



Behavioural Interactions and Hormones in Naturally and Hatchery-Spawned Chinook Salmon

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Received: April 20, 2010

Initial acceptance: June 1, 2010

Final acceptance: September 6, 2010

(L. Ebensperger)

doi: 10.1111/j.1439-0310.2010.01837.x

Abstract

Artificial breeding programmes commonly lead to domestication, which is associated with many behavioural differences that can reduce the success of animals released into natural environments. To better understand the factors contributing to domestication, we used a captive population of Chinook salmon (*Oncorhynchus tshawytscha*) to partition hormonal and behavioural differences to effects of the breeding method and rearing environment. We compared 9-mo-old juveniles from three lines that shared a common genetic background: (1) the Channel line produced by natural spawning and reared in a low-density environment with a natural substrate for approx. 6 mo before being transferred to the hatchery; (2) the Hatchery line produced by artificial spawning; and (3) the Transfer line produced by natural spawning but reared in the hatchery from the eyed-egg stage. Plasma concentrations of 11-ketotestosterone (11-KT) and cortisol were measured in groups of 150 fish and again after 4 d of social interactions in groups of six fish. There was no difference in 11-KT among lines in large groups, but in small groups, Transfer fish had lower 11-KT concentrations and were significantly less aggressive than both Channel and Hatchery fish. Regardless of group size, concentrations of the stress hormone cortisol were nearly twofold higher in Channel fish than in Hatchery and Transfer fish. Furthermore, the elevated cortisol concentrations in Channel fish were associated with 35% lower feeding rates than in the other two lines. Our study details complex behavioural and hormonal responses to breeding method and rearing environment in juvenile salmon.

Introduction

In animals that are artificially propagated, behaviour is among the first traits to show signs of domestication. For example, golden lion tamarins (*Leontopithecus rosalia*) that are born in captivity have reduced foraging efficiency, select different habitats and fall out of their tree habitat more often than tamarins that are born in the wild (Stoinski et al. 2003). In oldfield mice (*Peromyscus polionotus subgriseus*), populations raised in captivity for many generations show

a diminished response to predator cues and spend less time in refuges (McPhee 2004). In salmon, domestication has led to divergence in a number of key behavioural traits that may reduce the viability of artificially propagated individuals in natural environments (Huntingford 2004). Indeed, hatchery-raised salmon show reduced foraging efficiency on live prey, reduced sensitivity to predation risk and increased juvenile aggression relative to wild individuals (Fenderson et al. 1968; Sundström & Johnsson 2001; Álvarez & Nicieza 2003; Sundström et al.

2003; Fritts et al. 2007). Understanding and mitigating these behavioural differences between naturally and artificially bred animals is a key goal of supplementation programmes that aim to improve the survival of individuals following release into natural habitats (Brown & Laland 2001).

Behavioural changes associated with domestication may result from differences in the breeding method (e.g. natural mating compared to artificial propagation) and rearing environment. For example, hatchery programmes typically select breeding individuals either haphazardly from the pool of mature fish or select specific fish that have desirable characteristics for production (i.e. growth rate in captivity, age at maturation) and then give little attention to the specific pairings. The offspring are then reared at high densities and are provided with abundant food. In contrast, reproductive success in the wild varies considerably among individuals, with more competitive individuals (e.g. those with larger body size, higher androgen levels, brighter integument colouration) having a greater chance of spawning than their less competitive counterparts (Foote 1990; Fleming & Gross 1994; Quinn & Foote 1994; Petersson et al. 1999; Neff et al. 2008). Specific pairings are often non-random and involve mate choice, which can produce offspring that have higher growth rate and are more heterozygous or diverged at gene loci such as the major histocompatibility complex than the offspring of non-preferred individuals (Landry et al. 2001; Petersson & Järvi 2007; Neff et al. 2008). Juveniles in the wild also develop at low densities and may compete intensely for feeding territories (Keenleyside & Yamamoto 1962). Thus, there are considerable differences in both the breeding method and rearing environment between artificial and natural breeding.

Breeding method can affect behaviour by either altering the genetic constitution of the offspring (e.g. heterozygosity, genes underlying body size and competitiveness) or altering maternal environmental effects. For example, in Atlantic salmon (*Salmo salar*), high levels of microsatellite heterozygosity were associated with high levels of juvenile aggression (Tira et al. 2003). The maternal environment can affect hormone provisioning to eggs, and in tropical damselfish (*Pomacentrus amboinensis*), for example, egg androgen levels affect growth and development (McCormick 1999). However, only a couple of studies have examined the effects of breeding method on behaviour (Fenderson et al. 1968; Sundström et al. 2003). These studies compared naturally and hatchery bred salmon from the same stock to exclude pre-

existing genetic divergence among stocks and found higher aggression in hatchery than in wild origin fish. However, these studies did not separate the effects of breeding method from those of the rearing environment.

The effects of rearing environment on behaviour have been demonstrated in many studies. For instance, in a pair of studies, hatchery bred salmon were reared in either a natural stream or hatchery tank, and when observed together, the hatchery-reared fish were more dominant and aggressive than the naturally reared fish (Rhodes & Quinn 1998; Metcalfe et al. 2003). These differences in behaviour may result from effects of early rearing environment on brain development, as it was observed that steelhead trout (*Oncorhynchus mykiss*) that hatched in tanks with a natural substrate had significantly larger cerebella and were less active than fish that hatched in tanks without a natural substrate (Kihlslinger & Nevitt 2006). Differences in behaviour may also occur because fish reared in the hatchery are commonly exposed to high levels of disturbance, which can lead to an attenuated stress response (Woodward & Strange 1987; Salonius & Iwama 1993; but see Shrimpton et al. 1994). Conversely, fish reared in the wild develop a normal stress response and subsequently display elevated cortisol concentrations when disturbed. Elevated cortisol concentrations can lead to reduced aggression and activity (Øverli et al. 2002), and thus an altered stress response may contribute to the behavioural differences between hatchery and wild fish. Regardless of the mechanism, behaviour frequently differs between fish reared in artificial and natural environments.

