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# Effect of parental mate choice and semi-natural early rearing environment on the growth performance and seawater tolerance of Chinook salmon *Oncorhynchus tshawytscha*

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To assess whether parental mate choice and early rearing in a semi-natural spawning channel may benefit the culture of Chinook salmon *Oncorhynchus tshawytscha*, 90 day growth trials were conducted using hatchery *O. tshawytscha* (hatchery), mate choice *O. tshawytscha* (*i.e.* the offspring of parents allowed to choose their own mate) that spent 6 months in a spawning channel prior to hatchery rearing (channel) and mate choice *O. tshawytscha* transferred to the hatchery as fertilized eggs (transfer). During the growth trials, all *O. tshawytscha* stocks were reared separately or in either mixed channel and hatchery or transfer and hatchery groups for comparison of performance to traditional practices. After 60 days in fresh water, all *O. tshawytscha* were transferred to seawater for an additional 30 days. Reared separately, all stocks grew *c*. 4·5 fold over 90 days but specific growth rate (*G*) and food conversion efficiency were higher in fresh water than after seawater transfer on day 60. In contrast, hatchery *O. tshawytscha* from mixed hatchery and channel and hatchery and transfer growth trials had a larger mass and length gain than their counterparts on day 60, but reduced *G* in seawater. In general, plasma levels of growth hormone, insulin-like growth factor I and cortisol did not differ among any *O. tshawytscha* groups in either the separate or mixed growth trials. Despite some differences in gill $Na^+$,$K^+$-ATPase activity, all *O. tshawytscha* had a high degree of seawater tolerance and experienced virtually no perturbation in plasma chloride following seawater transfer. Overall, all *O. tshawytscha* exhibited similar growth and seawater performance under traditional hatchery conditions and any benefit derived from either parental mate choice or semi-natural early rearing environment was only observed in the presence of mutual competition with hatchery *O. tshawytscha*.


Key words: breeding strategy; early rearing habitat; hatchery; smolt physiology.

## INTRODUCTION

Studies of parental mate choice in vertebrates are providing increasing evidence that non-random selection of mates at the time of breeding can generate benefits to the fitness of offspring (Bernatchez & Landry, 2003; Clutton-Brock & McAuliffe, 2009).

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For example, several studies have now shown that parental mate choice in Chinook salmon *Oncorhynchus tshawytscha* (Walbaum 1792) and Atlantic salmon *Salmo salar* L. 1758 leads to an increase in the diversity of the major histocompatibility complex (Landry *et al*., 2001; Consuegra & Garcia de Leaniz, 2008; Neff *et al*., 2008; Evans *et al*., 2012), which in turn can confer higher pathogen resistance (Arkush *et al*., 2002; Grimholt *et al*., 2003). There is also some evidence that parental mate choice in salmonids can produce offspring with higher growth rates (Petersson & Järvi, 2007; Pitcher & Neff, 2007). Yet commercial hatcheries producing smolts for the aquaculture industry and breeding programmes that are aimed at augmenting wild salmonid populations, artificially cross parents and deprive the progeny from the potential benefits of parental mate choice.

The relative fitness of salmonid smolts can also be influenced by early rearing habitat. In general, hatchery-reared fishes have lower return rates than wild fishes (Jonsson *et al*., 2003; Araki *et al*., 2008). Moreover, although the results are equivocal (Berejikian *et al*., 1999; Fast *et al*., 2008), some studies have shown that the post-release survival of *O. tshawytscha* reared in raceways with semi-natural habitat can be significantly higher than that of *O. tshawytscha* reared in conventional raceways (Maynard *et al*., 1995, 2004). Differences in the early rearing environment of salmonids can also lead to divergence in a variety of behavioural traits, including social aggression (Rhodes & Quinn, 1998; Metcalfe *et al*., 2003; Huntingford, 2004; Garner *et al*., 2011) and competitive ability (Doyle & Talbot, 1986; Ryer & Olla, 1995; Berejikian *et al*., 2001). Ultimately, such behavioural differences may affect higher order processes such as growth performance (Berejikian *et al*., 2000).

The preparation of juvenile salmonids for the transition from fresh water to seawater involves complex behavioural, morphological and physiological changes that are under endocrine control (Hoar, 1988; Clarke & Hirano, 1995; Björnsson *et al*., 2011). For example, the plasma levels of growth hormone (GH) and insulin-like growth factor I (IGF-I) increase in late spring in association with the development of salinity tolerance in smolts (McCormick, 1995; Dickhoff *et al*., 1997) and injections of either hormone promote growth and seawater adaptation (Clarke *et al*., 1977; Miwa & Inui, 1985; McCormick *et al*., 1991). Similarly, plasma cortisol levels increase during the parr to smolt transformation (Specker & Schreck, 1982; Shrimpton *et al*., 1994*a*) and stimulate hypo-osmoregulatory functions (Bisbal & Specker, 1991). In part, these hormones contribute to the increase in euryhalinity of smolts by stimulating the activity of chloride cell $Na^+$,$K^+$-ATPase, a key enzyme involved in ion transport across the gill (Richman & Zaugg, 1987; McCormick, 1996). Several studies have identified differences in plasma hormone levels and in gill $Na^+$,$K^+$-ATPase activity between wild and hatchery-reared smolts (McCormick & Björnsson, 1994; Shrimpton *et al*., 1994*b*; Sundell *et al*., 1998). Similarly, higher levels of plasma GH and greater seawater tolerance in hatchery-raised steelhead *Oncorhynchus mykiss* (Walbaum 1792) transferred to semi-natural raceways than in hatchery-raised *O. mykiss* kept in traditional raceways suggest that rearing habitat may affect the endocrine control of smoltification (Zydlewski *et al*., 2003). In contrast, as far as is known, the potential effect of parental mate choice on the hormonal profile of salmonid smolts and their seawater tolerance has not been investigated.

Besides the potential to increase growth performance and seawater tolerance, the use of parental mate choice and semi-natural early rearing is associated with lower labour costs, a lower risk of mortality resulting from equipment failure and a reduced

need for infrastructure relative to traditional hatchery techniques. Mate choice breeding and semi-natural early rearing, however, are rarely used for *O. tshawytscha* smolt production because the egg-to-fry survival is relatively poor compared to that observed in the hatchery. For example, while 10 *O. tshawytscha* females can produce *c*. 15 000 fry in the hatchery, the same broodstock will yield *c*. 1500 fry in spawning channels (D. D. Heath, unpubl. data). Whether parental mate choice and semi-natural early rearing result in performance gains that outweigh the costs associated with lower egg-to-fry survival remains to be determined.

Therefore, the objective of this study was to assess if parental mate choice and early rearing habitat enhance the future growth performance and seawater tolerance of *O. tshawytscha* smolts when reared under common hatchery conditions. For this purpose, *O. tshawytscha* offspring were produced (1) using standard hatchery techniques (hatchery), (2) from parental mate choice followed by rearing in a semi-natural channel for 6 months (channel) and (3) to differentiate between the effects of breeding from those of early rearing, from parental mate choice followed by transfer to the hatchery as fertilized eggs (transfer). Ninety-day growth trials were then conducted in a hatchery and relative growth rate, length gain, condition factor, feed conversion efficiency and plasma levels of GH, IGF-I and cortisol were quantified. Seawater tolerance was also assessed by quantifying gill $Na^+$,$K^+$-ATPase and plasma chloride values prior to and 24 h after seawater transfer on days 60 and 61 of the growth trials, respectively. Finally, to assess the relative performance of the different *O. tshawytscha* lines with and without competition, growth trials were carried out with separate groups of *O. tshawytscha* (channel, hatchery and transfer) as well as with mixed groups of channel and hatchery and transfer and hatchery *O. tshawytscha*.

