

PRELIMINARY RESEARCH PROPOSAL (COE) (FY07)

I. BASIC INFORMATION

Title: Estimate of hydrosystem latent mortality associated with barge and in-river life-history strategies of Snake River spring/summer Chinook salmon

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Study Code: BPS-W-00-10

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II. PROJECT SUMMARY

The objectives of the proposed study are to: (1) quantify the incidence of latent mortality in juvenile Snake River spring Chinook with differing outmigration strategies that are held in the estuary within net pens; (2) partition the incidence of mortality between disease and ‘other factors’, and (3) develop methods of accounting for disease-induced mortality associated with hatcheries, and not the FCRPS, in the estimate of *D*. We intend to repeat the proposed work for three consecutive years to establish reproducibility in the results. The objectives of the proposed study are subdivided into five primary tasks: (1) characterize the impact of barging conditions on disease-induced direct and latent mortality; (2) assess the spatial and temporal distribution of pathogens and contaminants in outmigrants of hatchery-origin; (3) estimate the incidence of latent mortality associated with infectious diseases relative to other factors (e.g., smoltification timing, temperature, etc) in salmon with barged and in-river life-history strategies; (4) examine the relevance of immunological and stress-associated gene expression on latent mortality; and (5) compare SAR rates for in-river and barged fish based on modified survival estimates of smolts and adults.

In the study proposed herein, we hypothesize that the differential incidence of latent mortality in barged fish arises from infectious disease present in fish prior to transportation. Central to the proposed hypothesis is that hatchery fish in the Snake River have a high incidence of infectious disease immediately prior to release. The subgroup of diseased fish that remain in-river die during outmigration. The subgroup of diseased fish that are transported do not die prior to release below Bonneville, yet they are predisposed to mortality given their morbid status. Hence, any mortality of transported fish that occurs below Bonneville associated with infectious disease present in fish prior to transportation is implicitly incorporated into the transportation SAR even though it is unrelated to transportation. Within this context, barging would not necessarily represent a significant stressor that results in latent mortality; rather, infectious diseases associated with hatchery operations, potentially exacerbated by transmission vectors associated with barging operations, represent a significant stressor resulting in a quantifiable incidence of ‘latent’ mortality in barged fish. Latent is placed in quotes because the incidence of mortality is not necessarily a reflection of the FCRPS, and hence, should likely be accounted for in some manner prior to the calculation of *D*.

The proposed study will provide critical information for implementing management and control practices as part of the US Army Corps of Engineers’ obligations under the Endangered Species Act. The proposed study is consistent with NOAA Fisheries 2004 Biological Opinion (NOAA Fisheries, 2004) and Action Agencies Updated Proposed Action Plan (USACE et al., 2004). The proposed project relates directly to the following regional programs and recommendations:

- Reasonable and Prudent Alternative measure 141 (UPA, Appendix A) is specifically aimed toward investigations that acquire a better base of information to understand the sources of fish diseases and mortality during critical fish migration periods.
- Strategy 3 of the Research, Monitoring, and Evaluation (UPA, Chapter IV) states that action agencies will fund studies to (a) reduce or resolve key uncertainties related to delayed mortality of which disease has been specifically identified as a potential factor for both juvenile and adult fish passage (Biological Opinion, Sections 5.2.2.3.1 and 5.4.5.1), and (b) reduce or resolve key uncertainties related to survival differences between transported and in-river fish.

Finally, the proposed study is consistent with Federal trust and treaty responsibilities.

III. PROJECT DESCRIPTION

Background

The Columbia River provides critical habitat for threatened and endangered salmon species in the Pacific Northwest. Thirteen stocks, or evolutionarily significant units (ESUs; Waples 1991) from this region are threatened or endangered; Chinook (*Oncorhynchus tshawytscha*) ESUs include Snake River spring/summer and fall run, Lower Columbia River, Upper Willamette River, and Upper Columbia River spring-run (NRC 1996).

Factors contributing to the decline of salmon populations in the Pacific Northwest include habitat degradation, over harvest, hydropower operation, and hatchery production (NRC 1996). Although the Federal Columbia River Power System (FCRPS) has become instrumental in providing irrigation water, flood protection, navigation, and recreation, the system has critically affected salmon migration, with some of the Columbia River Basin ESUs migrating past as many as 8 dams. Aside from restricting access to adult reproductive habitat (Raymond 1988), the FCRPS contributes to stock losses in juveniles during river rearing and outmigration. In the absence of the FCRPS, salmonid juvenile mortality in the river and estuary may occur due to predation, disease, chemical toxicity, water quality, nutrition, injury, and physiological stresses associated with smoltification (Figure 1a). The FCRPS may further exacerbate these 'natural' causes of mortality, as well as impose additional sources of direct mortality (Figure 1b).

Direct mortality is considered to occur when death takes place during the same life stage as the stressor, and delayed mortality is considered to occur at a life stage subsequent to the stressor. Evidence of delayed mortality associated with the FCRPS is captured in smolt-to-adult (SAR) rates that decrease with the number of dams bypassed even though direct mortality should decrease with the number of bypasses (Sandford and Smith 2002). Delayed mortality of juvenile salmonids

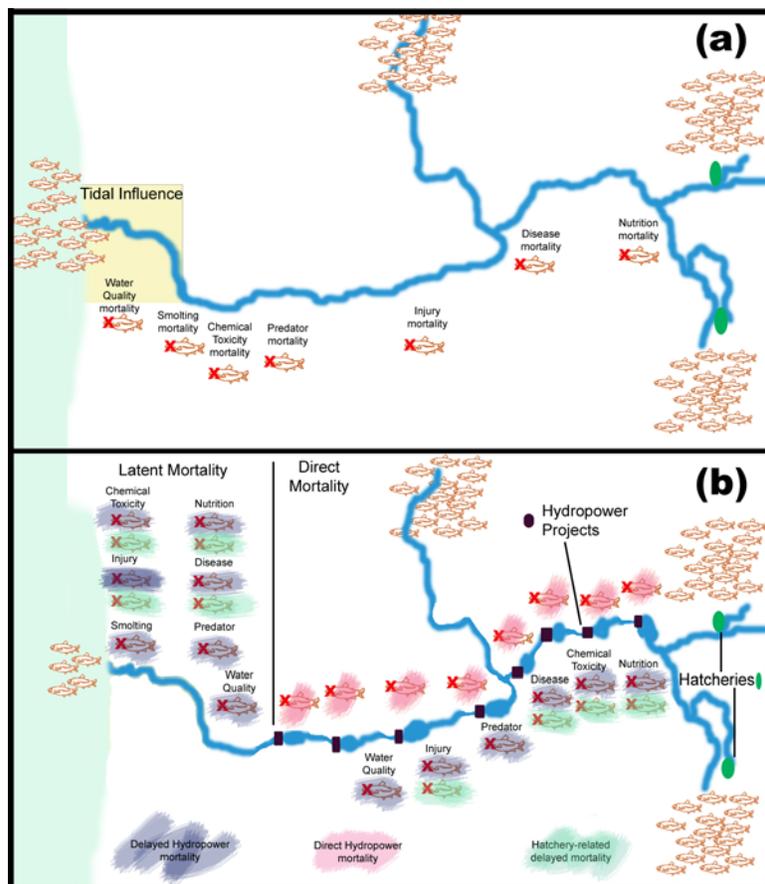


Figure 1. (a) Natural and (b) hydrosystem factors contributing to juvenile salmon mortality during outmigration.

due to the existence of the FCRPS that occurs after the last dam, i.e. Bonneville Dam, is termed latent mortality.

A number of actions have been undertaken to mitigate direct and delayed effects on salmon survival, including construction of juvenile fish-passage and collection facilities at all the mainstream dams except Ice Harbor and The Dalles Dams (Muir et al. 2001), predator control, transportation (barging), flow augmentation, and reservoir drawdown (Ruckelshaus et al. 2002). However, the specific impacts of these remediation strategies on population numbers are currently not well quantified. In addition, the remediation strategies themselves may induce levels of stress that exacerbate delayed health effects associated with predator response, disease susceptibility, and growth. For example, barging juvenile salmon is believed to induce stress associated with handling and crowding, and passage through either the turbines or bypass systems can cause mechanical injuries such as bruising and descaling or exposure to supersaturated gasses (NRC 1996; Budy et al. 2002).

