Residualism in wild and domesticated broodstock steelhead trout (*Oncorhynchus mykiss*): growth modulation during juvenile rearing can reduce rates of residualism

by

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EXECUTIVE SUMMARY

- The hatchery-produced offspring of wild steelhead (WWR) residualize at a higher rate than offspring of traditional domesticated hatchery broodstock (HWR). Based on the number of residuals in our samples and the numbers of yearling fish planted, the offspring of wild fish were 25 times more likely to become residuals than the offspring of hatchery fish. We speculate that altering the feeding regimen earlier in the rearing cycle and imposing the differential feeding rates for a longer time will further decrease residualism rate but caution that the offspring of wild broodstock steelhead may always residualize at a higher rate than offspring of domesticated origin. To the extent that residuals pose genetic and ecological risks to endemic fishes, the phenomenon of residualism should be considered when establishing wild broodstock programs.

- WWR residuals were bimodally distributed by size. The two modes were at approximately 170 mm and 220 mm. Small residuals were approximately as abundant as large residuals in our samples.

- Increasing the size of small fish by placing them on an aggressive feeding regimen decreases the rate of residualism of those fish.

- We were unsuccessful at limiting the growth of large fish and did not determine if modulating (reducing) growth of large fish alters the rate of residualism of those fish. That work should still be done.

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ABSTRACT

Residualism is the failure of some hatchery-reared salmonid juveniles to outmigrate as smolts with the rest of their cohort. We present results of an evaluation of residualism in wild and traditional broodstock hatchery steelhead in the Kalama River, Washington and discuss the potential for genetic and ecological consequences of high rates of residualism. Following the release of 1998 and 1999 broods, we found that the hatchery-reared offspring of wild broodstock residualized at a rate greater than that of offspring of traditional domesticated broodstock and that residuals were bimodally distributed by size. For the 2001 brood, we experimentally manipulated growth trajectories during juvenile rearing to decrease size variance among released fish. Our expectation was that fewer fish would be too small or too large to smolt as yearlings. Small fish placed on an aggressive rearing regimen residualized at a lower rate than comparable control fish. We saw no effect on residualism rate of large fish placed on a lower feeding regimen but the lower feeding regimen did not significantly affect size at release. Physiological parameters (sodium-potassium ATPase and insulin-like growth factor) were monitored throughout rearing and release. All fish showed expected patterns of change associated with smoltification in both parameters and small fish placed on the aggressive rearing regimen showed slightly but significantly higher IGF-1 levels than comparable control fish.

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INTRODUCTION

Wild salmonids are increasingly being incorporated into hatchery programs because their offspring may pose fewer genetic and ecological risks to the extant wild population than the naturally spawning offspring of domesticated, non-indigenous broodstock (Waples 1991, Krueger and May 1991). Residuals, defined here as hatchery-reared juveniles that fail to outmigrate with the remainder of their cohort pose ecological and genetic risks to indigenous fish (McMichael et al. 1997) and have an economic impact on a hatchery program. Ecologically, the residuals will compete directly with native con-specifics and other species in the watershed with overlapping ecological requirements. Two genetic issues arise with residualism. First, residualism is likely an expression of the natural phenotypic diversity of many salmonids and must have, in part, a genetic basis. High over-wintering mortality of residuals may result in a loss of genetic diversity within that part of the cohort that does successfully outmigrate and eventually return to spawn. Second, residual fish (especially the common precociously maturing male fish) may spawn directly with native resident rainbow trout or, conceivably, with native coastal cutthroat (*O. clarki*). Economically, the residuals represent a waste of fish culture effort since there is little evidence that they contribute substantially to adult returns.

A project to evaluate wild steelhead as broodstock is underway in the Kalama River, Washington. After two smolt releases (brood years 1998 and 1999), it became apparent that the rate of residualism of wild broodstock fish was greater than that of the traditional domesticated strain. Large numbers of residuals were noted during summer snorkel surveys and most were from our wild broodstock program despite large numbers of fish from our traditional stock released at the same time and place. The residuals were bimodally distributed by size with approximately equal numbers of small (fork length < 160mm) and large (>200mm) fish remaining in the stream after active migration of the rest of the cohorts had ceased (unpublished WDFW data; Figure 1).

Given the bimodal size distribution of the residuals, we reasoned that the phenomenon might have arisen via two different growth-mediated mechanisms: First, a fish may fail to reach an adequate size in its first year to trigger the parr-smolt transformation. Insufficient growth is a mechanism of particular importance to wild broodstock hatchery programs since wild fish typically spawn much later than domesticated strains leaving less time for fish to grow to an adequate size in time for release as smolts in the spring. In addition, the offspring of wild broodstock appear to accept feed less readily than domesticated fish, especially when hand-fed. Second, faster growing fish, especially males, may become precociously mature.

Figure 1. Size distributions of residuals from brood year 1998 and 1999 WWR released as juveniles in 1999 and 2000, respectively.

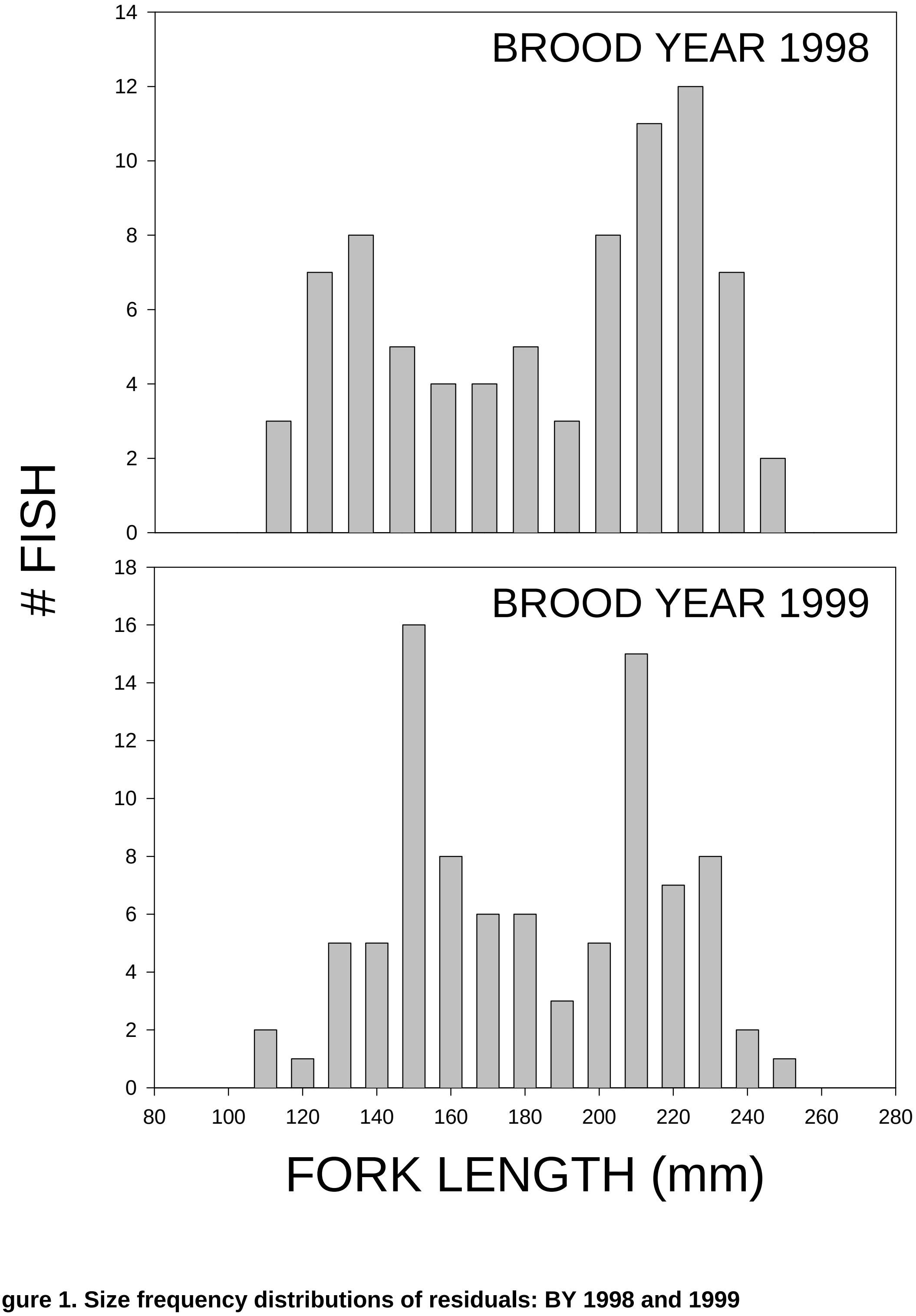


Figure 1. Size frequency distributions of residuals: BY 1998 and 1999

As a working hypothesis, we reasoned that it might be possible to reduce residualism by modifying the rearing regimen in the hatchery to decrease size variance within the smolt cohort. After size grading in the summer prior to smolting, small fish could be reared in isolation from their larger peers, releasing them from sub-optimal feeding conditions induced by competitively dominant larger individuals (McCarthy et al. 1992). The new environment could induce compensatory growth in the smaller fish, allowing them to "catch-up" and either match the size of the larger cohort (Jobling and Johansen 1999) or, at least, increase the abundance of individuals in the cohort that achieve a large enough size to undergo the parr-smolt transformation.

Additionally, the growth trajectory of the larger fish could be modified to reduce summer - fall growth, limiting adoption of a resident life history strategy such as precocious maturation the following year, while still achieving the wild-like growth trajectory (rapid growth in the spring prior to smolting) thought to contribute to successful smolting (Beckman et al. 1998, 1999).

The objectives of the work were to (1) develop a method to reduce residualism of hatchery-reared wild-broodstock steelhead, (2) assess physiological status of residual steelhead to determine mechanisms promoting residual behavior, and (3) compare growth, physiological parameters, smolt development and migration patterns of wild broodstock and domesticated broodstock steelhead juveniles to determine if differences in smolt development promote residualism.

Our goal is to develop a practical, logistically feasible, and effective method to reduce residualism of cultured salmonids, especially among the burgeoning number of programs using wild fish as broodstock.

STUDY AREA

The Kalama River, Washington is a westerly flowing tributary to the lower Columbia River entering the Columbia at river-kilometer (rkm) 117. The watershed drains approximately 531 km^2 with flows ranging from 8.3 to 88.1 m^3/s mean monthly minimum and maximum, respectively (1946-74; United States Geological Survey data). Water temperatures range from 5^0 C (January) to 15^0 C (July). Two barriers to anadromous adult migration exist in the system: one at the site of the Kalama Falls Hatchery (KFH) at rkm 17 and one at rkm 59. A hatchery fishway terminating in an adult trap bypasses the lower falls and provides access to virtually all anadromous adults attempting to enter the upper watershed to spawn. The upper falls is a complete barrier to upstream migration. An earthen pond in the upper watershed adjacent to Gobar Creek, a tributary to the Kalama at rkm 31, is used for final rearing and acclimation prior to release.

Two endemic run forms of steelhead exist in the Kalama: winter-run (entering the river from November to June just prior to spawning) and summer-run (which enter the river from April through December, over-winter, and then spawn). Other endemic fish species include resident and anadromous cutthroat trout (*O. clarki*), mountain whitefish (*Prosopium williamsoni*), peamouth (*Mylocheilus carinus*), largescale sucker (*Catastomas macrocheilus*), Pacific lamprey (*Lampetra tridentatus*), and a number of cottids. In addition, resident rainbow trout (*O. mykiss*) are present in the system. Hatchery programs in the Kalama plant coho (*O. kisutch*) and chinook (*O. tshawytscha*) salmon as well as domesticated strains of both steelhead run types (summer-run: Skamania stock; winter-run: Beaver Creek stock).

