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Smolting in coastal cutthroat trout *Onchorhynchus clarkii clarkii*

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Gill Na^+, K^+-ATPase activity, condition factor and seawater (SW) challenges were used to assess the development of smolt characteristics in a cohort of hatchery coastal cutthroat trout *Oncorhynchus clarkii clarkii* from the Cowlitz River in Washington State, U.S.A. Gill Na^+, K^+-ATPase activity increased slightly in the spring, coinciding with an increase in hypo-osmoregulatory ability. These changes were of lesser magnitude than are observed in other salmonine species. Even at the peak of tolerance, these fish exhibited notable osmotic perturbations in full strength SW. Condition factor in these hatchery fish declined steadily through the spring. Wild captured migrants from four tributaries of the Columbia River had moderately elevated gill Na^+, K^+-ATPase activity, consistent with smolt development and with greater enzyme activity than autumn captured juveniles from one of the tributaries, Abernathy Creek. Migrant fish also had reduced condition factor. General linear models of 7 years of data from Abernathy Creek suggest that yearly variation, advancing photoperiod (as ordinal date) and fish size (fork length) were significant factors for predicting gill Na^+, K^+-ATPase activity in these wild fish. Both yearly variation and temperature were significant factors for predicting condition factor. These results suggest that coastal *O. c. clarkii* exhibit weakly developed characteristics of smolting. These changes are influenced by environmental conditions with great individual variation. The data suggest great physiological plasticity consistent with the variable life-history tactics observed in this species.

Key words: condition factor; gill Na^+, K^+-ATPase; gill NKA; osmoregulation.

INTRODUCTION

The parr–smolt transition is a well characterized ontogenic process in most migratory juvenile salmonines. Changes in behavioural, morphological and physiological characteristics are presumed to be adaptive for both juvenile migration and subsequent entry into seawater (SW; McCormick, 2013). Life histories of salmonine species vary widely, as does the degree to which freshwater (FW) habitat is relied upon (Rounsefell, 1958; McCormick, 1994). For some species, smolt development is rudimentary or absent [*e.g.* brook trout *Salvelinus fontinalis* (Mitchill 1814)] and SW entry relies on physiological acclimation rather than preparation. Smolting species prepare and have

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distinct increases in SW tolerance and decreases in condition factor (K) that occur in advance of migration. Among species, this timing is also variable and can be dependent upon size. Species such as pink salmon *Oncorhynchus gorbuscha* (Walbaum 1792) and chum salmon *Oncorhynchus keta* (Walbaum 1792) migrate soon after emergence. Other species such as coho salmon *Oncorhynchus kisutch* (Walbaum 1792), steelhead trout *Oncorhynchus mykiss* (Walbaum 1792) and Atlantic salmon *Salmo salar* L. 1758 reside in FW for at least one winter before migration (McCormick, 1994, 2013).

Sea-run coastal cutthroat trout *Oncorhynchus clarkii clarkii* (Richardson 1836) generally reside in FW for multiple winters and migrate at age 2+ or 3+ years in the spring (Giger, 1972; Sumner, 1972; Trotter, 1989). This species is extremely plastic and sympatric individuals can be resident or migratory (Johnston, 1982; J. A. June, unpubl. data). While the behaviour of migrants is distinct, there are no apparent morphological (H. J. Fuss, unpubl. data; T. Tomasson, unpubl. data) or biochemical (Johnson *et al.*, 1999) distinctions between juveniles that are resident or anadromous. Additionally, there is no apparent genetic distinction between migrant and non-migrant fish within two tributaries of the Columbia River (Johnson *et al.*, 2010). These uncertainties withstanding, some of the observed characteristics of migrant fish are stereotypical of smolting salmonines. Sea-run juveniles move downstream in the spring and the likelihood of these fish to migrate is linked to growth during their first year (Zydlewski *et al.*, 2009). The timing of this movement peaks in the spring, overlapping with the timing of *O. kisutch* and *O. mykiss* smolt migration. Telemetry work in the lower Columbia River has further demonstrated that migrants reaching the main stem moved directly into the ocean environment rather than lingering in the lower river and estuary as might be expected from a fish acclimating to a saline environment (Zydlewski *et al.*, 2008). Although migrants can (and do) enter SW, these fish do not venture far from the estuary (Pearcy, 1997; Goetz *et al.*, 2013); therefore, exposure to full salinity beyond the river plume may be intermittent.

Sea-run *O. c. clarkii* juveniles are commonly referred to as smolts (Armstrong, 1971; Giger, 1972; Tipping & Blankenship, 1993; Kennedy *et al.*, 2009) but the degree to which these fish express the developmental traits associated with the parr–smolt transformation is unclear. Physiological assessment of this species is limited. Work by Yeoh *et al.* (1991) demonstrated poor salinity tolerance in juveniles after transfer to SW with a salinity of 28; juveniles experienced marked osmotic perturbation and mortality. Larger size was associated with a greater probability of survival in SW, consistent with observations that large sea-run *O. c. clarkii* had better juvenile to adult returns (Tipping, 1986). Zydlewski *et al.* (2008) reported a modest association between gill Na^+, K^+-ATPase (NKA) activity and downstream migratory speed in the Columbia River. Neither study, however, addressed the basic question of the ontogeny of smolt-associated characteristics.

Smolting is a complex synthesis of endogenous and exogenous factors. An individual's developmental decision relies on growth (Thorpe *et al.*, 1998; Zydlewski *et al.*, 2014) but photoperiod is generally accepted as the driver for the initiation (McCormick & Saunders, 1987; McCormick *et al.*, 1999; Handeland *et al.*, 2004). Increased water temperature accelerates the process (Sigholt *et al.*, 1998; McCormick *et al.*, 2002) and hastens its regression (McCormick *et al.*, 1997; McCormick, 2013). Thus, the thermal experience (accumulated thermal units or degree days) of an individual defines a smolt window during which the fish is optimally prepared for SW entry. In a

controlled setting, salinity tolerance can be directly assessed in fishes through isothermal transfers from FW to SW to assess mortality and osmotic perturbation (Clarke & Blackburn, 1977). In smolting salmonines, gill NKA activity has been extensively used to assess the preparatory changes in salinity tolerance associated with smolt development (McCormick, 1993) as well as assessing acclimation to SW (Hoar, 1988). A decrease in both *K* and increased gill NKA are generally accepted indicators of smolt quality and are correlated with seaward migration, hypo-osmoregulatory ability and high survival rates in SW (McCormick & Saunders, 1987; Handeland *et al.*, 2003).

These measures were used to study the development of smolting in *O. c. clarkii* in the hatchery and in the field in order to better understand the degree to which *O. c. clarkii* smolt. To characterize the timeline of osmoregulatory development, hatchery fish were sampled from sea-run broodstock using SW challenges measuring gill NKA activity and change in morphology (*K*) through the period of presumed smolting. In the field, actively migrating *O. c. clarkii* were sampled from four tributaries of the Columbia River to assess gill NKA activity and *K* compared with opportunistically sampled fish in autumn. Lastly, in one of the four tributaries, Abernathy Creek, 7 years of sampling of active migrants were used to model the influence of known smolting predictive factors on individual gill NKA and *K*.

MATERIALS AND METHODS

ASSESSMENT OF HATCHERY-REARED *O. C. CLARKII*

Oncorhynchus clarkii clarkii were sampled at the Cowlitz Trout Hatchery, Washington State, from the general production of 17 month (age 1+ years) smolt release programme. These fish were the progeny of sea-run adult returns to the Cowlitz River, of presumed hatchery origin. Fish were reared under ambient light conditions (46·5° N) in raceways supplied with river water that fluctuated naturally through the season from 6 to 14° C. Beginning in August 2001 through to June 2002, *c.* 30 juveniles were sampled from a single production raceway (Table I).

TABLE I. Mean ± S.E. fork length (L_F) and mass (M) of coastal *Oncorhynchus clarkii clarkii* juveniles sampled at the Cowlitz Trout Hatchery from autumn of 2001 to the summer of 2002. Shared lower-case letters designate no statistical difference among groups ($P > 0.05$)

Date	*n*	L_F (cm)	M (g)
20 August 2001	30	13·9 ± 0·2a	31·4 ± 1·5a
6 November 2001	30	16·8 ± 0·2b	49·2 ± 1·8b
18 January 2002	33	19·0 ± 0·2c	70·1 ± 2·9c
14 March 2002	25	20·7 ± 0·2d	84·0 ± 3·3cd
28 March 2002	29	21·5 ± 0·2df	91·0 ± 3·3d
14 April 2002	30	20·8 ± 0·4d	81·9 ± 4·4cd
27 April 2002	29	21·7 ± 0·2def	92·7 ± 3·5def
11 May 2002	29	21·7 ± 0·3def	90·2 ± 3·8def
25 May 2002	29	22·3 ± 0·2ef	104·8 ± 2·9ef
7 June 2002	29	22·6 ± 0·2f	99·9 ± 3·2df
25 June 2002	30	22·6 ± 0·4f	100·0 ± 4·5f

n, sample size.

