# Evaluation of Bacterial Kidney Disease (BKD) Impacts on the Canadian Salmon Aquaculture Industry

## FINAL REPORT

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White Paper Prepared by:
BC Centre for Aquatic Health Sciences

Prepared for:
Fisheries and Oceans Canada (DFO)

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**Appendix I – BKD Questionnaire**

**Appendix II – Balfry and Brown (2006)**

**Foreword**

Bacterial kidney disease (BKD) has been identified as an emerging disease of concern for Canadian aquaculture by the members of the National Fish Health Management Working Group for Aquaculture. BKD has substantial health impacts in cultured salmonids and significant economic impacts for commercial salmonid culture. The unique features of the causative bacterium, *Renibacterium salmoninarum*, make disease treatment and control difficult. As such, control relies on managing the disease to minimize its negative impact, fish death and economic loss rather than elimination of the disease. For example, in British Columbia, mortalities and downgrades due to BKD in cultured Pacific salmonids was a significant factor resulting in salmon farmers switching to Atlantic salmon as the primary culture species.

The aquaculture industry has been able to mitigate many of the negative impacts of BKD through good husbandry and management. BKD is a disease that industry has learned to live with and in the face of more pressing health and/or business concerns, the impact of BKD both as a disease affecting fish health and economically has received less attention than is likely warranted. However, the ability to mitigate the negative impacts of BKD doesn't detract from the need to re-evaluate and clarify current status of this disease with regards to: prevalence, species/strains of salmon affected, significance (as a fish health concern and economically), geographic location, environmental factors, husbandry practices and changes in fish feed formulation.

The current project stems from a need to gather, collate and report on BKD disease information from each of the major salmonid producing regions of Canada including commercial and enhancement facilities. The project utilized the expertise of federal, provincial and industry fish health and operations managers to characterize the status of BKD in British Columbia, Ontario, Quebec, New Brunswick, Nova Scotia and Newfoundland. To this end, a questionnaire was produced, trialed, revised and distributed to experts across Canada. Follow-up was done by phone to answer respondent's questions and to ask additional questions that further identify and evaluate the disease's effect. Results were compiled to provide an overview of BKD status in Canada. The attached report gives an overview of the disease, presents the findings of the questionnaire and provides a current BKD disease profile that can be used to clarify the direction of BKD management in Canada.

## Executive Summary


- Bacterial kidney disease (BKD) is caused by a non-motile, rod-shaped bacterium, *Renibacterium salmoninarum*. The disease has global significance and affects both wild and cultured salmonids and is found in both fresh and saltwater environments.

- BKD often establishes as a chronic disease causing continuous mortalities throughout the lifecycle but especially after the first year and as fish reach market size.

- *R. salmoninarum* can be transmitted vertically from female to progeny via eggs and horizontally from fish to fish. Transmission is direct: there are no intermediate hosts.

- Culture requires specialized media and takes 6-8 weeks. Thus, culture is a poor diagnostic tool and measurement of bacterial resistance is very difficult.

- The bacterium's ability to live and replicate within host cells makes antibiotic treatment difficult and hinders production of good efficacy vaccines.

- There are limited therapeutic tools available and their efficacy is unknown.

- Diagnosis is generally done by direct or indirect fluorescent antibody test (DFAT or IFAT), enzyme-linked immunosorbent assay (ELISA), nested polymerase chain reaction (PCR) assay or quantitative PCR. Histology can be used to identify systemic disease.

- The disease is controlled or prevented by broodstock screening, pharmaceutical treatment, and good husbandry practices that limit exposure to the bacteria and reduce fish stress.

- A questionnaire to better understand the effects of BKD in the Canadian aquaculture industry was responded to by 12 of 14 (86%) recipients. Respondents represented commercial and enhancement interests in the West and East Coast and Central Canada regions.

- Questionnaire results were compiled and presented according to four objectives that assessed importance, prevalence, diagnostic techniques and factors contributing to prevalence.

- Questionnaire results identified BKD as a highly significant disease across Canada which affects both Pacific and Atlantic salmon. In particular, in British Columbia it is a significant health issue of Pacific salmon in private and public facilities. Central Canada did not consider the disease to be significant, but East and West Coast facilities estimated prevalence in Atlantic salmon at about 3%. On the West Coast, Pacific salmon

prevalence was estimated as 5%. Within species there appears to be variation in prevalence by stock/strain.

- Significant stressors contributing to BKD prevalence during grow-out were low dissolved oxygen, predation, temperature and handling.

- Lack of knowledge about water hardness (in Central and East Coast Canada) and changing feed formulations and how these may contribute to increased BKD prevalence was evident.

Key areas of future research and/or development were identified as follows:

- Development of diagnostic tools that are easy to use and provide consistent, accurate, rapid diagnosis for multiple samples. The consistent application of these diagnostic tools in fish health management plans throughout Canada would ensure a better understanding of BKD disease and management.

- Development of vaccines and the need to use vaccines in combination with other preventative tools as part of integrated fish health management programs.

- Research studies to trial new potential drugs.

- Streamlined processes for getting new drug(s) approved for use.

- In the short term: establishing maximum residue limits (MRL's) and withdrawal periods for commonly used extra-label therapeutants.

- In the long term: more registered products made available for fish with MRL and withdrawal period to reduce the need for extra-label therapeutant use.

- Research into how water hardness in early fish rearing affects short and long term fish health.

- Research into feed formulation changes and the resulting effects on fish health.

- Characterization of the persistence and viability of BKD in hatchery facilities and in the marine environment.

## Introduction

Bacterial kidney disease (BKD) is caused by a non-motile, rod shaped bacterium, *Renibacterium salmoninarum*. The disease affects both wild and cultured salmonids and is found in both freshwater and saltwater environments. While susceptibility to disease varies among the salmonid species, *Oncorhynchus spp*. particularly *O. tshawytscha* (Chinook salmon) and *O. mykiss* (rainbow trout) are the most susceptible. In *Oncorhynchus spp*. losses can be as high as 80% and in Atlantic salmon losses of 40% have been reported (Evenden et al. 1993). Non-salmonids appear resistant to natural infection (Fryer and Sanders 1981) though Kent et al. (1998) described the bacterium's presence in moribund hake in British Columbia.

BKD is a significant health concern among cultured salmonids globally, so much so that it was an Office International des Epizooties (OIE) listed disease in 2003, but has been removed from that status in subsequent aquatic disease listings. Often BKD establishes as a chronic disease resulting in continual mortalities throughout the lifecycle but especially after the first year and as the fish reach market size. As a result, economic impact can be considerable and even when individuals survive to harvest, downgrade or culling of infected fish further reduces economic return.

BKD was first described during the 1930's in Atlantic salmon (*Salmo salar*) from the Dee River system in Scotland (Smith 1964). Since that time, BKD has been reported in salmonids throughout North America, Western Europe, Chile, Japan and Iceland. *R. salmoninarum* can be transmitted vertically from females to progeny via eggs (Evelyn et al. 1986a) and horizontally from fish to fish (Balfry et al. 1996). Vertical transmission occurs when the bacterium is carried within the ovum of an infected female. Since the bacterium is protected within the egg, surface disinfection prior to and during fertilization may not eliminate disease transfer to the next generation. In cultured stocks with BKD, vertical transmission may be controlled by injecting broodstock with antibiotics like erythromycin (Brown et al. 1990, Evelyn et al. 1986, Lee and Evelyn 1994). Timing and location of the injection needs to coincide with those stages of egg development that allow incorporation of the antibiotic into the egg. Vertical transmission from brood males to progeny does not occur. Transmission is always direct: there are no intermediate hosts.

### *Renibacterium salmoninarum*

The bacterium is small (0.1-0.3 μm by 1.0-1.5 μm), Gram-positive, non-acid-fast, and non-spore forming (Sanders and Fryer 1980). Its distinctive diplobacillus shape combined with a positive Gram stain distinguishes it from most other disease-causing organisms in fish. Culture requires specialized media (selective kidney disease media or SKDM) and patience as colony growth can

take 6-8 weeks or longer at 15°C. Slow growth of the bacteria negates quick and simple plate culture methods to measure bacterial resistance to various antibiotics. This slow growth is also characteristic of *R. salmoninarum* infections which are systemic and chronic in nature. *R. salmoninarum*'s ability to live and replicate within its host's cells (Bandin et al. 1993, Bruno 1986, Gutenberger et al. 1997) makes treatment difficult.

While fish with BKD infection may not show any external disease signs, individuals exhibiting clinical disease may have one or more of the following signs: exophthalmia, lethargy, darkened skin colouration, abdominal swelling from ascites, pales gills, haemorrhaging around the vent, and small sores or ulcers on the skin surface. These shallow sores or ulcers on the skin are known as 'belly rash' and may present on adults, particularly spawning adults. In 1991, Speare et al. described severe, pyogranulomatus menigoencephalitis that was associated with BKD even in absence of other lesions. Species demonstrating these brain lesions include: rainbow trout, brook trout, Chinook, Coho and Atlantic salmon.

Internally, signs include: focal to multifocal greyish-white granulomatus lesions in the kidney (distinctive disease feature) and sometimes in the spleen and liver; possible greyish-white pseudomembrane on the spleen or other organs; ascites in the abdominal cavity; haemorrhages on the abdominal wall or internal organs; and 'pockets' of haemorrhaging in the musculature. BKD is one of the few salmon diseases whose internal signs could be considered pathognomonic. Microscopically, bacteria are usually visible in the kidney lesions as well as the white blood cells or macrophages.

**BKD Diagnosis**

Traditionally, BKD diagnosis relied on clinical signs combined with visual presence of *R. salmoninarum* in kidney smears or imprints or bacterial culture. Presumptive diagnosis of smears and imprints could be done with Gram stain, but direct or indirect fluorescent antibody test (DFAT or IFAT) provided more definitive results (Bullock and Stuckey 1975). The need to quickly assess a larger number of samples (for broodstock screening) resulted in a switch to enzyme-linked immunosorbent assays (ELISA) for identification. Other immunodiagnostic tests that have been used include immunoblot assay (Sakai et al. 1987) and immunodiffusion assay (Chen et al. 1987). More recent, nested polymerase chain reaction (PCR) assay and now quantitative PCR provide excellent sensitivity and specificity for bacterium identification (Powell et al. 2005).

Histology, alone or in coordination with other diagnostic assays, is another traditional diagnostic tool that is particularly useful in the identification of subclinical tissue and cellular disease signs. Through the sampling and analysis of multiple organs, histology can confirm the systemic nature of the disease.

In 2003, when BKD was still a listed OIE disease, the organization suggested ELISA and FAT for BKD screening tools and culture and/or PCR for confirmations (OIE, 2003). In its Manual of Compliance, the Canadian Fish Health Protection Regulations recommend that Gram stains of kidney or other tissue lesions are presumptive and suggests that immunodiffusion, DFAT and IFAT be used for confirmations (Fisheries and Oceans Canada 2004). The American Fisheries Society Fish Health Section Blue Book (AFS-FHS 2007) recommends FAT for presumptive findings and confirmation with plate culture and nested PCR.

**Control and Treatment of BKD**

The high prevalence in some populations and intracellular nature of BKD infection challenges its control and treatment. As with other diseases that are difficult to treat, care is taken to avoid the development of disease in the first place. For cultured salmonids, avoidance methods include broodstock screening; antibiotic injections for particularly susceptible species; and good husbandry practices that limit opportunities for exposure to the bacterium (e.g. site fallowing, single year-class sites) and reduce stress to stock (e.g. lower densities). While broodstock screening and culling of positive individuals can effectively reduce BKD prevalence at the hatchery, some assays, such as ELISA, do not necessarily provide clear cut yes or no [the disease is present] results. In some populations, the high incidence of subclinical or carrier disease means that high positive individuals will be culled, but low positive individuals may not.

The unique features of *R. salmoninarum* deter vaccine production as well. Immunotolerance to *R. salmoninarum* infection can be established in individuals that are infected via vertical transmission. Humoral antibodies are slow to develop and do not appear to be very protective (Kaattari et al. 1988). Even so, vaccines have been developed, but their effectiveness is less than vaccines developed for other common bacterial fish pathogens such as *Listonella* (*Vibrio) spp*. and *Aeromonas salmonicida* (causative agent for furunculosis). This is supported by a 2005 study by Alcorn et al. which didn't find any mortality differences between fish groups that were vaccinated (Renogen vaccine produced by Aqua Health Ltd. in Prince Edward Island, Canada) and a control group which was vaccinated with saline solution.

