POLICY, MANAGEMENT, AND PRODUCTION STRATEGIES TO REDUCE THE RISKS OF HATCHERY-WILD INTERACTIONS IN ALASKA SALMON HATCHERIES

A Thesis

Presented to the Faculty of

Alaska Pacific University

In Partial Fulfillment of the Requirements

For the Degree of

Master of Science in Environmental Science

By

Sabrina Larsen

December 2015

December 2015

POLICY, MANAGEMENT, AND PRODUCTION STRATEGIES TO REDUCE THE RISKS OF HATCHERY-WILD INTERACTIONS IN ALASKAN SALMON HATCHERIES

by

Sabrina Larsen

THESIS

APPROVED: Thesis Committee

_____	_____
Chairperson Bradley Harris, Ph.D. Assistant Professor Alaska Pacific University	Date
_____	_____
Thesis Committee Suresh A. Sethi, Ph.D. Adjunct Assistant Professor Alaska Pacific University	Date
_____	_____
Thesis Committee Meagan Krupa, Ph.D. Research Professional University of Alaska Anchorage	Date

APPROVED:	_____	_____
	Tracy M. Stewart, Ph.D. Academic Dean	Date

ACKNOWLEDGEMENTS

I am extremely grateful to my advisor, Dr. Brad Harris, who has guided me through many challenges over the past three years. I want to thank Dr. Suresh Sethi for helping me through the struggle of sampling design, regression techniques, and for spending hours in the hatchery triploidizing fish. Finally, I want to thank Dr. Meagan Krupa for sharing her expertise on environmental policy and management.

The staff of the William Jack Hernandez Sport Fish Hatchery was instrumental to the completion of this project. In particular, I want to acknowledge Diane Loopstra for sharing her time and knowledge, and Andrea Tesch for allowing this project to become a reality.

I am grateful for the advice and assistance given by many biologists and technicians at the Alaska Department of Fish and Game, and by researchers at the University of Windsor. And thank you to John and Anne Heath from the Yellow Island Aquaculture Ltd. Facility for welcoming me into your research community and for offering guidance throughout this project.

I am greatly indebted to Alex Benecke, Anne Benolkin, Aileen Nimick, Sioned Sitkiewicz, Mariah Tengler, Kelley Voss, Sarah Webster, and Angel Wilkinson for volunteering their time in the hatchery.

Lastly, I appreciate the moral support given by my friends and family. Particularly from my father who encouraged me to enter graduate school but never saw the final product. Thank you all.

ABSTRACT

POLICY, MANAGEMENT, AND PRODUCTION STRATEGIES TO REDUCE THE RISKS OF HATCHERY-WILD INTERACTIONS IN ALASKAN SALMON HATCHERY PROGRAMS

Global hatchery production of all five species of Pacific salmon has increased in response to declining wild stocks and increasing pressures from sport and commercial fisheries. Modern hatchery regulations in Alaska identify the risks of hatchery production and state that hatcheries must contribute to common property fisheries while avoiding significant negative impacts to wild stocks. We reviewed the history of Alaskan hatchery policy and analyzed its ability to adequately address the adverse impacts of hatchery production. Given the variable nature of wild returns, and the stable level of hatchery production, there is cause for concern that the proportion of spawning hatchery origin fish in streams has exceeded the proposed maximum thresholds, potentially negatively impacting wild stocks. We also examined modern hatchery practices to mitigate for hatchery-wild genetic interactions by producing sterile, triploid Chinook salmon. Although optimal ranges of triploid induction parameters have been suggested, variability in triploidy success rates and survival through triploidy induction are typical outcomes, likely a strong reflection of maternal influences. The results of this study indicate that maternal effects do impact the survival rates of triploidized salmon, however we were unable to successfully assess triploid rates.

TABLE OF CONTENTS

LIST OF TABLES

Chapter 1

Chapter 2

LIST OF FIGURES

Policy Overview

Chapter 1

Chapter 2

GENERAL INTRODUCTION

Global farm and hatchery production of all five species of Pacific salmon has increased in response to declining wild stocks and increasing pressures from sport and commercial fisheries. Fish farms and hatcheries are both forms of aquaculture. However, a hatchery is a location where fish are spawned and reared until they are large enough to be transferred to a fish farm or released into the wild, and fish farms are locations in which fish are raised until consumption (e.g. pond culture and marine net pens) (NOAA 2015). Some species of Pacific salmon are commercially farmed in the U.S. (coho salmon) (NOAA 2015), however most Pacific salmon production, including Alaskan production, is limited to hatcheries.

Alaskan hatcheries released approximately 1.76 billion Pacific salmon for fishery enhancement, providing 34% of the statewide commercial harvest of salmon in 2014 (Vercessi 2015) and making these hatcheries some of the most prolific fish production facilities in the nation. The success in terms of production and management of Alaska's salmon hatchery program is often attributed to the statutes, regulations, and policies that protect wild stocks while also facilitating the production of cultured stocks (Vercessi 2013).

The overarching goal of Alaskan hatchery production is to supplement common property Pacific salmon fisheries, although other species of salmonids are produced. (In Alaska, a common property fishery is defined as "any fishery in which the general public is allowed to harvest fish" (5 AAC 40.990)). The Alaska Department of Fish and Game (ADF&G) has maintained this goal since production began in the 1800s (Heard 2014).

Although there is limited knowledge about the impacts of hatchery production in Alaska (Grant 2011), hatchery produced salmon could potentially impact wild fish populations through competition for food resources or spawning sites, disease transfer, and genetic introgression (e.g. Grant 2011, Zaporozhets and Zaporozhets 2005, Ruggerone et al. 2003, Cooney and Brodeur 1998). The legislative history of hatchery management in Alaska demonstrates attempts to limit these impacts. However, the current management guidelines do not adequately address hatchery risks.

The objectives of this thesis are to 1) review the history of Alaskan hatchery policy and analyze its ability to adequately address the adverse impacts of hatchery production; and 2) to examine modern hatchery practices to induce triploidy for the mitigation of hatchery-wild genetic introgression. The remaining sections of the introduction discuss the evolution of Alaska's hatchery policies and management. Subsequently, Chapter 1 provides an interpretation of the effectiveness of modern hatchery policies to avoid potentially harmful hatchery-wild genetic introgression caused by hatchery straying. Finally, Chapter 2 examines the variability in survival to the emergent fry stage of development explained by maternal effects and pressure shock duration of triploidized hatchery Chinook salmon.

POLICY OVERVIEW

PRE - STATEHOOD: THE RISE AND FALL OF SALMON HATCHERIES

In Alaska, hatchery salmon production to supplement fisheries began shortly after the federal Alaska Fisheries Service enacted the 1889 Fisheries Act. This policy was an attempt by the U.S. Congress to avoid potentially detrimental impacts of commercial fishing methods, specifically in the Alaska territory. The initial Fisheries Act banned the use of stream barricades as a means to capture salmon, and called for further investigations about salmon ecology and distribution (Roppel 1982). The act did not, however, explicitly call for the enhancement of wild stocks with hatchery produced fish.

The first salmon hatchery in Alaska was voluntarily established in 1891 on the Karluk River. Multiple cannery operators owned and operated the Karluk River hatchery and produced sockeye salmon to supplement the popular commercial fishery (Roppel 1982). A new hatchery was constructed on the Karluk River in 1896, replacing the 1891 hatchery. Many other voluntary sockeye salmon hatcheries were also constructed between 1892 and 1917 (Figure P.1).

The Fisheries Act was amended in 1900 to reflect the growing presence of hatcheries in Alaska. In an attempt to offset fishery impacts, the amended act mandated that any entity (person, company, or corporation) that harvested any species of wild salmon in Alaska must establish a hatchery to produce sockeye salmon (Roppel 1982). These mandatory hatcheries were required to produce sockeye salmon fry at levels four times greater than the harvested wild fish.

Although the 1900 amendment to the Fisheries Act led to the construction of two hatcheries, the Quadra and Fortmann hatcheries in Southeast Alaska (Figure P.1), there were still inadequacies in the legislation, and the efforts of many mandatory hatcheries were unsuccessful (Roppel 1982). This enhancement program created an imbalance between species production and harvest because only sockeye salmon runs were being enhanced despite the harvest of other species of Pacific salmon. The amended 1902 Fisheries Act required that ten sockeye salmon were produced for every wild salmon harvested, regardless of species (Roppel 1982). Despite mandatory production of salmon, funding was not available for hatchery owners, and facilities were still owned and operated by private canneries, thus increasing the production costs of participating in salmon fisheries in Alaska. Although the Fortmann and Quadra hatcheries operated for many years, most mandatory hatcheries were closed within one year of construction. Unsuccessful mandatory hatcheries were also constructed on Freshwater Bay, Ketchikan Creek, Karta Lake, Auke Creek, Lake Bay, and Shipley Bay (Figure P.1).

In 1905, the federal Sundry Civic Bill was the first piece of legislation that provided funding for the construction of federal salmon hatcheries in Alaska (Roppel 1982). The funding provided by the Sundry Civic Bill was used to construct the Yes Bay hatchery and Afognak hatchery (Figure P.1). After federal funds were established for hatchery development, the former United States Commission of Fisheries, housed under the Department of Commerce and Labor, loosened restrictions on private cannery operators.

The 1906 amendments to the Fisheries Act eliminated the requirement of mandatory hatcheries and started an inspection program for private hatcheries that operated in

Alaska (Roppel 1982). These amendments also reflected the high costs associated with mandatory hatcheries and the imbalances of production set in motion with the 1900 enhancement program. Taxes were levied for cannery products and rebates were issued to hatcheries for every 1000 sockeye salmon or Chinook salmon fry produced (Roppel 1982). Rebates incentivized the continued construction of private hatcheries despite the lack of direct funding from federal sources.

Federal funding for hatcheries continued with the passing of Senate Bill 10 in 1919. This bill not only allocated funds for the construction and operation of federal hatcheries, but it also called for research into the protection of wild stocks and artificial propagation of salmon (Roppel 1982). Despite efforts from the federal government to offset operating costs, hatcheries in Alaska were still too expensive for early producers to operate. All hatcheries, both private and federal, were closed by 1936 (Roppel 1982). In 1947, the federal government attempted to establish one last experimental hatchery operated by the newly formed United States Fish and Wildlife Service, but the facility closed after one year of operation (Roppel 1982). By the late 1940s, the lack of interest in propagation of hatchery salmon and the high operating costs of hatcheries were significant barriers to entering the salmon hatchery industry.

POST-STATEHOOD: REESTABLISHMENT OF SALMON HATCHERIES

Alaska obtained statehood on January 3, 1959, however efforts to establish a post-statehood governing body began before statehood was achieved. In preparation for statehood, the territorial legislature changed the name of the Alaska Fisheries Service to

the Alaska Department of Fish and Game (ADF&G) and began determining the duties of the new agency (ADF&G 2015). The ADF&G existed in name by 1957, but the legislature did not officially establish the agency until after statehood in 1959. Full authority over the governance of Alaska's fisheries was granted to the ADF&G on January 1, 1960 (ADF&G 2015). Afterwards, the Alaska Legislature created the Board of Fish and Game, which was later divided into the Board of Fisheries and the Board of Game in 1974. The Board of Fisheries is responsible for establishing regulations and harvest allocations (ADF&G 2015).

With harvest allocations in place, participants of the sockeye salmon fishery continued to harvest large quantities of fish. As the available biomass of sockeye salmon decreased, the commercial harvest of sockeye salmon steadily declined as well, falling from 126.4 million fish in 1936 to 21 million in 1967 (ADF&G 1983). As a result, the ADF&G established the Fisheries Rehabilitation, Enhancement and Development (FRED) Division (later merged with Commercial Fish Division in 1993) in 1973. The primary purpose of the FRED Division was to develop rehabilitation and enhancement plans for the state's wild fisheries, but the division was also responsible for stimulating hatchery development.

To avoid similar hatchery closures as those experienced in the 1930s, the FRED Division took proactive measures to encourage production. The division promoted the expansion of the hatchery industry by encouraging and sponsoring research that would facilitate the development of hatcheries in Alaska [AS 16.05.092]. Another innovative component of the FRED Division was the private investment program. Rather than

discourage private sector involvement in the fisheries industry, the FRED division encouraged private investment for technological development and economic utilization of fisheries resources [AS 16.05.092]. The encouragement of private sector involvement in fish production became increasingly important as the Alaskan hatchery industry expanded.

The Board of Fish and Game, was divided into the Board of Fisheries and the Board of Game in 1974. The Board of Fisheries is responsible for establishing regulations and harvest allocations, and is comprised of seven members who are appointed by the governor and confirmed by the legislature (ADF&G 2015). Today, the Board of Fisheries considers biological and socioeconomic data provided by the ADF&G, and public comments to make decisions on regulations and harvest allocations (ADF&G 2015).

The State of Alaska's 1974 Private Non-Profit Hatcheries Act promoted the industry further when it initiated a permitting process for private non-profit groups to establish hatcheries. With a permit, non-profit hatcheries could now produce salmon for harvest in common property fisheries. Since the original permitting statute in 1974, a series of statutes outlining the terms and conditions of permits and regulations pertaining to brood stocks and fish releases have been put into effect [AS 16.10.400 - 16.10.480].

Other important amendments to the 1974 Private Non-Profit Hatcheries Act were Alaska's 1976 Regional Planning and Regional Aquaculture Association Statutes. The Regional Planning Statute started the use of regional plans for hatchery production [AS 16.10.375]. This allowed management to target production based on the needs of specific

regions. The establishment of Regional Aquaculture Associations initiated a community-based component to Alaska's hatcheries. As stipulated in the statute, a Regional Association operates as a collective of private non-profit hatcheries. However, Regional Aquaculture Associations must be comprised of representatives of commercial fishermen in the region and at least one representative from each fishery user group [AS 16.10.380]. The 1976 statutes remain important components to modern hatchery management in Alaska.

By the 1980s, hatchery production of salmon was well established in Alaska for the second time, and concerns of releasing mass numbers of hatchery fish increased. Two concerns of particular importance were the effects of gene flow between cultured and wild stocks and the preservation of genetic diversity within and between populations (ADF&G 1985). To address these concerns, the ADF&G promulgated the first comprehensive Genetics Policy for Aquaculture Programs in 1985 (Genetics Policy). The overarching goal of the state's Genetics Policy was to preserve the genetic integrity of wild stocks (ADF&G 1985). In order to meet this goal, the authors of the Genetics Policy identified a series of guidelines to assist managers in making genetically sound production decisions. With the basic principles of salmon hatchery production in place, the ADF&G began writing guidelines for the stocking procedures of state operated sport fish hatcheries.

Although the Genetics Policy is fundamental to modern salmon hatchery production in Alaska, another policy, the state's Lake Stocking Policy, is equally important to production. The Lake Stocking Policy was first enacted in 1998 and was

later revised in 2013. The goal of the Lake Stocking Policy is to provide better recreational opportunities for anglers by guiding the ADF&G Sport Fish Division stocking projects (ADF&G 2013). If a stocking project has the potential to create self-reproducing or hybridized populations, the risks for potential impacts to wild stocks are considered high. In these cases, the ADF&G encourages hatcheries to stock with sterile fish whenever practical (ADF&G 2013). Although the ADF&G recommends that hatcheries stock with sterile, triploid fish (fish with three sets of chromosomes), it is not mandatory. Sport Fish hatcheries produce and stock with triploid fish as outlined by each Fish Transport Permit (FTP). FTPs are required for all entities releasing live fish or eggs into waters of Alaska, and a separate FTP must be obtained for every project involving the release of live fish or gametes (5 AAC 41.005).

Although every policy and statute passed since the original 1889 Fisheries Act is important for the management of Alaska's salmon fisheries, the current management system is not without flaws. Despite considerable efforts to avoid negative impacts of hatcheries in Alaska, there is still a considerable level of risk associated with hatchery production in the state.

