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EVALUATION OF CONSERVATION HATCHERY REARING AND RELEASE STRATEGIES FOR STEELHEAD RECOVERY IN THE HAMMA HAMMA RIVER

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ABSTRACT

The Hamma Hamma River supplementation project is designed to evaluate the demographic and genetic effects of a supplementation program on the abundance of a depressed winter steelhead (Oncorhynchus mykiss) population. Survival, behavior, otolith microchemistry, genetic, and fish husbandry data are being evaluated to determine the relative effectiveness of two smolt-rearing strategies and two release strategies (adult vs. smolt). Growth profiles of cultured smolts have closely mimicked those of wild fish.

Steelhead released as smolts (i.e., smolt release group, SRG) and as age-4 adults (adult release groups, ARG) have been observed spawning between 2002 and 2004. An average (± s.d.) of 11 (± 5.7) steelhead redds were observed annually between 1995 and 2001 in the Hamma Hamma River. After the release of steelhead from the SRG in 2000, 2001, and 2002 and ARG in 2002 (81 females, 116 males), 2003 (2 females, 2 males), and 2004 (35 females, 41 males) the number of redds observed in the Hamma Hamma River has averaged 114 (± 37) between 2002 and 2004. By comparison, the number of redds recorded in the control streams has remained fairly stable from 1995 through 2004.

Courtship was more frequently observed between ARG males and females or anadromous (either SRG or wild) males and females than between ARG and anadromous fish. The apparent assortative pairing by rearing type appears to be consistent with differences in spawn timing, which was earlier for ARG than anadromous females in both 2002 and 2004. Combined otolith and genetic analyses will aid in determining the adultto-parr reproductive success of ARG and anadromous steelhead. To date, the increased redd abundance in the Hamma Hamma River and evaluations of reproductive behavior and adult-to-fry reproductive success under experimental conditions indicate a substantial contribution of ARG steelhead to the Hamma Hamma River steelhead population.

EXECUTIVE SUMMARY

Conservation hatcheries for anadromous salmonids have the dual role of supplementing depleted populations, while at the same time minimizing genetic and ecological risks to the extant wild population(s). Conservation hatchery practices designed to aid in population recovery continue to evolve. This project aims to reduce numerous uncertainties currently encountered in the implementation of conservation hatchery strategies.

A collaborative group comprised of several agencies (Washington Department of Fish

and Wildlife, National Marine Fisheries Service, Point No Point Treaty Council, and the U.S. Fish and Wildlife Service) and non-profit salmon enhancement organizations (Long Live the Kings and the Hood Canal Salmon Enhancement Group) initiated the Hamma Hamma Steelhead (Oncorhynchus mykiss) Supplementation Project in 1998. This report describes findings to date of a multi-year scientific evaluation of the project that was initiated in 2000 with funds provided by the Hatchery Scientific Review Group (HSRG).

The captive population has been established by hydraulic removal of eyed steelhead eggs (embryos) in spring/summer of 1998 (4,683 embryos), 1999 (2,588 embryos), 2000 (1,662 embryos), 2001 (1,271 embryos), and 2003 (4,105 embryos). Methods for collection have been described in previous annual reports. In 2002 and 2004, no embryos were collected for broodstock because of the large number of redds observed in those years. Thus, returns of age-4 adults in 2006 and 2008 will be comprised of offspring of wild and cultured steelhead and will provide an opportunity to estimate the natual productivity of the population after a single generation of supplementation.

The cultured steelhead are reared and released at two different life history stages (age-2 smolts and age-4 adults). For the ‘smolt release group’ (SRG), emergent fry are divided equally for rearing at two locations: 1) off-channel earthen ponds near John Creek, which is a tributary of the Hamma Hamma River, and 2) circular rearing vessels at the Long Live the Kings (LLTK) Lilliwaup Hatchery. Prior to supplementation (1995 through 2001), an annual average (± s.d.) of 11 (± 5.7) steelhead redds were observed in the Hamma Hamma River. After the release of artificially propagated smolts in 2000, 2001, and 2002 and captively-reared adults in 2002 (81 females, 116 males), 2003 (2 females, 2 males), and 2004 (35 females, 41males) the number of redds observed in the Hamma Hamma River has markedly increased. The adjusted redd abundance in the Hamma Hamma River has averaged 114 (± 37) between 2002 and 2004 (Fig. 2). By comparison, the number of redds recorded in the control streams has remained fairly stable from 1995 through 2004. An increase in the frequency of redd surveys in the Duckabush River in 2004 did not result in a marked increase in the number of redds observed in 2004 (Figure 2).

A combination of otolith microchemistry and genetic (DNA) analyses are being performed to estimate and monitor genetic changes in the anadromous and resident

components of the O. mykiss population in the Hamma Hamma River and to estimate the

contribution of the adult release program to the production of juvenile steelhead.

Purpose and Objectives

Conservation hatcheries have the dual role of providing a demographic boost to depleted anadromous salmonid populations, while at the same time minimizing genetic and ecological risks to the extant wild population(s). Conservation hatchery practices designed to supplement natural populations and thereby aid in population recovery continue to evolve. By and large, supplemented populations have not been effectively monitored to determine the effects of supplementation programs on population abundance, productivity, and genetic diversity. In 1998, a steelhead supplementation program was initiated in the Hamma Hamma River, which flows into Hood Canal near Eldon, WA. The program's goal is to rebuild the depressed native population to the estimated carrying capacity of between 119 and 323 spawning adults. The supplementation plan involves the application of conservation hatchery protocols (Flagg and Nash 1999), which are currently being implemented to maintain or restore depleted salmon populations throughout the Pacific Northwest. This research project was designed to scientifically evaluate the demographic and genetic effects of the supplementation program on the steelhead population in the Hamma Hamma River. Specifically, the project aims to reduce numerous uncertainties currently encountered in the implementation of conservation hatchery strategies. First, it will determine whether a supplementation program incorporating genetic and ecological safeguards can contribute to the rebuilding of a depressed steelhead population by increasing natural spawner abundance. Second, the project will estimate the effects of an evolving but experimental reintroduction strategy (i.e., release of adult captively-reared fish) on population abundance. Third, the effects of providing fish with more naturalistic experiences (i.e., exercise) have been examined and a manuscript has been prepared for publication. Fourth, new feeding methodologies have been developed, which provide a template for rearing steelhead smolts at more natural growth rates. These rearing protocols are being developed in conventional hatchery tanks and in more natural 'conservancy' ponds.

