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Ionoregulatory changes in different populations of maturing sockeye salmon *Oncorhynchus nerka* during ocean and river migration

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Summary

We present the first data on changes in ionoregulatory physiology of maturing, migratory adult sockeye salmon *Oncorhynchus nerka*. Fraser River sockeye were intercepted in the ocean as far away as the Queen Charlotte Islands (~850 km from the Fraser River) and during freshwater migration to the spawning grounds; for some populations this was a distance of over 700 km. Sockeye migrating in seawater toward the mouth of the Fraser River and upriver to spawning grounds showed a decline in gill Na^+,K^+-ATPase activity. As a result, gill Na^+,K^+-ATPase activity of fish arriving at the spawning grounds was significantly lower than values obtained from fish captured before entry into freshwater. Plasma osmolality and chloride levels also showed significant decreases from seawater values during the freshwater migration to spawning areas. Movement from seawater to freshwater increased mRNA expression of a freshwater-specific Na^+,K^+-ATPase isoform (α1a) while having no effect on the seawater-specific isoform (α1b). In addition, gill Na^+,K^+-ATPase activity generally increased in active spawners compared with unspawned fish on the spawning grounds and this was associated with a marked increase in Na^+,K^+-ATPase α1b mRNA. Increases in gill Na^+,K^+-ATPase activities observed in spawners suggests that the fish may be attempting to compensate for the osmotic perturbation associated with the decline in plasma chloride concentration and osmolality.

Key words: sockeye salmon, *Oncorhynchus nerka*, ionoregulation, migration, salinity, spawning.

Introduction

The migrations of anadromous fish between freshwater and seawater represent remarkable transformations in the mechanisms that control ionoregulation. Fish actively take up ions in freshwater and must excrete excess ions in seawater (Hoar, 1988). Additionally, migration is an energetically demanding and physiologically challenging phase of life history (McKeown, 1984; Hinch et al., 2005) and the transition between freshwater and seawater is often a time of increased mortality (Fisher and Pearcy, 1988; S. J. Cooke, S. G. Hinch, G. T. Crossin, D. A. Patterson, K. K. English, J. M. Shrimpton, G. Van Der Kraak and A. P. Farrell, manuscript submitted for publication). While we know much regarding the morphological, behavioural and physiological changes that occur during the downstream migration of juvenile salmon smolts as they prepare for entry into seawater (McCormick and Saunders, 1987), comparatively little is known regarding migration of adults from seawater back to the freshwater spawning grounds. Increases in gill Na^+,K^+-ATPase activity occur in fish in freshwater and are preparatory for the increased ionic concentration of seawater as juvenile salmon migrate to the marine environment (McCormick and Saunders, 1987). In fact, the elevation in enzyme activity has been proposed as predictive of the entry date into seawater (Nielsen et al., 2004). Recently, Richards et al. (2003) demonstrated differential mRNA expression of Na^+,K^+-ATPase α-subunit isoforms in response to seawater transfer and speculated that isoform switching between a freshwater-responsive Na^+,K^+-ATPase isoform (α1a) and a seawater-responsive Na^+,K^+-ATPase isoform (α1b) is important for ionoregulatory competence during salinity transfer. Isoform switching potentially offers a highly sensitive indicator of osmoregulatory status.

The elevated gill Na^+,K^+-ATPase activity limits ionic perturbation in plasma of fish following movement into seawater. Fish transferred to seawater that are not prepared for the increased salinity showed large perturbations in plasma ions, decreased survival (Shrimpton et al., 1994) and reduced swimming performance (Brauner et al., 1992). While similar preparatory physiological changes are expected to occur in

preparation for maturing adult salmon returning to freshwater, their nature and their consequences on plasma osmolality and ion levels are unknown. Whatever these changes are, they are unlikely to exactly mirror the parr–smolt transformation. Juvenile salmon that are prevented from migrating into the ocean survive in freshwater (Shrimpton et al., 2000); in contrast, there is evidence that maturing adult salmon cannot remain in seawater, but must move into freshwater. Hirano et al. (1978) showed that maturing chum salmon *O. keta* did not survive transfer from freshwater into full strength seawater, and the physiological changes in ionoregulatory ability that accompany maturation appear to be irreversible.

In the present study, we assessed gill Na^+,K^+-ATPase activity, plasma osmolality and chloride concentration, and the mRNA levels of two isoforms of the Na^+,K^+-ATPase α-subunit, to evaluate physiological and molecular changes that occur in naturally migrating salmon. To examine the dynamics of ionregulation during migration, we intercepted wild adult sockeye salmon homing to the Fraser River watershed, British Columbia, Canada and collected gill tissue and plasma samples from these maturing fish migrating in seawater and in-river, and on the spawning grounds. We captured fish from as far away as ~850 km to ~25 km from the mouth of the Fraser River to examine physiological changes that occur during migration in seawater and whether physiological changes necessary for ionoregulation in freshwater were completed before moving into the river. Sockeye migrating upriver and on the spawning grounds were also sampled to assess the effect of migration and final maturation on ionoregulation.

For this analysis, we focussed our sampling efforts on four geographically isolated spawning aggregates, each comprising multiple spawning populations of sockeye salmon: Harrison, Late Shuswap, Chilko and Quesnel. Most populations of sockeye that spawn in the Fraser River watershed (Chilko and Quesnel) enter the river within a week of arriving at the mouth of the estuary. In contrast, late-run sockeye salmon (Harrison and Late Shuswap), named for their late summer arrival, normally congregate in the Fraser River estuary for 3–6 weeks prior to entering freshwater and initiating their upriver migration to natal spawning grounds (Cooke et al., 2004). The stocks we selected also varied in their migration distances in the freshwater of the Fraser River watershed, ranging from less than 120 km (Harrison) to over 700 km (Quesnel).

Materials and methods

Fish capture and sampling

The four stocks of sockeye salmon *Oncorhynchus nerka* Walbaum that we targeted were intercepted in the coastal waters of British Columbia and at various points during their upstream migration through the Fraser River system (Fig. 1). Migrating sockeye were sampled while still in seawater off the Queen Charlotte Islands (QCI, ~850 km; the mouth of the Fraser River is 0 km), off Port Renfrew on Vancouver Island (PR, ~300 km), in Johnstone Strait (JS, ~200 km), and in Georgia Strait (GS, ~25 km). Salinities off the north coast of British Columbia are greater than 32‰. In Johnstone Strait, salinities show a seasonal fluctuation due to river run-off in the spring, but generally exceed 27‰ in August when the sockeye migrate through this region. For the majority of Georgia Strait the sea surface salinity is also greater than 27‰ during August and September when sockeye were migrating through this area. In Georgia Strait close to the mouth of the Fraser River, however, surface water salinities are lower and fluctuate with the tide. In this area sockeye seem to prefer to

