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

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**DRAFT**

# Overview

TEFFS is a large-scale 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; and
(C) Develop disease & genomic profiles on smolts that are or are not exposed to fish farms. (A pathological assessment of each smolt has not been budgeted for, but would be a desirable addition).
(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.

# A. Experimental Design

The core component is a direct test of the effect of fish farm exposure on the survival of wild Fraser River sockeye. This component will be done by contrasting the survival of groups of free-ranging smolts that were first held either 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).

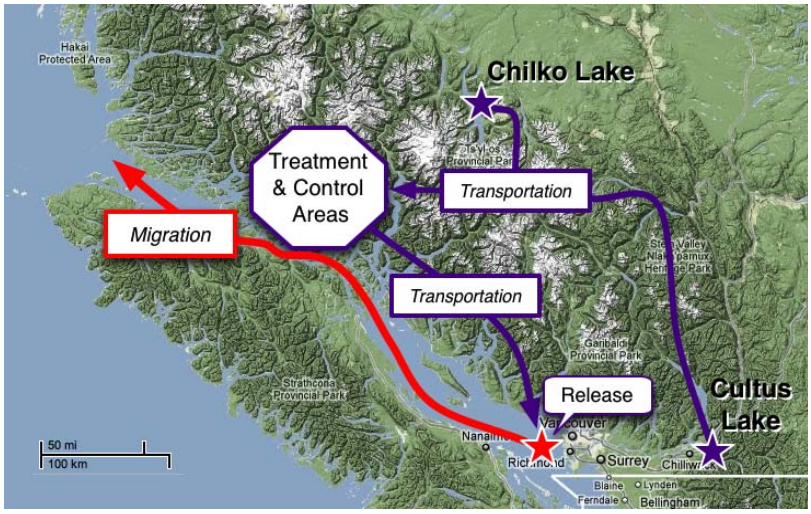



*Figure 1. Overview of the TEFFS manipulative experiment. Arrows show the movement of fish from the source populations.*

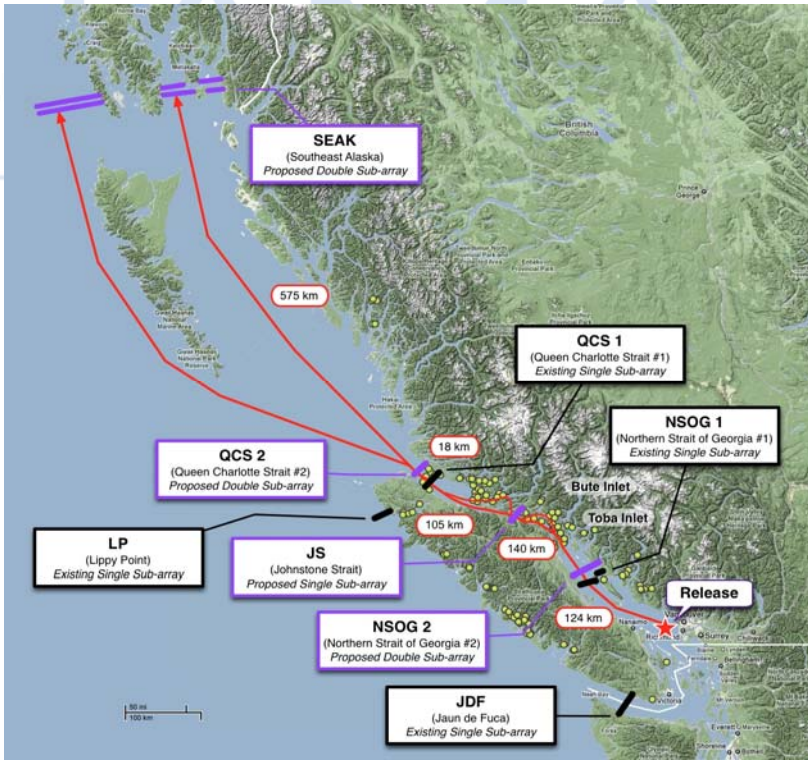



*Figure 2. Overview of possible acoustic sub-array configurations discussed in the Appendix on array design; black lines indicate existing POST sub-arrays, purple lines indicate additional proposed locations for sub-arrays, and yellow dots indicate existing fish farm tenures.*

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 as they migrate through Johnstone Strait. 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 a modified version of the original POST acoustic array (Figure 2) as the smolts migrate out of the area.

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 will still arise. For example, is the exposure level used in the study representative of the exposure actually experienced by wild smolts?

To address this, 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 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.

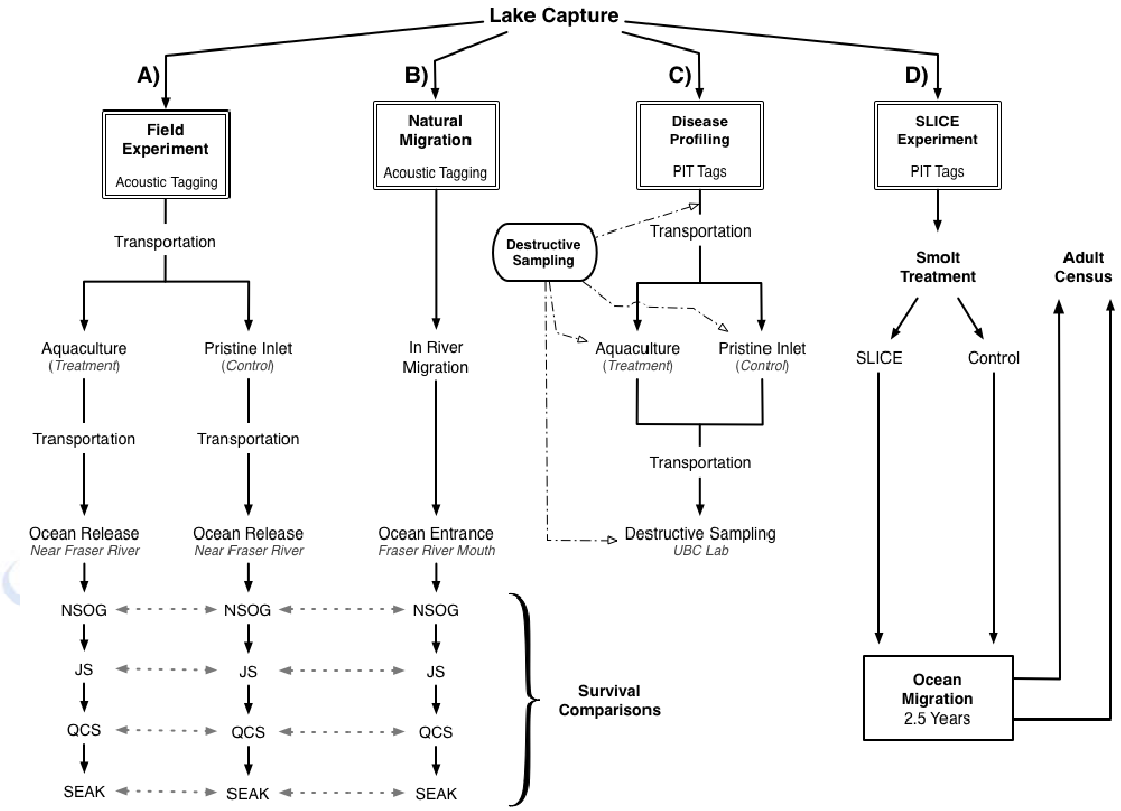



*Figure 3. Handling and treatment of smolts from the overall experiment. Columns A & B represent acoustic tag- based experiments while C represents smolts held for disease profiling. Column D represents a direct experiment to measure whether treating smolts with SLICE™,* **a sea lice prophylactic, improves 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). 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. A detailed pathological examination of some smolts (following the disease monitoring protocol at farm sites) would also be desirable; we have not budgeted for this as yet in this proposal but smolts will be collected and retained for potential future use.

