Conservation Supplementation Plan for Lake Sammamish Late-run (Winter-run) Kokanee

Lake Sammamish late-run kokanee fry at Issaquah Creek Hatchery (Photo: R. Tabor, USFWS)

Lake Sammamish Kokanee Work Group
Supplementation Technical Working Group

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Disclaimer

This plan was developed for the Lake Sammamish Kokanee Work Group through the collaborative efforts of the U.S. Fish & Wildlife Service, King County Department of Natural Resources and Parks, and Washington Department of Fish and Wildlife. This plan was developed to provide operational guidance for the implementation of the Lake Sammamish late-run kokanee supplementation program starting with the 2010-2011 spawning run. This plan reflects the current understanding among technical experts from the author agencies regarding effective supplementation techniques for salmon and their application within the specific circumstances and environment of the native Lake Sammamish kokanee salmon population. This plan will be revised on at least an annual basis to reflect information gained from program implementation, new technical information regarding effective techniques for artificial propagation of kokanee salmon, relevant changes in the habitat context into which fish from the program are released, relevant changes in the overall strategy to recovery Lake Sammamish kokanee, and other important drivers of the program.

The most current version of this plan is available at:

http://www.kingcounty.gov/environment/animalsAndPlants/salmon-and-trout/kokanee.aspx

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Overall Lake Sammamish Kokanee Conservation Strategy Goal:

"Prevent the extinction and improve the health of the native kokanee population such that it is viable and self-sustaining, and then supports fishery opportunities" (LSKWG 2009).

Supplementation Program Goal

In response to the decline of late-run Lake Sammamish kokanee, a comprehensive conservation strategy is needed to achieve the overall goal of rebuilding the population such that it is viable and supports fishery opportunities. The Lake Sammamish Kokanee Work Group (LSKWG) has established a goal for rebuilding the population: prevent the extinction and improve the health of the native kokanee population such that it is viable and self-sustaining, and then supports fishery opportunities. The LSKWG's highest priority near-term action in support of this goal is to employ aggressive artificial propagation methods.

The supplementation program will involve collecting gametes from spawners in Lake Sammamish tributaries and incubating them in hatcheries and then using a system of recirculating remote site incubators (RSIs) to increase egg-to-fry survival rates. All steps of the supplementation program will be designed to minimize the influence of domestication and thereby allow natural environmental processes to drive natural selection of Lake Sammamish kokanee population attributes such as emergence timing and maturation schedules. Additionally, the program will be designed to manage the risk of potential program failure during all operational stages. For instance, rearing of fertilized eggs at more than one hatchery and/or in multiple rearing incubators is desired.

Temporal Supplementation Program Goals:

Three-year (2010/2011 - 2012/2013) program goals:

- Maintain existing spatial distribution, abundance, population age-structure, and genetic diversity.
- Evaluate and improve the effectiveness of supplementation techniques employed

Twelve-year (2010/2011 – 2021/2022) program goals:

- Expand spatial distribution to additional Lake-Sammamish tributaries, increase abundance, maintain population age-structure, and genetic diversity.
- Evaluate and improve the effectiveness of supplementation techniques employed
- Terminate the supplementation program after three full generations (i.e.,12 years/2022)

Program Objectives and Metrics

a. Abundance – measured by returning adults

b. Spatial distribution – measured by number of tributaries with established populations[1]
c. Age-structure – measured by otolith analyses and cross referenced with any known otolith marks from the supplementation program indicating a stable age structure
d. Genetic diversity – measured by DNA sampling indicating adequate heterozygosity

Background Information on Late-run Population

Once numbering in the tens of thousands, Lake Sammamish kokanee have experienced a precipitous decline during the latter half of the 20th century. Historically, spawning was observed from August through January representing three distinct run timings (early/summer-run, middle/fall-run, winter/late-run), but by 2001, the only spawning aggregations of kokanee in Lake Sammamish were considered to be late-run, spawning in only four tributaries during November, December, and January. Since 1996, a high degree of variability has been observed in spawning ground escapement of late-run kokanee (Figure 1), prompting special concern from stakeholders in the watershed. This variability reflects, in several recent years, estimates of run size we

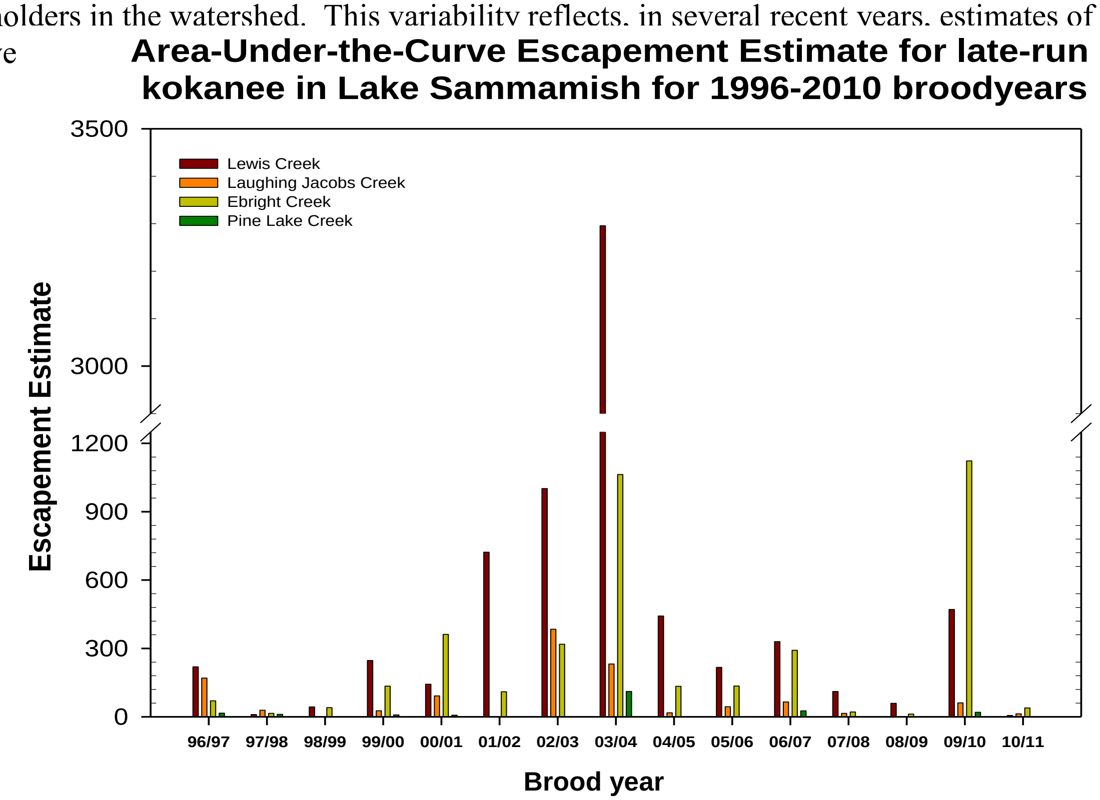

Figure 1. Area-under-the-curve escapement estimates of late-run kokanee for the four primary spawning tributaries to Lake Sammamish (1996-2010 broodyears).

[1] Currently it is unknown if there is significant or consistent kokanee spawning use of the lake shore. Therefore, this metric may also include areas of the Lake Sammamish shoreline in the future if surveys result in the detection of beach spawning.

Kokanee spend their entire lives in freshwater. Typically, kokanee fry emerge from redds in streams, immediately emigrate to a nursery lake, rear in the lake until maturity (typically three to four years), and return to natal tributaries to spawn. In the Lake Sammamish Basin, late-run kokanee currently spawn in three main tributaries (Lewis, Laughing Jacobs, and Ebright Creeks; Figure 2) with occasional observations of returning adults made in Pine Lake, Vasa, Zaccuse and George Davis Creeks.

Figure 2. Current distribution of major spawning sites of late-run kokanee in tributaries of Lake Sammamish.

Possible Overall Supplementation Strategies

In developing an overall supplementation strategy it was necessary to first determine what level of intervention may be required over time. Given recent late-run kokanee escapement (Figure 1), there were essentially two primary courses of action the program could consider:

1) supplementation using gametes from some proportion of annual adult returns, or
2) supplementation using captive broodstock developed from adult returns.

These courses of action are influenced by a number of factors, including but not limited to implementation cost, availability of facilities, program feasibility, annual escapement estimates, domestication concerns, and extirpation risk. Ultimately, it was decided annual collection of gametes was the best conservation supplementation option for Lake Sammamish kokanee under the current conditions, and developing a captive broodstock was not warranted at this time. The option of developing a captive broodstock may be pursued in the future should population conditions decline to a level where this may be the only option to maintain species persistence.

A range of incubation and rearing strategy options was considered before selecting the final strategy that will be implemented under the supplementation program. A number of factors relevant to the ultimate success of the program were considered during the evaluation of each strategy. These factors included the degree of possible influence from domestication, overall feasibility, the likelihood of implementation success, the likelihood of meeting program objectives, minimizing risk, and program costs (startup and annual). The merits and weaknesses of each of the options considered under incubation and rearing strategies are discussed below.

Incubation Strategy

Four basic incubation strategies were considered for the program, 1) complete incubation (from egg to fry stage) in a hatchery environment; 2) complete incubation in remote site incubators (RSIs) located at spawning tributaries; 3) complete incubation in a recirculating RSI system using tributary waters at Issaquah Creek Hatchery, and 4) some combination of the these strategies.

i. Hatchery Only

Incubating eggs within a hatchery environment has the distinct advantage of controlling for stochastic environmental events, particularly high-flow storm events and sediment transport dynamics that significantly increase the risk to eggs incubating in the wild. In addition to reducing environmental stochastic risk, it provides a more stable environment that allows for more control/manipulation of the incubation setting and period, and allows for a greater variation in capacity than an incubation system set up on location within individual spawning tributaries.

