MECHANISTIC BASIS OF INDIVIDUAL MORTALITY IN PACIFIC SALMON DURING SPAWNING MIGRATIONS

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Abstract. Reproductive-based migration is a challenging period for many animals, but particularly for Pacific salmonids, which must navigate from the high seas to freshwater natal streams. For the first time, we attempt to answer the question as to why some migratory adult Pacific salmon die en route to spawning grounds. Summer-run sockeye salmon (*Oncorhynchus nerka*) were used as a model, and the migration behavior of 301 fish was followed by intercepting them in the ocean about 215 km from the mouth of the Fraser River, British Columbia, Canada, and implanting a gastric radio transmitter. Before release, telemetered fish were also bio-sampled, which included drawing a blood sample, collecting a gill biopsy, and quantifying energetic status with a microwave energy meter. We tested the predictions that the fish that died prematurely would be characterized by low energy reserves, advanced reproductive development, elevated indicators of stress, and low osmoregulatory preparedness compared with fish that completed their river migration. Just over half (52.3%) of the sockeye tagged were subsequently detected in the Fraser River. Salmon that failed to enter the river had exhibited indicators of stress (e.g., elevated plasma lactate, glucose, and cortisol). Contrary to our prediction, fish that failed to enter the river tended to have higher gross somatic energy and be larger at the time of sampling in the ocean than fish that successfully entered the river. Of the fish that were detected in the river (i.e., 134 fish excluding fishery removals), 9.7% did not migrate beyond the lower reaches (~250 km from ocean), and a further 14.2% reached the upper reaches but failed to reach natal sub-watersheds, whereas the remainder (76.1%) reached natal sub-watersheds. Of these, fish unsuccessful in the lower reaches tended to have a high plasma osmolality in the ocean, whereas fish failing in the upper reaches had lower levels of reproductive hormones in the ocean.

Key words: biotelemetry; individual variation; mortality mechanisms; Oncorhynchus *spp.; reproduction; sockeye salmon; survival during spawning migration.*

INTRODUCTION

Migrations are some of the most energy-demanding and physiologically challenging phases of a fish's life history and represent one of the most complex interplays between behavior and physiology (McKeown 1984, Lucas and Baras 2001). This is particularly salient for the Pacific salmon (*Oncorhynchus* spp.) during their reproductive migration to natal streams. Pacific salmon must navigate from feeding grounds in the open ocean to coastal waters, eventually homing to their natal watershed. They then initiate spectacular migrations up rivers to the streams of their birth (Groot and Margolis 1991), with the ultimate goal to reproduce (Dingle 1996). As Pacific salmon are generally semelparous, failure to migrate to spawning grounds and reproduce means that the fish do not contribute their genes to future generations, resulting in negligible lifetime fitness (Dingle 1980). Mortality during reproductive migrations is not unique to salmonids and extends to animals in a variety of taxa (Gauthreaux 1980).

A number of non-anthropogenic factors could lead to mortality of Pacific salmonids en route to the spawning grounds. First, adult Pacific salmon must move from

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saltwater to freshwater. To do so marine fish must either remodel their osmoregulatory apparatus to deal with a hyperosmotic freshwater environment (Clarke and Hirano 1995) or face certain death upon entering freshwater. Second, Pacific salmon must power migration and complete maturation (Crossin et al. 2004, Patterson et al. 2004) on a fixed energy budget (Brett 1995, Crossin et al. 2004). Again, premature depletion of energy reserves can cause en route mortality. Unusual deviations in environmental river conditions (e.g., flow, turbidity, temperature) or timing can elevate the cost of migration (Rand and Hinch 1998), deplete energy faster, and even challenge fish to the limits of their swimming and cardiorespiratory capabilities (Lee et al. 2003). At times fish must also evade capture by predators, a situation that may require anaerobic activity (Rand and Hinch 1998), leading to the accumulation of metabolites and oxygen debt, the depletion of tissue energy stores, and acid–base imbalances (Kieffer 2000). Parasite infections can also compromise performance and lead to migration failure (Wagneretal 2005).

Given all of these challenges, it is perhaps not surprising that there is always a component of every Pacific salmon stock that dies en route to the spawning grounds. In fact, mortality during migration is a natural and expected outcome for a segment of any population and serves as a major selective force (Dingle 1980). Yet, we know little about what characterizes a successful migrant relative to one that dies en route to spawning grounds, despite the large body of work evaluating salmonid migrations, (Lucas and Baras 2001, Hinch et al. 2005). This knowledge gap reflects a paucity of techniques that can combine information on individual fate of free-swimming migratory fish with its behavior and physiology at a broad (i.e., watershed) scale (Bennett 1987). Consequently, to address this deficiency, we developed a novel approach of combining biotelemetry and biopsy. By intercepting sockeye salmon (*O. nerka*) in the coastal ocean environment as they headed toward the Fraser River estuary, where they would begin their upriver migration, we implanted fish with radio transmitters to follow their subsequent migration behavior and to determine their fate with radio receiving systems that were deployed throughout the Fraser River and its tributaries over a distance of 1200 km. These same individuals were also biopsied, which included drawing blood from the caudal vessels, removing some gill filament tips, and quantifying energetic status using a microwave energy meter. By comparing the physiological and energetic status of the fish that successfully reached spawning grounds with those that died en route to the spawning grounds, we were able to assess the physiological and energetic correlates of mortality.

We tested the prediction that fish that died prematurely would be characterized by low energy reserves, advanced reproductive development, elevated stress indices and low osmoregulatory preparedness for the freshwater environment. These predictions are based on our current understanding of salmonid migration biology (Hinch et al. 2005). For example, endogenous energy reserves are used to fuel migration so energetic exhaustion may explain migration failure (Brett 1995). Similarly, stress, as indicated by elevated metabolites and cortisol, may impede migration (Black 1958, Carruth et al. 2002). Fish that are moving from the marine to freshwater environment must remodel their osmoregulatory apparatus (Clarke and Hirano 1995) so low osmoregulatory preparedness for freshwater should lead to mortality (Hinch et al. 2005). Finally, fish are on a fixed reproductive clock, and so advanced reproductive development may indicate early maturity and potential for premature senescence (Carruth et al. 2002, Hinch et al. 2005).