Finally, for small populations in which a portion is taken into captivity, there are concerns that artificial propagation may result in stochastic changes in allele frequencies, loss of heterozygosity and loss of rare alleles. The genetic monitoring plan will evaluate these parameters. This report does not include statistical analyses, and the results should

be considered preliminary. Statistical anlalyses will be conducted after all data for multiyear objectives have been collected.

Materials and Methods

Embryo collections

The captive population was established by hydraulic removal of eyed steelhead eggs (embryos) in spring/summer of 1998 (4,683 embryos), 1999 (2,588 embryos), 2000 (1,662 embryos), 2001 (1,271 embryos), and 2003 (4,105 embryos). Methods for collection are described in previous annual reports. In 2002 and 2004, no embryos were collected for broodstock because the large number of redds observed in those years (see below). Thus, returns of age-4 adults in 2006 and 2008 will be comprised of offspring of

wild and cultured steelhead and will provide an opportunity to estimate the productivity of the population after one generation of supplementation.

Fish Culture

Smolt release groups (SRG)

Juvenile steelhead from five of the seven brood years (1998-2004) were divided equally for rearing to age-2 smolts at two locations: 1) off-channel earthen ponds near John Creek, a tributary of the Hamma Hamma River, and 2) circular rearing vessels at the LLTK Lilliwaup Hatchery. Steelhead destined for smolt release (i.e., the smolt release group, SRG) are reared so that body size characteristics are similar to those of wild fish

from a stream in the same region for which substantial seasonal size data is available (Snow Creek, Jefferson County, WA). To mimic these growth profiles, feed schedules were developed based on an assumed 1:1 conversion ratio. Rations were fed to fish in individual rearing containers three times per week. Generally, the juveniles were reared in separate family groups until October and then combined. Juveniles from each brood year (BY) were sorted by size and fed different rations as necessary to reduce size variation and maximize the number of fish attaining the targeted smolt size at age 2. Approximately every 3 months, the fish are sampled for length and weight and feeding rates are recalculated to mimic the natural growth template.

All SRG steelhead have been externally marked by removal of the adipose fin. Steelhead reared at Lilliwaup received a coded-wire-tag (CWT) approximately two months prior to release. Those reared at John Creek have not received CWTs.

Adult release groups (ARG)

A portion of the 296 fish collected in 1998 and all of the 150 fish collected in 2000 were allocated for rearing to age-4 (or later) and release as maturing adults (i.e., the adult release group, ARG). For BY 1998, groups of fish from eight individual redds were cultured in separate 1.8-m diameter tanks until each fish could be PIT-tagged for individual identification. The 296 steelhead, proportionately representing the eight reddspecific groups, were divided equally into four circular rearing tanks (6 m diameter) in October 1999. Pumps (2.0 HP) and variable speed controllers were installed in two tanks (high velocity, HV) to re-circulate water and create current velocities up to 0.64 m/sec for 12 hr each day between 22 October 1999 and 27 March 2000, after which the duration of HV rearing was increased to 23 hr/day (Figure 1). Thus, fish in the HV tanks experienced current velocities near 1 body length per second (BLPS), depending on their location in the tank. The velocities were similar to those implemented by Patterson et al. (2004) to determine the effects of exercise on reproductive characteristics of wild sockeye salmon during upstream migration. Current velocities were held at less than 0.25 BLPS in the two remaining low velocity (LV) tanks. Fish in all tanks were fed similar rations of BioProducts Brood Diet according to the manufacturer's recommendations. We have combined the HV and LV groups as a single group for analysis in this report (but see Berejikian et al. 2003).

On 24 February 2002, 81 maturing females (37 LV and 47 HV) and 116 maturing males (62 LV and 54 HV) were marked with yellow (HV treatment) or white (LV treatment) individually numbered anchor tags (3 cm long), weighed, and measured. All fish were released into the Hamma Hamma River for natural spawning on 28 February 2002. An additional 64 fish were transferred to the NMFS Manchester Research Station for research on breeding behavior and reproductive success (see Berejikian et al. 2003). Two males and two females that did not mature until age-5 were released in 2003.

The BY 2000 ARG is comprised of a subsample of individuals from the seven redds hydraulically sampled in 2000. These family groups were reared separately until 18 October 2001 when they were combined into a single 6.1-m diameter tank. On 31 October 2002, all remaining fish (n = 133) were PIT-tagged and split into two 6.1-m tanks by body size (n = 38 'small', n = 95 'large') in attempts to increase the growth rates of the smaller fish by removing competition from larger fish. Fish were fed three times per week until age-2, following the same protocol developed for the SRG. Beginning in May 2002, fish in both tanks were fed five times per week to apparent satiation.

The BY 2000 ARG steelhead population was sampled on 12 February 2004 to record

weight and length and determine maturation status. Each maturing fish received a uniquely numbered green anchor tag. Eight females had ovulated and nine males were spermiating; these fish were released into the Hamma Hamma River at river km 2.6 the following day. The remaining 59 maturing BY 2000 ARG fish (27 females and 32 males) were released on 3 March 2004 at river km 2.9. The remaining 24 fish that did not mature will be cultured to maturity and released in 2005.

Abundance of redds and adult steelhead

Redd surveys

Steelhead redds were counted by foot and snorkel surveys from January through June, 2004. At least once per week surveyors walked the entire study reach of the Hamma Hamma River to identify new redds. Snorkel surveys of the Hamma Hamma were conducted weekly. A foot survey was conducted immediately before each snorkel survey to compare the two methods and adjust the redd counts in the Hamma Hamma River for comparison to control streams, where only foot surveys have been conducted. John Creek, a major tributary of the lower Hamma Hamma River, was surveyed once weekly by foot only.