Our study represents one of the first attempts to separate the effects of breeding method and rearing environment on juvenile behaviour. We focussed on aggression and feeding in part because specific hormones have been associated with the expression of these behaviours. In fishes, 11-ketotestosterone (11-KT) is a prominent androgen (Borg 1994) and has been linked to aggression and social dominance in mature individuals of both sexes, although the role of 11-KT in shaping juvenile behaviour remains largely unexplored (e.g. Oliveira et al. 1996; Elofsson et al. 2000; Desjardins et al. 2006; Parikh et al. 2006). Cortisol mediates the hormonal response to stress, and elevated concentrations have been linked to impaired feeding through reduced appetite (Gregory & Wood 1999) and to other negative effects that include decreased feed conversion efficiency and compromised immune function

(reviewed by Mommsen et al. 1999; Bernier et al. 2004; Gilmour et al. 2005). Cortisol may also affect social behaviour, as salmonids with high plasma cortisol concentrations have been shown to have an increased likelihood of being subordinate in subsequent social interactions (Sloman et al. 2001; Di-Battista et al. 2005). Thus, measuring 11-KT and cortisol concentrations allows the hormonal differences that may mediate differences in aggression and feeding behaviour to be explored.

Our study employed two breeding methods by allowing some fish to spawn naturally in a freshwater channel (Channel fish) and spawning other fish artificially using standard hatchery techniques (Hatchery fish). A third line consisted of Channel fish that were transferred to the hatchery environment as fertilized eggs (Transfer fish). These latter individuals allowed the effects of breeding method to be separated from the effects of rearing environment. Limited access to freshwater channels precluded the inclusion of hatchery-spawned fish reared in channels, but the three lines enabled effects of the spawning protocol and rearing environment to be separated. Specifically, our study compared aggression, feeding and plasma concentrations of 11-KT and cortisol in the three lines both in large assemblages (hormone levels only) and in small groups after social hierarchy formation. The large assemblages are typical of hatchery environments in which aggressive territorial behaviours and stable social hierarchies are inhibited (Brown et al. 1992), and measuring basal hormone concentrations in these groups allowed us to explore the hormonal consequences of the subsequent social interactions in small groups. Additionally, we used observations of mixed groups of Channel and Hatchery or Transfer and Hatchery fish to examine the relative performance of these lines of fish. These tests allowed us to explore the factors contributing to behavioural domestication and evaluate the merits of incorporating elements of natural spawning in hatchery supplementation programmes and commercial aquaculture.

Methods

Brood Stock

This study conformed to animal care guidelines outlined by the Canadian Council on Animal Care. Experiments were conducted using Chinook salmon at Yellow Island Aquaculture Ltd. (YIAL) (Quadra Island, BC, Canada). The YIAL population was

founded with gametes from the Robertson Creek hatchery on Vancouver Island, which has been maintained since 1986. Previous studies of this population have observed normal breeding behaviour (i.e. female nest construction and defence, male courtship and mate guarding) and found that, as in wild salmon, mating patterns were affected by both competition and mate choice (Neff et al. 2008; Garner et al. 2010). The use of a captive population may provide a somewhat conservative estimate of the consequences of natural spawning, but all of the major aspects of sexual selection that occur in wild salmon were expressed in our population, so examining these fish would allow us to detect any biologically significant differences associated with the breeding protocol. YIAL has eliminated the male sex chromosome through the use of hormonal sex-reversal in female XX salmon, and as a result, all juvenile salmon examined in this study were female. An all-female population was selected to reduce the variance in hormones and behaviour within lines caused by differences between the sexes. This design would not identify differences between lines if they were present only in males, but otherwise offers a sensitive test of the factors affecting juvenile phenotype and is especially relevant for aquaculture, in which homogametic salmon are commonly raised to prevent early maturation by males (Piferrer 2001; Fitzpatrick et al. 2005). To create phenotypic males for breeding, a subset of newly hatched larvae were immersed in re-circulating, oxygenated water with the androgen 17 α -methyltestosterone (400 $\mu\text{g}/\text{l}$) for 2 h at 520 accumulated thermal units (ATUs) and for another 2 h at 620 ATUs (Heath et al. 2002). One study found that homogametic (XX) males were more likely to mature at 2 yr of age and had an approx. 10% lower gonadosomatic index (GSI) than heterogametic (XY) males (Heath et al. 2002). However, XX and XY males grew to a similar body size and had similar circulating concentrations of testosterone and 17 β -estradiol (Heath et al. 2002). Moreover, XX males displayed the same range and frequency of spawning behaviours as XY males and had similar courtship success when in direct competition with XY males in the spawning channels used in the current study (Garner et al. 2010).

