APPENDIX D. DISEASE HISTORY AND PATHOGEN RISKS AT MAKAH NFH¹

I. Fish Health Issues

Makah NFH faces many fish health challenges associated with warm summer temperatures, a surface water source containing adult salmon, and increased frequency of summer droughts. For example, parasitic infections of *Ichthyophthirius* (Ich) *and Ichthyobodo* sp. (aka Costia) require frequent treatments with formalin to avoid significant disease outbreaks; otherwise, high fish mortalities would most likely result. Chinook salmon (subyearlings) have typically been released earlier than desired to allow outmigration to saltwater before rising water temperatures and high pathogen loads in the Tsoo-Yess River pose a significant disease risk. Despite that precaution, *Ichthyobodo* sp. and furunculosis have often been significant pathogen issues for Chinook salmon subyearlings in the spring prior to release from Makah NFH.

Makah NFH also relies on the repeated use of antibiotics to prevent or treat bacterial infections of coho salmon and steelhead during the summer months (June-September) when average water temperatures are greater than 15°C (60°F) and often approach 21°C (70°F). In particular, steelhead had to be treated recently for furunculosis for the first time. These warm water temperatures, coupled with an open water supply known to contain many different fish pathogens, have the potential for culminating into disease epidemics. If left untreated, the majority of the fish on station during the summer (coho salmon and steelhead) would most likely die based on past history of rearing these two species at the hatchery. Hatchery management and fish health staffs do not believe the heavy reliance on drugs and chemicals is sustainable or a best management practice (BMP).

II. Primary pathogens of concern and current treatment/mitigation measures

The two pathogens of greatest concern during the summer months are (a) the parasite that causes Ich (*Ichthyophthirius multifiliis*) and (b) the bacterium that causes furunculosis (*Aeromonas salmonicida*). In addition, *Flavobacterium psychrophilum*, the causative agent of Bacterial Cold Water Disease (BCWD), has been a cause of significant acute mortality of steelhead during early rearing in the nursery building and after ponding into the raceways. Mortalities due to chronic BCWD have also been an issue for yearling steelhead during the winter months and in the early spring prior to release.

A. Ichthyophthirius multifiliis (Ich)

This is the parasite that causes "White Spot Disease". The life cycle of Ich is temperature dependent; as water temperatures increase from 12°C (54°F) to 22°C (72°F), the faster the

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parasite completes its life cycle (Table B2, Appendix B). If untreated, thousands of additional infective parasites are formed and released into the environment with the completion of each new life cycle. One way of combating Ich is to increase water flow or decrease fish density (increase volume), thus decreasing the encounter probability between the parasite and host. Unfortunately, this strategy is difficult, if not impossible, during periods of low water availability (e.g., during the summer months when water temperatures favor Ich). In addition, the immune system of salmonid fishes is significantly less effective at combatting Ich at the warmer temperatures that favor the parasite.

Ich has been a particular problem for steelhead at Makah NFH during the summer months. In 2009, Makah NFH lost approximately 80% of the juvenile steelhead because of an Ich infestation. The hatchery avoided that level of mortality in subsequent years through careful monitoring and substantial use of formalin. For example, in 2013 and 2014, Makah NFH used over 500 gallons of formalin to treat steelhead for Ich and increased its usage to 2,900 gallons in 2017. Under warmer conditions (and lower summertime water flows), we would expect Ich to become increasingly difficult to manage, resulting in more formalin usage or higher fish mortality (or both).

Measures taken to address Ich:

- Water flow and turnover rates have been maximized within the physical constraints of the hatchery and watershed by reducing the rearing volume during the summer months, thereby increasing water turnover rates for specific flows.
- The density index is maintained below or at the general recommendation for steelhead of 0.20 lbs./ft³/inch throughout the rearing cycle.
- Frequent and regular monitoring (microscopic) of steelhead by the Assistant Hatchery
 Manager and biologists from the Pacific Region Fish Health Program has greatly
 improved the ability of hatchery staff to deliver formalin early in the infection, thus
 increasing the likelihood of controlling the infestation and reducing mortalities.
- Use of an *extra-label prescription* for a low-dose, longer-duration formalin treatment has led to better success treating the fish than results obtained previously with the approved labeled dose. Occasionally, a 1-hour static bath followed by a long, low-dose flow-through treatment is implemented when warranted by the abundance of the parasite.
- Measures have been taken to reduce predation and pathogen transfer between raceways by fabrication of new crow deterrents.
- Hatchery staff are able to bypass the settlement pond by adjusting the incoming water supply. The retention time of the settlement pond has been documented to increase water temperatures by as much as 1°C (1.8° F) above river temperatures due to solar radiation.
- When the less-extreme measures listed above are no longer able to control Ich and fish are in imminent danger of high mortality, steelhead are transferred to freshly-cleaned raceways to interrupt the life cycle of the parasite and to increase effectiveness of chemical treatments.