When a new steelhead redd was observed it was given a unique identifying number and marked with flagging on the bank and a flagged rock placed within the depression of the redd. The dimensions of the redd (length, width, and depth) were measured. Length was the total extent of the excavated gravel. Widths were measured at ¼, ½, and ¾ the length of the redd. Depths and velocities were measured at the midpoint of each width transect. Additionally, the maximum depth within the redd was measured. Redd measurements were generally conducted within two days of their identification before any significant changes in flow occurred. The redd location was identified with a Global Positioning System (Garmin GPSmap 76S) and estimated on a map. Redds that had been enlarged within three to four days after the first

observation were considered to be an extension of the existing redd and were re-measured. Likewise, excavated areas that appeared on the same date that were not separated by at least 1 m were considered part of the same redd. Redds that were expanded or superimposed after 4 days, were recorded as separate, new redds. Only redds that exceeded 0.76 m long by 0.46 m wide (based on the minimum size of steelhead redds measured in previous years) were recorded as belonging to a female steelhead. .

Redd counts are conducted by the Washington Department of Fish and Wildlife (WDFW)

on the Duckabush, Dosewallips, and Dewatto rivers. Trends in redd abundance in these unsupplemented streams are compared with trends in the Hamma Hamma River to evaluate the efficacy of the supplementation program. In 2004, we increased our survey effort on one stream, the Duckabush River, to more closely match the effort expended on the Hamma Hamma River. In collaboration with WDFW and the Hood Canal Salmon Enhancement Group, two reaches totalling 4.6 miles of Duckabush River was surveyed 16 times between 11 February and 8 June, 2004. In comparison, only four surveys were conducted of the lower 2.3-mile reach, and one of the upper 2.3-mile reach between 11 March and 29 April, 2003. The objective of this increase in effort was to provide an unbiased estimate of redd abundance for comparing the Hamma Hamma River to control streams.

Redds counted in the Hamma Hamma River were divided into two categories; those visible during foot surveys, and those visible only by snorkeling. The Duckabush, Dosewallips, and Dewatto rivers were inventoried by foot surveys only. Only the number of redds seen during foot surveys in the Hamma Hamma River were compared with totals from the non-supplemented rivers, because those rivers were not snorkelsurveyed.

The number of redds observed in 2002 and 2003 in the Hamma Hamma River were not differentiated by survey method (foot vs. snorkel), so the 2002 and 2003 totals were each multiplied by the proportion of redds seen by foot surveys alone in 2004 for comparison to control streams.

Adult Hook and Line Sampling

Hook-and-line sampling was conducted at least once weekly. The objectives of this sampling were to 1) quantify the number of returning fish reared in John Creek versus Lilliwaup and released as smolts (SRG), 2) obtain accurate measurements of fork length, girth, and reproductive status (prespawn, mature, post-spawn) of all fish captured, and 3) collect DNA and scale samples from wild and SRG fish for genotyping and size-at-age information. Each captured fish was checked for the presence or absence of an adipose fin or anchor tag and measured for fork length and girth. Scales and fin tissue samples were collected for life history and genetic information. A CWT reader was used to determine the presence (Lilliwaup-reared) or absence (John Creek-reared) of a CWT.

Unique anchor tag numbers were recorded to identify ARG fish and recaptures of SRG or wild fish. Each female captured was determined to be pre-ovulated, ovulated (eggs easily expressed), or in post-spawning condition. Males were determined to either be spermiating or not. Untagged wild fish were marked with blue anchor tags.

Snorkel Surveys

Surveys conducted with a mask and snorkel provided additional data on the spatial distribution and relative abundance of steelhead from the various rearing groups throughout the spawning season. Snorkel surveys were conducted weekly from river-km 3.8 downstream to the area of tidal influence (river-km 1.4) to count fish and locate new redds. Surveyors walked upstream along the entire study section before snorkeling to look for fish and count redds. Surveyors kept track of the number of redds visible while on foot and those that would only have been visible by snorkeling. Surveyors recorded the river level (gauge height), weather conditions, and water clarity for each survey.

Once at the upstream end the study section, surveyors floated downstream to look for fish. When a steelhead was spotted, surveyors attempted to identify gender, the presence or absence of an adipose fin, anchor tag presence or absence and color, spawning condition, and size category. Size categories were ‘small’ (30-41 cm), ‘medium’ (42-51 cm), and ‘large’ (greater than 52 cm total length). Observations of spawning behavior were also recorded whenever possible. Data were recorded on underwater wrist-slates.

Reproductive behavior and breeding success

Breeding Behavior

The behavior of adult steelhead in the Hamma Hamma River was monitored several days each week between 1 March and 31 May in 2002, 2003, and 2004. Each of the population segments present during the spawning season were uniquely identifiable.

Wild fish had an adipose fin intact and no tags. Returning SRG fish had no adipose fin and no tag, and all ARG had adipose fins intact and external tags. In 2002, exercised ARG fish received yellow anchor tags and non-exercised fish received white anchor tags (see Berejikian et al. 2003). In 2003, when only two male and two female ARG fish (age-5) were released, each received two anchor tags, either yellow or white depending on their rearing treatment. In 2004, all released ARG fish received a single green anchor tag.

To collect data on breeding interactions among the rearing groups, observers walked along stream banks to locate females that were digging in a manner consistent with nest construction, covering nests containing recently deposited eggs, or holding positions over a developing nest or redd (assumed to be the females nest site). When a nesting female

was encountered, an attempt was made to determine its origin and rearing treatment based on the presence or absence of an adipose fin and anchor tag. The dominant male, which obtains and defends the position alongside a nesting female, and any 'satellite' males, which hold positions off to the side or downstream of the courting pair, were also identified to origin and rearing group.

