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Distribution, prevalence and severity of *Parvicapsula minibicornis* infections among anadromous salmonids in the Fraser River, British Columbia, Canada

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ABSTRACT: Polymerase chain reaction (PCR) and microscopic examination of stained kidney sections were used to diagnose infections with the myxozoan parasite *Parvicapsula minibicornis* in maturing Fraser River salmon. In 2 series of collections, the parasite was detected in 109 of 406 migrating sockeye salmon *Oncorhynchus nerka* belonging to Early Stuart, Early Summer and Summer run-timing groups, mainly upper Fraser River stocks. However, the parasite was detected neither in fish at sea nor once they had migrated several 100 km upstream. Prevalence then increased to 95% or greater at the spawning grounds. Histological examination of kidney was less sensitive than PCR in detecting the parasite in salmon collected from the earliest sites in both collections found positive by PCR. Severity of infection was greatest at the spawning grounds. Development of infection in sockeye, measured by prevalence, severity or by the rate of false-negative histological diagnoses, appeared to be a useful estimate of in-river residence time. Prevalence and severity of infections in sequential samples of Harrison River and Weaver Creek sockeye stocks collected from the Harrison River indicated that more time had elapsed since parasite transmission than would be predicted based on migration distance alone. Pink salmon *Oncorhynchus gorbuscha*, coho salmon *O. kisutch* and chinook salmon *O. tshawytscha* were found to be infected with the parasite. Development of *P. minibicornis* in pink salmon was most similar to that in sockeye. Pink and coho salmon may be at risk to the pathological consequences of *P. minibicornis* infection.

KEY WORDS: *Parvicapsula minibicornis* · *Oncorhynchus nerka* · Fraser River · Histology · Polymerase chain reaction

INTRODUCTION

Sockeye salmon *Oncorhynchus nerka* that spawn in tributaries of the Fraser River, British Columbia, Canada, are classified into 4 run-timing groups according to the timing of their spawning migration in freshwater, i.e. Early Stuart, Early Summer, Summer, Late. Each run-timing group is further divided into stocks, based on the nursery stream or lake of origin. *Parvicapsula minibicornis* is a myxozoan parasite of Fraser River sockeye that matures within glomerular capillaries (Kent et al. 1997, St-Hilaire et al. 2002). In severe infections, developmental stages and mature spores also occur within the renal tubules. Glomerulonephritis that is characterised by diffuse thickening of the basement membrane, occlusion of the glomerular capillaries and necrosis of the tubular epithelium is associated with severe infection (Raverty et al. 2000). This renal pathology has focused attention on the role of the parasite in elevated prespawn mortality recently

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observed among certain stocks of Late run-timing Fraser River sockeye (St-Hilaire et al. 2002).

Surveys for the parasite were conducted among several Fraser River stocks of sockeye salmon in 1999 and 2000 by using histological analysis and polymerase chain reaction (PCR) (St-Hilaire et al. 2002). While no evidence of *Parvicapsula minibicornis* was found in adults prior to river entry or shortly thereafter, prevalence increased during the upriver migration and ranged from 48 to 100% among all stocks examined at or near the spawning grounds. In addition, the parasite did not occur in juvenile salmon collected from nursery streams and lakes but was detected in sockeye smolts of Fraser River origin collected in the Strait of Georgia (St-Hilaire et al. 2002). This suggested that transmission occurred at or near the river estuary and that adult and juvenile salmon acquired the infection as they migrated through this site. All species of *Parvicapsula* have been reported from marine or anadromous fish (Shulman 1953, Kabata 1962, Evdokimova 1977, Kovaleva & Gaevskaya 1981, Hoffman 1984, Lester & Sewell 1989, Landsberg 1993, Padma-Dorothy & Kalavati 1993, Kent et al. 1997, Zhao et al. 2000). A hypothesis of estuarine transmission would therefore be consistent with the location of an intermediate host that occupies a marine or brackish water habitat and would explain the widespread distribution of the parasite throughout the Fraser River drainage basin. The objectives of the present study were 2-fold: to further describe the relationship between freshwater migration and the prevalence and severity of *P. minibicornis* infections in adult Fraser River sockeye salmon and to document the occurrence of the parasite in new host species.