CHAPTER 1

ASSESSING THE CONCEPTUAL FRAMEWORK OF HATCHERY-WILD INTERACTIONS CAUSED BY HATCHERY STRAYING OF PACIFIC SALMON IN SOUTHCENTRAL ALASKA

1.1 INTRODUCTION

Hatcheries have a significant presence in Alaska; however, little has been confirmed about the effects of hatchery fish on Alaska's wild salmon (Grant 2011). The driving force behind Alaska's hatchery production is that resources should be developed to provide maximum benefit to the public (Alaska constitution, Article VIII Sections 2 and 4). Deriving the maximum benefit from fish production is a common theme throughout the legislative history of Alaskan hatcheries, but there is another goal to production. Modern hatchery regulations identify the risks of hatchery production and state that hatcheries must contribute to common property fisheries (5 AAC 40.860(3)) while avoiding significant negative impacts to wild stocks (5 AAC 40.860(4)).

There are many interpretations of these standards and there is no metric to evaluate a hatchery's effectiveness at contributing to a fishery without impacting wild fish. Hatchery policies have been developed to guide managers in meeting program goals (e.g. 1985 Genetics Policy); however, until clear, measurable standards for hatchery impacts are established, it is not possible to accurately assess the success of hatchery production in Alaska.

Alaskan hatcheries release billions of Pacific salmon annually, greatly contributing to common property fisheries (Heard 2012). However, the risks to wild stocks associated with this level of production are still under investigation. Impacts of hatchery fish on wild populations can be extensive and involve both ecological and genetic interactions (Table 1.1). For example, wild and hatchery juveniles often compete for food in freshwater, estuarine and ocean environments (Cooney and Brodeur 1998, Ruggerone et

al. 2003). Increased competition for food can result in decreased body size over time (Cooney and Brodeur 1998, Unwin and Glova 1997), ultimately leading to diminished survival and reproductive potential (Cooney and Brodeur 1998). In addition, hatchery and wild adults compete for spawning sites and mates (Grant 2011, Zaporozhets and Zaporozhets 2005, Belyaev et al. 2000). All of these examples of ecological competition have the potential to effect the spawning potential and overall production of wild populations (Grant 2011).

Straying, or fish migrating to non-natal streams to spawn, is one of the major drivers of concern associated with hatchery-wild interactions (Grant 2011). Concerns about hatchery straying are based on ecological mechanisms that ultimately reduce the size of wild populations (Grant 2011). Decreases in wild populations reduce the effective population size, and ultimately lead to a loss of genetic diversity in wild stocks (Huusko and Vehanen 2011; Weber and Fausch 2003). Losses in genetic diversity can lead to a variety of population-wide effects including a decrease in the ability of a population to adapt to changing environmental conditions (Grant 2011), ultimately threatening survival. Preventative measures are taken to avoid hatchery-wild interactions caused by hatchery straying (e.g. segregating the release of hatchery fish from streams containing wild stocks, altering release location and timing). However, hatchery fish continue to stray into wild systems throughout Alaska (Brenner et al. 2012, Lin et al. 2008, Gilk et al. 2004, Mortensen et al. 2002, Wertheimer et al. 2000, Hard and Heard 1999, Sharp et al. 1994).

Controlling the factors that lead to hatchery-wild interactions is difficult given the current state of science (ADF&G 1985), and current hatchery management recognizes the difficulty of making production decisions without having complete knowledge of the system. However, research into the effects of hatchery practices on straying, and assessments of observed stray rates are currently underway. Although many hatcheries are collaborating on research efforts to reduce the number of strays, straying is a complex process and the answers to fundamental questions (e.g. How do hatchery practices affect stray rates?) may not be achievable in time to avoid irreparable damage to the genetic integrity of wild stocks.

Until critical knowledge gaps are filled, precautionary management would dictate proactive efforts to avoid hatchery-wild interactions by adjusting factors that are under mangers' control. Predicting and managing for the number of hatchery strays and the impact this has on wild stocks is not presently possible. However, managers have several controls that could mitigate hatchery-wild interactions including changing the number of hatchery fish released or by releasing sterile (triploid) fish when conditions allow.

This chapter will describe the conceptual framework for the management of hatchery-wild interactions caused by hatchery straying and provide policy and research recommendations to reduce the impacts of hatchery straying.

1.2 CONCEPTUAL FRAMEWORK OF HATCHERY STRAYING

Addressing the genetic impacts (i.e. genetic introgression) caused by hatchery straying is complex and requires large amounts of genetic marker data from both wild

and cultured stocks (Taranger et al. 2015). Nevertheless, effective management recognizes and mitigates the risks of hatchery practices to the extent possible. A precautionary approach assumes that any hatchery origin fish that strays has the potential to spawn with wild fish and therefore can contribute to genetic introgression (Taranger et al. 2015).

Previous studies have established that stray rates differ by species and location (Westley et al. 2013, Keefer and Caudill 2012), and "safe" levels of hatchery strays in wild systems have been proposed (Grant 2011). In Alaska, the maximum level of spawning hatchery fish in a system is determined on a case by case basis by Alaska Department of Fish and Game (ADF&G) Genetics staff. The ADF&G sets the acceptable level of spawning hatchery fish in most streams to between 2% - 10% (Brenner et al. 2012). Setting acceptable maximum levels of hatchery straying is beneficial because it recognizes that straying is unavoidable, and it provides management with a target to avoid.

Although setting straying thresholds is a key component in upholding Alaska's hatchery management goals, it is important to first understand the principle behind straying thresholds. What does it mean to allow up to 10% hatchery strays in a system? In this context, straying limits reflect the *proportion* of spawning adults in any given system that can be of hatchery origin. For example, if there are 10 000 spawning Chinook salmon in a stream, up to 1 000 of those fish can be of hatchery origin. The total number of hatchery fish straying to non-natal streams may exceed 10% of the total hatchery fish released. There is also no indication that strays are evenly distributed

amongst all streams in a region (i.e. Brenner et al. 2011, Grant 2011), so a significant portion of strays may return to one stream while another stream receives none. Although setting maximum hatchery straying thresholds recognizes the potential risk of hatchery-wild interactions, technology and the current state of scientific knowledge may prevent management from truly achieving this goal.

Given the previous research on stray rates (Keefer and Caudill 2012), we can estimate the number of hatchery origin fish straying into non-natal streams, but acceptable straying thresholds involve more factors than the number of hatchery strays. More importantly, the hatchery origin spawners threshold is a proportion of the population of spawning adults and is implicitly connected to the number of wild fish returning to spawn in a system (also referred to as the escapement level). Therefore, in order to maintain a given hatchery:wild spawner proportion in a system, as the wild escapement level decreases, the number of hatchery fish would need to decrease concurrently. Consider an example stream and let:

w_A = number of spawning adults from Stream A that are of wild origin

h_A= number of spawning adults from Stream A that are of hatchery origin.

If the acceptable level of hatchery origin fish spawning in a stream is 10%, and w_A is 10 000 fish in Year 1, then h_A in Year 1 is 1 000 fish. Now, suppose w_A falls to 5 000 fish in Year 2. To keep the percent of hatchery origin spawning fish at 10% of the total spawning population of this stream, h_A must decrease to 500 fish. From a production

standpoint, one of the fundamental questions to this oversimplified scenario is: *How should the number of fish produced be adjusted to reflect the changes in wild salmon escapement?*

The ability to forecast returns (both hatchery and wild) is poor, especially multiple years in advance (which is particularly important because depending on the species, fish won't return to spawn for many years). In addition, there are other fundamental questions for hatchery management in terms of controlling hatchery straying (e.g., How can release timing and release location reduce stray rates?). Although there are uncertainties about the impacts of hatchery production on wild stocks and the effects of hatchery practices on straying rates, hatchery production remains a significant component to commercial and sport fisheries in Southcentral Alaska.

1.3 HATCHERY PRODUCTION IN SOUTHCENTRAL ALASKA

There are numerous hatchery operators in Southcentral Alaska, both private, non-profit (PNP), and the ADF&G Sport Fish Division. All five species of Pacific salmon are produced in this region. Fish are produced, reared, and released in the following major geographic regions: Prince William Sound (PWS), Cook Inlet (CI), and Kodiak.

In PWS, there are six facilities operating as PNP hatcheries (Figure 1). Together, these six facilities released 0.03 million Chinook salmon, 33.46 million sockeye salmon, 2.72 million coho salmon, 672.94 million pink salmon, and 151.5 million chum salmon in 2014 (Vercessi 2015). Hatcheries contributed 45.2% of the fish harvested by the PWS commercial sockeye salmon fishery, 28.7% of the PWS coho salmon fishery, 92.5% of

the PWS pink salmon fishery, and 67.9% of the PWS chum salmon fishery in 2014 (Vercessi 2015).

Kodiak Regional Aquaculture Association operates two PNP hatcheries (Figure 1). Together, these facilities released 0.27 million Chinook salmon, 3.76 million sockeye salmon, 1.05 million coho salmon, 191.5 million pink salmon, and 21.9 million chum salmon in 2014 (Vercessi 2015). Kodiak hatcheries were responsible for producing 9.3% of the commercially harvested sockeye salmon in the region, 48.6% of the Kodiak commercial coho salmon fishery, 51% of the Kodiak commercial pink salmon fishery, and 13.5% of the Kodiak commercial chum salmon fishery in 2014 (Vercessi 2015).

In CI, Cook Inlet Aquaculture Association operates two PNP hatcheries, and ADF&G Sport Fish Division operates one hatchery in Anchorage (Figure 1.1). In 2014, these three facilities released 1.97 million Chinook salmon, 9.37 million sockeye salmon, 1.17 million coho salmon, and 51.3 million pink salmon (Vercessi 2015). Hatcheries produced 1.5% of the commercially harvested sockeye salmon and 6% of the commercially harvested pink salmon in the CI region in 2014 (Vercessi 2015).

Although both PNP and ADF&G hatcheries are present in Southcentral Alaska, PNP hatcheries were responsible for 100% of the releases of sockeye salmon, pink salmon, and chum salmon, and 81% of coho salmon production in Southcentral Alaska (Vercessi 2015). The ADF&G Sport Fish Division is responsible for approximately 86% of Chinook salmon production in the region (Vercessi 2015).

The combined (PNP and ADF&G) number of hatchery releases in Southcentral Alaska has remained relatively stable over the past 10 years (Figure 1.2), but escapement

estimates are variable. For example, Figure 1.3 depicts the reported escapement estimates of all five species of Pacific salmon for three systems located on Kodiak Island between 2009 and 2012. These data show considerable variability in escapement by species over time, a scenario that is not unique to these three systems. Furthermore, comprehensive escapement monitoring can be cost prohibitive and therefore escapement data are not available for every species, year, and system in the region. There are 41 escapement projects in Southcentral Alaska (ADF&G 2015), and none of the species of Pacific salmon are monitored by every project (Table 1.2). There are also different levels of accuracy associated with different methods of assessing escapement (e.g. counting fish using a video weir is more accurate than counting fish from a tower). It is not logistically or financially practical to monitor the escapement of every species of Pacific salmon on every system, however a method of accounting for escapement data should be developed to manage the overall potential impact of hatchery production.

1.4. DISCUSSION

A key component to evaluating the success of any environmental management program is the ability of that program to meet the objectives outlined in the governing statutes, codes, and policies. Alaska's salmon hatchery statutes maintain that the intent of the state's hatchery program is to "provide substantial public benefits" without "jeopardizing natural stocks" of salmon (AS 16.10.400(g)). Hatchery regulations also use these objectives as conditions for performance reviews of fish production facilities, reaffirming their importance. Without standardized metrics to evaluate the contributions

and impacts of hatcheries, current management is forced to rely on precautionary guidelines to protect wild populations from hatchery production. Despite these efforts, straying is a reality, and large hatchery returns make it impossible to avoid harmful hatchery-wild interactions. Even if only a fraction of the population strays, there are still millions of fish interacting with wild stocks every year (Brenner et al. 2012).

Due to technological and temporal constraints, adverse genetic impacts associated with current levels of hatchery production are not readily evident, and some suggest that production levels should continue to incrementally increase until more information on genetic impacts is available (Heard 2012).

This particular approach to hatchery production can be interpreted as a form of reactive management, because it requires managers to avoid mitigating hatchery-wild interactions until after harmful impacts have occurred. This is contrary to the proactive components of Alaska's hatchery policies, many of which are based on the idea that management should anticipate negative hatchery-wild interactions and take proactive steps to avoid them. The proactive principles of Alaska's hatchery management recognize that it is a misconception to believe that a lack of evidence of an impact equates to the lack of an impact (Pearsons 2008). Although quantifying the severity of hatchery-wild interactions is imperative to successful hatchery management, this should not be the primary long-term management strategy. Instead, long-term management will be more effective if we develop a concrete strategy to proactively reduce the potential for harmful genetic impacts now before mitigation is no longer possible.

What can be done to mitigate hatchery-wild interactions given the knowledge gaps associated with straying and genetic introgression? Understanding about the degree and subsequent outcomes of hatchery-wild interactions will be a long-term scientific challenge. However, one obvious control on hatchery-wild interactions under managers' jurisdiction is the number of hatchery origin fish released into the wild.

Assuming that the average stray rates reported by Keefer and Caudill (2012) can be used to estimate the number of hatchery strays in Southcentral Alaska, then the expected number of hatchery fish straying into streams has also remained relatively constant. The naturally variable component in the hatchery-wild interaction conceptual framework is the number of wild-origin fish returning to spawn. Based on the number of hatchery fish that have been released, an average of 4.12 million pink salmon, 0.80 million chum salmon, 0.05 million coho salmon, 0.06 million sockeye salmon, and 0.002 million Chinook salmon have strayed into streams in Southcentral Alaska over the past 10 years (Figure 1.4).

Given the variable nature of wild returns, and the results of a study published by ADF&G biologists (Brenner et al. 2012) there is cause for concern that the proportion of spawning hatchery origin fish in Southcentral streams has exceeded the proposed maximum 10% threshold. For example, Brenner et al. (2012) reported that the proportion of spawning hatchery fish in PWS streams in the 1990s ranged from 0% - 98% in pink salmon, 0% - 63% in chum salmon, and 0% - 93% in sockeye salmon, far exceeding the 10% threshold.

Although hatchery production has remained constant over time, the proportion of hatchery fish to wild fish spawning in these streams has been significantly altered. In order to avoid permanent detrimental effects of hatchery-wild interactions caused by straying, key efforts for the future include:

- Improving the accuracy and representation of escapement monitoring to improve the estimation of w
- Continuing to assess the appropriateness of a maximum hatchery origin to wild origin spawner ratios to avoid genetic introgression and other potentially harmful hatchery-wild interactions
- Restructuring hatchery production so the proportion of straying hatchery fish remains below the maximum threshold of wild fish, ensuring that $h < w$
- Continuing to assess observed stray rates to adjust h if necessary
- Continuing research into the effects of hatchery release practices on straying (e.g. time and location of releases, attempts to improve imprinting, and sterilization measures such as triploid induction) to better control h in the future

CHAPTER 2

MATERNAL EFFECTS IN THE SURVIVAL AND TRIPLOIDIZATION OF HATCHERY CHINOOK SALMON (*Oncorhynchus tshawytscha)*

2.1 INTRODUCTION

The illegal transportation of stocked hatchery fish has become increasingly common (McNeill 1995; McMahon and Bennet 1996) and can negatively impact native populations of salmonids. When an introduction occurs, ecological impacts are experienced first (i.e. competition for food and mates), and genetic impacts subsequently develop (i.e. reduction of genetic diversity) (Grant 2011). Sterilizing prior to release greatly reduces the possibility that illegally transported fish will establish new populations or hybridize with native stocks (Kozfkay et al. 2006).

Stocking with triploid salmon, which are inherently sterile, is an effective strategy for minimizing the potential harm caused by hatchery-wild interactions. To induce triploidy, an external hydrostatic pressure or temperature shock is applied to fertilized eggs prior to the second meiotic division, forcing the retention of the second polar body (Loopstra and Hansen 2008). Research to date indicates that triploidization in Pacific salmon is maximized when applying a hydrostatic pressure shock greater than 9,000 pounds per square inch (PSI) within one hour post-fertilization, for a duration lasting three to five minutes (Loopstra and Hansen 2008, Wickwire 2000, O'Keefe and Benfey 1995, Teskeredzic et al. 1993).