METHODS

Spawning and Incubation: Two groups of steelhead, one using the traditional hatchery source and one using natural origin winter-run steelhead were used for these experiments. The hatchery origin adults were derivatives of the Chambers Creek stock (Crawford 1979) and were returns to the Kalama from earlier smolt plants out of the Beaver Creek Hatchery (Elochoman River, Washington; Figure 2). Origin of the fish was established by the absence of an adipose fin. Twenty-five females and 26 males were captured in the adult trap at KFH between 27 December 2000 and 23 January 2001, checked for ripeness, and spawned using a partial factorial mating design (2M X 2F) except that in a single case two of the males were spawned with a single female. Eggs were incubated in vertical stacks following normal hatchery protocols with a daily formalin treatment to control fungus growth.

Fifteen female and 16 male natural-origin spawners were captured in the adult

Figure 2. Area map of the lower Columbia River.

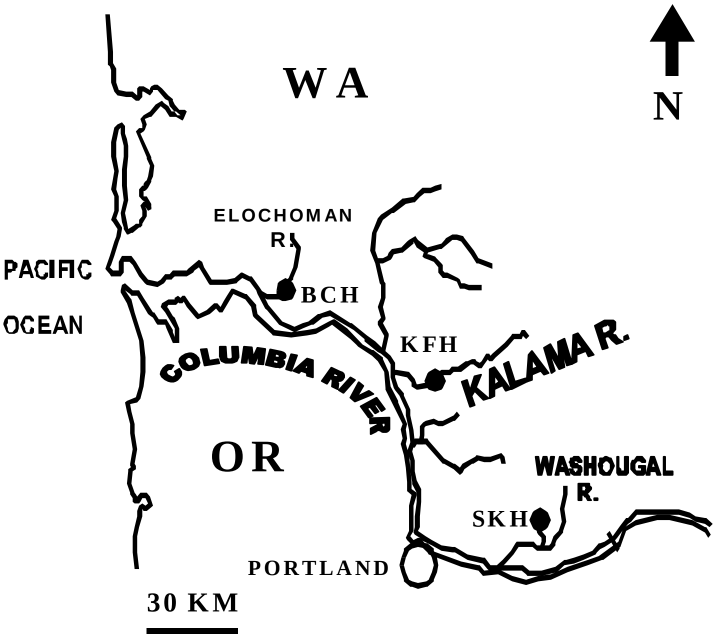


Figure 2. Area map of the lower Columbia River.

trap between 10 April and 17 April 2001. Origin of these fish was established by the presence of an adipose fin and absence of a stubbed dorsal fin. Most hatchery-origin steelhead returning to the Kalama have a markedly eroded dorsal fin. Because the adult trap is 17 km upstream of the confluence with the Columbia, we assumed that all the wild broodstock were of Kalama origin. The wild fish were, as with the hatchery fish, checked for ripeness and spawned using a partial factorial mating design (2M X 2F) except that again, coincidentally, in a single case two of the males were spawned with a single female. Eggs were incubated in egg baskets in shallow troughs following normal hatchery protocols with a daily formalin treatment to control fungus growth.

Early Rearing: After absorption of the yolk sack, fry were transferred to intermediate rearing vessels (L X W X D = 4.7 X 1 X 0.5m). At approximately 2 gm total weight, fry were transferred to standard hatchery ponds and fed to satiation by hand several times/d.

Pond Split and Marking of Fish: Beginning on 3 October 2001, a sample of 200 WWR parr was dipnetted from the hatchery pond and fork length and weight were recorded. The median fork length of this sample was used as a threshold for sorting the rest of the fish in the pond. Approximately two-thirds of the fish smaller than the median size received snout-implant coded wire tags (CWT) and were placed in a standard hatchery pond. These became the small treatment (SMTMT) fish. The remaining one-third of the fish smaller than the median size received a different CWT and were placed in another standard hatchery pond (small control; SMCNT). Approximately one-third of the parr larger than the median size received a different CWT, and were placed in the hatchery pond containing the SMCNT fish (LGCNT). The remaining two-thirds of the large fish received their own unique tags and were placed in their own pond to become the large treatment release group (LGTMT). Tagging of WWR fish was completed by 24 October 2001. HWR juveniles were marked with blank wire tags inserted in the musculature of the left cheek in January 2002, placed in their own hatchery pond and reared using normal hatchery protocols. In addition to tagging, all HWR and WWR juveniles were adipose fin clipped. All size sorting, tagging, and fin clipping of WWR fish was accomplished in a Worldmark ^{R} automated tagging trailer. HWR fish were tagged and clipped by hand by trained WDFW staff.

Imposition of Differential Rearing Regimens: In general, all WWR fish were fed to satiation several times/d. Additionally, to ensure that the SMTMT fish were given the maximum opportunity to feed and grow, their pond was equipped with standard bell-shaped surface demand feeders and an automatic underwater feeding apparatus fitted with two belt feeders slowly delivering a constant supply of pelleted feed. The LGTMT pond and the control pond (containing SMCNT and LGCNT fish) were equipped with the surface demand feeders only. HWR were fed by hand only. Throughout rearing at KFH, the fish were fed Moore-Clark^{R} Nutrafry^{R}, Clark's Fry^{R}, and Trout AB^{R}. Mass of food of each type delivered to each rearing vessel was recorded throughout rearing. An estimate of digestible energy within each food type was obtained from the manufacturer to allow standardization among feeding regimens using different amounts of food with differing nutritional content. Table 1 provides feeding schedules for the treatment and control groups expressed as percent body weight/d/pond weighted by the estimates of digestible energy in each feed type.

Final Rearing, Acclimation and Release: Beginning on 13 March 2002, five months after imposition of the differential feeding regimens, all HWR and WWR fish were crowded in their respective ponds, pumped into fish transport trucks and transferred to Gobar Pond. Other steelhead rearing in the pond included adipose clipped but otherwise unmarked winter-run trucked and planted into Gobar Pond on 19 March 2002 from the Elochoman hatchery and adipose clipped/right cheek tagged summer-run trucked and planted from KFH on 13 March 2002. The Elochoman winter-run and KFH summer-run are not explicitly part of this project and results from monitoring growth and migratory behavior of those fish will be reported at another time. Fish in Gobar pond were fed to satiation once each day by hand. Additionally, two floating docks, each mounted with two bell-shaped surface demand feeders were cabled to the center of the pond. The floating docks were fitted with solar powered electric fences to discourage use of the docks as feeding stations for great blue herons (Ardea herodias). Demand feeders were refilled with pelleted feed as necessary, generally every 1 - 2d. Feed types used at Gobar pond were Moore-Clark^R Nutrafry^R, Clark's Fry^R, and Trout AB^R.

Fish were released from Gobar Pond by removing a screen from the pond outflow on 2 May 2002 and incrementally removing dam boards on the 3rd, 9th,

Table 1. Bi-weekly feeding rates of experimental fish. Values are percent body weight/d adjusted for digestible energy of the various food types provided to the fish.

Table 1

2-WKS STARTING:	SMALL TREATMENT	CONTROL	LARGE TREATMENT	HATCHERY WINTER-RUN
10/24/01	1.33%	1.22%	2.06%	0.44%
11/7/01	2.68%	2.05%	1.18%	0.14%
11/21/01	1.28%	1.00%	0.52%	0.09%
12/5/01	1.64%	1.16%	1.11%	0.23%
12/19/01	1.42%	1.31%	1.00%	0.18%
1/2/02	1.41%	1.23%	1.08%	0.14%
1/16/02	2.76%	2.41%	1.34%	0.17%
1/30/02	0.81%	1.06%	0.93%	0.18%
2/13/02	0.52%	0.49%	0.38%	0.16%
2/27/02	0.77%	0.62%	0.87%	0.15%
AVERAGE	1.46%	1.25%	1.05%	0.19%

15th, 22nd, and 28th May 2002. National Marine Fisheries Service guidelines mandate that all hatchery steelhead be released by 1 June. On 30 May 2002, all fish remaining in the pond were forced out by passing a beach seine down the length of the pond.

Growth and Physiological Sampling: Samples were collected approximately monthly from the standard hatchery ponds by lowering the pond level to about half normal depth, crowding the fish by walking downstream along the central pond divider, and dipnetting at least 100 fish/pond. Fish sampled from the standard ponds were processed immediately, generally between 10AM and 3PM on each sampling day.

Samples from fish rearing in Gobar Pond were obtained by cast-net, always from multiple locations around the perimeter of the pond. Feed was thrown onto the surface of the pond and a 2m weighted, circular cast net with 1 cm nylon mesh was tossed over the baited fish. Captured fish were placed in a $1m^3$ live box with fresh circulating water. Fish were dipnetted from the live box, sorted by noting presence or absence and location of CWTs using a hand-held tag detector (Northwest Marine Technology^R), and placed into separate $0.5\ m^3$ aerated vessels. The water in the sorting vessels was frequently replenished to avoid undue stress to the specimens.

Volitional migrants were obtained by placing a fish screen in the downstream portion of the exit channel and dipnetting fish trapped between the screen and the counter. Migrant samples were placed in submerged, weighted live boxes until they could be processed as noted for Gobar Pond samples. When adequate numbers of snout tagged (WWR) and left cheek tagged (HWR) fish were sequestered in their own live boxes, the screen was removed and remaining fish in the exit channel were allowed to leave. Capture and sorting of the volitionally migrating fish generally occurred between 0200 and 0800 hours on each sampling day. The forced migrants were sampled and processed in a similar fashion except they were collected by dip net from the head end of the exit channel and that sampling occurred at 1100 hours.

Growth and physiological samples were obtained from fish rearing at KFH by placing 100 fish (approximately 10 at a time) in an anesthetic dose of MS222, recording fork length (FL) to the nearest mm, weight (WT) to the nearest 0.1gm, and a smolt index (SI: degree of smolt coloration including silvery skin, lack of coloration in anal, dorsal and paired fins, black band on caudal fin). Every fifth specimen from the LGTMT, SMTMT, and HWR groups was sampled for blood and gill tissue using standard methods until a sample size of 15 fish was obtained for each group on each date. Every third specimen from the control pond (LGCNT and SMCNT combined) was sampled for blood and gill tissue until a total sample size of 30 was obtained. Sex and degree of gonadal maturation was noted for all euthenized fish. Fish sampled only for FL, WT, and SI were returned to their respective hatchery ponds to recover.

Gobar pond, volitional exit, and forced exit samples occurred in similar fashion except that the first 15 HWR fish and first 100 WWR fish were euthenized for physiological sampling and determination of sex.

Migrant Sampling: The exit channel for the pond was fitted with an automatic fish counter manufactured by Smith-Root^R to constantly enumerate volitionally emigrating fish and forced migrants. The counter was placed at the head end of the exit channel approximately 1m below the water surface and 0.2m above the bottom of the exit channel. This placement provided adequate flow to ensure one-way movement of the fish through the counting device. Timing of volitional emigration from the pond was established by applying the proportion of WWR and HWR fish in each weekly sample to the total number of emigrants during that week. A separate sample on 30 May 2002 was used to estimate abundance of WWR and HWR fish remaining in the pond after volitional migration (forced migrants).

In addition, a rotary screw trap placed adjacent to KFH at rkm 17 was used to establish total numbers of WWR and HWR fish emigrating from the system. Smolt-trapped WWR fish were not euthenized so we are not able to partition WWR fish into the four different control and treatment groups.

Residual Sampling: After active migration of WWR and HWR fish from the Kalama system had ceased as indicated by the lack of tagged fish captured in a rotary screw trap, WWR and HWR fish remaining in Gobar Creek were presumed to have residualized. Specimens were obtained on 17 and 18 July 2002 by a combination of electrofishing and angling. Electrofishing was accomplished by multiple passes of electrofishing equipment between block nets placed in riffles above and below pools where, in earlier work, we noted that large numbers of residuals accumulate. Angled fish were captured with bait and spinners.