METHODS

Sampling strategy

The present investigation was part of a larger telemetry study in which sockeye salmon ($n = 559$ fish) were intercepted near the southern end of Johnstone Strait, British Columbia, Canada (Fig. 1), ~215 km from the mouth of the Fraser River (see English et al. 2004). We used protocols to biopsy a portion ($n = 301$ fish) of these sockeye salmon. Preliminary findings from earlier research on sockeye salmon suggested that use of anesthetics increased handling and holding time and elevated mortality relative to implanting non-anesthetized fish (English et al. 2005). In addition, anesthetics currently approved for use in fish should not be ingested by humans, and because the possibility existed that the fish released might be subsequently caught by fishers or other animals and consumed, we worked with unanesthetized fish. Our protocols, which were approved by the University of British Columbia Animal Care Committee, were validated in a parallel study, in which three independent assessments were used to demonstrate that it was possible to biopsy and tag sockeye salmon without causing deleterious effects to behavior or survival (Cooke et al. 2005).

Fish were collected using a large purse-seine net deployed from a commercial fishing vessel, which also served as the platform for biopsy, radio-tagging, and fish release. Fish were sampled, tagged and released over a three-week period between 11 August and 28 August 2003 at surface water temperatures of 10–13°C. Fish were first detected (i.e., arrival in the Fraser River) by two radiotelemetry stations ~300 km from the release site, 85 km upstream from the mouth of the river at Mission, British Columbia (Fig. 1) and beyond the tidal boundary. We defined a "river-entry fish" as one that was detected at or above the Mission telemetry station. Seven additional telemetry stations were strategically deployed on the mainstem Fraser River and at the entrances to natal sub-watershed (Fig. 1). Four additional receivers were deployed to assess behavior of late-run sockeye stocks in other sub-watersheds and are not discussed in this paper (English et al. 2004). Receiving
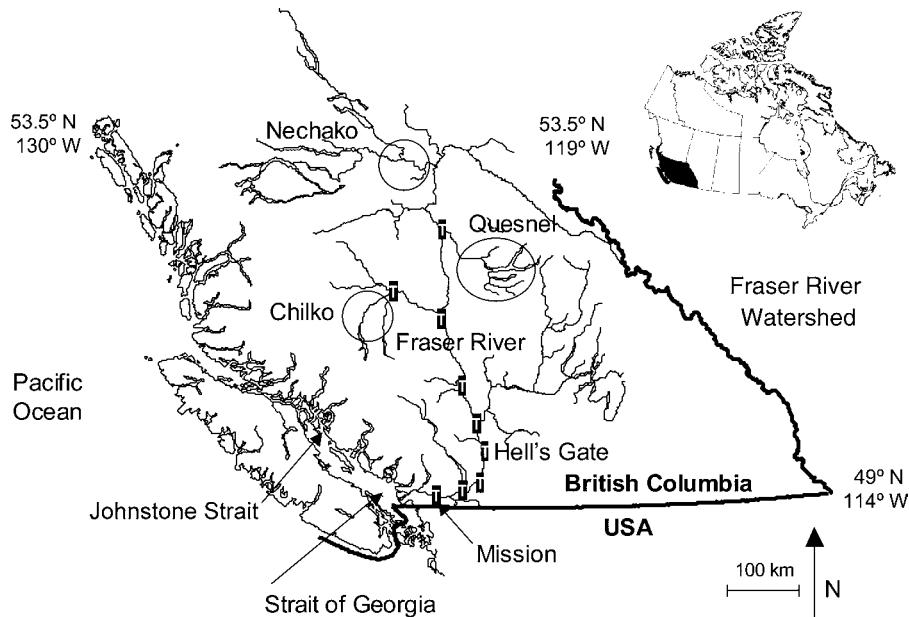
Fig. 1. Map of Canada with an inset of the Fraser River Watershed of British Columbia. Key locations are identified on the map including the river-entry telemetry station at Mission. Additional telemetry stations are indicated by the "T" in black boxes. Natal spawning watersheds and general terminal spawning locations are circled.

stations were equipped with up to three antennas and a data-logging radio receiver (SRX_400; Lotek Engineering, Newmarket, Ontario, Canada), as detailed in English et al. (2004). The detection efficiency of the individual receiving stations ranged between 87.5% and 100%, with increased efficiency as fish approached natal spawning grounds where the rivers became shallower and narrower (English et al. 2004). Mobile tracking was also conducted on foot and by boat. To encourage reporting of fish harvested by recreational anglers, commercial fishers, and First Nations fishers, we implemented a public-awareness campaign and offered a small reward for information and transmitter return. Receivers were also used to scan for transmitters at three of the largest (by volume) fish-processing plants in British Columbia. Reporting compliance was believed to be high (English et al. 2004), but any unreported harvesting would result in an overestimate of our mortality percentages.

