Metabolic rates and swimming performance of adult Fraser River sockeye salmon (*Oncorhynchus nerka*) after a controlled infection with *Parvicapsula minibicornis*

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Abstract: Adult sockeye salmon (*Oncorhynchus nerka*) acquire infections with the myxosporean kidney parasite *Parvicapsula minibicornis* during their spawning migration in the Fraser River, British Columbia. Controlled infections with this parasite in wild sockeye salmon had no significant impact on plasma ionic status, metabolic rates, and initial maximum prolonged swimming performance (U_{crit}) for fish ranked as either strongly, weakly, or noninfected by polymerase chain reaction analysis of kidney tissue. However, strongly infected fish had significantly lower second U_{crit} and recovery ratio (8%) values, indicating decreased ability to recover from exercise. As the present study shows that the severity of infection is affected by time and temperature, the accumulated thermal units (ATU) of exposure in this study were compared with those experienced by naturally migrating sockeye salmon. A parallel telemetry study revealed that early-timed sockeye experienced significantly more ATU (741.4 ± 29.4 °C) than normally migrating salmon (436.0 ± 20.0 °C) prior to spawning because of a significantly longer holding period in the lake system. The present data are discussed in the context of a threshold of >450 °C ATU for severe infection that would first manifest in early-timed fish in the upper reaches of the Fraser River and certainly on the spawning grounds.

Résumé : Les saumons rouges (*Oncorhynchus nerka*) attrapent des infections à *Parvicapsula minibicornis*, une myxosporidie parasite du rein, durant leur migration de fraye dans le Fraser, Colombie-Britannique. Des infections contrôlées de ce parasite chez les saumons rouges sauvages restent sans effet sur le statut ionique du plasma, les taux métaboliques et la performance initiale maximale de nage soutenue (U_{crit}) chez des poissons classés comme fortement, légèrement ou aucunement infectés par une analyse de la réaction en chaîne de la polymérase de leurs tissus rénaux. Cependant, les poissons fortement infectés ont un U_{crit} secondaire et des valeurs du rapport de récupération (8 %) significativement plus faibles, ce qui indique une capacité réduite à récupérer après l'exercice. Puisque notre étude montre que la sévérité de l'infection est affectée par le temps et la température, nous avons comparé les unités

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thermiques cumulées (ATU) de l'exposition durant notre étude à celles subies par des saumons durant leur migration naturelle. Une étude de télémétrie faite en parallèle montre que les saumons précoces accumulent significativement plus de ATU (741,4 ± 29,4 °C) que les saumons à migration normale (436,0 ± 20,0 °C) avant la fraye, à cause d'une période significativement plus longue de rétention dans le système de lacs. Nous discutons de nos données dans le contexte d'un seuil de > 450 °C ATU pour une infection sévère qui se manifesterait d'abord chez les poissons précoces dans le cours supérieur du Fraser et certainement sur les sites de fraye.

[Traduit par la Rédaction]

Introduction

Most stocks of sockeye salmon (*Oncorhynchus nerka*) that spawn in the Fraser River watershed in British Columbia, Canada, enter the river within a week of arriving at the mouth of the estuary. In contrast, late-run sockeye salmon stocks, named for their late-summer arrival, normally congregate in the ocean near the mouth of the Fraser River for 3–6 weeks prior to initiating their upriver migration to natal spawning grounds. However, this historical migratory behaviour began to deviate in 1995 when a large portion of late-run stocks congregated for a shorter period of time and initiated upriver migration earlier than normal. Most of these early-timed fish died before reaching spawning grounds; in some years, mortality exceeded 90% (Cooke et al. 2004). Early-timed fish that survived the migrations attempted to spawn at their historically normal dates, which meant that their freshwater residence time was increased by 3–6 weeks (Cooke et al. 2004). Thus, increased freshwater residence time was associated with increased mortality prior to spawning.

Adult sockeye salmon entering the Fraser River develop detectable infections with *Parvicapsula minibicornis* (Myxozoa, Myxosporea), a microscopic waterborne kidney parasite endemic to the lowermost reaches of the river (Pacific Salmon Commission 2001; Jones et al. 2003). Outmigrating smolts also are exposed to the parasite, but it is unknown whether it is pathogenic during their marine phase or if it causes latent infection when fish mature and return to fresh water (St-Hilaire et al. 2002; Jones et al. 2003). Trophozoites of *P. minibicornis* aggregate within the kidney glomeruli (Kent et al. 1997; Raverty et al. 2000). As the infection progresses, the number of parasitic stages observed in the glomeruli increases. With severe infections, parasite development occurs in the lumen of the renal tubules, causing glomerulonephritis that leads to renal failure and mortality (Raverty et al. 2000). The infection progresses with time after first entry into the Fraser River such that it is initially only weakly detectable in kidney sections, using polymerase chain reaction (PCR) methods, in fish that have recently entered fresh water. With increased migration time, the infection is detected more intensely by PCR and eventually is detectable using histological examination of kidney glomeruli (Jones et al. 2003). Thus, the increase in freshwater residence time of early-return late-run sockeye may allow *P. minibicornis* to complete sporulation and cause damage to the glomerular capillaries and tubular epithelium sooner before spawning than for normal-timed migrants. During normal migration behaviour, maturation of the parasite coincides closely with the time of spawning (Jones et al. 2003). This led us to hypothesize that *P. minibicornis* infection may contribute directly to the accelerated mortality in early-timed fish. A loss of kidney function associated with severe *P. minibicornis* infection would be problematic for fish in fresh water because of the resulting osmotic imbalance. This loss of function would be exacerbated in migrating adult salmon because increased water uptake across the gills during swimming (Wood and Randall 1973; Gonzalez and McDonald 1994; Gallaugher et al. 2001) places additional loads on kidney function. The level of *P. minibicornis* infection that will adversely impact the physiology of sockeye salmon is unknown, but the prediction is that the intensity of kidney infection will be correlated with impaired omoregulatory ability and swimming ability. Neither prediction has been tested experimentally, but sublethal infections of fish with other parasites such as haemoflagellates (Woo 2003), sea lice (Wagner et al. 2003, 2004), and trematodes (Coleman 1993) have been shown to negatively impact host physiology during exercise.

