

Juvenile growth and aggression in diploid and triploid Chinook salmon *Oncorhynchus tshawytscha* (Walbaum)

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Multilocus heterozygosity, aggressive and feeding behaviour, plasma cortisol levels and growth rate were evaluated among three groups of juvenile Chinook salmon *Oncorhynchus tshawytscha*: diploid, triploid and mixed groups of diploid and triploid fish. There was no difference between diploid and triploid fish in measurements of heterozygosity calculated using seven microsatellite loci, and these measurements did not correlate with performance measurements including feeding rate and growth rate. Aggression trials that examined small groups of fish revealed that after 4 days together in tanks, triploid fish were significantly less aggressive during feeding than diploid fish or fish in mixed groups. At the end of the trials, however, plasma cortisol levels did not differ among the three groups. Thirty-day growth trials in duplicate tanks of 60 fish revealed no difference in growth rate among diploid, triploid and mixed groups, but plasma cortisol levels were significantly lower in triploid fish than in either diploid fish or the mixed fish. Overall, independent of the above differences in aggressive behaviour and cortisol levels, these results suggest similar performance in diploid and triploid Chinook salmon, and thus provide support for the viability of triploid Chinook salmon culture in commercial aquaculture.

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Key words: aggressive behaviour; cortisol; heterosis; heterozygosity; social stress; triploidy.

INTRODUCTION

Worldwide, salmonid aquaculture is a multi-billion pound industry that has led to the emergence of novel breeding and rearing techniques, including the culture of triploid fishes. Triploid salmonids are typically cultured because they lack the levels of reproductive hormones needed to initiate maturation, and thus they maintain the characteristics of immature fishes throughout life (Schafhauser-Smith & Benfey, 2001). Triploid salmonids thereby circumvent the decline in flesh quality and value associated with maturation, which provides

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greater freedom in the timing of harvest and reduces the risk that fishes will mature before they can be harvested. The rearing of triploid salmonids, however, has seen limited adoption in aquaculture, largely due to concerns about reduced growth rate and increased sensitivity to stressors relative to diploid fishes (Benfey, 1999).

In contrast to these concerns, triploid fishes may actually show improved performance relative to their diploid counterparts due to the presence of a third set of chromosomes. For example, it has been speculated that an improvement may arise from a higher rate of transcription owing to the presence of three copies of each gene instead of two (the 'gene dose' hypothesis: Zouros *et al.*, 1996; Magoulas *et al.*, 2000). There is some uncertainty, however, over the relative expression of the three chromosome sets because of possible silencing or unequal expression following polyploidization (Adams *et al.*, 2003). Indeed, one study on Chinook salmon *Oncorhynchus tshawytscha* (Walbaum) showed that diploid and triploid fish had similar levels of gene expression (Shrimpton *et al.*, 2007). Alternatively, triploids may show higher levels of heterozygosity and the potential associated fitness benefits from overdominance and reduced inbreeding depression (Leary *et al.*, 1985; Coltman & Slate, 2003). Positive correlations between growth rate and heterozygosity have been observed in Atlantic cod *Gadus morhua* L. (Pogson & Fevolden, 1998), Atlantic salmon *Salmo salar* L. (McCarthy *et al.*, 2003) and rainbow trout *Oncorhynchus mykiss* (Walbaum) (Danzmann *et al.*, 1987; Ferguson, 1992). In addition, in Pacific oysters *Crassostrea gigas* (Thunberg) higher heterozygosity was found in triploid than in diploid individuals, and heterozygosity was correlated with measurements of individual performance including feeding rate and absorption efficiency (Hawkins *et al.*, 2000). A similar analysis examining the relationship between heterozygosity and growth performance in triploid fishes has not yet been performed.

A second source of improved performance in triploid fishes may arise through behavioural differences relative to diploid fishes. Specifically, several studies have documented evidence for reduced aggression in triploid fishes. For example, higher levels of dorsal fin damage in triploid Atlantic salmon in groups of mixed ploidy were inferred to result from reduced aggression in triploid fish relative to diploid fish (Carter *et al.*, 1994). In rainbow trout, lower fin damage in triploid groups than in diploid groups also suggests lower aggression in triploids, although direct observations of behaviour did not confirm this difference (Wagner *et al.*, 2006). Lower aggression by triploid fish was also suggested in brook trout *Salvelinus fontinalis* (Mitchill), as triploid fish held in mixed groups had lower positions in the dominance hierarchy than diploid fish, although this trend weakened as the fish grew (O'Keefe & Benfey, 1997).

Reduced aggression in triploid fishes may contribute to performance in aquaculture *via* two mechanisms. First, subordinate individuals are typically excluded from feeding by dominant individuals and consequently show lower growth rate (McCarthy *et al.*, 1992; Kadri *et al.*, 1996). Reduced aggression may facilitate a more even distribution of food and consistent growth among all individuals. Second, subordinate fishes within social hierarchies consistently display elevated levels of the stress hormone cortisol (Øverli *et al.*, 1999; Sloman *et al.*, 2001; Höglund *et al.*, 2002). These elevated cortisol levels are in turn associated with reduced growth rate and survival through lowered appetite,

decreased conversion efficiency and compromised immune function (Gilmour *et al.*, 2005). Reduced aggression can weaken hierarchies and thereby reduce the physiological consequences for subordinate fishes.

The present study compared performance between diploid and triploid Chinook salmon. Specifically, this study evaluated differences in genetic diversity, aggressive and feeding behaviour, and plasma cortisol levels between diploid and triploid fish, and then assessed the degree to which these factors related to growth rate. Higher heterozygosity is predicted to occur in triploid fish, which will lead to improved feeding or growth in triploid fish provided there is a positive relationship between individual heterozygosity and performance. Observations of aggressive interactions were used to investigate behavioural differences between diploid and triploid fish, and this study thereby provides one of the first direct tests of reduced aggression in a triploid salmonid. Reduced aggression is predicted to lead to lower levels of the stress hormone cortisol, which may in turn lead to differences in feeding behaviour and growth rate. Growth observations are then compared to the mixed results found in previous comparisons of diploid and triploid fishes (Withler *et al.*, 1995; O'Keefe & Benfey, 1999; Johnson *et al.*, 2004; Wagner *et al.*, 2006). Finally, the potential merits of using triploid Chinook salmon in aquaculture are discussed.

