Influences of Stocking Salmon Carcass Analogs on Salmonids in Klickitat River Tributaries
Yakama Nation Fisheries Program

Influences of stocking salmon carcass analogs on salmonids in Klickitat River tributaries

BPA PROJECT # 2001-055-00

Contract # 7534

Final Contract Report

September 2006

Prepared For: Bonneville Power Administration
P.O. Box 3621
Portland, Oregon 97208-3621

Prepared By: Joe Zendt
Bill Sharp
Yakama Nation Fisheries Program
P.O. Box 151
Toppenish, Washington 98948
Executive Summary

This report describes the work completed by the Yakama Nation Fisheries Program (YNFP) in the Klickitat subbasin in south-central Washington under BPA innovative project # 200105500 - Influences of stocking salmon carcass analogs on salmonids in Columbia River Tributaries. Salmon carcasses historically provided a significant source of marine-derived nutrients to many stream systems in the Columbia basin, and decreased run sizes have led to a loss of this nutrient source in many streams. Partners in this project developed a pathogen-free carcass analog and stocked the analogs in streams with the following objectives: restoring food availability to streams with reduced anadromous salmon returns; mimicking the natural pathways and timing of food acquisition by salmonids; minimizing unintended negative ecological effects; and increasing the growth and survival of salmonids.

In the Klickitat subbasin, carcass analogs were stocked in two streams in 2002 and 2003; a third stream was used as a control. Salmonid fish abundance, growth, and stomach contents were monitored in all three streams before and after carcass analog placement. Fish, invertebrate, and periphyton samples were also collected for stable isotope analysis (to determine if nutrients from carcass analogs were incorporated into the stream food web). Water quality samples were also collected to determine if nutrient overloading occurred in streams.

Significant differences in growth were found between fish in treated and untreated stream reaches. Fish in treatment reaches exhibited higher instantaneous growth rates approximately one month after the first carcass analog stocking. Stomach contents sampling indicated that salmonid fish routinely consumed the carcass analog material directly, and that stomach fullness of fish in treatment reaches was higher than in untreated reaches in the first few weeks following carcass analog stockings. No significant differences were detected in fish abundance between treatment and control streams after carcass analog stocking. Stable isotope analysis provided some evidence that nutrients (primarily nitrogen) were incorporated into periphyton and invertebrates, although this evidence is not strong. No significant differences in water quality were observed between treatment and control streams after analog stocking.

Although no significant changes were observed in fish abundance, this study does provide evidence that carcass analogs provide a viable and potentially useful alternative to stocking salmon carcasses. The analogs provide a direct food source to salmonids, and show some potential for providing nutrients for stream food webs. They can also increase stomach fullness and growth rates of individual fish. This nutrient source may very well improve individual fish condition sufficiently to improve overwintering or smolt survival. Further refinement of stocking densities and timing, treatment duration, and tailoring analog placement to individual stream characteristics (such as channel confinement and flow) will further improve the usefulness of carcass analogs.

Introduction

This report describes the work completed by the Yakama Nation Fisheries Program (YNFP) in the Klickitat subbasin in south-central Washington under Bonneville Power Administration (BPA) innovative project # 200105500 - Influences of stocking salmon carcass analogs on salmonids in Columbia River Tributaries. This project also included work in the Salmon and Yakima River basins; work completed in those basins is described in other publications (e.g., Pearsons et al. in review [a], Pearsons et al. in review [b], Pearsons et al. in review [c]). The
relationship between work in the Klickitat, Yakima, and Salmon River basins can be found in a combination of project proposal #’s 22002 and 22047.

Salmon carcasses historically provided a significant source of marine-derived nutrients to many stream systems in the Columbia basin. Decreased run sizes and subsequent reductions of carcass abundance in many streams and rivers has represented a significant loss of this nutrient source, and may hinder full recovery of stream ecosystems and salmon populations (Gresh et al. 2000). Recent research has suggested that addition of salmon carcasses to streams can provide some of these lost nutrients, and can influence stream productivity and increase growth and survival of juvenile salmonid fish (e.g., Bilby et al. 1998, Kiffney et al. 2005).

Potential ecological risks of adding salmon carcasses to streams include pathogen transfer (since most carcasses would come from hatchery sources that may have higher disease prevalence), nutrient overloading, and increased predation. To address the disease risk, partners in this project (Bio-Oregon, Inc. and Washington Department of Fish and Wildlife) developed a pathogen-free carcass analog made primarily from ground pasteurized fishmeal (derived from fall Chinook carcasses from Spring Creek Hatchery, Underwood, Washington). Details of the development of this product are described in Pearsons et al. (in review [c]).