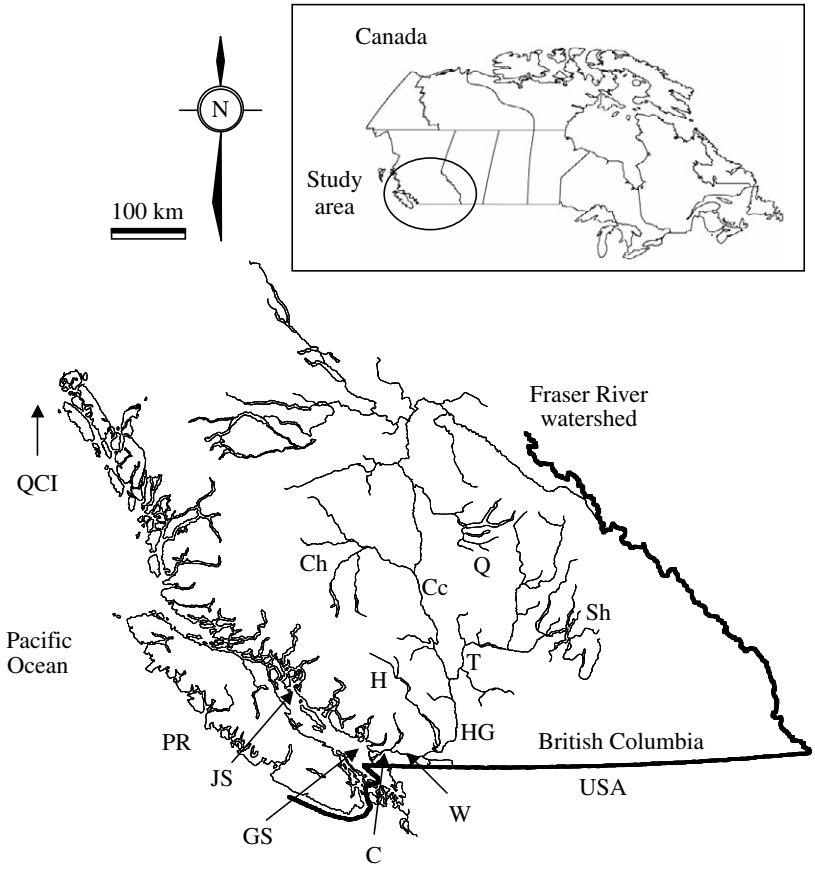

Fig. 1. Map of Fraser River watershed and coastal British Columbia showing locations where fish were intercepted during migration. Samples were collected off the Queen Charlotte Islands (QCI), Port Renfrew (PR), Johnstone Strait (JS), and Georgia Strait (GS) in seawater. Sample locations within the Fraser River were Cottonwood (C), Whonnock (W), Hells Gate (HG), near Churn Creek (Cc), and on the Thompson River (T) near Ashcroft. Spawning sockeye from Harrison stock (H) were sampled from Weaver Creek; Late Shuswap stock (Sh) from the Adams River; Chilko stock (Ch) from the Chilko River; and Quesnel stock (Q) from the Horsefly River.

be below the halocline as they migrate and were caught with deeper fishing gear.

During their upstream migration, sockeye were caught in the lower Fraser River at Cottonwood (C, 11 km), which is estuarine and at Whonnock (W, 50 km), which is beyond the saltwater intrusion. Lower Fraser sockeye of the Harrison stock were sampled on the spawning grounds in Weaver Creek (H, 117 km). Mid-Fraser sockeye of the Late Shuswap stock were sampled in the Thompson River near Ashcroft (T, 270 km), and on the spawning grounds in the Adams River (Sh, 484 km). Two stocks of Upper Fraser sockeye were sampled, Chilko and Quesnel. Chilko stock was sampled sampled at Hells Gate (HG, 200 km) and on the spawning grounds in the Chilko River (Ch, 562 km). Quesnel stock was sampled at Hells Gate, near Churn Creek on the Fraser River (Cc, 440 km) and on the spawning grounds in the Horsefly River (Q, 748 km).

Fish were collected using a variety of methods specific to each location. The following methods were employed: trolling off the west coast of the Queen Charlotte Islands and in Georgia Strait; purse seine off the coast of Vancouver Island near Port Renfrew and in Johnstone Strait; gill net near the mouth of the Fraser River at Cottonwood and at Whonnock; long dip nets at Hells Gate; beach seines at Ashcroft and Churn Creek; beach seines to capture sockeye arriving at the spawning grounds; and small dip nets to capture actively spawning fish. All groups were sampled on the spawning grounds at least twice in each year and fish were assessed for stage of maturation.

There is considerable knowledge of run timing for each of the stocks sampled; therefore, sampling was timed to intercept the stocks of interest. Stock identification for each fish sampled was determined by variation in genetic markers, as outlined by Beacham et al. (1995). Genetic determination, however, meant that stock identification was determined *a posteriori*. As a result, sample size varied considerably for each stock examined, among locations, and also between years. The fish caught during migration were grouped based on spawning aggregate and represent multiple spawning populations within a watershed. In contrast, the fish sampled on the spawning grounds were from a single spawning population in that aggregate. In all cases, however, the single spawning populations chosen were the largest spawning population for that aggregate in both sampling years.

Tissue sampling

In most cases these fish were sampled as part of normal test fishery or stock assessment operations and specific treatment of the fish was dependant on the gear type. Every effort was made to minimize the time from capture to tissue sampling. In those cases involving seine nets, fish remained in the ocean constrained by the seine net until they were individually dip-netted out for sampling. Fish caught using a troll line or dip net were landed and sampled within minutes. Where possible the soak time for gill nets was reduced to less than 15 min and only fish that were still vigorous at capture were sampled. Fish were killed with a single blow to the head and immediately sampled for blood and gill tissue. 10 ml of blood was collected from the caudal vasculature using a vacutainer syringe (1.5″, 21 gauge) for assessing plasma chemistry, and the tips of 6–8 filaments from the first gill arch were removed for analysis of Na^+,K^+-ATPase activity. Gill tissue and centrifuged plasma samples were stored on dry ice for several days before transfer to a –80°C freezer where they were held until analysis. Fork length (*FL*, cm) was measured and an adipose fin clip was removed for DNA stock identification.

Analysis of gill Na^+,K^+-ATPase activity

Gill Na^+,K^+-ATPase activity was measured according to the microassay protocol of McCormick (1993). Gill filaments were homogenized in SEI buffer (150 mmol l^{-1} sucrose, 10 mmol l^{-1} Na_2EDTA, 50 mmol l^{-1} imidazole, pH 7.3) containing 0.1% sodium deoxycholate. Following centrifugation (3000 ***g*** for 0.5 min), the supernatant was used to determine Na^+,K^+-ATPase activity by linking ATP hydrolysis to the oxidation of nicotinamide adenine dinucleotide (NADH), measured at 340 nm for 10 min at 25°C, in the presence and absence of 0.5 mmol l^{-1} ouabain. Protein content in the gill homogenate was measured using a bicinchoninic acid (BCA) protein assay (Pierce, Rockford, IL, USA). Specific activities were expressed as mol ADP mg^{-1} protein h^{-1}.

Determination of plasma osmolality and chloride

Plasma samples were thawed, vortexed and centrifuged for 5 min immediately before analysis. Osmolality was measured in duplicate on 10 μl samples using a model 5500 Wescor Vapour Pressure meter (Logan, UT, USA). Plasma chloride concentrations were measured in duplicate using a model 4425000 Haake Buchler digital chloridometer (Kansas City, MO, USA); values were checked against a chloride standard (100 mmol l^{-1} NaCl) before and after approximately every 10 duplicates.

Gill Na^+,K^+-ATPase α1a and α1b isoform expression

An analysis of Na^+,K^+-ATPase α-subunit isoforms expression, as described by Richards et al. (2003), was performed on a subset of gill samples from Upper Fraser stocks caught (i) in the Georgia Strait, (ii) at Whonnock, (iii) during migration at Hell's Gate (river 200 km), (iv) while the fish were holding on the spawning grounds before spawning (green), and (v) fully mature fish (spawners). Fish captured on the spawning grounds were sampled from the Horsefly River, Quesnel Watershed.

