

Specific Pathogen Control Plan for *Renibacterium salmoninarum*, at BC federal enhancement hatcheries and affiliates

The goals of a specific pathogen control plan are:

- To minimize the expression and transmission of pathogens during rearing to facilitate the release of healthy fish
- To adapt hatchery management measures to minimize the amplification of endemic pathogens, limiting the impact on both wild and cultured fish populations.

It should be anticipated that these guidelines will change in response to advances in diagnostic capabilities, disease prevalence changes over time/geographic ranges, and as program objectives change, but represent the best management practise at the time of writing.

Specific Pathogen Control Plan - *Renibacterium salmoninarum*

Renibacterium salmoninarum, the causative agent of Bacterial Kidney Disease (BKD), is an endemic pathogen in the Pacific Northwest. BKD is a slowly progressing, lifelong infection of salmonids. The bacterium may be horizontally transmitted between fish and vertically transmitted to the next generation. Clinical signs of BKD include exophthalmia (pop-eye), ascites (fluid-filled abdominal cavity), granulomas (whitish-grey nodules) within the kidney, pinpoint haemorrhages (rash) or vesicles of the skin, although fish may die exhibiting no clinical signs. Fish infected with *R. salmoninarum* will not normally exhibit clinical signs until the fish are at least 6 – 12 months old. However being infected with *R. salmoninarum*, even without clinical signs of disease, will increase susceptibility to other pathogens, such as *Flavobacterium psychrophilum* (Coldwater Disease) or *Aeromonas salmonicida* (Furunculosis). Treating *R. salmoninarum* infected fish with antibiotics may lower signs of disease and the associated mortality levels, but will fail to cure the disease. The best control measure for this disease is avoidance, through methods intended to prevent its vertical transmission.

Specific control measures:

- Egg disinfection for a minimum of 10 minutes using a buffered 100ppm povidone-iodine solution prior to incubation or placement into a hatchery water supply if eggs are collected off-site or transferred to the facility at the eyed egg stage. *Note: multimillion-egg pink and chum salmon incubation facilities with no disease history during rearing are exempt from egg disinfection requirements at this time.*
- Pre-spawning antibiotic injections given to females within 3-4 weeks prior to egg collection (additional prior injections may be given at 30 day intervals for stocks undergoing prolonged pre-spawning holding). A veterinary prescription is required for antibiotic procurement and use.
- Progeny segregation (keeping egg lots separate during water hardening, egg disinfection and incubation in Heath trays until test results from female parents are complete) and culling based on levels of soluble *R. salmoninarum*-antigen detected using the Enzyme Linked Immunosorbent Assay (ELISA).
 - Fertilized eggs/progeny from females that have an optical density (OD) value less than the mean OD value (minimum of 6 replicates) plus 2 standard deviations of a negative control fish kidney sample are to be reported as 'negative' and may be used for yearling programs or be eligible for transfer to another facility.
 - Fertilized eggs/progeny from females with an OD value greater than the mean negative control OD value (and therefore are positive for the *R. salmoninarum*

antigen) but less than 0.1 are to be reported as 'low level of detection'. Ideally, only eggs from negative females will be used for yearling programs, however, if needed to achieve production targets, low level of detection eggs are considered to present an acceptably low risk for the horizontal transmission of *R salmoninarum* may be included in yearling programs. If space allows, progeny should be kept separate from negatives if possible.

- Fertilized eggs/progeny from females with an OD value greater or equal to 0.1 but less than 0.25 are to be reported as 'low positive' and should be released early, as unfed fry to help maintain low levels of *R salmoninarum* within the hatchery and lower the risk of horizontal transmission between fish.
- Fertilized eggs/progeny from females with an OD value greater or equal to 0.25 but less than 0.6 are currently classified as 'moderately positive' and should be outplanted as eyed eggs if available rearing habitat is available downstream from the water intake of the facility or buried in dry ground or in wet ground with quicklime if appropriate habitat is unavailable.
- Fertilized eggs/progeny from females with an OD value greater or equal to 0.6 are currently classified as 'high positive'. It is recommended that these egg lots be destroyed via burial in dry ground or in wet ground with quicklime. In extreme circumstances (i.e. conservation concerns outweigh ecological consequences of propagating disease-positive fish) these eggs could be outplanted, but only after mutual agreement of the hatchery management, enhancement operations support staff and the DFO veterinarian.

