Parvicapsula minibicornis infections in gill and kidney and the premature mortality of adult sockeye salmon (*Oncorhynchus nerka*) from Cultus Lake, British Columbia

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Abstract: In recent years, large losses of migrating adult sockeye salmon (*Oncorhynchus nerka*) from the Fraser River, British Columbia, Canada, have resulted in reductions in catches and productivity. We investigated patterns of mortality and the occurrence of the myxosporean parasite *Parvicapsula minibicornis* in adult sockeye salmon from Cultus Lake, tributary to the Fraser River. Using data from a captive broodstock program, we found that early migrants to Cultus Lake had less severe *P. minibicornis* infections and were more likely to survive to maturity than those fish that arrived later after they had presumably held in the warmer Fraser River. We found *P. minibicornis* in kidneys and gills of spawners. In some fish, significant histopathology in the gills that included severe inflammation and hyperplasia of the gill lamellae was observed; the severity of the disease was correlated with the severity of *P. minibicornis* infections. Kidney and gill pathologies were more prevalent and more severe in fish that died before spawning compared with those that matured successfully. Gill disease associated with *P. minibicornis* infections had not been previously identified in Fraser River sockeye salmon, and its role in the loss of spawners needs further investigation.

Résumé : Ces dernières années, de lourdes pertes de saumons rouges (*Oncorhynchus nerka*) adultes migrateurs dans le fleuve Fraser, Colombie-Britannique, Canada, ont causé des réductions des prises et de la productivité. Nous examinons les patrons de mortalité et l'occurrence de la myxosporidie parasite *Parvicapsula minibicornis* chez les saumons rouges adultes du lac Cultus qui se déverse dans le fleuve Fraser. À l'aide de données provenant d'un programme impliquant un stock reproducteur captif, nous observons que les migrateurs précoces dans le lac Cultus ont des infections à *P. minibicornis* moins sévères et qu'ils sont plus susceptibles de survivre jusqu'à la maturité que les poissons qui arrivent plus tard après avoir vraisemblablement été retenus dans les eaux plus chaudes du fleuve Fraser. Nous avons trouvé *P. minibicornis* dans les reins et les branchies des reproducteurs. Nous avons observé chez certains poissons une importante histopathologie des branchies et, en particulier, une inflammation sévère et une hyperplasie des lamelles branchiales; la gravité de la maladie est en corrélation avec la sévérité des infections de *P. minibicornis*. Les pathologies des reins et des branchies sont plus fréquentes et plus graves chez les poissons qui sont morts avant la fraie, par comparaison à ceux qui ont frayé avec succès. On n'avait pas signalé auparavant la maladie des branchies associée à *P. minibicornis* chez les saumons rouges du fleuve Fraser et il faudra étudier plus au fond son rôle dans la perte des reproducteurs.

[Traduit par la Rédaction]

Introduction

The Fraser River in British Columbia, Canada, is home to numerous sockeye salmon (*Oncorhynchus nerka*) populations that are the mainstay of commercial, sports, and aboriginal fisheries in the region. Since 1995, the timing of entry of many adult sockeye salmon into the Fraser River from the ocean has advanced a month or more, for reasons that are still unknown (Cooke et al. 2004). A group of populations known as the "late runs" have been most affected by the change in behaviour, and high rates of adult mortality have been observed in some years (Cooke et al. 2004). Prior

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to 1995, late runs arrived at the mouth of the Fraser River in August but held in the estuary and delayed their entry into the river until late September. However, many of these fish now enter the Fraser River without delay in August and early September. This change in migration timing exposes migrants to higher river temperatures and flows than would have occurred historically (Patterson et al. 2007). English et al. (2005) and Farrell et al. (2008) both found that earliest migrants of the run were the least likely to reach their spawning grounds compared with those whose timing most resembled the historic pattern. Temperature stress, energy depletion, and disease have all been hypothesized as mechanisms linking the change in migration timing to adult mortality (Cooke et al. 2004; Farrell et al. 2008).

Included among late-run populations are sockeye salmon that spawn in Cultus Lake, located in the lower Fraser valley. This population had been trending downwards since the mid-1970s but took a precipitous decline in the late 1990s largely because of two successive years of >80% mortality of adults prior to spawning in the lake (Cooke et al. 2004). The myxosporean parasite *Parvicapsula minibicornis* is very prevalent in spawners from Cultus Lake and was thought to have contributed to that mortality (St-Hilaire et al. 2002). *Parvicapsula minibicornis* becomes abundant in the glomeruli of the kidney and renal failure has been speculated as cause of death (Raverty et al. 2000); however, the exact mechanism has not been established. The population has since been assessed and is now considered endangered by the Committee on the Status of Wildlife Species in Canada (2003). The reoccurrence of adult mortality events will likely imperil the already diminished population (Committee on the Status of Endangered Wildlife in Canada 2003), so a captive broodstock and hatchery supplementation program was initiated in 2001 to increase the number of spawners returning to the lake.

In the fall of 2006, field crews noticed that fish being collected at Cultus Lake for the hatchery program were particularly lethargic, and spawners were observed swimming at the surface of the lake, behaviours not normally observed. Subsequently, a large number of dead unspawned fish were collected from the lake, suggesting that another bout of premature adult mortality was occurring. These observations led to some opportunistic sampling of fish in 2006 and a more structured sampling program in 2007 in an attempt to understand the patterns of mortality and its causes.

The objective of this paper is to describe the mortality and histopathology of maturing Cultus Lake sockeye salmon. The presence of the captive broodstock program allowed us to follow the progression of *P. minibicornis* infections and disease in a more controlled environment than was previously possible from the examination of field collections. We used broodstock fish to compare the severity of infection and disease in fish that successfully matured relative to those that died before spawning.

Materials and methods

Study site

Cultus Lake (49°03′N, 121°59′W) is a small monomictic (6.4 km^2) lowland lake (elevation 46 m) located in southwestern British Columbia (Fig. 1). It has a maximum depth of 48 m and a relatively limited littoral zone. Surface water of the lake reaches temperatures >20 °C in the summer and a strong thermocline develops at 15–20 m depth. Temperatures below the thermocline remain at 6–8 °C (Shortreed 2007). In recent years, hypolimnetic oxygen depletion occurs over the fall, and by late fall, oxygen levels decrease to <6 $mg \cdot L^{-1}$ at 30 m depth (<50% of saturation; Shortreed 2007). The lake becomes isothermal and mixes in late fall (Pon et al. 2010).

Cultus Lake is drained by Sweltzer Creek, which flows 3 km north from the lake to the Vedder River. The Vedder River flows a further 13 km to its confluence with the lower Fraser River 99 km upstream from the ocean (Fig. 1). Water temperatures in the Fraser River can reach 20 °C in August but begin to decline in September (Fig. 2). The Vedder River is generally cooler than the Fraser River at the time of migration. Sweltzer Creek is very warm (>20 °C) during the summer and fall, as it is supplied by surface water from the lake (Fig. 2).