## MATERIALS AND METHODS

### EXPERIMENTAL ANIMALS

Experiments were conducted at Yellow Island Aquaculture Ltd. (YIAL; http://www.yellowislandaquaculture.com/) following the principles of the Canadian Council for Animal Care and using all female juvenile *O. tshawytscha* taken from the YIAL population. This population was founded with gametes from the Robertson Creek hatchery in Vancouver Island, BC, Canada, and has been maintained at YIAL since 1986. The YIAL broodstock does not contain the male sex chromosome. Males instead are produced through the use of hormonal sex-reversal on female XX *O. tshawytscha* (Garner *et al*., 2011). Note, however, that XX males grow to a similar body size and have similar circulating levels of sex steroids as XY males (Heath *et al*., 2002). Moreover, XX and XY males have similar spawning behaviours and courtship success in the spawning channels used in this study (Garner *et al*., 2010). Previous studies using the YIAL *O. tshawytscha* population have observed characteristic breeding behaviour and found that, as in wild *O. tshawytscha*, mating patterns are affected by both competition and parental mate choice (Neff *et al*., 2008; Garner *et al*., 2010). An analysis of genetic diversity across seven loci in 24 males from the YIAL and wild Quinsam River (Vancounver Island, BC) broodstocks revealed a high level of genetic diversity in both stocks, with an equivalent number (mean ± S.E.) of alleles (YIAL, 20·12 ± 0·82; Quinsam, 21·41 ± 0·97) and comparable heterozygosity (YIAL, 0·88 ± 0·02; Quinsam, 0·91 ± 0·02).

For this study, three groups of juvenile *O. tshawytscha* were generated: (1) hatchery *O. tshawytscha* were the product of hatchery spawning and rearing, (2) channel *O. tshawytscha* were the result of natural spawning and semi-natural rearing in spawning channels and (3) transfer *O. tshawytscha* resulted from natural spawning and hatchery rearing [Fig. 1(a)–(c)]. Hatchery spawning was timed to coincide with peak spawning in the channel. In brief, 10

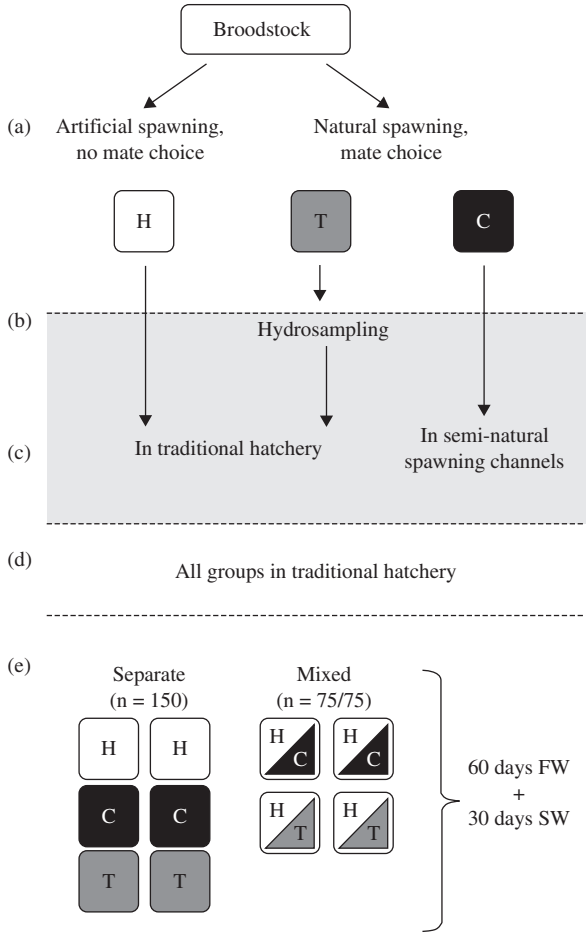


FIG. 1. Summary of the methods used to generate the three groups of juvenile *Oncorhynchus tshawytscha* used in this study: hatchery (H), transfer (T) and channel (C). Schematic shows (a) the timing and strategies used for breeding (breeding strategy, October to November), (b) the timing of pneumatic egg sampling in the transfer fish (eyed eggs, December), (c) the differences in early rearing environments (hatch and early rearing, January to April), (d) the common rearing period prior to the growth trials (common rearing, May to June) and (e) the experimental setup to assess the effects of parental mate choice, early rearing environment and competition on the growth performance in fresh water (FW) and seawater (SW) (growth trials, July to September).

male and female broodstock were used to produce 20 families by fertilizing half of the brood from each female with the milt of a different male, and by using each pair of males to fertilize the eggs of two females. The fertilized eggs were incubated by family in a Heath tray and at *c*. 110 days post-fertilization (dpf), 70 fry from each family (1400 total) were pooled and transferred to a 3000 l tank for rearing at a density of $<2\,g\,l^{-1}$. *Oncorhynchus tshawytscha* were fed pellet food *ad libitum* both manually thrice daily and continuously using a belt feeder. Natural springs provided common water supply to both the spawning channels and the hatchery over the duration of rearing as well as the growth trials. Seasonal variation in ground water temperature up to the growth trials occurred with a range of $4–10^{\circ}$ C.

For the channel *O. tshawytscha*, 18 male and 12 female broodstock were transferred into a 15 m × 3·5 m channel. The channel had a water depth of *c*. 1 m, a partially re-circulating

water flow of *c*. 300 l min$^{-1}$, gravel that measured 3–6 cm in diameter and was protected by netting to exclude predators. Broodstock were allowed to spawn without interference and were removed from the channel once they died. Genetic analysis of the parents and progeny revealed that 17 of 18 males and all 12 females mated successfully (Garner *et al*., 2010). At *c*. 110 dpf (*c*. mid-February), once the fry had emerged, pellet food was provided *ad libitum* both manually thrice daily and continuously using belt feeders installed above the channel. Once the fry reached 1–2 g in size, after 2·5 months of feeding in the channel at a density $<0{\cdot}05$ g l$^{-1}$ (*c*. late-April), *c*. 1400 were collected by seine and transferred to a 3000 l tank in the hatchery. In total, including the time spent at the egg and alevin stage and at the fry stage, the channel *O. tshawytscha* spent *c*. 6 months in the channel prior to being moved to the hatchery.

The transfer *O. tshawytscha* were collected from the channel by pneumatic sampling at the eyed-egg stage, *i.e*. at *c*. 48 dpf, and incubated in the hatchery. This technique and its effectiveness have previously been described in detail (Berejikian *et al*., 2011). In brief, eggs were netted as they were forced into the water column by compressed air injected into the channel gravel. Sampling was conducted in the entire channel area, which allowed eggs from all redds to be sampled. Approximately 1400 eggs were pooled and incubated in a Heath tray as described for hatchery *O. tshawytscha*. At *c*. 110 dpf, all fry were pooled and transferred to a 3000 l tank and fed *ad libitum* using the same feeding regime as the hatchery and channel *O. tshawytscha*.