One study (Balfry and Brown 2006 –this paper is included as Appendix II as it provides an excellent review of the disease) suggested that breeding for disease resistance based on indirect or direct selection criteria in cultured fish was premature due to the lack of information on the mechanism of pathogenesis. This report suggested that the British Columbia salmon farming industry continue with its current BKD control methods (broodstock selection programs, site fallowing, separation of year classes, prophylactic chemotherapy etc.) rather than attempt to breed for BKD resistance.

**BKD Questionnaire**

The BKD questionnaire (appendix 1) was developed by a veterinary epidemiologist and fish health experts and is divided into BKD observations and outcomes in freshwater and saltwater facilities (including commercial and enhancement facilities) with respect to four key areas: prevalence, environmental practices, husbandry practices, and costs. The initial questionnaire was trialed with one commercial salmon producer's hatchery manager and biologist – their comments were used to make revisions. The final questionnaire was distributed to fish health professionals representing both commercial and enhancement interests in the following regions and provinces: West Coast (British Columbia), Central Canada (Ontario and Quebec) and East Coast (Nova Scotia, Prince Edward Island, New Brunswick, and Newfoundland). Focus for the questionnaire was salmon – Pacific species and Atlantic. Additionally, some enhancement trout in Ontario and Quebec were included.

Respondents from the three geographic regions provided information on the following culture interests.

- East Coast – the four respondents represented both commercial and enhancement culture of Atlantic salmon for New Brunswick, Nova Scotia, and Newfoundland.
- Central Canada - The respondent from Ontario addressed provincial enhancement hatcheries and the Quebec respondent provided information on 'tanks and ponds' used in both enhancement and commercial activities. Both provinces are freshwater only. Atlantic salmon are grown in both provinces, but Ontario also grows Chinook and Coho. Lake and brook trout are the predominant salmonid species raised in each province respectively.
- West Coast – Respondents represented both commercial culture of Atlantic and Pacific species salmon and enhancement culture of Pacific species.

The following tables summarize: the number questionnaires and respondents (table 1); and whether respondents represented commercial and/or enhancement interests and had knowledge/responsibility for freshwater and/or growout (freshwater or saltwater) facilities (table 2).

| | **Questionnaires** | | |
|---|---|---|---|
| | Sent out | Returned | % Returned |
| West Coast | 6 | 6 | 100% |
| Central Canada | 2 | 2 | 100% |
| East Coast | 6 | 4 | 67% |

Table 1 – number of distributed questionnaire and responses

| | Facility | | | | |
|---|---|---|---|---|---|
| | Freshwater | Growout | Commercial | Enhancement | Both |
| West Coast | 4 | 5 | 4 | 1 | 1 |
| Central Canada | 2 | 1 | | 1 | 1 |
| East Coast | 4 | 3 | 4 | | 1 |

Table 2 – respondent's responsibility for commercial and/or enhancement, freshwater and/or growout facilities

Questionnaires were followed-up by phone and email to answer respondent's questions and to ask additional questions that further identified and evaluated the disease's effect. Results were compiled by region and presented according to the study's four objectives:

1. relative importance of BKD in each salmonid producing region in relation to costs associated with treatment, mortality and production/quality losses;
2. current prevalence in each region and whether there has been a change in disease occurrence over the last 5-10 years;
3. current techniques used to control/prevent BKD including husbandry, preventative and treatment practices and diagnostic tools; and
4. factors that may contribute to changes in observed prevalence.

Results of the questionnaire are summarized by region in each of these four areas and further summarized to present a national perspective on BKD prevalence and importance to cultured salmonids in Canada.

## Questionnaire Results

### Current Prevalence and Changes during Past Five to Ten Years

East Coast

The four respondents from the East Coast represent commercial and enhancement facilities. The predominant species raised is Atlantic salmon, specifically the St John River stock. The respondents rated the importance of BKD relative to other fish health concerns as high to very significant at their facilities and on a provincial scale.

Hatcheries least affected by BKD had 1-3% prevalence whereas hatcheries with greater BKD concerns had an estimated prevalence greater than 5%. At saltwater facilities, prevalence ranged from less than 1% (at low prevalence sites) to greater than 5% (at high prevalence sites)

with one respondent estimating a prevalence of 30% at his/her highest prevalence saltwater location. Generally, these respondents felt that there was no to some increase in BKD prevalence at freshwater and saltwater facilities during the past 5 and 10 years. A decrease in BKD prevalence was noted by several respondents at those sites with the highest BKD prevalence. One respondent with over 15 years of aquaculture experience felt that during his/her early career BKD was a manageable problem, but that as interprovincial fish movements have increased that control and management of this disease has been lost.

Central Canada

The two respondents from the Central region represent commercial and enhancement facilities: neither respondent regarded BKD as a significant disease for the region. The severity of BKD is marginal in Quebec, compared to it being a very significant problem in Ontario in one particular stock of Atlantic salmon (the LeHave River stock).

BKD has a low prevalence in Central Canada: only one hatchery was reported to have clinical BKD. This facility has been in operation for 24 years and has experienced an increase in BKD prevalence in one strain of Atlantic salmon (LeHave) during the past 5 and 10 years. In general, older fish (7-8 years) are reported to have higher mortality and more clinical signs of disease than younger fish (2 years). It was also noted that commercial facilities in the Central region (Quebec) have reported fish death as a result of BKD; however, only one case in the past two years has been confirmed via laboratory testing.

Western Canada

There were six respondents from British Columbia: 3 representing commercial Atlantic salmon producers and three representing Pacific salmon producers (commercial and enhancement).

The commercial Atlantic salmon production revolves around two European-derived stocks — Mowi and McConnells. Bacterial Kidney Disease was considered only low to moderate significance relative to other diseases in Atlantic salmon and it was felt that prevalence during the past 5 to 10 years either had not changed or had decreased slightly. In freshwater, prevalence tends to be very low and site specific. Lake-rearing sites showed both the lowest and highest occurrences. In the marine environment, BKD had a low prevalence (1-3%) and respondents indicated that occurrence was related to source (i.e. which freshwater facility they came from) and growout management practices. Although prevalence changes by year class and location, overall prevalence has decreased during the past 5 to 10 years. Even though there is no clinical disease (low ELISA reading) in freshwater, respondents noted prevalence increases with fish age (i.e. S1 fish have higher prevalence than S0).

Commercial Pacific salmon production in western Canada involves predominantly Chinook and Coho salmon while enhancement facilities raise other Pacific salmon species as well. In contrast to Atlantic salmon producers, respondents involved in rearing Pacific salmon consider BKD an extremely significant disease both at commercial facilities and at the provincial level. Chinook and Coho salmon are affected the most. However, even within these species there is considerable variability among the different stocks.

In hatchery facilities, Pacific salmon (commercial and enhancement) have a much higher prevalence of BKD than Atlantic salmon with respondents identifying stocks with a consistently higher prevalence of BKD that are more susceptible to infection and; therefore, more difficult/costly to culture. On a scale of 1 to 5 (with 5 being the most significant), Pacific salmon producers gave a 5 rating to BKD severity. Within enhancement stocks with a consistently higher prevalence of BKD, the prevalence of the disease over the past 5 to 10 years has varied considerably on a year-to-year basis. The respondent representing these operations stated that though coldwater disease (Flavobacterium) kills more fish (1.5-3.0g size), BKD has much greater impact.

During growout, Pacific salmon producers rated BKD significance as moderate to severe, again identifying stocks with a consistently higher prevalence of BKD. BKD prevalence in Pacific salmon was over 5%. Over the past 5-10 years, Coho producers have seen a decrease in prevalence and Chinook producers have not seen any changes. Respondents that commercially produce Pacific salmon noted that high BKD prevalence in specific Pacific salmon restricts commercial production of these species and limits opportunities to capitalize on specialized markets for niche strains. If BKD disease (and consequently costs) were controlled, these niche strains would be able to take advantage of full market potential.

**BKD Prevention/Management Associated Costs**

East Coast

BKD associated costs during freshwater rearing are high relative to other fish health concerns according to all respondents. This is primarily related to the costs associated with broodstock management (broodstock antibiotic treatment and screening). In saltwater, compared to other fish health issues, BKD associated costs were rated as "the same" by one respondent, "higher" by another respondent, and "significantly higher" by different respondent. Most cited the following as serious costs associated with BKD: treatment, management modifications, production loss, and production management (such as reduced densities). These costs have not changed in the last 5-10 years.

Central Canada

Bacterial Kidney Disease was not considered a significant disease; however, both the Quebec and Ontario survey participants rank costs associated with production losses as the most significant at the hatchery level. Ontario also ranks cost of treatments and costs associated with broodstock management as high. In Quebec, the most serious cost associated with BKD during growout is the cost of management modifications.

Western Canada

Overall, costs associated with broodstock management and production losses were the most significant for all producers. The least significant costs associated with BKD were vaccination (most respondents do not vaccinate) and harvest quality. Pacific salmon producers (commercial or enhancement) rated broodstock management and production loss costs higher than Atlantic salmon producers. Pacific salmon producers felt that costs associated with BKD were much higher than other fish diseases and they did not feel that these costs had changed significantly over the past 10 years. These same producers reported there is variation in BKD prevalence between different species and stocks of Pacific salmon. In contrast, Atlantic salmon producers felt that costs associated with BKD were lower than costs associated with other fish health diseases. For these producers, BKD disease costs have remained the same over the past 5 years and decreased over the past 10 years.

**Current Techniques to Control and Prevent BKD**

East Coast

Survey respondents indicated that of the techniques commonly used at facilities on the East Coast to prevent BKD, broodstock management was the most costly. This includes broodstock antibiotic treatment (erythromycin injection), broodstock screening and egg disinfection with iodine. Half the respondents who elaborated on broodstock screening mentioned IFAT as a screening tool, but one respondent elaborated that ELISA, IFAT and PCR were not "very good, with 3-5% declared as false negatives." This respondent mentioned a desperate need for new diagnostics. It was noted that broodstock on the East Coast are being raised in freshwater with the exception of one saltwater site (in Nova Scotia). This change in broodstock management (from saltwater to freshwater rearing) occurred during the past five years and was in response to containment and prevention of Infectious Salmon Anemia (ISA) disease. None of the respondents felt that management practices had changed in the past 5 years.

While one respondent mentioned smolt vaccination as a preventative measure, the other respondents did not and none of the respondents utilized broodstock vaccination. All respondents indicated that mortalities are routinely checked for BKD and fish groups were screened prior to release or transfer to another facility.

Regarding measures to control BKD in hatcheries, all respondents commonly used pharmaceutical treatment (Oxytetracycline added to feed - extra-label dose and duration) and biosecurity. Half the respondents use density management and handling management as well. Handling management included reduced grading, or grading fish at a younger age and ensuring proper handling at appropriate temperatures. None of the respondents used feed supplements or feeding regime modification as a method of controlling the disease. Half (2) respondents were unaware of water hardness parameters in the hatcheries and how water hardness may have changed over the past 5 or 10 years. One respondent stated that water was soft (with acidity varying with geography) and that there had been no changes to this parameter over the past 5 or 10 years. Two-thirds of the respondents felt that feed formulations had changed but had few specifics.

In saltwater, BKD control measures include antibiotic treatment (in feed OTC treatments) with one respondent noting that early detection and treatment was important. Biosecurity was mentioned by all respondents, density management by two respondents, and feeding regime modification by one (noting that water temperatures may lead to feeding changes). Furthermore, all companies maintain single year class sites, have only one finfish species per site (even where lease permits allow multiple species) and generally fallow between year classes (it was noted that in New Brunswick this is mandated by regulation).

Central Canada

Survey respondents from Central Canada report techniques commonly used to prevent BKD include broodstock management techniques such as broodstock antibiotic treatment, broodstock screening, egg screening, and egg disinfection. Prior to 2010, Ontario utilized oxytetracycline treatment to reduce mortality; however, at present broodstock are injected with erythromycin prior to spawning. Furthermore, in Ontario all wild fish broodstock are screened (fluid samples), and routine monitoring is conducted annually on broodstock in the hatchery. Egg screening is also conducted, and eggs from adults with high levels of *Renibacterium spp.* are culled. The only husbandry practice to change in the last 5 years is the addition of egg disinfection during water hardening in Ontario. Prior to fall of 2009 there was no clinical BKD and no need to use antibiotics. In Quebec, broodstock antibiotic treatment, egg screening, egg disinfection and prophylactic treatment are all used.

Regarding measures to control BKD, both of the Central Canada respondents report the use of pharmaceutical and biosecurity practices to try to control BKD at hatcheries; however, Ontario also uses density and handling management strategies. Quebec does not check mortalities or screen fish for BKD prior to release/transfer to other facilities. Ontario facilities are annually monitoring for pathogens, including *Renibacterium spp*. Neither of the respondents reported the use of feed supplements or feeding regime modification as a method of controlling the disease.