Embryo viability analysis

To determine the viability of eggs deposited by ARG females, stream-bank observations of reproductive behavior identified redds that likely contained eggs deposited by ARG

females. Redds in which ARG females were either observed digging or holding while accompanied by a courting male were selected for the embryo viability analysis in 2002 and 2004. At the eyed stage of embryonic development, eggs were removed by a hydraulic pump or by hand digging with a shovel. Eggs were weighed and measured (diameter), and the proportion live and dead were counted. We calculated the proportion of live eggs for comparison with embryo viability of wild fish obtained during broodstock collection from 1998 – 2000, and 2003. In 2002, five redds were likely constructed by BY 2000 ARG females, and eggs were hydraulically removed from all 5.

In 2004, eggs were collected from 12 of 19 redds where ARG females were observed holding or digging. Seven redds produced no eggs, five of which experienced high flows between spawning and egg retrieval, changing the redd characteristics and making it difficult to excavate. Two redds were in an areas of high water flow making it difficult to retrieve eggs by shovel and a hydraulic pump could not be used in those locations. Two redds produced either 3 or 4 eggs and were excluded from the analysis because of the small sample size.

Otolith microchemistry analyses

In 1996 and 1997, the WDFW Otolith lab analyzed the otoliths of 50 O. mykiss taken below the anadromous barrier falls on the Hamma Hamma River. Results showed that there was a clear dichotomy between otolith core strontium signatures for anadromous versus resident O. mykiss progeny, and that 35% of samples in 1996 and 47% specimens in 1997 were the progeny of resident female parents. In 2000, a similar analysis was conducted on a sample of 50 fish taken below the falls and we concluded that 37% of specimens were the progeny of anadromous females. Analyses conducted in the freshwater zones of otoliths were uniformly low, showing that elevated core strontium

could not be explained by residence in the Hamma Hamma River. Analyses of otoliths
from fish taken above the falls revealed no progeny of anadromous females, which should be expected from populations above this anadromous barrier. In 2001, we concluded that anadromous parents spawned 30% of specimens captured below the falls,
fairly consistent with previous results.
For 2002 specimens, we also analyzed otoliths using Laser Ablation Inductively Coupled
Plasma Mass Spectrometry (LA-ICP-MS) as no electron microprobe was available to us.
One advantage to the use of this instrument was that we were able to analyze entire life
history scans of otoliths in a time efficient manner rather than selecting several analysis
points in the core region and several in a zone presumed to represent freshwater residence. Results of these analyses indicated that at least 94% of fish originated from
anadromous females, a far higher proportion than we had seen in past years. This large
discrepancy, coincident with a shift in analysis technique, requires further investigation to
determine if the results are real, or somehow related to this change in analytical methods.
One possibility is that previous microprobe analyses we presumed represented residence
in freshwater were actually taken in an otolith region where juvenile fish experienced
some exposure to elevated salinities in the estuary. This would inflate the distribution of
“freshwater” otolith Sr/Ca values in the sampled population and result in an underestimate
of the proportion of fish we assigned to anadromous parents. We have found

evidence from laser scans that this may be occurring near the otolith edge where we
observed significantly elevated strontium count rates.
In order to resolve these apparent discrepancies, we are currently analyzing some samples
by both techniques. Access to the electron microprobe at Oregon State University has
been difficult, resulting in delays in the otolith analyses. The following four steps are
currently being taken to determine the female parentage of parr sampled from the Hamma

Hamma River:
1. Re-analyze the 2002 specimens with the electron microprobe, providing a comparison to the same specimens analyzed with LA-ICP-MS. We are using similar methods to those in years past as discussed in previous reports and work statements. We
are analyzing 25 specimens collected above the falls in 2002 by both methods.
2. Analyze 2003 below-falls samples (N=90) with the electron microprobe, as well as specimens taken from above the falls.
3. Analyze all of the 2003 specimens with LA-ICP-MS to provide a second methodological comparison.

4. Determine ages for all specimens, including a re-analysis of the ages for the 2002 samples.

Genetic Monitoring

A genetic monitoring program was initiated in 2000 at the request of the HSRG to determine whether the supplementation project will result in a loss of heterozygosity, loss of rare alleles, or accelerate stochastic changes in allele frequencies. Samples were collected for genetic analyses from the Hamma Hamma River both above and below a barrier to upstream migration of anadromous O. mykiss. Downstream from the barrier, tissue samples were collected in summer 2003 from the same juvenile steelhead sampled for otolith microchemistry (n = 90). The age-1 parr in this sample will represent fish from the post-supplementation population. Upstream from the barrier, approximately 35 O. mykiss were sampled in 2003 to represent a baseline for the resident rainbow trout population, which received annual outplants of hatchery-reared rainbow trout until 1997.

Allele frequency data for these samples have been collected for 8 microsatellite DNA loci. Allele frequencies and heterozygosities of variable loci of the population below the anadromous barrier will be compared to data collected from 2000 to 2003, before any possible changes caused by the supplementation efforts could have occurred. The genetic analyses for the 2003 samples have been conducted. However, the samples collected in 2003 consist of fish in both the pre-supplementation (age-2 parr), and postsupplementation (age-1 parr) datasets. Therefore we cannot complete the test for genetic change in the population until the final otolith analyses, including ageing, have been completed.

The same areas will be sampled annually throughout the duration of the project. Genetic data from these samples will be collected in the same manner as the baseline samples. These data will be compared to the baseline data in order to monitor any possible genetic changes to the population. Changes in allele frequencies or heterozygosities, or the loss of rare alleles at rates greater then those expected by natural processes such as genetic drift, could indicate that the supplementation efforts are altering the genetic structure of the population.

Project management

The following lists organization and individual responsibilities for the abovedescribed work:

Name	Agency	Responsibilities
Barry Berejikian	NMFS	Project oversight, project design, data analysis, coordination, and proposal and report writing
Julie Scheurer	NMFS	Redd surveys, behavioral observation, embryo

collection, data collection, report writing
Skip Tezak NMFS Implementation of ARG exercise system and data collection
Don VanDoornik NMFS Genetic sampling, analysis, and reporting
Eric Volk WDFW Otolith sampling, analysis, and reporting
Thom Johnson WDFW Redd survey, redd pumping, permitting, project oversight for WDFW, and project planning
Joy Lee LLTK Fish culture, redd surveys (Hamma Hamma R.), parr collections, embryo collection, behavioral observation, other data collection, reporting
Rick Endicott LLTK Fish culture, redd surveys, parr collection embryo collection, hatchery management, project planning
Kathy Hopper LLTK Project oversight for LLTK and project planning
Lee Boad HCSEG Redd surveys, snorkeling, location of redds with GPS
Nate Ackley HCSEG Snorkel surveys, embryo collection, redd surveys
Chris Weller PNPTC Technical work group representative for PNPTC and project planning

Results

Fish Culture

Smolt release groups (SRG)

No SRG steelhead were collected in 2002, and therefore, none were released in 2004.

