FALLOWING AS A TOOL FOR DISEASE MITIGATION IN MARINE FINFISH FACILITIES IN BRITISH COLUMBIA

Context

Fisheries and Oceans Canada's (DFO) Aquaculture Management Division (AMD) is the lead regulatory body for managing aquaculture in British Columbia (BC). The aquaculture management regime in BC is robust and complex, with oversight from provincial and other federal regulatory agencies. Licences are the primary tool used to manage this fishery, and are issued under the authority of the Pacific Aquaculture Regulations and the *Fisheries Act*. AMD licences marine finfish, marine shellfish, and land-based hatchery and production facilities, including approximately 111 marine finfish farms which are the focus of this review.

Currently, AMD requires farms to fallow prior to re-stocking a farm when benthic impacts are beyond acceptable levels to ensure seabed recovery. There is also an interest in determining if a fallow period based on the presence of pathogens or disease would be beneficial to reduce the risk of transmission between production cycles, in addition to other health management tools. This fallow period could be required by all farms as a precautionary approach, or could be performance based and only occur if active disease was present. To support AMD decision-making, a science-based understanding is needed to determine the factors that contribute to the benefits of fallowing in a BC context.

The use of farm-and area-level fallowing has been used in other countries as a strategy to reduce transmission of infectious pathogens between production cycles (Bron et al. 1993, Kilburn et al. 2012, McVicar 1987, Murray 2006; Rae 2002; Werkman et al. 2011, Wheatley et al. 1995). For example, Price et al. (2017) found that a 3-month farm-level fallowing was effective at significantly reducing the risk of Salmonid Rickettsial Septicaemia (SRS) between production cycles at farm sites in Chile. However, the effect of farm- and area-level fallowing has not been assessed for most pathogens that are endemic to BC waters. While fallowing is practiced voluntarily in BC, it is not regulated.

AMD has requested that the DFO Science Branch assess what is currently known about infectious pathogens found at BC Atlantic and Chinook salmon farms, in order to determine if fallowing can successfully minimize the transmission of pathogens between production cycles. Decreasing pathogens and disease on farms may also reduce the risk of transmission to wild salmon.

The assessment and advice arising from this Canadian Science Advisory Secretariat (CSAS) Science Response (SR) process will be used to inform AMD on the use of fallowing as an effective management tool to minimize the transmission of pathogens at finfish aquaculture farms in BC. The advice may be reflected in the conditions of license scheduled to be updated in 2022, and could inform area based management.

This Science Response results from the Regional Science Response Process of June 15, 2021 on Fallowing as a tool for disease mitigation in marine finfish facilities.

Background

As outlined in the Terms of Reference (ToR), the following will be reviewed and provide the basis for discussion and advice on the specific objectives outlined below.

1. For BC Atlantic and Chinook salmon farms, summarize what is known about the effectiveness of fallowing between production cycles to minimize transmission, considering the survivability and life cycle of the following infectious agents:
    - *Aeromonas salmonicida* (associated with Furunculosis)
    - *Piscirickettsia salmonis* (associated with Salmonid Rickettsial Septicaemia [SRS])
    - *Renibacterium salmoninarum* (associated with Bacterial Kidney Disease [BKD])
    - *Yersinia ruckeri* (associated with Enteric Red Mouth disease [ERM])
    - Piscine Orthoreovirus [PRV-1](associated with Heart and Skeletal Muscle Inflammation [HSMI] and Jaundice Syndrome)
    - *Moritella viscosa* (associated with Winter Ulcer)
    - *Tenacibaculum maritimum* (associated with Mouth Rot)
    - Viral Hemorrhagic Septicemia Virus (associated with Viral Hemorrhagic Septicemia [VHSv])
    - Infectious Hematopoietic Necrosis Virus (associated with Infectious Hematopoietic Necrosis (IHN))
    - Sea lice (*Lepeophtheirius salmonis*)
2. Provide information on the appropriate length of time required for the fallow to be successful for each agent.
3. Provide information on the controllable (e.g. farm practices) and uncontrollable (e.g. environmental conditions, host reservoirs) factors that will contribute to the effectiveness of the fallow.
4. Examine and identify uncertainties in the data and methods

The response considers the nine pathogens assessed for Discovery Island farms in response to Cohen 2012 recommendation #19 (1. *Aeromonas salmonicida*, 2. *Piscirickettsia salmonis*, 3. *Renibacterium salmoninarum*, 4. *Yersinia ruckeri*, 5. Piscine Orthoreovirus, 6. *Moritella viscosa,* 7. *Tenacibaculum maritimum*, 8. Viral Hemorrhagic Septicemia Virus, 9. Infectious Hematopoietic Necrosis Virus), as well as sea lice. Contributing factors to fallow success that can be controlled on the farm (e.g. net cleaning or removal) versus those that cannot be (e.g. water temperature or wild fish vectors) shall be identified.

