PRELIMINARY PROPOSAL FOR FY2007 FUNDING

Title: Mechanisms of delayed mortality in juvenile salmon outmigrating in the Columbia River Basin

Study codes: BPS-00-10 (This proposal addresses Objectives 1.b. and 2.)
(In FY05 this was Study Code: BPS-W-05-1)

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PROJECT SUMMARY

RESEARCH GOAL

The goal of this study is to explore the influence of two probable mechanisms (i.e., increased vulnerability to disease and size-selective predation) of delayed mortality of transported or bypassed juvenile salmonids migrating below Bonneville Dam. The results of this study will inform managers of the ultimate causes of delayed mortality. This information can be considered when making management decisions about operation of the Columbia River hydropower system, including the transportation and spill programs.

STUDY OBJECTIVES

Objective 1. Assess the physiological and health status of juvenile spring Chinook salmon that have been barged and those that have experienced different bypass histories during their in-river migration.

We will use new technologies, such as real-time, quantitative polymerase chain reaction (qPCR) assays and salmonid-specific cDNA microarrays to document the health and physiological status of different groups of fish at Bonneville Dam. The cDNA microarray will identify genes that are differentially up or down regulated and control physiological systems (e.g., osmoregulation, stress, metabolism, immunity) that may be impacted by route of migration of the fish. Using these techniques, we will also identify and develop molecular probes that are sensitive and reliable indicators of stress and fish health. Such information will provide much needed insight into whether disease, behavioral or developmental dysfunction may contribute significantly to delayed mortality in different groups of fish. Similar methods were used to identify physiological processes that allow fish to survive fluctuating daily water temperatures (Prodrabsky and Somero 2004) and the effects of contaminants on fish in laboratory and field studies (Hogstrand et al. 2002; Williams et al. 2003). For 2007, we have no plans for sampling more fish and will focus almost entirely on our cDNA assays for samples collected in 2005 and 2006.

Objective 2. Assess the relevance of size-selective predation on juvenile spring Chinook salmon that have been barged and those that have experienced different bypass histories during their in-river migration.

Passage of juvenile salmonids through or around the Columbia River Basin (CRB) hydrosystem exposes fish to numerous, possibly cumulative, stressors. Examples of stressors include handling and dewatering, passage through bypass systems at dams, loading, crowding and unloading from barges and exposure to elevated temperatures and contaminants. Exposure to these stressors may lead to delayed mortality of fish during the estuary or ocean life stage. This delayed mortality may claim 37 to 68% of juvenile salmon that pass Bonneville Dam (Budy et al. 2002). Also, rate of delayed mortality may differ for hatchery and wild fish and for fish

that have been transported compared to fish that migrated in-river because of developmental, physiological or size-related differences. The ratio of smolt-to-adult survival comparing transported and in-river migrants (D) may be < 1.0, suggesting delayed mortality of transported fish (Budy et al. 2002). Ultimately, delayed mortality could lead to reductions in smolt-to-adult survival rate and reduce the number of recruits per spawner.

For 2007, we will continue to conduct laboratory experiments with spring Chinook salmon designed to explore the relative seriousness of increased vulnerability to predation as a contributing mechanism of delayed mortality. In particular, we will focus on the influence of size-selective predation on delayed mortality. Size selective predation occurs in the wild and can influence the structure and dynamics of prey populations. This is of particular interest because fish that are transported are generally smaller than fish migrating in-river but it is not known whether such differences in size lead to differential predation. If size selective predation does occur, it could be one of the most parsimonious explanations for delayed mortality. This research will use well-established and powerful predator-prey bioassays in freshwater to determine the vulnerability of different groups of fish to predation by common piscivores. In addition, we will continue our analyses of predator diets below Bonneville Dam to assess the extent of size selective predation in the wild.

RELEVANCE TO BIOLOGICAL OPINION

The objectives of this proposal are relevant to Reasonable and Prudent Alternatives (RPAs) 5, 7 and 16, and Conservation Recommendation 2 in the NMFS 1995 Biological Opinion for Operation of the Federal Columbia River Power System (FCRPS), and in Terms and Conditions 1.j, 1.k, 2.d, 2.f, and 2.h in the 1998 Supplemental Biological Opinion. Addressing the relations between delayed mortality and hydropower operations is a critical factor in the 2000 FCRPS Biological Opinion RPAs, as indicated in Section 9.6.1 (page 9.4):

> "In hydro, for example, the projected effects of the hydro measures, or of the alternative of breaching dams, depends largely on the degree to which there is delayed mortality associated with juvenile fish passage at those dams, either in-river or with barge transportation, and the degree to which that delayed effect would be mitigated with breaching of any particular dam or dams."

