Exxon Valdez Oil Spill
Restoration Project Annual Report

Feasibility of Otolith Marking Wild Pink Salmon using Tetracycline in Prince William Sound

Restoration Project 94320C
Annual Report

This annual report has been prepared for peer review as part of the *Exxon Valdez* Oil Spill Trustee Council restoration program for the purpose of assessing project progress. Peer review comments have not been addressed in this annual report.

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Study History: Tetracycline marking was initiated in 1994 as an integral part of Restoration Project 94320C. This study was originally designed to implement thermal mass marking of otoliths and investigate small scale chemical otolith marking in Prince William Sound. The project was reduced to only investigate small scale chemical otolith marking.

Abstract: Emergent pink salmon *Onchorhynchus gorbuscha* fry were immersed in a 400 mg/l solution of tetracycline at three different temperatures and four durations of exposure (18 hr max) to test the usefulness of the procedure for field marking otoliths. Both treated and untreated fry were then reared in saltwater netpens for 4 weeks after which their otoliths were removed, processed, and examined for fluorescent marks. An initial examination of 10 fry from the highest treatment group found discernable fluorescent bands were present on the otoliths of all 10 fish. No significant difference in short-term mortality between treatment and control groups was detected.

Key Words: Marking, *Onchorhynchus gorbuscha*, otoliths, pink salmon, Prince William Sound, tetracycline.

Citation:

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EXECUTIVE SUMMARY

This study was designed to test the feasibility of marking pink salmon *Onchorhynchus gorbuscha* in a field setting by immersion in tetracycline. Coded wire tags have been the tool of choice for applying unique marks to populations of pink salmon in Prince William Sound. Because of the cost and problems associated with coded wire tag technology, other alternatives for marking larger portions of populations with relatively inexpensive non-intrusive methods are being investigated. Non-intrusive marks which cannot be shed and which do not affect survival or behavior will eliminate important sources of error in mark-recapture population and straying rate estimates. This data would provide essential information to fishery managers to reduce fishery exploitation rates on *Exxon Valdez* Oil Spill damaged wild salmon stocks. Tetracycline has been used successfully to apply chemical marks in many other fish species. Marks on fish otoliths from tetracycline have been shown to be permanent, easily applied, recognizable, and do not appear to alter fish survival.

Our objectives in this study were threefold; (1) test and refine remote field camp methods and equipment to be used for immersing wild pink salmon fry in tetracycline solutions for up to 18 hours at varying temperatures, (2) determine the minimum immersion time and temperature required to insure that otoliths from 100% of the individuals immersed have a unique fluorescent tetracycline mark which is distinguishable from otoliths selected randomly from a pool of individuals which are not immersed, and (3) compare short-term growth and survival among pink fry which are treated with tetracycline following capture versus those which are not.

Pink salmon fry from the Prince William Sound Aquaculture Corporation's Cannery Creek Hatchery in Unakwik Inlet, Prince William Sound, were donated for this study. All equipment used in test marking fry were identical to that proposed for field camp use. Fry were treated in polyethylene bags suspended in warm freshwater baths and transferred to mesh cylinders in saltwater for rearing. After 4 weeks, fry were enumerated and shipped to the Alaska Department of Fish and Game Otolith Processing Laboratory in Juneau for otolith removal and processing.

Ten fry from the first replicate of the maximum treatment group were examined in a preliminary study and all had marks. A systematic search was initiated and is underway to find the minimum treatment level that insures a recognizable mark. There was no significant short-term mortality due to treatment. Further study should be done to test for long-term mortalities before marks are introduced on wild streams.

INTRODUCTION

Pink salmon *Onchorhynchus gorbuscha* returns to Prince William Sound (PWS) are dominated by hatchery produced fish. In addition to their dominance in the catch, hatchery stocks may also complicate fisheries management by straying into streams and spawning with wild fish. The magnitude and range of straying by both hatchery and wild pink salmon stocks may significantly influence the success of restoration efforts. If straying of hatchery fish is significant and does lower the fitness of wild populations, restoration efforts which concentrate on insuring that spawning escapement goals are met may fail if no attention is given to the origins of the escapement. The definition of what constitutes a wild population and the scale of restoration efforts may change if significant straying also occurs among wild populations.

