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THE DESIGN AND ANALYSIS OF SALMONID TAGGING STUDIES IN THE COLUMBIA BASIN

VOLUME III: EXPERIMENT DESIGNS AND STATISTICS MODEL TO ESTIMATE THE EFFECT OF TRANSPORTATION ON SURVIVAL OF COLUMBIA RIVER SYSTEM SALMONIDS

Technical Report

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THE DESIGN AND ANALYSIS OF SALMONID TAGGING STUDIES IN THE COLUMBIA BASIN'

VOLUME III

EXPERIMENT DESIGNS AND STATISTICAL MODELS TO ESTIMATE THE EFFECT OF TRANSPORTATION ON SURVIVAL OF COLUMBIA RIVER SYSTEM SALMONIDS

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PREFACE

Project 9 l-05 1 was initiated in response to the Endangered Species Act (ESA) listings in the Snake River Basin of the Columbia River system. Primary objectives and management implications of this project include: (1) to address the need for further synthesis of historical tagging and other biological information to improve understanding and to help identify future research and analysis needs; (2) to assist in the development of improved monitoring capabilities, statistical methodologies, and software tools to assist management in optimizing operational and fish passage strategies to maximize the protection and survival of listed threatened and endangered Snake River salmon populations and other nonlisted stocks in the Columbia River Basin; and (3) to design better analysis tools for evaluation programs.

The following report addresses measure 5.0F.5 of the 1994 NPPC Fish and Wildlife Program with emphasis on improved design and analysis capabilities related to the conduct of salmonid tagging studies in the Columbia River Basin. In this report, alternative designs for conducting experimental manipulations of smolt tagging studies to study effects of river operations such as flow levels, spill fractions, and transportation are presented. The principles of study design discussed in this report have broad implications' for the many studies proposed to investigate both smolt and adult'survival relationships. The concepts are illustrated for the case of the design and analysis of smolt transportation experiments. The merits of proposed transportation. studies should be measured relative to these principles of proper statistical design and analysis. It is hoped that this statistical evaluation will help investigators better utilize available resources in the study of Columbia River fisheries issues and result in more timely and useful information for management of our natural resources.

Executive Summary.

Experiment designs to estimate the effect of transportation on survival and return rates of Columbia River system salmonids are discussed along with statistical modeling techniques. Besides transportation, river flow and dam spill are necessary components in the design and analysis, otherwise questions as to the effects of reservoir drawdowns and increased dam spill may never be satisfactorily answered.

Criteria for design comparison and the extremes of the design spectrum

Four criteria for comparing different experiment designs are:

1. feasibility;
2. clarity of results;
3. scope of inference;
4. time to learn.

A controlled experiment with treatments that are a combination of transport status (transported or left in-river), river flow level, and dam spill level should provide the clearest results of transport effect. The potential for bias due to interactions between year effects and the treatments is minimieed by running as many treatments as possible within a single outmigration year. Relatedly, the most rapid learning will occur if several different treatments are implemented at randomly chosen time periods within the *same* outmigration season. If the range of flow and dam manipulation includes scenarios of interest to managers, the scope of inference should be satisfactory. On the other hand these designs may be the least feasible; trying to manage the river system under a sequence of deliberately chosen flow regimes within a single season, for example, may be quite impractical.

At the other end of the spectrum are designs that simply have two treatment combinations, transportation and being left in-river, and the influence of flow and spill are controlled for, if possible, in after-the-fact statistical analysis. Because of possible confounding influences of flow and spill on the transportation effect, these designs could yield the most ambiguous results and require the most years of experimentation to learn. If flows and spill are not manipulated in a planned, well defined, and impartial manner the scope and quality of inference may not be satisfactory. On the other hand, these designs are the simplest to implement.

Implementation issues

1. The nature of flow and spill level manipulations will need clear definition, either in absolute terms, cfs, or relative terms, such as spilling 10% of the water.
2. Relatedly, systemwide implementation of flow and spill levels will provide simpler interpretation of results than will mixing spill rates, for instance, between dams. Transporting fish from just one location will also simplify interpretation.
3. Tagging of experimental fish should be done well upstream of the dams with random assignment to transport or in-river groups done later, near the dams, to minimize biases from delayed tagging mortality.
4. Tagging with PIT tags and CWTs in combination will provide evidence of any potential homing problems.
5. High PIT tag retention rates are important to minimizing potential analysis problems (thus on-going research to improve retention is vital).
6. Approximate sample sizes to achieve a desired level of precision can be calculated fairly easily using formulas provided in the report.

Contents

1 Introduction .

Experiments by the National Marine Fisheries Service (NMFS) to study the effect of transporting outmigrating juvenile salmonids around dams in the Columbia River system began in 1965. Transportation by barge or truck of large numbers of outmigrants, particularly those from the Snake River system, has been a routine management practice now for over twenty-years (Raymond 1988, Mundy, et al. 1994). The assumption behind transportation is that transported fish have a higher survival rate than fish remaining in the river that are exposed to potential turbine, spill, and predator mortality.

Transported fish do avoid in-river mortality factors, but questions have been raised about the net effect on return rates to spawning areas, which are functions of both survival rates and homing ability (Ebel 1980; Olney, et al. 1992; USFWS 1993). Do transported fish experience other types of mortality and suffer greater mortality rates at a later time (such as natural mortality in the ocean) than do fish that remain in the river? Does the reduced time-in-river and failure to travel the entire distance downstream interfere with a returning fish's ability to find its natal area?

Additional questions have been raised about the effect of river conditions on the transportation effect. Clearly an interaction exists between transport effect and river conditions- if flow and volume are diminished enough, outmigrant survival will certainly decrease: Conversely, are there situations in which fish left in the river will fare better than transported fish? For example, if the river flows are relatively high, do fish left in the river have higher return rates than transported fish? Would increasing the-amount of water spilling over the dams be more effective than transportation in increasing survival and return rates?