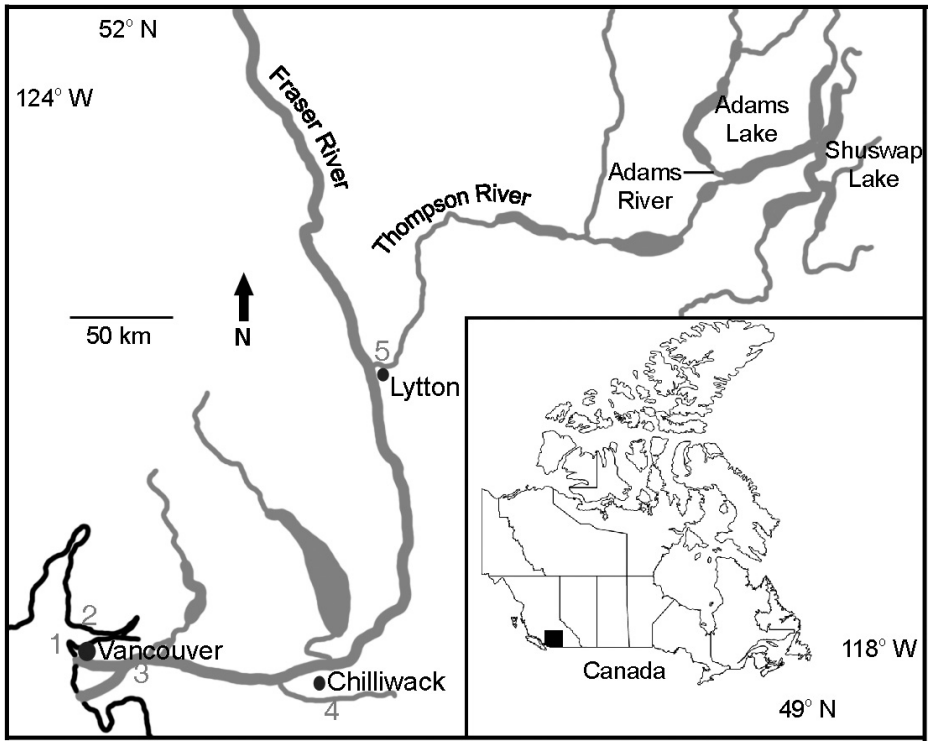
In the present study, we exposed wild sockeye salmon to sublethal infections of *P. minibicornis* and examined their plasma ionic status, metabolic rates, and swimming ability (maximum prolonged swimming performance, U_{crit}). By swimming fish twice with only a short recovery period (a repeated U_{crit} test; Jain et al. 1998), the expectation was that any physiological disturbance accrued in the first swim would be exacerbated in the second one and hamper performance (Tierney and Farrell 2004). Ionic and osmotic status was measured from blood samples collected after the second swim. Data from a parallel telemetry study were used to compare the estimated parasite impact on migration ability with actual migrations of early-timed and normally behaving sockeye.

Materials and methods

Fish capture and holding

The geographic locations of all sockeye capture and experimental sites for the present study are provided in Fig. 1. The fish used in experimental trials were part of a larger group of approximately 240 wild sockeye salmon captured by a commercial seine boat in the Strait of Georgia near Vancouver, British Columbia, on 26 and 28 August 2003. All fish were landed at the Fisheries and Oceans Canada West Vancouver Laboratory. To avoid exposure to the main stem of the Fraser River and hence prevent adult salmon from encountering *P. minibicornis*, we immediately transferred a group of fish that we termed "control, unexposed" (mean ± SE: 61.0 ± 0.9 cm, 2.57 ± 0.16 kg, $n = 10$) to the Fisheries and Oceans Canada Cultus Lake Laboratory and

Fig. 1. Map of the lower Fraser River, Thompson River, and Shuswap Lake areas in British Columbia indicating the location of ocean capture (1), the West Vancouver Laboratory (2), the Annacis Island facility (3), the Cultus Lake Laboratory (4), and the Thompson River capture site (5). The solid region in the inset map of Canada indicates the study area.



placed in 20 000-L holding tanks with a flow-through supply of fresh water (12.7 ± 0.3 °C) obtained directly from Cultus Lake. To provide a controlled exposure to lower Fraser River water, we transported a group of fish (60.7 ± 1.0 cm, 2.63 ± 0.18 kg, n = 10) from the West Vancouver Laboratory to Fisheries and Oceans Canada Annacis Island and placed in two 7000-L holding tanks where they became naturally infected with *P. minibicornis* over a 7-day period through the flow-through supply of water directly from the Fraser River (15.6 ± 0.1 °C). Water at this location has been shown to carry infectious stages of the parasite (Jones et al. 2003). Infected fish subsequently were transferred to the Cultus Lake Laboratory and kept in conditions similar to those of control fish. The number of accumulated thermal units (ATU) (daily cumulative temperature above 0 °C commencing from initial exposure to Fraser River water) was calculated because progress of the myxozoan life cycle is positively correlated with temperature and incubation time (Hendrickson et al. 1989; McGeorge et al. 1996; Redondo et al. 2002). We collected an additional 10 adult late-run sockeye (60.1 ± 1.2 cm, 2.61 ± 0.15 kg) from the Thompson River canyon on 20 September 2003 and transported them to the West Vancouver Laboratory where they were kept in a 7000-L holding tank with a flow-through supply of fresh water (12.6 ± 0.2 °C). These fish had completed 300 km of their 500-km spawning migration. Late-run sockeye salmon carrying transmitters were tracked by radiotelemetry from the Fraser River mouth using fixed stations until they entered Shuswap Lake, adjacent to the spawning grounds (English et al. 2004). Using this information, we estimate these fish had been migrating upriver for 13 days and thus were exposted to approximately 203 °C ATU. Four of these fish were lost prior to testing from unidentified causes.