These fish were anaesthetized (100 mg l^{-1} of MS-222 buffered with 0·2 mM $NaCO_3$, pH = 7·0) and measured for L_F and M. Additionally, a non-lethal gill biopsy was taken for subsequent measurement of gill NKA activity (enzyme code 3.6.3.9; International Union of Biochemistry and Molecular Biology, 1992). Four gill filaments were removed and immersed in 100 µl of ice-cold sucrose–EDTA–imidazole buffer (150 mM sucrose, 10 mM EDTA and 50 mM imidazole, pH 7·3), snap frozen and stored at −80° C.

During the presumed period of smolting (March to June of 2002), a series of isothermal SW challenges were conducted. Fish ($n = 30$) were transferred into either a FW (control) or SW (salinity of 35; Instant Ocean; www.instantocean.com) 125 l tank 24 h prior to sampling. Temperatures of tanks matched source water temperatures and ranged from 5 to 11° C over the course of study. Temperatures of FW and SW tanks did not differ by >1·5° C at any time point. At sampling, 24 h after transfer, fish were anaesthetized, measured and biopsied as described above. Mortalities from groups were only measured for L_F. Additionally, a blood sample was collected from the caudal vein into a 1 ml ammonium-heparinized syringe. The needle was removed and the blood was expelled into a 1·8 ml centrifuge tube. This tube was kept on ice for <10 min and was spun at 2000*g* for 5 min; the plasma was removed into a 0·5 ml tube, frozen and stored at −80° C for subsequent analysis. Plasma osmolality was measured using a Wescor Model 5520 vapour pressure osmometer (www.wescor.com).

ASSESSMENT OF WILD *O. C. CLARKII*

Wild *O. c. clarkii* were captured from four tributaries of the Columbia River; Chinook River [river kilometre (rkm) 6], Mill Creek (rkm 87), Abernathy Creek (rkm 88) and Germany Creek (rkm 91; Fig. 1). The Chinook River is a tidal system that is regulated by a tide gate at its confluence with the Columbia River. This system experiences salinity fluctuations from 0 to full strength SW and empties into an estuarine mixing zone of the Columbia River. Germany, Mill and Abernathy Creeks are third-order systems that experience tidal fluctuations at their confluences with the Columbia River, but do not experience salinity fluctuations.

In 2002, migrating juvenile *O. c. clarkii* were captured at the mouths of Germany, Abernathy and Mill Creeks in 1·5 m screw traps [operated by the Washington Department of Fish and

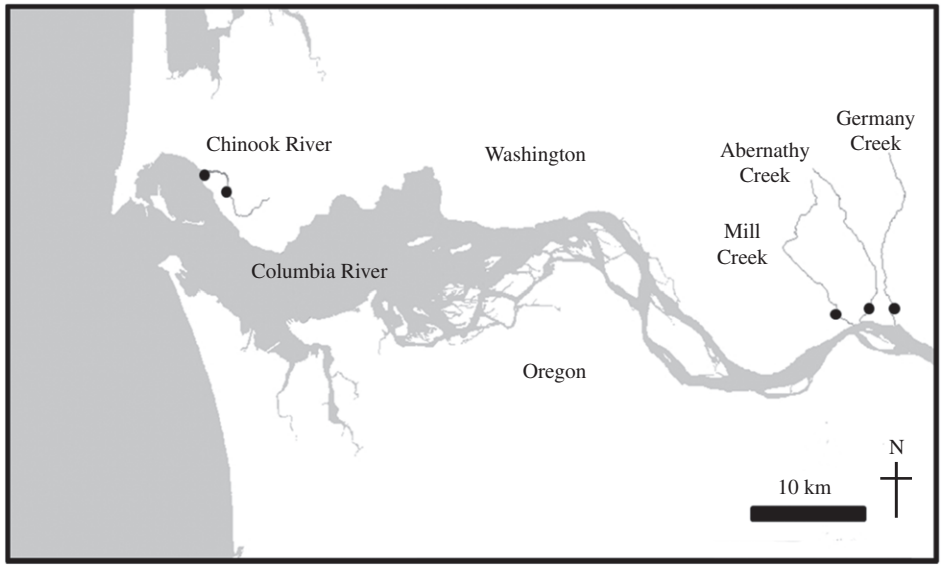

FIG. 1. Map of the Columbia River and tributaries (●, locations of screw traps used to capture migrating coastal *Oncorhynchus clarkii clarkii*).

Wildlife (WDFW)] and in a 2·4 m screw trap at the mouth of the Chinook River (operated by Sea Resources, Incorporated, Chinook, Washington). In Abernathy Creek, *O. c. clarkii* were also captured in springs of 2003–2008. All sampling of migrants included the presumed period of smolt migration from early April (range of 4 April to 9 April) to the end of June (range of 19 June to 20 June). In order to capture *O. c. clarkii* prior to migration in Abernathy Creek, 18 juveniles were electrofished from the lower part of the system on 7 November 2001. All captured wild fish were anaesthetized, measured and a gill biopsy was taken as described above.

MEASUREMENT OF GILL NKA ACTIVITY

Gill NKA activity was determined using the microplate method described by McCormick (1993). Kinetic analysis of ouabain-inhibitable ATP hydrolysis was measured in duplicate at 25° C by monitoring the nicotinamide adenine dinucleotide (NADH) concentration at 340 nm. Protein concentration of the gill homogenate was determined in triplicate using the bicinchoninic acid (BCA) method (Smith *et al.*, 1985; BCA Protein Kit, Pierce; www.piercenet.com) using bovine serum albumin as the standard. Activity of gill NKA is expressed as micromoles of inorganic phosphate per milligramme of protein per hour (μmol ADP mg protein^{-1} h^{-1}).

TEMPERATURE DATA

Reported Cowlitz Trout Hatchery temperatures are averages of daily recorded maximum and minimum values. For Abernathy Creek, water temperatures collected between 4 April 2002 and 30 June 2008 were measured using hand-held thermometer (in 2002) or temperature loggers operated by the U.S. Fish and Wildlife Service and Washington State University, Department of Ecology.

STATISTICS AND CALCULATIONS

For all comparisons reported, an inclusive one-way analysis of variance (ANOVA) was run for all groups. In all analyses, significance with a one-way ANOVA was followed by a Tukey's *post hoc* test. Least square regressions were used to assess correlation between two factors. Significance was reported at an α of 0·05. All intervals about a mean are reported as ±S.E. unless otherwise indicated.

General linear models (GLMs) were used to predict either gill NKA activity or K as indicators of smolt development in actively migrating *O. c. clarkii* captured *via* screw trap on Abernathy Creek from 2002 to 2008. Predictive variables included year of capture (Y), L_F, ordinal date (D_O), temperature on date of capture (T) and degree days (summation of daily temperatures from January 1 to date of capture; T_{DD}). Both T and T_{DD} were assessed as both linear and quadratic factors.

For the period of interest (January to June of each year), a complete daily temperature record was available from 2004 to 2008. In 2002, temperature data were absent for 1 January to 3 April, and daily temperatures were available only for 65% of the days during screw trap sampling (4 April to 21 June). In 2003, data from 1 January to 18 February were absent, but was otherwise complete. Where single daily temperatures were absent, the arithmetic mean of temperatures of the previous and following days were used to calculate a value for the missing date. For both 2002 and 2003, where a series of dates had missing data early in the year, the mean value of available data from other years was used. Akaike information criterion (AIC) values were used for model selection.

RESULTS

HATCHERY-REARED *O. C. CLARKII*

The cohort of juvenile *O. c. clarkii* sampled from the Cowlitz Trout Hatchery between 20 August and 25 June increased in size from 13·9 to 22·6 cm and tripled in mass over