In situations where escapements are low, or where the escapement BKD prevalence is so high that prescribed culling recommendations will compromise production targets, or where unexpectedly high levels of incubation mortalities occur; it may be desirable to rear progeny from females testing 'low positive' for BKD by ELISA. The disease transmission risk assessment must be made in consultation with the DFO fish health veterinarian, site management and hatchery support biologist using the following criteria:

- Fish Health Management Plan documenting personnel skills and respect for site biosafety objectives
- Physical containment to compartmentalize stocks on site
 - Separate areas with appropriate staff traffic flow, separate rearing units, separate water flow, separate nets and equipment with established disinfection protocols, separate dedicated staff as needed, etc.
 - To protect the progeny of females testing 'negative' from the potential spread of *R salmoninarum*
 - Separation between year classes - to protect all juvenile fish on site from broodstock of unknown disease status
- Availability of adequate type and volume of water for the duration of proposed rearing
 - Pathogen-free water is preferable
- Early recognition and mitigation of a problem
 - BKD is a slowly progressing, lifelong disease. Fish infected with *R salmoninarum* will not normally show clinical signs (pop-eye, fluid distended abdomen, white nodules within the kidney, 'rash' or small red, external skin lesions, etc.) until the fish are at least 6 – 12 months old. However, infection with the bacteria will make juvenile fish more susceptible to other common pathogens, such as *Flavobacterium psychrophilum* (Coldwater Disease) or *Aeromonas salmonicida* (Furunculosis). For these reasons, staff rearing progeny from females testing low positive will need increased vigilance with regards to early detection and reporting to site management and the veterinarian of any potential disease development.

The Fish Health Database at the Pacific Biological Station maintains records of BKD screening and disease detection results for federal and federal-affiliated Pacific salmon enhancement hatcheries in BC. Based on these historical records, stocks can be classified into two categories with regards to the vertical transmission of BKD, **low and high risk**. Annual compulsory BKD screening will be required to mitigate the risk of vertical transmission of high risk stocks and to maintain an accurate prevalence estimate for each stock at low risk.

The frequency and intensity of screening will depend on a number of factors: risk status, duration of rearing, capacity for egg segregation, enhancement of nonindigenous stocks, site biosecurity capabilities with regards to managing multiple species and year classes, and proportion of escapements being enhanced.

High risk stocks:

All Coho and Chinook brood females should be screened annually; with culling as indicated by the ELISA results as described above. Additionally, sites must disinfect eggs and should consider broodstock antibiotic therapy if pre-spawning holding times allow.

If sites are incapable of egg segregation, all female broodstock (or 60 brood females taken over the course of the run, whichever is lower) should be screened annually to maintain an estimate of BKD prevalence. Additionally, eggs must be surface disinfected, broodstock antibiotic treatment should be discussed with the DFO veterinarian if pre-spawning holding times allow and efforts should be directed to increase egg segregation capacity.

For hatcheries rearing nonindigenous stocks to be returned to their native systems, all efforts should be made to ensure biosecurity measures are applied to effectively compartmentalize stocks, minimizing the risk of the horizontal transmission of *R salmoninarum* and/or other pathogens.

Low risk stocks:

Minimum standards required will be to screen all females or 60 selected over the timing of the run (whichever is less) on 3 year schedule to maintain an accurate BKD prevalence estimate. However, at the hatchery manager's discretion, any site may request full screening annually on all Coho and Chinook stocks. This should be considered for all yearling release programs, at sites which rear nonindigenous stocks, and at sites which lack the capacity for egg segregation. The PBS Fish Diagnostic Laboratory will make every effort to comply with increased testing requests by Salmon Enhancement Program (SEP) facility management.

Juvenile Pre-release Disease Screening

Disease outbreaks in juveniles and/or significantly high levels of *R salmoninarum* detected during broodstock screening at facilities which can not segregate egg lots and/or poor performance prior to release which requires veterinarian intervention will necessitate a pre-release disease prevalence sampling of 60 juvenile fish to obtain release authorization.

Poor performance may be defined as having total cumulative mortalities attributable to any disease of equal to or greater than 5% in the 90 day period, one month prior to the planned release date; regardless of the definitive diagnosis, as subclinical infection with *R salmoninarum* increases the susceptibility to other pathogens.

In the event of poor performance and/or the suspicion of a BKD outbreak during rearing, a 60 fish sample of random, apparently healthy fish from the stock in question will be submitted to the PBS Fish Diagnostic Laboratory two weeks before the proposed release date. Routine necropsies will be performed if indicated, however, in the absence of abnormal clinical signs, examination may be limited to kidney samples using *R salmoninarum*-specific direct fluorescent antibody test (dfat) to estimate the prevalence of BKD in the juveniles. The detection of moderately high levels of *R salmoninarum* in at least 25% (15 of 60) of pre-released screened juveniles may prohibit release

and trigger the destruction of the stock. Additionally, increased monitoring of other stocks on site will be needed. The stock will be designated as a high risk for subsequent spawning seasons. It will be recommended that the site participate in antibiotic administration to broodstock and broodstock screening and segregation for the next lifecycle, if feasible, and vaccination options may be considered.