SUMMER CHUM SALMON CONSERVATION INITIATIVE

An Implementation Plan To Recover Summer Chum Salmon in the Hood Canal and Strait of Juan de Fuca Region

Supplemental Report No. 6

Protocols For Summer Chum Salmon Supplementation Recovery Projects

Steve Schroder
Jim Ames
Washington Department of Fish and Wildlife

July 2004

Report Availability

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Department of Fish and Wildlife
600 Capitol Way North
Olympia, Washington 98501-1091

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FOREWORD

The original version of the following report was prepared by Dr. Steve Schroder; a Research Scientist with the Washington Department of Fish and Wildlife. The hatchery protocols presented below were originated in part from requirements of the *Summer Chum Salmon Conservation Initiative* (SCSCI), and in part from Dr. Schroder's extensive knowledge of salmon reproductive biology. The protocols were developed to facilitate the operation of artificial production recovery programs for various chum salmon populations in western Washington State. Jim Ames, a member of the Summer Chum Supplementation Technical Workgroup, edited the chum salmon protocols to tailor them to the needs of the programs being used to recover summer chum salmon populations in Hood Canal and the Strait of Juan de Fuca.

The SCSCI Supplementation Technical Workgroup thanks Dr. Schroder for his invaluable contributions to the summer chum recovery program. The SCSCI Supplementation Workgroup includes the following participants.

WDFW	**PNPT Tribes**	**NMFS**	**USFWS**
Kyle Adicks	Chris Weller	Tim Tynan	Tom Kane
Jim Ames			Dave Zajack
Thom Johnson			

INTRODUCTION

In April of 2000 the Washington Department of Fish and Wildlife and the Point No Point Treaty Tribes published a resource management plan designed to preserve and recover the summer chum salmon stocks of Hood Canal and the Strait of Juan de Fuca. That recovery plan, the *Summer Chum Salmon Conservation Initiative* (SCSCI - WDFW and PNPTT 2000), identified the status of regional stocks, factors for their decline, and recommended a series of artificial propagation, habitat, and fisheries harvest management measures that were necessary to recover summer chum salmon in the region to healthy self-sustaining levels.

A key element in the recovery plan is the use of hatchery supplementation to assist in the recovery of wild summer chum populations that are at risk of extinction. The objective is to use artificial propagation to preserve and expeditiously recover extant summer chum salmon populations, and to re-establish returns where stocks have been extirpated. The SCSCI provides a rigorous suite of operational and monitoring standards that are designed to minimize the risk of deleterious genetic, ecological, and demographic effects to supplemented and un-supplemented stocks.

The following report presents specific protocols for conducting a science based artificial production and monitoring project for the recovery of a summer chum salmon stock. The SCSCI requires that project sponsors operate within the bounds of various criteria, which guide facility operation and monitoring, however, requirements can vary depending on the status of the stock involved and the degree of monitoring and evaluation needed (see SCSCI Section 3.2). The following individual protocols may not be required of every project, and project sponsors should be guided by the conditions in their specific Hatchery and Genetic Management Plan (HGMP).

This report is presented in two parts. Part I describes the methods used to spawn, incubate, and track various biological parameters from adult collection through fry emergence and ponding. Part II delineates the procedures that are used to culture juvenile chum salmon, track their survival, growth, and document their size at release. These protocols are taken mainly from instructions provided to WDFW biologists and technicians, and citizen volunteer groups, that are participating in summer chum salmon recovery projects currently taking place in Jimmycomelately Creek, Hamma Hamma River, Lilliwaup Creek, Union River, Tahuya River, and Big Beef Creek. Recovery projects implemented for Columbia River fall chum salmon are also utilizing these protocols for the supplementation of Grays River, Chinook River, and Duncan Creek chum salmon. The report can be used for two purposes; to provide detailed background information about how supplementation project data are collected and how various procedures are carried out for individual chum salmon recovery projects, and secondly, it can also serve as a reference for the staff that will be conducting these projects

A companion report, *Monitoring and Evaluation Techniques for Summer Chum Salmon* (SCSCI – Supplemental Report No. 7; in press), presents a variety of techniques that can be used to evaluate the contributions of artificial production programs, and to assess the status of wild summer chum populations.

PART I: SPAWNING AND INCUBATION PROTOCOLS

The spawning and data collection procedures presented below expand upon the artificial production criteria contained in the *Summer Chum Salmon Conservation Initiative* (WDFW and PNPTT 2000), and are designed to achieve six goals. First, to assure that fish selected for broodstock are representative of the wild population; second, to ensure that the genetic heritage of a supplemented population is maintained; third, to track a variety of adult attributes through time in an effort to detect any consequences of inadvertent domestication selection; fourth, to document stock-specific traits; fifth, to monitor and evaluate the survival of fish produced from each conservation strategy implemented; and sixth, to provide information that can be used to shape recovery programs in the future.

PROTOCOL 1: Adult Broodstock Collection - Timing & Abundance

The first objective of broodstock collection is to obtain a sample of the adults that is representative of the wild population. The genetic complexity of a stock being placed into a conservation hatchery can be compromised if fish are collected over a narrow temporal period. The intent is to collect and proportionately use adults throughout the duration of a spawning run. In those instances where a temporary or permanent weir has been established this will not be a problem because fish can be captured and removed throughout the duration of a spawning run on a proportional basis. However, for projects not using a weir, it will be necessary to capture chum salmon brood stock in a variety of locations, within the stream or in marine areas adjacent to the stream mouth. The goal of these collection efforts is twofold, to use adults that have been collected over the course of the spawning period, and to collect enough fish to avoid deleterious effects from genetic drift and inbreeding depression.

To accomplish the first objective, summer chum should be collected from donor populations across the breadth of the freshwater return (mid August through October 15 – end date selected to preclude egg takes from fall chum), and at weekly levels proportional to average escapement timings for the returning population (SCSCI Appendix Report 3.1.1). Historical run timing data should be consulted to establish weekly capture goals so that a proportional sample can be extracted from the entire run.

The SCSCI has adopted specific numeric standards for brood stock collection for the summer chum populations of Hood Canal and the Strait of Juan de Fuca (see table below, and SCSCI section 3.2.2.3 f). These objectives are based on donor population size, and are applied to help retain genetic diversity. Minimum broodstock collection objectives are set to minimize the loss of alleles and reduce the fixation of others. Maximum collection levels are set to allow for at least 50% of escaping fish to spawn naturally each year (populations > 200). For small populations, no maximum is set as an emergency measure.

SCSCI allowable broodstock collection levels as determined by donor stock population size. (From: SCSCI – Table 3.2, page122)

Donor Population Size (Number of individual fish)	Allowable Broodstock Collection Levels Minimum	Maximum
< 100	25 pairs	none
100 - 200	25 pairs	50 pairs
> 200	50 pairs	50 % of total return

The use of a permanent or temporary weir and trap, which has preferably been located near the mouth of the stream, is the most effective method for the collection of a representative broodstock. Captured fish will be placed in fish tubes and held in an adjacent holding area until they are either artificially spawned or placed into a protected spawning area (e.g. a spawning channel). Sex, and date of capture will be written on each tube. An example of the form that will be used at the weir to record information on captured fish is shown below (some hypothetical entries are also provided to indicate the type of data desired). In the event that a portion of the returning chum salmon are scheduled for release above the weir, or that other salmonids are trapped at the weir; the occurrence of these fish will be noted in the miscellaneous observation field. All non-target fish will be released upstream of the weir. Data will be collected on any fish captured at the weir using the following form.

XYZ CREEK SUMMER CHUM SALMON
WEIR CAPTURE RECORD

Location___River Mile 0.3 – XYZ Cr.__

One-line represents one fish **Page _____of _____**

Date m-d-yr	Time	Sex	Condition[1] Ex, G, F, & P	Fish Tube No.	Release Location Hatchery or Stream	Misc. Observations (e.g. trap in or out of operation)
11-7-03	08:30	&	Ex	1	Hatchery	Green, held in tube # 1 in trap
11-8-03	09:00	%	Ex	2	Channel A	Rain, high flows trap is out. Two coho released upstream of weir.

[1] Condition definitions:

Ex = Excellent: obviously newly arrived fish, no fin erosion or scale loss, no sign of disease, sea lice often present.
G = Good: slight fin erosion on lower lobe of caudal, anal, ventral, and pectoral, no fungus or scale loss.
F = Fair: obvious fin erosion, some scale loss, some patches of fungus.
P = Poor: heavy fin erosion, scale loss, heavy patches of fungus, often close to death.
P Spent = Partially Spent: female that has spawned at least once but is not through spawning.
Spent = Spent: female that has completed egg deposition .

It is anticipated that broodstock will most often be trapped at a weir, however, beach seines or tangle nets may also be used to capture chum salmon brood stock for projects without a weir, and other types of gear may be tried and found to be effective. As in the above weir example, fish captured by alternative methods will be placed into individual fish holding tubes. The tubes will be 10" in diameter (25.4 cm) by 3 feet long (91 cm) PVC pipe, perforated with 1.5 " (4 cm) diameter holes, and equipped with removable end pieces or caps. The sex of the fish, and where, and when it was captured will be written on the tube in pencil. In addition, these and other field data will be recorded on the form that is shown below, which includes examples of typical entries.

XYZ CREEK SUMMER CHUM SALMON
BROODSTOCK COLLECTION FORM - NET CAPTURE RECORD

Date (M-D-Y)_____**Location**__Sunrize Beach – XYZ Bay_____

Personnel_____

One-line represents one fish **Page** _____**of** _____

Time	Type of Collection Gear	Sex	Condition[1] Ex, G, F, P, PS, & S	Tube #	Miscellaneous Observations
09:00	Beach Seine	%	Ex	1	Teeth caught in net
09:00	Beach Seine	&	G	2	
10:30	Tangle Net	&	PS	3	May be partially spent

[1] Condition definitions:

Ex = **Excellent:** obviously newly arrived fish, no fin erosion or scale loss, no sign of disease, sea lice often present.
G = **Good:** slight fin erosion on lower lobe of caudal, anal, ventral, and pectoral, no fungus or scale loss.
F = **Fair:** obvious fin erosion, some scale loss, some patches of fungus.
P = **Poor:** heavy fin erosion, scale loss, heavy patches of fungus, often close to death.
P S = **Partially Spent:** female that has spawned at least once but is not through spawning.
S = **Spent:** female that has completed egg deposition .

