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National Capital Region

Canadian Science Advisory Secretariat Science Response 2022/045

ASSOCIATION BETWEEN SEA LICE FROM ATLANTIC SALMON FARMS AND SEA LICE INFESTATIONS ON WILD JUVENILE PACIFIC SALMON IN BRITISH COLUMBIA

Context

Fisheries and Oceans Canada (DFO), under the Sustainable Aquaculture Program, is committed to deliver science-based decision making related to sustainable aguaculture activities. In British Columbia (BC), DFO Aquaculture Management Division (AMD) is the regulatory body for managing aguaculture. Under the authority of the Fisheries Act and the Pacific Aquaculture Regulations, DFO issues marine finfish aquaculture licences that authorizes the licence holder to carry out aquaculture activities under prescribed conditions.

Sea lice management is one of the finfish conditions of licence with prescribed monitoring windows and frequencies, regulatory response thresholds, and reporting requirements (DFO, 2022b). AMD requested that DFO Aquaculture Science provide science advice to inform the development and application of adaptive management approaches to address interactions between sea lice infestations on farmed Atlantic Salmon (Salmo salar) and wild Pacific Salmon populations in BC. While there are several species of sea lice, the focus of this request for advice was Lepeophtheirus salmonis.

This science advice is expected to inform DFO's management of sea lice on Atlantic Salmon farms. This science advice will address the following objectives:

- 1. Estimate the number of *Lepeophtheirus salmonis* copepodids (infective sea lice larval stage) produced by Atlantic Salmon farms under current farm management practices;
- 2. Summarize counts of *Lepeophtheirus salmonis* on wild juvenile Pacific Salmon in BC; and
- 3. Determine the statistical strength of association between sea lice infestation pressure on Atlantic Salmon farms and sea lice prevalence on wild juvenile Pacific Salmon populations in BC.

This Science Response Report results from the National Peer Review process on the Association between sea lice from Atlantic Salmon farms and sea lice infestation on juvenile wild Pacific Salmon in British Columbia held on June 24, 2022.

Background

Sea lice are naturally occurring copepods in the genera Lepeophtheirus and Caligus that are parasites of marine fishes. While hundreds of species of sea lice occur over a broad geographic distribution and range of host species, the most common species reported on salmonids in British Columbia (BC) are Lepeophtheirus salmonis and Caligus clemensi. Of these two species, *L. salmonis* is larger and more commonly responsible for host damage caused by attachment and feeding activities (Johnson et al., 2004) and therefore the focus of management.



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The role of farmed salmon, particularly farmed Atlantic Salmon, as potential reservoirs of *L. salmonis* is accepted (Saksida et al., 2015). What is still debated is the effect of sea lice infestations on wild salmon populations, and the importance of the contribution of *L. salmonis* originating from farms to the overall sea lice burdens observed on wild salmon (Marty et al., 2010; Saksida et al., 2011; Saksida et al., 2015; Shephard and Gargan, 2017).

Life cycle of Lepeophtheirus salmonis

The life cycle of *Lepeophtheirus salmonis* has been well described (Hamre et al., 2013). There are eight developmental stages, each of which is separated by a moult. Egg strings, which are carried by the adult female, release first stage nauplii, which moult to second stage nauplii, then to copepodids, which are infective to the fish host. The naupliar stages and the copepodid stage, prior to attachment to a host, are free swimming, non-feeding and disperse in the water column. Temperature and salinity are the major environmental factors which regulate egg development and hatching rates, as well as the development rates and survival of the free-swimming stages.

Following settlement and attachment (infestation) to a host and a period of feeding, the copepodid moults to the first of two chalimus stages both of which are physically attached to the host by the frontal filament. The second chalimus stage moults to the first of two preadult stages of which the second preadult stage moults to the reproductive adult stage. The preadult and adult stages are considered motile as they lack a frontal filament and are not restricted to one site on the host.

Effects of temperature

Water temperature is the primary regulator of egg and larval developmental rates and the duration, prior to host attachment, over which copepodids survive and are infective (Table 1).

The naupliar and copepodid stages of *L. salmonis* are non-feeding and rely upon finite endogenous yolk (energy) reserves to support their development and survival. With respect to the copepodid stage, the rate of depletion of energy reserves is increased at higher temperatures which means that they are infective for a shorter period of time at high versus lower temperatures (Table 1).

Table 1. The effects of temperature on the duration of the free-swimming stages of Lepeophtheirus salmonis and the duration over which copepodids are infective. Data are from laboratory studies conducted at a salinity of 34 ppt (Samsing et al., 2016, 2018).

Water temperature (°C)	Average time before hatching (days)	Average duration of sea lice naupliar stages I and II (days)	Copepodid infective window (days)	Dispersal time (days)
5	13.0	11.5	10.2	21.7
7	7.6	7.0	12.7	19.7
10	4.6	3.8	13.2	17.0
15	2.9	2.2	9.7	11.9
20	1.8	1.7	6.7	8.4

The dispersal of sea lice in the water column occurs primarily during the free-swimming naupliar and copepodid stages. Temperature controls the rate of development through these stages, as well as the duration of time copepodids can survive off a host, with lower temperatures slowing rates of development and extending the survival time of copepodids. For this reason, the period

of time over which dispersal can occur is much higher at lower temperatures (approx. 20 days @ 7°C) versus higher temperatures (approx. 8 days @ 20°C) (Samsing et al., 2016, 2018) (Table 1). Lower temperatures delay the development of sea lice (Johnson and Albright, 1991; Samsing et al., 2016; Hamre et al., 2019; Skern-Mauritzen et al., 2020), hence the longer it takes to advance to the next stage and consequently the further the sea lice can travel through water currents prior to developing to the infective copepodid stage and infesting a host (Samsing et al., 2016; Hamre et al., 2019).

Effects of salinity

Salinity has a non-linear effect on *L. salmonis* egg development and hatching success, development and survival of naupliar and copepodid stages, and the capacity of copepodids to attach to and establish themselves on hosts (Tucker et al., 2000; Bricknell et al., 2006; Groner et al., 2016).

Johnson and Albright (1991) reported no egg development at 10 ppt salinity and that eggs developed but failed to produce active nauplii at 15 ppt. At salinities of 20–30 ppt, active nauplii were produced, but copepodids were only obtained at 30 ppt.

Copepodids survived for less than one day in waters with a salinity of 10 ppt or less. At salinities of 15–30 ppt and temperatures of 5,10, and 15°C average survival times ranged between two and eight days (Johnson and Albright, 1991). Bricknell et al. (2006) reported that the survival of free-swimming *L. salmonis* copepodids did not differ significantly with salinity between 29 and 36 ppt. Below 29 ppt, survival decreased with decreasing salinity with very low rates of survival, with mortality of >50% within 1 h of exposure to salinities <16 ppt.

Finally, salinity also affects infestation success. Decreased salinity has been shown to reduce the attachment of copepodids to salmonid hosts, some of which is likely due to morbidity at lower salinities (Tucker et al., 2000; Bricknell et al., 2006).

Sea lice management on farms in British Columbia

Sea lice management in British Columbia focuses on *Lepeophtheirus salmonis* (Jones and Johnson, 2015). Conditions of licence have been established to reduce the risk of infestation of juvenile Pacific Salmon with *L. salmonis* originating from salmon farms in BC.

In BC, marine Atlantic Salmon aquaculture licence holders must manage sea lice according to the timing of wild juvenile Pacific Salmon migration: non-migration window (July 1 to January 31), pre-migration window (February 1 to February 29), and out-migration window (March 1 to June 30).

During the out-migration window, current licence conditions require that average counts of more than three motiles *L. salmonis* per fish be reported to DFO and licence holders must take measures to reduce sea lice levels below this threshold (DFO, 2022b). This threshold is applied regardless of farm inventory.

British Columbia's coastal waters are broadly divided into Fish Health Surveillance Zones (FHSZ) based loosely on watershed boundaries (Figure 1). Zones 2-3 and 2-4 represent the west coast of Vancouver Island and those within Zones 3-1 to 3-5 represent the east coast of Vancouver Island or the mainland coast from the Fraser River north to the Alaska border.



Figure 1. Fish Health Surveillance Zones (FHSZ) in British Columbia. FHSZ descriptions: 2.1: Southeast Vancouver Island, 2.2: Northeast Vancouver Island, 2.3: Southwest Vancouver Island, 2.4: Northwest Vancouver Island, 3.1: Sunshine Coast, 3.2: Discovery Islands, 3.3: Broughton Archipelago, 3.4: Queen Charlotte Strait, and 3.5: Central Coast. Source: <u>DFO, Aquaculture Management, Pacific Region</u>.

Analysis and Response

This section addresses the main objectives of this advice: (1) estimate the number of *Lepeophtheirus salmonis* copepodids (infective sea lice larval stage) produced by Atlantic Salmon farms in British Columbia; (2) summarize counts of sea lice numbers on wild juvenile Pacific Salmon; and (3) determine the statistical strength of association in between sea lice infestations on Atlantic Salmon farms and prevalence on wild juvenile Pacific Salmon populations in British Columbia.

Estimates of number of copepodids produced by Atlantic Salmon farms in British Columbia

The estimation of the number of infective *L. salmonis* copepodids produced by Atlantic Salmon farms during the period of juvenile Pacific Salmon out-migration under current farm management practices was achieved in two steps:

- 1. estimating the total number of adult *L. salmonis* (ovigerous and non-ovigerous) females on Atlantic Salmon farms in each FHSZ (Appendix A); and
- 2. estimating the total number of copepodids derived from those adult *L. salmonis* females based on published peer-reviewed modeling approaches (Appendix B) and considering environmental conditions on Atlantic Salmon farms (see Appendix C).