This proposal addresses many RPAs in the 2000 FCRPS Biological Opinion. Specifically, RPAs 45, 46, and 47 under Section 9.6.1.3 "Juvenile Fish Transportation" require studies to address delayed mortality as a result of transportation. Moreover, virtually all of the RPAs in Section 9.6.1.4 "Fish Passage" (RPAs 54 through 98) address evaluations of operations and modifications of the FCRPS with the goal of increasing smolt-to-adult survival (i.e., decreasing delayed mortalities).

Importantly, this proposal addresses Research, Monitoring and Evaluation substrategy 2.1 Action Effectiveness Research: Hydrosystem (page 93):

> "Determine juvenile fish transportation effectiveness through evaluation of: (1) survival and adult return rates of juvenile salmonids transported compared to in-river migrating fish; (2) post-release losses and barging strategies that minimize post-release mortality; (3) benefits of trucking juvenile salmonids; (4) late-season transportation at McNary Dam; and (5) evaluate D of transported fish relative to in-river migrants."

and Research, Monitoring and Evaluation Strategy 3: Critical Uncertainties Research (pages 100 – 101):

> "**Updated Proposed Actions**: The Action Agencies will continue to fund studies to address the following critical uncertainties.
> • Uncertainty of in-river juvenile migration survival.
> • Relative survival difference of in-river versus transported fish.
> • Effect of ocean entry timing.
> • Delayed mortality related to hydrosystem passage.
> • Uncertainty of different dam passage histories relative to health and delayed mortality.
> • Extra mortality and its causes."

of the Final Updated Proposed Action for the FCRPS Biological Opinion Remand (2004).

PROJECT DESCRIPTION

BACKGROUND AND JUSTIFICATION

The Pacific Northwest is currently in the midst of an unprecedented decline of many stocks of anadromous salmonids. This crisis has lead to numerous research and management actions attempting to halt or reverse this decline. One factor potentially limiting salmonid production in the CRB is delayed mortality of juvenile fish after they have passed Bonneville Dam and entered the estuary and ocean. Delayed mortality, as defined by Budy et al. (2002), refers to mortality of fish that occurs in the estuary or ocean that is related to their earlier experiences in the hydrosystem. For example, fish may die in the estuary after leaving the hydrosystem (below Bonneville Dam) as a result of numerous, possibly cumulative stressors they experienced while passing as many as eight dams. The proximate causes, or specific mechanisms, of delayed mortality are speculative, but exposure of fish to hydrosystem-related stressors may make them more vulnerable to predation (see Mesa 1994) or lead to clinical expression of disease. *Renibacterium salmoninarum*, the causative agent of bacterial kidney disease (BKD) and a focus of this proposal, is prevalent in salmonid outmigrants from the Columbia and Snake River basins (Sanders et al. 1992; Maule et al. 1996; Elliott et al. 1997). Although evidence exists that anadromous salmonids infected with *R. salmoninarum* while in freshwater may die of BKD during downstream migration (Pascho et al. 1993) or after entry into seawater (Banner et al. 1986; Sanders et al. 1992; Elliott et al. 1995), more information is needed

on the incidence and severity of BKD in fish with different bypass histories to better link BKD with delayed mortality. In addition, little is known about the possible contribution of other fish pathogens to delayed mortality.

Recently, a workshop was convened by the Comparative Survival Study Oversight Committee and the U. S. Fish and Wildlife Service to organize and integrate scientific information on the indirect effects (i.e., delayed mortality) of hydrosystem configuration and operation on salmon and steelhead survival. One conclusion of this workshop was that increased vulnerability to predation and disease were likely contributors to delayed mortality, but confirmatory evidence for this hypothesis was lacking. Specifically, information is needed on whether mechanisms of delayed mortality may be differentially affecting groups of fish with different bypass histories. Budy et al. (2002) demonstrated an almost 2-fold greater smolt-to-adult survival (SAR) of summer and spring Chinook salmon that were not bypassed or bypassed at one dam as compared to those bypassed at two or more dams. These differences raise questions about the mechanisms of delayed mortality. For example, are there differences in vulnerability to predation or disease among fish that were: (1) never detected in a bypass system, (2) detected one or more times, or (3) barged around the dams? Determining the answer to this question will increase our understanding of the effects of hydrosystem passage and barging on delayed mortality of juvenile salmonids and is the focus of our proposal.