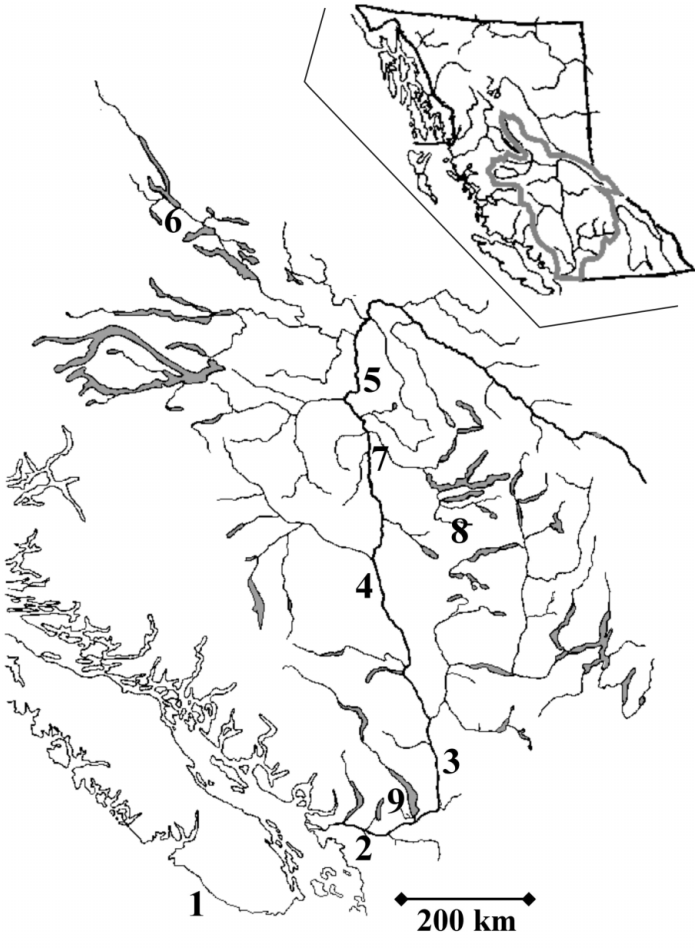

Fig. 1. Map of the Fraser River drainage basin showing marine and freshwater sockeye sampling sites: (1) Port Renfrew; (2) Whonnock; (3) Yale and Hell´s Gate; (4) Churn Creek; (5) Stoner; (6) Gluskie Creek; (7) Quesnel River; (8) Lower Horsefly River and McKinley; (9) Harrison River and Weaver Creek

MATERIALS AND METHODS

Collection of fish. Surveillance of migrating adult sockeye was conducted over 3 collections in 2001 (see map, Fig. 1). In the first 2 (June to July and July to September), fish were collected prior to river entry from a Pacific Salmon Commission test fishery at Port Renfrew, off the west coast of Vancouver Island, British Columbia and at several in-river sites leading up to and including the nursery streams. In the third (August to October), 7 sets of samples were collected from 1 location on the Harrison River, a tributary of the Fraser River, and 2 sets from the Fisheries and Oceans Canada (FOC) spawning channel at Weaver Creek, a tributary of the Harrison River. Collections were also made of sexually mature pink salmon *Oncorhynchus gorbuscha* from the mainstem Fraser River, recently spawned chinook salmon *O. tshawytscha* and sexually mature, pre- and post-spawned coho salmon *O. kisutch* held at FOC enhancement hatcheries in the Fraser River drainage basin.