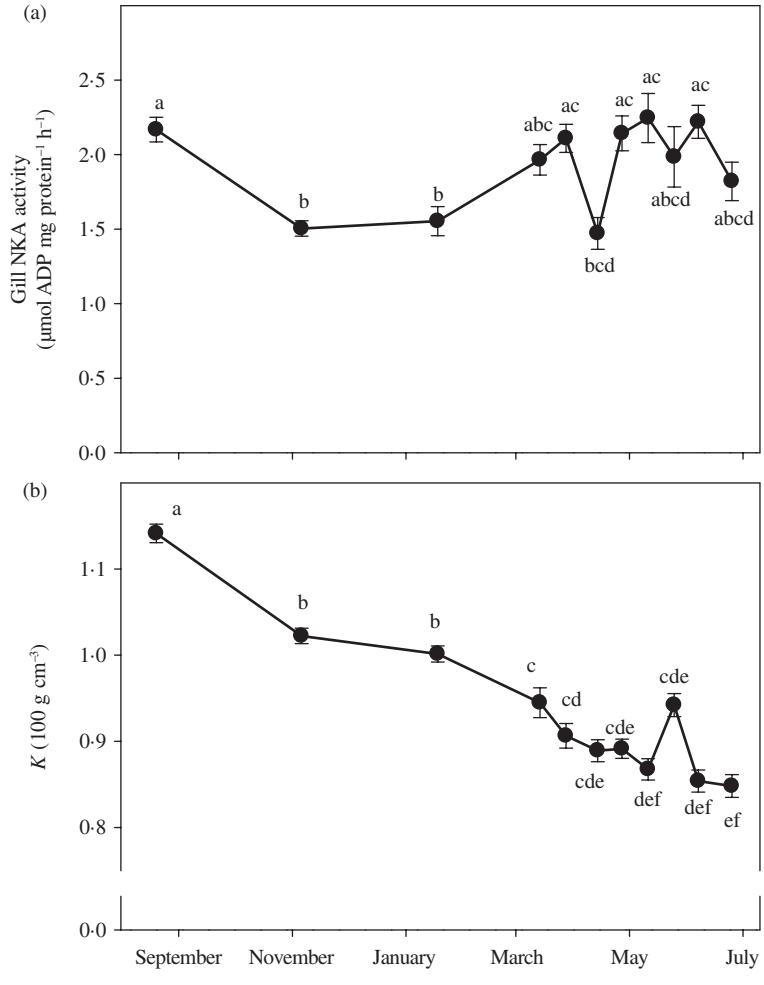

FIG. 2. Mean ± S.E. (a) gill Na^+, K^+-ATPase activity and (b) condition factor (K) of coastal *Oncorhynchus clarkii clarkii* juveniles sampled at the Cowlitz Trout Hatchery from autumn of 2001 to the summer of 2002. Different lower-case letters designate statistical differences among groups ($P < 0{\cdot}05$). See Table I for n, fork length (L_F) and mass data.

this period (from 31·4 to 100·0 g; Table I). Changes in gill NKA activity were of small magnitude among all time points, only ranging between 1·5 and 2·5 μmol ADP mg protein^{-1} h^{-1} (Fig. 2). The initial gill NKA activity on 20 August was relatively high and declined by 30% as fish entered the winter period. Enzyme activity then increased in the spring (with the exception of the 14 April sample) and declined to winter levels by late-June. The K of these same fish steadily declined through the study (Fig. 2). Fish in August had an initial K value of 1·14 while fish during the period of migration had K values of <0·90. For the last sample in late-June, K had dropped below 0·85.

Fish from the same cohort used for transfer to FW exhibited the same general trends of increasing size and gill NKA activities at or near 2·0 μmol ADP mg protein^{-1} h^{-1}, and decreasing K through the time course (Table II). Fish transferred to FW and SW

TABLE II. Mean ± S.E. fork length (L_F), mass (M), condition factor (K) and gill Na^+, K^+-ATPase (NKA) activity of coastal *Oncorhynchus clarkii clarkii* juveniles from the Cowlitz Trout Hatchery, 24 h after isothermal transfer to fresh water (FW) or seawater (SW) at a salinity of 35

		n	L_F (cm)	M (g)	K	Gill NKA activity
14 March	FW	15	20·5 ± 0·3	86·0 ± 4·2	0·99 ± 0·02*	2·14 ± 0·14*
	SW	15	21·1 ± 0·3	81·3 ± 4·4	0·88 ± 0·02	1·74 ± 0·11
28 March	FW	15	21·4 ± 0·4	93·1 ± 4·8	0·94 ± 0·02*	2·38 ± 0·08*
	SW	15	21·6 ± 0·3	88·8 ± 4·4	0·87 ± 0·01	1·81 ± 0·13
14 April	FW	15	21·0 ± 0·5	89·2 ± 6·8	0·93 ± 0·02*	1·58 ± 0·15
	SW	15	20·5 ± 0·5	74·6 ± 5·0	0·85 ± 0·01	1·36 ± 0·15
27 April	FW	15	21·6 ± 0·4	94·4 ± 5·6	0·92 ± 0·01*	2·34 ± 0·18
	SW	15	21·6 ± 0·4	91·0 ± 4·3	0·86 ± 0·01	1·93 ± 0·12
11 May	FW	15	21·9 ± 0·4	93·7 ± 5·3	0·89 ± 0·02	2·46 ± 0·29
	SW	16	21·4 ± 0·5	86·7 ± 5·1	0·84 ± 0·01	2·02 ± 0·12
25 May	FW	15	22·2 ± 0·3	106·4 ± 3·5	0·97 ± 0·02*	1·82 ± 0·37
	SW	15	22·2 ± 0·4	103·1 ± 4·6	0·91 ± 0·02	2·16 ± 0·14
7 June	FW	15	22·6 ± 0·3	104·7 ± 4·0	0·90 ± 0·01*	2·17 ± 0·14
	SW	15	22·7 ± 0·3	95·0 ± 4·5	0·81 ± 0·01	2·27 ± 0·17
25 June	FW	15	22·6 ± 0·6	105·0 ± 6·8	0·89 ± 0·02*	1·71 ± 0·15
	SW	15	22·6 ± 0·5	95·0 ± 5·9	0·81 ± 0·02	1·92 ± 0·22

n, sample size.

*A difference between FW and SW groups within a sample.

did not differ in L_F and M at any point when compared 24 h post-transfer. For 14 March and 28 March time points, gill NKA activity was higher in the FW groups than the SW. While neither M nor L_F differed, K was lower in all SW transfer groups *v*. FW, except for 11 May ($P > 0·05$).

Transfer to SW resulted in a total of five mortalities in the two earliest occasions (14 March and 28 March; Fig. 3). There were no mortalities in any transfers to FW. At all time points, fish transferred to SW had markedly higher plasma osmolalities than FW controls, with perturbations ranging from 50 to 110 mOsmol kg^{-1}. While plasma osmolality was elevated in all SW transfers, the greatest perturbations were observed at the earliest and latest time points; from mid-April to mid-June, perturbations of plasma osmolality decreased, although values remained between 374 and 375 mOsmol kg^{-1}. There were no differences in plasma osmolality among FW-transferred fish (Fig. 3).

WILD FISH FROM MULTIPLE RIVERS

As would be expected, autumn electrofished *O. c. clarkii* juveniles sampled in Abernathy Creek were markedly smaller (less than half the mass and at least 6·5 cm shorter) than migrants from Abernathy Creek (or any of the other three systems) captured months later (Table III). Based on size, many autumn sampled fish were probably of the same cohort as sampled migrants the following year (Zydlewski *et al.*, 2009) and two autumn sampled individuals were passive-integrated transponder (PIT) tagged and observed migrating the following spring (J. Zydlewski, unpubl. data). Migrants from different systems ranged in mean L_F from 18·5 to 20·1 cm and mean M of 50·2–75·2 g.

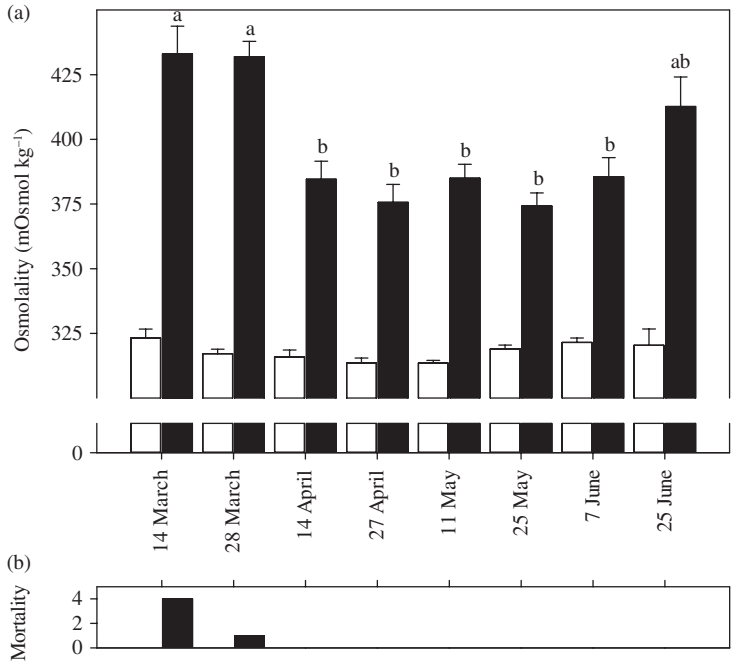

FIG. 3. Mean ± S.E. (a) plasma osmolality and (b) mortality of coastal *Oncorhynchus clarkii clarkii* juveniles from the Cowlitz Trout Hatchery, 24 h after isothermal transfer to fresh water (□) or seawater (■; salinity of 35). Lower-case letters designate statistical differences between groups ($P < 0{\cdot}05$). See Table II for *n*, fork length (L_F), mass, condition factor (K) and gill Na^+, K^+-ATPase activity of these fish.

Migrants from Abernathy Creek were the smallest while Chinook River fish were the largest. Autumn electrofished *O. c. clarkii* juveniles from Abernathy Creek had 45–55% lower gill NKA activity than all migrant *O. c. clarkii* from Abernathy and other rivers (Fig. 4). While the gill NKA activity of migrants from all four streams were similarly elevated, values were only between 3·5 and 4·5 μmol ADP mg protein^{-1} h^{-1}. Abernathy Creek migrants had 20% higher activity than migrants from the other three systems.