Estimates of Direct and Latent Mortality. Passive integrated transponder (PIT)-tagged hatchery reared salmonids in the FCRPS provide a mechanism for assessing the magnitude of direct and latent mortality. The Comparative Survival Study (CSS) has tagged salmon from up to 6 hatcheries each year, beginning in 1996, and monitored their passage through the hydropower projects equipped with detectors both as outmigrants and returning adults (Berggren et al., 2005). The CSS study has estimated the survival rate (V_c) of in-river outmigrants from the Lower Granite Dam tailrace to the Bonneville Dam tailrace as the product of individual survival rate estimates through 6 interior reaches by the Cormack-Jolly-Seber methodology (Berggren et al., 2003; Jolly 1965; Seber 1965; Cormack 1964). The mean in-river survival estimate for Dworshak (DWOR), Rapid River (RAPH), and McCall (MCCA) Hatcheries from 1997-2003 is 51-54%. An estimate of the magnitude of latent mortality is less straightforward due to multiple factors contributing to juvenile mortality below Bonneville Dam (Figure 1). SAR data has been used to estimate post-FCRPS mortality of one migratory group relative to another. The T/C ratio is a direct comparison of the SAR for smolts that are transported through the FCRPS to the SAR of in-river smolts experiencing 0–3 Columbia River bypasses during outmigration.

$$\frac{T}{C} = SAR_2(T_0)/SAR(C_0) \quad 1.$$

Between 1997 and 2003, the T/C Ratio has been generally greater than 1.0 (Table 1) for DWOR, RAPH, and MCCA Hatcheries, suggesting that transportation of fish around the dams increases the return rate of adults relative to an in-river life-history. Differential mortality (D) is an alternative parameter for comparing transportation and in-river life-histories:

$$D = \frac{SAR_2(T_0)}{V_t} \bigg/ \frac{SAR(C_0)}{V_c} \quad 2.$$

where, V_c is the survival rate of in-river outmigrants to Bonneville, and V_t is the survival rate of transported juveniles (assumes a fixed 98% barge survival that is further corrected for survival to a barge collection site other than Lower Granite Dam (Berggren et al., 2003)). The differential mortality parameter (D) differs from the T/C ratio in that the SARs used in the calculation of D are normalized by mortality in the FCRPS (V_t or V_c). The ratios otherwise are identical. By correcting the SARs for mortality experienced by smolts in the FCRPS, a value of 1.0 for D implies that there is no difference in post-FCRPS mortality (e.g., mortality after Bonneville) in either group. And if ocean conditions and drop-out rates are equivalent for each group, a value

of D equal to 1.0 further implies either that (1) there is no latent mortality associated with smolt transport and the FCRPS, or (2) the latent mortality is equivalent for each group. The values of D for CSS-tagged hatchery fish have consistently been less than 1.0 from 1997-2003 (Table 1), implying either that (1) latent mortality exists for both groups but is greater for transported fish or (2) latent mortality is only present in transported fish. In either case, latent mortality is greater in transported fish than fish with an in-river life-history. Hence, one can conclude from the CSS annual reports that despite the greater rate of returning adults from barged smolts, barging operations represent a significant stressor that results in latent mortality.

Table 1. Comparisons of Adult Returns for In-River and Transported Hatchery Spring/Summer Chinook with the T/C^a and D^b parameters.

Year	Dworshak Hatchery		Rapid River Hatchery		McCall Hatchery	
	T/C	D	T/C	D	T/C	D
1997	1.77	0.89	1.72	0.60	1.39	0.63
1998	0.72	0.38	1.67	1.02	1.96	1.16
1999	0.99	0.61	1.29	0.80	1.50	0.86
2000	0.99	0.53	1.32	0.82	1.90	1.24
2001 ^c	9.00	2.23	21.80	7.42	31.00	8.72
2002	1.24	0.84	1.51	1.14	1.45	0.88

^a T/C = Smolt-to-adult rate (SAR) for transported smolts ($SAR_2(T_0)$) divided by the SAR of in-river smolts ($SAR(C_0)$) defined as per Berggren et al. (2005). SAR values used were determined at Lower Granite Dam and reported in the 2005 Comparative Survival Study Annual Report (Berggren et al., 2005).

^b The delayed mortality ratio: $D = \frac{SAR_2(T_0)}{V_t} / \frac{SAR(C_0)}{V_c}$; where V_c and V_t are estimates for survival of in-river and barged smolts, respectively, as per Berggren et al. (2003). SAR values used were determined at Lower Granite Dam and reported in the 2005 Comparative Survival Study Annual Report (Berggren et al., 2005).

^c Year 2001 was anomalous year due to diminished spill at the hydropower projects. Consequently, the SAR estimate of bypassed fish was used as an estimate of in-river SAR (Berggren et al., 2005).

In the study proposed herein, we hypothesize that the differential incidence of latent mortality in barged fish arises from infectious disease present in fish prior to transportation. Central to the proposed hypothesis is that hatchery fish in the Snake River have a high incidence of infectious disease immediately prior to release. The subgroup of diseased fish that remain in-river die during outmigration, and thus their death affects the value of V_c . The subgroup of diseased fish that are transported do not die prior to release below Bonneville, and hence, their survival is incorporated into the value of V_t , yet they are predisposed to mortality given their morbid status. Hence, any mortality of transported fish that occurs below Bonneville associated with infectious disease present in fish prior to transportation is implicitly incorporated into the transportation SAR even though it is unrelated to transportation, or the FCRPS for that matter. Within this context, barging would not necessarily represent a significant stressor that results in latent mortality; rather, infectious diseases associated with hatchery operations, potentially exacerbated by transmission vectors associated with barging operations, represent a significant stressor resulting in a quantifiable incidence of ‘latent’ mortality in barged fish. Latent is placed in quotes because the incidence of mortality is not necessarily a reflection of the FCRPS, and hence, should likely be accounted for in some manner (e.g., through V_t) prior to the calculation of D . For example, if 10% of the fish released from Dworshak Hatchery in 1997 were morbid such that they would eventually die, the corrected value of D would equal 1 (Figure 2) which is a

modest increase over the uncorrected D -value of 0.89 (value associated with a 0 incidence of infectious disease in Figure 2). If roughly 50% of the fish released from Dworshak were morbid such that they would eventually die, the value of D in 1997 would have approached a value of 1.8 (Figure 2). Hence, the value of D is strongly influenced by the health and fitness of hatchery fish released into the river system. Key aspects of the proposed research include establishing: (i) incidence of infectious disease in outmigrant Snake River spring/summer Chinook salmon at selected locations between the point of hatchery release and Bonneville Dam (this is not a system-wide survey that would likely be under the purview of BPA, but rather a survey focused on providing key data necessary in modifying the value of D); (ii) rates of disease transmission in raceways used for loading barges and the barges themselves; and (iii) incidence of latent mortality in the estuary associated with infectious diseases relative to other factors (e.g., smoltification timing, temperature, etc), further subdivided according to outmigrant life-history strategy (e.g., barge versus in-river). ‘Other factors’ collectively represent such aspects as temperature, timing of estuary arrival, seasonal changes in estuary water quality, and physiological health status not related to infectious disease.

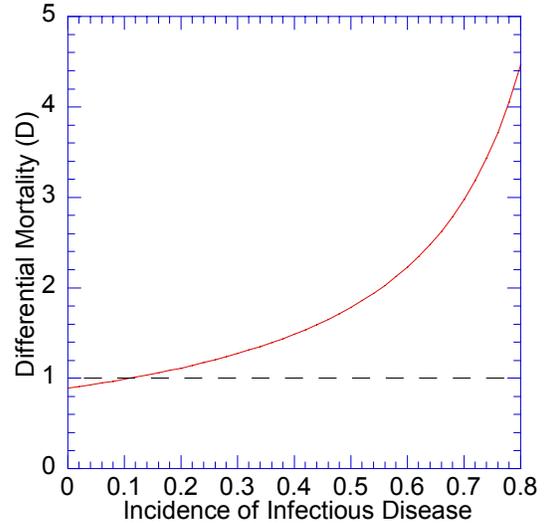


Figure 2. The potential impact of the incidence of infectious disease on delayed differential mortality (D) from smolts from the Dworshak Hatchery (1997) that die after transport.

Preliminary Data

Based on SAR rates and D -values, it appears that both transported and bypassed Snake River spring/summer Chinook salmon are experiencing greater delayed mortality than in-river fish (not bypassed fish). To address this issue, we conducted an AFEP-funded study in FY02 entitled “A study to compare long-term survival and disease susceptibility of yearling hatchery chinook salmon smolts with different juvenile migration histories” to examine the health of hatchery Snake River spring/summer Chinook salmon relative to bypass history and transportation. The health of outmigrants was assessed in terms of the difference in the incidence of mortality among fish, categorically grouped into no-bypass, bypass, and transportation life-histories, in response to challenge with the pathogenic marine bacterium *Listonella anguillarum* during seawater holding. Within the disease challenge study design, the incidence of disease-induced mortality was viewed as a direct measure of physiological health and the potential for delayed mortality among the three groups of fish representing different outmigrant life-histories. We are currently in the process of repeating the FY02 study with an FY06 AFEP funded study entitled “Disease susceptibility of hatchery-reared yearling Snake River Chinook salmon with different migration histories in the Columbia River.” Finding from each of these two studies (FY02 and FY06) relevant to the proposed study (FY07) are discussed in more detail below.

