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Development of a Natural Rearing System to Improve Supplemental Fish Quality

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DEVELOPMENT OF A NATURAL REARING SYSTEM TO IMPROVE SUPPLEMENTAL FISH QUALITY

FINAL REPORT

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EXECUTIVE SUMMARY

The National Marine Fisheries Service (NMFS), Northwest Fisheries Science Center (NWFSC), Resource Enhancement and Utilization Technologies (REUT) Division has been cooperating with the Bonneville Power Administration (BPA) on the Natural Rearing Enhancement System (NATURES) project (Project 199105500) to develop and evaluate new fish culture techniques designed to produce Pacific salmon (*Oncorhynchus spp.*) which are behaviorally, physiologically, and morphologically similar to their wild counterparts.

The major efforts of the NATURES program are directed at developing and evaluating rearing strategies for the production of hatchery salmonids with wild characteristics and increased postrelease survival. In mitigation, enhancement, and conservation hatcheries, salmonids are maintained in a protective environment for less than half their life cycle before being released to survive in the wild environment. Hatchery reared fish may have high migratory and marine mortality, with often considerably less than 1% of the fish surviving to recruit to the fishery or spawning population. This low survival partially stems from traditional fish culture practices failing to prepare fish for survival in their natural environment. NATURES addresses this problem by developing and evaluating more natural fish culture practices that equip fish with the behavioral, physiological, and morphological attributes they need to survive in the migratory corridor and sea.

NATURES studies conducted from 1992-1994 indicated that the instream survival of chinook salmon *Oncorhynchus tshawytscha* reared in raceways with seminatural habitat composed of gravel substrates, instream structure, and overhead cover may be up to about 50% higher than that of salmon reared in conventional raceways. In 1996, a new experiment was initiated at the Washington Department of Fish and Wildlife (WDFW) Forks Creek Hatchery to determine if seminatural raceway habitat also increases smolt-to-adult survival. From 1997 to 2000, fall chinook salmon were reared from swimup fry to zero-age smolt in 9.75 m long raceways. Each year, half of the raceways were fitted with seminatural habitat composed of gravel paver substrate, conifer instream structure, and camouflage net overhead cover, while the other half were maintained as conventional controls. The raceways fitted with seminatural raceway habitat can be cleaned with conventional vacuum technology and require only a minor increase in maintenance effort. In most years, chinook salmon reared in seminatural raceway habitat were slightly smaller than controls. The coloration of fish in the two rearing treatments always diverged, with NATURES fish developing a color pattern that seemed more cryptic in the postrelease environment. In laboratory bioassays, fish from seminatural raceway habitat were attacked by hooded mergansers less frequently than conventionally reared chinook salmon. The health of fish reared in seminatural raceway habitat equaled or exceeded that of their conventional controls. The instream survival of smolts reared in seminatural raceway habitat averaged 3.8, 10.0, 24.0, and 1.0% higher than their conventional counterparts in 1997, 1998, 1999, and 2000, respectively. The recovery of coded-wire-tagged salmon released to the sea will be assessed over the next five years to determine if seminatural raceway habitat rearing produces similar increases in smolt-to-adult survival.

In 2000, a four year study examining the effect of seminatural raceway habitat consisting of gravel-paver substrate, fir tree structure, and camouflage net cover on coho salmon (*O. kisutch*) smolt-to-adult survival was initiated at five WDFW hatcheries, with each facility having one modified (experimental) and one unmodified raceway. The study has successfully generated covers and concrete pavers well suited for use in a large variety of production hatchery rearing vessels (standard raceways, Burrows ponds, and large ponds). Fish are reared in the seminatural raceway habitats for at least the last two months prior to release. In the first two study years, there were no significant differences in fish size or health at release. However, similar to previous NATURES studies, the seminaturally reared fish developed a significantly different base skin coloration as measured by hue, saturation, and intensity. This skin color difference should enhance their crypticity in the natural environment to make them less vulnerable to visually hunting predators (fish, birds, mammals). In future years, the recovery of coded-wire tag data from the fishery and returns to the hatchery will be used to compare the effect of the two rearing strategies on coho salmon smolt-to-adult survival.

Ecological interactions between hatchery and wild salmonids, including competition and predation, have been identified as important factors that may negatively affect wild salmonid populations. We performed several experiments comparing naturally-reared juvenile steelhead to conspecifics reared in conventional and NATURES hatchery environments in order to estimate potential effects of hatchery-reared steelhead on wild steelhead in streams. In 2001, we quantified the feeding rates, agonistic interaction rates, and space use of naturally-reared steelhead at two densities alone and in the presence of NATURES and conventionally-reared steelhead from the same stock in order to examine competitive effects of hatchery-reared fry on wild conspecifics. In 2002, we quantified the agonistic behavior of age-0 steelhead from the three rearing treatments (conventional, NATURES, natural) in the presence and absence of predators (hatchery-reared age-1 steelhead) at three densities under laboratory conditions, and compared the results to agonistic behavior observed when fish from the hatchery rearing environments were releases into two natural streams. In 2003, we quantified habitat use, shelter use, and aggression of age-0 steelhead in the presence of predators and competitors in the laboratory. We found little evidence for consistent differences in aggression among the rearing treatment combinations, and we also found little evidence to suggest that NATURES rearing was responsible for differences in space use, habitat use, or shelter use of age-0 steelhead. In general, rearing environment appeared to have relatively little influence on the behavior of steelhead fry, particularly when compared to the effects of density and predator presence. In 2002 and 2003, we examined the effects of rearing environment on growth and survival of age-0 steelhead in an outdoor stream channel. In contrast to results of NATURES rearing on Chinook, we found few consistent, significant differences in growth or survival between steelhead fry reared in a conventional or enriched hatchery environment or between hatchery- and naturally-reared steelhead fry.

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PREFACE

Support for this research came from electricity rate payers of the Pacific Northwest through the Bonneville Power Administration.

DISCLAIMER

Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA

INTRODUCTION

The National Marine Fisheries Service (NMFS) has been conducting Natural Rearing Enhancement System (NATURES) research since the early 1990s. NATURES studies have looked at a variety of mechanisms to enhance production of wild-like salmonids from hatcheries. The development of such techniques is called for in the Columbia Basin Fish and Wildlife Program. The goal of NATURES research has been to develop fish culture techniques that enable hatcheries to produce salmon with more wildlike characteristics and increased postrelease survival. Over the history of the project, the effects of seminatural raceway habitats, automated underwater feeders, exercise current velocities, live food diets, and predator avoidance training have been investigated (Maynard et al. 1996a, 2001, 2003). The Bonneville Power Administration (BPA) terminated funding for the NATURES project at the end of FY2004. This document is the final report for the Supplemental Fish Quality Contract DE-AI79-91BP20651. The report covers work elements described in FY 2001 - 2004 work plans. Additionally, the report summarizes major conclusions of NATURES research throughout project history. Traditionally, salmon (*Oncorhynchus* spp.) are reared in barren concrete raceways that lack natural substrate, structure, or overhead cover. The fish are fed in an unnatural manner with artificial feeds mechanically or hand broadcast across the water surface. This traditional approach has increased the egg-to-smolt survival of hatchery-reared fish by an order of magnitude over that experienced by wild-reared salmon. However, once hatchery-reared fish are released into the wild their smolt-to-adult survival is usually much lower than wild-reared salmon.

The reduced postrelease survival of hatchery-reared fish may stem from differences in their behavior and morphology compared to wild-reared salmon. After release, hatchery-reared fish are inefficient foragers and are often found with empty stomachs or stomachs filled with indigestible debris (Miller 1953, Hochachka 1961, Reimers 1963, Sosiak et al. 1979, Myers 1980, O'Grady 1983, Johnsen and Ugedal 1986). Their social behavior also differs, with hatchery-reared fish congregating at higher densities, being more aggressive, and displaying less territory fidelity than wildreared fish (Fenderson et al. 1968, Bachman 1984, Swain and Riddell 1990). In the natural environment this results in hatchery-reared fish spending more time in high-risk aggressive behavior and less time in beneficial foraging behavior than their wild-reared counterparts. Hatchery-reared fish are also more surface oriented than wild-reared salmonids (Mason et al. 1967, Sosiak 1978). This increases their risk of being attacked by avian predators, such as kingfishers (*Ceryle* spp.), which search for fish near the surface. Although some of the differences between wild and hatchery-reared fish are innate (Reisenbichler and McIntyre 1977, Swain and Riddell 1990), many are conditioned and can be modified by altering the hatchery rearing environment. NATURES studies are aimed at developing a more natural salmon culture environment to prevent the development of these unnatural attributes in hatchery-reared fish. NATURES fish culture practices have the potential to increase survival. Conservation and supplementation programs can use NATURES-reared salmonids to help reduce their impacts to wild populations

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Section 1

SUMMARY OF 1991-2002 RESEARCH[1]

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1) Maynard, D.J., T.A. Flagg, and C.V.W. Mahnken (editors). 1996. Development of a natural rearing system to improve supplemental fish quality, 1991-1995. Report to Bonneville Power Administration, Contract DE-AI79-91BP20651, 216 p.

2) Maynard, D.J., B.A. Berejikian,T.A. Flagg, and C.V.W. Mahnken (editors). 2001. Development of a natural rearing system to improve supplemental fish quality, 1996-1998. Report to Bonneville Power Administration, Contract DE-AI79-91BP20651, 175 p.

3) Maynard, D.J., B.A. Berejikian, T.A. Flagg, and S. C. Riley (editors). 2003. Development of a natural rearing system to improve supplemental fish quality, 1999-2002. Report to Bonneville Power Administration, Contract DE-AI79-91BP20651, 188 p.

In this section we provide a summary of research results from our 1991-2002 NATURES studies to provide background of research leading to the studies described in this report.

I. The 1991-1995 research efforts were a collaboration between NMFS, BPA, the Washington State Department of Fish and Wildlife (WDFW), and the U.S. Fish and Wildlife Service (USFWS) to develop and evaluate innovative culture techniques to increase post-release survival of hatchery fish (see Maynard et al. 1996 for full description and findings for 1991-1995 research). During this period:

1--As an initial step in the research process, the WDFW Planning and Research Group (Olympia, Washington) identified fish marking and tagging procedures potentially suitable for NATURES. Identifying (i.e., marking) fish is an essential component of evaluating the effects of various NATURES rearing strategies. Marking methods identified as best suited for NATURES studies were those which that would not affect their behavior, growth, locomotion, or survival, and meet other general requirements such as long-term retention and readability. Mutilation and external tags were not considered acceptable marking methods for NATURES studies because of their adverse effects on behavioral and physiological factors. Branding techniques were evaluated, since they had been used in experiments for several decades and can provide a long-lasting external mark. Recent advancements in laser technology had improved the potential for laser marking as a viable tool; making this mark potentially more benign than branding and may last through the lifetime of some fish, particularly if methods can be developed that mark the soft fin rays. Visual implant (V.I.) tags were also shown to have promise for use in NATURES studies. Injection of fluorescent materials have the advantage of being invisible until revealed by remote interrogation, thus eliminating observer bias and interactions between fish that might be associated with an externally visible tag. Panjet marks were also evaluated because they may have a long retention time (i.e., several years) and can be used to mark young (30-40 mm) salmon fry.

Perhaps the two greatest concerns regarding all marking techniques are their degree of underwater visibility and their influence on fish behavior. Preliminary field evaluations of various marks were undertaken in 1992 to help establish protocols for evaluating these concerns. These observations indicated that laser marks were not retained as long as V.I. tags or adipose clips, and that the visibility of V.I. marks depended on light intensity and location of the mark on the fish. Unfortunately, branding, laser techniques, V.I. tags, and panjet marks required further research to determine their effects on physiology and fish behavior. Therefore, PIT tags were chosen for mark/recapture studies described in this report, since these tags allow non-intrusive identification of treatment fish at recapture weirs.

Several pilot investigations were undertaken prior to commencement of full-scale NATURES research.

2--The NMFS Newport Laboratory conducted laboratory research on the feasibility of conditioning salmonids to avoid predators. In these studies, coho salmon

(*O. kisutch*) disturbed by physical stressors demonstrated higher blood cortisol levels and vulnerability to predation by lingcod (*Ophiodon elongatus*) than non-stressed fish. Spring chinook salmon that had prior exposure to predation were less vulnerable to predation when compared with those that had not been previously exposed. Antipredator conditioning and stress reduction appeared to be keys for ameliorating the negative impacts of hatchery rearing on postrelease survival for juvenile salmon. Nevertheless, a subsequent experiment failed to demonstrate the efficacy of predator conditioning in improving postrelease in-stream survival of fall chinook salmon. For this study, fish were reared to age-0 smolts using standard fish culture techniques. Test groups were then allocated to one of two identical 2.2-m diameter circular tanks; the "training" tank held two predatory cutthroat trout (*O. clarki*) whereas the control tank had no predators. The fish were held under these conditions for 16 hours prior to release into a small coastal stream. This procedure was replicated six times. There was no significant difference in the proportion of trained and untrained smolts recovered at a downstream weir. It is possible that antipredator training procedures used in this study were not extensive enough to improve antipredator recognition or antipredation responses.

3--The USFWS Abernathy Salmon Culture Technology Center reviewed information regarding feeds and feed delivery systems designed to reduce stress in hatchery fish. Factors controlling feeding behavior of wild salmon include vision, olfaction, taste, diet and seasonal feeding patterns, and prey characteristics. All of these factors must be addressed in developing a new generation of hatchery fish food, and this can be done by use of live feeds and/or developing artificial feeds with diverse shapes, textures, colors, and scents that elicit wild-like feeding responses in the fish. It was determined that feed extrusion technology offers the ability to produce commercial feeds with wild food attributes. For instance, long, thin pellets can be produced, which have been shown to elicit stronger feeding responses than standard pellet shapes. Ideally, feed should be delivered below the surface in a drift form with enough current to keep it in suspension. Feed should also be delivered in low volumes, at high frequency, and at random subsurface locations throughout the raceway to simulate invertebrate drift patterns and to minimize territorial behavior and aggression in fish.

4--The NMFS Manchester Research Station initially investigated the use of live-food supplementation to increase the postrelease foraging ability of hatchery-reared fall chinook salmon. Replicate groups of fry were reared in six 2.4-m diameter circular tanks and fed on two different diets. Fish in three tanks received a standard, commercially available, pelletized diet, while those in the other tanks were given the opportunity to forage on natural live prey (mysids, mosquito larvae, chironomid larvae, and daphnia) prior to their daily ration of pellets. When foraging ability of individual fish was examined in 200-L observation tanks, the trained salmon were found to feed on twice the number of familiar prey (chironomids) and novel prey (mayfly larvae) as untrained fish. This work suggested that live-food supplementation could be used to increase the postrelease foraging ability of hatchery-reared salmon.

5--A subsequent experiment conducted by the NMFS Manchester Research Station failed to demonstrate the efficacy of live-food supplementation in improving instream foraging efficiency of spring chinook salmon. In this experiment, 24 replicate groups of yearling fish were held in 400-L tanks for approximately the last 60 days of rearing. The fish in all tanks received an equal volume of feed pellets each day. Fish in 12 tanks were given an additional ration of brine shrimp or tubifex worms prior to being fed pellets. At the end of the rearing period, the foraging efficiency of groups of test and control fish was evaluated in both freshwater and marine test arenas by allowing the fish to forage on natural prey for about 1 week.

Comparison of stomach contents from fish in the experiments indicated no significant difference in trained and untrained fish. Given observations of successful forage training with other species, it is surprising that habitat enrichment in this study had no effect on postrelease foraging ability. However, this observation may have been the result of very few fish in the study feeding, since many fish had little digestible material in their stomachs and most did not appear to have been feeding as well as they should. For habitat enrichment to enhance foraging ability, it may be necessary to instill a preference for live food diets earlier in the rearing cycle of salmonids.

6--The NMFS Manchester Research Station evaluated the effectiveness of various components of NATURES habitat concepts in three postrelease survival experiments conducted on chinook salmon. In the first experiment, fall chinook salmon were reared for 4 months from swim-up to smoltification. These fish, which were cultured in 400-L raceways outfitted with cover, structure, and substrate, survived in-stream travel to a collection weir 2.2 km downstream at a rate 50% higher than conventionally-reared salmon. In the second experiment, spring chinook salmon were reared for 3 months in 400-L raceways outfitted with cover, structure, and substrate. In clear water, these fish survived at a rate 24% higher than controls after release and traveling 225 km downstream to a collection weir. However, when fish were released in turbid water, there was no significant difference in postrelease survival between test fish and controls. In the final experiment conducted in conjunction with WDFW, culture vessel size was increased to 5,947 L, and fall chinook salmon were reared for about 4 months from swim-up to smoltification. NATURES raceways were outfitted with similar types of cover, structure, and substrate used in the other two experiments. However, in this study, an underwater feed delivery system was added to the NATURES treatment. In this experiment, NATURES fish averaged 27% higher postrelease survival to a collection weir 21 km downstream than their conventionally-reared counterparts.

In these studies, the NATURES variables tested succeeded in producing more "wild-like" fish than conventional rearing models. NATURES fish developed light and dark mottled body camouflage coloration patterns that were cryptic for the diverse stream bottom background over which these fish were released. In contrast, the uniformly light colored, conventionally-reared fish were cryptically mismatched for their release environment and required over 1 week of stream residence to begin development of the long-term color adaptation that can provide cryptic camouflage coloration for the stream background. By our subjective observations, the NATURES fish also displayed a greater fright response to overhead movement than the conventionally-reared groups. The high prerelease survival (98%+) of both conventionally- and NATURES-reared fish in all studies suggested that the NATURES culture techniques that were tested did not adversely affect fish health.

The 25-50% survival advantage during migration in the stream corridor for most groups of NATURES fish observed in the 1991-1995 studies appeared primarily due to the external camouflage color patterns of NATURES fish, which probably reduced their susceptibility to predation by visually hunting predators (e.g., birds and other fish). This may be why survival advantages were not noted for NATURES fish released in turbid water conditions where relative visibility was reduced. However, in the last experiment, it is probable that the automated underwater feeding system also lessened predator vulnerability of NATURES fish by inducing benthic orientation.

The 1991-1995 research demonstrated that rearing-habitat modification techniques developed in pilot-scale NATURES studies could be implemented in production fish rearing. We demonstrated that modification of the culture environment can produce significant positive differences in behavior and postrelease survival of hatchery fish in streams. The research demonstrated that rearing chinook salmon in NATURES environments with substrate, in-stream structure, and overhead cover increased in-stream postrelease survival. Our research also suggested that providing feed in the water column instead of at the surface could enhance fish foraging behavior. Vacuuming substrates was the only NATURES raceway operation procedure requiring a significant increase in maintenance effort. This was an important step in developing NATURES culture habitats for producing "wild-like" fish from hatcheries for use in genetic conservation and supplementation programs.

II. The 1996-1998 research efforts were a collaboration between NMFS, BPA, and WDFW to develop and evaluate innovative culture techniques to increase post-release survival of hatchery fish (see Maynard et al. 2001 for full description and findings for 1996-1998 research). During this period:

Research was continued on evaluation of potential of NATURES for increasing hatchery salmon post-release survival and for producing fish with more wild-like behavior, physiology, and morphology prior to release. Experiments were conducted evaluating automatic subsurface feeders; natural diets; exercise systems; seminatural raceway habitat enriched with cover, structure, and substrate; and predator avoidance conditioning for hatchery salmonids. Automatic subsurface feed delivery systems did not affect chinook salmon depth distribution or vulnerability to avian predators. Live-food diets only marginally improved the ability of chinook salmon to capture prey in stream enclosures. A prototype exercise system that could be retrofitted to raceways was developed, however, initial testing indicated that severe amounts of exercise may increase in-culture mortality. Rearing chinook salmon in seminatural raceway habitat with gravel substrate, woody debris structure, and overhead cover improved coloration and post-release survival without impacting in-culture health or survival. Steelhead fry reared in enriched environments with structure, cover, and point source feeders dominated and out competed conventionally reared fish. Exposing chinook salmon to caged predators was shown to increase post-release survival. Chinook salmon were shown to have an anti-predator response to chemical stimuli from injured conspecifics and exhibited acquired predator recognition following exposure to paired predator-prey stimuli.

A critical question for NATURES research was how rearing in structurally complex habitats affected both in-culture health and survival and post-release survival of the fish. During the 1996-1998 research period more complete results became available from initial experimental release groups (see above for 1991-1995 research period). These are summarized below. Fish were reared at either NMFS facilities (Tests 1-2) or WDFW Hatcheries (Tests 3-5). For fish health evaluations, subsamples of kidney tissue were examined for common fish pathogens by 1) culture on trypticase soy agar (TSA) media, 2) fluorescent antibody test (FAT) to determine *Renibacterium salmoninarum* incidence, and 3) microscopic examination to determine *Nanophyetus salmincola* parasite loads (Test 4 only). Juvenile in-river out-migration survival was evaluated through recapture of fish at downstream weirs.

- **1991-1992 fall chinook study**. Fish were reared from swim-up to smolt in 400-L raceways (3 replicates/treatment) fitted with cover, structure, and substrate. There was no significant difference (P>0.05) in ending length (74.5 vs 74.6 mm) or weight (4.4 vs 4.2 g) of conventionally (C) vs NATURES (N) reared fish. Documented inculture mortality was about 1% for each group. TSA and FAT tests were negative (0/36 C vs 0/76 N). Relative post-release survival to a collection weir about 2 km downstream was about 50% higher (40 versus 60%; P=0.007) for NATURES fish.

- **1994 spring chinook study**. Fish were reared for 3 months in 400-L raceways (6 replicates/treatment) fitted with cover, structure, and substrate. Conventionally reared fish were larger (P<0.05) in ending length (133.4 vs 131.3 mm) and weight (24.7 vs 23.4 g) than NATURES fish. Documented inculture mortality was less than 1% for each group. TSA tests were negative (0/43 C vs 0/45 N), while FAT tests indicated a single positive NATURES fish (0/43 C vs 1/48 N). Relative post-release survival to a collection weir about 225 km downstream was about 23% higher (22 versus 27%; P<0.05) for NATURES fish under clear water conditions, but not in turbid water conditions (34 versus 31%; P=0.285).

- **1994** fall chinook study. Fish were reared hatchery from swim-up to smoltification in pilot scale 5,947-L raceways (3 replicates/treatment) fitted with cover, structure, substrate, and an underwater feed delivery system. About two-thirds of the way through rearing it was noted that conventional fish were significantly (P<0.05) larger (64.0 vs 61.9 mm) than NATURES fish; ration was adjusted to produce comparable size fish (about 75 mm) at release. Documented inculture mortality was about 1% for each group. TSA tests were negative (0/109 C vs 0/112 N), while FAT tests indicated a small number of positives in each group (8/109 C vs 3/112 N). Relative post-release survival to a collection weir about 20 km downstream was 26% higher (38 versus 48%; P=0.001) for NATURES fish.

- **1997** fall Chinook study. Fish were reared to smolt for about 3 months in 18,000-L production scale raceways (2 replicates/treatment) fitted with cover, structure, and substrate at a WDFW hatchery. Conventionally reared fish were significantly ($P<0.001$) larger in ending length (65.9 vs 63.9 mm) and weight (3.3 vs 2.9 g) than NATURES fish. Extreme turbidity during much of the rearing cycle made exacting information on inculture mortality unreliable; however release information suggests it was less than 5% for each group. TSA tests were negative (0/60 C vs 0/60 N), while FAT tests indicated a small number of positives in each group (6/30 C vs 7/30 N). Nanophyetus screenings also indicated similar positive (55/60 C vs 58/60 N) and incidence (3.8 metacercariae per sample C vs 3.5 N) levels. Relative post-release survival to a collection weir about 20 km downstream was about 3.5% higher (73 versus 76%; $P>0.05$) for both groups.

- **1998**. Fall chinook were reared to smolt in the 18,000-L raceways (3 replicates/treatment). Conventionally reared fish were significantly ($P<0.001$) larger in ending length (84.8 vs 82.3) and weight (6.7 vs 6.2 g) than NATURES fish. Documented inculture mortality was less than 1% for each group. TSA tests were negative (0/90 C vs 0/90 N). Visual inspection of kidneys indicated no evidence of clinical bacterial kidney disease. Relative post-release survival to a collection weir about 20 km downstream was 11% higher (60 versus 67%; $P<0.001$) for NATURES fish.

As is apparent from the studies described above, NATURES rearing appeared to produce a slightly smaller smolt. NATURES rearing appeared to have no adverse effects on fish health or inculture survival. Importantly, NATURES rearing appeared in most cases to increase post release survival.

III. From 1999 to 2002 NATURES researchers continued evaluations of the effect of exercise, seminatural raceway habitat, and predator avoidance conditioning as tools that can be used to increase the postrelease survival of hatchery salmonids. In addition researchers examined how NATURES rearing effects ecological interactions between hatchery and naturally reared salmoids. NATURES research in 1999-2002 was a collaboration effort between NMFS, BPA, and the Congressionally established Western Washington Hatchery Scientific Review Group (HSRG).

In 1999 and 2000, two studies evaluating the effect of exercise on zero age fall chinook salmon were conducted. In these studies, fish were exercised for no more than two hours a day and the exercise program was suspended during disease outbreaks. Exercised fish in 1999 became longer and heavier than controls fed an equivalent ration. However, in 2000 this growth advantage of exercise failed to reoccur with fish in both rearing treatments growing at similar rates. In both years, the tested exercise protocols successfully reduced cumulative mortality during the primary experimental period. The post-release survival effect of exercise was evaluated during the summer of 1999 by releasing study fish into a Puget Sound tributary stream. Although some fish at release were observed to better able to hold position against the current than others, the downstream travel time of fish in both treatments was similar. Significantly more control than exercised fish were recovered at the weir in this post-release survival evaluation. Unless the exercised fish possessed an enhanced ability to hold position against the current and lacked a migrational urge, their downstream survival must have been diminished by the exercise program. Although exercise slightly increased resistance to hooded merganser attacks, the results were not significantly different. In summary, these finding indicated that exercise protocols under investigation needed further refinement before the 62% smolt-to-adult survival advantage attributed to them by Burrows (1969) could be realized on a consistent basis.

In 2000, a four year study examing the effect of seminatural raceway habitat consisting of gravel-paver substrate, fir tree structure, and camouflage net cover on coho salmon smolt-to-adult survival was initiated. The experiment was conducted at five hatcheries, with each facility having one modified (experimental) and one unmodified raceway. The study successfully developed covers and concrete pavers well suited for use in a large variety of production hatchery rearing vessels (standard raceways, Burrows ponds, and large ponds). Fish were reared in the seminatural raceway habitats for at least the last two months prior to release. In the first two study years, there were no significant differences in fish size or health at release. However, similar to previous NATURES studies, the seminaturally reared fish developed a significantly different base skin coloration as measured by hue, saturation, and intensity. This skin color difference should enhance their crypticity in the natural environment to make them less vulnerabile to visually hunting predators (fish, birds, mammals). Although funding from BPA for these studies was terminated in 2004, some support for the studies continues through NMFS, WDFW, and the HSRG. In future years, the recovery of coded-wire tag data from the fishery and returns to the hatchery will be used to compare the effect of the two rearing strategies on coho salmon smolt-to-adult survival.

During 1999-2002, researchers also conducted several studies to evaluate the efficacy of chemical conditioning as a predator conditioning tool. Items investigated included whether: 1) populations which evolved in sympatry and allopatry with northern pikeminnows possess innate predator recognition; 2) fright responses to predator odor can be increased by pairing conspecific extract with predator odor; 3) handling affects conditioned responses; 4) acquired predator recognition is retained after transport to a novel environment; and 5) vulnerability to live predators in a stream channel can be improved by chemical conditioning. Ecological interactions between hatchery and wild salmonids, including competition and predation, were also studied as important factors that may negatively affect wild salmonid populations. We performed several experiments comparing naturally-reared juvenile steelhead to conspecifics reared in conventional and NATURES hatchery environments in order to estimate potential effects of hatchery-reared steelhead on wild steelhead in streams.

In 2000, studies compared fin quality, competitive ability, aggressive behavior, and growth of age-0 steelhead from the same parent population reared in conventional and NATURES (structurally enriched) hatchery environments and a natural stream. Conventionally reared fish had lower mean dorsal fin quality than fish reared in the NATURES environment or the natural stream. Fish reared in the NATURES tanks and the natural stream had similar dominance ranks, and both of these treatments had significantly greater dominance ranks than conventionally reared fish. Dominant fish from the NATURES tanks, conventional tanks and the natural stream differed in the frequency of displays, but not in the frequency of aggressive attacks. Dorsal fin quality did not have a significant effect on dominance. Instantaneous growth rates of fish from conventional and NATURES environments did not differ significantly after approximately one month of rearing in the quasi-natural stream channel, but the growth rate of naturally-reared fish differed depending on whether they were stocked into the channel with NATURES or conventional fish. The studies suggested that hatchery-reared juveniles released for conservation purposes may acquire increased competitive ability through rearing in enriched habitats, and they may be better able to compete and establish territories in the presence of progeny of non-local hatchery strays.

NATURES studies conducted in 2001 and 2002 focused on differential competitive effects of NATURES and conventionally-reared age-0 steelhead on naturally-reared conspecifics. In 2001, studies quantified the feeding rates, agonistic interaction rates, and space use of naturally-reared steelhead at two densities alone and in the presence of NATURES and conventionally-reared steelhead from the same stock in order to examine competitive effects of hatchery-reared fry on wild conspecifics. In 2002, studies quantified feeding, agonistic behavior, growth and survival of age-0 steelhead from the three rearing treatments (conventional, NATURES, natural) in the presence and absence of predators (hatchery-reared age-1 steelhead) under laboratory conditions. Results of these studies are presented in this report.

A continuing critical question for NATURES research was how rearing in structurally complex habitats affected both in-culture health and survival and post-release survival of the fish. During the 1999-2002 research period more complete results became available from additional experimental release groups (see above for 1996-1998 research period). These include:

- **1999 fall chinook study**. Fish were reared to smolt in the 18,000-L raceways (3 replicates/treatment). Relative post-release survival to a collection weir about 20 km downstream was 24% higher (59 versus 73%; $P<0.001$) for NATURES fish.

- **2000 fall chinook study**. Fish were reared to smolt in the 18,000-L raceways (3 replicates/treatment). Relative post-release survival to a collection weir about 20 km downstream was 1% higher (80 versus 81%; $P>0.604$) for NATURES fish.

  As in earlier studies (see II, above) NATURES rearing appeared to produce a slightly smaller smolt. However, NATURES rearing appeared to have no adverse effects on fish health or in-culture survival. Importantly, NATURES rearing appeared in most cases to increase post release fitness.

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Section 2

A REVIEW OF RECENT STUDIES INVESTIGATING SEMINATURAL REARING STRATEGIES AS A TOOL FOR INCREASING PACIFIC SALMON POSTRELEASE SURVIVAL[1]

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Introduction

Seminatural rearing strategies that promote the development of natural behavior, physiology, and morphology may provide fish culturists the tools needed to increase the postrelease survival of ocean ranched salmonids. Mitigation, enhancement, and conservation programs all rear juvenile salmonids in protective environments, ensuring high inculture survival (usually greater than 95%). However, hatchery fish typically suffer very high mortality after release, with less than 1% of the chinook (*Oncorhynchus tshawytscha*) and 10% of coho salmon (*O. kisutch*) produced by hatcheries normally surviving to recruit to the fishery or spawning population. This large prerelease-postrelease survival difference dictates that fish culture practices which enhance postrelease survival will have a much greater impact on recruitment than those that increase inculture survival. As an example, a 5% increase in inculture survival (e. g., from 95% to 100%) for a hatchery stock with 2% recruitment will produce only one additional recruit for the fishery or spawning population per 1,000 individuals released (Figure 1). Whereas a 5% boost in postrelease survival (from 2 to 7%) will yield an additional 48 fish for harvest or restoration of the natural spawning population. Even a 1% (2-3%) increase in postrelease survival will generate an order of magnitude more recruits to the fishery and spawning population than a 5% increase in inculture survival. This relationship dictates researchers will make their greatest gains at increasing the number of recruits to the fishery and spawning population by focusing their efforts on developing fish culture practices that increase postrelease survival.

Hatcheries provide fish little experience for the peril-filled natural postrelease environment they must reside in during most of their life cycle. As an example, ocean type chinook salmon are typically cultured in slack water environments lacking natural substrates and structure. In this manmade environment, they are protected from predators and fed pellets for up to nine months before being released to survive on their own for the remaining 24 to 72 months of their life cycle. This artificial environment produces fish that are: 1) unfamiliar with the predators they must evade after release; 2) inexperienced with the natural habitat they must migrate through to reach the sea, 3) unprepared to swim in swift turbulent currents on their downstream migration; and 4) inexperienced hunters that are inept at searching out and capturing elusive prey.

These observations have led many behavioral biologists to hypothesize that exposing hatchery fish to more natural rearing conditions prior to release might help prepare them for life on their own. Seminatural rearing habitats, automated underwater feeders, exercise current velocities, live food diets, predator avoidance training, lower rearing densities, and oxygen supplementation have all been suggested as ways to provide hatchery fish with “heads up” training for life in the natural world (Butler 1981, Wiley et al. 1993, Suboski and Tempelton 1989, Olla et al. 1994, Maynard et al. 1995, Olla et al. 1998, Brown and Laland 2001). These approaches are all based on the central paradigm that rearing fish in a more natural hatchery environment helps promote the expression of traits enhancing the fish’s survival in the wild. The behavioral expression of these traits may include learning to recognize and evade predators, becoming skilled at swimming in a turbulent flow, and developing appropriate hunting techniques. The physiological expression of these traits may involve the cardiovascular and skeletal muscle conditioning required to swim in turbulent currents. Morphological expression may produce fish with the appropriate camouflage colorations for the habitat backgrounds found in streams, rivers, lakes, and estuaries. Maynard et al. (1995) reviews the theoretical approaches for seminatural conditioning of fish prior to release. Over the last decade, research evaluating the effectiveness of these theoretical approaches has expanded considerably. The present paper reviews current research examining the efficacy of seminatural rearing strategies as a tool for increasing the postrelease survival of ocean ranched fish.

Seminatural Rearing Habitat

Seminatural rearing habitat incorporates components of natural substrate, structure, and cover into fish culture vessels. This seminatural habitat provides fish the opportunity to experience natural environmental complexity prior to release. It offers fish the opportunity to develop the skills needed to rapidly swim through woody debris type structure, seek shelter, and develop appropriate camouflage coloration before these skills become vital to their postrelease survival. Seminatural raceway habitat is one subset of seminatural rearing habitat that is designed to make raceways resemble the natural fluvial habitat which most salmonids rear in (Maynard and Flagg 2001). This approach incorporates natural substrate (e.g., sand, gravel, epoxy resin rock pavers, or exposed aggregate pavers), structure (e.g., plastic aquarium plants or conifers), and overhead cover (e.g., solid opaque or camouflage net) simulating natural stream and riverine environments.

In the last few years, we have conducted four studies examining the effect of seminatural raceway habitat on chinook salmon behavior, growth, morphology, health, and survival. In addition we have a fifth study examining the effect of seminatural raceway habitat on coho salmon growth, morphology, health, and smolt-to-adult survival currently underway. The experiments ranged in scale from 400-l rectangular tanks to standard production raceways with a rearing volume of more than 28,320-l (Maynard et al. 1996 a, b, d; 2001a; 2003a; 2003c). Over the course of these experiments seminatural raceway habitat has evolved from a somewhat difficult to maintain loose sand or gravel substrate, plastic aquarium plant structure, and opaque overhead cover to an easier to work form consisting of gravel embedded in concrete pavers that can be vacuumed, with conifers suspended from cables that can be easily moved when working the raceways, and self lifting covers fitted with military specification camouflage net that can easily be opened during fish culture operations (Maynard et al. 1996d, Maynard et al. 2003c). Although in most experiments, fish have been reared full term from the swimup fry to smolt stage in seminaturally raceway habitat, an acclimation approach has also been successfully used where fish are placed in the experimental habitat for only the last few months preceding their release.

The general results from these five experiments are surprisingly similar given the wide range of experimental conditions under which they were conducted. In seminatural raceway habitat chinook salmon were observed to engage in natural aggressive activity more often and strike at decaying debris in the water column less often than conventionally reared fish (Maynard et al. 1996d). However, the inculture depth distribution behavior of chinook salmon reared in seminatural raceway habitat appears to be similar to controls (Maynard et al. 2003c). The growth of chinook salmon reared in seminatral raceway habitat usually lagged slightly behind that of fish grown in the conventional raceway environment (Maynard et al. 1996 a, b, c, 2003 a). However, the health of chinook salmon reared in seminatural raceway habitat is equivalent to or better than that of conventionally reared fish (Maynard et al. 1996a, b, d, 2003a). Both the growth and health of coho salmon reared in seminatural raceway habitat is similar to that of controls (Maynard et al. 2003c). The skin color of seminaturally and conventionally reared fish in all five experiments diverged during culture (Maynard et al. 1996 a, b, d, 2003 a, c). These color differences appear to enhance the ability of seminaturally reared fish to blend into stream and river backgrounds. In addition to this coloration advantage, predation bioassays suggest seminatural rearing may improve the ability of chinook salmon to evade predators (Maynard et al. 2003a). Importantly in 16 out of 17 releases, the instream survival of chinook salmon reared in seminatural raceway habitat was higher than that of their respective controls (Figure 2).

Some other studies have also observed that seminatural rearing habitat produces salmonids with more natural behavior and better fin condition. For instance, the addition of natural substrate to the bottom of grey fiberglass tanks has been shown to increase the number of Atlantic salmon (*Salmo salar*) exhibiting territorial behavior (Mork et al. 1999). Several experiments have demonstrated that covering the bottom of concrete ponds with natural cobble substrates usually improves trout fin condition (Bosakowski and Wagner 1994, Bosakowski and Wagner 1995, Wagner 1996, Arndt et al. 2001). However, the addition of cobble substrate tends to reduce trout condition factor, fat levels, and total length (Wagner 1996). The addition of structure to the rearing environment of rainbow trout (*O. mykiss*) has been shown to produce visual isolation that reduces territory size, but does not result in an increase in volitional density (Imre et al. 2002).

Other research has also demonstrated that rearing salmonids in seminatural rearing habitat may also lead to increased postrelease survival. As an example, brown trout (*Salmo trutta*) reared in natural ponds were found to have higher survival than those reared in conventional tanks (Naslund 1992). Similarly, the smolt-to-adult survival of cutthroat trout (*O. clarki*) reared in gravel bottom ponds was observed to be higher (60%) than fish reared in standard concrete raceways (Tipping 1998, 2001). Rearing coho salmon in seminatural ponds with gravel substrate, woody debris structure, and cover was found to produce a slight (but not significant) increase in their smolt-to-adult survival above that observed for fish reared in conventional concrete raceways (Fuss and Byrne 2002). Preliminary data indicate coho salmon reared in ponds with camouflage net covers and plastic crate structure have increased smolt-to-adult survival (Vander Hagen and Appleby 1998, Vander Hagen personal communication). When challenged to survive on their own the growth of age-0 steelhead cultured in tanks enriched with camouflage net cover and conifer structure was greater than that of conventionally grown fish, suggesting the possibility of a survival advantage (Berejikian et al. 2000). Unfortunately, for most of the studies mentioned above, the effects of density and the presence of natural feeds can not be separated out from the effect of seminatural rearing habitat.

In summary, most research conducted over the last two decades indicates seminatural rearing habitat leads to increased postrelease survival. In most studies, this rearing strategy appears to produce salmonids with more natural territorial behavior and skin coloration without any reduction in fish health. Seminatural raceway habitat, has been developed into a form that can be readily retrofitted to existing raceways.

Developing Hunting Skills

Wild salmonids are skilled predators successfully hunting for a variety of elusive invertebrate and vertebrate prey. Coevolution of salmonids and their prey has resulted in prey organisms being well camouflaged, exhibiting cryptic behavior, and possessing a variety of predator evasive behaviors. In sharp contrast, artificial feeds are designed to be highly visible and easy to consume to ensure maximum feed conversion which minimizes economic and environmental waste. Unfortunately, this ease of detection and capture of artificial feeds may result in hatchery salmonids failing to develop vital hunting skills they will need after release. This potential inability of hatchery fish to hunt may explain why they have often been observed to starve for prolonged periods after release (Miller 1953, Hochackka 1961, Reimers 1963, Sosiak et al. 1979, Myers 1980, O'Grady 1983). Behavioral research suggests that pellet-reared fish usually have some difficulty developing successful hunting techniques when they first encounter live prey (Coughlin 1991, Maynard et al. 1996e, Reiriz et al. 1998, Munakata et al. 2000, Sundstrom and Johnson 2001, Ellis et al. 2002, Kahilainen and Lehtonen 2002). Hypothetically, it may be possible to develop natural hunting skills in hatchery fish by supplementing or replacing their artificial diet with live prey. A number of experiments have evaluated the validity of this concept during the last two decades.

We have investigated the use of live food or live food supplemented diets as a tool to improve the hunting ability of hatchery reared chinook salmon. In the first study the diet of ocean type chinook salmon was supplemented with live feeds. When the fish were tested in laboratory aquaria, it was observed that fish whose diet had been supplemented with live food fed on twice the number of familiar and novel prey as fish reared only on commercial fish food (Maynard et al. 1996e). In the second study, when stream type chinook salmon reared on a similar live food supplemented diet were challenged to forage in freshwater and marine enclosures for a week, it was the pellet only fed fish that were most successful (Maynard et al. 1996f). Many fish from both rearing treatments in this second study had empty stomachs and it is possible that both contagious (trained fish teaching naïve fish) and despotic behavior (one fish dominates the food supply) effects may have confounded the results. In a subsequent third study, the behavior of individual fish was again observed in laboratory tanks where it was noted that fish reared on live food diets showed greater interest in live prey, while fish reared on pellets were more interested in nonfood items (Maynard et al. 2001e). Field trials, where individual fish foraged in cages suspended in a large coastal stream for a week, found that the gut contents of fish reared on live food diets were not significantly different by weight than those of fish reared only on a pellet diet (Maynard et al. 2001e).

Live food diets have been used with some success to improve the foraging skills of other species. Providing tiger muskellunge (hybrid *Esox lucius* x *E. masquinongy*) with the opportunity to hunt live prey enhanced their foraging behavior by decreasing the time and number of strikes required to capture natural live prey (Gillen et al. 1981). Similarly, the hunting skill of naïve sockeye salmon (*O. nerka*) tested in the laboratory improved with increasing experience (Vineyard 1982). Laboratory evaluations indicated that the foraging success of pellet-reared brown trout challenged to capture live crickets was lower than that of wild reared trout, but improved with experience (Sundstrom and Johnson 2001). Although the development of hunting skills in Atlantic salmon raised on pellets lags behind that of those raised on live food, the hunting skills of pellet reared fish improved in subsequent encounters (Coughlin 1991, Reiriz et al. 1998, Brown and Laland 2002).

Intriguingly, it has been shown in the laboratory that the image of experienced demonstrators is sufficient to accelerate this learning process in Atlantic salmon (Brown and Laland 2002). This contagious behavior suggests it may be possible to train a small group of hatchery fish to serve as demonstrators that can be used to rapidly train the remainder of the population in successful hunting tactics. Laboratory trials with hatchery reared turbot (*Scophthalmus maximus*) have found they are also less successful hunters than wild-reared fish (Ellis et al. 2002). As with salmonids, providing hatchery-reared turbot experience with live foods improved their hunting skills, although nonfood items, like stones, continued to be attacked for at least six weeks due to their pellet-like visual characteristics. Although these results suggest live food diets may be used to improve hatchery fish hunting skills, further research is needed to refine the techniques before they are ready for implementation at production hatcheries.

Conditioning Appropriate Anitpredator Behavior

Postrelease survival of hatchery fish may be improved by fish culture practices that encourage the proper development of strategies fish use to counter predation. These strategies usually include: 1) stealth (e.g., cryptic coloration); 2) avoiding habitats predators use; 3) adopting appropriate behavior when detecting predators (freezing, hiding, flight, etc.); 4) evolving better swimming and maneuvering ability than their predators, and 5) outgrowing their predators gape. As previously discussed, seminatural rearing habitat is a culture strategy that can be used to encourage fish to develop appropriate cryptic coloration. This section focuses on reviewing techniques to condition fish to recognize predators. The following sections will then discuss fish culture approaches that train fish to avoid habitats where they are most vulnerable to predators and to exercise salmonids to enhance their speed and maneuverability.

In the hatchery, fish are unlikely to experience the various visual, acoustic, and chemical cues emitted by most of the predators they will encounter after release. Hatchery fish may develop some experience with predation from the small suite of avian predators such as kingfishers (*Ceryle alcyon*), crows (*Corvus caurinus*), gulls (*Laurus sp*), and herons (*Ardea herodias*). However, they usually have no prerelease exposure to predacious fish such as trout, pikeminnow (*Ptychocheilus oregonensis*), and sculpins and most of the piscivorous birds such as mergansers (*Mergus merganser, Lophodytes cucullatus*), terns (*Sterna caspia*), and cormorants (*Phalacrocorax species*) that attack them on their postrelease migration. This predator naivety may be alleviated by conditioning hatchery fish to recognize and respond appropriately to the cues given off by the various predators they will encounter after release. Ideally, this conditioning process will result in little or no mortality during the hatchery rearing phase.

The predator recognition behavior of salmonids has both innate and learned components. The innate component produces reactions such as the fright response of artic charr (*Salvelinus alpinus*) to the cues given off by predacious burbot (*Lota lota*) and pike (*Esox lucius*) (Hiroven et al. 2000). The learned component has been demonstrated in the many studies where salmonids have been observed to rapidly associate danger with the specific visual, chemical, and acoustic cues given off by a predator (Thompson 1966, Patten 1977, Olla and Davis 1989, Jarvi and Uglem 1993, Healey and Reinhardt 1995, Brown and Smith 1998, Berejikian et al. 1999, Brown 1999, Yamamoto and Reinhardt 2003, Hiroven et al. 2000). This learned component of predator recognition provides fish culturists the opportunity to train their fish to recognize predators prior to release.

The efficacy of predator avoidance conditioning has been examined in several studies conducted over the last four decades. Thompson (1966) pioneered this concept with a series of laboratory experiments demonstrating that salmonids modify their behavior after being exposed to predation events. He then applied this concept at the hatchery level by exposing chinook salmon to an electrified model of a steelhead trout (*O. mykiss*). As predicted, the instream survival of predator conditioned chinook salmon to a downstream weir was higher than that of predator naïve controls. A similar study was conducted in Japan where an electrified model of a predacious goby was used to condition chum salmon (*O. keta*) to avoid predators (Kanayama 1968). However, the efficacy of this goby model at increasing survival in an enclosed stream section varied with fish size.

Direct exposure to predators usually produces a positive result. Exposing sockeye salmon to predacious rainbow trout increased their survival in predator laden stream channels by more than 16% (Ginetz and Larkin 1976). Similarly the direct exposure of coho salmon to predacious torrent sculpin (*Cottus rhotheus*) increased their relative survival in subsequent encounters by 67% (Patten 1977).

Field trials have shown that exposing chinook salmon to caged predators such as hooded mergansers, largemouth bass (*Micropterus salmoides*), and brown bullhead catfish (*Ictalurus nebulosus*) during raceway rearing increases their instream survival by 26% (Maynard et al. 2001c). Exposure to caged predators (blue crab, *Callinectes sapidus*) also increased the survival of hatchery-reared summer flounder (*Paralichtheys dentatus*) by more than 50% in subsequent encounters (Kellison et al. 2000).

Laboratory research has demonstrated that coho salmon may only need exposure to the visual, acoustic, and chemical cues given off by lingcod (*Ophidon longatus*) during a predation event to increase their survival in subsequent encounters (Olla and Davis 1989). The success of predator avoidance conditioning generally seems to improve as the number of cues the learner is exposed to increase. Thus, laboratory studies have found that directly exposing Atlantic salmon to predators generated a better response than exposure to caged predators (Jarvi and Uglem 1993). Nonetheless, because of the ease of application, efforts have even been made to use single cues, like video images or odors alone, as tools to condition hatchery reared salmonids to avoid predators (Berejikian et al. 1999, BBC News 2001). Field trials using stream enclosures have shown that chemosensory predator recognition training can be used to successfully increase the survival of brook trout faced with chain pickerel (*Esox niger*) predation by 5% (Mirza and Chivers 2000).

Unfortunately, not all predator avoidance conditioning research has been able to develop successful training protocols. As an example, one recent experiment determined that coho and chinook salmon both modify their behavior after being exposed to predation (Healey and Reinhardt 1995). However, this behavioral modification only improved the survival of coho salmon during open field trials. Similarly exposing chinook salmon to cutthroat predation did not increase their instream survival (Berejikian 1996), nor did predator avoidance training reduce the poststocking mortality of tiger muskies (Koupal 2000). These studies indicate that individual exposure protocols need to be developed for each species of concern. Properly developed, predator avoidance conditioning should be a very useful tool for enhancing the postrelease survival of hatchery salmonids.

Conditioning Natural Habitat Preference

The types of postrelease habitat fish utilize may markedly affect predator vulnerability. Fish culturists maybe able to improve the postrelease survival of fish by conditioning them to use the subset of natural habitats where they are less susceptible to predators. Surface orientation is one of the most notable attributes of hatchery salmonids that may increase their vulnerability to predation (Sosiak 1978, Mason et al. 1967, Uchida et al. 1989, Reinhardt 2001). Although this surface orientation has innate components, it is also known to be partially conditioned. As an example, although farmed and ocean ranched masu salmon (*O. masou*) are innately more surface oriented than wild stocks, they all become increasingly more surface oriented the longer they are fed pellets from the surface (Reinhardt 2001). This surface orientation of hatchery fish can markedly increase vulnerability to surface feeding predators, such as terns (Collis et al. 2001).

In a study conducted during the mid 1990s, we hypothesized that fish could be conditioned to be more benthic oriented by feeding via automated underwater feed delivery systems that do not positively reinforce surface orientation behavior. This study incorporated an automated underwater feeder system into one of the previously described seminatural raceway habitat experiments (Maynard et al. 1996b). Subjective observations conducted during this experiment suggested chinook salmon reared with the automated subsurface feed delivery system exhibited more natural territorial behavior and seemed less likely to strike at debris falling on the surface than did hand fed fish. However, during this study the effects of the feeder could not be separated from those of the other seminatural raceway habitat components.

In the follow up experiment, ocean type chinook salmon were reared in raceways where they were either fed by hand or using the automated underwater feed delivery system (Maynard et al. 2001b). As in the first experiment, the hand fed fish rapidly became conditioned to swim towards fish culture personnel and would swarm at the surface when humans approached the raceway. However, testing revealed no depth preference, innate fright response, or predator vulnerability differences had developed between fish in the two feeding treatments. Only the response hand fed fish gave to the visual image of a human standing beside the raceway differed between fish in the two rearing treatments. This suggests hand feeding at the surface may not increase the postrelease predation risk for chinook salmon.

As noted above, for other species the results may be quite different. Research conducted with sea run cutthroat trout indicates elimination of hand feeding can have beneficial postrelease survival effects (Tipping 2001). In this case, the smolt-to-adult survival of fish reared on demand feeders exceeded (but nonsignificantly) that of traditional hand fed fish by 10% (Tipping 2001). Since both feeding methods disperse food at the surface, the survival benefit must be attributed to not deconditioning the fish to fear large moving objects at the surface.

Developing Stamina

Fish culturists may be able to increase the postrelease survival of salmonids by improving their swimming performance. Swimming performance is not only a key factor in bursting away from and outmaneuvering predators, but is also critical in avoiding injuries from turbulent currents during downstream migration. Most hatchery salmonids gain little experience with any form of flow prior to release. This is because raceways and rearing ponds velocities are normally less than 1 cm/sec. This low flow environment fails to challenge the fish to swim as they would in their natural fluvial habitat.

There is ample evidence that exercising salmonids provides fish culture benefits. In one study examining exercise as a fish culture tool, brook trout (*Salvelinus fontinalis*) were reared for 10 weeks in circular tanks with or without exercise (Leon 1986). This exercise significantly increased fish growth and swimming stamina over that experienced by unexercised controls. Other investigations have observed exercise routinely improves food conversion (Christiansen et al. 1989, Christiansen and Jobling 1990, Christiansen et al. 1992, Nielsen et al. 2000, Azuma 2001) and swimming performance (Besner and Smith 1983, Schurov et al. 1986, McDonald et al. 1998). Regular exercise also improves the ability of salmonids to adapt to seawater and reduce their ion loss in epinephrine challenge tests (Khovanskiy et al. 1993, McDonald et al. 1998).

Burrows developed the rectangular circular pond as a tool to improve the quality of ocean ranched salmonids. His pioneering work indicates that the exercise velocities these rearing vessels generate improves fall chinook salmon smolt-to-adult survival by 62% (Burrows 1969). Similar results were observed with brown trout with the instream survival of exercised fish being more than 50% higher than that of unexercised trout (Cresswell and Williams 1983). Prerelease exercise also seemed to increase the instream survival of Atlantic salmon (Schurov et al. 1986). However, exercise does not always lead to increased postrelease survival. Coho salmon reared full term in the exercise velocities generated by Burrows ponds did not experience higher smolt-to-adult survival than controls reared in nonexercise standard raceway velocities (Lagasse et al. 1980). Exercise also did not improve the return of adult steelhead to Coles River Hatchery (Evenson and Ewing 1993).