Overall project objectives were as follows:

1. Restore food availability to streams with reduced anadromous salmon returns.
2. Mimic the natural pathways and timing of food acquisition by salmonids.
3. Minimize unintended negative ecological effects.
4. Increase the growth and survival of salmonids.

The first objective was addressed through development and production of the carcass analog, and by stocking of the carcass analogs into streams in the Klickitat, Yakima, and Salmon River subbasins. Progress towards the last three objectives was monitored via sampling for fish abundance, growth, and stomach contents, as well as stable isotope sampling to determine nutrient uptake in the stream food web (e.g., Bilby et al. 1996) and water quality sampling to monitor unintended nutrient overloading. The following sections describe the results of this work in the Klickitat subbasin.

**Methods**

**Study sites**

Three Klickitat River tributary streams were used in this study: Bear Creek, Trout Creek, and Summit Creek. All three streams are located in the middle part of the subbasin and are east bank tributaries (see Figure 1 for a map of study area). The Trout and Bear Creek study sites were located between 1900 and 2150 ft. in elevation; the Summit Creek sites were 2700-2800 ft. Trout and Bear creeks have primarily rainbow trout (*Oncorhynchus mykiss*) populations; Summit Creek has rainbow trout and brook trout (*Salvelinus fontinalis*) populations. All study sites are located above passage barriers; fish populations at the study sites are exclusively (or nearly so) resident. Resident fish sites were chosen to minimize any confounding effects in population size.
and growth that may result from anadromous outmigration. Bear Creek was used as a control stream (no carcass analogs were placed); analogs were stocked into Trout and Summit Creeks (treatment streams) in fall 2002 and 2003. Two 1000-meter reaches were used on each stream (one upstream reach and one downstream reach). Carcass analogs were placed in the downstream reaches of the treatment streams while the upstream reach served as a stream-specific reference reach. Upstream and downstream reaches were adjacent to each other; each reach end contained a 100-m buffer (one exception occurred on Summit Creek, where reach-end buffers were 50 m). Within each 1000-m reach, three 100-m population sites and two 250-m collection sites were sampled throughout the study. Population sites were used for abundance/population estimate sampling; sampling for stomach contents, length/growth, and stable isotopes was conducted in the collection sites. See Figure 2 for a detailed layout of each 1000-m reach. Sampling generally followed a before-after control-impact study design.

Figure 1. Map of study sites.
Figure 2. Layout of each 1000-meter reach used in study streams. Two adjacent 1000-m reaches were used in each stream; carcass analogs were placed in the downstream reach.

Stocking of carcass analogs

Carcass analogs were stocked into the downstream reaches of Trout and Summit creeks in October 2002 and October 2003. This timing would coincide with spawn timing of Chinook or coho salmon. The analogs consisted of pellets that were about 5 cm in diameter and weighed approximately 11.9 g each. They were stocked into streams at approximately 30 g of analog per square meter of bankfull width. Average bankfull width was 9.14 m in Trout Creek and 6.37 in Summit Creek. The streams received 604 and 421 lbs of analog, respectively, on each treatment occasion. Stocking densities were derived from published relationships between salmon carcass densities and maximum stable isotope compositions (Bilby et al. 2001).

Fish abundance

Salmonid population estimates were made via multiple-pass removal backpack electrofishing in the three 100-m population sites of each upstream and downstream reach of each stream. Sampling was conducted in the summer/early fall in 2002 (before carcass analog stocking), 2003 (one year after 2002 analog stocking), and 2004 (one year after 2003 stocking). Population estimates were derived using Program Capture (White et al. 1982). Population estimates were averaged in each upstream or downstream reach for each time period. The differences between average upstream and downstream population estimates in a given stream for a given year were used in a two-way analysis of variance to determine if significant differences occurred between streams (treatment and control) and time.

Fish growth and stomach contents

In 2002, rainbow trout were tagged with passive integrated transponder (PIT) tags in the collection sites of upstream and downstream reaches of all three streams to assess growth following carcass analog stocking. A total of 407 fish were tagged. Stream and reach totals were as follows: 119 in Bear Creek (68 upstream, 51 downstream), 150 in Summit Creek (75 upstream, 75 downstream), and 138 in Trout Creek (66 upstream, 72 downstream). Fish were
captured via backpack electrofishing. Tagged fish were recaptured during subsequent sampling for stomach contents and population estimates. Lengths and weights of all fish were measured at the initial tagging and at subsequent recaptures. Instantaneous growth rates (IGR) for length and weight were calculated for individual fish using the following equation:

\[
IGR = \frac{\ln Y_2 - \ln Y_1}{t_2 - t_1}
\]

where \( Y_2 \) = weight or length at the end of the time period, \( Y_1 \) = weight or length at the beginning of the time period, and \( t_2 - t_1 \) = number of days at large (Ricker 1975). Growth rates of fish recaptured after each time period (about one month and one year after carcass analog stocking) were analyzed with a two-way analysis of variance to determine if there were significant differences between treatment and control streams and upstream and downstream reaches. A Tukey HSD pairwise comparison test was used if significant differences were detected by the analysis of variance. If fish moved between reaches, they were placed in upstream or downstream groups based on where they were recaptured.