The primary drawbacks to using a "hatchery only" strategy include inferior location (e.g., outside of watershed, logistical difficulties, potential for different incubation temperature regimes), higher potential cost relative to more passive strategies for incubation, high likelihood of domestication effects, and ordinary risks of supplementation programs (e.g., disease transmission).

Two hatcheries have been identified as potential candidates for this kokanee supplementation program, Quilcene National Fish Hatchery (NFH) and Issaquah Creek Hatchery.

Quilcene National Fish Hatchery

Quilcene NFH is located on the Olympic Peninsula, within the Quilcene River watershed located on the west side of Hood Canal. This federal hatchery has the isolation facilities required to rear out-of-basin stocks, the capacity to meet our projected needs for the supplementation program, adequate water supply and temperature control, and is available during at least part of the timeframe necessary to incubate late-run kokanee eggs. Because the facility costs are partly shared by other fish rearing programs implemented at the hatchery, a significant cost savings in staffing can be realized. One drawback to using any out-of-basin facility is the additional logistics necessary to transport gametes, fertilized eggs, or fry to and from the facility, as well as the increased risk of possible loss of any of these life stages during transport.

Issaquah Creek Hatchery

Issaquah Creek Hatchery is located within the Lake Sammamish watershed on Issaquah Creek. This WDFW (Washington Department of Fish and Wildlife) hatchery will not require isolation facilities, has the capacity to meet our projected needs for the supplementation program, adequate water supply and temperature control, and is available during the timeframe necessary to incubate late-run kokanee eggs. Issaquah Creek surface water and Darigold coolant water were both successfully tested for their ability to incubate kokanee eggs.

Alternative facility

Two other facilities were considered for the supplementation program, Cedar River Hatchery in southern Lake Washington Basin and Chambers Creek Hatchery in southern Puget Sound. Both of these facilities were utilized in 2009 to successfully incubate and rear late-run kokanee eggs from Lake Sammamish tributaries. However, due to limitations with long-term availability and potential conflicts with other programs at these facilities, temperature control and pathogen treatment at Chambers Creek, and costs, it was determined that continued use of these facilities is problematic or impractical at this time.

Combination of facilities

Similar to 2009, using a combination of hatcheries to implement the supplementation program spreads risk of catastrophic failure at any one facility, and allows the program more options for incubation approaches. However, using multiple facilities does create additional logistical issues related to splitting brood collections and increases overall program cost.

ii. Remote Site Incubators (RSIs) Located at Tributaries

RSIs have successfully been used as an out-of-stream device for incubating eggs for a number of salmonid species and in a number of locations. The primary advantages of using RSIs are a significant reduction in sedimentation and other threats that developing embryos and alevins may encounter instream (especially when RSIs are supplied with cold, clear, off-channel water sources such as springs); imprinting upon natal water sources; more natural timing of egg incubation, hatching, and fry emigration; and maintenance of natural imprinting to natal streams. However, at unstable tributary sites where alternative water sources are not available, these systems are potentially more vulnerable to intake failures and sedimentation. Furthermore, RSIs may be more vulnerable to vandalism than hatchery facilities.

Although it is recognized that RSIs may be used in the future at some of the target Lake Sammamish tributaries, it was currently determined to not be feasible. Specific issues that make their use a poor alternative at this time include moderate to high risk of sedimentation, risk of potential water intake failures due to peak flow events, inability to otolith mark release groups, and logistical difficulties in adequately monitoring/securing sites.

iii. Recirculating RSI System at Issaquah Creek Hatchery

Using a similar concept to stream-side RSIs, a series of RSI systems would be constructed at the Issaquah Creek Hatchery. Each unit would run independently on a closed, filtered, recirculating system with water collected from the natal tributary of interest (Lewis Creek, Ebright Creek, or Laughing Jacobs Creek). These recirculating systems are anticipated to largely maintain all the biological advantages of streamside RSIs, but eliminate the risk of system failures due to sedimentation, peak flow events, and vandalism. This system could allow for either immediate release of hatched fry to natal tributaries or for transfer to alternate facilities with or without the use of natal water (e.g., stream-side raceways or hatchery troughs/raceways, respectively) for some period of holding and/or rearing.

The additional advantage of the recirculating RSI system is it provides the capability to imprint kokanee fry to other Lake Sammamish tributaries in the future where/when it is determined distribution needs to be expanded.

iv. Hatchery and RSIs

If using streamside RSIs, it was determined that some reduction in risk could be achieved by incubating eggs to the eyed stage in a controlled hatchery setting and then placing eyed eggs into streamside RSIs to complete incubation through hatching. This would be especially important for the late-run kokanee tributary sites, which can be extremely vulnerable to high flow events that typically occur early in the incubation period. In addition, it would allow for otolith marking of release groups, providing the opportunity to monitor the success of the program when the released fry return to the spawning grounds as adults.

However, concerns with adequate imprinting to natal streams, and ongoing concerns with less than ideal site availability, logistical difficulties to adequately monitor sites, moderate to high risk of sedimentation, and the continued risk of potential water intake failures it was determined that even this more limited use of RSIs at tributary sites was still not a preferred alternative at this time.

If using the recirculating RSIs, it was also determined that some reduction in risk could be achieved by incubating eggs to the eyed stage in a controlled hatchery setting and that it would allow for otolith marking of release groups. However, the recirculating systems did not have the continued risk that streamside RSIs would have after the eyed stage since recirculating RSIs eliminate the risk of system failures due to sedimentation, peak flow events, and vandalism.

Preferred Incubation Strategy

The supplementation planning partners' evaluation came to the conclusion that the preferred incubation strategy for the Lake Sammamish kokanee supplementation program was to use a combination of hatchery facilities and the recirculating RSI system at Issaquah Creek Hatchery. However, due to current timing constraints at Quilcene NFH (i.e., determined during pilot season they cannot accommodate late season broodstock collections of Lake Sammamish kokanee until after 2014 due to conflict with NOAA Fisheries' Hood Canal steelhead supplementation program efforts), planning partners have decided to complete all incubation at the Issaquah Creek Hatchery for the forseeable future. In addition, it was discovered that incubation water temperatures were significantly higher at Quilcene NFH than Issaquah Creek Hatchery, which lead to accelerated egg development and hatching. This resulted in significantly earlier hatch timing than naturally produced fry, which necessitated an extended rearing period (see Preferred Rearing Strategy section below). Since Issaquah Creek Hatchery is located closer in proximity to the broodstock creeks, incubation temperatures are more similar to the natural temperature conditions, and emergent fry require a much shorter extended rearing period in this case compared to fry incubated at Quilcene NFH. The highly successful incubation efforts conducted during the pilot program (2010/2011) at Issaquah Creek Hatchery provide strong indication that risk will not be significantly increased with the revised program strategy. This decision will be revisited for brood year 2012/2013.

The current program will transport all eggs collected from Lake Sammamish tributaries to Issaquah Creek Hatchery where they will be incubated to the eyed stage. Otolith marking of release groups will also occur at this facility, to provide the opportunity to evaluate the program's effectiveness. Once reaching the eyed stage, all eggs will be divided and placed in the appropriate recirculating RSI system until hatching. The supplementation planning partners believe that this approach currently provides the highest likelihood of program success, has an acceptable level of risk[2] , and is the most cost effective over the duration of the program.

One exception to this preferred incubation strategy applies to broodstock collected for reintroduction into Issaquah Creek. One objective of the supplementation program is to expand the distribution of kokanee into previously occupied streams. Issaquah Creek has been identified as one of the primary candidates for these efforts. Surface waters from Issaquah Creek along with existing hatchery capacity could be used specifically for this effort, since a reintroduction of kokanee into Issaquah Creek does not necessitate the use of the recirculating RSI system for incubation and imprinting.

Rearing Strategy

Two basic rearing strategies were considered for the program: 1) no rearing; and 2) some level of extended rearing

i. No Rearing

No rearing would require minimal holding time of kokanee fry prior to their direct release into the appropriate natal tributaries. A no rearing strategy has the advantage of most closely matching the life cycle and fry

[2] An individual risk assessment of the single-hatchery incubation strategy was developed to evaluate the level of risk to a brood year and population. This is a separate document available upon request.

size of wild fish, no risk of altering imprinting, no potential addition of domestication effects (see extended rearing), and no added cost associated with feeding/rearing.

The primary drawbacks to using a "no rearing" strategy include releasing individuals of smaller size/poorer fitness than fed fry, releasing fry at less than optimal times, potential logistical difficulties with frequency and numbers of fry released, and increased predation risk with smaller sized fish and possible small release groups.

ii. Extended Rearing (fed fry)

Extended rearing could range from several weeks to months depending on the objective. In order to minimize domestication effects and avoid altering imprinting, it was concluded that any rearing should generally be minimized. Any rearing would have to take place at Issaquah Creek Hatchery. However, because the recirculating RSI systems are not expected to be sufficient to maintain water quality while holding rearing fry, newly hatched fry emerging from the recirculating RSIs would require transfer to other holding tanks on Issaquah Creek surface water or possibly water from Darigold. An extended rearing strategy has the advantage of increasing fry size and fitness prior to release, allowing selection of optimal times for fry releases, minimizing the number of release groups, and maximizing the size of release groups.