Analysis and Response

In the scientific literature, as well as in provincial, national and international fish health regulations, the practice of fallowing marine open net pen sites between generations of fish is commonly identified as a preventative measure, that reduces the risk of transmission of infectious agents from one generation of fish to the next. In situations where coordinated fallowing of production sites within a particular geographical area occurs, fallowing is also commonly identified as method to significantly reduce the abundance of specific pathogens or disease within areas. Although it is logical to assume that fallowing will to some extent achieve the above stated goals there is very limited scientific evidence to assess the effectiveness of

fallowing as a health management strategy to minimize the risk of transmission of infectious agents between production cycles at sea cage sites.

With respect to the establishment of the duration of fallowing for sea cage sites it is generally not specified how decisions on the durations of fallowing found in regulations and licenses were arrived at and to what extent science-based evidence was used to inform those decisions. In many cases the regulations leave it up to the competent authority to determine the appropriate length of fallowing, which varies depending on the situation. Most regulations make reference to the use of risk-based decisions to determine whether fallowing should be used and if so how long the fallowing period should be.

As an example the Office International des Epizooties (OIE) states that “The length of the statutory fallowing period should be based on scientific evidence of the likelihood of a pathogenic agent remaining infective outside its aquaculture host(s) in the local environment, at a level likely to cause an unacceptable risk of re-infection of the aquaculture establishment. Account should be taken of the extent of the disease outbreak, local availability of alternative hosts, the survival and infectivity characteristics of the pathogenic agent and the local climatological, geographical and hydrographical factors. In addition, the level of risk to the local aquaculture industry and wider aquatic resources may be included. A scientifically based risk assessment approach should be used to determine the length of the fallowing period” (OIE 2021).

This document reviews what is known about the effectiveness of fallowing as a disease control method to reduce risk of transmission of infectious agents between production cycles in marine open net pen systems. Factors which need to be considered when establishing the length of fallowing periods and that contribute to the effectiveness of fallowing are also reviewed.

Review of the Effectiveness of Fallowing

Only a few studies have examined the effectiveness of fallowing as management practice to reduce transmission of infectious pathogens between production cycles in marine sea cage aquaculture. These studies are reviewed below.

Viral Diseases

Rodger and Mitchell (2007) conducted an epidemiological analysis of Pancreas Disease (PD) on Irish Salmon farms over a 2 year period. In their study fallowing was defined as the site being depopulated for at least 4 to 6 weeks. These authors reported an increased likelihood of PD occurring and of elevated mortality levels associated with fallowing. They proposed several reasons for these observations including: that sites which had recently undergone outbreaks of PD were more likely to have been fallowed, that factors other than breaking the disease cycle and reducing the accumulation of infective material are more important with respect to initiating PD outbreaks, and that the Pancreas Disease Virus may have been retained on adjacent farms which were not simultaneously fallowed.

Bacterial Diseases

Wheatley et al. (1995) examined associations between site management practices and mortalities on Irish salmon farms over a 34 year period. In their study fallowing was defined as removal of all stocks for at least 3 weeks prior to smolt introduction. These authors reported that total mortality was significantly higher in years where sites were: 1) not fallowed, 2) held multiple generations, 3) on site slaughtering was practiced and 4) when staff move between sites during their work shift. However they were unable to determine the individual effects of these practices on mortality rates as these practices often occurred together.

These authors also reported that fallowed years demonstrated significantly lower Vibriosis mortality than un-fallowed years and there was some evidence for a reduction in mortalities due to Pancreas Disease which was not significant. However there was no significant difference between fallowed and un-fallowed sites with respect to the mean numbers of occasions of antibiotic treatment and the mean number of isolations of *Vibrio* spp. and *Aeromonas salmonicida.* These authors offered several mechanisms for lower total mortality and Vibriosis mortality at fallowed sites which included: improvements in sedimentary conditions, a reduction in pathogen load on site or reductions in the need for chemotherapy for treatment of sea lice infestations.

Olivares and Marshall (Olivares and Marshall 2010) used real time polymerase chain reaction (PCR) to detect and quantify the amount of *Piscirickettsia salmonis* DNA (bacterial units) in seawater samples from affected farm sites. Water samples from the surface and 5 meters depth were taken every 10 days over a 40-day period at a depopulated farm located in an area of active disease in southern Chile. These authors reported that the number of bacterial units in farm water decreased to zero at day 50 leading the authors to report that a “fallowing period of 50 days after depopulation appears to be appropriate” under the conditions which occurred during their study.

In Chile SRS control strategies include coordinated stocking and a fallowing period of 3 months after harvesting in well-defined areas where coordinated health management activities are undertaken (Anonymous 2008, 2009).