MATERIALS AND METHODS

BROOD STOCK

Experiments were conducted using Chinook salmon at Yellow Island Aquaculture Ltd (YIAL), Quadra Island, British Columbia, Canada. Selective breeding at YIAL has eliminated the male sex chromosome, resulting in a homogametic (XX) stock. To create phenotypic males for breeding, a sub-set of newly hatched larvae are immersed in re-circulating, oxygenated water with the androgen 17 α -methyltestosterone ($400 \mu\text{g l}^{-1}$) for 2 h at 520 accumulated thermal units (ATUs) and for another 2 h at 620 ATUs (Heath *et al.*, 2002). Fish were spawned in October 2004 using standard aquaculture techniques (Sedgwick, 1982). A female's eggs were combined with the pooled milt of two males, with a different pair of males used for each female. Triploid fish were generated by hydrostatic pressure shock at 6.89×10^4 kPa for 5 min, 30 min after fertilization, and then pooled and transferred to Heath trays (Johnson *et al.*, 2004). Genetic analysis indicated that the triploidization success rate was *c.* 83%. For diploid fish, the fertilized eggs were immediately pooled and transferred to Heath trays with a constant water flow of *c.* 15 l min^{-1} . During incubation, dead eggs were regularly removed to prevent *Saprolegnia* spp. growth. After hatching in March 2005, the fry were transferred to 180 l circular tanks in the hatchery building at a density of 500 fish per tank and fed pellet food *ad libitum* (Micro Crumble Starter Feed; EWOS, Oslo, Norway).

GROWTH TRIALS

Growth trials were initiated on 18–20 July 2005, at which time diploid fish were between 3.2 and 9.7 g in mass and triploid fish were between 3.1 and 8.8 g in mass. Two weeks before the start of the growth trials, 360 fish were anaesthetized with buffered MS-222 and injected with an individually numbered passive integrated transponder (PIT) tag for identification. The PIT tag was inserted into the epaxial musculature anterior to the dorsal fin. Also at this time, the diet was changed to Silver Cup salmon

crumbles #3 (Nelson & Sons Inc., Murray, UT, U.S.A.). Three treatments were each replicated in two tanks, consisting of pure diploid fish ($n = 60$), pure triploid fish ($n = 60$) and a mixture of equal numbers of diploid fish and triploid fish ($n = 30$ of each). To begin a trial, the fish were anaesthetized with buffered MS-222 and their mass (M ; ± 0.05 g) and fork length (L_F ; ± 0.5 mm) were measured. The fish were then released into 180 l circular tanks. At 10, 20 and 30 days into each growth trial, all fish were again anaesthetized so that M and L_F could be re-measured. On day 30, 10 fish from each replicate of the diploid and triploid treatments and 20 fish from each replicate of the mixed treatment (10 diploid and 10 triploid fish) were quickly removed from the tanks and euthanized with an overdose of buffered MS-222 (0.3 g l^{-1}). The average time from immersion in the anaesthetic until blood was obtained was 4.4 min. Blood was sampled by severing the caudal fin and collected in a haematocrit tube. The time to obtain blood did not correlate with the cortisol concentration across individuals ($r_s = 0.08$, $P > 0.05$, $n = 110$). Care was taken to ensure that blood sampling procedures were standardized between treatments and experiments. The blood was then centrifuged to isolate the plasma, which was frozen at -20°C for later analysis of the cortisol concentration using a radioimmunoassay (Bernier *et al.*, 2008). The cortisol assay was validated with serial dilutions of Chinook salmon plasma that ran parallel to the standard curve.

Fish were hand-fed to satiation twice daily during the growth trials. The water temperature was maintained at 10°C , range $\pm 1^\circ \text{C}$, with a 16L:8D cycle. Specific growth rate (G) was calculated based on M for the interval from day 0–30, as $\ln M$ at day 30 minus $\ln M$ at day 0; g was then expressed as a % change day^{-1} .

AGGRESSION TRIALS

Following the 30 day growth trials, aggressive behaviour was examined in a randomly selected sub-set of the fish from the growth trials. The mean mass of triploid fish used for these trials was 7.7 g (range 2.8–15.5 g), and the mean mass of diploid fish was 6.9 g (range 2.7–12.5 g). The variance in mass within trials did not differ among the three trial compositions (ANOVA, d.f. = 2, 23, $P > 0.05$). Aggression trials were conducted in 24 l tanks with either eight fish per tank (3 l per fish; density equal to that in the growth trials) or two fish per tank (12 l per fish; density of one quarter that in the growth trials). Tanks were 300×400 mm in size, with a water depth of 200 mm and inflow of *c.* 0.5 l min^{-1} . The water temperature was maintained at 10°C , range $\pm 1^\circ \text{C}$, with a 16L:8D cycle. Four replicates were performed for each combination of density (*i.e.* two or eight) and treatment (*i.e.* diploid, triploid or mixed), for a total of 24 trials ($4 \times 2 \times 3$). Aggression trials were staggered to start between 19 and 26 August 2005, with individuals kept until needed in the 180 l tanks from the growth trials and fed pellet food (Silver Cup salmon crumbles #3) to satiation twice daily.

To begin a trial, fish were anaesthetized and then weighed, photographed with a Fujifilm (Tokyo, Japan) Finepix 4900 camera next to a ruler to determine L_F and tagged with a uniquely coloured 6 mm disc tag inserted below the dorsal fin to visually identify individuals. The fish were then placed into the test tank and allowed to acclimate for 24 h. A camcorder [Sony (Tokyo, Japan) DCR-TRV140 or DCR-TRV250] was then switched on and the fish were recorded for a total of 30.0 min, with an average of 12.5 min (range 10.3–16.6 min) recorded before feeding, followed by the addition of 0.1 g of food per fish and an additional recording of 17.5 min (range 13.6–19.7 min). Fish were maintained in these tanks and fed 0.1 g of food per fish, twice daily (*c.* 3% of body mass per fish per day) for the next 72 h, at which time they were recorded for an additional 30 min using the same protocol. Immediately following the second recording, all individuals in a trial were netted within 25 s and euthanized as a group with an overdose of buffered MS-222. Blood was sampled as above.

After all trials were completed, an observer who was blind to trial composition analysed the video recordings. Each trial was analysed for four observation periods, day 1 before feeding, day 1 during feeding, day 4 before feeding and day 4 during feeding.

The mean frequency of aggressive acts by each individual were calculated as the sum of charges (a rapid and direct movement towards another fish) and nips (a biting motion directed towards another fish) and divided by the duration of the observation period (Taylor & Larkin, 1986). Feeding rates were calculated from the number of food pellets obtained using the same method.

MICROSATELLITE ANALYSIS

DNA was extracted from 118 fish used in both the growth and aggression trials using a proteinase K digestion method (Neff *et al.*, 2000). Genetic variation was then evaluated using seven previously described microsatellite loci: *Omy325*, *Ots3*, *Ots68*, *Ots78b*, *Ots83b*, *Ots249* and *Ots432* (Olsen *et al.*, 1996; Williamson *et al.*, 2002). Each forward primer was tagged with a fluorescent dye, allowing the amplified products to be compared against size standards using an automated sequencer (LI-COR Biosciences, Lincoln, NE, U.S.A.). In one fish, genotype data were obtained for only five loci, while in three more fish genotype data were obtained for only six of seven microsatellite loci.

Two measurements of an individual's microsatellite variability were calculated: the proportion of loci at which they were heterozygous [multilocus heterozygosity (MLH)] and the average of the squared distances [in base pairs (bp)] between an individual's alleles at each locus (d^2) (Goldstein *et al.*, 1995; Coulson *et al.*, 1998). To facilitate MLH analysis of triploids, a locus was scored as heterozygous if an individual had either two or three alleles. For loci with three alleles, d^2 was calculated using the two alleles with the greatest divergence.