After a collection episode has been completed, the tubes will be placed into a live tank and transported to the spawning site and held in running water until the fish are artificially spawned. The location, date of capture, and other field data associated with a female will be linked to the biological information collected on that fish at the time it is spawned if the egg viability of individual females can be tracked. This would occur if the eggs from each female were incubated in iso-buckets or individual Heath trays. On the other hand, if eggs from multiple females will be combined, e.g. in Remote Site Incubator (RSI) trays, this will not be done because it would be impossible to link this information to individual performance. The information written on a tube will be erased once a fish has been removed and spawned so that the tube can be reused.

PROTOCOL 2: Collection Of Gametes From Broodstock Adults

The summer chum recovery plan requires that all supplementation programs meet two main goals: 1) that every adult in the broodstock contributes to the population, and 2) that the genetic

contribution from every fish is as equal as possible (SCSCI – Appendix Rpt 3.1; page A3.4). Protocols 2 and 3 present methodologies for spawning summer chum salmon that address the SCSCI objectives.

Once adults that will be used as broodstock have been collected and it is determined that they are ripe and can be spawned, the following procedures and forms will be used:

1) Adults will be killed with a sharp blow to the head.
2) Females will be killed first, and either have their vents closed by clamps or placed ventral-side up to prevent egg loss.
3) Females will be bled by severing a gill arch. This procedure significantly reduces the number of blood clots present in the eggs and helps ensure fertility.
4) Males do not have to be bled.
5) Capture data, i.e. location, date, condition, and tube number for each fish will be recorded on a field form filled out at the time the fish are killed (see sample of form below).

XYZ CREEK SUMMER CHUM SALMON
BROODSTOCK DATA

One-line represents one fish **Page _____of _____**

Fish #	Capture Location	Date of Capture	Condition at Capture	Tube Number
F-1	Union River	11 Nov 03	Ex	12
M-1	Union River	13 Nov 03	Ex	3

6) Before any eggs are taken the following data (examples indicated in the form below) should be collected on each female.

XYZ CREEK SUMMER CHUM SALMON
FEMALE BROODSTOCK DATA

Date_____ Personnel_____

One-line represents one fish **Page _____of _____**

Genetic #	& #	Wt (g)	Fork Length & Post-Eye Hyp	Condition	Egg Mass Wt.	Egg Sample Wt.	# Of Eggs In Sample	Mean Green Egg Wt	Est. Fecundity	%% Crossed With
00EN1	F-1	5,205.0	765-724	Ex	814.2	8.43	32	0.2634	3,091	M-1,2,3

See descriptions below for explanations for how each of these values or data were collected and what they mean. Blue entries (first five right hand columns) represent what can be collected prior to egg removal and red entries (last six columns) are data that must be collected after egg removal.

7) The **Genetic Numbers** will be assigned by the WDFW Genetics Lab (see Protocol 4 - Biological Sampling discussion below).
8) Each female should have a numbered Rite-in-the-Rain paper label stapled to one of her opercles. This label allows post-spawning data to be unambiguously linked to a specific female. Females should be numbered consecutively throughout a spawning season. That is, the first female spawned for a given year should be labeled as F-1, and the last female, for example, in a thirty-nine female project, would be labeled as F-39.
9) When handled, females should be supported under the head and also at the caudal peduncle and vents may be clamped shut with metal clips to help reduce egg loss. Fork length to the nearest mm should be obtained, and when possible a posterior-orbit-of-the eye to hypural plate measurement (POH) should be collected (optional). To determine the edge of the hypural plate, bend the caudal fin up and a crease will occur at its posterior edge. Then simply measure the distance between the posterior orbit of the eye to the crease edge. 10) To obtain adult weights use an electronic balance, place the fish in a pre-tared pan and read the weight off to the nearest 0.5-gram (depending upon the balance used). In all cases, protect the balance from the wind and place it on a level table. If a hanging spring-loaded balance is used, wait for the balance to stabilize and record the weight to the nearest unit, e.g. oz.
11) At the time a fish is being measured and weighed, examine the fins and body and make a condition assessment ranging from excellent to poor depending upon fin wear, scale loss, and fungus infestation. For females that have spawned, designate them as spent (no eggs left) or partially spent (some eggs remaining). In a few cases, it is difficult to assess whether a female has spawned without first weighing her egg mass, so some condition designations may change after eggs have been collected. For weighing and determining lengths, males and females should be treated in the same way. A separate data sheet is used to record biological information on the males, see below for column titles:

XYZ CREEK SUMMER CHUM SALMON
MALE BROODSTOCK DATA

Date_____ **Personnel**_____

One-line represents one fish **_ Page** _____**of** _____

Genetic #	% #	Wt. (g)	Fork Length Post-Eye Hyp. (mm)	Condition	&& Crossed With
00EN2	M-1	3,670.0	685/650	Good	F-1, 2, & 3

12) Prior to spawning, each female should be wiped down with paper toweling to remove any water, blood, or other contaminates. Eggs should be extracted via a spawning knife and allowed to gently fall into a plastic container. This container needs to be bone dry, and a label indicating the female's number placed on top of her eggs.

a) If the eggs are going to be transported to a hatchery for later fertilization they can be directly spawned into a plastic bag that has been placed inside the plastic container, e.g. a square two-gallon bucket. The bag should then be filled with O^2, sealed, and held in a cooler. The cooler should have a layer of crushed ice covered with soaked burlap, paper

towels or other light insulating material. A label with the Female's number should be inserted into the bag containing her eggs.

b) If a female's eggs are not going to be transported to another location her eggs can be directly deposited into the 2 gallon bucket and temporarily stored in a cooler supplied with crushed ice. As in the above case, a light layer of insulating material should be placed over the ice to prevent the eggs from freezing.

13) Milt should not be collected from males until either: a) all the females that will be used in a cross have been spawned (when fertilization takes place on site) or b) all the females that will be spawned on a given day have had their eggs extracted (when fertilization takes place at another location). If multiple trips are planned to the hatchery, then milt can be collected from the number of males needed to fertilize the eggs taken on each trip, but milt should always be collected after eggs have been obtained.

14) Males should be thoroughly wiped clean of all water, slime, blood, or other debris and milt should be expressed into clean, dry, one-liter capacity beakers. Before expressing milt into the beaker, one or two gentle squeezes should occur to extract any water residing in the cloaca. When little or excessively watery or congealed milt is obtained discard the sample and use another male.

a) If a male's milt is going to be transported to a hatchery, it should be poured into a large zip-lock bag. The greater the surface area of the bag the better, milt depth in the bag should not exceed ½ inch. A label with the male's number should be placed into the bag and O^2 should then be added to inflate the bag like a balloon. The bagged milt sample should then be stored in a cooler supplied with crushed ice. A layer of toweling should separate the samples from making direct contact with the ice.

b) If milt will be added to the eggs at the gamete collection area, then the one-liter beakers should be kept cold and away from light by placing them in a cooler supplied with crushed ice and a layer of insulating material.

15) Two electronic balances will be used to collect the egg data in columns 6-10 in the above table. One of the balances should have the capacity to weigh up to 1,200 g with 0.1 g accuracy (hereafter referred to as the 1,200 g balance), while the other should weigh items to the nearest 0.01gram. Typically, this balance will be able to weigh items up to 200 g (hereafter referred to as the 200 g balance). a) To determine the total weight of a female's eggs **(Egg Mass Wt)** the following steps have to occur.

i) Cut the bottom off a clean plastic pail (4 L plastic "dairy" pails are typically used) and place it on top of the 1,200 g balance. This bottom piece can be used repeatedly throughout the spawning season.

ii) Take a clean, dry plastic colander and place it on top of the pail bottom piece and tare the balance to zero.

iii) Next take another plastic colander and insert it inside a dry, clean plastic bag (for example a white trash bag) and then place the colander that was just tared on top of the colander that is inside the plastic bag. When assembled, the bare colander will be stacked on top of one that is lined with a plastic sack.

iv) Pour the eggs and ovarian fluid from a single female into the bare colander. Lift up the colander and very gently swish the green eggs around the colander in an effort to get the ovarian fluid to fall through the colander and land on top of the plastic bag covering the lower colander. After most of the ovarian fluid has been decanted, remove the plastic lined colander with the ovarian fluid; place the colander with the eggs on top

of the plastic pail bottom and record the total egg weight to the nearest 0.1 g. (Note: The retained ovarian fluid in the plastic bag will be recombined with the eggs during the fertilization process. See Protocol 3b below). Since the pail bottom and colander were previously tared, the balance will only register the egg mass weight. In a few instances, the total egg mass may weigh more than the 1,200 g balance's capacity. When this occurs, pour some of the eggs into another clean and dry colander. Reweigh the remaining eggs and record that number on the form. Then pour the weighed eggs into a dry plastic pail. Take the remaining eggs (that were placed in the new colander) and gently pour them back into the tared colander and get their weight. Record this number. The total egg mass weight can be obtained by adding these two values. Combine all the eggs from a female into the same pail so that they can be divided up into equal lots (see Protocol 3)

b) To get the **Egg Sample Weight** and **Number of Eggs In Sample** values the following steps need to be taken:

i) Place a plastic weighing boat (14 cm square by 2.5 cm deep polystyrene dish) on the 200 g balance and tare the balance to zero.

ii) Take a clean dry plastic spoon and collect about 6 to 10 grams of eggs (a spoonful) from the egg mass just weighed and place it in the tared weighing boat. Record the sample weight to the nearest 0.01 g.

iii) Use the spoon handle and gently count the eggs in the sample. One approach for doing this is to place the eggs into groups of five and after this is done count these sets and add the remaining eggs (1 - 4) that didn't make up a set to get a final sample count. These counts are critical so after placing the eggs into sets of five, double count the eggs to make sure that a correct count has been made.

c) Use an electronic calculator to determine a **Mean Green Egg Weight** by dividing the sample weight by the number of eggs in a sample. For example, suppose that an egg sample weighed 8.43 grams and that there were 32 eggs in the sample. Mean green egg weight would then equal 8.43/32 or 0.2634 grams.

d) Once the egg mass and mean green egg weight are known it is possible to estimate **Fecundity** by dividing total egg mass weight by mean green egg weight. In the table above, female F-1 had an egg mass weight of 919.4 g and a mean egg weight of 0.2634. Therefore her fecundity was estimated to be 919.4/0.2634 or 3,491 eggs. Invariably some ovarian fluid will remain in the egg mass and consequently when fecundity is calculated in this fashion it is often overestimated by about 3 to 4%. If the daily egg take total is adjusted by about 4% a close approximation of the actual number of eggs collected is obtained.