Synopsis of biopsy and tagging techniques

Following capture, individual fish were netted from the purse (which remained in the water and was gathered at the side of the vessel) and placed in large-flow through totes on deck. The entire netting process took ~30 min, but fish were only held in the drawn purse for <5 min prior to transfer to onboard holding totes. Individuals were then removed from the tote, placed ventral side up in a V-shaped trough that was lined with foam, and provided with a continuous supply of fresh ambient seawater via a tube placed near the mouth. Fish were manually restrained for less than three minutes during

which time fork length (FL) was measured, tissues were biopsied and a radio transmitter was inserted. The biopsy procedure involved: (a) removing a small piece (0.5 g) of the adipose fin for DNA stock identification, (b) removing one scale for ageing, (c) removing 3 mL of blood from the caudal vessel using a vacutainer syringe (3.8 cm [1.5 inches], 21 gauge; Houston 1990) for assessing plasma chemistry, and (d) removing <4 mm from the tips of 6 to 8 filaments (0.3 g) from the first gill arch (McCormick 1993) for assessing gill enzyme activity. Gill tissue and centrifuged plasma samples were stored on dry ice for several days until being transferred to a −80°C freezer where they were held until analysis. A hand-held microwave energy meter (Distell Fish Fatmeter model 692; Distell, West Lothian, Scotland, UK) was placed on the left side of the fish in two locations to quantify somatic energy levels (see Crossin and Hinch 2005). Radio transmitters, which measured 16 mm in diameter and 51 mm in length and weighed 16.1 g in air and 6.2 g in water (MCFT-3A; Lotek, Newmarket, Ontario, Canada), were orally inserted into the stomach using a plastic applicator. Fish were returned to the holding tote to recover for <1 h after this procedure. All fish in the tote were released as a group in an attempt to minimize predation by marine mammals.

Assays

The present analysis focused on fish from the three largest summer-run sockeye salmon stock complexes from three different natal sub-watersheds: the Chilcotin, Nechako, and Quesnel (Fig. 1). Migrating summer-run

fish are faced with some of the longest distances and highest elevation gains of all sockeye salmon (i.e., >1000 km distance and >1000 m gain from the estuary) making them a suitable model to evaluate en route mortality. Of 559 sockeye sampled and released in the larger investigation, 301 fish were identified as summer run. Stock origin was ascribed to all individual fish by DNA analyses (Beacham et al. 1995, 2004) and consistently confirmed by the tracking of telemetered fish to spawning grounds. Stock assignment through DNA analysis is a standard technique in fisheries management and research for Fraser River sockeye salmon and has 96% accuracy in stock assignment (Terry Beacham [Pacific Biological Station], *personal communication*). In our study, we only focused on differentiating fish into three primary stock groupings (i.e., Chilcotin, Nechako, and Quesnel) which are each comprised of a number of differentiated populations. Thus, for our purposes DNA accuracy may have been higher than 96%. For this study, the physiological analysis was restricted to 278 summer-run fish, of which 243 individuals had had tissue biopsies and energy measurements, while a further 35 fish had only energy measurements before release. The remaining fish were released with transmitters but no biological data collection. Plasma testosterone (T), 17β-estradiol (E_2), and 11-keto testosterone (11-KT) levels were measured by radioimmunoassay (McMaster et al. 1992) and used to assign gender. Plasma ion (K^+, Cl^-, Na^+), cortisol, lactate, glucose, and osmolality measurements followed the procedures described by Farrell et al. (2001). Gill tissue Na^+/K^+-ATPase activity was determined with a kinetic assay (McCormick 1993) and expressed as micromoles of adenosine diphosphate (ADP) per milligram of protein per hour. Detailed description of all assays presented here including the inter-assay variability and quality-control criteria are provided in Farrell et al. (2001).

Statistical analysis

Multivariate analysis of variance (MANOVA) on log_{10}-transformed data (McGarigal et al. 2000) was conducted to assess differences in biological characteristics (i.e., all physiological, energy and size data aside from reproductive hormones) among stock groups. Because canonical-variates analysis revealed that all stocks differed (Wilks' lambda = 0.729, $P < 0.001$), stock was included as a factor in the two-way analyses of variance (ANOVA). Two-way ANOVA was used to examine specific differences among sockeye grouped according to their fate during two phases of migration, with fate as the main effect. Fate was assessed during (1) the river-entry migration phase (i.e., excluding fishery removals, we compared fish that entered the Fraser River vs. those that failed to do so) and (2) the river-migration phase (i.e., of fish that entered the river and were not harvested, we compared those that did not pass the lower river, those that did not pass the upper river,

or those that successfully reached their natal sub-watershed). For these analyses, fish defined as "river-entry fish" were those that were detected at a downstream receiving station at Mission. Of fish that successfully entered the river, the "lower river" was defined as the reach between Mission and the downstream end of the Hell's Gate fishway. The "upper river" was defined as the reach upstream of Hell's Gate to the confluence of the different stocks' natal sub-watersheds. All variables were included in these analyses except for reproductive hormones. Reproductive-hormone assays were restricted to fish that entered the river, so statistical analysis was only conducted for the in-river migration phase. For these analyses, sexes were analyzed separately, so stocks were pooled to maintain adequate sample sizes and one-way ANOVA was used to assess differences among fate. Instances where the homogeneity-of-variances assumption was violated (assessed using the Levene's test; Zar 1996), a Welch's ANOVA was utilized.

We evaluated whether capture and handling affected physiological values by looking for relationships (Spearman's correlation) between order of sampling and plasma stress values (plasma lactate, cortisol, and glucose). In total, summer-run fish were captured and released in 43 separate events, with the number tagged in each seine haul ranging from 1 to 18 fish. For all releases where blood samples existed for at least 10 summer-run fish ($n = 9$ releases), there were typically no relationships between plasma stress indicators and order of release. However, in one release there was a significant ($P < 0.05$) negative relationship between order of release and plasma lactate, and in two releases there was a significant positive relationship between these variables ($P < 0.05$). Therefore, we assumed that order of capture and sampling did not play a significant or consistent role in plasma stress variables. We also used logistic regression to evaluate the role of tagging order on failure to arrive at the river mouth using the same nine capture/tagging sessions and found no significant relationships ($P > 0.05$).

All analyses were conduced using JMP 4.0 (SAS Institute 2001) and were assessed for significance at α = 0.05. However, because of multiple comparisons we conducted sequential Bonferroni corrections (see Tables 2 and 3; Rice 1989). Note that there is substantial debate on whether or not to use Bonferroni corrections as they can require very conservative levels of significance (e.g., Cabin and Mitchell 2000, Moran 2003). Therefore, we present uncorrected P values in tabular form and enable the reader to perform their own assessment as to which significance criteria to observe (as per recommendations in Cabin and Mitchell [2000] and Moran [2003]).

