Vol. 126: 89–98, 2017
https://doi.org/10.3354/dao03158

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published October 18

Myxobolus arcticus and *Parvicapsula minibicornis* infections in sockeye salmon *Oncorhynchus nerka* following downstream migration in British Columbia

A. M. Mahony, S. C. Johnson, C. M. Neville, M. E. Thiess, S. R. M. Jones*

Pacific Biological Station, Fisheries and Oceans Canada, Nanaimo, British Columbia V9T 6N7, Canada

ABSTRACT: Factors influencing the health of sockeye salmon *Oncorhynchus nerka* in British Columbia, Canada, are important for fisheries management and conservation. Juvenile salmon originating from the Fraser River were screened for 3 enzootic parasites (*Myxobolus arcticus*, *Parvicapsula minibicornis*, *Ceratonova shasta*) and the bacterium *Renibacterium salmoninarum*. Fish were collected from the Strait of Georgia in 2010, 2011 and 2012 and genotyped to stock of origin. Trends in infection status were estimated by year, spawning zone and catch area. The annual prevalences of *P. minibicornis* (n = 1448) were 23.3, 6.5 and 8.1%, and for *M. arcticus* (n = 1343), annual prevalences were 40.4, 66.3 and 27.4%, respectively. Logistic regression showed that *P. minibicornis* was most strongly associated with salmon from the lower Fraser River spawning zone and increased with distance caught from the mouth of the Fraser River. In contrast, infection with *M. arcticus* was most strongly associated with salmon from the middle Fraser River spawning zone, and there was no trend related to distance from the Fraser River. Neither *R. salmoninarum* nor *C. shasta* were detected. These observations are discussed in the context of salmon life history and pathogen biology.

KEY WORDS: Sockeye salmon · Juveniles · Fraser River · *Parvicapsula* · *Myxobolus*

INTRODUCTION

Sockeye salmon *Oncorhynchus nerka* from the Fraser River in British Columbia (BC), Canada, derive from approximately 90 genetically distinct spawning populations or stocks which for management purposes are divided among 4 run-timing groups (Early Stuart, Early Summer, Summer, Late) defined by their spawning migrations (Beacham et al. 2004). Typically, sockeye rear in freshwater during their first summer, and although 24 nursery lakes are known, approximately 90% of the production occurs in fewer than 10 lakes (Thomson et al. 2012). For these 'lake-type' stocks, 1 yr old juveniles begin a seaward migration with peak abundance in the lower Fraser River in May, and enter the Strait of Georgia east of Vancouver Island, where they remain resident for 43 to 56 d (Preikshot et al. 2012, Neville et al. 2016). In contrast, 'sea-type' sockeye salmon, represented in the Fraser River principally by the Harrison River stock, enter the Strait of Georgia in their hatch year approximately 8 wk after the lake-type juveniles (Beamish et al. 2016).

The Strait of Georgia has been described as the most important rearing area for juvenile Pacific salmon on Canada's west coast (Beamish et al. 2012). Key to salmon survival in this body of water is the coincidence of the outmigration of juveniles with optimal plankton productivity, which is driven by several oceanographic and meteorological factors

*Corresponding author: simon.jones@dfo-mpo.gc.ca

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(Thomson et al. 2012). By mid-July, most lake-type juvenile sockeye have departed the Strait of Georgia to the northwest into Queen Charlotte Strait and ultimately, to the Gulf of Alaska whence most return to spawn 2 yr later. In contrast, juvenile Harrison River sockeye remain in the Strait of Georgia throughout much of the autumn (Beamish et al. 2016). Generally for given cohorts of Pacific salmon, there is evidence that supports a critical size/critical period hypothesis in which brood year strength is predicted from growth occurring in the first summer at sea: fish that fail to thrive soon after entering the ocean are subject to early-marine mortality due to unknown causes (Beamish & Mahnken 2001, Farley et al. 2007). Additional mortality during the first winter in the ocean occurs among fish below a critical size that have not accumulated sufficient energy reserves (Beamish & Mahnken 2001).

The contribution of infection and disease to early-marine mortality or critical-size-associated mortality among juvenile sockeye salmon is not well understood. Sockeye salmon are exposed to microbes and parasites as juveniles in fresh water, as sub-adults and adults during ocean residence and as mature salmon upon return to fresh water during the spawning migration. Infections are most frequently reported in adult fish captured as part of commercial, recreational or test fisheries either at sea or during the spawning migration. Limited efforts to survey and catalogue the biological characteristics of juvenile sockeye salmon (Preikshot et al. 2012) are reflected in the relatively few reports of infections during early freshwater and marine life history. The objective of this study was to describe spatial and temporal variations in infection with potentially disease-causing microbes and parasites known to be enzootic in sockeye salmon in BC (i.e. *Renibacterium salmoninarum*, *Parvicapsula minibicornis*, *Myxobolus arcticus* and *Ceratonova shasta*) in an effort to understand the contribution of infectious disease to early marine mortality.