Stomach contents were collected via gastric lavage from approximately 30 rainbow trout longer than 60 mm from each upstream and downstream reach during four sampling periods (before carcass analog placement, 2-3 weeks after, 4-6 weeks after, and 1 year after analog placement) for the two analog placement occasions. Stomach contents samples were then dried in a drying oven and weighed. Stomach contents were expressed as % full based on methods described by Herbold (1986) and Pearsons et al. (2005). This method estimates stomach fullness for each fish by dividing the observed stomach content weight by a predicted maximum stomach content weight for a fish of that length. The predicted maximum stomach content weight is modeled with regression methods using the maximum stomach content weights of fish of different lengths from the total sample. For this maximum predicted weight regression, stomach content weights of fish from all streams, reaches, and time periods were used, but weights of carcass analog material in stomach samples were excluded. For the final estimation of stomach fullness, the total observed stomach content weight (including any carcass analog material present) was used. Stomach fullnesses were tested for significant differences between fish collected in upstream and downstream reaches in each sampling period. Because these data showed evidence of a non-normal distribution, a Kruskal-Wallis analysis of variance test was used.

**Stable isotope analysis**

Samples of rainbow trout, aquatic invertebrates, and periphyton were collected for stable isotope analysis during three sampling periods (before carcass analog placement, about 1 month after, and 1 year after analog placement) in upstream and downstream reaches for the two analog placement occasions. Total sample sizes were generally divided evenly between upstream and downstream reaches in each sampling period. Fish were captured via backpack electrofishing in the collection sites, sampled for stomach contents, and then euthanized. A portion of dorsal muscle tissue from each fish was later excised for analysis. The total number of fish sampled was 256 (88 in 2002; 120 in 2003; 48 in 2004). Aquatic invertebrates were collected with a D-frame kick net and held alive for 24 hours to allow for evacuation of gut contents. A total of 36 invertebrate samples were collected (18 in 2002; 12 in 2003; 6 in 2004); each sample consisted of 30-60 individuals. Samples consisted primarily of mayflies (Ephemeroptera) and were primarily grazers. Periphyton samples were collected from streambed rocks using a wire brush.
and were washed into sample bags with a small volume of water. A total of 30 periphyton samples were collected (12 in 2002 and 2003; 6 in 2004).

Frozen samples were sent to the National Marine Fisheries Service (NMFS) Northwest Fisheries Science Center for sample preparation and then to the University of Alaska for stable isotope analysis. Values for δ¹⁵N and δ¹³C were determined using mass spectrometry. These values represent differences in parts per thousand between the proportion of ¹⁵N or ¹³C in the sample and those proportions in a standard (Peterson and Fry 1987). Higher δ values represent higher amounts of the heavy isotope component in the sample, and higher amounts of marine-derived (in this case, carcass analog) nutrients.

The δ¹⁵N and δ¹³C values were averaged in each upstream or downstream reach for each time period. The differences between average upstream and downstream values in a given stream for a given time period were used in a two-way analysis of variance to determine if significant differences occurred between streams (treatment and control) and time. A Tukey HSD pairwise comparison test was used if significant differences were detected by the analysis of variance.

Water quality

Water quality samples were collected at 2- to 3-week intervals (3 sampling occasions before, 3 sampling occasions after carcass analog placement) in 2002 and 2003. Samples were collected in the upstream and downstream reach of each stream. The post-stocking samples were collected from 20 to 48 days after stocking in 2002, and 9-44 days after stocking in 2003. Samples were sent to the University of Washington for analysis. Water quality parameters measured were: total nitrogen, total phosphorus, PO₄-P, SiO₂-Si, NO₃-N, NO₂-N, and NH₄-N. Total N and total P concentrations were averaged in upstream and downstream reaches in treatment and control streams for each sampling occasion. The differences between average upstream and downstream values in treatment and control streams for time periods before and after stocking were used in a two-way analysis of variance to determine if significant differences occurred between streams (treatment and control) and time.

