For the value of  $c = 1$ , survival rates are identical (the null hypothesis),  $c < 1$  indicates fish farm smolts have a lower survival rate per 100 kilometers and  $c > 1$  indicates a higher survival rate for farm-exposed smolts.

To assess power, we considered six different acoustic array geometries (see Table II.1 and Figure 2) and investigated the statistical power of these designs to detect changes in marine survival using different numbers of tagged smolts. The baseline design is the POST permanent array infrastructure, Design A, and we looked at how much additional power could be obtained by strategic additions to this core. (In the financial analysis, we assumed additional infrastructure would be operated as temporary lines in place only during the May-July smolt migration period, to reduce costs).

Changes in marine survival can be expressed in a number of different ways. It can be expressed in terms of differences in the survival rate of the fish farm exposed group relative to the control group's survival rate (Figure II.1), that is  $\gamma_{rate} = (\zeta - c \cdot \zeta)/\zeta = 1 - c$ . It may also be expressed in terms of absolute differences in survival to a given sub-array (i.e.,  $S_{control} - S_{farm}$ ; Table II.2). Finally, we may also express an effect as a difference in overall survival of fish farm exposed smolts relative to the control smolt survival, calculated as  $\gamma_{survival} = (S_{control} - S_{farm})/S_{control}$  or  $1 - c^{D/100}$ . In terms of overall survival, for a given  $\gamma_{rate}$  we can expect to see a larger value of both the absolute difference ( $S_{control} - S_{farm}$ ) and relative difference  $\gamma_{survival}$  the further along the migration pathway that we measure overall survival, because more time will have elapsed, allowing mortality differences to grow.

Most designs include double lines at the end of the last migration segment to allow direct estimation of survival in the final segment, overcoming the technical issue that survival in the last segment is confounded with detection<sup>5</sup>. Including double array lines at the end of the final migration segment resolves this issue completely.

The first design (A, Table 1) is the current POST array and uses the existing permanent receiver lines at NSOG and QCS. Survival can be measured to NSOG without any simplifying assumptions and to QCS with assumptions. The second design (B) uses the existing line at NSOG1 and adds a second line immediately afterwards. Survival can only be measured to NSOG1 in this case, but no assumptions are required. Both these designs measure survival only to the northern Strait of Georgia region and avoid any possible repeated exposure to fish farm that may occur in Discovery Passage. However, both designs also have the shortcoming that smolts are expected to pass the NSOG line about two weeks after release, which may not be enough time for some diseases affecting survival to be fully expressed. (Smolts would reach NSOG just under three weeks after initial exposure to the fish farms, assuming a one-week holding period and 13 cm long smolts). An encouraging aspect of the original POST array design is that statistical power is considerably higher than would be obtained using the alternate Design B, which would hypothetically have moved all receiver assets in Queen Charlotte Strait into the Strait of Georgia to form a second sub-array;

*Table II.1 - Array configuration for the six proposed designs. A is the existing POST array; in all designs we assume the operation of the JDF sub-array as well, which is not listed.*

Design	NSOG 1	NSOG 2	JS	QCS 1	QCS 2	SEAK 1	SEAK 2
		<i>Considered</i>	<i>Proposed</i>		<i>Proposed</i>	<i>Considered</i>	<i>Considered</i>
A	✓			✓			
B	✓	✓					
C	✓		✓	✓			
D	✓		✓	✓	✓		
E	✓			✓	✓		
F	✓			✓		✓	✓

the existing design of a more geographically widespread infrastructure is clearly superior (in the early design phase for the original POST architecture, a number of

<sup>5</sup>At the last line, if a smolt is not detected we cannot determine whether the smolt died before the passing the line, or was not detected. Two closely spaced sub-arrays, a "double-line", resolves this uncertainty. However, it is also possible to overcome this limitation if mathematical assumptions are made. For example, we may assume treatment and control have identical detection probabilities (a reasonable assumption) or we can model survival as a function of time or distance travelled.

individuals had suggested that B would be superior). In the remaining designs that we considered, we thus look only at strategic seasonal additions to POST, not revisions to the core design.

The next four designs (C, D, E, and F) measure survival to northern Queen Charlotte Strait (beyond the majority of fish farm sites) and to Southeast Alaska (SEAK). In these designs, smolts could potentially be exposed twice to aquaculture, once during the experimental exposure phase and once as smolts migrate through Discovery Passage. The two groups will, however, differ in their level of fish farm exposure because of the first exposure phase and any survival differences should still be attributed to this difference.

Design C (Table II.1) uses the existing NSOG and QCS lines and adds a third line in Johnstone Strait (JS). Survival can be measured to JS without assumptions and to QCS with simplifying assumptions. Smolts are expected to cross the JS and QCS lines approximately four and five weeks after release. Design D (Table II.1) adds one extra sub-array after QCS allowing us to estimate survival from release to the current QCS line without any assumptions, allowing approximately six weeks for diseases to be expressed after the initial fish farm exposure. Design E (Table II.1) is similar to design D, except it excludes the intermediate JS line that occurs between the existing NSOG and QCS lines. The final design (F) again uses the existing NSOG and QCS sub-arrays, but places a final double line in Southeast Alaska. This design allows us to track smolts for almost fourteen weeks after release and potentially allow us to assess how survival may change over a 945 km long migration route.