MATERIALS AND METHODS

Collection and genetic identification of salmon

Sockeye salmon specimens were collected by purse seine between May and June in 2010, 2011 and 2012 from the Strait of Georgia, Discovery Islands and Johnstone Strait (Fig. 1). An August survey was included only in 2010. Fish included in this study were collected from 182 individual seine sets (29 in 2010, 95 in 2011, 58 in 2012) and were killed by immersion in MS222 and frozen whole to −80°C for later dissection. Dissected tissues were stored in liquid nitrogen or in 70% ethanol until PCR analysis.

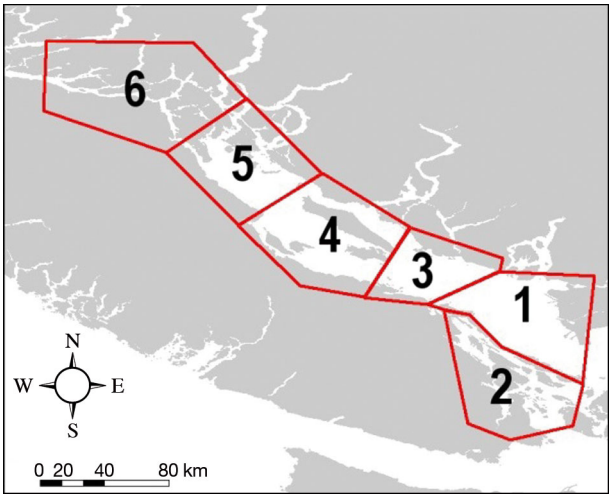

Fig. 1. Boundaries for sockeye salmon *Oncorhynchus nerka* catch areas in the Strait of Georgia, British Columbia, Canada (see Fig. 2 for location of study area in Western Canada)

Individuals were assigned to stock of origin based on the 2013 sockeye baseline dataset using procedures outlined by Beacham et al. (2014). Individual fish with a probability of assignment less than 0.50 were not included in the analysis.

PCR screening for microbes

DNA was obtained from the organic-layer of whole-brain Trizol (Thermo Fisher Scientific) extracts by adding 200 µl (or 300 µl in 2012) of TNES-6U (Tris-HCl pH 7.5 10 mM, 125 mM NaCl, 10 mM EDTA pH 8.0, 1% SDS, 6 M urea) and warming to 40°C. Samples were vigorously shaken, incubated at 20°C for 10 min and centrifuged at 4700 × *g* for 15 min. The aqueous layer (~200 µl) was transferred to a new tube and DNA extracted using a DNeasy blood and tissue kit (Qiagen) as per the manufacturer's instructions. *Myxobolus arcticus*-specific DNA was amplified following Mahony et al. (2015). Similarly, DNA was extracted from ethanol-preserved intestine or mid-kidney using the DNeasy Tissue kit, described above. *Ceratonova shasta*-specific DNA was amplified from the intestinal extract following Palenzuela et al. (1999), whereas *P. minibicornis*- or *R. salmoninarum*-specific DNA was amplified as described by Kent et al. (2000) or Brown et al. (1994), respectively, from kidney extracts. DNA quality was assessed by

amplification of small subunit (SSU) rDNA using 18E and 18G primers (Hillis & Dixon 1991), and controls (tissue-positive and no template) were included in all reactions. PCR products (8 µl) were visualized on 1.5% agarose gels stained with SYBR Safe.

Data partitioning and statistical analysis

In each year, infection data were analysed according to catch region in the Strait of Georgia and to spawning zone based on stock identity (Figs. 1 & 2). For descriptive statistics, each sample was assigned to 1 of 6 spawning zones within the Fraser River basin: 1, north central Fraser–Bowron; 2, northern Fraser–Stuart/Stellako; 3, central Fraser–Horsefly; 4, central Fraser–Chilko; 5, mid-Fraser–Thompson/Shuswap; and 6, lower Fraser (see Fig. 2 for stocks within each spawning zone). Logistic regression analysis (*plyr* package and *glm* function in R; Wickham 2011) was used separately for each microbe or parasite to investigate potential main effects of the explanatory variables year, spawning zone and/or catch region. Significant effects were obtained through iterative runs in which model complexity was sequentially reduced and statistical power in the model was improved by aggregating spawning zones into northern Fraser (zones 1 and 2), central Fraser (zones 3 and 4) and lower Fraser (zones 5 and 6) regions. Results of the logistic regression are presented as odds ratios (ORs), which indicate the risk of infection relative to sockeye salmon caught in 2010, originating from the upper Fraser River spawning zone and caught in the Fraser River plume (catch area 1). Stock-specific infection prevalence was examined for better represented stocks, and chi-squared tests were used to test the statistical significance of differences in prevalence across years ($\alpha = 0.05$). Confidence intervals of prevalence for each stock were calculated using the formula:

