AN ABSTRACT OF THE THESIS OF

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Title: Assessing Disease Impacts of Hatcheries on Downstream Salmonids in the Willamette River Basin, Oregon.

Abstract approved: _____

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Hatcheries are often perceived as a source of pathogen amplification, potentially increasing disease risk to free-ranging populations; at the same time, free-ranging fishes may introduce pathogens into hatcheries through untreated water sources. Many pathogens exist naturally within the environment (with the exception of introduced pathogens) and the presence of a pathogen does not guarantee infection or disease (Naish, Taylor III, Levin, Quinn, Winton, Huppert & Hilborn 2007). Infections can be acute, chronic, or asymptomatic, fish may die, recover, or become carriers (Naish *et al.* 2007), and pathogens may be shed from any of these stages (Scottish Executive 2002).

Most salmon and trout hatcheries along the Willamette River Basin, Oregon, USA, utilize an untreated river water supply for their rearing ponds and release this water, untreated, back into the river. This creates a potential for waterborne pathogens present in free-ranging hosts to be transmitted through the water supply to hatchery populations. Moreover, any hatchery epizootic can amplify pathogens and release these into the water, which could have a direct impact on free-ranging populations exposed to those pathogens in hatchery effluent. The goal of this thesis was to assess transmission of the pathogens *Flavobacterium columnare, F. psychrophilum, Aeromonas salmonicida, Renibacterium salmonicida,* and Infectious Hematopoietic Necrosis

Virus (IHNV), at selected hatcheries in the Willamette River Basin. To accomplish this, I considered historical data and hatchery-specific and pathogen-specific factors involved in transmission and disease. Additionally, I conducted sentinel fishes exposures (*Oncorhynchus mykiss* and *O. tshawytscha*) at hatcheries during both epizootics and non-epizootic periods. Naïve sentinel fish were placed in hatchery influents and effluents to determine transmission direction and pathogen prevalence associated with hatcheries.

I found that sentinel fishes developed infections downstream of hatcheries that were undergoing specific bacterial epizootics, or had low levels of pathogen prevalence within the hatchery, but not at any other time. Infections and mortality were due to the same pathogens responsible for hatchery epizootics, indicating the hatchery as a potential source. This may be a limited effect dependent on distance, dilution, and pathogen. The presence of large numbers of returning, congregating adult fishes may also contribute pathogens to the river in hatchery areas. Sentinel fishes held in hatchery influents did not, at any point, become infected with target pathogens, even during hatchery epizootics. Although I was unable to identify the pathogen entry point leading to hatchery epizootics, I determined that pathogen transmission appeared to be dependent on the pathogen, species, and location where sentinel fish were held. This thesis identifies routes and risks of pathogen transmission at selected Oregon hatcheries, with applications to inform state-wide fish health management.

Assessing Disease Impacts of Hatcheries on Downstream Salmonids in the Willamette River Basin, Oregon.

by
Michelle Jakaitis

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Michelle Jakaitis, Author

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CONTRIBUTION OF AUTHORS

Dr. Jerri L. Bartholomew served as the major advisor and was involved in all aspects of the research presented in this dissertation. Dr. Sascha L. Hallett assisted with experimental design and editorial feedback.

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CHAPTER 1: INTRODUCTION

DISEASE INTERACTIONS BETWEEN AQUACULTURE AND FREE-RANGING FISHES

As worldwide fish stocks have been increasingly depleted, aquaculture has become a major source of food and trade worldwide, seen as a viable solution for addressing economic demand and ecological impacts (FAO 2012). Salmon aquaculture, the artificial rearing of fish in net-pens or hatcheries, developed from the mid-1970's onward with a global harvest of 2.2 million tons in 2010 and continuing to increase (FAO 2012). Net-pens are a source of fish that are strictly for commercial harvest while hatcheries are utilized to improve commercial as well as recreational and tribal harvests and to mitigate the decline of wild ("naturally-reared") populations (Brannon et al. 2004; Waples 1999; Amos and Thomas 2002; Kostow 2009). Despite the pervasiveness of salmon aquaculture, this industry has received negative public opinion due to perceived pathogen transmission from net-pens and hatcheries to wild fish (Meyers 2004). At the same time, free-ranging fishes (artificially or naturally-reared) may serve as a reservoir for pathogens that may be introduced into aquaculture facilities through untreated water sources. Reviewed below is the evidence, methods, and implications of bidirectional pathogen transmission.

EVIDENCE FOR PATHOGEN TRANSMISSION BETWEEN FREE-RANGING AND HATCHERY SALMONID STOCKS

Viral, bacterial, and parasitic transmission between free-ranging fishes and aquaculture fishes has been documented in Europe and North America. In Europe, Infectious Salmon Anemia Virus (ISAV), Infectious Pancreatic Necrosis Virus (IPNV), and Viral Hemorrhagic Septicemia Virus (VHSV), have been demonstrated to be transmitted from free-ranging salmonids to those reared in seawater net-pens, with theorized freshwater transmission (Raynard et al. 2007). The

monogenean parasite *Gyrodactylus salaris* is thought to have led to the extinction of wild salmon populations after it was transmitted from artificially-reared Baltic salmon to susceptible Norwegian fish (Raynard et al. 2007), and another parasite, *Lepeophtheirus salmonis* (sea lice), has caused disease in wild and net-pen populations. However, *L. salmonis* transmission remains contentious. Some argue that wild salmon and sea trout smolts are most at risk for sea lice infection in fjord systems where they pass by net-pens, while others argue that wild fishes are the source for net-pen infections (Scottish Executive 2002; Krkošek et al. 2006). Besides these listed events, it is theorized that there are more undocumented cases of transmission and additional pathogens, an example being Salmon Alphavirus (SAV). SAV causes pancreatic disease in farmed freshwater and saltwater salmonids. SAV antibodies have been detected in free-ranging fish collected from areas where net-pen fish had sub-clinical infections, but the direction of pathogen transmission has not been verified (Raynard et al. 2007). There is circumstantial evidence for bidirectional transmission of the pathogens listed above, but the bacterium *Aeromonas salmonicida* is the only pathogen for which this has been demonstrated (Scottish Executive 2002). Net-pen escapees were thought to be responsible for the rapid spread of *A. salmonicida* in Norway which, once established in free-ranging populations, re-infected net-pen stocks (Johansen et al. 2011; Naylor et al. 2005).

Similarly to Europe, there are various salmon aquaculture facilities in North America, including ocean net-pen facilities and freshwater hatcheries. Introduced *Salmo salar* are reared off-shore in net-pens in the areas surrounding British Columbia for commercial harvest, as well as on the east coast where they are native. Trout hatcheries are present in New England and the western states of Colorado, Utah, and Montana. The highest numbers of salmonid aquaculture facilities are concentrated in Oregon, Washington, California, and Idaho. Pathogen transmission has been reported in isolated incidents from free-ranging salmonids to fish held in these facilities: *R. salmoninarum* transmission from wild *Salvelinus fontinalis* to hatchery-stocked *S. fontinalis*,

Salmo trutta, and *O. mykiss*, in southeast Wyoming (Mitchum and Sherman 1981), and Infectious Hematopoietic Necrosis Virus (IHNV) transmission from naturally-reared *O. nerka* to hatchery salmonids in Oregon (Mitchum and Sherman 1981; Anderson et al. 2000). These records demonstrate that free-ranging fishes can transmit pathogens to hatchery fishs, leading to infection and disease, while the European examples demonstrate that bidirectional pathogen transmission can occur. However, the events leading to transmission are complicated by different factors.

FACTORS INVOLVED IN TRANSMISSION

Factors that can impact pathogen transmission dynamics and contribute to infection and disease are broadly described as the host-pathogen-environment triad (Snieszko 1974). These include intrinsic properties such as fish health, immunological status, age, and species; environmental factors such as water quality, temperature, flow rate; and other properties such as pathogen encounter rate, pathogen virulence, fish density, and other potential stressors, all of which can impact fish health outcomes (Hedrick 1998; Snieszko 1974). Infections can be acute, chronic, or asymptomatic, fish may die, recover, or become carriers (Naish et al. 2007), and pathogens may be shed from any of these stages (Raynard et al. 2007). Additionally, many pathogens exist naturally within the environment (with the exception of introduced pathogens) and the presence of a pathogen does not guarantee infection or disease (Naish et al. 2007). Determining the direction of pathogen transmission and possible outcomes is difficult, especially if the pathogen is widespread and occurs in both free-ranging and hatchery salmonids and non-salmonids (Noakes et al. 2000).

Infection and disease surveillance can be utilized to detect emerging and exotic pathogens, to assess pathogen presence, abundance, and distribution, and to determine the success of disease control programs and fish health management (Oidtmann et al. 2011). An example of this application is a study conducted in British Columbia, in which a variety of salmonid and non-

salmonid fishes were sampled from the open ocean and in areas surrounding net-pens, and were tested for salmonid-specific pathogens (Kent et al. 1998). One of these pathogens, IHNV, causes mortality in net-pen *S. salar*, and was initially thought to be contracted from the environment. However, results from this survey demonstrated IHNV infections in Pacific herring and other species caught in the open ocean, suggesting that non-salmonids may be reservoirs (Kent et al. 1998).

Studies have been conducted to explore the risks of pathogen transmission to and from free-ranging fishes to hatchery fishes. For example, researchers have experimentally infected fish and cohabitated them with naïve fish as a proxy for this free-ranging to hatchery interaction. In one experiment, there was no mortality in naïve fish cohabited with IHNV-infected fish (Ogut and Reno 2004b), though sub-clinical infection did occur. In another, there was no IHNV transmission between wild and IHNV-infected hatchery Chinook smolts cohabited for short periods of time (Foott et al. 2006). On the other hand, researchers demonstrated that *Aeromonas salmonicida* infection was possible from one infected fish to cohabited naïve fish (Ogut and Reno 2004a). Researchers argue that the dynamics of IHNV suggest low ecological risk, while those involved in the *A. salmonicida* study suggest that it is an efficient pathogen that can infect one host and rapidly spread to others, resulting in an epizootic (Ogut and Reno 2004a). These studies investigating pathogen transmission often involve collecting wild fishes, hatchery fishes, and water, or experimentally infecting fishes and sampling the tissue and tank water. However, there are a variety of methods to assess pathogen presence and prevalence, each with their own advantages and disadvantages.

METHODS FOR ASSESSING PATHOGEN PREVALENCE AND TRANSMISSION

Pathogen presence and abundance can be determined through culturing and molecular or serological techniques. Culture methods involve sampling specific tissue, such as the gill, kidney,

spleen, (and lesions present on these tissues types), and inoculating these samples onto bacterial media. The time until growth, colony phenotype, and biochemical properties can be used to determine bacterial identiy. Tissue can also be processed and inoculated onto cell culture lines, such as *Epithelioma papillosum cyprini* (EPC) and Chinook salmon embryo (CHSE-214) cell lines (AFS-FHS 2012), to determine virus presence. Positive bacterial or viral identification may be achieved using biochemical or molecular techniques (AFS-FHS 2012). Samples can also be collected from the environment, such as from water or sediments, and tested in this same manner. *Flavobacterium columnare*, the causative agent of columnaris, can be transmitted indirectly through water without fish-to-fish contact (Welker et al. 2005; Kunttu et al. 2009), and has been cultured from environmental water samples and fish ladders in the Columbia River, Oregon (Fujihara and Hungate 1971). It has also been demonstrated that hatchery IHNV and *A. salmonicida* epizootics can begin with relatively low pathogen levels, as detected through water sampling combined with either tangential flow filtration (TFF) or PCR combined with a DNA probe (Zhang and Congleton 1994; Batts and Winton 1989; O'Brien et al. 1994). *F. psychrophilum* could also be detected from water samples assayed with PCR and DNA probes (Madetoja and Wiklund 2002). Molecular techniques have also been combined with serological methods to detect low levels of *F. psychrophilum* from fish tissue and the water from fish farms (Wiklund et al. 2000; Madetoja and Wiklund 2002).Sentinel fish exposures are another tool that can be utilized to investigate pathogen presence. This involves the placement of confined, naïve, susceptible fish at a given location for a given time, then monitoring for disease (Stocking et al. 2006). Time to infection or mortality can be indicative of pathogen presence and dose. Sentinel fish can be standardized by age and species, and can be held at a given location for a given time, all factors that cannot be easily manipulated or controlled when sampling free-ranging fishes. However, for all these methods listed, there are differences in sensitivity that can impact the interpretation of results, and researchers often will chose one technique, or combine several, to

best answer their specific questions. However, despite a variety of methods, there are still difficulties in accurately monitoring disease and pathogen prevalence in free-ranging populations, and this can impact management strategies (Oregon Dept. of Fish and Wildlife 2010).

STUDY OBJECTIVES

Pathogen transmission from free-ranging fishes to hatchery fishes is a concern to fish health organizations worldwide (OIE 2013). Objectively comprehending pathogen transmission involves understanding the historical presence and impact of a given pathogen and the methods involved to successfully determine its presence. Here we focus on selected hatcheries in the Willamette River Basin, Oregon, combining qualitative and quantitative methods to assess the risks of pathogen transmission. In Chapter 2, we discuss how we monitored four pathogens at three hatchery facilities over three years in the upper Willamette River Basin, OR, both during hatchery epizootics and non-epizootic periods. These pathogens were selected on the basis of historical occurrence at these facilities and the recommendations of Oregon Department of Fish and Wildlife (ODFW) fish health specialists. These pathogens have caused disease at Oregon hatcheries, and understanding when disease epizootics occur, and under what conditions, may contribute to understanding transmission risks and improving hatchery management practices. We address these factors in Chapter 3.

My objectives for this thesis were to:

- Determine the temporal occurrence and factors that have contributed to past disease epizootics
- Determine baseline pathogen prevalence within hatchery influents and effluents through sentinel fish exposures and water sample analysis
- Determine the potential for pathogen entry or release during active hatchery epizootics and periods of baseline health.

We hypothesized the following: (1) pathogen levels would be sufficiently high during hatchery epizootics to infect downstream sentinel fish, and that the pathogens could be detected through water sampling during epizootics; (2) sentinel fish would not contract infections when hatcheries were not undergoing epizootics; (3) sentinel fish infected from placement in hatchery influent would indicate pathogen entry into hatcheries, and this would be expected to occur during the late summer and fall when epizootics historically occurred at the facilities.

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CHAPTER 2:

ASSESSING DISEASE IMPACTS OF HATCHERIES ON DOWNSTREAM SALMONIDS

Michelle Jakaitis, Sascha L. Hallett, and Jerri L. Bartholomew

ABSTRACT

We demonstrated that during epizootics, pathogens present in hatchery effluents can infect sentinel fishes held immediately downstream, but this may be a limited effect dependent on distance, dilution, and pathogen. Downstream sentinel fishes did not contract infections or develop disease when hatcheries were not undergoing epizootics, indicating that hatcheries are not an important contributor of pathogens during non-epizootic periods. To examine disease risks for juvenile salmonids associated with pathogen transmission into and out of hatcheries, we exposed sentinel fishes and sampled water for four endemic pathogens at four hatcheries in the Willamette River Basin, Oregon, USA. We placed naïve juvenile rainbow trout *Oncorhynchus mykiss* and spring Chinook salmon *O. tshawytscha* in liveboxes in hatchery influents and effluents during hatchery epizootics and during non-epizootic periods from 2011 – 2013. Fishes held downstream of hatcheries experiencing *Flavobacterium columnare* and *Aeromonas salmonicida* epizootics became infected and developed disease signs. We detected sub-clinical infections in fish held downstream during a *Flavobacterium psychrophilum* epizootic. However, sentinel fishes did not become infected when held downstream of a *F. psychrophilum* epizootic at a different facility, and were not infected after cohabitation with Infectious Hematopoietic Necrosis Virus (IHNV)-infected adults. IHNV was not detected in tangential flow filtration (TFF)-concentrated water samples. *A. salmonicida* was detected from cultured water samples collected downstream of one epizootic in 2011 but not during baseline monitoring. Although we were unable to determine the source of hatchery epizootics, the causative agents likely enter with the incoming water. Infected adult salmonids may be a pathogen source.

INTRODUCTION

One of the major criticisms of aquaculture is the potential for pathogen transmission between captive fishes and free-ranging fishes, the latter of which may be wild ("naturally-reared") or artificially reared (Johansen et al. 2011; McVicar et al. 2014). Within hatcheries, dense rearing conditions may facilitate pathogen transmission, increased virulence, pathogen shedding, and downstream release into surrounding waterways (FIGURE 2.1) (Coutant 1998; Naylor et al. 2005; Mennerat et al. 2010). Thus, in addition to the economic cost of managing hatchery disease and fishes loss, epizootics could have direct ecological impact on free-ranging populations exposed to pathogens in hatchery effluent, leading to a higher overall pathogen prevalence and increased risk of infection and disease in free-ranging fishes (Naylor et al. 2005; Raynard et al. 2007). Free-ranging fishes can also contribute to disease in captive populations, as infected, diseased, or subclinical carriers may shed pathogens into hatchery water supplies, potentially leading to hatchery epizootics (FIGURE 2.1) (Arechavala López et al. 2013; Raynard et al. 2007).

Instances of this free-ranging to hatchery salmonid transmission were documented for *Renibacterium salmoninarum* in Wyoming (Mitchum and Sherman 1981), and for Infectious Hematopoietic Necrosis Virus (IHNV) in Oregon (Anderson et al. 2000). Although the physical construction of net-pens differs from hatcheries, they share the same risks of pathogen entry, reservoirs, and potential epizootics as a result of untreated incoming water (Scottish Executive 2002). Cases of free-ranging to net-pen transmission are reported for Infectious Salmon Anemia Virus (ISAV), Infectious Pancreatic Necrosis Virus (IPNV), and Viral Hemorrhagic Septicemia Virus (VHSV) (Raynard et al. 2007), while *Aeromonas salmonicida* and *Lepeophtheirus salmonis* (sea lice), have been implicated in bidirectional transmission (Scottish Executive 2002; Krkošek et al. 2006). These examples highlight the potential for pathogen transmission, between free-

ranging and captive fishes, which remains a key concern within the industry (Noakes et al. 2000; Kostow 2012). Despite these risks, salmonid aquaculture is considered to be a viable solution for addressing economic demand and ecological pressure on wild stocks (FAO 2012). Although international and national agencies strive to develop aquaculture health plans to minimize and mitigate disease (APHIS 2008), continuing research is needed to better understand the risks of pathogen transmission between free-ranging fishes and captive fishes and to inform management decisions.

Here we examine the potential for pathogen transmission at four salmonid hatcheries publically managed by the Oregon Department of Fish and Wildlife (ODFW). The hatcheries, located in the South Willamette Watershed District, Willamette River Basin, were monitored from 2011 – 2013 for four endemic pathogens: *Flavobacterium columnare* (columnaris disease), *Flavobacterium psychrophilum* (bacterial coldwater disease), *A. salmonicida* (furunculosis), and infectious hematopoietic necrosis virus (IHNV). These pathogens are both historically and currently responsible for disease and fishes loss at our study hatcheries and throughout the greater Pacific Northwest. We utilized sentinel fish exposures and water sampling to determine pathogen prevalence and the risk of transmission. We hypothesized that infection of sentinel fishes from exposure in hatchery influent and positive water samples would indicate pathogen entry into hatcheries, pathogen levels would be sufficiently high during hatchery epizootics to infect downstream sentinel fishes and be detected in water samples, and downstream transmission would not occur during non-epizootic periods.

FIGURE 2.1 Pathogen transmission pathways.

MATERIALS AND METHODS

Field sites

We monitored transmission of the four pathogens at four Willamette River Basin hatcheries, OR, USA; South Santiam Hatchery (SH), Leaburg Hatchery (LH), McKenzie Hatchery (MH), and Dexter Ponds Rearing Facility (DP) (FIGURE 2.2). Monitoring efforts were categorized as follows: epizootic (>0.1% loss per day for 5 consecutive days, for any given rearing unit), infection (≤0.1% loss per day), and baseline (negligible loss— for example, <50 fishes per day out of 800,000 for a given raceway at Dexter Ponds, approximately 0.006%). Fishes were exposed in facility influents and effluents between 2011-2013 as described below (TABLE 2.1). Hatcheries and pathogens were chosen based on historical occurrence of epizootics and recommendations of ODFW fish health specialists. SH is located on the south Santiam River, rears *Oncorhynchus mykiss* and *O. tshawytscha*, and receives water from Foster Reservoir. LH rears *O. mykiss,* a small number of *O. tshawytscha,* and receives water from Leaburg Reservoir. MH is approximately two miles downstream of LH, rears *O. tshawytscha,* and receives water from Cogswell Creek and Leaburg Canal. The canal diverts water from Leaburg Reservoir upstream of LH, thus ensuring that MH does not receive effluent water from LH. DP is on the middle fork Willamette, raises *O. tshawytscha*, and receives water from Dexter Reservoir. SH, LH, MH, and DP receive untreated, single-pass surface water that is released, untreated, back into the river.