Results from FY02 Study. The cumulative incidence of mortality of salmon with different bypass histories exposed to *L. anguillarum* after a 10-day observational period is graphically depicted in Figure 3 in reference to: (a) pooled data collected in the first disease challenge study using concentrations of *L. anguillarum* of 2.8×10^6 and 1.4×10^7 cfu/mL (concentrations bracket the LC_{50} value) and (b) data collected in the second study using a 6×10^6 cfu/mL concentration of *L. anguillarum* (equal to the LC_{50} value). In the first disease challenge, the cumulative incidence of mortality associated with barged fish was statistically less than in-river fish with no-bypass ($p \leq 0.005$) life-history. The cumulative incidence of mortality associated with barged fish was statistically different than in-river fish with a single ($p \leq 0.157$; data not shown) or multiple ($p \leq 0.121$) bypass life-history. However, the cumulative incidence of mortality was not statistically different between single and multiple bypass life-histories ($p \leq 0.381$; data not shown). In the second disease challenge, the cumulative incidence of mortality associated with barged fish was statistically less than in-river fish with both a no-bypass ($p \leq 0.001$) and multiple bypass ($p \leq 0.001$) life-history. The cumulative incidence of mortality was not statistically different between no-bypass and multiple bypass life-histories ($p \leq 1$). Differences in the p-values associated with the pair-wise comparisons of life-history strategies in the first and second disease challenge reflect, in part, differences in the sample size: the first disease challenge utilized roughly three times fewer fish per life-history category than the second study. The first disease challenge was largely intended to identify the LC_{50} -value for the second study.

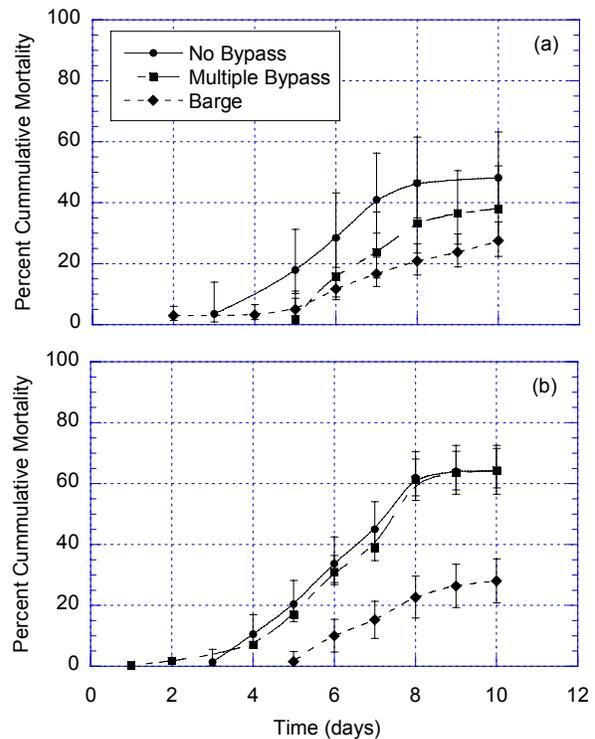


Figure 3. FY02: Kinetics of cumulative mortality over a 10-day observational period resulting from a 1-hour exposure of Snake River spring/summer Chinook salmon to a LC_{50} concentration of *L. anguillarum* in the (a) first and (b) second disease challenge study.

Preliminary Results of FY06 Study. The FY06 study was designed to: (a) confirm the reproducibility of the FY02 study, and (b) evaluate the impact of the hatchery of origin (RAPH and DWOR) on the incidence of delayed disease-induced mortality of fish categorically grouped according to outmigrant life-history strategy. The cumulative incidence of mortality over the 10-day observational period post-exposure to *L. anguillarum* is graphically depicted in Figure 3 in reference to data collected in the: (a) first disease challenge experiment and (b) second experiment with roughly seven times the number experimental fish. The cumulative incidence of mortality for barged and in-river fish was pooled for RAPH and DWOR fish in Figure 4. The cumulative incidence of mortality for in-river and barged outmigrants was significantly different in both the first ($p \leq 0.0142$) and second ($p \leq 0.0134$) disease challenge; results in FY06 are identical to FY02.

The cumulative incidence of mortality of outmigrants from Rapid River and Dworshak Hatcheries post-exposure to *L. anguillarum* in the second disease challenge study is graphically depicted in Figure 5 in reference to: (a) in-river and (b) transport life-history strategies. There was little to no statistical difference in the cumulative incidence of mortality between RAPH and DWOR fish with an in-river life-history ($p \leq 0.3865$). In contrast, there was a significant statistical difference in the cumulative incidence of mortality between RAPH and DWOR fish with barged life-histories ($p \leq 0.0929$). Although not depicted graphically, the statistically significant trends in the pooled analysis (Figure 4) were similar for a particular hatchery. For example, the cumulative incidence of mortality of in-river RAPH fish was statistically greater than RAPH fish barged through the hydropower system ($p \leq 0.0274$).

Preliminary Discussion. Results from the FY02 and FY06 AFEP funded studies indicate that in-river outmigrants (traveling through one or more bypass structures) are more susceptible to disease than juveniles transported through the hydropower network. These studies suggest that barging outmigrants reduces the impact of the FCRPS on latent mortality associated with marine pathogen exposure below Bonneville Dam (Figure 1; Arkoosh et al. in press), and more generally, improves the overall health status of the fish upon entry into the estuary.

Our findings are consistent with the following observations made by other researchers. The FCRPS has increased the time it takes for juvenile salmon to migrate down the Columbia River by decreasing water velocity and delaying fish at dams (Williams et al. 2005). Physiological changes brought about by this prolonged migration include a depletion of energy reserves that can contribute to an increase in disease susceptibility and ultimately lower survival rates in fish that are not barged (Congleton et al. 2000, Wedemeyer et al. 1990).

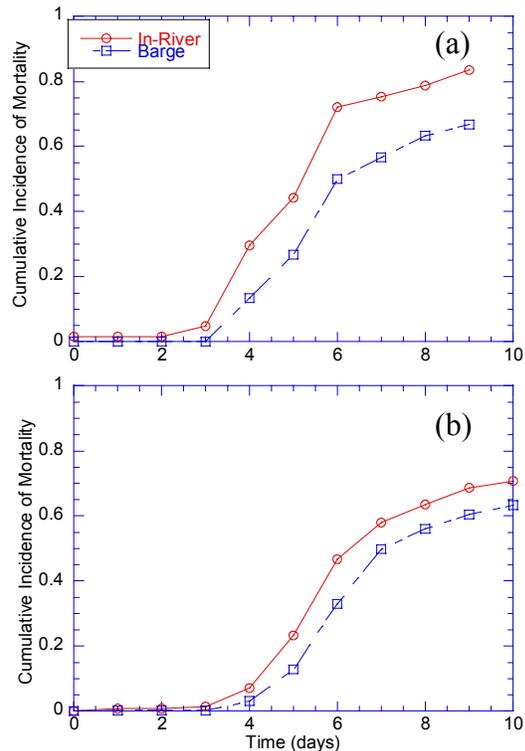


Figure 4. FY06: Kinetics of cumulative mortality over a 10-day observational period resulting from a 1-hour exposure of Snake River spring Chinook salmon to *L. anguillarum* in the (a) first and (b) second disease challenge study.

Results from this study are in contrast to studies focusing on SAR rates or D -values that indicate a higher incidence of delayed mortality in bypassed and barged Snake River spring/summer Chinook salmon relative to an in-river non-bypass migration strategy (Budy et al. 2002, Sandford and Smith 2002, Williams et al 2005). Collectively, the results are not mutually exclusive, but rather highlight the importance of understanding the relative significance of various components influencing the SAR rates, of which delayed disease-induced mortality is only one. We found that in-river migration strategies elevate the incidence of delayed disease-induced mortality over transported fish. To offset the reduction in stock losses associated with in-river migration strategies, other components influencing SAR rates must preferentially impact fish with a transportation migration history. The SAR ratio is, in fact, an estimate of salmon survival (V_s ; Eq. 3):

$$SAR = \frac{\#adults}{\#smolts} = \frac{V_s \cdot \#smolts}{\#smolts} = V_s \quad 3.$$

and can be expanded to include factors that may contribute to smolt and adult mortality after initial smolt detection (Eq. 4):

$$V_s = V_{FCRPS} \cdot V_{predator} \cdot V_{disease} \cdot V_{smoltification} \cdot V_{nutrition} \cdot V_{chemical} \cdot V_{WQ} \cdot V_{injury} \cdot V_{stray} \cdot V_{ocean} \quad 4.$$

Where V_{stray} and V_{ocean} are survival parameters specific to life stages subsequent to FCRPS exposure, hence, are not considered to contribute to latent mortality. We hypothesize in the study proposed herein that the differential incidence of latent mortality in barged fish arises from infectious disease present in fish prior to transportation. Alternatively stated, $(V_{disease})_{Barge} < (V_{disease})_{In-river \text{ post-Bonneville}}$; because the preponderance of diseased fish entering the river system at the hatchery have died prior to Bonneville with an in-river life-history such that the remaining subgroup has a higher likelihood of surviving, whereas the majority of disease fish in the barge survive until release into the estuary, at which time the preponderance of these fish die. In the proposed experimental design, the significance of disease will be evaluated in reference to ‘other factors’. The ‘other factors’ will specifically represent mortality that occurs in the estuary collectively associated with smoltification, chemicals, water quality (WQ), and