Electrofished and angled fish were sequestered in separate submerged and weighted live boxes and processed separately so that we could evaluate the potential for confounding effects of size bias imposed by the sampling methods. Specimens were processed in a manner identical to the Gobar Pond and exit samples except that no gill tissue was collected and all fish were euthenized to obtain blood samples and to note sex and degree of sexual maturation.

Assignment of Specimens to Release Group: All euthenized WWR fish from the KFH control pond, Gobar Pond and residual sampling were individually numbered, bagged, and frozen for later extraction and reading of the CWTs. Tags were dissected from the snouts of the specimens, cleaned, and read under a dissecting microscope.

Physiological Sample Processing: Blood samples were obtained by placing fish into a lethal dose of MS-222; tails were severed and blood was collected in heparinized glass tubes. The whole blood was placed on ice until all samples were obtained, then the blood was centrifuged at 3,000 X g for 5 minutes. Plasma was removed and stored at –80^{o} C until analyzed. Gill tissue was cut from gill arches, placed in a buffer solution (McCormick 1993), frozen and stored at -80^{o} C. Plasma levels of acid ethanol extracted IGF-I were determined by RIA using components obtained from GroPep Ltd. (Adelaide, Australia) as described by Shimizu et al. (2000). Plasma 11-KT levels were determined using an enzyme-linked immunosorbant assay (ELISA) according to Cuisset et al. (1994). Gill ATPase activities were measured using the method of McCormick (1993).

Statistics: For statistical comparisons, physiological and size parameters were analyzed using t-tests and ANOVA and, as necessary, their non-parametric equivalents. Sex ratios and frequencies of residuals from the different release groups were compared using the log-likelihood ratio test (G-test). A significance level of 0.05 was adopted throughout.

RESULTS

Rearing: WWR fish were successfully sorted by size into control and treatment groups and marked with coded wire tags. Immediately after the pond split SMTMT and SMCNT fish were of equal size and LGTMT and LGCNT were of equal size with a statistically significant difference between the large and small groups (2-way ANOVA, Tukey's multiple comparison test: $P<0.05$). Imposition of the different feeding regimens was successful in increasing growth of the SMTMT fish relative to the SMCNT group (Figure 3) with fork length of SMTMT fish being, on average, 13 mm greater than that of SMCNT fish immediately prior to transporting the fish to Gobar pond for acclimation, final rearing and release. However, we were not successful in limiting the growth of the LGTMT fish relative to the LGCNT group. Large treatment fish received less feed in terms of percent body weight/d than any of the other WWR groups (Table 1) but were only 5 mm on average smaller than LGCNT fish, a difference that was not significant statistically.

Figure 3. Mean FL (mm) + SEM from before pond split and imposition of differential feeding regimens to just before transport to Gobar Pond final rearing, acclimation, and release. Bars with letters in common are not significantly different (Duncan's Multiple Range Test; $P < 0.05$).

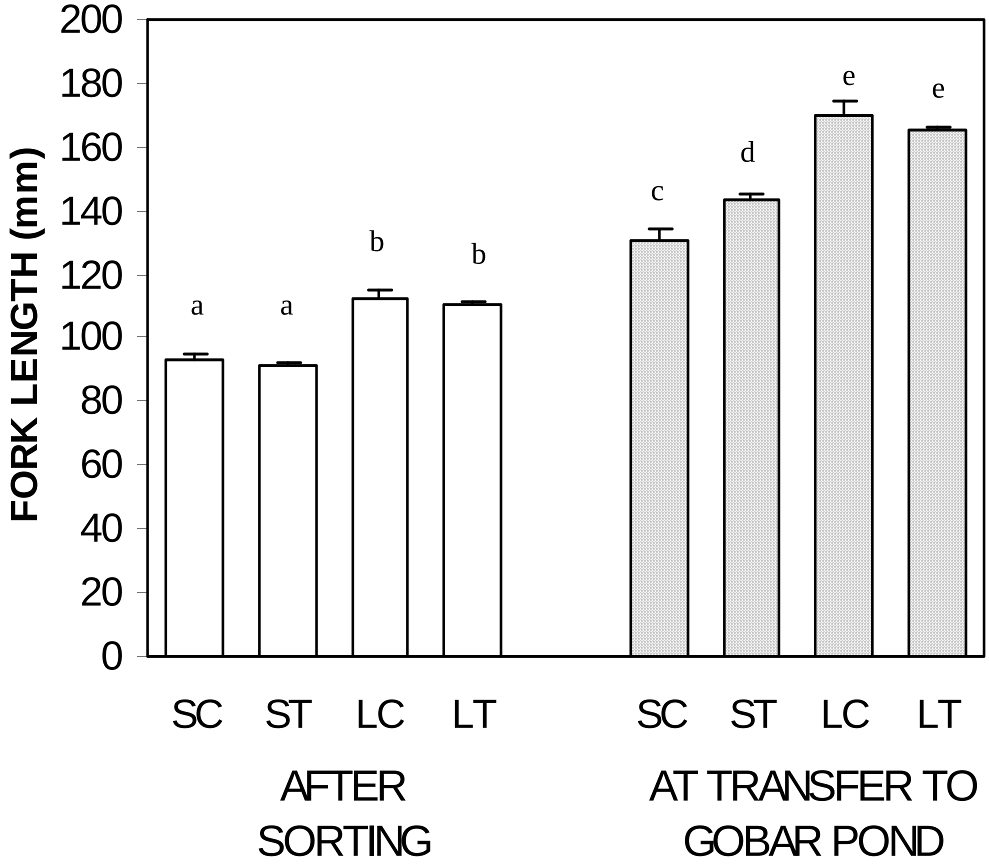
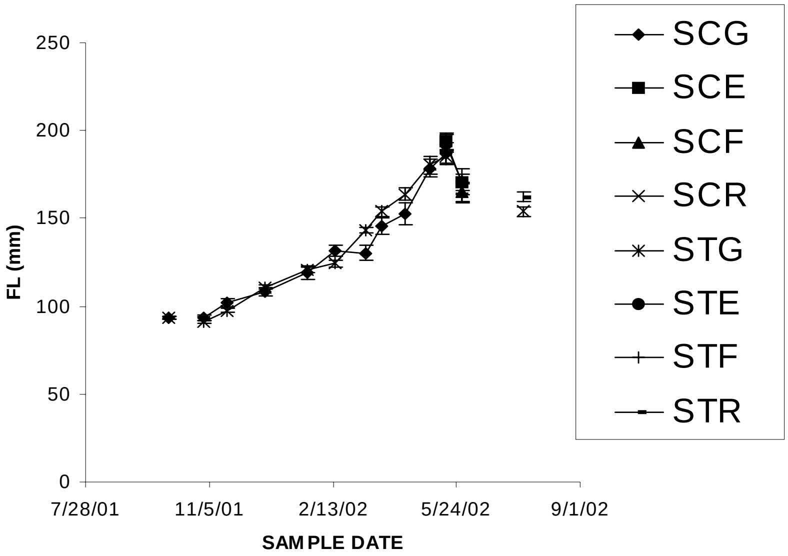


Figure 3. Fork lengths before and after different feeding regimens.

Figure 4. Fork Length (FL ± SEM) of WWR fish throughout rearing and release. The data for small treatment (SMTMT) versus small control (SMCNT) fish are presented in the top panel and large treatment (LGTMT) versus large control (LGCNT) are presented in the bottom panel. In legend, G, E, F, and R correspond to Growth, Exit, Forced migrant, and Residual samples, respectively.



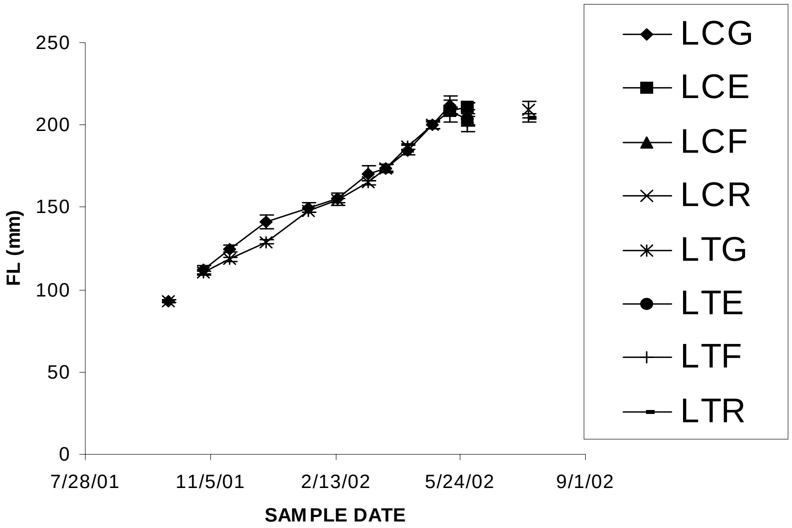


Figure 4. Fork Length (mm) in WWR.

Figure 5. Weight (WT ± SEM) of WWR fish throughout rearing and release. The data for small treatment (SMTMT) versus small control (SMCNT) fish are presented in the top panel and large treatment (LGTMT) versus large control (LGCNT) are presented in the bottom panel. In legend, G, E, F, and R correspond to Growth, Exit, Forced migrant, and Residual samples, respectively.