The timing of marine migrations of adult Cultus Lake sockeye salmon is only known from tagging studies conducted in the 1930s (Foerster 1936). Those studies found that adults arrived at the mouth of the Fraser River in August, but their progression up the Fraser was delayed to September, as was the case for other late-run populations. Historically, sockeye arrived at Cultus Lake in October and November (Fig. 3). However, since 1995, fish now enter the lake in August and September (Fig. 3). After entering the lake, spawners hold below the thermocline in 6–8 °C water (Pon et al. 2010). Spawning peaks in late November (Brannon 1987) and takes place on submerged gravel beds along the margins of the lake at depths ranging from 1 to 30 m. Little, if any, creek spawning takes place.

Fish sampling

A salmon counting weir is located in Sweltzer Creek near Cultus Lake and it has been operated annually since 1923 (Foerster 1936). Adults were collected from the weir for the captive broodstock program and we utilized these fish to observe the progression of disease and mortality. Fish were collected throughout the run and were immediately transferred to large, circular concrete ponds at the adjacent Cultus Lake Salmon Research Laboratory. These ponds are supplied with 6–8 °C aerated hypolimnetic water from Cultus Lake. Each fish received a unique passive integrated transponder tag for tracking purposes and was injected with 0.3 mL of Liquamycin LA 200 (oxytetracyline). The holding ponds were treated with Parasite S formalin solution (Syndel Laboratories, 100 ppm for 60 min) three times per week to minimize infections from external protozoans and fungi.

Holding ponds were examined each weekday for mortalities, which were then removed for passive integrated transponder tag recovery. In 2007, but not 2006, samples from premature mortalities were taken for assessment with histopathology. Once fish began to ripen, they were examined for maturity once per week from mid-November to early January. Females were killed at the time of spawning; males were returned to the holding ponds for subsequent spawning. Ovarian fluid from spawned females was tested for infectious haematopoetic necrosis virus using the cell culture method described by Traxler et al. (1997). Ovarian fluid was used,

Fig. 1. Map of the study area in southwestern British Columbia (BC), Canada.

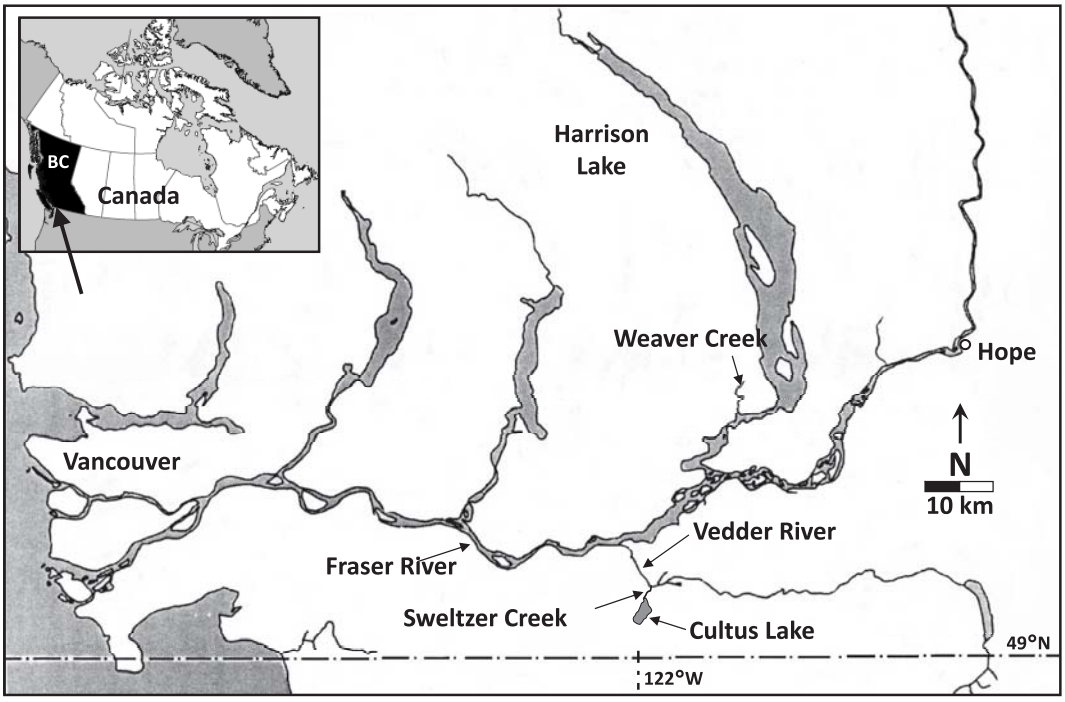
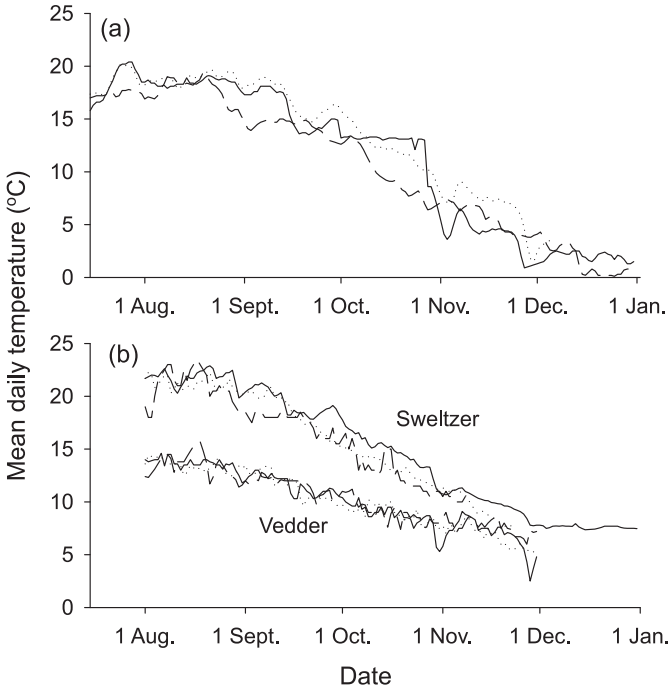


Fig. 2. Mean daily temperatures of (*a*) Fraser River (near Hope, British Columbia) and (*b*) Sweltzer Creek and the Vedder River. Solid line, 2006; dotted line, 2007; dashed line, 2008.



as viral particles are more stable than in tissue samples. All fish sampled were negative for this virus. Kidney tissues were also tested for bacterial kidney disease using the ELISA assay (Pascho et al. 1991). In 2006, 110 of 121 females were considered negative (pptical absorbance <0.08); the remaining 11 females had low-level infections (absorbances ranged from 0.086 to 0.13; Pascho et al. (1991) considered optical densities <0.2 to be a low-level infection). In 2007, all 75 females tested negative. Spawned-out fish in both years were retained for disease examinations.