To assess the triploidization success rate, individuals with three alleles at one or more of the seven microsatellite loci were considered triploids (42 of 58 fish subjected to pressure shock). These 42 individuals represent a minimum estimate because some triploids will contain only one or two alleles at all seven of the microsatellite loci. To estimate the number of triploids that have only one or two alleles, the known triploids were first used to calculate the proportion of triploids having three alleles at each microsatellite locus. This proportion was then used to calculate the probability that a triploid individual would receive three alleles at none of the seven loci. This probability was then multiplied by the group size to determine the expected number of triploid fish not containing three alleles at any of the loci sampled, which was added to the number of known triploids to determine the overall estimate of triploidization success rate.

STATISTICAL ANALYSIS

MLH values were transformed using $\arcsin(\text{MLH}^{0.5})$ (Zar, 1999), and mean d^2 (\bar{d}^2) was transformed using $\log_{10}(\bar{d}^2)$ (Neff, 2004); these values were then compared between diploid and triploid fish using *t*-tests. In the aggression and feeding trials, data were \log_{10} transformed to achieve a normal distribution using either $\log_{10}(\text{frequency of aggressive acts} + 0.006)$ or $\log_{10}(\text{feeding rate} + 0.032)$ (Berry, 1987). The transformed data were then analysed using repeated measures ANOVA with observation period as the repeated measure, density (two and eight) and trial composition (diploid, triploid and mixed) as fixed factors, the relative mass of each fish as a covariate, and tank number as a nested factor within density and trial composition to address possible tank effects. Relative mass was calculated as an individual's mass minus the average mass of all fish in the tank. Differences indicated by the repeated measures ANOVA were identified using one-way ANOVA and Tukey's HSD test for each observation period. Because measurements of diploid and triploid individuals in mixed trials were not independent, these data were analysed as a single level in the ANOVAs. These data, however, were graphed separately and *t*-tests were used to examine differences between diploid and triploid individuals within the mixed trials. Observations from one trial were removed from analyses of behaviour on day 4 because the water flow to that tank failed at that time.

In the growth trials, differences in mass were analysed using a repeated measures ANOVA with day of trial (0, 10, 20 and 30) as the repeated measure, trial composition (diploid, triploid and mixed) as a fixed factor and tank number as a nested factor within trial composition to address possible tank effects. An ANCOVA was used to analyse differences in overall G based on trial composition, with tank number as a nested factor within trial composition, and initial mass as a covariate. Plasma cortisol concentrations were transformed using $\log_{10}(\text{cortisol concentration} + 0.1)$ to achieve a normal distribution. Plasma concentrations in the aggression trials were then analysed using an ANOVA with trial composition and density as fixed factors and tank as a nested factor within trial composition and density. Plasma concentrations in the growth trials were analysed using an ANOVA with trial composition as a fixed factor and tank as a nested factor within trial composition.

The effects of heterozygosity measures (MLH and \bar{d}^2) were examined through rank correlations with feeding rate on day 1, feeding rate on day 4 and with overall G during the growth trials. Rank correlations were likewise used to examine the relationship between the average frequency of aggression on day 4 (before and during feeding) and plasma cortisol levels and between plasma cortisol levels and individual feeding rate on day 4. Statistical analyses were performed using SPSS (v 14.0, SPSS Inc., Chicago, IL, U.S.A.) or JMP (v4.0.4, SAS Institute Inc., Cary, NC, U.S.A.). Means are reported ± 1 s.e. or with the range.

RESULTS

Multilocus heterozygosity did not differ between diploid fish (mean \pm s.e. MLH = 0.93 ± 0.01) and triploid fish (mean \pm s.e. MLH = 0.91 ± 0.01) (t -test, d.f. = 116, $P > 0.05$). Similarly, \bar{d}^2 also did not differ between diploid fish (mean \pm s.e. $\bar{d}^2 = 980 \pm 84$) and triploid fish (mean \pm s.e. $\bar{d}^2 = 1113 \pm 94$) (t -test, d.f. = 116, $P > 0.05$).

A repeated measures ANOVA identified eight terms with significant effects on the frequency of aggressive acts (Table I). The observation period had a significant effect on aggression, with aggressive acts significantly more frequent during observations on day 4 than during observations on day 1 (ANOVA, d.f. = 3, 457, $P < 0.001$). An interaction between density and observation period revealed a higher frequency of aggression in groups of two fish than in groups of eight fish on day 1 after feeding (ANOVA, d.f. = 1, 117, $P < 0.05$), but no trend during any other observation period (ANOVAs, all $P > 0.05$). The frequency of aggressive acts was also affected by the interaction between trial composition and observation period. Comparing fish based on trial composition, aggressive acts were significantly less frequent in triploid than in either diploid or mixed trials on day 4 during feeding (ANOVA, d.f. = 2, 107, $P < 0.05$; Fig. 1). Conversely, there were no differences in aggressive acts based on trial composition before feeding on day 1, during feeding on day 1 or before feeding on day 4 (ANOVAs: all $P > 0.05$). In mixed trials, the frequency of aggressive acts was not significantly different between diploid and triploid fish during any observation period (t -tests, all $P > 0.05$). Additionally, aggression was significantly affected by the three-way interaction between density, trial composition and observation period. Triploid fish were more aggressive in groups of two than in groups of eight on day 1 before feeding (ANOVA, d.f. = 1, 37, $P < 0.05$), a trend also seen in diploid fish on day 1 after feeding (ANOVA, d.f. = 1, 38, $P < 0.01$), but opposite to the trend in mixed fish, which were more aggressive in groups of eight than in groups of

TABLE I. Summary of repeated measures ANOVA results for frequency of aggressive acts and feeding rate during aggression trials of Chinook salmon

	Variable	d.f.	<i>F</i>	<i>P</i>
Aggression	Obs. per. (repeated measure)	2.1, 180	8.01	< 0.001
	Density	1, 86	0.39	0.54
	Obs. per. × density	2.1, 180	3.67	0.026
	Trial comp.	2, 86	0.63	0.53
	Obs. per. × trial comp.	4.2, 180	3.79	0.005
	Trial comp. × density	2, 86	1.18	0.31
	Obs. per. × trial comp. × density	4.2, 180	2.50	0.023
	Tank [density, trial comp.]	17, 86	1.80	0.041
	Obs. per. × tank [density, trial comp.]	35.6, 180	1.53	0.038
	Relative mass (covariate)	1, 86	4.01	0.048
Feeding	Obs. per. (repeated measure)	2.1, 180	5.09	0.006
	Density	1, 86	17.93	< 0.001
	Obs. per. × density	1, 86	18.97	< 0.001
	Trial comp.	1, 86	0.02	0.89
	Trial comp. × density	2, 86	3.07	0.052
	Obs. per. × trial comp.	2, 86	2.85	0.064
	Trial comp. × density	2, 86	0.07	0.93
	Obs. per. × trial comp. × density	2, 86	4.44	0.015
	Tank [density, trial comp.]	17, 86	1.62	0.076
	Obs. per. × tank [density, trial comp.]	17, 86	3.91	< 0.001
Relative mass (covariate)	1, 86	1.24	0.27	
Obs. per. × relative mass (covariate)	1, 86	17.99	< 0.001	