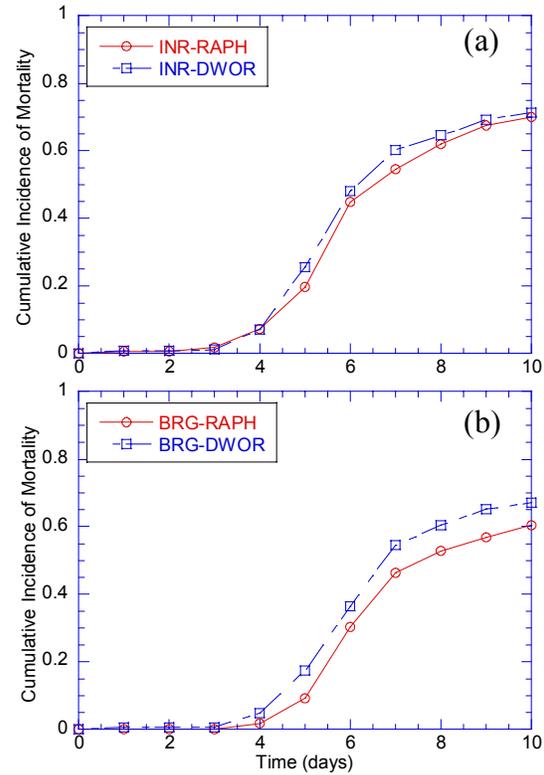


Figure 5. FY06: Kinetics of cumulative mortality over a 14-day observational period resulting from a 1-hour exposure of Dworshak and Rapid River hatchery salmon to *L. anguillarum* for (a) in-river and (b) barged smolts.

injury. Latent mortality associated with predation and nutrition, and ocean survival and adult straying will not be addressed as part of this study.

Objectives

The objectives of the proposed study are to: (1) quantify the incidence of latent mortality in juvenile Snake River spring Chinook with differing outmigration strategies that are held in the estuary within net pens; (2) partition the incidence of mortality between disease and ‘other factors’, and (3) develop methods of accounting for disease-induced mortality associated with hatcheries, and not the FCRPS, in the estimate of D . We intend to repeat the proposed work for three consecutive years to establish reproducibility in the results. The objectives of the proposed study are subdivided into five primary tasks:

1. Characterize the impact of barging conditions on disease-induced direct and latent mortality;
2. Assess the spatial and temporal distribution of pathogens and contaminants in outmigrants of hatchery-origin;
3. Estimate the incidence of latent mortality associated with infectious diseases relative to other factors (e.g., smoltification timing, temperature, etc) in salmon with barged and in-river life-history strategies;
4. Examine the relevance of immunological and stress-associated gene expression on latent mortality; and
5. Compare SAR rates for in-river and barged fish based on modified survival estimates of smolts and adults.

Methodology

Task 1.0: Characterize the impact of barging conditions on direct and disease-induced latent mortality. The CSS has provided estimates for direct mortality associated with the FCRPS (i.e. with V_c and V_t representing V_{FCRPS} for the in-river and transported smolts, respectively). However, the V_t estimate only accounts for the survival of the smolts to the site of transport and ignores any direct mortalities that may occur in the project raceways prior to barge loading or on the barge itself (assumes a fixed 98% survival (Berggren et al., 2003)). Barging conditions, such as loading density and water volume exchange rates in the barge hold, may also contribute to latent mortality in barged fish. For example, fish are held in the barge at a maximum loading density of 0.5 lb fish/gal water with a 10-minute volume exchange rate (Dave Hurson, personal comm.). The extent of disease transmission with this density or volume exchange rate is currently unknown.

In the proposed study, we are specifically interested in quantifying the extent of disease-induced direct and latent mortality associated with barging operations. Earlier studies have demonstrated that *Renibacterium salmoninarum*, the causative agent of bacterial kidney disease, can be transmitted after a 48-hr exposure, suggesting that, during the length of time it takes the barge to arrive at Bonneville Dam, transmission of pathogens may occur within the barge hold (Elliot and Pascho, 1994). Maule et al. (1996) suggested that decreasing loading densities in hatchery raceways and ponds improves the immune response in juvenile salmon potentially enhancing their ability to respond to pathogens. Similar improvements in transported fish may be observed by modifying current barging conditions.

Task 1.1: Collect information on standard operating procedures associated with barging. To effectively design laboratory experiments that simulate barging operations and estimate associated disease-induced direct and latent mortality, a number of operational control variables associated with raceways and barging must be identified (Table 2).

Raceway	Barge
Raceway fish densities	Barge fish densities
Raceway daily mortalities	Barge mortalities
Raceway flow rate	Barge volume exchanges
Raceway water quality	Barge aeration
	Barge water quality

All (or some portion) of these parameters may already be recorded on a daily basis, but to our knowledge, they have not been incorporated into estimates of direct and latent mortality. The loading density and exchange rate data will serve as the basis for selecting specific levels (herein referred to as factors) of the experiments described below. To complete this task, appropriate USACE points of contact (POC) will be consulted regarding data that is routinely compiled. For elements in Table 2 that are not routinely monitored by USACE (or other agencies), field data will be collected at Lower Granite, Little Goose, and Lower Monument Dams on a periodic basis between April – June. Monitoring events will coincide with site visits associated with Tasks 1.3, 2, and 3, discussed below. When reporting and using the information collected, an emphasis will be placed not only on the actual operating conditions of a given day, but also on the range of conditions that occur throughout the transport season.

Task 1.2: Assess the impact of loading density and water volume exchange rates on disease dynamics using a factorial design with laboratory-raised spring Chinook salmon. Various fish densities and water volume exchange rates will be examined in the laboratory to determine the effect of these parameters on both disease transmission and immune function, and ultimately their contribution to direct/latent mortality. A factorial design will be used to determine the number of fish densities and exchange rates to use, as well as the number of tank replicates required for appropriate statistical error rates (e.g., α and β). Snake River spring/summer Chinook stocks will be raised from the egg stage at the Hatfield Marine Science Center and certified disease free prior to use. Fish will be exposed to a freshwater pathogen through one or more infected fish (Ogut et al. 2004) to determine the effect of various fish densities on disease transmission and immune function. Mortality will be assessed directly throughout the duration of the experiment. Morbidity will be assessed by examining the gills, skin mucus, and kidneys for the pathogen (Arkoosh et al. 2005). Immune function will be assessed in terms of the activities of complement, lysozyme, and toll-like receptors (TLR) in response to the pathogen. The TLR signaling pathway is a critical pathway against microorganisms that activates both innate and adaptive immunity (Iwasaki and Medzhitov, 2004). These receptors recognize highly conserved sequences of microbial and viral pathogens known as pathogen associated molecular patterns. Fourteen TLRs have been described in teleosts (Bilodeau et al. 2006). We will collect liver, kidney, spleen, and stomach over time. Real-time PCR will be performed on these tissues to determine the expression of TLRs (Bilodeau et al. 2006). Complement plays critical roles in phagocytosis, respiratory burst, chemotaxis cell lysis (Boshra et al. 2006) and initiation of the

adaptive immune response. Crowding stress has been demonstrated to have immunosuppressive effects upon complement activity in fancy carp (*Cyprinus carpio* L.; Yin et al. 1995) and in gilthead seabream (*Sparus aurata*; Oruno et al. 2001). Whole blood will be collected for this analysis and an ELISA-based approach will be used for the evaluation of the three pathways of complement activation, classical, alternative, and MBL (Seelen et al. 2005). Lysozyme helps protect fish against pathogens (Bilodeau et al. 2006) and plays a critical role in innate immunity against gram-negative bacteria by stimulating phagocytosis of the pathogen (Grinde 1989). Whole blood will be collected over time for this analysis and lysozyme activity determined with an EnzChek Lysozyme assay kit (Molecular Probes, Eugene, Or.).

Task 1.3: Assess the impact of loading density and water volume exchange rates on disease dynamics using a factorial design with hatchery-raised spring Chinook salmon collected from the field. Approximately 2500 PIT-tagged spring/summer Chinook salmon from both Dworshak and Rapid River Hatcheries will be collected with the sort-by-code system at Lower Granite Dam (total of 5000 fish) and transported back to the FDL at HMSC. The transmission of a range of indigenous diseases will be assessed (e.g., Warsen et al., 2004) as a function of time under a limited subset of loading densities and volume exchange rates (refined in Task 1.2). In addition, immune function will be characterized using techniques outlined in Task 1.2. Pending findings from the laboratory studies, the impact of specific loading densities and exchange rates can be evaluated in the field in either the barge or loading raceways in Year 3.

Task 2.0: Assess the spatial and temporal distribution of pathogens and contaminants in outmigrants of hatchery origin. Our research suggests that stocks from various hatcheries may be entering the river system or estuary with varying degrees of fitness. We have demonstrated that barged fish from the Dworshak National Fish Hatchery were more susceptible to disease than fish from the Rapid River Hatchery. Also, pathology reports from 60 fish collected at Bonneville Dam in our AFEP 2006 study indicated that fish from the Dworshak National Fish Hatchery had a greater prevalence of a very important salmonid pathogen, Infectious Hematopoietic Necrosis Virus (IHNV), than fish from Rapid River Hatchery. Previous researchers have demonstrated that fish from Dworshak National Fish Hatchery are smaller with less lipid and protein reserves than those from the Rapid River Hatchery (Congleton et al. 2000). If hatcheries produce fish that are not fit and less likely to survive once released from the barges below Bonneville Dam, the mortalities will be viewed as latent even though it is not necessarily a reflection of the FCRPS. By contrast, the unfit hatchery fish that remain in-river may die prior to Bonneville Dam, due to differences in transport time, hence included in the estimate of direct mortality associated with FCRPS. The source, incidence, and location (during or after the FCRPS) of disease mortality, may significantly affect estimates of SARs, *D*, and latent mortality.