The 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 1~2 months after exposure (the study period). If disease transfer occurs but takes longer to develop, these studies will not detect them. Sea lice have been frequently identified as a potential 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. (The Jackson et al. 2011 study on Irish Atlantic salmon found that SLICE™ had very little effect on ocean survival).

# Pilot Study

While the general concept of TEFFS is straightforward, some components involve novel procedures. We are proposing 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. All major elements of this study have previously been successfully carried out by the proponents, but not 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.

*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$^{rd}$ 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 a re-designed 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 results do not apply to smaller individuals. This would provide both the numbers needed for the experiment and also expand the sample population to include smolts as small as 95 mm, much smaller than smolts we have previously tagged (≥125 mm).

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 5 of 6 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 possible that the tag's output signal may weaken with 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 modified array geometry we have outlined will allow disentangling these complex factors and our analysis (Appendix II) indicates that given the difference in survival rates apparent in Fig. 4 we should have high statistical power to resolve the effect of fish farms if they are the cause of the observed lower survival rates in the northern area.

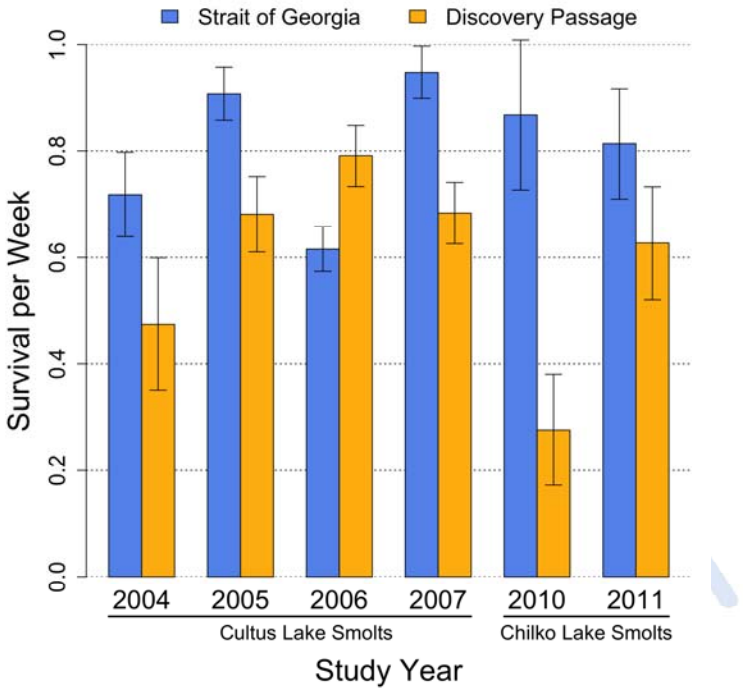



*Figure 4. Sockeye survival in the Strait of Georgia (blue) and in Discovery Passage/Queen Charlotte Strait (gold). 2004-2007 survival data is from (Welch et al. 2009; Welch et al. 2011); 2010 & 2011 results are for 2-year old wild Chilko Lake sockeye smolts, which are substantially smaller (unpublished). 2011 Chilko data is preliminary, as not all data from the NSOG sub-array has been recovered. In 5 of 6 study years, survival rates were lower in the northern area. Error bars are ±1 SE.*

*Timelines*

A timeline for work elements is presented in Appendix IV.

Limitations

*i)* This experimental design is initially focused on measuring potential short term acute disease expression, where a sudden and rapid onset of disease 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).

*ii)* If the early experimental results indicate no difference in mortality for farm-exposed smolts, then we would switch to using the more sophisticated tag programming and measure survival to adult return. However, these tags are larger, and will require using larger smolts (≥17 cm). Depending upon availability, either large hatchery-reared sockeye smolts or steelhead would be of appropriate size. Although the primary focus of the fish farm debate has shifted to sockeye, other species of salmon (including steelhead) have also undergone similar dramatic declines in marine survival since 1990. Steelhead would be an appropriate surrogate in the event that hatchery-reared sockeye of appropriate size were unavailable.

*iii)* During development of this proposal, it has been suggested that the control smolts will be exposed to fish farms during their migration after release and are therefore not "true" controls. In our assessment, this risk is low because: (a) if the control fish undergo rapid mortality soon after exposure to fish farms, then the treatment groups will have manifest this same mortality by the time they reach the NSOG sub-array, ca. 3 weeks after initial exposure to the farms (and before the control groups will be exposed); (b) In practice, migrating smolts are potentially subjected to repeated periods of exposure to multiple fish farms as they migrate through the Discovery Passage/Queen Charlotte Strait region; however, their exposure history is uncertain. Without data of the type collected in this study, a more refined understanding is not possible. The siting of additional receivers at each of the fish farms has a good likelihood of allowing us to reconstruct the prior exposure history of each smolt to the farms, and allow us to assess whether higher farm exposure during migration reduces survival. This approach should also be extensible to the control groups.

# 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). *PLoS Biology* 8(6); http://www.plosbiology.org/article/info%3Adoi%2F10.1371%2Fjournal.pbio.1000412).

# Participants

Drs Scott Hinch (UBC), Tony Farrell (UBC), Kristi Miller (DFO), Carl Schwartz (SFU), 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. Farrell will be responsible for the SLICE™ experiment. Kintama will take overall responsibility for design and operation of the revised array, management of the collected data, and will be responsible for survival analyses jointly with Carl Schwartz and our post-doctoral fellow, Dr Wendell Challenger. A copy of all telemetry data will be submitted to the POST public access database and will have no restrictions 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

Likely Budget: Pilot: $2.6M; Full Project: $3M~$3.5M/yr, depending upon scope (includes cost of running existing POST lines as well as additional acoustic lines needed to increase statistical power).

# Acknowledgements

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) disease assessments will be made using gill, kidney, liver, heart, and brain tissue. 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. 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 five 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. 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[1]. Including double array lines at the end of the final migration segment resolves this issue completely.