$$95\%\ \mathrm{CI} = \pm \sqrt{[(p)(1-p)/n]} * Z \qquad (1)$$

where p = mean proportion of positive samples, $Z = 1.96$, and n = the total number of samples tested.

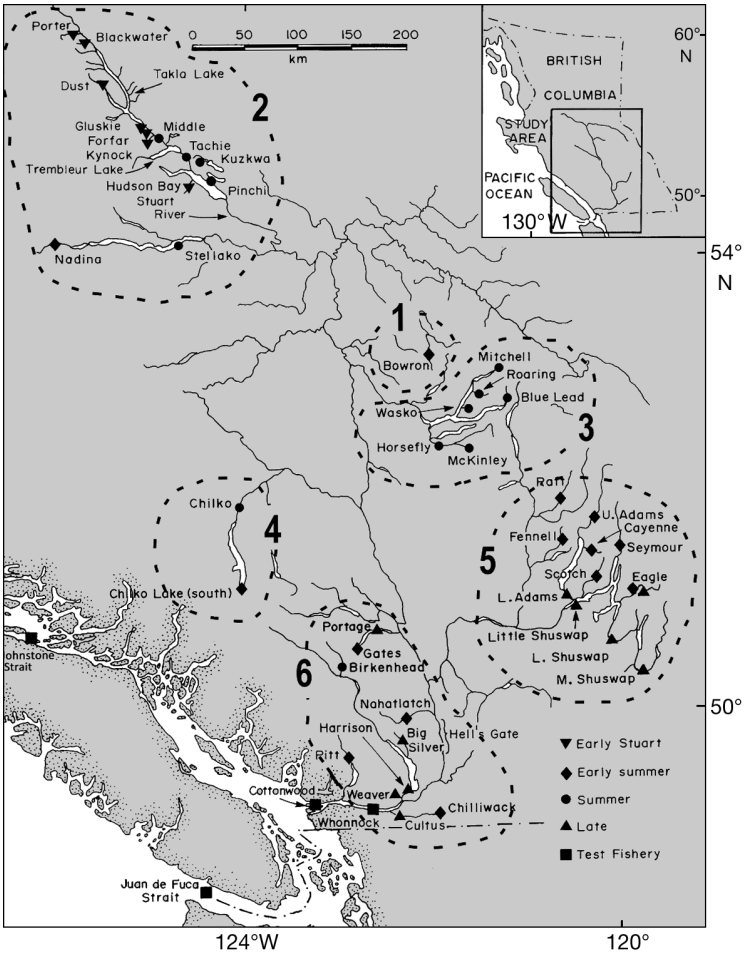

Fig. 2. Spawning zones within the Fraser River basin to which sockeye salmon *Oncorhynchus nerka* were assigned based on stock of origin. For statistical analyses (see Tables 1–3), zones were aggregated into Northern (1 and 2), Central (3 and 4) and Lower (5 and 6) (see 'Materials and methods')

RESULTS

Stock of origin

A total of 1530 sockeye salmon belonging to 45 stocks were identified in our samples. Five of these stocks (Chilko, Lower Adams, Lower Shuswap, Harrison and Stellako) accounted for 53.5% of all samples.

Prevalence of infection

Over 3 years, the prevalence of *Parvicapsula minibicornis* was 12.4% (n = 1448) and that of *Myxobolus arcticus* was 47.8% (n = 1343). The annual mean prevalence (95% confidence intervals) of *P. minibicornis* was 23.3% (19.4–27.1%; n = 473) in 2010, 6.5% (4.5–8.5%; n = 569) in 2011 and 8.1% (5.5–10.8%; n = 406) in 2012 (Table 1). Similarly, the annual mean prevalence of *M. arcticus* was 40.4% (35.0–45.7%; n = 327),