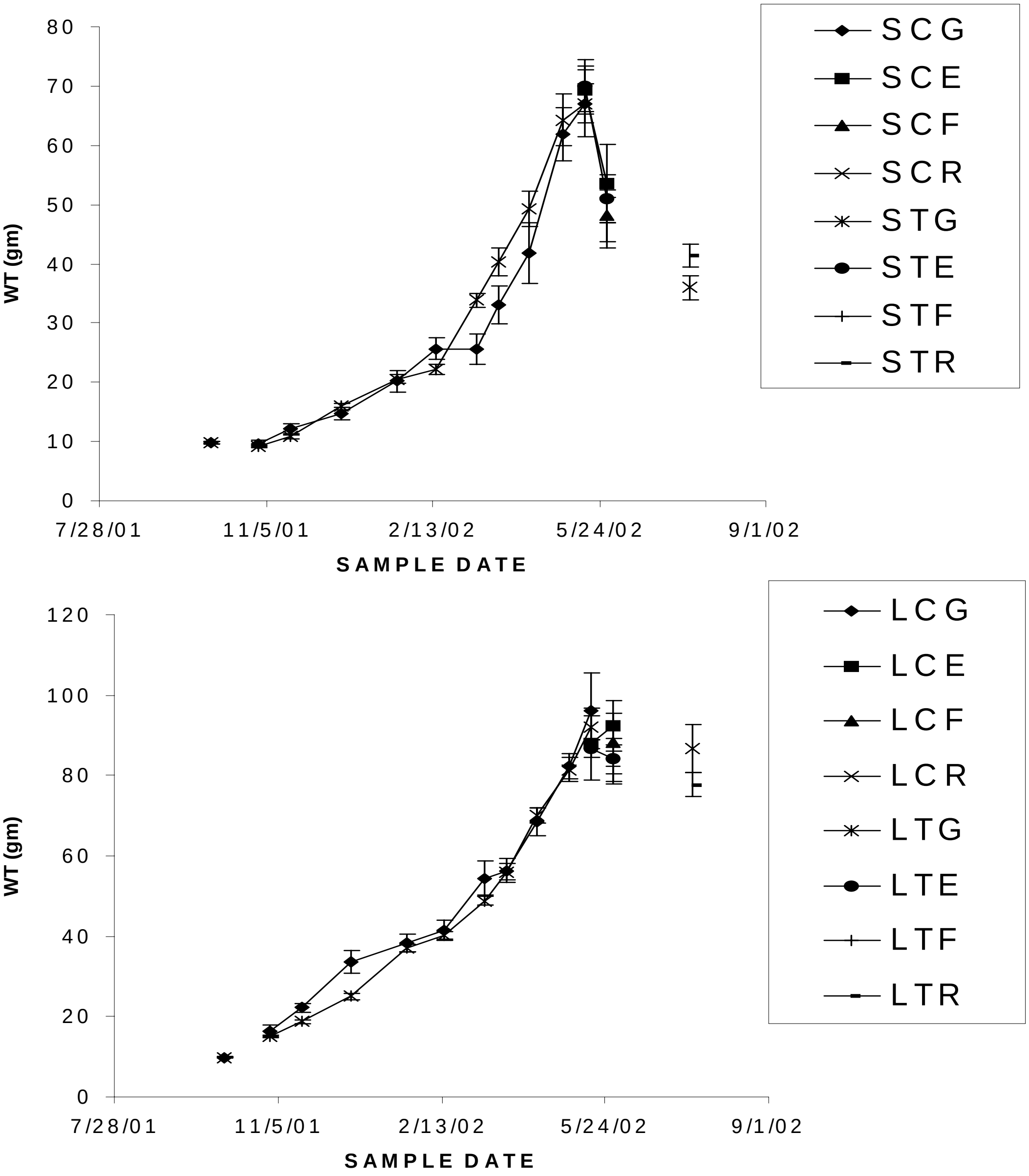
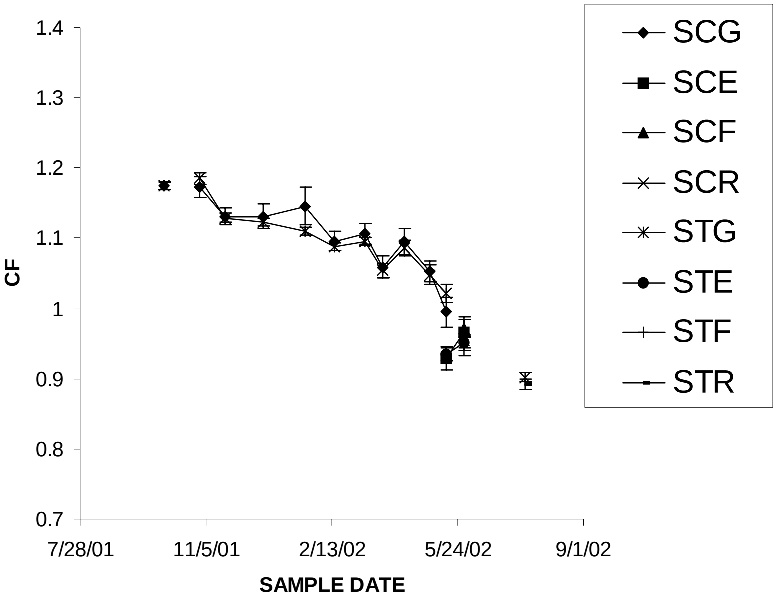


Figure 5. Weight (gm) in WWR.

Figure 6. Condition Factor (CF ± SEM) of WWR fish throughout rearing and release. The data for small treatment (SMTMT) versus small control (SMCNT) fish are presented in the top panel and large treatment (LGTMT) versus large control (LGCNT) are presented in the bottom panel. In legend, G, E, F, and R correspond to Growth, Exit, Forced migrant, and Residual samples, respectively.



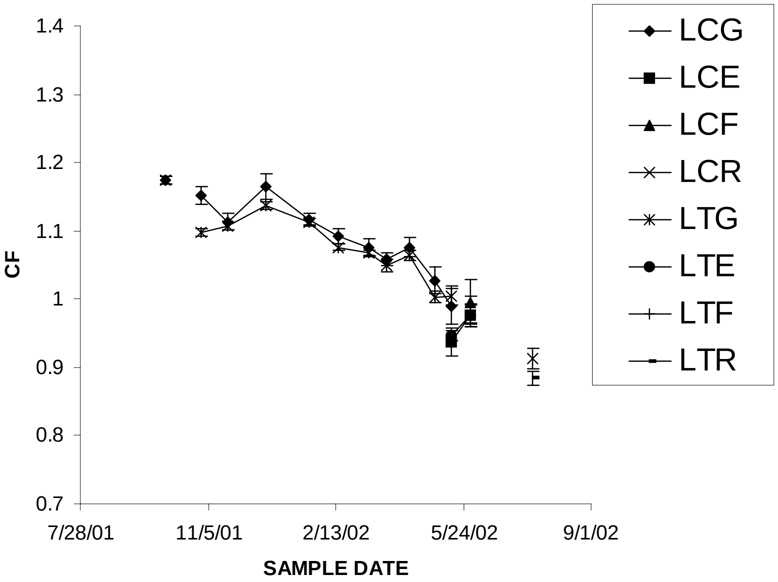
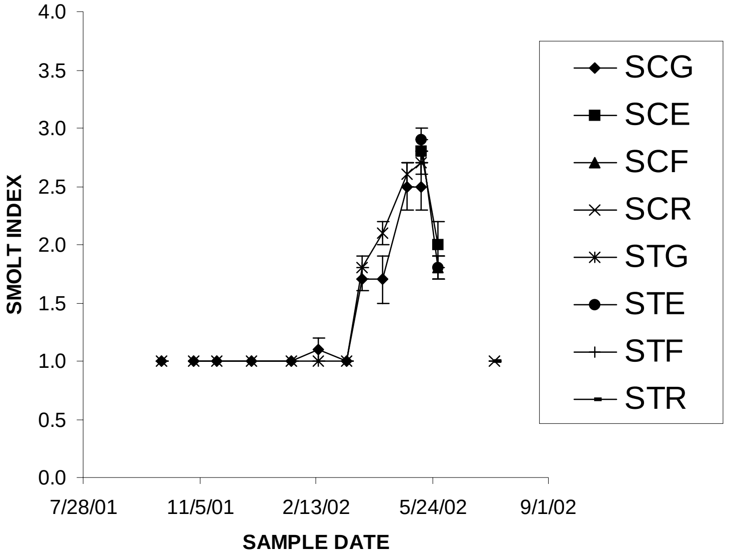


Figure 6. Condition Factor (CF) in WWR.

Figure 7. Smolt Index (SI ± SEM) of WWR fish throughout rearing and release. The data for small treatment (SMTMT) versus small control (SMCNT) fish are presented in the top panel and large treatment (LGTMT) versus large control (LGCNT) are presented in the bottom panel. In legend, G, E, F, and R correspond to Growth, Exit, Forced migrant, and Residual samples, respectively.



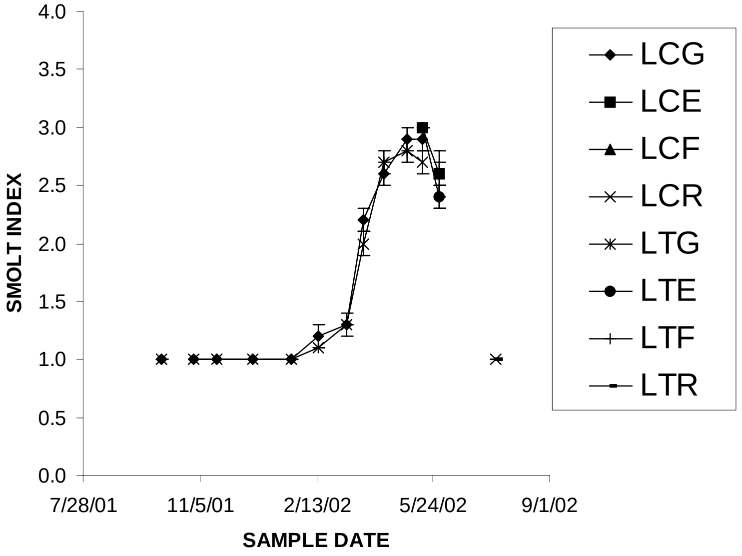


Figure 7. Smolt Index (SI) in WWR.

Figure 8. Fork length (FL), weight (WT), Condition Factor (CF) and Smolt Index (SI) for HWR fish throughout rearing and release. All values are ± SEM. In legend, G, E, F, and R correspond to Growth, Exit, Forced migrant, and Residual samples, respectively.

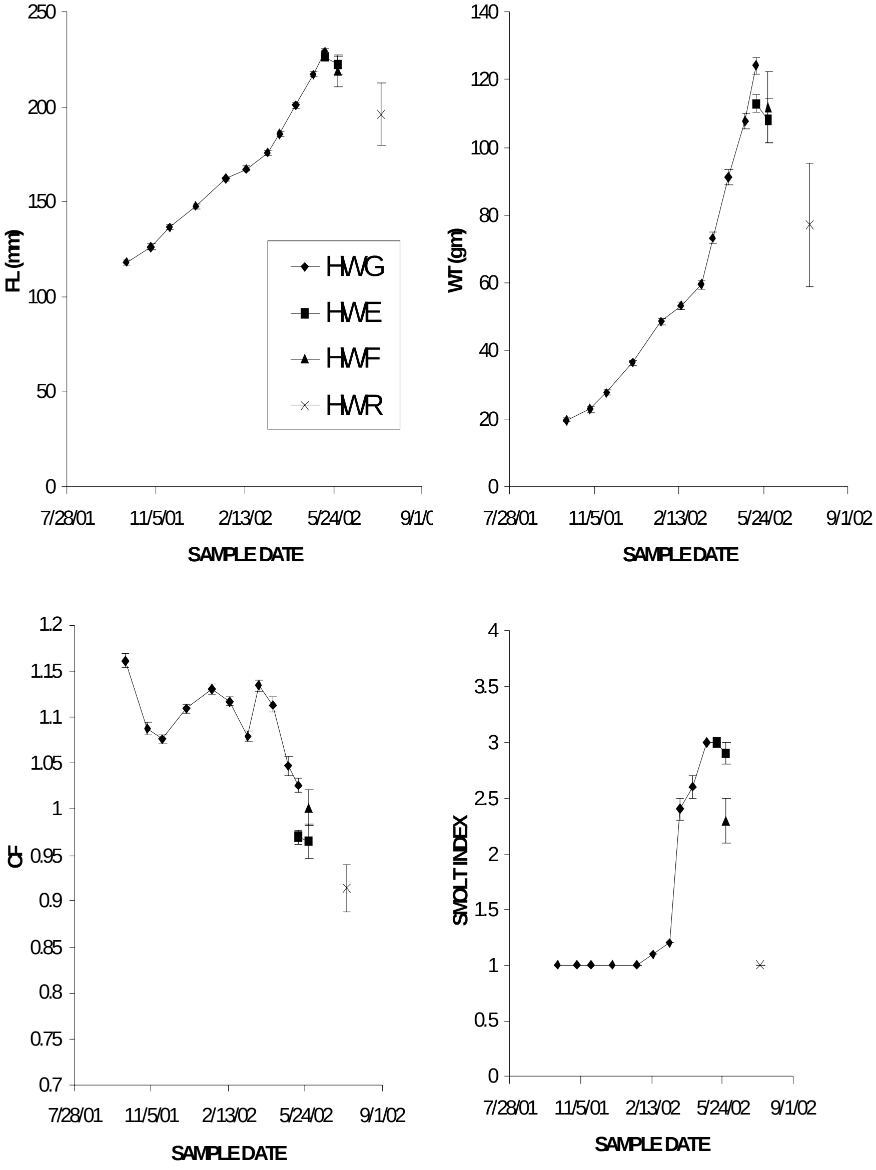


Figure 8. Fork Length (mm), weight (gm), condition factor (CF), and smolt index (SI) in HWR.