Coded wire tags (CWT) have been the tool of choice for applying unique marks to populations of pink salmon in PWS. The methodology has been used extensively to estimate hatchery and wild stock contributions to commercial harvests and has also been used in preliminary straying research. Trustee council projects F/S 3, R60A, 93067, and 94320b have all incorporated this technology to estimate contributions of wild and hatchery pink salmon returns to PWS since the *Exxon Valdez* Oil Spill (Sharr et al. 1995a, 1995b, 1995c). Despite its usefulness, there are drawbacks to coded wire tag technology. Approximately one million coded-wire tags must be applied to pink salmon fry each year to obtain catch contribution estimates for returning adults. Tagging and recovery are both very labor intensive and the number of tags applied and recovered are sometimes inadequate for the levels of accuracy and precision desired. Coded wire tags are also intrusive: tags can be shed, and tagging may affect subsequent survival. Tag loss affects subsequent estimates of adult returns based on tag recoveries. There is also recent evidence that poor placement of coded-wire tags in salmon may affect their ability to home (Habicht *et.al. personal communication*).

Because of the cost and problems associated with coded wire technology, other alternatives which mark a larger portion of the population with relatively inexpensive non-intrusive methods should be investigated. By marking all of the fish in a population, more accurate and precise contribution estimates for adult returns can be obtained at a lower cost than is possible with the current CWT technology. Non-intrusive marks which cannot be shed and which do not affect survival or behavior will eliminate important sources of error in mark-recapture population and straying rate estimates.

This study is designed to test the feasibility of chemically marking fish otoliths or skeletal parts by short term immersion in a dilute solution of tetracycline during the emergent fry life stage. Tetracycline has been used successfully to apply chemical marks in many other fish species. Tetracycline is now regularly permitted by the United States Food and Drug Administration (FDA) for use as an antibiotic and otolith marking agent on fish destined for human consumption. Marks from tetracycline have been shown to be permanent, relatively easy to

apply, easily recognizable, and at low dosages do not appear to alter fish survival. While the most widely reported means of applying tetracycline is by feeding, several investigators have reported successful marking of fish species by immersion in dilute solutions of the chemical. Spot and pinfish, coregonids, and striped bass, have all been successfully marked using immersion methods (Hettler 1984, Dabrowski and Tsukamoto 1986, and Secor *et al.* 1991). There are fewer documented instances of pink and chum salmon having been successfully marked by immersion as well (R.C. Johnson, National marine Fisheries Service, retired, personal communication; Short and Sharp-Dahl 1991). While probably not cost effective for large hatchery releases reared in massive flow through incubator systems, tetracycline immersion is an attractive alternative for marking much smaller wild populations of pink salmon as they migrate out of their natal streams as fry. Marking the total fry population in a stream provides an accurate and precise tool for estimating total adult returns and survival. As a non-intrusive method which does not appear to alter fish behavior, chemical otolith marking may also provide a powerful tool for investigating straying among wild populations.

Although laboratory tests with tetracycline marking of pink and chum fry have shown some success, results of experimentation for operational studies were not available. In a preliminary test of 10 fry from the highest treatment, marks were found in all fry with no significant difference in mortalities between the treatment and control groups in this feasibility study.

OBJECTIVES

Our objectives in identifying a feasible methodology for otolith marking wild pink salmon outmigrant fry using tetracycline were threefold;

a. Test and refine remote field camp methods and equipment to be used for immersing wild pink salmon fry in tetracycline solutions for up to 18 hours at varying temperatures,
b. determine the minimum immersion time and water temperature used for immersion of pink salmon fry in tetracycline solution to insure that otoliths from 100% of the individuals treated have a unique fluorescent tetracycline mark,
c. compare short-term growth and survival between pink salmon fry treated with tetracycline following capture and those which are not.

METHODS

Pink salmon fry from the Prince William Sound Aquaculture Corporation's Cannery Creek Hatchery in Unakwik Inlet, PWS, were donated for this study. All equipment used in test marking fry were identical to that proposed for field camp use.