In Quebec, mortalities are not routinely checked for BKD nor are fish screened prior to release or transfer to another facility. This is likely a reflection of the very low reported prevalence of the disease in the province.

West Coast

Survey respondents from the West Coast indicate that both enhancement and commercial facilities (Atlantic and Pacific salmon) utilize disease screening/culling and egg surface disinfection with iodophor in an attempt to prevent BKD. The broodstock programs are extensive in the commercial facilities and include: broodstock receiving one or more antibiotic treatments (erythromycin injections) prior to spawning; screening of female brood for BKD; and culling of eggs based on positive test results. The standard screening tools include ELISA and IFAT. Some facilities are able to isolate eggs from the rest of the population while screening results are pending. As a result of the nature of salmon enhancement (i.e. broodstock spend very limited amounts of time in the hatchery facilities), broodstock injection and egg screening is not as ubiquitous as in the commercial facilities.

Other techniques used to try and reduce prevalence are: smolt vaccination with Renogen – one respondent reported using this –with poor results; repeated antibiotic treatments (most commonly OTC) of Pacific salmon smolt which are destined for brood (results still pending); and genetic selection/breeding; however, the success of this program, with respect to BKD, is not well defined.

In freshwater, primary differences in husbandry practices are generally related to enhancement or commercial culture objectives. All West Coast commercial freshwater facilities have soft water (less than 35ppm); however, recirculation facilities (commercial Atlantic salmon operations) are able to adjust hardness to 70-85ppm or greater than 150ppm (one respondent). Enhancement operations and some commercial production of Pacific salmon utilize flow-through and; therefore, have minimal ability to treat water or adjust water parameters. This is also true of commercial lake-based facilities. Freshwater treatment options include sand, ozone and UV depending upon water source (well or surface). For all respondents (commercial or enhancement), density management and reduced handling are standard BKD control procedures. When BKD mortality increases, all respondents rely on pharmaceutical treatment (OTC or erythromycin). Biosecurity is applied by all commercial facilities and in some specific enhancement situations (though not to the same level as commercial facilities). Major enhancement facilities are in the process of developing Fish Health Management Plans and biosecurity training for personnel. Monitoring mortalities for BKD is an ongoing practice for commercial respondents. For enhancement facilities, stock loss thresholds must be reached in order for staff to submit samples for lab analysis. At commercial facilities, smolts are screened via ELISA, IFAT or PCR prior to transfer from the facility. This same screening is not done for fish released from enhancement facilities.

In commercial saltwater operations, BKD control measures include: antibiotic treatment (in feed OTC treatment - extra-label), single species and single year class site policies, and fallowing between production cycles. Lower densities and reduced handling have been increasingly used to control BKD over the past 5 years. Feeding regime management and feed supplements are not commonly used management strategies. Although in a commercial Pacific salmon facility, additional supplement of vitamins C and E is added to feed following a stress event in seawater (such as a harmful plankton bloom or predator attack) in attempt to prevent BKD.

**Factors that May Contribute to Increased Prevalence Levels**

East Coast

Changes in feed practices have been reported for the last five and ten year periods. Respondents (3 out of 4) reported an increase in plant protein proportion, and half of respondents reported a change in the type of plant protein along with replacing animal fats (from fish to poultry).

Two of the respondents also identified a lack of knowledge regarding the persistence of BKD in the environment. For example, one respondent reported a higher prevalence of BKD at certain production sites regardless of which hatchery the fish come from and a need to investigate how BKD might persist in the local environment.

With regards to stressors that may influence BKD prevalence, all three respondents felt that low dissolved oxygen, temperature and density were contributing factors. One respondent cited predation, harmful plankton (at saltwater facilities) and genetics. This respondent elaborated that some sites had high BKD regardless of which hatchery the fish came from and that this was a consistent problem.

Central Canada

Ontario has changed feeding practices and feed formulations, including more automated feeding systems at hatcheries in the last 5 years. Specifically, feed formulations have changed as well as the type of plant protein and increased plant protein proportions. During grow-out (in Quebec) significant stressors suspected to contribute to BKD prevalence include: low DO, temperature, pollution, density, handling management, and other concomitant diseases.

Western Canada

Some commercial respondents see improved overall fish health at freshwater facilities with harder water. Only one respondent had detailed knowledge of feed composition changes during the past 10 years, though two Atlantic salmon producers noted an increase in plant protein in feed. Most respondents were unaware of feed ingredient changes over time. Respondents stated that feeding practices were always changing and being fine-tuned based on research and site-specific requirements. Most respondents highlighted low dissolved oxygen,

predation, harmful plankton, density and handling as stressors that may contribute to BKD prevalence. While all respondents indicated that these stressors changed over the past 5 and 10 years, there was no indication of how.

**Questionnaire Summary**

Bacterial Kidney Disease has been identified as a highly significant disease across Canada affecting both Pacific and Atlantic salmon. In British Columbia, BKD is a significant health issue in Pacific salmonids both in private and in public facilities that raise these species. Although BKD affects all stocks, there are stocks that have a higher apparent prevalence than others. In a commercial setting, BKD can limit the development of other stocks/strains as potential commercial stock. In enhancement facilities, while cold-water disease kills more fish, BKD has a greater impact. This is mainly attributable to the disease's chronic nature which results in mortalities throughout all life stages. There is very limited data on fish health status of enhanced fish once they leave the hatchery facility so the effects of chronic disease mortality in the open ocean are not well understood.

Atlantic salmon are raised in all three regions of Canada and BKD is considered a significant health issue in the West and East Coast regions. Central Canada did not consider the disease to be a significant disease within their provinces. The prevalence during the past generation on each coast of Canada is estimated to be around 3% in Atlantics among each of the four main stocks that are cultivated including: St. John, LeHave, and the European-derived Mowi and McConnell. This prevalence is higher, about 5%, in cultured Pacific salmonids. Stock variations in prevalence can be seen in both commercial and enhancement facilities but are most notable in the enhancement hatcheries.

Costs associated with management modifications, production loss, treatment with pharmaceuticals and harvest quality issues were found to be the most substantial associated with BKD. In the freshwater phase, costs associated with broodstock management were considered by West and East Coast regions to be among the top two expenses. These brood costs include: pharmaceutical treatments, possible site segregation for brood only, and screening at spawning. The next highest cost associated with BKD in the freshwater phase was production losses due to the disease. In the Central region, the most serious cost associated with BKD is production losses and this cost was rated higher relative to other factors. An additional cost of BKD is the labour demand for handling each fish during injection and during sample collection for screening.

On the West Coast, costs associated with preventing or managing BKD are considered lower relative to other fish health issues. In contrast, in the Eastern region the costs associated with preventing or managing BKD are considered about the same to significantly higher relative to other fish health issues.

Sources considered to be significant stressors contributing to BKD prevalence in the growout phase were similar for the West and East Coasts: low dissolved oxygen, predation, temperature, density and handling. Quebec also identified pollution as a significant stressor. To mitigate the effects of these stressors, production / management alterations are necessary throughout the growout phase.

The questionnaire also highlighted the lack of knowledge within the industry with regards to the feed formulation used throughout the life stages of their fish. It is known that feed formulations have changed over the past 5-10 years with a switch to more plant protein. Many respondents indicated they did not know if and how their feed had changed.

Hatcheries in Atlantic and Central Canada do not exploit hatchery water treatment to the extent that hatcheries on the West Coast do. Recirculation is used to some degree on both coasts but not in central Canada. Two private companies on the West Coast (both with recirculation) treat the water to increase hardness. East Coast respondents generally did not know the hardness of their hatchery water and treatment consisted of sand filter or UV. One respondent from the Atlantic Coast indicated that the water was 'soft' and there was some indication that water softness had changed over the past 10+ years as acidity has increased. Longstanding research has indicated that the severity of BKD is greater in facilities supplied with soft water compared to hatcheries operating with hard water (Warren 1963). Consistently, an Atlantic salmon producer on the West Coast has seen a significant improvement in overall fish health when the water hardness at their facility was increased.

**Conclusion**

The commercial and enhancement production potential of key salmon stocks and species is being limited by BKD. Furthermore, a higher prevalence of BKD at some enhancement facilities raises questions with regards to the health of the released fish and the ensuing effects on wild stocks. However, both commercial and enhancement facilities nationwide have the same challenges of limited diagnostic methods and treatment options.

The success of a BKD control program lies with the application of reliable diagnostic methods that can detect low levels in a variety of samples. There is a need for a more reliable and rapid method for detecting *R. salmoninarum*. A single ideal test has not been developed for the evaluation of multiple samples. Being able to detect *R. salmoninarum* may help identify if BKD is the primary cause of mortality or if BKD infection weakens fish making them prone to other diseases that cause death. Detection of low *R. salmoninarum* levels and subsequent monitoring of changes in levels may allow earlier intervention in the management of BKD. Early intervention, such as feed additives (vitamins/minerals/nucleic acids/immunostimulants), prophylactic treatments etc. may reduce the long term, chronic consequences of BKD infection. These are areas of potential research.

Respondents from all regions rely on similar management strategies to keep BKD under control. Prophylactic measures to minimize the effects of BKD include immunization (not effective yet), chemotherapeutics and production strategy management (reduced handling, density management, reducing stressors, etc). The key to controlling BKD is to develop a fully integrated disease control approach that uses all available techniques.

At present, no drugs are known to cure BKD disease in fish and few effective licensed pharmaceutical options are available. Both Erythromycin and Oxytetracycline are used extra-label for treatment and control of BKD. Comprehensive drug-sensitivity assays are impeded due to the slow-growing nature of the bacterium; consequently, accurate characterization of effective dosage and detection of changes in drug efficacy is virtually impossible. Moreover, because salmon stop feeding as they mature, the only treatment option to control vertical transmission of BKD in broodstock is injectible erythromycin administered via the dorsal sinus, and less commonly, into the body cavity (intraperitoneal). However, despite being a registered product for other food animals, the formulation which is most effective (200 mg/ ml) is no longer manufactured in North America. Consequently, veterinarians have three options for administering erythromycin to broodstock: 1) prescribing a less dilute formulation (100 mg/ ml) that is still manufactured in North America but requires that double the fluid volume be injected into an animal; however, with limited internal fluid capacity, it is less likely that optimal therapeutic levels are reached 2) having the preferred dosage (200 mg/ ml) produced (from scratch) locally by a compounding pharmacy. However, these products have a very short shelf-life compared to commercially manufactured products and 3) obtaining the 200mg/ ml dosage from off-shore manufacturers (i.e. in Australia) which can be costly and time consuming. Overall, the availability of this treatment option is highly limited for aquaculture and enhancement producers.

Long standing research and current anecdotal information indicates that water hardness in early rearing could play a significant role in overall fish health. A coordinated effort to evaluate the validity of this observation at the commercial scale needs to be undertaken. Furthermore, the persistence and viability of BKD in and around aquaculture and enhancement facilities is unknown. Research is needed to determine how long the bacterium can remain viable in hatchery facilities (depending on varying hatchery conditions) or in the pelagic and/or benthic environment at marine production sites (depending on season, and ecological traits of different coastal environments).

Lastly, fish feed formulation has changed substantially over the past decade with the switch to more plant proteins in the feed. Even so, little research has gone into evaluating the effects on fish health and in particular, BKD. Changes in food composition need to be monitored in order to evaluate their potential impact on BKD prevalence. Studies that define and assess the health effects of these changes would contribute to our understanding of this disease and others that may be particularly influenced by nutritional changes.

Key areas of future research and/or development are:

- Development of diagnostic tools which are easy to use and provide consistent, accurate, and rapid diagnosis for multiple samples. The consistent application of these diagnostic tools in fish health management plans throughout Canada would ensure a better understanding of BKD disease and treatment.
- Development of vaccines and their use in combination with other prophylactic preventative tools as part of integrated fish health management programs.
- Research studies to trial new potential drugs.
- Streamlined processes for getting new drug(s) approved for use.
- In the short term: establishing maximum residue limits (MRL's) and withdrawal periods for commonly used extra-label therapeutants.
- In the long term: more registered products made available for fish with MRL and withdrawal period to reduce the need for extra-label use.
- Research into how water hardness in early fish rearing affects short and long term fish health.
- Research into feed formulations changes and the resulting affects on fish health.

- Characterization of the persistence and viability of BKD in hatchery facilities and in the marine environment.

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# Evaluation of Bacterial Kidney Disease (BKD) Impacts on the Canadian Salmon Aquaculture Industry

The National Fish Health Management Working Group has identified Bacterial Kidney Disease as an emerging fish health concern for the Canadian aquaculture industry. The objective of this questionnaire is to characterize the state of BKD in the major salmonid producing regions in Canada (including British Columbia, Ontario, Quebec, New Brunswick, Nova Scotia and Newfoundland) according to federal, provincial and industry fish health experts. The results will be compiled to generate a reference document outlining the perceived severity and costs associated with BKD to the Canadian Aquaculture Industry.