To assess these designs, we calculated the smallest difference in survival between control and fish farm exposed groups expected to be measurable to each sub-array location with 80% statistical power, for differing numbers of tag releases (Table II.2 and Figure II.1). The worst array design (B) required about twice as many acoustic tags to measure a given survival difference as the best<sup>6</sup>. Designs covering large geographic distances could measure smaller differences in survival by the last measurable point in the design. However, the design that covered the largest geographic distance (F) also required larger number of tags to detect survival

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<sup>6</sup> *Beyond the purely economic cost of tags, there are important ethical issues concerning animal care, which stipulate that the use of animals in research studies be minimized when possible, and practical issues involved in collecting and competently tagging large numbers of migrating wild smolts in a short time period. Both issues dictate putting more money into monitoring the smolts well, rather than the brute force approach of using large numbers of (potentially poorly) tagged smolts.*

differences to the outer end of the array design due to the expected mortality before reaching Alaska.

In order to conduct the power analysis we had to predict marine survival to different sub-array locations, including Alaska. This was accomplished by modeling baseline smolt survival as 60% per 100 kilometers of migration travel, a value found in our sockeye research. In addition to investigating power in regards to measuring difference in survival, we also considered the ability of each design to detect differences in survival rate between the control and treatment groups (Figure 3). Generally, all designs performed similarly, with higher tag releases allowing detection of smaller differences in survival rate. The main difference between the proposed designs is the length of time that changes in survival rate may be detected, ranging from just under two weeks (A and B), to almost 14 weeks (E). All else being equal, Array design D will provide the most insight.

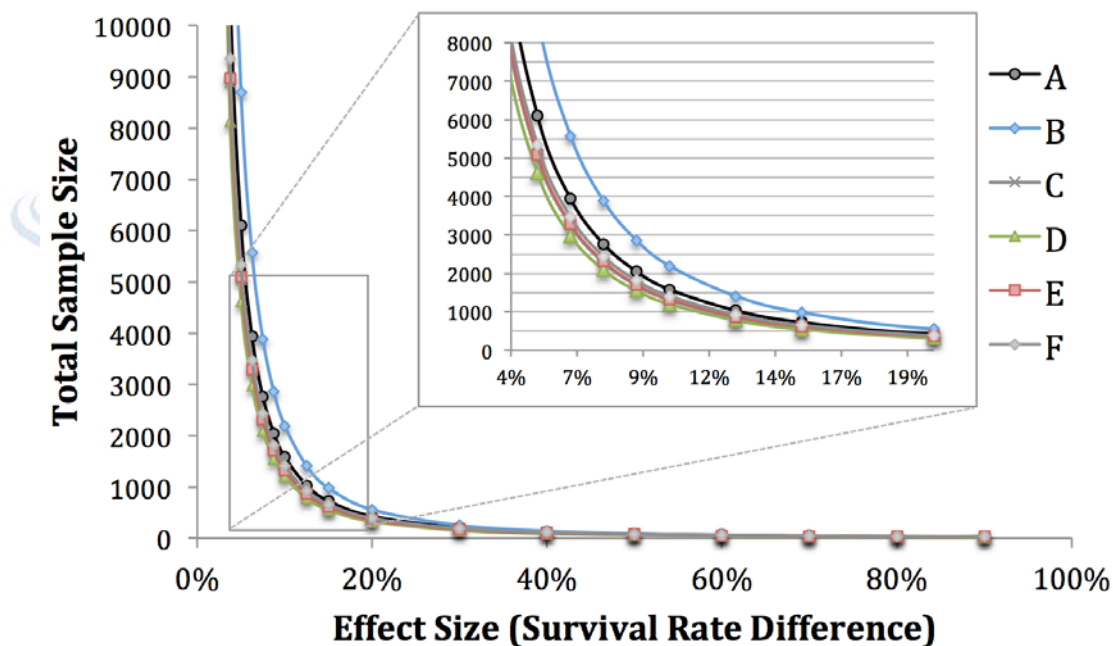


Figure II.1 - Total tag number required to identify a given percent difference in survival rate (effect size), with 80% power, for the six proposed array designs considered. Total tag releases are equally divided between control and treatment groups. The inset shows an expanded view of the area outlined on the main graph. Design A is the core POST array (see Table II.1); Design D would augment the core POST lines with additional temporary lines in Johnstone Strait and QCS 2, and has the highest power. The 2012 pilot study should have high power to identify a survival rate difference of 15% or greater using 500 tags.



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**Table II.2 - Smallest difference in overall survival between control and treatment groups that can be detected with 80% power for a given array design and number of tags released. Tag releases are equally divided between control and treatment groups. Blank values indicate too few smolts expected to reach that point in the array to reliably calculate a percent difference.**

		Design A		Design B	Design C			Design D			Design E		Design F		
Array Lines Used		NSOG	QCS 1	NSOG 1,2	NSOG	JS	QCS 1	NSOG	JS	QCS 1,2	NSOG	QCS 1,2	NSOG	QCS	SEAK 1,2
Base Survivorship		52.8%	15.1%	52.8%	52.8%	25.8%	15.1%	52.8%	25.8%	15.1%	52.8%	15.1%	52.8%	15.1%	0.8%
Distance from Release		125 km	370 km	125 km	125 km	265 km	370 km	125 km	265 km	370 km	125 km	370 km	125 km	370 km	945 km
Tags Released (N)	100	27.7%	13.4% <sup>1</sup>	29.0%	24.7%	19.1%	12.8% <sup>1</sup>	23.8%	18.6%	12.6%	25.7%	13.0%	26.8%	13.2%	-
	250	17.0%	10.3% <sup>1</sup>	18.9%	15.2%	13.3%	9.6% <sup>1</sup>	14.7%	12.9%	9.3%	15.6%	9.8%	16.3%	10.0%	-
	500	11.8%	8.0% <sup>1</sup>	13.5%	10.6%	9.8%	7.3% <sup>1</sup>	10.2%	9.4%	7.1%	10.8%	7.4%	11.2%	7.7%	-
	750	9.6%	6.7% <sup>1</sup>	11.1%	8.6%	8.1%	6.2% <sup>1</sup>	8.3%	7.8%	6.0%	8.8%	6.3%	9.1%	6.5%	0.6%
	1000	8.2%	6.0% <sup>1</sup>	9.6%	7.5%	7.1%	5.5% <sup>1</sup>	7.1%	6.8%	5.3%	7.5%	5.5%	7.8%	5.7%	0.6%
	2500	5.2%	4.0% <sup>1</sup>	6.1%	4.7%	4.6%	3.6% <sup>1</sup>	3.1%	4.4%	3.5%	4.7%	3.7%	4.8%	3.7%	0.4%