From May to mid-June, prior to the commencement of the growth experiment, all *O. tshawytscha* stocks were maintained in a common hatchery rearing environment in separate 3000 l tanks and fed *ad libitum* both manually and using a belt feeder as above [Fig. 1(d)].

## EXPERIMENTAL SETUP OF SEPARATE AND MIXED GROWTH TRIALS

Two weeks prior to the beginning of the experiment (mid-June), once all *O. tshawytscha* had reached a size of 5–7 g, each *O. tshawytscha* group was moved from the 3000 l tanks to duplicate 700 l tanks at a density of 15 kg m$^{-3}$ to allow proper acclimation time to the density and feeding rations of the growth trial. In the separate group growth trials, each tank contained 150 fish from the hatchery, channel or transfer group (six tanks in total). In the mixed group growth trials, each tank contained equal numbers (*i.e*. 75 *O. tshawytscha* from each group) of either hatchery and channel fish, or hatchery and transfer *O. tshawytscha* [four tanks in total; Fig. 1(e)]. In addition, *O. tshawytscha* in the mixed group growth trials were anaesthetized in buffered ($NaHCO_3$, 0·2 g l$^{-1}$) tricaine methanesulphonate (0·1 g l$^{-1}$, Syndel International; www.syndel.com) and injected with a passive integrated transponder tag for identification. All growth trials started on 1 July (day 0) when the *O. tshawytscha* were *c*. 6 months old, lasted 90 days and consisted of an initial 60 days in fresh water (flow rate: 6 l min$^{-1}$; mean ± s.e.: 10 ± 2° C; pH mean ± s.e.: 7·6 ± 0·1; dissolved oxygen: $>7{\cdot}5$ mg l$^{-1}$) followed by 30 days in full-strength seawater (salinity: 30; flow rate: 6 l min$^{-1}$; mean ± s.e.: 10 ± 2° C; pH mean ± s.e.: 8·1 ± 0·1; dissolved oxygen: $>7{\cdot}5$ mg l$^{-1}$). The transition from fresh water to seawater did not involve any *O. tshawytscha* handling and was accomplished within *c*. 1 h of switching the water supply (the seawater flow rate was 12 l min$^{-1}$ for the first hour and returned to 6 l min$^{-1}$ thereafter). There were no mortalities associated with the 24 h seawater challenge. During the acclimation and experimental period, *i.e*. between mid-June and the end of September, all *O. tshawytscha* were manually fed a commercial salmonid diet (EWOS Micro 1·2-2 mm; www.ewos.com) thrice daily and constantly supplied with feed using a 24 h automatic belt feeder with up to 3% mean tank biomass. All *O. tshawytscha* were kept on a 16L:8D photoperiod cycle during the growth experiment.

## SAMPLING PROCEDURE

Twenty *O. tshawytscha* from each group were sampled on days 0, 30, 60, 61 (*i.e*. 24 h post-seawater transfer) and 90. All *O. tshawytscha* were euthanized by lethal overdose of

buffered MS-222. Mass ($M$) and fork length ($L_F$) were measured on all fish and on days 60 and 61 a gill sample was collected for future analysis. Ten *O. tshawytscha* from each duplicate tank were quickly and randomly netted, terminally anaesthetized so that all *O. tshawytscha* were sampled for blood within 5 min and retained on ice for further tissue analysis. Blood collected *via* caudal puncture was immediately centrifuged at 10 000 *g* for 3 min in a counter-top centrifuge (IEC Centra CL3, Thermo Fisher Scientific; www.thermoscientific.com). The recovered plasma and gill tissue were initially frozen in liquid nitrogen and later stored at −80° C until further analysis.

## PLASMA ANALYSES

Plasma cortisol concentrations were measured in triplicate by radioimmunoassay (RIA) as specified by Bernier *et al*. (2008). Plasma IGF-I levels were assayed as described in Shimizu *et al*. (2000). Briefly, IGF-I was first extracted from plasma by acid-ethanol according to Breier *et al*. (1991) and quantified using recombinant *O. mykiss* IGF-I as standard and tracer, and anti-recombinant barramundi *Lates calcarifer* (Bloch 1790) IGF-I as primary antibody (1:7000; GroPep Bioreagents; www.gropep.com/au). Plasma GH levels were assayed as described by Swanson (1994) using recombinant *O. mykiss* GH as standard and tracer and anti-recombinant *O. mykiss* GH as primary antibody (1:25 000; GroPep Bioreagents). IGF-I and GH were iodinated by the chloramine-T method. For both the IGF-I and GH RIAs, *c*. 7000 counts $min^{-1}$ of tracer in 50 µl was added to tubes containing 50 µl of sample, 50 µl of primary antibody and 150 µl of RIA buffer [30 mM $NaH_2PO_4$, 0·02% protamine sulphate, 10 mM EDTA, 0·025% $NaN_3$, 0·05% (v/v) Tween-20, pH 7·5]. After a 48 h incubation at 4° C, the antibody-bound IGF-I or GH were complexed with secondary antibody (1:15, goat anti-rabbit immunoglobulin G (IgG); AbD Serotec; www.abdserotec.com) and polyclonal rabbit anti-human IgG (1:200; Dako, Glostrup; www.dako.com), and precipitated by using ice-cold polyethylene glycol 6000 (PEG-6000; Sigma-Aldrich; www.sigmaaldrich.com) and centrifugation at 4000 *g* for 30 min. Plasma IGF-I and GH analyses were each performed in a single assay. The lower detection limit of the IGF-I RIA was 40 pg $ml^{-1}$ and the intra-assay coefficient of variability was 3·9% ($n = 6$). The least detectable concentration of the GH RIA was 400 pg $ml^{-1}$ and the intra-assay coefficient of variability was 6% ($n = 6$). Concentrations of plasma cortisol, IGF-I and GH were determined using three-parameter sigmoidal curve regression equations (SigmaPlot 10, SPSS; www.sigmaplot.com) obtained from standard curves. Serial dilutions of *O. tshawytscha* plasma paralleled the standard curves in each RIA. Plasma chloride measurements were performed using a chloride titrator (CMT 10, Radiometer; www. radiometer.com) and assayed in duplicate for each *O. tshawytscha*.

## GILL $NA^+$,$K^+$-ATPASE ACTIVITY

Gill filament tissue was extracted and $Na^+$,$K^+$-ATPase activity assayed using the microassay method of McCormick (1993). Both $Na^+$,$K^+$-ATPase and bicinchoninic acid protein assays (Pierce; www.piercenet.com) were run on a SpectraMAX 190 microplate reader using SOFTmax software 4.6 (Molecular Devices; www.moleculardevices.com).