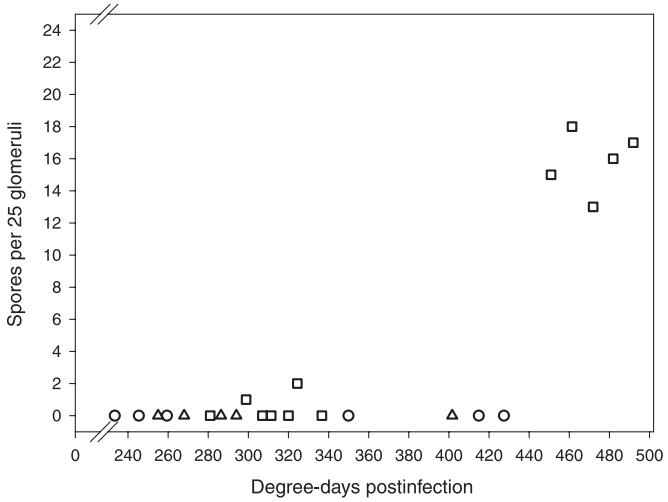
Routine blood samples

Blood was sampled from a subgroup of cannulated infected and uninfected fish to assess ionic status (plasma chloride, potassium, sodium glucose, lactate, and cortisol levels). Sockeye salmon were individually netted and anaesthetized in a 60 mg tricaine methanesulfonate·L^{-1} (MS 222) bath and placed on a surgery table with a flow-through maintenance bath of 30 mg MS 222·L^{-1}. A dorsal aorta cannula was inserted using an internal trochar, anchored in place using 3-0 silk suture, and filled with 0.9% heperanized saline. Fish were then placed into one of four 80-L isolation chambers with a water flow rate of 10 L·min^{-1} to recover for 14–20 h. Four 1-mL blood samples were taken over a 2-day period. Each blood sample was centrifuged and the plasma stored at –80 °C until the analyses were performed (as described in Farrell et al. 2001). Samples of gill filaments were frozen for enzymatic analysis of Na^+/K^+-ATPase activity performed according to McCormick (1993). Opercular tissue DNA was analysed according to Beacham et al. (2004) to confirm that fish were from the Adams River stock. As well, somatic energy levels were measured on each fish using a Distell fat meter (Distell.com, Old Levenseat, Fauldhouse, West Lothien, Scotland, UK) following the methods of Crossin and Hinch (2005).

Swimming performance

Owing to the constraint that only one fish could be tested daily, control and exposed fish were tested in split batches

Fig. 2. Infection levels of sockeye salmon (*Oncorhynchus nerka*) exposed to *Parvicapsula minibicornis* in the Fraser River for varying accumulated thermal units (ATUs) (cumulative daily water temperature above 0 °C in four locations along the migration route). Infection severity was estimated histologically from the mean number of infected glomeruli per 25 glomeruli (*y* axis) and by polymerase chain reaction amplification of 1091 base pair fragments of the *P. minibicornis* 18S rRNA gene present in kidney tissue (circles, uninfected; triangles, weak infection; squares, strong infection). All uninfected fish are controls not exposed to the parasite.



consisting of five salmon over an 8-week period that resulted in a graded level of infection among the infected group (see Fig. 2). Fish were netted individually and anaesthetized in a 60 mg MS 222·L^{-1} bath for approximately 5 min prior to taking body measurements and transferred to the swim tunnel (471-L Brett-type as described in Farrell et al. (2003) with a modified net plug to prevent drafting in the turbulent area near the upstream end). Fish were introduced into the swim tunnel in early afternoon and allowed to acclimate for 2 h at a water velocity of 0.45 body length·s^{-1} prior to performing a conditioning U_{crit} test (Jain et al. 1997). For this test, water velocity was increased by 0.15 body length·s^{-1} every 2 min until the fish could no longer swim against the current and rested for more than 20 s on the downstream grid. Fish were allowed to recover overnight (14–16 h) at 0.45 body length·s^{-1}. The next morning, routine oxygen uptake ($M_{O_2 routine}$) was estimated from two 15-min recordings taken twice during a 1-h period. The lowest value was used for $M_{O_2 routine}$, although the two values rarely differed by more than 5%. To measure oxygen uptake, we pumped swim tunnel water past an oxygen meter (Mark IV Oxyguard; Point Four Systems, Richmond, British Columbia) connected to a computer that logged oxygen measurements every 5 s using Labview software. A ramped U_{crit} protocol (Jain et al. 1997, 1998) was initiated by increasing water velocity 0.15 body length·s^{-1} every 5 min to ~50% of initial U_{crit} and subsequently every 20 min until fish fatigued. The U_{crit} values were calculated using the formula of Brett (1964) and corrected for solid blocking effects when necessary (10%–20% difference) according to Bell and Terhune (1970). Active M_{O_2} values were measured for every velocity increment of the U_{crit} test. Fish were permitted to recover for 45 min at 0.45 body length·s^{-1}, and $M_{O_2 routine}$ was remeasured for 15 min immediately prior to a second ramped U_{crit} test to examine the ability of fish to recover from the initial exhaustive exercise. Recovery ratios for U_{crit} and $M_{O_2 max}$ were calculated by dividing the results of the two swim tests ($U_{crit2} \cdot U_{crit1}^{-1}$ and $M_{O_2 max2} \cdot M_{O_2 max1}^{-1}$, respectively) for each fish. The recovery ratios of wild sockeye salmon typically equal unity (i.e., recovery ratios = 1.00) (Farrell et al. 2003; Lee et al. 2003). Therefore, if a recovery ratio was significantly <1.00, fish were considered to have failed to fully recover from the initial swim trial. The M_{O_2} recordings were continued for 30 min after the end of U_{crit2} to determine rates of recovery from exhaustive exercise. At the end of the second recovery period, fish were removed immediately from the swimming chamber and euthanized in 140 mg MS 222·L^{-1}. Blood was sampled by caudal puncture for analyses of the plasma. Plasma and all other tissues were analysed similar to cannulated fish.