Samples of posterior kidney and gill were dissected from freshly dead specimens and placed immediately into 95% ethanol. Samples of anterior and posterior kidney, gill, heart and liver were also fixed in Davidson's solution (Humason 1979) for histological assessment. One scale was collected from above the lateral line of each fish for stock identification (Gable & Cox-Rogers 1993).

PCR. DNA was extracted from approximately 25 mg of ethanol-fixed kidney using the method of St-Hilaire et al. (2002). The primers and conditions under which the PCR was run were as described by St-Hilaire et al. (2002) and the reaction amplified a 1091 bp fragment of the *Parvicapsula minibicornis* 18S rRNA gene.

Histological assessment. Tissues were processed for routine histological examination and duplicate 5 µm sections, mounted on glass slides were stained with a modified Pappenheim (MP) stain (Humason 1979) or with haematoxylin and eosin (H&E), respectively. Tissues initially fixed in 95% ethanol for PCR were post-fixed in Davidson's solution and also processed for histology as described above. The severity of infections at each collection site was estimated from the mean number of infected glomeruli per 25 glomeruli examined in MP-stained histological sections from infected fish.

RESULTS

Parvicapsula minibicornis was detected by PCR in 49 of 242 (20.2%) sockeye from the first collection. Racial analysis indicated that these fish belonged to the Early Stuart and Early Summer run-timing groups. The parasite was also detected in 60 of 164 (36.6%) sockeye from the second collection, found to be Summer run-timing sockeye. The parasite was first detected in these groups by using PCR, following upriver migrations of 440 and 619 km, respectively (Table 1). Thereafter, the prevalence increased to 95% or greater at the spawning grounds. Kidney was considered infected with *P. minibicornis* when parasite stages were identified within stained glomeruli using light microscopy. The parasite stages were more clearly evident in kidney sections stained with MP than in those stained with H&E. In both collections, significantly fewer infections were detected by histology than by PCR at downstream sites where salmon were first found to be infected ($\chi^2 = 10.99$, $p < 0.001$ and $\chi^2 = 7.06$, $p < 0.01$, respectively). In the Early Stuart/Early Summer groups, these false-negative histological results occurred in 11 of 13 PCR-positive samples at 440 km and in 1 of 18 at 718 km. In the Summer group, false-negative histological results occurred in 6 of 20 PCR positive samples at 619 km, in 2 of 20 at 750 km and in 1 of 21 samples at 805 km (Table 1). In contrast, myxozoan infections within glomeruli were observed in 2 samples (718 km, Early Stuart and Early Summer groups; 805 km, Summer group) in which *P. minibicornis* infections were not detected by PCR. In all other samples, histological diagnoses agreed with those obtained using PCR. The severity of infections ranged from 1.0 to 21.4 in the first and from 4.6 to 12.6 in the second collection (Table 1). In both groups, the severity of infection increased with distance migrated upstream and was greatest at the spawning grounds.

Most sockeye collected from the Harrison River belonged to the Harrison River (n = 52) or Weaver Creek (n = 66) stocks (Table 2). Of the remaining sockeye, 3 belonged to the Birkenhead River stock and 3 were unclassifiable. All 40 salmon in 2 collections from the spawning channel belonged to the Weaver Creek stock. Only 1 of 16 sockeye collected in the first 2 sets was of Weaver Creek stock. *Parvicapsula minibicornis* was detected in Harrison River sockeye in all sample sets but was not detected in Weaver sockeye until the fourth sample set. The overall prevalence by PCR (by histology) was 76.9% (65.4%) in Harrison River stock and 63.6% (56.1%) in Weaver stock sockeye collected from the Harrison River (Table 2). The mean severity in Weaver stock sockeye collected from the Harrison

Table 1. *Parvicapsula minibicornis* in 3 run-timing groups of adult Fraser River sockeye salmon *Oncorhynchus nerka* in 2001. Distance: km upstream from the mouth of the Fraser River. Date: date of collection. PCR: (sample size) of kidney samples from which 1091 bp product was amplified. Histology: (sample size) of kidney samples with recognizable *P. minibicornis*. Severity: mean number of affected glomeruli per 25 glomeruli examined from infected fish (sample size)