The primary drawbacks to using an "extended rearing" strategy include risk of altering imprinting, change from natural emigration timing, potential addition of domestication effects, and additional costs associated with feeding/rearing. We expect that fed fry will have a higher survival rate than unfed fry but we are uncertain if this will result in a higher number of returning adults to their natal stream.

Because success of the supplementation program is highly reliant on kokanee imprinting back to their natal streams, we will continue to evaluate this aspect of the program and make adjustments as necessary and as possible. Fry releases from 2009 offer an excellent opportunity to evaluate this because fed and unfed fry were released on the same day. However, Tilson and others' (1994) evaluation of artificial imprinting of Lake Roosevelt kokanee indicated that the swim-up stage has the highest percentage of fish that are attracted reliably to their exposure odor in behavioral tests. In addition, recently hatched eggs and alevins also displayed accurate homing in their behavioral tests. In contrast, pre-eyed eggs, pre-hatch eggs, and four fry stages ranging from post-swim-up fry to near fingerling-sized fry, all displayed poor homing ability or did not evidence selective attraction to their exposure odor. This would suggest the current supplementation program strategy captures the critical imprinting stage to natal streams, and that post-swim-up fry rearing in non-natal water (Darigold water) will likely have minimal adverse effects on imprinting. This issue will be evaluated as hatchery origin kokanee are collected as adults or carcasses on the spawning grounds beginning in brood year 2012/2013.

Preferred Rearing Strategy

The supplementation planning partners' evaluation initially came to the conclusion that the preferred rearing strategy for the Lake Sammamish kokanee supplementation program was to have no extended rearing/feeding of fry.

However, during the 2010/2011 pilot year it was determined that significantly warmer incubation water at Quilcene NFH and slightly warmer incubation water at Issaquah Creek Hatchery compared to the broodstock creeks resulted in accelerated emergence times. As a result, the release timing of any unfed fry would have been suboptimal due to a high likelihood of low food availability in the lake at that time of year (Berge 2009).

Therefore, the revised rearing strategy will collect the unfed fry that have emerged from the recirculating RSIs operating on natal waters and place them in rearing troughs on Darigold water (or Issaquah Creek surface water for any fry destined for Issaquah Creek) at Issaquah Creek Hatchery. Fry will be fed/reared from 2-4 weeks depending on emergence timing and then released into their natal tributary in several groups as close as possible to the optimum release period (i.e., high food availability in the lake and low predation risk).

The supplementation planning partners believe that this approach provides the highest likelihood of program success given current constraints, has the advantage of increasing fry size and fitness prior to release, has an acceptable level of risk[3] related to imprinting and domestication effects, and is cost effective at this time. An extended rearing strategy will continue to be revaluated as the program proceeds to determine if any improvements can be made to this aspect of the program and if any unanticipated risks need further evaluation.

Broodstock Collection

Once goals and objectives have been set for a supplementation plan, the first step in implementation is to collect fish to be used as broodstock. For Lake Sammamish kokanee, broodstock will be collected in an attempt to match what occurs on the spawning grounds through natural selective pressures (e.g., spawn timing). This includes collecting a representative sample of adults to be used as broodstock from across the run (from November through January), and from each of the natal tributaries.

[3] An individual risk assessment of an extended rearing strategy was developed to evaluate the level of risk to a brood year and population. This is a separate document available upon request.

a. Overall Collection Strategy

The overall collection strategy is to collect a representative sample of the spawning population of kokanee from the three major spawning aggregations (Lewis, Ebright, and Laughing Jacobs Creek) and transport them to the Issaquah Hatchery for spawning. Of equal importance is to not detrimentally affect the spawning success of the naturally spawning population of kokanee that are not incorporated into broodstock. This relies upon accurate accounting of fish as they enter tributaries across the entire spawning period, a method of collection that is unbiased, and avoiding harassment and disruption to redds.

Although the current broodstock collection strategy focuses on the three primary late-run tributary spawning aggregations, collections at additional tributaries (e.g., Pine Lake Creek, Zaccuse Creek, George Davis Creek, Issaquah Creek) may be considered by this program in the future (for supplementation and reintroduction efforts) depending on observed escapement and additional genetic analyses in other tributaries. Should a situation arise where kokanee are observed in other creeks, but not in the three primary spawning streams, the program may consider holding these fish and implementing a "rapid genetics assignment" to determine their likely origin. If it is determined that these fish genetically assign to one of the three primary spawning tributaries, then they may be used in the program under a low return scenario. If a fish does not assign to one of the three primary spawning tributaries, we would return it back to the creek from which it was collected.

b. Collection Locations

Collections will include sites in Lewis, Ebright, and Laughing Jacobs Creeks (Figure 2). Adult fish will be collected from the entire area representing the spawning grounds in an effort to reduce sampling bias and gather a representative sample of adult kokanee in each stream. Both males and females will be collected in Lewis Creek from the mouth to the I-90 culvert; in Ebright from the mouth to the passage barrier on the Pereyra Property; in Laughing Jacobs from the mouth to the upstream extent of spawning (in the vicinity of the pavilion within the Hans Jensen State Park).

c. Collection Timing

Broodstock will be collected across the run to represent the spawning population of kokanee. Males and females will be collected once fish start to enter tributaries, and will continue until the last fish arrive on the spawning grounds (as late as February).

d. Collection Method

The primary method for collecting broodstock will be the same as in 2009, using a backpack electrofisher (see Jackson 2010). This technique has proven to be effective in collecting kokanee that are used as broodstock from across the State, and worked relatively well for collecting kokanee in 2009 and 2010 in these same streams. In some instances, kokanee may be collected using a combination of dipnets or seines, especially if they are aggregated at the mouth of tributaries within the littoral zone of Lake Sammamish. In all instances, an attempt will be made to collect kokanee that are not actively spawning or partially spawned (particularly females). Once kokanee are collected and determined to be mature but not actively spawning, they will be transferred into coolers with aeration and transported to the Issaquah Creek Hatchery for subsequent spawning. Kokanee spawners may also be collected at the Issaquah Creek Hatchery if they naturally stray into Issaquah Creek or progeny return after a reintroduction effort has been initiated specifically for Issaquah Creek. The collection of spawners in this case would primarily be focused on reestablishing an Issaquah Creek population.

e. Collection Goal

A decision to collect broodstock will be made on a weekly basis following spawning ground surveys that identify the number and distribution of kokanee in Lewis, Ebright, and Laughing Jacobs Creeks. Given the variability in the size of the return, spawn timing, and the imprecision in forecasting an in-season return of kokanee (Figure 1; Jackson 2009, 2010), a weekly egg take goal cannot be applied. Given this constraint, we propose the following matrix (Table1) as a compromise that accomplishes the major goals of this program, collecting a representative sample of the spawning population, and improving the production of kokanee fry that enter Lake Sammamish on an annual basis.

Of course it is impossible to have knowledge of the final escapement estimate in-season, so we reviewed several potential scenarios for the timing of the return of kokanee (Figure 3). Each of these examples represents an example of a broodyear we have observed. If we had the knowledge outlined in Table 1 at the beginning of the season, our strategy would be easy to identify. In the “Ideal Return”, we would attempt to sample between 25% and 50% of the returning adults per week, and we would accomplish the goal outlined in Table 1. In the “Early Return”, we would need to sample 75% of the run, and complete all broodstock collection in November. In the “Late Return”, we would be successful if we sampled across the run between 25% and 50% per week. And finally, in the “Bi-Modal Return” scenario, we would target sampling 50% of the return per week, across the entire run. This strategy reflects an interest to leave more fish on the spawning grounds during years of higher returns, while being more aggressive at collecting broodstock for the supplementation program during years of low returns.

Table 1. Overall collection goals by tributary under different run return scenarios. Percent collected is expressed as the percent of annual spawning ground escapement. These goals will likely be adjusted bi-monthly to refine in-season estimates of spawning ground escapement.

	Size of Return/Escapement		
	Low	Medium	Good
Laughing Jacobs Creek	<50	50-100	>100
Collection goal (%)	***75%***	***50%***	***25%***
Lewis Creek	<50	50-200	>200
Collection goal (%)	***75%***	***33%***	***15%***
Ebright Creek	<50	50-100	>100
Collection goal (%)	***75%***	***33%***	***15%***

Given our uncertainty, and the fact that we will only collect broodstock one to two times per week, we feel the best strategy to accomplish our goals is to set collection goals based on the abundance of spawning kokanee by week using real-time data, and attempt to collect 75% of the fish on the spawning grounds in November, 25-50% in December, and 75% in January. These goals will be influenced by weekly calls with technical experts on the LSKWG, and will be adjusted according to what spawning ground surveyors are seeing on these streams.