Results

Stocking of carcass analogs

Carcass analogs were retained fairly well in the stream channel after placement, although a small portion of analogs were transported out of the treatment reach in the stream current. Roughly 1/3 to 1/2 of the analogs floated initially (analogs used in 2003 floated slightly less than those used in 2002), but most of these floaters became waterlogged in a short time and sank slowly or became trapped in eddies and backwaters along the channel margin. Downstream transport was more of a problem in Summit Creek due to a more confined channel and current. In 2003, very few analogs were placed directly in the lowermost 300 meters of the Summit treatment reach; instead extra analogs were placed directly in the current upstream (in the middle sections of the treatment reach) and these seeded the lower end adequately. This minimized the loss of analogs out the downstream end of the treatment reach. In Trout Creek, due to slower current, wider channel, and more backwater areas, analogs stayed roughly where they were placed. Analogs
decomposed over several weeks, with a fungal or algal film forming on their surface (which appeared several days to a week after stocking) before finally dissolving or breaking down.

Figure 3. Photo of carcass analogs on streambed in Trout Creek.

Fish abundance

No significant differences were detected in salmonid abundance between treatment and control streams after carcass analog placement (Figure 4; P > 0.10 for all factors [treatment, time, treatment x time]).

Fish growth

A total of 143 PIT-tagged fish were recaptured (108 in 2002, 33 in 2003, and 2 in 2004). In Summit Creek, 2 fish moved from upstream to downstream reaches and 5 fish moved from downstream to upstream reaches. All other fish were recaptured in the same reach in which they were tagged.
Figure 4. Average salmonid densities (± 2 SE) in the 3 study streams before (2002) and one year after each of two stockings of carcass analogs (2003 and 2004).
Fish recaptured about 1 month (average 29 days, range 16-47 days) after carcass analog stocking exhibited higher instantaneous growth rates for weight in 2002, but not in 2003. For fish recaptured in treatment streams in 2002, growth rates were significantly higher in downstream reaches than in upstream reaches (P < 0.05; Figure 5). There was no statistically significant difference in growth between upstream and downstream reaches in the control stream in 2002 (P > 0.20; Figure 5). No significant factors were found for fish recaptured 1 month after stocking in 2003 (P > 0.10; Figure 5).

Fish recaptured 1 year (average 374 days, range 367-384 days) after carcass analog stocking did not exhibit significant differences in instantaneous growth rates between treated and untreated areas (P > 0.10; Figure 5).

*Fish stomach contents*

Stomach contents collection indicated that rainbow trout fed directly on carcass analogs in treatment reaches (carcass analog material was frequently observed in stomach contents during gastric lavage). In 2002 after carcass analog stocking, 44% of the fish sampled in downstream reaches of treatment streams had carcass analog material in their stomachs; in 2003, this figure was 20%.

In both treatment streams in 2002 and 2003, a significant difference (P < 0.05) in stomach fullness was detected between upstream and downstream reaches in the sampling period 2-3 weeks after carcass analog stocking (Figure 6). In one treatment stream (Trout Creek), a significant upstream-to-downstream difference was also detected in the sampling period 6 weeks after stocking in 2002, and in the period 1 year after stocking (the first sampling period of 2003; Figure 6). In all of these instances, the fullness of downstream-reach fish was higher than that of upstream-reach fish. There was one instance of significant upstream-to-downstream difference in the control stream (in the last sampling period of 2003), however in this case the upstream fullness was higher than the downstream fullness (Figure 6). The dry weight of carcass analog material was higher than other stomach contents; however in most cases trout that consumed analog material also consumed more invertebrates than fish in untreated reaches.

*Stable isotope analysis*

There was some indication from stable isotope analysis that carcass analog nitrogen was incorporated by periphyton. There was some evidence that the upstream-downstream difference in δ¹⁵N values was higher in treatment streams one month after carcass analog placement (Figure 7; P < 0.10). No significant differences were detected in δ¹³C values, nor in δ¹⁵N values for any other time for periphyton (P > 0.10). No significant differences were detected in δ¹³C or δ¹⁵N values for invertebrates (P > 0.10), but missing samples prevented a treatment x time term in the analysis of variance. Graphical interpretation of invertebrate data indicates a possible increase in δ¹³C and δ¹⁵N values one month after carcass analog placement (Figure 8). No significant differences were detected in δ¹³C or δ¹⁵N values for fish (Figure 9; P > 0.10).
Figure 5. Mean instantaneous growth (± 2 SE) of PIT-tagged rainbow trout recaptured in upstream and downstream reaches of treatment and control streams. Asterisk indicates significant difference (P < 0.05) between upstream and downstream reaches.
Figure 6. Average percent stomach fullness (± 2 SE) for fish in upstream and downstream reaches of treatment (Summit and Trout) and control (Bear) streams in periods before (Period 1 in 2002), 2-3 weeks after, 4-6 weeks after, and 1 year after (Period 1 in 2003) carcass analog stocking in 2002 and 2003. Asterisk indicates significant difference (P < 0.05) between upstream and downstream reaches.
Figure 7. Stable isotope concentrations in periphyton before, approximately one month after, and one year after carcass analog stocking.
Figure 8. Stable isotope concentrations in invertebrates before, approximately one month after, and one year after carcass analog stocking.
Figure 9. Stable isotope concentrations in fish muscle tissue before, approximately one month after, and one year after carcass analog stocking.
**Water quality**