Task 2.1: Monitor pathogen prevalence and disease incidence in the barge holds and hydropower bypass facilities along the Snake – Columbia River migration corridor. To determine the potential contribution of pathogens to direct and latent mortality, and their ultimate influence upon the *D*-value, temporal and spatial pathogen surveys will be performed on PIT-tagged juvenile spring Chinook salmon from both Dworshak and Rapid River Hatcheries. Note that this is not an extensive system wide survey that would likely fall under the purview of BPA, but rather a focused survey specific to providing a basis for assessing barging operations. Sixty fish will be collected from each hatchery as well as from Lower Granite, McNary and Bonneville

Dams three times throughout the migration period. In addition, barged fish will be collected upon arrival at Bonneville Dam at times concurrent with the collection of fish from the other locations. Sixty animals from each hatchery will be tested at each time period and at each location to ensure that we will detect at least one infected animal in a population with 95% confidence (Thoesen et al. 1994). A 16S ribosomal DNA PCR and DNA microarray will be used to simultaneously detect and discriminate between a number of bacterial and viral fish pathogens (Warsen et al., 2004). The following is a minimum list of fish pathogens that will be probed for on the array: *Aeromonas hydrophila*, *A. salmonicida*, *Edwardsiella ictaluri*, *Flavobacterium branchiophilum*, *F. columnare*, *F. psychrophilum*, *Flexibacterium maritimus*, *Listonella anguillarum*, *Mycobacterium chelonae*, *M. fortuitum*, *M. marinum*, *Renibacterium salmoninarum*, *Streptococcus iniae*, *Vagococcus salmoniarum*, Infectious pancreatic necrosis virus, Infectious hematopoietic necrosis virus, Viral hemorrhagic septicemia virus, and *Myxobolus cerebralis*. High volume water samples will also be collected from the barge holds during transport at Lower Granite, McNary, and Bonneville and compared to water samples collected from the river at these locations to help determine the source of these pathogens. Additional pathogens and analytical techniques will be explored as necessary.

Task 2.2: Monitor contaminant body burden along the Snake – Columbia River migration corridor. Since contaminants can influence salmon survival and disease susceptibility (Loge et al. 2005; Arkoosh et al., 1991, 1994, 1998, 2002) we will collect two composites of 10 whole bodies each during each sampling event to estimate the concentrations of PBDE, PCB congeners, PAHs, DDTs (Krahn et al. 1988; Sloan et al., 2004), and biliary fluorescent aromatic hydrocarbons (FAC; Krahn et al. 1986). The whole body, stomach contents and bile samples will be collected and stored at -80°C until chemical analysis.

Task 3.0: Estimate the incidence of latent mortality associated with infectious diseases relative to other factors in salmon with barged and in-river life-history strategies. To our knowledge, the survival of barged and in-river smolts in the lower Columbia River and estuary has not been assessed in terms of outmigration life-history strategy, or further subdivided based on the prevalence of infectious diseases relative to other factors. Due to the difference in travel times of barged (e.g., 2 days) and in-river outmigrants (e.g., 2 to 7 weeks) between Lower Granite and Bonneville Dams, each group of fish is likely to (a) encounter different estuary and early ocean conditions (Williams et al. 2005) and (b) be at different physiological states upon entry into the estuary and/or ocean. The extent of latent mortality in cohorts of salmon with differing outmigration life-history strategies will be quantified using in-situ net-pens located in two spatially distinct areas within the Columbia estuary. Data collected in this manner provides (a) a logical extension of past studies wherein salmon were held in the laboratory to estimate latent mortality, and (b) a basis for the design of future acoustical tag studies with the intent of estimating latent mortality inclusive of predation.

Task 3.1: Placement and design of in-situ net-pens. Due to the heterogeneity of chemical contaminants in the estuary, in-situ net-pens will be installed at two locations in the estuary; one at a site known to have significant chemical contamination and one with minimal levels of contamination. Proposed locations are (Cathlamet Bay/Russian Island and Young's Bay). Additional site selection criteria for the in-situ net-pens include adequate water flow and quality,

tidal fluctuation, accessibility, and lack of conflicting uses. All necessary permits will be obtained prior to installing the temporary net-pens in the estuary.

Each in situ net-pen location will consist of four floating net pen docks, 3 meters wide by 9 meters long. A total of ten - 1m³ nylon mesh net pens will be attached to each floating dock. A 5 mm mesh size will be used for net pens to ensure adequate water circulation through the pens and to allow macro invertebrate and zooplankton food sources to enter the pens. The floating docks will have a 1 meter wide wooden walkway down the middle with net pens attached on either side. The net pens will be placed so that there is a 1 meter gap between each pen. This will ensure adequate circulation around each pen and minimize interaction between pens. Arranging the four floating docks in a square configuration will ensure that each treatment group will be exposed to varying water circulation patterns. A total of forty 1m³ net-pens will be used at each of the two locations.

Task 3.2: Collection and distribution of hatchery-reared experimental fish. The net-pens will be loaded at a maximum density of 8 kg/m³ (Linley 2001). A total of 100 juvenile salmon (average weight 30g) will be added to each net pen. At each of the two locations in the estuary, (a) 18 net-pens will be stocked with barged fish, (b) 18 pens will be stocked with in-river fish, and (c) the remaining four pens will be stocked with PIT-tagged hatchery fish collected at Lower Granite Dam that have experienced one bypass. The barge and in-river cohorts of salmon will be further subdivided into early, middle, and late estuary arrival groups with six net-pens used for each subdivision. For salmon in each net-pen, survival, health status, immune function, and pathogen prevalence (see above for methods) will be monitored for 28 days after arrival. Of the four control net-pens per site stocked with fish from Lower Granite, one will be stocked and monitored for the entire experimental period, and the remaining three will be stock with fish collected during early, middle, and late periods during outmigration. A total of 600 fish will be stocked for each treatment group (e.g., barge-early, mid, late) for a total of 4000 per site.

Task 3.3: Monitoring environmental conditions. At each of the two net-pen sites in the estuary, current, temperature, salinity, and water depth will be monitored continuously. Additional water quality measurements will be monitored on a daily basis, including, but not limited to, dissolved oxygen, pH, turbidity, nitrate/nitrite, ammonia, and CO₂. Sediment core samples will be collected from under the net-pens for chemical contaminant analysis once over the study period. The concentration of chemical contaminants in food and prey sources located near the net-pens will be quantified three times over the study period. And finally, water samples will be collected for contaminant and pathogen analysis three times over the study period. The measures of physical, chemical, and biological characteristics at each site will be used as covariates in addition to estuary arrival timing, life-history strategy, and pathogen prevalence data, in the analysis of statistical relationships (e.g., using one-way ANOVA or other comparable techniques) with latent mortality.

Task 3.4 Care and sampling of hatchery-reared net-pen salmon. Although the net-pens are designed to allow prey items to enter, the fish may need to be fed a maintenance ration once per day to ensure adequate nutrition (this concept will be explored in much more detail). Survival in the net pens will be monitored and mortalities removed on a daily basis. A full pathology analysis will be completed on each dead fish to determine if the cause of death is associated with

disease or ‘other factors.’ Tissue samples for pathogen prevalence, immune function, chemical body burden, and condition indices will be collected and analyzed three times over the 28-day study period.

Task 4.0: Examine the relevance of immunological and stress-associated gene expression on latent mortality. The intention of this task is to identify expression level biomarkers (e.g., proteins) that can be used to non-destructively assess the health of outmigrants and the corresponding population-level incidence of latent mortality. Potential biomarkers will be identified using gene expression profile data obtained with a DNA microarray with liver and kidney samples collected from laboratory-reared (at HMSC) Snake River stock and field-collected hatchery-reared Snake River spring Chinook salmon with different outmigrant life-histories, all challenged with the marine pathogen *Listonella anguillarum*. The protein biomarkers will subsequently be measured, along with array data, in fish exposed to natural pathogens in the river system, and the results will be used to predict the incidence of disease-induced latent mortality; model predictions will be confirmed with actual mortality data collected from holding the fish either in the laboratory or in-situ net pens located in the estuary.