Using company records Price et al. (Price et al. 2017) evaluated the effect of the duration of the fallow period on the hazard of piscirickettsiosis (SRS) during the first 24 weeks of the production cycle over a 7 year period in Chile. They also examined the hazard of SRS for production cycles on farms that did, and did not, report the disease immediately in the cycle which preceded fallowing. After controlling for external infectious pressure from neighboring farms these authors reported that there was no significant difference between the hazard of SRS for Atlantic salmon and rainbow trout farms that reported the disease in the previous cycle and the comparison group, when these farms fallowed for more than three months. Shorter fallow periods could only be assessed for rainbow trout farms and the trend in their data suggested fallowing fewer than 3 months was associated with a higher hazard of SRS. These authors concluded that “fallowing for 3 months is likely adequate to reduce the hazard of SRS from a carry-over effect of bacteria originating in the previous production cycle”.

Boerlage et al. (Boerlage et al. 2018) examined the risk factors associated with time to first clinical case of BKD in the Bay of Fundy, New Brunswick for the period of 2006 to 2012. In their study neither the duration of fallow period and/or presence of BKD in the previous site cycle had any detectable effect on BKD hazard. Wallace et al. (2011) examined the effectiveness of cage-level fallowing to break *Rennibacterium salmoninarum* infection cycles in freshwater trout production in Scotland. These authors suggested that eradication of infection using fallowing at a cage level is not feasible.

Murray et al. (Murray et al. 2012) examined the epidemiology of *R. salmoninarum* in Scotland and the factors required for control of BKD in salmon. With respect to marine rearing of Atlantic salmon these authors state that “fallowing at the farm-level has a mixed history, it appears to be successful on most salmon farms as BKD mostly does not recur after fallowing”. The variability in success they believe is related whether the farms are re-stocked with asymptomatic carriers of *R. salmoninarum* or not.

Parasitic Diseases

Douglas-Helders et al. (2004) examined the effectiveness of fallowing on mortality from amoebic gill disease (AGD) in Atlantic Salmon in Tasmania. Durations of fallowing ranged from 4 to 97 days and these authors reported that there was no significant differences in mortality rates after restocking of fallowed and un-fallowed sites.

Bron et al. (1993) examined the effectiveness of fallowing to control sea lice numbers on Atlantic salmon at 3 farm sites on the west coast of Scotland between 1990 and 1992. These authors reported that fallowing was effective in reducing the rate of infection of new fish when compared to a non-fallowed site. Since this initial work, fallowing has become an important component of sea lice (*Lepophtheirus salmonis* and to a lesser extent *Caligus* spp.) management and control in Norway, Scotland, Ireland and New Brunswick (DFO 2014). Fallowing is most effective when applied in a coordinated fashion at all sites which are hydro-dynamically connected and in areas where wild reservoir hosts are not abundant. The duration of fallowing periods at the end of production, range from 4 weeks in Scotland to at least 2 months in Norway (reviewed in Sitjà-Bobadilla and Oidtmann 2017). However, it is important to recognize that these fallow periods are not set specifically for the control of sea lice but are set to control all diseases between one production cycle to the next. Fallowing has been shown to reduce densities of free-living sea lice stages as well as reduce infection pressures on wild salmonids in the north Atlantic (Jevne et al. 2021 and references therein, Murray and Salama 2016 and references therein).

Modelling Studies

Werkman et al. (2011) modelled the effects of different fallowing strategies on disease transmission of infectious pancreatic necrosis virus and pancreas disease virus within management areas in Scotland. These authors predicted that synchronized fallowing would not prevent epidemics even when transmission rate of the virus is low ($\beta = 0.10$) if long-distance contacts (directed movements both between and within management areas) between farms are high. Reducing the number of long-distance contacts improve the chance that synchronized fallowing will contribute positively to the control of outbreaks even at higher rates of virus transmission.

Groner et al. (2016b) reviewed and synthesized the mathematical and statistical models that have been developed to study the epidemiology of sea lice and salmon. These authors noted that a strategy of coordinated fallowing among farms at spatial and temporal scales chosen to break infection cycling among neighboring farms along with coordination of treatments and agreements on treatment thresholds all help to regulate sea lice abundance at regional levels. The duration of fallowing was not examined.

The following analysis is provided for each of the TOR objectives.

Objective 1 : For BC Atlantic and Chinook salmon farms, summarize what is known about the effectiveness of fallowing between production cycles to minimize transmission, considering the survivability and life cycle of the following infectious agents

Although fallowing for disease control between production cycles has been practiced by BC salmon farming companies there are no publically available data on the effectiveness of fallowing.

In the case of Bacterial Kidney Disease (BKD) fallowing along with other health management practices and veterinary interventions was identified by BC Salmon Farmers in 2010 as being used to control this disease (Anonymous 2010).