The Alaska Department of Fish and Game (ADF&G) Sport Fish Division endorses the use of triploid fish for stocking whenever practical (ADF&G 2013). Recommendations for the stocking of sterile salmonids are outlined in the Lake Stocking Policy for Sport Fish Division (Lake Stocking Policy), established in 1998 and revised in 2013. This policy sets requirements for the certification of triploid stocks prior to release.

Mixed-sex triploid populations must be certified as 99% triploid with a 95% confidence level (ADF&G 2013). Despite improvements in induction procedures and technology, a triploid rate of 99% can be difficult to produce consistently. Although optimal ranges of triploid induction parameters have been suggested, variability in triploidy success rates and survival through triploidy induction are typical outcomes.

Variability in survival and triploidy is likely a strong reflection of maternal influences, including ripeness, female condition, and the maternal chromosomes retained in the second polar body (Blanc et al. 2005). Furthermore, genotype effects (e.g. family and female effects) have been documented as affecting survival (to the emergent fry stage of development) in diploid fish (Heath et al. 1996), survival through triploidization (emergent fry stage of development) (Johnson et al. 2004) and triploidization success (e.g. Johnson et al. 2004, Withler et al. 1995, Malison et al. 1993, Frankhauser and Watson 1942). Despite growing evidence that female effects can impact survival and triploidization success, the specific agents of these effects are unknown. Proposed explanations of female effects in the survival through, and success of triploidization include differences in the susceptibility for polar body retention (Diaz et al. 1993) and variable egg development rates (Dunham 2004).

The objective of this chapter is to determine the role of maternal effects and shock duration in the survival and triploidization of the ADF&G William Jack Hernandez Sport Fish Hatchery (WJHSFH) Chinook salmon (*Oncorhynchus tshawytscha*) triploid program. In accordance with the Lake Stocking Policy, the WJHSFH produces and releases many species of triploid fish into lakes of Southcentral and Interior Alaska,

including Chinook salmon (*O. tshawytscha*), coho salmon (*O. kisutch*), rainbow trout (*O. mykiss*), Arctic char (*Salvelinus alpinus*), and Arctic grayling (*Thymallus arcticus*). Even slight variations in triploid rates across female groups can hinder the achievement of 99% triploid rates that the Lake Stocking Policy requires.

In addition to assessing the role of maternal effects in this program, the effects of pressure shock duration on survival rates and triploidy success was also tested. Triploid induction protocols for Chinook salmon at the WJHSFH require that eggs be pressurized at 10,000 PSI for 4 minutes, 400 Centigrade Temperature Minutes (CTMs) after fertilization. CTMs are defined as the number of minutes elapsed between fertilization and pressure shocking, multiplied by holding water temperature. This standard was adopted following a 2008 study conducted by the ADF&G assessing survival to the eyed egg stage of development and triploidization rates in response to different induction parameters. However, pressure shock durations of 5 minutes were not assessed (Loopstra and Hansen 2008). It is possible that shocking eggs for longer periods of time will lead to higher triploidization rates (potentially at the risk of increased mortality). In addition to the effects of longer pressure shock durations, the variability in survival and triploid rates explained by maternal influences (female effects) has not been evaluated for WJHSFH Chinook salmon.

2.2 METHODS

All fish handling and laboratory methods for this project were based on current protocols used by the ADF&G (e.g. ADF&G 2013).

2.2.1 FEMALE CARCASS EXAMINATION

Carcasses of female spawners were transported to the Fisheries, Aquatic Science and Technology Laboratory on 25 July 2014 and stored on ice until they were examined on 26 July 2014. The standard length was recorded, and both sagittal otoliths were extracted using the "chop" method for whole salmon (University of Alaska Southeast Fisheries Technology Program 2014). Once extracted, otoliths were cleaned of any tissue and placed in manila envelopes until processing. Scales for aging were collected from an area above the lateral line that runs diagonally from the posterior base of the dorsal fin to the anterior base of the anal fin (following standard scale ageing recommendations, e.g. ADF&G 2014) on 16 September 2014. All otoliths and scales were read and processed at the ADF&G Mark, Tag and Age Laboratory in Juneau, Alaska. Otoliths were mounted to slides and ground using a LaboPol-5 grinder and 30 uμ grain sandpaper. Otoliths were ground on both sides (sulcus side up and down) to expose the core and determine the presence of thermal marks.

2.2.2 EGGTAKE AND FERTILIZATION

Eight males and eight females were randomly selected for spawning from the WJHSFH spawning raceway on 25 July 2014. Eggs from each female were separated into Female x Treatment groups (0 shock minute controls, and 3, 4, and 5 minute shock groups) prior to fertilization. The target sample size for each Female x Treatment group was 300 eggs, which was measured using volumetric techniques. The actual sample size at fertilization can be found in Table 2.1. Female x Treatment group egg weights were

measured prior to fertilization. Fertilization was staggered to maximize the use of hatchery equipment (Table 2.2). Eggs were fertilized using milt from two randomly selected males, and sperm was activated with a 7 ‰ saline (7 g NaCl/L H_2O) solution. Eggs soaked in activated sperm for two minutes and were rinsed with water. To prevent the spread of disease, eggs were transferred to incubation trays and disinfected for 15 minutes with a 1:100 iodophor solution. Disinfected eggs were rinsed and held in water until pressure shocking.

All groups underwent a treatment of 400 CTMs. The temperature of the holding water was 9° C, resulting in a wait period of just over 44 minutes. Eggs were pressurized using a 2.7-liter TRC-APV hydraulic pressure chamber (TRC Hydraulics Inc.). There were three pressure shock duration treatments in this study: 3 minute, 4 minute and 5 minute treatments. Zero minute control groups consisted of diploid eggs collected from each female. Replicate trials were conducted for a subset of Female x Treatment groups due to limited incubation space. In instances where Female x Treatment replicate groups were included (Females 1-4 & Female 8), there was a potential for survival to vary between Trial and Replicate Treatment groups due to the effects of delayed fertilization. To address this concern, two separate zero minute Female x Treatment groups were collected (one to represent the original Trial Treatment groups, another to represent the Replicate Treatment groups). All eggs were shocked at 10,000 PSI. Eggs from Control groups were left in an inactivated pressure chamber for four minutes to mimic the handling procedures experienced by the rest of the eggs. Eggs were placed in incubation trays for the remainder of the water hardening process. Water hardening was completed

two hours after fertilization. Eggs were transferred to four-ounce incubation containers following the water hardening process. Female x Treatment groups were divided approximately in half using volumetric techniques (Table 2.1). The locations of the incubation containers within the incubation stack and incubation trays were randomized to account for potential Tray and Position effects. Each container was assigned a tray number (1 - 8) corresponding to incubation tray (Figure 2.1). Each container was also assigned a position number (1 - 12), which indicated the relative position of each container within a tray (Figure 2.2).

2.2.4 EYED EGG SURVIVAL

Egg picking, or the removal of dead eggs from incubation trays, occurred at the eyed-egg stage of development on 3 September 2014 (40 calendar days after fertilization). Egg picking prevents further mortality by removing decomposing embryos from the system and improving the overall water quality within incubation trays and containers. Blank (embryo was unsuccessfully fertilized) and dead eggs were removed from individual incubation containers using forceps. Survival to the eyed egg stage of development was also recorded at this time.

2.2.5 TOTAL SURVIVAL

For the purposes of this project, total survival refers to the number of fish that survived to the emergent fry stage of development, which corresponded to the time of blood collection for the purposes of triploidy assessment (14 November 2014 - 11

December 2014; 112 - 139 calendar days after fertilization). Total survival data were analyzed using generalized linear mixed effects models implemented in the R statistical programming environment (R Core Team 2015; version 3.2.2). Binomial regression models with a logit link and inclusion of both fixed and random terms were considered. Initial model validation and model selection were conducted with package lme4 (Bates et al. 2014).

In the first step of regression analysis, we fit models to assess whether there was a meaningful effect on survival associated with a Replicate effect. Because Replicate data were only available for a subset of females (Females 1-4 & Female 8), models with Replicate effects were tested on a subset of the total dataset. Models with Replicate main effect terms (R formula notation: Survived ~ Treatment + Replicate + (1|Female) + (1|Position) + (1|Tray)) and models with Treatment x Replicate interaction terms (Survived ~ Treatment*Replicate + (1|Female) + (1|Position) + (1|Tray)) were tested. The data were ultimately pooled into one dataset because results indicated that there was a weak and inconsistently significant effect attributed to both Replicate main terms and Replicate x Treatment interaction terms (Table 2.3). Final sample sizes for Treatment x Female groups are given in Table 2.1.

After pooling data across Replicate trials, we next attempted to assess the adequacy of the full model with all measured potential sources of variability on survival (Survival ~ Treatment + (1|Female) + (1|Position) + (1| Tray)). Treatment was treated as a fixed effect because of the specific interest in 3, 4, or 5 minute shock assays. Female origin, age, and length were considered covariates in order to test their impact on

survival, but they were not included in the final model. There was an insufficient range in data for each covariate (e.g. all females were identical in age, all but one female was of hatchery origin, and there was a narrow range in female length). Preliminary analysis also indicated collinearity between these covariates and a categorical "Female" term. Therefore a generic categorical variable was used in the final model. Female, Position, and Tray were treated as random effects, reflecting their interpretation as samples from the statistical population of Ship Creek female Chinook salmon, and laboratory trays and positions. Furthermore, preliminary model fits treating Female as a fixed effect failed to converge due to complete separability issues (e.g. some combinations of Female and Treatment exhibited complete mortality or complete survival; Gelman et al. 2008).

The model due diligence routine included a series of preliminary checks. First, models were assessed for convergence. Models that failed to converge were removed from the analysis at this stage of the model selection process. Next, the predicted survival for remaining candidate models (conditional of fitted random effects) was simulated and quantile-quantile (QQ) plots of the residual error were assessed to determine overall goodness of fit of the models. QQ plots were visually appraised and models whose residuals quantiles considerably departed from the expected quantiles were removed from the analysis (e.g. Zuur et al. 2009).

The full model failed to converge due to sparseness in data when parsing out samples into Female-Tray-Position combinations. Subsequently, we examined empirical total survival estimates by Tray and by Position, finding that the average survival by Position was more variable than by Tray (Figure 2.3). Thus a decision was made to omit Tray

from subsequent model formulations. The implication of this decision is that some portion of variability attributable to Tray effects may be incorporated by other variance components in the model, although the degree to which this occurred is probably slight owing to the relative stability in average total survival noted across Trays (Figure 2.3). Upon completion of the model validation process, a single candidate model passed adequacy testing. This model included Treatment as a fixed effect and Female and Position as random effects (Survival ~ Treatment + (1|Female) + (1|Position). This remaining model was selected as the final model for interpretation. For computational convenience, this final selected survival model was subsequently implemented in a Bayesian framework using the MCMCglmm package (Hadfield 2010). This allowed for characterization of uncertainty for both random and fixed effects components in the final fitted model.

The binomial glmm model was fit without overdispersion by fixing the residual variance term to a value of 0.1 in MCMCglmm model specification, and then subsequently translating parameter estimates to be equivalent to the case of zero residual variance following protocols provided in Hadfield (2014). The final model was fit with a single MCMC chain run with 100 000 iterations, a 25% thin rate, and a 75 000 burn period. This resulted in 1 000 stored posterior parameter draws. Trace plots of parameter draws indicated that the model was well mixed, and there were bell-shaped posterior parameter distributions for all fitted and random effect variances.

Model results for predicted survival of individual Treatments are reported on the probability scale and marginalize across Female and Position random effects (e.g. Atkins

2013). Similarly, predicted survival pertaining to Female x Treatment groups marginalizes across Position random effects, and predicted survival of Position x Treatment groups marginalizes across Female random effects.

2.2.6 PLOIDY DETERMINATION

Blood was collected from the caudal vein at the emergent fry stage of development (14 Nov 2014 - 11 Dec 2014; 112 - 139 calendar days after fertilization). Fry were anesthetized using a lethal solution of MS-222, and caudal fins were removed to expose the caudal vein. Once caudal fins were removed, fry were placed cut end first into 1.5 ml snap cap tubes containing 1.00 ml of detergent/4', 6-diamidino-2-phenylindole (DAPI) solution (ADF&G 2013). Fry remained in the solution for approximately five minutes to allow for maximum absorption of DNA. Fry were then removed, and DNA-solutions were stored in a -20°C chest freezer.

Although ploidy determination protocols recommend analyzing DNA-solutions as soon after blood collection as possible (ADF&G 2013), short-term storage of samples was essential due to practical constraints (i.e. time required to run 3 022 samples and equipment use). Solutions were thawed and filtered prior to processing.

The final solutions were processed individually using a Partec PAS-III flow cytometer (27 February 2014 - 15 April 2014; 79 - 141 days frozen). A flow cytographic histogram was generated for each solution, and peaks in the histograms represented the average nucleus diameter of cells in each sample. Because triploid cells contain 50% more DNA than diploid cells (Dunham 2004), peaks for triploid samples are located

approximately 50% further to the right of diploid cells on the x-axis of the histogram (ADF&G 2013). A total of 3 022 blood samples were analyzed using flow cytometry.

2.3 RESULTS

2.3.1 FEMALE CARCASS EXAMINATION

Eight female carcasses were sampled for standard length, age, and origin (hatchery vs. wild). Standard lengths ranged from 74 to 81 cm (Table 2.4). All females sampled were 5 years old, and all but one female were hatchery fish (Table 2.4). Females 1 - 7 were Ship Creek brood produced in 2009 at the Fort Richardson Hatchery (replaced by the WJHSFH in 2011). The thermal mark was absent on both the left and right otoliths from Female 8, indicating that this female was of wild origin.

2.3.2 SURVIVAL

A high proportion of mortality occurred subsequent to the eyed egg stage of development (Figure 2.4); therefore, all reported model predictions reflect the total survival (corresponding to the fry stage of development). All three sources of variability modeled (Treatment, Female, and Position) had comparable levels of influence on survival to the emergent fry stage.

The predicted total survival after marginalizing out Female and Position random effects increased as pressure shock duration increased (Figure 2.5; Table 2.5). Excluding Controls (predicted total survival = 79%), there was a positive relationship between pressure shock duration and predicted total survival. Of the treatment groups that

experienced an external pressure shock, predicted total survival after marginalizing out the effects of Female and Position ranged from 38% (3 minute shock; Figure 2.5) to 60% (5 minute shock; Figure 2.5).

The ranges of predicted total survival attributed to Female and Position random effects were slightly larger than the ranges of predicted total survival attributed to Treatments (Table 2.6; Table 2.7). Both Female and Position explained similar amounts of the variation in predicted total survival and had similar sized random effect variance estimates (Female random effect variance estimate = 3.546, Figure 2.6, Table 2.5; Position random effect variance estimate = 2.599, Figure 2.7, Table 2.5). For example, predicted total survival for the 5 minute shock treatment across Females but marginalizing out the Position effect ranged from 9% to 63% (Figure 2.6; Table 2.6), and similarly ranged from 6% to 55% across Positions when marginalizing out the Female effect (Figure 2.7; Table 2.7).

2.3.3 PLOIDY DETERMINATION

Of the 3 022 samples analyzed, 266 were confirmed as triploid, 1 409 were degraded (a result of cell lysis during the thawing process), and 1 347 exhibited cell diameters that were indistinguishable as either triploid or diploid (Table 2.8). In some cases, these samples contained more than one peak, but neither size group fell within the acceptable size range of triploid or diploid cells. Of the 266 confirmed triploid samples, 15 were from 3 minute shock treatments, 83 were from 4 minute shock treatments and 168 were from 5 minute shock treatments (Table 2.8).

Although the proportion of degraded and undetermined samples from each Treatment were similar, the proportion of samples that was confirmed triploid was higher for the 5 minute shock duration than for the others (Table 2.8). The specific cause of the anomalies in the flow cytometric results is undetermined, however it is likely due to the inability of DAPI to retain fluorescence after the freezing process (Kamiya et al. 2006).