Mortality of juvenile salmonids migrating to the ocean in the CRB can be divided into two broad categories: direct and indirect (i.e., delayed) mortality. Direct mortality is mortality that is measurable and occurs within the hydrosystem, i.e., at or upstream of Bonneville Dam. Some examples of direct mortality include mortality that occurs during turbine passage or is due to predators feeding in the slack-water reservoirs. Delayed mortality, in contrast, is mortality that occurs during the near-estuarine, estuarine or ocean phases of juvenile salmon life histories and can be attributed to earlier hydrosystem experiences. For example, fish migrating through several dams on the Columbia River might be exposed to multiple, cumulative stressors that manifest themselves as an increased vulnerability to predation in the marine environment. Thus, exposure to stressors occurs during passage through the hydrosystem, but mortality occurs sometime later when fish are in the estuary or ocean. Currently, delayed mortality of juvenile salmonids, as defined here, seems to be more of a theoretical idea because little direct evidence exists to document its occurrence. Budy et al. (2002) argue that SARs of various groups of fish that experience different passage routes during their migration in the CRB is direct evidence of delayed mortality. For example, SARs of juvenile fish that migrated in-river and went through three or more bypass systems at different dams (assumed to be relatively stressful) were lower than SARs of fish that never got detected in a bypass system. Presumably, fish that did not get detected at various bypass systems passed the dams via the more natural, less stressful route of spill, thus minimizing delayed mortality and leading to an increase in SARs.

There is, however, a plethora of indirect evidence supporting the existence of delayed mortality and Budy et al. (2002) provide an excellent summary of this topic. Much of the indirect evidence for the existence of delayed mortality centers around the impact of stress on fish and is based on a voluminous literature. Figure 3 in Budy et al. (2002) summarizes it this

way: (1) juvenile fish can migrate past as many as eight dams; (2) fish passing a dam will either die, experience no effect, or be exposed to stress; (3) if exposed to stress, fish may recover completely, as in an acute physiological stress response, or they may experience cumulative or chronic effects; (4) cumulative or chronic effects of stress can lead to poor health, diminished physical abilities, and, presumably, delayed mortality.

The problem with much of this evidence, however, is that it was not generated with the specific objective of evaluating delayed mortality. For example, much of the literature on stress in fish results from trying to understand the effects of aquaculture-related stressors on fish physiology and health. Thus, although such work contributes to a theoretical and physiological framework for understanding the potential of stress to elicit delayed mortality in fish, it seems to be an incomplete surrogate for actual experimentation addressing the specific issue of delayed mortality. Does passage through several dams lead to metabolic or immune dysfunction and increased vulnerability to disease? Does passage through several dams compromise the physical abilities of fish to a point where they may be more vulnerable to predation in the marine environment? To our knowledge, studies addressing these specific questions have not been conducted, despite their obvious contributions to our understanding of the effects of stress on fish and delayed mortality. One reason for the paucity of information may be the lack of sensitive and reliable indicators of longer-term (chronic) stress in fish. Most of the commonly used end-points of stress (such as plasma cortisol, glucose and lactate levels) are highly variable and influenced to a large extent by the immediate stressful activity. Consequently, these plasma end-points of stress may provide limited information about the stress history (e.g., different passage routes) of the animal.

For this research, we will conduct laboratory studies and field sampling to determine whether vulnerability to predation and increased susceptibility to disease contribute significantly to delayed mortality of juvenile salmonids. Test animals for our fish health and physiology work will be collected at Bonneville Dam from several migration-history groups, including fish that have not previously been detected in a bypass system, fish that have been detected one other time, fish detected more than two other times, and those that have been barged. Our predation experiments will be conducted in large freshwater mesocosms and will be ecologically realistic because they address a scenario that juvenile salmon probably encounter frequently once they pass Bonneville Dam. Sampling fish with different bypass histories at Bonneville Dam will provide documentation of their health and physiological status at the time of passage, requisite data for making predictions about the fate of fish in certain groups, and the ability to correlate health status of PIT-tagged fish with adult returns.