The BC salmon farming industry also references fallowing in their Salmonid Health Management Plan (SHMP) Standard Operating Procedures (SOP) where it is used in situations where large pieces of infrastructure, which are too large to disinfect, are being moved between sites. The SOP states that these structures must be cleaned and fallowed for a minimum of two weeks before moving to the new location (Wade 2017).

Fallowing is also referenced in the Viral Management Plan 2011 which is primarily focused on the control of IHN. The plan states that “Biosecurity standards should include, amongst others, recommendations for low stress husbandry practices, vaccination of smolts in common areas which are by definition high risks areas, and minimum fallow periods between cycles as indicated by the site.” (Wade 2017).

As part of the Viral Disease Outbreak Management Plan companies have agreed that positive sites will remain fallow for a minimum of three months or one month after release from Canadian Food Inspection Agency (CFIA)-imposed quarantine, whichever is longer. (Wade 2017).

Objective 2: Information on the appropriate length of time required for the fallow to be successful for each agent

With the exception of sea lice there are insufficient data/information to determine based on scientific evidence whether fallowing is an effective health management practice and if so what is the appropriate length of time of fallow to protect sites from reinfection with the agents identified in this Science Response.

Sea lice: Fallowing for sea lice is generally not applied on a single farm basis or used to control sea lice species other than *Lepeophtheirus salmonis*. In the New Brunswick, Ireland, Scotland and Norway the use of area management with synchronized production and coordinated fallowing and sea lice treatments successfully reduces population growth in the first year thereby limiting the need for sea lice treatments, as well as reduces the risk of *L. salmonis* from salmon farms impacting wild stocks (reviewed in DFO 2014, Sitjà-Bobadilla and Oidtmann 2017).

The duration of fallowing to prevent reinfection of a site with *L. salmonis* originating from the site, or to apply in the case of coordinated fallowing, can be calculated using the time of development from egg to infectious copepodid stage, plus the duration that the copepodid stage remains viable/infectious without a host (infective window). The duration of fallowing could be adjusted based on water temperatures with longer periods of fallowing required during colder times of the year when development rates are slower and copepodid survival in the absence of hosts is longer (Table 1) (Groner et al. 2016a, Samsing et al. 2018).

Establishing a fallow period for *Caligus clemensii* is likely to be of limited benefit as this species is very common on non-salmonid fish that associate with sea cage sites and in addition to the copepodid stage infects hosts as preadults and adults. Further, development times for *C. clemensii* and the duration of copepodid, preadult and adult survival off hosts are not known.

Fallowing for *Caligus elongatus* in the North Atlantic is generally not effective due to the presence of large numbers of non-salmonids hosts for this species (Sitjà-Bobadilla & Oidtmann 2017).

Table 1. Fallowing period to prevent reinfection of a site with Lepeophtheirus salmonis originating from the site. The minimum fallowing period is the time in days from egg string extrusion to end of the infective window for the copepodid stage. Data are from laboratory studies conducted by Samsing et al. (Samsing et al. 2018).

Temperature °C	Average time before hatching (days)	Average duration of salmon lice naupliar stages I and II (days)	Copepodid infective window (days)	**Fallowing period (days)**
5	13.0	11.5	10.2	**34.7**
7	7.6	7.0	12.7	**28.7**
10	4.6	3.8	13.2	**21.6**
15	2.9	2.2	9.7	**14.8**
20	1.8	1.7	6.7	**10.2**

Objective 3: Provide information on the uncontrollable (e.g. environmental conditions, host reservoirs) and controllable (e.g. farm practices) factors that will contribute to the effectiveness of the fallow

Uncontrollable - Environmental Conditions

The effectiveness of the fallow is dependent on it being of long enough duration to ensure that infectious agents originating from the site are not present (or are present only at low abundance) when the site is restocked. The length of time required for fallowing depends on the infectious agent and conditions in the environment. Physio-chemical and biological factors which affect the capacity of pathogenic agents to survive and remain infectious outside of their host/s have been investigated under laboratory conditions. These are described below.

Water Column

Temperature: With respect to survival in the water column the effects of temperature have been most commonly studied with temperature recognized as having major effects on rates of inactivation and infectivity of viral and bacterial agents. Across all studies it has been demonstrated that survival is reduced in both of these groups at higher temperatures (Table 1). For example, the average time for a 3-log reduction in VHSv infectivity in natural seawater (31 ppt) held in the dark at 4, 10, 15 and 20°C was 12, 7, 4 and 2 d, respectively (Hawley and Garver 2008).

Table 2. Effects of temperature and salinity on survival of the viral and bacterial agents of concern in natural (non-sterile) waters under laboratory conditions.