Briefly, total RNA was extracted from gill tissue using TriPure Isolation Reagent (Roche Diagnostics, Montreal, QC, Canada) and quantified spectrophotometrically. First-strand cDNA was synthesized from 5 μg of total RNA using oligo(dT_{15}) primer and RevertAid^{TM} H-Minus M-MuLV reverse transcripase (MBI Fermentas Inc., Burlington, ON, Canada). The expression of Na^+,K^+-ATPase -α1a and -α1b isoforms was estimated using quantitative real-time PCR (qRT-PCR; ABI Prism 7000 sequence analysis system; Applied Biosystems Inc., Foster City, CA, USA). PCR

reactions contained 1 μl of cDNA, 4 pmoles of each isoform-specific primer and Universal SYBR green master mix (Applied Biosystems Inc.) in a total volume of 20 μl. Primers were designed from trout Na^+,K^+-ATPase α1a (GenBank Accession No. AY319391), Na^+,K^+-ATPase α1b (Accession No. AY319390) and elongation factor 1α (Accession No. AF498320). Primer sequences were as follows: Na^+,K^+-ATPase -α1a forward, 5′ GGC CGG CGA GTC CAA T 3′; Na^+,K^+-ATPase -α1a reverse, 5′ GAG CAG CTG TCC AGG ATC CT 3′; Na^+,K^+-ATPase -α1b forward, 5′ CTG CTA CAT CTC AAC CAA CAA CAT T 3′; Na^+,K^+-ATPase -α1b reverse, 5′ CAG CAT CAC AGT GTT CAT TGG AT 3′; and elongation factor-1α forward, 5′ GAG ACC CAT TGA AAA GTT CGA GAA G 3′; elongation factor-1α reverse, 5′ GCA CCC AGG CAT ACT TGA AAG 3′. All qRT-PCR reactions were performed as follows: 2 min at 50°C, 10 min at 95°C, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Melt curve analysis was performed following each reaction to confirm the presence of only a single product of the reaction. The melting temperature of the amplicon obtained with cDNA from sockeye salmon was identical to that of the trout amplicon, strongly indicating that the primers developed for trout also amplify the expected target in salmon. Negative control reactions were performed for a selection of samples using RNA that had not been reverse transcribed to control for the possible presence of genomic contamination. Genomic DNA contamination never constituted more than 1:4096 starting copies for any gene examined. One randomly selected sample was used to develop a standard curve relating threshold cycle to cDNA amount for each primer set and all results are expressed relative to these standard curves. mRNA amounts are normalized relative to elongation factor 1α and expressed relative to the gill samples collected from fish caught in Georgia Strait. Freshwater migration did not affect the expression of elongation factor 1α when expressed relative to total mRNA reverse transcribed; therefore, any changes in gene expression are due to changes in Na^+,K^+-ATPase and not due to changes in the control.

Statistical analysis

Sockeye collected at the peak of the run were compared for gill Na^+,K^+-ATPase activity, plasma chloride and osmolality using a one-way analysis of variance (ANOVA) to determine whether location had a significant effect on these variables for the 2002 and 2003 samples. In 2002, our sampling was more extensive in freshwater than seawater. In 2003, we sampled migrating sockeye more extensively in seawater than freshwater. As a consequence, fish were not captured from the same locations in both years. We could not, therefore, compare the data directly between the 2 years for all locations. However, comparisons between years and among stocks were made for fish captured at the common sampling sites of Whonnock and the spawning grounds using a two-way ANOVA. For each year, fish sampled on the spawning grounds were separated into those that were not fully mature (green) and those that were fully mature fish ready to spawn (spawners). When factors were found to be statistically significant, Tukey's test was used to determine differences between the samples collected from the different locations. Statistical significance was taken at a level of $P<0.05$. All values are expressed as means ± 1 S.E.M. Specific activity of gill Na^+,K^+-ATPase activity, α1a mRNA gene expression and α1b mRNA gene expression were examined for differences by location using a one-way ANOVA, followed by Tukey's test if significant differences were found.

Results

Physiological changes were observed in adult sockeye salmon returning from the ocean to spawning areas. We found

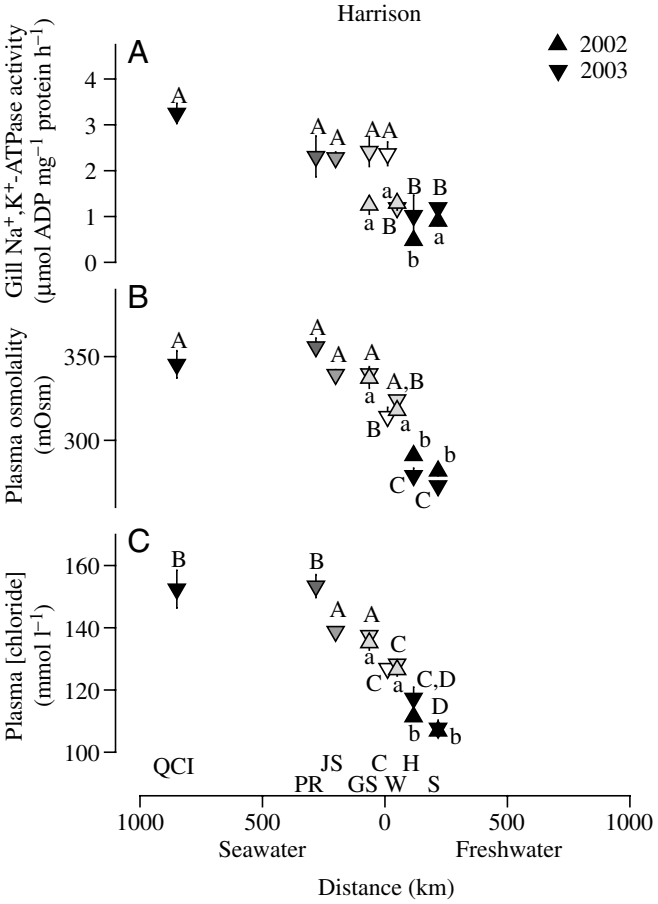

Fig. 2. (A) Gill Na^+,K^+-ATPase activity (μmol ADP mg^{-1} protein h^{-1}), (B) plasma osmolality (mOsm), and (C) plasma [chloride] (mmol l^{-1}) levels for Harrison sockeye salmon captured in seawater and freshwater during migration to the spawning grounds (distance, km). The mouth of the Fraser River is defined as 0 km. Sampling locations as identified in Fig. 1. Spawners were sampled in Weaver Creek, a major spawning tributary in this system. The last two samples were collected on the spawning grounds (117 km; H, holding, S, spawners), but sexually mature fish are offset to prevent overlap of the data points. Sample size ranged from 54 (JS, 2003) to 3 (holding, 2003; W, 2002). Values are means ± S.E.M. Symbols with a common letter do not differ significantly.