1.1 Previous studies

Many controlled experiments have been conducted, primarily by NMFS, using paired releases to evaluate the effect of transportation (see Ebel (1980) and the references therein). Several critical reviews are available and the reader is referred to Olney, et al. (1992), USFWS (1993), Mundy, et al. (1994), and Mundy (1995).

In particular, the returns of freeze-branded and coded-wire tagged fish released into the river system above hydroelectric projects have been compared with those transported below the projects (e.g., Ebel (1980)). The primary objective-of these experiments has been to estimate the ratio of return rates of transported fish to non-transported or in-river fish (sometimes referred to as controls). Denoting the estimate by &,-it is calculated by

$$\hat{\phi} = \frac{N_T/R_T}{N_C/R_C}, \tag{1}$$

where N and R are the number of returns and releases with subscripts T and C for transport and control. Ebel (1980), in a study of fish transported from Little Goose Dam to below Bonneville Dam, concluded that transportation was beneficial; $\hat{\phi}$ ranging from 1.1 to 15, and there was no significant diminishing of homing ability of transported fish.

One criticism of these studies is that mortality induced by handling and tagging/branding of the control group leads to overestimates of ϕ (Olney, et al. 1992; USFWS 1993). The observational studies, discussed below, by Raymond (1988), Harza and Associates (1994), and Newman (1997a) are less plagued by this particular problem. Observational studies in general, however, are more problematic than randomized controlled experiments, because non-random assignment of experiment units-to a given treatment makes arguments for causation more difficult. E.g., the treatment group does better than the control group because of differences in a background or confounding factor not because of a treatment effect.

Raymond (1988) estimated return rates to Lower Granite Dam (LGR) for many cohorte from 1962 to 1984 based on ratios of counts of adults at dams and interceptions in Columbia River fisheries to estimated indices of smolts reaching LGR. Be-made general observations linking trends in the rates to transportation activities and other enhancement measures, such as installation of fingerling bypasses at dams. Raymond concluded that the enhancements, in total, had reduced the decline of steelhead (*Oncorhynchus mykiss*) but not of chinook salmon (*Oncorhynchus tshawytscha*). Hi work did not directly measure the effect *of* transportation, however, and the estimated counts of smolte and counts of adults were based on several

Table 1: Estimates of ϕ based on data in Harsa and Associates (1994) on PIT-tagged spring and summer chinook released from the Snake River trap.

Year	N_T	N_C	$R_T/R.$	ϕ
1988	15	3	0.952	0.24
1989	13	8	0.950	0.09
1990	26	3	0.953	0.43
1991	18	2	0.936	0.62

somewhat coarse estimates, including, e.g., survival rates from hatcheries to dams, age of returns based on length data, and in-river harvest- rates.

Passive Integrated Transponder (PIT) tags were introduced to the Columbia River system in the late 1980s. PIT tags provide much more accurate and detailed information than was available to Raymond (1988) and have largely eliminated the need for such intermediate calculations as length to age conversions. Harsa and Associates (1994) analyzed returns to LGR of all Snake River salmonids that were PIT-tagged and outmigrated during the years 1988 through 1991 to estimate ϕ. The estimation of ϕ was made conditional on fish surviving to the lower Snake River dams. Since the fish were PIT-tagged above the dams, the problem of delayed handling and tagging mortality was somewhat controlled for. The effect of transportation was reported in a somewhat different manner than ϕ,

$$\frac{\%adult}{\%juvenile}|Transported = \frac{N_T/N.}{R_T/R.} \tag{2}$$

$$\frac{\%adult}{\%juvenile}|Control = \frac{N_C/N.}{R_C/R.} \tag{3}$$

N. is the total number of returning adults. $R.$ is the total number of juveniles surviving to the downstream dams, R_T and R_C are the numbers transported and left in-river. The ratio of (2) to (3) estimates ϕ. Through .a sequence of calculations that included hypothetical values for the percentage of fish going through turbines and over spillways, the fractions, $R_T/R.$ and $R_T/R.$, not the absolute numbers, were estimated. They partitioned the transport groups into three subgroups, those transported at LGR, at Little Goose, and at McNary. Ratios of (2) for each transport group to (3) were estimated to be greater than 1 for all 4 years and all 3 transport sites. They concluded that "true 'in-river' migrants may outperform transported fish by two to one, in good water years". Table 1 presents a slightly different summary of their results for spring and summer chinook tagged and released from Snake River trap and then recovered at LGR; I have collapsed over the three transport sites (there might be slight rounding errors).

Newman (1997a) performed a similar analysis. He examined, for the outmigration years 1989-91, all hatchery and wild spring chinook salmon PIT-tagged and released from the Clearwater, Salmon; and Grande Ronde rivers, as well as all hatchery and wild steelhead PIT-tagged and released from the Clearwater and Salmon rivers and the Snake River trap. Excluded. from the analysis were PIT-tagged salmonids that could not be identified as being hatchery or wild fish. Adult returns were few in number, a total of 41 spring chinook and 205 steelhead for the three outmigration years. Of the 41 spring chinook, 33 had been detected at one of the lower Snake River dams during outmigration; for the 205 steelhead, 199 were detected during outmigration.

Assuming that all fish detected as juveniles were subsequently transported, the effect of transportation was estimated using a range of estimates for probability of juvenile detection. The assumption that all fish detected as juveniles were, transported is known to be false, but the percentage over this time period is quite large[1]. Let S_J denote the probability of a juvenile surviving to any of these dams, p_J be the probability of juvenile detection at the dams, θ_T and θ_C be the probabilities of survival to adulthood conditional on reaching the dams es juveniles for transported and control fish, and p_A be the probability of detection at

[1] According to Harsa and Associates (1994) only one of the 33 adult spring chinook detected as a juvenile was returned to the river.