Table 2. Numbers of HWR and WWR marked at the time of the pond split number of residuals captured from each release group, and the relative residualism rate of fish from each release group. Letters in the far right column indicate statistically significant differences between rows that do not share a letter (e.g. small treatment [“a”] differs from all other release groups [“b”, “c”, and “d”] but large treatment does not differ from large control [both “c”]).

RELEASE GROUP	# RESIDUALS IN SAMPLE	# TAGGED	RELATIVE RESIDUALISM RATE	
SMALL TREATMENT	125	10928	0.0114	a
SMALL CONTROL	87	3810	0.0228	b
LARGE TREATMENT	57	10099	0.0056	c
LARGE CONTROL	23	5268	0.0044	c
HWR	7	17608	0.0004	d
TOTALS	299	47713	--	

Figures 4 through 8 provide details on fish size and condition throughout rearing, release, and residualism for WWR and HWR, respectively. Figure 9 provides size frequency distributions of WWR fish at important points throughout this study. The overall size frequency distributions were unimodal and symmetrical before and after size-grading. Subsequent distributions are either skewed left or multi-modal. Only fish larger than approximately 160 mm were captured in the downstream rotary screw trap. The size distribution of fish forced from the pond (Figure 6F) is heavily skewed left but is dominated by a large fish mode at approximately 210 mm. The majority of those fish apparently emigrated after being forced into Gobar Creek because the relative abundance of fish in that size range decreases among residuals captured from Gobar Creek. Two size modes of residuals are apparent in Figure 6G: one at approximately 160 mm and one at approximately 210 mm. The large mode closely matched the size distribution of LGTMT fish. The small mode closely matched the size distribution of SMTMT and the pooled SMCNT and LGCNT fish.

Outmigration From Gobar Pond: All fish were counted as they volitionally exited or were forced from Gobar Pond. Figure 10 provides the cumulative percent emigration from the pond. In earlier observations of emigration from the pond and this study, removal of dam boards did not result in immediate emigration ("flushing"). Fish left the pond beginning in the evening after removal of the dam boards after the pond had re-equilibrated at the lower level. We believe that fish exiting the pond prior to 30 May 2002 were in fact volitional outmigrants and their

Figure 9. Size frequency distributions for WWR at important points throughout rearing, migration, and residualism. The upper solid line in each panel represents the pool of all WWR of that sample type. The thick dashed line represents LGTMT. The thin dotted line represents SMTMT. The lower solid line represents pooled LGCNT and SMCNT fish. “VOLITIONAL EMIGRANTS” (panel D) represents a pool of all fish volitionally emigrating from Gobar Pond. Panel E represents fish captured in the rotary screw trap at KFH through the migration season. Panels F and G represent residuals captured from Gobar Pond and Gobar Creek, respectively.

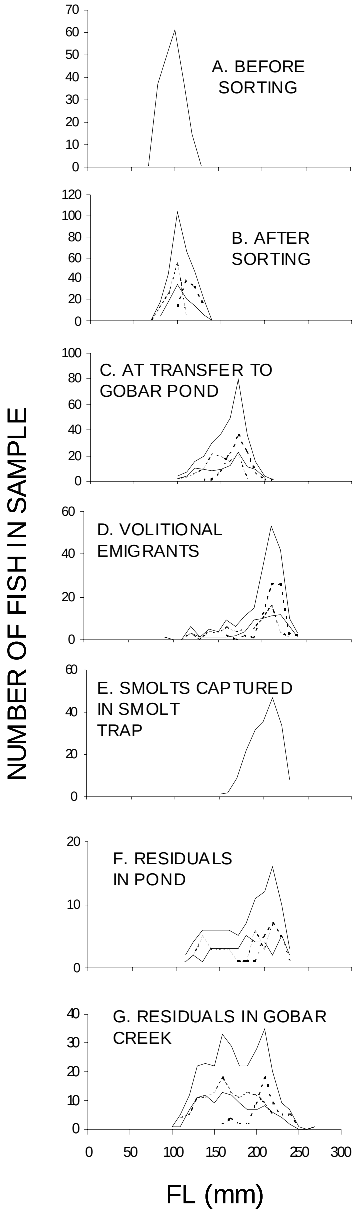


Figure 9. Size frequency distributions of WWR throughout study period.

Figure 10. Cumulative percent migration of WWR and HWR juveniles from Gobar Pond and past smolt trap adjacent to KFH. Percentages apply to each sampling week prior to the sample date except that the smolt trap estimate on 5/30/02 applies to all smolts captured after on and after 5/30/02.

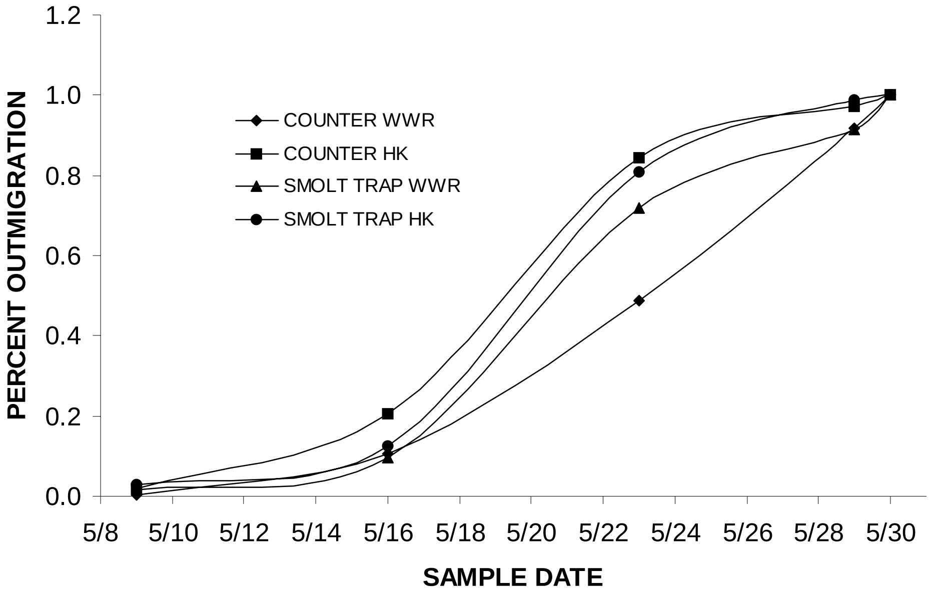


Figure 10. Migration of WWR and HWR out of Gobar Pond and capture in smolt trap.

exit is stimulated by the increase in flow and/ or change in pond level. HWR fish began leaving the pond earlier than WWR fish and a larger proportion of HWR fish exited the pond volitionally.

Smolt Trapping at KFH: Figure 11 provides details on timing of successful emigration of experimental fish and the size distribution of those smolts, respectively. No differences were noted in the timing of migration of WWR and HWR fish past the smolt trap. HWR smolts were significantly larger than WWR smolts (T-test: $P<0.05$)

Residual sampling: Nearly 300 WWR and HWR residuals were captured on 17 and 18 July 2002. Overall, WWR residuals were not normally distributed by size and the distribution does appear to be bimodal (Figure 12 and Figure 9g) but not as markedly so as in earlier years (Figure 1). The sampling methods were size biased with fish captured by angling ($N=227$) significantly larger than electroshocked ($N=65$) fish (Mann-Whitney: $P=0.003$). The size frequency distributions of fish captured by either method were, however, similar and the samples were pooled for further analysis. Too few HWR residuals were captured to adequately describe the shape of the size distribution of those fish.

Coded-wire tags were excised and read and all WWR residuals were assigned to their control or treatment release groups. Table 3 provides information on abundance of residuals from each release group (J_{GR}), numbers of juveniles originally marked (J_{GM}), and an expression of the relative residualism rate of fish from each release group ($R_G=J_{GR}/J_{GM}$)). HWR fish showed the lowest R_G of any

Figure 11. Temporal (a) and size frequency (b) distributions of HWR and WWR smolts captured in rotary screw trap at KFH. Data on emigration of naturally produced fish is provided for reference.

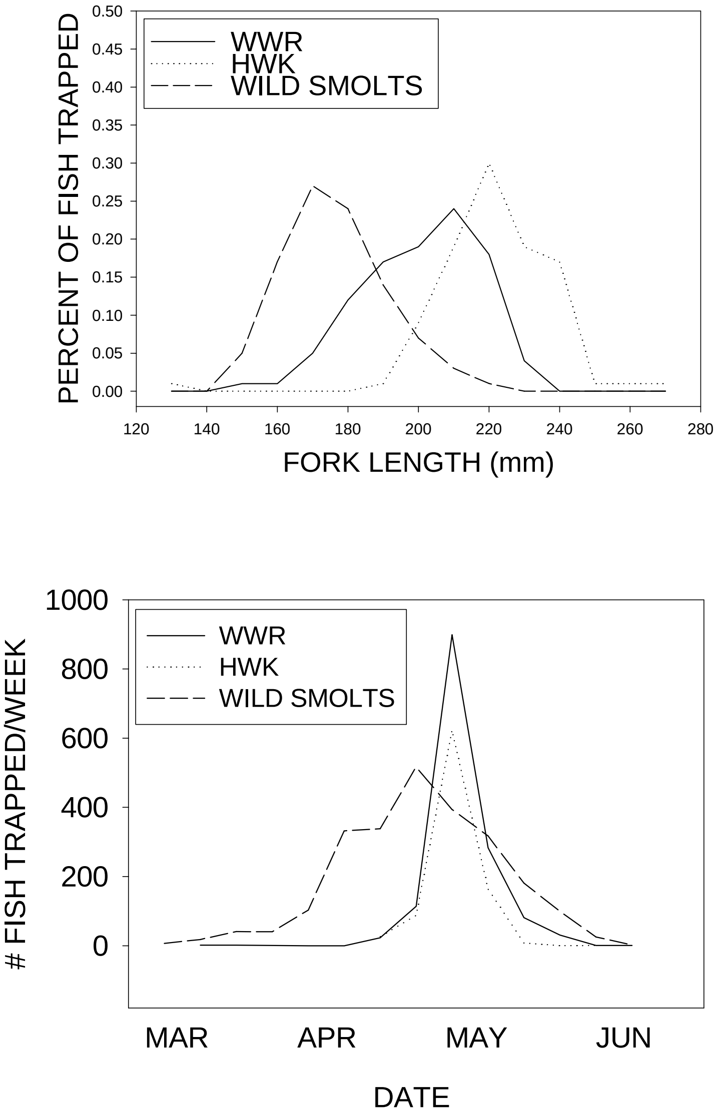


Figure 11. Temporal (a) and size frequency (b) distributions of HWR and WWR smolts captured in rotary screw trap at KFH.

Figure 12. Size frequency distributions for WWR and HWR residuals.

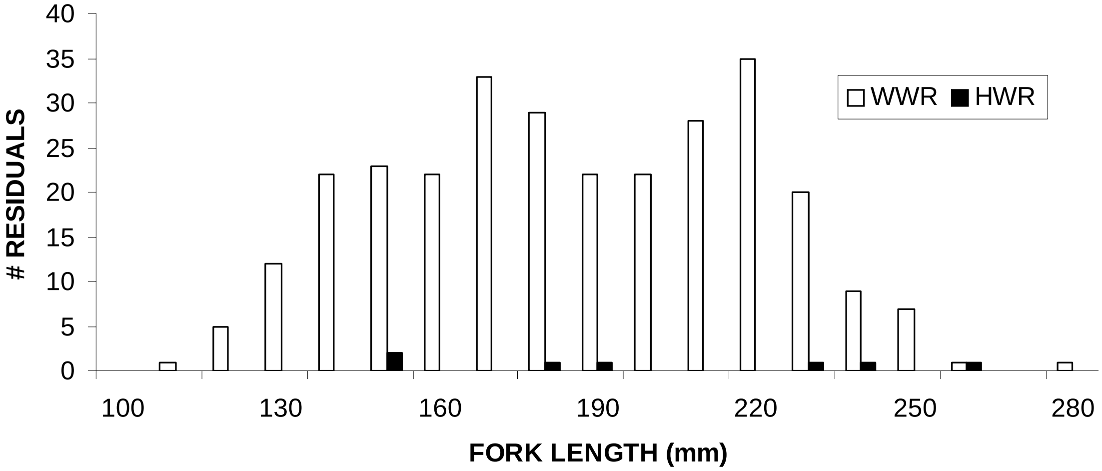


Figure 12. Size frequency distributions for WWR and HWR residuals.

group. Of the four WWR release groups, the lowest relative residualism rate was shown by LGTMT and LGCNT fish followed by SMTMT and SMCNT.