PROTOCOL 3: Creation Of Factorial Crosses And Fertilization Procedures

Whenever possible, factorial crosses will be used during the fertilization process. This approach helps maintain the genetic diversity of a supplemented population by reducing the risk that any individual will be mated with an unfertile partner. An unlimited number of factorial mating schemes are possible, but the ones most commonly used are 2&& x 2%%, 3&& x 3%%, and sometimes when uneven numbers of males and females are available 2 x 3 combinations. To create a factorial cross the following rules need to be followed:

1) Divide the egg mass of each female into the same number of portions as the number of males used to create her cross. For example, when three males will be used to fertilize eggs, divide each female's egg mass into three equal portions. To produce equal portions, divide the total egg mass weight by the number of males that will be used (three in this case). Pick up a dry and clean plastic pail (4 liter "dairy" pails are typically used); place it on the 1,200 g balance and tare to zero. Then gently pour some eggs into the pail until it is four or five grams less than the desired value. (As eggs sit in a colander, ovarian fluid will continue to drip off, so by deliberately creating "underweight samples" it is possible to create egg lots with approximately the same weight). Next take some (≅ 33% in this case) of the ovarian fluid that is retained in the plastic lined colander and add it to the egg sample. Place a label in the pail with the female's number on it, and put the pail in a cooler supplied with crushed ice covered with toweling until needed. Repeat this two more times to get the three egg lots. While dividing up the eggs, look for broken or over-ripe eggs. If any are seen, note their presence on the female form under miscellaneous observations. Over ripe eggs have a clearly defined polar body (usually a highly colored oval spot) while the rest of the egg tends to be less well pigmented and broken eggs can be detected by observing split shells. Both broken- and over-ripe eggs can significantly reduce viability. Also, if obvious blood clots are present in the eggs, remove them by touching them with a dry paper towel, they too will reduce fertility.
2) Repeat this process for each female that will be used in a cross. When a 3 x 3 cross is being created there will be 9 pails of eggs, three from each female. In the case of a 2 x 2 cross, only 4 pails will be present, when a 2&& x 3%% cross is made there would be 6 pails, three from each female (the number of pails needed equals number of females x number of males)
3) Once all the egg lots needed for a cross have been weighed out, the pails should be retrieved from their coolers and placed into an array on a table. See below for an example of 3 x 3 Factorial Cross Array.

An Example of A 3 X 3 Factorial Cross Array Showing the Location of Egg Lots From Each Female

	Column 1 & F-1	**Column 2 & F-2**	**Column 3 & F-3**
	O	O	O
Row 1 % M - 1	Pail #1 **M – 1 x F - 1**	Pail # 1 **M – 1 x F - 2**	Pail #1 **M-1 x F - 3**
	O	O	O
Row 2 % M – 2	Pail # 2 **M – 2 x F – 1**	Pail # 2 **M – 2 x F – 2**	Pail # 2 **M – 2 x F – 3**
	O	O	O
Row 3 % M – 3	Pail # 3 **M – 3 x F – 1**	Pail # 3 **M – 3 x F – 3**	Pail # 3 **M – 3 x F – 3**

4) Note that in the above array the egg pails from each female have been laid out in columns and the males are in the rows. This type of array allows each female to have one-third of her eggs fertilized by each male in her cross. And conversely, each male is able to fertilize one third of every female's eggs. Similar arrays can be set up for 2 x 2 and 2 x 3 crosses.
5) As mentioned above, milt is expressed into liter containers and stored or placed into oxygen filled bags if it was transported. Use a 10 cc syringe to collect milt from each sample, and then distribute equal amounts of a male's milt into each pail he is supposed to fertilize. The syringe makes it easy to distribute milt. In a few cases, only a small amount of milt may be obtained from a male. When this occurs the gradations on the syringe make it possible to equally distribute milt among the egg lots a male is suppose to fertilize.
6) After milt has been added to each egg lot, pour in enough water to completely cover the eggs by about a half-inch to an inch and then gently swirl or stir them for about 30 seconds. If back-up males are going to be used, add their milt to each pail about 30 seconds after water has been added and then gently stir or swirl the eggs again. In the factorial crosses, the males can back up one another, e.g. M-1 can be used to back up M – 2, M – 2 for M – 3, and M – 3 for M – 1. If one-to-one crosses are made, milt from another male will have to be added.
7) Let the newly fertilized eggs remain in their pails for approximately 2 minutes at that time the eggs from a female should be recombined and either placed into a heath tray (one female per tray), iso-bucket, or a RSI egg tray. The eggs are then soaked for 60 minutes in a PVP solution. After which they are moved into the incubation system where they will remain until eyeing. When eggs from a single female are placed into a heath tray or iso-bucket, the tray or bucket should be labeled with the female's number and spawning date. Plastic flagging can be used to label Heath Trays and iso-buckets.
8) Whenever possible each female's eggs should be incubated separately. This allows her fertility to be assessed, provides another opportunity to obtain a more accurate fecundity estimate, and also makes it possible to record the type and quantity of any abnormal offspring produced.
9) After spawning operations have been completed all adult carcasses should be returned back to their natal streams and the number of such carcasses should be reported to WDFW for departmental records.

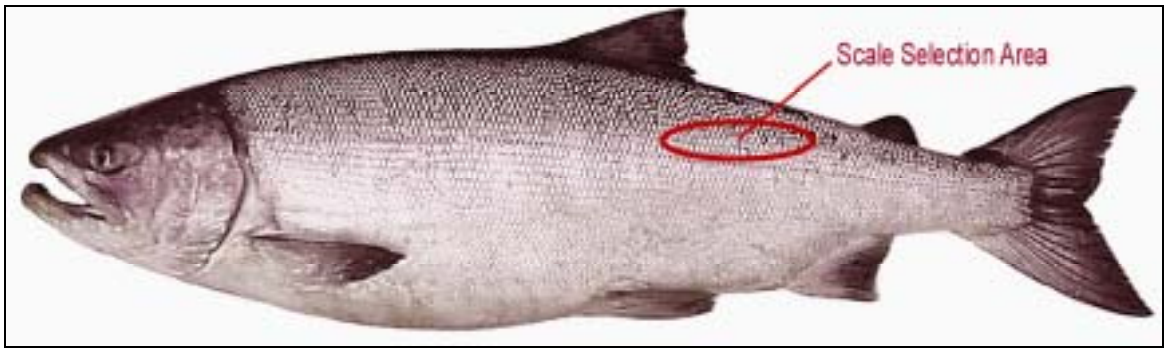
PROTOCOL 4: Collection Of Biological Samples From Broodstock Adults

The Summer Chum Recovery Plan recognizes that monitoring and evaluating the effects of hatchery supplementation on the recovery of natural summer chum populations are critical objectives of the conservation plan. The purpose of monitoring and evaluation is to collect information that will help determine: 1) the degree of success of each project; 2) if a project is unsuccessful, why it was unsuccessful, 3) what measures can be implemented to adjust a program that is not meeting objectives set forth for the project (SCSCI - 3.2.2.4 Monitoring and Evaluation).

To provide the needed information to meet the SCSCI monitoring and evaluation goals, a suite of biological samples will be collected from each spawned fish. In order to link these samples to an individual fish each spawned animal will be provided with a **Genetic Number** provided by WDFW's Genetic Lab. In the example table shown on page 13, female F-1 was given 00EN1

(Brood Year/Stream Code/Fish Number) as her Genetic Number. Depending upon the species and population, different biological samples may be collected. However, some samples, such as scales will be collected from all fish regardless of their origin. All of these samples should be collected after gamete extraction has occurred. Directions on how to collect the samples are provided below:

1) Scale samples—Lay the fish on its right side and remove three scales from the preferred area by using forceps or modified hemostat scissors. A preferred area scale is located directly below the posterior edge of the dorsal fin and three rows up from the lateral line. If these scales look like they have been regenerated (e.g. obvious body scars in the area) collect scales slightly above, below or to one side of the area, or flip the fish over and collect scales on the left side of the fish in the preferred area. Scale removal can be challenging, particularly in males that have been in freshwater for a while. Take the lower part of the forceps or hemostat scissor blade and slip it underneath a scale, grasp the scale and forcefully jerk it toward the fish's head and then quickly jerk it toward the tail. Scales have two surfaces, the top is ridged with circuli and the bottom is smooth. Take the scale and place it ridge side up on a gummed scale card. If the forceps or hemostat scissors are not rotated the ridged surface will be facing up, simply place the scale face up on the card. The mucus on the scale is enough to cause it to firmly adhere to the card. If a scale is clearly damaged collect another one to take its place. The cards are not really designed for recovery project purposes, so for future data entry add the female or male number in pencil adjacent to the set of three scales removed from that fish.