LGR as a returning adult. The probabilities of juvenile fish returning as adults and being detected at LGR can be written as:

$$\text{Adult recovery rate for transported fish} = S_J p_J \theta_T p_A$$
$$\text{Adult recovery rate for control fish} = S_J (1 - p_J)\theta_C p_A$$

Then $\phi = \theta_T/\theta_C \cdot p_J$ equates to $R_C/R.$ in the Harsa and Associates (1994) analysis. The ratio of adult returns detected as juveniles to adult returns not detected as juveniles estimates $(p_J\theta_T)/((1-p_J)\theta_C)$ or $p_J/(1-p_J)\phi$. The ratio for spring chinook was $33/8 = 4.125$, and for steelhead, $199/6 = 33.167$. Multiplying these ratios by various estimates of $(1 - p_J)/p_J$ estimates ϕ. If p_J is less than 80%, $\hat{\phi} > 1$ for spring chinooks; for steelhead, if p_J is less than 97%, $\hat{\phi} > 1$. Pooling over years had little effect on the ratios for steelhead, but for the 1989 spring chinook ratio was 10/6 meaning $p_J > 62.5\% \Rightarrow \hat{\phi} < 1$.

The Harsa and Associates (1994) data can be used in the same manner. E.g., 15/3, 13/8, 26/3, and 18/2 are the ratios of transported to in-river fish and values of p_J greater than 83%, 62%, 90%, and 90%, respectively, lead to estimates of $\phi < 1$. Clearly the Harsa and Associates (1994) conclusions are quite sensitive to estimates of the percentage of juveniles actually transported: their estimates, $R_T/R.$, were all greater than 90%, hence estimates of ϕ were less than 1. If anything, their analysis and Newman's (1997a) point out the need to precisely know the number of PIT-tagged fish being transported and being left in the river.

1.2 Goals of this report

Mundy, et al. (1994), in a review of transportation studies, recommends that studies be designed to evaluate the benefit of transportation relative to other mitigative measures such as increased spill rates and increased flows (p. 62 of the report). A follow-up report (Mundy 1995), currently undergoing review and revision, presents a specific experiment design and discussion of analysis procedures. The design proposal advocates PIT and CWT tagging Snake River hatchery spring chinook, releasing the fish from the hatcheries, and randomly allocating the survivors to LGR into transport and in-river groups, with no further transportation sites beyond that point. Many of the ideas from these two reports are incorporated into this report.

The goals of this report are summarized below and the remainder of the report follows the same order.

1. Formally define the primary transportation experiment objectives;
2. Formulate a framework for designing experiments to evaluate the effect of transportation and other factors such as flow and spill on fish survival and return rates;.
3. Present particular designs of varying efficiency and practicality;
4. Sketch subsequent analysis procedures;
5. Discuss some practical experiment protocol issues;
6. Compare various designs in terms of feasibility, clarity of results, scope of inference, and time to learn.

2 Experiment objectives

The management objective is, of course, to maximize survival and return rates for outmigrating salmonids. Given a particular year, species, race, and rearing type, what is the 'best' management action? Transport as many as possible? Leave all fish in the river but increase flow to a particular rate (X cfs, say) and spill at a particular volume (Y cfs)?

[2] The 'breakeven' point for p_J is the ratio $N_T/(N_T + N_C)$.

In light of this management objective, the experiment objective is, per species, race, and rearing type, to estimate the effect of transportation, flow and spill levels on survival and return rates. Additional objectives may include comparing the effects for different regions of origin and different time of outmigration. To reduce notation and verbage, species, race, and rearing type distinctions will not be made in the remainder of the report. However, if different species, races, or rearing types benefit from different flow, spill, and transport actions, value judgments will be required to choose a management action.

Other factors can influence the survival and return rates, such as individual fish characteristics (e.g., size, degree of smoltification) and other environmental conditions (river water temperature, predator abundance). Random assignment of fish to transport or in-river destination will average out individual fish characteristics. Dealing with the environmental conditions will be addressed in the discussion of statistical models and experiment design

3 Experiment design framework

Basic components of any experiment design are the experimental units, the influential factors and covariates, the treatment definitions, the variables measured, and the parameters of interest. Definitions of each are given below.

Experimental Unit: The individual fish is the experimental unit. An argument can be made for using groups of fish instead and this is discussed below.

Response Variables: The primary response variable of interest is a binary response variable, whether a fish returns to its natal area or not. Some other response variables are juvenile capture history and adult capture history (say detection at dams or caught in a fishery),' i.e., a more complex categorical variable.

Influential variates and Treatments: Below are listed some of the variables that may influence the response variables.

- Transport
- Flow
- Spill
- Hatchery or Wild
- Region of origin
- Ocean conditions: e.g., sea surface temperature
- Other river conditions: e.g., water temperature (outmigrating and upon return)
- Fishery harvest levels
- Individual (juvenile) fish characteristics
  - sise (length, weight)
  - degree of smoltification
  - time of arrival at Lower Granite Dam

Treatments would be combinations of variables set at particular levels. Spill and flow could be defined categorically, e.g., high or low, or as continuous variables, a particular cfs. Variatesoutside the control of the manager might be viewed as covariates to adjust for.

Parameters and contrasts: These are a function of response and design variables and characterize the abjectives of the experiment. They will usually be functions of probabilities; e.g., the ratio of the probability of return for a transported fish to the probability of return for a fish left in the river. ϕ is an example.

3.1 Defining the experimental unit .

The definition of experimental unit is crucial to both the implementation of the experiment as well as the subsequent analysis. Quoting D.R. Cox (p.2, 1958), 'The formal definition of an experimental unit is that it corresponds to the smallest division of the experimental material such that any two units may receive different treatments in the actual experiment'.

If individual fish are the experimental units, for analysis purposes two vital issues are:

- the degree of independence between experimental units;
- whether or not experimental units receiving identical treatment combinations follow the same probability distribution.

In the case of simply measuring return to a point, the latter issue can be restated as a problem of homogeneity of return probabilities.

Treating the individual fish as the experimental unit has been the most common approach for Pacific salmon data (eg., Burnham, et al. 1986, Skalslci and Cormack 1992), for both randomized experiments and observational studies. With a binary response variable, assuming homogenous probability of return within a treatment group and independence, the response variable is Bernoulli and estimates of the probability and variance estimates are easily calculated. Uodeliig the probabilities as functions of various factors and covariates may be done using generalized linear models; e.g., logistic regression.