RESULTS

River-entry migration phase

Of the 301 summer-run sockeye salmon released, five fish were recovered from marine fisheries. Of the

TABLE 1. Summary information on sample sizes and fates for three stocks of summer-run sockeye salmon from the Fraser River, British Columbia, Canada.

	Sockeye salmon stock					
	Chilko		Quesnel		Nechako	
Fish data characteristic	No.	%	No.	%	No.	%
Tagged	166		95		40	
Biosampled	128		78		37	
Fat probed	151		89		38	
Ocean-fishery removals	4		1		0	
Ocean mortality†	77	47.53	51	54.73	13	32.5
Reached the Fraser River	85	52.47	43	45.74	27	67.5
In-river fishery removals	10		7		4	
En route mortality‡						
Lower river	6	8.6	5	13.89	2	8.6
Upper river	14	18.67	4	11.11	1	4.34
Reached natal sub-watershed§	55	73.33	27	75.00	20	87.06

† Ocean mortality excludes all fish that were fishery removals before fish reached the Fraser River; this is a measure of the number of fish released relative to those that made it to the Fraser River.

‡ En route mortality in river excludes all fish that were fishery removals in the Fraser River; it is measured by taking the total number of fish that reached the Fraser River relative to those that died in different locales en route.

§ Note that the percentages of salmon that reached the natal watershed is only relative to the total number of fish that entered the river and avoided in-river fisheries.

remaining 296 fish, 141 (47.6%) individuals were not detected at the Mission receiver station and considered to have failed to enter the Fraser River. Failure rate was not stock specific, ranging between 32% and 55% among stocks (Table 1). Sockeye salmon that failed to enter the Fraser River had tended to be larger and to have had a higher energy density in the ocean than fish that entered the river because these results approached statistical significance without Bonferroni corrections (i.e., $P = 0.051$ and $P = 0.077$; Tables 2 and 3). However, when interpreted in the context of Bonferroni corrections, these values were not close to significant. Stock-specific significant differences in both size ($P = 0.009$) and energy density ($P = 0.003$) were noted (Table 3). Indicators of osmoregulatory status in the ocean were not consistent in their association with either river entry or stock origin. Plasma Na^+ had been higher in sockeye salmon that entered the river (Table 2). No stock or entry-related differences were observed for Cl^-, K^+, or gill Na^+/K^+-ATPase. Both plasma glucose and plasma lactate had been higher in fish that failed to enter the river with stock-specific differences noted for glucose (Table 2). There was a significant interaction between fate and stock for plasma cortisol concentrations ($P < 0.001$; Table 3). For the Quesnel and Chilcotin stocks, cortisol had been higher in fish that failed to reach the

river, whereas lower cortisol was associated with failure of the Nechako stock to reach the river.

River migration phase

Excluding in-river fishery removals ($n = 21$ fish), 134 summer-run sockeye salmon passed the lower river telemetry station during river migration (Table 1). Of these 134 fish, 13 (9.7%) sockeye were not detected past Hell's Gate (i.e., en route mortality in the lower river) and 19 (14.2%) sockeye successfully passed Hell's Gate but were not detected in natal sub-watershed stations (i.e., en route mortality in the upper river) (Table 1). Thus, 102 summer-run sockeye (76% of fish that entered the river and avoided harvest) reached their natal sub-watershed and were considered to be successful migrants, with a total in-river en route mortality of 24%. Of the 301 summer-run sockeye tagged in the Johnstone Strait, 33.9% reached their natal sub-watershed.

Many factors including fork length, ions (K^+, Cl^-, Na^+), gill Na^+/K^+-ATPase, lactate, and cortisol did not vary significantly among fish with different migratory fates and among stocks (Tables 4 and 5). The only suggestion of stock-specific differences for fish that successfully reached the river were for gross somatic energy ($P = 0.093$) and glucose ($P = 0.079$; Table 5). An elevated plasma osmolality was associated with lower-river mortality, but not upper-river mortality as compared with fish reaching natal sub-watersheds ($P = 0.043$; Table 5). Reproductive hormones varied with migratory fate; 11-KT (11-keto testosterone) in females ($P = 0.004$) was lower for upper-river mortalities than both lower-river mortalities and successful migrants. Testosterone in males followed a similar pattern; however, results were not significant even prior to Bonferroni correction ($P = 0.064$) (Table 6). No differences were observed for other reproductive hormones.

DISCUSSION

Why do some upriver-migrating Pacific salmon die en route to spawning grounds? Although a fascinating question, the answer has eluded researchers. Using summer-run sockeye salmon in the Fraser River (British Columbia, Canada) as a model, we investigated this question by coupling individual telemetry with physiological variables in fish intercepted in the ocean during their homeward migration. Our findings suggest that several physiological characteristics measured while the fish were still in the ocean provide insight into the subsequent migration success during two phases of migration. These assessments are based on extensive observations on 301 fish, of which ~50% failed to enter the river. Of the fish that did reach the river, ~75% reached the spawning areas. Potential alternative explanations to the "loss" of fish during migration, which we attribute to en route mortality, include transmitter regurgitation and fisheries removals. Transmitter regurgitation was unlikely because fish stop feeding some 500 km seaward from where they were

TABLE 2. Comparison of biological variables for three stocks of summer-run sockeye salmon with two fates: those that died before entering the Fraser River at Mission (British Columbia, Canada) with those that survived to Mission.