Breeding Design

For this study, three lines of fish were created: (1) the Channel line combined natural spawning and semi-natural rearing; (2) the Transfer line combined natural spawning and hatchery rearing; and (3) the

Hatchery line combined hatchery spawning and hatchery rearing. The Channel line used a freshwater channel to facilitate natural spawning by mature fish. On 7–14 Oct. 2005, 18 mature males and 12 mature females were collected from the saltwater pens and transferred into the channel. The channel was 15 × 3.5 m in size, with a water depth of approx. 1 m, and a partially recirculating flow of approx. 300 l/min. The gravel composition was similar to wild streams in the area and measured 3–6 cm in diameter. The channel was located outside and was thus exposed to natural patterns of light and temperature for Quadra Island, BC, but was surrounded on all sides by netting to exclude predators. Fish were allowed to spawn without interference and were removed from the channel as they died. Behavioural observations documented intra- and intersexual aggression, while genetic analysis showed that 17 of 18 males and all 12 females mated successfully (Garner et al. 2010). Peak spawning occurred from 25 Oct. to 1 Nov. 2005. Pellet food was provided *ad libitum* starting in Feb. once the fry emerged from the gravel. On 26 Apr. 2006, when the fry reached 1–2 g in size, approx. 1400 were collected by seine net and transferred to the hatchery. Thus, the progeny had been feeding exogenously in the channel for approx. 2 mo and in total had been in the channel for approx. 6 mo at temperatures that ranged between approx. 4 and 10°C and at a density (ρ : mass of fish per volume of water) below 0.05 g/l.

The Transfer line used eyed-eggs collected from the channel and incubated in the hatchery. Eggs were collected with pneumatic sampling from 12 to 16 Dec. 2005. Briefly, a metal probe was used to direct compressed air into the channel gravel, and the eggs were netted as they were forced upwards and into the water column. 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 with a constant water flow of approx. 15 l/min. During incubation, dead eggs were regularly removed to prevent *Saprolegnia* spp. growth. On 18 Feb. 2006, the fry were transferred to the hatchery.

The Hatchery line used standard aquaculture spawning techniques (Sedgwick 1982) and incubation. Mature fish were collected from the saltwater pens and transferred to freshwater tanks to complete maturation. Families were then produced between 31 Oct. and 3 Nov. 2005 to coincide with peak spawning in the channel. Mature females were euthanized, and eggs were harvested by cut-

ting open the abdomen. Milt was collected from mature males by applying pressure to the abdomen. A female's brood was then divided in half, with each half fertilized by the milt of a different male. Each pair of males was used to fertilize the eggs of two females. Overall, 10 males and 10 females were used to produce 20 families. Fertilized eggs were incubated by family in Heath trays as above-mentioned. On 18 Feb. 2006, 70 fry from each family (1400 total) were pooled and transferred to the hatchery.

All fish in the hatchery were held in 3000-l tanks, kept on a 16:8 light:dark cycle at temperatures between approx. 6 and 10°C and fed pellet food *ad libitum* (Micro Crumble Starter Feed; EWOS, Surrey, BC, Canada). We found no difference between duplicate tanks in subsequent measures of behaviour and hormone concentrations (all $p > 0.41$), and thus did not include tank in our analyses (for details see Garner et al. 2008). On 13 Jun. 2006, 150 fish from each line were transferred to two replicate 750-l tanks in the hatchery. These fish were maintained at a density ($\rho = 2$ g/l) that was similar to the density of the subsequent behavioural trials ($\rho = 3$ g/l). A random subset of these fish were sampled 1 mo later at body sizes similar to the fish in the behavioural trials (mean \pm SE; Channel: mass = 9.4 \pm 0.4 g, length = 9.5 \pm 0.1 cm; Hatchery: mass = 10.2 \pm 0.4 g, length = 9.6 \pm 0.1 cm; Transfer: mass = 11.4 \pm 0.5 g, length = 9.9 \pm 0.2 cm) and were used to measure basal hormone concentrations.

Behavioural Trials

Behavioural trials were conducted between 17 Jul. and 17 Aug. 2006, with trials from each composition staggered to start throughout the sampling period. All fish were approx. 9 mo old during these trials, and Channel fish had been feeding in the channel for approx. 2 mo and then in the hatchery for 3 mo, whereas Hatchery and Transfer fish had been feeding in the hatchery tanks for approx. 5 mo. The trials were conducted in 24 l tanks with six fish per tank, which is within the range of group sizes commonly used to observe social interactions in juvenile salmonids (e.g. Fenderson & Carpenter 1971; Brown & Brown 1993; Garner et al. 2008). The trial tanks were 30 × 40 cm in size, with a water depth of 20 cm and freshwater inflow of approx. 1 l/min. For each tank, the composition of fish was Channel ($n = 27$ tanks), Hatchery ($n = 25$ tanks), Transfer ($n = 23$ tanks), Mixed Channel (half Channel and half Hatchery; $n = 25$ tanks) or Mixed Transfer (half

Transfer and half Hatchery; $n = 22$ tanks). Behaviour was not assessed in the groups of 150 fish because social interactions are predicted to be less important in groups of this size (Brown et al. 1992), and there are considerable technical challenges associated with measuring individual behaviour in large shoals of fish.

To begin a trial, the fish were anaesthetized with buffered MS-222 (Sigma-Aldrich, St. Louis, MO, USA) and their mass (± 0.05 g) and body length (fork length, ± 0.5 mm) were measured. To visually identify individuals within a trial, fish were tagged with a uniquely coloured 6-mm disc tag inserted below the dorsal fin. The fish were then placed into the test tank and fed at a rate of 1% of body mass, twice daily for 4 d. Four days should allow social hierarchies to develop (e.g. Fenderson et al. 1968; Garner et al. 2008). On the fourth day of the trial, a camcorder (Sony DCR-TRV140, DCR-TRV250, Tokyo, Japan or Panasonic PV-GS180, Osaka, Japan) was positioned above the tank. After allowing the fish 2 h to acclimate to the camcorder, the fish were recorded for a total of 30 min, with an average of 12.5 (range 11.2–14.0) min recorded before feeding, followed by the addition of food equal to 1% of body mass and an additional recording of 17.5 (range 16.0–18.8) min. Immediately following the recording, all individuals were rapidly netted and euthanized as a group with an overdose of buffered MS-222. Blood was sampled by severing the caudal fin and collecting blood in a haematocrit tube. The blood was centrifuged to isolate the plasma, which was frozen at -20°C for hormone analyses.