[1]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.

The first design (A, Table 1) is the current POST configuration and uses a receiver line 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).

*Table II.1 – Array configuration for the four proposed designs.*

| Design | NSOG 1 | NSOG 2 *Proposed* | JS *Proposed* | QCS 1 | QCS 2 *Proposed* | SEAK 1 *Proposed* | SEAK 2 *Proposed* |
|---|---|---|---|---|---|---|---|
| **A** | ✓ | | | ✓ | | | |
| **B** | ✓ | ✓ | | | | | |
| **C** | ✓ | | ✓ | ✓ | | | |
| **D** | ✓ | | | ✓ | ✓ | | |
| **E** | ✓ | | | ✓ | | ✓ | ✓ |

The next three designs (C, D, and E) 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 can still be attributed to this.

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) uses the existing NSOG and QCS lines and places 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. The final design (E) 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. Finally, it should be noted that in all three designs survival can be assessed just to NSOG, in case there are concerns over secondary fish farm exposure.

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. 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 (E) also required larger number of tags to detect survival 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 (D). All else being equal, Array design C will provide the most insight.

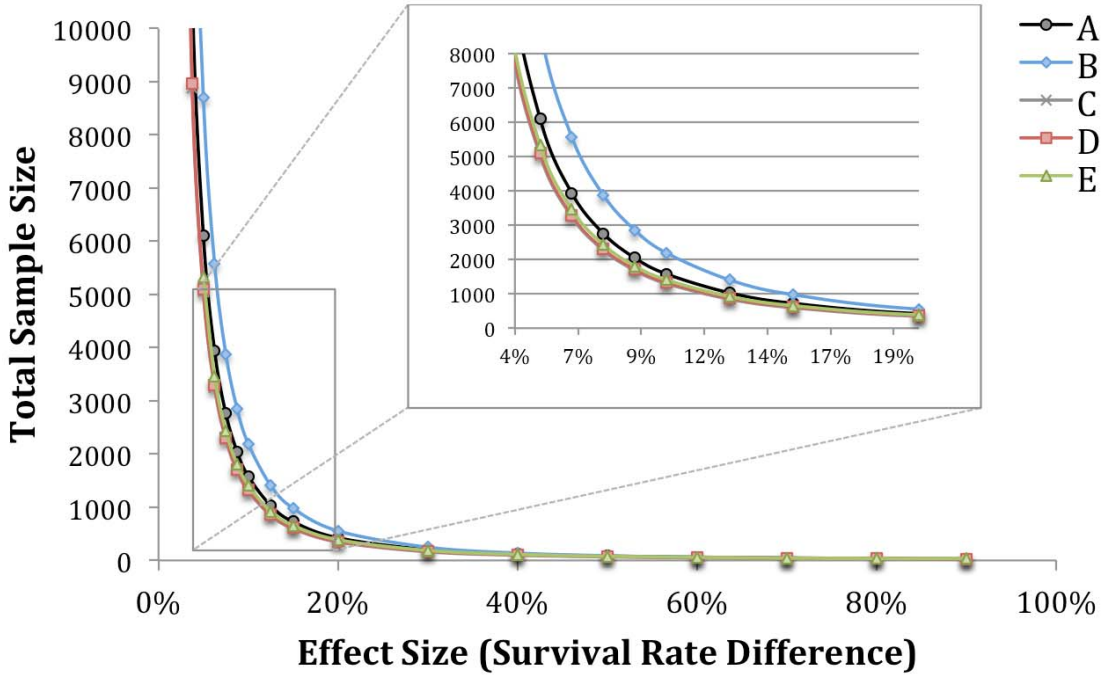



*Figure II.1 – Total tag number required to identify a given percent difference in survival rate (effect size), with 80% power, for the five proposed array designs. Total tag releases are equally divided between control and treatment groups. The inset shows an expanded view of the area outlined on the main graph.*

*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 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Array Lines Used | | NSOG | QCS | NSOG 1,2 | NSOG | JS | QCS 1 | 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% | 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 | 370 km | 125 km | 370 km | 945 km |
| Tags Released (N) | 100 | 27.7% | 13.4%[1] | 29.0% | 24.7% | 19.1% | 12.8%[1] | 25.7% | 13.0% | 26.8% | 13.2% | - |
| | 250 | 17.0% | 10.3%[1] | 18.9% | 15.2% | 13.3% | 9.6%[1] | 15.6% | 9.8% | 16.3% | 10.0% | - |
| | 500 | 11.8% | 8.0%[1] | 13.5% | 10.6% | 9.8% | 7.3%[1] | 10.8% | 7.4% | 11.2% | 7.7% | - |
| | 750 | 9.6% | 6.7%[1] | 11.1% | 8.6% | 8.1% | 6.2%[1] | 8.8% | 6.3% | 9.1% | 6.5% | 0.6% |
| | 1000 | 8.2% | 6.0%[1] | 9.6% | 7.5% | 7.1% | 5.5%[1] | 7.5% | 5.5% | 7.8% | 5.7% | 0.6% |
| | 2500 | 5.2% | 4.0%[1] | 6.1% | 4.7% | 4.6% | 3.6%[1] | 4.7% | 3.7% | 4.8% | 3.7% | 0.4% |

[1]*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.