Hypothetical scenarios for the spawn timing of Lake Sammamish kokanee

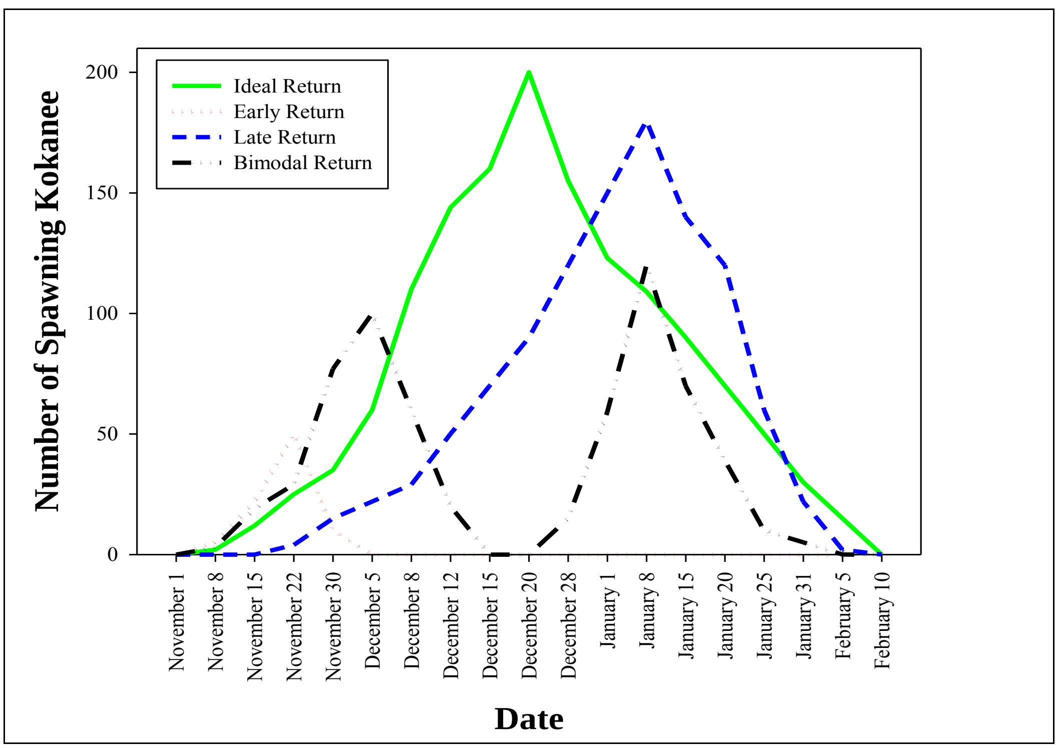

Figure 3. Hypothetical spawn timing curves for Lake Sammamish kokanee.

f. Egg Collection Cap

The overall collection goals outlined in Table 1 indirectly target the number of eggs to be collected throughout the season in the supplementation program. However, other constraints within the program ultimately set a limit or cap on the total number of eggs that can be collected and incubated in the program.

Genetic guidance limits the number of spawning pairs collected from each creek[4]. Assuming an average fecundity of 1,000 eggs per female, the maximum number of eggs that could be collected in total from all three current donor creeks is approximately 300,000 (no more than 100,000 from any one creek). However, current flow rates within the recirculating RSI systems limit the program to approximately 10,000 eggs per unit (5 units per RSI x 3 systems) or a total of 150,000 eggs to ensure high rates of incubation success. Accounting for a possible 5% egg loss during incubation, we have set the collection/incubation cap for the three RSIs at 160,000 eggs.

Any broodstock incubated on Issaquah Creek surface water (i.e., for potential reintroduction into Issaquah Creek), do not have the same physical capacity constraints that have been identified for the recirculating RSI systems. Therefore, the egg collection cap can largely be based on the biological constraints already established for other parts of the program. These include the previous guidance to not exceed collections from more than 100 pairs and to limit collections based on run-size as described in Table 1. Under scenarios where these collections are from outside of Issaquah Creek, a total of 100 pairs could be collected from all acceptable donor populations (up to 100,000 eggs total). However, donor populations will be assessed prior to collecting additional broodstock for Issaquah Creek reintroduction efforts to ensure no undesirable impacts occur to the donor population. Alternatively, late-run kokanee that may naturally stray into Issaquah Creek or return to Issaquah Creek after initiation of reintroduction efforts may also be used as broodstock to further reestablish/supplement kokanee within this system. In these circumstances, up to 100 pairs could be taken following the supplementation plan's established genetics guidance, or if insufficient spawners return to Issaquah Creek to acquire 100 pairs, an option is to add pairs from other donors (Laughing Jacobs, Ebright, and Lewis creeks) if available and appropriate, to bring the total up to 100 pairs (100,000 eggs). At this time, we believe establishing a collection cap of 100,000 eggs for surface water incubation is appropriate. However, we will continue to reevaluate on an annual basis. Limiting the number of eggs supplemented into Issaquah Creek aligns with our objective to largely allow natural environmental processes to drive natural selection of Lake Sammamish kokanee populations.

g. Disease Sampling and Management Response

[4] Overall program spawning guidance under Mating section directs the program not to exceed collection of 100 pairs from each creek.

Spawning ground surveyors will note the presence of parasites and external abnormalities on all fish collected for broodstock and carcasses collected from natural spawners. For example, parasitic copepods (e.g., *Salmincola*) are common in kokanee in Lake Sammamish and further understanding of this parasite and other parasites may be important in evaluating the health of this population (Figure 4).

Figure 4. Parasitic copepods on the gills of adult kokanee in Lewis Creek.

h. Adult Biosampling

For all kokanee collected for broodstock and carcasses from spawning ground surveys, fork (mm) and post-orbit to hypural plate (POH; mm) lengths will be recorded (Figure 5). In addition, fin tissues (preferably clips from any rayed fin, or alternatively opercle punches or adipose fin clips) will be collected and preserved in ETOH for analysis and archival at the WDFW Genetics Lab. In some cases, this analysis may be performed in-season in order to make decisions regarding spawning protocols. Otoliths will be collected from all kokanee broodstock and carcasses for subsequent aging. Fecundity of female kokanee collected for broodstock will be estimated, and pre-spawning mortality in naturally spawning kokanee will be examined by spawning ground surveyors (Figure 6). In addition, weekly counts will be made of live kokanee, kokanee redds, and other adult salmonids present in each stream (Figure 7).

Figure 5. Measuring fork length of a male kokanee in Ebright Creek.

Figure 6. 100% egg retention of a female kokanee collected in Lewis Creek. No external marks or signs of a source for mortality were observed.

Figure 7. Spawning ground survey in Laughing Jacobs Creek.

Mating

Population Structure

Based on existing WDFW genetic analyses (Warheit 2010; Warheit and Bowman 2008), there are four key findings that inform the supplementation program's long-term mating strategies. These are:

1) Genetic differences exist among the three main Lake Sammamish tributaries that support late-run spawning kokanee (Lewis, Ebright, and Laughing Jacobs Creeks).
2) Within each tributary there are also differences among annual collections, particularly between 2000/2003 and 2001. These differences may be due to small spawning populations for a given brood year
3) Kokanee that spawn in Ebright and Laughing Jacobs Creeks are more similar to each other than either is to kokanee that spawn in Lewis Creek.
4) Natural straying has been documented (genetically) between Ebright and Lewis Creeks.

a. Overall Program Spawning Guidance

In further consultation with geneticists from WDFW and USFWS, the following general spawning recommendations were developed to guide implementation of the supplementation program's mating strategies.

1) Collect genetic samples of all fish handled during collection of broodstock.
2) Maintain records of final distribution of all fish.
3) Live spawn males (if fewer than 20 males per tributary are currently being held) and tag after first use for visual identification. Males can be held or released back into tributary after use in the hatchery in the event of extremely low escapement, but should be placed in the tributary that matches their hatchery contribution (e.g. If an Ebright male is used in crosses with Laughing Jacobs females, the Ebright male, if released, should be released into Laughing Jacobs). If an extremely low return is not anticipated, males should be lethally spawned.
4) Only use males in two different spawning matrices (Note: males are not anticipated to be limiting. However, when an extremely low return is expected, supplementation partners will determine at that time if an exception to this guideline is warranted).
5) Use up to a maximum of 3 X 3 matrix crosses of available ripe fish (see Figure 8).[5] The primary objective is to keep mating numbers balanced, unless conditions dictate otherwise.
6) Maintain records of specific males and females used in each cross.
7) Do not exceed 100 spawning pairs per tributary even in the event of large escapements.
8) If the gametes collected on a specific day are to be split between Issaquah and another hatchery(ies), up to 3x3 matrix crosses should be planned and eggs and milt should be divided at Issaquah and transported separately on insulated ice in the dark. Fertilization should be carried out at the destination hatchery.
9) No changes in spawning recommendations will be implemented as a response to a single low escapement year.
10) If escapement remains at fewer than 20 fish within one of the individual tributaries for three years in a row, potential changes to some spawning protocols will be discussed, and any warranted changes implemented during the next spawning season (year 4).
11) If fish are transferred and used in spawning matrices outside of the tributary of collection, they become part of that population by definition and all resulting progeny should be released in the new tributary.
12) No fish and therefore no spawning pair should have their progeny released in more than one tributary or their gametes split between tributaries.

[5] At this time factorial crosses are not considered necessary for purposes of disease containment or monitoring.