After all trials were completed, an observer who was blind to trial composition analysed the video recordings. Each trial was analysed for two observation periods: before feeding and during feeding. The frequency of aggressive acts by each fish was calculated as the sum of charges (a rapid and direct movement towards another fish) and nips (a biting motion directed towards another fish) divided by the duration of the observation period (Taylor & Larkin 1986). These aggression observations were also used to calculate dominance (total aggressive acts performed by a fish – the number of aggressive acts directed at that fish) and to categorize the social status of each fish within a tank. Individuals that had the highest dominance rating within a group of six fish were categorized as dominant, the remaining fish that had positive dominance rating were classified as sub-dominant, and all fish with dominance ratings less than or equal to zero were classified as subordinate. Food pellets were clearly visible in the

video recordings, which allowed us to measure the feeding rate for each fish as the number of food pellets obtained divided by the duration of the observation period. A small proportion of food pellets were expelled before they were swallowed and were then typically ingested again by the same fish. Ingested pellets were included in the feeding rate regardless of whether they were swallowed, but subsequent ingestion of the same pellet by a fish was not counted.

Hormone Concentrations

Basal hormone concentrations were first measured prior to the behavioural trials on 13 Jul. 2006 using 5–11 fish from each trial composition. Hormone concentrations were determined in fish from six behavioural trials for each of the Channel, Hatchery and Transfer trials; hormone concentrations in the Mixed Channel or Mixed Transfer trials were not analysed. Cortisol and 11-KT concentrations were measured in different trials because individual fish contained insufficient plasma to run both assays. Plasma cortisol was measured in unextracted plasma using the radioimmunoassay of Bernier et al. (2008). Using a pooled plasma sample stock, the intra- and interassay variations for the cortisol assay were 3.2% ($n = 6$) and 5.3% ($n = 6$), respectively. Plasma 11-KT was analysed using the extraction and radioimmunoassay protocols of McMaster et al. (1995). The intra- and interassay variations for the 11-KT assay were 3.5% ($n = 4$) and 7.0% ($n = 6$), respectively. Both hormones were assayed in triplicate, and the radioimmunoassays were validated with serial dilutions of Chinook salmon plasma that ran parallel to the standard curves.

Statistical Analysis

The analysis first focussed on the pure groups from each breeding method and used a repeated measure ANOVA to examine the aggression data with observation period (before or during feeding) as the repeated measure, line (Channel, Hatchery and Transfer) as a fixed factor, tank number as a nested factor within line to address possible differences in behaviour among groups of six fish, and the body mass of each fish as a covariate. Differences in aggression among lines were further analysed using a *post hoc* least significant difference test. The feeding data were similarly analysed using an ANOVA based on line, tank number and mass. Hormone concentrations were analysed using ANOVAs with line and

group size (150 fish and six fish) as fixed factors. The 11-KT analysis revealed a near significant interaction between line and group size, so the pattern in the 11-KT data from the behavioural trials (six fish) was further analysed using an ANOVA that included line, tank number and mass. ANCOVAs were used to examine the relationships between the frequency of aggression, feeding rate, plasma cortisol concentrations and 11-KT concentrations, while controlling for differences in line, tank number and mass. A chi-square test was used to compare the number of fish with each social status in our three lines. ANOVAs that included line, tank number and mass were then used to examine the effect of social status on feeding rate, cortisol concentrations and 11-KT concentrations.

We next examined behaviour in the mixed line trials, which were analysed separately from the pure groups to simplify the interpretation of these data and avoid the need for a statistical model with multiple hierarchically nested factors. The aggression data were analysed independently for the Mixed Channel and Mixed Transfer trials using repeated measure ANOVAs that again included observation period, line, tank number and mass. The feeding data were similarly analysed using ANOVAs. We used chi-square tests to compare the social status of Channel and Hatchery or Transfer and Hatchery fish in mixed trials.

Prior to statistical analysis, aggression and feeding data were logarithm transformed to achieve a normal distribution using either $\log_{10}(\text{frequency of aggressive acts} + 0.045)$ or $\log_{10}(\text{feeding rate} + 0.4)$ (Berry 1987). Constants were added to remove 0 values and chosen to minimize skew and kurtosis. Hormone concentrations were similarly transformed using $\log_{10}(\text{hormone concentration} + 0.1)$ to achieve a normal distribution. Statistical analyses were performed using SPSS (v 14.0, SPSS Inc, Chicago, IL, USA) or JMP (v4.0.4, SAS Institute Inc, Cary, NC, USA). All data are reported as $\bar{x} \pm 1$ standard error of the mean.