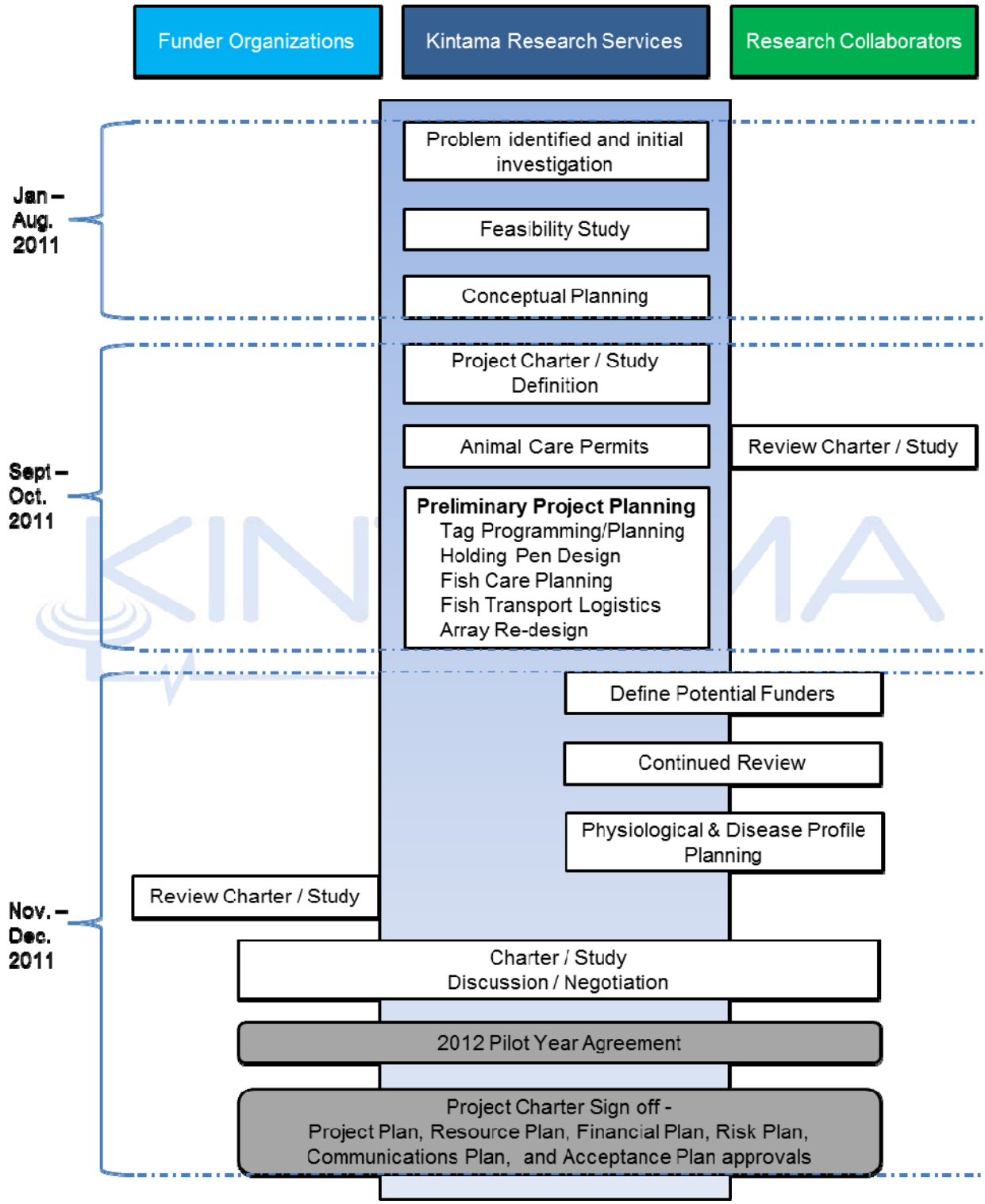
# Appendix III. Pilot Phase Target Smolt Numbers

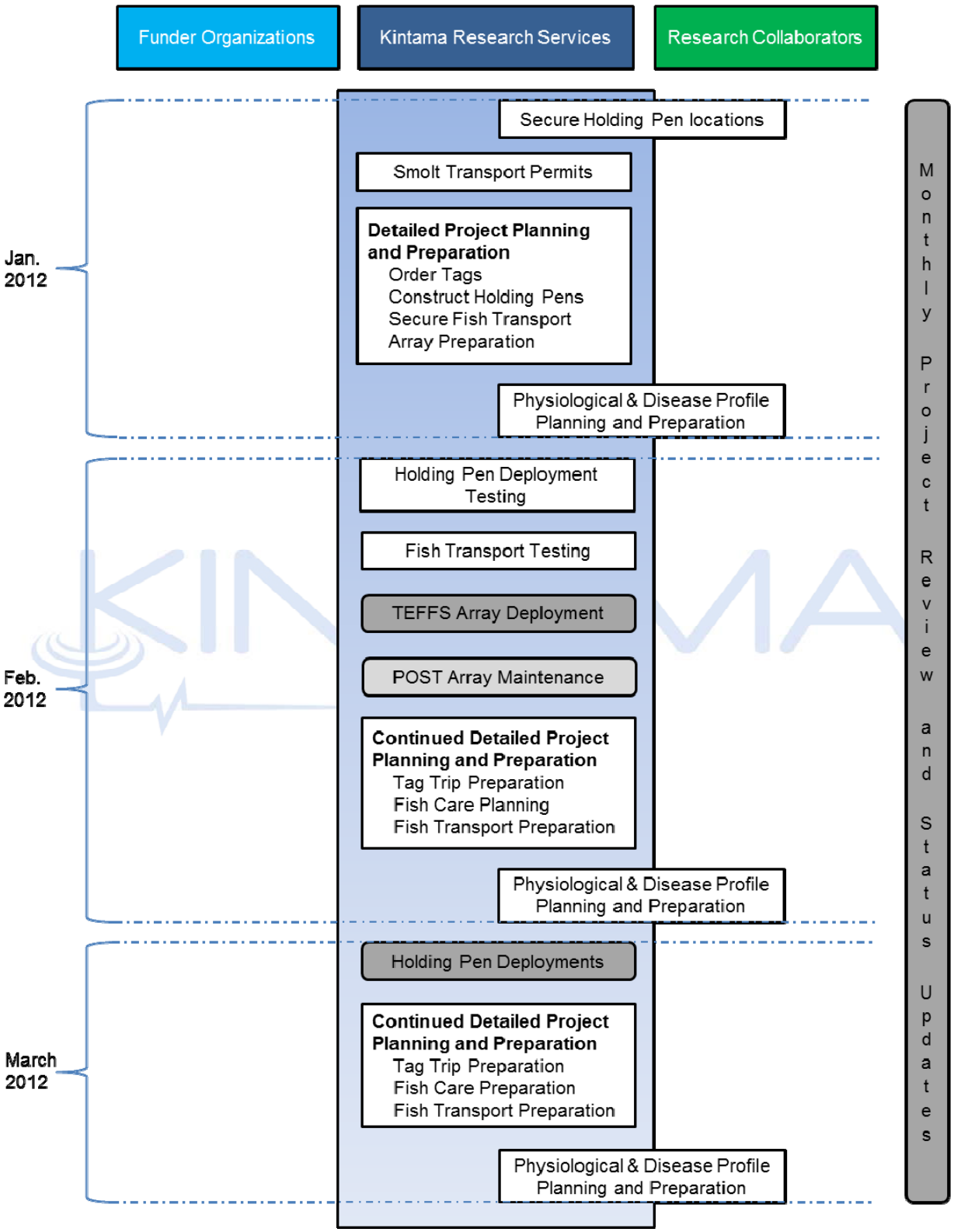
We designed the pilot phase around the goal of having 20 treatment and 20 control group smolts detected on the Queen Charlotte Strait sub-array. Based on our previous sockeye work in Cultus (2004-07) and Chilko lakes (2010-11), survival from the Fraser River mouth to the Queen Charlotte Strait sub-array 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.