Preliminary data collected in FY06 study. Although not part of our ongoing AFEP FY06 study, a limited number of liver samples collected from fish on day 0, 1, 3, and 7 post-challenge were analyzed on a DNA microarray containing roughly 1400 immunologically and toxicologically relevant genes (Gerwick et al, 2000). The microarray study was supported with funding internal to NOAA and was conducted on a limited basis to illustrate (1) that there are in fact discernable differences between the two life-history strategies on a DNA microarray, (2) the differences are dependent on whether or not the animal has been exposed to a pathogen, and (3) methods of statistically combining array data to identify a set of potential candidate biomarkers. To date, we have completed the analysis of gene expression in the liver on day 2 of Rapid River Hatchery fish exposed and not-exposed to *L. anguillarum*. Without pathogen challenge, roughly 4 genes were differentially expressed between barged and in-river fish (Table 3). A cursory review of the gene annotations would suggest regulatory differences between the two life-history strategies associated with nutritional status. Under pathogen challenge, roughly 9 genes were differentially expressed between barged and in-river fish (Table 4). A cursory review of gene annotation would suggest regulatory differences associated with nutritional status, as well as osmotic regulation and the innate immune system. Importantly, even at this early stage of analyses, we can say with some confidence that (1) there are in fact discernable differences between the two life-history strategies on a DNA microarray,

Table 3. Gene expression profile in liver of barged and in-river Spring Chinook not exposed to *Listonella anguillarum*

Array ID name	Accession number	Gene function	Fold difference ^a
OSUrbt2_686_MCH1	TC12400	Gene for melanin concentrating hormone. Plays a role in skin pigmentation by antagonizing the action of melanotropin alpha. Induces melanin concentration within melanophores. May participate in the control of the hypothalamo-pituitary adrenal gland axis by inhibiting the release of ACTH.	-3.08
GSR_Omy_C8908	Not available	Glutathione reductase (GSR)	-2.51
OSUrbt2_20_ACAT1	TC8908	Acetyl-CoA acetyltransferase mitochondrial precursor	1.24
OSUrbt2_909_P11	TC8556	Serine protease inhibitor, weakly similar to Thyroxine-binding globulin precursor	0.31

a Fold difference in the gene expression in barged fish relative to in-river fish. Value calculated as the Log of the ratio of the quantity of a particular gene expressed in the barged group divided by the quantity of the same gene expressed in the in-river group. As such, a negative fold difference means that less of that particular gene was expressed in the barged group than in the in-river group; a positive fold difference reflects a larger quantity of a particular gene expressed in the barged group relative to the in-river group. The magnitude of the ‘fold difference’ represents the magnitude in the difference in gene expression.

and (2) the differences are dependent on whether or not the animal has been exposed to a pathogen. We do not have sufficient data with our salmon array to illustrate methods of analyzing temporal expression data to identify potential biomarkers, so we illustrate the approach below (Tasks 4.2.6 and 4.4) with data collected by a collaborating group using an oyster (*Crassostrea gigas*) DNA microarray. Overall, in

our mind the preliminary results hold promise in identifying a set of protein biomarkers that would permit the non-destructive assessment of the health of outmigrant salmon, and the possible linkage between these biomarkers and population-level projections of latent mortality. The tasks outlined below build upon our preliminary data towards these longer term research goals, and focus primarily on (i) assessing the variability and reproducibility in expression profiles within a given stock and between stocks, (ii) identification of protein biomarkers, (iii) linking protein biomarkers to incidence of disease-induced latent mortality, and (iv) field validation. Note that one of the novel aspects in our approach is that we use controlled laboratory studies not only to develop candidate biomarkers for field validation, but also to develop mathematical models that link the expression of the biomarker to disease-induced mortality. The mathematical models then serve as a method of translating the field data comprising measures of the biomarker in an individual fish to population-level estimates of stock numbers.

Table 4. Gene expression profile in liver of barged and in-river Spring Chinook exposed to *L. anguillarum* after 2 days post-challenge.

Array ID name	Accession number	Gene function	Fold difference
DRTP1_Omy_AF281355	AF281355	Differentially regulated trout protein 1. Unknown function.	-2.38
OSUrbt2_124_C1qASP	TC39588	C1q-like adipose specific protein, related to carbohydrate metabolism.	-4.01
DRTP1_Omy_AF281355	AF281355	Differentially regulated trout protein 1. Unknown function.	-2.33
OSUrbt2_401_FBG	TC8727	Fibrinogen gamma-A chain precursor. Fibrinogen is a highly soluble, elongated protein complex found in blood plasma and involved in clot formation.	0.27
OSUrbt2_645_LC2	TC8718	Chondrogenesis associated lipocalin this gene is transcribed during pathological acute phase response.	0.69
OSUrbt2_27_AFP	TC8237	Serum albumin 1 precursor. Serum albumin, the main protein of plasma, has a good binding capacity for water, Ca(2+), Na(+), K(+), fatty acids, hormones and bilirubin. Its main function is the regulation of the colloidal osmotic pressure of blood.	-0.22
OSUrbt2_153_CATHEPSINK	TC8871	Cathepsin K precursor. Proteolysis and peptidolysis.	0.98
Cblnl_Omy_AF192969	AF192969	Precerebellin-like protein is an acute phase protein.	-0.55
OSUrbt2_117_BTD2	TC16810	Biotinidase precursor. Catalytic release of biotin from biocytin, the product of biotin-dependent carboxylases degradation.	-2.22

Task 4.1 Disease challenge of hatchery-reared barged and in-river outmigrants. PIT-tagged Dworshak and Rapid River fish will be collected from the FCRPS and transported to the FDL at HMSC in Newport OR. Experimental fish will include hatchery-reared juveniles collected from the bypass at Bonneville Dam (in-river) and juveniles transported in net-pens within barge holds and off-loaded at Bonneville Dam. A total of 300 fish will be collected per hatchery at each of the two locations. A subset of fish from each group will be exposed to an LC₅₀ concentration of *L. anguillarum*, with the remaining fish within the group used as a control (no pathogen exposure). On days 1, 3 and 7 post-challenge, a total of 30 fish exposed to the pathogen will be lethally harvested. In addition, 30 control fish will be lethally harvested. Head kidneys and livers will be collected and stored in liquid nitrogen until RNA extraction. Control and experimental samples will be collected at the same time during the day to avoid circadian effects on gene transcription. In Year 1 of the proposed study, we will focus on identifying a unique expression profile and the development of protein assays to quantify gene products. In Years 2 and 3, we will assess gene expression profiles, as well as protein profiles of candidate biomarkers. Collectively, the results will be used to assess the variability and reproducibility in expression profiles, and in the development of appropriate mathematical equations to link the expression of biomarkers to disease-induced latent mortality.

Task 4.2 Disease challenge of laboratory-reared fish. As part of the disease challenge taking place in *Task 1.2*, on days 1, 3 and 7 post-challenge, a total of 10 fish exposed to the pathogen will be lethally harvested. In addition, 10 control fish will be lethally harvested. Head kidneys and livers will be collected and stored in liquid nitrogen until RNA extraction. Control and experimental samples will be collected at the same time during the day to avoid circadian effects on gene transcription. Data collected under *Task 4.2* will undergo similar analyses as *Task 4.1*.

Task 4.3 Microarray methods and data analyses. Oligonucleotide array (OSUrbt ver. 2.0) containing 1,672 features representing approximately 1,400 genes was created at Oregon State University as described in (Tilton et al, 2005). The experimental microarray work involves: (1) RNA extraction; (2) reverse transcription of RNA to cDNA; (3) primary hybridization to microarray; (4) secondary hybridization to microarray; (5) microarray scanning; and finally (6) data analysis. Each element of this work is briefly described below.

Task 4.3.1 RNA extraction. RNA will be extracted individually by adding 1ml of trizol solution to the liver samples (0.5-1g), pestles will be treated with RNase away (Molecular Bio Products) and DEPC-treated water (Sigma-Aldrich) will be used to homogenize the tissue. Total RNA will be dissolved in 50-150ul of DEPC-treated water, concentrations will be measured spectrophotometrically at 260nm by adding 2 ul in a nanodrop ND-1000 spectrophotometer. Measurements will be calculated in nanodrop 3.1.0 software. The quality of the RNA will be determined with a 2100 Bioanalyzer (Agilent) and all samples that meet the quality criteria with RNA integrity number between 9 and 10 indicative of clean intact RNA will be selected. RNA will be extracted from 4 individual fish per treatment exposed to bacterium and 4 individual fish per treatment not exposed to *L. anguillarum*. A reference RNA will be made by combining kidneys livers pooled from the different treatments and control. The pooled RNA will be aliquoted and stored at -80°C until use.

Task 4.3.2 Reverse transcription to cDNA. Total RNA 2.5ug of sample and reference will be used for each reaction (Gerwick et al, unpublished) and reverse transcribed using 200 units of superscript III® (Invitrogen) that contains specific primers with tails that complement secondary binding with either CY3 or CY5 label fluorophore (Genisphere 900™). To each reaction 1µl of alien, 1ul of primer and RNase free water will be added. Aliens oligos® (Stratagene, La Joya, CA) will be used as positive control for reverse transcription quality. A master mix with standard amounts as recommended by Genisphere 900™ of 5X superscript III first strand buffer, 0.1M DTT, Superase-in Rnase inhibitor, dNTP mix and superscript III enzyme (Invitrogen) will be prepared in one stock and 4.5µl and will be added in to the RNA reverse transcription master mix. cDNA will be stored at -80°C until use.