This research should provide much needed information regarding two probable mechanisms of delayed mortality in juvenile salmonids. The cutting-edge techniques proposed here have the potential to provide valuable insight into the health, well being, and physiological status of fish after they have been barged or passed through the hydrosystem. Also, these techniques will allow us to develop sensitive and reliable molecular tools with which to detect chronic stress in juvenile salmonids. Collectively, this work will help substantiate or refute hypotheses concerning disease or predation, and perhaps lead to new hypotheses, as to the causes

of delayed mortality in Columbia River juvenile salmonids. Identifying the causes of delayed mortality is a critical step in understanding the phenomenon and will allow managers to make informed decisions when considering hydropower operations, including the transportation and spill programs

Work conducted in FY05 and FY06

Prior to the 2005 outmigration, 3000 summer Chinook salmon at McCall State Fish Hatchery (Idaho) were PIT tagged for our re-collection after release. Using sort-by-code (SbyC) systems, we collected 72 of these McCall migrants at John Day Dam between 10 and 26 May, and an additional 13 fish at Bonneville Dam between 27 and 31 May. We also collected non-PIT-tagged, hatchery Chinook salmon which had been loaded into a transport barge at Lower Granite Dam. We sampled fish from the barge as it moved between John Day and The Dalles dams, collecting 18, 22, and 31 fish on 4 May, 16 May and 24 May. All sampled fish were euthanized in an overdose of anesthetic and we collected gill filaments, blood, mucus, spleens, livers, kidneys, intestines, and fins, all of which were assayed for physiological activity associated with stress, smolt development, and fish pathogens. We are currently working on assays associated with gene expression.

Prior to the 2006 outmigration, we obtained permission to sample fish from the large number of PIT tagged fish used for the Comparative Survival Study. The fish we targeted this year were from Rapid River (RR) and Dworshak (DWK) hatcheries. First, we sampled 10 fish from each hatchery prior to their release. Using sort-by-code (SbyC) systems, we collected 17 of these migrants at Lower Granite Dam on 16 and 17 April, another 20 on 27 and 28 April, and a final sample of 20 fish on 8 May. We also sampled PIT-tagged RR and DWK fish that had been loaded onto a transport barge at Lower Granite Dam. We sampled fish from the barge as it moved between The Dalles and Bonneville dams, collecting 27, 25, and 31 fish on 25 April, 2 May, and 10 May. Finally, we completed our sampling by collecting PIT tagged RR and DWK fish from the SbyC system at Bonneville Dam. We sampled 30 fish on 16 May and 29 fish on 22 May. All sampled fish were euthanized in an overdose of anesthetic and we collected gill filaments, blood, mucus, spleens, livers, kidneys, intestines, and fins, all of which were assayed for physiological activity associated with stress, smolt development, and fish pathogens. We are currently working on assays associated with gene expression.

OBJECTIVES AND METHODOLOGY

Objective 1. Assess the health and physiological status of juvenile spring Chinook salmon that have been barged and those that have experienced different bypass histories during their in-river migration.

This work will involve testing tissues from individuals that have (1) been barged, (2) experienced one bypass system, or (3) experienced multiple bypass systems to provide an indication of the general health and well being of each population before leaving Bonneville Dam and migrating to the estuary. For 2007, our focus will be on completely finishing our fish

health and cDNA microarray assays from fish collected in 2005 and 2006. No new sampling is planned. In total, just from our sampling in 2006, we have about 221 fish samples to process and analyze. Methods are detailed below.

Fish health assessments.—We collected tissues from fish in each group (lethal sampling) and will assay them for various pathogens known to occur in the CRB, including the bacteria *R. salmoninarum*, *Yersinia ruckeri*, *Aeromonas salmonicida* and *Flavobacterium psychrophilum*, infectious hematopoeitic necrosis virus (IHNV), the myxosporean parasite *Ceratomyxa shasta*, and the microsporidian parasite *Nucleospora salmonis*. For detection of these pathogens, samples (25 mg minimum per sample) of kidney (two samples), liver and intestine will be removed from each fish. A portion of the kidney will be placed in an RNA stabilization reagent for the IHNV assay. These stabilized samples can be held at room temperature for up to one week but will be transferred to –80°C for longer storage before processing and testing for IHNV RNA by reverse transcriptase-PCR (LaPatra et al. 2001). An additional kidney sample will be preserved in 95% ethanol at room temperature for real-time quantitative PCR (qPCR) detection of *R. salmoninarum* (Elliott and Pascho 2004), nested PCR detection of *R. salmoninarum* (Chase and Pascho 1998), and nested PCR detection of *Nucleospora salmonis* (Giorgiadis et al. 1998). Liver tissue will be preserved in ethanol for detection of *Yersinia ruckeri*, *Aeromonas salmonicida*, and *Flavobacterium psychrophilum* by multiplex PCR (del Cerro et al. 2002). Intestinal tissue will also be preserved in ethanol for nested PCR detection of *Ceratomyxa shasta* (Palenzuela et al. 1999; Fox et al. 2000). We will also collect blood and a small gill sample from each fish to measure levels of thyroid hormones (T4 and T3), cortisol, glucose, and sodium-potassium ATPase.