TABLE III. Mean ± S.E. fork length (L_F) and mass (M) of coastal *Oncorhynchus clarkii clarkii* juveniles captured in Abernathy Creek (AB), Chinook River (CH), Germany (GR) and Mill (ML) Creeks as active migrants from early April to the end of June. Shared lower-case letters designate no statistical difference among groups

		n	L_F (cm)	(M) (g)
Autumn 2001	AB	18	11·9 ± 0·37a	18·5 ± 1·80a
Spring 2002	AB	130	18·5 ± 0·17b	50·2 ± 1·76b
	CH	23	20·1 ± 0·60c	75·2 ± 7·23c
	GR	22	18·8 ± 0·35bc	60·2 ± 3·53bc
	ML	52	18·4 ± 0·19bc	53·4 ± 1·81b

n, sample size.

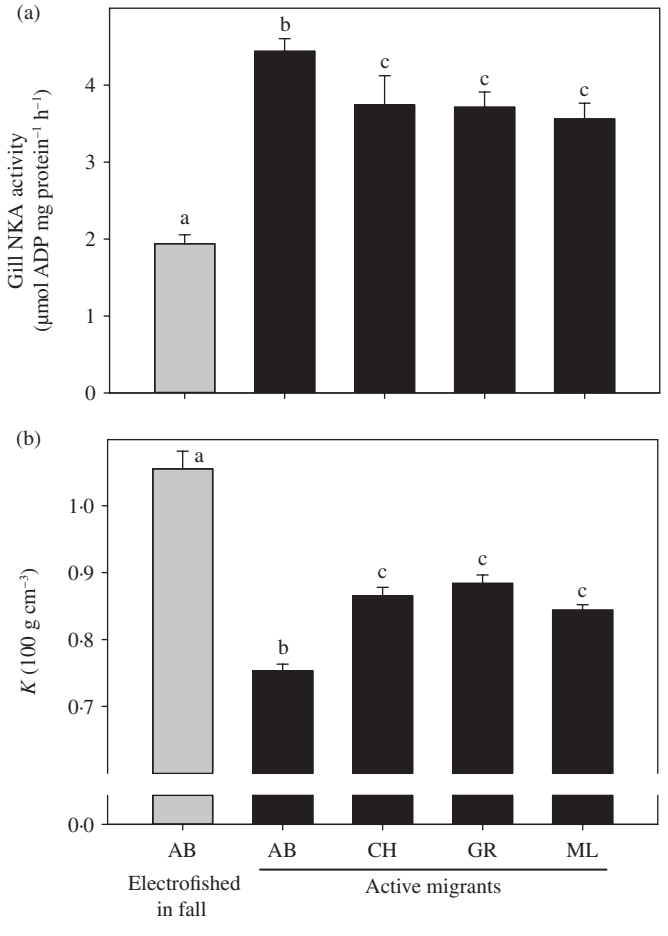

FIG. 4. Mean ± S.E. (a) gill Na^+, K^+-ATPase activity and (b) condition factor (K) of coastal *Oncorhynchus clarkii clarkii* juveniles sampled at Abernathy Creek (AB), Chinook River (CH), Germany (GR) and Mill (ML) Creeks during 2001–2002. Shared lower-case letters designate no statistical difference among groups. See Table III for fork length (L_F) and mass data.

Autumn electrofished *O. c. clarkii* juveniles from Abernathy Creek had a markedly higher K (1·06) than all migrant groups sampled. Although the K values of all migrants were relatively low, Abernathy Creek had lower average K than the other systems with an average of 0·75 *v.* 0·84–0·89 (Fig. 4). More than 10% of migrants in Abernathy Creek were observed in 2002 to have a K lower than 0·65. Gill NKA was correlated with K ($P < 0{\cdot}05$) but explained little of the variance ($r^2 = 0{\cdot}038$).

WILD ABERNATHY MIGRANTS FROM 2002 TO 2008

Between 2002 and 2008, a total of 807 coastal *O. c. clarkii* were sampled from Abernathy Creek. The average L_F of migrants was similar among years (from 17·4 to 18·5 cm) except for 2003 when migrants were smaller than those captured in 2002, 2004

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TABLE IV. Mean ± S.E. fork length (L_F) and mass (M) of coastal *Oncorhynchus clarkii clarkii* juveniles captured during rotary screw trap sampling in Abernathy Creek from 2002 to 2008 as active migrants during early April to the end of June. Lower-case letters designate statistical differences among years

	n	L_F (cm)	M (g)
2002	132	18·5 ± 0·2a	50·2 ± 1·8a
2003	110	17·4 ± 0·2b	46·5 ± 1·3ab
2004	164	18·3 ± 0·2a	56·1 ± 2·1ac
2005	130	17·7 ± 0·2ab	48·2 ± 1·3abd
2006	123	18·3 ± 0·2a	58·4 ± 2·1c
2007	127	17·9 ± 0·2ab	53·3 ± 1·6abcd
2008	21	18·2 ± 0·6ab	54·9 ± 5·2abcd

n, sample size.

and 2006 (Table IV). The M of migrants was also similar (46·5–58·4 g), although 2006 migrants were greater in M on average than those captured in 2002, 2003 and 2005.

Among years, average gill NKA activity of *O. c. clarkii* sampled in 2002 was the highest (4·5 μmol ADP mg protein^{-1} h^{-1}) and was >25% greater than 2003–2008 (Fig. 5). While enzyme activity of 2005 migrants was greater than that of migrants in 2003, 2004 and 2007, the difference was modest (3·22 *v.* 2·60–2·81 μmol ADP mg protein^{-1} h^{-1}). Likewise for K, 2002 fish differed from all other years, with a mean K much lower than all other years (0·75 *v.* 0·85–0·91). The 2006 migrants had a K that was higher than 2003, 2004 and 2005, although this difference was slight.

Within each year, *O. c. clarkii* migrants in Abernathy Creek exhibited great variability in measures of NKA over the course of sampling. Qualitatively, gill NKA activity had a pattern of being lowest in the earliest and latest fish caught, with the highest enzyme activities being observed at the mid- to late-May (Fig. 6). This general pattern was not apparent in 2003. At the qualitative peak, however, when some fish had the highest gill NKA activities, fish captured in the same time interval were observed with some of the lowest observed gill NKA values. Results from the GLM to predict gill NKA activity indicated that Y, L_F and D_O were the critical factors in the top linear model (Table V). All three factors were significant ($P < 0·001$). Inclusion of temperature and degree days (as either linear or quadratic functions) results in less favoured models with little support.

The K of migrants had no obvious qualitative trend among years. In 2002, there was some indication that K declined in captured *O. c. clarkii* migrants from May to June (Fig. 7). In other years (2003–2007), some of the latest sampled fish had equally high or higher K than earlier migrants. Results from the GLM for K were similar to that of NKA in that year, L_F and ordinal date were critical factors in the top model (Table VI). In contrast to the gill NKA model, however, both degree days and temperature as quadratic functions were also included in the top model. Interestingly, removal of either degree days or temperature resulted in a competing model with far less support. In this model, only year and temperature (both T and T^2) were significant factors ($P < 0·001$) with D_O and L_F having $P > 0·05$.

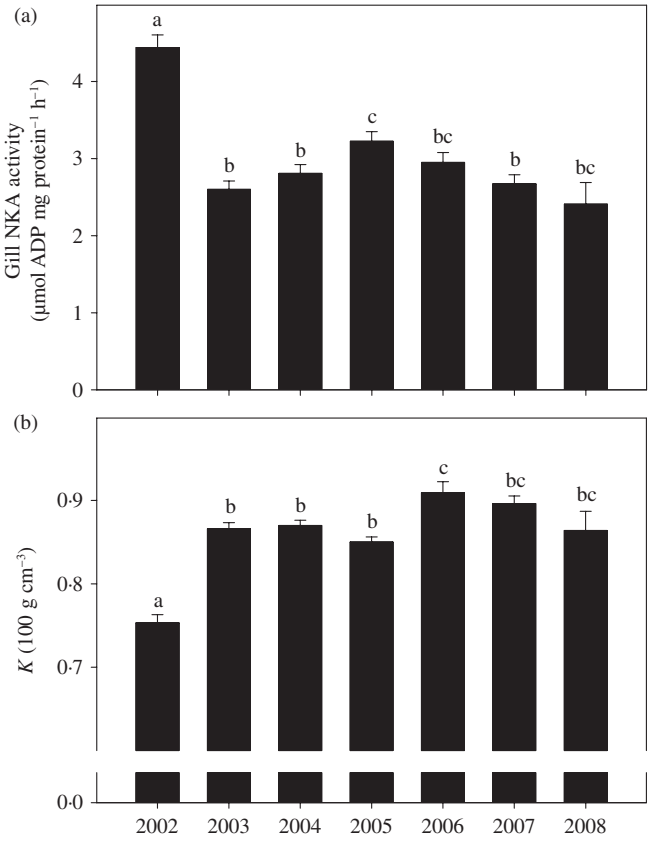

FIG. 5. Mean ± S.E. (a) gill Na^+, K^+-ATPase activity and (b) condition factor (*K*) of coastal *Oncorhynchus clarkii clarkii* juveniles captured as active migrants in Abernathy Creek from 2002 to 2008. Lower-case letters designate statistical differences between groups ($P < 0{\cdot}05$). See Table IV for *n*, fork length (L_F) and mass data.