The growth of SRG steelhead from previous brood years is reported in Berejikian et al. (2003). Growth and survival rates of the BY 2003 SRG steelhead will be reported after rearing is complete and the fish have been released in May 2005. Table 1 summarizes

steelhead smolt and pre-smolt (unintentional) releases into the Hamma Hamma River for each year of the study.

Adult release groups (ARG)

Of the 133 ARG steelhead surviving to age-3 (March 2003), a total of 100 survived to age-4 (March 2004). Four fish died as a result of either natural causes or handling effects and the remaining mortalities (n = 30) were a result of illegal poaching from the rearing tanks. The proportion of males and females from the BY 2000 ARG maturing at age-3 and age-4 varied substantially among redd-groups (Table 2). The proportion of specific redd groups that matured as age-3 females ranged between 0% and 25%, and the proportion that matured as age-3 males ranged between 0% and 36% (Table 2). The majority of the population (76%) matured at age-4 and repeat maturation rates among families ranged between 0% and 23% for females and 0% and 38% for males (Table 2).

The growth of steelhead that did not mature in 2004 began to exceed that of steelhead that matured as either age-4 males or females approximately 6 months prior to the onset of maturation in February 2004 (Fig. 1).

Abundance of redds and adult steelhead

Redd abundance and characteristics

The number of redds observed in the Hamma Hamma River (including John Cr) has increased markedly since age-4 steelhead from the supplementation program (both ARG and SRG) first began spawning in 2002. Prior to supplementation, an average (± s.d.) of 11 (± 5.7) steelhead redds were observed annually between 1995 and 2001 in the Hamma Hamma River. The unadjusted number of redds recorded in the Hamma Hamma River (including John Creek) has averaged 25 (± 40) from 2002 through 2004. In 2004, all 30 redds recorded in John Creek were observed during foot surveys, and 89% of the redds (91 of 102 redds) observed in the Hamma Hamma River were visible by foot surveys alone. Thus, the abundance of redds in the Hamma Hamma River (including John Creek) was adjusted for years 2002 – 2004 with the following equation:

Adjusted redd abundance = ((0.89 · Hamma Hamma River redds) + (1.00 · John Creek redds))

The adjusted redd abundance in the Hamma Hamma River has averaged 114 (± 37) between 2002 and 2004, an approximate 10-fold increase over pre-supplementation abundance (Fig. 2). By comparison, the number of redds recorded in the control streams has remained fairly stable from 1995 through 2004 (Figure 2). The increased frequency of redd surveys in the Duckabush River did not result in a marked increase in the number of redds observed in 2004 (Figure 2).

Table 3 shows the average length, width, velocity and depth, and calculated area, for the 99 of 132 redds for which measurements were taken. It also shows the average values for

both the Hamma Hamma River and John Creek. In addition, the values for redds measured and constructed by females of known origin (wild or ARG) are shown.

Adult steelhead abundance and characteristics

Between 30 January and 3 June 2004, 43 fish were sampled by by hook-and-line (190 angler-hours). Twenty-five BY 2000 ARG fish (12 females and 13 males) were captured. One BY 1998 ARG (exercised) and one BY 1998 ARG (non-exercised) fish released in 2002 were also captured. Eight of the females captured were in a postspawning condition (all eggs deposited; none could be expressed), two were in a prespawning condition, and two were ovulated (eggs easily expressed). Thus, females appear to have been very successful at depositing eggs in the Hamma Hamma River. Of the 13 males captured, 8 were spermiating, 4 were considered in post-spawning condition because of their deteriorated condition, lack of milt, and the condition of the other male was undetermined.

Similar numbers of wild, SRG-Lilliwaup, and SRG-John Creek steelhead have been captured between 2002 and 2004 (Table 4). Data on the spawning condition of these fish is still being compiled.

Scales collected from O. mykiss captured by hook and line during the spawning season in 2002, 2003, and 2004 have provided some preliminary information on size-at-age for this population. Scales were not collected from all fish. Some scales were regenerated, and age could not be reliably determined. Fish spending two summers in the ocean (n = 13) ranged between 420 and 750 mm FL. Six fish captured (347, 360, 370, 385, 404, and 420 mm) had ‘plus’ growth indicating they’d spent a single summer in the ocean and returned less than a year after smolting. All six of these fish had spent three years in freshwater. The scales of six additional fish captured indicated they had not yet migrated to sea. Four apparent smolts (based on appearance) measured 210, 215, 260, and 333 mm and ranged in freshwater age from 2 to 4 years. Two age-3 fish measured 360 and 390 mm. Planned scale analyses from fish captured in 2005 will allow us to construct an improved size-at-age relationship.

An average of 21 fish (range 6-54) was observed on each of 19 weekly snorkel surveys between 23 January and 3 June 2004. No survey was conducted the week of 20 February due to weather and flow conditions. We began snorkel surveys one month earlier than in 2003 and detected steelhead on the first survey. A total of 400 individual observations were recorded of O. mykiss estimated to be greater than 30 cm total length, without parr marks, and not smolting.

A total of 144 observations were made of BY 2000 ARG steelhead released in 2004, and 18 observations were made of BY 1998 ARG released in 2002 (Fig 3). It is clear that repeat observations were made of the same fish because only 76 BY 2000 ARG steelhead were released. Ten adult SRG fish were observed between 28 January and 28 April, 2004, and no more than three were seen in a single survey (Fig. 3). Five ARG fish of

unknown rearing treatment and brood year and 38 steelhead of unknown origin were also recorded.