Variable	Stock†	Died prior to river entry			Survived to river entry		
		Mean	SE	n	Mean	SE	n
Nose fork length (cm)	C	60.1[a]	0.3	77	60.2	0.2	85
	Q	61.6[b]	0.4	51	60.7	0.4	43
	N	60.5[a]	1.0	13	59.2	0.6	27
Gross somatic energy (MJ/kg)	C	9.55[a]	0.07	71	9.47	0.07	73
	Q	9.21[b]	0.08	50	9.08	0.08	38
	N	9.59[a]	0.14	13	9.52	0.07	25
Plasma Na$^+$ (mmol/L)	C	172.2	1.2	68	183.2	0.8	55
	Q	178.2	2.0	43	183.6	1.5	32
	N	175.0	1.7	12	183.7	2.2	23
Plasma K$^+$ (mmol/L)	C	1.62	0.26	68	1.54	0.11	55
	Q	2.19	0.68	43	1.74	0.17	32
	N	1.90	0.25	12	1.29	0.12	23
Plasma Cl$^-$ (mmol/L)	C	145.3	0.5	67	146.5	0.5	56
	Q	146.5	0.8	45	146.7	0.7	31
	N	147.0	1.1	13	147.2	0.7	24
Plasma osmolality (milliosmol/kg)	C	345.5	1.6	68	343.5	2.1	58
	Q	347.8	2.4	45	344.3	2.8	32
	N	342.8	5.0	13	340.4	2.3	25
Plasma cortisol (ng/mL)	C	627.4	53.2	66	473.2	39.4	55
	Q	745.9	74.2	43	456.1	41.2	32
	N	417.6	56.8	13	514.6	67.6	25
Plasma lactate (mmol/L)	C	9.82	0.39	67	8.93	0.42	58
	Q	10.54	0.55	45	8.59	0.43	32
	N	9.64	0.87	13	6.89	0.36	25
Plasma glucose (mmol/L)	C	7.37[a]	0.16	67	6.93	0.14	58
	Q	7.17[a]	0.18	45	6.52	0.17	32
	N	7.94[b]	0.41	13	7.15	0.19	25
Na$^+$/K$^+$ATPase (µmol ADP·mg protein^{-1}·h^{-1})	C	2.65	0.15	30	2.80	0.11	52
	Q	2.32	0.11	30	2.61	0.12	32
	N	2.96	0.26	12	2.50	0.22	20

Notes: Fishery losses from the ocean were excluded from analyses. Data are means ± SE; n = number of fish. For each fate, means with different lowercase superscript letters indicate significant differences between stocks for that variable ($\alpha = 0.05$). ANOVA results are shown in Table 3.

† Stock groupings were: C, Chilcotin; Q, Quesnel; and N, Nechako.

TABLE 3. Results of a two-way ANOVA, with fate, stock, and the fate × stock interaction as effects, comparing three stocks (C, Q, N) of summer-run sockeye salmon and their fates: those that died before entering the Fraser River at Mission, British Columbia, Canada, and those that survived to river entry at Mission.

Variable	Fate			Stock			Fate × stock		
	F	df	P	F	df	P	F	df	P
Nose fork length (cm)	3.16	1	0.077	4.80	2	0.009	1.76	2	0.173
Gross somatic energy (MJ/kg)		1	0.051	**2.89**	2	**0.003**	1.80	2	0.168
Plasma concentrations									
Na$^+$ (mmol/L)	**15.46**	**1**	**<0.001**	1.42	2	0.244	1.01	2	0.367
K$^+$ (mmol/L)	1.81	1	0.180	0.41	2	0.665	0.15	2	0.858
Cl$^-$ (mmol/L)	0.31	1	0.580	2.10	2	0.125	0.18	2	0.833
Osmolality (milliosmol/L)	3.07	1	0.082	0.58	2	0.559	0.09	2	0.916
Cortisol (ng/mL)	7.22	1	0.008	3.16	2	0.045	**7.27**	**2**	**<0.001**
Lactate (mmol/L)	**24.93**	**1**	**<0.001**	2.80	2	0.064	0.14	2	0.865
Glucose (mmol/L)	**10.36**	**1**	**0.002**	4.64	2	0.011	0.15	2	0.864
Na$^+$/K$^+$-ATPase	0.22	1	0.643	2.67	2	0.072	1.34	2	0.266

Notes: Data tested by ANOVA are presented in Table 2. Because we conducted multiple comparisons, we applied Bonferroni corrections; significant values based on this criterion ($\alpha = 0.005$) are in boldface type.

TABLE 4. Comparison of biological variables for three stocks of summer-run sockeye salmon that survived entry into the Fraser River at Mission, British Columbia, Canada, but with three fates: they either died in the lower river (between Mission and Hell's Gate), died in the upper river (between Hell's Gate and natal sub-watersheds), or survived to reach the natal sub-watersheds.