We took two approaches to analyzing differences in abundance of residuals from each of the release groups – the first comparing rate of residualism within WWR (control versus treatment) and the second contrasting residualism of pooled small and large WWR to HWR. For all comparisons, observed values were actual counts of residuals from each release group. Expected values were derived by assuming equal likelihood of residualism across groups and multiplying the total number of residuals by the proportion of fish planted from each release group. For fish that were small at the time of the pond split, STMT fish residualized at approximately one-half the rate of SMCNT fish and we conclude that the treatment had the desired effect of increasing the proportion of those fish that successfully smolted and emigrated from the system (G-test; $p<0.05$). Among fish that were large at the time of the pond split, we noted no difference in relative residualism rate between LGTMT and LGCNT fish (G-test; $p>0.05$).

For the second set of tests, we pooled counts of SMTMT with SMCNT (SMALL) and LGCNT with LGTMT (LARGE). Using the G-test for unplanned tests of homogeneity (Sokal and Rohlf 1982, p. 728) with the probability per comparison adjusted to account for multiple comparisons, LARGE, SMALL, and HWR groups all showed statistically significant differences in relative residualism rates. The LARGE group residualized at a rate less than one-third that of the SMALL groups. HWR fish residualized at a rate more than one order of magnitude less than the WWR groups in combination.

Sex Ratios: Table 3 provides information on sex ratios from WWR and HWR fish during rearing, migration and after residualism. For WWR fish, sex ratios are provided for samples from individual control and treatment groups and for pooled samples as appropriate.

Among WWR fish, the initial sex ratio (in presort sample) did not depart from the expected 50%M:50%F. During rearing (after sorting by size) fish that were large at the time of the pond split were slightly skewed towards being male but fish that were smaller were not. Similarly, among fish volitionally leaving Gobar pond, initially large fish were skewed male while initially small fish were not. Pond residuals (the fish that were forced from the pond) did not depart significantly from the expected 50%M:50%F although that may have been a consequence of an inadequate sample size (N=28) for initially large fish. All WWR residuals sampled from Gobar Creek (large and small, individual control and treatment groups and combined) were strongly skewed male.

Among HWR fish, sex ratio was not skewed in samples taken from fish rearing at KFH or Gobar pond but was skewed male in “Pond Residuals” (fish forced from the pond on 5/31/03). HWR residuals captured from Gobar Creek were not skewed male but sample size was only seven fish.

Physiology: Differences in levels of sodium-potassium ATPase (NaK-ATPase) and insulin-like growth factor-1 (IGF-1) were analyzed using two-way ANOVA.

Table 3. Proportions of males among WWR fish during rearing, release, and as residuals. N is the sample size. P(M) is the proportion of male fish in the sample. In the column labeled “P < 0.05” a “Y” indicates a statistically significant deviation from the expected ratio of 1:1 (M:F).

GROUP	REARING			EXIT			POND RESIDUALS			CREEK RESIDUALS		
	N	P(M)	P<0.05?	N	P(M)	P<0.05?	N	P(M)	P<0.05?	N	P(M)	P<0.05?
HWR	174	0.47		29	0.48		15	0.93	Y	7	0.57	
WWR PRESORT	48	0.54	--	--	--	--	--	--	--	--	--	--
WWR LGCNT	148	0.59	Y	27	0.63		7	0.71		23	0.74	Y
WWR LGTMT	243	0.54		74	0.59		21	0.62		57	0.75	Y
WWR SMCNT	151	0.48		34	0.44		31	0.52		87	0.59	
WWR SMTMT	211	0.53		62	0.56		35	0.66		125	0.68	Y
LARGE	391	0.56	Y	101	0.60	Y	28	0.64		80	0.75	Y
SMALL	362	0.51		96	0.52		66	0.59		212	0.64	Y
All WWR	753	0.53		197	0.56		94	0.61	Y	292	0.67	Y

For WWR, within each size category, grouping factors were control or treatment and sample date (i.e. SMCNT compared to SMTMT over time) to test for treatment effects. Both NaK-ATPase activity and IGF-1 levels varied significantly over time ($P < 0.0001$) with a gradual increase to a peak in early to mid-May just as active migration from the pond commenced followed by a precipitous decline among late migrants and fish residualized in the pond (Table 4 and Figures 13 and 14). NaK-ATPase activity did not differ between control or treatment fish within either large or small groups ($P > 0.05$). IGF-1 levels did differ significantly between SMCNT and SMTMT ($P = 0.005$), an outcome in agreement with the differences in fork length between those two groups at transfer to Gobar Pond. IGF-1 levels did not differ between LGCNT and LGTMT fish ($P = 0.93$).

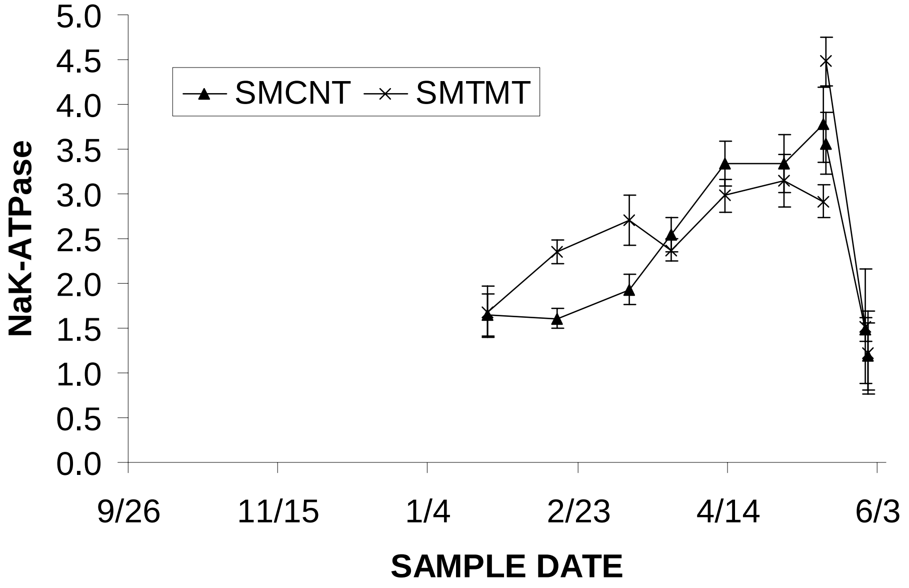
We also used two-way ANOVA to compare physiological parameters between HWR and WWR (with all WWR data pooled within sampling dates) to test for differences between offspring of our traditional and wild broodstocks. Both parameters varied significantly over time ($P < 0.001$) but neither parameter differed (NaK-ATPase: $P = 0.25$; IGF-1: $P = 0.76$) between WWR and HWR fish (Figure 15).

Circulating levels of 11-Ketotestosterone (11KT), in combination with visual scores of the degree of sexual maturation of the male residuals, indicated that a substantial portion of both small and large residuals were either precociously mature males at the time of release or were becoming sexually mature.

Table 4. Mean values for physiological parameters among WWR fish by release group. Values in parentheses are sample size and SEM.

DATE	TYPE	LARGE CONTROL (LC)		LARGE TREATMENT (LT)		SMALL CONTROL (SC)		SMALL TREATMENT (ST)	
		IGF-1	NaK-ATPase	IGF-1	NaK-ATPase	IGF-1	NaK-ATPase	IGF-1	NaK-ATPase
10/4/01	PS	13.5	--	13.5	--	13.5	--	13.5	--
		(48, 0.874)		(48, 0.874)		(48, 0.874)		(48, 0.874)	
11/1/01	R	19	--	18.3	--	12.9	--	19.1	--
		(11, 1.693)		(15, 2.265)		(18, 1.074)		(15, 1.243)	
11/21/01	R	18.8	--	20	--	18.2	--	21.3	--
		(20, 1.262)		(15, 1.804)		(8, 2.338)		(15, 1.457)	
12/21/01	R	26.1	--	23.9	--	14.9	--	27.6	--
		(13, 2.403)		(15, 2.189)		(17, 1.995)		(15, 1.389)	
1/24/02	R	27.3	1.5	32	1.8	26.7	1.7	24	1.7
		(18, 1.676)	(18, 0.126)	(15, 1.889)	(15, 0.257)	(11, 2.972)	(10, 0.231)	(15, 1.545)	(15, 0.29)
2/16/02	R	29.6	1.6	29.8	1.6	28	1.6	27.1	2.4
		(15, 2.142)	(15, 0.16)	(15, 1.862)	(15, 0.19)	(15, 2.018)	(15, 0.11)	(15, 2.988)	(15, 0.135)
3/12/02	R	34.7	2.3	31.8	1.7	27.5	1.9	31.7	2.7
		(13, 1.656)	(13, 0.141)	(15, 1.457)	(15, 0.135)	(16, 2.423)	(16, 0.163)	(15, 2.34)	(15, 0.28)
3/26/02	R	36.5	2.4	34	2.2	28.9	2.5	34.8	2.4
		(19, 1.947)	(19, 0.228)	(32, 1.738)	(32, 0.962)	(17, 2.907)	(17, 0.19)	(33, 2.144)	(33, 0.129)
4/13/02	R	45.3	3.5	47.2	2.9	41.1	3.3	40.4	3
		(14, 3.259)	(14, 0.279)	(47, 1.411)	(47, 0.19)	(15, 4.453)	(15, 0.246)	(24, 2.727)	(24, 0.188)
5/3/02	R	49.8	3.4	47.8	3.6	44.8	3.3	50.9	3.1
		(18, 2.559)	(17, 0.237)	(39, 1.831)	(39, 0.192)	(18, 4.837)	(17, 0.33)	(23, 4.094)	(23, 0.294)
5/16/02	R	29.2	3	29.3	3.5	23.9	3.8	30.8	2.9
		(7, 1.486)	(7, 0.413)	(35, 1.476)	(35, 0.227)	(15, 1.951)	(15, 0.42)	(40, 1.77)	(40, 0.181)
5/17/02	E	26.7	3.6	28.3	3.8	31.4	3.6	30.1	4.5
		(9, 2.967)	(9, 0.312)	(47, 0.86)	(47, 0.171)	(17, 3.457)	(17, 0.349)	(25, 2.363)	(26, 0.271)
5/30/02	E	14.9	1.5	15.5	1.4	13.4	1.5	13.5	1.5
		(18, 0.869)	(18, 0.754)	(27, 1.041)	(27, 0.19)	(18, 0.729)	(18, 0.13)	(36, 0.678)	(36, 0.638)
5/31/02	F	8.5	1.3	10.4	1.2	9.9	1.2	9.5	1.2
		(7, 0.601)	(7, 0.143)	(21, 0.682)	(21, 0.692)	(31, 0.528)	(31, 0.376)	(35, 0.413)	(35, 0.459)

Figure 13. Sodium-Potassium ATPase (NaK-ATPase) levels in WWR fish.