Wild O. mykiss accounted for 185 of the 400 observations. Because repeat observations of the same fish are known to have occurred for BY 2000 ARG, they are likely to have occurred for wild fish as well. Of the 185 wild O. mykiss observations, we were able to estimate the approximate length of 158. Of these, 76 observations (48%) were of fish

between 30 and 41 cm, 48 observations (30%) were of fish between 42 and 50 cm, and
34 observations (22%) were of fish greater than 51 cm total length (Fig. 3). Based on the
scale analysis, we preliminarily assume that fish estimated to be between 30 and 41 cm
were either age-3 or age-4 pre-migrant steelhead, resident rainbow trout, or steelhead that
have spent only one summer in the ocean. Fish greater than 42 cm were steelhead that
had spent two summers in the ocean.

Breeding Behavior and Success

Breeding Behavior

In 2002, a total of 24 courting pairs of steelhead were observed in the Hamma Hamma

River. Fifty-eight percent of the pairs consisted of an ARG female and an ARG male
(Table 5). In 2004, a total of 20 courting pairs were observed, 50% of which consisted of
an ARG female and an ARG male (Table 5). Thus, there was a tendency for ARG
females to pair with ARG males and for anadromous fish to pair with each other (Table
5). A male's ability to court females explained a significant portion ($P < 0.05$, $r^2 = 0.39$)
of the variability in adult-to-fry reproductive success of ARG males in an experimental
stream channel (Berejikian et al. unpublished). Given the numerous other factors that
contribute to reproductive success (e.g., spawn timing, sperm quality, number of
peripheral males, female egg quality, nest location, among others), pairing combinations
provide a substantial indication of eventual matings. Thus, ARG female x ARG male
and anadromous female x anadromous male matings appear slightly more likely to occur
than matings between groups. Spawn timing (determined by redd construction timing)
was earlier for ARG females than for anadromous females (Table 6), and may explain the
assortative pairing by rearing type. Spawn timing of the combined ARG, SRG, and wild
population did not appear to be linked to streamflows (Fig. 5).

Embryo viability

The viability of embryos hydraulically removed from redds that were constructed by
captively reared females averaged 91% in 2002 and 75% in 2004. The percentage of
viable embryos in the two years combined averaged 83% (Table 7), which was less than
for wild fish spawning in the Hamma Hamma River (86% to 98% annual average)
between 1998 and 2001 (Berejikian et al. 2002).

Otolith microchemistry

The analysis of the 2003 samples and re-analysis of samples from previous years (see
Methods) is not yet complete. Results from these analyses will be presented at the

project annual review and reported in the 2005 annual report.

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Genetic monitoring

The genetic analyses of the 2003 samples will take place when results of the otolith analyses become available. The samples have been catalogued and supplies purchased.

Results of genetic monitoring through 2002 are presented in prior annual reports.

Conclusions/Future work

The number of redds observed in the Hamma Hamma River has increased markedly as a result of the supplementation program. Females from the ARG appear to be contributing substantially to those increases in the year they are released. ARG fish have also been observed during the spawning season up to two years after they were released, indicating their potential to contribute as repeat spawners. Although only a small number of measured redds could be assigned to either an ARG or anadromous female, the size, depth, and flow conditions of the redds were similar for the two groups. Work planned for 2005 will provide a fourth year of data on redd abundance and characteristics in the Hamma Hamma River. By the end of the 2005 spawning season, data will have been collected for two years in which fairly large numbers of ARG steelhead were released (197 in 2002 and 76 in 2004) and two years in which few ARG steelhead were released (4 in 2003 and 24 in 2005).

Wild and ARG steelhead were observed (by snorkeling) in similar numbers in 2004. However, the majority of the wild steelhead were fairly small (<42 cm TL). Some of these fish are confirmed to have gone to sea for a single summer, but their gender and reproductive status were not clear (most appeared bright and without obvious signs of secondary sex characteristics). Few SRG steelhead were observed in 2004, probably because so few were released in 2002. Further analysis of scales to be collected from steelhead in 2005 will provide a greater understanding of the life history characteristics of the Hamma Hamma steelhead population. In particular size-at-age information will allow us to refine abundance estimates of wild fish obtained from snorkel surveys.

All four combinations of pairing between anadromous and ARG steelhead were observed in 2002 and 2004, when substantial numbers of ARG steelhead were released. However, the majority of pairing combinations consisted of ARG x ARG pairs or anadromous x anadromous pairs. The documented timing of redd construction by ARG and

anadromous females suggests that the indications of assortative pairing may be caused by differences in spawn timing, with ARG females as a group completing redd construction earlier than anadromous fish. A final statistical analysis of these data will be completed following the release of the last ARG steelhead in 2005.

The majority of ARG females captured by hook-and-line had deposited all of their eggs. The others were either in the process of spawning or had not yet matured. The proportion of eggs that produced viable embryos from ARG females was less in 2004 than in 2002, and overall slightly poorer than documented for wild fish. The comparison is confounded by the fact that the data on wild and ARG embryo viability were collected in different years. Nevertheless, the ability to produce viable eyed embryos does not

appear to be a major impediment to the reproductive success of ARG or wild steelhead in the Hamma Hamma River.

Data analyses to test for genetic changes in the Hamma Hamma steelhead population and otolith analyses designed to test for adult-to-parr reproductive success of females from anadromous and ARG females have been slightly delayed because of problems associated with use of otolith diagnostic equipment.

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Table 1. The number off eggs collected from wild steelhead in the Hamma Hamma River for each year of the study, number of fry stocked into the hatchery tanks at the Lilliwaup Hatcher (LH) and John Creek pond (JCP), number of smolts released at age-2, and

number of fish estimated to have escaped from the JCP in each year of the study. The
JCP escape estimate assumes that mortality rate in the JCP was the same as in the LH.
The escape estimates are revised from those reported in prior years.
No. stocked
No. smolts released
Brood year
Eggs Collected LH JCP LH JCP JCP Escapes
JCP escape date
1998 4,683 1,678 1,884 1,524 0 1,711 Nov 1999
1999 2,588 1,150 1,150 901 435 466 Nov 2000

2000 1,622 591 747 481 8 601 Nov 2001
2001 1,2711 1,000 1,000 727 150 577 Mar 2003
2003 4,105 1,570 1,570 --
1 SRG was supplemented with BY 1998 3 year-old mature fish to increase the total group size to 2000 fish.