SENTINEL FISH EXPOSURES

Stock fishes and controls

Juvenile *Oncorhynchus mykiss* and *O. tshawytscha* were obtained from Oregon hatcheries (Roaring River and Marion Forks, respectively) and held at the Salmon Disease Lab

(SDL), Oregon State University (OSU), Corvallis, OR. All fishes were held in pathogen-free water at 13°C prior to exposure and were transported to exposure sites in insulated coolers supplemented with oxygen. Water temperature was measured prior to fish placement to ensure no more than a 5°C temperature change; fishes were acclimated over 30 minutes prior to transfer to temperatures warmer or cooler than this range. We did not observe mortality due to these temperature changes. In addition, it is possible that there was some stress associated with fish transport and fish exposure, but our methods were consistent so any such stressors were standardized across groups. Sentinel fishes were held in 0.3m x 1m cylindrical, mesh liveboxes for one to two weeks without feeding, then transported to the Salmon Disease Lab at Oregon State University (SDL-OSU) and held at average river site temperatures in either 25L or 100L tanks. All sentinel exposures were conducted when no antibiotics, medicated food, or other chemicals were in use at the hatchery that could potentially inhibit pathogens in order to maximize the chance of pathogen detection. Moribund fish and mortalities were removed frequently to minimize the effects of horizontal transmission. In 2011, one livebox of 50 *O. mykiss* and one livebox of 50 *O. tshawytscha* were exposed per site. In 2012 and 2013, this total remained the same, but fishes were split into two groups of 25 fish per livebox. Following exposure, all fishes were checked three times daily. Survivors were euthanized (0.04% tricaine methanesulfonate + 0.1% sodium bicarbonate) after the post-exposure holding period and sampled for bacterial and viral pathogens to determine target pathogen prevalence (see below for specifics). Groups of 25 fish per species were held as controls at the same temperatures, and water flows as exposed fishes and processed in the same manner. Experiment protocols were approved per IACUC guidelines, ACUP #4189, OSU.

Baseline Monitoring

Sentinel fishes were exposed at hatcheries during periods when no pathogens were detected and loss was negligible to determine baseline pathogen prevalence. In 2011, fishes were exposed in hatchery influents and effluents for one week during June, July, and August at LH, MH, and DP (FIGURE 2.2, TABLE 2.2). In January 2012, *O. mykiss* fry were placed in the LH influent and effluent for virus-specific baseline exposures. From the beginning of August until the second week of October 2012, baseline monitoring was conducted at DP, LH, and MH. During this time, sentinel fishes were held in the influent and effluent of each hatchery for two weeks, and then replaced with new groups. Fishes were checked weekly for survival. Following exposures, fishes were returned to the SDL. There was a two-week period (the middle to end of August) when no sentinel fish were placed at DP, as river temperatures had increased from 19°C to 24°C. Fish were placed again in the river when temperatures had decreased to 18°C. In October 2012, sentinel fish exposures were conducted at SH when there were asymptomatic IHNV infections in adult *O. mykiss* (infection prevalence 88 % – 100%) being held for spawning. Sentinel *O. mykiss* were held in the hatchery influent to determine if there was an incoming pathogen source, with the infected adults to determine the effects of cohabitation, and in the effluent to determine and the potential for downstream dilution. Baseline monitoring was not conducted in 2013.

Infection monitoring

Starting in late August and continuing through late October 2013, *F. columnare* was consistently the cause of low levels of fish loss at DP. Sentinel *O. tshawytscha* were placed for one-week exposures at varying downstream sites (FIGURE 2.3) during three exposure periods: Sept 13-20, Sept. 27-Oct 2, and Oct 17-24. Sites below the hatchery were selected to determine if

there was a dilution effect based on distance, and across the river to determine if downstream transmission was attributed to the hatchery or due to another source, such as migrating adult salmonids. Site selection across the river was based on location of a sandbar that split the river, segregating hatchery effluent water from the opposite side of the river. Sentinel fish were retrieved and held at river temperatures, 18°C, 16°C, and 14°C, respectively, for the three exposures, and were monitored for 30 days following exposure.

Epizootic Monitoring

Sentinel fishes were exposed for one week at hatcheries when loss was greater than 0.1% per day for five consecutive days. In 2011, epizootic monitoring was conducted at DP during an epizootic of *A. salmonicida* at the end of August and the beginning of September, and at LH during an *F. psychrophilum* epizootic in October (TABLE 2.2). Also in September 2011, fish in the DP facility experienced sudden mortality and sentinel fish were exposed in the hatchery influent and effluent immediately after this mortality event, even though no etiological agent (virus, bacterium, ectoparasite) was identified as the cause. In October 2012, sentinel fishes were exposed during hatchery epizootics of *F. psychrophilum* at MH, when temperatures were approximately 10°C, and fish were held for 60 days post-exposure, instead of 30 days post-exposure for all other groups, in case it would take longer for disease signs to manifest. We also placed sentinel fish during *F. columnare* and *A. salmonicida* at DP (October 2012), when temperatures were 15°C. In addition to exposure locations in the influent and effluent, fish were placed at additional sites 200 ft. and 400 ft. below the hatchery effluents to determine if infection prevalence and severity remained consistent (indicative of a river source of pathogens), or decreased due to dilution (indicative of the hatchery as a main pathogen source).

PATHOGEN SAMPLING

Sentinel fish sampling

One week prior to sentinel exposures, 20 *O. tshawytscha* and 20 *O. mykiss* were processed for bacteriology and virology (described below) to confirm they were not infected with target pathogens prior to field exposures. Following field exposures in 2011 only, all dead fishes and enough live fishes to bring the total to 10 fish were sampled upon field retrieval of liveboxes and fishes (day 0) to determine if one-week exposures resulted in bacterial, viral, or ectoparasite infection (see below). Control fish (10) were also sampled at this time.

Parasitology

Gill arches and skin scrapings were collected and examined for ectoparasites using light microscopy.

Bacteriology

All fishes (euthanized, dead, or moribund) were examined for disease signs and sampled as follows: kidneys and lesions were streaked onto tryptic soy agar (TSA) and tryptone yeast extract salts agar (TYES) media for bacterial isolation, incubated at 18˚C, and examined at days 1, 3, and 7 post-inoculation. Representative colonies were identified by PCR using primers specific for each pathogen (AFS-FHS 2010). Additionally, gills were inoculated onto TYES from fish exposed during *F. columnare* epizootics or infections. Moribund fishes were lethally sampled as a proxy for mortality. Mortalities from the field or laboratory minimally had to have an intact body cavity and pale pink gills to be suitable for pathogen sampling, indicating that the fish died recently, and that pathogens present were not due to post-mortem bacterial colonization. Some

fishes that died during field exposures were not sampled because of poor carcass quality which made it difficult to determine cause of death.

Virology

Virology was performed on all fishes in 2011 and virology-specific exposures in 2012. The gill, kidney, and spleen were collected and pooled in groups of 5 fish of the same species and field exposure group, prepared, and inoculated onto *Epithelioma papulosum cyprini* (EPC) cells and Chinook embryonic stem cells (CHSE 214) (Zhang and Congleton 1994; AFS-FHS 2012). Inoculated cell plates were monitored for cytopathic effects at day 1, 3, 7, 10, and 14, and then re-passaged for an additional two weeks.

Water sampling: virology

Water was collected, filtered by tangential-flow filtration (TFF) to concentrate viral particles, and inoculated onto cell culture for IHNV detection and quantification. Water samples were collected during sentinel fish placement and retrieval during baseline monitoring at LH in January 2011 and at SH in late October 2012 and the beginning of January 2013. There was no infection in juvenile hatchery fishes in January 2011 – 2013, but there were asymptomatic IHNV infections in adult *O. mykiss* held for spawning at SH in late October and early November. Water was also collected in early January (infection prevalence 26%), but we did not expose sentinel fish at this time. Ten liters of water per site, collected in 1L aliquots, were sampled from the hatchery influent, effluent, and adult holding area at the beginning and end of the one-week exposures. Immediately after collection, 1 mL fetal bovine serum (FBS) was added to each liter, which was then mixed by inverting four times. Water samples were immediately placed on ice for transport, then pooled in a sterile 5 gallon container and kept chilled during TFF (Thurber et al. 2009); samples were processed within 24 hours. The final volume was roughly 50 mL retentate

per site, concentrated from 10 L. Of this, 200 µL was inoculated per well onto 12-well EPC plates for a total of 10 mL per site. Samples were passed through a 0.2 µm filter to remove bacteria prior to inoculation onto cell culture. Virus culture plates were monitored as previously described for two weeks, re-inoculated on both EPCs and CHSE214 cells, and monitored for an additional 2 weeks.

Water sampling: bacteriology

Water samples were collected to supplement sentinel fish exposures at DP during the 2011 *F. columnare* and *A. salmonicida* epizootic. One liter was collected per exposure site, and of this, 200 µL of water was inoculated in triplicate, 10-fold dilutions ($10^0 – 10^5$ with sterile phosphate-buffered saline (PBS)), onto TSA and TYES plates and incubated and monitored for two weeks for detection of *A. salmonicida* and *F. columnare*. Additionally, from August through the first week of October 2012, water was collected weekly at DP from the facility influent and effluent and processed as described above.

Molecular characterization of F. columnare isolates

We collected bacterial isolates from hatchery fish and sentinel fish for genetic comparison, theorizing that colonies isolated from hatchery and sentinel fish would be genetically similar or identical if the pathogen source was the hatchery. If they were different, this might indicate an alternative source, while mixed strains could indicate contributions from multiple sources. Individual *F. columnare* colonies were selected from plates inoculated with samples from hatchery and sentinel *O. tshawytscha* that died within two-weeks of each other to minimize temporal effects. Colonies were chosen from sentinel fish mortalities exposed on the side of the river opposite of that of the hatchery to maximize the likelihood of detecting genetically distinct *F. columnare* isolates. Colonies were collected from hatchery fish (4 fish, 4 samples: two gill,

two kidney), sentinel fish held across the river from the facility at 200 ft. (8 fish, 10 samples: 6 gill, 4 kidney) and 400 ft. downstream (6 fish, 9 samples: 3 gill, 6 kidney). Individual colonies were transferred to individual microfuge tubes and held at -20°C prior to DNA extraction. Colonies were extracted with a DNeasy® kit (Qiagen). Colonies were verified as *F. columnare* through morphology and PCR (AFS-FHS 2010). PCR reagents and cycling parameters were as described by (Zavaleta et al. 1996; AFS-FHS 2012) with the following modifications: we did not use the 50 mM KCl, 1.5 mM $MgCl_2$, or 0.5 mM Triton X-100, as these were contained in the Invitrogen RediLoad™. We used Promega GoTaq®, 5X colorless GoTaq™ Flexibuffer, GeneMate dNTP Master Mix, but concentrations remained the same as those listed in the above procedure. Reactions were cycled in a PTC-200 Peltier Thermal Cycler, amplified products were purified using ExoSAP-IT® (Affymetrix) and submitted for sequencing at the OSU Center for Genome Research and Biocomputing (CGRB) with reverse primer FCISSR1 (AFS-FHS 2010), to amplify the 16S-23S rDNA internal ribosomal spacer (IRS). IRS sequences were manually aligned in BioEdit (Hall 2005) and any ambiguous base pair differences were clarified with reference to the chromatogram.

Statistical analysis

Kruskal-Wallis tests were performed to analyze differences in mean pathogen infection or mortality between sites at two time periods: upon sentinel fishes retrieval (zero days post-exposure (0 DPE)), and at 30 DPE at experiment termination. Replicates were tested individually. Ad-hoc tests were performed to evaluate pairwise differences between groups, with an adjusted p-value of 0.1, 0.15, and 0.2. Analyses were performed in R 2.13.2 using the pgirmess package 1.5.2 (Giraudoux 2014).

Hatcheries	*Flavobacterium columnare*	*Flavobacterium psychrophilum*	*Aeromonas salmonicida*	Infectious hematopoietic necrosis virus
	Pathogens			
South Santiam (SH)		X	X	
Leaburg (LH)		X	X	X
McKenzie (MH)		X	X	
Dexter (DP)	X		X	

FIGURE 2.2 Study hatcheries in the Willamette River Basin, Oregon, USA, and pathogens responsible for historical loss denoted with an "x". Map courtesy of Stephen Atkinson.

TABLE 2.1 Dates and fish species exposed for one-week baseline, infection, and epizootic monitoring at each hatchery, as defined respectively as negligible loss, ≤ 0.1% loss per day per five consecutive days, or > 0.1% loss per day per five consecutive days. Rbt = *Oncorhynchus mykiss*, Chs = *Oncorhynchus tshawytscha*, N = negligible pathogen presence or not detected.

	Hatchery	Pathogen	Year 2011	2012	2013	Fish species
Baseline monitoring	Leaburg	N	Jun-Aug	Aug-Oct*	–	Rbt, Chs
	Leaburg	N	–	Jan	–	Rbt
	McKenzie	N	Jun-Aug	Aug-Oct.*	–	Rbt + Chs
	Dexter	N	Jun-Aug	Aug-Oct*	–	Rbt + Chs
Infection Monitoring	South Santiam	Infectious hematopoietic necrosis virus†	–	Oct	–	Rbt
	Dexter	*Flavobacterium columnare*	–	–	Sept-Oct	Chs
Epizootic Monitoring	Leaburg	*Flavobacterium psychrophilum*	Oct	–	–	Rbt
	McKenzie	*Flavobacterium psychrophilum*	–	Oct	–	Rbt + Chs
	Dexter	*Aeromonas salmonicida*	Aug-Sept	–	–	Chs
	Dexter	*Aeromonas salmonicida* + *Flavobacterium columnare*	–	Oct	–	Chs
	Dexter	Unknown cause	Sept	–	–	Chs

* Continuous, two-week long exposures with weekly monitoring
† Infected adult *Oncorhynchus mykiss* held for spawning

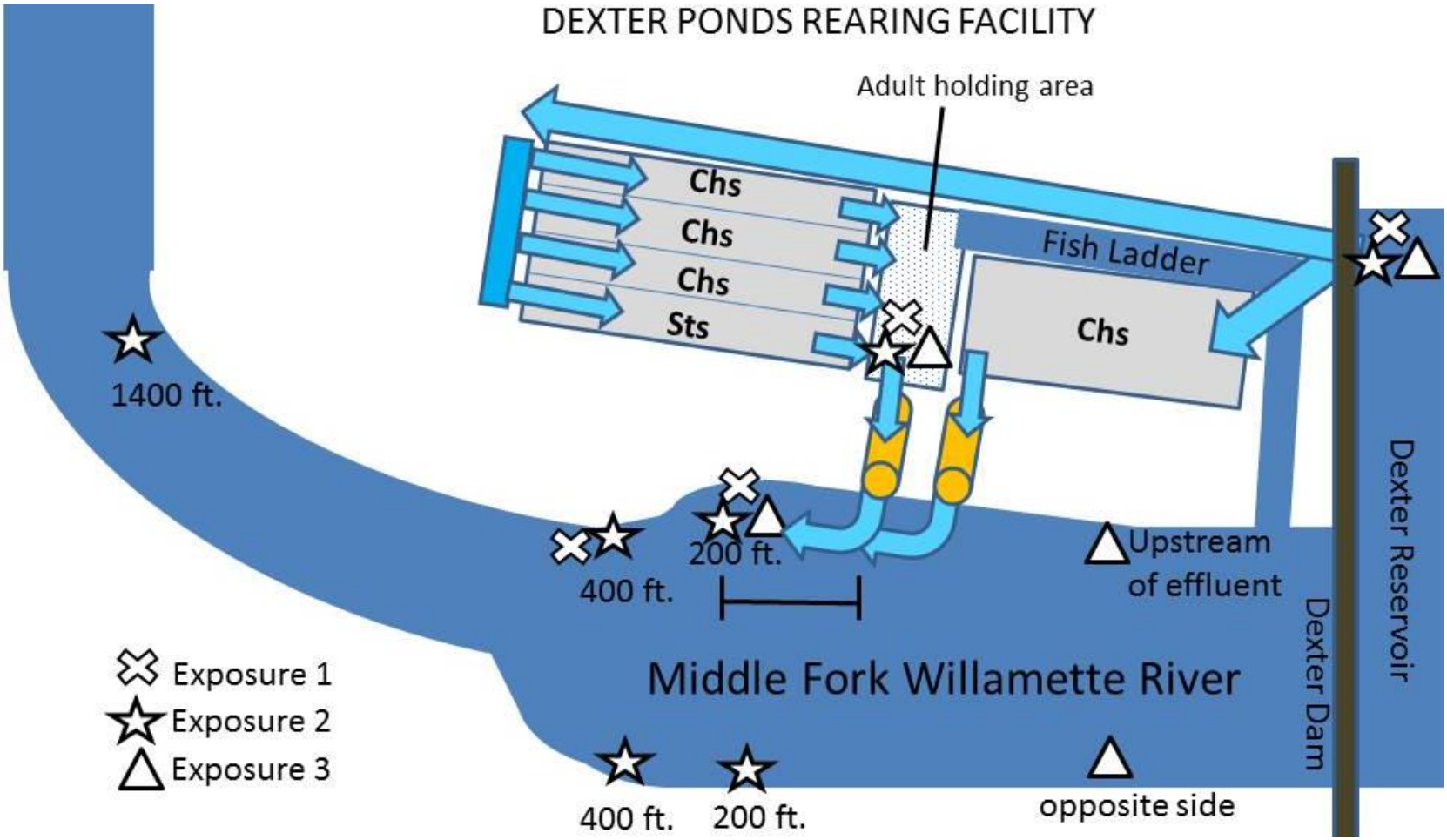

FIGURE 2.3 Dexter Ponds (DP) hatchery schematic and infection monitoring sites, 2013. Exposure 1 = Sept. 13-20; exposure 2 = Sept. 27 – Oct. 2; exposure 3 = Oct. 17 – 24. Chs = *Oncorhynchus tshawytscha*, Sts = summer steelhead *Oncorhynchus mykiss*. Arrows indicate water flow, cylinders indicate water transport pipes. Not to scale.

RESULTS

From 2011 – 2013 we processed 12,600 sentinel fishes from 24 baseline exposures, 800 sentinel fishes from three infection exposures, and 900 sentinel fishes from five epizootic exposures. Sixty-two liters of river water and hatchery water were collected. Of this, approximately 500 mL was inoculated onto media in 200 µL aliquots and processed for bacteriology. An additional sixty liters were collected and filtered through TFF for virus detection. All stock fishes and controls were negative for target pathogens.

Baseline monitoring

Sentinel fishes survival was high (98% – 100%); low levels of nonpathogenic bacteria were detected in exposed sentinel fishes and there were no mortalities due to target pathogens. We did not detect ectoparasites from the gill or skin in any sentinel fishes sampled at the end of the week-long field exposures in 2011. All sentinel fish held to detect IHNV at LH in January and SH in October 2012 were negative for the virus. IHNV was not detected in water samples collected from LH in January and MH in October and November. Target pathogens were not detected in water samples collected during baseline monitoring, either from samples that were inoculated onto bacterial media or those concentrated through TFF and inoculated onto cell culture for IHNV detection.