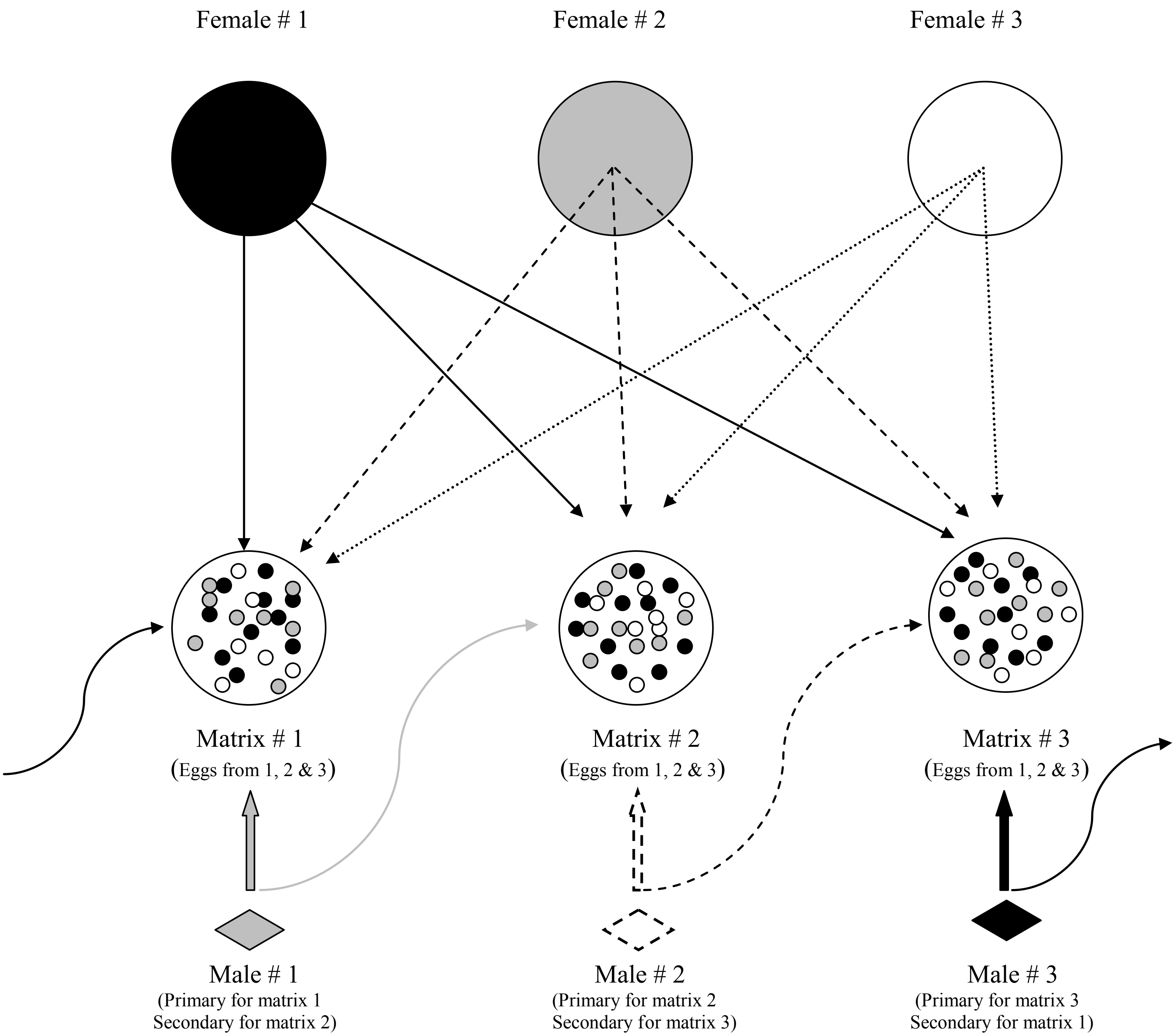

Figure 8. Diagram of recommended matrix spawning.

Tributary-Specific Spawning Guidance

Lewis Creek

1) Maintain Lewis independently from Ebright and Laughing Jacobs.
2) If escapement is fewer than 20 fish for three years in a row, fish from Ebright or Laughing Jacobs may be used in spawning matrices to establish equal sex ratios in the next spawning year.
3) In the event of bringing in fish from outside Lewis, each Lewis fish should have at least one Lewis partner if possible. Note that Cross 4 F2xM2 should not occur unless Lewis escapement is VERY low (see #6).

	Lewis Female 1	Ebright Female 2
Lewis Male 1	Cross 1 F1xM1	Cross 3 F2xM1
Ebright Male 2	Cross 2 F1xM2	Cross 4 F2xM2

	Lewis Female 1	Lewis Female 2
Lewis Male 1	Cross 1 F1xM1	Cross 3 F2xM1
Ebright Male 2	Cross 2 F1xM2	Cross 4 F2xM2

4) Early in the return, if only one fish is ready to spawn (i.e., no mate available) it should be released back into the creek (males could be held longer than females, but should be released with a good potential for surviving to spawn with any undetected fish still in the creek).
5) Late in the return (within the three-year low return time frame), if only one fish is ready to spawn (i.e., no mate available) it should be released back into the creek (males could be held longer than females, but should be released with a good potential for surviving to spawn with any undetected fish still in the creek).
6) Late in the return (after a three-year low return time frame), if only one fish is ready to spawn (i.e., no mate available) and the number of pairs spawned to date for that tributary is fewer than 20, and there are ripe fish of the opposite sex available from Ebright Creek, the fish should be retained and used in a cross tributary spawning matrix, otherwise it should be released back into the creek (males could be held longer than females, but should be released with a good potential for surviving to spawn with any undetected fish still in the creek).

Ebright and Laughing Jacobs Creeks

1) Maintain Ebright and Laughing Jacobs independently from Lewis.
2) If the combined Ebright and Laughing Jacobs escapement is fewer than 20 fish for three years in a row, fish from Lewis can be can be used in spawning matrices to establish equal sex ratios in the next spawning year.
3) Maintain Ebright and Laughing Jacobs independently from each other with the following exceptions:
   i. If escapement is fewer than 20 fish, fish can be exchanged between Ebright and Laughing Jacobs to establish equal sex ratios.
   ii. In the event of bringing in fish from the other tributary, each fish should have at least one partner from the same tributary if possible. Note that Cross 4 F2xM2 (Ebright Male x Ebright Female) should not occur unless Laughing Jacobs escapement is VERY low.

	Laughing Jacobs Female 1	Ebright Female 2
Laughing Jacobs Male 1	Cross 1 F1xM1	Cross 3 F2xM1
Ebright Male 2	Cross 2 F1xM2	Cross 4 F2xM2

	Laughing Jacobs Female 1	Laughing Jacobs Female 2
Laughing Jacobs Male 1	Cross 1 F1xM1	Cross 3 F2xM1
Ebright Male 2	Cross 2 F1xM2	Cross 4 F2xM2

   iii. Early in the return, if only one fish is ready to spawn (i.e., no mate available) it should be released back into the creek (males could be held longer than females, but should be released with a good potential for surviving to spawn with any undetected fish still in the creek).
   iv. Late in the return, if only one fish is ready to spawn (i.e., no mate available) and the number of pairs spawned to date for that tributary is fewer than 20, and there are ripe fish available from the other tributary of the opposite sex, the fish should be retained and used in a cross tributary spawning matrix, otherwise it should be released back into the creek (males could be held longer than females, but should be released with a good potential for surviving to spawn with any undetected fish still in the creek).

b. Holding facility

Prior to spawning (gamete collection), all broodstock will be transported to Issaquah Creek Hatchery. When necessary, broodstock will be held at the facility until they have reached spawning condition (i.e., ripe). It is not anticipated that gamete collection will be required in the field at this time. At Issaquah Creek Hatchery, gametes will be collected from spawners and fertilized when they are to remain on site, or they will be collected and transported to Quilcene NFH or other facility(ies) in the program (if need), where they will be fertilized. In either case, matings will be consistent with the spawning guidance outlined above.

Gamete and Eyed-Egg Transport

(No longer being implemented at this time, see Incubation Strategy section)

The following transport procedures will be utilized for moving gametes and eyed-eggs in the supplementation program to and from Quilcene NFH or other facility(ies) that are involved in this program.

Transporting Eggs:

Place block ice in a cooler, then put a layer of burlap or heavy cloth over the ice to prevent the eggs from coming into contact with the ice. Place a temperature logger on top of the burlap/heavy cloth layer. Place 1 female's eggs into the sandwich container and cover with the lid. Label the lid with the fish's unique identification number. Put the sandwich container on top of the burlap/heavy cloth layer.

Transporting Milt:

Place block ice in a cooler, and then put a layer of burlap or heavy cloth over the ice to prevent the milt from coming into contact with the ice. Place a temperature logger on top of the burlap/heavy cloth layer. Spawn a male into a Ziplock bag. Label each bag with its own unique identification number. Close the bag with a half inch gap, insert the O2 hose and fill the bag up with O2. Close the bag as you remove the tube. Place the milt on top of the burlap/heavy cloth layer.

Transporting Eyed Eggs back to Issaquah SFH:

A layer of ice and wet burlap will be placed in the bottom of a cooler. The "isolation baskets" containing the eggs will be placed on the burlap. A rigid "shelf" will be placed above the baskets and another layer of wet burlap and ice will cover the shelf. The eggs will be disinfected in iodophore solution upon arrival at Issaquah SFH.

Hatchery Incubation and Thermal Marking

a. Quilcene National Fish Hatchery Procedures **(No longer being implemented at this time, see Incubation Strategy section)**

Gametes will be combined just inside the door of the hatchery's Isolation Building. Appropriate matrix crosses will be made in accordance to recommendations as outlined in section # 6 (Mating) of the supplementation plan.

Eggs will be put into previously-disinfected 6-quart stainless steel buckets and then milt added. Water from the Quilcene NFH pathogen-free well #1 water supply (i.e., Isolation Building water supply) will be added to a point that the eggs are fully covered then thoroughly swirled to mix the contents. After 2 minutes, the eggs from each female may be combined into a single isolation basket. Each tray can accommodate 4 isolation baskets, or thus 4 females. Egg trays will have a 100 ppm iodophor solution added and isolation baskets containing eggs will water harden for 30 minutes. At this point, the tray will be pushed in to rinse and begin incubation at a 4 g.p.m. flow rate. Formalin treatment will be administered Monday, Wednesday and Friday at the same rate.