This questionnaire is being conducted by the British Columbia Centre for Aquatic Health Sciences under contract with Fisheries and Oceans Canada. If you have any questions please contact: Elan Downey (elan.downey@cahs-bc.ca) or Alexandra Eaves (alex.eaves@cahs-bc.ca), by phone at 250.286.6102, or by fax at 250.286.6103. Thank you very much for your time and participation.

**Please return your completed questionnaire no later than March 12, 2010 by email or fax to the above contacts**

| | | | |
|---|---|---|---|
| **Name** | | **Position:** | |
| **Email:** | | **Phone #:** | |
| **Name of company or organization:** | | | |
| **Years of experience with facility:** | | | |
| **In what province does your facility operate?** | | | |

## Section 1. General

| | | | |
|---|---|---|---|
| 1.1 | Type of Facility: | | Commercial / Enhancement/ Both |
| 1.2 | What species of salmonid do you raise and how many stocks/ strains of each? | | Species: Stock: |
| 1.3 | What is the predominant salmonid raised? | | Species: Stock: |
| 1.4 | Do you raise fish in freshwater or saltwater? | | FW / SW / Both |
| 1.5 | Do you consider BKD to be a significant disease within a particular strain or stock relative to other fish health concerns? 1= not significant; 5= very Significant | | Strain or Stock:<br>Severity: |
| 1.6 | Do you consider BKD to be a significant disease within your province? 1= not significant; 5= very significant | | |

## Section 2. Hatchery/ Early rearing

**For operations with a single hatchery:**

| | | |
|---|---|---|
| 2.1 | Do you see BKD at the hatchery? If yes, please explain: | Y / N |
| 2.2 | How long has this facility been in operation? | |
| | Do you see a difference in prevalence according to strain, stock, site or other? | Strain / stock / site / other (please specify) |

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| | | |
|---|---|---|
| 2.3 | What has been the prevalence (% of population) of BKD among these fish over the last generation (year): | 0%<br>1-3%<br>3-5%<br>Greater than 5% |
| 2.4 | Has there been a change in the prevalence of BKD over the last 5 years?<br>Please elaborate. | Strain / species<br>Increase / decrease / no change |
| 2.5 | Have you noticed a change in the prevalence of BKD over the last 10 years (or indicate the number of years if 10 year history is not available)? | Strain / species<br>Increase / decrease / no change |
| **For operations with multiple hatcheries:** | | |
| **Site or region with the LOWEST prevalence of BKD:** | | |
| 2.6 | How long has this facility been in operation? | |
| 2.7 | Do you see a difference in prevalence according to strain, stock, site or other? | Strain / stock / site / region/ other (please specify) |
| 2.8 | What has been the prevalence (% of population) of BKD among these fish over the last generation (year): | 0%<br>1-3%<br>3-5%<br>Greater than 5% |
| 2.9 | Has there been a change in the prevalence of BKD over the last 5 years?<br>Please elaborate. | Strain / species<br>Increase / decrease / no change |
| 2.10 | Have you noticed a change in the prevalence of BKD over the last 10 years (or indicate the number of years if 10 year history is not available)? | Strain / species<br>Increase / decrease / no change |
| **Site or region with the HIGHEST prevalence of BKD:** | | |
| 2.11 | How long has this facility been in operation? | |
| | Do you see a difference in prevalence according to strain, stock, site or other? | Strain / stock / site / region/ other (please specify) |
| 2.12 | What has been the prevalence (% of population) of BKD among these fish over the last generation (year): | 1-3%<br>3-5%<br>Greater than 5% |
| 2.13 | Has there been a change in the prevalence of BKD over the last 5 years?<br>Please elaborate. | Strain / species<br>Increase / decrease / no change |
| 2.14 | Have you noticed a change in the prevalence of BKD over the last 10 years (or indicate the number of years if 10 year history is not available)? | Strain / species<br>Increase / decrease / no change |
| **Site or region with AVERAGE prevalence of BKD:** | | |
| 2.16 | How long has this facility been in operation? | |
| | Do you see a difference in prevalence according to strain, stock, site or other? | Strain / stock / site / region/ other (please specify) |
| 2.17 | What has been the prevalence (% of population) of BKD among these fish over the last generation (year): | 1-3%<br>3-5%<br>Greater than 5% |
| 2.18 | Has there been a change in the prevalence of BKD over the last 5 years?<br>Please elaborate. | Strain / species<br>Increase / decrease / no change |
| 2.19 | Have you noticed a change in the prevalence of BKD over the last 10 years (or indicate the number of years if 10 year history is not available)? | Strain / species<br>Increase / decrease / no change |

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## Environmental Practices

### For operations with a single hatchery:

| | | |
|---|---|---|
| 2.20 | What is the water source? Well, river, etc. | |
| 2.21 | Is the water recirculated or flow-through? | |
| 2.22 | Is incoming water treated, if yes – how? | Y / N |
| | sand filter<br>UV<br>Ozone<br>Other (please specify) | |
| 2.23 | What is the water hardness? | |
| 2.24 | Has there been a change in this in the last 5 yrs? | Y / N / unknown |
| 2.25 | Has there been a change in this in the last10 yrs? | Y / N / unknown |

### For operations with multiple hatcheries:

**Site or region with the LOWEST prevalence of BKD:**

| | | |
|---|---|---|
| 2.26 | What is the water source? Well, river, etc. | |
| 2.27 | Is the water recirculated or flow-through? | |
| 2.28 | Is incoming water treated, if yes – how? | Y / N |
| | Sand filter<br>UV<br>Ozone<br>Other (please specify) | |
| 2.29 | What is the water hardness? | |
| 2.30 | Has there been a change in this in the last 5 yrs? | Y / N / unknown |
| 2.31 | Has there been a change in this in the last10 yrs? | Y / N / unknown |

**Site or region with the HIGHEST prevalence of BKD:**

| | | |
|---|---|---|
| 2.32 | What is the water source? Well, river, etc. | |
| 2.33 | Is the water recirculated or flow-through? | |
| 2.34 | Is incoming water treated, if yes – how? | Y / N |
| | Sand filter<br>UV<br>Ozone<br>Other (please specify) | |
| 2.35 | What is the water hardness? | |
| 2.36 | Has there been a change in this in the last 5 yrs? | Y / N / unknown |
| 2.37 | Has there been a change in this in the last10 yrs?<br>If yes, please elaborate. | Y / N / unknown |

**Site or region with an AVERAGE prevalence of BKD:**

| | | |
|---|---|---|
| 2.38 | What is the water source? Well, river, etc. | |
| 2.39 | Is the water recirculated or flow-through? | |
| 2.40 | Is incoming water treated, if yes – how? | Y / N |
| | Sand filter | |
| | UV | |
| | Ozone | |
| | Other (please specify) | |

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| | | |
|---|---|---|
| 2.41 | What is the water hardness? | |
| 2.42 | Has there been a change in this in the last 5 yrs? | Y / N / unknown |
| 2.43 | Has there been a change in this in the last10 yrs? If yes, please elaborate. | Y / N / unknown |
| **Husbandry Practices** | | |
| 2.44 | What practices are currently used to try to prevent BKD? (please elaborate for each method selected) | |
| | Broodstock vaccination | |
| | Broodstock antibiotic treatment | |
| | Broodstock screening | |
| | Egg screening | |
| | Egg disinfection | |
| | Smolt vaccination | |
| | Other (please specify) | |
| 2.45 | Have any practices changed in the last 5 years? If yes, please elaborate. | |
| 2.46 | What husbandry practices do you use to try to control BKD? (indicate all applicable): | |
| | Pharmaceutical treatment | |
| | Feed supplements | |
| | Feed modification | |
| | Density management | |
| | Handling management | |
| | Biosecurity practices | |
| | Other husbandry practices (please specify) | |
| 2.47 | Have any feeding practices changed in the last 5 years? If yes, please elaborate. | Y / N |
| 2.48 | Have you changed feed formulation in the past 5 years? If yes, please elaborate. | Y / N |
| | Change in type of plant protein | |
| | Increase in plant protein proportions | |
| | Change in vitamin/minerals | |
| | addition of immunostimulants | |
| 2.49 | Do you check your mortalities for BKD? | |
| 2.50 | Do you screen your fish for BKD prior to release/transfer to another facility? | |
| **Costs** | | |
| 2.51 | What do you consider the most serious cost associated with BKD? (rank all applicable): | 1= greatest cost |
| | Cost of vaccination | |
| | Cost of treatment | |
| | Cost of management modifications | |
| | Costs associated with production losses | |
| | Costs associated with production (i.e. Maintain low densities, not handling) | |
| | Costs associated with harvest quality | |
| | Costs associated with broodstock management | |

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| | | |
|---|---|---|
| | Other (specify) | |
| 2.52 | Are the cost totals associated with preventing or managing BKD relative to other fish health issues: | Significantly higher / Higher/ About the same/ Lower |
| 2.53 | Has this amount increased/ decreased or stayed the same in the last 5 years? 10 yrs? | 5yr: increased/ decreased/ stayed same<br><br>10yr: increased/ decreased/ stayed same |
| 2.56 | Describe the costs associated with the downgrades attributable to BKD relative to other factors: | Significantly higher / Higher/ About the same/ Lower |
| 2.57 | Has this amount increased/ decreased or stayed the same in the last 5 years? | 5yr: increased/ decreased/ stayed same<br><br>10yr: increased/ decreased/ stayed same |

# Section 3. Later Life stages (grow-out facilities)

| | | |
|---|---|---|
| **For operations with a single facility** | | |
| 3.1 | Do you see BKD at the facility? | Y / N (if yes, please elaborate) |
| 3.2 | How long has this facility been in operation? | |
| | Do you see a difference in prevalence according to strain, stock, site or other? | Strain / stock / site / other (please specify) |
| 3.3 | What has been the prevalence (% of population) of BKD among these fish over the last generation (year)? | 0%<br>1-3%<br>3-5%<br>Greater than 5% |
| 3.4 | Has there been a change in the prevalence of BKD over the last 5 years?<br>Please elaborate. | Strain / species<br>Increase / decrease / no change |
| 3.5 | Have you noticed a change in the prevalence of BKD over the last 10 years (or indicate the number of years if 10 year history is not available)? | Strain / species<br>Increase / decrease / no change |
| **For operations with multiple facilities -** | | |
| **Site or region with the LOWEST prevalence of BKD:** | | |
| 3.6 | How long has this facility been in operation? | |
| | Do you see a difference in prevalence according to strain, stock, site or other? | Strain / stock / site / region/ other (please specify) |
| 3.7 | What has been the prevalence (% of population) of BKD among these fish over the last generation (year)? | 1-3%<br>3-5%<br>Greater than 5% |
| 3.8 | Has there been a change in the prevalence of BKD over the last 5 years?<br>Please elaborate. | Strain / species<br>Increase / decrease / no change |
| 3.9 | Have you noticed a change in the prevalence of BKD over the last 10 years (or indicate the number of years if 10 year history is not available)? | Strain / species<br>Increase / decrease / no change |
| **Site or region with the HIGHEST prevalence of BKD:** | | |
| 3.10 | How long has this facility been in operation? | |
| | Do you see a difference in prevalence according to strain, stock, site or other? | Strain / stock / site / region/ other (please specify) |

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| | | |
|---|---|---|
| 3.11 | What has been the prevalence (% of population) of BKD among these fish over the last generation (year)? | 1-3%<br>3-5%<br>Greater than 5% |
| 3.12 | Has there been a change in the prevalence of BKD over the last 5 years?<br>Please elaborate. | Strain / species<br>Increase / decrease / no change |
| 3.13 | Have you noticed a change in the prevalence of BKD over the last 10 years (or indicate the number of years if 10 year history is not available)? | Strain / species<br>Increase / decrease / no change |
| **Site or region with AVERAGE prevalence of BKD:** | | |
| 3.14 | How long has this facility been in operation? | |
| | Do you see a difference in prevalence according to strain, stock, site or other? | Strain / stock / site /region/ other (please specify) |
| 3.15 | What has been the prevalence (% of population) of BKD among these fish over the last generation (year)? | 1-3%<br>3-5%<br>Greater than 5% |
| 3.16 | Has there been a change in the prevalence of BKD over the last 5 years?<br>Please elaborate. | Strain / species<br>Increase / decrease / no change |
| 3.17 | Have you noticed a change in the prevalence of BKD over the last 10 years (or indicate the number of years if 10 year history is not available)? | Strain / species<br>Increase / decrease / no change |
| **Husbandry** | | |
| **General** | | |
| 3.18 | Do you maintain single year class sites? | Y / N |
| 3.19 | Do you fallow between year classes? | Y / N |
| 3.20 | Do you maintain single species sites? | Y / N |
| 3.21 | What practices are currently used to try to prevent BKD? (please select applicable options): | |
| | Broodstock antibiotic treatment | |
| | Broodstock vaccination | |
| | Broodstock screening | |
| | Genetic selection for disease resistance | |
| | Egg screening | |
| | Egg disinfection | |
| | Smolt vaccination | |
| | Prophylactic treatment | |
| | Other (please specify) | |
| 3.22 | Have any practices changed in the last 5 years? | Y / N (if yes, please elaborate) |
| 3.23 | What husbandry practices do you use to try to control BKD? Please elaborate for all 'yes' selections. | |
| | Treatment if problem | |
| | Feed supplements | |
| | Feed modification | |
| | Density management | |
| | Biosecurity practices | |
| | Other husbandry practices | |
| | | |