Infection monitoring

Sentinel exposure groups held at DP in 2013, when *F. columnare* was detected at low prevalence in the hatchery population, had varying infection and mortality over all three exposures (FIGURE 2.4). *F. columnare* was not detected at any time in sentinel fish held in the influent. At 0 DPE there were low levels of mortality in groups held in the effluent and at 200 ft.

and 400 ft. downstream during the first and second exposures but not during the third exposure. At 30 DPE, mortality had increased across all downstream sites during all exposures (FIGURE 2.4). There was no difference in mean *F. columnare* mortality during the first exposure among sites at 0 DPE: χ^2 (3, N = 25) = 3.5, p = 0.32 or at 30 DPE: χ^2 (3, N = 25) = 4.99, p = 0.17. No pairwise differences were indicated by ad-hoc tests. There were no differences in mean *F. columnare* mortality during the second exposure among sites at 0 DPE χ^2 (6, N = 25) = 4.75, p = 0.58 or 30 DPE: χ^2 (6, N = 25) = 4.96, p = 0.55.There was no initial mortality due to *F. columnare* during the 3rd exposure at 0 DPE, and there were no differences in mean *F. columnare* mortality at 30 DPE: χ^2 (4, N = 25) = 6.29, p = 0.18. No pairwise differences were indicated by ad-hoc tests. There were no differences in IRS sequences between *F. columnare* colonies from infected *O. tshawytscha* DP fish and sentinel fish held across the river at 200 ft. and 400 ft. downstream. Sequences were deposited in GenBank (temporary ID number 1769500), and were 100% similar with to *F. columnare* sequences isolated from *O. mykiss* (GI527460411), *O. tshawytscha* (GI527460398), and non-salmonids *Ictalurus punctatus* (AY842904) and *Notemigonus crysoleucas* (GU07993.1).

Epizootic monitoring

During the furunculosis epizootic at DP (August 2011), there was 30% mortality attributed to *A. salmonicida* in fish held in effluent at 0 DPE. At 30 DPE cumulative mortality was 54% (FIGURE 2.4). Fish developed disease signs, including darkening, lethargy, and were inappetent. *A. salmonicida* was isolated from all mortalities from both the gill and kidney. From water sampling we detected *A. salmonicida* at levels of 2,000 colony-forming units (CFU) per mL (minimum detection level) at DP at the following sites: the hatchery raceways experiencing the epizootic, 200 ft., 400 ft., and 1,400 ft. downstream, but not at 800 ft. downstream. We did not detect pathogens during any other epizootic or during baseline monitoring in 2013.

Sentinel fish exposed in the influent and effluent after the mortality event of unknown cause at DP (September 2011) did not develop infections. No target pathogens were detected in water samples collected from the hatchery influent at any time.

During the concurrent epizootic of *A. salmonicida* and *F. columnare* at DP (mid-September 2012), no mortality occurred as a result of infection by either pathogen in sentinel fish held in the facility influent. There were low levels of mortality in groups held in the effluent and at 200 ft. and 400 ft. downstream at 0 DPE during the first and second exposures but not during the third exposure. After 30 days, mortality had increased across all downstream sites during all exposures (FIGURE 2.5**)**. There were no significant differences in mean mortality of fish infected with *A. salmonicida* among sites at 0 DPE: χ^2 (3, N = 25) = 6.05, $p = 0.11$, or at 30 DPE: χ^2 (3, N = 25) = 6.58, $p = 0.087$. Fish displayed clinical disease signs characteristic of furunculosis. There were no significant differences in mean mortality of fish infected with *F. columnare* among sites at 0 DPE or 30 DPE; χ^2 (3, N = 25) = 6.86, $p = 0.07$. Infected fish displayed clinical disease signs characteristic of columnaris. No pairwise differences were indicated by ad-hoc tests. *A. salmonicida* infection prevalence was greater than *F. columnare* and we did not detect dual infections in sentinel fish.

No mortality occurred as a result of *F. psychrophilum* infection in sentinel fishes exposed at LH (2011) or MH (2012) during bacterial coldwater epizootics at these facilities. However, *F. psychrophilum* infections were detected in downstream of MH in *O. mykiss*: 16% infection in one group held in the effluent, and 4% infection in one group each held at 200 ft. and 400 ft. downstream (FIGURE 2.6). *O. mykiss* did not display gross clinical disease signs and we did not detect infections in the *O. tshawytscha*. A Kruskal-Wallis test indicated that there was no difference in mean *F. psychrophilum* infection among sites at 60 DPE: χ^2 (4, N = 25) = 2.63, $p = 0.62$.

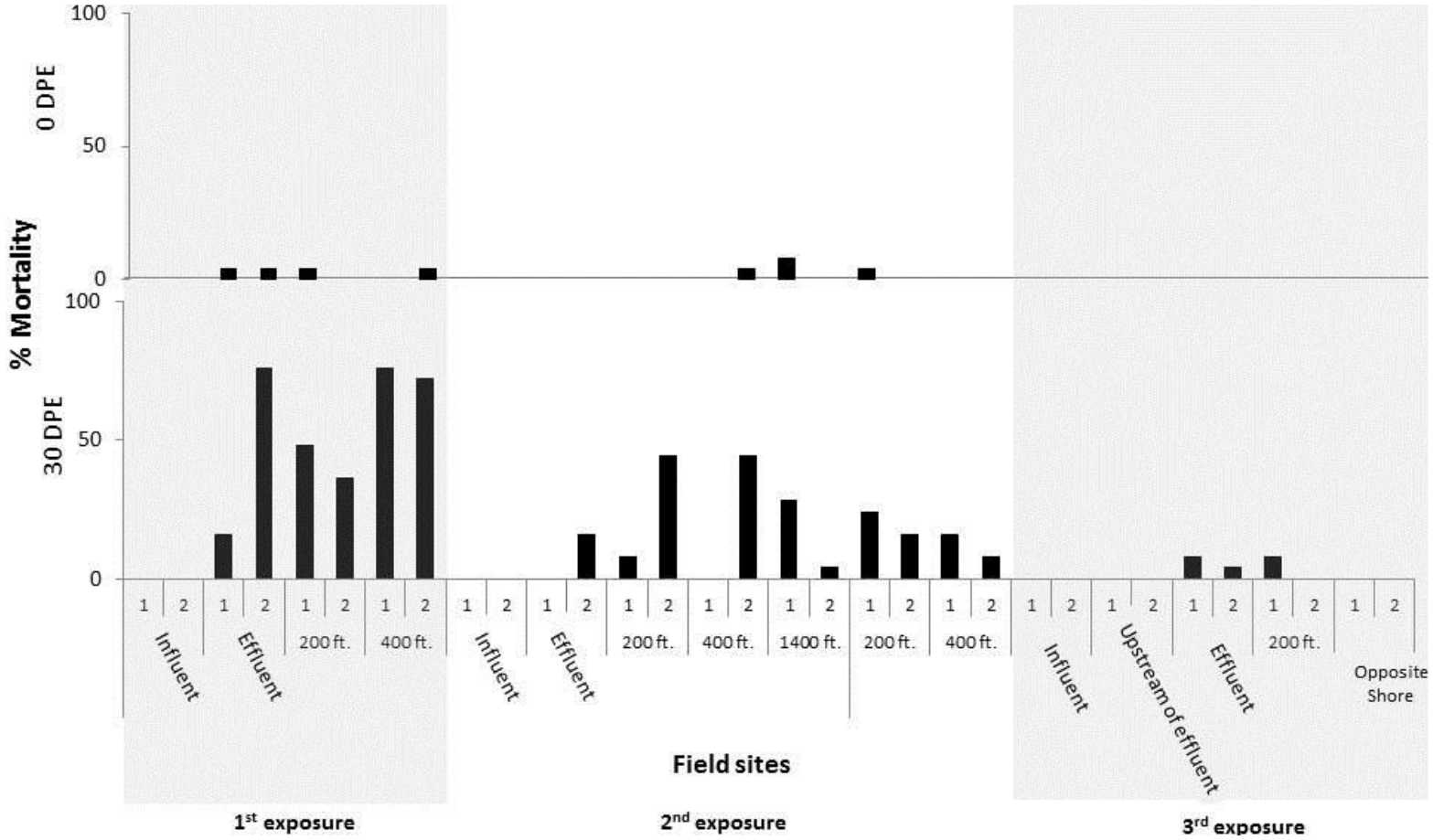

FIGURE 2.4 Initial and final mortality attributed to *Flavobacterium columnare* in *Oncorhynchus tshawytscha* sentinel fish exposed at Dexter Ponds, 2013, with two groups per site. 1st exposure = Sept. 13-20; 2nd exposure = Sept. 27 – Oct. 2; 3rd exposure = Oct. 17 – 24. Chs = *Oncorhynchus tshawytscha*, Sts = summer steelhead *Oncorhynchus mykiss*. Arrows indicate water flow, cylinders indicate water transport. 0 days post-exposure (DPE) is defined as day 7 of the field exposure, when fish were retrieved. 30 DPE is when the experiment was terminated. N = 25 fish per group. Numbers indicate feet downstream of hatchery effluent.

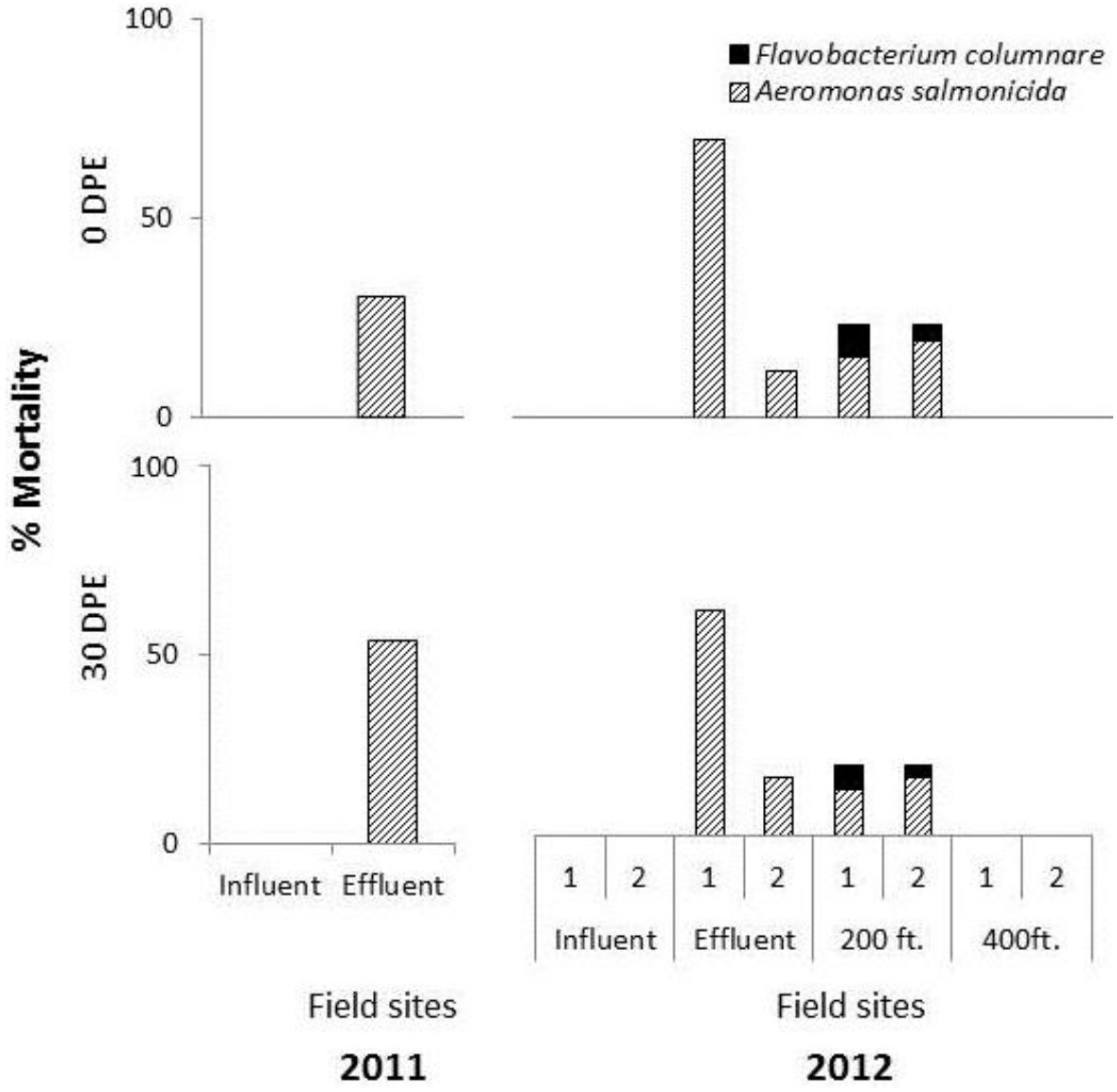

FIGURE 2.5 Initial and final mortality attributed to *Flavobacterium columnare* and *Aeromonas salmonicida* (dominant infection) in *Oncorhynchus tshawytscha* sentinel fish exposed at Dexter Ponds. N = 50 fish per group in 2011, and two replicate groups of 25 fish in 2012, as indicated by either a "1" or "2."

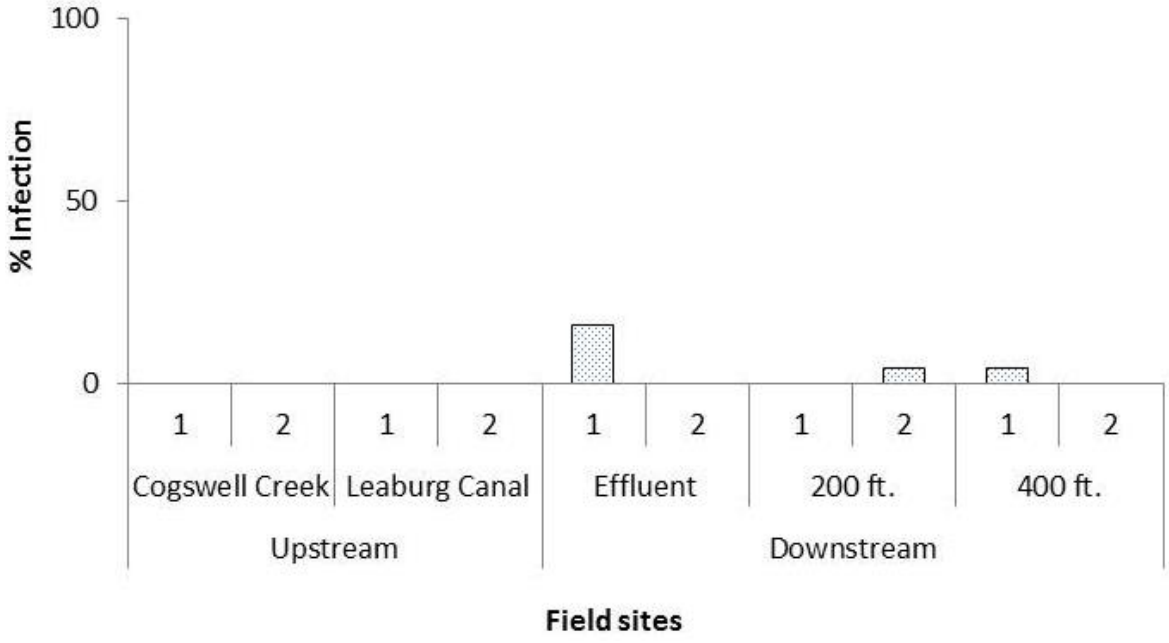

FIGURE 2.6 Percent infection of *Flavobacterium psychrophilum* in *Oncorhynchus mykiss* at 60 DPE at McKenzie River Hatchery. N = 25 fish per two replicate groups of 25 fish in 2012, as indicated by either a "1" or "2."

DISCUSSION

We demonstrated that pathogen transmission can occur from a hatchery to sentinel fishes held immediately downstream during hatchery epizootics, but this may be a limited effect dependent on distance, dilution and pathogen. Here we explore these and other risk factors that may have contributed to our findings; for example, the presence of returning adults at or near the facilities. We also address the implications of pathogen transmission to and from free-ranging and hatchery fishes, pathogen release during epizootics, and management options for both.

The risks of downstream pathogen transmission may depend on hatchery health status, distance from hatchery, pathogen, and characteristics of the physical location. Sentinel fishes did not contract infections either upstream or downstream of hatcheries at any point during baseline monitoring. Only during periods that pathogens were detected and causing mortality in a hatchery (infection and epizootic monitoring) did we observe downstream infection and mortality. However, even during these times there were instances where we did not observe downstream infection and mortality. Differences in pathogen-attributed mortality in fish among downstream sites were insignificant and thus did not support downstream dilution as a factor affecting infection rate, contrary to our prediction. The rivers at our study facilities are on a greater size and flow scale than the effluent, which we expected would offset the risk of pathogen transmission during epizootics. However, the Kruskal Wallis test is fairly conservative in ascertaining significant differences and the environmental data are highly variable. The effects of downstream dilution may have been more evident had there been larger numbers of sites or replicates.

We also observed differences in transmission depending on pathogen. Infection and mortality were highest in sentinel fish exposed downstream of facilities experiencing epizootics due to *A. salmonicida* and *F. columnare*, but there was no loss due to *F. psychrophilum*, although low levels of infection were detected. Furthermore, we did not detect IHNV in sentinel fish

cohabited with infected adults at SH or in water samples from the adult holding area. These differences indicate that different pathogens pose different transmission risks. *F. columnare* and *A. salmonicida* appeared to be more efficient in infecting fish than *F. psychrophilum* and IHNV. Thus, not all hatchery disease epizootics may pose a transmission risk to free-ranging fishes. This is further supported by laboratory studies that reported high bacterial concentrations and extended exposures were necessary to cause an infection with *F. psychrophilum* by bath exposure (Holt et al. 1989; Garcia et al. 2000). IHNV did not cause infections in sentinel fish even when cohabited with infected adults, and the virus was not detected from TFF-filtered water during the period when the pathogen was detected in fish. Although IHNV infection prevalence was high in the adult *O. mykiss* held at SH (88% - 100%), none displayed disease signs and they may not have been shedding virus. This corroborates results from another study investigating the risks of IHNV transmission from captive to free-ranging populations, in which fish did not contract IHNV when cohabited with infected cohorts (Foott et al. 2006). The authors of that study suggest that there is low ecological risk of IHNV transmission from infected hatchery fishes to free-ranging fishes. However, it is important to note that both studies involved sub-clinically infected fishes. Other studies have found that large-scale IHNV epizootics in free-ranging salmonids, complete with disease signs verified through histology, can result in transmission of the virus to hatchery salmonids (Anderson et al. 2000).

Time of year and physical location may also impact transmission. Our study hatcheries were downstream of dams, which substantially alter both thermal and hydrological regimes in the Willamette River basin (Keefer et al. 2010). Reservoirs are often temperature-stable, resulting in extended periods of warmer waters in the summer and fall (Keefer et al. 2010). These periods of thermal stress could increase fish susceptibility to infection and disease (pers. comm. Tony Amandi, Craig Banner, Tim Wright). Rivers may experience greater temperature fluctuation, and nightly cooling may normally allow fishes to recover from thermal stress. Dams are also

migration barriers for returning adult salmon and steelhead, which arrive annually between mid-May and November (Oregon Dept. of Fish and Wildlife 2014). The presence of large numbers of returning, congregating adult fishes may contribute pathogens to the river in hatchery areas. Returning adult salmon and steelhead may spawn in the river below the dam, or move up fish ladders to traps on hatchery premises to be spawned for hatchery production or be transported to tributaries upstream to spawn naturally. All of these groups may contribute pathogens to the river around hatcheries, supported by our infection monitoring results from DP in 2013. Sentinel fish were exposed in the effluent, varying downstream sites, and across the river at sites that were isolated by a sandbar that split the river, segregating hatchery effluent water from that water channel. Sentinel fish became infected at all downstream locations, suggesting that there was either a different source or an additional source that contributed pathogens, or that the upstream pathogen levels were below detection through water sampling or sentinel fish exposures. Sequencing of a diagnostic, 16-23S intergenic spacer region (ISR) of the genome of *F. columnare* isolates from these sites and from infected fish in the facility did not support the theory regarding genetically distinct infection sources or host. Although other studies have found that *F. columnare* strains may be different depending on host species, such as 95.99% sequence similarity between strains isolated from *O. nerka* and *Ictalurus punctatus,* but we did not note differences between salmonid and non-salmonid *F. columnare* strains compared in GenBank. This may indicate that either the hatchery was the source, or that the source of infection was the same. We hypothesize that pathogens shed by adult salmonids contributed to infections in both the hatchery and sentinel fishes, although we were unable to determine the pathogen source that led to hatchery epizootics.