In 2007, moribund or fresh dead (defined as having pink or red gills) prespawning mortalities that were recovered from field surveys of the lake were sampled for histopathology. We also present data on the arrival and mortality of spawners in 2008, but disease investigations were not conducted in this year.

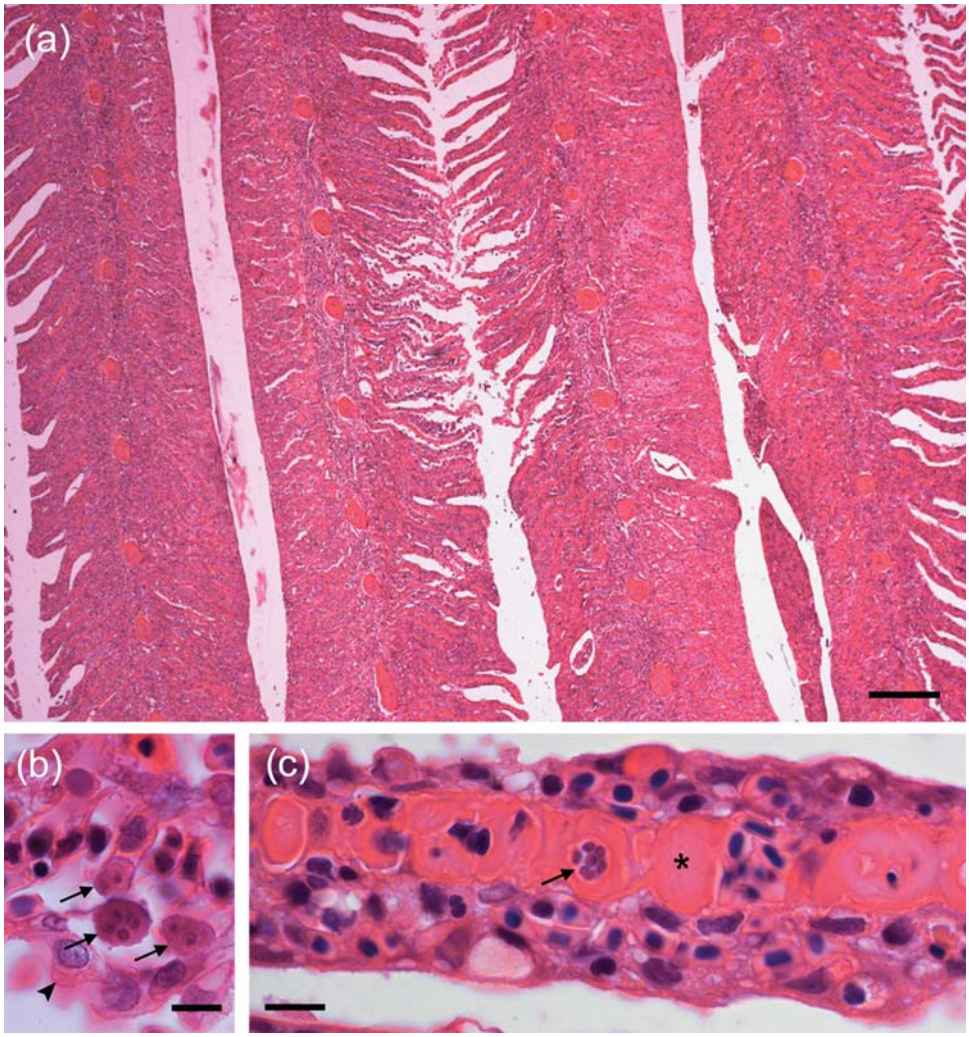
Histopathology

In 2006, only gill and posterior kidney samples from successfully spawned fish from the captive broodstock program were taken. In 2007, samples of posterior kidney, gill, liver, spleen, and heart were taken from all fish. All tissues were initially fixed in formalin for 24 h and were then transferred to 70% ethanol or isopropanol. Tissues were then dehydrated through a graded series of ethanols and were embedded in parafin wax. Five micrometre sections were mounted on glass slides and stained with haematoxylin and eosin. In 2006, the slides were interpreted at the Pacific Biological Station (Nanaimo, British Columbia); the 2007 material was read at the Atlantic Veterinary College (Charlottetown, Prince Edward Island). In both cases, the readers were blind to the origins of the fish. Unfortunately, scoring systems used each year were different, which precluded comparisons across years.

For the 2006 samples, the gills and kidneys were examined for the presence of abnormalities. A 0–3 scoring system was used that depended on a visual assessment of the the proportion of abnormal tissue on the slide. Scores were assigned as 0: no abnormalities, 1: <10%, 2: 10%–50%, and 3: >50%.

In 2007, a more quantitive scheme was devised to describe histopathology. For kidney, 25 glomeruli from each fish were examined and the number of glomeruli that contained *P.*

Fig. 3. Sockeye salmon gills. (*a*) Severely affected gills with diffuse lamellar fusion and inflammation in both the gill filaments and lamellae; this gill would receive a score of 3 in the quantitative analysis. Scale bar = 200 μm. (*b*) Developmental stages of the parasite (arrows) localized beneath the gill epithelium (arrowhead). Scale bar = 10 μm. (*c*) Gill lamella with a parasite within a pillar channel (arrow) and eosinophilic material filling the lumen of the channel (asterisk). Scale bar = 10 μm.



minibicornis parasites was recorded following the procedure of Jones et al. (2003). A glomeruli score was defined as the proportion of the 25 that were positive for the parasite. Additionally, 100 renal tubules were examined in each fish by analyzing about 10 microscope fields under the 40× objective, and the renal tubule score was defined as the proportion that contained *P. minibicornis* in the lumen.

Analysis of gill tissue collected in 2007 was done by a 0–3 scoring system. A gill score of 0 was given if no inflammation or lamellar fusion was observed, a 1 if focal lamellar fusion affecting four or less lamellae and mild infiltration of inflammatory cells was observed in <10% of the tissue on the slide, a 2 if focal lamellar fusion extended to over four lamellae and an inflammatory infiltrate was observed in the gill filament affecting between 10% and 25% of the tissue, and a 3 for a severe inflammatory response that included both the gill filaments and lamellae and lamellar fusion that affected over 25% of the gill tissue (see Fig. 3). Additionally, roughly 10 lamellae of a gill filament were observed with the 40× objective to quantify the number of parasites present in the gills. A score of 0 was given if no parasites were observed, a 1 if one to nine parasites were observed, a 2 if 10–19 parasites were observed, and a 3 if over 20 parasites were seen. In a subsample of fish, the in situ hybridization procedure of Jones et al. (2004) was used to confirm the presence of *P. minibicornis* in the gills.