Variable	Stock†	Died in lower river			Died in upper river			Reached natal sub-watershed		
		Mean	SE	n	Mean	SE	n	Mean	SE	n
Nose fork length (cm)	C	59.7	1.2	6	59.62	0.5	14	60.2	0.3	55
	Q	60.0	0.5	5	60.0	1.7	4	60.9	0.6	27
	N	59.5	1.5	2	64.0	NA	1	58.7	0.7	20
Gross somatic energy (MJ/kg)	C	9.50	0.46	5	9.54	0.12	12	9.48	0.08	48
	Q	9.29	0.14	5	9.62	0.07	4	8.95	0.11	22
	N	9.54	0.00	2	9.09	NA	1	9.58	0.08	18
Plasma Na^+ (mmol/L)	C	188.0	2.7	4	182.6	1.3	10	183.3	1.1	36
	Q	184.8	2.0	4	176.7	9.9	3	183.2	2.0	19
	N	179.5	1.5	2	200.0	NA	1	183.8	2.6	16
Plasma K^+ (mmol/L)	C	1.08	0.08	4	1.66	0.32	10	1.53	0.13	36
	Q	2.08	0.34	4	1.97	0.26	3	1.61	0.25	19
	N	2.25	0.05	2	1.30	NA	1	1.11	0.13	16
Plasma Cl^- (mmol/L)	C	146.0	1.9	4	145.9	1.2	10	146.7	0.6	37
	Q	149.7	0.9	4	142.5	1.3	2	147.1	0.9	19
	N	148.5	2.1	2	147.8	NA	1	147.2	0.8	17
Plasma osmolality (milliosmol/kg)	C	352.6	3.7[x]	4	340.9	4.3[y]	10	343.4	2.7[y]	39
	Q	363.1	5.7	4	340.7	13.0	3	341.2	3.5	19
	N	349.5	3.5	2	346.0	NA	1	339.1	3.0	18
Plasma cortisol (ng/mL)	C	640.4	265.4	3	352.2	54.5	10	492.5	51.1	37
	Q	415.9	60.4	4	573.5	159.9	3	431.5	43.0	19
	N	513.2	261.6	2	638.2	NA	1	440.9	61.3	18
Plasma lactate (mmol/L)	C	10.05	1.00	4	8.26	0.64	10	8.93	0.57	39
	Q	9.50	0.95	4	8.13	0.44	3	8.41	0.69	19
	N	6.36	0.54	2	6.69	NA	1	7.04	0.48	18
Plasma glucose (mmol/L)	C	7.05	0.28	4	7.23	0.33	10	6.85	0.17	39
	Q	6.51	0.48	4	6.07	0.26	3	6.54	0.22	19
	N	6.19	1.11	2	6.36	NA	1	7.31	0.21	18
Na^+/K^+-ATPase (μmol ADP·mg protein^{-1}·h^{-1})	C	3.02	0.54	4	2.60	0.22	9	2.81	0.14	34
	Q	2.94	0.21	5	2.99	0.21	3	2.46	0.17	18
	N	2.53	0.12	2	2.55	NA	1	2.72	0.24	14

Notes: Fishery losses were excluded from analyses. Data are means ± SE; n = number of fish. For each fate, means with different lowercase superscript letters indicate significant differences for that variable. ANOVA results are shown in Table 5.

† Stock groupings were: C, Chilcotin; Q, Quesnel; and N, Nechako.

intercepted in this study (Hinch et al. 2005) and their stomachs were thus shrinking, which promotes transmitter retention among migratory adult sockeye salmon (Ramstad and Woody 2003). In fact, in a preliminary study, we observed no transmitter expulsion during a 24-hour period despite the fact that sockeye were confined and exposed to stressful holding conditions (Cooke et al. 2005). Although fisheries removals were possible, the antennas protruding from the mouths of the fish were affixed with external tags. A comprehensive awareness and monitoring program was mounted, which included placement of signs at fishing sites, presentations to fishing (recreational, First Nations, and commercial) groups, and placement of telemetry receivers at fish-processing plants (English et al. 2004, 2005). We believe that compliance was high, although we cannot eliminate this as a potential source of loss, and hence a false assignment of mortality. Based on the premise that the losses we observed are a result of en route mortality, the following discussion focuses on the physiology associ-

ated with failure to complete either the ocean migration or the river migration. Recall that the findings represent data collected from fish sampled in the marine environment.

The first migratory phase that we examined was ability to complete the ocean migration (about 215 km), migrate through the lower, tidal stretch of the river, and reach the first receiving station 85 km upstream. Just under half of the summer-run sockeye tagged (47%) failed to enter freshwater beyond the boundary of tidal influences. We predicted that fish that failed to reach this point would be characterized by a lack of osmoregulatory preparedness delaying river entry and increasing susceptibility to marine predation. One expectation was that the activity of an important gill enzyme, Na^+-K^+ATPase, would be elevated (reflecting the normal marine status rather than a lower level typical of freshwater fishes). However, this was not the case. We were initially surprised to find that fish intercepted some 200 km from the river mouth in the marine environment had already

TABLE 5. Results of two-way ANOVA, with fate, stock, and the fate × stock interaction as effects, comparing three stocks (C, Q, N) of summer-run sockeye salmon and their fates along the Fraser River, British Columbia, Canada.

Variable	Fate			Stock			Fate × stock		
	F	df	P	F	df	P	F	df	P
Nose fork length (cm)	1.80	2	0.171	0.95	2	0.390	0.88	4	0.479
Gross somatic energy (MJ/kg)	0.96	2	0.388	2.45	2	0.093	1.23	4	0.307
Plasma concentrations									
Na$^+$ (mmol/L)	1.27	2	0.286	0.58	2	0.558	1.42	4	0.235
K$^+$ (mmol/L)	1.11	2	0.333	0.66	2	0.521	1.25	4	0.298
Cl$^-$ (mmol/L)	1.12	2	0.330	0.45	2	0.650	0.702	4	0.593
Osmolality (milliosmol/L)	3.26	2	0.043	0.01	2	0.991	0.60	4	0.661
Cortisol (ng/L)	0.20	2	0.816	0.30	2	0.740	0.53	4	0.719
Lactate (mmol/L)	0.41	2	0.668	1.48	2	0.235	0.34	4	0.849
Glucose (mmol/L)	0.66	2	0.521	2.62	2	0.079	1.80	4	0.137
Na$^+$/K$^+$-ATPase	0.22	2	0.805	0.20	2	0.823	0.73	4	0.565

Notes: Data tested by ANOVA are presented in Table 4. Because we conducted multiple comparisons, we applied Bonferroni corrections; significance was thus assessed at $\alpha = 0.003$.

down-regulated gill Na$^+$-K$^+$ ATPase below the level found for adult sockeye salmon ($\sim$3.5–5 µmol ADP·mg protein^{-1}·h^{-1}; Hinch et al. 2005) captured 500 km from the river mouth several weeks earlier in their 2003 migration. However, the telemetry results revealed that summer-run sockeye took just under 7 days (6.9 ± 0.2 days, [mean ± SE; range: 3.9–14.0 days) to reach the Fraser River after ocean tagging, suggesting that they would have had little time to prepare for freshwater entry en route after release. Given that a freshwater surface layer exists in the Georgia Strait to varying depths (Thomson 1981), it is entirely possible that migrating salmon use this as a cue to initiate freshwater preparedness as they migrate towards the Fraser River. Instead of elevated gill-enzyme activity, failed migrants were characterized by low plasma Na$^+$, a characteristic we cannot explain at this time. Interestingly, failed migrants also tended ($P = 0.082$) to have high

osmolality, which may be coupled with stress since commercially captured coho salmon show elevated osmolality (Farrell et al. 2001).