2.4 DISCUSSION

The objective of this chapter was to determine the role of maternal effects and pressure shock duration in the survival and triploidization of hatchery Chinook salmon. While maternal effects did impact survival, results of this study indicate that conditions during the incubation process (reflected by Position effects) are also critical for survival to the emergent fry stage of development. For example, there was a positive trend in predicted survival as the Position number increased (i.e. as eggs were incubated closer to the front of the tray and away from the water source located at the back of the tray, corresponding to Positions 7 - 12; Figure 2.2; Figure 2.7). We hypothesize that Position effects were likely due to altered water flow caused by the presence of individual incubator containers, and future research employing similar incubation procedures should preliminarily investigate the impacts of incubation position (both within and among incubation trays) prior to beginning experimental procedures.

Diploid Control groups had higher survival than eggs that were pressure shocked, however survival did vary by Female across all Treatments (Table 2.5; Figure 2.5; Figure 2.6). This overall trend in survival by Female group indicates that Female effects are

apparent. Furthermore, our results corroborate and add to the findings of a previous study by Johnson et al. (2004), which reported variations in survival to the emergent fry stage of development between family groups.

Although it is impossible to predict survival solely based on the variation explained by spawning female, maternal influences do affect the success of triploid programs and warrant investigation. Previous studies have also found variable survival rates to the emergent fry stage of development by family groups both through a triploidy assay (e.g. Johnson et al. 2004), and under controlled hatchery conditions in general (Heath et al. 1996); however, the source of this variation beyond the female level has not been identified.

Egg quality is one potential source of variation attributed to females, and positive correlations have been observed between egg quality and survival through triploidization (Taylor et al. 2011). Egg quality is conceptually defined as the ability of an unfertilized egg to be fertilized and develop into an embryo (Bobe and Labbe 2009), and there are many characteristics that describe egg quality. Egg size is a common and easily measured indicator of egg quality. Many hypothesize that egg size is positively correlated to diploid survival to various stages of development (e.g. Heath et al. 1996, Bobe and Labbe 2009). However, others have found that smaller eggs often have similar survival rates as larger eggs, indicating that size is not a reliable predictor of survival (Bromage et al. 1992). Other indicators of egg quality include the distribution of lipid droplets (Mansour et al. 2007) and the pH of ovarian fluid (Lahnsteiner 2000, Aegerter and Jalabert 2004). Despite evidence that egg quality impacts survival, these factors are

often difficult to measure and control (Aegerter and Jalabert 2004, Bobe and Labbe 2009, Ciereszko et al. 2009) and were not assessed in this study.

The environment of the spawning female before reproduction can also have significant impacts on offspring survival (Chambers and Leggett 1996), although this is likely also a factor in the ability of a female to develop "high quality" eggs. Due to the nature of hatchery production, we are unable to determine or control the environment experienced by females during the appropriate time scale before reproduction. Furthermore, salmon are known to travel between estuarine and freshwater environments, and throughout a stream before entering spawning grounds (in this case, the spawning raceway). Without significant tracking efforts, we are only able to speculate the environments of females prior to being contained in raceways.

While assessing the sources of variation in survival is important for triploid programs, assessing the actual triploid rate is imperative, especially for programs with certification requirements. Despite our inability to assess triploid rates, important lessons can be learned about the practical constraints of hatchery research. Protocols for many triploid programs, including the program at the WJHSFH, emphasize the importance of running samples for ploidy determination promptly after blood collection (ADF&G 2013), however this was not possible in our study due to large sample sizes (approximately 3 000 samples). Under optimal efficiency (using the resources available for this project), approximately 200 samples can be assessed for ploidy using flow cytometry in one business day. Continuing to rear these fish and assessing ploidy 200

fish at a time was not an option due to feeding costs and the availability of rearing tanks and other hatchery equipment.

Cell degradation was a common result of the ploidy analysis, but many samples remained intact (but indistinguishable as triploid or diploid) after the freezing and thawing process (Table 2.8). For example, flow cytographic histograms indicated that the cells being measured were uniform in size but smaller than both triploid and diploid cells (based on the amount of DAPI stained DNA being reflected by wavelengths generated by the flow cytometer). Although the inability to determine ploidy using reported cell size was not investigated further, Kamiya et al. (2006) reported similar findings after using flow cytometry to measure the count of marine bacteria in samples that were fixed and stained with DAPI prior to freezing. Kamiya et al. (2006) suggest that this result was a consequence of DAPI losing its fluorescence during the freezing process. The reported cell size of a DAPI-stained sample that has been compromised in this manner may be smaller than the actual cell size, because the amount of detectable DNA in a cell is reduced. Consequently, the limitations of using DAPI and flow cytometry to determine the ploidy of large numbers of samples need to be addressed in experimental designs of hatchery triploid research.

Although the triploid rates were unsuccessfully assessed in this study, inferences can be made about the impact of shock duration on the overall success of triploid induction. The positive relationship between predicted survival and shock duration indicates that using longer shock durations may be beneficial for triploid induction. Furthermore, while flow cytometry was mostly unreliable, the small selection of eggs

identified as triploid did correspond positively with the length of pressure shock duration (Table 2.8). This suggests that a 5 minute shock treatment may result in higher triploidization rates, although additional investigation is needed before this speculation can be confirmed.

There may also be disparities in triploid rates across females due to the timing of the second meiotic division, a consequence of variable embryonic development rates (Dunham 2004, Withler et al. 1995, Malison et al. 1993, Diaz et al. 1993, Frankhauser and Watson 1942). There is also limited evidence that the origin of spawning female (hatchery vs. wild) may influence the susceptibility of an egg to triploid induction (Solar and Donaldson 1985). The effect of female origin was not assessable due to sample size, although the observed survival of eggs from Female 8 (wild origin) was among the highest (Figure 2.8).

The results of this study indicate that maternal effects do impact the survival rates of WJHSFH triploidized Chinook salmon. Female effects can make it difficult to predict offspring survival and triploidization rates, and measurable sources of variation need to be identified to determine the feasibility of selecting for high performing offspring (Johnson et al 2004). Regardless of a facility's ability to predict the impacts of maternal effects, female level variation should be accounted for in program objectives and triploid certification requirements.

In some instances it may be beneficial to increase the number of broodstock used in triploid programs in order to account for female level variability in survival. However, increasing the number of brood fish used for a triploid program can have larger hatchery-

level consequences. For example, some facilities employ quarantine procedures (at the female level) during incubation to prevent the spread of disease. Increasing the number of brood fish used in a triploid program may consequently increase the amount of incubation space needed for that program (e.g. 8 females results in the use of 8 incubation trays and 16 females results in 16 incubation trays). This scenario can lead to significant trade offs between the number and type (species or ploidy) of fish produced during a season. Ultimately, individual facilities need to determine the costs of female level variability in survival (or triploidization rates) and identify the appropriate method to mitigating impacts in their program.

While the triploid rate in this study was undetermined, the relationship between survival and pressure shock treatment (taking into account the variation explained by Female and Position effects) warrants further investigation into the efficacy of using five minute shock durations for the WJHSFH Chinook salmon triploid program. The purpose of this project was to investigate the effects of pressure shock duration and the magnitude of maternal effects in the WJHSFH Chinook salmon triploid program, and our results help inform triploid research and practices. We established that maternal effects impact survival, but we were unable to determine a measurable trait to assist hatcheries in selecting for high performing (high survival and triploidization rates) offspring. Subsequently, we identified a detrimental effect to long-term storage of samples before assessing ploidy using flow cytometry.

As a result, we recommend that future research 1) investigates the ability to predict survival and triploidization based on measurable characteristics of egg quality

(e.g. egg size, pH of ovarian fluid) and 2) determines the effects of long term storage on DAPI stained DNA samples, in an effort to determine (a) potential correction factors to adequately assess cell size in frozen samples and (b) improve technology to account for time and equipment constraints. Furthermore, we recommend that pilot studies be conducted prior to large-scale triploid production to 1) determine the optimal shock duration treatment, 2) assess the impacts of maternal effects, especially for programs with strict certification requirements, and 3) determine the impact of incubation position on survival for programs that plan to segregate eggs during incubation. Despite the advances that have already been made in triploid research, the renewed interest in triploidy as a means of preventing hybridization between hatchery and wild origin fish motivates the need for future research to further optimize this method of hatchery production.

GENERAL DISCUSSION

Alaskan hatcheries release billions of Pacific salmon every year, greatly enhancing the state's common property fisheries (Heard 2012). The initial intent of Alaska's hatchery production was to provide maximum benefit to the public through fisheries enhancement (Alaska Constitution, Article VIII Sections 2 and 4), but modern legislation also recognizes (and calls for the avoidance of) the risks to wild populations created by hatchery production (5 AAC 40.860(3)). Although preventative measures are taken to avoid potentially harmful hatchery-wild interactions (i.e. altering the timing and location of hatchery releases), the straying of hatchery-origin fish into streams throughout Alaska (Brenner et al. 2012, Lin et al. 2008, Gilk et al. 2004, Mortensen et al. 2002, Wertheimer et al. 2000, Hard and Heard 1999, Sharp et al. 1994) may indicate that hatchery-wild interactions occur despite our best mitigation efforts. In some cases (e.g. stocking salmon for recreational angling opportunities) releasing sterile, triploid salmon is an effective strategy for minimizing the potential harm caused by hatchery-wild interactions.

Given the mass numbers of hatchery fish being released into wild systems, is triploid induction a feasible method to reduce the overall risk of hatchery-wild interactions in Alaska? The two most important factors to consider when discussing the practical use of triploid technology is 1) the scale of production and 2) the goal of the hatchery program it will be applied to.

Although a majority of hatchery-wild interactions involve fish produced by private, non-profit hatcheries, triploid induction may not be a feasible tool for this sector

of the Alaskan hatchery industry. The certification requirements for releasing triploid fish in Alaska (99% sterility) is high enough that it can be considered logistically impractical and cost prohibitive for facilities that stock more than a handful of streams with triploids (Kozfkay et al. 2006). The major benefit of using triploid fish is the reduction of potential hybridization between hatchery and wild fish, which is reduced by an amount equal to the triploidization rate (Kozfkay et al. 2006). While reducing the hybridization potential is a considerable benefit, a program cannot be evaluated on benefits alone. There are many costs to consider before determining the efficacy of large-scale triploid induction.

Embryonic and juvenile mortality is usually higher in triploids, which is likely caused by a combination of increased handling during the induction process and disruptions in cellular development and function (Johnson et al. 2004). For example, the observed survival to the emergent fry stage for diploid Control groups in this study was 34% greater on average than the survival of triploidized groups, although there was variability across treatment groups (Table 2.9; Figure 2.4). To maintain production levels of triploidization, facilities would need to mitigate the costs of increased mortality by fertilizing approximately 5 to 15% more eggs (Kozfkay et al. 2006). Increasing the number of fertilized eggs may require the use of additional brood fish (removing fish from the fishery), and has the potential to increase rearing and feeding costs (Kozfkay et al. 2006). In addition to rearing and feeding costs, the equipment used in triploid induction is expensive. One hydrostatic pressure chamber cost upwards of $15 000 in 2006 (Kozfkay et al. 2006) and can hold a maximum of approximately 6 000 Chinook

salmon eggs. Although a per/egg production cost for triploidization is not available, mixed-sex triploid rainbow trout eggs were sold for approximately $0.02 / egg, and all-female triploid rainbow trout eggs were sold for approximately $0.03 / egg in 2006 (Kozfkay et al. 2006). More important than the production costs, are the costs that would accrue if triploid fish do not meet the performance goals of large-scale private, non-profit hatcheries.

The purpose of private, non-profit hatchery production is to supplement common property fisheries (mainly commercial fisheries) in Alaska (Heard 2014). Commercial fisheries are located in marine waters, so it is imperative that privately produced hatchery fish migrate to the ocean following release. Little information exists regarding the performance of triploids when released into natural environments (Fraser et al. 2012), or on sterilized salmonid behavior in general (Utter et al. 1993). It has been hypothesized that triploid salmonids have impaired sensory perception (Tiwary et al. 2004), and reduced olfactory bulbs (Fraser et al. 2012). These developmental impairments may prevent the migration of triploid salmonids, rendering them useless in completing the goals of private, non-profit hatcheries.

Although the Alaska Department of Fish and Game Sport Fish Division successfully implements triploid technology, extending these efforts to the private non-profit hatcheries may not be possible. The differences between the scale and purpose of Sport Fish and private non-profit hatchery production are large. It is feasible to produce triploid fish at relatively small scales (on the order of hundreds of thousands) for recreational angling purposes (fish that cannot and should not migrate). However, the

costs of producing triploids salmonids in private non-profit hatcheries (including increased productions costs and the costs of producing fish that do not meet performance goals) make this technology impractical to implement under the current framework of Alaskan hatchery production.

REFERENCES

Aegerter, S., and B. Jalabert (2004). Effects of post-ovulatory oocyte ageing and temperature on egg quality and on the occurrence of triploid fry in rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*. 231: 59-71.

Alaska Department of Fish and Game. *A basic guide to aging and identification of Pacific salmon scales* [PowerPoint slides]. Retrieved from http://www.ADF&G.alaska.gov/static/education/educators/curricula/pdfs/salmon_scales_guide_aging_identification.pdf

Alaska Department of Fish and Game. Division of Fisheries, Rehabilitation, Enhancement and Development. (1983). A Review of Alaska's Fisheries Rehabilitation, Enhancement and Development (FRED) Program 1971 - 1982.

Alaska Department of Fish and Game. Division of Fisheries, Rehabilitation, Enhancement and Development. (1989). Background of the Genetics Policy of the Alaska Department of Fish and Game.

Alaska Department of Fish and Game (2015). *Fish Count Data Search*. Retrieved from https://www.ADF&G.alaska.gov/sf/FishCounts/

Alaska Department of Fish and Game. Genetic Policy Review Team. (1985). Alaska Department of Fish and Game Genetic Policy.

Alaska Department of Fish and Game (2015). *Hatcheries and Stocking*. Retrieved from http://www.ADF&G.alaska.gov/index.cfm?ADF&G=SportStockingHatcheriesSearch.main

Alaska Department of Fish and Game. (2013). Lake Stocking Policy for Sport Fish Division.

Alaska Department of Fish and Game (2015). *Our History*. Retrieved from http://www.ADF&G.alaska.gov/index.cfm?ADF&G=about.history

Alaska Department of Fish and Game. "Procedures Manual: Triploid Induction and Ploidy Certification." Procedures Manual, Alaska Department of Fish and Game, 2013.

Alaska Department of Fish and Game (2015). *Welcome to the Board of Fisheries*. Retrieved from http://www.ADF&G.alaska.gov/index.cfm?ADF&G=fisheriesboard.main

Atkins DC, Baldwin SC, Zheng C, Gallop RJ, and C Neighbors (2013). A tutorial on count regression and zero-altered count models for longitudinal substance use data. *Psychology of Addictive Behavior*. 27: 166-177.

Barrieu, Pauline and Bernard Sinclair-Desgagne (2006). On Precautionary Policies. *Management Science*, 52: 1145-1154. doi: 10.1287/mnsc.1060.0527

Bates D., Maechler M., Bolker B. and Walker S. (2014). *lme4: Linear mixed-effects models using Eigen and S4*. R package version 1.1-7, <URL: http://CRAN.R-project.org/packages=lme4>.

Benfey, Tillmann (1998). *Use of Triploid Atlantic Salmon (Salmo salar) for Aquaculture* (Canadian Stock Assessment Secretariat Research Document). Ottawa, ON: Fisheries and Oceans.

Belyaev, VA, Probatov, NS, Zolotukhin, SF, and TN Mironova (2000). Current issues in

salmon management in Khabarovsk Krai. *In: Proceedings of the Russian-American Conference on Salmon Conservation*. Khabarovsk, October 4-8, 1999. Pp 25.