Data sources

In BC, active facilities must conduct sea lice monitoring following prescribed protocols and frequency based on the wild juvenile Pacific Salmon migration windows as described above.

Licence holders must count sea lice on farms at prescribed frequencies during the different windows. During the non-migration window, sea lice must be counted at each farm in a minimum of three stocked containment structures once a month. During the pre-migration window, all containment structures must be counted at least once. Finally, during the out-migration window, sea lice must be counted in a minimum of three stocked containment structures within the first week and then once every two weeks. Licence holders must submit the results to DFO by the 15th of the following month during the non-migration window; and within 48 hours of each sea lice counting event during the pre-migration and the out-migration windows.

The average *L. salmonis* motile (female and male preadult and adult stages) per fish, the average *L. salmonis* females per fish and the average chalimus (*L. salmonis* and *C. clemensi* together) per fish are reported to DFO. Sea lice counts and monthly inventories are stored in DFO's internal Aquaculture Integrated Information System (AQUIIS)), which is a business confidential data set. Monthly average sea lice counts on farms are available online (DFO, 2022a). The adult female sea lice counts from Atlantic Salmon farms on which this analysis is based include data from March through July at various sites and points in time between 2016 and 2021.

Assumptions

The following assumptions were made in estimating the number of *L. salmonis* females on Atlantic Salmon farms and the number of copepodids produced on Atlantic Salmon farms:

• *L. salmonis* counts on Atlantic Salmon farms provided a reliable estimate of adult female abundance for that farm and for that week;

- Linear interpolation is an appropriate method to estimate adult female abundance and the number of salmon on farms between sampling events;
- Spline interpolation is an appropriate method to smooth temperature and salinity data;
- The Norwegian sea lice development model from Samsing et al. (2016), which describes the temperature dependence of sea lice development (see Appendix B) is applicable in British Columbia; and
- Mortality of the free-swimming stages of *L. salmonis* is due only to salinity, other causes of mortality were not considered.

Adult female sea lice on farms

The number of adult female *L. salmonis* on each Atlantic Salmon farm was determined based on the monthly Atlantic Salmon inventories reported by the industry and available through DFO's internal Aquaculture Integrated Information System (AQUIIS) and industry reported sea lice counts available through the same system. Refer to Appendix A for details.

The median number of *L. salmonis* adult females per week on a given Atlantic Salmon farm varied by years, zones and migration windows (Table 2). Among years, the highest median weekly estimates of *L. salmonis* adult females per farm occurred in 2015, 2020 and 2021, and the lowest median weekly estimates occurred in 2014 and 2018 (Table 2). Among zones, the median weekly estimates of *L. salmonis* adult females per farm was highest in FHSZ 3.5 (Central Coast) and lowest in FHSZ 3.1 (Sunshine Coast) (Table 2).

Of most relevance to this advice are the differences observed among the different wild juvenile Pacific Salmon migration windows. Overall, the median weekly number of *L. salmonis* adult females was highest in the non-migration window and lowest in the out-migration window (Table 2). This pattern reflects the combined influences of parasites originating from returning wild salmon in the late summer and autumn and a more rapid population growth in the warmer months; as well as the effects (reductions) of on-farm sea-lice treatments prior to and during the wild juvenile Pacific Salmon out-migration window in the late winter and early spring.

Figure 2 provides weekly point estimates of total adult females *L. salmonis* on Atlantic Salmon (*Salmo salar*) farms in the seven FHSZ in British Columbia between 2013 and 2021.

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Table 2. Minimum, median and maximum weekly estimates of Lepeophtheirus salmonis adult females on Atlantic Salmon (Salmo salar) farms in British Columbia between 2013 and 2021. Data consist of a total of 19,422 sea lice counting events from 84 farms, and are summarized here by year, Fish Health Surveillance Zone (FHSZ) and migration window. The number of farms indicates how many Atlantic Salmon farms were active in each year, FHSZ, or migration window. Aggregation of results by FHSZ and migration window included all sea lice counting events from 2013 to 2021. The same data were used to generate Figure 2. FHSZ: 2.3: Southwest Vancouver Island which includes Clayoquot Sound, 2.4: Northwest Vancouver Island which includes Quatsino Sound, 3.1: Sunshine Coast, 3.2: Discovery Islands, 3.3: Broughton Archipelago, 3.4: Queen Charlotte Strait, and 3.5: Central Coast. Migration windows are defined as pre-migration (February), out-migration (March to June), and non-migration (July to January).

		Number of farms	Number of counting events	Minimum	Median	Maximum
Year	2013	54	1,588	0	91,190	17,267,338
	2014	59	1,986	0	83,297	4,832,039
	2015	68	2,318	0	187,379	7,700,548
	2016	66	2,065	0	123,046	5,931,768
	2017	66	2,126	0	123,906	12,826,529
	2018	64	2,190	0	81,410	17,379,755
	2019	71	2,415	0	98,165	9,600,534
	2020	62	2,213	0	188,183	6,630,405
	2021	62	2,166	0	179,984	7,419,493
FHSZ	2.3	15	3,808	0	148,147	15,354,943
	2.4	13	3,479	0	196,750	17,379,755
	3.1	6	1,232	0	32,583	1,558,183
	3.2	10	2,309	0	156,971	5,931,768
	3.3	20	4,937	0	84,976	7,709,777
	3.4	12	2,277	0	234,262	13,065,948
	3.5	8	1,380	0	301,470	20,121,870
Window	Non-migration	83	10,954	0	181,608	20,121,870
	Pre- migration	82	1,674	0	136,257	14,212,333
	Out-migration	84	6,794	0	78,853	13,221,418

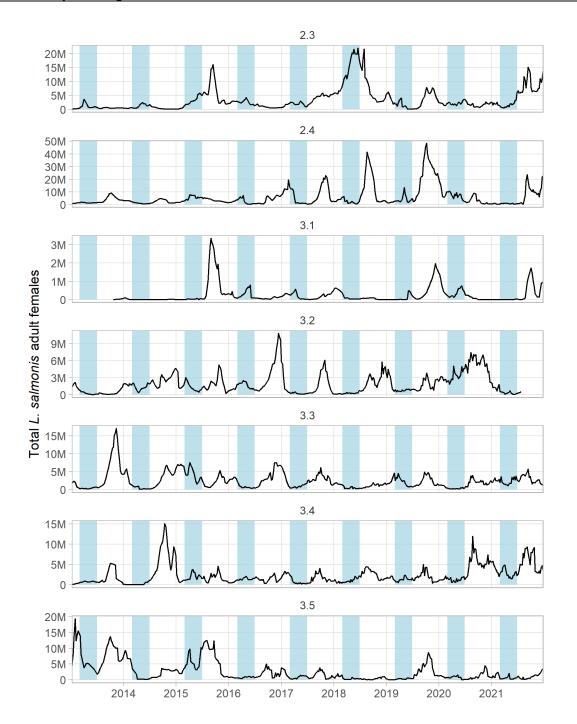


Figure 2. Weekly point estimates of total adult female Lepeophtheirus salmonis on Atlantic Salmon (Salmo salar) farms in the seven Fish Health Surveillance Zones (FHSZ) in British Columbia between 2013 and 2021. Blue areas indicate juvenile out-migration period (March to June inclusively). Note the scale of the y-axis varies among FHSZ. Data consist of a total of 19,422 observations from 84 farms. The same data were used to generate Table 2. FHSZ descriptions: 2.3: Southwest Vancouver Island which includes Clayoquot Sound, 2.4: Northwest Vancouver Island which includes Quatsino Sound, 3.1: Sunshine Coast, 3.2: Discovery Islands, 3.3: Broughton Archipelago, 3.4: Queen Charlotte Strait, and 3.5: Central Coast.

Infective copepodids from farms

The total number of infective *L. salmonis* copepodids produced by adult female sea lice on Atlantic Salmon farms were derived from the number of adult females estimated in the previous section and based on published peer-reviewed modeling approaches (refer to Appendix B for details) and considering environmental conditions on Atlantic Salmon farms in British Columbia (refer to Appendix C for details).

The median number of farm-derived *L. salmonis* copepodids per week varied by years, zones and migration windows (Table 3). Among years, the highest median weekly estimates of *L. salmonis* copepodids per farm occurred in 2015, 2020 and 2021, and the lowest median weekly estimates occurred in 2014 and 2018 (Table 3). Among zones, the median weekly estimates of *L. salmonis* copepodids per farm was highest in FHSZ 3.5 (Central Coast) and lowest in FHSZ 3.1 (Sunshine Coast) (Table 3).

Of most relevance to this advice are the differences observed among the different juvenile Pacific Salmon migration windows. Overall, the median weekly number of *L. salmonis* copepodids produced on Atlantic Salmon farms was highest during the non-migration window and lowest during the out-migration window (Table 3).

Figure 3 provides weekly estimates of total farm-derived *L. salmonis* copepodids (infective larvae) in the seven FHSZ in British Columbia are shown between 2013 and 2021. Generally, the pattern displayed by the number of farm-derived copepodids show similarity to the number of estimated adult females on farms.