Table 1. *Parvicapsula minibicornis* and *Myxobolus arcticus* in juvenile Fraser River sockeye salmon *Oncorhynchus nerka* by catch area in the Strait of Georgia and by spawning zone (see Fig. 2 for zone definitions); n = number tested; PCR+: number positive by polymerase chain reaction

Catch area	Spawning zone	*Parvicapsula minibicornis*						*Myxobolus arcticus*					
		2010		2011		2012		2010		2011		2012	
		n	PCR+	n	PCR+	n	PCR+	n	PCR+	n	PCR+	n	PCR+
1-Fraser Plume	Northern	1	0	20	0	2	0	0	0	20	0	1	0
	Central	2	0	57	1	9	2	2	2	57	50	9	8
	Lower	48	21	18	0	65	0	3	1	18	4	65	0
2-Gulf Islands	Northern	0	0	1	0	0	0	0	0	3	1	0	0
	Central	0	0	55	1	11	1	0	0	67	63	11	10
	Lower	0	0	4	2	45	2	0	0	12	5	45	6
3-Middle/Lower	Northern	0	0	10	1	0	0	0	0	12	3	0	0
	Central	0	0	46	0	13	0	0	0	51	43	15	14
	Lower	2	2	25	5	62	3	0	0	28	8	68	8
4-Middle/Upper	Northern	16	1	23	0	9	2	16	2	23	4	9	6
	Central	29	4	60	1	26	2	29	20	60	57	26	21
	Lower	22	1	32	3	69	7	22	7	32	11	73	9
5-Upper	Northern	63	6	15	1	22	0	47	5	15	5	22	7
	Central	75	12	66	6	17	2	51	47	64	57	18	13
	Lower	62	12	24	9	56	12	49	11	21	7	57	13
6-Lower Johnstone Strait	Northern	49	17	14	2	0	0	38	3	14	2	0	0
	Central	49	14	81	2	0	0	30	24	81	73	0	0
	Lower	55	20	18	7	0	0	40	10	18	2	1	0

66.3% (62.5–70.1%; n = 596) and 27.4% (23.1–31.6%; n = 420; Table 1). Neither *Renibacterium salmoninarum* nor *Ceratonova shasta* were detected in any of 1744 or 1761 samples, respectively.

The mean prevalence of *M. arcticus* and *P. minibicornis* across catch areas and by spawning zone for each of 2010, 2011 and 2012 is illustrated in Fig. 3. For each parasite, logistic regression was used to estimate the significance of year, catch area and spawning zone as a predictor of risk of infection. For both parasites, OR is expressed relative to year = 2010, spawning zone = Northern Fraser and catch area = 1 (Fraser River plume). These analyses showed that the risk of *M. arcticus* was significantly higher in 2011 (OR = 1.84) and among salmon spawning in the Central Fraser zone (OR = 34.00; Table 2). Similarly, the risk of infection was significantly elevated among salmon caught in catch areas 2, 4 and 5 (Table 2). For *P. minibicornis*, the risk of infection was significantly less (OR = 0.38 and 0.33) in 2011 and 2012, respectively (Table 2) and was significantly greater (OR = 2.6) for salmon spawning in the Lower Fraser zone. Likewise, the risk of infection was significantly greater (OR = 2.6) for fish caught in the lower Johnstone Strait (Catch Area 6).

When all years were combined, the prevalence of *M. arcticus* varied significantly (χ^2 = 382.0, p < 0.001) among the Chilko, Lower Shuswap, Lower Adams and Stellako salmon stocks. There was significant variation in the prevalence of *P. minibicornis* among stocks when Harrison stock salmon were included (χ^2 = 73.4, p < 0.001), but not when they were excluded (χ^2 = 4.2, p = 0.123). A prevalence of 45% for *P. minibicornis* in Harrison River sockeye from the August 2010 survey was the highest observed for this parasite in this study. In this survey, the fish were collected in catch areas 1 (18 of 37 positive) and 3 (1 of 1 positive). Infected Harrison sockeye were also collected during surveys in May (1 of 1 infected in catch area 4) and June (2 of 8 infected in catch area 1; 1 of 4 infected in catch area 6). The extent of annual variability in parasite prevalence was species specific (Table 3). In Chilko salmon, the annual prevalence of *M. arcticus* was relatively high and moderately variable: 83.8% (76.5–91.1%), 91.1% (87.4–94.6%) and 78.4% (65.1–91.7%; χ^2 = 7.0, p = 0.03), respectively while that of *P. minibicornis* was relatively low and highly variable: 20.1% (13.3–26.9%), 2.5% (0.5–4.5%) and 8.6% (0–17.9%; χ^2 = 32.7, p < 0.001), respectively.