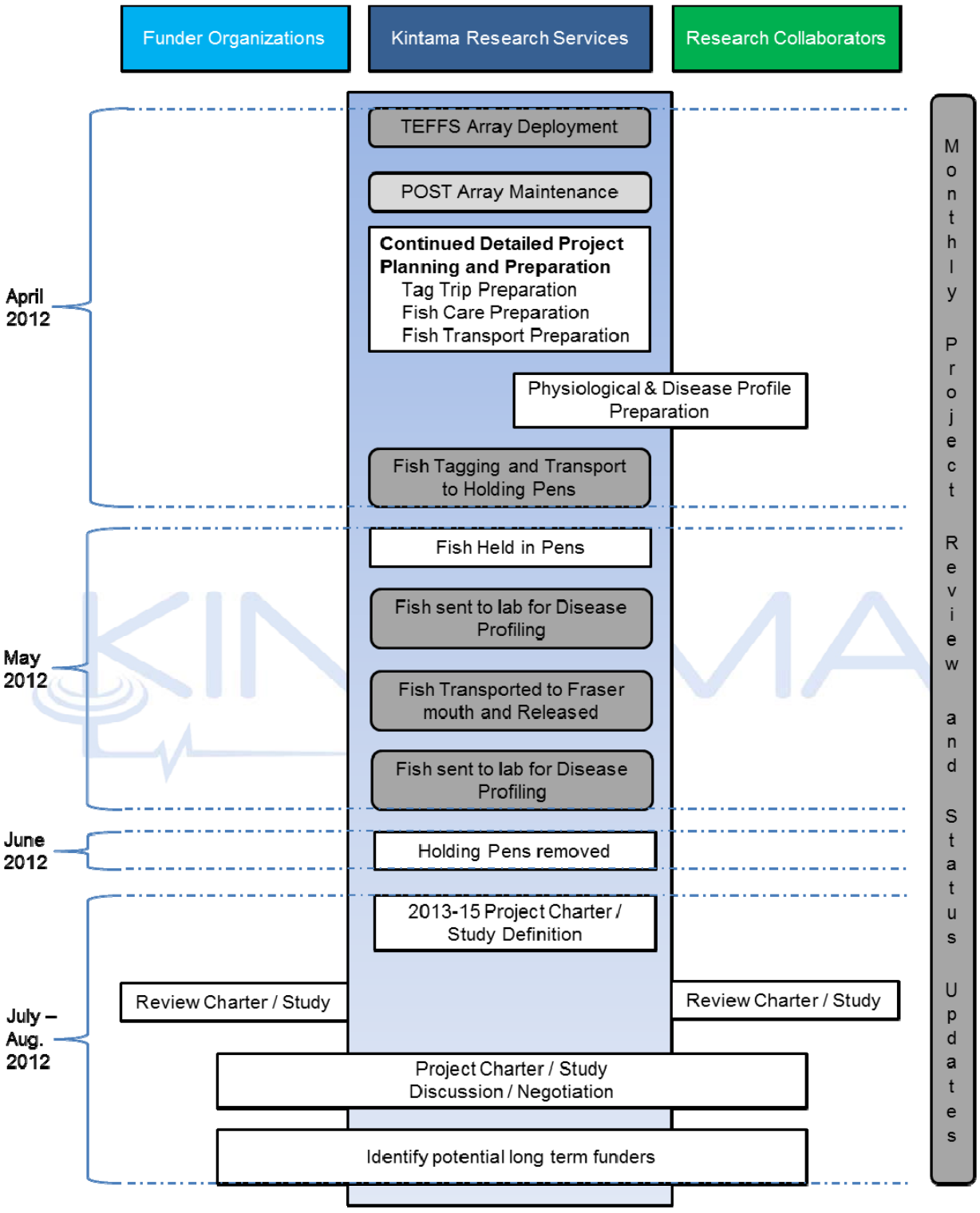
If logistically feasible, we will hold 50 tagged smolts at each of 10 replicate treatment sites (5 fish farm and 5 control sites in inlets lacking fish farms), so that we can estimate the variability expected among treatment groups. These data can then refine the design for the full-scale project.

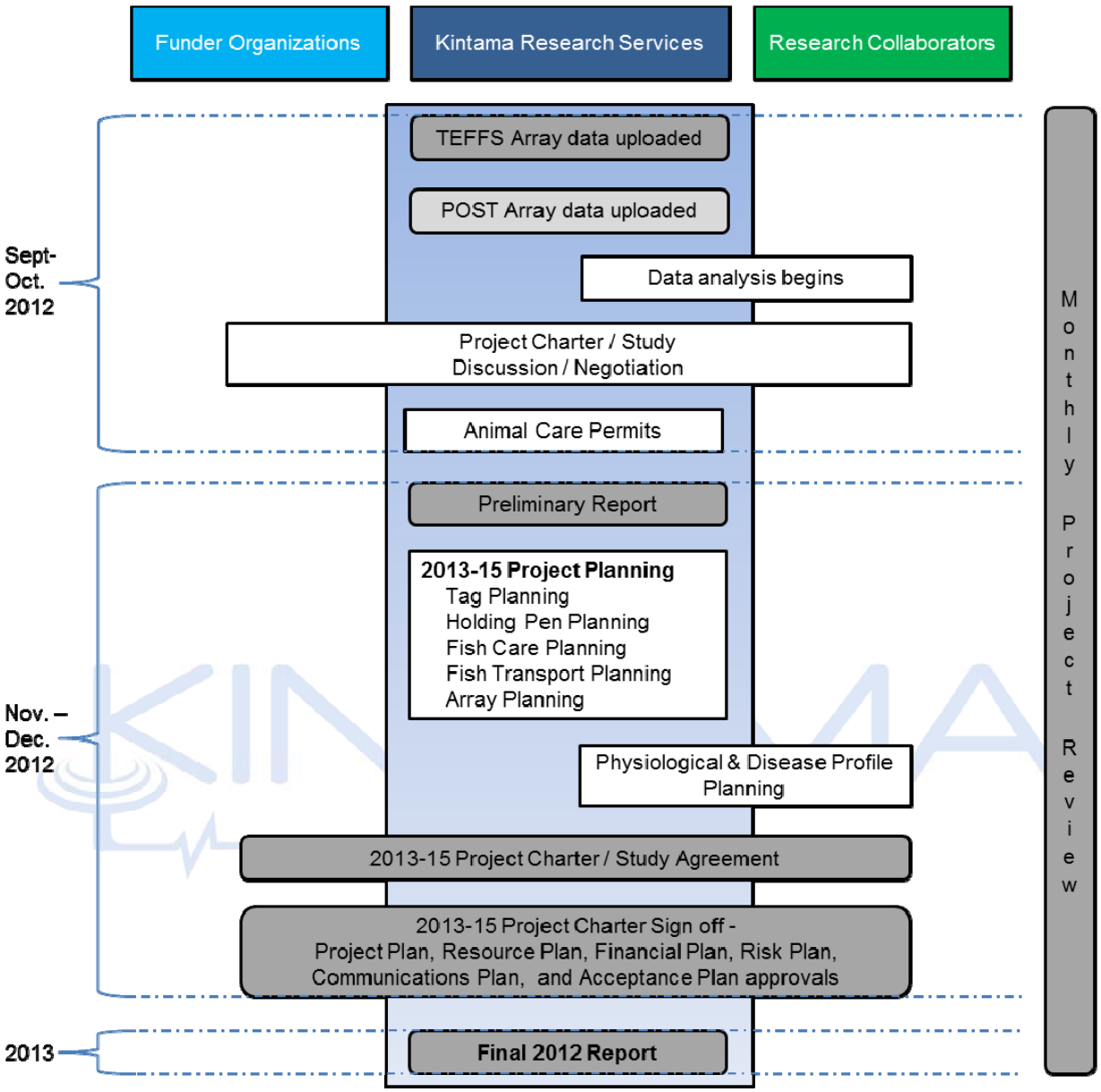
To further ground truth the results from the experimental study, we can compare the post-release marine survival of these smolts to the marine survival from freely migrating smolts released at the Lake. Taking Chilko as the example, 2011 survival to the Fraser River mouth was 33% and from the river mouth 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.