Untreated influent water was considered the most likely transmission pathway, but we were unable to detect target pathogens with sentinel fishes or water sampling. This could have been attributed to our sampling techniques, as sentinel fishes may not have been present at the

time of pathogen entry, as in 2012 when an epizootic occurred three weeks after baseline monitoring concluded, or had not received a sufficient dose to become infected. For example, we determined that the minimum detection threshold of water sampling on bacterial media for *A. salmonicida* was 2,000 CFU per mL, equivalent to $2x10^6$ CFU per liter, but disease and mortality can be caused by doses as low as $10^{4.8}$ CFU per mL. Due to this limited sensitivity, we may have not detected incoming pathogens. A likely sources of pathogens are reservoir hosts located upstream of the hatcheries. For example, populations of largescale suckers *Catostomus macrocheilus* living in Dexter Reservoir may be the source of *F. columnare* and *A. salmonicida* epizootics at DP (pers. comm. Craig Banner Sept 2013), as fishes in the Catostomidae family serve as reservoirs for infection for salmonids (Bullock et al. 1986). And as discussed above, adult salmonids are transported upstream to bypass the dams, and these fishes may be in spawning condition and immune suppressed, thus bearing a high pathogen load (Schouten et al. 2013). In addition, nets and barriers may not prevent predators and other wildlife from entering hatchery raceways, which may be potential transmission pathways or may cause injury to the fishes making them more susceptible to pathogens. It is also possible that infection could have occurred at another location, as fish transfers are common between ODFW hatcheries, and our study hatcheries receive fish stocks from other locations. Although fish health must be certified prior to transportation, subclinical carriers or low pathogen prevalence may go undetected. These fishes may shed and transmit pathogens for an undetermined time while remaining apparently healthy and indistinguishable from other fishes. *F. psychrophilum* was isolated from subclinical *O. mykiss* held downstream of a bacterial coldwater disease epizootic at MH, and other studies support the possibility of a carrier state (Madetoja et al. 2000). There are many pathways for pathogen entry into hatcheries, and methods for reducing disease risk will depend on understanding transmission routes. Although utilizing fish-free water sources, such as spring or

well water, could reduce the risk of transmission, this option is not available to the hatcheries in this study, and water treatment should be considered.

UV irradiation and ozonation, combined with filtration, may reduce disease issues and overall pathogen prevalence within hatcheries. However, these treatment systems are not available at our study hatcheries or at other hatcheries in Oregon. In addition, in the event that epizootics occur, treating the effluent prior to release would minimize the risks of transmission to free-ranging fishes. UV irradiation inactivates IHNV and other viruses (Afonso et al. 2012) in freshwater (but not saltwater) systems (Liltved et al. 2006) and preceding UV irradiation with ozonation is the most effective method for removing pathogenic bacteria (Sharrer and Summerfelt 2007). Filtration may be coupled with ozonation or irradiation to remove particles that block treatment (Summerfelt 2003). Other pathogens, such as the myxozoan *Ceratonova shasta*, can be inactivated with chlorination and irradiation (Bedell 1971; Hoffman 1974). Water filtration and ozonation is in use at select aquaculture facilities, such as the Northwest Fishery Center in Lama, PA, in which this system successfully and consistently removed and inactivated pathogenic bacteria (Summerfelt et al. 2008). However, this is a research facility, and such water treatment systems are complicated to run, maintain, and may be above budgeting costs for hatcheries (Summerfelt 2003). The Green Lake National Fish Hatchery in Maine and the Cowlitz Hatchery in Washington also filter and UV irradiate their water to minimize the risk of incoming pathogens (U.S. Fish and Wildlife Service 2014; Deere & Ault Consultants, Inc. 2014), although the Cowlitz Hatchery used ozonation in the past (Tipping 1988). However, cleaning or maintenance, if done improperly, can allow pathogens to enter (Bedell 1971; Morgan and Withalm 2008), and such treatment systems are expensive. Over $30 million was spent at the Cowlitz Hatchery for remodeling and water treatment installations in 2010, and water treatment is not the norm for hatcheries. It is likely it will take time to develop and implement such systems at hatcheries, but these modifications may be worthwhile given the risks to valued wild fishes.

Although sentinel fish in this study were used to detect pathogens rather than to mimic responses of free-ranging fishes, they provided information on pathogen transmission and prevalence. Free-ranging fishes are not confined to the hatchery effluent and can seek out their ideal habitats and escape areas of high current, low oxygen, high temperature, or other stressors: thus the actual risk to free-ranging fishes may be lower than that measured by sentinels. In addition, free-ranging fishes may not be as chronically stressed as our sentinel fish, which were confined and fasted for one or two weeks. The high mortality in sentinel fish during the *A. salmonicida* and *F. columnare* epizootics may have been influenced by horizontal transmission during the post-exposure holding period, which could be reduced in free-ranging fish that are not restricted in tanks. Furthermore, the outcome of exposure of free-ranging fish to these pathogens is difficult to predict – especially when several of these pathogens are endemic, and exposure does not necessarily result in infection or disease (Naish et al. 2007).

Pathogen transmission and subsequent disease are multifactorial and there are different factors that complicate risk (Snieszko 1974; Hedrick 1998; Lhorente et al. 2014). Although downstream pathogen transmission was demonstrated during hatchery epizootics, this did not occur during all epizootics. Other information that would be useful to interpret our results and understand transmission include studies on pathogen dispersion rates (such as McVicar et al. 2014), pathogen prevalence in salmonid hosts, and reservoirs in free-ranging fishes. Finally, we understand that large-scale changes in hatchery facilities, such as water treatment, may not be feasible in the interim, but could be a long-term goal. As aquaculture develops worldwide, continued research is needed to better inform and reduce disease risk to both captive fishes and free-ranging fishes.

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CHAPTER 3: DISEASE RISKS ASSOCIATED WITH HATCHERIES IN THE WILLAMETTE RIVER BASIN

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ABSTRACT

Hatcheries are often perceived as a source of pathogen amplification, potentially increasing disease risk to free-ranging populations during epizootics; at the same time, free-ranging fishes in the water supply of hatcheries may introduce pathogens into hatcheries. Incoming and exiting hatchery water is generally not treated, creating a potential pathogen entry and exit portal, but not much is known about this dynamic. Here we assess transmission of the pathogens *Flavobacterium columnare, F. psychrophilum, Aeromonas salmonicida, Renibacterium salmoninarum*, and infectious hematopoietic necrosis virus (IHNV), at selected hatcheries in the Willamette River Basin. We generated historical epizootic trends from past ODFW data and also obtained data and personnel communications from the ODFW fish health management plan (2003) and hatchery managers. We found differences in disease incidence and pathogens at these hatcheries which are likely influenced by hatchery design, location, and water source. Facility-wide water treatment of the influent and effluent may be an option for reducing pathogen entry as well as pathogen release during epizootics.

INTRODUCTION

Most salmon and trout hatcheries located in the Willamette River Basin, Oregon, USA, utilize an untreated river water supply for their rearing ponds and release this water, untreated, back into the river. Thus waterborne pathogens present in free-ranging fishes (naturally-reared or hatchery-reared) can be transmitted through the water supply to hatchery populations and vice versa with pathogens released from the hatchery to free-ranging fishes in the effluent. Moreover, a hatchery epizootic may amplify pathogens, resulting in their release into the water, which could have a direct impact on free-ranging populations. Here we selected five pathogens and examined the presence, prevalence, and potential of entry and exit of these pathogens at three hatcheries in the Willamette River Basin (FIGURE 3.1), and discuss management implications.

METHODS

The following analysis was developed based on historical data, hatchery data, and personal communications from experts including ODFW fish health specialists and hatchery managers. We first generated historical epizootic trends from Oregon Department of Fish and Wildlife (ODFW) Fish Health Records (FileMaker Pro v.11 © Database) for disease comparison between hatcheries. The ODFW considers an epizootic as loss greater than 0.1% per day over five consecutive days for any given rearing unit, and epizootics in the database are listed as incidents during which there was abnormal loss and fish treatment was required. Second, to determine the potential for pathogen transmission into and out of select facilities, we held juvenile rainbow trout *Oncorhynchus mykiss* and juvenile spring Chinook salmon *O. tshawytscha* in liveboxes for week-long exposures at study hatcheries during three times categorized as: epizootic, infection, and baseline monitoring (see Chapter 2 for details). Infection monitoring occurred when loss was at or less than 0.1% per day, and baseline monitoring occurred when loss

was negligible (for example, fewer than 50 fish out of 800,000 per rearing unit, or 0.006% loss, at Dexter Ponds). Sentinel fishes were transferred to the Oregon State University Salmon Disease Lab (OSU-SDL), for post-exposure disease monitoring. Exposures were conducted from 2011 – 2013 and the results are described within each hatchery section below. Finally, we investigated hatchery design and disease factors, including environmental data such as time of year, water temperature, water source, and species of free-ranging fishes within the surrounding waterways. Together, these findings help us better understand disease risks at Willamette River basin hatcheries.

ETIOLOGY OF SELECTED PATHOGENS

Flavobacterium columnare

The causative agent of columnaris, it is a yellow-pigmented, gram-negative bacterial rod that typically causes gill erosion and yellow-pigmented lesions on the gills and body surface (AFS-FHS 2010). For this pathogen as well as the others listed below, there are a range of doses that cause mortality and disease. Pathogenicity varies by strain but some strains may cause up to 90% mortality (AFS-FHS 2010). Generally, *F. columnare* is most virulent in younger fish and epizootics typically occur when water temperatures rise to 18˚C or higher (Bullock et al. 1986; Wakabayashi 1991). This pathogen has a broad host range, infecting both salmonids and non-salmonids worldwide (AFS-FHS 2012). Some fish, such as suckers and others in the Catostomidae family, are considered as reservoirs of infection for salmonids (Bullock et al. 1986). *F. columnare* can be transmitted horizontally from live and dead fish (Kunttu et al. 2009) and can survive in sterile water and mud, suggesting extended exposure and infection potential. *F. columnare* can be isolated by streaking material from lesions onto media. Bacteria on fish or in lesions are presumptively identified by the presence of columnar bacterial structures on the gills

under wet mount through light microscopy. Colonies are yellowish, flat, dry, and rhizoid, with irregular edges adhering to the agar surface, and grow on tryptic yeast extract soy agar (TYES), cytophaga agar, Hsu-Shotts agar, or modified Shieh media (AFS-FHS 2012). PCR can be used to confirm bacteria as *F. columnare* (AFS-FHS 2012).

Flavobacterium psychrophilum

The causative agent of bacterial coldwater disease, *F. psychrophilum* is a filamentous rod that is yellow-pigmented, gram-negative, and infects salmonids, causing acute septicemia that manifests in skin and muscle lesions and erosion, commonly in the peduncle area (LaFrentz and Cain 2004; AFS-FHS 2010). *F. psychrophilum* has caused serious losses in aquaculture, is believed to be ubiquitous in freshwater systems worldwide (Nicolas et al. 2008; AFS-FHS 2010), and can survive for long periods of time in the water although it may lose virulence (Madetoja et al. 2003). Epizootics may occur below 12˚C, although infections can persist at 16˚C (AFS-FHS 2012), and transmission is waterborne and horizontal (AFS-FHS 2010). *F. psychrophilum* is also shed from dead fish (Madetoja et al. 2000) and has been isolated from within surface-sterilized eggs (Brown et al. 1997), suggesting vertical transmission. *F. psychrophilum* has a broad geographical distribution (Nicolas et al. 2008), likely worldwide in temperate regions, and has been found in salmonids and non-salmonids. Rainbow trout and steelhead *O. mykiss* and coho salmon *O. kisutch* are the most susceptible (AFS-FHS 2010). Juvenile fish are most susceptible to infection and subsequent disease (AFS-FHS 2010). Laboratory studies have determined that infectious dose varies depending on age of fish, bacterial strain, and challenge method, with injections more effective in causing infections and disease than immersion challenges (Garcia et al. 2000). Survivors may develop spinal deformation following recovery (Nematollahi et al. 2003). The bacterium can be isolated from lesions or the kidney on TYES agar and verified with PCR (AFS-FHS 2012).

Aeromonas salmonicida

The causative agent of furunculosis is a short, non-motile, gram-negative rod, and infection can result in upraised lesions (furuncles) (AFS-FHS 2010). It is found in North and South America, Europe, Asia, and Africa, and has a broad host range, impacting both freshwater and saltwater fish (AFS-FHS 2010). Disease can occur at elevated temperatures (15-20˚C) or chronic infections can occur at temperatures below 13˚C (AFS-FHS 2010; (Groberg Jr. et al. 1978). It can be horizontally transmitted and shed from live and dead fish (Rose et al. 1989). The bacterium can be isolated from lesions and kidney tissue inoculated onto tryptic soy agar (TSA) and some strains produce a characteristic brown-diffusing pigment. The bacterium can also enter a non-culturable but viable state off-host but may lose virulence (Morgan et al. 1992).

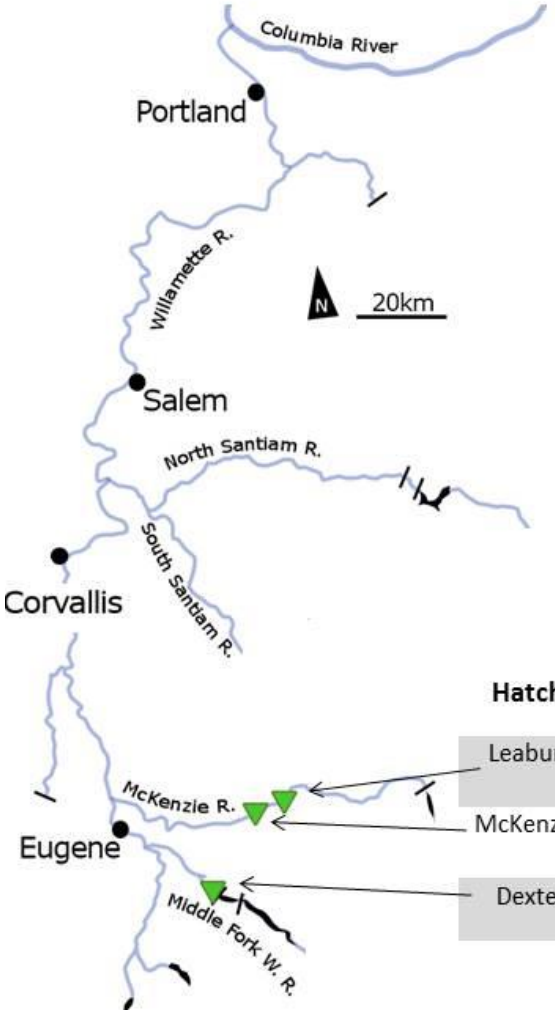
Renibacterium salmoninarum

The causative agent of bacterial kidney disease, it is a gram-positive diplobacillus that can occur in salmonids at any life stage, at times causing chronic disease and manifesting as white lesions in the kidney (AFS-FHS 2010). Disease can occur over a wide temperature range from 4˚C -20˚C (AFS-FHS 2012). Although it can be cultured on KDM2 medium (AFS-FHS 2012), its growth is slow. Thus it is typically detected by enzyme-linked immunosorbant assays (ELISAs) or direct fluorescence antibody tests (DFAT) on kidney smears and microscopic examination of gram stained tissue smears. It is transmitted among fish both horizontally and vertically (Elliott et al. 1997).

Infectious Hematopoietic Necrosis Virus (IHNV)

The causative agent of infectious hematopoietic necrosis, IHNV is a rhabdovirus that infects salmonids. It can cause high mortality in younger fish (up to 2 months) and can also kill

older fish, but survivors may serve as virus carriers. Disease signs include anemia, hemorrhaging, and internal necrosis (AFS-FHS 2010). Its range includes North American, and was introduced into Europe and Asia. Disease often develops at or below 15˚C (AFS-FHS 2010), and transmission may be horizontal, vertical, or waterborne (AFS-FHS 2010; Mulcahy and Pascho 1985; Mulcahy et al. 1983). IHNV can survive at least 2 weeks at 15˚C in water, and up to several months at 5˚C (Pietsch et al. 1977). Pathogenicity can vary between virus strains, fish age, size, and species (AFS-FHS 2012). Presumptive diagnosis is performed by homogenizing the gill, kidney, spleen, reproductive fluids, incubating the tissue in an antimycotic/antibacterial media, and then inoculating the supernatant onto *Epithelioma papillosum cyprini* (EPC) and Chinook salmon embryo (CHSE-214) cell lines (AFS-FHS 2012). Cytopathic effects can be observed between 48-96 hours and presents as grape-like clusters of refractile cells. Confirmatory diagnosis may be verified through serum neutralization assays or PCR on presumptively diagnosed samples (AFS-FHS 2012).



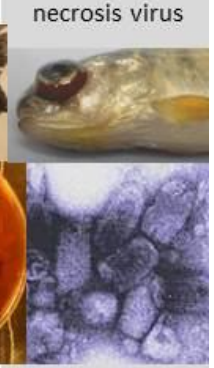
Hatcheries	*Flavobacterium columnare*	*Flavobacterium psychrophilum*	*Renibacterium salmoninarum*	*Aeromonas salmonicida*	Infectious hematopoietic necrosis virus
					
Leaburg (LH)		X	X	X	X
McKenzie (MH)		X		X	
Dexter (DP)	X		X	X	

FIGURE 3.1 Study hatcheries in the Willamette River Basin, Oregon, USA, pathogens responsible for historical loss, and disease signs. X indicates past pathogen epizootics for a given hatchery. Map courtesy of Stephen Atkinson. (Images from AFS-FHS 2012; Kurath 2012; http://www.fws.gov/pacific/ fisheries/fishhealth/Images/4_a_ii%20 Rsal%20&%20Asal%20Gram%20StainL.jpg).

DISEASE FACTORS AT DEXTER PONDS REARING FACILITY (DEXTER PONDS)

Location and hatchery plan

Dexter Ponds, a satellite of Willamette Hatchery, is situated on the middle fork Willamette River immediately downstream of Dexter Dam. Both facilities were constructed to mitigate fishery losses caused by the Hills Creek, Lookout Point, and Dexter hydroelectric and flood control projects (Oregon Dept. of Fish and Wildlife 2012). Dexter Ponds receives its water from Dexter Reservoir and incoming and exiting water is not treated. Water temperatures throughout the year range between 4˚C-20˚C (U.S. Army Corps of Engineers 2014a; U.S. Army Corps of Engineers 2014b). The raceways receive single-pass water which flows into the adult holding pond then out of the facility. There is no settling pond (Oregon Dept. of Fish and Wildlife 2012). Within the facility, there is one adult holding pond, four raceways, and one large asphalt rearing pond (FIGURE 3.2), and there is no settling pond (Oregon Dept. of Fish and Wildlife 2012). Raceways are 41.1m long, 5.5m wide, 1.8m deep, with a unit volume of 4,444m^3. The asphalt rearing pond is 52.4m long, 19.5m long, 1.8m deep, with a unit volume of 20,131m^3. Dexter Ponds rears juvenile spring Chinook salmon and a small number of steelhead (FIGURE 3.2). No eggs are incubated at Dexter Ponds. Adult fish are trapped at Dexter Ponds and transferred to Willamette Hatchery where they are spawned and eggs are incubated. Returning adult salmon that are not needed for egg production are transported to streams above Lookout Point Reservoir in the upper watershed. Juvenile fish are transferred from Willamette Hatchery to Dexter Ponds starting early to mid-May. Additional yearling steelhead and spring Chinook salmon from Willamette Hatchery are transferred to this facility in November (Oregon Dept. of Fish and Wildlife 2012); pers. comm. Tim Wright, Dexter Ponds Rearing Facility, 2013). These fish are reared and then liberated in November, February, and March for spring Chinook salmon and April for steelhead.