<sup>1</sup> Assumptions on detection or survival are required to estimate survival difference over the final segment of the array.

It should be noted that by repeating the study design over several years, and amalgamating across years, the final test can be made much more powerful than in any one year. Adding the total number of tags across years allows us to approximate the statistical power. From Figure II.1, tagging 1,500 smolts per year should provide sufficient statistical power to distinguish differences in the survival rate as small as 6% and 4% after 3 and 5 years of study respectively. To place these survival differences in context, small wild Chilko sockeye smolts took 5 weeks (35 d) to migrate from the Fraser River mouth to the Queen Charlotte Strait sub-array in 2011, a total distance of 370 kilometers. We would expect  $S_{control} = 0.6^{3.7} = 0.151$  (15.1%) of released smolts to survive to the Queen Charlotte Strait sub-array. If fish farms reduce the survival rate by 5% ( $\gamma_{rate} = 0.05$ ) then  $c = 0.95$  and we would expect fish farm smolts to exhibit  $S_{farm} = 0.125$  (12.5%) survival to Queen Charlotte Strait. We can express this in terms of a 2.6% difference in overall survival (see Table II.2) or as change in overall survival relative to control smolts of  $\gamma_{survival} = 0.173$  (17.3%). This difference should be identifiable with high statistical power in a 3 or 5 year study using 1,500 tags per year. Figure 4 in the main text shows that in 4 of 5 previous years of study, the survival rate per 100 km of travel in the Discovery Passage region was clearly reduced by about 17% ( $\gamma_{rate} = 0.17$ ) relative to the Strait of Georgia “control” rate. (In 2006, relative survival was reversed, and survival was better in the Discovery Passage region). In terms of survival rate per week of travel, again excluding 2006, this effect was even larger ( $\gamma_{rate} = 0.38$ ). So if the fish farms are causing the observed difference in survival rates seen between the Strait of Georgia and Discovery Passage regions, it should be measurable.

The levels of fish farm-related reduction in overall survival that are potentially measurable by the time the smolts reach Queen Charlotte Strait are relatively minor when compared to BC’s commercial sockeye fishery, which induced a ~70% harvest (mortality) of the adults prior to the 1990s. Although the level of harm deemed unacceptable and ultimately requiring governmental regulation is a political decision, we believe that the statistical power of the design we have identified is high enough that by the end of 3~5 years a clear decision can be made about whether fish farm impacts are unacceptably large and the industry should be regulated to minimize interaction with wild stocks.