significant changes in gill Na^+,K^+-ATPase activity between seawater and freshwater locations in both years of our study and for all populations of sockeye examined. For the Harrison stock, gill Na^+,K^+-ATPase activity showed significant change with location in 2002 ($F_{3,45}$=8.06, P<0.001) and 2003 ($F_{7,156}$=11.20, P≪0.001) (Fig. 2). Plasma osmolality (2002, $F_{3,46}$=37.42, P≪0.001; 2003, $F_{7,163}$=46.35, P≪0.001) and [chloride] (2002, $F_{3,46}$=32.19, P≪0.001; 2003, $F_{7,164}$=69.10, P≪0.001) also showed highly significant relationships with sampling location. For the Shuswap stock, gill Na^+,K^+-ATPase activity similarly showed significant change with location in 2002 ($F_{4,175}$=19.98, P<0.001) and 2003 ($F_{8,171}$=22.17, P≪0.001) (Fig. 3), as did plasma osmolality (2002, $F_{4,186}$=48.85, P≪0.001; 2003, $F_{8,175}$=42.71, P≪0.001) and [chloride] (2002, $F_{4,187}$=70.01, P≪0.001; 2003, $F_{8,175}$=19.86, P≪0.001). Likewise, for the Chilko stock, gill Na^+,K^+-ATPase activity showed significant change with location in 2002 ($F_{3,42}$=7.48, P≪0.001) and 2003 ($F_{6,116}$=6.58, P≪0.001) (Fig. 4), as did plasma osmolality (2002, $F_{3,42}$=15.57, P≪0.001; 2003, $F_{6,119}$=47.32, P≪0.001) and [chloride] in 2003 ($F_{6,119}$=52.95, P≪0.001), but not in 2002 ($F_{3,42}$=1.13, P=0.34). For the Quesnel stock, gill Na^+,K^+-ATPase activity showed significant change with location in 2002 ($F_{5,93}$=4.95, P<0.001) and 2003 ($F_{6,86}$=6.20, P≪0.001) (Fig. 5), as did plasma osmolality (2002, $F_{5,96}$=51.04, P≪0.001; 2003, $F_{6,92}$=13.65, P≪0.001) and [chloride] (2002, $F_{5,95}$=19.33, P≪0.001; 2003, $F_{6,92}$=6.74, P≪0.001).

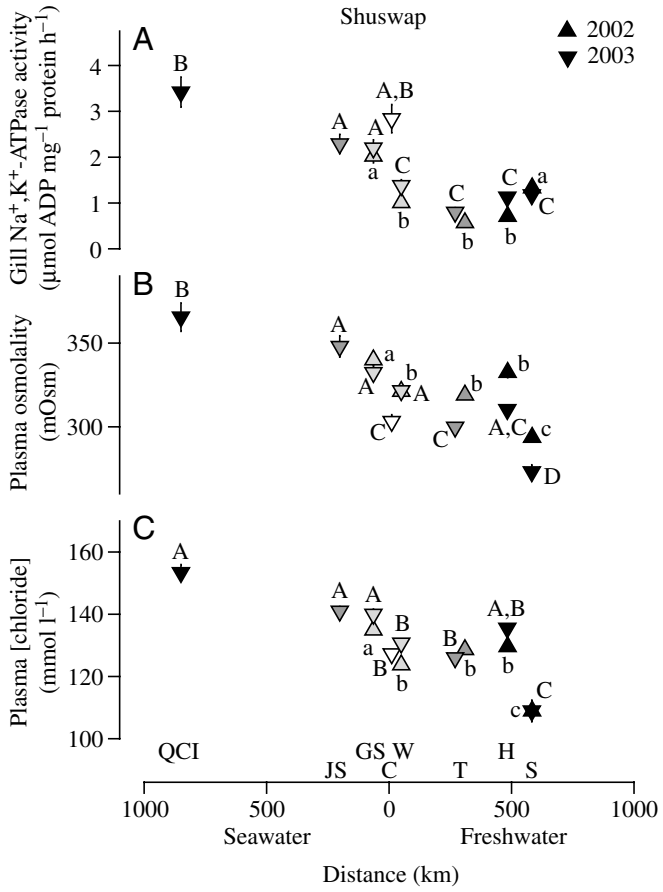

Fig. 3. (A) Gill Na^+,K^+-ATPase activity (μmol ADP mg^{-1} protein h^{-1}), (B) plasma osmolality (mOsm), and (C) plasma [chloride] (mmol l^{-1}) levels for Shuswap sockeye salmon captured in seawater and freshwater during migration to the spawning grounds (distance, km). The mouth of the Fraser River is defined as 0 km. Sampling locations as identified in Fig. 1. Spawners were sampled in Adams River, a major spawning tributary in this system. The last two samples were collected on the spawning grounds (480 km; H, holding, S, spawners), but sexually mature fish are offset to prevent overlap of the data points. Sample size ranged from 74 (GS, 2002) to 6 (QCI, 2003). Values are means ± S.E.M. Symbols with a common letter do not differ significantly.

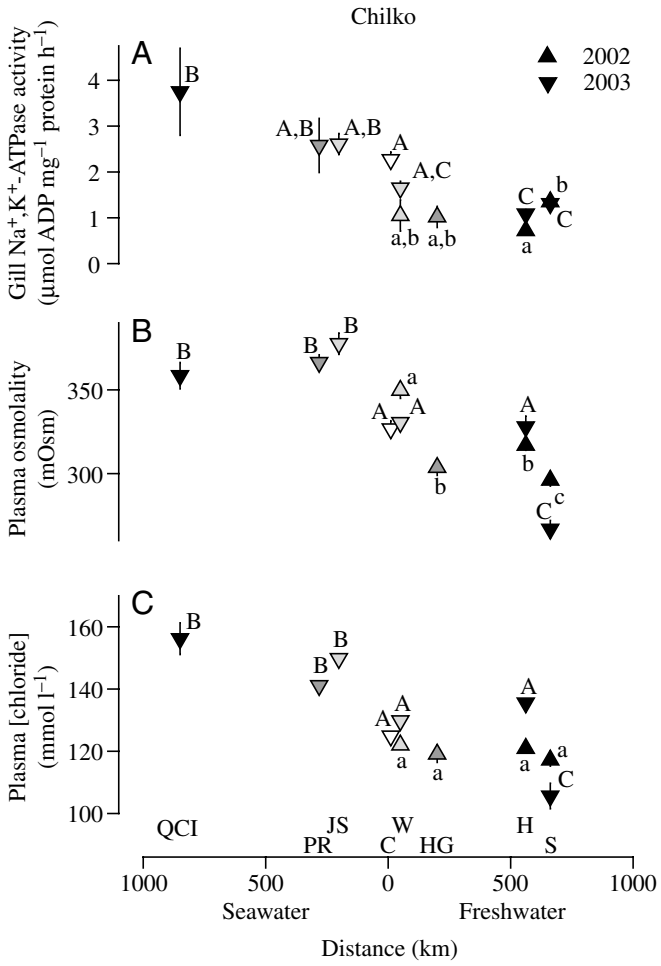

Fig. 4. (A) Gill Na^+,K^+-ATPase activity (μmol ADP mg^{-1} protein h^{-1}), (B) plasma osmolality (mOsm), and (C) plasma [chloride] (mmol l^{-1}) levels for Chilko River sockeye salmon captured in seawater and freshwater during migration to the spawning grounds (distance, km). Sampling locations as identified in Fig. 1. The mouth of the Fraser River is defined as 0 km. Spawners were sampled in Horsefly River, a major spawning tributary in this system. The last two samples were collected on the spawning grounds (562 km; H, holding, S, spawners), but sexually mature fish are offset to prevent overlap of the data points. Sample size ranged from 30 (W, 2003) to 2 (HG, 2002). Values are means ± S.E.M. Symbols with a common letter do not differ significantly.