The bulbus arteriosus of the heart was examined for the presence of *Loma* xenomas and all other tissues were screened for abnormalities.

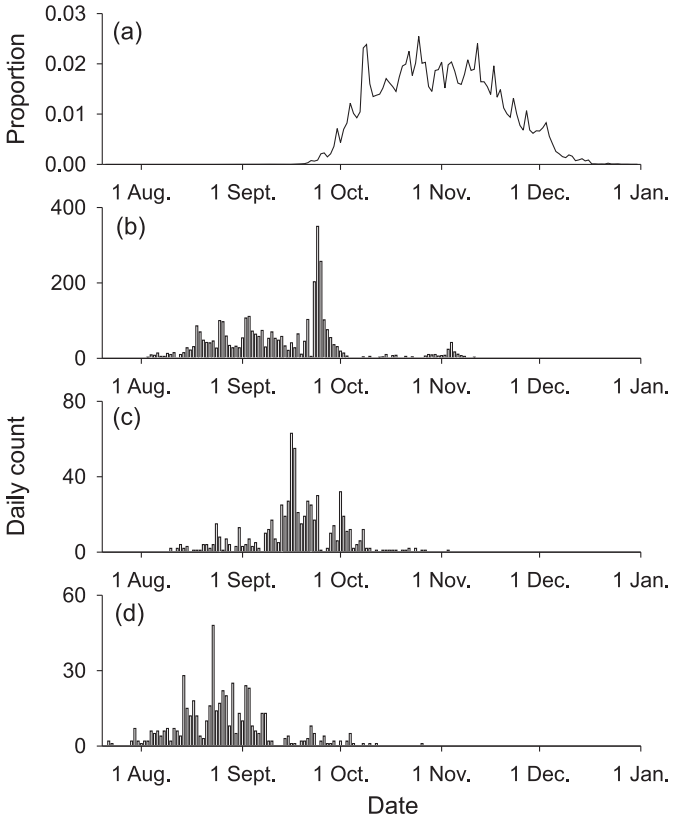
Results

Spawning in Cultus Lake

A total of 3797 sockeye salmon arrived at the counting fence in 2006, with the first arrival on 31 July. Most of the run had entered the lake by the beginning of October (Fig. 4). The broodstock program retained 277 fish that were taken between 25 August and 21 November.

Field cre ws made the first observations of abnormal behaviour of spawners in the lake in mid-October 2006. Adults were observed swimming slowly at the surface of the lake and along its margins. Fall rains increased flows to small

Fig. 4. Timing of the arrival of spawners at the Sweltzer Creek counting weir. (*a*) Historical data are averaged over 1941–1994 (Schubert et al. 2002). Recent counts are for (*b*) 2006, (*c*) 2007, and (*d*) 2008 and are Fisheries and Oceans Canada unpublished data (M. Bradford, Fisheries and Oceans Canada, Burnaby, British Columbia).



streams draining into the lake and salmon were observed attempting to swim into the rapidly flowing water. A total of 78 dead unspawned females were collected from the lake between 31 October and 24 November by boat and foot surveys. Heavy rains in late November raised lake levels and added turbidity and debris to the water, which largely prevented the recovery of carcasses after that point. Because spawning takes place deep in the lake and spawned-out carcasses are not easily captured, obtaining a reliable estimate of the rate of prespawning mortality is not possible for the Cultus Lake population.

A total of 649 spawners returned to Cultus Lake in 2007; the run began on 10 August and was complete on 4 November. Most fish arrived at the fence in mid-September (Fig. 4). The hatchery program retained 151 fish. Only nine unspawned females were found in the lake; field crews did not observe any abnormal behaviours or surface swimming in 2007.

The 2008 return was the earliest on record. The run began in July and was largely complete by early September. A total of 360 fish arrived at the lake and 159 were retained for the captive broodstock program. This was the only year in which broodstock were collected in early August. Eight unspawned females were collected from the lake.

Mortality in the captive broodstock

In 2006, 81 salmon (29% of the total) died before spawning in the broodstock holding ponds. In 2007, the mortality rate was 12%, and in 2008, 21% died prematurely. Mortality was a function of the date of arrival at the counting fence: fish arriving before October generally suffered low mortality (Fig. 5). When analyzed with a logistic regression, the timing of mortality in 2006 and 2007 was similar ($P > 0.3$). The arrival date that resulted in an expectation of 50% mortality for these years was 25 October. In 2008, the mortality pattern was somewhat different, as fish collected in early August suffered high mortality but those collected after that followed a similar pattern to the other years. Logistic regression fitted to the 2008 data was significantly different from that of 2006 and 2007 ($P < 0.001$ using data after 15 August) with the predicted date of 50% mortality of 30 September. There was no effect of sex on the timing of mortality ($P > 0.3$).

Histopathology

Parvicapsula minibicornis infections were frequent in kidneys, and in many instances, every glomerulus that was inspected was heavily infected with parasites. *Parvicapsula minibicornis* was observed both in the visceral layer of Bowman's capsule and within Bowman's space. There was no noticeable cellular host response around or within infected glomeruli, although the infection was associated with eosinophilic material throughout Bowman's capsule and into Bowman's space (Fig. 6). Both parasites and eosinophilic material were also observed in the lumen of renal tubules (Fig. 6).

In gills, pathological lesions ranged from absent to severe. In severe cases, lesions included multifocal to diffuse areas of lamellar fusion and an inflammatory infiltrate composed of neutrophils, macrophages, and lymphocytes in the gill filament and lamellae (Fig. 3*a*). *Parvicapsula minibicornis* was found in three anatomical locations within the gill. The first was within the hyperplastic epithelium of the gill lamellae and in this case were accompanied by an inflammatory infiltrate. The second location was in unfused gill lamellae and in these situations were localized underneath the lamellar epithelium sandwiched between the lamellar epithelium and the basement membrane of the pillar system (Fig. 3*b*). Finally, the parasite was observed directly within pillar channels of the lamellae (Fig. 3*c*), and in these instances, the lumen of the pillar channels contained eosinophilic material, which appeared to occlude the lumen (Fig. 3*c*). In situ hybridization confirmed that the commonly observed parasite observed within gills was *P. minibicornis* (Fig. 7).

Although the myxosporean *Ceratomyxa shasta* is present in the lower Fraser River (Ching and Munday 1984) and uses the same invertebrate host as *P. minibicornis* (Bartholomew et al. 2006), it was not found in fish examined in this study.