Benfey, Tillmann J. (2001). Use of sterile triploid Atlantic salmon (*Salmo salar* L.) for aquaculture in New Brunswick, Canada. *Journal of Marine Science*. doi: 10.1006/jmsc.2000.1019

Blanc, J.M., Maunas, P. and F. Vallee (2005). Effect of triploidy on paternal and maternal variance components in brown trout, *Salmo trutta* L. *Aquaculture Research*. 36: 1026-1033.

Bobe, Julien and Catherine Labbe (2009). Egg and sperm quality in fish. *General and Comparative Endocrinology*. 165:535-548.

Botz, J., R. Brenner, G. Hollowell, B. Lewis, and S. Moffitt (2008). 2006 Prince William Sound Area finfish management report. Alaska Department of Fish and Game, Fishery Management Report No. 08-30, Anchorage.

Brenner, Richard E., William S. Grant and Steve D. Moffitt (2012). Straying of hatchery salmon in Prince William Sound, Alaska. *Environmental Biology of Fishes*. doi: 10.1007/s10641-012-9975-7

Bromage, N., Jones, J., Randall, C., Thursh, M., Davies, B., Springate, J.R.C., Duston, J., and G. Barker (1992). Broodstock management, fecundity, egg quality and the timing of egg production in the rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*. 100: 141-166.

Chourrout, D. (1980). Thermal induction of diploid gynogenesis and triploidy in the eggs of the rainbow trout (*Salmo gairdneri Richardson*). *Reproduction Nutrition Development*, 20.

Ciereszko, A., Wojtczak, M., Dietrich, G.J., Kuzminski, H., and S. Doboxz (2009). A lack of consistent relationship between distribution of lipid droplets and egg quality in hatchery-raised rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*. 289: 150-153.

Cooney RT and RD Brodeur (1998). Carrying capacity and North Pacific salmon production: stock enhancement implications. *Bulletin of Marine Science*. 62: 443-464.

Diaz, N.F., Iturra, P., Veloso, A., Estay, F., and N. Colihueque (1993). Physiological factors affecting triploid production in rainbow trout, *Oncorhynchus mykiss*. Aquaculture. 114:33-40.

Dunham, Rex A. (2004). R.A. Dunham; Chapter 3 Polyploidy. *Aquaculture and fisheries biotechnology.*

Ford, M. (2002). Selection in captivity during supportive breeding may reduce fitness in the wild. *Conservation Biology*. 16: 815-825.

Fraser, T.W.K., Fjelldal, P.G., Skjaeraasen, J.E., Hansen, T. and I. Mayer (2012). Triploidy alters brain morphology in pre-smolt Atlantic salmon *Salmo salar*: possible implications for behaviour. *Journal of Fish Biology*. 81(7): 2199-2212.

Fraser, Thomas W.K., Fjelldal, P.G., Hansen, T. and I. Mayer (2012). Welfare considerations of triploid fish. *Reviews in Fisheries Science*. 20(4): 192-211.

Frankhauser, G., and R.C. Watson (1942). Heat-induced triploidy in the newt, *Triturus virdescens*. Proceedings of the National Academy of Sciences U.S.A. 28: 436-440.

Gelman, A, Jakulin, A, Pittau, MG, Pittau and Yu-Sung Su (2008). A weakly informative default prior distribution for logistic and other regression models. *Annals of Applied Statistics*. 2(4): 1360-1383.

Gilk S., Wang, I., Hoover, C., Smoker, W., Taylor, S., Gray, A., and A. Gharret (2004). Outbreeding depression in hybrids between spatially separated pink salmon, *Oncorhynchus gorbuscha*, populations: marine survival, homing ability, and variability in family size. *Environmental Biology of Fishes*. 69: 287-297.

Grant, Stewart W. (2011) Understanding the Adaptive Consequences of Hatchery-Wild Interactions in Alaska Salmon. *Environmental Biology of Fishes*. doi: 10.1007/s10641-011-9929-5

Hadfield, Jarrod (2014). MCMCglmm Course Notes. May 24 2014.

Hadfield, Jarrod (2010). MCMC Methods for Multi-Response Generalized Linear Mixed Models: The MCMCglmm R Package. Journal of Statistical Software, 33(2), 1-22. http://www.jstatsoft.org/v33/i02

Hard, J. and W. Heard (1999). Analysis of straying variation in Alaskan hatchery Chinook salmon (*Oncorhynchus tshawytscha*) following transplantation. *Canadian Journal of Fisheries and Aquatic Sciences*. 56: 578-589.

Heard, William (2014). Alaska Salmon Enhancement: A Successful Program for Hatchery and Wild Stocks. National Oceanic and Atmospheric Administration/National Marine Fisheries Service-Auke Bay Laboratory. Juneau.

Heard, William (2012). Overview of salmon stock enhancement in southeast Alaska and compatibility with maintenance of hatchery and wild stocks. *Environmental Biology of Fishes*. 94: 273-283.

Heath, D., Fox, C., and John Heath (1999). Maternal effects on offspring size: variation through early development of Chinook salmon. *Evolution*. 53(5): 1605-1611.

Hino H, Iwai T, Yamashita M, Ueda H (2007). Identification of an olfactory imprinting-related gene in the lacustrine sockeye salmon, *Oncorhynchus nerka*. *Aquaculture*, 273: 200-208.

Huusko A and T Vehanen (2011). Do hatchery-reared brown trout affect the growth and habitat use of wild congeners? *Fisheries Managemetn and Ecology*. 18: 258-261.

Johnson, Rachel M., Mark J. Shrimpton, John W. Heath and Daniel D. Heath (2003). Family, induction methodology and interaction effects on the performance of diploid and triploid Chinook salmon (*Oncorhynchus tshawytscha*). *Aquaculture*, 234. doi: 10.1016/j.aquaculture.2004.01.024

Kamiya, Eriko, Shinji Izumiyama, Masahiko Nishimura, James Mitchell, and Kazuhiro Kogure (2006). Effects of fixation and storage on flow cytometric analysis of marine bacteria. *Journal of Oceanography*, 63: 101-112.

Keefer, Matthew and Christopher Caudill (2013). Homing and straying by anadromous salmonids: a review of mechanisms and rates. *Reviews in Fish Biology and Fisheries*, doi: 10.1007/s11160-013-9334-6

Kron, Tom (1983). Proceedings of the twenty-second U.S.-Japan aquaculture panel symposium. Prince William Sound salmon enhancement programs and considerations relative to wild stocks. Homer, Alaska

Lahnsteiner, F. (2000). Morphological, physiological and biochemical parameters characterizing the over-ripening of rainbow trout eggs. *Fish Physiology and Biochemistry*. 23: 107-118.

Lichatowich, James (1999). *Salmon Without Rivers: A history of the Pacific Salmon crisis*. Washington D.C.: Island Press.

Lin, J., Quinn, T., Hilborn, R., and L. Hauser (2008). Fine-scale differentiation between sockeye salmon ecotypes and the effect of phenotype on straying. *Heredity*. 101: 341-350.

Loopstra, D., and P.A. Hansen (2008). Induction of triploidy in Chinook salmon (Oncorhynchus tshawytscha) using hydrostatic pressure. Alaska Department of Fish and Game, Fishery Data Series No. 08-23, Anchorage.

Malison, J.A., Procarione, L.S., Held, J.A., Kayes, T.B., Amundson, C.H. (1993). The influence of triploidy and heat and hydrostatic pressure shocks on the growth and reproductive development of juvenile yellow perch (*Perca flavescens*). *Aquaculture*. 116: 121-133.

Mansour, N., Lahnsteiner, F., and R.A. Patzer (2007). Distribution of lipid droplets is an indicator of egg quality in brown trout, *Salmo trutta fario*. *Aquaculture*. 273: 744-747.

Mortensen, D., Wertheimer, A., Maselko, J., and S. Taylor (2002). Survival and straying of Auke Creek, Alaska pink salmon marked with coded wire tags and thermally induced otolith marks. *Transaction of the American Fisheries Society*. 131: 14-26.

Moyle, Peter and Joseph Cech (2004). *Fishes: An Introduction to Ichthyology*. San Francisco, CA: Pearson Benjamin Cummings.

National Oceanic and Atmospheric Administration. National Marine Fisheries Service (2015). *Basic Questions about Aquaculture*. Retrieved from http://www.nmfs.noaa.gov/aquaculture/faqs/faq_aq_101.html

National Oceanic and Atmospheric Administration. National Marine Fisheries Service (2014). *Endangered and Threatened Marine Species under NMFS' Jurisdiction*. Retrieved from http://www.nmfs.noaa.gov/pr/species/esa/listed.htm#fish

O'Keefe, R and T. Benfey, (1995). The Production of Triploid and Sex-Reversed Arctic Charr (Salvelinus alpinus). *Aquaculture,* 137.

Pearsons, Todd N. (2008). Misconception, reality, and uncertainty about ecological interactions and risks between hatchery and wild salmonids. *Fisheries*. 33(6): 278-290.

Quinn, TP (1993). A review of homing and straying of wild and hatchery produced salmon. *Fisheries Research*, 18:29-44.

R Core Team (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Roppel, Patricia (1982). *Alaska's Salmon Hatcheries 1891-1959*. U.S.A: Library of

Congress.
Ruggerone GT, Zimmermann M, Meyers KW, Nielsen JL, and DE Rogers (2003). Competition between Asian pink salmon (*Oncorhynchus gorbuscha*) and Alaskan sockeye salmon (*O. nerka*) in the North Pacific Ocean. *Fisheries Oceanography*. 12: 209-219.
Rutz, David S. and Craig C. Baer (1996). Comparative performance of stocked mixed-sex and all-female rainbow trout and diploid and triploid coho salmon in landlocked lakes in Southcentral Alaska, 1994 and 1995. Alaska Department of Fish and Game, Fishery Data Series No. 96-23, Anchorage.
Seeb, J.E., C. Habicht, and G.D. Miller (1983). Proceedings of the twenty-second U.S.-Japan aquaculture panel symposium. Use of Triploids for Gene Conservation of Salmonids. Homer, Alaska.
Sharp, D., Sharr, S., and C. Peckham (1994). Homing and straying patterns of coded wired tagged pink salmon in Prince William Sound. *Proceedings of the 16th Northeast Pacific chum and pink salmon workshop*. Alaska Sea Grant College Program. 77-82.
Solar, I.I. and E.M. Donaldson (1985). Studies on genetic and hormonal sex control in a domesticated rainbow trout. II. Use of methyltestosterone for masculinization and sterilization cultured rainbow trout (*Salmo gairdneri Richardson*). Canadian Technical Report of Fisheries and Aquatic Sciences. 1380: 9.
Solar, I.I., E.M. Donaldson, and G.A. Hunter (1984). Induction of Triploidy in Rainbow Trout (*Salmo gairdneri Richardson*) by Heat Shock, and Investigation of Early Growth. *Aquaculture* 42: 57-68.
Sutterlin, A. M., J. Holder and T. J. Benfey (1987). Early Survival Rates and Subsequent Morphological Abnormalities in Landlocked, Anadromous and Hybrid (Landlocked X Anadromous) Diploid and Triploid Atlantic Salmon. *Aquaculture, 64*.
Taylor JF, Preston AC, Guy D, and H Migaud (2011). Ploidy effects on hatchery survival, deformities, and performance of Atlantic salmon (*Salmo salar*). *Aquaculture*. 315: 61-68.
Taranger, G., Karlsen, O., Bannister, R., Glover, K., Husa, V., Karlsbakk, E., Kvamme, B., Boxaspen, K., Bjorn, P., Finstad, B., Madhun, A., Morton, H., and Terje Svasand (2015). Risk assessment of the environmental impact of Norwegian Atlantic salmon farming. *ICES Journal of Marine Science*, 72(3): 997-1021.
Teskeredzic, E., E.M. Donaldson, Z. Teskeredzic, I.I. Solar and E.M. Lean (1993). Comparison of Hydrostatic Pressure and Thermal Shocks to Induce Triploidy in Coho Salmon (*Oncorhynchus kisutch*). *Aquaculture, 117*.
Tiwary, Basant, R. Kirubagaran and Arun Ray (2004). The Biology of Triploid Fish. *Reviews in Fish Biology and Fisheries, 14*. doi: 10.1007/s11160-004-8361-8
University of Alaska Southeast Fisheries Technology Program. *Extraction of otoliths: "chop" method for whole salmon*. Retrieved from http://elearning.uaf.edu/cc/otolith/extraction/chop.htm
Unwin MJ and GJ Glova (1997). Changes in life history parameters in a naturally

spawning population of Chinook salmon (*Oncorhynchus tshawytscha*) associated with releases of hatchery-reared fish. *Canadian Journal of Fisheries and Aquatic Sciences*. 54: 1235-1245.

Utter, Fred, Kjetil Hindar, Nils Ryman. "Genetic Effects of Aquaculture on Natural Salmonid Populations". *Salmon Aquaculture*. Ed. Knut Heen, Ed. Robert L. Monahan, Ed. Fred Utter. New York: Halsted Press, 1993. 144-159. Print.

Utter, Fred, Orlay Johnson, Gary Thorgaard and Peter Rabinovitch (1983). Measurement and Potential Applications of Induced Triploidy in Pacific Salmon. *Aquaculture, 35*.

Vercessi, L. (2012). Alaska salmon fisheries enhancement program 2011 annual report. Alaska Department of Fish and Game, Fishery Management Report No. 12-04, Anchorage.

Vercessi, L. (2013). Alaska salmon fisheries enhancement program 2012 annual report. Alaska Department of Fish and Game, Fishery Management Report No. 13-05, Anchorage.

Vercessi, L. (2014). Alaska salmon fisheries enhancement program 2013 annual report. Alaska Department of Fish and Game, Fishery Management Report No. 14-12, Anchorage.

Vercessi, L. (2015). Alaska salmon fisheries enhancement program 2014 annual report. Alaska Department of Fish and Game, Fishery Management Report No. 15-15, Anchorage.

Weber ED, and DK Faush (2003). Interactions between hatchery and wild salmonids in streams: differences in biology and evidence for competition. *Canadian Journal of Fisheries and Aquatic Sciences*. 69: 1018-1036.

Wertheimer AC, Heintz RA, Thedinga JF, Maselko JM, Rice SD (2000). Straying of adult pink salmon from their natal stream following embryonic exposure to weathered *Exxon Valdez* crude oil. *Transactions of the American Fisheries Society*, 129: 989-1004.

White, B. (2005). Alaska salmon enhancement program 2004 annual report. Alaska Department of Fish and Game, Fishery Management Report No. 05-19, Anchorage.

White, B. (2006). Alaska salmon enhancement program 2005 annual report. Alaska Department of Fish and Game, Fishery Management Report No. 06-19, Anchorage.

White, B. (2007). Alaska salmon enhancement program 2006 annual report. Alaska Department of Fish and Game, Fishery Management Report No. 07-04, Anchorage.

White, B. (2008). Alaska salmon enhancement program 2007 annual report. Alaska Department of Fish and Game, Fishery Management Report No. 08-03, Anchorage.

White, B. (2009). Alaska salmon enhancement program 2008 annual report. Alaska Department of Fish and Game, Fishery Management Report No. 09-08, Anchorage.

White, B. (2010). Alaska salmon enhancement program 2009 annual report. Alaska Department of Fish and Game, Fishery Management Report No. 10-05, Anchorage.

White, B. (2011). Alaska salmon enhancement program 2010 annual report. Alaska Department of Fish and Game, Fishery Management Report No. 11-04, Anchorage.

White, B. (2011). Alaska salmon enhancement program 2010 annual report. Alaska Department of Fish and Game, Fishery Management Report No. 07-04, Anchorage.

Wickwire, R (2000). Salmonid triploiding experiment. Alaska Department of Fish and Game. Found at www.kokaneepower.org/articles/triploid.pdf

Withler, R.E., Beacham, T.D., Solar, I.I., Donaldson, E.M., 1995. Freshwater growth, smolting, and marine survival and growth of diploid and triploid coho salmon (*Oncorhynchus kisutch*). *Aquaculture*. 136: 91-107.

Zaporozhets, OM and GV Zaporozhets (2004). Interaction between hatchery and wild Pacific salmon in the Far East of Russia: A review. *Reviews in Fish Biology and Fisheries*. 14: 305-319.