Table 3. Minimum, median and maximum weekly estimates of the number of infective (or viable) Lepeophtheirus salmonis copepodids produced by infestations on Atlantic Salmon (Salmo salar) farms in British Columbia between 2013 and 2021. Data consist of a total of 19,422 sea lice counting events from 84 farms, and are summarized here by year, Fish Health Surveillance Zone (FHSZ) and migration window. The number of farms indicates how many Atlantic Salmon farms were active in each year, FHSZ, or migration window. Aggregation of results by FHSZ and migration window included all sea lice counting events from 2013 to 2021. The same data were used to generate Figure 3. FHSZ: 2.3: Southwest Vancouver Island which includes Clayoquot Sound, 2.4: Northwest Vancouver Island which includes Quatsino Sound, 3.1: Sunshine Coast, 3.2: Discovery Islands, 3.3: Broughton Archipelago, 3.4: Queen Charlotte Strait, and 3.5: Central Coast. Migration windows are defined as pre-migration (February), outmigration (March to June), and non-migration (July to January).

		Number of farms	Number of counting events	Minimum	Median	Maximum
Year	2013	54	1,588	0	60,686,582	10,257,985,037
	2014	59	1,986	0	51,162,601	3,769,483,342
	2015	68	2,318	0	115,834,439	5,067,450,151
	2016	66	2,065	0	75,162,796	4,391,710,046
	2017	66	2,126	0	77,925,846	8,193,749,841
	2018	64	2,190	0	59,414,155	10,076,254,197
	2019	71	2,415	0	68,574,393	5,900,434,861
	2020	62	2,213	0	122,292,399	3,608,308,890
	2021	62	2,166	0	116,235,931	4,168,147,778
FHSZ	2.3	15	3,808	0	72,668,796	7,352,045,836
	2.4	13	3,479	0	129,313,561	10,076,254,197
	3.1	6	1,232	0	12,325,875	570,816,102
	3.2	10	2,309	0	113,288,093	4,391,710,046
	3.3	20	4,937	0	54,810,126	5,487,126,436
	3.4	12	2,277	0	183,598,711	8,325,268,909
	3.5	8	1,380	0	251,022,083	10,957,682,266
Window	Non-migration	83	10,954	0	112,602,435	10,957,682,266
	Pre-migration	82	1,674	0	96,621,746	10,257,985,037
	Out-migration	84	6,794	0	53,106,438	9,334,247,617

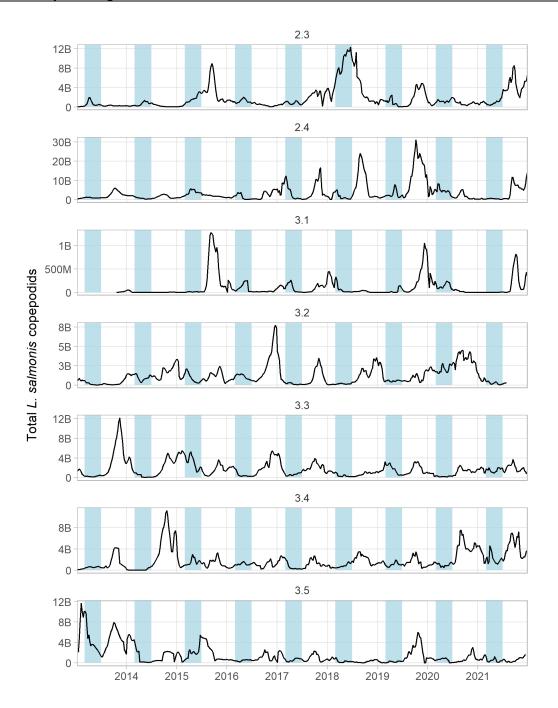


Figure 3. Weekly estimates of total farm-derived Lepeophtheirus salmonis copepodids (infective larvae) in the seven Fish Health Surveillance Zones in British Columbia between 2013 and 2021. Blue areas indicate juvenile out-migration period (March to June inclusively). Note the scale of the y-axis varies across FHSZ. Note the scale of the y-axis varies among FHSZ. Data consist of a total of 19,422 observations from 84 farms. The same data were used to generate Table 3. FHSZ descriptions: 2.3: Southwest Vancouver Island which includes Clayoquot Sound, 2.4: Northwest Vancouver Island which includes Quatsino Sound, 3.1: Sunshine Coast, 3.2: Discovery Islands, 3.3: Broughton Archipelago, 3.4: Queen Charlotte Strait, and 3.5: Central Coast.

Sea lice counts on wild juvenile Pacific Salmon in British Columbia

This section summarizes the *Lepeophtheirus salmonis* counts on juvenile Pacific Salmon species in British Columbia.

Data sources

The summary of *L. salmonis* numbers on wild juvenile Pacific Salmon were based on reports which used consistent methods of fish collection and sea lice counts using microscopic examination of each fish and reported sea lice abundance on individual fish. Other datasets which did not meet these criteria were not included in this analysis.

Some companies operating marine finfish aquaculture sites in BC, sometimes in partnership with First Nations, contract third parties to conduct monitoring of sea lice on out-migrating wild juvenile salmon. All reports are available online on respective company websites.

As of January 2022, reports were available for surveys conducted in six different coastal regions of British Columbia (Broughton Archipelago, Discovery Islands, Port Hardy, Central Coast, Clayoquot Sound, and Quatsino Sound) for some or all years between 2014 and 2021. All reports include summary statistics related to sea lice observed on fish captured during the surveys; however, only the reports about Broughton Archipelago, Discovery Islands, Quatsino Sound and Clayoquot Sound from 2016 to 2021 which met the above criteria and were included in this analysis (Table 4). Consequently, the sea lice counts on wild fish on which this analysis is based include data from March through July in four regions between 2016 and 2021.

Table 4. Summary of wild juvenile Pacific Salmon sea lice monitoring reports in British Columbia based
on studies that conducted microscopic examination of fish-level counts. Data summarized in January
2022.

Region	Surveyed years	References	Years with fish-level counts
Broughton Archipelago	2015-2021	MBC (2016a, 2017a, 2018a, 2019a, 2020c)	2016-2021
Discovery Islands (Campbell River)	2016-2021	MBC (2018b, 2019b, 2020a, 2021a)	2017-2021
Quatsino Sound	2015-2021	MBC (2016b, 2017b, 2018c, 2019c, 2020d, 2021b)	2016-2021
Clayoquot Sound	2016-2021	MBC (2016c, 2017c, 2018d, 2019d, 2020b, 2021c)	2016-2021

Assumptions

The following assumptions were made in summarizing the *L. salmonis* counts on wild juvenile Pacific Salmon:

- *L. salmonis* counts as derived from used datasets represent true estimates of populationlevel infestation; and
- Fish sampled within the same week were assumed to belong to one sampling event.

Sampling area and fish description

Figure 4 indicates the four areas for which fish-level counts of sea lice on wild fish were available: Clayoquot Sound in FHSZ 2.3, Quatsino Sound in FHSZ 2.4, Discovery Islands as FHSZ 3.2 and Broughton Archipelago as FHSZ 3.3.

Sampling was carried out from March through July at various sites and points in time between 2016 and 2021. A total of 18,824 wild juvenile Pacific Salmon were caught using beach seines at various sites in the above areas (Table 5).

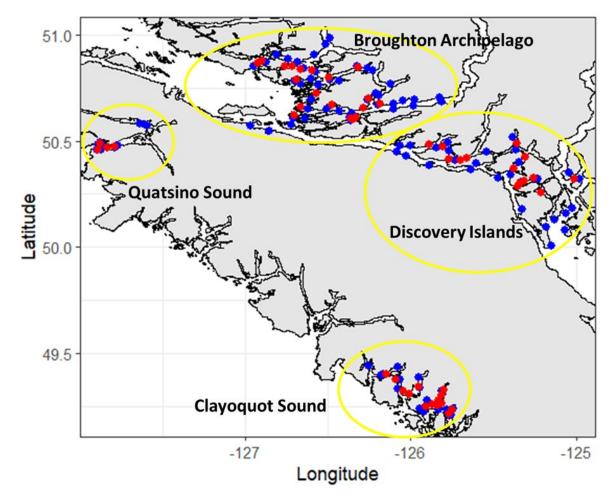


Figure 4. Wild juvenile Pacific Salmon sea lice monitoring areas, farms and sampling sites. Red points: locations of salmon aquaculture sites, blue points: wild salmon sampling sites. The monitoring areas overlap with some of the Fish Health Surveillance Zones (FHSZ): Clayoquot Sound (in FHSZ 2.3), Quatsino Sound (in FHSZ 3.3), Discovery Islands (FHSZ 3.2) and Broughton Archipelago (FHSZ 3.3). See Table 4 for sources of data for fish-level counts. See Appendix D for close-up of each area.

 Table 5. Number of wild juvenile Pacific Salmon caught and examined microscopically for fish-level sea

 lice counts in four regions of British Columbia from 2016 to 2021. See Table 4 for sources of data.