A buffered solution of tetracycline hydrochloride (Tetra-bac) diluted to 400 ppm in fresh water was used to mark all treatment groups. A preliminary test conducted by the Cordova ADF&G staff in March 1994 using emergent hatchery pink salmon fry immersed in this solution for 24 hours showed no short-term mortalities and exhibited no signs of stress during exposure. This concentration has also been used with success in chum salmon (Short and Sharp-Dahl 1991). Short and Sharp-Dahl also reported that results improved to a point with increasing temperature and length of immersion. This study tested 12 treatments (t_{ij}); each a unique combinations of immersion time (i) and temperature (j). Immersion times of 3, 6, 12 and 18 hours (i = 1, 2, 3, and 4) were tested at 2°, 5°, and 8° C (j = 1, 2, and 3). Each treatment was replicated five times (r = 1, 2, 3, 4, and 5).

Two 750 L water baths were prepared in large insulated fish totes. Water was heated and maintained at temperature by thermostatically controlled electric immersion heaters supplied by a gasoline powered generator. Fry emerging from hatchery incubators were initially divided into 60 groups (12 treatments x 5 replicates) of 600 individuals. Each 600 fish group was placed in a clear polyethylene bag containing 4 L of hatchery water at ambient stream temperature. Compressed air was supplied to each bag by air stones to insure that fry receive adequate oxygen. A pre-mixed 135 ml. buffered tetracycline solution prepared by dissolving 2.25g of Tetra-bac and 2.0 g dibasic sodium phosphate in 135 ml of warm (~30°C) fresh water was cooled to stream temperature and added to the each of 60 treatment bags. Fifteen additional bags were left untreated and used as controls (c_{ij}) to test the effects of tetracycline on survival at different temperatures and exposure times. Treatment bags and control bags were transferred in equal numbers to each of the three heated water baths. The water temperature in treatments bags was monitored and when all bags in a tote reached the desired immersion temperature, timing for duration of immersion began. At the endpoints of each time treatment, five treatment bags were removed from each of the totes, transferred to a saltwater enclosure in front of the hatchery and allowed to cool to ambient seawater temperature. Fry from each bag were then transferred to separate saltwater rearing cylinders constructed of fine meshed plastic screen (vexar). In addition, at the start of the treatment day fifteen groups of 600 fry each were transferred directly from the hatchery into saltwater rearing cylinders. These fry acted as controls for testing the marking effectiveness of each of the 12 treatments.

All treatment and control groups were held and fed Rangen fry powder in saltwater rearing pens to insure that the treatment band was deposited on the otolith and that otolith growth occurred beyond the marking band. After 4 weeks, fry from each rearing cylinder, which represented one replicate of a treatment group, were transferred to a light-proof black plastic

bottle containing 90% ethyl alcohol and shipped to the ADF&G Otolith Processing Laboratory in Juneau (Otolith Lab) for otolith removal and processing.

Mortalities were enumerated for each treatment and control group before, after, and during rearing periods.

A sample of 10 otoliths from the first replicate of the maximum treatment group (18 hours at 8° C) was mounted and processed to determine if the maximum treatment resulted in a tetracycline mark. After processing, otoliths were examined under a Leitz Laborlux microscope with UV light source and a filter block D(BP 355-425). If any of the 10 otoliths examined bore no mark it would be assumed that lesser treatments were equally or more ineffective, that tetracycline marking procedures tested were not effective, and that the experiment should be terminated with no further expenditure of funds for otolith processing. To find the minimum effective treatment a random sample of 30 otoliths from the first replicate of the maximum treatment group t_{341} will be mounted and processed to determine if the maximum treatment resulted in a tetracycline mark. If all 30 otoliths from t_{341} bear marks, then a systematic search for the minimum required treatment from among those having no effect on survival will proceed according to the following steps:

(1) Thirty otoliths from each replicate of t_{11} will be processed and examined by a trained observer.

(2) If all 30 in each replicate are marked, 30 more otoliths from the first replicate t_{111} will be extracted, randomly mixed and mounted on slides with 30 similarly prepared otoliths from the control group of fish c_0. The trained observer will examine this pool of 60 otoliths and attempt to correctly identify the treated individuals.