Obs. per., observation period; trial comp., trial composition; square parentheses denote nested factors; a Greenhouse–Geisser correction that leads to non-integer values was applied to the d.f. when the condition of sphericity was not met.

two before feeding on day 4 (ANOVA, d.f. = 1, 38, $P < 0.05$). Significant effects of both tank and the interaction between tank and observation period indicated differences in aggression among trials. An individual's mass relative to the other fish in the tank as well as the interaction between relative mass and observation period were the final factors that affected the frequency of aggressive acts. Relative mass did not predict aggression on day 1 before feeding (rank correlation, $r_s = 0.08$, $P > 0.05$, $n = 119$) or during feeding (rank correlation, $r_s = -0.02$, $P > 0.05$, $n = 119$), but there was a significant positive correlation between relative mass and the frequency of aggression on day 4 before feeding (rank correlation, $r_s = 0.23$, $P < 0.05$, $n = 110$) and during feeding (rank correlation, $r_s = 0.30$, $P < 0.01$, $n = 110$). All other factors included in the model were not significant.

Similarly, a repeated measures ANOVA identified five terms with significant effects on feeding rate (Table I). Feeding rate differed significantly among observation periods, with higher feeding on day 4 than on day 1 (ANOVA, d.f. = 1, 227, $P = 0.001$). Feeding rate was significantly affected by density (Fig. 2), with higher feeding rates in groups of two than in groups of eight (ANOVA, d.f. = 1, 227, $P < 0.001$). Feeding rate was also significantly affected

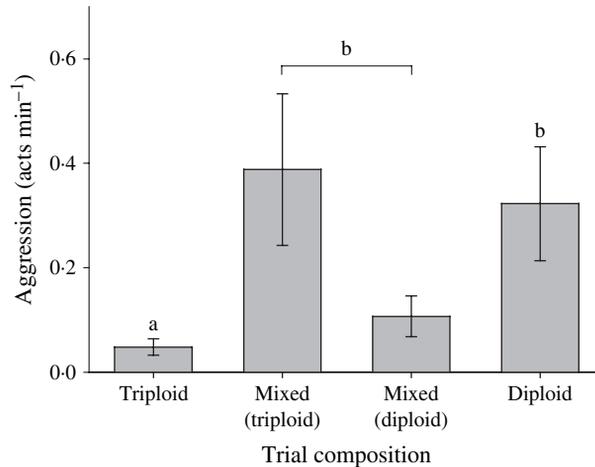


FIG. 1. Frequency of aggressive acts in diploid and triploid Chinook salmon. Means \pm S.E. are presented for observations made during feeding, 4 days after the start of the trial. Aggressive acts were significantly less frequent in triploid than in diploid or mixed trials. Because no significant differences in aggression were observed based on density, data from the groups of two and eight fish were pooled together. Different lower case letters are used to indicate significant differences ($P < 0.05$) between groups.

by the three-way interaction between density, trial composition and observation period. In groups of eight fish measured on day 4, feeding rate was significantly lower in triploid groups than in diploid groups (ANOVA, d.f. = 2, 83, $P < 0.05$; Fig. 2). No trend in feeding rate based on trial composition was observed in groups of eight fish on day 1 or in groups of two fish on either day 1 or day 4 (ANOVAs, all $P > 0.05$). Feeding rate was not significantly affected by trial composition, and in mixed trials, feeding rate was not significantly different between diploid and triploid fish on either day 1 or day 4 (t -tests, both $P > 0.005$). A significant effect of the interaction between tank and observation period indicated differences in feeding rate among trials. Relative mass within a tank was not a significant predictor of feeding rate, although a significant effect of the interaction between relative mass and observation period was observed. On day 1, feeding rates were higher in the smaller fish within a tank (rank correlation, $r_s = -0.20$, $P < 0.05$, $n = 119$), but no trend was observed on day 4 (rank correlation, $r_s = 0.13$, $P > 0.05$, $n = 110$). All other factors included in the model were not significant.

Mass increased significantly as the 30 day growth trials progressed, with significant differences in mass based on trial composition and the interaction between trial composition and day [Table II and Fig. 3(a)]. Triploid fish were significantly heavier than diploid or mixed fish at day 0 (ANOVA, d.f. = 2, 340, $P < 0.001$) and day 10 (ANOVA, d.f. = 2, 334, $P < 0.001$). Both diploid and triploid fish were significantly heavier than mixed fish at day 20 (ANOVA, d.f. = 2, 301, $P < 0.01$) and day 30 (ANOVA, d.f. = 2, 264, $P < 0.01$). In mixed trials, diploid fish were significantly heavier than triploid fish at the start of the trial (t -test, d.f. = 118, $P < 0.05$), but this difference was not found on

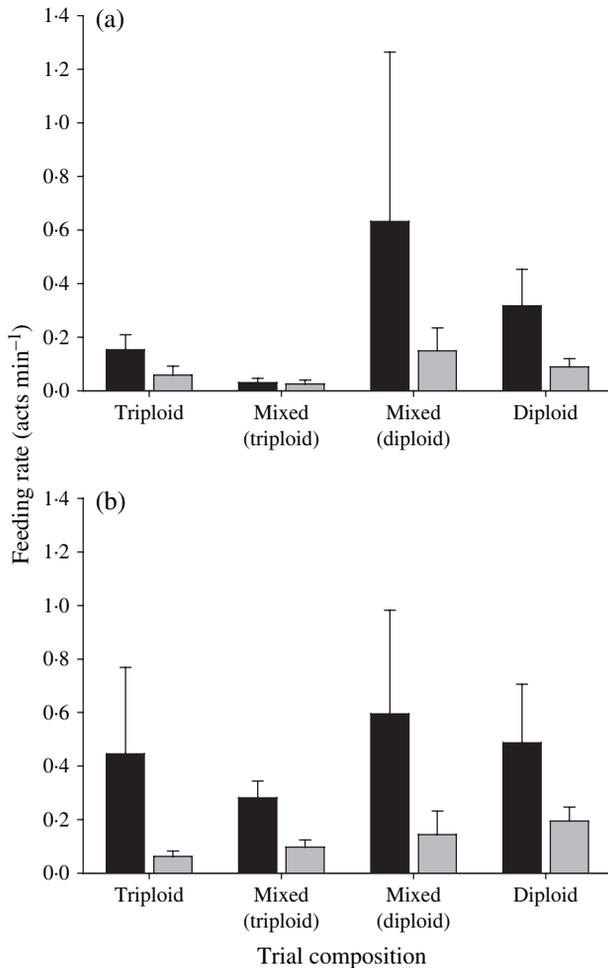


FIG. 2. Feeding rate in diploid and triploid Chinook salmon. Means \pm S.E. are presented for day (a) 1 and (b) 4, with observations from groups of two fish (■) and groups of eight fish (◻) shown separately. There were no significant differences ($P > 0.05$) in feeding rate based on trial composition, although feeding rate was significantly higher ($P < 0.05$) in groups of two fish than in groups of eight fish.

later sampling dates (all $P > 0.05$). In addition to the effects of trial composition, significant differences in mass were also observed among tanks (Table II). Nevertheless, overall G did not differ based on tank (ANCOVA, d.f. = 3, 245, $P > 0.05$), initial mass (ANCOVA, d.f. = 1, 245, $P > 0.05$) or trial composition [ANOVA, d.f. = 2, 245, $P > 0.05$; Fig. 3(b)]. Likewise, G in mixed trials did not differ between diploid fish and triploid fish (t -test, d.f. = 78, $P > 0.05$). Similar results were found when L_F and G based on L_F were analysed.