The assumption of independence could be relaxed by using quasi-likelihood models (McCullagh and Nelder 1989) and allowing for overdispersion parameters (for fisheries examples, see Cormack and Skalski 1992, Pascual et al. 1995). These statistical techniques allow one to at least partially account for schooling or shoaling behavior of fish. The assumption of constant probability within a treatment combination could perhaps be relaxed by specifying probability distributions for the probabilities (such as a Beta distribution).

Viewing a group of fish as the experimental unit is an interesting alternative. de Libero (1986) took this perspective when analyzing the returns of CWT chinook salmon to the Abernathy National Fish Hatchery on the lower Columbia River. The proportion returning within a group became the response variable. Using an arcsin square root transformation he carried out a mixed effects analysis of variance to assess the impact of the different release timings and years, among other things. The assumption of independence of fish within a group becomes less relevant, perhaps, under this approach. Furthermore the use of random effects for 'like' treatment groups incorporates to some degree the possibility of random probabilities. The assumption of independence *between* groups, however, is usually made. The trade-off between the group and single fish approaches deserves further study, but for the remainder of this report, I will assume that a single fish is the experimental unit.

3.2 Restricting possible treatment combinations

There many possible ways to influence river conditions and affect the possible route past dams for an outmigrating fish. For example, spill at one dam but not another. Or to manipulate flow, release water from upstream dams in short periodic pulses, or slow increases, etc. One can in theory *estimate* the probability of survival under a particular set of dam and reservoir manipulations by simply looking at the number returning divided by the number released. However, this overlookz the practical problem of knowing exactly the path taken by a fish left in the river and may lead to confusion when interpreting results. I concur with Mundy (1995) that a systemwide simplification of manipulations is necessary to increase the feasibility of implementing an experiment and facilitating interpretation of the results. Along these lines, transportation should be done at just one location and if spill is manipulated, spill in a consistent manner at all dams encountered by in-river fish.

Suppose systemwide consistencies are not maintained. For example let spill be manipulated at one of two levels, 10% and 40%, and assume that 40% spilling iebetter than 10%. But control over spilling is only done at Little Goose Dam, while dams downstream are operated in a haphazard fashion with regard to spilling. For example, in 1998 40% spilling is done at Little Goose, but all downstream dams do not spill. Then in

1999, 10% spilling is done at Little Goose, but all downstream dams spill at 40%. Then the effect of spilling at Little Goose is confounded with downstream dam spilling and correct interpretation of results is made difficult or impossible.

3.3 Defining the treatment combinations

Determining what is manipulable is necessary to the formulation of treatment combinations and subsequent analysis. Three categories of factors can be defined. The first category is those factors that are completely within the control of man, such as transportation. The second category includes those factors that are clearly are not, such as species. The third category is a complex combination of natural and man-made influences, such as flow and spill, both only partially controllable and interacting. E.g.; if a dam turbine fails and must be blocked and flow is relatively high, spill must be done to protect the dam. Likewise if spring runoff is high, upstream reservoirs may have to release water and thereby increase flow; and if the flow is high enough, spill may be necessary. This third category complicates the design and analysis because man may try to control a factor such as flow by upstream dam drawdowns, but nature will limit the extent of his control.

The management question is 'Given what nature is doing this year, what flow and spill manipulations should we do to maximize return rate?'. A related question is 'given what I can do with flow and spill, should I transport or not?'. The experiment design and subsequent analysis should incorporate flow and spill effects to provide unambiguous answers to these questions. What is meant by high and low spills, or minimum attainable levels of flow needs to be clearly defined by biologists and engineers. Below I outline several different perspectives to take on flow and spill in the design and analysis.

1. As two separate factors with absolute levels: level x flow and level y spill are so many cfs.
2. As two separate factors with continuous relative levels: level x flow is a specified percentage increase among a minimum attainable level, and level y spill is a particular percentage of the currently available amount for spilling.
3. As two separate factors with binary relative levels: high flow is as much as physically possible with low flow as little as physically possible; likewise for high spill and low spill.
4. As combined treatment components: allowing for the possibility that not all factor level combinations may be possible (eg, very high flow with no spill may not be possible); the possible combinations (based on absolute, relative, or binary relative levels) are viewed as in-river treatments in a non-factorial sense.
5. As two separate covariates: the degree of manipulation possible is ignored, and flow and spill are simply covariates to be controlled for after the fact.

The first perspective is an idealized one that ignores the fact that nature in some years may not allow such control. The second and third perspectives are quite similar in that all factor levels can be crossed (run in any combination), but the definition of the levels is given in relative terms.

Three possible definitions of treatment and covariate combinations of transport (T), flow (F), and spill (S) are now given.

Treatment Definition 1 (6 Treatments): Treatment combinations are formed with spill and flow levels defined in relative binary terms and completely crossed. In particular assume that flow can be (somewhat) controlled within a given year by upstream reservoir drawdowns and two levels can be selected. Likewise, two levels of spill are clearly-defined and manipulable. Denote the spill levels S_1 and S_2, likewise the flow levels F_1 and F_2. Assuming that spill does not interact with transported fish, there are six treatments, TF_1, TF_2, S_1F_1, S_1F_2, S_2F_1, and S_2F_2. If spill did interact with transport, this would be a full 2^3 factorial or 8 treatments.

Treatment Definition 2 (5 Treatments): Spill and flow levels are binary and relative but not completely crossed, but, for instance, low spill-low flow (S_1F_1), high spill-low flow (S_2F_1), and high spill-high flow (S_2F_2), say, are possible, with high and low defined in relative terms. The missing combination is high

Table 2: 6 treatments within a year with two replications of TF_1 and TF_2

Period 1	Period 2	Period 3	Period 4
TF_1	TF_2	TF_2	TF_1
S_1F_1	S_2F_2	S_1F_2	S_2F_1

Table 3: Latin Square design for Trt Definition 1 (Transport implicit)

Year	Period 1	Period 2	Period 3	Period 4
1996	S_1F_1	S_2F_2	S_1F_2	S_2F_1
1997	S_2F_2	S_1F_1	S_2F_1	S_1F_2
1998	S_1F_2	S_2F_1	S_2F_2	S_1F_1
1999	S_2F_1	S_1F_2	S_1F_1	S_2F_2

flow-low spill in thii case. With no spill-transport interaction, there are 5 treatments (including TF_1 and TF_2).