Microsporidial infections likely caused by *Loma salmonae* were observed in many fish. *Loma* infections were most frequently found in the bulbus arteriosus of the heart, although they were also observed in gills and spleen. Infections in the bulbus arteriosis ranged from a single xenoma to heavy infections of up to 20 xenomas within a single section. In some cases, the bulbus arteriosis was positive for *Loma*,

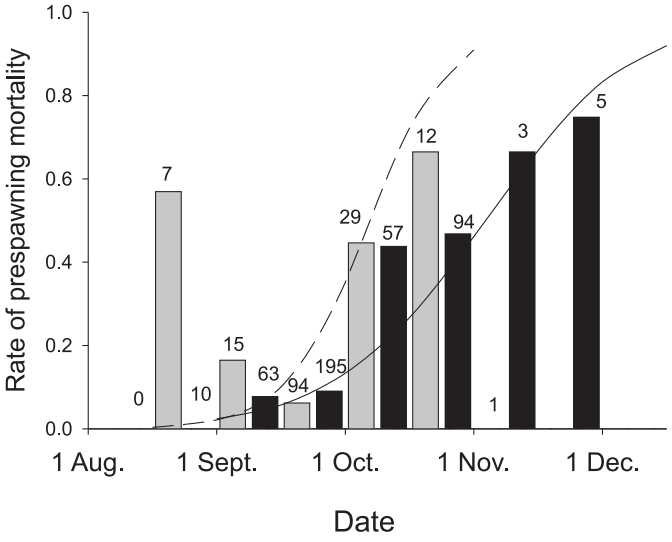


Fig. 5. Relationship between the probability of a fish in the captive broodstock dying before spawning and the date of its collection from the Sweltzer Creek weir. Lines are logistic model fits to individual fate data; histograms are the proportion of fish that died before spawning by semimonthly interval. Sample sizes are indicated above the bars. Data from 2006 and 2007 are combined (solid line and solid bars), as there was no statistical difference between years; the dashed line and shaded bars are for 2008. Data from the first half of August 2008 were not used to fit the logistic model.

while the parasite was not detected in the gills of the same fish.

There was little liver pathology throughout the sample. One interesting finding was the observation of single cell necrosis through the liver of nine fish. We observed necrotic hepatocytes in various stages of degeneration surrounded by healthy cells. The cause of this condition is unknown.

Quantitative analysis of infection and pathology

In 2006, tissue samples were only collected from successfully spawned fish from the captive broodstock program. Abnormalities were observed in 64% of kidneys and 89% of gills that were examined. Both kidney and gill scores were positively correlated with the date of capture at the fence (kidney: $r = 0.32$, $P = 0.003$, $N = 86$; gill: $r = 0.51$, $P < 0.001$, $N = 110$), suggesting that the severity of disease at the time of spawning was related to the timing of migration to Cultus Lake. Gill and kidney scores were positively related to each other within fish ($r = 0.49$, $P < 0.001$, $N = 84$).

After the 2007 slides were read, the data were organized into three groups: broodstock fish that matured successfully, broodstock fish that died prematurely, and fish recovered from the lake that had died before spawning. The prevalence of *P. minibicornis* in kidney and gill and the prevalence of pathology were consistently highest in the two groups of fish that died before spawning (Table 1). Nonetheless, *Parvicapsula* was identified in nearly half of the fish that were successfully spawned. The prevalence of *Loma* in the heart and spleen was low and unrelated to spawner fate.

There were significant differences in mean scores between the three groups of fish for glomeruli, renal tubule, gill pathology, and gill parasite scores (one-way ANOVA, all $P < 0.001$, $N = 81$–96; in Fig. 8, successful broodstock fish are separated by the date of spawning). In all cases, a post hoc contrast indicated that scores were significantly lower for the successful spawners compared with fish that had died prematurely in the broodstock or the lake (all $P < 0.001$).

Egg takes for the captive broodstock program spanned a 7-week period (Fig. 8). There was no significant variation in mean pathology scores among sampling periods (ANOVA, all $P > 0.2$, $N = 56$–59) but there appeared to be a weak increasing trend in some measures (Fig. 8). These weak trends were confirmed with regressions of pathology scores on the date of sampling (glomeruli: $r = 0.21$, $P = 0.12$; renal tubules: $r = 0.22$, $P = 0.10$; gill pathology: $r = 0.26$, $P = 0.06$; gill parasites: $r = 0.27$, $P = 0.04$).

For captive broodstock fish, three pathology scores were positively correlated with the date that they were captured at the counting fence (glomeruli: $r = 0.62$, $P < 0.001$; gill pathology: $r = 0.59$ $P < 0.001$; gill parasites: $r = 0.48$, $P = 0.002$). Fish arriving at Cultus Lake after mid-October had heavy *P. minibicornis* infections in the glomeruli at the time of sampling (Fig. 9). All three scores were negatively correlated with the date of death (all $P < 0.001$) largely because most of the premature broodstock mortalities, which had the highest disease scores, occurred before the egg takes took place. Scores for renal tubules were not related to the date of arrival ($r = 0.14$, $P = 0.23$, $N = 74$) or the date of sampling ($r = 0.06$, $P = 0.64$).

The severity of infections in renal tubules and gill scores were positively correlated with glomeruli scores (all $r > 0.6$, $P < 0.001$, $N > 80$) (Fig. 10). Those relationships tended to take on a triangular form, as there were fish with high levels of infection in the glomeruli that did not have high levels of other pathologies, but rarely did the reverse situation occur. Most fish that died before spawning had high levels of infection in glomeruli and nonzero renal and gill scores. Gill pathology and gill *P. minibicornis* scores were also positively correlated (Spearman's $\rho = 0.75$, $P < 0.001$, $N = 82$).

Discussion

Our investigations have extended our understanding of causes of mortality in late-run Fraser River sockeye salmon in a number of ways. Our data from the captive broodstock are the first that show a clear difference in *P. minibicornis* infections and related pathology between fish that died prematurely and those that successfully matured, providing correlative evidence for the role of *P. minibicornis* as a factor in premature mortality. Our finding of significant inflammation of gill tissue in many fish associated with *P. minibicornis* raises the possibility that impaired gill function may play a role in the premature mortality of Fraser River sockeye salmon.

The freshwater habitats used by adult Cultus Lake salmon have changed considerably with the change of timing of entry to the river that began in 1995. Although direct information on the timing of migration is not available because of their relative rarity compared with other late-run populations, observations of fish first arriving at Cultus Lake in late July suggest that their migration to freshwater has

Fig. 6. Sockeye salmon kidney infected with *P. minibicornis*. (*a*) A renal tubule and the two glomeruli with abundant eosinophilic material (asterisk). Scale bar = 50 µm. (*b*) A glomerulus at higher magnification with a *P. minibicornis* infection (arrows) and dense eosinophilic material (asterisk) throughout. Scale bar = 10 µm.