An energetically efficient exercise system that can be retrofitted to standard raceways was recently developed by Maynard et al. (2001d). Exercising ocean type Chinook salmon for 24-h a day for a week in this system did not increase postrelease survival. However, the exercise regime did significantly increase inculture mortality probably because of high rearing water temperatures (to 18° C) encountered during that study (Maynard et al. 2001d). Adopting a 2-h a day exercise protocol and suspending the exercise program at the first sign of a disease outbreak significantly increased growth and decreased inculture mortality of exercised fish relative to that of unexercised controls (Maynard et al. 2003b). While this exercise protocol may have slightly increased resistance to hooded merganser attacks, it did not increase downstream survival. Further refinement and evaluation of exercise protocols are needed before the smolt-to-adult survival advantage observed by Burrows (1969) can be realized on a consistent basis.

Conclusion

A number of seminatural rearing strategies exist for increasing hatchery fish postrelease survival. As previously reviewed, reducing rearing density appears to consistently increase smolt-to-adult survival (Maynard et al. 1995). Seminatural rearing habitat and predator avoidance training are beginning to prove their worth as tools for increasing hatchery fish instream survival. Data from fish now at sea will help determine if these strategies also lead to increased recruitment to the fishery and spawning population. Unfortunately, other seminatural strategies, like subsurface feed delivery systems, live food diets, and exercise protocols have proven to be less useful as tools for increasing postrelease survival. These techniques require further refinement before they can be generally adopted as fish culture tools to enhance recruitment to the fishery and spawning population.

The continued development of fish culture techniques that improve postrelease survival is mandatory if recruitment to the fishery and spawning population is to be improved. Economic and social factors suggest it unlikely that the reduction in both the quantity and quality of freshwater rearing habitat can be totally reversed. Thus, we believe hatcheries will continue to be a necessity for the maintenance of anadromous salmonids stocks. The inculture survival of fish in hatcheries is already so high that further increases will have only minor impacts on the number of fish recruiting to the fishery and spawning population. Only fish culture techniques that increase the postrelease survival of fish released to sea offer any meaningful hope of improving recruitment. Fishery managers may use these new techniques to generate more recruits to the fishery, more returning spawners to listed populations, or simply to improve economic efficiency of hatchery operations. They may also use this increased postrelease survival tool to reduce the impact of mitigation and enhancement hatcheries on wild salmonids. This can be done since increased postrelease survival will enable hatcheries to release fewer smolts that negatively interact with wild fish, while maintaining stable recruitment levels to the fishery.

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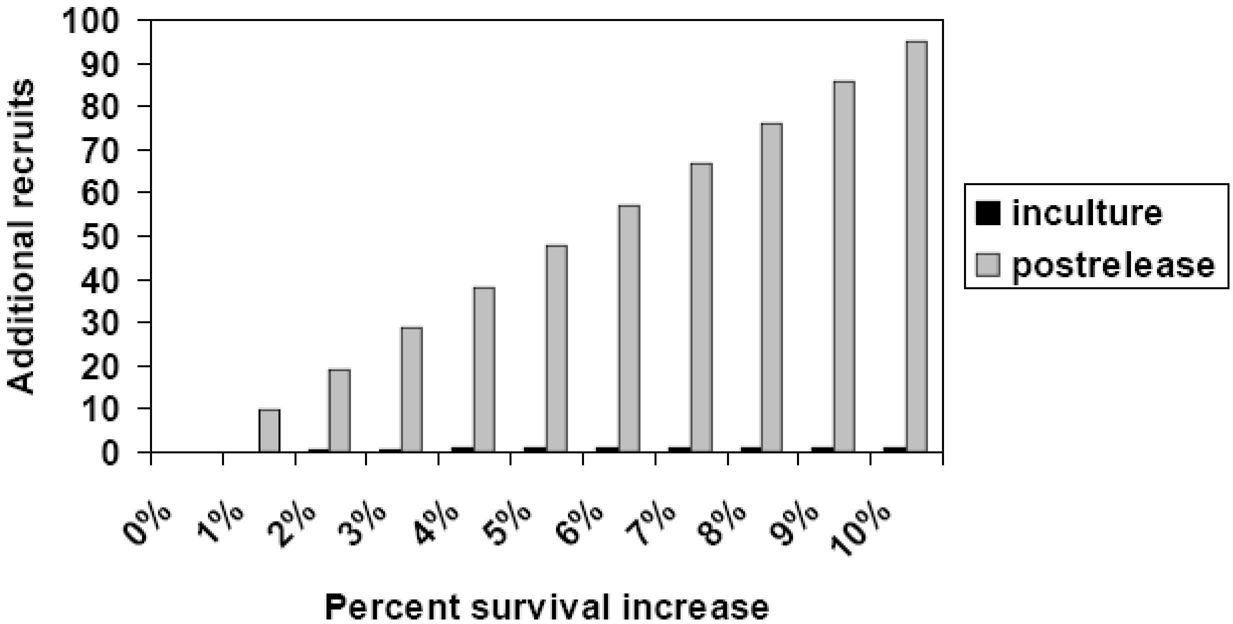

Figure 1. The number of additional recruits generated by equivalent increases in pre- and postrelease survival. (Assumptions: base in culture survival = 95%; base postrelease survival = 2%)

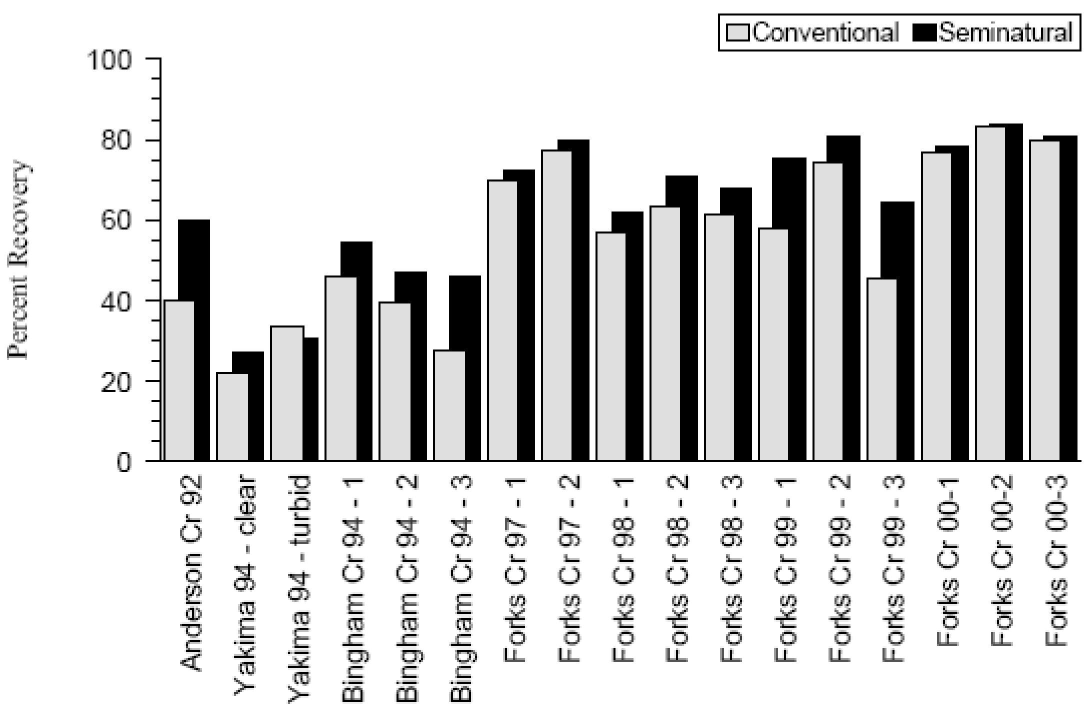

Figure 2. Summarized instream recovery of Chinook salmon reared in conventional and seminatural raceway habitat (Combined from Maynard et al. 1996a, 1996b, 1996c, 2003a, 2003c).

Section 3

Effects of density and rearing environment on the behavior of steelhead *Oncorhynchus mykiss* fry in a laboratory stream[1]

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Abstract

We quantified the aggression, feeding, dominance, position, and space use of naturally-reared steelhead fry stocked with two types of hatchery-reared fry (conventional and enriched rearing environments) at two densities in order to determine how rearing environment and fish density affect the behavior of steelhead fry in an experimental flume. Rates of threats and attacks by steelhead fry were higher at high density, but there were no effects of rearing treatment on aggression. The overall feeding rate was higher at low density, but this was not observed for dominant or subdominant fry; there were no significant effects of rearing treatment on feeding rate. Naturally-reared fry were dominant more often at low density, while hatchery-reared fry were more often dominant at high density; there was no effect of hatchery rearing treatment on dominance. The only significant effect of rearing treatment that we observed was on the upstream position of naturally-reared fry, which occupied more upstream positions when stocked with conventional than enriched hatchery-reared fry. There were no effects of rearing treatment on space use. Our results indicate that hatchery-reared steelhead fry stocked at low densities may have effects on similar-size wild fish that are comparable to the effects of a similar increase in density of wild fish. Rearing environment had relatively little influence on the behavior of steelhead fry at low to moderate densities; the density at which hatchery fry are released may be more important in this respect than rearing environment. Releasing steelhead fry similar in size to wild fish at low densities may be a supplementation strategy that entails few risks to wild steelhead populations.

Introduction

The listing of a number of salmonid populations as threatened or endangered under the U.S. Endangered Species Act has resulted in increased interest in using artificial propagation to enhance, or 'supplement', wild salmonid populations. The ultimate goal of hatchery supplementation programs is to restore wild salmonid stocks to a self-sustaining state. There are currently a number of salmonid supplementation programs underway (BPA 1992; Waples et al. in press), but there is little evidence that any have been successful (Winton & Hilborn 1994; IMST 2000, Waples et al. in press).

Two potential reasons for the lack of success of salmonid supplementation programs are high post-release mortality of hatchery fish (Olla et al. 1998; Brown & Laland 2001) and negative ecological interactions between hatchery-reared and wild fish in streams (Nickelson et al. 1986; Flagg et al. 1995). Hatchery-reared fish may use different habitats than wild fish (Mason et al. 1967; Dickson & MacCrimmon 1982), may not feed efficiently (Sosiak et al. 1979; Sundstrom & Johnsson 2001), and may suffer reduced survival as a result (Olla et al. 1998). Hatchery-reared salmonids are often more aggressive than wild conspecifics (Fenderson et al. 1968; Swain & Riddell 1990; Mesa 1991; Rhodes & Quinn 1998), and it has been suggested that energetic expenditures associated with increased aggression may contribute to the low survival observed for hatchery-reared fish (Fenderson et al. 1968; Bachman 1984; Mesa 1991; Deverill et al. 1999; Bettinger & Bettoli 2002).

Increased aggression by hatchery-reared fish might also result in negative effects on wild fish resident in streams where hatchery-reared fish are released, which might undermine the success of supplementation programs. Competition from hatchery-reared fish has been suggested as a mechanism contributing to the decline in abundance of natural salmonid populations (Nickelson et al.1986; Flagg et al. 1995). Hatchery-reared fish may disrupt natural social patterns in streams through competitive interactions (Bachman 1984; Nielsen 1994; McMichael et al. 1999), and may affect the abundance, growth, and survival of natural salmonids (Fresh 1997).

It has been suggested that changes in hatchery rearing practices may be necessary to produce fish suitable for supplementation (Stickney 1994). Modifications to rearing density or water velocity have sometimes been shown to increase the performance (e.g., swimming speed) or survival of hatchery-reared salmonids (Cresswell & Williams 1983; Martin & Wertheimer 1989), but results are equivocal (Maynard et al. 1995) and it has been suggested that it is unlikely that such modifications will produce fish that are similar to wild fish in performance (McDonald et al. 1998). Other experiments have shown that structural modifications to hatchery rearing environments may increase the survival or predator avoidance of juvenile salmonids (Bams 1967; Leon 1975; Fuss & Johnson 1988; Maynard et al. 1996), and may affect the agonistic behavior, dominance, growth, and space use of steelhead fry in novel experimental environments (Berejikian et al. 2000; 2001).

If hatchery rearing environments affect the dominance and agonistic behavior of hatchery-reared salmonids, they might also be expected to alter any effects that hatchery-reared fish may have on wild fish. This experiment was conducted to determine how rearing environment and fish density affect the aggression, feeding, dominance, position, and space use of steelhead *Oncorhynchus mykiss* fry in an experimental flume.

Methods

Study population

We obtained artificially-spawned steelhead eggs from the Skookumchuck River, a tributary of the Chehalis River in western Washington (Lewis County). This hatchery population was derived from the local wild population and spawning protocols have continued to incorporate naturally produced steelhead each year. Eyed eggs were transported from their initial incubation site (Bingham Creek Hatchery, Washington Department of Fish and Wildlife, Mason County, WA) to the University of Washington's Big Beef Creek Research Station (Kitsap County, WA) on 8 May 2001 and incubated in 10°C well water.

Rearing treatments

At the initiation of exogenous feeding, 650 steelhead fry were stocked into each of six 1.8-m diameter circular tanks (water volume = 1,520 L; flow = 28 L min^{-1}) on 9 June 2001. Three enriched tanks contained the tops of two fir trees to provide structure. Military camouflage netting on top of each enriched tank provided approximately 60% overhead shade cover, and each tank had an underwater food-delivery system that delivered commercial food pellets at the mid-water depth at two locations on opposite sides of the tanks (see Berejikian et al. 2000 for photographs of the tanks). Three conventional tanks contained no cover or structure other than a center standpipe. Fish in the conventional tanks were hand-fed by scattering food across the water surface; fish in all tanks were fed with equal frequency.

At the time emergent fry were stocked into the rearing tanks, 1,950 fry were released into a screened-off section of a side channel of Big Beef Creek (Kitsap County, WA); we refer to these as naturally-reared, or natural, fry. The 35-m long channel received 0.05 m^3s^{-1} flow from springs and the main channel of Big Beef Creek, and was augmented with 0.025 $m^3 s^{-1}$ well water. The channel had a gravel-cobble substrate; riparian vegetation included red alder (*Alnus rubra*), western cedar *(Thuja plicata*), and salmonberry (*Rubus spectabilis*). Woody debris was added to provide structure and cover from avian and terrestrial predators; few fish predators were present. Steelhead fry fed on natural food produced within the channel.

Experimental facility

Experimental trials were conducted in two 10-m long by 1.5-m wide flumes previously described by Berejikian et al. (2000; 2001). Screens were placed perpendicular to the flow to produce five 1.5-m long by 1.5-m wide sections in each flume. Each flume received 30 L min^{-1} of 12°C well water recirculated at a flow of approximately 1,700 L min^{-1} by 2-horsepower submersible pumps. Water depth was maintained at approximately 20 cm. Light was provided by wide-spectrum fluorescent lights on a photoperiod of 14 hours light/10 hours dark. The sides of the flumes are double-paned glass, which allowed continuous viewing of all fish in each section.

The substrate of each flume section was a sheet of fiberboard marked with a 10 cm grid to facilitate the estimation of space use and position by overhead video cameras. Two velocity refuges (9 cm high by 10 cm wide pieces of aluminum) were placed on the substrate of each section, one 40 cm from the upstream screen and 10 cm from the center, and another 80 cm from the upstream screen and 50 cm from the center, both on the left side of the section.

Experimental design and protocol

We used a substitutive experimental design (Fausch 1998) to examine the relative effects of three types of steelhead fry (natural, conventional hatchery and enriched hatchery) on the behavior of natural steelhead fry at two densities. In the low density trials, two fry (one natural and one conventional, enriched, or natural fry; total density = 0.9 fry m^{-2}) were matched for body weight (less than 5% difference) and simultaneously introduced into a flume section. Four fry (two natural and two conventional, enriched, or natural fry; total density = 1.8 fry m^{-2}) were similarly matched for body weight and stocked for the high density trials; hereafter we refer to these rearing treatment combinations as CN, EN, and NN, respectively. Fish were allowed to acclimate in the flume sections for 44 hours with minimal feeding (an average of one bloodworm every 2 hours). Fry used in this experiment ranged from 48 to 82 mm in length and from 1.08 to 6.02 grams in weight; fish size increased over the duration of the experiment, but rearing treatment combinations were balanced over time. A total of 120 trials (20 replicates of three treatment combinations at two densities) were conducted between 8 August – 26 September, 2001.

Frozen bloodworms were thawed and introduced into each section at a rate of two per minute during observation periods; this averages to approximately 642 mg m^{-2} of food per hour, which is within the range of food abundance observed in juvenile steelhead territories in a British Columbia stream (Keeley & McPhail 1998). The worms entered each section through a PVC tube that was located in the center of the section at the upstream end and turned at a 45 degree angle to the left such that fish which positioned themselves in the upstream-most position on the left side of the section had first access to food.

Feeding and agonistic behavior

The number of food items captured (successful feeding strikes) and the number of attacks (nips, charges, and chases) and threats (lateral and frontal displays) initiated by each fish were recorded simultaneously in each flume section for 10 minutes by two observers. We defined the dominant fish in each trial as the fish that obtained the most food particles during the observation period (cf. Fausch 1984; Metcalfe 1986). The subdominant fish in each trial was defined as the fish that consumed the second-largest number of food particles or (if only the dominant fish in the trial fed) the fish that initiated the second-largest number of threats and attacks.

It was not necessary to mark steelhead fry because naturally- and hatchery-reared fry differed in color and observers kept track of individual fish during the course of observations. The order of observation was from downstream to upstream in each flume, and treatments and densities were balanced among flumes, flume sections, and observation dates. All observations took place between 8:00 and 13:00 PST.

Position and space use

Fish in each flume section were recorded by a remotely-operated overhead video camera during the 10-minute periods when feeding and agonistic interactions were observed. The positions of individual fish were frequently noted by observers on the videotape audio track, which allowed us to determine position and space use for each fish. Video tapes were played back at a later date and the position of each fish was recorded (as x, y coordinates on the grid that made up the cell substrate) at ten second intervals (60 observations per fish). These coordinates were used to produce 95% confidence ellipses of space use using SAS; space use for each fish was estimated as the area of the ellipse. The mean crosswise (x) and upstream (y) positions of each fish within the flume section were also estimated from the x, y coordinates.

Data analysis

The substitutive design used here allows one to compare the effects of adding fry reared in conventional or enriched hatchery environments to the effects of adding equal numbers of naturally-reared fry. For example, if aggression was significantly higher when enriched or conventional fry were stocked with naturally-reared fish compared to when natural fish are stocked alone at the same density, then the behavioral effects of adding hatchery fish would likely be greater than adding the same number of wild fish. In this case, rearing treatment *combinations* (NN, EN, CN) are tested against one another. Within this design framework, we performed a number of different analyses on subsets of the data to address planned comparisons (Table 1).

We first tested the hypothesis that the overall levels of aggressive interactions (threats and attacks) and feeding were not different among the three rearing treatment combinations and between the two densities. We compared the mean number of threats, attacks, and successful feeding strikes per fish, the proportion of fish feeding, and the proportion of food eaten among rearing treatment combinations (CN, EN, and NN) and between densities using two-way multivariate analysis of variance (MANOVA) of ranked data. Data were ranked using PROC RANK and analyzed using PROC GLM in SAS (SAS 1990). This procedure is essentially equivalent to performing a Kruskal-Wallis test, and was undertaken because of significant departures from normality in the data.

We next compared individual aggression, feeding, position, and space use among rearing treatment combinations and between densities for dominant and subdominant fry separately. We first ranked the data (as above) and then performed two-way MANOVA (with rearing treatment combination and density as main effects) on the ranks. This analysis tests the hypothesis that aggression, feeding, position, and space use were not different among rearing treatment combinations and between densities for dominant or subdominant fry.

The foregoing analyses allow one to determine if differences exist among rearing treatment combinations or between densities, but do not provide direct comparisons between hatchery and natural fry, enriched and conventional fry, or natural fry in the presence of enriched or conventional fry. In order to explore these comparisons, we performed several analyses using only the treatments with hatchery fish (EN, CN). We first tested the null hypothesis that the relative dominance of hatchery and natural fish is unaffected by density and rearing treatment combination using a log-linear model with three dependent variables – density, rearing treatment combination (EN or CN), and fish type (natural or hatchery). This analysis was performed using PROC CATMOD in SAS (SAS 1990).

We next used a three-way MANOVA on ranked data to test the hypothesis that the aggression, feeding, position, and space use of steelhead fry were not affected by rearing treatment combination (EN or CN), density, or fish type (natural or hatchery). This analysis allowed us to make direct comparisons between natural and hatchery-reared fry. MANOVA was performed on ranked data, as above.

Using only hatchery-reared fry from the EN and CN treatment combinations, we tested the hypothesis that the aggression, feeding, position, and space use of hatchery-reared fry were not affected by rearing treatment or density. Similarly, we tested the hypothesis that the aggression, feeding, position, and space use of naturally-reared fry were not affected by the rearing treatment of the hatchery fish that they were stocked with or by density. In these analyses, a significant treatment effect would indicate that either hatchery or natural fish, respectively, differed in behavior depending on the rearing treatment of hatchery fish. We performed two-way MANOVA (with rearing treatment combination and density as main effects) for these analyses, using only hatchery or natural fish. These analyses were performed on ranked data, as above.

Results

The numbers of threats and attacks given by hatchery- or naturally-reared fry in this experiment were not significantly related to body length ($P > 0.2$ in all cases, by linear regression). Space use of steelhead fry in this experiment was also not significantly related to body length ($P > 0.6$, by linear regression).

Comparisons of aggression and feeding among treatment combinations

There was a significant overall effect of density on aggression and feeding (MANOVA, Wilks' $\lambda = 0.0980$, F = 202.46, df = 5, 110, $P < 0.0001$) when all rearing treatment combinations were considered together; no significant effects of rearing treatment combination (Wilks' $\lambda = 0.8980$, F = 1.22, df = 10, 220, $P = 0.2817$) or the interaction (Wilks' $\lambda = 0.9751$, F = 0.28, df = 10, 220, $P = 0.9853$) were evident. The mean number of attacks and threats per fish was significantly higher in the high density trials, while the number of successful feeding strikes per fish was lower (Table 2). The proportion of fish feeding was significantly greater in low density than in high density trials, and the proportion of food consumed was lower (Table 2). There were no significant effects of rearing treatment combination or the interaction between rearing treatment combination and density on any of these variables (Table 2).

Dominant and subdominant fry

There was a significant overall effect of density on aggression, feeding, and space use of dominant (MANOVA, Wilks' λ = 0.8609, F = 2.16, df = 8, 107, $P = 0.0362$) and subdominant (Wilks' λ = 0.8005, F = 3.33, df = 8, 107, $P < 0.0019$) steelhead fry, but there was no significant effect of rearing treatment combination (dominant - Wilks' λ = 0.8637, F = 1.02, df = 16, 214, $P = 0.4403$; subdominant - Wilks' λ = 0.8345, F = 1.27, df = 16, 214, $P = 0.2212$) or the interaction (dominant - Wilks' λ = 0.8270, F = 0.66, df = 16, 214, $P = 0.8270$; subdominant - Wilks' λ = 0.8974, F = 0.74, df = 16, 214, $P = 0.7469$). Dominant and subdominant fry initiated significantly more threats and attacks in high density trials; there were no significant effects of rearing treatment combination or the interaction between rearing treatment combination and density on threats or attacks for dominant or subdominant fry (Table 3). There were no significant effects of rearing treatment combination, density, or their interaction on the number of successful feeding strikes, upstream or crosswise position, or space use of dominant or subdominant fry (Table 3).

Dominance

Regardless of rearing treatment combination (EN or CN), natural fish were dominant in more than half of trials at low density; hatchery fish were more often dominant in high density trials (Table 4). Dominance, analyzed by log-linear models, was significantly affected only by an interaction between density and fish type (natural or hatchery; $P = 0.0261$); no main effects or other interactions were significant ($P > 0.3$).

Hatchery vs. natural fry

There was a significant overall effect of density on aggression, feeding, and space use (MANOVA, Wilks' λ = 0.8339, F = 7.53, df = 6, 227, $P < 0.0001$), but no significant effects of rearing treatment combination (Wilks' λ = 0.9730, F = 1.05, df = 6, 227, $P = 0.3932$), fish type (Wilks' λ = 0.9710, F = 1.13, df = 6, 227, $P = 0.3467$), or any of the interactions (treatment by density - Wilks' λ = 0.9850, F = 0.58, df = 6, 227, $P = 0.7501$; treatment by type - Wilks' λ = 0.9771, F = 0.89, df = 6, 227, $P = 0.5046$; density by type - Wilks' λ = 0.9625, F = 1.47, df = 6, 227, $P = 0.1886$; treatment by density by type - Wilks' λ = 0.9873, F = 0.48, df = 6, 227, $P = 0.8193$) were evident. Univariate analyses revealed significant effects of density on threats, feeding strikes, and space use (Table 5). A significant density by type interaction was apparent for feeding strikes, and a significant treatment by type interaction was noted for upstream position (Table 5).

Enriched vs. conventional hatchery fry

We observed a significant overall effect of density on aggression, feeding, and space use of hatchery fish from the CN and EN treatments (MANOVA, Wilks' λ = 0.8390, F = 3.55, df = 6, 111, *P* = 0.0030), but no significant effects of rearing treatment (Wilks' λ = 0.9680, F = 0.61, df = 6, 111, *P* = 0.7209) or the interaction (Wilks' λ = 0.9698, F = 0.58, df = 6, 111, *P* = 0.7488) were evident. A significantly greater number of threats were initiated by hatchery-reared fry in high density than low density trials (Table 6). There were no significant effects of density, rearing treatment combination, or their interaction on attacks, feeding strikes, position, or space use of hatchery-reared fry (Table 6).

Comparisons of natural fry from EN and CN treatment combinations

For natural fish from the CN and EN treatment combinations, there was a significant overall effect of density on aggression, feeding, and space use (MANOVA, Wilks' λ = 0.7707, F = 5.50, df = 6, 111, *P* < 0.0001), but no significant effects of the rearing treatment of the hatchery fish they were stocked with (Wilks' λ = 0.9331, F = 1.33, df = 6, 111, *P* = 0.2514) or the interaction (Wilks' λ = 0.9772, F = 0.43, df = 6, 111, *P* = 0.8566). Univariate analyses revealed that the number of feeding strikes and space use by natural fry was greater in low density than in high density trials; there were no significant effects of treatment combination or the interaction on these variables (Table 6). Natural fry in the CN treatment combination tended to occupy positions further upstream than those in the EN combination; there were no significant effects of density or the interaction on the upstream position of natural fry (Table 6). There were no significant effects of any factor on threats, attacks, or crosswise position of natural fry (Table 6).

Discussion

The use of artificially propagated fish to assist in the recovery of wild salmonid populations is increasing, although concerns have been raised about potential negative ecological effects of hatchery-reared fish on wild salmonids in streams (Nickelson et al. 1986; Flagg et al. 1995; Levin et al. 2001). We found significant effects of fish density on the aggression, feeding, and space use of mixed groups of steelhead fry in a laboratory flume, but few significant effects of fish type (natural or hatchery) or the type of hatchery environment (conventional or enriched) that fry were reared in. These results suggest that the effects of stocking hatchery steelhead fry on the behavior of wild fry are not likely to be different than the effects of adding similar numbers of wild fry, and that enriched rearing environments have little effect on the behavior of steelhead fry at the densities tested here.

Aggression

At the densities used here, mixed groups of natural and hatchery-reared steelhead fry showed similar levels of aggression as the same number of natural fry. The relative aggressiveness of steelhead fry reared in different environments depended on fish density and dominance status, and the presence of hatchery-reared steelhead fry did not necessarily increase the level of aggression of dominant or subdominant steelhead fry at the densities used here (0.9-1.8 fry m^{-2}). Compared to density, rearing environment had little effect on aggressive behavior.

Berejikian et al. (2000) similarly observed no significant difference in the aggressive behavior (frequency of attacks and displays) of steelhead fry reared in conventional and enriched hatchery environments when the treatments were stocked together at 10.7 fry m^{-2}. When naturally-reared steelhead fry were stocked with both hatchery treatments at a lower density (5.3 fry m^{-2}), however, there were significant differences among rearing treatments in the frequency of threat displays by dominant fish (conventionally-reared fry delivered significantly fewer displays than enriched or natural fry), but not attacks (Berejikian et al. 2001). When fry from each of the three rearing treatments were observed separately at a density of 4.0 fry m^{-2}, Berejikian et al. (2001) observed a significant difference in the relationship between body size and total aggression among the three treatments – aggressiveness increased with body size in the enriched and conventional hatchery treatments, but not in naturally-reared fry. The fact that we observed no effects of rearing treatment on aggressive behavior of steelhead fry may be because we observed fish at lower densities, but our results clearly demonstrate that enriched hatchery environments had little effect on the aggression of steelhead fry at the densities tested here.

A number of studies have suggested that hatchery-reared salmonids are more aggressive than wild-reared conspecifics (Fenderson et al. 1968; Fenderson & Carpenter 1971; Bachman 1984; Mesa 1991; Peery & Bjornn 1996; Deverill et al. 1999). Our results indicate that this is not always the case, since we found no significant effects of fish type (hatchery or natural) on the number of attacks or threats initiated by steelhead fry. Our results suggest that the relative aggressiveness of hatchery and natural steelhead fry may depend on fish density; further research is necessary to understand how fish density and other factors may interact to affect the relative aggressiveness of natural and hatchery-reared steelhead fry.

Feeding

We observed that mixed groups of natural and hatchery-reared steelhead fry showed similar levels of feeding as the same number of natural fry. Several authors have suggested that increased aggression by hatchery fish is not energetically efficient and might result in decreased feeding and growth of hatchery-reared salmonids released into streams (Fenderson et al. 1968; Bachman 1984; Mesa 1991; Deverill et al. 1999; Bettinger & Bettoli 2002). We estimated the proportion of food eaten in each trial because we hypothesized that increased aggression by hatchery-reared fry might cause feeding opportunities to be foregone by natural or hatchery fry, resulting in decreased overall feeding efficiency. We saw no evidence that hatchery rearing treatment affected the feeding efficiency of groups of steelhead fry, but efficiency was significantly greater at high density. These results suggest that the feeding efficiency of groups of steelhead fry is not affected by the presence of hatchery-reared fry or their rearing environment at the densities used here.

Hatchery-reared fry from the EN and CN treatment combinations appeared to feed at similar rates at both densities, while natural fish fed at a significantly higher rate at low density. We observed a significant density by type interaction for feeding strikes in the three-way MANOVA, which reflects that fact that natural fry tended to feed more than hatchery fry at low density, while the opposite was true at high density, although the type effect was not significant. This suggests that the feeding behavior of natural fry may be more strongly affected by increases in fish density than that of hatchery-reared fry. Further research using a wider range of densities is necessary to determine the relative effects of fish density on feeding in natural and hatchery-reared steelhead fry.

Dominance

We found that hatchery-reared steelhead fry were more likely to dominate naturally-reared fry when overall fish density was higher, regardless of hatchery rearing treatment, while naturally-reared fry were more likely to be dominant at lower density. In previous experiments, however, steelhead fry from enriched rearing environments were significantly more likely to be dominant over conventionally-reared fry when the two were observed together (Berejikian et al. 2000), and fry reared in natural and enriched hatchery environments were more likely to dominate conventionally-reared fish, but not each other, when all three treatments were observed together (Berejikian et al. 2001). Based on the results of these previous experiments, we hypothesized that rearing treatment would have a significant effect on dominance – specifically, that fry reared in conventional hatchery environments would be dominant over naturally-reared fry significantly less often than would fry from enriched environments.

Although the experiments reported by Berejikian et al. (2000; 2001) were conducted in the same experimental flume using steelhead fry of the same size range and stock as this experiment, methodological differences may have contributed to the different results observed. Berejikian et al. (2000; 2001) determined dominance based on agonistic behavior, while we defined the dominant fish as the individual that acquired the most food particles (although in most cases these individuals initiated more attacks than they received, a criterion for dominance used by Berejikian et al.). Berejikian et al. (2000; 2001) stocked fry from the two hatchery rearing treatments together, either with (2001) or without (2000) naturally-reared fish, and their experiments were conducted at higher densities than used here (2000 - 10.7 fry m^{-2}; 2001 - 5.4 fry m^{-2}).

The experiment reported here was designed to determine the relative effects of the two hatchery rearing treatments on competitive interactions with naturally-reared fry, while the previous experiments were designed to assess differences between conventionally-reared and enriched hatchery fry (Berejikian et al. 2000) or differences among the two hatchery rearing types and naturally-reared fry (Berejikian et al. 2001). In this experiment, we stocked fry from conventional or enriched hatchery environments with equal numbers of naturally-reared fry, and our results suggest that hatchery rearing environment has little effect on the tendency for hatchery steelhead fry to dominate naturally-reared fry at the relatively low densities (0.9-1.8 fry m^{-2}) used here.

Metcalfe et al. (2003) found that wild-reared juvenile Atlantic salmon tended to be dominant over hatchery reared fish from the same stock when tested in pairs in a laboratory stream. Our results suggest that the relative dominance of hatchery- and naturally-steelhead fry depends on fish density, with hatchery-reared fry being more likely to be dominant at higher densities. This may have important implications for supplementation stocking practices, as we discuss below.

Position

We observed no significant effects of rearing treatment combination, density, or their interaction on the upstream or crosswise position of dominant or subdominant steelhead fry in this experiment, suggesting that density or the presence of hatchery fry had little effect on the position choice of steelhead fry. Although we included velocity barriers in our flume sections in order to provide energetically profitable positions (Fausch 1984) near the food source, there was a high degree of variability in position choice of fry that may have hindered our ability to identify significant differences in position choice of fry among rearing treatment combinations.

Several authors have found hatchery fish to be distributed differently than wild fish in laboratory streams or aquaria (Dickson & MacCrimmon 1982; Mesa 1991; Deverill et al. 1999). We found no significant effects of fish type on the upstream or crosswise positions occupied by fry. A significant treatment by type interaction observed for upstream position, however, illustrates that natural fish tended to occupy positions closer to the food source than hatchery fish in the CN treatment. This effect was also detected when natural fish were analyzed separately; the rearing treatment of hatchery-reared fry appeared to affect the position of natural fry in the experimental flume sections when natural fry from the EN and CN treatments were considered separately, since a significant effect of rearing treatment was observed for upstream position of natural fry. At both densities, natural fry in the CN treatment combination occupied more upstream positions (closer to the food source) than natural fry in the EN treatment combination. This may indicate that fry reared in enriched and conventional hatchery environments chose different positions relative to naturally-reared fry.

Space use

Several studies provide evidence that the territory size of juvenile salmonids decreases with fish density (McNicol & Noakes 1984; Keeley & McPhail 1998; Keeley 2000), although others have found no relationship (Keeley & Grant 1995). Space use by dominant fry in this study was very similar (approximately 3,000 cm^2) at low and high density, and we found no significant effect of density on space use by dominant fish. Although space use was generally lower for subdominant fry at high density, we also observed no significant effect of density on space use by subdominant fry. We did observe a significant effect of density on space use in the three-way MANOVA analysis, which indicates that space use was lower in high density trials when all fish were included. When natural and hatchery-reared fish were considered separately, space use was lower at high density, but this was only significant for natural fish. These results suggest that the effect of density on space use may be stronger for natural than hatchery-reared fish, and that space use by dominant fry may not be strongly affected by density within the range of densities considered here.

We observed no significant effects of rearing treatment on space use in any of the analyses. In our experiment, space use by dominant fry was very similar among rearing treatments and between densities, and we observed no significant effects of either factor on space use by dominant fry. Berejikian et al. (2001) similarly found no difference in mean space use (territory size) among steelhead fry from natural, enriched, or conventional hatchery environments when the treatments were observed separately at 4.0 fry m^{-2}. These results suggest that rearing environment may have few effects on space use in steelhead fry.

Management implications

In a number of cases where hatchery salmonids were reported to be more aggressive than wild salmonids (Bachman 1984; Mesa 1991; Peery & Bjornn 1996; Deverill et al. 1999), hatchery fish were from different populations, and differences in aggression could have been a result of genetic factors and not rearing environment. Other studies have found increased aggression of hatchery compared to natural salmonids from different genetic backgrounds but reared in the same environments (Swain and Riddell 1990; Berejikian et al. 1996; Einum & Fleming 1997). The experiments reported here were designed to isolate environmental (rearing) and density-dependent effects and to remove potentially confounding genetic factors by evaluating juveniles from the same parent population reared in different environments. Because modern supplementation strategies call for the use of local native broodstocks and the minimization of genetic differences between wild and artificially-produced fish (Anders 1998; Flagg & Nash 1999), it is likely that in this respect the results from this study will be applicable to releases of steelhead from ‘conservation hatcheries’ (Flagg & Nash 1999).

Hatchery-reared salmonids and wild conspecifics are nearly ecologically identical (i.e., they share the same niche) and are therefore virtually certain to compete at some density (Weber & Fausch in review). If hatchery-reared fish are more aggressive and more likely to be dominant, however, the density at which competitive effects on wild fish become evident might be lower for combinations of wild and hatchery-reared fish than for wild fish alone. Fenderson & Carpenter (1971) observed a dramatic increase in nipping rates of hatchery-reared Atlantic salmon fry at 9.6 fry m^{-2} compared to 4.8 fry m^{-2}. Because the densities at which we observed fish were less than half of the lowest density used in other studies (Fenderson & Carpenter 1971; Berejikian et al. 2000; 2001), we may not have had the opportunity to observe the highest rates of aggression by hatchery-reared fry, although the densities that we used are within the range likely to be observed in Pacific Northwest streams in summer (Platts & McHenry 1988; Bjornn & Reiser 1991).

The density at which competitive effects between conspecific salmonids would be expected to be observed in the wild would depend on the carrying capacity (e.g., productivity, food availability, habitat quality) of the stream in question (e.g., Weiss & Schmutz 1999). The rate of food delivery used in our experiment was similar to the rate of natural drift observed in a stream in British Columbia (Keeley & McPhail 1998), and our results are therefore comparable to what might be expected in the wild with respect to food availability.

We observed few significant effects of rearing environment (natural, conventional, or enriched) on the feeding, aggression, position, or space use of steelhead fry at the low to moderate densities used here. The only variable that showed a significant effect of rearing treatment combination was the upstream position of naturally-reared fry (natural fry in the CN treatment were more likely to take positions closer to the upstream food source than those in the EN treatment). No effects of rearing treatment combination were observed for aggression, feeding, crosswise position, or space use. Therefore, with the exception of the effect on upstream position of natural fry, hatchery fish from both conventional and enriched environments appear to be similar in behavior to each other and to natural fish at the densities used here. Our results indicate that hatchery-reared steelhead fry stocked at low densities are unlikely to have effects on similar-size wild fish that are different from the effects of a similar increase in density of wild fish, suggesting that hatchery-reared fry could be stocked at densities approaching the carrying capacity of a stream with relatively low risk to wild fish. Our results also suggest that fry from conventional and enriched rearing environments are unlikely to have differential effects on natural fry at low densities.

The results of our experiments may not apply directly to conservation releases of hatchery-reared fish into natural streams for several reasons. We did not allow natural fish to acclimate in the flume before introducing hatchery fish, and natural fish therefore did not enjoy prior residence as they probably would in the wild. Prior residence may give juvenile salmonids an advantage in foraging and territorial disputes (Huntingford & Garcia de Leaniz 1997; Deverill et al. 1999; Johnsson et al. 1999; O'Connor et al. 2000; Metcalfe et al. 2003), and wild steelhead fry in streams might be expected to fare better than natural fry in these experiments. Conversely, hatchery fish that are released into streams are usually larger than wild conspecifics, and larger body size might be expected to give them an advantage compared to the results (from size-matched fish) presented here. Modern conservation hatcheries, however, might be expected to release fish at a size more similar to wild salmonids (Flagg & Nash 1999).

Although steelhead fry have been released into streams for research purposes (Hume & Parkinson 1987; 1988; Close & Anderson 1992), fry releases are not a common hatchery practice in the Pacific Northwest. For example, of 71 Columbia River basin salmonid supplementation projects reviewed by BPA (1992), only 6 (8%) used releases of fry or parr. This is presumably due to the fact that larger fish generally have higher survival rates in the wild (Hume & Parkinson 1988; Brown & Day 2002; Miyakoshi et al. 2003), but this is not always the case (Borgstrøm et al. 2002) and we are aware of no published experiments that have quantified the survival advantage associated with release strategies (e.g., fry, pre-smolt and smolt) for juvenile steelhead. Some studies suggest that survival rates of steelhead fry stocked at low density may be similar to wild salmonid fry survival, particularly when wild fish densities are low (Hume & Parkinson 1987; Rhodes & Quinn 1999). Our results suggest that steelhead fry released at a similar size to wild conspecifics and at low density may have similar effects on the ecology of wild fry as the same number of wild fry. Further research should be conducted to determine if steelhead fry release is a viable strategy for supplementing imperiled wild steelhead stocks while minimizing effects on wild steelhead fry.

Summary

1. Juvenile steelhead *Oncorhynchus mykiss* from the same genetic stock were reared in a conventional hatchery environment, an enriched hatchery environment (with cover, structure, and underwater feeders), and a natural stream channel. We quantified the aggression, feeding, dominance, position, and space use of naturally-reared steelhead fry stocked with the two types of hatchery-reared fry in a laboratory stream at two densities in order to determine how rearing environment and fish density affect the behavior of steelhead fry in an experimental flume.

2. Rates of threats and attacks by steelhead fry were higher at high density, but there were no effects of rearing treatment on aggression. The overall feeding rate was higher at low density, but there were no significant effects of rearing treatment on feeding rate. Naturally-reared fry were dominant more often at low density, while hatchery-reared fry were more often dominant at high density; there was no effect of hatchery rearing treatment on dominance. The only significant effect of rearing treatment that we observed was on the upstream position of naturally-reared fry, which occupied more upstream positions when stocked with conventional than enriched hatchery-reared fry. There were no effects of rearing treatment on space use.

3. Our results indicate that hatchery-reared steelhead fry stocked at low densities may have effects on similar-size wild fish that are comparable to the effects of a similar increase in density of wild fish. Rearing environment had relatively little influence on the behavior of steelhead fry at low to moderate densities; the density at which hatchery fry are released may be more important in this respect than rearing environment. Releasing steelhead fry similar in size to wild fish at low densities may be a supplementation strategy that entails few risks to wild steelhead populations.

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Table 1. Summary of data analyses performed. Variables: A = attacks, T = threats, F = Feeding, X = crosswise position, Y = upstream position, S = space use, PF = proportion of fish feeding, PE = proportion of food eaten. Factors: Trt = rearing treatment, Dens = Density, Type = fish type (natural or hatchery).

Analysis type	Variables	Treatments	Rearing types	Dominance status	Factors	Results in Table
MANOVA	A, T, F, PF, PE	All	All	All	Trt, Dens	2
MANOVA	A, T, F, X, Y, S	All	All	Dominant	Trt, Dens	3
MANOVA	A, T, F, X, Y, S	All	All	Subdominant	Trt, Dens	3
Log linear models	Dominance	EN, CN	All	Dominant	Trt, Dens, Type	4
MANOVA	A, T, F, X, Y, S	EN, CN	All	All	Trt, Dens, Type	5
MANOVA	A, T, F, X, Y, S	EN, CN	E, C	All	Trt, Dens	6
MANOVA	A, T, F, X, Y, S	EN, CN	N	All	Trt, Dens	6

Table 2. Feeding and aggressive behavior of steelhead fry from three rearing treatment combinations (see text) at two densities in experimental trials in sections of a laboratory flume. Significance levels (P) of two-way MANOVA tests between densities and among treatment combinations are shown to the right. Treatment = rearing treatment combination. Model comparisons are df 5, 114; density comparisons are df 1, 114; treatment and interaction comparisons are df 2, 114.

	Low Density			High Density			P			
Variable	NN	CN	EN	NN	CN	EN	MODEL	TREATMENT	DENSITY	INTERACTION
Threats per fish	1.65	1.08	0.98	1.91	1.70	1.26	0.0487	0.2380	0.0054	0.7414
Attacks per fish	1.93	3.00	1.65	3.58	4.70	2.01	0.0229	0.2230	0.0016	0.9047
Feeding strikes per fish	4.26	4.35	4.11	1.13	1.20	1.08	0.0001	0.0802	0.0001	0.6910
Proportion of fish feeding	0.78	0.70	0.78	0.54	0.48	0.46	0.0001	0.4432	0.0001	0.6453
Proportion of food eaten	0.86	0.86	0.83	0.92	0.97	0.89	0.0148	0.1159	0.0035	0.4590

Table 3. Aggression, feeding, position and space use of dominant and subordinate steelhead fry from three rearing treatment combinations at two densities in sections of a laboratory flume. The significance levels (P) of two-way ANOVA tests among rearing treatment combinations and between densities appear to the right. Treatment = rearing treatment combination. All model tests are df 5, 114; treatment are 2, 114; density are 1, 114; interaction are 2, 114. Upstream position is cm from downstream end; crosswise position is cm from left side.

	Low Density			High Density			P			
Variable	NN	CN	EN	NN	CN	EN	MODEL	TREATMENT	DENSITY	INTERACTION
					Dominant fry					
Threats	1.9	1.9	1.4	3.0	2.3	1.8	0.2560	0.3333	0.0439	0.8670
Attacks	2.2	4.4	2.2	7.8	7.9	3.6	0.0162	0.2597	0.0009	0.9391
Feeding strikes	15.2	15.3	14.4	14.0	16.4	14.2	0.4209	0.1924	0.8893	0.4435
Upstream position	54.1	65.9	61.0	57.3	78.3	61.8	0.7358	0.2968	0.7166	0.9155
Crosswise position	52.0	52.4	54.2	51.0	43.8	61.4	0.1252	0.0694	0.9216	0.1907
Space use (cm^2)	2896	3018	3272	3377	3088	2820	0.9415	0.7996	0.7631	0.7103
					Subdominant fry					
Threats	1.4	0.3	0.6	2.6	2.7	1.7	0.0001	0.0605	0.0001	0.4409
Attacks	1.7	1.6	1.2	5.0	7.9	2.6	0.0125	0.4911	0.0004	0.7897
Feeding strikes	1.9	2.2	2.1	3.3	2.2	2.8	0.6077	0.6712	0.1199	0.8366
Upstream position	30.5	55.9	27.3	33.0	32.3	30.9	0.1926	0.0856	0.8426	0.2934
Crosswise position	63.8	84.6	72.6	61.2	84.1	64.0	0.3699	0.0847	0.5548	0.9741
Space use (cm^2)	3836	3778	1359	3046	2318	1629	0.3999	0.1189	0.4072	0.9286

Table 4. Rearing type (natural or hatchery) of dominant steelhead fry in experimental trials in laboratory flume sections.

		Dominant fish	
Treatment	Density	Natural	Hatchery
Conventional	Low	14	6
	High	9	11
Enriched	Low	12	8
	High	7	13

Table 5. Aggression, feeding, position and space use of hatchery- and naturally-reared steelhead fry from three rearing treatment combinations at two densities in sections of a laboratory flume. The significance levels (P) of three-way ANOVA tests among rearing treatment combinations, between densities, and between fish types appear to the right. Model tests are df 7, 232; all others are 1, 232.

	Low Density				High Density				P							
	CN		EN		CN		EN									
	Natural	Hatchery	Natural	Hatchery	Natural	Hatchery	Natural	Hatchery	Model	Treatment	Density	Type	Tr*D	Tr*T	D*T	Tr*D*T
Threats	1.7	0.5	1.4	0.6	1.4	2.0	1.4	1.2	0.1144	0.7135	0.0154	0.3909	0.1238	0.2971	0.2432	0.7713
Attacks	4.4	1.6	1.6	1.7	1.3	8.0	2.4	1.6	0.2978	0.4065	0.1051	0.3136	0.5445	0.0981	0.6819	0.3668
Feeding strikes	11.0	6.5	8.9	7.6	3.7	6.0	3.5	5.1	0.0006	0.7717	0.0001	0.5714	0.7818	0.5748	0.0180	0.2885
Upstream position	70.3	51.6	40.9	47.4	66.7	40.1	44.1	50.5	0.1302	0.0761	0.2838	0.2615	0.3907	0.0298	0.8530	0.6576
Crosswise position	62.2	74.8	56.6	70.2	79.1	66.3	70.9	60.4	0.8055	0.2749	0.9158	0.7037	0.9974	0.8869	0.1318	0.7505
Space use (cm^2)	2726	4069	2240	2391	1823	2315	1096	1688	0.0783	0.1296	0.0040	0.4187	0.3885	0.4342	0.6620	0.9941

Table 6. Aggression, feeding, position and space use of hatchery- and naturally-reared steelhead fry from three rearing treatment combinations at two densities in sections of a laboratory flume. The significance levels (P) of two-way ANOVA tests among rearing treatment combinations and between densities appear to the right. Treatment = rearing treatment combination. Model tests are df 3, 116; all others are 1, 116. Upstream position is cm from downstream end; crosswise position is cm from left side.

	Low density		High density		P			
Variable	CN	EN	CN	EN	MODEL	TREATMENT	DENSITY	INTERACTION
				Hatchery fry				
Threats	0.5	0.6	2.0	1.2	0.0242	0.3056	0.0097	0.1838
Attacks	1.6	1.7	8.0	1.6	0.1157	0.0875	0.1617	0.2984
Feeding strikes	6.5	7.6	6.0	5.1	0.4053	0.5665	0.1838	0.3676
Upstream position	51.6	47.4	40.1	50.5	0.6275	0.7739	0.3717	0.3554
Crosswise position	74.8	70.2	66.3	60.4	0.6709	0.4935	0.3123	0.8206
Space use (cm^2)	4069	2391	2315	1688	0.1316	0.1163	0.0928	0.5509
				Natural fry				
Threats	1.7	1.4	1.4	1.4	0.6328	0.6423	0.3838	0.3906
Attacks	4.4	1.6	1.3	2.4	0.7572	0.5493	0.3801	0.8299
Feeding strikes	11.0	8.9	3.7	3.5	0.0001	0.8402	0.0001	0.5590
Upstream position	70.3	40.9	66.7	44.1	0.0460	0.0062	0.5335	0.7705
Crosswise position	62.2	56.6	79.1	70.9	0.5648	0.3927	0.2639	0.8239
Space use (cm^2)	2726	2240	1823	1096	0.0905	0.5914	0.0162	0.5332

Section 4

Growth and survival of hatchery- and naturally-reared steelhead fry in a semi-natural stream channel[1]

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Abstract

Steelhead *Oncorhynchus mykiss* fry reared under natural conditions were introduced into a stream channel alone and with fry reared in conventional and enriched hatchery environments in 2002 and 2003 to determine whether the growth and survival of naturally-reared steelhead were affected differentially by the introduction of fry from different rearing environments. There were no significant differences in survival or growth in length or weight among rearing treatment combinations. Significantly higher survival of fry reared in an enriched hatchery environment compared to those reared naturally was observed in 2002 but not 2003; no differences in survival between fry reared in conventional hatchery environment and those reared naturally were found in either year. Naturally-reared fry grew faster than fry reared in a conventional hatchery environment in 2002, but the opposite was true in 2003. No differences in growth were observed between fry reared naturally and those reared in an enriched hatchery environment. Fry from the enriched rearing treatment survived at a higher rate than fry from the conventional rearing treatment in 2002 but not in 2003. There were no significant differences in the growth of hatchery- or naturally-reared fry from the rearing treatment combinations that included hatchery-reared fry in either year. These results suggest that stocking hatchery-reared steelhead fry at moderate densities may have similar effects on fry growth and survival as stocking the same number of naturally-reared fry, and that there were no consistent significant differences in growth or survival between steelhead fry reared in a conventional or enriched hatchery environment or between hatchery- and naturally-reared steelhead fry.

Introduction

Supplementation programs for salmonids are designed to use hatchery-reared fish to assist in the recovery of imperiled salmonid populations. Although salmonid supplementation programs are common, particularly in the Columbia River basin (ISAB 2003), there is limited evidence that these programs have been successful (Winton and Hilborn 1994; Waples et al. 2003) and few studies have assessed ecological risks to wild populations associated with stocking of juvenile salmonids in rivers (ISAB 2003). Recent studies suggest that hatchery-reared fish may affect the survival and production of wild salmonid populations (Levin and Williams 2002; Nickelson 2003), and previous work has indicated that hatchery-reared fish may affect wild salmonids in streams through competitive interactions (Bachman 1984; Nielsen 1994; McMichael et al. 1999).