Results

In the pure trials, there was no significant difference in mass or body length among Channel, Hatchery and Transfer fish (ANOVAs: $p > 0.18$ for all; Table 1). Examining hormone concentrations, 11-KT concentrations were significantly higher in groups of six fish than in groups of 150 fish, which was driven by higher 11-KT in Channel and Hatchery fish after the behavioural trials (ANOVA: $F_{1,125} = 15.09$, $p < 0.001$;

Table 1: Summary of body size measurements for the Chinook salmon (*Oncorhynchus tshawytscha*) used in the behavioural trials

| Trial composition | n | Mass (g) | Body length (cm) |
|---------------------------|-----|------------|------------------|
| Channel | 162 | 11.8 ± 0.3 | 10.1 ± 0.1 |
| Hatchery | 150 | 11.8 ± 0.4 | 10.0 ± 0.1 |
| Transfer | 138 | 12.8 ± 0.6 | 10.0 ± 0.2 |
| Mixed Channel – Channel | 75 | 9.4 ± 0.3 | 9.5 ± 0.1 |
| Mixed Channel – Hatchery | 75 | 9.5 ± 0.3 | 9.5 ± 0.1 |
| Mixed Transfer – Transfer | 66 | 14.0 ± 0.8 | 10.5 ± 0.2 |
| Mixed Transfer – Hatchery | 66 | 13.7 ± 0.7 | 10.4 ± 0.2 |

Data are broken down by trial composition and comprise sample size (n), average mass (±1 SE) and average body length (±1 SE).

Fig. 1a). No significant differences in 11-KT concentrations were observed among lines (ANOVA: $F_{2,125} = 0.82$, $p = 0.44$), and there was a non-significant interaction between group size and line (ANOVA: $F_{2,125} = 2.67$, $p = 0.073$). An expanded analysis of 11-KT concentrations following the behavioural trials revealed that Channel fish had intermediate 11-KT concentrations that were not significantly different from either Hatchery or Transfer fish, but that 11-KT concentrations were significantly lower in Transfer fish than in Hatchery fish (ANOVA: $F_{2,85} = 5.72$, $p = 0.005$; Fig. 1a). After the behavioural trials, concentrations of 11-KT did not differ among tanks (ANOVA: $F_{15,85} = 1.08$, $p = 0.39$), but there was a significant relationship between mass and 11-KT concentrations, with higher 11-KT concentrations in smaller fish (ANOVA: $F_{1,85} = 8.33$, $p = 0.005$). Cortisol concentrations were significantly higher in Channel fish than in either Hatchery or Transfer fish (ANOVA: $F_{2,107} = 4.28$, $p = 0.016$; Fig. 1b). Cortisol concentrations were also significantly higher in groups of six fish than in groups of 150 fish (ANOVA: $F_{1,107} = 86.8$, $p < 0.001$), although no interaction between group size and line was observed for cortisol concentrations (ANOVA: $F_{2,107} = 0.05$, $p = 0.95$).

Examining behaviour in the pure trials, the repeated measure ANOVA identified one term with a significant effect on the frequency of aggressive acts (Table 2). There was a significant difference among lines, with aggressive acts significantly less frequent in Transfer fish than in Channel or Hatchery fish (Fig. 2a). All other factors included in the model were not significant. Among the three lines, there was no significant difference in the number of fish achieving each social status (chi-square test: $\chi^2 = 1.23$, $df = 4$, $p = 0.87$; Table 3). Examining feeding rates, Channel fish fed significantly less than Hatchery fish or Transfer fish (ANOVA: $F_{2,374} = 7.03$,

Fig. 1: Hormone concentrations in Channel (■), Hatchery (□) and Transfer (▨) groups of Chinook salmon (*Oncorhynchus tshawytscha*). \bar{x} (\pm SE) are presented for (a) plasma 11-ketotestosterone and (b) plasma cortisol. Hormone concentrations were first determined in large groups ($n = 150$) before starting behavioural trials and again after groups of six fish were allowed to form social hierarchies. Different letters denote significant differences between groups. Note that the cortisol axis contains a break to better display the data.

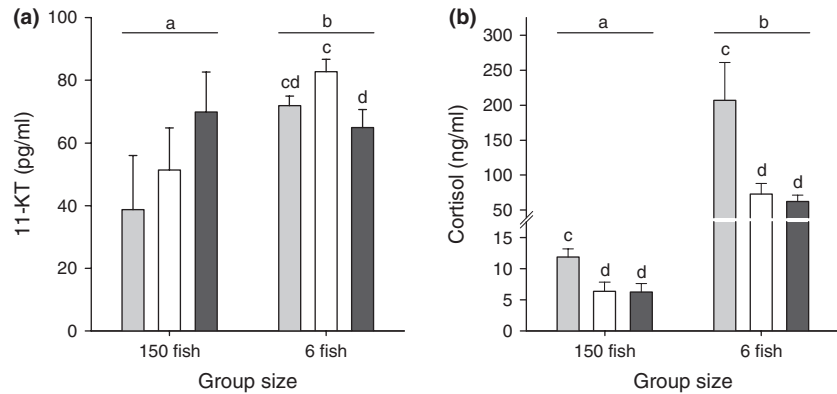


Table 2: Summary of repeated measure ANOVA results for the frequency of aggressive acts in behavioural trials of Chinook salmon (*Oncorhynchus tshawytscha*)

| Trial composition | ANOVA term | df | F | p |
|-------------------|---------------------------------------|--------|-------|-------|
| Pure | Obs. period (repeated measure) | 1,374 | 0.24 | 0.63 |
| | Line | 2,374 | 3.60 | 0.028 |
| | Obs. period \times Line | 2,374 | 0.12 | 0.88 |
| | Tank [Line] | 72,374 | 1.07 | 0.34 |
| | Obs. period \times Tank [Line] | 72,374 | 0.95 | 0.60 |
| | Mass (covariate) | 1,374 | 0.73 | 0.39 |
| Mixed Channel | Obs. period (repeated measure) | 1,99 | 1.52 | 0.22 |
| | Line | 1,99 | 1.22 | 0.27 |
| | Obs. period \times Line | 1,99 | 0.01 | 0.93 |
| | Tank [Line] | 48,99 | 1.20 | 0.22 |
| | Obs. period \times Tank [Line] | 48,99 | 1.46 | 0.058 |
| | Mass (covariate) | 1,99 | 2.72 | 0.10 |
| Mixed Transfer | Obs. period (repeated measure) | 1,84 | 0.17 | 0.68 |
| | Line | 1,84 | 0.55 | 0.46 |
| | Obs. period \times Line | 1,84 | 0.90 | 0.34 |
| | Tank [Line] | 42,84 | 0.77 | 0.82 |
| | Obs. period \times Tank [Line] | 42,84 | 1.50 | 0.057 |
| | Mass (covariate) | 1,84 | 0.53 | 0.47 |
| | Obs. period \times Mass (covariate) | 1,84 | 0.004 | 0.95 |