## CALCULATIONS AND STATISTICAL ANALYSES

Briefly, condition factor ($K$) was calculated from $M$ (g) and $L_F$ (cm) as: $K = 10^2 M L_F^{-b}$, where $b$ was obtained by regressing $\ln M$ on $\ln L_F$ using the data from all sampled *O. tshawytscha* ($b = 3{\cdot}24$). Specific growth rate ($G$) was defined as the relative percentage growth rate between sample intervals and calculated as: $G = 100(\ln M_t - \ln M_{t-x})\, x^{-1}$, where $M_t$ is the individual mass (g) from sample point $t$, $M_{t-x}$ is the average mass (g) $x$ days prior to the current sampling point and $x = 30$ days. Relative $L_F$ gain was calculated as: $L_F$ gain $= (L_{Ft} - L_{Ft-x})\, x^{-1}$, where $L_{Ft}$ is the individual $L_F$ (mm) from sample point $t$, $L_{Ft-x}$ is the average $L_F$ (mm) $x$ days prior to the current sampling point and $x = 30$ days. Relative food conversion efficiency ($E_{FC}$) was calculated as: $E_{FC} = M' R^{-1}$, where $R$ is the mean food rationed per tank for the sampling interval and corrected for total biomass and $M'$

is the relative mass increase of individual *O. tshawytscha* during that interval (*i.e.* $M'_t - M'_{t-x}$). All results are presented as mean ± S.E. There were no significant differences between treatment replicates throughout the experiment. Data were analysed in SigmaStat 3.5 (SPSS) using two-way ANOVAs and time and treatment-group type as factors followed by a pairwise Holm–Sidak *post hoc* multiple comparison test with a sequential Bonferroni adjustment based on the number of treatments and sample points being considered (*e.g.* the comparison of parameters among hatchery, channel and transfer *O. tshawytscha* sampled at 0, 30, 60, 61 and 90 days was analysed using a Bonferroni correction of $\alpha = 0{\cdot}05\,k^{-1}$, where $k = 8$ and $\alpha_{adj} = 0{\cdot}0063$). Data that did not meet both the assumptions of the ANOVA (normality and equal variance) were either $\log_{10}$ or arc-sine transformed to a normal distribution prior to parametric analysis. Global differences and interactions among variables denoted within the ANOVA are described in following text.

## RESULTS

### GROWTH AND FEEDING

Equivalent mass ($M$) was gained by the hatchery, channel and transfer *O. tshawytscha* in separate growth trials [$P > 0{\cdot}05$; Fig. 2(a)]. Although the hatchery *O. tshawytscha* were larger than either the channel [$P < 0{\cdot}001$; Fig. 2(b)] or transfer [$P < 0{\cdot}001$; Fig. 2(c)] *O. tshawytscha* on day 60, all *O. tshawytscha* had similar mass ($P > 0{\cdot}05$) by the end of the mixed growth trials. On average, the *O. tshawytscha* from all the three groups in the separate growth trials increased body mass by *c*. 4·5 fold over 90 days. $K$ of channel *O. tshawytscha* in the separate growth trials, although lower on day 0 than in the other two groups ($P < 0{\cdot}001$), increased over the next 30 days to where all *O. tshawytscha* had comparable $K$ values after day 30 [$P > 0{\cdot}05$; Fig. 2(d)].

In the hatchery and channel mixed growth trial, channel *O. tshawytscha* generally had lower $K$ than hatchery cohorts [$P < 0{\cdot}001$; Fig 2(e)] and the difference was significant on days 0 and 90 ($P < 0{\cdot}05$). Both hatchery and transfer *O. tshawytscha* had similar $K$ values in the hatchery and transfer mixed growth trials [$P > 0{\cdot}05$; Fig. 2(f)].

Although the transfer *O. tshawytscha* had lower $G$ [$P < 0{\cdot}001$; Fig. 3(a)], $L_F$ gain [$P < 0{\cdot}001$; Fig. 3(d)] and $E_{FC}$ [$P < 0{\cdot}001$; Fig. 3(g)] than the hatchery and channel *O. tshawytscha* during the initial 30 day period of the separate growth trials, these differences were not maintained over the next 30 days in fresh water nor during the seawater phase ($P > 0{\cdot}05$). In general, $G$ and $E_{FC}$ were higher during the freshwater period than following seawater transfer, while $L_F$ gain in transfer *O. tshawytscha* following seawater remained elevated relative to the other stocks.

In the mixed growth trials, while the $G$ of hatchery *O. tshawytscha* was consistently lower in seawater than in fresh water, the $G$ of either channel [Fig. 3(b)] or transfer [Fig. 3(c)] *O. tshawytscha* remained unchanged throughout the experiment ($P > 0{\cdot}05$). During the seawater phase, hatchery *O. tshawytscha* $G$ was lower than either channel ($P < 0{\cdot}001$) or transfer ($P < 0{\cdot}001$) *O. tshawytscha* $G$. $L_F$ gains were lower in channel and transfer *O. tshawytscha* than hatchery cohorts during mixed trials in fresh water [Fig. 3(e), (f)]. Channel *O. tshawytscha* continued to increase $L_F$ gain after the seawater exposure, surpassing gains by hatchery counterparts [$P < 0{\cdot}001$; Fig. 3(e)], however, this was not mirrored in the mixed hatchery and transfer reared *O. tshawytscha* [$P > 0{\cdot}05$; Fig. 3(f)]. The $E_{FC}$ values did not