DISCUSSION

Overall, *O. c. clarkii* do exhibit characteristics of the parr–smolt transformation. In the hatchery and field, gill NKA is elevated, SW tolerance is increased and *K* is decreased in the spring. In the hatchery, increases in gill NKA activity and increased hypo-osmoregulatory ability are reversed in fish sampled in June, consistent with de-smolting in other salmonines. Thus, for the remainder of the paper, identifying *O. c. clarkii* juveniles as smolts is probably justified. The magnitude of these changes indicates, however, that smolt development is both poorly developed and variable. This is consistent with the general conclusion that *O. c. clarkii* display a lower degree of anadromy than other species in the genus (McCormick, 2013).

At the peak in hatchery fish, gill NKA activity ranged from 1·5 to 2·5 μmol ADP mg protein^{-1} h^{-1}. In wild fish, migrant activity was higher (mean of 2·5–4·4 μmol ADP mg protein^{-1} h^{-1}). The difference between enzyme levels of hatchery and wild fish may be due to the influence of rearing and age of smolting. In other species, wild fishes exhibit stronger smolt characteristics than those in a hatchery (*e.g.* brown trout *Salmo trutta*

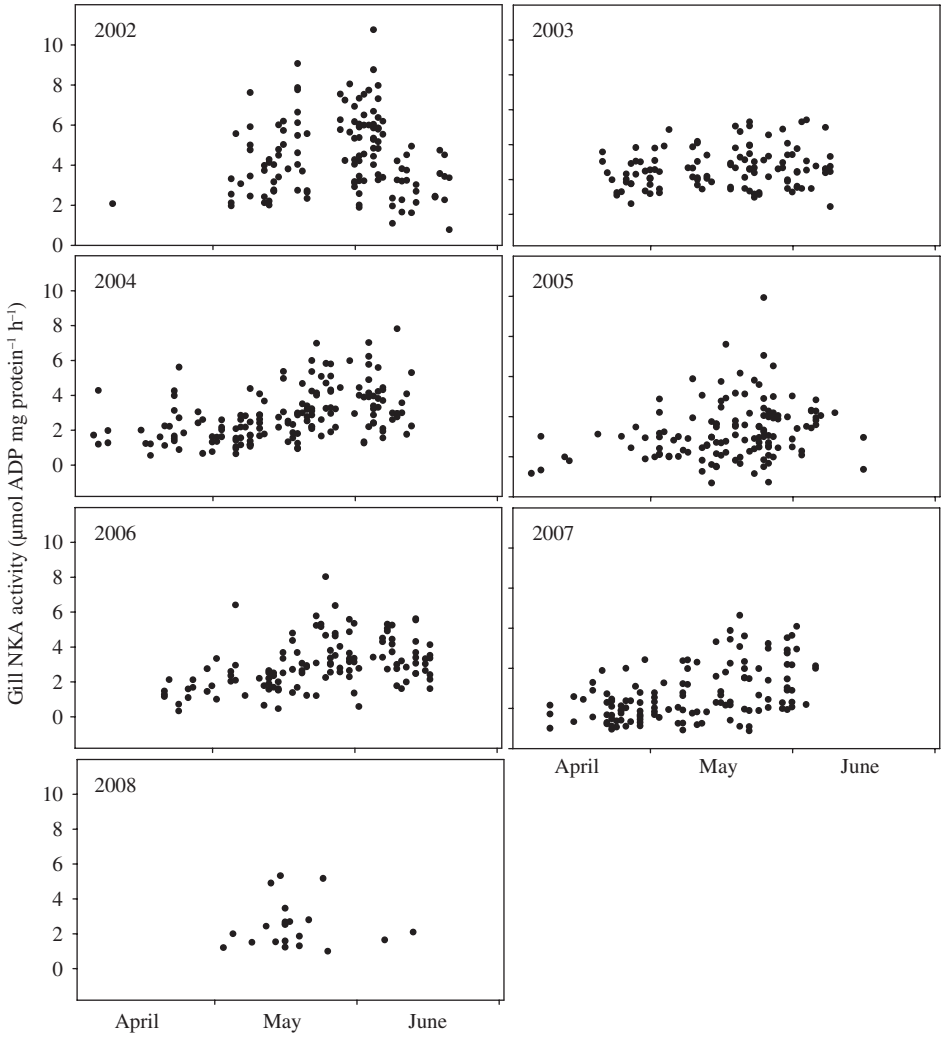

FIG. 6. Scatter plots of gill Na^+, K^+-ATPase activity of coastal *Oncorhynchus clarkii clarkii* juveniles captured as active migrants by screw trap in Abernathy Creek from 2002 to 2008.

L. 1758, Sundell *et al.*, 1998; *O. kisutch*, Shrimpton *et al.*, 1994). The hatchery fishes were of larger size than the wild fishes and this may also negatively influence expression of smolt characteristics (McCormick & Björnsson, 1994; Sundell *et al.*, 1998). Regardless, whether in wild or hatchery fish, the expression of smolt development in the *O. c. clarkii* studied here was modest.

The observed changes in gill NKA activity are notably lower than those measured in many smolt species (McCormick & Saunders, 1987). In closely related *O. mykiss*, gill NKA of smolts was two-fold greater than *O. c. clarkii* (mean of 5·5 μmol ADP mg protein^{-1} h^{-1}) and hybrids exhibited an intermediate activity more close to *O. c. clarkii* (Kennedy *et al.*, 2009). Other species have even higher values (*e.g. S. salar*, 8–10 μmol ADP mg protein^{-1} h^{-1}; Zydlewski *et al.*, 2010). Increased gill NKA activity is

TABLE V. Comparison of predictive general linear models for gill Na^+, K^+-ATPase (NKA) activity from 7 years of data collected on Abernathy Creek coastal *Oncorhynchus clarkii clarkii* captured *via* screw trap during springs of 2002–2008 ($n = 807$). Year (Y) was included as a categorical predictive factor and fork length (L_F), ordinal date (D_O), daily temperature (T) and degree days (T_{DD}) were included as continuous predictive factors. The fully parameterized model is underlined. Corrected Akaike information criterion (AIC) values, multiple r^2 and ΔAIC values are presented to demonstrate model ranks

Rank	Model	AIC_c	Multiple r^2	ΔAIC
1	$Y + L_F + D_O$	2825·2	0·243	0·0
2	$Y + L_F + T_{DD} + T$	2828·8	0·241	3·7
3	$Y + L_F + D_O + T_{DD} + T$	2829·2	0·243	4·0
4	$\underline{Y + L_F + D_O + T_{DD} + T_{DD}^2 + T + T^2}$	2830·2	0·246	5·0
5	$Y + L_F$	2905·2	0·162	80·0
6	Y	2915·8	0·155	90·7
7	$T_{DD} + T_{DD}^2 + T + T^2$	2987·0	0·072	161·9
8	T_{DD}	3017·9	0·028	192·7
9	L_F	3023·7	0·012	198·6
10	T	3036·2	0·006	211·1
11	$T + T^2$	3036·8	0·008	211·7

Factors in top model	Coefficient	d.f.	F-ratio	P
D_O	165·6	1	85·411	<0·001
Y	180·1	6	15·486	<0·001
L_F	22·4	1	11·578	<0·001

frequently used as an indicator of smolting and increased number of mitochondrion-rich cells in the gills that function to increase salinity tolerance (Hoar, 1988; Uchida *et al.*, 1996). Such changes are generally related to increases in osmoregulatory competence.

Coincident with the small increase in gill NKA activity, hatchery *O. c. clarkii* exhibited an increase in hypo-osmoregulatory ability in the spring. Transfer to full strength SW resulted in mortality in March with significant perturbations in plasma osmolality after SW challenge. Although there is a clear pattern of increased hypo-osmoregulatory ability during the spring period, it is notable that perturbations of plasma ions in SW, even at the peak of smolting, are >374 mOsmol kg^{-1}. This is indicative of severe osmotic stress. *Onchorhynchus mykiss* (Hill *et al.*, 2006) and *S. salar* (Zydlewski *et al.*, 2010) transferred at the peak of smolting generally regulate between 325 and 340 mOsmol kg^{-1}. Such poor osmoregulatory ability in *O. c. clarkii* smolts is consistent with the observations of Yeoh *et al.* (1991) who reported low survival and regulatory ability at a salinity of only 28 even at the presumed peak of smolting.