Treatment Definition 3 (2 Treatments): In line with previous transportation experiments, fish arc either transported, say T_1 or left in the river, say T_2. To gain additional precision, spill and flow are covariates (possibly qualitative) and are not deliberately manipulated.

4 Particular designs

Each of the following designs incorporates transportation, flow, and spill in -the design and analysis using one of the three treatment definitions.

4.1 Latin Square Designs

All six treatments in Treatment Definition 1 are run each year. Because of the impossibility of running some treatments simultaneously, 'e.g., low flow and high flow, replication is sought across time. The outmigration season is partitioned into four disjoint-time periods. Low flows and high flows are randomly assigned to two periods each; and within each low flow pair spill/no spill are randomly assigned and the same for the high flow pair. For an example see Table 2. Note that transportation can be done for all time periods under any river conditions and can be viewed as a benchmark for comparison of in-river releases in all the river conditions. In fact the response variable could be viewed as the ratio of return rates for transported and in-river fish.

Time will likely have an effect, e.g., larger fish arriving in the fourth period may have higher chance of survival, and could be viewed as a blocking factor in the analysis. To achieve balance in ordering, and thus attempt to control for possible time affects within and between years, a modified Latin Square design is used. The key assumption is that treatments do not interact with the temporal blocks, neither within the year nor between years. Transportation will always be done and can be viewed as a 'separate' factor. A single replication of the Latin Square design for the four in-river treatment combinations would require four years of experimentation to achieve the necessary balance over within season intervals. Table 3 shows one possible configuration. See p. 145 of Cochran and Cox (1957) for 3 other configurations. Similarly a Latin Square design using Treatment Definition 2 could be implemented with sequential in season changes as shown in Table 4. Here only three time periods are necessary and a full replication can be achieved in three years.

There is the practical problem of spatial-temporal overlap between fish receiving different treatment combinations. For instance fish in time period 1 may be getting S_1F_1 and fish in period 2 get S_1F_2, but 'stragglers' from period 1 may still be in the system when the increased flow for the second period comes

Table 4: Latin Square design for Trt Definition 2 (Transport implicit)

Year	Period 1	Period 2	Period 3
~~1996~~	~~$S_L F_L$~~	~~$S_H F_H$~~	~~$S_H F_L$~~
1997	$S_H F_L$	$S_L F_L$	$S_H F_H$
1998	$S_H F_H$	$S_H F_L$	$S_L F_L$

Tabldete' Randomized Block design witl design with Trt Definition 1

Year	Treatment combinations	
1996	$T_1 S_1 F_1$	$T_2 S_1 F_1$
1997	$T_1 S_2 F_1$	$T_2 S_2 F_1$
1998	$T_1 S_1 F_2$	$T_2 S_1 F_2$
1999	$T_1 S_2 F_2$	$T_2 S_2 F_2$

through. One alternative is to insert windows between the periods where no experimentation is taking place. Fish are of course moving through the system, but they would not be considered part of the study. Alternatively, one could have the periods end and start on consecutive days, but during later analysis either remove .the fish falling within a specified window or use analytical procedures that would recognize the overlap (a statistical research problem).

4.2 'Incomplete 'Randomized Block Designs,

Suppose that such in-river manipulations as required by Treatment Definitions 1 and 2 cannot be done *within* a season, but can be done over an entire outmigration season. Because transportation can always be done, at least two treatment combinations can be carried out each year. Using treatment definition 1, the four river combinations could be randomly assigned to four different years, blocking by year effects. Table 5 gives an example (T_1 and T_2 denote transport and in-river, respectively). If the experimental units were. viewed as groups of fish, then thii might be viewed as a balanced incomplete block design.

Similarly, under Treatment Definition 2, an incomplete block design could be carried if three in-river manipulations within a single season was not possible (Table 6).

4.3 Randomized Block Design using Trt Dkfinition 3

Using definition 3, each year fish would be randomly assigned to transport or in-river but spill and flow would not be design factors. Years would serve as blocks in the analysis and spill and flow would be covariatea to adjust for. A key assumption of this design is that that whatever potential interactions exist between year and treatment can be accounted for by the covariates flow and spill, i.e., the assumption of no block by treatment interaction is satisfied.

Table 6: 'Incomplete' Randomized Block design with Trt Definition 2

Year	Treatment combinations	
1996	$T_1 S_L F_L$	$T_2 S_L F_L$
1997	$T_1 S_H F_L$	$T_2 S_H F_L$
1998	$T_1 S_H F_H$	$T_2 S_H F_H$

5 Statistical models

Given a 'good' experiment design, namely a design utiliiing randomization and replication principles to minimize biases and allow estimation of errors, many different statistical analyses will be possible. In this sense, developing proper data collection and generation procedures are the more critical concerns, but possible statistical models are worth discussing.

To simplify discussion just consider a single binary response, namely the adult fish returns to point P or it does not, say Lower Granite Dam. Only the effects of transport, spill, and flow are considered. For a group of fish experiencing the same treatment-and covariate combination, the relevant data will simply be number released, R, and number returning to P, Y, and the parameter of interest is the probability of return, 8.. The statistical problem is to model θ as a function of transport, spill, and flow.