Run-timing group Location	Distance (km)	Date (d/mo)	Prevalence PCR (%)	 Histology (%)	Severity
Early Stuart/Early Summer					
Port Renfrew	–	30/6	0 (29)	0 (29)	0 (29)
Whonnock	50	05–6/7	0 (61)	0 (27)	0 (27)
Yale	170	09–10/7	0 (51)	0 (27)	0 (27)
Hell's Gate	200	12–14/7	0 (41)	0 (23)	0 (23)
Churn Creek	440	18–19/7	65 (20)	10 (20)	1.0 (20)
Stoner	718	25/7	95 (20)	95 (19)	3.9 (19)
Gluskie Creek	1086	31/7	100 (20)	100 (20)	21.4 (20)
Summer					
Port Renfrew	–	28/7	0 (23)	0 (23)	0 (23)
Whonnock	50	10/8	0 (40)	0 (24)	0 (24)
Hell's Gate	200	15/8	0 (40)	0 (27)	0 (27)
Quesnel River	619	24/8	100 (20)	70 (20)	4.6 (20)
Lower Horsefly River	750	28/8	100 (20)	90 (20)	5.1 (20)
McKinley Creek	805	05/9	95 (21)	95 (21)	12.6 (21)

Table 2. Prevalence and severity of *Parvicapsula minibicornis* in adult sockeye salmon *Oncorhynchus nerka* migrating in the Harrison River and Weaver Creek in 2001. Date: date samples collected. The first 7 collections were made at 1 location on the Harrison River. Collections of 4/10 and 15/10 were made at the Weaver Creek spawning channel. Stock: determined by scale analysis. PCR: number of posterior kidney samples from which 1091 bp product was amplified. Histology: number of posterior kidney samples with *P. minibicornis*. Severity: mean number of affected glomeruli per 25 glomeruli examined from infected fish

Date (d/mo)	Stock	Sample size	Prevalence PCR (%)	Prevalence Histology (%)	Severity
17/08	Harrison	7	3 (42.9)	3 (42.9)	3.3
	Weaver	0	–	–	–
20/08	Harrison	8	5 (62.5)	5 (62.5)	7.4
	Weaver	1	0	0	–
30/08	Harrison	7	6 (85.7)	4 (57.1)	5.0
	Weaver	7	0	0	–
06/09	Harrison	12	8 (66.7)	8 (72.7)[a]	8.4
	Weaver	9	3 (33.3)	1 (12.5)	5.0
13/09	Harrison	4	4 (100)	4 (100)	11.3
	Weaver	16	9 (56.3)	8 (50.0)	17.1
20/09	Harrison	3	3 (100)	3 (100)	7.0
	Weaver	19	16 (84.2)	16 (84.2)	22.5
27/09	Harrison	11	11 (100)	7 (63.6)	5.4
	Weaver	14	14 (100)	12 (85.7)	18.7
04/10	Harrison	0	–	–	–
	Weaver	20	20 (100)	20 (100)	20.3
15/10	Harrison	0	–	–	–
	Weaver	20	20 (100)	20 (100)	20.1

[a]Sample size for histology was 11

River was 19.5 (range 5.0 to 22.5) and in Harrison River stock sockeye was 7.0 (range 3.3 to 11.3). The parasite was detected in all 40 sockeye collected in 2 sets from the spawning channel with mean severities of 20.3 and 20.1, respectively (Table 2). Myxozoan infections within the glomeruli were observed in 1 sample (Harrison stock) in which *P. minibicornis* was not detected by PCR.