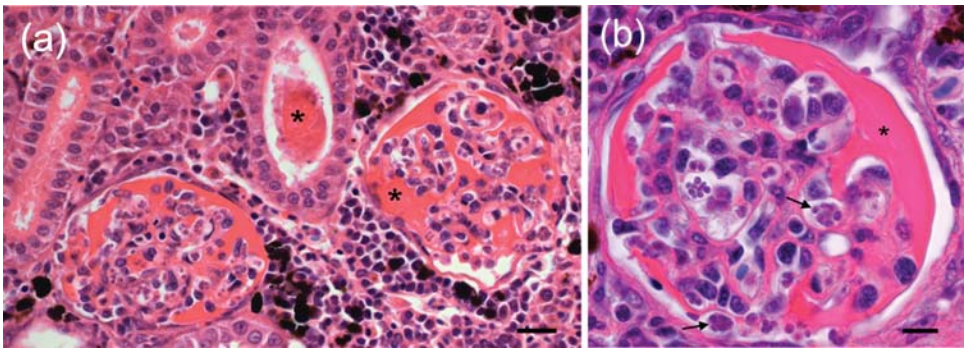


Fig. 7. In situ hybridization and staining of *P. minibicornis* (in blue) in gills of adult sockeye salmon with mild hypertrophy of the lamellar epithelium.

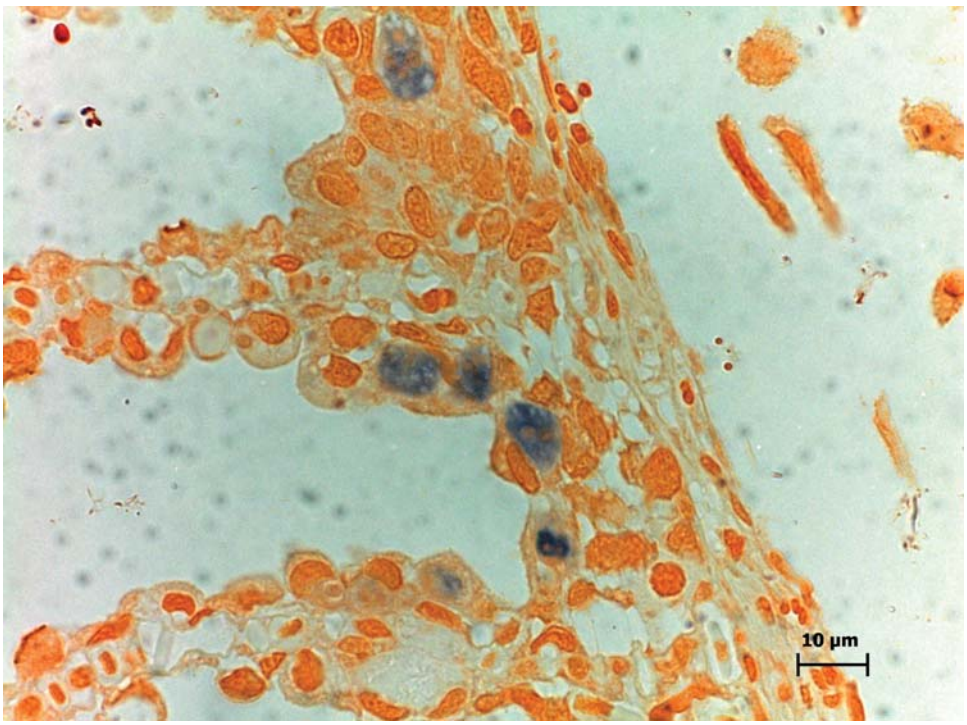


Table 1. Prevalence of *P. minibicornis*, *Loma*, and related pathologies in adult sockeye salmon from Cultus Lake in 2007.

	Egg take	Brood PSM	Lake PSM
Mean date of sampling	8 December	7 November	8 November
P. minibicornis in glomeruli	27/60 (45%)	16/16 (100%)	17/17 (100%)
P. minibicornis in renal tubules	13/59 (22%)	16/17 (94%)	8/8 (100%)
Gill pathology	15/57 (26%)	12/13 (92%)	11/12 (92%)
P. minibicornis in gill	7/58 (12%)	11/15 (73%)	13/15 (87%)
Loma in heart	7/59 (12%)	1/17 (6%)	3/20 (15%)

Note: Egg take refers to spawners that were successfully spawned in the captive broodstock program; the other columns are data from prespawning mortalities in the captive broodstock (brood PSM) and in the lake (lake PSM). Sample sizes vary among metrics because of poor tissue preservation.

advanced much like other late runs (Cooke et al. 2004). Upon entry to the Fraser River, fish are immediately exposed to warm water and *P. minibicornis* actinospores (Wagner et al. 2005) produced by its alternate invertebrate host (Bartholomew et al. 2006). We infer from the timing of arrival at Cultus Lake that most individuals now move

Fig. 8. Mean pathology scores (±SE) for 2007 adult Cultus Lake sockeye salmon. Data are organized by lake and broodstock prespawning mortality (PSM) and for fish sacrificed for egg takes from the captive broodstock program.

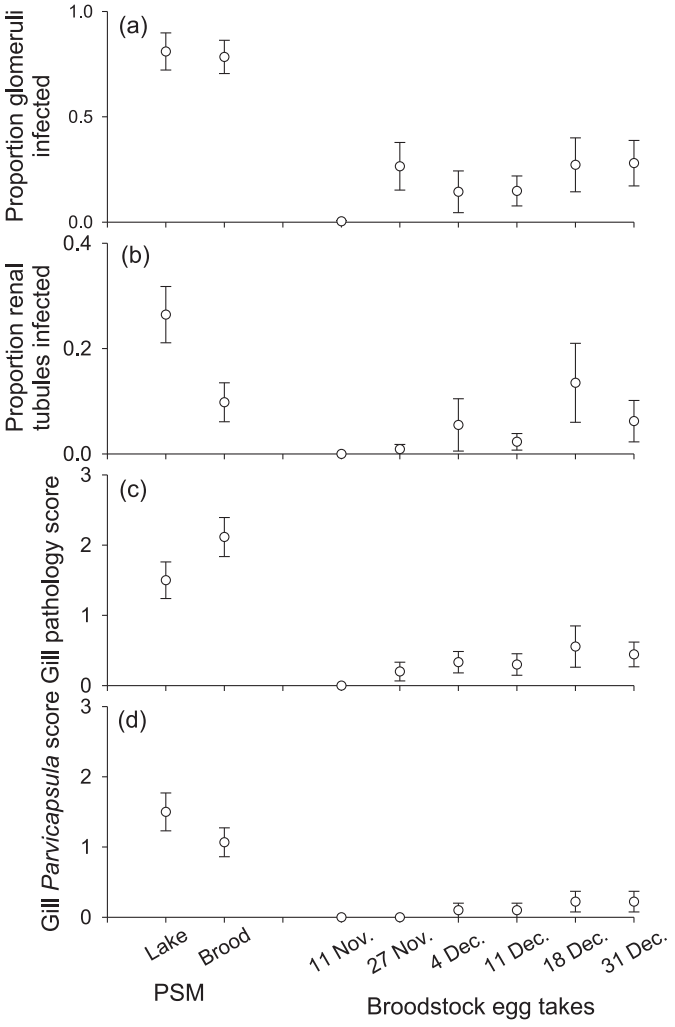
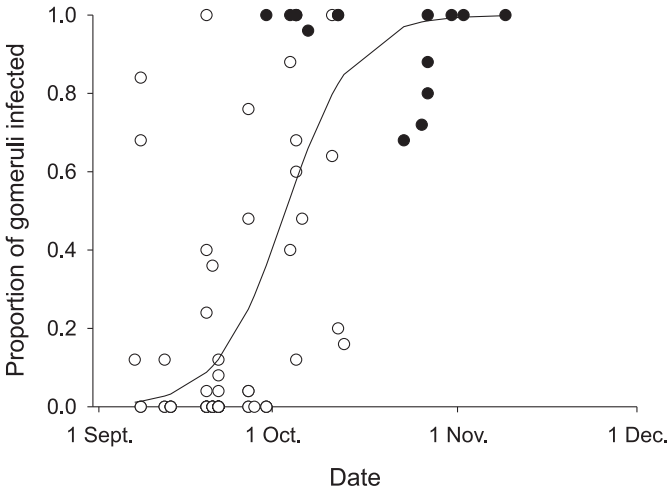