Viral

Agent	**Description**	**References**
Infectious Hematopoietic Necrosis Virus	High concentrations (106 to 107 PFU) of IHNv are rapidly inactivated in natural seawater (8-12oC, 31ppt) in < 6 days under conditions of darkness.	(Garver and Wade 2017, Garver et al. 2013)
Piscine Orthoreovirus-1	The extent to which PRV-1 can survive and remain infectious in the natural seawater is unknown.	-

Agent	Description	References
Viral Hemorrhagic Septicemia Virus	In natural seawater (31 ppt) 99.9% of VHSv was rendered inactive at 13 days @ 4ºC, 3.0 d @ 15°C and by 1.5 d @ 20°C under conditions of darkness. The average time for a 3-log reduction in VHSv infectivity at 4, 10, 15 and 20°C was 12, 7, 4 and 2 d, respectively. Remains infectious longer in freshwater than in seawater with 99.9% of VHSv rendered inactive by 3 d in seawater at 15°C versus 11 d in freshwater.	(Garver and Hawley 2021, Hawley and Garver 2008)

Bacterial

Agent	Description	References
Aeromonas salmonicida	Survives from 2 – 26 days (median = 6 days) @ 11 to 15ºC at salinities of 25 – 35 ppt. These values are from only those studies which used culture-based techniques.	(Boily et al. 2019)
Moritella viscosa	Survives and proliferates in an oligotrophic environment similar to marine water. Cell growth reaches and maintains higher densities for a longer period at 4º when compared to 15ºC. Poor stability at 15ºC is thought to be why infections are not seen at this temperature. Cell growth and survival was higher at 30-40 ppt when compared to 10-15 ppt.	(Wade and Weber 2020)
Piscirickettsia salmonis	In natural seawater at 20°C viable bacteria were not detected after one week, whereas at 5 and 10°C viable bacteria were detected after 3 weeks. Survival of *P. salmonis* in natural seawater over a period of 10 to 15 days was equal to or greater than that in tissue culture medium at 5°, 10° or 15°C. In a field based study conducted in Chile *P. salmonis* DNA was undetectable in the water column after 50 days when cages were empty. Temperature and salinity were not specified. These results led Olivares and Marshall (2010) to recommend a fallow period of 40–50 days for sea farms before restocking.	(Jones 2019) (Olivares and Marshall 2010)
Renibacterium salmoninarum	Viable cells could be recovered for up to seven days in natural (non-sterile) seawater @ 10ºC and 22‰.	(Rhodes and Mimeault 2019)
Tenacibaculum maritimum	Naturally occurs in seawater in BC. Survival in seawater has not been examined.	(Levipan et al. 2019, Nowlan et al. 2021, Shea et al. 2020, Wynne et al. 2020)
Yersinia ruckeri	In un-supplemented natural water incubated in the dark at 8-10ºC there were no detectable changes in colony forming units during the first three days and only a small decrease over the next four months at low salinities (0-20 ppt.). Under the same conditions, the survival was decreased below detection limits (3 CFU mL^{-1}) after 32 days in 35 ppt. water.	(Thorsen et al. 1992)

Salinity: The impact of salinity has received less attention with few studies comparing survival across a range of salinities. Pathogens such as IHNv, VHSv and *Y. ruckeri*, are reported to have higher levels of survival over time at low when compared to high salinities (Garver and Hawley 2021, Hawley and Garver 2008, Pietsch et al. 1977). In the case of *L. salmonis,* survival is greatly reduced at salinity of < 20 ppt (Groner et al. 2016a).

Organic Material: The presence of organic material in seawater increases virus and bacterial survival. For example, Kocan et al. (2001) reported that the presence of ovarian fluids in natural seawater, such as would occur during spawning events, significantly increases survival of VHSv.

Solar Radiation: It has been demonstrated that IHNv is very sensitive to exposure to solar radiation with infectious virus concentrations reduced by six orders of magnitude compared to those of dark controls over a 3 hour experiment (Garver et al. 2013). It is known that VHSv is sensitive to UV light and it is expected that VHSv may have a similar sensitivity to solar radiation exposure as IHNv (Garver and Hawley 2021). There are no data for the other agents of interest to this Science Response.

Microbial Community: It is well recognized that the presence of microbial biota effects both viral and bacterial survival with survival times reduced in natural fresh and seawater when compared to sterile waters. For example IHNv and VHSv can remain infectious for up to a year at 4^{o}C in sterile waters in the dark (Garver and Hawley 2021, Garver and Wade 2017, Hawley and Garver 2008). However, it is not known to what extent seasonal or other changes in the composition and abundance of microbes in seawater would effect viral and bacterial survival rates in natural conditions.

Sediments

Sediments are considered an important environmental reservoir for infectious agents arising from sea-caged farmed salmon. Of the viral and bacterial agents of interest for this Science Response only *A. salmonicida*, *R. salmoninarum*, *T. maritimum* and *Y. ruckeri* have been reported as being found in estuarine/marine sediments. However with exception of *A. salmonicida*, for which there is a single study, there are no data for survival in marine sediments. In the case of *A. salmonicida* survival is reduced in non-sterile sediments (sand, mud) (11 days) when compared to sterile sediments (>22 days) at 15^{o}C and 25 ppt. (Effendi and Austin 1994).