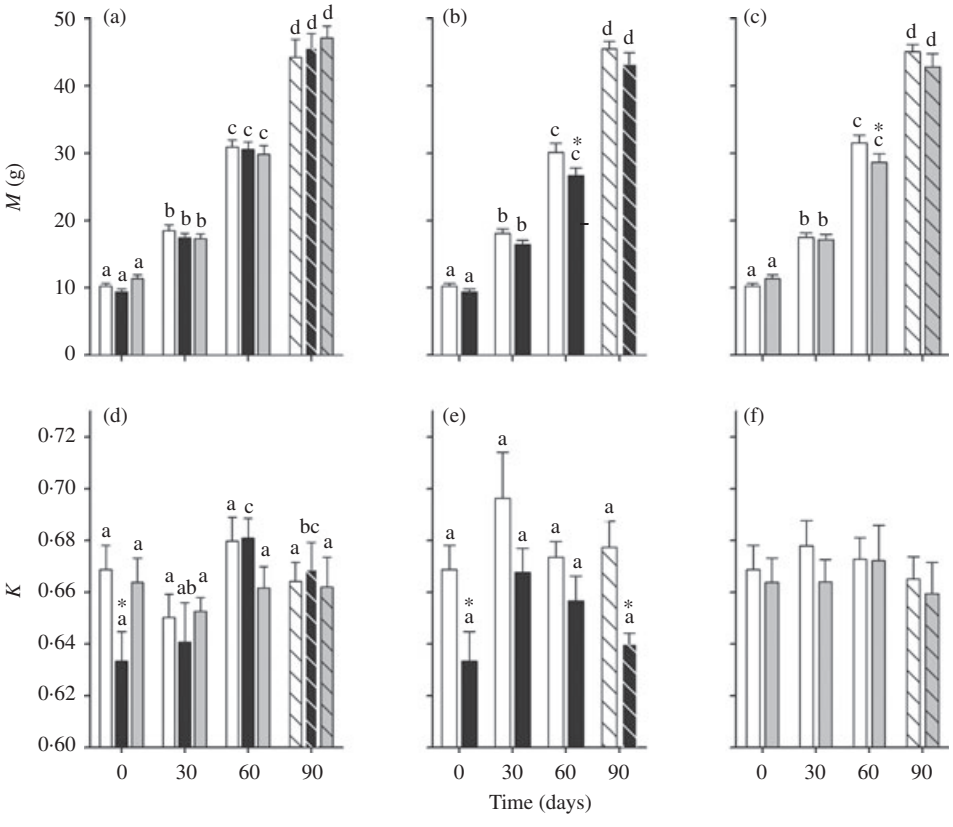


FIG. 2. (a–c) Mass ($M$) and (d–f) condition factor ($K$) of hatchery (□, ▨), channel (■, ▩) and transfer (▩, ▨) juvenile *Oncorhynchus tshawytscha* reared for 90 days either as (a, d) separate or mixed groups: (b, e) mixed hatchery and channel and (c, f) mixed hatchery and transfer. *Oncorhynchus tshawytscha* were reared in fresh water (non-hatched bars) between days 0 and 60, and in seawater (hatched bars) between days 61 and 90. Day 0 corresponds to 1 July. Values are mean ± S.E. ($n = 20$ per group at each time point). Bars that do not share a common lower case letter are significantly different ($P < 0{\cdot}05$) from each other within a specific treatment group of *O. tshawytscha*. *, a significant difference from the hatchery group at a given time (ANOVA, Holm–Sidak, $P < 0{\cdot}05$).

differ among the different groups ($P > 0{\cdot}05$) and consistently decreased between the freshwater and seawater phase in the mixed group growth trials [Fig. 3(h), (i)].

## PLASMA HORMONES

Throughout both the separate and mixed growth trials, plasma GH levels did not differ among the hatchery, channel or transfer *O. tshawytscha* at any given sampling time [$P > 0{\cdot}05$; Fig. 4(a)–(c)]. Other than an increase in the channel *O. tshawytscha* at day 90 in the separate trial [$P < 0{\cdot}001$; Fig. 4(a)] and a decrease in the transfer *O. tshawytscha* at day 30 in the mixed trial [$P < 0{\cdot}05$; Fig. 4(c)], plasma GH levels remained relatively unchanged throughout.

In the separate trials, although plasma IGF-I did not differ among the three groups at any given sampling time ($P > 0{\cdot}05$) the levels were transiently increased on day 30

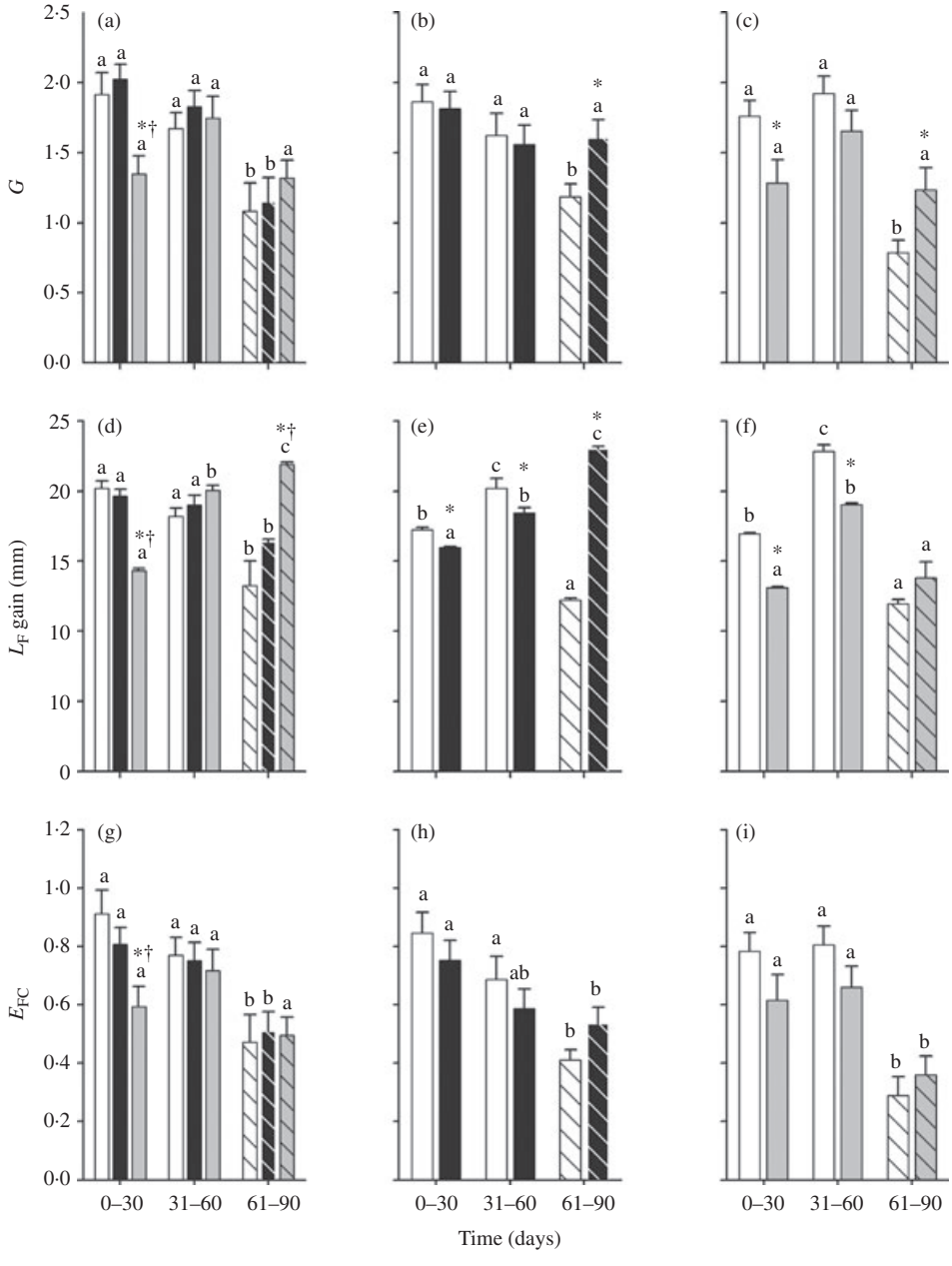


FIG. 3. (a–c) Relative specific growth rate ($G$), (d–f) fork length ($L_F$) gain and (g–i) food conversion efficiency ($E_{FC}$) of hatchery (□,▨), channel (■,▩) and transfer (▩,▨) juvenile *O. tshawytscha* reared for 90 days either as (a, d, g) separate or mixed groups: (b, e, h) mixed hatchery and channel and (c, f, i) mixed hatchery and transfer. *Oncorhynchus tshawytscha* were reared in fresh water (non-hatched bars) between days 0 and 60, and in seawater (hatched bars) between days 61 and 90. Day 0 corresponds to 1 July. Values are mean ± S.E. ($n = 20$ per group at each time point). Bars that do not share a common lower case letter are significantly different ($P < 0{\cdot}05$) from each other within a specific treatment group of *O. tshawytscha*. *and †, a significant difference from the hatchery and channel groups, respectively, within a given time period (ANOVA, Holm–Sidak, $P < 0{\cdot}05$).