Fig. 9. Relationship between the proportion of kidney glomeruli infected with *P. minibicornis* at the time of death and the date of collection from the Sweltzer Creek weir for captive broodstock fish in 2007. Open circles represent fish that were spawned successfully and solid circles represent fish that died before spawning. The line is a logistic regression fit by nonlinear parameter estimation.



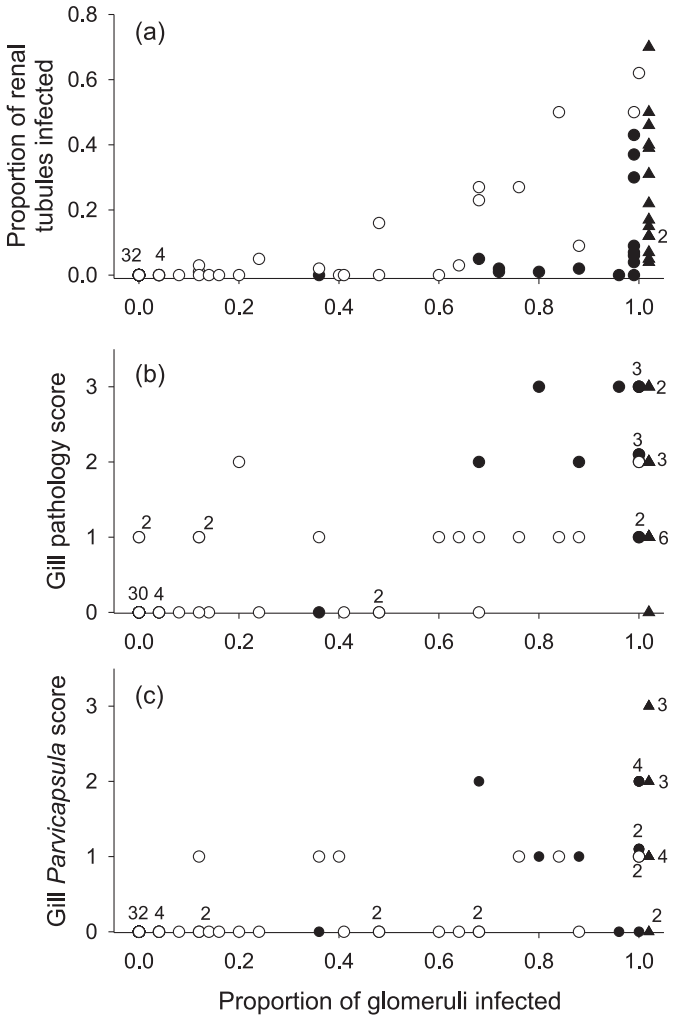
relatively quickly to the lake and hold in the hypolimnion up to 3 months before spawning. Based on our observations on captive broodstock fish, this behaviour appears to retard the progression of disease and increase the probability of survival. Whether the recent change in the location of holding for Cultus Lake sockeye is an adaptive response to disease or a fortunate outcome of the overall change of migration timing from the ocean is currently unknown; however, it would appear to be advantageous from a disease perspective, as most fish enter the lake early enough to be able to mature successfully.

We think that fish that arrived at the lake in late October or November (which is the historical timing) probably held in warmer downstream habitats and that allowed *P. minibicornis* and possibly other infections to develop, reducing the likelihood of successful reproduction in the lake. This behaviour is somewhat different from that observed for the nearby Weaver Creek population where later migrants were found to be more likely to spawn than early ones (Mathes et al. 2010). Mathes et al. (2010) proposed that successful migrants delayed their entry into the Fraser River until later in the season and were exposed to lower water temperatures in the river, which reduced the risk of disease in these fish. Our disease data suggest that most Cultus Lake fish enter the river relatively early in the season, and variation in survival is a consequence of variation in the length of residence in the Fraser River.

The increased mortality of 2008 broodstock fish that arrived at Cultus Lake before mid-August suggests that other agents of mortality may also be involved. These early migrants are unlikely to have been in the Fraser River long enough to allow *P. minibicornis* to fully develop, although they would have been exposed to peak temperatures in the Fraser River and Sweltzer Creek during their migration. Most of these fish died within 2–3 weeks after capture, despite being held in the relatively cool water of the holding ponds. For other late-run sockeye populations, the earliest migrants suffer high mortality and it has been conjectured that this is the result of exposure to higher than normal river flows and temperature (Gilhousen 1990; English et al. 2005; Farrell et al. 2008). It is also possible that these very early fish are in a stressed condition as a result of their abnormal migration timing from the ocean and may be susceptible to a number of factors that could contribute to their death (Mathes et al. 2010). A larger sample and a thorough examination of the physiology and histology of the earliest migrants are needed to determine the cause of death.

We found a large difference in the intensity of infection by *P. minibicornis* for adults that died before spawning compared with those that successfully matured. Previous investigators have not been able to identify differences in prevalence or intensity of infections between successful spawners and premature mortalities (St-Hilaire et al. 2002; Jones et al. 2003); however, those studies relied on samples from postspawning fish for which the disease may have progressed significantly after the start of spawning. Sockeye salmon can survive for a week or more after spawning (Foerster 1968), and the rapid senescence of fish during

Fig. 10. Relationship between the (*a*) proportion of renal tubules infected with *P. minibicornis*, (*b*) gill pathology score, and (*c*) gill *P. minibicornis* score and the proportion of kidney glomeruli infected with *P. minibicornis* for individual Cultus Lake sockeye salmon in 2007. Open circles represent fish that were spawned successfully in the captive brood stock, solid circles represent captive broodstck fish that died before spawning, and triangles represent prespawning mortalities from Cultus Lake. Numerals indicate the number of overlapping points. In some cases, points were jittered on the *x*-axis to reduce overlap.



their final days may contribute to an increase in the severity of disease.