## Appendix III. Pilot Phase Target Smolt Numbers

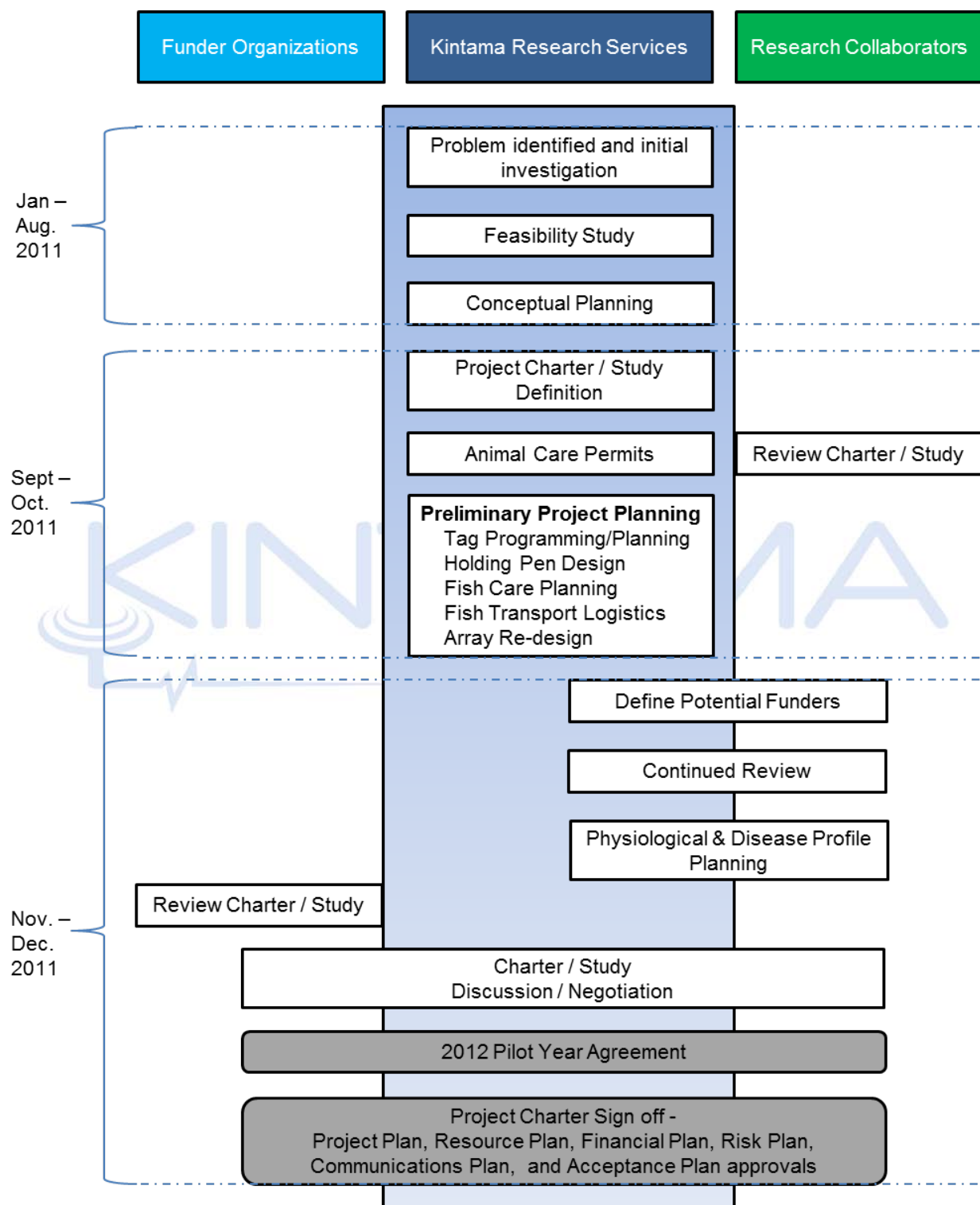
We designed the pilot phase around the goal of having 20 treatment and 20 control group smolts detected on the Queen Charlotte Strait sub-array. Based on our previous sockeye work in Cultus (2004-07) and Chilko lakes (2010-11), survival from the Fraser River mouth to the Queen Charlotte Strait sub-array is ca. 15% (2010:13%; 2011:17%). The number of fish released has to be further increased to compensate for the 30% chance that the V7 tags will not be detected by the sub-array. Thus, we will need to release ca. 500 V7 tagged smolts in order to meet our goal.

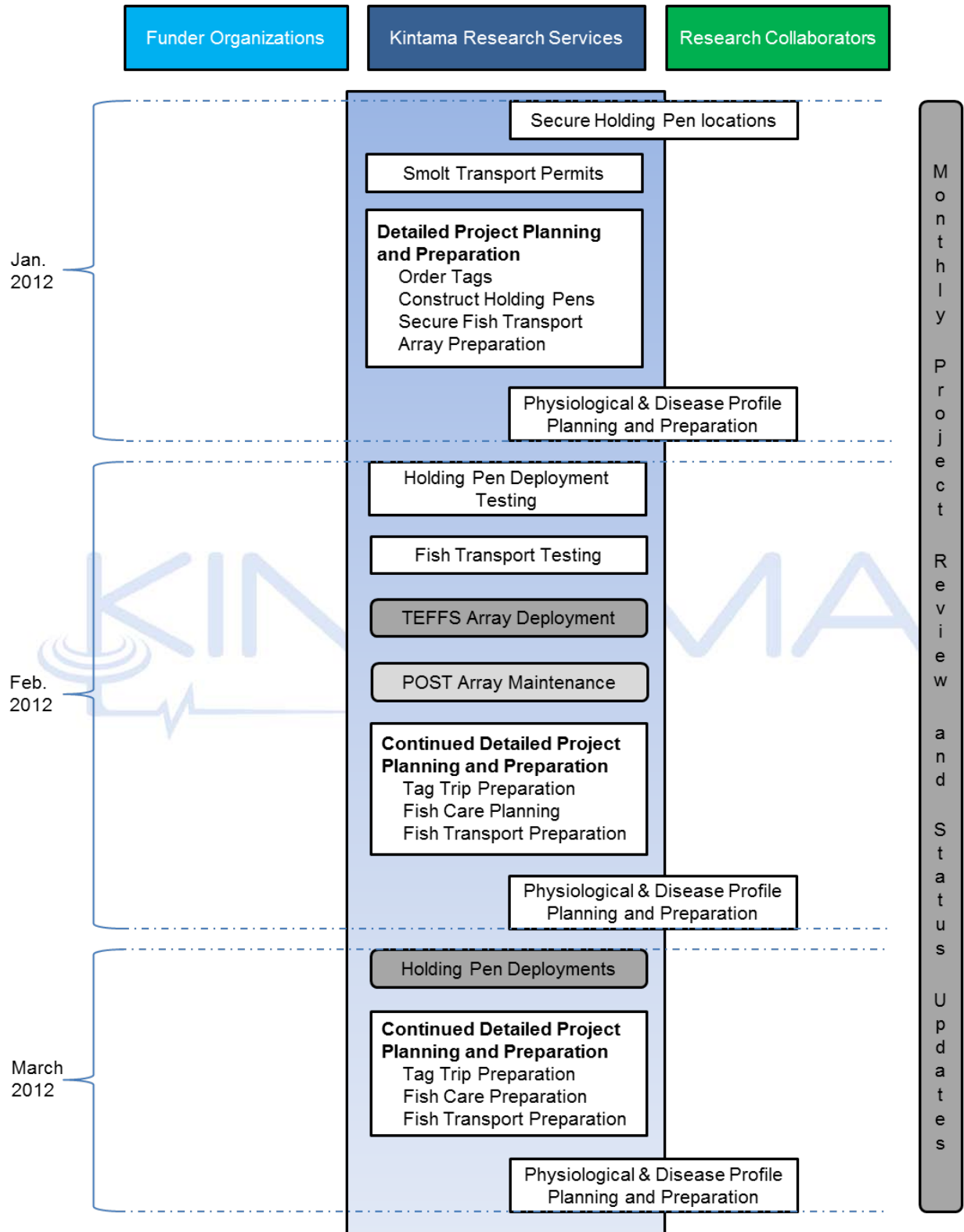
If logistically feasible, we will hold 50 tagged smolts at each of 10 replicate treatment sites (5 fish farm and 5 control sites in inlets lacking fish farms), so that we can estimate the variability expected among treatment groups. These data can then refine the design for the full-scale project.