During the aggression trials, cortisol levels did not differ based on density (ANOVA, d.f. = 1, 87, $P > 0.05$), trial composition [ANOVA, d.f. = 2, 87, $P > 0.05$; Fig. 4(a)] or the interaction between density and trial composition (ANOVA, d.f. = 2, 87, $P > 0.05$); however, cortisol levels did differ significantly

TABLE II. Summary of repeated measures ANOVA for mass during growth trials of Chinook salmon

	Variable	d.f.	<i>F</i>	<i>P</i>
Mass	Day (repeated measure)	1·4, 328	37·40	<0·001
	Trial composition	2, 239	6·68	0·002
	Day × trial composition	2·7, 328	3·36	0·022
	Tank [trial composition]	3, 239	6·89	<0·001
	Day × tank [trial composition]	4·1, 328	2·28	0·058

Square parentheses denote nested factors; a Greenhouse–Geisser correction that leads to non-integer values was applied to the d.f. when the condition of sphericity was not met.

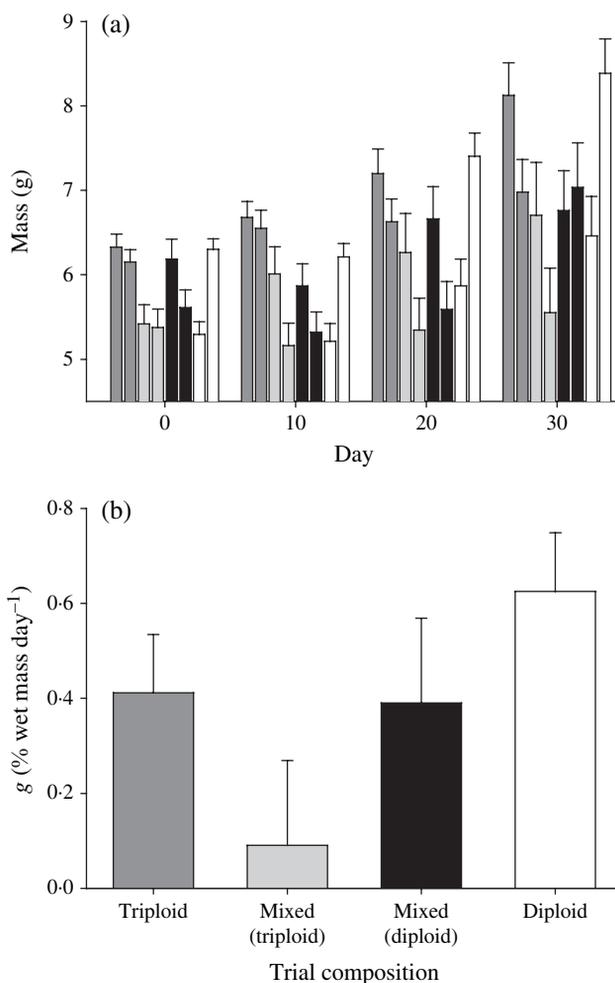


FIG. 3. Means \pm s.e. mass (*M*) and growth rate (*G*) in triploid (■), mixed (triploid) (□), mixed (diploid) (■) and diploid (□) Chinook salmon. (a) Values for *M* on each sampling day and (b) for overall *G* during the 30 day trials. (a) data are presented with each replicate tank as a separate bar. In (b), *G* did not differ among tanks, so these data are presented as a single bar for each trial composition.

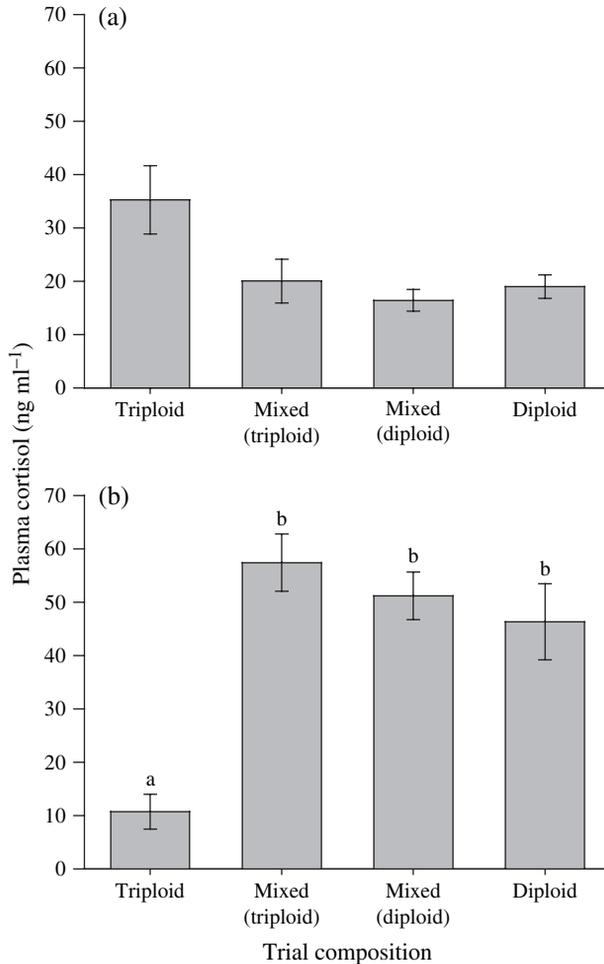


FIG. 4. Means \pm S.E. cortisol concentrations in diploid and triploid Chinook salmon for the (a) aggression and (b) growth trials. In the aggression trials, plasma cortisol levels did not differ ($P > 0.05$) based on trial composition. During the growth trials cortisol levels were significantly lower ($P < 0.05$) in triploid trials than in either diploid or mixed trials. Different lower case letters are used to indicate significant differences ($P < 0.05$) between groups.

among tanks (ANOVA, d.f. = 17, 87, $P < 0.001$). In contrast, during the growth trials plasma cortisol levels were significantly lower in triploid trials than in either diploid or mixed trials [ANOVA, d.f. = 2, 70, $P < 0.001$; Fig. 4(b)] and also differed significantly among tanks (ANOVA, d.f. = 3, 70, $P < 0.01$). In mixed trials, plasma cortisol levels were not significantly different between diploid and triploid fish during either the aggression or growth trials (t -tests, both $P > 0.05$).