DISCUSSION

This study confirmed that infections with *Myxobolus arcticus* and *Parvicapsula minibicornis* occur among juvenile sockeye salmon following their migration from the Fraser River and provided insight into spatial and temporal variations in the overall and stock-specific prevalence. *M. arcticus* is a parasite of

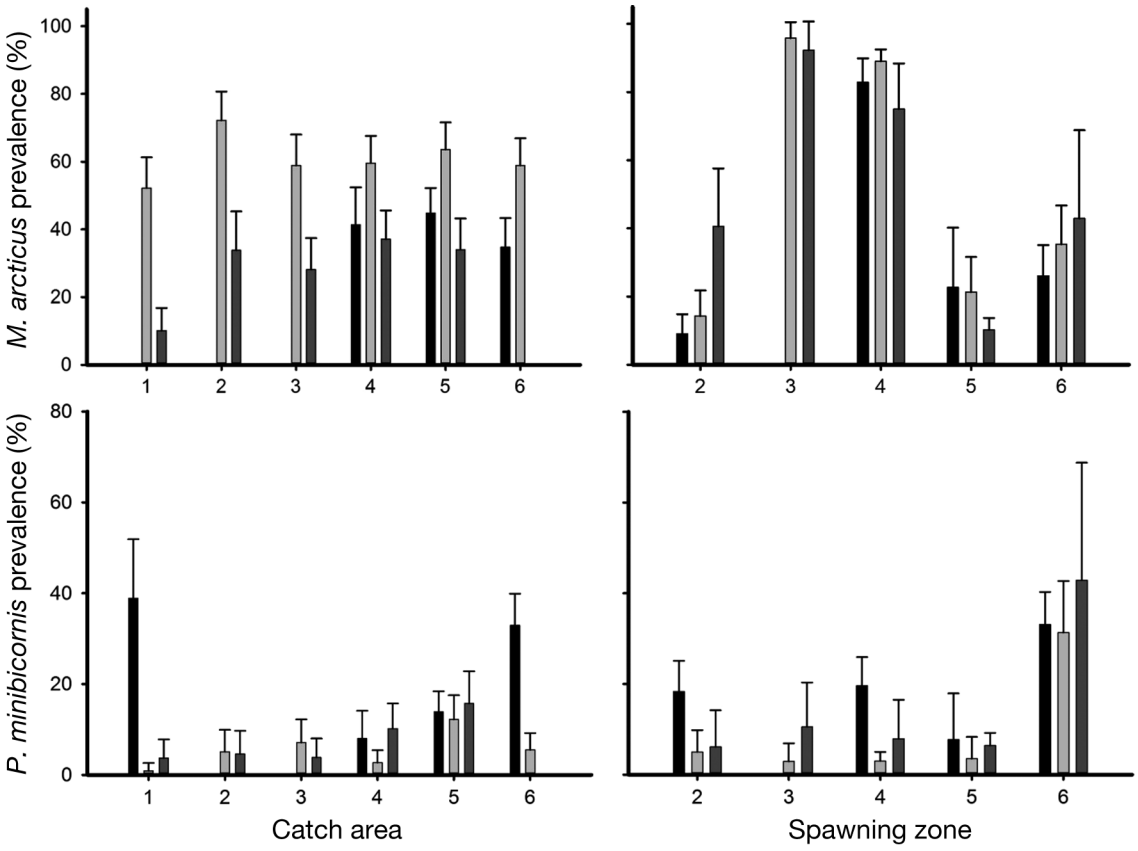

Fig. 3. Mean prevalence (error bars are 95% confidence intervals) of *Myxobolus arcticus* and *Parvicapsula minibicornis* in juvenile Fraser River sockeye salmon *Oncorhynchus nerka* by catch area and spawning zone (see 'Materials and methods') in 2010 (black), 2011 (light gray) and 2012 (dark gray). For any year, prevalence was not calculated when n < 10 (missing bars). In no year was n ≥ 10 for either parasite in spawning zone 1

the anterior spinal cord or hindbrain of sockeye salmon that elicits negligible overt pathology. Our finding of *M. arcticus* in 27 to 66% of juvenile Fraser River sockeye collected from the Strait of Georgia over 3 yr was consistent with Mahony et al. (2015), who reported the parasite in 53 and 71% of histological brain preparations from juvenile sockeye salmon collected at the Chilko Lake smolt fence in 2010 and 2011, respectively. Nearly all (96%) of the latter group were found to be infected by using PCR (Mahony et al. 2015). There was no evidence in the present study for trends in the prevalence of *M. arcticus* among catch areas in the Strait of Georgia during the 6–8 wk residency of juvenile sockeye within the Strait of Georgia. The prevalence would be expected to drop if infected fish were preferentially removed, as shown for poor-condition juvenile salmon under avian predation pressure (Tucker et al. 2016). This may indicate that the infections in Fraser River sockeye were not as severe as those in the Alaskan sockeye associated with reduced swimming speed (Moles & Heifetz 1998).