Assessments of infection prevalence and severity

Duplicate posterior kidney samples were fixed and analysed for the presence of *P. minibicornis* by histology and PCR methods according to Jones et al. (2003). For histological analysis, duplicate 5-µm sections of kidney fixed in Davidson's solution were mounted on glass slides and stained with haematoxylin and eosin. Histological samples were scored infected when myxozoan trophozoites were observed in glomeruli, and the severity of infection was estimated from the mean number of infected glomeruli per 25 glomeruli examined under a light microscope. A severe level of infection was >10 spores per 25 glomeruli (Jones et al. 2003). For PCR analysis, kidney samples fixed in 95% ethanol had DNA extracted and amplified using the same primers and conditions described by St-Hilaire et al. (2002). Samples were scored positive if a 1091 base pair fragment of the *P. minibicornis* 18S rRNA gene was detected. Results for the PCR analysis were classified into three categories by an individual technician: uninfected (no PCR product amplified), weak (barely detectable 1091-bp product amplified), and strong (an intense 1091-bp product amplified). Levels of infection severity are compared as a function of ATU for both assessment methods (Fig. 2).

Modelling thermal exposure

River migration travel rates for 51 sockeye salmon from the Adams River late-run stock were obtained from a multi-stock radiotelemetry study conducted on Fraser River sockeye in 2003 (English et al. 2004). In their study, sockeye were captured using a commercial seine boat in Johnstone Strait near Campbell River, British Columbia (50.12°N, 125.21°W). These fish were gastrically implanted with radiotransmitters (16.1 g, 16 mm × 51 mm, MCFT-3A; Lotek Wireless Inc., Newmarket, Ontario) and tracked using SRX_400 receiver stations located in the lower Fraser River (Mission, British Columbia, 49.08°N, 122.18°W, 10 m above sea level), at the entrance to the holding lake adjacent to the main spawning stream (Shuswap Lake, 50.42°N, 119.16°W, 345 m above sea level), and at the entrance to the

spawning grounds (Adams River, 50.90°N, 119.55°W, 345–360 m above sea level). Water temperatures encountered by migrating sockeye were calculated from temperature stations located just upstream from the mouth of the Fraser River (Whonnock, British Columbia, 49.17°N, 122.46°W), at the midpoint of their migration in the Fraser River (Hope, British Columbia, 49.38°N, 121.43°W), in the Thompson River near its confluence with the Fraser River (Lytton, British Columbia, 50.23°N, 121.58°W), and at completion of river migration at the entrance to Shuswap Lake (Chase, British Columbia, 50.82°N, 119.68°W) at the entrance to Shuswap Lake where the sockeye hold prior to entering their spawning grounds in the Adams River. As the exact location of sockeye migrating through Shuswap Lake is unknown, we used both a maximum exposure temperature (assuming fish that held in the epilimnion, which was estimated from the temperature of water flowing from the lake entrance) and a minimum exposure temperature (assuming that fish held in the hypolimnion, which is normally stable at 8 °C from September through November within a few metres of the bottom (Nidle and Shortreed 1996)). Migrants were classified as early-timed if they entered the Fraser River 13 days or fewer after initial capture and tagging in Johnstone Strait (English et al. 2004).

Statistical analyses

Values presented are means ± SE and statistical significance was assessed at $P < 0.05$ using a standard statistical program (JMP 4.0.4; SAS Institute Inc., Cary, North Carolina). Histological, PCR, and blood data were analysed by one-way ANOVA. Oxygen consumption and U_{crit} data were analysed by one-way ANOVA for comparisons between treatments and one-way repeated-measures ANOVA for comparisons between U_{crit1} and U_{crit2}. Recovery ratios were transformed for statistical analysis and compared with a value of unity using one-sample t tests followed by Bonferroni adjustments to account for multiple comparisons.

In all cases, parasite infection level was the independent variable, and fish length, mass, and water temperature were tested for covariation and included if significant. Tukey's multiple comparison method was used to determine which treatments differed if ANOVA indicated statistically significant differences. Migration data were analysed by Mann–Whitney rank sum tests because of unequal variances for migration days and ATU of early and normal-behaving sockeye.