B. Aeromonas salmonicida (A. sal)

This bacterial species causes furunculosis. This organism is also highly temperature dependent. Disease outbreaks typically do not occur when the water temperature is below 13°C (55°F), but outbreaks are very likely to occur at temperatures above 20°C (68°F), and somewhat likely to occur between those two temperatures (13-20°C) depending on other conditions.

A.sal has been a summertime problem for decades at Makah NFH. As water temperatures approach and exceed 15°C (60°F) in the spring, Chinook salmon are more likely to manifest the disease. In the past, Chinook salmon have been released early at a smaller size than their target release size due to the threat of this disease when water temperatures began to increase in the late spring. In recent years, furunculosis has been an increasing concern for steelhead, one of the more resistant salmonid species to this disease: A.sal has been isolated from steelhead with increasing frequency during the summer months and associated with lesions suggestive of the disease. The summer of 2017 was the first time in many years that the level of disease warranted treatment of steelhead with Florfenicol, a recently approved antibiotic. With the predicted increases in water temperatures, furunculosis is likely to have greater impacts on steelhead at Makah NFH than in the past. Coho salmon are more susceptible to this disease than steelhead and have been treated annually (2012-2017) with Florfenicol (Table D1).

Table D1. Florfenicol (antibiotic) treatments of coho salmon for furunculosis, 2012-2017:

Month	2012	2013	2014	2015*	2016*	2017
May					X	
June		X	X	X		
July	X		X	X		
Aug	X	X	X	Early release euthanized	X	X
Sept	X	X				

^{*}Coho salmon were also treated for Bacterial Cold-Water Disease (BCWD) in 2015 in 2016 with Florfenicol.

Antibiotic resistance for treating furunculosis has become a critical issue over the past 20 years at Makah NFH. In 1994, the strain of *A.sal* at Makah NFH had become resistant to the two antibiotics available for fish at that time (Romet and Oxytetracycline), and mortalities due to *A.sal* reached one percent (approx. 3000 fish) per day. Since then, the antibiotic Florfenicol has been approved, and it has been effective to date for controlling mortality with no apparent antibiotic resistance. However, increasing use of this antibiotic increases the risk of the bacteria developing resistance over time. One of our alternative disease management strategies

is to withhold feed to lessen the impacts of the fecal-oral route of transmission. However, as water temperatures increase, the nutritional impacts (i.e., decreased immune system function) of withholding feed from coho salmon for weeks at a time will likely become more severe.

Measures taken to address A.sal:

- The feeding strategy for coho salmon was changed to increase feed schedule in the cooler months. This practice allows fish to be taken off feed periodically during warmer periods in the summer when disease is a problem. It also decreases fecal-oral transmission of *A.sal*. However, this strategy is counter to the natural growth cycle of fish as a function of water temperature.
- Fish Health staff conducted several trials to assess the value of vaccinating fish via immersion baths, with or without use of feed immunostimulants. Little benefit was realized from those immersion vaccines.
- Hatchery staff have maximized flow and turnover rates in raceways to improve the environmental conditions for juvenile coho salmon.
- Measures have been taken to reduce stress, and pathogen transmission between raceways has been reduced via new crow deterrents.

C. Flavobacterium psychrophilum (Fpsy)

This bacteria, the causal agent of BCWD, has caused disease in Chinook salmon, steelhead and coho salmon at Makah NFH. Steelhead is the most susceptible species to this pathogen. The name Bacterial "Cold Water" Disease is somewhat misleading because we have cultured this species of bacteria in water as warm as 20°C (68°F) from steelhead showing acute (or chronic) signs of the disease at Makah NFH.

Measures taken to help address Fpsy:

- Good fish culture practices in the nursery building prior to ponding to outside raceways are a key aspect of disease prevention and are in place to ensure a clean environment, adequate nutrition, and daily removal of dead fish.
- In typical years, fish are treated with the antibiotic Florfenicol after transfer to the raceways to control disease outbreaks.
- In 2016, fish were pumped instead of netted when transferred from the nursery building
 to the outdoor raceways; this action reduced stress and physical trauma, and no antibiotic
 treatment was required after ponding. The efficacy of this latter practice will be assessed
 over the next few years.