Comparing performance measurements in individual fish, there was no significant relationship between MLH and overall g (rank correlation, $r_s = 0.04$, $P > 0.05$, $n = 92$), MLH and feeding rate on day 1 (rank correlation, $r_s = 0.03$, $P > 0.05$, $n = 118$) or MLH and feeding rate on day 4 (rank correlation,

$r_s = 0.06$, $P > 0.05$, $n = 110$). Likewise, there was no significant relationship between \bar{d}^2 and overall G (rank correlation, $r_s = 0.08$, $P > 0.05$, $n = 92$), \bar{d}^2 and feeding rate on day 1 (rank correlation, $r_s = -0.03$, $P > 0.05$, $n = 118$) or \bar{d}^2 and feeding rate on day 4 (rank correlation, $r_s = 0.03$, $P > 0.05$, $n = 110$). Individuals that performed aggressive acts at a higher frequency on day 4 (average of aggressive acts performed before and during feeding) had lower levels of cortisol (rank correlation, $r_s = -0.329$, $P < 0.001$, $n = 110$). High plasma cortisol levels were in turn correlated with lower feeding rates on day 4 (rank correlation, $r_s = -0.279$, $P < 0.01$, $n = 110$).

DISCUSSION

This study examined genetic diversity, aggressive behaviour and plasma cortisol as possible determinants of performance in diploid and triploid Chinook salmon. Measurements of heterozygosity did not differ between diploid and triploid fish, and neither of these measurements correlated with growth or feeding rate. The aggression trials revealed a lower frequency of aggressive acts during feeding in triploid fish compared to diploid fish, but no difference in aggression before feeding. Plasma cortisol levels did not differ among treatments in the aggression trials, but during the growth trials the cortisol levels were significantly lower in triploid fish than in either diploid or mixed fish. Aggressive fish had lower cortisol levels, and lower cortisol levels were in turn associated with improved feeding rate. Neither overall growth rate nor feeding rate, however, differed between diploid and triploid fish as a consequence of the reduced aggression and lower cortisol levels in triploid fish.

Heterozygosity may be a potential source of performance differences among individuals or between diploid and triploid fishes. Indeed, some studies have identified a positive relationship between growth and heterozygosity (Ferguson, 1992; Pogson & Fevolden, 1998), although others have found no relationship (Borrell *et al.*, 2004; Pujolar *et al.*, 2006). In the present study, heterozygosity measurements did not correlate with individual performance, nor did heterozygosity differ between diploid and triploid fish. Triploidy probably failed to increase heterozygosity because of the high heterozygosity in the diploid population ($MLH > 0.90$), which was already close to the maximum level of 1.0. Likewise, the high levels of heterozygosity found in all fish may have reduced the ability to detect a relationship between individual heterozygosity and performance because of reduced statistical power. The failure to detect a trend is consistent with recent reports that morphological traits like growth are not consistently associated with microsatellite heterozygosity across studies (Wang *et al.*, 2002; Coltman & Slate, 2003).

Variation in behaviour may also contribute to differences in performance between diploid and triploid fishes. In the present study, triploid Chinook salmon held in pure groups displayed significantly lower levels of aggression during feeding than diploid groups. Although this trend was absent in observations collected on the first day after handling the fish, the reduced feeding and aggression seen in all fish at this time suggests that a single day of acclimation was not enough for the fish to resume typical patterns of behaviour. Indeed, the lower levels of aggression during feeding 4 days after handling are consistent

with previous findings of reduced aggression in triploid fishes (Kavumpurath & Pandian, 1992; Carter *et al.*, 1994). This difference in aggression may relate to cognitive differences resulting from the reduced cell number in triploid fishes (due to increased cell size) (Benfey, 1999). Alternatively, as androgens have a well-known role as regulators of aggressive behaviour (Nelson, 2005), lower reported androgen concentrations in triploid fishes than in diploid fishes may lead to reduced aggression (Benfey, 1999; Schafhauser-Smith & Benfey, 2001). Unfortunately, the present study was unable to test this hormonal explanation of behavioural differences due to insufficient plasma to measure both cortisol and androgen levels. It is interesting to note, however, that triploid fish held in groups of mixed ploidy displayed elevated levels of aggression that were comparable to diploid fish (see Fig. 1). This trend suggests that triploid fish are capable of high aggression when housed with more aggressive fish, although they may not experience the external cues (*e.g.* other aggressive individuals) that lead to higher aggression when housed with only triploid fish.

Behavioural differences between diploid and triploid fishes are likely to result in differences in the social environment experienced by these two groups, which in turn may lead to differences in the stress they experience. A majority of studies have shown that the stress response, as measured by plasma cortisol levels, is generally comparable between diploid and triploid fishes (Biron & Benfey, 1994; Sadler *et al.*, 2000; Leggatt *et al.*, 2006). A few studies, however, have shown lower cortisol levels in triploid than in diploid fishes (Benfey & Biron, 2000; Peruzzi *et al.*, 2005). Results of the growth trials conducted here likewise demonstrated lower cortisol levels in triploid fish than in diploid fish or the mixed groups. In salmonids, aggressive fishes that achieve dominance have low plasma cortisol levels, whereas subordinate fishes typically have elevated levels (Øverli *et al.*, 1999; Sloman *et al.*, 2001; Höglund *et al.*, 2002; Bernier *et al.*, 2008). This result parallels that found in the aggression trials conducted here, in which lower cortisol levels were found in more aggressive fish. The lower aggression in groups of triploid fish observed here may have resulted in weaker dominance hierarchies and less cortisol elevation in subordinate fish, thus leading to the lower overall cortisol levels in the triploid fish than in the diploid or mixed fish. The failure to detect a trend in cortisol levels following the aggression trials may indicate that the effects of social environment on stress are most pronounced after a prolonged period in a group. Regardless, accumulating data now suggest a difference in cortisol concentrations between diploid fish and triploid fishes.

Higher stress may lead to differences in 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 the present study, an effect of stress on feeding was observed during the aggression trials, with lower feeding rates in fish with higher cortisol levels. Despite significant differences in cortisol levels among groups, however, no significant difference in overall feeding or growth rate was observed between diploid and triploid fish. Although the 30 day duration for growth measurements may have limited the power to detect subtle differences between groups, this observation is consistent with previous studies that similarly failed to provide clear support for a difference in growth rate between diploid and triploid fishes (Benfey,

1999). For example, in juvenile coho salmon *Oncorhynchus kisutch* (Walbaum), diploid fish grew faster than triploid fish (Withler *et al.*, 1995), while in juvenile rainbow trout the opposite result was found (Sheehan *et al.*, 1999; Wagner *et al.*, 2006). In juvenile Atlantic salmon, diploid fish initially grew faster than triploid fish, but this difference did not persist for > 5 months in any study (Carter *et al.*, 1994; McGeachy *et al.*, 1995; Cotter *et al.*, 2002). In brook trout and in Chinook salmon, no difference in juvenile growth rate was observed between diploid and triploid fishes (O'Keefe & Benfey, 1999; Johnson *et al.*, 2004). Thus, there appears to be no consistent difference in growth performance of diploid and triploid fishes.