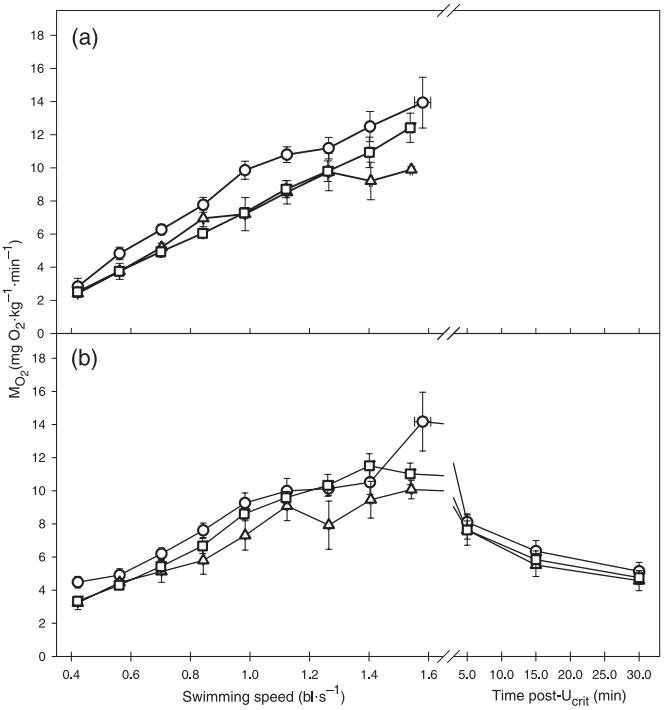
Results

Parasite infection

Histological analyses determined that spores were absent from kidney glomeruli of all control fish and all fish collected from the Thompson River. Fish exposed at Annacis Island became infected with *P. minibicornis* and those exposed and held for more than 450 °C ATU averaged 15.8 spores per 25 glomeruli. In contrast, fish tested prior to the 350 °C ATU incubation period had either very low or no spores in the glomeruli.

PCR analyses detected *P. minibicornis* infection much earlier than histological analysis. All fish exposed at Annacis Island were PCR positive for *P. minibicornis*, as were all fish captured from the Thompson River (Fig. 2). However, only a weak PCR response was observed in fish tested prior to 300 °C ATU incubation. A strong parasite presence was always indicated by PCR for all exposed fish with >300 °C ATU incubation and spores were observed in these fish. One control fish had a weak parasite presence based on PCR analysis and was therefore reassigned to the weak PCR parasite grouping for subsequent analysis. This fish was tested at 400 °C ATU and is the one fish with weak PCR presence above 300 °C ATU. The remaining control fish were PCR negative.

Fig. 3. Rates (mean ± SE) of oxygen uptake (M_{O_2}) for sockeye salmon (*Oncorhynchus nerka*) with uninfected (circles) or weak (triangles) or strong (squares) kidney infections of *Parvicapsula minibicornis* (as measured by polymerase chain reaction analysis) during the (*a*) first and (*b*) second swim test. The 30-min recovery period during the second swim test is also shown. Routine oxygen uptake ($M_{O_2\,routine}$) was taken from the slowest swimming speed (0.45 $m \cdot s^{-1}$). While M_{O_2} increased with swimming speed, values were not significantly different ($P > 0.05$) between groups for either swimming trial.



Metabolic rates and swimming performance

The M_{O_2} values increased with swimming speed in all groups, as expected (Fig. 3), but the level of parasite infection had no significant effect on either $M_{O_2\,routine}$ or $M_{O_2\,max}$ (Table 1). Similarly, active M_{O_2} during swimming and recovery did not differ significantly with infection.

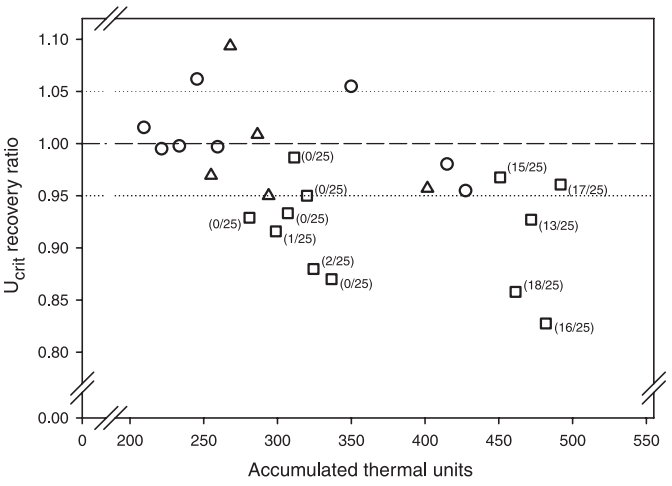
While U_{crit1} and U_{crit2} values did not differ significantly between treatments, sockeye with strong PCR parasite infection levels performed significantly poorer during U_{crit2} compared with U_{crit1} (Table 1). Only strongly infected fish had recovery ratios significantly below unity (Table 1), and a comparison of the U_{crit} recovery ratios and the ATU for each

Table 1. Routine metabolic rates, maximum metabolic rates (M_{O_2max}), repeated maximum prolonged swimming performance (U_{crit}), and recovery ratios ($M_{O_2max2} \cdot M_{O_2maxl}^{-1}$ and $U_{crit2} \cdot U_{crit1}^{-1}$) for sockeye salmon (*Oncorhynchus nerka*) with different levels of *Parvicapsula minibicornis* infection (measured by polymerase chain reaction).

Parasite presence	Routine metabolic rates (mg $O_2 \cdot kg^{-1} \cdot min^{-1}$)	M_{O_2maxl} (mg $O_2 \cdot kg^{-1} \cdot min^{-1}$)	M_{O_2max2} (mg $O_2 \cdot kg^{-1} \cdot min^{-1}$)	M_{O_2} recovery ratio	U_{crit1} (body lengths$\cdot s^{-1}$)	U_{crit2} (body lengths$\cdot s^{-1}$)	U_{crit} recovery ratio
Uninfected (n = 9)	2.83±0.50	13.94±1.54	14.18±1.78	1.02±0.04	1.56±0.07	1.57±0.07	1.01±0.01a
Weak (n = 5)	2.42±0.23	9.89±0.18	10.08±0.56	1.02±0.04	1.59±0.07	1.55±0.03	0.99±0.03a
Strong (n = 12)	2.52±0.20	12.50±1.08	11.70±0.70	0.95±0.03	1.52±0.06	1.40±0.06*	0.92±0.01b

Note: Data are means ± SE. Fish were swim tested in two separate trials with a 45-min recovery period between them. The asterisk indicates that the U_{crit2} and recovery ratio of strongly infected fish were significantly ($P < 0.001$) lower than U_{crit1}. Dissimilar letters indicate significant ($P < 0.05$) differences between treatments.