2) Pathogen samples—A minimum of 60 males and 60 females will be sampled through the spawning season to collect tissues and fluids used for pathogen screening. **Kidney** and **spleen** samples are collected from both the male and female fish, and **ovarian fluid** samples are also obtained from females. Combining the tissues and fluids from five fish creates pooled samples. First, use a spawning knife to open up the body cavity of the males (the females have already been opened for egg removal). Next, cut pencil eraser size chunks of spleen and kidney from five fish of the same sex and place them in a twirl pack plastic bag. These tissue samples are to be segregated by sex, so that samples from males are not mixed with those from females. Use one scalpel per fish, after using it place it in a beaker filled with PVP (an iodine based disinfectant): to disinfect it, pull out another scalpel and use it on the next fish, and continue to rotate scalpels as needed. WDFW's Pathology Lab has a sample form that needs to be filled out indicating the stock origin and number of fish present in each pooled sample. Five-cc syringes will be provided to collect about 1cc of ovarian fluid from each female. Place five samples into each syringe. Cap the syringes to prevent spillage. The twirl packs and

syringes can be labeled by using a Sharpie pen, place the samples in a cooler supplied with ice and return to WDFW's Pathology Laboratory.

3) Genetic samples—DNA samples are collected from all fish spawned for a recovery project**.** WDFW's Genetic Lab will provide sample labels (see above), sample tubes, ethanol, and a paper punch. The labels are printed on "rite-in-the- rain" paper and need to be cut so that they can fit inside the sample vials. Fill the vials with ethanol from the supplied squeeze bottle. Use the paper punch to collect six samples from the operculum of each fish, and insert the six plugs into a vial, and cap. Allozyme samples—In rare instances, allozyme samples will also be taken for Genetic Stock Identification. When allozyme samples are required, the WDFW Genetics Lab should be contacted to obtain instructions for sample collection.

4) Egg samples—Five eggs will be collected from each female and placed into a plastic vial containing water and a label indicating the female's number, date, and stock. Place the egg samples in the same cooler containing the pathology samples and ship them to WDFW's Pathology Laboratory. After the eggs have water hardened they will be individually weighed to the nearest mg by WDFW staff.

5) Otolith samples—Adult salmon produced from supplementation projects will return to spawn either in the stream they were released into as fry or possibly into adjacent watersheds. To estimate their survival and abundance, the origin of fish returning to these streams has to be determined (see SCSCI Suppl. Rpt. No 7, *Monitoring and Evaluation Techniques for Summer Chum Salmon*; in press). Once that has been done it will be possible to: a) assess the contributions project fish may be making to their local population, b) to determine their straying rates, and, c) to estimate the number of natural strays entering a stream. To facilitate this identification, every fry released from summer chum salmon recovery projects will either be thermally marked or fin clipped. External marks such as fin clips can be easily seen and recorded. Thermal marks, on the other hand, are bar codes that have been induced into otoliths during early ontogeny by purposeful water temperature manipulations. Thus, they can only be detected after an otolith has been processed in a laboratory. To detect these marks, otoliths from all the fish used as brood stock will be collected at the time of spawning. In addition, otoliths will be removed from the carcasses of the fish placed into spawning channels. Up to 500 such samples will be randomly collected. Furthermore, when feasible, otoliths will be obtained from a representative sample of fish that have naturally spawned in streams that potentially could have received project-origin adults. These collections will begin when the first three-old fish produced from a project are expected to return. Otoliths will be placed in plastic vials supplied with a label. The labels associated with otoliths collected from fish used as brood stock will possess the Genetic designation number of the fish from which it was collected. This will make it possible to link the information contained in the otolith to all the biological information that was collected at the time the fish was spawned, and during the subsequent incubation and rearing periods. Specimens obtained from individuals placed into channels or collected from fish spawning in natural streams will be placed into small plastic vials and labeled with the fish's sex, POH length, and date of collection. The channel name (e.g. A, B, etc.) will also be included on this label if the otolith came from an adult placed into a spawning channel. For otoliths obtained from naturally spawning fish the label would include the stream name and an approximate location of collection. When carcasses are sampled in streams GPS (Geographical Positioning System) coordinates should be collected if possible. These location data will be used to assess the in-stream distribution patterns of project fish.

PROTOCOL 5: Evaluations At The Eyed-Egg Stage Of Development

When eggs from each female have been incubated separately from one another, egg viability and refined fecundity estimates can be made. In those cases where eggs from multiple females have been combined, e.g. in RSI trays, less precise green-egg to eyed-egg mortality rates can be measured to provide an overall average survival rate for this life-history stage. At the eyed stage of development it also is possible to split eggs into multiple treatment groups when different rearing or release strategies are planned.

When Females Have Been Incubated Separately

The following form will be used when each female's eggs have been incubated separately.

XYZ CREEK SUMMER CHUM SALMON
SURVIVAL TO EYEING/EGG NUMBER - INDIVIDUAL FEMALE EGG INCUBATION

Date_____ **Personnel**_____

& No.	# Of Dead Eggs	Total Wt. of Eyed Eggs Wt.	Residue	Wt. of Egg Sample Wt.	Residue	No. of Eggs In Sample	Est. # of Eyed Eggs
& F-1	40	872.9	0.7	6.70	0.02	22	2,873
		-.7		6.69	0.01	22	2,873
		872.2		8.09	0.02	27	2,918
				8.66	0.01	29	2,924
				6.71	0.02	22	2,868

Explanation of the data entered in each column:

Column 1 "& No": Number of the female whose eggs were examined.

Column 2 "# Of Dead Eggs": Not every egg will produce an embryo and unfertilized eggs or eggs that have died during early development need to be removed because they may become infested with pathogens that can attack adjacent live eggs. Generally, salmon eggs are sensitive to mechanical shock until the early eyed-stage of development. Consequently, eggs should not be disturbed prior to eying. However, shortly after reaching this stage, they will need to be "shocked". This is commonly accomplished by shaking them or dropping them a short distance. Twenty-fours after this treatment, dead eggs will turn white and they can be removed. This color change occurs because the shocking procedure ruptures the yolk membrane allowing a protein called globulin to escape from the yolk and form a white precipitate in the perivitelline space of the egg. The number in Column 2 equals the quantity of dead eggs removed from a Heath Tray or Iso-bucket after shocking has occurred.

Column 3 "Total Wt of Eyed Eggs": To obtain the data requested in columns 3 and 4, set up the 1,200 g and 200 g balances. Once again, place a cut-off plastic pail bottom and a clean colander on the 1,200 g balance and tare to zero. After all the dead eggs have been removed from a Heath Tray or Iso-bucket, pour the remaining eyed eggs into the colander that was tared, and rotate the eggs in the colander to remove as much water as possible. Place the colander and eggs on the balance and record its weight. Pour the recently weighed eggs into another colander and then re-weigh the colander that held the eggs. If the colander and plastic

pail bottom do not contain any water residue, this new weight will equal zero. However, up to several grams of water may have fallen down into the plastic pail bottom or adhered to the holes or slots of a colander and it is important to subtract this weight from the total egg weight value to get a better estimate of the total weight of a female's eyed-eggs. After the residue weight has been recorded, wipe the plastic pail bottom dry, and lightly tap the colander on a tabletop or other surface to extract any water. Re-tare pail bottom and colander to zero prior to weighing another female's eyed-egg mass.

Column 4 "Weight of Egg Sample": After the eyed egg mass and water residue weights have been obtained use the 200 g capacity balance to obtain five independent egg sample weights from the recently weighed egg mass. In this instance, place a weighing boat on the balance and tare the scale to zero. Take a plastic spoon and randomly collect about 6 to 12 grams of eggs (a spoonful) and place them on the tared weighing boat. Record the sample weight. The residue weight will be determined after the eggs are counted in the next step of this procedure.

Column 5 "No. Of Eggs in a Sample": Next, count the number of eggs in a sample at least twice to ensure that an accurate count has been made; place this value in Column 5. Again, segregating the eggs into groups of five helps speed up this process and allows a rapid recount to occur. After the eggs have been counted twice, return them to their egg mass. Next, take the weighing boat and re-weigh it on the 200 g balance. Again, some water residue will undoubtedly be adhering to the weighing boat; record the weight of this water in sub-column labeled "residue" in Column 4. Repeat this process four more times so that a total of five independent egg sample weights have been obtained. Either blot the weighing boat dry or use another one each time a sample is weighed. Always re-tare the balance to zero before weighing a new sample of eggs but do not tare the balance until a water residue weight has been obtained, and the value entered in Column 4.

Column 6 "Est. No. of Eyed Eggs": The data in this column are often not directly entered in the field. Instead, a computer spreadsheet program, e.g. Excel, is used to calculate an estimate by first determining an average eyed-egg weight for each sample [(Egg Sample Wt. - Weight of Water Residue for that sample)/(Number of eggs in the sample)]. For instance, in the above form, the first egg sample weighed 6.70 g – 0.02 g or 6.68 g. There were 22 eggs in the sample, thus average egg weight equaled 6.68/22 or .304 g. Once the average eyed-egg weight in a sample has been ascertained, then an estimate of the total number of eyed eggs produced by a female is calculated by dividing the total weight of all her eyed eggs by their average weight, or in this instance 872.2g/0.304g. Each eyed-egg sample is then used to calculate an independent estimate of the number of eyed-eggs produced by the female. These five values are then used to produce an overall mean estimate with 95% confidence intervals. For the example above, the overall mean estimate was 2,891 (95% C.I. = "40 eggs). A fecundity estimate is obtained by simply adding the number of dead eggs and any sampled eggs to the eyed-egg estimate (e.g. 2,891 + 40 dead + 5 sampled = 2,936). These fecundity estimates appear to be less subject to error than those produced at spawning and can be used to calculate survival rates from one life-history stage to the next.

Splitting Egg Lots To Produce Different Treatment Groups When Egg Lots Are Incubated Separately

In some instances, multiple rearing treatments or transplants of eggs are planned as part of an overall recovery strategy. When this occurs, the offspring produced from each fish should be equally represented in every cultural strategy. When the eggs from each female are incubated separately from one another, precise allocations of eggs into each planned treatment group can occur at the eyed-stage of development. This can be accomplished by dividing the total weight of a female's eyed eggs into the number of treatment groups required. In a situation where there are two planned treatments, the total weight of the eyed-egg mass is simply divided in half. Whenever possible, split egg lots should be incubated on a female-by-female basis so that additional survival and other offspring traits can continue to be monitored.