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| | | | | |
|---|---|---|---|---|
| 3.24 | Have any feeding practices changed in the last: | 5 years / 10 years / Both | | |
| 3.25 | Have you changed feed formulation in the past: (Please check all that apply) | 5 years | 10 years | Both |
| | Change in type of plant protein | | | |
| | Increase in plant protein proportion | | | |
| | Change in vitamin/minerals | | | |
| | Addition of immunostimulants | | | |
| 3.26 | Do you check your mortalities for BKD? | | | |
| 3.27 | Do you screen your fish for BKD prior to release/transfer to another facility? | | | |
| 3.28 | How do you feed? | | | |
| | To Satiation | | | |
| | Tables | | | |
| | Meals (set interval for feeding) | | | |
| | Other (please specify) | | | |
| 3.29 | Do you consider any of these significant stressors to contribute to BKD prevalence? | Y / N (if yes, please elaborate) | | |
| | Low DO | | | |
| | Predation | | | |
| | Harmful plankton | | | |
| | Low/High salinity | | | |
| | Temperature | | | |
| | Pollution | | | |
| | Density | | | |
| | Handling management | | | |
| | Genetics | | | |
| | Other (please specify) | | | |
| 3.30 | Have you seen changes in any of these variables in the past: | 5 years / 10 years / Both | | |
| **Costs** | | | | |
| 3.31 | What do you consider the most serious cost associated with BKD? | | | |
| | Cost of vaccination | | | |
| | Cost of treatment | | | |
| | Cost of management modifications | | | |
| | Costs associated with production losses | | | |
| | Costs associated with production (i.e. Maintain low densities, not handling) | | | |
| | Costs associated with harvest quality | | | |
| | Costs associated with biosecurity | | | |
| | Costs associated with broodstock management | | | |
| | Other | | | |
| 3.32 | What are the cost totals associated with preventing or managing BKD relative to other fish health issues? | Significantly higher / Higher/ About the same/ Lower | | |
| 3.33 | Has this amount increased/ decreased or stayed the same in the last 5 years? 10 yr | 5yr :increased/ decreased/ stayed same<br>10yr:increased/ decreased/ stayed same | | |

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| | | |
|---|---|---|
| 3.34 | Describe the costs associated with the downgrades attributable to BKD relative to other factors: | Significantly higher / Higher/ About the same/ Lower |
| 3.35 | Has this amount increased, decreased, or stayed the same in the last 5 years? 10yrs? | 5yr :increased/ decreased/ stayed same<br>10yr:increased/ decreased/ stayed same |

**Are there any other comments pertaining to the impact of BKD on your organization you would like to add, and/or is there some component of the disease you would like to discuss that was not a part of this questionnaire? Please describe:**

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# Feasibility of Selective Breeding for Resistance to Bacterial Kidney Disease: Current State of Knowledge

**Shannon K. Balfry[1*] and Laura L. Brown[2]**

**[1] University of British Columbia, DFO/UBC Centre for Aquaculture and Environmental Research, 4160 Marine Drive, West Vancouver, British Columbia, V7V 1N6**
**balfry@interchange.ubc.ca**

**[2] Institute for Marine Biosciences, National Research Council, 1411 Oxford Street, Halifax, Nova Scotia B3H 3Z1**
**Laura. Brown@nrc-cnrc.ca**

**[*] Author responsible for correspondence**

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# Executive Summary

Bacterial kidney disease (BKD) is a serious disease of both wild and farmed salmon in British Columbia. BKD is a chronic disease that results in mortalities up to and including harvest, when the financial investment and loss is significant. There is no known treatment for the disease, due primarily to the unique characteristics of the causative agent, *Renibacterium salmoninarum*. The indigenous Pacific salmon are more susceptible to BKD and is a major reason why the BC salmon farming industry primarily produces Atlantic salmon.

The industry relies on avoidance methods to prevent BKD infections. This includes avoiding vertical transmission of the pathogen by practicing broodstock selection, which removes infected females from the population. This method has been found to be successful both here in BC and also in the various hatcheries in the US Pacific Northwest. Lowered rearing densities and minimal handling have had significant positive impacts on reducing BKD outbreaks, even among groups of Chinook salmon which are very susceptible to BKD. However, despite these and other fish health management practices, there is the question of whether the BC aquaculture industry should look towards implementing a selective breeding program to increase resistance to BKD. The focus of this report is to examine the feasibility of this approach.

The report includes an extensive literature review on the latest research on BKD and the development of breeding programs for producing disease resistant fish. Information was also obtained from discussions with local and international experts. The peer-reviewed

literature was the primary source of information, and when further details were required the authors were contacted. Industry information and expert opinions were also obtained and included in the report to provide insight and aid in the interpretation of the various published reports and data.

The conclusions of this report suggest that there is still not enough information available to initiate a selective breeding program for BKD. The pathogenesis and epizootiology of BKD is extremely complex and at this time, no genetic marker – direct or indirect – exists to form the basis of a selective breeding program. The use of survival data for breeding resistance is not a scientifically valid approach because of the many factors that affect survival especially when considering the chronic nature of this disease. In addition, due to environmental and fish welfare concerns, BKD affected fish should be treated with antibiotics. There are many fish health management practices currently in place that appear to be effective in reducing both the vertical and horizontal spread of BKD. There are great advances being made in the identification of genetic markers associated with disease resistance. However, we are many years away from being able to apply this technology to select for BKD resistance in a commercial aquaculture setting.

In summary, there is no clear basis in evidence to suggest that breeding for BKD resistance is a viable and defendable fish health management practice.

# Introduction:

This report addresses the question of whether it is feasible for the British Columbia (BC) aquaculture industry to develop a broodstock program designed to select salmon for resistance to Bacterial Kidney Disease (BKD). BKD is a chronic, systemic disease of that affects all species of salmonids, however the chinook salmon (*Oncorhynchus tshawytscha*) are particularly susceptible. BKD is of extreme economic importance to the aquaculture industry because the chronic progression of the disease, often results in mortalities of market-size fish. Current controls methods have failed to eradicate the disease and the salmon farming community of BC have turned their attention to using a stock selection program to reduce BKD prevalence and associated mortality. This report was therefore prepared, to assess the feasibility of this approach in controlling BKD among farmed salmon in BC.

The first part of this report provides the reader with the most current state of knowledge on bacterial kidney disease. This was deemed necessary to ensure that those reading this document are completely informed on the latest research and information on BKD. This literature review in needed to understand the unique complexity of the disease and why it is such a difficult disease to control. Following this literature review is an introduction to how genetics influence disease resistance in fish, and how genetic variation in disease resistance factors can be used to breed fish with enhanced abilities to resist infectious diseases. The genetic variation of BKD resistance is introduced with a literature review and the feasibility of breeding for BKD resistance. Various approaches that could be used as selection criteria for breeding BKD resistant fish are discussed, and include the advantages and disadvantages of these approaches. The final section of this report is a summary of literature review within this report and recommendations/comments on the feasibility of breeding fish for BKD resistance. These recommendations are based on the cited information from the literature and from

personal communications with experts in the field of BKD research, breeding programs, and the BC aquaculture industry. Local, national and international expertise was solicited to gain the most relevant information to evaluate the feasibility of developing a breeding program to select for BKD resistant fish in BC.

# Bacterial Kidney Disease: Background Review

Bacterial kidney disease (BKD) is a systemic disease of wild and cultured salmonids, caused by the bacterium, *Renibacterium salmoninarum* (Sanders & Fryer 1980). BKD was first reported in the 1930's in Atlantic salmon (*Salmo salar*) from the Dee River system of Scotland (Smith 1964). BKD was first reported on the Pacific coast of North America in 1936 (Earp et al. 1953), and in cutthroat trout (*Oncorhynchus clarkii*) in British Columbia by 1937 (work by Duff et al., reported by Evelyn 1988). BKD has now been reported in salmonids from North America, Western Europe, Chile, and Japan. The *Oncorhynchus* genus (Pacific salmon and rainbow trout, *O. mykiss*) are considered to be the most susceptible of the salmonid species. No natural cases of BKD have been reported in non-salmonids (Fryer & Sanders 1981).

## *Renibacterium salmoninarum*

The causative organism of BKD, *R. salmoninarum*, is a small (0.3-0.1 μm by 1.0-1.5 μm), Gram-positive, non-motile, non-acid-fast, and non-spore-forming diplobacillus (Sanders and Fryer 1980). *R. salmoninarum* is a slow-growing bacterium requiring incubation times of 6-8 weeks (at 15 °C) for primary isolations. A result of this slow growing characteristic of *R. salmoninarum* is that BKD infections progress slowly, and are generally considered to be chronic in nature. This relatively long infection process results in mortalities that occur usually after the fish are one year old and considerable financial investment has been made. The economic impact of these mortalities is therefore significant. The ability of *R. salmoninarum* to survive and replicate within fish leucocytes (including macrophages) has been reported (Bandin et al., 1993, Bruno 1986, Gutenberger et al. 1997, Young and Chapman 1978). The intracellular survival of *R.*

*salmoninarum* is considered to be a critical feature of the pathogenesis of the disease and one of the main reasons why it is so difficult to develop effective treatments for BKD.

Isolates of *R. salmoninarum* are generally similar to each other, however, there are some differences noted in the virulence of some of these strains. Non-autoagglutinating strains of *R. salmoninarum* have been shown to be less virulent, which has been attributed to low levels of the p57 protein on the cell surface (Bruno 1988, 1990). Research by Dale et al. (1997) has shown differences in virulence between strains of *R. salmoninarum,* and corresponding differences in resistance between species of salmon (rainbow trout, *O. mykiss* and coho salmon, *O. kisutch*). Similar research examining attenuated strains of *R. salmoninarum* (MT 239) has shown that they produce fewer mortalities in both rainbow trout and the relatively more susceptible, chinook salmon (O'Farrell et al. 2000). The virulence of *R. salmoninarum* is related to the presence of a p57 kDa protein believed to be associated with the peritrichous fimbriae that extends from the capsule surrounding the cell (Dubreuil et al., 1990a, b). In addition to lower levels of p57 on these strains, these authors suggest avirulence may also be attributed to a slower *in vivo* growth rate of altered strains and/or differences in the ability to survive intracellularly.

## Immunopathological responses to BKD infections

External signs of BKD include exophthalmia, abdominal distension, hemorrhagic areas and superficial blisters or ulcers on the skin (aka 'spawning rash'). Internally, the kidneys appear to be the primary target organ, and infected fish typically exhibit kidneys which are swollen, gray and contain visible white lesions. Other organs such as the spleen and liver may also have these white lesions. Histological examination of infected tissues (predominantly the hematopoietic kidney tissue) reveals necrosis, tubule damage, and the presence of numerous bacteria. Bruno (1986) has published a description of the pathogenesis of BKD in laboratory-infected fish. His observations include the rapid uptake (45 min post-injection) of bacterium within kidney and spleen phagocytes, and he reported that after 6-10 days the bacterium showed evidence of multiplication within these phagocytes as well as within monocytes. The experimentally infected fish displayed similar histopathological changes as the naturally infected fish, and indicated a

chronic systemic infection. The cause of mortality in fish infected with BKD was attributed to the destruction of normal kidney and liver tissue structure and function, heart failure due to infiltration of heart tissue with *R. salmoninarum* containing phagocytes, and soft-tissue damage caused by liberation of substances from the bacteria, as well as host macrophages.