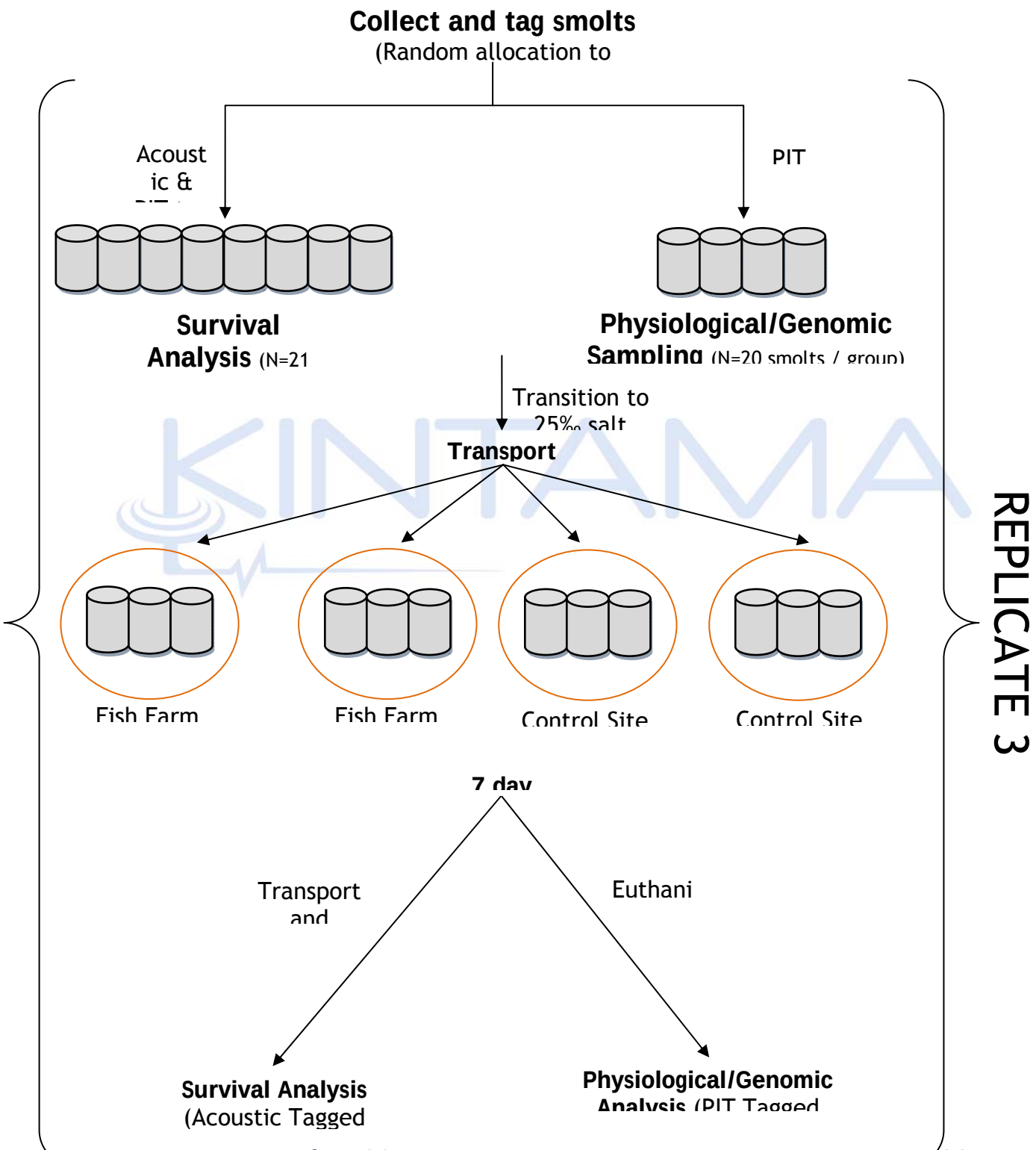



Funder Organizations
Kintama Research Services
Research Collaborators
Secure Holding Pen locations
Smolt Transport Permits
Detailed Project Planning and Preparation
Order Tags
Construct Holding Pens
Secure Fish Transport
Array Preparation
Physiological & Disease Profile Planning and Preparation
Jan. 2012
Holding Pen Deployment Testing
Fish Transport Testing
TEFFS Array Deployment
POST Array Maintenance
Continued Detailed Project Planning and Preparation
Tag Trip Preparation
Fish Care Planning
Fish Transport Preparation
Physiological & Disease Profile Planning and Preparation
Feb. 2012
Holding Pen Deployments
Continued Detailed Project Planning and Preparation
Tag Trip Preparation
Fish Care Preparation
Fish Transport Preparation
Physiological & Disease Profile Planning and Preparation
March 2012
Monthly Project Review and Status Updates


Funder Organizations
Kintama Research Services
Research Collaborators
April 2012
May 2012
June 2012
July – Aug. 2012
TEFFS Array Deployment
POST Array Maintenance
Continued Detailed Project Planning and Preparation
Tag Trip Preparation
Fish Care Preparation
Fish Transport Preparation
Physiological & Disease Profile Preparation
Fish Tagging and Transport to Holding Pens
Fish Held in Pens
Fish sent to lab for Disease Profiling
Fish Transported to Fraser mouth and Released
Fish sent to lab for Disease Profiling
Holding Pens removed
2013-15 Project Charter / Study Definition
Review Charter / Study
Review Charter / Study
Project Charter / Study Discussion / Negotiation
Identify potential long term funders
Monthly Project Review and Status Updates


Funder Organizations
Kintama Research Services
Research Collaborators
Sept-Oct. 2012
Nov. – Dec. 2012
2013
TEFFS Array data uploaded
POST Array data uploaded
Data analysis begins
Project Charter / Study Discussion / Negotiation
Animal Care Permits
Preliminary Report
2013-15 Project Planning
Tag Planning
Holding Pen Planning
Fish Care Planning
Fish Transport Planning
Array Planning
Physiological & Disease Profile Planning
2013-15 Project Charter / Study Agreement
2013-15 Project Charter Sign off -
Project Plan, Resource Plan, Financial Plan, Risk Plan,
Communications Plan, and Acceptance Plan approvals
Final 2012 Report
Monthly Project Review

# 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 need additional collection permits for fish to be used for diagnostics;
- 20 March 2012 – Confirm all tagging staff surgical refresher and fish handling courses completed;
- 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 and PIT tag) and N=240 PIT tagged smolts (to be sacrificed for physiological/genomic sampling) will be used in 2012 pilot study. If possible, smolt tagging will occur from the two locations for which we have prior acoustic tagging experience, Cultus Lake (2004-2007) and Chilko Lake (2010-2011).