No significant differences after carcass analog placement between treatment and control streams were detected in total nitrogen or total phosphorus concentrations (Figures 10, 11, and 12; \( P = 0.45 \) for treatment x time [total N]; \( P = 0.63 \) for treatment x time [total P]; \( P > 0.10 \) for all factors). In addition, no consistent differences were evident in PO\(_4\)-P, SiO\(_4\)-Si, NO\(_3\)-N, NO\(_2\)-N, and NH\(_4\)-N concentrations (Figures 13, 14, and 15).

![Graph of 2002 Bear Creek Total P](image)

**Figure 10.** Total phosphorus and total nitrogen concentrations in Bear Creek (no carcass analogs placed).
Figure 11. Total phosphorus and total nitrogen concentrations in Trout Creek before and after carcass analog placement (analogs placed in downstream reach).

Figure 12. Total phosphorus and total nitrogen concentrations in Summit Creek before and after carcass analog placement (analogs placed in downstream reach).
Figure 13. PO$_4$-P, SiO$_4$-Si, NO$_3$-N, NO$_2$-N, and NH$_4$-N concentrations in Bear Creek (no carcass analogs placed).
Figure 14. PO_4-P, SiO_4-Si, NO_3-N, NO_2-N, and NH_4-N concentrations in Trout Creek before and after carcass analog placement (analogs placed in downstream reach).
Figure 15. PO$_4$-P, SiO$_4$-Si, NO$_3$-N, NO$_2$-N, and NH$_4$-N concentrations in Summit Creek before and after carcass analog placement (analogs placed in downstream reach).
Discussion

Although no significant changes were observed in fish abundance, this study does provide evidence that carcass analogs provide a viable and potentially useful alternative to stocking salmon carcasses. The analogs provide a direct food source to salmonids, as evidenced by the frequent observation of carcass analog material in stomach content sampling. Fish in treatment reaches had significantly higher stomach fullness than those observed in untreated reaches in the first few weeks following carcass analog stockings. Fish in treatment reaches also exhibited significantly higher growth rates approximately one month after the first carcass analog stocking. And although the evidence from this study is not statistically strong, carcass analogs show some potential for providing nutrients for stream food webs via uptake by periphyton and grazing invertebrates. It is also evident that stocking carcass analogs in similar streams at the densities used in this study is not likely to degrade water quality.

Several reasons could be explored for not finding significant changes in salmonid abundance or stronger evidence of nutrient uptake in this study. Stocking densities may have been too low for these particular streams. The duration of the study or the treatment may not have been long enough. The short-term application of carcass analogs may not closely mimic natural availability of salmon carcasses (which may be supplied over a spawning period of several weeks). A longer availability of the nutrient source may allow the stomach fullness and growth changes observed in this study to translate into increases in survival and abundance. And sample sizes (especially for stable isotope samples) may have been too small.

Carcass analogs may very well provide a nutrient source that improves individual fish condition sufficiently to improve overwintering or smolt survival. Further refinement of stocking densities and timing, and tailoring analog placement to individual stream characteristics (such as channel confinement and flow), will further improve the usefulness of carcass analogs.

Acknowledgements

Christopher Johnson, Todd Pearsons, and Michael Schmuck of WDFW provided assistance with study design, field methods, and data analysis. Many of the analyses in this report were patterned after those in Pearsons et al. (in review [a]) and Pearsons et al. (in review [b]). The majority of the field data collection was performed by Sandy Pinkham, Rodger Begay, Isadore Honanie, Roger Stahi, William Wesley, Chelsey Moss, Bill Flett, and Ernie Reynolds. Rolf Evenson assisted with field crew oversight. Stable isotope analysis was conducted by NMFS Northwest Fisheries Science Center (Beth Sanderson, Holly Coe, and Chau Tran) and by University of Alaska. Water quality analysis was conducted by Kathy Krogslund at University of Washington. Stomach contents analysis was conducted by Brenda Ben James of Cascade Aquatics, L.L.C.
References