Fig. 4. Critical swimming speed recovery ratios for sockeye salmon (*Oncorhynchus nerka*) with uninfected (circles) or weak (triangles) or strong (squares) kidney infections of *Parvicapsula minibicornis* (as measured by polymerase chain reaction analysis) exposed to a given number of accumulated thermal units (ATUs) (cumulative daily water temperature above 0 °C in four locations along the migration route). Parenthetical data next to strongly infected fish indicate the number of kidney glomeruli, determined out of 25 by histological analysis, with spores present. The dashed line indicates the line of unity (i.e., performances in the first and second swim tests were identical) and the dotted lines indicate the expected resolution for recovery ratios (±5%).



fish shows that the majority of these fish fell below the expected recovery ratio resolution (±5%) (Fig. 4).

Tissue variables

Routine and postexercise plasma ion levels (chloride, potassium, and sodium) did not differ significantly with disease or with exercise (Table 2). Routine plasma glucose and cortisol levels also did not differ significantly with disease or with exercise. Plasma lactate levels increased significantly ($P < 0.01$) with exercise for the uninfected and strongly infected treatments but not for fish with weak infections. Significant differences in plasma osmolality occurred between infected fish at rest and uninfected fish after exercise. Gill Na^+/K^+-ATPase activity after exercise was significantly lower (weak, $P < 0.01$; strong, $P < 0.03$) in infected fish compared with resting uninfected fish but was unchanged by exercise in control fish. Whole-body energy levels did not differ significantly between the three treatments after exercise, thereby eliminating the possibility that infected fish were in a poor physical condition.

Modelling thermal exposure

Late-run sockeye salmon that began river migration earlier than normal required a similar number of days (24.4 ± 1.2) as normally timed fish (22.2 ± 0.7) to migrate from the mouth of the Fraser River to the Shuswap Lake holding area (Fig. 5). Early migrants also held in Shuswap Lake for a significantly longer period (20.0 ± 1.9 days) ($P < 0.001$) than normally migrating salmon (6.5 ± 0.7 days) before migrating to their spawning grounds (Fig. 5). Early timing also results in exposure to warmer temperatures and as a result, early migrants were exposed to significantly more ATU (429.7 ± 13.3 °C) ($P < 0.001$) than normal-timed fish (333.0 ± 10.1 °C) during upriver migration (Fig. 6). Thus, prior to entering the spawning grounds, early-timed fish had experienced significantly ($P < 0.001$) higher ATU (minimum = 592.4 ± 21.8 °C, maximum = 741.4 ± 29.4 °C) compared with normally migrating salmon (minimum = 384.9 ± 11.2 °C, maximum = 436.0 ± 20.0 °C) prior to entering the spawning grounds (Fig. 6). Based on the dates and migration times of fish in the telemetry study (English et al. 2004), sockeye captured from the Thompson River for the physiological study were deemed to exhibit normal migration behaviour.

Discussion

Our primary purpose was to determine if a controlled *P. minibicornis* infection affects the repeated prolonged swimming performance of adult wild sockeye salmon and, if a negative effect occurred, to assess the potential impact on sockeye migratory ability. Although a controlled experimental infection with *P. minibicornis* has never been performed previously with adult salmon, the ability to infect with lower Fraser River water is consistent with the prediction that this is the natural site of transmission of *P. minibicornis* (St-Hilaire et al. 2002; Jones et al. 2003). The life cycle of *P. minibicornis* is not fully understood but like other myxosporeans is expected to include an invertebrate alternate host that releases actinospores into the water column, in this case the lower Fraser River. The absence of detectable infections in most unexposed salmon further confirmed that salmon acquire infections following entry into the Fraser River during the spawning migration rather than in a latent fashion via

Table 2. Routine and postexercise (taken after 30 min of recovery from U_{crit2}) plasma variables, gill enzyme activity, and whole-body energy of sockeye salmon (*Oncorhynchus nerka*) with different levels of *Parvicapsula minibicornis* infection (measured by polymerase chain reaction).

	Routine*		Post-U_{crit}		
Blood variable	Uninfected (n = 8)	Strong (n = 8)	Uninfected (n = 4)	Weak (n = 5)	Strong (n = 12)
Sodium ($mmol \cdot L^{-1}$)	153.7±1.6	152.2±1.0	162.6±4.7	161.8±11.6	157.9±3.7
Chloride ($mmol \cdot L^{-1}$)	124.2±3.6	124.6±1.4	117.0±3.5	120.2±2.2	119.0±0.8
Potassium ($mmol \cdot L^{-1}$)	2.6±0.5	2.3±0.2	1.4±0.1	2.0±0.5	3.3±0.4
Osmolality ($mosmol \cdot kg^{-1}$)	288.8±4.8ab	274.0±3.4a	326.8±23.3b	301.6±10.7ab	290.8±3.0ab
Glucose ($mmol \cdot L^{-1}$)	6.4±0.4	6.3±0.4	7.0±0.7	7.5±0.9	7.4±0.7
Lactate ($mmol \cdot L^{-1}$)	2.0±0.9a	1.4±0.3a	6.1±1.2b	2.9±0.9ab	4.6±0.9b
Cortisol ($ng \cdot mL^{-1}$)	125.4±17.9	156.0±24.4	228.9±82.0	225.8±19.5	188.7±41.3
Na^+/K^+-ATPase (μmol ADP$\cdot mg$ protein$^{-1} \cdot h^{-1}$)	1.8±0.1a	1.3±0.2ab	1.4±0.3ab	0.7±0.2b	1.1±0.2b
Somatic energy ($MJ \cdot kg^{-1}$)	6.8±0.5	6.5±0.3	5.9±0.3	6.2±0.3	6.5±0.2

Note: Data are means ± SE. Dissimilar letters indicate significant differences ($P < 0.05$) between treatments.
*Samples taken from cannulated fish in 80-L isolation chambers.