The similarity in disease scores between prespawning mortalities in the lake and captive brood ponds as well as the similarity in the median time to death suggest that our conclusions from broodstock data can be cautiously extrapolated to wild fish. However, there are a number of factors that could cause the progression of disease in the rearing ponds to be different from that in the lake. Confinement in ponds likely induces stress in these fish and requires the use of prophylactic drug treatments to prevent the outbreak of bacterial and fungal infections. In other fish, oxytetracycline has been shown to cause a depression in the immune response (Lundén et al. 1998), which may affect susceptibility to disease. Nonetheless, there were both successful spawners and premature mortalities, large variations in the severity of infection and disease, and associations between the timing of arrival, disease, and fate that suggest that the drug treatments did not significantly affect the range of outcomes in the broodstock population. Unfortunately, it is very difficult to sample actively spawning fish in Cultus Lake to evaluate infection and disease because of the depth at which they spawn.

The prevalence and severity of infection of *P. minibicornis* in kidneys were very high among many of our samples. The eosinophilic material seen throughout glomeruli was first reported by Kent et al. (1997) as an eosinophilic fibrinoid exudate that resembles basement membrane thickening. In order for the parasite to become released into Bowman's space and into the renal tubules and urine, it must cause a breach in the glomerular basement membrane, and that may be the cause of the exudate. Diffuse thickening of the glomerular basement membrane is observed with other diseases such as viral hemorrhagic scepticaemia and bacterial kidney disease (Reimschuessel and Ferguson 2006). However, in our samples, we did not observe the wire loop pattern of membrane thickening often associated with those diseases. Presporogonic stages and myxospores were visible in renal tubules once the parasite was widespread in the glomeruli, and this was a condition that was commonly associated with premature death.

Parvicapsula minibicornis has previously been observed in gills of sockeye salmon (St-Hilaire et al. 2002), but the present investigation is the first to observe an association between the severity of the infection and significant histological changes in gills. We observed diffuse lamellar fusion and inflammatory infiltrate throughout the gill filament and lamellae in severe cases. Lamellar fusion is a common response in gills to a number of pathogens and irritants, likely to reduce the surface area exposed to pathogens or toxins (Mallatt 1985; Lewis et al. 2009), although it is often difficult to determine the cause of these changes in an uncontrolled environment. In this study, much of the affected gill tissue contained developmental stages of *P. minibicornis*, and the severity of the gill pathology was correlated with *P. minibicornis* levels in the kidney and gills. Thus, the evidence suggests that the parasite could be the cause of gill disease, although we cannot rule out some other unidentified agent. Controlled infections in an experimental environment such as those conducted by Wagner et al. (2005) are needed to confirm our hypothesis.

Based on observations of developmental stages of a myxosporean parasite in gill tissue and a positive polymerase chain reaction test for *P. minibicornis*, St-Hilaire et al. (2002) proposed that gills may serve as a point of entry for the parasite as is observed for other myxosporeans (Kallert et al. 2009). Our observation that heavy gill infections and associated pathologies occurred after the parasite first appeared in kidney suggests that parasites were accumulating in gills during later stages of infection. The presence of parasite stages within pillar channels of gills demonstrates that the pathogen is in blood, but it is unclear if *P. minibicornis* stages that we found in gills are the result of direct transmission from the environment or were carried from kidneys through the blood to the gills. Pillar channels that were affected by the parasite were fully obstructed with an eosino-

philic material, which is probably the result of parasite presence in the blood as well as basement membrane damage caused by the parasite crossing the membrane when travelling to or from gill epithelium.

Our finding of gill pathology that is potentially related to the presence of *P. minibicornis* raises the potential for respiratory impairment to affect migration and survival. Wagner et al. (2005) found that adult Fraser River sockeye salmon that were infected with *P. minibicornis* took longer to recover from exercise, which they ascribed to a deterioration in renal function. However, these effects may also have been the consequence of reduced function of gills for either gas exchange or osmoregulation. Reductions in the maximal uptake of oxygen during exercise have been observed in rainbow trout (*Oncorhynchus mykiss*) when gill surface area was experimentally reduced (Duthie and Hughes 1987). Similar reductions in performance were observed in trout exposed to zinc or nickel, which were attributed to a reduction in gas exchange caused by lamellar thickening (Lappivaara et al. 1995; Pane et al. 2004). In 2006, we observed fish attempting to swim up highly aerated ephemeral creeks and at the surface of Cultus Lake. Surfacing behaviour was confirmed in a concurrent telemetry study (Pon et al. 2010) that found that death usually followed surface swimming. These observations suggest that gill impairment may have been extremely significant for some fish.

The temperature of the hypolimnetic water in Cultus Lake (<8 °C) appears to suppress further development of the disease, as we found little effect of the length of holding in the captive broodstock ponds in similar water on disease scores. Farrell et al. (2008) also found that sockeye salmon adults from nearby Weaver Creek that held in the hypolimnion of Harrison Lake were more likely to spawn successfully than those that held in much warmer streams near the spawning ground. In Cultus Lake, adults entering the lake remain almost entirely in cold water until the time of spawning, at which point the lake has become isothermal at <8 °C (Pon et al. 2010). Temperature plays a major role in the development of myxosporidian diseases, although threshold temperatures for development have not yet been identified for *P. minibicornis*. Further work using degree-day accumulations to relate river water temperatures to disease progression (e.g., Wagner et al. 2005) should consider using a threshold in the 8 °C range in those calculations.

In summary, our investigations provide correlative evidence for the link between the severity of *Parvicapsula*-related pathology in kidney and gill and premature mortality in adult sockeye salmon. In the case of the Cultus Lake population, negative implications of early entry into the Fraser River for disease development experienced by many late-run populations were offset by the extended use of the hypolimnion of the lake for holding before spawning. Our finding of a relationship between survival and date of arrival at the lake provides some guidance for managers wanting to reduce risk to the population from fishing or habitat impacts in the migration corridor. We recognize that *Parvicapsula* is unlikely to be the sole source of mortality for adult sockeye, and there are likely complex interactions between the parasite, its invertebrate host, the state of fish entering the river, and environmental factors that create the conditions for increased mortality in some years but not in others. Given our findings and the continuing large losses of late-run sockeye salmon in recent years, further investigation of the dynamics of the parasite and its impacts on salmon physiology and performance seem warranted.

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