We also predicted that failure to enter the river would be associated with an energy limitation. However, the trend was contrary to our prediction and the prevailing belief that energetic limitations play a key role in migration failure (e.g., Brett 1995, Crossin et al. 2004). Fish that successfully migrated to the river tended to have had lower gross somatic energy ($P = 0.051$) and be shorter ($P = 0.071$), but these data were marginally nonsignificant even prior to Bonferroni corrections. One possible interpretation is that indeed there were no significant differences and that, surprisingly, energy did not play a role in mortality. If one accepts the notion that these differences are biologically relevant and not spurious, it is possible that the size selectivity (for bigger fish) of gill nets in the Lower Fraser River created a bias,

TABLE 6. Reproductive hormone levels and results of ANOVA for summer-run sockeye salmon (pooled stocks C, Q, N) with different fates along the Fraser River, British Columbia, Canada.

Variable	Died in lower river			Died in upper river			Reached natal sub-watershed			ANOVA, Fate		
	n	Mean	SE	n	Mean	SE	N	Mean	SE	F	df	P
Testosterone (pg/mL)												
Male	2	11 414^x	1556	6	9600^y	358	40	12 127^x	393	9.10†	2	0.064
Female	7	13 620	1062	9	13 199	1227	39	13 615	612	0.05	2	0.954
11-keto testosterone (pg/mL)												
Male	2	5136	1068	6	4551	371	40	5670	243	1.55	2	0.222
Female	8	1053^x	65	8	817^y	31	36	975^x	43	7.61†	2	0.004
Estradiol 17β (pg/mL)												
Male	2	294	118	6	313	72	40	354	27	0.24	2	0.786
Female	8	4760	477	8	5060	399	36	4884	281	0.07	2	0.929

Notes: Fishery losses were excluded from analyses. Stock from Chilcotin, Quesnel, and Nechako were pooled. Within each gender, means with different superscript letters x, y, or z indicate significant differences between fates for that variable ($\alpha = 0.05$). Fish losses in the lower Fraser River were between Mission and Hell's Gate. Losses in the upper Fraser River were between Hell's Gate and the natal sub-watersheds; survivors reached their natal sub-watersheds. n = number of fish.

If variances were homogeneous for these data, analyses were conducted with one-way ANOVA; otherwise, Welch ANOVA was used. Because we conducted multiple comparisons, we applied Bonferroni corrections; significance was thus assessed at $\alpha = 0.003$.

† Denotes use of Welch ANOVA.

but our length analyses included in-river fishery harvests. An alternative explanation, however, is that fish with lower somatic energy had committed to river entry and spawning, and had already shifted substantial energy from somatic reserves to gonadal maturation (Patterson et al. 2004). Unfortunately this subtlety of interpretation, while extremely important, cannot be resolved without a nonlethal assessment of gonadal energy investment (a lethal assessment precludes information on the fate of the fish). Instead, a somatic energy limitation may be a more commanding selective force specifically during river migration when fish are contending with variable and often arduous river hydrologies.

Fish that failed to enter the river also had been more physiologically stressed than successful fish, as revealed by both acute (plasma glucose and lactate) and chronic (plasma cortisol) stress indicators. Stress can have a variety of negative consequences relevant to migration including elevated energy expenditure (Barton and Schreck 1987), suppression of reproductive hormones and other endocrine complications (Kubokowa et al. 2001, Carruth et al. 2002), fitness impairments (Schreck et al. 2001), and in some cases death (Wood et al. 1983, Farrell et al. 2000). Thus, a possible explanation for this association is that different levels of stress imparted by the capture and sampling of fish in the ocean resulted in differential mortality. However, we noted no consistent relationship between the order of sampling within a capture-and-tagging session and the subsequent plasma stress values (i.e., plasma lactate, glucose, and cortisol).

Clearly, capture and handling of fish results in stress that may have affected our results. The most stressful components of capture and handling were undoubtedly the final stages of pursing the seine beside the vessel and transferring individual fish to the onboard holding tanks where they were crowded and exposed to air. A complimentary study on late-run Thompson/Shuswap sockeye conducted in river using First Nations dip nets to capture fish yielded data that had the same magnitude of variation we observed in this study, but the actual values were much lower (Young et al. 2006). The additional stress from the biopsy procedure (relative to simply tagging the fish) was likely negligible because the validation study by Cooke et al. (2005) of the techniques used here revealed that no difference in mortality for biopsied and tagged fish relative to control fish when the fish were held in sea pens after the tagging. Plasma glucose, lactate, and cortisol are acute indicators of stress and would likely be influenced by capture and handling (Milligan 1996, Mommsen et al. 1999, Farrell et al. 2000). Plasma cortisol levels in excess of 400 ng/mL may not have been entirely due to capture and handling stress and instead may also reflect a cumulative stress because cortisol can also be indicative of chronic stress (Mommsen et al. 1999). Furthermore, the fact that mortality was associated with elevated cortisol levels for Quesnel and Chilcotin stocks, but lower cortisol levels

for the Nechako stock, opens the possibility that different stocks may respond differently to cumulative stresses. Since we cannot ascribe the relative contribution of capture, handling and background stress to the values we observed, future studies should focus on utilizing techniques where fish can be captured, handled, and released more rapidly to eliminate this uncertainty.