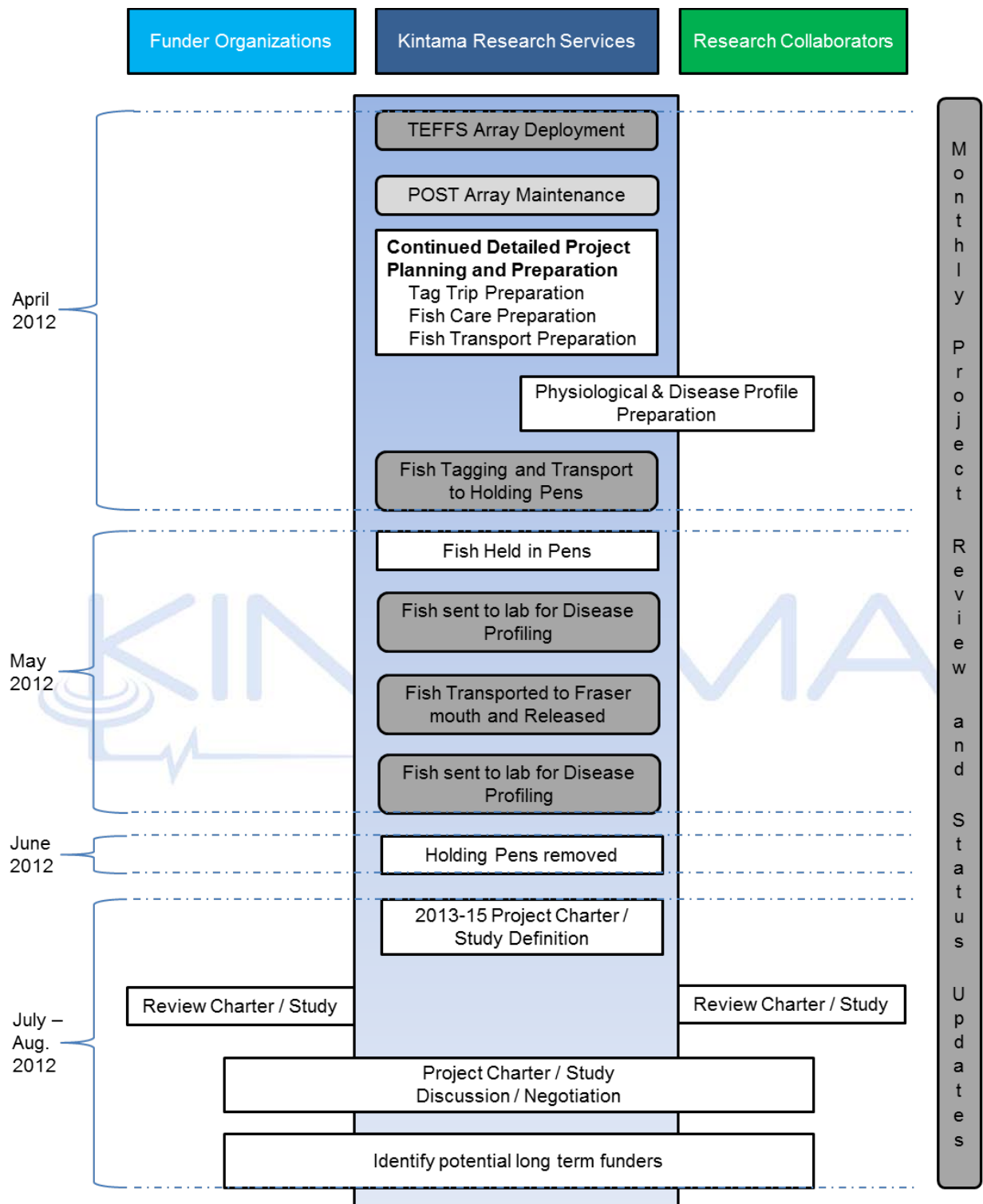
To further ground truth the results from the experimental study, we can compare the post-release marine survival of these smolts to the marine survival from freely migrating smolts released at the Lake. Taking Chilko as the example, 2011 survival to the Fraser River mouth was 33% and from the river mouth to QCS was 15% (5% overall). To have 20 free-migrating smolts detected at QCS would thus require 400 smolts released at the lake. Transporting the tagged smolts below Chilko River before release to avoid an area of high mortality, as was done in 2011, should approximately double survival to Queen Charlotte Strait.