Region	Species	2016	2017	2018	2019	2020	2021	Total
	Chinook Salmon	0	0	0	0	0	0	0
	Chum Salmon	905	1,122	696	428	696	854	4,701
Clayoquot Sound	Coho Salmon	0	84	45	1	29	32	191
(in FHSZ 2.3)	Pink Salmon	0	0	1	0	0	0	1
	Sockeye Salmon	0	38	0	0	0	0	38
	Total	905	1,244	742	429	725	886	4,931
	Chinook Salmon	19	0	6	6	5	3	39
	Chum Salmon	235	479	325	441	302	417	2,199
Quatsino Sound	Coho Salmon	1	58	37	35	79	42	252
(in FHSZ 2.4)	Pink Salmon	2	0	0	7	0	1	10
	Sockeye Salmon	0	0	31	2	0	0	33
	Total	257	537	399	491	386	463	2,533
	Chinook Salmon	0	26	79	9	6	0	120
	Chum Salmon	0	942	722	599	564	918	3,745
Discovery Islands	Coho Salmon	0	88	34	21	33	0	176
(FHSZ 3.2)	Pink Salmon	0	374	434	510	578	848	2,744
	Sockeye Salmon	0	0	1	2	0	0	3
	Total	0	1,430	1,270	1,141	1,181	1,766	6,788
	Chinook Salmon	0	2	0	1	0	0	3
	Chum Salmon	512	562	281	246	497	249	2,347
Broughton Archipelago	Coho Salmon	25	19	11	24	5	0	84
(FHSZ 3.3)	Pink Salmon	430	411	356	230	402	309	2,138
. ,	Sockeye Salmon	0	0	0	0	0	0	0
	Total	967	994	648	501	904	558	4,572
TOTAL IN ALL REGIONS		2,129	4,205	3,059	2,562	3,196	3,673	18,824

The remainder of this analysis focused on Chum and Pink salmon given that together, they represented 95% (17,885 of 18,824) of sampled wild juvenile Pacific Salmon (Table 5). More specifically, this analysis focused on Chum Salmon in Clayoquot Sound and Quatsino Sound as they represented 95% (4,701 of 4,931) and 87% (2,199 of 2,533) of sampled fish, respectively in those areas. However, this analysis focused on both Chum and Pink salmon in Discovery Islands and Broughton Archipelago, as Chum and Pink salmon represented 55% (3,745 of 6,788) and 40% (2,744 of 6,788), respectively in the Discovery Islands and 51% (2,347 of 4,572) and 47% (2,138 of 4,572), respectively in the Broughton Archipelago.

Juvenile Chum and Pink salmon captured between March and July at various sampling locations in BC between 2016 and 2021 ranged from 0.08 to 35 g with a median weight of 0.60 g and a mean weight of 0.99 g (Figure 5). Overall, 95% of all sampled fish in the four regions weighed less than 3 g. In Discovery Islands and Broughton Archipelago, the weights of sampled Chum Salmon tended to be heavier than Pink Salmon. More specifically, median weights of

Chum Salmon were 0.60, 0.57, 0.69 and 0.70 g in Clayoquot Sound, Quatsino Sound, Discovery Islands and Broughton Archipelago, respectively, while median weights of Pink Salmon were 0.48 and 0.52 g in Discovery Islands and Broughton Archipelago, respectively.

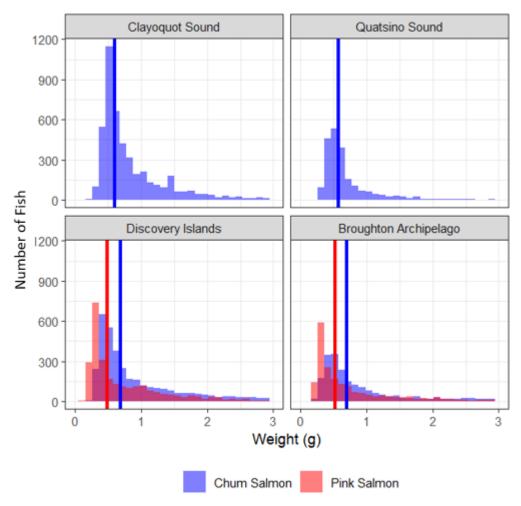


Figure 5. Weight distribution of Chum Salmon (Oncorhynchus keta) *and Pink Salmon* (Oncorhynchus gorbuscha) *sampled between 2016 and 2021 presented by monitoring areas. Clayoquot Sound and Quatsino Sound include Chum Salmon only, while Discovery Islands and Broughton Archipelago include Chum and Pink salmon. Right-skewed distributions are observed in all the distributions. As 95% of all sampled fish in the four regions weighed less than 3 g, histograms are truncated at 3 g. Blue and red vertical lines represent the median weights of Chum and Pink salmon, respectively. The monitoring areas partially overlap with some Fish Health Surveillance Zones (FHSZ): Clayoquot Sound (in FHSZ 2.3), Quatsino Sound (in FHSZ 3.3), Discovery Islands (FHSZ 3.2) and Broughton Archipelago (FHSZ 3.3).*

Sea lice on wild juvenile Pacific Salmon

Figure 6 reports on the prevalence of sea lice infestation on wild juvenile Pacific Salmon (Chum Salmon only in Clayoquot Sound and Quatsino Sound and Chum and Pink salmon in Discovery Islands and Broughton Archipelago). Each point represents a prevalence, that is the number of infested fish divided by the total number of fish, for each sampling event between 2016 and 2021. Every year, fish samples were collected from multiple sites during two to four sampling events in each of the four regions.

Relatively higher levels of *L. salmonis* infestation prevalence were observed in Clayoquot Sound compared to the other three regions (Table 6). Infestation levels on Chum Salmon in Clayoquot Sound also varied by year with the highest levels reported in 2018.

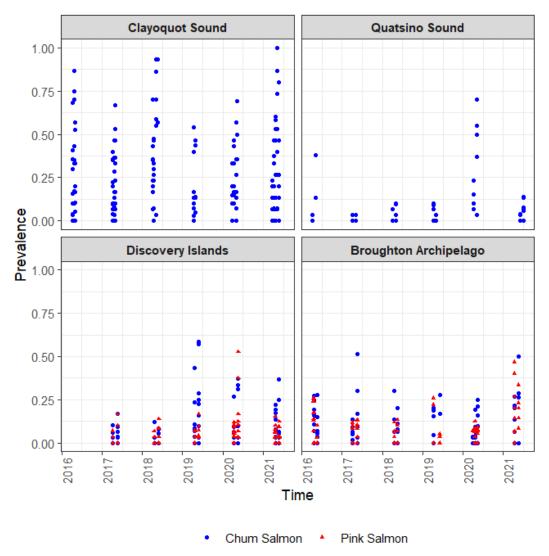


Figure 6. Prevalence of Lepeophtheirus salmonis *on juvenile Chum Salmon* (Oncorhynchus keta) *and Pink Salmon* (Oncorhynchus gorbuscha) *for each sampling event between 2016 and 2021. Clayoquot Sound and Quatsino Sound include Chum Salmon only, while in Discovery Islands and Broughton Archipelago include both Chum (blue dots) and Pink salmon (red triangles). Prevalence is the number of infested fish divided by the total number of fish in a sampling event. Source of data: Wild juvenile salmon sea lice monitoring reports conducted by Mainstream Biological Consulting (see Table 4 for references).*

Table 6. Prevalence of Lepeophtheirus salmonis infestation on juvenile Chum Salmon (Oncorhynchus keta) and Pink Salmon (Oncorhynchus gorbuscha) during sampling events between 2016 and 2021. Prevalence is the proportion of the infested fish out of the total number of sampled fish in a sampling event. Prevalence in Clayoquot Sound and Quatsino Sound are from Chum Salmon only, while prevalence in Discovery Islands and Broughton Archipelago are from Chum and Pink salmon. The same data were used in in Figure 6. The total number of fish examined in each year, species and regions are included in Table 5. Source of data: Wild juvenile salmon sea lice monitoring reports conducted by Mainstream Biological Consulting (see Table 4 for references).

Decien	Salmon					Year			
Region	species		2016	2017	2018	2019	2020	2021	All
		Min	0	0	0	0	0	0	0
Clayoquot	Chause	Mean	0.29	0.19	0.41	0.18	0.22	0.23	0.25
Sound	Chum	Max	0.87	0.67	0.93	0.54	0.69	1	1
		N	30	39	25	15	26	56	191
		Min	0	0	0	0	0.03	0	0
Quatsino	Chum	Mean	0.09	0.02	0.02	0.02	0.29	0.05	0.07
Sound	Chum	Max	0.37	0.03	0.1	0.1	0.7	0.17	0.7
		N	8	15	11	16	10	15	75
		Min	-	0	0	0	0	0	0
	Churre	Mean	-	0.03	0.02	0.17	0.09	0.06	0.07
	Chum	Max	-	0.17	0.12	0.58	0.37	0.37	0.58
Discovery		N	-	33	22	19	20	31	125
Islands		Min	-	0	0	0	0	0	0
	Pink	Mean	-	0.02	0.02	0.05	0.1	0.03	0.05
	PINK	Max	-	0.17	0.14	0.17	0.53	0.15	0.53
		N	-	15	16	17	22	28	98
		Min	0	0	0	0	0	0	0
	Chum	Mean	0.11	0.1	0.1	0.09	0.07	0.15	0.1
	Chum	Max	0.28	0.52	0.3	0.28	0.25	0.5	0.52
Broughton		N	16	19	9	11	18	14	87
Archipelago		Min	0	0	0	0	0	0	0
	Pink	Mean	0.11	0.06	0.06	0.07	0.06	0.15	0.09
	PILIK	Max	0.26	0.13	0.13	0.26	0.12	0.47	0.47
		N	14	15	11	8	16	18	82

Overall, 88% of *L. salmonis* observed on wild juvenile salmon were copepodids and chalimus, and 12% were pre-adults or adults (Figure 7). This suggests a short period between the time that the fish were infested with the parasites and the moment the fish were caught.