At the same time as lethal samples are being taken, additional micro-samples will be taken from the same fish to test their potential value for non-lethal monitoring of fish for *R. salmoninarum* infections. Small gill samples will be removed according to the non-lethal method of Schrock et al. (1994) and preserved at room temperature in an individually numbered tube containing 95% ethanol. Additional samples taken from each fish will include fin clips and surface mucus samples. All of these samples will be tested for *R. salmoninarum* by qPCR (Elliott and Pascho 2004) and nested PCR (Chase and Pascho 1998) for comparison with results from kidney samples, the standard tissue used for *R. salmoninarum* assays. Although preliminary research indicates that non-lethal sampling of gill tissue may be a feasible alternative to lethal sampling of kidney tissue for this pathogen (Elliott and Pascho 2004), the potential value of other sample types for this purpose is unknown. Documenting the health status of fish via non-lethal sampling could be useful for studying PIT-tagged fish and adult returns. It is possible that non-lethal techniques could be developed for other pathogens. Collectively, this information will provide an indication of the likelihood that fish from certain groups will develop clinical diseases during their estuary or ocean life stage.

Molecular techniques (PCR and qPCR) were selected for the pathogen surveys in this project for several reasons. First, samples for PCR analysis are relatively easy to collect and preserve in the field for later laboratory analysis. Samples for PCR can be stored indefinitely (DNA) or for up to one week (RNA) at room temperature in the appropriate solutions. Samples

for ELISA can be stored at 4°C if they are processed within 1 or 2 days of sampling; otherwise they must be frozen at –70°C (AFS-FHS 2004). Pathogen cultures must be made shortly after collection, be refrigerated but not frozen (AFS-FHS 2004), and contamination can be a problem in the field. Second, only a small amount of tissue is needed for PCR testing. For PCR or qPCR, only 25 mg of tissue is required for nucleic acid extraction (Pascho et al. 2002; AFS-FHS 2004), and multiple PCR analyses for several pathogens can be performed with a single sample of that weight. For detection methods such as ELISA, larger tissue samples (80 mg minimum, with 250 mg preferred) are needed for a single analysis (AFS-FHS 2004). Thus, a tissue sample from a single juvenile salmonid is only of sufficient weight for one ELISA analysis for detection of a single pathogen. Third, PCR techniques are sensitive for detection of fish with active pathogen infections, and frequently demonstrate sensitivity equal to or greater than conventional methods (Miller et al. 1998; Palenzuela et al. 1999; Williams et al. 1999; Fox et al. 2000; del Cerro et al. 2002). For *R. salmoninarum* detection, for example, nested PCR procedures can be more sensitive than ELISA (Chase and Pascho 1998) and of equal or greater sensitivity than culture (Miriam et al. 1997).

Although the PCR procedures proposed for this research cannot distinguish live from dead pathogenic organisms, attempted culture of those organisms is not a reasonable option for this study. Culture procedures are not available for the parasitic pathogens in this survey (*Ceratomyxa shasta* and *Nuclospora salmonis*). Additionally, the different media types and incubation conditions necessary for the various bacterial pathogens and the cell culture procedures required for IHNV would be unwieldy, and further testing of isolated organisms would be required for confirmation of identity. Other common pathogen detection procedures such as ELISA and FAT also cannot be used for viability determinations. An additional problem with the ELISA for *R. salmoninarum* is that the soluble antigens detected by the test can persist for at least 110 days in the absence of live bacteria (Pascho et al. 1997), whereas the DNA fragments detected by PCR disappear at a faster rate when the infection is cleared (Elliott and Pascho 2004).

cDNA microarray.—We will use the same tissues collected during the lethal sampling of fish for the health assessment to determine the status of various physiological systems using a salmonid cDNA microarray. The advent of DNA microarray technology in the past decade has changed the field of physiological experimentation. The spotting of hundreds and thousands of genes on a single coated glass slide has opened up new possibilities in our ability to investigate and compare changes in gene expression. Basically, cDNA microarrays are glass slides upon which are placed small segments of DNA that encode for genes associated with the production of specific biochemical products. For example, the 530 base pair (bp) segment of rainbow trout cDNA, hsp70i, encodes for the inducible form of heat shock protein 70, a product related to the cellular stress response. Heat and other stressors will increase the expression of this gene. Thus, by hybridization of RNA from various tissues on the cDNA microarray slide, we will be able to determine which genes, and therefore which physiological systems, are up- or down-regulated at the time of capture (for a detailed microarray protocol see Lou et al., 2001).