The wide distribution of *M. arcticus*, combined with its spatial heterogeneity and apparent stability over time (Moles & Heifetz 1998, Moles & Jensen 2000) has in the past supported use of this parasite, then referred to as *M. neurobius*, as a biological tag to aid in the discrimination of sockeye salmon stocks in the Pacific Northwest of North America (Bailey & Margolis 1987, Quinn et al. 1987). The variable prevalence of *M. arcticus* among salmon populations is related to conditions in salmon natal lakes that are suitable for the annelid intermediate host *Stylodrilus heringianus* (Kent et al. 1993, Moles & Jensen 2000). Not surprisingly, our analyses showed statistically significant variation in prevalence among salmon stocks and that salmon from the central Fraser spawning zone (Quesnel, Chilko and Thompson River drainages) were 34 times more likely to be infected than those from the lower Fraser zone (Pitt, Harrison and Chilliwack Rivers). This is consistent with Bailey & Margolis (1987), who reported the parasite in 62.0 and 66.0% of smolts from Quesnel and Bowron Lakes, respectively. Interestingly, unlike

Table 2. Logistic regression analysis of main effects (Year, Geographic Spawning Zone, Run Timing and Catch Area) related to prevalence of infection with *Myxobolus arcticus* or *Parvicapsula minibicornis* in juvenile Fraser River sockeye salmon *Oncorhynchus nerka*. OR: odds ratio; Intercept: fish caught in 2010 from the Northern Fraser River geographic spawning zone, and caught in the Fraser River plume catch area. **Bold** values: significant

	OR	CI	p
M. arcticus			
(Intercept)	0.07	0.04–0.14	**<0.001**
Year			
2011	1.84	1.22–2.78	**0.004**
2012	0.84	0.53–1.33	0.456
Geographic Spawning Zone			
Central Fraser	34.04	22.02–53.85	**<0.001**
Lower Fraser	1.33	0.86–2.07	0.205
Catch Area			
2-Gulf Islands	2.79	1.44–5.45	**0.002**
3-Middle Strait of Georgia	1.83	1.00–3.38	0.051
4-Upper/Middle Strait of Georgia	2.39	1.39–4.17	**0.002**
5-Upper Strait of Georgia	2.84	1.63–5.01	**<0.001**
6-Lower Johnstone Strait	1.74	0.95–3.22	0.074
Observations	132		
P. minibicornis			
(Intercept)	0.13	0.07–0.23	**<0.001**
Year			
2011	0.38	0.25–0.58	**<0.001**
2012	0.33	0.20–0.54	**<0.001**
Geographic Spawning Zone			
Central Fraser	0.91	0.55–1.53	0.726
Lower Fraser	2.56	1.61–4.16	**<0.001**
Catch Area			
2-Gulf Islands	0.78	0.27–1.92	0.608
3-Middle Strait of Georgia	0.90	0.40–1.91	0.787
4-Upper/Middle Strait of Georgia	0.81	0.43–1.53	0.522
5-Upper Strait of Georgia	1.50	0.89–2.60	0.139
6-Lower Johnstone Strait	2.56	1.48–4.55	**0.001**
Observations	136		

here or in Mahony et al. (2015), Bailey & Margolis (1987) found no evidence of the parasite in 244 juvenile sockeye sampled from Chilko Lake between 1976 and 1982. Evidently, the distribution of the parasite among natal lakes is not stable over decadal time scales. The mechanisms of parasite introduction into previously naïve populations are not known but may be related to infected salmon straying among natal lakes (Quinn et al. 1987) or to vector-mediated movement of infected annelid or fish hosts among lakes (Moles & Jensen 2000).