Zuur, AF., Ieno, EN., Walker, NJ., Saveliev, AA., and GM Smith (2009). Mixed Effects Models and Extensions in Ecology with R. New York, NY: Springer Science & Business Medial LLC.

TABLES

Chapter 1

Table 1.1- Specific examples of drivers and mechanisms of hatchery-wild interactions

Driver	Definition	Mechanisms	Management Questions	Proposed Preventative Measure	References
Straying	Adult migration, and attempted reproduction at non natal sites	Imprinting Olfaction Genetics Memory formation Sexual maturation Location of release site Intermingling of wild and hatchery fish in marine environment	What is the natural stray rate?	Increase complexity of rearing environment Match release timing to natural pre-smolt timing, Avoid releasing juveniles near streams containing wild populations	Quinn 1993; Keefer and Caudill 2013; Brenner et al. 2011; Hino et al. 2007; Wertheimer et al 1994
Illegal Transportation of Stocked Fish	Relocation of a stocked fish to an unauthorized waterbody	Anthropogenic sources	What is the current rate of illegal transportation of stocked fish?	Triploidization	ADFG 2013

Table 1.2- Number of escapement monitoring projects located in Southcentral Alaska

Method of Escapement Monitoring	Number of Projects
Weir	26
Tower	7
Creel	2
Sonar	6
Total	41

Species Monitored

	Pink Salmon	Chum Salmon	Sockeye Salmon	Coho Salmon	Chinook Salmon
Number of Projects	21	20	37	21	24

Chapter 2

Table 2.1 - Summary output of generalized linear mixed effects models, which included Replicate effect terms, conducted with R package lme4.

Formula Fitted		Replicate Main Effect Coefficient	S.E	p-value
Survived ~ Treatment + Replicate + (1|Tray) + (1|Position) + (1|Female)		4.39	0.10	0.15
Survived ~ Treatment + Replicate + (1|Position) + (1|Female)		4.24	0.08	< 0.01
Formula Fitted		**Replicate*Treatment Effect Coefficients**	**S.E**	**p-value**
Survived ~ Treatment*Replicate + (1|Tray) + (1|Position) + (1|Female)				
	Replicate*0 Minute Shock	3.92	0.29	0.24
	Replicate*3 Minute Shock	4.38	0.40	0.76
	Replicate*4 Minute Shock	6.85	0.42	< 0.001
	Replicate*5 Minute Shock	2.01	0.31	< 0.001
Survived ~ Treatment*Replicate + (1|Position) + (1|Female)				
	Replicate*0 Minute Shock	3.90	0.22	0.32
	Replicate*3 Minute Shock	3.84	0.32	0.37
	Replicate*4 Minute Shock	7.38	0.31	< 0.001
	Replicate*5 Minute Shock	2.13	0.28	< 0.001

*Analyses only included data from Females 1-4 and Female 8
**All values are reported on the log scale

Table 2.2- Treatment and pooled sample sizes

Treatment	Number of Eggs		Treatment	Number of Eggs		Pooled Sample Size
Female 1 3 Minute Shock	227		Female 1 3 Minute Shock Replicate	196		423
Female 1 4 Minute Shock	257		Female 1 4 Minute Shock Replicate	211		468
Female 1 5 Minute Shock	187		Female 1 5 Minute Shock Replicate	261		448
Female 1 Control	170		Female 1 Replicate Control	273		443
Female 2 3 Minute Shock	240		Female 2 3 Minute Shock Replicate	218		458
Female 2 4 Minute Shock	232		Female 2 4 Minute Shock Replicate	197		429
Female 2 5 Minute Shock	177		Female 2 5 Minute Shock Replicate	228		405
Female 2 Control	279		Female 2 Replicate Control	210		489
Female 3 3 Minute Shock	209		Female 3 3 Minute Shock Replicate	161		370
Female 3 4 Minute Shock	214		Female 3 4 Minute Shock Replicate	169		383
Female 3 5 Minute Shock	221		Female 3 5 Minute Shock Replicate	174		395
Female 3	215		Female	179		394

Control			3 Replicate Control			
Female 4 3 Minute Shock	292		Female 4 3 Minute Shock Replicate	230		522
Female 4 4 Minute Shock	262		Female 4 4 Minute Shock Replicate	230		492
Female 4 5 Minute Shock	271		Female 4 5 Minute Shock Replicate	292		563
Female 4 Control	275		Female 4 Replicate Control	325		600
Female 5 3 Minute Shock	309					309
Female 5 4 Minute Shock	238					228
Female 5 5 Minute Shock	229					229
Female 5 Control	210					210
Female 6 3 Minute Shock	250					250
Female 6 4 Minute Shock	393					393
Female 6 5 Minute Shock	140					140
Female 6 Control	292					292
Female 7 3 Minute Shock	269					269
Female 7 4 Minute Shock	157					157
Female 7 5 Minute	255					255

Shock						
Female 7 Control	262					262
Female 8 3 Minute Shock	315		Female 8 3 Minute Shock Replicate	218		533
Female 8 4 Minute Shock	168		Female 8 4 Minute Shock Replicate	268		436
Female 8 5 Minute Shock	151		Female 8 5 Minute Shock Replicate	247		398
Female 8 Control	269		Female 8 Replicate Control	249		518
Total Egg Sample Size 12,171						

Table 2.3- Egg take and triploid induction sampling schedule

Treatment	Fertilization Time	Time Pressure Shocked	Time of Final Incubation
Female 1 (3 Minute Shock)	14:40	15:24	16:40
Female 1 (4 Minute Shock)	14:40	15:24	16:40
Female 1 (5 Minute Shock)	14:50	15:34	16:50
Female 1 Control	14:50	15:34	16:50
Female 1 (3 Minute Shock) Replicate	15:00	15:44	17:08
Female 1 (4 Minute Shock) Replicate	15:00	15:44	17:08
Female 1 (5 Minute Shock) Replicate	15:10	15:54	17:15
Female 1 Replicate Control	15:10	15:54	17:15
Female 2 (3 Minute Shock)	14:40	15:24	16:40
Female 2 (4 Minute Shock)	14:40	15:24	16:40
Female 2 (5 Minute Shock)	14:50	15:34	16:50
Female 2 Control	14:50	15:34	16:50
Female 2 (3 Minute Shock) Replicate	15:00	15:44	17:08
Female 2 (4 Minute Shock) Replicate	15:00	15:44	17:08
Female 2 (5 Minute Shock) Replicate	15:10	15:54	17:15
Female 2 Replicate Control	15:10	15:54	17:15
Female 3 (3 Minute Shock)	16:00:10	16:44	18:00
Female 3 (4 Minute Shock)	16:00:10	16:44	18:00
Female 3 (5 Minute Shock)	16:10	16:54	18:10
Female 3 Control	16:10	16:54	18:10
Female 3 (3 Minute Shock) Replicate	16:20	17:06	18:20
Female 3 (4 Minute Shock) Replicate	16:20	17:06	18:20
Female 3 (5 Minute Shock) Replicate	17:30	18:14	18:30
Female 3 Replicate Control	17:30	18:14	18:30
Female 4 (3 Minute Shock)	16:00:10	16:44	18:00
Female 4 (4 Minute Shock)	16:00:10	16:44	18:00
Female 4 (5 Minute Shock)	16:10	16:54	18:10
Female 4 Control	16:10	16:54	18:10
Female 4 (3 Minute Shock)	16:20	17:06	18:20

Replicate			
Female 4 (4 Minute Shock) Replicate	16:20	17:06	18:20
Female 4 (5 Minute Shock) Replicate	17:30	18:14	18:30
Female 4 Replicate Control	17:30	18:14	18:30
Female 5 (3 Minute Shock)	17:40	18:24	19:42
Female 5 (4 Minute Shock)	17:40	18:24	19:42
Female 5 (5 Minute Shock)	17:50	18:34	19:53
Female 5 Control	17:50	18:34	19:53
Female 6 (3 Minute Shock)	17:40	18:24	19:42
Female 6 (4 Minute Shock)	17:40	18:24	19:42
Female 6 (5 Minute Shock)	17:50	18:34	19:53
Female 6 Control	17:50	18:34	19:53
Female 7 (3 Minute Shock)	18:00	18:44	20:01
Female 7 (4 Minute Shock)	18:00	18:44	20:01
Female 7 (5 Minute Shock)	18:10	18:54	20:10
Female 7 Control	18:10	18:54	20:10
Female 8 (3 Minute Shock)	18:00	18:44	20:01
Female 8 (4 Minute Shock)	18:00	18:44	20:01
Female 8 (5 Minute Shock)	18:10	18:54	20:10
Female 8 Control	18:10	18:54	20:10
Female 8 (3 Minute Shock) Replicate	18:20	19:04	20:35
Female 8 (4 Minute Shock) Replicate	18:20	19:04	20:35
Female 8 (5 Minute Shock) Replicate	18:30	19:14	20:35
Female 8 Replicate Control	18:30	19:14	20:35

Table 2.4- Results of female carcass examination

Female	Age (years) (freshwater.saltwater)	Origin	Standard Length (cm)	Average Egg Weight (g)	Average Fry Weight (g)
1	5 (1.3)	Fort Richardson	79.06	0.317	0.478
2	5 (1.3)	Fort Richardson	76.84	0.319	0.442
3	5 (1.3)	Fort Richardson	73.66	0.336	0.477
4	5 (1.3)	Fort Richardson	76.20	0.253	0.366
5	5 (1.3)	Fort Richardson	80.96	0.278	0.362
6	5 (1.3)	Fort Richardson	74.93	0.242	0.334
7	5 (1.3)	Fort Richardson	74.93	0.309	0.415
8	5 (1.3)	Wild	79.69	0.315	0.450

Table 2.5- Summary output of predicted survival to the emergent fry stage of development marginalizing across Female and Position effects. Data were analyzed with generalized linear mixed effects models using the R package MCMCglmm.

Fixed Effect Parameter	Posterior Mean*	Median Predicted Survival**	95% Credibility Interval**	
			Lower Bound	Upper Bound
0 Minute Shock	2.4129	0.80	0.58	0.91
3 Minute Shock	-0.8852	0.38	0.21	0.61
4 Minute Shock	-1.4612	0.53	0.32	0.73
5 Minute Shock	0.7197	0.60	0.38	0.78

Random Effect Parameter	Posterior Mean*	95% Credibility Interval**	
		Lower Bound	Upper Bound
Female	3.589	67%	99%
Position	2.70	70%	99%

*linear predictor scale
**probability scale

Table 2.6- Results of ploidy analysis determined using flow cytometry

	Degraded	% Degraded	Undetermined	% Undetermined	Triploid	% Triploid	Total
Control	324	65%	176	35%	0	0%	**500**
3 Minute Shock	208	45%	242	52%	15	3%	**465**
4 Minute Shock	446	45%	455	46%	83	6%	**984**
5 Minute Shock	431	40%	474	44%	168	16%	**1073**
Total	**1409**	**47%**	**1347**	**45%**	**266**	**8%**	

Table 2.7- Summary of predicted survival by Female across Treatments marginalizing across Position effects. Data were analyzed with generalized linear mixed effects models using the R package MCMCglmm.

0 Minute Shock

Random Effect	Predicted Survival		
	Prediction	Min	Max
Female 1	0.91	0.79	0.96
Female 2	0.66	0.49	0.78
Female 3	0.69	0.52	0.81
Female 4	0.53	0.36	0.67
Female 5	0.98	0.92	0.99
Female 6	0.90	0.77	0.95
Female 7	0.83	0.67	0.90
Female 8	0.94	0.85	0.98

3 Minute Shock

Random Effect	Predicted Survival		
	Prediction	Min	Max
Female 1	0.14	0.05	0.30
Female 2	0.03	0.01	0.10
Female 3	0.03	0.01	0.10
Female 4	0.01	0.00	0.06
Female 5	0.36	0.18	0.60
Female 6	0.12	0.04	0.29
Female 7	0.07	0.02	0.19
Female 8	0.20	0.08	0.39

4 Minute Shock

Random Effect	Predicted Survival		
	Prediction	Min	Max
Female 1	0.27	0.11	0.48
Female 2	0.06	0.02	0.19
Female 3	0.07	0.02	0.20
Female 4	0.03	0.01	0.11
Female 5	0.55	0.33	0.76
Female 6	0.25	0.10	0.45
Female 7	0.15	0.05	0.33
Female 8	0.35	0.17	0.58

5 Minute Shock

Random Effect	Predicted Survival		
	Prediction	Min	Max
Female 1	0.34	0.16	0.57
Female 2	0.09	0.03	0.23
Female 3	0.10	0.04	0.25
Female 4	0.05	0.01	0.15
Female 5	0.63	0.42	0.82
Female 6	0.31	0.15	0.53
Female 7	0.20	0.08	0.39
Female 8	0.43	0.24	0.66

*Female estimates reflect the predicted survival marginalizing across Position effects

Table 2.8- Summary of predicted survival by Position across Treatments marginalizing out Female effects. Data were analyzed with generalized linear mixed effects models using the R package MCMCglmm.

0 Minute Shock

Random Effect	Predicted Survival		
	Prediction	Min	Max
Position 1	0.73	0.53	0.85
Position 2	0.52	0.34	0.70
Position 3	0.42	0.24	0.61
Position 4	0.78	0.57	0.89
Position 5	0.84	0.66	0.93
Position 6	0.87	0.69	0.94
Position 7	0.91	0.74	0.96
Position 8	0.88	0.71	0.95
Position 9	0.88	0.71	0.95
Position 10	0.95	0.82	0.98
Position 11	0.92	0.76	0.97
Position 12	0.96	0.84	0.99

3 Minute Shock

Random Effect	Predicted Survival		
	Prediction	Min	Max
Position 1	0.04	0.01	0.14
Position 2	0.01	0.00	0.07
Position 3	0.01	0.00	0.05
Position 4	0.06	0.02	0.16
Position 5	0.09	0.04	0.22
Position 6	0.11	0.04	0.24
Position 7	0.16	0.07	0.30
Position 8	0.12	0.05	0.27
Position 9	0.13	0.05	0.26
Position 10	0.26	0.13	0.42
Position 11	0.17	0.08	0.32
Position 12	0.30	0.17	0.47

4 Minute Shock

Random Effect	Predicted Survival		
	Prediction	Min	Max
Position 1	0.10	0.04	0.23
Position 2	0.04	0.01	0.13
Position 3	0.02	0.01	0.09
Position 4	0.13	0.06	0.27
Position 5	0.19	0.09	0.34
Position 6	0.22	0.11	0.37
Position 7	0.29	0.16	0.45
Position 8	0.24	0.12	0.39
Position 9	0.24	0.12	0.40
Position 10	0.42	0.26	0.59
Position 11	0.31	0.17	0.47
Position 12	0.47	0.31	0.65

5 Minute Shock

Random Effect	Predicted Survival		
	Prediction	Min	Max
Position 1	0.14	0.06	0.28
Position 2	0.06	0.02	0.16
Position 3	0.03	0.01	0.12
Position 4	0.17	0.08	0.32
Position 5	0.25	0.13	0.41
Position 6	0.28	0.15	0.44
Position 7	0.36	0.22	0.52
Position 8	0.30	0.17	0.46
Position 9	0.31	0.17	0.46
Position 10	0.49	0.34	0.67
Position 11	0.38	0.23	0.55
Position 12	0.55	0.39	0.73

*Position estimates reflect the predicted survival marginalizing across Female effects