Ionoregulatory changes in seawater

Fish were sampled in the sea at four locations, one ~850 km, a second 300 km, a third 200 km, and a fourth <25 km from the mouth of the Fraser River. Depending on stock, there were significant decreases in gill Na^+,K^+-ATPase activity, plasma osmolality and chloride levels as the sockeye salmon moved between these three saltwater locations (Figs 2–5); enzyme activity declined significantly for Shuswap sockeye between the Queen Charlotte Islands and Georgia Strait. The pattern was similar for the other stocks, but small sample size and no Quesnel and Chilko sockeye caught in Georgia Strait for 2003 limited statistical power and sample comparisons. Plasma osmolality and [chloride] values declined significantly for Shuswap and Harrison stocks, respectively, between Queen Charlotte Island and Georgia Strait; but not Chilko and Quesnel. These data provide evidence for pre-freshwater ionoregulatory preparations occurring before fish encountered and entered the Fraser River.

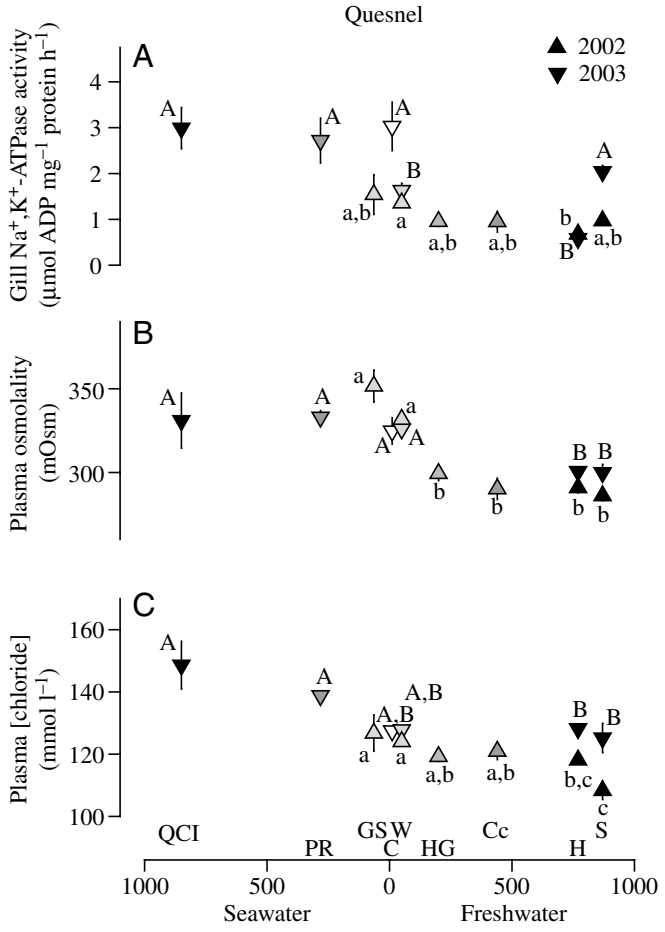

Fig. 5. (A) Gill Na^+,K^+-ATPase activity (μmol ADP mg^{-1} protein h^{-1}), (B) plasma osmolality (mOsm), and (C) plasma [chloride] (mmol l^{-1}) levels for Quesnel River sockeye salmon captured in seawater and freshwater during migration to the spawning grounds (distance, km). Sampling locations as identified in Fig. 1. The mouth of the Fraser River is defined as 0 km. Spawners were sampled in Horsefly River, a major spawning tributary in this system. The last two samples were collected on the spawning grounds (749 km; 749; H, holding, S, spawners), but sexually mature fish are offset to prevent overlap of the data points. Sample size ranged from 53 (W, 2002) to 2 (QCI, 2003). Values are means ± S.E.M. Symbols with a common letter do not differ significantly.

Ionoregulatory changes with freshwater entry

The Cottonwood sample site (river, 11 km) is estuarine. For the Shuswap and Harrison stocks intercepted at Cottonwood, both plasma osmolality and chloride had declined significantly compared with Strait of Georgia samples (Figs 2 and 3).

At the Whonnock sample site (river, 50 km) fish are in freshwater. Here gill Na^+,K^+-ATPase activity had significantly declined in three of the four stocks compared with the Cottonwood and Georgia Strait samples. Two-way ANOVA indicated that there were no significant differences among stocks (P=0.097) or between years (P=0.378) for gill Na^+,K^+-ATPase activity. Except for plasma osmolality in the Shuswap stock, which showed a significant increase, there was no significant change in plasma osmolality and [chloride] between the Cottonwood and Whonnock samples (Fig. 3). Gill Na^+,K^+-ATPase activity differed significantly between Georgia Strait and Whonnock for Shuswap (2002) and Harrison and Shuswap stocks in 2003.

Ionoregulatory changes during freshwater migration

For all stocks examined, gill Na^+,K^+-ATPase activity, as well as plasma osmolality and [chloride] values, of fish arriving at the spawning grounds were significantly lower than values from the Georgia Strait. Thus, from the Queen Charlotte Islands to the spawning areas, there was a fairly consistent decline in gill Na^+,K^+-ATPase activity, with the exception of values from fish captured shortly after entry into freshwater.

Because the different fish stocks migrated different distances in freshwater, it was possible to examine the effects of freshwater migration time and distance on the ionoregulatory changes. The loss of Na^+,K^+-ATPase activity per unit distance was highest for the fish stock that migrated the shortest distance (Harrison stock), and least for the fish stock migrating the longer distances (over 750 km in freshwater for the Quesnel stock; Figs 2–5). Regardless, all stocks arrived at the spawning grounds with levels of gill Na^+,K^+-ATPase activity that were very low (approximately 1 μmol ADP mg^{-1} protein h^{-1}). There were significant differences among the stocks and between years (Fig. 6A); however, there was no apparent trend and Na^+,K^+-ATPase activities of fish holding on the spawning grounds were not related to migration distance. Instead, the decline in enzyme activity appeared to be dependent on time in freshwater. This is evident when the absolute change in enzyme activity is plotted against the estimated time in freshwater for fish sampled at the spawning grounds (Fig. 6B). The exception to this trend was the Harrison, 2003 data, but these data were limited in that only three green fish were sampled on the Weaver Creek spawning grounds in 2003.

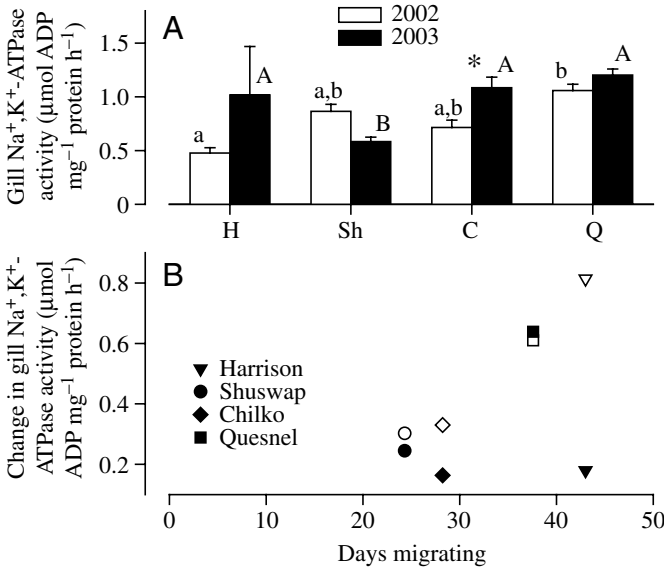

Fig. 6. (A) Gill Na^+,K^+-ATPase activity (μmol ADP mg^{-1} protein h^{-1}) for fish arriving on the spawning grounds in the Harrison (H), Shuswap (Sh), Chilko (C), Quesnel (Q) Watersheds in 2002 and 2003. *Significant difference between years for the same stock. Samples with a common letter do not differ significantly. Sample size ranged from 15 to 30, except for Harrison, 2003 where sample size was 3. (B) The absolute change in gill Gill Na^+,K^+-ATPase activity between fish captured at Whonnock and fish arriving on the spawning grounds for the different stocks examined plotted as a function of migration time within freshwater. Migration rates are based on estimates from English et al. (2004) and differences in dates between sampling at the two locations.