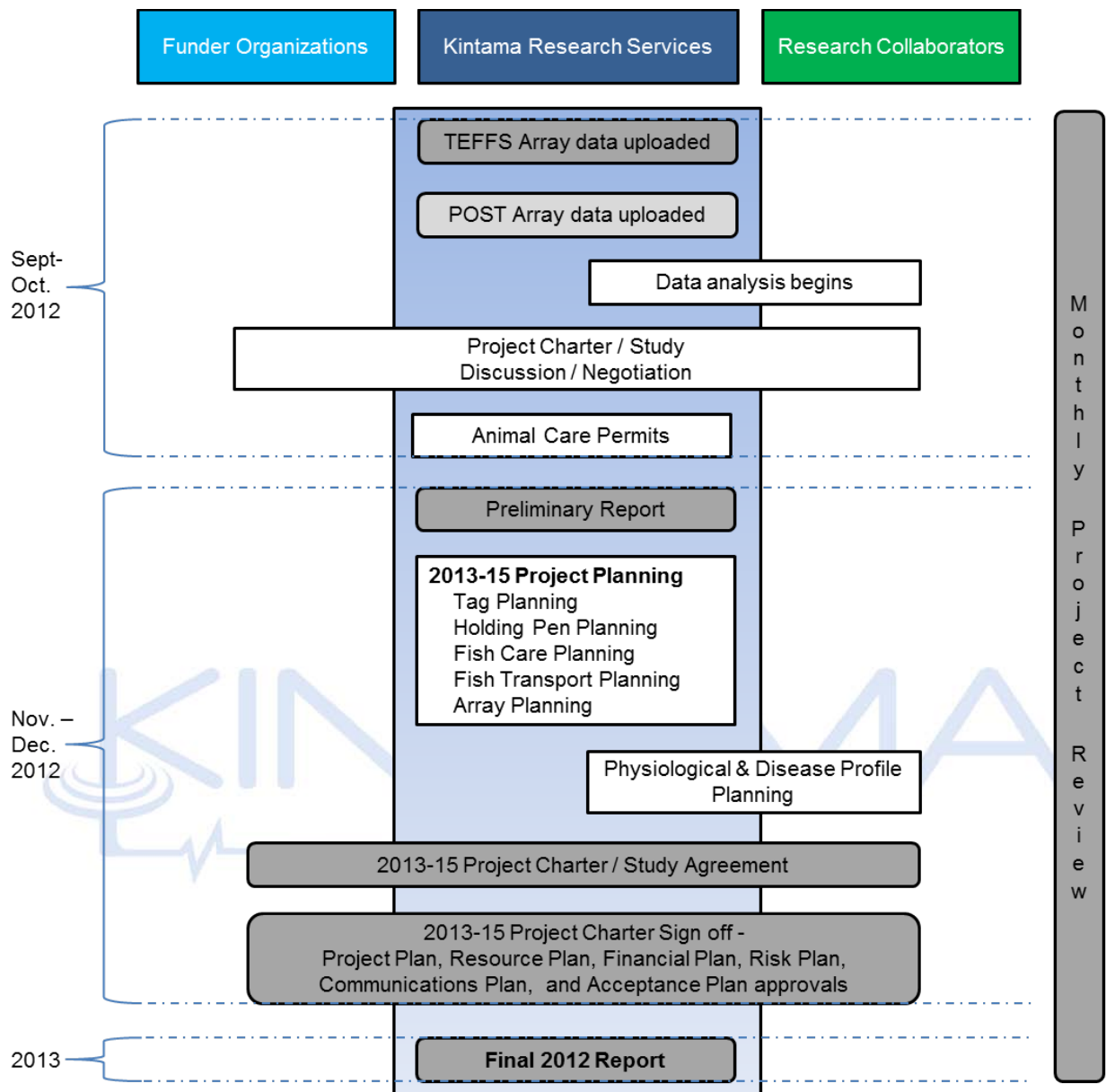


## Appendix IV. 2012 Operations Timeline.



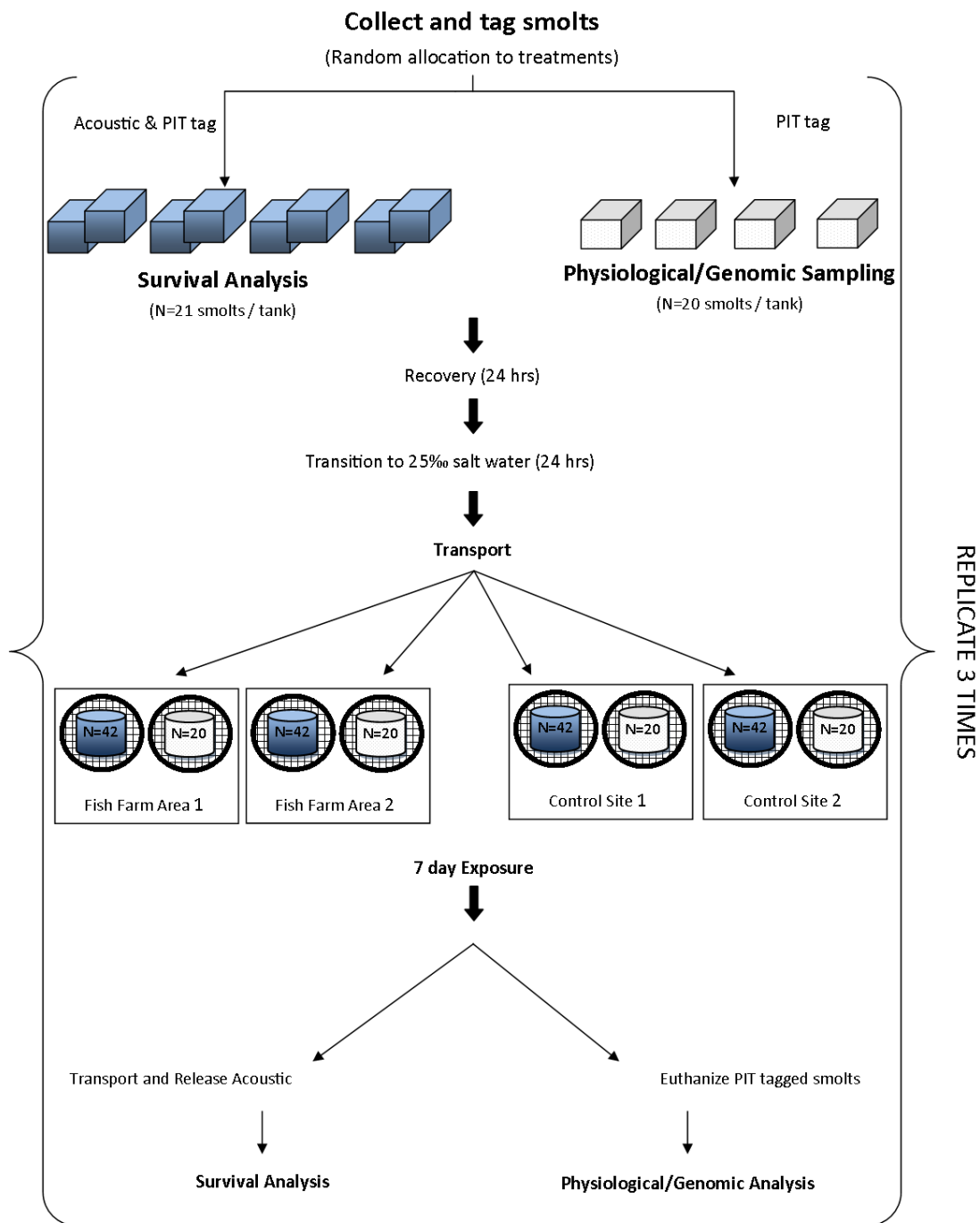








## Appendix V. Smolt Handling Flowchart for paired release groups.



## Appendix VI. TEFFS Fish Handling SOP

### Tagging logistics and major milestones

- 28 Feb 2012 - secure off-label prescription for Metomidate;
- 15 March 2012 - Secure Animal Care approval from Vancouver Island University;
- 15 March 2012 - Secure collection permits for migrating smolts from Fisheries and Oceans;
- 15 March 2012 - Secure transfer permits for migrating smolts from Fisheries and Oceans;
- 30 March 2012 - Confirm if additional collection permits are necessary for fish to be used for diagnostics;
- 20 March 2012 - Confirm all tagging staff have completed surgical refresher and fish handling courses;
- 01 April 2012 - Confirm tagging location, facilities available, and number staff allowed on site.

### Tagging locations

A total of N=500 double tagged smolts (each with an acoustic transmitter and PIT tag) and N=240 PIT tagged smolts (to be sacrificed for physiological/genomic sampling after exposure) will be used in 2012 pilot study. If possible, smolt tagging will occur at the two locations for which we have prior acoustic tagging experience, Cultus Lake (2004-2007) and Chilko Lake (2010-2011). In addition, approximately 100 untagged smolts will be collected and sacrificed for baseline disease measurements.

#### Chilko Lake

- Collect smolts at Chilko Lake outlet traps; fence operational mid April to late May;
- Will tag smolts greater than 125mm over the entire run time.

#### Cultus Lake

- If smolts from this COSEWIC listed stock are made available, half the total tagged smolts will be sourced from this stock. No physiological samples will be taken from this stock, as it would require euthanizing the smolts.

### Collection and handling

- Smolts will be collected from the weir at the outlet from the lakes and dip-netted into a sorting tank;
- Collected smolts will be sorted to identify the required number of smolts >125mm;
- Smolts will be held in a flow through net pen within the lake until sufficient numbers for tagging are available.