Contributing disease factors

Juvenile Chinook are susceptible to all five pathogens, but historically, epizootics or infections at Dexter Ponds have been caused by *F. columnare*, *A. salmonicida,* and there have been rare instances of low levels (non-epizootic) of clinical disease caused by *R. salmoninarum* (FIGURE 3.3). Epizootics typically occur at Dexter Ponds at the end of the summer and the beginning of the fall when water temperatures are at their highest, between 15˚C-19˚C (FIGURE 3.3). This has occurred annually from 1997 to 2012, with several years in between where there were no epizootics (FIGURE 3.3). Fish from Dexter Ponds are originally transferred from Willamette Hatchery, but the facilities have different health profiles. Willamette Hatchery rears both rainbow trout and Chinook salmon, but, in contrast to Dexter Ponds, *F. psychrophilum* frequently causes epizootics in the rainbow trout, up to several times a year during the summer when water temperatures average 14°C (FIGURE 3.4). However, sporadic *A. salmonicida* and *F. columnare* epizootics occur in the Chinook salmon (FIGURE 3.4). These differences in health problems are likely a result of the different temperature regimes at the two facilities: Dexter Ponds is downstream of a reservoir and temperatures average 16°C-19°C, in comparison to 14°C on average at Willamette Hatchery. Water temperature monitors (Onset HOBO® data temperature loggers) placed at Dexter Ponds through the summer and fall 2013 demonstrated that influent temperatures were warmer than the effluent (FIGURE 3.5). These elevated temperatures (≥18˚C) can be stressors and exacerbate disease (Baker et al. 1995; AFS-FHS 2012). In addition to the effects of temperature, susceptibility of species reared also affects pathogen occurrence. Although *F. psychrophilum* likely infects all salmonids, rainbow trout are known to be most susceptible to disease (Nematollahi et al. 2003), and this is the species reared primarily at Willamette Hatchery.

Pathogens may also be introduced into Dexter Ponds through untreated water sources. Largescale suckers, *Catostomus macrocheilus,* live in Dexter Reservoir and may be a pathogen source, as they are known to contract *F. columnare* infections (Craig Banner pers. comm.) and may be carriers (Bullock et al. 1986). Migrating adult salmonids may also contribute pathogens. Dexter Dam blocks the passage of adult spring Chinook salmon and summer steelhead (typically 10,000-11,000 fish annually). Spring Chinook arrive between mid-May through November and summer steelhead arrive between mid-May through June or later (Oregon Dept. of Fish and Wildlife 2014). Some of these fish move up Dexter Pond's fish ladder and are held in a trap on the hatchery premises (FIGURE 3.2). These adults are ultimately transported upstream to Willamette Hatchery for broodstock, or to tributaries upriver of Dexter Dam/Lookout Point Dam, although some may remain immediately below the dam. The adults arrive with a variety of pathogens (pers. comm. Craig Banner) and all groups may be contributing pathogens downstream into the river. The stress associated with spawning depresses immune function and inhibits the ability to fight infection (Schouten et al. 2013), which may be exacerbated with elevated water temperatures during summer and fall (Keefer et al. 2010). Furthermore, adults trapped at Dexter Ponds are held in the hatchery effluent (FIGURE 3.2) and there is a risk of them becoming infected, in their immune-suppressed state, if hatchery fish are experiencing disease. Adult females are injected with antibiotics prior to spawning to limit *R. salmoninarum* from being vertically transmitted to the eggs (pers. com. Sarah Bjork), but other pathogens can still be released into the water. The influent and effluent are not treated at Dexter Ponds or Willamette Hatchery, and so there is risk of pathogen transmission from adults transported to upstream tributaries or from those held and spawned at Willamette Hatchery. These adults could be an infection source to juvenile hatchery fish.

There is potential for disease outbreaks to occur as a result of pathogens entering the hatcheries in untreated influent. In addition, there are also risks of downstream pathogen

transmission from hatchery fish and trapped adult fish to free-ranging fishes. We found that sentinel fish became infected when held downstream of Dexter Ponds during *A. salmonicida* and *F. columnare* epizootics (see Chapter 2). Although this is suggestive of transmission of pathogens from the facility, adult salmon congregating below Dexter Dam may also have contributed to these infections. However, adults were also present during baseline studies when infections did not occur in sentinel fish, indicating that pathogens were likely transmitted from hatchery fish during epizootic conditions. Generally, the adults arrive at Dexter Ponds in June, and most are transported to Willamette Hatchery by July, before epizootics occur at Dexter Ponds, (pers. comm. Rich Holt) although there may still be adults congregating immediately downstream of Dexter Dam.

Management implications

F. columnare and *A. salmonicida* routinely cause epizootics at Dexter Ponds, likely due to the elevated water temperatures. In contrast to other facilities, *F. psychrophilum* has not been a cause of loss, perhaps due to temperatures warmer than the threshold associated with bacterial coldwater disease epizootics (AFS-FHS 2012). *F. columnare* and *A. salmonicida* are hypothesized to enter into Dexter Ponds through untreated water, although we were unable to demonstrate this using either sentinel fish or water sampling (see Chapter 2). Utilizing a water treatment system, such as UV irradiation or ozonation, could minimize pathogen entry through incoming water.

Ozonation is more complicated and expensive due to the infrastructure requirements, such as enriched oxygen gas to produce ozone, gas-phase to liquid phase converters, and off-gassing compartments to prevent the release of ozone (Summerfelt 2003). Extensive training is required to maintain this system and to monitor it to prevent toxicity to fish (Summerfelt 2003). UV systems are much simpler and less costly in comparison, and do not generate toxic residuals

as ozonation does (Summerfelt 2003). UV systems and ozonation are often combined with filtration to remove particles that would interfere with water treatment. Another option for reducing disease incidence in both Dexter Ponds fish and adults held at the facility would be to install a water chiller. This would reduce thermal stress associated with higher temperatures in the summer and fall, thus reducing disease incidence and improving survival. This was done at the American Rivers Hatchery in California but cost close to one million dollars (Taylor 2014) which would be cost prohibitive at Dexter Ponds. Annual operations costs at Willamette Hatchery were approximately one million dollars in 2013 (Oregon Dept. of Fish and Wildlife 2013), and Dexter Ponds is a smaller satellite facility of Willamette Hatchery. Another, perhaps cheaper, option would be to move the influent pipe into deeper, cooler water to provide thermal relief, providing this would not interfere with normal dam operations. Finally, we suggest providing clean, single-pass water to the adults held within the Dexter Ponds trap. These adults are held in the effluent water from the facility raceways (FIGURE 3.2), and could contract pathogens from this contact, if, for example, the facility fish are experiencing an epizootic.

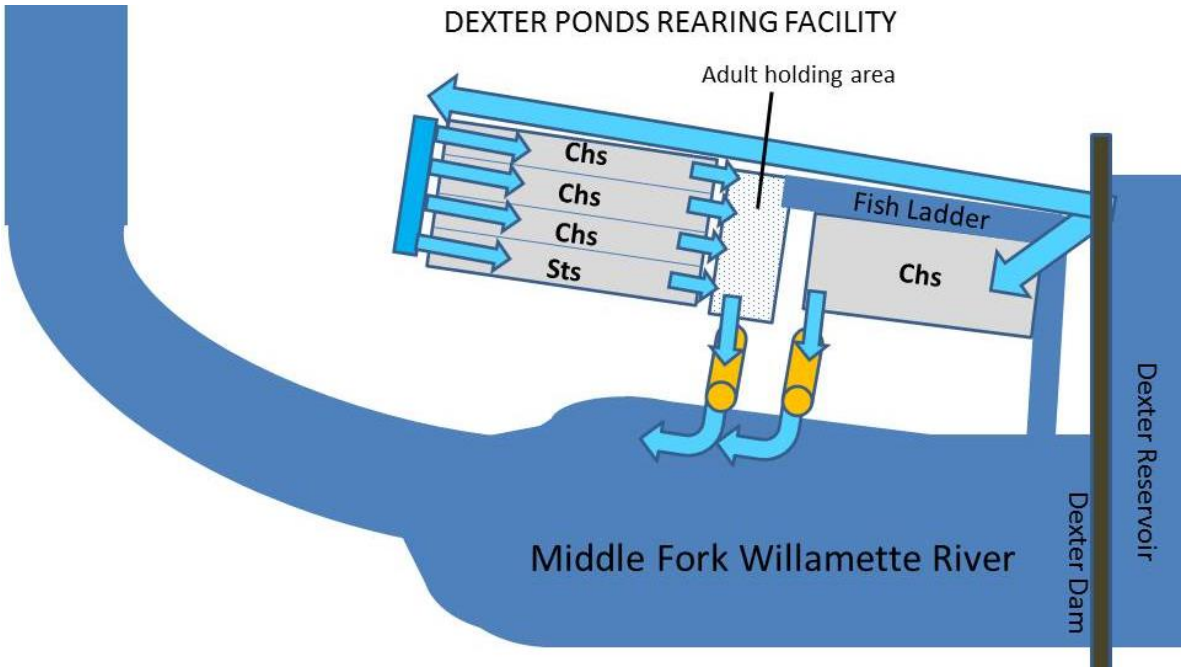


FIGURE 3.2 Dexter Ponds Rearing Facility schematic and species reared per raceways. Arrows indicate water flow, cylinders indicate water transport pipes. Not to scale.

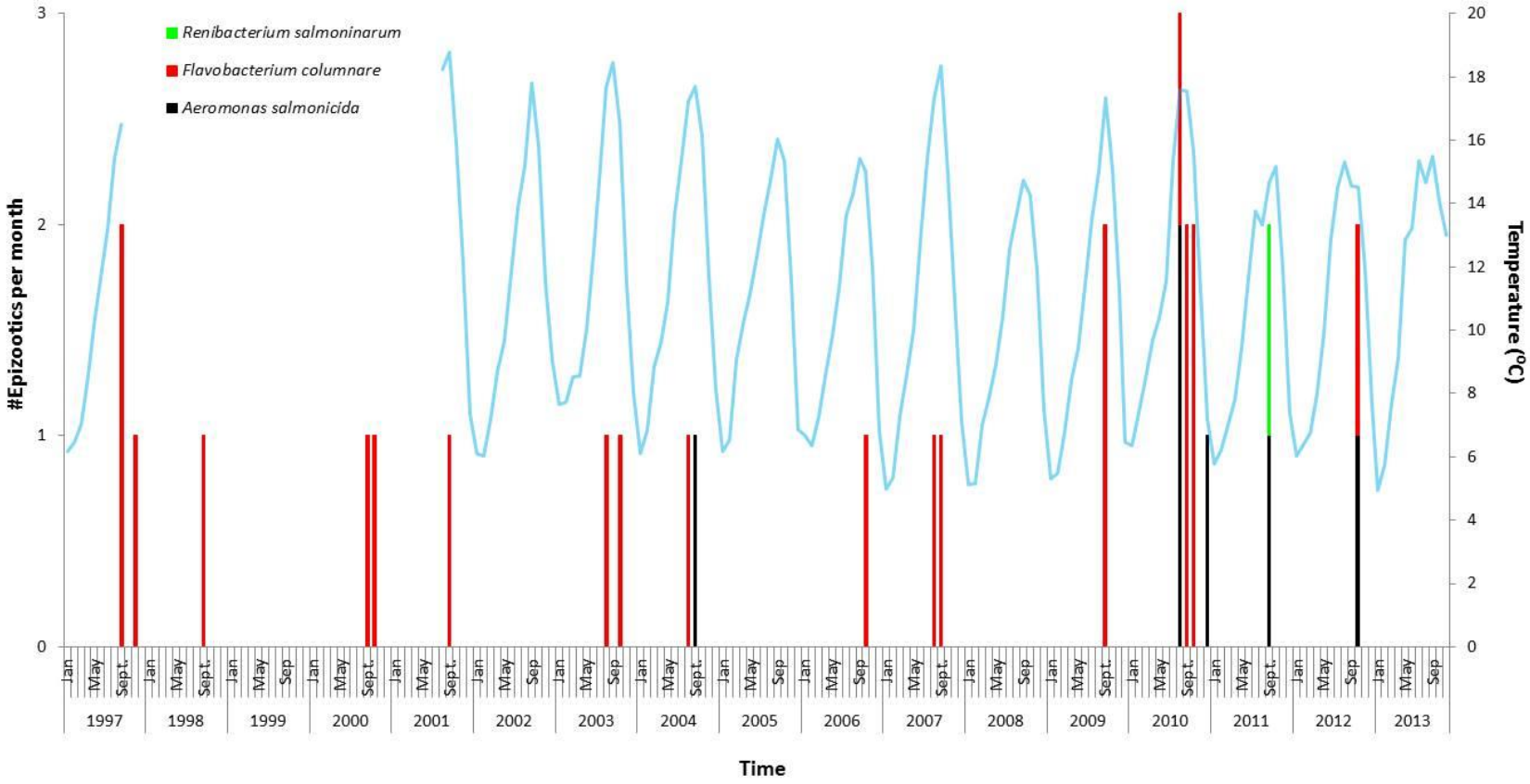


FIGURE 3.3 Historical pathogen epizootics (abnormal loss and treatment) at Dexter Pond in spring Chinook salmon and average monthly downstream water temperatures (blue line). Temperatures obtained from USGS water gauge 2.6 miles downstream of the dam (U.S. Army Corps of Engineers 2014b).

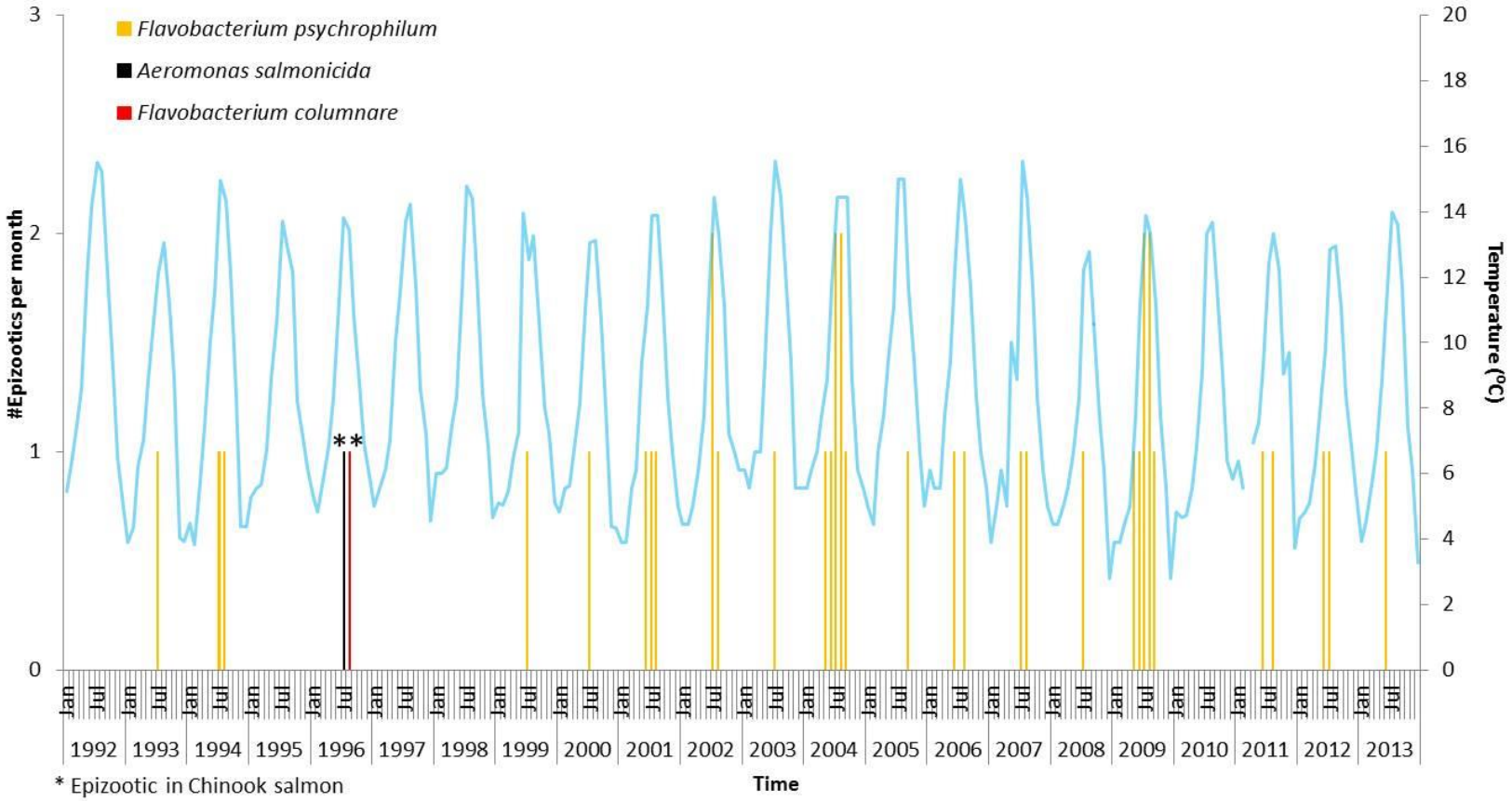


FIGURE 3.4 Historical pathogen epizootics at Willamette Hatchery in rainbow trout and Chinook salmon, and average monthly hatchery water temperatures (blue line).

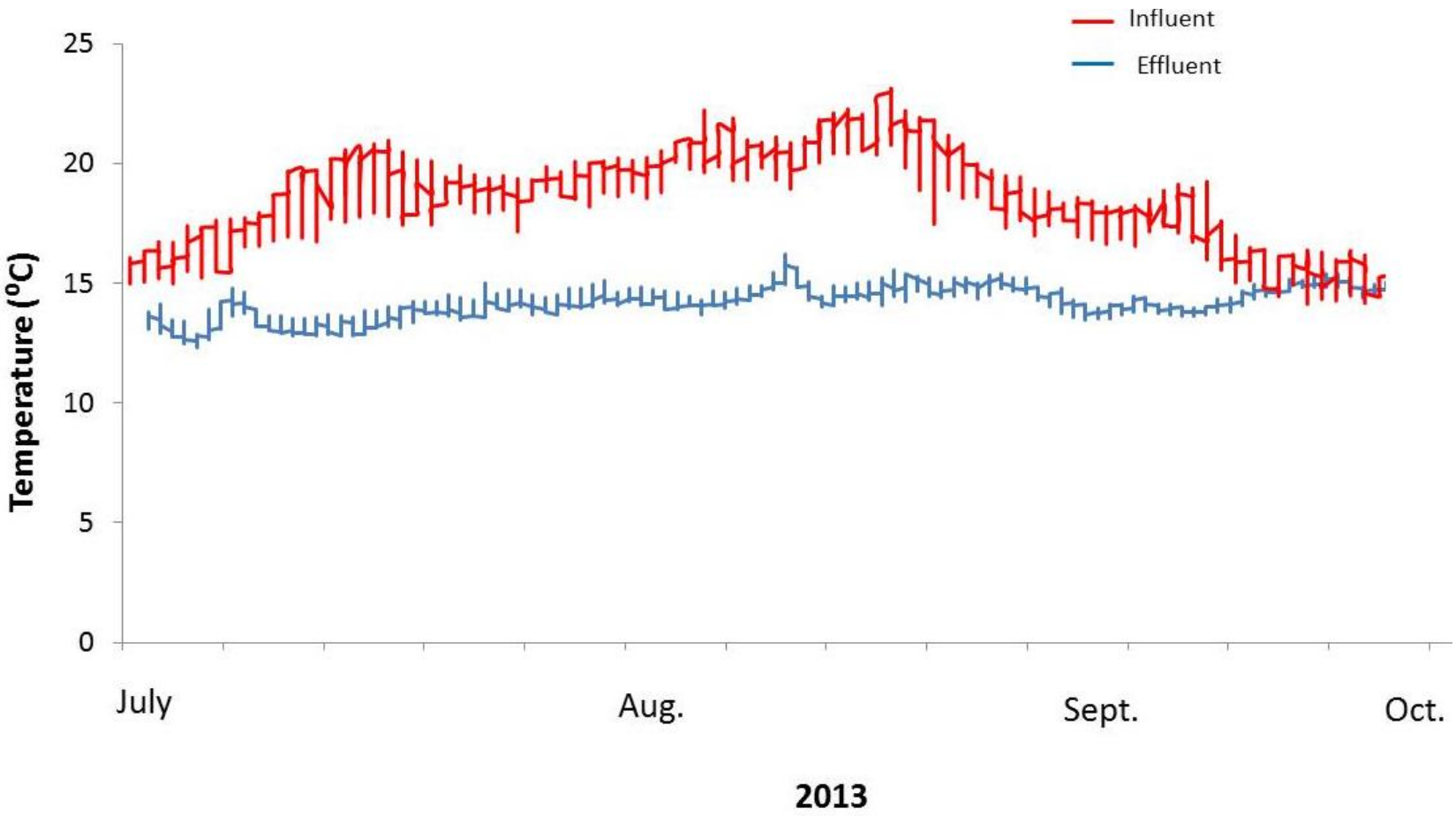


FIGURE 3.5. Dexter Ponds Rearing Facility influent and effluent water temperatures (Onset HOBO® temperature logger).