For 2007, we will explore the efficacy of three different microarray systems and choose

the one that works best in terms of ease of procedures, throughput, analysis, and information gained. These three array systems are (1) a targeted rainbow trout cDNA microarray from the University of Waterloo [the array we proposed using in 2005]; (2) an array developed and produced by the Zoology Department at Oregon State University; and (3) the array produced by the consortium Genomics Research for All Salmon, or GRASP. The array from Waterloo contains about 150 genes, the one from Oregon State University has about 1500 genes, and cGRASP contains over 15,000 genes. We are currently testing each array and will choose the one that works best and proceed from there.

For our analyses, we will group genes according to how their products relate to various physiological systems, including (1) stress physiology; (2) metabolism; (3) exposure to contaminants; (4) reproduction; (5) immune function and disease resistance; (6) osmoregulation or smoltification; (7) growth and development; and (8) respiration. The utility of cDNA microarray technology is immense and will be especially important for this proposal because it will provide not only insight into the physiological responses to the stressors experienced by fish, but also will provide a tool to identify molecular probes that are indicative of homeostatic dysfunction associated with environmental stressors in the CRB. Such information could provide valuable insight into the physiological status of different groups of fish before they leave Bonneville Dam, would be useful for predicting the fate of fish as they continue their migration, and could be used to develop testable hypotheses about the physiological basis of delayed mortality.

Prodrabsky and Somero (2004) used high density microarray technology to identify physiological processes that allow an annual killifish *Austrofundulus limnaeus* to survive large daily fluctuations in water temperature (20 to 37º C). They were able to detect changes in the expression of genes which are responsible for cell growth and proliferation, metabolic functions (e.g., carbohydrate, intermediary, and nitrogen metabolism), and immune responses. For the microarray hybridization and analysis for this proposal, we will pool RNA from all liver samples and use this RNA pool as a universal reference sample (Prodrabsky and Somero 2004). Thus, each sample will be pooled with the reference sample and hybridized to a slide. The normalized data for each fish, which will be expressed as percent-fold change in the unknown sample relative to the reference sample, will then be treated as a sample size of one. All fish will be normalized in exactly the same way (to the same reference sample) and differences between groups will be analyzed using a two-way analysis of variance with treatment (barged, single bypass, multiple bypasses) and time (early, mid, late) as the factors. We will confirm the microarray results by randomly picking several genes, that are either consistently up- or down-regulated, and verifying the results using quantitative real-time PCR (Vijayan et al., 2003; Sathiyaa & Vijayan 2003).

Objective 2. Assess the relevance of size-selective predation on juvenile spring Chinook salmon that have been barged and those that have experienced different bypass histories during their in-river migration.

These experiments are designed to explore any overt differences in predator avoidance ability between fish that have been barged and those that remained in-river during migration and experienced different bypass histories. If differential predation exists, it could be caused by two factors, acting either independently or synergistically: (1) differences in size of fish within the two groups; or (2) differences in the types and severity of stressors experienced by fish. For example, if barged fish are smaller than run-of-river fish, vulnerability to predation may be mostly a size-related phenomenon. On the other hand, if the stressors experienced by one group of fish were more severe than those experienced by fish in other groups, the more severely stressed fish may be significantly more vulnerable to predation. For 2007, we propose to continue experiments to explore the influence of size selectivity. In subsequent years, we will pursue work addressing differences in vulnerability to predation between barged and run-of-river fish due to physiological stress.