Data comprise trial composition, ANOVA term, degrees of freedom (df), *F* statistic and *p* value. Obs. period, observation period; square brackets denote nested factors.

Fig. 2: Behaviour in individuals from the Channel, Hatchery and Transfer groups of Chinook salmon (*Oncorhynchus tshawytscha*). \bar{x} (\pm SE) are presented for (a) the frequency of aggression before feeding (□) and during feeding (▨) and (b) feeding rate. Different letters denote significant differences between groups.

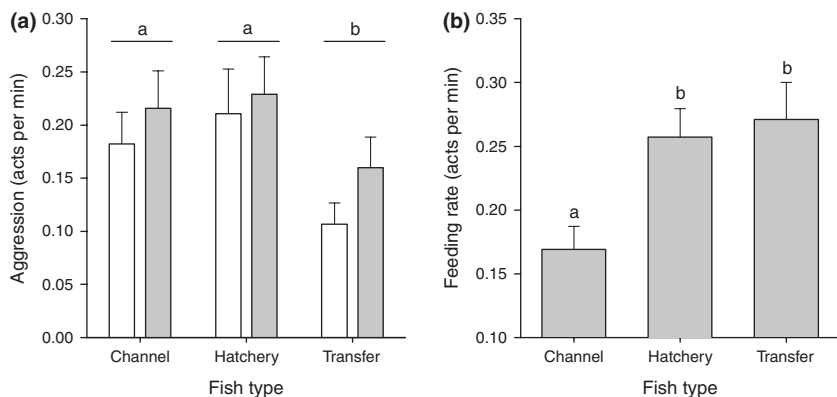


Table 3: Summary of social status for the Chinook salmon (*Oncorhynchus tshawytscha*) used in the behavioural trials

| Trial composition | Dominant | Sub-dominant | Subordinate |
|-------------------|----------|--------------|-------------|
| Channel | 19.8 | 16.7 | 63.6 |
| Hatchery | 17.3 | 14.0 | 68.7 |
| Transfer | 17.4 | 17.4 | 65.2 |
| Mixed Channel | | | |
| Channel | 17.3 | 22.7 | 60.0 |
| Hatchery | 17.3 | 20.0 | 62.7 |
| Mixed Transfer | | | |
| Transfer | 18.5 | 9.2 | 70.8 |
| Hatchery | 18.5 | 10.7 | 72.3 |

Dominance (total aggressive acts performed by a fish minus the number of aggressive acts directed at that fish) was used to categorize social status as dominant (highest dominance within its tank), sub-dominant (other fish with dominance >0) or subordinate (dominance ≤0). Data are broken down by trial composition and show the percentage of fish with each social status.

Each Mixed Channel trial consisted of three Channel and three Hatchery fish, whereas Mixed Transfer trials used three Transfer and three Hatchery fish.

$p = 0.001$; Fig. 2b). Feeding rate showed significant variation among tanks (ANOVA: $F_{72,374} = 1.34$, $p = 0.046$), but was not related to body mass (ANOVA: $F_{1,374} = 0.81$, $p = 0.37$). Feeding rate was significantly related to the frequency of aggressive acts after food was added, with higher feeding in more aggressive fish (ANCOVA: $F_{1,373} = 62.5$, $p < 0.001$). Similarly, feeding rate was also significantly related to social status, with the highest feeding rate in dominant fish (0.37 ± 0.03 acts/min), followed by sub-dominant fish (0.28 ± 0.03 acts/min) and then subordinate fish (0.18 ± 0.02 acts/min; ANOVA: $F_{2,372} = 22.5$, $p < 0.001$).

The differences in aggressive frequencies paralleled the differences in 11-KT concentrations following the behavioural trials, with the lowest values in Transfer fish, the highest values in Hatchery fish and intermediate values in Channel fish. However, no significant relationship between 11-KT concentrations and aggression was detected at the level of individual fish (repeated measure ANCOVA: $F_{1,84} = 0.54$, $p = 0.47$). Concentrations of 11-KT did not differ based on social status, as no significant difference was observed among dominant fish (72.1 ± 6.5 pg/ml), sub-dominant fish (76.3 ± 6.4 pg/ml) and subordinate fish (72.5 ± 3.3 pg/ml; ANOVA: $F_{2,83} = 0.25$, $p = 0.78$). The feeding rates mirrored the plasma cortisol concentrations, with higher cortisol and lower feeding in the Channel group, and lower cortisol and higher feeding in the Hatchery and Transfer groups. This relationship was

also significant at the level of individual fish, with lower feeding rates in fish with higher cortisol concentrations (ANCOVA: $F_{1,76} = 15.6$, $p < 0.001$). Cortisol concentrations were not significantly correlated with the frequency of aggressive acts within individual fish (repeated measure ANCOVA: $F_{1,76} = 3.58$, $p = 0.062$). Cortisol concentrations also did not differ based on social status, as no significant difference was observed among dominant fish (103 ± 34 ng/ml), sub-dominant fish (114 ± 32 ng/ml) and subordinate fish (117 ± 29 ng/ml; ANOVA: $F_{2,75} = 0.57$, $p = 0.57$).