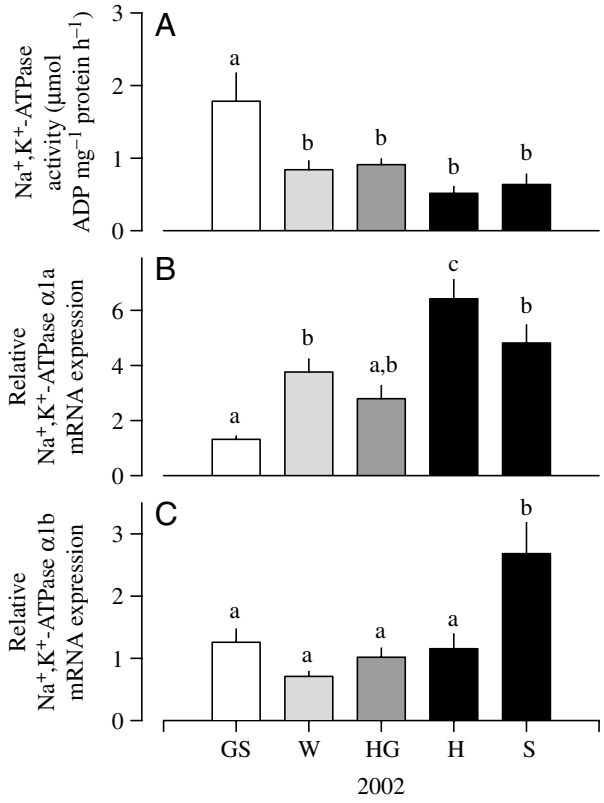

Fig. 7. (A) Gill Na^+,K^+-ATPase activity (μmol ADP mg^{-1} protein h^{-1}), (B) Na^+,K^+-ATPase α1a expression (relative to EF-1α) and (C) Na^+,K^+-ATPase α1b expression (relative to EF-1α) for fish captured in seawater and freshwater during migration to the spawning grounds in 2002. Sample locations as indicated in Fig. 1. Fish holding on the spawning grounds (H) and spawners (S) were sampled on the spawning grounds of the Horsefly River (Quesnel Watershed). Sample size ranged from 9 to 13. Values are means ± S.E.M.

Spawning salmon

After arrival at the spawning areas in each river system, sockeye salmon typically hold for approximately 4–8 days before they become fully ripe and begin spawning. Once spawning is completed, fish rapidly senesce and die after a further 7–15 days (McPhee and Quinn, 1998; Healey et al., 2003). Given the progressive declines in gill Na^+,K^+-ATPase activity as the fish migrate upriver, it is surprising that gill Na^+,K^+-ATPase activities increased while fish were holding on the spawning grounds for many of the populations (Figs 2–5). For peak run fish, the relative increase varied, but was often significant. Overall, the increased enzyme activity, however, failed to prevent further significant declines in plasma osmolality and chloride as fish completed maturation.

Na^+,K^+-ATPase mRNA expression

Expression of Na^+,K^+-ATPase α1a and α1b subunits showed significant differences in fish sampled at different locations of the migration (Fig. 7). Movement into freshwater, as exhibited by the samples collected at Whonnock, was characterised by a significant increase in α1a subunit mRNA expression. The highest levels of gill Na^+,K^+-ATPase α1a mRNA were measured in fish arriving at the spawning grounds. In contrast, fish that were maturing on the spawning grounds were characterised by a significant increase in α1b subunit mRNA, despite there being no difference in expression between seawater and freshwater samples.

Discussion

The results presented in this study represent the first examination of ionoregulatory changes in wild migratory adult salmon as they move from seawater to freshwater and return to spawning grounds. Sampling adult wild fish during a 1600 km migration path is technically challenging and requires that resources are focused on key sampling locations at appropriate times. The sampling locations that we selected for this study allowed us to attempt to answer four questions that have not been previously addressed.

Do sockeye salmon prepare for freshwater in advance of arrival at the estuary?

A decrease in gill Na^+,K^+-ATPase activity indicates that seawater ionoregulatory capacity of migrating sockeye declines before the fish enter freshwater. The changes in

enzyme activity that occur in seawater are probably preparatory for movement into freshwater. Uchida et al. (1997) also suggest that physiological and histological changes in the gill of chum salmon are preparatory for upstream migration. These authors showed that gill Na^+,K^+-ATPase continued to decrease in maturing chum captured before river entry and held in seawater; chum held in seawater showed elevated plasma osmolality and increased mortality. Although physiological changes in seawater appear adaptive for river entry, there is also evidence that hypo-osmoregulatory function is directly impaired in sexually maturing adult salmon (Clarke and Hirano, 1995). Changes observed in seawater salmon may be associated with elevated reproductive hormones. Endocrine factors associated with maturation impair ionoregulatory ability in seawater (Lundqvist et al., 1989; Madsen and Korsgaard, 1989). In a parallel study, an increase in reproductive hormones for Fraser River sockeye tagged in Johnstone Strait was found before entry into freshwater (S. J. Cooke, S. G. Hinch, G. T. Crossin, D. A. Patterson, K. K. English, M. C. Healey, S. Macdonald, J. M. Shrimpton, J. L. Young, A. Lister, G. Van Der Kraak and A. P. Farrell, manuscript submitted for publication). These authors, however, found no significant differences in reproductive hormones among the populations of sockeye, suggesting that differences between populations are not a function of reproductive state.

Despite the decline observed in gill Na^+,K^+-ATPase activity, changes in osmolality and chloride values for seawater fish do not suggest an impairment in ionoregulatory performance. Plasma osmolality and chloride did not differ among seawater sampling sites for the Chilko and Quesnel stocks (Figs 4 and 5). There was a parallel decline in plasma osmolytes with the changes in gill Na^+,K^+-ATPase activity for the Shuswap sockeye migrating from the Queen Charlotte Islands (850 km from the Fraser River mouth) to Johnstone Strait (210 km from the Fraser River mouth) (Fig. 3). Lower enzyme activities would be expected to correspond with an increase in plasma osmolality for fish maintained in seawater, as has been demonstrated in juvenile salmon during the parr–smolt transformation (McCormick and Saunders, 1987; Shrimpton et al., 1994). Waters for these two areas are hyperosmotic to plasma levels, so it is difficult to interpret the declines in chloride and osmolality. Harrison sockeye are the only population to show a significant change in plasma chloride for seawater fish; there is a significant decline in values for fish caught in Johnson Strait compared to Georgia Strait (Fig. 2). There is considerable influence of the Fraser River within this region and the declines in [chloride] may reflect lower environmental salinities (Thompson, 1981).

Our sampling of fish in seawater for 2003 was much more extensive than in 2002, but we sampled fish in Georgia Strait in both years. The troll fishery in Georgia Strait is selective for late-run fish and few Quesnel and no Chilko fish were caught. Values in Georgia Strait were similar for both years, with the exception of gill Na^+,K^+-ATPase activity for Harrison fish (Fig. 2). The difference between years may reflect differences in holding time for fish within Georgia Strait. S. J. Cooke, S. G. Hinch, G. T. Crossin, D. A. Patterson, K. K. English, M. C. Healey, S. Macdonald, J. M. Shrimpton, J. L. Young, A. Lister, G. Van Der Kraak and A. P. Farrell (manuscript submitted for publication), however, examined Fraser River late-run sockeye that showed variable delay in migration and found no difference in gill Na^+,K^+-ATPase activities with delay time in Georgia Strait.