### Tagging of treatment and control groups

- Smolts will be removed from the flow through net pen and implanted with both an acoustic and PIT tag as per Kintama's published tagging SOP (Appendix A). These smolts will be referred to as "acoustic tagged" smolts;
- Acoustic tagged smolts will be allocated to transport groups as per the randomization protocol below;
- Concurrently, a subset of untagged smolts will be sacrificed for physiological/genomic sampling.

### Tagging of disease profiling groups

- Smolts for physiological/genomic sampling will be removed from the flow through net pen and implanted with a single PIT tag as per Kintama's published tagging SOP (Appendix A). These smolts will be referred to as "PIT tagged" smolts;
- The full size range of smolts will be PIT tagged (PIT tags can be used on all smolts >65mm);

### Randomization

- Acoustic tagging will be done to achieve three experimental replicates of farm exposed and control groups for transportation by float plane;
- Following surgical implantation, acoustic tagged smolts will be successively allocated to groups of N=21 smolts per transport tote;
- Individual transport totes of smolts will be randomly allocated either to a treatment or a control site destination.
- Smolt treatment (i.e., fish farm exposed or control site) will not be revealed to the analysts until after survival analysis is complete;

### Holding post tagging and transition from fresh water to sea water

- All acoustic and PIT tagged fish will be held in aerated transport totes for 24 hours after tagging before altering water salinity;
- The acoustic and PIT tagged fish held in transport totes will then be transitioned to 25ppt salt water over 24 hours;
- Salinity will be increased by adding 6g of Instant Ocean sea salt per hour to each holding tote;
- After the acoustic and PIT tagged fish have recovered, they will be fed a mixture of chopped krill and pellet food frozen in cubes once a day until release;
- Transport totes will be periodically monitored and cleaned;
- Transport totes will be the same or similar to the Hauling tank (HT6) on page 254 of the 2008 Aquatic Eco-systems Inc catalog;
- Transport totes will be lined with large heavy duty clear plastic bags, which will reduce over handling, and simplify transfer into net pens and equilibrate water temperatures.