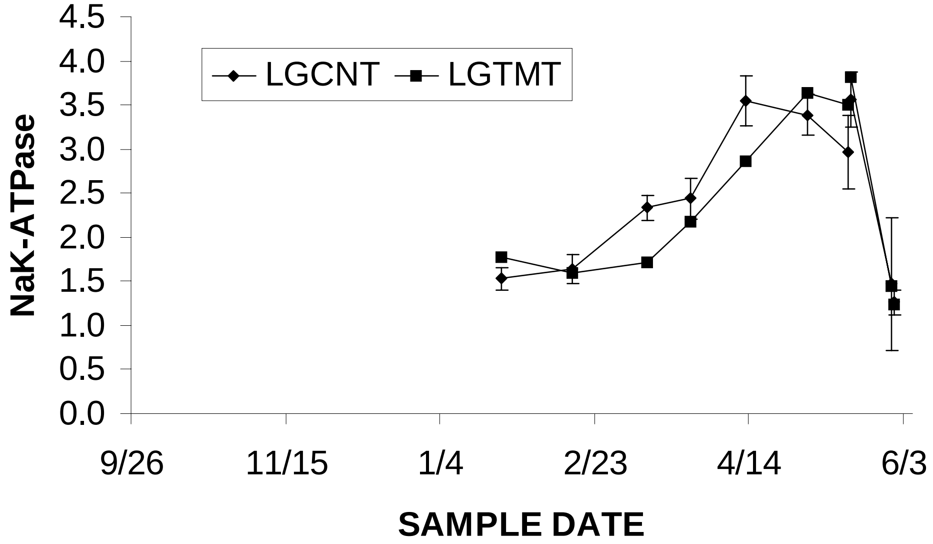
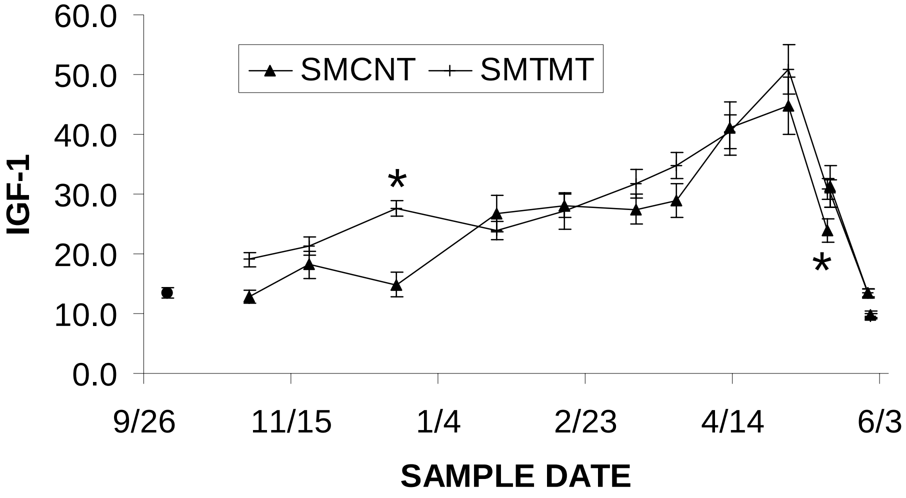


Figure 13. Sodium-Potassium ATPase (NaK-ATPase) levels in WWR.

Figure 14. Insulin-like Growth Factor 1 (IGF1) in WWR fish. Asterices indicate statistically significant differences at that sample time ($P < 0.05$; Tukey's multiple comparison test).



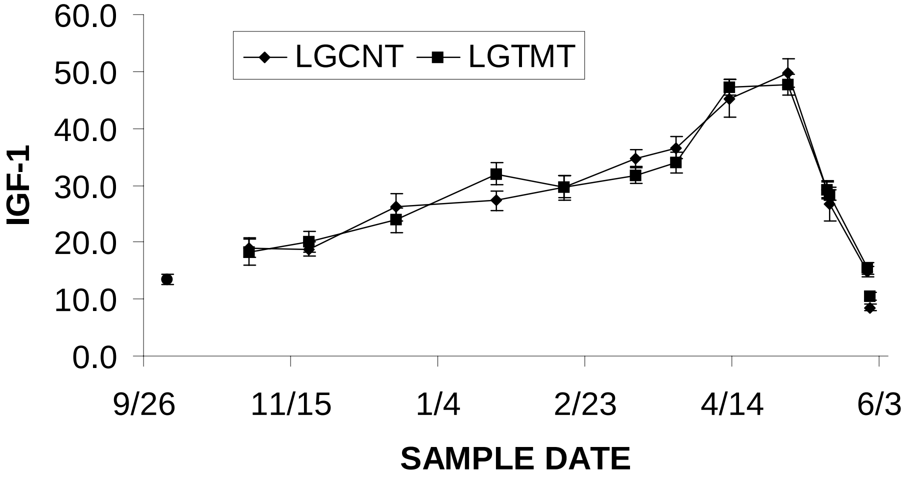
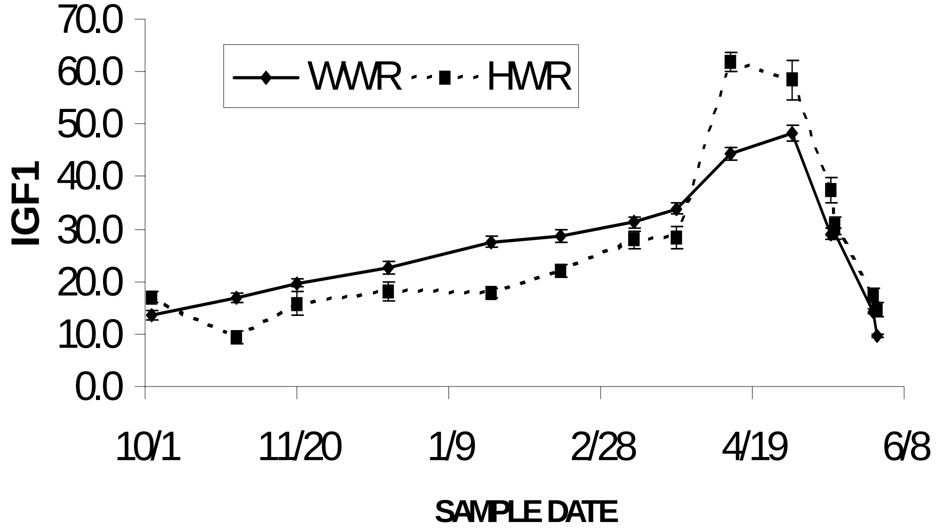


Figure 14. Insulin-like Growth Factor 1 (IGF1) in WWR.

Figure 15. Sodium-Potassium ATPase (NaK-ATPase) and IGF-1 levels in HWR and pooled WWR fish.



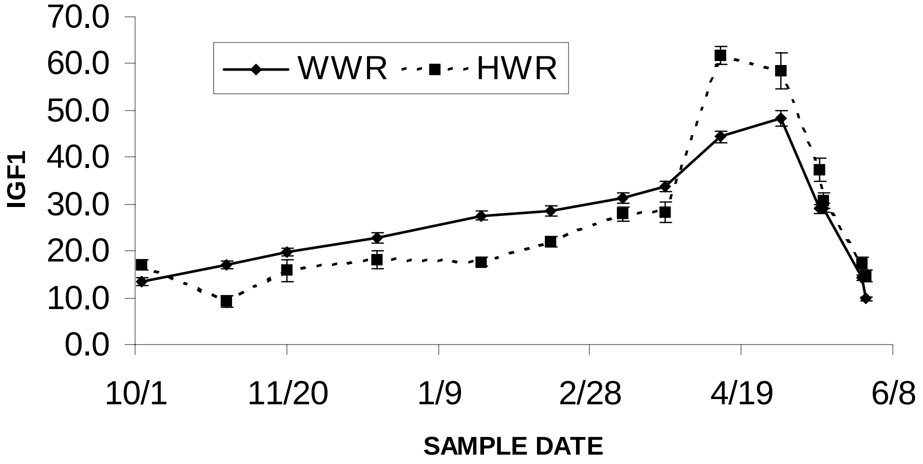


Figure 15. Sodium-Potassium ATPase (NaK-ATPase) and IGF-1 levels in HWR and pooled WWR

Male residuals were visually scored as undeveloped male (M; threadlike testes), developing male (MD; anterior half of testis thickened), or precocious male (MP; greatly enlarged testes). We used one-way ANOVA followed by Tukey's pairwise multiple comparison procedure to test for differences in circulating levels of 11KT among males showing differing degrees of sexual maturation. 11KT values were log (base-10) transformed to increase normality and equalize variance among groups. The three groups (M, MD, and MP) showed significant differences in circulating levels of 11KT (M<MD<MP; $P < 0.05$) (Figure 16).

We used two-way ANOVA (with the Tukey test for multiple comparisons) with initial size (LARGE vs. SMALL) and CONTROL vs. TREATMENT as factors to test for treatment effects (differences between CONTROL and TREATMENT within LARGE or SMALL) and for underlying differences between degree of sexual maturation of initially LARGE and SMALL males. We found no treatment effects: within initially SMALL or LARGE fish, CONTROL values for 11KT did not differ from TREATMENT values ($P > 0.05$). However, initially LARGE fish showed significantly elevated circulating levels of 11KT relative to initially SMALL fish ($P < 0.001$). We conclude that even early in life when the absolute size of the juvenile fish is small, the larger individuals within the cohort are more likely to mature precociously.

We also compared the incidence of male maturation among residuals from the four release groups. We used the G-test (G-test for Independence; Sokal and Rohlf p. 745) to determine if male maturation occurs independently of the initial

Figure 16. Frequency distributions of 11-ketotestosterone levels for undeveloped, developing, and precocious males. Statistically significant differences ($P < 0.05$) were apparent among all three groups (ANOVA followed by Tukey’s pairwise multiple comparison procedure).

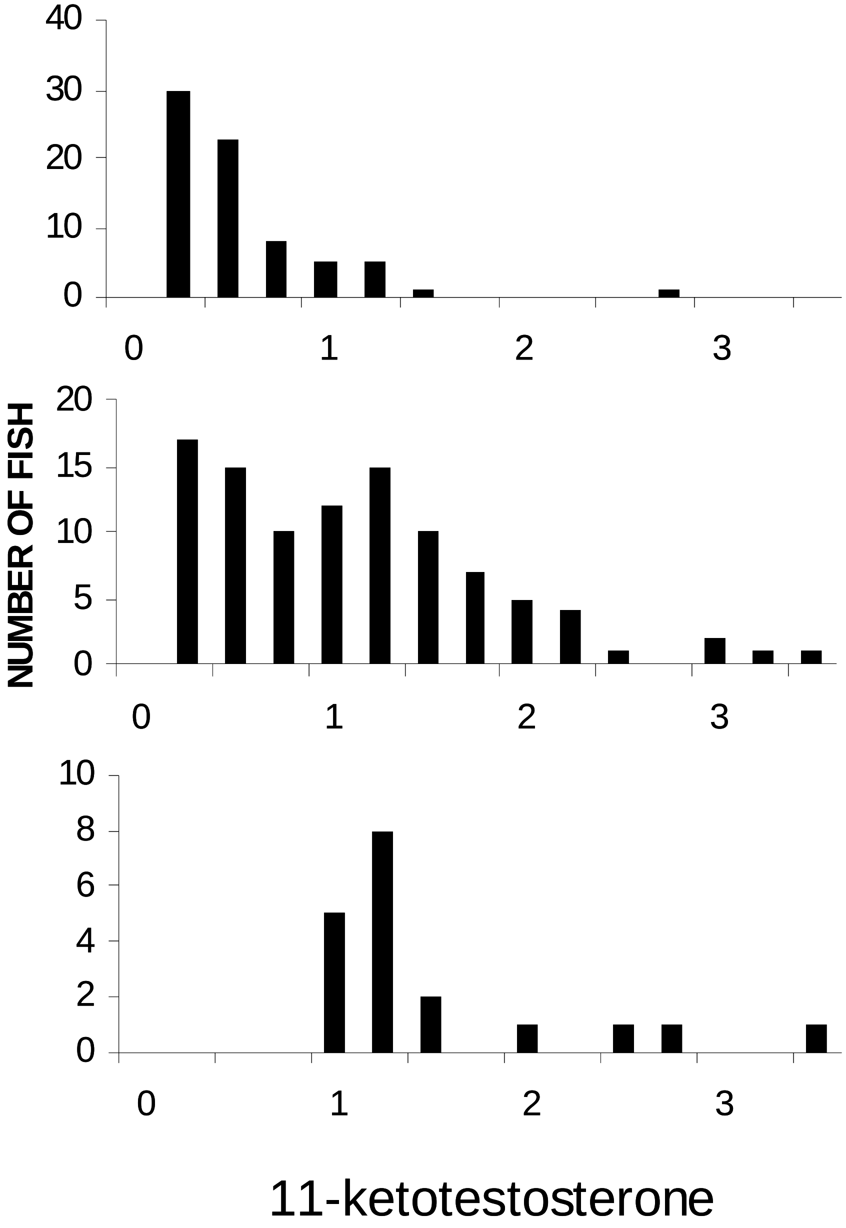


Figure 16. Frequency distributions of 11-ketotestosterone levels for undeveloped, developing, and precocious males.