Eyed-eggs will be moved to a different series of trays after shocking and cleaning so they are no longer receiving formalin treatments along with green eggs still in the previous trays. Dead eggs will be hand-picked prior to moving them out of the Isolation Building at eye-up. Surface water from Penny Creek will be the emergency water source should pumps/backup generator ever fail during incubation. This water source contains resident fish, but there are no anadromous fish that have access to the creek.

Once thermal marking has been completed the eggs would be prepared for transport to Issaquah Creek Hatchery and transferred to the recirculating RSI systems and incubated on the appropriate natal stream waters.

b. Thermal Marking Procedures/Quilcene National Fish Hatchery

TBD

c. Issaquah Creek Hatchery Procedures

Gametes from male and female kokanee targeted for this facility will be collected and fertilized at Issaquah Creek Hatchery. Appropriate matrix crosses will be made in accordance to recommendations as outlined in section # 6 (Mating) of the supplementation plan: a proportional number of each female's eggs will be fertilized by each male within the matrix. Gamete origin will be from any of the three primary late-run kokanee spawning tributaries, Ebright, Laughing Jacobs and Lewis creeks. A record of the matrix crosses for each spawning event for each creek system will be maintained.

Fertilized eggs will be transferred to Issaquah Creek surface water in egg baskets, suspended in shallow troughs. Eggs will be incubated on filtered Issaquah Creek water (at ambient temperature) from fertilization to shocking (agitating eggs to turning the infertile eggs white so they can be separated from the fertile ones). Because most of the egg-to-fry mortality takes place from fertilization to eyeing, collected eggs are being incubated in egg baskets, where dead eggs can be easily removed and counted. Heath Trays will be used to facilitate thermal marking.

During the thermal marking period, eggs would be transitioned to Darigold water (see Thermal Marking Procedures below). Once marking has been completed the eggs would be transferred to the recirculating RSI systems and incubated on the appropriate natal stream waters.

d. Thermal Marking Procedures/Issaquah Creek Hatchery

After eggs have been shocked and picked, Darigold water will be gradually added to the water stream used to incubate the eyed eggs to increase ambient temperature over a 24-hour period until the water stream is 100% ambient temperature Darigold water.

Four gallons-per-minute of Darigold water will be delivered to the water chilling boxes where the ambient temperature would be reduced by 4^{o}C. This chilled water would be plumbed into a half stack of Heath Trays. The trays of kokanee eggs now receiving ambient Darigold water would be moved into the Heath Tray receiving chilled water and left there for 24 hours to induce a thermal band. Egg trays would be moved back and forth between the Heath stacks receiving chilled and ambient Darigold water to create the desired thermal codes (per the thermal marking maps).

e. Pathogen/Disease Testing and Management Response

WDFW will conduct viral sampling for the program and FWS's Olympia Fish Health Center will be the alternative backup as needed. The primary concern is the detection of IHNV positive adults, because historically sockeye in the Lake Sammamish Basin have been positive for IHNV and WDFW manages the basin as an IHNV-positive basin.

The testing goal is to sample 100% ovarian and tissues from female broodstock and 100% tissues from males. For spawning adult salmonids it is generally accepted that IHNV is associated with the surface of the egg, and hatchery disinfection protocols can typically stop the vertical (parent to progeny) transmission of the virus. However, there are some unexplained epidemics in sockeye in Alaska where the same disinfection protocols are practiced, so a conservative approach will be adopted by the program.

For Issaquah Creek Hatchery, after combining the gametes, eggs will be placed into iso-incubation buckets until virology test results are completed. Standard egg disinfection protocols will be implemented for water hardening and again after picking. If any adults test positive for IHNV, their eggs will be separated, incubated separately, and should be released only as unfed fry.

If gametes are taken to Quilcene NFH, they will come on station and be combined in the quarantine facility. Standard egg disinfection protocols will be followed and the green eggs will be placed into separate vertical flow incubators. If any adults test positive for IHNV their eggs will be separated, incubated separately, and should be released only as unfed fry when returned to Issaquah Creek Hatchery.

Specific disease handling protocols will be developed and incorporated into this plan for any other facility(ies) that may become part of this program. Eggs from parents that test negative for IHNV can either be released as unfed fry or reared at Issaquah Creek Hatchery.

Recirculating RSI Incubation

a. Design and Operation

The recirculating RSI system will be located at Issaquah Creek Hatchery, and located on station where it will largely be exposed to ambient air temperatures. This exposure to local ambient temperature/climate conditions is expected to help further mimic natural incubation and emergence timing for the area. It was determined during the 2010/2011 pilot season that heat transfer from the recirculating pumps to the incubation water was increasing the water temperature in the recirculating system and needed to be compensated for in order to maintain the local ambient water temperatures. Early in the pilot season, a passive cooling exchange system using water from Issaquah Creek was successfully installed at the hatchery which will now help maintain near local ambient water temperatures. Up to three recirculating systems will be constructed and operated for the three primary spawning groups (Ebright Creek, Laughing Jacobs Creek, and Lewis Creek). Each of the three recirculating systems will incubate eyed eggs on surface water from the specific creek in which resultant fry are targeted for release. The number of incubators used in any one year will be determined by the number of egg takes conducted during broodstock collection efforts.

Water in these systems will be filtered and treated for pathogens, however, no water treatments (e.g., charcoal filtration) are implemented that could significantly alter the tributary “signature” of the water source (Figures 9 and 10). Water will be exchanged at a rate sufficient to maintain adequate incubation water quality within the recirculating RSI systems. Weekly water exchanges have been anticipated during the pilot stage of operation, but may be more or less frequent depending on evaluation and testing during this initial period of operation.

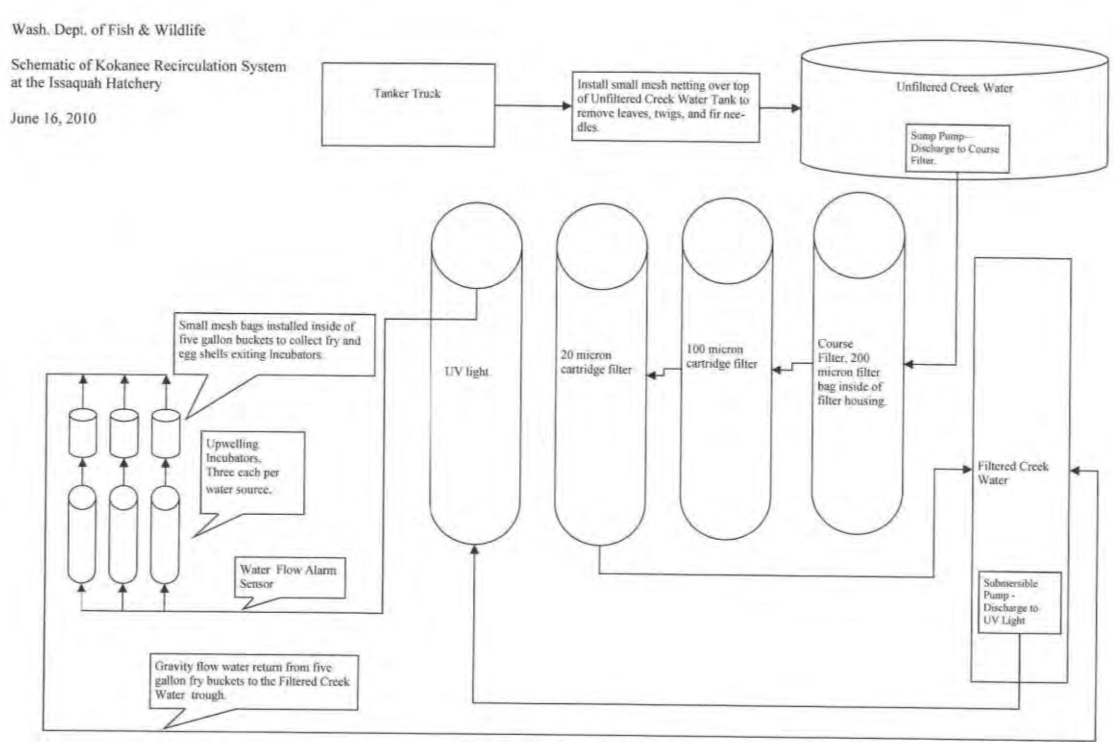

Figure 9. General schematic of the recirculating RSI system.