The importance of sediments as a reservoir of infectious agents will ultimately depend on whether agents deposited to the sediments will undergo resuspension into the water column at a concentration which is sufficient to establish a new infection.

Fomites

Fomites are defined as objects or materials which can retain and transmit infectious agents. With respect to net-pen culture systems such objects include the physical objects which make up the structure of the site (e.g. cage structures, floats, ropes etc.), equipment (e.g. boats, equipment for fish handling etc.), as well as organisms found living on or in association with the site.

Virus

There are no reports of PRV-1 being found in association with fomites. In the case of VHSv Pham *et al*. (2012) examined the survival on various substrates in freshwater under laboratory conditions, VHSv was able to be retained and remain infectious on plastic, metal and glass objects in fresh water for various amounts of time. This suggest that fomites in marine waters

have the potential to retain VHSv but the extent to which this occurs and the duration that VHSv would remain viable of such substrates in not known.

Joiner et al. (2020). examined the survival (recoverability) of IHNv and VHSv at 4 – 25°C after drying on stainless steel discs. They reported that survival was inversely related to temperature with longest survival at 4°C. Over this range of temperatures these viruses became undetectable between 6 weeks and > 7 weeks for VHSv, and 3 days to > 8 weeks for IHNv depending on the strain tested.

Vennerström et al. (2020) examined the distributions of VHSv in brackish water environmental compartments. These authors report that although traces of VHSv were found in blue mussels collected from a site with active disease outbreak it doesn't appear to replicate in blue mussels suggesting that they are not an important carrier of VHSv.

Bacteria

Yersinia ruckeri adheres to hard surfaces and readily forms biofilms in freshwater (Coquet et al. 2002a, Coquet et al. 2002b) and biofilms have been suggested to be the source of recurrent infections in freshwater rainbow trout farms (Tobback et al. 2007). Whether *Y. ruckeri* forms biofilms on surfaces in marine waters is not known.

Tenacibaculum maritimum is adhesive and can therefore create biofilms on hard surfaces in seawater. Levipan et al. (2019) examined the kinetics and stages of biofilm formation in T. maritimum. They confirmed the ability of *T. maritimum* to rapidly develop profuse biofilms consisting of dead and live cells on polystyrene *in vitro*. They suggest that such biofilms could serve as transient reservoirs of *T. maritimum* which once detached could trigger new disease outbreaks.

Nowlan et al. (2021) examined the occurrence and distribution of *T. maritimum* and *Tenacibaculum dicentrarchi* at BC Atlantic salmon farms during disease outbreaks using quantitative PCR. In addition to being found on fish and in water samples both species were also found on farm infrastructure and fouling organisms. These authors provide evidence that these species can be retained on farm infrastructure after disease has resolved.

It has been suggested that the tendency of *P. salmonis* to form biofilms in marine environments may enhance its survival (Marshall et al. 2012). However examination of macro and micro algae, zooplankton, farm infrastructure, and animals associated with infected farm sites in Chile failed to detect *P. salmonis* (Olivares and Marshall 2010). This led these authors to suggest that fomites are not an important source of *P. salmonis* for re-infection of marine salmon farms.

One study has examined the survival of *A. salmonicida* on sediments (including wood fragments), macroalgae and invertebrates under laboratory conditions (Effendi and Austin 1994). In the presence of other aquatic micro-organisms (non-sterile system) at an unspecified temperature, *A. salmonicida* demonstrated an initial increase in numbers within 1 — 3 days on sediment particles, followed by a progressive decline. Colony-forming units were recoverable over a period of <10 days from the surfaces of the seaweeds *Ascophyllum nodosum* and *Fucus vesiculosus*, but for >10 days from mud, sand and wood fragments. Wood fragments supported the longest survival with colony-forming units recoverable over a period of 2 weeks. With 1 exception, colony-forming units were not recovered from invertebrates after 2 days (Effendi and Austin 1994).

With the exception of the occurrence of *R. salmoninarum* in feces from infected fish which can serve as a route of horizontal transfer between individuals (Balfry et al. 1996) no other associations with fomites have been described.

There is no information on the capacity of *M. viscosa* to form biofilms and/or associations with fomites.

Factors that require consideration when establishing fallowing periods and that will contribute to the success of fallowing

For the purpose of this document “infectious dose” is defined as the amount of pathogen required to cause infection under environmental conditions favorable to the host and “minimum lethal dose” as the smallest number of pathogens required to establish an infection that results in disease and death of any individual within a population. Unfortunately, for the majority of the viral and bacterial agents of interest for this science response we do not know what their infectious dose or minimum lethal dose are. For this reason we do not know what levels of infectious agents we need to achieve at the end of the fallow period to reduce risk of re-infection of the site to an acceptable level.