It has been suggested that hatchery fish reared in environments that are more similar to natural stream environments may be more suitable for use in supplementation programs (Stickney 1994; Flagg et al. 2000; Brown and Day 2002). There is some evidence that structural modifications to hatchery rearing environments may increase the survival or predator avoidance of juvenile salmonids (Bams 1967; Leon 1975; Fuss & Johnson 1988; Maynard et al. 1995), but results are equivocal (Maynard et al. 1996). Recent work has shown that enriched rearing environments may affect the agonistic behavior, dominance, growth, and space use of steelhead fry (Berejikian et al. 2000; 2001), although other studies have shown little effect of enriched rearing environments on steelhead fry behavior (Riley et al. in review).

The experiments reported here were conducted to determine whether the growth and survival of naturally-reared steelhead fry were affected differentially by the introduction of fry from hatchery (conventional and enriched) or natural rearing environments, and to determine if the survival or growth of hatchery-reared steelhead fry differed from natural fry, and if the survival and growth of fry from enriched hatchery environments were different from those of fry reared in conventional hatchery environments.

Methods

Rearing treatments

Steelhead eggs obtained from artificially spawned steelhead from the Skookumchuck River, a tributary of the Chehalis River, were initially incubated at the Washington Department of Fish and Wildlife's Bingham Creek Hatchery in spring 2002 and 2003. Eyed embryos were transported to the University of Washington's Big Beef Creek Research Station (Kitsap County, WA) for final incubation in 10^{o}C well water.

After emergence, steelhead fry were reared in conventional and enriched circular tanks and in a semi-natural stream channel using slightly different methods in 2002 and 2003. In 2002, 1667 emergent fry were stocked into each of twelve 1.2-m diameter circular tanks on 21 May; 2500 fry were stocked into each of six of the same tanks on 12 May 2003. In each year, half of the tanks (6 in 2002, 3 in 2003) were conventional tanks that contained no cover or structure other than a standpipe. The other tanks were 'enriched' and contained the tops of two fir trees to provide structure, had military camouflage netting on top, and had an underwater food-delivery system which distributed commercial food pellets from automatic feeders through two 2.5-cm diameter nylon tubes near mid-water depth (four outlets per tank). Fish in the conventional tanks were fed by automatic feeders which scattered the pellets across the water surface. In 2003, two 34 X 48 X 15 cm plastic and metal shopping baskets filled with cobbles were added to each enriched tank. All tanks received a constant flow of 40 l/min of well water. Fluorescent lights controlled by a timer mimicked the natural photoperiod.

When fry were stocked into the rearing tanks each year, 5,000 fry were simultaneously released into a screened-off section of a side channel of Big Beef Creek; these are referred to as naturally-reared, or natural, fry. The 35-m long channel received approximately 0.05 m^3/s flow from springs and the main channel of Big Beef Creek, and was augmented with 0.025 m^3/s well water. The channel had a gravel-cobble substrate and natural early successional riparian vegetation. Woody debris was added to provide structure and cover from predators. Steelhead fry fed on natural food produced within the channel.

Experimental facility

This experiment was conducted in a 45-m long by 6-m wide outdoor stream channel previously described by Berejikian et al. (2000). Well water was recirculated through the channel at approximately 6,800 L/min, and 350 L/min of channel water was continuously routed through a chiller to reduce temperature. The substrate consisted of 2 – 10 cm diameter gravel graded to create similar depth and velocity profiles among replicate channel sections. The channel supported abundant aquatic insect populations which provided a natural food source for fry. Avian predators were excluded by a net placed over the entire channel. Water temperatures in the channel ranged from 10.6-15.7^{o}C in 2002 and from 11.0-19.7^{o}C in 2003.

In 2002, 16 replicate 5 by 3 m sections were created in the stream channel using a wooden barrier which divided the channel along its entire length, and with seven wire mesh (3 mm) screens set on top of weirs situated across the channel perpendicular to the flow. In 2003, 32 replicate 5 by 1.5 m sections were created by further subdividing the 2002 sections longitudinally down the middle using plywood boards secured into the substrate, with vexar attached to the top of each board. All screens and dividers were sealed to prevent fry from moving among sections. Two small boulders and the denuded tops of two Douglas Fir trees were added to each section to provide cover for fry.

Experimental protocol

In both years, natural fry were stocked into the channel alone and in combination with steelhead fry reared in enriched and conventional environments. On 23 July 2002, 14 natural fry were stocked into each of 15 channel sections simultaneously with 14 fry from natural, conventional, or enriched rearing environments; these are hereafter referred to as NN, CN, and EN rearing treatment combinations, respectively (five replicate sections per rearing treatment combination, 28 fry per section, 1.87 fry/m^2). On 10 July 2003, ten natural fry were stocked with ten fry from natural, conventional, or enriched rearing environments into each of 30 channel sections (ten sections per rearing treatment combination, 20 fry per section, 2.67 fry/m^2). In both years, two yearling steelhead (205-237 mm fork length) reared in enriched environments were simultaneously stocked into all sections as predators. All fry were measured (fork length, nearest mm) and weighed (nearest 0.1 gram) before introduction, and half of the fry in each section were marked by removal of the adipose fin for later identification; for mixed treatments (EN and CN), the rearing treatment that was marked was chosen randomly. Rearing treatment combinations were balanced among stream channel sections. In 2002, fry averaged 54.7 mm (range 39 – 70) in length and 1.77 g (0.71-3.44) in weight at stocking; in 2003, fry averaged 49.3 mm (range 46 – 55) and 1.25 g (1.01 – 1.70) g.

All steelhead fry were removed from stream channel sections on 1-3 October 2002 and 5-7 August 2003. In both years, fish were captured by multiple seine hauls in each section on the first day, followed by multiple electrofishing passes on the second and third days. All fry were measured and weighed (as above) upon removal.

The survival of steelhead fry was estimated by expressing the number of fry of each type (N, C, or E) removed from each section as a proportion of the total number stocked. Instantaneous growth in length (IGL) of fry in each section was estimated as:

$$(\log(L1) - \log(L2))/t$$

and instantaneous growth in weight (IGW) as:

$$(\log(W1) - \log(W2))/t$$

where: L1 = mean length at stocking, L2 = mean length at removal, W1 = mean weight at stocking, W2 = mean weight at removal, t = time (days) between stocking and removal.

Data analysis

A substitutive design (Fausch 1998) was used to compare the effects of adding fry reared in conventional or enriched hatchery environments on the survival and growth of groups of steelhead fry to the effects of adding equal numbers of naturally-reared fry. The hypothesis tested was that the survival and growth of steelhead fry were not different among the three treatment combinations (NN, EN, CN). Arcsine-transformed survival data were analyzed using a balanced one-way analysis of variance (ANOVA) design with rearing treatment combination as the experimental factor, while growth data (IGL and IGW) were analysed using a one-way analysis of covariance (ANCOVA) with initial length (IGL) or weight (IGW) as covariates.

The analysis described above allows one to determine if differences exist among rearing treatment combinations, but does not provide direct comparisons between hatchery and natural fry or enriched and conventional fry. In order to explore these comparisons, several analyses were performed using only the treatment combinations with hatchery fry (EN and CN). First, the null hypothesis that the survival of hatchery- and naturally-reared steelhead fry was not significantly different was tested by performing several one-way ANOVA analyses within each treatment combination (EN, CN). Second, the hypothesis that the growth (IGL, IGW) of hatchery- and naturally-reared fry were not significantly different was tested by performing ANCOVA (with rearing treatment as the main effect and initial length or weight as the covariate) within each treatment combination.

Several one-way ANOVA analyses within each fish type (natural or hatchery) were used to test the hypothesis that steelhead fry survival was not significantly different between rearing types (enriched or conventional), and then ANCOVA (as above) was used to test the hypothesis that fry growth was not significantly different between rearing types.

Results

Differences among rearing treatment combinations

There were no significant effects of adipose fin removal on steelhead fry survival (2002: $F_{1, 29} = 0.24$, $P = 0.6313$; 2003: $F_{1, 59} = 1.31$, $P = 0.2592$, by one-way ANOVA) or growth rate in terms of length (IGL – 2002: $F_{1, 29} = 0.39$, $P = 0.5405$; 2003: $F_{1, 59} = 0.73$, $P = 0.3975$) or weight (IGW – 2002: $F_{1, 29} = 0.29$, $P = 0.5962$; 2003: $F_{1, 59} = 0.94$, $P = 0.3392$) in the stream channel in either year. Survival was not significantly correlated with IGL (2002: $r = 0.207$, $P = 0.4779$; 2003: $r = -0.266$, $P = 0.1639$) or IGW (2002: $r = 0.426$, $P = 0.1293$; 2003: $r = -0.171$, $P = 0.3754$) in either year.

The highest proportion of fry survived in the EN rearing treatment combination in 2002 and in the NN in 2003 (Table 1). There were no significant differences in the proportion of fish surviving among treatment combinations in 2002 ($F_{2, 14} = 3.03$, $P=0.0862$) or 2003 ($F_{2, 29} = 1.26$, $P = 0.3002$). Significant effects of initial length on IGL were observed in both years (2002: $F_{1, 14} = 9.32$, $P = 0.0110$; 2003: $F_{1, 29} = 7.61$, $P = 0.0105$), but no significant differences in the slope of the initial length vs. IGL relationship were observed among rearing treatment combinations in either year (2002: $F_{2, 14} = 0.21$, $P = 0.8152$; 2003: $F_{2, 29} = 2.35$, $P = 0.9502$). There were no significant differences in IGL among rearing treatment combinations in either year (2002: $F_{2, 14} = 0.35$, $P = 0.7139$; 2003: $F_{2, 29} = 2.48$, $P = 0.1036$). Growth rates were very similar among rearing treatment combinations in 2002; growth in the NN treatment combination was lower than in the others in 2003, but not significantly so (Table 1).

A significant effect of initial weight on IGW was observed in 2002 ($F_{1, 14} = 13.10$, $P = 0.0040$) but not in 2003 ($F_{1, 29} = 0.68$, $P = 0.4182$). There were no significant differences in the slope of the initial weight vs. IGW relationship among rearing treatment combinations in either year (2002: $F_{2, 14} = 0.20$, $P = 0.9522$; 2003: $F_{2, 29} = 0.54$, $P = 0.5878$). There were no significant differences in IGW among rearing treatment combinations in either year (2002: $F_{2, 14} = 0.06$, $P = 0.9443$; 2003: $F_{2, 29} = 2.86$, $P = 0.0771$).

Hatchery- vs. naturally-reared fry

Hatchery-reared steelhead fry survived at a significantly higher rate than naturally-reared fry in the EN treatment combination in 2002 ($F_{1, 9} = 6.80$, $P = 0.0312$), but not in 2003 ($F_{1, 19} = 1.14$, $P = 0.2993$). No differences in survival between hatchery- and naturally-reared fry were found in the CN treatment combination in either year (2002: $F_{1, 9} = 0.33$, $P = 0.5807$; 2003, $F_{1, 19} = 1.1$, $P = 0.3078$; Table 2).

Naturally-reared fry in the CN treatment combination grew faster in weight (but not in length) than hatchery-reared fry in 2002 (Tables 2 and 3). In 2003, however, hatchery-reared fry in the CN treatment combination grew faster than naturally-reared fry in terms of length, but not weight. No differences in growth in terms of length or weight were observed between hatchery- and naturally-reared fry in the EN treatment combination in either year (Tables 2 and 3).

EN vs. CN

Hatchery-reared steelhead fry from the enriched rearing treatment survived at a higher rate than hatchery-reared fry from the conventional rearing treatment in 2002 ($F_{1, 9} = 6.63$, $P =$

0.0328), but not in 2003 ($F_{1, 19} = 0.87$, P = 0.3620; Table 2). There was no significant difference in survival of naturally-reared fry from enriched and conventional environments in either year (2002: $F_{1, 9} = 0.03$, P = 0.8627; 2003: $F_{1, 19} = 0.19$, P = 0.6644; Table 2). There were no significant differences in the growth of hatchery- or naturally-reared from the EN and CN treatment combinations in either year (Tables 2 and 4).

Discussion

The ecological risks associated with the stocking of salmonids for conservation purposes must be understood before salmonid supplementation programs can be properly evaluated (ISAB 2003). If hatchery-reared salmonids have ecologically significant negative effects on wild salmonids in streams, one would expect to see reduced survival or growth of wild salmonids in streams where hatchery fish are stocked. In this experiment, the potential for such effects on growth and survival was assessed using hatchery- and naturally-reared steelhead fry in an artificial stream channel.

No statistically significant differences in growth or survival of mixed groups of naturally- and hatchery-reared steelhead fry were observed in the stream channel in either year. These results suggest that stocking hatchery-reared steelhead fry at moderate densities may have similar ecological effects as adding similar numbers of wild fish, and are in agreement with the results of previous studies that have found no effect of stocked hatchery fish on wild fish growth (Petrosky and Bjornn 1988; Bohlin et al. 2002). McMichael et al (1997), however, found that the growth of wild rainbow trout in stream enclosures was reduced in the presence of hatchery-reared steelhead, but the effect was confounded by increased density in the enclosures with hatchery fish. Because salmonid growth may often be density-dependent (Jenkins et al. 1999; Bohlin et al. 2002), the release of hatchery-reared fish at high densities might be expected to result in decreased growth of wild and hatchery fish in streams. The densities used in the stream channel were within the range observed for wild salmonids in Pacific Northwest streams (Platts & McHenry 1988; Bjornn & Reiser 1991). The results of this study suggest that stocking hatchery-reared steelhead fry at these natural densities may not have significant effects on the growth or survival of wild fry in streams.

Previous work has indicated that juvenile Chinook salmon reared in enriched environments may have higher survival rates in streams than those reared in conventional environments (Maynard et al. 1995; 1996), but no previous studies have examined the effect of enriched rearing environments on steelhead fry survival. In the present study, steelhead fry reared in an enriched environment survived at a significantly higher rate than those reared in a conventional hatchery environment in 2002, but not in 2003. These results indicate that enriched rearing may have benefits for steelhead fry survival, but also suggests that these benefits may only accrue under specific circumstances. For example, Maynard et al. (1996) suggest that a survival advantage to Chinook fry reared in enriched environments may not occur when water is turbid.

No differences in growth were observed between steelhead fry reared in enriched and conventional hatchery environments in either year. Berejikian et al. (2001) similarly found no difference in growth between steelhead fry reared in conventional and enriched hatchery environments when they were stocked into the same stream channel with naturally-reared fry at a density of 3.2 fry/m^2. Berejikian et al. (2000), however, again using the same stream channel, found that steelhead fry reared in an enriched hatchery environment grew faster than those from a conventional environment when the two treatments were stocked together at 2.8 fry/m^2, but not when they were stocked separately. Taken together, these results suggest that steelhead fry reared in enriched hatchery environments may have a growth advantage in some situations but not in others. Further research is necessary to determine the conditions under which growth or survival benefits are associated with enriched hatchery rearing of steelhead fry.

Although it is a common perception that hatchery-reared salmonids may perform poorly in terms of growth and survival in natural streams (Miller 1954; Mason et al. 1967; Wiley et al. 1993; Bohlin et al. 2002; Borgstrøm et al. 2002), some authors have documented higher survival or growth of hatchery than wild fish or no difference between the two (Adelman and Bingham 1955; Symons 1969; Rhodes and Quinn 1999; Reinhardt et al. 2001). Hatchery-reared fry in the EN rearing treatment combination survived at a significantly higher rate than natural fry in 2002, but no difference was observed in 2003 or in the CN combination in either year. Hatchery-reared fry in the CN treatment combination grew significantly faster than natural fry in 2003, but the opposite was true in 2002, and no differences in growth of hatchery and natural fry were observed in the EN treatment combination in either year. These results therefore suggest that there is no consistent survival or growth advantage for hatchery- or naturally-reared steelhead fry under the semi-natural conditions in the stream channel used here.

In summary, stocking hatchery-reared steelhead fry into a semi-natural stream channel at moderate densities had similar effects on the growth and survival of naturally-reared fry to stocking the same number of naturally-reared fry, and there were no consistent significant differences in growth or survival between steelhead fry reared in a conventional or enriched hatchery environment or between hatchery- and naturally- reared steelhead fry. These results suggest that stocking hatchery-reared steelhead fry at moderate densities may be a supplementation strategy that poses few risks to wild steelhead fry in streams, and that growth or survival benefits from enriched rearing may not always occur and may be dependent on variables that are currently incompletely understood.

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Table 1. Survival and instantaneous growth in length (IGL) and weight (IGW) of steelhead fry from three rearing treatment combinations in a semi-natural stream channel in summer 2002 and 2003. n = 5 for all treatment combinations in 2002; n=10 in 2003. SE = standard error. DF for 2002 tests = 2, 14; DF for 2003 tests = 2, 29.

Treatment combination	Proportion surviving		IGW		IGL	
	Mean	SE	Mean	SE	Mean	SE
			2002			
NN	0.72	0.06	0.0103	0.0005	0.0029	0.0002
EN	0.88	0.09	0.0106	0.0010	0.0032	0.0003
CN	0.79	0.04	0.0103	0.0011	0.0030	0.0003
			2003			
NN	0.84	0.03	0.0009	0.0019	0.0018	0.0005
EN	0.75	0.03	0.0058	0.0012	0.0027	0.0003
CN	0.77	0.05	0.0042	0.0010	0.0022	0.0002

Table 2. Survival and instantaneous growth in length (IGL) and weight (IGW) of hatchery- and naturally-reared steelhead fry from two rearing treatment combinations in sections of a semi-natural stream channel in summer 2002 and 2003. n = 5 for all treatment combinations in 2002; n = 10 in 2003. SE = standard error.

	Survival		IGL		IGW	
Treatment combination	Hatchery Mean (SE)	Natural Mean (SE)	Hatchery Mean (SE)	Natural Mean (SE)	Hatchery Mean (SE)	Natural Mean (SE)
2002						
EN	0.97 (0.04)	0.78 (0.07)	0.0030 (0.0003)	0.0034 (0.0002)	0.0095 (0.0012)	0.0115 (0.0009)
CN	0.81 (0.06)	0.77 (0.05)	0.0028 (0.0002)	0.0034 (0.0004)	0.0091 (0.0009)	0.0119 (0.0014)
2003						
EN	0.79 (0.03)	0.70 (0.05)	0.0029 (0.0004)	0.0024 (0.0004)	0.0068 (0.0010)	0.0041 (0.0021)
CN	0.82 (0.06)	0.72 (0.07)	0.0027 (0.0003)	0.0015 (0.0003)	0.0058 (0.0017)	0.0011 (0.0009)

Table 3. ANCOVA results for comparisons of instantaneous growth in length (IGL) and weight (IGW) between hatchery- and naturally-reared steelhead fry in a semi-natural stream channel in summer 2002 and 2003. df = degrees of freedom, F = F statistic.

Variable	Rearing treatment combination	Year	Effect	df	F	p
IGL	EN	2002	initial length	1, 9	0.19	0.6735
			slope	1, 9	2.18	0.1904
			treatment	1, 9	0.90	0.3756
IGL	CN	2002	initial length	1, 9	5.11	0.0583
			slope	1, 9	2.33	0.1777
			treatment	1, 9	3.17	0.1184
IGL	EN	2003	initial length	1, 19	8.73	**0.0089**
			slope	1, 19	0.05	0.8338
			treatment	1, 19	1.49	0.2385
IGL	CN	2003	initial length	1, 19	4.72	**0.0442**
			slope	1, 19	0.39	0.5390
			treatment	1, 19	5.53	**0.0311**
IGW	EN	2002	initial weight	1, 9	2.07	0.1935
			slope	1, 9	0.42	0.5432
			treatment	1, 9	1.74	0.2292
IGW	CN	2002	initial weight	1, 9	8.94	**0.0202**
			slope	1, 9	1.44	0.2747
			treatment	1, 9	7.79	**0.0268**
IGW	EN	2003	initial weight	1, 19	2.84	0.1104
			slope	1, 19	0.01	0.9520
			treatment	1, 19	1.73	0.2061
IGW	CN	2003	initial weight	1, 19	0.94	0.3462
			slope	1, 19	3.44	0.0822
			treatment	1, 19	4.43	0.0506

Table 4. ANCOVA results for comparisons of instantaneous growth in length (IGL) and weight (IGW) between steelhead fry reared in enriched and conventional hatchery environments and naturally-reared steelhead fry stocked with the two treatments in a semi-natural stream channel in summer 2002 and 2003. df = degrees of freedom, F = F statistic.

Variable	Rearing type	Year	Effect	df	F	p
IGL	Hatchery	2002	initial length	1, 9	3.59	0.0998
			slope	1, 9	0.65	0.4493
			treatment	1, 9	2.57	0.0998
IGL	Natural	2002	initial length	1, 9	1.58	0.2487
			slope	1, 9	3.69	0.1030
			treatment	1, 9	0.66	0.4445
IGL	Hatchery	2003	initial length	1, 19	6.80	**0.0184**
			slope	1, 19	0.16	0.6943
			treatment	1, 19	0.30	0.5904
IGL	Natural	2003	initial length	1, 19	7.20	**0.0157**
			slope	1, 19	0.02	0.8860
			treatment	1, 19	1.91	0.1843
IGW	Hatchery	2002	initial weight	1, 9	5.47	0.0519
			slope	1, 9	0.84	0.3959
			treatment	1, 9	2.42	0.1635
IGW	Natural	2002	initial weight	1, 9	4.84	0.0637
			slope	1, 9	0.52	0.4993
			treatment	1, 9	0.67	0.4406
IGW	Hatchery	2003	initial weight	1, 19	6.85	**0.0180**
			slope	1, 19	0.03	0.8669
			treatment	1, 19	1.13	0.3022
IGW	Natural	2003	initial weight	1, 19	1.36	0.2604
			slope	1, 19	1.52	0.2361
			treatment	1, 19	0.10	0.7520

Section 5

EXTENDED ABSTRACT – do not cite

Aggression and feeding of hatchery- and naturally-reared steelhead fry in a laboratory flume and natural streams[1]

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Extended abstract

Hatchery-reared salmonids have been observed to be more aggressive than wild conspecifics, and increased aggression by hatchery-reared fish might be expected to result in negative effects on wild fish in streams where hatchery-reared fish are released. In 2002, we quantified the aggression of naturally-reared steelhead fry stocked into a laboratory flume with hatchery-reared fry from conventional and enriched rearing environments at three densities in the presence and absence of predators (yearling steelhead) in order to determine the effects of density and predation risk on the agonistic behavior of fry from the three rearing environments. We also compared the level of aggression of steelhead fry observed in the flume with that observed in two natural streams.

Steelhead fry were reared in 'conventional' (standard circular tanks), 'enriched' (the same tanks outfitted with structure, overhead cover, and an underwater feeding system), and 'natural' (an outdoor stream channel with natural substrate and food) rearing environments. The three rearing treatments were combined such that natural fry were size-matched with fry from the natural, conventional and enriched environments and introduced into a section of an experimental flume for behavioral observations. Rearing treatment combinations are referred to as CN (conventional and natural), EN (enriched and natural), and NN (natural only), and were designed to compare the effects of introducing fish from the two hatchery rearing environments to the effects of adding the same number of natural fish over a range of densities (1.8 – 7.1 fry/m^2) in the presence and absence of predators. The number of attacks (nips, charges, and chases) and threats (lateral and frontal displays) initiated by each fry were recorded in each flume section for ten minutes. We also estimated the frequency of aggressive interactions by fry from the two hatchery rearing treatments that were stocked into two natural streams.

The rate of attacks by steelhead fry increased with fry density and was reduced in the presence of yearling steelhead, but was not affected by rearing treatment (Table 1). The rate of threat displays also increased with density but was not significantly affected by yearling presence. Differences among rearing treatments in threat rate were apparent, but were confounded by a significant density by yearling interaction. Our results suggest that there was no consistent difference in aggression among the three rearing treatment combinations. The rate of aggression by steelhead fry from the two hatchery rearing treatments was lower in two natural streams than in the laboratory, did not increase with density, and was not significantly greater than that of wild steelhead fry in the streams.

Previous work has provided equivocal evidence regarding the effects of rearing environment on the aggressive behavior of steelhead fry (Berejikian et al. 2000; 2001). Berejikian et al. (2000) observed no difference in the aggressive behavior of steelhead fry reared in conventional and enriched hatchery environments when the treatments were stocked together at 10.7 fry/m^2, but observed that conventionally-reared fry delivered significantly fewer threat displays than enriched or natural fry when natural steelhead fry were stocked with both hatchery treatments at 5.3 fry/m^2. When fry from the three rearing treatments were observed separately at a density of 4.0 fry/m^2, Berejikian et al. (2001) observed that aggressiveness increased with body size in the enriched and conventional hatchery treatments, but not in naturally-reared fry. In the present study, rearing treatment combination appeared to affect the rate of threats, but not attacks, by groups of steelhead fry in the laboratory. Groups of naturally-reared fish initiated more threats than groups including fish reared in conventional or enriched hatchery environments in some cases, but threat rate was not consistently lowest for either mixed rearing

treatment combination across densities. These results suggest that the introduction of hatchery-reared steelhead fry may not increase the level of aggression in mixed groups of hatchery-reared and wild steelhead fry under laboratory conditions, and that the relative aggressiveness of groups containing conventional or enriched fry is dependent on density and predation risk.

We observed significant positive effects of fish density on steelhead fry aggression, and we observed a significant reduction in the number of attacks, but not threats, by steelhead fry in the laboratory when predators were present. Because predators are probably rarely absent from habitats where wild salmonids occur, including predators in laboratory experiments may make results more relevant to natural streams.

In summary, our results suggest that levels of aggression in mixed groups of hatchery-reared and naturally-reared steelhead fry were not consistently higher than in groups of naturally-reared fry, and that aggression generally increased with fry density and decreased in the presence of potential predators. These results also suggest that aggression levels of steelhead fry in the laboratory may not reflect those in the wild.

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Table 1. Mean numbers of threats and attacks initiated by steelhead fry from three rearing treatment combinations at three densities in the presence and absence of predators in experimental laboratory flumes in summer 2002. Standard errors appear below the means in italics. See text for explanation of rearing treatment combinations.

		Rearing treatment combination					
	Density	Predator absent			Predator present		
Behavior	(fry/m^2)	NN	EN	CN	NN	EN	CN
Threats	1.8	4.35	1.55	5.9	2.8	0.7	3.95
		2.247	*0.693*	*3.336*	*2.371*	*0.467*	*1.905*
	3.6	8.225	6.225	12.275	5.8	4.7	3.675
		2.511	*1.976*	*2.874*	*2.893*	*1.674*	*1.279*
	7.1	13.675	18.4875	16.3375	10.3125	11.3	5.6125
		1.207	*1.143*	*2.609*	*2.193*	*2.745*	*1.546*
Attacks	1.8	1.85	1.45	1.35	3.45	1.1	2.1
		0.895	*0.721*	*0.646*	*2.08*	*0.452*	*0.714*
	3.6	8.175	4.6	8.6	12.25	6.5	3.6
		2.327	*1.763*	*4*	*4.117*	*1.609*	*1.156*
	7.1	24.7375	17.0625	10.1375	16.075	13.85	6.5625
		2.351	*1.733*	*1.773*	*4.844*	*3.695*	*1.558*

Section 6

Effects of density and rearing environment on the agonistic behavior and habitat use of steelhead *Oncorhynchus mykiss* fry in the presence of competitors and predators in a laboratory stream

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Introduction

It is widely recognized that artificial propagation programs for Pacific salmonids may pose risks to wild salmonid populations, but there is currently insufficient information to allow a quantitative determination of the potential impacts (ISAB 2003). The National Research Council (NRC 1996), the Independent Scientific Group (ISG 1996), and the Independent Scientific Advisory Board (ISAB 2003) have identified ecological interactions between hatchery and wild salmonids, including competition and predation, as important factors that may negatively affect wild salmonid populations.

Competition from hatchery fish may be one of several factors causing declines in wild salmonid populations (Nickelson et al. 1986; Flagg et al. 1995). Hatchery fish can disrupt natural social patterns in streams through competitive interactions (Bachman 1984, Nielsen 1994, McMichael et al. 1999), and hatchery fish may affect the abundance, growth or survival of natural salmonids (Hillman and Mullan 1989, Fresh 1997, Weiss and Schmutz 1999; but see Riley et al. 2004a). Although there is evidence to suggest that hatchery fish may have negative effects on wild salmonid populations, few experiments have been conducted to determine the ecological mechanisms which may cause these effects. Competition impacts on wild fish may be caused by unnatural behavior of hatchery-reared fish, the increase in density that accompanies the release of hatchery fish, or both. Maximizing the success of supplementation programs will require information on how the behavior of hatchery fish differs from that of wild fish, whether behavioral changes can be minimized by enriched rearing, and what effects behavioral changes imparted on the hatchery fish will have on wild fish.

Several authors have reported that hatchery-reared salmonids were more aggressive than wild conspecifics (Fenderson et al. 1968; Fenderson and Carpenter 1971; Bachman 1984; Swain and Riddell 1990; Mesa 1991; Rhodes and Quinn 1998; Deverill et al. 1999; Sundström et al. 2003), although other studies have reported no difference in aggression (Dickson and MacCrimmon 1982; Weber and Fausch 2003). Increased aggression by hatchery-reared fish might cause negative ecological effects on wild fish in streams where hatchery-reared fish are released, and might therefore negatively affect supplementation efforts for declining salmonid populations.

It has been suggested that rearing juvenile salmonids in enriched environments may produce fish that behave in a more natural manner (Berejikian et al. 2000). Juvenile salmonids reared in enriched environments have been shown to have increased survival or predator avoidance in some cases (Bams 1967; Leon 1975; Fuss and Johnson 1988; Maynard et al. 1996), but results are equivocal (Maynard et al. 1995; Riley et al. 2004c). Other experiments have shown that structural modifications to hatchery rearing environments may affect the agonistic behavior, dominance, and growth of steelhead fry in novel experimental environments (Berejikian et al. 2000; 2001). In the experiment reported here, we evaluate the effects of steelhead fry reared in conventional and enriched (NATURES) environments on the aggressive behavior and habitat use of naturally-reared steelhead *Oncorhynchus mykiss* in the presence of chinook salmon juveniles and yearling steelhead in a laboratory stream.

Methods

Rearing of experimental fish

Twenty-five thousand eyed eggs were obtained from artificially spawned steelhead from the Skookumchuck River, WA. Eggs were initially incubated at the WDFW Bingham Creek Hatchery, and were transported at the eyed stage of development to the University of Washington's Big Beef Creek Research Station for final incubation in constant 10^{o}C well water. Emergent fry (2,500) were stocked into each of 6 1.8-m diameter circular tanks on 12 May 2003. Three enriched (NATURES) tanks contained submerged commercially grown Douglas fir trees to provide structure, two 34 X 48 X 15 cm plastic and metal shopping baskets filled with cobbles, a double layer of brown, and green camouflage netting hung on an aluminum frame to provide overhead shade cover, and each was outfitted with an underwater food-delivery system. Three "conventional" tanks contained no overhead cover or structure, and received 40 L/min of water from above the water surface. Fish in the conventional tanks were fed by automatic feeders that scattered the food across the surface of the water. Fish in all tanks were fed with equal frequency.

Stream-type Chinook salmon *Oncorhynchus tshawytscha* fry were obtained from the Carson National Fish Hatchery (Carson, WA) in February 2003 and transported to the NMFS Manchester Research Station. Chinook salmon fry were reared in a single conventional 1.8 m diameter circular tank.

Experimental facility

This experiment was conducted in two 10-m long by 1.5-m wide flumes that have been previously described (Berejikian et al. 2000; 2001). Each flume received identical flows (1600 l/min) and had similar water depths. Light was provided by wide-spectrum fluorescent lights on a photoperiod of 16 h light to 8 h dark. The side walls of the flumes consisted of double-paned glass and allowed observers to view all fish in each section.

Fine wire mesh screens were placed parallel and perpendicular to the flow to produce six 2.25-m long by 0.75-m wide sections in each flume. The substrate of each flume section was gray PVC installed at an angle to provide a velocity gradient (shallow and fast upstream; deeper and slower downstream). Each flume section was divided into three subsections by marking on the viewing glass, and a line was drawn along the length of the glass equidistant between the substrate and the water surface to delineate the upper and lower water column. Mean depths were 0.14, 0.21, and 0.27 m in the upstream, middle and downstream subsections, respectively. Mean water velocities were 0.18, 0.03, and 0.01m/s in the upstream, middle, and downstream subsections, respectively.

Three plastic shelters (simulated hyporheic refuges) for juvenile steelhead and Chinook salmon were placed on the substrate of each section (one in each subsection) against the viewing glass. The shelters were 61 cm long and were divided into four chambers that fish could enter via 3 cm diameter holes; fish that used the shelters were visible through the viewing glass.

Experimental design and protocol

We used a substitutive experimental design (Fausch 1998) to examine the relative effects of three types of steelhead fry (natural, conventional hatchery and enriched hatchery) on the behavior of natural steelhead fry at three densities. In the lowest density trials, two fry (one natural and one conventional, enriched, or natural fry; total density = 1.2 fry/m^2) were matched for body weight (less than 5% difference) and simultaneously introduced into a flume section; hereafter these rearing treatment combinations are referred to as CN (conventional and natural), EN (enriched and natural), and NN (natural only), respectively. Four (two natural and two conventional, enriched, or natural fry; total density = 2.4 fry/m^2) or eight (four natural and four conventional, enriched, or natural fry; total density = 4.8 fry/m^2) fry were similarly matched for body weight and stocked for higher density trials. One hatchery-reared yearling steelhead from the same stock (mean length = 212 mm, range = 175-245 mm) and two hatchery-reared juvenile Chinook salmon were simultaneously stocked into all cells on each observation date. Fish were allowed to acclimate in the flume sections for approximately 44 hours with minimal feeding. Steelhead fry used in this experiment ranged from 40 to 62 mm in length and from 0.58 to 2.48 grams in weight; fish size increased over the duration of the experiment, but rearing treatment combinations were balanced over time. Chinook fry ranged from 59 to 85 mm in length and from 2.24 to 7.42 g in weight. A total of 90 trials (10 replicates of three treatment combinations at three densities) were conducted between 25 June – 13 August, 2003. The allocation of treatments and fish densities to flume sections was randomized.

Frozen bloodworms (chironomid larvae) were thawed and introduced into each section as prey for steelhead and Chinook fry. One chironomid larva per fish was introduced into each section at the onset of observation and every two minutes thereafter (a total of five larvae per fry) during observation periods. The larvae entered each section through a PVC tube that was located in the center of the section at the upstream end such that fish which positioned themselves in the upstream-most position on the left side of the section had first access to food.

Agonistic behavior

The number of attacks (nips, charges, and chases) and threats (lateral and frontal displays) initiated by fish of each type (Chinook, hatchery steelhead, natural steelhead) were recorded simultaneously in each flume section for 10 minutes by three observers. It was not necessary to mark steelhead fry because naturally- and hatchery-reared fry differed in color and observers kept track of individual fish during the course of observations. The order of observation was from downstream to upstream in each flume, and treatments and densities were balanced among flumes, flume sections, and observation dates. All observations took place between 8:00 and 13:00 PST.

Habitat and shelter use

In this experiment, the flume sections were divided into three subsections with varying habitat (see above). At the end of each observation period, observers noted the number of fish of each type (natural and hatchery-reared steelhead and Chinook) in the upper water column, the number in the upstream subsection, and the number occupying the simulated hyporheic shelters.

Data analysis

The substitutive design used here allows one to compare the effects of adding fry reared in conventional or enriched hatchery environments to the effects of adding equal numbers of naturally-reared fry. For example, if aggression was significantly higher when enriched or conventional fry were stocked with naturally-reared fish compared to when natural fish are stocked alone at the same density, then the behavioral effects of adding hatchery fish would likely be greater than adding the same number of wild fish. In this case, rearing treatment *combinations* (NN, EN, CN) are tested against one another. Within this design framework, we performed a number of different analyses on subsets of the data to address planned comparisons.

We first tested the hypothesis that levels of agonistic interactions (threats and attacks) and habitat and shelter use of steehead and Chinook fry were not different among the three rearing treatment combinations and the three densities. We compared these variables among rearing treatment combinations (CN, EN, and NN) and densities using two-way analysis of variance (ANOVA) of ranked (threats, attacks) or arcsine-transformed (habitat use, shelter use) data. Agonistic behavior data were ranked using PROC RANK and analyzed using PROC GLM in SAS (SAS 1990). This procedure is essentially equivalent to performing a Kruskal-Wallis test, and was undertaken because of significant departures from normality in the data.

In order to compare enriched vs. conventionally-reared steelhead fry, we performed a similar analysis using only the treatments with hatchery fish (EN, CN). Using only hatchery-reared fry from the EN and CN treatment combinations, we tested the hypothesis that the agonistic behavior and habitat and shelter use of hatchery-reared fry were not affected by rearing treatment or density. Similarly, we tested the hypothesis that the agonistic behavior and habitat and shelter use of naturally-reared fry were not affected by the rearing treatment of the hatchery fish that they were stocked with or by density. In these analyses, a significant treatment effect would indicate that either hatchery or natural fish, respectively, differed in behavior depending on the rearing treatment of hatchery fish. We performed two-way ANOVA (with rearing treatment combination and density as main effects) for these analyses, using only hatchery or natural fish. These analyses were performed on ranked (threats, attacks) or arcsine-transformed (habitat use, shelter use) data, as above.

Results

Agonistic behavior

The number of threats and attacks initiated by juvenile Chinook showed a strong increase with steelhead density (Figs. 1, 2). Threats and attacks by steelhead fry also tended to increase with density, but to a lesser degree (Figs. 1, 2). There was a significant effect of density on the number of threats and attacks initiated by steelhead fry and by juvenile Chinook (Table 1).

There was little evidence that aggressive behavior by juvenile Chinook or steelhead varied among rearing treatment combinations (Figs. 1, 2). There were no significant effects of rearing treatment combination or the interaction (rearing treatment combination by density) on the number of attacks or threats initiated by steelhead fry or juvenile Chinook (Table 1).

When natural and hatchery steelhead fry from the EN and CN treatment combinations were analyzed separately, the number of threats per fry appeared to increase with density in the CN treatment combination, but not in the EN (Fig. 3). The number of attacks per fish generally appeared to increase with density in both treatment combinations (Fig. 3). There were significant effects of density on the number of attacks initiated by natural and hatchery steelhead fry, and a significant effect of density on threats initiated by hatchery, but not natural, steelhead fry (Table 2). There were no significant effects of rearing treatment combination or the interaction (rearing treatment combination by density) on the number of threats or attacks initiated by natural or hatchery steelhead fry (Table 2).

Habitat and shelter use

There was little evidence that habitat use by steelhead or Chinook fry varied consistently with density or rearing treatment combination. The proportion of steelhead or Chinook fry in the upper water column showed no consistent pattern with respect to density or rearing treatment combination (Fig. 4), and no significant effects of either factor were observed (Table 3). The proportion of steelhead and Chinook fry in the upstream subsection appeared to be relatively constant across densities and showed no consistent differences among rearing treatment combinations; there were no significant effects of density or rearing treatment combination for this variable (Fig. 5, Table 3).

Very few Chinook used the shelters; Chinook were observed in shelter only in the CN treatment combination at the two lower densities (Fig. 6). There were no significant effects of density or rearing treatment combination on shelter use by Chinook (Table 3). Steelhead more frequently used the shelters, and there was a significant effect of density, but not rearing treatment combination, on shelter use by steelhead fry (Fig. 6, Table 3).

When natural and hatchery steelhead fry from the EN and CN treatment combinations were analyzed separately, there was no evidence that habitat use or shelter use differed among rearing treatment combinations (Fig. 7, Table 4). There was a significant effect of density on the proportion of natural, but not hatchery-reared, steelhead fry in shelters, with shelter use tending to increase with density. There were no significant effects of density on the proportion of fry in the upper water column or the proportion in the upstream subsection for either natural or hatchery-reared fry (Table 4).

Discussion

We found little evidence for differences in agonistic behavior or habitat use among mixed groups of steelhead fry from the three different rearing environments, or among Chinook fry cohabiting experimental cells with these mixed groups of steelhead fry. There were no significant effects of rearing treatment combination observed for any variable examined. When steelhead fry from the EN and CN rearing treatment combinations were considered separately, there was also no evidence that hatchery fry from the conventional or enriched (NATURES) rearing environments differed in agonistic behavior or habitat use, nor any evidence that natural fish inhabiting the cells with the two types of fish differed in agonistic behavior or habitat use.

Overall, the results of our research on the effects of enriched (NATURES) rearing on agonistic behavior, all of which was conducted in the same laboratory flumes, have produced equivocal results. No significant difference in the agonistic behavior (frequency of attacks and displays) of steelhead fry reared in conventional and enriched hatchery environments was observed when the treatments were stocked together at 10.7 fry/m^2 (Berejikian et al. 2000), but significant differences were observed among rearing treatments (C, E) in the frequency of threats (but not attacks) by dominant fish (conventionally-reared fry delivered significantly fewer threat displays than enriched or natural fry) when naturally-reared steelhead fry were stocked with both hatchery treatments at 5.3 fry/m^2 (Berejikian et al. 2001). When fry from each of the three rearing treatments were observed separately at a density of 4.0 fry/m^2, Berejikian et al. (2001) observed a significant difference in the relationship between body size and total aggression among the three treatments – aggressiveness increased with body size in the enriched and conventional hatchery treatments, but not in naturally-reared fry. Riley et al. (in review) observed no significant effect of rearing treatment combination on the number of threats initiated by steelhead fry, but did observe a barely significant effect of rearing treatment combination on the number of threats initiated by steelhead fry; this was confounded by a density by rearing treatment combination interaction, suggesting that any differences among rearing types may depend on density. In the present study, we observed no significant effects of rearing treatment combination on the aggression rate of steelhead fry. These results suggest that enriched hatchery environments may have some effect on the aggression of steelhead fry under some circumstances, but that this effect depends on density, the presence or absence of predators and competitors, and other potential factors.

When data from the present study were compared with data that were collected in 2002 (Riley et al. in review), it is apparent that different patterns were observed in the two years, although the numbers of threats and attacks per fish were within similar ranges (Fig. 8). In 2002, steelhead fry in the NN treatment combination initiated the most threats, while the most threats were observed in the EN or CN treatment combinations (depending on density) in 2003 (Fig. 8). Patterns in the number of attacks per fish were also different between the two years; for example, steelhead fry in the CN treatment combination initiated the fewest attacks in 2002 at the medium density level, while steelhead fry in this treatment combination initiated the most threats at the same density in 2003. Differences in the experimental setup between the two years included the presence of Chinook salmon fry in 2003, larger flume sections in 2003, and a much greater range of depths and velocities in the flume sections in 2003. The fact that we observed such variation in the relative aggression level in the three rearing treatment combinations both within and between the two years suggests that that there were no *consistent* differences in agonistic behavior among the three rearing treatment combinations in 2002 and 2003, that variables such as the presence of competitors and the nature of the habitat may affect the relative level of agonistic behavior in the rearing treatment combinations, and also that any differences in the relative level of agonistic behavior among the rearing treatment combinations that are observed in a particular situation (i.e. at a single density under a certain set of conditions) may not apply to other situations.

We observed significant effects of fish density on the agonistic behavior of steelhead fry, with the numbers of attacks and threats per fish generally increasing as density increased. Increases in the level of aggression with density have been previously observed for steelhead fry in previous NATURES research (Riley et al. in review; 2004b) and in juvenile salmonids (Fenderson and Carpenter 1971; Keeley 2000) and other fish species (Jones 1983) in laboratory or culture settings. Other studies, however, have not found a direct positive relationship between fish aggression and density (Keenleyside and Yamamoto 1962; Fenderson and Carpenter 1971; Sale 1972; Cole and Noakes 1980; Jones 1983; McNicol and Noakes 1984; Syarifuddin and Kramer 1996; Hedenskog et al. 2002; Caballero and Castro-Hdez 2003). If agonistic behavior in steelhead fry does increase with density, then the density at which fry are stocked may be an important determinant of aggression levels. Riley et al. (in review), however, found no relationship between fry density and aggression in natural streams. Further research on aggression n wild and hatchery salmonids in natural streams is necessary to evaluate the potential effects of aggression by hatchery fish on the ecology of wild juvenile salmonids.

Although several authors have reported differences in habitat use between hatchery-reared and wild salmonids (Mason et al. 1967, Dickson and MacCrimmon 1982; Mesa 1991; Reinhardt 2001), we observed no significant effect of rearing treatment on the habitat use of steelhead fry or Chinook salmon in the flumes. Similarly, Riley et al. (2004b) observed no significant effects of rearing treatment combination on the position of steelhead fry in experimental flume sections. Riley et al. (2004b) did, however, observe that natural fry in the CN treatment combination occupied more upstream positions than natural fry in the EN treatment combination. Berejikian et al. (2000) found that steelhead fry reared in conventional and enriched environments used structured and unstructured habitats in similar proportions. Overall, the results of NATURES research provide little evidence that enriched rearing environments lead to differences in habitat use by steelhead fry under laboratory conditions.

There was little evidence that fish density affected the habitat use of steelhead fry. No significant effects of density were observed for the proportion of fry in the upper water column or the proportion in the upstream flume subsection. Bult et al. (1999) found that habitat selection by Atlantic salmon parr varied with density, with relatively more parr being found in pools at higher densities. Bohlin (1977, 1978) and Elliott (1986) also reported that brown trout habitat varied with population density. The fact that we did not observe patterns in habitat use with density may be due to the fact that we used a fairly narrow range of densities.

Salmonids have been observed to use hyporheic refuges, particularly at low temperatures (Griffith and Smith 1993; Fraser et al. 1995; Gregory and Griffith 1996), and the proportion of Atlantic salmon fry sheltering has been observed to decrease with population density, probably because refuge space may be limited (Armstrong and Griffiths 2001). In the present study we observed that the proportion of natural (but not hatchery-reared) steelhead fry that were in shelters tended to increase with density. Differences between the two studies may be due to interspecific behavioral differences, but might also be due to a number of factors, including water temperature (higher in the present study), water quality (Armstrong and Griffiths [2001] used single-pass water, while this study used recirculated water), fish density (lower densities in the present study), and the relative number of shelters (more shelters in the present study).

Because the use of shelters by natural fish tended to increase with density in this study, while shelter use by hatchery-reared fish did not, these results suggest that the propensity of naturally- and hatchery- reared steelhead fry to seek shelter may differ. Griffiths and Armstrong (2002) noted that a higher proportion of hatchery-reared than wild Atlantic salmon fry occupied shelters. The results of this study show no consistent pattern in this respect, i.e. the proportions of naturally- and hatchery-reared fry in shelters varied with density and were not notably higher for either group overall. Further research on this topic would be beneficial.

In summary, our results suggest that the introduction of hatchery-reared steelhead fry does not significantly affect aggression, habitat use, or shelter use in mixed groups of hatchery- and naturally-reared steelhead fry under laboratory conditions, and that the relative aggression level of these mixed groups may be dependent on density. Further, these results suggest that there were few differences in aggression, habitat use, or shelter use between steelhead fry reared in enriched and conventional environments. For management purposes, it is therefore likely that stocking hatchery-reared steelhead fry at low densities will have similar effects on wild fish as an increase in density of wild fish, suggesting that hatchery-reared fry could be stocked at densities approaching the carrying capacity of a stream with relatively low risk to wild fish. Our results also suggest that fry from conventional and enriched rearing environments are unlikely to have differential effects on natural fry at low densities. We suggest that releasing hatchery-reared steelhead fry similar in size to wild fish at low densities may be a supplementation strategy that entails few risks to wild steelhead populations.

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Table 1. ANOVA results of comparisons of the number of threats and attacks per fish among rearing treatment combinations and densities. Values shown are significance levels (p) of ANOVA tests. Tests for rearing treatment combination and density are DF 2, 81; tests for the interaction are DF 4, 81.

		FACTOR		
Variable	Species	Rearing treatment combination	Density	Interaction
Threats	Steelhead	0.9839	**0.0076**	0.6831
	Chinook	0.2437	**<0.0001**	0.4743
Attacks	Steelhead	0.9312	**0.0004**	0.8801
	Chinook	0.8301	**<0.0001**	0.8224

Table 2. ANOVA results of comparisons of the number of threats and attacks per fish among rearing treatment combinations and densities for steelhead fry from the EN and CN. rearing treatment combinations. Values shown are significance levels (p) of ANOVA tests. Tests for rearing treatment combination and density are DF 1, 54; tests for the interaction are DF 2, 54.

		FACTOR		
Variable	Type	Rearing treatment combination	Density	Interaction
Threats	Natural	0.4882	0.3161	0.6430
	Hatchery	0.4236	**0.0024**	0.1272
Attacks	Natural	0.9900	**0.0141**	0.6821
	Hatchery	0.8088	**0.0021**	0.5956

Table 3. ANOVA results of comparisons of habitat use among rearing treatment combinations and densities. Values shown are significance levels (p) of ANOVA tests. Tests for rearing treatment combination and density are DF 2, 81; tests for the interaction are DF 4, 81.

		FACTOR		
Variable	Species	Rearing treatment combination	Density	Interaction
Proportion in upper water column	Chinook	0.1597	0.4599	0.2848
	Steelhead	0.8397	0.8845	0.2110
Proportion in upstream subsection	Chinook	0.3853	0.7167	0.4088
	Steelhead	0.3916	0.1928	0.5952
Proportion in shelter	Chinook	0.1630	0.6093	0.6093
	Steelhead	0.6430	**0.0196**	0.6291

Table 4. ANOVA results of comparisons of habitat use among rearing treatment combinations and densities for steelhead fry from the EN and CN rearing treatment combinations. Values shown are significance levels (p) of ANOVA tests. Tests for rearing treatment combination and density are DF 1, 54; tests for the interaction are DF 2, 54.

		FACTOR		
Variable	Type	Rearing treatment combination	Density	Interaction
Proportion in upper water column	Natural	0.1275	0.8843	0.4107
	Hatchery	0.2224	0.7230	0.1730
Proportion in upstream subsection	Natural	0.2437	0.0557	0.4062
	Hatchery	0.9298	0.7836	0.5638
Proportion in shelter	Natural	0.5900	**0.0040**	0.2433
	Hatchery	0.7795	0.5777	0.9240

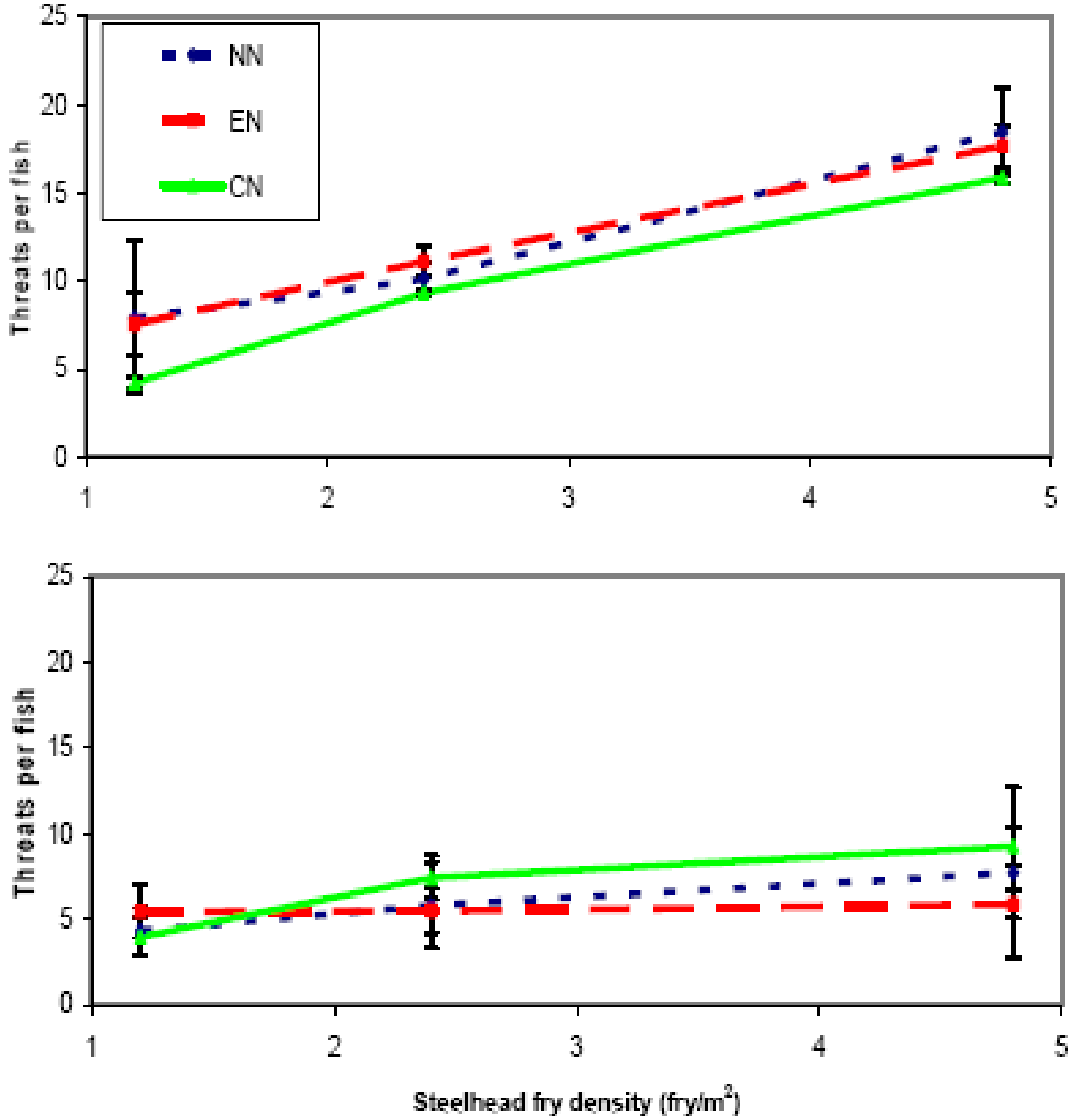

Figure 1. The number of threats observed per fish for Chinook (top panel) and steelhead (bottom) fry for three rearing combinations of steelhead fry at three densities in experimental flumes. Diamond symbols represent the NN treatment combination, squares the EN, and triangles the CN; see text for more details of rearing treatment combinations. Error bars are 95% confidence limits.