Ultimately, the widespread culture of triploid fishes in salmonid aquaculture depends on whether these fishes can be farmed more profitably than diploid fishes. Reduced maturation in triploid females represents a considerable advantage because it provides greater freedom in the timing of harvest and reduces the risk that fishes will mature before they can be harvested. The present study contributes to the growing evidence that growth rate in triploid fishes is comparable to that in diploid fishes and thus there may be no reduction in performance by using triploid fishes. This study also identified additional traits that may improve performance in triploid fishes, including reduced aggression and differences in the stress response as measured by plasma cortisol levels. In particular, lower cortisol levels found in the triploid fishes may prove a source of additional benefits as elevated cortisol is generally associated with increased susceptibility to pathogens (Gilmour *et al.*, 2005). Behavioural and hormonal differences were absent in triploid fish held in mixed groups, which suggests that triploid fish benefit from being reared apart from diploid fish. Overall, comparable performance coupled with lower maturation by triploid fish provides further evidence that triploid fish culture can be a profitable addition to salmonid aquaculture.

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References

- Adams, K. L., Cronn, R., Percifield, R. & Wendel, J. F. (2003). Genes duplicated by polyploidy show unequal contributions to the transcriptome and organ-specific reciprocal silencing. *Proceedings of the National Academy of Sciences of the United States of America* **100**, 4649–4654.
- Benfey, T. J. (1999). The physiology and behavior of triploid fishes. *Reviews in Fisheries Science* **7**, 39–67.
- Benfey, T. J. & Biron, M. (2000). Acute stress response in triploid rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*). *Aquaculture* **184**, 167–176.
- Bernier, N. J., Bedard, N. & Peter, R. E. (2004). Effects of cortisol on food intake, growth, and forebrain neuropeptide Y and corticotropin-releasing factor gene expression in goldfish. *General and Comparative Endocrinology* **135**, 230–240.

- Bernier, N. J., Alderman, S. L. & Bristow, E. N. (2008). Heads or tails? Stressor-specific expression of corticotropin-releasing factor and urotensin I in the preoptic area and caudal neurosecretory system of rainbow trout. *Journal of Endocrinology* **196**, 637–648.
- Berry, D. A. (1987). Logarithmic transformations in ANOVA. *Biometrics* **43**, 439–456.
- Biron, M. & Benfey, T. J. (1994). Cortisol, glucose and hematocrit changes during acute stress, cohort sampling, and the diel cycle in diploid and triploid brook trout (*Salvelinus fontinalis* Mitchell). *Fish Physiology and Biochemistry* **13**, 153–160.
- Borrell, Y. J., Pineda, H., McCarthy, I., Vázquez, E., Sánchez, J. A. & Lizana, G. B. (2004). Correlations between fitness and heterozygosity at allozyme and microsatellite loci in the Atlantic salmon, *Salmo salar* L. *Heredity* **92**, 585–593.
- Carter, C. G., McCarthy, I. D., Houlihan, D. F., Johnstone, R., Walsingham, M. V. & Mitchell, A. I. (1994). Food consumption, feeding behavior, and growth of triploid and diploid Atlantic salmon, *Salmo salar* L., parr. *Canadian Journal of Zoology* **72**, 609–617.
- Coltman, D. W. & Slate, J. (2003). Microsatellite measures of inbreeding: a meta-analysis. *Evolution* **57**, 971–983.
- Cotter, D., O'Donovan, V., Drumm, A., Roche, N., Ling, E. N. & Wilkins, N. P. (2002). Comparison of freshwater and marine performances of all-female diploid and triploid Atlantic salmon (*Salmo salar* L.). *Aquaculture Research* **33**, 43–53.
- Coulson, T. N., Pemberton, J. M., Albon, S. D., Beaumont, M., Marshall, T. C., Slate, J., Guinness, F. E. & Clutton-Brock, T. H. (1998). Microsatellites reveal heterosis in red deer. *Proceedings of the Royal Society of London Series B* **265**, 489–495.
- Danzmann, R. G., Ferguson, M. M. & Allendorf, F. W. (1987). Heterozygosity and oxygen-consumption rate as predictors of growth and developmental rate in rainbow trout. *Physiological Zoology* **60**, 211–220.
- De Boeck, G., Alsop, D. & Wood, C. (2001). Cortisol effects on aerobic and anaerobic metabolism, nitrogen excretion, and whole-body composition in juvenile rainbow trout. *Physiological and Biochemical Zoology* **74**, 858–868.
- Ferguson, M. M. (1992). Enzyme heterozygosity and growth in rainbow trout: genetic and physiological explanations. *Heredity* **68**, 115–122.
- Gilmour, K. M., DiBattista, J. D. & Thomas, J. B. (2005). Physiological causes and consequences of social status in salmonid fish. *Integrative and Comparative Biology* **45**, 263–273.
- Goldstein, D. B., Linares, A. R., Cavalli-Sforza, L. L. & Feldman, M. W. (1995). An evaluation of genetic distances for use with microsatellite loci. *Genetics* **139**, 463–471.
- Gregory, T. R. & Wood, C. M. (1999). The effects of chronic plasma cortisol elevation on the feeding behaviour, growth, competitive ability, and swimming performance of juvenile rainbow trout. *Physiological and Biochemical Zoology* **72**, 286–295.
- Hawkins, A. J. S., Magoulas, A., Héral, M., Bougrier, S., Naciri-Graven, Y., Day, A. J. & Kotoulas, G. (2000). Separate effects of triploidy, parentage and genomic diversity upon feeding behaviour, metabolic efficiency and net energy balance in the Pacific oyster *Crassostrea gigas*. *Genetical Research* **76**, 273–284.
- Heath, D. D., Rankin, L., Bryden, C. A., Heath, J. W. & Shrimpton, J. M. (2002). Heritability and Y-chromosome influence in the jack male life history of Chinook salmon (*Oncorhynchus tshawytscha*). *Heredity* **89**, 311–317.
- Höglund, E., Balm, P. H. M. & Winberg, S. (2002). Behavioural and neuroendocrine effects of environmental background colour and social interaction in Arctic charr (*Salvelinus alpinus*). *Journal of Experimental Biology* **205**, 2535–2543.
- Johnson, R. M., Shrimpton, J. M., Heath, J. W. & Heath, D. D. (2004). Family, induction methodology and interaction effects on the performance of diploid and triploid Chinook salmon (*Oncorhynchus tshawytscha*). *Aquaculture* **234**, 123–142.
- Kadri, S., Huntingford, F. A., Metcalfe, N. B. & Thorpe, J. E. (1996). Social interactions and the distribution of food among one-sea-winter Atlantic salmon (*Salmo salar*) in a sea-cage. *Aquaculture* **139**, 1–10.
- Kavumpurath, S. & Pandian, T. J. (1992). Effects of induced triploidy on aggressive display in the fighting fish, *Betta splendens* Regan. *Aquaculture and Fisheries Management* **23**, 281–290.