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Table 2. Maturation rates for each of the seven redd-groups collected in 2000 for rearing
to adult (i.e., the adult release group, ARG). The proportion of the population surviving
at age-4 (N = 97) that were mature males (M), females (F), or not mature (B) at age-3,
and age-4. The proportions of each redd-group that matured at both age-3 and age-4 are
shown separately for males (Rep. M) and females (Rep. F).

Reddgroup	Age 3				Age 4					
	M	F	B	n	M	F	B	n	Rep. F	Rep. M
1	0.15	0.00	0.85	13	0.46	0.46	0.08	13	0.00	0.15
2	0.38	0.23	0.38	13	0.46	0.31	0.23	13	0.15	0.38
3	0.00	0.11	0.89	18	0.17	0.50	0.33	18	0.11	0.00
4	0.33	0.25	0.42	12	0.54	0.23	0.23	13	0.23	0.31
5	0.36	0.00	0.64	11	0.55	0.27	0.18	11	0.00	0.36
6	0.19	0.13	0.69	16	0.38	0.31	0.31	16	0.06	0.19
7	0.25	0.25	0.50	12	0.38	0.46	0.15	13	0.23	0.23

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Table 3. Measurements from redds observed in the Hamma Hamma River (HHR) and John Creek (JCR). The measurements from redds constructed by wild steelhead and adult release group (ARG) steelhead are also shown. Thirty-three of the redds observed
in 2004 were not measured.
Location
n
Mean Length (m)

		Mean Width (m)	Mean Area (m2)	Mean Depth (m)	Mean Velocity (m/s)
HHR	70	2.06	0.86	2.05	0.48	0.83
JCR	29	1.52	0.72	1.18	0.22	0.42
Total	99	1.90	0.82	1.79	0.40	0.71
Wild	3	2.62	0.71	1.88	0.43	0.42
ARG	6	3.10	1.14	3.77	0.38	0.64

Table 4. The number of adult steelhead greater than 420 mm fork length (assumed to have spent two summers at sea) captured by hook-and-line between 2002 and 2004. Three groups are shown: natural orign fish (i.e., wild), fish reared to age-2 at John Creek and released as smolts, and fish reared to age-2 at Lilliwaup and released as smolts. Unknown (Unk) gender refers to fish that had not matured to the point where gender could be reliably determined, or data were not recorded by anglers.

	Wild				John Creek				Lilliwaup			
Year	Male	Female	Unk	Total	Male	Female	Unk	Total	Male	Female	Unk	Total
2002	2	1	1	4	0	2	2	4	1	0	2	3
2003	0	0	4	4	0	0	1	1	0	1	0	1
2004	0	0	0	0	1	0	0	1	0	3	0	3
Totals	0	1	5	8	1	2	3	6	1	4	2	7

Table 5. Pairing combinations of adult steelhead exhibiting reproductive behavior in the Hamma Hamma River in 2002 and 2004. Fish were either from the adult release group (ARG) or anadromous fish (AN) from the smolt-release group or natural-origin. The pairing combinations were similar in 2002 and 2004, so the combined data for the two years are shown.

	ARG	AN	Totals
2002			
ARG	14	3	17
AN	3	4	7
Totals	17	7	24
2004			
ARG	10	1	11
AN	3	6	9
Totals	13	7	20
Combined			
ARG	24	4	28
AN	6	10	16
Totals	30	14	44

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Table 6. The earliest, latest, and mean date of nesting activity by ARG and anadromous
females in the Hamma Hamma River in 2004. Anadromous females may have either been natural-origin or from the SRG. The difference in mean date of nest construction is
calculated as anadromous – captive and expressed as days (d).

2002	2004			
Anadromous	Captive	Difference (d)	Anadromous	Captive	Difference (d)
N	7	17	9	11
Earliest	6-Mar	1-Mar	5	9-Mar	8-Mar	1
Latest	4-Jun	23-Apr	42	11-May	14-Apr	27
Mean	22-Apr	24-Mar	29	16-Apr	20-Mar	27

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Table 7. Viability of eggs likely deposited by ARG females in 2002 and 2004 in the
Hamma Hamma River (HHR) and John Creek (JC). A total of 838 eggs were collected for analysis in 2002 and 2004. The sample size (N) refers to the number of eggs examined from each redd.

Year	Location	N	Prop. live
2002			
	HHR	30	1.00
	HHR	129	0.95
	HHR	84	0.73
	HHR	29	0.90
	HHR	30	0.97
	Ave proportion 5a		0.91
2004			
	JC	62	0.79
	JC	42	0.95
	JC	86	0.21
	HHR	141	0.48
	HHR	44	0.98
	HHR	51	0.77
	HHR	85	0.84
	HHR	25	0.96
	Ave proportion 8a		0.75
	Total Ave proportion 3a		0.83

a The sample size (N) refers to the number of redds sampled

27
0
100
200
300
400
500
600
Jun-00 Apr-01 Feb-02 Dec-02 Oct-03
Length (mm)
0

500
1000
1500
2000
2500
Jun-00 Apr-01 Feb-02 Dec-02 Oct-03
Sampling Dates
Weight (g)
Figure 1. Length and weight of the BY 2000 adult release group (ARG) steelhead. Data points are means of individual mature age-4 females (.), mature age-4 males (X) and non-maturing (.) fish at age-4.

28

29
0
20
40
60
80
100
120
140
160
1995 1996 1997 1998 1999 2000 2001 2002 2003 2004
Year
Number of redds
Dosewallips R.
Duckabush R.
Hamma Hamma R
Dewatto R
Figure 2. The number of redds observed in the Hamma Hamma River (supplemented) and control streams. The Hamma Hamma River data from 2002 – 2004 has been adjusted to exclude redds observed during snorkeling (see Methods). The number of redd surveys in the Duckabush River was increased from 5 in 2003 to 18 in 2004.