In the mixed trials, there was no significant difference in mass or body length between Hatchery fish and Channel or Transfer fish (ANOVAs: $p > 0.80$ for all; Table 1). The repeated measure ANOVAs identified no terms with a significant effect on the frequency of aggressive acts in Mixed Channel or Mixed Transfer trials (Table 2, Fig. 3a). Although not significant in mixed trials, the Hatchery fish were again 50% more aggressive than Transfer fish, with fewer than half as many of these fish examined in Mixed Transfer trials ($n = 132$) when compared to pure trials ($n = 288$). In both Mixed Channel and Mixed Transfer trials, there was no difference in social status between the fish from different lines (chi-square tests: $p > 0.99$ for both; Table 3). Feeding rate in mixed trials did not differ between Channel and Hatchery fish (ANOVA: $F_{1,99} = 0.65$, $p = 0.42$; Fig. 3b) or between Transfer and Hatchery fish (ANOVA: $F_{1,84} = 0.57$, $p = 0.45$; Fig. 3b). There was a significant difference in feeding rate among tanks in Mixed Channel trials (ANOVA: $F_{48,99} = 1.55$, $p = 0.034$), but not in Mixed Transfer trials (ANOVA: $F_{42,84} = 0.76$, $p = 0.84$). There was a significant negative relationship between mass and feeding rate in Mixed Channel trials (ANOVA: $F_{1,99} = 7.92$, $p = 0.006$), and a similar relationship in Mixed Transfer trials, although it was not significant (ANOVA: $F_{1,84} = 2.98$, $p = 0.088$).

Discussion

Our study provided mixed evidence for an effect of breeding method on patterns of juvenile behaviour. We found that the frequency of aggressive acts was higher in Hatchery fish than in Transfer fish, which is consistent with an effect of the breeding method because both lines were reared in the same environment from an early egg stage. However, the frequency of aggressive acts did not differ between Hatchery and Channel fish, which suggests that the effects of the breeding method on aggressive

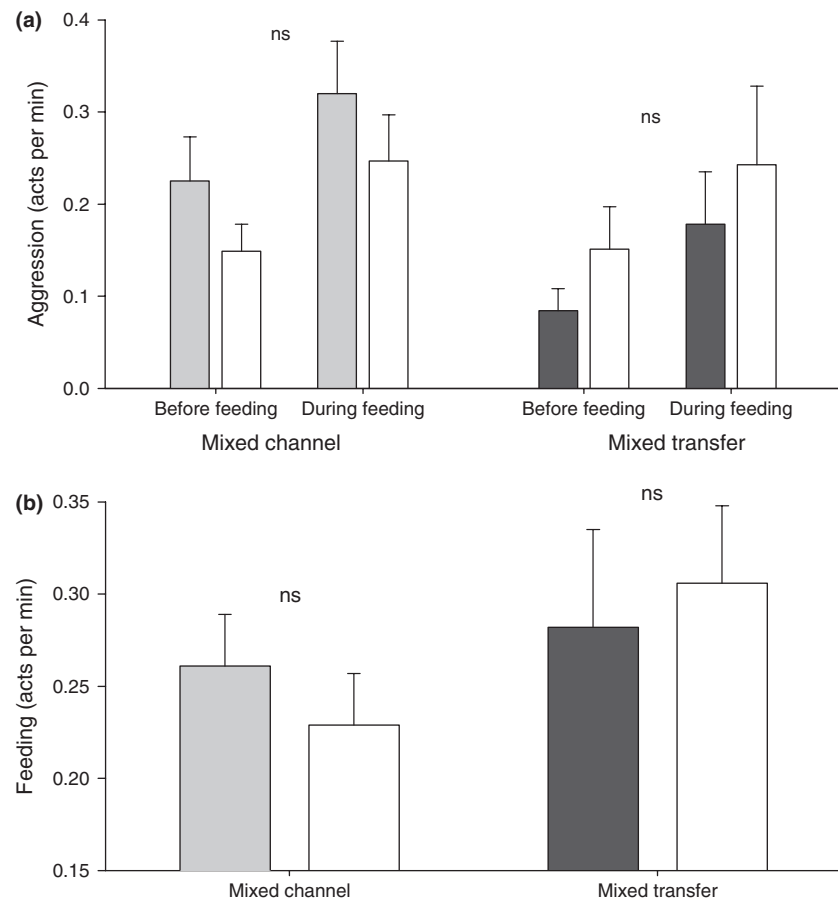


Fig. 3: Behaviour in individuals from mixed groups of Chinook salmon (*Oncorhynchus tshawytscha*). Mixed groups contained Hatchery (□) fish as well as either Channel (▒) or Transfer (■) fish. \bar{x} (\pm SE) are presented for (a) the frequency of aggression before and during feeding and (b) feeding rate. No significant differences were observed between groups.

behaviour were most apparent when individuals were reared in a common environment. Furthermore, fish from all three lines achieved similar social status when forming dominance hierarchies in mixed groups, which suggests that the breeding method was not a major determinant of the outcome of aggressive interactions. Previous studies of salmonids have likewise failed to identify an independent effect of the breeding method on patterns of aggression (Fenderson et al. 1968; Sundström et al. 2003), so the breeding method is unlikely to be a major cause of the differences in aggression that are often associated with hatchery programmes (Huntingford 2004). Instead, adaptation to hatchery conditions over multiple generations and environmental differences between natural streams and hatchery tanks are likely the primary contributors to high aggression in hatchery bred salmonids (Swain & Riddell 1990; Einum & Fleming 1997; Rhodes & Quinn 1998; Metcalfe et al. 2003; Wessel et al. 2006).