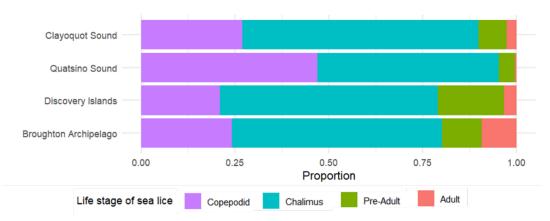


Figure 7. The proportion of Lepeophtheirus salmonis *life stages observed on wild juvenile Chum Salmon* (Oncorhynchus keta) *and Pink Salmon* (Oncorhynchus gorbuscha) *captured in four regions of British Columbia between 2016 and 2021. Counts in Clayoquot Sound and Quatsino Sound are from Chum Salmon only, while counts in Discovery Islands and Broughton Archipelago are from Chum and Pink salmon.*

Association between sea lice infestations from Atlantic Salmon farms and infestations on wild juvenile Pacific Salmon populations

We explored the statistical association between Atlantic Salmon farm-derived *L. salmonis* copepodids and the occurrence of copepodid and chalimus stages of *L. salmonis* on wild juvenile Pacific Salmon in Clayoquot Sound, Quatsino Sound, Discovery Islands, and Broughton Archipelago (Figure 4). Refer to Appendix D for higher resolution maps of wild juvenile Pacific Salmon sampling sites and Atlantic Salmon farms in each region (Figure 12, Figure 13, Figure 14 and Figure 15).

Data sources

Data from the first two sections were compiled together for the analyses in this section. Consequently, this analysis required time periods with both sea lice counts from Atlantic Salmon farms and on wild Pacific Salmon hence is based on sea lice counts during the months of March to July between 2016 and 2021.

Refer to Appendix E for methods of how the overall *L. salmonis* infestation pressure was estimated at each sampling site and to Appendix F for details on the mixed-effects logistic model.

Assumptions

The following assumptions were made in evaluating the association between the infestation pressure of *L. salmonis* from Atlantic Salmon farms and the probability of *L. salmonis* infestation on wild juvenile salmon in BC:

- Wild juvenile salmon sampling site is the location of *L. salmonis* infestation;
- The total number of copepodids on any given week is comprised of the nauplii that became copepodids that week and the copepodids from previous weeks that have survived up to that week and remained infective;
- The environmental conditions observed on the farms are typical of environmental conditions in the regions;

- The naturally occurring background sea lice infestation pressure is uniform within an area; and
- Juvenile Pink and Chum salmon are equally susceptible to *L. salmonis* infestation.

Infestation pressure from farms and prevalence on wild fish

To associate the sea lice infestation pressure from Atlantic Salmon farms and the probability of *L. salmonis* infestation on wild juvenile salmon, we estimated the number of infective copepodids resulting from the infestations on farmed salmon.

First, infestation pressure was estimated based on the number of infective copepodids derived from infestations on Atlantic Salmon farms at a specific time and based on the distance between wild salmon sampling locations and neighboring Atlantic Salmon farms within 30 km of seaway distance. Hence, infestation pressure is an estimation of the sea lice load attributable to surrounding Atlantic Salmon farms within 30 km at a specific sampling location and time.

Second, prevalence of wild Pacific Salmon was calculated by using the sampling data of outmigrating wild juvenile salmon (see previous section). For each wild juvenile salmon sampling event, an average of 23 fish were caught and examined. The fish captured in each sampling event (location, week of the year, and year) were considered as a unique combination of wild salmon sampling site, sampling year and sampling week. Prevalence was calculated by dividing the number of infected fish with the number of sampled fish at each sampling occasion.

After calculating the prevalence at each timing and each sampling location, infestation pressure corresponding to the timing and location of the prevalence was obtained. Therefore, each analytical unit represents a unique combination of sampling site-year-week with a value of infestation pressure and prevalence of wild Pacific Salmon.

Figure 8 presents the number of unique combinations with zero prevalence or non-zero prevalence on wild Pacific Salmon. The proportion of zero prevalence, in other words, the proportion with no fish infested with *L. salmonis*, in all unique combinations were 0.17, 0.41, 0.45, and 0.28 for Clayoquot Sound, Quatsino Sound, Discovery Islands, and Broughton Archipelago, respectively.

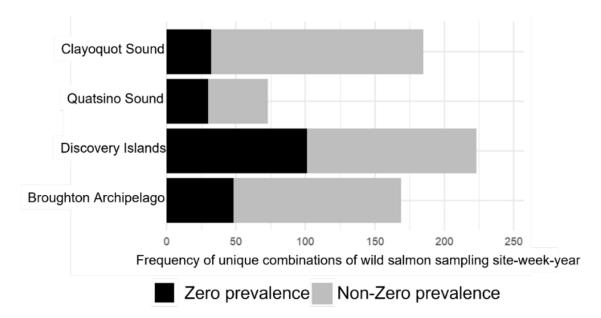


Figure 8. Frequency of zero prevalence and non-zero prevalence of Lepeophtheirus salmonis infestation on juvenile Chum Salmon (Oncorhynchus keta) and Pink Salmon (Oncorhynchus gorbuscha) for unique combinations of sampling site-week-year. Clayoquot Sound and Quatsino Sound include Chum Salmon only while Discovery Islands and Broughton Archipelago include Chum and Pink salmon. Black and gray areas represent zero prevalence and non-zero prevalence, respectively.

Logistic regression model

The high proportion of zero prevalence values on wild Pacific Salmon indicated the application of a logistic regression model to explore the relationship between the infestation pressure from the Atlantic Salmon farms (infestation pressure) and the prevalence of sea lice on wild salmon. In this model, prevalence is expressed as either zero (if prevalence = 0) or non-zero (if prevalence > 0) at each given sampling site-year-week.

While Chum Salmon is the dominant species caught in Clayoquot Sound and Quatsino Sound, Chum and Pink salmon represent together at least 95% of fish caught in Discovery Islands and Broughton Archipelago. The effect of fish species on the predicted probability of infestation on out-migrating wild juvenile Pacific Salmon was initially included in the model and found to be not significant in both Discovery Islands and Broughton Archipelago (P = 0.83 and P = 0.94, respectively). In these regions, Chum and Pink salmon were therefore analyzed together for the remaining analyses.

With increasing values of infestation pressure, the predicted probability of occurrence of infestation approaches one, which means that wild Pacific Salmon collected under these conditions are more likely to contain at least one infested fish. However, given the wide confidence intervals, this association should be interpreted with caution.

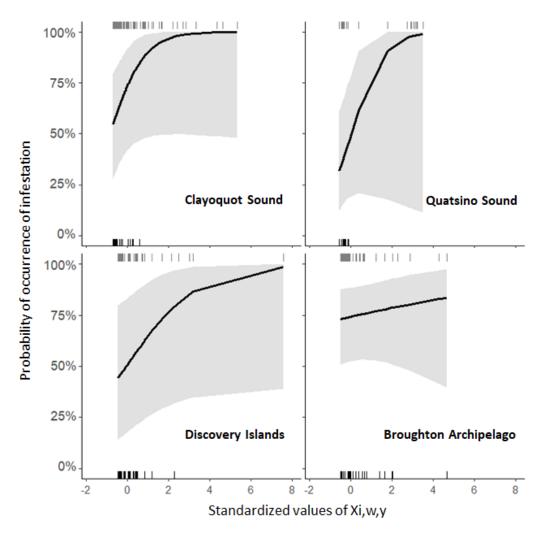


Figure 9. Margins plots based on logistic regression illustrating the relationship between the standardized Lepeophtheirus salmonis infestation pressure (the main predictor of interest, Xi,w,y) from Atlantic Salmon (Salmo salar) farms (X-axis) on the predicted probability of occurrence of infestation on out-migrating wild juvenile Chum Salmon (Oncorhynchus keta) and Pink Salmon (Oncorhynchus gorbuscha) (Y-axis). The grey area represents the 95% confidence interval around the prediction line (black). The ticks at the top and bottom of the plots mark zero prevalence (bottom) and non-zero prevalence (top) of L. salmonis infestation at unique wild salmon sampling event. Clayoquot Sound and Quatsino Sound include infestations on Chum Salmon only while Discovery Islands and Broughton Archipelago include infestations on Chum and Pink salmon.

An increasing probability of occurrence of infestation with increasing standardized values of infestation pressure was observed in all four regions, although all four regions had considerably wide confidence intervals (i.e., grey regions in Figure 9). This finding indicated that a positive association (i.e., positive values of coefficient in Table 7) was observed between infestation pressure attributable to copepodids originating from Atlantic Salmon farms and the probability of occurrence of *L. salmonis* infestations in any given sampling event of out-migrating juvenile salmon (Figure 9). However, this association did not reach the 0.05 threshold for statistical significance in the four regions (p-values in Table 7), hence the occurrence of *L. salmonis* infestation

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pressure from farm-sourced copepodids. Further work is required to verify the validity of model assumptions and to identify additional factors which may be relevant to understanding this association.

Table 7. Results of coefficients of infestation pressure (Xi,w,y) in logistic regression models evaluating the effect of Lepeophtheirus salmonis infestation pressure (Xi,w,y) from Atlantic Salmon (Salmo salar) farms on the log-odds of the occurrence of infestation with the same species of sea lice on out-migrating juvenile Chum (Oncorhynchus keta) and Pink (Oncorhynchus gorbuscha) salmon (Y). Clayoquot Sound and Quatsino Sound include Chum Salmon only, while Discovery Islands and Broughton Archipelago include Chum and Pink salmon.

Region	Coefficient	95% Confidence Interval	p-value
Clayoquot Sound	1.19	-0.06 ~ 2.43	0.06
Quatsino Sound	1.30	-0.34 ~ 2.95	0.12
Discovery Islands	0.57	-0.03 ~ 1.17	0.06
Broughton Archipelago	0.12	-0.25 ~ 0.50	0.52

Discussion

Our analyses provided quantitative estimates of the association between the weekly number of farm-origin *L. salmonis* copepodids in the marine environment in British Columbia and their contribution to infestations on wild juvenile Pacific Salmon under current farm management thresholds. We saw a positive but statistically insignificant association in all four regions studied.