Fig. 5. Mean (±1 SE) cumulative number of days taken by late-run sockeye salmon (*Oncorhynchus nerka*) that entered the Fraser River early (≤13 days after capture and tagging (hatched bars)) and those that held in the ocean prior to river entry (>13 days after capture and tagging (open bars)) to migrate from the mouth of the Fraser River to Shuswap Lake and from the Fraser River mouth to their final spawning grounds in the Adams River. The asterisk indicates that the value is significantly ($P < 0.05$) higher for early-migrating sockeye.

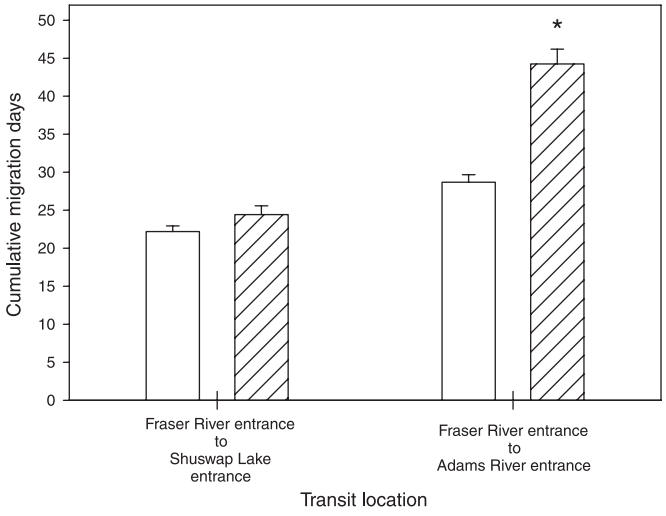
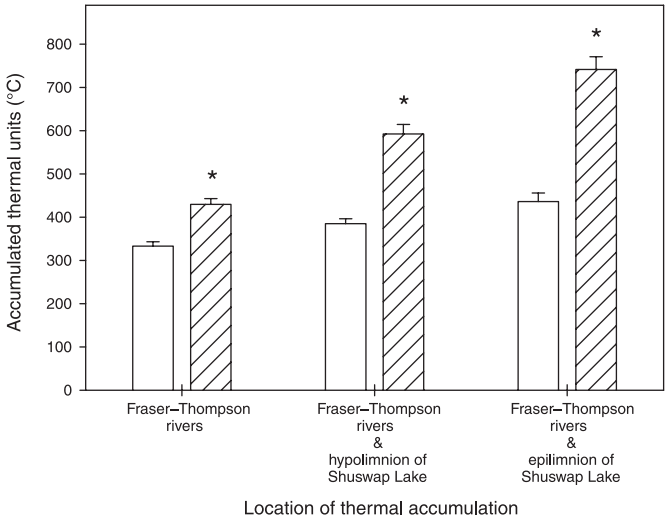


Fig. 6. Mean (±1 SE) accumulated thermal units (ATUs) (cumulative daily water temperature above 0 °C in four locations along the migration route) by early-timed (hatched bars) and normal-timed (open bars) late-run sockeye salmon (*Oncorhynchus nerka*) passing through the river segments of their migration (Fraser River mouth to Shuswap Lake) and passing through the entire migration route including river and lake segments (Fraser River mouth to the spawning ground entry). Because fish locations in Shuswap Lake are unknown and the lake thermally stratifies in the summer, minimum (hypolimnion exposure) and maximum (epilimnion exposure) ATUs are presented for the cumulative river and lake segments. Asterisks indicate significant difference ($P < 0.05$).



contact during the smolt out-migration. The infection in the single unexposed salmon may have been due to the chance exposure of this fish to actinospores that had entered the Strait of Georgia from the river or to the swimming behaviour of this fish having brought it closer to the river estuary. Previous studies failed to find evidence of infection in over 540 adult sockeye that had been collected from the west coast of Vancouver Island or the lower Fraser River prior to accruing sufficient ATU for parasite development (St-Hilaire et al. 2002; Jones et al. 2003). Also in agreement with earlier studies was the finding that PCR analysis identified *P. minibicornis* infections much earlier than histological analysis (St-Hilaire et al. 2002; Jones et al. 2003). In the present study, a weak PCR infection consistently appeared at ~150 °C ATU before the appearance of spores, an interval that translates to about 2 weeks at 12 °C. Infected sockeye that experienced >450 °C ATU had more severe PCR infections and histological infections (13–18 glomeruli infected; according to Jones et al. (2003), severe ≥10 per 25 glomeruli infected), while those with <350 °C ATU had a weak PCR infection and few spores. Therefore, the period between 350 and 450 °C ATU seems to be necessary for the proliferation of spores in the glomeruli of late-run sockeye salmon under the present conditions. By implication, any significant im-

pact associated with infection likely will require exposure levels of >500 °C ATU to reach glomeruli infection levels nearing 25, which are commonly seen in wild fish (Jones et al. 2003). Fish in the present study did not reach the level of ATU exposure needed to produce the severe kidney damage found by Raverty et al. (2000).