Are freshwater ionoregulatory changes complete before fish leave the ocean?

The first freshwater sampling point was at Cottonwood, which is 11 km from the mouth of the Fraser River, yet there are still saltwater intrusions at this location. As a consequence, fish sampled from Cottonwood may still have access to increased salinity, but the decrease in plasma osmolality and [chloride] in the four populations examined (Figs 2–5) suggest that fish sampled were in full freshwater. The switch from seawater ionoregulation to chloride uptake for fish directly transferred from seawater to freshwater has been shown to take approximately 4 days (Battram and Eddy, 1990). Perturbations in plasma osmolality and [chloride], although in the opposite direction, are similar in magnitude to those seen in smolting (Blackburn and Clarke, 1987); suggesting ionoregulatory changes that are preparatory for freshwater entry have already occured in seawater. Gill Na^+,K^+-ATPase activity showed little change between the Georgia Strait and Cottonwood samples (Figs 2 and 3); however, Na^+,K^+-ATPase α1a mRNA expression indicates that physiological changes continue after movement into freshwater (Fig. 7).

In the 2003 fish sampled, we found a signficant decline in gill Na^+,K^+-ATPase activity between fish sampled at Cottonwood and Whonnock for three of the populations. We do not know the actual temporal difference for fish caught between the two locations, but fish may travel the 40 km in less than 24 h (English et al., 2004). Modifications of enzyme activity have adaptive significance and occur when euryhaline teleosts move between changing salinities (McCormick, 1995). The response normally takes several days, suggesting transcriptional regulation. Short-term regulation of this enzyme, however, has been demonstrated in killifish transferred between salinities (Mancera and McCormick, 2000). Elevated cyclic AMP levels in brown trout have also been found to decrease maximal Na^+,K^+-ATPase activity in gills of brown trout (Tipsmark and Madsen, 2001). The authors suggest that phosphorylation may regulate Na^+,K^+-ATPase activity in teleosts. The significant differences in gill Na^+,K^+-ATPase activity between fish sampled at Cottonwood and Whonnock could also be due to rapid modulation of the enzyme (Tipsmark and Madsen, 2001).

Isoform switching between two Na^+,K^+-ATPase α-subunit isoforms (α1a and α1b) during salinity transfer is thought to be an important component in changing the salmonid gill from an ion-absorbing epithelium in freshwater to an ion-secreting epithelium in seawater (Richards et al., 2003). Consistent with this notion, the expression of Na^+,K^+-ATPase α1a increased in

wild sockeye salmon following movement from seawater in Georgia Strait to freshwater in Whonnock and is associated with a decrease in Na^+,K^+-ATPase activity (Fig. 7). These results suggest that the gill ionoregulatory changes necessary for freshwater acclimation, at least in terms of mRNA expression and enzyme activity, are not complete before the fish enters freshwater (Fig. 7). The lack of change in Na^+,K^+-ATPase α1b mRNA in the gills of wild sockeye salmon following movement from seawater to freshwater are consistent with the results of Richards et al. (2003), who found that expression of Na^+,K^+-ATPase α1b increases transiently following seawater transfer, and returns to the levels observed in freshwater acclimated individuals within 10 days post-transfer. Overall, enhanced expression of Na^+,K^+-ATPase α1a during freshwater migration is consistent with the isoform switching proposed by Richards et al. (2003).

Concurrent with the changes in enzyme activity at Whonnock is a rebound in plasma osmolality and [chloride]. Based on changes in plasma parameters and gill Na^+,K^+-ATPase activity, the chloride uptake is not likely to have achieved freshwater values (Battram and Eddy, 1990), but may be adjusted as the fish move upriver.

Are maturing sockeye in an ionoregulatory steady state during migration?

Gill Na^+,K^+-ATPase activity, plasma osmolality and [chloride] declined with the time and distance covered while migrating upriver. Migrating adult sockeye, therefore, are not in an ionoregulatory steady state. Generally the lowest values of gill Na^+,K^+-ATPase were observed for fish approaching or first arriving on the spawning grounds. For all stocks, enzyme activities and plasma osmolalities had significantly declined from seawater values (Figs 2–5). The factors measured in the present study of freshwater migrating fish suggest continued physiological changes throughout migration. Physiological changes associated with migration distance have previously been observed in smolting salmonids with distance moved downstream (Muir et al., 1994). Therefore, the decline in gill Na^+,K^+-ATPase activity as sockeye migrate to spawning areas may reflect physiological adjustments that continue to occur as the fish migrate. The absolute changes in gill Na^+,K^+-ATPase activity were related to estimated time in freshwater. English et al. (2004) used radiotelemetry to determine migration rates in maturing late-run sockeye salmon in freshwater. They found that sockeye after entering freshwater migrated at a fairly consistent rate and tagged sockeye maintained their chronological order during migration as assessed by detection at monitoring stations along the Fraser River. The Harrison sockeye, however, hold in freshwater for a relatively longer period given their short river migration distance (117 km).

Does final maturation affect ionoregulatory status?

Plasma osmolality and chloride levels measured in spawning fish suggest that they are no longer able to maintain adequate homeostasis in freshwater. Sockeye are semelparous and die shortly after spawning. The physiological perturbation that the fish experience may be of little consequence, however; gill Na^+,K^+-ATPase activities in spawners are generally higher than for fish holding on the spawning grounds (Fig. 6). The higher gill Na^+,K^+-ATPase activities observed in spawners suggests that the fish may be attempting to compensate for the osmotic perturbation.

Spawning sockeye salmon are characterized by a marked increase in gill Na^+,K^+-ATPase α1b mRNA compared to individuals holding on the spawning grounds. Previous work demonstrated a transient increase in the expression of this isoform following transfer to seawater (Richards et al., 2003) and these authors speculated that the expression of Na^+,K^+-ATPase α1b might be under the regulation of circulating glucocorticoid levels. No change in Na^+,K^+-ATPase α1b expression was observed in response to seawater to freshwater movement; however, large increases in Na^+,K^+-ATPase α1b mRNA levels were observed in spawning sockeye. Previous work has shown that circulating cortisol levels increase during maturation (Donaldson and Fagerlund, 1972; Carruth et al., 2000) and the gene is possibly responsive to the higher cortisol levels.

In conclusion, in the present study we found that ionoregulatory changes preparatory for freshwater residence occur in adult sockeye salmon while they are migrating in seawater. The entry into freshwater was accompanied by further physiological adjustments. Fish from the four stocks caught at Whonnock, the first sampling location that was fully freshwater, however, did not differ in our measures of ionoregulatory performance. A large difference in freshwater migration distance and time in freshwater before spawning existed in the four stocks of sockeye examined. All stocks arrived on the spawning grounds in similar physiological condition, as indicated by our measurements and consistent with measures of energy partitioning reported by Patterson et al. (2004) and Crossin et al. (2004). Physiological changes observed in spawners suggest that sockeye attempt to minimize the osmotic perturbation that is associated with final maturation.

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References

Battram, J. C. and Eddy, F. B. (1990). Recovery of chloride uptake in seawater-adapted rainbow trout (*Salmon gairdneri*) after transfer to freshwater. *J. Exp. Biol.* **148**, 489-493.

Beacham, T. D., Withler, R. E. and Wood, C. C. (1995). Stock identification

of sockeye salmon by means of minisatellite DNA variation. *N. Am. J. Fish. Man.* **15**, 249-265.