Previous research conducted in British Columbia has modeled sea lice interactions between farmed salmon and juvenile out-migrating wild salmon. Nekouei et al. (2018) used a similar approach to analyze sea lice counts from farmed and wild Pacific Salmon over 10 years (2007-2016) in the Muchalat Inlet region, located in FHSZ 2.4, of British Columbia (Appendix E). These authors reported a significant positive association between the sea lice abundance on farms and the likelihood that wild fish would become infested. Data for Muchalat Inlet were not available for the present analysis. Rees et al. (2015) used a statistical model to describe the variables affecting spatial patterns of sea lice (L. salmonis and C. clemensi) infestations among wild and captive salmon in the Broughton Archipelago region of BC. On average they found infestations on farmed and wild salmon were correlated within 30 km. Similar to our analysis, these earlier studies were based on extensive data of many years and multiple farms and wild salmon sampling locations under various conditions but were each conducted for only one region. All regions in the current study have their own unique geographic and hydrodynamic characteristics and number and distributions of wild salmon natal streams and host-parasite ecological interactions which will influence the relationship between sea lice on farms and on wild salmon.

In contrast, a number of studies used counts of sea lice on wild Pacific Salmon sampled before and after migration past salmon farms to estimate the impact of farms on sea lice abundance on wild salmon (Morton et al., 2004; Krkosek et al., 2005; Price et al., 2011). In general, these studies used modeling or statistical approaches to assess the effects of salmon farms on sea lice infestations on wild fish. With or without farm sea lice infestation or environmental data, these studies identified increases in sea lice (*L. salmonis* and/or *C. clemensi*) infestation on wild salmon after migration past one or more salmon farms which they attributed to the farm(s). In the present study, availability of farm-level data provided an opportunity to better estimate the association between sea lice on salmon farms and sea lice infestations on wild Pacific Salmon. To evaluate this association over several years, we used sea lice data from multiple Atlantic Salmon farms and sea lice counts on wild fish sampled at known distances from nearby farms in the four study areas. However, assumptions were made in the development of this advice and there are uncertainties associated to some of those assumptions. For example, the wild salmon sampling sites may not have been the site of infestation in all cases; mortality of free-swimming stages of *L. salmonis* due to causes other than salinity likely occurred; or environmental conditions experienced by wild salmon and sea lice larval stages most likely differed from those reported on farms. Furthermore, our analyses were limited by not including factors such as the influence of hydrodynamic processes in the vicinity of salmon farms and the occurrence of nonfarm sources of sea lice.

Conclusions

The number of *Lepeophtheirus salmonis* adult females on Atlantic Salmon farms and the corresponding number of copepodids released from Atlantic Salmon farms were lowest during the out-migration window compared to the rest of the year across all Fish Health Surveillance Zones.

The mean prevalence of *L. salmonis* on wild juvenile Pink and Chum salmon in Clayoquot Sound, Quatsino Sound, Discovery Islands and Broughton Archipelago varied by year and sampling area. Across all years, the mean prevalence of *L. salmonis* infestation was highest on wild juvenile Chum Salmon in Clayoquot Sound and Iowest on Chum and Pink salmon in Discovery Islands. Overall, most *L. salmonis* observed on wild juvenile salmon were copepodids and chalimus.

No statistically significant association was observed between infestation pressure attributable to Atlantic Salmon farms and the probability of *L. salmonis* infestations on wild juvenile Chum and Pink salmon in Clayoquot Sound, Quatsino Sound, Discovery Islands, and Broughton Archipelago. However, the data suggests a positive trend in all studied areas. The lack of statistical significance implies that the occurrence of *L. salmonis* infestation on wild migrating juvenile Pacific Salmon cannot be explained solely by infestation pressure from farm-sourced copepodids.

Further work is required to determine how sea lice monitoring activities of wild salmon may be refined to improve modeling approaches. Key uncertainties could be addressed by verifying the validity of model assumptions by identifying additional relevant environmental, physical and biological factors. Together with this science response, these analyses will contribute to further work on assessment of risk to and to considerations of thresholds on minimizing impact to wild fish.

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Appendices

Appendix A: Estimating total *Lepeophtheirus salmonis* adult females on Atlantic Salmon farms

The number of adult female *L. salmonis* on each Atlantic Salmon farm was determined from:

- Monthly Atlantic Salmon inventories reported by the industry and available through DFO's internal Aquaculture Integrated Information System (AQUIIS);
- Industry reported sea lice counts available through AQUIIS.

DFO's conditions of licence for marine finfish aquaculture include monitoring and intervention requirements to minimize the potential exposure of wild and farmed fish to sea lice. Industry sea lice count data available through AQUIIS are reported monthly and show which Atlantic Salmon farms were actively raising fish during the month and the results of industry's monthly sea lice monitoring.

The sea lice count data in AQUIIS included pen-level counts of motile stages of *L. salmonis* and *Caligus* spp. reported separately by species, and all chalimus stages grouped together. All stages were reported as mean abundance (total sea lice observed divided by total fish examined). Within motile stages, adult females were reported separately by species. An estimate of weekly total adult female *L. salmonis* on Atlantic Salmon farms was obtained by grouping the AQUIIS abundance data by count event and multiplied by the estimated inventory at the day of the event. For this purpose, end of the month inventories were used to estimate the inventory on the day the count event started by using Eq. 1:

$$y_{ij} = x_{j-1} - \frac{x_{j-1} - x_j}{D_j} D_i$$
 Eq. 1

Where the fish inventory y_{ij} on the *i*th day of the *j*th month was estimated using the final inventory on the previous month x_{j-1} minus the difference between the final inventories of the previous month x_{j-1} and the current month x_j divided by the number of days in the current month D_i multiplied by the number of days elapsed until the *i*th day of the current month D_i .

Only Atlantic Salmon data were used and broodstock inventory was excluded. The total adult female sea lice data were then grouped by farm, calendar year and week (ISO specification). An average was calculated when more than two counts were made in a week, typically associated with pre-treatment and post-treatment counts. To derive weekly totals of adult females, missing values were made explicit by adding every possible week and year combination for each farm and the total number on weeks without counts was interpolated from the two most proximal values. A maximum of six weeks was allowed for the interpolation. Although there was at least one case during the interpolation where two production cycles were separated only by six weeks and the abundances were bridged, using a maximum of six weeks for interpolation minimized errors in identifying production cycles. A higher gap was not selected to avoid overlaps between production cycles.

Results are presented at the Fish Health Surveillance Zone (FHSZ)-level for which farm-level estimates, on a given ISO week in a given FHSZ, were added.

Appendix B: Estimating total *Lepeophtheirus salmonis* copepodids on Atlantic Salmon farms in British Columbia

The total number of infective *L. salmonis* copepodids produced by adult female sea lice on Atlantic Salmon farms were modelled for each of the farms in British Columbia based on the estimated female sea lice abundances and considering existing models.

Four models from peer-reviewed literature were used to estimated weekly totals of infective copepodids produced by the females on Atlantic Salmon farms in British Columbia.

Exponential-quadratic models from Samsing et al. (2016) were used to determine the number of eggs produced by each female and the duration of copepodid infectivity (Eq. 2). In addition, a linearized power law model from the same publication was used to estimate the duration of the nauplius stages (Eq. 3).

$$\ln(y) = \beta_0 + \beta_1 \times \ln\left(\frac{T}{T_c}\right) + \beta_2 \times \left[\ln\left(\frac{T}{T_c}\right)\right]^2$$
 Eq. 2

$$\ln(y) = \beta_0 + \beta_1 \times \ln\left(\frac{T}{T_c}\right)$$
 Eq. 3

Where y is the outcome of interest, *T* is water temperature (°C), and *Tc* is a centering parameter (set at 10 °C).

To estimate mortality of eggs (Eq. 4) and naupliar stages (Eq. 5) resulting from exposure to suboptimal salinities, and ultimately the number of these stages which molted to become infective copepodids, we used models developed by Groner et al. (2016).

$$M_{egg} = \frac{1}{1 + \left(\frac{S}{20.82}\right)^{13.98}}$$
Eq. 4
$$M_{copepodid} = \frac{1}{1 + \left(\frac{S}{19.09}\right)^{7.11}}$$
Eq. 5

Where *M* is mortality and *S* is salinity (ppt)

To utilize these models, temperature and salinity data obtained at five meters of depth from each farm were provided by industry (Appendix C). A smoothing spline with 10 knots was fitted for each farm to replace missing and unlikely values.

The proportion of female *L. salmonis* that will produce a pair of egg strings each week was estimated from weekly total adult female values by assuming that all adult females are fertilized and that over a week, 80% will become ovigerous. Heuch et al. (2000) found that at 7.2°C, most females will produce another pair of strings within 10 days after the release of an egg string.

To determine the number of eggs per string across the range of observed temperatures at each farm, the Samsing et al. (2016) exponential-quadratic temperature-dependent model was applied while the Groner et al. (2016) model was used to determine the proportion of eggs that hatched based on salinity values.

The development time from hatching to the infective copepodid stage was estimated using the linearized power model developed by Samsing et al. (2016), and mortality rates were estimated using the model of Groner et al. (2016). Together, these models permitted an estimate of the number of nauplii that survive to the copepodid stage for the observed range of salinities.