Evaluating Mortality When The Eggs of Females Have Been Combined Immediately After Fertilization

In a few instances, it will not be possible to incubate the eggs from different females separately from one another. When this occurs, eggs from multiple females will be incubated together and it is not possible to track individual mortality rates or gather other female-specific data on offspring performance. Nonetheless, at the eyed-stage the eggs will be shocked and mortalities removed and counted. To obtain an overall estimate of egg to eyed-egg survival, the number of mortalities found are subtracted from the sum of all the green eggs placed into an incubation device that held eggs from more than one female, e.g. a Remote Site Incubator (RSI). RSIs may have multiple trays of eggs in this case survival estimates can be made on a tray-by-tray basis. Here the number of mortalities observed on a tray is subtracted from the number of newly fertilized eggs placed on that tray. Mortality estimates done on a tray-by-tray basis may be quite informative. Salmon embryos are sensitive to mechanical shock during early embryonic development. The first eggs placed into an RSI will be located in the lowest tray, as eggs are added more trays are stacked one on top of the other. A tray-by-tray analysis of mortality would allow one to determine if the placement of trays and other activities associated with adding additional eggs to a RSI inadvertently causes mortalities to occur. If that were the case, higher mortalities would be expected in the lowest trays.

In those instances where eggs incubating on RSI trays need to be subdivided into separate treatment groups, gravimetric estimates of egg numbers will need to be calculated so that allocations can be made. The following form should be used to gather the data needed to make a gravimetric estimate of the number of eyed-eggs present on each RSI tray or other comparable incubation device. An explanation of how the data will be used to estimate egg numbers follows.

XYZ CREEK SUMMER CHUM SALMON
SURVIVAL TO EYEING/EGG NUMBER - MIXED FEMALE EGG LOT INCUBATION

Date_____

Personnel _____

RSI #	Tray #	# Of Dead Eggs	Wt. of Eyed Eggs		Egg Sample Wt.		# Of Eggs In Sample	Est. # of Eyed Eggs
			Wt.	Residue	Wt.	Residue		
1	1	254	467.8	0.9	6.52	0.02	22	8,249
			678.0	1.2	6.07	0.02	19	7,654
			702.6	1.5	6.41	0.01	23	8,759
			593.2	0.7	6.68	0.02	22	8,051
			Corrected Sum for Tray					Average
			2437.3					8,178

Explanation of data entries:

Column 1 "RSI #": Number of RSI, Heath Tray Stack, or other incubation device used to incubate the inspected eggs.

Column 2 "Tray #": Number of the individual tray in the RSI, Heath Tray stack, etc.

Column 3 "# Of Dead Eggs": Number of dead eggs removed from the tray.

Column 4 "Wt. of Eyed Eggs, sub-columns Wt. & Residue": These values are calculated exactly as before, using the 1,200 g balance. A colander and pail bottom are tared to zero, eggs are removed from the tray placed in the tared colander to remove excess water and the sample is then weighed. The weight of any residual water adhering to the colander and pail bottom is determined by re-weighing them on the balance. Both weights are placed on the form as shown. Weigh all the eggs on a tray in a similar fashion until done. The form for this work will have ten spaces instead of four shown in the above example to accommodate large groups of eggs. The "Corrected Sum for Tray" simply represents the sum of all the weight values minus the sum of all the residue weights. This can be rapidly calculated by using an electronic calculator.

Column 5 "Egg Sample Wt, sub-columns Wt. & Residue": Randomly remove four or more samples of eyed-eggs and weigh them on the 200 g capacity balance using the same procedures outlined for egg lots from individual females. Residue weight will be measured and recorded as a part of step #6 below.

Column 6 " # Of Eggs In Sample": Double count the number of eggs in each sample and record that number in Column 6. Weigh any residual water for each sample and record the value in the Residue sub-column of Column 5.

Column 7 "Est. # of Eyed Eggs": These values will most likely be calculated by using a spreadsheet program such as Excel. Each egg sample will provide an independent estimate of the number of eggs on a tray. These values will be used to calculate an overall mean egg number per tray and 95% confidence intervals around that estimate (Note: The more samples collected the smaller the confidence interval will be. For this type of information it appears that four to five samples will provide enough information to create relatively tight confidence intervals). Overall survival to the eyed-stage can be calculated by adding the mortality counts obtained from a tray to its estimated egg number value and then dividing the live egg value by the number of the live eggs plus dead eggs. For example, suppose it was estimated that a tray

had 10,215 eggs and that 386 dead eggs had been removed from that tray. Survival to the eyed-stage of development for that tray would then equal 10,215/(10,215+386) or 96.36%.

Splitting Egg Lots To Produce Different Treatment Groups When Egg Lots Have Been Combined

Once the total weight of the eyed-eggs incubating on a tray has been determined, divide that sum by the number of groups needed. For example, the corrected weight of all the eyed eggs in the Table immediately above was 2,437.3 grams. If two equal-sized groups are desired simply divide this sum in half and use the 1,200 g balance and colanders to weigh out the groups. Other proportional splits can be easily obtained as well. The weighing process, which will thoroughly mix the eggs, should ensure that offspring from each adult fish is represented in every treatment group.

PROTOCOL 6: Marking All The Fish Produced From Each Recovery Program

All summer chum salmon liberated from a recovery program must be uniquely marked so that their survival and distribution patterns can be evaluated (SCSCI- Section 3.2.2.4; page129).. Moreover, many recovery programs utilize several different incubation and rearing procedures. Marked fish will make it possible to ascertain the costs and benefits of each of these strategies. So far, two marking methods have been used to identify summer chum salmon from recovery projects, thermal marking and fin clips. (See SCSCI Suppl. Rpt. No 7; *Monitoring and Evaluation Techniques for Summer Chum Salmon***;** in press).

Thermal marks are created by purposefully manipulating water temperatures experienced by embryos and alevins from the eyed-stage through yolk absorption. Each time water temperatures are dropped about 2 – 4 degrees C a distinctive black band is deposited in the microstructure of a developing otolith. Every year in Washington State, approximately 25 to 30 million embryonic salmonids are marked using this technique. Worldwide, the procedure is used to mark over a billion salmon every year, more than any other marking tool.

Thermal events (exposure to chilled water for 8 to 48 hrs) are used to create systematic patterns in otoliths, essentially bar codes that are produced by following some simple rules. WDFW Otolith Lab personnel will develop the codes, and provide each facility with a schedule that indicates when and for how long developing eggs should be exposed to chilled incubation water.

Prior to thermal marking, portable electric chillers, chiller boxes, pumps, flex hoses and other equipment will be established at a hatchery or field incubation location. Flow rates out of the chiller boxes will be regulated and monitored. If equipment failures occur, at least one backup chiller will be on site and WDFW Otolith Lab personnel will make repairs as needed. Because of the number of thermal marking projects that are occurring around the state it is not possible for lab personnel to move hoses, so local staff will be asked to follow the marking schedules that have been generated: essentially just moving chilled water from one location to the next at predetermined times.

Salmonid embryos can be thermally marked both before and after hatching. In those programs that require eggs to be incubated in RSIs, simple pre-hatch marks will be used. When fish can be marked for longer periods (e.g. throughout the alevin stage) more complex codes are possible. Voucher samples will be removed from each batch of marked fish to determine mark quality and form. In a few instances, electrical problems or scheduling conflicts may prevent fish from being marked precisely as planned and it is therefore important that a known sample of the produced mark is obtained so it can be used to help interpret subsequent otolith recoveries. Different thermal marks will be applied each year and will be designed so that project and treatment origins can be identified.

To date, only the Big Quilcene summer chum supplementation program has used a fin clip mark to identify project fish. This marking technique has utility when large numbers of adult fish are to be screened for the mark. All adult capture projects should routinely examine summer chum for the occurrence of clipped adipose fins.

PROTOCOL 7: Ponding Procedures And Evaluating The Occurrence Of Mortalities And Abnormalities From The Eyed-Stage Through Yolk Absorption

When eggs have been incubated in RSIs, the incubating fish emerge on their volition and thus it is not necessary to determine when they should be placed in rearing vessels. Judging when to liberate fry held in Heath Trays or other incubation devices that physically prevent natural emigration is a little more challenging. Typically, chum fry emerge from redds with condition factor values (K_D) (Bams 1970) that range from 1.8 to 2.0 and these values should be used to guide when ponding will occur. Fry condition factors (K_D) are discussed in Part 2 below.

Quantifying Mortalities And Occurrence Of Monstrosities At Ponding When Female Egg Lots Have Been Incubated Separately From One Another**.**

When the eggs from each female have been incubated separately from one another it is possible to obtain mortality data (from eyeing to ponding) and document the occurrence of any abnormalities present in her offspring at the time her fry are being ponded. This can be accomplished by pouring all the fry from an incubation tray into a shallow trough filled with running water that has been lined with fine meshed nylon netting. Dead eggs and alevins adhering to the incubation tray are removed and counted. Small lots of fry are then dipped out of the netting and inspected for obvious deformities. All deformed fish are described, counted, and retained. In addition, five normal fry from each tray will have their lengths (fork length to the nearest mm) and weights (to the nearest mg) measured. These data are used to calculate a mean K_D value (see Ponding Protocol 1 below for formula*)*. The remaining fish are then either ponded or transported to a rearing location. The form below is used to collect the mortality and monstrosity data.

XYZ CREEK SUMMER CHUM SALMON
SURVIVAL AND CONDITION OF EGGS/ALEVINS

Date_____

Personnel _____

One-line represents one fish **Page** _____**of** _____

& No.	No. of Dead Eggs	No. of Dead Alevins	Description of Monstrosities, e.g. scoliosis, twins, large yolks, albinos, mosaics, fin eruptions, abnormal fins, etc.	Total Loss
F-1	6	0	1 twin, 3 Large Yolks, 2 Bent Backs	12
F-2	7	11	8 twins, 4 Large Yolks, 8 Truncated Caudal Fins, 1 Albino	39
F-3	18	3	5 Large yolks, 13 Bent Backs, 7 Scoliosis	46

Explanation of data entries:

Column 1 "& No.": Number of the Female whose offspring were examined and ponded.
Column 2 "No. Of Dead Eggs": The number of dead eggs observed in a tray.
Column 3 "No. Of Dead Alevins": Number of dead alevins observed in a tray.
Column 4 "Description of Monstrosities etc.": Simple descriptions and counts of any observed abnormalities in the fry. Most of the abnormalities seen fall into several discrete classes, spinal coiling, fry with very large yolks and undeveloped bodies, fry with v-shaped backs and protruding yolks, and Siamese twins, which can range from almost two complete individuals to a slight splitting of the head. Albinos, mosaics (fish with patches of no pigment), and individuals with various fin deformities are not frequently seen but can be present. The occurrence and types of these fish should be documented to see if recovery efforts increase their abundance as the recovery program proceeds through time.