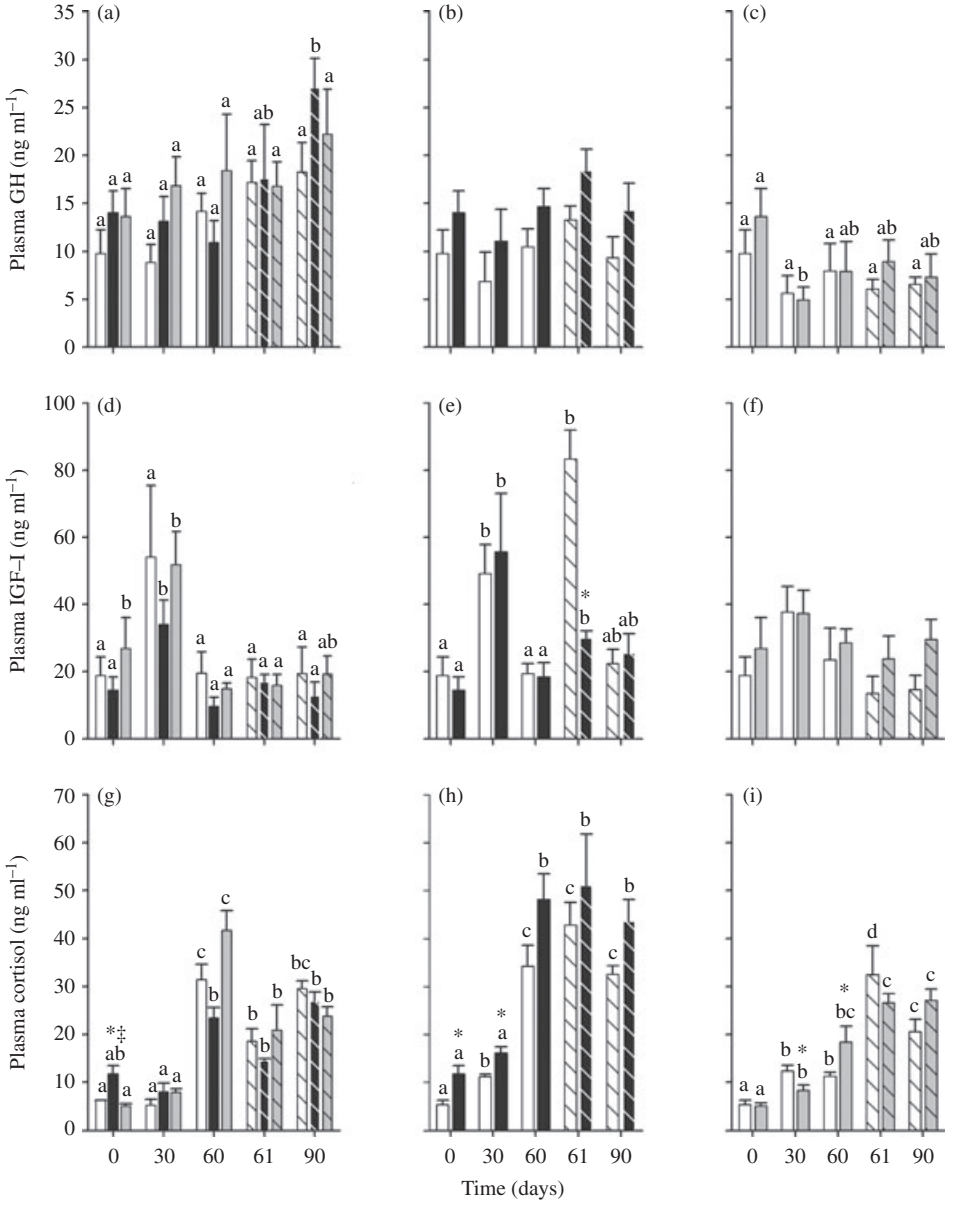


FIG. 4. (a–c) Plasma growth hormone (GH), (d–f) insulin-like growth factor I (IGF-I) and (g–i) cortisol concentrations of hatchery (□, ▨), channel (■, ▩) and transfer (▤, ▧) juvenile *Oncorhynchus tshawytscha* reared for 90 days either as (a, d, g) separate or mixed groups: (b, e, h) mixed hatchery and channel and (c, f, i) mixed hatchery and transfer. *Oncorhynchus tshawytscha* were reared in fresh water (non-hatched bars) between days 0 and 60, and in seawater (hatched bars) between days 61 and 90. Day 0 corresponds to 1 July. Values are mean ± S.E. ($n = 6$ per group at each time point). Bars that do not share a lower case common letter are significantly different ($P < 0{\cdot}05$) from each other within a specific treatment group of *O. tshawytscha*. * and ‡, a significant difference from the hatchery and transfer groups respectively (ANOVA, Holm–Sidak, $P < 0{\cdot}05$).

[$P < 0{\cdot}05$; Fig. 4(d)]. Similarly, both mixed trials were characterized by increases in plasma IGF-I levels on day 30 [Fig. 4(e), (f)] but the increase only reached significance in the hatchery and channel comparison [$P < 0{\cdot}01$; Fig. 4(e)]. Other than a marked difference in plasma IGF-I levels between the hatchery and channel *O. tshawytscha* on day 61 [*i.e.* 1 day after seawater transfer; $P < 0{\cdot}01$; Fig. 4(e)], hatchery *O. tshawytscha* had similar IGF-I plasma levels as either the channel or transfer *O. tshawytscha* in the mixed growth trials [$P > 0{\cdot}05$; Fig. 4(e), (f)].

Plasma cortisol levels in the separate trials were initially relatively low in fresh water, rose *c.* three to seven-fold to a peak on day 60 and remained *c.* two to five-fold higher during the seawater period than during the first 30 days in fresh water [$P < 0{\cdot}001$; Fig. 4(g)]. Similarly, in the hatchery and channel mixed trial, plasma cortisol levels rose by *c.* three-fold between days 30 and 60 in fresh water and remained elevated during the seawater period [Fig. 4(h)]. In the hatchery and transfer mixed trial, cortisol levels rose more gradually in fresh water and peaked after transfer to seawater at levels that were *c.* 4–5 higher than on day 0 [$P < 0{\cdot}001$; Fig. 4(i)]. While plasma cortisol levels did not differ among the three groups of *O. tshawytscha* during the seawater period of the growth trials ($P > 0{\cdot}05$), channel *O. tshawytscha* had higher basal values than hatchery *O. tshawytscha* in the first 30 days of freshwater rearing. In the hatchery and transfer mixed trial, there were significant differences in plasma cortisol between the two groups on sampling days 30 and 60 [$P < 0{\cdot}01$; Fig. 4(i)].

### GILL $NA^+$,$K^+$-ATPASE AND PLASMA CHLORIDE

In the separate trials, although gill $Na^+$,$K^+$-ATPase activity of the channel *O. tshawytscha* before and after seawater transfer did not differ ($P > 0{\cdot}05$), both hatchery ($P < 0{\cdot}001$) and transfer ($P < 0{\cdot}01$) groups experienced a near two-fold increase in activity [Fig. 5(a)]. In contrast, while gill $Na^+$,$K^+$-ATPase activity of hatchery *O. tshawytscha* in both mixed group trials did not change between days 60 and 61, the enzymatic activity decreased in both the channel and transfer *O. tshawytscha* following entry into seawater [$P < 0{\cdot}05$; Fig. 5(b), (c)]. The transition between fresh water and seawater had no effect on plasma chloride levels in the separate trials [$P > 0{\cdot}05$; Fig. 5(d)] and in the hatchery and transfer mixed trial [$P > 0{\cdot}05$; Fig. 5(f)], but resulted in a small overall increase in the hatchery and channel mixed trial [$P < 0{\cdot}05$; Fig. 5(e)]. Either before or after seawater transfer, however, plasma chloride concentrations did not differ among any of the groups in either the separate or mixed growth trials.