P. minibicornis was originally described from spawning sockeye salmon in the Fraser River (Kent et al. 1997) where it also occurs in coho *Oncorhynchus kisutch* and pink salmon *O. gorbuscha* (Jones et al. 2003). In the adult sockeye, severe infection is associated with pathological changes in affected glomeruli and renal tubules (Raverty et al. 2000). The parasite has also been reported from adult Pacific salmon in the Columbia and Klamath Rivers (Jones et al. 2004, Bartholomew et al. 2007, Atkinson et al. 2011). Our detection of *P. minibicornis* confirms an earlier study in which the parasite was reported in juvenile Fraser River sockeye salmon in the Strait of Georgia. St-Hilaire et al. (2002) used PCR to demonstrate the infection in approximately 60% of 67 juveniles collected from an unreported location in the lower Strait of Georgia. *P. minibicornis* was reported in juvenile Chinook salmon *O. tshawytscha* in the Klamath River, California, USA (Foott et al. 2004), where infective actinospores are shed by the polychaete host, *Manayunkia* sp. (Bartholomew et al. 2006). In the present study, the risk of infection with *P. minibicornis* was significantly reduced in 2011 and 2012, a pattern strongly influenced by the elevated prevalence in juvenile sockeye from the Fraser River plume and the lower Johnstone Strait/western Discovery Islands in 2010. In 2010, most infected salmon were collected in August, and these belonged to the Harrison River stock. Harrison River sockeye display a sea-type behaviour in which they spend their first spring and summer in the lower Fraser River before migrating to the ocean, a behaviour which evidently increases their risk of exposure to the parasite (St-Hilaire et al. 2002).

Table 3. Occurrence of *Myxobolus arcticus* and *Parvicapsula minibicornis* among 5 Fraser River sockeye salmon *Oncorhynchus nerka* stocks from 2010 to 2012; n = number tested; %: percent positive by PCR

Stock	*Myxobolus arcticus*						*Parvicapsula minibicornis*					
	2010		2011		2012		2010		2011		2012	
	n	%	n	%	n	%	n	%	n	%	n	%
Chilko	99	83.8	246	91.0	37	78.4	134	20.1	236	2.5	35	8.6
Shuswap	0	0	0	0	100	11.0	0	0	0	0	94	4.3
Adams	0	0	21	19.0	109	9.2	1	100.0	20	0.0	105	6.7
Stellako	46	8.7	8	50.0	24	37.5	60	15.0	7	0.0	24	4.2
Harrison	4	25.0	0	0	0	0	51	45.1	0	0	0	0

Epidemiological evidence indicates transmission of *P. minibicornis* to salmon as they migrate through the lower Fraser River. In adult salmon, prevalence and severity progressively increase with distance migrated in the river (St-Hilaire et al. 2002, Jones et al. 2003). In the present study and with the exception of the aforementioned 2010 catches, there was a trend of increasing prevalence in juvenile salmon with distance from the Fraser River plume, and this was statistically significant for the Lower Johnstone Strait. This pattern of increasing prevalence and severity, whether in spawning adult salmon or in ocean-migrating juveniles, may be explained by the proliferative development of the parasite following transmission. In adult salmon, the most severe infections coincide with post-spawning mortality (Raverty et al. 2000, Jones et al. 2003), and more severe pathological consequences occur when the spawning migration is delayed in-river (Cooke et al. 2004) or when water temperatures increase during the in-river migration (Wagner et al. 2005, Crossin et al. 2008). The outcome of the infection in juveniles is not known, but the infections are not detectable in returning adults until they re-enter the river on the spawning migration (Jones et al. 2003). In this context, Miller et al. (2014) found quantitative PCR evidence for *P. minibicornis* in juvenile sockeye salmon from Queen Charlotte Sound, approximately 100 km WNW of catch area 6. In that study, the parasite occurred at a higher prevalence (46%) in fish that had been predated by rhinoceros auklets *Cerorhinca monocerata* compared with a reference population (24% prevalence).

Ceratonova shasta is a parasite of the intestinal epithelium of Pacific salmon that occurs among coastal rivers and their tributaries in northern California, Oregon, Washington, Idaho, BC and Alaska (Follett et al. 1994, Bartholomew et al. 1997). In the American rivers studied, salmon are infected in freshwater by exposure to waterborne actinospores which are shed from the polychaete host *Manayunkia* sp. (Bartholomew et al. 1997). Infection with the parasite causes ceratomyxosis, which presents as a severe enteritis or in later stages as a systemic infection. The severity of ceratomyxosis is host-specific and increases with water temperature (Bartholomew 1998, Foott et al. 2004). In the Fraser River drainage basin, the parasite occurs in Chinook salmon, coho salmon, pink salmon, rainbow trout *O. mykiss* and cutthroat trout *O. clarkii* (McDonald 1983, Bell & Traxler 1985). Failure to detect *C. shasta* in juvenile Fraser River sockeye salmon in the present study may have been related to an apparently lower susceptibility of sockeye salmon (Zinn et al. 1977) or to the early timing of smolt outmigration relative to the spring increase in infectivity of river water (Ching & Munday 1984). Margolis et al. (1992) suggested the relatively low prevalence (3.3–9.1%) of *C. shasta* in Chinook salmon smolts examined over 3 yr in the Fraser River was related to most salmon having left the river before the period of elevated infectivity in mid-May. The mean date of sockeye smolt migration in the Fraser River from late April to mid-May (Preikshot et al. 2012, Neville et al. 2016) suggests that migration in this species also preceded the infectious period.