- Leary, R. F., Allendorf, F. W., Knudsen, K. L. & Thorgaard, G. H. (1985). Heterozygosity and developmental stability in gynogenetic diploid and triploid rainbow trout. *Heredity* **54**, 219–225.
- Leggatt, R. A., Scheer, K. W., Afonso, L. O. B. & Iwama, G. K. (2006). Triploid and diploid rainbow trout do not differ in their stress response to transportation. *North American Journal of Aquaculture* **68**, 1–8.
- Magoulas, A., Kotoulas, G., Gérard, A., Naciri-Graven, Y., Dermitzakis, E. & Hawkins, A. J. S. (2000). Comparison of genetic variability and parentage in different ploidy classes of the Japanese oyster *Crassostrea gigas*. *Genetical Research* **76**, 261–272.
- McCarthy, I. D., Carter, C. G. & Houlihan, D. F. (1992). The effect of feeding hierarchy on individual variability in daily feeding of rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Biology* **41**, 257–263.
- McCarthy, I. D., Sánchez, J. A. & Blanco, G. (2003). Allozyme heterozygosity, date of first feeding and life history strategy in Atlantic salmon. *Journal of Fish Biology* **62**, 341–357.
- McGeachy, S. A., Benfey, T. J. & Friars, G. W. (1995). Freshwater performance of triploid Atlantic salmon (*Salmo salar*) in New Brunswick aquaculture. *Aquaculture* **137**, 333–341.
- Neff, B. D. (2004). Mean d^2 and divergence time: transformations and standardizations. *Journal of Heredity* **95**, 165–171.
- Neff, B. D., Fu, P. & Gross, M. R. (2000). Microsatellite multiplexing in fish. *Transactions of the American Fisheries Society* **129**, 584–593.
- Nelson, R. J. (2005). *An Introduction to Behavioral Endocrinology*. Sunderland, MA: Sinauer Associates.
- O'Keefe, R. A. & Benfey, T. J. (1997). The feeding response of diploid and triploid Atlantic salmon and brook trout. *Journal of Fish Biology* **51**, 989–997.
- O'Keefe, R. A. & Benfey, T. J. (1999). Comparative growth and food consumption of diploid and triploid brook trout (*Salvelinus fontinalis*) monitored by radiography. *Aquaculture* **175**, 111–120.
- Olsen, J. B., Wenburg, J. K. & Bentzen, P. (1996). Semiautomated multilocus genotyping of Pacific salmon (*Oncorhynchus* spp.) using microsatellites. *Molecular Marine Biology and Biotechnology* **5**, 259–272.
- Øverli, Ø. Harris, C. A. & Winberg, S. (1999). Short-term effects of fights for social dominance and the establishment of dominant-subordinate relationships on brain monoamines and cortisol in rainbow trout. *Brain Behavior and Evolution* **54**, 263–275.
- Peruzzi, S., Varsamos, S., Chatain, B., Fauvel, C., Menu, B., Falguière, J. C., Sévère, A. & Flik, G. (2005). Haematological and physiological characteristics of diploid and triploid sea bass, *Dicentrarchus labrax* L. *Aquaculture* **244**, 359–367.
- Pogson, G. H. & Fevolden, S. E. (1998). DNA heterozygosity and growth rate in the Atlantic cod *Gadus morhua* (L.). *Evolution* **52**, 915–920.
- Pujolar, J. M., Maes, G. E., Vancoillie, C. & Volckaert, F. A. M. (2006). Environmental stress and life-stage dependence on the detection of heterozygosity–fitness correlations in the European eel, *Anguilla anguilla*. *Genome* **49**, 1428–1437.
- Sadler, J., Pankhurst, N. W., Pankhurst, P. M. & King, H. (2000). Physiological stress responses to confinement in diploid and triploid Atlantic salmon. *Journal of Fish Biology* **56**, 506–518.
- Schafhauser-Smith, D. & Benfey, T. J. (2001). The reproductive physiology of three age classes of adult female diploid and triploid brook trout (*Salvelinus fontinalis*). *Fish Physiology and Biochemistry* **25**, 319–333.
- Sedgwick, S. D. (1982). *The Salmon Handbook*. London: André Deutsch Ltd.
- Sheehan, R. J., Shasteen, S. P., Suresh, A. V., Kapuscinski, A. R. & Seeb, J. E. (1999). Better growth in all-female diploid and triploid rainbow trout. *Transactions of the American Fisheries Society* **128**, 491–498.
- Shrimpton, J. M., Sentlinger, A. M. C., Heath, J. W., Devlin, R. H. & Heath, D. D. (2007). Biochemical and molecular differences in diploid and triploid ocean-type Chinook salmon (*Oncorhynchus tshawytscha*) smolts. *Fish Physiology and Biochemistry* **33**, 259–268.

- Sloman, K. A., Metcalfe, N. B., Taylor, A. C. & Gilmour, K. M. (2001). Plasma cortisol concentrations before and after social stress in rainbow trout and brown trout. *Physiological and Biochemical Zoology* **74**, 383–389.
- Taylor, E. B. & Larkin, P. A. (1986). Current response and agonistic behavior in newly emerged fry of Chinook salmon, *Oncorhynchus tshawytscha*, from ocean- and stream-type populations. *Canadian Journal of Fisheries and Aquatic Sciences* **43**, 565–573.
- Wagner, E. J., Arndt, R. E., Routledge, M. D., Latremouille, D. & Mellenthin, R. F. (2006). Comparison of hatchery performance, agonistic behavior, and poststocking survival between diploid and triploid rainbow trout of three different Utah strains. *North American Journal of Aquaculture* **68**, 63–73.
- Wang, S. Z., Hard, J. J. & Utter, F. (2002). Genetic variation and fitness in salmonids. *Conservation Genetics* **3**, 321–333.
- Williamson, K. S., Cordes, J. F. & May, B. (2002). Characterization of microsatellite loci in Chinook salmon (*Oncorhynchus tshawytscha*) and cross-species amplification in other salmonids. *Molecular Ecology Notes* **2**, 17–19.
- Withler, R. E., Beacham, T. D., Solar, I. I. & Donaldson, E. M. (1995). Freshwater growth, smelting, and marine survival and growth of diploid and triploid coho salmon (*Oncorhynchus kisutch*). *Aquaculture* **136**, 91–107.
- Zar, J. H. (1999). *Biostatistical Analysis*. Upper Saddle River, NJ: Prentice Hall.
- Zouros, E., Thiriou-Quievreux, C. & Kotoulas, G. (1996). The negative correlation between somatic aneuploidy and growth in the oyster *Crassostrea gigas* and implications for the effects of induced polyploidization. *Genetical Research* **68**, 109–116.