size of the fish. To simplify the test, we pooled counts of developing males (MD) with counts of precocious males (MP) and tested for variation in the frequency of males showing any sign of maturation among SMCNT, SMTMT, LGCNT, and LGTMT. Highly significant differences ($P < 0.05$) were apparent. We again used the G- statistic (Unplanned Tests of Homogeneity; Sokal and Rohlf p. 728) to determine which release groups differed in their incidence of maturing males. SMCNT and SMTMT groups did not differ from each other and LGCNT and LGTMT did not differ from each other but both small groups differed significantly from both large groups with initially large fish more than twice as likely to exhibit signs of precocious maturation (Figure 17).

Figure 17. The proportion of mature or maturing males (pooled developing and precocious males divided by total number of males) among WWR release groups. Different letters above bars indicate statistically significant differences between release groups. Error bars are 95% confidence intervals.

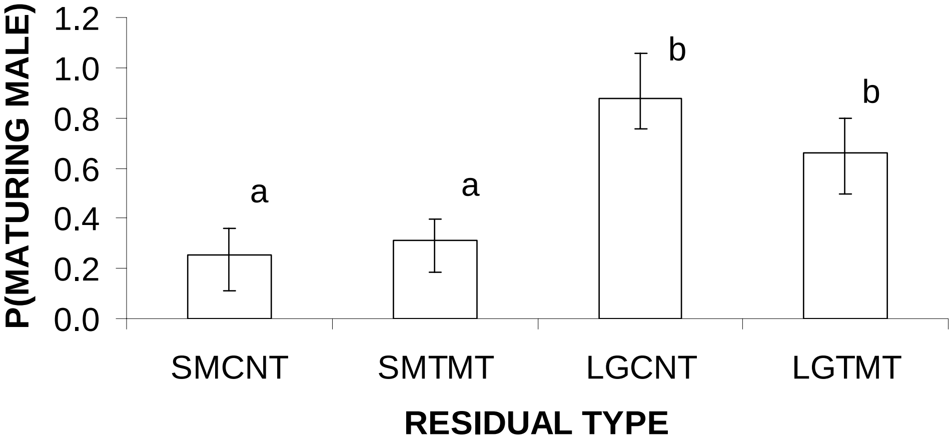


Figure 17. Mature or maturing male WWR among residuals.

DISCUSSION

Our work has shown that the rate of residualism can be reduced by accelerating growth of the part of the cohort that on average would have been too small at the time of release to outmigrate as smolts. Residualism was not eliminated nor was it reduced to the level expressed by offspring of the traditional stock. The group that was successfully reared under the aggressive feeding regimen (SMTMT) residualized at a rate lower than that of its comparable control (SMCNT) but still failed to migrate as efficiently as the HWR fish.

From an aquaculture perspective, grading of the juveniles by size early in their life and aggressively feeding the smaller individuals in the cohort will be effective in reducing the incidence of residualism. Costs associated with that approach would include increased manpower, wastage of food, and specialized feeding equipment. A key element to success may be the time available for rearing small and large fish under different feeding regimens. We were constrained by our facilities and the existing program to sorting the fish by size late in the rearing cycle because October is the earliest time when water temperatures allow sorting and tagging of fish. Further, the steelhead must be moved to a common rearing vessel (Gobar Pond) in March to make room at KFH for other salmonids on station. We speculate that we might have further reduced the rate of residualism had we been able to impose the aggressive feeding regimen earlier and maintained the separate rearing longer. We did not succeed in determining if decreased growth among larger individuals results in fewer large residuals so we cannot speculate whether earlier and more protracted rearing on a restricted diet would decrease residualism among large fish. That work should still be done.

The abundance of WWR juveniles in Gobar Pond at the end of the release period suggests that some of the risks of high rates of residualism might be contained by using an acclimation pond as a “filter”, retaining fish that fail to volitionally outmigrate for further rearing or some other purpose (Viola and Schuck 1995). Success using this as an approach is complicated by the composition of the fish in the pond. Approximately 30% of the WWR juveniles forced from Gobar pond appeared to be fully smolted (SI =3). Another approximately 30% were scored as transitional smolts (SI=2). Conservatively, one-half or more of the fish remaining in the pond may eventually have volitionally exited had they been given more time. The logistics involved in rearing in the hatchery for an additional year are complex but tractable. Some hatchery programs routinely use a two-year rearing program for steelhead. That might be expected to at least increase the successful outmigration of fish that would otherwise become small residuals. Using an acclimation pond as a device to sort juveniles into migrants and non-migrants will be more beneficial if some way can be found to permit a more protracted emigration season.

Under some circumstances, is seems likely that the risks posed by residual steelhead are low and far outweighed by benefits. For example, consider systems with natural fish populations persisting at very low abundance and markedly under-seeded juvenile rearing habitat. A wild broodstock program might contribute a significant number of returning adults from smolts that migrate at release and juveniles that successfully rear for an additional year.

Alternatively, in systems with relatively healthy endemic populations, large numbers of residuals would be expected to compete with and supplant naturally produced conspecifics and other species with overlapping ecological requirements. However, in either case, the potential for negative ecological interactions would be in addition to the various potential genetic interactions (domestication selection and interbreeding with endemics).

A central issue is whether rearing in the hatchery environment amplifies expression of protracted juvenile residency: does hatchery rearing of the offspring of wild fish increase the rate at which those offspring adopt residency? We have shown that our wild broodstock programs produce more residuals than our traditional program but it is unknown if the fish that became residuals would have adopted residency had they reared naturally. Other work has shown that with O. mykiss, some offspring of anadromous parents do not migrate as smolts and, conversely, some offspring of resident fish express anadromy (Zimmerman 2000). Given the positive relationship between artificially high juvenile growth rates and incidence of parr maturity (Thorpe 1987), it does seem likely that hatchery rearing increases both the absolute and relative abundance of at least the precocious males and, speculatively, the developing males that are heavily represented among the large residuals. Work with Atlantic salmon (Salmo salar) has suggested that increases in numbers of precociously maturing fish may disrupt breeding systems in that species (Fleming 1996, 1998).

The potential for reproductive interactions between residualized steelhead and native fishes including resident rainbow and cutthroat trout is another issue of concern. The results of both the androgen analyses and gonadal development scores presented here show that some of the residuals were sexually mature at the time of release. Many more residuals showed evidence of intermediate testes development and androgen levels, suggesting that they would become sexually mature in the winter and spring following release. Overlap in time and space with spawning resident O. mykiss and O. clarki may have occurred and might be expected to result in interbreeding. Determining if this actually occurs is a tractable problem and we are pursuing opportunities to do so. We envision a genetic survey of fry captured from one or more Kalama tributaries with residuals and coastal cutthroat present during the spawning season. The fry could be screened for a suite of loci including loci useful for detecting hybrids between cutthroat and rainbow trout. Given that we DNA sample all our broodstock and have known matings it should be possible to determine with high statistical certainty if one of the parents of a rainbow-cutthroat hybrid is the offspring of one of our wild broodstock.

Spawning interactions between residuals and endemics represents a direct genetic risk. A less direct genetic risk is the potential for domestication selection. High rates of residualism coupled with high incidence of mortality during natural rearing might be expected to result in some degree of genetic change within the remainder of the cohort if the tendency to residualize has a large genetic component in its expression. One explanation for the lack of residuals among the

HWR release group is that, over successive generations of culture, hatchery programs select against the phenotype of protracted juvenile residency. Such selection will occur if fish that become residuals are not incorporated in subsequent generations. We noted far fewer HWR residuals than large WWR residuals despite an almost complete overlap in size of the WWR large residuals and all the HWR juveniles released. The potential for this phenomenon to pose a genetic risk is amplified in hatchery programs that incorporate returning hatchery fish as broodstock because successive generations of selection should increase the loss of diversity within the population.

Reisenbichler et al. (In Press) showed that the hatchery-reared offspring of wild broodstock migrated downstream after release at a much lower rate (seven-fold) than comparable offspring of domesticated broodstock. They suggest that the phenomenon is a potent source of domestication selection in steelhead. They noted that most of the difference could be attributed to non-migration of small fish because of a failure of offspring of wild fish to thrive in the hatchery environment leading to a lack of smoltification (small residuals). In addition, they noted that some of the discrepancies in migration rates were independent of size of the released fish -- an outcome that could be explained by a failure of some of their large fish to migrate (large residuals). Fewer large wild broodstock juveniles were detected as migrating smolts than hatchery origin juveniles of the same size migrate.

Residuals that do survive and outmigrate as two-year-olds (or older) buffer somewhat against what might otherwise be a source of domestication in a hatchery stock. The offspring of wild fish might in fact survive at a high enough rate to effect such a buffering. We did not estimate overwintering survival of the residuals in this study but in other work in the basin, we have noted that some offspring of wild broodstock do overwinter and outmigrate in their second spring (authors' unpublished data). In 2001, we captured in our rotary screw trap 60 wild broodstock summer-run steelhead smolts from a plant of approximately 60,000 juveniles in the Spring of 2000. Our trap efficiency in that year was approximately 10% and thus, if we assume (arbitrarily) an absolute residualism rate of 10%, 600 two-year old smolts emigrated out of a cohort of 6,000 juveniles that residualized or died soon after release.

Other work has shown that, in general, hatchery fish exhibit high mortality when rearing in freshwater under natural conditions. For example, Reisenbichler and McIntyre (1977) showed substantial differences between hatchery- and wild-origin fish rearing naturally with the offspring of wild fish exhibiting higher survival.

Tipping et al. (1995) reported the capture of a single residual three months post-release and a single two-year old migrant steelhead in a study in Snow Creek, WA when they estimated that 28% of the steelhead planted failed to migrate.

From a management perspective, the results of the research presented here become most significant when viewed in the context of ongoing changes in the development and management of salmonid hatchery programs in the region. Management agencies at all levels are actively promoting the use of locally derived wild broodstocks as an alternative to using domesticated, often non-locally derived fish. There is a growing consensus that use of wild fish in hatchery programs poses fewer genetic and ecological risks to their parent populations. We argue that genetic and ecological risks of a different form will emerge and must be considered when implementing new or modifying existing programs. As one example, our work suggests that more residuals will be present in systems planted with juvenile offspring of wild broodstock. We argue that new or existing wild broodstock programs should invest every effort in maximizing growth of fish that would otherwise be too small to emigrate at release, that rearing protocols be developed to minimize residualism of larger individuals, and that much greater effort be expended in rigorously evaluating the potential for genetic and ecological risks posed by wild broodstock programs.

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