The cortisol-mediated stress response appears to be consistently lower in hatchery-reared salmon than in their naturally reared counterparts. Although,

hatchery and wild salmon often have similar resting cortisol concentrations, wild salmon typically respond to external stressors with significantly greater increases in cortisol concentrations (Woodward & Strange 1987; Salonius & Iwama 1993; but see Shrimpton et al. 1994). Like previous studies, we found that resting cortisol concentrations were low in all three lines, albeit resting cortisol concentrations were higher in the Channel fish than in Hatchery and Transfer fish. Regardless of individual social status, fish from all lines had significantly higher cortisol concentrations after forming social hierarchies. However, the magnitude of the increase in cortisol concentrations was considerably higher in Channel fish than in fish from either of the hatchery-reared lines. Our results are thus consistent with an attenuated response to stressors in hatchery-reared salmon, as was similarly shown in a study of Coho salmon (*Oncorhynchus kisutch*) that partitioned differences in the stress response to environmental rather than genetic effects of hatchery spawning (Salonius & Iwama 1993). The attenuated sensitivity to stress in hatchery-raised salmon likely arises from

greater familiarity and habituation to the high densities and disturbance associated with hatchery environments. Interestingly, the Channel fish in our study were held in a common hatchery environment for approx. 3 mo before trials began and similarly the differences in the stress response between wild and hatchery Coho salmon persisted through 7 mo in a common hatchery environment (Salonius & Iwama 1993). These data collectively suggest that sensitivity to stressors in salmon is laid down early in life and may become fixed thereafter. Thus, a low-density, low-disturbance environment may help to maintain a wild-type stress response in fish, and this may be especially important when the fish are intended for release into streams to enhance wild populations. Conversely, the attenuated stress response in hatchery fish may be beneficial for fish intended to stay in the high-disturbance aquaculture setting and suggests that aquaculture fish benefit from early exposure to the hatchery environment.

Differences in the stress response may also have consequences for foraging behaviour and growth. Increased stress, as measured by consistently elevated cortisol concentrations, has been associated with reduced growth rate through reduced appetite and lower efficiency in converting food into body mass (Gregory & Wood 1999; De Boeck et al. 2001; Bernier et al. 2004; Gilmour et al. 2005). In our study, a negative relationship between cortisol concentration and feeding rate was observed both within fish and among breeding methods, with higher cortisol concentrations and reduced feeding in the Channel fish relative to the other two groups. These relationships were not driven by differences in social status because dominant and subordinate fish did not differ in cortisol concentrations and Channel fish as a group were equally dominant to the other two lines. Our results are thus consistent with a role of cortisol in mediating feeding behaviour and also implicate differences in cortisol levels as a factor contributing to the differences in feeding behaviour among lines.

There is mounting evidence that the social environment can affect feeding behaviour in salmon. For example, a study of Coho salmon found that individual fish fed most when they observed groups of conspecifics feeding, fed less when they observed non-feeding groups and fed least when they observed alarmed groups (Ryer & Olla 1991; for a study on Atlantic salmon, see Brown & Laland 2002). In our study, groups of Channel fish fed significantly less than groups of Hatchery fish, but no difference in feeding rate was observed when Channel fish were

housed in mixed groups with Hatchery fish. Channel fish may have displayed relatively higher feeding in mixed groups because they observed high feeding rates in the Hatchery fish, and this social stimulation encouraged the Channel fish to also feed at a higher rate. The social stimulation may occur because observing conspecifics feeding alerts other fish to the presence of a food source, or it may suggest that the environment is safe and that vigilance can be reduced. Interestingly, previous research in brown trout has shown that wild fish respond to the presence of another fish by increasing their feeding rate, whereas feeding by hatchery-raised fish is not altered in the presence of another fish (Sundström & Johnsson 2001). This difference in sensitivity between wild and hatchery-raised fish (and our Channel and Hatchery fish) may occur because the predictability of the hatchery environment lowers the value of responding to social cues. Thus, although social cues are an important factor affecting foraging behaviour in the wild, individual sensitivity to these cues may depend on the environment experienced early in life.

In conclusion, our data implicate the breeding method, rearing environment and the interaction between the two as factors affecting juvenile hormones and behaviour in salmon. Additionally, our results have broader implications for supportive breeding and aquaculture. The greater sensitivity to social stress and lower feeding rate in Channel fish may not be beneficial in an aquaculture environment, but the attenuated social stress response and higher feeding rate on food pellets in hatchery fish are unlikely to benefit fish that are released into natural environments. Supportive breeding programmes are thus the most likely to benefit from breeding methods that incorporate elements of natural spawning to minimize behavioural divergence from wild stocks.

Acknowledgements

We thank Yellow Island Aquaculture Ltd (YIAL) for generous access to fish and infrastructure. We also thank Glen Van Der Kraak for his generous donation of the 11-ketotestosterone (11-KT) antiserum and standard and Andrea Lister for technical advice with the 11-KT radioimmunoassay. Three anonymous reviewers and editor Luis Ebensperger provided valuable comments on this manuscript. This research was supported by a Natural Science and Engineering Research Council (NSERC) of Canada strategic grant (BDN, NJB), an Ontario Graduate Scholarship

(BNM) and an NSERC postgraduate scholarship (SRG).

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