The summer-run sockeye traverse long, challenging, and energy-demanding river reaches. Total freshwater migration distances are: Chilcotin, ~629 km; Quesnel, ~723 km; and Nechako, ~965 km. To reach the upper river, all fish must migrate through Hell's Gate, a long (~30 km) and steep (1.2 m/km) river canyon located ~250 km from the river mouth. Hell's Gate is the single most demanding reach in a series of demanding river constrictions encountered when migrating through the Fraser River canyon (Hinch et al. 1996) and is a logical site to differentiate between lower river and upper river en route mortality. Here, many fish can die as they attempt to pass through turbulent waters, with energy exhaustion, disease, and high stress levels all being implicated as mortality agents (Hinch and Bratty 2000, Macdonald 2000, Macdonald et al. 2000). Indeed, 9.7% of in-river migrants in our study failed to pass Hell's Gate. This level of mortality is considered moderate, which is consistent with the conditions at Hell's Gate in 2003 being relatively benign in terms of extreme water flows but somewhat high water temperatures (mean water temperature of 18.5°C in river during summer-run migration; David Patterson [Fisheries and Oceans Canada], *unpublished data*). This compares to past years like 1997 or 1998 (Macdonald 2000, Macdonald et al. 2000) when river conditions were more extreme and when in-river mortality rates were believed to be considerably higher. Unfortunately, without telemetry it has not been possible to reveal mortality rates in a reach-specific manner as we have done here. Not surprisingly, few physiological differences were observed for fish that passed and those that did not. Importantly, energy status in the ocean was not a good predictor of mortality during this phase of migration for these sockeye stocks and this particular year. However, osmolality was highest in fish that failed to pass Hell's Gate, but none of the individual ions (i.e., Na^+, K^+, Cl^-) were significantly elevated, suggesting that this was a combined ionic effect—but this will only be elucidated with additional in-river tagging and biopsy.

We also assessed en route mortality in the upper river above Hell's Gate, i.e., the ability to reach the spawning ground. Of the tagged summer-run fish that were detected in river, 14.2% passed Hell's Gate but failed to reach their natal sub-watershed. Unexpectedly, an indicator of en route mortality in the upper river was depressed plasma sex-hormone concentrations: males that failed to reach spawning grounds tended to have had lower concentrations of plasma testosterone and females had lower 11-ketotestosterone. Both of these hormones normally become elevated during the spawn-

ing migration as part of the gonadal maturation process, but the major hormonal rise occurs close to the time of spawning (Truscott et al. 1986, Leonard et al. 2002). We interpret this observation as some individuals being less sexually advanced when intercepted in Johnstone Strait. Interestingly, this finding is contrary to recent work (Young et al. 2006) that involved capturing sockeye salmon in the Thompson Canyon of the Fraser River Watershed (about halfway to spawning grounds for a late-summer stock) and conducting the same telemetry and biopsy procedure we used here. In that study, failed migrants tended to be more reproductively advanced. This then leads to the possibility that mortality in the final phase of migration may have been related to premature maturity and perhaps an early onset of senescence (Patterson et al. 2004), or expression of secondary sexual characteristics (e.g., kype, and change in body morphology prior to arrival making an arduous river journey even more challenging; Hendry and Berg 1999). However, because we captured and sampled fish in the ocean, it is possible that those characteristics were not yet expressed. It is also possible that the mortality factors vary among stocks (as we noted here), with differences potentially even more pronounced between run timing groups (e.g., summer run vs. late-summer run). In this study, it was not possible to determine which fish actually spawned successfully, so we used arrival to the natal sub-watershed as a proxy for migration success. This seemed to be a reasonable assumption for 2003 because, although premature mortality among fish that reach spawning grounds is a common phenomenon within sockeye salmon (Gilhousen 1990), in 2003 the estimate of prespawning mortality on spawning grounds was <1% (David Patterson [Fisheries and Oceans Canada], *unpublished data*). Despite the en route mortality that we observed, 76.1% of in-river migrants successfully reached their natal sub-watershed.

The factors associated with migratory failure of individuals are clearly multifactorial and are likely expressed differently during different phases of the entire migration. Here we summarize the characteristics associated with failure to reach the river and failure to reach the natal sub-watershed in a two-phase model. For the freshwater-entry phase, indicators of stress (cortisol and anaerobic metabolites) and possibly cumulative stress (cortisol) seem to be important. Cumulative stress may make it difficult for migrants to partition somatic energy into gonadal development. A less advanced reproductive condition of the fish sampled in the Johnstone Strait was associated with subsequent mortality during their upper-river migration phase. A general model for Pacific salmonids and other anadromous fishes is undoubtedly more complex, but the present results could apply more generally and serve as the basis for future hypothesis testing. Moreover, the associations that we discovered are novel and should be added to those factors suggested previously as being important

for individual-sockeye migration success, for example environmental factors such as salinity in the ocean (Heifetz et al. 1989) and water temperature, silt, and discharge in the river (Macdonald 2000, Macdonald et al. 2000), as well as choice of migratory route and path (Hamilton 1985, Hinch et al. 2002), timing of migration (Cooke et al. 2004a), and parasite burden (Barber et al. 2000).

Future studies that use biopsy and telemetry of salmon during yearly migrations will be needed to fully reveal how environmental conditions and migratory phase interact with physiology and energy variables to determine whether a salmon will successfully reach its natal spawning grounds or die en route. This type of approach may prove particularly valuable in identifying stock-specific differences because, although stock-specific differences were not a significant factor for the most part in the present study, they were not completely eliminated. Ultimately, this type of information will enable fisheries managers to develop more robust predictive models to ensure the sustainable use and conservation of salmonid fish stocks as we reveal more about the correlates of migration success in Pacific salmonids. The approach utilized in this paper is at the frontier of modern biology as we take a more mechanistic and integrative approach to ecology (Altman and Altman 2003), particularly in difficult field environments (Cooke et al. 2004b).

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