The following notation is used:

- i denotes a year
- j denotes a period (within a given year)
- k denotes a treatment (within a given year and period)
- I denotes a replicate level (within a year, period, and treatment)
- R_{ijkl} is the size of replicate l getting treatment k during period j in year i
- Y_{ijkl} are the returns from R_{ijkl}
- θ_{ijkl} is the probability of return for each fish in R_{ijkl}
- $X_{r,ijkl}$ is the rth block, factor, or covariate value for release group R_{ijkl}

A Poisson distribution is assumed for Y_{ijkl} and a generalized linear model based on a log link function is used to relate the probability of return to various factors and covariates. What the log link function implies is that each factor or covariate has a multiplicative effect on return rate.

$$\begin{aligned} Y_{ijkl} &\sim \text{Poisson}(R_{ijkl}\theta_{ijkl}) \\ \log(\theta_{ijkl}) &= \beta_0 + \beta_1 X_{1,ijkl} + \ldots + \beta_p X_{p,ijkl} \end{aligned}$$

Suppose, the Latin Square design is used with Definition 1 of treatment combinations. A possible model for θ, written symbolically:

$$\log(\theta_{ijkl}) = \beta_0 + \beta_i Yr_i + \beta_j Season_j + \beta_k Trt_k$$

The resulting fitted model would provide estimates of the average return rate for each of the 6 treatment combinations. As written there are no assumptions about the functional relationship of return rate to flow level or spill level nor issues of interaction, but .it is assumed that the treatments do not interact with year and period. This means, for example, that the ratio $\phi(x, y)$ for flow level x and spill y, defined by

$$\phi(x, y) = \frac{\theta_{x,y,T}}{\theta_{x,y,C}}$$

would not depend upon the period within a outmigration year. Suppose Trt 1 is transport at F_1 (and spill S_2) and Trt 2 is in-river at the same levels, then

$$\phi(F_1, S_2) = \exp(\beta_1 - \beta_2)$$

However, if the relative effect of transportation varies between juveniles with different levels of maturation, the above assumption would be violated and the transportation effect would have to be estimated on a per period basis.

Another example is the Randomized Block design using Definition 3 of treatments with flow and spill as covariatee that vary in some haphazard or unplanned way and spill does not affect transported fish:

$$\log(\theta_{ikl}) \quad = \quad \beta_0 + \beta_i Yr_i + \beta_1 I(Transport) + \beta_2 Flow_i + \beta_3 I(In - river) \times Spill_i \qquad (4)$$

where $I(\cdot)$ is an indicator function equalling 1 when the argument is matched. Allowing for possible interaction between treatment and flow:

$$\begin{aligned} \log(\theta_{ikl}) &= \beta_0 + \beta_i Yr_i + \beta_1 I(Transport) + \beta_2 Flow_i \text{ --} \\ &+ \beta_3 I(Transport) \text{ x } Flow_i + \beta_4 I(In - river) \text{ x } Spill_i \end{aligned} \qquad (5)$$

In both (4) and (5) itis assumed that the treatment effect (transportation) does not interact with the year effect. To assess the effect of transportation for given flow (x) and spill (y) conditions and same year under equation (5), the transportation effect would be measured by

$$\phi(x,y) \quad = \quad \exp(\beta_1 + \beta_3 x - \beta_4 y) \qquad (6)$$

If hypothesis tests suggest that flow and spill have no effect, this reduces to estimating the historical Transportation Benefit Ratio (1):

$$\phi \quad = \quad \exp(\beta_1)$$

Several complications and extensions are mentioned. First, the treatment of the year effect may be more involved than is apparent. In the above approach year is treated as a fixed effect, but year effect is a random variable, and should be treated as a random effect which averages out to zero. Mixed effects generalized linear models are considerably more involved to analyze, unfortunately. A yet even more realistic model, and consequently more difficult to analyze, is to recognize the time series nature of the year effects, e.g., the cyclic nature of ocean conditions, and incorporate some type of dependency structure in the model. Which of these three approaches to use will require additional work and is not addressed further.

Second, several alternatives to the Poisson model with a log link function exist for modeling the survival over *a* given time interval. The statistical package SURPH (Smith, et al. 1994) offers the options of proportional hazards models (some similarity with a log-log link function) as well as a Binomial model with a logistic link function. Furthermore SURPH allows modeling the effect of individual fish covariates, such as size at time of release, on, survival in addition to the group covariatee described above, such as a commonly experienced flow or spill regime, or region of origin, for example.

Third, a more comprehensive modeling approach, and subsequently more complex parameter estimation problem, is to model the survival rates over several time intervals simultaneously. In other words, multivariate responses are modeled as contrasted to the univariate response of survival to a single point or not. An underlying probability model is the multinomial distribution, and so-called polytomous regression models may be used to link the set of survivals to covariates and treatments simultaneously (McCullagh and Nelder, 1989).

6 Experimental protocol issues

6.1 Tags

Until an adult PIT tag interrogation system iz installed at Bonneville Dam, CWTs and PIT tags should be used in combination. The primary benefit of CWTs will be the information provided by lower river fisheries' recoveries of tagged fish. Without an adult PIT tag interrogation system at Bonneville Dam, CWT recoveries in the lower river fisheries may be the only means of eztimating differences in homing ability of transported and control groups. If the ocean fisheries are examined, CWTs will provide additional information on differences between the two groups. See Newman (1997b) for more on the combined use of CWTs and PIT tags.

PIT tag retention, especially to the adult stage, is an issue that can affect the success of the experiment. There have been concerns expressed about loss of PIT tags by maturing fish and research is underway by Earl Prentice (personal communication) to modify PIT tags to increase retention, such as acid etching the otherwise smooth PIT tags. This is a critical issue and problems with adult retention rates will bias estimates of return rates. Another reason for double tagging with CWTs is to assess tag loss rates.

6.2 Timing and location of tagging

Following Mundy (1995) I recommend tagging at the hatchery, and in the case of wild outmigrants, in the upper reaches of the river systems. Combined with random assignment to transport and in-river groups at a later point in time (the next topic), the potential bias of delayed tagging mortality is minimized. Simultaneously, greater control is maintained over getting representative samples of outmigrants; e.g., one can ensure that fish from Dworshak and Rapid River hatcheries are in the study. Tagging at thii stage will diminish the problem of handling fish at widely varying degrees of smoltification, as can be the case with tagging at a downstream dam, for instance, as well as provide more control over the problem of handling wild outmigrants unintentionally.