Renibacterium salmoninarum is a Gram-positive diplobacillus and the causative agent of bacterial kidney disease (BKD), a chronic condition causing morbidity and mortality in cultured salmonid fishes in many parts of the world (Evelyn 1993). In western North America, infections are widespread among Pacific salmon in fresh water and in the ocean (Banner et al. 1986, Meyers et al. 1993, Kent et al. 1998) and also in cultured salmon (Evelyn 1993). The bacterium has frequently been detected in juvenile Pacific salmon in fresh and coastal waters of the western USA (Sanders et al. 1992, Arkoosh et al. 2004, Van Gaest et al. 2011, Sandell et al. 2015). The mean prevalence of *R. salmoninarum* in juvenile Chinook salmon from 12 coastal locations ranged from 10 to 68% (Arkoosh et al. 2004). At Alsea Bay, annual prevalence over 6 yr ranged from 0 to 44% (Arkoosh et al. 2004). Infection with *R. salmoninarum* has repeatedly been diagnosed in adult sockeye salmon returning to the Fraser River. The prevalence in adult Cultus Lake sockeye between 2002 and 2015 ranged from 0.6% (n = 160) to 89.1% (n = 101) (Department of Fisheries and Oceans Canada [DFO] unpublished data). Similarly, the prevalence of infections in adult sockeye salmon from other Fraser River sites was 75% at Weaver Creek in 2010 (n = 20), 1.7–56.1% at Inch Creek between 2011 and 2014 (n = 54–139) and 2.9–7.5% at Henderson Lake between 1996 and 1998 (n = 35–63; DFO unpublished). Thus *R. salmoninarum* was absent in juvenile sockeye salmon in the present study despite evidence for its widespread occurrence in spawning Fraser River sockeye salmon, the susceptibility of sockeye salmon to the infection and to BKD (Sanders et al. 1978, Bell et al. 1984) and despite the tendency for the bacterium to be vertically transmitted within the ovum (Evelyn et al. 1984). Our inability to detect *R. salmoninarum* may have been related to sample sizes that were too small, to low-level infections that occurred below

the limit of detection or to the loss from the population of infected juveniles prior to migration to the ocean. In the present study, we did not screen juvenile sockeye for *R. salmoninarum* during their period of freshwater residence, and this knowledge gap should be remedied by focussing on the epidemiology and outcome of infections in juvenile sockeye salmon in fresh and marine waters.

In conclusion, this study furthers our understanding of parasite dynamics in juvenile sockeye salmon belonging to a complex mix of genetic stocks migrating from the Fraser River drainage basin. Foci of transmission of myxosporean parasites are related to the distribution and abundance of the invertebrate host. In migratory species like anadromous salmon, duration of residence within waters occupied by the invertebrate host increases the risk of infection. The observed patterns of infection supported the hypothesis that some natal systems within the drainage basin are more conducive than others to the transmission of *M. arcticus*. In addition, prolonged residency of Harrison River stock sockeye salmon in the lower Fraser River is consistent with increased risk of *P. minibicornis* infection. Data supporting the impacts of these infections on salmon survival were less clear. The prevalence of *M. arcticus* and *P. minibicornis* either remained constant or increased following salmon migration to the ocean, neither result supporting a hypothesis of infection-induced early marine mortality. In contrast, the possibility of lethal infections with *C. shasta* and *R. salmoninarum* resulting in their absence from populations sampled in the present study reinforced a need for improved surveillance in juvenile sockeye during their period of freshwater residency.

Acknowledgements. We thank J. Bradshaw and G. Prosperi-Porta for assistance with sample preparation and PCR. Thanks also to J. Bradshaw, Y. Jung, D. Conover and numerous volunteers and technicians, along with the captains and crews of the CFV 'Nordic Queen' and CFV 'Ocean Venture' for assistance with sample collections. Funding was provided by the DFO's Program for Aquaculture Regulatory Research.

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Editorial responsibility: Dieter Steinhagen, Hannover, Germany

Submitted: May 11, 2017; Accepted: July 18, 2017
Proofs received from author(s): September 16, 2017