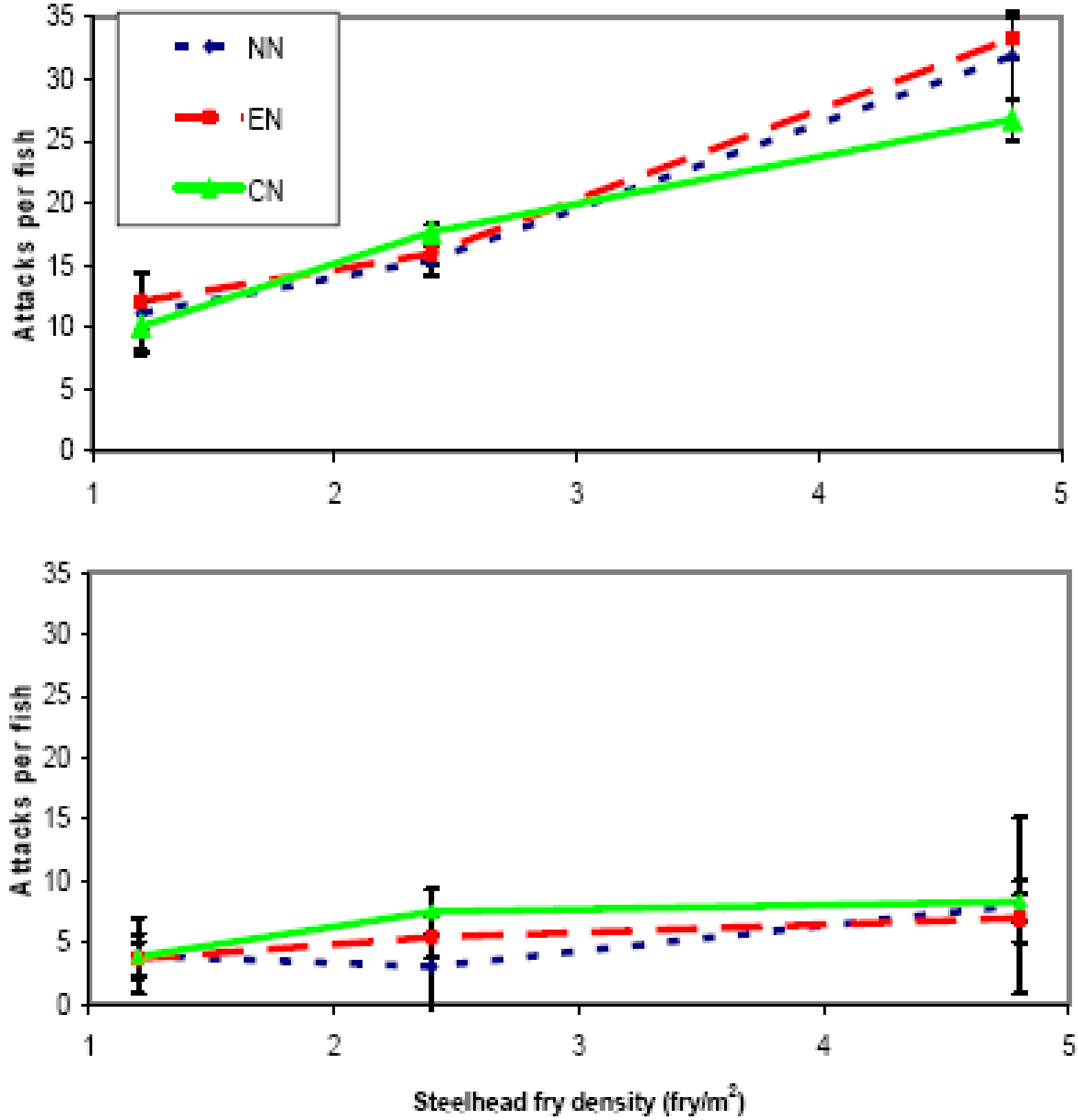

Figure 2. The number of attacks observed per fish for Chinook (top panel) and steelhead (bottom) fry for three rearing combinations of steelhead fry at three densities in experimental flumes. Diamond symbols represent the NN treatment combination, squares the EN, and triangles the CN; see text for more details of rearing treatment combinations. Error bars are 95% confidence limits.

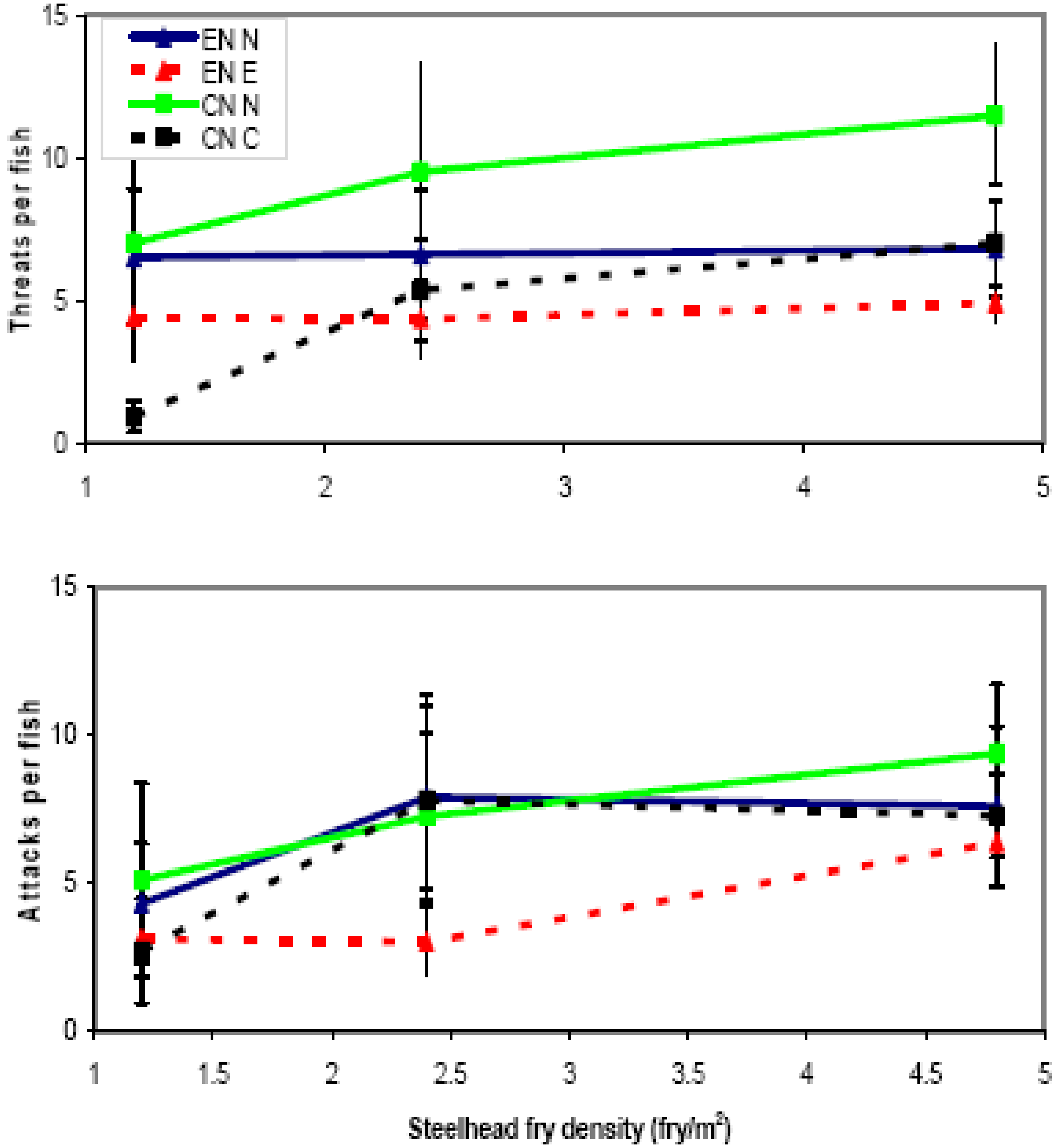

Figure 3. The number of threats (top panel) and attacks (bottom panel) initiated by steelhead fry from the EN and CN rearing combinations at three densities in experimental flumes. Solid lines represent naturally-reared fry, and dashed lines represent hatchery-reared fry. Squares represent the CN rearing treatment combination, and triangles the EN; see text for more details of rearing treatment combinations. Error bars are 95% confidence limits.

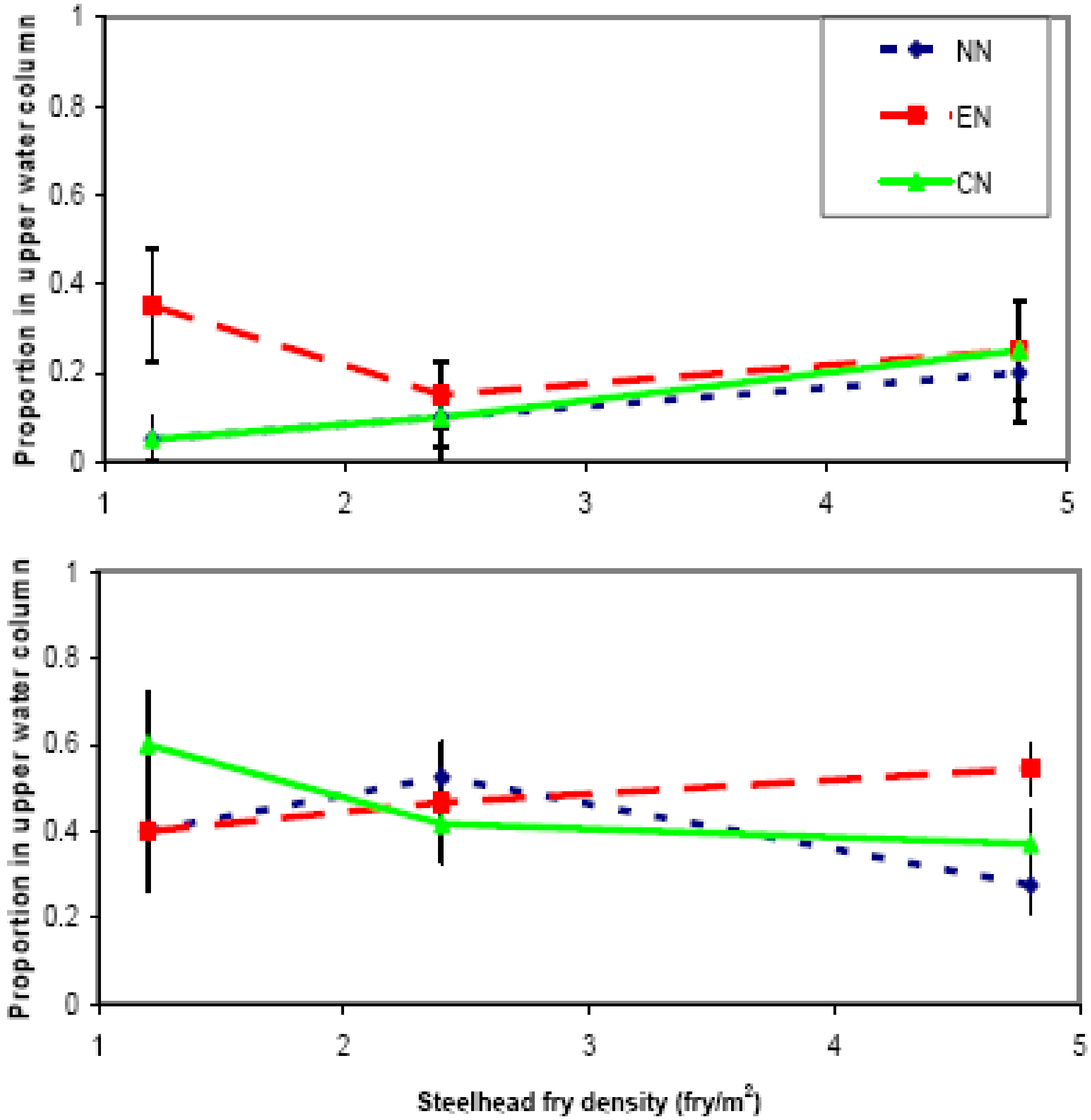

Figure 4. The proportion of Chinook (top panel) and steelhead (lower panel) fry in the upper water column for three rearing combinations of steelhead fry at three densities in experimental flumes. Diamond symbols represent the NN treatment combination, squares the EN, and triangles the CN; see text for more details of rearing treatment combinations. Error bars are 95% confidence limits.

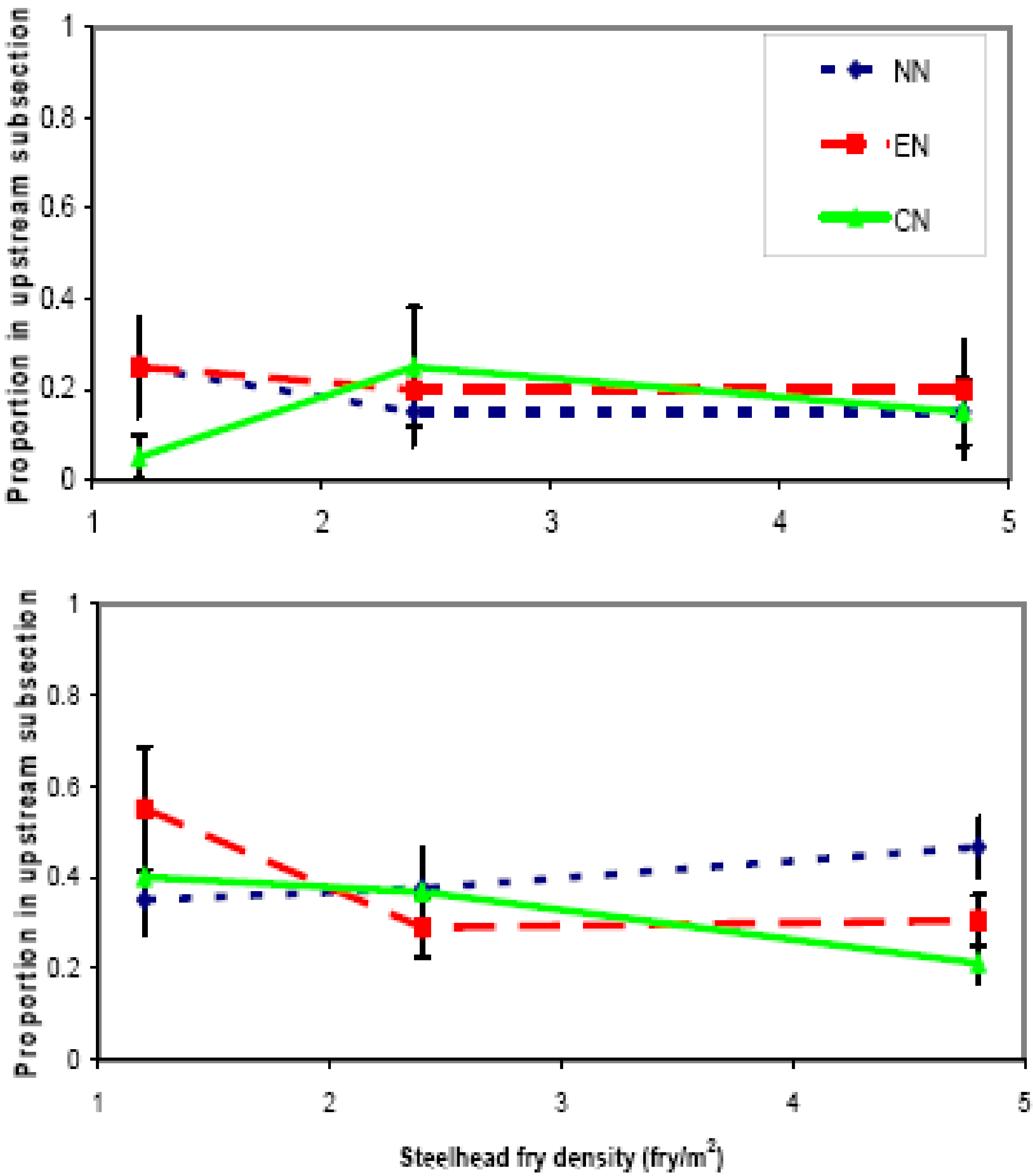

Figure 5. The proportion of Chinook (top panel) and steelhead (lower panel) fry in the upstream flume subsection for three rearing combinations of steelhead fry at three densities in experimental flumes. Diamond symbols represent the NN treatment combination, squares the EN, and triangles the CN; see text for more details of rearing treatment combinations. Error bars are 95% confidence limits.

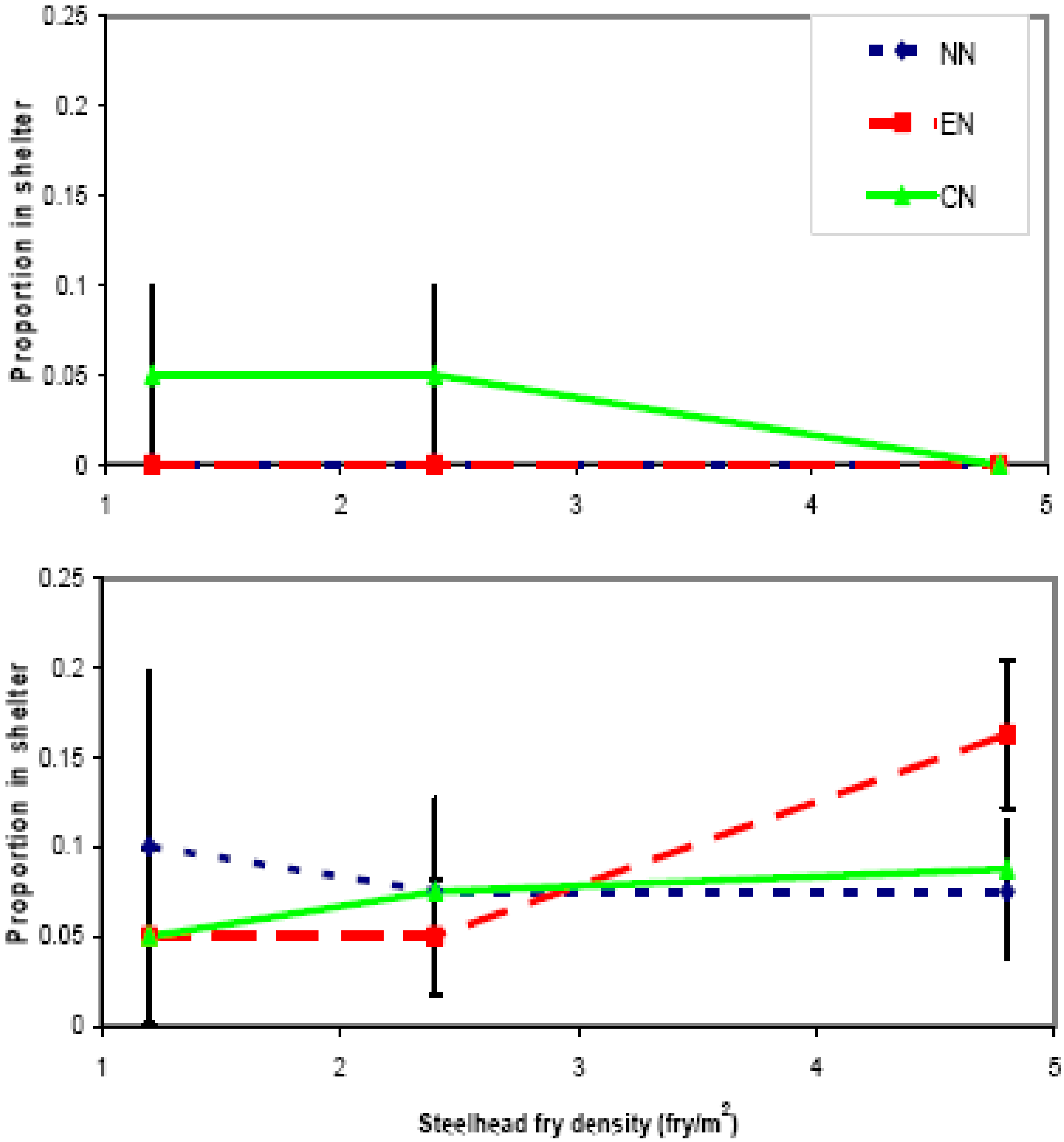

Figure 6. The proportion of Chinook (top panel) and steelhead (lower panel) fry using simulated hyporheic shelters for three rearing combinations of steelhead fry at three densities in experimental flumes. Diamond symbols represent the NN treatment combination, squares the EN, and triangles the CN; see text for more details of rearing treatment combinations. Error bars are 95% confidence limits.

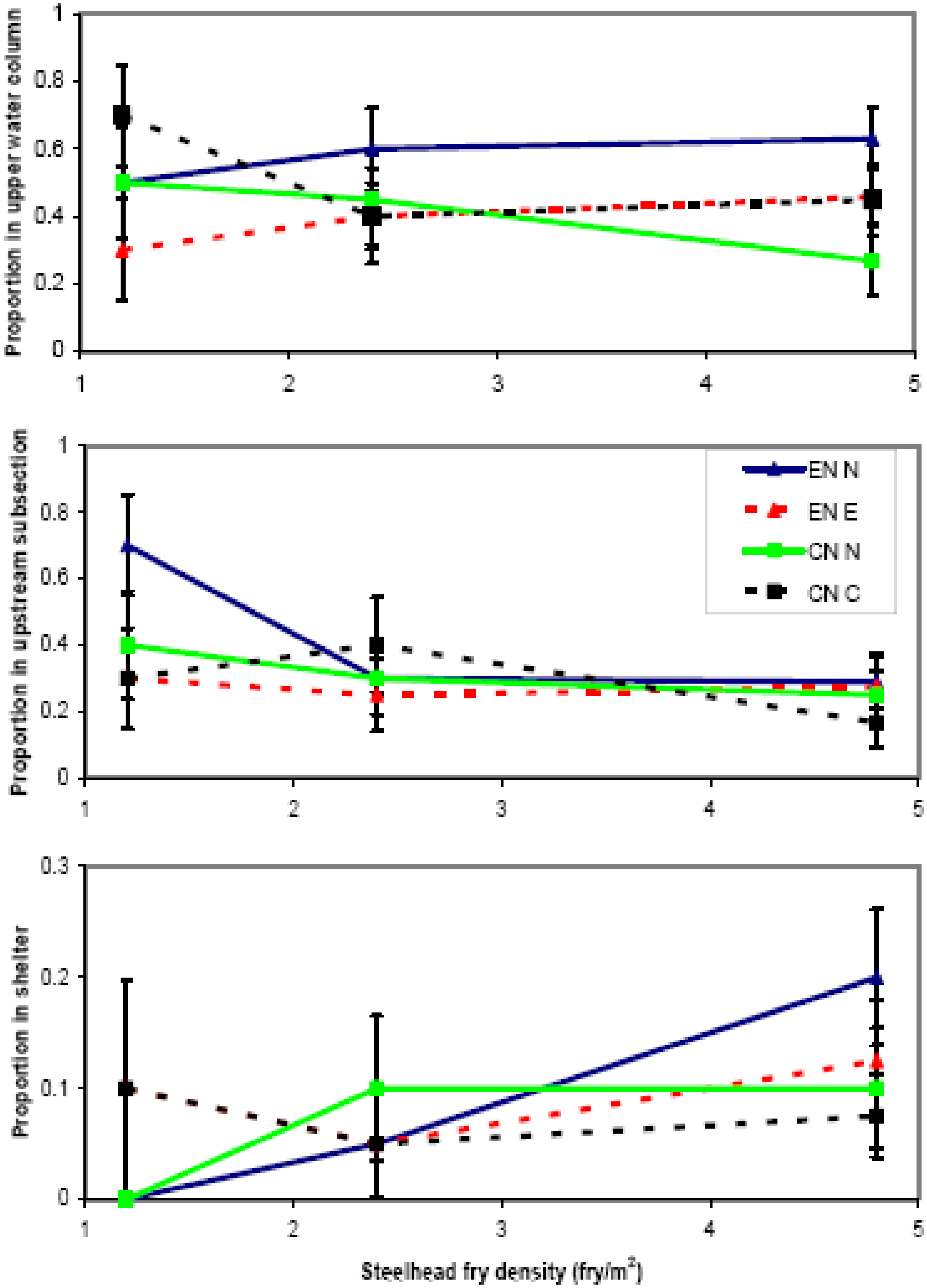

Figure 7. The proportion of steelhead fry in the upper water column (top panel), the proportion of steelhead fry in the upstream flume subsection (middle panel), and the proportion of steelhead fry using simulated hyporheic shelters (bottom panel) for steelhead fry from the EN and CN rearing combinations at three densities in experimental flumes. Solid lines represent naturallyreared fry, and dashed lines represent hatchery-reared fry. Squares represent the CN rearing treatment combination, and triangles the EN; see text for more details of rearing treatment combinations. Error bars are 95% confidence limits.

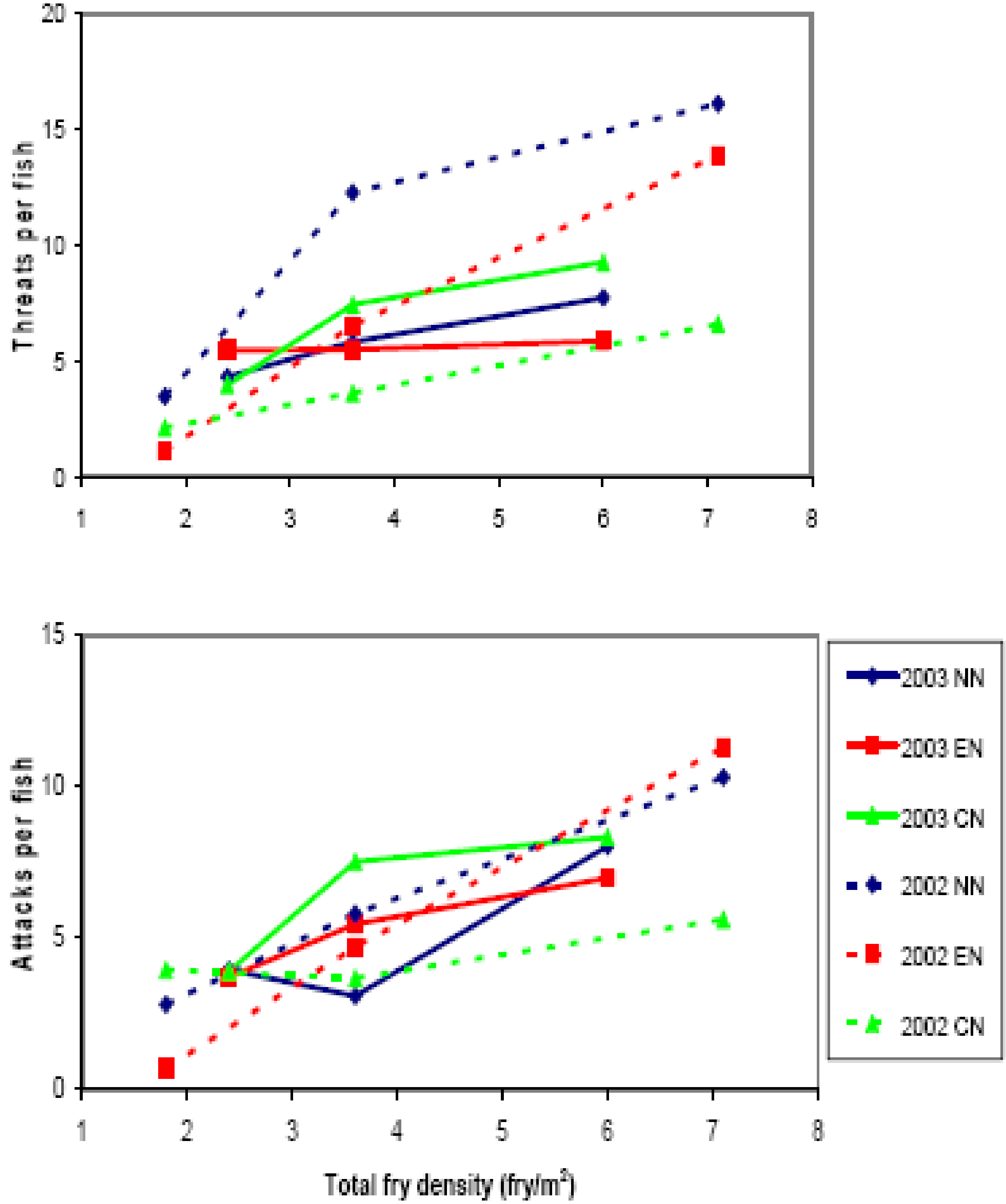

Figure 8. The number of threats (top panel) and attacks (bottom panel) initiated by steelhead fry from all rearing combinations at three densities in experimental flumes in 2002 and 2003. Solid lines represent 2003 data; dashed lines represent 2002. Diamond symbols represent the NN treatment combination, squares the EN, and triangles the CN; see text for more details of rearing treatment combinations. Data for 2002 are from trials which included yearling steelhead as potential predators. Total fish density (x axis) includes Chinook salmon in 2003 trials.

Section 7

EFFECTS OF SEMINATURAL HABITAT REARING ON COHO SALMON, 2000-2003

by

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Introduction

New salmon culture techniques must be developed if the survival of hatchery coho salmon (*Oncorhynchus kisutch*) recruiting to the fishery or spawning population is to be increased. Lower than optimal rates of survival are unacceptable as public hatcheries produce up to 80% of the salmon available for harvest and are often the only broodstock source available for restoring depleted natural runs. In addition, low survival rates requires hatcheries to release more smolts that could negatively interact with ESA listed stocks than would be necessary with higher survival rates. Fortunately, there appears to be scope for improvement as the smolt-to-adult survival of hatchery fish is often much lower than that of wild-reared salmon. It appears that salmonids produced with traditional fish culture techniques lack many of the behavioral, physiological, and morphological characteristics needed to survive in the wild immediately after release (see the review by Maynard et al. 1995). It may be possible to promote the expression of these wild characteristics by rearing salmonids in a hatchery environment that resembles natural stream and river habitats. This is the paradigm behind the NATURES seminatural raceway habitat concept.

The National Marine Fisheries Service (NMFS) and Washington Department of Fish and Wildlife (WDFW) have been cooperatively developing a Natural Rearing Enhancement System (NATURES) consisting of seminatural raceway habitat, live food diets, exercise systems, predator avoidance training, underwater feed delivery systems, and oxygen supplementation (Maynard et al. 1995; Maynard et al. 1996a,b,c,d; Maynard and Flagg 2001; Maynard et al. 2001 a,b). The most successful component of NATURES rearing has been the development of seminatural raceway habitat. Seminatural raceway habitat is composed of gravel substrates, inwater structure, and overhead cover that are installed in raceways to produce a rearing environment that resembles the stream and river habitats utilized by juvenile chinook and coho salmon. This differs from the environmental enrichment research previously conducted with coho salmon, which included structure and cover, but lacked substrate (Vander Haegen and Appleby 1998). Studies conducted with chinook salmon (*O. tshawytscha*) have shown that seminatural raceway habitat rearing can improve instream postrelease survival up to 50% (Maynard et al. 1996c). The following study was initiated to determine if seminatural raceway habitat rearing produces similar increases in coho salmon postrelease survival (Maynard et al. 2001b).

The study's experimental design tests the hypothesis that rearing coho salmon in seminatural raceway habitat increases their smolt-to-adult survival. The experimental approach is to rear coho salmon in standard-sized control and seminatural raceways for at least the last two months of their culture, release them into the wild, and then compare their smolt-to-adult survival. A unique attribute of the study is that it is being conducted with paired raceways at five Puget Sound hatcheries spread over a large geographic range. This approach enables the findings to be extrapolated throughout western Washington and removes the specter of the findings being unique to a single facility and stock.

Project Objectives

1. Starting with designs used in previous experiments, construct and install NATURES rearing habitat that is compatible with daily activities at a salmon hatchery.

2. Compare color development of fish reared using conventional techniques to the color development of fish reared using NATURES habitat.

3. Compare growth of fish reared using conventional methods to fish reared using NATURES habitat.

4. Compare the health of fish reared using conventional methods to fish reared using NATURES habitat.

5. Compare the smolt-to-adult survival of fish reared using conventional methods to fish reared using NATURES habitat.

Approach

Study Sites and Experimental Design

The research is being conducted at WDFW Kendall Creek Hatchery near Kendall (WA), Soos Creek Hatchery near Auburn (WA), Minter Creek Hatchery near Purdy (WA), Sol Duc Hatchery near Sappho (WA), and Issaquah Hatchery near Issaquah (WA). Seminatural raceway habitat research was initiated in the first three hatcheries during the 1999 broodyear experimental period, with Issaquah and Sol Duc hatcheries being added into the study during the 2000 broodyear experimental period. The ponds at Kendall, Soos Creek, and Issaquah Hatcheries are standard raceways that are about 3 m wide by 30.5 m long, while Minter Creek Hatchery has much larger raceways that are about 6.1 m wide and 36.6 m long. The rearing units at Sol Duc Hatchery are large operational Burrow's ponds. At Kendall Creek Hatchery, Soos Creek Hatchery, Issaquah Hatchery, and Sol Duc Hatchery the fish are reared in first-pass water, while at Minter Creek Hatchery they are reared on aerated second-pass water. There are marked differences in the distance the fish must migrate downstream to reach the estuary. At Minter Creek Hatchery, the fish are released almost directly into the estuary, while at Kendall Creek, Soos Creek, Issaquah, and Sol Duc Hatcheries they are released many kilometers inland. Land use along the banks of these migratory corridors also markedly differs. The fish released from Kendall Creek and Sol Duc Hatcheries migrate down medium size rivers that pass through rural, agricultural, and forest land corridors. In contrast, the fish released from Soos Creek and Issaquah Hatcheries primarily migrate through suburban-urban corridors. In addition to these differences, the Issaquah coho salmon smolts must migrate through a large lake before reaching Puget Sound via the Lake Washington Ship Canal. These differences enable the study to determine if the NATURES seminatural raceway habitat concept can be usefully employed over a large array of geographic and hatchery conditions.

At each facility, two similar raceways were selected for the experiment. One of these serves as an unmodified control, while the second is fitted with seminatural raceway habitat.

This is an acclimation study, in which fish are conventionally-reared until their second year of life when the experimental fish are introduced into the raceways equipped with seminatural raceway habitat for final rearing. A control and experimental group of coho salmon will be reared at each of the five facilities through 2004 to provide a total of 18 paired releases for experimental evaluation of smolt-to-adult survival.

It was necessary to refine previous seminatural raceway habitat components for installation in the production raceways at each WDFW facility prior to initiating the experiment. The first step was to develop a concrete gravel paver that was lower in cost, more durable, and easier to install than the resin-rock pavers used in an earlier study at the WDFW Forks Creek hatchery with chinook salmon (Maynard et al. 2001b). The new paver was fabricated by first covering the bottom of a 46 x 46-cm mold that is 5 cm deep with 2.2-cm gravel similar in color to the stream and river bottoms where the fish are released. Concrete (colored to match river sand) was then poured in the mold and allowed to cure. After curing, the pavers were removed from the mold and stored on pallets until installation. The pavers were installed by simply laying them down side by side until the entire raceway bottom was covered. Paver weight alone is sufficient to hold them in place. A brick saw is required to cut pavers into smaller pieces to fill in gaps along the raceway wall edge. Using this technique, pavers were successfully installed at all five hatcheries. In addition, uncolored concrete pavers (20.5 cm wide x 41 cm long x 3 cm thick) were installed over the control pond bottom at Sol Duc Hatchery because aging had resulted in the control pond bottom resembling the experimental treatment.

Camouflage net covers were developed, constructed, and installed at all the hatcheries. As in previous seminatural raceway habitat studies, military camouflage netting is suspended within 90 cm of the water surface. The netting is suspended from aluminum frames and covers from 50 to 80% of the raceway surface. The camouflage net frame design is similar at four of the hatcheries, but different at Minter Creek Hatchery because of longer side span of its double width ponds. The design used at Issaquah Creek, Kendall Creek, Sol Duc River, and Soos Creek hatcheries is a rectangular 1.67 x 3.1-m frame constructed from 2.5-cm square aluminum tube. The frame is attached by pins to a galvanized piece of 10 x 10-cm angle iron to form a hinge. The angle iron is then bolted to the concrete wall on one side of the raceway. When lowered, the opposing frame end is supported by a 2.5 x 2.5-cm piece of angle-aluminum attached to the other raceway side. Two hydraulic lift struts are attached to the cover frame to reduce the effort required to lift the frame to the fully open position. This design enables fish culturists to quickly and easily open the covers when they need to feed fish or vacuum the raceways. The Minter Creek Hatchery covers are built from 5-cm square aluminum tube and are 6.4 x 6.4-m squares that span the raceway. Each aluminum frame is supported by wheels that rest on a track running the length of the raceway. Stainless steel cable spans the frame diagonally for extra support, and the camouflage net was initially draped over these cables and fastened to the cover frame. However, after the covers failed due to snow load, the camouflage net was suspended below the frame on wire ties that should break away when loaded with snow. There are three covers per raceway and each cover can be moved back and forth on the rail as necessary for raceway vacuuming.

The instream structure used in this experiment is similar to that used in previous NATURES research. At all hatcheries structure has been installed by suspending a stainless steel cable the length of the raceway and hanging denuded fir trees weighted with rebar from that cable. Trees are attached to the cable with carabiner-type clips and can be readily removed for cleaning or replacement. This structure differed from that used in previous WDFW research, where plastic containers were sunk to the raceway bottom with sandbags (Vander Haegen and Appleby 1998).

In the BY 1999 experimental rearing period, the fish were transferred into the NATURES raceways as soon as modifications were completed. Both control and experimental fish were maintained in similar conditions until this time. After being placed into the experiment, the fish were reared similarly except for the presence or absence of seminatural raceway habitat.

In 2002, BY 2000 fish were transferred into the NATURES raceways when they were coded-wire tagged, when no other rearing space was available, or when modifications were completed. Both control and experimental fish were maintained in similar conditions until this time. After being placed into the experiment, the fish were reared similarly except for the presence or absence of seminatural raceway habitat.

The BY 2001 coho salmon were transferred into the NATURES study raceways at the time of coded-wire tagging, or when rearing space availability dictated. At some hatcheries, fish which would not receive coded-wire tags were placed into the study raceways earlier on, then coded-wire tagged fish were added to the raceway at the time of tagging. Both control and experimental fish were maintained in similar conditions until this time. After being placed into the experiment, the fish were reared similarly except for the presence or absence of seminatural raceway habitat.

Experimental Rearing

BY 1999 Experimental Rearing Period

The control and experimental raceways at Kendall Creek Hatchery each received approximately 51,000 BY1999 coho salmon on 11 January 2001. Of the fish in the control raceway, 7,656 were coded-wire tagged and adipose fin-clipped and an additional 7,656 were coded-wire tagged alone. The seminatural raceway had 7,656 coded-wire tagged and adipose fin-clipped fish, as well as 7,658 coded-wire tagged only fish. These fish were tagged on 28 June 2000, prior to initiation of experimental rearing.

Soos Creek Hatchery was the second facility to begin NATURES rearing in 2001. On 25 January, approximately 55,000 BY1999 coho salmon were transferred into the seminatural raceway at Soos Creek Hatchery. In both the control and experimental raceways, 5,100 fish were coded-wire tagged and adipose fin-clipped, with an additional 5,100 fish coded-wire tagged only. All tagging was completed prior to initiation of the experiment.

Minter Creek Hatchery was the last facility to initiate experimental rearing. Coded-wire tagging took place prior to experimental rearing, on 21 and 22 June 2000, when 10,040 of the coho salmon designated as controls received coded-wire tags and adipose fin clips, and 10,124 of those designated to be NATURES fish received coded-wire tags and adipose fin clips. The control raceway at Minter Creek Hatchery initially contained 298,200 BY1999 coho salmon, and 300,075 coho salmon were transferred into the seminatural raceway on 28 February 2001.

BY 2000 Experimental Rearing Period

BY 2000 coho salmon were placed in the control and experimental raceways at Kendall Creek Hatchery beginning in July 2001. On 2 and 3 August 2001, the coded-wire tagged fish were loaded into the raceways as they were tagged. Each raceway received approximately

51,400 BY2000 coho salmon. A total of 20,064 fish from the conventional raceway were coded-wire tagged (half of them adipose clipped), and a total of 20,007 fish from the seminatural raceway were coded-wire tagged (half of them adipose clipped). Roughly 30,000 coho salmon, which were adipose clipped but not coded-wire tagged, were also put into each raceway.

Sol Duc River Hatchery coded-wire tagged BY00 coho salmon into the conventional and seminatural raceways on 12 December 2001. For the conventional raceway, 12,629 coho salmon were coded-wire tagged and adipose clipped; 12,533 were coded-wire tagged only; and an additional 64,900 were adipose clipped only, and received no coded wire tag. The seminatural raceway received 12,805 coho salmon with coded-wire tags and adipose clips; 12,691 coded-wire tagged only fish; and an additional 64,900 adipose-clipped only coho salmon.

At Minter Creek Hatchery, 283,600 BY00 coho salmon were placed into experimental seminatural raceway habitat on 13 December 2001, of which a total of 20,320 fish were coded-wire tagged. A total of 283,600 (20,294 coded-wire tagged) conventionally-reared fish were placed into the matching control raceway on 21 December 2001.

Soos Creek coho salmon were placed into the seminatural raceway the second half of December 2001, during coded-wire tagging. Of those reared in the conventional raceway, 20,899 were coded-wire tagged, while 20,885 of those reared in the seminatural raceway were coded-wire tagged.

Issaquah Hatchery had 25,012 coho salmon tagged for controls and 25,514 tagged for seminatural rearing. Tagging took place 27 February through 1 March 2002. Fish were first placed into the experimental rearing habitat after coded-wire tagging.

BY 2001 Experimental Rearing Period

Untagged BY 2001 coho salmon were placed in the control and experimental raceways at Sol Duc River Hatchery in July 2002. Additional coho were coded-wire tagged into both control and experimental raceways in December 2002. There was a problem with the initial coded-wire tagging (the same tag codes ended up in both raceways), so fish were removed, scanned, and only those fish with no coded-wire tag in them received new tags near the end of December 2002.

Kendall Creek Hatchery also received fish in two batches – those not receiving coded-wire tags in September 2002, and those being coded-wire tagged the week of 11-15 November 2002. The control raceway received 10,056 (untagged) and 10,026 (coded-wire-tagged) fish, while the seminatural raceway received 10,055 (untagged) and 10,007(coded-wire-tagged) fish.

Issaquah Hatchery coded-wire tagged 25,051 coho salmon into the control raceway and 25,134 into the seminatural raceway. Tagging took 18 to 21 November 2002. Fish were held in similar (control) raceways prior to tagging, and loaded into the experimental raceway at time of tagging.

At Soos Creek Hatchery, fish were loaded into the control and experimental raceways at the time of coded-wire tagging. On 12 December 2002, the control raceway received 20,656 tagged coho salmon. On 13 December 2002, the experimental raceway received 20,881 tagged coho salmon.

Finally, fish which had already been coded-wire tagged in July 2002 and reared in similar (conventional) raceways were loaded into the control and seminatural raceways at Minter Creek Hatchery on 16 and 17 December 2002. A total of 21,118 tagged fish were loaded into the control raceway, and 21,183 into the seminatural raceway.

Growth, Coloration, and Health

BY 1999 Experimental Rearing Period

At all experimental sites a sample of 100 fish was removed monthly from each raceway, weighed (to the nearest 0.001 g), measured (fork length to the nearest 1 mm), and means compared with *t*-tests. At least 30 fish in each sample were photographed with 400 ASA color slide film using a Nikon 8008S single lens reflex camera equipped with a micro lens (60 mm) and circular polarizing filter. The camera was mounted on a photographic light stand equipped with two quartz halogen lamps (300 W). The light was filtered through photographic gel to simulate daylight.

Before being photographed, the fish were anesthetized in tricaine methane-sulfonate (MS 222) solution in black dishpans, and then placed individually on a clear acrylic angled stand over a standardized blue background. The fish were photographed at least twice.

Each photograph was mounted in a standard plastic slide mount and placed on a PVC plate (with the center drilled out) attached to the stage of a stereoscopic binocular microscope. A fiber-optic light illuminated the slide from below. The image was then recorded by a Hyper HAD RGB color video camera, captured, and processed by image analysis software. For skin color analysis, a rectangular section of the caudal fin was examined on each fish for its hue, intensity (brightness), and saturation value. These values make up the three axis of the color solid used to define solid colors (Rossotti 1983, Parker 1994). The values were compared with *t*-tests.

Near the time of release, 30 fish were sacrificed from each raceway for fish health examinations. In 2001, these examinations occurred on 10 May at Kendall Creek Hatchery, on 27 April at Soos Creek Hatchery (on a subsample of fish held back from the main release), and on 4 May at Minter Creek Hatchery. In each examination, the fish were first euthanized in MS 222 and then the external condition of the fish assessed using the Goede Index (Adams et al. 1993). Blood samples were then drawn to assess each fish's hematocrit, leukocrit, and serum protein profile. The coelomic cavity was then opened and the condition of major internal organs assessed using the Goede Index. A kidney smear was then plated on TSA agar to assay pathogen presence. Morphological and pathogen presence data were compared with 2 x 2 contingency table analysis. Blood parameters were arcsine transformed (hematocrit only) and compared with *t*-tests.

BY 2000 Experimental Rearing Period

Growth sampling and photography methods were the same as in the BY 1999 experimental rearing period, with the exception that a digital camera (Nikon D1) was used instead of the 35 mm slide film for some sampling dates. All Kendall Creek Hatchery photosampling was done using the digital camera. Other hatcheries were sampled with a mix of digital and slide technology. Slides were digitized using a Nikon LS-2000 slide film scanner.

Fish health examinations in 2002 took place on 10 May at Kendall Creek Hatchery, 12 April at Sol Duc River Hatchery, 1 May at Minter Creek Hatchery, 4 April at Soos Creek Hatchery, and 9 April at Issaquah Hatchery. All samples were taken at or immediately prior to release. Both hematocrit and leukocrit values were arcsine transformed prior to analysis. Otherwise fish health assessment was identical to 2001.

BY 2001 Experimental Rearing Period

Growth sampling and photography methods were the same as in the BY 1999 and BY 2000 study cycles. A digital Nikon D1 camera was used for all photographs for BY 2001.

Fish health examinations in 2003 took place on 11 April at Sol Duc River Hatchery, 14 April at Kendall Creek Hatchery, 17 April at Issaquah Hatchery, 15 April at Soos Creek Hatchery, and 2 May at Minter Creek Hatchery. All samples were taken at or immediately prior to release. Both hematocrit and leukocrit values were arcsine transformed prior to analysis. Otherwise fish health assessment was identical to 2001 and 2002.

Additionally, in 2003 photographs were taken of the raceway bottoms (conventional and seminatural) at the time of release. Once fish were out of the raceways, and water drained, the same set-up (camera and light stand) used for fish coloration photography was used under a black plastic tarp to photograph 20 different locations of the raceway bottom surface. This data will be used to compare final coloration values of fish at time of release to their rearing background. This data has not been analyzed at this time.

Smolt-to-Adult Survival Evaluation

BY 1999 Experimental Rearing Period

Fish were released on site at each of the facilities following standard WDFW protocols. The fish at Kendall Creek Hatchery were released on 16 May 2001 following 18 weeks of experimental rearing. On 19 April 2001, following 8 weeks of experimental rearing, fish were released from Soos Creek Hatchery. At Minter Creek Hatchery fish were volitionally released overnight on both 15 and 16 May 2001, and all remaining fish were direct (non-volitional) released on 17 May 2001 after 11 weeks of experimental rearing.

BY 2000 Experimental Rearing Period

Fish releases in 2002 were done as in 2001. Fish were released from Kendall Creek Hatchery on 15 May 2002, after 41 weeks in experimental rearing. Fish were released from Sol Duc River Hatchery on 15 April 2002, after 18 weeks of experimental rearing. At Minter Creek Hatchery fish were released on 1 May 2002, after 19.5 weeks of experimental rearing. Soos Creek Hatchery released the fish on 8 April 2002, following 17 weeks of experimental rearing. Finally, Issaquah Hatchery fish were released on 15 April 2002, following 6.5 weeks in experimental habitat. All releases were direct (non-volitional) releases.

BY 2001 Experimental Rearing Period

Fish releases in 2003 were done as in 2001and 2002. Fish were released from Sol Duc River Hatchery on 17 and 18 April 2003. Fish were released from Kendall Creek Hatchery on 14 May 2003, after 26 weeks of experimental rearing (from addition of coded-wire tagged fish to raceways). Issaquah Hatchery released fish on 21 April 2003, following 22 weeks of experimental rearing. Fish were released from Soos Creek Hatchery on 20 April 2003, after 18 weeks of experimental rearing. Minter Creek released fish on 7 May 2003, following 20 weeks of rearing in experimental habitat. All releases were direct (non-volitional) releases.

Project Management

This project was collaboratively managed by NMFS and WDFW. Coded-wire tagging and fish rearing was primarily performed by WDFW. Seminatural raceway habitat development and installation was primarily performed by NMFS. Data collection and analysis were conducted collaboratively by the two agencies.

Findings

Growth, Coloration, and Health

BY 1999 Experimental Rearing Period

All of the growth, coloration, and fish health samples for the first rearing year have been processed and statistically analyzed. From tagging until they were placed into the experimental raceways the fish were reared in separate, but similar, rearing units. The staff at each hatchery was advised to rear the fish in an identical fashion during this time period. None the less, the lag between tagging and placement into experimental raceway habitat provided an opportunity for differences to develop between the paired groups prior to experimentation. At all three facilities, sampling did not begin until at least 1 week after fish were ponded into the experimental raceways.

At all three hatcheries, some size differences already existed between the paired rearing treatments at first sampling. Fish at Kendall Creek Hatchery did not differ significantly in length one week after the initiation of experimental rearing (Fig. 1), though the control fish did weigh significantly more than their seminatural counterparts ($P = 0.021$; Fig. 2). By the second sampling period, five weeks after the beginning of the experiment, fish did not differ in either length or weight, and this similarity continued throughout the duration of rearing. At experiment initiation, fish at Soos Creek Hatchery differed significantly in length ($P = 0.025$; Fig. 3), but not in weight (Fig. 4). Size had equalized by the second sampling period, and fish remained similar in size for the duration of rearing. Minter Creek Hatchery fish differed in weight ($P = 0.029$), but not in length ($P = 0.076$) two weeks after initiation of experimental rearing (Figs. 5 and 6). These differences had disappeared by the second sampling period and did not develop again. In summary, the trend at all three rearing facilities was for the size difference between the treatments to close by time of release.

Even at first sampling, the color of the fish in the two rearing treatments differed on at least one of the three color axes (hue, saturation, and intensity). In general, hue relates to the selective reflectance of the surface for particular wavelengths of light, intensity relates to the overall reflectance of white light, and saturation depends primarily on the purity of the band of wavelengths reflected by the surface (Parker 1994). At Kendall Creek, both hue and intensity were statistically similar one week after ponding, but saturation was significantly different ($P = 0.000$; Figs. 7, 8, and 9). The difference in this variable was maintained throughout rearing. Significant differences developed in hue by the second sampling period, and differences in intensity did not appear until the third sampling period. One week prior to release, again only two of the three variables differed significantly, with intensity being no longer significant ($P = 0.053$).

At Soos Creek saturation was also the only one of the three coloration variables to differ significantly at the first sampling period (P = 0.005; Figs. 10, 11, and 12). Differences in hue were not detected until the last coloration sample, but intensity differences were significant (P = 0.008) by the second sampling period. All three color axes were significantly different at the final sample.

At Minter Creek neither hue nor saturation differed significantly in the first coloration sample (Figs. 13 and 14), though intensity was significantly different (P = 0.000; Fig. 15). All three color axes were significantly different one month later. These color differences were maintained through the final coloration sample 14 May 2001.