Such weak development of salinity tolerance may be reflective of the variable life history and migratory patterns of this species. Because there is no genetic difference between sympatric resident and sea-run forms (Johnson *et al.*, 2010), the expression of anadromy is consistent with the expression of phenotypic plasticity (Scheiner, 1993). While increased salinity tolerance at the time of smolting is generally associated with increased swimming ability, predator avoidance and growth (McCormick *et al.*, 2009),

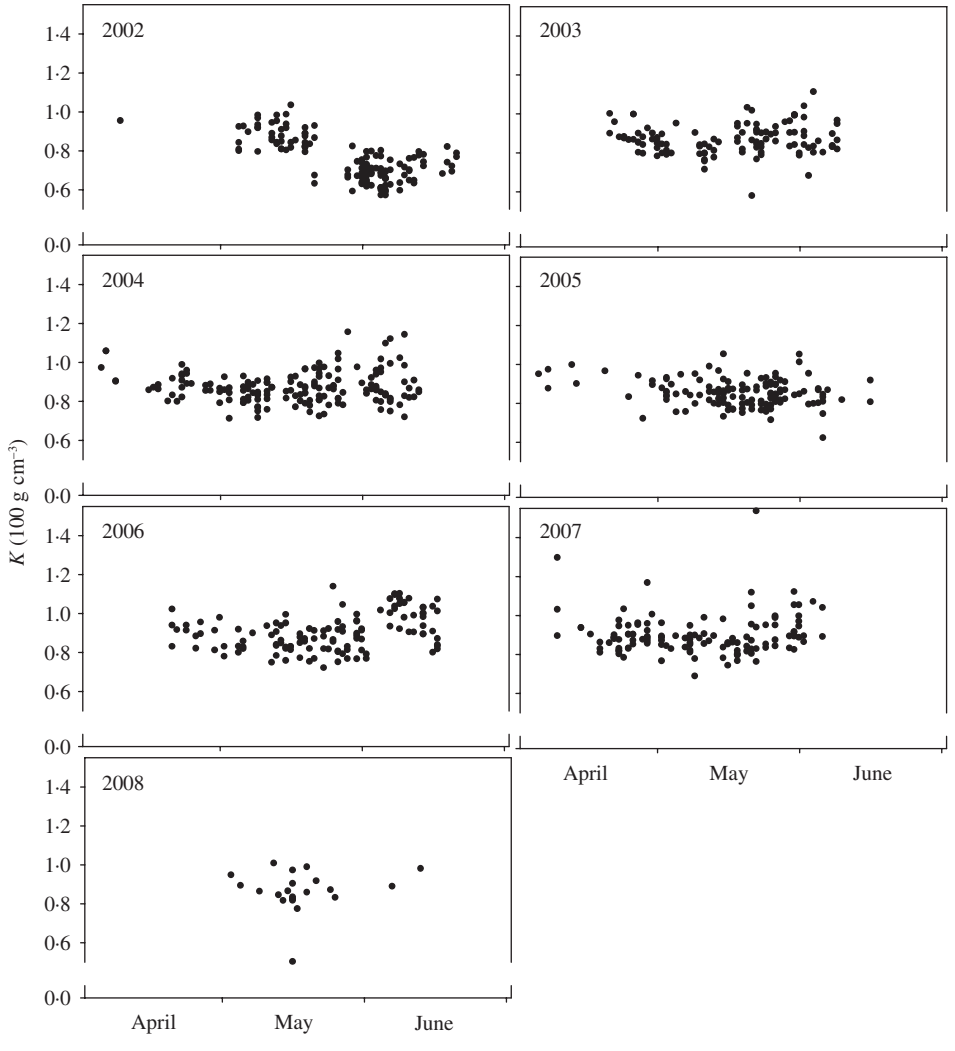

FIG. 7. Scatter plots of condition factor (*K*) of coastal *Oncorhynchus clarkii clarkii* juveniles captured as active migrants by screw trap in Abernathy Creek from 2002 to 2008.

O. c. clarkii have been postulated to select lower salinities in the estuary (Loch & Miller, 1988; Northcote, 1997). From the tributaries of the Columbia River studied here, telemetry data demonstrate a clear seaward movement that is directed and rapid. Even so, this movement may take 4–7 days for fish to reach the Columbia River plume (Zydlewski *et al.*, 2008). For fish that do not have strongly developed salinity tolerance, time to acclimate may be critical.

The hypothesis that *O. c. clarkii* migrating downstream may require further acclimation for full strength SW tolerance would be consistent with the reported association between increase gill NKA and migration speed (Zydlewski *et al.*, 2008). Such rapid acclimation after exposure to salinity is well characterized in numerous teleost species (Holmes & Donaldson, 1969; Jacob & Taylor, 1983; Evans, 1984). The absence of a

TABLE VI. Comparison of predictive general linear models for condition factor (K) from 7 years of data collected on Abernathy Creek coastal *Oncorhynchus clarkii clarkii* captured *via* screw trap during springs of 2002–2008 ($n = 807$). Year (Y) was included as a categorical predictive factor and fork length (L_F), ordinal date (D_O), daily temperature (T) and degree days (T_{DD}) were included as continuous predictive factors. The fully parameterized model is underlined. Corrected Akaike information criterion (AIC) values, multiple r^2 and ΔAIC values are presented to demonstrate model ranks

Rank	Model	AIC_c	Multiple r^2	ΔAIC
1	$\underline{Y + D_O + L_F + T_{DD} + {T_{DD}}^2 + T + T^2}$	−1616·4	0·286	0·0
2	$Y + D_O + L_F + T_{DD}$	−1607·6	0·273	8·9
3	$Y + D_O + L_F + T + T^2$	−1607·3	0·274	9·1
4	$Y + D_O + L_F + T_{DD} + T$	−1605·8	0·273	10·6
5	$Y + D_O + L_F + T_{DD} + {T_{DD}}^2$	−1605·7	0·273	10·7
6	$Y + L_F + T_{DD} + T$	−1556·3	0·225	60·1
7	$Y + D_O + L_F + T$	−1555·4	0·224	61·0
8	$Y + D_O + L_F$	−1555·1	0·222	61·3
9	Y	−1554·9	0·217	61·5
10	$Y + L_F$	−1553·7	0·218	62·7
11	$T_{DD} + {T_{DD}}^2 + T + T^2$	−1473·1	0·129	143·4
12	T	−1375·2	0·009	241·2
13	T_{DD}	−1372·1	0·005	244·4
14	L_F	−1368·0	0·000	248·5
14	$T + T^2$	−1368·0	0·072	248·5

Factors in top model	Coefficient	d.f.	F-ratio	P
Y	1·071	6	23·21	<0·001
L_F	0·001	1	0·07	>0·05
D_O	0·022	1	2·89	>0·05
T_{DD}	0·009	1	1·21	>0·05
${T_{DD}}^2$	0·000	1	0·01	>0·05
T	0·113	1	14·72	<0·001
T^2	0·110	1	14·36	<0·001

time series describing the physiological acclimation of *O. c. clarkii* to SW remains a conspicuous hole in present understanding.

While gill NKA activity and hypo-osmoregulatory ability are increased slightly during *O. c. clarkii* smolting, the observed decreases in K were striking. Parr captured in Abernathy Creek had a mean $K > 1{\cdot}00$ but K of active migrants was <0·90 and as low as 0·75 (Fig. 5). Individual migrants were observed with K values <0·60 without any indication of injury or illness. Even in the hatchery, with no change in food availability, K declined from >1·10 to 0·85. Such observations are consistent with Kennedy *et al.* (2009) who reported *O. c. clarkii* migrants to have lower mean K than *O. mykiss* (0·86 *v.* 0·93, respectively). Reported K values for smolts of other species are often >1·00 [*e.g.* Chinook salmon *Oncorhynchus tshawytscha* (Walbaum 17920), Beckman *et al.*, 1999; *S. salar*, Sigholt *et al.*, 1998]. Reduced K in smolts is thought to be the result of the energetic demands of development (Dickhoff & Sullivan, 1987; Maxime *et al.*, 1989) and increased activity (McCormick & Saunders, 1987; Leonard & McCormick,

2001). These processes probably interact with feeding in a complex way as spring temperatures increase (Handeland *et al.*, 2008). Thus, it is unclear if the drastic K reduction in *O. c. clarkii* is exclusively a natural shift in form.

Using either gill NKA activity or K as an indicator of smolting, there was a remarkable variation within and among years. Notably, Abernathy Creek fish samples had the highest gill NKA of all the four sampled runs in 2002, and among years, 2002 Abernathy fish had the highest enzyme activity. Conversely, this group had the lowest K among streams and among years during which Abernathy Creek was sampled. For both of these measures, there is also considerable variation within each year, without any obvious trends (Figs 6 and 7). The application of linear models to 7 years of Abernathy Creek migrant data was useful in assessing the influence of probable determining factors. The hypothesized influence of annual variation (Y), size (L_F), photoperiod (D_O) temperature (T) and thermal history (T_{DD}) were examined.