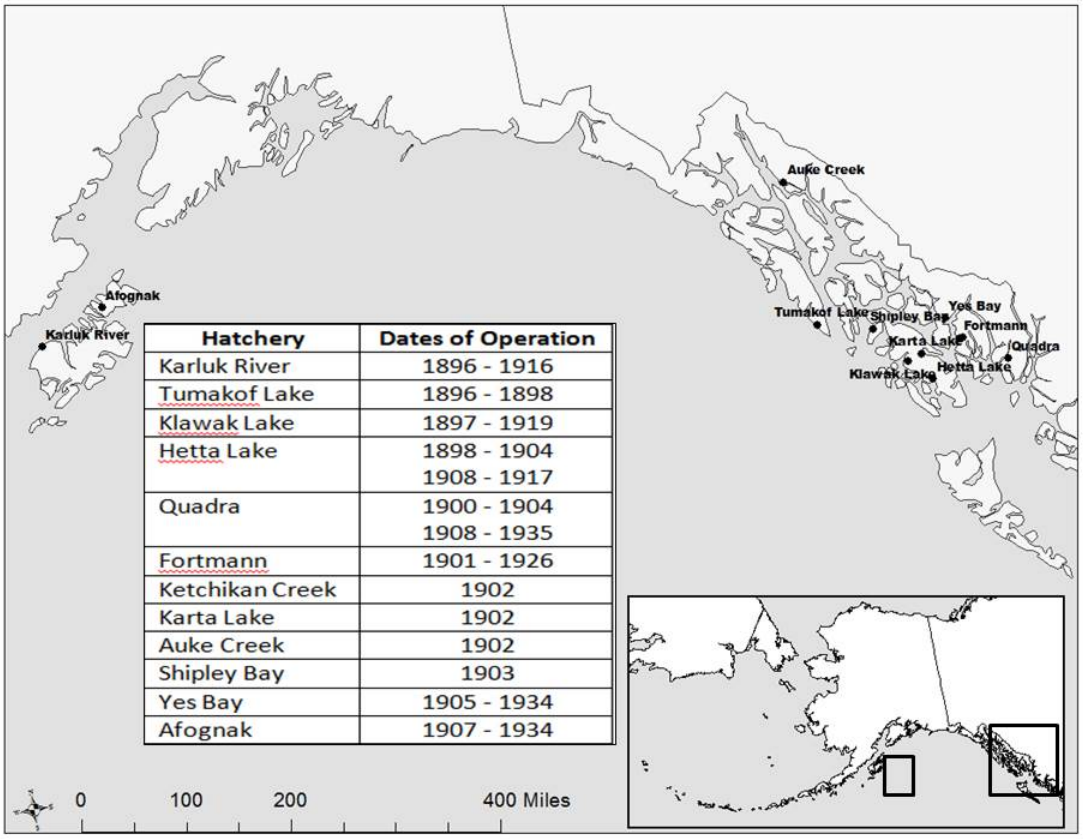
Table 2.9- Summary of observed survival to the emergent fry stage across Treatment groups

Shock Treatment	Percent Survival	Percent Difference from Control Survival
3 Minutes	26	54
4 Minutes	58	22
5 Minutes	52	28
Control	80	
	Average Difference	**35**

FIGURES

Policy Overview

Figure P.1- Map of historic salmon hatchery locations

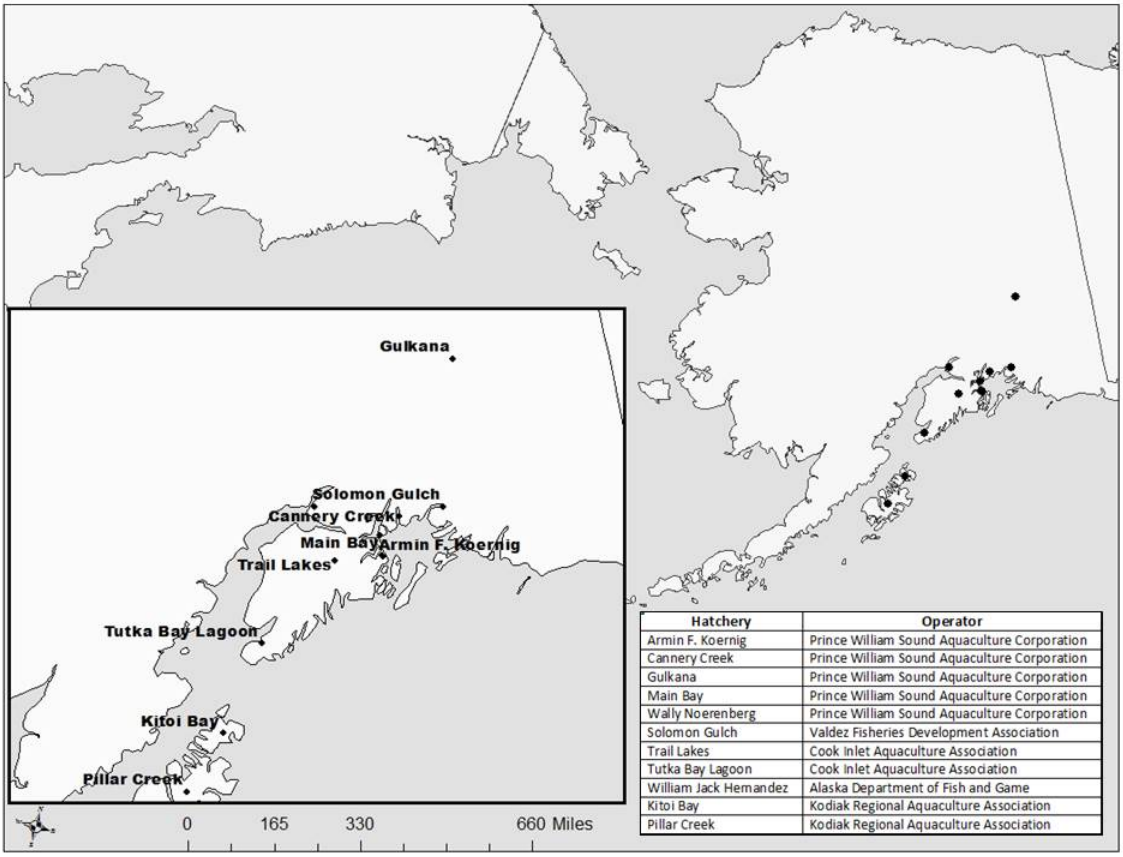


Hatchery	Dates of Operation
Karluk River	1896 - 1916
Tumakof Lake	1896 - 1898
Klawak Lake	1897 - 1919
Hetta Lake	1898 - 1904 1908 - 1917
Quadra	1900 - 1904 1908 - 1935
Fortmann	1901 - 1926
Ketchikan Creek	1902
Karta Lake	1902
Auke Creek	1902
Shipley Bay	1903
Yes Bay	1905 - 1934
Afognak	1907 - 1934

(Created using ESRI ArcMap v. 10.2)

Chapter 1

Figure 1.1- Map of modern salmon hatchery locations



Hatchery	Operator
Armin F. Koernig	Prince William Sound Aquaculture Corporation
Cannery Creek	Prince William Sound Aquaculture Corporation
Gulkana	Prince William Sound Aquaculture Corporation
Main Bay	Prince William Sound Aquaculture Corporation
Wally Noerenberg	Prince William Sound Aquaculture Corporation
Solomon Gulch	Valdez Fisheries Development Association
Trail Lakes	Cook Inlet Aquaculture Association
Tutka Bay Lagoon	Cook Inlet Aquaculture Association
William Jack Hernandez	Alaska Department of Fish and Game
Kitoi Bay	Kodiak Regional Aquaculture Association
Pillar Creek	Kodiak Regional Aquaculture Association

(Created using ESRI ArcMap v. 10.2)

Figure 1.2- Number of juvenile Pacific salmon (reported in millions) released from hatcheries in Southcentral Alaska between 2004 and 2014

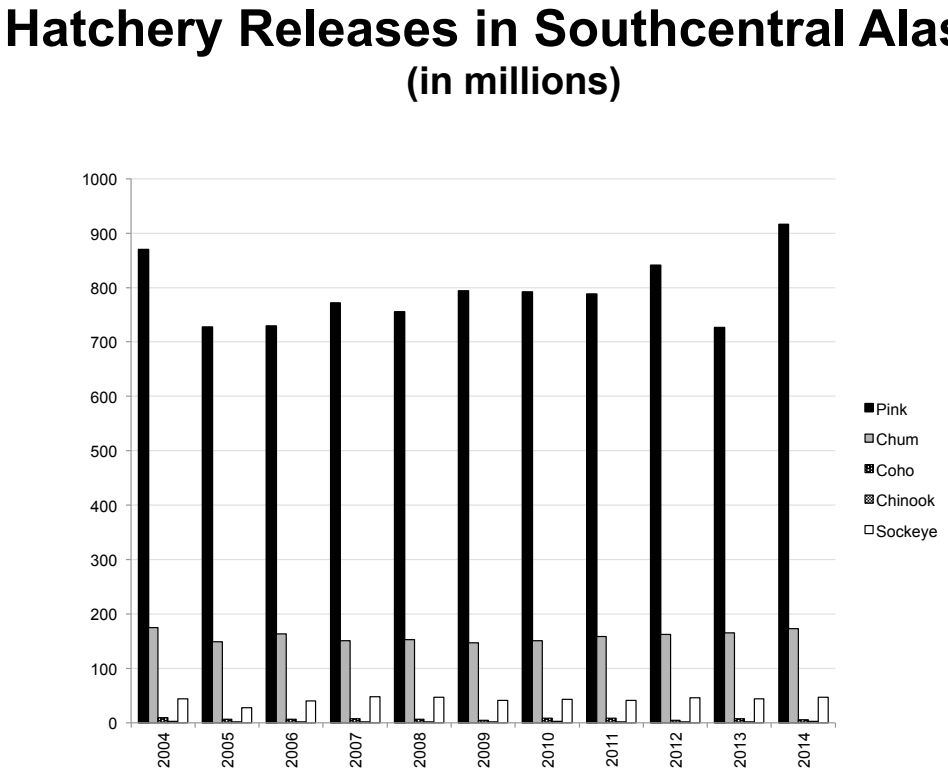


Figure 1.3- Reported escapement of all five species of Pacific salmon from 2009 - 2012. The Ayakulik, Dog Salmon, and Karluk Rivers are all located on Kodiak Island, Alaska. Escapement on all three rivers was assessed using weir technology.

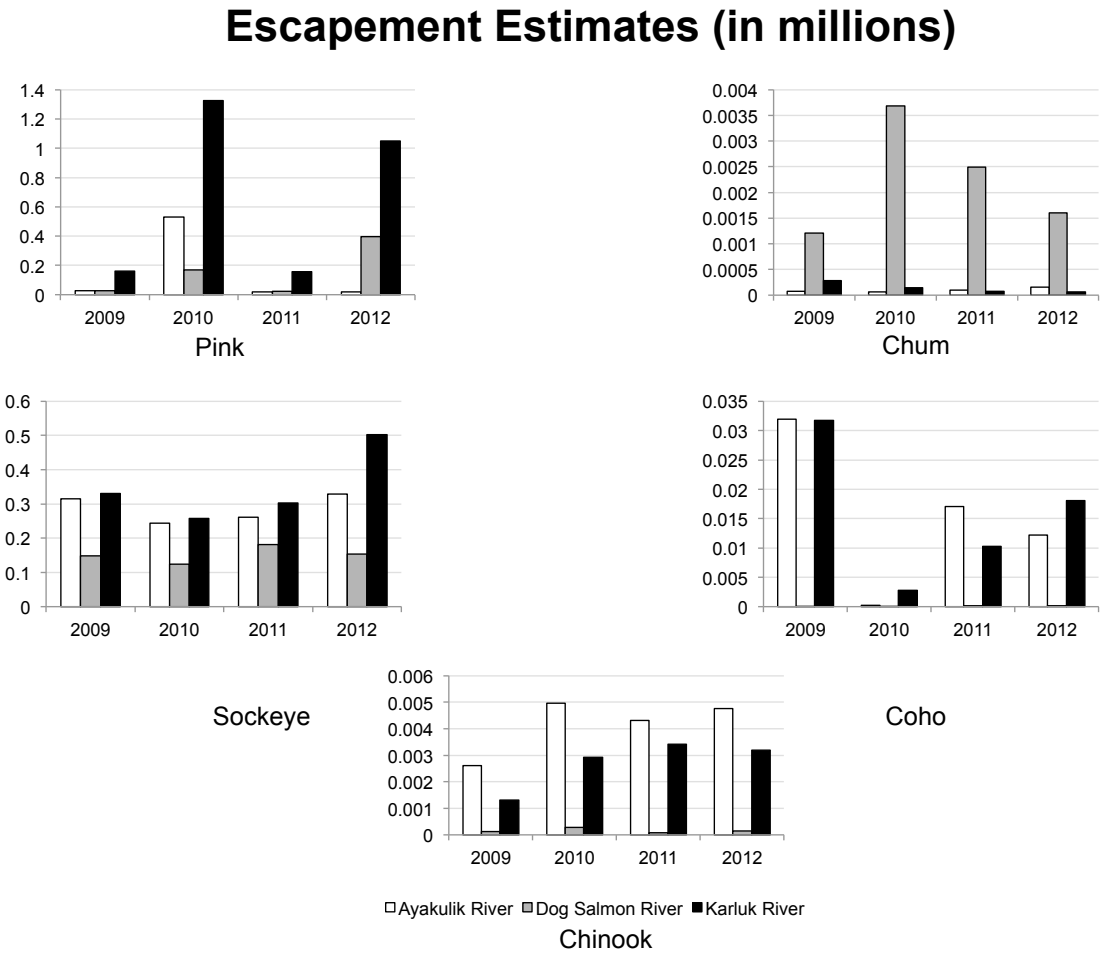
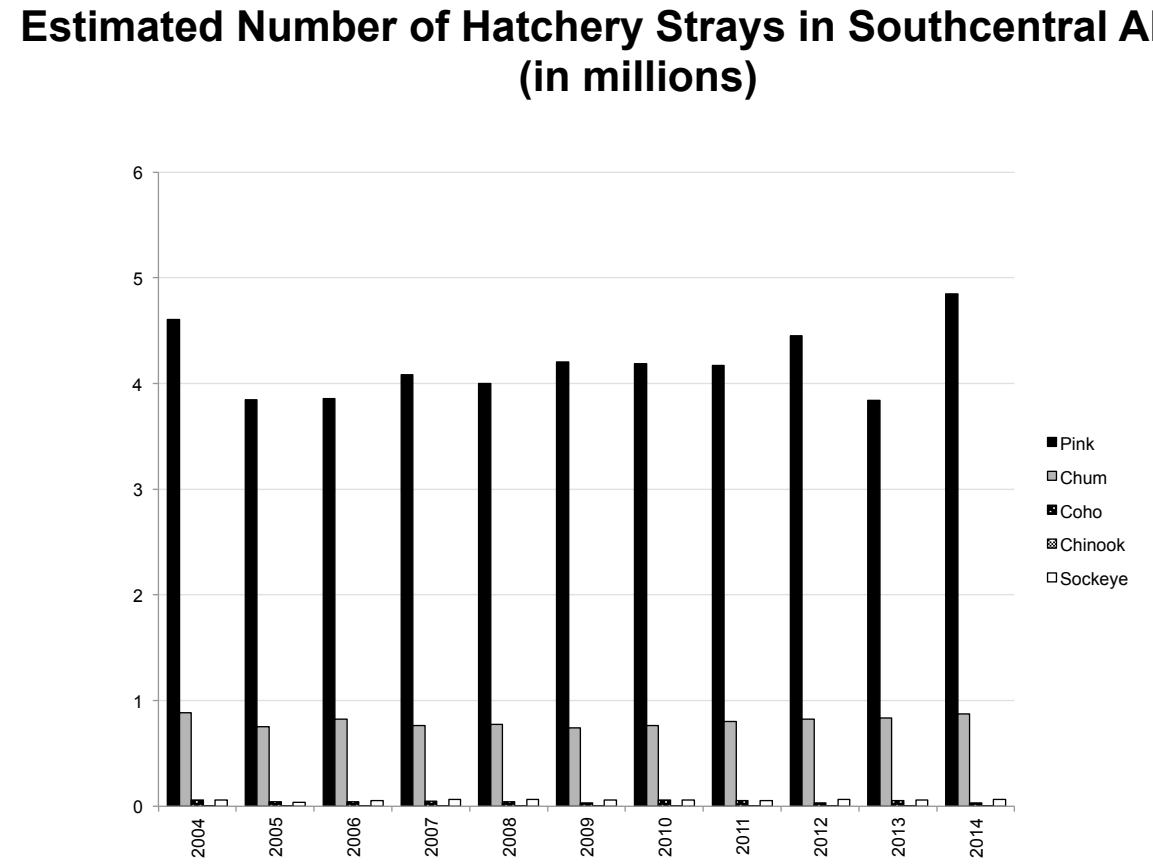


Figure 1.4- Estimated number of salmon hatchery strays (reported in millions) in Southcentral Alaska. Average stray rates (Keefer and Caudill 2012) were applied to the estimated number of salmon hatchery returns (White 2004 - 2010 and Vercessi 2011 - 2014) to obtain estimated number of strays.