Blackburn, J. and Clarke, W. C. (1978). Revised procedure for the 24 h seawater challenge test to measure seawater adaptability of juvenile salmon. *Can. Tech. Report Fish. Aquat. Sci.* **1515**, 35.

Brauner, C. J., Shrimpton, J. M. and Randall, D. J. (1992). The effect of short duration seawater exposure on plasma ion concentrations and swimming performance in coho salmon (*Oncorhynchus kisutch*). *Can. J. Fish. Aquat. Sci*. **49**, 2399-2405.

Carruth, L. L., Jones, R. E. and Norris, D. O. (2002). Cortisol and pacific salmon: A new look at the role of stress hormones in olfaction and homestream migration. *Integ. Comp. Biol.* **42**, 574-581.

Clarke, W. C. and Hirano, T. (1995). Osmoregulation. In *Physiological Ecology of Pacific Salmon* (ed. C. Groot, L. Margolis and W. C. Clarke), pp. 317-377. Vancouver, BC, Canada: UBC Press.

Cooke, S. J., Hinch, S. G., Farrell, A. P., Lapointe, M., Jones, S. R. M., Macdonald, S., Patterson, D., Healey, M. C. and Van Der Kraak, G. (2004). Abnormal migration timing and high en route mortality of sockeye salmon in the Fraser River, British Columbia. *Fisheries* **29**(2), 22-33.

Crossin, G. T., Hinch, S. G., Farrell, A. P., Higgs, D. A., Lotto, A. G., Oakes, J. D. and Healey, M. C. (2004). Energetics and morphology of sockeye salmon: effects of upriver migratory distance and elevation. *J. Fish Biol.* **65**, 788-810.

Donaldson, E. M. and Fagerlund, U. H. M. (1972). Corticosteroid dynamics in Pacific salmon. *Gen. Comp. Endocrinol.* **3**, 254-265.

English, K., Sliwinski, C., Labelle, M., Koski, W. R., Alexander, R., Cass, A. and Woodey, J. (2004). Migration timing and in-river survival of Late-run Fraser River sockeye using radio-telemetry techniques. Report prepared by LGL Limited, Sidney, BC for the Pacific Biological Station of Fisheries and Oceans Canada.

Fisher, J. P. and Pearcy, W. G. (1988). Growth of juvenile coho salmon (*Oncorhynchus kisutch*) off Oregon and Washington, USA, in years of differing coastal upwelling. *Can. J. Fish Aquat. Sci.* **45**, 1036-1044.

Healey, M. C., Lake, R. and Hinch, S. G. (2003). Energy expenditures during reproduction by sockeye salmon (*Oncorhynchus nerka*). *Behaviour* **140**, 161-182.

Hinch, S. G., Cooke, S. J., Healey, M. C. and Farrell, A. P. (2005). Behavioural physiology of fish migration. In *Behaviour and Physiology, Fish Physiology Series*, vol. 24 (ed. K. Sloman, S. Balshine and R. Wilson), pp. 239-295. Amsterdam: Elsevier Science.

Hirano, T., Morisawa, M. and Suzuki, K. (1978). Changes in plasma and coelomic fluid composition of the mature salmon (*Oncorhynchus keta*) during freshwater adaptation. *Comp. Biochem. Physiol.* **61A**, 6-8.

Hoar, W. S. (1988). The physiology of smolting salmonids. In *Fish Physiology*, vol. 11B (ed. W. S. Hoar and D. J. Randall), pp. 275-344. New York: Academic Press.

Lundqvist, H., Borg, B. and Berglund, I. (1989). Androgens impair seawater adaptability in smolting Baltic salmon (*Salmo salar*). *Can. J. Zool.* **67**, 1733-1736.

Madsen, S. S. and Korsgaard, B. (1989). Time-course effects of repetitive oestradiol-17β and thyroxine injections on the natural spring smolting of Atlantic salmon, *Salmo salar* L. *J. Fish Biol.* **35**, 119-128.

Mancera, J. M. and McCormick, S. D. (2000). Rapid activation of gill Na^+,K^+-ATPase activity in the euryhaline teleost *Fundulus herteroclitus*. *J. Exp. Zool.* **287**, 263-274.

McCormick, S. D. (1993). Methods for the nonlethal gill biopsy and measurements of Na^+,K^+-ATPase activity. *Can. J. Fish Aquat. Sci.* **50**, 656-658.

McCormick, S. D. (1995). Hormonal control of gill Na^+,K^+-ATPase activity and chloride cell function. In *Fish Physiology*, vol. 14 (ed. C. M. Wood and T. J. Shuttleworth), pp. 285-315. San Diego, CA: Academic Press.

McCormick, S. D. and Saunders, R. L. (1987). Preparatory physiological adaptations for marine life of salmonids: osmoregulation, growth, and metabolism. *Am. Fish Soc. Symposium* **1**, 211-229.

McKeown, B. A. (1984). *Fish Migration*. London: Croom Helm Publishers.

McPhee, M. V. and Quinn, T. P. (1998). Factors affecting the duration of nest defence and reproductive life span of female sockeye salmon, *Oncorhynchus nerka*. *Envir. Biol. Fish.* **51**, 369-375.

Muir, W. D., Zaugg, W. S., Giorgi, A. E. and McCutcheon, S. (1994). Accelerating smolt development and downstream movement in yearling chinook salmon with advanced photoperiod and increased temperature. *Aquaculture* **123**, 387-399.

Nielsen, C., Aarestrup, K., Norum, U. and Madsen, S. S. (2004). Future migratory behaviour predicted from premigratory levels of gill Na^+/K^+-ATPase activity in individual wild brown trout (*Salmo trutta*). *J. Exp. Biol.* **207**, 527-533.

Patterson, D. A., Macdonald, J. S., Hinch, S. G., Healey, M. C. and Farrell, A. P. (2004). The effect of exercise and captivity on energy partitioning, reproductive maturation and fertilization success in adult sockeye salmon. *J. Fish Biol.* **64**, 1039-1059.

Richards, J. G., Semple, J. W., Bystriansky, J. S. and Schulte, P. M. (2003). Na^+/K^+-ATPase α-isoform switching in gills of rainbow trout (*Oncorhynchus mykiss*) during salinity transfer. *J. Exp. Biol.* **206**, 4475-4486.

Shrimpton, J. M., Bernier, N. J., Iwama, G. K. and Randall, D. J. (1994). Differences in measurements of smolt development between wild and hatchery-reared juvenile coho salmon (*Oncorhynchus kisutch*) before and after saltwater exposure. *Can. J. Fish Aquat. Sci.* **51**, 2170-2178.

Shrimpton, J. M., Björnsson, B. T. and McCormick, S. D. (2000). Can Atlantic salmon smolt twice? Endocrine and biochemical changes during smolting. *Can. J. Fish. Aquat. Sci.* **57**, 1969-1976.

Thomson, R. E. (1981). Oceanography of the British Columbia Coast. *Can. Spec. Pub. Fish. Aquat. Sci.* **56**, 251.

Tipsmark, C. K. and Madsen, S. S. (2001). Rapid modulation of Na^+/K^+-ATPase activity in osmoregulatory tissues of a salmonid fish. *J. Exp. Biol.* **204**, 701-709.

Uchida, K., Kaneko, T., Yamaguchi, A., Ogasawara, T. and Hirano, T. (1997). Reduced hypoosmoregulatory ability and alteration in gill chloride cell distribution in mature chum salmon (*Oncorhynchus keta*) migrating upstream for spawning. *Mar. Biol.* **129**, 247-253.