DISEASE RISKS AT LEABURG HATCHERY

Location and hatchery plan

Leaburg Hatchery was constructed to mitigate for lost trout habitat caused by the Blue River Dam, Cougar Dam, and other McKenzie River valley projects. It is situated on the south fork of the McKenzie River, immediately below Leaburg Dam, and receives single-pass water from Leaburg Reservoir. The facility has 42 raceways and a hatch house with egg incubation trays, and rears summer steelhead, rainbow trout, and spring Chinook salmon (FIGURE 3.6). Three raceways are 15.2m long, 6.1 m wide, 1.1m deep, with a unit volume of 1,116m^3. The other 39 raceways are 30.5m long, 6.1m wide, 1.1m deep, with a unit volume of 2,231m^3. There is no settling pond. Leaburg Hatchery also receives juveniles from Willamette Hatchery. Since 2007, a UV irradiation system has been installed to disinfect hatch house water for egg and fry stages. Water temperatures range between 4 -14˚C (FIGURE 3.6). We obtained water temperature data from the U.S. Geological Survey (USGS), as yearly Leaburg Hatchery temperatures were not available. The USGS gauge is at their Vida station, approximately two miles upstream of Leaburg Hatchery (U.S. Geological Survey 2014b).

Contributing disease factors

F. psychrophilum historically caused epizootics in rainbow trout in the spring to early summer when water temperatures ranged between 6 -14˚C (FIGURE 3.7). Leaburg Hatchery receives rainbow trout from Willamette Hatchery, and it is possible that fish contract *F. psychrophilum* at this facility prior to transfer. IHNV epizootics and infections are rare, although the epizootics in 2003 led to the destruction of the infected rainbow trout (FIGURE 3.7). Both IHNV and *F. psychrophilum* epizootics have since decreased in frequency (FIGURE 3.7), which may be due to changes in management and hatchery structure. Historically, the ODFW used to

transport adult summer steelhead upstream of Leaburg Reservoir, which were suspected to be IHNV carriers (Withalm 2007) (and perhaps *F. psychrophilum* carriers), but the ODFW halted this practice in the mid 1990's. Summer steelhead are now recycled back downriver for angling opportunities. The hatchery also underwent extensive repairs to the facility and raceways. Prior to this, water could leak or be back-flushed among the raceways. As such, containing an epizootic to one raceway would be difficult (pers. com. John Kaufman), and repairs could have made epizootics more manageable. Although *F. psychrophilum* epizootics have declined, infection and some chronic loss continue to occur, but not at levels to warrant treatment or to be defined as an epizootic. Of our target pathogens, *R. salmoninarum* is seldom detected in fish at Leaburg Hatchery and has not led to epizootics (FIGURE 3.7). *A. salmonicida* has sporadically caused epizootics in spring and summer (FIGURE 3.7). Other health issues also arise sporadically. Spring floods often result in suspended sediments in incoming water causing gill inflammation which could predispose fish to bacterial and viral infections. Heavy particulates in the water during these periods can interrupt routine peroxide or formalin therapeutic treatments, allowing the incidence and severity of ectoparasites to increase (pers. com. Erik Withalm, Jerry Jones). This combination of physical skin and gill irritation coupled with poor respiration can allow secondary bacterial infections to establish and develop into epizootics. The immediate proximity of Leaburg Reservoir could be responsible for silting and ectoparasite presence. The reservoir accumulates silt that is agitated during floods (pers. com. Erik Withalm) and parasites may also enter into the facility during these times.

Management implications

F. psychrophilum is the pathogen most commonly detected at Leaburg Hatchery, although it has led to only sporadic epizootics at Leaburg Hatchery for the past six years (FIGURE 3.7). Similarly, IHNV has not been detected since 2006, which could be attributed to

specific management changes. Rearing densities are reduced, and fish are reared off-site at IHNV negative hatcheries until they are larger and less susceptible to the virus (Withalm 2007). These fish are then transferred to Leaburg Hatchery for stocking during January – July, when there are higher flows and temperatures are moderate. Furthermore, IHNV-positive adults are not passed upstream of the facility (pers. comm. Craig Banner). This appears to have reduced IHNV incidence, and the facility experiences few epizootics. However, we suggest that there may be the risk of pathogen entry into Leaburg Hatchery through untreated water.

Adult salmon move upstream of the dam through the fish ladder at Leaburg Dam. These fishes and upstream free-ranging fishes above hatchery waterway are likely pathogen reservoirs. Furthermore, *F. psychrophilum* can survive for up to 300 days in water and sediment, suggesting an extended exposure risk (Madetoja et al. 2003). There may also be the risk of downstream transmission from hatchery fish to free-ranging fish during hatchery epizootics of *F. psychrophilum*. Laboratory experiments demonstrate difficulties in initiating *F. psychrophilum* infections through bath exposures (Holt et al. 1989; Garcia et al. 2000). The high levels of pathogens required to initiate infection ($1x10^6$ colony-forming units per mL) (Garcia et al. 2000) are unlikely to be present in the hatchery effluent, even during an epizootic. Our studies corroborate this, as sentinel fish did not become infected downstream of the *F. psychrophilum* epizootic in 2011 (see Chapter 2), and we did not detect target pathogens in sentinel fish held downstream at any point. Thus we suggest that there is low risk of downstream pathogen transmission of *F. psychrophilum* from hatchery fish to free-ranging fishes. It is important to note that laboratory challenges may not encompass all the environmental variables that can lead to infection and disease. Implementing a facility-wide water treatment system, such as UV irradiation, could both minimize pathogen entry into the facility and exit from the effluent.

Leaburg Hatchery incorporated a UV treatment system for their hatch house water for $50,000 with an operating cost of $10 per day (Morgan and Withalm 2008). A facility-wide water

treatment operation is estimated cost be five to six million dollars, which may be cost-prohibitive (pers. comm. Craig Banner). However, *F. psychrophilum* has not caused extensive disease issues in recent years, occurring rarely from 2007 – 2013, and, given the difficulty in transmitting the pathogen horizontally, perhaps a water treatment system is not necessary. Conversely, it is possible that low, chronic exposures lead to disease, which would argue for water disinfection. Leaburg Hatchery experiences yearly flooding and a facility-wide water treatment system, such as UV irradiation or ozonation, would need to be combined with filtration to remove silt due to flooding and swift flows.

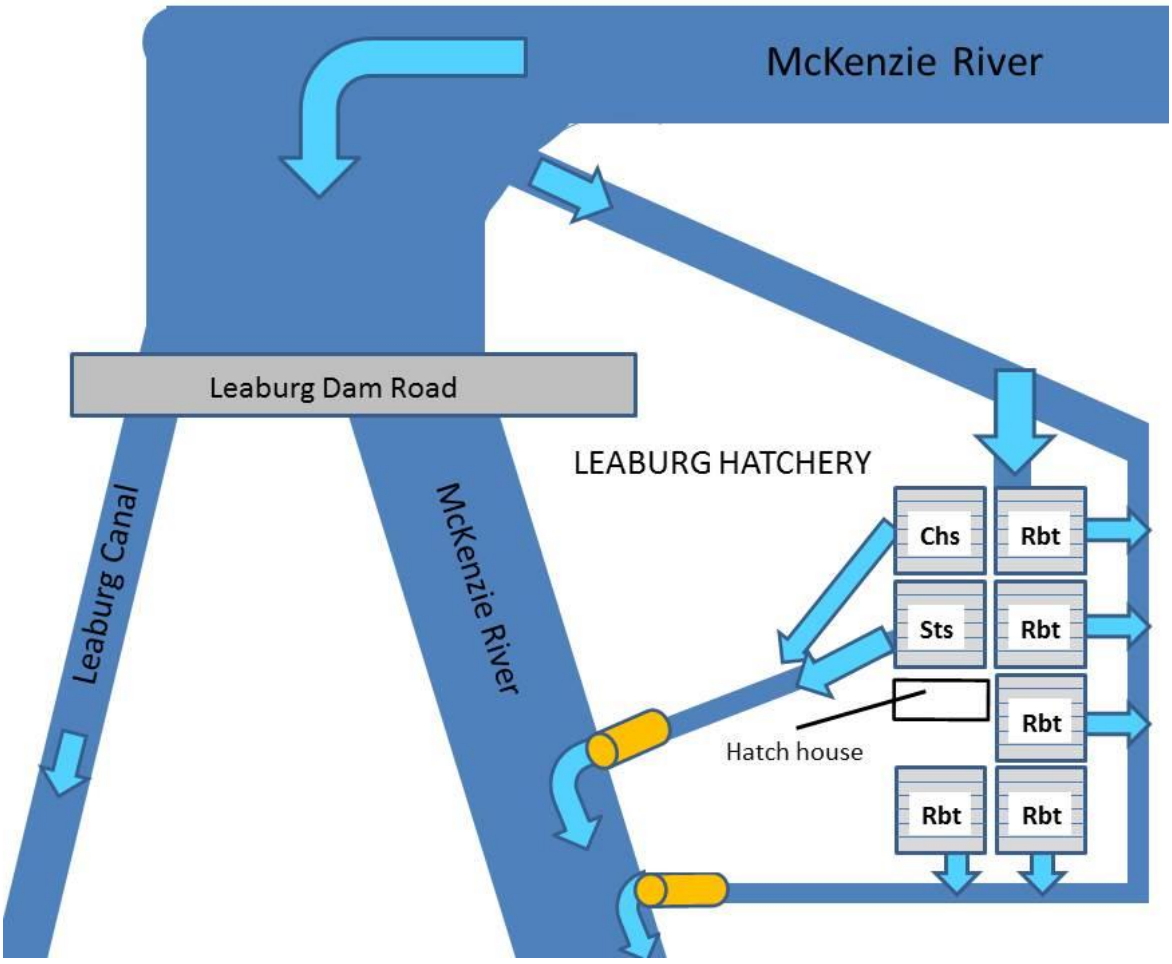


FIGURE 3.6 Leaburg Hatchery schematic and species reared per raceways. Arrows indicate water flow, cylinders indicate water transport pipes. Not to scale.

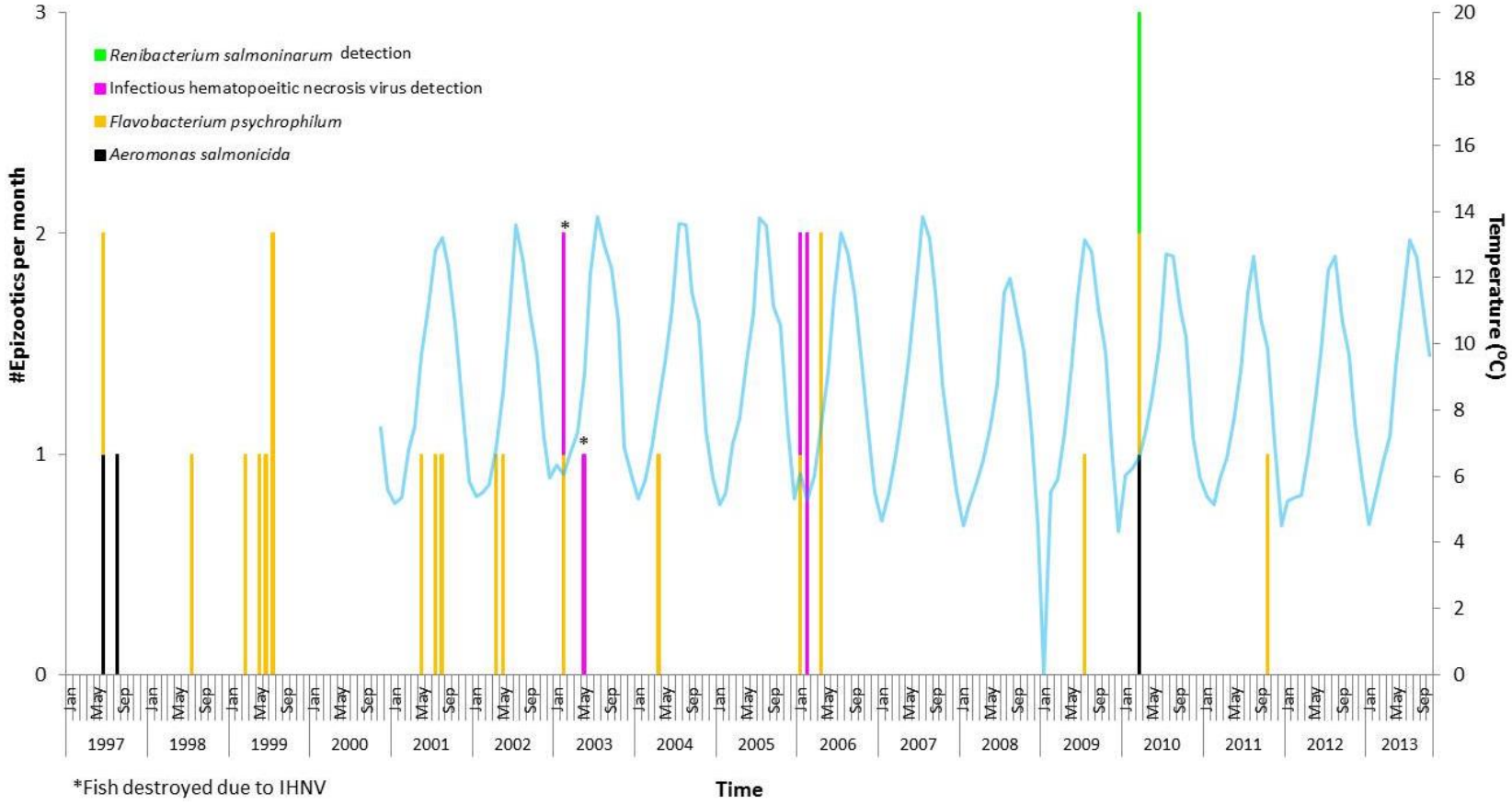


FIGURE 3.7 Historical pathogen epizootics at Leaburg Hatchery, rainbow trout. and average monthly hatchery water temperatures (blue line). Temperatures from USGS gauge, Vida station, approximately two miles upstream (U.S. Geological Survey 2014b).

DISEASE RISKS AT MCKENZIE RIVER HATCHERY

Location and hatchery plan

Similar to the previously discussed facilities, this hatchery was constructed to mitigate for lost trout and salmon runs on the McKenzie River due to hydroelectric projects in the surrounding area. McKenzie River Hatchery is situated on the south fork of the McKenzie River, roughly two miles downstream of Leaburg Dam and Leaburg Hatchery, and it receives single-pass water from Cogswell Creek and Leaburg Canal. The canal diverts water from Leaburg Reservoir upstream of Leaburg Hatchery; thus McKenzie River Hatchery does not receive effluent water from Leaburg Hatchery but both share the same water supply. Migrating adult and juvenile salmonids are blocked from entering Leaburg Canal and thus are not present in Cogswell Creek (Eugene Water and Electric Board 2014), although other non-salmonids fish species are present (Williams 2014). McKenzie River Hatchery has 30 raceways used for rearing spring Chinook salmon, an adult holding area, and a hatch house for rearing egg and fry stages (FIGURE 3.8). The raceways are 22.9m long, 5.1m wide, 0.81m deep, with a unit volume of 1,017m^3, and there is no settling pond at the facility. Hatchery influent and effluent are not treated and water temperatures range between 4˚C -18˚C (FIGURE 3.9).

Contributing disease factors

A. salmonicida epizootics often occur during or immediately after water temperature increases, though they also may occur during cooler temperature periods (FIGURE 3.9). These epizootics have decreased in frequency since 2008, while *F. psychrophilum* epizootics have increased. Pathologists have suggested that this could be due to fluctuation in pathogen prevalence or reduced fish handling at the facility (pers. com. Tony Amandi). *F. psychrophilum* epizootics and erythrocytic inclusion body syndrome virus (EIBSV) have occurred together when

temperatures were approximately 4˚C (FIGURE 3.9). *F. psychrophilum* often presents as a secondary pathogen when fish are infected with EIBSV (pers. com. Tony Amandi; (AFS-FHS 2012) and these co-infections may synergistically compound disease (Densmore et al. 2004). Salmonids upstream in Leaburg Dam and the upper McKenzie River likely contribute pathogens into hatchery waterways. However, in contrast to Leaburg Hatchery, McKenzie River Hatchery has few issues with ectoparasites and silting during seasonal floods. Although both facilities share water from Leaburg Reservoir, Leaburg Canal may allow particulates to settle from the water before flowing into McKenzie River Hatchery, thus reducing the risk of silting and ectoparasite entry. *R. salmoninarum* has not caused epizootics at this facility, although infections have occurred. Juvenile fish are fed erythromycin-treated feed to manage this pathogen (pers. com. Sarah Bjork).

Management implications

Disease risks at McKenzie River Hatchery are similar to Leaburg Hatchery. Both receive untreated incoming water, and free-ranging fish upstream could contribute pathogens. Utilizing a facility-wide UV filtration system could minimize pathogen entry through incoming water, and may reduce the incidence of *F. psychrophilum* and *A. salmonicida* epizootics. McKenzie River Hatchery has two water sources and we suggest that incoming water from Leaburg Canal be treated, as adult and juvenile salmonids are present upstream and are a hypothesized pathogen source. It may not be necessary to treat incoming water from Cogswell Creek, as salmonids may not be present in this source.

We did not detect target pathogens in sentinel fish held downstream during baseline monitoring (see Chapter 2). A low percentage of rainbow trout held downstream during a *F. psychrophilum* epizootic became infected, but these fish did not display gross clinical disease signs (see Chapter 2). Infections do not necessarily lead to a disease state, and it is difficult to

transmit *F. psychrophilum* through bath exposures (Holt et al. 1989; Garcia et al. 2000), and *A. salmonicida* epizootics are infrequent. As such, we suggest that there is low risk of pathogen transmission from McKenzie River Hatchery fish to downstream, free-ranging fishes.

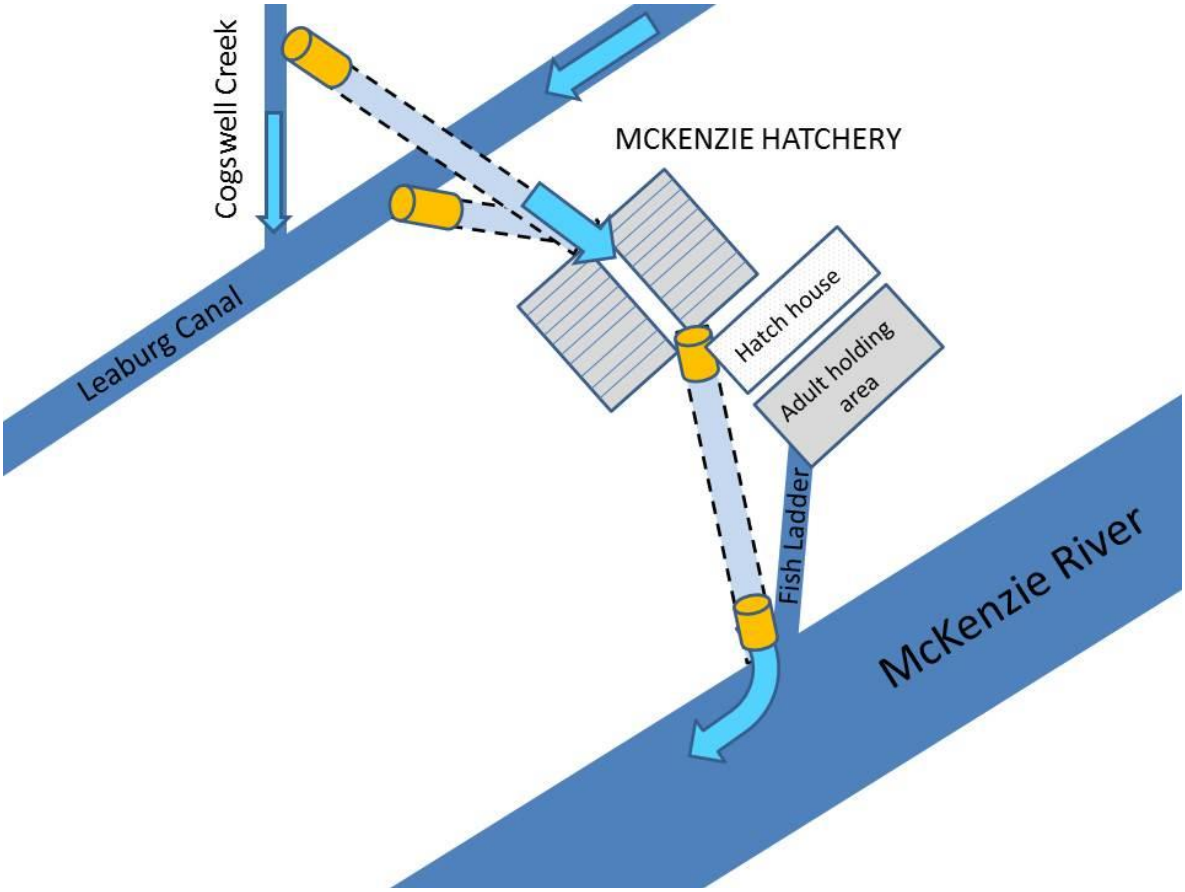


FIGURE 3.8 McKenzie River Hatchery schematic. Arrows indicate water flow, cylinders indicate underground pipes. Not to scale.