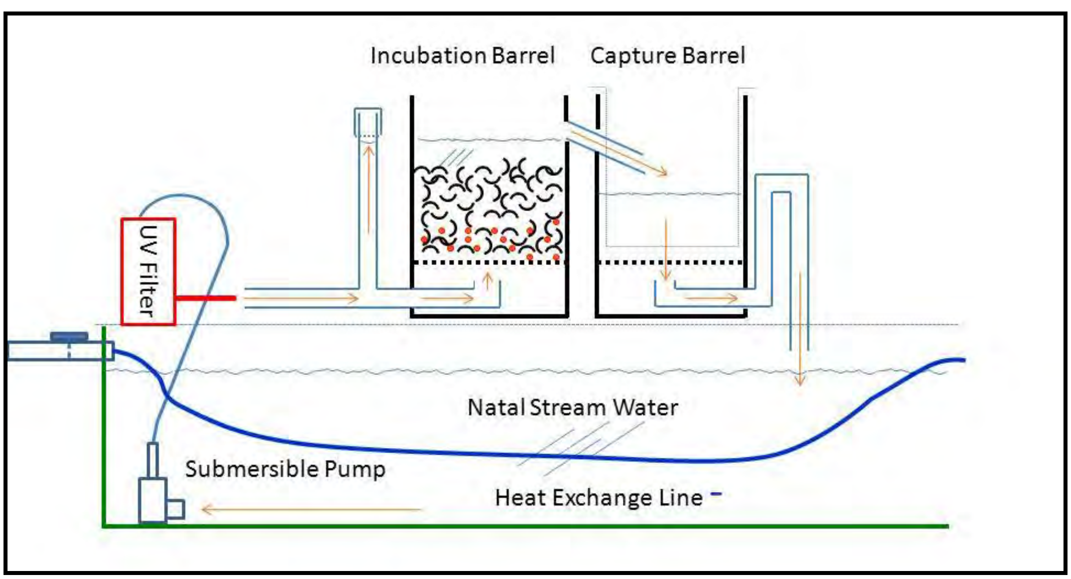

Figure 10. Setup details of incubators in recirculating RSI system.

b. Water Withdrawal Locations and Volumes

Water withdrawal locations for the three primary spawning tributaries have been selected and are listed below. Primary criteria used to evaluate site selection were vehicle (water tanker) access and actual feasibility to withdraw water from the creek at that location. Required permitting from Washington Department of Ecology for these withdrawals (i.e., Short Term Water Use Authorization) will be acquired on an annual basis (WDOE 2010). Continued authorization of short term water use will require a new application submission for each withdrawal site (i.e., creek) no later than September 1 of each year the project is in operation. Per the requirements of the permit, WDFW/Issaquah Creek Hatchery will record how much water is used from each site and these totals will be submitted with the annual application. Permissions and conditions to access sites for water withdrawals have been secured from the relevant entities.

Ebright Creek: Walter Pereyra residence or East Lake Sammamish Trail.

Laughing Jacobs Creek: Lake Sammamish State Park or East Lake Sammamish Trail.

Lewis Creek: West Lake Sammamish Parkway or Bridge crossing on 187th Avenue.

Additional withdrawal locations on other Lake Sammamish tributaries will likely be required in 3 to 5 years, when the supplementation program transitions its efforts towards the longer-term supplementation program goals (i.e., increase population distribution and abundance). These potential reintroduction or introduction sites for late-run kokanee will be thoroughly evaluated by the kokanee supplementation planning partners for their suitability and likelihood of success prior to their inclusion into the program. These tributary assessments will be incorporated into the supplementation planning document at the time they have been completed.

Each recirculating system requires 300-400 gallons of water to operate. This withdrawal volume would likely be required from each of the creeks on at least a weekly basis during the period of operation (February to June). However, these exchanges may be more or less frequent depending on the monitoring results of this year’s pilot operation.

c. Monitoring/Oversight

The daily operation and maintenance of the recirculating system will be implemented by Issaquah Creek Hatchery staff. Water quality will be tested on a regular basis to help determine how frequently system water exchanges are required. The recirculating system has been integrated into the hatchery’s existing alarm system to alert staff of any major system failures. A temporary backup water system/strategy has been designed should the system experience a catastrophic loss of recirculating water.

Release Strategies

a. Tributaries and Numbers Released

The supplementation program's general release strategies are expected to evolve over time and may depend on number of broodstock collected in any one year. Release strategies under the three-year (2010/2011 – 2012/2013) program goal are expected to focus only on the three current, primary spawning tributaries, Lewis Creek, Ebright Creek, and Laughing Jacobs Creek. Typically release numbers to each tributary will be proportioned based on annual escapement/broodstock collection numbers from each tributary in any one year.[6] Under the twelve-year (2010/2011 – 2021/2022) program goal, release strategies are expected to include other tributaries that currently have infrequent use or are part of the historical distribution for late-run kokanee (e.g., Pine Lake Creek, Zaccuse Creek, Vasa Creek), and possibly tributaries that were not part of their historical distribution (e.g., Issaquah Creek and/or its tributaries). These future release sites will ultimately be selected based on the likelihood of reestablishing or establishing a self-sustaining spawning group. Tributaries will be evaluated over the next several years based on their existing and anticipated future water quantity and quality, expected habitat protection, and the effectiveness of ongoing and future restoration actions. Before supplementation is implemented in a candidate stream, it will be shown that identified impediments to a self-sustaining population have largely been addressed or will be addressed prior to first-time spawners returning to that stream.

b. Release Sites within Streams

Release sites were selected based on following objectives: 1) maintain previously established stream imprinting; 2) minimize the predation risk to fry; and 3) minimize logistical challenges to accessing the stream. All fry will be released into the tributary assigned to the corresponding recirculating RSI system for that tributary. Site selection avoids or at least minimizes the presence of artificial lighting and minimizes the out-migrant travel time for fry to reach the lake while taking other objectives into account. Sites were stable and easily accessible by vehicle and or foot to minimize travel times and release logistics.

Based on these objectives, the following primary and backup sites have been selected for the three main spawning tributaries.

[6] As discussed in the Brood Stock Collection section, under very low escapement scenarios some spawners from a more productive tributary may be permanently "adopted" by a low or no escapement tributary in order to maintain at least some annual production to be released by the program into that tributary.

Lewis Creek:	Primary – West Lake Sammamish Parkway at the upstream extent of current spawning Backup – TBD
Ebright Creek:	Primary – 100 meters upstream of Pereyra residence Backup – East Lake Sammamish Parkway crossing
Laughing Jacobs Creek:	Primary – Pavilion at upstream end of State park Backup – East Lake Sammamish Parkway crossing

*If identified release sites become unsuitable in the future, mutually agreed upon alternate sites will be selected after an evaluation by the supplementation program partners.

c. Timing

Under the extended rearing strategy, fry will likely be pooled and held/reared for 1 to 4 weeks in order to reach an optimal release period. This pooling will result in fewer but larger release groups, which will likely help reduce predation rates on released fry. Depending on the annual broodstock collection, fry releases will typically occur between mid to late April based on general timing of the optimal rearing conditions within the lake. This timing coincides with increasing zooplankton densities within Lake Sammamish (Figure 11).

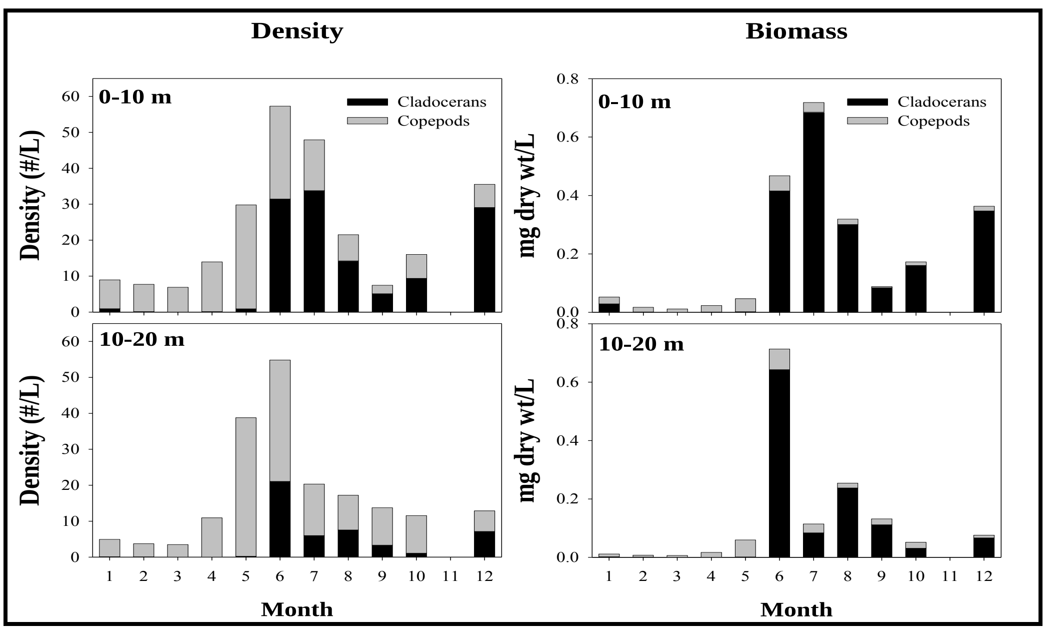

Figure 11. Zooplankton (cladoceran and copepod) density and biomass in Lake Sammamish by month (Berge 2009).

Hatchery coho smolt releases and the kokanee fry releases have been coordinated to avoid possible increased species interactions (predation on kokanee fry) within Lake Sammamish. Kokanee fry will be released at least 2 weeks prior to the annual scheduled (May 10) coho smolt releases from Issaquah Creek Hatchery to allow fry to move offshore into deeper waters. We will continue to reevaluate timing of kokanee fry releases to ensure the strategy balances optimal rearing conditions within the lake, reduced predation rates, and any advantages gained by releasing larger fry.

d. Methods

The following methods will be employed for fry releases:

Equipment: Appropriately sized holding container/tank with supplemental aeration will be used. Containers will be covered.

Transport: Fry will be hauled to the release site in their designated recirculating RSI creek water in covered/shaded containers; anesthetic will not be used in the hauling container/tank.

Time of day: All fry releases will be conducted at night, at least an hour after dusk, to minimize their risk to predation. When possible, target releases on a cloudy or rainy night, as opposed to clear nights, especially during full moon cycle.