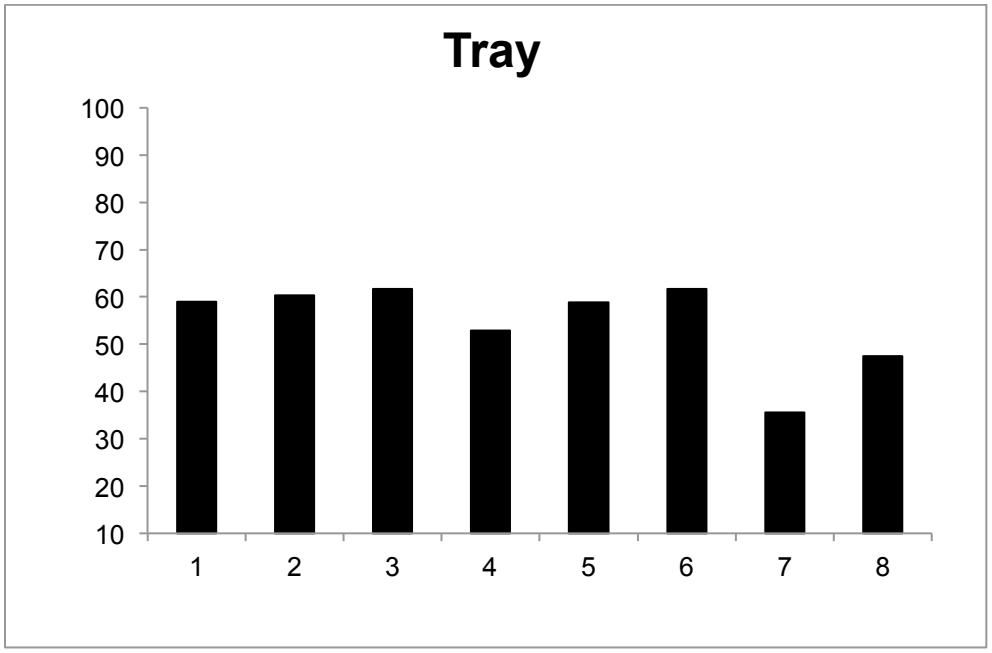


Chapter 2

Figure 2.1- Numbering of incubation trays

Figure 2.2- Numbering of incubation location within trays

Figure 2.3- Observed percent total survival by Tray and Position



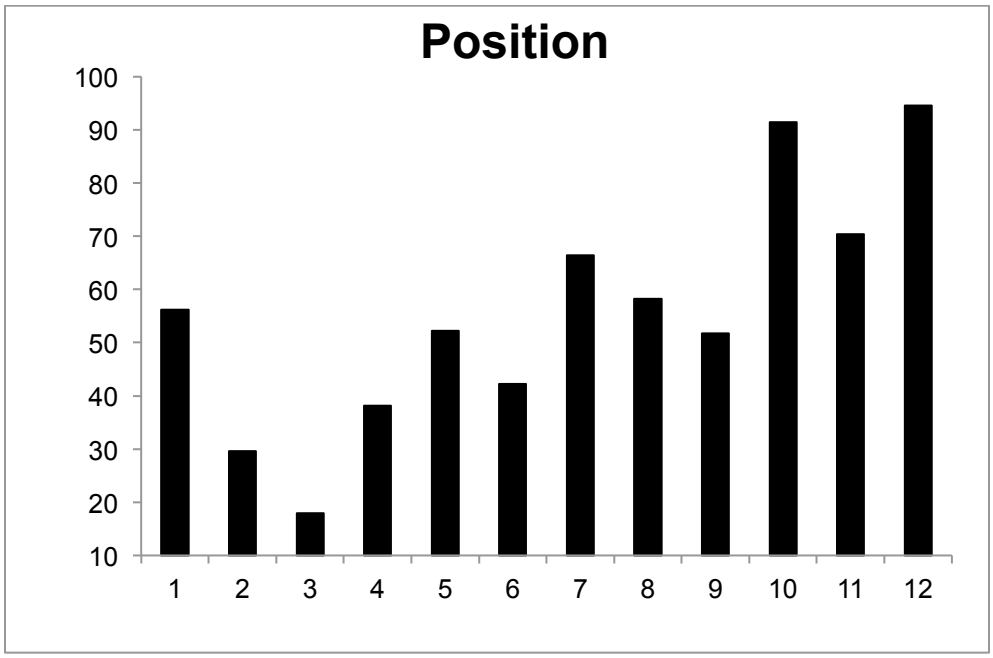


Figure 2.4- Observed percent survival by Treatment at the eyed egg and fry (Total) stages of development

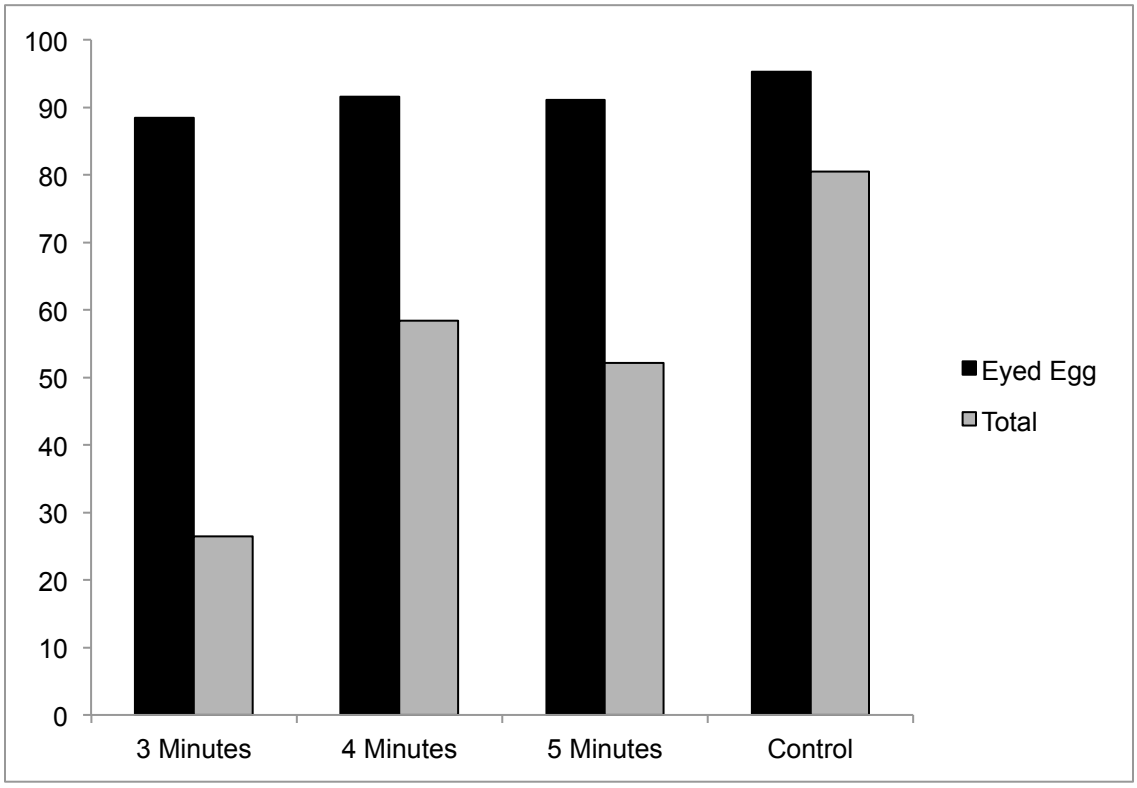


Figure 2.5- Predicted median total survival by Treatment with 95% credibility intervals

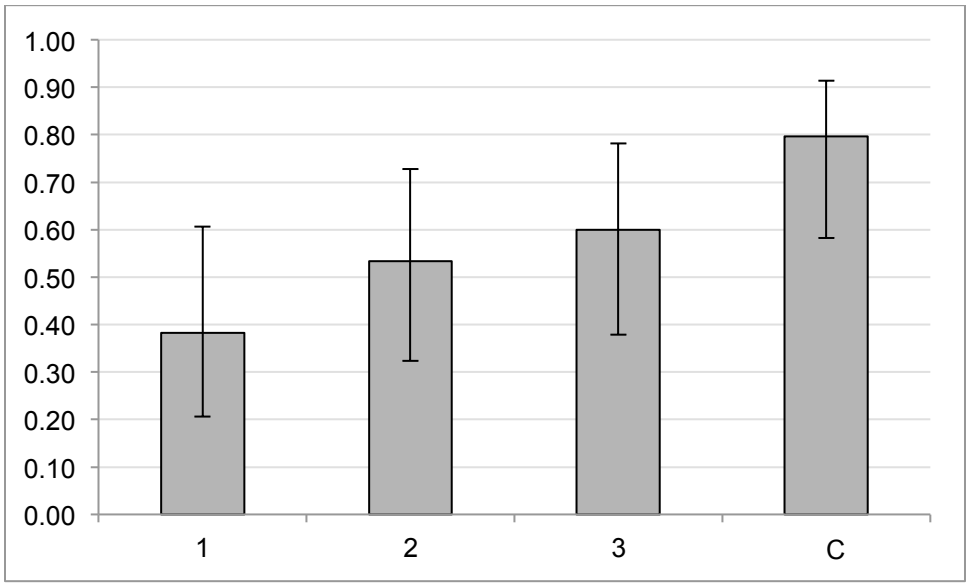


Figure 2.6- Predicted median total survival by Female for each Treatment with 95% credibility intervals marginalizing across Position

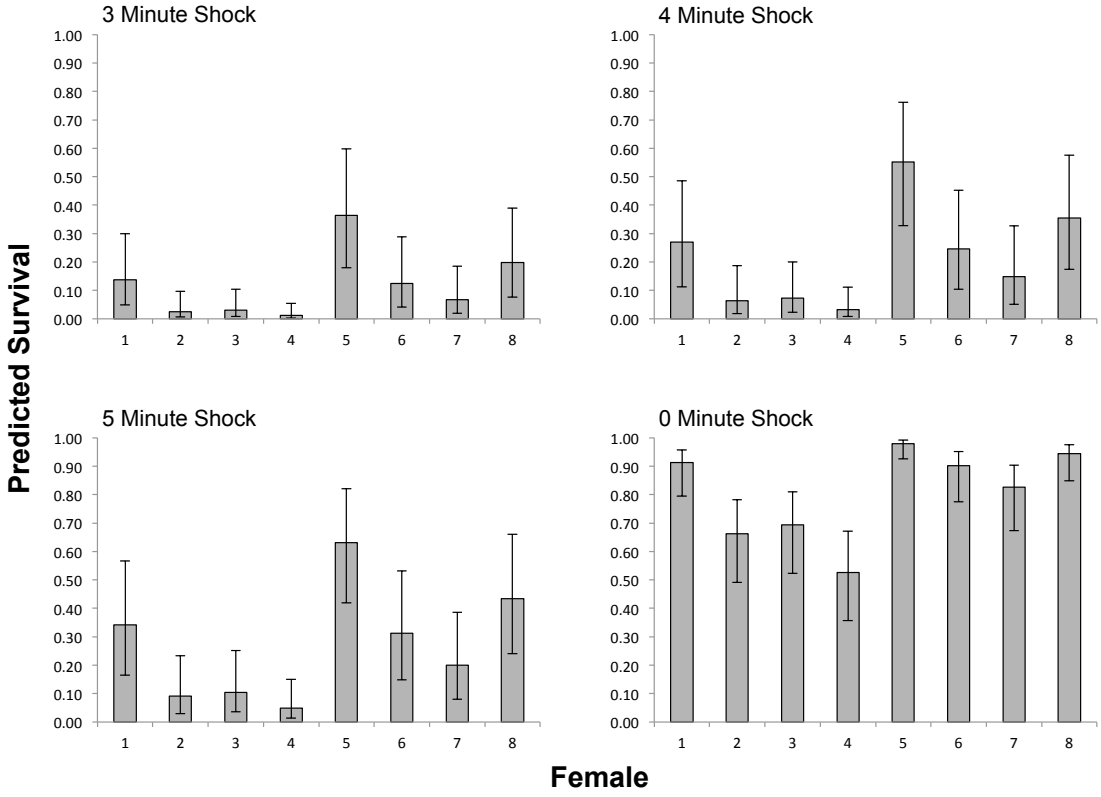


Figure 2.7- Predicted median total survival by Position for each Treatment with 95% credibility intervals marginalizing across Females

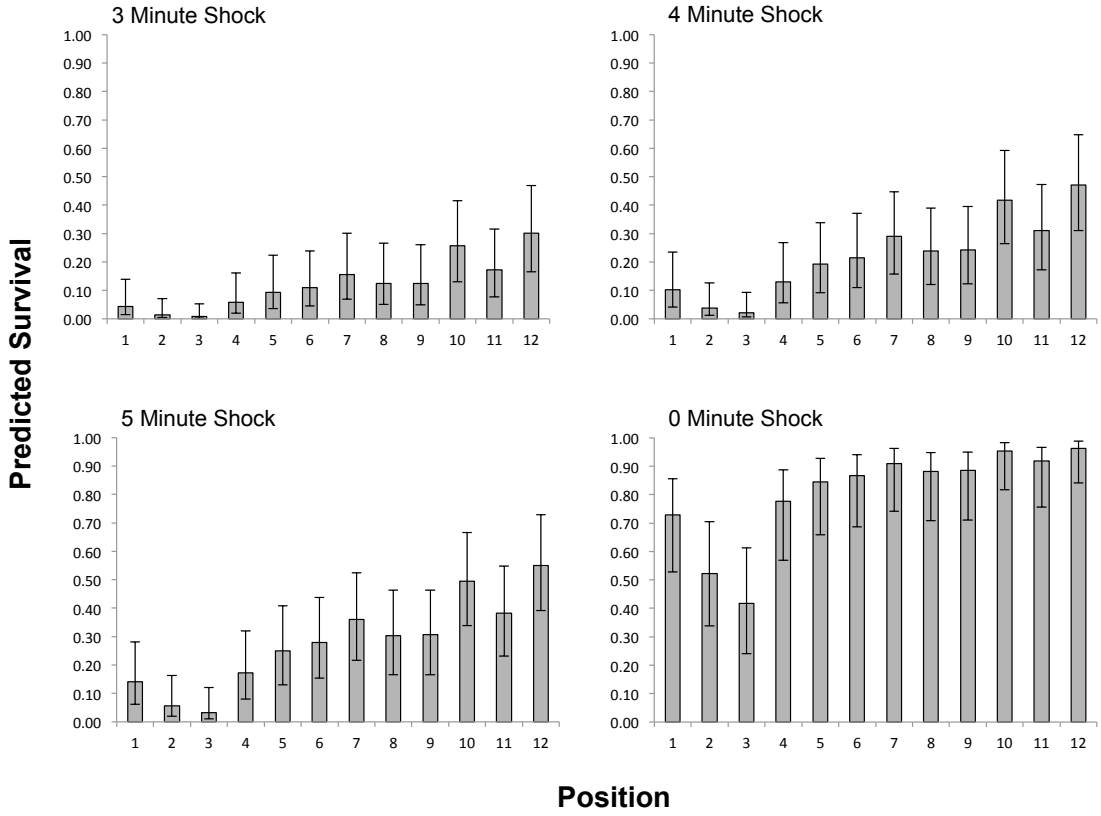


Figure 2.8- Observed percent total survival by Female