Until they attach to a host, the planktonic copepodid is non-feeding and therefore dependent on maternally derived energy sources. The availability of this endogenous lipid reserve limits the duration of copepodid viability prior to host detection and attachment. The rate at which this reserve is depleted is primarily dependent on temperature and activity levels. We used the model from Samsing et al. (2016) (see Table 8) to estimate the duration of the copepodid viability window, and assigned the surviving copepodids to the corresponding weeks.

Model	β0	β1	β2
Number of eggs per string	5.6	-0.43	-0.78
Duration of naupliar stages	1.4	-1.48	NA
Infective window	2.6	-0.26	-1.03

Table 8. Parameter values estimated by Samsing et al. (2016).

At temperatures and salinities reported in BC the duration of copepodid survival is often in excess of 7 days. Weekly copepodid numbers were estimated from the number of nauplii that develop into copepodids that week taking into consideration temperature and salinity and added the number of copepodids remaining alive from the previous week. We have assumed that all viable copepodids are infective regardless of their age. This is conservative approach as the capacity of copepodids to infect hosts declines with age at a rate which depends on temperature and salinity.

Appendix C: Environmental conditions on Atlantic Salmon farms in BC

Industry provided temperature and salinity measurements at five meters of depth on farms for year from 2016 to mid-2021. A smoothing spline with 10 knots was fitted to the existing data for each farm and the predictions for an "average" year were used as a replacement if an observation had missing or unlikely values.

Temperature

Weekly distributions of water temperature (°C) recorded at five-meter depth on active Atlantic Salmon farms in BC over five years (2016-2020) are presented in Figure 10. The water temperatures vary both seasonally and regionally with overall temperatures ranging between 4.8 and 21.5°C (Table 9).

Salinity

Weekly distributions of seawater salinity (ppt) recorded at five-meter depth on active Atlantic Salmon farms in BC over five years (2016-2020) are presented in Figure 11. Salinity varies seasonally and regionally with overall salinities ranging between 10.6 and 35.9 ppt (Table 10). Salinities vary the most (10.6 to 34.9 ppt) on farms located in FHSZ 2.4 (Northwest Vancouver Island) and the least (26.0 to 31.4 ppt) on farms located in FHSZ 3.5 (Central Coast).

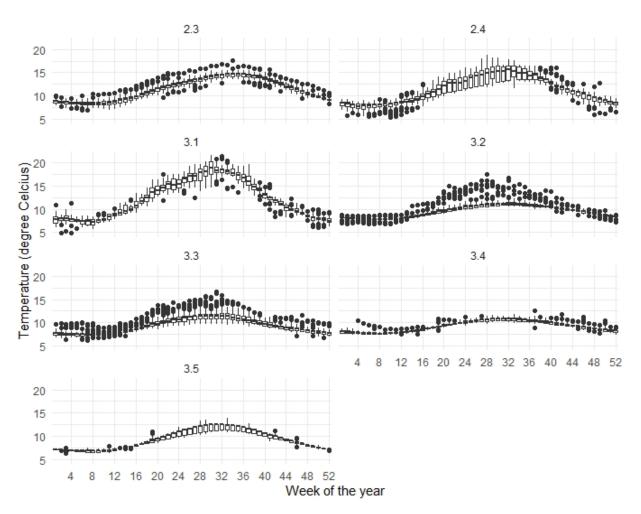


Figure 10. Distribution of weekly water temperatures (°C) at five meters of depth on Atlantic Salmon farms in British Columbia between 2016 and 2020. Temperatures are a mix of recorded measurements and predicted values when data were unavailable or unlikely for a given farm and week. Each panel represents a Fish Health Surveillance Zone (2.3: Southwest Vancouver Island; 2.4: Northwest Vancouver Island; 3.1: Sunshine Cost; 3.2: Discovery Islands; 3.3: Broughton Archipelago; 3.4: Queen Charlotte Strait; 3.5: Central Coast). Each box represents the interquartile range including the median line for the values in the week of the year. Whiskers indicate the upper and lower adjacent values. Dots represent outliers, defined as observations outside the range between the upper and lower adjacent values. Note that values in iso week 53 (only in 2020) are not plotted for clarity. Data source: BC Salmon Farmers Association, 2021. The same data were used in Table 9.

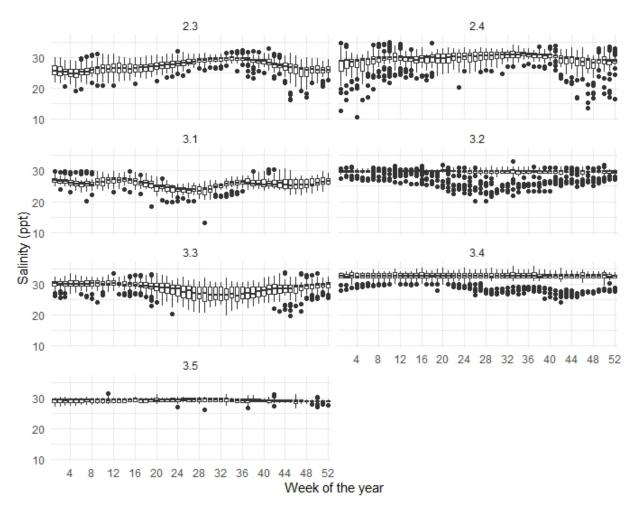


Figure 11. Distribution of weekly salinity (ppt) at five meters of depth on Atlantic Salmon farms in British Columbia between 2016 and 2020. Salinities are a mix of recorded measurements and predicted values when unavailable or unlikely for a given farm and week. Each panel represents a Fish Health Surveillance Zone (2.3: Southwest Vancouver Island; 2.4: Northwest Vancouver Island; 3.1: Sunshine Cost; 3.2: Discovery Islands; 3.3: Broughton Archipelago; 3.4: Queen Charlotte Strait; 3.5: Central Coast). Each box represents the interquartile range including the median line for values in the week of the year. Whiskers indicate the upper and lower adjacent values. Dots represent outliers, defined as observation outside the range between the upper and lower adjacent values. Note that values in iso week 53 (only in 2020) are not plotted for clarity. Data source: BC Salmon Farmers Association, 2021. The same data were used in Table 10.

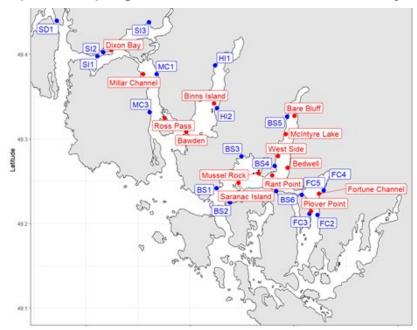
Science Response: Sea lice on Atlantic SalmonNational Capital Regionfarms and wild Pacific Salmon in British Columbia

Table 9. Water temperature (°C) at five meters of depth on Atlantic Salmon farms in British Columbia from 2016 to 2022. Temperatures are a mix of recorded measurements and predicted values when data were not available for a given farm and week. FHSZ: Fish Health Surveillance Zone (2.3: Southwest Vancouver Island; 2.4: Northwest Vancouver Island; 3.1: Sunshine Cost; 3.2: Discovery Islands; 3.3: Broughton Archipelago; 3.4: Queen Charlotte Strait; 3.5: Central Coast). The same data were used in Figure 10.

FHSZ	Temperature (degree Celsius)								
FHSZ	Minimum	Median	Maximum	Variation (maximum - minimum)					
2.3	6.9	11.4	17.5	10.6					
2.4	5.4	10.8	18.9	13.5					
3.1	4.8	11.4	21.5	16.7					
3.2	6.8	9.6	17.4	10.6					
3.3	6.2	9.1	16.7	10.5					
3.4	7.3	9.2	12.5	5.2					
3.5	6.2	8.9	13.9	7.7					

Table 10. Salinity (ppt) at five meters of depth on Atlantic Salmon farms in British Columbia from 2016 to 2022. Salinities are a mix of recorded measurements and predicted values when not available for a given farm and week. FHSZ: Fish Health Surveillance Zone (2.3: Southwest Vancouver Island; 2.4: Northwest Vancouver Island; 3.1: Sunshine Cost; 3.2: Discovery Islands; 3.3: Broughton Archipelago; 3.4: Queen Charlotte Strait; 3.5: Central Coast). The same data were used in Figure 11.

FHSZ				
FHSZ	Minimum	Median	Maximum	Variation (maximum - minimum)
2.3	16.2	27.4	33.0	16.8
2.4	10.6	29.8	34.9	24.3
3.1	13.2	25.7	30.6	17.4
3.2	20.2	29.9	33.1	12.9
3.3	19.6	29.3	33.9	14.3
3.4	24.3	32.6	35.9	11.6
3.5	26.0	29.1	31.4	5.4



Appendix D: Maps of sampling sites and Atlantic Salmon farms by area

Figure 12. Wild juvenile Pacific Salmon sea lice sampling sites and Atlantic Salmon (Salmo salar) farms in Clayoquot Sound, British Columbia. Red labels: locations of Atlantic Salmon farms, blue labels: wild juvenile salmon sampling sites. The monitoring area is in the Fish Health Surveillance Zones 2.3.

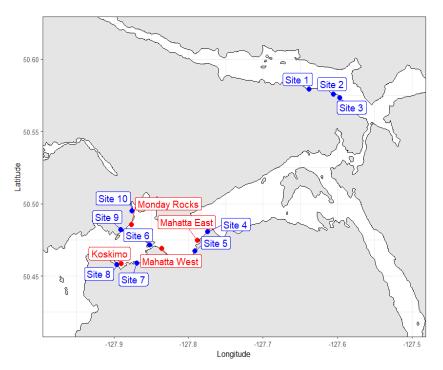


Figure 13. Wild juvenile Pacific Salmon sea lice sampling sites and Atlantic Salmon (Salmo salar) farms in Quatsino Sound, British Columbia. Red labels: locations of Atlantic Salmon farms, blue labels: wild juvenile salmon sampling sites. The monitoring area is in the Fish Health Surveillance Zone 2.4.