In summary, by release, it appears measurable color differences had developed between the fish in the control and NATURES rearing treatments at all three experimental facilities.

In 2001, there were no consistent or major differences in the health of the fish in the two rearing treatments. At Kendall Creek Hatchery no statistically significant differences were detected in fish condition by the Goede Index (Figs. 16 and 17), except in the amount of hematocrit in the blood. Fish from the seminatural raceway displayed higher red blood cell counts (P = 0.005; Fig. 17). At Soos Creek Hatchery, the only variable to show significant differences from the fish condition profile was the bile (P = 0.010; Fig. 18). None of the blood variables were statistically different (Fig. 19). At Minter Creek Hatchery there were no detectable differences in the fish condition profile (Figs. 20 and 21).

BY 2000 Experimental Rearing Period

All the growth, coloration, and fish health samples for the BY 2000 experiment have now been processed and statistically analyzed. From tagging until they were placed into the experimental raceways, the fish were reared in separate, but similar, rearing units. The staff at each hatchery was advised to rear the fish in an identical fashion during this time period. None the less, the lag between tagging and placement into experimental raceway habitat provided an opportunity for differences to develop between the paired groups prior to experimentation. The time from fish distribution into the experimental raceways to first sampling varied between hatcheries.

In 2002, unlike in 2001, fish in the two treatments were similar in size at four of the five hatcheries at the beginning of sampling. Only Kendall Creek Hatchery had size differences, which was also the only hatchery where fish had been in the raceways for longer than 1 month prior to sampling initiation. Both length (P = 0.050; Fig. 22) and weight (P = 0.035; Fig. 23) were significantly different at this time. These size differences disappeared by the second sampling and were not observed again through release. Fish at both Sol Duc and Minter Creek hatcheries remained similar in size throughout the duration of the study (Figs. 24, 25, 26, and 27). Fish size did not differ significantly at Soos Creek Hatchery at either the first or second sample (Figs. 28 and 29). Both length (P = 0.005) and weight (P = 0.004) were significantly different in March, but these differences were removed by release. Issaquah Hatchery coho salmon were not significantly different in size at the beginning of experimental rearing (Figs. 30 and 31), but both length (P = 0.007) and weight (P = 0.011) were significantly different at the final sample.

At Kendall Creek Hatchery at the first sampling the coloration of fish in the two rearing treatments differed significantly on two of the three color axes (Figs. 32, 33, and 34). Hue and saturation were significantly different (P = 0.000 for both), but intensity was not (P = 0.368).

This trend was repeated one month later, and reversed the following month. At each of the last two samples, including 1 week prior to release, all three color axes differed significantly.

Sol Duc Hatchery coloration was significantly different for all three color axes at first sampling (Figs. 35, 36, and 37). One month later, hue was not significantly different ($P = 0.464$), but saturation ($P = 0.000$) and intensity ($P = 0.000$) still differed. By the third month, there were no longer any coloration differences between treatments, and likewise for the fourth sample. At the time of release, hue ($P = 0.002$) and saturation ($P = 0.000$) again differed significantly, but intensity was not significantly different ($P = 0.088$).

On the day after fish were placed into seminatural rearing habitat at Minter Creek Hatchery, saturation differed significantly ($P = 0.000$; Fig. 38) between treatments, but not hue or intensity (Figs. 39 and 40). One month later, significant differences in hue had developed ($P = 0.004$), saturation differences remained, but intensity differences had not developed. Inversely, at the third sampling date, neither hue nor saturation differed significantly, but intensity did ($P = 0.000$). By the fourth sampling date, hue and saturation differences returned, with intensity again being similar. None of the three color axes differed significantly at the fifth sampling. On the day of release, hue was statistically similar, but saturation ($P = 0.000$) and intensity ($P = 0.018$) were significantly different.

At Soos Creek Hatchery, hue differences ($P = 0.000$; Fig. 41) had developed by first sampling, approximately three weeks after initiation of experimental rearing, while saturation and intensity were similar (Figs. 42 and 43). Of the three color axes, only intensity differed significantly ($P = 0.005$) at the time of the second sample. Both hue ($P = 0.000$) and intensity ($P = 0.001$) were significantly different by the third sample. All three color axes differed significantly by the final sample.

Coho salmon at Issaquah Hatchery did not differ in coloration at the first sampling date, less than 1 week after placement into experimental rearing habitat (Figs. 44, 45, and 46). At the time of final sampling, however, all three color axes differed significantly.

In 2002, there were no consistent or major differences in the health of the fish in the two rearing treatments. At Kendall Creek Hatchery no statistically significant differences were detected in fish condition by the Goede Index (Figs. 47 and 48). There were also no statistically significant differences in fish condition or blood variables at Sol Duc River Hatchery (Figs. 49 and 50), nor at Minter Creek Hatchery (Figs. 51 and 52). At Soos Creek Hatchery no statistically significant differences were detected in fish condition by the Goede Index (Figs. 53 and 54), except in the amount of hematocrit in the blood. Fish from the seminatural raceway displayed lower red blood cell counts ($P = 0.000$; Fig. 54). Issaquah Hatchery coho salmon displayed no differences in fish condition (Fig. 55), except for elevated plasma protein in the blood of seminaturally-reared coho salmon ($P = 0.009$; Fig. 56). Hematocrit was not significantly different (Fig. 56).

BY 2001 Experimental Rearing Period

All the growth and fish health samples, and the vast majority of the coloration samples for this third rearing year have now been processed and statistically analyzed. From tagging until they were placed into the experimental raceways the fish were reared in separate, but similar, vessels. The staff at each hatchery was again advised to rear the fish in an identical fashion during this time period. None the less, the lag between tagging and placement into experimental raceway habitat provided an opportunity for differences to develop between the paired groups prior to experimentation. The time from fish distribution into the experimental raceways to first sampling varied between hatcheries.

At experiment initiation in 2003, fish at three of the five hatcheries were similar in size. Fish at Sol Duc Hatchery were statistically similar in size at initial placement into experimental raceways. By the second sampling weights differed statistically, and by the third sampling both length and weight were significantly different (Figs. 57 and 58). At the fifth sampling, size differences no longer existed, and fish remained similar in size throughout the remainder of rearing. Kendall Creek Hatchery fish were similar at experiment initiation, but differences in weight developed in the first month (Fig. 60). Sizes were similar again one month later, but both length (Fig. 59) and weight differed at the fourth sampling. Sizes evened out by the next sample, and had changed direction by the seventh sample (when both length and weight again significantly differed). These differences remained one month later, but were gone by the time of release. Issaquah Hatchery had control fish which weighed significantly less than treatment fish at the beginning of the experiment (Fig. 62). Both length (Fig. 61) and weight differed at the fourth sampling, but fish sizes were similar by the time of release. Soos Creek Hatchery fish were similar in size at first sampling, but weights were different by the second sampling (Fig. 64). Length also differed by the third sampling (Fig. 63). Sizes were similar one month prior to release, but both length and weight were significantly different at release. Finally, Minter Creek Hatchery control fish weighed more in the beginning than did seminatural rearing fish (Fig. 66). This had reversed by the next month, when both length (Fig. 65) and weight were higher for treatment fish. By the third month, however, sizes at Minter Creek Hatchery were similar and remained that way through release.

At Sol Duc River Hatchery at the first sampling the coloration of the fish in the two treatments were statistically similar on two of the three color axes (Figs. 67, 68, and 69). Only saturation ($P = 0.009$) differed at this time. One month later hue was significantly different ($P = 0.031$), but neither saturation nor intensity differed. Results from the third sample showed differences in both saturation ($P = 0.041$) and intensity ($P = 0.001$), but not hue. Only intensity differed at the fourth sampling ($P = 0.000$), when coded-wire tagged fish were added to the population, and all three color axes were similar at the fifth sampling. There was only one sampling when all three values differed significantly, and that was the sixth. All color axes were again similar 10 days prior to release.

When fish were first placed in to the study raceways at Kendall Creek Hatchery, no color differences existed in any of the three color axes (Figs. 70, 71, and 72). At the third sampling, when coded-wire tagged fish only were sampled (as they were being added to the population), hue was significantly different between treatments ($P = 0.022$), but saturation and intensity were similar. Hue differences persisted one month later, when the sample was taken from the entire population (tagged and untagged), hue still differed significantly ($P = 0.000$), and saturation also differed ($P = 0.009$) but intensity was similar. These results were reversed at the fifth sampling, when only intensity differed ($P = 0.004$). Saturation was the only color variable which differed significantly ($P = 0.007$) in month 6. Intensity was again the only different color axis in the seventh and eighth samples ($P = 0.000$ and 0.005, respectively). All three color axes were significantly different on the day of release (hue, $P = 0.004$; saturation, $P = 0.000$; intensity $P = 0.046$).

Coloration at Issaquah Hatchery was initially sampled the day after they were tagged in to the study raceways. Two of three color axes, saturation and intensity ($P = 0.001$ for both), were significantly different at this time (Figs. 73, 74, and 75), but hue was not. Differences in saturation had disappeared one month later, when only intensity differed significantly (P = 0.034). No differences existed at the third sampling. All three color axes were significantly at month 4, but those differences were gone in both hue and saturation one month later, when only intensity was still different (P = 0.001). No color differences were detected 4 days prior to release.

Soos Creek Hatchery coloration was similar on all three color axes five days after tagging in to the study raceways (Figs. 76, 77, and 78). Hue differences developed by the second sample (P = 0.002), and remained through the third sample (P = 0.005) when intensity also differed (P = 0.007). No differences existed for any of the three color axes at the fourth sampling. Saturation (P = 0.001) and intensity (P = 0.000) were significantly different 5 days prior to release, but hue was similar.

Two days after being moved in to the study raceways at Minter Creek Hatchery, one of three color axes was significantly different (Figs. 79, 80, and 81). Hue and intensity were similar, but saturation differences were statistically significant (P = 0.018). One month later, saturation differences were gone, but intensity differences (P = 0.000) had developed, and remained for the remainder of rearing. Hue differences had developed (P = 0.001) by the third sampling, were not apparent in month four, but had reappeared (P = 0.027) 5 days prior to release. Saturation was again significantly different at the third sample (P = 0.001), but disappeared after that for the remainder of rearing.

In 2003, there were again no overall major differences in the health of the fish in the two rearing treatments. At Sol Duc River Hatchery, the Goede Index detected no statistical differences in any parameter except bloodwork (Figs. 82 and 83). Fish in the seminatural raceway had a lower red blood cell count (P = 0.000), a higher white blood cell count (P = 0.013), and less plasma protein in the blood (P = 0.003). There were no differences in any parameter detected at either Kendall Creek Hatchery (Figs. 84 and 85) or Issaquah Hatchery (Figs. 86 and 87). Seminaturally reared fish at Soos Creek Hatchery had higher red blood cell count (P = 0.030) and lower white blood cell count (P = 0.005) than their conventionally reared counterparts, but no other differences in health (Figs. 88 and 89). Minter Creek coho salmon from the seminatural raceway had a lower red blood cell count that from the control raceway (P = 0.011), but no other differences in health parameters (Figs. 90 and 91).

Smolt-to-Adult Survival Evaluation

Data on returns of coho salmon released in 2001 will not be available until fall 2004. Return data of coho salmon released in 2002 should be available for analysis beginning in 2005. Return data for 2003 coho salmon releases should be available for analysis beginning in 2006.

General Observations

During all three study years, the pavers at Kendall Creek Hatchery, Soos Creek Hatchery, and Minter Creek Hatchery retained their basic coloration during the experimental rearing period. This was evidenced by looking down through the water and being able to observe the variety of colored stones that covered the pavers' surface. Although the difference was greatest when fish were initially ponded into the study raceways, they were clearly distinguishable when the fish were released. This was not the case at Sol Duc Hatchery where pavers in both the control and experimental Burrow's ponds were rapidly covered by a lush brown algae mat in both rearing years. In both years, the development of white fungus growth on decaying material that collected under the NATURES structure (trees) in the raceways and lodged between stones on the paver surface at Sol Duc resulted in the bottom color of the seminatural pond being visually lighter than the control pond. A thick layer of fine light colored sand usually covered at least half the length of the control and seminatural pond at Issaquah Hatchery during both study years. Although the ponds were frequently vacuumed, each new spring freshet would recover the raceway bottom with a new layer of sand. Except for Issaquah, differences in substrate rugosity were maintained throughout the experimental rearing period. With the exception of Sol Duc algal growth on the pond walls was minimal.

Discussion

Our results demonstrate that the structural components of seminatural raceway habitat retain their durability and compatibility with daily fish culture activities at salmon enhancement facilities. Even after three years of active use, the concrete pavers exhibited very little stone loss, no cracking, and had no noticeable color fading. Their rugosity continued to aid in settling solids (waste and sediment) within the raceway, while still providing a mottled background. The hydraulic lifts used on the hinged covers at facilities except Minter Creek Hatchery are easily operated by hatchery staff. The structural failure of covers was limited to broken frame welds on the first frame design. The second generation frames exhibited no weld cracks and the problem with the older frames was easily solved with a simple reinforcement. Snow load problems did not reoccur with the Minter Creek Hatchery rolling frames after the camouflage net was suspended below, rather than above, the frame. The fir tree instream structure has remained problem-free, requiring no other maintenance than the replacement of trees at some of the facilities after 2-3 years of use. In conclusion, the structural elements of seminatural raceway habitat have developed to the point that additional engineering development is not needed.

Seminaturally-reared coho salmon outgrew their conventionally-reared counterparts in six out of thirteen cases. In four of thirteen cases, the growth rates of fish in the two treatments was similar from beginning to end. In only three of thirteen cases, the conventionally-reared fish outgrew seminaturally-reared fish. Overall, seminatural raceway habitat seems to have no effect or actually improves growth of coho salmon. This contrasts with our earlier chinook salmon studies where seminatural raceway habitat seemed to cause a slight reduction in growth (Maynard et al. 1996a,b,d). The difference, may relate to the reluctance of Chinook salmon to feed on food that has fallen into spaces between the gravel in the seminatural raceways. Hatchery coho salmon are known to have no reluctance to pick food up off the bottom, thus we would expect both rearing types to grow at similar rates. It has also been suggested that the 0.1° C lower water temperature that camouflage net shading produced in the seminatural habitat raceways may have reduced the growth of Chinook salmon. It is unknown if similar temperature reductions occurred in the seminatural raceways in the current coho study. The improved growth in coho salmon in seminatural raceways might also be related to a stress reduction associated with the cover and inwater structure.

The development of color differences between the two rearing treatments in this third study year is less pronounced than observed in the two previous years and with chinook salmon (Maynard et al. 1996b,d). At release, the base coloration of seminautral reared coho salmon at Issaquah and Sol Duc did not significantly differ from the controls, while those at Minter, Soos Creek, and Kendall did. Presumably this is the result of the former two facilities having similar colored bottoms in control and experimental raceways, while the latter three vessels had different colored bottoms. The short duration Issaquah and Sol Duc ponds were operated in 2002 may may have resulted in the ponds retaining some of their color differences. This might explain why significant color differences were detected in the earlier year. If release coloration is a driving force behind survival we can predict there will be no difference in adult returns for BY 2001 coho salmon from Sol Duc and Issaquah, but there should be between treatments at Kendall, Soos Creek, and Minter.

As in past experiments, the health of the fish in seminatural and conventional raceway habitat appeared to be similar. The differences that did occur were not consistent across all rearing facilities, suggesting major treatment differences do not exist.

The preliminary study findings indicate that seminatural raceway habitat can be operated at production scale facilities and produce apparent beneficial biological rearing effects in coho salmon similar to those that seem to improve the instream postrelease survival of chinook salmon. Fishery managers can use this increased postrelease survival to improve hatchery efficiency, increase harvest, speed the rebuilding of self-sustaining natural runs through supplementation, or simply reduce the ecological impact of hatchery fish by lowering release numbers while maintaining recruitment.

The BPA-funded portion of these NATURES studies terminated in February 2004. However, portions of this research will continue into the future with support provided by the western Washington Hatchery Scientific Review Group (HSRG), WDFW, and NOAA-Fisheries. This work will include release of smolts in spring 2004 and analysis of adult returns in 2004-2008.

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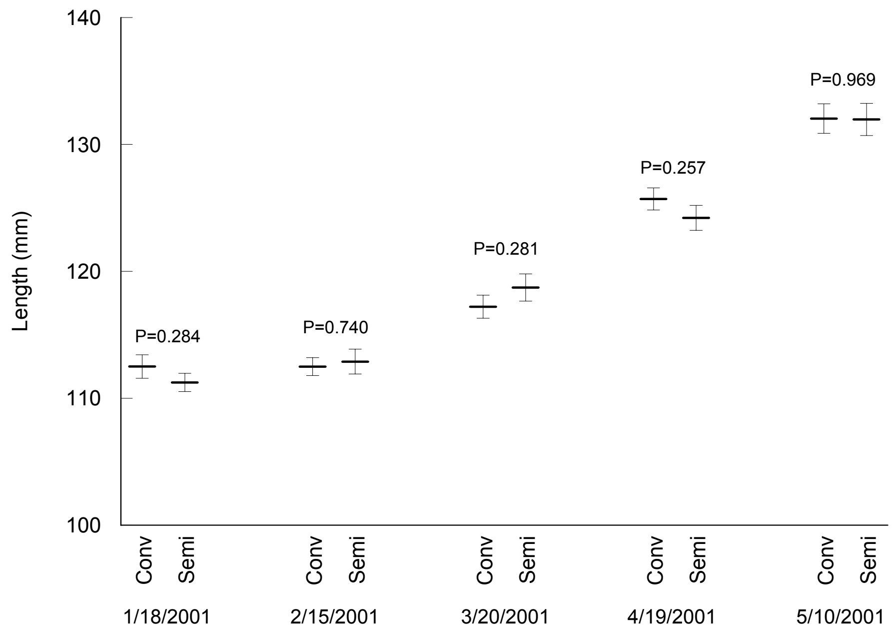

Figure 1. Mean length (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Kendall Creek Hatchery in 2001 (N = 100 per treatment, except N = 30 per treatment on 5/10/2001). P values are based on *t*-tests.

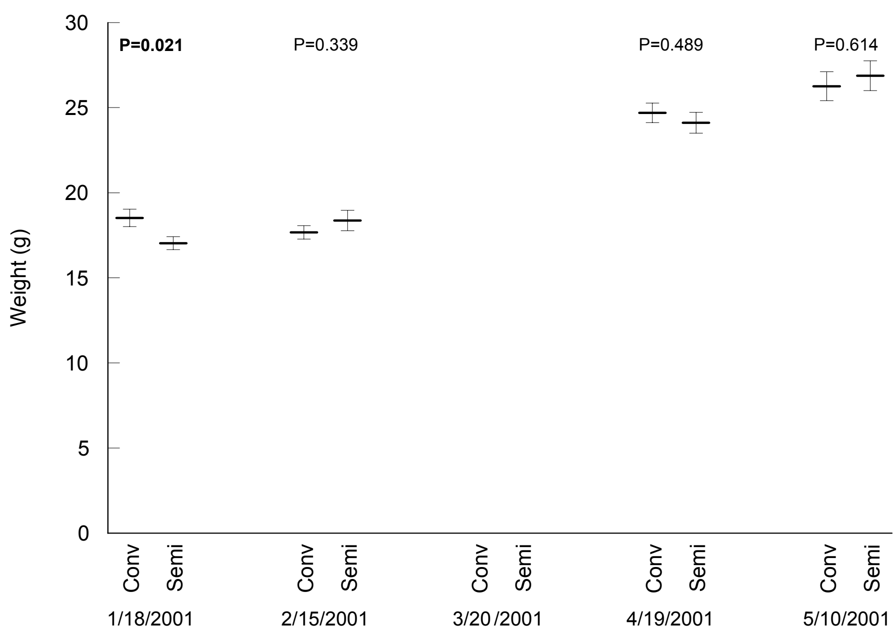

Figure 2. Mean weight (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Kendall Creek Hatchery in 2001 (N = 100 per treatment, except N = 30 per treatment on 5/10/2001). P values are based on *t*-tests.

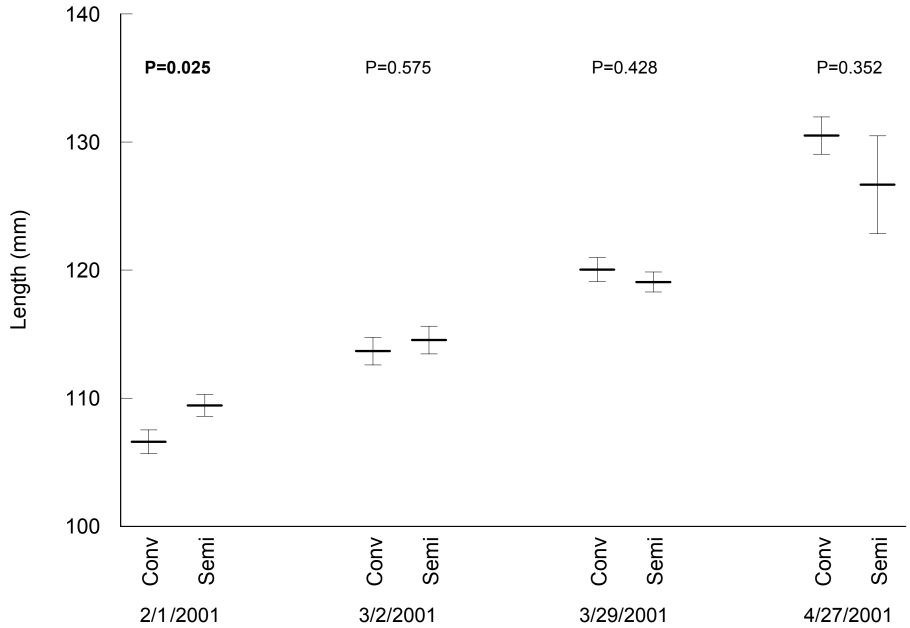

Figure 3. Mean length (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Soos Creek Hatchery in 2001 (N = 100 per treatment, except N = 30 per treatment on 4/27/2001). P values are based on *t*-tests.

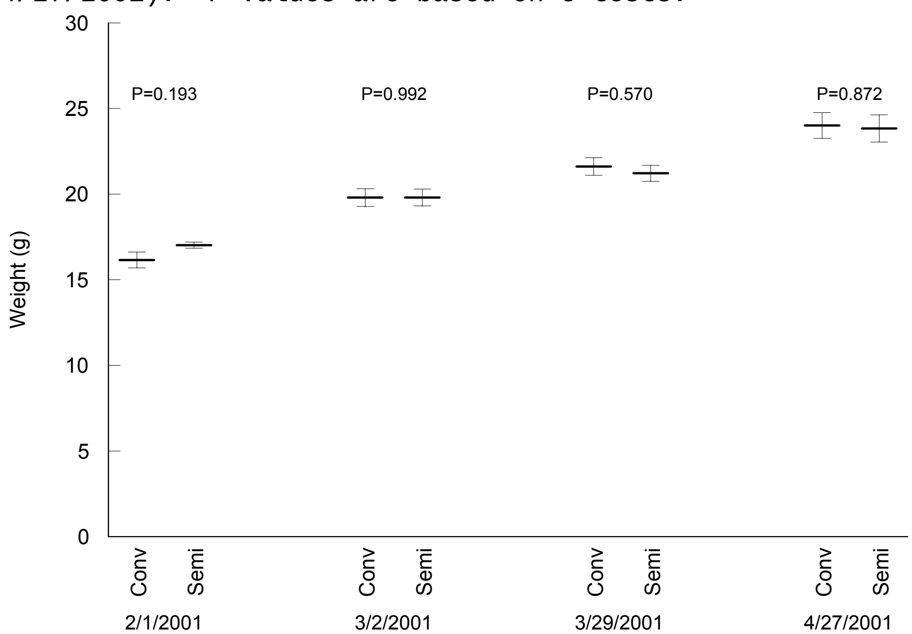

Figure 4. Mean weight (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Soos Creek Hatchery in 2001 (N = 100 per treatment, except N = 30 per treatment on 4/27/2001). P values are based on *t*-tests.

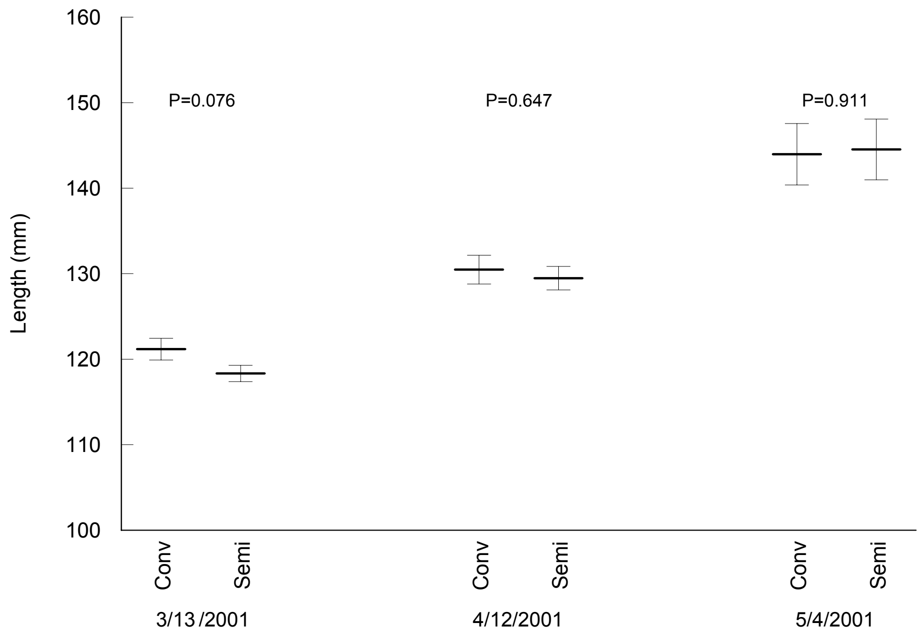

Figure 5. Mean length (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Minter Creek Hatchery in 2001 (N = 100 per treatment, except N = 30 per treatment on 5/4/2001). P values are based on *t*-tests.

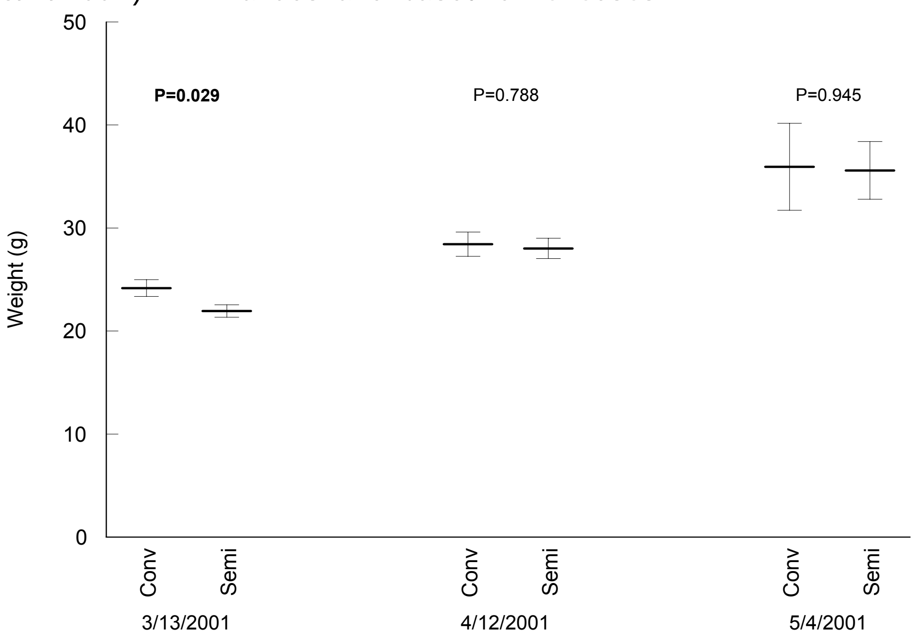

Figure 6. Mean weight (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Minter Creek Hatchery in 2001 (N = 100 per treatment, except N = 30 per treatment on 5/4/2001). P values are based on *t*-tests.

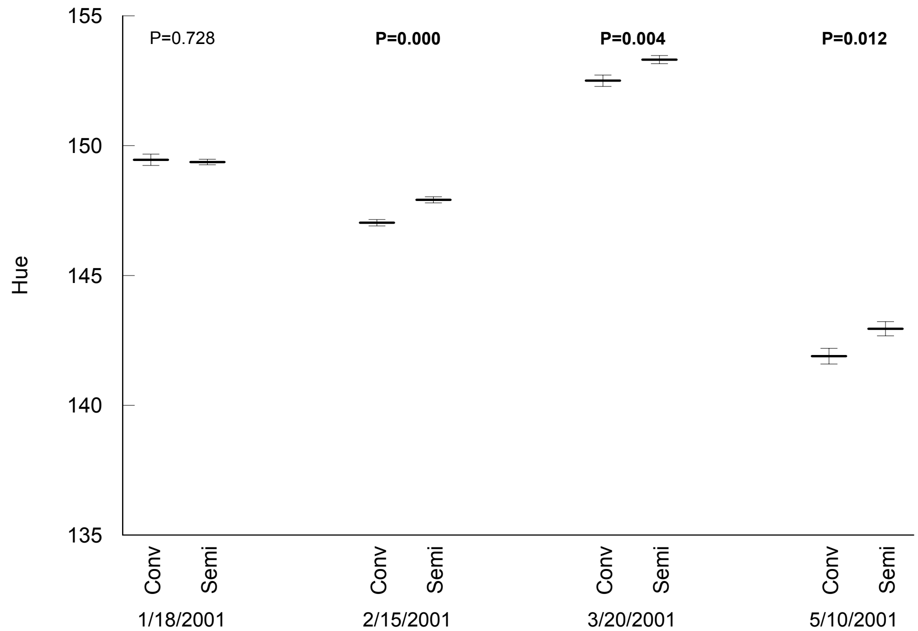

Figure 7. Mean hue values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Kendall Creek Hatchery in 2001 (N = 30 per treatment). P values are based on t-tests.

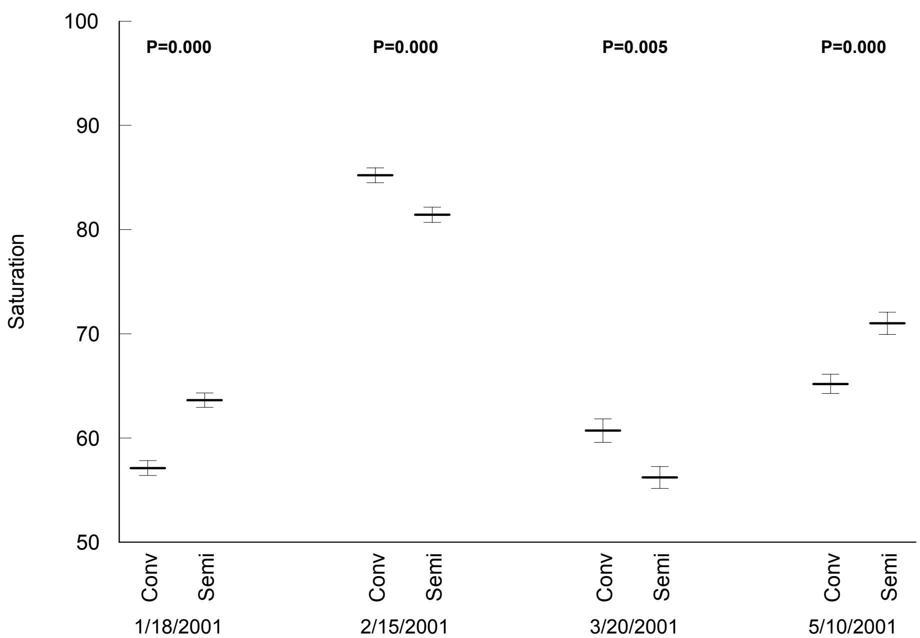

Figure 8. Mean saturation values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Kendall Creek Hatchery in 2001 (N = 30 per treatment). P values are based on *t*-tests.

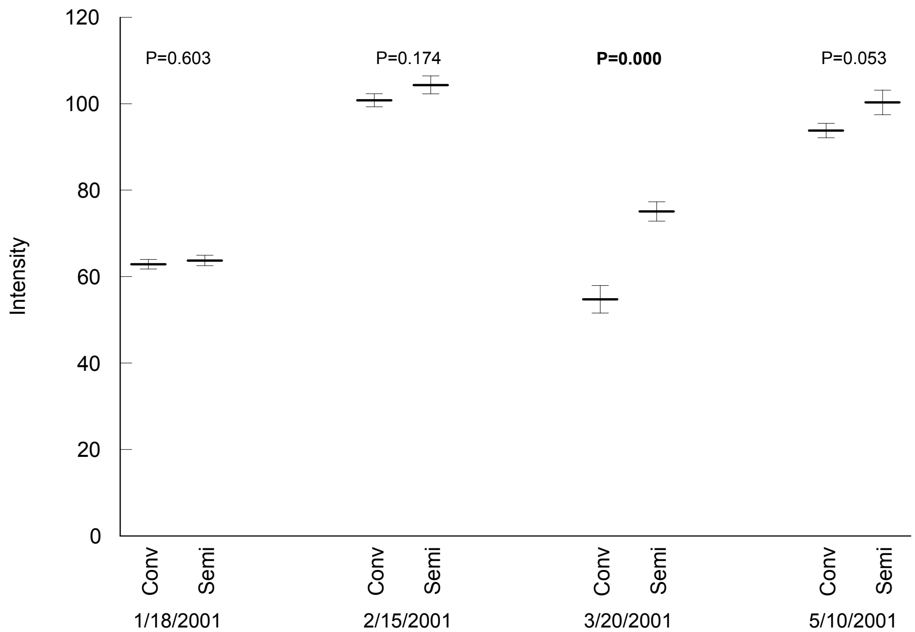

Figure 9. Mean intensity values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Kendall Creek Hatchery in 2001 (N = 30 per treatment). P values are based on *t*-tests.

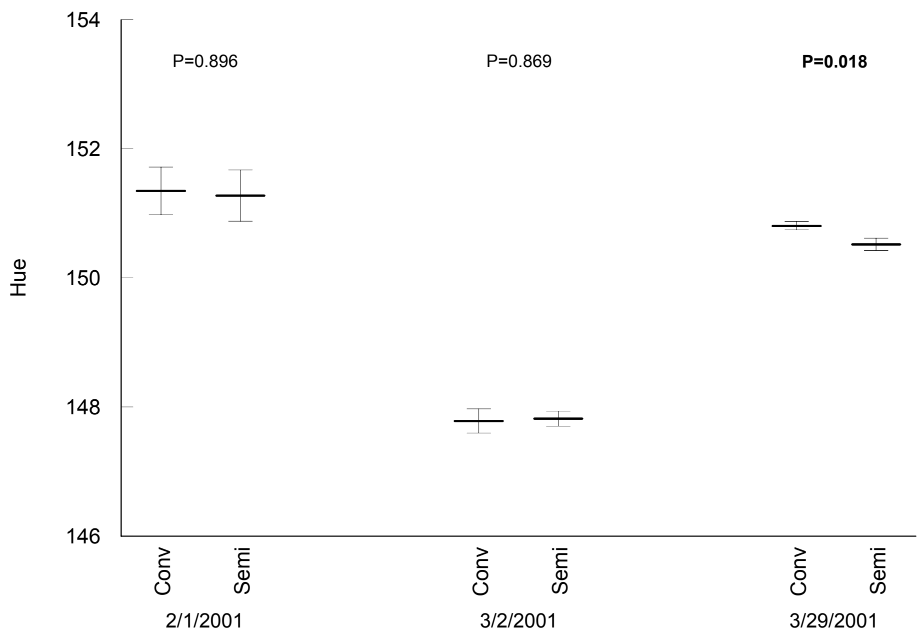

Figure 10. Mean hue values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Soos Creek Hatchery in 2001(N = 30 per treatment). P values are based on *t*-tests.

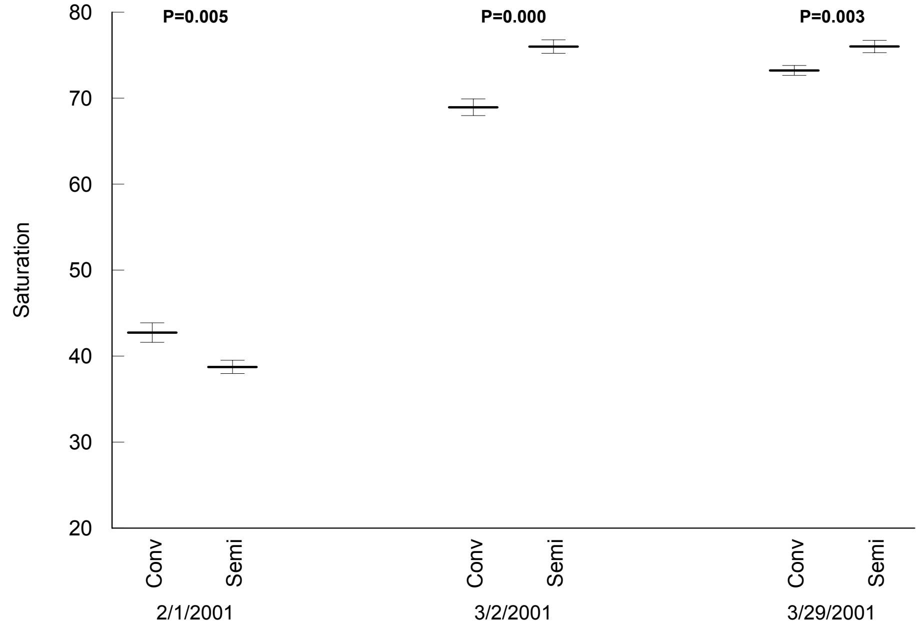

Figure 11. Mean saturation values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Soos Creek Hatchery in 2001 (N = 30 per treatment). P values are based on *t*-tests.

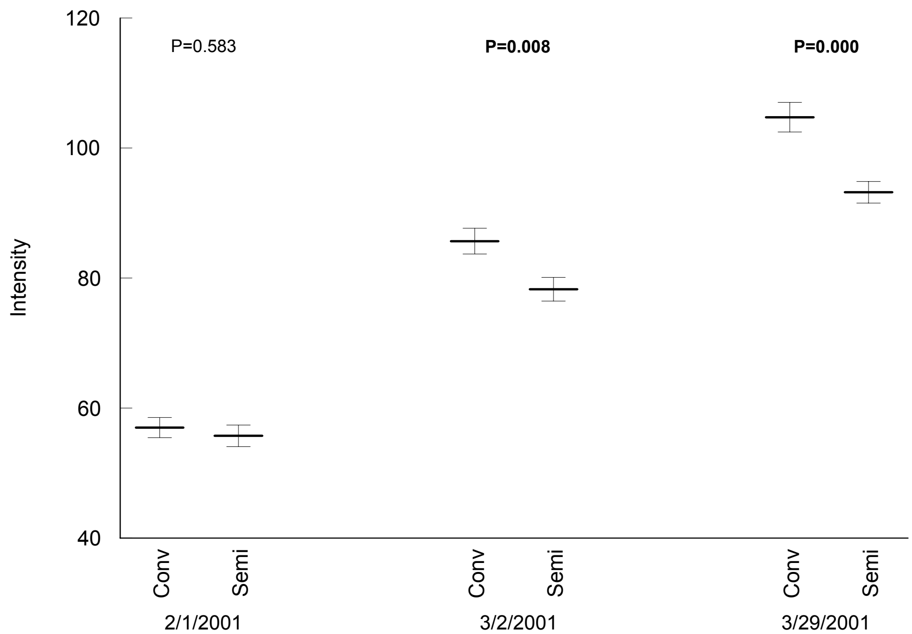

Figure 12. Mean intensity values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Soos Creek Hatchery in 2001(N = 30 per treatment). P values are based on *t*-tests.

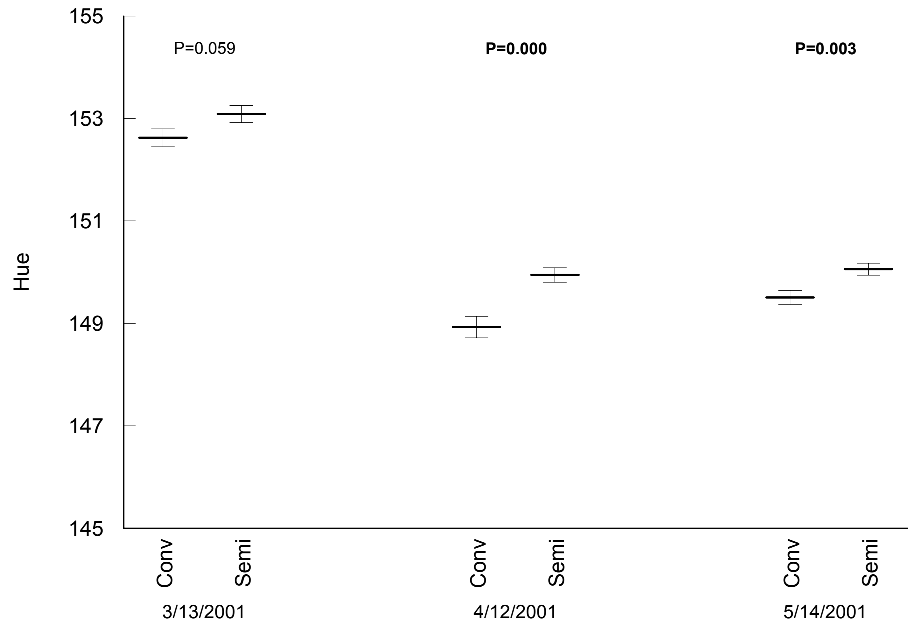

Figure 13. Mean hue values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Minter Creek Hatchery in 2001 (N = 30 per treatment). P values are based on *t*-tests.

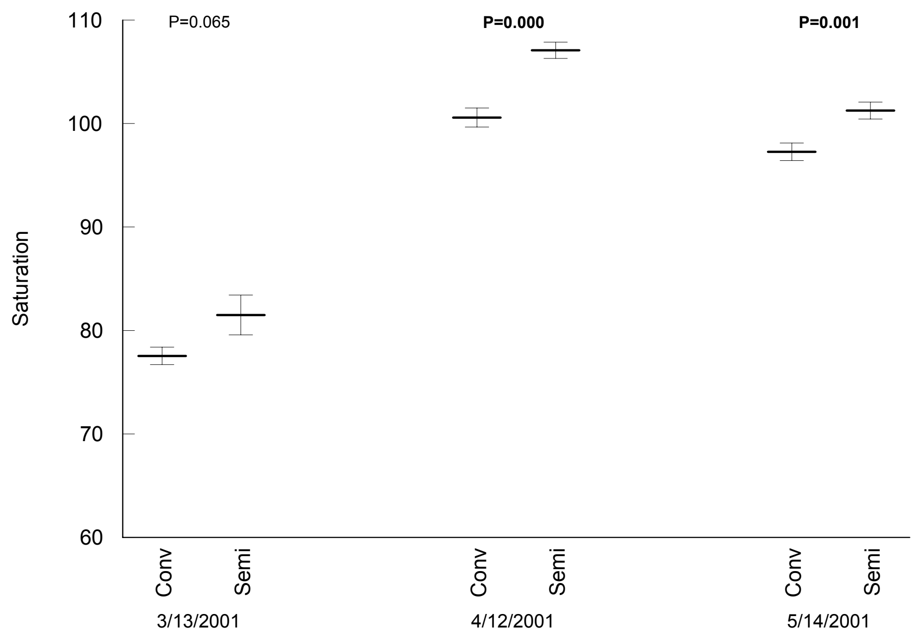

Figure 14. Mean saturation values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Minter Creek Hatchery in 2001 (N = 30 per treatment). P values are based on *t*-tests.

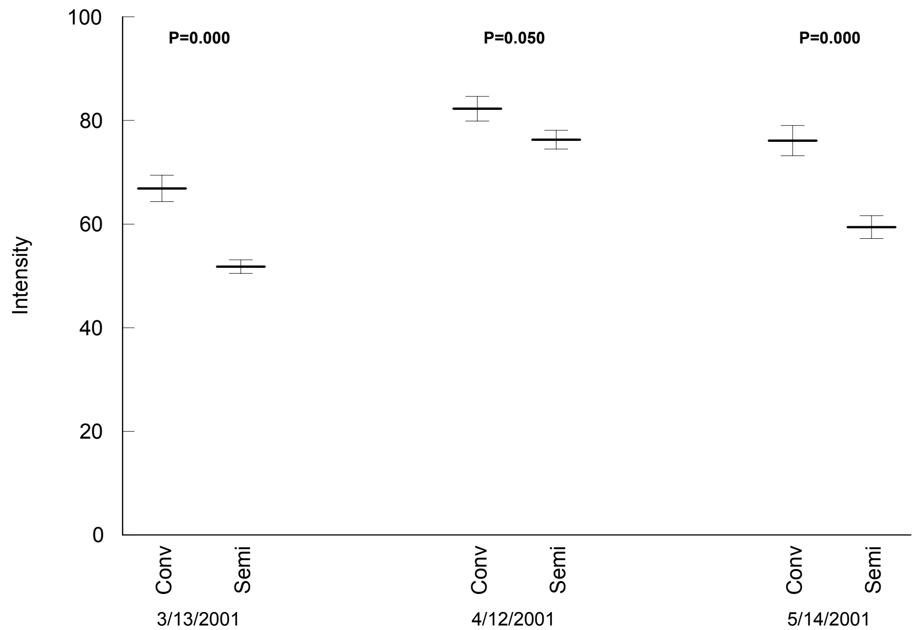

Figure 15. Mean intensity values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Minter Creek Hatchery in 2001 (N = 30 per treatment). P values are based on *t*-tests.

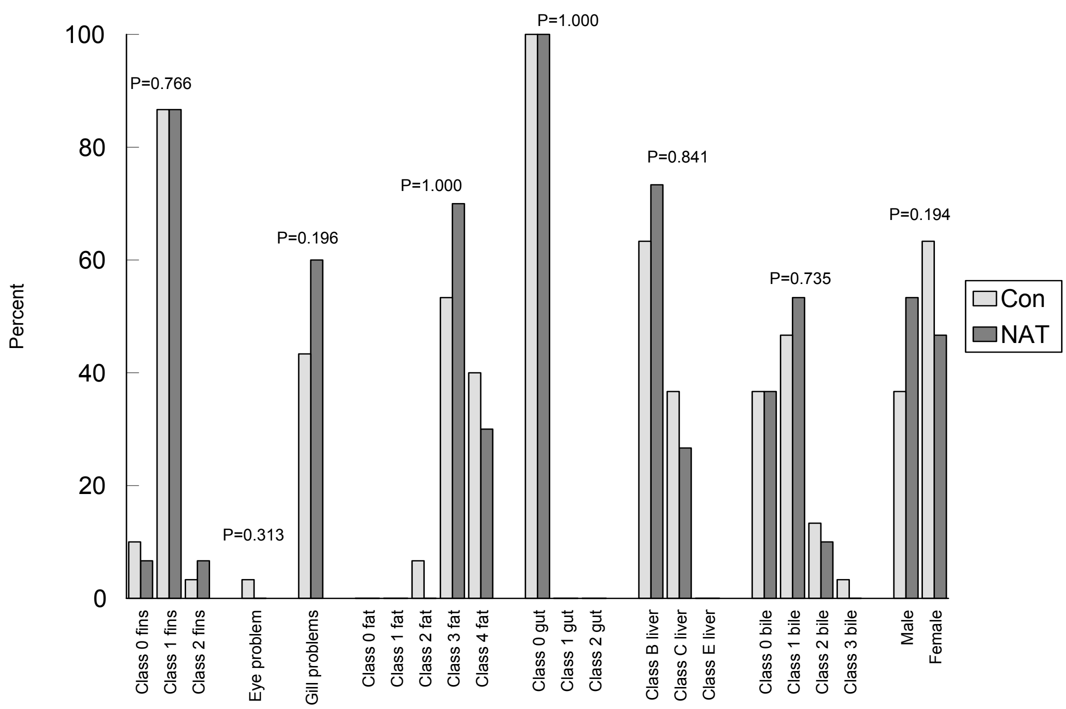

Figure 16. Percentage of coho salmon in different Goede Index classes in the 10 May 2001 Kendall Creek fish condition profile. Fish were reared in seminatural (NAT, N = 30) or conventional (Con, N = 30) raceways. P values are based on contingency table analysis.
A) B) C)

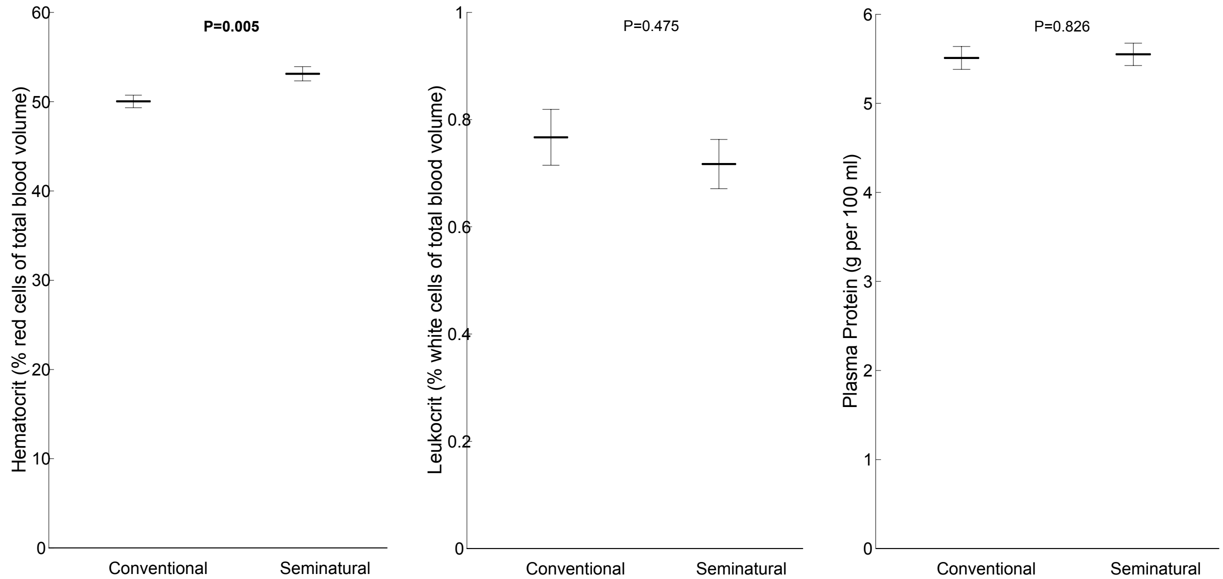

Figure 17. Means (with standard error bars) of blood variables from coho salmon reared in seminatural or conventional raceways at Kendall Creek Hatchery sampled on 10 May 2001. A) hematocrit (N = 30 per treatment) P value based on *t*-tests of arcsine transformed data; B) leukocrit (N = 30 per treatment) P value based on *t*-tests; and C) plasma protein (N = 29 conventional and 28 seminatural) P value based on *t*-tests.