D. Conclusions

Survival of coho salmon and steelhead at Makah NFH have relied extensively on chemicals and antibiotics to minimize disease and mortalities. FWS staffs at Makah NFH and the Pacific Region Fish Health Program have concluded that the current situation is not sustainable over

the long term, both in terms of fish health and environmental safety. Fish health staff further believe that bacterial resistance to the antibiotic Florfenicol is inevitable at the current rates of treatment. In addition to Ich and furunculosis, early outbreaks of BCWD typically occur in a few steelhead tanks in the nursery building during April and May and typically for the entire population after ponding into the raceways. BCWD also affected coho salmon in the spring of 2015. As surface water temperatures rise in the spring and summer and reach 16°C (60°F), coho salmon are challenged with *A.sal* and the threat of an outbreak of furunculosis. Moreover, steelhead are challenged every year with Ich, and 2009 was a particularly bad year when greater than 80% mortality occurred among the brood-year juveniles due to infection with Ich.

III. Other pathogens detected at Makah NFH

A. Ichthyobodo sp. (aka Costia)

In the spring, it is routine for Chinook salmon at Makah NFH to become infected with the parasite *Ichthyobodo sp.* If left untreated, mortality will increase and remain elevated until formalin treatment is applied. When water temperatures are lower (≤10°C or 50°F), formalin treatment can be applied every 1-2 weeks to keep parasite levels low enough not to cause daily mortality until the fish are released. When water temperatures exceed 14°C (57°F), formalin may need to be applied as often as every four days to keep parasite levels manageable. With projected increases in future mean temperatures, this disease is likely to have a greater impact on Chinook salmon at Makah NFH, either resulting in increased use of formalin or higher mortality or both.

B. Nucleospora salmonis

This parasite has been identified by histology in steelhead at Makah NFH in past years. The detections were associated with increased mortality and histopathological lesions (disease at a microscopic level) in the fish examined. We do not know whether increased temperatures will increase the prevalence of this parasite in the Tsoo-Yess River. However, as water temperatures during the summer increase above 60°F, the immune systems of the fish are less likely to combat the parasite successfully if it is present.

IV. Potential future pathogens at Makah NFH

A. Ceratonova shasta

This parasite has been identified in watersheds to the north and south of the Tsoo-Yess River (British Columbia, Alaska, Washington, Oregon, and California). Since 1998, attempts to detect this pathogen at Makah NFH have yielded negative results; however, fish are not routinely examined for this parasite because disease symptoms have not been observed. *C. shasta* can cause significant disease in hatchery and natural-origin fish, but no FDA-approved

treatment exists currently. This parasite requires an intermediate host, a polychaete worm, and its presence or absence in the Tsoo-Yess River is unknown at this time. The disease is associated with warm water and low flows, and predicted warmer temperatures and lower flows of the Tsoo-Yess River in future years are expected to enhance the environmental conditions that could favor the parasite over the fish.

B. Vibrio spp.

The bacterial species *Vibrio anguillarum* and *V. ordalii* have been identified in disease outbreaks of salmonid fishes reared in saltwater net pens in the Pacific Northwest. These species are largely marine species that are associated with salt and brackish water. While fish can be successfully vaccinated for these bacteria, the fish must be immune competent; i.e., they must be of sufficient age and minimum size to have a functional immune system (approximately 5-10 grams in body weight). If saltwater intrusion into the hatchery or water supply occurs in the future due to sea level rise and/or storm surges up the Tsoo-Yess River, these pathogens could become problematic at Makah NFH.

C. Flavobacterium branchiophilum

This bacterium, the causative agent of Bacterial Gill Disease, has not been a significant disease issue for many years at Makah NFH. However, as low summer-time flows increase in duration and magnitude, and water is re-used multiple times, this disease may become more prevalent.

D. Flavobacterium columnare

This bacterium, the causative agent of Columnaris disease, has not been observed at Makah NFH. However, this bacterium is believe to be ubiquitous in the aquatic environment. Survival and growth of this bacterium is temperature and water chemistry dependent. Increased water hardness, organic matter, and a low-level salinity provide better environment for this bacterium. This disease may also become more prevalent with expected environmental changes.