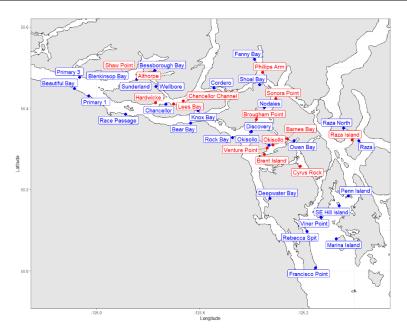


Figure 14. Wild juvenile Pacific Salmon sea lice sampling sites and Atlantic Salmon (Salmo salar) farms in Discovery Islands, British Columbia. Red labels: locations of Atlantic Salmon farms, blue labels: wild juvenile salmon sampling sites. The monitoring area overlaps with the Fish Health Surveillance Zone 3.2.

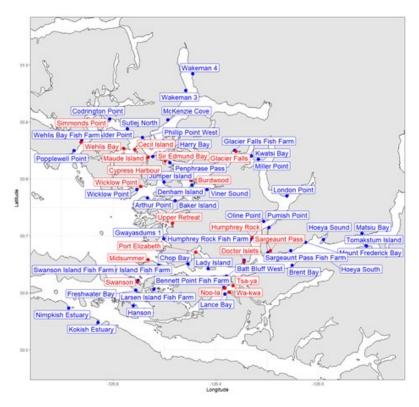


Figure 15. Wild juvenile Pacific Salmon sea lice sampling sites and Atlantic Salmon (Salmo salar) farms in Broughton Archipelago, British Columbia. Red labels: locations of Atlantic Salmon farms, blue labels: wild juvenile Pacific Salmon sampling sites. The monitoring area overlaps with the Fish Health Surveillance Zone 3.3.

Appendix E: Overall sea lice pressure on a sampling site

Nekouei et al. (2018) evaluated the association between *Lepeophtheirus salmonis* infestation on Atlantic Salmon farms and wild Pacific Salmon in Muchalat Inlet in BC. In this study, the infestation pression attributable to the farms was based on the load of farm-derived adult females one month before the wild fish were sampled. The outcome of interest was the prevalence of infestation with any life stages of sea lice on the sampled out-migrating Chum salmon.

Building on this approach, we evaluated the association between sea lice infestation levels on Atlantic Salmon farms and wild Pacific Salmon and hypothesized that the *L. salmonis* prevalence on out-migrating wild salmon in a certain week (w) and sampling site (i) was a function of the sum of the weighted (by seaway distances) load of farm-derived copepodids (infestation pressure) one week prior; i.e., at (w-1). This one-week lag time was applied in order to approximate the average time needed for infective copepodids on farmed fish to disperse to nearby areas and to infest wild salmon (Eq. 6). To build the final models, the following variables were defined and used:

 $Y_{i, w, y}$ (the outcome of interest): the prevalence of infestation with immature sea lice (copepodid and chalimus stages) on the sampled out-migrating wild juvenile Pacific Salmon (Table 11) at week 'w' and at year 'y' (per site-year-week of sampling), calculated as the number of wild salmon with at least one sea louse at week 'w' and at year 'y' in sampling site 'i' divided by the total wild salmon sampled at the same week, year, and site. Sampling events with fewer than 10 fish (i.e., the number of fish of Y_{i, w, y} < 10) were not included in the regression analysis.

- N_{j,w-1, y}: the total number of copepodid on farm 'j' at week 'w-1' and year 'y'.
- d_{i,j}: seaway distance (km) between each pair of wild site 'i' and farm 'j'.
- W_{i,j}: Gaussian kernel density estimated weight for the seaway distance 'd_{i,j}'.
- Year: sampling year (for farmed and wild fish); 2016–2021.
- Week: sampling week. Iso week from 12 to 27.
- i: wild salmon sampling site.
- j: Atlantic salmon farm.

To define our main predictor of interest $(X_{i,w,y})$, the following formula was used:

$$X_{i,w,y} = \sum_{j=0}^{n} W(d_{i,j}) \times N_{j,w-1,y}$$
 Eq. 6

where, X _{i,w,y} is the overall sea lice pressure received by a wild site 'i' at week 'w' and year 'y' from the neighboring farm/s 'j'; 'n' is the number of farms located within a radius (i.e., bandwidth) of 30 km from a wild sampling site. The 30 km bandwidth was chosen based on both biological plausibility and statistical considerations (Nekouei et al., 2018). The expected traveling distance for sea lice particles from a source farm to its surrounding water environment has been investigated in previous studies. 'N_{w-1,y'}, and d_{i,j} were defined earlier, under the variables of interest. W(d_{i,j}) or the Gaussian kernel density weight for 'd_{i,j}' was calculated using Eq. 7:

$$W(d_{i,j}) = \frac{1}{\sqrt{2\pi\sigma}} e^{-\frac{d_{i,j}^2}{2\sigma^2}}$$
 Eq. 7

where, π = 3.1416; and σ is standard deviation or ½ of bandwidth = 7.5 km.

The number of wild Pacific Salmon sampling locations are 18, 10, 28 and 46 in Clayoquot Sound, Quatsino Sound, Discovery Islands and Broughton Archipelago, respectively. The number of salmon aquaculture farms are 14, 4, 14 and 19 in Clayoquot Sound, Quatsino Sound, Discovery Islands and Broughton Archipelago, respectively. For the wild salmon data, the number of sampled fish during the study period were calculated for the four regions by years and by ISO weeks (Table 10).

Seaway distances between each combination of wild sampling sites and farms were calculated with the 'gdistance' package in the R statistical language, using each site's geographical location and a vector map outlining the coastline provided by DFO Aquaculture Management Division. The seaway distances were stored in a matrix and later retrieved for further analyses.

The effect of $X_{i,w,y}$ on Y was evaluated using a mixed-effects logistic regression model (Nekouei et al., 2018) was built, with Y being either zero (if prevalence = 0) or 'one' (if prevalence > 0) at each given wild site-year-week. The random effects of years and fixed effects of weeks were included in both models (random intercept models) to account for the potential confounding effects of time.

The main predictor of interest, $X_{i,w,y}$, had a very wide range of values (0 ~ 267,257,569). It was, therefore, standardized (centered to its mean, and divided by its standard deviation) to provide more meaningful interpretations.

Table 11. Numbers of sampled wild juvenile Pacific Salmon by region, ISO week and year. ISO weeks in ordinal weeks of the year.

	Year											
Region	ISO week	2016	2017	2018	2019	2020	2021	Total				
	12	0	0	0	0	0	179	179				
	13	0	0	0	0	228	0	228				
	14	351	0	224	0	0	241	816				
	15	0	366	0	297	222	0	885				
Clayoquot	16	311	483	226	0	0	194	1,214				
Sound	17	0	0	7	0	0	0	7				
	18	243	395	211	132	198	148	1,327				
	20	0	0	0	0	69	124	193				
	21	0	0	74	0	0	0	74				
	22	0	0	0	0	8	0	8				
	Total	905	1,244	742	429	725	886	4,931				
	14	193	202	110	0	0	0	505				
	15	0	0	0	250	125	0	375				
Quatsino	18	64	335	289	241	0	0	929				
Sound	19	0	0	0	0	261	0	261				
	22	0	0	0	0	0	227	227				
	27	0	0	0	0	0	236	236				
	Total	257	537	399	491	386	463	2,533				
	14	0	245	0	0	0	0	245				
	15	0	295	0	0	0	0	295				
	16	0	0	776	0	643	1,050	2,469				
Discovery	17	0	0	0	594	0	0	594				
Islands	20	0	0	0	0	0	2	2				
	21	0	890	167	0	538	714	2,309				
	22	0	0	327	547	0	0	874				
	Total	0	1,430	1,270	1,141	1,181	1,766	6,788				
	14	0	431	0	0	261	0	692				
	15	189	0	239	320	0	376	1,124				
Broughton Archipelago	16	366	0	85	0	349	0	800				
	20	412	563	324	0	294	182	1,775				
	23	0	0	0	181	0	0	181				
	Total	967	994	648	501	904	558	4,572				

Appendix F: Mixed-effect logistic model

This model was built upon the final data set to evaluate the association between the overall infestation pressure of sea lice from the farms $(X_{i,w,y})$ and the log-odds of the occurrence of an infestation (Y). Here is the final model equation:

 $Logit(P) = \beta_0 + \beta_1(X_{i,w,y}) + \beta_2(F) + \beta_3(1st Week) + \beta_4(2nd Week) + \dots + \beta_{n+2}(nth Week) + u$

where, 'P' is the probability of infestation, with any sea lice, at any given 'site-week-year (or the probability that prevalence in wild salmon is non-zero); if F is Chum Salmon, then F is 0, otherwise 1; β_0 is the constant; β_s are regression coefficients; and 'u' is the random effect of 'year'. In the case of Clayoquot Sound and Quatsino, the term of $\beta_2(F_f)$ was removed, because in the two regions only Chum Salmon was sampled in considerable numbers. The interaction between Xi,w,y and week was not significant and was not included in the final model. The linearity assumption between the standardized *Lepeophtheirus salmonis* infestation pressure and the logit of the predicted probability of the occurrence of infestation on a sampling group of out-migrating wild juvenile Pacific Salmon was met.

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