Parvicapsula minibicornis was detected by PCR in 19 of 20 (95%) spawning pink salmon from the Fraser River near Chilliwack, in all 30 (100%) spawning coho salmon from 3 sites and in 21 of 40 (53%) spawning chinook salmon from 2 sites within the Fraser River drainage basin (Table 3). The prevalence of the parasite, determined histologically, was 85% in pink salmon, 66% in coho salmon, 0% in 1 chinook salmon sample and 20% in the second sample (Table 3). PCR analysis failed to detect the parasite in 3 of 4 chinook samples with histological evidence of infection in glomeruli. The severity of infections in spawning pink, coho and chinook salmon was considerably lower than those measured in spawning sockeye salmon (Table 3).

DISCUSSION

Parvicapsula minibicornis infections were detected in the kidney of sockeye salmon using PCR. While not quantitative, this method was shown to be specific for *P. minibicornis* and more sensitive in detecting the parasite than histological examination (St-Hilaire et al. 2002). Histological examination of kidney sections, stained with a MP stain (Humason 1979), were used here, however, to quantitate the severity of infection and to document how severity changed during the freshwater migration of adult salmon. The detection of *P. minibicornis* using PCR concurred with that obtained using histological examinations in most specimens. Despite this, it was not always possible to confirm the identity of sporoblasts and plasmodia observed in the glomeruli, tubules or renal interstitium. Thus, myxozoan infections were observed within glomeruli in a total of 3 sockeye samples that were negative by PCR, indicating either infection with another parasite or false-negative PCR results. Although the high prevalence of *P. minibicornis* in these

Table 3. *Parvicapsula minibicornis* in post-spawned coho salmon *Oncorhynchus kisutch*, chinook salmon *O. tshawytscha* and pink salmon *O. gorbuscha* collected from the Fraser River Drainage basin. PCR: number of kidney samples from which 1091 bp product was amplified. Negative, faint and strong refer to the detectability or relative intensity of the amplicon in ethidium bromide stained gels. Severity: mean number of infected glomeruli per 25 glomeruli examined from infected fish

Species	Location	Sample size	PCR Negative	PCR Faint	PCR Strong	Histology No. infected	Histology Severity
Coho	Deadman Creek	10	0	0	10	7	2.1
Coho	Coldwater River	10	0	0	10	6	1.3
Coho	Salmon River	10	0	0	10	7	1.9
Chinook	Chilliwack River	20	8	1	11	0	0
Chinook	Chehalis River	20	11	8	1	4	2.0
Pink	Fraser River	20	1	0	19	17	3.7

populations favoured the latter possibility, at least 3 other genera of myxozoa are known to parasitise the kidney of sockeye salmon in British Columbia (McDonald & Margolis 1993).

The patterns of distribution and prevalence of *Parvicapsula minibicornis* in migrating Fraser River sockeye in 2001 were similar to those observed in 1999 and 2000 (St. Hilaire et al. 2002). Infections in both Early groups and in the Summer group adult sockeye were not evident either at sea or prior to several 100 km of in-river migration. Transmission of the parasite to salmon has been hypothesized to occur in the lower river or estuary (St-Hilaire et al. 2002). While cage-exposure studies using juvenile naïve salmonids confirmed that transmission occurs at the river mouth and approximately 10 km upstream (S. Jones unpubl.), additional research is necessary to fully identify the limits of this area. Failure to detect the parasite until several days after river entry is probably due to the low numbers of developing parasites that are present immediately following transmission. PCR detected *P. minibicornis* earlier than histological examination of kidney in a significant number of salmon, as reported earlier by St-Hilaire et al. (2002). Therefore the initially high numbers of false-negative histological results from both Early groups and from Summer group sockeye were likely due to the relative insensitivity of this method in detecting early infections. It follows that subsequent increases in prevalence and severity observed in both stocks were the result of the proliferative development of the parasite. Infection with *P. minibicornis*, measured by prevalence, severity or the rate of false-negative histological diagnosis, may therefore provide a useful measure of time elapsed since transmission within a migrating population, in this case equated with time since the salmon re-entered the Fraser River. To fully develop this relationship, however, further research is necessary to better understand the factors that affect the rate of parasite development.