As a first step in exploring the influence of size selective predation on fish migrating from Bonneville Dam to the estuary, we have obtained and summarized some data on size differences between barged and run-of-river spring Chinook salmon. First, data collected by NOAA-Fisheries in 2002 and 2003 from PIT tagged groups of wild fish showed that the difference in length between barged and river-run fish ranged from 5-13 mm and averaged 8 mm. In all cases, barged fish were smaller than run-of-river fish. Differences in length of PIT tagged hatchery fish were smaller, with barged fish being from 5-6 mm smaller than run-of-river fish. Second, we are currently collecting and analyzing data on fish lengths from the run at large at Bonneville dam and fish released from barges below the dam. These data, combined with those from NOAA-Fisheries, will allow us to derive some descriptive statistics on the difference in size between barged and run-of-river fish. We will then test whether such differences in mean size of fish within the two groups is sufficient to elicit significant selective predation.

For our size selection experiments in 2007, we are proposing to use the five concrete raceways located at the Fingerling Experimental Research Laboratory (FERL) at Bonneville Dam. In 2006, we conducted preliminary predation experiments in 12 and 5-foot-diameter circular tanks, which allowed us to test our methods and design. The raceways at the FERL will provide good replication, are large, have flowing freshwater, structure in the form of rocks and woody debris, and will be under an ambient photoperiod. We will conduct these experiments using common freshwater predators, such as Northern pikeminnow *Ptychocheilus oregonensis* or smallmouth bass *Micropterus dolomieui*. Since these experiments are designed to explore size selective predation only, we propose to use juvenile spring Chinook salmon from the Little White Salmon National Fish Hatchery as prey. A large group of fish will be taken from the hatchery in the spring, 2007, and we will rear subgroups of them on different rations to achieve different sized fish by mid-summer. Previously, there has been some concern about the realism of these experiments because of our desire to use hatchery-reared fish as prey. We think such concern is relatively minor because the focus of these experiments is on size-selective predation. Further, this is the only way to test size-selective predation independent of variable migration histories. If required, we could conduct these experiments with fish from the wild; however, some things to consider include difficulty collecting enough fish for the tests, establishing

appropriate size groups, and logistics of holding run-of-river fish. We anticipate predation experiments will take place from July through September, 2007.

For the trials, we will weigh and measure individuals from each general size group to create populations of test prey that differ in size by a prescribed amount. This difference in size between the two groups will be based on our findings from the analysis of lengths as described above. We plan on testing a few multiples of the mean difference in size between barged and run-of-river fish (e.g., 5, 10, and 20 mm length differences between groups). All fish will be briefly anesthetized using 50 mg/L tricaine methanesulfonate (MS-222) buffered with sodium bicarbonate and we will randomly select and mark one group by removing the adipose fin. The other group will be sham marked. Marking procedures will rotate between groups for different sets of trials. After fish are marked, they will be held for 24 h before being subjected to predation.

After 24 h, 20 fish from each size group will be water brailed simultaneously to the predation raceways. Predators will be allowed to consume juvenile salmon until 50% of the prey are eaten. We will observe fish behavior from overhead and estimate the number of prey eaten to determine when a trial should end. Afterwards, the remaining prey will be seined from the raceways and placed in a lethal dose (200 mg/L) of MS-222. Lengths and weights will be recorded and fish will be identified with a group based on presence of a mark. For each size difference being tested, we will attempt to conduct enough replicates to achieve a total of 180 prey eaten (see power analysis below).

We will analyze all data in a manner identical to that of Mesa (1994). First, data will be subjected to a heterogeneity π^2 analysis to determine if the individual tests were homogenous (Sokal and Rohlf 1981). Chi-squared goodness-of-fit tests will then be used on pooled data to determine if predation was random (i.e., 50:50) on the different groups of fish. Because the π^2 test has low statistical power (i.e., low probability of correctly rejecting the null hypothesis) when relatively small sample sizes are used, we will set the significance level at 0.10, the detectable difference in predation rates (i.e., the effect size) at 20%, and target sample sizes at about 180 to increase power and reduce the probability of the more serious type II error (Fairbairn and Roff 1980, Peterman 1990).

In addition to the predation experiments described above, we also plan to continue an analysis we started in 2005 evaluating the diets of Northern pikeminnow below Bonneville Dam. In 2006, we collected over 130 adult Northern pikeminnow from the Sport Reward Fishery below Bonneville dam. The stomachs were removed from these fish and the contents chemically digested to leave only diagnostic bones of prey fish that were consumed. We also intend to obtain stomach contents (diagnostic bones only) from pikeminnow collected by the Oregon Department of Fish and Wildlife during electrofishing surveys below Bonneville Dam in 2004. For all of these samples, we will identify any juvenile salmonids that were consumed and estimate their original length using established regression equations (Hansel et al. 1988). To explore the influence of size selective predation in the wild, we will compare the sizes of fish eaten by Northern pikeminnow to the sizes of fish available in the environment.