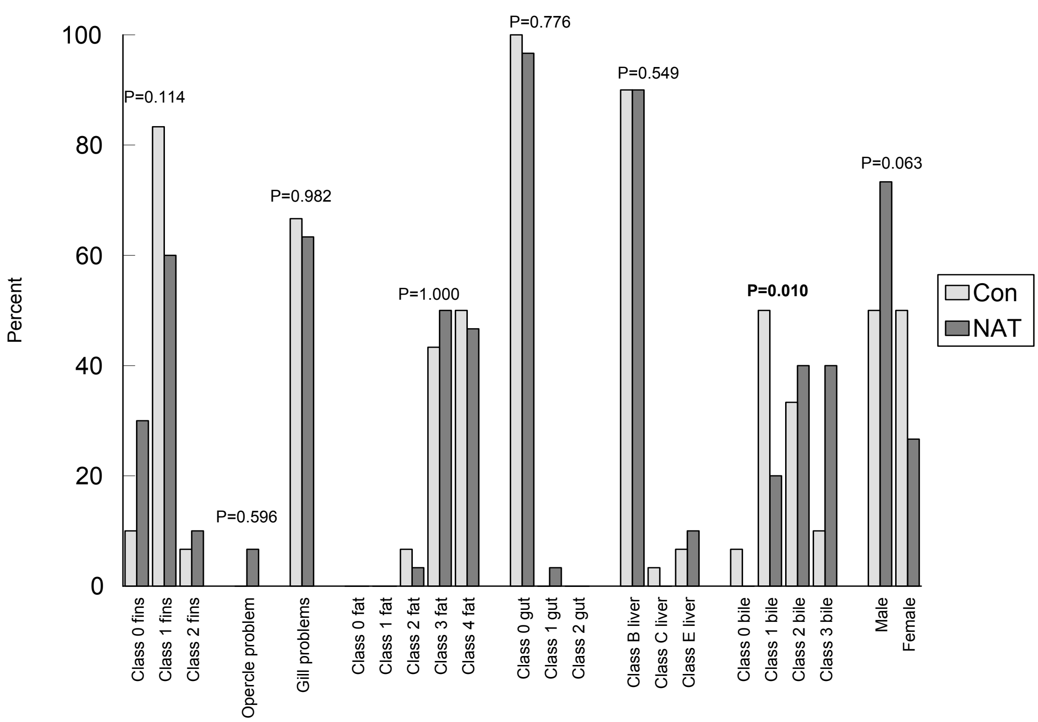

Figure 18. Percentage of coho salmon in different Goede Index classes in the 27 April 2001 Soos Creek fish condition profile. Fish were reared in seminatural (NAT, N = 30) or conventional (Con, N = 30) raceways. P values are based on contingency table analysis.
A) B) C)

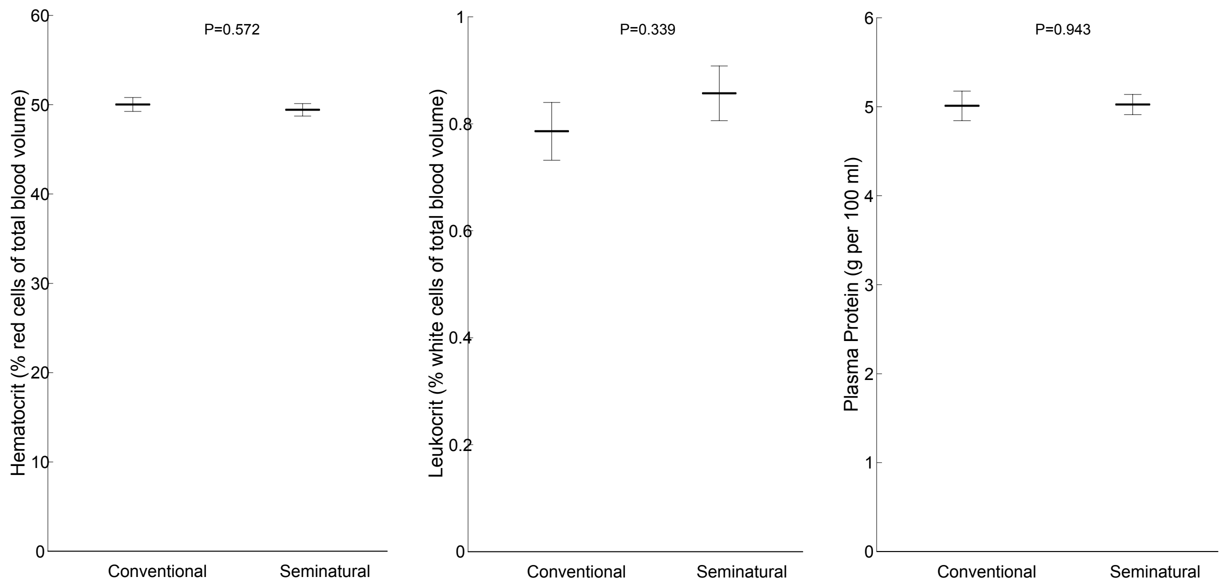

Figure 19. Means (with standard error bars) of blood variables from coho salmon reared in seminatural or conventional raceways at Soos Creek Hatchery sampled on 27 April 2001. A) hematocrit (N = 30 per treatment) P value based on *t*-tests of arcsine transformed data; B) leukocrit (N = 28 per treatment) P value based on *t*-tests; and C) plasma protein (N = 28 per treatment) P value based on *t*-tests.

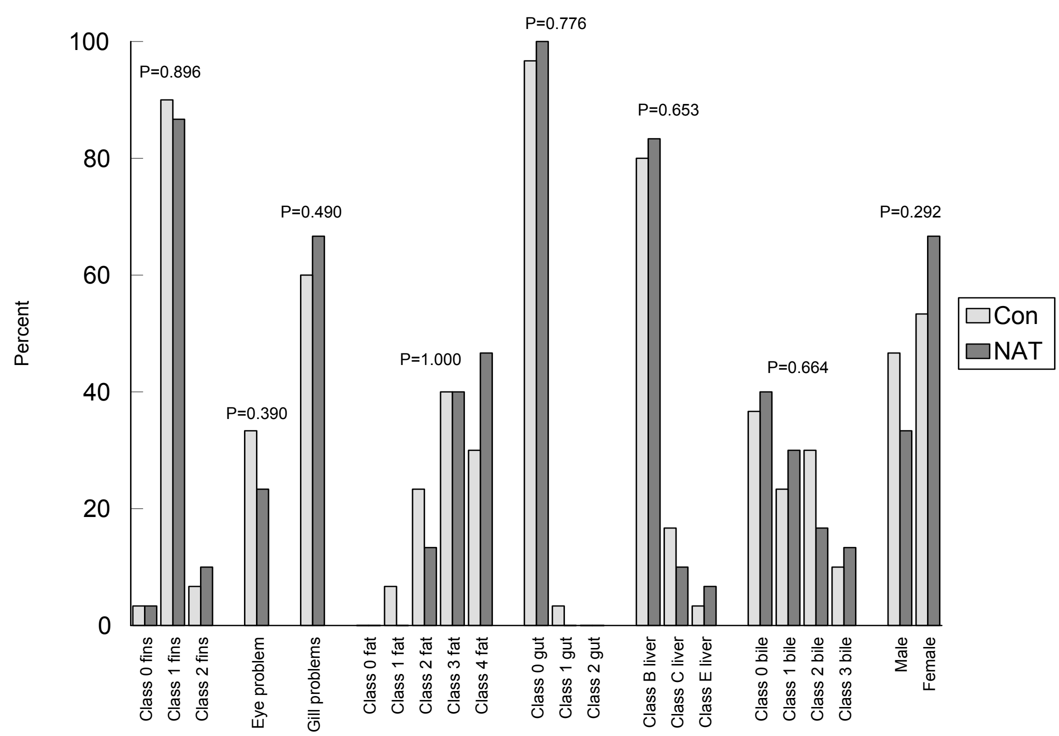

Figure 20. Percentage of coho salmon in different Goede Index classes in the 4 May 2001 Minter Creek fish condition profile. Fish were reared in seminatural (NAT, N = 30) or conventional (Con, N = 30) raceways. P values are based on contingency table analysis.

A) B) C)

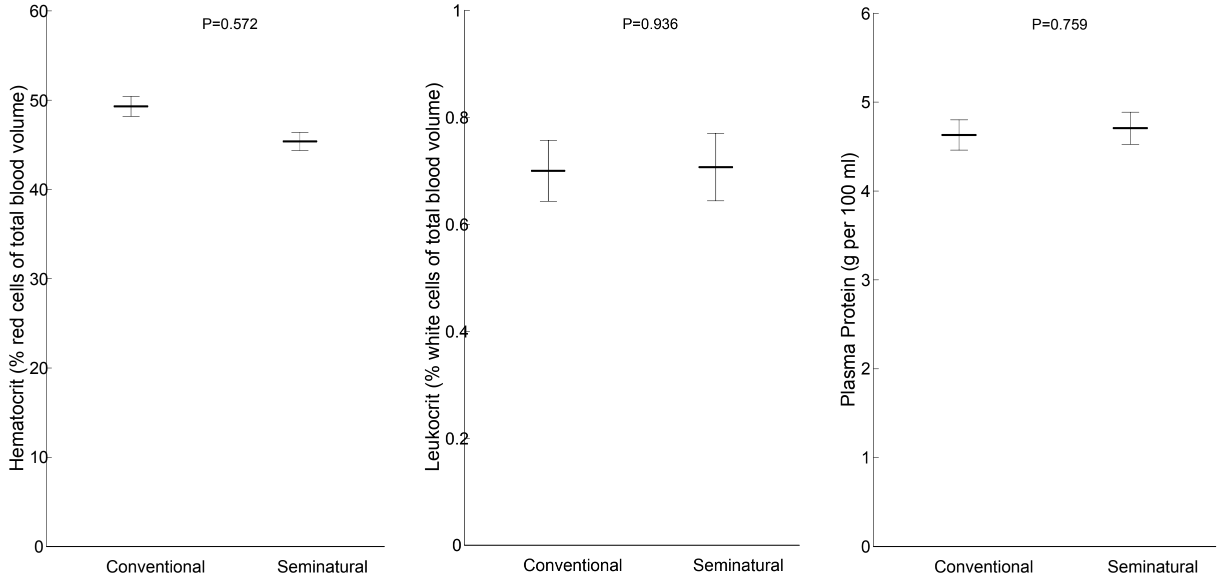

Figure 21. Means (with standard error bars) of blood variables from coho salmon reared in seminatural or conventional raceways at Minter Creek Hatchery sampled on 4 May 2001. A) hematocrit (N = 30 conventional and 29 seminatural) P value based on *t*-tests of arcsine transformed data; B) leukocrit (N = 30 conventional and 29 seminatural) P value based on *t*-tests; and C) plasma protein (N = conventional and 30 seminatural) P value based on *t*-tests.

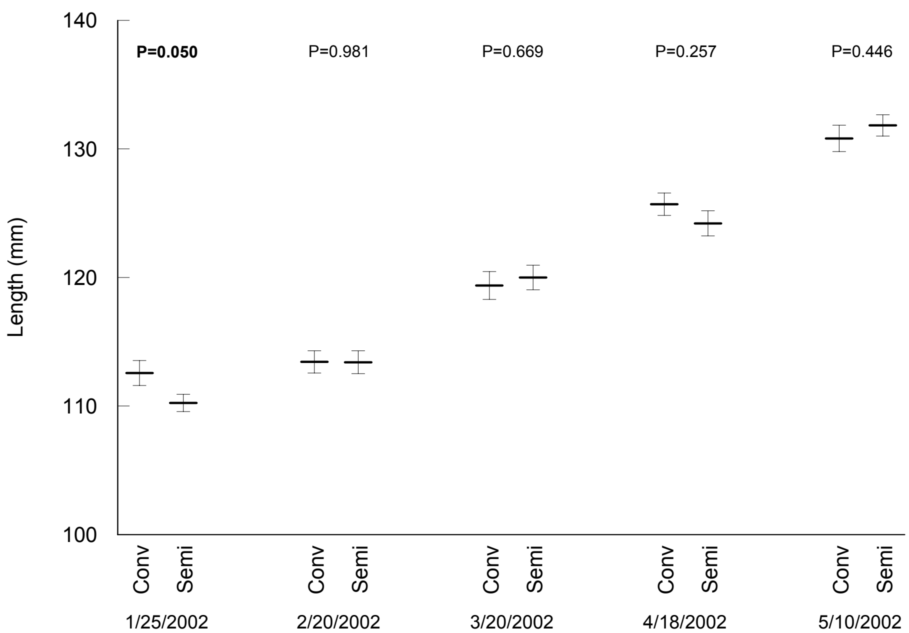

Figure 22. Mean length (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Kendall Creek Hatchery in 2002 (N = 100 per treatment). P values are based on *t*-tests.

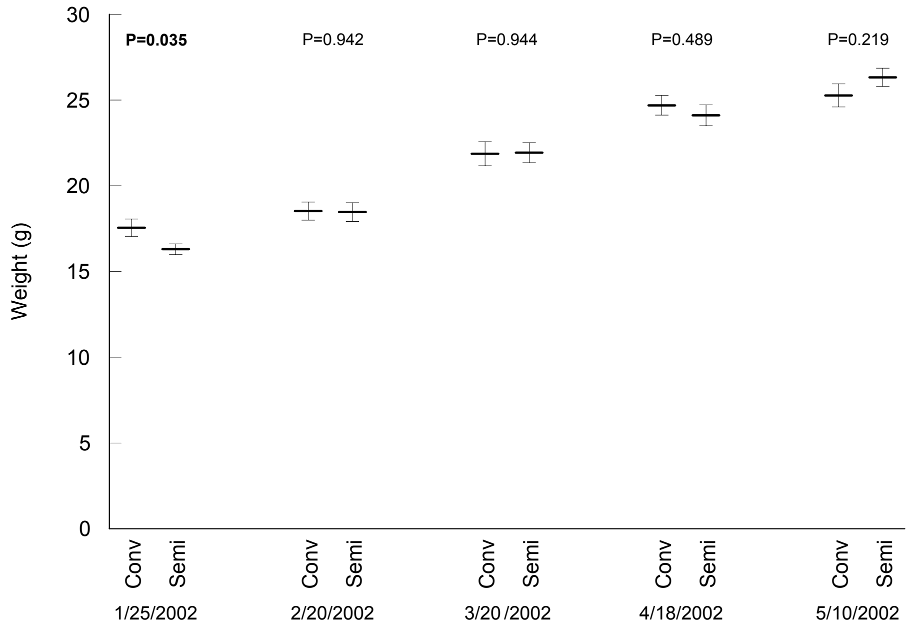

Figure 23. Mean weight (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Kendall Creek Hatchery in 2002 (N = 100 per treatment). P values are based on *t*-tests.

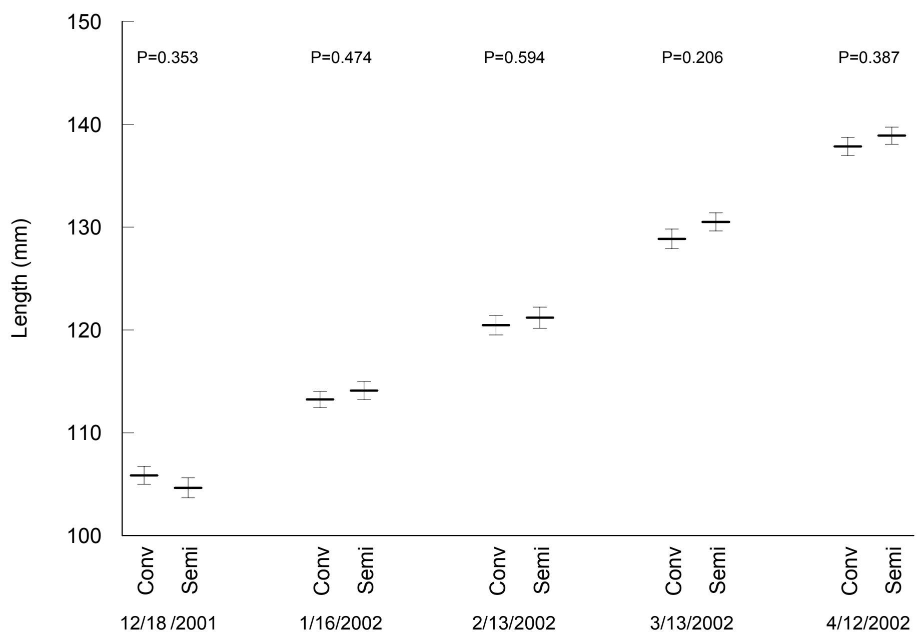

Figure 24. Mean length (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Sol Duc Hatchery in 2002(N = 100 per treatment). P values are based on *t*-tests.

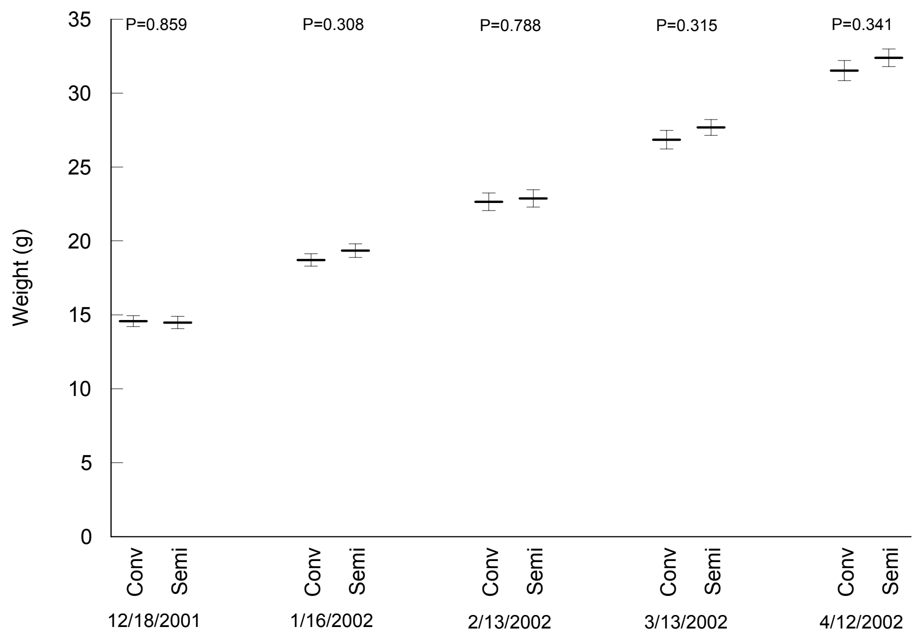

Figure 25. Mean weight (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Sol Duc Hatchery in 2002 (N = 100 per treatment). P values are based on *t*-tests.

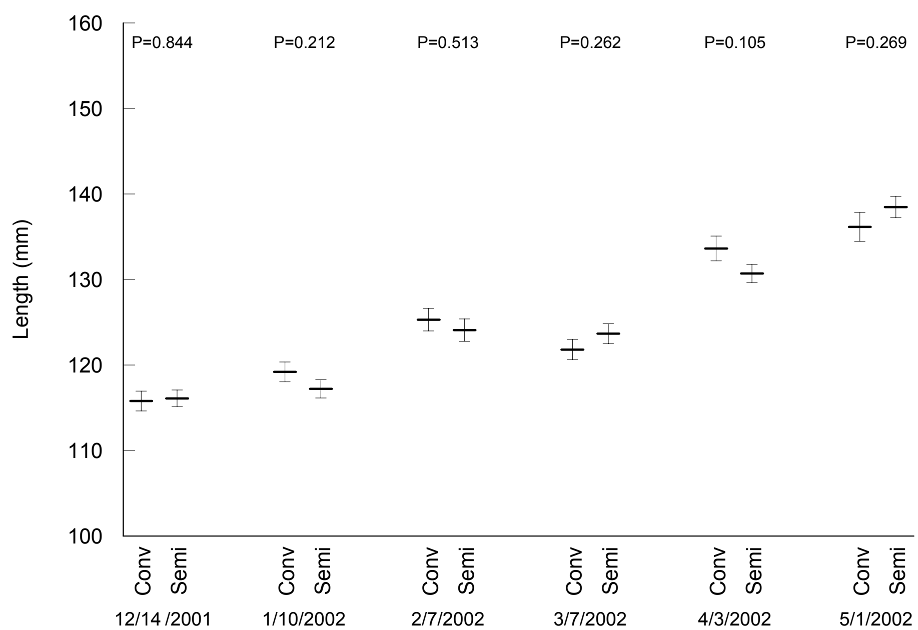

Figure 26. Mean length (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Minter Creek Hatchery in 2002 (N = 100 per treatment). P values are based on *t*-tests.

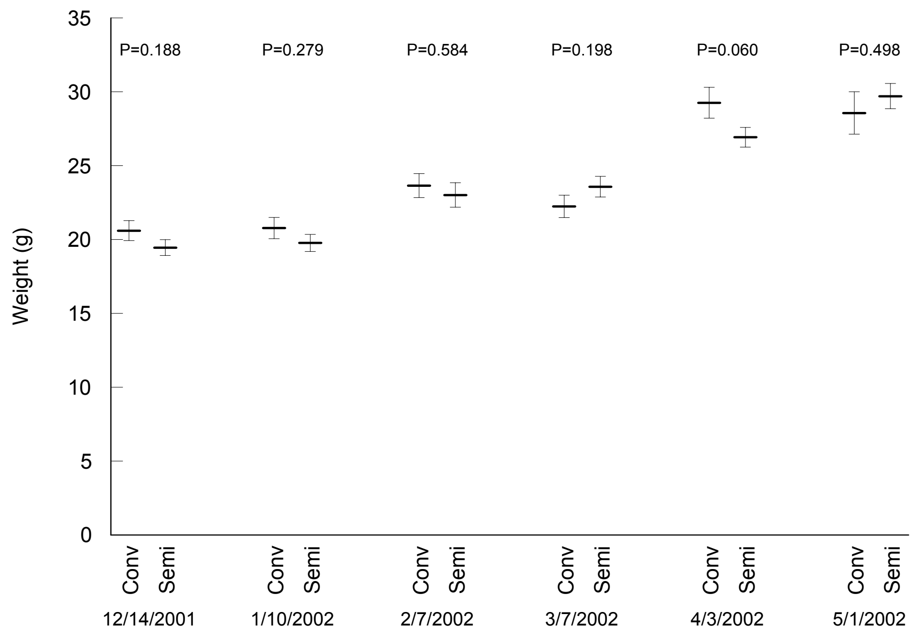

Figure 27. Mean weight (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Minter Creek Hatchery in 2002 (N = 100 per treatment). P values are based on *t*-tests.

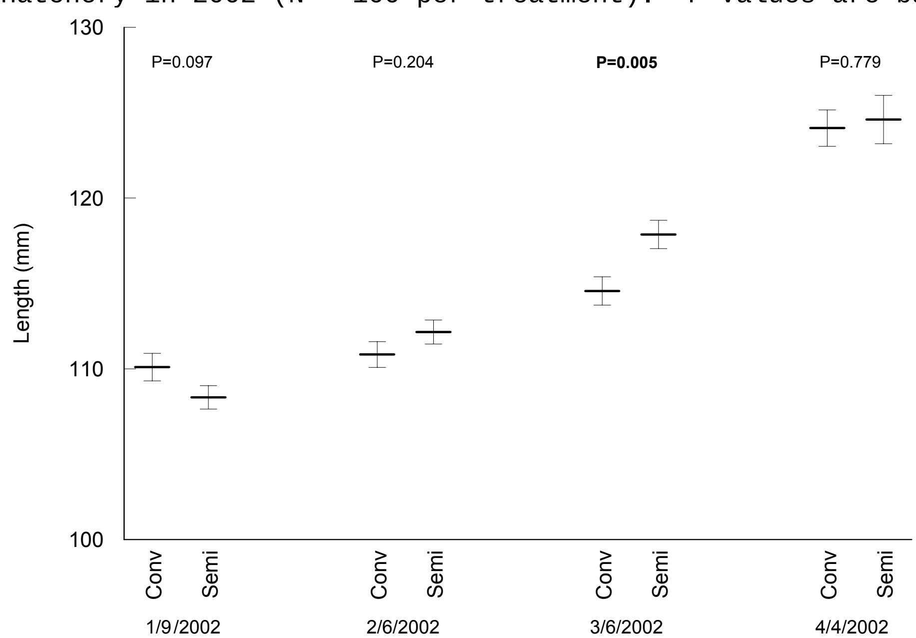

Figure 28. Mean length (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Soos Creek Hatchery in 2002 (N = 100 per treatment, except N = 30 per treatment on 4/4/2002). P values are based on *t*-tests.

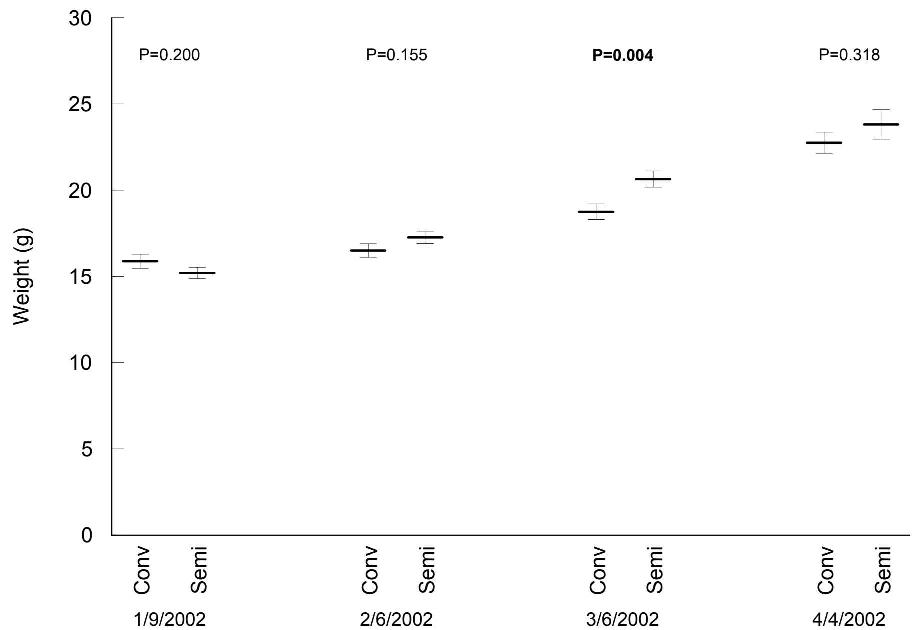

Figure 29. Mean weight (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Soos Creek Hatchery in 2002 (N = 100 per treatment, except N = 30 per treatment on 4/4/2002). P values are based on *t*-tests.

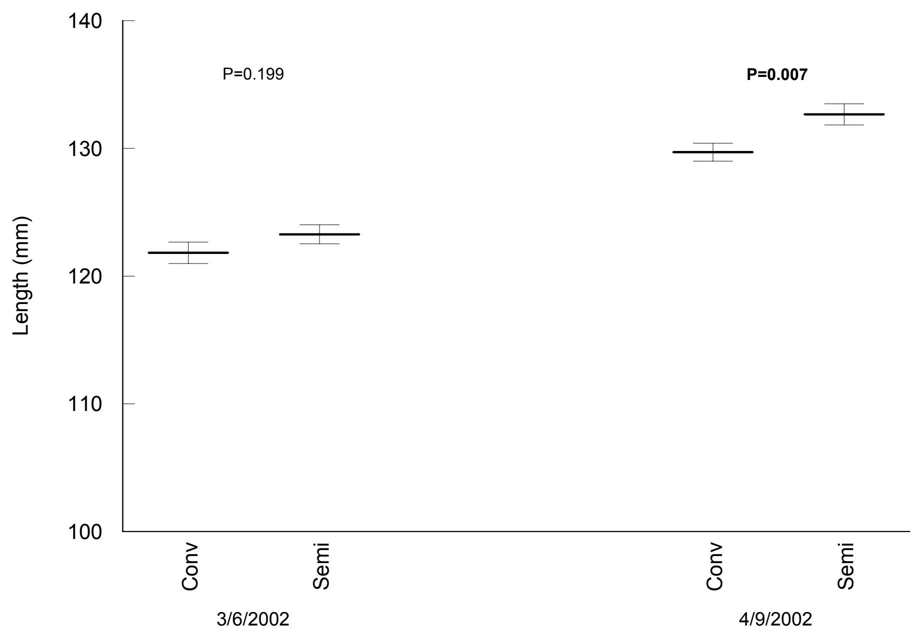

Figure 30. Mean length (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Issaquah Hatchery in 2002 (N = 100 per treatment). P values are based on *t*-tests.

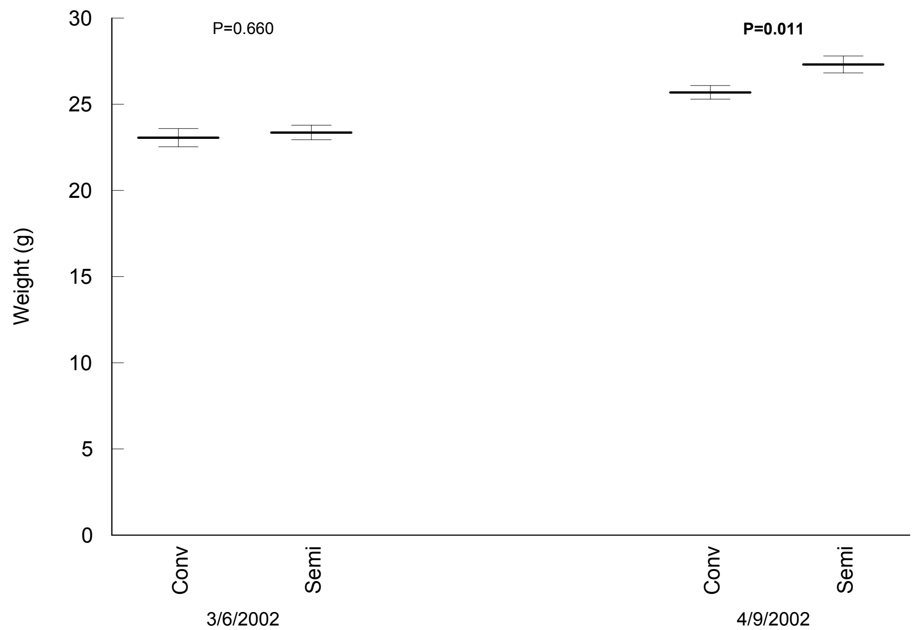

Figure 31. Mean weight (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Issaquah Hatchery in 2002 (N = 100 per treatment). P values are based on *t*-tests.

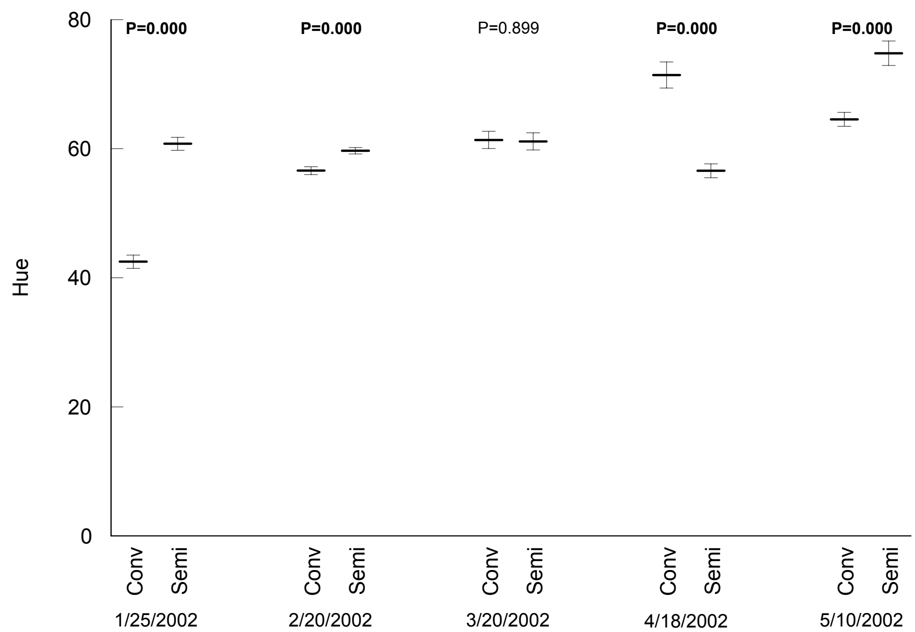

Figure 32. Mean hue values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Kendall Creek Hatchery in 2002 (N = 30 per treatment). P values are based on *t*-tests.

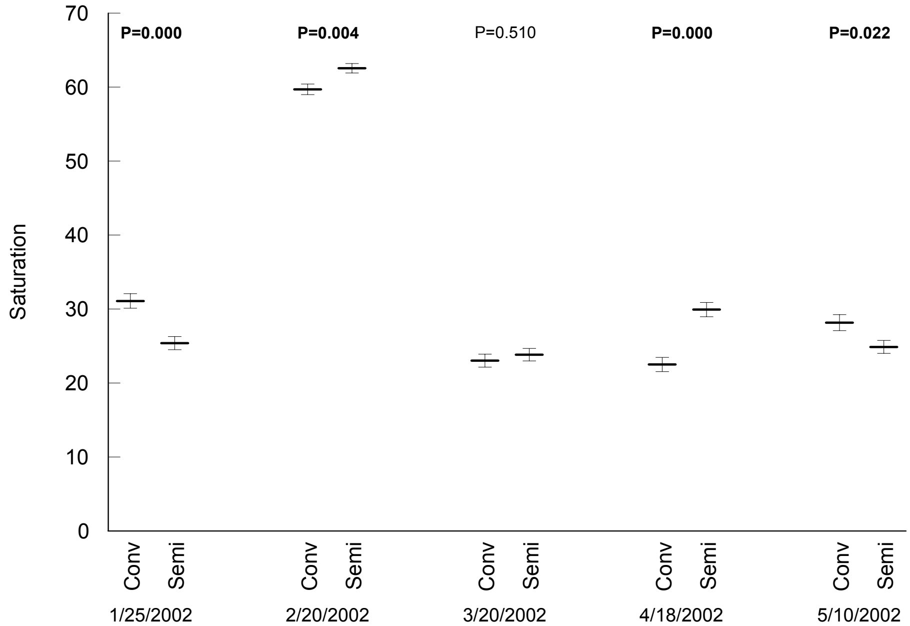

Figure 33. Mean saturation values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Kendall Creek Hatchery in 2002 (N = 30 per treatment). P values are based on *t*-tests.

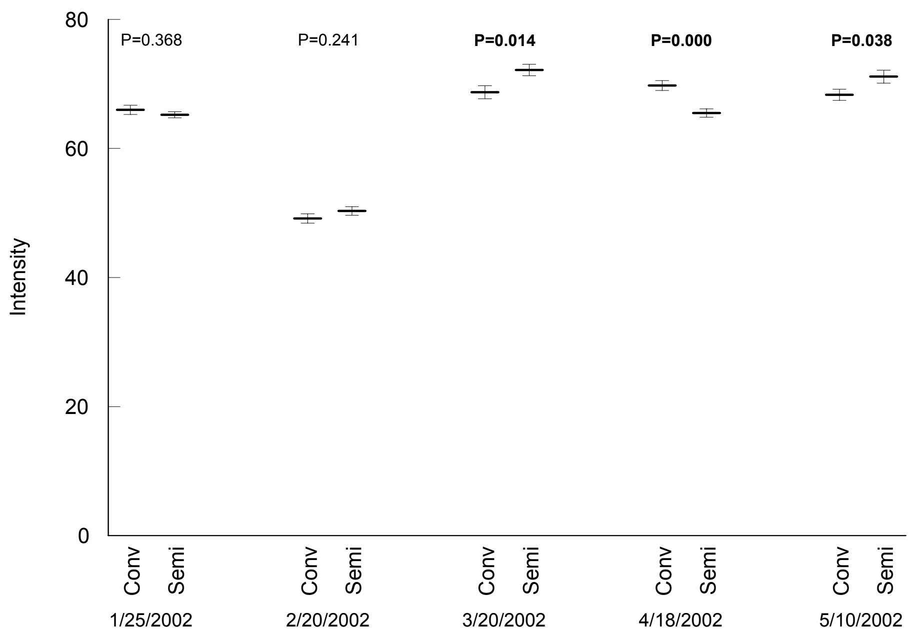

Figure 34. Mean intensity values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Kendall Creek Hatchery in 2002 (N = 30 per treatment). P values are based on *t*-tests.

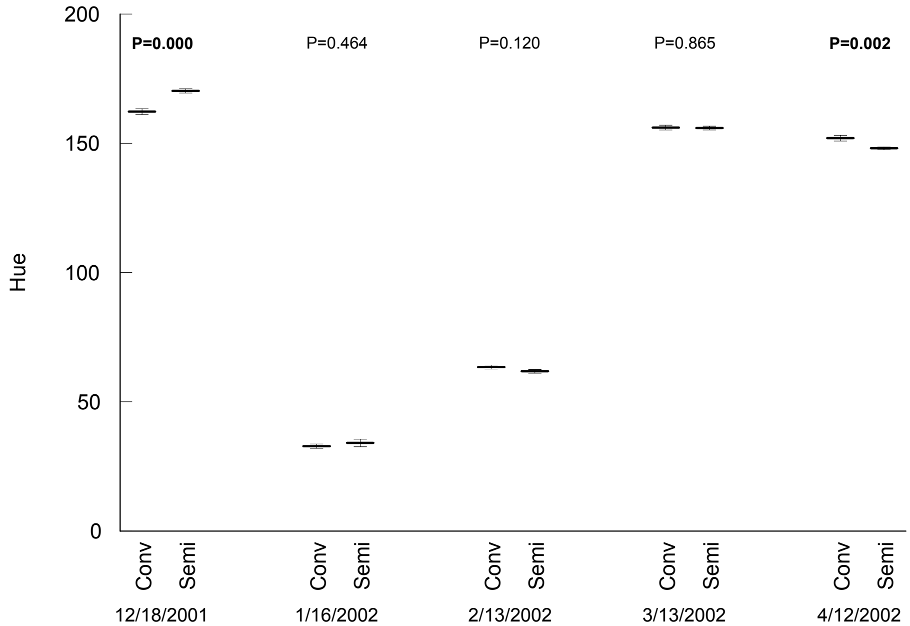

Figure 35. Mean hue values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Sol Duc Hatchery in 2002 (N = 30 per treatment). P values are based on *t*-tests.

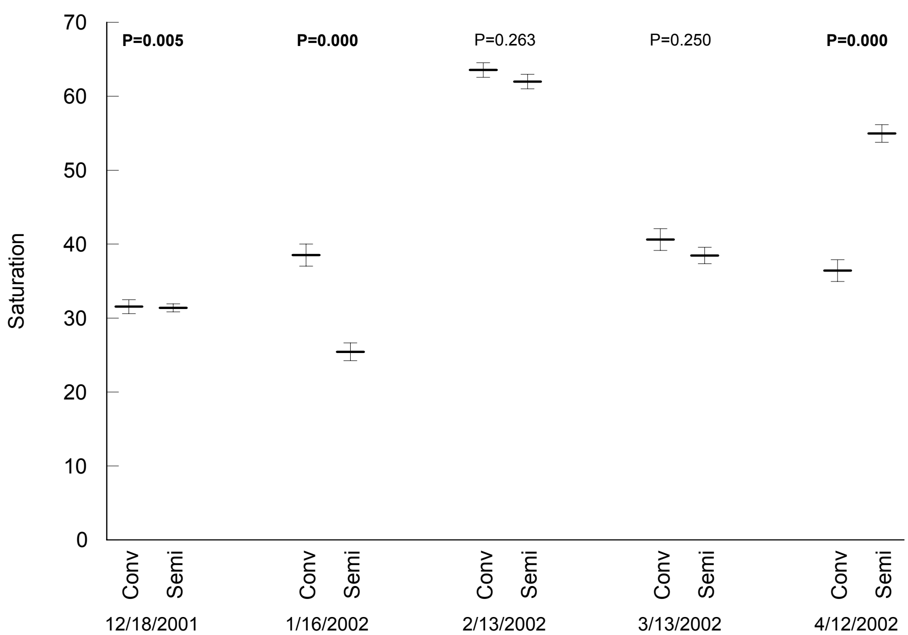

Figure 36. Mean saturation values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Sol Duc Hatchery in 2002 (N = 30 per treatment). P values are based on *t*-tests.

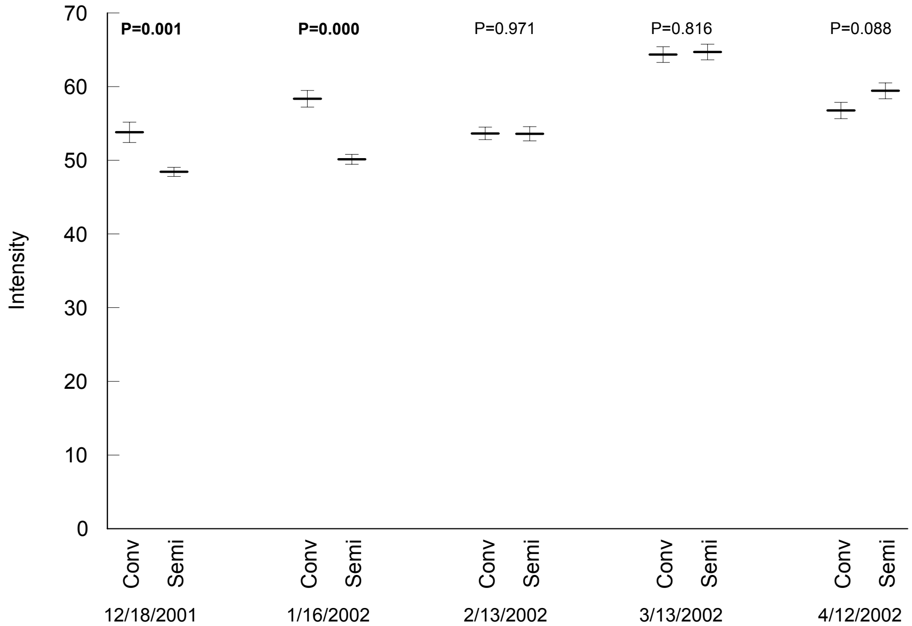

Figure 37. Mean intensity values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Sol Duc Hatchery in 2002 (N = 30 per treatment). P values are based on *t*-tests.

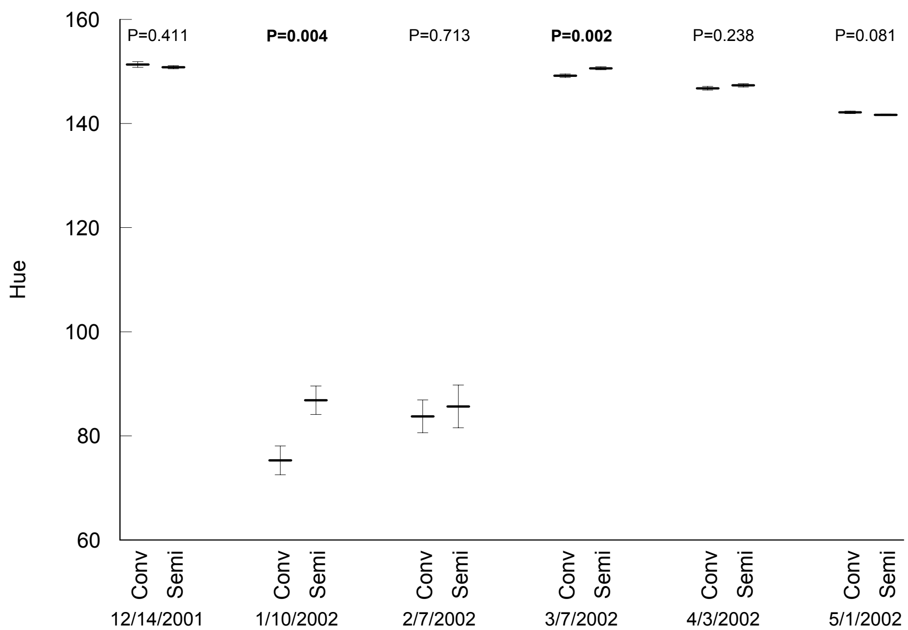

Figure 38. Mean hue values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Minter Creek Hatchery in 2002 (N = 30 per treatment). P values are based on *t*-tests.

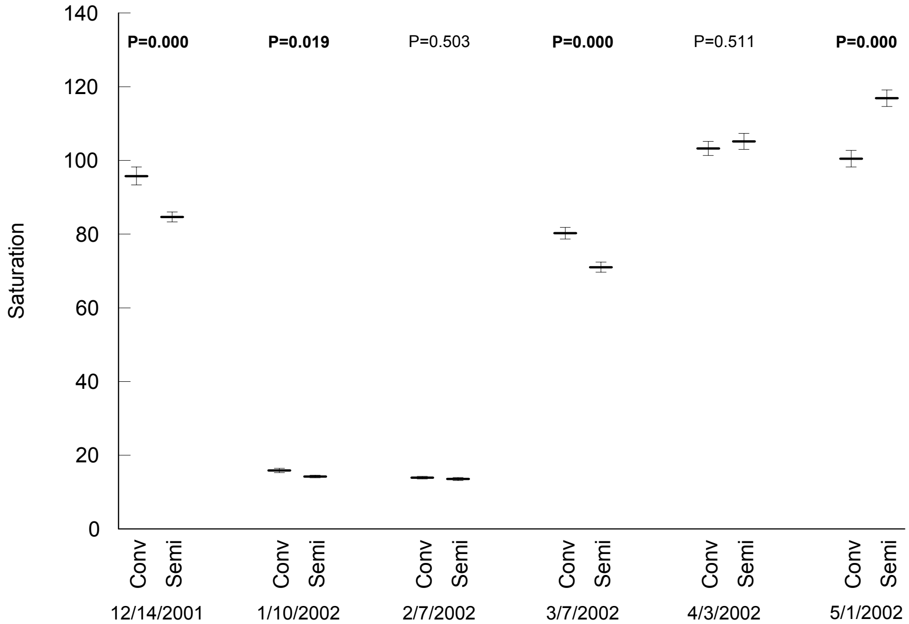

Figure 39. Mean saturation values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Minter Creek Hatchery in 2002 (N = 30 per treatment). P values are based on *t*-tests.

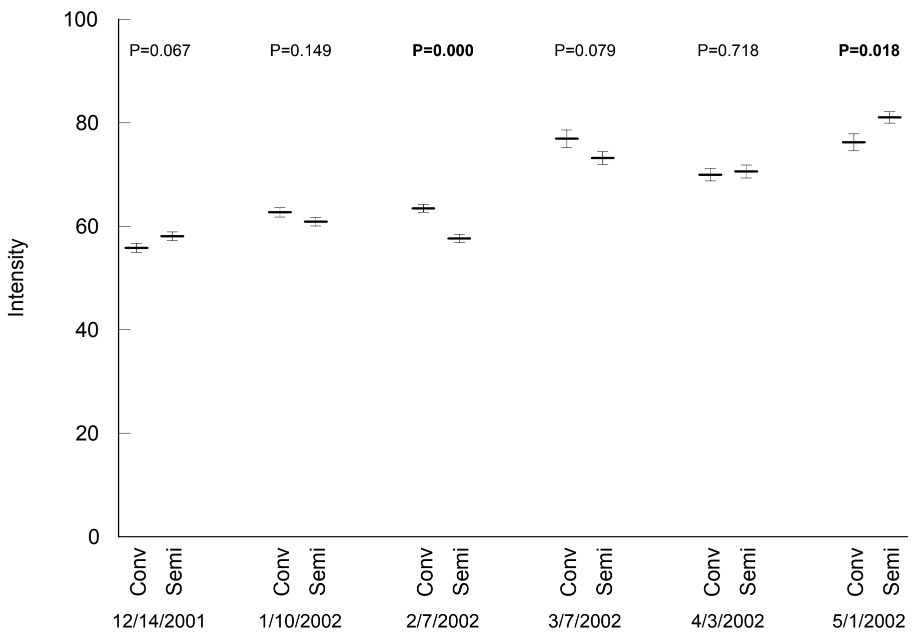

Figure 40. Mean intensity values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Minter Creek Hatchery in 2002 (N = 30 per treatment). P values are based on *t*-tests.

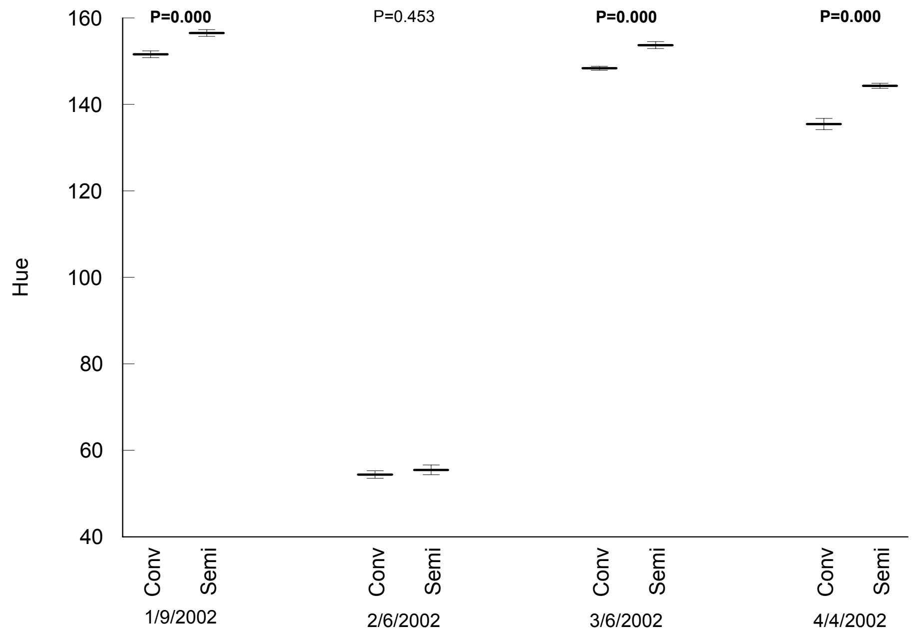

Figure 41. Mean hue values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Soos Creek Hatchery in 2002 (N = 30 per treatment). P values are based on *t*-tests.

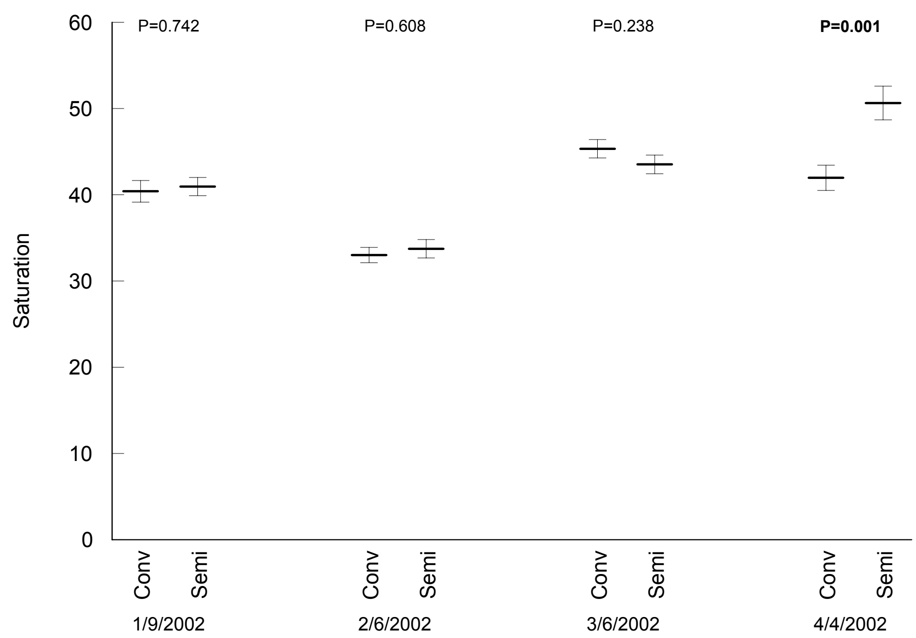

Figure 42. Mean saturation values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Soos Creek Hatchery in 2002 (N = 30 per treatment). P values are based on *t*-tests.

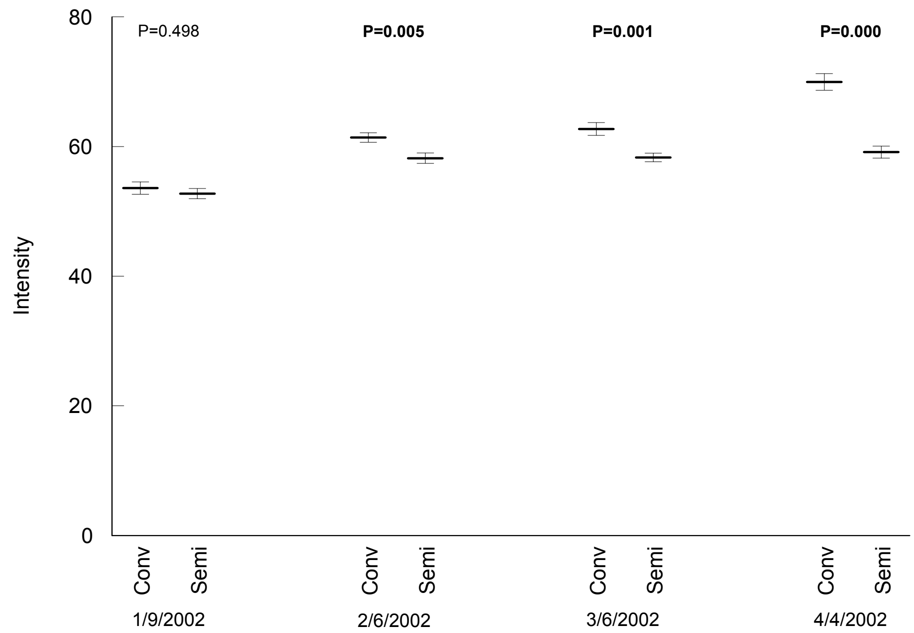

Figure 43. Mean intensity values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Soos Creek Hatchery in 2002 (N = 30 per treatment). P values are based on *t*-tests.