The overall high prevalence and relatively low rates of false-negative histology among sockeye salmon collected from the Harrison River were similar to those observed in both Early groups and in Summer groups after 600 to 700 km migrations, despite the relatively short distance (~110 km) between the Harrison River and the Fraser River estuary. Together, these observations suggested that more time had elapsed since parasite transmission than would be predicted based on migration distance alone. This further supported the view that the severity of *Parvicapsula minibicornis* infection was exacerbated in some stocks by prolonged river residence times before spawning due to their premature migration into the river (Raverty et al. 2000, St-Hilaire et al. 2002). Weaver Creek is one of several Late run-timing stocks that has experienced premature river entry dates since 1995 (Pacific Salmon Commission 2001). This early migratory behaviour prolongs river residency, since dates of spawning have not changed. The present data also suggest, however, that a proportion of this stock retained an historical pattern of migration with typical river residence times. The initial absence of detectable infection among Weaver Creek stock sockeye suggested that any delay in migration since entering the Fraser River had been minimal for the first salmon of this stock to be captured in the Harrison River. Subsequent increases in prevalence and severity within the Weaver stock indicated a progressive increase in the period of freshwater residence. Thus, Weaver Creek sockeye that were late entering the Fraser River appeared to migrate with less delay into the Harrison River, evidently ahead of some of the same stock that had entered the Fraser River earlier. Whether the spawning success of the relatively less infected early migrants differed from that of the more heavily infected later migrants, however, was not determined here. The significantly lower severity of infection among Harrison stock sockeye probably related to the last collection from this stock (on September 29) being made approximately 44 d before peak spawning (November 11 to 15). The apparent fluctuations in severity observed in Harrison stock sockeye were due to small sample sizes (e.g. September 13 and 20).

Infection with *Parvicapsula minibicornis* in pink salmon represents a new host record. In addition, the use of PCR confirmed for the first time the identity of the parasite in coho salmon as *P. minibicornis*. These fish were mature adults collected at or near the spawning grounds and in most cases had already spawned. The occurrence of parasite stages in the glomeruli of 85% of PCR-positive pink salmon suggested that parasite development was similar to that in sockeye. The high proportion of glomerular infections in coho salmon also suggested similar tissue tropism and development in this host species. In contrast, *P. minibicornis* was rarely detected by histological examination in chinook salmon. Thus, sockeye and pink salmon were evidently the more suitable host species for *P. minibicornis*, followed by coho salmon. The failure of the parasite to develop infections within the glomeruli in most chinook salmon indicated that while susceptible, this species is not a suitable host. Undescribed *Parvicapsula* species have been reported from wild and farmed coho salmon in British Columbia and from net pen reared coho salmon in Puget Sound, Washington (USA) (Hoffman 1984, Johnstone 1984, Kent 1998). Johnstone (1984) also reported *Parvicapsula* sp. in farmed chinook salmon, cherry salmon *Oncorhynchus masou*, Atlantic salmon *Salmo salar* and cutthroat trout *S. clarki* in Puget Sound. The specificity of the PCR

used in the present study suggested that the parasites previously observed in coho and chinook salmon were *P. minibicornis*.

Parvicapsula minibicornis has been associated with significant pre-spawning mortality among certain Late run-timing stocks of Fraser River sockeye (for example Weaver Creek and Cultus Lake) (St-Hilaire et al. 2002), which entered the Fraser River earlier than normal (Pacific Salmon Commission 2001). The occurrence of this parasite in adult pink, coho and chinook salmon from the Fraser River suggested that under appropriate conditions some or all of these species may also be at risk to elevated pre-spawn mortality associated with *P. minibicornis*. Further study to understand factors contributing to the pathogenicity of *P. minibicornis* in its various hosts is warranted.

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