These mortality data, in combination with that obtained at the eyed-stage, will be used to calculate an overall egg-to-fry survival value for each female. This information will be used to help evaluate whether the spawning and incubation conditions the fish were exposed to are adequate or need to be modified.

Quantifying Mortalities And Occurrence Of Monstrosities At Ponding When Female Egg Lots Have Been Combined**.**

Essentially the same form can be used to quantify mortalities and monstrosities when eggs from multiple females have been combined. When RSIs have been used, it is necessary to go through the incubation substrate and also to examine the pea gravel pressure plate for mortalities and live, abnormal fish. Since there should be a fairly accurate estimate of the number of eyed-eggs that were placed into a RSI it will be possible to calculate an eyed-egg to fry survival value for that RSI. In addition, the overall frequency of various abnormalities can be determined. When the eggs of multiple females have been placed into Heath Trays, the same documentation should occur on a tray-by-tray basis.

PART II: PONDING, REARING AND RELEASE PROTOCOLS

All of the summer chum salmon recovery projects currently taking place in Hood Canal and Strait of Juan de Fuca streams employ a short rearing period before fish liberation. The genesis of this requirement is the belief that reared fish will have enhanced early survival rates (Whitmus 1985; Sakuramoto and Yamada 1980). Whitmus (1985), for instance, found that chum salmon released at 53 mm survived almost four times higher than those measuring 45 mm when both groups were released together into Hood Canal. It is possible to culture this species in freshwater for extended periods of time, however, Iwata et al. (1982) suggest that osmo-regulatory competence in chum salmon will decline as they increase in size. Consequently, the *Summer Chum Salmon Conservation Initiative* (WDFW and PNPTT 2000) requires that chum be reared until they reach 1 to 1.5 grams or 50 to 55 mm in fork length prior to being released. (See SCSCI Section 3.2.1.2, and Appendix Rpt. 3.1) The artificial propagation of chum salmon at WDFW hatcheries has shown that such fry will realize significant survival advantages and not suffer any loss in their osmo-regulatory capacity. This size standard will be followed until data specific to a release location or stock indicates that an alternative size may have an increased survival potential.

The following survival rate objectives for each life stage are the SCSCI guidelines for measuring the effectiveness of each program. (See SCSCI – Appendix Rpt. 3.1)

Chum Life Stage	% Survival by Life Stage	Cum. % Survival from Green Egg
Green egg to eye-up	90.0 %	90.0 %
Eye-up to Swim-up	99.5 %	89.5 %
Swim-up to release	95.0 %	85.0 %

PROTOCOL 1: Ponding Procedures

Chum salmon typically emerge from streambeds with condition factor values (K_D) (Bams 1970) that range from 1.8 to 2.0 (see formula below). Consequently, fry incubated in Heath trays, iso-buckets or other devices that do not permit volitional residency should be placed into rearing environments when they have achieved 1.8 to 2.0 K_D values. Periodic visual inspections should be made on cultured chum salmon. When only a small slit of yolk material is visible, individual weight and length samples on 10 to 20 fry should be made to determine their K_D values. When these values average around 1.9 the fish can be ponded. On the other hand, when chum fry volitionally exit their incubation environment (e.g. RSIs, Kitoi boxes, egg incubation channels, spawning channels, etc.) they can be placed into rearing vessels immediately after emergence.

In order to use proper rearing densities and appropriate daily feeding rations two pieces of information need to be obtained at ponding; i.e. 1) how many fish are being placed into a rearing container, and 2) what is their mean weight. How these parameters will be determined depends upon how the eggs were incubated.

Determining The Mean Weight And Number Of Fry Placed Into Rearing Vessels When The Eggs Of Each Female Have Been Incubated Separately From One Another

When the egg complements of each female are incubated separately the following data tables will be used (hypothetical data have been included):

XYZ CREEK SUMMER CHUM SALMON
NUMBER OF FRY PONDED BY INDIVIDUAL FEMALES

Date_____

Personnel _____

One-line represents one fish **Page** _____**of** _____

♀ #	No. of Eyed Eggs	Total Mortalities and Monstrosities from Eying to Emergence	Estimated No. of Fry	Mean Fry Weight (grams)	Total Wt of Female's Fry
F-1	2,891	12	2,879	.463	1,333.0
F-2	3,006	32	2,974	.445	1,323.4
F-3	2,867	46	2,821	.445	1,255.3

Explanation of data entries:

Column 1 Female #: A consecutive and unique number assigned to every female used in a supplementation project.

Column 2 No. of Eyed Eggs: A gravimetric estimate of the number of eyed eggs produced by a female (see above for details on how that value is determined.

Column 3 The number of dead eggs, alevins, and live monstrosities observed in a female's incubation tray at the time of ponding.

Column 4 Number of eyed eggs minus mortalities and monstrosities will provide an estimate of the number of fry produced by a female.

Column 5 Mean wet weight of five individually weighed fry randomly removed from the female's incubation tray. Values are obtained from the following form titled: "Fry Length, Weight, And Condition Factor At Ponding."

Column 6 Mean fry weight times the estimated number of fry in a tray
i.e. (Column 4 x Column 5 = Column 6).

The form shows the number of fry produced from each female and also the total weight of those fish. To obtain such data, fry originating from a female are typically poured into a fine mesh net placed into a trough supplied with running water. Small groups of fry are then inspected for monstrosities, and dead eggs and alevins are removed and counted. After being inspected the fry are then placed into five-gallon buckets and immediately released into a rearing vessel. The data on the form can be used to determine how many groups of fry, should be placed into a given raceway. Suppose for example that the total number of fry produced from females 1 – 7 equals 20,000 fry. If the project was using fiberglass raceways that are 3 foot wide x 3 foot deep x 16 feet long (0.9 m x 0.9 m x 4.9 m), each raceway can hold up to 20,000 fry at appropriate flow rates.

The fry from those seven fish could be combined into a single raceway. The total biomass of those fish would be calculated by summing the weight of the fry produced by each female (column 6, rows 1 – 7). In this manner, the number of fry and biomass of fish placed into a rearing container can be determined at the onset of a rearing program.

The mean body weights and K_D values of fry will also be obtained at ponding by randomly selecting five fry from each female. Fry will be anesthetized in MS222, individually measured to the nearest mm (fork length) and weighed on an electronic balance to the nearest hundredth of a gram or mg depending upon the accuracy of the electronic balance. The form below provides examples of the type of data desired.

XYZ CREEK SUMMER CHUM SALMON
FRY LENGTH, WEIGHT, AND CONDITION FACTOR AT PONDING

Date_____ **Personnel** _____

♀ #	Length	Wt.	♀ #	Length	Wt	♀ #	Length	Wt.
F-1	40	.472	F-2	39	.449	F-3	39	.441
Mean Wt.	41	.478		39	.450		38	.444
0.4632	40	.476		39	.443		39	.444
	40	.462	Mean Wt.	39	.440	Mean Wt.	39	.438
	40	.428	0.4450	39	.443	0.4452	40	.459

Mean fry weights are calculated in the field with an electronic calculator, K_D values are determined on each individual by using a spreadsheet program and the following formula taken from Bams (1970):

$$K_D = \frac{\sqrt[3]{10 \text{ Body Wt mg}}}{\text{Length mm}}$$

Mean fry weights are added to the form titled "Number Of Fry Ponded By Individual Females" and are used to help determine the biomass of the fry produced from each female.

Determining The Mean Weight And Number Of Fry Placed Into Rearing Vessels When The Eggs Of Each Female Have Been Combined With One Another During The Incubation Period

In some circumstances, eggs from multiple females will be combined and incubated with one another, for example in RSIs. In this situation the survival and size of fry originating from individual females will not be tracked. Instead, overall mean weight and length values will be determined once a day throughout the entire emergence period.

When fry emergence first begins, a few to several hundred individuals may exit an RSI for a number of days. Eventually these daily numbers will increase to thousands of fry. This peak may last for a week or longer and then fry abundance will decrease until only a few fish are observed. Fiberglass tote boxes placed directly below the outfall pipes of project RSIs will be used to capture

and hold all the fish leaving these incubators. Each tote shall be lined with knotless 1/8 inch nylon netting and equipped with an exterior standpipe. The standpipe will maintain the water level in the tote while the net liner is used to gently crowd the fry to facilitate their capture by dip nets. On those days when less than a thousand fish have exited a RSI, they can be counted by hand by using a small fine mesh screen scoop and a hand tally-counter. In this instance, uncounted fish will be held in 5-gallon buckets partially filled with water that have been supplied with air stones. The scoop is used to capture groups of 1 to 4 fry, which are then counted, one-by-one, by using the tally-counter. Counted fish will be immediately placed into an adjacent 5-gallon bucket and held until transferred into their rearing vessel. When numbers allow, three groups of 20 fry will be counted out and placed into separate pails. These samples will be used to obtain a daily mean wet weight for the fry captured on that day. To obtain these weights, a one-liter beaker or small plastic pail partially filled with water will be placed on an electronic balance (accurate to 0.1 g) and tared to zero. A single group of twenty fry will then be poured into a metal-screened strainer. Any water adhering to the fry and strainer will be wicked off by lightly pressing the strainer against a moist sponge three times. The fry will then be decanted into the container resting on the balance and its weight can be recorded. The form below, which shows some hypothetical data, will be used to collect mean fry weights.