Despite the severe levels of kidney infection in some fish, the initial metabolic rates at rest and during exercise were within the range of those found in previous studies on wild adult sockeye (Farrell et al. 2003; Lee et al. 2003). This lack of impairment also was true for the initial U_{crit} of fish regardless of infection severity. However, fish with strong *P. minibicornis* infections averaged a significantly lower second U_{crit} and reduced recovery ratio, indicating a decreased the ability to recover from exercise. This 8% reduction in U_{crit} recovery ratio is similar to that found by Jain et al. (1998) for sockeye salmon exposed to the toxicant dehydroabietic acid. The decrease in recovery ratio was not dependent on exposure time because the majority of strongly infected fish fell below the expected recovery ratio resolution regardless of ATU. Therefore, it appears that fish can experience a physiological impact similar to that of a toxicant before *P. minibicornis* spores reach high numbers. However, ATU exposure of fish in the present study is below that experienced in-river by wild sockeye salmon, and it is most likely that this impact will be much greater with longer ATU exposure. Given that metabolic rates were similar between treatments, a reduction in swim performance may be the first indicator that physiological problems associated with *P. minibicornis* are beginning.

Given that *P. minibicornis* causes kidneys damage, poorer recovery of severely infected fish in the absence of significant effects on metabolic rates could mean that the osmoregulatory abilities of fish are beginning to fail. Raverty et al. (2000) previously hypothesized that a relationship exists between kidney tissue damage and an impairment of renal function during severe infections, possibly leading to mortality. Osmoregulatory failure was implicated in a significant decline in swimming performance without a change in cardiac output for Atlantic salmon infected (*Salmo salar*) with sea lice (Wagner et al. 2003). Even so, the strong *P. minibicornis* infection produced here (12–18 of 25 glomeruli infected) had no significant effect on the plasma ion and osmolality levels for sockeye salmon at rest. Ion and osmotic disturbances can be delayed in their appearance, but no such disturbances were observed after exercise. Plasma lactate levels also did not differ between treatments postexercise, indicating that muscle activity likely was comparable.

The early stages of glomerulonephritis that precede eventual renal failure in severely infected fish (Kent et al. 1997; Raverty et al. 2000) could be compensated for by increased reliance on the gills for ionoregulation, thereby maintaining plasma ionic status as observed here. The activity of chloride cells in the gills normally increases to compensate for the passive ion loss and water entry in fresh water (Schreck 1990; Wang et al. 1994; Wendelaar Bonga 1997). However, an up-regulation of the Na^+/K^+-ATPase ion pump was not observed in infected fish. In fact, Na^+/K^+-ATPase activity was significantly lower in infected fish postexercise compared with uninfected resting fish but was not significantly different from that of uninfected fish postexercise. It is important to note that in the present study, postexercise tissue samples were not taken until after a 30-min recovery period (which was needed to measure excess postexercise oxygen consumption). We cannot exclude the possibility that this amount of time may have been long enough for the tissue variables to stabilize from exercise and account for the lack of any significant postexercise changes.

We are able to place the results for the present controlled infection in the context of naturally infected early- and normal-migrating late-run salmon. The radiotelemetry tracking study (English et al. 2004) clearly revealed that both normal- and early-timed late-run sockeye travelled from the mouth of the Fraser River to their holding lake in about the same amount of time. Therefore, the present results clearly show that the primary difference in incubation ATU lies in the extra time that early-timed fish hold in the lake before spawning. Early migrants also experience higher river temperatures, further increasing the difference in incubation ATU. Given the observed threshold of 450 °C ATU for fish to begin to manifest severe *P. minibicornis* infection (i.e., a strong PCR result plus histological presence of ≥10 of 25 infected glomeruli), this threshold would have been approached but not exceeded when early migrants attempted to pass through the Thompson River canyon. This stretch of river is the last major hydraulic challenge during the migration of this stock. Therefore, the present results suggest that the severity of *P. minibicornis* at this stage of the migration would not impact on repeat swimming performance. Whether the progression of the infection may influence spawning physiology in the lake and beyond, when energy still is required to hold position in the river while competing for mates and defending redds (Healey et al. 2003), cannot be determined by the present study.

We did not assess for graded effects of increasing incubation between 300 and 490 °C ATU, nor were longer incubation periods studied. Nevertheless, the present analysis suggests that such studies are needed to fully assess the physiological impact of *P. minibicornis*. Depending on where fish hold in the lake, incubation could reach minimally 600 °C ATU (i.e., holding in the hypolimnion) and maximally 750 °C ATU (i.e., holding in the epilimnion) by the time early-timed fish reach the spawning grounds in the Adams River. Obviously, fish holding in the lake hypolimnion could benefit substantially in terms of retarding parasite infection compared with fish holding in the epilimnion. In comparison, the successfully spawning normal-timed late-run sockeye are estimated to have approximately 400 °C ATU of incubation regardless of location within the lake. This period of incubation corresponds to the point where spore proliferation likely begins to become severe, although apparently not enough to negatively affect spawning ability. Furthermore, the PCR signal would likely indicate a strong infection for normal-timed spawning ground sockeye, suggesting that while PCR may be an advantageous tool for early detection of the parasite, spore appearance in the glomeruli may be more useful in predicting adverse consequences of infection likely to occur at higher ATU exposure.

Based on our findings and the observations of naturally migrating late-run sockeye salmon, we have eliminated the

possibility that *P. minibicornis* impact the metabolic rates, repeat swimming performance, or osmotic disruption of these fish within the main stem of the Fraser and Thompson Rivers. However, we only assessed the impact for up to 490 °C ATU, and yet it is clear from the telemetry data that early-timed migrants of these stocks can experience an additional incubation period of 100–250 °C ATU. During this time, the ability of sockeye salmon to recover from exercise is likely to deteriorate and possibly affect their ability to avoid predators, traverse the spawning grounds, hold station, and compete for mates. Therefore, further studies using extended exposure intervals and a broader range of assessment endpoints are required to fully evaluate the impact of this parasite on the physiology of early-timed sockeye salmon in the Fraser River.

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