D_O, L_F and Y can be used to predict gill NKA activity. The role of D_O in predicting gill NKA activity is consistent with the demonstrated function of photoperiod in the timing of smolt development (Hoar, 1988; Duston & Saunders, 1995; McCormick, 2013). The variability in gill NKA activity observed during May to June probably reflects the development of individuals on discordant developmental tracks. The role of L_F in characterizing some of this variability in gill NKA activity is consistent with a size threshold for smolting. Presumptive smolts must attain a threshold size during the winter prior to migration in order to be responsive to a photoperiod cue in the spring (Dickhoff *et al.*, 1997; McCormick *et al.*, 2007; Zydlewski *et al.*, 2014). This threshold may not be size *per se*, but may be indicative of growth (Beckman *et al.*, 2007; McCormick, 2013) or energy status (McCormick, 1994).

It is interesting that neither absolute temperature nor degree days (tested as linear and quadratic functions) leveraged any predictive influence for gill NKA activity. Increased temperature can accelerate the development of smolt characteristics (Solbakken *et al.*, 1994) but the thermal experience (quantified as degree days) can define the rate of smolting (and its regression) in some species (McCormick *et al.*, 1997, 2002). In spite of this result, it remains likely that temperature is important in this species' smolt development. The modelled data were necessarily derived from those fish that were actively migrating. Thus, those fish that may have exhibited regression of smolt characteristics may have simply ceased migration and not been captured. Additionally, the inclusion of Y as a factor was strongly supported through model selection. It is likely that year to year differences represent an integration of notable environmental factors such as water clarity (Jonsson *et al.*, 1991), flow (McCormick *et al.*, 1998) and their interactions with temperature, thermal history and therefore the expression of migration.

The decreases in K observed in *O. c. clarkii* through smolting were of great magnitude with considerable individual variation. K characteristically decreases during the parr–smolt transformation (Hoar, 1976), but the use of K as a measure of smolt development can be confounded. Some studies have successfully used K as a predictive factor (Saunders & Henderson, 1970; Wagner, 1974) while others do not report any predictive power for this measure (Beckman *et al.*, 1999). Variation in the utility of this measure centres on the influence of feeding and growth on K. Changes in behaviour and food availability may therefore obscure a developmental decrease in K.

These cautions withstanding, in predicting K in migrating *O. c. clarkii* smolts, the model that included all tested factors (the global model) had the highest support.

The role of photoperiod and size may be explained by the rationale invoked above for smolt development. In this model, both degree days and temperature were also included in the model. It is important to note that of all the factors included, only year and temperature (as a quadratic function) were significant. The significance of year further underscores the complex influence of annual conditions on the expression of *K* with respect to smolting. The quadratic influence of temperature in this model captures the subtle U shape observed in the *K* of migrant *O. c. clarkii* and probably captures the integration of reduced *K* associated with smolt development and an increase in *K* that is reversed in later migrants that begin to feed prior to or during migration.

In summary, *O. c. clarkii* do exhibit some characteristics of smolting, but the preparatory adaptations observed in these fish are weakly developed. Gill NKA increases are lower than those observed in smolts of other highly anadromous species and hypo-osmoregulatory ability is poorly developed even at the peak of migration. These observations are intriguing in the context of the exceptionally complex life histories of this species and of the evolutionary derivation of this lineage. Such complexity invites comparative assessments of both the physiology and ecology of these poorly studied fish. Specifically, characterizing the dynamics of SW acclimation of wild fish would greatly inform the current view of this species.

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References

Armstrong, R. H. (1971). Age, food, and migration of sea-run cutthroat trout, *Salmo clarki*, at Eva Lake, southeastern Alaska. *Transactions of the American Fisheries Society* **100,** 302–306.

Beckman, B. R., Dickhoff, W. W., Zaugg, W. S., Sharpe, C., Hirtzel, S., Schrock, R., Larsen, D. A., Ewing, R. D., Palmisano, A., Schreck, C. B. & Mahnken, C. W. (1999). Growth, smoltification, and smolt-to-adult return of spring chinook salmon from hatcheries on the Deschutes River, Oregon [Review]. *Transactions of the American Fisheries Society* **128,** 1125–1150.

Beckman, B. R., Gadberry, B., Parkins, P., Cooper, K. A. & Arkush, K. D. (2007). State dependent life history plasticity in Sacramento River winter-run chinook salmon (*Oncorhynchus tshawytscha*): interactions among photoperiod and growth modulate smolting and early male maturation. *Canadian Journal of Fisheries and Aquatic Sciences* **64,** 256–271.

Clarke, W. C. & Blackburn, J. (1977). A seawater challenge test to measure smolting of juvenile salmon. *Canada Fisheries and Marine Service Technical Report* **705**.

Dickhoff, W. W. & Sullivan, C. V. (1987). Involvement of the thyroid gland in smoltification, with special reference to metabolic and developmental processes. *American Fisheries Society Symposium* **1,** 197–210.

Dickhoff, W. W., Beckman, B. R., Larsen, D. A., Duan, C. & Moriyama, S. (1997). The role of growth in endocrine regulation of salmon smoltification. *Fish Physiology and Biochemistry* **17,** 231–236.

Duston, J. & Saunders, R. L. (1995). Advancing smolting to autumn in age 0+ Atlantic salmon by photoperiod, and long-term performance in sea water. *Aquaculture* **135,** 295–309.

Evans, D. H. (1984). The roles of gill permeability and transport mechanisms in euryhalinity. In *Fish Physiology*, Vol. XB (Hoar, W. S. & Randall, D. J., eds), pp. 239–283. New York, NY: Academic Press.

Giger, R. D. (1972). Ecology and management of coastal cutthroat trout in Oregon. *Fisheries Research Report Number 6*. Corvallis, OR: Oregon State Game Commission.

Goetz, F. A., Baker, B., Buehrens, T. & Quinn, T. P. (2013). Diversity of movements by individual anadromous coastal cutthroat trout *Oncorhynchus clarkii clarkii*. *Journal of Fish Biology* **83,** 1161–1182.

Handeland, S. O., Arnesen, A. M. & Stefansson, S. O. (2003). Seawater adaptation and growth of post-smolt Atlantic salmon (*Salmo salar*) of wild and farmed strains. *Aquaculture* **220,** 367–384.

Handeland, S. O., Wilkinson, E., Sveinsbo, B., McCormick, S. D. & Stefansson, S. O. (2004). Temperature influence on the development and loss of seawater tolerance in two fast-growing strains of Atlantic salmon. *Aquaculture* **233,** 513–529.

Handeland, S. O., Imsland, A. K. & Stefansson, S. O. (2008). The effect of temperature and fish size on growth, feed intake, food conversion efficiency and stomach evacuation rate of Atlantic salmon post-smolts. *Aquaculture* **283,** 36–42.

Hill, M., Zydlewski, G., Zydlewski, J. & Gasvoda, J. (2006). Development and evaluation of portable PIT tag detection units: PIT packs. *Fisheries Research* **77,** 102–109.

Hoar, W. S. (1976). Smolt transformation: evolution, behaviour and physiology. *Journal of the Fisheries Research Board of Canada* **33,** 1234–1252.

Hoar, W. S. (1988). The physiology of smolting salmonids. In *Fish Physiology*, Vol. 11B (Hoar, W. S. & Randall, D. J., eds), pp. 275–343. New York, NY: Academic Press.

Holmes, W. N. & Donaldson, E. M. (1969). The body compartments and the distribution of electrolytes. In *Fish Physiology*, Vol. I (Hoar, W. S. & Randall, D. J., eds), pp. 1–79. New York, NY: Academic Press.

International Union of Biochemistry and Molecular Biology (1992). *Enzyme Nomenclature 1992*. San Diego, CA: Academic Press.

Jacob, W. F. & Taylor, M. H. (1983). The time course of seawater acclimation in *Fundulus heteroclitus* L. *Journal of Experimental Zoology* **228,** 33–39.

Johnson, O. W., Ruckelshaus, M. H., Grant, W. S., Waknitz, F. W., Garrett, A. M., Bryant, G. J., Neely, K. & Hard, J. J. (1999). Status review of coastal cutthroat trout from Washington, Oregon and California. *NOAA Technical Memorandum NMFS-NWFSC*-**37**.

Johnson, J., Baumsteiger, J., Zydlewski, J., Hudson, M. & Ardren, W. (2010). Evidence of panmixia between sympatric life history forms of coastal cutthroat trout in two Lower Columbia River Tributaries. *North American Journal of Fisheries Management* **30,** 691–701.

Johnston, J. M. (1982). Life histories of anadromous cutthroat with emphasis on migratory behavior. In *Proceedings of the Salmon and Trout Migratory Behavior Symposium* (Brannon, E. L. & Salo, E. O., eds), pp. 123–127. Seattle, WA: University of Washington.

Jonsson, B., Jonsson, N. & Hansen, L. P. (1991). Differences in life history and migratory behaviour between wild and hatchery-reared Atlantic salmon in nature. *Aquaculture* **98,** 69–78.