The immune response of the fish to BKD infections show that the humoral and cell-mediated immune system does mount a limited protective response, however, little is known of the mechanisms of this response. A recent publication by Grayson et al. (2002) suggests that the host immune response is directed against the p57 protein liberated by *R. salmoninarum*. The p57 protein appears to initially suppress host inflammatory responses, however the expression of tissue necrosis factor–α (TNF-α) and major histocompatibility II (MH II) genes remain elevated. Grayson et al. (2002) suggested this elevated stimulation as an explanation for the observed chronic pathology associated with BKD infections. Molecular approaches to study the pathogenesis of BKD were used by Booy et al. (2005), who reported that Atlantic salmon infected with *R. salmoninarum* showed an upregulation in the expression of liver and kidney interferon-induced viral resistance protein (IFI-Mx). This Mx protein is generally associated with viral infections, and indicates that the intracellular nature of *R. salmoninarum* infections adds complexity to the immune response and our understanding of host responses.

The pathogenesis of BKD in salmonids is further complicated due to the immunotolerance of fish that have been exposed in the early life history stage (i.e. via vertical transmission in the egg) to *R. salmoninarum*, or more specifically to the p57 protein associated with this bacterium (Brown et al. 1996). This research suggested that the coho salmon exposed to the p57 protein at the egg stage demonstrated at least partial immunosuppression and an increased susceptibility to BKD infections. The mechanism for this immunosuppression/immunotolerance is related to the presence of the bacterium within the egg, long before immunity is developed and the ability to differentiate self from non-self.

## Epizootiology

*R. salmoninarum* is known to be transmitted horizontally from fish to fish, however the exact mechanism is unclear. It has been demonstrated that ingestion of feces from BKD infected fish can result in the infection being transmitted (Balfry et al. 1996). The horizontal transmission of this disease has been repeatedly demonstrated in the laboratory. The co-habitation challenge method is an accepted method used successfully in the laboratory to study BKD infection, and relies on the horizontal transmission of *R. salmoninarum* from experimentally infected fish (*via* intraperitoneal injection) to naïve un-infected fish (McKibben and Pascho 1999, Murray et al. 1992).

As indicated above, *R. salmoninarum* is also transmitted vertically from females to her progeny via the eggs (Evelyn et al. 1986a). This route was demonstrated by Evelyn et al. (1984), who found *R. salmoninarum* within the eggs of females with the bacterium present in the ovarian fluid. The exact route(s) and timing by which the bacterium is capable of entering the gametes is unclear. It appears that *R. salmoninarum* present in infected ovarian fluid and/or coelomic fluid that is in contact with the maturing eggs (Evelyn et al. 1986a) is the major pathway for entry. The intra-ovum survival of *R. salmoninarum* is the reason why surface disinfection of eggs prior to and during fertilization (i.e. with the water hardening phase) is not completely effective in reducing BKD infections in fry. Injection of broodstock with antibiotics such as erythromycin has resulted in reduced infections of eggs and fry, if the antibiotics are injected at a time when they can be incorporated into the eggs (Brown et al. 1990). There is no evidence that males contribute to the vertical transmission of the disease.

## Diagnosis of Bacterial Kidney Disease

Bacterial kidney disease was originally diagnosed on the basis of clinical signs, the presence of *R. salmoninarum* in kidney tissue smears or from culture. Confirmation of R. salmoninarum in the smears and culture was obtained from Gram stains, which revealed the characteristic Gram-positive diplobacillus morphology. These methods were highly specific but not very sensitive. Primary isolation of the bacterium using culture methods have been problematic and difficult due to the slow growth rate of *R. salmoninarum*, and the need for specialized, selective media to prevent contamination of culture plates due to the extended time required for isolation. Benediktsdóttir et al.

(1991) reported that up to 19 weeks may be required for primary isolation from subclinically infected fish. Atypical growth in subclinically infected fish using selective media (SKDM; Austin et al. 1983) has been recently described as a possible explanation for the lack of sensitivity of the culture method (Hirvelä-Koski et al. 2006).

During the 1980's there was significant progress in the development of more sensitive and specific assays to detect *R. salmoninarum*, with the general acceptance of the direct and indirect fluorescent antibody test (DFAT and IFAT, respectively; Bullock & Stuckey 1975). Modification of these methods soon evolved to provide methods that were both specific and quantitative – the membrane filtration fluorescent antibody test (MF-FAT; Elliott and Barila 1987), and the quantitative fluorescent antibody test (QFAT; Cvitanich 2004). Several immunodiagnostic tests have been developed with varying degrees of sensitivity and specificity. Examples of these include: the enzyme-linked immunosorbent assays (ELISA; Pascho et al. 1987), the immunoblot assay (Sakai et al. 1987), and the immunodiffusion assay (Chen et al. 1974). The nested polymerase chain reaction method (PCR) has proven to be a very sensitive method to detect the pathogen in tissue samples (Chase and Pascho 1998). Recently a quantitative polymerase chain reaction (PCR) method has been described that offers excellent sensitivity and specificity (Powell et al. 2005). A review and discussion of the various detection methods is provided by Wiens and Kattaari (1998).

Recommendations by the Office International des Epizooties (OIE), suggest that the ELISA and FAT be used for BKD screening, and culture and/or PCR be used for confirmations (OIE 2003). The Canadian Fish Health Protection Regulations recommends in the Manual of Compliance (Fisheries and Oceans Canada 2004) that Gram stained kidney or lesion tissue be used as presumptive evidence of BKD infections, while the immunodiffusion, DFAT or IFAT be used for confirmation. The American Fisheries Society, AFS Fish Health Section Blue Book (AFS-FHS 2004) also suggests Gram stain as a presumptive indicator of infections, and as confirmation they recommend the immunodiffusion, DFAT, IFAT, ELISA or nested PCR. Thus it can be seen that there is some agreement between these various organisations as to the appropriate test to be used. This agreement would be helpful in developing assays for breeding and monitoring programs that were recognised internationally.

## Factor affecting susceptibility to BKD

As was indicated above, salmonid species vary in their susceptibility to BKD. Other factors that have been reported to have significant impact on susceptibility and the transmission of BKD among fish are: temperature, water quality, nutrition, salinity. The relationship between water temperature and the progression of BKD was reported by Belding and Merrill (1935). Increases in water temperature have long been associated with increased BKD-related mortality. This is likely due to positive effect of increased temperature on the growth of the pathogen. This was demonstrated by Fryer and Sanders (1981) who found a mean time to death of 25 days at 15-20.5°C, compared to a much longer mean time to death of 70 days reported for fish reared at 4 °C.

Nutrition appears to have an effect on susceptibility to BKD. Diet composition has been shown to affect innate and specific immune function (Waagbo 1994). In addition, feed ration has been shown to impact some cellular immune responses (Alcorn et al. 2003). The quality and source of dietary lipids can affect overall disease resistance (Balfry and Higgs, 2001) and even the quantity of dietary lipid has been suggested to have an effect on BKD resistance. Austin (1985) suggested that reductions in BKD infections could be achieved by feeding salmon low fat diets. The mineral and trace element composition of the diet has also been shown to have an effect of BKD infections. Studies conducted by Paterson et al. (1985) and Lall et al. (1985) report that diets supplemented with iodine and fluorine were effective at reducing BKD infections in Atlantic salmon. The mechanism for these diet effects could not be determined and the authors suggest that a combination of dietary, water quality, physiological condition, and environmental factors contributed to the infection status of the fish. Bell et al. (1984) reported that sockeye salmon (*O. nerka*) fed diets low in zinc and manganese, showed survival time following BKD infections was inversely related to dietary ascorbate levels.

Water quality has been reported to have an impact on BKD susceptibility, as increased water hardness has been associated with increased resistance to BKD outbreaks (Warren 1963). The author of that report could not specify which constituent exerted the greatest effect on the severity of BKD outbreaks, and suggested the results could be

attributed to either the ability of the pathogen to survive in the water, or the ability of the fish to resist infections.

Stress can increase the susceptibility of fish to diseases and this includes BKD. The mechanism for this is related to the effect of cortisol on reducing the number of circulating leucocytes (Maule et al. 1989), which results in a diminished capacity to mount and effective immune response. High rearing densities have been shown to increase the prevalence of BKD infection in farmed chinook salmon (Mazur et al. 1993). The higher rates of infection were attributed to the physiological stress response (i.e. cortisol) and the enhanced potential for horizontal transmission via the fecal-oral route. Another factor that may contribute to the progression of BKD is the marine environment. There are several reports published that indicate a higher incidence of BKD amongst salmon reared in seawater versus freshwater environments (Sanders et al. 1992, Fryer and Sanders 1981). The role smoltification plays in the physiological response and increased disease susceptibility however has not been investigated as a possible explanation for the reported results of the impact of salinity (i.e., marine versus freshwater) on BKD resistance.

As can be seen, many external, environmental factors influence the ability of fish to resist disease. It is therefore extremely important to consider all these extrinsic factors when examining the feasibility of any widescale breeding program. The last factor that appears to play a role in susceptibility of salmonids to BKD is genetics. This topic will be discussed in detail later in this report.

## Control and Treatment of BKD

The use of chemotherapeutics to control BKD infections in salmon has been met with limited success (Elliot et al. 1989). The intracellular survival of *R. salmoninarum* is considered to be the primary reason for the lack of success (Fryer & Sanders 1981). An early report by Austin (1985) compared over 70 antimicrobial compounds and found that the most useful compound was rifampicin, however this antibiotic is a potent treatment for tuberculosis and not appropriate for treating BKD. Erythromycin is the most common antibiotic used to treat BKD, and is generally administered by injection to broodstock and

orally to juvenile fish. The injection of erythromycin into pre-spawning broodstock has been shown to reduce the levels of *R. salmoninarum* within the egg and consequently reduces the process of vertical transmission (Evelyn et al. 1986b, Brown et al. 1990). The practice of treating fish with feed containing therapeutic levels of erythromycin (200 mg/kg body weight) has been shown to reduce BKD mortalities when administered for a 21 day period (Moffitt 1992). The difficulty in treating already infected fish via oral administration, however, is that infected fish generally do not have the appetite required to ingest the therapeutic dose of antibiotic. This problem has been studied by Pirhonen et al. (2000) in chinook salmon naturally infected with BKD. The authors found that both healthy and infected fish eat more readily following a period of fasting (i.e., when feed was withheld). Therefore they recommend starving fish prior to antibiotic treatments, or alternatively, during a treatment, to alternate feeding days with fasting days. Another use of chemotherapeutics to control of BKD is to immerse newly fertilized eggs in erythromycin or iodophor solutions. This method however, does not appear to be effective in preventing the vertical transmission of BKD as the bacterium appears to be able to enter the eggs prior to ovulation (Bruno and Munro 1986).

There has been a great deal of effort put towards developing effective vaccines for BKD. The unique nature of the pathogenesis of BKD has made this an extremely difficult task. The immune response of fish to *R. salmoninarum* infections is complicated by the issues of immunotolerance due to vertical transmission. In addition, the humoral antibody response though slow, does result in high titres. However these antibodies do not appear to be highly protective (Kaattari et al. 1988). A commercial vaccine for BKD has recently become available. This vaccine is called Renogen and is produced by Aqua Health Ltd. (Novartis) in PEI, Canada. This vaccine is based on an attenuated strain of *Arthrobacter sp.*, and has shown some promise in reducing BKD mortalities. However, a study published by Alcorn et al. (2005) did not find differences in mortality between groups of fish vaccinated with Renogen, an experimental vaccine (i.e., a recombinant p57 vaccine) or a group of control fish vaccinated with a saline solution (phosphate buffered saline with and without Freud's incomplete adjuvant). Other researchers are examining the attenuated strains of *R. salmoninarum* for their usefulness as vaccines against BKD (Daly et al. 2001, Piganelli et al. 1999).

The control of BKD appears to be best accomplished through culling of infected fish. The ability to detect infected fish is therefore critical to the success of this method. As described above, there is an abundance of methods to detect *R. salmoninarum*. The critical question with all of these detection methods is how well do these diagnostic resuls correlate with the risk of infectivity. A recent article published by Hamel & Anderson (2002) addresses this problem, by examining the relationship between antigen load (specifically to the p57 protein) and bacterial load. The results indicate a complex and non-linear relationship, as there appears to be a decrease in antigen secretion rate with increasing antigen concentration. An explanation for these results will be pursued with further research, but these findings indicate the complexities involved in the pathogenesis of this organism and the reasons why BKD is so difficult to control.

Despite the problems associated with the inability to predict risk of infections, particularly in subclinically infected fish, the avoidance method does appear to be one of the best options for controlling BKD. The avoidance method being a combination of methods designed to avoid initial and/or new infections from occurring. This avoidance method used in combination with monitoring programs, and prophylactic chemotherapy treatments at various life history stages (even first feedings, Evelyn 1988) can significantly reduce BKD infections. Husbandry practices that include good sanitation and handling of mortalities, separation of year classes, maintenance of good nutrition, and reducing stress to fish associated with high densities, handling, and transportation, will all help maintain general health and immunocompetence. Providing a stress-free environment for the rearing of fish is particularly important in the spring when water temperatures are rising and pathogen growth is favoured.