(3) If the observer correctly identifies all of the treated individuals from a pool of t_{111} and c_0, the procedure in step (2) will be repeated three more times for similar t_{111}, t_{112}, t_{113}, t_{114}, t_{115} and control pools.

(4) If at any point in these tests the observer fails to detect a mark on an otolith which has been treated, the procedure will terminate for $i=1$ and begin anew at step (1) for $i=2$ through 4.

(5) If the observer fails to classify any time treatments of temperature $j=1$ with 100 percent accuracy the steps (1) through (4) will be repeated for treatments t_{12} through t_{34}.

(6) The first instance of the observer correctly identifying all marked individuals in all replicates for a treatment will be defined as the minimum treatment suitable for marking.

After the minimum suitable treatment is identified, 30 otoliths from each of the remaining untested treatment groups may be examined to determine if more readily identifiable marks are available and if accidentally elevated temperature in the field may adversely affect marking. If a more readily identifiable mark is found, steps one through three listed above will be repeated

for that treatment. If 100 percent classification accuracy is achieved by the observer for all replicates of the treatment, this new treatment will be designated as the minimum treatment of choice and the former selected treatment will become the alternate treatment of choice. The treatment used in future field studies will be the one which produced the lowest mortality rate during treatment and subsequent rearing.

Mortalities from each replicate for each treatment (both controls and tetracycline treated fish) were estimated before and after the rearing phases of the experiment. Analysis of variance was used to test for significant differences among treatments.

RESULTS

Short-term mortalities and visible stress in groups of pink salmon exposed to the various tetracycline and thermal treatments were minimal. Only one replicate from the highest temperature and longest treatment showed any immediate mortalities. In that replicate 100 percent of the fry were dead after treatment.

Estimation of rearing mortalities and subsequent comparisons of treatments and controls were complicated by fry escaping the rearing cylinders and the quick decomposition of dead fry. Without an accurate evaluation of the number of live and dead fry, we could not adequately evaluate the long-term mortality effects of the treatments.

Otoliths from a sample of ten fry from the highest treatment were examined and found to contain discernable marks when compared to a sample of control otoliths. A systematic search has been initiated to find the minimum treatment where a 100% mark is obtained.

DISCUSSION

Our short-term mortality study results were consistent with the findings of other studies marking with 400 mg/L tetracycline (Short and Sharp-Dahl 1991; Hettler 1984). The methodology used for dissolving the chemical and buffer, heating large baths of warm water, and aerating individual polyethylene treatment bags was efficient and seemed to cause little stress to the fry. Other studies using tetracycline and similar buffers also recorded low short-term mortalities at concentrations up to 1,000 mg/L (Page 1989). The one replicate at the maximum treatment in which all fry died seems to have been due to a mix up in the buffer or a lack of air to this bag for a short time. Other replicates in this treatment group had no mortalities.

Problems with assessing long-term mortalities in the rearing cylinders stemmed from several factors that were discovered during the experiment. Fry cylinders used for the otolith project were made of 1/8" vexar screening. This size screen was successfully used in live box

constructions to hold fry at wild streams for CWT studies during F/S Study #3 (Sharr et al. 1995a) Some fry used in this study from both treatment and control groups were observed swimming through the screen out of the cylinders. No weights were collected to compare wild and hatchery fry during this study, but the hatchery fry visually appeared to be smaller. Dead fry may have been eaten by live fry before they could be enumerated. Dead fry also decomposed quickly and were often difficult to distinguish when removed from the cylinders. These factors prevented us from examining long-term mortality. Without accurate long-term rearing mortality estimates we cannot estimate the treatment effect on overall fry survival.

Our initial evidence of readable marks on otoliths at the highest treatment level is consistent with the literature. Although pink salmon marking with tetracycline is not common, results reported in the literature provided us with initial confidence that we could obtain a readable mark at our maximum treatment.

CONCLUSIONS

Evidence of readable marks under UV light in this initial examination along with a lack of short-term mortality indicates that tetracycline is a viable means of mass marking pink salmon fry in field conditions. Further study should be done on assessing long-term mortality effects.

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