Kennedy, B. M., Baumsteiger, J., Gale, W. L., Ardren, W. R. & Ostrand, K. G. (2009). Morphological, physiological, and genetic techniques for improving field identification of steelhead, coastal cutthroat trout, and hybrid smolts. *Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science* **1,** 45–56.

Leonard, J. B. K. & McCormick, S. D. (2001). Metabolic enzyme activity during smolting in stream and hatchery-reared Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences* **58,** 1585–1593.

Loch, J. J. & Miller, D. R. (1988). Distribution and diet of sea-run cutthroat trout captured in and adjacent to the Columbia River plume, May-July 1980. *Northwest Science* **62,** 41–48.

Maxime, V., Boeuf, G., Pennec, J. P. & Peyraud, C. (1989). Comparative study of the energetic metabolism of Atlantic salmon (*Salmo salar*) parr and smolts. *Aquaculture* **82,** 163–171.

McCormick, S. D. (1993). Methods for non-lethal gill biopsy and measurement of Na^+,K^+-ATPase activity. *Canadian Journal of Fisheries and Aquatic Sciences* **50,** 656–658.

McCormick, S. D. (1994). Ontogeny and evolution of salinity tolerance in anadromous salmonids: hormones and heterochrony. *Estuaries* **17,** 26–33.

McCormick, S. D. (2013). Smolt physiology and endocrinology. In *Fish Physiology*, Vol. 32 (McCormick, S. D., Farrell, A. P. & Brauner, C. J., eds), pp. 199–251. Amsterdam: Elsevier.

McCormick, S. D. & Björnsson, B. T. (1994). Physiological and hormonal differences among Atlantic salmon migrants and non-migrants reared in the wild, and hatchery smolts. *Aquaculture* **121,** 235–244.

McCormick, S. D. & Saunders, D. (1987). Preparatory physiological adaptations for marine life of salmonids: osmoregulation, growth and metabolism. *American Fisheries Society Symposium* **1,** 211–229.

McCormick, S. D., Shrimpton, J. M. & Zydlewski, J. D. (1997). Temperature effects on osmoregulatory physiology of juvenile anadromous fish. In *Global Warming: Implications for Freshwater and Marine Fish* (Wood, C. M. & McDonald, D. G., eds), pp. 279–301. Cambridge: Cambridge University Press.

McCormick, S. D., Hansen, L. P., Quinn, T. P. & Saunders, R. L. (1998). Movement, migration, and smolting of Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences* **55,** 77–92.

McCormick, S. D., Cunjak, R. A., Dempson, B., O'Dea, M. F. & Carey, J. B. (1999). Temperature-related loss of smolt characteristics in Atlantic salmon in the wild. *Canadian Journal of Fisheries and Aquatic Sciences* **56,** 1649–1658.

McCormick, S. D., Shrimpton, J. M., Moriyama, S. & Björnsson, B. T. (2002). Effects of an advanced temperature cycle on smolt development and endocrinology indicate that temperature is not a zeitgeber for smolting in Atlantic salmon. *Journal of Experimental Biology* **205,** 3553–3560.

McCormick, S. D., Shrimpton, J. M., Moriyama, S. & Björnsson, B. Th. (2007). Differential hormonal responses of Atlantic salmon parr and smolt to increased daylength: a possible developmental basis for smolting. *Aquaculture* **273,** 337–344.

McCormick, S. D., Lerner, D. T., Monette, M. Y., Nieves-Puigdoller, K., Kelly J. T. & Björnsson, B. Th. (2009). Taking it with you when you go: how perturbations to the freshwater environment, including temperature, dams and contaminants, affects seawater performance of anadromous fish. In *Challenges For Diadromous Fishes in a Dynamic Global Environmen*t (Haro, A. J., Smith, K. L., Rulifson, R. A., Moffitt, C. M., Klauda, R. J., Dadswell, M. J., Cunjak, R. A., Cooper, J. E., Beal, K. L. & Avery, T. S., eds), pp. 195–214. *American Fisheries Society Symposium* **69**.

Northcote, T. G. (1997). Why sea-run? An exploration into the migratory/residency spectrum of coastal cutthroat trout. In *Sea-run Cutthroat Trout: Biology, Management, and Future Conservation* (Hall, J. D., Bisson, P. A. & Gresswell, R. E., eds), pp. 20–26. Corvallis, OR: American Fisheries Society.

Pearcy, W. G. (1997). The sea-run and the sea. In *Sea-Run Cutthroat Trout*: *Biology*, *Management and Future Conservation* (Hall, J. D., Bisson, A. & Gresswell, R. E., eds), pp. 29–36. Corvallis, OR: Oregon Chapter, American Fisheries Society.

Rounsefell, G. A. (1958). Anadromy in North American Salmonidae. *Fisheries Bulletin* **58,** 171–185.

Saunders, R. L. & Henderson, E. B. (1970). Influence of photoperiod on smolt development and growth of Atlantic salmon (*Salmo salar*). *Journal of the Fisheries Research Board of Canada* **27,** 1295–1311.

Scheiner, S. M. (1993). Genetics and evolution of phenotypic plasticity. *Annual Review of Ecology and Systematics* **24,** 35–68.

Shrimpton, J. M., Bernier, N. J., Iwama, G. K. & 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. *Canadian Journal of Fisheries and Aquatic Sciences* **51,** 2170–2178.

Sigholt, T., Åsgård, T. & Staurnes, M. (1998). Timing of parr-smolt transformation in Atlantic salmon (*Salmo salar*): effects of changes in temperature and photoperiod. *Aquaculture* **160,** 129–144.

Smith, P. K., Krohn, R. I., Hermanson, G. T., Mallia, A. K., Gartner, F. H., Provenzano, M. D., Fujimoto, E. K., Goeke, N. M., Olson, B. J. & Klenk, D. C. (1985). Measurements of protein using bicinchoninic acid. *Analytical Biochemistry* **150,** 76–85.

Solbakken, V. A., Hansen, T. & Stefansson, S. O. (1994). Effects of photoperiod and temperature on growth and parr-smolt transformation in Atlantic salmon (*Salmo salar* L) and subsequent performance in seawater. *Aquaculture* **121,** 13–27.

Sumner, F. H. (1972). *A Contribution to the Life History of the Cutthroat Trout in Oregon with Emphasis on the Coastal Subspecies*, Salmo clarki clarki *Richardson*. Corvallis, OR: Oregon State Game Commission.

Sundell, K., Dellefors, C. & Björnsson, B. Th. (1998). Wild and hatchery-reared brown trout, *Salmo trutta*, differ in smolt related characteristics during parr–smolt transformation. *Aquaculture* **167,** 53–65.

Thorpe, J. E., Mangel, M., Metcalfe, N. B. & Huntingford, F. A. (1998). Modelling the proximate basis of salmonid life-history variation, with application to Atlantic salmon, *Salmo salar* L. *Evolutionary Ecology* **12,** 581–599.

Tipping, J. (1986). Effect of release size on return rates of hatchery sea-run cutthroat trout. *Progressive Fish-Culturist* **48,** 195–197.

Tipping, J. M. & Blankenship, H. L. (1993). Effect of condition factor at release on smolt-to-adult survival of hatchery sea-run cutthroat trout. *Progressive Fish-Culturist* **55,** 184–186.

Trotter, P. C. (1989). Coastal cutthroat trout: a life history compendium. *Transactions of the American Fisheries Society* **118,** 463–473.

Uchida, K., Kaneko, T., Yamaguchi, A. & Hirano, T. (1996). Morphometric analysis of chloride cell activity in the gill filament and lamellae and changes in gill Na^+,K^+-ATPase activity during seawater adaptation in chum salmon fry. *Journal of Experimental Zoology* **276,** 193–200.

Wagner, H. H. (1974). Photoperiod and temperature regulation of smolting in steelhead trout. *Canadian Journal of Zoology* **52,** 219–234.

Yeoh, C.-G., Kerstetter, T. H. & Loudenslager, E. J. (1991). Twenty-four hour seawater challenge test for coastal cutthroat trout. *Progressive Fish-Culturist* **53,** 173–176.

Zydlewski, J., Johnson, J., Brunzell, J., Hogle, J., Karnowski, M., Clements, S. & Shreck, C. (2008). Rapid seaward migration of coastal cutthroat trout in the Columbia River. *Proceedings of the Coastal Cutthroat Trout Symposium* **2005,** 65–74.

Zydlewski, G., Zydlewski, J. & Johnson, J. (2009). Patterns of migration and residency in coastal cutthroat trout. *Journal of Fish Biology* **75,** 203–222.

Zydlewski, J., Zydlewski, G. & Danner, R. (2010). Descaling impairs the osmoregulatory ability of Atlantic salmon smolts. *Transactions of the American Fisheries Society* **138,** 129–136.

Zydlewski, J., Cox, O., O'Malley, A., Ruksznis, P. & Trial, J. (2014). The fate of lower mode Atlantic salmon, *Salmo salar*, stocked into the Penobscot River, Maine. *North American Journal of Fisheries Management* **34,** 147–158.