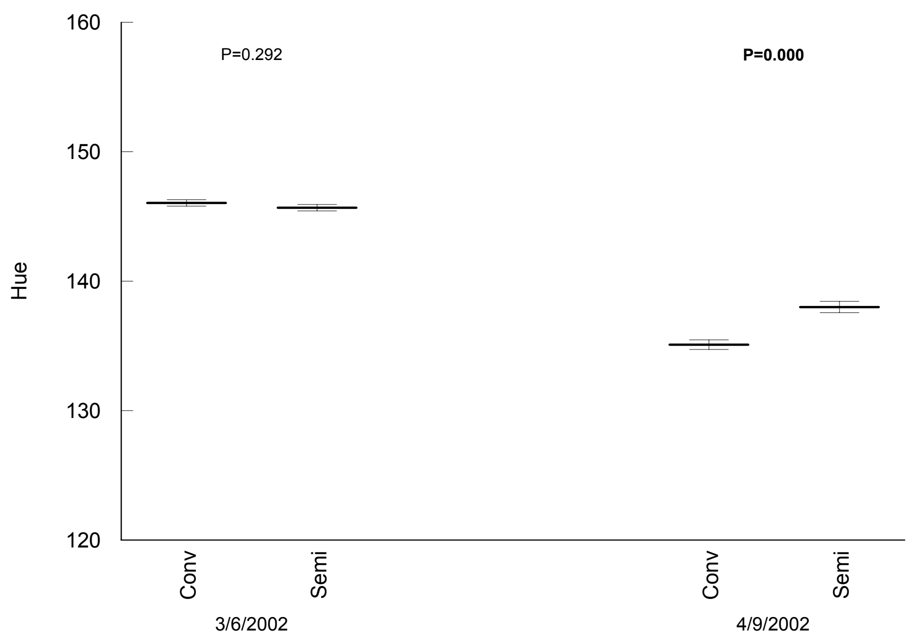

Figure 44. Mean hue values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Issaquah Hatchery in 2002 (N = 30 per treatment). P values are based on *t*-tests.

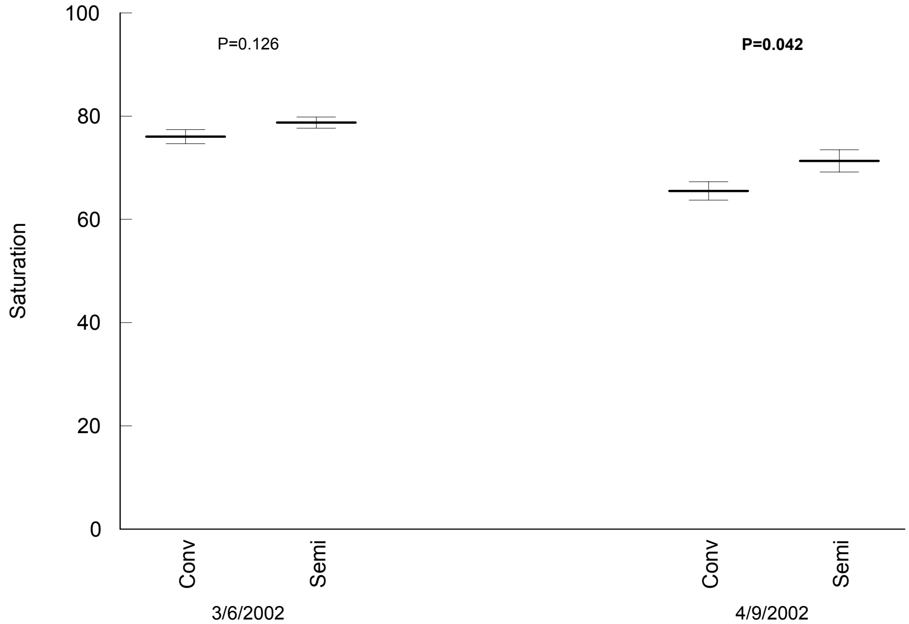

Figure 45. Mean saturation values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Issaquah Hatchery in 2002 (N = 30 per treatment). P values are based on *t*-tests.

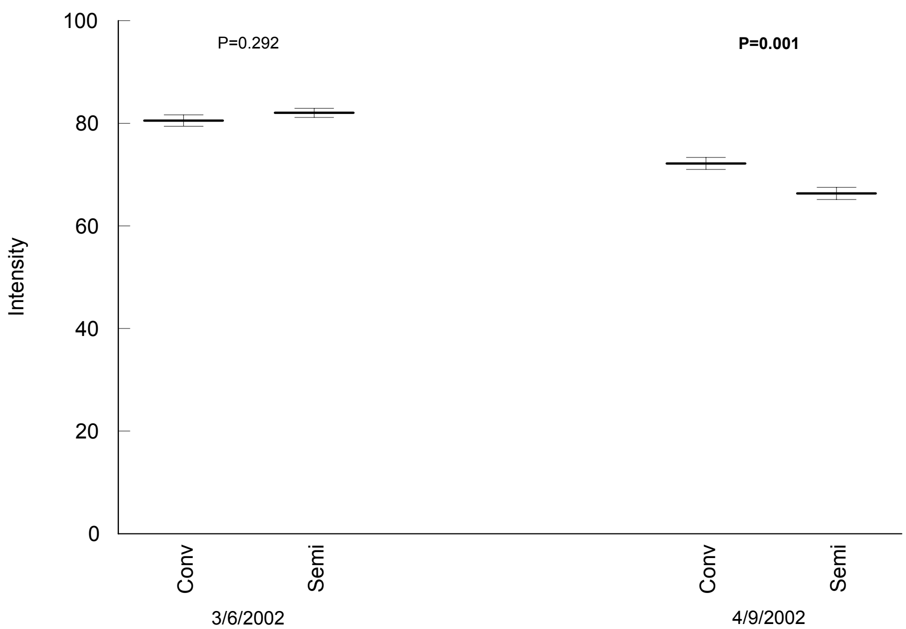

Figure 46. Mean intensity values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Issaquah Hatchery in 2002 (N = 30 per treatment). P values are based on *t*-tests.

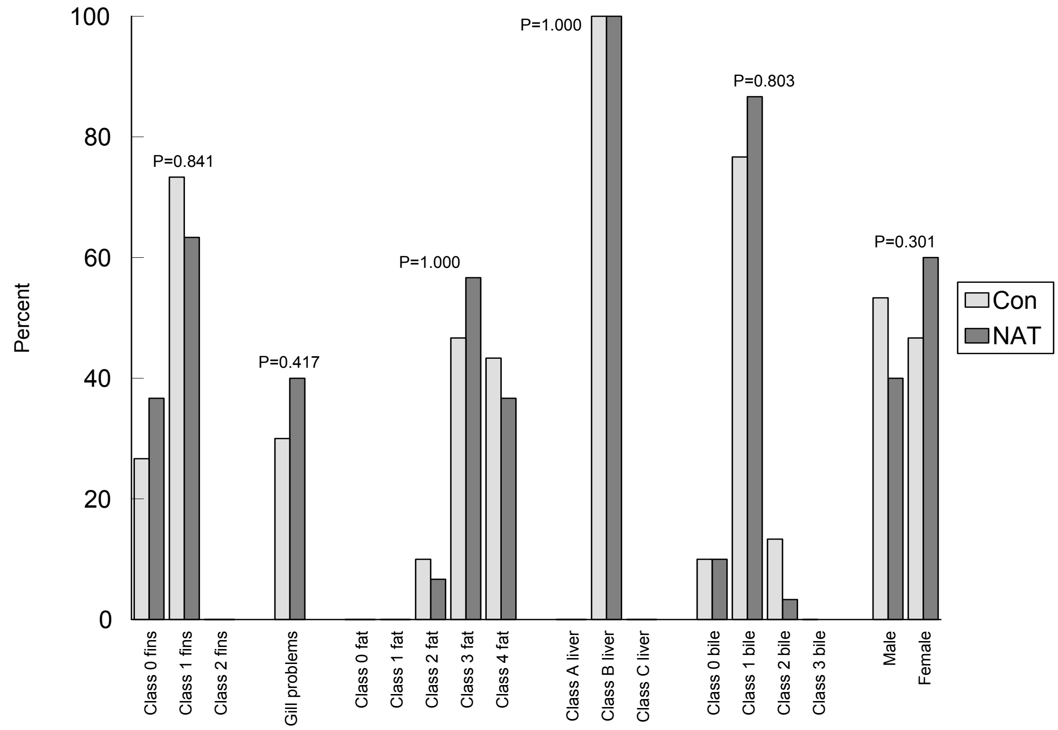

Figure 47. Percentage of coho salmon in different Goede Index classes in the 10 May 2002 Kendall Creek fish condition profile. Fish were reared in seminatural (NAT, N = 30) or conventional (Con, N = 30) raceways. P values are based on contingency table analysis.

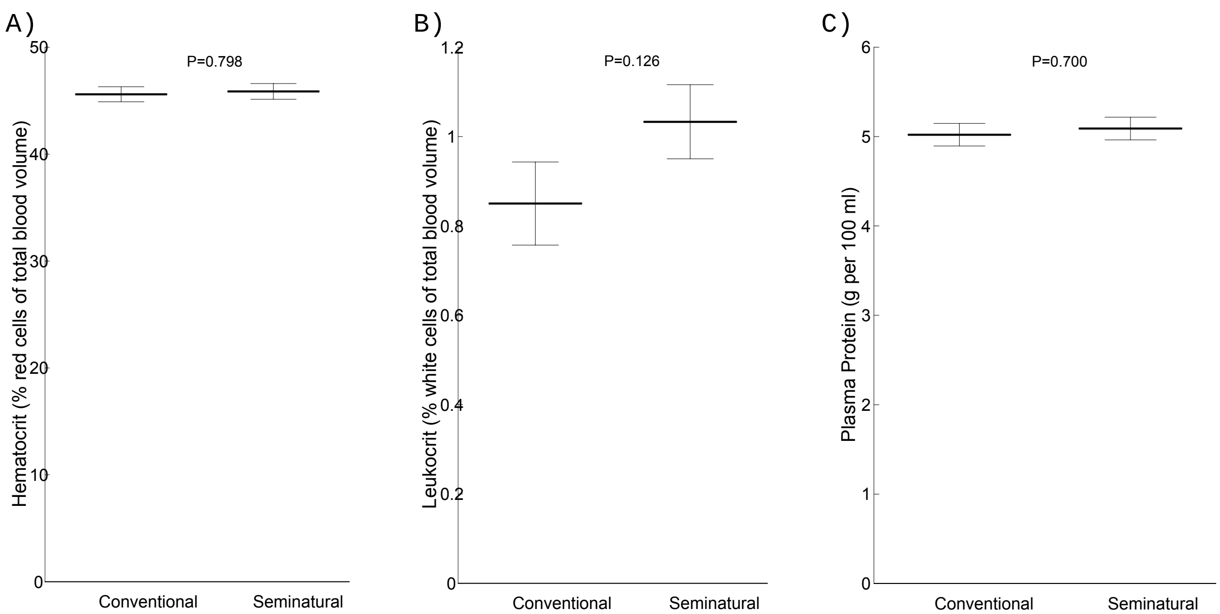

Figure 48. Means (standard error bars) of blood variables from coho salmon reared in seminatural or conventional raceways at Kendall Creek Hatchery sampled on 10 May 2002. A) hematocrit and B) leukocrit (N = 30 per treatment) P values based on *t*-tests of arcsine transformed data; and C) plasma protein (N = 28 conventional and 30 seminatural) P value based on *t*-tests.

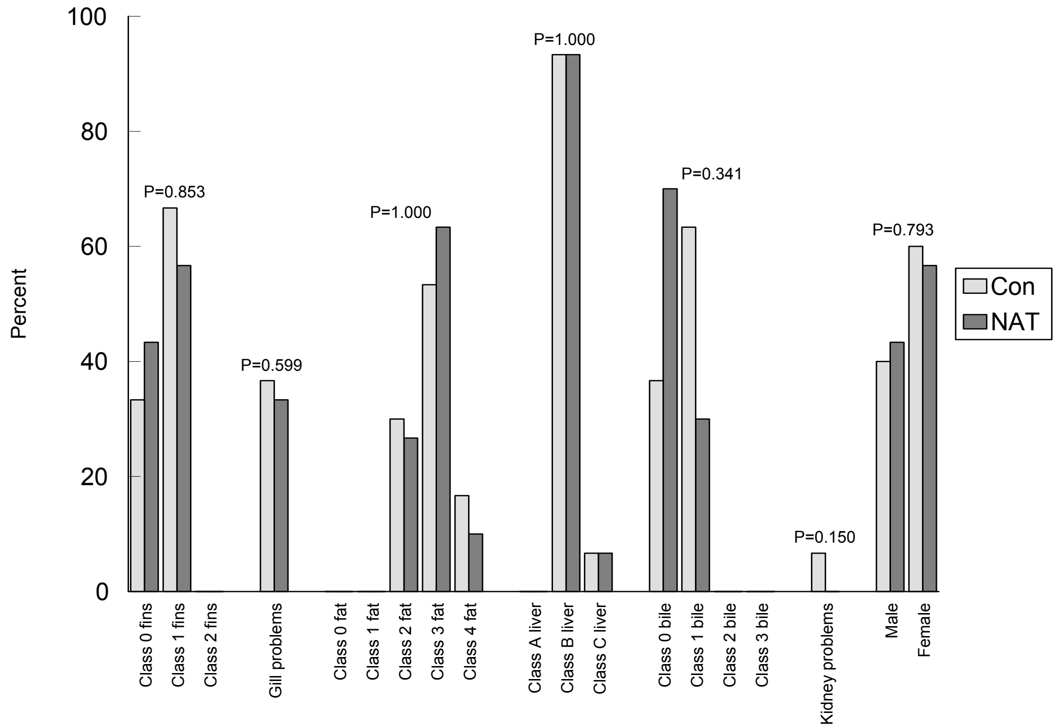

Figure 49. Percentage of coho salmon in different Goede Index classes in the 12 April 2002 Sol Duc River Hatchery fish condition profile. Fish were reared in seminatural (NAT, N = 30) or conventional (Con, N = 30) raceways. P values are based on contingency table analysis.

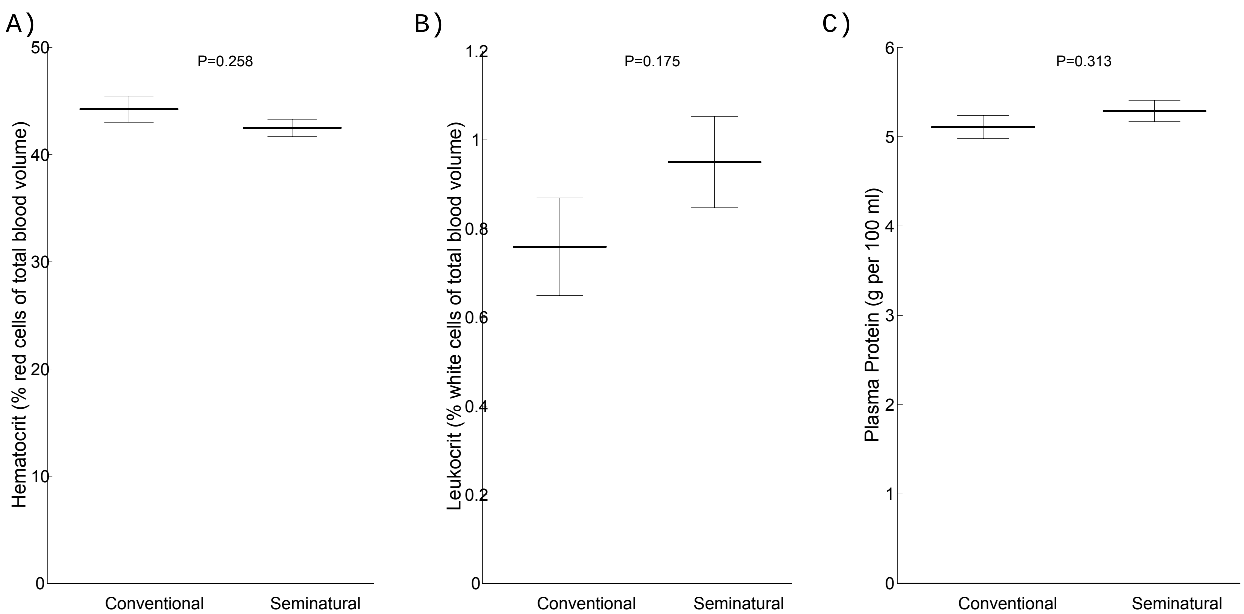

Figure 50. Means (standard error bars) of blood variables from coho salmon reared in seminatural or conventional raceways at Sol Duc River Hatchery sampled on 12 April 2002. A) hematocrit and B) leukocrit (N = 29 conventional and 30 seminatural) P values based on *t*-tests of arcsine transformed data; and C) plasma protein (N = 28 per treatment) P value based on *t*-tests.

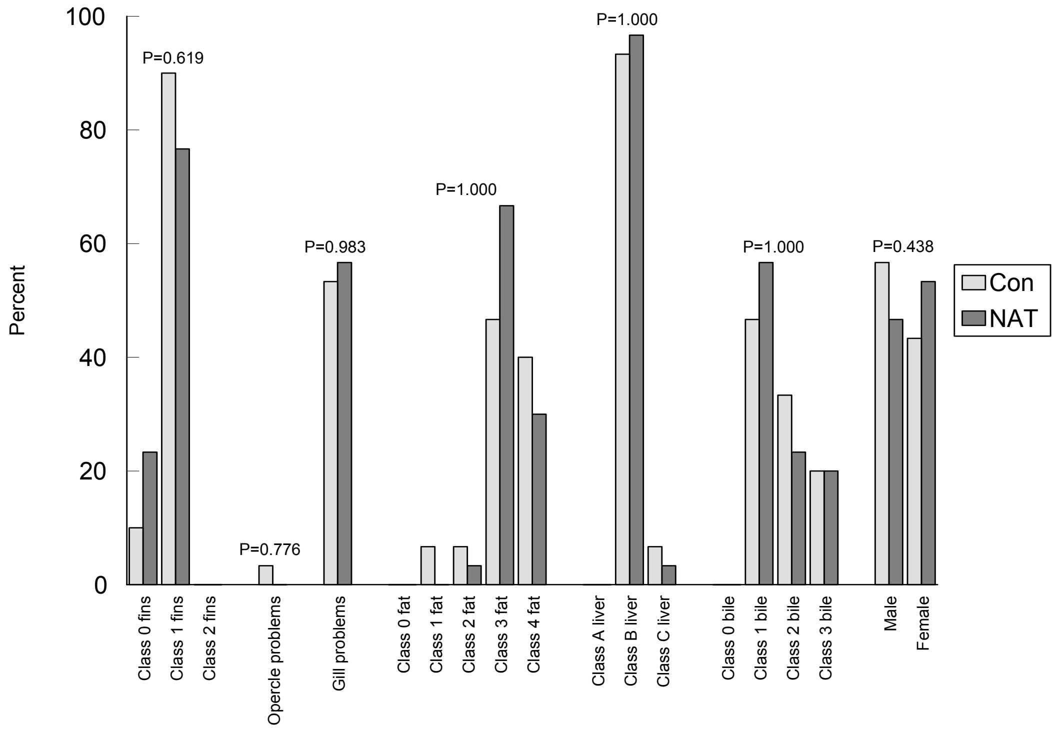

Figure 51. Percentage of coho salmon in different Goede Index classes in the 1 May 2002 Minter Creek fish condition profile. Fish were reared in seminatural (NAT, N = 30) or conventional (Con, N = 30) raceways. P values are based on contingency table analysis.

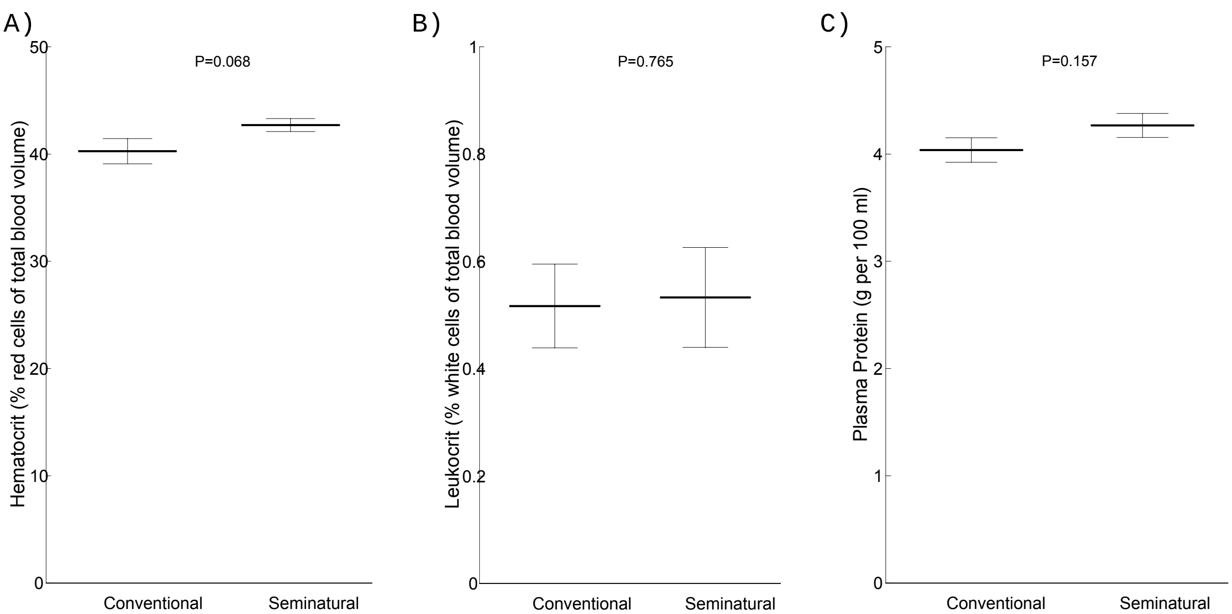

Figure 52. Means (with standard error bars) of blood variables from coho salmon reared in seminatural or conventional raceways at Minter Creek Hatchery sampled on 1 May 2002. A) hematocrit and B) leukocrit (N = 30 per treatment) P values based on *t*-tests of arcsine transformed data; and C) plasma protein (N = 27 per treatment) P value based on *t*-tests.

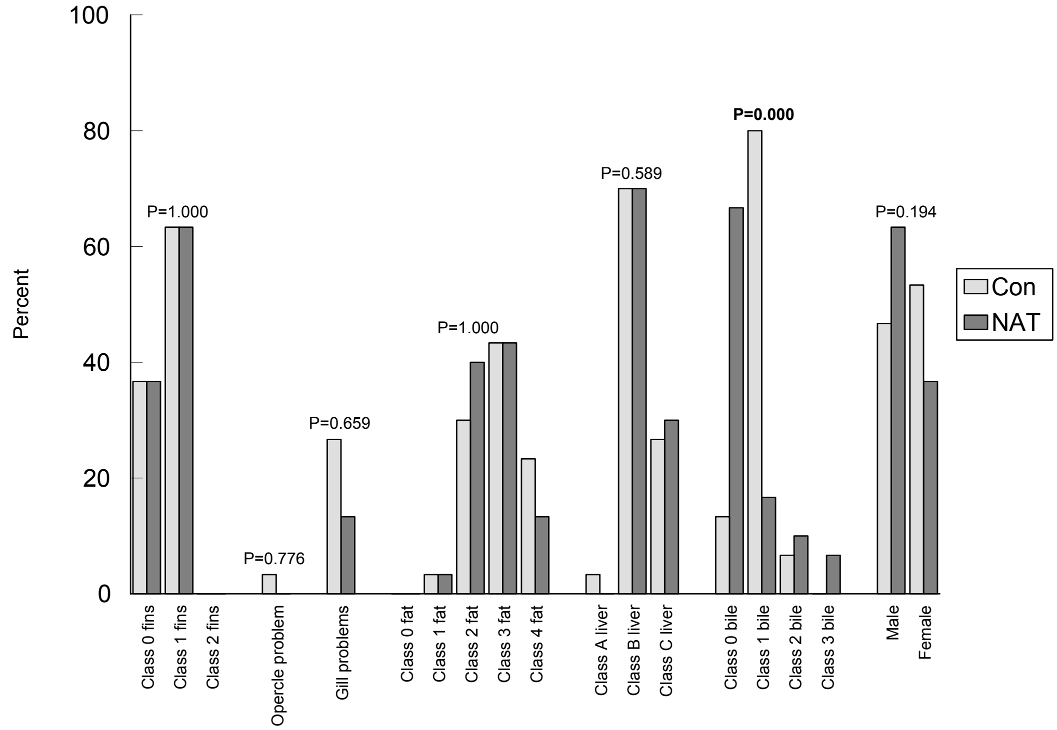

Figure 53. Percentage of coho salmon in different Goede Index classes in the 4 April 2002 Soos Creek fish condition profile. Fish were reared in seminatural (NAT, N = 30) or conventional (Con, N = 30) raceways. P values are based on contingency table analysis.

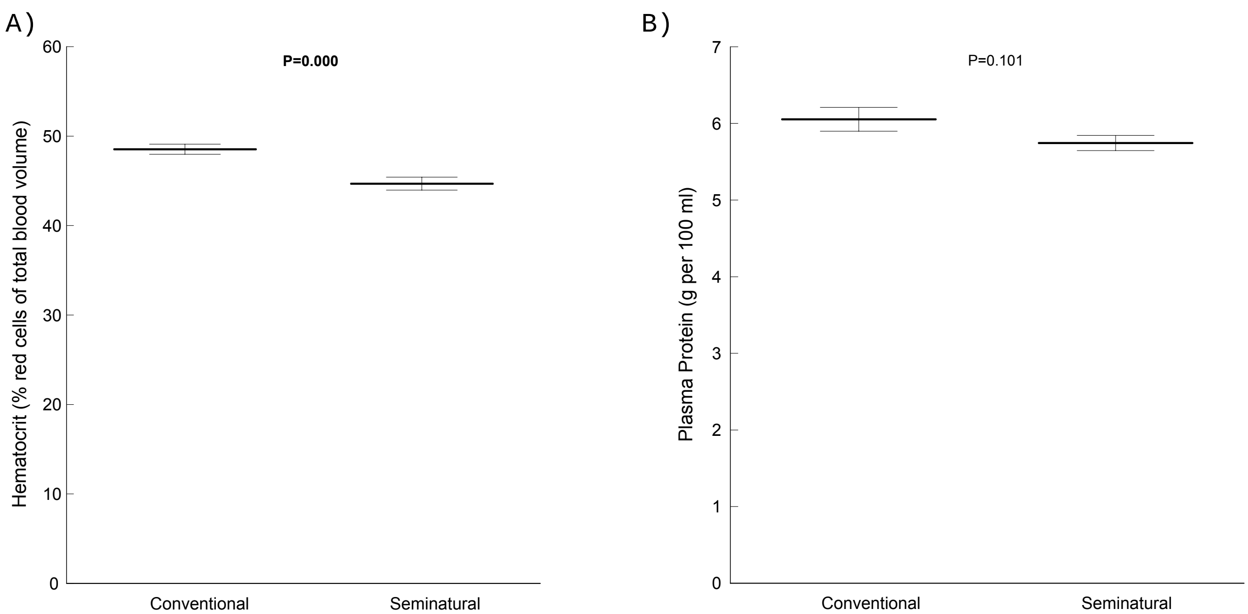

Figure 54. Means (with standard error bars) of blood variables from coho salmon reared in seminatural or conventional raceways at Soos Creek Hatchery sampled on 4 April 2002. A) hematocrit (N = 30 conventional and 29 seminatural) P value based on *t*-tests of arcsine transformed data; and B) plasma protein (N = 28 conventional and 27 seminatural) P value based on *t*-tests.

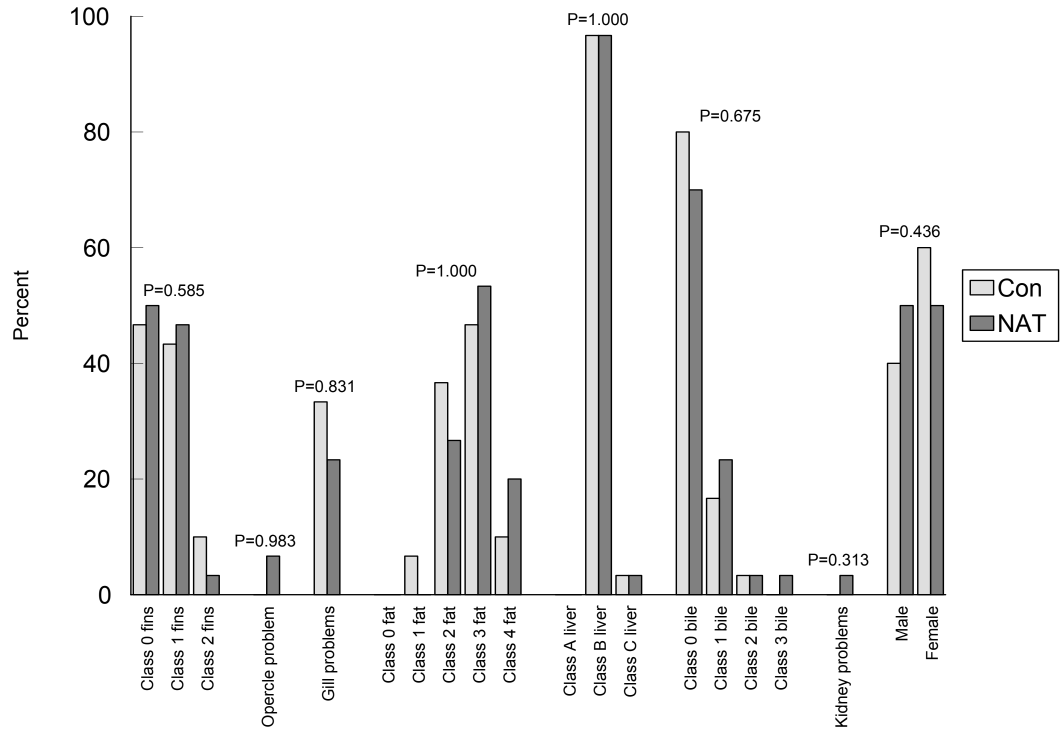

Figure 55. Percentage of coho salmon in different Goede Index classes in the 9 April 2002 Issaquah Hatchery fish condition profile. Fish were reared in seminatural (NAT, N = 30) or conventional (Con, N = 30) raceways. P values are based on contingency table analysis.

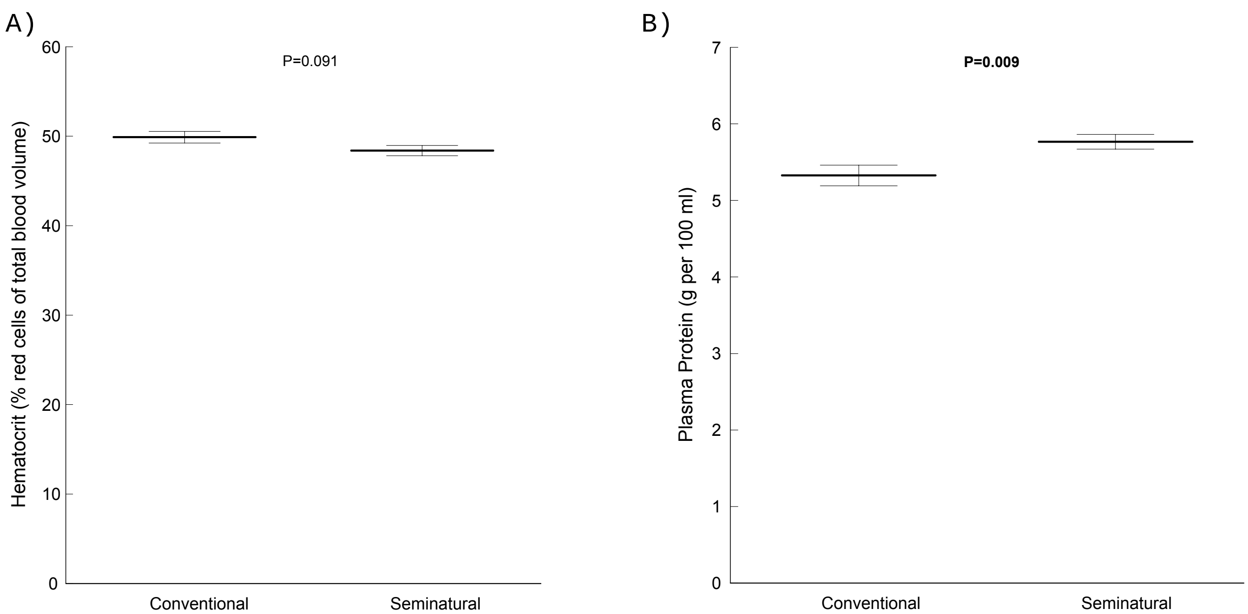

Figure 56. Means (with standard error bars) of blood variables from coho salmon reared in seminatural or conventional raceways at Issaquah Hatchery on 9 April 2002. A) hematocrit (N = 30 conventional and 28 seminatural) P value based on *t*-tests of arcsine transformed data; and B) plasma protein (N = 26 conventional and 30 seminatural) P value based on *t*-tests.

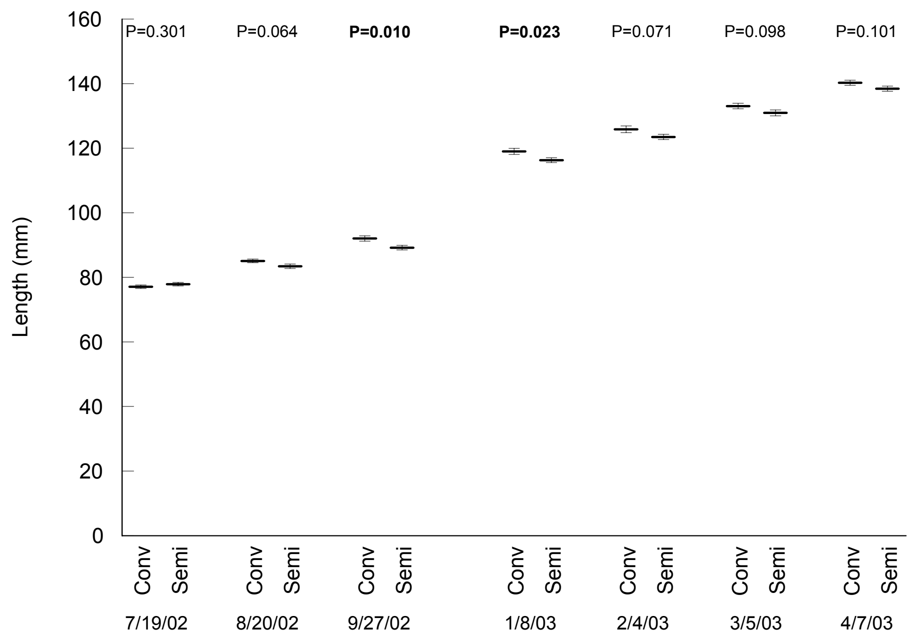

Figure 57. Mean length (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Sol Duc River Hatchery in 2003 (N = 100 per treatment). P values are based on *t*-tests.

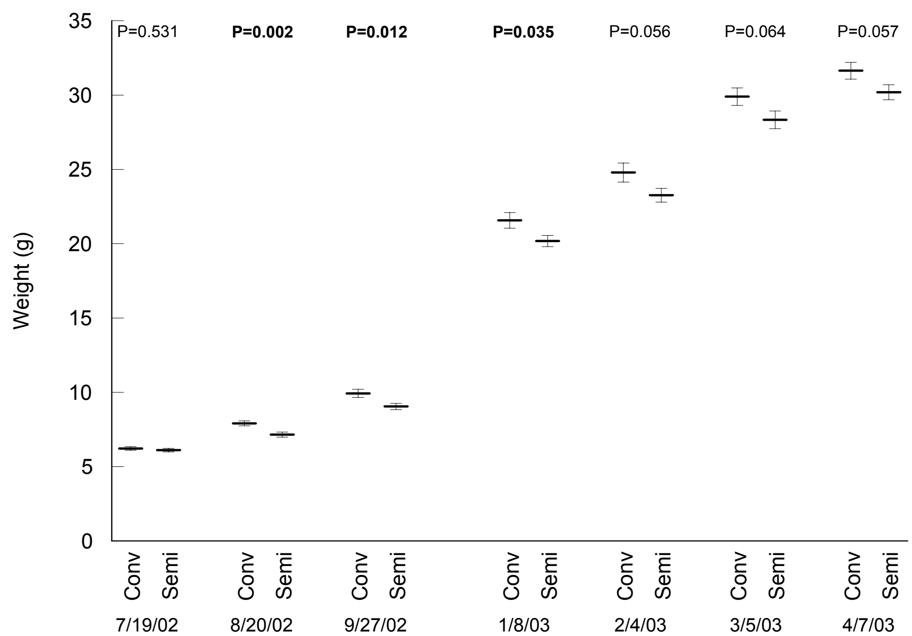

Figure 58. Mean weight (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Sol Duc River Hatchery in 2003 (N = 100 per treatment). P values are based on *t*-tests.

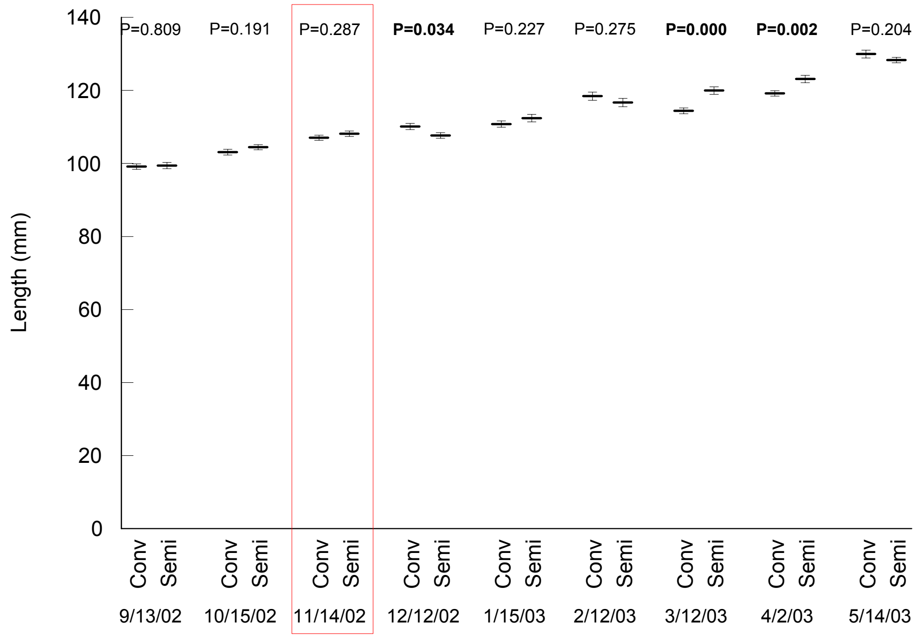

Figure 59. Mean length (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Kendall Creek Hatchery in 2003 (N = 100 per treatment). P values are based on *t*-tests.

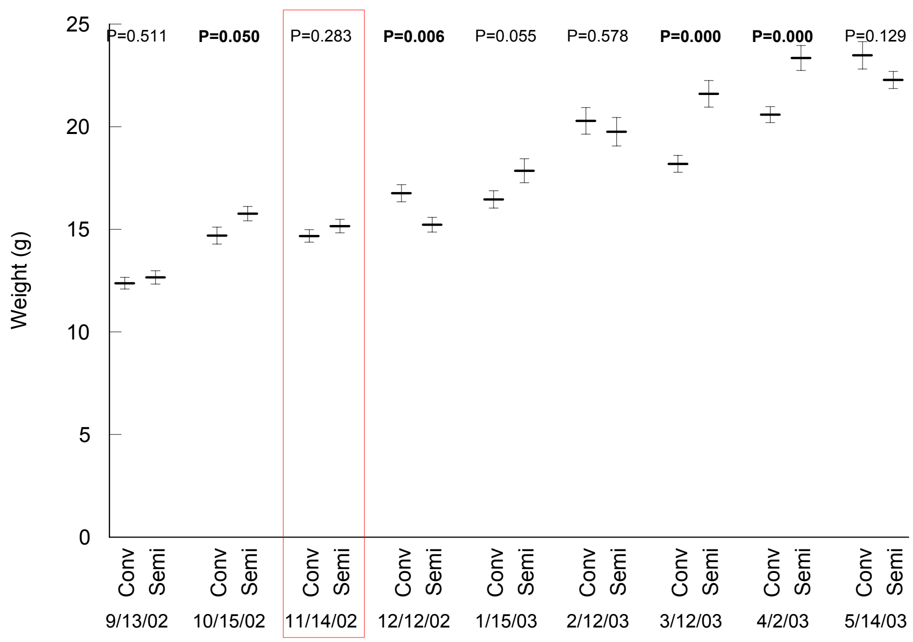

Figure 60. Mean weight (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Kendall Creek Hatchery in 2003 (N = 100 per treatment). P values are based on *t*-tests.

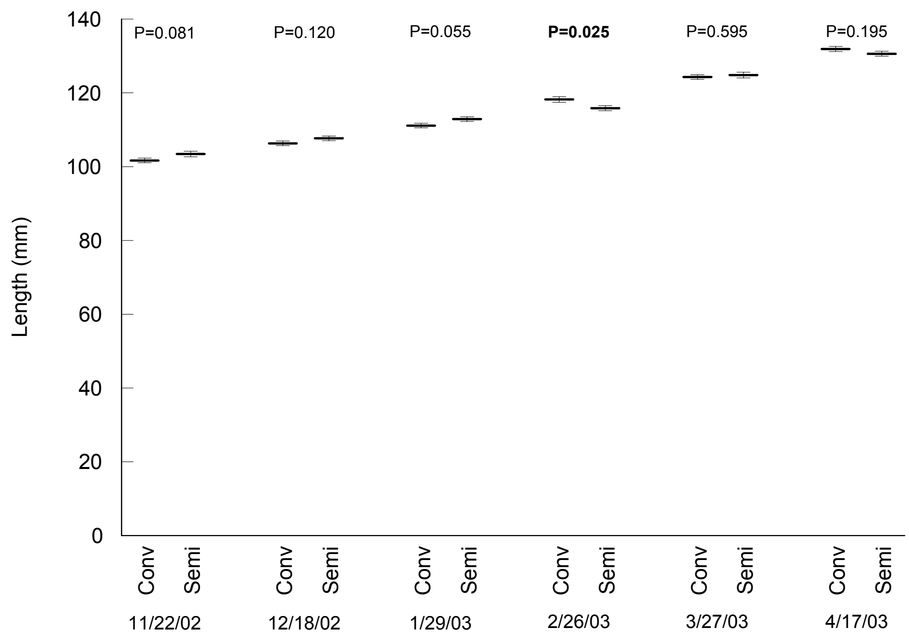

Figure 61. Mean length (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Issaquah Hatchery in 2003 (N = 100 per treatment). P values are based on *t*-tests.

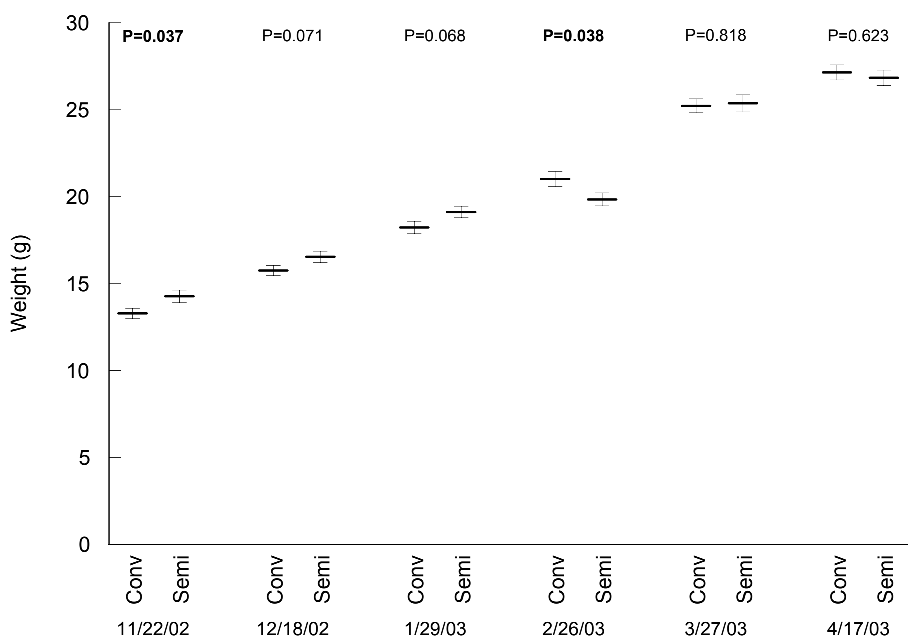

Figure 62. Mean weight (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Issaquah Hatchery in 2003 (N = 100 per treatment). P values are based on *t*-tests.

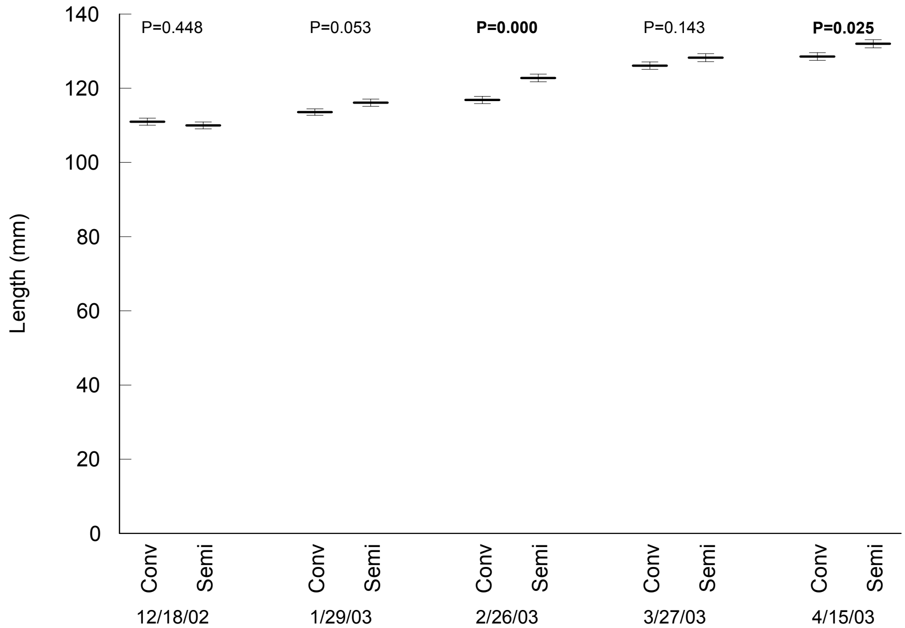

Figure 63. Mean length (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Soos Creek Hatchery in 2003 (N = 100 per treatment). P values are based on *t*-tests.

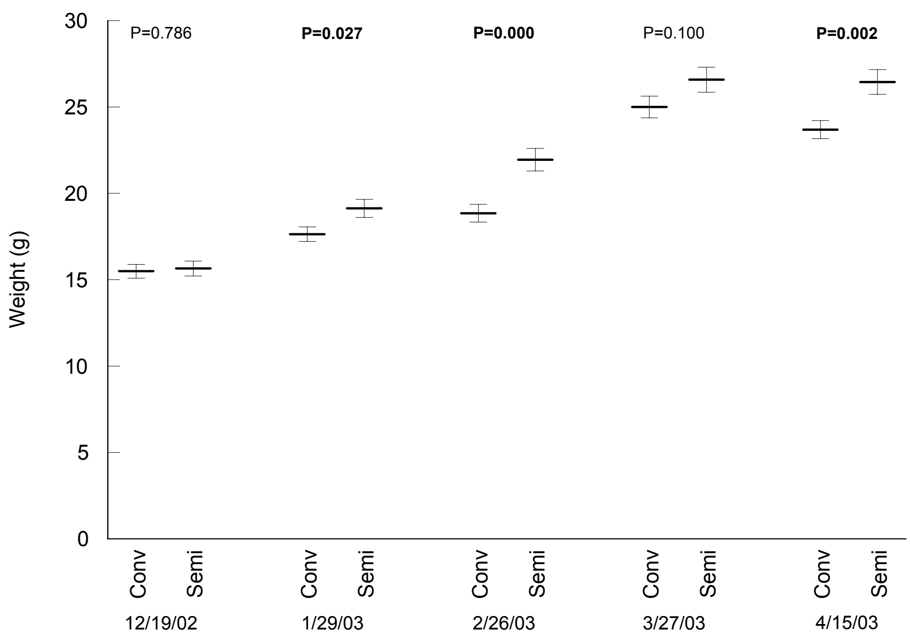

Figure 64. Mean weight (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Soos Creek Hatchery in 2003 (N = 100 per treatment). P values are based on *t*-tests.

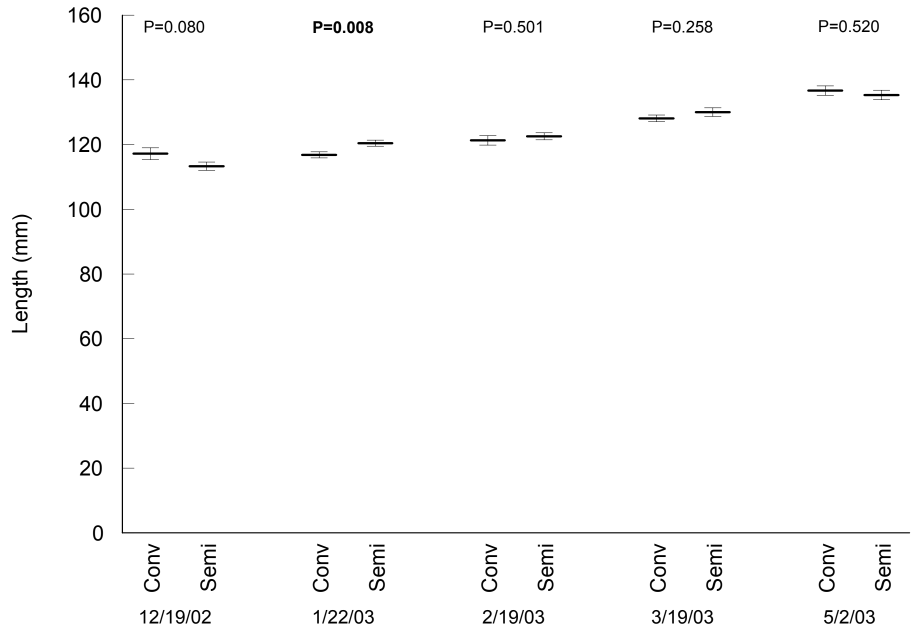

Figure 65. Mean length (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Minter Creek Hatchery in 2003 (N = 100 per treatment). P values are based on *t*-tests.

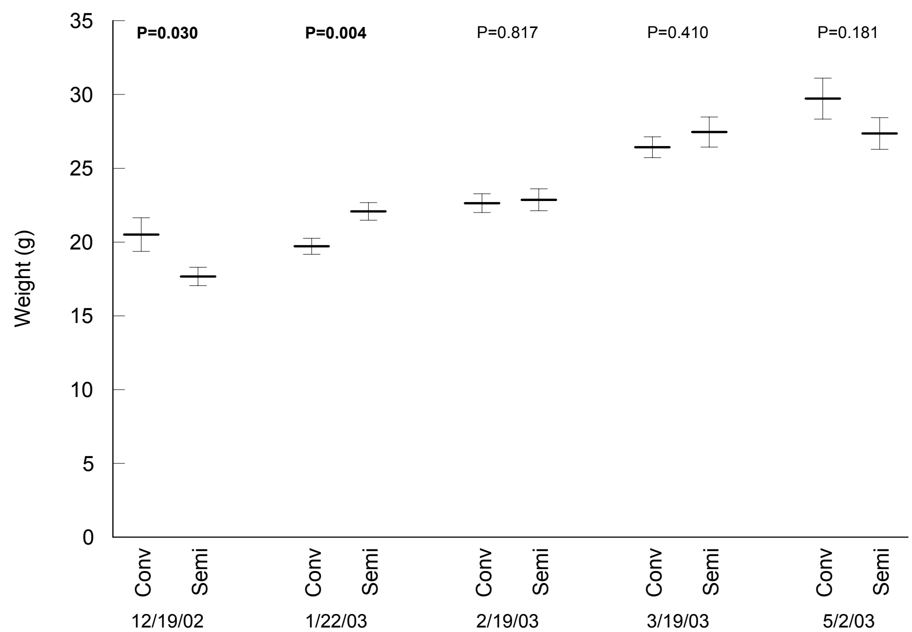

Figure 66. Mean weight (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Minter Creek Hatchery in 2003 (N = 100 per treatment). P values are based on *t*-tests.

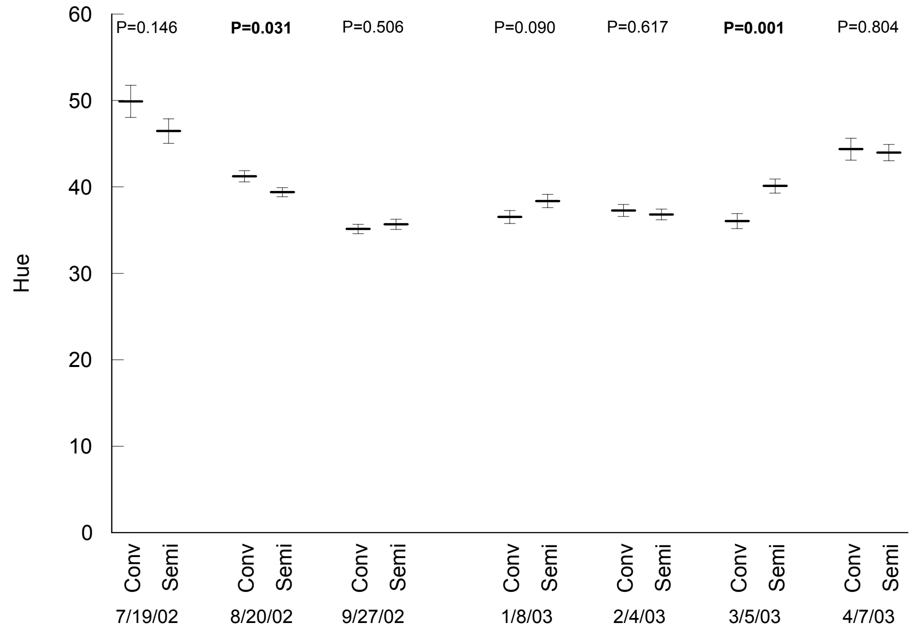

Figure 67. Mean hue values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Sol Duc Hatchery in 2003 (N = 30 per treatment). P values are based on *t*-tests.