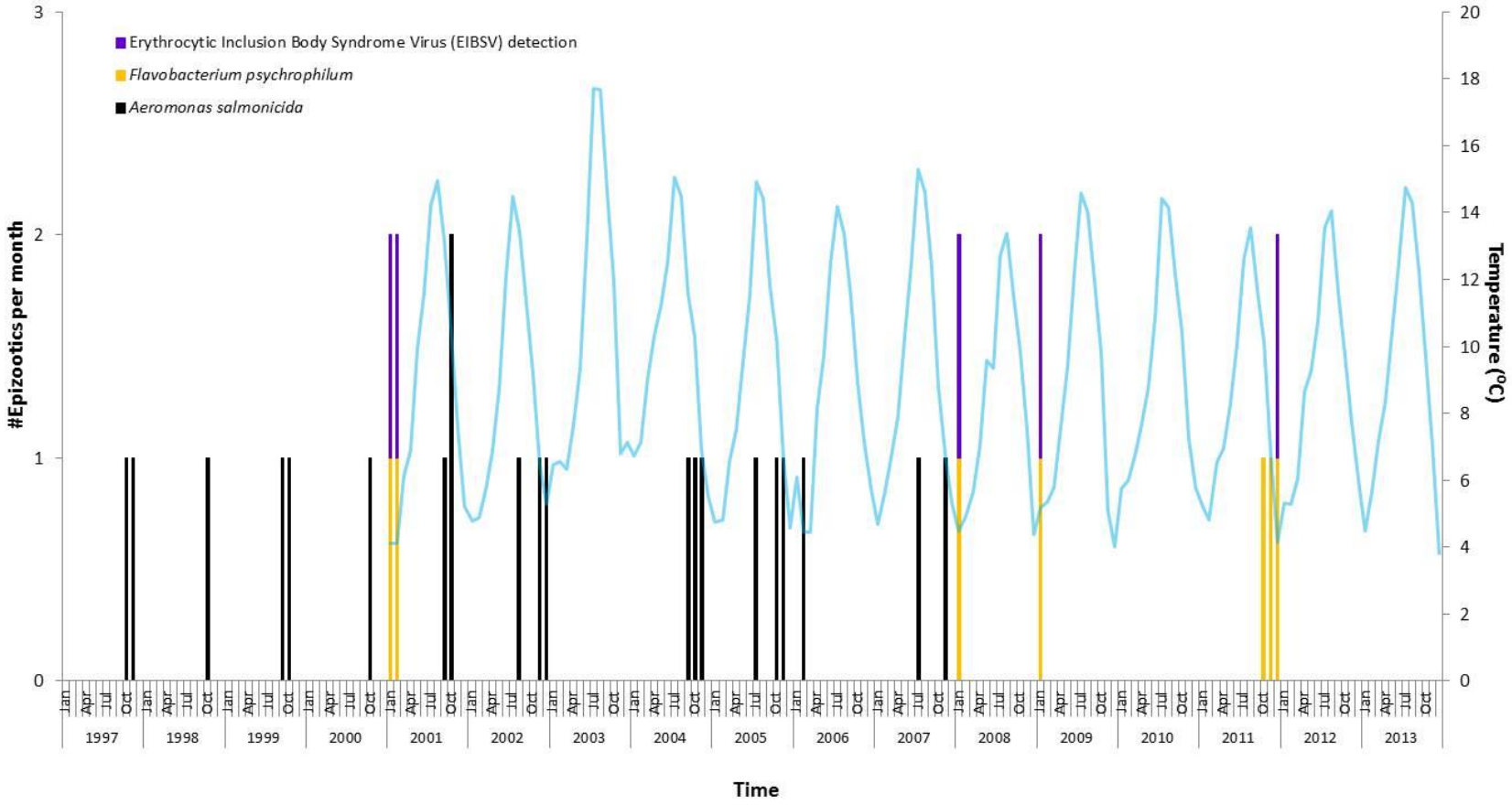


FIGURE 3.9 Historical epizootics at McKenzie River Hatchery, Chinook salmon. Temperature data from McKenzie River Hatchery.

DISEASE MANAGEMENT AT OREGON HATCHERIES

We have examined disease factors based on historical data, hatchery data, ODFW reports on hatchery and fish health regulations, and personal communications from ODFW fish health specialists and hatchery managers. Hatcheries have a variety of preventative methods and regulations in place to minimize pathogen transmission. These include appropriate biosecurity measures such as restricting equipment for use in a given area or raceway, preventing cross-contamination, maintaining an adequate cleaning schedule, performing monthly health checks, and monitoring and recording daily mortality (Oregon Dept. of Fish and Wildlife 2003). Managers also follow fish Density Index and Flow Index guidelines for rearing. Stressors such as handling, fish transfer, or transport are limited, as these activities may exacerbate disease. Horizontal and vertical transmission of *R. salmoninarum* and IHNV are minimized through egg surface sterilization and by testing select populations of female spring Chinook salmon for pathogen presence (by ELISA and cell culture, respectively) and culling eggs from heavily infected individuals (Oregon Dept. of Fish and Wildlife 2003). Injection of adult salmon with antibiotics prior to spawning is done to reduce both bacterial transmission and adult mortality. However, despite these efforts, infections may develop into disease and further treatment may be required.

Bacterial infections caused by *F. columnare* or *A. salmonicida* can be treated with antibiotics. However, this should be carefully managed as with increased use there is potential for antibiotic resistance (Cabello 2006), which has been reported in both species (Tsoumas et al. 1989; Schmidt et al. 2000). Antibiotic resistance could allow pathogens to spread unchecked through a hatchery population, resulting in high levels of loss. Resistance has been observed in *A. salmonicida* isolated from Dexter Ponds (pers. com Tony Amandi). In the case of IHNV and

other viruses, once fish are infected there is no treatment, so hatcheries will often cull eggs or fish (Harrod and Sall 2011).

Despite these treatment options and preventative measures, pathogens that enter through untreated water, fish transfer, or through compromised biosecurity can result in disease epizootics in the hatchery. The wildlife surrounding a hatchery may contribute to disease transmission, and even nets and barriers may be insufficient for preventing predators from entering hatchery raceways. In addition, transmission of pathogens by asymptomatic carriers may be difficult to control, as these fish may shed the bacterium for a long period of time while remaining apparently healthy and indistinguishable from other fish.

A potential consequence of an epizootics occurring in a hatchery is the release and transmission of pathogens to downstream fish. We found that pathogen transmission, resulting in infection and clinical disease, occurred during *A. salmonicida* and *F. columnare* epizootics. In contrast, during *F. psychrophilum* epizootics, sentinel fish held downstream of hatcheries either did not become infected (Leaburg Hatchery), or became subclinically infected (McKenzie River Hatchery). This indicates that there are different transmission risks associated with our target pathogens. However, it is difficult to predict the health outcome of free-ranging fishes downstream of epizootics at hatchery facilities, as these are likely a variety of age classes and species with different susceptibilities.

There are several options for reducing the risk of pathogen entry and release from hatchery influents and effluents. Utilizing fish-free water sources, such as spring or well water, could reduce the risk of transmission, but this option is not available to the hatcheries in this study. UV irradiation or ozonation, combined with filtration, inactivate bacterial and viral pathogens (Sharrer and Summerfelt 2007). However, UV irradiation is available only to Leaburg Hatchery's hatch house, and facility-wide treatment of all incoming water may be cost-prohibitive and mechanically difficult. In the event of facility epizootics, there is also the risk of

pathogen discharge. Therefore, treating the effluent as well as the influent could reduce the risk of transmission to downstream, free-ranging fishes. We suggest that the effluent should be treated during *F. columnare* and *A. salmonicida* epizootics, as these pathogens appear to be more efficiently transmitted in comparison to *F. psychrophilum*. Our data suggest the potential for *F. columnare* and *A. salmonicida* being released in the hatchery effluent during epizootics, and treatment of hatchery effluent during those epizootics could eliminate that possibility. However, it is likely that it will take time to implement facility-wide water treatment systems due to cost and construction. Until then, hatchery management should continue to focus on prevention to reduce pathogen transmission.

Preventative methods include reducing rearing densities and administering vaccines. Fish rearing densities were reduced at Leaburg Hatchery and are credited for improving fish health and reducing IHNV epizootics (pers. comm. Erik Withalm). Vaccines are available for *A. salmonicida*, *F. columnare*, and *R. salmoninarum* (Purcell et al. 2004; American Fisheries Society Fish Culture Section 2011), and a DNA vaccine for IHNV was recently approved in March 2013 (USDA-APHIS 2014). However, vaccinations are not in use at our study hatcheries, one reason being that it may be cost prohibitive to administer vaccines at facilities that rear millions of fish (pers. comm. Rich Holt) or have variable efficacy. Oral or immersion delivery is easier than injection but may not be as effective, depending on the vaccine (Brudeseth et al. 2013). In addition, fish cannot be released or consumed within a certain period of time after being vaccinated, to prevent human or animal exposure to the vaccine. These factors may limit the use of vaccinations at hatcheries.

The pathogens that impact Dexter Ponds, Leaburg Hatchery and McKenzie River Hatchery are transmissible through a variety of vertical and horizontal pathways. Despite hatchery cleaning, maintenance, and routine health checks, facilities continue to experience epizootics by these pathogens. Although water treatment and vaccination may further reduce

these risks, these measures are costly and do not preclude transmission. Best management practices should be adhered to in order to maintain the health of hatchery fish populations.

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CHAPTER 4: SUMMARY

Chapter 2: Assessing disease impacts of hatcheries on downstream fishes

- Downstream pathogen transmission can occur during hatchery epizootics but is pathogen dependent. *A. salmonicida* and *F. columnare* were the most efficient in infecting downstream fish, while *F. psychrophilum* was less efficient.
- We did not detect infections in sentinel fish held downstream of Leaburg Hatchery during a *F. psychrophilum* epizootic, nor when cohabited with IHNV-infected adults.
- We did not detect infections in sentinel fish held in the hatchery influent or effluent during baseline monitoring. The highest risk of downstream pathogen transmission from hatchery fishes to free-ranging fishes is during epizootics.
- Incoming, untreated water could be a pathogen source but we were unable to verify this.

Chapter 3: Disease risks associated with hatcheries in the Willamette River Basin

- Our target pathogens present different disease risks at our study hatchery facilities. These are likely influenced by hatchery location, species reared, and water source.
- Changes in fish husbandry and infrastructure improvements have reduced disease incidence.

- Treating the hatchery influent and effluent with UV irradiation or ozonation, combined with filtration, is an option for removing pathogens, but may be cost-prohibitive.

FUTURE RESEARCH

- Data gaps remain in respect to the risks of pathogen transmission from free-ranging fishes to hatchery fishes, and vice versa. Transmission studies, determining parameters such as dose, water velocity, temperature, and pathogen dispersion rates, would be relevant to this study.
- The role of carrier fish is still not well understood, and it is a difficult factor to control in aquaculture. Non-lethal sampling may be useful in laboratory challenges determining shedding rate in sub-clinically infected individuals.
- Additional data is needed on pathogen prevalence in juvenile and adult salmonids, especially in spatial and temporal relation to hatchery epizootics.

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APPENDICES

WATER ISOLATION OF *FLAVOBACTERIUM COLUMNARE*

INTRODUCTION

As party of our study on pathogen transmission dynamics in the Willamette River Basin (Chapter 2), we collected environmental water samples to test for the presence of *Flavobacterium columnare*. In order to determine which media to utilize for detecting environmental *F. columnare*, we cultured two *F. columnare* strains, diluted them in various water substrates and then inoculated the culture onto four types of media. The media with the greatest number of bacteria at the highest dilution would indicate the highest sensitivity.

MATERIALS AND METHODS

Bacterial growth and culture

F. columnare was isolated from the kidney of an *Oncorhynchus tshawytscha* in 2013. Bacteria were inoculated into 600 mL tryptic yeast extract soy (TYES) broth and grown for 24 hours to 0.2 optical density (OD) at 525 nm wavelength. Bacteria were diluted from 10^{0} to 10^{-8} in the following reagents: sterile TYES broth, non-sterile Willamette River water, and sterile phosphate-buffered saline (PBS). Bacteria were then inoculated in triplicate onto the following media: TYES (AFS-FHS 2012), *F. columnare* growth medium (FCGM) (Farmer 2004), modified Cytophaga (Hawke and Thune 1992), modified Shieh's (Bullock et al. 1986), and dilute Mueller-Hinton (DMH)(Hawke and Thune 1992) agar. Plates were incubated at 15°C and examined daily for bacterial growth for seven days.

RESULTS

At 10^{-5} and 10^{-6} dilution in TYES broth, *F. columnare* growth was highest on Hsu-Shotts media and comparable among TYES, FCGM, modified cytophaga, and modified Shieh's media (FIGURE 5.1). At $^{10-7}$ dilution, there was more growth on Hsu-Shotts media. At $^{10-5}$ dilution in river water, *F. columnare* growth was highest on modified cytophaga agar and comparable among TYES, FCGM, and modified Shieh's media. At $^{10-6}$ dilution in river water, *F. columnare* growth was highest on Hsu-Shotts media and comparable between modified cytophaga and modified Shieh's media. At $^{10-7}$ dilution, there was more growth on modified Shieh's media. At $^{10-5}$ dilution in PBS, *F. columnare* growth was highest on modified Shieh's media but was comparable to Hsu-Shotts media. At $^{10-6}$ and $^{10-7}$ dilution, *F. columnare* growth was highest on TYES media.

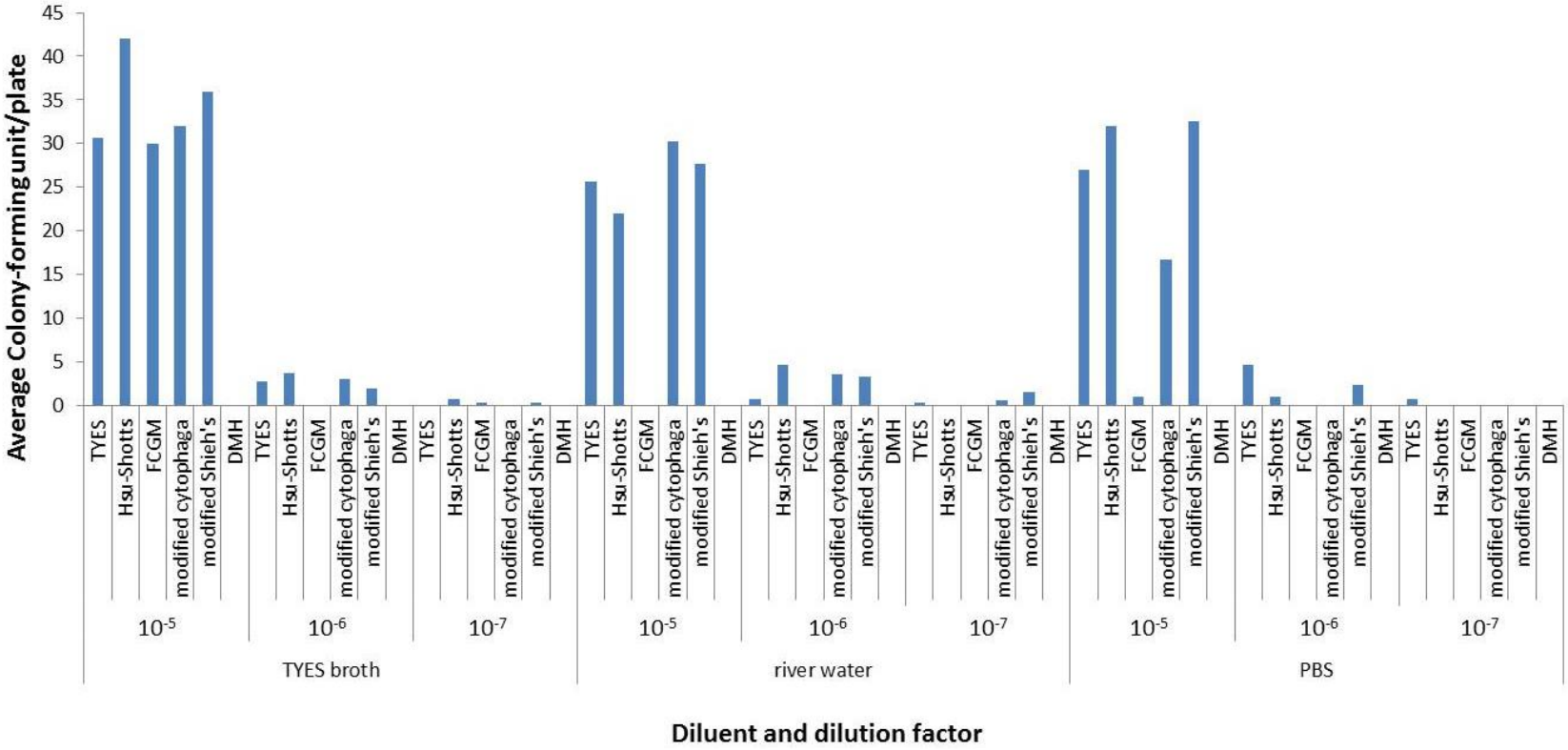

FIGURE 5.1 Average *F. columnare* colony-forming units (CFU) per media and diluent.

DISCUSSION

We have determined that TYES is the preferable media for us to use for environmental water samples due to ease of use and rapid *F. columnare* growth. *F. columnare* colonies grew fastest on TYES and modified Shieh's and were visible within 24 hours, while colonies took longer to grow on modified cytophaga and were translucent and difficult to visualize. *F. columnare* did not grow up on samples diluted in river water or PBS that were then inoculated onto FCGM, and DMH did not work under any condition. We do not know the reasons for this, but other studies have noted that both FCGM and DMH are poor at isolating *F. columnare* (Farmer 2004). Modified Shieh's may be more sensitive than TYES at lower *F. columnare* concentrations, but it is more time intensive to make and it is recommended for same-day use. Our field experiments take multiple days to transport sentinel fishes into and out of our field sites, and we are unable to adhere to the time constraints required to make modified Shieh's media.

FLAVOBACTERIUM COLUMNARE COHABITATION STUDY

INTRODUCTION

We are studying pathogen transmission the hatchery systems in the Willamette River Basin in Oregon, and establishing horizontal transmission is very important to understanding disease dynamics. We constructed an experiment to establish horizontal, water-borne bacterial transmission rates. Our experiment is based on the experiments and data collected by Ogut and Reno in 2005, where they performed a transmission study with *A. salmonicida*. Ogut and Reno experimentally infected salmonids with *A. salmonicida*, exposed the fish to naïve fish through cohabitation, and monitored infection rates. There have been various studies testing pathogenicity and lethal doses of *F. columnare*, but none exploring cohabitation and water transmission. Here we experimentally infected *Oncorhynchus tshawytscha* and cohabited these donor fish with naïve fish, at a ratio of one donor fish to 25 naïve fish per tank, and monitored them for infection and disease signs every day for ten days.