Task 4.3.3 Primary hybridization to microarray. For each slide the hybridization consists of 6.5 µl of sample cDNA that represents individual fish that will be labeled with Cy5 dye and mix with 6.5ul of reference cDNA labeled Cy3 and 17ml of 2x formamide buffer for a total volume of 30 µl. Dye swabs for each hybridization will be performed with the reference and the sample labeled inversely. We will have a total of 16 slides per time-point. Genisphere 900™ protocol will be followed with the same modifications made by (Bravo et al, 2005). Hybridization temperature will be 49°C for 24h.

Task 4.3.4 Secondary hybridization to microarray. All steps will be performed under low intensity light. Tagged Cy3 and Cy5 dyes from Genisphere 900™ will be mixed with 2X formamide buffer and anti-fade reagent (Genisphere 900™). A second hybridization will be done in the dark with this mixture for 4h at 49°C. Slides will be washed as described in (Bravo et al, 2005). Slides will be dried by centrifugation (1000 X g for 3 min) and dip in dye saver™ 2 anti-fade coating (Genisphere™) for 2 sec followed by another centrifugation (1000 X g for 3 min). Slides will be immediately scanned.

Task 4.3.5 Microarray scanning. Slides images will be acquired with scanner Axon 4200A (Molecular devices, Union City, CA) at an excitation of 532nm for Cy3 and 635nm for Cy5. The photomultiplier tube (PMT) settings and percent power of the laser for each fluor will be set base on intensity of spike internal aliens controls so that the alien control shows an overall ratio of medians close to one. (Cy5 to Cy3 signal of the entire array) should be close to one. This is a first manual correction for differences in intensity between arrays due to technical errors, and it can be seen as “normalization” between arrays step, aiming at making the arrays comparable with each other. The scan array files will be examined individually for spots that are saturated failed to yield good signals or are artifacts and will be flagged as “bad spots”. The GenePix Pro 6.0 files will be imported into S-plus array analyzer program to be analyzed.

Task 4.3.6 Data analysis. The intensity files will be analyzed for differential gene expression with S+ArrayAnalyzer 2.0 software, in S+ environment (S+®6.2 for Windows Professional Edition, Lucent Technologies, Inc.). The context of the analysis is a pair-wise comparison (unpaired t-test), with dye swap and reference design: every array contains the reference (pool of samples from all treatments at all days, liver and kidney, which thus carries no biological meaning) on one channel, and each biological replicate will be represented by two arrays (one with sample on green channel and reference on red, and vice versa for the dye-swapped array). To complete the analysis, quality diagnostics, filtering, and normalization operations will be performed to assess gene expression. Signal intensity data will be captured, and background-subtracted. Variance-versus-mean dependence of signal intensity data will be assessed by and corrected using a robust generalized log transformation, variance stabilized normalization (Figure 6) (Huber et al. 2002).

Genes that are differentially expressed will be

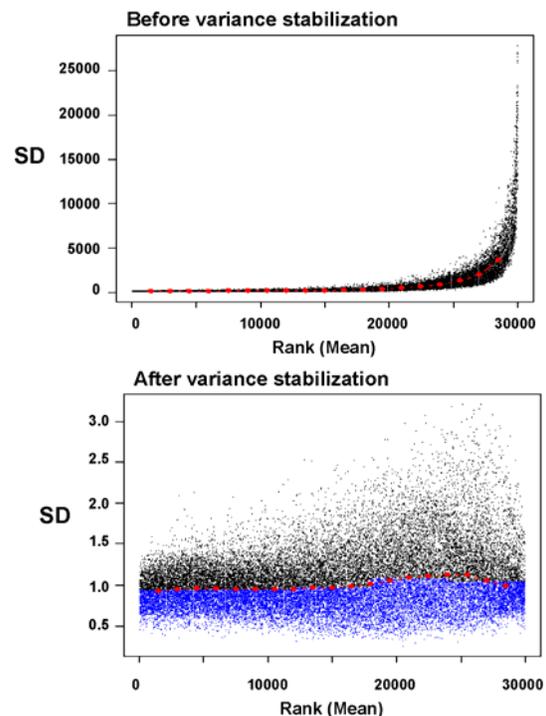


Figure 6. Variance stabilization after Normalization. Variance-versus-mean dependence is apparent in the top chart where the standard deviation (SD) of raw intensity values (after background subtraction) increase dramatically as the ranked mean intensity increases. The bottom chart illustrates the stabilization of the variance after VSN transformation. More details can be found in (Huber et al., 2002).

categorized based on function using TIGR database (www.tigr.org), gene ontology (www.geneontology.org) and pub med (www.ncbi.nlm.nih.gov) databases for descriptions.

Task 4.4 Biomarker identification. After the gene expression profile in the liver and kidneys of sample fish is determined, the physical relevance of those genes will be compared to (1) cluster analysis of functional proteins, and (2) the physiological parameters measured in *Task 1.2*. Genes will be clustered using a smoothing spline clustering method (Ma et al. 2006), which assigns genes into clusters based upon similar temporal profiles, and predicts their behavior in spite of experimental and biological noise. This program is run in the R environment, and provides a visual representation of each clusters function using a mean curve and confidence interval (Figure 7).

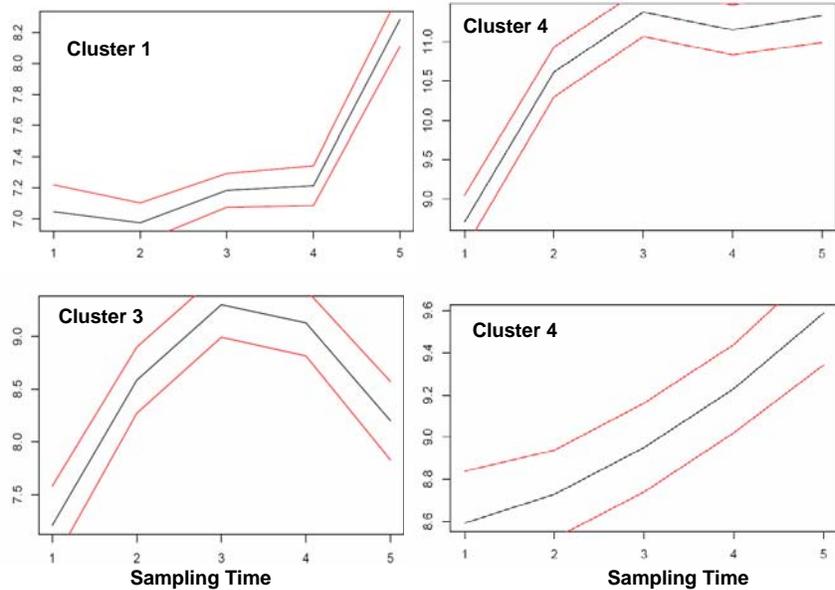


Figure 7. Estimated mean expression curves (solid lines) and 95% confidence bands (red bands) for four of 7 clusters discovered by SSC in heat-shocked *Crassostrea gigas* (Lang, unpublished) time course microarray data.

Based on this comparison, the criteria for selecting a putative functional biomarker will be: (1) temporal expression profile, which will indicate whether the gene is inducible, and is thus relevant to the applied stimuli; (2) biological function of the gene according to the Gene Ontology Database; and (3) its expression relative to other genes known to be stress-induced.

Task 4.5 Ground truth the biomarker. PIT-tagged outmigrants will be collected during *Tasks 1.3, 2.1, 3.4*. The levels of the biomarker(s) will be obtained and compared to: (1) the gene expression profile in the livers and kidneys, based on the completion of microarray analysis discussed in *Task 4.3*; (2) cluster analysis of functional proteins; and (3) the physiological parameters measured in the respective tasks. The mathematical equations developed as part of *Tasks 4.1* and *4.2* will then be used to predict the incidence of disease-induced latent mortality based on biomarker(s) expression, and the results will be independently validated with mortality data collected experimentally.

Task 5.0: Estimate smolt-to-adult rates and latent mortality for in-river and barged fish based on modified survival estimates of smolts and adults. As presented in Figure 1 and Equation 4, several factors contribute to salmon mortality during river and estuary migration and ocean residence. The intent of the laboratory and field work discussed above, in part, is to generate estimates of: (i) latent and direct disease-induced mortality associated with barging; (ii)

delayed disease-induced mortality associated with hatcheries; and (iii) latent mortality for in-river and barged juveniles. Additional estimates of the impacts of predation, nutrition, and ocean conditions on survival may be derived from a thorough review of existing scientific literature. In this task, a literature review will be completed to collect these estimates of mortality and identify any gaps in the published research. These mortality estimates, as well as published jack rates, will be combined with the results found in Tasks 1–4 of this study, to estimate smolt survival (V_s) in Eq. 4, and subsequently SAR values as expressed in Eq. 3. A detailed breakdown of the various factors influencing SARs will permit a comparative analysis of the relative importance of each factor, as well as a quantitative estimate of the relative impact of a particular remediation strategy on SAR values.