30
0
5
10
15
20
25
30
35
40

45
1/23
1/30
2/62/13
2/20
2/273/5
3/12
3/193/26

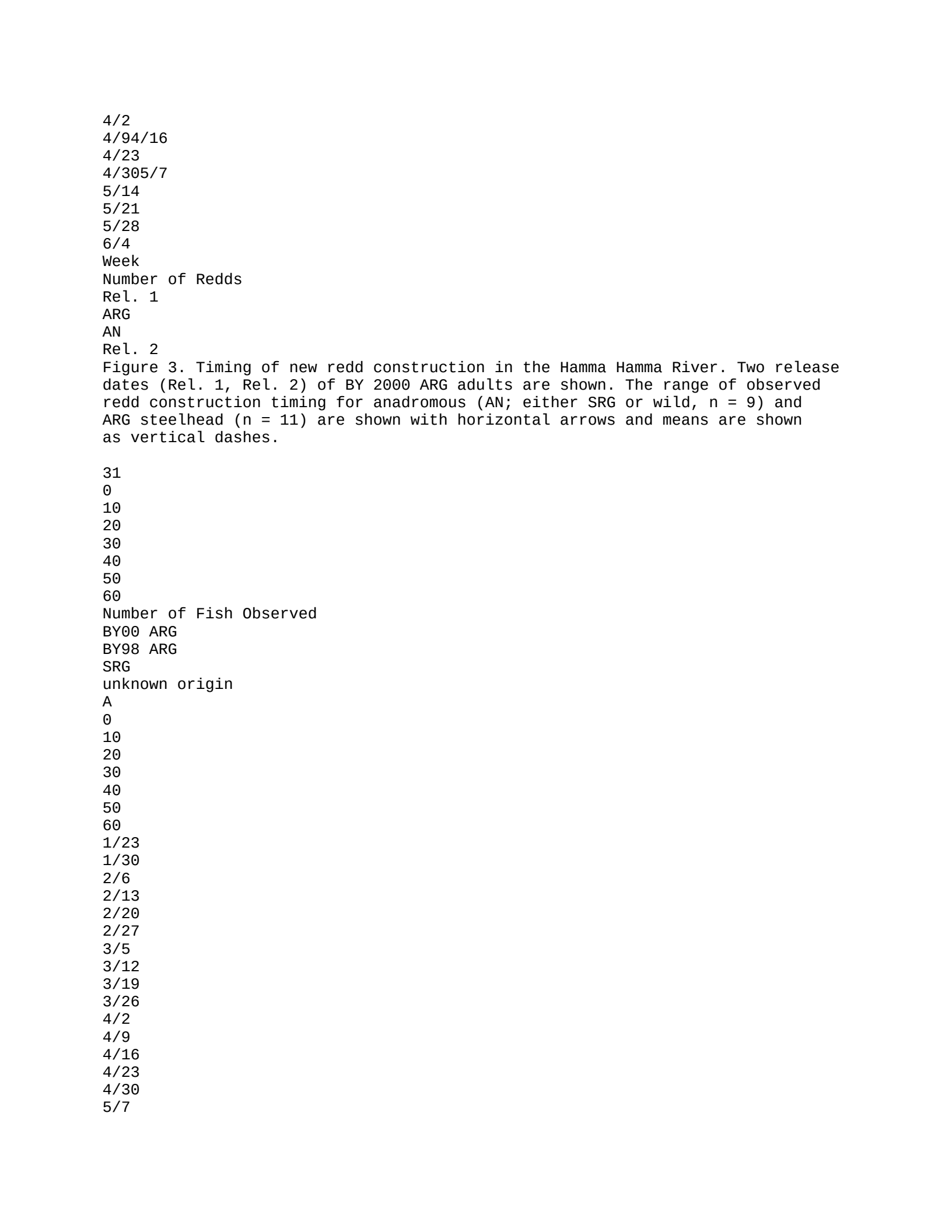

Figure 3. Timing of new redd construction in the Hamma Hamma River. Two release dates (Rel. 1, Rel. 2) of BY 2000 ARG adults are shown. The range of observed redd construction timing for anadromous (AN; either SRG or wild, n = 9) and ARG steelhead (n = 11) are shown with horizontal arrows and means are shown as vertical dashes.

5/14
5/21
5/28
6/4
Week of (2004)
Number fish observed
BY00 ARG
Wild
Unknown
B
Figure 4. Number of steelhead observed by snorkeling in the Hamma Hamma River in 2004. A total of 400 observations were made between 23 Jan and 4 June, 2004. Observation of all adults from the supplementation program are shown in panel A. Observations of wild and BY 2000 ARG adults are shown in panel B, and wild fish size categories (total length in cm) are shown in panel C (next page). Repeat observations of the same fish are known to have occurred for BY 2000 ARG, and thus likely to have occurred for the other groups as well.

32
0
10
20
30
40
50
60
1/23
1/30
2/6
2/13
2/20
2/27
3/5
3/12
3/19
3/26
4/2
4/9
4/16
4/23
4/30
5/7
5/14
5/21
5/28
6/4
Week of (2004)
Number fish observed
Large (>42cm total length)
Small (<42cm total length)
Undetermined size
C
Figure 4. Continued

33
0
5

10
15
20
25
30
35
40
1/23
1/30
2/6
2/13
2/20
2/27
3/5
3/12
3/19
3/26
4/2
4/9
4/16
4/23
4/30
5/7
5/14
5/21
5/28
6/4
Date
Discharge (cms)
0
5
10
15
20
25
30
35
40
45
Number of new redds
Fig. 5. Hydrograph and new steelhead redd construction (solid diamonds) by week in the
Hamma Hamma River and John Creek in 2004. BY 2000 ARG were released on
13 February (8 females) and 3 March 2004 (27 females). Actual stream discharge
(solid lines) was calculated from daily gauge readings using a regression equation
derived from stream gauge calibrations conducted during 2003. For dates without
gauge readings, discharge was estimated (dashed lines) from the actual discharge
readings.