MATERIALS AND METHODS

Pilot study

We conducted a pilot study to verify *F. columnare* strain virulence and dose for donor fish. 25 *O. tshawytscha*, between 5 g to 13 g per fish, were held in 100L tanks at 2L/min flow at 13°C. Over a 1-2 hour period, the temperature was raised to 15°C, and fish were held at this temperature for 24 hours. After this time, the temperature was raised to 18°C over another 1-2 hours, and fish were allowed to recover for 24 hours. Fish were bath-challenged to varying doses of *F. columnare* (TABLE 5.1). *F. columnare* was grown in TYES broth for 12 hours at18°C and then was transferred to a shaker and agitated at moderate speed for two additional hours at 18°C.

The optical density was determined and the culture was diluted accordingly with TYES broth (TABLE 5.1). For the bath challenges, water flow was halted during the exposure and tanks were supplemented with aeration for the duration of the time (10 – 45 minutes), after which point the water flow was resumed. Fish were monitored 3x daily for disease sign development. Fish displaying clinical disease signs such as lesions, darkening, lethargy, loss of equilibrium, and inappetence, were lethally sampled (0.04% tricaine methanesulfonate + 0.5% sodium bicarbonate) and processed.

Donor fish

Donor fish were held at 13°C and were not fed for 24 hours prior to elastomer tagging. We sedated the fish (0.01% tricaine methanesulfonate + 0.025% sodium bicarbonate) and injected an elastomer tag into the base of the caudal fin. Fish were allowed to recover for 24 hours, and then were acclimated to 18°C as described above. Fish were not fed 24 hours prior to *F. columnare* exposure. As determined by our pilot study, donor fish were bath challenged to *F. columnare* at 0.2 OD, 1:20 dilution, for 45 minutes. The following day, the donor fish were transferred into tanks to begin the cohabitation study.

Cohabitation

O. tshawytscha, between 5 g to 13 g per fish, were held in 100L tanks at 2L/min flow at 13°C and acclimated to 18°C as described above. Fish were held at 18°C for 48 hours prior to the experimental start. After this, one bath-challenged, elastomer-tagged donor fish was added per tank. Each day for 10 days, three randomly selected tanks were euthanized (TABLE 5.2); the tank water level was reduced and we administered an overdose of 0.01% tricaine methanesulfonate + 0.025% sodium bicarbonate. Three control tanks of 25 fish were also sampled at day 10. Gills, head kidney, and external lesions were streaked for bacterial isolation on tryptic yeast extract soy

agar (TYES) (AFS-FHS 2012). Control fish were held at the same conditions as experimental fish, and treatment and control groups were randomly assigned to tanks. Fish displaying clinical disease signs, including lesions, darkening, lethargy, loss of equilibrium, and inappetence, were lethally sampled and processed.

Water sampling

One mL of surface water was collected each day from the three randomly selected tanks. Of this, 200 µL was diluted to 10^{0}, 10^{-1} – 10^{-4} and inoculated in triplicate on TYES media. Plates were incubated at 15°C and examined at day 3 and 7 and counted for *F. columnare* colonies.

TABLE 5.1 Pilot studies to determine *Flavobacterium columnare* LD_{50}.

Tank #	Optical density of *F. columnare* culture	Dilution factor	# fish	Exposure time
1	0.2 at 525 nm	1:20	25	45 min.
2	0.2 at 525 nm	1:20	25	45 min.
3	0.2 at 525 nm	1:20	25	30 min.
4	0.2 at 525 nm	1:20	25	30 min.
5	0.2 at 525 nm	1:20	25	15 min.
6	0.2 at 525 nm	1:20	25	15 min.
7	0.2 at 525 nm	1:20	25	45 min.
8	0.15 at 525 nm	1:20	25	30 min.
9	0.15 at 525 nm	1:20	25	30 min.
10	0.15 at 525 nm	1:20	25	15 min.
11	0.15 at 525 nm	1:20	25	15 min.
12	0.1 at 525 nm	1:20	25	45 min.
13	0.1 at 525 nm	1:20	25	45 min.
14	0.1 at 525 nm	1:20	25	30 min.
15	0.1 at 525 nm	1:20	25	30 min.
16	0.1 at 525 nm	1:20	25	15 min.
17	0.1 at 525 nm	1:20	25	15 min.
18	0.1 at 525 nm	1:20	25	10 min.
19	0.1 at 525 nm	1:20	25	10 min.
20	0.1 at 525 nm	1:40	25	10 min.
21	0.1 at 525 nm	1:40	25	10 min.
22	0.1 at 525 nm	1:80	25	10 min.
23	0.1 at 525 nm	1:80	25	10 min.
24	0.1 at 525 nm	1:100	25	10 min.
25	0.1 at 525 nm	1:100	25	10 min.
26	0.1 at 525 nm	1:200	25	10 min.
27	0.1 at 525 nm	1:200	25	10 min.
Control	PBS	1:20	25	30 min.
Total fish			700	

TABLE 5.2 Cohabitation exposure and daily sampling, at a *F. columnare* dose of 0.2 OD at 525 nm, 1:20 dilution, for 45 minute static exposure. After this time, water flow was resumed.

Sample Day Post-Exposure (DPE)	Tanks	Exposure	Donor Fish per tank	Exposed Fish per tank	Replicates	Total Fish
1	1	Cohabitation	1	25	3	75
2	1	Cohabitation	1	25	3	75
3	1	Cohabitation	1	25	3	75
4	1	Cohabitation	1	25	3	75
5	1	Cohabitation	1	25	3	75
6	1	Cohabitation	1	25	3	75
7	1	Cohabitation	1	25	3	75
8	1	Cohabitation	1	25	3	75
9	1	Cohabitation	1	25	3	75
10	1	Cohabitation	1	25	3	75
10	1	Cohabitation	1	25	3	75
Fish Total:						825

RESULTS

Pilot study

We had difficulty determining *F. columnare* dose for donor fish. There was inconsistent mortality among tanks, optical density, and exposure time.

Cohabitation study

We noted varying, inconsistent levels of infection in donor fish (FIGURE 5.4, 5.5, 5.6, TABLE 5.3) between the two experiments. No naïve, cohabited fish became infected with *F. columnare* in either experiment.

Water inoculation

Plates inoculated with water from experiment 1 became rapidly overgrown with fungus and we did not detect the bacterium. In experiment 2, we detected varying levels of bacteria (FIGURE 5.6a) among tanks, which suggested a trend that water detection was dependent on donor fish status (FIGURE 5.6b).

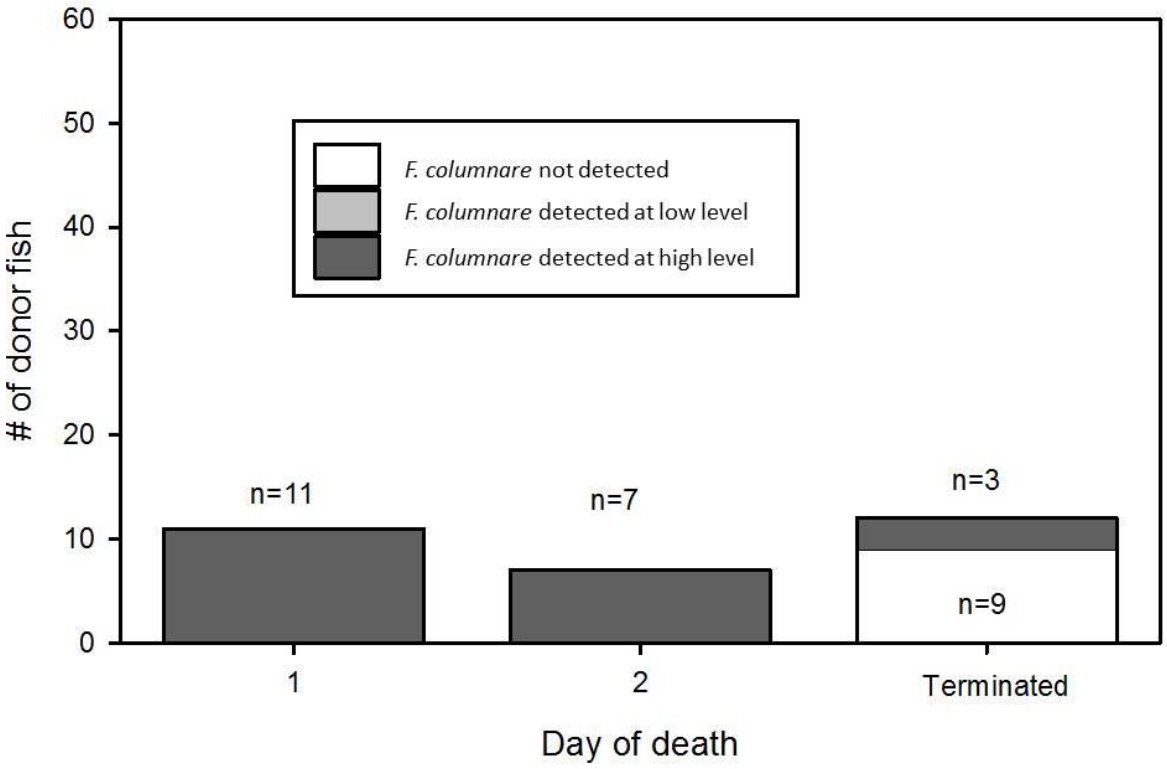

FIGURE 5.2 Experiment 1 number and relative proportions of donor fish in the three categories of infection per score. Terminated indicates fish survived until sample date. Figure courtesy of Julie Alexander.

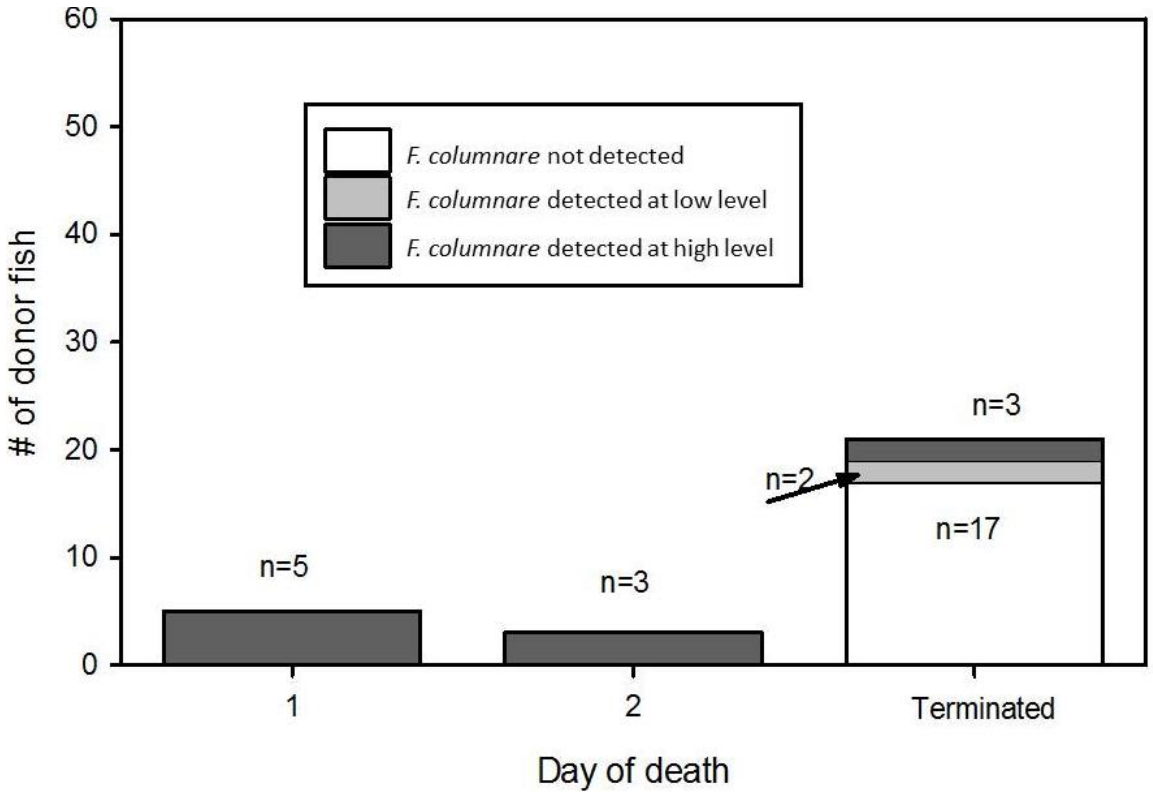

FIGURE 5.3 Experiment 2 number and relative proportions of donor fish in the three categories of infection per score. Terminated indicates fish survived until sample date. Figure courtesy of Julie Alexander.

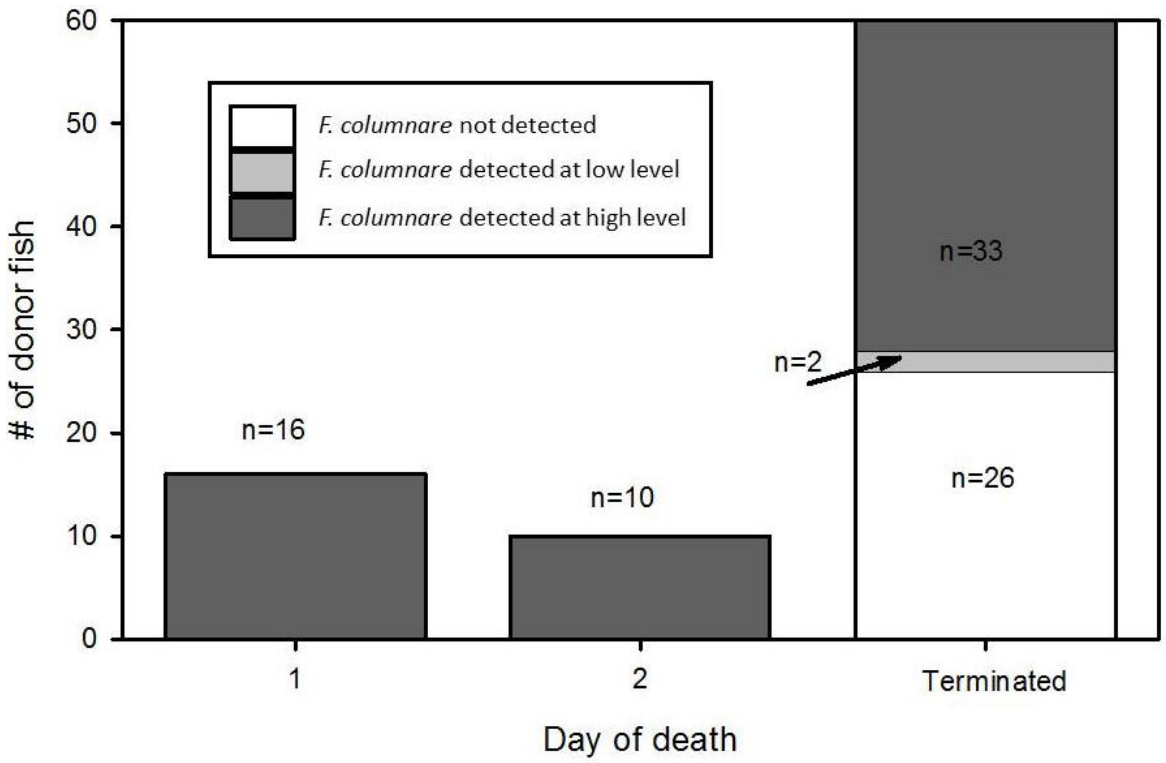

FIGURE 5.4 Number and relative proportions of donor fish in the three categories of infection per score for both experiments. Terminated indicates fish survived until sample date. Figure courtesy of Julie Alexander.

TABLE 5.3 Number of donor fish shown by experiment, score, and survival category.

Survival	Donor score	Experiment 1	Experiment 2
Died within 48 hrs.	High	18	8
	Low to not detected	0	0
Survived until termination	High	3	3
	Low to not detected	9	19

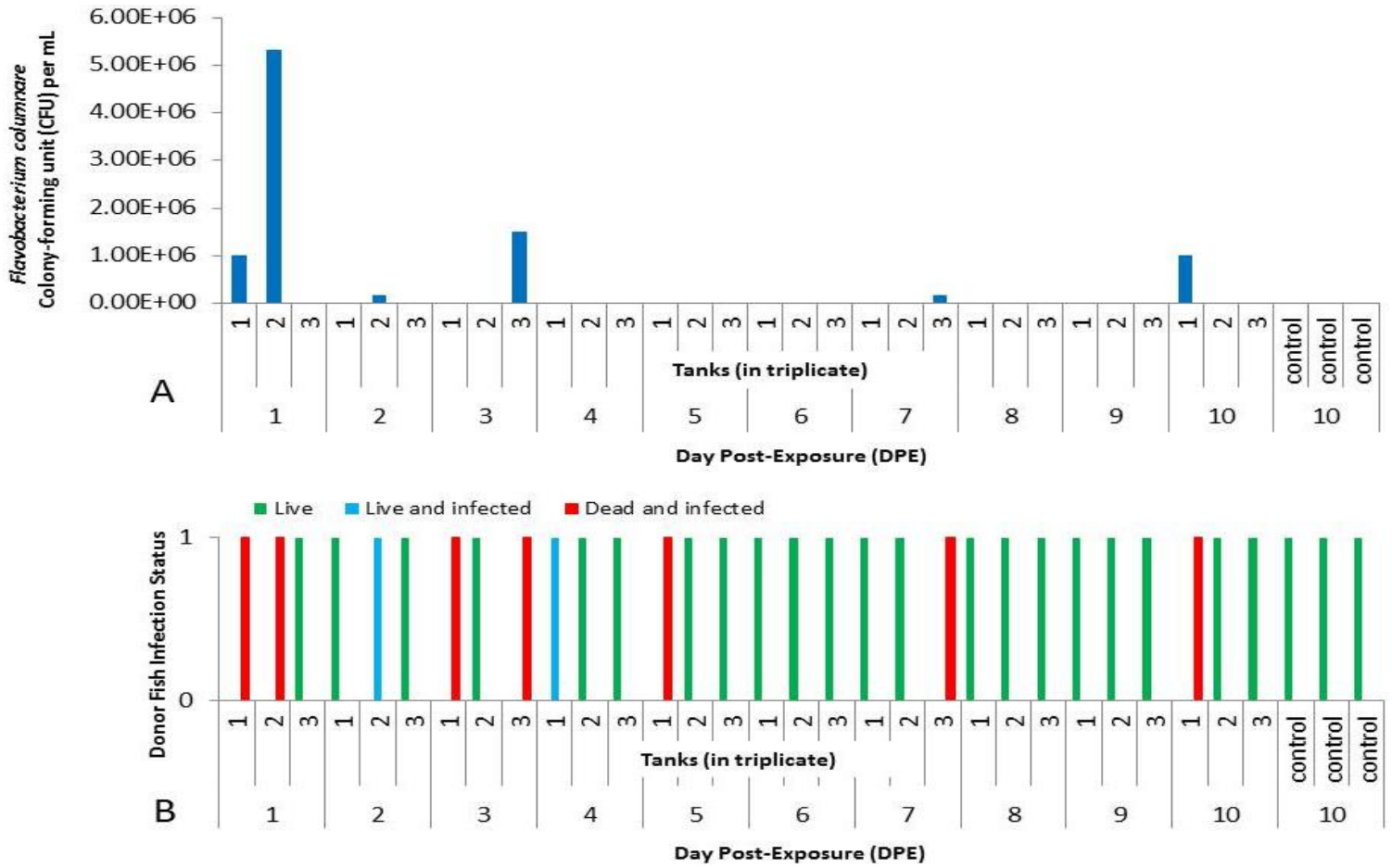

FIGURE 5.5 (A) *Flavobacterium columnare* detection in water and (B) donor fish infection status. Dead and infected indicated that the donor fish had died prior to sampling and was positive for the infection.

DISCUSSION

We were unable to consistently infect donor fish and establish a dose for donor fish. Due to this, we were only able to conduct two full experiments before we did not have sufficient fish to continue with a third exposure, as planned. Possible explanations for this are that *F. columnare* can be difficult to grow and may form long filamentous clumps that can interfere with OD readings. As such, we would only use the upper fraction of *F. columnare* grown in broth culture in flasks. We used the same spectrophotometer to determine OD per each exposure, and used the same formulas for TYES broth. *F. columnare* growth conditions were standardized across all groups. Despite this, we observed inconsistent mortality, even across groups that had been challenged with the same dose. However, similar experiments such as these would be valuable to determine transmission efficiency, if *F. columnare* dose could be standardized.

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