XYZ CREEK SUMMER CHUM SALMON
MEAN FRY WEIGTHS

Date_____ **Location** RM 0.2 Trib. # 0215 to XYZ Creek

Personnel _____

Number of Hand-Counted Fry 35. + 60 weighed fish = 95 total fish

RSI # (Origin of Sampled Fish)	Sample No.	Total Weight of Sample (In grams)	No. of Fish In Sample	Mean Fry Wt.
1	1	6.5	20	.325
1	2	6.7	20	.335
1	3	6.7	20	.335
Mean Fry Wt. = sum of all the fry wt. samples/total number of fish weighed				.3317

On those days when less than sixty fish are present, all the fish can be weighed in one group and a mean weight can be determined by dividing their total weight by the number of fish in the sample.

As mentioned above, many thousands of fry may emerge from an RSI over a twenty-four hour period. When this happens it is impractical to hand count the fish and a gravimetric method will be used to estimate their abundance. In this instance, groups containing one hundred fry will be hand counted, placed in separate pails, and weighed as previously described. The form shown above can be used to record these data. Three such groups should be weighed when the day's catch equals approximately 1,000 fry. When daily catches are estimated to be higher than 1,000 fry, weigh out another group of 100 fry for every additional estimated 1,000 fish. For example, suppose that a quick visual inspection suggests that six thousand fry are being held in a tote. In

this case a total of six groups of 100 fry should be weighed out, three for the first 1,000, and an additional three groups for the remaining five thousand fry (this will result in six groups for ~6,000 fry). In those cases where it appears that more than 10,000 fry are present, weigh out ten groups of 100 fish. Once these samples have been weighed, calculate an overall mean wet weight for the fry. Next use a high weight capacity (e.g. a 10 to 15 kilo capacity) top-loading balance that is accurate to 0.5 grams to weigh out the remaining fry. A five-gallon bucket partially filled with water should be placed on this larger balance and tared to zero. Groups of fry will be placed into the large metal strainer, blotted as before on the sponge, and then added to the tared bucket and weighed. Record their weight on the form shown below, as in previous tables, examples of data have been included on the form.

XYZ CREEK SUMMER CHUM SALMON
GRAVIMETRIC COUNTS OF FRY ADDED TO REARING VESSELS
Total Weight of Fry Added to Each Raceway or Rearing Tank

Date_____ **Personnel** _____

Number of 100 Fish Samples Weighed __5_____

All Five Groups were added to Raceway 1

Mean Fry Wt.	RSI#	Raceway or Tank No.	Date Loaded With Fry	Wt of Fry Added to Raceway or Tank	Estimated Number of Fry
0.333	1	R-1	1-2-01	99	297
0.333	1	R-1	1-2-01	740	2,222
0.333	1	R-1	1-2-01	985	2,958

These data plus the number of fry hand-counted (e.g. in the one hundred fry samples) should be added together to determine the biomass and numbers of fry placed into a raceway. Using the above table, it can be seen that 5, 477 fry were added to Raceway 1 and that their biomass equaled 1,824 grams. In addition, five samples of 100 fish each were used to produce a mean wet weight. These counted fish were also added to Raceway 1. So in total, Raceway 1 received 5,977 fry [5,477 + 500 (total number of counted fry)] that weighed 1,990.5 grams [1,824 + 166.5 (total wt. of the five groups of 100 fry)]. Records on the number and weight of the fry added to each rearing vessel need to be kept until fry loading has been completed.

Placing Fry Into Their Rearing Vessels

Generally, enough fry will emerge from RSIs to load up a raceway in just a few days, however, if necessary fry can be placed into a raceway over a 10 to 12 –day period.

Collecting Voucher Samples of Fry

Ten fry should be collected from each RSI at the time of emergence and placed into a labeled vial containing 100% ethanol. Otoliths from these fish will be extracted and processed to verify that the thermal codes placed into the fish resemble those that were established for that group of fish.

A similar random sample of fry should be collected on any fry incubating in heath trays. A total of 20 fry should be collected from the trays receiving the same code. A few individuals can be removed from each tray at ponding and placed into a labeled vial containing 100% ethanol. These samples should be sent to WDFW's Otolith Laboratory for processing. Each label should have location, date, species, stock, RSI or Heath Tray number, and collector information placed on it.

PROTOCOL 2: Initial Feeding Strategy, Daily Feeding Frequency, Food Size, And Weekly Ration

Newly ponded chum in each raceway will exhibit a fright response to overhead shadows and movements. Within a few days or sooner, however, they will become habituated to these stimuli and take food from the surface. Some fish culturists like to start chum with finely ground food or mash; however, this type of food can lead to gill abrasions. Consequently, a semi-moist diet that does not have fines should be used. For the first day or so, a small amount of food should be thrown onto the surface of each rearing vessel. Most of the fish will probably sound or dart away but some will begin to feed after this has been done a few times. Repeat the introduction of small amounts of food at least once every hour during an eight-hour day. Don't throw in too much because most of the food will not be eaten and will have to be cleaned out of the raceway. After a few days the fish should readily come to the surface and vigorously feed. Once that happens the fish can be fed approximately 3% of their body weight per day.

Alternative feeding approaches are possible. For example, during the last ten years feeders that deliver food pellets into the mid-water column, have been developed by NOAA Fisheries and WDFW researchers. Fry fed in this manner readily accept food, do not exhibit a fright response, nor do they become surface orientated. The ones currently in use consist of a spring-driven conveyer belt that delivers a constant stream of pellets to a pipe that vertically descends into a raceway or circular tank. A continuous stream of water is added to the pipe. The pipe ends in a "T" that has two arms that are approximately 1.5 meters long and about 13 mm in diameter. The ends of each pipe are furnished with 45^{o} elbows and caps with 6 mm holes. The elbows are used to direct the water and food delivered by the pipe in an upward direction. Pellets released from the pipes swirl up into the water column before they eventually start to sink. Presumably the underwater movement of the food pellets facilitates normal feeding behavior. Moreover, the lack of surface movement caused by humans throwing food into a rearing vessel allows the fish to begin feeding without having to overcome fright responses to surface shadows. An evaluation of the effects of using such feeders on chum fry has not yet been completed. Nonetheless, the method appears promising and should be considered as a possible rearing strategy in recovery programs.

The starting number and weight of the fish added to each raceway should be known so calculating a % body weight ration is a straightforward exercise. Suppose that a raceway has been loaded with 20,000 fry and that the total biomass at loading equaled 6,000 grams. Three percent of that value equals 180 grams. Consequently, during the first rearing week the fry should receive no more than 180 grams of food per day. Chum grow rapidly, therefore adjustments in the quantity of food they receive per day will have to occur on a regular basis. This should continue to equal about three percent of their body weight throughout the entire rearing period.

To adjust the amount of food the fish receive, fry should be sampled once a week to determine how much they weigh. The number of fry present at the beginning of each new rearing week must also be known. To estimate fish weight at the beginning of a rearing week the following steps should be used:

1) Seven days after the fish have been placed into a raceway, randomly collect three groups of 25 fry. Do this in the morning before the fish have been fed as this will provide the most accurate weights and will also minimize stress. The samples should be obtained by using a dip net and grabbing fry from the head, middle, and end of each raceway. Once a sample has been collected, gently remove twenty-five fish and place them in a plastic five-gallon bucket (or similar container) partially filled with water. Repeat this two more times to get a full complement of samples.
2) Set up an electronic balance (should be accurate to 0.01 g), place a pail partially filled with water on the balance and tare to zero.
3) Take one of the sample buckets and pour the 25 fish into a fine mesh strainer. Gently blot the strainer three times onto a slightly moist sponge to absorb excess water from the fish. Pour the fish into the tared container and record their total weight. Perform this process on the other two samples.
4) Calculate a mean body weight value from the three samples. An example of the form used to record these data is presented below:

XYZ CREEK SUMMER CHUM SALMON
WEEKLY CHANGES IN FRY MEAN BODY WT. & RATIONS

Date_____ **Personnel** _____

Raceway No.	No. of Fry In Raceway	Wet Wt. of 25 Fry Samples			Mean Fry Wt.*	Wt. of All Fry In Tank**	Weekly Ration (3% of Total WT***
		Sample 1	Sample 2	Sample 3			
1	17,520	22.0	21.9	21.8	0.87	15,242.4	457
2	17,453	21.4	21.0	21.0	0.84	14,660.5	439
3	17,399	17.7	18.1	17.2	0.70	12,179.3	365
4	22,417	13.4	13.8	13.7	0.54	12,105.2	363

The instructions listed below are printed at the bottom of the form for instant referral.

* To calculate **mean fry weight**, add the weights of samples 1, 2, and 3 and divide by 75 (the total number of fry weighed)
Randomly collect fry from each raceway as indicated in the protocol section, count out three groups of 25 fry. Place a tared beaker of water on the electronic balance. Gently pour 25 fry into a metal meshed strainer and blot off excess water on a sponge (3 to 4 times) and pour the fry into the tared beaker, record weight on form. Repeat until three weights of 25 fry have been obtained for each raceway. After the fry have been weighed return them to their raceway.

** To calculate **Total Wt of all Fry in Tank,** subtract the number of mortalities observed in a raceway from the number of fish present in the raceway at the beginning of a rearing week. This will give the number of fry in the raceway at the time of sampling. Multiply this number by the mean weight of the fry in that raceway; this value will equal the total number of grams of fry in the raceway.

*** To determine **Weekly Ration**, multiply the number of fry in a raceway by the mean weight of the fish. This will give the total weight of all the fish in that raceway. Multiply the total weight value by .03, and that will give 3% of the total weight. That is the amount of food that raceway should receive once a day.

5) Use the Daily Fry Feeding form (see example below) to determine how many mortalities occurred during the past seven-day rearing period and subtract that value from the number of fish present at the beginning of the rearing period. For example, imagine that 17,000 fry were initially loaded into Raceway 1. After seven days the total mortality noted in this raceway was found to be 15 fish. Therefore, the number of fish present when sampled seven days later would be 17,000 - 15, or 16,985.