6.3 Location and mechanics of randomization

Given that the fish are tagged upstream of the transportation site, random assignment to transport or being left in-river is necessary to mihmise bias. The simplest approach logistically is to take those surviving outmigrants that enter the juvenile bypass system at Lower Granite Dam and randomly assign individual fish to one group or the other using "flip-gates". The use of fish going through the bypass is convenient since PIT tag detectors can fairly easily separate out tagged from untagged fish and minimize handling of untagged fish.

A criticism of randomizing over these-fish is that these are fish that 'chose' to go through the bypass and into the collection facility to begin with and thereby excludes that portion of the population that 'chose' to go through the turbines, or if possible, go over the spillway. Furthermore, one can argue that those fish that go through the bypass and into the collection facility are weakened by this experience and may later suffer negative effects. If that is the case, these bypassed and collected fish are not representative of those fish which go over the spillway, say, and perhaps are not so weakened.

An alternative is to capture fish above the dam, scan the captures for PIT tags and then randomly assign to transport or in-river, letting the in-river fish procede through the dam however they 'choose', and transporting fish from above the dam now. This would lead to unintentional handling of untagged fish and, depending on the capture method (purse seine, screw trap, etc.), may stress those fish adversely. This has the drawback that the mechanics of loading fish on barges for transport is now more difficult.

6.4 Sample size

Meeting the objective of estimating the effects of transportation, spill, and flow depends upon having sufficiently accurate and precise estimates. Accuracy is a function of using randomiration to assign the- experimental units to particular treatment combinations. Precision is a function of the experiment design, the inherent variabiiity of the responses, the magnitude of the treatment effects, and sample size. The better the design, the smaller is the necessary sample size and the fewer are the years of experimentation. Likewise as the inherent variability of the responses decreases and as the magnitude of the treatment effects increases, the smaller the sample size.

In this section an approach to sample size determination within 'a single year is given. The problem is further simplified by only considering the problem of estimating the ratio of the transport, return rate to a dam with a PIT tag interrogation system to the in-river return rate to the same location-for a fixed spill and flow regime, namely $\phi(x, y)$. Thii ignores sample size issues related to using CWTs and PIT tags in combination- more complicated tagging mixtures may be more cost effective (see Newman (1997b) for examples).

Table 7: Sample sise per group to estimate ϕ with a specified CV

	θ_C=0.001		θ_C=0.0005	
	CV		CV	
ϕ	10%	20%	10%	20%
0.6	266,467	66,617	533,133	133,283
0.8	224,800	56,200	449,800	112,450
1.0	199,800.	49,950	399,800	99,950
1.5	166,467	41,617	333,133	83,283
2.0	149,800	37,450	299,800	74,950
3.0	133,133	33,283	266,467	66,617

The probiem is to determine the sample size necessary to estimate $\phi(x, y)$ with a -specific precision. Assume that ϕ is estimated by the ratio of observed return rates for both groups, dropping the flow and spill subscripts and letting θ_T and θ_C denote return probabilities for transported and in-river fish, respectively.

$$\hat{\phi} = \frac{\hat{\theta}_T}{\hat{\theta}_C}$$

A Taylor series approximation to the estimate as a function of the true rates can be written as follows:

$$\hat{\phi} \approx \frac{\theta_T}{\theta_C} + \frac{1}{\theta_C}(\hat{\theta}_T - \theta_T) - \frac{\theta_T}{\theta_C^2}(\hat{\theta}_C - \theta_C)$$

and then the variance is, assuming equal sample sires per group (R) and a Binomial model for returns:

$$\begin{aligned} Var(\hat{\phi}) &\approx \frac{1}{\theta_C^2} Var(\hat{\theta}_T) + \frac{\theta_T^2}{\theta_C^4} Var(\hat{\theta}_C) \\ &= \frac{\phi + \phi^2(1 - 2\theta_C)}{\theta_C R} \end{aligned}$$

Suppose desired precision is expressed in terms of coefficient of variation, $CV = \frac{\sqrt{Var(\hat{\phi})}}{\phi}$, then the sample size can be found from

$$R = \frac{1/\phi + 1 - 2\theta_C}{\theta_C CV^2}$$

Table 7 gives sample &es for a range of ϕ, CV, and θ_C. The Poisson model serves as a close approximation to the Binomial for the hinds of return rates expected and would provide similar sample size results.

7 Comparing experiment designs

To compare competing designs four criteria are considered:

1. feasibility ;
2. clarity of results;
3. scope of inference;
4. time to learn.

7.1 Feasibility

By feasibility is meant whether or not particular treatment combinations could be implemented using random assignment. The feasibility of the Latin Square design with Treatment Definitions 1 or 2 depends on whether or not the dams could be operated in any of the ways required despite electrical and irrigation demands, for example. The Randomized **Block** design with Treatment Definition 3 is clearly feasible because such transportation studies have been conducted for overtwenty years, perhaps with various experimental protocol problems, but no design problems.

7.2 Clarity of results

By clarity of results is meant the accuracy and the precision of estimates of the ratio of transported fish return rates and **in-river** fish return rates, ϕ, under ***identical*** environmental, biological, and physical conditions.

For any given set of environmental, biological and physical conditions, randomization of fish to the particular treatments will minimize the potential for bias by making the fish in both groups relatively homogenous, thus the estimate of ϕ under this particular set of conditions would be (relatively) unbiased. Ideally one would like to know how ϕ varies with varying environmental, biological and physical conditions; e.g., flow, spill, maturation level, rearing type, region of origin. The formulation thus far has aimed at simply trying to determine how flow and spill levels alter the transportation effect, i.e., the **ratio** $\phi(x, y)$ may depend on flow level x and spill level y. In any case, randomization again provides the means of unbiasedly estimating $\phi(x, y)$ for specified levels of flow and spill simply by creating treatment combinations based on these levels.

However, because of the physical and practical constraints on transportation experiments, such as the impossibility of diierent river flows existing **simultaneously,** randomization at best can be done over sub-groups within temporal blocks. Randomization of fish is done ***within a*** given migration season or period within a season. This restriction on randomization creates a potential for bias if interactions exist between the factors blocked on (such as year or period) and the treatments.