## DISCUSSION

Independent of breeding strategy and early rearing environment, when comparisons are made under common hatchery conditions, results from this study suggest that parental mate choice and early rearing in a semi-natural habitat do not affect the overall growth performance, hormonal profile and seawater tolerance of *O. tshawytscha* smolts. In contrast, when groups of *O. tshawytscha* from different breeding and early rearing strategies are mixed with traditional hatchery *O. tshawytscha* and reared in the hatchery, it was observed that parental mate

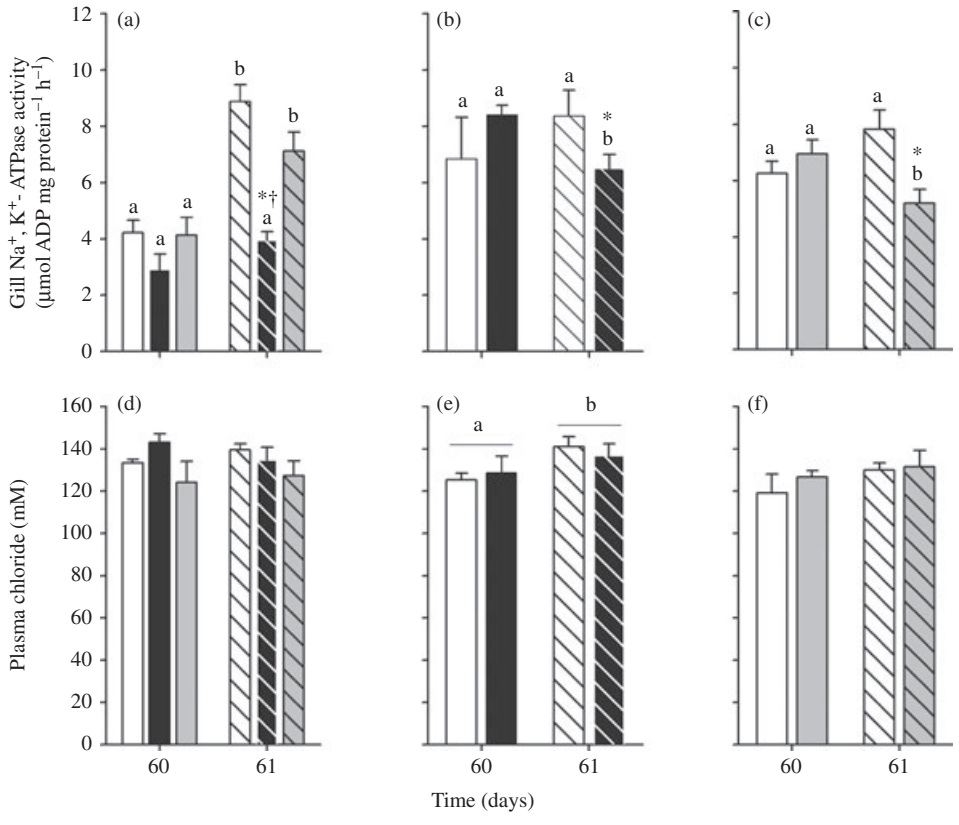


FIG. 5. (a–c) Gill $Na^+$,$K^+$-ATPase activity and (d–f) plasma chloride concentration of hatchery (□,▨), channel (■,▩) and transfer (▤,▧) juvenile *O. tshawytscha* reared either as (a, d) separate or mixed groups: (b, e) mixed hatchery and channel and (c, f) mixed hatchery and transfer. Enzymatic activity and chloride levels were assessed after 60 days of rearing in fresh water (non-hatched bars) and 24 h after transfer to seawater (hatched bars). Values are mean ± S.E. ($n = 6$ per group at each time point). Bars that do not share a common lower case letter are significantly different ($P < 0{\cdot}05$) from each other within a specific treatment group of *O. tshawytscha*. * and †, a significant difference from the hatchery and transfer groups, respectively, at a given time. Global differences between sampling times are indicated by dissimilar letters (ANOVA, Holm–Sidak, $P < 0{\cdot}05$).

choice and early rearing habitat can have significant effects on growth performance. Interestingly, however, the differences in growth performance observed in the mixed trials were not associated with consistent changes in plasma GH, IGF-I or cortisol levels or due to differences in seawater performance. Overall, no lasting advantage in growth performance was observed in *O. tshawytscha* resulting from parental mate choice and reared in a semi-natural environment.

Reared under identical hatchery conditions, the hatchery, channel and transfer *O. tshawytscha* grew equally well during the 90 day separate growth trials. Early rearing in the spawning channel did, however, produce *O. tshawytscha* smolts with a lower *K* than in either hatchery or transfer *O. tshawytscha*. Similarly, stream-reared *S. salar* (McCormick & Björnsson, 1994), wild Coho salmon *Oncorhynchus kisutch* (Walbaum 1792) (Shrimpton *et al*., 1994*b*) and wild brown trout *Salmo trutta* L. 1758

(Sundell *et al*., 1998) smolts all have lower $K$ values than their hatchery counterparts. Although the factors responsible for the smaller $K$ of channel *O. tshawytscha* are not known, it is important to note that all three groups of *O. tshawytscha* were fed the same diet *ad libitum* from first feeding. The fact that transfer and hatchery *O. tshawytscha* had similar $K$ value and that channel *O. tshawytscha* $K$ no longer differed from the $K$ of the two other groups after 30 days of common hatchery rearing suggests that the cause for this difference in $K$ is environmental and not genetic. All three *O. tshawytscha* groups also experienced a similar relative decrease in $G$ and $E_{FC}$ between days 31–60 in fresh water and days 61–90 in seawater. While growth rate is known to be inversely related to body size (Brett, 1979), the reduction in $G$ following transfer to seawater is also probably a result of the physiological challenge associated with seawater adaptation (Clarke *et al*., 1981; Zaugg & Beckman, 1990). In *S. salar*, transient decrease in $G$ and $E_{FC}$ is a characteristic response to seawater transfer and typically lasts 2–7 weeks (Usher *et al*., 1991; Stead *et al*., 1996; Handeland *et al*., 2003). Overall, in the absence of any interaction among the channel, hatchery and transfer *O. tshawytscha*, no evidence was found that either parental mate choice or early rearing in a semi-natural habitat has a sustained effect of the growth performance of *O. tshawytscha* within a conventional hatchery setting.