In the few cases where laboratory challenge trials have been conducted these data must be used with caution as it is difficult to extrapolate their results to potential outcomes in the field due to the inherent variability of natural systems, ranging from the strain of fish, strain of pathogen, the effects of handling, fish rearing and environmental conditions at the farm.

The abundance and retention of pathogenic agents in the various environmental compartments at the start of the fallowing period should be considered when establishing the duration of fallowing. For example, in situations where there has been active disease and therefore a higher number of infectious agents shed into the environment, a longer duration of fallow may be advisable and required to reduce pathogen levels below the level required to establish infections in newly introduced fish. The current fallowing periods which are used are not science based, they are more of an adaptive management approach where things are tried and if they seem to work they are continued.

Patterns of water movement and the amount of water which is exchanged during the fallow period will control the abundance of infectious agents (including sea lice) retained on site in the water column. It is likely that for most sites which have high rates of water exchange that few if any pathogens originating from the site which have been shed into the water will be retained on site.

Infectious agents are more likely to be retained on site in association with sediments and fomites (e.g. farm infrastructure). As described previously, all of the species of concern with exception of PRV-1 and *M. viscosa* are known to associate with sediments and/or fomites. However, the conditions under which, and to what extent these agents are available to re-infect the site is not known.

Fallowing is likely to not be effective or even necessary for those infectious agents such as *M. viscosa* and *T. maritimum* which are present as members of the bacterio-plankton community or those which are present in wild reservoirs (e.g. VHSv in herring) which reside in the vicinity of the site. In the case of sea lice, a requirement to fallowing for *Caligus clemensii* is likely not appropriate due to their presence on non-salmonids hosts throughout the year, as well as their ability to infect new hosts as preadults and adults in addition to the copepodid stage.

Controllable - Farm Practices

In addition to having a satisfactory length of time for fallowing for the agent/s of concern the success of fallowing at a site is dependent on there being appropriate geographical separation between sites and/or synchronous fallowing of adjacent sites. Development of agreements

between farms to synchronously fallow have provided benefits in the control of agents such as sea lice and Infectious Salmon Anemia Virus (ISAv) (Sitjà-Bobadilla and Oidtmann 2017).

Health management practices which reduce the incidence of disease on farms throughout the production cycle will limit the shedding of infectious agents into the environment thereby reduce the need for, or the duration of fallowing.

The risk of disease occurring following fallowing will be reduced if the site is restocked with genetically resistant stocks or vaccinated smolts. In situations where the fish to be restocked are vaccinated against the agent/s of concern fallowing may not be necessary. Regardless of how effective fallowing is the use of stocks which are genetical resistant or vaccinated against the agent of concern will further limit whatever risk there is of transmission of that agent between production cycles. The source of the agent (nets sediments, wild fish etc.) will not matter. For vaccines which have a high effectiveness there may be no need to fallow regardless of what the source of infection is, especially as the numbers of pathogens retained in the environment will be low.

It is necessary to remove all susceptible species for the disease/s of concern from the site prior to fallowing. In BC where the majority of sites are single year class this isn't a factor to consider.

Removal of contaminated materials (e.g. net removal) and cleaning and disinfection of sites prior to fallowing may reduce the need for and/or reduce the duration of the fallowing period. Most regulations identify net removal and site disinfection as a requirement before the start of a fallow period.

Objective 4: Examine and identify uncertainties in the data and methods

There is limited standardization with respect to the best process to follow when establishing a fallowing period to prevent re-infection of sites. As noted previously the OIE recommends that

"The length of the statutory fallowing period should be based on scientific evidence of the likelihood of a pathogenic agent remaining infective outside its aquaculture host(s) in the local environment, at a level likely to cause an unacceptable risk of re-infection of the aquaculture establishment. Account should be taken of the extent of the disease outbreak, local availability of alternative hosts, the survival and infectivity characteristics of the pathogenic agent and the local climatological, geographical and hydrographical factors. In addition, the level of risk to the local aquaculture industry and wider aquatic resources may be included. A scientifically based risk assessment approach should be used to determine the length of the fallowing period" (OIE 2021). To use this approach there needs to be agreement on what is an "unacceptable risk of re-infection" as well as recognition that the duration of fallowing will be highly influenced by the species of infectious agent and features of the site and local environment.

There are no standardized procedures to establish a fallowing period that would work for all agents of concern across a broad geographical area. As identified above, the length of fallow periods and fallowing success is highly dependent on the characteristics of the agent, environment conditions, the site (geographical and hydrographic features) and farm management practices which interact in complex and unpredictable ways. For this reason many regulations specify that decisions whether fallowing occurs and the duration of the fallow is the responsibility of veterinary/regulatory authority and is made on a case by case basis. In other cases fallowing times which were originally set for the control of specific agents of high concern (e.g. ISAv) are specified in regulations.