**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.

**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 & 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 (implantation of VEMCO tag)

- 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);
- Tagged smolts will be allocated to transport groups as per the randomization protocol below;
- A subset of smolts will be sacrificed for physiological/genomic sampling at time of tagging.

## Tagging (PIT tags only)

- 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);
- The full size range of smolts will be tagged (PIT tags can be used on all smolts >65mm).

## Randomization

- Tagging will be done to achieve three experimental groups for transportation by float plane;
- After surgical implantation, smolts will be successively allocated to groups of N=20 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;
- Transportation by float plane will consist of 8 groups of 21 acoustically 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.

## Holding post tagging and transition from FW to SW

- All tagged fish will be held in aerated transport totes for 24 hours after tagging prior to altering water salinity;
- The 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 tagged fish have recovered, we will feed to satiation 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

- 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 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 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 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 to hold smolts at a fish farm site);

- Two transport totes of tagged smolts will be combined into a single floating net pens, providing 2 replicates of approximately N=42 tagged smolts at each fish farm exposure area (ideally, geographically distinct for each replicate);
- Smolts to be sacrificed for physiological/genomic sampling will be handled identically to the acoustically tagged smolts.

**2) Control area:**

- Will be chosen to 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 tagged smolts will be combined into a single floating net pens, providing 2 replicates of approximately N=42 tagged smolts at each control site (ideally, geographically distinct for each replicate);
- Smolts to be sacrificed for physiological/genomic sampling will be handled identically to the acoustically tagged smolts.

## 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 & 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 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

Jackson, D., et al. (2011). "An evaluation of the impact of early infestation with the salmon louse *Lepeophtheirus salmonis* on the subsequent survival of outwardly migrating Atlantic salmon, *Salmo salar* L., smolts." Aquaculture **320**(3-4): 159-163. DOI: 10.1016/j.aquaculture.2011.03.029.

Welch, D. W., et al. (2011). "*In situ* Measurement of Coastal Ocean Movements and Survival of Juvenile Pacific Salmon." Proc. Nat. Acad. Sci. USA **108**(21): 8708-8713 DOI: 10.1073/pnas.1014044108.

Welch, D. W., et al. (2009). "Freshwater and marine migration and survival of endangered Cultus Lake sockeye salmon smolts using POST, a large-scale acoustic telemetry array." Can. J. Fish. Aquat. Sci **66**(5): 736-750. DOI: doi:10.1139/F09-032.

# Appendix VIII. Letters of Support

See following.

December 11, 2011

Dear Sir/Madam:

It is my pleasure to provide a letter of support for the proposal by Welch and colleagues to determine whether and to what extent sea lice emanating from aquaculture facilities are interacting with Pacific salmon. Needless to say, this study is sorely needed. The current "war" that is being waged in the media regarding aquaculture and wild salmon interactions requires credible "big" science to address the uncertainty that currently confuses the public and makes it nearly impossible for industry and regulators to determine how to proceed. I am very familiar with biotelemetry and both fish behaviour and physiology such that I feel qualified to comment on the proposal. After reviewing the proposal I am convinced that such a large-scale experimental approach using biotelemetry is the only way to definitively address the "sea lice issue" given available technology. The project is costly but it is even more costly to proceed without credible science. The research team, led by Dr. David Welch from Kintama, is populated with experts that have the skill and track record to succeed with the project. Moreover, the project team is comprised of dispassionate researchers that can objectively address this socially complex problem. Although in due course (e.g., after the Cohen Commission concludes) it is highly likely that funding could be rallied to support the project, the sooner that the project can begin, the better. If possible, I would encourage bridge funding to initiate the project in 2012.

I ask that you give the project the strongest consideration.

Dr. Steven Cooke

Canada Research Chair in Fish Ecology and Conservation Physiology
Associate Professor of Biology and Environmental Science
Carleton University, Ottawa, Canada
and
President of the Canadian Aquatic Resources Section of the American Fisheries Society

Steven_Cooke@carleton.ca
Cell: 613 867 6711

SEYMOUR SALMONID SOCIETY
P.O. Box 52221 North Vancouver, BC V7J 3V3 PHONE 604 288 0511

9th Dec 2011

Dear Dr. Welch,

The Seymour Salmonid Society is fully supportive of the proposed study to investigate the effect of fish farms on migrating sockeye. Should it be that Cultus Lake sockeye are unavailable in 2012, as we understand might be the case, steelhead smolts from the Seymour hatchery would be available as a substitute. We know from 4 years worth of acoustic tracking studies carried out by the Society in collaboration with Kintama and other groups that the migration route, timing, and pattern of early marine mortality of Seymour steelhead are very similar to those of Cultus sockeye. Health monitoring of Seymour steelhead, performed by Dr. Shannon Balfry in parallel with the acoustic tracking studies, has provided valuable baseline data on the condition of the fish. These studies are described in more detail in the paper below (1): it is also described there how vaccination of hatchery steelhead against a trio of common saltwater-borne diseases resulted in a significant improvement in early marine survival; also how marine rather than river releases result in greatly improved early marine survival.

We feel it's very important that these studies continue and are extended, as in the proposed study, to try and determine the cause(s) of mortality of salmonids in the marine environment, and look forward to playing a role in this major study if possible.

Sincerely,

Stephen Vincent, D.Phil.

Vice-President,
Seymour Salmonid Society

spvincent@shaw.ca
604-433-7096

(1) http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0014779

Dr. David Welch,
Kintama Research,
10-1850 Northfield Rd.
Nanaimo. BC , V9S 3B3
david.welch@kintama.com

24 Nov 2011

Dear David,

I have read your proposal on "**Testing the Effects of Fish Farms on Salmon Survival**" and have the following comments.

In the history of fisheries science there has been much reliance on mathematical models and very little original work on fish and their environment. Consequently fisheries science has repeatedly failed to be predictive or explain events largely because of this lack of real time observations. Unlike other successful fields of biological science, such as medicine and agriculture, fisheries science has lagged behind in methodology. Too many fisheries scientists spend their time behind computers and never go to sea or conduct experiments. The proposal that you are making takes us into real-time science.

Your objectives are well planned and currently very important. The problem of fish farms and wild stocks can only be solved by the type of approach that you have outlined. The use of a formal experiment and a control is the right way to do science in order to find practical solutions to on-going problems. The proposal that you are tackling is of enormous importance to the fishing industry and should be supported by those who make money from marine resources.