In contrast, the mixed growth trials revealed specific differences in growth performance among the channel, hatchery and transfer *O. tshawytscha*. For example, the smaller $K$ of channel *O. tshawytscha* contrasted to hatchery *O. tshawytscha* at the beginning of the growth trial and was still present 90 days later when these *O. tshawytscha* were allowed to interact in a common hatchery environment. This persistent difference in $K$ suggests that as a result of early rearing environmental effects channel *O. tshawytscha* may have been conditioned to respond to food and to conspecifics in a different manner than hatchery *O. tshawytscha*. Indeed, as previously observed in steelhead *O. mykiss* (Berejikian *et al*., 1996, 2000), the increased complexity of semi-natural environments and the opportunity for additional natural food resources may have enabled channel *O. tshawytscha* to develop innate territorial behaviour that persisted even after they were placed in the hatchery environment. Similarly, the differences in day 60 $M$, seawater $G$ and $L_F$ gain within the hatchery and channel, and the hatchery and transfer group pairings suggest that in the presence of competitive interactions parental mate choice can affect the growth performance of juvenile *O. tshawytscha*. These results are consistent with the observation that *S. trutta* parr and smolts resulting from mate choice have different growth rates than non-mate choice offspring when the experiment is conducted on small mixed groups of *S. trutta* fed at restricted rates and allowed to establish dominance hierarchies (Petersson & Järvi, 2007).

Reared under intensive hatchery conditions and as separate groups, the channel, hatchery and transfer *O. tshawytscha* had similar circulating levels of plasma GH and IGF-I. These results suggest that within a hatchery setting and in fast growing *O. tshawytscha*, neither parental mate choice nor early rearing in a semi-natural environment affect the seasonal changes in plasma GH and IGF-I that are associated with smolting. The ocean-type *O. tshawytscha* stocks from south-western British Columbia such as the YIAL stock migrate to sea as underyearlings after spending a few months in the river and estuary (Healey, 1991; Clarke & Hirano, 1995). Although juvenile ocean-type *O. tshawytscha* retain their hypo-osmoregulatory ability throughout the summer (Clarke *et al*., 1989), smolt migration in these stocks is

generally complete by the end of June (Healey, 1991). As such, the GH and IGF-I hormonal profiles of the *O. tshawytscha* in this study are characteristic of smolts that are maintained in fresh water and transferred to seawater beyond their natural smolt window. The GH levels were maintained at moderately elevated levels throughout the summer (Clarke *et al*., 1989; Young *et al*., 1989; Dickhoff *et al*., 1997) and although the IGF-I levels were lower than maximum springtime levels (Beckman *et al*., 2000; Shrimpton *et al*., 2007*a*), the peaks in plasma IGF-I levels on day 30 correlated well with the fast growth rates that characterized the freshwater phase of the growth trials as previously observed in juvenile *O. tshawytscha* (Beckman & Dickhoff, 1998).

In the mixed growth trials, with the exception of one time point, no differences in plasma GH or IGF-I were found between the paired groups of *O. tshawytscha*. Moreover, although the difference in IGF-I levels between the hatchery and channel *O. tshawytscha* on day 61 was marked, the physiological basis for this difference is not clear. While acute stressors can result in a decrease in plasma IGF-I levels in other freshwater fish species (Davis & Peterson, 2006), the transfer to seawater did not elicit a stress response and both groups of *O. tshawytscha* had similar plasma cortisol levels. In addition, previous studies on *S. salar* have found that plasma IGF-I levels do not change in response to a 24 h seawater challenge (Nieves-Puigdoller *et al*., 2007; Monette *et al*., 2008). Therefore, the results suggest that the observed differences in growth performance associated with either early rearing habitat or parental mate choice in the mixed growth trials cannot be attributed to differences in the circulating levels of GH or IGF-I. Although GH and IGF-I play important roles in the regulation of somatic growth in fishes, additional physiological processes such as food intake, digestion and assimilation also influence growth (Beckman, 2011; Johnston *et al*., 2011) and may have contributed to the differences in growth performance observed in this study.

The results suggest that early rearing of juvenile *O. tshawytscha* in a semi-natural environment leads to higher resting cortisol concentrations when evaluated under typical hatchery conditions. Similarly, wild *O. kisutch* smolts consistently have higher resting cortisol levels than hatchery-reared *O. kisutch* (Shrimpton *et al*., 1994*a*). These differences in resting cortisol levels may also translate into differential responses to stressors. Indeed, when channel *O. tshawytscha* from this experiment were allowed to form social hierarchies in small groups of fish, the magnitude of the increase in plasma cortisol was significantly higher than in fish from either of the hatchery-reared lines (Garner *et al*., 2011). Interestingly, the differences in basal cortisol levels between the channel and hatchery *O. tshawytscha* in either the separate or mixed growth trials were not maintained in the hatchery environment beyond day 30, *i.e*. at a time when cortisol levels increased in all groups. While a previous study with the YIAL stock during the natural spring smolt window reported peak plasma cortisol values of *c*. 35 ng ml$^{-1}$ on 31 May (Shrimpton *et al*., 2007*b*), sharp increases in plasma cortisol concentrations during the summer months have previously been reported in *O. kisutch* that were retained in fresh water (Young *et al*., 1989). Conceivably, as previously observed by Shrimpton *et al*. (1994*a*), differences in basal cortisol concentrations resulting from early rearing environment in this study were masked by the dynamic changes in cortisol levels that are associated with maintaining the hypo-osmoregulatory ability of smolts. Similarly, the variable but significant differences in basal cortisol levels that were observed

between the hatchery and transfer *O. tshawytscha* in the mixed growth trial were no longer observed post-seawater transfer.

Despite significant differences in $Na^+,K^+$-ATPase activity post-seawater transfer among the different groups of *O. tshawytscha* in the separate and mixed group growth trials, all *O. tshawytscha* retained a high degree of hypo-osmoregulatory adaptability and experienced virtually no ion perturbation as a result of the change in salinity. In fact, all groups retained $Na^+,K^+$-ATPase activity levels that were $\geq 4\,\mu$mol ADP mg protein$^{-1}$ h$^{-1}$, the peak value reported in the YIAL stock during the natural spring smolt window (Shrimpton *et al*., 2007*b*). Given the key role of GH and cortisol in stimulating gill $Na^+,K^+$-ATPase activity during the smoltification process (McCormick, 2009), it is suggested that the moderately elevated levels of these hormones in all groups prior to seawater transfer contributed to the high degree of seawater tolerance. Moreover, in spite of having lower $Na^+,K^+$-ATPase activity levels than the hatchery *O. tshawytscha* 24 h after the switch to seawater, the channel and transfer *O. tshawytscha* had higher *G* during the seawater phase of the mixed group growth trials. Therefore, independent of breeding strategy or early rearing environment, juvenile *O. tshawytscha* reared for several months in a hatchery environment prior to seawater transfer maintain equivalent seawater tolerance.

Parental mate choice and early rearing in a semi-natural channel for *c*. 6 months, or parental mate choice and transfer to the hatchery at the eyed-egg stage, do not have any sustained effects on the growth performance, hormonal profile or seawater tolerance of juvenile *O. tshawytscha* reared under traditional hatchery conditions. While the lower *K* and higher basal cortisol levels of channel *O. tshawytscha* at the beginning of the growth trials indicate that early rearing in a semi-natural channel does have an impact on their physiology, the differences were eliminated within a couple of months of hatchery rearing. Although the possibility that early rearing of *O. tshawytscha* in a semi-natural channel can lead to enhanced smolt characteristics as previously observed by Zydlewski *et al*. (2003) for steelhead *O. mykiss* cannot be excluded, the results show that any benefit accrued from early rearing in a semi-natural environment are not sustained under traditional hatchery conditions. As such, the findings have practical significance for both production hatcheries and facilities involved in stock enhancement.

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