Pathogenic agents from sea cage sites are released into the water column through shedding or upon death and decomposition of the host. A proportion of these agents may be deposited in sediments, become associated with fomites (farm structures and associated organisms), and/or

establish infections in alternative host species. There is evidence that the relative importance of these reservoirs as potential sources of re-infection varies for the infectious agents we are considering. However, there are limited data to predict:

1. the duration that infectious agents survive and the viability of infectious agents in these reservoirs.
2. the risk that pathogenic agents will be released from these reservoirs at a level sufficient to establish an unacceptable risk of infection.
3. how effective disinfection procedures are with respect to removing the targeted infectious agent/s from farm structures.

A number of laboratory studies have examined the potential of infectious agents to survive in these reservoirs, as well as describe some of the physical, chemical and biological factors which influence their survival. These studies provide information on the duration over which infectious agents could be recovered by culture or detected by molecular methods and for some agents estimates of rates of decline. It is important to remember: 1) that these studies used different techniques and measured different aspects of survival, 2) results based on culture and molecular methods are not always in agreement when applied to the same samples, and 3) that detection by some of the methods is not a good predictor of cell viability/infectivity.

For the majority of the infectious agents of concern the dose and duration of exposure required to establish infections in Atlantic and Chinook salmon is unknown. In addition we do not know to what extent unfavorable environmental and/or husbandry conditions will have an effect on susceptibility.

Lack of knowledge in all of these areas limits our ability to establish fallowing periods based on science evidence as well as to predict whether a particular fallowing strategy will work.

Conclusions

It is not possible to establish, based on scientific evidence, a single fallow period suitable for all of the viral and bacterial agents of concern.

For viral and bacterial agents which are common or endemic to the surrounding environment (e.g. *M. viscosa*, *T. maritimum*), vertically transmitted (e.g. *R. salmoninarum*) and/or that originate from non-farmed sources (IHNv (source wild salmon), VHSv and *C. clemensi* (source non-salmonid hosts) fallowing to prevent infection between cycles is likely to be of limited benefit.

For viral and bacterial agents and sea lice which are broadly distributed across hydro dynamically linked farm sites the success of single site fallowing is likely to be limited in the absence of synchronous fallowing of all sites.

For those viral and bacterial agents which are shed into the water column, but not found in other reservoirs, a fallowing period to minimize the transmission of pathogens between production cycles may not be necessary. Under most circumstances such agents would be rapidly dispersed from the site by water currents.

Fallowing to minimize the transmission of pathogens between production cycles for those viral and bacterial pathogens which form associations with farm structures (fomites) may be beneficial, especially for those for which effective vaccines are not available.

A fallowing period after a production cycle is not necessary if there has been no active disease in that time period. However, in situations where there has been active disease, a fallowing

period along with other management actions (e.g. site disinfection) may be appropriate, based on the pathogen.

For the reportable agents such as IHNv, VHSv the decision of whether to fallow prior to restocking rests with the Canadian Food Inspection Agency. Setting conditions of license related to fallowing for agents over which the CFIA has jurisdiction would need to be discussed.

For non-reportable agents the requirements for fallowing should be considered on a case by case basis with any decision on fallowing and/or management actions made following review of the particular circumstances by regulatory authorities.

Fallowing times for *L. salmonis* can be based on water temperature with longer periods of fallowing needed at lower temperatures. For farms sites where salinities are <20 ppt. there is no need to fallow for *L. salmonis* as survival of the free living stages are poor in low salinity water. Setting up a fallowing for *Caligus* spp. makes little sense due to the large number of non-salmonid hosts which reside in the vicinity of farm sites.

Table 3. Summary of potential fallowing options.

Agent *	Fallow recommended	Disinfection needed	Coordination required	Minimum fallow length
Infectious Hematopoietic Necrosis Virus	No (CFIA responsibility)	-	-	-
Piscine Orthoreovirus	No (endemic)	-	-	-
Viral Hemorrhagic Septicemia Virus	No (CFIA responsibility)	-	-	-
Aeromonas salmonicida	No (effective vaccine**)	-	-	-
Moritella viscosa	No (endemic)	-	-	-
Piscirickettsia salmonis	Yes	Yes	No (limited evidence of far field effects)	21 days (provisionally)
Renibacterium salmoninarum	No (vertical transmission)	-	-	-
Tenacibaculum maritimum	No (endemic)	-	-	-
Yersinia ruckeri	No (limited horizontal transmission)	-	-	-
Lepeophtheirus salmonis	Yes	No	Yes (area TBD)	Temperature-dependent (worst-case scenario?)

* Assuming presence of agent at time of depopulation
** Against the typical strain

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