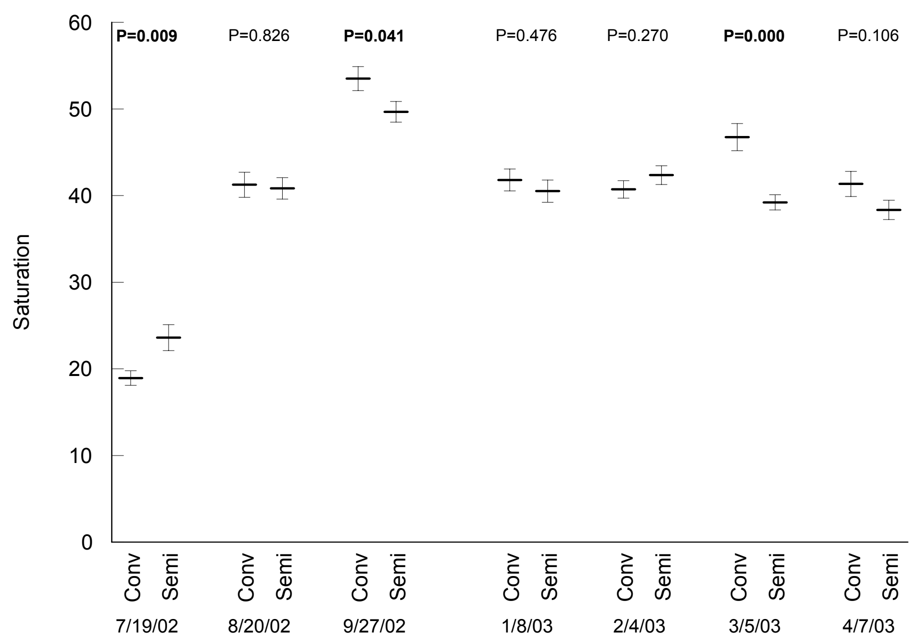

Figure 68. Mean saturation values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Sol Duc Hatchery in 2003 (N = 30 per treatment). P values are based on *t*-tests.

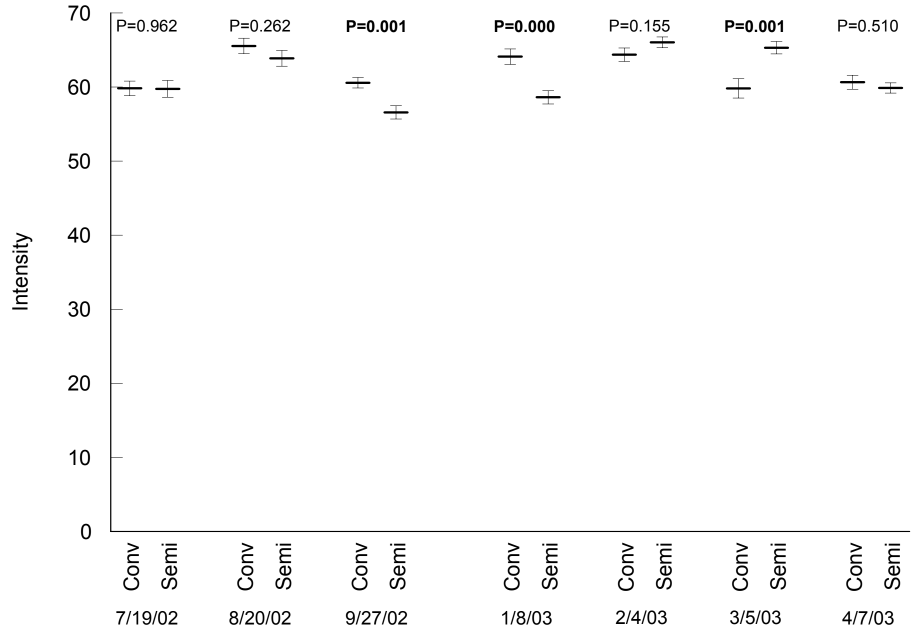

Figure 69. Mean intensity values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Sol Duc Hatchery in 2003 (N = 30 per treatment). P values are based on *t*-tests.

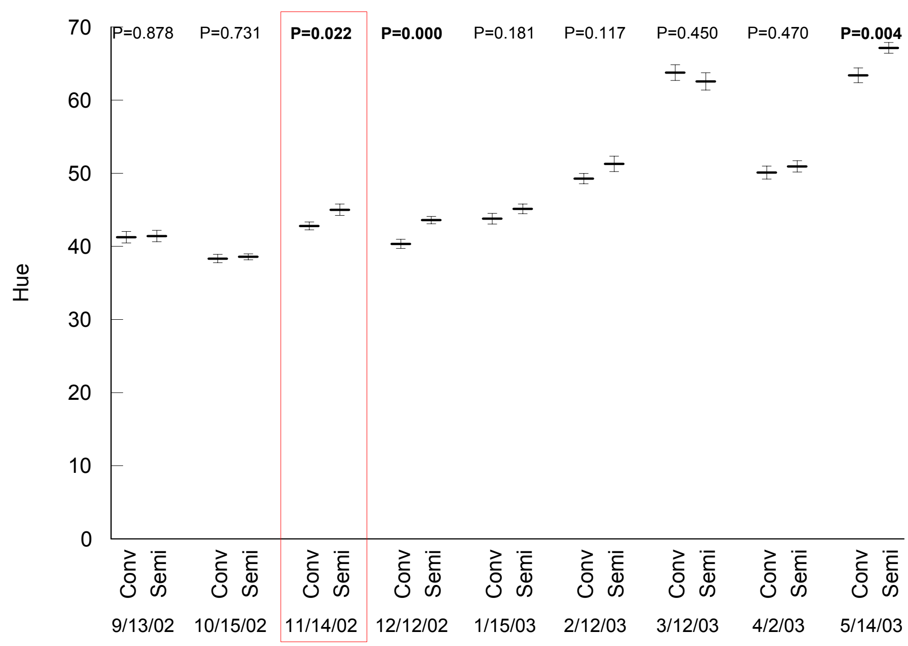

Figure 70. Mean hue values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Kendall Creek Hatchery in 2003 (N = 30 per treatment). P values are based on *t*-tests.

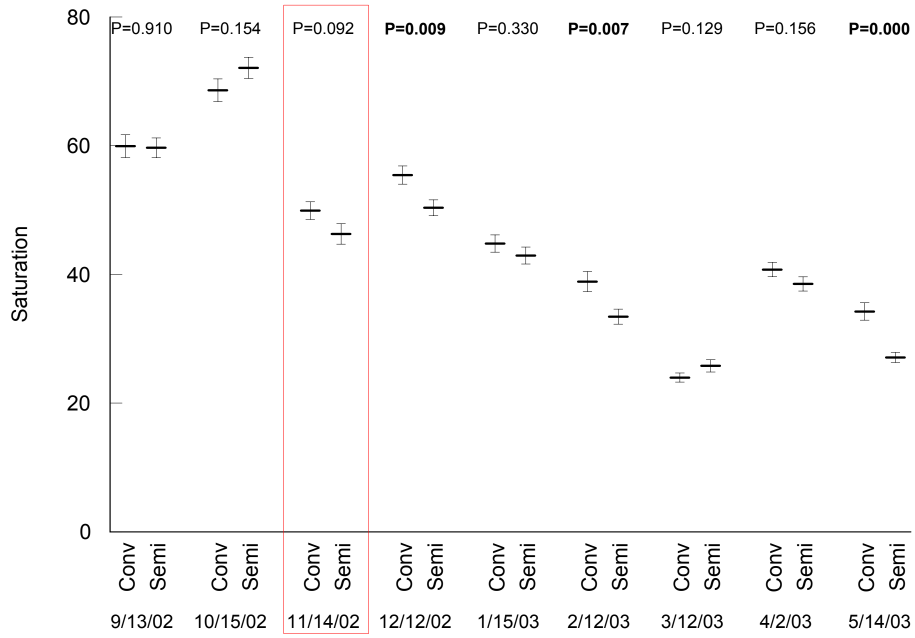

Figure 71. Mean saturation values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Kendall Creek Hatchery in 2003 (N = 30 per treatment). P values are based on *t*-tests.

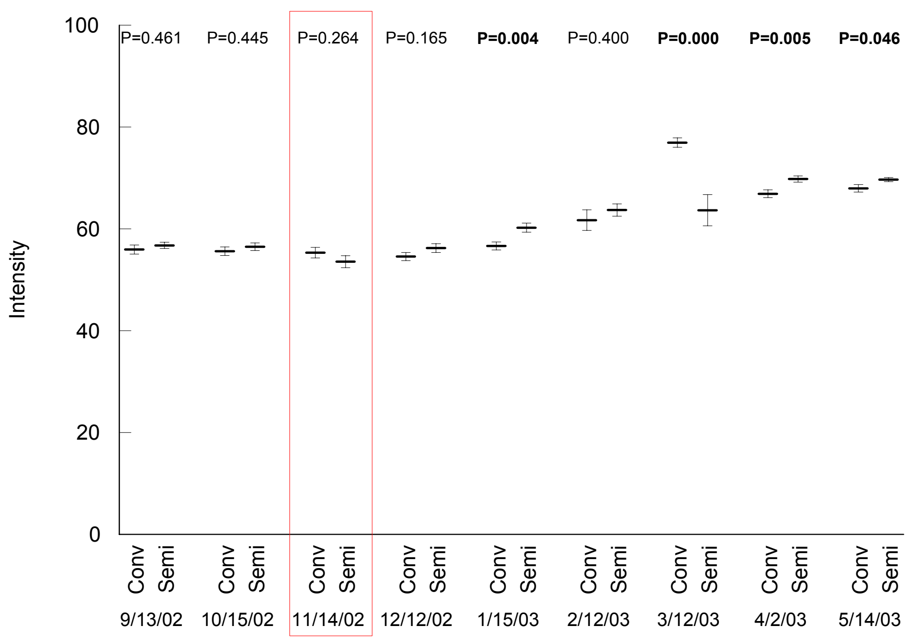

Figure 72. Mean intensity values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Kendall Creek Hatchery in 2003 (N = 30 per treatment). P values are based on *t*-tests.

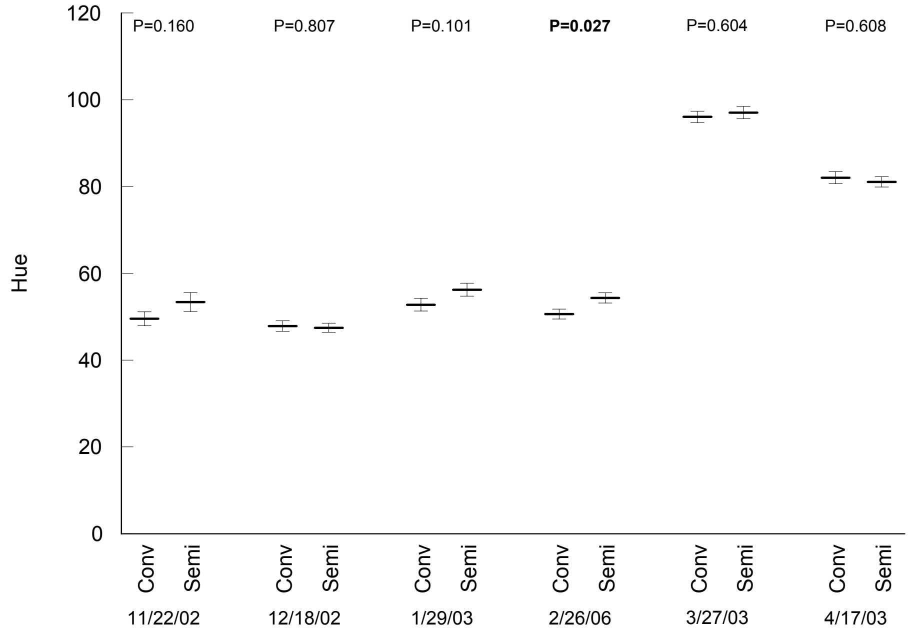

Figure 73. Mean hue values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Issaquah Hatchery in 2003 (N = 30 per treatment). P values are based on *t*-tests.

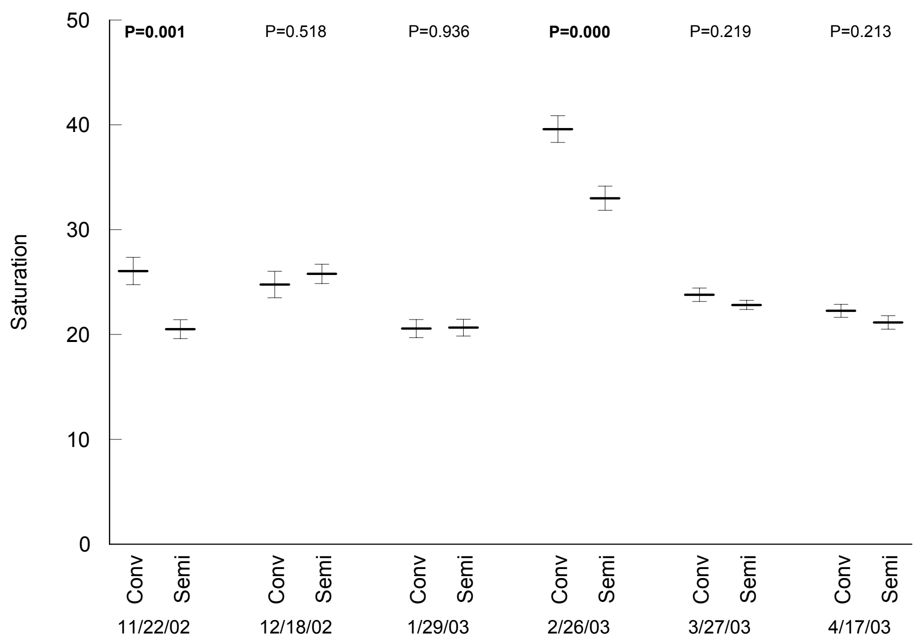

Figure 74. Mean saturation values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Issaquah Hatchery in 2003 (N = 30 per treatment). P values are based on *t*-tests.

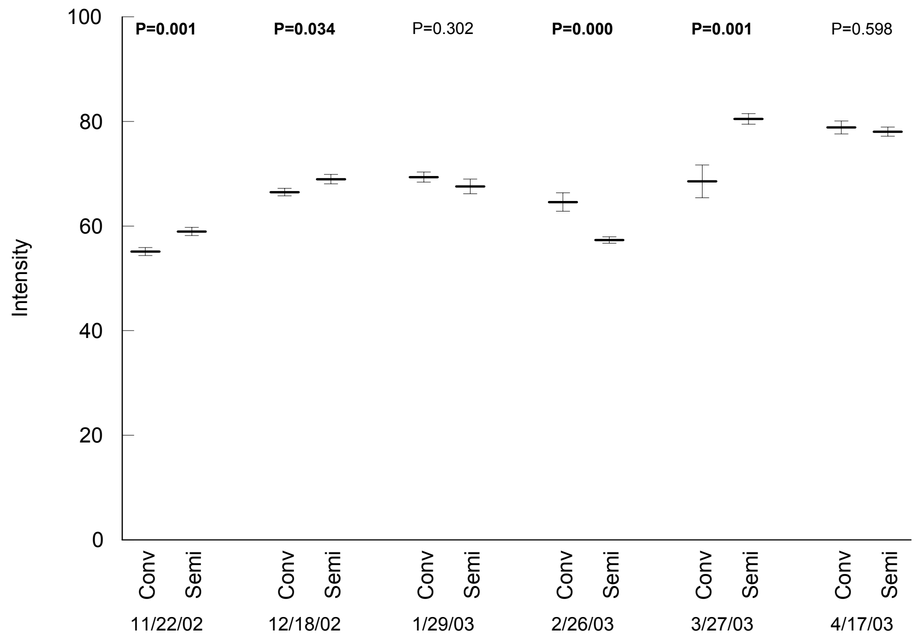

Figure 75. Mean intensity values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Issaquah Hatchery in 2003 (N = 30 per treatment). P values are based on *t*-tests.

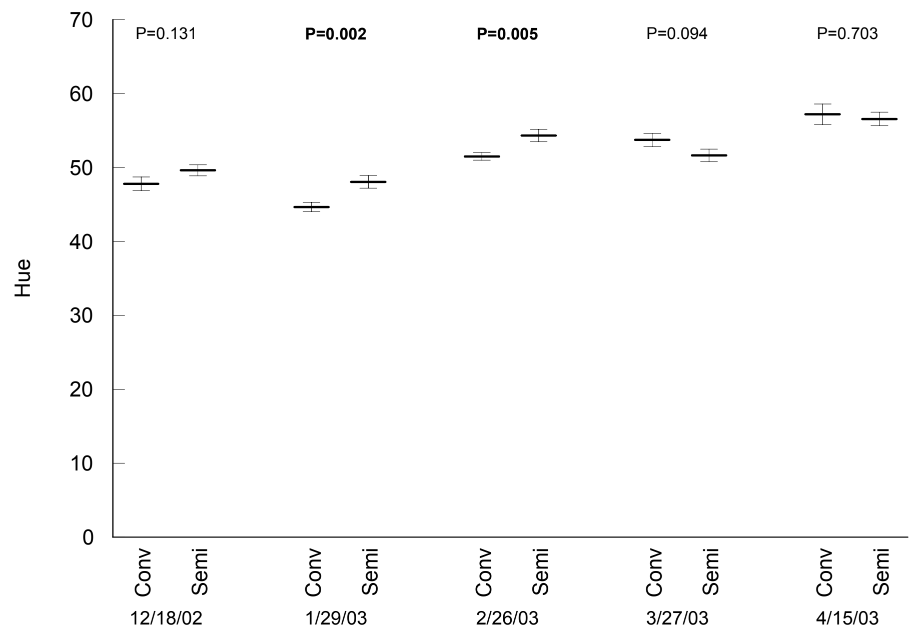

Figure 76. Mean hue values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Soos Creek Hatchery in 2003 (N = 30 per treatment). P values are based on *t*-tests.

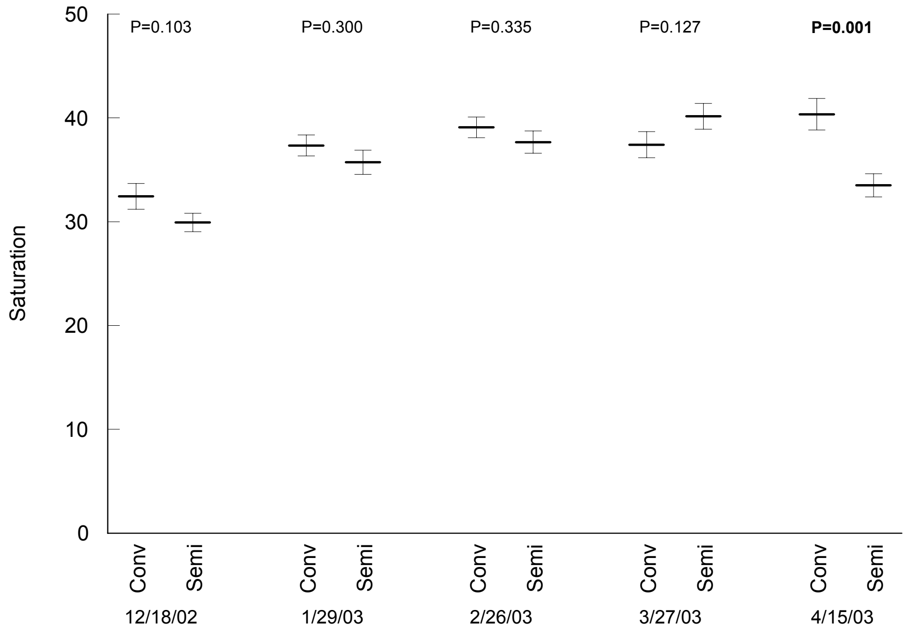

Figure 77. Mean saturation values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Soos Creek Hatchery in 2003 (N = 30 per treatment). P values are based on *t*-tests.

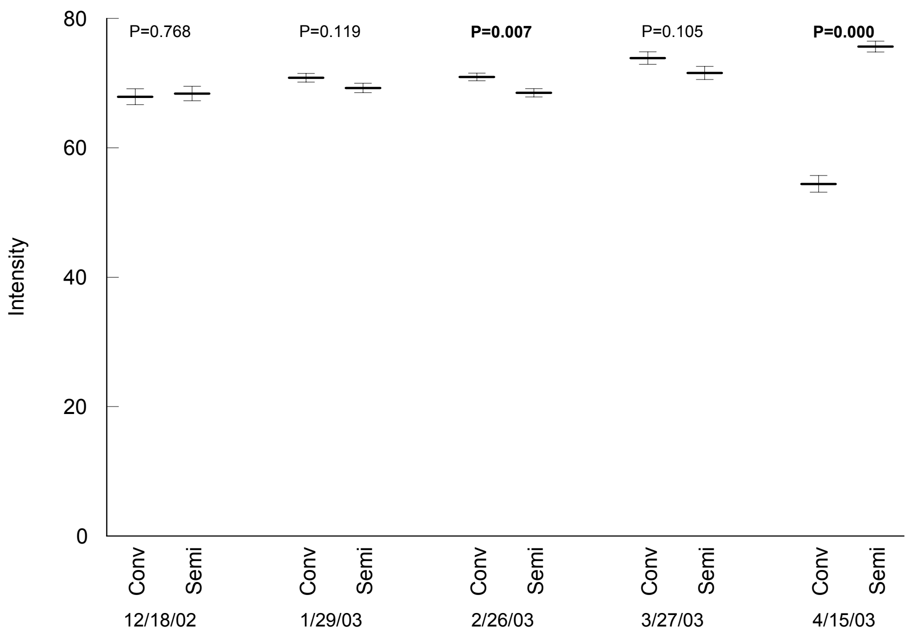

Figure 78. Mean intensity values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Soos Creek Hatchery in 2003 (N = 30 per treatment). P values are based on *t*-tests.

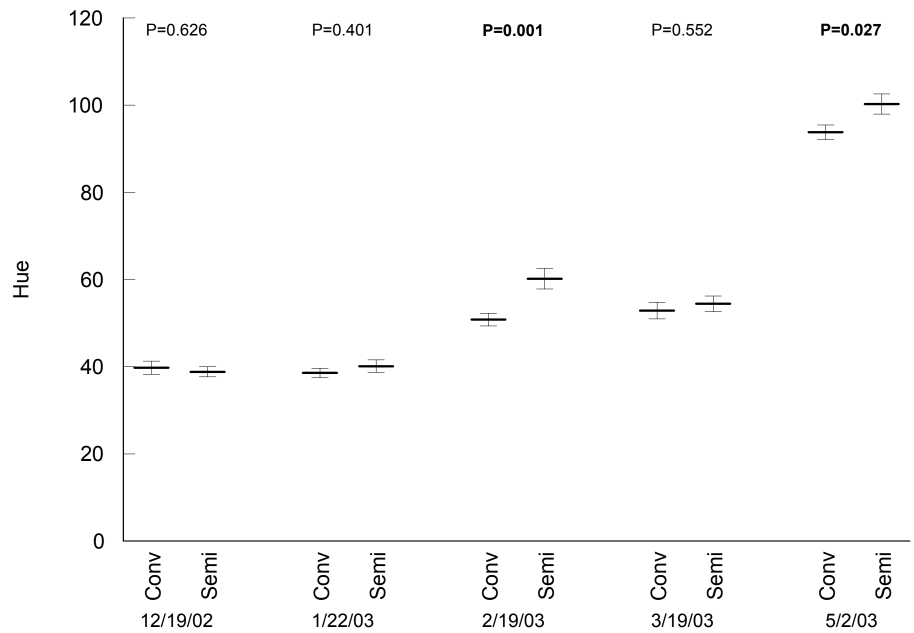

Figure 79. Mean hue values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Minter Creek Hatchery in 2003 (N = 30 per treatment). P values are based on *t*-tests.

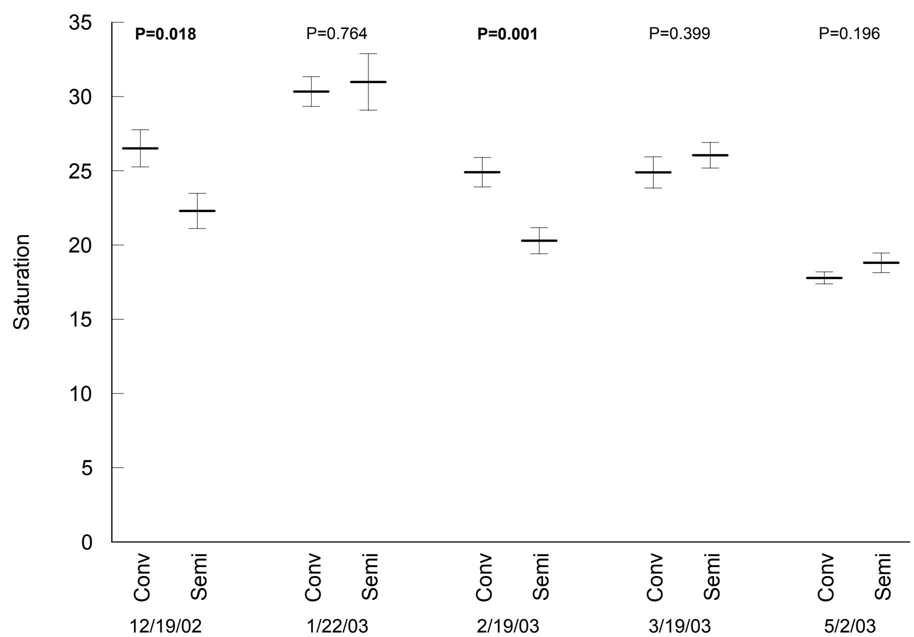

Figure 80. Mean saturation values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Minter Creek Hatchery in 2003 (N = 30 per treatment). P values are based on *t*-tests.

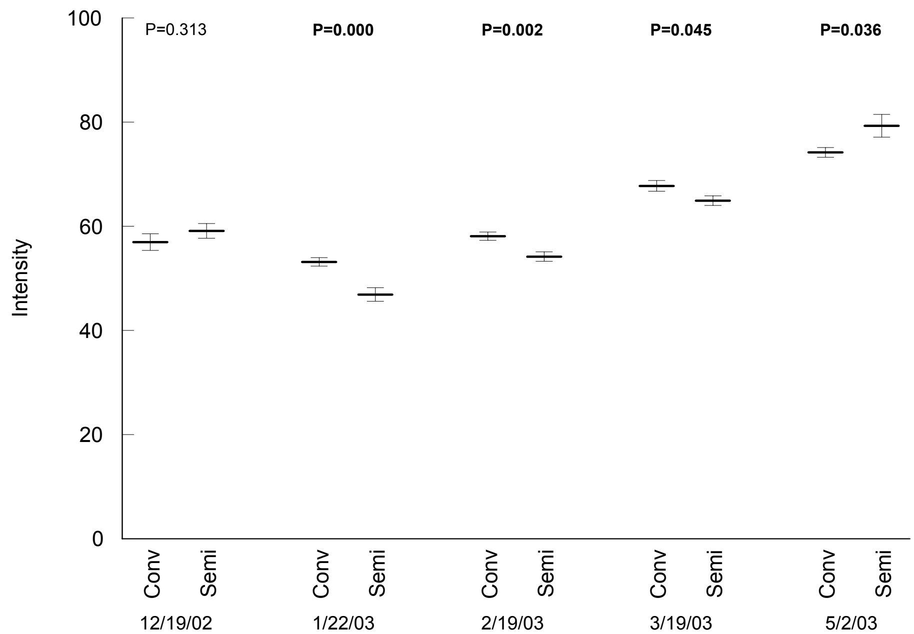

Figure 81. Mean intensity values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Minter Creek Hatchery in 2003 (N = 30 per treatment). P values are based on *t*-tests.

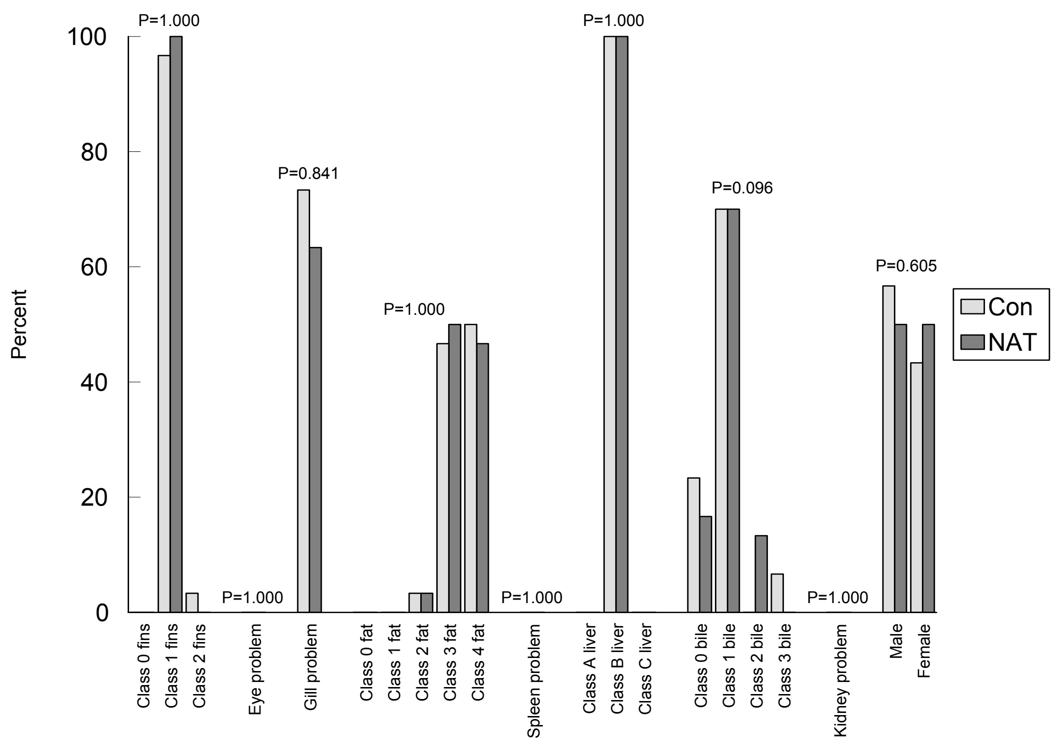

Figure 82. Percentage of coho salmon in different Goede Index classes in the 7 April 2003 Sol Duc River Hatchery fish condition profile. Fish were reared in seminatural (NAT, N = 30) or conventional (Con, N = 30) raceways. P values are based on contingency table analysis.

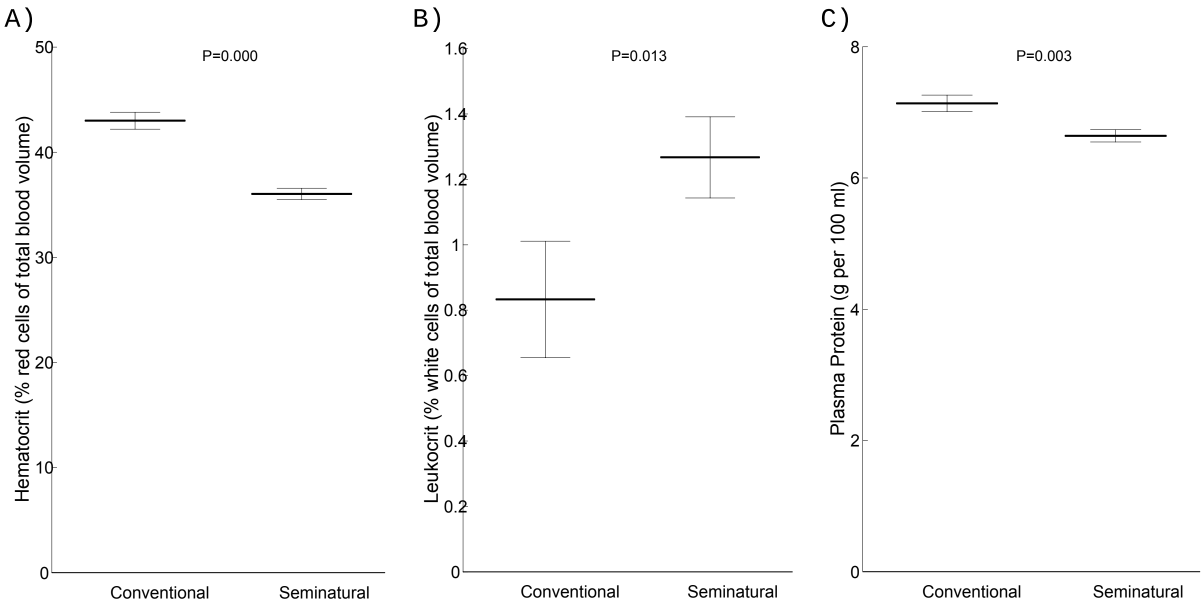

Figure 83. Means (with standard error bars) of blood variables from coho salmon reared in seminatural or conventional raceways at Sol Duc River Hatchery sampled on 7 April 2003. A) hematocrit and B) leukocrit (N = 30 per treatment) P values based on *t*-tests of arcsine transformed data; and C) plasma protein (NAT, N = 28; CON, N = 29) P value based on *t*-tests.

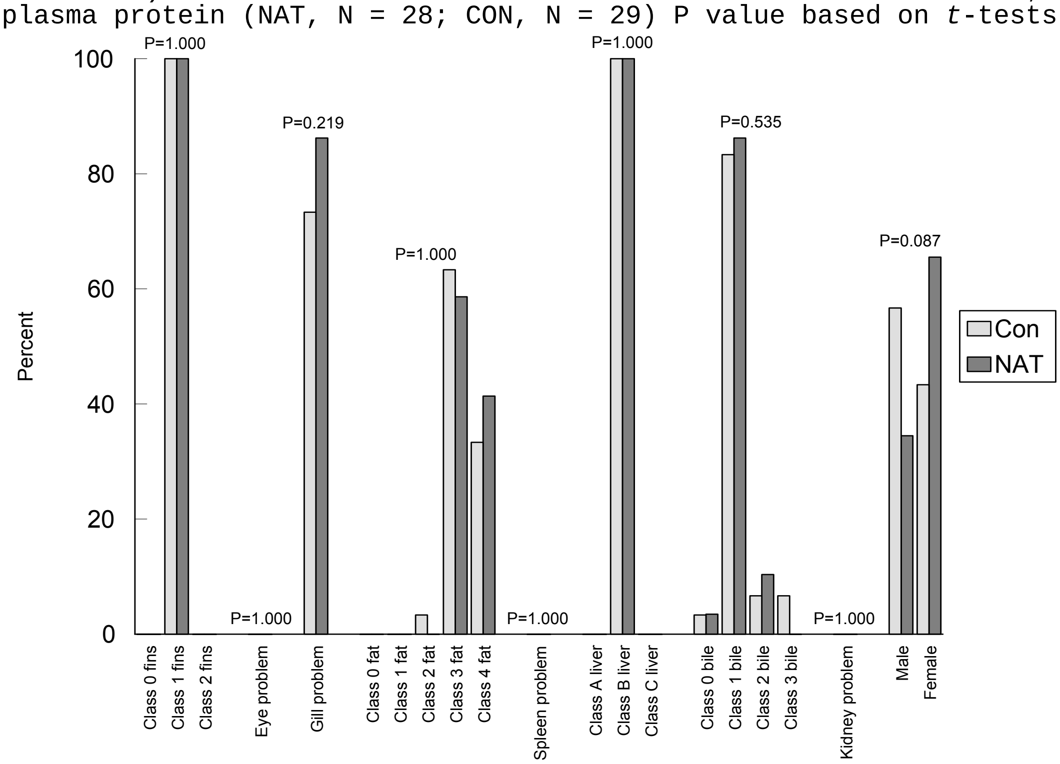

Figure 84. Percentage of coho salmon in different Goede Index classes in the 14 May 2003 Kendall Creek fish condition profile. Fish were reared in seminatural (NAT, N = 30) or conventional (Con, N = 30) raceways. P values are based on contingency table analysis.

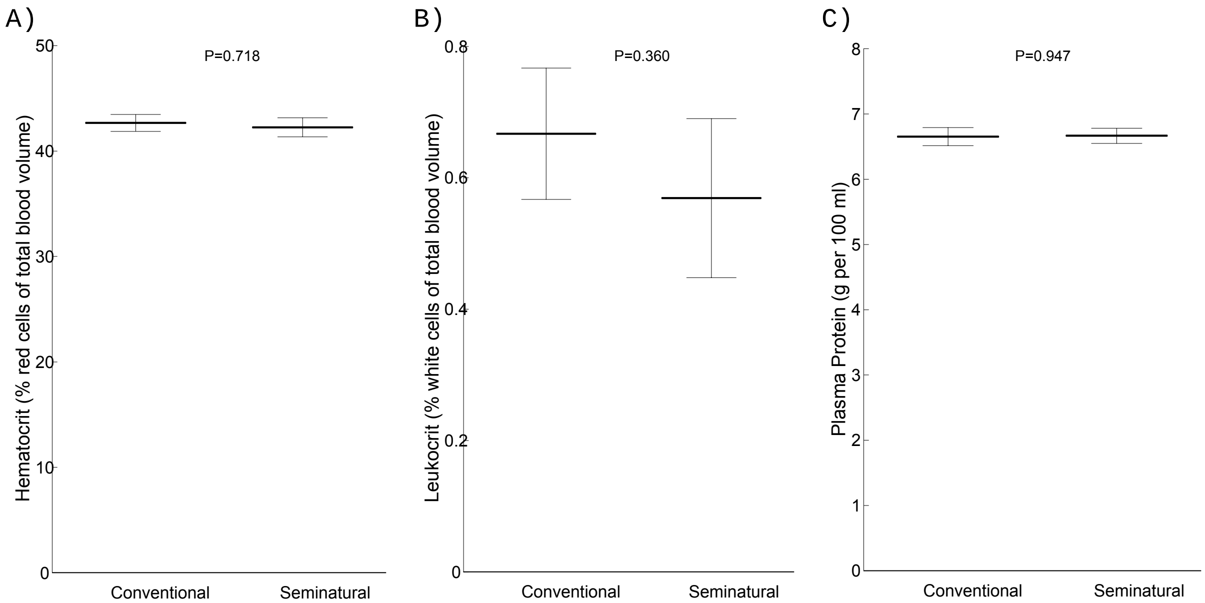

Figure 85. Means (with standard error bars) of blood variables from coho salmon reared in seminatural or conventional raceways at Kendall Creek Hatchery sampled on 14 May 2003. A) hematocrit and B) leukocrit (NAT, N = 29; CON, N = 30) P values based on *t*-tests of arcsine transformed data; and C) plasma protein (NAT, N = 29; CON, N = 30) P value based on *t*-tests.

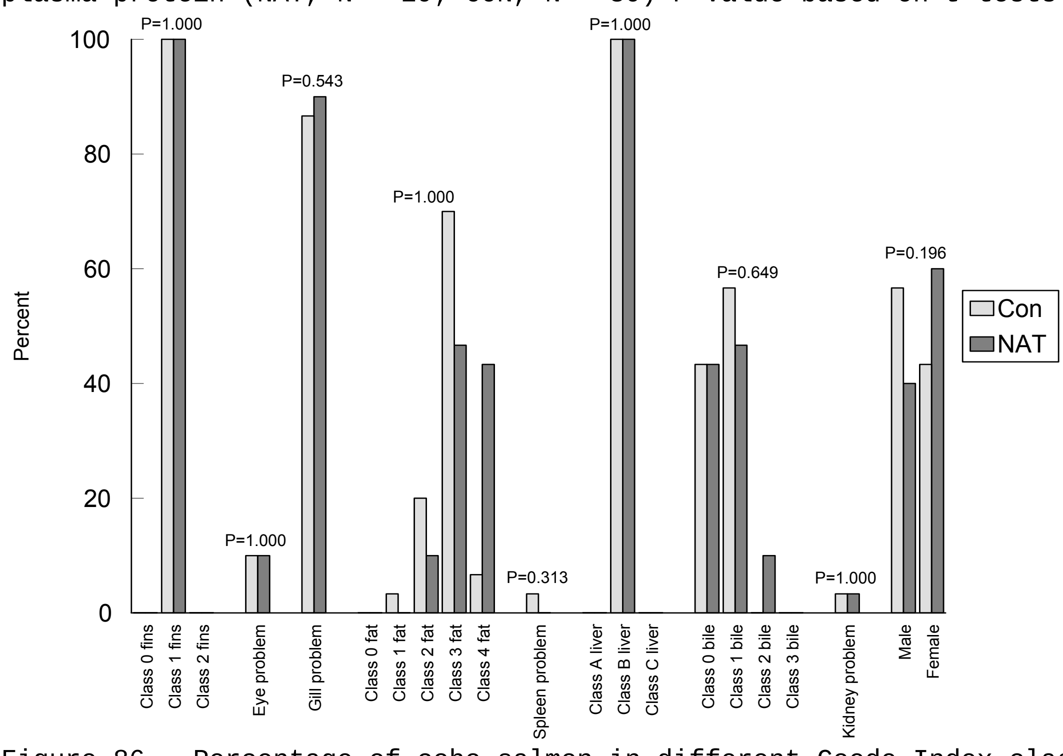

Figure 86. Percentage of coho salmon in different Goede Index classes in the 17 April 2003 Issaquah fish condition profile. Fish were reared in seminatural (NAT, N = 30) or conventional (Con, N = 30) raceways. P values are based on contingency table analysis.

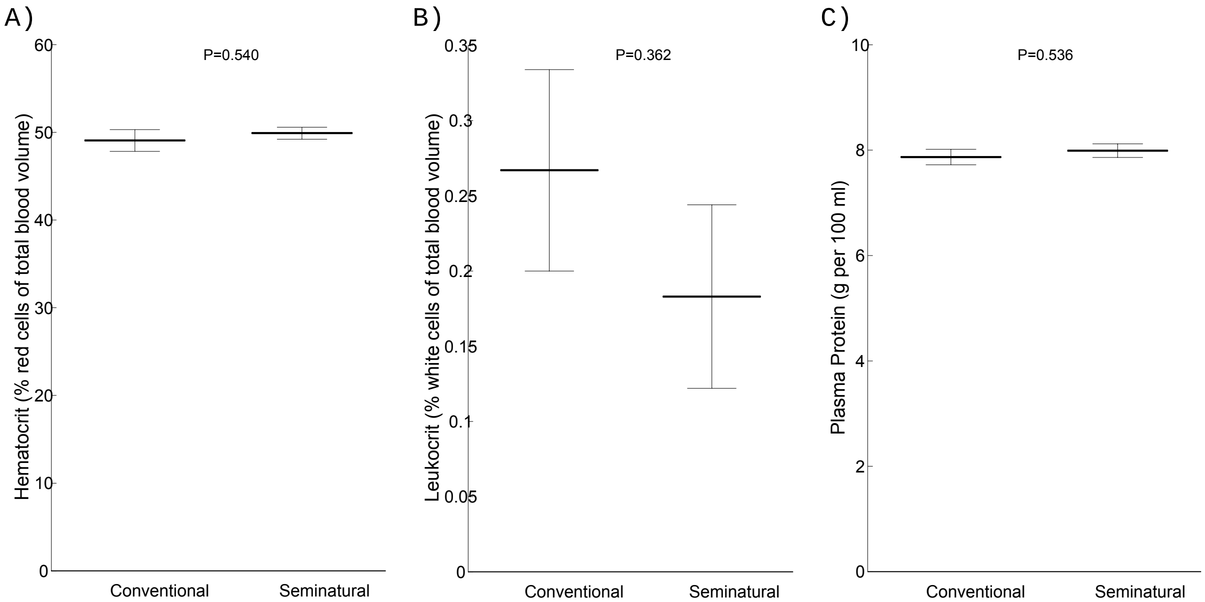

Figure 87. Means (with standard error bars) of blood variables from coho salmon reared in seminatural or conventional raceways at Issaquah Hatchery sampled on 17 April 2003. A) hematocrit and B) leukocrit (N = 30 per treatment) P values based on *t*-tests of arcsine transformed data; and C) plasma protein (NAT, N = 30; CON, N = 29) P value based on *t*-tests.

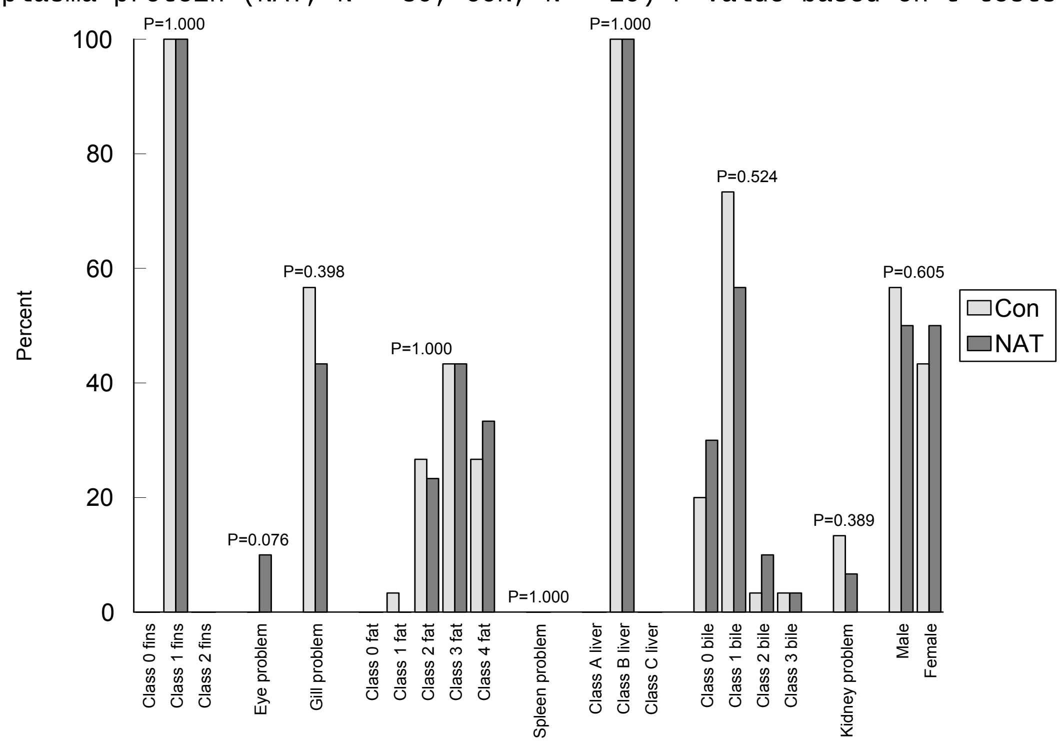

Figure 88. Percentage of coho salmon in different Goede Index classes in the 15 April 2003 Soos Creek fish condition profile. Fish were reared in seminatural (NAT, N = 30) or conventional (Con, N = 30) raceways. P values are based on contingency table analysis.

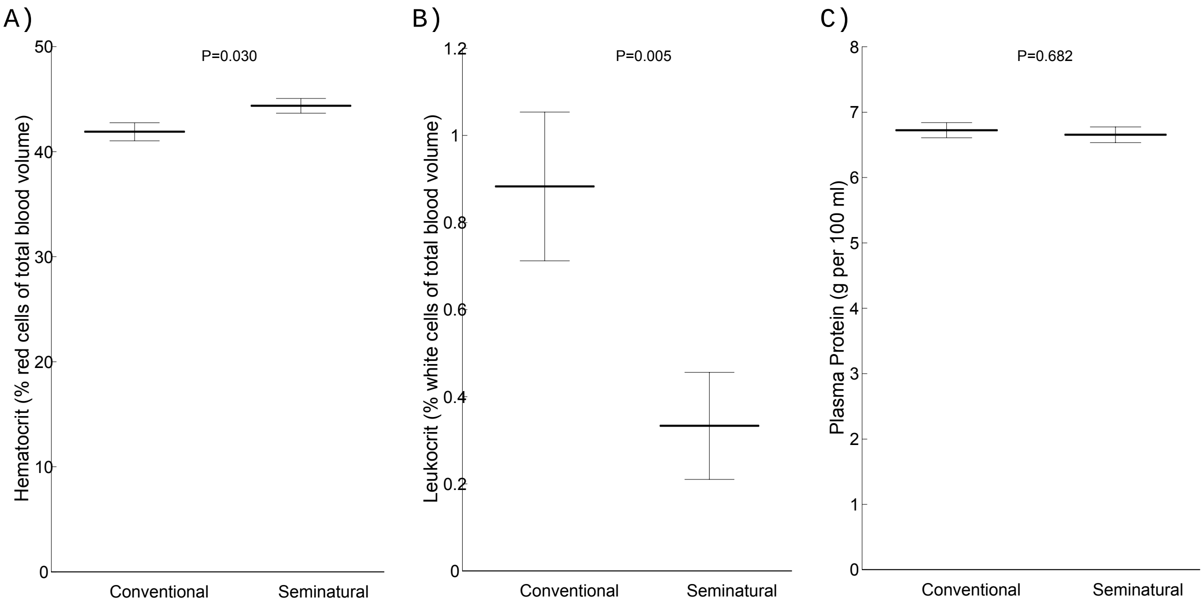

Figure 89. Means (with standard error bars) of blood variables from coho salmon reared in seminatural or conventional raceways at Soos Creek Hatchery sampled on 15 April 2003. A) hematocrit and B) leukocrit (N = 30 per treatment) P values based on *t*-tests of arcsine transformed data; and C) plasma protein (N = 29 per treatment) P value based on *t*-tests.

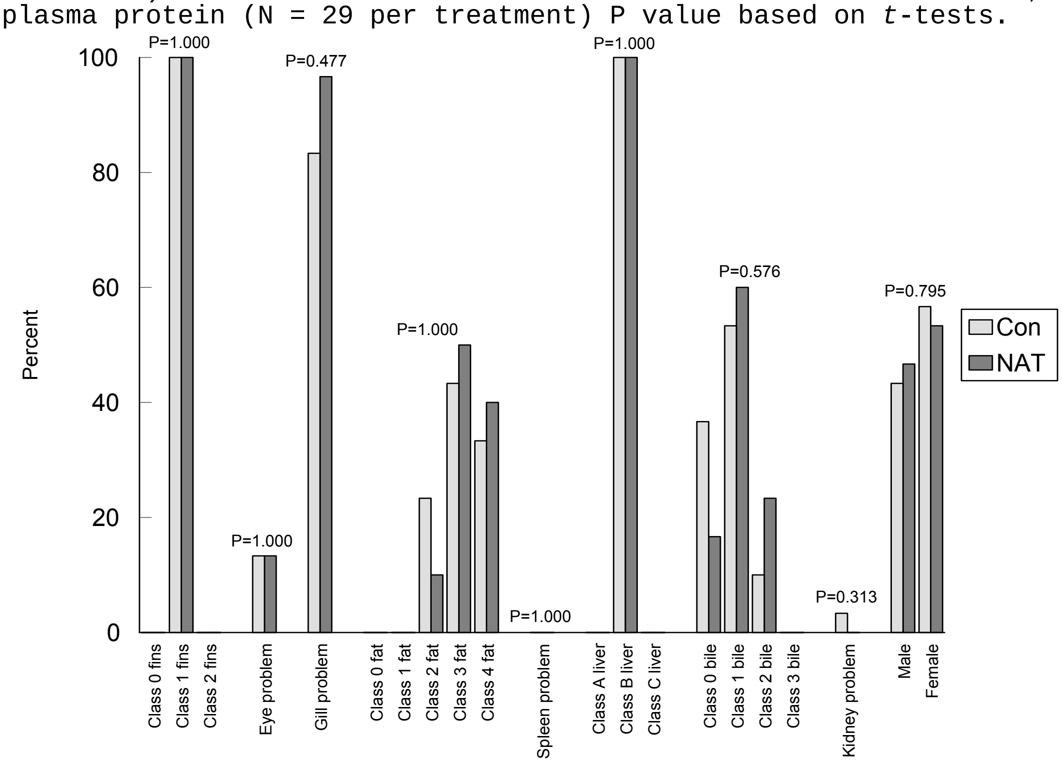

Figure 90. Percentage of coho salmon in different Goede Index classes in the 2 May 2003 Minter Creek fish condition profile. Fish were reared in seminatural (NAT, N = 30) or conventional (Con, N = 30) raceways. P values are based on contingency table analysis.