### Transport in float plane

- Transportation by float plane will consist of 8 groups of 21 acoustic tagged smolts destined for either a fish farm (N=84) or control site (N=84); and 4 groups of 20 PIT tagged smolts for physiological/genomic analysis, treated similarly.
- Transport totes will be aerated and monitored during the flights;
- Upon arrival, each transport tote will be assigned to control or fish farm sites according to a pre-determined randomized allocation.

### Floating net pens

- Floating net pens holding acoustic and PIT tagged smolts will have an outer predator exclusion cage;
  - Floating net pens will be constructed of ¼ inch knotless mesh and will be the same or similar to the Fish Cage Kit (C2) on page 209 of the 2008 Aquatic Eco-systems Inc catalog;
  - Floating net pens will have:
    - Flotation;
    - A secure top and bottom lid that will allow feeding fish;
    - The ability to open from the top and bottom (to facilitate fish transfer between totes);
    - Rigid sides;
- Outer predatory cage will be:
- Assembled at each location;
  - One predator pen per floating net pen;
  - Constructed of ¼ knotless mesh inside wire mesh enclosure;
  - Top will be hinged and raised above the water to exclude birds and mink.

### Treatment areas

#### 1) Fish farm sites:

- Will be chosen to have easy access via boat or plane with good water circulation and shelter from waves;
- Smolts may be held at either a fish farm site (if permission is obtained) or at a "friendly dock" near a farm site (if permission is refused);
- Two transport totes of acoustic tagged smolts (N=21) will be combined into a single floating net pen, providing 2 replicates of approximately N=42 acoustic tagged smolts at each fish farm exposure area (ideally, geographically distinct for each replicate);
- PIT tagged smolts to be sacrificed for physiological/genomic sampling will be handled identically to the acoustically smolts.

#### 2) Control area:

- Will ideally have easy access via boat or plane with good circulation and shelter from waves;

- Control areas will be distant from fish farms, possibly in fjords (e.g., Bute or Kingcome Inlet);
- Two transport totes of acoustic tagged smolts will be combined into a single floating net pens, providing 2 replicates of approximately N=42 acoustic tagged smolts at each control site (ideally, geographically distinct for each replicate);
- PIT tagged smolts will be handled identically to the acoustic tagged smolts prior to being sacrificed for physiological/genomic sampling.

#### Transfer from water to boat

- After 7 days exposure, staff will collect and transport smolts from each treatment area to the release location in the Strait of Georgia north of the Fraser River using a seine vessel;
- Each net pen holding approximately N=42 smolts will be lifted out of the water into a large transport tank using a lift bag to keep them continuously in water;
- After collection, boat will deliver all smolt groups to the release site, holding each group separately to prevent cross contamination.

#### At release from boat

- Treatment groups will be released after dark, randomly alternating exposure and control groups;
- At time of release, smolts will be enumerated for PIT tags (to measure surviving smolt numbers released) and videotaped to record physical condition (lesions), healing of incisions, and parasite load (prevalence and intensity).

#### Genomic and physiological samples

- Weekly samples of smolts will be collected at each treatment location to observe potential disease responses;
- Diagnostics staff will euthanize the PIT tagged smolts, remove tissues and freeze samples for future analysis (following Miller/Hinch/Farrell/Marty protocols; these protocols have been reviewed by Fisheries and Oceans and University of British Columbia Animal Care Review Committees and are not part of Kintama's protocol).



## Appendix VII. References

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## Appendix VIII. Letters of Support

See attached.

1. Dr Stephen Cooke, Canada Research Chair in Fish Ecology & Conservation Physiology, Carleton University
2. Prof. Paul H. LeBlond, Ph.D., FRSC, Prof. Emeritus, Dept. Earth & Ocean Sciences, University of British Columbia
3. Prof. Tim Parsons, Ph.D., FRSC, OC, Prof. Emeritus, Dept. Earth & Ocean Sciences, University of British Columbia
4. Prof. L. Neil Frazer, Professor of Geophysics, School of Ocean and Earth Science and Technology, University of Hawaii at Mānoa
5. Dr Steve Strand, Dept. of Life Sciences, UCLA (Retired)
6. Dr Stephen Vincent, Vice-President, Seymour Salmonid Society, Vancouver, BC
7. Dr. Fred Whoriskey, Executive Director, Ocean Tracking Network, Dalhousie University, Halifax, N.S.
8. John Werring, M.Sc., Aquatic Habitat Specialist, Marine & Freshwater Conservation Program, David Suzuki Foundation, Vancouver, B.C.
9. Paris Gaudet, Executive Director, MISTIC (Mid-Island Science, Technology & Innovation Council), Nanaimo, BC
10. Stan Proboszcz, M.Sc., Watershed Watch Salmon Society, Coquitlam, BC
11. Prof. John Reynolds, Tom Buell BC Leadership Chair in Salmon Conservation & Management, Simon Fraser University, Vancouver, BC
12. Prof. Larry Dill, FRSC., Prof. Emeritus, Dept. of Biological Sciences, Simon Fraser University, Vancouver, BC
13. Oba Harding, Director, Business Development, MITACS, Vancouver, B.C.