Manner/technique: Fry will be gently released directly (no acclimation period) within the thalweg of the stream, versus the stream margins, to encourage emigration and further minimize their risk to predation.

Coordination with other activities: No fry trapping will be conducted during the night of releases.

Monitoring and Adaptive Management

To assess the success of supplementation efforts, some type of monitoring of the kokanee population is needed. Because supplementation groups of kokanee have their otoliths thermally-marked, a sample of the overall population of kokanee needs to be taken to determine what percent of the overall population consist of fish from the supplementation program and determine if there is any difference between supplementation groups. Removal of otoliths requires lethal sampling of fish; therefore, the best method is to collect otoliths from kokanee carcasses during spawning grounds surveys or from broodstock after they have had their gametes removed.

a. Sampling/Marking Design

It is currently anticipated that at least six different thermal otolith marks will be used annually for kokanee that are released by the program when a two-hatchery incubation stategy is employed, and at least three different thermal marks will be used when a single-hatchery strategy is employed. Three different marks, one for each of the three primary tributary groups, will be used at each of the incubation hatcheries. Since the thermal marks generated at each hatchery will be different, this will result in six unique otolith marks that allow for future monitoring of these release groups through time.

b. Otolith Sampling

Otolith collections will begin in the fall of 2012, when the first group of supplementation fish will be returning as 3-year old adults. Collection of otoliths will continue each year until the last group of supplemented fish has returned as 5-year old fish. Because the stream life of kokanee carcasses appears to be relatively short and often the number of available carcasses is low, all carcasses will have their otoliths removed if heads are intact. The current plans are to survey each of the three major creeks four times each week from late October to the end of January. If needed, additional surveys will be conducted to ensure an adequate sample of kokanee carcasses is obtained. Otoliths will also be removed from all adult kokanee collected for broodstock. If under some circumstance too many carcasses are available, some type of subsampling will need to occur. The exact level of subsampling will depend on the expected ratio of naturally-produced and supplemented adults.

c. Otolith Analysis

After annual field collections have been completed, all otoliths will be sent to the WDFW otolith lab for analysis. Annual results will be provided to supplementation partners before each spawning season to assess the need for any changes to the program. Before each spawning season, a detailed monitoring plan will need to be developed. The monitoring plan will include overall monitoring objectives and otolith collection goals.

d. Using Genetic Analyses to Monitor Supplementation Success

Genetic parentage assignment could be used as an alternative method, or in addition to otolith analyses, to monitor the success of the Lake Sammamish kokanee supplementation efforts. The technique, termed parentage-based tagging (PBT; initial description: Anderson and Garza 2005), involves comparing the genotype of offspring (either as juveniles or returning adults) to a database of potential parent genotypes. If all hatchery broodstock are included in the database of potential parents, then any potential offspring that do not assign to parents in the database are assumed to be of natural origin. Once the parents of an individual have been identified, the population of origin and the supplementation group of that individual is known as long as the entire family is maintained in the same treatment group. Because this method of monitoring provides assignment to family, fine scale information about differences in success of specific crosses made in the hatchery can be obtained. Additionally, all fish produced by the supplementation program are essentially tagged by genotyping the broodstock.

Fin clips are being collected and analyzed from all kokanee used as broodstock in the supplementation program to determine their population of origin using a suite of microsatellites, therefore the genetic signature of all potential parents of kokanee produced by the program is known (the potential parent database). An assessment of the power of this suite of microsatellites to exclude incorrect parent assignments should be carried out prior to use in parentage. If this assessment indicates that the suite of microsatellites is sufficient for parentage assignment, the only additional data that will be required for this assessment is the collection and analysis of potential progeny.

Project Duration

The maximum duration of the supplementation program will be based on criteria that minimize the likelihood that potentially deleterious genetic changes occur in the wild population.

a. Number of Generations Objective

The duration of the supplementation program is targeted for three generations, or approximately 12 years based on age 4 spawners. This generation objective is consistent with other recent conservation supplementation programs developed for at risk salmonids (Summer Chum Conservation Initiative). It is believed that a maximum duration of three generations will limit the risk of adverse, within and among, population genetic effects that could harm the target or conspecific wild populations (S. Phelps, WDFW, pers. comm., April, 1998 cited in WDFW and Point No Point Treaty Tribes 2000). As with the Summer Chum Conservation Initiative, this limit will also provide two generations (eight years) of adult returns to assess the program, prior to stopping egg takes.

It is recognized that an exception to this three-generation maximum, leading to an increase in the duration of a program, may be acceptable if there have been catastrophic declines in habitat condition, or if other uncontrollable factors affecting kokanee survival emerge during the course of a supplementation effort, making sustainable natural production unlikely. In such a situation, the risk of the project would be re-evaluated and measured against jeopardy to the status of the targeted stock that is likely if the program were terminated. A consideration of whether the supplementation program should be shifted to a gene pool conservation or captive brood program would be made.

b. Run-size Objective

TBD

c. Early Termination Criteria

TBD

Project Partners and Roles

A number of project partners are involved in implementation of the Kokanee Conservation Supplementation program. These partners will continue to be involved in future monitoring and evaluation of the program, as well as in any decisions regarding proposed program changes that result from new information gained either through program implementation, other related research, or other best available information. Each partner and their specific supplementation program role are described below.

a. Washington Department of Fish and Wildlife: Primary role is participation in overall supplementation program planning and implementation of program components located at Issaquah Creek Hatchery. This also includes general oversight/coordination of kokanee fry releases.

b. King County: Primary role is overall supplementation program planning and implementation of broodstock collection component of the program. Role may also include general oversight/coordination of kokanee fry releases.

c. U.S. Fish & Wildlife Service: Primary role is participation in overall supplementation program planning and implementation of program components located at Quilcene NFH, as needed. Also responsible for transport of gametes and eyed-eggs, respectively from and to Issaquah Creek Hatchery.

d. Local governments: Primary role is project coordination support (e.g., access for fry releases, local permitting, public outreach).

e. Other (Trout Unlimited, Friends of Issaquah Salmon Hatchery): Primary role of these organizations is additional on-the-ground program support where and when needed during implementation (e.g., broodstock collection surveys, fry releases, etc.). Trout Unlimited also collects ongoing outmigrant data for naturally produced kokanee fry, which provides important information to assist in the long-term monitoring of the supplementation program.

References

Anderson, E.C. and Garza, J. C. 2005. A Description of Full Parental Genotyping. pp 80-90 *In* Hankin et al. 2005. Report of the expert panel on the future of the coded wire tag recovery program for Pacific salmon. Report Submitted to the Pacific Salmon Commission. Available at: http://www.psc.org/pubs/CWT/EPfinalreport.pdf

Berge, H.B. 2009. Effects of a temperature squeeze on distribution, feeding, growth, and survival of kokanee (*Oncorhynchus nerka*) in Lake Sammamish. Master's thesis, University of Washington, Seattle.

Bosworth, A. and J. Spinelli. 2011. Lake Sammamish late-run kokanee 2010-11 spawning ground survey summary and escapement estimate. Washington Department of Fish and Wildlife, Region 4, Mill Creek, Washington.

Jackson, C. 2009. 2008/2009 Lake Sammamish late-run kokanee survey and escapement summary. Washington Department of Fish and Wildlife, Region 4. Mill Creek, Washington.

Jackson, C. 2010. 2009/2010 Lake Sammamish late-run kokanee spawning ground survey summary and escapement summary. Washington Department of Fish and Wildlife, Region 4. Mill Creek, Washington.

LSKWG (Lake Sammamish Kokanee Work Group). 2009. Goals and Priorities for the Lake Sammamish Kokanee Conservation Strategy. April 17.

Tilson, M.B., A.T. Scholz, R.J. White, and J.L. Hendrickson. 1995. Artificial imprinting and smoltification in juvenile kokanee salmon: implications for operating Lake Roosevelt kokanee salmon hatcheries. Prepared by Upper Columbia United Tribes Fisheries Research Center, Department of Biology, Eastern Washington University, Cheney, WA. Annual Report (1994) submitted to U.S. Department of Energy, Bonneville Power Administration, Portland, OR. Project No. 88-63; Contract No. DE-B179-88BP91819. 125 pp.

Warheit, K.I. 2010. Genetics report-Lake Sammamish Kokanee. 2009 spawners. February 12. Washington Department of Fish and Wildlife, Genetics Laboratory. Olympia, Washington.

Warheit, K.I. and C. Bowman. 2008. Genetic structure of kokanee (*Oncorhynchus nerka*) spawning in tributaries of Lake Sammamish, Washington. Report submitted to King County Department of Natural Resources and Parks, Water and Land Resources Division, and Trout Unlimited-Bellevue/Issaquah as partial fulfillment for Contracts 07-2047 (King County) and 07-2098 (Trout Unlimited). Washington Department of Fish and Wildlife, Genetics Laboratory. Olympia, Washington.

WDOE (Washington Department of Ecology). 2010. 2010/2011-short term water use authorization no. S1-28675 (Lewis Creek), S1-28676 (Ebright Creek), and S1-28677 (Laughing Jacob’s Creek). Northwest Regional Office, Bellevue, Washington.

WDFW (Washington Department of Fish and Wildlife) and Point No Point Treaty Tribes. 2000. Summer Chum Salmon Conservation Initiative. An Implementation Plan to recover Summer Chum Salmon in the Hood Canal and Strait of Juan de Fuca Region. J. Ames, F. Graves, and C. Weller, editors. Washington Department of Fish and Wildlife, Olympia, Washington.