The above avoidance methods will help prevent the horizontal transmission of BKD. Methods to avoid the vertical transmission of BKD include the injection of female broodstock with erythromycin, and the surface-disinfection of fertilized eggs to eliminate surface-born *R. salmoninarum*. Another method commonly used to prevent BKD infections is the screening of ovarian fluid and/or kidney tissue of the female broodstock for *R. salmoninarum*. This method relies on the accurate detection of *R. salmoninarum*, and the subsequent culling of BKD infected females and progeny. In the 1980's this screening was performed widely in BC, using the IFAT procedure. There were however,

at the time there were problems associated with quality control that could explain why this method was not overly effective (Armstrong et al. 1989).

Recent developments in diagnostics have increased the sensitivity of detection and the subsequent culling of *R. salmoninarum*-positive broodstock has been shown to dramatically reduce BKD prevalence. In Iceland, this broodstock culling procedure was used to reduce BKD prevalence in farmed Atlantic salmon from 35% to below 2% after just a few years (Gudmundsdottir et al. 2000). A recent article supports this, and suggests selective culling of infected females is effective in controlling BKD in the hatchery, and may result increased disease resistance of the progeny (Hard et al. 2007). The effectiveness of this method of controlling BKD, was also demonstrated by Maule et al. (1996) who reported that broodstock culling, progeny separation and lowered rearing densities, contributed to a reduction in BKD prevalence from 100% to 3%, in 6 of the 8 hatcheries participating in this 5-year study. The husbandry practices used by these hatcheries were aimed at reducing both vertical and horizontal transmission of *R. salmoninarum*.

Another practice that appears to be effective in controlling BKD infections at farm sites is the fallowing of sites between production cycles. The fallowing of sites to break disease cycles has been practiced in Scotland now for several years, and has been cited as a primary reason for significant reductions in BKD outbreaks at marine sites (Bruno 2004).

Breeding for BKD resistance is another possible method to control and prevent infections. The feasibility of this method will be explored in the last half of this report.

# The role of genetics on disease resistance in fish

## Introduction

Evidence of a genetic basis for disease resistance was documented and demonstrated several years ago, for both plants (Wallace 1961) and animals (Hutt 1970). There is also strong evidence for a genetic basis to disease resistance in fish (see reviews by Chevassus and Dorson 1990, Fjalestad et al. 1993, Wiegertjes et al. 1996). The use of genetics to improve disease resistance or any other economically important trait is generally achieved by applying a breeding program based on selection of individuals, families, or groups that possess the desirable trait. It is imperative however, that for any selective breeding program to be successful (i.e., achieve genetic improvement or gain) there must exist sufficient genetic variation (additive variation) of the trait between individuals. The potential for genetic gain can be estimated from experiments that examine and measure the presence of this trait in parents and then progeny. The potential for genetic gain can be expressed mathematically as the heritability ($h^2$), which is the ratio of additive genetic variance to phenotypic variance. The closer the $h^2$ value is to 1.0, the greater the potential for making significant genetic gains for that particular trait. The method involved in the calculation of heritability estimates is very complex (and beyond the scope of this report, please refer to Falconer 1981 for further details). Estimating heritability of disease resistance is further complicated by the important relationship between host, pathogen and environment. Each of these three components are involved in the disease process and must be controlled in experiments designed to accurately predict heritability. In addition, the ultimate measure of disease resistance is survival, which is a trait that is extremely difficult to quantify because of the problems associated with associating cause of death with full certainty.

Selective breeding programs designed to improve disease resistance are generally based on survival data following a disease outbreak (natural or an experimental laboratory challenge). Survival rates, however, are under the influence of many factors, such as pathogen virulence, water temperature, fish stress and prior exposure to the pathogen. This complex relationship between the host, pathogen and environment often

results in inaccurate estimates of the actual genetic contribution to disease resistance (Gavora and Spencer 1983, Van Muiswinkel et al. 1999). Survival data collected from natural disease outbreaks are problematic due to the inability to control or accurately determine the cause of the mortality. Open net pen situations are particularly highly variable with respect to environmental conditions, pathogen prevalence/virulence, and the interactions of these factors with possible stressors such as handling, predators, etc. Experimental laboratory derived survival data is usually more controlled with respect to the previously mentioned factors, but it can be difficult to extrapolate results to natural or commercial settings. Researchers have therefore been directing their focus to finding other markers of disease resistance that can be correlated (statistically and biologically) with disease resistance in commercial fish farms.

## Selection for disease resistance in fish

A great deal of effort has been directed towards establishing a link between the activity of the innate immune system and disease resistance. There are several reasons why the innate immune system has been the subject of so much research effort to establish a genetic trait linked with disease resistance. The most important reason for this focus is because the innate immune system represents an in-born, immediate, and permanent form of protection against infections caused by bacteria, parasites, fungi, and viruses (for a review of the innate immune system, refer to Magnadottir 2006). The innate immune system is preferred over the specific immune system because there is less impact of environment on protection. Environmental effects such as previous pathogen exposure and water temperature have a significant negative impact on antibody production, memory formation, and T cell activity, which are key to the functioning of the specific immune system (Rijkers 1982, Clem et al. 1984). In addition, the innate immune system appears to be the dominant form of protection during early development and prior to the fish being fully immunocompetent (approximately 4 g mean weight, Ellis 1988). The innate humoral immune system functions at a much earlier age; for example, fish eggs have been found to contain lysozyme, lectins and hemagglutinins (Ingram 1980, Yousif et al. 1991). The innate cellular immune system also becomes functional at a very

early age. Tatner and Manning (1985) have shown that the phagocytic system begins to function at 4 d post-hatch and is fully functional by 14 d post-hatch.

The specific, adaptive immune response has also been investigated as a source for disease resistance selection criteria. Camp et al. (2000) working with channel catfish (*Ictalurus punctatus*) and the bacterial disease caused by *Edwardsiella ictaluri*, but found that levels of specific antibody and lymphocyte numbers were not clearly associated with the infection process or subsequent disease resistance. An alternative approach to finding appropriate criteria for selective breeding is to utilize the link between stress susceptibility and disease susceptibility and/or activity of different components of the innate immune system. Fevolden et al. (2002) examined the relationship between low and high cortisol – responding rainbow trout, plasma lysozyme activity and disease resistance. In general, the high cortisol-responding fish appeared to be less capable of performing and surviving than their low-cortisol-responding cohorts. However, the authors cautioned that it is essential to correlate these traits (lysozyme and cortisol) carefully with other production traits (i.e., growth rate, flesh quality). Their later work (Fevolden et al. 2003) indicated that the decreased survival attributed to the high-cortisol responders may be related to an inability to adapt to the environment of a marine netpen (saltwater and confinement). These results further suggested that survival data is not an appropriate method to determine and evaluate the resistance of fish populations, particularly in the uncontrolled environment of a commercial marine net pen.

Lund et al. (1995) found no correlation between innate hemolytic activity and survival to furunculosis, cold-water vibriosis, or bacterial kidney disease. The lack of a clear relationship between hemolytic activity and disease resistance may be related to the actual contribution of hemolytic activity (presumed to be due to alternative complement pathway activity) to the overall protection of fish from disease. This difficulty in distinguishing the contribution of a particular trait such as hemolytic activity to disease resistance is exacerbated in the case of bacterial kidney disease, because the pathogenesis of the disease is still largely unknown.

There has been much focus in the literature on using lysozyme activity as an indirect marker for disease resistance. Wiegertjes et al. (1996) in his comprehensive review of the literature on the immunogenetic basis for disease resistance, states that

lysozyme appears to be the most promising of all the indirect traits for use as a selection criteria to improve disease resistance. Balfry et al. (1997) reported a correlation between plasma lysozyme activity and survival following an outbreak of vibriosis in netpen-reared chinook salmon. However, despite these correlations, Balfry and Iwama (2004) cautioned on the interpretation of lysozyme data, because variations in lysozyme activity appeared with different size- and life history stages of coho salmon. In addition, lysozyme activity has also been shown to vary with stress (Mock and Peters, 1990), and during the infection process as the immune response becomes activated (Balfry et al. 2001). Johnson et al. (2003) found that chinook salmon with high kidney lysozyme activity were BKD-positive fish (as detected using PCR). However, the causal relationship of this association could not be determined. The pathogenesis of BKD in salmon is still unclear and the use of lysozyme activity or other indirect selection markers is not recommended until significant correlations with resistance are established.

Another approach to genetic selection for disease resistance in farmed fish, is to use genetic markers as selection criteria. The majority of this type of research, has focused on those genes that are associated with disease resistance – notably the MH genes that produce proteins involved in antigen recognition (Van Muiswinkel et al. 1999). There are two classes of MH genes that exist in mammals and fish. MH class I and II genes are involved in the presentation and subsequent protection against viral and bacterial antigens, respectively. The functional differences in the role of each of these two genotypes may explain why selection based on MH gene effects can enhance resistance to a bacterial disease (i.e. furunculosis) and decrease resistance to a viral disease (i.e., infectious salmon anemia) (Kjøglum et al. 2006). The ability to predict the gain from a selective breeding program based on the presence and expression of certain genes requires a clear correlation between the gene of interest and enhanced survival (i.e., resistance). There is also a requirement for sufficient additive genetic variation for improvements to be made through selective breeding. Pitcher and Neff (2006) examined the genetic variation of MH Class I and II genes in chinook salmon and found the lowest levels of MH gene diversity when compared to all other vertebrates. This result would suggest therefore that MH genes would not be useful for selection criteria when breeding for disease resistance in chinook salmon.

Recently there has been much research on using molecular tools to identify traits that might be correlated with disease resistance. The major focus of this approach has been to identify Single Nucleotide Polymorphisms (SNPs) and quantitative trait loci (QTL) associated with quantitative traits such as disease resistance. The power and usefulness of this approach has been discussed by Hayes et al. (2006), and its usage cautioned (Ferguson and Danzmann 1998). In BC, the Consortium on Genomics Research for All Salmon Program (CGRASP ; http://GRASP.ca) has been involved in the development of a bacterial artificial chromosome (BAC) and Expressed Sequence Tag (EST) libraries that have aided in the mapping and sequencing of the Atlantic salmon genome (see Thorson et al. 2005). Other projects are currently underway in Canada and internationally with similar approaches and goals but directed to alternate species such as the Atlantic halibut (*Hippoglossus hippoglossus*) and the Canadian Atlantic cod (*Gadus morhua*).

## Genetic variation in BKD resistance

Early work examining the genetic basis for BKD resistance was performed by Suzumoto et al. (1977) who compared BKD mortality between stocks of coho salmon possessing different transferrin genotypes. The focus of this research was to determine if the iron-binding properties of transferrin would confer resistance to BKD because transferrin would reduce circulating iron levels and thus limit bacterial growth, pathogenicity and thus disease. These authors did find that there were transferrin genotypes associated with increased resistance to BKD, however they suggested that this result was likely not due to the transferrin protein itself. Later, work by Winter et al. (1980) found similar results to Suzumoto et al. (1977), stating that the importance of the transferrin genotype was stock-specific and its association with BKD resistance was likely due to gene linkage with a particular transferrin genotype. These authors cautioned against selecting for BKD resistance based on transferrin genotype. Investigations examining serum iron levels and BKD resistance in different Atlantic salmon families, revealed correlations between survival and serum iron levels of close to zero (Ravndal et al. 1994). Recent work by Stafford and Belosevic (2003) has demonstrated in goldfish that transferrin may enhance bacterial killing of some fish bacterial pathogens by

activating macrophage antimicrobial responses. The relationship between this previously unknown role of transferrin in the immune response and BKD resistance has not been investigated.