Suppose there are 4 treatment **combinations, transport+low** flow, in-river+low flow, **transport+high** flow, and in-river+high flow. An Incomplete Randomized Block design is carried out with 2 years **as** blocks and low flow randomly assigned to 1998 and high flow to **1999.** Assume that flow has no effect, but there is an interaction between the transportation factor and year (Figure 1). For example, transported fish enter the ocean earlier, ocean currents at the mouth of the Columbia **River** change radically between entry times of transported fish and in-river fish in 1998 but remain constant in 1999. Flow may be wrongly attributed to the difference in return rates because ocean conditions are a confounding factor. Repeating the experiment over many years will possibly show that flow has no effect.

Each of the three experiment designs, Latin Square, Incomplete Randomized Block, **and** ordinary Ban-domized Block, have the potential for **bias** if interactions exist between the treatments and the blocks'. The Latin Square using Treatment Definition 1 could have a bias if $\phi(x, y)$ depends upon fish maturation level. Return rates can differ for both transported and in-river fish for differing maturation levels, but the ratio of the rates needs to stay constant to avoid biases. The previous hypothetical example shows the potential bias in the Incomplete Randomized Block Design. With the Randomized Block design using Treatment Definition 3, flow and spill are covariates, and two critical assumptions are that the treatments (transportation and in-river) do not interact with the blocks nor do the covariates interact with the blocks. Interaction between treatments and covariates could be dealt with using a model like (5). A situation similar to that depicted in Figure 1 represents a violation of the no block by treatment interaction.

The other factor affecting **clarity is** the precision of estimates. For a given design this is purely a sample size issue and may be simple to specify, but practically it may be expensive. The precision depends upon the number of replicates at the level (x, y) and the size of $\phi(x, y)$ (see Table 7). Assuming independence between fish and no year by treatment interactions,, for a given value of $\phi(x, y)$ a single year at level (x, y) with number of **fish** in transported group and number of fish left in-river matching corresponding values in Table 7 would give the dezired precision.

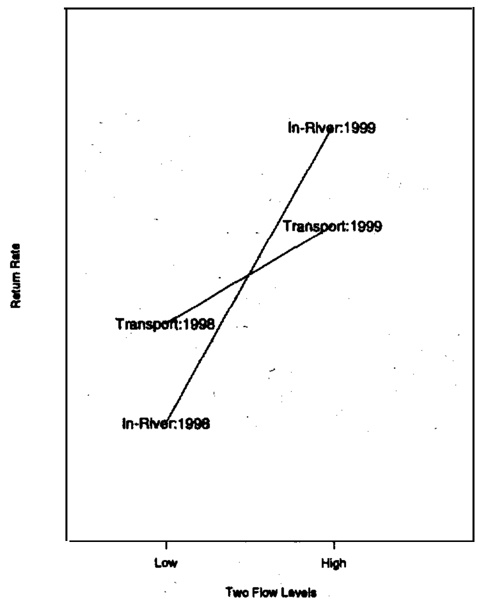

Figure 1: Hypothetical: Year effects confounded with flow levels.

7.3 Scope of inference

The scope of inference depends upon the range-of possible values of each factor manipulated or observed and the degree of inter&ion between the transportation effect and the factors. For instance if rearing type is a factor and only hatchery fish are used, then clearly extending inference about ϕ to wild fish may be risky. Similarly the range of flow and spill levels manipulated or observed will affect the scope of inference, assuming that flow and spill affect the transportation effect.

Even if observations made at the extreme ranges of the factor levels, the scope of inference may be limited if intermediate values are not observed depending on the relationship between the factor and the transportation effect. The Latin Square and 'Incomplete' Randomized -Block designs using binary levels to flow, say, would only allow for fitting linear relationship between with flow and return rate, with no ability to test for lack of fit; likewise for spill. A functional model of the form 5 can, however, be fit *assuming* linearity, thus yielding an predictive model for intermediate values. An expanded definition of the treatments would be preferred,, say to at least three levels in order to test the assumption of linearity.

On the other hand the haphazard flows and spills under the simple Randomized Block Design with Treatment Definition 3 will yield different flow and spill levels between years of experimentation and may provide information about nonlinear relationships. However, it may not cover a wide enough range of flows and spill levels to detect real effects.

7.4 Time to learn

One would like to learn as quickly as possible the effect of transportation and various river conditions on return rates. The Latin Square designs are in this sense ideal- one manipulates river conditions as much as possible within a single year, randomly assigning fish to all of the different treatment combinations.' Allowing for year effects and repeating this strategy for several years, one could quickly learn the effects. At the other extreme the slowest learning may occur with the Randomized Block design using Treatment Definition 3, if flow and spill have an effect but the range of flows and spills do not vary appreciably.

8 · Summary

Studies to determine the effect of transportation on survival and return rates relative to fish left in the river need to account for the possible influence of flow and spill on the magnitude of the effect. The cleanest interpretation of the effect under various flow and spill combinations will come from designed experiments in which flow and spill are deliberately manipulated factors applied in an impartial manner, i.e., randomly assigned. The most rapid learning, and understanding of the effect of transportation under differing flow and spill levels will happen when different river manipulations can be done within a single outmigration year.

On the other hand, the designs simplest to implement are those which view only transportation or being left in-river as the defined treatments and attempt to adjust for the potential effects of flow and spill through statistical methods. Such designs would be somewhat similar to some of historical transportation studies. Given haphazard flow and spill regimes, however, the scope of inference may not be satisfactory, and the interpretation of experiment results may not be as clear as for designs with deliberately manipulated flows and spills applied using an impartial chance mechanism.

Interactions between treatments, however defined, and blocks remains problematic for all designs- the impossibility of running several of the treatment combinations simultaneously makes this unavoidable. Designs running the most possible treatments within a single outmigration season are the best insurance against such complications, but are the most logistically difficult to implement.

One issue left unresolved in this report is an exact specification of the nature of flow and spill manipulations. Once resolved a more precise definition of possible treatment combinations could be given.

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