SCHEDULE

Work on both objectives would start in October 2006. All of this work is basically a continuation of that started in FY 2006. Assays, data proofing, analysis, and report preparation should be completed by December 2007.

RELATION OF PROPOSED RESEARCH TO OTHER ONGOING OR PROPOSED RESEARCH

We are unaware of any similar delayed mortality work ongoing currently in the basin. Our proposed research should compliment past or future work on delayed mortality (e.g., the NOAA-Fisheries work of Lyle Gilbreath). We have communicated with Bill Muir (NOAA-Fisheries) who is submitting a proposal addressing size differences between barged and in-river fish, and our Objective 2 is relevant to that proposal.

IMPACTS

We anticipate that any impacts from this study on other ongoing or proposed research will be negligible, particularly since we have no plans to sample fish this year. We will need to gain and coordinate access to the FERL. We are unaware of any other biological impacts from this study.

LIST OF KEY PERSONNEL AND PROJECT DUTIES

Matthew Mesa, principal investigator: oversight of predation studies, tissue sampling, analysis and writing

Alec Maule, principal investigator: general project oversight, analysis and writing

Diane Elliott, principal investigator: oversight of fish health studies, analysis and writing

FACILITIES AND EQUIPMENT

Predation work will be conducted at either the Columbia River Research Laboratory (CRRL) or the Bonneville JFF. Assays for this study will be conducted at the CRRL, and the Western Fisheries Research Center in Seattle. All facilities are well supplied with all the modern equipment, computers, and analysis software necessary to complete this research.

The CRRL in Cook, WA, is part of the USGS's Western Fisheries Research Center (WFRC). The CRRL has three state-of-the-technology analytical laboratories dedicated to enzymology, immunology and cell culture, and general physiology. In addition to standard equipment such as centrifuges, pH meters, and balances, the laboratories are equipped with VIS-UV and reflectance spectrophotometers, enzyme-linked-immunosorbent assay (ELISA) plate readers,

flame photometers, and ultracold freezers. The laboratory is staffed with trained technicians and biologists proficient in these techniques with backgrounds in fish behavior, immunology, physiology, and endocrinology. The 1500 sq. ft. wet lab facility is adequate to conduct a variety of studies, including investigations into fish development or behavior, disease resistance, reproduction, predator avoidance, thermal preference, osmoregulation, swimming performance and bioenergetics. The CRRL has a modern computer network that services over 100 users at the facility and a GIS laboratory. Computer software available for data analyses includes SAS, Excel, SigmaPlot, Statgraphics, and a variety of other word processing and data management software. The CRRL has its own T-1 line for fast Internet access.

The WFRC represents a state-of-the-art center for work on infectious diseases of fish that includes over 16,000 square feet of laboratory space for cell culture, virology, bacteriology, immunology, histology, parasitology, and molecular biology. The laboratory also houses a 9,000 square foot wet laboratory supplied with pathogen-free fresh water to 20 individual bays (each with temperature control from 4-25°C) containing a total of more than 300 tanks of various sizes. The laboratory effluent is treated with chlorine gas. Within the dry lab complex is a restricted access Biosafety Level 3 laboratory containing dry and wet laboratories for work with exotic fish pathogens. Also in the dry lab are a walk-in cold laboratory (4°C), walk-in cold storage (4 and -20°C), fluorescence microscopy rooms, a common computer room and an animal care facility meeting NIH guidelines. The laboratory is equipped with 2 ultracentrifuges, 4 refrigerated centrifuges, 4 refrigerated microfuges, automated equipment for enzyme-linked immunosorbent assays (ELISA), more than 10 PCR machines including an ABI 7900 sequence detection system, automated DNA sequencer, peptide synthesizer, DNA synthesizer, pulsed-field, protein, and nucleic acid electrophoresis equipment, 4 spectrophotometers, luminometer, fluorometer, scintillation counter, 5 chemical fume and 10 laminar flow hoods, 10 ultrafreezers, 5 research microscopes, networked and stand-alone computers with internet and both DNA and image analysis capabilities, and other large and small equipment items commonly found in microbiology and molecular biology laboratories.

TECHNOLOGY TRANSFER

Results from this study will be disseminated in the form of annual reports of research, oral presentations and briefings, and peer-reviewed journal publications.

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