Stock differences in BKD resistance have also been demonstrated by other researchers who have reared different stocks of coho salmon and experimentally infected them with *R. salmoninarum*. McGeer et al. (1991) compared six BC coho stocks (Capilano, Chehalis, Chilliwack, Quinsam, Tenderfoot, and Eagle Rivers) and found that the Capilano River had the lowest mortality following experimental challenge with *R. salmoninarum*. Withler and Evelyn (1990) compared coho salmon from the Kitimat River and Robertson Creek for differences in BKD resistance. These researchers examined the genetic variation within and between the two stocks using quantitative genetics to evaluate the potential for increasing BKD resistance within a strain by selective breeding. The Kitimat River coho salmon stock was found to be more resistant to BKD, based on higher post-challenge survival and longer mean time to death. Heritability estimates based on survival data were calculated to be 0.53 (sire component, binomial character), which was considered to be indicative of sufficient levels of additive genetic variation to achieve positive results if a selective breeding program was initiated to increase BKD resistance. This interpretation of these results, however, should be considered cautiously because this conclusion was based on survival data that was collected from fish that were not challenged using a natural method. In that particular study, the fish were infected using an intraperitoneal injection of virulent *R. salmoninarum*. Important innate factors responsible for resisting infections and disease such as mucus antimicrobials (complement, lysozyme), were by-passed and therefore a true measure of disease resistance was not measured. Later work published by Beacham and Evelyn (1992a) demonstrated the importance of these external factors in determining disease resistance following experiments on chinook salmon families challenged by both immersion and injection. Different survival rates were obtained for the same families, and no correlation in mortality could be detected. These authors therefore reported that external barrier such as mucus or skin appear to be important in providing initial protection against *R. salmoninarum* infections. Balfry (1997) demonstrated the importance of external innate factors when comparing coho salmon stocks for differences

in disease resistance. In this work, five coho salmon stocks (Kitimat, Quinsam, Robertson, Chehalis, Capilano Rivers) were challenged with *Listonella (Vibrio) anguillarum* using two different challenge methods - intraperitoneal injection (two doses) and immersion. Stock differences in survival were only found when the fish were infected using the more natural immersion challenge method.

Further research examining the role of genetics in BKD resistance was carried out by Beacham and Evelyn (1992b). In this study, they performed immersion challenges on subgroups of families from three stocks of chinook salmon (Kitimat, Quinsam, Nitinat). The subgroups were challenged with one of three bacterial pathogens – *R. salmoninarum, Aeromonas salmonicida* (causes furunculosis) and *Listonella anguillarum* (causes vibriosis). Variations in mortality and mean time to deaths were determined and heritabilities estimated for each of the diseases. Heritabities were low for all parameters for each disease. With respect to BKD, the authors reported much lower heritability estimates (0.00-0.38) for BKD than the 0.53 estimate previously reported by Withler and Evelyn (1990) in coho salmon. Low heritability estimates of 0.00-0.05 for BKD survival (sire component, binomial character) were also reported by Beacham and Evelyn (1992a) when families from three different salmon species (chum, *O. keta*, chinook and coho) were experimentally infected with *R. salmoninarum*.

Other researchers have calculated heritability estimates based on family analyses of non-specific innate immune factors as hemolytic activity and lysozyme activity (reviewed by Wiegertjes et al. 1996). In general, these estimates indicate that due to the low heritability and correlation with disease resistance, selection based on these traits would not result in a significant increase in disease resistance. Johnson et al. (2003) examined different families of chinook salmon for genetic variation in plasma lysozyme activity, PCR detectable levels of *R. salmoninarum*, and a necropsy-based health assessment index. These authors were unable to detect significant heritability estimates for the PCR detectable levels of *R. salmoninarum*, nor were they able to find a correlation between this parameter and performance variables such as growth and survival. The difficulty in determining an association between the presence of *R. salomoninarum* and fish survival may be attributed to the variability in the cause of mortality experienced by the open seawater-reared chinook salmon families. The environment in seawater netpens

is highly variable and causes of mortalities are often unknown. As indicated earlier, environmental interactions have long been recognized as a source of problems when attempting to distinguish genotype from environmental effects, especially when considering the complex issue of disease.

It is extremely difficult to establish the role of genetics on fish survival under farming conditions, because the fish are under the influence of many environmental factors (Fjalestad et al. 1993). Therefore, while survival is ultimately the most telling measure of disease resistance because it indicates the outcome of all the host responses to infection, it is difficult to distinguish cause of mortality, the extent of interactions between the host, pathogen and environment.

## Feasibility of breeding for resistance to BKD

Breeding for resistance to diseases caused by parasites has been demonstrated and appears to be feasible because of the strength of the relationship between parasitic infections, innate immunity and the role of genetics in conferring resistance to infections. There are more opportunities to select for resistance to parasitic diseases than those caused by bacteria and viruses, because many of these innate immune traits follow simple Mendelian inheritance rules (Jones 2001). For example, breeding for resistance against parasites such as *Cryptobia* spp. is possible to achieve because resistance is determined largely by innate alternate complement activity (Woo 2003). In contrast, as described above, the factors involved in resistance to BKD are complex. Host, pathogen and environmental factors involved in BKD resistance/susceptibility are still largely unknown.

The results from the research presented herein, suggests that while there appears to be some stock differences in BKD resistance, the actual genetic contribution in the form of additive genetic variation is very low. In conclusion, there is no evidence to suggest that a selective breeding program for BKD resistance would be a viable method for reducing BKD mortality. Beacham and Evelyn (1992a) suggest that judicious stock selection rather than selective breeding would result in greater improvement in BKD resistance.

The simplest and most practical approach to examine genetic variation is to compare different genetic strains (Price 1985). A strain can be defined as a genetically distinct population, produced from either naturally or artificially segregated breeding (Larkin 1972). Genetic variation between coho salmon strains from BC has been demonstrated by electrophoretic variation (Utter et al. 1970, Wehrhahn & Powell 1987), and microsatellite DNA probes (Beacham et al. 1996). Withler et al. (2005) recently examined the genetic variation in the three strains of Atlantic salmon currently used in the aquaculture industry in BC (Mowi, McConnell, and Cascade). There appears to be a lack of diversity in quantitative traits within and between the fish strains. This may be due to the introgression of the Cascade strain with the European strains which likely had reduced diversity prior to or during importation into BC. This lack of genetic diversity would therefore likely result in low heritabilities of traits such as BKD resistance, however this would have to be examined and studied further.

Pacific salmon are more susceptible to BKD than Altantic salmon, and this is one reason why the BC aquaculture industry moved away from farming Pacific salmon and toward the Atlantic salmon in the late 1980's. There are few companies in BC currently farming Pacific salmon and these farmers have developed husbandry techniques (i.e., reduced density and handling) that appear to be successful in reducing BKD outbreaks. It would therefore appear that an effective approach to BKD control is to concentrate on health management, rather than attempting selective breeding programs described above.

McIntyre and Johnson (1977) recommend that caution be applied to selective breeding programs because of the need to maintain genetic variability required to meet the demands of a variable environment. This cautionary note, even applies to the current practice of culling broodstock with 'high' positive ELISA titres (ie. antigen loads). However, because this type of selection is applied only to females, there should be limited impact on genetic diversity. Recent research in the Columbia River (Hard et al. 2007) suggests that the process of culling females with high ELISA titres is an effective method to control BKD in the hatchery, by reducing the vertical transmission of the bacterium. The observed enhanced BKD resistance in the progeny, was based on survival of young hatchery-reared fish and therefore cannot be extrapolated to include seawater, commercially-reared fish. The body of evidence to date suggests that

broodstock culling is effective in reducing BKD in the hatchery. In enhancement facilities and especially in commercial aquaculture operations, broodstock culling is a sound and valid management practice from a financial perspective because by reducing BKD-related mortalities, economic loss will be reduced.

## Is breeding for BKD resistance feasible?

Breeding for resistance to BKD can be based on indirect or direct selection criteria. There are no known indirect criteria at this time. Molecular approaches to finding selection criteria, appear to be the most promising, and currently there is research on host gene expression associated with BKD resistance. However the identification of the appropriate genes/QTL correlated with BKD resistance, is likely many years away and even when (and if) implemented, the impact on the aquaculture industry is questionable due to the highly variable environment, pathogen prevalence and interactions of these factors in a production setting.

Selective breeding for BKD resistance based on the direct measure of survival may appear to be an efficient way to select for resistance, but survival is a very complex trait to use as a breeding goal. Fjalestad (1993) has stated that while this may be the most efficient method, there are numerous problems associated with this approach. Most of these problems are concerned with the inability to define the cause of the mortality (which could be attributed to such things as an accident, predator, disease, plankton, non-smolting, etc.) and control the environment (which is highly variable even within a single farm site). The cause of mortalities that occur in farmed fish should be determined as part of the fish health management plan. However, it is extremely difficult to say with certainty that the cause of each mortality was or was not due to BKD. Therefore collecting mortality/survival data for selection criteria is not a viable approach for breeding for BKD resistance.

There is also concern in collecting mortality data in farmed fish because infected fish require treatment if diseased. This treatment includes antibiotic therapy, even if the fish are deemed organic fish. In BC, the Pacific Organic Seafood Association (POSA) guidelines (POSA, 2004) state that in order to meet the animal health and welfare

requirements for organic farming, sick fish must be treated to minimise the harmful effects on the environment and animal health. In the case of BKD affected fish, the treatment would likely involve the oral administration of antibiotics because there is no natural treatment available. If the fish are treated with antibiotics, the farmers must either withdraw the fish from the 'organic' market, or use a withdrawal time of at least twice the time as required by farmers of fish for the conventional market.

With respect to the treatment of sick fish, the OIE at the 2004 global animal welfare conference, presented a paper (Halstein 2004) stating that like all other animals, the 'Five Freedoms' should also be applied to fish in an aquaculture situation. This includes "freedom from pain, injury and disease, and hence all efforts should be made to reduce disease in cultured fish". There has been controversy surrounding the issue of whether fish feel pain, but Chandroo et al. (2004) provide convincing evidence that fish do have the capacity to suffer and therefore the concept of reducing pain and suffering in fish should be considered in the capacity of animal welfare. A recent review paper by Huningford et al. (2006) includes how disease (along with reduced water quality, handling, density, etc.) can have adverse affects of fish welfare. The authors recommend that in the interest of maintaining optimal fish welfare conditions, disease should be prevented, rapidly diagnosed and treated when necessary. It is also possible that future international trade and sales of cultured fish will have to meet designated welfare standards, and is an important area of concern by the National Aquatic Animal Health Program (NAAHP).

With respect to BKD and fish welfare, there are recent reports that BKD infections can negatively impact the welfare of the affected fish, and if the affected fish is a sexually mature female, there can be negative impacts on her progeny. Mesa et al. (1998) demonstrated that the chronic progression of BKD in juvenile chinook salmon (particularly in the later stages of the disease) is associated with physiological changes indicative of a stressful event. This information coupled with the recent work by Eriksen et al. (2006) demonstrates that prespawning stress (as would occur in BKD infected fish), can adversely affect offspring survival, growth and incidence of malformations. These reports therefore indicate that BKD infections even at a subclinical level should be considered harmful and every attempt must be made to reduce infection levels.

It is important to make every effort to reduce BKD infection levels in cultured fish from not just a fish welfare perspective, but also from the perspective of reducing point sources of infection that could threaten wild fish. Cubitt et al. (2006) have examined the issue of the possible threat of disease transfer from farmed to wild fish, and concluded that there is a very remote chance of disease transfer, even in the event that diseased fish escape in to the open ocean environment. The controversial issue of disease transfer between wild and farmed fish will likely continue because of the difficulty in determining the direction of disease transfer. There are reports that support both sides of this contentious issue. Halstein and Lindstad (1991) have reported that BKD outbreaks on fish farms could threaten wild fish. However, it is generally considered that wild fish populations are a primary source of infectious agents and contribute to disease outbreaks in farmed fish (Olivier 2002). It is certain that to protect both wild and cultured populations of fish, BKD infection levels should be monitored and every effort made to reduce outbreaks or even chronic levels of infection.

# Recommendations on Best Management Practises to control BKD in farmed fish: Consideration of selective breeding to enhance resistance

In summary, it can be seen that BKD is a complex disease. The pathogenesis of the disease is influenced by genetic factors, however, there are also many other factors (likely many still unknown) that also influence the virulence of *Renibacterium salmoninarum*, host responses and ultimately the ability of a fish to resist the disease The intrinsic and extrinsic complexities of BKD resistance in farmed fish is further complicated by the endemic nature of the disease and high prevalence in some wild Pacific salmon populations. It is a daunting task to identify genetic traits that are clearly additive and heritable, and that are tightly linked to BKD resistance. It will likely require significant financial commitments and many years of research involving numerous generations of salmonids. It is therefore recommended that the BC salmon farming industry continue with current BKD control methods (broodstock selection programs, site fallowing, separation of year classes, prophylactic chemotherapy, etc.) rather than attempt to breed for BKD resistance. The lack of information regarding the mechanisms of pathogenesis, suggest that it is premature to breed for disease resistance based on indirect or direct selection criteria. Research should be encouraged to develop a better understanding of BKD resistance, and short term focus on the development of cost-effective and efficacious vaccines, standardisation of husbandry procedures and formulations of diets to enhance salmonid immune responses.

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