In addition to the modification of SAR rates, estimates of delayed disease-induced mortality associated with hatcheries and latent mortality for in-river and barged juvenile salmon will be used in an improved calculation of D , the delayed differential mortality of transported and in-river juveniles:

$$D = \frac{SAR_2(T_0)}{(1 - M_{h,t}) \cdot V_t} \bigg/ \frac{SAR(C_0)}{(1 - M_{h,c}) \cdot V_c} \quad 5.$$

Specifically, hatchery contributions to delayed disease-induced mortality ($M_{h,t}$ and $M_{h,c}$ for transported and in-river smolts, respectively) will be included in the denominator of Eq. 2, as shown in Eq. 5. Our intent is to extend the project over a sufficient number of years (we have proposed 3) to obtain a solid estimate of $M_{h,t}$ and $M_{h,c}$ such that they can be used as fixed values in the calculation of D , similar in concept to the approximately constant value of $V_c = 51$ to 54%. If the values of $M_{h,t}$ and $M_{h,c}$ are found to vary widely from year to year, the range of methodologies and technologies employed in this study should elucidate a low cost method of explicitly assessing the impact of hatchery-induced latent mortality on the annual value of D .

Fish Requirements. The fish requirements will vary over the course of the study as specific tasks are completed, repeated, or modified based on knowledge gained from the previous year. As described above, a total of 70 hatchery salmon, 2160 barged salmon, 685 river-run salmon at Lower Granite Dam, 210 river-run salmon at McNary Dam, and 2160 river-run salmon at Bonneville Dam will be collected for each of the respective two hatcheries (a total of 5,285 salmon per hatchery per year). In addition, in the third year, 2,500 salmon destined for transport at Lower Granite Dam will be collected for each of the respective two hatcheries (a total of 7,785 salmon per hatchery for the final year). The above fish numbers do not include the laboratory-raised fish discussed in Task 1.2.

Tagging Requirements. Assuming a 50% incidence of mortality prior to Lower Granite Dam, an additional 50% mortality between Lower Granite and Bonneville Dams, and a 25% collection efficiency in the PIT-tag separation-by-code system at Lower Granite, McNary and Bonneville, we anticipate roughly 60,000 salmon will have to be PIT-tagged at each hatchery in years 1 and 2, and 80,000 salmon in year 3. We recognize this approach is conservative, and the numbers can be modified with consultation with USACE.

Schedule. Laboratory activities will commence January 1, 2007 and extend through December 31, 2009. Tasks involving field activities will encompass the outmigration period, and likely extend from April through August of a given year, depending on the specific activity. In acknowledging that USACE may not be interested in all the tasks outlined in this proposal, we have designed each task to be autonomous. We also recognize that there is synergy and associated cost savings in performing all the tasks as a collective unit.

Table 4. Time-Line.

Task	2007				2008				2009			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
1.0 Barging conditions												
1.1 Barging SOPs												
1.2 Lab-reared disease transmission												
1.3 Field fish, disease transmission												
2.0 Pathogen prevalence												
2.1 Field pathogen survey												
2.2 Contaminant body burden												
3.0 Estuary survival												
3.1 Placement of net-pens												
3.2 Collection and distribution of fish												
3.3 Monitoring environmental conditions												
3.4 Care & sampling of experimental fish												
4.0 Microarray												
4.1 Collection and challenge of in-river and barged fish												
4.2 Challenge of lab-reared fish												
4.3 Microarray analyses												
4.4 Biomarker analyses												
4.5 Ground truth biomarker use												
5.0 Revised SAR calculation and data analyses												

Facilities and Equipment

The HMSC has research facilities that support investigations in marine fisheries, aquaculture, water quality, marine biology, botany, microbiology, zoology and oceanography. The Center is well equipped with appropriate specialized instruments (e.g., Zeiss phase plus fluorescence Axioskope, Zeiss epifluorescence and phase Axiovert, laminar flow hood and cytospin, centrifuges, PCR equipment; sterile tissue culture work, fish necropsy, and sequencing facilities) constant temperature rooms, and computer equipment. Wet laboratories are provided with running fresh and sea water. The Fish Disease Laboratory at the Hatfield Marine Science Center

(HMSC) has the capacity of pumping 250 gpm of pathogen free sea water and 75 gpm of dechlorinated pathogen-free freshwater. Seawater, at 33ppt salt, is pumped from Yaquina Bay, sand filtered, UV treated, and chilled to 10°C prior to delivery to the tanks. Fresh water (obtained from the city of Newport) is dechlorinated and chilled to 10°C prior to delivery into the tanks. There are 42-3' circular tanks and 4-6' circular tanks in the disease laboratory. The effluent is treated prior to discharge back into Yaquina Bay. Meeting and lecture rooms are located in close proximity to the laboratories. Administrative support for this project is available through the Environmental Conservation Division within NOAA Fisheries.

During the first year we will expand and optimize the capability of our state of the art fish disease laboratory to hold more fish from the Columbia River Estuary. ODF&W requires us to treat the effluent of any fish from the Columbia River Estuary for the parasitic protozoan, *Myxobolus cerebralis*, the causative agent of whirling disease. Currently the effluent treatment system limits our experiments with Columbia River fish. We will also purchase a 'live-haul' tank to minimize the stress to fish while they are being transported. It is critical that fish transport is performed in such a way as not to influence delayed mortality. Transport tanks are equipped with insulation, have flow meters, oxygen regulators, aerators, diffusers and dump chutes.

Impacts to Ongoing or Proposed Research or Other Projects

The proposed study will require access to: (a) the fish collection facilities at Lower Granite, McNary, and Bonneville Dams, (b) CSS-tagged fish to help reduce the numbers of fish tagged as part of this study, (c) coordination of fish collection with PTAGIS, and (d) access to barges and coordination with barge operations. No other impact to proposed or ongoing research/projects is anticipated. All permits necessary for the proposed study will be obtained by NOAA Fisheries. Any ESA-listed salmon captured at Bonneville will be handled in accordance with established protocols and permits. All by-catch will be released. It is not anticipated that these activities will result in additional impacts to ESA listed salmon.

Collaborative Arrangements and Subcontracts

The proposed study will be conducted in collaboration with the University of California at Davis, with contract personnel hired by NOAA through Frank Orth (or equivalent). Subcontracts will be issued to both organizations through existing federally negotiated contracts. We have been in contact with Christopher Peery at the University of Idaho regarding his energetics proposal that will be submitted this AFEP cycle, and we both feel there is substantial merit in working together in both the tagging and collection of fish, as well as coordinating selected physiological methods/measures and the analyses of data. If both projects are selected for funding, the PIs will discuss in detail how to integrate the two projects.

IV. LIST OF KEY PERSONNEL AND PROJECT DUTIES

Dr. Mary Arkoosh, Principal Investigator of the proposed study, is the team leader of Immunology and Disease in the Ecotoxicology Branch of the NWFSC's Environmental Conservation Division. She has authored over 30 scientific articles related to immune suppression, infectious disease, and disease challenge studies. She oversees the state-of-the-art Fish Disease Laboratory at the Hatfield Marine Science Center in Newport, OR. Dr. Arkoosh will provide oversight of all aspects of the proposed study, and will specifically focus on the disease challenge study.

Dr. Frank Loge, co-PI of the proposed study, is an Associate Professor in the Dept. of Civil and Environmental Engineering at the University of California Davis. He has worked for the past year on sabbatical leave with the NWFSC's Environmental Conservation Division developing quantitative techniques in risk assessment to characterize the incidence of delayed disease-induced mortality in outmigrant juvenile salmon in the Columbia River Basin associated with chemical and in-river stressors. Dr. Loge will assist in the experimental design of the disease challenge study, and will take the principal lead on the analysis of data and coordination of field work.

Dr. Joseph Dietrich, co-PI of the proposed study, has been a contract scientist in the Ecotoxicology Branch of the NWFSC's Environmental Conservation Division for two years. He is currently contracted through the University of California Davis as a post-doctoral scholar. As part of the Immunology and Disease team, he has (1) investigated the impacts of chemical and life-history stressors on latent mortality associated with disease, and (2) developed population lifecycle models of salmon in the Columbia River Basin to comparatively assess ocean climate conditions, fishing, and in-river stressors on population numbers. Dr. Dietrich will provide oversight and coordination of sample collection and data analyses.

Dr. Chris Bayne, co-PI of the proposed study, is a Professor Emeritus in the Department of Zoology at Oregon State University. He has authored over 100 scientific articles and chapters; served on an ad hoc committee to review the Cell Biology program at NSF; served on the editorial board of numerous journals, the most recent being *Developmental and Comparative Immunology* and *Journal of Parasitology*; and developed the DNA microarray that will be used in Task 4 of the proposed study. Dr. Bayne will serve as a technical advisor for all DNA microarray and proteomics work that will be performed as part of the proposed study.

Dr. Tracy Collier, co-PI of the proposed study, is currently director of the NWFSC's Environmental Conservation Division, which includes the Ecotoxicology Program. He has authored over 100 scientific articles and chapters, served as a guest editor for *Human and Ecological Risk Assessment* and *Marine Environmental Research*, and, in the past two years, organized two international symposia assessing contaminant fluxes and risk. Dr. Collier will provide oversight and coordination of sample collection and analyses of data, and report production.

V. TECHNOLOGY TRANSFER

Technology transfer will be in the form of written reports and oral presentations as required. Results will also be published in appropriate peer-reviewed scientific journals and presented at national and international conferences.

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