I wish you all the best in your endeavors.

Sincerely,

Tim Parsons

Tim Parsons, PhD, FRSC, OC,
Prof. Em. Dept. Earth and Ocean Sci.
UBC, Vancouver

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Dr. David Welch,
Kintama Research,
10-1850 Northfield Rd.
Nanaimo. BC , V9S 3B3
david.welch@kintama.com

22 November 2011

Dear David,

Thank you for the tour of your facility last Saturday and the update on your recent work on acoustic fish tracking. You have achieved great success over the past decade and demonstrated the practicality of a revolutionary new technology for monitoring salmon migrations.

I have read the draft of your proposal for "**Testing the Effects of Fish Farms on Salmon Survival**". The impact of fish farms on wild salmon populations has been a subject of heated controversy for many years now and has figured prominently within the scope of the Cohen Commission on Fraser salmon stocks.

Evidence for or against the hypothesis that fish farms, especially in the Broughton Archipelago, have had a significant negative impact on wild salmon populations remains unconvincing. Arguments are put forward with great emotion but are poorly based in solid science. Your proposal for conducting a controled experiment - a rare instance in fisheries science, where most inferences are based on correlations - offers a real possibility of providing direct and reproducible results with significant relevance to policy and management of both the wild fishery and the aquaculture industry.

"**Testing the Effects of Fish Farms on Salmon Survival**", as your proposal outlines, could save years of further bickering and argumentation and presents, at last, an opportunity for providing a scientific answer to the question of the impact of net-pen fish farms on wild salmon stocks.

With best wishes and good luck in your further endeavours,

Paul H. LeBlond, Ph.D., FRSC
Prof. Emeritus,
Dept. Earth & Ocean Sciences, Univ. of BC

,

**UNIVERSITY OF HAWAI'I AT MĀNOA**

School of Ocean and Earth Science and Technology
**Department of Geology and Geophysics**

Dr. David Welch
Kintama Research
10-1850 Northfield Rd.
Nanaimo, BC V9S 3B3

November 26, 2011

Re: "**Testing the Effects of Fish Farms on Sockeye Salmon**"

Dear David:

Thank you for the opportunity to review draft 15 of your proposal entitled "Testing the Effects of Fish Farms on Sockeye Salmon." It addresses a problem that is important not just to British Columbia, which has been deeply troubled by questions regarding the effects of fish farms on wild fish ever since the sea lice epidemic of 2001, but to all localities with intensive sea cage aquaculture.

Time-tested principles of ecology show that predators control disease in prey populations. Therefore, marine monocultures that are protected from predators, but not from the pathogens carried by wild stocks, inevitably become unintended culture facilities for such pathogens. In Norway, the nation that invented salmon farming, government scientists now accept that wild Atlantic salmon and sea trout are declining in every fiord with salmon farms, and even in fiords adjoining those with farms. The only question is: How great a separation is required to protect wild fish? Of course, the answer depends on (a) the migration routes of wild stocks relative to farms, (b) coastal hydrodynamics and (c) the level of farm production; but the work you propose is an essential first step toward answering such questions for Fraser River sockeye.

The great thing about your methods, which I hope will be obvious to funding agencies, is that they eliminate the confounding inherent in traditional escapement-based studies of salmon. There is no other method I know of that can parse ocean mortality either spatially or temporally, and your methods do both. They represent – try not to get a swelled head here – a watershed in fisheries research because they do not require that a fish be captured in order to learn where it is in the ocean. They take advantage of the fact that sound is the preferred sensory modality in the ocean, because water transmits sound so much more efficiently than light, and they have the obvious potential to turn fish into moving reporters on the marine environment. (It seems likely that in the not-too-distant future you will be able to equip your fish with sensors for temperature, salinity and biochemical proxies for stress, with data offloaded to transponders along the way – the high 'carrier' frequencies used by your transponders provide more than enough bandwidth.) Moreover, your methods are bound to improve with time as transponders and receivers become more sensitive, more compact, and less costly. In short, even if there were no questions regarding the effects of fish farms on Fraser sockeye, there would still be great scientific value in the proposed work.

The proposed work is low-risk from a technical point of view because your prior work at Kintama has already demonstrated the methods and equipment. It is also low-risk with regard to research products because you have shown repeatedly that you are able to publish your results in high-profile journals such as PNAS and PLoS ONE. The power calculations that you use to estimate the relative effectiveness of different experimental geometries show that you have

thought carefully and quantitatively about the deployment of your hydrophone assets, and that you understand statistical techniques better than most scientists. The prior success of your earlier projects has repeatedly demonstrated your ability to manage a project with critical timelines and widely dispersed technical assets in a difficult marine environment. Your collaborators Kristi Miller and Scott Hinch are similarly distinguished in their respective fields. In short, the reward-risk ratio of the proposed work seems to me to be as high as it could possibly be.

Sincerely,

L. Neil Frazer
Professor of Geophysics
School of Ocean and Earth Science and Technology
University of Hawaii at Mānoa
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808-956-3724

Dr. David Welch,
Kintama Research,
10- 1850 Northfield Rd.
Nanaimo. BC, V9S 3B3

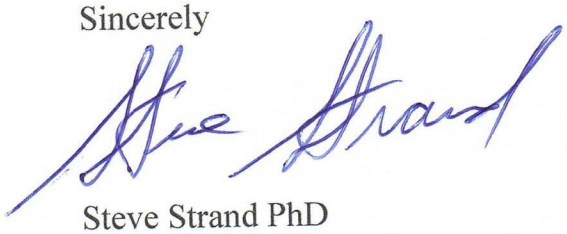
7 December 2011

Dear David,
After reading your proposal on "Testing the Effects of Fish Farms on Salmon Survival" I was both pleased and impressed. I was pleased because this is exactly the right approach to the questions of the effects of fish farming on wild salmon stocks. I was, and am, impressed with the thoughtfulness and scope of your proposal.

As a long-term proponent of fish farming who has in the past several years become increasingly skeptical of the overall benefits of current farming techniques employed in British Columbia, I am eager to see the results of your experiments. I believe that your research will be able to answer many of the questions which have made the controversy so heated. It is entirely possible that this research resulting from this proposal will actually put to rest many of the questions surrounding the effects of salmon farms on native salmon populations. The fact that the work will be done under the auspices of a scientist of your stature with the resources of Kintama should help to negate criticisms from both the pro and anti-fish farm factions.

As you know, the fate of juvenile fishes has been the impenetrable black box for most studies of fisheries biology. The ability of Kintama to follow these animals and determine their survival over long distances is truly exciting. The historical successes of the POST technologies suggest that your current proposal is likely to yield data that is both scientifically and environmentally important. Having controlled experiments to address specific questions is science at its best. I expect that reviewers from funding agencies will agree.

Sincerely



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