XYZ CREEK SUMMER CHUM SALMON
DAILY FRY FEEDING AND MORTALITY RECORDS

Date_____**Personnel** _____

Raceway Number	**Wt. of Daily Ration**	**Number of Times Fed Per Day**								**Mortalities**
1	450									2
2	400									0
3	325									0
4	315									1
Daily observations and comments: Fish are feeding well, mortalities in raceways 1 and 4 were pinheads, flows were increased from 20 gallons per minute to 25, increased flow had no affect on feeding rate.										

6) To determine the total weight of fry in each raceway simply multiply the number of fry present times their mean body weight.

7) To calculate how much food that raceway should receive each day over the next seven days multiply the total fry weight value obtained in (f) above by three percent. Feed that amount to the fish in the raceway for the next seven days. The fish should be fed six to eight times/day at one-hour intervals. The food should be distributed evenly throughout a raceway so that all fish have access to food. Feed on an ad libitum basis; i.e. give them what they want but don't over feed. Typically the fish will take a little more food in the morning after not feeding during the night. As the fish grow, their food size should increase. Pellet diameter should, however, not exceed one-fortieth of the length (fork length) of the fish, which is approximately equal to the diameter of the esophagus.

PROTOCOL 3: Monitoring Rearing Mortalities

As mentioned above, it is very important that the mortalities in each rearing vessel be recorded so that fry numbers and biomass can be tracked over time. The form entitled "Daily Fry Feeding And Mortality Records" has a column to record daily mortalities. Generally, mortality should be low, if a sharp rise in mortality occurs, contact the WDFW regional pathologist for a diagnosis and possible treatment regime.

PROTOCOL 4: Rearing Density

A variety of formulas or "rules" have been developed (WDFW 1996) that establish appropriate rearing densities for salmonids. These approaches typically rely on information about flow rates into the rearing container, on how large the rearing container is, upon the size (length and weight), and species of reared fish, plus the water temperature and elevation of the rearing location. The raceways used in most of WDFW's chum salmon recovery projects are sixteen feet long (4.9 m), have a mean water depth of twenty-two and a-half inches (57 cm), and are on average thirty-three inches wide (84 cm) and therefore have 82.5 cubic feet or 617.1 gallons of rearing space.

The first, and simplest rule proposes that no more than three pounds of chum salmon should be reared per gallon of inflow. At the end of a rearing period, the fish should average around 1.25 to 1.5 grams in weight. If it is assumed that the maximal number of fry placed into a raceway equals 20,000 then at the conclusion of the rearing period total fish weight should equal approximately 66.2 lbs or [(1.5 x 20,000)/453.6]. Where 1.5 equals mean fry weight in grams, 20,000 equals total number of fish in the raceway, and 453.6 is the number of grams present in a pound. Given that scenario, the minimum inflow into the raceway would equal 66.2/3 or 22.05 gallons per min at the end of the rearing period.

The second method is one referred to as the Flow Index method. It relies on the following formula to determine the minimum flow needed to rear a set number of fish:

$$I = W/(F \times L)$$

Where: I = inflow, or amount of water entering the rearing container per minute
W= Total weight of the fish in the rearing container,
F = A flow index value that varies according to water temperature and rearing site elevation. For example, at 50^{o} F and sea level, F = 1.80, and
L = Average length of the reared fish in inches, in the example below it equals 58 mm or 2.2835 inches.

Minimum inflow in a raceway containing twenty thousand 1.5 gram by 58 mm long chum fry held in 50^{o} F rearing water at sea level would then equal:

$$I = 66.2/(1.8 \times 2.2835) \text{ or } 16.1 \text{ gallons/min}$$

Where 66.2 equals total fry weight in the rearing vessel, 1.8 is the Flow Index value, and 2.2835 is fish length in inches.

The third method looks at how quickly a total water exchange occurs in a rearing vessel. At a minimum at least one and preferably two complete volume exchanges per hour should occur. The formula for this rule is:

$$R = (I \times 60)/V$$

Where: R = Number of container volumes exchanged per hour,
I = Inflow rate or gallons per minute of flow into the rearing container,
60 = Number of minutes in an hour, and
V = Volume in gallons of the container (recall that one cubic foot contains 7.48 gallons of water).

This rule indicates that the minimum flow entering a vessel per minute must equal the amount needed to fill it in one hour. As mentioned above, the raceways used in WDFW's salmon recovery projects typically hold 617.1 gallons of water so a minimum flow rate in this type of raceway would equal 10.28 gallons/min or (617.1/60 = 10.28). Two complete water exchanges would occur if 20.56 gallons were added to each raceway.

Given the above rules, flows should be set at 15 to 20 gallons per minute when fry are first added to a raceway. As the fish grow, flows should be slowly increased and may reach 30 gallons per minute at the end of a rearing period. If water is available, higher flows should be used at the end of the rearing period as a greater water exchange will provide the fish with better rearing conditions. Flows greater than 20 gallons per minute should not be used initially since they may fatigue the fry or impinge them on the exit screen of a raceway. Moreover, if water is limited, it appears that 20 to 25 gallons/minute would provide a reasonable flow rate in a 617-gallon capacity raceway for an entire rearing period.

PROTOCOL 5: Raceway Cleaning

How often raceways should be cleaned commonly reflects a compromise between the desire to have the fish living in a clean environment and the need to minimize stressors during the rearing phase. Mortalities should be removed as soon as detected and the bottom of the raceways should be cleaned on a once-a-week basis with a vacuum or siphon. Clean the raceways in the morning prior to feeding, as this will reduce stress. If necessary, tank sidewalls can be cleaned with a stiff brush if algae begin to entrap food or feces. To prevent disease or pathogen transfer from one raceway to the next, use a separate set of brushes, siphon, and nets for each rearing container. Moreover, set up a container holding a strong solution of PVP (an Iodine based disinfectant) for each rearing vessel and soak the brushes, nets, and vacuum or siphon head in the PVP solution until its next use. Prior to reusing these tools, thoroughly rinse them with freshwater.

PROTOCOL 6: Monitoring Environmental Conditions During the Rearing Period

Four environmental parameters, flow rates, water temperature, dissolved oxygen, and Total Settleable Solids (TSS) will be monitored on a routine basis throughout the rearing period. Flow rates will be checked on a weekly basis. This will be accomplished by capturing outflow water from each tank for a ten second period of time and then measuring the volume of this sample.

Three such samples should be taken on each raceway at every sampling period. The form shown below will be used to record these data.

XYZ CREEK SUMMER CHUM SALMON
WEEKLY REARING VESSEL FLOW RATES

Date m-d-yr	Raceway or Tank No.	Gallons Per Ten Seconds			Mean Flow (Gallons/Min)
		Obs 1	Obs 2	Obs 3	
1-5-04	R-1	2.7	2.7	2.6	16.0
1-12-04	R-1	3.6	3.8	3.8	22.3

TSS values and dissolved oxygen levels in each rearing vessel will also be determined on a weekly basis. Water temperatures will be recorded continuously with Tidbit recorders and will also be taken with thermometers twice a day, once in the morning and again at the last feeding period. Imhoff Cones will be used to record TSS values. Samples of incoming water will be collected and poured into three Imhoff cones and allowed to stand for one hour. TSS values will be read from the cone. A portable Dissolved Oxygen Meter will be used to record two DO levels in each tank. The meter will be calibrated before each use and measurements will be taken at the end of the raceways. The form shown below will be used to record these data.

XYZ CREEK SUMMER CHUM SALMON
ENVIRONMENTAL CONDITIONS DURING THE REARING PERIOD

Date m-d-yr	Raceway or Tank No.	Water Temperature		Dissolved Oxygen		Imhoff Cone Readings TSS		
		Morning	Afternoon	Sample 1	Sample 2	Cone #1	Cone # 2	Cone # 3

If local weather conditions are not being recorded, a rain gauge and min-max air temperature thermometer need to be established at the incubation and rearing sites to help track local weather conditions. The rainfall and air temperature data should be recorded daily.

PROTOCOL 7: Determining Mean Fry Size At Release

The length, weight, and Fulton's Condition (K) of fish about to be released will be determined on the day they are liberated. A dip net will be used to collect random samples of fry from the head, middle, and end of each raceway. The collected fish will be held in an aerated five-gallon bucket until sampling has been completed. A small wire mesh strainer will remove some of the fry, which will be placed into MS222 (at a dosage of 1 g of MSS22 per 19 Liters of water). Once a fish has been anesthetized, it will be measured to the nearest mm (fork length) and then weighed to the nearest 0.01 g on a top loading electronic balance. Prior to being weighed, the fish will be placed into a wire-mesh strainer and gently blotted on a moist sponge three times to wick off any water adhering to its surface. A beaker containing water will be placed on the electronic balance and tared to zero. Once a fry has been added its weight will be recorded and the beaker will be re-tared

to zero so that another fish can be weighed. A total of fifty fry from each raceway will be measured and weighed. The resulting data will be used to produce mean weights, lengths, K values, and coefficient of variation statistics for each of these parameters, and frequency distributions for lengths and weights. Fulton's K values are calculated by using the following formula K = body weight in grams/(fork length in mm)^3. This value is often multiplied by 100,000 to provide values that vary around 1. As the formula implies, Fulton's K examines the relationship between fish length and weight, fish having relatively high K values are more robust than those with lower ones. The sampled fish will be returned to their raceway. The form below shows how these size data will be recorded:

XYZ CREEK SUMMER CHUM SALMON
LENGTH AND WEIGHT OF LIBERATED FRY

Date _____ **Personnel**_____

Number	Length (fk)	Wt. (g)	Number	Length (fk)	Wt. (g)
1			26		
2			27		
3			28		
4			29		
↓			↓		
25			50		

PROTOCOL 8: Liberation Of Fry

Whenever possible fry releases should occur on out-going tides and after sunset. Twenty-four to forty-eight hours before a release, feeding will cease. This will help reduce stress when the fish are transported to their release location.

The following form will be used to record release information.

XYZ CREEK SUMMER CHUM SALMON
LIBERATION OF FRY

Date m-d-yr	Release Location	Time	Tide	Fry Number	Mean Weight	Mean Length	Comments/Condition At Release

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